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Analyzing TEMPO-Oxidized Cellulose Fiber Morphology: New Insights into Optimization of the Oxidation Process and Nanocellulose Dispersion Quality

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ABSTRACT: High-definition automated optical analysis was used to observe the morphological changes of bleached hardwood dissolving pulp during oxidation mediated by (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO). The effects of TEMPO treatment kinetics on fiber morphology and fibrillation degree at a constant reagent loading were studied. The pulp underwent significant swelling, and carboxyl groups were introduced in which the fiber cell walls loosened, which contributed to fibrillation and subsequently nanocellulose dispersion quality in terms of both viscosity and visible light transmittance. The use of an automated optical fiber analyzer facilitates process control, as it allows controlling the fiber morphology and preventing major losses in the form of pulp fines stemming from an unnecessarily long TEMPO oxidation time.

KEYWORDS: biomass, fiber morphology, nanocellulose, optical analysis, TEMPO oxidation

INTRODUCTION

The production of cellulose nanofibrils is largely based on certain micronization techniques, namely, microfluidization, high-pressure homogenization, or high-speed friction grinding, using, for example, a Masuko Supermasscolloider.1−3 Although these mechanical means are efficient at producing the desired nanofibrillated material, several pretreatment strategies have been employed to facilitate the fibrillation of feedstock (i.e., cellulose fibers) and lower the energy demands of the fibrillation process.4 Partial enzymatic hydrolysis,5,6 (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) or similar surface oxidations,7−10 carboxymethylation,11,12 or fiber swelling in nonderivatizing solvents13,14 are among the most common pretreatments investigated to render the cell wall less resistant to mechanical forces and, consequently, allow the liberation of nanofibrils.15,16

One of the most popular chemical pretreatments in the manufacture of cellulose nanofibrils is oxidation mediated by TEMPO. It is generally accepted that the main mechanism facilitating fibrillation in TEMPO-oxidized cellulose is a negative surface charge introduced to the fibers.8 The electrostatic repulsion forces help liberate individual fibrils and keep them in a stable colloidal dispersion.17 At a high enough carboxyl group content, even a mild mechanical treatment is enough to fibrillate the pretreated cellulose fibers, at least partially. Recently, it was also proven that self-fibrillation or autorelease of nanofibrils is feasible, provided that a sufficient surface charge is present.18 Similar results have been previously noted in the case of cellulose sulfonation during partial hydrolysis with sulfuric acid.19,20 TEMPO oxidation of native cellulosics has been widely studied.21 Studies examining the influence of carboxyl content on the fibrillation characteristics22 and the use of alternative reagents,23−27 techniques such as ultrasound to assist the oxidation kinetics,28,29 pulp pretreatments to enhance oxidation,30 different cellulose feedstocks,31 and variations thereof have been published in the last decade. A few studies have focused on how the TEMPO oxidation process affects fiber morphology. Besbes et al.,22 for example, used an automated fiber analyzer to study the most basic fiber morphologies, their width and length. Similarly, Chinga-Carrasco et al.32,33 used an optical analyzer to determine the residual fiber fractions in fibrillated cellulose samples (cellulose nanofibril dispersions) using a STFI FiberMaster machine. FiberMaster can analyze the basic characteristics of the fibers, such as fiber length, width, fines, and deformations, such as fiber shape, curl, and kink with a coarse image resolution of 26 μm and maximum fine resolution of 6 μm.34 Rol et al.35 used a

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MorFi (4 μm resolution) optical analyzer to observe the fiber content and generation of fines in relation to a twin-screw extrusion process of producing phosphorylated nanocelluloses as well. However, the higher optical resolution of the recently launched Valmet UHD machine can gauge and classify the finer fractions that are produced during pretreatment and during fibrillation in more detail. Advances in charge-coupled device (CCD) technology now allow a more detailed observation of more parameters that include quantification of fiber particles such as flake-like and fibrillar-type fines as well as the properties of the fiber surface (fibrillation).

While TEMPO oxidation is rather forgiving on a laboratory scale, where the processing efficiency is not of utmost importance, the quality of the final product (i.e., cellulose nanofibrils) in its industrial applications demands process optimization and product consistency control in terms of both carboxyl group content and fiber morphology. While there are no applicable online measurement tools to quantify the liberation of nanofibrils from lignocelluloses, the equipment to assess fiber reactivity to pretreatment chemicals or mechanical disintegration has been developed substantially during the recent years. Nothing short of electron microscopy suffices to visualize cellulose nanofibrils but micrometer-scale changes in fiber structure and liberation of fibrils as a result of delamination and swelling are sound indicators of chemical reactivity that can already be monitored.

Excessive pretreatment leading to unwarranted disintegration of fibers and generation of fines is a potential problem for the subsequent processing stages. To optimize the pretreatment's effect on the fiber cell wall and prevent significant generation of fines in order to keep the treated pulp quality and yield at a high level, morphological analysis of fibers and measurement of fine content can be beneficial. Connecting these as online analyses to the micronized pulp properties allows more precise process control on an industrial scale.

In this study, we investigated the effect of TEMPO treatment kinetics on the quality parameters of TEMPO-oxidized cellulose fibers in terms of carboxyl group content, fiber characteristics, and fibrillation degree of the pulp. For the first time, optical analysis of fibers and nanofibrillated products was performed on a micrometer scale (effective resolution of 1 μm) to quantify the fiber changes (fiber width and length and
Experimental Section

Materials. The never-dried softwood dissolving pulp (SWDP) was supplied by Domjö Fabriker AB (Omröksöviken, Sweden) and had a minimum of 92% α-cellulose content, less than 8% hemicelluloses, and 0.2% lignin. Additionally, sheets of bleached hardwood Kraft pulp (BSKP) and hardwood dissolving pulp (HWDP), supplied by Stora Enso Ltd. (Finland), were also subjected to TEMPO mediated oxidation. The chemical composition of BSKP and HWDP was as follows: BSKP consisted of 75% α-cellulose, 24.3% hemicelluloses, and 0.7% lignin. HWDP on the other hand had 95% α-cellulose, 4.8% hemicellulose, and 0.2% lignin. TEMPO reagent, sodium bromide, sodium hypochlorite, sodium hydroxide, and hydrochloric acid were supplied by Sigma-Aldrich (Darmstadt, Germany). All compounds were of reagent grade and were used as obtained.

Pulp samples were subjected to TEMPO pretreatment followed by homogenizing and systematic product testing with fiber and fines analyzer, scanning electron microscopy (SEM) imaging, conductometric titration, rheological measurements, and light transmission to assess the pretreatment performance. A schematic presenting the experiment and analysis flow is shown in Figure 1.

TEMPO Oxidation of Cellulose Fibers and Fibrillation.

TEMPO oxidation was performed according to a well-established procedure described by Isogai et al. Briefly, for each batch of TEMPO-oxidized cellulose fibers, 125 mg of TEMPO and 1250 mg of sodium bromide were used for 10 g of pulp (dry equivalent weight). A quantity of 37 mL of sodium hypochlorite solution was used per batch, which was equivalent to 5 mmol of sodium hypochlorite per 1 g of dry equivalent pulp. The pH was maintained between 10.0 and 10.1 using 0.5 M sodium hydroxide for the duration of the reaction. The reaction was terminated by the addition of 30 mL of denatured ethanol. The variable component in this experiment was the reaction time. The different pulp batches were treated for 30, 40, 50, 60, and 120 min. Fibrillation was performed on a PandaPLUS 2000 high-pressure homogenizer (GEA NS, Parma, Italy). Three passes of the fiber suspension, with a solid content of 0.5 wt % through the homogenizer at 800 bar, were sufficient to produce highly translucent and viscous TEMPO-oxidized cellulose nanofibril dispersion (TCNF). Samples were taken prior to homogenization and after every pass. The BSKP and HWDP samples were only treated by the TEMPO oxidation system and were not further processed into nanocellulose. As a control, the colloidal stability of the micronized samples was determined by measuring the zeta potential using a NanoBrook ZetaPALS (Brookhaven Instruments, USA). The measured dispersions had a TCNF concentration of 0.035 wt %.

Optical Analysis of Fibers and Fibrillated Products.

Morphological characterization of the fibers and fines following TEMPO pretreatment were assessed using an FSS UHD Fiber Image Analyzer (Valmet, Kajaani, Finland) with an image resolution of 1 µm. The analysis of fiber suspensions was done on 500 mL of sample suspension with a solid content of 250–300 mg/L. The analysis is based on the tube-flow fractionation of the dilute pulp suspension and imaging of particles on water media with an ultra-high-definition CCD camera, followed by automated image analysis and particle classification based on 2D projection size and morphological features (e.g., shape, form, and fibrillation). The optics module and the optical resolution of the analyzer allow analysis of object features down to 1 µm in diameter and a new classification scheme for fines, including categories A and B for flake-like and fibrillar fines, respectively. Flake-like fines are defined as particles with a projection area shorter than 0.2 mm and are divided into five subcategories depending on their size (A1 to AS). Fibrillar fines are defined as particles no longer than 0.2 mm with a diameter less than 10 µm. No fewer than 4,500,000 particles were measured, at least 600,000 of which were optically identified as fibers, that is, elongated particles with sizes above the fines threshold. The samples were analyzed in six replicate batches for each treatment. Comprehensive statistical analysis of data obtained by optical analysis was conducted with R statistical software. The data was analyzed by a Welch two sample t-test, ANOVA, or Tukey’s HSD where appropriate.

SEM Imaging. Fiber suspensions with a solid content of 0.5 wt % were used for SEM imaging using an FEI Quanta 200 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to observe the formed films with both completely and partially fibrillated fragments. Amounts of 50 µL were vacuum-deposited on a polycarbonate membrane with a pore size of 200 nm. The samples were dried under low vacuum, sputtered with gold, and observed under high vacuum at an acceleration voltage of 2 kV.

Rheological Measurements. Dispersion behavior under an increasing shear rate was investigated using an Ares G2 rheometer (TA Instruments, New Castle, Delaware, USA). A 25 mm stainless steel parallel plate geometry with a testing gap of 0.5 mm was used. Before testing, the samples were conditioned to 25 °C, after which the viscosity measurements were performed in a range from 0.1 to 100.0 s⁻¹. The shear rate was ramped up linearly within 300 s. The tested dispersions had a solid content of 0.5 wt %.

UV–Vis Measurement of Light Transmittance through Dispersions. The TCNF dispersions were all diluted to 0.5 wt % solid content and analyzed using a UV–vis spectrophotometer (LAMBDA 2, PerkinElmer, Waltham, Massachusetts, USA) in the visible light wavelength range (800–400 nm). Polystyrene cuvettes were used, which were degassed under vacuum before the analysis.

Results and Discussion

Conductometric Titration. Conductometric titration was performed on TEMPO-oxidized cellulose fibers to determine the effect of the time component on the amount of carboxyl groups in the final product. Table 1 presents the results of three measurements per batch.

In all cases, the TEMPO oxidation reaction produced the majority of carboxyl groups in the first 30 min (approximately 1.2 mmol/g), with the once-dried pulps achieving a slightly lower carboxyl group content. Over the next 30 min, the carboxyl group content gradually increased to approximately 1.35, 1.25, and 1.33 mmol/g for SWDP, BSKP, and HWDP, respectively. An additional hour did not appear to have a significant impact on the carboxyl contents, except in the case of BSKP where it increased by 0.05 mmol/g. A 60 min reaction time thus seems to be sufficient to produce the maximum amount of carboxyl groups for this treatment.

Morphological Properties of Fibers. After TEMPO oxidation, a small sample of cellulose fibers was taken from the
original reaction medium, while the rest were purified, rinsed, and immersed in distilled water. Both the nonpurified and purified samples were analyzed to measure their dimensions and assess other visual characteristics, such as the degree of fibrillation. The samples were analyzed in six replicates for each treatment, for which the averages for 4,500,000 fibers and fine particles are presented.

The morphological traits of the fibers were strongly influenced by TEMPO oxidation. The softwood dissolving pulp fiber width increased from 27.71 µm in the control fibers to around 28.71 µm after 2 h of oxidation. The consistent difference in fiber swelling between raw and purified samples indicates that the oxidation medium itself influenced the fiber dimensions. An increase in fiber swelling was observed when the original medium was replaced with distilled water. We attribute this to the ionic strength of the medium and its higher pH. In TEMPO-oxidized cellulose, the introduction of a surface charge occurs on the individual nanofibril level, as the oxidation medium can freely penetrate the amorphous structure of the cellulose down to the level of the highly ordered crystalline regions. Thus, the nanofibrils within the cell wall are governed by strong repulsive forces due to the carboxylate anion. Increasing the ionic strength of the medium with sodium chloride (a reaction byproduct) weakens the repulsive forces due to a higher concentration of sodium counterions near the deprotonated carboxyl groups, thus also reducing the fiber width. Decreasing the ionic strength strengthens the repulsive forces and pushes the cell walls apart, thereby increasing their lateral dimension.

Table 1. Carboxyl Group Contents of TEMPO Cellulose Fibers Determined by Conductometric Titration (Values of Three Consecutive Measurements for Each Batch)†

| TEMPO oxidation time (min) | carboxyl group content (mmol/g) | carboxyl group content (mmol/g) | carboxyl group content (mmol/g) |
|---------------------------|-------------------------------|-------------------------------|-------------------------------|
|                           | TEMPO SWDP                   | BSKP                          | HWDP                          |
| 30                        | 1.21 ± 0.01                  | 1.15 ± 0.00                   | 1.16 ± 0.03                   |
| 40                        | 1.27 ± 0.00                  | 1.13 ± 0.03                   | 1.25 ± 0.02                   |
| 50                        | 1.31 ± 0.01                  | 1.25 ± 0.02                   | 1.26 ± 0.03                   |
| 60                        | 1.36 ± 0.00                  | 1.25 ± 0.01                   | 1.33 ± 0.01                   |
| 120                       | 1.35 ± 0.01                  | 1.30 ± 0.01                   | 1.32 ± 0.02                   |

†SWDP = never-dried softwood dissolving pulp. BSKP = bleached softwood Kraft pulp. HWDP = hardwood dissolving pulp.

Fiber length is another indirect indicator of cell wall swelling. As the fiber swelled, a significant reduction of kinks (bends) and curls caused an apparent elongation of fibers in correlation with the increasing TEMPO oxidation time. Pulp washing further decreased the curl percentage, indicating significant swelling (Table 2). For SWDP, a high statistical significance was observed in relation to nonpurified and purified samples regarding fiber width (p-value of 0.0017), number of kinks (p-value of 0.0039), and fiber curl (p-value of 2.27 × 10⁻⁶). Additionally, both the HWDP and BSKP samples showed similar statistically significant differences between nonpurified and purified samples.

Once-dried BSKP exhibited the same phenomenon of fiber width increases when the solvent was changed from a higher ionic strength medium to deionized water, the other parameters also followed the same trend as outlined by the never-dried SWDP. Observing the HWDP fibers showed an initial difference between fiber width in the nonpurified and purified samples, but as the reaction progressed, these differences diminished. This was observed especially for fiber width, fiber curl, and number of kinks. The lack of a clear difference between a nonpurified and purified HWDP sample makes it possible to establish the end point more accurately for TEMPO oxidation based on the fiber analyzer data. For example, the fiber width of the HWDPs corroborates the finding that after 50 min of oxidation time, at a hypochlorite loading of 5 mmol/g, the oxidation is nearing its end and that no further benefit in terms of fiber wall swelling can be expected. Full data sets for fiber characteristics of softwood Kraft and HWDPs are available in the Supporting Information.

**Generation of Flake-like Fines during Chemical Pretreatment.** The fiber analyzer is capable of distinguishing two types of fines particles (Figure 2): the so-called flake-like fines (type A) of different sizes (from A1 to A5: 0.00–0.04, 0.04–0.08, 0.08–0.12, 0.12–0.16, and 0.16–0.20 mm) and fibrillar fines (type B, see the next section). Flake-like fines were defined as the percentage of the projection area of measured particles shorter than 0.2 mm.

During TEMPO oxidation, there is always mechanical agitation that ensures homogeneous reaction conditions, even though the reaction itself is heterogeneous. This results in the generation of fines, as any mechanical action on fibers will eventually lead to the disruption of their cell wall and

Table 2. Fiber Width, Length, Curl, and Kink and Fibrillation of Never-Dried SWDP Presented as Functions of TEMPO Oxidation Time and Medium Composition

| sample | fiber width (µm) | fiber length (mm) | fiber curl (%) | fiber kink (1/1000) | fibrillation (%) |
|--------|-----------------|------------------|---------------|--------------------|-----------------|
| SWDP—Control | 27.71 ± 0.01 | 1.41 ± 0.01 | 28.50 ± 0.05 | 6937 ± 65 | 0.23 ± 0.00 |
| TEMPO SWDP 30' (nonpurified) | 28.07 ± 0.28 | 1.31 ± 0.11 | 20.95 ± 0.73 | 5871 ± 138 | 0.30 ± 0.09 |
| TEMPO SWDP 30' (purified) | 28.40 ± 0.18 | 1.42 ± 0.09 | 17.22 ± 0.33 | 5419 ± 92 | 0.23 ± 0.05 |
| TEMPO SWDP 40' (nonpurified) | 27.85 ± 0.34 | 1.26 ± 0.13 | 19.72 ± 0.67 | 5404 ± 88 | 0.30 ± 0.07 |
| TEMPO SWDP 40' (purified) | 28.50 ± 0.31 | 1.44 ± 0.12 | 16.62 ± 0.24 | 5332 ± 88 | 0.25 ± 0.02 |
| TEMPO SWDP 50' (nonpurified) | 27.95 ± 0.47 | 1.26 ± 0.15 | 18.64 ± 0.75 | 5327 ± 56 | 0.34 ± 0.09 |
| TEMPO SWDP 50' (purified) | 28.23 ± 0.34 | 1.36 ± 0.17 | 14.71 ± 0.27 | 4658 ± 102 | 0.27 ± 0.02 |
| TEMPO SWDP 60' (nonpurified) | 27.85 ± 0.31 | 1.26 ± 0.18 | 18.91 ± 0.76 | 5306 ± 28 | 0.35 ± 0.04 |
| TEMPO SWDP 60' (purified) | 28.26 ± 0.34 | 1.39 ± 0.13 | 16.01 ± 0.34 | 5065 ± 36 | 0.31 ± 0.05 |
| TEMPO SWDP 120' (nonpurified) | 28.43 ± 0.49 | 1.41 ± 0.14 | 14.99 ± 0.69 | 4868 ± 85 | 0.26 ± 0.05 |
| TEMPO SWDP 120' (purified) | 28.71 ± 0.66 | 1.46 ± 0.12 | 14.92 ± 0.69 | 4931 ± 140 | 0.28 ± 0.10 |

Data for HWDP and softwood Kraft pulp are available in the Supporting Information as Tables S1 and S2. The values presented are average values of six replicate batches of pulp.
liberation/cleavage of small particles from the bulk of the material.

Flake-like fines were abundant in all specimens; however, with increasing processing time, a reduction in their abundance was observed. This could come as a result of a secondary disintegration beyond the analyzer’s resolution (below 1 μm of size) because of the shearing effect of the mechanical stirrer on the fibers during TEMPO oxidation. The purified samples had a significantly smaller number of flake-like fines, likely attributable to losses during washing.

The classification and proportions of fines for never-dried softwood dissolving pulp is presented in Table 3. The

![Figure 2. Classification of fines that the machine is capable of distinguishing between, including different size grades for flake-like fines.](https://dx.doi.org/10.1021/acssuschemeng.0c05989)

Table 3. Classification and Proportions of Fines in Nonhomogenized Samples of TEMPO-Oxidized SWDP

| sample                  | total fines (%) | flake-like fines (%) | fibrillar fines (%) |
|-------------------------|-----------------|----------------------|---------------------|
| SWDP—control fibers     | 81.28 ± 1.23    | 20.50 ± 1.35         | 0.76 ± 0.04         |
| TEMPO SWDP 30'          | 78.59 ± 1.61    | 18.07 ± 1.23         | 1.01 ± 0.06         |
| TEMPO SWDP 30' (purified)| 76.27 ± 0.75    | 15.32 ± 0.57         | 1.07 ± 0.06         |
| TEMPO SWDP 40'          | 78.23 ± 0.85    | 18.72 ± 0.90         | 1.61 ± 0.18         |
| TEMPO SWDP 40' (purified)| 73.75 ± 1.20    | 13.88 ± 1.04         | 1.39 ± 0.15         |
| TEMPO SWDP 50'          | 78.74 ± 0.44    | 19.48 ± 1.07         | 2.06 ± 0.08         |
| TEMPO SWDP 50' (purified)| 80.33 ± 1.10    | 19.13 ± 1.76         | 2.15 ± 0.24         |
| TEMPO SWDP 60'          | 81.85 ± 0.94    | 21.59 ± 1.69         | 2.08 ± 0.14         |
| TEMPO SWDP 60' (purified)| 78.37 ± 0.35    | 17.29 ± 0.63         | 1.88 ± 0.14         |
| TEMPO SWDP 120'         | 73.50 ± 0.99    | 14.72 ± 1.06         | 1.80 ± 0.20         |
| TEMPO SWDP 120' (purified)| 74.13 ± 0.44    | 14.10 ± 0.55         | 1.54 ± 0.13         |

*Data for HWDP and BSKP are available in the Supporting Information (Tables S3 and S4). The values presented are average values of six replicate batches of pulp.

The formation of fines is a time-dependent process which, at the same time, includes the formation of carboxyl groups and the duration of mechanical agitation in the reaction slurry. As a function of time and carboxyl group content, the generation of total fines shows the following statistical significance: treatment time 40′—30′ (p-value of 0.6153, not statistically significant), 50′—40′ (p-value of 0.1522, not statistically significant), 60′—50′ (p-value of 0.0000, statistically very significant), and 120′—60′ (p-value of 0.0000, statistically very significant). This shows that the generation of fines begins after about 50 min of treatment time, when the carboxyl group content has begun to level off. The degree of oxidation has, which is related to oxidation time, a strong influence on the formation of fines, with a p-value of 1.01 × 10⁻¹⁰.

Flake-like fines follow the formation of total fines, with statistically significant differences beginning after 50′ min of treatment time (60′—50′ and 120′—60′, p-values of 0.0001 and 0.0000, respectively).

We also analyzed the nonpurified TEMPO suspension for different sizes of flake-like fines to observe their formation dynamic. The mass fractions of flake-like fines in the nonpurified TEMPO suspension according to their sizes are presented in Figure 3.

A noticeable drop in the mass fraction of all fines was observed in the 120 min nonpurified TEMPO-oxidized SWDP suspension sample (Figure 3). As there were no fiber or fines losses in this case, we attribute this drop to the disintegration of the fines beyond the detection capability of the fiber analyzer, that is, particles with sizes below 1 μm. The generation of different classes of flake-like fines in BSKP was different, after 60 min of oxidation, there was an increase in the number of flake-like fines. A small number of fines was cleaved from the fiber surface; however, they were not further disintegrated to beyond the capability of the machine. HWDP samples on the other hand showed a steady decrease in both A1 and A2 (the two smallest fractions) classes of flake-like fines. The decrease however was not as abrupt as in the case of the never-dried softwood pulp.

**Generation of Fibrillar Fines during TEMPO Oxidation.** As mentioned before, the fiber analyzer can distinguish between flake-like and fibrillar fines, this section deals with fibrillar fines formation. Fibrillar fines or type B are particles that are no longer than 0.2 mm but thinner than 10 μm. An increase in this group was observed in all samples compared to the virgin pulp (Table 3). This is attributed to fiber wall swelling and delamination, partly because of TEMPO oxidation, which loosened the cell wall, and partly because of the action of the mechanical agitation, which released the loosened fibrils into the suspension. The never-dried softwood dissolving pulp is especially prone to swelling and ballooning when the carboxyl group content is above 0.7 mmol/g.¹⁵ The amount of these fines seemed greatest after 50 min of TEMPO oxidation, after which it started to gradually decrease, most likely because of fibrillation of the fines into particles smaller...
than the machine could resolve. Statistical analysis shows that in all pulp types (never-dried softwood dissolving, once-dried bleach softwood Kraft, and once-dried HWDP), the formation of fibrillar fines does not seem to be dependent on treatment time as was the case for flake-like fines.

**Comments on Fines Generation.** A different dynamic of fines generation became apparent when different grades of cellulose pulp were used. Softwood Kraft pulp was more resistant to fiber disintegration, while the total amount of fines did increase; the fines that were generated were not further disintegrated into smaller particles. This is likely the result of a different chemical composition of the pulp. Kraft pulp has roughly 3 times as much lignin and a significantly higher hemicellulose content as the dissolving pulps, making the fibers effectively stronger and less prone to breakage and cleaving of fines particles from their surface. Dissolving pulps on the other hand are by design purer, as they are primarily intended for dissolution and derivatization, both of which require high α-cellulose and low lignin contents.

Both dissolving pulps showed a stronger decrease in the total amount of fines and flake-like fines, while the increase in fibrillar fines was comparable between all pulp grades. An important consideration is also the use of a never-dried pulp or pulp that comes in dry sheets. During drying, hornification, or the reformation of an extensive hydrogen bond network, occurs. This lowers the pulp’s water retention value and decreases the reactivity.40 The extensive hydrogen bond network likely contributes to fines formation dynamic during the reaction,15 but at the cost of reactivity and possibly final product quality.

**Scanning Electron Microscopy.** Low-magnification SEM images of softwood dissolving pulp were obtained to observe the characteristics of the dried fiber suspensions on a polycarbonate membrane. Direct comparisons between sample features obtained by the fiber analyzer and those obtained by SEM are difficult, as the samples are in suspension and in dried form, respectively. A comparison of virgin nontreated fibers to TEMPO-oxidized ones revealed clear morphological differences and intricate surface details. As suggested by the optical analysis and the formation of fines, many of these finer particles were lost during TEMPO oxidation and mechanical stirring. The effect of prolonged oxidation is evident in Figure 4, which shows clear changes in the shape and thickness of fibers. With longer oxidation times, the fibers tended to unfold, indicating a curl and kink reduction, which affected the structural integrity of the fiber wall, as the fibers appeared to flatten significantly when deposited on the polycarbonate membrane. With a higher carboxyl group content (Table 1), a reduction in fiber surface roughness was also observed. Moreover, a homogeneous film of material covering the nanosized pores of the polycarbonate membrane was noticed next to the TEMPO-oxidized fibers. Its presence might indicate that at least a fraction of the cellulose fibers had
already been converted to nanofibrils prior to any strong mechanical treatment. Autoliberation of nanofibrils or self-fibrillation during sequential TEMPO/periodate oxidation was recently proven possible, provided that the surface charge is sufficient. A similar phenomenon may have occurred in our case, though on a smaller scale and aided by agitation of the mechanical stirrer.

**Optical Analysis of Homogenized Samples.** A suspension of TEMPO-oxidized softwood dissolving pulp (TO-SWDP) was passed through a high-pressure homogenizer several times to fibrillate the pulp. Thus, in addition to the fiber suspensions of TEMPO-oxidized pulps, homogenized softwood dissolving pulp dispersions were also analyzed with the fiber analyzer. Although the machine is not capable of analyzing nanoscale particles, it can provide insights into the behavior of particles down to the 1 μm threshold, that is, fragments of fibers and fines retained in the suspension after homogenization (Figure 5).

While the total fines content in the dispersions after the third pass was high, approaching 100%, some clear differences were observed in the size distribution of flake-like fines. Class A1 fines were the most numerous detectable fines, indicating strong fibrillation, and their mass fraction of total fines increased with subsequent homogenization passes but also as a function of TEMPO oxidation time. The number of A1 fines formed during the first homogenization pass was quite similar in all samples, between 14.5 and 17.5%. Differences could be observed after the second homogenization pass, where A1 formation in the 30’ sample was around 26%, whereas the rest had already more than 34.5% of A1 fines in the samples. The number of A1 fines after the third homogenization step increased in all samples but following the same trend with the 30’ trailing behind the rest. Another important observation is that larger fines (A2−A5) exhibited a decreasing trend. ANOVA of the data showed that formation of flake-like fines is primarily governed by the number of homogenization passes (Pr (>f) of 2 × 10−16, highly significant). The degree of oxidation, in a suspension system where the total carboxyl group content of the particles ranges from 1.21 to 1.36 mmol/g, plays a less significant role in the fibrillation (Pr (>f) of 0.1380, approaching statistical significance). The formation of fibrillar fines on the other hand expresses strong dependence on both the number of homogenization passes as well as the carboxyl group content (a Pr (>f) of 1 × 10−16 and 0.0120, respectively). Total fines formation shows a similar dependence as flake-like fines, with a Pr (>f) value of 4 × 10−15 and 0.2440 for a number of homogenization passes and carboxyl group content, respectively.

**Cellulose Nanofibril Dispersion Properties.** Only never-dried softwood dissolving pulp was used for further processing into cellulose nanofibrils as its fibrillation characteristics are generally accepted as being better compared to dried bale pulps because of the lack of hornification and general higher α-cellulose content when compared to Kraft pulp. Regardless of the oxidation time and severity of mechanical fibrillation, the majority of the micronized samples exhibited a good colloidal stability with zeta potential on average being below −60 mV, with the exception of 120’ Pass 3 sample, where the average value was below −40 mV. The values for zeta potential are available in the Supporting Information in Table S6. The degree of pulp oxidation and the severity of mechanical fibrillation procedure on the other hand affected the fibrillation and dispersion properties (viscosity and light transmittance). The rheological behavior of the dispersions was analyzed with respect to their pretreatment regime, whereas the mechanical fibrillation was performed under the same conditions for all samples.

The rheological behavior of nanocellulose dispersions is a complex system influenced by many factors. These include the aspect ratio, surface area, and density of surface hydroxyl groups and consequently interactions between the fibers and fibrils, which lead to floc formation via entanglement and adhesive interactions.54 Regarding colloidal dispersions, the movement of colloidal particles is governed by the Brownian motion of particles randomly oriented within the dispersion medium. In the case of nanocelluloses, this means that beyond a certain concentration, the fibrils collide and start forming networks, increasing the viscosity of the dispersion to near-infinity.55 If these dispersions are subject to shear forces, hydrodynamic shear will start breaking the network apart, and
particles within the dispersion will start moving and eventually align in the direction of shear, thus exerting a major impact on the flow characteristics of the dispersions, including shear thinning behavior. Higher shear rates are more efficient at breaking this fibrillar network into decreasing flocs, after which a profound decrease in viscosity occurs due to a more efficient momentum transport within the dispersion.

In the case of TCNF dispersions studied in this paper, the initial pass of the 30° TEMPO-oxidized softwood dissolving pulp fibers suspension through the homogenizer produced a dispersion with a large number of unbrillated fragments that are seen on the curve as an inconsistency (Figure 6). However, with an increasing shear rate, the loose flocs broke up, and the fibrils and other fiber fragments began to orient themselves in the direction of shear, resulting in a smooth decrease in viscosity. Increasing the TEMPO oxidation time resulted in a higher carboxyl group content on the fibrils. Moreover, the samples’ viscosity increased after a single pass through the homogenizer in treatments of up to 50 min. The viscosity graphs show that this minute difference in carboxyl group content between the shortest and longest oxidation times (only 0.15 mmol/g) had a profound effect on the dispersion properties in terms of viscosity. The flow curves themselves were rather uniform in treatments of 40 min or longer, indicating that even though fibrillation may not have been complete, the fragments were much smaller, and the dispersion behaved like a more homogeneous fluid (Figure 6).

Interestingly, after three passes through the homogenizer, the samples’ viscosity was not that dissimilar regardless of treatment time (Table 4). Even though, the fibrillation degree differed between the samples, as observed by ultraviolet-visible (UV–vis) spectroscopy and the differences in fibrillated material reach a point where the differences in viscosity are no longer significant. For example, after three homogenization passes, a difference of less than 100 mPa·s can be observed in the 30, 40, and 50° samples, even though the transmittance values suggest that the 30° sample should clearly be inferior because of a larger proportion of larger particles present. It suggests that the mass fraction of a highly fibrillated cellulose dominates the viscosity behavior of the gel and differences in more coarse fractions become less apparent. It is important to note that further treating the dispersions with high shear forces by passing them through the homogenizer repeatedly would ultimately cause a decrease in viscosity because of a deterioration of the nanofibrillated product.

The transmittance of these dispersions was also investigated, as it is related to particle sizes and the turbidity of the dispersion. To gauge the effectiveness of fibrillation, UV–vis spectra were recorded from 800 to 400 nm for dispersions with a solid content of 0.5 wt % (Figures 7 and 8). Namely, light Table 4. Viscosity (mPa·s) at 10 s⁻¹ for TCNF Dispersions after Three Passes through the High-Pressure Homogenizer

| TEMPO oxidation time (min) | homogenizer pass 1 | homogenizer pass 2 | homogenizer pass 3 |
|---------------------------|-------------------|-------------------|-------------------|
| 30                        | 89                | 1323              | 2793              |
| 40                        | 178               | 1138              | 2804              |
| 50                        | 300               | 1444              | 2874              |
| 60                        | 266               | 1740              | 4261              |
| 120                       | 332               | 1970              | 3326              |

“*The TCNF dispersion consistency was 0.5 wt %.

Figure 7. Appearance of TEMPO-TCNF dispersion in polystyrene cuvettes at a solid content of 0.5 wt %. An increase in the clarity of the dispersions can be observed even with the unaided eye.

Figure 8. Light transmittance values for TEMPO-TCNF dispersions at different light wavelengths after 3 passes through the homogenizer at 500 bar. With longer oxidation time and higher carboxyl group content, a higher transmittance value was obtained under the same mechanical treatment conditions. The respective results of the first and second passes are displayed as Figures S3 and S4 in the Supporting Information.
transmittance is related to particle size or, in this case, nanofibril width. Larger particles, un fibrillated fiber fragments or agglomerates, scatter incident light more effectively, thus reducing the transmittance value. In the case of highly fibrillated TEMPO-oxidized celluloses, the fibril diameters are in the low nanometer ranges (3–10 nm). Such small particles do not effectively scatter incident light; hence, most will pass through the dispersion, contributing to the high light transmittance values. 

Increasing the TEMPO oxidation time and subsequent homogenization steps had a profound effect on the light transmittance values of TEMPO-TCNFs (Table 5). An increase in transmittance was observed across the visible spectrum. After three passes through the homogenizer, the samples exhibited only marginally worse transmittance than distilled water, indicating a highly fibrillated product. After the first pass, the 30 min sample had the lowest transmittance (4.06 % at 600 nm). Treatments of 50–120 min produced TCNFs with comparable transmittance. The second pass produced dispersions with consistently higher transmittance and more pronounced differences between specimens. The third and final pass through the homogenizer resulted in a product that exhibited high light transmittance values. The specimen pretreated for 30 min again had the lowest value. The transmittance curves (Figures 8, S3, and S4 in the Supporting Information) also showed a dependency of transmittance on the light wavelength. Light transmittance percentages for 600 nm light are presented in Table 5.

The findings indicate that in the preparation of well-dispersed TCNFs, strong mechanical treatment can, to a certain degree, compensate for the TEMPO oxidation time and the carboxyl group content.

**Table 5. Light Transmittance (%) for TEMPO-Oxidized Softwood Dissolving Pulp Nanofibril Dispersions at 0.5 wt % Consistency and at 600 nm Wavelength**

| TEMPO oxidation time (min) | homogenizer pass 1 | homogenizer pass 2 | homogenizer pass 3 |
|---------------------------|--------------------|--------------------|--------------------|
| 30                        | 4.1                | 12.7               | 55.3               |
| 40                        | 5.8                | 25.8               | 71.5               |
| 50                        | 7.1                | 33.3               | 75.7               |
| 60                        | 6.7                | 34.3               | 75.6               |
| 120                       | 6.3                | 34.4               | 79.4               |

*Both the TEMPO oxidation time and the number of passes through the homogenizer had a strong influence on the material’s optical properties.*

Conductometric titration, in turn, is the preferred direct method of carboxyl group determination after the reaction has been concluded. In an industrial setting, understanding the product itself—for example, morphologically—is crucial for determining the process efficiency.

Morphological changes on the fibers were strongly influenced by TEMPO oxidation. While swelling increased the fiber width to 29.5 μm in the 120 min sample, this seemed to have little impact on the quality of the dispersions. While swelling of the fiber wall is considered an important pretreatment, the total carboxyl group content in TEMPO cellulose can be considered the dominant factor of fibrillation. Optical analysis revealed that fiber width was directly influenced by the type of medium in which the fibers were stored. Washing the fibers with distilled water further increased their width, most likely because of the decreased ionic strength of the medium. The swelling of fibers during TEMPO oxidation and further swelling during washing were corroborated by fiber length, curl, and kink measurements. The generation of fines was also influenced by the treatment time. Analysis of the fiber suspensions in the TEMPO medium showed a notable decrease in flake-like fines in the suspension, which can only have been the result of mechanical action causing them to fibrillate to a size below the machine’s detection level, that is, below 1 μm in size.

An important aspect must also be considered when choosing the type of pulp for chemical modification. The softwood Kraft pulp has higher lignin and hemicellulose contents than dissolving pulps, which are generally intended for dissolution and regeneration or chemical derivatization, both of which benefit from material purity and higher α-cellulose content. Optical analysis of TEMPO-oxidized softwood Kraft pulp showed that the fibers are less prone to breakage and fines formation. Both dissolving pulps however had similar characteristics, and they were oxidized to a similar degree and behaved similarly in terms of fines formation, albeit the once-dried pulp was somewhat more resistant to cleavage of finer particles from the bulk of the fibers. Additionally, the use of never-dried or dried pulp also plays a role. The reformation of the hydrogen bond network, or hornification in the dried pulps, reduces the water retention value and reactivity of the pulp. As this hydrogen bond network is extensive, it likely also plays a role in the fines formation dynamic as the observed differences suggest.

While the viscosity of the final nanocellulose dispersion continued to increase with subsequent homogenization passes, the differences became less pronounced with increasing mechanical treatment intensity. A similar observation can be made for the transmittance of visible light through the dispersion. While further increasing the carboxyl group content did ease fibrillation (i.e., it resulted in a product with higher viscosity or a dispersion with higher light transmittance), the differences between samples were negligible.

**CONCLUSIONS**

Under the tested conditions, an optimal time for the TEMPO oxidation of never-dried softwood dissolving pulp would be 50–60 min with a sodium hypochlorite loading of 5 mmol/g. However, the 120 min process did produce a slightly better product in terms of both fiber characteristics (e.g., fiber width) and degree of fibrillation (increase in viscosity and visible light transmittance). Furthermore, an important aspect was highlighted in this paper; during extended TEMPO oxidation
times, a generation of small fines particles was observed, suggesting that a breakdown of the fibers has begun because of the presence of the surface charge coupled with mechanical agitation of the reaction slurry. However, on a laboratory scale, such phenomena are not of concern, and large-scale TEMPO cellulose production, for example, that takes place in Japan, could benefit from data stemming from fines generation in the reaction slurry. Such data could provide means to optimize filtration processes and increase fiber and particle recovery.

The automated optical analysis of the TEMPO cellulose can be considered a viable option to control the fiber response to treatment and the quality of the nanodispersion obtained. The degree of fibrillation of the final nanofibrillated cellulose greatly depends on the carboxyl content and mechanical fibrillation for which the optical analysis provides a quick and reliable method for process optimization and increase in material efficiency.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge at [https://pubs.acs.org/doi/10.1021/acssuschemeng.0c05989](https://pubs.acs.org/doi/10.1021/acssuschemeng.0c05989).

Rheological measurements, light transmittance, and numerical values for different grades of pulp during TEMPO oxidation, fines production, and zeta potential of the micronized pulp (PDF)

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### Author Contributions

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### Notes

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