

A comparative study of enzymatic and Fenton pretreatment applied to a birch kraft pulp used for MFC production in a pilot scale high-pressure homogenizer

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ABSTRACT: Microfibrillated cellulose (MFC) was produced in pilot scale from a bleached birch (*Betula verrucosa*) kraft pulp that was pretreated with either Fenton's reagent or with a combined mechanical and enzymatic method used at the Centre Technique du Papier (CTP; Grenoble, France). The change in fiber fibrillation during the homogenization treatment was monitored by analyzing the fiber and the fines content, size fractionation, rheological properties and visualization by light- and scanning electron microscopy (SEM). The Fenton pretreatment resulted in MFC suspensions that contained a high amount of small sized elements. After five passes through the high-pressure homogenizer, the amount of particles smaller than 20 μm was 37% for the Fenton pretreated MFC compared to 13% for the enzymatically (endoglucanase) pretreated MFC. Altogether, the Fenton pretreatment enabled preparation of MFC with a higher degree of fibrillation after the same number of passes through the high-pressure homogenizer. Another option is to produce MFC of the same amount of fibrillation as after an enzymatic stage, but at significantly lower energy consumption.

Application: Fenton pretreatment may be used as an alternative to enzymatic hydrolysis in the production of MFC and give an opportunity to further reduce the energy demand or produce a product with higher amount of small sized elements.

In Europe and North America, the forest-based industry is struggling with high prices for wood raw material and energy, while the demand for paper is simultaneously declining. To address these changes, an intensive work is performed to find new opportunities to utilize wood and pulp fibers as raw materials in new application areas and products. Being renewable and biodegradable, wood is a very attractive feedstock. One area of interest is the use of lignocellulosic materials for the production of micro- and nanofibrillated cellulose (MFC and NFC). MFC was first produced by Turbak et al. in the early 1980s [1] through the intense mechanical treatment of wood fibers by several passes through a pressurized homogenizer. The resulting product is a gel-like mixture consisting of unaffected fibers, fiber fragments, fibrils, and microfibrils [2]. The width of the fibrils ranged from the size of the elementary fibril, 3-5 nm, and upwards to about 20-50 nm. A variety of important industrial uses for MFC/NFC have been suggested, from traditional forest products applications like additives in paper and packaging to non-traditional areas like cement, composites, electronics, and pharmaceuticals [1,3-8].

The industrial production of MFC/NFC is hampered due to problems that include clogging of the equipment and high

energy requirements, with reported values in the range of 12-65 MWh/metric ton [5,9,10]. During the second half of the last decade, interest in MFC/NFC increased dramatically because the protection set by the earliest patent applications had expired [11]. Several pretreatment methods have been proposed to facilitate production at larger scale, including enzymatic [12,13] and chemical modification such as carboxymethylation [4,14] or TEMPO-mediated oxidation (TEMPO = 2,2,6,6-tetramethylpiperidine-1-oxyl) [15-17]. With the use of TEMPO-mediated oxidation as pretreatment, the energy requirement can be reduced to 500-1500 kWh/metric ton [18], but high chemical charges are needed and up to several hundred kg/metric ton of pulp have been reported [17]. Homogeneity, average size of the fiber fibrils, and charge density are properties that are affected by the chosen pretreatment method and mechanical processing (equipment and time), which in turn will influence the appropriate application area. Enzymatic hydrolysis combined with mechanical treatment is one prevailing pretreatment method for MFC preparation [19]. The product will contain larger fibrils with a broader size distribution and a charge density much lower than MFC prepared using carboxymethylation or TEMPO-mediated oxidation.

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The use of Fenton's reagent (i.e., treatment with acidic hydrogen peroxide in the presence of ferrous ions producing hydroxyl radicals) has also been suggested as a pretreatment in MFC production [20,21]. In a study by Hellström et al. [21], a fully bleached birch (*Betula verrucosa*) kraft pulp was treated with Fenton's reagent followed by mechanical treatment in a colloid mill to produce MFC. The result indicated that the Fenton treated pulp was easier to process mechanically, i.e., reached a higher specific surface area (BET) at a given mechanical processing time. To confirm these findings, MFCs in the present study were produced in pilot scale from a commercial birch kraft pulp pretreated with either Fenton's reagent or the combined mechanical and enzymatic pretreatment used at the Centre Technique du Papier (CTP; Grenoble, France). The final mechanical treatment to MFC was performed by homogenization at high pressure in multiple passes. The change in the fibrillation degree during mechanical processing was monitored by measurement of fiber and fines content, rheological measurements, size fractionation, and examinations in light- and scanning electron microscopes (SEMs).

MATERIAL AND METHODS

Preparation of MFC

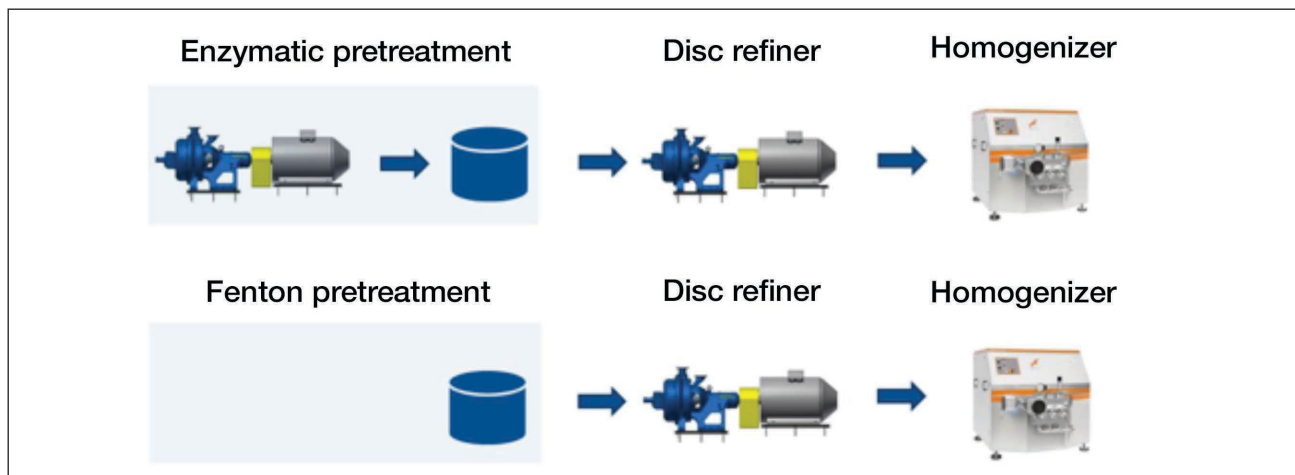
The MFCs were prepared from a fully bleached birch (*Betula verrucosa*) kraft pulp with the following properties: ISO brightness 88.6%, kappa number 0.62, and intrinsic viscosity 929 dm³/kg. Two batches of each 8.5 kg pulp (oven dry) were treated enzymatically or with acidic hydrogen peroxide in the presence of ferrous ions (Fenton's reagent). After pretreatment, the pulps were refined in a disc refiner to cut and weaken the fibers so they could pass through the homogenizer without causing clogging problems. The fiber suspension passed through the homogenizer five times, and samples were collected from each process step to be characterized. A schematic overview of the trial setup can be seen in **Fig. 1**, and a more detailed description of the preparation methodology follows.

The pulp destined for enzymatic pretreatment was refined

at 4.5% consistency in a 12-in. single-disc refiner equipped with hardwood plate pattern (specific edge load of 0.69 Ws/m and a speed of 1500 rpm) to a targeted drainage index of 25°SR. The purpose of the pre-refining was to open the fiber structure and facilitate the penetration of the enzyme, thus improving the efficiency of the enzymatic treatment. The enzymatic treatment was applied on the pre-refined pulp using a commercial endoglucanase solution (FiberCare R, Novozymes; Bagsvaerd, Denmark) at pH 5, 50°C for 60 min. The charge was 1 L/b.d. metric ton, which corresponds to about 4-5 endocellulose units per gram of oven dry pulp.

Prior to the Fenton pretreatment, the pulp was disintegrated as described in ISO standard 5263-1:2004 "Pulp — Laboratory wet disintegration — Part 1: Disintegration of chemical pulps," drained on a Büchner funnel and thereafter reacted (at 10% consistency, 150 min, 90°C) with acidic hydrogen peroxide in the presence of ferrous ions as described in Hellström et al. [21]. The amount of hydrogen peroxide (KR 59, AkzoNobel; Amsterdam, Netherlands) was 50 kg/b.d. metric ton, and the charge of ferrous ions (charged as Fe₂SO₄ x 7 H₂O, Scharlau; Sentmenat, Spain) was 0.2 kg/b.d. metric ton. Afterwards, the pulp was washed and dewatered to a consistency of about 30% and stored in a refrigerator until further processed.

To reduce the risk of clogging in the homogenizer, both the enzymatic and Fenton pretreated pulps were refined in a 12-in. single-disc refiner equipped with hardwood plate pattern at a specific edge load of 0.08 Ws/m and a speed of 1500 rpm until a mean fiber length of 300 µm was achieved. The pulp sampled after this refining stage will be denoted P0 in the text. Microfibrillated cellulose was obtained by mechanical disintegration of a fiber suspension (2% consistency) in a high-pressure homogenizer (model NS3045, GEA Niro Soavi; Parma, Italy) with a capacity of 1000 L/h. At the output of the homogenization zone, the suspension was cooled with cold water to minimize the temperature increase. Each pulp was first homogenized at 1000 bar and denoted P1, and then four additional passes at 1500 bar were applied, denoted P2-P5.



1. Schematic illustration of the trial setup.

Pretreatment	DP	Total Carbonyl Groups, $\mu\text{mole/g}$	Calculated REG, $\mu\text{mole/g}$
Pulp without pretreatment	2720	13	2
Enzymatically pretreated pulp	1600	20	4
Fenton pretreated pulp	430	107	14

I. The degree of polymerization (DP), amount of carbonyl groups, and calculated amount of reducing end groups (REG) for untreated and pretreated pulps.

Characterization and analyses

In this study, the diluted fiber suspensions prepared from the refined birch kraft pulp were pretreated with either Fenton's reagent or enzymatic hydrolysis before entering the homogenizer (P0). The products from each pass through the high-pressure homogenizer (P1-P5) are referred to as MFC, and the fines/fibrils produced during the mechanical processing are referred to as small sized elements.

The intrinsic viscosity measurement was based on the standard method ISO standard 5351-1:2010 "Pulps — Determination of limiting viscosity number in cupriethylenediamine (CED) solution," utilizing cupricethylenediamine as solvent. A departure from the applied method was made for the Fenton pretreated pulp since the efflux time was outside the specified limit. The determination of the carbonyl group content was performed by reaction with hydroxylammonium chloride, which forms an aldoxime with the carbonyl groups in the pulp sample. After reaction, the content of carbonyl groups is proportional to the amount of nitrogen in the sample, which is determined by chemiluminescence using an Antek MultiTek (PAC; Houston, TX, USA) after catalytic combustion.

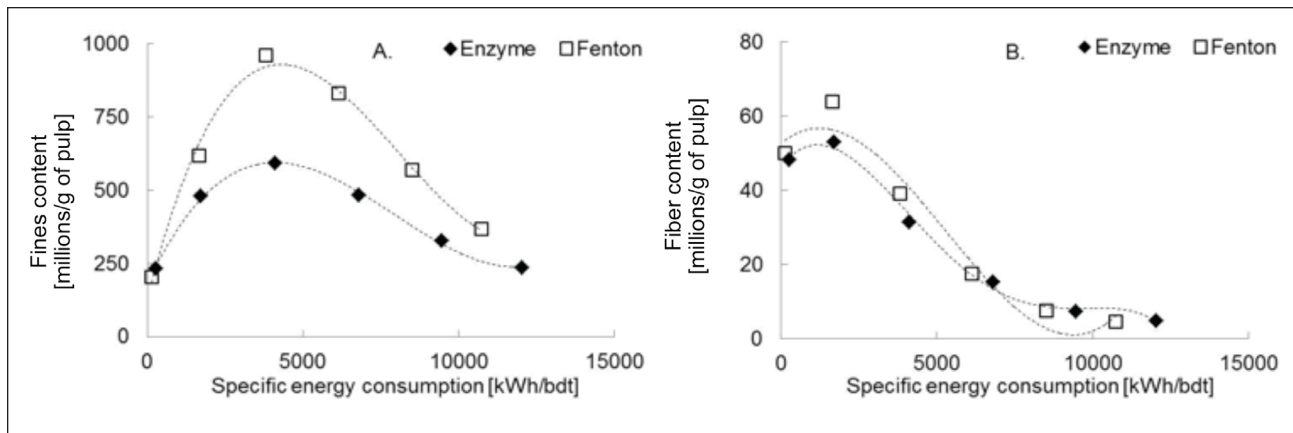
The amount of fiber and fines is often used as a quick and easy parameter to follow the development of small sized elements during MFC preparation. In this study, the laboratory version of the MorFi analyzer (Techpap; Grenoble, France) was used to measure the fiber and fines content during pre-refining and homogenization. Fibers in this context are defined as elements with a length $>80\ \mu\text{m}$; fines between $5\text{--}80\ \mu\text{m}$ and elements $<5\ \mu\text{m}$ are not detected. More detailed information on the mechanical processing is obtained by size fractionation of fiber suspensions after mechanical treatment in the refiner, as well as after several passes through the homogenizer. The size fractionation was performed on highly diluted samples (0.016%) by successive fractionation through filters/membranes with finer and finer apertures, resulting in four fractions: $>112\ \mu\text{m}$, $20\text{--}112\ \mu\text{m}$, $0.1\text{--}20\ \mu\text{m}$, and $<0.1\ \mu\text{m}$. The procedure is described in detail in Hellström et al. [21]. The rheological behavior of MFC suspensions was characterized by Brookfield measurements at room temperature (23°C – 24°C) and 2% consistency using a control-stress rheometer with plate-plate geometry (Brookfield Engineering; Middleboro, MA, USA). The energy used in the MFC production was measured with a wattmeter in the pre-refining stages, as well as in each pass through the homogenizer. In

the homogenizer, the energy requirement corresponds to the energy needed for increasing the hydraulic pressure into the homogenizing chamber. Light microscope (Axio Imager Z2; Zeiss, Oberkochen, Germany) examinations were performed on pretreated pulps and MFC suspensions after dying with Congo red according to [22]. Enzymatic and Fenton pretreated MFC samples after five passes through the high-pressure homogenizer were, after freeze drying and metallization with 2 nm Au/Pd, examined in a high resolution scanning electron microscope (SEM) equipped with a field emission gun (FEG). The SEM-FEG microscope used was an Ultra 55 from Zeiss used at 3000x magnification and with an acceleration voltage of 300 kV.

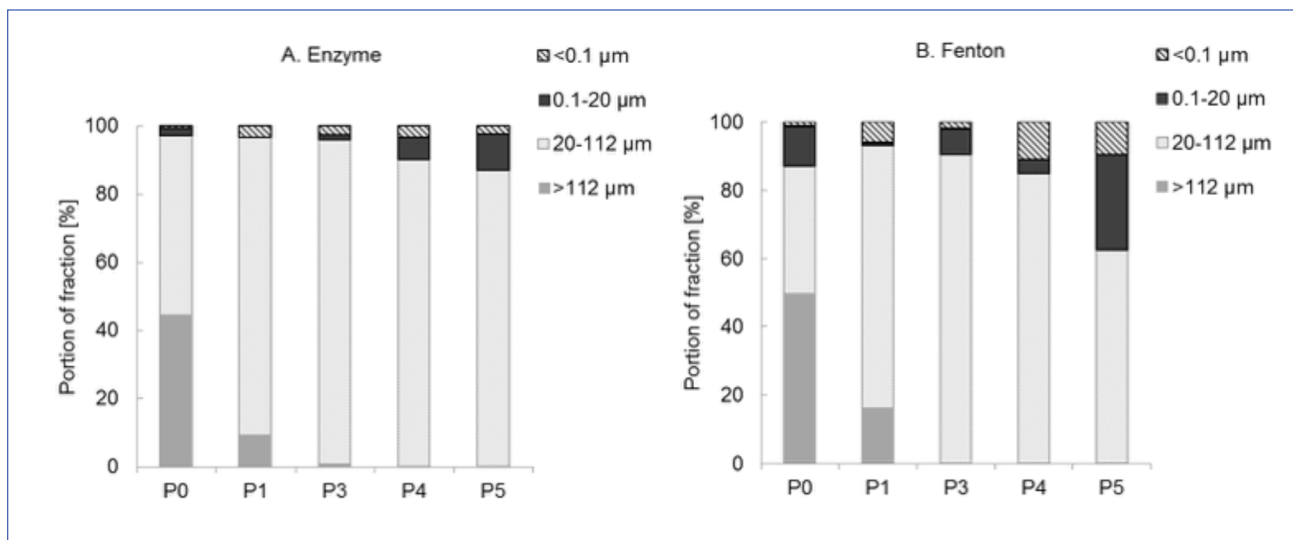
RESULTS AND DISCUSSION

Both enzymatic and Fenton pretreatments caused cleavages in the cellulose chain, i.e., a decrease in the degree of polymerization (DP). The pretreatment with endoglucanase as performed in this study resulted in a DP of 1600, which should be compared with 2720 for the pulp without pretreatment and 430 for the Fenton pretreated pulp (**Table I**). The degree of polymerization was calculated from intrinsic viscosity data using formulas as described in [23]. The chain cleavage after Fenton pretreatment is not caused by alkaline degradation of the cellulose through β -elimination by the alkalinity of the cupriethylenediamine solvent used when measuring intrinsic viscosity, because neither oxidation with sodium chlorite nor reduction with sodium borohydride before measurement affected the intrinsic viscosity [21]. A decrease in DP results in an increased number of cellulose chains and, consequently, an increased number of reducing end groups (REGs). REGs will contribute to the measured carbonyl groups in the pulp. For the pulp without pretreatment, as well as the enzymatically pretreated pulp, the measured amount of carbonyl groups is in the same range as the amount of REGs based on DP calculations. Fenton pretreatment of the pulp resulted in an additional formation of carbonyl groups along the cellulose chains (**Table I**). Similar effects (i.e., a decrease in molar mass and an increase in carbonyl group content) have been reported when hydroxyl radicals were introduced by γ -radiation to cotton linter, bleached beech sulfite pulp, bleached eucalyptus kraft pulp, and softwood kraft pulp [24]. Furthermore, in a previous study using the same pulp and pretreatment conditions, the carboxylic acid groups increased from 40 to 58 $\mu\text{mole/g}$ after Fenton pretreatment [21].

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2. MorFi fiber (A) and fines (B) content after pre-refining in a disc refiner (P0) and after one to five passes through a high-pressure homogenizer (P1-P5) as a function of specific energy consumption in kWh/b.d. metric ton of birch kraft pulp.

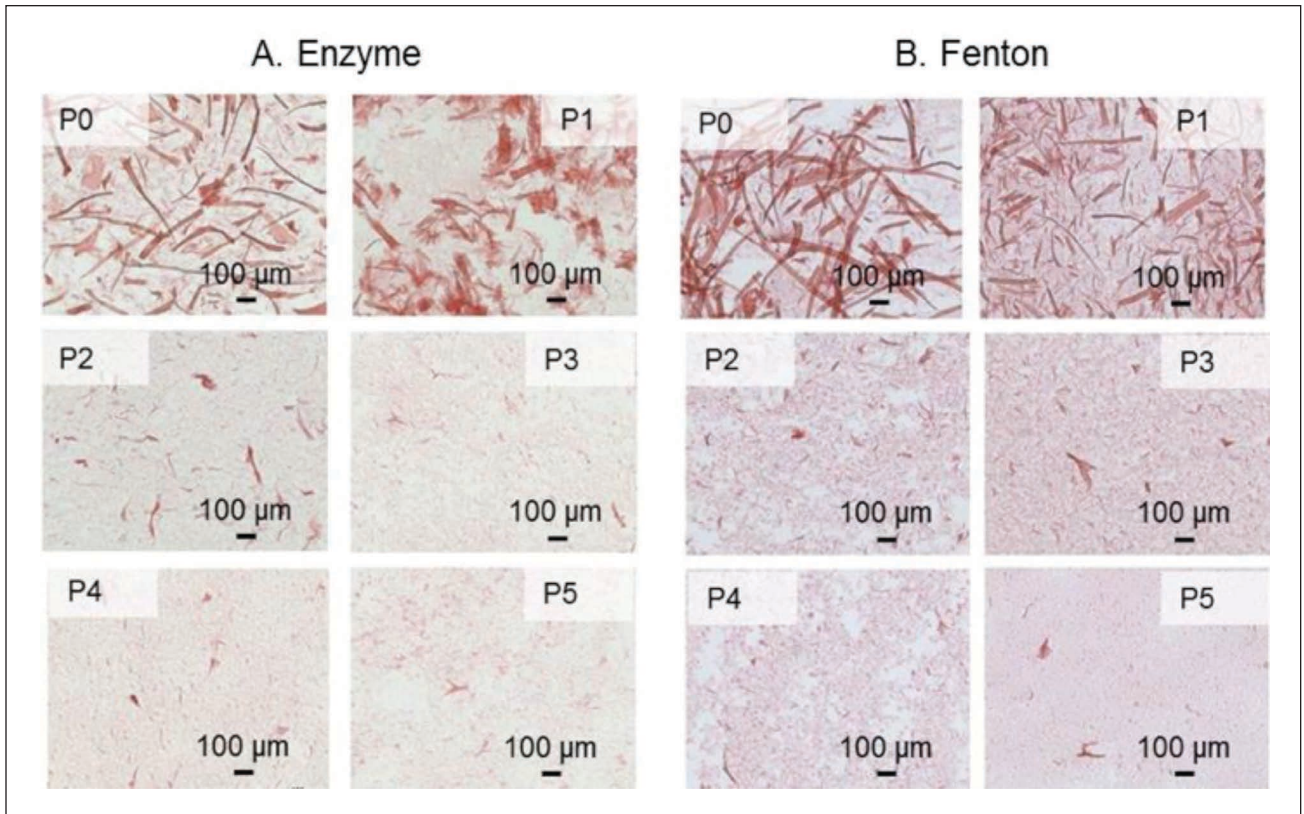


3. The weight in percent of enzymatic (A) and Fenton (B) pretreated pulps after successive fractionation with finer and finer filters/membranes resulting in fractions >112 μm , 20-112 μm , 0.1-20 μm , and <0.1 μm . The fractionation was performed after pre-refining in a disc refiner (P0) and after one (P1), three (P3), four (P4), and five (P5) passes through a high-pressure homogenizer.

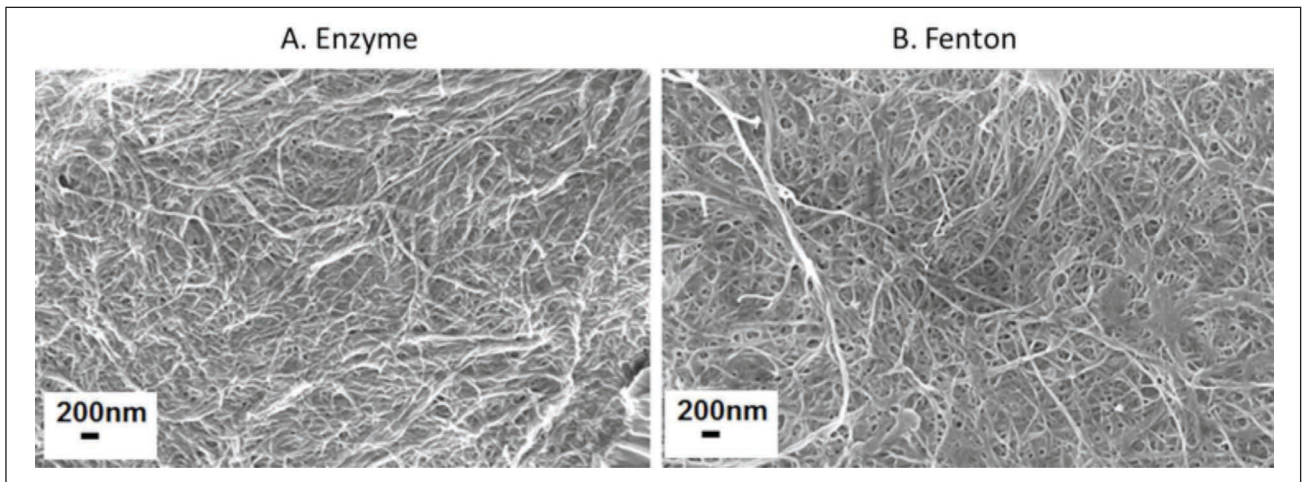
During the mechanical treatment in the high-pressure homogenizer, the fibers are subjected to a large pressure drop under high shearing forces and will be disintegrated into fiber fragments, fines, and smaller fibrils [25,26]. The impacts of increased mechanical processing are detected as changes in fiber and fines content and measured by the MorFi analyzer. After the first pass through the homogenizer (P1), the number of fibers increased due to fiber shortening and then decreased in the subsequent homogenizing steps (P2-P5). The number of elements detected as fibers were in the same magnitude for both enzymatic and Fenton pretreated MFCs (Fig. 2A). The amount of fines increased with increasing degree of mechanical processing and reached the highest level after two passes through the homogenizer, and thereafter a substantial amount of fines were shifted to elements too small to be detected with the MorFi analyzer, which is reflected in a decreased number of fines (Fig. 2B). The fines content was considerably higher

in the Fenton pretreated MFCs compared to MFCs pretreated with the endoglucanase enzyme solution.

The observed higher amount of fines in the Fenton pretreated MFCs as indicated with the MorFi analyzer was confirmed by fractionation of the MFC suspensions. After the refining stage (P0), the Fenton pretreatment already resulted in a higher amount of material in fractions, 0.1-20 μm and <0.1 μm , as well as a higher amount in fractions >112 μm (Fig. 3). As the mechanical treatment in the homogenizer proceeds, coarser fiber fragments are transformed into smaller sized elements that can be followed as a decrease of the amount in fractions >112 μm and an increase in fractions 20-112 μm , 0.1-20 μm , and <0.1 μm . The sum of the fractions 0.1-20 μm and <0.1 μm are significantly higher for the Fenton pretreated pulps compared to the enzymatically pretreated pulps after refining (P0) and after each pass through the homogenizer (P1, P3, P4, and P5). The results indicate the



4. Light microscope images of microfibrillated cellulose (MFC) suspensions after dying with Congo red for pulps pretreated with enzyme (A) and Fenton's reagent (B) after mechanical treatment in a disc refiner (P0) and after one to five passes (P1-P5) through the high-pressure homogenizer.

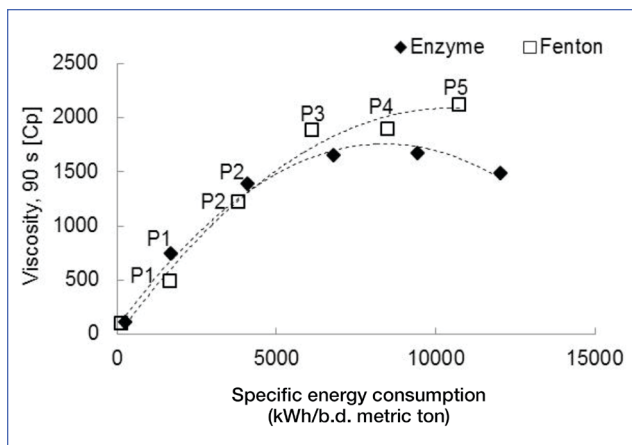


5. Scanning electron microscope images of enzyme (A) and Fenton (B) pretreated birch kraft pulp after five passes (P5) through the high-pressure homogenizer.

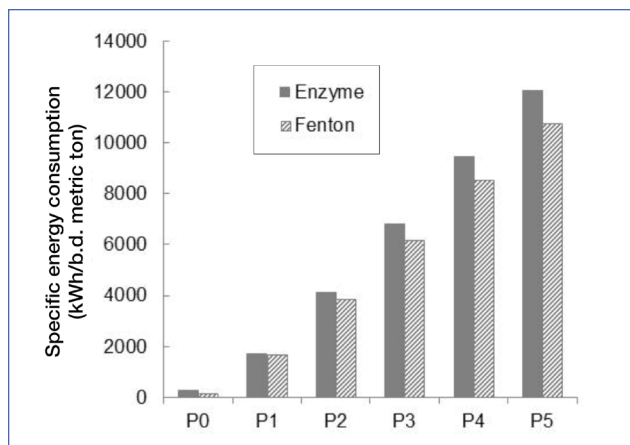
possibility to achieve a product with a higher amount of small sized elements at the same number of passes through the homogenizer or to produce an MFC with the same amount of small sized elements after a reduced number of passes.

Visual inspection of the fiber suspensions in a light microscope after dying with Congo red (**Fig. 4**) revealed that larger elements were present in the Fenton pretreated pulp after pre-refining (P0) compared to the enzymatic pretreated pulp. After

the first homogenizing pass at 1000 bar, the Fenton pretreated pulp contained more cut and less fibrillated fiber segments and more individualized fines compared to enzymatic pretreated pulp. After five passes through the homogenizer, a few coarse elements with lengths of about 100 µm can still be seen in suspensions from both pretreatment methods. Small sized elements are seen as cloudy transparent red aggregates in the images and seem to be more abundant and more flocculated



6. Brookfield viscosity after 90 s as a function of specific energy consumption (kWh/b.d. metric ton) in the pre-refining stage (P0) and after one to five passes through a high-pressure homogenizer (P1-P5) for microfibrillated cellulose produced after enzymatic and Fenton pretreatment of a birch kraft pulp.



7. Specific energy consumption (kWh/b.d. metric ton) during pre-refining in a disc refiner (P0) and after one to five passes through a high-pressure homogenizer (P1-P5) of a water suspension containing 2% of an enzymatic or a Fenton pretreated birch kraft pulp.

in the Fenton pretreated samples. SEM examinations of both enzymatically and Fenton pretreated MFCs after five passes through the homogenizer confirmed that Fenton pretreatment resulted in an MFC with less coarse elements and thinner fibrils, i.e., a higher degree of fibrillation (**Fig. 5**).

The highly entangled network formed by MFC in water dispersions results in an increased viscosity of the suspension when the concentration of MFC increases [27,28]. Rheological measurements may therefore be used as an indirect measure of MFC evolution during homogenization [29]. For the evaluated pretreatment methods, the viscosity development as a function of energy consumption differentiated after two passes (P2) through the homogenizer. For the Fenton pretreated MFC, the viscosity continued to increase while the enzymatic pretreated MFC reached an optimum after three passes and then slightly decreased during pass number four and five (**Fig. 6**). The increased viscosity may be a result of a higher amount of small sized elements present in the solution but can also be derived from a more fibrillated product or otherwise different in structure. The decline in viscosity as seen for the enzymatically pretreated pulp (**Fig. 6**) may be a result of a reduced ability to form a network due to shortening of the fibril length.

Fenton pretreatment resulted in slightly lower energy requirement in P0, P2, P3, P4, and P5, while the energy requirement was comparable in P1 (**Fig. 7**). The size distribution for the Fenton pretreated MFC after three passes through the homogenizer is about the same as for the enzyme pretreated MFC after four passes (**Fig. 3**), indicating a possibility to reduce the energy requirement. To determine the extent of energy reduction, the Fenton pretreated pulp needs to be further explored and compared with MFC prepared after enzymatic pretreatment for the MFC product's intended application area. However, it is obvious that the number of passes has a strong influence on the energy requirement, because

each pass consumes approximately 2.5 MWh/b.d. metric ton for the raw material and equipment used in this study.

CONCLUSIONS

MFC was produced in pilot scale from a bleached birch (*Betula verrucosa*) kraft pulp pretreated with either Fenton's reagent or a combined mechanical and enzymatic pretreatment. The final mechanical treatment to MFC was performed by homogenization at high pressure. Samples from each process step were characterized in order to follow the formation of MFC during processing. Fiber and fines content, size distribution, and light- and scanning electron microscopic examinations indicate an increased formation of small fibrillated elements, i.e., an MFC with higher degree of fibrillation after Fenton pretreatment compared to enzymatic (endoglucanase) pretreatment. Thus, Fenton pretreatment enables the possibility to achieve an MFC with a higher amount of small sized elements after the same number of homogenizer passes to alternatively produce an MFC with comparable amounts of small sized elements at significantly lower energy consumption. **TJ**

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ABOUT THE AUTHORS

We chose to conduct this research to explore the possibility of using Fenton's reagent as an alternative to enzymatic pretreatment when producing MFC in a high pressure homogenizer. Compared with conventional enzymatic hydrolysis, our results indicate that Fenton pretreatment enables a reduction in energy demand at comparable amounts of MFC in the product or a higher amount of MFC at equal energy consumption.

Since no standardized methods are available, the most difficult aspect of this research was to estimate the amount of small sized elements and to ensure that a fibrillated product was produced. In this study, we used a combination of commonly used methods, i.e., rheological and particle size measurements, together with microscopic characterizations.

The work presented here may provide the forest-based industry with an opportunity to reduce energy consumption when preparing MFC with the use of an environmentally-friendly pretreatment method.

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