

## PRODUCTION OF NANOCELLULOSE FROM NATIVE CELLULOSE – VARIOUS OPTIONS UTILIZING ULTRASOUND

Shree P. Mishra,<sup>a,\*</sup> Anne-S. Manent,<sup>a</sup> Bruno Chabot,<sup>a</sup> and Claude Daneault<sup>a</sup>

In this study three different ways of applying ultrasound for the production of nanocellulose from native cellulose were explored. In the first option bleached hardwood kraft pulp was oxidized with the ultrasound (US) assisted TEMPO/NaBr/NaOCl-system (US-TEMPO-system) followed by mechanical separation of nanocellulose. The pulp oxidized by the US-TEMPO-system had higher carboxyls content and ca. 10% increase in nanocellulose yield when compared to the TEMPO-system without sono catalysis. In the second option ultrasound pretreated pulp was oxidized using the TEMPO-system. Although there was no gain in carboxyls content in this process versus the oxidation with TEMPO-system without ultrasound treatment, a higher degree of fibrillation was obtained after ultrasound treatment. In the third case the TEMPO oxidized pulp was subjected to mechanical and ultrasound treatments for nanocellulose production. Under similar treatment time the subsequent ultrasound treatment achieved higher nanocellulose yield than the subsequent mechanical treatment. However, in comparison, the ultrasound treated nanocellulose had lower Rheometer Stresstech viscosity. Furthermore, it was observed that cellulose nanofibrils produced by ultrasound treatment were slightly thinner compared to those produced using the mechanical method.

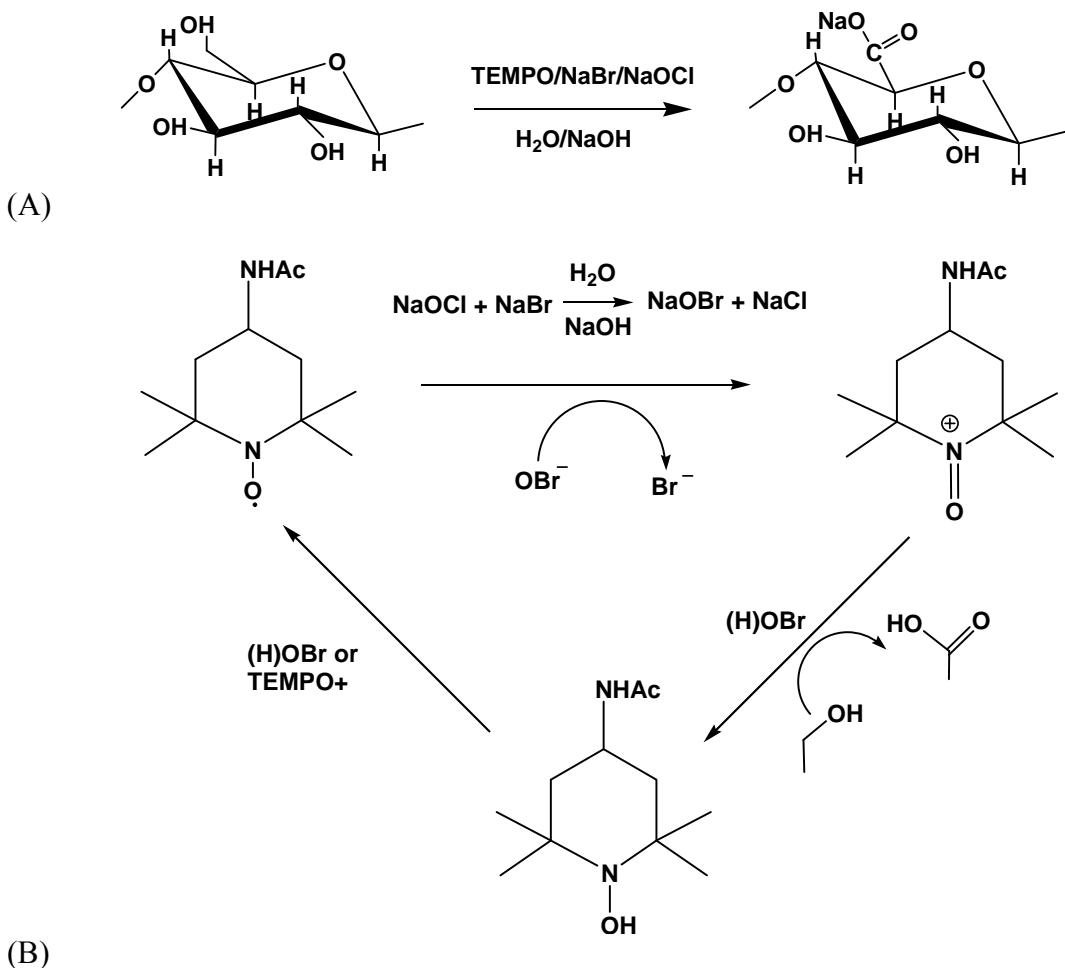
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*Contact information* a: Centre de Recherche sur les Matériaux Lignocellulosiques (CRML), 3351, boulevard des Forges, Trois-Rivières (Québec) Canada G9A 5H7; \* Corresponding author: Shree.Prakash.Mishra@uqtr.ca

### INTRODUCTION

In the search for alternative applications of lignocellulosic biomass, the concept of biorefinery, and production of cellulose nanostructures (microfibrillated cellulose or cellulose nanofibres or nanocellulose and cellulose nanocrystals or whiskers) from native cellulose have been the subject of intensive investigation nowadays (Cherubini 2010; Chinga-Carrasco 2011; Habibi and Dufresne 2008; Iwamoto et al. 2007; Janardhnan and Sain 2006; Kamm and Kamm 2007; Paakko et al. 2007; Saito et al. 2007; Taniguchi and Okamura 1998; Wang and Sain 2007). Fundamentally, lignocellulosic biomass is made up of nanometer-scale cellulose building blocks that provide mechanical, optical, and other properties to wood and other types of renewable lignocellulosic and cellulosic biomaterials. These nanosized cellulose building units, which are networked and irreversibly fixed in the supramolecular cellulose structure, and determine the product properties and functionality, have been described as nanocellulose (Klemm et al. 2005). The nano-dimensions of nanocelluloses can be determined using transmission electron

microscopic (TEM), field emission scanning electron microscopic (FE-SEM), and atomic force microscopic (AFM) techniques.



**Fig. 1.** Reaction scheme of the oxidation of C6 hydroxyl group of cellulose by TEMPO-system. (A) Oxidation of C6 hydroxyl to carboxylate by TEMPO-system and (B) Oxidation cycle of 4-acetamido-TEMPO during TEMPO-mediated oxidation.

Nanocellulose can be prepared from lignocelluloses by various methods such as mechanical (Dufresne et al. 2000; Habibi and Dufresne 2008; Iwamoto et al. 2007; Wang and Sain 2007), chemical + mechanical (Saito et al. 2007), enzyme pretreatment + mechanical (Janardhnan and Sain 2006; Paakko et al. 2007), etc. During the past few years TEMPO-mediated oxidation using a TEMPO-NaBr-NaOCl system followed by mechanical or ultrasound (US) treatment has been employed to produce nanocellulose from native cellulose (Johnson et al. 2009; Saito et al. 2007). Oxidation of the primary alcohol groups (C6 hydroxyls in the cellulose chain) to carboxylate in the presence of secondary and tertiary hydroxyls is the main mechanism of TEMPO-NaBr-NaOCl oxidation (de Nooy et al. 1995). Such regioselective conversion of the primary hydroxyl groups to carboxylate makes it possible to weaken the adhesion between cellulose fibrils,

preventing the formation of strong interfibril hydrogen bonds (Saito et al. 2007). A general oxidation reaction scheme is shown in Fig. 1. The detailed reaction mechanism has been described elsewhere (Li 2006).

Ultrasound has been widely used for catalyzing chemical reactions containing carbohydrate compounds, for example, hydrolysis and depolymerisation of starch, dextran, and other di- and polysaccharides including cellulose derivatives (Kardos and Luche 2001; Suslick 1995; Suslick and Price 1999). Modification of cellulosic fibres using ultrasound has also been reported. Detailed information on applications of ultrasound in papermaking has been reviewed by Thompson and Manning (2005). The effect of ultrasound on pulp fibrillation has been reported by Iwasaki et al. (1962). According to these authors the modification of fibre morphology by ultrasound (20 kHz, 40 W) could take place in four stages: deformation of the cell wall, removal of the S1 layer, swelling of the fibres, and fibrillation. The application of ultrasound (20 kHz, 1500 W) for the deinking of papers printed with thick films of UV-cured screen process printed inks has been reported (Manning and Thompson 2004). In another study Thomson and Manning (2005) observed no chemical changes on the cellulose chain due to ultrasound treatment. In liquid phase reaction medium the principal mechanism utilizing high intensity ultrasound waves is the acoustic cavitation (the formation, growth, and collapse of bubbles) (Leighton 1994), leading to a sonochemical effect. During cavitation, the collapsing bubble produces intense local heating and high pressure for a very short lifespan. These transient, localized hot spots drive high energy chemical reactions, yielding temperatures of ca. 5000°C, pressures of about 1000 atm, and heating and cooling rates above  $10^{10}$  K/s (Suslick and Price 1999).

The aim of this study was to use high-intensity ultrasound (68 and 170 kHz frequency) in combination with a TEMPO system to oxidize the primary hydroxyl groups on the cellulose polymer chains to carboxylate groups. The oxidized pulps were subjected to mechanical and ultrasonic treatments for the production of nanocellulose. Low frequency ultrasonic waves (ca 20 kHz and 40 kHz) were used to produce nanocellulose.

## EXPERIMENTAL

### Materials

A mill-bleached, machine-dried hardwood kraft pulp, which had a carboxyl content of  $61 \pm 0.4$  mmol/kg and a DP<sub>v</sub> of  $1025 \pm 4$ , was used in our oxidation experiments. Sodium bromide (NaBr), 4-acetamido-TEMPO (2,2,6,6-tetramethyl-piperidin-1-oxyl) (termed as TEMPO for brevity), and sodium chlorite (NaClO<sub>2</sub>) were obtained from Sigma-Aldrich and used as received. Sodium hypochlorite (NaOCl) was procured locally, and assayed for its molar concentration before use. Cupriethylenediamine (CED) solution 1M was purchased from Anachemia and was used as received.

Deionised water was produced in our laboratory by reverse osmosis method and had a conductivity between 0.7 and 0.9 μS/cm. The ultrasonic cleaning bath and ultrasonic generators were supplied by Ultrasonic Power Corporation (USA).

## Methods

### *US (ultrasound)-TEMPO-NaBr-NaOCl-oxidation*

The detailed oxidation protocol and experimental setup were reported earlier (Mishra et al. 2011). In brief, the oxidation was carried out in a specially designed glass reactor placed in an ultrasonic bath. The glass reactor held 20 g (wt. %) pulp sample at ca. 1% pulp consistency in deionized water. Two ultrasonic frequencies, 68 kHz and 170 kHz, at 1000 W of ultrasonic power intensity were used. 4-acetamido TEMPO (0.46 g, 0.11 mmol per g cellulose fibre), and NaBr (1.27 g, 0.617 mmol per g cellulose fibre) were dissolved in 50 mL of de-ionized water and added to the fibre suspension. A pH-stat was used to maintain the pH using 0.5 M NaOH or 0.5 M HCl. No detrimental effect was observed on the pH electrode due to ultrasound treatment at the given US frequencies and intensity. The oxidation process was started by adding the desired amount of the NaOCl solution (3.75 mmol NaOCl per g of cellulose fibre), at 25 °C. Deviations related to reaction conditions are specified in the respective sections. The reaction was stopped after 90 minutes by adding 50 mL of ethanol, and the final pH of the solution was adjusted to 7.0 by adding 0.5 M NaOH or 0.5 M HCl as required. The US-TEMPO-oxidized cellulose slurry was filtered, thoroughly washed with de-ionized water, and preserved at 4 °C for further treatments and analyses. Similar TEMPO-mediated oxidations were also performed in the absence of ultrasonic waves. It should be noted that because of the strong swelling of the oxidized cellulosic fibres in water, the filtration and washing of the oxidized pulps were longer in both, US-TEMPO- and TEMPO-pulps and there was not much difference in their filtration efficiency. The filtration was more difficult in the final washing stages than in the beginning. Caution should be taken while applying vacuum during the filtration. In the beginning, once the filter paper was well settled at the surface of the Buchner, transfer of the fibre suspension on the filter paper should be done under minimum or nil vacuum to avoid the blockage of the pores of the filter paper.

The yield of the oxidized fibres was calculated as follows,

$$\text{Yield (\%)} = 100 \times [W_2 - 37 \times 10^{-6} W_2 (C_2 - C_1)] / W_1 \quad (1)$$

where  $W_1$  is the o.d. weight of the untreated fibre,  $W_2$  is the o.d. weight of the oxidized fibre,  $C_2$  is the carboxyls content of the oxidized fibre, and  $C_1$  is the carboxyls content of the untreated fibre (61 mmol/kg).

### *Measurement of carboxyls content*

The carboxylate content of the TEMPO-oxidized cellulose was determined by a conductometric titration method using a Dosimat 765 (Metrohm) titrator according to the technique of Katz et al. (1984). The sodium carboxylate groups in the TEMPO-oxidized celluloses were converted to the free carboxyl form by treating the sample with 0.1 M HCl solution three times followed by thorough washing with de-ionized water to remove the excess acid before the conductometric titration, which was carried out in 0.001 N NaCl solution with 0.1N NaOH solution. Five mL of 0.1 N HCl was added to the fibre suspension before starting the titration. At the end of the process the fibres were filtered,

washed, and dried in an oven at 105 °C to determine the sample weight. The carboxyl content expressed in mmol/kg was calculated by software.

#### *Post-oxidation with NaClO<sub>2</sub>*

Post oxidation of the TEMPO-oxidized pulp was carried out with NaClO<sub>2</sub> in acetic acid / sodium hydroxide buffer (pH 4.5-5.0) at 70 °C to convert the aldehyde groups, which were produced during the TEMPO-mediated oxidation, to carboxyl groups (Mishra et al. 2011). In this process 2 g of the oxidized pulp (wt. %) was first suspended in 160 mL of de-ionised water in a tightly closed 250-mL glass bottle (from SCHOTT DURAN, Germany). This was followed by the addition and mixing, sequentially, of 20 mL of 34 g/L NaClO<sub>2</sub> solution and 20 mL of acetic acid/ sodium hydroxide buffer, giving a final volume of 200 mL of the reaction mixture (i.e. 1% pulp consistency, w/v). The mixture was kept in a water bath at 70 °C for 2 h, after which it was cooled, filtered and washed thoroughly with de-ionized water prior to further analyses.

#### *Measurement of viscosity of oxidized pulp*

The relative viscosity of the TEMPO- and US-TEMPO-oxidized pulps, as well as reference samples was measured as per SCAN standard methods SCAN-CM 15:88. The degree of polymerization (DP<sub>v</sub>) was calculated as per the Sihtola method, using the following Mark-Houwink-Sakurada equation:  $DP_v^{0.905} = 0.75 [\eta]$  (Sihtola and Kyrklund 1963). The DP<sub>v</sub> calculation is based on the assumption that both cellulose and oxidized cellulose molecules have the same viscosity at the same DP.

#### *Production of nanocellulose*

The TEMPO- or US-TEMPO-oxidized cellulose sample (0.3 g) was suspended in de-ionized water (300 mL) at 0.1 % concentration. Three methods were used for the production of nanocellulose: (1) mechanical treatment in a blender (Saito et al. 2007); (2) with an ultrasonic probe at 20 kHz and at maximum intensity setting (Branson Sonifier 350), and (3) in an ultrasonic bath (Advanced Sonics, USA) at 40 kHz, 600 W for various span of time. The disintegrated suspension was centrifuged at 10,000 g during 12 min to separate the supernatant containing nanocellulose from the unfibrillated and partially fibrillated fractions. The quantity of nanocelluloses was obtained by drying three samples of 40 mL each from the supernatant at 105°C. The slurry was stored at 4°C for further characterization.

#### *SEM and TEM observation*

The samples were examined using a Phillips EM 208S transmission electron microscope (TEM) operated at 80 kV. Images were acquired electronically with an installed camera. Diluted nanofibril suspensions were deposited on formvar-coated copper grids (400 mesh). The excess liquid was absorbed with filter paper, and the remaining nanofibres were stained with 2% uranyl acetate negative stain before observation under TEM. Scanning electron microscopic analysis was done with a JEOL scanning electron microscope (SEM) model JSM-5500. The samples were lyophilized and coated with gold using a sputter coater prior to observation under SEM.

### Rheological measurements

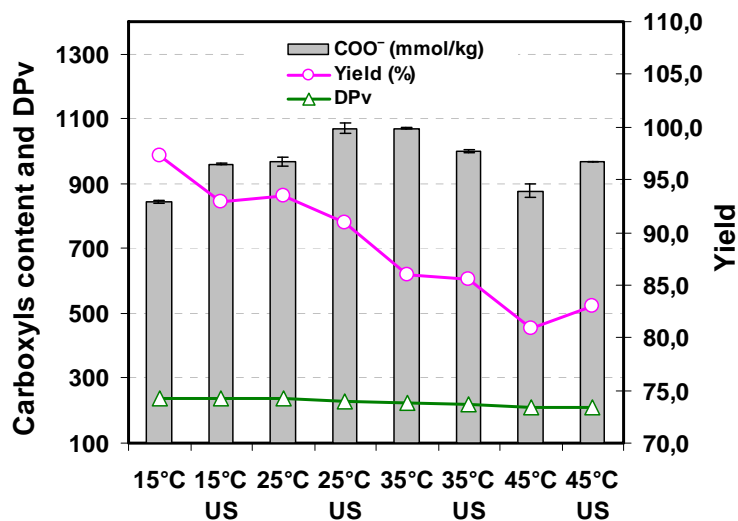
The viscosity of the nanocellulose solutions obtained by mechanical treatment and by ultrasound treatment was carried out with a Rheometer Stresstech instrument from ATS RheoSystems, NJ, USA. The measurement was done under constant rate mode using C40/ 4° head at 25°C. The concentration of the nanocellulose solution was maintained at 0.16% in both samples.

## RESULTS AND DISCUSSION

In an earlier work we noted that the use of ultrasound treatment alone (e.g. at 170 kHz & 68 kHz, 1000 W) did not affect the carboxyl content of pulp (Mishra et al. 2011). However, when the ultrasound was used in the TEMPO oxidation system, different results were obtained. We also observed that an ultrasound frequency of 170 kHz in combination with TEMPO-system gave better results when compared to the frequency of 68 kHz. As a result, we selected 170 kHz for further study.

### Oxidation of Pulp with Ultrasound-TEMPO System

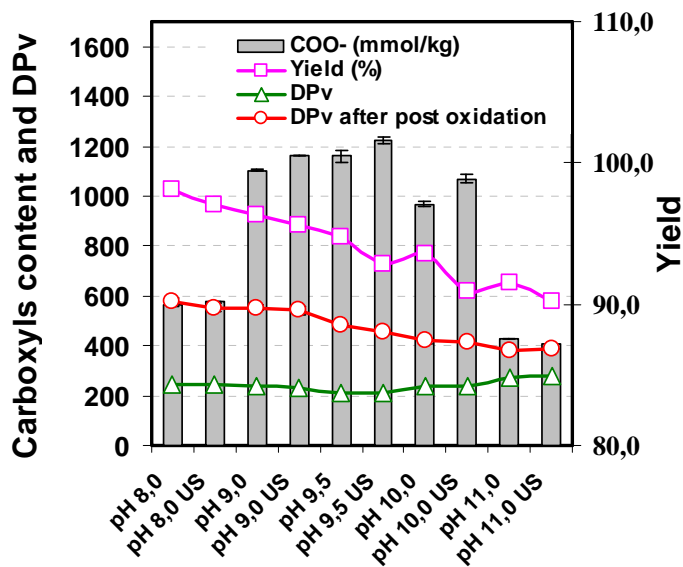
Ultrasonic cavitation generates a lot of heat, which could increase the reaction temperature and, consequently, the reaction rate. The US-TEMPO-oxidations were carried out at controlled temperature using a thermostated water bath to maintain an optimum reaction temperature. Temperature optimization was carried out at 15, 25, 35, and 45°C and at pH 10.0. The carboxyl content, nanocellulose yield, and DP versus temperature for the US-TEMPO-system (170 kHz, 1000 W) and the TEMPO-system (without ultrasound) are presented in Fig. 2. Based on the carboxyl content and nanocellulose yield a temperature of 25°C was found to be optimum for both oxidation methods (US-TEMPO- and TEMPO-systems). Figure 2 shows that at 25°C there was



**Fig. 2.** Carboxyl content, DPv and yield as a function of temperature for the US (170 kHz, 1000 W)-TEMPO and TEMPO oxidized pulp

a gain of about 10 % in carboxyl content by US-TEMPO-system (i.e. 1071 mmol/kg) over the TEMPO-system (i.e. 968 mmol/kg). The detailed results and discussion on various aspects of this study can be found in our earlier publication (Mishra et al. 2011).

In our temperature optimization study a pH 10 was selected, based on the information from the published literature on TEMPO/NaBr/NaOCl oxidations of cellulose. However, different pH optima have been reported for the oxidation of carbohydrates with TEMPO derivatives. A low pH an optimum of ca. 8.0 has been reported for a bromide free 4-acetamido-TEMPO/NaOCl oxidation system (Bragd et al. 2001). de Nooy et al. (1996) have reported better selectivity at pH 9.2 to 9.7 in a study carried out on pullulan with TEMPO. There is a lack of information on the pH optimum for the oxidation of native cellulose using 4-acetamido-TEMPO/NaBr/NaOCl. Hence, we conducted experiments at 25°C using a range of pH (i.e. 8.0, 9.0, 9.5, 10.0, and 11.0) to determine the optimum reaction pH in US-TEMPO- and TEMPO-systems. Other experimental conditions such as consistency and amount of reactants were the same as those used in the temperature optimization. The carboxyl content, yield and DPv versus pH are shown in Fig. 3. Both US-TEMPO and TEMPO-oxidized pulps showed higher carboxyl content at pH 9.0 to 9.5. At pH 9.5 the US-TEMPO gave a maximum carboxyl content of 1223 mmol/kg, and the TEMPO alone yielded 1161 mmol/kg; the former was about 5.0% higher than the latter. Note that the pulp yield decreased with increasing reaction pH. This might be due to the fact that the solubility of low molecular weight carbohydrates increased with increasing reaction pH under a given oxidation condition. On the other hand, the variation in pH showed no significant influence on the DPv for both processes. Interference from aldehyde groups in the oxidized pulps might have led to such results during the CED viscosity determination in these samples. The elimination or minimization of aldehyde groups by post oxidation with NaClO<sub>2</sub>, as discussed below, have given near true DPv for these samples and gave altogether different trend to what was obtained without post oxidation.



**Fig. 3.** Carboxyls content, DPv and pulp yield versus reaction pH for the US-TEMPO and TEMPO oxidized pulp

It has been discussed in our previous study that the CED viscosity measurements (viscosity measurements carried out using cupriethylenediamine solution as solvent) were affected by the interference from the residual aldehydes present in the TEMPO-oxidized pulps. The DP<sub>v</sub> calculated based on the viscosity of the pulp after post-oxidation increased by about two-fold in comparison with the DP<sub>v</sub> of the oxidized pulp samples before post-oxidation (Mishra et al. 2011). A possible reason to explain this effect could be the presence of aldehyde groups generated during the oxidation process, which may have rendered the cellulose polymers vulnerable to depolymerization in the highly alkaline CED solution used for the viscosity determination. A post-oxidation treatment converted the aldehyde groups to carboxyls and thus eliminated any kind of possible damage due to the highly alkaline conditions during CED viscosity measurement. Celluloses containing carbonyl groups were found to be depolymerised by CED solution during the viscosity measurement ( $\beta$ -elimination) (Chandra and Gratzl 1985; de Nooy et al. 1996; Godsay and Pearce 1984; Isogai 1998; Isogai and Kato 1998). The  $\beta$ -elimination causes chain rupture, decreasing the pulp viscosity and hence the DP<sub>v</sub>. Therefore, to minimize the interference from residual aldehydes, TEMPO-oxidized pulps were post-oxidized with NaClO<sub>2</sub>. It was assumed that the interference from the carboxyls on the CED viscosity is insignificant and, hence, the CED viscosity represents a true value of DP<sub>v</sub> (Mishra et al. 2011).

Figure 3 shows that the DP<sub>v</sub> values after post-oxidation were higher and showed different trends when compared to the DP<sub>v</sub> of the oxidized pulps without being post-oxidized. The DP<sub>v</sub> after post-oxidation indicated that the pH had a remarkable influence on the US-TEMPO and TEMPO-mediated oxidations. Despite the lower carboxyl content at pH 8.0 and pH 11.0, the DP<sub>v</sub> after post-oxidation was the lowest at pH 11.0 (Fig. 3), reflecting a greater depolymerization of cellulose at this pH under the given oxidation conditions. However, it should be noted that there was still a significant depolymerization of the pulp during the US-TEMPO or TEMPO oxidation in comparison to the original pulp (DP<sub>v</sub>: 1025). The exact mechanism of the depolymerisation of the cellulose during the TEMPO/NaBr/NaOCl oxidation under alkaline conditions is still not clearly known. The following three mechanisms for the depolymerisation of polysaccharides including cellulose proposed in the literature could be cited here. (1) The first reason could be the  $\beta$ -elimination of the glycoside bonds in the presence of intermediate aldehyde groups (C6) under alkaline reaction conditions (de Nooy et al. 1996; Isogai and Kato 1998), (2) depolymerisation of polysaccharides could occur due to sodium hypochlorite oxidation, leading to the 2,3-scission of glucose units resulting in the formation of aldehyde and dicarboxylic structures (Besemer 1993), and (3) the third reason could be the formation of active species such as hydroxyl radicals ( $\cdot$ OH) from the hydroxylamine structure of TEMPO and NaOBr, which could attack glycoside bonds of polysaccharides (Shibata and Isogai 2003). The findings in this study show that the US-TEMPO or TEMPO oxidations are very much dependent on the reaction conditions, where pH and temperature play a very important role in affecting the oxidation efficiency and pulp properties.

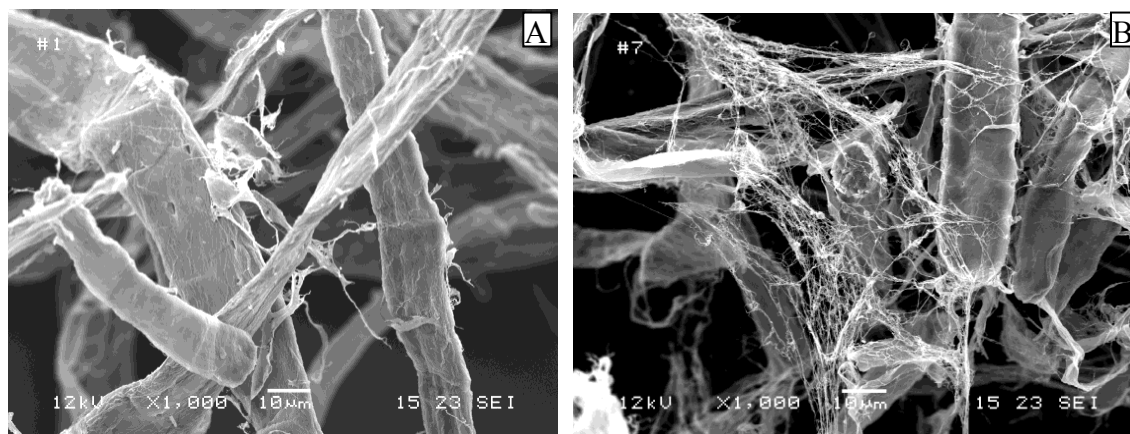
It has been reported that carboxyl contents of 800 to 1000 mmol/kg are sufficient for producing cellulose nanofibres from oxidized native cellulose with respectable yield (Isogai et al. 2011; Mishra et al. 2011). Hence, the extra gain in the carboxyl content by optimizing the pH (e.g. pH-9.5) could ultimately lead to a net reduction in the use of



active halogen species (NaOCl & NaOBr) and TEMPO, rendering the process more economical and less polluting.

### TEMPO-Mediated Oxidation of Ultrasound Pre-Treated Pulp

In the second part of this study the pulp was treated with a low frequency ultrasound (20 kHz) using an ultrasonic probe during 20 minutes at 25°C followed by oxidation with a TEMPO-system at the same temperature and pH 10. The objective was to examine the effect of ultrasonic pretreatment on TEMPO-mediated oxidation. This oxidized pretreated pulp (Fig. 4B) had a carboxyl content of 986 mmol/kg, while the US-TEMPO counterpart produced at the same temperature and pH had a higher carboxyl content of 1071 mmol/kg. The carboxyl content of the former was only slightly higher than that of the control (968 mmol/kg) i.e. TEMPO-oxidized pulp. However, the pulp pretreated with ultrasound showed a greater degree of fibrillation compared to the control as shown in Fig. 4. Because of its low carboxyl content, ultrasonic pretreated-TEMPO oxidized pulp was not analyzed for DP<sub>v</sub> and yield.



**Fig. 4.** SEM micrographs of the control pulp without any treatment (A) and treated using ultrasonic-probe (B)

There are two possible reasons for the low carboxyl content of the ultrasound-pretreated pulp:

(1) The oxidation environment for the ultrasound pre-treated pulp is the same as that for the original pulp used in TEMPO-mediated oxidation. Therefore, the oxidation mechanism, by and large, remains the same despite the fact that the ultrasound pre-treatment caused some fibre deformation which may permit a greater access of the reagents, resulting in a slightly higher carboxyl content in comparison to the control.

(2) There is a possibility of detachment and dissolution of the fines and fibrils from the highly fibrillated fibres during TEMPO-oxidation. Since the results of ultrasound pretreatment were not encouraging, no further study in this area was conducted.

## Production and Characterization of Nanocellulose

### *Production of nanocellulose from US-TEMPO- and TEMPO-oxidized pulps by mechanical treatment*

TEMPO-oxidized and US-TEMPO-oxidized pulps, both of which had similar carboxyl content (980 mmol/kg) were fibrillated under identical conditions in a home blender for 20 min to produce nanocellulose as per the protocol given in the experimental section. The nanocellulose yield was ca. 10% higher for the US-TEMPO-oxidized pulp (71 %) compared to the TEMPO-oxidized pulp (61%). The difference in nanocellulose yield could be attributed to the physical effects of the cavitating bubbles in the presence of ultrasound. It is assumed that the collapse of cavitation bubbles in the reaction medium with the presence of oxidants facilitated the access of the reactive species to the reaction site (turbulent mixing and mass transfer), C6 hydroxyls in this case, with a greater ease, increasing the reaction rate and, consequently, increasing the carboxyl content. Meanwhile, the simultaneous damage to the fibre surface caused by the shockwaves and micro-jets facilitated the separation of nanofibres when subjected to mechanical treatment (Mishra et al. 2011; Suslick and Price 1999).

### *Production of nanocellulose from TEMPO-oxidized pulps by mechanical and ultrasound treatments-comparison of nanocellulose yield*

In the second part a series of experiments were done to compare different methods for determining the production yield of nanocellulose. Since ultrasound was used in one of the methods to produce nanocellulose, only TEMPO-oxidized pulp was considered here. The TEMPO oxidized pulp having a carboxyl content ca. 1000 mmol/kg was subjected to (1) mechanical treatment in a home blender for 10, 20, 30, and 40 minutes and to (2) ultrasonic treatments for various spans of time. Ultrasound treatments were carried out in two different ways. In the first method an ultrasonic probe (20 kHz ultrasonic frequency and at maximum output intensity) was used, whereas in the second method the treatment was done in an ultrasonic bath (40 kHz, 600 W).

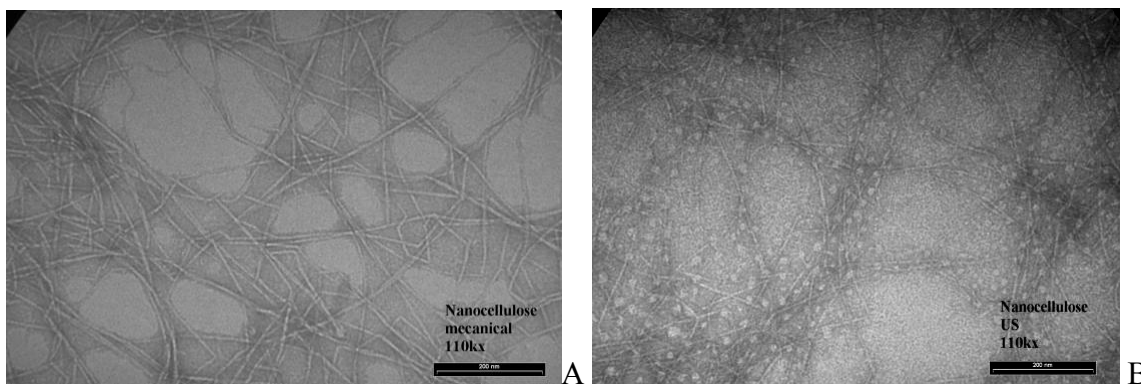
**Table 1.** Nanocellulose Yield as a Function of Treatment Times of TEMPO-Oxidized Pulp having a Carboxyls Content of ~1000 mmol/kg

Treatment Time (min)	Nanocellulose Yield (%)		
	Ultrasonic probe 20kHz	Ultrasonic bath 40kHz	Blender
5	25.4	9.0	-
10	56.7	9.5	50.8
15	78.1	9.8	-
20	82.1	10.0	79.8
25	98.42	-	-
30	-	24.4	83.7
40	-	-	92.5
60	-	50.9	-

The results are presented in Table 1. Table 1 shows that the nanocellulose production was very efficient using the ultrasonic probe, giving almost 100% yield in 25-min treatment time. The treatment in ultrasonic bath gave ca. 50% yield in 60 minutes and it took 40 minutes to yield ca. 90% nanocellulose with mechanical treatment in a blender. Since we used two different ultrasonic devices at different ultrasonic frequencies

and intensities for the nanocellulose production, their nanocellulose production efficiencies cannot be compared. However, it will help selecting a right device for the nanocellulose production. It is reported that ultrasonic probes can deliver much higher ultrasonication intensity (greater cavitation effect) than the ultrasonic bath because of their mode of application that is to say, for the ultrasonic treatment, the sample container is immersed in an ultrasonic bath whereas an ultrasonic probe is immersed directly into the sample container (Santos et al. 2009). That could be a reason for the greater efficiency of nanocellulose production with ultrasonic probes. Because of the higher yield, the nanocellulose produced by using ultrasonic probe and mechanical treatment were selected for further evaluations.

Nanocelluloses produced by mechanical treatment and by ultrasonic probe were observed under TEM and their diameters were measured manually using AnalySIS 2.1 from Soft imaging Germany. The TEM micrographs are shown in Fig. 5. As seen from Fig. 5, the nanocellulose produced by ultrasound treatment seemed slightly more deformed (Fig. 5B) compared to that produced by mechanical treatment (Fig. 5A). The length of cellulose nanofibres was not measured, but apparently the nanofibres produced with the blender looked slightly longer than those produced by ultrasound probe.



**Fig. 5.** TEM micrographs of the nanocellulose produced by Mechanical treatment (A) and by using ultrasonic-probe (B)

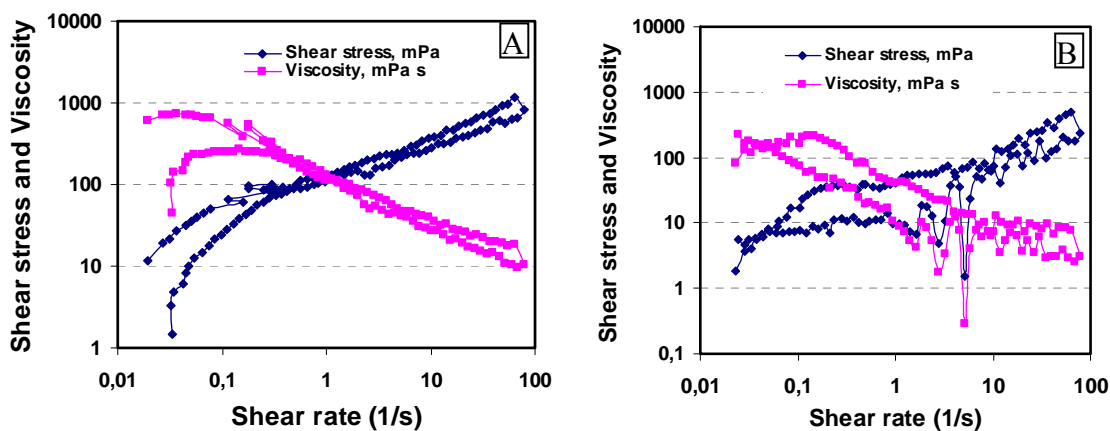
**Table 2.** Diameters of the Cellulose Nanofibres Produced by Mechanical and by Ultrasound Treatment

Diameter of the nanofibres (nm)			
Mechanical		US probe	
Average (11 meas.)	5.51	Average (15 meas.)	4.70
Max	6.38	Max	5.58
Min	4.21	Min	3.46

The diameters of the cellulose nanofibres are shown in Table 2. Measurement was carried out on 10-15 nanofibres selected randomly from the figures 5A & B. Table 2 shows a slightly lower average diameter for the nanocellulose produced by ultrasonic treatment compared to that produced by mechanical treatment. As these measurements were based on the above mentioned two figures, they may not be a representative one. A more elaborate study using other sophisticated techniques on multiple images might be

needed to come to any conclusion as to the diameters of nanocellulose produced using different techniques.

During the production of the nanocellulose by different methods, it was observed that nanocellulose produced using the ultrasound probe was apparently more fluid compared to its mechanical counterpart and hence it was decided to determine the viscosity of both the samples. For the viscosity measurements the concentration of the transparent nanocellulose suspensions (looks like solution) were kept constant ca 0.16%. The viscosity was measured with Rheometer Stresstech from ATS RheoSystems. The viscosity and shear stress were plotted against shear rate, and results are shown in Figs. 6 A and B. Figure 6 shows lower viscosity for the nanocellulose suspensions obtained by ultrasound treatment as compared to that obtained by mechanical treatment. At the same nanocellulose concentration both systems exhibited shear thinning behaviour. However, this tendency was higher in the case of nanocellulose produced by mechanical treatment at the same shear rate compared to the nanocellulose produced using ultrasound probe. A detailed rheological study on microfibrillar suspension from TEMPO-oxidized never-dried sulfite pulp could be found in Lasseguette et al. (2008). These authors have reported that besides the shear thinning behavior, nanocellulose suspensions produced from TEMPO-oxidized pulp also showed thixotropic behaviour and that the nanocellulose suspensions, produced mechanically, concentrated and homogenized by ultrasound treatment exhibited less thixotropic behavior (Lasseguette et al. 2008). Although the ultrasound treatment in our case was directly on the TEMPO-oxidized pulp to produce nanocellulose, our results almost conform to what was reported by Lasseguette et al. It is commonly believed that ultrasound treatment gives more fibrillated material compared to mechanical treatment. In that case the higher specific surface should give a higher viscosity if the fibril lengths are the same, which was not the case here. Therefore a possible reason for the lower viscosity in the case of nanocellulose obtained using ultrasound probe could be a lower aspect ratio. However, further studies are needed to completely understand the behavior of the nanocellulose obtained by both methods and its implications in further applications.



**Fig. 6.** Rheometer Stresstech viscosity of the nanocellulose produced by Mechanical treatment (A) and by using ultrasonic-probe (B)

## CONCLUSIONS

1. An ultrasound-assisted TEMPO system facilitates the oxidation of native cellulose for the production of nanocellulose. An additional carboxyl content of 5 to 15 % can be obtained using US-TEMPO system, depending upon the reaction condition compared to TEMPO alone. A temperature of 25°C and a pH of 9.5 seem optimum in the oxidations with 4-acetamido-TEMPO/NaBr/NaOCl system with or without ultrasound.
2. US-TEMPO-oxidized pulp yields ca. 10% more nanocellulose for the same duration of subsequent mechanical treatment compared to TEMPO-alone oxidized pulp that was later mechanically treated.
3. Treatment with an ultrasonic probe was found to be efficient compared to the mechanical treatment for the production of nanocellulose from TEMPO oxidized pulp. Nanocellulose suspension produced by ultrasound probe has lower viscosity when compared to that prepared by mechanical treatment probably because of the lower aspect ratio of the nanocellulose in the former.

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