



# Optimization of reagent consumption in TEMPO-mediated oxidation of *Eucalyptus* cellulose to obtain cellulose nanofibers

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**Abstract** Eucalyptus cellulose is usually pre-treated by oxidation with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), NaBr and NaClO at pH 10.5 and 25 °C before the mechanical process required to obtain cellulose nanofibers (CNFs). In this study, different aspects to improve the effectiveness and sustainability of the TEMPO-mediated oxidation are analyzed. The optimization was carried out at different reaction times by modifying both the concentration of the NaClO and the amount of the catalysts (TEMPO and NaBr). Results show that the carboxyl groups increased up to 1.1 mmol/g with 5 mmol NaClO/g after 50 min, and that the catalyst concentration can

be reduced to 0.025 mmol TEMPO/g and 0.5 mmol NaBr/g to minimize costs while maintaining the high fibrillation degree of the CNFs. The kinetic of the reaction can be considered as zero-order with respect to NaClO, and as first order with respect to cellulose. As a result of this work, the catalyst doses are reduced up to 75% compared to the most widely used catalyst doses (0.1 mmol/g TEMPO and 1 mmol/g NaBr), obtaining highly fibrillated CNFs with a lower environmental impact. This reduction of catalyst doses will reduce the costs and facilitate the implementation of CNF production at industrial scale.

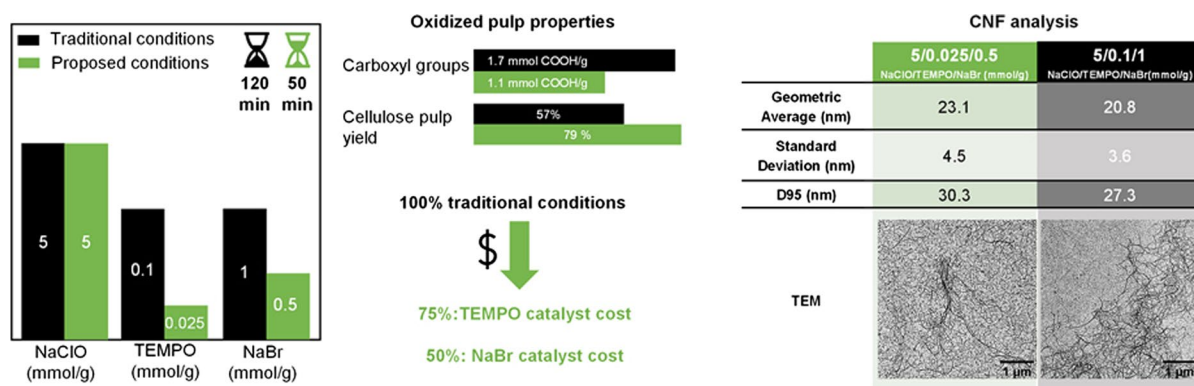
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## Graphical abstract



**Keywords** Cellulose nanofibers · TEMPO-mediated oxidation · Sustainability · Cost reduction · Nanocellulose · Reagent consumption

## Introduction

Cellulose nanomaterials have grown substantially in the last decades due to their great potential as green materials for several applications (Liu et al. 2021). Nanocellulose presents advantageous properties both in aerogel or hydrogel structure, such as sorption capacity, high mechanical strength, high surface area, functionalization possibility or light weight. Furthermore, they are obtained from renewable raw materials (Blanco et al. 2018; Klemm et al. 2018).

Cellulose nanofibers (CNFs) are generally produced through mechanical processes to break the cellulose structure down to the micro and nanoscale. However, the high energy costs of these processes require the use of pretreatments, which can be chemical, mechanical or enzymatic (Kargazadeh et al. 2018; Nechyporchuk et al. 2016). The pretreatment selection together with the intensity of the mechanical process affect considerably the fibrillation efficiency, resulting in CNFs of different qualities, which will define their final use (Sanchez-Salvador et al. 2020). Although there are several small commercial and pilot plants, the production of CNFs is still limited, due to the high initial investment and the high production cost related to the defibrillation of the cellulosic materials. An important factor to overcome

this problem is to increase the knowledge of the pretreatment stage to maximize its efficiency (Djafari Petroudy et al. 2021).

To obtain high quality nanofibers, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation (TMO) is the most common pretreatment due to the high homogeneity and fibrillation of the obtained CNFs. The oxidation of cellulose catalyzed by TEMPO was first used by Isogai and Kato (1998), guided by the oxidation of other polysaccharides using NaBr as co-catalyst and NaClO as oxidant (Chang and Robyt 1996; De Nooy et al. 1995). C6 hydroxyl groups of cellulose are oxidized first into aldehydes and then, to carboxyl groups using the system NaClO/TEMPO/NaBr (Saito and Isogai 2004). This reaction at pH ~ 10 produces the electrostatic repulsion between the oxidized C6 groups of cellulose and facilitates the separation of CNFs during the subsequent mechanical treatment (Isogai et al. 2018).

Table 1 summarizes the different conditions used for TMO by different researchers. Isogai and Kato (1998) studied the effects of TEMPO and NaBr in TMO of rayon cellulose using 18–24, 0.04–0.26 and 1.2–9.4 mmol NaClO, mmol TEMPO and mmol NaBr per gram of cellulose, respectively. They monitored the oxidation time required until the pH was constant without adding NaOH. The optimal TMO conditions were selected as 18/0.13/5.0 (NaClO/TEMPO/NaBr) to maintain the highest PD and the shortest oxidation time to minimize the cellulose degradation. However, these TMO conditions do not consider the final properties of CNFs, such as optical

**Table 1** Different reaction conditions used for TMO

NaClO (mmol/g)	TEMPO (mmol/g)	NaBr (mmol/g)	(NaClO/TEMPO/NaBr)	References
24	0.04	5.0	18–24/0.04–0.26/1.2–9.3	Isogai and Kato (1998)
24	0.13	5.0		
18	0.06	5.0		
18	0.13	5.0		
18	0.26	5.0		
18	0.13	1.2		
18	0.13	2.3		
18	0.13	9.3		
13.6	0.1	3.0	13.6/0.1/3.0	Tahiri and Vignon (2000)
0–9.7	0.016	0.25	0–9.7/0.016/0.24	Saito and Isogai (2004)
15	0.06	2.3	15/0.06–0.26/0.6–2.3	Sun et al. (2005)
15	0.16	2.3		
15	0.26	2.3		
15	0.16	0.6		
15	0.16	1.2		
15	0.16	3.5		
5	0.1	1	5/0.1/1	Saito et al. (2007), Fukuzumi et al. (2010), Balea et al. 2017, Sanchez-Salvador et al. (2021)
6	0.002–0.01	6	2–8/0.002–0.01/1.2–6	(Mao et al. 2010)
6	0.01	1.2–6		
2–8	0.01	6		
10	0.1	1	10/0.1/1	Okita et al. (2010)
1	0.016	0.25	1/0.016/0.24	Dai et al. (2011)
1.3–10	0.1	1	1.3–10/0.1/1	Shinoda et al. (2012)
5	0.1	0–1	5/0.1/0–1	Inamochi et al. (2017)
2–15	0.1	1	2–15/0.1/1	Serra et al. (2017)
15	0.1	13	15/0.1/13	Tang et al. (2017)
1–16	0.1	2	1–16/0.1/2	Lin et al. (2018)
5	0.2	1	5/0.2/1	This study
5	0.1	1	5/0.1/1	
5	0.05	1	5/0.05/1	
5	0.1	0.5	5/0.1/0.5	
5	0.05	0.5	5/0.05/0.5	
5	0.025	0.5	5/0.025/0.5	
5	0.05	0.25	5 /0.05/0.25	

transmittance or anionic charge. Tahiri and Vignon (2000) wider these studies considering different types of cellulose and using a similar TEMPO dose (0.1 mmol/g), but lower amount of NaBr (3 mmol/g), with an oxidant dose of NaClO of 13.6 mmol/g.

Later, Saito and Isogai (2004) studied the oxidation time of cotton using a low content of TMO catalysts (0.016 mmol TEMPO/g and 0.25 mmol NaBr/g)

and around 5 mmol of NaClO/g cellulose, obtaining an important increase of the carboxyl content until 2 h of reaction and a quick variation of PD during the first 30 min of reaction. After the extended oxidation up to 2 h, carboxyl groups were further formed up to about 0.6 mmol/g, whereas the content of aldehyde groups was around 0.2 mmol/g. They also studied the crystallinity and the crystal size of the oxidized CNFs

using different doses of NaClO, and observed that both parameters were nearly unchanged, indicating that the oxidation occurs in accessible or disordered regions of the cellulose.

Since then, most researchers have used the TEMPO and NaBr doses proposed by Saito et al. (2007) (0.1 mmol/g TEMPO, 1 mmol/g NaBr and 1% (w/w) cellulose pulp) while other parameters, such as NaClO dose and the pH of the reaction, have been widely studied for the oxidation of different raw materials (Balea et al. 2017; Fukuzumi et al. 2010; Inamochi et al. 2017; Lin et al. 2018; Okita et al. 2010; Saito et al. 2007; Serra et al. 2017). Therefore, there is the need to delve into the study of the catalyst dose effect.

Time oxidation during TMO has been also little studied. In general, the oxidation is left until a constant pH is obtained and all NaClO is consumed. Only Lin et al. (2018) reported the optimal oxidation time of wood dissolving pulp (*Pinus caribaea*) at 6 h resulting in a higher amount of carboxyl content (0.7 mmol/g). They observed that an extension of the reaction time barely enhanced the amount of carboxyl groups. Myja et al. (2018) tried to better understand the TMO conditions on thermomechanical pulp by using 4-acetamido-TEMPO as catalyst. Besides, Sanchez-Salvador et al. (2021) has recently proposed to reduce the oxidation time of cotton and eucalyptus pulp, below 1 h, although all the NaClO available in the medium is not consumed, avoiding an excessive cellulose degradation and allowing the reuse of the reagents in successive TMO cycles. Crystallinity and morphology of the raw material also affect the efficiency of the TMO pretreatment as well as the final properties of CNFs (Sanchez-Salvador et al. 2022; Sun et al. 2005; Tahiri and Vignon 2000). For example, cellulose fibers from eucalyptus pulp have more

disordered regions than fibers from cotton, favoring the TMO reaction due to the higher availability of C6 hydroxyl groups. Thus, by using the same TMO conditions, the amount of carboxyl groups obtained using cotton are lower than with eucalyptus pulp (Sanchez-Salvador et al. 2021). However, none of these studies analyzed the variation of the TEMPO and NaBr doses. Only Inamochi et al. (2017) combined NaBr co-catalyst with other salts as Na<sub>2</sub>SO<sub>4</sub> to reduce the presence of brominated compounds, observing that a reduction of NaBr dose up to 0.2 mmol NaBr/g cellulose, produces a slightly decrease on the carboxyl content after a reaction time of 100 min. Nevertheless, this study was focused on maintaining the properties of the oxidized cellulose combining both NaBr and Na<sub>2</sub>SO<sub>4</sub>.

Therefore, the aim of this study is to improve the TMO knowledge to develop a more efficient and sustainable process to obtain highly fibrillated CNFs. In other words, minimizing NaClO, NaBr and TEMPO doses, as well as the reaction time to achieve a higher cellulose recovery, the environmental impact and the process costs of TMO are reduced while the CNF properties are maintained. As the optimal TMO conditions are affected by the raw material, the Eucalyptus cellulose is selected in the present study since it is one of the most common raw materials to produce CNFs. In a first part, NaClO consumption has been evaluated with time for different NaClO doses. After selecting the NaClO dose, NaBr and TEMPO doses have been studied. On both cases, the evaluation of the TMO conditions (time, NaClO, NaOH consumption and cellulosic pulp yield), the oxidized cellulose (carboxyl and aldehyde groups) and the final properties of CNFs (transmittance, cationic demand (CD) and PD) have been quantified. The hypothesis is that a better integrated knowledge on all these factors will allow us to reduce the chemical doses and the oxidation time and, therefore, to improve the viability of the process. In addition, a kinetic study of the TMO reaction has also been considered in the determination of the optimal conditions, including the recovery of the pulp and the catalyst cost.

**Table 2** Chemical Composition of the Eucalyptus bleached kraft pulp

Compound	Percentage (w/w, %)
Cellulose	74.5 ± 0.4
Hemicellulose	16.9 ± 0.2
Soluble lignin	5.7 ± 0.2
Klason lignin	1.3 ± 0.1
Extractives	1.2 ± 0.1
Ashes	0.4 ± 0.1

## Materials and methods

### Materials

*Eucalyptus globulus* ECF bleached kraft pulp, kindly supplied by Torraspapel, S.A. (Zaragoza, Spain) was used as cellulose source. Table 2 shows the chemical composition of the pulp.

TEMPO reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at 4 °C before used. NaBr and 10% (w/v) NaClO solution (previously titrated to determine the actual concentration of NaClO) were purchased from Panreac AppliChem (Barcelona, Spain). Reagents used for monitoring the reaction and characterization of the products, NaOH, HCl, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, KI and bis(ethylenediamine) copper(II) hydroxide solution, were also supplied by Panreac AppliChem (Barcelona, Spain). 0.1% (w/w) Poly-L-Lysine solution was purchased from Electron Microscopy Sciences (Hatfield, PA, USA).

### TEMPO-mediated oxidation reaction

To prepare a batch of oxidized cellulose with certain chemical reagent conditions, 30 g of dry cellulose pulp was soaked in water for at least 24 h. The samples were disintegrated for 30,000 revolutions at 2% (w/w) consistency in a Messmer pulp disintegrator manufactured by Mavis Engineering Ltd. (London, UK) and then adjusted until a final concentration of 1% (w/w) with deionized water. Before adjusting the concentration at 1% (w/w), different doses of TEMPO (0.025–0.2 mmol/g pulp) and NaBr (0.25–1 mmol/g pulp) were stirred until complete the dissolution. The reaction starts with the addition of NaClO (2.5–10 mmol/g pulp) into the 5-L agitated reactor, maintaining the temperature at 25 °C, using a cooling jacket, and also the agitation at 400 rpm. The pH was adjusted in the range 10–10.5 with 2 M HCl and maintained constant with the addition of 2 M NaOH when necessary.

During the reaction, 250 mL of sample were extracted from the reactor at different times. The sample was washed through filtration steps using 500 mL of deionized water. The oxidized pulps obtained (OPs) at ~5% consistency were stored at 4 °C to be characterized and, finally, homogenized to produce the CNFs.

The reaction was monitored by the NaClO content, using a redox titration multi-step method (Lewin and Avrahami 1955). Firstly, three reactions take place in which NaClO, HCl, KI in excess and starch are combined to form a starch-triiodide complex. Then, the complex is titrated by Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> to form a colourless solution. For the determination of NaClO, 25 mL of sample from the reactor were added in an Erlenmeyer flask together with 15 mL of deionized water, 20 mL of 2 M HCl and 10% (w/w) KI solution. The mixture was titrated with 0.13 M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, adding 2 mL of 0.2 M starch solution, until the sample becomes transparent. The concentration of NaClO was determined by the following equations (Eqs. 1 and 2):

$$\text{mol Na}_2\text{S}_2\text{O}_3 = 0.13M \cdot V_{\text{Na}_2\text{S}_2\text{O}_3} \quad (1)$$

$$\text{mol NaClO} = \frac{\text{mol Na}_2\text{S}_2\text{O}_3}{2} \quad (2)$$

where  $V_{\text{Na}_2\text{S}_2\text{O}_3}$  is the volume of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> used for titration.

### Characterization of the initial and oxidized pulp

The composition of the initial pulp (Table 2) was determined according to TAPPI T204 (TAPPI 2017) (extractives), TAPPI T211 (TAPPI 2016) (ashes) and NREL/TP-510-42618 (NREL 2012) (cellulose and hemicellulose) laboratory analytical procedures. Briefly, the procedure consists of determining the extractives by Soxhlet extraction using acetone as solvent; the ashes by calcination at 525 °C; and the acid-insoluble lignin (or Klason lignin) by the following stages: (i) acid hydrolysis of 0.3 g of dry pulp with 3 mL of 72% (w/w) H<sub>2</sub>SO<sub>4</sub> during 1 h at 30 °C; (ii) dilution with 84 g of distilled water to avoid phase separation between high and low concentration acid layers; (iii) autoclaving at 121 °C for 1 h; (iv) collecting the precipitates by filtration; and (v) drying and weighting the filtration cake. The percentage of soluble lignin, cellulose and hemicellulose were determined by analyzing the filtrate. Soluble lignin was obtained from the absorbance of the filtrate at 240 nm measured with UV–Vis spectrophotometer. Cellulose and hemicellulose were determined by HPLC of the filtrate after neutralization with CaCO<sub>3</sub> and microfiltration through 0.2 µm filter.

To determine the solid content of OPs, samples were dried at 65 °C in a laboratory stove until constant weight. The amount of salts from TMO required its quantification by incineration at 525 °C according to the ISO 1762 standard (ISO 2019). The ash content of cellulose source was negligible under 0.2% of the dry pulp. Therefore, cellulose content of OPs was calculated subtracting the salt content from the solid content.

Carboxyl groups were determined by conductometric titration with 0.15 g of dry pulp. 5 mL of NaCl 0.01 M and deionized water were added to a total volume of 55–60 mL. Then, 0.1 M HCl were added to adjust the pH around 2.5–2.8 to protonate the carboxyl groups. NaOH 0.05 M was added in 0.2 mL increments and the conductimetry values were recorded. At the beginning, the conductivity decreases due to the formation of water molecules with de hydroxyl ions from the NaOH and the protons of the HCl. Then, the undissociated H<sup>+</sup> are attacked by OH<sup>-</sup> when the strong acid is neutralized. Thus, the conductivity is maintained at this stage. After the weak acid is neutralized, NaOH is in excess and the conductivity increases. The carboxyl groups are determined by Eq. 3.

$$\text{carboxyl groups} \left( \frac{\text{mmol COOH}}{\text{g dry pulp}} \right) = \frac{(V_e(\text{mL}) - V_i(\text{mL})) \cdot \text{NaOH molarity}(M)}{\text{dry pulp}(g)} \quad (3)$$

where,  $V_i$  and  $V_e$  are the volume of NaOH in the initial and end points in which the conductivity is constant.

Aldehyde groups were established by a Schiff base reaction using 0.05 g of dry pulp, hydroxylamine hydrochloride at 5 wt. % and pH 3.5. In this

condition, the aldehyde groups were converted to oximes and HCl. NaOH 0.1 M was added to control the pH at 3.5. The reaction was finished when the pH is maintained without adding more NaOH and the aldehyde groups were determined in the same proportion than the NaOH consumed.

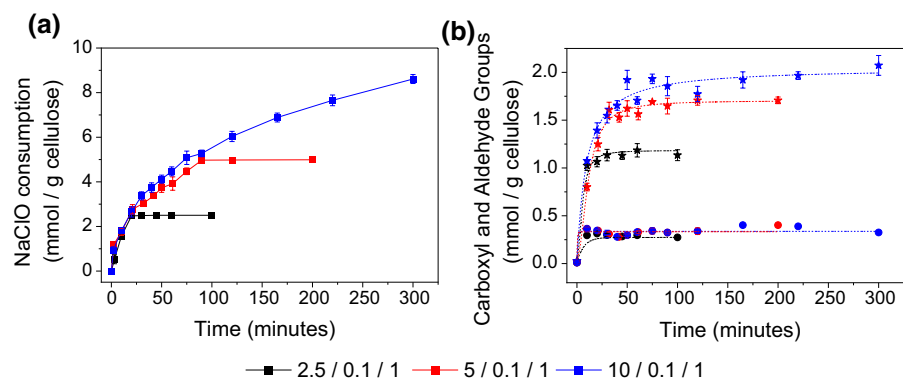
Micrographs of the OPs were displayed with a Zeiss Axio Lab.A1 optical microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany). PD was calculated from the intrinsic viscosity of the OPs, determined following the ISO 5351 standard (ISO 2010) using bis(ethylenediamine)copper(II) hydroxide solution as a solvent, based on Mark-Houwink-Sakurada (MHS) equation (Henriksson et al. 2008).

### Cellulose nanofibrils production and characterization

To produce CNFs, the OPs were homogenized using a high-pressure laboratory homogenizer PANDA PLUS 2000 (GEA Niro Soavy, Parma, Italy). First, consistency of OPs was adjusted to 1% (w/w) and then, samples were treated at 600 bar and 4 passes of homogenization. CNFs were stored at 4 °C until they were characterized.

Characterization of CNFs is detailed below. Transmittance measurements were carried out at 600 nm using 0.1% (w/w) CNF suspensions and a Cary 50 Conc UV–visible spectrophotometer (Varian Australia PTI LTD, Victoria, Australia). CD was measured by an inverse colloidal titration. An excess of

**Fig. 1** TEMPO-mediated oxidation parameters. **a** NaClO consumption and **b** Carboxyl groups (stars) and aldehyde groups (circles)





0.001 N polyDADMAC (30–40 mL) was added to 25 mL of a 0.1% (w/w) CNF suspension and stirred for 24 h. The unreacted polyDADMAC was titrated with 0.001 N PesNa using a Müttek PCD05 particle charge detector (BTG Instruments GmbH, Herrsching, Germany). As in the oxidized pulp, PD was also measured in CNFs. Finally, Transmission Electron Microscopy (TEM) of CNFs was carried out with a JEM 1400 microscope (JEOL, Tokyo, Japan) in the National Centre of Electronic Microscopy (Madrid, Spain) according to Campano et al. (2020).

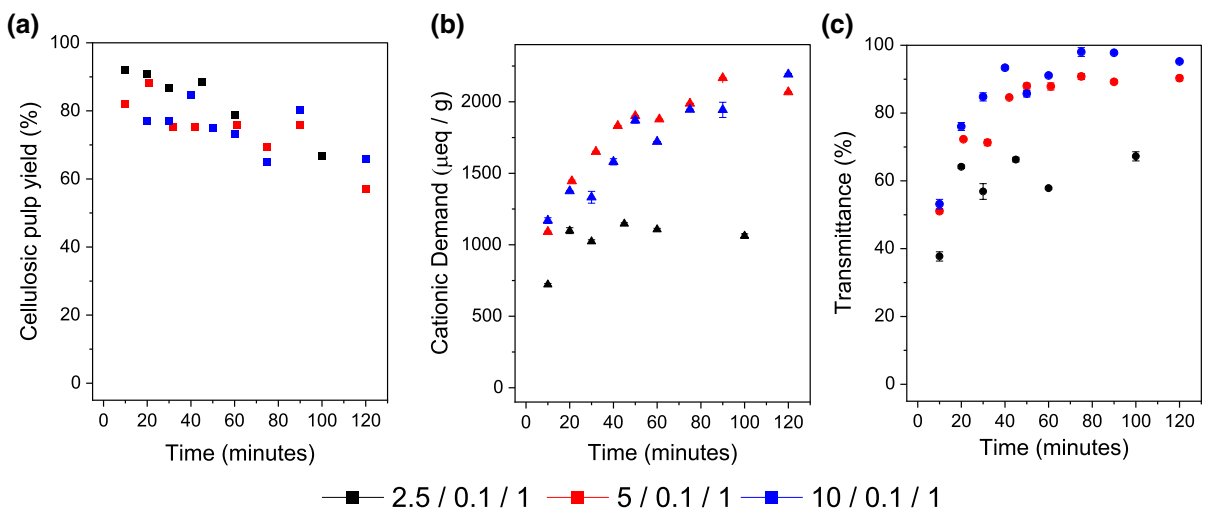
## Results and discussion

### Evaluation of NaClO dose with the oxidation time

Figure 1a shows the consumption of NaClO during TMO reaction using 2.5, 5 and 10 mmol of NaClO/g of cellulose fixing the doses of TEMPO and NaBr at 0.1 mmol/g and 1 mmol/g, respectively (2.5/0.1/1, 5/0.1/1 and 10/0.1/1). In the three cases, the consumption rate of the oxidant is not affected by the NaClO concentration obtaining similar results until all NaClO is consumed in the reaction. Thus, the reaction rate can be considered as zero-order with respect to NaClO (Mao et al. 2010). During the first minutes of the reaction, the consumption rate is very high due to the availability of C6 hydroxyl groups in

the accessible disordered regions of the raw material. Progressively, the reaction rate slows down due to the oxidation of superficial surface of the cellulose and to a limited access to deeper regions favours secondary reactions producing chlorates and bromates (Saito et al. 2010). Among these secondary reactions, the breakdown of the polymeric cellulose chains is one of them. The oxidant attacks the 1,4- $\beta$ -glycosidic bond of the cellulose, producing amorphous small chains that can be easily dissolved in the reaction medium (Saito et al. 2005).

The accessibility to C6 groups is observed in Fig. 1b, in which the number of carboxyl groups show a quick increase in the first part of the reaction, and, in a second phase they are almost stabilized, prior to the NaClO consumption, with a very slow rise until the oxidant depletion (Sanchez-Salvador et al. 2021). Then, the oxidation of hydroxyl groups to carboxyl and aldehyde groups reaches faster the plateau than NaClO consumption, due to the accessible disordered regions are easily attacked in the first minutes of the reaction obtaining carboxyl groups. After that, NaClO is mainly consumed in secondary reactions and chain cleavage. Different NaClO doses for the same reaction time produce a similar number of carboxyl groups if oxidant is available. Using 2.5/0.1/1, the slower part of the reaction does not occur because all the oxidant is consumed in the first part. When 5/0.1/1 and 10/0.1/1 are added to the pulp, the number of carboxyl groups increases



**Fig. 2** **a** Cellulosic pulp yield during TEMPO-mediated oxidation (TMO) reaction, **b** cationic Demand (CD) of cellulose nanofibers (CNFs) and **c** transmittance at 600 nm of CNFs

**Table 3** Statistical parameters of diameter of CNFs obtained at different oxidation times and NaClO doses

	Time (min)	D10 (nm)	D50 (nm)	D95 (nm)	Geometric Average (nm)	Standard deviation (nm)	Number of Samples
2.5/0.1/1	10	19.8	23.9	31.7	24.1	4.5	561
	30	20.4	23.2	27.3	23.2	2.6	431
	50	19.5	22.7	27.9	22.8	2.9	445
5/0.1/1	10	19.3	23.6	30.8	23.6	3.9	511
	30	19.4	23.1	29.5	23.1	3.6	488
	50	16.6	20.6	28.6	20.7	4.0	556
	120	17.3	20.6	27.3	20.8	3.6	455
10/0.1/1	10	18.4	23.6	34.7	23.7	5.3	488
	30	17.7	21.1	26.7	21.2	3.2	485
	50	17.0	20.8	26.9	21.0	4.2	523
	120	15.5	20.1	26.9	20.0	4.1	512

quickly up to 1.5 mmol COOH/g, 30 min after the reaction starts. Then, there is a slow increase stage from 30 to 60 min until the carboxyl groups reach 1.7 mmol COOH/g. From this moment, the carboxyl groups are constant when 5/0.1/1 is used at initial oxidant dose. However, the addition of 10/0.1/1 produces a further slowly increase of the carboxyl groups from 1.7 mmol COOH/g to 2.0 mmol COOH/g after 300 min. Therefore, an oxidant dose higher than 5/0.1/1 would not be required when TMO reaction last less than 1 h because the increase of the carboxyl groups is slow and remains almost constant. On the other hand, 1.7 mmol COOH/g is enough for the mechanical treatment.

The amount of aldehyde groups, generated in the intermediate reaction of the obtention of carboxyl groups, remains constant in the three cases with values around 0.3–0.4 mmol/g of cellulose. This is because aldehyde groups can form intra- and intermolecular hemiacetals with hydroxyl groups of cellulose. These hemiacetals are recalcitrant to further attack by the TEMPO-mediated oxidation due to steric hindrance (Saito and Isogai 2006).

Therefore, the rate of NaClO consumption was assumed similar in the three cases independent of the amount of NaClO. The higher rate, at first, is associated only with the higher availability of native cellulose and then, a progressive diminution of the reaction due to the oxidation, indicating a zero-order reaction rate in relation to NaClO (Mao et al. 2010). Therefore, NaClO does not interfere in the reaction rate and its presence is required at any concentration only during the time the cellulose is oxidized.

Cellulose pulp yield after TMO reaction is shown in Fig. 2a. Whenever the oxidation time increase,

there is a decrease in the pulp yield due to the dissolution of a part of the cellulosic fraction. However, scarce differences are observed among the doses studied. Only in the first minutes of reaction using 2.5/0.1/1 could be observed a higher pulp yield due to a less intensive oxidation. Besides, the final properties of CNFs homogenized at 4 passes and 600 bar were studied using the CD (Fig. 2b) and transmittance at 600 nm (Fig. 2c). CD increases with the oxidation time because the anionic surface charge of the fibers also increases at the same time as the carboxyl groups rise up. Once the carboxyl groups stabilize, the CD remains practically constant, only a slightly increase is observed due to an extended reaction time. The 5/0.1/1 and 10/0.1/1 series have a higher CD since a higher concentration of oxidant breaks to a greater extent the hydrogen bonds between the fibers, generating a higher repulsion between the nanofibers. In the same way as CD, transmittance values show the same trends due to is related with the proportion of nanofibrils in the sample. Using 5 or 10 mmol NaClO/g of pulp more than 85% of transmittance can be achieved after 40 min of reaction, whereas with a lower oxidant dose (2.5/0.1/1), 70% transmittance is not reached at any time.

Table 3 shows a diameter analysis of the three NaClO doses at 10, 30, 50 and 120 min of oxidation. 2.5/0.1/1 at 120 min has not been analysed since all NaClO has been consumed before. D10, D50 and D95 indicate the percentile 10, 50 and 95 of CNF diameter distribution when the samples are classified from the shortest to the largest. In all cases, the range of CNF diameters is on the nanometric scale with slight differences. The longer the oxidation time and



the higher the NaClO dose, the diameter of the CNFs is smaller. However, the maintaining of the carboxyl groups after 50 and 120 min of oxidation in both 5/0.1/1 and 10/0.1/1 series show no differences in the diameter range with an average of 20–21 nm. Therefore, it is not necessary to extend the TMO reaction more than 50 min, which also improves the cellulose pulp yield. On the other hand, Supplementary Information (Fig. S1) shows the diameter distribution of the CNFs at different times of oxidation. After 10 min of oxidation, the diameter distribution is similar in all cases. Higher oxidation times for 2.5/0.1/1 show higher diameter range compared to the other samples due to all NaClO has been consumed in the first minutes. TMO reaction using 5/0.1/1 and 10/0.1/1 and at least 50 min presents similar distributions. Since Fig. 1a indicates that the TMO reaction with 5/0.1/1 lasts about 90 min until the depletion of all NaClO. Then, the dose of 5 mmol NaClO/g has been selected in the second part of the study to have enough oxidant reagent to study the reaction without wasting it. Finally, Fig. 3 shows TEM images of CNFs after homogenization of the oxidized pulp at 10 and 50 min

of oxidation time with different doses of NaClO (2.5, 5 and 10 mmol NaClO/g).

Study of TEMPO and NaBr doses with the oxidation time

Figure 4 shows the NaClO consumption during TMO reaction using different TEMPO and NaBr doses. The TMO doses commonly used by Saito et al. (2007) are shown in blue colour (5/0.1/1). As expected, doubling the amount of TEMPO catalyst causes the increase of the NaClO consumption rate. Thus, the reagent is depleted after 60 min instead of 90 min with the traditional conditions, so the oxidation of the fibers, but also the secondary reactions, will occur in a shorter time, making more difficult its decoupling. On the other hand, reducing the amount of catalyst produces the deceleration of the oxidant consumption. This effect is more pronounced with the reduction of NaBr than of TEMPO.

The fact that the consumption of NaClO is higher does not mean that the oxidation reaction is improved, as it can be consumed in the degradation

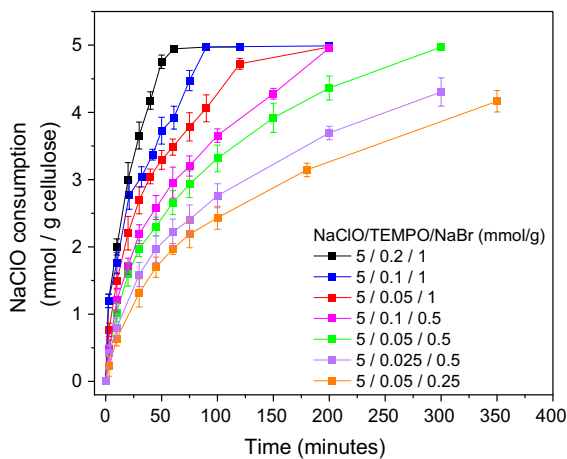
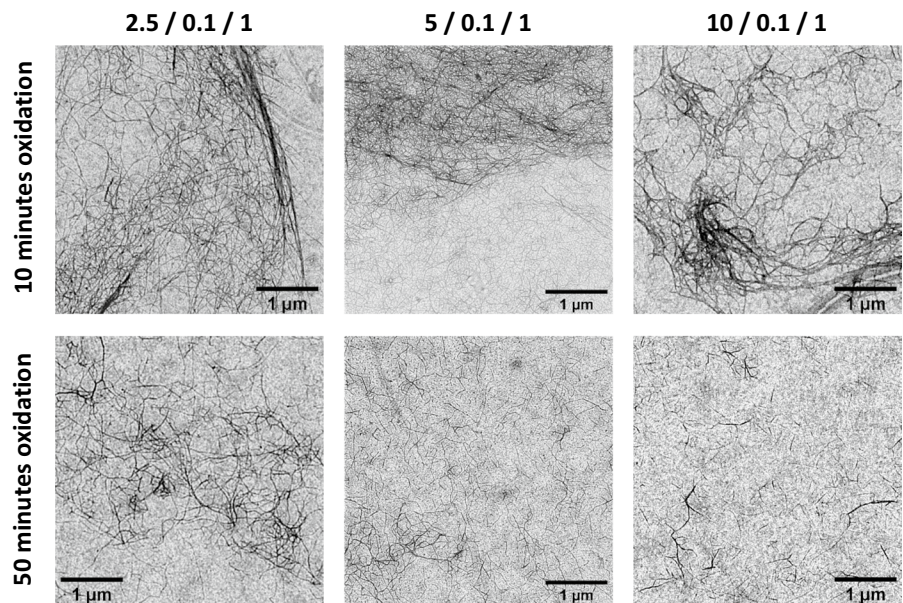
**Table 4** Statistical parameters of CNF diameter prepared from different catalyst doses and oxidation time

	Time (min)	D10 (nm)	D50 (nm)	D95 (nm)	Geometric Average (nm)	Standard Deviation (nm)	Number of Samples
5/0.2/1	10	16.1	20.2	27.3	20.2	4.3	464
	30	17.4	20.9	26.1	20.8	3.0	548
	50	16.4	20.3	26.1	20.3	3.4	432
5/0.1/1	10	19.3	23.6	30.8	23.6	3.9	511
	30	19.4	23.1	29.5	23.1	3.6	488
	50	16.6	20.6	28.6	20.7	4.0	556
	120	17.3	20.6	27.3	20.8	3.6	455
5/0.05/1	10	20.3	26.4	40.7	27.0	7.6	508
	30	20.2	25.2	32.4	25.1	4.5	526
	50	17.6	21.9	29.4	22.1	4.0	504
5/0.1/0.5	10	20.7	26.4	37.4	26.7	6.4	469
	30	20.5	25.0	32.9	25.1	4.7	425
	50	17.7	21.5	27.5	21.5	3.4	440
5/0.05/0.5	10	22.2	27.6	39.7	28.0	7.2	432
	30	20.9	25.4	32.7	25.4	4.5	459
	50	17.8	22.3	30.3	22.3	4.3	442
5/0.025/0.5	10	23.1	27.4	43.0	28.3	6.6	313
	30	20.1	24.7	34.0	24.9	4.9	510
	50	18.6	23.1	30.3	23.1	4.5	485
5/0.05/0.25	10	22.3	27.9	40.3	28.2	6.0	443
	30	20.9	25.7	34.3	26.0	4.7	393
	50	18.9	23.1	30.7	23.2	4.6	583

**Table 5** Polymerization degree of the oxidized cellulose and CNFs

	Original Cellulose	Oxidized Cellulose	CNF
(Results in number of monomers of anhydroglucose)			
5 / 0.2 / 1	931±17	240±15	175±6
5 / 0.1 / 1		206±11	168±5
5 / 0.05 / 1		239±6	206±8
5 / 0.1 / 0.5		212±7	200±9
5 / 0.05 / 0.5		221±7	199±6
5 / 0.025 / 0.5		316±6	256±16
5 / 0.05 / 0.25		303±6	216±4

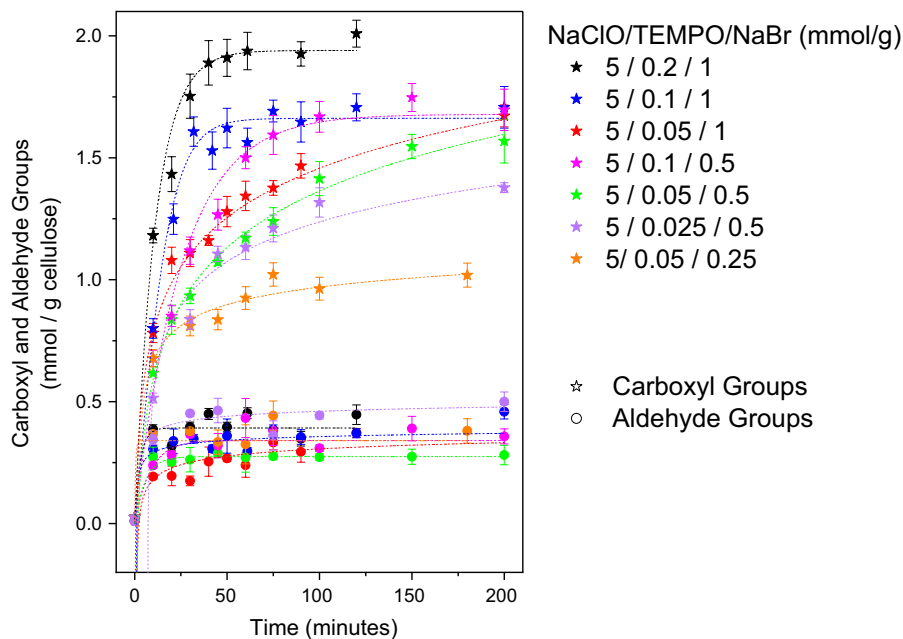
**Fig. 3** TEM images of CNFs at different oxidation times and NaClO doses



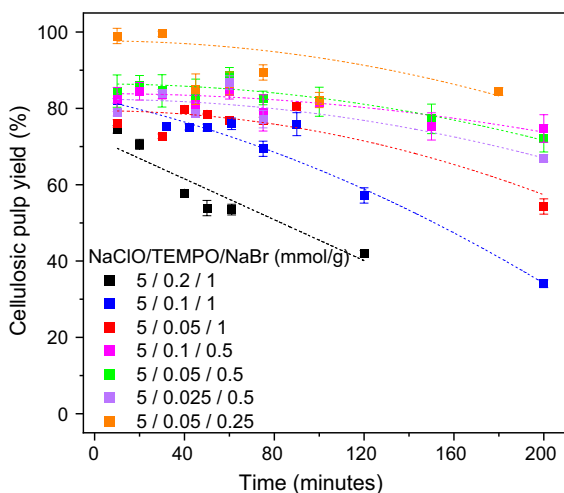
**Fig. 4** NaClO consumption during TEMPO-mediated oxidation with 5 mmol NaClO/g pulp

of cellulose, hemicellulose or in the side reactions (Saito and Isogai 2004). This effect can be observed by studying the amount of carboxyl groups or the cellulosic pulp yield. Figure 5 shows the carboxyl group content of the pulp fibers after TMO pretreatment varying TEMPO and NaBr doses. As in Fig. 4, the traditional conditions are shown in blue, in which a stability zone begins to observe after 30 min of oxidation reaching around 1.7 mmol of carboxyl groups/g cellulose and, from this time, carboxyl groups do not increase. Pulp yield decreases more noticeably with prolonged oxidation time, as shown in Fig. 6. The amount of carboxyl groups could be improved up to 1.9 mmol COOH/g by using 5/0.2/1, at the expense of higher catalyst costs and lower recovery of oxidized cellulose.

**Fig. 5** Carboxyl and aldehyde groups evolution during TEMPO-mediated oxidation



The reduction in the catalyst concentration is associated with the increase in the oxidation time. Nonetheless, setting an oxidation time in the range of 50 min and reducing the catalysts up to 5/0.025/0.5, the amount of carboxyl groups remains around 1.1 mmol COOH/g, while the cellulose recovery has increased slightly. However, the reduction of NaBr up to 0.25 mmol/g does not allow obtaining higher carboxyl groups even using 0.05 mmol TEMPO/g.



**Fig. 6** Cellulosic pulp yield during TEMPO-mediated oxidation

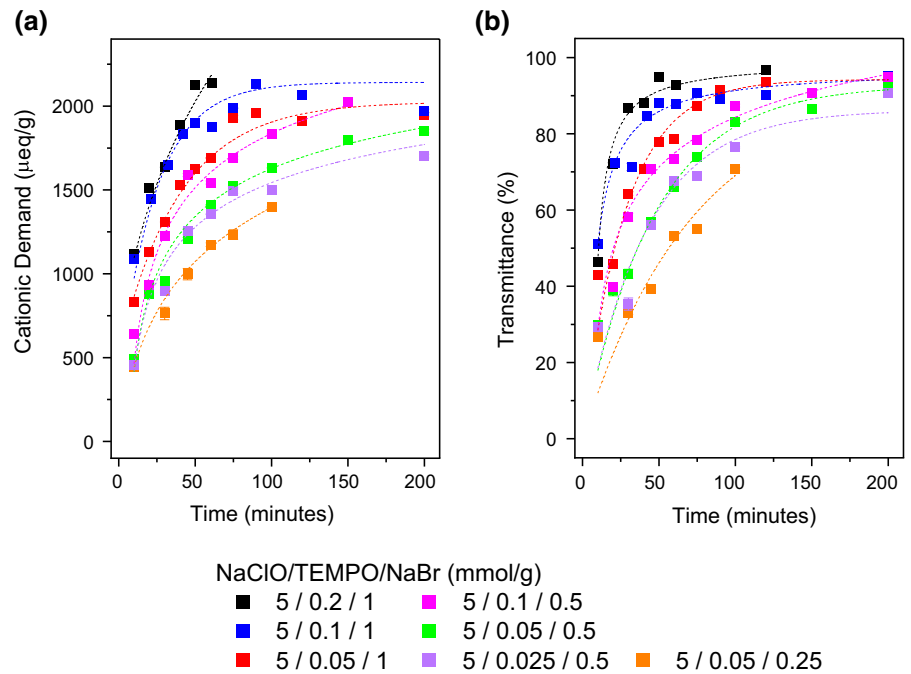
By varying the amount of NaBr while the TEMPO dose is maintained, such as in the case of 5/0.1/0.5 and 5/0.1/1, or even 5/0.5/1 and 5/0.5/0.5, the same carboxyl groups are reached but at different reaction times.

The content of aldehyde groups of each experiment, as explained above, remains constant due to the formation of hemiacetals with hydroxyl groups of cellulose. Therefore, some aldehyde groups are not converted to carboxyl groups in TMO (Saito and Isogai 2006).

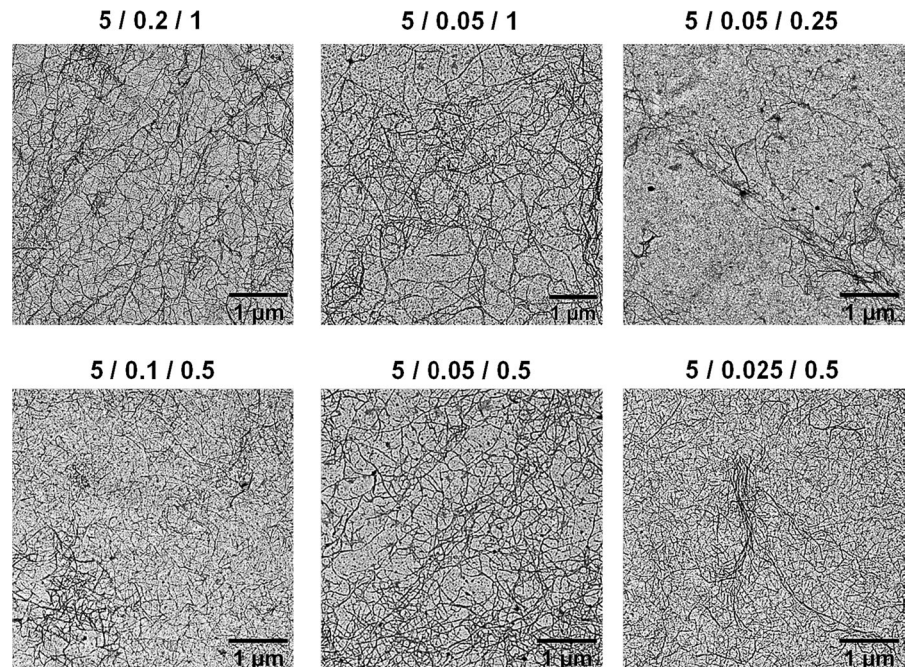
As for the properties of the final CNFs, CD and transmittance were studied as shown in Fig. 7. The CD and transmittance follow the same trend, increasing with catalyst concentration.

The variation of TEMPO dose affects less on the properties of the CNFs, whereas, the NaBr dose produce higher variations, decreasing both CD and transmittance in a higher extent. The three oxidation experiments with 1 mmol/g of NaBr (5/0.2/1, 5/0.1/1 and 5/0.05/1) show higher CD and also transmittance, followed by the ones with 0.5 mmol NaBr/g (5/0.1/0.5, 5/0.05/0.5 and 5/0.05/0.5) and finally the oxidation reaction with 0.25 mmol NaBr/g (5/0.05/0.25). With 1 mmol/g of NaBr, the CD can be reached up to 1600–2100  $\mu\text{eq/g}$  and the transmittance, to 80–95% after 50 min of oxidation. Whereas, using 0.5 mmol/g of NaBr, the CD is reduced to 1250–1600  $\mu\text{eq/g}$  and

**Fig. 7** **a** Cationic Demand (CD) of cellulose nanofibers (CNFs) after TMO and homogenization and **b** transmittance at 600 nm of CNFs



**Fig. 8** TEM images of cellulose nanofibers (CNFs) at different TEMPO and NaBr doses



the transmittance also is reduced to 60–70% at the same reaction time. Finally, with 0.25 mmol/g of NaBr, the CD can only achieve 1000  $\mu\text{eq/g}$  and 30% of transmittance after 50 min of reaction.

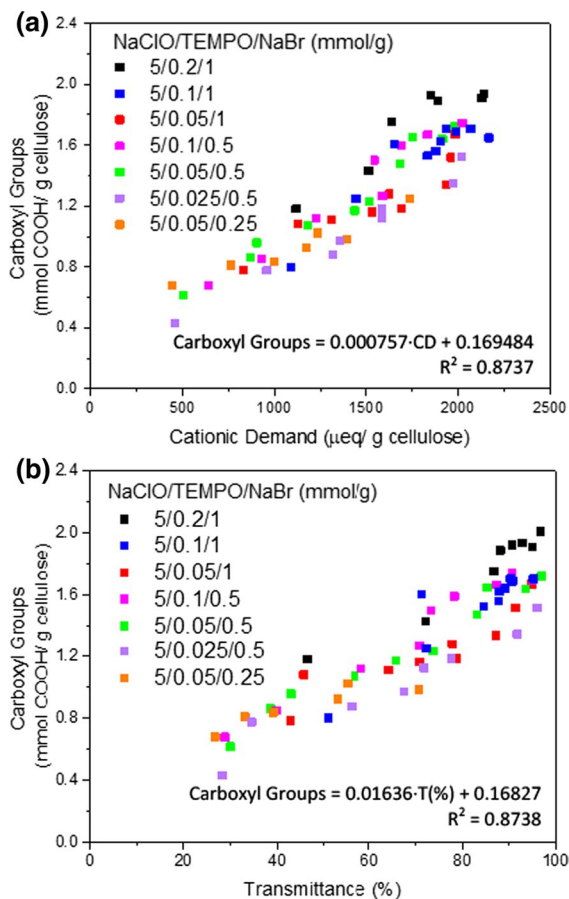
Table 4 shows the statistical parameters of CNF diameters obtained with different catalyst doses and oxidation time. It is observed that the most part of the fibers in all samples are on the nanometric scale even with an oxidation of only 10 min as D95 shows



with values under 43 nm. The shorter the oxidation time and the lower the catalyst dose, the CNFs are slightly wider. The two experiments with the higher amount of TEMPO (5/0.2/1 and 5/0.1/1) have a standard deviation around 4 for all oxidation times. In other words, the CNF diameter is relatively more homogeneous and slightly smaller than in the rest of cases, as shown in the distribution of diameter of the Supplementary Information (Fig. S2), in which the standard deviation was around 6–8 after 10 min of oxidation. For these last samples, as the oxidation time increased, the standard deviation resembled that of the samples with a higher amount of TEMPO, since they were more homogeneous. After 50 min of oxidation, the samples with lower doses of catalysts (5/0.025/0.5 and 5/0.05/0.25) had a geometric average of 23.1 and 23.2 nm, respectively, less than 3 nm above the average of the CNFs prepared with Isogai and co-workers conditions. In addition, the homogeneity of the CNFs is similar to the samples with higher catalyst doses as standard deviations indicate. Although the statistical parameters of 5/0.05/0.25 are similar to 5/0.025/0.5, the cost of catalysts in this last case is lower due less TEMPO dose is used. So, it is possible to reduce the 75% and 50% the doses of TEMPO and NaBr, respectively maintaining a high fibrillation of CNFs after 50 min of oxidation.

Finally, Fig. 8 shows TEM images of CNFs after homogenization, using 50 min of oxidation time with 5 mmol NaClO and different doses of TEMPO and NaBr. Previously, in Fig. 3 a micrograph using the same amount of oxidant agent and the traditional conditions of Saito and Isogai (2004) in terms of TEMPO and NaBr doses (5/0.1/1) has been plotted.

Another property that has been analyzed for both oxidized cellulose and CNFs is the PD. Table 5 shows that the PD decreases from  $931 \pm 17$  which is the value for the original wood pulp to 206–316 for OPs and 168–256 for the CNFs around 50 min of oxidation. The production of the hydroxyl radicals in the combination of TEMPO, NaBr and NaClO at basic pH brings a significant depolymerization of cellulose leading to a remarkable decrease in pulp viscosity (Shibata and Isogai 2003). The experiments performed with the lowest amount of catalysts (5/0.025/0.5 and 5/0.5/0.25) have the highest PD, around 320 for OPs and 220–260 for the CNFs, due to the catalyst doses. It is observed that the lower the



**Fig. 9** Carboxyl groups versus **a** cationic demand and **b** transmittance at 600 nm

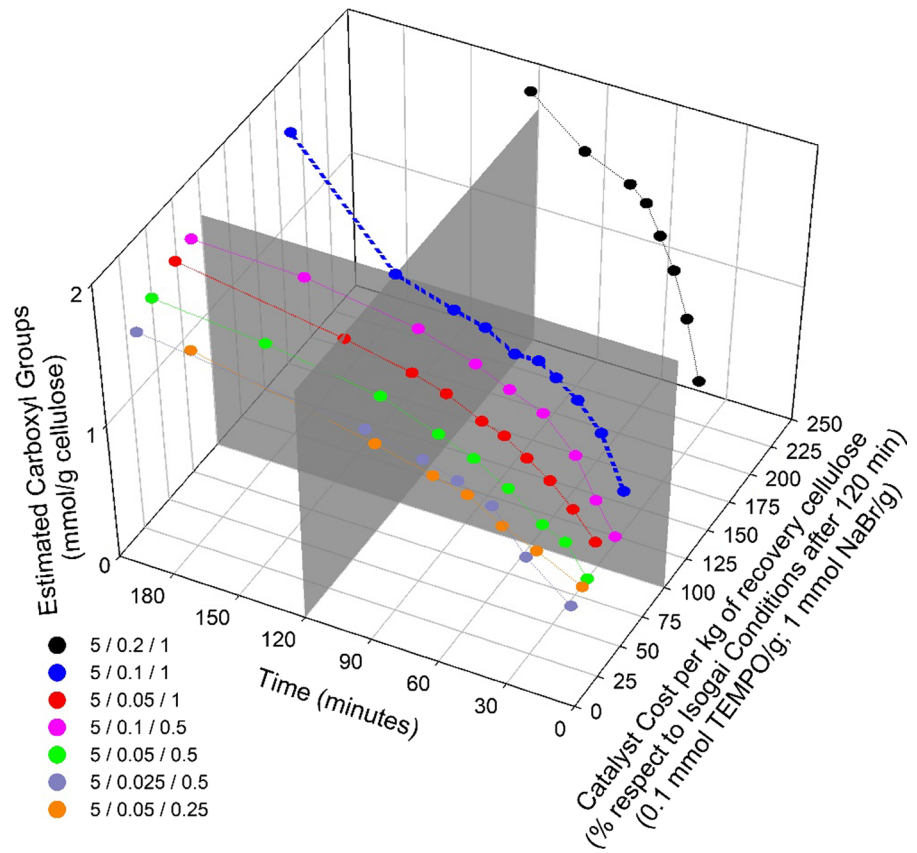
amount of the catalyst, the lower the consumption rate of the NaClO, thus, the degradation of the polymer chains is decreased.

On the other hand, PD of CNFs does not show a remarkable difference due to subsequent mechanical treatment using high pressure homogenizer, it also causes some depolymerization of wood cellulose (Funahashi et al. 2018).

Study of reaction rate coefficient as a function of catalyst doses

The chemical reactions that take place in TMO are divided into three main steps. First, the formation of HOBr from NaClO, then the formation of the ion TEMPO+ and finally the oxidation of the primary hydroxyl to aldehyde groups and then to carboxyl group (Sun et al. 2005). Some simplifications

**Fig. 10** Evaluation of catalyst cost of the TEMPO-mediated oxidation



have been considered according to previous studies reported by Sun et al. (2005) and Dai et al. (2011), describing a pseudo first-order reaction system as Supplementary Information shows.

In the first part of this study, the rate constant  $k_1$  for 2.5, 5 and 10 mmol of NaClO/g of pulp is around  $4.5 \times 10^{-3} \text{ min}^{-1}$  with an error deviation of 3%. Similar observations were found by Mao et al. (2010) who reported that the constant rate does not vary with the concentration of NaClO keeping constant the doses of NaBr and TEMPO at 6 and 0.01 mmol/g of pulp, respectively. However, they obtained a higher rate constant around  $8.7 \times 10^{-3} \text{ min}^{-1}$  because the dose of NaBr was six times higher. Therefore, the constant value in the reaction rate implies a zero-order kinetic with respect to NaClO as Eq. S3 indicates.

On the other hand,  $k_1$  values have been determined at different catalyst doses, maintaining the amount of oxidant at 5 mmol NaClO/g, as Supplementary Information shows. When the amount of TEMPO has been set to 0.05 mmol/g (Fig. S3a), there is a linear relationship between the rate constant with respect

to NaBr dose. Moreover, the rate constants obtained with different amount of TEMPO (Fig. S3b), it seems to also have a linear trend. This observation is consistent and in the same magnitude order than the results reported by Mao et al. (2010) as they found a linear relationship between  $k_1$  and the concentration of NaBr and TEMPO. However, they used higher concentrations of NaBr (until 6 mmol/g of pulp) and a lower amount of TEMPO than the current study. Nevertheless, a higher reaction rate with higher doses of catalysts does not imply an improvement in the TMO process due to the losses of cellulose are higher in the samples which show a higher reaction rate at the same time the catalysts are more expensive.

Study of catalyst costs and selection of the optimal doses

One of the main parameters for the selection of the optimal TEMPO and NaBr catalyst doses is the cost. However, it is not the only one, but also the reaction time and the properties of the CNFs and the



oxidized pulp to prepare the previous ones. Firstly, a study, which relates the different characterization parameters of the OPs and the CNFs, is carried out in order to reduce the number of variables involved in the study. Figure 9 shows that CD, transmittance and carboxyl groups obtained per gram of oxidized pulp are linearly dependent on each other, as long as they have the same subsequent homogenization. Therefore, it is possible to combine three different measurements, carried out using different methods, in one single parameter, thereby reducing the likelihood failure obtained through the variables independently. In other occasions, this fact could reduce the number of variables analyzed decreasing the labor time. The evaluation of these three variables allows us to use the parameter "Estimated carboxyl groups" as an average of the three variables. In other words, the "Estimated carboxyl groups" can be calculated using the equations in the plot with CD and transmittance obtained experimentally. Comparing the "Estimated carboxyl groups" calculated by the linear fits respect to the experimental carboxyl groups, the difference between them is below than 5%.

Thus, a 3D diagram to select the optimal conditions in TMO has been plotted in Fig. 10 using as variables the estimated carboxyl groups, the time of reaction and the catalyst costs, setting as reference the traditional conditions of Isogai, 5/0.1/1 at 120 min of oxidation (grey planes). Therefore, with these three variables plotted it is possible to join up seven parameters: carboxyl groups, transmittance, cationic demand, cellulosic pulp yield, reaction time and price and dose of both catalysts in relation to the traditional conditions. The cost of the catalysts has been obtained through the average of different companies on an industrial scale with estimated average prices of 180 \$/kg and 5000 \$/t for TEMPO and NaBr, respectively. However, these are indicative prices, since they depend on each country, quantity, and the specific market situation.

The use of high catalyst doses increases the carboxyl groups but at the same time, the pulp yield decreases, and the cost is higher. Thus, it is necessary to find a compromise solution which satisfies the properties of CNFs without wasting unnecessary amount of catalysts. It is seen that each experiment reaches a maximum point of estimated carboxyl groups and then almost stabilizes. From this point, larger oxidation times are negative due to a higher

degradation of the cellulose which reduce its recovery yield. In all cases, this time is much less than traditional conditions (approx. 120 min), which indicates a high margin for TMO improvement. In addition, the CNF diameter analysis of Table 4 shows that in all samples the CNF size is enough to obtain highly fibrillated materials similar to the traditional TMO conditions. Thus, the use of 5/0.025/0.5 after 50 min of oxidation reduce in an 80% the catalyst cost per kg of recovery cellulose, making TMO process more economically viable in the industrial sector.

## Conclusions

After studying the monitorization of the TEMPO-mediated oxidation reaction, the characterization of oxidized cellulose and homogenized CNFs is possible to conclude that the cellulose oxidation is performed at the same reaction rate, regardless the NaClO concentration, if there is oxidant available in the reaction medium. Moreover, reaction time above 1 h and excess addition of NaClO to the system are unnecessary due to cellulose losses and because carboxyl groups stabilise between 30 and 50 min, being these reaction times enough to obtain highly fibrillated CNFs. On the other hand, increasing the NaClO doses during the TEMPO reaction favours the degree of fibrillation in the samples, so the higher is the NaClO doses, the higher is the transmittance reaching up to 85%. As for the CD, the anions present in the CNFs are higher at 5/0.1/1 and 10/0.1/1 due to the higher specific surface area of the nanocellulose because of the increase in carboxyl groups. Therefore, it is concluded that the use of 5 mmol NaClO/g is enough to carry out TMO reaction up to 90 min of oxidation. Beyond this time, the CNF fibrillation do not improve and the cellulose pulp yield decrease.

Catalyst doses play an important role in the oxidation. They affect the rate of oxidant consumption as well as the oxidation time. However, an excess concentration of TEMPO and NaBr leads to an unnecessary over cost of catalyst to obtain a similar nanofibrillation degree of CNFs. It is concluded that the amount of catalysts could be reduced up to 75% (TEMPO) and 50% (NaBr), which means a significant cost reduction in comparison with the traditional conditions. Therefore, the chemical reagent costs and the oxidation time have been improved while the

CNF fibrillation is maintained which will facilitate the sustainability of the process and its implementation at industrial scale.

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### Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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### References

- Balea A, Merayo N, De La Fuente E, Negro C, Blanco A (2017) Assessing the influence of refining, bleaching and TEMPO-mediated oxidation on the production of more sustainable cellulose nanofibers and their application as paper additives. *Ind Crop Prod* 97:374–387. <https://doi.org/10.1016/j.indcrop.2016.12.050>
- Blanco A, Monte MC, Campano C, Balea A, Merayo N, Negro C (2018) Nanocellulose for industrial use: cellulose nanofibers (CNF), cellulose nanocrystals (CNC), and bacterial cellulose (BC). In: *Handbook of nanomaterials for industrial applications*. pp 74–126, Elsevier. <https://doi.org/10.1016/B978-0-12-813351-4.00005-5>.
- Campano C, Balea A, Blanco A, Negro C (2020) A reproducible method to characterize the bulk morphology of cellulose nanocrystals and nanofibers by transmission electron microscopy. *Cellulose* 27:4871–1887. <https://doi.org/10.1007/s10570-020-03138-1>
- Chang PS, Robyt JF (1996) Oxidation of primary alcohol groups of naturally occurring polysaccharides with 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion. *J Carbohyd Chem* 15(7):819–830. <https://doi.org/10.1080/07328309608005694>
- Dai L, Dai HQ, Yuan YC, Sun X, Zhu ZJ (2011) Effect of Tempo oxidation system on kinetic constants of cotton fibers. *BioResources* 6(3):2619–2631
- De Nooy AE, Besemer AC, van Bekkum H (1995) Highly selective nitroxyl radical-mediated oxidation of primary alcohol groups in water-soluble glucans. *Carbohyd Res* 269(1):89–98. [https://doi.org/10.1016/0008-6215\(94\)00343-E](https://doi.org/10.1016/0008-6215(94)00343-E)
- Djafari Petroudy SR, Chabot B, Loranger E, Naebe M, Shojaeiarani J, Gharekhani S (2021) Recent advances in cellulose nanofibers preparation through energy-efficient approaches: a review. *Energies* 14(20):6792. <https://doi.org/10.3390/en14206792>
- Fukuzumi H, Saito T, Okita Y, Isogai A (2010) Thermal stabilization of TEMPO-oxidized cellulose. *Polym Degrad Stabil* 95(9):1502–1508. <https://doi.org/10.1016/j.polymdegradstab.2010.06.015>
- Funahashi R, Ono Y, Tanaka R, Yokoi M, Daido K, Inamochi T (2018) Changes in the degree of polymerization of wood celluloses during dilute acid hydrolysis and TEMPO-mediated oxidation: Formation mechanism of disordered regions along each cellulose microfibril. *Int J Biol Macromol* 109:914–920. <https://doi.org/10.1016/j.ijbiomac.2017.11.078>
- Henriksson M, Berglund LA, Isaksson P, Lindström T, Nishino T (2008) Cellulose nanopaper structures of high toughness. *Biomacromol* 9(6):1579–1585. <https://doi.org/10.1021/bm800038n>
- Inamochi T, Funahashi R, Nakamura Y, Saito T, Isogai A (2017) Effect of coexisting salt on TEMPO-mediated oxidation of wood cellulose for preparation of nanocellulose. *Cellulose* 24(9):4097–4101. <https://doi.org/10.1007/s10570-017-1402-y>
- ISO (2010) ISO 5351:2010 Pulps — Determination of limiting viscosity number in cupri-ethylenediamine (CED) solution. Switzerland, Geneva
- ISO (2019) ISO 1762:2019 Paper, board, pulps and cellulose nanomaterials — Determination of residue (ash content) on ignition at 525 °C. Switzerland, Geneva
- Isogai A, Kato Y (1998) Preparation of polyuronic acid from cellulose by TEMPO-mediated oxidation. *Cellulose* 5(3):153–164. <https://doi.org/10.1023/A:1009208603673>
- Isogai A, Hänninen T, Fujisawa S, Saito T (2018) Catalytic oxidation of cellulose with nitroxyl radicals under aqueous conditions. *Prog Polym Sci* 86:122–148. <https://doi.org/10.1016/j.progpolymsci.2018.07.007>
- Kargarzadeh H, Mariano M, Gopakumar D, Ahmad I, Thomas S, Dufresne A (2018) Advances in cellulose nanomaterials. *Cellulose* 25(4):2151–2189. <https://doi.org/10.1007/s10570-018-1723-5>
- Klemm D, Cranston ED, Fischer D, Gama M, Kedzior SA, Kralisch D (2018) Nanocellulose as a natural source for groundbreaking applications in materials science: today's state. *Mater Today* 21(7):720–748. <https://doi.org/10.1016/j.mattod.2018.02.001>
- Lewin M, Avrahami M (1955) The decomposition of hypochlorite-hypobromite mixtures in the pH Range 7–10. *J Am*

- Chem Soc 77(17):4491–4498. <https://doi.org/10.1021/ja01622a014>
- Lin C, Zeng T, Wang Q, Huang L, Ni Y, Huang F (2018) Effects of the conditions of the TEMPO/NaBr/NaClO system on carboxyl groups, degree of polymerization, and yield of the oxidized cellulose. *BioResources* 13(3):5965–5975
- Liu S, Low ZX, Xie Z, Wang H (2021) TEMPO-Oxidized cellulose nanofibers: a renewable nanomaterial for environmental and energy applications. *Int J Adv Meter Technol*. <https://doi.org/10.1002/admt.202001180>
- Mao LS, Ma P, Law K, Daneault C, Brouillette F (2010) Studies on kinetics and reuse of spent liquor in the TEMPO-Mediated selective oxidation of mechanical pulp. *Ind Eng Chem Res* 49(1):113–116. <https://doi.org/10.1021/ie901039r>
- Myja D, Loranger É, Lanouette R (2018) TEMPO mediated oxidation optimization on thermomechanical pulp for paper reinforcement and nanomaterial film production. *BioResources* 13(2):4075–4092. <https://doi.org/10.15376/biores.13.2.4075-4092>
- Nechyporchuk O, Belgacem MN, Bras J (2016) Production of cellulose nanofibrils: a review of recent advances. *Ind Crop Prod* 93:2–25. <https://doi.org/10.1016/j.indcrop.2016.02.016>
- NREL (2012). NREL/TP-510–42618 Determination of Structural Carbohydrates and Lignin in Biomass.
- Okita Y, Saito T, Isogai A (2010) Entire surface oxidation of various cellulose microfibrils by TEMPO-mediated oxidation. *Biomacromol* 11(6):1696–1700. <https://doi.org/10.1021/bm100214b>
- Saito T, Isogai A (2004) TEMPO-mediated oxidation of native cellulose. The effect of oxidation conditions on chemical and crystal structures of the water-insoluble fractions. *Biomacromol* 5(5):1983–9. <https://doi.org/10.1021/bm0497769>
- Saito T, Yanagisawa M, Isogai A (2005) TEMPO-mediated oxidation of native cellulose: SEC–MALLS analysis of water-soluble and-insoluble fractions in the oxidized products. *Cellulose* 12(3):305–315. <https://doi.org/10.1007/s10570-004-5835-8>
- Saito T, Isogai A (2006) Introduction of aldehyde groups on surfaces of native cellulose fibers by TEMPO-mediated oxidation. *Colloids Surf A-Physicochem Eng Asp* 289(1–3):219–225. <https://doi.org/10.1016/j.colsurfa.2006.04.038>
- Saito T, Kimura S, Nishiyama Y, Isogai A (2007) Cellulose nanofibers prepared by TEMPO-mediated oxidation of native cellulose. *Biomacromol* 8(8):2485–2491. <https://doi.org/10.1021/bm0703970>
- Saito T, Hirota M, Tamura N, Isogai A (2010) Oxidation of bleached wood pulp by TEMPO/NaClO/NaClO<sub>2</sub> system: effect of the oxidation conditions on carboxylate content and degree of polymerization. *J Wood Sci* 56(3):227–232. <https://doi.org/10.1007/s10086-009-1092-7>
- Sanchez-Salvador JL, Balea A, Monte MC, Negro C, Miller M, Olson J (2020) Comparison of mechanical and chemical nanocellulose as additives to reinforce recycled cardboard. *Sci Rep* 10(1):3778. <https://doi.org/10.1038/s41598-020-60507-3>
- Sanchez-Salvador JL, Campano C, Negro C, Monte MC, Blanco A (2021) Increasing the possibilities of TEMPO-mediated oxidation in the production of cellulose nanofibers by reducing the reaction time and reusing the reaction medium. *Adv Sustain Syst* 5(4):2000277. <https://doi.org/10.1002/advsu.202000277>
- Sanchez-Salvador JL, Campano C, Balea A, Tarrés Q, Delgado-Aguilar M, Mutjé P, Blanco A, Negro C (2022) Critical comparison of the properties of cellulose nanofibers produced from softwood and hardwood through enzymatic, chemical and mechanical processes. *Int J Biol Macromol* 205:220–230
- Serra A, González I, Oliver-Ortega H, Tarrés Q, Delgado-Aguilar M, Mutjé P (2017) Reducing the amount of catalyst in TEMPO-oxidized cellulose nanofibers: Effect on properties and cost. *Polymers* 9(11):557. <https://doi.org/10.3390/polym9110557>
- Shibata I, Isogai A (2003) Depolymerization of cellouronic acid during TEMPO-mediated oxidation. *Cellulose* 10(2):151–158. <https://doi.org/10.1023/A:1024051514026>
- Shinoda R, Saito T, Okita Y, Isogai A (2012) Relationship between length and degree of polymerization of TEMPO-oxidized cellulose nanofibrils. *Biomacromol* 13(3):842–849. <https://doi.org/10.1021/bm2017542>
- Sun B, Gu CJ, Ma JH, Liang BR (2005) Kinetic study on TEMPO-mediated selective oxidation of regenerated cellulose. *Cellulose* 12(1):59–66. <https://doi.org/10.1023/B:CELL.0000049409.56806.da>
- Tahiri C, Vignon MR (2000) TEMPO-oxidation of cellulose: Synthesis and characterisation of polyglucuronans. *Cellulose* 7(2):177–188. <https://doi.org/10.1023/A:1009276009711>
- Tang Z, Li W, Lin X, Xiao H, Miao Q, Huang L (2017) TEMPO-oxidized cellulose with high degree of oxidation. *Polymers* 9(9):421. <https://doi.org/10.3390/polym9090421>
- TAPPI (2016). TAPPI T 211 om-16:2016 Ash in Wood, Pulp, Paper and Paperboard: Combustion at 525 Degrees C. Peachtree Corners, GA.
- TAPPI (2017). TAPPI T 204 cm-17:2017 Solvent Extractives of Wood and Pulp. Peachtree Corners, GA.

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