FULL PAPER

Increasing the possibilities of TEMPO-mediated oxidation in the production of cellulose nanofibers by reducing the reaction time and reusing the reaction medium

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Abstract: TEMPO-mediated oxidation of cellulose is the most effective pretreatment to obtain highly fibrillated cellulose nanofibers (CNFs). However, the associated environmental impact and the high operational costs have limited its implementation at large scale. This study aims to improve the effectiveness of this pretreatment by reducing the oxidation time and reusing the reaction medium, with the ultimate objective of upscaling a more viable sustainable process. Results show the possibility of reducing the reaction time by 85% and 60% in the case of cotton and eucalyptus pulps, respectively, without altering the properties of the obtained CNFs (carboxyl groups, nanofibrillation yield, cationic demand and morphology). The reuse of 75% of the reaction medium for ten cycles, did not alter the properties of the CNFs either. With this approach, the consumption of TEMPO and NaBr was reduced by 67%, while that of NaClO decreased by 42% and 22% in the oxidation of cotton and eucalyptus cellulose, respectively. At the same time, the environmental concerns derived from the disposal of the catalysts were considerably reduced.

Introduction

The use of cellulose nanofibers (CNFs) has grown substantially in the last decades due to their promising properties, as, for example, high mechanical strength, high surface area and lightweight, together with the fact that they are obtained from renewable resources[1]. The potential application of CNFs in different fields has grown considerably, including their use as reinforcement of paper-based products[2], nanocomposites[3], cement-based materials[4], in the food industry[5], for environmental treatments[6] or in biomedical applications[7]. However, their implementation at industrial scale is being developing very slowly in most sectors.

The production of CNFs has been generally conducted through a mechanical process that breaks down the structure of the fibers up to the nano-scale[8]. This process triggers high energy costs that can be often reduced with the use of a pretreatment, which can be chemical, mechanical or enzymatic[9]. As a function of the process configuration, different fibrillation efficiencies and CNF properties can be obtained[10]. Although a high fibrillation and homogeneity is not always needed, as for paper reinforcement[2a], it is a requirement for other applications, such as for the preparation of nanopapers [11] or for their use in biomedicine[12].

TEMPO-mediated oxidation is the most used pretreatment to reach both high fibrillation and good homogeneity of the CNFs. It aims to oxidize C6 hydroxyl groups of cellulose to aldehyde and then to carboxyl groups, using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) as catalyst, NaBr as co-catalyst and NaClO as oxidant[13]. This reaction, typically conducted under alkaline conditions, triggers the electrostatic repulsion between the C6 carboxyl groups of the oxidized cellulose, which facilitates the separation of the nanofibers during the subsequent mechanical treatment[14]. The use of the TEMPO-mediated oxidation pretreatment allows the CNFs to have a homogeneous diameter, since the primary structure of the fibers has been reached[15]. This is one of the main advantages of this process compared to the mechanical pretreatment, where a high heterogeneous product is normally obtained[16].

The preferential reaction of chemicals with the amorphous regions of the cellulose applies to almost all chemical reactions[17], since amorphous regions are more kinetically accessible[18]. Thus, cellulose fibers with high crystallinity and crystal size have shown resistance to oxidation through the reaction catalyzed by TEMPO, obtaining a low carboxyl content[19]. On the other hand, cellulose sources with a high fraction of disordered regions could suffer the dissolution of some oxidized amorphous parts[20]. The TEMPO-mediated oxidation reaction is usually controlled based on the monitoring of the carboxyl content of the oxidized cellulose. However, the dissolved fraction could hinder a high proportion of these groups, with would suppose the loss of cellulose to produce CNFs. This loss of material could be reduced by a better control of the reaction time and oxidant dose considering, at the same time, an appropriate carboxyl content to facilitate the subsequent fibrillation process.

Currently, the main drawbacks to scale-up the TEMPO-mediated oxidation process have been the high cost of the TEMPO reactant and the environmental impact caused by the disposal of the reaction media[21]. Although the reuse of the reaction medium could be a good strategy, it has been seldom studied. Mao, et al. [22] reused the reaction medium in the oxidation of TMP pulp adding in each cycle, 6 mmol/g of NaClO. However, they observed an increase in the reaction time up to 40% after 5 cycles and a reduction in the carboxyl content of 7%. These poor results could have discouraged further studies. However, we believe they could be improved with a better control of the reaction.

This study aims to facilitate the upscaling of the TEMPO-mediated oxidation as a pretreatment to produce CNFs by reusing the reaction medium, that would allow a reduction in the consumption of reagents, in the operational costs and, consequently, in the associated environmental impact. On the other hand, it is proposed a monitoring process, based on the consumption of NaClO, which would allow a direct interpretation of the role of NaClO in terms of its conversion to produce carboxyl groups or

other products derived from secondary reactions. Two cellulose sources were considered in order to assess the effect of crystallinity and morphology of the raw material on the reaction kinetic. First, we have selected the reaction time based on the CNFs properties. Then, the reuse of the reaction medium was studied by assessing the effects of the accumulated products on both the reaction effectivity and the CNFs properties.

Results and Discussion

Monitoring of the TEMPO-mediated oxidation

Figure 1 shows the evolution of the parameters used to monitor the TEMPO-mediated oxidation, namely available NaClO, consumed NaOH, recovery rate, aldehydes and carboxyl groups. The reaction starts with the oxidation of the hydroxyl groups in C6, positioned in the disordered regions of the cellulose, to aldehydes and then to carboxyl groups, causing the electrostatic repulsion between the substituted chains and, thus, releasing free C6 positions available to react. At this point, the reaction rate is so high that most of the NaClO available in the medium is consumed in a few minutes. However, once the pulp reached a certain oxidation degree, the reaction rate becomes slower. As expected, this evolution varied significantly with the type of cellulose. As observed in Figure 1, the NaClO consumption is more prolonged in the case of cotton, where the time spent in consuming 50% NaClO is around 30 min, compared to less than 20 min in the case of eucalyptus. Moreover, NaClO is completely consumed in the reaction with eucalyptus after 90 min, while for cotton, the reaction has to be prolonged up to 200 min until NaClO was not observed.

Considering only the main reactions involved in the system (Figure 2), the consumption of NaClO and NaOH should occur in a 2:1 ratio. This ratio is maintained at the beginning of the reaction, due to the easier accessibility of C6-groups. However, the NaClO:NaOH ratio decreased below 2, when the reaction time was 30 min for cotton and 50 min for eucalyptus. This fact may be due to parallel reactions, in which the oxidant was probably consumed to produce chlorates and bromates[23] but also ketones in the formation of C2 and C3 groups of the anhydroglucose units of cellulose[24]. The production of chlorates and bromates comes from the formation of HClO and HBrO, intermediates needed for the protonation of TEMPO, but that also react between them to produce these compounds together with hydronium ions, which are also neutralized by NaOH. The fact that the HBrO reacts with TEMPO or with HClO, depends mainly on the availability of TEMPO to be activated, i.e. the accessibility of hydroxyl groups in C6 and on the pH. At pH close to 10.5-11, the proportion of NaClO that is transformed into HClO is below 0.1%, low enough to be spent in the oxidation of hydroxyl groups at the beginning of the reaction, when the availability of TEMPO is very high due to the higher reaction rate. However, when the oxidation rate increases, the TEMPO regeneration is decelerated, favouring the production of secondary reactions[25]. Thus, although the consumption of NaOH and NaClO was still increasing, the ratio NaClO:NaOH was decreasing from 2 to 1.7 in both raw materials.

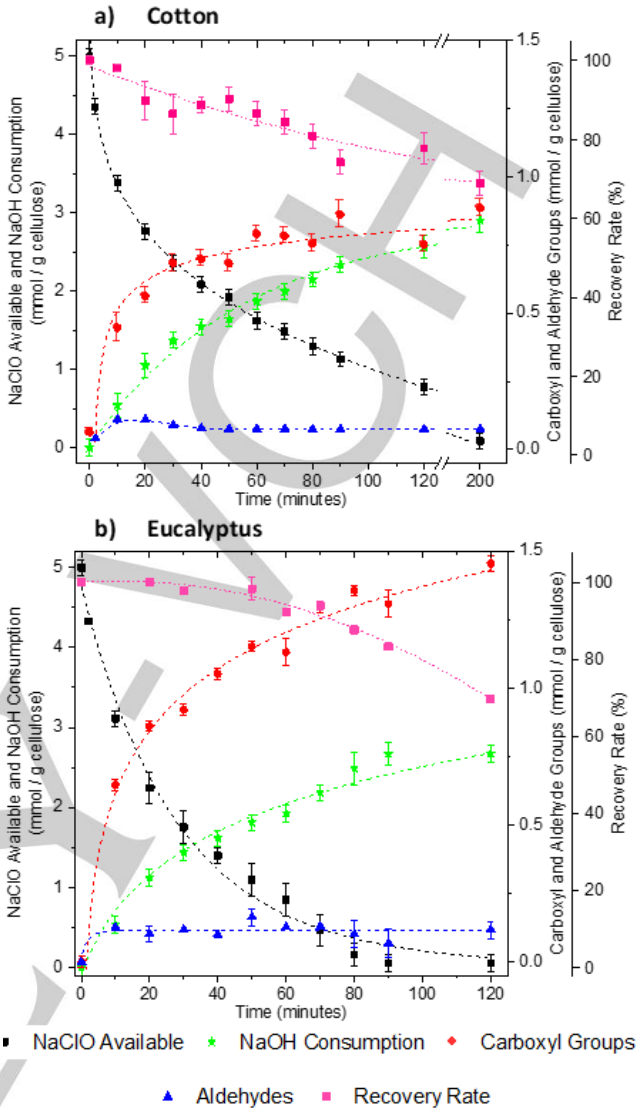

Figure 1. Monitoring of the TEMPO-mediated oxidation of a) cotton and b) eucalyptus

The carboxyl groups of Figure 1a showed a quick increase during the first part of the reaction in the case of cotton, reaching a stabilization in the second part. By times bands, after 30 minutes, the cotton sample had 0.68 mmol COOH/g (77% of the carboxyl groups obtained at the end of the reaction). At this time, only 2.6 mmol NaClO/g sample were consumed. In the case of eucalyptus (Figure 1b), the carboxyl groups increased in a higher extent than in the cotton sample. After 50 minutes, when the second part of the reaction began, the eucalyptus sample had 80% of the carboxyl groups obtained at the end of the reaction (~1.15 mmol COOH/g cellulose), with a NaClO consumption of 3.9 mmol/g. Then, when all NaClO was consumed, the number of carboxyl groups were around 1.4 mmol/g cellulose recovered. The number of aldehyde groups in both raw materials increased in the first minutes and then it was maintained during the rest of the reaction with a value under 0.15 mmol/g. This fact seems to indicate that most aldehyde groups were quickly oxidized to carboxyl groups.

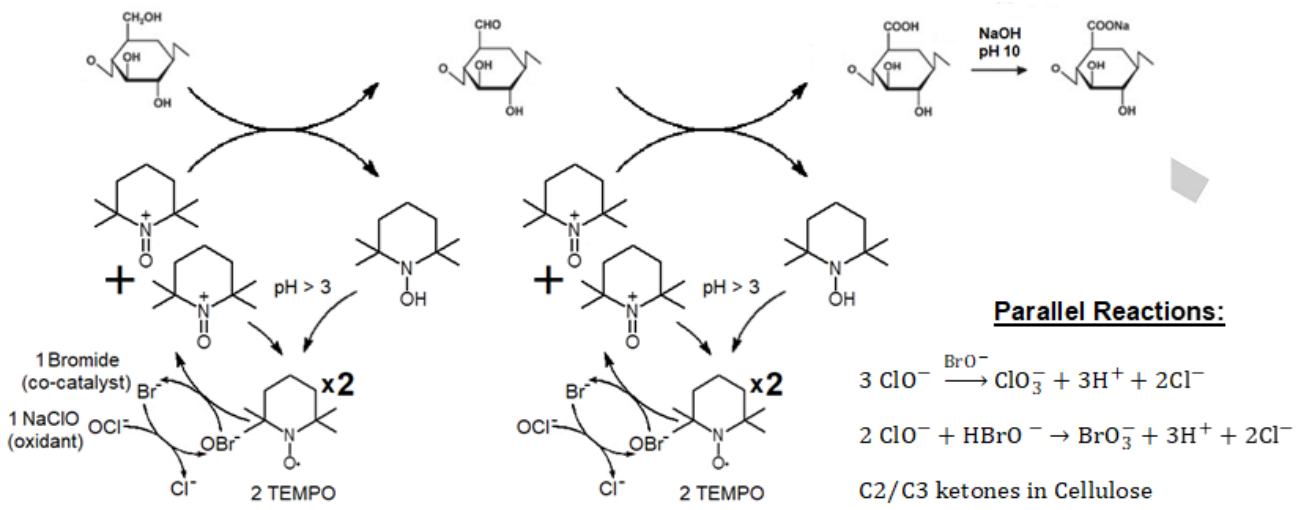

Figure 2. TEMPO-mediated oxidation scheme (Adapted from Carlsson, et al. [26])

The differences in the morphology of the raw materials might have influence on the higher number of carboxyl groups obtained in the eucalyptus samples. The lower crystallinity index of eucalyptus, 87.7% compared to 97.5% of cotton, that indicates more disordered regions, would justify the formation of more carboxyl groups. Additionally, the crystal size of hardwood plants, as eucalyptus, is lower than the crystal size of cotton, favouring the TEMPO-mediated oxidation of more C6-groups placed in the surface of the fibers [19c].

On the other hand, the increase of the reaction time induces the reduction of the fiber size, in which a part of the oxidized cellulose remains suspended in water, and also triggers the formation of a water-soluble fraction[13]. Despite, the oxidation process is scarce in the second part of the oxidation, as the number of carboxyl groups reveal, the recovery rate of cotton continues decreasing from 90%, at 50 minutes, to 70%, when all the NaClO is consumed, due to the fact that hydrodynamic conditions were maintained producing the cellulose degradation.

The recovery yield of eucalyptus cellulose at the end of the reaction was the same than with cotton, the 70% of the initial cellulose; although at intermediate times, the recovery rate is higher, reaching 95% at 70 minutes. Additionally, the cellulose loss due to the water-soluble and colloidal fractions, is not only a problem due to the loss of material itself, but also because of its accumulation in the reaction medium. Tahiri and Vignon [19a] proposed the addition of ethanol for the recovery of colloids that do not settle during centrifugation due to their small size and electrostatic stabilization. However, the presence of alcohol groups in the medium, in a new oxidation reaction would be counterproductive by competing directly with the hydroxyl groups of the cellulose. Therefore, a time-controlled oxidation would allow us to optimize both the number of carboxyl groups and the cellulose recovered fraction after TEMPO-mediated oxidation. Additionally, the no depletion of NaClO in the reaction would allow its later use in the case of reusing the reaction medium

As for the diameter distribution of the OPs, Figure 3 shows the median, the geometric meand and the percentile 5 and 95. In the case of cotton, a slow and progressive decrease of the median and the geometric mean of the OP diameter is observed, whereas in eucalyptus OPs is observed a quickly reduction of the average diameter at the first 30 minutes of reaction, reaching a stabilization afterwards. This fact shows the great effectiveness of this treatment during the first minutes of reaction.

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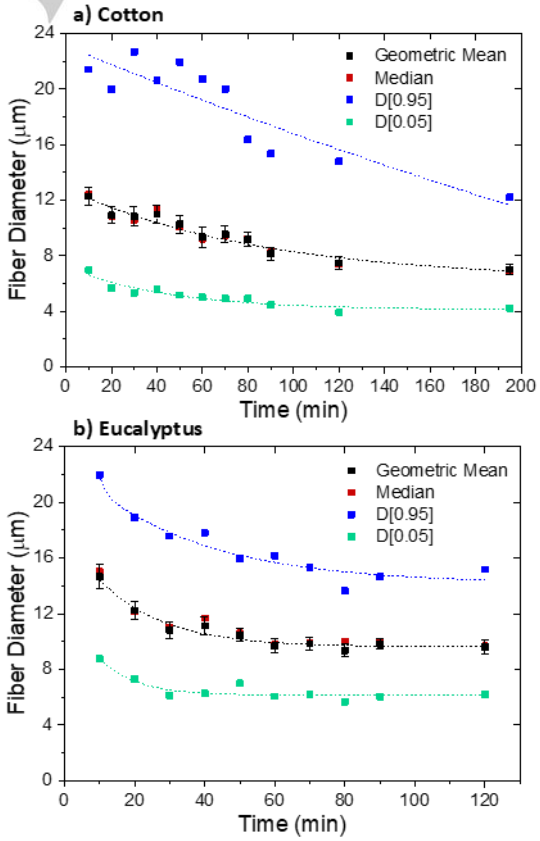

Figure 3. Oxidized pulp diameter distribution after different times of TEMPO-mediated oxidation: a) Cotton; b) Eucalyptus

Characterization of the CNFs

To optimize the oxidation time of the pulp, the characterization of the CNF properties plays an important role together with the OP properties and the composition of the reaction medium that would be reused. Therefore, nanofibrillation yield, transmittance and cationic demand of cotton and eucalyptus CNF were determined as shown in Figure 4. It is observed that the nanofibrillation yield of cotton samples after 2 passes of homogenization is almost 100% in the whole sequence of times. This behaviour could be due to the fact that the raw material was in a powder state favouring the small size at low reaction times. Transmittance at 600 nm showed increasing values for CNF with the oxidation time, reaching values above 90% from 30 minutes of reaction, what would indicate a great effectiveness of the oxidation up to that time. Finally, cationic demand indicates the increase in the anionic charge of the CNF with the oxidation time, which is due to the increase of both the carboxyl groups and the specific surface due to a higher repulsion between nanofibers since the hydrogen bonds between them are broken.

As for eucalyptus CNF, the yield at a low oxidation time was lower than for cotton, since the raw material was not powder and had a higher fiber length. As oxidation time increases, the eucalyptus CNFs show big differences reaching around 80% nanofibrilation, after 50 minutes of oxidation; or more than 90%, after 2 hours of oxidation, although in the latter case this fact is associated to a lower recovery (Figure 4b). Regarding the transmittance of eucalyptus samples, it shows the same trend as the nanofibrillation yield. As expected, cationic demand increases as the carboxyl groups do, and it has higher values than cotton that may be due to the differences in the CNF morphology. Cotton CNFs are shorter, which facilitate the interaction between fibers, but they are also more crystalline, meaning they are less reactive. However, the higher length and lower crystallinity of CNF from eucalyptus facilitated the repulsion between the CNFs due to the higher oxidation degree, which caused a decrease in the number of hydrogen bonds, increasing the anionic charge and, therefore, the cationic demand.

Figure 5 shows TEM images of the eucalyptus and cotton CNF at different oxidation times. As oxidation time progresses, the average diameter of the CNF nanofibers decreases. Cotton samples with 2 homogenization passes and only 10 minutes of TEMPO-mediated oxidation show nanofibers with a diameter up to 100-120 nm, which decreases to 20-60 nm after 30 minutes of oxidation. By increasing the oxidation time, the diameter of the fibers barely decreases up to 80 minutes of oxidation and only after 200 minutes of oxidation and the subsequent homogenization process allow a slight improvement of the CNF fibrillation with the decrease of the higher diameter of the CNFs up to 50 nm. In the case of eucalyptus, low reaction times, up to 30 minutes, show some big fibers that have not been fibrillated in the homogenizer. Nevertheless, from 50 minutes of reaction, the CNFs obtained shows a homogeneous diameter range from 25 to 40 nm that does not diminish at longer oxidation times.

From the results of the properties of OPs and CNFs, the oxidation times selected for the next study to reuse the reaction medium were 30 and 50 minutes for cotton and eucalyptus pulp, respectively. The high crystallinity of cotton and the limited amount of amorphous cellulose quickly oxidized all available cellulose; whereas eucalyptus, with more amorphous cellulose, requires higher times. However, more carboxylic groups were obtained. At the selected times, both OPs have a high content of carboxyl groups, around 80% of the obtained at the end of the reaction. At higher times, the rate of increase of carboxyl groups was reduced and, in addition, the recovery rate of cellulose was decreased. On the other hand, the CNFs properties at higher times were barely improved. Moreover, at the selected times, a part of the NaClO was still present in the medium and it can be reused in a new oxidation cycle.

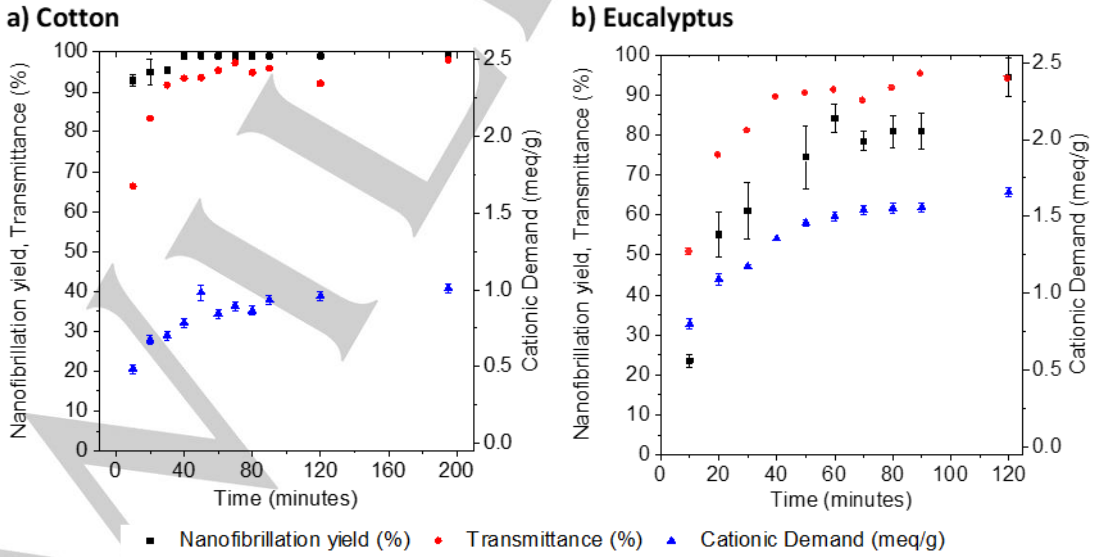

Figure 4. Evaluation of the CNF properties with the TEMPO-mediated oxidation time: a) cotton; b) eucalyptus

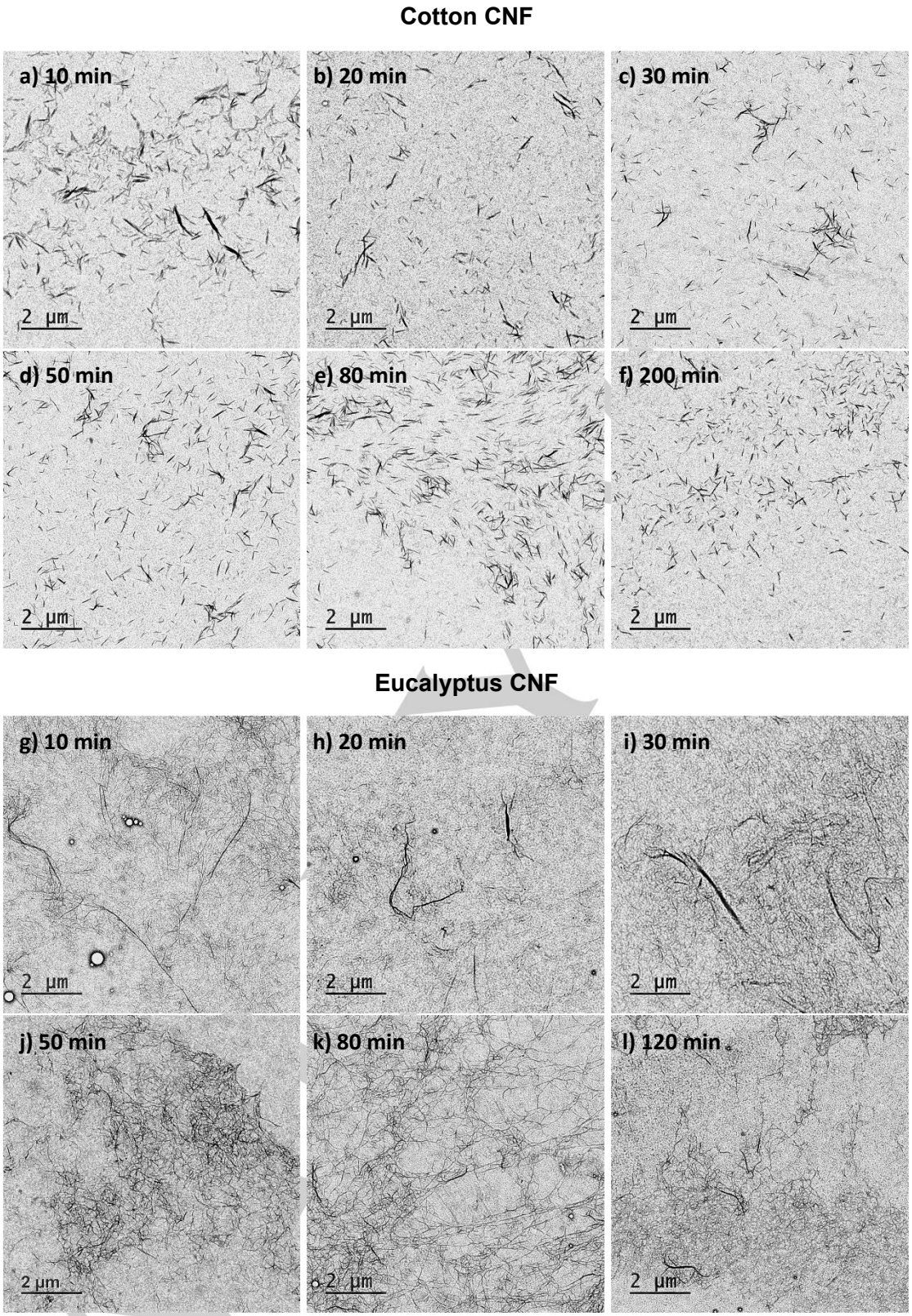

Figure 5. Transmission Electron Microscopy (TEM) images of cellulose nanofibers (CNF) produced from cotton (a-f) and eucalyptus (g-l) at different TEMPO-mediated oxidation times

Reuse of the TEMPO-mediated oxidation medium

After selecting the most adequate reaction time for the oxidation of cellulose fibers, the reuse of the reaction medium was studied. At the selected times, part of the added NaClO was still active and could be determined by titration and used in a subsequent oxidation reaction. Figure 6 shows the consumption of NaClO and NaOH in each cycle, the recovery rate and the proportion of carboxyl groups in each OPs.

In the case of cotton (Figure 6a), the consumption of NaClO was 2.1 mmol/g in the first cycle. Although it increased slightly to 2.6 mmol/g in cycle 4, the proportion of carboxyl groups in the OPs was almost invariable, close to 0.7 mmol/g. Since the recovery rate was kept almost constant, this may indicate that some NaClO was consumed in secondary reactions. After cycle 5, the number of carboxyl groups of OPs decreased slightly up to around 0.5 mmol/g. Although this value may not be enough to achieve a high fibrillation degree in woody celluloses, the fact that cotton has a high crystallinity and a low initial particle size indicates that this carboxyl value, although lower than the one in the first cycles, could be enough for reaching a high nanofibrillation degree (Figure 7a). This is indeed what is observed in the properties of CNFs, i.e. transmittance and nanofibrillation degree, which did not significantly vary with the number of cycles of the reused of the reaction medium. As observed in TEM images of CNFs obtained from cotton (Figure 8), they presented a rod-like morphology similar to cellulose nanocrystals [27]. Thus, the fact that they have a high fibrillation degree and transmittance, but slightly lower cationic demand could suppose a lower electrostatic stabilization in suspension.

In the case of eucalyptus, titration data shown a NaClO consumption of around 3.5 mmol/g in the first 6 cycles and, then, it decreased slightly to 3.2 mmol/g. However, in this case, the carboxyl groups were seldom affected, as observed in Figure 6b, fluctuating around 1.1 mmol/g. In the CNFs produced from eucalyptus, nanofibrillation yield and transmittance remained stable and over 90% with the number of cycles as occurred with cotton (Figure 7b). However, cationic demand was also slightly reduced. Since this cellulose is more amorphous, it is highly probable that cellulose fibers may break down due to the oxidation reaction, forming some shorter chains and others that could have a molecular weight low enough to remain in solution. The possibility that each of these options occur will depend on the conditions used for the study, such as agitation or temperature. Moreover, the presence of hemicellulose in this pulp could suppose that this loss of material could also be affected by the loss of oxidized hemicellulose [28]. This high number of effects could be responsible for the fluctuations found in the recovery rate of eucalyptus CNF, which varied in a high proportion from one cycle to the other.

The main reason for the decreased tendency observed in the cationic demand for both cellulose sources could be the accumulation of components. As observed in Figure 6a, although the cellulose recovery rate did not show a marked increasing or decreasing tendency during all oxidation cycles, they presented values from 82 to 87%, in the case of cotton, and from 84 to 95%, for eucalyptus OPs. The rest of cellulose will be either lost during the washing stages or will remain in the medium in a dissolved or colloidal form.

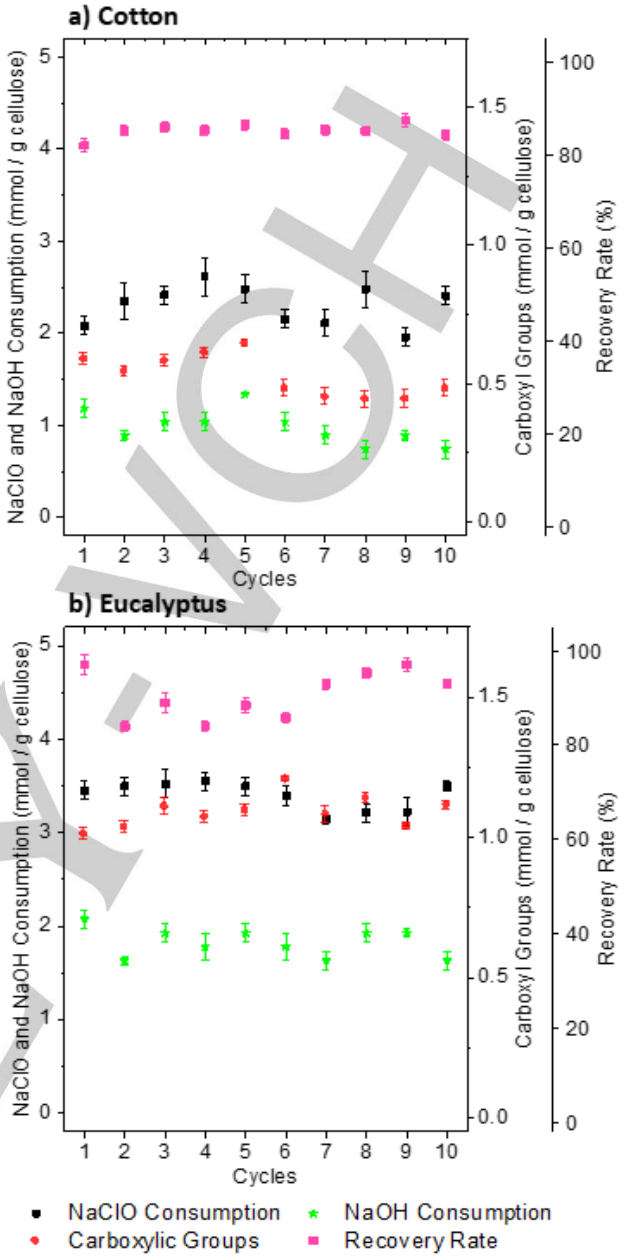

Figure 6. Effect on the number of cycles of the NaClO and NaOH consumption, carboxyl groups and recovery rate of the oxidized pulp: a) Cotton; b) Eucalyptus

Although it is more probable the first option, in which the smaller fibers are lost, the part of OPs that is being dissolved or suspended would mean an accumulation during the rest of the recycling cycles. In addition, it is important to note the accumulation of salts in the medium as NaCl obtained as by-product in the TEMPO-mediated oxidation of cellulose [22, 29]. Thus, despite introducing 25% of fresh medium in each cycle and washing the cellulose until pH 7, it was possible to detect an increasing percentage of NaCl in the dried CNFs from the third cycle, as well as in the reused medium. This accumulation could suppose a reduction in the value of cationic demand in the last cycles, due to the decrease of the thickness of the double electric layer and a partial neutralization of the CNF surface charge.

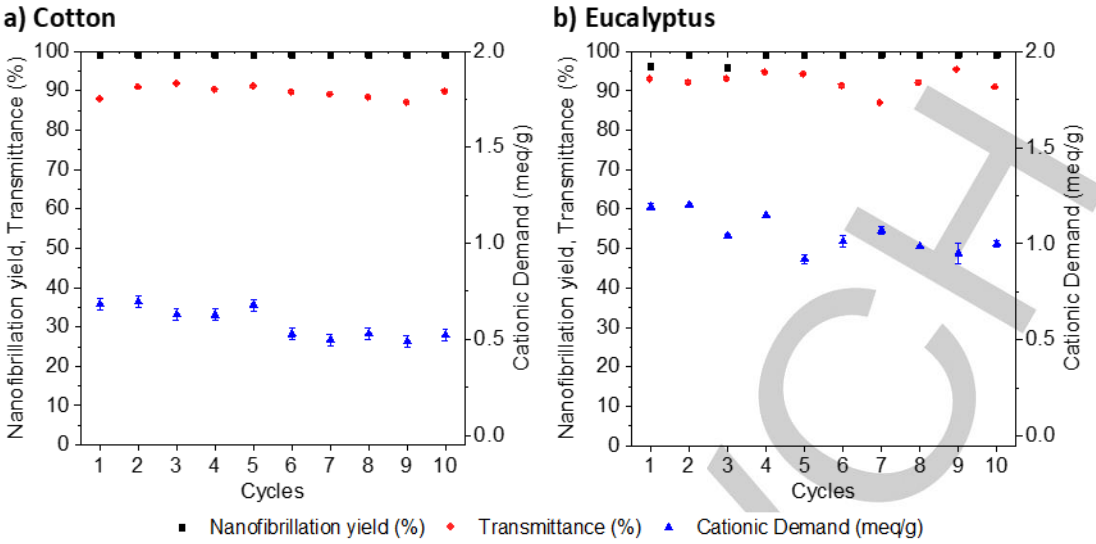

Figure 7. Evaluation of the CNF properties with the number of cycles of the TEMPO-mediated oxidation. a) Cotton; b) Eucalyptus

Although this effect is not very relevant for the properties of the CNFs, it could suppose operational problems, such as corrosion or clogging in the filters. In addition, a final treatment of wastes and purges should be included to remove both the dissolved and colloidal material, the chlorates, the bromates and the NaCl produced in the principal reaction.

Finally, Figure 9 shows TEM images of cotton and eucalyptus CNFs obtained from OPs after different cycles of reusing the reaction medium. As seen in the figure, the number of cycles barely shows any variation in the diameter and bulk morphology of the fibers. In the case of cotton CNFs, the diameter range varied from 20 to 50 nm with some nanofibers up to 60 nm. In the case of CNFs from eucalyptus, the diameter range was between 25 and 45 nm with some scarce fibers up to 55 nm. Thus, we could conclude that despite the presence of salts and some accumulated cellulosic material, neither the properties of CNFs nor their morphology was likely suffering any deterioration.

It is worth to mention that after 10 cycles in a row of oxidation, 42% of NaClO was saved with the reused medium in the case of cotton, and 22% for eucalyptus, compared to the NaClO that would be consumed using 5 mmol/g in each cycle. Regarding the use of TEMPO and NaBr, the 67.5% of both catalysts could be saved with the recovery process proposed in this study. Furthermore, not only reactant costs could be saved, but also those derived from the agitation and temperature controller, that would be considerably reduced as the reaction time was decreased from around 200 min to 30 min for cotton, and from 120 min to 50 min for eucalyptus. Finally, the loss of cellulose material has been reduced, from 32 and 35% in the case of exhausting the NaClO to 15 and 10% in each cycle for cotton and eucalyptus, respectively.

Conclusion

Results show the viability of reusing the reaction medium in the TEMPO-mediated oxidation pretreatment of cellulose used to obtain highly fibrillated CNF.

Thanks to the novel monitoring of the oxidation reaction, in which the availability of NaClO was determined during the process, it was possible to reduce the oxidation reaction time: from more than 2 h to 30 min, when using cotton cellulose, and to 50 min, when using eucalyptus cellulose. In this way, the losses of raw materials were reduced up to 35%, while the final properties of the CNFs remain practically constant. Differences between the results observed for both raw materials were assigned to their differences in crystallinity, morphology and composition.

By optimising the reaction time, the part of the NaClO that was not consumed in the first oxidation cycle can be reused in the subsequent one. After the reuse of the reaction medium in 10 cycles, the obtained CNFs showed properties very similar to those of the CNFs produced is the first cycle, in terms of transmittance, carboxyl groups, nanofibrillation yield and diameter. Moreover, the addition of 25% fresh medium allowed the reduction in the accumulation of salts and colloidal material.

With the proposed optimized treatment, 67% of TEMPO and NaBr can be saved after 10 cycles of reuse, while the consumption of NaClO is reduced by 42% and by 22% in the oxidation of cotton and eucalyptus cellulose, respectively. In this way, both chemical costs and operational costs are reduced as well as the environmental impact caused by the disposal of the catalysts, making the TEMPO-mediated oxidation process more sustainable and easier to upscale.

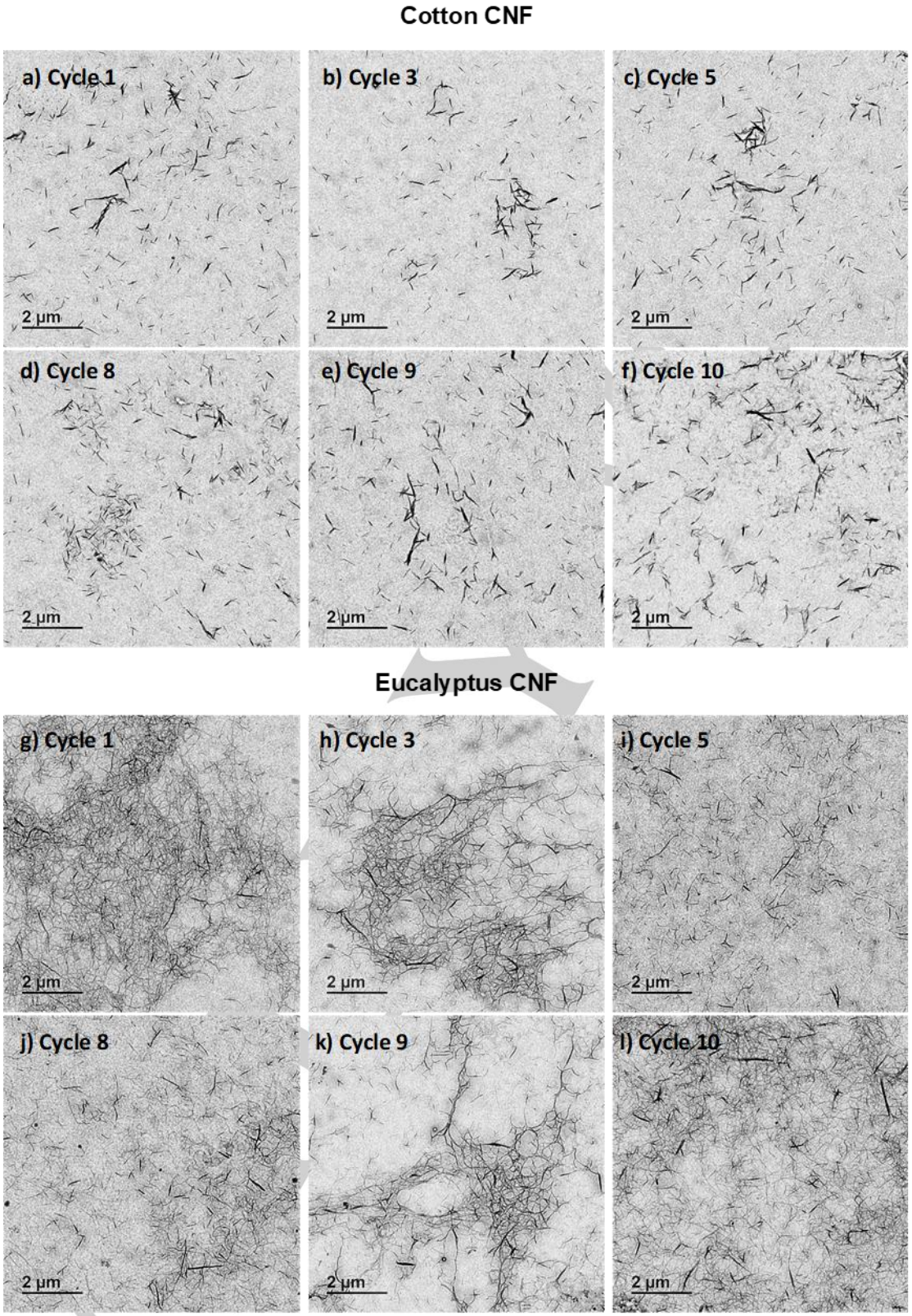

Figure 8. Transmission Electron Microscopy (TEM) images of cellulose nanofibers (CNFs) produced from cotton (a-f) and eucalyptus (g-l) at different reuse cycles of reaction medium

Experimental Section

Materials

Two cellulose sources of different crystallinity and morphology were selected: cotton linters, a commercial high purity cellulose acquired from Sigma-Aldrich (St. Louis, MO, USA); and a refined *Eucalyptus globulus* ECF bleached kraft pulp, kindly supplied by Torraspapel, S.A. (Zaragoza, Spain). Crystallinity of raw materials was determined according to Campano, et al. [27].

TEMPO reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at 4ºC before used. Sodium bromide and 10% w/v NaClO solution were purchased from Panreac AppliChem (Barcelona, Spain). Reagents used for monitoring the reaction and characterization of the products, NaOH, HCl, $Na_2S_2O_3$ and KI, were also supplied by Panreac AppliChem. 0.1% w/w Poly-L-Lysine solution was obtained from Electron Microscopy Sciences.

TEMPO-mediated oxidation

Cotton and eucalyptus (30g) were soaked in water for at least 24 h. In the case of eucalyptus, the sample at 3% consistency was disintegrated for 30,000 revolutions in a Messmer pulp disintegrator manufactured by Mavis Engineering Ltd. (London, UK). The pulp consistency was adjusted to 1%. Then, 0.1 mmol TEMPO/g pulp and 1 mmol NaBr/g pulp were added and the medium was stirred until reagents were completely dissolved[30]. Then, 5 mmol NaClO/g pulp were poured into the reactor to start the reaction. The reaction was conducted at a temperature of 25 ºC and stirring conditions. The pH was adjusted to 10 with 2M HCl and it was maintained constant with the addition of 2M NaOH. The reaction was monitored through the determination of the NaClO content as explained below.

Reaction time

Once the reaction was started, 250 mL of sample were extracted from the reaction beaker at different times: 10, 20, 30, 40, 50, 60, 70, 80, 90, 120 and 200 min. The sample was centrifuged for 5 minutes at 4480 *x g* using a 3–16L centrifuge of JP Selecta S.A (Barcelona, Spain). The NaClO content in the reaction medium was determined immediately after centrifugation from the supernatant, while the sediment, containing the oxidized pulp (OP), was washed with distilled water. The OPs with a consistency of ~ 10% were stored at 4 ºC to be characterized and to produce CNFs.

Reuse of the reaction medium

The reaction medium was recycled 10 times using the same proportion of cellulose in all of them. In the first cycle, the reaction was started using the protocol described above and was conducted for the time selected in the previous step. Then, the OP and the reaction medium were separated by centrifugation at 4480 *x g* for 5 min. The OP in the sediment was washed and stored. 75% of the initial reaction volume, collected in the supernatant, was used in the next cycle. This percentage was selected based on the maximum reaction medium that could be recovered after the removal of Ops that was around 80%. The remaining 25% was supplemented with fresh medium, prepared with NaBr and TEMPO at the initial conditions. The content of NaClO present in the reused medium was determined, and the part corresponding to the spent reactant in the previous cycle was supplemented to have always 5 mmol NaClO/g pulp. At the same time, the cellulose was added to the reaction beaker to start the oxidation reaction.

Determination of NaClO

A redox titration using a multi-step method was used for NaClO determination[23]. In the first step, three reactions take place in which NaClO, HCl, iodide ion in excess and starch are combined to form a starch-triiodide complex (starch-TC) (equations 1, 2 and 3).

$$NaClO\,(aq) + HCl\,(aq) \rightarrow HClO\,(aq) + NaCl\,(aq) \quad (1)$$

$$HClO\,(aq) + HCl\,(aq) + 3KI\,(aq) \rightarrow KI_3\,(aq) + 2KCl\,(aq) + H_2O\,(l) \quad (2)$$

$$KI_3\,(aq) + \text{Starch} \rightarrow \text{Starch-Triiodide complex} \quad (3)$$

In the second step, the produced starch-TC is titrated by sodium thiosulfate to form a colourless solution of iodide, dithionate and the uncomplexed starch (equation 4).

$$\text{Starch-TC} + 2Na_2S_2O_3\,(aq) \rightarrow 3KI\,(aq) + Na_2S_4O_6 + \text{Starch} \quad (4)$$

This method, which has been previously used to evaluate the available chlorine in a bleach solution[31], has been tested in preliminary experiments to allow the determination of NaClO during the TEMPO-mediated oxidation. In this reaction, NaClO reacts with the co-catalyst NaBr to form NaBrO. In this point, the content of both ClO^- and BrO^- was determined, but both ions come from the NaClO, so the quantification of this compound is not altered. Besides, the effect of TEMPO in the reaction medium did not affect in the NaClO determination.

To determine the NaClO content, 25 mL of the reaction medium were added in a 250 mL Erlenmeyer flask. Then, 15 mL of deionized water, 20 mL of 10% (w/w) KI and 20 mL of 2 M HCl were added. The sample was titrated with 0.25 M $Na_2S_2O_3$, adding 2 mL of 0.2 M starch solution, until the sample becomes transparent. The number of NaClO moles in the aliquot is determined as the number of thiosulphate moles consumed divided by two.

Characterization of the oxidized pulp

Carboxyl groups were determined by conductometric titration using 0.15 g of dry pulp, 5 mL of 0.01 M NaCl and deionized water until a final volume of 55 mL[2a]. The pH was adjusted to around 2.8 with 0.05 M HCl. The conductivity was recorded after each addition of 0.2 mL of 0.05 M NaOH, until the conductivity increases at a constant rate. The carboxyl groups were calculated through the volume of NaOH spent in the weak acids corresponding to the intermediate part of the curve[32].

Aldehyde groups were determined through a Schiff base reaction that converts the aldehydes to oximes. A suspension of 0.15 g of dry pulp in a total volume of 20 mL was prepared with deionized water and the pH was adjusted to 3.5. An excess amount of 5% (w/w) hydroxylamine hydrochloride, whose pH has been previously adjusted to 3.5, was added to the sample. The pH was maintained to 3.5 through the addition of 0.05M NaOH. The reaction was finished when no change in pH was observed. The moles of NaOH consumed were used to calculate the proportion of aldehyde groups in the pulp[33].

OP were visualized under a Zeiss Axio Lab.A1 optical microscope with a colour microscope camera Zeiss Axio Cam ERc 5s (Carl Zeiss Microscopy GmbH, Göttingen, Germany). ImageJ software was used to determine the diameter distribution of the OP.

Cellulose nanofibers production

The OPs were homogenized to produce CNFs using a high-pressure laboratory homogenizer PANDA PLUS 2000 (GEA Niro Soavy, Parma, Italy). The consistency of OPs was adjusted to 1% and the suspension was submitted to 600 bar. The criteria selected to choose the number of passes for each cellulose source was the non-visualization of cellulose under optical microscopy. It resulted to be 4 passes in the case of eucalyptus and 2 passes for the cotton sample.

Cellulose nanofibers characterization

Nanofibrillation degree was determined by centrifugation of 0.1% (w/w) CNF suspension at *4480 x g* for 30 min. The nanofibrillated fraction was isolated in the supernatant and the yield was calculated as the relationship between the dry pulp in the supernatant and the total dry pulp.

Transmittance measurements were carried out on 0.1% (w/w) CNF suspensions at 600 nm on a Cary 50 Conc UV-visible spectrophotometer supplied by Varian Australia PTI LTD (Victoria, Australia).

Morphology of CNFs was characterized by Transmission Electron Microscopy (TEM) with a JEM 1400 microscope from JEOL (Tokio, Japan). The microscopy analyses were carried out at the National Centre of Electronic Microscopy (Madrid, Spain). To prepare the samples, 15 µL of Poly-L-Lysine solution were added on a copper grid covered with a Formvar/carbon continuous layer, placed on filter paper. After drying, 12 µL of 0.005% (w/w) CNF suspensions were deposited on them [16].

Cationic demand was measured by an inverse colloidal titration using a Mütek PCD05 particle charge detector (BTG Instruments GmbH, Herrsching, Germany). 30 mL of 0.001 N polyDADMAC was added to 25 mL of a 0.1% (w/w) CNF suspension and stirred for 1 hour. The excess of polyDADMAC was titrated with 0.001N PesNa.

Acknowledgements

Authors want to acknowledge the Spanish Ministry of Economy and Competitiveness for the funding of the project CTQ2017-85654-C2-2-R, and the Community of Madrid for funding the RETO-PROSOST-2-CM (P2018/EMT4459), as well as the support of Universidad Complutense de Madrid and Banco de Santander for the grant of J.L. Sanchez-Salvador (CT17/17) Also thanks to the Spanish National Centre of Electronic Microscopy for the support during image acquisition.

Keywords: Cellulose nanofibers • nanoparticles • reuse of reaction medium • sustainable chemistry • TEMPO-mediated oxidation

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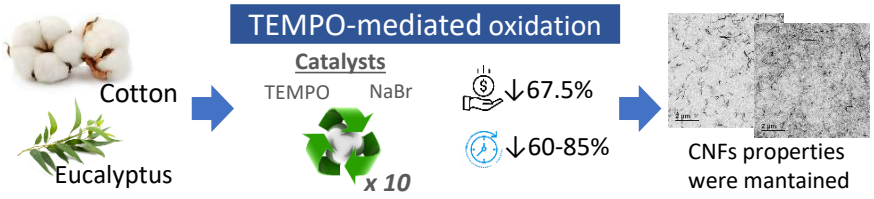

This study has improved the efficiency and sustainability of the TEMPO-mediation oxidation process to produce cellulose nanofibers (CNFs). The reaction medium has been reused for 10 times, observing no variation in the CNFs properties of cotton and eucalyptus. Results showed a reduction in the need of catalysts by 67.5% and in the reaction time by 60-85%. With this approach, the environmental impact and the operational costs would be reduced.