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ORIGINAL PAPER

TEMPO oxidized cellulose thin films analysed by QCM-D and AFM

Tuomas Hänninen · Hannes Orelma · Janne Laine

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Abstract 2,2,6,6-Tetramethylpiperidine 1-oxyl (TEMPO) oxidized cellulose has quickly become a highly utilized material in the production of nanofibrillar cellulose (NFC) and a functionalization strategy for cellulosic materials. In this study we have prepared oxidized cellulose films by TEMPO oxidation and used a quartz crystal microbalance with dissipation monitoring to analyse changes in the film properties. Oxidation was performed at different pH values and reaction times for amorphous and NFC surfaces were also varied. By varying TEMPO oxidation conditions the carboxylation efficiency, stability, and topographical sensitivity of TEMPO oxidation can be optimized for production of a required functionalization level without causing unwanted damage to the cellulosic material. The topographical changes on oxidized cellulose films were further characterized by AFM. Although both surfaces reacted in a similar manner to oxidation, the NFC film was found to be more stable during the oxidation than amorphous cellulose film. The results in this article can be utilized for the functionalization of cellulosic materials in a more controlled manner and for tuning the required carboxylation levels without causing permanent changes to the functionalized cellulosic material.

Keywords TEMPO mediated oxidation · Carboxylation · Cellulose · QCM-D · Cellulose thin film

Introduction

2,2,6,6-Tetramethylpiperidine 1-oxyl (TEMPO) is known to selectively oxidize primary alcohols (Semmelhack et al. 1983). TEMPO can also be applied to carbohydrates in an aqueous medium where it results in uronic acids via an intermediate aldehyde form. Although selective oxidation of primary alcohols in an aqueous solution of different carbohydrates by TEMPO oxidation has been reported already in 1990s (Davis and Flitsch 1993; de Nooy et al. 1995a, b), the method truly gained the attention of material science researchers when Saito et al. (2006) introduced a method for preparation of cellulose nanofibrils (CNFs) from TEMPO oxidized pulp. TEMPO oxidized CNFs have become one of the most industrially and scientifically intriguing CNF grades and it has been proposed for a variety of

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T. Hänninen (✉)
VTT Technical Research Centre of Finland,
P.O.Box 1000, 02044 Espoo, Finland
e-mail: tuomas.hanninen@vtt.fi

H. Orelma · J. Laine
Department of Forest Products Technology, School of Chemical Technology, Aalto University, P.O.Box 16300, 00076 Espoo, Finland

applications (Fukuzumi et al. 2009; Fujisawa et al. 2012; Koga et al. 2013; Rebouillat and Pla 2013; Trygg et al. 2014).

The crystalline structure of cellulose affects the degree of oxidation significantly. Oxidation can take place only on the surface primary alcohols of cellulose I crystals while the oxidation of cellulose II, cellulose III and amorphous cellulose mainly results water soluble polymers (Isogai and Kato 1998; da Silva Perez et al. 2003; Saito and Isogai 2004). The undisturbed crystallinity of cellulose enables easy isolation of individual CNFs due to electrostatic repulsion between the fibrils which is induced by the carboxylic acid groups.

TEMPO oxidation reaction rate is highly dependent on pH. The reaction reaches its maximum rate at pH 10 (de Nooy et al. 1995a, b). At alkaline pH's cellulose can be degraded severely if the oxidation is carried on too long, mainly due to the β-elimination. This is why also slower oxidation in neutral and acid conditions has been studied (Hirota et al. 2009; Saito et al. 2009).

In the present paper we have investigated the TEMPO oxidation of cellulose thin films with different morphologies. Trimethylsilyl cellulose (TMSC) (Kontturi et al. 2003, Kontturi et al. 2011) and nanofibrillar cellulose (NFC) (Ahola et al. 2008) were used to prepare surfaces with different morphologies to analyse their behaviour and stability during oxidation. The oxidation reaction was observed using a quartz crystal microbalance with dissipation monitoring (QCM-D). The topographical changes on the TEMPO oxidized cellulose thin films were examined by AFM.

Understanding the stability and behaviour of cellulosic materials with different morphologies and crystallinities during the oxidation helps to create oxidized cellulosic raw materials and it also provides tools to modify readymade cellulosic structures without causing severe changes in their morphology (Orelma et al. 2012; Trygg et al. 2014). Studies so far have mainly focused on the analysis of dissolved and undissolved fractions of oxidized cellulose. In our study we have observed the stability and morphology of the cellulosic structures during oxidation.

Materials and methods

Quartz crystal microbalance with dissipation monitoring (QCM-D) E4 equipment was supplied by Q-Sense Ab (Västra Frölunda, Sweden). The gold and silicon dioxide (SiO_2) coated QCM-D crystals were obtained from Q-Sense Ab (Västra Frölunda, Sweden). Cellulose thin films were prepared using a spin-coater (Model WS-650SX-6NPP, Laurell Technologies, PA, USA).

Amorphous cellulose thin films were prepared by spin-coating TMSC in toluene on a SiO_2 QCM-D crystal at 3,000 rpm for 60 s following a procedure presented by Kontturi et al. (2003). The TMSC-coated QCM-D crystals were converted to the cellulose form by a HCl-vapor treatment and stored in a desiccator.

Nanofibrillar cellulose model cellulose films were prepared following a procedure presented by Ahola et al. (2008). In brief, bleached hardwood pulp (birch) was mechanically treated (five times with a Masuko grinder) and then it was disintegrated by 20 passes through a microfluidizer. The cellulose nanofibrils sonicated with Branson S-450 ultrasonic probe (10 min, 25 % amplitude) and the resulting NFC suspension was centrifuged at 10,400 rpm for 45 min. The colloidal nanofibrils were then collected from the supernatant by pipetting and were spin-coated (0.148 wt% NFC in water) on PEI-treated gold QCM-D crystals with a spin-coater (Model WS-650SX-6NPP, Laurell Technologies, PA, USA) at 3,000 rpm and 90 s spinning time. The prepared NFC model cellulose films were stored in a desiccator.

The cellulose thin films were stored in water over night to improve their stability before use in a QCM-D instrument. Data presenting frequency and dissipation changes were constructed using the fifth overtone. In all measurements different overtones followed each other well, as illustrated in Figure S1 (Online Resource 1).

Cellulose films with and without TEMPO-oxidation were characterized with an AFM (Nanoscope IIIa Multimode scanning probe microscope from Digital Instruments Inc., Santa Barbara, CA, USA). The AFM scans were collected by using tapping mode in air with silicon cantilevers. At least three different areas on each sample were analyzed and no image processing was used except flattening.

The oxidation solution was prepared by dissolving TEMPO (0.1 mmol/l) and NaBr (1 mmol/l) in milli-Q water. Cellulose surfaces were stabilized in the oxidation solution prior to the measurements. Before starting the measurements NaClO (3 mmol/l) was added to activate TEMPO and the pH of the solution was adjusted and kept constant at selected pH using 0.1 M HCl and 0.1 M NaOH.

Results and discussion

Both of the cellulose surfaces used in this study reacted similarly to TEMPO-oxidation. When the cellulose surfaces came into contact with activated TEMPO solution, the frequency began to decrease and dissipation increase (Figs. 1, 2). The decrease in frequency indicated that the increase in mass of the surface is induced by introduction of carboxyl groups to cellulose. Carboxyl groups are also able to couple significantly with higher amounts of water molecules than hydroxyls, which cause the surfaces to swell more. Simultaneously, the swelling caused by absorbed water results in an increase in dissipation.

After the initial decrease, the frequency started to increase which indicates a decrease in mass. The introduced carboxyl groups increase the negative charge of the cellulose, which in turn causes electrostatic repulsion between the charged groups. Due to the repulsion, loosely bound material is removed from the films. Moreover, hemicelluloses have been shown to dissolve during TEMPO oxidation (Rodionova et al. 2013), which further boosts the increase in the frequency in oxidation of NFC surface. The amorphous cellulose films are likely to partially dissolve due to an increase in solubility by introduction of carboxyl groups. Removal of matter from the film causes dissipation to decrease as the film becomes thinner. However, in this case the dissipation kept increasing as the frequency increased. This can be explained by continuous swelling of the film due to increasing hydrophilicity and looseness. The oxidation takes place very rapidly and the effect of the loss of mass on dissipation is diminished by the drastic increase of hydrophilicity. As the oxidation proceeds, the effect of mass removal becomes more pronounced and dissipation begins to decrease. Subsequently, both frequency and dissipation levels off at levels that are higher than the starting levels, respectively. This indicates that a significant amount of cellulose is removed from the crystal surface, which can be confirmed from AFM images from fully oxidized surfaces (Fig. 5).

The pH effect on the oxidation rate was evaluated. As seen from Figs. 1 and 2, the reaction rate of TEMPO oxidation increases rapidly with increasing pH up to pH 10. Any further increase of pH causes only a minor change in frequency and dissipation profiles (Fig. 2). The initial decrease of frequency and increase of dissipation suggests that the reaction rate remained stable above pH 10. Similar results have been reported earlier by de Nooy et al. (1995a, b). Although the reaction rate remains fairly stable above pH 10, the steeper slopes of frequency and dissipation changes (Fig. 2a, b) indicate removal of mass from the QCM-D crystal. Instability of the NFC films at pH 11 could result from increased solvent power along with higher alkalinity and faster degradation of cellulose due to β-elimination. At pH 8.5 the oxidation affected the films very slowly, however, after measurement was

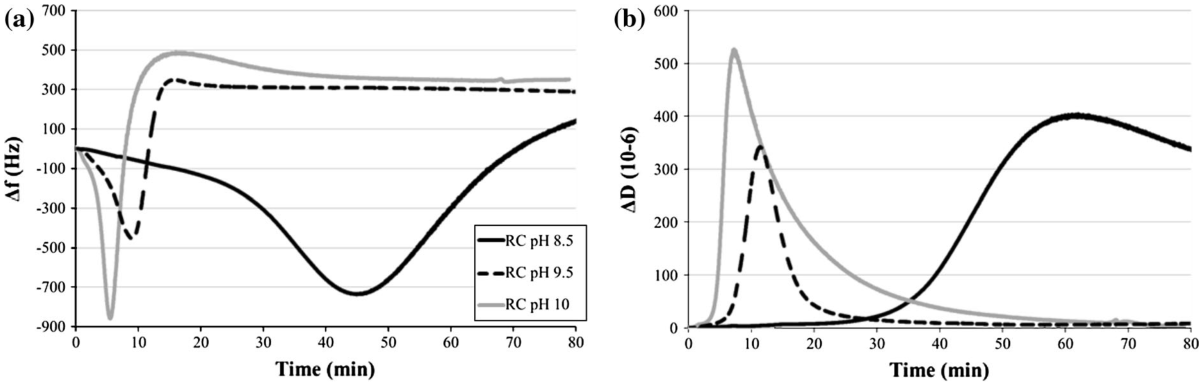


Fig. 1 **a** Frequency and **b** dissipation changes of amorphous cellulose film on QCM-D crystal during TEMPO oxidation in different pHs

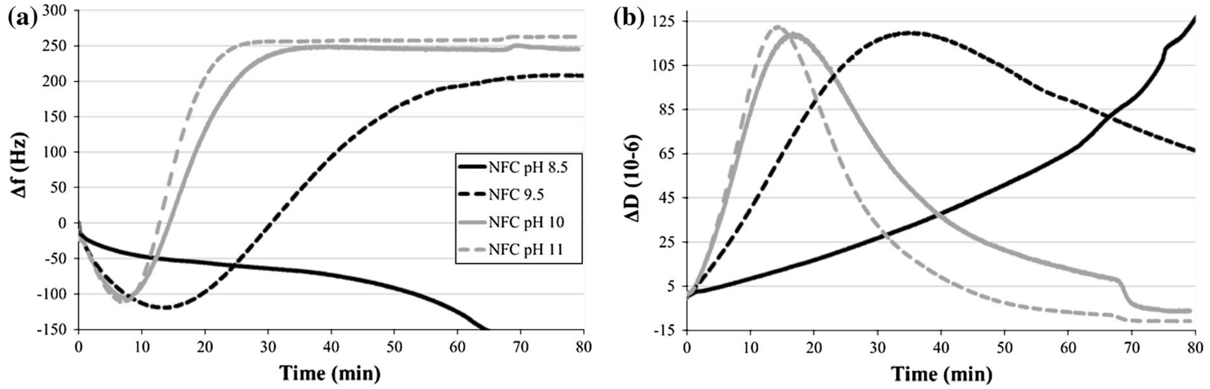


Fig. 2 **a** Frequency and **b** dissipation changes of NFC film on QCM-D crystal during TEMPO oxidation in different pHs

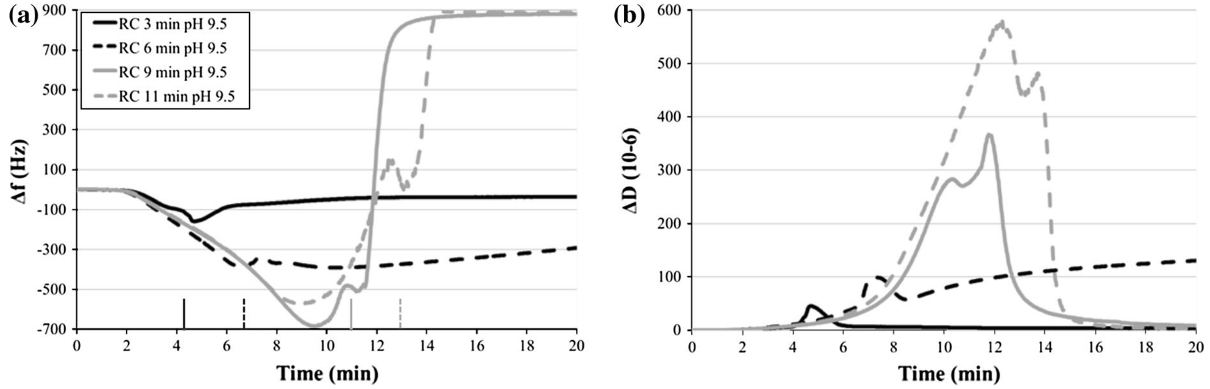


Fig. 3 **a** Frequency and **b** dissipation changes of amorphous cellulose film on QCM-D crystal during TEMPO oxidation. Oxidations were discontinued with addition of ethanol after 3, 6, 9 and 11 min of oxidation. *Vertical lines* on x-axis indicate points of ethanol addition. After 3 min, ethanol was changed to water and system was left to stabilise

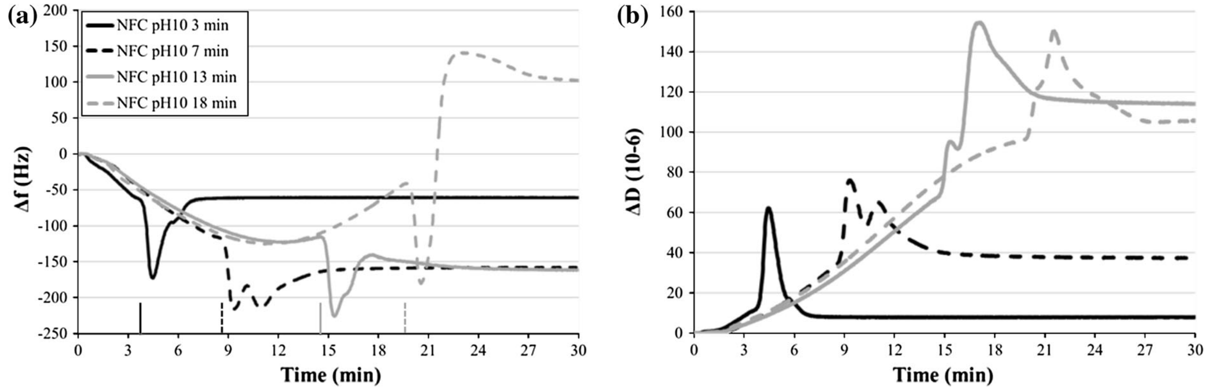
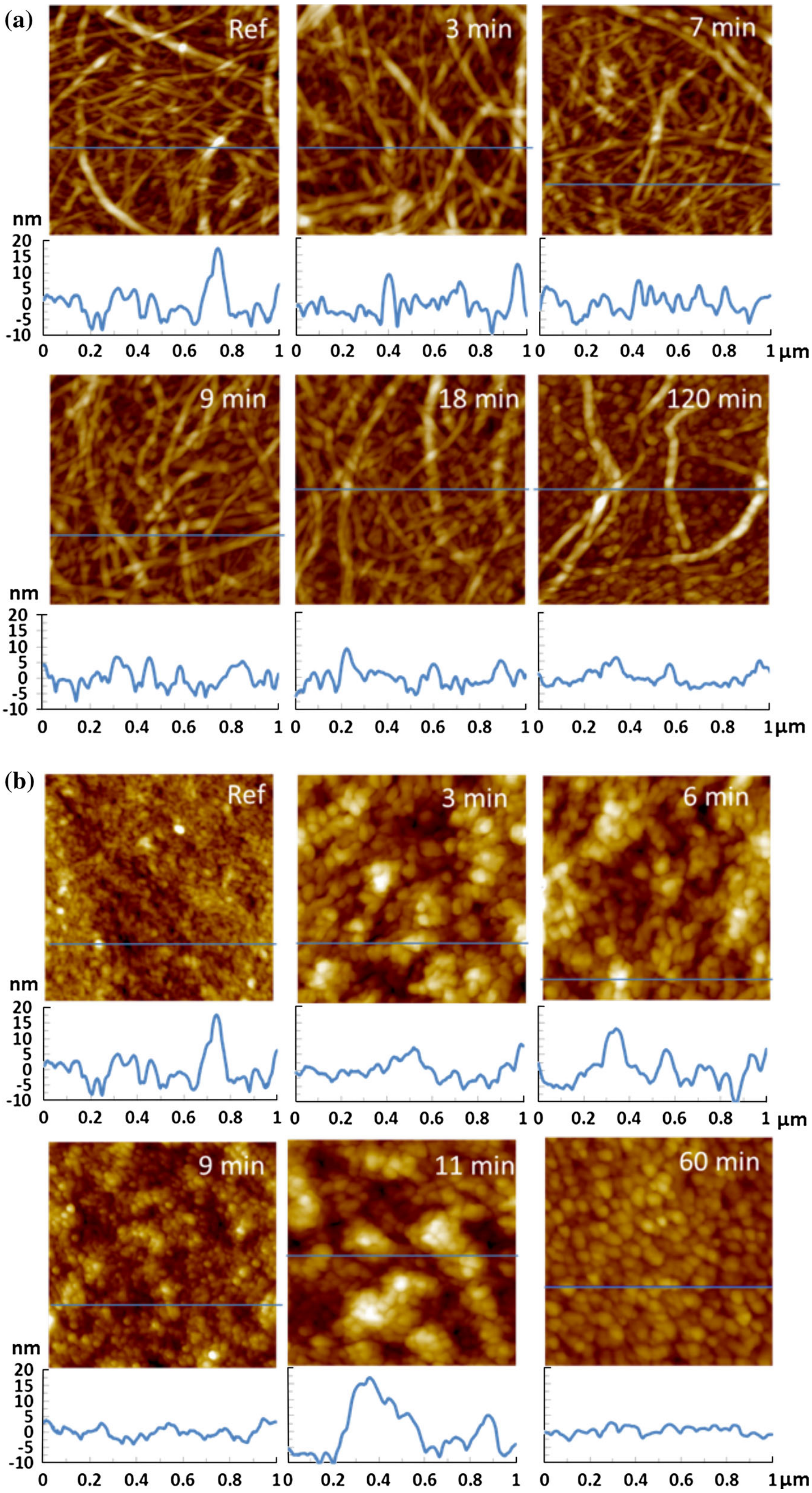


Fig. 4 **a** Frequency and **b** dissipation changes of NFC film on QCM-D crystal during TEMPO oxidation. Oxidations were discontinued with addition of ethanol after 3, 7, 13 and 18 min of oxidation. *Vertical lines* on x-axis indicate points of ethanol addition. After 3 min, ethanol was changed to water and system was left to stabilise

conducted for 40 min, the QCM-D crystals started degrading and data could not be obtained any further (Online Resource 1, Fig. S2).

Changes in frequency and dissipation profiles of amorphous cellulose surfaces followed each other more closely than the NFC surfaces, as shown by the

Fig. 5 AFM topography images and surface profiles of TEMPO oxidized **a** nanofibril and **b** amorphous cellulose surfaces. Images of empty sensors can be found from Online Resource 1 (Fig S3)



peak positions. Dissipation during the oxidation of amorphous film started to decrease soon after the frequency began to increase whilst the dissipation in NFC began to fall clearly later than the increase of frequency. This difference between the signals comes from the stability of NFC films during the oxidation. The effect of mass loss overcomes the effect of increased swelling on dissipation more quickly for amorphous cellulose film than for NFC films. This demonstrates that amorphous cellulose surfaces were oxidized much faster that NFC surfaces in similar conditions. It has been reported that TEMPO oxidation mechanism first oxidises the more accessible regions in a cellulose fibre, amorphous regions and surfaces of NFC. Hereafter, the TEMPO oxidation begins to cause permanent changes on crystalline regions of CNFs via breaking cellulose chains and opening the fibril surface (Hirota et al. 2010). Therefore, crystalline cellulosic materials need more time to reach the high oxidation level than more amorphous cellulosic materials.

Stability of oxidized surfaces were analysed by interrupting the reaction after a selected period of time by the addition of ethanol and rinsing of the surface with water (Fig. 3). In this study, pH 9.5 and pH 10 were chosen for oxidation of regenerated cellulose and NFC films, respectively, because the reaction rates at these pH's were similar and the reaction could be easily stopped at distinctly different levels. When the period of oxidation was 3 min the oxidation caused only a very small change in amorphous cellulose film properties. After 6 min the oxidation changes were much more prominent and cellulose surface had clearly swollen due to the oxidation, which could be seen as increased dissipation and decreased frequency. When the oxidation duration was 9 min or more most of the cellulose was removed from the surface, which is indicated by levelling of frequency to very high and dissipation close to zero after rinsing with water. AFM images (Fig. 5b), however, indicates that a thin layer of cellulose still remains on the surface of the QCM-D crystals after 11 min of oxidation. Difference in frequency change could be observed between measurements illustrated in Figs. 1a and 3a. This is likely caused by the differences in thickness of amorphous cellulose films prepared. Despite variations in thicknesses the frequency change profiles follow each other very well, which indicates that oxidation affects films in similar manner. Dissipation values were similar in both of the measurements.

NFC films had much higher stability after oxidation (Fig. 4). The properties of NFC surfaces showed clear differences at all of the reaction times. Although the mass of the surface varied due to removal of fibrils by electrostatic repulsion, the dissipation kept increasing with reaction time. After 18 min of oxidation a clear decrease of mass could be observed from frequency profile, however, a fully covering of the surface could still be seen in AFM images (Fig. 5a). Even after 2 h of oxidation some nanofibrils remained on the surface.

TEMPO oxidation can be used to oxidize readymade cellulosic structures; however, the crystalline structure of the material should be taken into account. A film of CNFs showed clearly higher stability towards the oxidation than one prepared with amorphous cellulose. The fundamental knowledge of the behaviour of different cellulosic materials during oxidation will help to create more elaborate structures for high value applications. Oxidized cellulosic structures have great potential in many different applications, especially in the field of medicine due to the good biocompatibility and biosorption (Bajerová et al. 2009).

Conclusions

Oxidized cellulose surfaces with differing properties were prepared from amorphous and NFC films by TEMPO oxidation. Reactivity of amorphous surface was higher than that for the NCF surface due to higher accessibility. pH 10 was found to be the optimum for oxidation without causing excess degradation of the films.

NFC films were found to be significantly more stable after oxidation than amorphous ones. After 18 min oxidation at pH 10 fully covering surface could be seen in AFM images. Most of the amorphous cellulose was dissolved and degraded from amorphous cellulose film after 9 min oxidation at pH 9.5.

The tuning of TEMPO oxidation condition opens new avenues for utilizing robust TEMPO oxidation to functionalize cellulosic materials in a controlled manner for use in high value add applications.

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