Creation of a new material stream from Japanese cedar resources to cellulose nanofibrils

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ABSTRACT

Japanese cedar is one of the most abundant plantation softwood in Japan, although it is not effectively utilized as wood resources. Japanese cedar cellulose was isolated and subjected to one-pot catalytic oxidation and reduction with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and NaBH₄, respectively. The TEMPO-oxidized and NaBH₄-reduced Japanese cedar celluloses (TOCs-NaBH₄) had carboxylate contents of up to 1.4 mmol/g and viscosity-average degrees of polymerization from 2000 to 3000. The X-ray diffraction patterns of the TOCs-NaBH₄ showed that the crystal widths were ~3 nm, indicating that the C6-OH groups present on the crystalline cellulose microfibril surfaces were selectively oxidized to C6-carboxylate groups. When the TOCs-NaBH₄ with carboxylate contents of 0.9–1.4 mmol/g were mechanically disintegrated in water, transparent TEMPO-oxidized cellulose nanofibril (TOCN) dispersions were obtained. The lengths of the TOCNs, determined from their atomic force microscopy images, varied from 800 to 1500 nm, depending on the oxidation conditions. The TOCNs prepared from Japanese cedar cellulose have the average of high aspect ratios (>300), which are greater than those (~150) prepared from wood pulps and thus advantageous.

Keywords: Japanese cedar; Cellulose nanofibril; TEMPO-mediated oxidation;
Nanofibrillation; Sugar composition

1. Introduction

Approximately 66% of Japan's total land area is forest. The cumulative stock of forest was 4.43 billion m³ in 2009, and has increased annually by 80 million m³ [1]. Japan therefore has abundant forest resources. The enhancement of the quantitative and qualitative use of these abundant wood biomass resources as materials (not fuels), together with adequate artificial management of forests and tree plantations, would help to create a sustainable and low-carbon society, decrease CO₂ emissions, and prevent global warming. In particular, softwood trees represent ~53% of the total trees in Japan, and with Japanese accounting for ~21% (or ~40% of softwood trees) [2]. Japanese cedar wood is therefore a significant potential source of materials.

Japanese cedar trees are generally felled ~60 years after planting, but they have not been efficiently and quantitatively used [1,2]. Moreover, the wood resources produced from forest thinning, which correspond to ~20 million m³ per year, have been left in forests as logging residues rather than being used. Japanese cedar is therefore one of the most abundant but unused softwoods in Japan [2]. Conventionally, wood resources have mainly been used in areas such as housing and furniture timbers, pulp and paper, and wood fuel, and these have been the driving forces in establishing a carbon-neutral society. However, quantitative and qualitative increases in these conventional uses of wood resources in the future are no longer expected, because of the decreasing population and growing paperless society in Japan [3,4].

Nanocelluloses, including nanocrystalline celluloses and nanofibrillated celluloses, with low and high aspect ratios, respectively, are categorized as new, wood-based, and crystalline nanomaterials. They have potential applications as biomaterials for commodity goods and high-tech materials [5,6]. Nanocelluloses are prepared from wood celluloses by various pretreatments and mechanical disintegration of the pretreated wood celluloses in water; they have widths from a few nanometers to several hundred nanometers [7]. In various pretreatments, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidation of wood cellulose fibers to papermaking-grade materials

provide typical TEMPO-oxidized cellulose nanofibrils (TOCNs) with homogeneous widths of ~3 nm and high aspect ratios, i.e., >100 [8,9]. Cast films of wood TOCNs, TOCN networks, TOCN hydrogels, TOCN aerogels, and wood- TOCN-containing composites have been reported to have unique oxygen barrier, mechanical, optical, thermal, hydrophilic/hydrophobic, electric, and catalytic properties, different from those of materials prepared from other nanocelluloses [10–16].

Japanese cedar wood is therefore a potential resource for the preparation of TOCNs for high-tech bio-based nanofibers, after isolation of cellulose from the wood, because softwood cellulose provides completely and individually nanofibrillated TOCNs more efficiently than hardwood celluloses do [11]. We previously prepared TOCNs from commercially available softwood bleached kraft pulps for papermaking. However, for environmental reasons, sulfur-free pulping of, and cellulose-isolation from Japanese cedar wood is necessary, because the odor of methyl mercaptan is a serious problem [17–19]. Puangsin et al. [20] and Kuramae et al. [21] used various wood and non-wood holocelluloses containing significant amounts of hemicelluloses for the preparation of TOCNs.

In this study, Japanese cedar holocellulose was extracted with 4% aqueous NaOH to remove as much of the hemicelluloses as possible, while maintaining the original cellulose I crystal structure. The alkali-treated Japanese holocellulose or Japanese cedar cellulose was oxidized using a TEMPO-mediated system under various conditions in water at pH 10. The obtained TEMPO-oxidized and the NaBH₄-reduced Japanese cedar celluloses (TOCs-NaBH₄) and TOCNs prepared from them were characterized to be used as bio-based nanomaterials for the creation of a new materials stream from forest to high-tech industry.

2. Materials and methods

2.1. Materials

Japanese cedar (*Cryptomeria japonica*) powder (80 mesh pass, particle size <0.2 mm) was used as the wood resource. It was delignified with NaClO₂ in water at pH 4–5 and 75 °C for 1 h, according to the Wise method [22]. This delignification treatment

was repeated five times with fresh chemicals to prepare Japanese cedar holocellulose [23], and the holocellulose was extracted with 4% aqueous NaOH at room temperature for 1 h to remove hemicelluloses. The obtained Japanese cedar cellulose was neutralized by washing it with acidic water at pH 4–5, washing it thoroughly with water, and keeping it in a wet state at 4 °C before use. TEMPO, NaBr, 70% NaClO₂ solution, 13% NaClO solution, and other chemicals and solvents were of laboratory grade (Wako Pure Chemicals Ind., Tokyo, Japan) and used without further purification.

2.2. TEMPO-mediated oxidation of Japanese cedar cellulose

Japanese cedar cellulose (1 g) was stirred in water (100 mL) containing TEMPO (0.016 g), NaBr (0.1 g), and NaClO (2.5–12.5 mmol/g) at pH 10 and room temperature. The mixture was maintained at pH 10 with 0.5 M NaOH until no NaOH consumption was confirmed using a pH stat [9]. Oxidation was stopped by adding a small amount of ethanol to the mixture, and NaBH₄ (0.1 g) was added to the slurry at room temperature for 3 h to reduce the small amounts of aldehyde and ketone groups present in the oxidized cellulose to hydroxyl groups, in a one-pot reaction [24]. The resulting TEMPO-oxidized and NaBH₄-reduced cellulose (TOC-NaBH₄) was washed thoroughly with water by filtration, and stored in a wet state for further treatment or analyses. The yields of the water-insoluble TOCs-NaBH₄ were calculated from their dry weights before and after the TEMPO-oxidation and NaBH₄-reduction. The carboxylate contents of the TOCs-NaBH₄ were determined using an electrical conductometric titration method [9].

2.3. Nanofibrillation of TOCs-NaBH₄ in water

The never-dried TOC-NaBH₄ was suspended in water (100 mL) at a 0.1% (w/v) solid content, and the slurry was homogenized at 7500 rpm for 6 min at room temperature using a double-cylinder-type homogenizer (Physoctron NS-56, Microtec). The slurry was sonicated for 6 min using an ultrasonic homogenizer equipped with a 26-mm probe tip (US-300T, Nihon Seiki) to prepare an aqueous dispersion of TEMPO-oxidized cellulose nanofibrils (TOCNs). The small amounts of partly

unfibrillated fractions present in the dispersion were removed by centrifugation at 7500 rpm for 20 min.

2.4. Analyses

The neutral sugar composition of the Japanese cedar holocellulose, Japanese cedar cellulose, and TOCs-NaBH₄ were determined using myo-inositol as an internal standard, according to a previously reported method [21]. A high-performance liquid chromatography system with a Shodex Asahipak NH2P-50 4E column (ψ 4.6 mm $\times$ 250 mm) and a refractive index detector (Optilab T-rEx, Shodex, Wyatt Technology, USA) was used for the sugar composition analysis; acetonitrile/250 mM H₃PO₄ (3:1 v/v) was used as the mobile phase, at flow rate of 1.0 mL/min, at 30 °C [20].

Freeze-dried samples (0.04 g each) were dissolved in 0.5 M cupriethylenediamine for 30 min. The intrinsic viscosities [η] of the solutions were measured using a capillary viscometer, and the viscosity-average degrees of polymerization (DP_v) were calculated from the [η] values using the Mark–Houwink–Sakurada equation: $[\eta] = 2.84 \times DP_v^{0.67}$ [25].

Solid-state ¹³C nuclear magnetic resonance (NMR) spectra were obtained using a JEOL JNM-ECA500 spectrometer operated at a ¹³C frequency of 500 MHz, with a combination of proton dipolar decoupling, magic-angle sample spinning (MAS), and cross-polarization (CP). The spectra were acquired at room temperature with a MAS spinning rate of 6 kHz. CP transfer was achieved using a ramped amplitude sequence for an optimized total time of 2 ms. The relaxation time was 5 s, and the average number of scans acquired for each spectrum was 1024.

X-ray diffraction (XRD) patterns of pressed disk-pellet samples (0.1 g each) were obtained in reflection mode, using an RINT 2000 diffractometer (Rigaku, Tokyo, Japan) with monochromator-filtered Cu K α radiation, at 40 kV and 40 mA. Scans were obtained from 2 θ values of 4° to 40° a scanning speed of 1°/min. The crystallinity index and (2 0 0) crystal size of cellulose I were calculated from the XRD patterns using conventional methods [20,26].

The mechanically disintegrated aqueous TOCN dispersion (without removal of the unfibrillated fraction) was placed in a disposable poly(methyl methacrylate) cuvette and

the optical transmittance of the dispersion was measured from 300 to 800 nm using an ultraviolet-visible (UV-Vis) spectrophotometer (JASCO V-670).

Atomic force microscopy (AFM) images of the TOCNs (after removal of the unfibrillated fraction) were obtained using a Nanoscope III Multimode (Digital Instruments, USA) instrument. Aqueous dispersions of TOCN (~0.0005%) were deposited on freshly exfoliated mica plates of dimensions $\sim 1 \times 1 \text{ cm}^2$, and subjected to tapping-mode AFM after drying in air. The widths and lengths of TOCNs in each sample were determined from the AFM height profiles.

3. Results and discussion

3.1. Chemical structures of TEMPO-oxidized Japanese cedar celluloses

Japanese cedar powder was delignified five times with fresh NaClO_2 and acetic acid to prepare holocellulose by the Wise method. The yield of the obtained holocellulose was about 71.5%, indicating that most of the lignin was removed [23]. The obtained never-dried holocellulose was extracted with 4% aqueous NaOH to remove hemicelluloses, keeping the original cellulose I crystal structure. The yields of the alkali-treated holocellulose were 72.4% and 51.1% based on the holocellulose and original wood powder, respectively. The alkali-treated holocellulose was regarded as Japanese cedar cellulose. The neutral sugar compositions of the Japanese cedar holocellulose and cellulose are discussed later.

The never-dried Japanese cedar cellulose was oxidized using a TEMPO/NaBr/NaClO system in water at pH 10, followed by reduction with NaBH_4 at the same pH in a one-pot procedure without isolation/purification of the TEMPO-oxidized cellulose. The post-reduction of TOCs with NaBH_4 is required to convert C6-aldehydes and C2/C3 ketones slightly present in TOCs to hydroxyls, which can prevent discoloration by heating and maintain high TOCN yields from completely dried TOCs in the following disintegration process in water [24]. The amounts of NaClO added varied from 2.5 to 12.5 mmol/g. As the amount of NaClO added increased from 2.5 to 12.5 mmol/g, the time required for oxidation increased from 40 min to ~4 h. The weight recovery ratios of TOCs- NaBH_4 decreased slightly, but still remained high,

i.e., >90%. The non-crystalline and low-molecular-mass hemicelluloses present in Japanese cedar cellulose are more susceptible to TEMPO-mediated oxidation than crystalline and high-molecular-mass cellulose, and were therefore probably oxidized and degraded to water-soluble low-molecular-weight compounds during oxidation with the TEMPO-system, and removed during washing of the TOCs-NaBH₄ with water.

Fig. 1 shows the carboxylate contents and DP_v values of the TEMPO-oxidized Japanese cedar celluloses prepared using various amounts of NaClO in the oxidation. The carboxylate contents of the TOCs-NaBH₄ increased linearly from 0.2 to 1.3 mmol/g with increasing amounts of added NaClO from 0 to 7.5 mmol/g, and then leveled off in the range 1.3–1.4 mmol/g on further addition of NaClO. The maximum carboxylate content of the TOCs-NaBH₄ prepared in this study (1.4 mmol/g) was lower than that achieved using TEMPO-oxidized softwood bleached kraft pulp (~1.7 mmol/g), which was post-oxidized with NaClO₂ to convert small amounts of C6-aldehyde groups present in the TEMPO-oxidized pulp [21]. Because the TOCs underwent one-pot reduction with NaBH₄ in this study, most of the C6-aldehydes present in the TOCs were reduced to C6-OH groups, resulting in lower carboxylate contents.

The DP_v values decreased to 1800, or approximately half, as the amount of NaClO added in the TEMPO-oxidation increased to 10–12.5 mmol/g. Significant depolymerization of the cellulose chains in Japanese cedar cellulose therefore occurred during oxidation and subsequent reduction with NaBH₄ in water at pH 10. The primary reason for the decrease in the TOC DP_v was β -elimination of the C6-aldehyde groups, formed as intermediates during TEMPO-mediated oxidation, under aqueous conditions at pH 10 [27].

The sugar compositions of Japanese cedar holocellulose, the alkali-treated holocellulose (or Japanese cedar cellulose), and TOCs-NaBH₄ prepared using various amounts of NaClO are listed in Table 1. The sugar compositions shown in Table 1 and the yields of the alkali-treated holocellulose suggest that more than half of the mannose and xylose in the hemicelluloses in Japanese cedar holocellulose was removed by 4% aqueous NaOH extraction. The glucose content of Japanese cedar cellulose was 86%, which corresponds to $72.4 \times 0.86 = 62.3\%$ of that in the holocellulose, because the weight recovery ratio of Japanese cedar cellulose based on the original holocellulose was 72.4%. The holocellulose had a glucose content of 63.2% (Table 1), and therefore

most of the glucose units present in the Japanese cedar holocellulose were not extracted, and remained in the alkali-treated holocellulose or Japanese cedar cellulose.

The xylose and mannose contents of the TOCs-NaBH₄ decreased to zero as the amount of NaClO added increased to 7.5 mmol/g or more. The glucose contents of the TOCs-NaBH₄ decreased from 62% to 57% with increasing amounts of added NaClO from 7.5 to 12.5 mmol/g; the C6-OH groups present on the crystalline cellulose microfibril surfaces in Japanese cedar cellulose were oxidized to C6-carboxylate groups by the TEMPO-mediated oxidation. Accordingly, the contents of other sugars (Table 1), including glucuronate units with C6-carboxylate groups, increased extent of oxidation. The contents of other sugars were calculated by subtracting the determined total neutral sugar content from the initial weight before acid hydrolysis in the sugar composition analysis. Thus, TOCs can be prepared from Japanese cedar through delignification, dilute NaOH extraction and successive one-pot TEMPO-oxidation/NaBH₄-reduction, which is advantageous for effective utilization of softwood resources in terms of local production for local consumption without using kraft pulping process.

3.2. Solid-state structures of TEMPO-oxidized Japanese cedar celluloses

The solid-state CP/MAS ¹³C-NMR spectra of the holocellulose, alkali-treated holocellulose (or Japanese cedar cellulose) and TOCs-NaBH₄ prepared with various amounts of NaClO are shown in Fig. 2. The holocellulose had the typical NMR pattern consisting of crystalline wood cellulose I. The weak signal at ~176 ppm indicates that a small amount of acetyl ester groups were present in the hemicelluloses of the holocellulose. Extraction with 4% aqueous NaOH resulted in disappearance of this carbonyl signal, showing that the acetyl groups were removed, together with part of the hemicelluloses, by the alkali extraction. The ratio of the signal intensities for crystalline C4-carbons to that of non-crystalline C4-carbons at 89 and 84 ppm, respectively, increased as a result of alkali extraction, showing that non-crystalline hemicelluloses were partly removed [28–30].

When Japanese cedar cellulose was oxidized using 5 and 12.5 mmol/g of NaClO in the TEMPO system, the intensity of the signal from C6-carboxylate carbons at ~176 ppm increased, and the intensity of the shoulder signal at ~63 ppm from non-crystalline

C6-OH carbons decreased. The TEMPO-oxidation therefore occurred in the C6-OH groups present on the crystalline cellulose microfibril surfaces and those in disordered regions in the cellulose. The ratio of the intensities of the crystalline C4-carbon to non-crystalline C4-carbon signals, at 89 and 84 ppm, respectively, decreased with increasing amounts of NaClO added, and therefore the TEMPO-mediated oxidation of Japanese cedar cellulose slightly decreased the crystallinity of cellulose I.

Fig. 3 (top) shows the XRD patterns of Japanese cedar cellulose before and after TEMPO-mediated oxidation with NaClO at 5.0, 10.0, and 12.5 mmol/g. All the TOCs-NaBH₄ had the original cellulose I structure, showing that the TEMPO-mediated oxidation of the C6-OH groups to C6-carboxylates mostly occurred on the crystalline cellulose microfibril surfaces. The crystallinity index and crystal size in the perpendicular direction of the (2 0 0) plane were determined from the XRD patterns, shown in Fig. 3 (bottom). The crystallinity index decreased slightly with increasing amounts of added NaClO, but the (2 0 0) crystal size was roughly constant, ~3 nm, for all the TOCs-NaBH₄. These results show that C6-carboxylation by TEMPO-mediated oxidation occurs on the crystalline cellulose microfibril surfaces and in the disordered regions periodically present along the longitudinal direction of the cellulose microfibrils [25].

3.3. Properties of TEMPO-oxidized Japanese cedar cellulose nanofibrils

The TOCs-NaBH₄ were mechanically disintegrated in water under the conditions described in Section 2.3; photographs of the dispersions and their UV-Vis spectra are shown in Fig. 4. Although the TOC-NaBH₄ prepared using 2.5 mmol/g of NaClO did not give a transparent dispersion containing unfibrillated particles, all the other TOCs-NaBH₄ containing 0.9–1.4 mmol/g of C6-carboxylates gave transparent dispersions at 0.1% solid contents and had light transmittances >90% at 600 nm, showing that complete nano-fibrillation to TOCNs was achieved from these TOCs-NaBH₄. The TOCs-NaBH₄ with carboxylate contents ≥ 0.9 mmol/g prepared from Japanese cedar cellulose can therefore be completely nanofibrillated in water.

Fig. 5 shows the AFM height and phase images of the TOCNs prepared from Japanese cedar cellulose with various amounts of NaClO. These images were obtained

for dispersions after centrifugation to remove unfibrillated fractions. Although the images for the TOCN using 2.5 mmol/g of NaClO show the presence of TOCN agglomerates, most of the nanofibrils were individually separated entities, without agglomeration, for the TOCNs prepared using ≥ 5 mmol/g of NaClO.

The number-average widths, and number-average and length-average lengths of the TOCNs, determined from the AFM images, are shown in Fig. 6. All the TOCNs had similar average nanofibril widths, ~ 2.5 nm, which were smaller than those determined from the XRD patterns (Fig. 3) [31]. When the widths were measured along each fibril length direction, the deviations in the number-average TOCN widths were small for each sample. In contrast, the length-average lengths of the TOCNs decreased from ~ 1500 to ~ 800 nm with increasing amounts of added NaClO from 5.0 to 12.5 mmol/g. The aspect ratios of the TOCNs prepared from Japanese cedar cellulose therefore varied from ~ 320 to ~ 600 , depending on the oxidation conditions. The number-average TOCN length for each sample had significantly large deviations.

It is noteworthy that the lengths of TOCNs prepared from Japanese cedar cellulose in this study were much greater than those prepared from wood kraft pulps and other plant holocelluloses by TEMPO/NaBr/NaClO oxidation in water at pH 10 with the same amount of NaClO used. For example, the length-average length of TOCNs prepared with NaClO of 5 mmol/g in this study was ~ 1500 nm, whereas those prepared from softwood bleached kraft pulp and plant holocelluloses were ~ 580 nm and 500–800 nm, respectively [21,27]. Because mechanical properties of fiber-reinforced composite materials are significantly influenced by the aspect ratios of fibers present in matrix polymers [32], the high aspect ratios of TOCNs prepared from Japanese cedar cellulose are potentially advantageous to be used as nanofillers.

The DP_v values of the TOCs-NaBH₄ prepared using 5.0–12.5 mmol/g of NaClO were in the range 2000–3000 (Fig. 1), which corresponds to cellulose chain lengths of 1000–1500 nm, assuming that all the cellulose and TEMPO-oxidized cellulose molecules are fully extended in each TOCN element. These viscosity-average TOCN chain lengths were similar to, or slightly smaller than, the average TOCN lengths of 800–1500 shown in Fig. 6. It is possible that the AFM method of measuring the TOCN lengths may have some limitations for long TOCN elements [33]. Transmission electron microscopy images of the TOCNs gave average TOCN widths of ~ 3.5 nm (data not

shown), i.e., larger than those determined from the AFM images, but close to those determined from the XRD patterns [31].

4. Conclusions

One-pot TEMPO-mediated oxidation and reduction of Japanese cedar cellulose gave TOCs-NaBH₄ with carboxylate contents of up to 1.4 mmol/g and *DP_v* values of 2000–3000. TOCs-NaBH₄ with carboxylate contents of 0.9–1.4 mmol/g were converted to transparent gels consisting mainly of individual TOCNs. The AFM images showed that the TOCNs had the same widths, i.e., ~2.5 nm, but different lengths, i.e., 800–1500 nm, depending on the oxidation conditions. TOCNs with high aspect ratios (>300) were therefore obtained from Japanese cedar cellulose by one-pot oxidation/reduction and subsequent mechanical disintegration in water. Thus, it may be expected to create a new material stream from unutilized softwood resources abundantly present in forest of Japan to TOCNs used in forefront industry.

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Table 1

Sugar compositions (%) of Japanese cedar holocellulose, Japanese cedar cellulose (alkali-treated holocellulose) and TEMPO-oxidized Japanese cedar celluloses (TOCs-NaBH₄).

Sample	NaClO added (mmol/g)	Sugar composition (%)						
		Glu	Xyl	Man	Rha	Ara	Gal	Others
Holocellulose	—	63.2	7.9	13.8	0	1.1	0	14
Cellulose	—	86.0	3.5	9.4	0	0	0	1.1
TOC-NaBH ₄	2.5	82.7	2.8	5.8	0	0	0	8.7
	5.0	72.8	2.0	1.7	0	0	0	23.5
	7.5	62.3	0	0	0	0	0	37.7
	10.0	59.3	0	0	0	0	0	40.7
	12.5	56.9	0	0	0	0	0	43.1

<Figure captions>

Fig. 1. Effect of amount of NaClO added in TEMPO-oxidation on carboxylate contents and viscosity-average degrees of polymerization (DP_v) of TOCs-NaBH₄.

Fig. 2. Solid-state ¹³C-NMR spectra of Japanese cedar holocellulose, Japanese cedar cellulose, and TOCs-NaBH₄ prepared using various amounts of NaClO.

Fig. 3. XRD patterns of TEMPO-oxidized celluloses (top), and crystallinity indices and (2 0 0) crystal sizes (bottom) of cellulose I for TOCs-NaBH₄ prepared using various amounts of NaClO.

Fig. 4. Photographs and corresponding light-transmittance spectra of 0.1% aqueous TOCN dispersions without centrifugation.

Fig. 5. AFM height (top) and phase (bottom) images of TOCNs prepared using various amounts of NaClO.

Fig. 6. Number-average widths, and number-average and length-average lengths of TOCNs prepared using various amounts of NaClO, determined from AFM images.

Fig. 1. Effect of amount of NaClO added in TEMPO-oxidation on carboxylate contents and viscosity-average degrees of polymerization (DP_v) of TOCs- NaBH_4 .

Fig. 2. Solid-state ^{13}C -NMR spectra of Japanese cedar holocellulose, Japanese cedar cellulose, and TOCs- NaBH_4 prepared using various amounts of NaClO .

Fig. 3. XRD patterns of TEMPO-oxidized celluloses (top), and crystallinity indices and (2 0 0) crystal sizes (bottom) of cellulose I for TOCs-NaBH₄ prepared using various amounts of NaClO.

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Graphic abstract

TEMPO-oxidized cellulose nanofibrils prepared from Japanese cedar cellulose

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