

Totally chlorine-free peracetic acid pulping for nanocellulose isolation from hemp and poplar

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ABSTRACT: Nanocellulose is a promising and sustainable feedstock for developing advanced and functional materials. However, the characteristics of nanocellulose, such as crystallinity, surface energy, and aspect ratio, can vary depending on biomass source and pretreatment methods, leading to variable performance of the nanocellulose-based materials.

In this study, cellulose nanocrystals (CNCs) were isolated from hemp and poplar using totally chlorine free (TCF) peracetic acid and sodium chlorite delignification and bleaching pretreatments to probe the influences of biomass source and treatment methods on the isolation and characteristics of CNCs. Our results showed that hemp and poplar were almost completely delignified by peracetic acid treatment, whereas sodium chlorite treatment left 5%–6% lignin in the pulp. The yields of CNCs from raw hemp and poplar biomass ranged from 9.8% to 21.9% and 10.9% to 28.3%, respectively, depending on the treatment methods. The dimensions of CNCs from TCF-treated biomass generally maintained a larger width and aspect ratio than those from sodium chlorite-treated biomass. The poplar-derived CNCs exhibited slightly higher crystallinity of 53%–58% than hemp-derived CNCs of 49%–54%. The zeta potential of the CNCs, ranging from -20.1 mV to -31.1 mV, ensured a well-dispersed aqueous solution. The surface energy (dispersive energy of 40–80 mJ/m² and specific energy of 2–10 mJ/m²), water interaction, and thermal stability of the CNCs were comparable, regardless of the biomass source and pretreatment methods. Our finding suggests that the TCF technique with peracetic acid treatment is a promising delignification and bleaching approach to obtain cellulose-rich pulps from herbaceous and hardwood biomass for nanocellulose isolation.

Application: This study investigated a TCF method using peracetic acid to isolate nanocellulose from two important bioenergy crops — poplar and hemp. We have found that both botanical origins and pretreatment procedures have influence on the geometry, crystallinity, zeta potential, and water interactions of nanocellulose.

The use and valorization of agroforestry waste through biorefineries is a sustainable strategy for satisfying the demands for food, raw materials, and energy. Cellulose, a major component of plant cell walls and occurring in animals and bacteria [1], is the most abundant natural polymer on land. It provides a renewable, biodegradable, and biocompatible resource for various value-added products such as fuels, chemicals, and materials. Nanocellulose, defined as a cellulose segment on the nanometer scale, can be derived from the plant cell wall through acid hydrolysis and mechanical shearing, yielding cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs), respectively. These CNCs and CNFs possess appealing characteristics that combine the features of both cellulose and nanomaterials, such as capabilities of chemical modification and functionalization, low density, high mechanical strength, high degree of crystallinity, and non-toxicity [2,3]. These desirable properties of nanocellulose have been explored in the production of fibers and textiles, packaging composites, aerogels, biomaterials, electrochemical energy storage, and many other applications.

Cellulose isolation from lignocellulosic biomass has been the subject of intensive research for scientists and engineers worldwide in recent years [4,5]. The pulping and bleaching processes are important steps in removing non-cellulosic materials (i.e., hemicellulose, pectin, and lignin) from the plant cell wall to liberate cellulose fibers. Recently, the industry typically has used kraft pulping of woody biomass, which involves using sodium sulfide and sodium chlorite to delignify the biomass, followed by elemental chlorine-free bleaching [6,7]. Bleaching with sodium chlorite (NaClO₂) can produce chlorine radicals, Cl•, that can generate highly toxic organochlorine [8]. Therefore, new technologies aimed at reducing or eliminating the use of chlorine in the pulping and bleaching process for cellulose isolation are highly desirable in the production of nanocellulose.

Peracetic acid (PAA) is a totally chlorine free (TCF) alternative to NaClO₂ and chlorine dioxide (ClO₂) that has gained increasing attention in the biorefining process for cellulose isolation [9,10]. Peracetic acid is a strong oxidizing agent that can selectively attack lignin with minimal altera-

tion to cellulose and hemicellulose [11,12]. In the presence of PAA and acidic conditions, lignin macromolecules react with the hydronium (H_3O^+) reactive species [13]. Therefore, PAA has been frequently used to isolate and determine the structures of cellulose and hemicellulose from various cell walls [14–17]. Another advantage of using PAA for cellulose isolation is that it can be recycled and eventually decomposes into water, offering additional benefits [18]. Previous studies have used PAA to bleach a diversity of biomass, such as sugarcane bagasse [19], pine, and Victorian ash [11], for production of high-value chemicals (e.g., ethanol, levoglucosenone, and 5-chloromethyl furfuryl). Researchers are also interested in using PAA pretreatment as a technique for nanocellulose isolation. For example, soybean hulls were delignified with PAA under mild conditions that enabled low-energy and environmental input before subsequent nanocellulose isolation [20]. Peracetic acid was used to reduce energy consumption for treating spruce bark and birch wood, while still retaining the intrinsic fiber properties of the materials [21]. Most of these studies have mainly focused on the isolation of CNFs following PAA pretreatment, while limited studies have examined the effect of PAA pretreatment on CNCs isolation.

The pretreatment approaches employed to isolate cellulose and the botanical origin of cellulose impact the characteristics of the ultimate nanocellulose. Pretreatments can affect the surface chemistry of cellulose fibers and the hydrophilicity of the nanocellulose. For example, 2,2,6,6-tetramethylpiperidine 1-oxyl-mediated oxidation and carboxymethylation can introduce carboxyl and carboxymethyl groups to the surface of CNFs, respectively [22]. Meanwhile, the characteristics of nanocellulose, such as crystallinity and thermal stability, have also been found to vary depending on the biomass source. For instance, rice straw with higher crystallinity has been shown to possess higher thermal stability than palm fiber, despite maintaining the same nanocellulose isolation conditions [23,24]. The reason lies in the fact that it is specific to the organism producing the fibrils in the plant [1,25]. During the synthesis process, the terminal complexes within the plasma membrane move in directions that align with the orientation of cellulose deposition, and the genetic controls of the organism result in differences in the way elementary fibrils of cellulose are packed together, ultimately affecting the overall structure of the cellulose chains [26]. Both hemp and poplar are promising energy crop sources for biofuels and bioproducts [27,28]. However, the difference in their fiber lengths, with hemp having a length of 8.46 mm and poplar having a length of 0.48 mm, suggests that there may be characteristic differences in the nanocellulose generated from these sources [29,30]. Therefore, it is essential to investigate the impact of both pretreatment approaches and biomass sources side-by-side to better understand their fundamental influence on the isolation and characteristics of nanocellulose.

In this study, we isolated CNCs from two representative biomasses, hemp (herbaceous) and poplar (hardwood), using a TCF technique (PAA delignification-bleaching) followed by sulfuric acid hydrolysis, as well as a commonly used bleaching approach in a laboratory with NaClO_2 as a benchmark for comparison. Our aim was to examine the influence of botanical origins and pretreatment procedures on biomass delignification, hemicellulose removal, cellulose preservation, and the ultimate isolation and characterization of the CNCs. We comprehensively characterized the major properties of CNCs, including morphological appearance, aspect ratio, crystallinity, surface energy, zeta potential, and water-nanocellulose interactions. The knowledge gained from this study has the potential to contribute to the development of sustainable and efficient methods for delignification and production of CNCs from lignocellulosic biomass.

MATERIALS AND METHODS

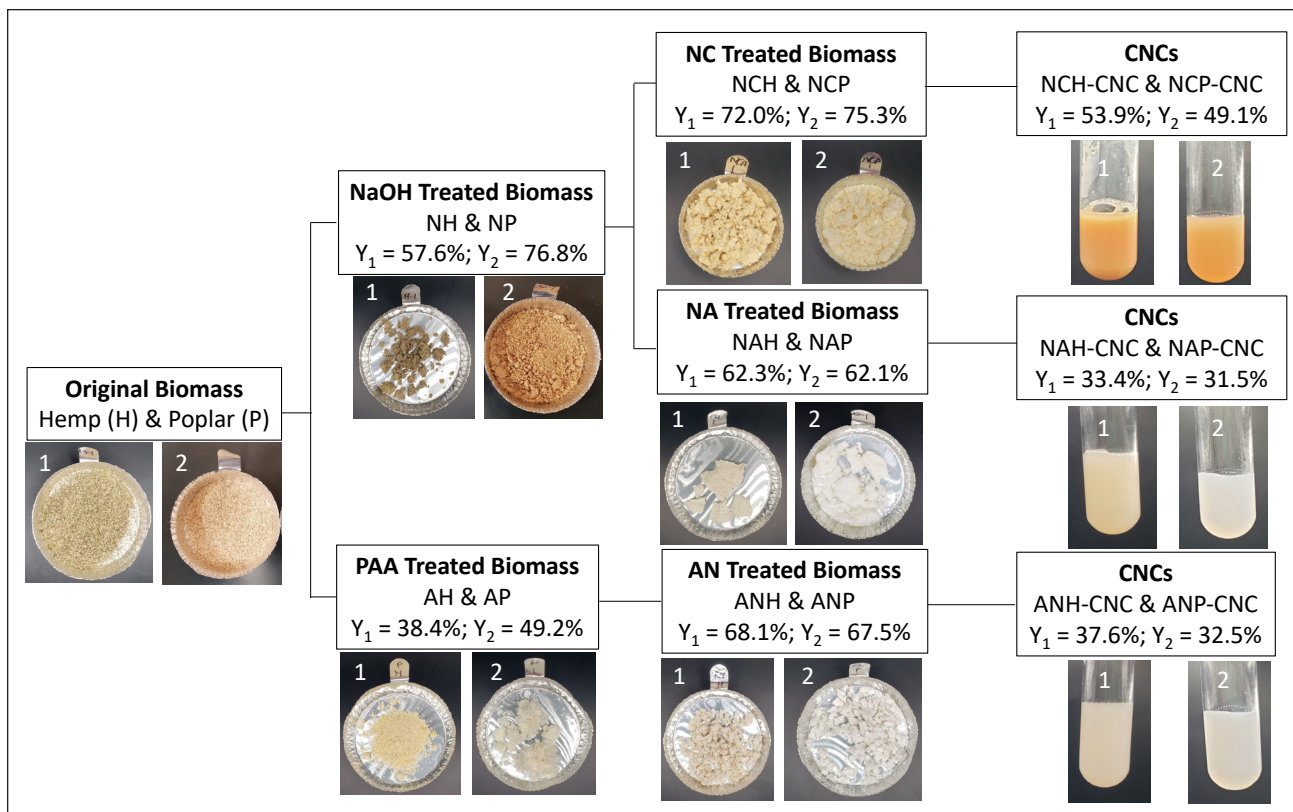
Materials and chemicals

The hemp stalks (*Cannabis sativa*) used in this study were provided by Tortuga Bioplastics. The hybrid poplar (*Populus spp.*) hardwood residues were collected from the University of Tennessee. Avicel PH-101 was purchased through Fluka BioChemika (Morris Plains, NJ, USA). Commercial CNCs (10.8% w/v) were purchased from the Forest Products Laboratory Process Development Center at the University of Maine (Orono, ME, USA). Used as received were: sodium hydroxide (NaOH , 95% purity) from Fisher Scientific (Waltham, MA, USA); glacial acetic acid from Fisher Scientific; hydrogen peroxide (H_2O_2 , 30% w/v) from Sigma Aldrich (St. Louis, MO, USA); sulfuric acid (H_2SO_4 , ACS grade 95%–98%) from VWR (Radnor, PA, USA); NaClO_2 (80%) from Acros Organics (Geel, Belgium); ethanol (200 proof) from Decon Laboratories (King of Prussia, PA, USA); and toluene (95%) from Fisher Scientific. Nano-pure water (18.0 M Ω) was obtained using a Barnstead MicroPure UV Ultrapure Water Purification System from Thermo Scientific (Waltham, MA, USA).

Size selection and chemical composition analyses

The air-dried hemp stalks and poplar chips were ground using a Wiley mill (standard model N 0.3) and sieved to a particle range between 0.18 mm and 0.5 mm. A portion of the ground biomass particles was subjected to dewaxing in a Soxhlet apparatus using a toluene/ethanol mixture (2:1, v/v) overnight. The dewaxed particles were then air-dried in a fume hood and used for chemical composition analysis.

The chemical analysis of dewaxed biomass and biomass treated at each stage (outlined in **Fig. 1**) was conducted according to the NREL protocol, with some modifications [31]. The sugar contents were quantified using the Dionex HPLC system following the previous method in triplicates [16].



1. Flowchart of pretreatment methods and cellulose nanocrystals (CNCs) isolation from hemp and poplar biomass. The yields (Y) of pulp or CNC were expressed as percentages of the starting materials used at each treatment stage instead of the original weight of the raw biomass. See Table I for acronym explanations.

Isolation of cellulose pulp from hemp and poplar

The PAA solution (~15%) was freshly produced by mixing glacial acetic acid and 30% H_2O_2 at a ratio of 1.5:1 [32]. Then, 1.5 wt% H_2SO_4 based on the total weight of the solution was added as a catalyst, and the solution was thoroughly mixed at room temperature before use.

To examine the influence of chemical treatments on the biomass delignification and nanocellulose isolation, we carried out three biomass pretreatment procedures with different bleaching agents and treatment orders as described below:

- NC treatment (benchmark): the biomass was treated with NaOH followed by $NaClO_2$.
- NA treatment: the biomass was treated with NaOH followed by PAA.
- AN treatment: the biomass was treated with PAA followed by NaOH.

In detail, 20 g ground biomass (i.e., hemp or poplar) was first treated with 2% NaOH at 10:1 (v/w) at 95°C water bath for 2 h with continuous magnetic stirring for the processes (a) and (b). Then, the biomass was filtered through Q-8 filter paper, washed with distilled water, and air-dried in a fume hood. The $NaClO_2$ treatment was conducted with ~7 g NaOH-treated biomass using ~70 mL 1.7% $NaClO_2$ at a 1:10 w/v ratio at 60°C for 2 h (glacial acetic acid was added

to adjust the mixture to pH 3–4 before heating). The $NaClO_2$ -treated biomass was then washed with deionized (DI) water, and this $NaClO_2$ process was repeated 3 times for each sample [33]. Process (a) led to the NC-treated biomass that was dried and stored in a -20°C freezer. The PAA treatment in process (b) was conducted with ~7 g NaOH-treated biomass with ~35 mL freshly prepared 15% PAA (reaction of acetic acid and 30% H_2O_2 volume ratio of 1.5:1 and 1.5% H_2SO_4 based on the total weight of the solution at room temperature for 12 h) at a 1:5 w/v at 70°C for 2.5 h with stirring [32]. Process (b) led to the NA-treated biomass that was then filtered, washed, dried, and stored in a freezer. Process (c) was conducted the same way with inverse treatment order of NaOH and PAA in process (b), leading to AN-treated biomass. These three treatments combined with two biomass sources afforded three groups of pulps (the third column in Fig. 1); namely, (i) NC-treated hemp and poplar (NCH and NCP, respectively), (ii) NA-treated hemp and poplar (NAH and NAP, respectively), and (iii) AN-treated hemp and poplar (ANH and ANP, respectively) for CNCs isolation.

Isolation of CNCs from treated hemp and poplar pulps

The CNCs were isolated from 2 g cellulosic pulps of hemp and poplar using 64 wt% H_2SO_4 at a 1:11 w/v ratio of pulp to acid at 45°C for 45 min. The acid hydrolysis was quenched

by diluting the reaction mixture with 50 mL of ice-cold deionized (DI) water. The resulting slurry was centrifuged at 6,000 rpm (4,186 rcf) for 15 min to precipitate the CNCs. The acidic supernatant was decanted, and the precipitate was washed by adding fresh DI water and centrifuging. This washing procedure was repeated three times. The obtained CNCs (gel-like slurry) were then dialyzed against DI water with a dialysis tube (cut-off molecular weight = 12,000 Da), with the water being changed out daily until the pH of the water was neutral. This led to six types of CNCs (the last column in Fig. 1), namely NCH-CNC, NCP-CNC, NAH-CNC, NPP-CNC, ANH-CNC, and ANP-CNC, corresponding to the type of biomass and the treatment procedures. The commercial CNCs (com-CNC) were used as a reference for the analysis.

Characterization

The dispersive and acid-base surface energies of the freeze-dried CNCs were assessed using an inverse gas chromatography-surface energy analyzer (IGC-SEA, Surface Measurement Systems; Alpertown, UK). Briefly, about 45 mg of each freeze-dried CNCs were packed into a 3 mm chromatographic column and stoppered using silanized glass wool at each end of the sample in the column. Each column was conditioned and dried under 2 h of anhydrous nitrogen flow before *n*-alkane probe molecules (C6 to C11) and polar probes (acetone, ethanol, acetonitrile, ethyl acetate, and dichloromethane) were introduced over a range of injection volumes. This method was used to determine the dispersive and specific components of surface energy as a function of fractional surface coverage and to investigate energetic heterogeneity using the Schultz method [34].

The attenuated total reflectance fourier transform infrared (ATR-FTIR, Thermo-Nicolet Nexus 670, Thermo Fisher Scientific) spectroscopy of CNCs was collected in transmittance mode for 16 scans per spectrum from 4,000 to 650 cm^{-1} with a resolution of 2 cm^{-1} using air as background.

The thermogravimetric analysis (TGA) was performed using a thermal analyzer (Themys One, Setaram KEP Technologies; Cranbury, NJ, USA) to assess the thermal stability of CNCs (~10 mg). The temperature ramp was set to increase from 25°C to 500°C at a heating rate of 10°C/min. An argon gas flow of 50 mL/min was employed during the analysis.

The microstructures and surface morphologies of the CNCs were examined using Scanning Electron Microscopy (SEM, Zeiss Auriga, Zeiss Group; Oberkochen, Germany). Before the SEM analysis, the CNC slurries (~2%) were filtered through a 2 mm syringe filter to remove unhydrolyzed residues and then diluted to 0.02%. A droplet of CNC suspension (20 μL) was placed onto a silica wafer with most of the water wicked away using filter paper, with the sample left to dry. The samples were sputter coated with gold under a vacuum at 20 mA for 20 s. The samples were observed and imaged at a 5 kV accelerating voltage. The fiber

dimensions and distribution were determined from more than 100 fibers by using ImageJ open-source software. The statistical analysis was performed using JMP Pro 15 software (JMP; Cary, NC, USA).

The zeta-potential (ζ) of the CNCs was measured using a Zetasizer (SZP-06 Zetasizer, Malvern Panalytical; Westborough, MA, USA) at 25°C. Before measurement, 0.5 wt% CNC suspensions were prepared in duplicate and bath-sonicated for 30 min. For each sample, six measurements were taken, and the mean and standard deviation were reported.

The crystallinity of the CNCs was measured using solid-state nuclear magnetic resonance (SS-NMR) with a Bruker Avance-400 (Bruker; Billerica, MA, USA) [16]. About 150 mg freeze-dried CNCs was packed into 4-mm cylindrical Zirconia rotors. The ^{13}C cross-polarization magic angle spinning (CP/MAS) NMR analysis of cellulose was carried out at a frequency of 100.59 MHz in a Bruker double-resonance MAS probe head at the spinning speed of 10 kHz. The CP/MAS experiments utilized a 5 μs (90°) proton pulse, 1.5 ms contact pulse, 4 s recycle delay, and 4,000 scans. The cellulose crystallinity index, *CrI*, was determined from the areas (*A*) of the crystalline (86–92 ppm) and amorphous (79–86 ppm) C4 signals using the following formula:

$$CrI = \frac{A_{86-92\text{ ppm}}}{A_{86-92\text{ ppm}} + A_{79-86\text{ ppm}}}$$

The water-CNCs interaction was determined using a low-field nuclear magnetic resonance spectrometer (LF-NMR, Bruker mq20-Minispec, 0.47 Tesla, 20 MHz H^1 resonance frequency). In brief, CNCs at three moisture contents were measured; namely, freeze-dried CNCs and CNCs equilibrated in 100% relative humidity over a desiccator for 24 h and 72 h according to the previous method [35].

RESULTS AND DISCUSSION

Biomass delignification and CNCs isolation

To study the impacts of pretreatment methods and biomass sources on the biomass delignification and CNCs isolation, we have designed the study consisting of three pretreatment procedures; namely, NC, NA, and AN for both hemp and poplar, followed by CNCs isolation using 64% H_2SO_4 , leading to a total of six CNCs products (Fig. 1). The yields of CNCs ranged from 25% to 50%, depending on the pretreatment procedures and biomass origin. These yields are comparable with the ~30% CNCs yield obtained from microcrystalline cellulose crystals [36]. The overall yields of CNCs based on the starting hemp and poplar biomass are 21.9% and 28.3% for NC, 12.6% and 15.3% for NA, and 9.8% and 10.9% for AN, respectively. These results indicate that NC treatment results in the highest CNC yield, while AN treatment produces the lowest yield for both hemp and poplar. Moreover, a higher yield of CNCs was isolated from poplar than from hemp.

	H0	P0	NH	NP	AH	AP	NCH	NCP	NAH	NAP	ANH	ANP
ASL	2.2±0.3	2.5±0.1	3.8±0.7	4.5±0.4	4.8±0.4	2.6±0.1	4.4±0.9	7.3±0.3	4.9±0.3	2.3±0.3	2.1±0.1	1.9±0.1
AIL	26.9±1.6	26.2±1.4	20.6±2.4	20.9±3.5	0	0	5.0±1.4	6.6±0.6	0	0	0	0
Glc	39.6±1.8	43.5±2.9	59.8±3.5	53.7±0.6	73.8±2.1	79.4±7.9	69.5±3.1	62.2±1.3	76.8±5.4	76.5±3.7	89.2±2.8	91.7±7.7
Xyl	12.6±0.4	13.8±0.9	11.8±1.2	13.3±0.5	13.6±0.5	10.4±1.2	17.3±2.1	16.4±0.3	16.7±1.2	15.2±1.1	10.3±0.5	7.9±0.7
Ara	3.1±0.1	1.1±0.1	1.5±0.1	1.6±0.2	1.3±0.1	0	1.3±0.1	1.5±0.1	0.6±0.1	0	0.1±0.1	0
Gal	2.7±0.1	1.3±0.1	1.1±0.2	0.7±0.2	1.5±0.1	0	1.1±0.1	0.7±0.2	0.8±0.1	0	0	0
Man	3.2±0.1	2.1±0.3	1.8±0.1	1.5±0.7	2.2±0.2	3.6±0.3	1.2±0.2	0.9±0.1	1.1±0.3	1.3±0.3	1.4±0.3	1.3±0.8
Total	90.3	90.5	100.4	96.3	97.2	95.9	99.8	96.6	100.9	95.4	103.1	102.8

H0 = raw hemp; P0 = raw poplar; NH = sodium hydroxide (NaOH) treated hemp; NP = NaOH treated poplar; AH = peracetic acid (PAA) treated hemp; AP = PAA treated poplar; NCH = NaOH & sodium chlorite (NaClO₂) treated hemp; NCP = NaOH & NaClO₂ treated poplar; NAH = NaOH & PAA treated hemp; NAP = NaOH & PAA treated poplar; ANH = PAA & NaOH treated hemp; ANP = PAA & NaOH treated poplar; ASL = acid-soluble lignin; AIL = acid-insoluble lignin; Glc = glucan; Xyl = xylan; Ara = arabinan; Gal = galactan; Man = mannan.

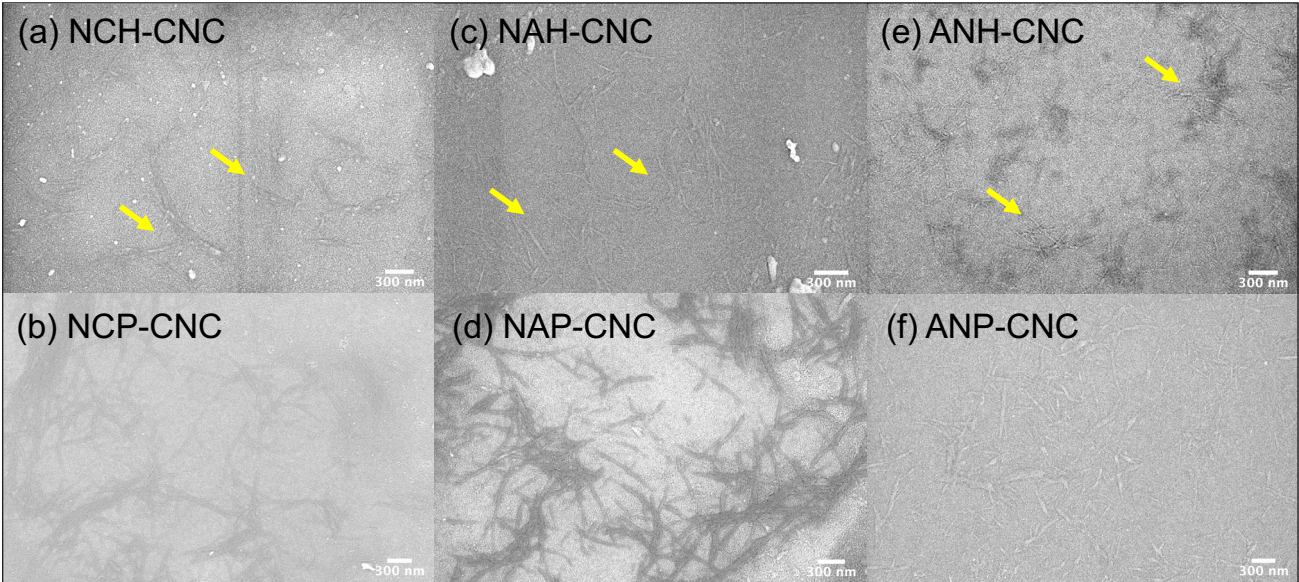
I. Chemical composition of raw and pretreated hemp and poplar (wt%).

The delignification and bleaching capacity of the three procedures can be visually compared through the colors of treated biomass and the resulting CNC suspensions (Fig. 1). The brownish color was mainly due to the presence of chromophores associated with lignin [37]. Therefore, a visually whiter pulp indicates better delignification and bleaching and a higher content of cellulose. The NA and AN treatments showed a much better delignification and bleaching effect on both hemp and poplar than the NC control. The effects of the pretreatment procedures on delignification and bleaching can also be observed through the pulp yields obtained from each treatment. For example, the NC treatment had higher pulp yields of 72.0% and 75.3% than the NA treatment of 62.3% and 62.1%, and the AN treatment of 68.1% and 67.5% for hemp and poplar, respectively. The CNCs isolated from the NA- and AN-treated biomass are pale white in color, while those isolated from the NC treatment had a brownish-yellow hue, indicating the NC-treated pulps retained more lignin. Similar to the yields of pulps, the yields of CNCs isolated from the NA- and AN-treated biomass were lower than those isolated from the NC-treated pulp. Therefore, the whitening color and lower yields of both pulps and CNCs suggest that the NA and AN treatments are more effective in delignifying and bleaching the biomass.

Considering the chemical compositions of the biomass and pulp are critical factors for understanding the yields and appearance of the isolated CNCs, we measured and compared the glucan, lignin, and hemicellulose contents for the original and treated biomass (Table I). The chemical composition data provided insights into how each treatment affected the distribution of cellulose, lignin, and hemicellulose in the original biomass and pulps. The glucan content indicated the cellulose content of the pulp at each stage. The NaOH treatment slightly removed lignin and

hemicellulose from the plant cell wall [38], which was evident from the decrease in acid-insoluble lignin (AIL), xylan, arabinan, galactan, and mannan contents in the treated biomass. Both the PAA and NaClO₂ treatments aimed to remove lignin from the biomass [18]. All pulps from the PAA treatment had a near-zero AIL content, whereas the NaClO₂ treatment also showed a substantial delignification effect. For example, the NC treatment resulted in a reduced AIL of 5.0% and 6.6% for hemp and poplar, respectively. We also found that the NA- and AN-treated biomasses had higher cellulose contents compared with the NC control treatment. The PAA treatment alone (AH and AP in Table I) also resulted in a higher cellulose content than the NC-treated hemp and poplar (NCH and NCP), with 73.8% and 79.4% for PAA-treatment and 69.5% and 62.2% for NC-treatments, respectively. These results suggest that PAA pretreatment is a more effective approach for delignifying hemp and poplar than NaClO₂ treatment. The treatment order also appeared to have an impact on cellulose content, such as 76.8% and 76.5% for NA-treated hemp and poplar and 89.2% and 91.7% for AN-treated hemp and poplar, respectively. This means that AN treatment resulted in the highest cellulose composition for both hemp and poplar.

The PAA treatment primarily delignifies biomass by oxidative cleavage of lignin bonds to substantially disrupt the matrix of the cell wall. The PAA treatment of biomass led to lignin solubilization and cell wall disruption, and therefore, increased the solubilization of hemicellulose during the following NaOH treatment. However, when the biomass was first treated with NaOH, the current conditions employed (i.e., 2% NaOH at 95°C for 2 h) were likely not strong enough to break down cell wall structure to the same degree as the PAA-treated biomass and to solubilize hemicellulose. This explanation was supported by the slightly lower hemicellulose sugar contents of the NaOH-treated



2. Scanning electron microscope (SEM) images of the isolated CNCs from hemp (top) and poplar (bottom). Scale bar 300 nm.

biomass (i.e., the NH and NP vs. raw biomass in Table I), and the much lower hemicellulose content in the AN-treated pulps in comparison to the NA-treated pulps.

Morphological analyses

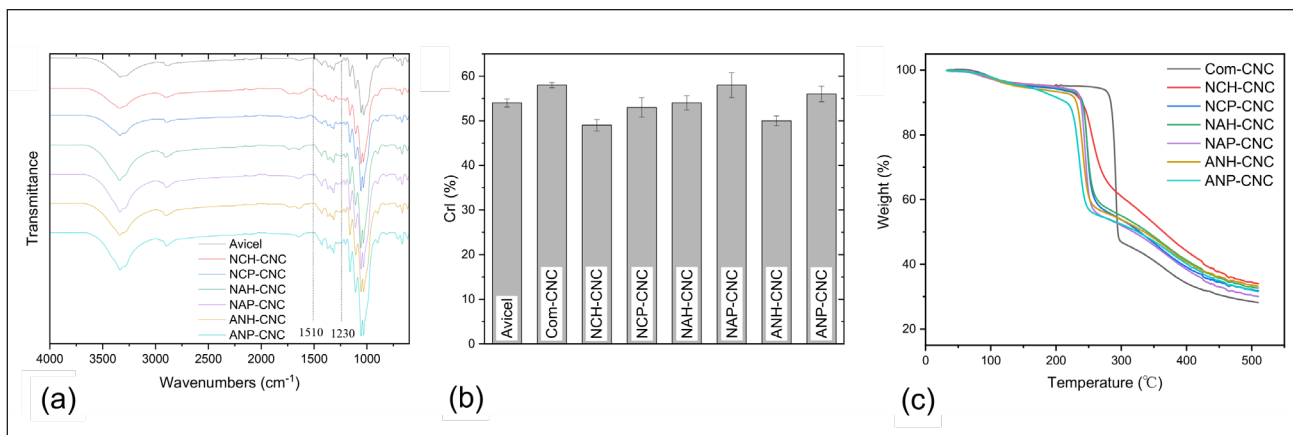
To quantify the morphology and geometry of the isolated CNCs, the six CNC suspensions were observed using SEM (Fig. 2). All the CNCs exhibited a characteristic whisker-like appearance, with lengths ranging from 350–550 nm and widths ranging from 30–50 nm. In addition, some of the CNCs appeared to be bundled and packed, which has also been reported in previous literature [34]. This bundling is likely due to the aggregation of individual CNCs during the air-drying process from an aqueous solution. We further quantified the geometry of the CNCs by measuring 100 randomly selected CNC individuals for each isolation method (Table II). First, the CNCs isolated using the same pretreatment method had similar fiber lengths for both hemp and poplar, despite the fact that hemp was documented to have much longer cellulosic fibers than

poplar (i.e., 8.46 mm and 0.48 mm, respectively [29, 30]). This suggests that the length difference in the native cellulose in the cell walls between hemp and poplar did not carry over into the isolated CNCs under each of the employed conditions. However, the lengths of CNCs varied significantly depending on the pretreatment procedures. The average lengths of CNCs for the NA, NC, and AN treatments were in the order of long-to-short for both hemp and poplar. For example, the NA-treated biomass had the longest CNCs, with lengths of 533.5 nm and 541.5 nm, while the AN treatment produced the shortest CNCs, with lengths of 342.1 nm and 345.1 nm for hemp and poplar, respectively.

The diameter of CNCs was primarily within the range from 20 to 40 nm (an average of 32 nm), and the ANP-CNC of 44.1 and NAP-CNC of 50.2 nm were exceptionally wider. Due to the tendency of CNCs aggregation during the drying process before microscopic analyses, we deduced that the actual diameters of the CNCs are possibly much less. The widths of the poplar-derived CNCs were found to be

CNCs	Length (nm)	Width (nm)	Aspect Ratio
NCH-CNC	480.6 ± 135.5	33.9 ± 6.6	14.7 ± 5.1
NCP-CNC	445.9 ± 141.5	33.4 ± 8.3	14.0 ± 5.0
NAH-CNC	533.5 ± 149.6	34.3 ± 6.0	15.9 ± 4.6
NAP-CNC	541.5 ± 106.5	50.2 ± 8.9	11.2 ± 3.2
ANH-CNC	342.1 ± 88.1	31.0 ± 7.9	11.5 ± 3.4
ANP-CNC	345.1 ± 107.5	44.1 ± 11.6	8.1 ± 2.7

II. Average dimensions of the isolated cellulose nanocrystals (CNCs). Aspect ratio equals length over width.



3. (a) Fourier transform infrared (FTIR) spectra; (b) cellulose crystallinity index (CrI) values, and (c) thermogravimetric curves of CNCs isolated from hemp and poplar in comparison to Avicel or commercial CNC (Com-CNC).

larger than the hemp-derived CNCs using the non-chlorine bleaching treatments (i.e., AN and NA). However, the difference in the widths between the CNCs from hemp and poplar diminished when NaClO_2 was used, which is in agreement with the observation that PAAs selectively attack lignin [11,12] can essentially maintain the structure of cellulose in comparison to NaClO_2 . The variation in lengths and widths of the isolated CNCs led to aspect ratios ranging from 8 to 16. Notably, the CNCs from hemp had much higher aspect ratios than those from poplar for the non-chlorine-treated batches. However, this difference in aspect ratio between hemp-derived and poplar-derived CNCs was not observed for the NaClO_2 treatment. These results suggest that the original structure of the longer hemp fibers (compared with the poplar fibers) may have partially contributed to the larger aspect ratios of CNCs, but varied on isolation approaches used. We would like to warn that the observed CNC particles from SEM may be aggregates of various numbers rather than individual elementary crystals.

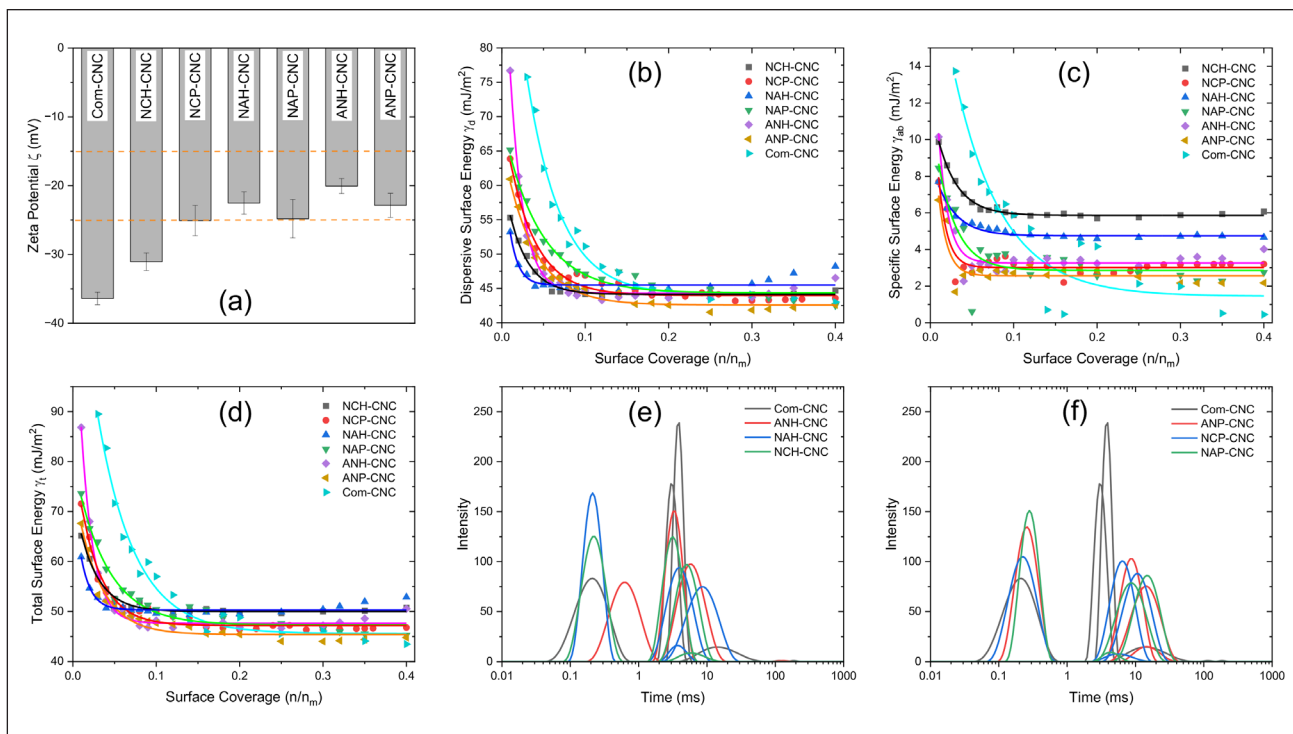
Our results indicate that it is possible to produce different geometry and dimensions of CNCs by varying the biomass source and the order of biomass pretreatment. For example, the hemp-derived CNCs yielded a relatively larger aspect ratio than the poplar-derived CNCs; the non-chlorine PAA bleaching maintained larger width of the isolated CNCs compared with the NaClO_2 bleaching; and biomass treatment with PAA followed by NaOH gave rise to much shorter (~200 nm) CNCs than the treatment with NaOH followed by PAA.

Chemical structure, crystallinity, and thermostability of isolated CNCs

The chemical structure and purity of CNCs isolated from hemp and poplar using different treatments were assessed through FTIR spectra (Fig. 3a). The spectra can be used to determine the remainder of non-cellulosic plant cell wall components (i.e., lignin and hemicellulose). The highly

superimposed spectra of CNCs over Avicel microcrystalline cellulose (MCC, Fluka BioChemika) indicate a high cellulose content, except for the shoulder peak at $1,230\text{ cm}^{-1}$ that is assigned to the sulfate half-ester on the CNCs [39]. The peaks of the CNCs were compared with Avicel and commercial CNCs. The lack of peaks at $1,510\text{ cm}^{-1}$, $1,436\text{ cm}^{-1}$, and 835 cm^{-1} , corresponding to the C=C stretching of aromatic rings of lignin [40], indicates that the isolated CNCs have non-detectable lignin. However, the NCH and NCP showed a small lignin absorbance band at $1,510\text{ cm}^{-1}$ that is consistent with the brownish colors of the CNCs (Fig. 1). Additionally, the presence of a small peak at $1,736\text{ cm}^{-1}$ that corresponds to the C=O stretching of hemicellulose [41] was observed in all hemp-derived CNCs but not in poplar-derived CNCs, suggesting that there is still hemicellulose present in the hemp-derived CNCs, which is in line with the relatively higher hemicellulose content in the bleached hemp than in the poplar (Table I).

Cellulose *CrI* can describe the degree of alignment and organization of the cellulose chains in the cellulose nanocrystals. The *CrI* index of the isolated CNCs were determined in comparison to Avicel and the commercial CNC using ^{13}C CP/MAS SS-NMR (Fig. 3b). The *CrI* of Avicel was 54%, slightly lower than the reported value in the previous literature (~59%) [15]. The commercial CNC had a higher *CrI* of 58% than Avicel. The isolated CNCs from hemp and poplar had slightly lower *CrI* index, in the range of 49%–58%, than the commercial CNC, likely due to the source of the cellulose. The softwood-originated commercial CNC appeared to have relatively higher *CrI* than the herbaceous- and hardwood-originated CNCs. Additionally, all poplar-derived CNCs (i.e., 53%–58%) had slightly higher *CrI* than the hemp-derived CNCs (i.e., 49%–54%) by 4%–6%. This means that the woody sample tends to have higher nanocellulose crystallinity than herbaceous biomass, which agrees with the previous literature, despite using a different *CrI* measurement technique (i.e., X-ray diffraction) [42]. However, it is difficult to draw a clear trend of



4. Surface characteristics of isolated and commercial CNCs: (a) zeta potentials, with lines of zeta potential value at -15 mV for agglomeration and at -25 mV for stable colloid; (b) dispersive energy; (c) specific (acid-base) energy; (d) total surface energy profiles over surface coverage; and time domain low-field nuclear magnetic resonance (LF-NMR) spectra of (e) hemp-derived CNCs and (f) poplar-derived CNCs.

the influence of treatment methods on the *CrI* of CNCs due to the slight variation of *CrI* indexes among the studied treatment methods.

The TGA curves of CNCs (Fig. 3c) revealed that all CNC samples undergo three distinct phases of degradation [43]. The initial stage manifested the dehydration of CNC occurring at 90°C–120°C. The second stage at 240°C–290°C featured the most significant degradation, primarily due to the pyrolysis of the cellulose backbone. Finally, a further degradation appeared at ~350°C, likely due to the volatilization of levoglucosan. The primary degradation temperatures for all CNCs were lower than that of the commercial CNC at 292°C. This is probably attributed to the neutralized sulfate half-ester group in the commercial CNC (i.e., sodium form in CNC can improve thermal stability) [44]. The degradation temperatures for the CNCs from hemp were 253°C for NCH, 248°C for NAH, and 241°C for ANH. The poplar-derived CNCs showed marginally lower degradation temperature, for example, 247°C for NCP, 244°C for NAP, and 235°C for ANP, compared with the hemp-derived CNCs. Our results showed that the thermal behavior of the CNCs is comparable despite the source and treatment procedures.

Surface chemistry analysis of isolated CNCs

The CNC suspensions are expected to have a colloidal suspension due to the introduction of negatively charged sulfate half-ester groups onto CNCs (FTIR spectra peak at

1,230 cm⁻¹, Fig. 3a) that afford electrostatic repulsive forces between the CNCs nanoparticles [45]. The zeta potential of CNCs is a crucial parameter that indicates the surface charge of the nanoparticles and their dispersibility and alignment in a solution. Longer acid hydrolysis time increases the number of sulfate groups attached to the CNCs, presumably resulting in a higher absolute value of zeta potential [34]. The stability of CNC suspensions is affected by the magnitude of zeta potential. Agglomeration of CNCs occurs when the zeta potential is between -15 and 15 mV [45]. For a CNC suspension to remain stable, a zeta potential with an absolute value above 25 mV is desired [46]. We determined the zeta potential of the isolated CNCs to investigate the effect of treatments and biomass sources on the surface charge of the CNC nanoparticles (Fig. 4a). In comparison to the zeta potential of commercial CNC of -36.4 mV, the zeta potential of the isolated CNCs was relatively lower, in the range of -20.1 mV to -31.1 mV, which is comparable with the CNCs of -31.4 mV produced through enzymatic treatment [47]. The CNCs derived from NaClO₂-treated hemp and poplar had a relatively higher magnitude of zeta potential (i.e., -25.1 to -31.1 mV) than the non-chlorine PAA-treated biomass (i.e., -20.1 to -24.8 mV). This result indicates that the CNCs derived from the NC-treated biomass can achieve a stable suspension (i.e., zeta potential of -25.1 mV and -31.1 mV for poplar and hemp, respectively, with an absolute value above 25 mV), whereas the CNCs

	γ_{ab} , mJ/m ²			γ_t , mJ/m ²			γ_{ab}/γ_t	SSA, m ² /g
	min	med	max	min	med	max		
Com-CNC	1.5	6.9	21.2	45.6	65.2	129.2	0.10	2.5
NCH-CNC	4.8	7.0	7.8	27.3	54.3	54.9	0.16	12.5
NCP-CNC	3.0	5.3	14.8	47.2	53.5	81.1	0.12	4.6
NAH-CNC	4.8	5.5	8.8	50.3	54.5	72.8	0.11	9.9
NAP-CNC	2.9	4.5	11.5	47.3	53.4	79.6	0.10	4.6
ANH-CNC	3.3	6.2	18.8	47.6	61.2	119.9	0.11	4.8
ANP-CNC	2.6	4.5	12.8	45.4	51.0	75.1	0.11	5.3
γ_d = dispersive surface energy; γ_{ab} = acid-base specific surface energy; γ_t = total surface energy; γ_{ab}/γ_t = hydrophilicity index; SSA = specific surface area.								

III. Minimum, median (50% percentile of surface energy), and maximum surface energies and specific surface area of CNCs measured by inverse gas chromatography (iGC).

derived from NA- and AN-treated biomass can prevent agglomeration but may not maintain a stable suspension (i.e., zeta potential of -20.1 to -24.8 mV that is below -15 mV). Additionally, the zeta potential of CNCs from NA- and AN-treated hemp of -22.5 mV and -20.1 mV, respectively, were lower than their poplar counterparts of -24.8 mV and -22.9 mV, respectively. The variation of zeta potential values of isolated CNCs suggests that the delignification treatments and biomass sources have a mild influence on the surface charges of the obtained CNCs, which may affect their dispersion stability in aqueous media.

The surface energy of nanocellulose is a critical factor in determining the surface chemistry and the compatibility of the nanocellulose with different polymer matrices to form optimal fiber-matrix interactions for enhanced performance. Inverse gas chromatography (iGC) is a highly sensitive and versatile gas phase technique to study the surface and bulk properties of solids [48]. Dispersive energy (γ_d) refers to the hydrophobic properties of the nanocelluloses associated with van der Waals force, while specific energy (i.e., nondispersive or acid-base, γ_{ab}) refers to the hydrophilic properties of the nanocelluloses associated with electron-donating/accepting forces. Total surface energy (γ_t) is the summation of dispersive energy and specific surface energy. The surface energy profiles in Figs. 4b–4d demonstrated that all CNCs showed an energy-heterogeneous property. It is featured by high surface energy at lower surface coverage, gradually decreasing and leveling off as the surface coverage increases. The surface energy heterogeneity of the CNCs is likely due to surface defects or impurities in the CNC samples. We also observed that the dispersive energy (40–80 mJ/m²) had much higher values than the specific energy (2–10 mJ/m²), indicating that the CNCs tend to have higher van der Waals forces than acid/base forces in particular interactions, which is

consistent with the previous literature [49].

However, the degree of heterogeneity of CNCs varies with different CNC isolation methods. For example, the commercial CNCs (Com-CNC in Fig. 4) showed a larger surface energy range than the CNCs isolated from hemp and poplar in our study for both dispersive and specific surface energies. This suggests that the CNCs isolated from our study have less surface energy heterogeneity than the commercial CNC. Additionally, the range of dispersive surface energy of all three poplar samples (43–70 mJ/m²) was narrower than that of the three hemp samples (26–101 mJ/m²) (Table III), which is consistent with nanocelluloses measured in literature in general [34]. This means that the surface chemistry of poplar-derived CNCs is more consistent and stable among the three treatment methods than the hemp-derived CNCs. On the other hand, all CNCs isolated from hemp had higher specific surface energies than poplar samples (Fig. 4c). This indicates that the hemp CNCs have higher hydrophilicity (i.e., stronger electron donating/accepting forces) than the poplar CNCs. This is also partially reflected in the hydrophilicity index, which is defined as the ratio of specific energy to total surface energy, that is slightly higher for the hemp CNCs than the poplar CNCs. Taken together, these results indicate that both the biomass source and the treatment methods play an important role in affecting the surface energy of CNCs. The biomass treatment methods have a more considerable impact on hemp-derived CNCs than on poplar-derived CNCs.

We also used iGC to determine the surface area of the isolated CNCs (Table III). The isolated CNCs in our study had a surface area ranging from 4.6 to 12.5 m²/g, which was higher than that of the commercial CNC. It is important to note that although the CNCs are nano-scaled materials, the surface area is primarily derived from the external area rather than the porous structure. This explains the low sur-

face area of the CNCs as nanoparticles. Similar to surface energy, the surface area for hemp-derived CNCs had a wider range than the poplar-derived CNCs, indicating the relative stability of poplar over hemp against the employed treatment methods.

We then studied the water interactions with the isolated CNCs at three different moisture levels (i.e., freeze-dried, equilibrated with water for 24 h and 72 h in a desiccator) using LF-NMR. In LF-NMR spectra, the spin-spin relaxation time (T_2) of hydrogen nuclei determines how tightly water is bound to the substrates [50], which has been frequently used to study water within solid substrates such as wood, pulp, and paper [35]. In general, the shorter the T_2 , the tighter the binding or the smaller the compartmentalization of water. All CNCs at three different stages were measured and compared in terms of their T_2 relaxation times (Fig. 4e and Fig. 4f for hemp- and poplar-derived CNCs, respectively). The T_2 of the freeze-dried CNCs from hemp (Fig. 4e), poplar (Fig. 4f), and commercial CNC appeared at 0.2–0.3 ms, which is tentatively assigned to be the primary bound water trapped within the network of hydroxyl groups of cellulose [51]. Interestingly, the freeze-dried ANH-CNC had a much larger T_2 at ~0.7 ms, indicating weaker water-CNC interactions than the other CNCs. This might be related to the large range of surface energy of ANH-CNC (Table III), indicating a more heterogeneous surface chemistry accompanied by relatively lower cellulose crystal regions (Fig. 3b) — more freedom of water motions. It could also be related to the lower zeta potential of ANH-CNC, indicating fewer sulfate groups introduced to the CNC surface, leading to weaker water molecule interaction.

When the freeze-dried CNCs were equilibrated with water, they absorbed water molecules to form capillary water. The second peak at 3–5 ms indicated the formation of capillary condensed water when the CNCs were equilibrated with water for 24 h. These peaks became broader and with longer relaxation times when the CNCs were equilibrated with water for a longer time (72 h). With more water molecules absorbed, the interaction between water and CNCs became weaker, approaching bulk-like free water. The CNCs derived from different treatments had slightly different T_2 relaxation times, reflecting different intensities of water-CNC interaction. This is likely related to the different surface chemistry, surface energy, and zeta potential of the CNCs. However, no clear trend could be obtained among these treatment methods for hemp and poplar.

For poplar, the first peak of the CNCs from the NA and AN treatments had longer T_2 times than the NC-treated sample (Fig. 4f), which indicates that the CNCs from the NC treatment held primary water more strongly than the NA and AN treatments. This finding is consistent with the hydrophilicity data (γ_{ab}/γ_t in Table III), with NCP-CNC having the highest hydrophilicity among all poplar-derived

CNCs. This trend was also observed for the capillary condensation water (the second peak) and bulk-like free water (the third peak) of the poplar-derived CNCs. However, the peaks for hemp-derived CNCs did not follow the same pattern as poplar-derived CNCs, indicating that the biomass source likely played a role in CNC-water interactions.

CONCLUSIONS

We used a TCF method to isolate CNCs from two different biomass sources, hemp and poplar, representing herbaceous and hardwood species, respectively. Three different chemical treatments, including two TCF PAA delignification methods and one NaClO_2 reference method, were employed on hemp and poplar. Under the current dosages of PAA and NaClO_2 , the PAA delignification was much more effective than NaClO_2 for both hemp and poplar, although the CNCs yields were 8%–18% lower. The CNCs isolated from the TCF methods showed preferential opaque color, whereas the counterparts from NaClO_2 are brownish for both hemp and poplar. Among the three chemical treatments, the AN treatment was better in the delignification and dissolution of hemicellulose than the NA and NC treatments. Both the cellulose source and the biomass pretreatment order influenced the dimensions of CNCs, with non-chlorine PAA treatment generally resulting in larger width and aspect ratio of the isolated CNCs for hemp and poplar than NaClO_2 . Poplar-derived CNCs had slightly higher crystallinity than hemp-derived CNCs. The aqueous suspension of these CNCs is well-dispersed and relatively stable due to the magnitude of the introduced sulfate groups with negative charges. The surface energy, water interaction, and thermal stability of the CNCs were comparable despite the different source and pretreatment procedures of the biomass. Our study demonstrates that the TCF technique with PAA treatment is a promising delignification approach for obtaining cellulose-rich pulps from herbaceous and hardwood biomass for nanocellulose isolation. **TJ**

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ABOUT THE AUTHORS

Nanocellulose is a promising feedstock for valuable products and applications, and cellulose isolation from biomass using a low-environmental impact approach is always desired, yet remains a challenge. Although a totally chlorine free (TCF) technique in pulp and paper is not new, we have uniquely applied TCF peracetic acid (PAA) pulping to two emerging lignocellulosic biomass sources (herbaceous hemp and woody poplar) for nanocellulose isolation and then compared the results.

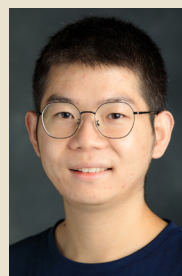
One key challenge in this study was that the biomass is overtreated by delignification and bleaching so that the air-drying process causes irreversible hornification of pulp. This can be addressed by increasing biomass size and mitigating pulping conditions.

We discovered that both hemp and poplar can be effectively and near-completely delignified by PAA. The high degree of lignin removal results in final production of pale white nanocellulose with potential high quality and without concerns about chlorine leaching. This TCF technique with PAA treatment proved a surprisingly effective and promising delignification and bleaching approach for obtaining cellulose-rich pulps from herbaceous and hardwood biomass for nanocellulose isolation.

For mills, our results can provide the fundamental knowledge and guidance for using PAA in hemp and poplar pulping and value-added nanocellulose isolation. As a next step, we would like to optimize the nanocellulose isolation conditions to achieve higher yield and to deploy the compounds left in the pulping liquor.



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