

Approaching zero cellulose loss in cellulose nanocrystal (CNC) production: recovery and characterization of cellulosic solid residues (CSR) and CNC

Q. Q. Wang • J. Y. Zhu • R. S. Reiner •
S. P. Verrill • U. Baxa • S. E. McNeil

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Abstract This study demonstrated the potential of simultaneously recovering cellulosic solid residues (CSR) and producing cellulose nanocrystals (CNCs) by strong sulfuric acid hydrolysis to minimize cellulose loss to near zero. A set of slightly milder acid hydrolysis conditions than that considered as "optimal" were used to significantly minimize the degradation of cellulose into soluble sugars that cannot be economically recovered, but resulted in CSR that is easily recoverable through conventional centrifuge. It was found that the window for simultaneous recoveries of CSR and producing high yield CNC in strong

acid hydrolysis was extremely narrow. However, we achieved significant CSR yield with near zero cellulose loss but without sacrificing CNC yield compared with that obtained at "optimal condition". The resultant CSR contains sulfate ester groups that facilitated subsequent mechanical nano-fibrillation to cellulose nanofibrils (CNFs), a potential high value nanocellulosic material for a variety of applications.

Keywords Nanocellulose materials/composites • Cellulose nanocrystals (CNCs) • Cellulose nanofibrils (CNFs) • Cellulose nanowhiskers (CNWs) • Acid hydrolysis

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Q. Q. Wang
State Key Lab of Pulp and Paper Engineering, South
China University of Technology, Guangzhou, China

Q. Q. Wang • J. Y. Zhu (✉) • R. S. Reiner • S. P. Verrill
USDA Forest Service, Forest Products Laboratory,
Madison, WI, USA
e-mail: jzhu@fs.fed.us

U. Baxa
Electron Microscopy Laboratory, SAIC-Frederick, Inc.,
NCI-Frederick, Frederick, MD 21702, USA

S. E. McNeil
Nanotechnology Characterization Laboratory, Advanced
Technology Program, SAIC-Frederick, Inc.,
NCI-Frederick, Frederick, MD 21702, USA

Introduction

Cellulose as a structural material is extremely strong with a theoretical modulus of around 250 GPa (Wegner and Jones 2009) and a specific tensile strength of about 5,200 kN-m/kg, or about 18 times that of titanium. However, most cellulose is naturally present in plant lignocellulosic biomass as a biocomposite made of cellulose, hemicelluloses, lignin, etc., with a hierarchical structure (Wegner and Jones 2009). Existing manufacturing technology has been limited to producing papermaking fibers as one of the largest utilization of lignocelluloses. Advanced separation techniques are required to effectively liberate cellulose from lignocellulosic biomass in the forms of

nanocrystals (CNCs) and nanofibrils (CNFs) made of elemental crystals or elemental fibrils, respectively. These CNCs or CNFs have very special mechanical and optical properties and have been identified as a powerful building block for producing high-quality, durable, light weight, and cost-effective products for a variety of applications (Eichhorn et al. 2010; Klemm et al. 2011).

There are several approaches to produce CNC and CNF from cellulose. The acid hydrolysis approach developed in the 1940s and 1950s (Battista 1950; Mukherjee and Woods 1953; Nickerson and Habrle 1947; Rånby 1951) remains the process for CNC production (Bondeson and Oksman 2006; Chen et al. 2009; Moran et al. 2008). Early studies used sulfuric acid at 2.5 N or 22 wt% for a long period of time at boiling conditions with low CNC yield of 30–40 % (Nickerson and Habrle 1947; Rånby 1951). A later study found cellulose can be hydrolyzed into colloidal nanowhiskers (or CNC) with good dispersing properties by using strong acid of approximately 65 wt% (Mukherjee and Woods 1953). However, it resulted in a very low CNC yield of approximately 30 %. Since then, most studies have used strong sulfuric acid approximately 64 wt% to produce stable CNC suspension (Araki et al. 1998; Marchessault et al. 1961; Revol et al. 1992) because of the sulfate groups formed impart electrostatic stability to the CNCs and facilitate aqueous processing. Reaction time can be reduced to a couple of hours or less at temperatures between 45 and 50 °C (Beck-Candanedo et al. 2006; Bondeson and Oksman 2006; Chen et al. 2009; Dong et al. 1998; Hamad and Hu 2010). Optimization of acid hydrolysis for CNC production using a commercial microcrystalline cellulose was also conducted (Bondeson and Oksman 2006). The study reported total cellulosic solids yield from 0 to 95 %; however, the resultant cellulosic solids from runs with solid yield higher than 47 % was agglomerates rather than colloidal suspension of CNCs. Separation of the agglomerates was not attempted to verify whether or not CNCs were produced. The maximal CNC yield was only approximately 30 % obtained at 63.5 wt% acid and 44 °C for 130 min using a cellulose concentration of 10 wt%.

The extent of sulfation and/or the degree of polymerization (DP) have been identified as indicators for the existence of CNC in acid hydrolysate (Hamad 2011). However, few studies have investigated the

relationship between sulfation and DP with CNC yield. When a softwood Kraft pulp was hydrolyzed using sulfuric acid of 16, 40, and 64 wt% at 45, 65, and 85 °C for periods between 5 and 25 min (Hamad and Hu 2010), the total cellulosic solid yield was found to be a constant of approximately 90 % obtained at the two low acid concentrations when the DP of the solids was above 120. The resultant cellulosic solid substrates were simply identified as partially hydrolyzed pulp without sulfate groups, suggesting no colloidal suspension of CNCs was produced. Attempts to separate the hydrolyzed pulp to verify the existence of CNC were not reported. Solid yield reduced abruptly when the DP was reduced to below 120, suggesting cellulose was significantly depolymerized into CNC. CNC with sulfate groups was produced only at an acid concentration of 64 % (w/w) but with yields below 40 %.

The above discussions indicate that maximal achievable CNC yield reported in the literature is between 30 and 50 %; that is lower than the reported amount of crystalline cellulose of 65 % in bleached wood (Stamm 1964). DP reduction, cellulosic solids sulfation, and the production of CNCs occurred abruptly and simultaneously when acid hydrolysis parameters were varied. This abrupt onset occurs when cellulose DP reaches 120. Past researchers have failed to identify this abrupt transition and the potential of producing good yields of CNC without hydrolyzing all of the remaining cellulose to soluble sugars. As a result, simultaneous recoveries of CNC and any remaining solid residues were not attempted in the literature. The objective of this study is to separately recover and characterize CNC and non-CNC cellulosic solid residues (CSR) under a range of acid hydrolysis conditions. The goal of this study is to demonstrate simultaneous production of CNC and easily recoverable CSR to minimize cellulose loss to near zero to improve the economics of CNC production.

Materials and methods

Materials

Sulfuric acid ACS reagent grade was used as received from Sigma-Aldrich (St. Louis, MO). A bleached Kraft eucalyptus dry lap pulp (Aracruz Cellulose,

Table 1 Chemical composition of the bleached Eucalyptus dry lap pulp

Component	Content (%)
Klason Lignin	0.1 ± 0.1
Glucan	78.1 ± 1.0
Xylan	15.5 ± 0.6

Other components are undetectable

Brazil) was used. The chemical composition of the eucalyptus pulp is listed in Table 1. The dry lap was soaked in water overnight and disintegrated for 5,000 revolutions at 312 rpm and 5 % consistency at room temperature (Model 73-06-01, TMI, Ronkonkoma, New York, USA). The pulp is then vacuum dewatered and air dried to approximately 8 % moisture content. The air dried pulp was used in acid hydrolysis.

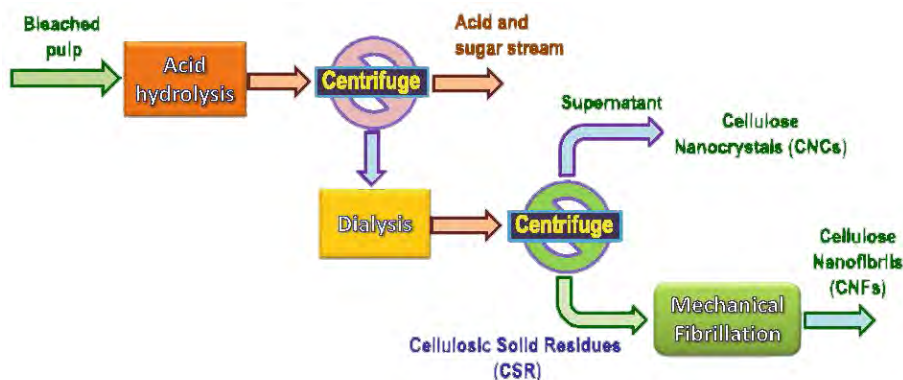
Acid hydrolysis

The entire experimental process flow is schematically shown in Fig. 1. The production of CNCs was performed according to the procedure described by Beck-Candanedo et al. (2006). Two sets of acid hydrolysis experiments were carried out using strong sulfuric acid solutions with a ratio of solution to pulp in oven dry (od) weight of 8:1 (v/w). The Box-Wilson central composite design of response surface methodology was used to design each set of 17 experiments in two sets of ranges of acid concentration, temperature, and hydrolysis duration. The moisture of the pulp was not included in determining the actual initial acid concentration in the reaction solution. The difference is very small (0.4 wt% at 62 wt% acid concentration) due to the low moisture content of 8 %. The first set of

experiments has larger acid concentration and temperature ranges than those of the second set of experiments to coarsely determine the desired acid hydrolysis parameters. Each set of experiments has 3 replicate runs at the center point. Strong sulfuric acid solutions were prepared by using sulfuric acid at 98.6 wt%. A set of the 17 acid hydrolysis runs were conducted individually in a flask with a capacity of 25 mL. Hydrolysis temperature was controlled using temperature controlled water baths. The pulp suspension was constantly mixed using a mechanical mixer at 200 rpm during hydrolysis. The reaction was quenched at the end of the hydrolysis by adding 10-fold of di-ionized (DI) water, and then vigorously stirred for 10 min. The suspension was washed and repeatedly centrifuged at 9,000 rpm or approximately 13,200 *g* (Sorvall Super-speed RC2-B, 5.75 inches rotator, Ivan Sorvall, Inc., Norwalk, CT) to remove excessive sulfuric acid until turbid. The resultant suspension was dialyzed in a regenerated cellulose tube (Fisher Science lot no: 11116-3) with a cutoff molecular weight of 12,000 Da for a week or until the pH of the dialysis water no longer changed. Then the suspension underwent an ultrasonic treatment in an ultrasonic bath (model FS21H, Fisher Scientific) with ice for 15 min to disperse the cellulose nanocrystals. The suspension was finally centrifuged for 10 min at 9,000 rpm, or approximately 13,200 *g* at maximal solids consistency below 0.3 % to separate CSR from CNCs.

Recovery and yield determination of CNC and CSR

The volumes and weights of the separated CNC and CSR were recorded. The consistencies of the final

**Fig. 1** Schematic process flow diagram of experiments

cellulose suspension before and after CNC and CSR separation were determined by a chemical oxygen demand (COD) method (Franson 1985) assuming detected organic materials were $(C_6H_{10}O_5)_n$. An aliquot of each fraction was digested in commercial COD test vials (Biosciences, Inc., Bethlehem, PA, ISO 9001:2000 certified). COD was measured from the amount of chromium consumed determined colorimetrically based on the spectral intensity at 600 nm using an UV—Vis spectrophotometer (Spectronic Genesys 5, Milton Roy Company, Warminster, PA), i.e., $COD\text{ (mg/L)} = 3,410.2 \times I^{600}$. The mass of cellulose can be calculated: $_{mc6H10O5}\text{ (mg/L)} = COD/1.185 = 2,877.6 \times I^{600}$ through calibration using avicel. The manufacturer specified COD range of the test kit was 20–900 mg/L. This corresponds to a lowest detection limit of CNC concentration of 16.9 mg/L. The CNC samples were diluted to within this linear calibration range before measurements. In separate laboratory verification studies, the COD measured CNC solids averaged over multiple runs were compared with that from gravimetric method through oven drying. The difference was 3.5 % which is within the error margin of the gravimetric method.

The separated CSRs were examined using SEM which revealed CSRs were in the form of cellulose fibers (CFs) or cellulose microfibrils (CMFs) rather than CNC. Obtaining a representative aliquot sample is difficult from high consistency CSR samples that contain large CFs or CMFs. The yields of the CSR samples with high consistencies were therefore also measured using gravimetric method by oven drying each whole sample at 105 °C overnight. The final oven dry weight of the sample is used to calculate the CSR yield. The COD yield of these CSR samples were reported as reference only and were not used for data analysis.

Mechanical fibrillation of CSR

The precipitated CSR from selected runs was further fibrillated to evaluate its potential for nanocellulose production. Deionized water was used to make a CSR suspension of approximately 2 % (wt). The 2 % suspension was passed through a Microfluidizer (Microfluidics, Newton, MA) 10 times using a 200 micron chamber and 5 additional times using an 87 micron chamber.

Sulfur content analysis

The sulfur contents of the CNC and CSR from each run were separately analyzed using ICP-MS. The CSR suspensions were shaken well before sampling. Aliquots of samples were digested at 145 °C for 15 min in a microwave (MDS-2000, CEM Corp., Matthews, North Carolina, USA) with approximately 5 mL of HNO_3 and 3 mL of 30 % H_2O_2 before ICP-OES analysis.

TEM imaging and CNC length distribution

Aqueous CNC samples at solids consistency of approximately 0.1 % were used for TEM imaging. Each aqueous sample was adsorbed to freshly glow-discharged carbon-coated grids for 30 s, then rinsed with water and negatively stained with 0.5 % uranyl acetate. Images were recorded on a Hitachi H7650 microscope at 80 kV with a 2k x 2k AMT CCD camera. Manual measurements with ImageJ (<http://rsbweb.nih.gov/ij/>) were used to determine the length distributions of CNC samples. Approximately 500 CNCs for each sample were measured.

Specimens for scanning electron microscopy (SEM) were prepared by drying drops of the aqueous slurry on polished aluminum mounts. All SEM samples were sputter-coated with gold to provide adequate conductivity. Samples were imaged and photographed using a Leo EVO 40 SEM.

Results

Verification of simultaneous production of CNC and CSR

The COD yield measurements can reveal the existence of cellulosic solids in the milky "CNC" (quotation marks represent unconfirmed existence of CNC) suspension after separation of CSR through centrifugation. The production of CSR can be easily seen from the precipitated cellulosic fibers in the post dialysis sample and the solid yield from oven dry measurements. The yields of the "CNC" and CSR separately measured by COD and gravimetric method through oven drying are listed in Table 2. The results indicate that a substantial amount (yield >10 %) of "CNC" was produced only under a few set of experimental

Table 2 Yields and sulfur contents of CNC and cellulose solid residues (CSR) from the wide range acid hydrolysis experiments for first experimental design listed in Table 2

Run order	Sample label ^a	CNC yield by COD (%)	CSR yield (%) by drying	Total yield (%)	CNC sulfur content (mg/g)
1	A45-T50-t75	0.46	83.4	83.9	5.69
2	A55-T65-t75	23.59	60.1	83.7	
3	A55-T50-t75	2.21	84.7	87.0	
4	A55-T50-t75	1.75	84.0	85.7	
5	A50-T55-t102	1.14	92.5	93.6	
6	A55-T50-t75	0.93	86.6	87.6	8.54
7	A55-T35-t75	0.23	91.8	92.0	
8	A50-T55-t48	0.36	92.6	92.9	
9	A50-T45-t48	0.44	90.1	90.6	
10	A55-T50-t30	0.47	88.4	88.9	
11	A60-T55-t102	39.54	31.7	71.2	7.91
12	A60-T45-t102	21.01	58.7	79.7	7.09
13	A50-T45-t102	0.63	88.8	89.4	
14	A60-T55-t48	30.67	48.3	79.0	
15	A65-T50-t75	27.31	5.1	32.5	
16	A55-T50-t120	6.87	69.5	76.4	
17	A60-T45-t48	4.12	77.1	81.2	0.97
Mean	A55-T50-t75	1.75	84.0	85.7	
STD	A55-T50-t75	0.65	1.35	0.97	

^a Axx stands for sulfuric acid concentration in wt%; Txx stands for temperature in °C; txx stands for hydrolysis duration in min

conditions (experiments No 2, 11, 12, 14, 15). For the temperatures and hydrolysis durations examined, acid concentrations below 55 wt% did not produce significant amounts of "CNC", suggesting an abrupt change in CNC production, in agreement with those reported in the literature (Bondeson and Oksman 2006; Hamad and Hu 2010). The maximal "CNC" yield of 40 % (COD estimate) was achieved in experiment A60-T55-t102 (No. 11) under a sulfuric acid concentration of 60 wt% and temperature of 55 °C for a hydrolysis period of 102 min (Table 2). We will use the format of (60, 55, 102) to represent experimental conditions of (acid in wt%, T in °C, hydrolysis duration in min) throughout the text. Furthermore, a substantial amount of CSR was produced and easily recovered with yields of 32 %. The "CNC" yield at (65, 50, 75) was 27 %, similar to those reported in the literature using an acid concentration of 64 wt% (Bondeson and Oksman 2006; Hamad and Hu 2010). However, there was no substantial amount of remaining cellulosic solids left. This indicates that remaining cellulose was completely hydrolyzed by acid to soluble sugars or other soluble byproducts that is very difficult to recover.

AFM imaging confirmed the existence of CNC in the form of nano whiskers. The CNC sample from (60,

55, 102) with a yield of 40 % are individually separated whiskers (not shown). The SEM imaging of the CSR obtained from five runs (experiments No 2, 11, 12, 14, 15) with significant CNC yield are in the form of cellulose fibers (CF). The CNC obtained in these five sets of acid hydrolysis conditions all contained significant sulfate ester groups based on sulfur measurements (Table 2). The sulfur content in these five CNC samples were comparable with those in the literature (Hamad and Hu 2010). The high acid concentration run (65, 50, 75) resulted in a high sulfur content but with almost zero CSR yield (Table 2).

To further verify simultaneous production of CNC and CSR, mg/g yields of CNC, CSR, and total of CNC and CSR were plotted against CNC sulfur content (Fig. 2). The results clearly indicate that CNC sulfur content, a measure of the degree of sulfation and the degree of acid hydrolysis severity, is inversely proportional to CSR yield. The CNC yield except for the third data point, however, first increased with sulfation and then decreased. This is because at very low acid hydrolysis severities, the cellulose has not been hydrolyzed to CNCs. Therefore, increased hydrolysis severity can increase CNC yield. However, further increase in hydrolysis severity beyond the

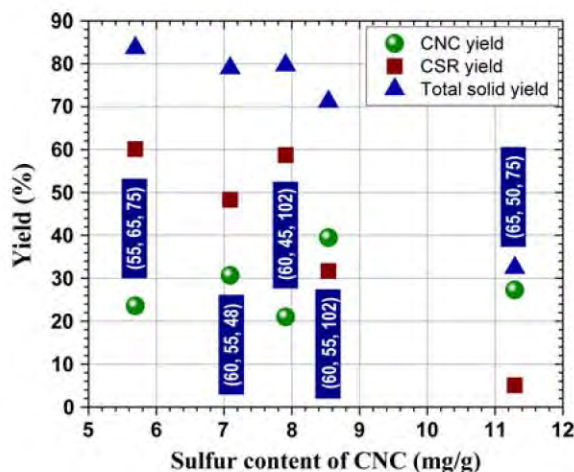


Fig. 2 Correlations between sulfur content in CNC with CNC, CSR, and total solid yield from the first set of acid hydrolysis experiments from Table 2

maximal CNC yield condition decreased CNC yield due to cellulose degradation to sugar. The total solid yield is also inversely proportional to CNC sulfur content. The results in Fig. 2 clearly indicate that there is a range of acid hydrolysis severities within which we can achieve simultaneous productions of CNC with yields of approximately 30 % or higher and good sulfation with sulfur content between 6 and 9 mg/g and CSR with yields of approximately 40 % or higher. The loss of solids (mainly hemicelluloses and cellulose) to soluble products was less than 25 %. Literature work has failed to identify the co-existence of both CNC and CSR (Bondeson and Oksman 2006; Hamad and Hu 2010).

Simultaneous recoveries of CNC and CSR: small ranges of acid concentration and temperature

The abrupt change in CNC production reported above prevented us from conducting linear regression analysis to accurately predict the optimal conditions for CNC and CSR production using the data presented in the previous section. A separate set of 17 experiments with narrowed ranges of acid concentration and temperature were conducted to fine tune the operating conditions for simultaneous CNC and CSR production. Again the yields of CNC were measured by COD, in at least duplicates. The yields of CSR were measured by both COD and gravimetric methods. Due to difficulties in obtaining representative sampling using aliquots of pulp suspension, the COD

results were listed as reference. The gravimetric CSR yields were used in data plotting and analysis. However, gravimetric measurements were not conducted on those CSR samples with very low yields, in those cases, the COD yields were used instead. The results indicate that 12 out of the 17 experiments, including the 3 replicate runs at the center point (62, 50, 70) produced significant amounts of CNCs with yields >10 % (Table 3). Furthermore, 7 out of the 17 experiments resulted in significant amounts of CSR. Under 5 different experimental conditions, both the yields of CNCs and CSR were significant (>10 %). While the center point has high CNC yield with a mean value of 55.8 %, the CSR yield is negligible at approximately 1 %. With cellulose content of the eucalyptus pulp of 78.1 % (Table 1), this indicates that the remaining cellulose of approximately 22 % of the pulp was degraded into soluble compounds such as sugars during hydrolysis.

The CNC yield was 55.7 % at (58, 56, 100), approximately equal to the yield obtained at the center point (62, 50, 70) of 55.8 %. However, the run at (58, 56, 100) resulted in a CSR yield of 17.4 %. The total yield for this run was 73.1 %, indicating only approximately 5 % of the cellulose was degraded to soluble sugars. Comparing to the center point run (62, 50, 70), a net of 17 % cellulosic solid can be recovered without reduction in CNC yield. When hydrolysis duration was reduced to 40 min at (58, 56, 40), the CNC yield was reduced to 27.5 %. This is approximately a reduction of 50 % compared with the CNC yield for the run (58, 56, 100) and the center point run (62, 50, 70). The total yield of CNC and C

reater than the cellulose content of 78.1 %, suggesting that the CSR may contain some hemicelluloses and that cellulose loss was minimal. When hydrolysis temperature was reduced to 44 °C at (58, 44, 100), CNC yield was reduced to 16.2 % and CSR yield increased to 61.3 % with a total cellulosic solid yield of 77.5 %. These experiments again demonstrated the co-existence of CSR with CNCs at near zero cellulose loss. Furthermore, CNC yield can be very close to optimal while producing CSR.

TEM images reveal the morphologies of the CNCs produced under various acid hydrolysis conditions. The CNC samples obtained at (62, 50, 70), (58, 56, 100), and (58, 56, 40) are in the form of dispersed colloidal nanowhiskers with length on the order of 200 nm and widths of 10 nm as shown in Figs. 3a, b, c.

Table 3 Yields2012 sulfur contents of CNC and cellulose solid residues (CSR) from the narrow range acid hydrolysis experiments for second experimental design listed in Table 3

Run order	Sample label ^a	CNC yield-I by COD" (%)	CNC yield-II by COD" (%)	CNC COD yield repeat (%)	Mean CNC yield (%)	STD CNC yield (%)	CSR yield by COD (%)	CSR COD yield repeat (%)	CSR yield by drying (%)	CSR yield (%)	Total yields (%)	CNC sulfur (mg/g)	CSR sulfur (mg/g)
1	A55-T50-t70	8.12	7.26		7.7	0.61	48.17	77.16	41.68	41.68	49.4	8.81	3.89
2	A62-T60-t70	51.80	50.03		50.9	1.25	1.97			1.97	52.9	10.47	8.65
3	A62-T50-t70	55.20	53.92		54.6	0.91	1.28			1.28	55.8	8.73	8.31
4	A62-T50-t70	54.06	52.34		53.2	1.22	0.68			0.68		8.77	153.54
5	A58-T56-t100	54.73	57.01	55.30	55.7	1.19	25.93		17.41	17.41	73.1	6.83	5.24
6	A62-T50-t70	58.87	60.48		59.7	1.14	0.96			0.96	60.6	7.92	6.26
7	A62-T40-t70	32.35	29.76		31.1	1.83	37.05	34.74	25.55	25.55	56.6	11.14	4.55
8	A58-T56-t40	27.45	27.45		27.5	0.00	66.53	64.60	56.69	56.69	84.1	6.29	2.96
9	A58-T44-t40	4.67	3.74		4.2	0.66	58.40	70.41	72.88	72.88	77.1	13.39	3.04
10	A62-T50-t20	36.13	36.62	34.57	35.8	1.07	26.78	26.21	18.61	18.61	54.4	9.36	5.25
11	A66-T56-t100	6.61	5.73		6.2	0.62	2.65			2.65	8.8	19.94	6.91
12	A66-T44-t100	2.81	7.51	2.76	4.4	2.73	0.18			0.18	4.5	28.09	11.67
13	A58-T44-t100	16.15	16.15		16.2	0.00	20.36	72.00	61.33	61.33	77.5	5.58	14.65
14	A66-T56-t40	7.97	11.95		10.0	2.81	1.39			1.39	11.4	12.67	3.01
15	A69-T50-t70	1.77	1.55	1.39	1.6	0.19	3.56			3.56	5.1	50.58	5.87
16	A62-T50-t120	51.95	50.24		51.1	1.21	1.73			1.73	52.8	9.77	9.09
17	A66-T44-t40	11.43	11.93	10.94	11.4	0.50	0.49			0.49	11.9	46.66	9.74
Mean	A62-T50-t70	56.04	55.58		55.8	3.17	0.97			0.97	56.8	8.47	7.29
STD	A62-T50-t70	2.51	4.32		3.17		0.30			0.30		0.48	1.44

^a Axx stands for sulfuric acid concentration in wt%; Txx stands for temperature in °C; txx stands for hydrolysis duration in min

^b Replicate COD analysis of the same aliquot sample

^c Repeat measurements of two aliquot samples of the same suspension

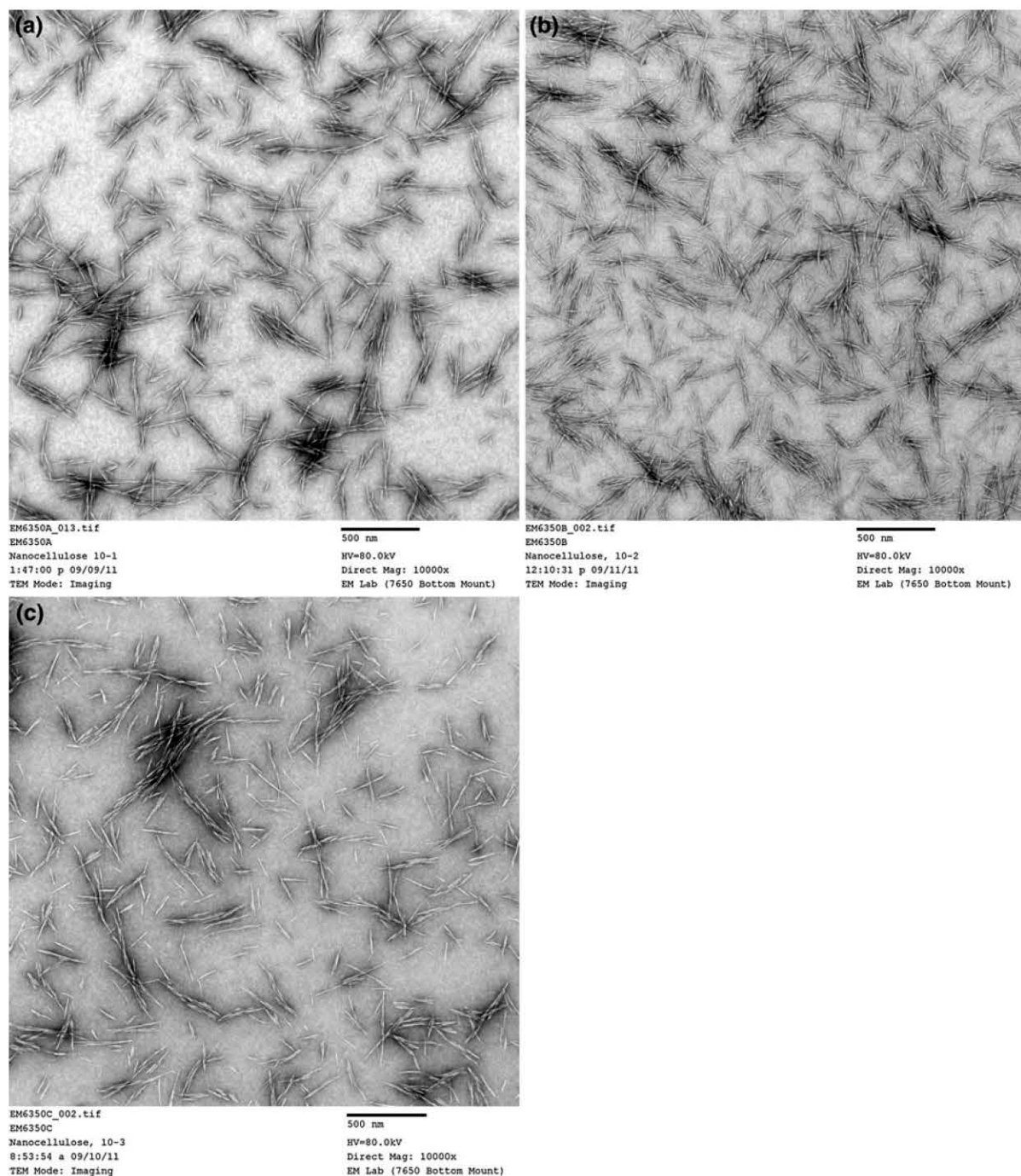


Fig. 3 TEM images of three CNC samples from a (62, 50, 70); b (58, 56, 100); c (58, 56, 40). Scale bar = 500 nm

Visual observations indicate no significant morphological differences among these three CNC samples obtained at different hydrolysis conditions with different CNC and CSR yields (Table 3). Image analyses obtained crystal length and width distributions

confirmed the visual observation. Both the crystal length and width distributions between the samples produced at (62, 50, 70) and (58, 56, 100) were almost identical (Figs 4a, b). Both the length and width distributions of the sample obtained at (58, 56, 40)

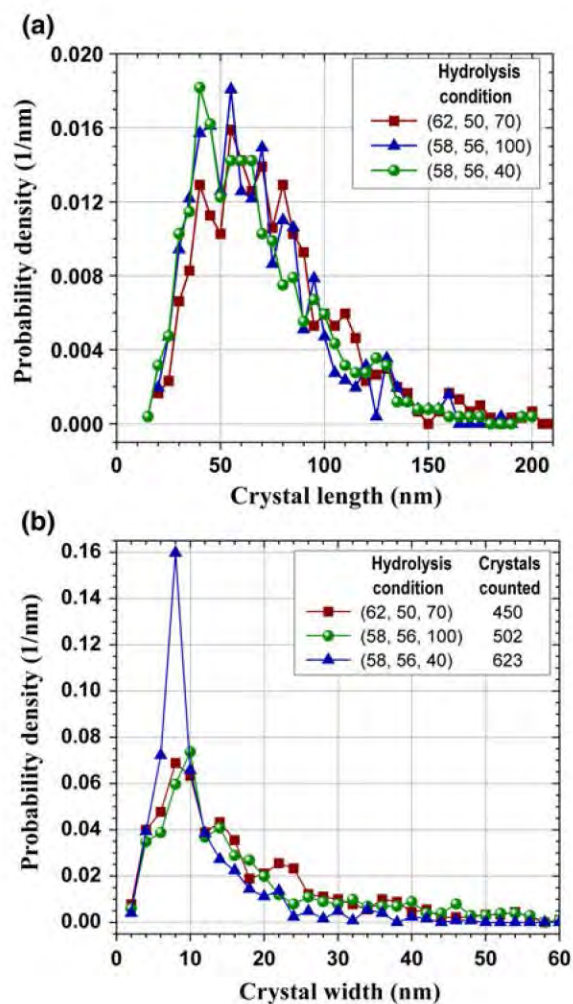


Fig. 4 Comparisons of crystal size distribution probability densities of CNC samples produced from different hydrolysis conditions. **a** CNC length **b** CNC width

slightly deviate (shorter and narrower) respectively from the distributions of the samples from (62, 50, 70) and (58, 56, 100). The calculated mean lengths and widths along with their respective standard deviations (Table 4) show no difference between sample (62, 50, 70) and (58, 56, 100) and slightly shorter and narrower crystals from (58, 56, 40). This indicates that experiment (58, 56, 100) produced almost identical CNC morphology and excellent yield to those obtained at the center point (62, 50, 70). However, the low acid condition (58, 56, 100) also produced CSR with a yield of 17 % (Table 3, drying method). The SEM images of the CSR samples from (58, 56, 100) and (58, 56, 40) indicated that the CSR samples are in the form of CF (Fig. 5a, b).

Table 4 Mean lengths and widths along with their respective standard deviations of the CNC samples produced at (62, 50, 70), (58, 56, 100), and (58, 56, 40)

	(62, 50, 70); Fig. 3a	(58, 56, 100); Fig. 3b	(58, 56, 40); Fig. 3c
Mean length (nm)	204	201	174
Length standard deviation (nm)	129	128	125
Number of crystals counted	496	497	510
Mean width (nm)	17.3	17.9	10.7
Width standard deviation (nm)	16.1	17.5	8.5
Number of crystals counted	450	502	623

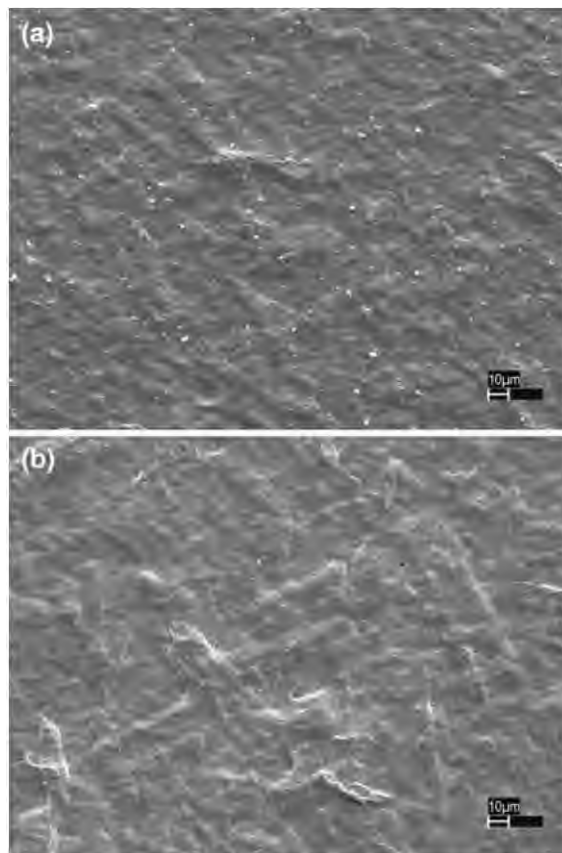


Fig. 5 SEM images of the cellulosic solid residual ($C_{\mu m}$) from **a** (58, 56, 100); **b** (58, 56, 40). Scale bar = 100 μm

Sulfation of CNC and CSR

The CNC yields in relation to sulfation was further evaluated by plotting the CNC yields from the second

set of experiments against the measured sulfur contents as shown in Fig. 6a. The results indicate, in general, decreased CNC yield and increased sulfation represented by the sulfur content of the CNC when acid hydrolysis severity was increased through a combination of increasing acid concentration, temperature, and hydrolysis duration. The half-filled symbols shown are runs with an acid concentration greater than 64 wt% (the center point value was 62 wt%). It is noted sulfuric acid becomes a solvent for cellulose at concentration above 65 wt%. Therefore, different symbols were used to represent process discontinuity. Results from the first set of experiments (Fig. 2) indicate CNC yield first increases with hydrolysis severity as represented by sulfur content (sulfation) to achieve a maximal value then decreases due to cellulose degradation to soluble products. To illustrate this, we zoomed in on the results in Fig. 6a with sulfur content between 4 and 14 mg/g, a similar range to that in Fig. 2. The zoom view indicates that CNC yield first increased with sulfur content to achieve a maximal sulfur content between 7 and 9 mg/g, then decreased (Fig. 6b). It is also noticed that there are two data points that do not conform to this general trend. These two runs happen to be the run with lowest temperature and shortest hydrolysis duration, respectively. This suggests that sulfur content may not be a good measure of hydrolysis severity for extreme conditions.

Similar yield and sulfation analysis can be carried out for CSR. The results indicate that CSR also contains sulfur (Table 3). The sulfur content of CSR from run 4 at (62, 50, 70) is apparently an outlier as the other two replicate experiments showed much lower sulfur contents of less than 10 mg/g. The sulfur content of CSR from run 13 at (58, 44, 100) may also contain large measurement errors as it is higher than that of the corresponding CNC sample. The difficulties in obtaining representative samples by aliquot sampling of pulp suspension may cause the amount of cellulose sampled to deviate from that prescribed, which produced errors in sulfur content of CSR. These two data points were removed in Fig. 7 which shows the correlation between yield and sulfur content of CSR. The yield can be fitted to an exponential decay curve with sulfur content. The half-filled symbols are runs with acid concentrations greater than 64 wt% when sulfuric acid becomes a solvent of cellulose. Better fit can be obtained when these data points are excluded. It is clear that CSR with good sulfation, i.e., sulfur content of

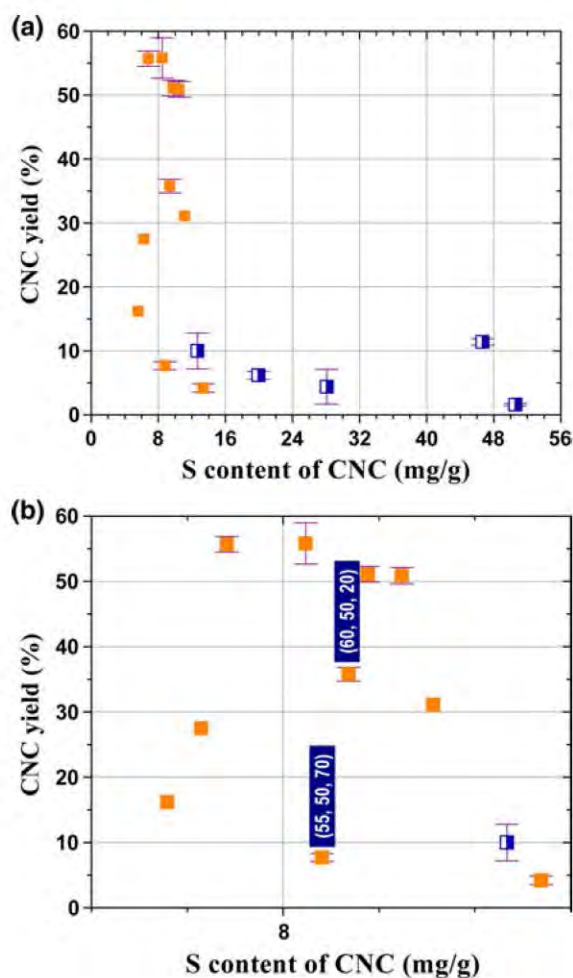


Fig. 6 Correlation between CNC yield and the sulfur content of the resultant CNC; **a** all data from Table 3; **b** zoom view of (a) with S content between 4 and 14 mg/g

4–5 mg/g can be produced with yields between 25 and 50 %. When the outlier data points are taken out, the sulfur content of CSR has a trend to be proportional to the sulfur content of CNC (Table 3).

CNC and CSR yields with hydrolysis severity

Kinetics studies can produce a combined hydrolysis or severity factor relevant to scale-up experiments (Chum et al. 1990; Zhu et al. 2012). This is especially true for CNC production due to a very narrow operating window for simultaneous production of CNC and CSR with good yields. No literature data are available on kinetics of strong acid hydrolysis of cellulose, which is different from acid hydrolysis of

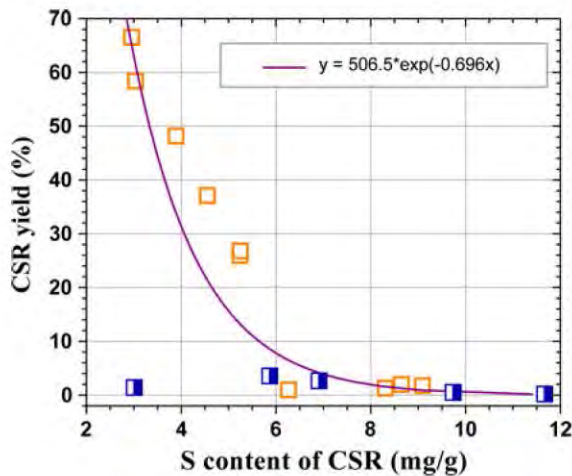


Fig. 7 Correlation between CSR yield and sulfur content of the resultant CSR

xylan. Therefore, previously developed combined hydrolysis or severity factors for xylan acid hydrolysis (Chum et al. 1990; Zhu et al. 2012) cannot be applied to this study. Developing such a factor for CNC production based on kinetics is not in the scope of the present study. Sulfur content failed to represent acid hydrolysis severity at certain conditions as discussed above. Therefore, we used an empirical combined hydrolysis severity (*ECHS*) defined below to correlate CNC and CSR yield,

$$ECHS = \log \left[\frac{t}{t_{cp}} \cdot T^6 \right] \cdot \left(\frac{A}{A_{cp}} \right)^\beta \quad (1)$$

where subscript *cp* stands for the center point value. *t* is acid hydrolysis time, $t_{cp} = 75$ min. *A* is acid concentration, $A_{cp} = 62$ wt%, and $\beta = 1$ for acid concentration $A \leq A_{cp}$; $\beta = 2$ when $A > A_{cp}$. The results clearly show that CNC yield is between 30 to 55 % at *ECHS* between 9.5 and 10.7, a very narrow window, as shown in Fig. 8a. Similarly, we plotted CSR yield against *ECHS* as shown Fig. 8b. Significant amounts of CSR can be obtained for *ECHS* between 9 and 10. By simultaneously examining Fig. 8a, b, it can be concluded that the *ECHS* range that can simultaneously produce significant amounts of CNC and negatively charged CSR is even narrower, between 9.5 and 10. This explains why literature studies have failed to identify the possibility of producing a high yield of CNC while recovering a significant amount of cellulose in the form of negatively charged solid materials, i.e., CSR

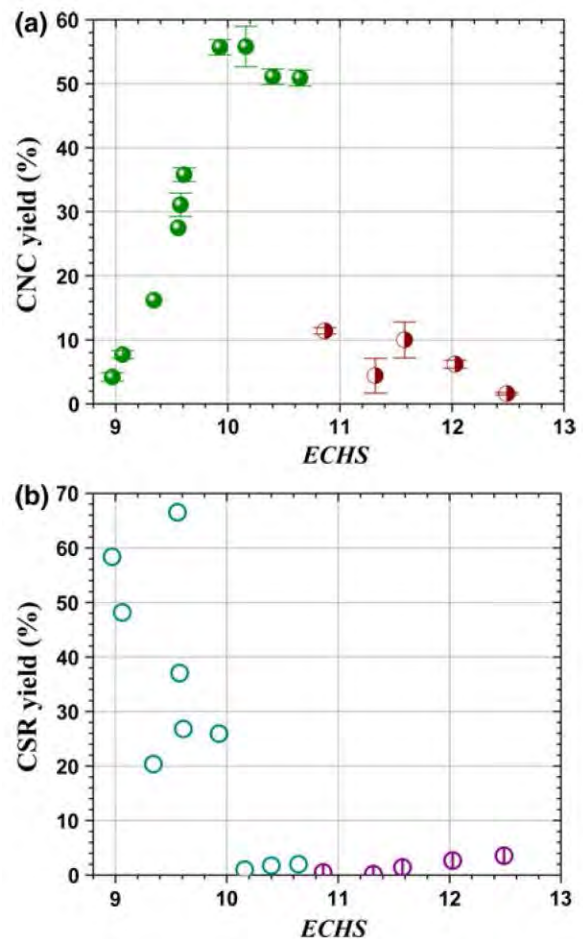


Fig. 8 Correlations of **a** CNC yield and **b** CSR yield with an empirical combined severity factor for acid hydrolysis A_{cp} , =

$\log \left[\frac{t}{t_{cp}} \cdot T^6 \right] \cdot \left(\frac{A}{A_{cp}} \right)^\beta$ "cp" stands for center point value. $t_{cp} = 75$ min and $k_{gs} = 62$ wt%. $\beta = 1$ for acid concentration $A \leq A_{cp}$; $\beta = 2$ when $A > A_{cp}$

Predictions for simultaneous productions of CNC and CSR

The following expressions for CNC and CSR yield predictions were obtained based on nonlinear regression of the CSR and CNC yield data in Table 3.

$$\begin{aligned} \text{CNC (\%)} = & 55.83 - 8.950A + 7.097T + 4.050t \\ & - 29.085A^2 - 5.340T^2 - 4.458t^2 \\ & - 7.800A \times T - 6.375A \times t + 2.425T \times t \end{aligned} \quad (2)$$

$$\begin{aligned} \text{CSR}(\%) = & 0.97 - 25.450A - 7.085T - 5.755t \\ & + 17.740A^2 + 4.603T^2 + 3.311t^2 \\ & + 7.935A \times T + 6.473A \times t - 3.270T \\ & \times t \end{aligned} \quad (3)$$

Where: $A = (\text{Acid}-62)/4$, $T = (\text{Temperature}-50)/6$, $t = (\text{Time}-70)/30$.

The adjusted R^2 were 0.974 and 0.965 for CNC (Eq. 2) and CSR (Eq. 3), respectively. The root mean squared errors were 3.46 and 4.73 for CNC and CSR respectively.

We determined the regions for simultaneous productions of CNC and CSR using the predictive Eqs. (2) and (3). Contour plots of CNC yield of 56–60 % with CSR yield ≥ 10 % is shown in Fig 9a. A hydrolysis time range of 25–115 min was used to determining the contours. Specific hydrolysis time or time intervals were not provided. The results indicate that as a desired CNC yield increases, the ranges of hydrolysis temperature and acid concentration to obtain ≥ 10 % CSR narrows. At CNC yield of 60 %, the region to obtain ≥ 10 % CSR practically narrows down to one point because temperature control to within ± 1 °C is very difficult. If a CNC yield of 56 % is desired, the predicted maximal CSR yield is at approximately 16.9 % (Fig. 9b), again only achievable practically at one point at sulfuric acid concentration of approximately 58.7 wt% and 56 °C. This qualitatively agrees with our experimental results of CNC yield of 55.7 % and CSR yield of 17.4 % achieved at acid concentration of 58 wt% and 56 °C for 100 min.

We also determined the regions for maximal CNC yield without considering CSR yield, i.e., using Eq. (2) alone, as most optimization studies would do. The predicted maximal CNC yield is approximately 63.5 % at acid concentration of 60.2 % and 57.5 °C (Fig. 9c). A yield of 63 % is more realistic due to difficulties in controlling temperature to within ± 1 °C. For a given desired CNC yield, the temperature range to achieve ≥ 10 % CSR yield (Fig. 9a) was similar to that without considered CSR yield or CSR yield ≥ 0 % (Fig. 9c) except at very high CNC yield of ≥ 60 %, however, the acid range is narrower and shifted toward a low acid concentration range. This suggests (1) the production of CSR is more sensitive to acid concentration; (2) the CSR yield is most likely low or is close to zero when an acid concentration is out of the region outlined in Fig. 9a but still within that outlined in Fig. 9c for a given desired

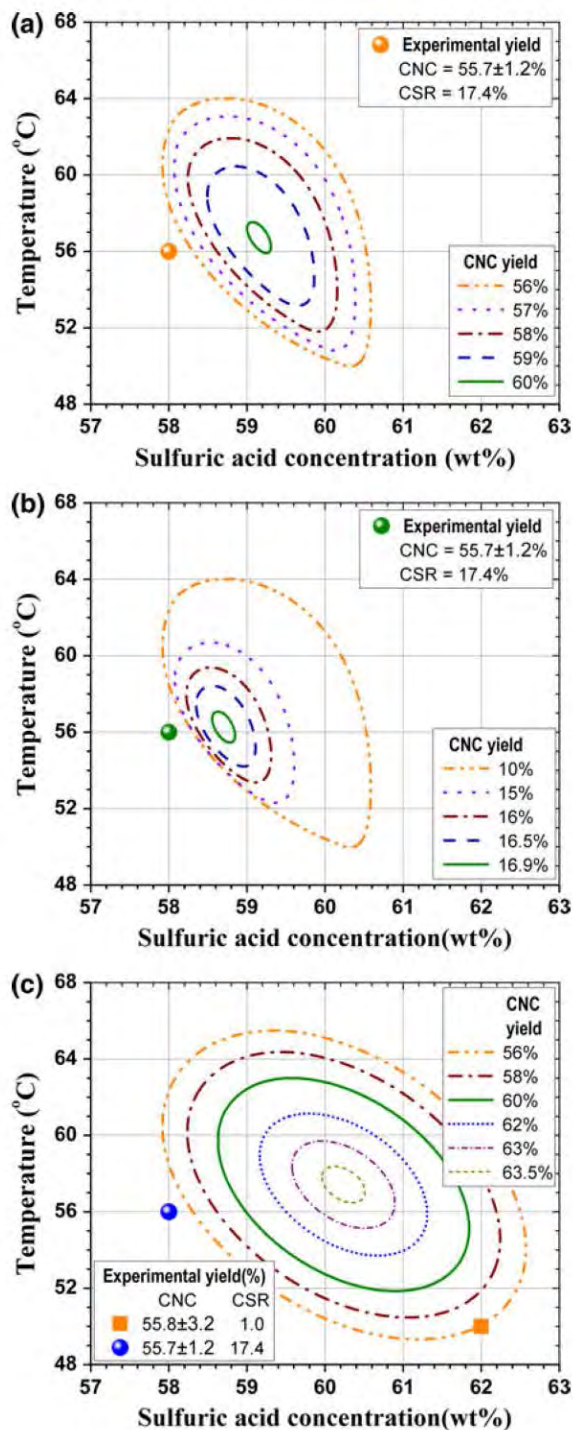


Fig. 9 Quadratic fits predicted ranges of sulfuric acid and temperature with hydrolysis time between 25 and 115 min for simultaneous recovery of CSR and CNC. **a** CNC yield varies between 56 and 60 % while CSR yield ≥ 10 %; **b** CNC yield ≥ 56 % while CSR yield varies between 10 and 16.9 %; **c** CNC yield varies between 56 and 63.5 % while CSR yield ≥ 10 %

CNC yield and temperature. One can overlay Figs. 9a, c to see this difference; (3) literature studies used experimental design out of the region shown in Fig. 9a but within that shown in Fig. 9c and therefore failed to achieve simultaneous production of CNC and CSR.

It should be noted that none of the experimental data point falls into the plotted contours shown in Figs. 9a, b, c. Only two data points (58, 56, 100) and (62, 50, 70) were very close to the most outer contours shown. We were not able to fine tune the experimental design even when narrow acid and temperature ranges were used in the second set of experiments due to the abrupt transition in strong acid hydrolysis of cellulose for CNC production. However, the predictions provided guide for future experimental design to use much narrow ranges of experimental parameters, especially sulfuric acid concentration to within 58–62 wt% for the bleached eucalyptus pulp used. The predictions also suggest that significant improvement in CNC and CSR recovery is difficult due to difficulties in accurate control of experimental conditions to a very narrow range, such as temperature to within ± 1 °C. The predictions suggest a maximum CNC yield of 60 % is achievable with simultaneous recovery of 10 % CSR yield (Fig. 9a). When without considering CSR yield, the practical achievable CNC

yield is approximately 63 % based on the predictions (Fig. 9c). The tradeoff is 3 % CNC versus 10 % CSR.

Production of nanofibrils from CSR through mechanical fibrillation

CSR contains sulfate ester groups, which is attractive for producing nanocellulose with good dispersibility using mechanical fibrillation. The CSRs from (58, 56, 40) and (62, 40, 70) with yields of 67 and 37 %, respectively, were mechanically fibrillated using a microfluidizer. It was found that CSR can be easily fibrillated to nanocellulose with less mechanical energy input or fewer passes (total of 15 passes at solids loading of 2 %) than that for the original bleached pulp before acid hydrolysis (over 100 passes). TEM imaging indicates that the morphologies of the resultant nanocellulose depend on CSR. Short fibrils with lengths between 100 and 200 nm were obtained from CSR produced at (62, 40, 70) or $ECHS = 9.58$ (Fig. 10a). Relatively longer nanofibers of 200–400 nm were obtained using CSR from (58, 56, 40) or $ECHS = 9.56$ (Fig. 10b). These CNFs are in the form of needles different from the fibril network structure of CNFs from different preparations, such as enzymatic (Zhu et al. 2011) or TEMPO-mediated

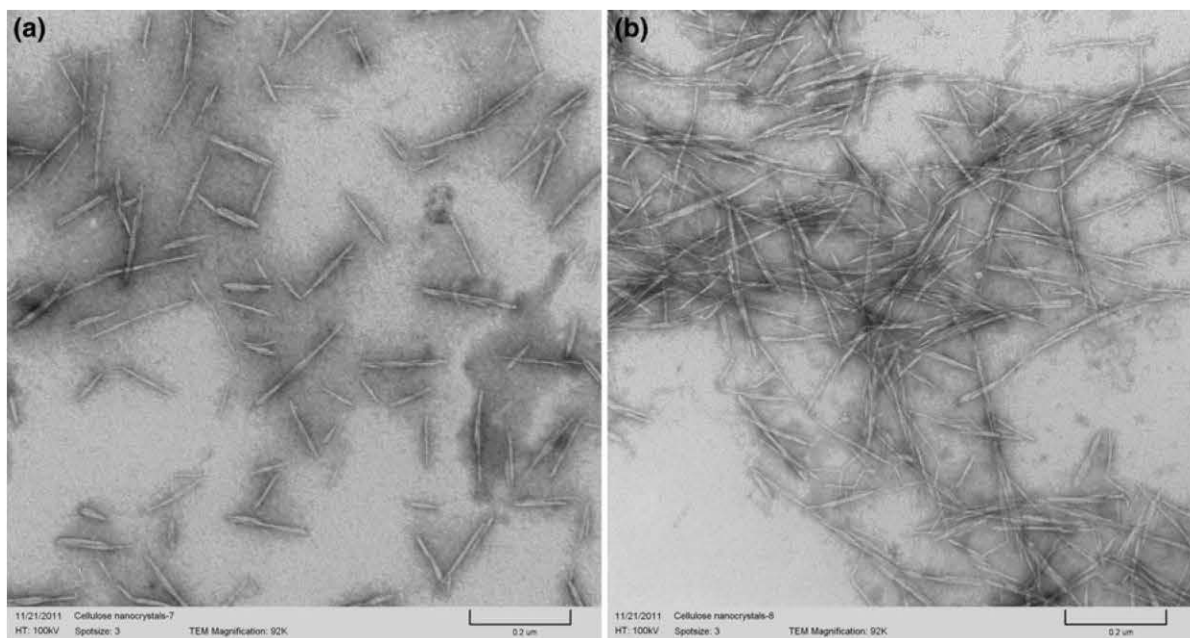


Fig. 10 TEM images of the CNFs produced from CSR samples produced at two acid hydrolysis conditions. **a** (62, 40, 70); **b** (58, 56, 40). Scale bar = 200 nm

oxidation treated CNFs (Saito et al. 2006). Further evaluation of the properties of these nanofibrils is needed in the future to assess their utility.

Conclusions

We demonstrated high yields of cellulose nanocrystals (CNCs) with simultaneous recovery of a significant amount of cellulosic solids residues (CSR) to achieve near zero cellulose loss in CNC production. The CNC produced has almost identical yields and morphologies as those obtained under conventionally considered "optimal" conditions. The recovered CSR contains sulfate ester groups that facilitated mechanical fibrillation to produce cellulose nanofibrils with good dispersibility for aqueous processing. The window for simultaneous production of CNC and recovery of CSR was extremely narrow, which may be why researchers in the past failed to recognize the possibility for reduced cellulose loss in CNC production. Further evaluations of the properties and utilities of CSR and nanofibrils produced from mechanical fibrillation of CSR are needed.

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