SUPPORTING INFORMATION FOR

ONE-STEP METHOD FOR THE PREPARATION OF CATIONIC NANOCELLULOSE IN REACTIVE EUTECTIC MEDIA

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CONTENTS

EXPERIMENTAL SECTION

Materials.

Commercially available short-fiber cellulose (Opitec Handel GmbH, Giebelstadt, Germany, cellulose content 100%), and bleached softwood Kraft pulp (MERCER Stendal GmbH, Arneburg, Sachsen-Anhalt, cellulose content > 80%) were used as cellulose raw material. Softwood pulp was disintegrated in deionized water overnight under constant stirring at room temperature. Disintegrated pulp was filtered and washed with deionized water and ethanol on a glass filter, and subsequently dried for 24 h at 60 °C. Shortfiber cellulose was used without any pretreatments. Ammonium formate (97 %, Alfa Aesar) was dried in the vacuum oven at room temperature overnight prior to use; glycolic acid (98 %) was obtained from Alfa Aeser, copper-ethylene diamine (CED)-solution (1 M in water), Levulinic acid (>98 %), L(+)-lactic acid (90 % solution in water) from Acros Organics.

Preparation of the reactive eutectic mixture (REM).

In order to obtain the eutectic, 50.4 g of preliminary dried ammonium formate was mixed with 30.4 g of glycolic acid (lactic or levulinic acids in corresponding amounts), resulting in the molar ratio 2:1 of the corresponding components. The mixture was grind thoroughly in an agar mortar. Formation of the eutectic was observed visually when the mixture gradually liquidized under the grinding. To facilitate the formation of the eutectic, the mixture was kept at 60 °C under occasional stirring for at least two hours or until the complete disappearance of the crystalline phases.

Nanocellulose extraction in REM.

Static reactor: Pulp or SFC were used as the cellulose source. The amount of the cellulose source for the reaction was calculated in order to obtain 5 wt% of pulp or 25 wt% of SFC in REM. The mixture was transferred into an autoclave, which was then kept at 180 °C for 4 h. After cooling the autoclave to RT, the dark brown upper layer of the product was removed. The residue was diluted with a few mL of distilled water, homogenized with a spatula, and ultra-sonicated for 30 minutes. Subsequent centrifugation at 10000 g for 5 min precipitated the colloidal particles.[1] The precipitate was washed using the following sequence: re-dispersion in water, vortexing for 30 s, ultra-sonication for 30 min, centrifugation at 12000 g (50000 g for last three runs) for 5 min, and decanting of the supernatant. The procedure was repeated with water and ethanol, until a clear washing solution was obtained.

Stirred reactor: Pulp or SFC were used as the cellulose source. The amount of the cellulose source for the reaction was calculated in order to obtain 5 wt% of pulp or 25 wt% of the SFC in REM. The mixture was placed in a PTFE beaker. The mixture stirred in an autoclave with stirring function at the desired temperature for the desired reaction time. After cooling the autoclave to RT, the mixture was diluted with ca. 20 mL of distilled water and dosed with 3 mL glacial acetic acid to facilitate removing of the dark brown reaction side products. The product was separated by centrifugation at 10000 g for 15 min. The supernatant was decanted, and the sediment was washed with a solution of acetic acid in water (5 vol.%) and ethanol consecutively until a clear supernatant

was obtained, using the following sequence: re-dispersion in water/acetic acid or ethanol, vortexing for 30 s, ultra-sonication for 30 min, centrifugation at 10000 g (20000 g after ethanol wash) for 15 min, and decanting of the supernatant.

Figure S1. Physical appearance of SFC (a), and extracted CNC in static conditions in the REM with glycolic (b), levulinic (c) and lactic (d) acids after washing and oven-drying.

Figure S2. Pulp starting material (left) and treated cellulose (right) observed with a polarized light microscope.

Cellulose characterization.

Particle size distribution of the product was measured with a LS 13320XR laser diffraction particle size analyzer and a Multisizer 4e, Beckman Coulter GmbH (Brea, California, USA), and Mastersizer 3000, Malvern Instruments (Malvern Panalytical, United Kingdom). **Zeta potential** and **dynamic light scattering** measurements were carried out on a Zetasizer Nano ZS, Malvern Instruments (Malvern Panalytical, United Kingdom). The measured electrophoretic mobility was converted to Zeta potential using Smoluchowski's approximation.

Scanning electron microscopy pictures were recorded on the Gemini 1550, Zeiss AG (Oberkochen, Germany), at an accelerating voltage of 3.00 kV. **Transmission electron microscopy** pictures were obtained on a 912 Omega TEM, Zeiss AG (Oberkochen, Germany) operating at 120 kV. Samples were negatively stained using an aqueous uranyl acetate solution.

ATR-IR was measured on the Nicolet iS5 with an iD5 ATR crystal, Thermo Fisher Scientific Inc. (Waltham, Massachusettes, USA), with a resolution of 0,5 cm^{-1}.

The crystallinity of the cellulose was analyzed by wide angle X-ray diffractometry (WAXD). The crystallinity index CrI was calculated from the intensity of the (200) signal ($2\theta = 22.6°$) and the minimum intensity between the signals (200) and (110) I_{am} ($2\theta = 18.8°$), corresponding to the amorphous part according to equation 1:

$$CrI = \frac{I_{200} - I_{am}}{I_{200}} \quad (1)$$

The **average degree of polymerization (DP)** of original cellulose pulp, REM-treated cellulose, and NFCs was evaluated from the limiting viscosity, measured in cupriethylenediamine (CED) solution according to the ISO 5351 standard. Samples were freeze-dried prior to the measurements. The evaluation followed the Mark-Houwink-Sakurada correlation between molecular weight and limiting viscosity (Staudinger index) using experimentally derived parameters by da Silva Perez [2]:

$$D_P = \left(\frac{1.65\ [\eta] - 116\ H}{C}\right)^{1.111} \quad (2)$$

, with the Staudinger index $[\eta]$, the mass fraction of cellulose C, and the mass fraction of hemicellulose H. This correction accounts for the contribution of eventual hemicellulose with an average D_P of 140 to the viscosity of the solution. In pure-cellulose sample, the equation thus simplifies to

$$D_P = (1.65\ [\eta])^{1.111} \quad (3)$$

. Due to the broad variety of parameters and standards for similar systems, the D_P shall only be considered a comparative value for starting material and pre-treated material samples within this work, not as an absolute.

Gel permeation chromatography (GPC). Cellulose samples were derivatized by a carbanilation reaction with phenyl isocyanate according to a procedure described by Evans et al.[3] to yield tricarbanilate cellulose which is soluble in organic solvents. To this end, 25 mg freeze-dried sample was dispersed in a vial with 10 mL DMSO and 1 mL phenyl isocyanate was added. The vial was placed in a 70 °C oil bath under occasional shaking for 40 h or until a clear solution was obtained. The reaction was terminated by adding 2 mL methanol to react with the excess phenyl isocyanate.

After the removal of methanol, GPC was carried out in NMP/LiBr (0.05 mol/L) on a GRAM-100/1000-7µ column (PSS Polymer Standards Service GmbH, Mainz, Germany) kept at 70 °C. The sample was detected with a refractive index detector (1260 Infinity II RID, Agilent, Santa Clara, California, USA). The molecular weight was evaluated against a pullulan standard.

Thermogravimetric analysis (TGA) measurements of the CNC samples were carried out using a thermo microbalance TG 209 F1 Libra (Netzsch, Selb, Germany) in nitrogen and air atmospheres, both with a constant flow of 100 mL min^{-1}. Each measurement was made using 10 $\pm$ 1 mg of the freeze-dried sample, which was heated from 25 to 600 °C at a scanning rate of

10 °C min^{-1} in an aluminum crucible. The temperature of degradation T_d was defined as the onset point temperature of weight loss in the obtained TGA curve.

The ninhydrin probe was used to determine the primary amino groups in the cellulose samples after the treatment in REM. The analysis was performed according to the procedure described by Wellings et al.[4] Prior to the determination, three test solutions were prepared according to the following procedure:

Reagent A:

1. Dissolve 16.5 mg of KCN in 25 mL of distilled water.

2. Dilute 1.0 mL of above solution with 49 mL of pyridine (freshly distilled from ninhydrin).

3. Pour it into a small reagent bottle and label it "A".

Reagent B:

1. Dissolve 1.0 g of ninhydrin in 20 mL of n-butanol.

2. Pour into a small reagent bottle and label it as "B".

Reagent C:

1. Dissolve 40 g of phenol in 20 mL of n-butanol.

2. Pour it into a small reagent bottle and label it "C".

Ninhydrin Test Procedure:

2-3 mg of dried CNC was placed in a dry glass tube and mixed with 1 mL of ethanol. The same procedure was repeated for SFC. One tube was left empty for the reference experiment. To each tube 2 to 3 drops of Reagent A, 2 to 3 drops of Reagent B and 2 to 3 drops of Reagent C were added in this order. The tubes were heated at 110°C for 5 minutes, and the developed colors were compared.

Characterization of REM

To detect the volatile decomposition products in REM, **TGA** was coupled with a Thermostar mass spectrometer (Pfeiffer Vacuum; Asslar/Germany) with an ionization energy of 75 eV. These **TGA-MS** measurements were conducted on 10 $\pm$ 1 mg of the sample in a helium flow of 10 mL min^{-1} and a heating rate of 2 °C min^{-1}.

Differential scanning calorimetry was performed on a DSC 204 F1 Phoenix (Netzsch, Selb, Germany) using an aluminum crucible with a pierced lid at a heating rate of 10 °C min^{-1}. Data for thermal analyses were recorded and analyzed using the Proteus (6.1.0) and Quadstar (7.03, MID modus) software package.

RESULTS

Determination of the D_P by GPC method

M_W values were converted into D_P following the common assumption that a complete carbanilation of the cellulose to cellulose tricarbanilate takes place,[3, 5] although this has not been proven here. This amounts to a M_W of 519 g/mol, which is the value for the repeat unit anhydroglucose with three substituted phenyl isocyanate groups. Minor changes in M_W due to the amination reaction were neglected. With the length of aanhydroglucose unit of 0.518 nm,[6] the range of the DP of a chain in a cellulose whisker in the nm range was determined to 192 – 1932, or a M_W of $9.97 \cdot 10^4$ to $1.00 \cdot 10^6$ Da, corresponding to a chain length of 100-1000 nm (grey rectangle).

Conclusions from the measured D_P on the CNC length are based on the assumption that all chains in the given range were actually part of nanocrystals before their dissolution, meaning that the DP, in literature referred to as "leveling-off DP", is related to the average crystallite length.[7]

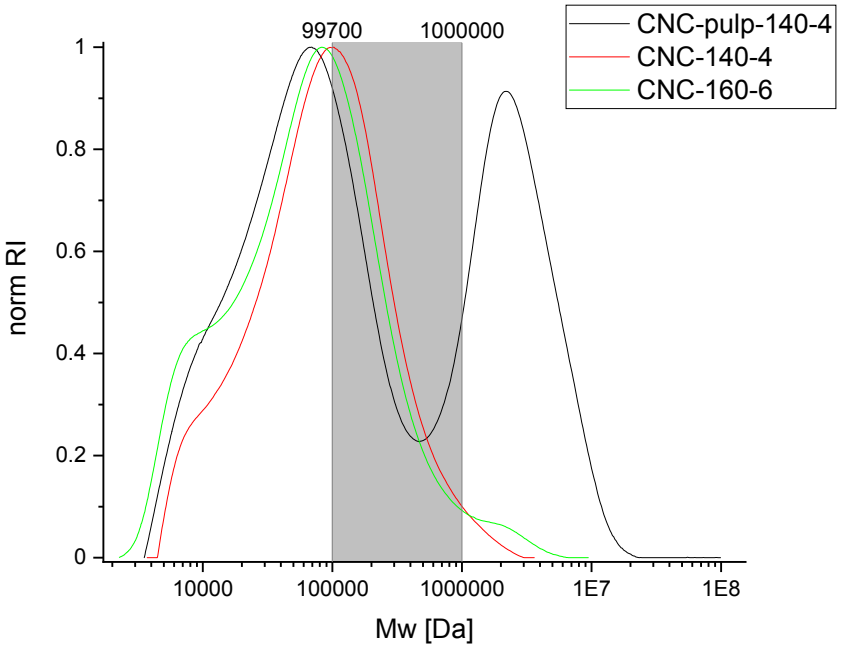

Figure S3. GPC analysis data for the CNC samples prepared under different conditions: the first number represents reaction temperature in °C; the second number represents the reaction time in h. The CNCs obtained from pulp exhibit a bimodal molar mass distribution, which arises from the presence of larger fibers in this starting material, compared to the short-fiber cellulose.

Elemental analysis, Zeta potential and crystallinity index of cellulose pretreated in REM

Table S1. Elemental analysis, Zeta potential and crystallinity index of cellulose pretreated in REM

Cellulose source, reaction conditions	***Composition of REM***	***ZP [mV]***	***C [%]***	***H [%]***	***N [%]***	***O [%]***	***CrI [%]***
*Short-fiber cellulose *, 180 °C, 4 h, static*	Glycolic acid:AF 1:2	35.0	43.29	6.12	0.37	50.19	83.8
Short-fiber cellulose, 180 °C, 4 h, static	Lactic acid: AF 1:2	35.3	43.17	6.18	0.36	50.27	86.5
Short-fiber cellulose, 180 °C, 4 h, static	Levulinic acid:AF 1:2	30.1	43.62	6.21	0.72	49.43	80.5
*Softwood pulp**, 180 °C, 4 h, static*	Glycolic acid:AF 1:2	6.4	42.55	6.27	0.41	50.69	80.2
Softwood pulp, 180 °C, 4 h, static	Lactic acid: AF 1:2	14.1	42.33	6.27	0.26	50.39	79.2
Softwood pulp, 180 °C, 4 h, static	Levulinic acid:AF 1:2	5.8	42.18	6.305	0.28	50.43	78.4
Short-fiber cellulose, 180 °C, 4 h, stirred	Glycolic acid:AF 1:2	21.5	42.39	6.28	0.35	50.94	73.0
Short-fiber cellulose, 180 °C, 2 h, stirred	Glycolic acid:AF 1:2	34.4	42.37	6.32	0.43	50.84	73.8
Short-fiber cellulose, 180 °C, 1 h, stirred	Glycolic acid:AF 1:2	31.9	41.93	6.20	0.40	51.42	77.3
Short-fiber cellulose, 160 °C, 6 h, stirred	Glycolic acid:AF 1:2	34.1	41.94	6.42	0.42	51.01	83.1
Short-fiber cellulose, 160 °C, 2 h, stirred	Glycolic acid:AF 1:2	34.6	41.95	6.23	0.35	51.41	82.0
Short-fiber cellulose, 140 °C, 4 h, stirred	Glycolic acid:AF 1:2	33.9	41.75	6.02	0.36	51.76	79.0
Short-fiber cellulose, 140 °C, 1 h, stirred	Glycolic acid:AF 1:2	35.9	42.03	6.23	0.28	51.39	79.2
Softwood pulp, 180 °C, 4 h, stirred	Glycolic acid:AF 1:2	29.6	41.93	6.15	0.28	51.61	82.4
Softwood pulp, 180 °C, 2 h, stirred	Glycolic acid:AF 1:2	26.2	41.95	6.22	0.27	51.53	83.6
Softwood pulp, 180 °C, 1 h, stirred	Glycolic acid:AF 1:2	32.2	42.25	6.29	0.32	50.98	81.5
Softwood pulp, 160 °C, 6 h, stirred	Glycolic acid:AF 1:2	28.2	41.98	6.24	0.32	51.41	83.1
Softwood pulp, 160 °C, 2 h, stirred	Glycolic acid:AF 1:2	31.5	42.05	6.47	0.30	51.10	84.5
Softwood pulp, 140 °C, 4 h, stirred	Glycolic acid:AF 1:2	35.6	41.93	6.39	0.26	51.20	83.3
Softwood pulp, 140 °C, 1 h, stirred	Glycolic acid:AF 1:2	-0.4	42.01	6.19	0.19	51.58	82.8

*Commercially available short-fiber cellulose (Opitec, cellulose content 100%), SFC

**Bleached softwood Kraft pulp (cellulose content>80%)

Determination of the DP of CNC with the limiting viscosity method

Table S2. Polymerization degree D_P determined from viscometric measurements according to ISO 5351 standard.

sample	***c*** **mg/mL**	**[η] mL/g**	D_P
Short-fiber cellulose	2.55	645.3012	2308
Short-fiber cellulose, 140 °C, 4 h	2.62	105.4777	309
Short-fiber cellulose, 160 °C, 2 h	2.50	110.4000	325
Softwood pulp, 140 °C, 4 h	2.55	108.2353	318
Softwood pulp, 180 °C, 4 h	2.48	111.1409	327

Laser diffraction measurements

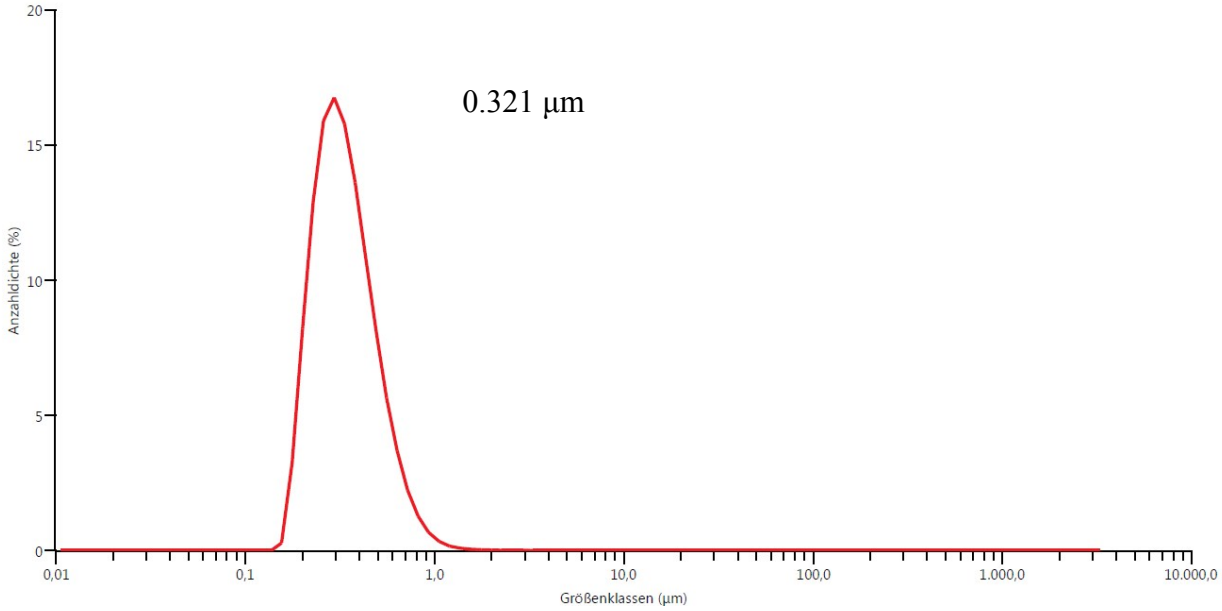

Figure S4. Laser diffraction determination of the cellulose particle sizes

Phase determination of the CNC

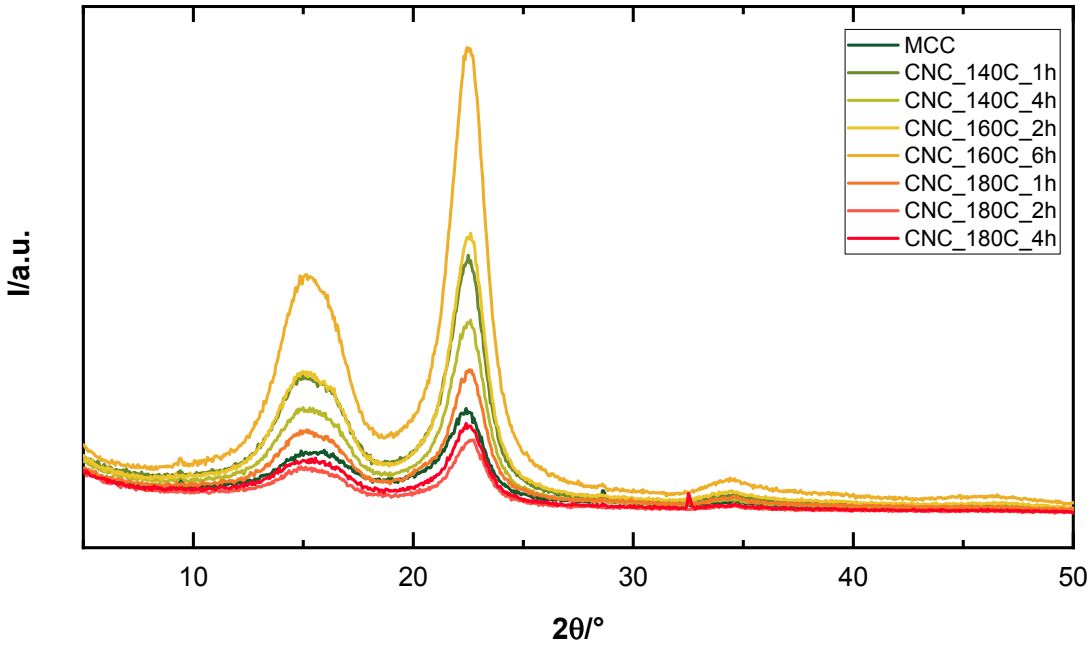

Figure S5. WAXD spectrum of SFC treated in REM1 in varying conditions.

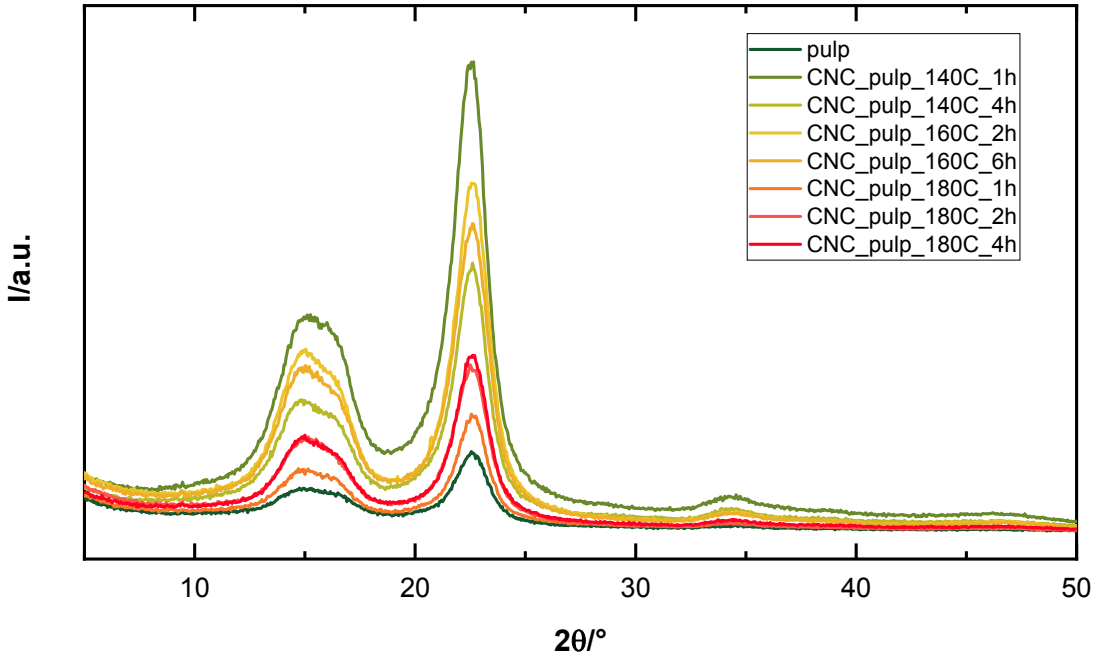

Figure S6. WAXD spectrum of pulp treated in REM1 in varying conditions.

FTIR of SFC treated with different REMs

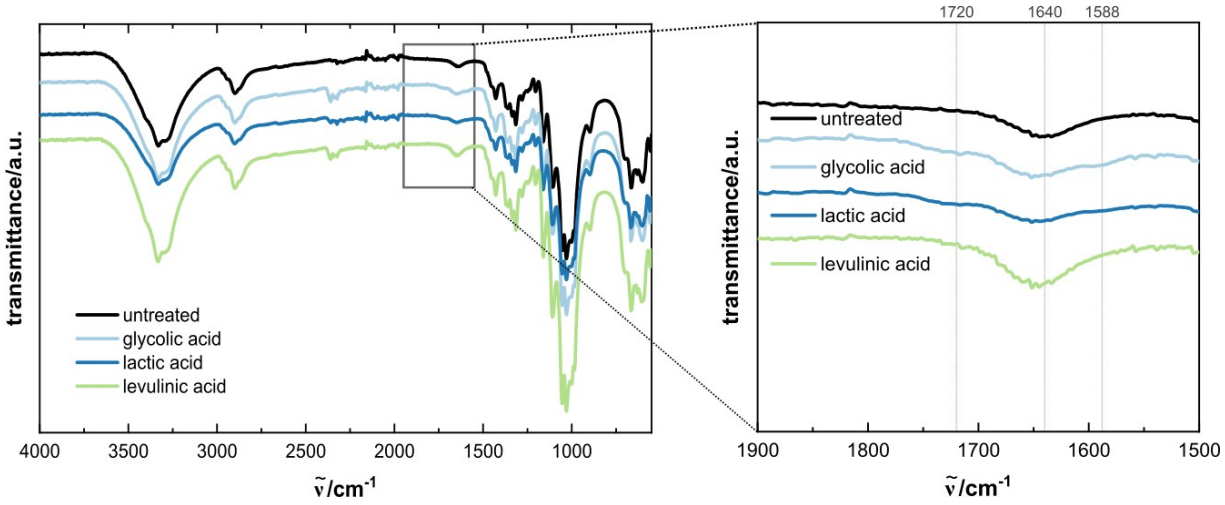

Figure S7. ATR-IR spectra of untreated starting material and cellulose products from the treatment of SFC with REM of ammonium formate and the respective acid in a ratio of 2:1.

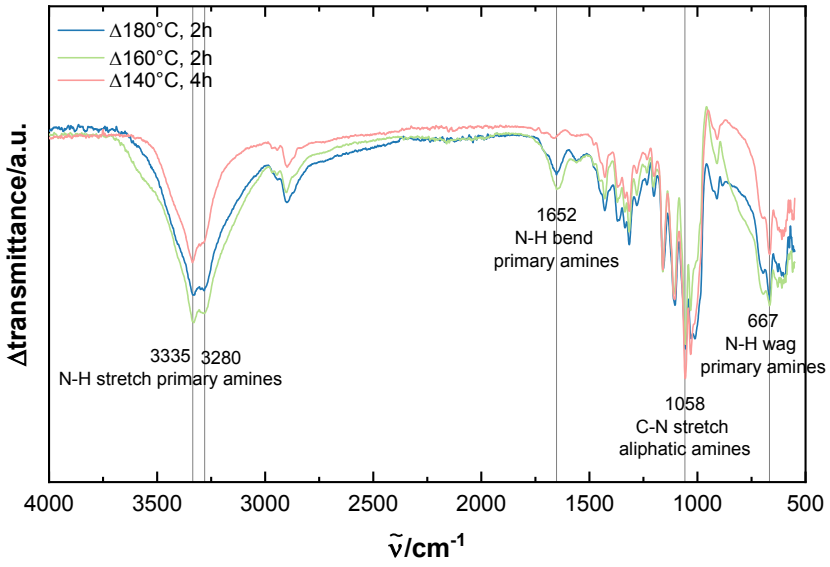

Figure S8. ATR-IR difference spectra between the REM-treated SFC samples and the starting material SFC.

Reaction conditions optimization regarding the crystallinity index of the CNC

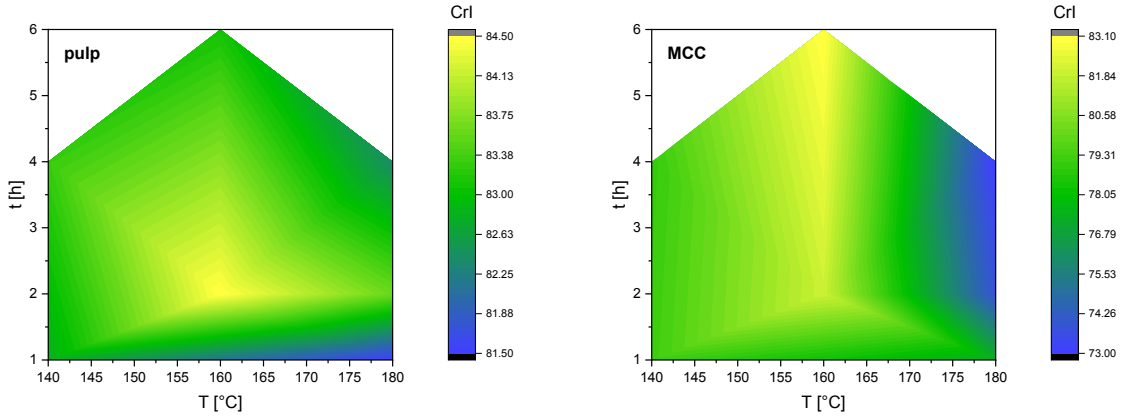

Figure S9. Degree of crystallinity *CrI* of pulp and SFC treated with REM1, dependent on the reaction time and temperature.

Chemical determination of the amino groups on CNC

The ninhydrin test proves the presence of primary amine groups. It is a sensitive enough probe to evidence the presence of the low amount of the amino groups in the treated CNC samples.

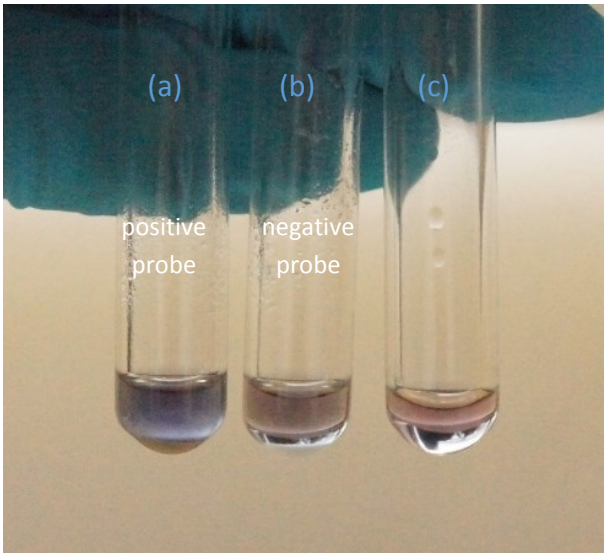

Figure S10. Ninhydrin probe of (a) SFC sample treated in the REM1 at 140 °C for 4h, (b) untreated SFC and (c) blank probe without sample.

Thermal behavior of REM

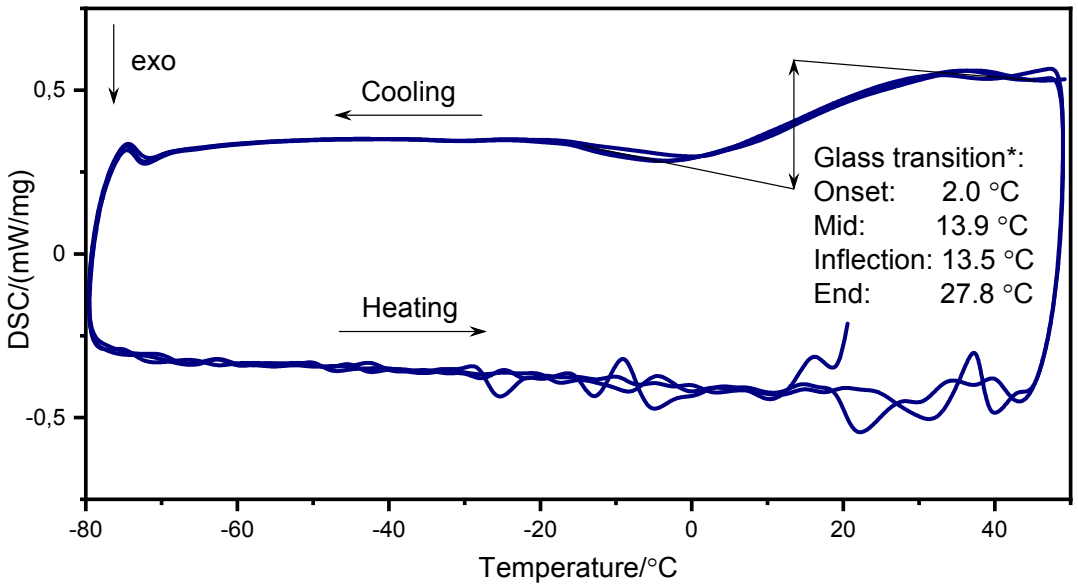

Figure S11. Differential scanning calorimetry (DSC) trace for the eutectic mixture REM1. *glass transition is calculated as average from the three measurements.

Physical properties of the reactive eutectic mixtures

Table S3. Physical properties of the reactive eutectic mixtures prepared from ammonium formate with corresponding organic acids in the molar ratio 2:1.

Entry	***Reactive eutectic medium***	***Organic acid***	***Density [g/cm^3]***		***Viscosity [mPa·s]***	
			at 25 °C	**at 40 °C**	**at 25 °C**	**at 40 °C**
1	REM1	Glycolic acid	1.239	1.284	151	63.3
2	REM2	Lactic acid	1.225	1.216	145	64.6
3	REM3	Levulinic acid	1.196	1.186	91	45.4

NMR spectroscopy of pulp starting material and CNC product

$^{13}C\{^{1}H\}$ CP/MAS and ^{1}H MAS measurements were carried out using a Bruker range Avance 400 MHz Solid State spectrometer and a Bruker 4 mm double resonance probe-head operating at a spinning rate of 6500 Hz. Acquisition time was 0.082 seconds for ^{1}H and 0.041 seconds for ^{13}C, and sweep-width was 50 kHz. The number of scans was 30,065 to 46,584 with a relaxation time of 1.0 seconds for ^{13}C.

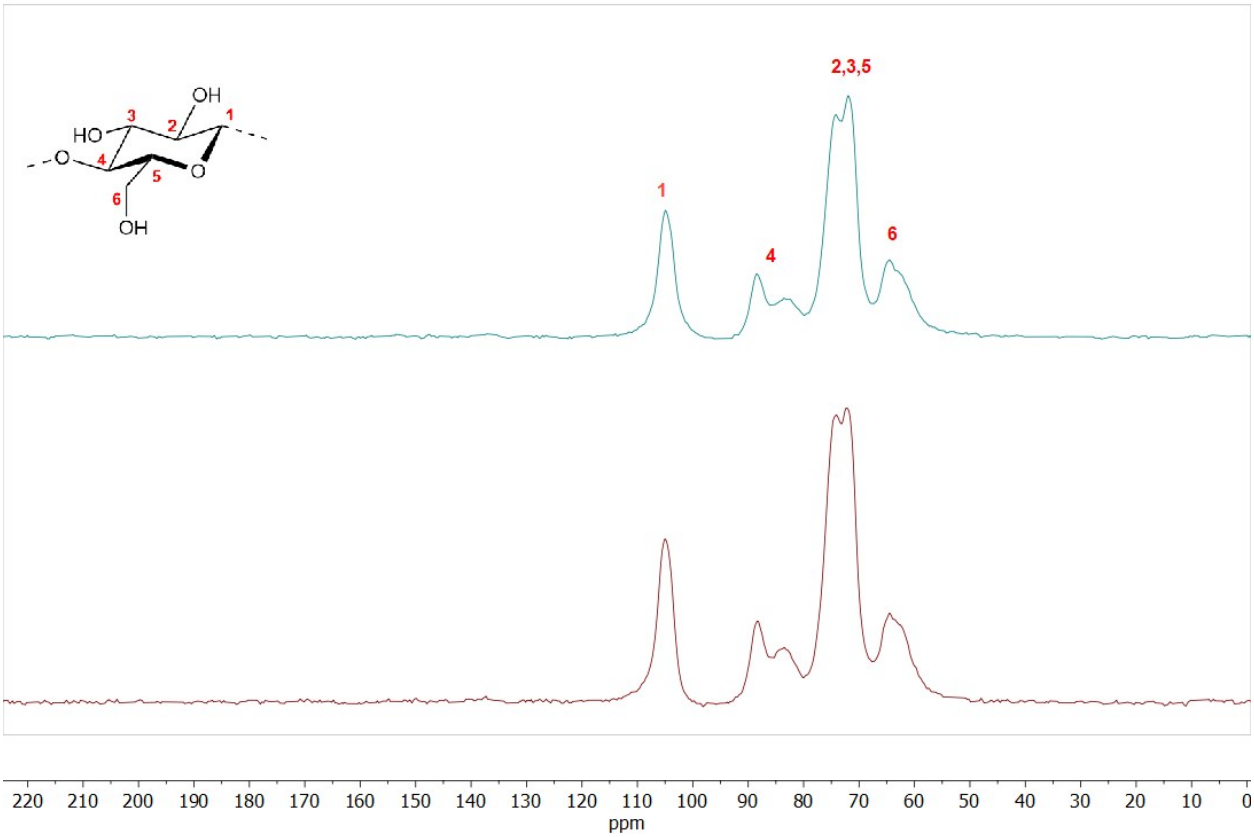

Figure S12. ^{13}C-NMR spectrum of the starting material pulp (blue line) and the REM-treated CNC sample (red line).

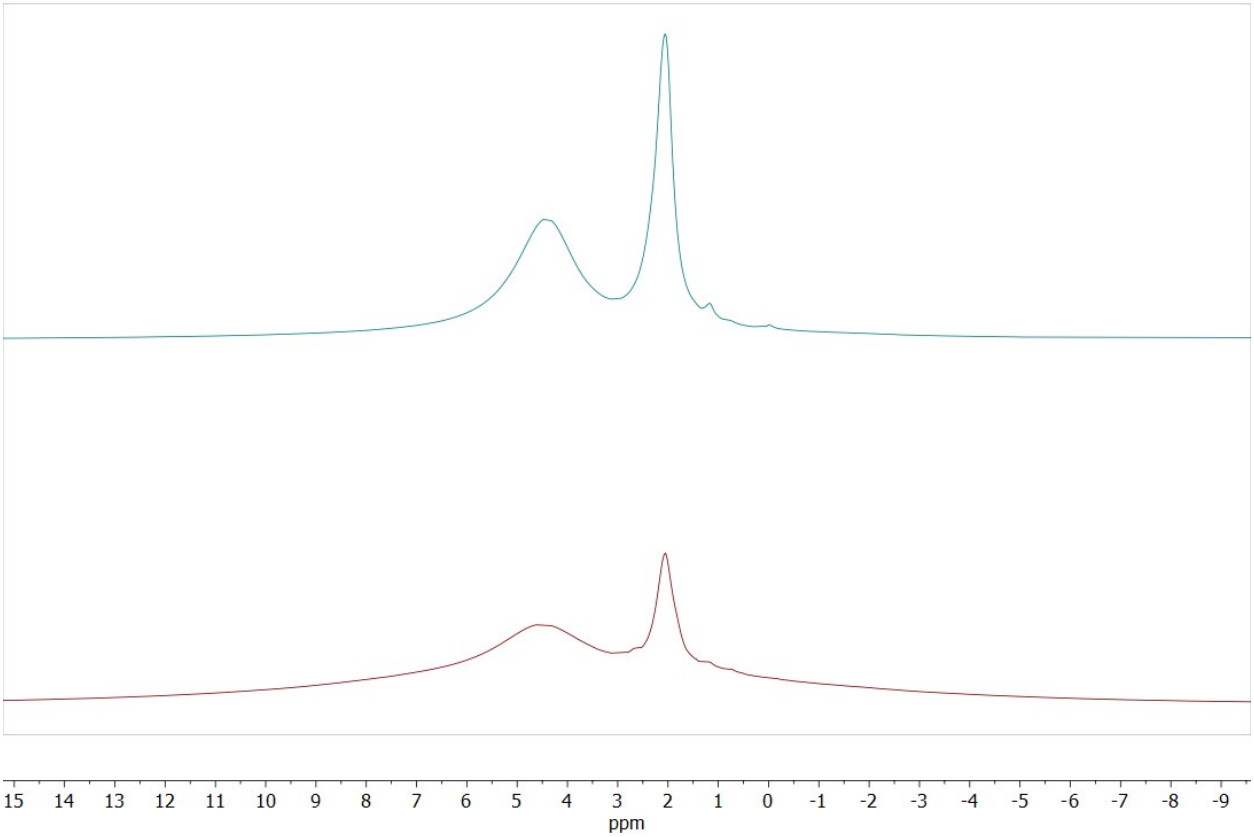

Figure S13. ^{1}H-NMR spectrum of the starting material pulp (blue line) and the REM-treated CNC sample (red line).

X-ray photoelectron spectroscopy

X-ray photoelectron measurements were performed with an ESCALAB 250Xi using a monochromated Al Ka line for excitation.

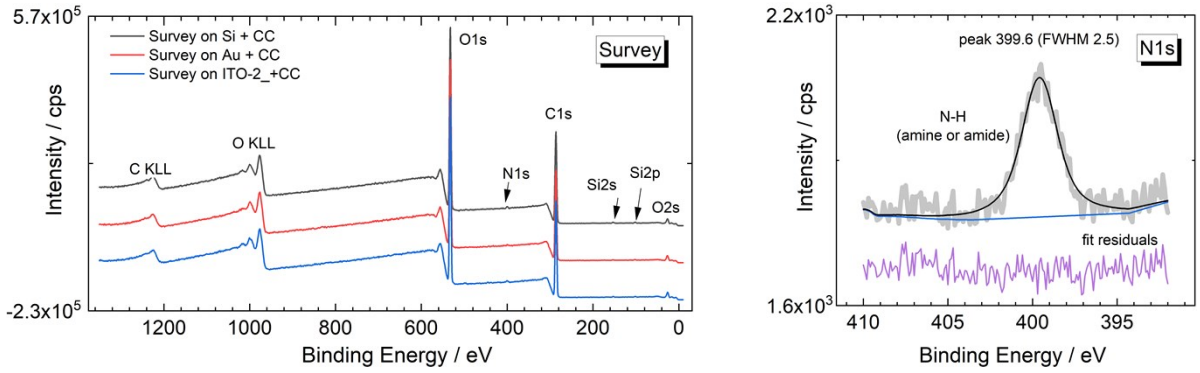

Figure S14. XPS of the REM-treated CNC sample.

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