ELECTRONIC SUPPORTING INFORMATION

Citrated cellulose nanocrystals from post-consumer cotton textiles

Maria-Ximena Ruiz-Caldas,[a] Varvara Apostolopoulou-Kalkavoura,[a] Anna-Karin Hellström,[b] Jutta Hildenbrand,[b] Mikael Larsson,[b] Aleksander Jaworski,[a] Joseph S.M. Samec,[c] Panu Lahtinen,[d] Tekla Tammelin,[d] Aji P. Mathew [a]*

[a] Department of Materials and Environmental Chemistry, Stockholm University, SE-10691 Stockholm, Sweden
[b] RISE—Research Institutes of Sweden, SE-431 53, Mölndal, Sweden
[c] Department of Organic Chemistry, Stockholm University, SE-10691 Stockholm, Sweden
[d] VTT Technical Research Centre of Finland, Biologinkuja 7, FL-02044 Espoo, Finland

*Corresponding author: aji.mathew@mmk.su.se

CHARACTERIZATION

Conductometric titration

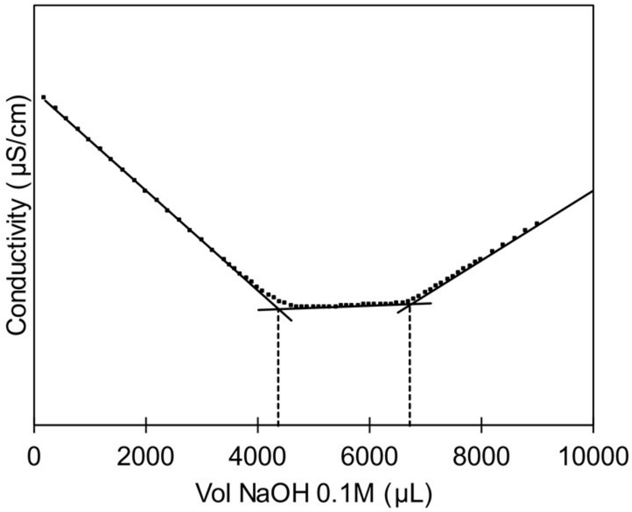


Figure S1. Example of conductometric titration curve of citrated cotton and CitCNCs. The flat region corresponds to the titration of carboxylic groups.

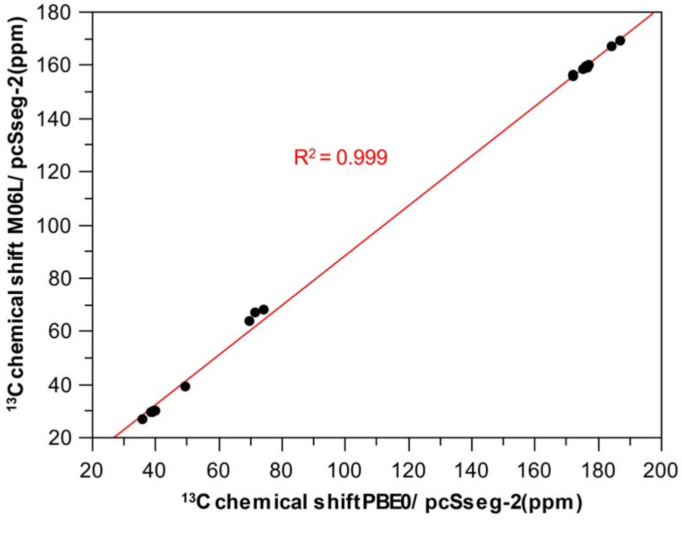
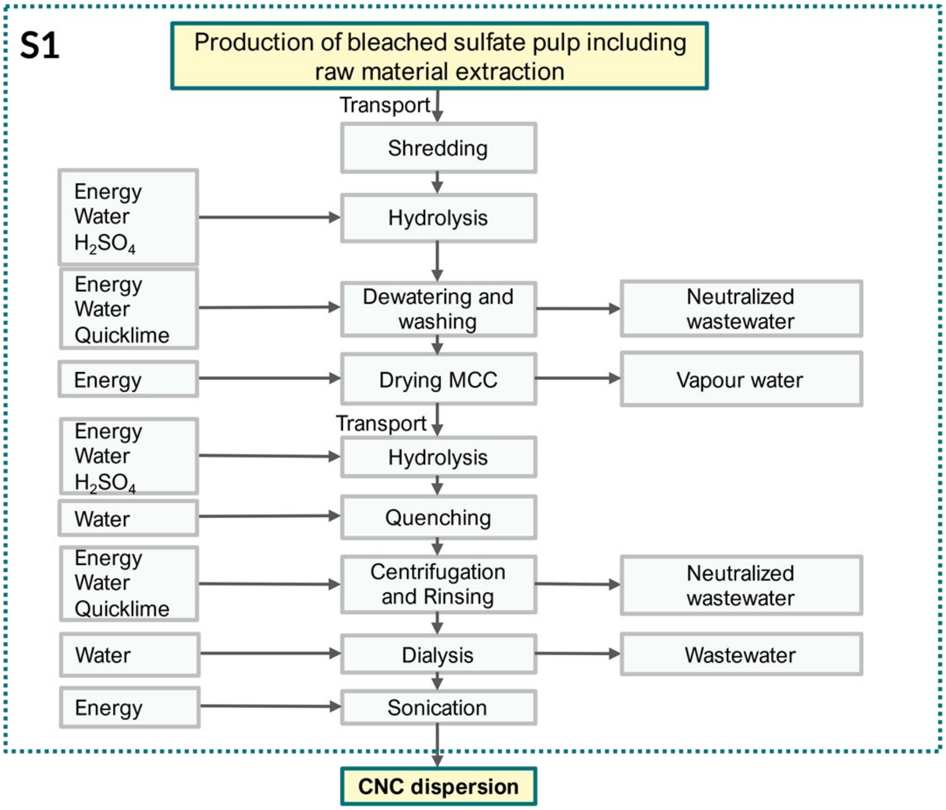
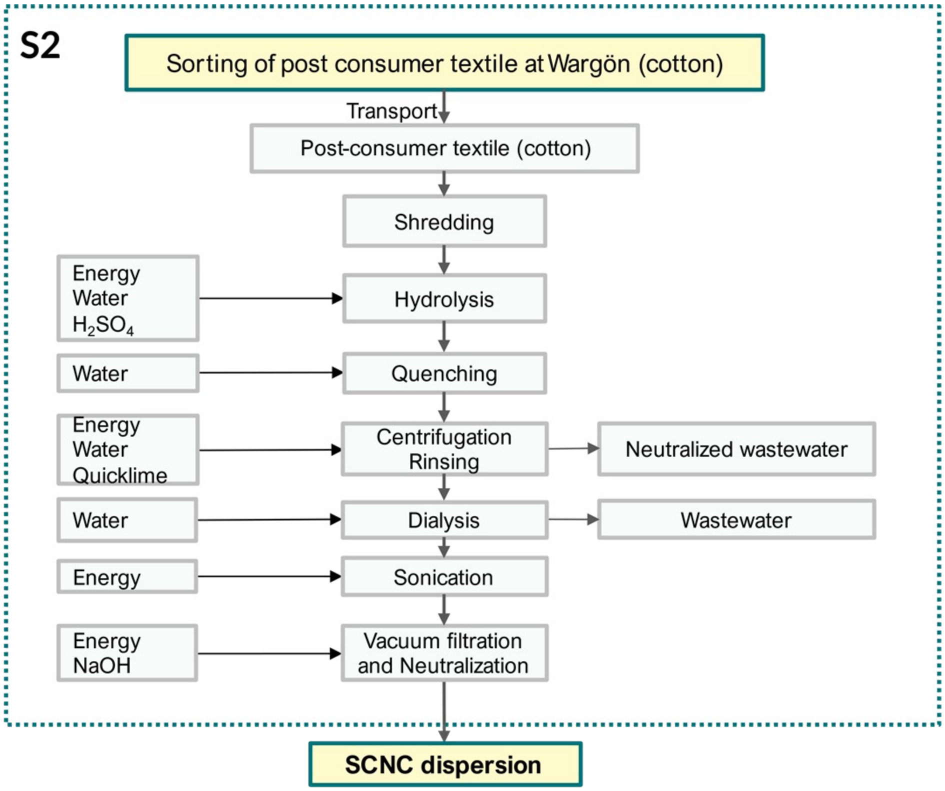


Figure S2. Correlation plot between the modeled configurations of citrate esters computed with M06L and PBE0 using pcSseg-2

23 Life Cycle Assessment





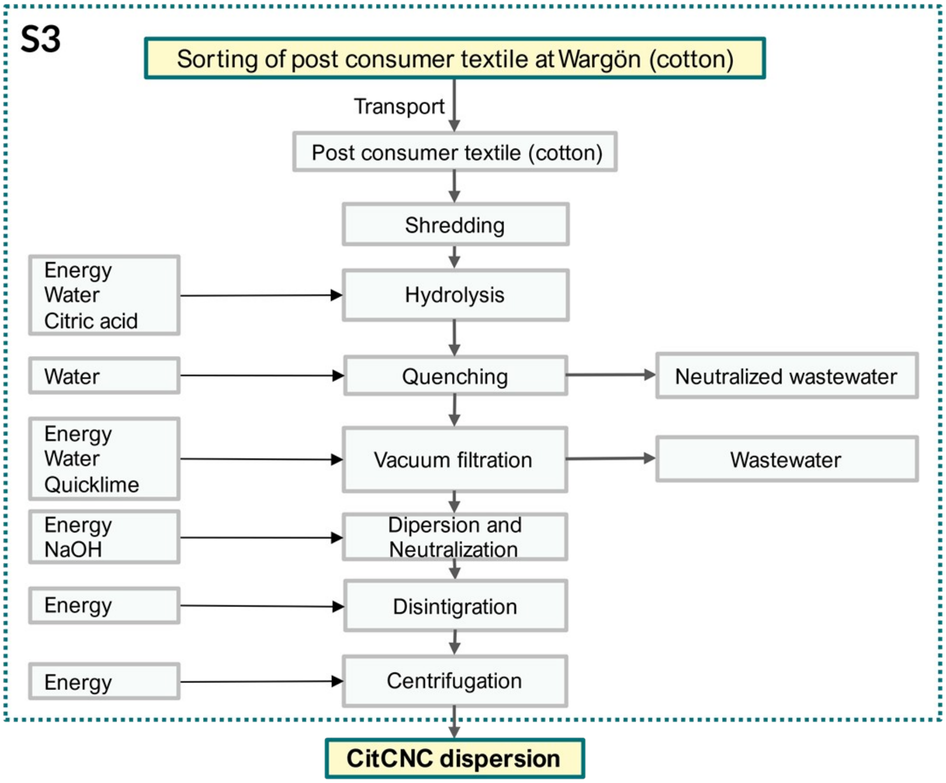


Figure S3. System boundaries for CNC preparation from a). S1 (wood pulp), b). S2 (SCNC) and S3 (CitCNC).

Table S1. Life cycle inventory (LCI) data per gram produced crystalline nanocellulose (CNC) for S1, S2, and S3. The functional unit (FU) is 1 gram of CNC in water.

Inputs and outputs	S1	S2	S3
Post-consumer cotton (g/FU)	-	1.8	1.3
Bleached sulfate pulp (g/FU)	4.3	-	-
Sulfuric acid (g/FU)	21	12	-
Citric acid (g/FU)	-	-	26
Sodium hydroxide (mg/FU)		12	48
Quicklime (g/FU)	22	12	13
Water, deionized water (kg/FU)	7.2	3.8	0.2
Wastewater (dm^3/FU)	7.3	3.8	0.2
Solid waste (g/FU)	27	13	37
Electricity mix (kWh/FU)	0.6	0.3	0.3
District heating (MJ/FU)	0.7	0.1	0.5
Transport (kgkm/FU)	1.8	0.8	0.5

Table S2. Background data

Input and output	Ecoinvent 3
Electricity1)	Sweden, market for electricity, medium voltage, Cut-off, S
Heating2)	RER, market for heat, district or industrial, other than natural gas, Cut-off, S
Sulfuric acid3)	RER, market for sulfuric acid, Cut-off, S
Citric acid4)	RER, production for citric acid, Cut-off, S
Quicklime	RER, market for quicklime, milled, Cut-off, S
Sodium hydroxide5)	Global, market for sodium hydroxide (50%), Cut-off, S
Bleached sulfate pulp	RER, market for sulfate pulp, bleached, Cut-off, S
Deionized water	RER, market for deionized water, Cut-off, S
Solid waste1)	Sweden, market for municipal solid waste
Wastewater	Europe, average treatment of wastewater

Transport	Europe, freight lorry EURO 5

1) Production of MCC was assumed to be located in Örnsköldsvik (Sweden) and CNC was assumed to be located at Stockholm´s University (Sweden).
2) District heating production, Sweden has a low share of fossil fuel, therefore, Europe, district heating has been chosen.
3) Sulfuric acid production was assumed to not induce any temporal mismatches in the near future since it is a well-established production process.
4) Citric acid might induce temporal mismatches since the research in this field has intensified in recent years. However, in the near future, a mismatch was assumed to be neglectable[1]
5) Sodium hydroxide production was assumed to not induce any temporal mismatches in the near future since it is a well-established production process.
6) Location of the production of MCC was assumed to be located in Örnsköldsvik (Sweden) and CNC at Stockholm´s University (Sweden).

Background processes

Wood pulp production

In the baseline scenario, CNC was produced from microcrystalline cellulose (MCC). The commercially available microcrystalline cellulose (MCC) is mainly produced by wood pulp. In general, any wood pulp can be used as raw material[2]. Dissolving pulp would have the highest cellulose content but is also the most expensive. The other main types of wood pulp are sulfite and kraft pulp (sulfate pulp). The main differences between these two types of pulps are the processing steps and the chemicals used. Furthermore, both unbleached and bleached pulp are available on the market. The bleached pulp can be either elementary chlorine-free (ECF) or totally chlorine-free (TCF). In the Ecoinvent database, three types of pulps are available: unbleached sulfate, bleached sulfate (mix of ECF and TCF), and bleached sulfite (mix of ECF and TCF) pulp. The data in the Ecoinvent database include production of the wood, transportation to the pulp mill, chemical pulping and bleaching, drying energy use, recovery of chemicals, and wastewater treatment[3] For the baseline scenario, bleached sulfate pulp was selected since this was proposed in the article by Vanhatalo et al[4]. Nevertheless, unbleached wood pulp was included in the sensitivity analysis.

Processing steps

Inventory data for citric acid, sulfuric acid, sodium hydroxide, quicklime, and deionized water were obtained from the Ecoinvent database, and these selections are presented in Table S2.

In some cases, the acids used during hydrolysis can be recovered after the reaction through distillation or filtering, but such recycling is rarely a part of the laboratory process. However, closed loops for circulating the acids can be modeled for the scale-up. Recycling the acid will reduce the acid input, but the energy needed for the recovery has to be taken into account. In the laboratory, the citric acid from the hydrolysis processing water has been recovered by evaporation of the water. The recovery of citric acid has been modeled as distillation (without vacuum) and the energy consumption has been calculated according to Piccinno et al.[5]

The sulfuric acid has not been recovered since the carbohydrates dissolve and accumulate in the acid-processing water and diminish the acid's hydrolytic reactivity. In future scenarios, the acid could potentially be recovered and used in other applications where the high purity of the recovered acid is not crucial[6].

A conventional laboratory process for the microcrystalline cellulose (MCC) without recovery of chemicals has been chosen for this case study[4] As no recovery of chemicals occurs, large amounts of wastewater and particularly acidic wastewater are produced due the sulfuric acid hydrolysis. The acidic water must be neutralized before it enters the wastewater treatment facilities. Quicklime was used as a neutralizing agent for acidic wastewater. The acidic wastewater also contained dissolved carbohydrates. During the neutralization reaction, $CaSO_4$ (calcium sulfate) sludge was formed and a yield of 100% was assumed according to Husgafvel et al.[7] The S2 (SCNC) and S3 (CitCNC) wastewaters were also neutralized with quicklime and a yield of the calcium sulfate and calcium citrate sludge was assumed to be 100%.

Transport

Due to the low technical readiness level of this study, the future mode of transport and distance are not known. It was assumed that the production site for CNC would be located at Stockholm University. A future production location in Sweden is not unrealistic since the production probably can take place in a Swedish pulp mill. MCC was assumed to be produced in Örnsköldsvik in Sweden. The pulp can potentially be produced in Sweden, Finland, or Russia. The chemicals were assumed to be produced in Germany where large chemical plants are located (except for sodium hydroxide). However, different locations were evaluated in the sensitivity analysis. For further details see Table S8.

Treatment

The energy required for the hydrolysis, distillation, and drying was calculated according to Piccino et al.[5] The heating energy consists of the energy needed to increase (Q heat) and maintain the reaction temperature (Q loss) for a specific time assuming reactor with glass fiber insulation with a thickness of 75 mm (see equation 1).

$$Q_{(reaction)} = (Q_{(heat)} + Q_{(loss)}) / \eta_{(eff)} \qquad (1)$$

where $\eta_{(eff)}$ is the efficiency. The heat capacity required to calculate Q(heat) was collected from the literature for sulfuric acid and citric acid.[8–11]

The distillation energy required for the evaporation of water for the recovery of citric acid was calculated according to the following equation:

$$Q_{(dist)} = (Q_{(heat)} + Q_{(dist)}) \times (R-1)) / \eta_{(eff)} \qquad (2)$$

where R is the reflux ratio.

Finally, the drying of MCC was calculated according to:

$$Q_{(dry)} = (Q_{(heat)} + \Delta H_{(vap)} \times m_{(vap)}) / \eta_{(eff)} \qquad (3)$$

where $\Delta H_{(vap)}$ is the enthalpy of vaporization. Data for centrifugation, homogenization, and filtration has been obtained from producers of the corresponding machinery.

The electricity mix and heat sources were acquired from the Ecoinvent database and further described in Table S2. Swedish electricity mix was assumed to be used in all scenarios and the effect of changes in the mix was tested in the sensitivity analysis. The heat source was chosen to Europe heat, district or industrial, other than natural gases due to the low share of fossil fuel[12]. The heat source was varied in the sensitivity analysis.

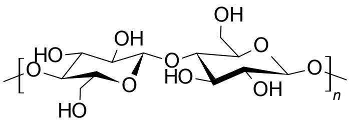
RESULTS

Preparation of citrated cotton

Table S3. Optimization reaction conditions functionalization of cotton fabrics.

Run	Reaction time (h)	Concentration citric acid (% wt)	Temperature (°C)	Carboxylate content (mmol/kg)
1	3	60	60	90
2	6			110
3	3	80		200
4	6			220
5	3	60	90	345
6	6			305
7	3	80		550
8	6			790
9	**7**	**85**	**100**	**1090**

Table S4. Characteristic vibrations observed in the FTIR spectra of cotton fabrics[13–16]

Wavenumber (cm^{-1})	Group	Structure
Broad in 3550–3100, peak in 3331	O–H stretching	
2900	C−H stretching	
1640	O–H bending, adsorbed H_2O	
1375	C−H bending	
1340	O–H *in-plane* bending	
1315	C−H wagging	
1158, 1105, 1029, 992	C−O stretching in C−O−C and C−O−H fragments	
1057	In-plane ring stretch	
898	Glycosidic linkage	

Fibrillation to give CitCNCs

Table S5. Citrate and carboxylate content of esterified cotton and extracted CitCNCs

Sample	Citrate content [mmol/kg]	Carboxyl content [mmol/kg]	Carboxyl groups from monoesters (%)
Cotton	345 ± 20	1090 ± 50	63
CitCNCs	295 ± 20	900 ± 55	66

Note: Averages and standard deviations were rounded to the nearest five

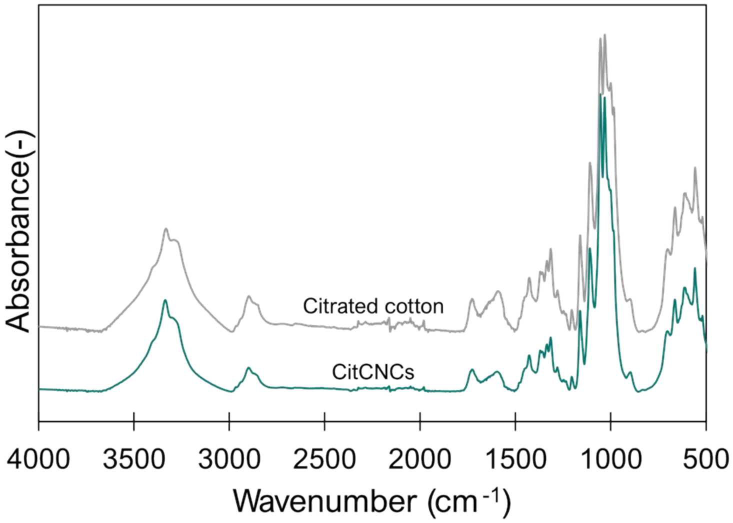


Figure S4. FTIR spectra of citrated cotton and CitCNCs.

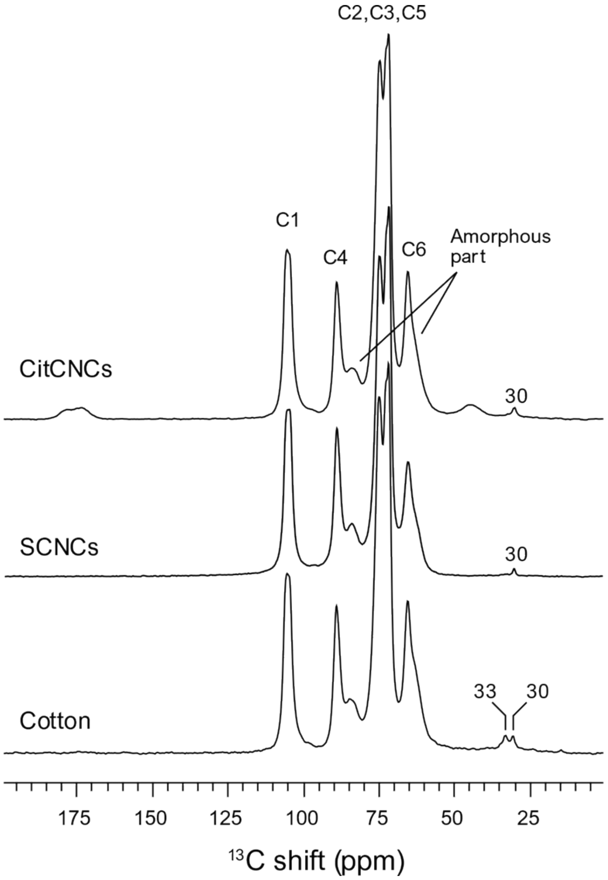


Figure S5. ^{13}C NMR spectra of cotton, SCNCs, and CitCNCs.

Table S6. Parameters peaks used for fitting of XRD diffractograms.

Sample	Center (2θ (°))	Height	Area	FWHM (°)
Cotton	22.6784	5865.24	10270.1	1.24727
	14.8499	1530.28	2874.97	1.67884
	20.4556	487.062	646.8	0.847648
	16.5537	1275.01	2252.25	1.34313
	34.2855	503.16	797.572	1.48826
	20.5477	202.736	1179.78	5.45525
SCNCs	22.6617	11900.1	22912.4	1.37376
	14.8038	2628.02	4537.35	1.62197
	16.5108	1937.18	3205.74	1.46329
	34.3999	366.508	622.145	1.59469
	20.6	564.966	3648.36	6.06657
CitCNCs	22.761	11286.3	23684	1.37589
	16.668	1936.12	4642.72	1.52868
	15.0053	1826.31	3245.1	1.66822
	34.4407	217.667	315.03	1.34322
	20.6	606.87	4052.34	4.27506

Guiding process development by life cycle assessment

Contribution analysis

Table S7. Results for environmental impact assessment (EF 3.0).

Impact category	**Unit**	**S1**	**S2**	**S3**
Climate change	kg CO_2 eq	0.12693417	0.041325	0.152084
Ozone depletion	kg CFC11 eq	8.27E-09	2.96E-09	1.16E-08
Ionizing radiation	kBq U-235 eq	0.29775153	0.121069	0.150992
Photochemical ozone formation	kg NMVOC eq	0.000331784	9.31E-05	0.000367
Particulate matter	disease inc.	8.94E-09	2.53E-09	8.24E-09
Human toxicity, non-cancer	CTUh	2.37E-09	9.59E-10	2.61E-09
Human toxicity, cancer	CTUh	9.15E-11	3.68E-11	8.80E-11
Acidification	mol H^+ eq	0.000887955	0.000269	0.001007
Eutrophication, freshwater	kg P eq	3.98E-05	1.24E-05	4.60E-05
Eutrophication, marine	kg N eq	0.000260546	0.000109	0.000255
Eutrophication, terrestrial	mol N eq	0.001243712	0.000353	0.002443
Ecotoxicity, freshwater	CTUe	45.443029	16.15892	4.812674
Land use	Pt	2.3941605	0.482084	1.703481
Water use	m^3 depriv.	0.12773799	0.056714	0.18061
Resource use, fossils	MJ	4.8207067	1.844051	3.265418
Resource use, minerals, and metals	kg Sb eq	8.05E-07	3.49E-07	9.57E-07

The contribution analysis of S1 is presented in Figure S7. For S2 (SCNC) the contribution analysis shows that centrifugation has the highest impact (Figure S8) due to the electricity required for the centrifugation.

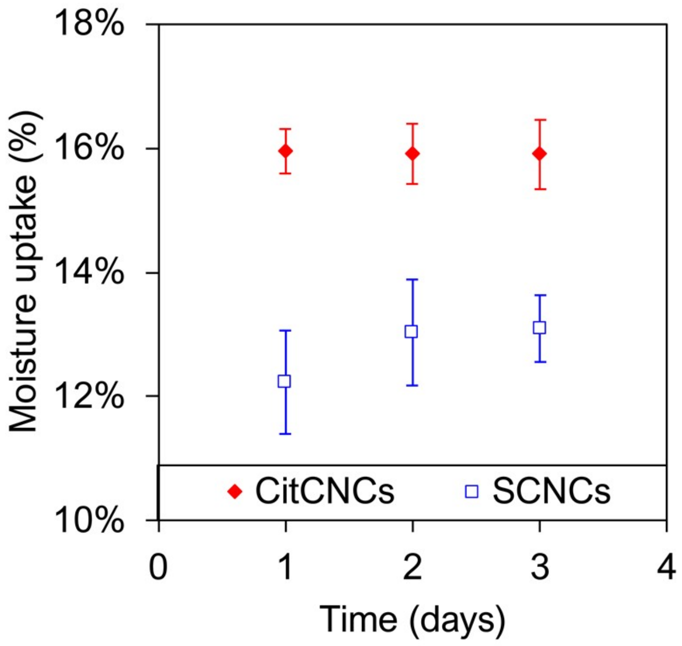


Figure S6. Moisture uptake of CNCs at a relative humidity of 84%, the error bars correspond to the standard deviation.

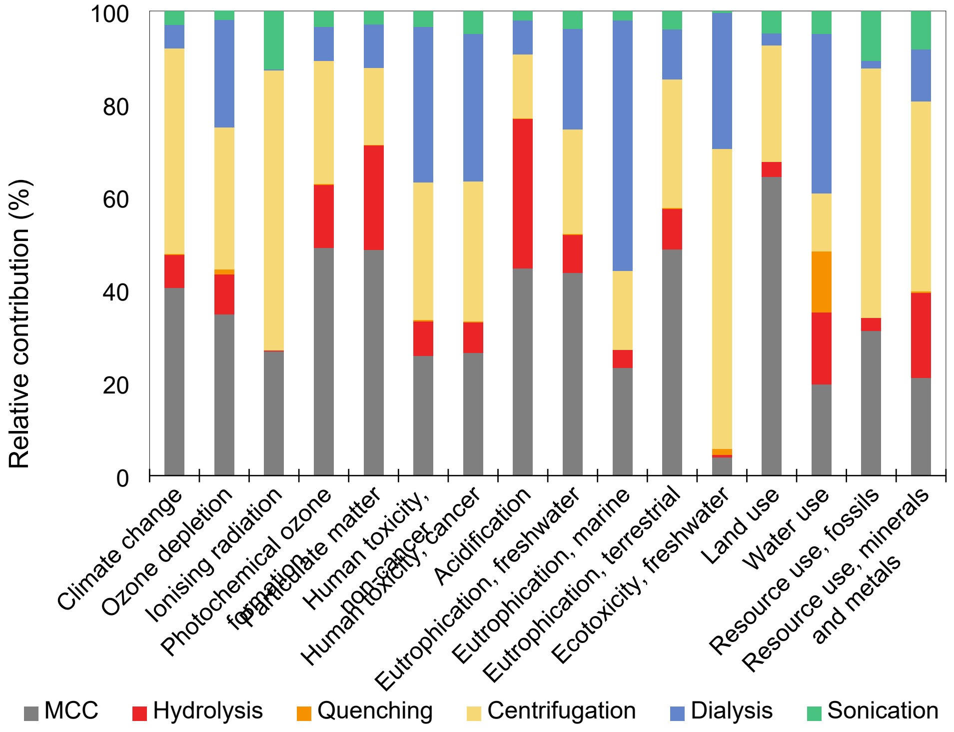


Figure S7. Contribution analysis of S1 (CNC from wood pulp) (EF 3.0 LCIA results)

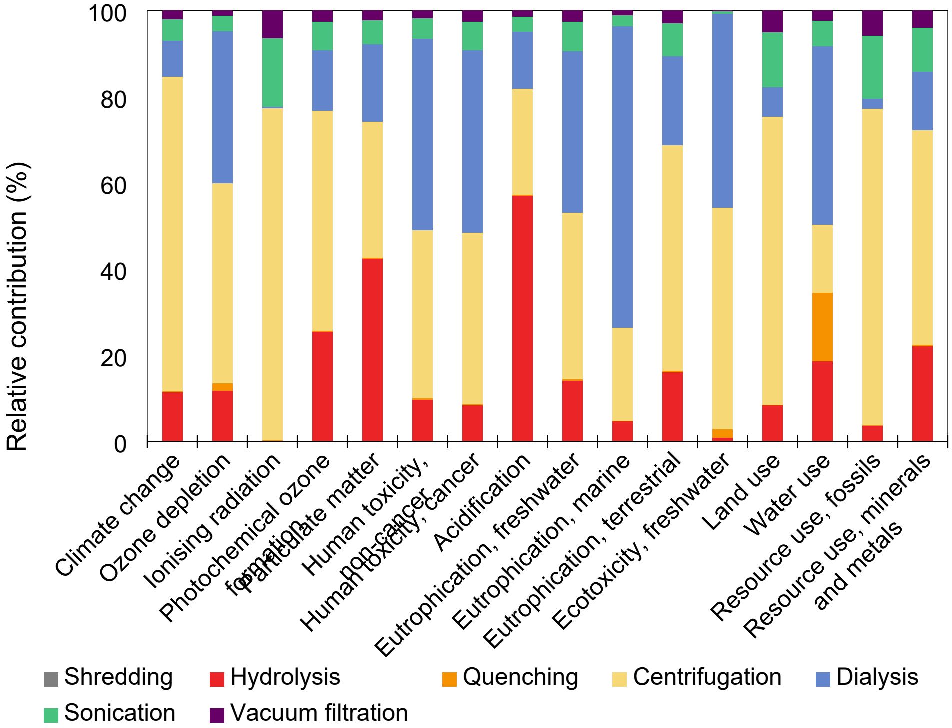


Figure S8. Contribution analysis of S2 (SCNC) (EF 3.0 LCIA results).

Sensitivity Analysis

Citric acid production, heat source, and electricity in S3 could potentially have a strong influence on the results and were included in the sensitivity analysis. Furthermore, pulp, electricity mix, transport, and citric acid recovery could also have a large influence on the results and are therefore included in the analysis. First, a perturbation analysis was performed according to Heijungs et al.[17] A sensitivity ratio (SR) was calculated for each parameter where the impact category result was recalculated for +/-10% variation of the initial parameter. This analysis showed the parameters that induced large relative variations in the scenarios. It is only a relative result and does not show anything about the uncertainty of the input value. Perturbation analysis is valuable for evaluating the sensitivity of the model to parameter uncertainties and selecting important parameters for further sensitivity analysis.

The SR values are presented in Figure S9-11. The analysis shows that citric acid has the highest SR values. All impact categories except resource use, fossil, and ionizing radiation were sensitive to a 10% increase by citric acid resulting in SRs between 0.4-0.7. The most sensitive impact category of district heating was particulate matter (0.43). The emissions of PM from district heating were one of the most important environmental factors contributing to negative health effects[18,19]. Electricity was most sensitive to resource use, fossil (SR=0.35), and ionizing radiation (SR=0.57). As expected, sulfuric acid production was most sensitive to acidification (SR=0.48) and particulate matter (SR=0.34) due to sulfur dioxide emissions during production. Quicklime was most sensitive to ozone depletion (0.41) and could potentially be replaced by limestone. Pulp was most sensitive to land use (SR=0.28) and sodium hydroxide induced negligible variation in the scenario.

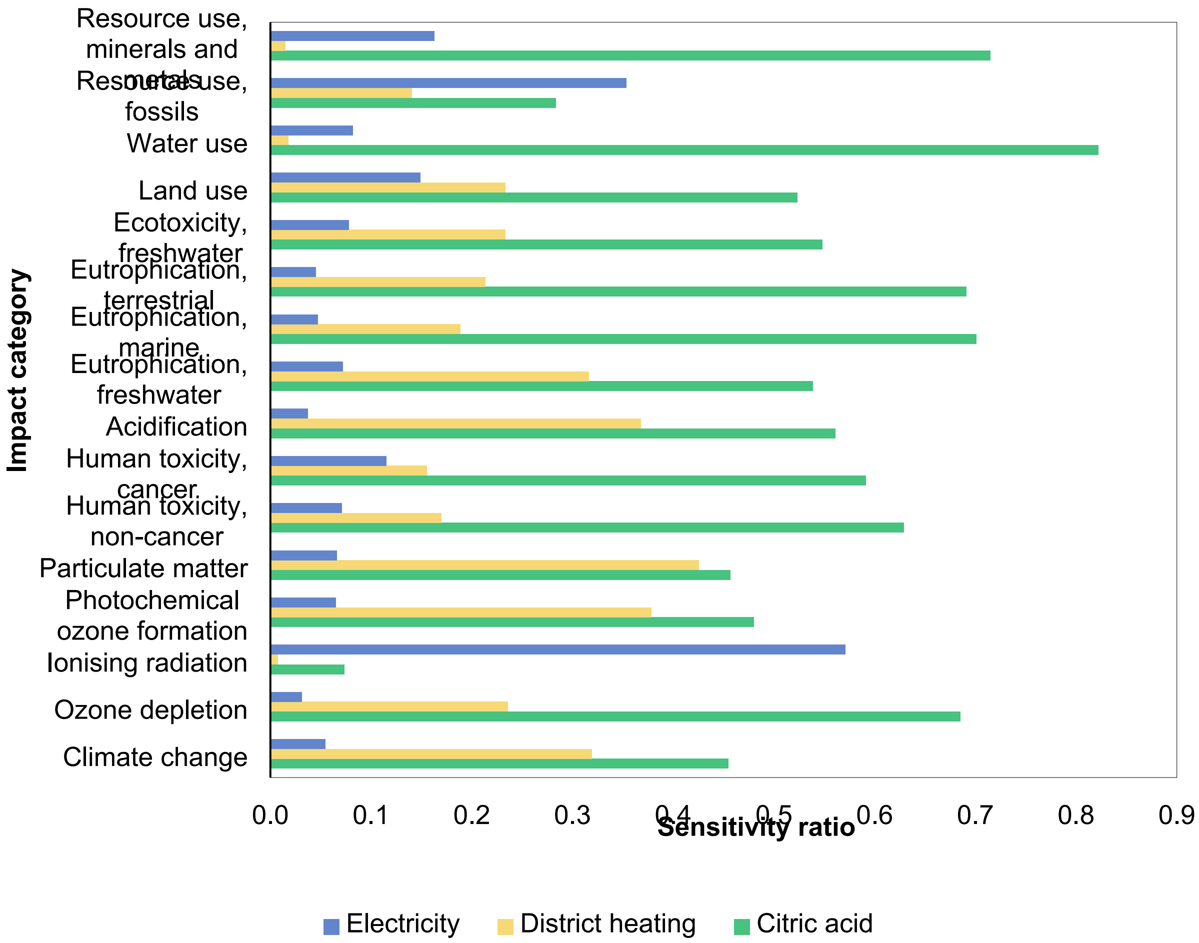


Figure S9. Perturbation analysis of citric acid, district heating, and electricity for S3 (CitCNC).

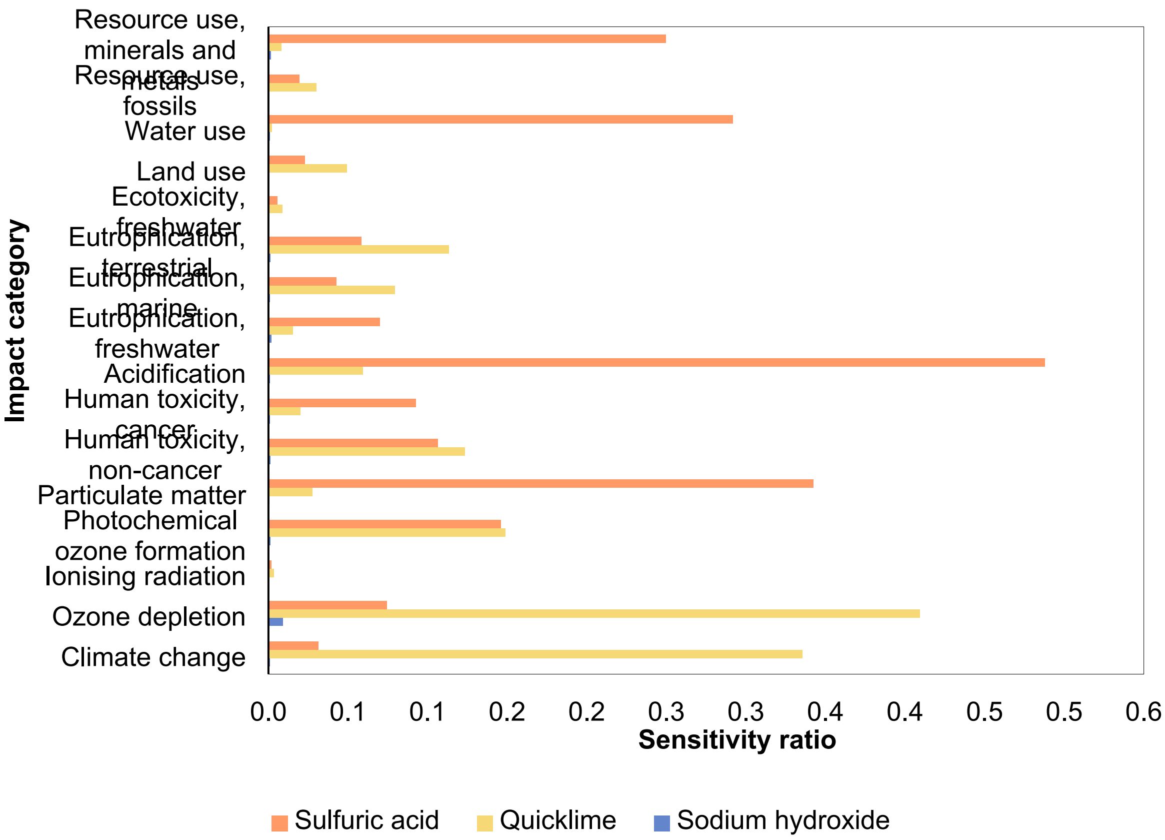


Figure S10. Perturbation analysis of sulfuric acid, quicklime, and sodium hydroxide for S2 (SCNC).

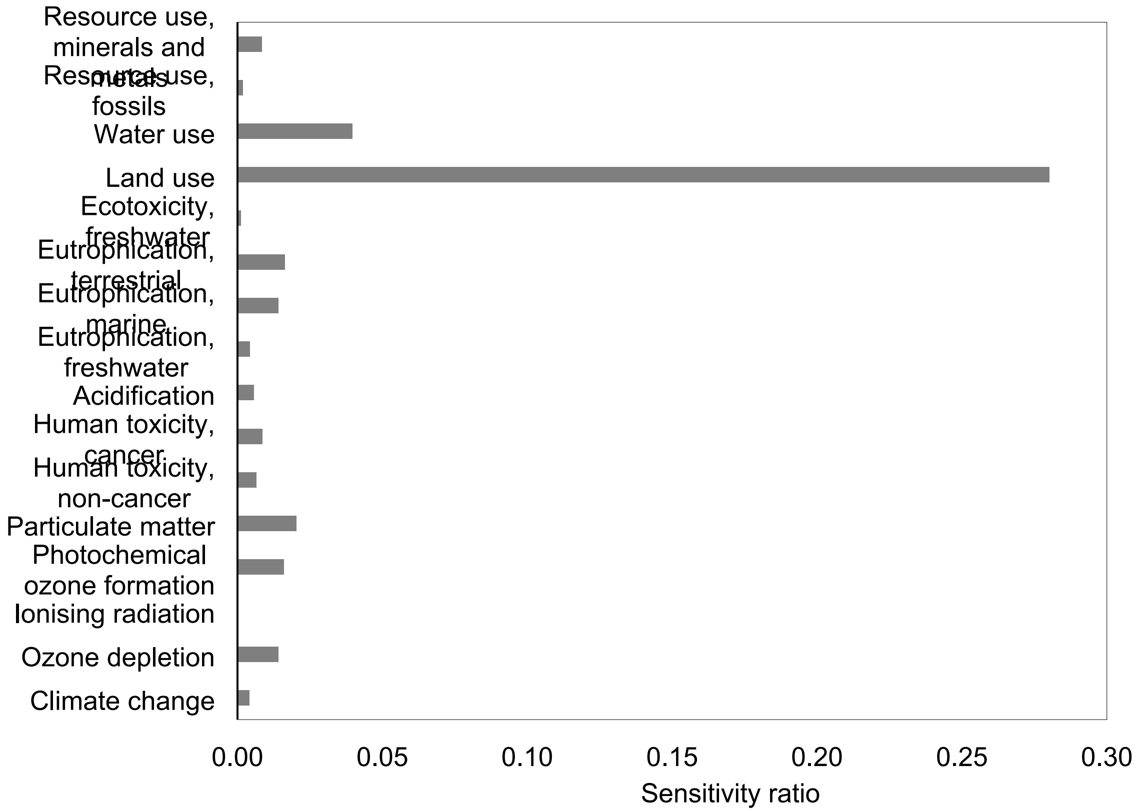


Figure S11. Perturbation analysis of the pulp for S1 (baseline)

Second, a sensitivity analysis was performed based on “what if” scenarios proposed by Börjeson et al to investigate the actual impact of the selected parameters[20]. Citric acid and transport, heat source, and electricity mix were analyzed according to the high-impact scenario. The baseline scenario was compared to the highest impact scenario of each of the parameters. High-impact scenarios of the parameters applied in this analysis were the production of citric acid in China (including transport from China to Sweden), coal gas for district heating, and a fuel oil boiler-predominated electricity mix (See Table S8). Citric acid showed changes in almost all the impact categories (except ionizing radiation) which was expected as the production was assumed to be located in China including transport from China to Sweden. Climate change is an important impact category and compared to the baseline scenario, the high-impact scenario of S3 (CitCNC), increased by 80% for citric acid production, 120% for the heat source, and 20% for electricity mix. For S1, climate change decreased only by ~1% when unbleached sulfate pulp was used compared to bleached sulfate pulp.

Table S8. Sensitivity analysis for S3 (CitCNC) a) citric acid b) electricity mix c) heating source

Impact category	**Impact change (%)**		
	Citric acid	**Electricity mix**	**Heating source**
Climate change	+80	+120	+20
Ozone depletion	+79	+37	+21
Ionizing radiation	-5	-70	-3
Photochemical ozone formation	+121	+58	+118
Particulate matter	+201	+18	+27
Human toxicity, non-cancer	+295	+42	+23
Human toxicity, cancer	+113	+23	+1150
Acidification	+110	+40	0
Eutrophication, freshwater	+86	+574	+82
Eutrophication, marine	+62	+42	+12
Eutrophication, terrestrial	+61	+35	+13
Ecotoxicity, freshwater	+129	+25	+41
Land use	+50	0	-6
Water use	+44	-11	0
Resource use, fossils	+21	+19	+24
Resource use, minerals, and metals	+428	+10	+1

Finally, a sensitivity analysis was performed on the emission of sulfuric acid to water in S2 (SCNC) assuming 100% emission according to Piccinno et al.[5,] and the ecotoxicity impact category increased by ~44%.

Citric acid recovery and its environmental impact

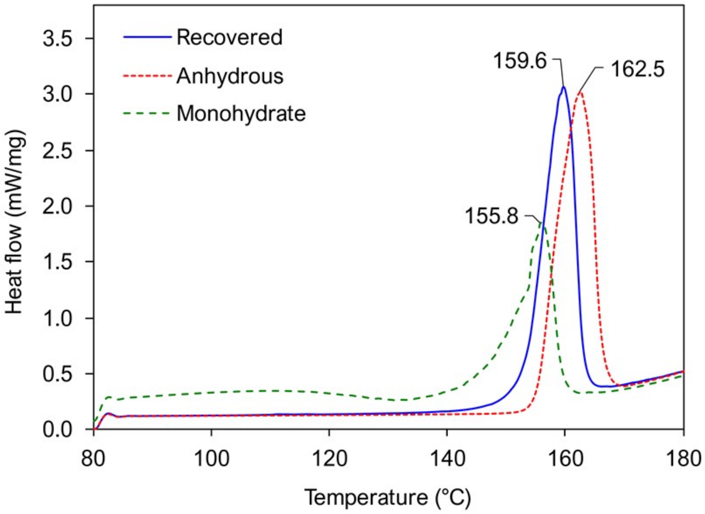


Figure S12. differential scanning calorimetry curves of recovered citric acid (solid line) and citric acid anhydrous and monohydrate (dashed line)

One method to calculate the purity of the recovered citric acid is by considering it an ideal eutectic mixture. By definition, a eutectic system has a lower melting point than its constituents, and according to the Schroder-van Laar equation, the molar fraction can be calculated as:

$$\ln x = \frac{\Delta H_{fus}}{R}\left(\frac{1}{T_{fus}} - \frac{1}{T}\right)$$

Where:

x = Mole fraction of citric acid
ΔH_{fus} = Molar enthalpy of fusion of pure citric acid: 43.92 kJ/mol
T_{fus} = Melting temperature (onset point) of pure citric acid: 428.4 K
R = Gas constant: 8.3144 J mol^{-1} K^{-1}
T = Melting temperature (onset point) of recovered citric acid

The molar enthalpy and the melting temperature of pure citric acid were obtained from the DSC curve of the citric acid used in this study (99 wt% Thermo Scientific™), and T from three different batches recovered: T_1 = 425.0 K, T_2 = 425.6 K, and T_3 = 425.5 K.

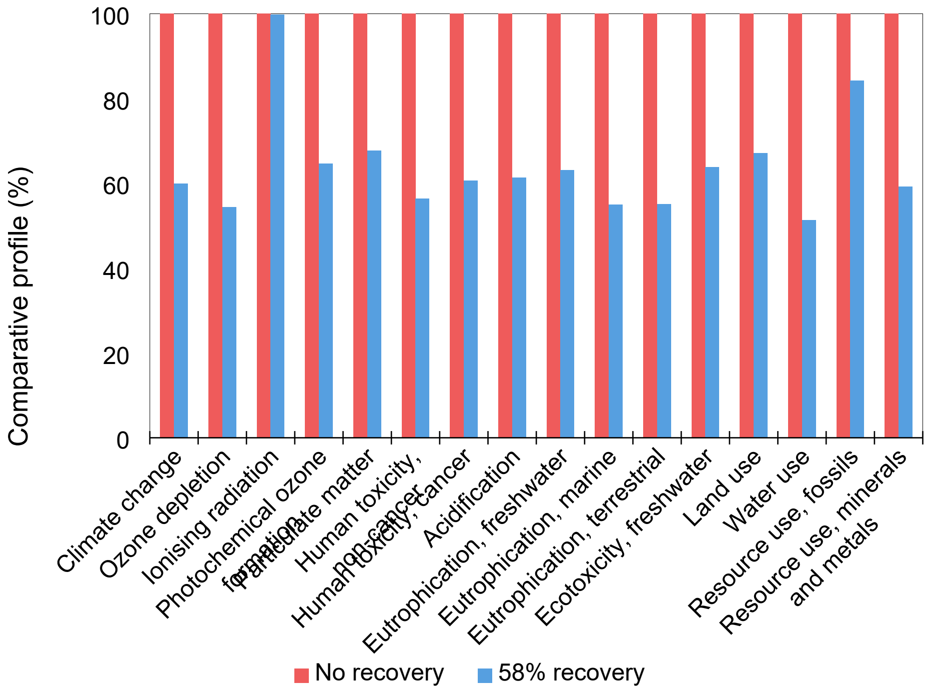


Figure S13. Environmental impact (EF 3.0 LCIA method) of S3 (CitCNC) with and without recovery of citric acid (58 w/w%) citric acid.

REFERENCES

1. Becker, M., Kohlheb, N., Hunger, S., Eschrich, S., Müller, R., and Aurich, A., Early-stage sustainability assessment of biotechnological processes: A case study of citric acid production. *Eng Life Sci*, 2020, **20**, 90–103.

2. Leppänen, K., Andersson, S., Torkkeli, M., Knaapila, M., Kotelnikova, N., and Serimaa, R., Structure of cellulose and microcrystalline cellulose from various wood species, cotton and flax studied by X-ray scattering. *Cellulose*, 2009, **16**, 999–1015.

3. Swiss center for life cycle inventories, Ecoinvent version 3. 2022.

4. Vanhatalo, K. M. and Dahl, O. P., Effect of mild acid hydrolysis parameters on properties of microcrystalline cellulose. *Bioresources*, 2014, **9**, 4729–4740.

5. Piccinno, F., Hischier, R., Seeger, S., and Som, C., From laboratory to industrial scale: a scale-up framework for chemical processes in life cycle assessment studies. *J Clean Prod*, 2016, **135**, 1085–1097.

6. Duan, C., Peng, Y., Fu, S., and Lucia, L., Investigation on the recycling of sulfuric acid from pretreatment of cellulose for nanocellulose preparation. *Tappi J*, 2020, **19**, 103–111.

7. Husgafvel, R., Vanhatalo, K., Rodriguez-Chiang, L., Linkosalmi, L., and Dahl, O., Comparative global warming potential assessment of eight microcrystalline cellulose manufacturing systems. *J Clean Prod*, 2016, **126**, 620–629.

8. Kunzler, J. E. and Giauque, W. F., Aqueous Sulfuric Acid. Heat Capacity. Partial Specific Heat Content of Water at 25 and -20° [1]. *J Am Chem Soc*, 1952, **74**, 3472–3476.

9. de Kruif, C. G., van Miltenburg, J. C., Sprenkels, A. J. J., Stevens, G., de Graaf, W., and de Wit, H. G. M., Thermodynamic properties of citric acid and the system citric acid-water. *Thermochim Acta*, 1982, **58**, 341–354.

10. Wang, J., Cui, Z., Li, Y., Cao, L., and Lu, Z., Techno-economic analysis and environmental impact assessment of citric acid production through different recovery methods. *J Clean Prod*, 2020, **249**, 119315.

11. Bates, R. G. and Pinching, G. D., Resolution of the Dissociation Constants of Citric Acid at 0 to 50°, and Determination of Certain Related Thermodynamic Functions. *J Am Chem Soc*, 1949, **71**, 1274–1283.

12. Werner, S., District heating and cooling in Sweden. *Energy*, 2017, **126**, 419–429.

13. Socrates, G., *Infrared and Raman Characteristic Group Frequencies: Tables and Charts* 3rd ed. Wiley, 2004.

14. Silverstein, R. M., Webster, F. X., and Kiemle, D., *Spectrometric Identification of Organic Compounds* 7th ed. Wiley, 2005.

15. Peets, P., Leito, I., Pelt, J., and Vahur, S., Identification and classification of textile fibres using ATR-FT-IR spectroscopy with chemometric methods. *Spectrochim Acta A Mol Biomol Spectrosc*, 2017, **173**, 175–181.

16. CHUNG, C., LEE, M., and CHOE, E., Characterization of cotton fabric scouring by FT-IR ATR spectroscopy. *Carbohydr Polym*, 2004, **58**, 417–420.

17. Heijungs, R. and Kleijn, R., Numerical approaches towards life cycle interpretation five examples. *Int J Life Cycle Assess*, 2001, **6**, 141.

18. Sippula, O., Hokkinen, J., Puustinen, H., Yli-Pirilä, P., and Jokiniemi, J., Particle Emissions from Small Wood-fired District Heating Units. *Energy & Fuels*, 2009, **23**, 2974–2982.

19. Ghafghazi, S., Sowlati, T., Sokhansanj, S., Bi, X., and Melin, S., Particulate matter emissions from combustion of wood in district heating applications. *Renewable and Sustainable Energy Reviews*, 2011, **15**, 3019–3028.

20. Börjeson, L., Höjer, M., Dreborg, K.-H., Ekvall, T., and Finnveden, G., Scenario types and techniques: Towards a user's guide. *Futures*, 2006, **38**, 723–739.