Process scale up and characterization of wood cellulose nanocrystals hydrolysed using bioethanol pilot plant

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ABSTRACT

The paper discusses the isolation of cellulose nanocrystals (CNC_{BE}) from wood resources by integrating the processing with pilot-scale bioethanol processing unit. The nanocrystals were isolated from cellulose obtained by acid pretreatment of wood chips in a bioethanol pilot-scale facility, followed by a series of chemical processes and subsequent homogenization using a lab-scale homogenizer. The isolated nanocrystals had diameters of 5–15 nm, cellulose I crystalline structure and formed a thick semi-transparent gel at low concentration (2 wt%). XPS data showed that these nanocrystals had predominantly O=C–O surface groups which also contributed to its high negative zeta potential. Casted CNC_{BE} films showed excellent mechanical performance (200 MPa of strength, 16 GPa of modulus) and transparency and were also found to be cytocompatible. The developed process route resulted in high-quality nanocellulose crystals with a yield of 600 g/day.

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1. Introduction

Nanocellulose was isolated from natural resources following a top-down approach, taking advantage of its natural hierarchical structure. Isolation and use of biobased nanomaterials has been an area of great interest in materials science research during the last few decades.

Since the first reports on cellulose nanocrystal isolation from wood pulp by Rånby in the 1950s, several natural resources like wheat straw, crab shell, tunicates, plant fibres, etc., have been used by researchers to prepare nanocrystals via chemical processes (Rånby, 1952; Dufresne, 2008; Marchessault, 1959; Samir, 2005; Eichhorn et al., 2010; Mathew et al., 2009; Siro and Plackett, 2010). The chemical process involves the removal of the amorphous region from cellulose using either sulphuric acid or hydrochloric acid and subsequent neutralization and individualizing of nanocrystals using ultrasonication. The earlier reports also point to the fact that the ease of isolation, yield of the isolation process as well as the dimensions of the isolated nanocrystals depend on the raw material source. Wood can provide good yield compared to lower plants and agricultural sources, as wood has about 40% cellulose content (Hon, 1996; Han and Rowell, 1996). Furthermore, refined cellulose raw material from industry which can be directly isolated

to cellulose nanocrystals without any specific purification step can provide higher yield. In this context, raw material sources of interest were wood pulp, MCC, etc, which are commercial grades of cellulose with high purity. Many studies report the use of these commercial cellulose sources as starting material for processing of cellulose nanocrystals and nanofibres with high yield efficiency (Edgar and Gray, 2003; Bondeson et al., 2006). Our earlier study on MCC (Bondeson et al., 2006) resulted in processing of CNC at a rate of 50 g/week (with 30% yield w.r.t. initial weight) and provided an improved yield compared to processing of CNC from bioresources like tunicates, wheat straw, etc.

In the recent years, there have been increased efforts to scale up the processing and enhance the efficiency and economy of nanocellulose isolation. CNC isolation in large scale has been reported in recent years because economical and efficient processing of CNC is essential for utilizing CNC as reinforcement or functional additive in commercial products. Most of these attempts to scale up processing have been made in Canada and the USA. For example, FP Innovations Canada reported a production rate of 3 kg/day, CelluForce Canada 1000 kg/day and FPL, US Forest Services 50 kg/week, following a sulphuric acid hydrolysis process (FutureMarkets, 2012; Chauve and Bras, 2014).

Bioresidues and industrial side-streams are of interest in this context and Oksman and co-workers have reported the use of different by-products or residues from forest industries as potential sources for nanocellulose production economically or even at negative cost (Oksman et al., 2011; Jonoobi et al., 2012; Herrera et al.,

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2012a,b). Cellulose nanocrystals were also isolated from bioethanol residue by mechanical treatments like homogenization or sonication and showed similar properties as nanocrystals isolated from other sources by acid hydrolysis (Oksman et al., 2011; Herrera et al., 2012b). These raw materials were considered as potential sources for isolation of CNCs in large scale.

The current study aims to prepare nanocellulose in bench scale by integrating the lab-scale process with pilot-scale processes existing in wood-based industries. The cellulose obtained from acid hydrolysis using a pilot-scale bioethanol unit was isolated into nanocellulose by mechanical processes in the laboratory. The isolation efficiency, scaling-up potential and quality of final product were evaluated. The properties of the isolated materials and their potential applications in reinforced composites or functional materials are evaluated using mechanical testing, surface characteristics, transparency and cytocompatibility studies. It is expected that scaled-up production of nanocellulose from residues economically and efficiently, with good mechanical and functional properties will facilitate the commercial scale processing of biobased nanostructured products.

2. Experimental

2.1. Materials

Unbarked wood chips of Norway spruce (*Picea abies*) with a dry matter content of 50–55% were used as the raw material for the processing of cellulose nanocrystals (CNC) following the bioethanol processing route at the pilot-scale facility at SEKAB, Örnsköldsvik, Sweden.

The other chemicals used, NaOH, NaClO₂, toluene, sulphuric acid (96%), etc., are of reagent grade and were purchased from VWR, Sweden.

2.2. Methods

2.2.1. Isolation of cellulose nanocrystals

The isolation process is summarised in Fig. 1. Unbarked wood chips of Norway spruce (*Picea abies*) with a dry matter content of 50–55% were treated in a continuous mode with sulphur dioxide in a 30 l reactor at a temperature of 200 °C, at a pressure of 20 bars, and with a residence time of 7 min. 3% (w/w) of SO₂ was used as catalyst. The pH after pre-treatment was about 2 and the total solids content was 28–30%. The slurry was then diluted with water to facilitate pumping and the water insoluble solids content of the diluted slurry was about 17%. The solid material of the slurry was then separated from the liquid using a membrane filter press. The solid material was stored in plastic bags at 4 °C for subsequent use.

The product obtained had cellulose and high amount of lignin and some extractives, which was refined to pure cellulose following TAPPI test method T204 for extraction of cellulose (TAPPI T204).

The first step to purify the solid matter obtained from the bioethanol plant, was the Soxhlet extraction for 6 h at 150 °C using toluene/acetone mixture (2:1 ratio), where by extractives were removed. This material was then bleached using a mixture of 700 ml deionised water, 1.5 ml acetic acid, 6.7 g sodium chlorite, at 70 °C for 12 h. Four further additions of acetic acid and sodium chlorite were made every 2 h and kept for another 12 h, where by a pure white material was obtained. After 24 h of bleaching the materials were washed with excess of deionized water and concentrated to 17 wt% solid content by centrifugation. The FTIR data of the product obtained after this process showed typical cellulose structure with peaks at 3350 cm⁻¹, 2929 cm⁻¹, 1312 cm⁻¹ and 1057 cm⁻¹ of C–H stretching, C–H bending and C–O stretching bonds of cellulose (spectrum not shown), being also verified in our earlier report

where cellulose was isolated from bioethanol residue following this process route (Oksman et al., 2011).

The purified cellulose was made into 2 wt% suspensions, mixed well with shear mixture and passed through the APV 2000 high-pressure homogenizer (Denmark) at a pressure of 500 bars. The suspension with a batch size of 2 l was passed through the homogenizer 10 times to obtain a thick gel of nanocrystalline cellulose, and the processing time was 40 min per batch. This product obtained is denoted as CNC_{BE}.

CNC was also prepared from wood pulp by sulphuric acid hydrolysis in lab-scale, following the procedure reported by Bondeson et al. (2006) and was used as reference. The sulphuric acid hydrolyzed nanocrystals prepared in lab-scale is denoted as CNC_{H₂SO₄}.

2.2.2. Nanofilms and nanopaper preparation

Thin films and nanopaper mats of CNC_{BE} were prepared using (a) casting and (b) filtration and pressing processes, respectively.

Casted films were prepared by drying 40 ml of 1% suspensions of CNC under ambient conditions for approximately 48 h in petri dishes. The resultant films had thicknesses in the range of 100–200 microns.

Nanopaper mats were prepared by vacuum filtration of 120 ml of 0.5% suspension using Whatman filter paper (Pore size number 3 and 125 mm diameter) at –1.0 bar. The CNC filter cakes were dried in ambient temperature under 30 N load for 3 days to keep the nanopaper wrinkle free. The resultant nanopaper mats had thicknesses in the range of 600–700 microns.

CNC_{H₂SO₄} films were prepared only via the casting method, due to experimental difficulties with the filtration and pressing process with this material.

2.3. Characterization

Optical microscopy images of the nanoparticle suspensions were collected using a Leica Optical Microscope using suspension of 0.1 wt%. An atomic force microscope, Nanoscope V, Veeco Instruments (Santa Barbara, CA, USA), was used to examine the morphologies of the CNC_{BE}. A drop of diluted suspensions of each sample was deposited onto freshly cleaved mica and left to dry at room temperature. All the samples were imaged in tapping mode. Height, amplitude and phase images were recorded. The instrument was operated at a resonance frequency of 350 kHz and a spring constant of 10–200 nm⁻¹. The diameter measurements were conducted by Nanoscope V software.

Viscosity of the CNC gels were measured using a Vibro Viscometer (SV 10/SV 100, A&D Company, Ltd, Japan) at 22 °C. Measurements were recorded until the viscosity values were stable and the values were reported after 30 min.

The thermal stability of the nanomaterials was studied by thermogravimetric analysis Q500 (TGA), TA Instruments (New Jersey, USA), at a temperature range of 30–500 °C in an air atmosphere. The temperature was increased at a rate of 10 °C/min and the sample weight was 5–10 mg. All thermal stability curves show the calculated average values and each sample was tested in triplicate.

The zeta potentials of the materials at different pH were measured using a Zetasizer nano ZS, Malvern, at 25 °C. The concentrations of all measured samples remained the same at 0.05 wt%.

X-ray photoelectron spectroscopy (XPS) was used to understand the surface groups on the produced nanocrystals. All XPS spectra were collected with Axis Ultra DLD electron spectrometer (Kratos Analytical Ltd, UK) using monochromatized Al K α (1486.6 eV) radiation. Survey spectra were collected from 1100 to 0 eV at pass energy of 160 eV. High resolution C 1s, O 1s, F 1s and Si 2p spectra were collected at pass energy of 20 eV with a scan step of 0.1 eV. In order to minimize possible X-ray induced degradation

Fig. 1. Schematic representation of the processing of CNC_{BE} and the gel obtained after the process.

of the samples, C 1s and O 1s spectra were measured within first 10 min of exposure. Processing of the spectra was accomplished by Kratos software. High-resolution XPS spectra were fitted using linear combinations of 70:30 Gauss–Lorentzian functions on Shirley background-subtracted spectra. Binding energy (BE) scale was calibrated using aliphatic C 1s component, set at 285.0 eV.

Casted films of CNC were analyzed at the same temperature by step scanning on the Siemens diffractometer D5000 (Berlin, Germany) to understand the crystallinity of the nanomaterials. The angle of incident monochromatic X-ray was in the range of $2\Theta = 10^\circ$ – 30° with a step size of 0.0263. The wavelength of the monochromatic X-ray was 1.540598 Å ($K\alpha 1$). The percentage crystallinity is calculated using Segals equation (Segal et al., 1959).

$$CI(\%) = \frac{I(22^\circ) - I(18^\circ)}{I(22^\circ)} \times 100\% \quad (1)$$

CI(%) in the equation (1) stands for crystalline index of cellulose. $I(22^\circ)$ (arbitrary units) represents the peak diffraction intensity corresponding to crystalline cellulose and $I(18^\circ)$ is the peak diffraction intensity corresponding to the amorphous sections in cellulose, at 22° and 18° respectively.

Tensile tests were performed using strips with dimensions of 5 mm × 50 mm on a universal testing machine, Shimadzu Autograph AG-X (Japan); with a load cell of 1 kN. The gauge length was 20 mm, at a strain rate of 2 mm/min. The elastic modulus was calculated from the initial part of the slope from stress–strain curves. At least five tests were performed for each sample and the average values are presented.

Transparency tests were performed using a UV-visible spectrophotometer (Perkin Elmer, Lambda 2S). CNC films of

approximately 50 × 10 mm were cut and fitted in the sample holder and transmittance was measured over the wavelength region from near ultraviolet (300–400) to visible light (400–800 nm). An empty sample holder was used as a reference. The films were also photographed digitally, against a printed surface to visually observe and compare transparency.

Biocompatibility of CNC and CNF casted films was studied by direct contact test systems. The material was fixed to cell culture dish and the cells (adipose derived stem cells and cell line L929) were seeded evenly throughout the cell culture dish. The impact of the biomaterial on cell growth and morphology was monitored and documented with photographs up to 15 days.

3. Results and discussion

3.1. Isolation process

Fig. 1 shows the scheme of the integration of the CNC_{BE} isolation process with the bioethanol pilot-scale production unit. In the case of bioethanol process after the acid hydrolysis step the liquid fraction of dissolved hemicellulose and amorphous cellulose is collected, where as in the case of CNC_{BE} process, the solid residue stream is collected for further processing. The solid residue obtained was purified to cellulose by bleaching and solvent extraction and thereafter isolated to nanocrystals by a simple mechanical process of homogenization. It may be noted that further acid hydrolysis is not required to obtain CNC_{BE} because it was done at the first step of bioethanol processing. Here the main advantage over starting from pulps or other purified cellulose sources as microcrystalline cellulose is that when acid hydrolysis occurs in

Fig. 2. Images of the studied materials from macro to nanoscale. (a) Photographs of wood chips (b) OM images of wood particles after mild acid hydrolysis (c) OM image after chemical refining (d) OM image after homogenization to nanocrystals and (e) the AFM image of isolated nanocrystals.

the bioethanol pilot-scale unit, the material handled in each step is significantly higher and difficulties related to handling of acids and neutralization in the lab is avoided.

The acid hydrolysis of wood using a bioethanol pilot-scale unit provided materials in large quantities for further processing to cellulose. The yield of the acid hydrolysis step was 46%. After the solvent extraction step to remove extractives, 85% yield was observed and 71–77% yield was recorded after the bleaching step, which removed lignin. This corresponds to 30% pure cellulose with respect to the wood chips at the start. CNC_{BE} isolation by homogenization process resulted in a 100% conversion rate with respect to purified cellulose.

Optical microscopy (OM) and atomic force microscopy (AFM) were used as a tool to study the extent of isolation of the raw material to nanoscaled cellulose and are given in Fig. 2. Figures on the left (Fig. 2a) show the starting materials used. Fig. 2b shows the OM image of the wood particles after the acid hydrolysis step in the pilot-scale facility and OM images of purified wood cellulose (after solvent extraction and bleaching) used for isolating

the nanoparticles are shown in Fig. 2c. Fig. 2d shows the OM of the suspension obtained after the homogenization, where microscaled particles are not visible, indicating that the isolation process has been effective in preparing nanoscaled materials. Atomic force microscopy (AFM) in Fig. 2e gives the nanoscaled information of the isolated materials. The isolated CNC_{BE} had diameters in the range of 5–15 nm. Accurate length determination was not possible from AFM images. The material mostly showed nanocrystal morphology, though some longer nanofibril like structures were also present to a limited extent.

The viscosity of the suspensions increased significantly during mechanical isolation of CNC_{BE} and formed thick gels at low concentrations (see Fig. 1). The viscosity of the CNC_{BE} suspensions at 2 wt% increased from 5 mPa s before grinding to 1440 mPa s after 10 passes through the homogenizer. Gel formation at low concentration is an indication of large surface area, which in turn suggests isolation into nanoscaled structures. CNC_{BE} gels at 2 wt% concentrations showed a semi-transparent nature (see Fig. 1).

Fig. 3. XRD, TGA and zeta potential data of CNC_{BE}.

3.2. Physical properties

The CNC_{BE} films showed high crystallinity and well defined peaks were visible in the X-ray diffractograms (See Fig. 3a). CNC diffractogram showed peaks at $2\theta = 14.1^\circ$, 16.2° and 22.3° , indicating typical cellulose I structure (Krässig, 1996; Klemm et al., 2005). The percentage crystallinity calculated using Segals's equation from the XRD curves was found to be 77% for CNC. Higher crystallinity of CNC indicates that the process of nanocrystal isolation was efficient to remove amorphous regions. This value is comparable to CNC crystallinity values obtained by acid hydrolysis of MCC (Herrera et al., 2012a; Bondeson et al., 2006).

CNC_{BE} is found to be thermally stable until 240°C (see Fig. 3b) and has higher stability than sulphuric acid hydrolyzed CNC. CNC isolated in the current study by homogenization does not have sulphate groups on the surface that induce the degradation of cellulose and therefore has better thermal stability than traditional acid hydrolyzed nanocrystals. (Roman and Winter, 2004; Bondeson et al., 2006; Herrera et al., 2012a,b).

Zeta potential of the prepared nanomaterial suspensions as a function of pH is shown in Fig. 3c. The zeta potentials of CNC_{BE} remained negative in the studied pH range, which was attributed to negative surface charge, possibly from carboxyl groups. The zeta

Table 1

A comparative study of C 1s and O 1s spectrum of CNC_{SL} and CNC_{BE}.

	CNC _{BE}		H ₂ SO ₄		
	BE, eV	AC, at. %	BE, eV	AC, at. %	
C 1s	285.0	10.62	285.0	24.17	C-(C, H)
	286.6	40.86	286.6	37.2	C-OH
	288.0	9.57	288.1	4.47	O-C-O
	289.2	2.21	289.5	3.45	COOH
O 1s	533.0	35.28	532.0	23.87	

potential was more negative for CNC_{BE} compared to CNC prepared by sulphuric acid hydrolysis in our laboratory (see the dotted lines in Fig. 3c for comparison).

Survey XPS spectra of treated CNC_{BE} and CNC_{H₂SO₄} show different core-level photoelectron peaks attributed to C and O atoms are the main components. Theoretically, pure nanocellulose exhibits two peaks (around 533 and 285 eV) in the full XPS spectra, which correspond to oxygen and carbon, respectively (Siqueira et al., 2010). Both these peaks are present in XPS spectra for CNC_{BE} and CNC_{H₂SO₄} shown in Fig. 4. Table 1 illustrates the introduction of more oxygen at 533 eV in CNC_{BE} compared to CNC_{H₂SO₄} indicating the more oxygen containing moieties on CNC_{BE} surface. Further examination of full XPS spectra of CNC_{H₂SO₄} (Fig. 4b) confirmed the presence of traces of sulfur in the spectrum, S 2p at approximately 165 eV and S 2s at approximately 230 eV (detectable only in detailed analysis and not visible here) and its absence in CNC_{BE}. This indicates the presence of $-\text{SO}_3^-$ groups on CNC_{H₂SO₄} as expected. A small amount of Na and F were also detected, which might be impurities.

The main C1s signals for both nanocrystals are decomposed and are shown in the inset. Theoretically, the deconvolution of C1s signal for pure cellulose should exhibit two peaks associated to C-O of alcohols and ethers groups and O-C-O for acetal moieties while in practice the XPS analysis of cellulose always reveals four C peaks: C-C/C-H (C1, 285 eV), C-OH (C2, 286.6 eV), O-C-O (C3, 288.0 eV) and COOH (C4, 289.2 eV) (Siqueira et al., 2010) (see Fig. 4 and Table 1). Both CNC_{BE} and CNC_{H₂SO₄} in Fig. 4 (inset) showed all peaks corresponding to carbon atoms C1, C2, C3 and C4. The magnitude of C2 and C3 peaks is however higher in CNC_{BE}, which indicate the presence of more C-OH and O=C-O on the surface of CNC_{BE} compared to CNC_{H₂SO₄}. The XPS study shows that CNC_{BE} has more surface groups with as carboxyl and carbonyl groups probably introduced during weak acid hydrolysis and the subsequent bleaching steps.

The O=C-O groups on CNC_{BE} can be considered responsible for its negative zeta potential where as SO_3^- and O=C-O on CNC_{H₂SO₄} contribute to its negative zeta potential. However, the O=C-O groups on CNC_{BE} is significantly higher (almost double) compared to CNC_{H₂SO₄}, which explains the high negative zeta potential of CNC_{BE}. Our recent studies also showed that negatively charged nanocellulose are capable of preferentially adsorbing positively charged entities as heavy metal ions or dyes from water (Liu et al., 2014; Karim et al., 2014) and therefore CNC_{BE} with high negative zeta potential have potential as functional nanomaterial in water cleaning.

Table 2

Mechanical properties of films and mats of the prepared materials.

Material	Stress (MPa)	Strain (%)	Young Modulus (GPa)
CNC _{BE} (Casting)	204.64 $\pm$ 20.06	2.23 $\pm$ 0.57	16.11 $\pm$ 1.33
CNC _{BE} (Filt and press)	97.58 $\pm$ 8.29	2.47 $\pm$ 0.59	7.25 $\pm$ 1.18

Fig. 4. XPS survey spectra of a) CNC_{BE} and CNC_{H₂SO₄} and the representative C1s photoemission spectra (inset).

Fig. 5. Stress-strain curves of the films and nanopapers of CNC_{BE}.

3.3. Mechanical properties

Tensile properties of the mats and films prepared from the nanomaterials were evaluated to understand the effect of the processing methods on the mechanical properties. The mechanical properties are also considered as an indirect measure of the degree of isolation to nanoscale. The data obtained are given in Table 2 and the stress-strain curves are shown in Fig. 5.

CNC_{BE} showed the best mechanical performance of all the studied materials. The casted films had strength as high as 204 MPa and modulus of 16.11 GPa. The strain at break was similar for CNC_{BE} materials prepared by casting as well as filtration and pressing. The big drop in tensile strength and modulus for the CNC_{BE} prepared by filtration and pressing compared to casted films was unexpected. One possible explanation for the higher mechanical strength for casted films of CNC may be due to less voids, which resulted from slow and organized compacting of nanoparticles during evaporation compared to filtered ones. The tensile data of CNC_{H₂SO₄} are

Fig. 6. Photographs of films of (a) CNC_{BE} (Casting) (b) CNC_{BE} (Filtration & pressing) (c) CNC_{H₂SO₄}, comparing the optical properties.

not presented here, due to the high brittleness of the films which resulted in unreliable values with high standard deviation.

3.4. Optical properties

The optical properties of the CNC_{BE} films (Fig. 6a) and mats (Fig. 6b) are shown in Fig. 6 and were compared with CNC_{H₂SO₄} (Fig. 6c). All the films showed transparency, but the highest transparency was observed for CNC_{H₂SO₄}. UV-visual data at 540 nm, showed 58% transparency for CNC_{H₂SO₄}, while it decreased to the range of 16 and 12%, respectively, for CNC_{BE} casted and CNC filtered and pressed (curves not shown). The reason for lower transparency for CNC from bioethanol process was attributed to nanocrystals with lengths higher than wavelength of light (Herrera et al., 2012b). It was also noted that casting resulted in films with better

transparency than filtration and pressing, even though the differences were minimal. The higher transparency for casted films may be due to less voids and compacted films and is in agreement with the mechanical data discussed in Section 3.3. However, in general, the transparency values obtained are lower than those found in some earlier reports for nanocellulose (Herrera et al., 2012b; Uetani and Yano, 2011).

3.5. Cytocompatibility

Fig. 7 shows the cell adhesion and growth of primary human cells and L929 on negative control and CNC_{BE}. The films surfaces of CNC_{BE} showed the growth of cells after 15 days of incubation indicating its non-toxic and cytocompatible nature. The study shows

Fig. 7. Images showing adhesion and growth of primary human cells and L929 cells on the negative control and films of CNC_{BE} after 15 days of incubation.

the potential of CNC_{BE} in biomedical products and implants where cell viability is crucial.

3.6. Process efficiency

The adopted processing route resulted in scaled-up production of CNC in the lab. The processing route achieved CNC_{BE} yield in the range of 600 g/day (excluding the pilot-scale acid hydrolysis process and the cellulose refining step), which is a major improvement compared to 50 g/week in lab-scale processing.

The energy consumption for the mechanical isolation step at laboratory scale was estimated to be 30 kWh/kg for CNC_{BE}. The total energy consumption for the scaled-up process including industrial/pilot-scale steps is not discussed here and will be reported in detail separately.

4. Conclusions

The isolation of cellulose nanocrystals from wood chips via bioethanol pilot-scale unit resulted in CNC_{BE} processing in the range of 600 g/day.

CNC_{BE} produced by the scaled-up processes had cellulose I structure and negative zeta potential being related to the surface groups providing interactions with positively charged entities. The CNC_{BE} films had mechanical strength as high as 204 MPa and modulus of 16 GPa, showing their potential as nanoreinforcement. The nanocrystals were also found to be cytocompatible towards primary human cells and L929 cells indicating potential in biomedical applications.

The new process route developed can be adopted easily by research laboratories and small-scale industries to produce nanocrystals in larger scale at the point of use, via mechanical isolation of cellulose from bioethanol production.

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