

Supporting Information

Enhancing aqueous dispersibility of uncharged cellulose through biosurfactant adsorption

Thuy-Linh Phi,^{a,b} Wenyang Xu,^a Petra Pernot,^c Niki Baccile,^{b,} Eero Kontturi^{a,*}*

^a Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University, P.O. Box 16300, 00076 Aalto, Finland

^b Sorbonne Université, Centre National de la Recherche Scientifique, Laboratoire de Chimie de la Matière Condensée de Paris, LCMCP, F-75005 Paris, France

^c ESRF – The European Synchrotron, 38043 Grenoble, France

Materials and Methods

Materials. Whatman 1 (W1) filter papers with a diameter of 150 mm (Catalog No. 1001 150) served as the cellulose substrate. As per the manufacturer's information, W1 filter papers are primarily comprised of purified cotton linters, boasting a cellulose content exceeding 98%. Hydrochloric acid was employed from a concentrated stock solution with a concentration of ~37% (Sigma-Aldrich). A commercial high sulfur content cellulose nanocrystals (CNC) suspension, acquired from CelluloseLab in Canada, was used as control at 1 wt%. Cellulose powder sourced from spruce, featuring fiber lengths ranging from 0.02 to 0.15 mm and obtained from Fluka, was used as the starting material for the synthesis of trimethylsilyl cellulose (TMSC). The glucolipid G-C18:1 biosurfactant was purchased from Amphistar (Gent, Belgium), and originated from production at the Bio Base Europe Pilot Plant in Gent, Belgium, under batch No. APS F06/F07, Inv96/98/99, and was used as received. This monounsaturated glucolipid G-C18:1 (with a molecular weight of $460 \text{ g}\cdot\text{mol}^{-1}$) consists of a β -D-glucose unit covalently bound to oleic acid. As specified by the producer, the batch, comprising 99.4% dry matter, was composed predominantly of 99.5% G-C18:1. The synthesis of this molecule is achieved through fermentation using the yeast strain *Starmerella bombicola* *AugtB1*, following a previously described protocol.¹ To adjust the pH value, sodium hydroxide and hydrochloric acid solutions (NaOH & HCl 0.1 M, Supelco - Sigma-Aldrich, Germany) are employed. In all instances of dilution, washing, and rinsing steps involving water, milli-Q water from Millipore Corporation, characterized by a resistivity of $18.2 \text{ M}\Omega \text{ cm}$, was employed.

Hydrolysis with liquid HCl. The preparation of cellulose nanocrystals (CNCs) within a conventional liquid-solid setting was derived from Klemm *et al.* method.² Initially, 10 grams of Whatman 1 filter paper were mixed with 300 milliliters of pre-heated 3 M hydrochloric acid (HCl) solution, and this mixture was continuously stirred at 1500 rpm at 80°C for 4 hours. The resulting suspension underwent multiple rounds of centrifugation at 2900 rpm during 10 minutes, until the pH reached a range of 4-5. Subsequently, the solution underwent a three-day dialysis process using a Spectra/Por® Dialysis Membrane (with a molecular weight cutoff of 6-8 kD).

Amorphous cellulose preparation. Trimethylsilyl cellulose (TMSC) was prepared following the procedure from Kontturi *et al.*^{3,4}

A thin film of regenerated cellulose was prepared from TMSC powder dissolved in toluene at 1 wt%. The solution was spin-coated using a WS-650SX-6NPP/LITE spin coater from Laurell

Technologies, US, either on Si wafers for XPS measurements or gold sensors for QCM-D analysis. Post-spin-coating, the substrates underwent HCl vapor hydrolysis in a desiccator saturated with 3M HCl, leading to the removal of the trimethylsilyl groups and yielding regenerated cellulose. This process is further detailed in Figure S7.

G-C18:1 preparation for CAC determination. Biosurfactant 2 mg/mL solution was prepared by gently mixing 20 mg of glucolipid powder in 10 mL of Milli-Q water. The pH value, initially below 4, was raised to 9 to obtain a clear solution (micellar phase). pH is eventually lowered to about 5. pH variations are performed with μL amounts of NaOH or HCl solutions of 0.5 M and 1 M. The solution was further diluted to attain the desired concentrations, ranging from 0.05 to 1 mg/mL.

Cellulose and biosurfactant sample preparation. The hydrolyzed cellulosic suspension was diluted at 1 mg/mL before performing tip sonication at a 20% amplitude for 10 minutes, with the container placed in an ice bath. Subsequently, a specific amount of G-C18:1 powder was weighed and combined with the pre-prepared CNC solution. The mixture was magnetically stirred at room temperature until the G-C18:1 powder was fully dissolved. An additional mixing step involving tip sonication at a 10% amplitude for 5 minutes was necessary. It should be noted that higher amplitude mixing could lead to the foaming of the biosurfactant, as shown in Figure S2b. The list of samples is presented in Table S1.

Tip sonication. The Branson Ultrasonics Sonifier™ S-450 Digital (20 kHz, 400 W; Branson Ultrasonics Corporation, USA) coupling with 1/8" Tapered microtip (n° 101-148-062) was achieved for bursts of 40 s with an interval of 20 s. Sonication and homogenization steps were accomplished in an ice bath to inhibit sample overheating.

Turbidity by light scattering (LS). The turbidity (mean count rate in kilocounts per second) was evaluated by means of a Zetasizer Nano ZS90 (Malvern Panalytical, UK) dynamic light scattering apparatus, employing a fixed measurement angle of 90°. Each measurement corresponds to an average of 10 runs, each lasting 60 seconds. The attenuation factor was constant set to 1, which corresponds to a nominal transmission of 0.0003%.

Optical microscopy. An Olympus BX53M microscope was chosen for the experiment. The objective lenses used were MPlanFL N with magnifications of 50x /0.80, 20x /0.45, 10x /0.30,

and 5x /0.15 FN26.5. A sample drop was placed directly onto a glass slide, and Halogen Bulbs served as the light source for illumination.

Scanning electron microscopy (SEM). A Zeiss Sigma VP FE-SEM (Germany) was used for SEM imaging, where acceleration voltages of 2 kV were applied. For effective SEM visualization, the cellulose sample required a sputtering treatment using an Au-Pd coating for 90 seconds at a current of 15 mA.

Atomic force microscopy (AFM). Samples for AFM were prepared by applying a cellulose suspension onto a newly cleaved silicon substrate. The Si wafers (purchased from Okmetic Oy, Finland) were cleansed with ethanol and distilled water, followed by a 15-minute UV treatment using a ProCleaner™ UV Ozone Cleaner (BioForce Nanosciences, US). The spin coating step was done using a WS-650SX-6NPP/LITE spin coater from Laurell Technologies, US. AFM imaging was performed utilizing a NanoScope IIIa Multimode instrument (Digital Instruments Inc., Santa Barbara, California) in tapping mode with the Standard Tapping Mode AFM Probe (HQ:NSC15/Al, MikroMasch, Estonia).

Colloidal stability experiments. The colloidal stability of cellulose suspensions was evaluated using a Turbiscan™ Classic MA 2000 Stability Analyzer (Formulation SA, France) apparatus, operated with Turbisoft software at room temperature. Real-time sedimentation data were continuously collected by measuring the backscattering and transmission intensity of pulsed near-infrared light ($\lambda = 880$ nm). For the initial 24 hours, the measurements were taken every 10 minutes, followed by 60-minute intervals for the subsequent 3 to 6 hours, and finally once every 2 days until a stable condition was achieved. For each measurement, 7 mL of sample were introduced in 20 mL cylindrical glass cells with an internal diameter equal to 12 mm. Intensity profiles were captured by scanning the full height of the sample at 40 μ m intervals. These transmission readings offer valuable insights into sedimentation changes over time, according to the Beer-Lambert law.⁵

Small angle X-ray scattering. SAXS experiments were performed at BM29 BioSAXS beamline (ESRF, Grenoble, France) under the proposal number MX2572. These experiments employed a synchrotron source at energy, $E = 12.5$ KeV and were performed with a sample-to-detector distance set at 2.83 meters. The standard automatic sample-changer system at the BM29 beamline was employed. For sample placement, a 96-well plate sample holder was used,

with each sample having an injection volume of 60 μL . All measurements were carried out at controlled room temperature of 23 $^{\circ}\text{C}$.

SAXS data were fitted using the model-independent Guinier function and parallelepiped form factor model available in the 5.0.6b2 version of SasView freeware. For the parallelepiped model, we have used the following parameters, optimized for the commercial sulfate-rich CNC control suspension at 1 wt%:

scale= 0.001 (fixed)

background= 0.0007 (fixed)

solvent scattering length density (sld)

sld_solvent= $9.4 \cdot 10^{-4} \text{ nm}^{-2}$ (fixed, typical of water)

parallelepiped sld, sld= $12.19 \cdot 10^{-4} \text{ nm}^{-2}$ (variable)

length_a= 5.0 nm (variable)

length_b= 21.3 nm (variable)

length_c= 400 nm (fixed)

In this model, the scale corresponds to the volume fraction, here 0.1 w%, “length_a” is the parallelepiped height, (h , in main text), and “length_b” is the parallelepiped width (w , in main text). The value of “length_c” is arbitrary and it defines an infinite length. “sld” was a fitted parameter and it was contained between the sld_solvent (water) and the sld of dry cellulose, the latter calculated at $13.5 \cdot 10^{-4} \text{ nm}^{-2}$ with the “SLD Calculator” tool provided in the SasView software and using a molecular formula of $\text{C}_6\text{H}_{10}\text{O}_5$ and a density, $d= 1.5 \text{ g/cm}^3$.

Fitting of Sample 0.2 SAXS profile was performed using the same set of parameters, with only length_a and length_b as free variables.

Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR). ATR-FTIR spectroscopy was employed to verify the adsorption of biosurfactants on the cellulose surface. Infrared spectra were obtained at ambient temperature using an FTIR spectrometer (PerkinElmer, MA, USA), coupled with a standard ATR sampling accessory (PerkinElmer, MA, USA). The samples were subjected to freeze-drying prior to measurement, and a self-leveling flat tip was used for the analysis. All spectra were measured in transmission mode in the range of $4000\text{--}500 \text{ cm}^{-1}$, with the baseline and the band at 1030 cm^{-1} normalized to 1 and 0, respectively.

X-ray photoelectron spectroscopy. XPS measurements were conducted to examine the success of cellulose regeneration from TMSC. A Kratos AXIS Ultra DLD X-ray photoelectron spectrometer with a monochromated AlK α source (1486.7 eV) was utilized at 100 W. Survey spectra employed a pass energy of 80 eV and a 1.0 eV step size, whereas high-resolution spectra used 20 eV pass energy and 0.1 eV step size. Photoelectrons were collected at a 90° angle in an ultra-high vacuum (below 1 x 10⁻⁹ Torr). The X-ray beam spot was 1 mm, analyzing areas of 300 μ m x 700 μ m. Both spectrum types were taken from three different sample spots for homogeneity and surface charge analysis. Charging effects were noted on the second sample's last position, influencing elemental but not chemical analysis. Spectra were charge-corrected to the C-O bond position of carbon at 286.5 eV.

Quartz crystal microbalance with dissipation (QCM-D) monitoring. Quantitative characterization of biosurfactant adsorption on cellulose surface was assessed using Quartz Crystal Microbalance with Dissipation monitoring (QCM-D). Regenerated cellulose substrates were affixed to polished gold sensors (14 mm diameter, 5 MHz, Ti/Au) sourced from AWSensors, Spain. Prior to experimentation, these sensors were subjected to a cleaning regimen utilizing a UV-ozonizer (Bioforce Nanosciences, CA) for 10 minutes to ensure pristine surface conditions. The interaction kinetics between amorphous cellulose and biosurfactants were elucidated by employing a Q-Sense E4 QCM-D system (Q-Sense AB, Gothenburg, Sweden). This apparatus facilitates real-time tracking of frequency (Δf) and dissipation (ΔD) shifts at 5 MHz, enabling inferences regarding the mass of the adsorbed entities and their viscoelastic characteristics. Adsorption dynamics were explored by first equilibrating the cellulose-coated sensors in QCM-D chambers with Milli-Q water for a duration of one hour. After stabilization, biosurfactant solutions of G-C18:1 at concentrations of 0.2 mg/mL and 1 mg/mL - filtered through a 0.45 μ m membrane - were prepared at pH= 4.5 and introduced at a flow rate of 100 μ L/min. The adsorption process was documented continuously over a three-hour period. Post-adsorption, the sensors were flushed with Milli-Q water to evaluate the desorption of the biosurfactant layer. The desorption effect was quantified by monitoring frequency and dissipation variations over time.

Analyzed data, supporting images and tables

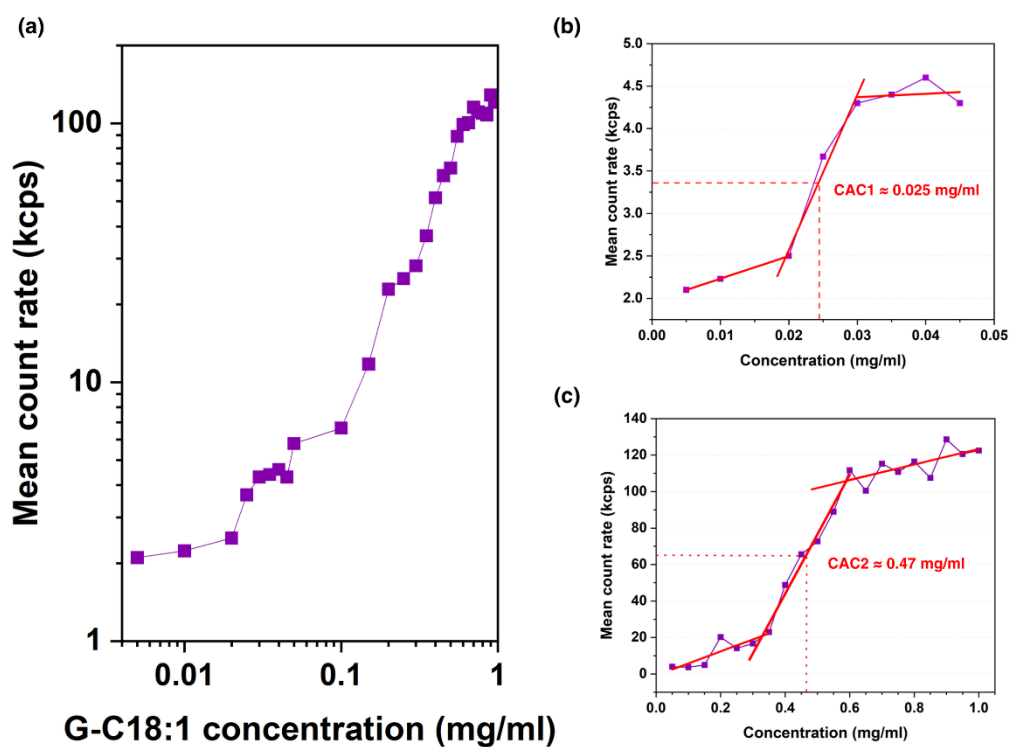


Figure S1 - A) Light scattering experiments to determine the critical aggregation concentration (CAC) of G-C18:1. For G-C18:1, two CACs are observed: around b) 0.025 mg/mL and c) 0.47 mg/mL.

Table S1 - The list of investigated samples. The mass of G-C18:1 is dispersed in 10 mL of a suspension of hydrolyzed cellulose at 1 mg/mL

Sample name	Mass ratio between cellulose and G-C18:1	G-C18:1 mass (mg)
0	1 : 0	0
0.2	1 : 0.2	2
0.4	1 : 0.4	4
0.6	1 : 0.6	6
0.8	1 : 0.8	8
1	1 : 1	10

Table S2. List of main FTIR peaks attributed to G-C18:1 and cellulose

Type of Vibration	Origin	~Band frequency/cm ⁻¹
O–H stretching	Carboxylic acid -COOH	3389
C–H stretching	Unsaturation -C=C-H	3010
C–H asymmetric stretching	Aliphatic carbon chain	2926
C–H symmetric stretching	Aliphatics carbon chain	2854
C=O stretching	Carboxylic acid -COOH	1701
C–H bending	Aliphatics carbon chain	1465
O-H bending	Alcohol -C-OH	1420-1323
C–O asymmetric stretching	O–C–C in sugar ring	1030-986
C–H rocking-bending	Aliphatic carbon chain	644

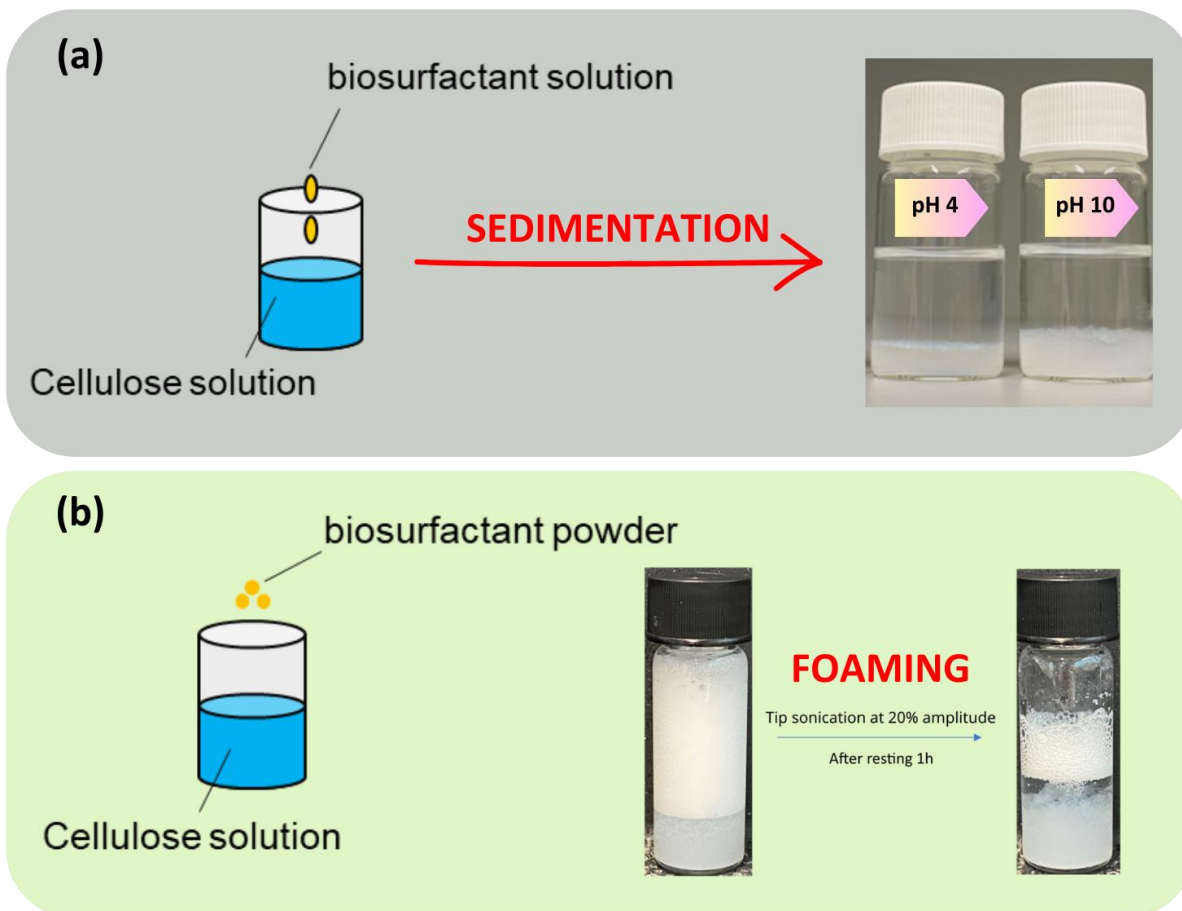


Figure S2 – Illustration of the two methods employed to disperse G-C18:1 in a hydrolyzed cellulose suspension (1 mg/mL). (a) Introduction of a G-C18:1 micellar solution drives immediate sedimentation; (b) introduction of G-C18:1 powder is followed by tip sonication, which can produce foam. Protocol in b) is chosen throughout this work.

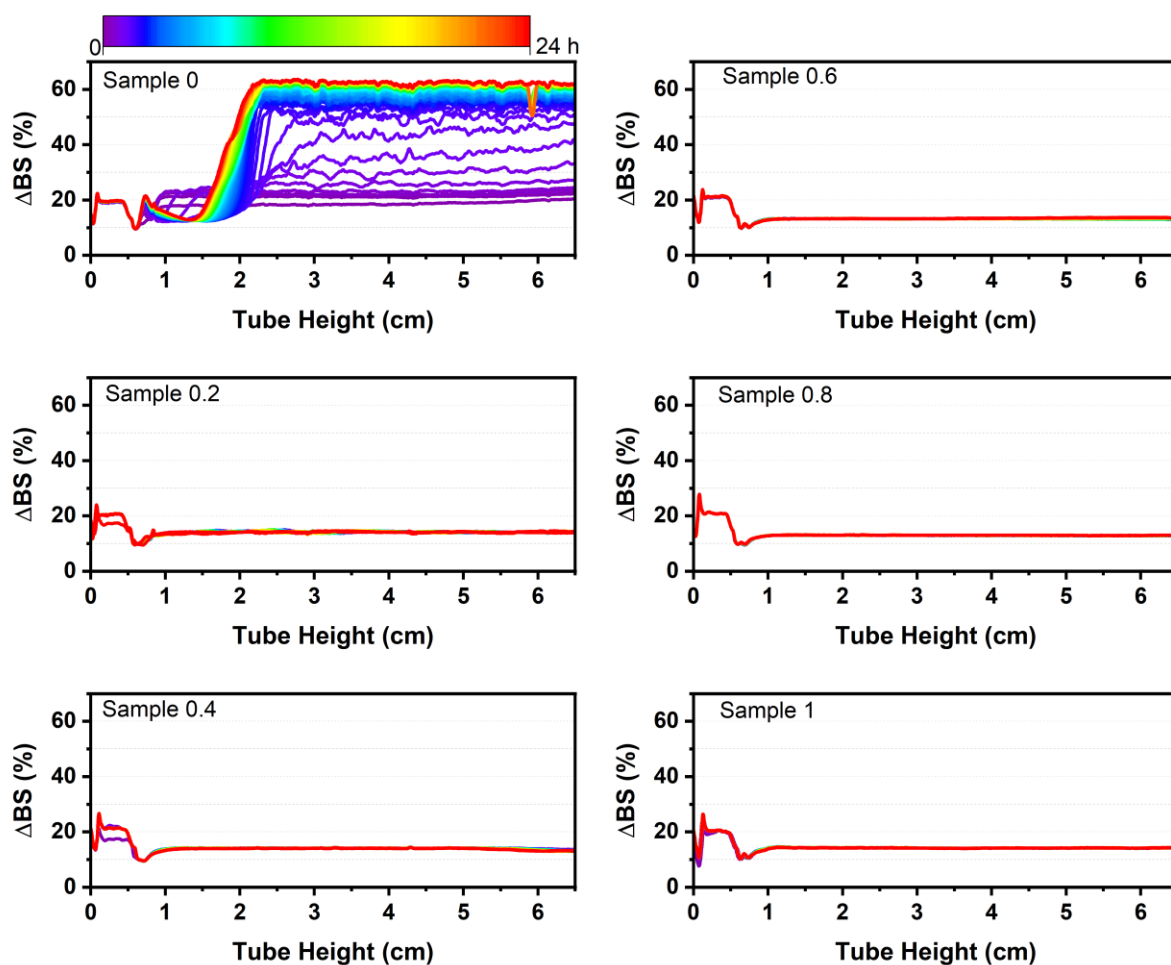


Figure S3 - Backscattering raw data of all samples were collected during 24 hours, with one scan every 10 minutes, including the first 0.7 cm of the tube height corresponding to the bottom black end of the vial. The profile of Sample 0 changed with every scan, indicating fast sedimentation, while the other samples remained stable over time.

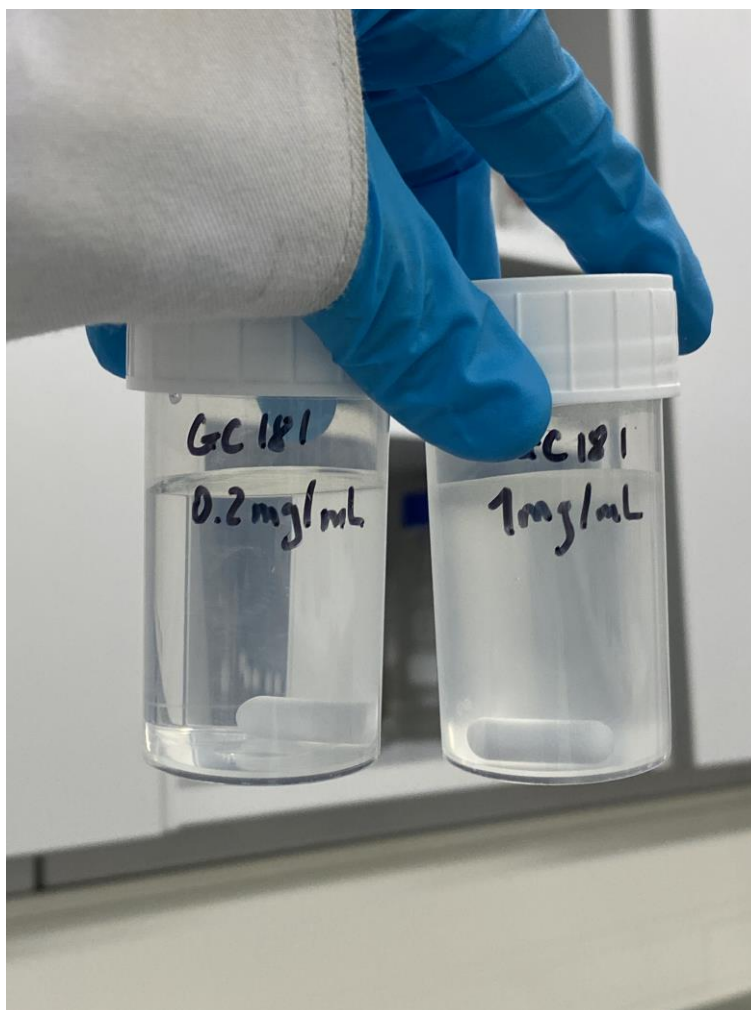


Figure S4 - Solutions of G-C18:1 prepared at two different concentrations: 0.2 mg/mL, below CAC2, and 1 mg/mL, above CAC2. Only the solution above CAC2 exhibits an opaque appearance.

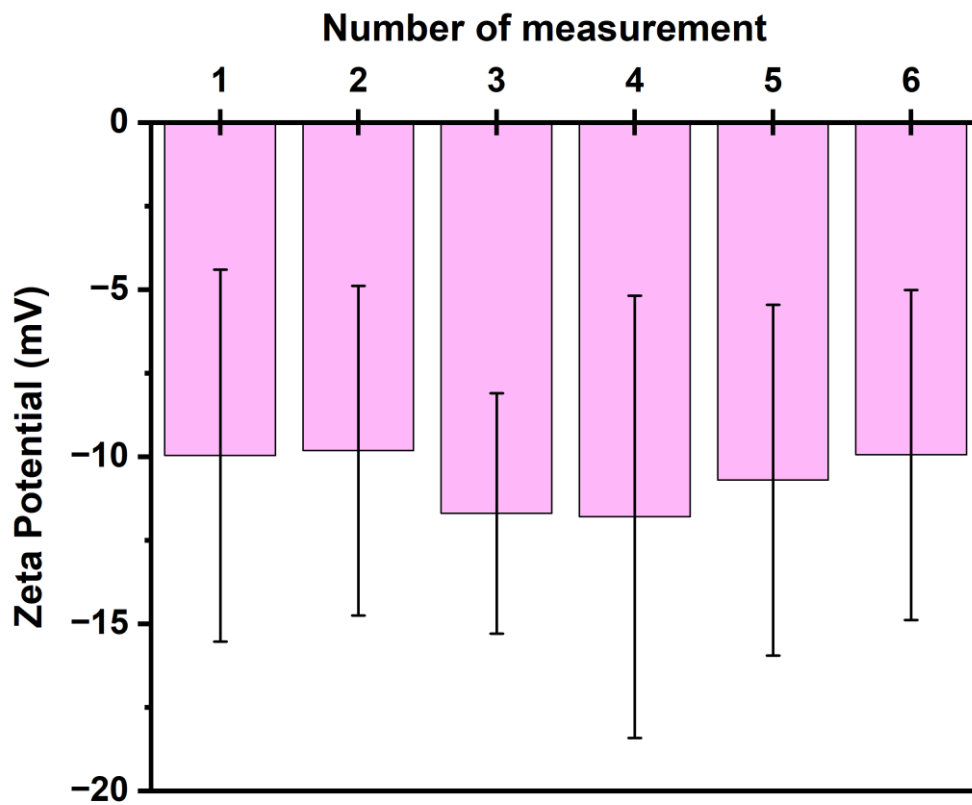


Figure S5 - Zeta potential measurements of Sample 0 (6 replicas) indicate a small negative charge and a large relative error (standard deviations given by the instrument) due to the instability of the system.

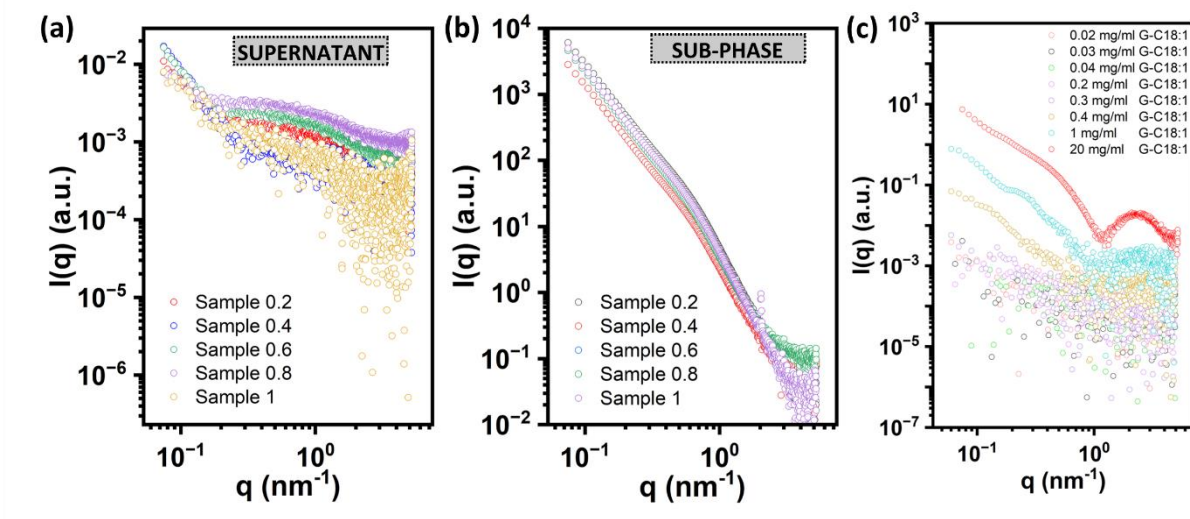


Figure S6 - SAXS profiles of all samples: Samples 0 through Sample 1 in the a) supernatant phase and b) sub-phase. c) Control G-C18:1 solutions at pH 5 and from 0.02 mg/mL to 20 mg/mL.

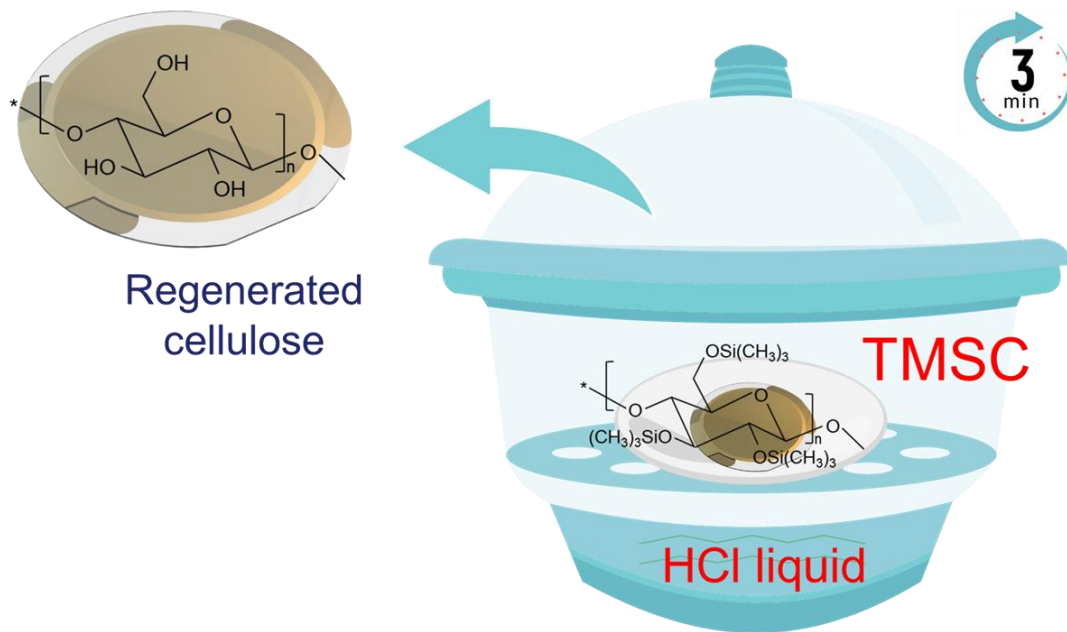
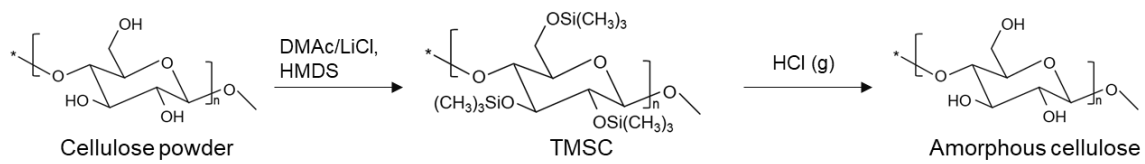


Figure S7 - Preparation protocol for trimethylsilyl cellulose (TMSC) from microcrystalline cellulose, leading to the production of a thin film of amorphous cellulose through acid vapor treatment.

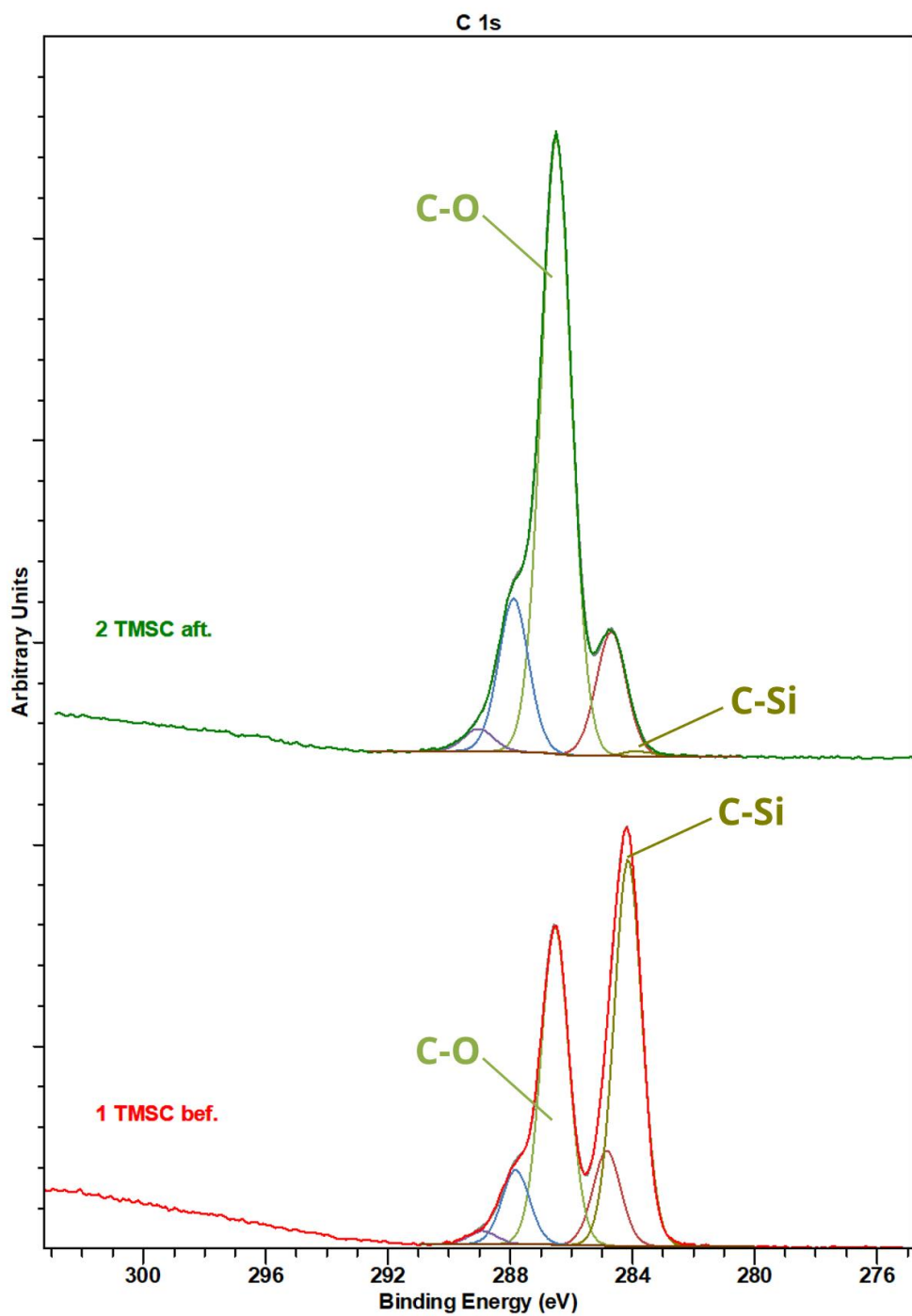


Figure S8 - XPS C 1s spectra of the TMSO thin film obtained both before and after vapor acid hydrolysis. The relative concentration of (C-Si) was 43.40 % before the hydrolysis and significantly reduced to 0.32 % after hydrolysis.

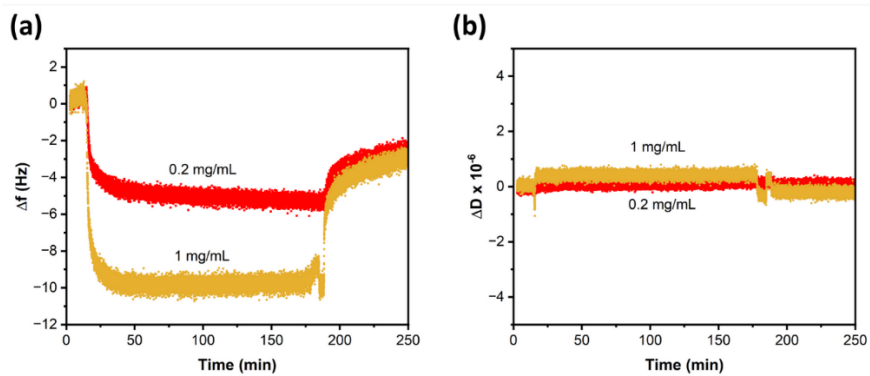


Figure S10 – QCM-D experiments showing the biosurfactant adsorption on amorphous cellulose: (a) frequency and (b) dissipation factor changes at 0.2 and 1 mg/mL G-C18:1. A baseline for regenerated cellulose surfaces was established, followed by the introduction of biosurfactant solutions at 60 minutes and a rinsing step at 240 minutes. $f_0 = 5$ MHz, $n = 3$.

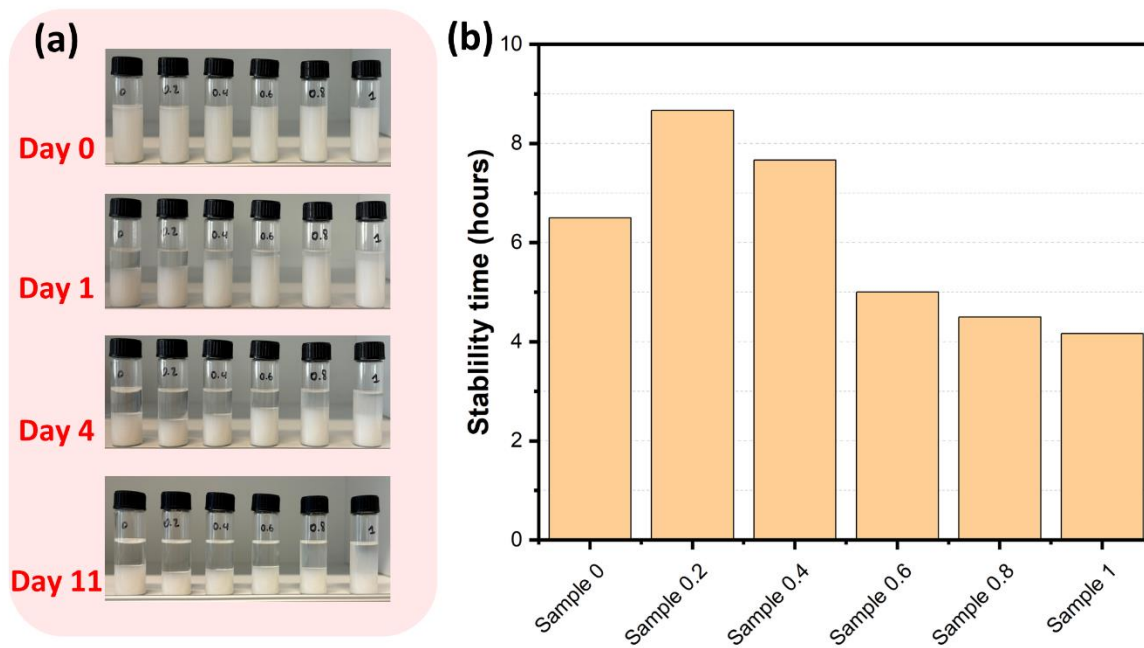


Figure S10 - (a) Visualization of colloidal stabilization of gaseous CNCs (hydrolysis following the method outlined by Kontturi *et al.*,⁶) and G-C18:1 mixtures, demonstrating behavior consistent with that of liquid acid hydrolysis CNCs previously examined in this study; (c) The recorded duration of sample stability prior to sedimentation was noted.

References

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