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Rapid quantification of cellulose nanocrystals by Calcofluor White fluorescence staining

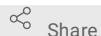
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ABSTRACT

Cellulose nanocrystals (CNCs) have gained worldwide interest for their properties. CNCs are mostly produced by acid hydrolysis of cellulose-rich biomass, but its' quantification is still complicated and tedious. Here, we describe a rapid method for the determination of CNCs concentration using Calcofluor White (CW) fluorescence dye. This method is robust, easy, selective for crystalline cellulose and suitable for routine measurement in CNCs production.

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GUIDELINES

1. CNC production procedure was performed on Whatman filter paper 1 as a model material as presented by Peretz et al. (2019), however it is suitable for a variety of lignocellulosic material, i.e. agricultural wastes, food leftovers, etc.
2. The pH must remain basic for the CW staining procedure. This is done by adding the 5M KOH which maintain pH levels at 12-13.

Materials and reagents

Purified CNC powder (Nanografi, Turkey)
 Whatman filter paper 1
 Calcofluor White M2R (Sigma- Aldrich, Israel, cat #18909)
 Potassium hydroxide (KOH)
 Sulfuric acid

Equipment

Flat-bottomed black 96 wells plate (Nunc™, Denmark)
 Spark 10M plate reader (Tecan, Switzerland)
 Direct-Q3 UV System (Millipore, France)
 250W laboratory blade mill (MRC Ltd., Israel)
 Jouan B4i Multifunction Centrifuge (Thermo Electron Corporation, USA)
 14 kDa MWCO dialysis bags (Sigma-Aldrich, Israel)

Software

SparkControl software

SAFETY WARNINGS

When you mix concentrated acid and water, it gets very hot and is dangerous!

Standard calibration curve

- 1 For calibration curve, prepare different CNC solutions in DI water (up to 0.5% w/v).
- 2 In flat-bottomed black 96 plate, place 100 µL of sample, 20 µL of 5M KOH and 80 µL of CW reagent (in this order). KOH is needed for basic conditions (pH=12-13).
- 3 Fluorescence signal can be quantified using plate reader and report in the instrument's Relative Fluorescence Units (RFU). Excitation is measured at 355 nm and emission at 433 nm. The plate should be continuously shaken to prevent CNC sedimentation and temperature should be kept at 30 °C.
- 4 Fluorescence is read in kinetic mode every 10 min with maximal incubation time of 60 min until plateau is reached. ^{1h}

CNC production from Whatman filter paper and total estimation

1h

- 5 Grind and dry Whatman filter paper 1.
- 6 Add the ground dried filter paper sample to 64% aqueous sulfuric acid at 1:20 ratio (w/w).
- 7 Incubate the mixture for 45 minutes at 45 °C.
- 8 Stop the reaction by diluting the mixture 10-fold with cold water.

- 9 Remove the acid from the CNCs by centrifugation (10,000 rpm) for 15 min at 10 °C. 15m
- 10 Decanting the liquid, and re-suspend the pellet in DIW for three repeating washes. 45m
- 11 Re-suspend the CNC in 30 ml DIW, and dialyze against deionized water until a constant pH is reached.
- 12 Follow the CNC fluorescence procedure as mentioned above, and compare the results to the calibration curve, in order to establish CNCs concentration.