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Bacterial cellulose thin-films for energy harvesting applications

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INTRODUCTION

Bacterial cellulose, also known as microbial cellulose, is a promising natural polymer synthesized by certain bacteria such as *Gluconacetobacter xylinum*. Bacterial cellulose binds large amounts of water - up to 99 wt% during biosynthesis in the aqueous culture media. Even though it is chemically identical to plant cellulose, its supramolecular structure and high purity cellulose content demonstrates unique properties such as high crystallinity, high water holding capacity and mechanical strength. Several studies have focused on the utilization of bacterial cellulose as reinforcement material, in biomedical applications or cellulose based smart material devices.

Recently the application of ultrasound assisted extraction of plant polysaccharides, ultrasound assisted delignification, ultrasound assisted size reduction of cellulose or intensification of enzymatic hydrolysis has gained much interest.

The isolation of cellulose nanoparticles without serious degradation, at low costs and using an environmentally friendly method is constantly being sought. Wang and Cheng examined the use of high intensity ultrasound to isolate fibrils from four cellulose sources: regenerated cellulose (lyocell), pure cellulose fiber, microcrystalline cellulose and pulp fiber. Wong et al. investigated the effect of ultrasound irradiation time on the depolymerization of plant and bacterial celluloses. Tischer at al. subjected bacterial cellulose pellicles to a high power ultrasonic treatment for 15, 30, 60 and 75 min; these were carried out in an ice bath for tissue engineering applications.

The aim of the present study was to examine the effect of two main ultrasound operating conditions, i.e the effect of temperature and distance of ultrasonic probe on defibrillation of previously chemically purified bacterial cellulose samples. The purification treatments used in this research were selected to: (i) maintain the native cellulose I form of bacterial cellulose, and (ii) act as chemical pretreatment and swelling of bacterial cellulose, to enhance the subsequent ultrasound treatment and (iii) to remove dead bacterial debris. The overall purpose of this research was to develop a method of obtaining highly crystalline, low anisotropy and thermodynamically more stable bacterial cellulose films suitable for energy harvesting devices, such as piezoelectric strain sensors.

MATERIALS AND METHODS

Purification of nata de coco

Nata de coco cubes (PT. Cocomas, Indonesia) were washed and soaked in distilled water until the pH was neutral (pH 5-7) to remove the citric acid and other components of syrup added for preservation. In order to improve purity of bacterial cellulose, nata de coco was further purified by alkaline treatment to remove any remaining bacterial cell debris, microorganisms and other soluble polysaccharides. The one step and two step purification methods were prepared by the procedure reported by Gea et al [18]. After being harvested, the nata de coco cubes were immersed in 2.5 wt% NaOH overnight. This process will be referred to hereafter as one step purification. Another sample was prepared in the same way and successively treated with 2.5 wt% NaOCl, hereafter referred to as two step purification. A third sample was prepared by heating nata de coco in 0.1 M NaOH at 70℃ for 2 h under continuous stirring; this will be called as 0.1 M NaOH purification.

Subsequently, nata de coco cubes were rinsed under distilled water several times at room temperature to remove any solvent until the pH of the water became neutral. Once neutral pH was reached, bacterial cellulose was mechanically ground in a laboratory blender for a few minutes, homogenized, poured into silicon trays and then dried in an oven at 50 ℃.

Ultrasonication of bacterial cellulose films

After drying at 50 ℃, the bacterial cellulose films were cut. The cut bacterial cellulosic materials (0.1 wt.%, immersed in 80 mL distilled water) were redispersed and subjected to further grinding, this time with a hand blender, prior to ultrasonication. Sonication was achieved at low frequency (20 kHz) using an ultrasonic horn (Tesla 150 WS) with a tip diameter of 18 mm dipped in the suspension. Two essential parameters of acoustic cavitation, i.e. temperature (no water bath, cold water bath, ice water bath) and distance of the ultrasonic probe (4 cm and 1 cm from the bottom of container) were considered to evaluate the effects of ultrasound on bacterial cellulose aqueous suspensions. Maximum intensity ($25 W/cm^2$) and ultrasonication time (30 min) were maintained constant.

Preparation of bacterial cellulose films

Thereafter, the liquid supernatant nano colloids were collected from treated materials and were poured once more into silicon trays. After this process, the ultrasonicated bacterial cellulose was reconstituted in the form of thin films after oven drying at 50 ℃ for a second time. Due to the evaporation drying method, properties of the bacterial cellulose films were independent of direction.

CHARACTERIZATIONS

Field Emission Scanning Electron Microscopy (FE-SEM)

FE-SEM micrographs were obtained using a Zeiss ULTRA Plus (Oberkochen, Germany) instrument at an acceleration voltages of 1 and 2 kV. The suspensions were filtered through a gilded PC membrane and dried for 1 h at r.t. All samples were coated with a highly conductive film of gold by Bal-Tec SCD 500.

Atomic Force Microscopy (AFM)

AFM experiments were performed using a MultiMode atomic force microscopy 8 with a Nanoscope Veeco V controller (Bruker Nano Surfaces, Santa Barbara, CA, USA) instrument. Small cut pieces of oven dried bacterial cellulose films were placed on magnetic slides and the scans were obtained in tapping mode using a V-shape Silicon Nitride cantilever. Prior to the measurements, the tip radius and geometry of the tip were calculated. Two repetitions of imaging (5x5 μm and 1x1μm) were carried out. These experiments were implemented in an environment with constant relative humidity and temperature. Width was measured by image analysis using ImageJ software (ImageJ 1.46, National Institute of Health (NIH), USA).

Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra of the bacterial cellulose films were obtained using a Jasco FT/IR6300 equipped with an ATR PRO 470-H spectrometer. A total of 50 cumulative scans were taken per sample with a resolution of 4 cm^{-1}, in the frequency range of 4000-400 cm^{-1}, in absorbance mode. ATR correction was applied in each measurement.

X-ray Powder Diffraction (XRD)

The X-ray diffraction patterns were recorded at room temperature in the 5–80° 2θ range using an MPD Pro Panalytical diffractometer equipped with a linear Xcelerator detector. Cu-K (1.54056˚A) radiation was used with the 0.016° recording step and the 1000 s per step counting time. The samples were powdered before the analysis.

XRD peak height method, developed by Segal and coworkers, was used to determine the crystallinity index by the following equation (Eq.1)

$$Cr.I = \frac{I_{200} - I_{am}}{I_{200}} \; 1oo \qquad (1)$$

where I_{200} is the peak intensity at the (200) ($2\theta \approx 22.5^{o}$) plane, and I_{am} is the minimum intensity at the valley between (200) and (110) peaks ($2\theta \approx 18^{o}$).

The interplanar distances of the crystallites (d-spacings) could be calculated with Bragg's law,

$$\lambda = 2d \; sin\theta \qquad (2)$$

where λ is the wavelength of the X-rays (and moving electrons, protons, and neutrons), d is the spacing between the crystal planes in the atomic lattice, and θ is the Bragg angle between the incident ray and the scattering planes

The crystallite sizes at d_1, d_2 and d_3, the three main peaks respectively, were determined using the Scherrer equation:

$$Cr.S. = \frac{0.9\,\lambda}{H_{hkl}\; cos\theta_{hkl}} \qquad (3)$$

where $Cr.S.$ is the crystallite size, λ is the wavelength of incident X-rays, H_{hkl} is the full-width at half-maximum (FWHM) and θ_{hkl} is the Bragg angle at the corresponding lattice plane.

Thermal analysis

Thermal analysis techniques, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were used to measure the thermal stability behavior of bacterial cellulose films. Thermogravimetric (TG) data were acquired between 0 and 500 °C using a Perkin Elmer Diamond thermal analyzer under nitrogen purging gas (100 cm^3min^{-1}) at a heating rate of 2 K min^{-1}. Differential scanning calorimetry (DSC) analysis was carried out on a Netsch DSC204 instrument under nitrogen purging gas (30 cm^3min^{-1}) at a heating/cooling rate of 2 K min^{-1}. Temperature and enthalpy were calibrated using the melting transition of standard materials (Hg, In, Sn).

SUMMARY OF THE RESEARCH

Structural (FT-IR and XRD), thermal (TGA and DSC) and morphological (FE-SEM, AFM) measurements justified that purification and ultrasound treatments resulted in a new purified and more crystalline bacterial cellulose films without significantly altering the native cellulose I polymorph of bacterial cellulose obtained from nata de coco. However, there were significant changes in the hydrogen bonding network that indicates changes in the physical properties of cellulose such as solubility, reactivity and crystallinity. Even more, there were observed small variations among the crystallite sizes of samples. Removal of bacterial cellulose impurities and ultrasonication resulted in the formation of a direct or closer contact between the cellulose fibrils and in the formation of strong intra- and interfibrillar hydrogen bonds. Total crystallinity index (TCI), lateral order index (LOI) and hydrogen bond intensity (HBI) were significantly altered after purification and ultrasound treatments. Initially, it was considered as the most favourable processes to produce bacterial cellulose films with higher crystallinity order and improved properties, the treatments which resulted in the following order combinations: lower LOI, higher TCI and lower HBI values. Based on this combination the following samples should be considered as the best process of each purification treatment: a) WP_CW_1cm, b) OSP_CW_4cm, c) TSP_IW_1cm and d) NaP_NoW_1cm. However, since there were no significant changes in the crystallinity index of cold treated samples, we would prefer to avoid the two extreme conditions (NoW and IW) and use milder conditions, i.e. cold bath as preferred temperature in all ultrasonication treatments. The mean values of energy hydrogen bond was calcultated to be 21.51 (□}1.5) kJ/g mol. This interfibrillar hydrogen bonding energy is necessary to overcome in order to achieve the separation of microfibrils into individual fibrils.

Effect of purification treatments

Results showed that alkali treatment played an important role in removing most of the non-cellulosic materials, bacterial cell and other impurities to obtaining pure cellulose. The most effective purification treatment was the one using 0.01 M NaOH. In one step and two step purification treatments bacterial debries were still predominant in cellulose network. This observation can be explained for several reasons. Boiling in sodium hydroxide solution is a common method for sterilization and has been used in a number of studies to treat bacterial cellulose prior to mechanical testing. Warm conditions allow

better alkali penetration into the fibers than the ambient temperatures. Additionally, it is well known that proteins and nucleic acids can be hydrolysed with alkaline solutions at warm temperatures. Alkaline treatments can cause cellulose to swell and even can dissolve cellulose above 8-10% (m/v) concentrations. Further, cellulose due to its nature is hydrophilic and swells in the presence of water. In our research, it is assumed that the used purification treatments caused a homogeneous, weak to very low swelling on bacterial cellulose samples. This type of swelling could result in an inter-crystalline swelling and the formation of new interactions between water and cellulose molecules or sodium hydroxide and cellulose, respectively. Sodium hydroxide aqueous solutions cellulose formed a Na+/water and cellulose system, which chemically forms stable hydrogen bonded network structures. Because of this interaction between cellulose and Na+ alkali treatment one step and two step purification it seems had a decrystallising effect on cellulose and resulted in reduced crystallinity. However, those changes preserved the native structure of cellulose I, while simultaneously facilitated isolation of microfibrils during ultrasound. In contrast, the degree of crystallinity using 0.01 M NaOH purification at 70 ℃ for 2 h was increased. This could be explained, by partial decomposition of amorphous regions.

Effect of ultrasonication

Our results suggests that ultrasonication could be very useful for isolating cellulose microfibrils and fabricating homogeneous nanocellulose films. High intensity ultrasound when was treated with purified bacterial cellulose solutions had a significant impact on morphology of bacterial cellulose nanofibrils and supramolecular properties of cellulose. Nevertheless, ultrasonication did not modified the native crystalline structure of bacterial cellulose. Ultrasound was transferred through shear forces due to cavitation to the glycan chains, enhancing the decomposition of amorphous domains in cellulose. However, longer treatment time could result in cellulose degradation. Cellulose and ultrasound, from their individual perspectives, seems to be simple, but are quite complex. Ultrasound is influenced by many operating parameters and even slight changes could attribute to significant variations in ultrasound efficiency. Our results indicate that ultrasonication is a very powerful technique for isolating cellulose fibrils from a dense, highly anisotropic structure, without damaging it. Some parameters, such as geometry of ultrasonic horn, operating frequency and intensity of

irradiation or ultrasound energy distribution are not easy to change or to control. On the contrary, the other parameters could be investigated in order to obtain the optimum ultrasound operating conditions. For this research, a frequency of 20 kHz was considered as the optimum ultrasound frequency. The intensity of ultrasound is proportional to the amplitude of vibration (Santos et al. 2009) and it was kept constant. Furthermore, 20 kHz was found to be effective for extraction of plant contents (Shirsath et al. 2012). Water is the most favourable solvent due to its low cost and it also displays good cavitation effect under ultrasound irradiation. To avoid stability problems, it was decided to use cylindrically shaped containers instead of conically shapes ones where ultrasound energy distribution is better. It was found that as the exposure time to cavitation increased, crystallinity of the cellulose decreased and faster degradation was observed (Pinjari & Pandit 2010). Moreover, ultrasonic power influences the number of cavitation bubbles formed, their lifetime and the generated cavitation intensity. In this study the operating time and power were 30 min and around 25 W/cm2 respectively. Considering the reaction for a cavitational process, bulk operating conditions such as distance of ultrasonic probe and temperature are crucial factors, which often interact with each other. Acoustic cavitation phenomenon due to ultrasound leads to an increase of solvent temperature. High temperatures are preferable to disrupt strong interaction forces such as Van der Waals, hydrogen bonds and dipole attraction between the solute molecules and solute-matrix. In contrast, cavitation is performed better at lower temperatures, in which the ultrasonic power is constant (Santos et al. 2009). Water bath treatments influenced the starting temperature which also affected ultrasound. In the absence of a water bath, colloids obtained rapidly high temperatures into the colloid biphasic system. In the case of an ice water bath, bacterial cellulose samples had the lowest starting temperature. Although the cavitation was more efficient, duration of 30 min was not enough to increase mass transfer in order to disrupt the strong hydrogen bonds existent in cellulose molecules. Taking into account these parameters, bacterial cellulose performed higher crystallinity in mild conditions, such as in a cold water bath. Ultrasonic intensity rapidly decreases mostly axially, but also radially from the ultrasonic probe. Minimizing dead zone areas below ultrasonic probe and the wall of the container, a maximum contact angle between the sample and the cavitation zones was achieved at 1 cm distance. Based on the experimental results, 1 cm distance attended better results compared to 4 cm distance from the bottom of the reactor container. It was observed that ultrasound irradiation generated a local

turbulence and liquid microcirculation in that biphasic system at 1 cm distance of horn tip to bottom, which helped in the liberation of the fibrils as well. Ultrasonication treatment in biphasic systems produced a colloidal solution and amplified the reduction of aggregates, due to drying and simultaneously improved the separation of microfibrils. This disaggregation phenomena was confirmed by the AFM and FE-SEM images.

Main conclusions of this research work (Thesis)

The main conclusions of this doctoral work can be summarized as follows:

1. The most favourable settings of ultrasonic treatment and NaOH concnetration found to be mild enough to preserve the initial crystalline structure (cellulose I) of bacterial cellulose, but simultaneously suitable to remove bacterial cellulose contaminations, achieve high purity, increase the crystallinity, facilitate separation of bacterial cellulose nanofibrils and thus enabling the film formation.

2. It can be stated, from FE-SEM and AFM images that the mild alkaline pretreated bacterial cellulose increased the swelling of amorphous regions compare to the control sample and enhanced the penetration of ultrasound mass transfer forces to bacterial cellulose samples. 3. It can be reported that compare to the control bacterial cellulose, the pretreated and sonicated samples showed variations in the total crystallinity index, lateral order index and hydrogen bond intensity values, which indicates changes in the physical properties and hydrophilic nature of bacterial cellulose films.

4. The selection of the most favourable parameters of ultrasound-treated bacterial cellulose thin film formation were based on the following measured criteria: low lateral order index > high total crystallinity index > low hydrogen bond intensity.

5. It can be stated that ultrasonication increased the overall crystallinity of the bacterial cellulose samples during the treatments based on the XRD analysis, which is a requirement for energy harvesting applications of cellulose based thin films.

6. It has been shown that d-spacing values (the planar distances of crystallites) did not change during the ultrasonication, but crystallite sizes of cellulose Iα and Iβ allomorphs existing in bacterial cellulose were altered.

7. It has been found that thermal onset temperature of degradation was increased when proteineous bacterial bodies were removed during the purification treatments and the thermal decomposition of cellulose occurred at higher temperatures. The DSC measurement proved that part of amorphous cellulose regions were rearranged to crystalline ordered regions around 50°C.

List of my selected publications

1. Dimitrios Tsalagkas, Rastislav Lagaňa, Ida Poljanšek, Primož Oven, Levente Csoka. Morphological, structural and thermal properties of ultrasound-assisted bacterial cellulose films formation. (submitted).

2. Koutsianitis Dimitrios, Mitani Constantina, Giagli Kyriaki, Tsalagkas Dimitrios, Halasz Katalin, Kolonics Otto, Gallis Christos, Csoka Levente. (2015). Properties of ultrasound extracted bicomponent lignocellulose thin films. *Ultrasonics Sonochemistry*, 23, 148-155, doi:10.1016/j.ultsonch.2014.10.014.

3. Nemeth R., Tsalagkas D., Bak M. (2015). Effect of soil contact on the modulus of elasticity of beeswax-impregnated wood. *Bioresources*, 10 (1), 1574-1586.

4. Dimitrios Tsalagkas, Rastislav Lagaňa, Levente Csoka. (2014). Morphological and structural changes of ultrasound treated bacterial cellulose. Proceedings of the 57th International Convention of Society of Wood of Science and Technology. 23-27 June 2014, Technical University in Zvolen, Slovakia, Part 1, 161-169.

5. Divos, F., Tsalagkas, D & Koutsianitis D. 2013. Wood density determination of trees by microwave impulse radar. Proceedings of the 18th International Symposium on Nondestructive testing and evaluation of wood, 24-27 September 2013, Madison Wi, USA, 143-149.

6. D. Tsalagkas, G. Grozdits, O. J. Rojas, L. Csoka. (2013). ZnO-Bacterial cellulose nanocrystal composite and its potential as energy harvesting material. Proceedings of TAPPI International Conference on Nanotechnology for Renewable Materials, Stockholm, Sweden, 25-27 June 2013, ISBN: 978-1-59510-225-6.

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