Proc. 14th Int. Conf. on Global Research and Education, Inter-Academia 2015
JJAP Conf. Proc. **4** (2016) 011109

Bio-Based Nanomaterials – Versatile Materials for Industrial and Biomedical Applications

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(Received September 28, 2015)

In this work, unmodified bacterial cellulose pellicles, biosynthesized by the bacterium *Komagataeibacter rhaeticus*, bleached birch Kraft pulp (Södra Cell AB, Sweden) and birch bark supplied by the plywood industry (JSC Latvijas Finieris, Latvia), were used to obtain the nanoparticles. The results showed that cellulose nanoparticles fabricated by the ammonium persulfate oxidation method, an alternative method developed at the Latvian State Institute of Wood Chemistry, are promising constituents for producing nanopaper. Cellulose and birch bark nanofillers show the potential to improve the physical-mechanical and biological properties of chitosan-matrix films.

1. Introduction

Nowadays, there has been extensive research in cellulose, cellulose-based particles and cellulose-based biocomposites [1,2]. This research area is becoming more and more privileged due to the increasing interest of industry and medicine. Among the available biopolymer resources, lignocellulose and wood residues are the most abundant materials in nature, making them natural candidates as renewable and sustainable feedstocks for eco-products [3] and biomaterials [4].
The cellulose extracted from wood sources contains impurities of other natural fibers, e.g. lignin and hemicelluloses, while bacterial cellulose, an exopolysaccharide produced by various bacteria, is made up of almost entirely pure cellulose [5]. Bacterial cellulose has proven to be a versatile biomaterial and can be used as a paper product, and in electronics and biomedical devices [6,7].
Natural nanofillers from cellulose and wood bark can provide strong reinforcement in chitosan-matrix biocomposites due to their similarities in molecular structures [8]. Chitosan, the only cationic polysaccharide of natural origin, has been extensively explored; its features highlight the suitability and extensive applications that it has in the food, textile and paper industries [9], as well as in biomedicine and pharmacology [10].
This research takes on the challenge, with attention to physical-mechanical and biological properties, of developing biopolymer films that can be used in the packaging industry or biomedicine.

2. Experimental section

2.1 Bacterial cellulose film

The bacterium *Komagataeibacter rhaeticus* (*K.rhaeticus*, P1463, deposited in the Microbial Strain Collection of Latvia), which had been previously isolated from local kombucha tea, was used to produce bacterial cellulose in a *Hestrin–Schramm* medium modified with glucose. The culture was incubated statically at 30 ºC for 14 days, and then purified by washing in deionized water and boiling

in 1 N NaOH solution for 1 h. After the alkaline treatment, the bacterial cellulose was washed in deionized water for several days to reach neutral pH. For testing the mechanical properties, bacterial cellulose was dried using the organic solvent exchange method. The organic solvent saturated pellet was pressed between two organic glass sheets and air-dried for 24 h.

2.2 Nanofillers-doped chitosan film

Nanofillers were obtained from bacterial cellulose (biosynthesized by the bacterium *Komagataeibacter rhaeticus*) and bleached birch Kraft pulp (Södra Cell AB, Sweden), as well as birch bark supplied by the plywood industry (JSC Latvijas Finieris, Latvia) using the ammonium persulfate oxidation method (OX); the preparation was based on the previous work [11]. These nanofillers (up to 1 wt%) were dispersed in deionized water or 2% chitosan-acetic acid solution (chitosan (CH), Bioprogress, Russia; low molecular weight with a deacetylation degree of 79%) and ultra-sonicated (Ultrasonic Homogenizer Ultrasonic Cell Crusher Sonic 650W, MRC Scientific Instruments, UK) for 15 min. The resulting solution was used to produce a film on glass substrate by a film-casting method and oven-dried (Memmert, Germany) at 45 °C for 48 h.

2.3 Characterization

An X-ray diffractometer (D8 Advance, Bruker, Germany) with a Cu Kα X-ray source (X-ray wavelength 0.154 nm) generated at 40 kV and 40 mA was used to determine the crystallinity and crystallite size. The X-ray diffraction pattern was produced in the range of 20° to 50° at a scan speed of 0.20°/min. The divergence slit was set at 0.6 mm, and the antiscatter slit – at 8.0 mm. The crystallite size was calculated using the BGMN Rietveld program [12].

The freeze-dried bacterial cellulose film was investigated using scanning electron microscopy (SEM) (TS Vega Tescan 5136M, Czech Republic and Vega TS software) at an accelerating voltage of 15 kV. Samples were sputtered with gold for increasing the surface conductivity in the scanning electron microscope. The morphology of cellulose and birch bark nanofillers was characterized with a high resolution scanning electron microscope (FEI Helios Nanolab 600, USA).

The mechanical properties of films were evaluated using equipment for testing tensile strength (Frank Tensile Tester, Germany, DIN EN ISO 1924-1 standard), and statistics were determined by the one-way analysis of variance (ANOVA). A film strip of dimensions 15 mm by 50 mm and free from physical imperfections was held between two clamps positioned at a distance of 3.5 cm. The film was pulled by a top clamp at a rate of 25 ± 5 mm/min. Measurements were run 8 times for each film.

The Saos-2 osteosarcoma cell line was used to assess the *in vitro* cytocompatibility of nanofillers-doped chitosan films by seeding 20 000 cells cm^{-2}. The cells were cultured in a 5% CO_2 incubator at 37 °C for 7 days. The cell viability was investigated with an AlamarBlue® cell viability assay (Life Technologies, Italy) by measuring the fluorescence (Tecan Genius Plus, Tecan Italia S.r.l., Italy) at an excitation wavelength of 540 nm and emission wavelength of 590 nm. Cytotoxicity was gauged by resorufin release according to the AlamarBlue® cell viability assay protocol.

3. Results and Discussion

The freeze-dried bacterial cellulose film was white and smooth; the fibril layers inside the bacterial cellulose film are shown in Fig. 1a. The bacterial cellulose film represented a three-dimensional network of micro- and nano-fibrils. In SEM micrographs, the length of the individual bacterial cellulose fibrils was up to 70 μm and width varied from 80 to 200 nm.

Native cellulose is usually composed of crystalline and amorphous regions, and the amount of each region depends on its source [13]. According to Abyshev *et al.*, birch bark contains high amounts of the natural compounds: terpenoids (75.2%) and their esters (fatty acid esters of betulinol and lupeol, 4.4%) [14]. The oxidized bacterial and bleached Kraft pulp birch showed a typical XRD pattern for crystalline cellulose [15], namely, sharp peaks at 2θ = 14.7, 16.2 and 22.4°; in contrast, for the

oxidized birch bark, there was a broad amorphous peak centered at 2θ = 20° (data not shown). Crystallinity and crystallite size are summarized in Table I; the highest crystallinity was found for bacterial cellulose, and its amorphous regions decreased during the oxidation with ammonium persulfate. After oxidation, the crystallinity increased due to amorphous regions being hydrolyzed. The crystallite size, varied from 4.0 to 6.0 nm, was very close to the source material and decreased with the ammonium persulfate treatment due to the depolymerization process. However, the crystallite size was partly comparable to the particle size viewed in SEM micrographs (Figs. 1b and 2); the bigger particles, approximately 100 nm, were obtained from bacterial cellulose compared to bleached birch Kraft pulp and birch bark.

Table I. Crystallinity and crystallite size of the source materials and nanofillers.

Abbreviation		Crystallinity, %*	Crystallite size, nm*
BC	Bacterial cellulose	86.0	6.5
KP	Bleached birch Kraft pulp	45.0	3.9
BB	Birch bark	–**	–**
OX-BC	Oxidized bacterial cellulose	87.5	6.1
OX-KP	Oxidized bleached birch Kraft pulp	59.0	4.6
OX-BB	Oxidized birch bark	–**	–**

**Calculated by Rietveld analysis*
***With a characteristic amorphous peak*

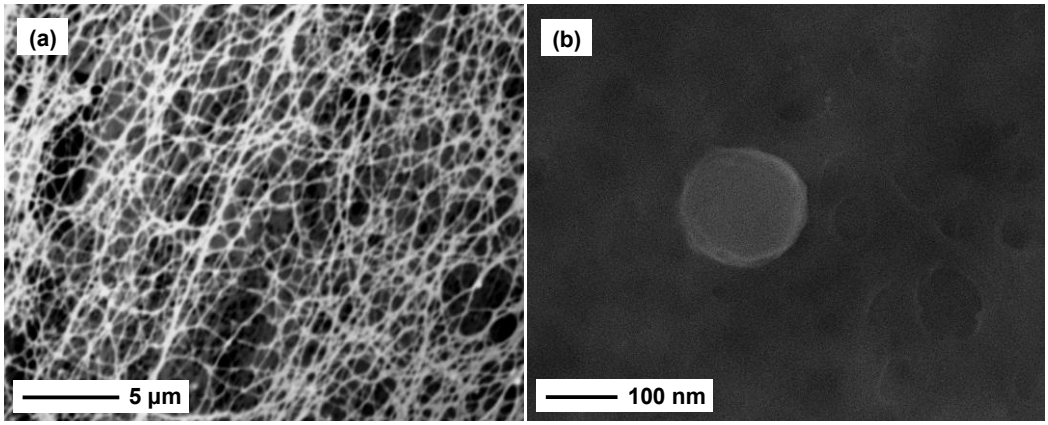

Fig. 1. Morphology of bacterial cellulose after: (a) alkaline treatment and freeze drying and (b) oxidation with ammonium persulfate.

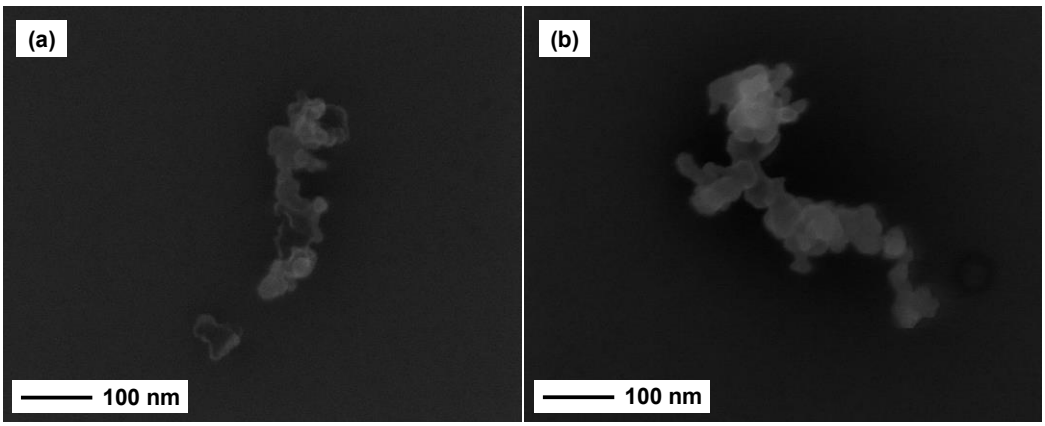

Fig. 2. Morphology of natural nanofillers prepared by the ammonium persulfate oxidation method: (a) bleached birch Kraft pulp and (b) birch bark.

The bacterial cellulose film obtained by the solvent exchange method was white and non-homogenous; bacterial and bleached birch Kraft pulp nanoparticles dispersed in water produced films that were transparent, uniform, and without any visible micro and macro agglomerates. Nanofillers-doped chitosan films were with a smooth surface topography and homogenous distribution of natural nanofillers; the chitosan film was transparent and colorless compared to nanofillers-doped chitosan films.

The mechanical properties of the films are shown in Fig. 3. Referring to Fig. 3, the bio-based films differed significantly ($p << 0.05$) in tensile strength. The highest tensile strength was found for the oxidized bacterial cellulose (OX-BC) film, followed by the chitosan-oxidized bacterial cellulose (CH-OX-BC) film, the chitosan (CH) film, the BC film (obtained by the solvent exchange method from biosynthesized cellulose), and finally oxidized bleached birch Kraft pulp (OX-KP), chitosan-oxidized bleached birch Kraft pulp (CH-OX-KP) and chitosan-oxidized birch bark (CH-OX-BB) films. The crystallinity of the nanofillers (Table I) influences their mechanical properties. The tensile strength of the OX-BC film and the CH-OX-BC film was significantly higher, and their stretch at break – the lowest, because there was a less amorphous phase in the oxidized bacterial cellulose than in oxidized bleached birch Kraft pulp and birch bark.

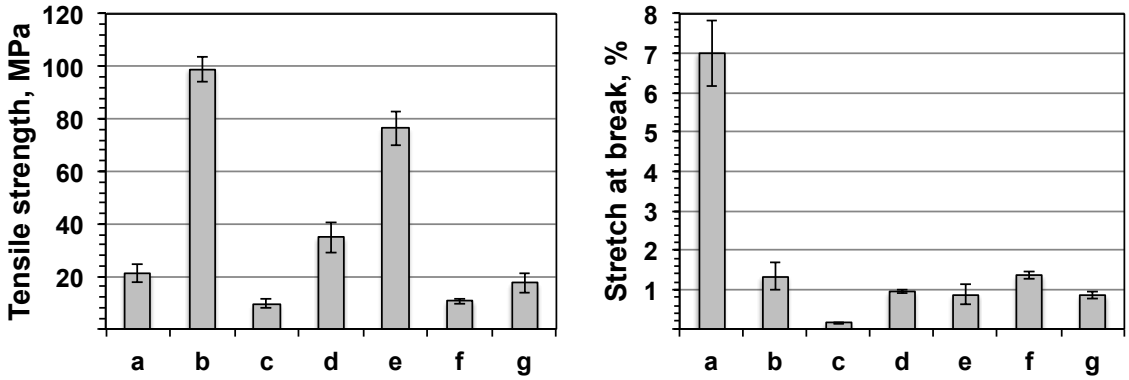

Fig. 3. Mechanical properties of bio-based films: (a) BC film (obtained by the solvent exchange method from biosynthesized cellulose), (b) OX-BC film, (c) OX-KP film, (d) CH film, (e) CH-OX-BC film, (f) CH-OX-KP film, and (g) CH-OX-BB film. Data represent the mean ± SD of 8 samples.

The cell viability was measured via the AlamarBlue® assay; the films affected the cell viability differently over time (data not shown). At day one, the viability of cells was higher on CH-OX bacterial cellulose films, followed by CH-OX bleached birch Kraft pulp and CH-OX birch bark films. At day 5, the viability of cells was decreased, which was maintained for 7 days. This behavior was not in close agreement with the results of cell viability, the number of cells, which can be seen in SEM micrographs (data not shown), increased up to 7 days of culture. The results showed that the films had a decreased cellular biocompatibility to osteosarcoma cells; it could be related to the interaction of resazurin – an active component of the AlamarBlue® reagent – to the films. Otherwise, all films showed a good biocompatibility to osteosarcoma cells and could be suitable candidates as biomaterials.

4. Conclusion

The influence of the obtained cellulose and birch bark nanofillers using the ammonium persulfate oxidation method on the physical-mechanical and biological properties of bio-based films was investigated. The results showed that bacterial cellulose and bleached birch Kraft pulp nanoparticles can be used to obtain nanocellulose film, and the chitosan matrix can be successfully tuned by

including these nanofillers to improve the physical-mechanical properties. The bacterial cellulose and bleached birch Kraft pulp nanofillers-doped chitosan films were non-toxic and had the ability to promote cell attachment and spreading. The viability of cells was lower on birch bark nanofillers-doped chitosan films than on cellulose nanofillers-doped chitosan films.

Acknowledgment

Financial support for this research from the National Research Program of Latvia is greatly appreciated. The authors are grateful to Madara Saka, Laboratory of Industrial Microbiology and Food Biotechnology, Institute of Microbiology and Biotechnology, University of Latvia, for production of bacterial cellulose. Linda Vecbiskena would like to express her appreciation to the COST Action FP1205 Grant Holder for the financial support to realize a Short-Term Scientific Mission at the Department of Chemistry, Materials and Chemical Engineering "G. Natta", Milan, Italy. Linda Vecbiskena is thankful to Lina Altomare, Luigi de Nardo and Gabriele Candiani, Department of Chemistry, Materials and Chemical Engineering "G.Natta", Milan, Italy, for the cooperation to perform the cell culture work, and Sergei Vlasov, Institute of Physics, University of Tartu, for the SEM micrographs.

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