U.P.B. Sci. Bull., Series B, Vol. 73, Iss. 2, 2011 ISSN 1454-2331

SYNTHESIS AND CHARACTERIZATION OF POLY(VINYL ALCOHOL)- BACTERIAL CELLULOSE NANOCOMPOSITE

Teodor G. CHICIUDEAN[1], Anicuța STOICA[2], Tănase DOBRE[3],
Michel van TOOREN[4]

A fost obținut un nou compozit pe bazã de pelicule de celulozã produse de Acetobacter Xylinum si APV. Pentru mediul de culturã ales s-a stabilit pH-ul optim de lucru asociat obținerii unei structuri uniforme a peliculelor ca fiind în domeniul de 3.5 - 4. S-a propus o nouã tratare alcalinã a peliculelor pentru ca ele sa fie mai bine utilizate în producerea de noi materiale compozite. Materialul obținut a fost testat la tracțiune rezultând o valoare a modulului de elasticitate de 42 GPa şi o rezistențã maximã la rupere de 370 MPa. Experimentele de sorbție de apã relevã o micã influențã asupra caracteristicilor mecanice ale noului material.

A new composite material has been obtained using cellulose pellicles produced by Acetobacter Xylinum and PVA. An optimal pH study for BC production was carried out at different initial pH values. A uniform structure was produced at pH 3.5 and 4. A new method for alkaline treatment of bacterial cellulose was proposed. The resulted cellulose was used to produce a new composite material which was subjected to tensile tests revealing a Young's modulus of 42 GPa and a tensile strength of up to 370 MPa. Experiments for water absorption showed that mechanical properties are slightly influenced after water absorption.

Keywords: Bacterial Cellulose Composites, *A. Xylinum*, poly(vinyl alcohol)

1. Introduction

Composites are a very important class of engineering materials. Nowadays many polymers and fibers which are used in composite materials are derived from petroleum industry. These polymeric materials are recalcitrant to environmental degradation. For this reason, there is an urgent need for the development of biodegradable materials. Various biodegradable polymers are being developed to replace non-degradable plastics materials. From this class of biodegradable polymers, natural polymers are the most attractive to replace synthetic materials.

---

[1] PhD eng., Ke-Works S.R.L., Bucharest, Romania e-mail: t.g.chiciudean@ke-works.ro

[2] Reader, Depart. of Chemical Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: anicuta_s@yahoo.com

[3] Prof., Depart. of Chemical Engineering, University POLITEHNICA of Bucharest, Romania, e-mail: tghdobre@gmail.com

[4] Prof., Depart. of System Engineering and Aircraft Design, Faculty of Aerospace Engineering, TU DELFT, The Netherlands e-mail: M.J.L.vanTooren@TUDelft.nl

Cellulose is the most common organic polymer on Earth. About 33 % of all biosphere matter consists of cellulose. It is environmentally friendly, 100% recyclable and in pure form biocompatible [1]. In nature it occurs in a combination with hemicelluloses and lignin mainly produced by plants with an annual production of over 1.5 x10^{12} tons [2]. However, there are some species of bacteria that can also produce cellulose. From these, the most studied and most used bacterial cellulose (BC)-producing bacterium species is *Acetobacter xylinum. A. xylinum* is the only one capable to produce cellulose in industrial quantities [3]. *A. xylinium* is a gram-negative, aerobic, rod-shaped organism producing cellulose at the air-medium interface as an assembly of interwoven ribbons that are chemically pure [4]. This bacterium has been used as a model in the exploration of cellulose biosynthesis [5, 6]. Even though it still remains a mystery for science, it is now, more then ever, investigated as a key source for high strength bio-degradable polymers [7, 8]. The reason for this are the unique properties of bacterial cellulose produced by *Acetobacter xylinum* compared to other natural polymers. Bacterial cellulose (BC) is a three-dimensional reticular network of fine fibrils (2-4 nm in diameter) coalesced into a ribbon (100 nm width, 1-9 µm length) one-hundred times smaller than wood fibers. Because of the extensive hydrogen bonding within the reticulated network, it can hold up to 700 times its dry weight in water. The crystallinity index is above 70% and a separate fibril can have a theoretical Young's modulus of 173 GPa and tensile strength in the order of 2 GPa [9]. These mechanical properties are based on a pure cellulose fiber and therefore, they are never reached in actual materials that consist of cellulose fibers. These unique properties emphasize the potential of BC as a new material for use in the production of quality paper [10], paint additives, diet and dessert foods, artificial skin [11], wound dressing [12] and ultrafiltration membranes [13].

Even if it is a synthetic polymer polyvinyl alcohol (PVA), a hydrolysis product of polyvinyl acetate, it is well suited for blending with natural polymers since it is a highly polar synthetic polymer and is considered biodegradable.

Despite its versatile properties and extensive use in different applications, BC has never been investigated as a potential bio-degradable polymer for aerospace applications like composite structure for micro satellites or high strength membranes for micro Unmanned Aerial Vehicles (UAV) with morphing wings [14]. The reason for this is the inconsistence of data available with respect to the mechanical properties of BC and its thermal and moisture behavior. For this type of application is important to quantify very precisely the consequences of a potential failure. More specifically, if a component X undergoes a water absorption process is it still able to preserve its designed stresses despite its geometrical deformation?

In the recent years several research groups have taken on the difficult task of quantifying the properties of a series of BC composite materials. The studies revealed an 18-28 GPa Young's modulus and a tensile strength of 260-425 MPa [9, 15, 16]. Despite the achieved result in terms of strength, there are a number of small, but important issues which were not fully addressed like water absorption and concise alkaline treatment of BC.

The aim of this paper is to present the preparation and characterization of BC- PVA laminate composites which can be used in aerospace applications. The paper is organized in two parts. The first part one starts with isolation of bacteria from its natural habitat, optimization of the cultivation medium, alkaline treatment and drying of BC pellicles. The second part presents the composite BC-PVA production and characterization. Because of the envisaged applications the new material was subjected to a tensile test before and after water absorption. Scanning electron microscopy (SEM) analysis was performed to study the morphology of PVA- BC composites.

2. Experimental

Reagents

The chemicals used throughout this work were purchased from Carl Roth GmbH + Co. KG. Only poly(vinyl alcohol) (PVA), average molecular weight (Mw) 85,000–124,000 g/mol, 87–89% hydrolyzed was purchased from Sigma–Aldrich and used without further treatment or purification.

Production of strater culture

The microorganism used in this research (a strain of *A. xylinum*) was isolated from pineapple origin from Costa Rica. Isolation and purification of the culture was accomplished empirically through repeated plate cultivation on 3.2 pH agar medium. The main composition of the isolation medium was agar-agar (1.6 % w/v), honey (30 g L^{-1}) (from a local market), peptone (3 g L^{-1}), yeast extract (3 g L^{-1}), K_2HP0_2 (1 g L^{-1}), adjusted to pH 3.5 with acetic acid.

The microorganism was maintained at 4 °C and transferred monthly on an in-house developed serum similar to the inoculation medium. The inoculum was grown in a medium containing: dextrose (20 gL^{-1}), peptone (5gL^{-1}), 5 gL^{-1} yeast extract adjusted to different pH values with acetic acid in static culture. The inoculation was performed in aerated culture for 72 h using a 4 L/min air flow.

Determination of optimum fermentation conditions

Based on previous experimental results, it was established that the factors which influence BC yield are operating temperature and carbon source concentration [15]. In this regard, the fermentation temperature was maintained constant at 24 °C and dextrose concentration at 20 g/L. In order to obtain uniform pellicles, only the initial pH medium and medium heights were considered as dependent parameters of the process. Using the same experimental set-up, several experiments were performed at initial pH values of 2.7, 3.0, 3.5, 4.0, 5.0 and 6.0. For each pH value a static culture was performed using square glass trays with an area of 600 cm^2. The volume was also varied for each pH value from 1 to 6 cm medium height with an increment of 0.5 cm/tray. It was concluded that for BC produced at liquid-air interface, the production was most favorable at pH 4 and 5 with a medium height of 4.5 cm. Even though the highest BC yield was obtained at pH 4 and 5, a uniform structure was produced at pH 3.5 and 4 for 4.5 cm medium height. These conditions were preferred for the next experiments.

Based on the revealed results, a set of 40 pellicles were produced in a static culture, the experimental parameters being presented in Table 1. Previous to static cultivation, the medium was aerated for 24 h in order to speed-up the bacteria multiplication within the medium. The resulted inoculated medium was qualitatively analyzed using optical microscopy before static cultivation.

Table 1

Experimental parameters for BC static culture

Medium	pH	Temperature (°C)	Growth time (h)	Tray dimensions	
				Volume (L)	Height (cm)
Dextrose (20g/L) Peptone (5g/L) Yeast Extract (5g/L)	3.5	24	250	9	4.5

Purification of BC pellicle

The obtained BC pellicles were harvested after 250 h of static cultivation. Apart from cellulose, the pellicles contain a large amount of water, bacteria and other constituents of the medium, mostly high concentrations of gluconic acid. In order to obtain a pure BC pellicle, an alkaline treatment must be performed. Despite the fact that the literature abounds in papers addressing the alkaline treatment of BC, a series of data inconsistency were revealed in practice, mostly regarding the alkaline solution volume [16]. To clarify this issues this work will present an alkaline treatment performed in two steps.

In the first step, each pellicle was weighted and soaked in 0.1 N NaOH aqueous solution. The pellicles were soaked individually in the alkaline solution according to their weight using a three times higher volume of solution than the volume of the pellicle. In this step, the alkaline solution was brought from room temperature to 80 °C and maintained for one hour under agitation. During this process, the pellicles changed color from white to brown-gold and the pH value, in some cases, grew up to pH=7. Before the second phase of the treatment, the pellicles were squeezed to 5 % of their initial volume. Then, the squeezed pellicles were again soaked individually in a ten times higher volume of 0.1 N NaOH solution than their volume before being squeezed. In this phase the solution temperature was maintained at 80 °C for 30 minutes followed by 20 minutes at 100 °C, then cooled down at room temperature. During this process, the pellicles regain their initial water holding capacity.

The alkaline treated pellicles were squeezed again to 5 % of their initial volume and soaked in a 3% (w/w) monohydrated citric acid solution for 24 h. The resulted pellicles were then squeezed and washed in distillated water until a neutral pH value was obtained.

Preparation of BC sheets

The obtained 10-mm thick bio-free gel-like pellicles of BC were uniform vacuum squeezed using a laboratory setup as depicted in Fig. 1. The squeezing process consisted of two phases. In first phase the squeezing is realized by applying a vacuum pressure on the water evacuation channels fallowed by a 6 bar pressure applied on the pellicle surface.

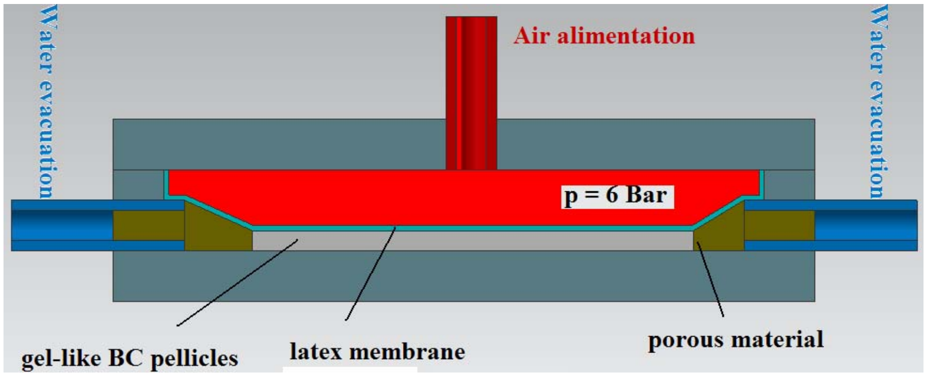

Fig. 1. Uniform vacuum squeeze

After uniform vacuum squeeze, BC pellicles of about 0.2 mm thickness were dried under air flow at 70 °C on a cylindrical surface and detached using acetone. The obtained sheets had approximately 40 µm thickness and 1.2 g/cm^3 in density.

Preparation of BC composites

Two types of BC composites were developed in this research using PVA as matrix and dried BC sheets, respectively hydro BC pellicles as reinforced fibers.

In the case of the composite having as reinforced fibers dried BC sheets, about six BC sheets were immersed in a solution containing 8% (w/w) PVA, 50% (w/w) ethanol and 42% (w/w) water for 30 min. Impregnated sheets were taken out from the solution and laminated by hand. During lamination, one of the sheet edges was clamped to the lamination board with tape to constrain the sheets not to shift as it can be observed from Fig. 2. The laminated composite was pressed by hand several times to eliminate air and wrinkles, then ethanol and water was left to evaporate at room temperature for 6 h. The resulted composite was then uniform vacuum squeezed to 0.3 mm thickness and a set of smaller probes were cut off in different sizes: (1a) 9 x 150 mm, (2a) 7.2 x 150 mm, (3a) 10 x 150 mm, (4a) 9.3 x 150 mm, (5a) 6.5 x 150 mm, (6a) 6.7 x 150 mm. Finally, the probes were dried at room temperature for 48 h. The dried probe measured 0.24 mm in thickness.

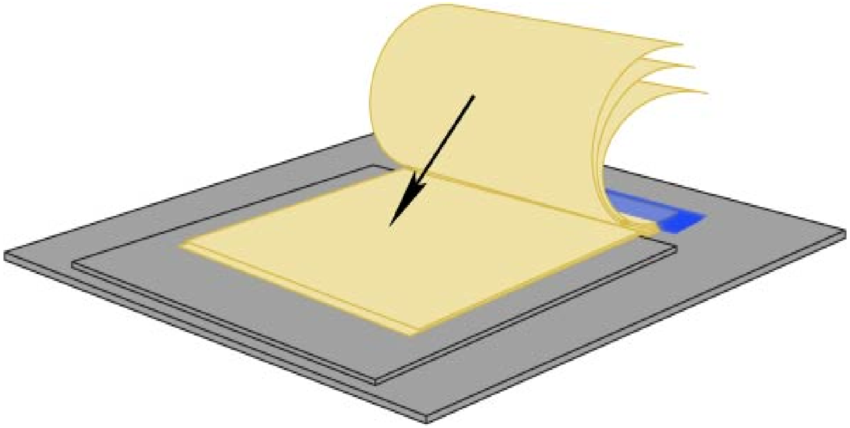

Fig. 2. Laminating the taped sheets

The second composite material was made using non-dried alkaline treated pellicles of BC with 0.2 mm thickness. The pellicles were immersed in a solution of 8% PVA, 50% ethanol and 42% water for 24 h. The impregnated BC pellicles were stacked together by hand lay-up in layers of about six BC pellicles. To avoid air insertion during lay-up, the BC pellicles were laid-up by submerging the sheets in PVA solution. The resulted probes were uniform vacuum squeezed till they measured 0.8 mm in thickness and dried at room temperature for 48 h. The dried probe measured 0.4 mm in thickness. The probe was cut into smaller pieces of different dimensions: (1b) 9 x 150 mm, (2b) 19 x 150 mm and (3b) 19x 150 mm.

Tensile test

The samples were subjected to tensile tests using the same Zwick 1455 at a strain rate of 1 mm/min over a spam of 75 mm. As guideline the standard tests method for tensile properties of polymers matrix composites materials D 3039/D 3039M-07 was used. The only deviation from the standard was the use of paper tabs for the sample grip area (see Fig. 3).

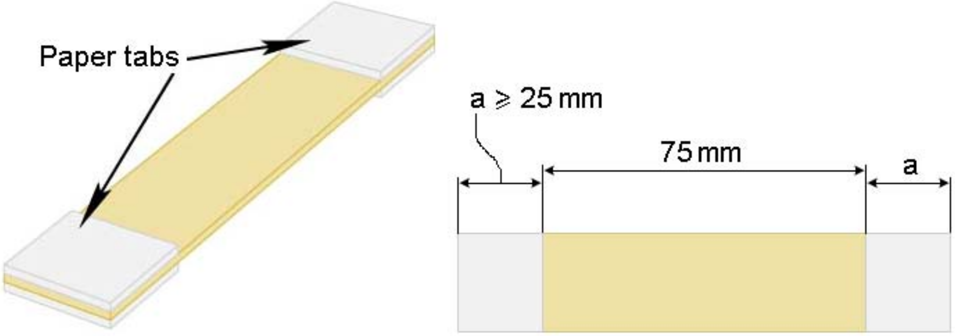

Fig. 3. Rectangular specimen with paper tabs

Water absorption

A simplified relation between the effect of water absorption and the mechanical properties of the composite was investigated. Since bacterial cellulose is highly hydrophilic, it will immediately start to absorb water from the air/environment when pellicles are dried or composites are cured at high temperature. This water can cause an increase in volume and directly affect the mechanical properties of the composite. To model the effect of water absorption it was assumed that the composite behaves as a sponge to which a pressure force was applied to reduce its thickness. This pressure force is analogous to the pressure applied to remove the water from the BC pellicles. In consequence, it can be assumed that the length and width of a specimen is constant and that the ratio of stress in a saturated specimen and dry specimen can be calculated as:

$$\frac{\sigma_{wet}}{\sigma_{dry}} = \frac{A_{wet}}{A_{dry}} \approx \frac{d_{dry}}{d_{wet}} = \frac{d_{dry}}{d_{dry} + d_{water}} = \frac{1}{1+a} \quad ; \quad a = \frac{d_{water}}{d_{dry}} \tag{1}$$

Where σ is the stress [N/mm^2], F is the force [N], A is the loaded area [mm^2], d is the thickness [mm]. The subscript '*wet*' indicates the BC with absorbed water, '*dry*' the dry BC and '*water*' the absorbed water in the BC. The increase of thickness caused by the water is:

$$d_{water} = \frac{V_{water}}{l \cdot b} = \frac{m_{water}}{\rho_{water} \cdot l \cdot b} = \frac{x_w \cdot m_{dry}}{\rho_{water} \cdot l \cdot b} \tag{2}$$

Where V is the volume [mm^3], l is the length [mm], m is the mass [g], ρ is the density [g/mm^3] and x_w is the weight ratio of absorbed water and BC dry weight. This makes the ratio of thicknesses <a> and ratio of stresses:

$$a = \frac{x_w \cdot m_{dry} \cdot \rho_{BC} \cdot l \cdot b}{m_{dry} \cdot \rho_{water} \cdot l \cdot b} = \frac{x_w \cdot \rho_{BC}}{\rho_{water}} \approx 1.6 x_w \tag{3}$$

$$\frac{\sigma_{wet}}{\sigma_{dry}} \approx \frac{1}{1 + 1.6 x_w} \tag{4}$$

Since it was considered that the water absorption does not affect the length or elongation according to the previous assumptions, the ratio of Young's modulus would become:

$$\frac{E_{wet}}{E_{dry}} = \frac{\sigma_{wet} / \varepsilon_{wet}}{\sigma_{dry} / \varepsilon_{dry}} \approx \frac{\sigma_{wet}}{\sigma_{dry}} \tag{5}$$

Where E is the Young's modulus [N/m^2] and ε is the strain. It is clear that this is a simple model, because the water absorption phenomenon is not a linear one. In addition to material volume increase, the material undergoes a series of physical and chemical transformations during water absorption which can alter its mechanical properties. To evaluate the validity of the model and to which level of saturation it can be applied, two of the tensile tests were performed using samples with 30% mass increase due to water absorption.

3. Results and discussions

In Fig. 4 the stress-strain behavior of the composite obtained using dried BC sheets, is presented together with the general effect of water absorption.

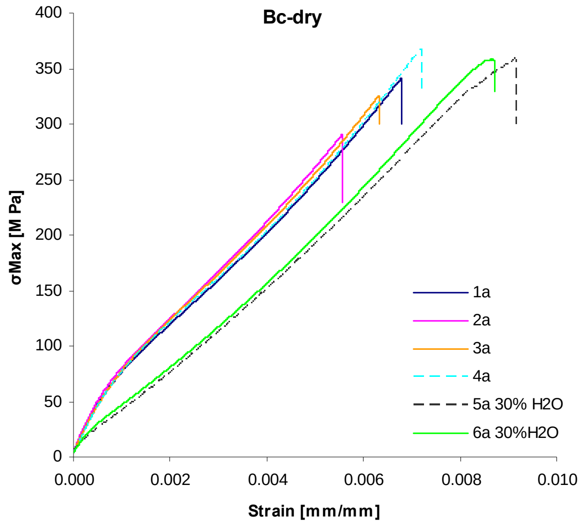

Fig. 4. BC- dry stress-strain diagram for samples 1a, 2a, 3a, 4a, 5a and 6a. The probes 5a and 6 a are measured after water absorption

Fig. 5 shows the consistency of the results considering the fact that the second composite, with non-dried BC pellicles, has with 60% less fiber than the one with dry BC sheets.

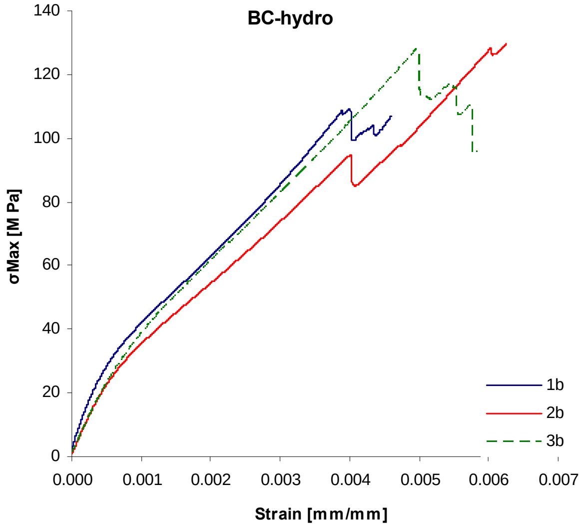

Fig. 5. BC-hydro stress-strain diagram for the composites BC-PVA 1b, 2b and 3b

In the diagram presented in Fig. 4, σMax was calculated for sample (5a) and (6a) by omitting the thickness increase as a result of water absorption. It was noticed that the material suffers a plasticizing effect which decreases its mechanical properties in addition to its thickness increase. Even though the material behaves well in strength, a more visible effect of plasticization is noticed in its elongation which directly affects Young's modulus, as shown in Fig. 6.

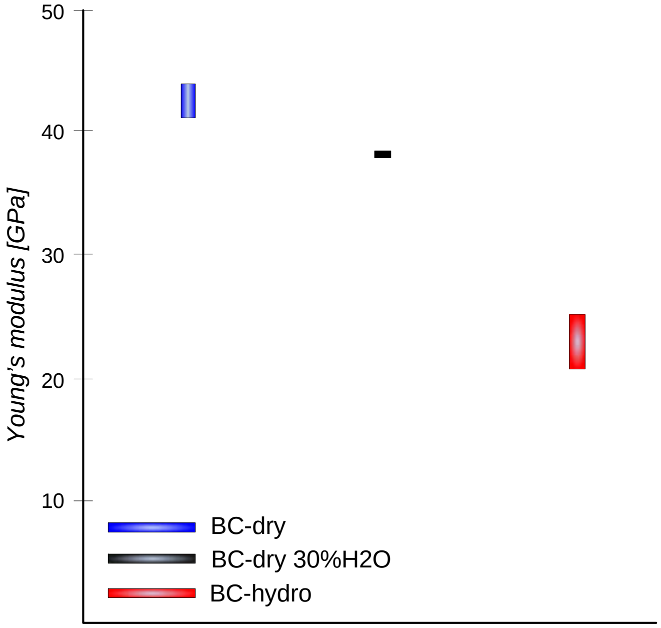

Fig. 6. Young's modulus of different BC composites

The data confirms the fact that water is a plasticizer for the composite, but does not affect the fiber strength at this water absorption level. Strength is attributed to the fact that the material, at this value of absorbed water, does not form high extents of hydrogen bonds with the water which means that the material is not in the saturation phase of water absorption.. In order to verify this assumption, a water absorption experiment was performed by placing samples identical with sample (5b) and (6b) in 1500 ml screw-cap flasks containing distilled water. Experiments were conducted at 25 °C with different immersion periods: from several minutes to about 250 h. Fig. 7 shows the water uptake of the BC-dry composite. One can observe that the water uptake increases linearly, then the rate slows down in the saturation phase, and finally leads to a plateau, corresponding to the water uptake in equilibrium. From the presented graphic it can be deduced that the tested material is in a linear phase until its mass increases to 80 % as a result of water uptake

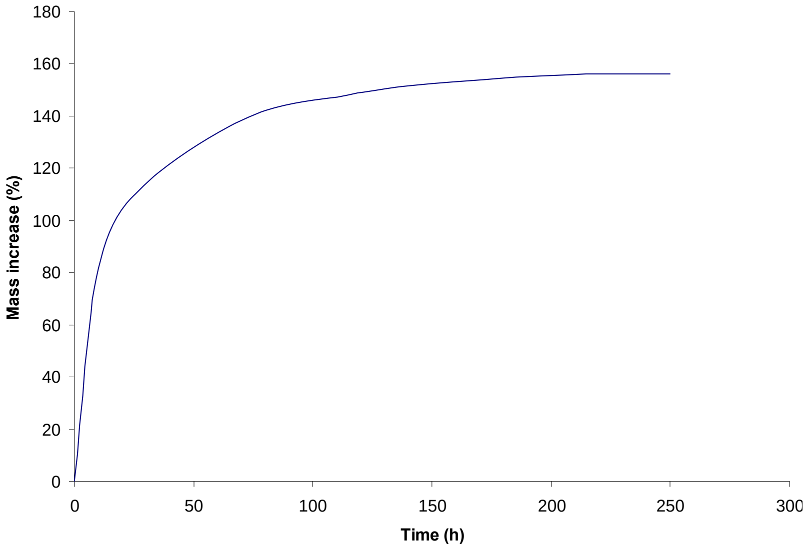

Fig.7. BC water absorption

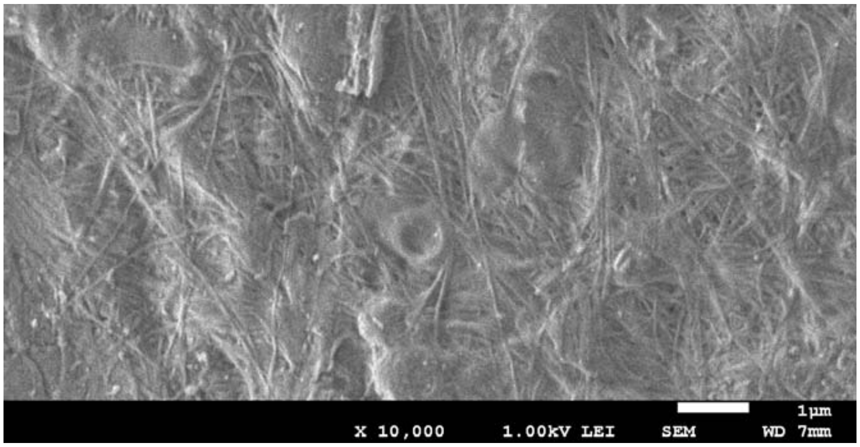

Fig. 8. SME micrographs of BC-dry composite; top view perpendicular to the pellicle growing area "air-medium interface"

The material elongation can be attributed to the fact that the water existing in the material matrix allows the cellulose fibers to slide over each other. As described frequently by many authors [9, 17], BC is a random in-the-plane orientation of nanofibrils as it can be seen in Fig. 8. It was also considered as 3D interwoven network of nanofibrils, "extremely fine, pure and dimensionally uniform" [18]. But a more close examination of a delaminated fracture from Fig. 9a revealed that the BC is far from dimensional uniformity. The fibrils resemble to tree branches in which a thicker fibril is orientated in-plane from which several thinner fibrils emerge with a small offset from in-plane orientated. This structure explains why the BC fracture resembles to a laminated nano composite (Fig. 9b) and also the creep effect which is amplified by water absorption [19].

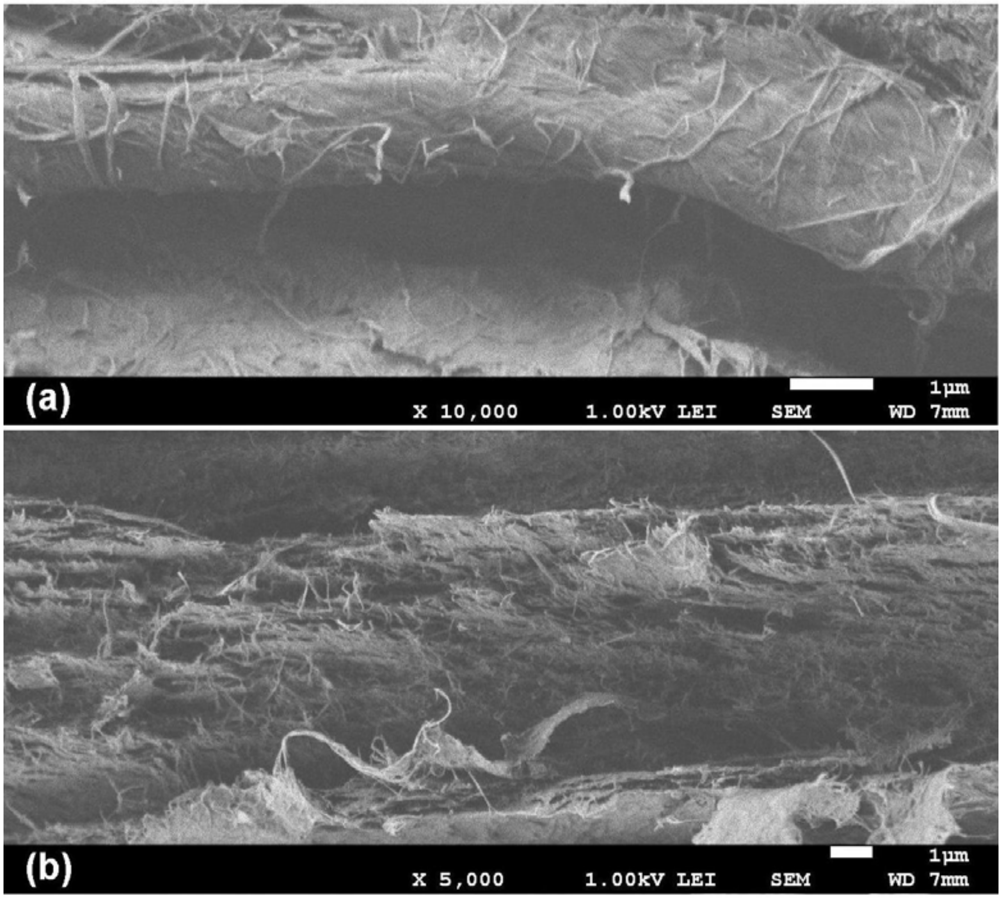

Fig. 9. SME micrographs of BC-dry composite: a) delaminated fracture b) section view

Based on these results it can be deduced that the equation (5) is partially valid. To account for this, a correction factor is introduced and the equation becomes:

$$(1+m)\cdot\frac{E_{wet}}{E_{dry}}=\frac{\sigma_{wet}/\varepsilon_{wet}}{\sigma_{dry}/\varepsilon_{dry}}\approx\frac{\sigma_{wet}}{\sigma_{dry}} \tag{6}$$

where m is the local slope of the water absorption curve. This empirical formulation is valid just for the linear phase that is until 80 % mass increase in the material due to water absorption.

Based on this facts, it can be assumed that a component X produced from BC it outstands a tension force F with or without absorbed water and suffers an elongation $L \cdot (1+m)$ with absorbed water and L without absorbed water. This unique polymorphic property of BC composites is a great value in applications that require a material that behaves rigid and plastic in dry and wet environment. For example, in UAV the wing morphs can be induced by water injection within the wing structure without compromising the structure's capacity to outstand the aerodynamic forces. In this way a wing bending can be achieved.

6. Conclusions

Bacterial cellulose was obtained in static culture using different conditions for cultivation in order to obtain pellicles with good mechanical properties. Alkaline treated BC pellicles were impregnated with PVA to produce high-strength biodegradable composites. Two types of composites were prepared using dried and wet bacterial cellulose sheets. The Young's modulus of the composite was significantly higher than any reported for similar composites by other authors, about 42 GPa. Despite the fact that the new composites absorb a considerable quantity of water until saturation, it was concluded that the material is able to outstand high stresses even in wet conditions or immersed in water for short periods.

REFERENCES

[1] *F.A. Bovey, Winslow,* Macromolecules, An Introduction to Polymer Science, Academic Press, New York. 2001

[2] *D.R. Ridley, F.R. Williams, K.D. Song, S.R. Yun, K.S. Kang, J. Kim*, Effect of Electrode Pattern on the Actuator Performance of Cellulose Electro-Active Paper, Journal of Intelligent Material Systems and Structures, **vol.21**, No 4, pp 401-406, 2010

[3] *Kiyoshi Toda,Tomoko Asakurab, Masahiro Fukaya, Etsuzo Entani, Yoshiya Kawamura* Cellulose production by acetic acid-resistant Acetobacter xylinum, Journal of Fermentation and Bioengineering, **Vol. 84**, Issue 3, Elsevier B.V., 1997

[4] *R. Mormino, H. Bungay*, Composites of bacterial cellulose and paper made with a rotating disk bioreactor, Applied Microbiology and Biotechnology, **Vol. 62**, Numbers 5-6, Springer, Berlin, 2003

[5] *L.R. Lynd, P.J. Weimer, W.H. van Zyl, I.S. Pretorius*, Microbial Cellulose Utilization: Fundamentals and Biotechnology ,Microbiology and Molecular Biology Reviews, p. 506-577, **Vol. 66**, No. 3, September 2002

[6] *R.M. Brown Jr, I.M. Saxena, Krystyna Kudlicka*, Cellulose biosynthesis in higher plants,Trends in Plant Science,**Vol. 1**, Issue 5, Elsevier Ltd, 1996

[7] *S.J. Eichhorn, A. Dufresne, M. Aranguren, N.E. Marcovich, J.R. Capadona, S.J. Rowan, C. Weder, W. Thielemans, M. Roman,S. Renneckar, W. Gindl, S. Veigel, J. Keckes, H. Yano, K.*

Abe, M. Nogi, A.N. Nakagaito, A. Mangalam, J. Simonsen, A.S. Benight, A. Bismarck, L.A. Berglund, T. Peijs, Review: current international research into cellulose nanofibres and nanocomposites,Journal of Materials Science, **Vol. 45**, Number 1, Springer Netherlands, 2010

[8] *O.M. Astley, Elisabeth Chanliaud, Athene M. Donald, M.J. Gidley,* Tensile deformation of bacterial cellulose composites International Journal of Biological Macromolecules, **Vol. 32**, Issues 1-2, Elsevier B.V., 2003

[9] *S. Yamanaka, K. Watanabe, N. Kitamura, M. Iguchi, S. Mitsuhashi, Y. Nishi, M. Uryu,* The Structure and Mechanical Properties of Sheets Prepared from Bacterial Cellulose , Journal of Materials Science, **Vol. 24**, pp3141-3145, 1989

[10] *D.C. Johnson, A.R. Winslow,* Bacterial cellulose has potential application as new paper coating. Pulp & Paper, pp. 105–107 May 1990

[11] *E. Cannon, S.M. Anderson,* Biogenesis of bacterial cellulose. Crit. Rev. Microbiol. 17 (1991), pp. 435–447. 1990

[12] *J.D. Fontana, A.M. Souza, C.K. Fontana, I.L. Torriani, J.C. Moreschi, B.J. Gallotti, S.J. Souza, G.P. Narcisco, J.A. Bichara, L.F.X. Farah,* Acetobacter cellulose pellicle as a temporary skin substitute. Appl. Biochem. Biotechnol. 24–25 (1990), pp. 253–26, 1990

[13] *M. Takai, F. Nonomura, T. Inukai, M. Fujiwara, J. Hayashi,* Filtration and permeation characteristics of bacterial cellulose composite. Sen'i Gakkaishi 47, pp. 119–129 1990

[14] *K. De Clercq, R. de Kat, B. Remes, B. van Oudheusden, H. Bijl,* "Flow Visualization and Force Measurements on a Hovering Flapping-Wing MAV 'DelFly II' ", 39th AIAA fluid dynamics conference, AIAA-2009-4035

[15] *T. Dobre, A. Stoica, O. Parvulescu, M. Stroescu, G. Iavorschi,* Factors influence on bacterial cellulose growth in static reactors, Revista de chimie, (Bucuresti), **Vol. 59 (5)**, pp. 591-594, 2008

[16] *J. George, K.V. Ramana., S.N. Sabapathy, J.H. Jagannath, A.S. Bawa,* Characterization of chemically treated bacterial (*Acetobacter xylinum*) biopolymer: Some thermo-mechanical properties, International Journal of Biological Macromolecules, **Vol. 37**, pp.189–194, 2005

[17] *S. Yamanaka, K. Watanabe, N. Kitamura, M. Iguchi, S. Mitsuhashi, Y. Nishi, M.Uryu,* The Structure and Mechanical Properties of Sheets Prepared from Bacterial Cellulose, Part 2: Improvement of the mechanical properties of sheets and their applicability to diaphragms of electroacoustic transducers, Journal of Materials Science, **Vol. 25**, pp. 2997-3001, 1990

[18] *A.N. Nakagaito, S. Iwamoto, H. Yano,* Bacterial cellulose: the ultimate nano-scalar cellulose morphology for the production of high-strength composite, Appl. Phys., A 80,pp. 93-97, 2005

[19] *J. Alfthan,* The Effect of Humidity Cycle Amplitude on Accelerated Tensile Creep of Paper Mechanics of Time-Dependent Materials, **Vol. 8**, Number 4, Springer Netherlands 2004.