

## Influence of Drying Temperature on Tensile and Bursting Strength of Bacterial Cellulose Biofilm

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The article presents an experimental study of mechanical properties of cellulose biofilm produced by bacterial fermentation process. Naturally derived biomaterial has great current and potential applications therefore the conditions of material preparation as well as control and prediction of mechanical properties is still a relevant issue. Bacterial cellulose was obtained as a secondary product from Kombucha drink. Presented technique for material preparation and drying is particularly simple and easy to access. The influence of drying temperature (25 °C, 50 °C and 75 °C) on the sample size (thickness and planar dimensions) and mechanical properties (tensile and bursting strength) of cellulose biofilm has been evaluated. It was estimated that during drying biofilm specimens lost up to 92 % of weight and up to 87 % of thickness therefore planar specimen dimensions varied insignificantly. The study showed that the drying temperature is important for optimum strength properties of bacterial cellulose biofilm. The maximum tensile strength (27.91 MPa) was recorded for the samples dried at temperature of 25 °C, when the moisture from the biomaterial is removed gradually and good deformation properties are ensured (respectively tensile extension 18.8 %). Under higher drying temperature biomaterial shows lower values of tensile strength and higher values of bursting strength. The maximum bursting strength (57.2 MPa) was recorded for samples dried at 75 °C when punch displacement changes were insignificant for all tested samples (from 17.8 mm to 21.7 mm).

**Keywords:** bacterial cellulose, drying temperature, mechanical properties, tensile test, bursting strength.

### 1. INTRODUCTION

Cellulose is the most abundant organic compound on Earth. This material is associated with plants, but certain type of bacteria can also produce it. Bacterial cellulose (BC) has unique structure and properties. It can be synthesized by bacteria *Gluconacetobacter xylinus*, which is found in fruits, vegetables, vinegar, fruit juices and alcoholic beverages [1].

Bacterial cellulose has porous structure of three-dimensional network of fibrils. These fibrils are around 100 nm in diameter and are composed of much finer nanofibrils of 2–4 nm in diameter [2].

Bacterial cellulose has great current and potential applications in food [3], medicine [4], electronics [5], nanocomposite industries [6], because of properties such as a high degree of purity, polymerization, crystallinity, tensile strength, water absorption, water retaining capacity, biological adaptability, biocompatibility, biodegradability and renewability.

Kombucha film as by-product of fermented sweetened tea [7] could be used as additional source of bacterial cellulose. An origin of China, Kombucha is composed of yeast and acetic acid bacteria, which forms a cellulose film. Kombucha drink is popular in many countries and many benefits of consuming this drink have been reported [8]. Some research found where purification process and

structure of bacterial cellulose obtained from Kombucha was investigated [9, 10].

Cellulose sheet is produced with the help of bacterial fermentation process, when the gel like biofilm floats on the liquid medium surface. Stable and solid film is obtained when wet membrane is dried. The biomaterial of bacterial cellulose is highly hydrophilic, therefore drying procedure is important in order to obtain certain material [11].

The significant factors that many researchers have proved as drying method, temperature and time, growing media and conditions, duration of growth, bacteria, carbon and nitrogen source can have an influence on mechanical properties of BC biofilm [12, 13, 14].

The research was conducted to identify suitable drying method to produce dry cellulose using three methods: freeze drying, spray drying, supercritical drying and oven drying as control method [15]. Spray drying was proposed as a technically suitable manufacturing process to dry cellulose nanofibrils. Other research [16] proved that using a tray dryer and changing temperature from 40 °C to 110 °C the moisture is effectively removed at 60 °C and higher drying temperatures.

The morphology test, swelling measurement, crystallinity and tensile strength investigations were performed for the biofilm samples that were dried using three physical drying methods such as oven, tray dryer or freeze-drying until samples reached 3–5 % moisture content. Different drying method gave different properties

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of biomaterial and freeze-dried sample showed the highest tensile strength up to 148 MPa [17].

Tensile strength properties were investigated for BC biofilm, which was dried in a room, oven at 60 °C, and dried by lyophilizer with rising temperature from 20 °C to 50 °C. The best tensile strength properties were obtained for samples dried at 60 °C [18].

Scientist M. Zeng with his colleagues obtained BC from two bacterial strains (*Gluconacetobacter Xylinus* (GX) and *Gluconacetobacter Europaeus*) and used three biofilm drying methods: solvent evaporation at the room temperature, freeze drying and supercritical drying [19]. It was concluded that drying method modifies the porosity, mechanical properties and water absorption capability of the BC films. The best mechanical properties for *Gluconacetobacter Xylinus* were obtained when material was dried at the room temperature.

Wet BC sheets of the same origin *Gluconacetobacter Xylinus* were freeze dried, dried at room temperature (25 °C) and dried at elevated temperature (50 °C) by M.Ul-Islam et al. [20]. It was shown that increasing temperature to a certain point has a positive effect on the mechanical properties of BC and the highest stress values (around 40 MPa) were obtained for samples dried at 50 °C.

Mostly known scientific researches of bacterial cellulose are based on the investigation of material obtained by standardized bacteria and growing media. Researchers use different techniques to prepare and treat bacterial cellulose. Generally, NaOH of various concentrations is used to purify BC film [21]. The growing interest for naturally fermented biomaterial as the source of bacterial cellulose and problems concerning material treatment and mechanical properties is a relevant issue that should be explored.

Bacterial cellulose biofilm as a secondary product of fermented Kombucha drink was investigated in this study. The aim of the research is to estimate the influence of oven drying temperature on the mechanical properties of dried BC biofilm – tensile and biaxial strength as well as deformation. The information of biomaterial's mechanical behaviour is important to predict the quality of the final product. The limits of biaxial tension may be valuable for the evaluation of processing smooth three-dimensional shape.

## 2. EXPERIMENTAL DETAILS

**Preparation of biofilms.** In this study the biofilm of bacterial cellulose (BC) was produced using Kombucha strains primarily referred as the gram-negative bacteria species *Gluconacetobacter xylinus* (formerly *Acetobacter xylinum*). Culture medium prepared of 1 L water, 4 g green tea, 100 g of sucrose and 100 mL of 6 % yeast extract. The prepared medium was incubated with Kombucha strain and fermentation was carried out under standard room conditions (20 ± 2 °C temperature and 65 ± 5 % relative air humidity) for 7 days in static cultivation conditions.

**Drying.** During Kombucha strain fermentation, floating on medium surface gel-like biofilm was produced. Removed film was washed several times with distilled water and squeezed to reduce its water content. The samples were covered with tissue paper to protect the film surface.

Washed film samples were dried on the horizontal surface in the laboratory oven SNOL 60/300 LFN at 25±1 °C (sample code BC25), 50±1 °C (sample code BC50) and 75±1 °C (sample code BC75) until it gained a constant specimen weight. Dried biofilm samples were used for the thickness, shrinkage and mechanical behaviour investigations.

**Size parameters of BC.** Thickness of each BC specimen was measured by a digital indicator DPT 60 with accuracy of 0.01 mm. The average value of 5 measurements was calculated for the investigations. The thickness was estimated from wet and dry material at each 1 hour step during drying process. The percentage thickness loss was calculated.

The shrinkage of biofilm was determined by measuring size of BC specimen with accuracy of 0.5 mm. For each drying procedure, the diameter of biofilm specimen was determined before and after drying and the percentage diameter loss was calculated.

**Hardness measurements.** The hardness of dried biofilm was measured by Durometer. Durometer hardness testing was performed using the Shore A scale. Shore hardness was used as an indication of biofilm stiffness.

**Tensile strength.** The tensile properties of dried BC samples were investigated by TINIUS OLSEN H10 KT tensile test machine according to ISO 3376:2011 standard [22]. Samples were cut with a standard pick form: overall length – 110 mm, initial distance between grips – 50 mm, width of narrow parallel-sided portion – 10 mm. The crosshead rate of 100 mm/min was set. To estimate the average result 7 specimens have been tested. The load-extension curve was recorded to determine tensile strength, tensile modulus and extension at break.

**Bursting strength.** The ball burst test was carried out with Instron tensile testing machine equipped with the original test unit (Fig. 1) according to ISO 9073-5:2008 standard [23].

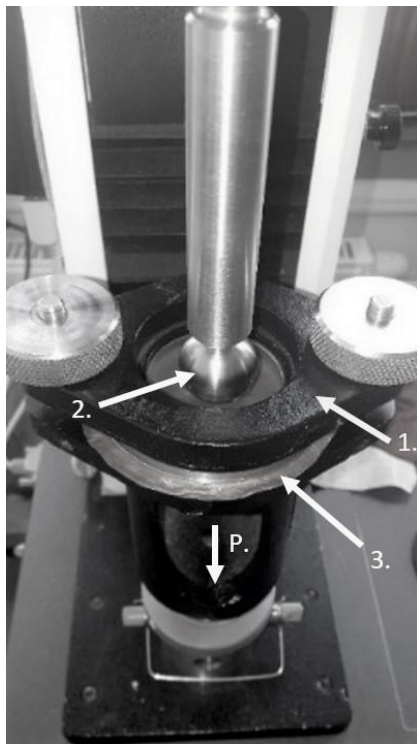
Disc-shaped specimens (specimen diameter 70 mm, internal clamp diameter 44.5 mm) were loaded by steel spherical punch (diameter 25.4 mm) up to rupture. Deformation rate was set 50 mm/min. Punch displacement (distance travelled by the plunger until specimen burst) and bursting load was estimated and the curve of whole sample deformation process was recorded.

## 3. RESULTS

### 3.1. The influence of drying on size parameters and hardness

The BC biofilm is extremely hydrophilic and drying characteristics demonstrate how efficiently moisture can be removed. The parameters of thickness loss and weight loss indicate the removal of water content in drying process. The dynamic of curves and its thickness loss showed (Fig. 2) different drying rate for each drying temperature. At higher temperature moisture is removed more effectively and drying process takes less time. A maximum weight loss (Table 1) of 91.8 % was determined for the samples dried at 50 °C and 88.3 % was estimated for the samples dried at 75 °C, and 85.5 % – for the samples dried at 25 °C temperature. In the study the water content of the

obtained bacterial cellulose film from Kombucha was up to 92 % when other researchers got results of 90–95 % [24]. Drying process at 50 °C until constant sample weight was observed to be 70 % shorter and drying at 75 °C was 58 % shorter than drying at 25 °C temperature process, which was running approximately 27 hours.



**Fig. 1.** Original ball burst test unit: 1–upper clamp; 2–punch; 3–biofilm specimen; P–bursting load direction

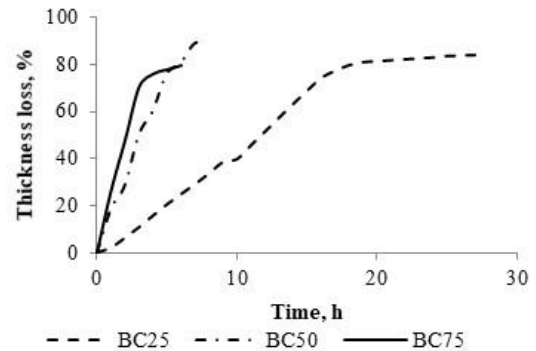
**Table 1.** Drying properties of BC

	BC25	BC50	BC75
Drying temperature, °C	25±1	50±1	75±1
Density of wet sample, g/m <sup>3</sup> (CV, %*)	1411 (6.4)	789 (5.8)	722 (5.2)
Density of dry sample, g/m <sup>3</sup> (CV, %*)	204.6 (2.6)	64.7 (1.9)	84.5 (4.9)
Weight loss, %	85.5	91.8	88.3
Thickness of dry sample, mm (CV, %*)	0.36 (2.3)	0.47 (3.1)	0.63 (4.6)
Thickness of wet sample, mm (CV, %*)	0.43 (8.9)	0.53 (5.7)	0.79 (7.8)
Thickness loss, %	83.9	88.6	79.6
Diameter of dry sample, mm (CV, %*)	13.9 (3.6)	17.6 (4.9)	15.3 (2.5)
Diameter of wet sample, mm (CV, %*)	14.6 (5.2)	17.7 (4.6)	15.4 (3.4)
Diameter loss, %	0.04	0.005	0.003
Shore hardness (CV, %*)	41 (10.1)	46 (12.8)	61 (10.5)

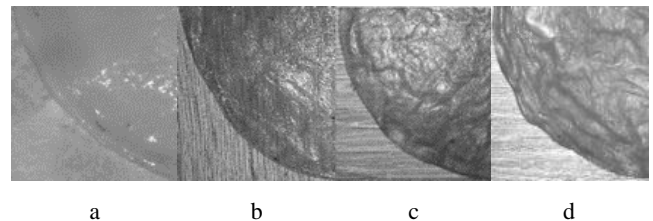
\* CV indicate result's variation

It's evident that heat applied during drying process has influence on the size parameters of BC sample and the most significant changes are recorded for the thickness and weight loss therefore planar dimensions vary insignificantly. Some physical appearance changes of

biofilm were captured also (Fig. 3). The surface of the dried BC sample shrunk more at higher drying temperature. This is confirmed by the recording of higher sample thickness values as well. The surface shrinkage might appear due to more rapid water evaporation at higher drying temperature that as was described in research [25] causes a closer contact between nanofibers and agglomeration.



**Fig. 2.** The dependence of the thickness loss of BC samples during drying



**Fig. 3.** Physical appearance for BC samples: a–wet; b–dried at 25 °C; c–dried at 50 °C, d–dried at 75 °C

Change of biomaterial hardness was estimated – BC material dried at lower temperature obtains more soft structure (Shore A value 41 for BC25) as samples dried at higher temperatures derive medium soft (Shore A value 46 for BC50) and medium hard (Shore A value 61 for BC75) nature.

### 3.2. The influence of drying on tensile properties

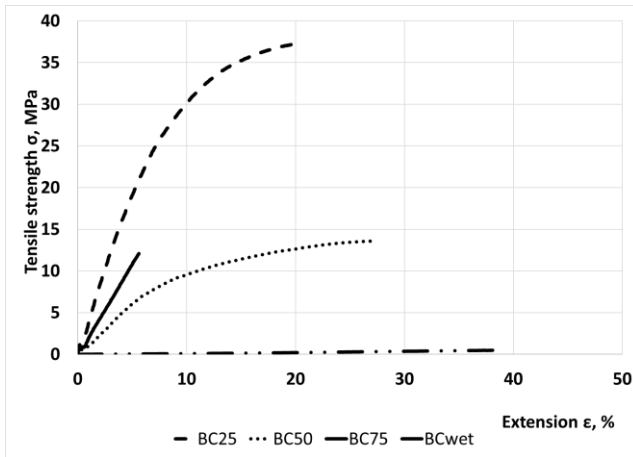
Mechanical properties are the key parameters of biofilm to be useful for industrial purpose. The results of tensile test proved that drying procedure has strong influence on the strength properties of biomaterial. Experiment confirmed that wet biomaterial is elastic (the highest extension value of 35.1 % and lowest  $E$  modulus obtained for gel-like sample BCwet) but strength is low ( $\sigma = 0.4$  MPa) (Table. 2).

**Table 2.** Tensile properties of BC biofilms

	BCwet	BC 25	BC 50	BC 75
Maximum force $F$ , N (CV, %*)	14.7 (3.1)	100.5 (3.1)	81.2 (5.9)	65.6 (19.9)
Tensile strength $\sigma$ , MPa	0.4	27.9	17.3	12.8
Extension $\varepsilon$ , % (CV, %*)	35.1 (15.9)	18.8 (12.5)	20.1 (19.1)	4.5 (4.3)
Tensile modulus $E$ , MPa	0.02	2.78	1.83	2.65

\* CV indicate result's variation

Dried biomaterial obtains different tensile behaviour – higher tensile strength but lower extension (Fig. 4). The highest breaking strength value ( $\sigma = 27.91$  MPa) was observed for samples dried at 25 °C, consequently the worst tensile properties were observed for the BC samples dried at 75 °C – the lowest values of breaking strength ( $\sigma = 12.8$  MPa) and extension (4.5 %) were recorded.



**Fig. 4.** Typical tensile strength – extension curves obtained for the BC samples

Tensile extension values obtained similar for the samples dried at 25 °C and 50 °C – respectively 18.8 % and 20.1 %. It shows that dried at a higher temperature material loses its strength therefore ability to deform does not change. Breaking strength values for samples dried at 50 °C were 38 % lower than for the samples dried at 25 °C. Dried samples acquired more stiff structure and increase of tensile modulus E was estimated. Tensile modulus for gel like BCwet sample is 0.02 MPa, as samples dried at 25 °C and 75 °C make much higher and similar modulus E (respectively 2.78 MPa and 2.65 MPa) and a lower value for sample BC50 is recorded – 1.83 MPa.

The results proved that BC samples are sensitive to drying temperature and under higher temperature biomaterial obtains more stiff structure. Lower drying temperatures help to preserve the porous structure, strength and deformation properties of material.

Comparing obtained results with other researches [15, 24, 25] it was confirmed that strong and resilient to mechanical impact dry bacterial cellulose biomaterial can be produced from Kombucha.

The high scattering of elongation result (up to 19.1 %) demonstrates the non-uniform structure of bacterial cellulose. The BC material is produced during natural fermentation process which strongly depends on many parameters [2, 7, 8] and it's extremely difficult to get material with even structure and constant mechanical parameters [26].

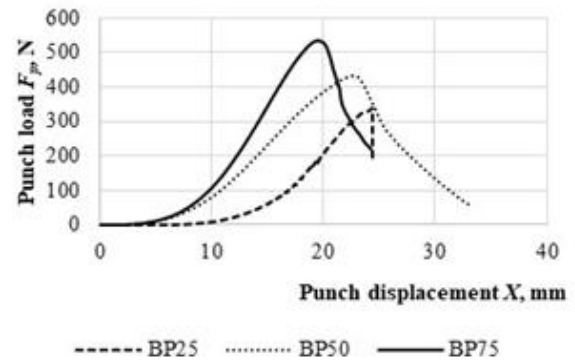
### 3.3. The influence of drying on biaxial deformation properties

The influence of drying on the biaxial deformation of biofilm (Fig. 5) was investigated by punch loading experiment. In punch experiment (Table 3) samples dried at 75 °C obtained the highest values of bursting strength (57.2 MPa) as the lowest value of strength was observed

for samples BC25 (37.1 MPa). Punch displacement changes were insignificant for all tested samples (from 21.7 mm for sample BC25 to 17.8 mm for sample BC75).

**Table 3.** Bursting properties of BC biofilm

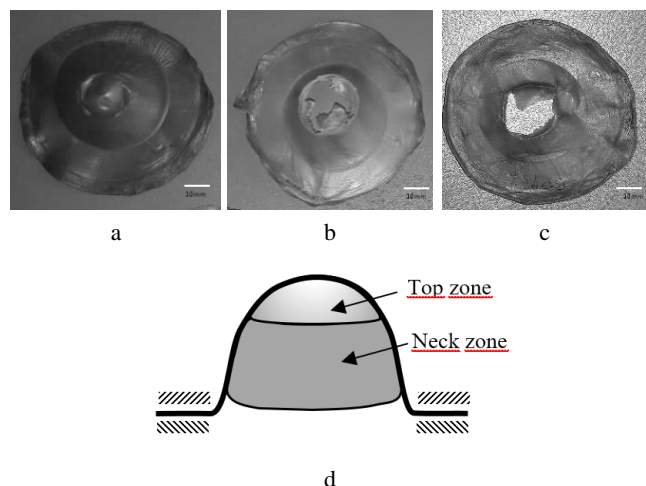
	BC 25 (CV, %)	BC 50 (CV, %)	BC 75 (CV, %)
Punch load $F_p$ , N	370.7 (13.28)	516.8 (43.56)	571.9 (38.9)
Bursting strength $\sigma$ , MPa	37.1	52.8	57.2
Punch displacement $X$ , mm	21.7 (22.89)	20.9 (23.51)	17.8 (15.68)



**Fig. 5.** Typical punch load – displacement curves obtained for BC samples

The estimated parameters showed biaxial behaviour of investigated BC similar to natural leather. The value of BC bursting strength was higher than natural leather [27] as BC punch displacement values were also higher [28].

In bursting deformation process a shell of complicated shape with two zones is formed [29]. The punch contact top zone and thinned neck zone of a biomaterial shell differ in length and breaking nature. In Fig. 6 presented samples of BC biofilm after a ball burst test.



**Fig. 6.** BC biofilm samples after ball burst test: a – dried at 25 °C; b – dried at 50 °C; c – dried at 75 °C; d – shell scheme

Samples dried at 25 °C have longer neck and rounded up cap, which was smaller than for samples dried at higher temperatures. Shells of samples dried at 50 °C and 75 °C temperatures have shorter neck zone and wider cap zone with a crack in the centre. It shows that dried at a higher

temperature BC biofilm loses its elasticity and exposed to the biaxial deformations mainly deforms and ruptures in the contact zone (punch contact top zone) when critical deformations are exceeded.

#### 4. CONCLUSIONS

The change in the sample size (thickness and planar dimensions) during dried BC biomaterial production is important for the material application. It is estimated that during drying procedure the biofilm loses up to 92 % of the weight and up to 87 % of the thickness therefore planar dimensions of material specimen varied insignificantly (up to 0.04 %). The appropriate procedure of biomaterial preparation (drying conditions) can prevent a surface shrinkage due to the rapid moisture evaporation.

BC biomaterial possesses useful mechanical properties, therefore due to the natural fermentation process, it is difficult to ensure even structure and constant mechanical parameters of biomaterial. The study proved the influence of drying temperature on the tensile and bursting behaviour of material.

The highest tensile strength (27.91 MPa) is recorded for the samples dried at temperature of 25 °C when the moisture out of the BC film is removed gradually. Dried at a higher temperature material loses its strength therefore elasticity for sample dried at 25 °C and 50 °C remains similar. In the biaxial punch loading of BC biofilm, a shell of complicated shape is formed. Shell zones and rupture character differ according to sample preparation procedure (drying temperature). The maximum bursting strength (57.2 MPa) is recorded for samples dried at 75 °C when punch displacement changes is insignificant for all tested samples (from 17.8 mm to 21.7 mm).

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