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Characteristics of Bacterial Cellulose Obtained from *Acetobacter Xylinum* Culture for Application in Papermaking

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Abstract
In this paper, a method of cultivation and characteristics of obtained bacterial cellulose is presented. It was stated that the greatest increase in the weight of bacterial cellulose takes place after 7 - 8 days of breeding Acetobacter xylinum at a temperature of 30 °C, using a Herstin-Schramm nutrient medium. The highest degree of polymerisation exists in bacterial cellulose synthesied with glucose and mannitol average degree of polymerisation (approx 1700), and xylose (approx. 1050), as a carbon source. In the photograph showing the structure of cellulose (taken under an AFM microscope), one can clearly see long, smooth and oriented fibrils and fibril bundles which have a width varying from 70 to 200 nm. Bacterial cellulose exhibits considerable thermal stability. The quick drop of a sample weight leading to its decomposition begins at a temperature of approx. 300 °C, and the maximum of this transformation occurs at 350 - 370 °C.

Key words: *bacterial cellulose, Acetobacter xylinum, carbon source, structure, thermal stability.*

Introduction

At the Institute of Papermaking and Printing, complex research was undertaken in order to develop a procedure for the modification of different papermaking semi-products with bacterial cellulose, and to determine the suitability of composite materials obtained in this way.

Both the literature and our preliminary studies indicate that the presence of bacterial cellulose in papermaking semi-products can lead to improvement of their strength properties and protect the surface of paper [1 - 5].

In this work, a method of cultivation and obtaining the characteristics of bacterial cellulose are presented. Besides this, the suitability of the procedure used and the properties of bacterial cellulose for preparing papermaking composites are also presented.

Aim and range of the research

The aim of the work was to study the process of bacterial cellulose formation in *Acetobacter xylinum* static culture. The process and bacterial cellulose produced were characterised assuming the following criteria: the yield of the biosynthesis process in variable conditions and the characteristics of cellulose, including the viscosity value and average degree of polymerisation, surface structure, chemical and physical structure, and thermal properties.

Experimental

Acetobacter xylinum bacterial culture

Acetobacter xylinum culture (coming from the Pure Culture Collection of the Institute of Microbiology and Fermentation, Technical University of Łódź) was cultivated in stationary conditions using a Herstin-Schramm nutrient (HS) medium composed of glucose – 2 w/v%, yeast extract – 0.5 w/v%, bacto-pepton – 0.5 w/v%, citric acid – 0.115 w/v%, Na_2HPO_4 – 0.27 w/v%, $MgSO_4 \cdot 7H_2O$ – 0.05 w/v% and ethanol – 1 v% added after sterilisation of the base [6].

Conic flasks (300 and 750 cm^3) were used, filled with an HS medium of different volumes. The bacterial breeding process was conducted within 3-10 days at 25, 30 and 35 °C, grafting inokulum of approx. 4 w% in relation to the medium prepared. In the process of bacterial cellulose biosynthesis, glucose as well as arabinose, mannose, galactose, xylose and mannitol were used as carbon sources. The film of bacterial cellulose obtained was then treated with NaOH (a concentration of approx. 5%, for 60 min., temp = 100 °C) in order to remove bacterial cells and substrate from the inner layers of the bacterial cellulose film. Then it was rinsed with tap water until a neutral reaction was achieved. The bacterial cellulose films prepared were studied, also after being dried using Rapid-Koethen apparatus (in the same manner as sheets of fibrous semi-products are dried).

Determination of the yield of the biosynthesis process and properties of bacterial cellulose

- **The yield** of the biosynthesis process (Y, w%) was calculated in the following way:

  $$Y = C/G \cdot 100 \text{ in } \%$$

  where:
  C – weight of dry film in g,
  G – weight of carbon source in substrate in g.
- **The average** degree of bacterial cellulose polymerisation was determined according to the method used in papermaking technology, using a cuprietylenediamine solution (CED) [7].
- **The structure** of bacterial cellulose was studied using optical microscopy (a BIOLAR PI microscope with CCD camera coupled with a computer equipped with a Multi-Scan Base v. 8.08. program) and an AFM (AFM-Solver P47 (NT-MDT) scanner (100×100). AFM pictures were taken with the use of a semi-contact module (taping mode), at room temperature and relative humidity of 65%. The cantilever used was the silica mash model: NSC16/50, with a force constant of 40 N/m.
- **IR** spectra of bacterial cellulose were performed by the FTIR-AR and FT-IR-GATR methods. In these studies a BIO-RAD spectrophotometer, model FST-175 equipped with an MCT linear detector and ATR max module (PIKE Technology) with variable angle incidence of beam, was used. The measurements were carried out at an angle of 45°. In addition, a GATR module was used.

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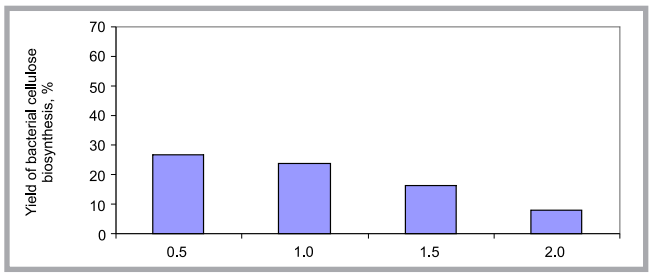

Figure 1. *Effect of the height of the substrate layer of the culture on the yield of bacterial cellulose biosynthesis, using glucose as a source of carbon.*

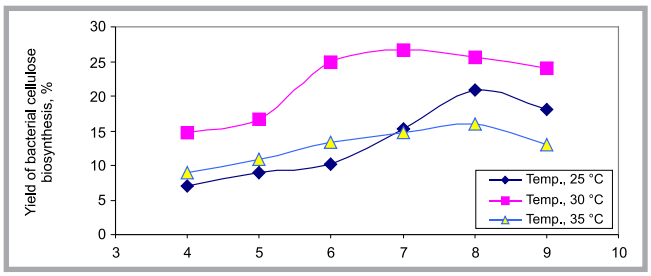

Figure 2. *Effect of the temperature and time of Acetobacter xylinum bacteria breeding, using glucose as a carbon source.*

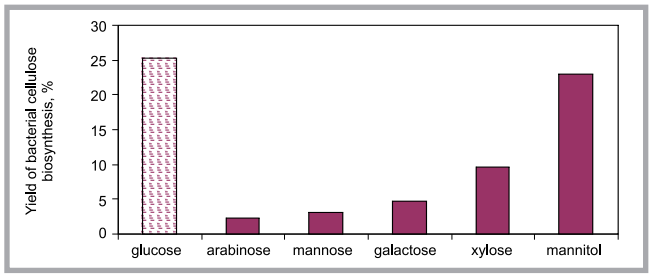

Figure 3. *The yield of bacterial cellulose biosynthesis obtained with the use of different carbon sources (temp. 30 °C, 7 days).*

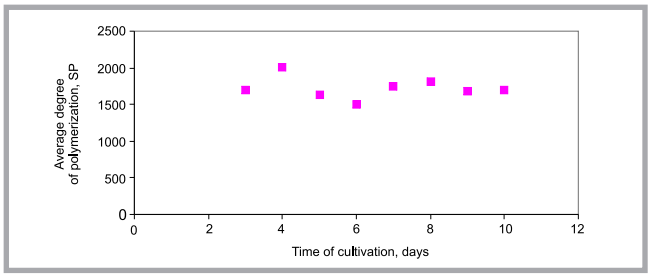

Figure 4. *The effect of breeding time on the average degree of polymerisation of bacterial cellulose, using glucose as a carbon source (temp. 30 °C, volume of flask 750 cm³).*

- **Thermal properties** of bacterial cellulose were studied using thermogravimetry (TG and DTG) and also dynamic differential calorymetry – DSC (NETZSCH-Geratebau GmbH Thermal Analysis), in a nitrogen atmosphere in the following conditions: sample weight = 2.7 - 6.1 mg, rate of heating = 10 °C/min., temperature range = -50 ÷ 500 °C.

Results and discussion

Production of bacterial cellulose

During the breeding of *Acetobacter xylinum* bacteria in stationary conditions, bacterial cellulose is synthesised in the form of film on the surface of the nutrient medium solution. The yield of the biosynthesis process depends on many factors i.e. temperature, time, and the relation of the surface area to the volume of substrate. The last parameter determines oxygen access because both its deficiency and excess adversely affect the bacterial cellulose synthesis process due to microorganisms [1].

During the experiments, there was a study of the progression of the bacterial cellulose biosynthesis process from *Acetobacter xylinum* culture in conic flasks, at a volume of substrate of 50, 100, 150 and 200 cm³, respectively. The height of the culture substrate layer was changed within the range of 0.5 - 2 cm. On the basis of preliminary research, the following conditions of breeding were assumed: a time of 7 days and temperature of 30 °C. The results obtained are presented in ***Figure 1***.

Similar experiments were carried out using 300 cm³ flasks and Petri glass plattes. In this case it was stated that the yield of biosynthesis decreased significantly (± 3% rel.). On the basis of the experiments performed, 700 cm³ flasks and a layer of breeding solution of 1 cm height were used.

Using chosen laboratory glass vessels, the optimal temperature of breeding and time of its duration were checked. The experiment was conducted within 4 - 9 days because in the preliminary research it was stated that after the first day of breeding only the turbidity of the solution was observed, whereas after the next two days, the washing and determining of the weight of the delicate polymer film caused a lot of difficulty.

The cellulose yield-breeding time relationship at temperatures of 25, 30 and 35 °C, respectively, is depicted in ***Figure 2***.

From the course of the curves in ***Figure 2***, we can observe that for the breeding conducted at 25 and 35 °C, the biosynthesis yield is highest after 8 days, whereas at 30 °C after 7 days the yield is approximately two times higher than the biosynthesis yield at 25 and 35 °C. For further research a temperature of 30 °C and time of 7 days were chosen as optimal conditions. These were in accordance with conditions determined in the preliminary studies.

Except for the parameters of the culture process affecting the amount of product, the choice of substrate is also important.

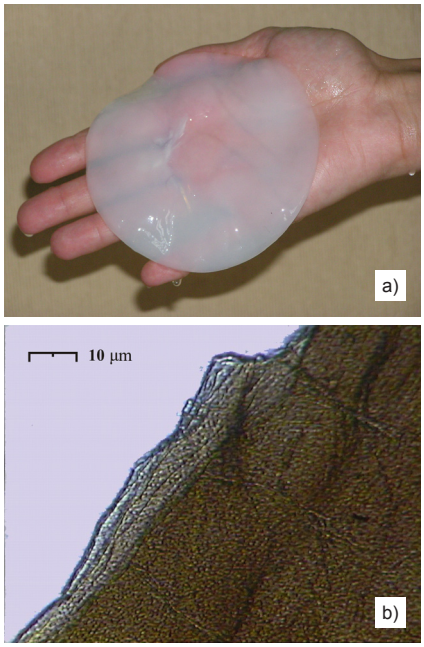

Figure 5. *Bacterial cellulose film; a – real image, b – fragment of film from an optical microscope.*

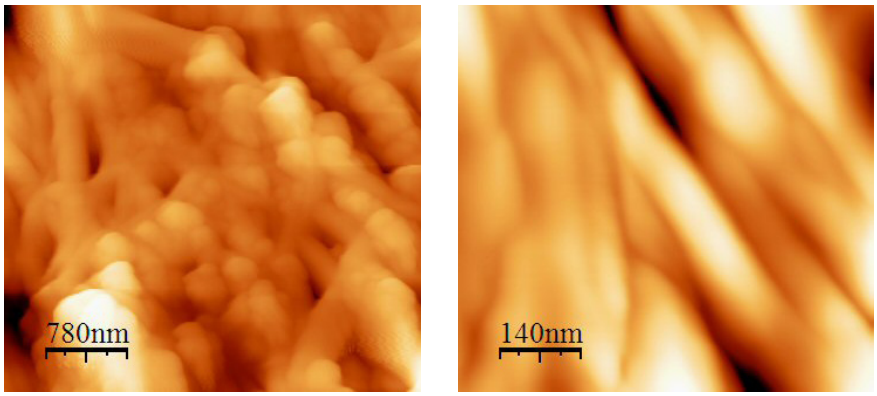

Figure 6. *The surface of bacterial cellulose under an AFM microscope.*

In addition to glucose - galactose mannose, xylose, arabinose (i.e. monosaccharides, of which vegetable polysaccharides are built) and mannitol were also used.

Figure 3 (see page 109) shows that the greatest yield of bacterial cellulose biosynthesis was achieved using glucose and mannitol as sources of carbon. The yield of biosynthesis using xylose as a carbon source was approximately two times lower in comparison with the yield taking place in the presence of glucose. The lowest yield was attained for arabinose, and subsequently mannose and galactose.

Structure and properties of bacterial cellulose

The average degree of polymerisation was determined for bacterial cellulose produced in/by stationary breeding within 3 - 10 days, using the viscometry method. The results are presented in ***Figure 4*** (see page 109).

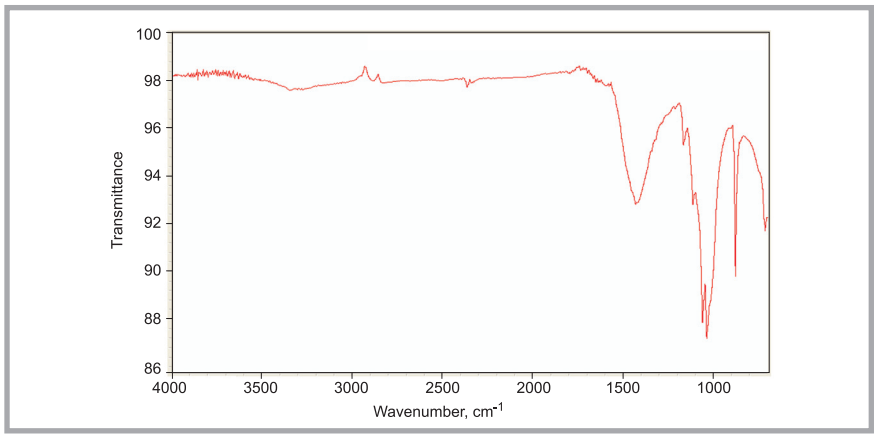

Figure 7. *Bacterial cellulose, FTIR-ATR spectrum.*

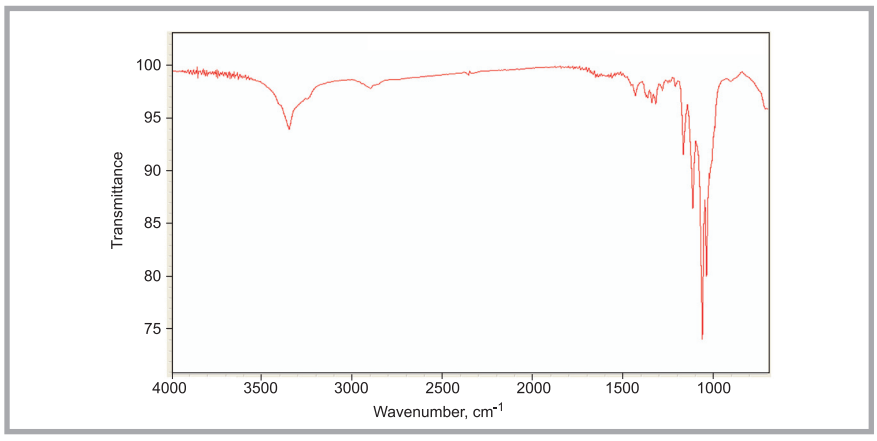

Figure 8*. Bacterial cellulose (FTIR-GATR spectrum).*

Figure 4 shows that the average degree of polymerisation of the synthesised bacterial cellulose varied in the time of breeding the *Acetobacter xylinum* from 4 - 10 days in the range of 1150 - 2000, and after 7 days it was 1750.

Similarly, the average degree of polymerisation of the bacterial cellulose was determined after 7 days breeding, using mannitol and xylose as carbon sources, for which the highest yield of the biosynthesis process was obtained after glucose. The average values of the DP obtained were: for mannitol approx. 1700 (like in case of glucose), whereas for xylose it was approximately 1050 (i.e. approx. 40% less).

For research of the structure and properties of bacterial cellulose the sample produced with glucose as a carbon source was used; because of the accessibility of this source and good yield of the biosynthesis process. In ***Figure 5*** (see page 109) the film of bacterial cellulose produced in *Acetobacter xylinum* culture is presented.

As shown in this photo, the structure of bacterial cellulose is characterised by great homogeneity. However, weakly marked ribbons (bundles of fibrils) produced in the first stage of the biosynthesis process can be distinguished. In ***Figure 6***, the surface of bacterial cellulose is presented. These images were performed using Atomic Force Microscopy. In the photos, taken at high resolution, long, smooth and oriented fibril bundles of width within the range of 70-200 nm can be seen.

Figures 7 and ***8*** show the spectrum of bacterial cellulose obtained by the FTIR-ATR and FTIR-GATR methods. The FTIR-ATR spectrum shows strong absorption in the range of 1490 cm^{-1}, which shows the presence of a carbonyl group in the bacterial cellulose. In turn, the FTIR-GATR spectrum shows the occurrence of hydroxyl groups in the region of 3400 cm^{-1}, together with bonds corresponding to those of glycoside, including C_1OC_4 at 1162 cm^{-1} (as in case of natural cellulose).

Thermal properties of produced and rinsed bacterial cellulose were studied by the TG and DSC methods (***Figures 9*** and ***10***).

The TG spectrum (***Figure 9***) shows a weak loss of weight due to the evapora-

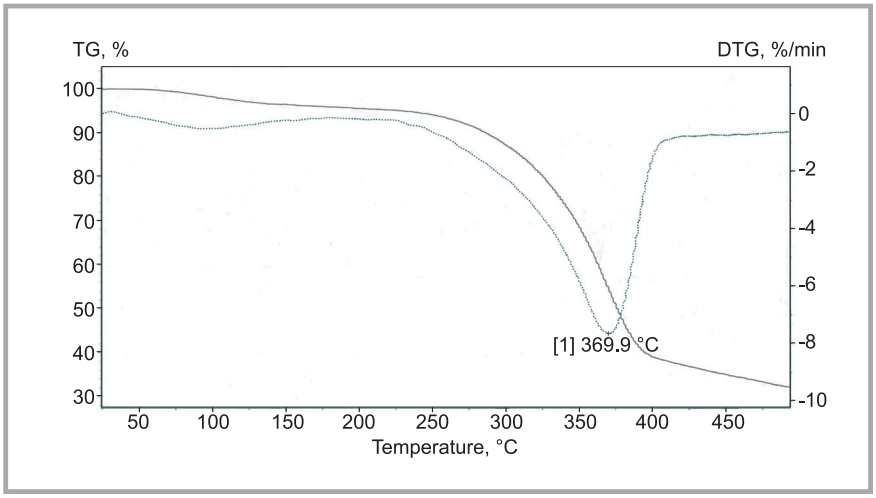

Figure 9. *TG and DTG spectrum of bacterial cellulose.*

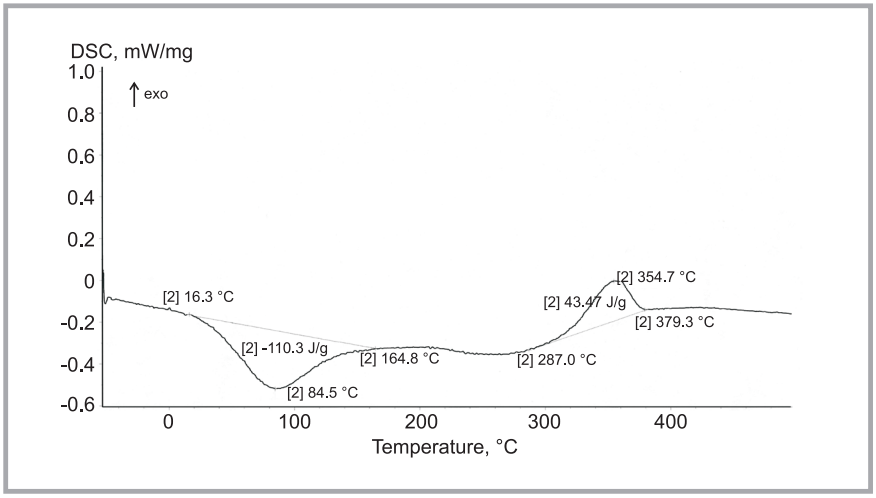

Figure 10. *DSC spectrum of bacterial cellulose.*

tion of water (at temp. 85 °C) and also quick drop in weight beginning at a temperature of approx. 300 °C, resulting from decomposition of the sample. The DTG curve shows that the maximum rate of this transformation occurs ata temperature of approx. 370 °C.

Figure 10 shows an image of thermal transformation obtained by the differential a colorimetry method – DSC. From the course of the curve in this Figure, one can see the transformation related to the evaporation of water at an endothermic maximum of 85 °C. According to literature, at a temperatures of 80 - 140 °C, there is known to be an occurrence of transformation related to the melting of the crystalline phase of cellulose [8]. After removing water, bacterial cellulose exhibits considerable thermal stability. The maximum of the next transformation, exothermic, occurs only at the temperature of approx. 355 °C, leading to the decomposition of the sample. This confirms the observations resulting from the DTG curve (***Figure 9***).

Conclusions

1. It was proved that the greatest increase in the weight of bacterial cellulose takes place after 7 - 8 days of breeding *Acetobacter xylinum* at a temperature of 30 °C, using a Herstin-Schramm nutrient medium containing: glucose – 2 w/v%, yeast extract – 0.5 w/v%, bacto-pepton – 0.5 w/v%, citric acid – 0.115 w/v%, Na_2HPO_4 – 0.27 w/v%, $MgSO_4 \cdot 7H_2O$ – 0.05 w/v% and w etanol – 1 v%, added after sterilisation of the base.
2. The highest degree of polymerisation exists in bacterial cellulose synthesised with glucose and mannitol as a carbon source, and then xylose. The values of the average degree of polymerisation obtained were: for glucose and mannitol – approx. 1700, whereas for xylose – approx. 1050.
3. In the photograph taken under an AFM microscope, showing the structure of cellulose, one can see clearly long, smooth and oriented fibrils and fibril bundles that have width varying from 70 to 200 nm.
4. The spectrums formed by the FTIR method show the occurrence of glycosides bonds in cellulose (including C_1OC_4 similar as in the case of natural cellulose) and hydroxyl groups - in the region of 3400 cm^{-1}.
5. Bacterial cellulose exhibits considerable thermal stability. A quick drop in sample weight begins at a temperature of approx. 300 °C, leading to its decomposition. The maximum of this transformation occurs at 350-370 °C.

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