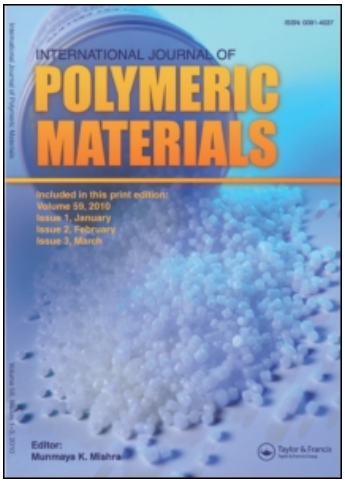

International Journal of Polymeric Materials
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713647664

GPC STUDIES ON BACTERIAL CELLULOSE

Grazyna Strobin[a]; Andrzej Wlochowicz[b]; Danuta Ciechanska[a]; Stefan Boryniec[b]; Henryk Struszczyk[a]; Serafina Sobczak[a]
[a] Institute of Chemical Fibres, Łódź, Poland [b] University of Bielsko-Biała, Bielsko-Biała, Poland

Online publication date: 16 August 2010

To cite this Article Strobin, Grazyna , Wlochowicz, Andrzej , Ciechanska, Danuta , Boryniec, Stefan , Struszczyk, Henryk and Sobczak, Serafina(2004) 'GPC STUDIES ON BACTERIAL CELLULOSE', International Journal of Polymeric Materials, 53: 10, 889 — 900
To link to this Article: DOI: 10.1080/009114030490502418
URL: http://dx.doi.org/10.1080/009114030490502418

International Journal of Polymeric Materials, 53:889–900, 2004

ISSN: 0091-4037 print/1543-5253 online
DOI: 10.1080/00914030490502418

Taylor & Francis
Taylor & Francis Group

GPC STUDIES ON BACTERIAL CELLULOSE

Grazyna Strobin
Institute of Chemical Fibres, Łódź, Poland

Andrzej Wlochowicz
University of Bielsko-Biała, Bielsko-Biała, Poland

Danuta Ciechanska
Institute of Chemical Fibres, Łódź, Poland

Stefan Boryniec
University of Bielsko-Biała, Bielsko-Biała, Poland

Henryk Struszczyk
Institute of Chemical Fibres, Łódź, Poland

Serafina Sobczak
Institute of Chemical Fibres, Łódź, Poland

The aim of the present study was to examine the effect of time duration of bacterial cellulose (BC) biosynthesis as well as the culture medium composition on molecular parameters of the obtained polymer. It was found that the degree of polymerization of BC increases as the duration of biosynthesis is prolonged up to 6 days. A further prolongation of the process to 28 days lowers the degree of polymerization (DP) value and increases polydispersity. An examination of the effect of culture medium composition on the biosynthesis pointed out that the course of the process is mainly influenced by the chemical nature of the carbon source. The best results for molecular parameters were obtained for a medium containing 4% of glucose.

Keywords: bacterial cellulose, molecular characteristics, gel permeation Chromatohraphy, biosynthesis, molecular weights, molecular weight distribution

Received 4 December 2002; in final form 11 December 2002.

The presented studies have been supported by The Polish State Committee for Scientific Research, Grant 7T08E 00917.

Address correspondence to Dr. Grazyna Strobin, Institute of Chemical Fibres, ul. Sklodowskiej Curie 19/27, Łódź, 90-570, Poland. E-mail: iwch@iwch.man.lodz.pl

INTRODUCTION

Cellulose, being the most abundant natural polymer, is an attractive initial material for various technologies. Wood cellulose dominates in paper and chemical fiber industries whereas in textile processing the basic raw material is cotton. Cellulose, formed in nature in the process of photosynthesis can also be produced by non–photosynthesized organisms such as bacterial strains, for example, *Acetobacter, Achromobacter, Aerobacter* or fungi [1–4]. The synthesis takes place under controlled conditions independent of atmospheric ones. Its course may be affected by changing various parameters of cultivation, for example, by adding different substances to the culture medium [5–6]. A significant and still growing interest in obtaining cellulose and other polymers by biosynthesis is mainly driven by the possibility of getting polymers with required unique properties [7–8].

In biosynthesis of cellulose the most efficient are gram-negative acetic bacteria *Acetobacter xylinum* [3–4,9–11]. The cellulose produced in a bacterial cell is liberated to the outside in the form of single macromolecules joined to one another in the form of microfibriles. Combination of microfibriles forms a thick gelatinous film (even up to 2 cm thick) on the surface of the liquid culture medium. The film, after its purification and drying resembles a paper-like sheet. It is worth mentioning that bacterial cellulose (BC) is almost pure (content of α-cellulose $>99\%$), thus the purification and bleaching can be avoided and the product of biosynthesis is ready for further processing or direct application.

BC is also characterized by its high tensile strength and Young modulus values as well as by biocompatibility, biodegradability, and selective permeability by liquids and gases [8,12]. The set of these unique properties of BC forms the basis of its potential applicability as headphone diaphragms, additives to special sorts of paper in the paper industry, wound dressing and artificial skin in medicine and a membrane in filtration of water [7–8,12]. Despite numerous attempts of many scientific centers carrying our studies on BC synthesis and modification, both the process and the formed product have not been yet sufficiently understood.

The interest of researchers concentrates on the mechanism of synthesis of cellulose macromolecules, the effect of various factors on the course of biosynthesis, on the properties of the product obtained, and BC modification. The higher aim of each study, however, is recognition and control of the process in order to obtain BC having various required and expected properties.

From this point of view, BC characterization on the molecular level is especially important because its unique physical properties result from exceptionally high molecular weight, high molecular homogeneity, and chemical purity. Thus, the attempts to find the correlation between biosynthesis parameters (such as duration of the process, temperature, composition, and pH value of the culture medium) and the productivity of the process, chemical purity of the product (expressed by α-cellulose content) have both theoretical and practical significance. In this article the effect of duration time of biosynthesis as well as culture medium composition on the aforementioned characteristics will be presented. The other data will be published in a later article.

To examine the molecular characteristics of BC samples synthesized under various conditions, gel permeation chromatography (GPC) method has been used. Due to the application of two detectors (refractive index and viscometric) the method enables the obtainment of comprehensive information concerning all important molecular parameters of the polymer, that is, number- and weight-average molecular weights (Mn and Mw), polydispersity (Mw/Mn), and molecular weight distribution (MWD), as well as some characteristics describing the properties of BC in its diluted solutions, that is, intrinsic viscosity [η] and radius of gyration of the macromolecular coil (Rg).

EXPERIMENTIAL

Biosynthesis of Bacterial Cellulose

Bacterial Strain

The *Acetobacter xylinum* strain produced at the Institute of Microbiology and Fermentation, Technical University of Łódź, Poland, was used in the studies on cellulose biosynthesis.

Culture Media

The following culture media based on Hestrin Schramm cultures were used [6]:

- **A**—Standard culture: glucose 2% (w/v), yeast extract 0.5% (w/v), disodium phosphate 0.27% (w/v), citric acid 0.12% (w/v), ethyl alcohol 2% (v/v), pH6.0.
- Cultures of changed composition:
  B—Fructose 4% instead of glucose 2%,
  C—Maltose 4% instead of glucose 2%,

D—Glucose 4% instead of glucose 2%,
E—Soytone extract 0.5% instead of yeast extract,
F—Beef extract 0.5% instead of yeast extract.

In all the (B to E) the remaining components were the same as in the standard culture. Extracts of yeast, soytone, and beef are typical ingredients of microbial cultures and the are commercially available (Difco, USA).

Inoculum Preparation

Solid components of the culture medium were dissolved in distilled water, sterilized in autoclave at 121°C and then ethyl alcohol was added in the amount of 2% (v/v). The culture medium was inoculated by *Acetobacter xylinum* strain, that is, liquid culture of *Acetobacter xylinum* was added to the culture medium in proportion 1:10 (v/v) and incubated for 3 days at 30°C in an incubator [10].

Biosynthesis of BC by Static Method

The inoculum prepared in this way was added to the new batch of the culture medium previously sterilized in an autoclave (in proportion 1:10 (v/v)). The synthesis of cellulose was carried out in glass crystallizers using a static method at 30°C within the time ranging from 4 to 28 days.

Separation of BC from Culture Medium

After filtration of the culture medium, the obtained cellulose film (BC) was washed first with distilled water, next 2% NaOH aqueous solution and then it was sterilized in an autoclave at 121°C for 15 min. After sterilization, the cellulose was washed with distilled water, treated with 1% aqueous solution of acetic acid and again washed with water. BC was spread to dry at 120°C [10].

Applied Analytical Methods

Determination of α-Cellulose Content

α-cellulose content in BC sample was evaluated using gravimetric method by determining the content of the fraction insoluble in 17.5% NaOH solutions is described in [13].

Gel Permeation Chromatography

Preparation of BC solutions for GPC analyses. BC samples were dissolved according to Ekmanis [14] and following further modifications

of his method [15,16] with the application of microwave heating. The procedure described in the mentioned article consists of four stages. First, cellulose is swollen in water to cause the opening of its structure; 20 mg of BC was placed in a 50 ml beaker in distilled water for 48 h. The sample was then wrung and immersed in 10 ml of dimethylacetamide (DMAC) and next placed in a microwave oven and heated at about 160°C for 1 min. The excess DMAC was removed and then a new portion of 10 ml of DMAC was introduced into the vessel. The operation of DMAC exchange in the oven was repeated twice. After removing DMAC the sample was transferred into a 25 ml flask in 7.5 ml of DMAC/8% LiCl. During the first several hours swelling of cellulose occurred in a static condition. After that the flask was gently shaken periodically (8 h per day) at ambient temperature (21 ± 1°C). Complete dissolution of BC in this system took place after 2 to 3 days. After dissolving, the solution was diluted with DMAC to obtain finally a solution having concentration of about 0.2 mg/l in DMAC/0.5% LiCl. The solution was vigorously shaken, then heated at 80°C for 0.5 h in order to bring about protein denaturation and possibly to dissolve precipitated macromolecules, and after 3 to 4 hours it was filtered using 1 μm filters (Gelman). After the dilution the solution of BC was injected into the GPC system within 3 to 6 hours.

The application of microwave heating considerably shortens the time of solvent exchange in the cellulose structure in comparison with other methods [14,17]. Microwave heating probably brings about a slight degradation of cellulose caused, however, rather by the action of high temperature (about 160°C) than by microwaves [18].

LiCl and DMAC were carefully dried before the preparation of DMAC/0.5%LiCl and DMAC/8%LiCl solutions. LiCl was dried in a vacuum at 105°C for 48 h. DMAC was dried on 4A molecular sieves.

Description of GPC system. Number and weight molecular weights (Mn and Mw), polydispersity (Pd), as well as intrinsic viscosity [η] and radius of gyration of BC were determined by GPC method. The GPC system consists of a DG-700 degasser (Viscotek, Houston Texas USA), HP 1050 pump (Hewlett-Packard, Waldrom, Germany), a sample injector (Rheodyne Inc. Model 7125 Cofati Texas USA), online filter (2 μm), a set of three columns PLgel Mixed A (300×7.5 mm) with a guard column (Polymer Laboratories Ltd. Shropshire, UK), differential viscometric detector H502B (Viscotek, Texas, USA), and refractive index detector HP 1047 (Hewlett-Packard). Temperatures of cells were maintained at 80°C (viscometric detector) and at 50°C (refractive detector). Detectors were connected in series. DMAC/0.5%LiCl was applied as an eluent. The temperature of column set was

80°C. The flow rate of the eluent was 0.4 ml/min. The volume of the injection was 150 μl. The registration of signals from detectors and their further processing was possible due to the application of Unical GPC Software, Version 4.06 (Viscotek, Texas, USA). Checking the correctness of chromatographic system performance as well as the calibration and analytical procedures were in accordance with ASTM D-5296-92 [19].

RESULTS AND DISCUSSION

The solvent used in GPC analyses has been applied for cellulose examination since the end of 1980s [17,19]. Its application enables direct dissolving of cellulose. Detailed studies showed that cellulose solutions in DMAC/LiCl system are stable, which is of great significance in cellulose examination by means of GPC method [19].

The analyses presented in this article were preceded by introductory trials in order to optimize analytical parameters such as flow rate, volume, and concentration of the dosed solution. It is well known that the aforementioned parameters are of importance when the molecular weight of the analyzed polymers is very high [20]. An introductory analysis of "broad" standard obtained from a mixture of "narrow" polystyrene standards led to the determination of an optimum flow rate of the eluent in GPC system at the level of 0.4 ml/min, volume of injection: 150 μl, and cellulose concentration: 0.18 ± 0.01 mg/ml.

Following previous studies [21–26] the polystyrene (PS) standards were applied to calibration of GPC system. The results of analyses were calculated using a universal calibration method [27]. In the studies, a set of 8 PS standards having Mp values from 7000 to 7100000 and polydispersity <1.1 (Polymer Laboratories Ltd. Shropshire, UK) was applied. The obtained Mark-Houwink equation for PS was $[\eta] = 19.6 \times 10^{-5} M^{0.638}$, which is in good accordance with the one obtained by Timpa $[\eta] = 17.3 \times 10^{-5} M^{0.642}$ [24]. It indicates that the GPC system has been properly prepared for BC analyses.

Two series of cultivation were carried out. In the first, BC samples were obtained after various cultivation periods from 4 to 28 days, at 30°C, pH 6.0, and with a standard composition of the culture medium. The medium contained, beside other components, 2% of glucose as the source of carbon and yeast extract as a nitrogen source. In the second series the cultivation process was carried out for various composition of the culture medium fro 7 days at 30°C and pH = 6.0.

The results obtained for the samples with different cultivation durations are presented in Table 1, and for a better illustration of

TABLE 1 Effect of Biosynthesis Time on Molecular Characteristics of BC

Biosynthesis time	DPn	DPw	DPw/DPn	Rg_z nm
4 days	2570 ± 60	7350 ± 70	2.8	85 ± 1
5 days	3150 ± 50	9260 ± 80	2.9	95 ± 2
6 days	3200 ± 30	10400 ± 60	3.2	99 ± 1
7 days	2930 ± 50	9820 ± 100	3.3	98 ± 2
14 days	2350 ± 50	8030 ± 80	3.4	92 ± 2
28 days	1620 ± 70	5680 ± 100	3.5	76 ± 2

DPn and DPw are number – and weight – average polymerization degrees and Rg_z values are mean of triplicates.

the particular parameter changes—also in the form of a histogram (Figure 1). On the basis of the results obtained it can be concluded that the time of cultivation distinctly affected the productivity of the process and the parameters of BC molecular characteristics. During 4 to 7 days of cultivation the productivity increased from about 1.6 to 3.2 g/l. A further prolongation of the period of cultivation to 28 days had no effect on the growth of productivity. The content of α-cellulose is independent of the cultivation time and for all the samples under examination exceeds 99.5%. The parameters of molecular characteristics reach maximum values for the sample cultivated for 6 days

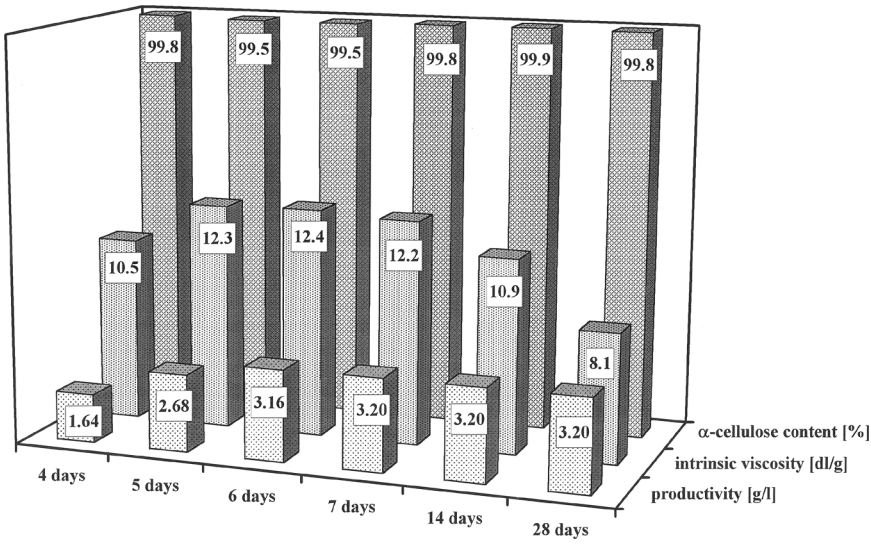

FIGURE 1 Effect of biosynthesis time on cellulose productivity, α-cellulose content and intrinsic viscosity.

(DPn = 3200, DPw = 10400). Further prolongation of the cultivation time up to 28 days brings about a continuous decrease of DPn an DPw values. After 28 days of cultivation DPn = 1620 and DPw = 5680. Polydispersity of BC rises from 2.8 after 4 days up to 3.5 after 28 days. Intrinsic viscosity [η] and Rg values also tend to grow with time. The maximum value of [η] after 6 days of cultivation is equal to 12.4 dl/g where Rg = 99 nm.

Figure 2 shows molecular weight distribution (MWD) curves for BC obtained after different cultivation periods. Only selected results for BC being synthesized for 4, 6, 14, and 28 days have been presented in order to make the illustration clear. The areas have been normalized. All curves are unimodal and almost symmetrical, yet a significant contribution of high molecular fractions in the sample obtained after 6 days is evident. Distinct lowering of the highest fraction content after 14 and 28 days of cultivation can easily be observed.

Table 2 shows the results of the examination of BC samples obtained from different compositions of culture media. For clarity, some results are presented in the form of a histogram (Figure 3). The culture media components were selected in such a way that it was

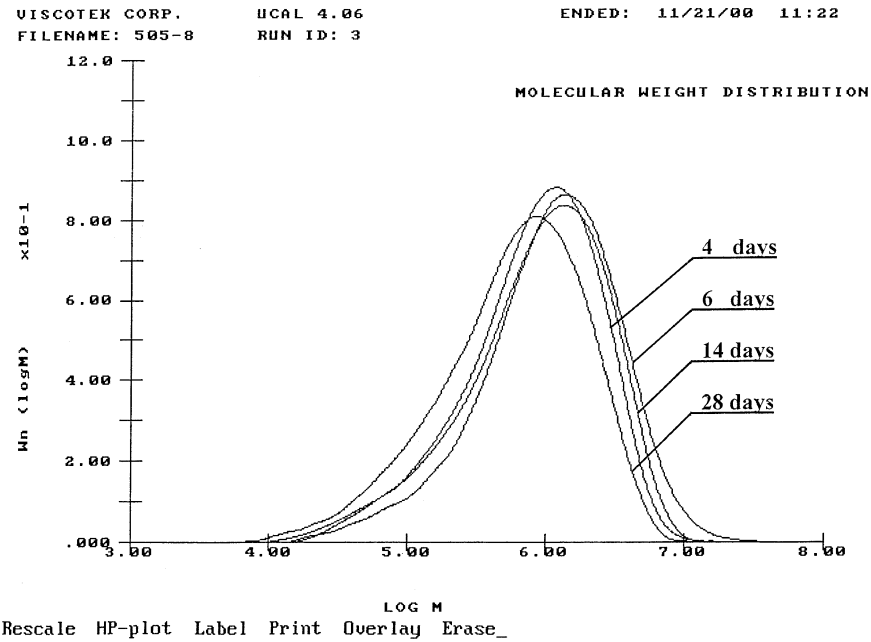

FIGURE 2 Effect of biosynthesis time on the molecular weight distribution of bacterial cellulose.

TABLE 2 Effect of Medium Composition on Molecular Characteristics of BC

Medium	DPn	DPw	DPw/DPn	Rg_z nm
C—Maltose 4%, yeast 0.5%	1940 ± 100	4630 ± 110	2.4	73 ± 1
B—Fructose 4%, yeast 0.5%	2120 ± 90	5960 ± 110	2.8	80 ± 2
F—Beef 0.5%, glucose 2%	1930 ± 50	7040 ± 90	3.6	90 ± 1
E—Soytone 0.5%, glucose 2%	2470 ± 70	8030 ± 100	3.2	93 ± 2
A—Glucose 2%, yeast 0.5%	2930 ± 70	98420 ± 100	3.3	98 ± 2
D—Glucose 4%, yeast 0.5%	2770 ± 60	11420 ± 80	4.1	103 ± 2

DPn, DPw, and Rg_z values are mean of triplicates.

possible to examine the effect of carbon and nitrogen sources on the process of biosynthesis. A standard culture medium containing 2% of glucose was the source of carbon and yeast extract—the source of nitrogen. Carbon source modification consisted in substitution of 2% glucose solution by 4% of maltose, 4% of fructose, or 4% of glucose. In case of a nitrogen source, the yeast extract applied in standard medium was substituted by beef or Soyton extract. As seen in Table 2, maltose is the least effective carbon source. The application of maltose resulted in the lowest productivity of the process and the obtained BC had the lowest values of molecular parameters (DPw = 4630,

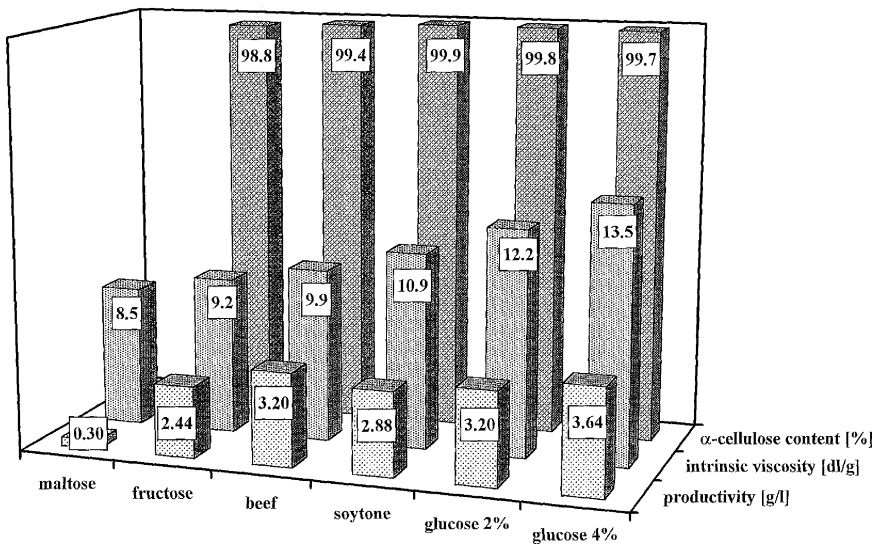

FIGURE 3 Effect of medium composition on cellulose productivity, α-cellulose content and intrinsic viscosity.

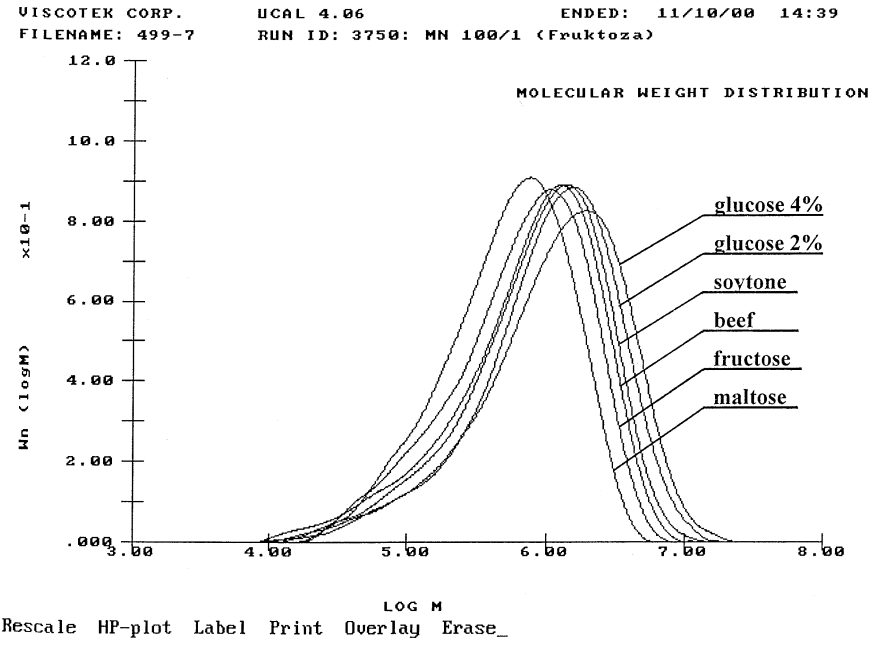

FIGURE 4 Effect of medium composition on the molecular weight distribution of bacterial cellulose.

$[\eta] = 8.5\,\mathrm{dl/g}$, $\mathrm{Rg} = 73\,\mathrm{nm}$). The highest productivity and best molecular parameters were found for BC obtained with 4% content of glucose in the culture medium ($\mathrm{DPw} = 11420$, $[\eta] = 13.5\,\mathrm{dl/g}$, $\mathrm{Rg} = 103\,\mathrm{nm}$). Thus, the results from Table 2 led to the conclusion that the process of bacterial biosynthesis of cellulose is significantly affected by the kind and amount of carbon source. The change of nitrogen source was of no importance.

Figure 4 shows MWD curves for cellulose samples obtained from different compositions of culture medium. The curves well illustrate a considerable contribution of the highest fractions in case of BC samples cultivated when glucose content in the culture medium was 2% or 4%, and their lowest contribution when 4% maltose was applied.

CONCLUDING REMARKS

The results of studies presented in this article show that a very high level of polymerization degree of cellulose, equal to 7350, and respectively low polydispersity $\mathrm{DPw/DPn} = 2.8$ are attained quite early, that is, after 4 days of biosynthesis. Prolongation of cultivation up to 6 days

causes a further growth of DPw up to its maximum value of 10400 and polydispersity to 3.2. At the same time an increase of the productivity is also observed. A further prolongation of the cultivation period lowers DPw value to the level of 5680 and increases polydispersity to a value of 3.5 for BC synthesized for 28 days. At the same time the productivity of the process remains unchanged at the level of 3.2 g/l. MWD curves have unimodel character and are relatively symmetrical. The BC samples under investigation are characterized by their high purity, which is demonstrated by high α-cellulose contents exceeding 99%.

The studies of the effect of culture medium compositions on biosynthesis point out that the chemical nature of the carbon source has the greatest influence on the course of the process. Considering different compositions of the culture medium, best results were obtained when 4% of glucose was used. The effect of a nitrogen source was less significant. In all cases, MWD functions were unimodal and symmetrical.

REFERENCES

[1] J. Glasser, *J. Biol. Chem.* 232, 627 (1958).
[2] J. W. Sutherland, *Biotechnology* 3, 531 (1983).
[3] S. Hestrin and M. Schramm, *Biochem. J.* 58, 345 (1954).
[4] A. J. Brown, *J. Chem. Soc.* 49, 432 (1986).
[5] H. Struszczyk, D. Ciechańska, K. Wrześniewska-Tosik, D. Linowiecka, and D. Woźniak, *Wlókna Chemiczne* 1, 41 (1992).
[6] R. W. Lenz, *Advances in Polymer Science* 107, 1 (1993).
[7] S. Yamanaka, K. Watanabe, N. Kitamua, and M. Iguchi, *J. Mat. Sci.* 24, 3142 (1989).
[8] D. Romano, G. Franzosi, A. Eves, and S. Sora, *Cell. Chem. Technol.* 23, 217 (1989).
[9] H. Struszczyk, K. Wrześniewska-Tosik, D. Ciechańska, and E. Wesołowska. In *Cellulose and Cellulose Derivatives*, J. Kennedy (Ed.) (Woodhead Publishing Ltd., England, 1995), p. 15.
[10] D. Ciechańska, H. Struszczyk, and K. Guzińska, *Fibres & Textiles in Eastern Europe* 6(2), 59 (1998).
[11] F. C. Lui, R. M. Brown, and J. B. Copper, *Science* 230, 822 (1985).
[12] J. D. Fontana, A. de Souza, C. K. Fontana, and J. L. Torrini, *Appl. Biochem. Biotechnol.* 24–25, 253 (1999).
[13] Polish Norm PN 62/P-50099.
[14] J. L. Ekmanis, *Am. Lab. News* Jan/Feb, 10 (1987).
[15] D. Ciechańska, G. Strobin, S. Boryniec, and H. Struszczyk, *Int. J. Polym. Anal. Charact.* 4, 205 (1997).
[16] S. Boryniec, G. Strobin, and K. Bartos, *Polimery* 5, 339 (1999).
[17] C. L. McCormick, P. A. Callais, and B. H. Hutchinson, *Macromolecules* 18, 2394 (1985).
[18] Q. Wang, P. J. Wood, and W. Cui, *Carbohydrate Polymers* 47, 35 (2002).
[19] ASTM D 5296-92, Standard Test Method for Molecular Weight Distribution of Polystrene by High Performance Size-Exclusion Chromatography.

[20] A. F. Turbak. In *Wood and Agricultural Residues* (ACS, vol. XIII), E. J. Soltes (ED.) (Academic Press, New York, 1983), p. 87.
[21] J. C. Moore, *Separ. Sci.* 7, 723 (1970).
[22] J. D. Timpa, *J. Agric. Food Chem.* 39, 270 (1991).
[23] J. D. Timpa, *ACS Symp. Ser.* 302 (1993).
[24] J. D. Timpa and B. A. Triplett, *Planta* 189, 101 (1993).
[25] J. D. Timpa, *Trends Polym. Sci.* 1, 105 (1993).
[26] M. Roussell and P. S. Hawley, *Textile Res. J.* 68, 606 (1998).