Solubility of Bacterial Cellulose and Its Structural Properties

B. ŁASZKIEWICZ

Technical University of Łódź, Department of Man-Made Fibers 90-924 Łódź, Poland

Received 16 July 1996; accepted 15 August 1997

ABSTRACT: Based on experiments conducted, it has been found that bacterial cellulose, like spruce cellulose, is soluble in an aqueous NaOH solution with the concentration of 8.5% at a temperature of −5°C if the polymerization degree of the cellulose does not exceed 400. When 1% of urea is added to the NaOH solution, the solubility of cellulose increases; and, in this solvent, bacterial cellulose may be dissolved so long as its polymerization degree is below 560. The results of these experiments are of great practical importance since they point to the possibility of the preparation of cellulose spinning solutions suitable for fiber formation. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci **67:** 1871–1876, 1998

Key words: cellulose; solubility; bacterial cellulose; cellulose structure; cellulose solutions

INTRODUCTION

The solubility of cellulose depends on many factors,[1–5] especially its structure, molecular weight, and origin.

At present, many scientific centers are interested in dissolving cellulose in inorganic and in organic solvents. This results from the necessity of replacing, in the nearest future, the viscose process of manufacturing fibers and films by a new method, completely nontoxic for the natural environment and based on inexpensive direct solvents, which do not require the preparation of cellulose derivatives.

Our experiments concerning the solubility of bacterial cellulose were based on our previously elaborated process,[1–3] in which spruce cellulose was dissolved in an aqueous 9% NaOH solution at a temperature of −5°C. In the course of our examinations of the solubility of bacterial cellulose, the latter was compared with spruce cellulose having a similar degree of polymerization.

The main aim of our experiments was to determine the solubility of bacterial cellulose and its susceptibility to degradation under alkaline conditions and to compare these properties with those of spruce cellulose, as well as to examine the bacterial cellulose structure by the X-ray method and cross-polarization–magic-angle spinning (CP–MAS) ^{13}C nuclear magnetic resonance (^{13}C-NMR).

EXPERIMENTAL

The bacterial cellulose used in our experiments was prepared in the form of gel using Gram-negative bacterium *Acetobacter xylinum*. For the purpose of comparison, spruce cellulose was also used. The polymerization degree of both the bacterial and spruce celluloses was determined by the conventional method described in the existing literature,[6] while their solubility was determined by our previous technique,[2] which consists of dissolving cellulose in an aqueous 8.5% NaOH solution at a weight ratio of 1 : 20 at a temperature of −5°C for 6 h. The undissolved cellulose was then centrifuged at 7000 rpm and washed using a 3% H_2SO_4 solution first, then water and acetone. The remains of undissolved cellulose were dried at a temperature of 80°C to a constant weight.

Journal of Applied Polymer Science, Vol. 67, 1871–1876 (1998)
 CCC 0021-8995/98/111871-06

Table I Solubility of Bacterial and Spruce Cellulose

Cellulose	Polymerization Degree: Initial Cellulose	Indissoluble Fraction of Cellulose	Dissolved Fraction of Cellulose	Fraction of Dissolved Cellulose (%)
Bacterial A	680	760	390	20.4
Bacterial B	679	734	388	17.8
Bacterial B[a]	679	859	561	48.6
Spruce	634	682	307	19.9
Spruce	640	675	362	25.8

[a] Cellulose was treated in an aqueous 8.5% Na OH solution with a 1% urea content.

The NaOH solution containing the dissolved cellulose fraction was neutralized with 3% H_2SO_4, then the cellulose was precipitated with a high excess of methanol. The precipitated fraction was separated from the solution by centrifuging at 7000 rpm, washed with acetone, and dried to a constant weight. In order to provide accurate results, the solubility experiments were repeated for several samples of both celluloses.

The ripeness rate of bacterial and spruce celluloses was determined under the same standard conditions used in the viscose process.[3]

To prepare alkalicellulose, bacterial cellulose with DP equal to 680 and spruce cellulose with DP equal to 640 were treated with an aqueous NaOH solution (17.5%). After the preparation of alkalicellulose, the excess of NaOH solution was pressed off and the product was disintegrated and subjected to ripening at 55°C. During this process, samples were taken in order to examine the changes in the degree of polymerization.

X-ray diffraction patterns of the cellulose samples were recorded on an X-ray diffractometer using the reflective method.

The relative crystallinity index was estimated by Segal's method,[7] using the following equation:

$$C_I = 100 \times [I(002) - I_{am}]I(002)$$

where $I(002)$ is the peak intensity corresponding to the (002) plane at $2\Theta = 22.8°$ for cellulose I, and I_{am} is the peak intensity of amorphous fraction at $2\Theta = 16°$ for cellulose I.

The results obtained show similar values of relative crystallinity index for spruce cellulose I, where C_I equals 64.1%, and bacterial cellulose, where C_I equals 62.3% (Fig. 4). After a steeping process in sodium hydroxide solution of a 17.5% concentration, the crystallinity index of bacterial cellulose decreases to C_I equal to 43.5%.

Solid-state CP–MAS ^{13}C-NMR spectra of the cellulose samples were recorded on a Bruker

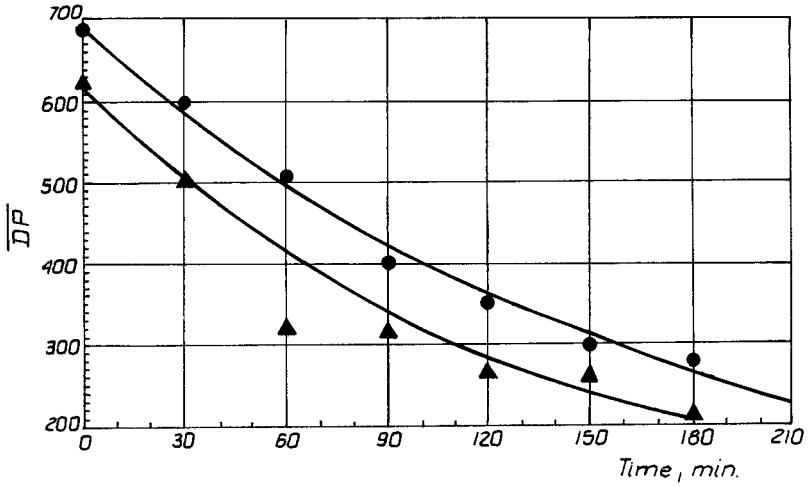

Figure 1 Ripening curves of bacterial and spruce celluloses: (●) bacterial cellulose; (▲) spruce cellulose.

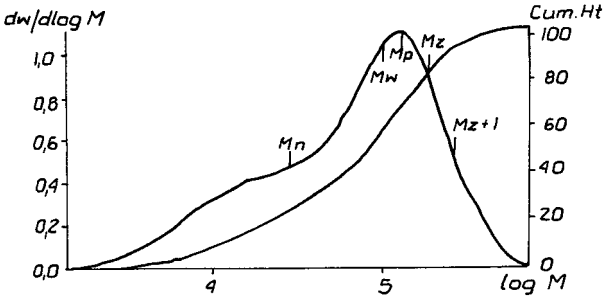

Figure 2 Molecular weight distribution of bacterial cellulose. Polydispersity is 3.691.

MSL-300 type Fourier transform NMR (FT-NMR). The following measurement conditions were employed: a rotation angle to the magnetic field of 56° at a frequency of 2.5 kHz, a relaxation time of 6 s, a resonance frequency of 75.968 MHz, a cross-polarization contact time of 1000 s, a measurement temperature of 25°C, a scanning number of 113–440 impulses, and a pulse width of 29.41 kHz. The molecular weight distribution of the cellulose samples was determined using Hullard–Pacard gel permeation chromatography (GPC) equipment. The following measurement conditions were employed in the determination of molecular weight distribution: cellulose solvent, DMAC containing 0.5% LiCl; concentrations of cellulose solutions, 0.25%; detector, RI; column PL gel mixed 5μ standard polystyrenes; and flow rate, 0.9 mL/min, at a temperature of 80°C.

RESULTS AND DISCUSSION

The solubility characteristics of bacterial and spruce celluloses are given in Table I. The results point to similar solubilities of all the cellulose samples, amounting to about 20%. A higher solubility, amounting to 48.6%, was obtained only when some low quantity of urea was added to the NaOH solution. These results confirm our previous observations[3] that urea increases the solubility of cellulose. From the experiments performed, it can be clearly seen that, irrespective of its origin (that is, either bacterial or spruce cellulose), cellulose may be dissolved in 8.5% NaOH if its polymerization degree is 300–400. These results indicate that 8.5% NaOH solution dissolves cellulose that has not been previously subjected to any structural modification, as was noted in the studies reported by Kamide and coworkers.[8]

From the relationships shown in Figure 1, it can be seen that the ripeness curve of bacterial alkalicellulose is the same as that of spruce cellulose. Hence, one can assume that the ripening mechanisms of both celluloses are also the same. Therefore, it can be concluded that the addition of bacterial cellulose to the viscose process of fiber manufacture should not present any difficulties, and the process can be carried out under the same conditions as those employed for spruce cellulose.

The GPC examinations of bacterial (Fig. 2) and spruce (Fig. 3) celluloses performed by us show that these celluloses are characterized by different molecular weight distributions. Bacterial cellulose contains more low-molecular-weight fractions when compared to spruce cellulose. This fact can be accounted for by the synthesis mechanism of bacterial cellulose, which is quite different from that of spruce cellulose, despite the fact that both synthesis mechanisms are associated with a gradual growth of cellulose macromolecules. The considerable content of low-molecular-weight fractions in bacterial cellulose is confirmed by the value of polydispersion, which is considerably higher for bacterial cellulose (3.691) than for spruce cellulose (3.195).

The X-ray diffraction examinations (Fig. 4) of bacterial cellulose (curve 2) show that its X-ray scattering is much the same as that of spruce cellulose I (curve 1). However, curve 2 shows a distinct deflection at $2\Theta = 20.8°$, which would testify to the fact that in the bacterial cellulose under investigation, there is a polymorphic fraction of cellulose II beside cellulose I. This is explainable by the fact that the synthesis of bacterial cellulose proceeds bidirectionally, so under the conditions used by us, cellulose II is formed beside cellulose I.

Bacterial cellulose II (curve 3) is formed only during the alkalization of bacterial cellulose I in a 17.5% NaOH solution, as is also the case of the alkalization of spruce cellulose II, previously ex-

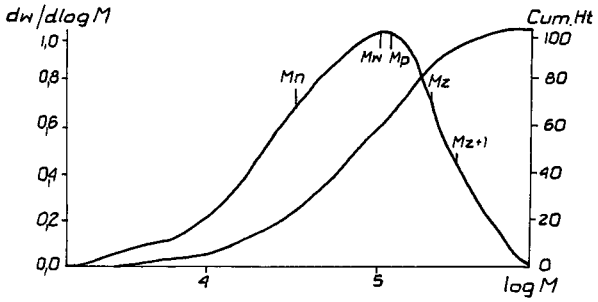

Figure 3 Molecular weight distribution of spruce cellulose. Polydispersity is 3.195.

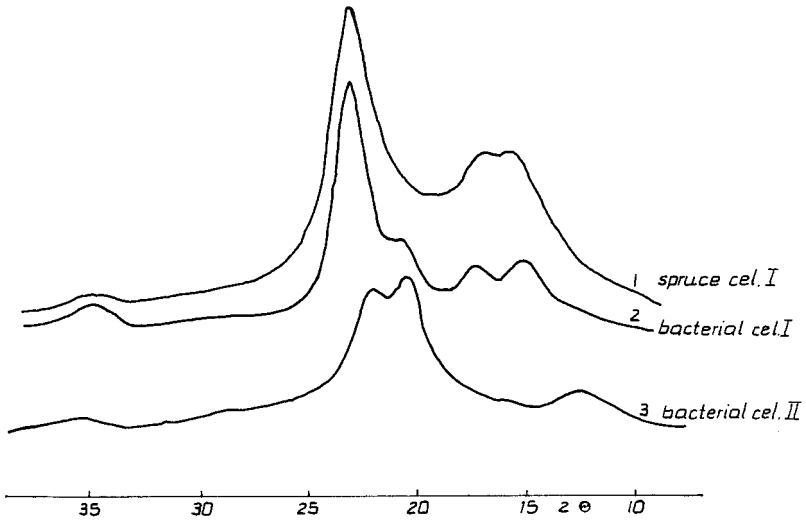

Figure 4 X-ray diffractogram of spruce (1) cellulose I, (2) bacterial cellulose I, and (3) bacterial cellulose II.

amined by us.[3] It should be noted that the presence of a cellulose II fraction beside cellulose I in the initial sample of bacterial cellulose is associated with the bidirectional mechanism of synthesis and not with the polycrystalline transformation during the purification of bacterial cellulose with 2% NaOH solution after the synthesis and separation of bacterial cellulose from the reaction medium. This assumption is based on our previous proof[3] that, at such a low NaOH concentration, the polymorphic transformation of cellulose I into cellulose II is impossible.

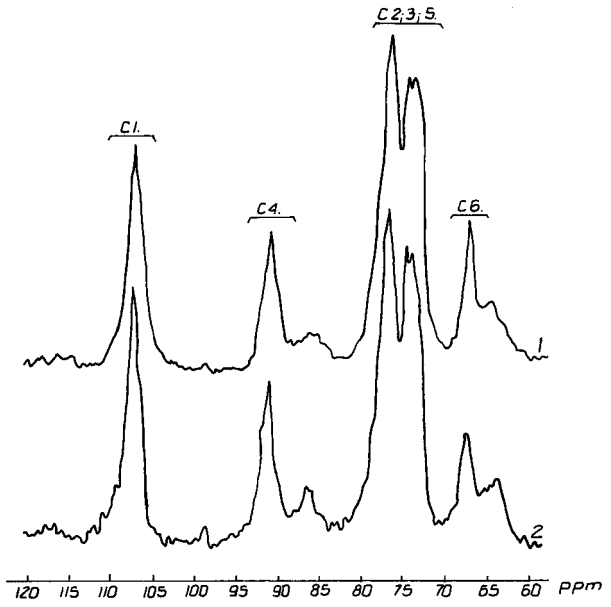

Figure 5 CP–MAS ^{13}C-NMR spectra of bacterial cellulose in (1) dry and (2) wet stage.

The CP–MAS ^{13}C-NMR examinations performed (Figs. 5 and 6) show that when bacterial cellulose is treated with 8.5% NaOH at a low temperature, considerable structural and conformational changes take place. From a comparison of the diagrams shown in Figure 5, one can see that bacterial cellulose shows slightly different quantities and types of hydrogen bonds, depending on its moisture content. The spectrogram of dry cellulose (Fig. 5, curve 1), containing about 2% moisture, differs from the spectrogram of wet cellulose (Fig. 5, curve 2), containing 250% moisture, within the range of characteristic peaks for C4, C2, C3, C5, and C6, correlating to the chemical shifts within the range of 60–100 ppm. The shift values (Table II) in this case are within the experimental error limits (except for peak C6), while the differences in the intensities of particular peaks for dry and wet celluloses are considerable, which testifies to significant differences in the association of water molecules with OH groups combined with particular C atoms in the glycopyranose units of cellulose. The highest association in the wet bacterial cellulose sample is observed at the OH group linked with C6, which is expressed by an extraordinarily high change in the peak intensity and a chemical shift towards a higher magnetic field from 65.2 to 63.7 ppm. In our opinion, the association of water in this case leads to a change in the conformation of HO—C(6) group from position "tg" to position "gt," with a simulta-

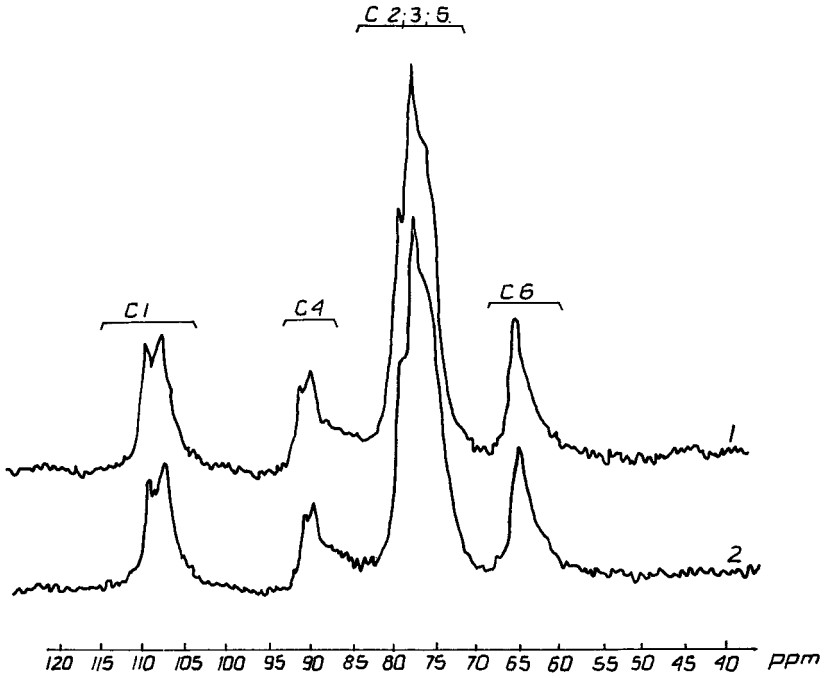

Figure 6 CP–MAS ^{13}C-NMR spectra of (1) soluble and (2) indissoluble fractions of bacterial cellulose after NaOH solution treatment.

neous interruption of the intermolecular hydrogen bond C(3)···HO(6).

Quite a considerable increase in the peak intensities is also observed for C4 and C2, C3, and C5, although, in this case, no chemical shifts take place. Therefore, it should be concluded that the association of water molecules with OH groups combined at positions C4 and C2, C3, and C5, brings about no intermolecular hydrogen bond breaking. Our conclusions concerning the changes in the association of OH groups in cellulose under the influence of water are fully confirmed by the previous observations made by Atalla and coworkers.[9] It should also be emphasized that even the high association of OH groups of cellulose observed in this case does not result in intermolecular H-bond breaking, which is due to the fact that in wet bacterial cellulose, no chemical shifts characteristic of particular C atoms take place when compared to dry cellulose (Table II).

During the treatment of bacterial cellulose with 8.5% NaOH at a temperature of −5°C, considerable inter- and intramolecular changes occur, which are associated first of all with the conformational changes in group HO—C(6). The polymorphic transformation of cellulose I into cel-

Table II Comparison of the CP–MAS ^{13}C-NMR Chemical Shifts of the Sharp Spectral Features in Bacterial Cellulose

Sample	C1	C4	C2, C3, C5	C6
Bacterial cellulose in dry form	107.3 (8.010)[a]	91.2 (4.781)	76.8 (11.901)	67.3 (4.683)
		86.7 (1.073)	74.7 (10.345)	65.2 (1.951)
Bacterial cellulose in wet form	107.3 (17.603)	91.2 (7.887)	76.9 (15.871)	67.5 (5.998)
		86.8 (2.597)	74.0 (15.310)	63.7 (4.266)
Dissoluble[b] fraction of bacterial cellulose	108.6 (6.957)	89.1 (5.398)	77.9 (13.727)	64.3 (7.899)
	106.6 (7.334)		76.2 (21.301)	
Indissoluble[b] fraction of bacterial cellulose	108.7 (6.728)	90.1 (4.572)	76.2 (19.898)	64.2 (7.128)
	106.7 (7.057)	89.1 (5.034)		58.6 (1.033)

[a] Intensity (in parenthesis).
[b] Following treatment in 8.5% NaOH solution.

lulose II (Fig. 4), determined by us by means of the X-ray method, results from the breaking of many inter- and intramolecular hydrogen bonds. It is this phenomenon that makes polymorphic transformation possible. It should be emphasized here that it is the breaking of numerous primary inter- and intramolecular hydrogen bonds in cellulose I that results in the polymorphic transformation of cellulose I into cellulose II, the transformation being a secondary phenomenon, which could not occur without breaking the primary hydrogen bonds in cellulose I.

Changes in the values of chemical shifts (Table II) towards higher magnetic fields are observed, first of all, for peak C(6), for both the fractions of bacterial cellulose dissolved and undissolved in 8.5% NaOH. These considerable changes in chemical shifts, from 67.3 and 65.2 to 64.3 ppm for the dissolved fraction in NaOH and to 64.2 and 58.6 ppm for the undissolved fraction, are undoubtedly the result of breaking the following bonds: intramolecular H-bond C(3)0′···HOC(6) and intermolecular H-bond C(2)—OH···0′(6), as well as of changing the "tg" conformation of the C(6)—OH group into a "gt" conformation, with the simultaneous formation of intramolecular H-bond C(2)0···0′(6), which is in complete agreement with the previously reported findings by Cuculo and coworkers.[10,11] A similar change in chemical shifts towards higher magnetic field values is observed for the nuclei of C4 for the fraction of bacterial cellulose dissolved, as well as undissolved, in 8.5% NaOH. This fact may be accounted for by the breaking of the intramolecular bonds between 0′···HOC(4), that is, glycoside oxygen, and the hydroxyl group belonging to the adjacent macromolecule.

CONCLUSIONS

As a result of the studies we performed, it has been found that bacterial cellulose, like spruce cellulose, is soluble in an aqueous NaOH solution with an 8.5% concentration at a temperature of −5°C if the polymerization degree of the cellulose does not exceed 400. When, however, 1% of urea is added to the 8.5% NaOH solution, the solubility of cellulose is considerably increased. In this solvent, bacterial cellulose may be dissolved so long as its polymerization degree is below 560.

Based on CP–MAS ^{13}C-NMR measurements, it has been found that during the process of dissolving bacterial cellulose in 8.5% NaOH solution, the cellulose is subject to transformation from "tg" to "gt" form, which is associated with the intermolecular hydrogen bond cleavage.

The experimental results described are of great practical importance since they point to the possibility of the preparation of cellulose spinning solutions suitable for fiber formation without the need for cellulose activation by the process of steam explosion[8] or enzymatic biotransformation of cellulose.[12]

The X-ray measurements carried out by us show that the synthesis of bacterial cellulose in the presence of Acetobakter xylinum results in a product that contains a small fraction of polymorphic cellulose II in addition to the main fraction of cellulose I.

The results presented in our study show that bacterial cellulose is subject to degradation in alkaline media in a similar way as spruce cellulose.

REFERENCES

1. B. Łaszkiewicz, *Włókna Chemiczne,* **13,** 167 (1987).
2. B. Łaszkiewicz and J. A. Cuculo, *J. Appl. Polym. Sci.,* **50,** 27 (1993).
3. B. Łaszkiewicz and P. Wcisło, *J. Appl. Polym. Sci.,* **39,** 415 (1990).
4. A. F. Turbak, R. B. Hammer, R. E. Davies, and H. L. Hergert, *CHEMTECH,* **10,** 51 (1980).
5. K. Gotze, *Chemifasern Nach Dem Viscose Verfahren,* Springer-Verlag, 1967.
6. G. Jayme and W. Bergmann, *Das Papier,* **12,** 187 (1958).
7. L. Segal, J. Creely, A. Martin, C. Conrad Jr., *Text. Res. J.,* **29,** 786 (1959).
8. T. Yamashiki, T. Matsui, S. Saitoh, K. Okajima, K. Kamide, and T. Sawada, *Brit. Polym. J.,* **22,** 73 (1990).
9. R. A. Atalla and J. D. Ellis, *TAPPJ Proceedings,* Boston, 1983, p. 64.
10. J. A. Cuculo, C. B. Smith, U. Sangwatanaroj, E. O. Stejskal, and S. S. Sankar, *J. Polym. Sci., Part A: Polym. Chem.,* **32,** 229 (1994).
11. J. A. Cuculo, C. B. Smith, U. Saugwatanaroj, E. O. Stjeskal, and S. S. Sankar, *J. Polym. Sci., Part A: Polym. Chem.,* **32,** 241 (1994).
12. H. Struszczyk, D. Ciechańska, and D. Wawro, *Fibers Text. East. Eur.,* **3,** 47 (1995).