# Control of the Crystal Structure of Microbial Cellulose During Nascent Stage

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**ABSTRACT:** The structure of the product, from an *Acetobacter* culture in the presence of Fluorescent Brightener, Direct Red 28, and Direct Blue 1, 14, 15 and 53, characterized by an X-ray diffractormeter is a crystalline complex. On the other hand, solid-state $^{13}$C-NMR spectroscopy reveals that the product is noncrystalline. However, the X-ray result of the product sample suggests that the dye molecule is included in the form of a monolayer between the cellulose sheets in the complex corresponding to the $(1\bar{1}0)$ plane of microbial cellulose. But the celluloses regenerated from the Fluorescent Brightener product, the Direct Red 28 product, and the rest of the dye products are celluloses I, IV, and II, respectively. More specifically, the $^{13}$C-NMR spectra revealed that the crystal types of cellulose from the Fluorescent Brightener and Direct Red 28 products are $I_\beta$ and $IV_I$, respectively. Thus, the crystal structure of the product and the regenerated cellulose depends mainly on the position and number of the sulfonate groups in a direct dye and the interactions of the dye with the noncrystalline microbial cellulose in the nascent stage. The conformation and arrangement of the nascent cellulose chain changes when a direct dye adheres to it. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 79: 1726–1734, 2001

**Key words:** *Acetobacter xylinum;* microbial cellulose; dye–cellulose complex; regenerated cellulose

## INTRODUCTION

Due to the extreme pressure on a biodegradable polymer and undesirable ecopolution by a synthetic polymer, microbial cellulose (MC) is now of growing interest to scientists. It is also of interest because it is mostly pure and its quality and quantity can be controlled. Since cellulose is an ubiquitous natural polymer, much effort has been spent to understand the mechanism by which it is formed. This problem has proved to be complex because it involves not only the biosynthesis of high molecular weight $\beta$-1,4 glucans but also the biogenesis of crystalline cellulose I fibrils. These most common natural cellulose I fibrils exist in a parallel cellulose chain crystalline lattice, although the antiparallel chain lattice, cellulose II, is thermodynamically favored.[1,2]

It is well known that MC is noncrystalline at its initial stage and then crystallized to be cellulose $I_\alpha$-rich. As the noncrystalline MC is crystallized normally to be cellulose $I_\alpha$-rich, this crystallization of cellulose may be controlled at this nascent stage. As no direct alteration of crystallization and fibril assembly has been accomplished, several fluorescent brightening agents

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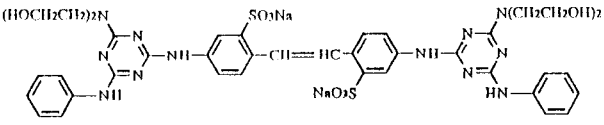

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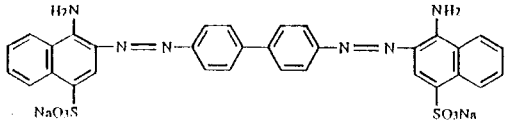

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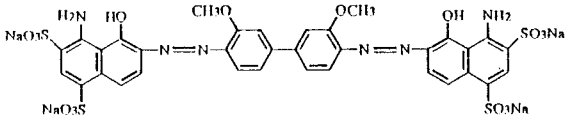

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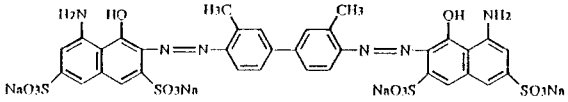

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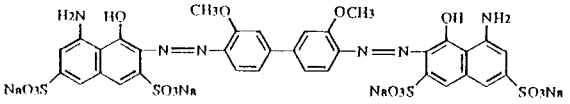

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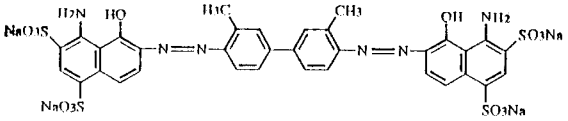

Direct Blue 53

**Figure 1** Chemical structure of direct dyes.

and direct dyes recently have been employed to alter the crystallization and fibril assembly. Keeping this point in mind, the pioneer groups of Brown et al.[3–5] and Kai and Mondal[6–9] started their investigations to control the structure of MC using direct dyes and cellulose derivatives as a probe. Most of their observations were carried out with an electron microscope and an X-ray micrograph. Much progress in elucidating the mechanism and control of cellulose fibril biogenesis has been made by studying a normal and an altered cellulose ribbon assembly: the Gram-negative bacterium *Acetobacter xylinum.*[3–5,9–11] But no worthwhile discussion is available in the light of an X-ray diffractogram and solid-state $^{13}$C-NMR spectroscopy.

In the present study, direct dyes, such as Fluorescent Brightener (FB), Direct Red 28 (DR28), and Direct Blue 1, 14, 15, and 53 (DB1, DB14, DB15, and DB53), were selected to control the structure of nascent cellulose produced by *A. xylinum.* The structures of the products from the *Acetobacter* culture in the presence of direct dyes and the cellulose regenerated from it were characterized by an X-ray diffractometer and solid-state $^{13}$C-NMR spectroscopy.

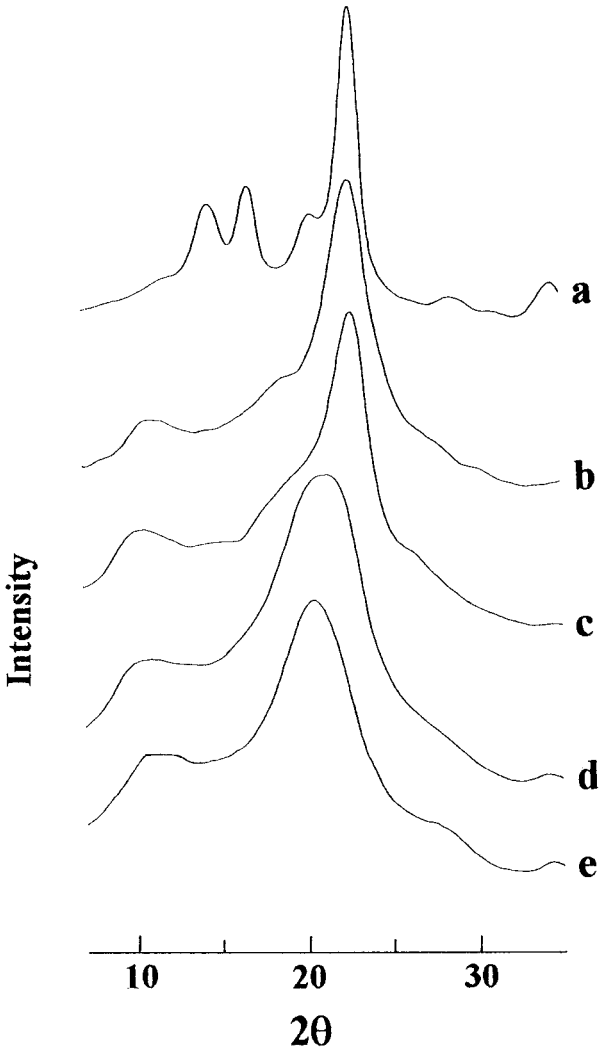


**Figure 2** X-ray diffractograms of MC and the product samples obtained from *Acetobacter* culture in the presence of a dye of 0.1 wt %: (a) MC; (b) FB product; (c) DR28 product; (d) DB1 product; (e) DB15 product.

**Table I X-ray Diffraction Angles of MC and Isotropic Product Samples Obtained from *Acetobacter* Culture in the Presence of Direct Dyes (0.1 wt %)**

| Sample | $2\theta$ (deg) ($d$-spacing in Å) | | | | |
|---|---|---|---|---|---|
| MC | — | 14.47 (6.12) | 16.77 (5.28) | 20.48 (4.33) | 22.70 (3.92) |
| FB | 10.60 (8.34) | — | — | — | 22.08 (4.02) |
| DR28 | 10.39 (8.51) | — | — | — | 22.71 (3.91) |
| DB1 | 10.45 (8.45) | — | — | 20.34 (4.36) | — |
| DB14 | 10.48 (8.43) | — | — | 20.47 (4.33) | — |
| DB15 | 10.36 (8.53) | — | — | 20.33 (4.36) | — |
| DB53 | 10.34 (8.55) | — | — | 20.16 (4.40) | — |

## EXPERIMENTAL

### Preparation of the Sample

About 60 mL of a cell suspension prepared from the *A. xylinum* culture (IFO 13693) was added to 140 mL of a Hestrin–Schramm medium[12] (pH 7.0) containing a direct dye of 0.1 wt % and this was incubated at 28°C for about 24 h in a static condition. The product was collected by centrifuging (6000 rpm, 5.15 g, 20 min) and washed well with a 0.1 wt % aqueous caustic soda solution to remove the medium components and the dye which is not related to the dyeing.[8,13–15] This was then rinsed well with distilled water until alkali-free and was preserved at 3°C for the next experiments. The chemical structure of the direct dyes used in this experiment are shown in the Figure 1.

The extraction of the dye was performed by boiling the product sample (product) in a 70 vol % aqueous EtOH solution for 18 h and the fresh ethanol solution was exchanged for every 3 h. This dye-extracted sample was again boiled in a 1.0 wt % aqueous caustic soda solution for 10 h under a $N_2$ atmosphere to remove proteinous matters. After neutralization with a 1.0 vol % acetic acid solution, the sample was rinsed sufficiently with distilled water.[13–15] This is the regenerated sample.

The standard cellulose I sample was prepared by washing MC with distilled water to remove the medium components and boiled in a 1.0 wt % aqueous sodium hydroxide solution for 10 h under a $N_2$ atmosphere. Mercerization of MC was done at 20°C for 25 h to the prepared standard cellulose II sample.[9–11] The standard cellulose $IV_{II}$ sample was obtained by which cellulose II was annealed in glycerol at 260°C for 30 min.

### Measuring Methods

To characterize the structure of the product from the *Acetobacter* culture in the presence of a direct

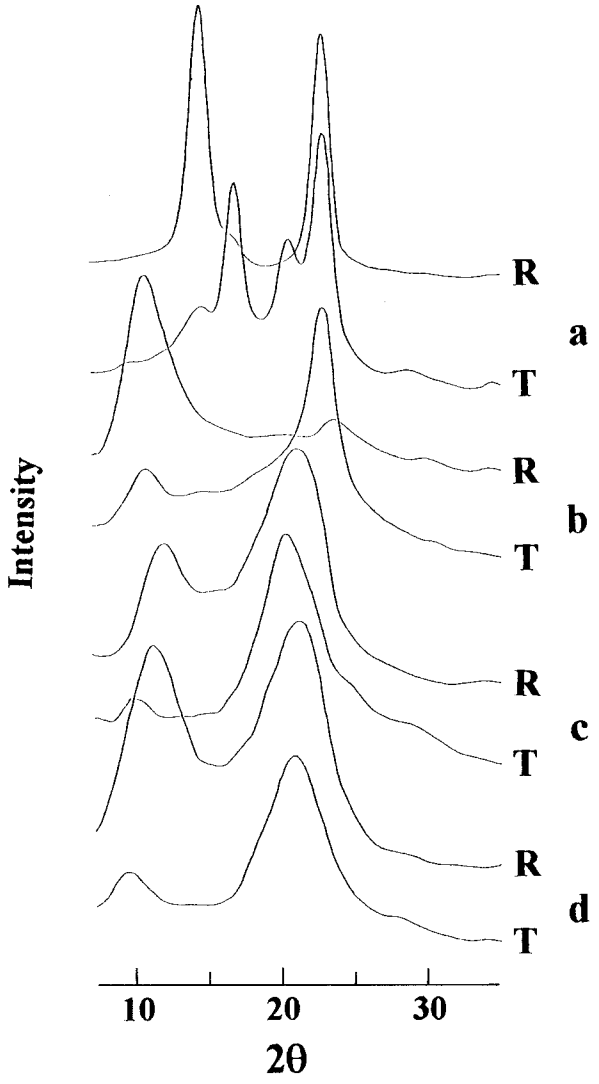


**Figure 3** X-ray diffractograms of an uniplanar oriented membrane of MC and the product samples measured by reflection (R) and transmission (T) methods: (a) MC; (b) DR28 product; (c) DB1 product; (d) DB15 product.

**Table II X-ray Diffraction Angles of Oriented Membrane of MC and Product Samples Measured by Reflection and Transmission Methods**

| | Reflection | | Transmission | |
|---|---|---|---|---|
| | 2$\theta$ (deg) ($d$-spacing in Å) | | | |
| Sample | Low Angle | High Angle | Low Angle | High Angle |
| MC | 14.46 (6.15) | 16.82 (5.27) | 14.65 (6.04) | 16.91 (5.24) |
| | Lower Angle | Higher Angle | Lower Angle | Higher Angle |
| FB | 9.21 (9.59) | 22.50 (3.95) | 11.04 (8.01) | 22.60 (3.93) |
| DR28 | 9.57 (9.23) | 22.99 (3.86) | 10.83 (8.16) | 22.84 (3.89) |
| DB1 | 12.34 (7.17) | 21.15 (4.19) | 9.46 (9.34) | 20.54 (4.32) |
| DB14 | 10.10 (8.75) | 21.90 (4.05) | 9.54 (9.26) | 19.77 (4.48) |
| DB15 | 11.17 (7.91) | 21.15 (4.19) | 9.46 (9.34) | 20.54 (4.32) |
| DB53 | 10.85 (8.15) | 21.07 (4.19) | 9.56 (9.24) | 20.30 (4.37) |

dye and cellulose regenerated from it, the following measurements were performed: An X-ray diffraction diagram of the product samples and cellulose regenerated from it were performed by an MXP18 diffractometer (MAC Science Co., Ltd., Tokyo) with Ni-filtered CuK$\alpha$ radiation. The measurement conditions were as follows: divergence slit, 1.0 deg; receiving slit, 0.15 mm; scanning speed, 4.0 deg/min; and X-ray radiation, 40 kV, 200 mA. The X-ray diffraction diagram of an uniplanar oriented membrane of the product sample was measured by the reflection and transmission methods. On the other hand, the diffraction diagram of an isotropic product sample and its regenerated cellulose was obtained by the transmission method. In this case, the sample was compressed as a pellet into the pore of a sample holder of 1-mm diameter and 1-mm thickness.

Solid-state $^{13}$C-NMR spectra of the wet sample were obtained at room temperature on a JEOL JNM EX 270 spectrometer operating at 6.35 T. The mass rates were 5.0–5.5 kHz and the chemical shifts relative to tetramethylsilane were determined by using the crystalline peak at 17.3 ppm of hexamethyl benzene as an external standard. The nondried samples were packed in an MAS rotor with an O-ring sealed to avoid the loss of water during NMR measurements. The spectrum of the crystalline component of the control and regenerated cellulose was measured selectively by taking NMR signals after a relaxation of the noncrystalline component. Therefore, to de-

**Table III X-ray Diffraction Angles of MC and Cellulose II, Cellulose $IV_{II}$, and Celluloses Regenerated from Product Samples**

| | 2$\theta$ (deg) ($d$-spacing in Å) | | |
|---|---|---|---|
| Sample | $(1\bar{1}0)$ | (110) | (020) |
| MC | 14.47 (6.12) | 16.77 (5.28) | 22.68 (3.92) |
| Cotton cellulose | 14.87 (5.95) | 16.13 (5.49) | 22.60 (3.93) |
| FB | 14.30 (6.19) | 15.99 (5.54) | 22.23 (3.99) |
| Cellulose $IV_{II}$ | 15.40 (5.75) | 15.40 (5.75) | 22.01 (4.04) |
| DR28 | 15.33 (5.77) | 15.33 (5.77) | 22.01 (4.04) |
| Cellulose II | 11.96 (7.39) | 19.75 (4.49) | 21.10 (4.21) |
| DB1 | 12.35 (7.16) | 20.21 (4.39) | 21.84 (4.07) |
| DB14 | 12.01 (7.36) | 20.04 (4.43) | 21.02 (4.22) |
| DB15 | 12.30 (7.19) | 20.66 (4.30) | 21.40 (4.15) |
| DB53 | 11.83 (7.47) | 19.87 (4.46) | 21.60 (4.11) |

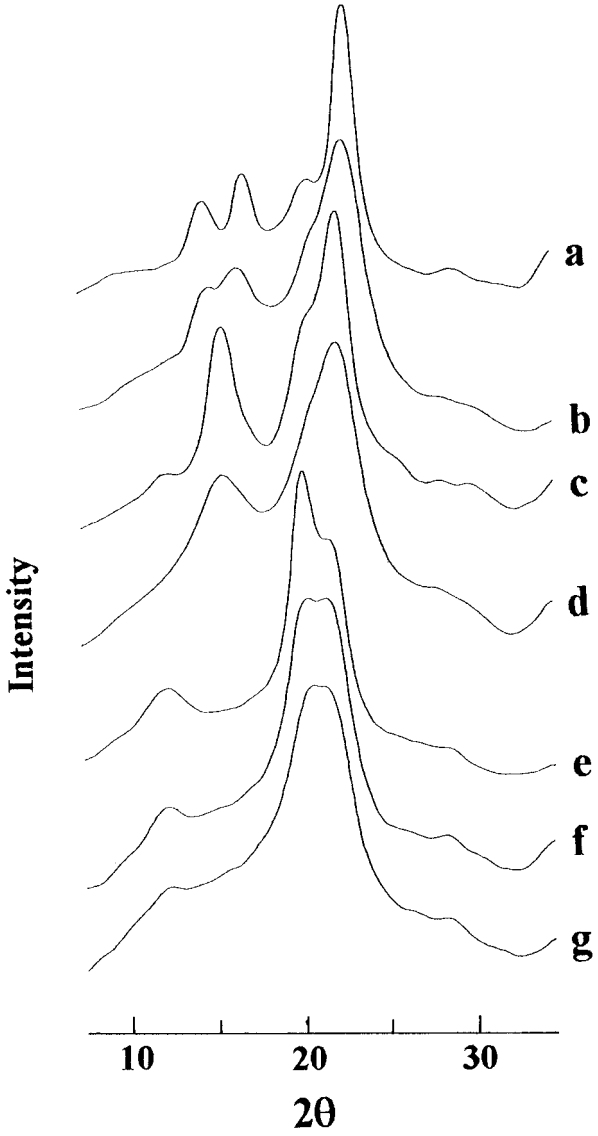


**Figure 4** X-ray diffractograms of standard celluloses and celluloses regenerated from the product samples: (a) MC; (b) FB cellulose; (c) cellulose $IV_{II}$; (d) DR28 cellulose; (e) cellulose II; (f) DB1 cellulose; (g) DB15 cellulose.

termine the crystalline component of cellulose samples, the signals obtained after $\lambda = 50$ s were measured by using NMR T1CP pulse sequence[16] with CP as the spectrum of the crystalline component.

## RESULTS AND DISCUSSION

*A. xylinum* in a glucose medium initially produces extracellular noncrystalline cellulose which is associated in a normal situation through van der Waals' forces and then through hydrogen bonding to form the crystalline cellulose $I_\alpha$ fibril. The noncrystalline cellulose, that is, nascent cellulose, interacts with the direct dyes in the medium to produce a product known as a dye–cellulose complex. X-ray diffraction of these products showed a characteristic diagram of a crystalline pattern having two peaks: one at the lower-angle side near 10° and the other at the higher-angle side near 20.5° for DB1, DB14, DB15, and DB53 products and near 22.5° for the FB and DR28 products. The $2\theta$ values of MC and the product samples obtained from Figure 2 are listed in Table I. The dissimilarities of the pattern from that of

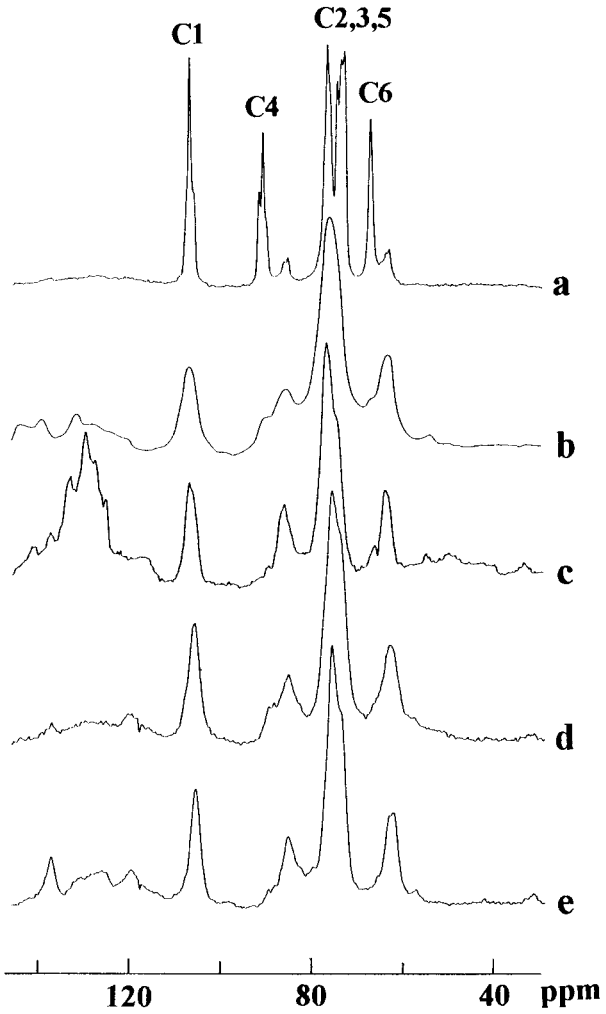


**Figure 5** Solid-state $^{13}$C-NMR spectra of the wet MC and wet product samples obtained from *Acetobacter* culture in the presence of a dye of 0.1 wt %: (a) MC; (b) FB product; (c) DR28 product; (d) DB1 product; (e) DB15 product.

**Table IV Solid-state $^{13}$C-NMR Chemical Shifts of MC and Product Samples Obtained from *Acetobacter* Culture in the Presence of Direct Dyes (0.1 wt %)**

| | Chemical Shifts (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample | C1 | C4 | | | C2, 3, 5 | | | C6 |
| MC | 106.3 | 90.2 | 84.7 | 75.9 | 73.8 | 72.3 | 66.5 | 62.5 |
| FB | 105.6 | — | 84.8 | 75.4 | — | — | — | 62.6 |
| DR28 | 106.0 | — | 85.2 | 76.1 | — | — | — | 63.4 |
| DB1 | 105.2 | — | 84.7 | 75.2 | — | — | — | 62.4 |
| DB14 | 105.2 | — | 85.0 | 75.2 | — | — | — | 62.6 |
| DB15 | 105.2 | — | 84.9 | 75.3 | — | — | — | 62.9 |
| DB53 | 105.6 | — | 85.0 | 75.5 | — | — | — | 62.9 |

MC, which has four peaks, indicates that the product is not an ordinary microbial cellulose; rather, it is a complex of direct dye and cellulose. For this, the normal hydrogen-bond formation between the cellulose sheets is hindered. The characteristics of this complex will become clearer in the latter discussion.

Figure 3 shows the X-ray diffraction diagrams of oriented membranes of MC and the product samples obtained by reflection and transmission methods (FB, DB14, and DB53 are not shown as they are very similar to DR28, DB15, and DB1, respectively) and $2\theta$ values of their strong and weak peaks are listed in Table II. The X-ray diffraction diagram of FB and the DR28 product membranes measured by reflection and transmission methods shows a very good uniplanar orientation. The membrane was prepared from the wet product sample on a Teflon plate and was dried at room temperature. The higher-angle plane of the oriented membrane of MC is perpendicular to the lower-angle plane and the orientation of the lower-angle plane is parallel to the surface of the membrane.[13,17] However, the reflection and transmission behavior of the X-ray diffraction diagrams of the oriented membrane of MC indicates that the higher-angle plane of the oriented product membrane of the dye is perpendicular to the lower-angle plane. The peak intensity of the product membrane also indicates the degree of orientation. However, that the *d*-spacing of the lower-angle plane of the product membrane (and also in the isotropic product) becomes broader than that of the low-angle plane $(1\bar{1}0)$ of MC suggests that the dye molecule is included between the lower-angle planes of MC. Due to the effect of dye inclusion between the lower-angle planes, that is, the $(1\bar{1}0)$ plane of MC at 14.47°, the van der Waals' forces between the cellulose chains of higher-angle planes, that is, (110) planes of MC at 16.77°, become stronger, and as a result, the (110) plane of MC in the complex shifted to narrower *d*-spacing at 3.8–4.5 Å (ref. 13) (Table II).

On the other hand, each of the membranes from the DB1, DB14, DB15, and DB53 products do not show a good uniplanar orientation like that of the MC, FB, or DR28 product membrane (Fig. 3). Among the dye products, the DB14 and DB15 product membranes attained a little better uniplanar orientation than that of the DB1 and DB53 product membranes. DR28, DB1, DB14, DB15, and DB53 have same biphenylenebis(azo) skeletal structure and FB has a different skeletal structure. The product from two sulfonate groups containing dye, for example, FB and DR28, shows good orientation. In contrast, the product from four sulfonate groups containing dye, for example, DB1, DB14, DB15, and DB53, shows poor orientation. Furthermore, the product from each DB14 and DB15 dye having four sulfonate groups at the 3 and 6 positions in the naphthalene nucleus shows a little better orientation than that of each DB1 and DB53 dye product. The latter two dyes contain their sulfonate groups at the 5 and 7 positions in the naphthalene nucleus. The structure and substituent groups of DB1 and DB15 are similar, but the only difference between these two dyes are the position of sulfonate groups in the naphthalene nucleus. Furthermore, methyl and methoxy groups in a dye have no effect on the uniplanar orientation.[19,20] Therefore, the better uniplanar orientation of the DB1 product than of the DB15 product is due to the position of sulfonate groups. Similarly, it is shown that the difference of the effect of uniplanar orientation of the DR28 product with that of the DB14, DB15, and DB53 products is due to the number of sulfonate groups. Anyhow, their X-ray results suggest

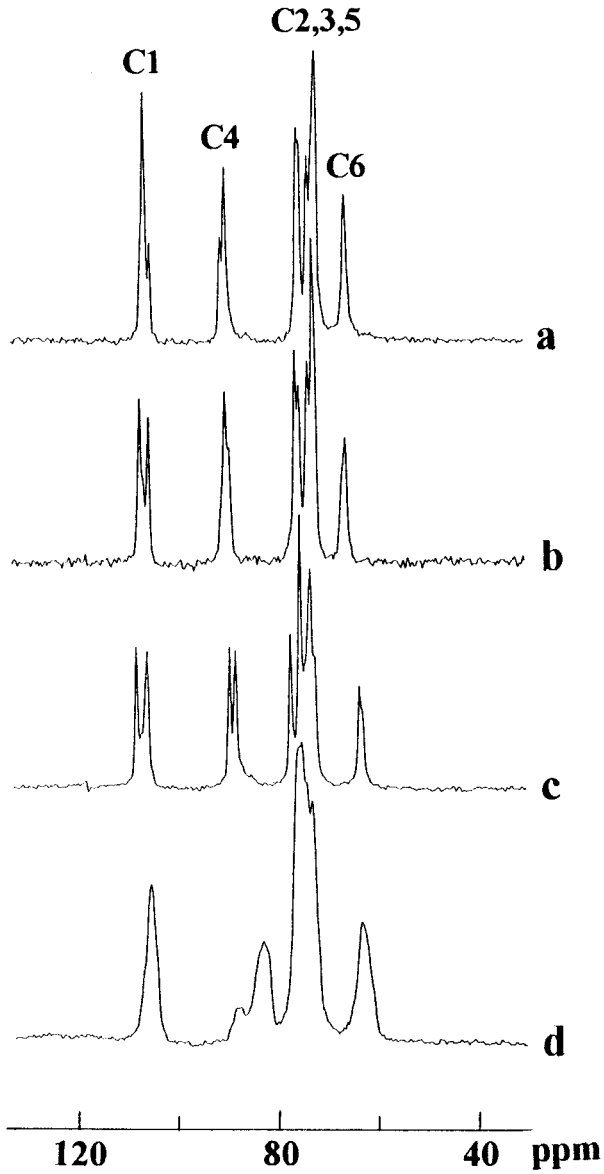


**Figure 6** Solid-state $^{13}$C-NMR spectra of the crystalline component of wet standard celluloses: (a) MC; (b) cotton cellulose; (c) cellulose II; (d) cellulose $IV_{II}$.

that the normal hydrogen-bond formation between the cellulose sheets corresponding to the $(1\bar{1}0)$ plane of MC and the dye molecule is hindered by the inclusion of dyes which may bind with the nascent cellulose chain through van der Waals forces and/or hydrogen bonding. Thus, the differences of the effect on the product structure are the results of the number and position of sulfonate groups in a dye, but not the other substituent groups.

The effect of the direct dyes is observed not only on the product structures but also on the dye-extracted samples. The X-ray diffraction diagram of the dye-extracted sample shown in the Figure 4 is different from that of their corresponding dye products, indicating that after dye extraction from the product some rearrangements of bonds, which had hindered by the dye molecules in the product, between the cellulose sheets are developed. The X-ray diffraction diagram of these dye-extracted samples suggest that these are celluloses.

Table III contains the $2\theta$ values of the diffraction angles of standard celluloses and celluloses regenerated from the product samples. The celluloses regenerated from the FB and DR28 products (FB and DR28 celluloses) are celluloses I and IV, respectively; those from the DB1, DB14, DB15,

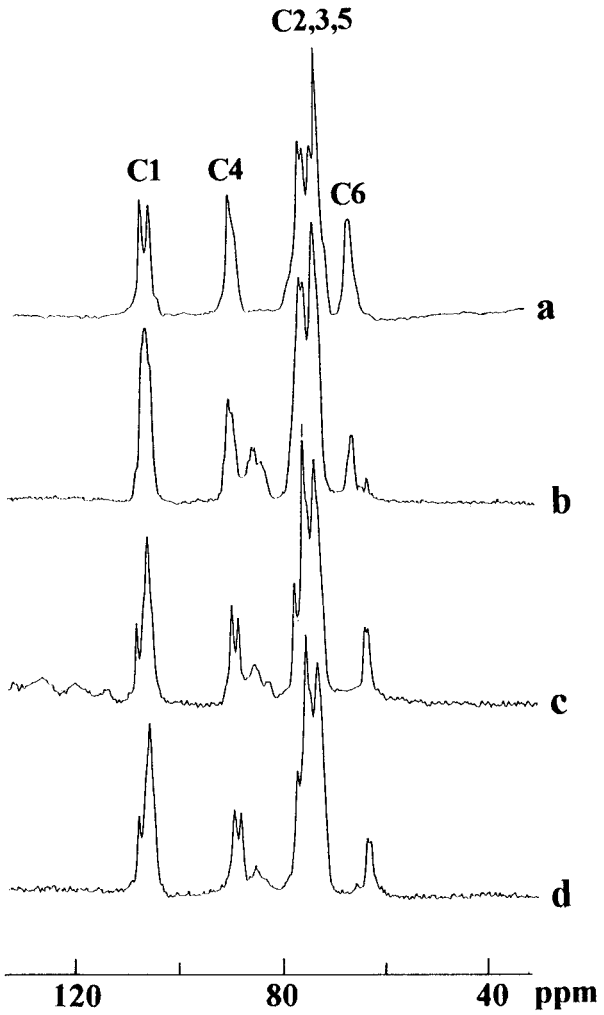


**Figure 7** Solid-state $^{13}$C-NMR spectra of the crystalline component of wet celluloses regenerated from the product samples: (a) FB cellulose; (b) DR28 cellulose; (c) DB1 cellulose; (d) DB15 cellulose.

**Table V Solid-state $^{13}$C-NMR Chemical Shifts of Crystalline Components ($\tau$ = 50 s) of MC, Cotton Cellulose, Cellulose II, Cellulose $IV_{II}$, and Celluloses Regenerated from Product Sample**

| Sample | Chemical Shifts (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C1 | | C4 | | C2, 3, 5 | | | C6 |
| MC | 106.7 | 104.9 | 90.7 | 89.9 | 75.6 | 73.5 | 71.9 | 66.5 |
| Cotton cellulose | 106.7 | 105.1 | 89.8 | 89.1 | 75.9 | 73.4 | 72.3 | 65.9 |
| FB | 107.8 | 105.2 | 89.8 | — | 75.9 | 73.6 | 72.5 | 66.2 |
| Cellulose $IV_{II}$ | 106.1 | — | 89.9 | 88.7 | 77.1 | 75.3 | 73.7 | 64.3 |
| DR28 | 106.2 | — | 89.9 | — | 76.1 | 75.4 | 73.5 | 66.1 |
| Cellulose II | 107.7 | 105.5 | 89.4 | 88.1 | 77.2 | 75.4 | 73.4 | 63.6 |
| DB1 | 107.9 | 105.7 | 89.6 | 88.3 | 77.4 | 75.7 | 73.4 | 63.2 |
| DB14 | 107.9 | 105.6 | 89.5 | 88.2 | 77.3 | 75.6 | 73.4 | 63.6 |
| DB15 | 107.6 | 105.3 | 89.2 | 87.9 | 77.0 | 75.3 | 73.1 | 63.4 |
| DB53 | 108.3 | 106.2 | 90.1 | 88.7 | 77.8 | 75.9 | 73.8 | 63.6 |

and DB53 products (DB1, DB14, DB15, and DB53 celluloses) are cellulose II.

From Figure 5, it is apparent that resonance lines of the solid-state $^{13}$C-NMR spectra of all the products appear as broad compared to that of MC. Moreover, the C4 and C6 lines of all the products are near 84.7 and 62.5 ppm, respectively, which are the corresponding chemical shifts of the noncrystalline component of MC. The chemical shifts of MC and products obtained from Figure 5 are listed in Table IV. No resonance lines of dye powder (not shown) overlap the lines of their corresponding dye product except only the downfield side of C1 lines of DB1, DB14, and DB15. This suggests that there is no free, that is, no unreacted dye, in the products. The downfield side of the C4 resonance lines of the DB1, DB14, DB15, and DB53 products has two small peaks near 88–90 ppm, but these are absent in the case of the FB and DR28 products. The uniplanar orientation of the former group has some similarities to that of cellulose II and is not as good as that of the latter group. As their regenerated cellulose is cellulose II, these small peaks indicate the presence of conceived cellulose II crystal in the products. The cellulose II crystal in the product might be developed during the rinsing process in the preparation of the sample and was affected by the planarity of the product.

However, there are discrepancies in the structure of the products between the X-ray measurement results and the solid-state $^{13}$C-NMR results. The former indicated that the product was a crystalline complex of a dye and cellulose, while the latter indicated that the cellulose component in the product was in a noncrystalline state. This discrepancy suggests that the mobility of the carbon of the cellulose component in the complex is almost the same as is the carbon in the noncrystalline region of microbial cellulose, because hydrogen bonding between the cellulose sheets is hindered due to the inclusion of a dye between the sheets in the complex. The spectrum of the cellulose component in each product sample is similar to each other, since a dye is included between the cellulose sheets.

The solid-state $^{13}$C-NMR spectra of the crystalline component of the standard celluloses and celluloses regenerated from the complex products are shown in Figures 6 and 7, and their corresponding chemical shifts are listed in Table V. The peak of the $^{13}$C-NMR spectra of all the dye-extracted cellulose become narrower and sharper. Moreover, the noncrystalline peaks that appeared at 84.7 and 62.5 ppm in the product are not observed in all the regenerated celluloses. The peak patterns and their chemical shifts of FB cellulose (Fig. 7) are very close to the $I_\beta$ structure of cotton cellulose (Fig. 6), suggesting that the FB cellulose has an $I_\beta$-rich crystal structure and this result strongly supports Kai et al.'s[9] results. Similarly, the peak patterns and the chemical shifts of the C4 and C6 resonance lines of DR28 cellulose indicate that the crystal structure of DR28 cellulose is the $IV_I$ type. The crystal structure of the rest of the dye celluloses is very similar to mercerized cellulose, that is, cellulose II. Although these four dye celluloses are cellulose II, their C1 resonance line differs from that of the standard mercerized cellulose II. The ratio of the strength of the downfield C1 resonance lines to that of the upfield side indicates the situation of hydrogen-bond forma-

tion.[18] The lower ratio suggests that less stable and less strong hydrogen bonds exist in the DB1, DB14, DB15, and DB53 celluloses than in standard cellulose II. Similarly, DB1 and DB53 celluloses have a little stronger hydrogen bond than those of the other two celluloses. This may be due to the position of sulfonate groups in the dyes. The sulfonate groups of DB1 and DB53 dyes are at the 5 and 7 positions in the naphthalene nucleus and that of DB14 and DB15 dyes are at the 3 and 6 positions.

## CONCLUSIONS

The product obtained from the *Acetobacter* culture in the presence of a direct dye is a crystalline complex containing a monomolecular layer of dye between the (110) planes of microbial cellulose. Celluloses $I_\beta$, $IV_I$, and II were regenerated from FB, DR28, and DB1, DB14, DB15, and DB53 complexes, respectively. The polymorphs seem to be the results of the interaction of sulfonate groups in a direct dye with noncrystalline nascent microbial cellulose, and its number and position affect the conformation of cellulose chains.

## REFERENCES

1. Gardner, K. H.; Blackwell, J. Biopolymers 1974, 13, 1975.
2. Sarko, A. Appl Polym Symp 1976, 28, 729.
3. Brown, R. M., Jr.; Haigler, C. H.; Suttie, J.; White, A. R.; Roberts, E.; Smith, C.; Itoh, T.; Cooper, K. Appl Polym Symp 1983, 37, 33.
4. Haigler, C. H.; Brown, R. M., Jr.; Benziman, M. Science 1980, 210, 903.
5. Haigler, C. H.; Chanzy, H. J Ultrastruct Mol Struct Res 1988, 98, 299.
6. Kai, A. Makromol Chem Rapid Commun 1984, 5, 653.
7. Kai, A. Makromol Chem Rapid Commun 1984, 5, 307.
8. Kai, A.; Mondal, Md. I. H. In Biomedical Functions and Biotechnology of Natural and Artificial Polymer; Yalpany, M., Ed.; ATL; Science: Shrewsbury 1996; p 77.
9. Kai, A.; Xu, P.; Horii, F.; Hu, S. Polymers 1994, 35, 75.
10. Cuisins, S. K.; Brown, R. M., Jr. Polymer 1995, 36, 3885.
11. Haigler, C. H.; White, A. R.; Brown, R. M., Jr.; Copper, K. M. J Cell Biol 1982, 94, 64.
12. Hestrin, S.; Schramm, M. Biomed J 1954, 58, 345.
13. Kai, A.; Mondal, Md. I. H. Int J Biol Macromol 1997, 20, 221.
14. Mondal, Md. I. H.; Kai, A. J Appl Polym Sci 1998, 71, 1007.
15. Kai, A.; Mondal, Md. I. H. Polym J 1998, 30, 398.
16 Torchia, D. A. J Magn Reson 1978, 30, 613.
17. Takai, M.; Tsuda, Y.; Hayashi, J.; Watanabe, S. Polym J 1975, 7, 157.
18. Horii, F.; Hirai, A.; Kitamaru, R. Polym Bull 1982, 8, 163.
19. Mondal, Md. I. H.; Kai, A. Polym J 1998, 30, 78.
20. Mondal, Md. I. H.; Kai, A. Polym J 1998, 30, 84.