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Spectral assignments and anisotropy data of cellulose I_α: ^{13}C-NMR chemical shift data of cellulose I_α determined by INADEQUATE and RAI techniques applied to uniformly ^{13}C-labeled bacterial celluloses of different *Gluconacetobacter xylinus* strains

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Solid-state ^{13}C-NMR spectroscopy was used to characterize native cellulose pellicles from two strains of *Gluconacetobacter xylinus* (ATCC 53582, ATCC 23769), which had been statically cultivated in Hestrin–Schramm (HS) medium containing fully ^{13}C-labeled β-D-glucose-U-$^{13}C_6$ as the sole source of carbon. For both samples, the ^{13}C-NMR chemical shifts were completely assigned for each ^{13}C-labeled site of cellulose I_α with the aid of 2D refocused INADEQUATE NMR. To determine the principal chemical shift tensor components, a pulse sequence based on the recoupling of anisotropy information (RAI) was applied at 10 kHz MAS. The detailed ^{13}C tensors of cellulose I_α from different bacterial celluloses are thus available now for the first time, and these results have been compared with previously published data of nonenriched material and with theoretical predictions.

Keywords: NMR; ^{13}C-NMR; bacterial cellulose; *Gluconacetobacter xylinus*; *Acetobacter xylinum*; recoupling of anisotropy information; RAI; INADEQUATE

Introduction

Solid-state NMR spectroscopy has been widely used to study the structure of cellulose from different sources such as algae, plants, and bacteria, and of its derivatives. Cellulose consists of 1–4 linked β-D-glucopyranose repeating units which form fibrous structures with regions of high crystallinity as well as amorphous parts. Elucidation of the crystal structure of native cellulose still remains one of the most challenging tasks in the field of cellulose research. Using cross-polarized/magic-angle spinning (CP/MAS) ^{13}C{^{1}H}-NMR on highly crystalline native cellulose I, VanderHart and Atalla[1] demonstrated the presence of two allomorphs, I_α and I_β. It was shown that the I_α/I_β ratio in native cellulose differs greatly from species to species. Cellulose I_α is the dominant form in algae and bacterial celluloses, while I_β is dominant in higher plants and animal celluloses.[2–5] The first ^{13}C-NMR resonance assignment was published for carbons C1, C4, and C6, and the cluster of signals between 70 and 80 ppm was attributed to C2, C3, and C5.[1] These conclusions were subsequently confirmed by solid-state INADEQUATE NMR.[6] Further details based on correlation spectroscopy were presented by Sakellariou *et al*.[7] and Cadars *et al*.,[8] and the three-dimensional cellulose structure and NMR investigations thereof were discussed by Sternberg *et al*.[9] and Witter *et al*.[10] The complete assignment of all carbon sites in the crystalline structure was finally possible using specifically ^{13}C-labeled D-glucose and D-glycerol for biosynthesis,[11,12] allowing to resolve the individual chemical shifts of C2, C3, and C5. Next, the structural assignment of all ^{13}C-NMR signals in the two different anhydroglucose rings were succeeded by solid-state INADEQUATE NMR of specifically prepared allomorphs I_α (purified *Cladophora*) and I_β (purified tunicate cellulose).[13] It was clearly demonstrated that both I_α and I_β phases contain two magnetically different D-glucose residues in the unit cells. Slightly different isotropic chemical shift values were found by Jaeger *et al*.,[14] who assigned all carbon sites in unpurified, uniformly ^{13}C-enriched bacterial cellulose (BC) of the strain *Gluconacetobacter xylinus* (previously

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referred to as *Acetobacter xylinum*) DSM 13 368 by RFDR and Double Quantum NMR techniques. More recently, Kono and Numata[15] studied the sequence of D-glucose residues A_1 and A_2 (originally referred to as A and A′, but the nomenclature has been adapted to the present work) within the same chain of cellulose I_α, and that of residues B and B′ in I_β, using RFDR experiments on uniformly ^{13}C-labeled bacterial cellulose samples. They concluded that the chains in cellulose I_α consist of $-A_1-A_2-$ repeating units, whereas cellulose I_β is composed of two independent $-B-B-$and $-B'-B'-$chains. This interpretation fully supports the crystallographic results on cellulose I_α and I_β, according to synchrotron X-ray and neutron diffraction data.[16,17] The structures are represented as a triclinic unit cell containing a single chain, and a monoclinic unit cell with two independent chains, respectively. This self-consistent model thus forms the basis of our present NMR analysis, aiming at a further structure refinement that is based on accurate ^{13}C chemical shift anisotropies.

Using nonenriched bacterial cellulose from *G. xylinus* DSM 14 666, the principal values of the ^{13}C chemical shift anisotropy (CSA) tensors in cellulose I have been determined by two complementary magic angle spinning NMR methods. Sideband separation was achieved using the 2D PASS experiment,[18] and alternatively the quasi-static powder patterns could be obtained from the recoupling of anisotropy information (RAI) experiment.[19] With regard to the technical possibilities, the 2D RAI sequence at 10 kHz MAS frequency proved to be the most suitable technique for cellulose. Both, 2D PASS and 2D RAI were also applied to nonenriched cellulose II[18,19] and revealed interesting results on the carbons of the glycosidic linkage. For all polymorphs it was found that C4 exhibited some of the biggest and almost identical tensor span values of about $\Omega(C4) = 44$ ppm for each carbon site of the 1–4 linked β-D-glucopyranose repeating units. Furthermore, C1 always possessed the smallest span values of about $\Omega(C1) = 28$ ppm, and particularly for this C1 position some drastic differences remained between the experimental results and theoretical calculations. The experimental values were generally much smaller than their theoretical predictions based on neutron diffraction crystal structures.[10] A crystal structure refinement utilizing NMR chemical shift constraints was finally able to yield a good agreement between the experimental and theoretical chemical shift tensor values. It turned out that the intrachain hydrogen bonds are responsible for the special glycosidic linkage. This effect also explains the characteristic isotropic chemical shift of C4 (90 ppm) that is observed in crystalline cellulose.[10]

In the present study, uniformly ^{13}C-labeled bacterial cellulose samples from two different strains of *G. xylinus* (ATCC 53 582 and ATCC 23 769) have been investigated. These strains were chosen because of their slightly different behavior in cellulose production. As described previously,[20] the bacterial motion due to the inverse force derived from the secretion of cellulose ribbons by the cell is influenced by the bacterial strain and by the kind of D-glucose used for the biosynthesis. The movement of a single *G. xylinus* ATCC 53 582 cell of about 10 µm length is decelerated by the ^{13}C-isotope to be only half the speed, while the movement of a single *G. xylinus* ATCC 23 769 cell of about 2 µm length is less influenced. Caused by their different sizes, *G. xylinus* ATCC 23 769 cells generally produce smaller cellulose microfibrils than *G. xylinus* ATCC 53 582 cells. Furthermore, differences in the morphological structures of BC pellicles produced by the two different *G. xylinus* strains occur.[21] To investigate the supramolecular structures of these bacterial celluloses, NMR experiments are indispensable. The corresponding ^{13}C isotropic chemical shift values of cellulose I_α could be assigned using 2D refocused INADEQUATE experiments. These results were then used to analyze several 2D iso-aniso spectra of the two samples to extract the respective chemical shift tensor values for each ^{13}C-labeled site for the first time. Accurate principal tensor components were obtained using an optimized version of the 2D RAI sequence.[10,22] Here, the influence of finite pulse length was taken into account, and proton decoupling with a ratio of $\omega_{1H}/\omega_{13C} \geq 2$ was achieved, which significantly improved the quality of the quasi-static powder patterns.

Experimental

Bacterial cultures

Two types of *G. xylinus* strains (NQ-5: ATCC 53 582 and AY-201: ATCC 23 769) were cultured in sterilized Hestrin–Schramm (HS) medium[23] at pH 6.0 using β-D-glucose-U-$^{13}C_6$ (^{13}C, 99%; Campro Scientific, Germany and Sigma-Aldrich Chemicals Co., USA). For cultivation, 200 µl of preparatory cultures of the respective *G. xylinus* strains grown with normal β-D-glucose (^{13}C, 1.1%) were inoculated per 8.3 ml of autoclaved HS media. The culture media were incubated statically at 30 °C for a period of 14 days, during which the prepolymer was not removed. After cultivation, the pellicles were washed with distilled water, treated with 0.1 N aqueous NaOH solution at 80 °C for 4 h, and washed again with running water to a neutral reaction of the rinsing agent. Finally, the pellicles were air dried (covered by aluminum foil) at 50 °C for 24 h. In the following, we will denote the samples as BC NQ-5 (**1**) from *G. xylinus* ATCC 53 582, and BC AY-201 (**2**) from *G. xylinus* ATCC 23 769. Further details about crystallinity and the morphological structure of these ^{13}C-labeled bacterial cellulose pellicles will be published elsewhere.[21]

NMR spectroscopy

1D CP/MAS ^{13}C{^{1}H}-NMR spectra were recorded on a Bruker AMX 400 MHz (AMX 400) spectrometer operating at 100.58 MHz for ^{13}C, with a 4-mm MAS double resonance probe and ZrO_2 rotors. The measurements were carried out at 6.5 kHz MAS. The cross polarization (CP) contact time was 1 ms; 64 scans were accumulated; and two-pulse phase modulation [TPPM: ±10°, 7 µs for BC DSM 13 368 and 5 µs for BC NQ-5 (**1**)/BC AY-201 (**2**), respectively] was applied for proton decoupling.

The 2D correlation experiments were carried out on Bruker Avance 500 MHz (Avance 500) and Bruker Avance 600 MHz (Avance 600) wide-bore spectrometers at room temperature (298 K), with a 4-mm MAS double resonance probe. The MAS frequency was set to 10 kHz for the 2D RAI experiments (Avance 500). The 2D refocused INADEQUATE measurements were carried out at 12.5 kHz for BC NQ-5 (**1**; Avance 500) and 10 kHz for BC AY-201 (**2**; Avance 600). Furthermore, a ramped rf field with 5 ms proton irradiation was used. During data acquisition a standard TPPM ^{1}H-decoupling scheme[24] was applied with a decoupling power of 50 kHz. For the 2D methods, quadrature phase detection was applied using the States method,[25] and $\pi/2$(^{13}C)-pulses of 2.4 µs and π(^{13}C)-pulses of 5 µs were used. The experiments were collected with respective numbers of scans of 256, 96, and 32. The indirect time dimension of the 2D experiments consisted of 128 data points with a dwell time of 80 µs for the 2D refocused INADEQUATE, and 64 points with a separation of 3 times the rotor period (300 µs) for the RAI powder pattern recoupling sequence.

The echo time of the 2D refocused INADEQUATE experiment was set to 3.04 ms (**1**) and 3.00 ms (**2**), respectively.

The recycle delay was set to 2 s for all experiments, and adamantane was used as an external reference having ^{13}C chemical shifts of 29.50 ± 0.10 ppm (CH) and 38.56 ± 0.10 ppm (CH_2) with respect to tetramethylsilane at 0.0 ppm.[26]

Results and Discussion

The ^{13}C chemical shifts of bacterial celluloses of different *G. xylinus* strains could be readily assigned by line shape analysis taking into account data from the literature. (e.g. Ref. [13]) The fact that the chains in cellulose I_α are constructed by $-A_1-A_2-\beta$-D-glucopyranose repeating units, while cellulose I_β is composed of two types of independent chains,[15] is fully consistent with the NMR data presented elsewhere by Hesse-Ertelt.[27] The investigated ^{13}C chemical shift data of nonenriched, never-dried bacterial cellulose pellicles from *G. xylinus* ATCC 53 582 (NQ-5) and *G. xylinus* ATCC 23 769 (AY-201) are comparatively given in Table 1.

For both samples, the cellulose I-type with a high content of I_α allomorph ($I_\alpha : I_\beta = 2.7 : 1$) could be proven. Amorphous cellulose parts were found near 83.9 ppm (C4 region), and between 62.3 and 61.5 ppm (C6 region) for the BC from *G. xylinus* ATCC 53 582, and near 84.0 ppm (C4 region) and between 62.5 and 61.4 ppm (C6 region) for the BC from *G. xylinus* ATCC 23 769.

It is noted that bacterial celluloses in the wet state, which have never been dried, exhibit ^{13}C-NMR spectra with much sharper and better resolved multicomponent lines than samples in the dried state; however, the drying procedure does not alter the isotropic values.[18] Thus, the previous assignments of the ^{13}C chemical shifts to the respective carbon atoms listed above could be used for further line shape analysis of the CP/MAS $^{13}C\{^1H\}$-NMR spectra of our air-dried cellulose pellicles from *G. xylinus* ATCC 53 582 and ATCC 23 769, which have been grown with β-D-glucose-U-$^{13}C_6$.

Figure 1 shows the CP/MAS $^{13}C\{^1H\}$-NMR spectra of ^{13}C-labeled bacterial celluloses from the different strains of *G. xylinus*. The samples are called BC DSM 13 368 (fat line), BC NQ-5 (**1**; thin line), and BC AY-201 (**2**; dots). BC DSM 13 368 represents another type of fully ^{13}C-enriched, natural bacterial cellulose that had been previously used for assignment[14] and for studies concerning the ^{13}C-enrichment and associated mechanisms.[27] Compared to NMR spectra of nonenriched material, the lineshape characteristics

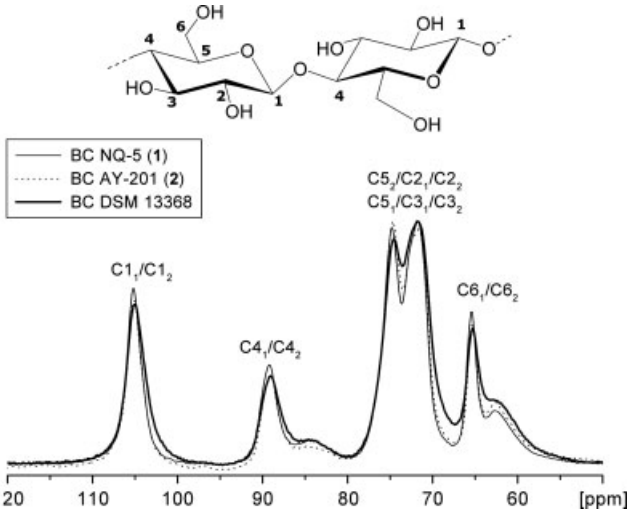


Figure 1. CP/MAS $^{13}C\{^1H\}$-NMR spectra (AMX 400, 4 mm, $\nu_R = 6.5$ kHz, ns = 64, $t_w = 2$ s, $t_{CP} = 1$ ms) of ^{13}C-enriched bacterial cellulose from *Gluconacetobacter xylinus* DSM 13 368 (fat line) and of highly crystalline, uniformly ^{13}C-labeled BC NQ-5 (**1**; thin line) and BC AY-201 (**2**; dots). The spectra are normalized to their maximum peak intensity.

and resolution of the CP/MAS $^{13}C\{^1H\}$-NMR spectra of the three fully ^{13}C-labeled samples in Fig. 1 are rather limited because of the strong homonuclear dipolar carbon–carbon couplings. Nevertheless, despite an inferior spectral resolution, lineshape analysis allowed the assignment of all ^{13}C chemical shifts of samples **1** and **2**, on the basis of the values of the nonenriched samples mentioned above.

Basically, it could be shown by Fig. 1 that the ^{13}C-labeled bacterial celluloses from *G. xylinus* ATCC 53 582 and ATCC 23 769 are of high crystallinity. The cellulose *I*-type with a high content of the I_α allomorph could be proven for samples **1** ($I_\alpha : I_\beta = 2.6 : 1$) and **2** ($I_\alpha : I_\beta = 2.5 : 1$). As for the nonenriched samples, the quantitative analysis of the C4 resonances of the crystalline (~90 ppm) and amorphous (~84 ppm) components indicated that only about one fourth of the ^{13}C-labeled material occurs in noncrystalline regions.[21,27] The data also show that the ^{13}C-enrichment of samples **1** and **2** was successful with about 94% yield, virtually uniformly at all carbon positions. In view of their rather well-resolved ^{13}C-NMR lines compared to BC DSM 13 368,

Table 1. Isotropic chemical shifts δ_{iso} [ppm] of nonenriched, never-dried bacterial cellulose pellicles from *G. xylinus* ATCC 53 582 (NQ-5) and *G. xylinus* ATCC 23 769 (AY-201)[27]

	Carbons of cellulose I_α of nonenriched, never-dried BC											
	G. xylinus ATCC 53 582 (NQ-5)						*G. xylinus* ATCC 23 769 (AY-201)					
	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
A_1	105.5	72.0	75.0	90.1	71.1	65.6	105.3	72.0	75.0	90.0	71.1	65.6
A_2	105.5	71.1	74.5	89.3	72.9	65.6	105.3	71.1	74.4	89.2	72.8	65.6
	Carbons of cellulose I_β of nonenriched, never-dried BC											
	G. xylinus ATCC 53 582 (NQ-5)						*G. xylinus* ATCC 23 769 (AY-201)					
	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
B	106.1	72.0	74.5	89.3	72.9	65.6	106.0	72.0	74.4	89.2	72.8	65.6
B′	104.4	72.0	75.9	88.5	72.0	65.6	104.3	72.0	75.9	87.8	72.0	65.6

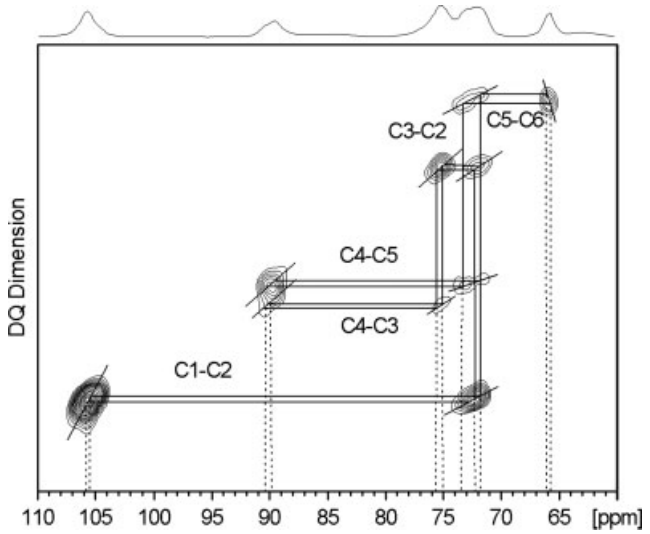


Figure 2. 2D refocused INADEQUATE spectrum for J(CC) = 83 Hz taken from Witter *et al.*[10] with slight modifications (Avance 500, 4 mm, ν_R = 12.5 kHz, ns = 256, t_w = 1 s, t_{CP} = 5 ms, TPPM: ±20°, 10 µs; echo time for J-couplings = 3.04 ms) of uniformly ^{13}C-labeled bacterial cellulose from *Gluconacetobacter xylinus* NQ-5 (**1**).

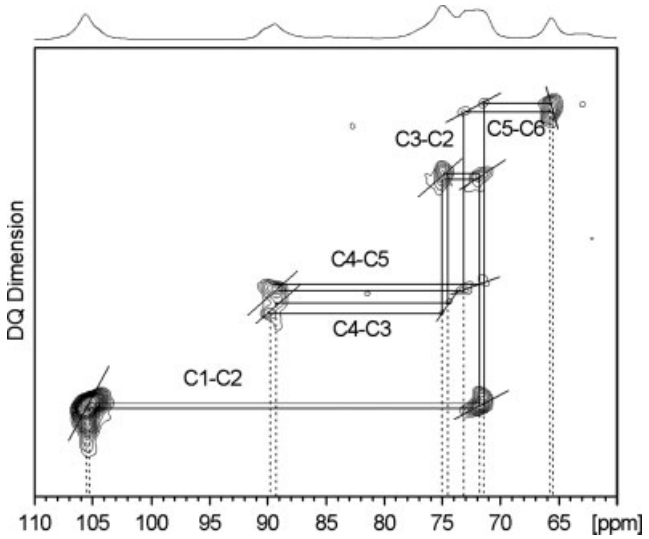


Figure 3. 2D refocused INADEQUATE spectrum for J(CC) = 83 Hz (Avance 600, 4 mm, ν_R = 10 kHz, ns = 96, t_w = 2 s, t_{CP} = 5 ms, cw-decoupling; echo time for J-couplings = 3.00 ms) of uniformly ^{13}C-labeled bacterial cellulose from *Gluconacetobacter xylinus* AY-201 (**2**).

samples **1** and **2** provide excellent structural characteristics for the analysis of crystalline properties. These uniformly ^{13}C-enriched samples are thus suitable to extract ^{13}C chemical shift data by correlation spectroscopy, which is fully representative of the cellulose I_α modification in a natural cellulose sample.

^{13}C chemical shift assignment of cellulose I_α by 2D refocused INADEQUATE experiments

The 2D refocused INADEQUATE experiments allow the resolution and structural assignment of all carbon signals to the two discrete anhydroglucose units of cellulose in either crystalline modification (I_α or I_β). INADEQUATE spectra display the single quantum ^{13}C chemical shift in the horizontal dimension, and the double quantum frequencies in the vertical dimension. The cross-peaks represent through-bond ^{13}C–^{13}C correlations between J-coupled carbons. The complete resonance assignments of all cellulose I_α signals of BC NQ-5 (**1**) and BC AY-201 (**2**) are shown in the 2D refocused INADEQUATE spectra of Figs 2 and 3, respectively.

The ring assignment for the crystalline allomorph I_α is straightforward for the two glucose units A_1 and A_2. Ability to detect the C-atom connections within each anhydroglucose unit now gives improved ^{13}C chemical shift data δ_{iso} for the dominant cellulose I_α modification of samples BC NQ-5 (**1**) and BC AY-201 (**2**). The illustration in Scheme 1 compares refined ^{13}C chemical shifts of samples BC NQ-5 (**1**) and BC AY-201 (**2**) with previously published data of celluloses from other sources.[13,14]

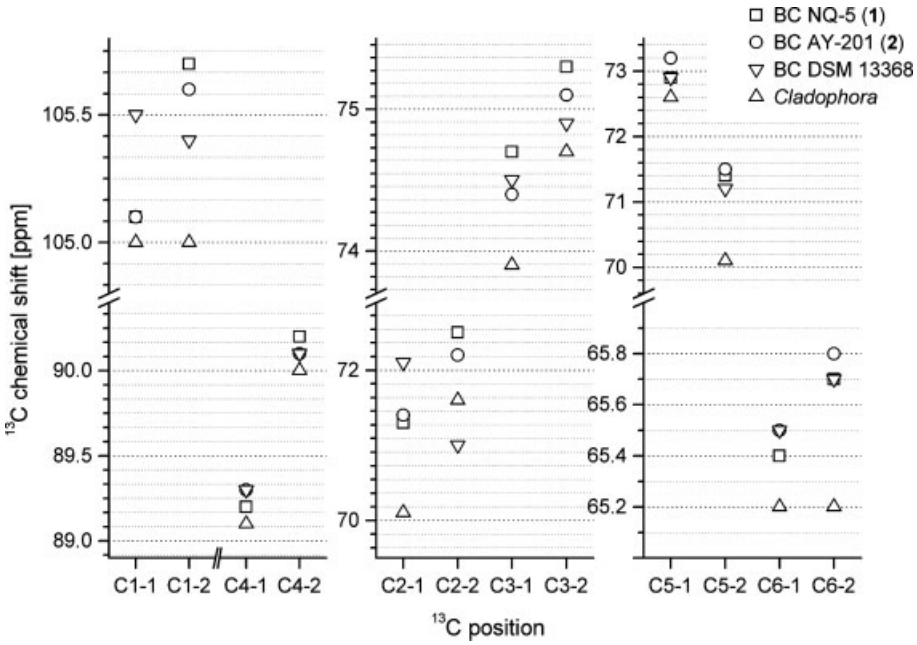
Scheme 1 clearly indicates that there are some significant differences in the ^{13}C chemical shifts of cellulose I_α from bacteria and *Cladophora* algae. There are even some differences between the values of uniformly ^{13}C-labeled bacterial celluloses **1** and **2** (grown here under the same conditions) compared to a ^{13}C-enriched sample from *G. xylinus* DSM 13 368 (biosynthesized under different conditions). It thus has to be concluded from the chemical shift data that the underlying supramolecular structure of the crystalline allomorph I_α is affected by the origin of the cellulose sample. In the special case of bacterial celluloses, it even appears to be dependent on the cultivation conditions and probably on the type of bacteria used for the biosynthesis as well.

^{13}C chemical shift anisotropy determination by 2D RAI experiments

To extract the full information about the principal chemical shift tensor components, an improved version of the recoupling of anisotropy information (RAI) experiment was used.[10,19,22] This sequence correlates the highly resolved isotropic chemical shift of each C-atom with the quasi-static powder pattern of the same site. The 2D RAI experiment can be applied at sufficiently high MAS frequencies to average out the homonuclear dipolar interactions between adjacent carbons in a uniformly ^{13}C-labeled sample. For cellulose, 10 kHz MAS turned out to be adequate. The heteronuclear dipolar couplings between ^{1}H and ^{13}C were suppressed by strong proton decoupling, for which the ^{1}H power was increased during the irradiation of ^{13}C pulses to satisfy the decoupling condition $\omega_{13C} \approx 2\omega_{1H}$, as outlined by Ishii *et al.*[28] The optimized RAI sequence is about a 4-pulse sequence with a separation of 3 times the rotor period avoiding the shearing of the 2D iso-aniso RAI spectra. Relevant parameters for the timings of the optimized RAI sequence are: $T_1^* = 0.148357 \cdot \tau$, and $T_2^* = 0.398357 \cdot \tau$, with a scaling factor of $\chi = -0.1256333$.

Figures 4 and 5 show the 2D iso-aniso RAI spectra of BC NQ-5 (**1**) and BC AY-201 (**2**). The isotropic ^{13}C chemical shifts are recorded with high resolution in the direct dimension, while the CSA powder patterns are recoupled in the indirect dimension.

The tensor values were extracted from the 2D iso-aniso RAI spectrum using the software DMfit,[29] applying the Haeberlen–Mehring–Spiess convention $|\delta_{33} - \delta_{iso}| \geq |\delta_{11} - \delta_{iso}| \geq |\delta_{22} - \delta_{iso}|$.[30,31] Critical examination reveals that the powder patterns are to some extent artificially broadened by the increasing number of recoupling pulses with increasing number of t_1 increments. However, this fact did not have any significant impact on the data extracted by fitting the powder patterns. The mean deviation of the chemical shift tensor values merely amounted 0.5 ppm.



Scheme 1. Comparison of the ^{13}C chemical shifts of different celluloses from bacteria (BC) and from *Cladophora* algae.

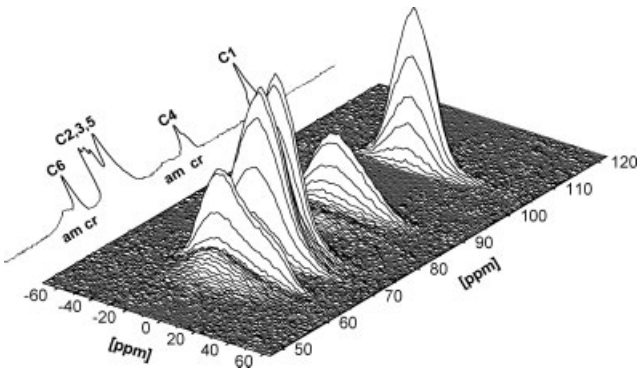


Figure 4. 2D iso-aniso RAI spectrum taken from Witter *et al.*[10] with slight modifications (Avance 500, 4 mm, $\nu_R = 10$ kHz, ns = 32, $t_w = 1$ s, $t_{CP} = 5$ ms, cw-decoupling; optimized 4-pulse sequence[19,22] without shearing, $T_1^* = 0.148357 \cdot \tau$, $T_2^* = 0.398357 \cdot \tau$, $\chi = -0.1256333$, with $\xi = 0$, $\tau = 3/2\ \tau_r$, 128 t_1-data points) of uniformly ^{13}C-labeled bacterial cellulose from *Gluconacetobacter xylinus* NQ-5 (**1**).

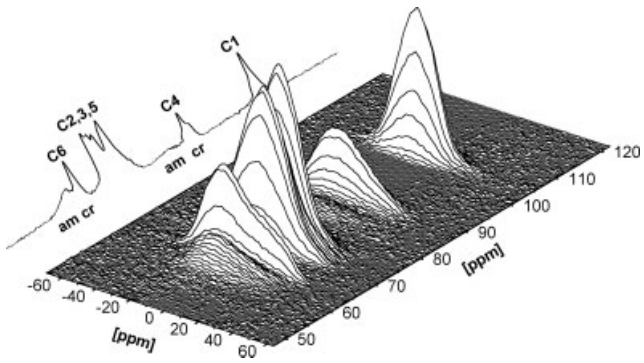


Figure 5. 2D iso-aniso RAI spectrum (Avance 500, 4 mm, $\nu_R = 10$ kHz, ns = 32, $t_w = 1$ s, $t_{CP} = 5$ ms, cw-decoupling; optimized 4-pulse sequence[19,22] without shearing, $T_1^* = 0.148357 \cdot \tau$, $T_2^* = 0.398357 \cdot \tau$, $\chi = -0.1256333$, with $\xi = 0$, $\tau = 3/2\ \tau_r$, 128 t_1-data points) of uniformly ^{13}C-labeled bacterial cellulose from *Gluconacetobacter xylinus* AY-201 (**2**).

For the accurate determination of the principal chemical shift tensor components, the data from the 2D refocused INADEQUATE experiments have been taken into account. The experimentally obtained values for the tensor span Ω, the skew κ, the tensor principal values, and the refined ^{13}C chemical shifts are given in Table 2.

Comparing the 2D iso-aniso RAI spectra of samples **1** and **2** (from *G. xylinus* ATCC 53 582 and ATCC 23 769, respectively), slight differences in the span values of C2, C3 and C5 are apparent, which differ up to 2 ppm. Nevertheless, no significant variation could be detected for the parameters Ω and κ of the several carbon positions. For both samples, C6 exhibits the biggest span values of about Ω(C6) = 52 ppm; C4 follows with about Ω(C4) = 44 ppm; and C1 always possesses the smallest values of about Ω(C1) = 30 ppm.

Further analysis shows that the results from samples **1** and **2** are in reasonably good agreement with the anisotropy data from the nonenriched bacterial cellulose of the strain *G. xylinus* DSM 14 666 determined by 2D PASS[18] and by a preliminary version of the 2D RAI sequence.[19] Previous span values obtained by RAI were on average smaller than the corresponding PASS data of cellulose I, with a maximum deviation of 10–20%. Using the optimized RAI sequence here, the span values now turn out to be similar to, and even marginally greater than the 2D PASS data of the nonenriched bacterial cellulose (BC DSM 14 666). Even though the ^{13}C-enrichment causes line broadening for the carbon signals of cellulose, which drastically affects the subtle resolution of the crystalline modifications, the influence of homonuclear dipolar carbon–carbon couplings in case of ^{13}C-labeled material is negligible for the chemical shift anisotropy parameters. As mentioned above, the powder patterns obtained by the optimized RAI pulse sequence are already to some extent artificially broadened. This fact, however, does not have any significant impact on the data extracted by the fitting procedure. Dipolar carbon–carbon couplings are about 7.6 kHz for bond

Table 2. Isotropic chemical shifts δ_{iso} [ppm], span values Ω [ppm], skew parameters κ, and tensor principal values $\delta_{11,\,22,\,33}$ [ppm] of BC NQ-5 (**1**) based on the measurements of Witter *et al.*[10] and BC AY-201 (**2**) determined in this work. Results were obtained using an optimized version of the RAI sequence.[19,22] The tensor principal values are listed according to $|\delta_{33} - \delta_{iso}| \geq |\delta_{11} - \delta_{iso}| \geq |\delta_{22} - \delta_{iso}|$

	Carbons of cellulose I_α of BC NQ-5 (**1**)											
	Anhydroglucose unit A_1						Anhydroglucose unit A_2					
	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
δ_{iso}	105.1	71.3	74.7	89.2	72.9	65.4	105.7	72.5	75.3	90.2	71.4	65.7
δ_{11}	90.3	88.5	91.3	109.2	91.1	87.6	90.9	89.7	91.4	110.6	88.6	87.9
δ_{22}	105.0	72.2	75.3	93.1	74.5	73.2	105.6	74.2	75.8	94.1	72.3	73.5
δ_{33}	120.0	53.2	57.5	65.3	53.1	35.4	120.6	53.6	58.7	65.9	53.3	35.7
Ω	29.7	35.3	33.8	43.9	38.0	52.2	29.7	36.0	32.8	44.7	35.3	52.2
κ	−0.02	0.08	0.05	0.27	0.13	0.45	−0.02	0.14	0.05	0.26	0.08	0.45
	Carbons of cellulose I_α of BC AY-201 (**2**)											
	Anhydroglucose unit A_1						Anhydroglucose unit A_2					
	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
δ_{iso}	105.1	71.4	74.4	89.3	73.2	65.5	105.6	72.2	75.1	90.1	71.5	65.8
δ_{11}	89.9	88.9	90.5	109.3	91.5	88.1	90.4	89.9	91.1	110.3	89.2	88.4
δ_{22}	105.0	72.1	75.1	93.3	74.9	73.4	105.5	73.7	75.6	94.5	73.0	73.7
δ_{33}	120.4	53.2	57.6	65.3	53.2	35.0	120.9	53.0	58.6	65.4	52.3	35.3
Ω	30.4	35.7	32.9	44.1	38.4	53.1	30.4	37.2	32.5	44.9	37.0	53.1
κ	−0.01	0.06	0.06	0.27	0.13	0.45	−0.01	0.13	0.05	0.30	0.13	0.45

lengths of 1 Å. In our studies, however, the bond lengths are even greater than 1.3 Å, whereby $^{13}C-^{13}C$ couplings of less than 3.5 kHz are expected, which can be averaged out at spinning speeds of 10 kHz. In the 1D CP/MAS $^{13}C\{^1H\}$-NMR spectra of ^{13}C-labeled bacterial celluloses received at 10 kHz MAS, no spinning sidebands were found, which meant that neither CSA nor carbon–carbon couplings sufficiently remained to be detectable. Nevertheless, the chemical shift anisotropy information of 2D RAI experiments at 10 kHz MAS is still available through the T_2 spin–spin relaxation (H–C) allowing the determination of the principal chemical shift tensor components. Finally, the revised values of cellulose obtained by an optimized version of the 2D RAI sequence conform well with the theoretical investigations.[10] The slight discrepancies found for bacterial celluloses from *G. xylinus* ATCC 53 582/ATCC 23 769 and BC DSM 14 666 can thus be attributed not only to the different cultivation conditions and the different bacteria used for the biosynthesis, but probably also to the different NMR techniques used for analysis in the past. Notably, these overall equivalent results could now be achieved by a drastically reduced measuring time using uniformly ^{13}C-enriched crystalline material in combination with an optimized RAI pulse sequence.

Conclusions

Refined ^{13}C-NMR chemical shift data of the dominant cellulose I_α modification were obtained using 2D refocused INADEQUATE experiments on uniformly ^{13}C-labeled bacterial cellulose from two different strains of *G. xylinus* (ATCC 53 582 and ATCC 23 769), referred to as BC NQ-5 (**1**) and BC AY-201 (**2**), respectively. Their respective isotropic ^{13}C chemical shifts exhibited only slight differences, but there were major discrepancies compared to the ^{13}C chemical shift data of bacterial cellulose from another *G. xylinus* strain (DSM 13 368) grown at different cultivation conditions. Apparently, the structure of the crystalline allomorph I_α does not only depend on its origin (algae or bacterial cellulose, etc.), but also on the cultivation conditions and possibly on the type of bacterium used for biosynthesis. These insights on the distinctive character of cellulose samples from different strains of *G. xylinus* have also been confirmed by analytical methods other than solid-state NMR and will be published elsewhere.[21]

Using 2D refocused INADEQUATE experiments on uniformly ^{13}C-labeled samples, accurate ^{13}C chemical shifts δ_{iso} were obtained for all carbons of the cellulose allomorph I_α in BC NQ-5 (**1**) and BC AY-201 (**2**). These values were taken into account when determining the principal chemical shift tensor components with the aid of an optimized 2D RAI experiment. Having optimized the RAI technique, our results are in good agreement with previously published 2D PASS data of nonenriched BC DSM 14 666.[18] Even though the span values of samples **1** und **2** are marginally greater than the 2D PASS data, in either case carbon C6 exhibits the largest span of about $\Omega(C6) = 52$ ppm. Previously reported results on the glycosidic linkage of the anhydroglucose units could also be confirmed here: C4 always exhibits second largest span of about $\Omega(C4) = 44$ ppm, while C1 has the minimum value of about $\Omega(C1) = 30$ ppm. Given the pronounced structural dependence of the chemical shift tensor components[10] it can, nevertheless, be concluded that both, BC NQ-5 (**1**) and BC AY-201 (**2**) biosynthesized under similar cultivation conditions, have a very similar crystal structure and intrachain hydrogen bond scheme. Slight differences compared to previously published data are attributed to different cultivation conditions, different bacterial strains, and different NMR techniques used for the analysis.

Overall, a drastic reduction in measurement time was achieved here compared to previous studies, and the ^{13}C-labeled bacterial celluloses were well characterized with regard to their cultivation process and structural properties.[20,21] The optimized RAI sequence thus proves to be the most appropriate method for de-

termining the full information on ^{13}C chemical shift anisotropies of cellulose in moderate experimental time.

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