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Preparation of Arabinoxylan and its Sorption on Bacterial Cellulose During Cultivation

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Summary: The sorption of arabinoxylan (AX) on bacterial cellulose was investigated by adding AX to the culture medium of *Gluconacetobacter xylinus*. The starting AX material was produced by alkaline extraction of oat spelts. To investigate the impact of varying AX quality, the residual lignin was reduced by ClO_2 bleaching. Furthermore, bleached and unbleached xylans were subjected to xylanase hydrolysis in order to produce fractions of varying molar mass. Of all samples only the water soluble fractions were used for sorption experiments. A reduced molar mass resulted in a lower sorption of AX to the cellulose, while the lignin content increased the sorption of AX on bacterial cellulose. The sorption of AX resulted in a reduction of bacterial cellulose crystallinity and cellulose Iα content. In combined treatments of AX with xyloglucan and β-glucan no synergistic effect of those polysaccharides on the AX sorption was found.

Keywords: arabinoxylan; bacterial cellulose; β-glucan; cellulose Iα/Iβ; crystallinity; molar mass; sorption; xyloglucan

Introduction

Bacterial cellulose (BC) is often used as a model system to elucidate influencing factors on the formation of cellulose.[1,2] The lack of lignin, hemicelluloses, pectin and extractives is a major advantage of bacterial cellulose for these purposes. In contrast to that cellulose in lignified plant cell walls is always contaminated by other components and modified by isolation procedures. BC formation is subdivided into several steps. Glucan chains are enzymatically synthesized from activated D-glucose at linear terminal complexes located in the bacterial plasma membrane. The chains extruded into the extracellular phase are immediately assembled into one elementary fibril per terminal complex. As a next step several elementary fibrils are combined to microfibrils, then associating to form bundles which subsequently configure the final bacterial cellulose ribbons.[3–7] Except for the biosynthesis of single chains, all further steps occur by self-assembly forming highly crystalline structures by intra- and intermolecular hydrogen bonds. Up to 30 min after extrusion, weak alkali suffices to transform the native cellulose I into technical cellulose II, while later on only strong alkali has this effect.[8] This behavior indicates that crystallite stabilization is not finished at the time of extrusion but can be influenced for a short-term period. The presence of water soluble polysaccharides in the culture medium can affect the structure and formation of BC as well. If these polysaccharides bind to cellulose, they can interfere with the assembly of microfibrils and bundles. Carboxymethylcellulose[4,9,10], glucomannan[6,9,11], xyloglucan (XG)[9,10,12], pectin[6] and

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xylan[6,9] influence nascent BC in different manner and extent. In general the sorption results in less ordered bacterial cellulose proven via electron microscopy, X-ray diffraction, FTIR- and solid-state NMR spectroscopy. Besides crystallinity, the ratio of cellulose I_α and cellulose I_β can be affected. The cellulose I_α content of BC is reduced when cultivation is accomplished in the presence of some polysaccharides.[6,13,14] Accordingly, some of the cellulose from BC/polysaccharide composites is more akin to plant cellulose concerning their crystalline characteristics.[9] Based on various model systems, the role of structural hemicelluloses in plant cell walls is redefined. Hemicelluloses are not only a link between cellulose and lignin but also a major factor for the formation of cellulose structure. In view of that, it can be expected that they influence the formation of lignin structures as well.[15,16] In the present study several arabinoxylan (AX) fractions varying in molar mass and lignin content were produced and added to the culture medium of Gluconacetobacter xylinus. The sorption effect of these AX fractions on the cellulose and their effect on the BC structure were investigated. A focal point was the importance of AX molar mass for these experiments. Since xylans from lignocellulosics plants are not available in a pure form, the effect of lignin as a major impurity was investigated as well in this study. For testing synergistic effects on sorption rates AX was also added in combination with xyloglucan or β-D-glucan to the cultivation medium. β-glucan consists of cellotriosy and cellotetrasyl units which are bound by β-(1,3) linkages. Xyloglucan is made up of a β-(1,4)-glucan backbone which is highly substituted with α-D-xylose. Some of the xylose units are further linked to β-D-galactose.[17]

Experimental

Xyloglucan (XG), β-Glucan (β-Gl) and Arabinoxylan (AX)

XG from tamarind seeds (Megazyme, LOT 00401) and β-Gl from oat (medium viscosity, Megazyme, LOT 60501) were purchased from Megazyme. AX samples were produced by a successive extraction and fractionation procedure (Figure 1). The starting material (AX_{raw}) was extracted from oat spelts with 5% (w/v) NaOH at 90 °C.[18] AX_{raw} was further purified by washing with methanol/water (60/40, v/v), methanol and ether. A part of the purified sample (AXMeOH) was bleached with 3% ClO_2 at 70 °C and 25% consistency for 3 h ($AXClO_2$). AXMeOH and $AXClO_2$ were both dissolved in water (concentration: 5%, w/v; 100 °C for 20 min, shaken over night at 20 °C) and centrifuged (20 min, 3500 rpm) to remove the insoluble fractions. The supernatants were freeze dried (Alpha 2-4 LSC, Christ), yielding the water soluble fractions AX1 (from AXMeOH) and AX2 (from $AXClO_2$).

AX1 and AX2 were fragmented by xylanase (78 nkat/g, Ecopulp TX-200C, Röhm Enzyme) at a concentration of 0.4% in ammonium acetate buffer (0.1 M, pH 6) at

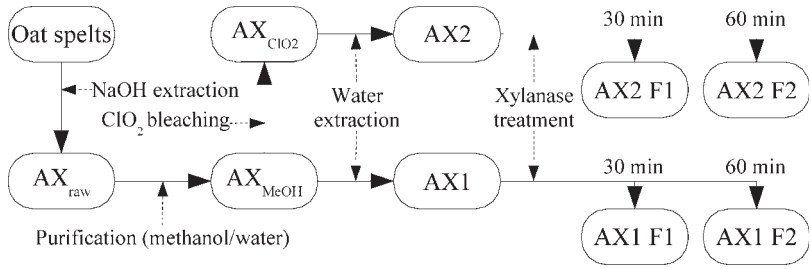

Figure 1.
Diagram of the extraction and fractionation steps of the oat spelt arabinoxylan also illustrating the abbreviations used for the different arabinoxylan fractions.

55 °C for 30 min (fraction F1) and 60 min (fraction F2). After enzyme denaturation (100 °C) and centrifugation (4400 rpm, 60 min) the supernatant was lyophilized.

Cultivation of Bacterial Cellulose (BC)

BC was produced by *Gluconacetobacter xylinus* (DSM 2004, ATCC 23768) in a static medium (pH 5, 28 °C) for 10–12 days in flat poly-carbonate boxes (26/15.3/4.5 cm).[19,20] The culture medium contained 5 g/l of tryptone, 5 g/l yeast extract, 5 g/l Na_2HPO_4, 1.15 g/l citric acid, 0.1 g/l cycloheximide, 20 g/l glucose and 1g/l hemicellulose except for the reference. Inoculating was performed with 1% bacteria from a pre-culture. As a pretreatment for the carbohydrate analysis residues from the culture medium were removed from BC mats by a washing procedure (distilled H_2O, 60 °C). Subsequently the samples were freeze dried. For FTIR analysis an additional intense purification was applied.[6] A part of each mat was treated with 0.25 M NaOH (100 °C, 6 h) followed by washing and incubation with 2.5 M HCl (100 °C, 4 h). Finally the samples were washed (distilled H_2O) and freeze dried after solvent exchange with ethanol and tert-butanol.

Monosaccharide Analysis

Individual AX fractions were hydrolyzed in a 1-step procedure applying 1% of sulphuric acid for 40 min at 120 °C. Monosaccharides were determined according to Puls *et al.* on MCI Gel CA08F in the borate form.[21,22] BC mats were ground in a ball mill (MM 2000, Retsch) and subjected to a 2-step hydrolysis: pre-hydrolysis: 72% H_2SO_4, 1 h, 20 °C; post-hydrolysis: 9.8% H_2SO_4, 2 h, 100 °C. Hydrolysates were analyzed by anionic exchange chromatography with pulsed amperometric detection (AEC-PAD) on CarboPac PA1 columns (analytical 4×250 mm and guard column 4×50 mm, Dionex).

^{1}H NMR Spectroscopy

30 mg arabinoxylan were dissolved in D_2O, freeze dried and re-dissolved in 1% NaOD. Acquisitions were performed at 80 °C on a Mercury spectrometer (400 MHz, Varian, CA) using a 5 mm probe, a pulse angle of 45° and a relaxation delay of 12 sec. Signal analysis was carried out according to Teleman and co-workers.[23]

Size Exclusion Chromatography (SEC)

Separation was performed on GRAM columns (10 μ; 30, 1000, 3000 Å, each 8×300 mm; Polymer Standard Service) using DMSO:H_2O (9:1) with addition of 0.05 M LiBr as eluent at 60 °C with flow rate of 0.4 ml min^{-1}.[24] Molar masses were determined from a viscosimetric (H502B, Viscotek) and refractive index detector (RI-71, Shodex) using a universal calibration with pullulan standards.[25]

Nitrogen Determination

The determination of nitrogen in bacterial cellulose was carried out with an N-Protein NA2000 unit (Fisons Instruments). Samples were weighed on a microbalance (M2P, Sartorius) and the system was calibrated with atropine ($C_{17}H_{23}NO_3$).

FTIR Spectroscopy

FTIR spectra were recorded on a Vector 33 spectrometer (Bruker) employing the KBr technique. 2–3 mg carefully defibrated freeze dried BC were blended with 350 mg pre-ground KBr without exerting pressure to prevent changes of crystalline properties. Baselines were corrected manually at 4000, 3800, 1895, 850 and 400 cm^{-1} (the last only at half intensity). The highest peak in the range from 2000 to 800 cm^{-1} was scaled to 1. Base lines, absorption intensities (A) and crystallinity indices were determined applying the methods of O'Conner *et al.*[26] ($A_{1429\ cm^{-1}}/A_{897\ cm^{-1}}$), Nelson *et al.*[27] ($A_{1372\ cm^{-1}}/A_{2900\ cm^{-1}}$) and Hulleman *et al.*[28] ($A_{1280\ cm^{-1}}/A_{1200\ cm^{-1}}$). For the last method the spectra were additionally deconvoluted (half bandwith = 20 cm^{-1}, peak-width narrowing factor = 1.5).[28] Cellulose I_α and I_β ratios were determined according to the method of Yamamoto *et al.* using $A_{750\ cm^{-1}}$ (A_α) for cellulose I_α and $A_{710\ cm^{-1}}$ (A_β) for cellulose I_β.[13] The

I_α content was calculated according to the equation suggested by Yamamoto: $I_\alpha = [2{,}55^* A_\alpha/(A_\alpha + A_\beta)] - 0{,}32$. The standard deviation (s) for most of the FTIR methods was determined by repeated measurements on the starting BC material: crystallinity index O'Conner: ±1.1%, crystallinity index Nelson: ±3.3%, cellulose I_α/I_β ratio: ±1.0%.

Results and Discussion

Preparation and Characterization of Water Soluble Arabinoxylans

Arabinoxylan obtained from alkaline extraction of oat spelts (AXraw) was subjected to various purification and fragmentation treatments in order to produce samples of varying lignin content and molar mass. The carbohydrate composition was determined after a mild 1-step hydrolysis. The resulting hydrolysis residue is considered as a measure for lignin content analogous to the Klason procedure.[29] This procedure is based on a complete acid hydrolysis of the polysaccharides and a gravimetrical determination of the insoluble lignin condensation products. Additionally the kappa number of samples was determined.[30] In this method the residual lignin in AX is oxidized and the required amount of $KMnO_4$ is measured as a more sensitive procedure for lignin determination. A purification of AXraw was performed by a successive washing in order to remove lignin impurities and low molar mass components. Both, the hydrolysis residue and the kappa number indicated a reduced lignin content of the resulting fraction (AXMeOH) while the carbohydrate composition of the material changed only slightly (Table 1). A part of the sample was subjected to a bleaching stage with ClO_2 ($AXClO_2$) leading to a further reduction of the lignin content corresponding to a Kappa number of 13.3 and a hydrolysis residue of 4.7%. The bleaching resulted as well in a change of the carbohydrate composition, respectively a lower arabinose and higher xylose content.

From both samples, AXMeOH and $AXClO_2$ the water soluble fractions were isolated by dissolution, centrifugation and lyophilization (Figure 1). The resulting fractions were obtained in yields of 33% (AX1) and 26% (AX2). Both samples had a significantly higher arabinose content compared to the previous fractions (Table 1), indicating an enrichment of polysaccharides with high arabinose substitution. It had been previously reported that substituents in AX improved water solubility and impeded aggregation.[17]

The amount of 4-*O*-methylglucuronic acid substituents (4-*O*-MeGlucA) was determined by ^{1}H-NMR. The ClO_2 bleaching had no significant effect on 4-*O*-MeGlucA, while higher amounts were found in the water soluble fractions AX1 and AX2. This indicated again that water soluble fractions exhibited a higher branching (Table 2). The amount of arabinose linked to the xylan backbone was deter-

Table 1.
Carbohydrate composition, hydrolysis residue and kappa number of arabinoxylan fractions.

Sample[a]	Lignin content		Carbohydrate composition[b]			
	Kappa-no.	Hydrolysis residue	Arabinose	Galactose	Glucose	Xylose
		% abs.	% rel.	% rel.	% rel.	% rel.
AXraw	48.9	10.7	13.3	3.5	1.0	82.0
AXMeOH	42.0	7.6	12.1	1.9	2.4	83.4
$AXClO_2$	13.3	4.7	8.6	2.0	2.7	86.6
AX1	—	5.4	18.0	5.4	2.8	73.6
AX2	—	4.9	16.1	7.7	5.1	71.1

[a] see Figure 1 for abbreviations
[b] determination after 1-step hydrolysis and borate anion exchange chromatography

Table 2.
4-*O*-methylglucuronic acid and arabinose substituents on the xylan backbone compared to the total arabinose content of the samples.

Sample[a]	4-*O*-MeGlucA[b]	Arabinose[b]	Arabinose[c]
	based on xylan backbone		total sample
	mol-%	mol-%	mol-%
AXMeOH	2.3	8.7	14.6
$AXClO_2$	2.4	7.2	10.0
AX1	5.3	12.8	24.5
AX2	8.2	11.4	22.7

a) see Figure 1 for abbreviations
b) determined by ^{1}H-NMR
c) determined by 1-step hydrolysis and borate anion exchange chromatography

mined by ^{1}H-NMR as well. These values were lower compared to the arabinose determined by hydrolysis and chromatography. Accordingly a part of the total arabinose was not incorporated into the arabinoxylan, an indication for significant impurities most likely due to arabinogalactan (Table 2). A detailed comparison of arabinose mol-% from NMR and hydrolysis gave further insight into the fractionation procedure. The ClO_2 bleaching reduced the arabinose content in AX by 17% while the total arabinose content was reduced by more than 30%. Therefore most of the degradation occurred in the postulated arabinogalactan impurity. The water soluble fractions AX1 and AX2 on the other hand show a higher increase of arabinose in the hydrolysis results. Accordingly, it can be still stated that the water soluble arabinoxylan fraction has a higher proportion of arabinose substituents. Nevertheless, the higher results for arabinose determined by hydrolysis indicate an enrichment of the proposed arabinogalactan impurity in the water soluble fractions.

Xylanase Degradation of Water Soluble Arabinoxylans

Various approaches have been attempted in order to produce arabinoxylans of varying molar mass. An ultrafiltration treatment failed due to a clogging of membranes in the first pre-filtration steps. A fractionated precipitation resulted into fraction with varying molar masses from 19,000 to 24,000 g/mol (Mp in peak maximum). Nevertheless, the chemical characterization revealed that the major differences existed in the substitution pattern of the various fractions obtained.[31] Finally an enzymatic degradation of the water soluble fractions AX1 and AX2 with xylanase was performed. The resulting size exclusion chromatography (SEC) elution profiles were somehow broad but showed a clear shift to lower elution volume (Figure 2). For the starting materials AX1 and AX2 it became

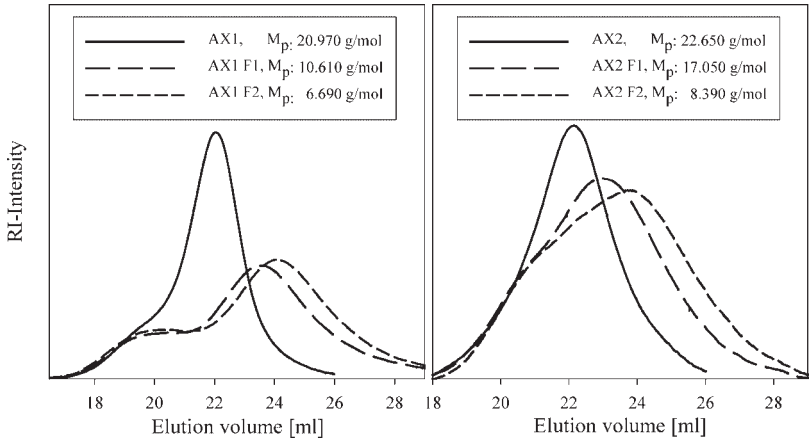

Figure 2.
SEC elution profiles of the water soluble fractions of unbleached (AX1) and bleached (AX2) arabinoxylan before and after xylanase hydrolysis (F1 30 min; F2 60 min).

apparent that AX1 had a more pronounced high molar mass shoulder in the range from 18 to 20 ml. This material could not be degraded by the xylanase, indicating a different chemical structure. It could be assumed that this fraction represented the impurity postulated from the carbohydrate analysis data. This impurity was less pronounced in the fraction AX2 supporting the previous conclusion that the impurity was preferably degraded during the ClO_2 bleaching. Due to the broad molar mass distribution, the degradation intensity was best characterized by the change of molar mass in the peak maximum (Mp). For the unbleached sample AX1, the Mp was reduced from 20,970 g/mol to 10,610 g/mol for AX1 F1 and to 6,690 g/mol for AX1 F2.

For the bleached AX2 sample the corresponding Mp values were 22,650 g/mol for AX2, 17,050 g/mol for AX2 F1 and 8,390 g/mol for AX2 F2. It was somewhat surprising that Mp values were slightly higher for the fractions produced from the bleached sample. This might be at least partly due to the different yields in the generation of the water soluble fractions. As an outcome of this fractionation and degradation scheme six water soluble AX samples with varying lignin content and molar masses were obtained. The carbohydrate composition of these samples was determined by a 2-step hydrolysis which was more intense compared to the protocol applied previously. This procedure was chosen since a carbohydrate essay was needed for sorption experiments which could degrade not only AX but also bacterial cellulose (BC), xyloglucan (XG) and β-Glucan (β-Gl). This could only be achieved with a procedure using concentrated acid (72% H_2SO_4) in the first step (Table 3).

Applying the 2-step hydrolysis procedure to AX1 and AX2 a slightly lower arabinose content and a higher proportion of galactose, glucose and xylose was determined compared to the 1-step hydrolysis (Table 1). Within each fractionation series (AX1 → AX1 F1 → AX1 F2 and AX2 → AX2 F1 → AX2 F2) no significant differences in the carbohydrate composition were found. Therefore it could be stated that within each series the samples only varied with regard to their molar mass and not regarding their substitution pattern or the level of impurities. The XG sample consisted not only of xylose and glucose but also of galactose and arabinose. This complicated the calculation of sorption rates for mixed experiments with AX and XG. For the β-Gl only glucose could be detected.

Sorption of Arabinoxylan on Cellulose During the Cultivation of *Gluconacetobacter Xylinus*

The various AX fractions were added to the culture medium of *Gluconacetobacter xyli-*

Table 3.
Carbohydrate composition of water soluble arabinoxylan fractions, xyloglucan, and β-glucan as determined by a 2-step hydrolysis procedure and AEC-PAD chromatography.

Sample[a)]	Xylanase hydrolysis	Carbohydrate composition			
	min	Arabinose	Galactose	Glucose	Xylose
		% rel.	% rel.	% rel.	% rel.
AX1	—	15.5	6.1	3.7	74.8
AX1 F1	30	16.3	6.3	3.6	73.9
AX1 F2	60	16.1	7.5	4.8	71.6
AX2	—	14.5	7.7	5.5	72.5
AX2 F1	30	15.2	8.2	5.9	70.8
AX2 F2	60	15.9	7.2	4.9	72.1
XG	—	1.6	16.8	50.8	30.8
β-Gl	—	—	—	100.0	—

[a)] see Figure 1 for abbreviations

nus in order to investigate the sorption of polysaccharides to the BC. Besides the AX fractions XG and β-Gl were included into this study. To determine the sorption of polysaccharides, only a gentle washing of the BC mats was performed in order to remove the constituents of the culture medium. Afterwards the material was freeze dried and hydrolyzed followed by carbohydrate analysis. A direct extraction of hemicelluloses from the BC mats was not pursued because even with alkali a complete recovery of hemicelluloses can not be achieved.[11] However, with the procedure applied in this study only hydrolysis yields in the magnitude of 40–50% could be achieved. The nitrogen contents of samples indicated that likewise 40–50% of proteins were contained in the samples. This indicates that the gentle washing procedure could remove the low molar mass components of the culture medium but not bacterial residues which are the source of the protein contamination. The combined yield of hydrolysis and proteins amounted to ~90% for all samples indicating a complete hydrolysis of the polysaccharides. The normalized carbohydrate data showed 99.9 % of glucose for the BC reference which confirmed that no carbohydrate impurities originated from the bacteria which would interfere with the determination of AX or XG (Tabel 4). For all experiments performed with AX samples especially an increased amount of xylose, arabinose and galactose was found. XG resulted in an increase of xylose and galactose (Table 4). The polysaccharide sorption rates were now calculated from the normalized carbohydrate data under consideration of the composition determined for the various starting materials.

In order to calculate the sorption of AX1 and AX2 fractions onto BC, the xylose percentage from Table 4 and the arabinose/xylose ratio from the ^{1}H-NMR measurements (Table 2) were used. This procedure enabled a good estimation of the AX sorption without interference from the impurities. The situation became more complicated for the combined treatments

Table 4.
Normalized carbohydrate composition of bacterial cellulose (BC) mats produced in the presence of various polysaccharide fractions.

Sample[a)]	Carbohydrate composition			
	Arabinose	Galactose	Glucose	Xylose
	% rel.	% rel.	% rel.	% rel.
BC	0.0	0.1	99.9	0.0
BC/AX1	2.1	0.9	83.2	13.8
BC/AX2	2.4	0.9	85.4	11.3
BC/AX1 F1	1.1	0.7	91.1	7.1
BC/AX1 F2	1.1	0.9	91.8	6.2
BC/AX2 F1	1.1	1.1	90.7	7.2
BC/AX2 F2	0.7	1.0	92.7	5.6
BC/XG	0.0	2.3	94.5	3.2
BC/XG/AX1	2.0	2.3	82.4	13.3
BC/XG/AX2	1.7	2.2	84.0	12.1
BC/β-Gl	0.0	0.1	99.9	0.0
BC/β-Gl/AX1	1.5	0.9	86.0	11.5
BC/β-Gl/AX2	1.1	0.9	86.9	11.1

[a)] see Figure 1 for abbreviations

with the two other polysaccharides. The β-Gl consisted only of glucose and could not be differentiated from the cellulose. Therefore AX sorption could only be calculated based on the total glucan (BC plus β-Gl) and not solely on its BC component. For the XG even more assumptions had to be made in order to calculate an estimate of the AX sorption. For all experiments incorporating AX fractions and BC, the galactose values were rather stable between 0.7 and 1.0%. As discussed before the galactose in AX fractions must result from impurities, most likely an arabinogalactan component. All BC mats cultivated in the presence of XG had a higher galactose content of 2.2 to 2.3% due to the galactose within the XG polymer. It was assumed that for combined treatments 0.7% galactose was due to the AX fraction and the remaining galactose due the XG fraction. Subsequently a sorption rate of XG was calculated from the galactose/xylose ratio in Table 3. Considering that the lowest galactose value for impurities from BC/AX experiments was applied, the calculated XG sorption should represent a rather high level. The remaining xylose percentage was used now for the calculation of AX sorption and should represent a minimum value which

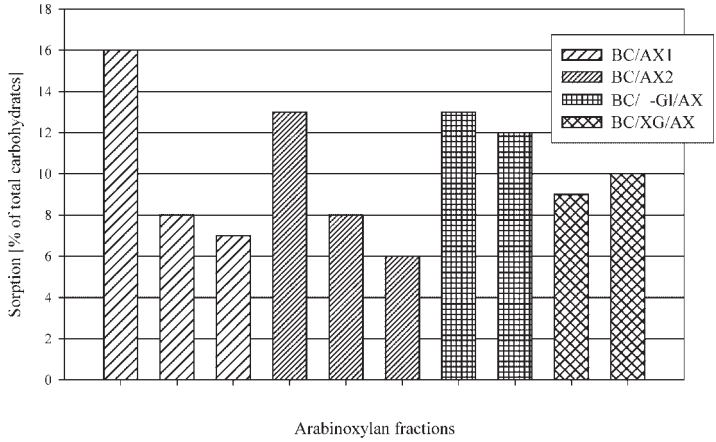

Figure 3.
Polysaccharide sorption rates onto BC. For experiments with AX fractions an accurate calculation was performed, while data for combined treatments with β-Glucan (β-Gl) and xyloglucan (XG) estimate a minimum level of the sorption rate.

might be exceeded by the real sorption level.

From the experiments with the various AX fractions it became apparent that the unbleached AX1 sample had a higher sorption rate compared to the bleached AX2 (Figure 3). It could be possible that the higher lignin content favored the aggregation of AX and consequently the retention of larger particles within the BC mat. An increased sorption of xylan aggregates to cellulose was reported previously.[32] Further, it became evident that enzymatic degraded fractions F1 and F2 were always adsorbed to a far lower content by comparison to the starting materials AX1 and AX2. Apparently high molar masses were beneficial for the sorption of AX onto BC. For the combined treatments with β-Gl a lower sorption rate of AX1 and AX2 occurred. As discussed before this value could not be related exclusively to the BC component of the fiber mat. Nevertheless, it is at least unlikely that a strong synergistic effect existed between β-Gl und AX fractions.

The sorption rate determined for XG without AX fractions was about 10% and rather low compared to literature data of 35% and 47%.[10,11] These differences might result from variations in solubility, molar mass or substitution. For combined treatments with XG and AX the estimated sorption rates were lower compared to the single experiments. Considering the assumptions made, these calculations can not be used as proof for a reduction of AX sorption by XG. Nevertheless, it is on the other hand unlikely that a positive synergistic effect existed between AX fractions and the XG polymer

Effect of Polysaccharide Sorption on the Structure of Cellulose

The changes of cellulose structure resulting from polysaccharides in the cultivation medium were investigated by FTIR spectroscopy. This method could not provide absolute information but a qualitative comparison between the various samples. For these investigations it was crucial to further purify the cellulose mats in order to remove all impurities from the bacteria. A purification procedure from Tokoh was applied, which enables the determination of cellulose I_α and I_β ratios.[6,7] The crystallinity index was determined as well. However, the applied purification using HCl treatments at 100 °C resulted in a hydrolysis of amorphous cellulose regions. From viscosimetric determinations in cupriethylenediamine the degree of polymerisation was calculated for the various samples. The results varied between 400 and 500 indicat-

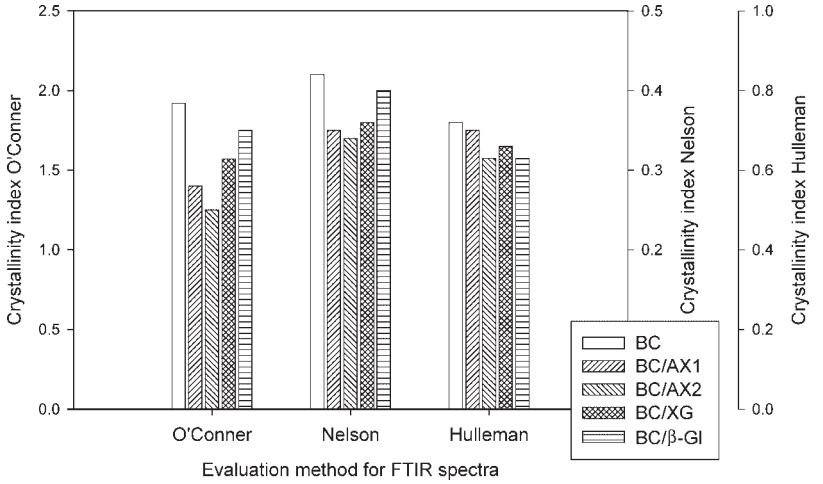

Figure 4.
Crystallinity index of cellulose from various BC/polysaccharide mats as determined by FTIR spectroscopy according to the methods of O'Conner (A_{1429}/A_{897}), Nelson (A_{1372}/A_{2900}), and Hulleman (A_{1280}/A_{1200}).[26–28] (see Figure 1 for abbreviations).

ing a severe hydrolysis of cellulose chains.[31] Therefore the information on crystallinity should be considered with caution. In future studies a milder purification with sodium dodecyl sulfate solutions should be applied in order to avoid hydrolytic degradation.[33]

The evaluation of crystallinity was performed by various methods which all indicated a reduction of BC crystallinity as a result of AX sorption (Figure 4). This reduction was more pronounced for the data obtained by the methods of Nelson and O'Conner compared to the procedure of Hulleman.[26–28] In experiments applying XG or β-Gl a reduction of crystallinity occurred as well. However, at least for the determination method according to Nelson and O'Conner this reduction was less pronounced than for experiments with AX fractions. The bleached AX2 sample had always a higher effect compared to the unbleached AX1 sample. This was somehow surprising, since AX1 showed the higher sorption rates. It has already been discussed that the higher sorption of AX1 might result from a more intense aggregation caused by the higher lignin content. It could be postulated that AX2 was better integrated into cellulose microfibrils and bundles, exhibiting a larger effect on crystallinity, even at lower sorption rates. The effect of the lower molar mass fractions F1 and F2 on the crystallinity of BC was not conclusive, since results from the various determination methods were somehow contradicting (data not shown).

The ratio of cellulose I_α was significantly reduced for BC cultivated in the presence of the AX of high molar mass (Figure 5). Again it can not be excluded that the intense purification procedure might effect the magnitude of the values determined here. Various authors have stated that Cellulose I_α was easier degraded compared to cellulose I_β by both, enzymatic and acid hydrolysis procedures.[34,35] However, considering that within this study all samples have been submitted to the same experimental procedures a comparrsion between the various samples should be legitimate. In accordance with the crystallinity index, the effect of the unbleached AX1 was slightly lower compared to the bleached AX2 sample. This might again support the assumption that lower lignin contents favored the integration of polysaccharides within the cellulose microfibrils and bundles. Again the results on fractions with reduced molar mass were not univocal.

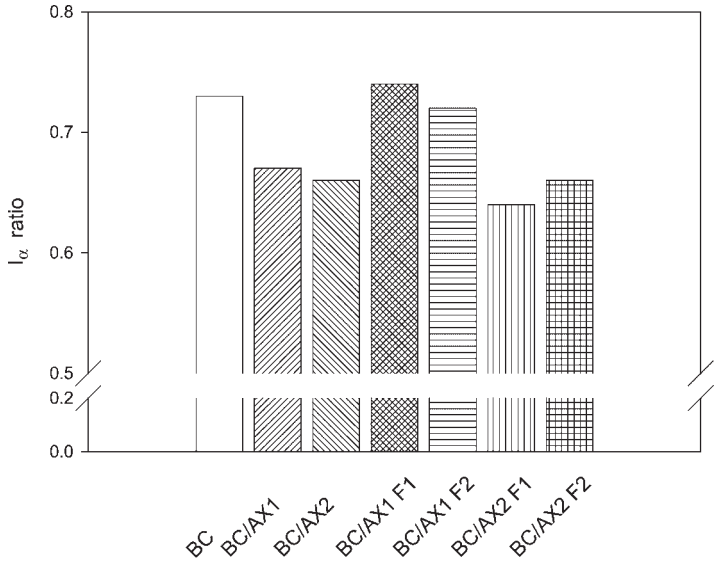

Figure 5.
Ratio of cellulose Iα in various BC polysaccharide mats as determined by FTIR spectroscopy according to the methods of Tokoh.[6,7]

Enzymatic degraded fractions from unbleached AX1 (AX1 F1, AX1 F2) hardly had any effect on the cellulose I_α proportion compared to the reference BC sample. The fraction AX2 F1 and AX2 F2 obtained from the bleached sample had a similar effect as the corresponding high molar mass sample AX2. In general the magnitude of these results was in accordance with the data of Tokoh obtained after sorption of 4-*O*-methylglucuronoxylan from birch.[6] The author determined cellulose I_α values of 0.73 for the reference BC and 0.62 for BC cultivated in the presence of birch xylan.

Conclusion

A method for the production of water soluble AX fractions with varying molar masses and lignin content was developed. This enabled for the first time to investigate the effect of these two characteristics on the sorption of AX onto BC. In cultivation experiments of BC with the different AX fractions it became apparent that higher molar masses of AX increased the sorption rate significantly.

A higher lignin content resulted as well in a higher sorption rate of AX to the BC mat. Further, BC cultivation was performed combining AX fractions with XG and β-Gl. Here the determination of sorption rates was less accurate. For experiments with β-Gl no differentiation between BC and β-Gl could be made from the carbohydrate analysis. For combined treatment with β-Gl and AX the differentiation of theses components was based on assumptions, permitting only an estimated minimum sorption rate. Nevertheless, the results gave a strong indication that no positive synergistic effect existed between those polysaccharides regarding their sorption on BC. Crystallinity and cellulose I_α/I_β ratio were qualitatively investigated by FTIR spectroscopy. A sample purification was applied, which somehow obscure the crystallinity data due to the hydrolysis of amorphous cellulose regions. Nevertheless, the comparison of the various samples indicated that unbleached and bleached AX (AX1, AX2), XG and β-Gl all reduced the crystallinity of the BC formed during cultivation. This tendency was already stated previously for various polysacchrides.[6,9,10]

For two of the three evaluation methods applied to the FTIR spectra the effect of AX was more pronounced compared to XG and β-Gl. Surprisingly the bleached sample AX2 caused a higher reduction of crystallinity, although the sorption rates were higher for the unbleached sample (AX1). Both AX fractions resulted as well in a reduction of the cellulose I_α proportion within the BC. The cellulose I_α values for the BC reference, BC/AX1 and BC/AX2 resemble to literature.[6] Again the findings indicate a higher effect for the bleached AX2 sample. Based on these results it might be hypothesized that lignin impurities could favor aggregation and higher sorption rates, which might be preferential on outer surfaces of microfibrils and bundles. On the other hand a lower lignin content might improve incorporation into the structure of BC, due to lower aggregation. Therefore AX2 could exhibit a stronger effect on crystallinity and cellulose I_α/I_β ratio. Additionally the present study demonstrated that the sorption of xylans is strongly dependent on their structure, molar mass and level of impurities. The developed procedure for the production of bleached, water soluble arabinoxylan might be a suitable model for future studies.

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