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Degradation of Wheat Straw by *Fibrobacter succinogenes* S85: a Liquid- and Solid-State Nuclear Magnetic Resonance Study

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Wheat straw degradation by *Fibrobacter succinogenes* was monitored by nuclear magnetic resonance (NMR) spectroscopy and chemolytic methods to investigate the activity of an entire fibrolytic system on an intact complex substrate. In situ solid-state NMR with ^{13}C cross-polarization magic angle spinning was used to monitor the modification of the composition and structure of lignocellulosic fibers (of ^{13}C-enriched wheat straw) during the growth of bacteria on this substrate. There was no preferential degradation either of amorphous regions of cellulose versus crystalline regions or of cellulose versus hemicelluloses in wheat straw. This suggests either a simultaneous degradation of the amorphous and crystalline parts of cellulose and of cellulose and hemicelluloses by the enzymes or degradation at the surface at a molecular scale that cannot be detected by NMR. Liquid-state two-dimensional NMR experiments and chemolytic methods were used to analyze in detail the various sugars released into the culture medium. An integration of NMR signals enabled the quantification of oligosaccharides produced from wheat straw at various times of culture and showed the sequential activities of some of the fibrolytic enzymes of *F. succinogenes* S85 on wheat straw. In particular, acetylxylan esterase appeared to be more active than arabinofuranosidase, which was more active than α-glucuronidase. Finally, cellodextrins did not accumulate to a great extent in the culture medium.

Fibrobacter succinogenes is a major rumen fibrolytic bacterium that is found in large numbers when ruminants are fed a poor diet (10). In vitro, *F. succinogenes* S85 digested more cellulose from intact forages than did several strains of *Ruminococcus albus* and *Ruminococcus flavefaciens*, the two other predominant cellulolytic bacterial species in the rumen (14). *F. succinogenes* also demonstrated a qualitative advantage for degrading hemicellulose compared to *R. flavefaciens* (13). *F. succinogenes* does indeed have a strong ability to solubilize xylans (12, 38, 39), although it is not able to use xylose (30, 34). The enzymatic system of *F. succinogenes* S85 has been extensively studied by molecular and biochemical approaches, and many different cellulases have been identified, as well as xylanases, ferulic acid, acetylxylan esterase, α-arabinofuranosidase, and α-glucuronidase (18). Although many of these enzymes have been characterized, little is known about their concurrent modes of action on solid substrates. In addition, our group has extensively studied the metabolism of *F. succinogenes* S85 (5, 20, 29-33), and in particular, it has been shown that no cellodextrins accumulated in the extracellular medium of resting cells that were incubated with glucose (35).

For the present work, the degradation and metabolism of wheat straw by *F. succinogenes* cells growing on this substrate were studied by the use of nuclear magnetic resonance (NMR). Cellulose, the sole substrate for *F. succinogenes* among the polysaccharides of the plant cell wall, is embedded in a matrix of hemicelluloses and lignin. Lignin and lignin-carbohydrate complexes in cell walls have been shown to limit the access of enzymes and microbes to their substrates (3, 46). Microscopic examinations of the cell walls of Graminaceae undergoing microbial attack showed that some wall types and some parts of individual walls are more susceptible to attack than others (12). Thus, a kinetic analysis of the degraded polysaccharides and solubilized sugars should help to define the compounds or linkages that limit the degradation process.

The challenge of this work was to study both a complex substrate (natural fibers) and a complex enzymatic system (whole cells). Most of the results reported in the literature deal with isolated enzymes and/or model substrates (such as carboxymethylcellulose) or purified compounds (xylans, cellulose, etc.). Therefore, to assess the mode of action of *F. succinogenes* fibrolytic enzymes on wheat straw, we chose specific tools adapted for direct in situ investigations. In situ solid-state NMR with ^{13}C cross-polarization magic angle spinning (^{13}C CP-MAS), which has been proven to be a suitable technique for monitoring the degradation of wood and cell walls (19, 21), was used to monitor the action of the *F. succinogenes* S85 fibrolytic system on lignocellulosic fibres (^{13}C-enriched wheat straw). Liquid-state two-dimensional (2D) NMR experiments and chemolytic methods were used to analyze in detail the various released sugars.

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MATERIALS AND METHODS

***F. succinogenes* S85 growth on a solid substrate.** *F. succinogenes* S85 cells (ATCC 19169) were grown at 38°C on 10 ml of mineral medium (20) with

unlabeled or ^{13}C-labeled wheat straw (10 mg/ml; 10% total ^{13}C enrichment). Wheat straw was ground into fine particles (<500-μm diameter) in a blender before incubation. Cell cultures (in triplicate) were harvested after 8, 16, 24, 48, 56, 72, and 96 h of growth. The extracellular medium was separated from cells and the solid substrate by centrifugation (15 min at 20,000 × *g*) before analysis. Both supernatants and pellets were freeze-dried and analyzed by chemolytic methods as well as by 2D liquid-state NMR and solid-state ^{13}C CP-MAS NMR spectroscopy, respectively.

NMR experiments. **(i) Solid-state NMR.** For solid-state measurements, 50 mg of freeze-dried ^{13}C-enriched wheat straw (with or without cells) was mixed with 10 mg of polypropylene and 50 μl of water. The 4-mm-long ZrO_2 rotors were filled with these mixtures. High-resolution solid-state ^{13}C CP-MAS NMR spectra were measured in a commercial Bruker double-bearing probe on a Bruker Avance DSX spectrometer operating at 75.46 MHz. The acquisition of 2,000 scans for each sample was performed at 10 kHz at room temperature by use of a variable amplitude cross-polarization sequence, a standard pulse program of the Bruker library, with a 3.3-μs proton 90° pulse, 1-ms contact time, and 5-s relaxation delay. Chemical shifts were referenced to an external standard glycine (δ 176.03 ppm).

(ii) Liquid-state NMR. After pellet separation, the pHs of cell-free supernatants were corrected to 7.40 and the supernatants were freeze-dried two times with D_2O. Samples were further dissolved in a mixture of 470 μl of 99.98% D_2O, with 20 μl of 10 mM sodium 3-(trimethylsilyl) propionate (TSP-d_4) (δ 0.0) and 10 μl of 50 mM 1-*O*-methyl-β-D-xylopyranose (δ 4.331/104.79) used as standards. Samples were subjected to liquid-state NMR measurements in 5-mm TXI inverse probes (^1H, ^{13}C, and ^{15}N) on a Bruker Avance DSX spectrometer operating at 300 and 500 MHz, with z-gradients at 27°C. The following techniques were used for the assignment of NMR signals: two-dimensional gradient-enhanced proton-homonuclear shift correlation spectroscopy (ge-COSY), one-dimensional transient gradient-enhanced nuclear Overhauser effect spectroscopy (44), one-dimensional gradient-enhanced total correlation spectroscopy, gradient-enhanced single quantum coherence ^1H-^{13}C (ge-HSQC), and heteronuclear single quantum coherence-distortionless enhanced polarization transfer (HSQC-DEPT) (41). To maintain the same quantity of salts, we dissolved samples of standards in the buffer used for incubation, and after pH correction to pH 7.4, freeze-dried the samples and then dissolved them in D_2O.

^1H-^{13}C correlated experiments were performed with supernatants issued from incubations with ^{13}C-enriched straw due to the low sensibility of the ^{13}C nucleus. Nonlabeled straw was used for ^1H-^1H correlated experiments to avoid interferences with ^{13}C satellites.

Chemolytic methods. **(i) Sugar compositional analysis.** The constituent monosaccharides of wheat straw materials were identified after prehydrolysis by 13 M H_2SO_4 at room temperature for 1 h followed by hydrolysis with 1 M H_2SO_4 at 100°C for 3 h or with 2 M trifluoroacetic acid at 120°C for 1 h and reduction in the form of trifluoroacetates (42). Products were analyzed by gas chromatography on a Hewlett-Packard model 5890 series II chromatograph equipped with a PAS-1701 column (0.32 mm by 25 m), with a temperature program of 80°C for 2 min, ramping from 80 to 115°C at 15°C/min, and ramping up to 160°C at 4°C/min and with a hydrogen flow rate of 20 ml/min. Heptitol was used as an internal standard for determinations of carbohydrate content. The uronic acid content was determined spectrophotometrically by use of the 3-hydroxybiphenyl reagent (7). Thin-layer chromatography was performed on Kieselgel 60 in a solvent system of *n*-butanol–ethanol–water (10:5:2).

(ii) Sugar linkage analysis. Dry samples of supernatant issued from incubations of *F. succinogenes* (~10 mg) were solubilized in dry dimethyl sulfoxide (1 ml) and methylated by the Hakomori method (23). The methylated products were isolated by partitioning with dichlormethane, dried, and concentrated. The permethylated products were hydrolyzed with 2 M trifluoroacetic acid at 120°C for 1 h, reduced with sodium borodeuteride, acetylated, and analyzed by gas-liquid chromatography (GLC)-mass spectrometry (24).

Metabolite assays. Succinate and acetate were quantified from 1D ^1H NMR spectra, with TSP-d_4 used as an internal reference.

Production of ^{13}C-enriched wheat straw. Durum wheat (cv. Ardente) was cultivated in air-tight chambers (750 liters) which allowed accurate regulation of the atmospheric gas composition and the following environmental parameters (2): a 16-h photoperiod, a photon flux rate of 1,200 μmol m^{-2} s^{-1} (mid-height of the plants), temperatures of 21°C (day) and 17°C (night), relative humidities of 65% (day) and 70% (night), and watering with half-strength Hoagland and Arnon nutrient solution (the nutrient solution was replaced with water after 77 days of culture). The culture substrate was made of a mixture of one-fourth sand and three-fourths perlite to avoid the release of $^{12}CO_2$ by soil respiration. The CO_2 (containing 10% of $^{13}CO_2$) concentration in the chamber was maintained at 350 μl liter^{-1} during the light period by automatic injections of CO_2 to compensate for photosynthetic assimilation. Plants were harvested after 104 days of culture and were then dried.

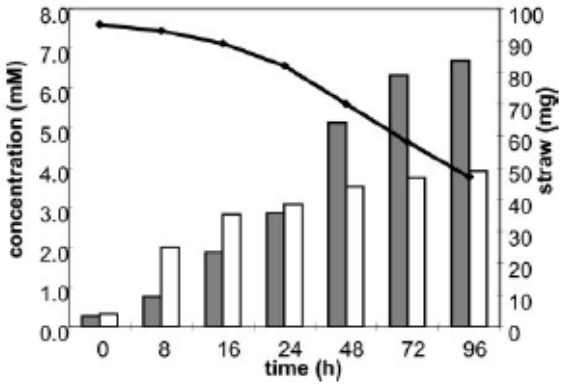

FIG. 1. Growth of *F. succinogenes* S85 on wheat straw. *F. succinogenes* S85 cells were grown at 38°C on 10 ml of mineral medium with 100 mg of wheat straw. The time dependence of the dry weight of the solid residue (♦) and of the succinate (gray bars) and acetate (white bars) concentrations are shown.

Chemicals. TSP-d_4 and $^{13}CO_2$ were purchased from Eurisotop (France). 1-*O*-Methyl-β-D-xylopyranose was purchased from Sigma. Polypropylene (PP) was purchased from Aldrich.

RESULTS

Growth of *F. succinogenes* S85 on wheat straw. *F. succinogenes* was grown for up to 4 days with 100 mg of wheat straw (^{13}C labeled or unlabeled). Growth was monitored by succinate and acetate quantification, and straw consumption was measured as the difference in dry weight (Fig. 1). About 50 mg of straw was biotransformed after 4 days.

Monitoring wheat straw degradation by analysis of solid residue. *F. succinogenes* cells grown in the presence of ^{13}C-labeled wheat straw were harvested after 8 and 16 h and 2, 3, and 4 days of growth, and pellets containing bacteria and solid fibers, obtained after centrifugation, were freeze-dried and analyzed further by ^{13}C CP-MAS NMR. To quantify the ^{13}C signals, we chose PP as an internal reference due to its very well separated CH_2 signal at δ 43.8 ppm (Fig. 2). Figure 2 shows the ^{13}C CP-MAS NMR spectrum of ^{13}C-enriched native straw, i.e., before incubation with *F. succinogenes*. First, in agreement with data in the literature, signals of the cellulose glucose units, which are the most intense, were observed. Note that C-4 resonances were distributed between two zones, corresponding to C-4 of crystalline cellulose and that of amorphous cellulose and hemicelluloses (22, 28, 45). Signals corresponding to hemicellulose polysaccharides and lignin could be seen. The intensities of the signals resonating between 30.1 and 107.1 ppm, except those at 72.2 and 75 ppm, were measured for samples taken at different time intervals and compared to that of the internal reference (PP). The results showed that the ratio of the ^{13}C signal intensities in each spectrum remained constant, indicating that all components of cellulose and hemicellulose were degraded at the same rate. In particular, the amorphous regions of cellulose were not degraded faster than the crystalline ones in straw (not shown). Because of the relatively low crystallinity of cellulose in the wheat straw used for these experiments, as already observed with this type of mate-

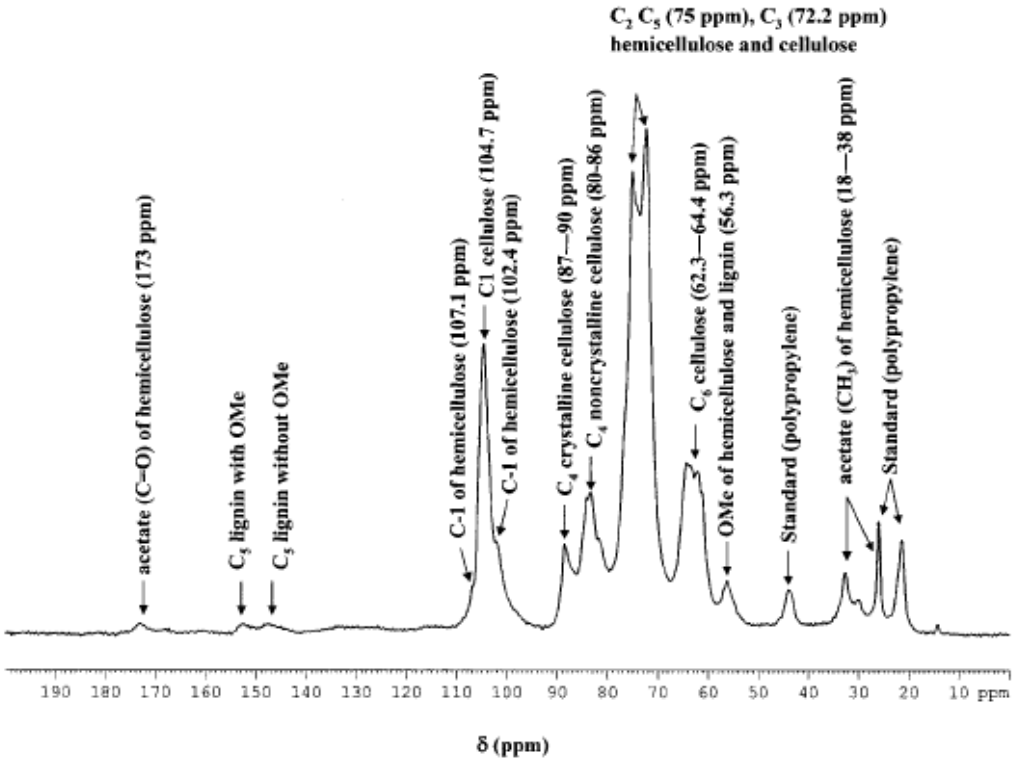

FIG. 2. ^{13}C CP-MAS NMR spectrum of native ^{13}C-enriched straw before incubation with *F. succinogenes*. Polypropylene was used as an internal reference.

rial (45), the same type of experiment was performed with Sigmacell 20 cellulose, which is more crystalline (not shown). The same results were obtained.

The monosaccharide composition of wheat straw and the monosaccharide composition of hemicellulose polysaccharides were determined as a complementary experiment for native wheat straw and for wheat straw after 4 days of incubation with *F. succinogenes* (Table 1). The sugar composition of the native wheat straw was similar to that published elsewhere (13, 38). In agreement with the CP-MAS NMR results, the relative percentages of monosaccharides in the native straw and in the residual samples did not vary to a great extent. However, in the sample taken after 4 days of bacterial growth, the total carbohydrate content was decreased by a factor of 2. This fact is consistent with the decreased dry weight of the solid residue after 4 days of incubation.

Monitoring wheat straw degradation by analysis of culture medium. Degradation of the wheat straw by *F. succinogenes* and the appearance of solubilized components were monitored by NMR. Incubations were carried out with labeled and unlabeled wheat straw, and samples were taken at various time intervals. After discarding the cell and straw pellets, we analyzed the culture medium by ^1H-^1H COSY and ^1H-^{13}C HSQC NMR experiments and chemolytic methods. The identified signals were mainly due to free xylose, arabinose, and arabinoglucuronoxylan oligosaccharides. ^1H and ^{13}C NMR chemical shifts of compounds that were identified or searched for are presented in Table 2. The results of sugar composition and

TABLE 1. Sugar composition and uronic acid and carbohydrate contents of straw samples

Sample[a]	Monosaccharide composition (% [wt/wt])								% Uronic acid	% Carbohydrate
	Rha	Fuc	Rib	Ara	Xyl	Man	Glc	Gal		
H NatStraw	1.1			12.2	79.2	1.3	4.4	1.8	ND	31.0
H FSStraw	1.5			10.7	78.4	1.6	6.0	1.8	ND	24.5
T NatStraw	0.4	TR	TR	4.2	33.7	2.8	58.4	1.4	3.2[b], 4.0[c]	81.0
T FSStraw	TR	TR	TR	2.7	29.6	2.2	65.5	TR	2.6[b], 5.6[c]	46.8

[a] H NatStraw, hemicellulose hydrolysis of autoclaved wheat straw; H FSStraw, hemicellulose hydrolysis of autoclaved wheat straw after 4 days of incubation with *F. succinogenes* S85; T NatStraw, total hydrolysis of autoclaved wheat straw; T FSStraw, total hydrolysis of autoclaved wheat straw after 4 days of incubation with *F. succinogenes* S85

[b] Calculated for all material.

[c] Calculated for the carbohydrate content. TR, traces; NO, not determined.

TABLE 2. Chemical shifts of metabolites found or searched for in culture medium of *F. succinogenes* S85 grown on wheat straw[a]

Residue	Chemical shift (δ/ppm)		
	H-1	C-1	H-2
αGlc	5.24	92.93	3.54
βGlc	4.65	96.75	3.24
αGlc6P	5.24	93.07	3.58
βGlc6P	4.65	96.92	3.28
Glc1P	5.46	94.16	3.52
Malt-1P$_{ar}$	5.43	100.41	3.58
Malt-1P$_{red}$	5.46	94.28	3.52
MD$_{term}$	5.41	100.59	3.59
MD$_{int}$	5.41	100.41–100.37	3.63
αMD$_{red}$	5.24	92.74	3.58
βMD$_{red}$	4.66	96.60	3.28
CB$_{ar}$	4.52	103.31	3.32
αCB$_{red}$	5.23	92.68	3.59
βCB$_{red}$	4.67	96.61	3.30
CD$_{int}$[b]	4.53	102.18	3.36
CD$_{term}$[b]	4.51	102.35	3.32
α*Arap*	4.53	97.60	3.52
β*Arap*	5.25	93.41	3.82
αXyl	5.20	93.32	3.53
βXyl	4.59	97.64	3.23
Xyl$_{int}$	4.47	102.6	3.27
βXyl$_{red}$	4.60	97.24	3.26
αXyl$_{red}$	5.20	92.79	3.56
αGlcA[b]	5.25	93.01	3.58
βGlcA[b]	4.65	96.66	3.30
Xyl^{GlcA}	4.63	102.4	3.43
Xyl$_2^{GlcA}$	4.58	ND	3.34
Xyl$_3^{GlcA}$	4.61	ND	3.41
GlcA^{Xyl}	5.32	98.30	3.58
Araf^{Xyl}	5.3–5.1	110–107	4.1–4.0
I-OMe-Xyl*p*	4.33	104.79	3.26

[a] Data were collected at 27°C after pH correction to 7.4. The H-1 signal of 1-*O*-methyl-β-D-xylopyranose was used as the standard. ND, not determined.
[b] Data from the work of Flugge et al. (17).

sugar linkage analyses of the supernatant obtained after 4 days of incubation are presented in Table 3. A large quantity of free xylose was identified, as well as free α-arabinopyranose and β-arabinopyranose. Xylooligosaccharides were also detected. Signals corresponding to internal xylose units (Xyl$_{int}$) and to reducing-end α- and β-xylose units of xylan oligosaccharides were present, while those of acetylated xylan oligosaccharides could be excluded (26). Resonances of arabinose (in the form of arabinofuranose) attached to O-2 and/or O-3 of xylose (Ara*f*$^{-Xyl}$) in arabinoxylans were detected. In agreement, the sugar linkage analysis showed relatively large proportions of 1,4-linked xylose residues (25%), some of which were substituted at position O-2 or O-3, indicating the presence of branched xylooligosaccharides. Characteristic signals due to a xylose unit being replaced at O-2 with 4-OMe-αGlcA (4-*O*-methyl-α-D-glucuronic acid) (Xyl^{-GlcA}) and those of the glucuronyl moiety attached to Xyl (GlcA^{-Xyl}) in glucuronoxylan oligosaccharides were also found (27). A comparison with data in the literature suggested the presence of xylobiose and xylotriose, the terminal Xyl of which is replaced at O-2 with 4-OMe-αGlcA. Characteristic H1/H2 signals of a βXyl^{GlcA}(1→4)βXyl(1→4)Xyl tetramer with a terminal xylose being replaced with 4-OMe-αGlcA (named Xyl$_3^{-GlcA}$) (11) and of a trimer Xyl^{-GlcA}(1→4)Xyl (named Xyl$_2^{-GlcA}$) (4) are listed in Table 3. The presence of oligosaccharides with 1,4-linked xylose residues branched at position O-2 was also supported by the sugar linkage analysis data (Table 3). This analysis also showed the presence of 2.8% 1,4-linked glucose, which might have originated from cellodextrins (Table 3). However, the major product (28.6%) suggested the presence of free or terminal 1-linked glucose. Since no H1/H2 cross peak due to free glucose was detected in the COSY spectra, this product should represent terminal glucose in some glucose derivative with a

TABLE 3. Sugar linkage analysis of culture medium obtained after 4 days of growth of *F. succinogenes* S85 on wheat straw

Sugar derivative[a]	Mol %	Mode of linkage	Possible original structure
2,3,5-Me$_3$-Ara	7.6	Araf-(1→	Free arabinose (or substituted at C-1)
2,3,4-Me$_3$-Ara	3.2	Arap-(1→	Free arabinose (or substituted at C-1)
3,5-Me$_2$-Ara	2.6	2)-Arap-(1→	Arabinoxylan oligosaccharides
2,3-Me$_2$-Ara	2.8	4)-Arap-(1→ or →5)-Araf-(1→	Arabinoxylan oligosaccharides
Total	16.2		
2,3,4-Me$_3$-Xyl	16.6	Xylp-(1→	Free xylose (or substituted at C-1)
2,3-Me$_2$-Xyl	13.1	→4)-Xylp-(1→	Nonsubstituted xylooligosaccharides
2-Me-Xyl	4.1	→3,4)-Xylp-(1→	Xylooligosaccharides with substitution of Araf
3-Me-Xyl	1.4	→2,4)-Xylp-(1→	Xylooligosaccharides with substitution of GlcA or Araf
1,2,3,4,5-Ac$_5$-Xyl	6.6	2,3,4)-Xylp-(1→	Xylooligosaccharides with substitution of Araf
Total	41.8		
2,3,4,6-Me$_4$-Glc	28.6	Glcp-(1→	Free glucose (or substituted at C-1)
2,3,6-Me$_3$-Glc	2.8	→4)-Glcp-(1→	Glucans
2,3,4-Me$_3$-Glc	1.9	→6)-Glcp-(1→	Glucans
Total	33.3		
2,3,4,6-Me$_4$-Gal	2.8	Galp-(1→	Free galactose (or substituted at C-1)
2,3,4-Me$_3$-Gal	0.8	→6)-Galp-(1→	Heteropolysaccharide
2,3,6-Me$_3$-Gal	2.4	→4)-Galp-(1→	Heteropolysaccharide
Total	6.0		
3,4,6-Me$_3$-Man	3.2	→2)-Manp-(1→	Heteropolysaccharide

[a] 2,3,5-Me$_3$-Ara = 1,5-di-*O*-acetyl-2,3,5-tri-*O*-methyl-arabinitol. Other names were abbreviated in the same manner.

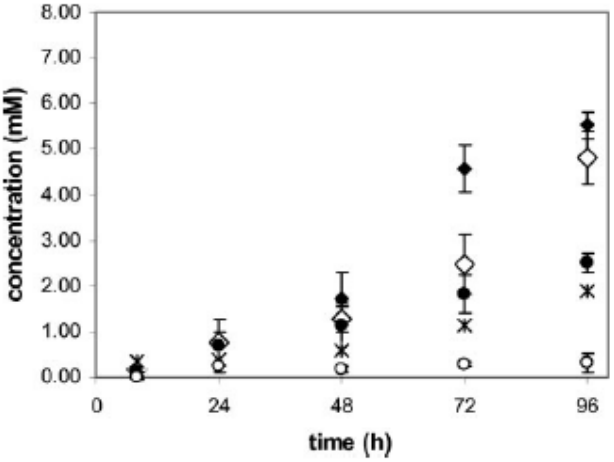

FIG. 3. Time dependence of concentrations of metabolites released during the growth of *F. succinogenes* S85 on nonenriched wheat straw, as determined from COSY NMR spectra. ♦, free xylose (Xyl); ◇, internal xylose unit of xylooligosaccharides (Xyl_{int}); *, xylose units replaced at O-2 with 4-OMe-GlcA (Xyl^{-GlcA}); ○, arabinosyl moiety in xylooligosaccharides ($Araf^{-Xyl}$); ●, free arabinopyranose. Values are means ± standard deviations of two experiments.

substitution at position 1. In addition, an H1/H2 cross peak corresponding to a glucose derivative with a substitution at position 1 was actually detected in the COSY spectra (not shown). Sugar linkage analysis data also identified about 6% galactose, which might have originated from arabinogalactan or galactan oligosaccharides.

Kinetic analysis. The NMR signal intensities of identified metabolites were quantified relative to those of the internal standard 1-*O*-methyl-β-D-xylopyranose (H1/H2 at 4.325/3.256 in the COSY-DQF spectra). The time dependence of the concentration of selected metabolites issued from hemicellulose degradation is presented in Fig. 3. Free xylose and xylooligosaccharides were the major metabolites released, with concentrations reaching 5.7 and 5.2 mM, respectively, after 4 days. These xylooligosaccharides may be substituted or not. The release of substituted xylooligosaccharides was monitored by the quantification of Xyl^{-GlcA} and Ara^{-Xyl}. The concentration of xylooligosaccharides with substitutions of arabinose remained constant over time (about 0.2 mM), while that of xylooligosaccharides with substitutions of 4-OMe-αGlcA increased (up to 2 mM). Free arabinose was produced all along and reached 2.8 mM after 4 days.

DISCUSSION

In this work, the use of NMR spectroscopy combined with chemolytic methods to monitor wheat straw degradation by *F. succinogenes* gave new insight into the mode of action of this organism's complex fibrolytic system.

The first important question addressed was whether *F. succinogenes* cellulases preferentially degrade the amorphous regions of cellulose in wheat straw. An analysis of ^{13}C CP-MAS NMR spectra did not show a preferential degradation of amorphous regions of cellulose versus crystalline ones in wheat straw or in pure Sigmacell cellulose. This suggests either simultaneous degradation of the amorphous and crystalline parts of cellulose by the enzymes or degradation at the surface on a molecular scale that cannot be detected by NMR. Studies with pure cultures of nonruminal cellulolytic microorganisms or purified cellulases have shown that fine structural features such as crystallinity, in addition to other properties such as pore volume and surface area, are strong determinants of pure cellulose degradability (16, 47). However, it was previously shown that when *Clostridium cellulolyticum*, a nonruminal cellulolytic bacterium, was grown in batch culture on cellulose, the relative crystallinity index of the cellulose was not modified during the course of degradation, suggesting that the crystalline and amorphous regions of cellulose were digested at the same rate (15). In addition, a study of the digestion of model crystalline celluloses by isolated *Clostridium thermocellum* cellulosomes showed a relative invariability in their crystallinities throughout the degradation process (8). Finally, our results are in agreement with those of Weimer et al. (48), who suggested several years ago that in ruminal cellulolytic bacteria, the gross surface area of the fibers is a major determinant of the hydrolytic rate, while the substrate's crystallinity appears to be relatively unimportant. The second important question raised was as follows: are there preferential bond cleavages within the polysaccharides of the plant cell wall by *F. succinogenes* enzymes? CP-MAS NMR and chemolytic analyses showed that there was no preferential degradation of cellulose versus hemicellulose in wheat straw. Again, the simultaneous degradation of cellulose and hemicelluloses by the *F. succinogenes* enzymatic system or degradation at the surface may be proposed to explain the NMR results. These results could be confirmed by the use of techniques that allow surface analysis, such as Fourier transform infrared photoacoustic spectroscopy (9).

An analysis by liquid-state NMR of the compounds released during *F. succinogenes* growth gave much more information on the mechanism of cell wall degradation by the enzymatic system. On the one hand, glucose, the product of cellulose degradation, did not accumulate in the culture medium, suggesting its rapid utilization by the cells. On the other hand, in agreement with the fact that *F. succinogenes* is not able to use xylose and arabinose (30, 34) or xylanes (unpublished results), hemicellulose hydrolysis products, composed mainly of arabinoglucuronoxylan oligosaccharides, accumulated in the medium. A kinetic analysis showed the production of xylose and xylooligosaccharides, indicating the action of xylanases. The identification of Xyl_3^{-GlcA} tetramers and Xyl_2^{-GlcA} trimers with a terminal xylose being replaced with 4-OMe-αGlcA indicated the activity of an endoxylanase cleaving the linkage between nonsubstituted Xyl and Xyl^{-GlcA} in glucuronoxylan. This is a characteristic property of xylanases belonging to family 10 glycosylhydrolases (GH10) (6). Three GH10 xylanases from *F. succinogenes* were recently characterized (25). These enzymes, acting on oat spelt xylans and arabinoxylans, produced xylobiose and xylotriose as main hydrolysis products. In contrast, xylanases from family 11, which have also been identified in *F. succinogenes* (40), cleave only the linkage between two nonsubstituted xyloses and may lead to the release of nonsubstituted xylooligosaccharides.

No acetylated xylan oligosaccharides (at both the O-2 and O-3 positions of xylose) could be detected, showing the high activity of the *F. succinogenes* acetylesterase (36). This may

have resulted from the activity of the acetylxylan esterase that was previously purified from *F. succinogenes* S85, which showed a unique ability to deacetylate birchwood acetylxylan in the absence of xylanase action (36).

Free α- and β-arabinopyranose were also detected, and their concentrations increased during the course of straw degradation. Their release reflects a high α-arabinofuranosidase activity, and their accumulation in the culture medium confirms that strain S85 is not able to use arabinose (34). In parallel, the concentration of $Araf^{-Xyl}$ did not vary much over time. This suggests that the linkages between arabinose and xylose are cleaved as soon as the $Araf^{-Xyl}$ oligomers are produced from the xylans. Until now, arabinofuranosidase activity was only demonstrated by the use of *p*-nitrophenyl-α-L-arabinofuranoside or purified substrates (37, 43). Recently combined purified arabinofuranosidase and xylanases from *Clostridium stercorarium* were shown to completely hydrolyze arabinoxylan (1). Our work documents for the first time the activity of the *F. succinogenes* S85 arabinofuranosidase on a natural substrate.

It was not possible to identify free 4-OMe-αGlcA in the culture medium, so we could not evaluate the α-glucuronidase activity during straw degradation. α-Glucuronidase was previously characterized from *F. succinogenes* S85. This enzyme was unable to cleave 4-OMe-αGlcA from intact xylans but could act on low-molecular-weight glucuronoxylan fragments created by xylanases (43). The accumulation of Xyl^{-GlcA} oligomers over time shown in the present work suggests that the α-glucuronidase activity in *F. succinogenes* S85 is lower than that of the other esterases, arabinofuranosidase and acetylxylan esterase.

Finally, although it was not possible to detect cellodextrins by NMR due to the complexity of the spectra, the results of sugar linkage analysis suggest that only a small amount of 1,4-linked β-glucans are present in the culture medium after 4 days. These results suggest that cellodextrins are rapidly used by *F. succinogenes* S85 after their release from straw.

In conclusion, this is the first study of the degradation of a natural substrate, wheat straw, by *F. succinogenes* by the use of in situ liquid- and solid-state NMR in combination with chemolytic analysis. The main advantage of this approach was its ability to investigate the activity of an entire fibrolytic system on an intact complex substrate, which should give more insight into what happens under physiological conditions. It also allowed a comparative analysis of the concurrent activities of xylanases and esterases on the wheat straw.

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