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Degradation and Utilization by *Butyrivibrio fibrisolvens* H17c of Xylans with Different Chemical and Physical Properties

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Hemicelluloses, mainly xylans, can be a major component of diets consumed by ruminants and undergo various degrees of microbial digestion in the rumen. The ability of *Butyrivibrio fibrisolvens*, a major xylanolytic ruminal species, to degrade and utilize nine chemically and physically different xylans for growth was examined. The arabinoxylans used included two isolated from corncobs (CCX-A and CCX-B), a native xylan excreted by corn cell tissue cultures (CX), an oxalic acid-treated, arabinose-depleted CX, and oat spelt xylan. Except for CCX-A, these xylans were extensively converted within 3 h of growth to acid-alcohol-soluble forms that remained at high levels for the duration of culture growth. These xylans contain mainly xylose and arabinose with small amounts of uronic acids. For a given xylan, all three components were used at about the same rate and extent. During the early stages of growth *B. fibrisolvens* also rapidly solubilized glucuronoxylans from birchwood, larchwood, 4-*O*-methylglucuronoxylan, and the xylose homopolymer xylan isolated from beechwood (BEWX). In contrast to the findings for the arabinoxylans, little acid-alcohol-soluble carbohydrate remained in these cultures after 9 h of growth, except for BEWX. Initially, with birchwood, larchwood, and 4-*O*-methylglucuronoxylan the uronic acid components were preferentially used over the xylose. Final xylan utilization measured at 72 h for all xylans varied from 57% for CCX-A to 92% for BEWX and was correlated with the initial 12-h utilization rate for a given xylan. Since CCX-A and BEWX are both highly water insoluble, this aspect did not appear to influence overall utilization. Thus, the type and distribution of xylan side chains are more important to xylan utilization by *B. fibrisolvens*.

The digestion of feedstuffs by ruminal microorganisms results in production of fermentation acids and microbial cells, which provide the host animal with its main sources of energy and protein. Cellulose, hemicellulose, and pectin are the main carbohydrates of forages, a major component of many ruminant diets. Although hemicelluloses (mainly xylans) represent about 30 to 40% of the total forage carbohydrate, their contribution to dietary energy available to the animal is often decreased because of low overall (40 to 60%) digestion (45). Dehority and colleagues showed in a series of studies that hemicellulose degradation varies greatly with species and strains of ruminal bacteria (12, 13) and with the source of xylan used (7, 14). For a given species, such as *Ruminococcus albus*, digestion can vary from a low of 5% to a high of 88% for corn hull and oat hull hemicelluloses, respectively (7). One of the most numerous and commonly isolated ruminal xylanolytic (hemicellulolytic) bacteria is *Butyrivibrio fibrisolvens*. This organism was also shown to vary significantly in its ability to degrade and use hemicelluloses from different, intact forages (7, 36a, 36b).

Xylan-degrading enzymes have been isolated from a variety of microorganisms and characterized (6, 11, 19, 29, 36, 37). Although genes encoding these enzymes have been cloned from several ruminal bacterial species (16, 17, 30, 32, 34, 38, 42, 47–49), in most cases it is not known if the cloned gene product has the same properties as the native enzyme. Structures within specific xylans have been elucidated by methods involving enzymatic degradation followed by analysis of products by carbohydrate chemistry (1, 4, 6, 25, 28, 39). However, most of these studies have been limited to use of a single xylan, enzyme mixtures containing only one or two xylan-degrading enzymes, or mixtures of enzymes isolated or cloned from different microbial species. As a result, very little information is available on the abilities of any one microbial species to degrade different xylans and to utilize the products for growth. In the present study, corn- and wood-derived xylans with different chemical and physical properties were used as substrates for growth of *B. fibrisolvens* under identical conditions. Xylan degradation and utilization of breakdown products over time were determined to gain insights into factors that could affect overall xylan utilization.

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MATERIALS AND METHODS

Growth conditions. All cultures were grown at 39°C in a Trypticase-yeast extract-mineral medium (24) containing 0.3 to 0.4% (wt/vol) xylan or other carbohydrates as the energy substrate. Growth was monitored by measuring optical densities (660 nm) of cultures and/or cell protein concentrations (31) after cell hydrolysis (0.1 M NaOH, 70°C, 20 min) with horse heart cytochrome *c* as the standard. Time course experiments examining the utilization of specific xylans were initiated by inoculating cultures (5%, vol/vol) from late-logarithmic-phase (16 to 18 h) oat spelt xylan-grown cultures. Samples (1 to 2 ml) were removed at various times during growth, subdivided into portions, and processed as follows. One portion was frozen and lyophilized to dryness, and the residue was then analyzed for neutral-sugar composition. A second portion was stored frozen until it was analyzed for total pentose (orcinol reaction), hexuronic acids, and cell protein. A third portion was mixed with 3 volumes of ice-cold acid-alcohol (95% ethanol containing 5% [vol/vol] glacial acetic acid), and the mixture was kept on ice for 30 min. This mixture was then centrifuged at 16,000 × *g* at 4°C for 20 min. The pellet (acid-alcohol-insoluble materials), resuspended in water, and supernatant fluid (acid-alcohol-soluble materials) were both analyzed for total pentose and hexuronic acid contents. No effort was made to remove bacterial cells from any of the samples (e.g., by centrifugation) since much insoluble or cell-adhering xylan would also be removed in the process. Cellular carbohydrate contents of cultures grown to similar extents on completely utilized soluble sugars indicate that the contribution of microbial carbohydrate to the residual carbohydrate is small (less than 30 μg of xylose equivalents per ml as measured with the orcinol reaction).

TABLE 1. Composition and properties of xylans

Xylan	% Soluble in[a]			%[b]		% of recovered neutral sugars[c]					
	Water	Acid-water	Acid-alcohol	Hexuronic acids	Neutral sugars	Xyl	Arab	Glu	Gal	Mann	Rham
CCX-A	0.4		1.8	3.9	86.3	89	7	4	Tr	0	0
CCX-B	95.1	72.5	7.9	7.6	75.3	76	15	5	5	0	0
CX	95.4		59.2	5.4	57.8	35	34	21	11	0	0
CX-OX	78.6		17.6	8.0	56.0	66	23	0	11	0	0
OSX	28.5		4.5	2.4	69.7	84	9	7	1	0	0
BWX	91.7		2.6	10.2	87.7	98	Tr	4	Tr	0	Tr
LWX	56.5	4.1	5.6	12.0	85.3	96	Tr	1	1	Tr	1
4-MGX	96.4	12.8	5.1	13.0	84.8	93	1	3	1	Tr	1
BEWX	5.5	4.9	0.6	2.7	96.0	97	Tr	Tr	0	0	0

[a] Dry weight.
[b] The remaining material consisted of protein, ash, and cellulosic materials.
[c] Xyl, xylose; Arab, arabinose; Glu, glucose; Gal, galactose; Mann, mannose; Rham, rhamnose; Tr, trace.

Sources and analysis of xylans. Oat spelt xylan (OSX), birchwood xylan (BWX), larchwood xylan (LWX), and 4-*O*-methylglucuronoxylan (4-MGX) were purchased from Sigma Chemical Company (St. Louis, Mo.). Beechwood xylan (BEWX) was obtained from Lenzing Aktiengesellschaft (Lenzing, Austria). Corn xylan (CX) was purified from cell-free fluids of maize endosperm cell cultures provided by J. A. Miernyk (National Center for Agricultural Utilization and Research, Peoria, Ill.) (15), which were then lyophilized to dryness. The dried residue was suspended in a small amount of water, dialyzed against distilled water to remove salts and sugars, and then lyophilized to dryness to yield the CX residue. A portion of CX was treated with 20 mM oxalic acid for 5 h at 80°C, and then the mixture was dialyzed against distilled water and lyophilized to dryness to obtain the low-arabinose CX-OX residue. This treatment removed 50 to 60% of the arabinose present in the untreated CX. Two xylans were prepared by S. N. Freer (National Center for Agricultural Utilization and Research) from ground corncobs (Andersen's Feeds, Danville, Ill.) by the methods outlined by Whistler et al. (46). Lignin materials were removed from the gritty corncob powder to obtain a holocellulose which was alkaline extracted to obtain the acetic-acid-insoluble corncob A xylan (CCX-A) and the acetic acid-ethanol-insoluble corncob B xylan (CCX-B).

Solubilities of xylans in water. The solubilities of various xylans were determined by adding the xylan with stirring to warm (45°C) distilled water to a final concentration of 5% (wt/vol) and incubating with stirring for 60 min. After cooling to room temperature, the xylan mixture was centrifuged at 26,400 × *g* for 30 min at 22°C. The amount of water-soluble xylan in the supernatant fluid was estimated by determining the amount of orcinol-positive material in the fluid. A similar procedure was used to measure solubilities of xylans in acidified water, but the xylans were dissolved in 5% (vol/vol) acetic acid. For acid-alcohol solubility measurements, the room temperature xylan mixture was diluted into 3 volumes of an ice-cold acetic acid-ethanol solution and centrifuged as described above for culture samples. The xylan content of the alcohol supernatant fluid was determined as orcinol-positive material present.

Enzymatic pretreatment of BWX. *B. fibrisolvens* H17c was grown on RGM medium (24) containing 0.3% OSX until stationary phase was reached, and the cells were removed by centrifugation (10,000 × *g*, 20 min, 4°C). About 100 ml (4 U of xylanase per ml) of the cell-free culture fluid was mixed with 10 g of BWX, placed into an ultrafiltration cell with a 10-kDa cutoff filter (Amicon, Danvers, Mass.), and incubated at 37°C for 24 h with stirring. The fluid was removed by filtration. The retained xylan was washed by filtration with an equal volume of water and then lyophilized to dryness. About 50% of the initial BWX weight was recovered, and this xylan had an orcinol material/hexuronic acid material ratio of 20.2 compared with 5.5 for the initial BWX.

Analytical methods and assays. Xylosidase and arabinofuranosidase activities were determined by release of *p*-nitrophenol from *p*-nitrophenyl glycoside substrates (24, 48). Xylanase activity was measured by release of acid alcohol-soluble pentoses from water-soluble OSX (24). Total sugars and pentoses in samples were determined by the phenol-sulfuric acid (2) and orcinol (40) methods with glucose and xylose, respectively, as the standards. Uronic acids were determined by the dimethylphenol procedure (41) using glucuronic acid as the standard. One unit of enzyme activity was defined as 1 μmol of *p*-nitrophenol or sugar formed per h.

The neutral sugars of various samples and of xylans (after hydrolysis in 2 M trifluoroacetic acid for 60 min at 100°C) were determined as the alditol acetate derivatives (52). Xylooligosaccharides, sugars, and xylan degradation products were analyzed by thin-layer chromatography by procedures previously described (9, 48).

RESULTS

Characteristics of xylans. The solubilities in water or acid-alcohol and the sugar compositions varied considerably among the xylans studied (Table 1). The corncob CCX-A was a particulate material that was insoluble in both water and acid-alcohol. This xylan was composed of xylose and arabinose in a 13:1 ratio and small amounts of uronic acids. In contrast, this ratio decreased to 5:1 in the corncob CCX-B, which also contained more uronic acids. CCX-B was almost completely water soluble but was acid-alcohol insoluble. Corn endosperm cells secreted CX, a highly water- and acid-alcohol-soluble xylan having equal amounts of xylose and arabinose. Treatment of CX with oxalic acid removed much of the arabinose, and the resulting CX-OX was considerably less soluble in acid-alcohol or in water (Table 1). Oxalic acid also removed all the glucose, much of which could also be removed by amylase treatment, suggesting contamination of the CX with starch-like material.

The four wood-derived glucuronoxylans studied were composed of only xylose and various amounts of uronic acids. In general, these xylans were essentially insoluble in acid-alcohol but varied in water solubility (Table 1). BWX and 4-MGX had xylose-to-uronic acid ratios of about 8:1 and 6:1, respectively, and were more than 90% soluble in water. A similar ratio of 7:1 was found for LWX, but water solubility of this xylan was only 56%. The water solubilities of the glucuronate-containing LWX and 4-MGX were greatly reduced under acidic conditions, but solubilities of the non-glucuronate-containing BEWX and CCX-B were not significantly affected. OSX appeared to be a mixture of xylan polymers or particles. While OSX is acid-alcohol insoluble, the extent of OSX solubility in water varied greatly, depending mainly on temperature and extent of centrifugation (43). Under the given conditions (see Materials and Methods), no significant differences could be detected in uronic acid levels or in neutral-sugar composition between the water-soluble and water-insoluble fractions of OSX.

Growth on CCX-A and CCX-B. The particulate, insoluble CCX-A rapidly settled out of cultures that were not shaken. However, visual observations of cultures indicated that many of the particles disappeared at early stages of growth. This was confirmed by chemical measurements. Within the first 3 h following inoculation, *B. fibrisolvens* cultures converted much of the highly insoluble CCX-A into an acid-alcohol-soluble form, but little overall utilization of the CCX-A was observed (Fig. 1A). During the next 6 h, the levels of the acid-alcohol-

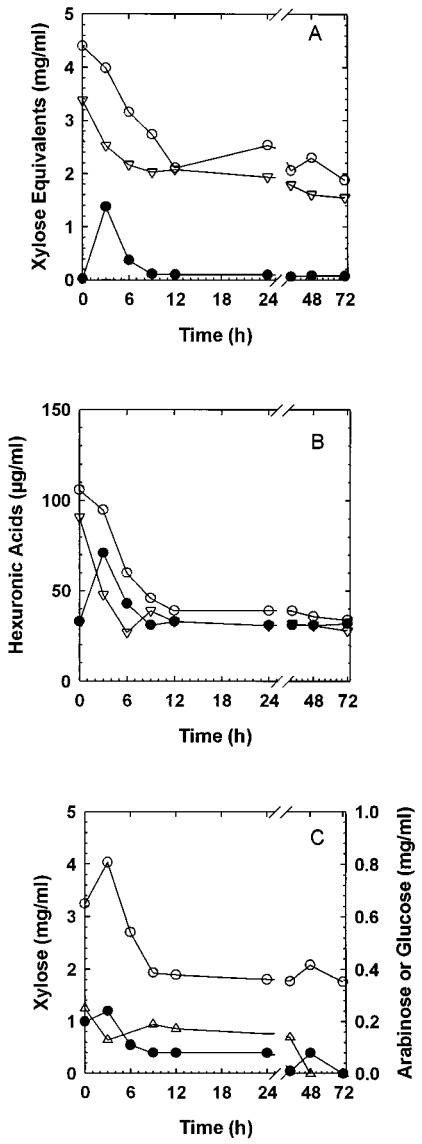

FIG. 1. Utilization of CCX-A by *B. fibrisolvens*. (A) Total xylan utilization (orcinol reaction); (B) hexuronic acid utilization. ○, whole xylan; ▽, acid-alcohol-precipitable xylan; ●, acid-alcohol-soluble xylooligosaccharides. (C) Neutral-sugar utilization. ○, xylose; ●, arabinose; △, glucose.

soluble materials decreased back to the initial levels (about 5% of the total xylan present), and there was extensive disappearance of the total xylan in the culture during the first 12 h of growth (Fig. 1A). While hexuronic acids were quantitatively minor components of CCX-A (Table 1), the usage patterns of these acids (Fig. 1B) were similar. However, some differences were noted, as about 50 to 60% of the hexuronic acids remained in an acid-alcohol-soluble form after rapid xylan usage ceased at around 12 h (Table 2). The usage of xylans by cultures was also monitored by neutral-sugar levels (Fig. 1C). Both xylose and arabinose disappeared from the culture until about 12 h and then remained stable. A small amount of glucose, a minor CCX-A component (Table 1), disappeared during initial growth, but the remainder was not used until after 36 h (Fig. 1C).

In contrast to CCX-A, the other corncob xylan, CCX-B, was highly soluble in water. Growth of *B. fibrisolvens* on CCX-B paralleled that observed with CCX-A except for a few differences. CCX-B was converted to acid-soluble materials more quickly than CCX-A, and these materials represented 50% or more of the total xylan present in the culture at all times after 9 h of growth (Table 2). Both orcinol-positive and hexuronic acid materials were used to the same or greater extent by 9 h relative to CCX-A. Constant and rapid declines of xylose and arabinose levels occurred from the time of inoculation to 12 h, after which only slight declines were noted (data not shown). The rates of disappearance of both sugars were approximately the same.

TABLE 2. Utilization of xylan and changes in acid-alcohol-soluble material with growth on various arabinoxylans

Sample[a]	% Used (% acid-alcohol soluble)				
	0 h	3 h	6 h	9 h	12 h
CCX-A					
OPM	0 (2)	10 (35)	32 (15)	38 (6)	52 (5)
HXA	0 (26)	10 (55)	43 (62)	66 (51)	64 (50)
CCX-B					
OPM	0 (3)	3 (61)	40 (74)	70 (55)	71 (54)
HXA	0 (18)	4 (66)	37 (82)	70 (59)	71 (59)
CX					
OPM	0 (53)	16 (84)	48 (95)	66 (91)	76 (94)
HXA	0 (49)	20 (71)	51 (91)	63 (91)	68 (90)
CX-OX					
OPM	0 (35)	11 (75)	45 (94)	76 (92)	83 (91)
HXA	0 (15)	12 (70)	61 (65)	70 (66)	72 (65)
OSX					
OPM	0 (5)	3 (47)	15 (45)	25 (34)	60 (23)
HXA	0 (12)	15 (40)	47 (33)	48 (34)	63 (12)

[a] OPM, orcinol-positive material; HXA, hexuronic acid material.

Growth on CX and CX-OX. Water-soluble xylans CX and CX-OX were fermented rapidly and extensively by *B. fibrisolvens* H17c. The orcinol-positive and hexuronic acid materials were both converted extensively to acid-soluble forms by 6 h of growth (Table 2). The remaining xylan persisted in these soluble forms for the remaining incubation time of the culture. Neutral-sugar analyses (data not shown) indicated that arabinose, xylose, and galactose levels in the culture remained constant for the initial 3 h and then declined at a rapid rate until 9 h, after which only slight decreases were noted. On the other hand, the contaminating glucose component declined at a much slower, linear rate from 0 to 24 h, after which slight decreases occurred. About 90% of the initial glucose was utilized.

CX-OX was not used during the initial few hours after inoculation (Fig. 2A), but there was rapid conversion into acid-alcohol-soluble materials. By 6 h, about 94% of the remaining CX-OX material was acid soluble (Table 2), and this situation persisted for the remaining time of culture incubation (Fig. 2A). Monitoring of the hexuronic acids of the culture revealed a slightly different pattern (Fig. 2B). Lesser amounts of the hexuronic acids were associated with the acid-alcohol-soluble materials present after 6 h (Table 2). During the first 3 h, no detectable loss of arabinose and only small losses of xylose were observed (Fig. 2C). Thereafter, there were rapid declines in the levels of both sugars until 12 h, after which utilization almost ceased. The small galactose component was also extensively used during the first 12 h of growth (Fig. 2C).

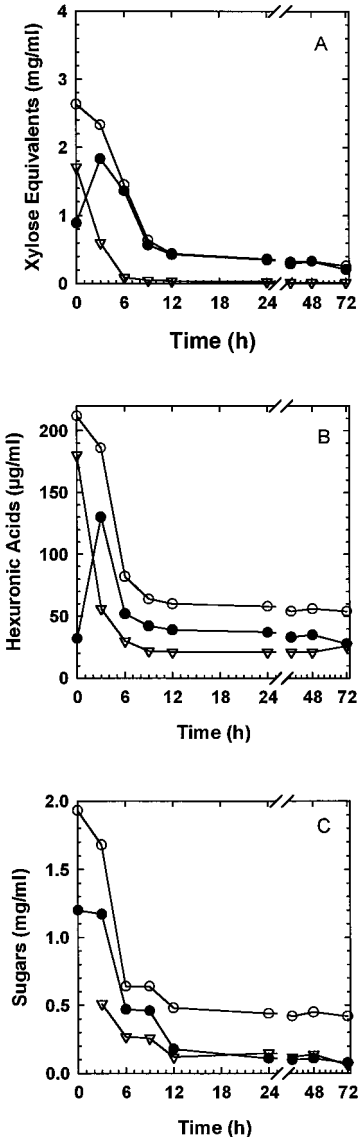

FIG. 2. Utilization of CX-OX by *B. fibrisolvens*. (A) Total xylan utilization (orcinol reaction); (B) hexuronic acid utilization. ○, whole xylan; ▽, acid-alcohol-precipitable xylan; ●, acid-alcohol-soluble xylooligosaccharides. (C) Neutral-sugar utilization. ○, xylose; ●, arabinose; ▽, galactose.

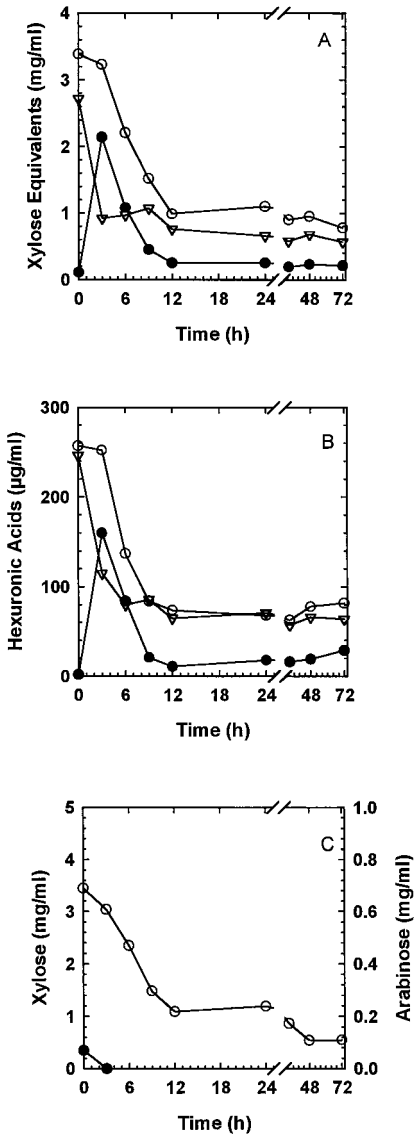

FIG. 3. Utilization of 4-MGX by *B. fibrisolvens*. (A) Total xylan utilization (orcinol reaction); (B) hexuronic acid utilization. ○, whole xylan; ▽, acid-alcohol-precipitable xylan; ●, acid-alcohol-soluble xylooligosaccharides. (C) Neutral-sugar utilization. ○, xylose; ●, arabinose.

Growth on OSX. Cultures of *B. fibrisolvens* growing on OSX initially appeared cloudy and opaque but became visually clear during the first 6 to 9 h of growth. In the first 3 h, little or no utilization of xylan was observed, but about half of the xylan was converted into acid-alcohol-soluble material (Table 2). Between 3 and 12 h, there was a rapid loss of total xylan and decline in the percentage of the remaining xylan that was acid-alcohol soluble. After 12 h, further disappearance of xylan was small. OSX contains only minor amounts of hexuronic acids (Table 1), but the use of these materials followed patterns similar to those for the orcinol-positive material (Table 2). During the first 9 h, there appeared to be a greater disappearance of the hexuronic acid materials than of the orcinol-positive materials. Neutral-sugar analyses of the cultures (data not shown) indicated no loss of arabinose and only a slight decrease in xylose content of the culture in the first 3 h but then rapid, parallel losses of both sugars until 12 h, by which time only about 50% of the initial levels remained. Over the next 48 h, a slow, gradual loss of these sugars occurred, and about 30% of the initial sugars remained at 60 h. Growth of *B. fibrisolvens* on the water-soluble portion (see Materials and Methods) of OSX resulted in xylan usage patterns that were almost the same as those found for whole OSX. However, at 3 h some losses (10 to 15%) of sugars could be detected and a greater proportion (60%) of the xylan was acid-alcohol soluble.

Growth on 4-MGX, BWX, and LWX. Growth of *B. fibrisolvens* on 4-MGX resulted in very rapid degradation and utilization of the xylan (Fig. 3A). The xylan was converted from an almost totally acid-alcohol-insoluble form to a soluble form within 3 h after inoculation, but little overall loss of orcinol-positive material occurred. This material then rapidly disappeared from the culture until 12 h. After this time, the amount of xylan in the acid-soluble form remained at about 30% of the total xylan present. The levels of xylose in the culture indicated

TABLE 3. Utilization of xylan and changes in acid-alcohol-soluble material with growth on various glucuronoxylans

Sample[a]	% Used (% acid-alcohol soluble)				
	0 h	3 h	6 h	9 h	12 h
BWX					
OPM	0 (2)	0 (51)	24 (37)	32 (19)	48 (9)
HXA	0 (9)	16 (62)	51 (56)	69 (43)	69 (42)
LWX					
OPM	0 (3)	0 (54)	26 (36)	43 (18)	52 (14)
HXA	0 (0)	8 (66)	48 (54)	72 (12)	74 (13)
4-MGX					
OPM	0 (4)	5 (70)	35 (53)	55 (30)	71 (25)
HXA	0 (1)	8 (58)	47 (51)	67 (20)	71 (15)
BEWX					
OPM	0 (3)	12 (32)	63 (68)	81 (78)	82 (50)[b]
HXA	0 (6)	10 (37)	15 (65)	42 (71)	65 (95)[b]

[a] OPM, orcinol-positive material; HXA, hexuronic acid material.
[b] Samples taken at 24 h.

a pattern of usage similar to that found for the orcinol material (Fig. 3C). The level of hexuronic acid materials in the culture also indicated a rapid conversion to acid-soluble forms (Fig. 3B) but exhibited a greater rate of decrease. By 6 h, much more of the initial hexuronic acid materials had disappeared compared with the percentage of orcinol materials that had disappeared (Table 3). The extents of disappearance of hexuronic acids and orcinol materials were the same (71%) by 12 h.

The results from growth on another wood glucuronoxylan, BWX, indicated a slow utilization of orcinol-positive material (Fig. 4A) compared with growth on 4-MGX. Only 48% of this material was used by 12 h compared with 71% for 4-MGX (Table 3). As noted for 4-MGX, there was a more rapid loss of hexuronic acid materials than orcinol materials from the culture with BWX (Fig. 4B), but the extents of losses of the two types of material were the same by 12 h (Table 3). Neutral-sugar analyses indicated nearly constant loss of xylose from 0 to 12 h (Fig. 4C), and the low levels of glucose present did not significantly change during these incubations.

The growth and utilization of a third wood glucuronoxylan, LWX, were very similar to those of BWX. Rapid disappearance of hexuronic acid materials similar to that for the other glucuronoxylans was observed (Table 3). In addition, the loss of orcinol-positive materials from LWX cultures in the first 12 h was about the same as those for BWX.

B. fibrisolvens also was grown on a BWX (BWX-D) which had been predigested with a crude mixture of xylan-degrading enzymes present in the *B. fibrisolvens* culture fluid. Unlike BWX, BWX-D solutions were particulate, as the xylan was insoluble in water. Cultures grown with BWX-D (data not shown) showed a very slow rate of loss of orcinol-positive material, with only about 45% of the initial amount used after 36 h of growth. BWX-D contained only about 3% hexuronic acid material, and about 60% of this material was used in the first 14 h of growth. At all culture sampling times, less than 5% of the orcinol material was acid-alcohol soluble. The hexuronic acid material was about 2% soluble at 0 h and increased to only about 12 to 15% after a 14-h incubation.

Growth on BEWX. BEWX consisted of essentially only xylose and, when added to cultures, appeared as fine particles that rapidly sedimented out in cultures. However, in shaken cultures almost all of these particles disappeared during the first few hours of growth, leaving an opaqueness in the cultures. *B. fibrisolvens* growth on BEWX was rapid, and within 6 h almost two-thirds of the xylan had been utilized and much of the remaining BEWX was acid-alcohol soluble (Table 3). Maximal rates of utilization continued up to 9 h, after which residual xylan disappeared slowly.

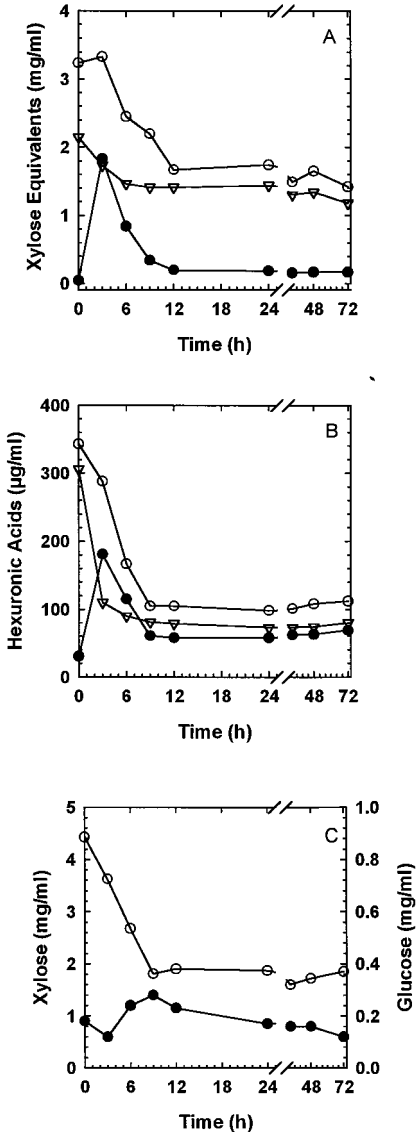

FIG. 4. Utilization of BWX by *B. fibrisolvens*. (A) Total xylan utilization (orcinol reaction); (B) hexuronic acid utilization. ○, whole xylan; ▽, acid-alcohol-precipitable xylan; ●, acid-alcohol-soluble xylooligosaccharides. (C) Neutral-sugar utilization. ○, xylose; ●, glucose.

Comparison of utilization rates and extents of xylan usage. Measurements of the levels of orcinol-positive materials present in a culture during the first 12 h of growth were used to calculate the initial rate of xylan utilization. This rate varied over an almost fourfold range (Table 4). The slowest rate was with CCX-A, and the fastest rate was with BEWX; both of these xylans are water insoluble. The extent of maximum overall xylan utilization measured after 72 h of growth varied from about 57 to 92%. These overall usage values correlated (r = 0.96) with the initial utilization rates. The faster initial utilization rates were associated with greater overall xylan usage.

TABLE 4. Initial xylan degradation rate and extent of utilization

Xylan	Orcinol-positive material	
	Rate of disappearance[a] (h^{-1})	% Loss[b]
CCX-A	0.068	57
BWX	0.073	57
OSX	0.082	72
LWX	0.091	67
4-MGX	0.131	77
CX	0.139	86
CCX-B	0.176	85
CX-OX	0.193	91
BEWX	0.261	92

[a] During the first 12 h of growth.
[b] Over 72 h of growth.

Xylan-degrading activities. The levels of xylanase, xylosidase, and arabinosidase present in cultures of *B. fibrisolvens* grown with various substrates were determined (Table 5). Substantial levels of xylanase were present when glucose, other sugars, or nonxylan polymers such as arabinogalactan were used as growth substrates. Growth on xylans increased xylanase levels about three- to fourfold. Xylanase activities were more than 95% extracellular, except when water-insoluble xylans like CCX-A were used as substrates. In these cases, about 10 to 20% of the xylanase was cell associated during logarithmic growth (data not shown). Xylosidase activities were always cell associated, and high levels were detected only during growth on xylans (Table 5). High levels of arabinofuranosidase were present in cultures grown with xylose or xylans as substrates but not with arabinose or glucose as the substrate. About 85 to 90% of the total culture arabinofuranosidase activity was cell associated, regardless of the substrate used for growth or stage of growth.

DISCUSSION

B. fibrisolvens is one of the most metabolically versatile species of ruminal bacteria. Almost all strains are able to grow on many simple sugars as well as on starches, pectins, mannans, hemicelluloses (20), and intact forages (7, 14, 36a, 36b). Although all strains are xylanolytic, *B. fibrisolvens* strains can be divided into four or five groups or species (33, 35) that display different patterns of expression of xylanolytic enzymes (23, 24). Representatives from each of these groups can grow on 4-MGX and utilize its hexuronic acid components (21), indicating that glucuronidase activity is also common among *B. fibrisolvens* strains. Extracellular esterase activity is found with most *B. fibrisolvens* strains, and strain H17c possesses an acetylxylan esterase activity (22). *B. fibrisolvens* H17c possesses all five of these xylan-degrading activities and is able to degrade crude forage hemicelluloses (13, 14) and a variety of chemically and physically different xylans (Table 1). This strain's xylanase activity is expressed constitutively, whereas xylosidase and arabinosidase activities are inducible or increase greatly with growth on a variety of xylans (Table 5). Xylosidase activity is low with growth on xylose, suggesting that xylobiose or other xylooligosaccharides are probably the inducers. Early studies by Howard et al. (26) with an undesignated *Butyrivibrio* strain and some recent work with other strains (50) support this suggestion.

TABLE 5. Xylan-degrading activities of *B. fibrisolvens* H17c grown on various substrates

Substrate	μmol/h/ml[a]		
	Xylanase	Xylosidase	Arabinofuranosidase
Glucose	15.1	ND[b]	0.5
Xylose	18.9	0.1	4.3
Arabinose	11.7	ND	0.6
Arabinogalactan	6.7	ND	0.3
OSX	32.6	3.1	1.5
BWX	33.3	2.6	3.5
4-MGX	33.8	2.0	4.0
CCX-A	32.4	2.3	3.0

[a] Late logarithmic phase; 0.22 to 0.29 mg of protein per ml.
[b] ND, not detected.

An unusual finding of the current study was that the arabinofuranosidase activity does not increase with growth on arabinose but increases greatly with growth on xylose or BWX and 4-MGX, xylans which contain essentially no arabinose (Table 1). These data are consistent with xylose being an arabinosidase-inducing substrate. However, digestion of BWX (and OSX) with a crude *B. fibrisolvens* xylanolytic enzyme mixture (see Materials and Methods) produced only xylobiose or larger oligosaccharides as products but not xylose. Thus, these products may also induce arabinofuranosidase activities.

In its simplest structure, a xylan is a β-1,4-linked polymer of xylopyranose residues that can form twisted ribbon-like strands and have extended chains. These chains can associate with one another via hydrogen bondings (3) to form semicrystalline aggregates or fibers that have some properties similar to cellulose. As was clearly shown by BEWX (Table 1), such xylan fibers are highly water insoluble. In many xylans, the backbone xylose residues can be substituted at positions 2 and 3. L-Arabinofuranose, 4-*O*-methylglucuronic acid, and acetyl groups are the most common components of the side chains. As has been shown for L-arabinose (3), the side groups can project outward from the xylan backbone and give a pinwheel appearance when viewed parallel to the backbone. Arabinose side chains block aggregation of the xylan backbone chains (10) and increase the water solubility of the xylan as has been shown clearly in studies on wheat arabinoxylans (1) and evidenced by the solubility of CCX-B, CX, and CX-OX (Table 1). As shown by our experiments with corn xylans, the oxalic acid removal of arabinose from CX also resulted in the less water-soluble CX-OX (Table 1). The presence of glucuronic acid side chains also affects xylan chain aggregation and water solubility properties of BWX, LWX, and 4-MGX (Table 1). These xylans are relatively water soluble, unless suspended in acetic acid solutions in which ionization of the carboxyl groups is suppressed. Compositional studies with various xylans (8, 25, 39) indicate that the side groups or side chains are often irregularly distributed, yielding backbone regions of either high or low side chain substitution. Differences in side chain distribution may explain our findings that BWX and LWX, which have similar uronic acid contents (or CCX-A and OSX, which have similar arabinose contents), have greatly different water solubilities (Table 1) and digestibilities (Table 4).

On the basis of the xylans studied, initial water solubility appears not to be an important determinant of xylan utilization by *B. fibrisolvens* H17c. BEWX is almost totally water insoluble but was the most rapidly attacked and most extensively utilized xylan (Tables 3 and 4). BEWX contains only xylose (Table 1), is a short-chain xylose polymer of about 40 residues, and has a molecular weight of about 5,000 (18). Aggregation of the chains into cellulose-like semicrystalline fibers presents little impediment to xylanase attack by *B. fibrisolvens* enzymes, which rapidly converted BEWX into acid-alcohol-soluble xylooligosaccharides during growth (Table 3). While the water-

insoluble CCX-A and BEWX are converted to about the same extent to acid-soluble forms in the first 3 h, further solubilization of the CCX-A and extensive utilization do not occur (Fig. 1). Extensive digestion of corncob xylan by *Streptomyces* sp. xylanases demonstrated that the residual arabinoxylooligosaccharides include unusual ones consisting of a side chain of xylotriose with an arabinoxylose residue (28). Similar side chains may be present in CCX-A and may block *B. fibrisolvens* xylanase attack since the residual xylan after 3 h appears to be increasingly more xylanase resistant.

In addition to requiring xylanase, the degradation and use of the corn xylans and OSX require arabinofuranosidase activity. With *B. fibrisolvens* H17c, this activity is produced with growth on various xylans (Table 5) and is essentially cell associated. Free arabinose was not detected in samples of culture fluids taken during the first 12 h of incubation as determined by thin-layer chromatography (data not shown), although arabinose was still present in these samples in the form of residual xylan and xylan degradation products (e.g., Fig. 1C and 2C). Bifunctional xylanases that also have arabinofuranosidase activity and remove arabinose side chains from xylan prior to significant attack on the xylose backbone have been reported (36). The absence of free arabinose in culture fluids suggests that such bifunctional xylanases may not be present in culture fluids of strain H17c. The disappearance of total arabinose from the cultures (Fig. 1C and 2C) suggests that uptake of the arabinoxylooligosaccharides generated during the initial xylan degradation may occur. Alternatively, if one of the arabinose transport mechanisms (42a) of some strains of *B. fibrisolvens* is present in xylan-grown strain H17c, rapid uptake of this sugar may account for the absence of free arabinose.

Studies with other microorganisms have shown a synergistic effect between arabinofuranosidase and xylanase activities in that their combined activity can be 5- to 20-fold greater than the predicted sum of their individual activities (19, 22, 29). Recent reviews indicate that some xylanases may involve a binding region that may encompass four or five xylose residues (4). For such xylanases, arabinose side groups may sterically hinder binding to the xylan. As has been shown for the *Polyporus tulipiferae* xylanase (6), these side groups block cleavage of the xylose backbone within several residues around an arabinose side group. According to this model, side chain removal by arabinofuranosidase would be expected to greatly enhance overall xylanase activity. CX is a highly substituted xylan, having equal amounts of arabinose and xylose (Table 1), but was still degraded at an intermediate rate (Table 4). Oxalic acid treatment of CX removes much of the arabinose, and CX-OX was degraded at a higher rate than CX by *B. fibrisolvens* H17c. Strain H17c appears to have several xylanase activities which include at least a 73-kDa enzyme (30) and most probably a 47-kDa enzyme originally found in strain 49 (34). Thus, it would appear that one of the *B. fibrisolvens* H17c xylanases is not hindered by arabinose side chains and can cleave at or near xylose residues that contain arabinose side chains.

The utilization of wood glucuronoxylans by *B. fibrisolvens* H17c differs from its usage of arabinoxylans in several ways. First, while the arabinose and xylose components of the corn xylans were used at about the same rate, there was clearly a greater disappearance of the glucuronic acid components with the glucuronoxylans compared with xylose, beginning to disappear as early as 3 h after inoculation (Table 3; Fig. 3 and 4). Secondly, although substantial amounts of both types of xylans are rapidly converted to acid-alcohol forms, most of the glucuronoxylans remaining after 9 h are acid-alcohol insoluble. Thirdly, the overall rates of glucuronoxylan utilization are slower than those for the corn xylans (except for CCX-A), and the residual glucuronoxylan is quite resistant to further degradation, resulting in an overall poor utilization of these xylans, particularly BWX (Table 4).

The results with growth of *B. fibrisolvens* on glucuronoxylans suggest that the initial degradative attack may occur at certain glucuronate-containing regions of the glucuronoxylans. Glucuronate side groups can be irregularly distributed along the backbone, especially with BWX, in which unsubstituted backbone regions can be 2 to 18 xylose residues long (39). Most likely, the initially attacked glucuronate-containing regions are ones that are more important in determining overall solubility of these xylans since the enzyme-digested BWX-D was insoluble, as was much of the residual, glucuronate-depleted xylan present in the cultures after 9 h of growth. The enzyme responsible for the initial attack by *B. fibrisolvens* may be a xylanase similar to one that has been purified from *Bacillus subtilis* enzyme mixtures. This xylanase preferentially cleaves the xylan backbone near xylose residues that contain single glucuronate side groups (37) in contrast to the more distal attack required by a xylanase from *Clostridium thermolacticum* (11). While *B. fibrisolvens* apparently has an α-glucuronidase activity, the cellular location of this activity is unknown. Attempts to show release of glucuronic acid from 4-MGX or BWX or from aldobiouronate with concentrated culture fluids or cell extracts have been unsuccessful (data not shown). The reason for the inability to show glucuronidase activity might be that the substrates for the *B. fibrisolvens* enzyme could be glucuronoxylooligosaccharides, as has been found for the *Streptomyces olivochromogenes* enzyme (27).

Several studies have examined the degradation and utilization of hemicelluloses and xylans present in plant materials and feedstuffs. The earlier studies by Dehority (13) with various pure cultures of ruminal species (mainly cellulolytic species) and xylans isolated from flax, corn hull, or fescue grass showed that overall utilization varied with the xylan used and xylan degradation often preceded significant utilization, findings both of which agree with our data with *B. fibrisolvens*. Since utilization of xylan was always accompanied by an accumulation of acid-alcohol-soluble products, it appears that the assimilation or further degradation of these intermediate products may limit the utilization of xylan. However, the rate and extent of xylan utilization still varied with xylan source, even though all the xylans used in our studies gave rise to a pool of soluble products. Thin-layer chromatography analysis of these soluble intermediates demonstrated the presence of xylose-containing oligosaccharides, but this method is not satisfactory for the identification of oligosaccharides containing uronic acids or other side groups. The nature of these side group substituents in oligosaccharide intermediates likely affects their uptake or susceptibility to enzymatic attack as discussed for whole xylan.

Incubations with mixed microorganisms from rumen contents exhibited a lag time prior to digestion of intact alfalfa and orchard grass plant materials (44). This lag time probably occurs in the rumen and contributes to the overall decrease in ruminal xylan degradation. The lag time may reflect the need to degrade other cell wall polymers such as lignin, cellulose, and pectin to allow for enzyme access to the xylan. The degradation rates of 0.049 to 0.056 h^{-1} and 54 to 62% utilization found for the xylans in these plant materials compare well with values obtained in our studies for CCX-A (Table 4). In addition, both arabinose and uronic acids were found to be used to a greater extent than xylose, whereas our results showed only a possible preference for uronic acids by *B. fibrisolvens* (Table 3). However, these two preferential uses are supported by data

from animals fed various forage diets (5, 44, 45, 51). Further work with growth of *B. fibrisolvens* on intact plant materials may indicate a similar effect and find other plant cell wall factors that may influence xylan digestion.

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