JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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In Vitro Fermentation of Bacterial Cellulose Composites as Model Dietary Fibers

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ABSTRACT: Plant cell walls within the human diet are compositionally heterogeneous, so defining the basis of nutritive properties is difficult. Using a pig fecal inoculum, in vitro fermentations of soluble forms of arabinoxylan, mixed-linkage glucan, and xyloglucan were compared with the same polymers incorporated into bacterial cellulose composites. Fermentation rates were highest and similar for the soluble polysaccharides. Cellulose composites incorporating those polysaccharides fermented more slowly and at similar rates to wheat bran. Bacterial cellulose and cotton fermented most slowly. Cellulose composite fermentation resulted in a different short-chain fatty acid profile, compared with soluble polysaccharides, with more butyrate and less propionate. The results suggest that physical form is more relevant than the chemistry of plant cell wall polysaccharides in determining both rate and end-products of fermentation using fecal bacteria. This work also establishes bacterial cellulose composites as a useful model system for the fermentation of complex cell wall dietary fiber.

KEYWORDS: arabinoxylan, bacterial cellulose, in vitro fermentation, mixed-linkage glucan, short-chain fatty acids, xyloglucan

INTRODUCTION

Dietary fiber (DF) from cereals, fruits, and vegetables has diverse nutritional benefits resulting from effects in both the small and large intestines of humans. In the small intestine, these include slowing of the rate of uptake of carbohydrates, reducing bile salt reabsorption and thereby controlling plasma cholesterol levels, and promotion of feelings of satiety.[1−3] In the large intestine, DF fermentation results in the production of short-chain fatty acids (SCFA; principally acetate, propionate, butyrate), which in the short term have been associated with stimulation of gastrointestinal tract (GIT) microorganisms beneficial to health.[4,5] In the longer term, SCFA have also been related to protection against inflammatory bowel diseases, such as ulcerative colitis, as well as protection against colorectal cancers.[6−8]

DFs are varied depending on plant source and food processing and range from soluble polymers (e.g., pectins, fructo-oligosaccharides, guar gum), to hydrated cellular particles (e.g., fruit and vegetable pieces), intractable lingocellulosic materials, and resistant starch.[1] It is known that plant cell wall components of DF, for example, are based on cellulose, with various amounts of non-cellulosic polymers. In fruits and vegetables, these are typically pectins and xyloglucans (XG), whereas in cereal cell walls, arabinoxylans (AX) and mixed-linkage glucans (MLG) are the predominant noncellulosic polymers.

DF, by definition, is indigestible by mammalian enzymes.[9] However, various components are fermentable by the complex microbiota within the GIT. A number of studies have investigated the fermentation of DF components by selected probiotic and intestinal bacterial species,[10] as well as fermentation by the intestinal microbiota as a whole.[11−14] To the best of our knowledge, materials studied so far either have been isolated polymers, which can be well-defined structurally but have limited relevance in most natural foods, or originate from plant-based foods (e.g., wheat bran) that are difficult to define structurally.

A construction approach is available to create cellulose-based composites as potential model DFs, which can be examined in vitro. The formation of these composites involves the growth of the cellulose-producing bacterium *Gluconacetobacter xylinus* (previously known as *Acetobacter xylinus* or *Acetobacter xylinum*), in a strictly controlled environment of solutions containing plant polysaccharides of interest, such as pectins and XG. These cellulosic composites have previously been shown to be a useful model system for plant cell walls, as they are typical of plant cell wall supramolecular structure.[15−17]

For human nutritional studies, it is often convenient to use the pig as a model,[18] especially for those studies that investigate the diet and large intestinal function.[19,20] In this study, feces were collected from pigs fed a semipurified diet, so that the fecal bacteria had not been exposed previously to the substrates being tested. The use of such an inoculum has advantages over human samples, both of consistency between animals and of the absence of adaptation by bacteria to the DFs being used as test substrates.

In this study, cellulose composites were constructed, containing the plant polysaccharide AX, MLG, or XG. The in vitro fermentability of these potential model DFs was compared with that of control substrates including the purified plant polysaccharides (AX, MLG, and XG) and selected natural plant substrates (wheat bran (WtBr) and cotton wool (Cott)). Fermentation characteristics of all substrates were assessed using the in vitro batch culture as described by Williams et al.[21] During fermentation, cumulative gas production was measured at regular intervals as an indicator of the kinetics of the reaction. On completion of fermentation, end-products such as SCFA and ammonium (NH_4^+) were analyzed. The hypotheses tested were

Received: December 21, 2010
Revised: March 17, 2011
Accepted: March 18, 2011
Published: March 18, 2011

Table 1. Substrates Used for Fermentation

substrate no.	substrate	abbreviation	source	% incorporation
1	arabinoxylan, medium viscosity	AX-med	wheat flour	na[a]
2	mixed-linkage glucan, medium viscosity	MLG-med	barley	na
3	xyloglucan	XG	tamarind seed	na
4	bacterial cellulose	BC	*Gluconacetobacter xylinus* bacterium	na
5	AX composite	BC−AX	*G. xylinus*	12.1% AX
6	MLG composite	BC−MLG	*G. xylinus*	26.9% MLG
7	XG composite	BC−XG	*G. xylinus*	28.0% XG
8	cotton	Cott	cotton wool	na
9	wheat bran	WtBr	wheat	na

[a] na, not applicable.

(1) soluble plant polysaccharides (AX, MLG, and XG) differ in their fermentation kinetics and end-products; (2) incorporation of plant polysaccharides into cellulose composites changes the rate and products of fermentation compared with their soluble form; and (3) bacterial cellulose composites containing soluble polysaccharides are a useful model for the in vitro fermentation of plant DF.

MATERIALS AND METHODS

Substrates. The substrates used in this study included soluble polymers (AX, MLG, and XG), their assemblies (model DFs), and insoluble polymers (WtBr and Cott). Medium-viscosity wheat AX (with 37% L-arabinose, 61% D-xylose, and 2% other sugars; lot 40301) and barley MLG (lot 10301), as well as tamarind XG (with 35% D-xylose, 45% D-glucose, 16% galactose, and 4% L-arabinose; lot 00401a) were sourced from Megazyme International Ireland Ltd. (County Wicklow, Ireland). Cotton wool (medical grade, 100% cotton) and wheat bran were obtained from local suppliers.

Cellulose-based composites were produced as described by Mikkelsen et al.[22,23] Briefly, *G. xylinus* (strain ATCC 53524) was cultivated under static conditions at 30 °C in Hestrin Schramm medium, pH 5.0, containing 5 g/L peptone, 5 g/L yeast extract, 2.7 g/L Na_2HPO_4, 1.15 g/L citric acid, and 2% glucose (w/v).[24] The soluble cell wall polymers (AX, MLG, or XG; 0.5% w/v) were added to this medium, as appropriate, for composite formation. After 72 h of incubation, cellulosic material was harvested and washed at room temperature by gentle agitation (50 rpm) in ice-cold sterile deionized water, with frequent rinses to remove excess medium and bacterial cells. Once cleaned, these samples were subsampled, and the microarchitecture was visualized using field emission scanning electron microscopy (FESEM) as described by Mikkelsen et al.[22] The remaining hydrated composites were subsequently freeze-dried.

The cotton wool, wheat bran, and cellulose composites were cryo-ground in a 6850 SPEX freezer/mill (SPEX, Metuchen, NJ), using precooling for 5 min, followed by two cycles of grinding at an impactor speed of 10/s for 5 min each, with an intermediate cooling time of 2 min between cycles. Table 1 provides a summary of the substrates used in this study.

Cryo-ground composites were hydrolyzed to component sugars by Seaman hydrolysis.[25,26] The monosaccharides in the hydrolyzed preparations were converted to alditol acetates and determined by gas chromatography as described by Blakeney et al.[27] This allowed for the determination of the percentage of polysaccharide incorporated into the composites.

Collection and Preparation of Inoculum. Porcine feces were collected from five male Large White grower pigs of between 50 and 60 kg. The pigs had been offered a standard semidefined diet as described previously as the control diet[28] for at least 10 days prior to collection. This diet, based on highly digestible corn starch and fishmeal, was formulated to be as free as possible of potentially fermentable carbohydrates to avoid adaptation of the microbial population to any of the substrates being tested. Feces were collected per rectum with a gloved finger and placed immediately into a warmed vacuum flask previously flushed with CO_2. The feces from all animals were combined and then diluted 1:6 with prewarmed, sterile saline (0.9% NaCl). This mixture was then mixed for 60 s using a hand-held blender and filtered through four layers of muslin cloth. All procedures were carried out under a constant stream of CO_2.

Preparation of Substrates and Cumulative Gas Production. A weighed amount of approximately 0.16 g of each substrate was placed into five replicate serum bottles and the weight recorded. Cumulative gas production was measured according to the in vitro method of Williams et al.[29] In brief, five replicates of each substrate were randomized within a single tray and fermented at 39 °C. Two milliliters of inoculum was added per 60 mL serum bottle within 2 h of collection of the feces. Gas readings were taken at regular intervals over different time periods (two values below 1 mL were considered low, and the fermentation was stopped at the third low reading). The time periods were as follows: WtBr, 48 h (16 readings); soluble polysaccharide substrates, 48 h (17 readings); insoluble cellulose composites and Cott substrates, 72 h (21 readings); and BC, 96 h (24 readings). This variation in end-points was done to avoid "over-fermentation" of substrates as described by Awati et al.[30] At the end of fermentation, all bottles were plunged into iced water and then frozen at −20 °C to inhibit the bacteria and thereby prevent further fermentation. Two blanks (excluding any added substrate) were included. The blank BL−I, was medium only with no added inoculum, for which a sample was taken at 0 h; BL+I was medium with added inoculum, and for this, duplicate samples were taken at 0, 72, and 96 h.

Analyses of Fermentation Products. All substrates were analyzed for their dry matter (DM) and ash. The pH and ash and DM contents were also analyzed for the fecal inoculum. At the end of fermentation, samples of fermentation fluid were collected for the measurement of SCFA and ammonium (NH_4^+). The pH was also measured at this time.

The postfermentation samples were thawed, prepared by vacuum distillation, and analyzed by gas chromatography (Agilent 6890 series GC, Agilent Technologies, Wilmington, DE), using a fused silica column 30 m in length (J&W Scientific, supplied by Agilent), with a 1 μm coating. The carrier gas was helium at a flow rate of 6 mL/min, at a split injector and FID detector, each held at a temperature of 250 °C. For each run the oven was held at 90 °C for 1 min, then ramped to 190 °C at a rate of 10 °C/min, and held there for 1 min. All SCFA values were corrected to millimoles per gram DM weighed into the fermentation bottles prior to inoculation.

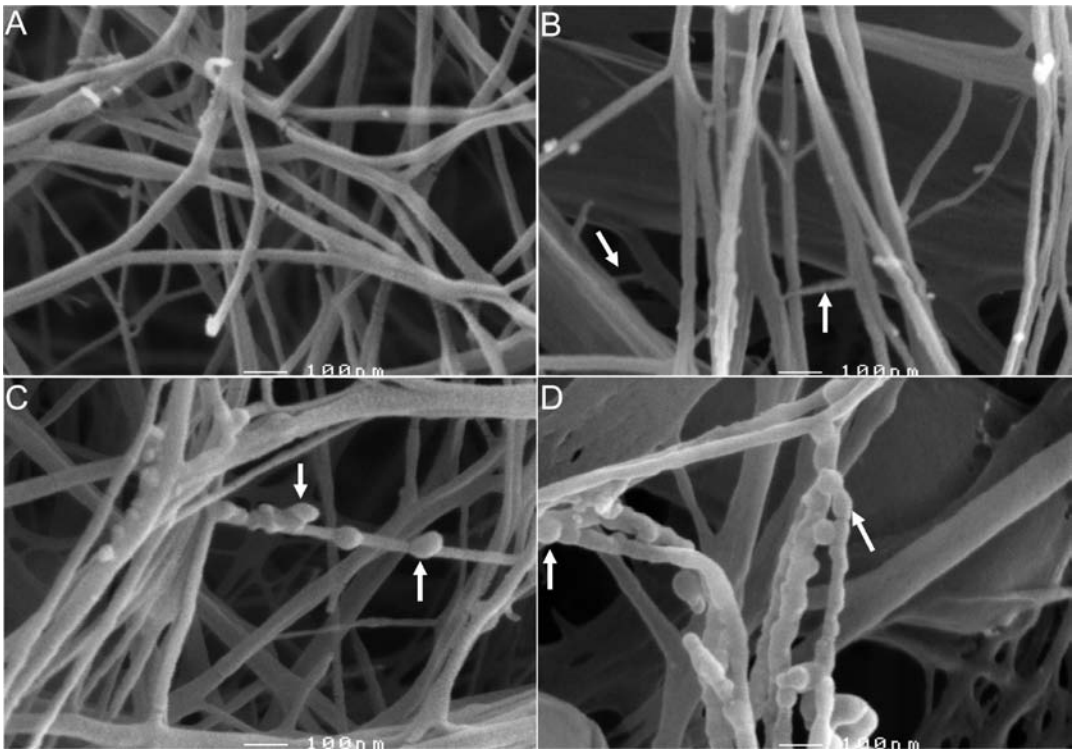

Figure 1. Representative FESEM micrographs illustrating the microarchitectural differences between BC (A) and the composites BC−XG (B), BC−AX (C), and BC−MLG (D). Incorporation of the various polysaccharides into the BC network is indicated with white arrows.

The branched-chain ratio (BCR) was also calculated. This is the ratio of the mainly branched-chain SCFA (branched chain and valeric acids) to the straight-chain acids (e.g., acetic, propionic, and butyric). The former (including valeric) are generally considered to be associated with protein degradation from the metabolism of amino acids,[31] whereas the latter originate from the degradation of carbohydrates. The BCR is therefore used as an indicator of relative protein fermentation.

Ammonium was analyzed using a method modified from Baethgen and Alley.[32] In brief, ammonium nitrogen was determined colorimetrically, utilizing the chemical reaction of NH_4^+ ions with sodium salicylate and nitroprusside in a weakly alkaline buffer, at a wavelength of 650 nm, using a UV−visible spectrophotometer (Automated Discrete Analyzer model AQ2+, SEAL Analytical Ltd., Fareham, U.K.).

Statistics. The data for cumulative gas production (as mL of gas accumulated per g DM with time) were fitted to the monophasic model described by Groot et al.[33] as

$$G = A/(1 + (C/t)^B) \quad (1)$$

where G = cumulative gas produced at time t (mL), A = asymptotic total gas production, B = switching characteristic of the curve, C = time at which half of the asymptotic value has been reached (h), and t = time (h).

The maximum rate of gas production (R_{MAX}) and the time at which it occurs (T_{RMAX}) were calculated according to the following equations:[29]

$$R_{MAX} = (A \times (C^B) \times B \times (T_{RMAX}{}^{(-B-1)}))/(1 + (C^B) \times T_{RMAX}{}^{-B})^2 \quad (2)$$

$$T_{RMAX} = C \times (((B-1)/(B+1))^{(1/B)}) \quad (3)$$

Differences between substrates were tested for significance using Tukey's Studentized range test of multiple comparisons according to

$$Y = \mu + S_i + \varepsilon_i \quad (4)$$

where Y is the result, μ the mean, S_i the effect of substrate i, and ε_i the error term. An effect of replicate was tested separately, but was not significant for any of the parameters. Blank values were excluded from the actual analysis, but are shown in the tables.

All statistical analyses were performed using the SAS NLIN (curve-fitting) and GLM (significant differences) procedures (Statistical Analysis Systems Institute 9.1, 2002/3).

RESULTS

Formation and Microarchitecture of Cellulose-Based Composites. With glucose as substrate in the fermentation medium, pure BC was produced. The microarchitecture of the cellulose microfibrils appeared to be a densely packed network, which was random in orientation at the micrometer length scale (Figure 1A).

The percentage of the respective polysaccharides incorporated into each model DF formed is listed in Table 1. For BC−XG, FESEM micrographs showed cross-bridges (indicated with white arrows; Figure 1B) between adjacent cellulose fibers and gave the impression of microfibril directionality, as reported previously.[16] In BC−AX (Figure 1C) and BC−MLG (Figure 1D), microfibrils appeared to support local deposition of polysaccharides (indicated with white arrows), and no cross-bridges were observed. In addition to physical entanglement, the cellulose fibers of BC-MLG appeared to coalesce more prominently (Figure 1D), compared to BC−AX (Figure 1C).

In Vitro Fermentation. The inoculum had a DM of 4.42%, an ash content of 1.43%, and a starting pH of 6.37.

Table 2 shows the results for the kinetics of cumulative gas production. When all substrates were analyzed together, the majority of results seemed to be grouped into the two categories of (i) soluble polysaccharide substrates and (ii) insoluble

Table 2. Cumulative Gas Parameters, Comparing Soluble Polysaccharides and Insoluble Complex Substrates (n = 5 per Substrate)[a]

substrate	DMCV (mL/g DM)	C (h)	R_{MAX} (mL/h)	T_{RMAX}(h)	pH
soluble polysaccharide substrates					
AX-med	393 bc	13.84 h	26.38 a	11.55 def	6.31 f
MLG-med	420 ab	20.32 efg	19.38 bc	16.43 c	6.36 ef
XG	371 cde	14.80 gh	22.50 ab	12.31 de	6.36 ef
insoluble complex substrates					
BC	361 de	43.97 a	6.78 fg	28.65 a	6.57 bcd
BC-AX	330 fg	26.15 cde	9.50 defg	13.51 cde	6.61 abc
BC-MLG	393 bc	29.21 cd	10.29 def	14.69 cd	6.58 bc
BC-XG	366 cde	30.98 bc	9.34 defg	11.25 def	6.60 abc
Cott	177 i	30.41 bcd	4.98 g	22.72 b	6.71 a
WtBr	263 h	24.47 de	8.93 defg	13.41 cde	6.65 ab
blanks[b]					
BL−I (0 h)					6.84
BL+ I (0 h)					6.77
BL+I (72 h)	15.2				6.75
BL+I (96 h)	15.5				6.73
prob	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MSD	28.6	6.0	5.1	3.8	0.12

[a] Different letters in the same column show significant differences ($P < 0.05$). [b] Blank bottles contained medium and inoculum, but no added substrate. Therefore, units are mL/bottle. They were not included in the analysis of variance nor were the gas profiles fitted.

complex substrates including the model DFs (BC, BC−AX, BC−MLG, and BC−XG) and plant cell wall materials (Cott and WtBr). Among the soluble polysaccharides, XG produced significantly less gas than MLG (DMCV). However, the maximum rate of fermentation was not significantly different from that of MLG or AX. The time at which the maximum rate of gas production occurred (T_{RMAX}) was significantly later for MLG compared with AX and XG. MLG shared a similar gas profile with WtBr up to 15 h, but then fermented more quickly than WtBr, reaching a higher end-point. This is illustrated in Figure 2A, where representative gas profiles for AX, MLG, and XG are shown, compared with BC and WtBr.

An examination of the gas data for the insoluble substrates showed that the cellulose composites were slower to ferment than the individual soluble polymers (Table 2). These results are reflected in the representative gas profiles shown in Figure 2B, where it is also evident that the cellulose composites with each of AX, MLG, and XG had a reduced lag compared to BC. Furthermore, these cellulose composites shared very similar gas fermentation kinetics with WtBr up to 36 h. However, whereas WtBr fermentation stopped soon after, cellulose composites continued fermenting and reached a higher end-point, having similar end fermentation kinetics to BC. Cott and BC were slowest in terms of the latest T_{RMAX} and the slowest R_{MAX} (Table 2). Of the cellulose composites, BC−AX was the slowest fermenting and had the lowest end-point.

Table 3 shows the end-product parameters measured for both soluble and insoluble substrates, including the molar percentages for acetic, propionic, and butyric acids. Generally, the soluble polysaccharide substrates had a lower pH (Table 2), a lower BCR, and lower NH_4^+ (Table 3) compared with the insoluble substrates except for AX-med, for which NH_4^+ was not significantly different. Although the total SCFA produced by the soluble fiber and the BC-containing substrates were similar, there

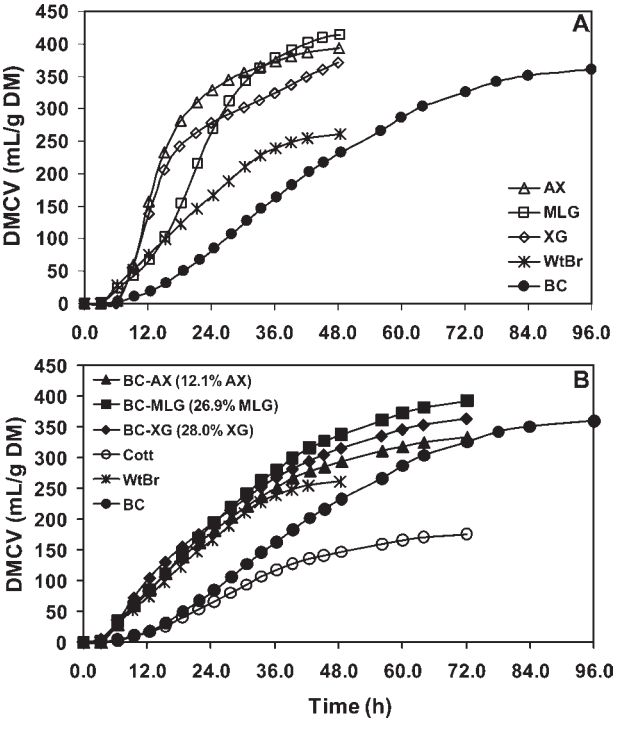

Figure 2. Comparison of the gas profiles of (A) the soluble polysaccharide substrates AX, MLG, and XG, with the insoluble model (BC) and plant (WtBr) fiber substrates and (B) the insoluble substrates including the model dietary fibers (bacterial cellulose composites) BC−AX, BC−MLG, and BC−XG, as well as the plant fibers cotton wool (Cott) and wheat bran (WtBr). Bacterial cellulose is included for comparison.

were differences in the SCFA ratios. The cellulose composites, despite fermenting more quickly than BC (Table 2), had SCFA

Table 3. End-Product Parameters, Comparing Soluble Polysaccharides and Insoluble Complex Substrates[a]

	mmol/g DM				mmol/L	% of total SCFA			
substrate	acetic	propionic	butyric	total SCFA	NH_4^+	BCR	acetic	propionic	butyric
soluble polysaccharide substrates									
AX-med	8.55 c	4.24 a	0.14 cd	14.33 bc	169.6 d	0.086 d	66.1 g	32.8 ab	1.1 d
MLG-med	8.16 cd	4.14 a	0.11 d	14.25 bc	189.9 cd	0.090 d	65.7 g	33.4 a	0.9 e
XG	8.10 cd	3.48 b	0.14 cd	13.21 de	167.6 d	0.094 d	69.1 ef	29.7 cd	1.2 c
insoluble complex substrates									
BC	10.36 a	1.56 f	0.28 a	14.30 bc	308.8 ab	0.175 c	84.9 ab	12.8 g	2.3 b
BC−AX	9.23 b	2.11 d	0.28 a	13.82 cd	232.6 abcd	0.191 b	79.4 c	18.2 f	2.4 b
BC−MLG	10.78 a	1.94 de	0.31 a	15.48 a	318.7 a	0.192 b	82.8 b	14.9 g	2.4 b
BC−XG	9.18 b	2.48 c	0.30 a	14.34 bc	288.1 ab	0.203 b	76.8 d	20.7 e	2.5 b
Cott	6.00 f	0.71 g	0.21 b	8.18 g	245.0 abcd	0.244 a	86.8 a	10.2 h	3.0 a
WtBr	7.09 e	2.06 de	0.21 b	11.06 f	301 ab	0.203 b	75.7 d	22.0 e	2.2 b
blanks[b]									
BL−I (0 h)	0.06	0.02	0.01	0.09	0.4	0.081			
BL+I (0 h)	0.09	0.02	0.02	0.14	0.4	0.079			
BL+I (72 h)	0.68	0.09	0.08	0.97	0.8	0.145			
BL+I (96 h)	0.69	0.09	0.08	0.99	0.5	0.151			
prob	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MSD	0.58	0.30	0.04	0.68	97.4	0.014	2.4	2.4	0.4

[a] Different letters in the same column show significant differences ($P < 0.05$). [b] Blank bottles contained medium and inoculum, but no added substrate. Therefore, units are mL/bottle. They were not included in the analysis of variance nor were the gas profiles fitted.

patterns similar to that of BC. Moreover, these substrates shared similar SCFA ratios with WtBr. Interestingly, proportions of butyric acid were significantly higher (approximately double) for the cellulose composite substrates compared with the soluble noncellulosic polymers, although overall concentrations remained low.

DISCUSSION

Construction of Cellulose-Based Composites. The microscopy results reported here showed that addition of soluble polysaccharides to the fermentation medium resulted in bacterial cellulose assemblies having varied microstructure according to the polysaccharide added. As expected,[16] XG formed a cross-linked network, whereas neither AX nor MLG did. Association of AX and MLG with cellulose seemed to be by surface deposition rather than the extensive molecular binding (including disruption of cellulose crystallinity) that is characteristic of XG.[16] This interpretation of SEM observations was supported by evidence from solid-state ^{13}C NMR (Mikkelsen et al., unpublished results).

Fermentation Characteristics. Generally, the soluble substrates (AX, MLG, and XG) were more rapidly fermented (Table 2; Figure 2) than the cellulose composites. This was not surprising as these plant cell wall polysaccharides were readily accessible to the enzymes of the fermenting microbiota. These three soluble substrates are chemically diverse and, therefore, require different combinations of bacterial glycosyl hydrolases to liberate the monosaccharide units (e.g., xylanase is needed to degrade AX but has no action on MLG or XG), which are then utilized as an energy source by bacteria. It is therefore not surprising that there are some differences in the fermentation properties of the three soluble polysaccharides, although these were minor compared to differences with the same polymer incorporated into their respective cellulose composite.

The SCFA patterns of AX and MLG from this study, where acetic acid was the dominant SCFA produced followed by propionic and then butyric acid, concurred with those of Williams et al.,[34] despite the use of a different batch of pig fecal inoculum. Williams et al. also showed that gas production from the relevant monosaccharides (arabinose, glucose, xylan) was not much faster than from soluble polymers, consistent with the relatively rapid conversion of soluble polymers into monosaccharides.[34]

The generally higher ammonium concentration for the cellulose composites, compared with the soluble polysaccharides, indicated more fermentation of protein, which is likely to occur in the absence of available carbohydrate.[35] This is confirmed by the values for BCR, which are also generally higher for the model DFs. Although this would suggest that the cellulose composites have less readily available carbohydrate for fermentation compared with the soluble polymers, it is interesting to note that the total SCFA for composites are generally not significantly different from the soluble polymers.

The reduced fermentation rate of the cellulose composites/model DFs (BC, BC−AX, BC−MLG, and BC−XG) and plant-derived DF substrates (WtBr and Cott) seems to be connected with their insolubility. This is likely due to the highly crystalline model DFs and cotton[36−38] and the less crystalline wheat bran not being hydrolyzed as rapidly by microbial enzymes compared with the soluble polymers. These substrates were also less completely fermented compared to the soluble polysaccharides, despite all substrates being soaked for 16 h in the medium prior to inoculation.

Cellulose composites were the most rapidly fermented of the insoluble substrates, presumably due to the presence of a

noncellulosic polymer (AX, MLG, or XG) within the composite. Interestingly, the percentage incorporation of polymer within each composite, which ranged from 12 to 28%, had no significant impact on the maximum rate of gas production (R_{MAX}). Despite the compositional differences between cellulose composites, it appears that once the microbial consortia were able to adapt their enzyme systems and commence fermentation of these substrates, the rates of fermentation were similar for all three model DFs.

The different microarchitecture of the cellulose composites, with polymers either physically entangled with the BC microfibrils (AX and MLG) or cross-linked with them (XG), had no bearing on the fermentation kinetics for these substrates. It has been reported that pig hind-gut microbiota contain both cellulolytic and hemicellulolytic bacterial species (required for depolymerizing AX/MLG/XG),[39] which increase in numbers and activity as the bacterial community adapts to DF.[40] Future work will focus on using fluorescence in situ hybridization to profile microbial community shifts as they adapt to ferment cellulose-based composites as model DF substrates.

The SCFA molar ratios of the cellulose composites were significantly different from those of the soluble polysaccharides. The high acetic acid production from the fermented model DFs was most likely due to the high cellulose (glucose) content, stimulating acetogenic fermentation.[41] The low propionic acid production was most likely due to the slow rate of fermentation of these complex substrates. High propionic acid production is typical of rapidly fermentable substrates such as rice cell walls.[42] Butyric acid production was significantly higher for the cellulose composites, suggesting proliferation of bacterial species that are efficient butyrate producers.

For WtBr, one of the least fermentable substrates, the probable presence of intractable lignocellulosic materials may explain its comparatively poor fermentability. Of particular interest was the similar fermentation kinetics of WtBr and the cellulose composites up to 36 h. Together with the similar SCFA end-products, this suggests that the cellulose composites produced by the *G. xylinus* bacterial system bear a sufficiently close resemblance to cereal plant cell walls to be a useful model system for studying their fermentation. As cellulose composites can be prepared from all major soluble cell wall polymers, this construction and deconstruction (i.e., fermentation) approach will be expanded in the future to address molecular and microbial aspects of DF benefits to human health.

Implications for Composites as a Model of Cellulosic Dietary Fiber. These composites are easy to produce and give a relatively consistent product.[23] They have previously been shown to be a useful model system for plant cell walls, as they are typical of plant cell wall supramolecular structure.[15−17] Furthermore, they provide a model based on a constructive approach rather than the more commonly used destructive approach such as chemical and/or enzymatic treatments to obtain plant cell wall fractions.[43,44] This may give a more realistic approximation of what occurs within the actual plant cell wall.

Implications for Dietary Fiber Fermentation in Vivo. In vivo, the site of fermentation of specific substrates is related to the rate of fermentation of those substrates, the microbial population present at that site, and the transit time through each site. For example, whereas transit time through the small intestine is usually estimated to be between 3 and 4 h, in the large intestine this can range from 48 to 96 h.[45] This variation is highly related to the diet composition, with "fiber" having an extremely important role. Thus, rapidly fermentable substrates are more typically fermented proximally, whereas very slowly fermented substrates will be fermented more distally or may even be passed in the feces before fermentation is complete. These results suggest that soluble AX/MLG/XG are examples of more rapidly fermentable substrates that may not remain intact beyond the proximal colon. In contrast, cellulose may be incompletely fermented in vivo,[39] consistent with the slow kinetics reported here. It is intriguing that neither the amount of incorporated soluble polymer (ranging from 12 to 28%) nor chemical form of the cellulose composites as model DFs had any effect on the rate, extent, or even the end-products of fermentation in this study. The inference is that it is the general physical form of composite materials that determines the fermentation behaviors studied here, rather than details of molecular structure.

Although the presence of soluble polymers in cellulose composites increased the rate of fermentation compared to BC, the end-product ratios were similar to those for cellulose alone. The inference is that similar bacterial populations ferment both cellulose and the model DFs but that the presence of noncellulosic polysaccharides in the latter leads to more rapid colonization and hence fermentation. Future characterization of the microbial species involved in these fermentation processes will allow this inference to be tested.

This study has shown how bacterial cellulose composites can be used as models to define structure−fermentability relationships, allowing the study of molecular features underlying the functionality of complex dietary fibers of relevance to human nutrition and health.

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Funding Sources

This study was supported in part by a grant from the CSIRO Flagship Collaboration Fund to the High Fiber Grains Cluster via the Food Futures Flagship.

ACKNOWLEDGMENT

We thank Dr. Dagong Zhang for preparation of pig diet and animal handling; Barbara Gorham for technical assistance; Cherie Walsh for monosaccharide analysis; Ian Brock for conducting the SCFA analysis; and the UQ Gatton Piggery for supply of pigs.

ABBREVIATIONS USED

A, asymptotic gas production; AX, arabinoxylan; *B*, switching characteristic of the curve; BC, bacterial cellulose; BCR, branched-chain ratio; *C*, time at which half of the asymptotic value has been reached (h); cSt, centistokes; Cott, cotton wool; DF, dietary fiber; DM, dry matter; DMCV, cumulative gas per gram dry matter; FESEM, field emission scanning electron microscope; *G*, cumulative gas produced at time *t* (mL); GIT, gastrointestinal tract; I, inoculum; MLG, mixed linkage (1,3;1,4)-β-glucan; MSD, minimum significant difference; MW, molecular weight; prob, probability; R_{MAX}, maximum rate of gas production; rpm, rotations per minute; SCFA, short-chain fatty acids; T_{RMAX}, time at which R_{MAX} occurs; WtBr, wheat bran; XG, xyloglucan.

REFERENCES

(1) Aleixandre, A.; Miguel, M. Dietary fiber in the prevention and treatment of metabolic syndrome: a review. *Crit. Rev. Food Sci.* **2008**, *48*, 905–912.

(2) Good, C. K.; Holschyh, N.; Albertson, A. M.; Eldridge, A. L. Whole grain consumption and body mass index in adult women: an analysis of NHANES 1999−2000 and the USDA pyramid servings database. *J. Am. Coll. Nutr.* **2008**, *27*, 80–87.

(3) Slavin, J. Whole grains and human health. *Nutr. Res. Rev.* **2004**, *17*, 99–110.

(4) Flint, H. J.; Bayer, E. A.; Rincon, M. T.; Larned, R.; White, B. A. Polysaccharide utilization by but bacteria: potential for new insights from genomic analysis. *Nat. Rev. Microbiol.* **2008**, *6*, 121–131.

(5) Jacobs, D. M.; Gaudier, E.; Duynhoven, J. v.; Vaughan, E. E. Non-digestible food ingredients, colonic microbiota and he impact on gut health and immunity: a role for metabolomics. *Curr. Drug Metab.* **2009**, *10*, 41–54.

(6) Galvez, J.; Rodriguez-Cabezas, M. E.; Zarzuelo, A. Effects of dietary fiber on inflammatory bowel disease. *Mol. Nutr. Food Res.* **2005**, *49*, 601–608.

(7) Lim, C. C.; Ferguson, L. R.; Tannock, G. W. Dietary fiber as "prebiotics": implications for colorectal cancer. *Mol. Nutr. Food Res.* **2005**, *49*, 609–619.

(8) Zhang, G. Y.; Hamaker, B. R. Cereal carbohydrates and colon health. *Cereal Chem.* **2010**, *87*, 331–341.

(9) Cummings, J. H.; Mann, J. I.; Nishida, C.; Vorster, H. H. Dietary fiber: an agreed definition. *Lancet* **2009**, *373*, 365–366.

(10) Crittenden, R.; Karppinen, S.; Ojanen, S.; Tenkanen, M.; Fagerström, R.; Mättö, J.; Saarela, M.; Mattila-Sandholm, T.; Poutanen, K. In vitro fermentation of cereal dietary fiber carbohydrates by probiotic and intestinal bacteria. *J. Sci. Food Agric.* **2002**, *82*, 781–789.

(11) Hernot, D. C.; Boileau, T. W.; Bauer, L. L.; Swanson, K. S.; Fahey, G. C., Jr. In vitro digestion characteristics of unprocessed and processed whole grains and their components. *J. Agric. Food Chem.* **2008**, *56*, 10721–10726.

(12) Rose, D. J.; Patterson, J. A.; Hamaker, B. R. Structural differences among alkali-soluble arabinoxylans from maize (*Zea mays*), rice (*Orysa sativa*), and wheat (*Triticum aestivum*) brans influence human fecal fermentation profiles. *J. Agric. Food Chem.* **2010**, *58*, 493–499.

(13) van Laar, H.; Tamminga, S.; Williams, B. A.; Verstegan, M. W. A. Fermentation of the endosperm cell walls of monocotyledon and dicotyledon plant species by faecal microbes from pigs − the relationship between cell wall characteristics and fermentability. *Anim. Feed Sci. Technol.* **2000**, *88*, 13–30.

(14) van Laar, H.; Tamminga, S.; Williams, B. A.; Verstegen, M. W. A. Fermentation characteristics of polysaccharide fractions extracted from the cell walls of maize endosperm. *J. Sci. Food Agric.* **2002**, *82*, 1369–1375.

(15) Chanliaud, E.; Gidley, M. J. In vitro synthesis and properties of pectin/*Acetobacter xylinus* cellulose composites. *Plant J.* **2002**, *20*, 25–35.

(16) Whitney, S. E. C.; Brigham, J. E.; Drake, A. H.; Reid, J. S. G.; Gidley, M. J. In vitro assembly of cellulose/xyloglucan networks: ultrastructural and molecular aspects. *Plant J.* **1995**, *8*, 491–504.

(17) Whitney, S. E. C.; Brigham, J. E.; Drake, A. H.; Reid, J. S. G.; Gidley, M. J. Structural aspects of the interaction of mannan-based polysaccharides with bacterial cellulose. *Carbohydr. Res.* **1998**, *307*, 299–309.

(18) Miller, E. R.; Ullrey, D. E. The pig as a model for human nutrition. *Annu. Rev. Nutr.* **1987**, *7*, 361–382.

(19) Bauer, E.; Williams, B. A.; Bosch, M. W.; Voigt, C.; Mosenthin, R.; Verstegen, M. W. A. Differences in microbial activity of digesta from three sections of the porcine large intestine according to *in vitro* fermentation of carbohydrate-rich substrates. *J. Sci. Food Agric.* **2004**, *84*, 2097–2104.

(20) Moughan, P. J.; Cranwell, P. D.; Darragy, A. J.; Rowan, A. M. The domestic pig as a model animal for studying digestion in humans. In *VIth International Symposium of Digestive Physiology in Pigs*; Souffrant, W.-B.; Hagemeister, H., Eds.; Forschungsinstitut für die Biologie Iandwirtschaftlicher Nutztiere: Dummerstorf, Germany, 1994; pp 389−396.

(21) Williams, B. A.; Bosch, M. W.; Boer, H.; Verstegen, M. W. A.; Tamminga, S. An in vitro batch culture method to assess potential fermentability of feed ingredients for monogastric diets. *Anim. Feed Sci. Technol.* **2005**, *123*, 445–462.

(22) Mikkelsen, D.; Flanagan, B. M.; Dykes, G. A.; Gidley, M. J. Influence of different carbon sources on bacterial cellulose production by *Gluconacetobacter xylinus* strain ATCC 53524. *J. Appl. Microbiol.* **2009**, *107*, 576–583.

(23) Mikkelsen, D.; Gidley, M. J. Formation of cellulose-based composites with hemicelluloses and pectins using *Gluconacetobacter* fermentation. In *Methods in Molecular Biology: The Plant Cell Wall: Methods and Protocols*; Popper, Z. A., Ed.; Humana Press: New York, USA, 2011, in press.

(24) Hestrin, S.; Schramm, M. Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochem. J.* **1954**, *58*, 345–352.

(25) Selvendran, R. R.; DuPont, M. S. An alternative method for the isolation and analysis of cell wall material from cereals. *Cereal Chem.* **1980**, *57*, 278–283.

(26) Seaman, J. F.; Moore, W. E.; Millet, M. A. Hydrolysis and quantative paper chromatography. In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Ed.; Academic Press: New York, 1963; Vol. 3, pp 54−69.

(27) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291–299.

(28) Awati, A.; Williams, B. A.; Bosch, M. W.; Gerrits, W. J. J.; Verstegen, M. W. A. Effect of inclusion of fermentable carbohydrates in the diet on fermentation end-product profile in feces of weanling piglets. *J. Anim. Sci.* **2006**, *84*, 2133–2140.

(29) Williams, B. A.; Bosch, M. W.; Awati, A.; Konstantinov, S. R.; Smidt, H.; Akkermans, A. D. L.; Verstegen, M. W. A.; Tamminga, S. In vitro assessment of gastrointestinal tract (GIT) fermentation in pigs: fermentable substrates and microbial activity. *Anim. Res.* **2005**, *54*, 191–201.

(30) Awati, A.; Williams, B. A.; Bosch, M. W.; Li, Y. C.; Verstegen, M. W. A. Use of the in vitro cumulative gas production technique for pigs: an examination of alterations in fermentation products and substrate losses at various time points. *J. Anim. Sci.* **2006**, *84*, 1110–1118.

(31) Macfarlane, G. T.; Gibson, G. R.; Beatty, E.; Cummings, J. H. Estimation of short-chain fatty-acid production from protein by human intestinal bacteria based on branched-chain fatty-acid measurements. *FEMS Microbiol. Ecol.* **1992**, *101*, 81–88.

(32) Baethgen, W. E.; Alley, M. M. A manual colorimetric procedure for measuring ammonium nitrogen in the soil and plant kjeldahl digests. *Commun. Soil Sci. Plant.* **1989**, *20*, 961–969.

(33) Groot, J. C. J.; Cone, J. W.; Williams, B. A.; Debersaques, F. M. A.; Lantinga, E. A. Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. *Anim. Feed Sci. Technol.* **1996**, *64*, 77–89.

(34) Williams, B. A.; Mikkelsen, D.; le Paih, L.; Gidley, M. J. In vitro fermentation kinetics and end-products of cereal arabinoxylans and (1,3;1,4)-β-glucans by porcine feces. *J. Cereal Sci.* **2011**, *53*, 53–58.

(35) Williams, B. A.; Verstegen, M. W. A.; Tamminga, S. Fermentation in the large intestine of single-stomached animals and its relationship to animal health. *Nutr. Res. Rev.* **2001**, *14*, 207–227.

(36) Cannon, R. E.; Anderson, S. M. Biogenesis of bacterial cellulose. *Crit. Rev. Microbiol.* **1991**, *17*, 435–447.

(37) Ross, P.; Mayer, R.; Benziman, M. Cellulose biosynthesis and function in bacteria. *Microbiol. Rev.* **1991**, *55*, 35–58.

(38) Ioelovich, M.; Leykin, A. Structural investigations of various cotton fibers and cotton celluloses. *Bioresources* **2008**, *3*, 170–177.

(39) Rodriguez, Z.; Lopez, A.; Riveri, Z. Effect of fiber level of the number and activity of the cellulolytic microflora in the caecum of pigs. *Cuban J. Agric. Sci.* **2000**, *34*, 47–55.

(40) Metzler, B. U.; Mosenthin, R. A review of interactions between dietary fiber and the gastrointestinal microbiota and their consequences on intestinal phosphorus metabolism in growing pigs. *Asian Aust. J. Anim. Sci.* **2008**, *21*, 603–615.

(41) Hungate, R. E. *The rumen and its microbes*. Academic Press: New York, 1996.

(42) Van Houtert, M. F. J. The production and metabolism of volatile fatty acids by ruminants fed roughages: a review. *Anim. Feed Sci. Technol.* **1993**, *43*, 189–225.

(43) Englyst, H. N.; Hudson, G. J. The classification and measurement of dietary carbohydrates. *Food Chem.* **1996**, *57*, 15–21.

(44) Van Soest, P. J.; Robertson, J. B.; Lewis, B. A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597.

(45) Granger, D. N.; Barrowman, J. A.; Kvietys, P. R. *Clinical Gastrointestinal Physiology*; Saunders: Philadelphia, PA, 1985.