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The degradation and utilization of wheat-straw cell-wall monosaccharide components by defined ruminal cellulolytic bacteria

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Abstract. Cell walls (CW) of untreated wheat straw and sulphur-dioxide (SO_2)-treated wheat straw were used as model substrates for the hydrolysis and utilization of CW carbohydrates by pure cultures or pair-combinations of defined rumen bacterial strains. *Fibrobacter succinogenes* S85 and BL2 strains and their co-cultures with D1 were the best degraders of CW among ruminal cultures, solubilizing 37.2–39.6% of CW carbohydrates of untreated straw and 62.2–74.5% of SO_2-treated straw. Complementary action between *Butyrivibrio fibrisolvens* D1 and the *F. succinogenes* strains was identified with respect to co-culture growth and carbohydrate utilization. However, the extent of CW solubilization was determined mainly by the *F. succinogenes* strains. In both substrates, utilization of solubilized cellulose by *F. succinogenes* S85 and BL2 monocultures was higher than that of xylan and hemicellulose: 96.5–98.3%, 34.4–40.5% and 33.5–36.2%, respectively. Under scanning electron microscopy visualization, S85 and BL2 cells of the co-cultures comprised the most dense layer of bacterial cell mass attached to and colonized on straw stems and leaves, whereas D1 cells were always nearby. Stems and leaves of the untreated straw were less crowded by attached bacteria than that of the SO_2-treated straw. In both materials, the cell surface topography of S85 and BL2 bacteria attached to CW particles was specified by a coat of characteristic protuberant structures, "polycellulosome complexes".

Introduction

The major obstacle in using wheat straw (WS) as a feedstuff for high-producing ruminants is their high cell-wall (CW) content, and the fact that this CW is rich in matrix hemicellulose and related phenolics and therefore its digestibility by ruminal microbes is very low. In this laboratory it has been shown that by treating WS with sulphur dioxide (SO_2) gas most of the hemicellulose and related phenolics are solubilized, and the residual CW are highly digestible by ruminal microbes in vitro and in vivo (Ben-Ghedalia and Miron 1981, 1983; Miron and Ben-Ghedalia 1982, 1987). However, there is lack of information about the mechanism of interaction between rumen bacterial species involved in straw CW degradation (Forsberg and Cheng 1990). Therefore, the use of untreated WS rich in hemicellulose or SO_2-treated WS (TWS) rich in cellulose, as model substrates for bacterial growth, might provide information about the predominant role of various rumen cellulolytic bacterial strains in determining the extent of CW degradation.

The objectives of this study were:

1. To examine the solubilization of WS and TWS CW-monosaccharide components by the defined ruminal strains *Fibrobacter succinogenes* S85 and BL2, *Ruminococcus flavefaciens* FD1 and C94 and *Butyrivibrio fibrisolvens* D1, grown on CW in monocultures.
2. To identify interactions in CW-monosaccharide solubilization and utilization between *B. fibrisolvens* D1 and each of the other strains when grown in pair combinations on WS or TWS CW.
3. To follow CW degradation studies with scanning electron microscopy (SEM) visualization of the bacterial strains.

Materials and methods

CW substrates. Wheat straw was treated by 5% SO_2 gas at 70° C for 72 h as previously described (Ben-Ghedalia and Miron 1983). Untreated WS and TWS samples were ground to pass through a 1-mm screen and used for CW preparation according to Goering and van Soest (1970), and determination of CW solubilization by defined monocultures and co-cultures of ruminal bacteria.

Organisms and maintenance conditions. The pure *F. succinogenes* S85 and BL2 strains were a generous gift from Dr. C. Stewart, Rowett Research Institute, Aberdeen, Scotland. The strains *R. flavefaciens* FD1 and C94 and *B. fibrisolvens* D1 were generously provides from the stock culture collection of M. P. Bryant, University of Illinois, Urbana, USA. All strains have been characterized in previous publications (Miron et al. 1989; Morris and van Glyswyk 1980; Stewart et al. 1981). Strains were maintained on glucose-cellobiose rumen fluid agar slants prior to inoculation into the growth medium. The composition of the rumen fluid growth

medium, medium preparation, and CW adaptation procedures were as described previously (Miron et al. 1989; Miron 1991). After adaptation to CW, a particle-free bacterial aliquot was employed (in four replicates) for growth, solubilization and utilization of CW monosaccharide assays by the monocultures or by combinations of pair cultures. In parallel, suspensions of CW particles plus attached bacteria in the mid-log phase of growth (48–72 h) were used for cationized ferritin (CF) pretreatment followed by SEM visualization.

Growth, CW solubilization and utilization assays. Each sample of CW-adapted monoculture or co-culture of bacterial inocula was grown in basal medium tubes (15 ml) containing 100 mg CW of either WS or TWS as the sole added carbohydrate substrate. The rate of bacterial growth of each monoculture or the combined cultures was estimated colorimetrically by the optical density at 640 nm (OD_{640}) in the liquid phase of each tube, according to Miron (1991). After 120 h of incubation at 38° C, the remaining CW particles and attached bacteria were sedimented by centrifugation (2000 *g*), and the upper liquid phase was sampled for quantitative determination of monosaccharides solubilized by bacteria but not utilized. Utilization of monosaccharide components was calculated as the ratio of solubilized monosaccharide consumed by bacteria during incubation, to total solubilized monosaccharide component $\times 100$.

The sedimented pellet was then extracted by neutral detergent solution (Goering and van Soest 1970) for adherent bacteria solubilization, washed on a sintered glass filter, freeze-dried, and used for quantitative determination of residual CW monosaccharides, and calculation of solubilization data (see Tables 3 and 4).

Tubes without bacterial inocula in the CW media were treated as above for determination of the monosaccharide components of the original undegraded CW or soluble fractions. Solubilization and utilization results were analysed statistically by a standard analysis of variance design. Duncan's multiple range test served to differentiate between means (Little and Hills 1978).

SEM visualization. A 0.2-ml sample of a suspension containing culture fluid + CW particles, was filtered through a Nucleopore membrane filter (0.6 μm), and then treated by CF prior to glutaraldehyde (5%) fixation. The procedures of CF treatment, glutaraldehyde fixation, dehydration and gold coating of preparation prior to visualization with a Jeol JSM-T330A SEM were as described previously (Miron et al. 1989; Miron 1991).

Analytical procedures. Monosaccharide analysis was performed on ball-milled CW samples and on the soluble fraction, after acidic hydrolysis, as their alditol acetate derivatives. Acid hydrolysis conditions for the CW monosaccharides were 12 M H_2SO_4 for 1 h followed by 1 N H_2SO_4 at 100° C for 5 h. The concentrated acid stage was omitted in the soluble monosaccharide hydrolysis for the utilization assay. The derivation to alditol acetates and detection by gas-liquid chromatography were as described by Miron (1991). The uronic acids in the acid hydrolysates were determined colorimetrically (Blumenkrantz and Asboe-Hansen 1973). Lignin was determined by the permanganate procedure (Goering and van-Soest 1970).

Results and discussion

Chemical composition of WS CW and TWS CW

The SO_2 treatment of WS reduced the CW content from 78.1 to 51.6 g/100 g dry matter, solubilizing mainly the hemicellulose fraction and related phenolic components. The composition (g/100 g dry CW) of the undegraded CW fraction supplied to the cultures is shown in Table 1. The TWS CW contained 39% more cellulose, 18% more lignin and 36.6% less hemicellulose than the WS CW.

Table 1. Chemical composition of cell-walls (CW) (g 100 g^{-1} dry CW) of untreated wheat straw (WS) and SO_2-treated WS (TWS)

CW component	WS	TWS
Glucose polymers (cellulose)	45.1	62.5
Xylose (xylan)	24.6	17.6
Arabinose	3.34	0.58
Uronic acids	3.20	1.60
Mannose	0.19	0.30
Galactose	0.63	0.19
Total non-glucose polysaccharides (NGP = hemicellulose)	32.0	20.3
Total polysaccharides	77.1	82.8
Lignin (permanganate)	12.8	15.1

Growth and CW solubilization by the bacterial cultures

Solubilization of WS and TWS CW by the bacterial monocultures and co-cultures is detailed in Table 2. This table also shows the maximal absorption values (OD_{640}) obtained in the liquid medium at the end of the log phase of bacterial growth on CW. Generally, the effect of the treatment was removal of the major biodegradation obstacle in the matrix of WS CW, i.e. the hemicellulose (Miron and Ben-Ghedalia 1982). Consequently, the CW solubilization by *F. succinogenes* monocultures S85 and BL2 and their co-cultures with D1 was increased by 51–90%. On the other hand, the *Ruminococcus* monocultures and pair combinations, which are

Table 2. Solubilization of CW (g 100 g^{-1} dry CW) and maximal growth [optical density ($OD_{640\,nm}$)] on CW of untreated WS and TWS by defined cultures of ruminal bacteria

Bacterial strain		Solubilization (%)		Growth (OD)	
		WS	TWS	WS	TWS
Fibrobacter succinogenes	S85	34.5^{a}	65.5^{a}	0.22^{b}	0.63^{c}
	BL2	32.1^{a}	59.1^{b}	0.21^{b}	0.46^{d}
Butyrivibrio fibrisolvens	D1	20.0^{b}	14.4^{d}	0.13^{c}	0.18^{f}
Ruminococcus flavefaciens	FD1	12.5^{d}	3.53^{ef}	0.08^{d}	0.09^{g}
	C94	10.2^{d}	2.71^{f}	0.16^{bc}	0.18^{f}
S85 + D1		34.9^{a}	61.2^{b}	0.37^{a}	1.18^{a}
BL2 + D1		32.9^{a}	49.8^{c}	0.38^{a}	0.93^{b}
FD1 + D1		15.4^{c}	6.25^{e}	0.16^{bc}	0.21^{ef}
C94 + D1		17.7^{bc}	2.69^{f}	0.22^{b}	0.23^{e}
SEM		0.98	1.01	0.02	0.01

SEM = standard error of the means
^{a,b,c,d,e,f,g} Within columns, means followed by a common letter do not differ statistically $P<0.05$

hemicellulolytic rather then cellulolytic by nature (Morris and van-Gylswyk 1980; Weimer et al. 1991), solubilized the hemicellulose-rich WS CW better than the cellulose-rich TWS CW.

Data in Table 2 show that *F. succinogenes* S85 and BL2 strains were the best CW solubilizers of both WS and TWS CW. The high hydrolytic ability of pure *F. succinogenes* to solubilize plant CW shown in this study was demonstrated also in previous studies employing different CW substrates (Cheng et al. 1983; Chesson et al. 1986; Dehority and Scott 1967; Miron 1991; Osborne and Dehority 1989; Varel et al. 1991). This high hydrolytic ability of S85 was explained (Miron 1991) by two characteristics of this strain: (i) the high attachment ability to, colonization on and penetration through CW particles, as demonstrated under electron microscopy visualization (Cheng et al. 1983; Miron et al. 1989, 1990; Varel et al. 1991), and (ii) the wide spectra of enzymatic capabilities of *F. succinogenes* employed in the degradation of cellulose and xylan (Forsberg and Cheng 1990).

With the pair-combinations S85 + D1 and BL2 + D1 grown on WS and TWS, the maximal absorption values obtained in the liquid phase were equal to or higher than the calculated sum of individual strains. This phenomenon implies a complementary pattern of growth, probably originating from better utilization of the solubilized CW carbohydrate by those co-cultures (see Tables 5, 6). However, this effect of complementary growth was not reflected in the overall extent of CW solubilization, which was determined mainly by the *F. succinogenes* strains during the 120 h of co-culture incubation.

The poor CW-solubilizing abilities of the *Ruminococcus* monocultures and co-cultures with D1 (Table 2), motivated the restriction of further monosaccharide solubilization and utilization analyses only to the *F. succinogenes* monocultures and co-cultures with D1.

Bacterial solubilization of CW monosaccharide components

To the best of our knowledge there are only a few studies demonstrating the interaction between ruminal strains in the degradation of CW down to the level of the monosaccharide components (Miron 1991; Varel et al. 1989).

The solubilization of untreated WS and treated WS CW monosaccharide components by the monocultures *F. succinogenes* S85 and BL2, *B. fibrisolvens* D1 and by the co-cultures S85 + D1 and BL2 + D1, is presented in Tables 3 and 4.

The data show that in both CW substrates there was similar solubilization values of cellulose (CW glucose) and xylan by the pure *F. succinogenes* strains and by their co-cultures with D1. *F. succinogenes* strains S85 and BL2 were the dominant cultures in determining the extent of all CW polysaccharides solubilization in their co-cultures with D1.

Table 3. Solubilization (%) of CW-monosaccharide components of untreated WS by the pure rumen bacterial strains *F. succinogenes* S85 or BL2 and *B. fibrisolvens* D1, and by the pair-combinations S85 + D1 and BL2 + D1

CW component	Bacterial strains					SEM
	S85	BL2	D1	S85 + D1	BL2 + D1	
Glucose (cellulose)	38.0^{a}	37.6^{a}	19.0^{b}	37.6^{a}	35.7^{a}	0.76
Xylose	38.5^{ab}	36.1^{b}	29.6^{c}	40.6^{a}	37.0^{b}	0.72
Uronic acids	35.6^{a}	36.1^{a}	29.0^{b}	35.8^{a}	36.1^{a}	0.76
Arabinose	55.2^{b}	52.2^{c}	44.6^{d}	59.5^{a}	56.4^{b}	0.51
Total NGP (= hemicellulose)	40.6^{b}	38.1^{b}	31.3^{c}	42.8^{a}	39.4^{b}	0.70
Total monosaccharides	39.0^{a}	37.8^{a}	23.8^{b}	39.6^{a}	37.2^{a}	0.73

SEM = standard error of the means
^{a,b,c,d} Within rows, means followed by a common letter do not differ statistically, $P<0.05$

Table 4. Solubilization (%) of CW-monosaccharide components of TWS by the pure rumen bacterial strains *F. succinogenes* S85 or BL2 and *B. fibrisolvens* D1, and by the pair-combinations S85 + D1 and BL2 + D1

CW component	Bacterial strains					SEM
	S85	BL2	D1	S85 + D1	BL2 + D1	
Glucose (cellulose)	74.7^{a}	70.1^{b}	26.9^{d}	72.6^{a}	61.3^{c}	0.70
Xylose	74.9^{a}	72.3^{b}	38.7^{d}	74.1^{ab}	67.0^{c}	0.63
Uronic acids	61.7^{a}	62.0^{a}	48.9^{d}	58.6^{b}	55.5^{c}	0.85
Arabinose	70.6^{a}	65.0^{b}	32.5^{d}	68.4^{a}	59.8^{c}	0.75
Total NGP (= hemicellulose)	74.0^{a}	71.3^{b}	38.5^{d}	73.1^{ab}	66.0^{c}	0.64
Total monosaccharides	74.5^{a}	70.3^{b}	29.2^{d}	72.7^{a}	62.2^{c}	0.69

SEM = standard error of the means
^{a,b,c} Within rows, means followed by a common letter do not differ statistically, $P<0.05$

The SO_2 treatment effect was expressed in increasing by 91% and 86% the solubilization of the total CW monosaccharide components by S85 and BL2, respectively, and by 67–84% in their co-cultures with D1. A lesser improvement effect of treatment was found with the D1 monoculture.

The WS and TWS CW monosaccharide-solubilizing ability of *F. succinogenes* monocultures are very similar to in-vivo digestibility data (Miron and Ben-Ghedalia 1987) suggesting that *F. succinogenes* strains might have an important role in determining the extent of CW degradation in the rumen.

Utilization of solubilized carbohydrates by bacterial strains

Table 5 shows the utilization of the bacterial solubilized monosaccharide components of WS CW by the S85, BL2 and D1 monocultures and by the pair combinations S85 + D1 and BL2 + D1. Table 6 presents the data on TWS CW carbohydrate utilization. Tables 5 and 6 show that solubilized cellulose was highly utilized (96.5–100%) by all bacterial monocultures and combinations. *B. fibrisolvens* D1 and its co-cultures with *F. succinogenes* strains utilized very efficiently (93.7–99.8%) the solubilized hemicellulose components of both substrates, whereas *F. succinogenes* strains utilized only 35–40% of the xylan and 34–36% of hemicellulose (Tables 5 and 6) of both substrates, despite the large differences in their extent of hemicellulose solubilization (Tables 3 and 4). Similar utilization values of larchwood xylan and rye-grass hemicellulose by S85 bacteria were reported also by Williams and Withers (1982). Notwithstanding, lower values of hemicellulose utilization were obtained with S85 bacteria grown on teff (*Eragrostis abyssinica*) cell-walls or wood xylan (Morris and van-Gylswyk 1980) and with *F. succinogenes* A3C grown on orchard-grass (Osborne and Dehority 1989). This discrepancy between the studies can be explained by the indirect analytical procedures used for the determination of hemicellulose fraction in the studies of Morris and van-Gylswyk (1980) and Osborne and Dehority (1989).

The higher utilization values found with the co-cultures S85 + D1 and BL2 + D1 (Tables 5 and 6) compared to S85 and BL2 monocultures, and the fact that the presence of D1 in co-cultures does not affect (Table 4) or only slightly reduced (Table 3) the extent of CW monosaccharide solubilization by the *F. succinogenes* strains, suggests that these strains can be considered complementary in the degradation of WS and TWS CW. Additional support to this is obtained from growth values (Table 2) and the SEM observations (Fig. 1), and from previous studies (Cheng et al. 1983; Chesson et al. 1986; Miron 1991; Miron et al. 1989).

Table 5. Utilization (%) of the solubilized CW-monosaccharide components of untreated WS by the pure rumen bacterial strains *F. succinogenes* S85 or BL2, and *B. fibrisolvens* D1, and by the pair-combinations S85 + D1 and BL2 + D1

CW component	Bacterial strains					SEM
	S85	BL2	D1	S85 + D1	BL2 + D1	
Glucose (cellulose)	98.2[a]	97.3[a]	99.0[a]	99.1[a]	99.8[a]	1.26
Xylose	40.5[b]	39.8[b]	98.0[a]	99.8[a]	98.7[a]	1.16
Uronic acids	27.2[d]	32.1[c]	86.5[a]	82.2[b]	81.0[b]	0.97
Arabinose	25.1[d]	32.4[c]	90.7[b]	95.7[a]	94.3[a]	1.10
Total NGP (= hemicellulose)	35.6[b]	36.2[b]	95.0[a]	94.1[a]	93.7[a]	1.08
Total monosaccharides	72.8[b]	73.3[b]	96.9[a]	97.0[a]	97.4[a]	1.19

SEM = standard error of the means
[a,b,c,d] Within rows, means followed by a common letter do not differ statistically, $P<0.05$

Table 6. Utilization (%) of the solubilized CW monosaccharide components of TWS by the pure rumen bacterial strains *F. succinogenes* S85 or BL2, and *B. fibrisolvens* D1, and by the pair-combinations S85 + D1 and BL2 + D1

CW component	Bacterial strains					SEM
	S85	BL2	D1	S85 + D1	BL2 + D1	
Glucose (cellulose)	98.3[a]	96.5[a]	100.0[a]	99.6[a]	98.9[a]	2.02
Xylose	34.4[b]	35.5[b]	99.7[a]	98.9[a]	99.1[a]	1.66
Uronic acids	49.5[b]	45.2[b]	96.1[a]	92.0[a]	91.5[a]	1.60
Arabinose	27.0[b]	18.3[c]	99.8[a]	99.1[a]	99.6[a]	1.60
Total NGP (= hemicellulose)	33.5[b]	33.6[b]	99.8[a]	99.7[a]	99.3[a]	1.63
Total monosaccharides	84.7[b]	83.0[b]	99.9[a]	98.6[a]	99.1[a]	1.93

SEM = standard error of the means
[a,b,c,d] Within rows, means followed by a common letter do not differ statistically, $P<0.05$

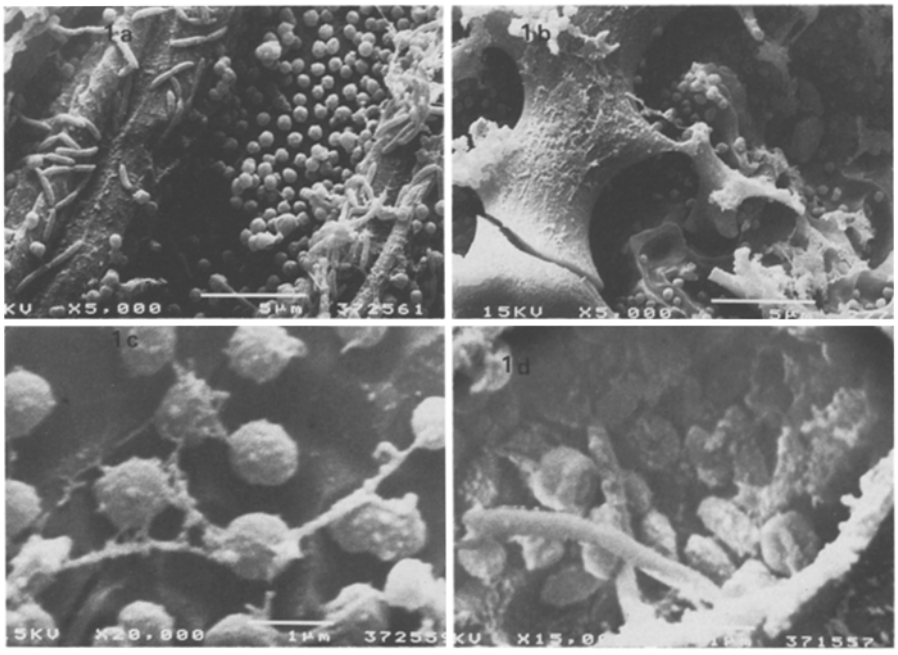

Fig. 1a–d. Scanning electron micrographs of cationized ferritin-pretreated bacterial cells of the following monocultures and co-cultures grown on cell walls (CW) of wheat straw (WS) or SO_2-treated WS (TWS). **a** *Fibrobacter succinogenes* S85 plus *Butyrivibrio fibrisolvens* D1 co-culture on leaf CW tissue of TWS. **b** S85 monoculture on leaf CW tissue of WS. **c** S85 monoculture on stem CW tissue of TWS. **d** *F. succinogenes* BL2 plus D1 co-culture inside a stoma of WS leaf tissue. Note the high density of bacterial mass of the S85 and BL2 cells colonizing damaged tissues of WS and TWS CW particles (**a–d**), the appearance of protuberant structures, "polycellulosome complexes", on surface topography of S85 and BL2 cells attached to CW of both substrates (**c, d**), the pit formation by S85 cells (**c**) and the mode of complimentary living of the co-cultures S85 + D1 (**a**) and BL2 + D1 (**d**). In **a** and **b**, *bar* = 5 μm; in **c** and **d**, *bar* = 1 μm

Electron microscopy visualization of bacterial cultures

The rumen bacterial monocultures and co-cultures examined were subjected to stabilization treatment with cationized ferritin, and the treated cells were visualized by SEM. *F. succinogenes* strains S85 and BL2 and their co-cultures with D1 colonized WS leaves and stems only in broken tissue unprotected by epidermal CW tissue (Fig. 1b) and also under open stomata (Fig. 1d). The TWS leaves and stems were characterized by a higher proportion of broken and unprotected tissues, and therefore were heavily colonized by a denser layer of *F. succinogenes* S85 and BL2 cells, as demonstrated in Fig. 1a and c.

In those unprotected tissues of both substrates the *F. succinogenes* S85 and BL2 cells were firmly attached to CW particles creating pits in the surounding CW tissue as shown in Fig. 1c, and their surface topography was characterized by the presence of protuberant structures (Fig. 1c and d). This surface topography of BL2 and S85 cells is similar to that found with S85 monoculture grown on lucerne CW (Miron et al. 1989) and supports the "polycellulosome complexes theory" suggesting that these complexes, induced by the presence of CW substrate, are involved in attachment to the substrate and the CW hydrolysis mechanism of ruminal cellulolytic bacteria (Miron et al. 1989, 1990; Forsberg and Cheng 1990).

SEM visualization of the S85 + D1 and BL2 + D1 co-cultures grown on leaves of WS and TWS (Fig. 1a and d) shows that *F. succinogenes* S85 and BL2 cells comprised a dense bacterial mass firmly attached to and colonizing CW tissue, whereas only few D1 cells were distributed among the *F. succinogenes* cells, and both strains of the co-cultures were living together in the same area. These findings support the hydrolysis values (Tables 2–4), showing the importance of *F. succinogenes* cells in determining the extent of CW hydrolysis in co-cultures with D1, and the complementation in carbohydrate utilization and growth of the co-cultures (Tables 2, 5 and 6).

Additional research employing wider spectra of CW substrates is needed to assess the findings of this study about the predominant role of *F. succinogenes* in the degradation and utilization of CW carbohydrates in the rumen.

References

Ben-Ghedalia D, Miron J (1981) Effect of sodium hydroxide, ozone and sulfur dioxide on the composition and *in vitro* digestibility of wheat straw. J Sci Food Agric 32:224–228

Ben-Ghedalia D, Miron J (1983) The response of wheat straw varieties to mild sulphur dioxide treatment. Anim Feed Sci Technol 10:269–276

Blumenkrantz N, Asboe-Hansen G (1973) New method for quantitative determination of uronic acids. Anal Biochem 54:484–489

Cheng KJ, Stewart CS, Dinsdale D, Costerton JW (1983) Electron microscopy of bacteria involved in the digestion of plant cell walls. Anim Feed Sci Technol 10:93–120

Chesson A, Stewart CS, Dalgarno K, King TP (1986) Degradation of isolated grass mosophyll, epidermis and fiber cell walls in the rumen and by cellulolytic rumen bacteria in axenic culture. J Appl Bacteriol 60:327–336

Dehority BA, Scott HW (1967) Extent of cellulose and hemicellulose digestion in various forages by pure cultures of rumen bacteria. J Dairy Sci 50:1136–1141

Forsberg CW, Cheng KJ (1990) Integration of rumen microorganisms and their hydrolytic enzymes during the digestion of lignocellulosic materials. In: Akin et al. (eds) Microbial and plant opportunities to improve lignocellulose utilization by ruminants. Elsevier, New York, pp 411–423

Goering HK, van Soest PJ (1970) Forage fiber analysis. USDA Agricultural Handbook no. 379. USDA, Washington, D.C., pp 1–19

Little TM, Hills FJ (1978) In: Agricultural experimentation, design and analysis. Wiley, New York, p 47–75

Miron J (1991) The hydrolysis of lucerne cell-wall monosaccharide components by monocultures or pair combinations of defined ruminal bacteria. J Appl Bacteriol 70:245–252

Miron J, Ben-Ghedalia D (1982) Effect of hydrolyzing and oxidizing agents on the composition and degradation of wheat straw monosaccharides. Eur J Appl Microbiol Biotechnol 15:83–87

Miron J, Ben-Ghedalia D (1987) Digestibility by sheep of total and cell-wall monosaccharides of wheat straw treated chemically or chemically plus enzymatically. J Dairy Sci 70:1876–1884

Miron J, Yokoyama MT, Lamed R (1989) Bacterial cell surface structures involved in lucerne cell wall degradation by pure cultures of cellulolytic rumen bacteria. Appl Microbiol Biotechnol 32:218–222

Miron J, Ben-Ghedalia D, Yokoyama MT, Lamed R (1990) Some aspects of cellobiose effect on cell surface structures involved in lucerne cell walls utilization by fresh isolates of rumen bacteria. Anim Feed Sci Technol 30:107–120

Morris EJ, van Gylswyk NO (1980) Comparison of the action of rumen bacteria on cell walls from *Eragrostis* tef. J Agric Sci 95:313–323

Osborne JM, Dehority BA (1989) Synergism in degradation and utilization of intact forage cellulose, hemicellulose, and pectin by three pure cultures of ruminal bacteria. Appl Environ Microbiol 55:2247–2250

Stewart CS, Paniagua C, Dinsdale D, Cheng KJ, Garrow SH (1981) Selective isolation and characteristics of *Bacteroides succinogenes* from the rumen of a cow. Appl Environ Microbiol 41:504–510

Varel VH, Richardson AJ, Stewart CS (1989) Degradation of barley straw, ryegrass and alfalfa cell walls by *Clostridium longisporum* and *Ruminococcus albus*. Appl Environ Microbiol 55:3080–3084

Varel VH, Jung HG, Krumholz LR (1991) Degradation of cellulose and forage fiber fractions by ruminal cellulolytic bacteria alone and in coculture with phenolic monomer degrading bacteria. J Anim Sci 69:4993–5000

Weimer PJ, French AD, Calamari TA (1991) Differential fermentation of cellulose allomorphs by ruminal cellulolytic bacteria. Appl Environ Microbiol 57:3101–3106

Williams AG, Withers SE (1982) The effect of carbohydrate growth substrates on the glycosidase activity of hemicellulose-degrading rumen bacterial isolates. J Appl Bacteriol 52:389–401