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Pretreatment of Sugar Cane Bagasse for Enhanced Ruminal Digestion

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ABSTRACT

Crop residues, such as sugar cane bagasse (SCB), have been largely used for cattle feeding However, the close association that exists among the three major plant cell-wall components, cellulose, hemicellulose, and lignin, limits the efficiency by which ruminants can degrade these materials Previously, we have shown that pretreatment with 3% (w/w) phosphoric acid, under relatively mild conditions, increased considerably the nutritional value of SCB. However, in this preliminary study, pretreated residues were not washed prior to *in situ* degradability assays because we wanted to explore the high initial solvability of low-mol-wt substances that were produced during pretreatment We have now studied the suitability of water- and/or alkali-washed residues to *in situ* ruminal digestion. Alkali washing increased substrate cellulose content by removing most of the lignin and other residual soluble substances As a result, the ruminal degradability of these cleaner materials had first-order rate constants five times higher than those substrates with higher lignin content (e.g., stem-exploded bagasse) However, alkali washing also increased the time of ruminal lag phase of the cellulosic residue, probably because of hemicellulose and/or lignin removal and to the development of substrates with higher degree of crystallinity. Therefore, longer lag phases appear to be related to low microbial adherence after extensive water and alkali extraction, as Novell as to the slower process of cellulase induction during ruminal growth. The kinetic data on ruminal digestion were shown to be very well adjusted by a nonlinear model. Although pretreatment enhances substrate accessibility, the occurrence of an exceedingly high amount of lignin byproducts within the pretreated material reduces considerably its potential degradability.

Index Entries: Sugar cane bagasse; autohydrolysis; phosphoric acid; *in situ* degradability.

INTRODUCTION

It has been suggested that the process of ruminal digestion depends on adherence of the rumen biota to the cellulosic substrate *(1)* Three main factors are known

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to influence this process: the degree of lignification of the cell wall, the degree of crystallinity of the cellulose component, and the substrate surface area available for microbial and/or enzymatic attack *(2,3)* Therefore, the structure and composition of the substrate must facilitate microbial adherence through interaction with large multicomponent complexes (cellulosomes) *(4,5)*, microbial exopolysaccharides *(6)*, and cellulose binding domains (CBD) of fungal hydrolases *(7,8)*. In general, microbial adhesion occurs rapidly and is strongly influenced by changes in pH, temperature, and ionic strength inside the ruminal tract *(9)*.

Several pretreatment methods have been shown to enhance the susceptibility of lignocellulosic residues to microbial degradation, even at relatively mild severities. This enhancement in degradability is usually triggered by partial hemicellulose solubilization, yielding water-soluble low-mol-wt xylooligomers, which can be readily utilized by the rumen biota *(10)*. However, insoluble pretreated residues often contain most of the metabolic energy available for animal growth and production. Therefore, the utilization of this residue for cattle feeding is of utmost importance, and will depend on pretreatment optimization with regard to substrate accessibility and production of growth inhibitors.

Pretreatment with phosphoric acid under relatively mild conditions increases the initial solubility of sugar cane bagasse (SCB) (fraction that was immediately available to the rumen microorganisms) from 6.8 to 27.5%, without having a similar effect on potential degradability *(10,11)*. This improvement in initial solubility was a result from the hydrolytic effect of pretreatment on the hemicellulose component, yielding low-mol-wt substances, which could be readily utilized by the rumen biota Steam explosion (autohydrolysis) has also been shown to improve both the potential degradability and the initials solubility of cellulosic residues by similar mechanisms *(12–15)*. Finally, the potential degradability of SCB can be considerably increased by alkali washing *(16)*. This has been attributed to the ability of this treatment to reduce both the lignin and hemicellulose content oaf the residue, yielding a cellulosic material that can be readily used for cattle feeding.

In this article, we have tried to identify the substrate-related factors that can be used to evaluate the suitability of several cellulosic residues for cattle feeding. Four pretreatment methods have been assessed: phosphoric acid treatment, alkali washing, steam explosion, and steam explosion followed by alkali washing. All of these residues, except the raw material, were washed thoroughly prior to any ruminal degradability assays in order to allow for the determination of their actual potential degradabilities.

MATERIALS AND METHODS

Pretreatment of SCB

A large batch of SCB was obtained from the processing stages of a sugar mill in southern Brazil (Porto Belo Distilleries, Itajaí, SC). The residue, which contained about 2% (w/w) of contaminating sucrose and had a moisture content of 48% (w/w), was conditioned in plastic bags md stored frozen at –20°C.

The sample named ALK was obtained by treating SCB with hot alkali. Approximately 30 g of SCB were milled to pass a 2-mm screen and treated with 900 mL of NaOH 2*N* for 2 h at room temperature. The suspension was then kept under reflux for l h, filtered through a nylon bag, dialyzed overnight against tap water, and dried for 48 h at 55°C. The alkali-extracted residue was then Willey-milled to

pass a 0.5-mm screen and kept for further analysis, yielding approx 40% (w/w) ion relation to the dry weight of the raw material.

Both solvolysis (SOL) and phosphoric acid (OPA) residues were obtained as described previously *(10,11)*. SCB was pretreated at temperatures up to 197°C (13.5 atm) in the presence and absence of OPA as an acid catalyst. OPA was used at 3% (w/w) in relation to the dry weight of the residue. SOL was defined as those experiments in which water was used as a replacement to the dilute-acid solution Both samples were washed thoroughly with ethanol at 70% ([v/v] aqueous) for 1 h at room temperature to eliminate low-mol-wt contaminants. Yields were around 78% for the SOL residue and 60% for the OPA residue, both in relation to their original oven-dried weights

The steam-exploded (SE) SCB was kindly supplied by André Luiz Ferraz (CEBIQ, FAENQUIL—Lorena, SP, Brazil). The SE fraction was prepared at the CEBIQ pilot plant facility by steam treating SCB at 190°C for 15 min. Recovery yield of the water-insoluble fraction was typically around 66.6%. Finally, SE alkali washing (SEALK) was carried out by treating the SE residue with alkali (NaOH 1%, w/w) at 100°C for 1 h and a water-to-solids ratio of 1:20 (dry basis). After alkali washing, the residual material was washed thoroughly with water until neutrality and stored at 4°C for further utilization SEALK had a yield around 39.6% in relation to the dry weight of the original, untreated SCB.

Substrate Analysis

The chemical composition of the residues was determined using the classical methods of neutral detergent fiber (NDF) and acid detergent fiber (A-DF) as described previously *(17)*. By definition, the NDF fraction includes lignin, cellulose, and hemicellulose, whereas the ADF fraction consists of only cellulose and lignin. The hemicellulose content was estimated by subtracting ADF from NDF, whereas cellulose was determined gravimetrically after treatment of the residue with the oxidative acetic/nitric acid reagent *(18)*.

The relative amount of low-mol-wt phenolic compounds within the cellulosic residue was estimated by UV/vis spectroscopy. Approximately 0.25 g of the washed residue was treated with 5 mL of methanol:HCl (1%, [v/v]) for 96 h at room temperature. The supernatant was collected by centrifugation, diluted in methanol (1:50), and scanned in a Zeiss Specord M-500 UV/vis spectrophotometer from 200 to 500 nm in wavelength

Substrate chemical composition was also assessed by Fourier-transformed infrared spectroscopy (FT-IR). After milling, each cellulosic material was compacted into KBr disks at 8 t of pressure for 3 min at room temperature and analyzed using a Bomem FT-IR spectrophotometer within the region of 800–1900/cm. Peak intensities at 897/cm were associated with cellulose *(19)*. Hemicelluloses were monitored by absorption bands at 1734/cm (axial stretch deformations of carboxyl groups) and within the region of 1160–1300 cm (axial stretch deformations of ether bonds) *(19,20)*. Finally, peak intensities at 1590/cm and within the region of 1590–1600/cm were collectively attributed to carbon-to-carbon axial stretch deformations of aromatic compounds found in lignin *(19,20)*.

The degree of crystallinity of the substrates was obtained by X-ray diffraction. Each sample was compressed into compact sheets and analyzed using a Philips X'Pert diffractometer. The wavelength of the Cu/Kaα radiation source was 0.154 nm and the spectra were obtained at 30 mA and 40 kV. Samples were

scanned on the automated diffractometer from 8 to 40° of 2Θ (Bragg angle), with an elapsed time of 1 s and data acquisition taken at intervals of 0.02° *(21)*.

The crystallinity index of cellulose was calculated by the empirical method described by Segal et al. *(22)*, using the following equation:

$$CrI\ (\%) = (I_{002} - I_{AM}/I_{002}) \times 100 \quad (1)$$

where I_{002} is the maximum intensity of the 002 lattice diffraction (reflection attributed to the crystalline region of the sample), and I_{AM} is the intensity of diffraction at 2Θ = 18° (reflection attributed to the amorphous region of the sample).

Determination of *In Situ* Degradability (Ruminal Digestion)

Samples were subjected to *in situ* ruminal digestion as described earlier *(11,23)*. Approximately 0.5 g of biomass (oven-dried weight) were placed in a 9 × 15 cm nylon bag with a pore size of 40 μm and incubated for several residence times in cattle fitted with rumen cannulae. After a given incubation time, the bag was withdrawn from the rumen cannulae and washed thoroughly with tap water. The residual, undigested material was then collected from the bag, air-dried at 55°C, weighed, and kept for further analysis.

Kinetics of ruminal digestion were determined after 0, 6, 10, 16, 24, 32, 48, and 72 h of incubation. Two animals were used in these experiments and each experiment was done in duplicate for each animal. Therefore, each data point was a result of a total of four replicates. Results derived from *in situ* ruminal digestion, expressed as weight loss in relation to the dry weight of the residue, were adjusted according to the following equation *(23)*:

$$p = a + b\ (1 - \exp - c{\cdot}t) \quad (2)$$

were p is the potential degradability of the substrate, t is the ruminal digestion time, a is the fraction of the substrate that is immediately available to the rumen biota (initial solubility), b is the degradability potential of the insoluble fraction, and c is the expected degradation rate of fraction b.

Based on the model, the *in situ* lag phase of each of the substrates used was calculated with regard to the amount of original material that remained after 6–32 h of incubation (undigested dry matter). For this, the weight loss data for each cellulosic substrate were fitted by linear regression analysis to a discontinuous first-order kinetic equation *(24,25)*:

$$\ln y = a + k{\cdot}t \quad (3)$$

were y is the undigested dry matter, α is the intercept at the ordinate axis, k is the first-order rate constant and t is the incubation time. In adjusting the data to the model, k was calculated by regressing ln y against t. The *in situ* lag phase was then calculated by solving the regression equation for time when ln y = ln 1.

The effective *in situ* degradability of each residue was determined according to the following equation *(23)*:

$$e = a + (b \times c)/(c + k) \quad (4)$$

where e is the material effectively degraded and k is the rate of passage of the diet through the rumen tract.

Table 1
Chemical Composition of the Various Cellulosic Residues

Sample[b]	NDF[b]	ADF[b]	Hemicellulose[c]	Cellulose[d]	CrI, %[e]
Native	87.0	53.0	34.0	39.5	36.2
ALK	94.7	85.6	9.1	88.3	57.5
SOL	89.7	67.0	22.7	55.5	42.2
OPA	92.0	85.5	6.5	68.1	42.3
SE	78.0	73.3	4.7	65.8	46.3
SEALK	98.5	95.8	2.7	92.3	53.7

[a]ALK, alkali washed; SOL, solvolyzed; OPA, phosphoric acid treated; SE, steam exploded; SEALK, steam exploded and alkali washed. All values are averages of triplicates with a coefficient of variation always lower than 3%.

[b]Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest *(17)*.

[c]Hemicellulose content was determined by subtracting ADF from NDF.

[d]Cellulose was determined gravimetrically after digestion of the residue with the acetic/nitric reagent according to Crampton and Maynard *(18)*.

[e]Crystallinity index was determined according to Segal et al. *(22)*.

RESULTS AND DISCUSSION

The suitability of several cellulosic residues for cattle feeding was evaluated with regard to their chemical composition, potential degradability, accessibility to rumen microorganisms, and time of lag phase during ruminal digestion. In addition, the rate of passage of the diet through the digestive tract was also considered in order to predict the effective degradability of the substrates.

Each of the substrates used in this study was shown to contain very little amount of detergent-extractable materials in their chemical composition (Table 1). Therefore, their NDF values were very high, except for the SE sample, which had a lower NDF content. The high recovery yield of both NDF and cellulose components after alkali washing also indicated that this extraction was very effective in removing both hemicellulose and lignin, leaving a cellulosic residue with nearly 90% of cellulose (ALK and SEALK).

The higher amount of hemicellulose in SOL has demonstrated that hemicellulose is more effectively removed when pretreatment is carried out in the presence of an acid catalyst, such as OPA *(10,11)*. This observation is also valid for the SE fraction, since several organic acids, such as acetic and formic acids, are released during the process of steam explosion (autohydrolysis).

FT-IR spectroscope showed that all pretreatment methods, except solvolysis, were effective in removing most of the hemicellulose component of SCB (Fig. 1). Compared to the native residue, a considerable decrease in intensity of the absorption band at 1734/cm (C = O stretching deformation in acetyl groups and/or 4-*O*-methylglucuronic acid units) was observed in relation to the peak intensities at 1510 and 1600/cm (aromatic compounds of lignin) *(20)*. The effective removal of lignin by alkali washing was further confirmed by the new disappearance of latter absorbance band in both ALK and SEALK spectra. It was also apparent that lignin removal by alkali washing was more effective after steam explosion,

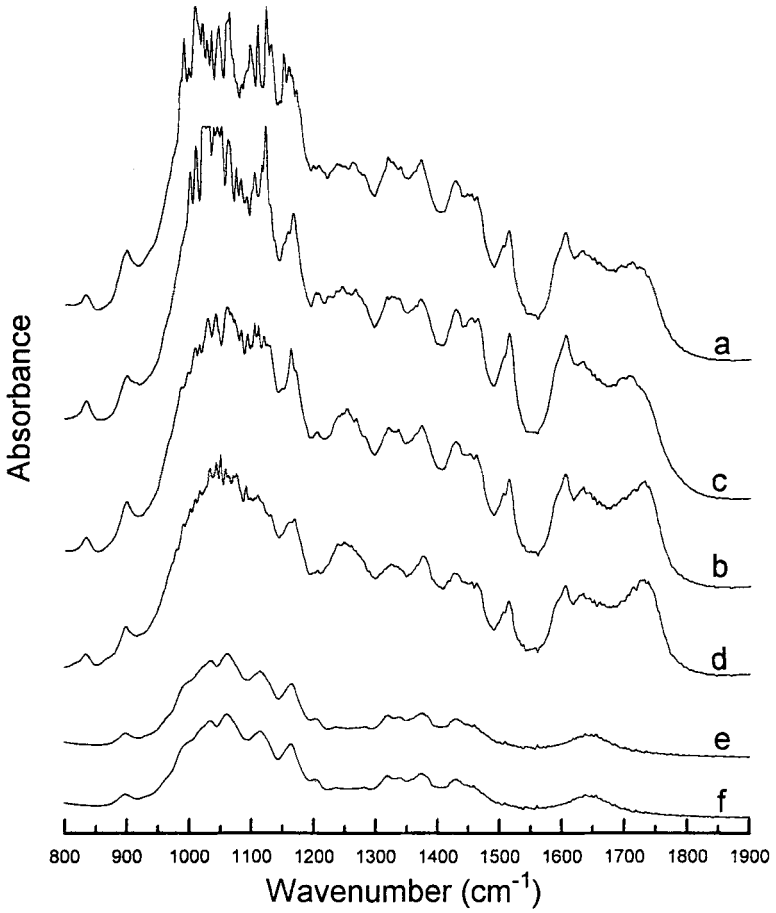

Fig. 1. FT-IR spectra of substrates derived from SCB **(a)** OPA, **(b)** SOL, **(c)** SE, **(d)** Native, **(e)** ALK, and **(f)** SEALK.

probably owing to the higher accessibility of the steam-exploded fibers to the alkaline reagent.

With regard to the kinetic parameters of ruminal digestion, all of the pretreated substrates were shown to have very low *a* values (Table 2). This result was expected, since *a* is usually low for extracted materials, which are mainly composed of polymeric compounds, such as cellulose, hemicellulose, and lignin (*23*). Nevertheless, even though SE had been water-washed after pretreatment, its initial solubility (3.4%) was slightly higher than that of other lignin-containing residues, such as OPA and SOL. This higher initial solubility SE was consistent with its lower NDF content of 78% (Table 1), indicating that a small amount of detergent-extractable materials (readily accessible, low-mol-wt lignin–carbohydrate complexes) still remained within the sample after water washing. It appeared that these low-mol-wt substances, particularly phenolic compounds, were more effectively removed from the substrates by ethanol/water extraction. In fact, both SOL and OPA were shown to have higher ruminal lag phases than SE (7:00, 6:32, and 1:08 h, respectively), supporting the concept that a simple water washing is not enough to remove most of the low-mol-wt substances from steam-treated pulps (Table 3).

Table 2
Kinetic Parameters of Ruminal Digestion

Sample	a[a]	b[b]	c[c]	r^2	t = 72[d]	k = 0.02[e]
Native	0.068	0.339	0.040	0.97	0.38	0.30
ALK	0.000	0.987	0.037	0.93	0.92	0.64
SOL	0.014	0.815	0.017	0.95	0.59	0.39
OPA	0.004	0.735	0.016	0.96	0.51	0.33
SE	0.034	0.623	0.026	0.96	0.56	0.38
SEALK	0.000	0.905	0.042	0.90	0.86	0.61

[a]Fraction that is immediately available to ruminal digestion.
[b]Degradability potential of the insoluble fraction.
[c]Expected degradation rate of fraction.
[d]Expected degradation after 72 h of ruminal digestion.
[e]Expected degradation when the rate of passage is included.

Table 3
Determination of *In Situ* Lag Phase by Linear Regression Analysis of the Amount of Original Material That Remained After 6–32 h of Ruminal Digestion (Undigested Dry Matter)

Sample	In $y = \alpha + k \times t$	r^2	Lag, h[a]	k_N[b]
Native	$0.02025 - 0.01016 \times t$	0.96	1:54	1.00
ALK	$0.31225 - 0.05640 \times t$	0.91	5:32	5.55
SOL	$0.12441 - 0.01773 \times t$	0.85	7:00	1.74
OPA	$0.08779 - 0.01341 \times t$	0.90	6:32	1.32
SE	$0.01516 - 0.01338 \times t$	0.70	1:08	1.32
SEALK	$0.32984 - 0.05189 \times t$	0.92	6:21	5.11

[a]Lag phase as determined according to the linear regression analysis.
[b]Normalized rate in relation to the native residue (k_{sample}/k_{native}).

Compared to the SE residue, both SOL and OPA showed higher potential degradabilities (62.3, 81.5, and 73.5%, respectively). It has been hypothesized that the hydrolytic action of the enzymes can be strongly influenced by lignin hydrophobicity *(3,26)*. Pretreatment methods, such as steam explosion, result in a partial depolymerization of both hemicellulose and lignin, thus exposing the cellulose component to microbial degradation *(13–15)*. However, the higher temperatures and pressures usually developed during steam explosion can modify the lignin component to coalescent spheres of high hydrophobicity. This pseudoplastic behavior of the modified lignin allows its redeposition onto the cellulosic matrix, causing a shielding effect, which translates into a steric hindrance to microbial interaction *(13,27–29)*.

It is also worth mentioning that raw bagasse showed higher initial solubility (6.8%) than any other substrate used. This can be easily explained by the presence of low amounts of sucrose and other soluble materials within the raw fibers. Nevertheless, raw bagasse does not behave as a good substrate for cattle feeding owing to the close association that exists within its three main polymeric components, cellulose, hemicellulose and lignin *(11)*. In fact, raw fibers have a very low potential degradability (parameter *b*) of only 33.9%.

The highest kinetic values of potential degradability (parameter *b*) were observed for the alkali-washed residues, ALK and SEALK. These residues also had high degradation rates (parameter *c*), even at considerably high rates of passage through the ruminal digestive tract (e.g., $k = 0.02$ or 2% passage of the bulk material/h) (Table 3). However, both ALK and SEALK had longer ruminal lag phases, as determined during the *in situ* degradability assay (Table 3). Similar results have been demonstrated for several purified cellulose preparations, which also showed relatively long lag phases *(25,30,31)*. This effect can be attributed to low microbial adherence after extensive water and alkali extraction of the substrate, as well as to the slow process of cellulase induction during ruminal growth.

It has been suggested by several authors that glucose and cellobiose accumulation can impose inhibitory effects on cellulose digestion because of catabolite repression of cellulases *(30,32,33)*. Other authors have attributed this negative effect to a strong interference on the binding capacity of cellulases through their cellulose binding domain *(34)*. Our observations during ruminal digestion of ALK and SEALK did not suggest any strong effect of sugar accumulation on the efficiency by which the rumen biota degraded residues with high cellulose content.

Low molecular weight phenolic compounds, such as vanillin, *p*-coumaric acid, and ferulic acid are also known for their inhibitory effects on the in vitro fiber digestion of pure and mix cultures of rumen microorganisms K35). These and other phenolic compounds appear to compromise microbial adhesion to the substrate *(36,37)*. To investigate this hypothesis, we have determined the relative amount of phenolic compounds within each of the cellulosic residues used in this study by UV/vis spectroscopy. The SE residue was shown to have the highest UV absorbance profile (Fig. 2). This was consistent with the previously observed low NDF values and relatively short lag phase of this residue. Depending on pretreatment conditions, the process of steam explosion produces microbial inhibitors that have to be water-washed prior to their utilization for bioethanol production through in vitro enzymatic hydrolysis and/or simultaneous saccharification and fermentation *(38)*. However, these low-mol-wt substances do not interfere with *in situ* degradability assays because they are either utilized by the diverse rumen microflora or quickly diffused through the digestive tract.

Various researchers have tried to obtain a better understanding of the relative importance of cellulose crystallinity on the ruminal degradability of pretreated cellulosic residues *(39)*. It has been suggested that, at least for pure cultures of *Fibrobacter succinogenes* ssp. succinogenes, crystallinity acts as a barrier for microbial attack, and this barrier must be disrupted before the substrate can be effectively utilized *(34)*. Other authors have suggested that the crystallinity barrier is not a problem for the rumen biota because it can be easily overcome by the strong synergism that occurs among the existing microorganisms *(25)*.

Our results have shown that, regardless of the higher crystallinity index (CrI) of both ALK and SEALK (57.5 and 53.7, respectively), these substrates had higher potential degradabilities than those of lower CrI, that had not been alkali washed (Table 1, Fig. 3). These were the same substrates that were characterized by having a long time of lag phase In light of these results, it seems plausible to assume that longer lag phases might have been required to overcome the stronger crystallinity barrier of both ALK and SEALK residues. However, it must be emphasized that this crystallinity effect may be just apparent, and that lignin and hemicellulose removal could be a factor of a greater importance.

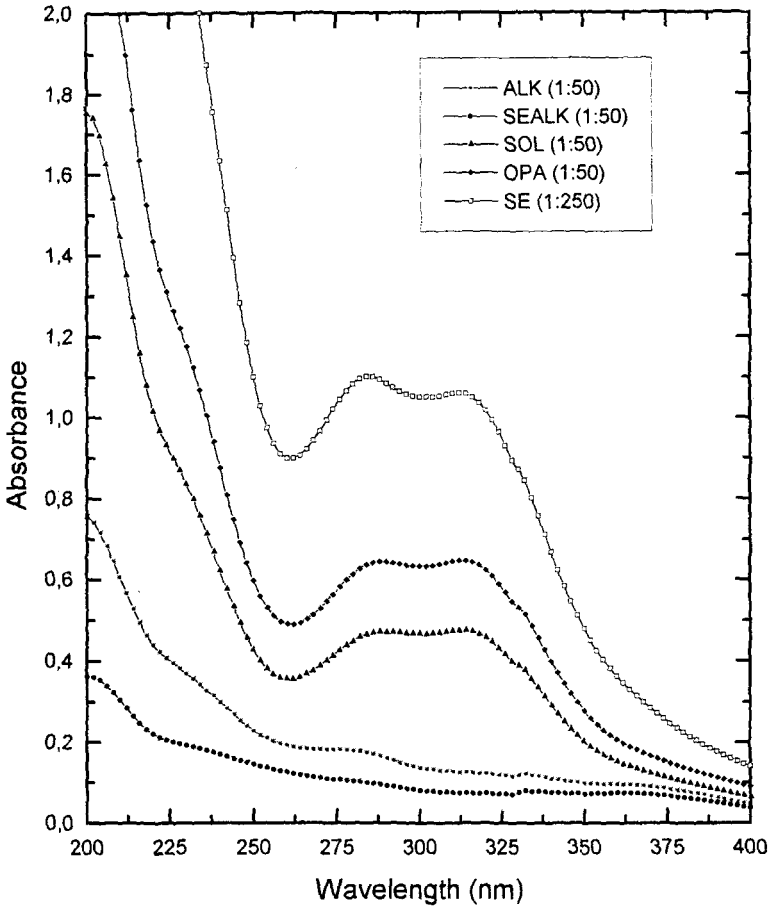

Fig. 2. UV/vis spectra of methanol:HCl extracts obtained from pretreated fractions of SCB. (◆) OPA, (▲) SOL, (□) SE, (*) ALK, (●) SEALK.

The two sharp diffraction intensities, observed at 2Θ (Bragg angles) of 20.89 and 26.65°, were attributed to the occurrence of silica within the residues. This was more apparent for the SE material, which was shown to contain approx 2% (w/w) of ash in its chemical composition.

A linear relationship between crystallinity and the rate of hydrolysis of different cellulose substrates have been often described in the literature *(40,41)*. Iran and coworkers *(41)* suggested that a pretreatment method should decrease the degree of crystallinity to be effective. Other authors *(13–16,40)* have related the improvement in enzyme digestibility of pretreated cellulosic residues to the removal of hemicellulose and lignin, which, according to Grethlein *(42)*, results in an increase in both the accessible pore volume and the specific surface area of the substrate. Stone and coworkers (43) showed that the median pore size is also strongly dependent on the degree of swelling. Therefore, it can be seen that the concepts of cellulose crystallinity, hemicellulose removal, substrate swelling (pore volume distribution), and accessible surface area are all strongly related, and any attempt to discuss one of these aspects in isolation can fall into a rough and inac-

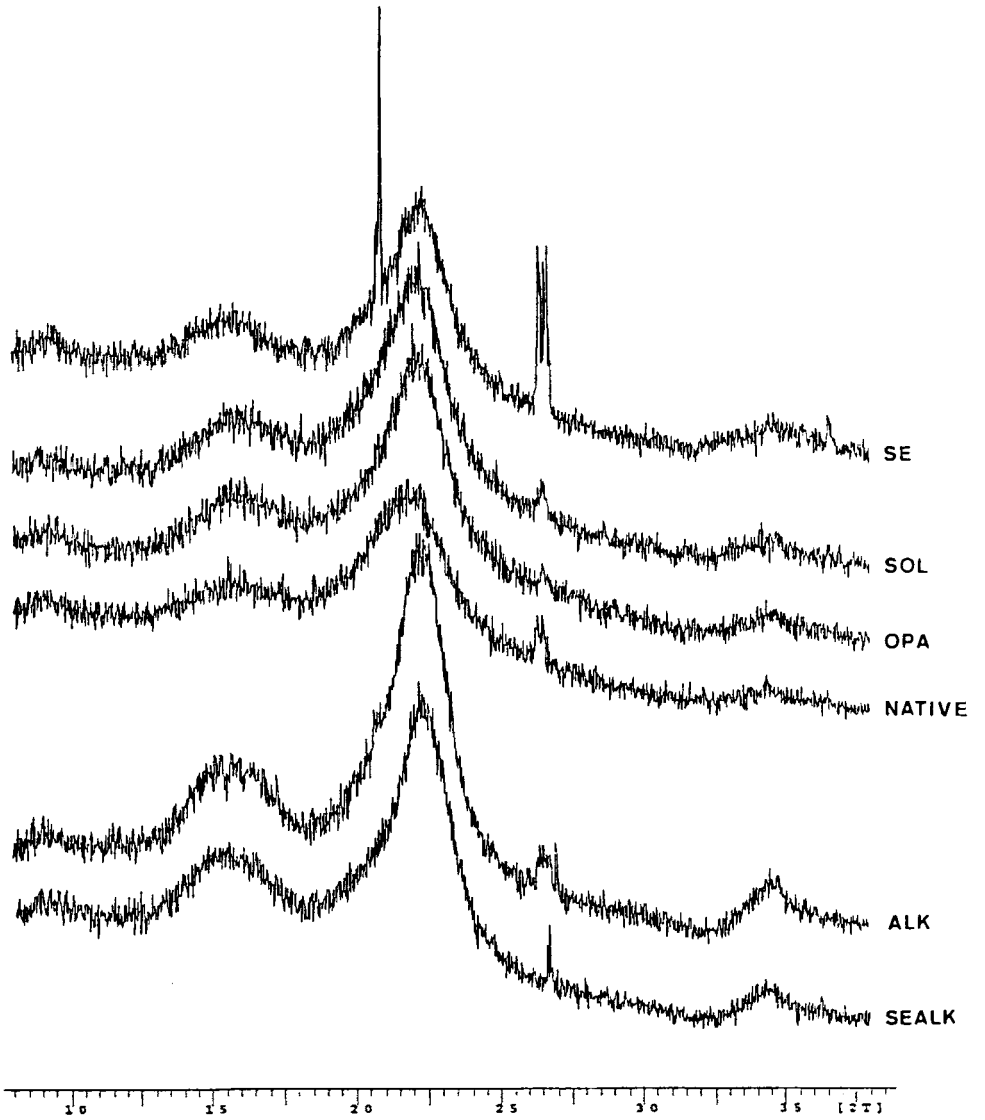

Fig. 3. X-ray diffraction analysis of substrates derived from SCB.

curate simplification of the complexity that the process of cellulose degradation actually represents.

Our work has shown that partial lignin and hemicellulose removal increases the potential degradability of SCB. However, this gain in potential degradability also increases the time of lag phase during ruminal digestion, unless the amount of soluble sugars that is released during pretreatment is not washed out of the substrate prior to their use for cattle feeding. If this is the case, the conditions used for pretreatment have to be optimized in order to avoid production of toxic byproducts and/or growth inhibitors for cattle feeding.

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REFERENCES

1. Akin, D. E. (1979), *J. Anim. Sci.* **48,** 701–710.
2. Chesson, A. and Forsberg, C. W. (1988), in *The rumen microbial ecosystem*, Hobson, P. N., ed., Elsevier, New York, pp. 251–284.
3. Kerley, M. S., Fahey, G. C., Gould, J. M., and Ianotti, E. L. (1988), *Food Microstruct.* **7,** 59–65.
7. Lamed, R. and Bayer, E. A. (1988), *Adv. Appl. Microbiol.* **33,** 1–46.
5. Leatherwood, J. M. (1973), *Fed. Proc.* **32,** 1815–1819.
6. Cheng, K. J. and Costerton, J. W. (1980), in *Digestive Physiology and Metabolism in Ruminants*, Ruckebush, Y. and Thivend, P., ed., AVI, Wesport, pp. 227–250.
7. McGavin, M. and Forsberg, C. W. (1989), *J. Bacteriol.* 17, 3310–3315.
8. Gilbert, H. J., Hall, J, Hazlewood, G. P., and Ferreira, L. M. A. (1990), *Mo. Microbiol.* **4,** 759–767.
9. Weimer, P. J. (1993), in *Forage Cell Wall Stucture and Digestibility*, Jung, H. G., Buxton, D. R., Hatfield, R. D., and Ralph, J., eds., Madison, WI, pp. 485–498.
10. Fontana, J. D., Correa, J. B. C., Duarte, J. H., Barbosa, A. M., and Blumel, M. (1984), *Biotechnol. Bioeng. Symp.* **14,** 175–184.
11. Fontana, J. D., Ramos, L. P., and Deschamps, F. C. (1995), *Appl. Biochem. Biotechnol.* **51/52,** 105–116.
12. Playne, M. J. (1984), *Biotechnol. Bioeng.* **26,** 426–433.
13. Brownell, H. H. and Saddler, J. N. (1987), *Biotechnol. Bioeng.* **29,** 228–235.
14. Ramos, L. P., Breuil, C., and Saddler, J. N. (1992), *Appl. Biochem. Biotechnol.* **34/35,** 37–48.
15. Saddler, J. N., Ramos, L. P., and Breuil, C. (1993), in *Bioconversion of Forest and Agricultural Plant Wastes*, Saddler, J. N. ed., C. A. B. International, London, pp. 73–92.
16. Rolz, C., de Arnola, M. C., Valladares, J., and de Cabrera, S. (1987), *Process Biochem.* **23,** 17–23.
17. Van Soest, P. J. (1967), *J. Anim. Sci.* **26,** 119–128.
18. Crampton, E. W. and Maynard, L. A. (1938), *J. Nutr.* **15,** 383–395.
19. Keller, R. J. (1986), The Sigma Library of FT-tR Spectra, Sigma, St. Louis, MO.
20. Reeves, J. B., III (1993), *Vibrational Spectroscopy* **5,** 303–310.
21. Ramos, L. P., Nazhad, M. M., and Saddler, J. N. (1993), *Enzyme Microb. Technol.* **15,** 821–831.
22. Segal, L., Creely, J. J. Martin, A. E., Jr., and Conrad, C. M. (1959), *Textile Res. J.* **27,** 30–41.
23. Orskov, E. R. and McDonald, I. (1979), *J. Agricultural Sci.* **92,** 449–453.
24. Mertens, D. R. (1977), *Fed. Proc.* **36,** 187–192.
25. Weimer, P. J., Guisa-Lopez, J. M., and French, A. D. (1990), *Appl. Environ. Microbiol.* **56,** 2421–2429.
26. Bacic, A., Harris, P. J., and Stone, B. A. (1988), in *The Biochemistry of Plants*, vol. 14, Preiss, J., ed., Academic, New York, pp. 297–371.
27. Donaldson, L. A., Wong, K. K. Y., and Mackie, K. L. (1988), *Wood Sci. Technol.* **22,** 103–114.
28. Wong, K. K. Y., Deverell, K. F., Mackie, K. L., Clark, T. A., and Donaldson, L. A. (1988), *Biotechnol. Bioeng.* **31,** 447–456.
29. Toussain, B., Excoffier, G., and Vignon, M. R. (1991), *Animal Feed Sci. Technol.* **32,** 235–242.
30. Smith, W. R., Yu, I., and Hungate, R. E. (1973), *J. Bacteriol.* **114,** 729–737.
31. Beveridge, R. J. and Richards, G. N. (1975), *Carbohydr. Res.* **43,** 163–172.
32. Russel, J. B. and Baldwin, R. L. (1978), *Appl. Environ. Microhiol.* **36,** 319–329.
33. Roger, V., Fonty, G., Komisarczuk-Bony, S., and Gouet, P. (1990), *Appl. Environ. Microbiol.* **56,** 3081–3087.
34. Huang, L. and Forsberg, C. W. (1990), *Appl. Environ. Microbiol.* **56,** 1221–1228.

35. Chesson, A., Stewart, C. S., and Wallace, R. J. (1982), *Appl. Environ. Microbiol.* **44,** 497–603.
36. Varel, V. H. and Jung, H. G. (1986), *Appl. Environ. Microbiol.* **52,** 275–280.
37. Akin, D. E., Rigsby, L. L., Theodorou, M. K., and Hartley, R. D. (1988), *Anim. Feed Sci. Technol.* **19,** 261–275.
38. Ramos, L. P., Breuil, C., and Saddler, J. N. (1992), *Enzyme Microb. Technol.* **15,** 19–25.
39. Hatfield, R. D. (1993) in *Forage Cell Wall Structure and Digestibility*, Jung, H. G., Buxton, D. R., Hatfield, R. D., and Ralph, J., eds., American Society of Agronomy, Madison, WI, pp. 285–313.
40. Sinitsyn, A. P., Gusakov, A. V., Vlasenko, E. Y. (1991), *Appl. Biochem. Biotechnol.* **30,** 43–59.
41. Fan, L. T., Gharpuray, M. M., and Lee, Y.-H. (1987), *Cellulose Hydrolysis*. Springer-Verlag, Berlin.
42. Grethlein, H. E. (1985), *Bio/Technology* **3,** 155–160.
43. Stone, J., Scallan, A., Donefer, E., and Ahlgren, E. (1969), *Adv. Chem. Ser.* **95,** 219–241.