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Pretreated Sugar Cane Bagasse as a Model for Cattle Feeding

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ABSTRACT

Pretreatment under mild conditions in the presence of water (solvolysis) or aqueous orthophosphoric acid (phosphorolysis) was used to increase the nutritional value of sugar cane bagasse for cattle feeding. The best pretreatment conditions were defined as those in which the highest *in situ* degradability rates (ruminal digestion) were achieved with the least energy consumption and/or production of inhibitory products. Heating sugar cane bagasse up to 197°C (13.5 atm) at a 4:1 (w/w) water ratio was shown to be a compromised condition for solvolysis, as higher temperatures would require more energy consumption without adding too much to the already high 60% ruminal degradability of the residue in relation to its dry weight. These rates of degradability were shown to be further enhanced to almost 70% by adding 2.9% (w/w) orthophosphoric acid as an acid catalyst. A mathematical treatment of the kinetic data describing ruminal digestion of each of the pretreated residues was also developed in this study.

Index Entries: Sugar cane bagasse; pretreatment; phosphoric acid; cattle feeding; mathematical modeling.

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INTRODUCTION

Crop residues, such as sugar cane bagasse, have been largely utilized as a source of nutrients 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 to about 35%. To improve this rate, additional treatments are needed.

Several pretreatment methods, ranging from biological to purely chemical, have been described in the literature as effective ways of increasing the accessibility of lignocellulosic residues to microbial and/or enzymatic attack (*1–3*). Among them, pretreatment with hot mineral acids is by far the most studied. Under relatively mild conditions, acid catalysis appears to result in almost complete solubilization of hemicelluloses with a concomitant increase in the rates at which cellulose is hydrolyzed. Several drawbacks have been associated with the use of strong mineral acids, such as increased sugar losses, production of inhibitors to microbial growth, and high capital cost (*4*). In contrast, the use of weaker acids appears to have several advantages, because they are less destructive to carbohydrates, therefore, decreasing the amount of dehydrated and/or oxidized byproducts that are produced as a result of pretreatment (*5*).

Hot aqueous orthophosphoric acid (OPA, pKa_1 = 2.1), under relatively mild conditions, has been shown to be particularly useful for pretreating agricultural residues (*6*). An additional advantage of this method is that the pretreated material does not have to be washed out prior to cattle feeding. The residual amount of phosphate, which remains within the fiber, can act as an important conutrient for microbial growth, particularly after partial neutralization with ammonia.

In this article, the potential of sugar cane bagasse for cattle feeding has been investigated after pretreatment under relatively mild conditions, with and without the addition of OPA as an acid catalyst. Rates of biomass degradability were assessed by *in situ* ruminal digestion of the various materials.

MATERIALS AND METHODS

Pretreatment of Sugar Cane Bagasse

A large batch of sugar cane bagasse was obtained from the processing stages of an alcohol plant in southern Brazil (Porto Belo Distilleries, Itajai, SC). The residue, which was kept frozen until usage, had a moisture content of 48% (w/w) and contained about 2% (w/w) of contaminating sucrose.

Pretreatment was carried out under several OPA concentrations (phosphorolysis), ranging from 1.5–4.4% (w/w) of the residue in relation to its

oven-dry weight. An aqueous solution, containing a known amount of OPA (85%, d = 1,71), was added to 100 g (dry wt) of the residue at 4:1 (w/w) ratio and the moist residue was heated in a 2-L stainless-steel reactor until it reached the desired temperature. The heating system of the reactor was stopped immediately after the temperature was reached and the reactor was depressurized by bleeding the built-up pressure down to atmospheric pressure. Three sets of experiments were carried out by heating the moist biomass up to 185°C (10 atm), 197°C (13.5 atm), and 208°C (17 atm). The heating times required to reach these temperatures were 20, 25, and 29 min, respectively. Solvolysis was defined as those experiments in which water was used to replace the dilute acid solution.

Every batch of unwashed pretreated material, derived from both solvolysis and phosphorolysis, was air-dried at 55°C and milled to pass a 2-mm screen for both *in situ* degradation (ruminal digestion) and chemical analysis. A small amount of the water-solubles produced at each pretreatment run was clarified by centrifugation and analyzed for its carbohydrate composition.

Chemical Analysis

The chemical composition of the residues was determined using the classical methods of neutral detergent fiber (NDF) and acid detergent fiber (ADF) as described previously (*7*). 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 (*8*).

Neutral sugar were determined in both water-soluble and water-insoluble fractions (acid hydrolysates) by HPLC, using a Bio-Rad (Richmond, CA) HPX-87H column, operated at 60°C, and eluted with 0.01*M* sulfuric acid at a flow rate 0.6 $mL \cdot min^{-1}$ (*9*). Qualitative thin-layer chromatography (TLC) was performed in SG-60 Merck chromatoplates, using a mobile phase that consisted of isopropanol, nitromethane, ethyl acetate, ethyl methyl ketone, methanol, and water in a 50:45:50:25:10:20 molar ratio (*10*).

Total sugars were determined by the phenol-sulfuric method (*11*), whereas reducing sugars were estimated using the method described by Nelson and Somogyi (*12,13*).

Determination of *In Situ* Degradability (Ruminal Digestion)

Samples were subjected to *in situ* ruminal digestion as described earlier (*14,15*). Approximately 3 g of biomass (oven-dry weight) were placed in a 9 × 15 cm nylon bag with a pore size of 45 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, 3, 6, 14, 24, 48, and 72 h of incubation. Three 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 six replicates. Time zero was obtained by measuring weight loss after incubation of the bag in water at 39°C for 2 min. Each bag contained enough material to correspond to a 1% (w/w) suspension of the residue. Results derived from *in situ* ruminal digestion, expressed as weight loss in relation to both dry matter and cellulose content of the residue, were adjusted according to the following equation (*16*):

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

were p = potential degradability, a = fraction that is immediately available, b = degradability potential of the insoluble fraction, c = expected degradation rate of fraction "b", and t = ruminal digestion time.

The effective *in situ* degradability of each residue was determined according to the following equation (*16*):

$$e = a + [(b{\cdot}c) / (c + k)] \tag{2}$$

were e = material effectively degraded and k = rate of passage.

Scanning Electron Microscopy (SEM) Observation

Pretreated bagasse samples (never-dried) were subjected to in vitro ruminal digestion at a 1% (w/w) consistency for 24 h at 39°C, using a cheesecloth-filtered rumen fluid at a 1:2 dilution with McDonald's buffer (*17*). The sample was fixed with glutaraldehyde/cacodylate buffer, dehydrated through an ethanol series, critical point dried, and finally covered with a thin layer of gold/palladium. SEM observation was carried out with a Philips microscope, model 505 (Netherlands).

RESULTS AND DISCUSSION

Pretreatment of sugar cane bagasse was performed with the aim of increasing its *in situ* ruminal degradability from 35–70% in relation to its dry weight. This goal was established based on the physiology of ruminal digestion, which indicates that a degradability around 70% results in the best possible turnover of the diet inside the ruminal tract and increased animal production. This optimal energy uptake also depends on the rate of passage of the diet through the whole digestive tract.

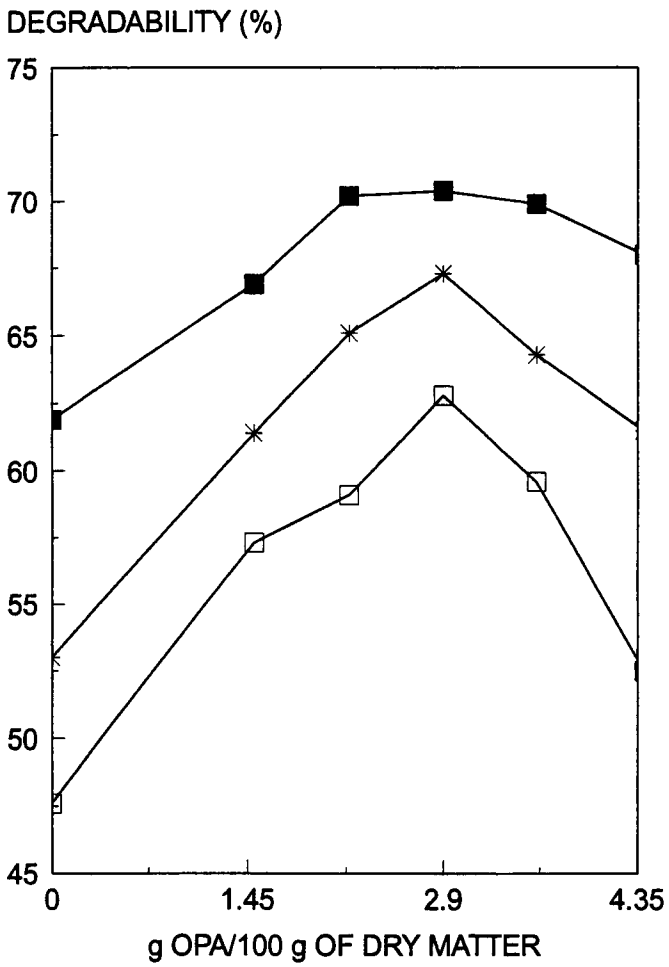

Fig. 1. *In situ* ruminal degradability of sugar cane bagasse pretreated at various OPA concentrations. Ruminal digestion of the residues was carried out for 48 h. (□) 185°C, 10 atm; (*) 197°C, 13.5 atm; (■) 208°C, 17 atm.

Pretreatment of sugar cane bagasse up to 208°C (solvolysis) increased the *in situ* ruminal degradability of the pretreated material to more than 60% of its dry weight (Fig. 1). Lower rates of ruminal degradability were observed when pretreatment was carried out at lower temperatures. There was also a progressive depolymerization of the hemicellulose fraction as pretreatment severity increased (Table 1). As a result, a relative increase in both ADF and cellulose content of the residue was observed. It seemed that hemicellulose degradation was a result of the same acetic acid-catalyzed, autohydrolytic mechanism described for other pretreatment methods, such as autohydrolysis (*18*) or steam explosion (*9,19*).

To investigate the effects of adding OPA as an acid catalyst, pretreatment of the OPA-impregnated residue (phosphorolysis) was carried out

Table 1
Chemical Composition of the Residues
Obtained by Pretreating Sugar Cane Bagasse Under Various Conditions

Pretreatment	NDF[a]	ADF[b]	HEMI[b,c]	Cellulose[d]
Untreated	87.3	47.6	39.7 (0.0)	52.4
Pretreated at 185°C				
(10 atm) solvolysis	82.1	50.3	31.8 (20.0)	52.4
OPA at 1.5% (w/w)[e]	79.4	51.0	28.4 (28.5)	55.5
OPA at 2.9% (w/w)	70.1	52.9	17.2 (56.6)	55.9
OPA at 4.4% (w/w)	73.5	54.7	18.8 (52.6)	59.6
Pretreated at 197°C				
(13.5 atm) solvolysis	84.0	54.6	29.4 (26.0)	55.1
OPA at 1.5% (w/w)	74.9	56.6	18.4 (53.7)	56.5
OPA at 2.9% (w/w)	69.0	57.6	11.4 (71.2)	57.0
OPA at 4.4% (w/w)	69.2	58.9	10.3 (74.0)	58.5
Pretreated at 208°C				
(17 atm) solvolysis	75.5	55.2	20.3 (48.9)	57.9
OPA at 1.5% (w/w)	75.3	58.5	16.8 (57.6)	58.7
OPA at 2.9% (w/w)	68.9	58.7	10.3 (74.1)	61.8
OPA at 4.4% (w/w)	64.6	58.2	6.3 (84.1)	57.2

[a]Both NDF and ADF were determined in four replicates and expressed in relation to the dry weight of the residue; coefficients of variation were never above 2–3%.
[b]Hemicellulose content was determined by subtracting ADF from NDF.
[c]Values in brackets correspond to the percent hemicellulose removal at each pretreatment condition.
[d]Cellulose was determined gravimetrically after digestion of the residue with the acetic/nitric acid reagent.
[e]OPA, orthophosphoric acid at 1.5% (w/w) concentration in relation to the dry weight of the residue (phosphorolysis).

at 197°C, using the same conditions used for solvolysis. Addition of this catalyst was performed within the range of 1.5–4.4% (w/w). The NDF content of the pretreated residue decreased with pretreatment severity, indicating a substantial increase in the amount of hemicellulose that was removed from the residue as a result of pretreatment (Table 1). For instance, pretreatment up to 197°C using 2.9% (w/w) OPA reduced the hemicellulose content of the untreated bagasse by 71% of its original content, whereas solvolysis, at the same pretreatment conditions, was responsible for a much slighter reduction of 26% in its hemicellulose content.

At increasing OPA loadings, there was a considerable appearance of glucose in the water-soluble fraction. This was shown by deriving glucose to xylose molar ratios after HPLC analysis of each water-soluble fraction produced (Table 2). This observation was probably a result from the hydrolytic action of the catalyst against either sacarose or the more disordered parts of the cellulose structure (4). Arabinose could not be detected in the

Table 2
Changes in Molar Ratio of the Three Main nonosaccharides Found in the Water-Soluble Fractions[a]

Pretreatment conditions	Water-insoluble[b]			Water-soluble[c]		
	Glc	Xyl	Ara	Glc	Xyl	Ara
Untreated residue	1.85	1	0.09	—	—	—
Solvolysis	2.06	1	0.06	0.92	1	0.17
OPA at 2.9% (w/w)[d]	6.14	1	—	0.37	1	0.12
OPA at 4.4% (w/w)	5.67	1	—	0.31	1	0.11

[a]Pretreatment was carried out at 197°C with and without the addition of OPA as an acid catalyst.
[b]Determined in the hydrolysates of a Klason lignin determination by HPLC.
[c]Determined by HPLC analysis of the water-solubles derived from each pretreatment condition.
[d]OPA, orthophosphoric acid at 2.9 and 4.4% (w/w) concentration in relation to the dry weight of the residue (phosphorolysis).

water-soluble fraction of any phosphorolysis run, suggesting that this sugar is more acid-labile than other pentoses and hexoses found within the native material. Phosphorolysis also led to a relative increase in both ADF and cellulose content of the residue.

TLC analysis of the water-solubles produced at each pretreatment condition also indicated that during phosphorolysis, the residual sucrose of the untreated material, which accounted for $<2\%$ of its dry weight, was totally converted to equivalent amounts of glucose and fructose, whereas solvolysis was not drastic enough to cause the same effect (data not shown). A considerable amount of hydroxymethylfurfural was produced when pretreatment was carried out at higher OPA concentrations probably because of fructose dehydration.

The best pretreatment conditions were defined as those in which the highest *in situ* degradability rates were achieved with the least production of inhibitory products to microbial growth. Heating bagasse up to 197°C (13.5 atm) was shown to be a compromised condition for solvolysis, since higher temperatures would required more energy consumption (longer heating times) without adding too much to the rumen degradability of the pretreated residue. A water ratio of 4:1 (w/w) was shown to be adequate for our homemade reactor, avoiding problems of uneven cooking of the residue (lower water ratios) or exceedingly high dilution of water-solubles (higher water ratios).

The increased hemicellulose removal that was associated with phosphorolysis also led to better yields of *in situ* degradability of the insoluble residue. The maximum values were obtained at OPA concentrations of 2.9% (w/w), regardless the maximum temperature reached during pretreatment (Fig. 1). Lower OPA loadings required higher pretreatment

Table 3
Mathematical Treatment of the Data Obtained for the Kinetics of *In Situ* Ruminal Digestion of Pretreated Sugar Cane Bagasse in Relation to Its Dry Weight

Pretreatment conditions	Parameters			
	a[a]	b[b]	c[c]	r^2
Untreated	0.068	0.339	0.040	0.97
Solvolysis[d]	0.159	0.511	0.040	0.98
OPA at 2.9% (w/w)[e]	0.275	0.461	0.032	0.96
OPA at 4.4% (w/w)[e]	0.261	0.470	0.023	0.94

[a]Fraction that is immediately available for ruminal digestion (time zero).
[b]Degradability potential of the insoluble fraction.
[c]Expected degradation rate of fraction "b."
[d]Solvolysis was carried out by heating the residue up to 197°C.
[e]Phosphorolysis was carried out by heating the residue up to 197°C after impregnation with either 2.9 or 4.4% (w/w) aqueous orthophosphoric acid (OPA).

temperatures to result in very similar degradability rates, whereas high OPA concentrations of 4.4% (w/w) did not result in better degradability rates probably because of the concomitant production of strong inhibitors to microbial growth, such as furfural and hydroxymethylfurfural (*20*).

In order to predict the degradability potential of these pretreated materials, each set of results, obtained for each pretreatment condition, was adjusted using a nonlinear model (*16*). Every set of data, based on *in situ* dry matter degradability of the residues, fitted very well in the exponential model, given r^2 values higher than 0.94 (Table 3 and Fig. 2).

The mathematical modeling of ruminal digestion allowed us to make several observations concerning the beneficial effect of pretreatment. For instance, adding OPA to the residue, prior to pretreatment, resulted in higher values for the variable *a*, which corresponds to time zero in the kinetics of ruminal digestion (*see* Materials and Methods). This is again related to a more extensive hemicellulose removal as a result of both higher heating times (solvolysis) or higher OPA concentrations (phosphorolysis) (Fig. 2). Therefore, the beneficial action of OPA resides in the fact that a greater amount of the OPA-treated residue was readily available to the digestive tract of the animal.

Several advantages of using OPA were apparent from this study, such as the more extensive hemicellulose solubilization and the better rumen degradation of the insoluble residue. However, the mathematical modeling of each kinetic of ruminal digestion predicted, after a long residence time in the rumen (up to 60–70 h), an almost equivalent degradability potential for residues derived from both solvolysis and phosphorolysis. This may indicate that OPA does not have an essential role in these pretreatment conditions, particularly if one considers that its addition may reduce the half-life of the reactor owing to acid corrosion. However, if a new variable is introduced to the model, that is, the rate of passage of the diet through

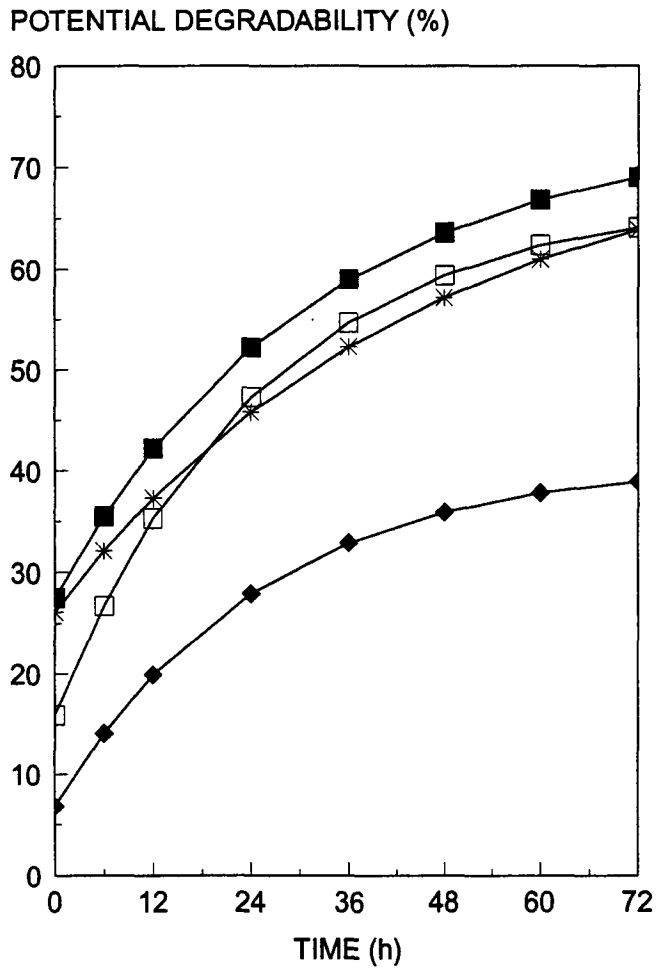

Fig. 2. Mathematical modeling of *in situ* degradability (ruminal digestion) of both native and pretreated sugar cane bagasse in relation to their dry weight. (◆) Native bagasse; (□) bagasse after solvolysis at 197°C (13.5 atm); bagasse after phosphorolysis at 197°C (13.5 atm), using OPA concentrations of (■) 2.9% and (*) 4.4% (w/w).

the digestive tract, the higher content of readily metabolized components within the OPA-treated residue may offer advantages in energy gain during ruminal digestion, particularly if more desirable high rates of passage are considered (Table 4). This can be already shown at a relatively low rate of passage of 2%/h (k = 0.02), where phosphorolysis at a 2.9% (w/w) was 11.8% more efficient than solvolysis in relation to dry matter degradability. Higher k values of 0.08 indicated that OPA catalysis can be as much as 24% more effective than solvolysis, therefore justifying the eventual use of this acid catalyst.

Both pretreatment methods, solvolysis and phosphorolysis, resulted in a residue that had a similar initial rate of degradation of the cellulose component (Fig. 3). However, when high OPA concentrations of 4.4%

Table 4
Determination of the Effective *In Situ* Degradability
of Several Pretreated Fractions in Relation to Their Dry Weight

k^a	Untreated	Solvolysis[b]	2.9% [OPA][c]	4.4% [OPA][d]
0.02	0.296	0.499	0.558	0.511
0.05	0.220	0.385	0.455	0.407
0.08	0.182	0.328	0:407	0.365

[a]Rate of passage of the diet through the digestive tract.
[b]Solvolysis was carried out by heating the residue up to 197°C.
[c]Phosphorolysis was carried out by heating the residue up to 197°C after impregnation with either 2.9 or 4.4% (w/w) aqueous orthophosphoric acid.

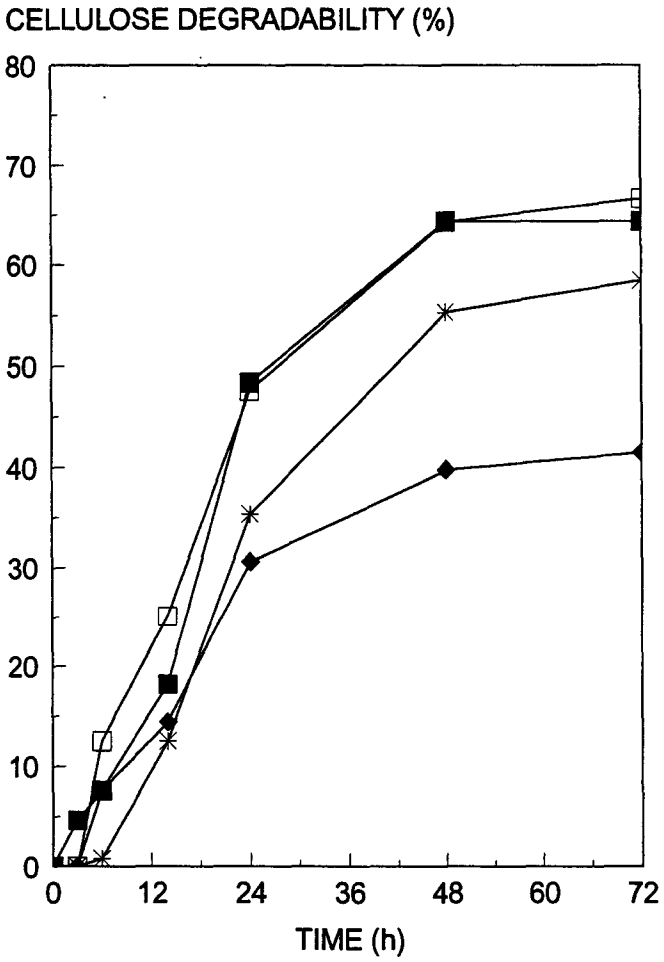

Fig. 3. *In situ* degradability (ruminal digestion) of both native and pretreated sugar cane bagasse in relation to their cellulose content. (◆) Native bagasse; (□) bagasse after solvolysis at 197°C (13.5 atm); bagasse after phosphorolysis at 197°C (13.5 atm), using OPA concentrations of (■) 2.9% and (*) 4.4% (w/w).

(w/w) were used, there was a more pronounced lag phase before cellulose was effectively utilized by the rumen biota. This was probably a result from the higher amount of microbial inhibitors, which was produced under these more drastic conditions. On the other hand, it appears that pretreatment at 4.4% (w/w) OPA had triggered a more extensive chemical modification of the lignin components, which, after drying, contributed to a substantial decrease in cellulose accessibility by acting as a recalcitrant shield to microbial and/or enzymatic attack.

It is known that ruminal digestion is triggered by the adherence of the rumen biota to the surface of the residue. Therefore, lower rates of ruminal digestion are usually associated with a decreased surface area available to enzyme/microbial interactions. In fact, SEM observation of partially digested materials has shown that particles of pretreated residues could be effectively colonized by the rumen microorganisms, whereas only the apical zone of untreated residue was consistently attacked (data not shown).

Although drying of pretreated residues can lead to collapsing of micropores, thus reducing the accessible surface area of the substrate (*3*), this was shown not to be as relevant for ruminal digestion as it is for the in vitro hydrolysis of other pretreated cellulosic materials (*21*). Furthermore, drying is a requirement for the *in situ* degradability assays.

CONCLUSION

The main focus of this work was to evaluate a pretreatment method that could be easily transferred to the field at a reduced cost. The homemade, easy-to-build pretreatment reactor was fairly effective and reproducible in increasing the energy value of sugar cane bagasse, one of the most abundant Brazilian secondary phytobiomasses, for cattle feeding. Pretreatment of bagasse under relatively mild conditions was shown to be an effective way of increasing its accessibility to *in situ* ruminal digestion. Heating sugar cane bagasse up to 197°C (13.5 atm) at a water ratio of 4:1 (w/w) was shown to be the best pretreatment condition, where the best degradability rates were achieved with the least energy consumption and/or release of inhibitory products. Adding 2.9% (w/w) of orthophosphoric acid as an acid catalyst helped enhance degradability rates by solubilizing most of the hemicellulose content of the residue. Since this catalyst does not have to be washed out of the residue prior to cattle feeding, this may be an attractive way of further increasing the nutritional value of this annual crop.

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