Sustainability challenges: conversion of fibrous agroindustrial waste from sugar cane in animal food

Llanes Gil López D. I. [1], Lois-Correa J. A.[1], Ramírez-Calzada C.[2], Jiménez-García E.[2], Sánchez-Pardo M. E.[2]

[1]Instituto Politécnico Nacional. Research Center on Applied Sciences and Advanced Technology (CICATA-IPN). Altamira, Tamaulipas, México,

[2]Instituto Politécnico Nacional. Escuela Nacional de Ciencias Biológicas, Departamento de Ingeniería Bioquímica. Unidad Profesional Adolfo López Mateos, Av. Wilfrido Massieu Esq. Cda. Manuel Stampa s/n, C.P.07738, Delegación Gustavo A. Madero, México

*corresponding author: SÁNCHEZ-PARDO, M. E.

e-mail: alimentoselena@hotmail.com

Abstract In Mexico, one of the problems that most concern in the industrial sector is the final destination of the waste, making this a challenge for sustainability; such is the case of fibrous waste from sugarcane, since being lignocellulose compounds need prior to its use a treatment to delignify the fibers. In this investigation, the bagasse of the grass *Saccharum officinarum* was subjected to an alkaline treatment based on sodium hydroxide. The development of a pre-digested base food from cane bagasse for protein-supplemented cattle is presented. In order to reduce the lignins content in the applied fibers an alkaline treatment with sodium hydroxide (2.0 Normal). *In vitro* digestibility tests, protein, and mineral content, percentage of moisture were performed. For the physical characterization of the integral and post-treatment fibers, the technique was used: confocal microscopy with laser scanning. According to the results obtained it was possible to corroborate that the alkaline treatment confers a greater digestibility to the bagasse and therefore, its results can be applied in bovine animal feeding technologies. One of the advantages of the formulation of an alkaline food is the reduction of lignin which facilitates the assimilation of nutrients in the ruminant digestive system.

Keywords: animal feed, bagasse, chemical composition, sustainability

1. Introduction

In recent years, care and preservation of the environment has become governmental main target. In this sense, the appropriate disposition of agro-industrial wastage as well as organic and inorganic residues has been taken upon. A limitation for the use of agro-industrial wastage is its complex structure.

Lignocellulosic substrates in sugar cane bagasse and tops, among other residues, are composed mainly by cellulose, hemicellulose and lignin. These fibers embed a lignin matrix and are present in various proportions depending on plant species, age and organs. Lignin is recalcitrant and inhibits hydrolytic enzymes (Di Girolamo *et al*, 2014). Pretreatments are necessary in order to degrade the matrix and favor vitamin and protein absorption in cattle's digesting cycle.

On the other hand, these agro-industrial products are rich in minerals and vitamins such as potassium, iron, calcium and magnesium. These properties are necessary by making of animal food with nutriments and fiber.

Nowadays a significant amount of different agro-industrial dry wastage matter, over 75.73 million tons from 20 crops, is generated in Mexico and of which scarcely 10% is put to a better use. The sugar cane industry alone produces 9 by-products from which a large number of raw materials, useful as co-products for commercial, pharmaceutical or energy, can be obtained.

In this investigation bagasse of the *Saccharum officinarum* (sugar cane) are treated with alkaline treatment based on sodium hydroxide (NaOH).

Pre-treatment of sugar cane include mechanical, biological and chemical (acid or alkaline) agents. These pre-treatments have advantages as well as disadvantages. Chemical treatments with acid require high temperatures and pressure during the process which provokes issues such as equipment corrosion and the need to process the residual effluents with fresh water translating this into an immense amount of water consumption and elevated non-sustainable costs.

In addition, alkaline pre-treatments are often favored in lignocellulosic substrates since they are performed at lower temperatures and pressures as those used in acid pre-treatments. Furthermore, alkaline pre-treatments require longer reaction process time instead of minutes required in acid pre-treatments. Also, acid pre-treatments are not applicable to animal food production because the residues of the process can evolve in further problems.

In time, various alkali agents have been used such as calcium and potassium hydroxide, but sodium hydroxide

has mostly been under study. Alkaline pre-treatments involve saponification of the ester bonds between hemicellulose and lignin, loosening the links between lignin and structural carbohydrates (Sun Y. and Cheng J. 2002). This causes a swelling of the pre-treated biomass, an increase of the porosity and internal surface and as consequence a concurrent decrease in the degree of polymerization.

In all, alkaline pre-treatments are performed under severe temperature conditions and alkali concentrations (Bruni, A.P. *et al* 2010; Taherdanak M. and Zilouei H. 2014) in spite of the high costs involved in this approach alkali treatments were initially used to increase biomass digestibility in animal feeding. Diluted alkali solutions lead to the disruption of the lignocellulosic cell walls by dissolving hemicellulose, lignin and silica through the hidrolization of uronic and acetic acid esters and cellulose swelling (Zhang Y. P. and Lynd L. R., 2014; Jackson M. G., 1977).

Lignin decomposition is usually attributed to the rupture of the alpha-aryl ester bonds from its polyphenolic monomer, while hemicellulose dissolution and cellulose swelling are a consequence of hydrogen bond weakening (Jackson M. G., 1977). Sodium hydroxide (NaOH) provokes the hugest degradation in comparison with other alkalis (Rodríguez-Vázquez R *et al*, 1992; Rodríguez-Vázquez R and Díaz-Cervantes D. 1994). Rodríguez-Vazquez *et al*, 1992 used a NaOH solution to treat the pith component of sugar cane bagasse (0.2 g of NaOH per pith gram) obtaining a maximum digestibility of 71% at 92 centigrade. In this work ambient temperature was used.

2. Methods

2.1. Title

2.1. Alkaline Pre-treatment.

The pre-treatment was applied to dry grinded bagasse (0.5 mm.). The alkaline pre-treatment was performed in order to increase the digestibility of bagasse by a solution of sodium hydroxide 2 molar through the method of spraying for 10 minutes, as retention time. A honey-urea solution was prepared to neutralize the bagasse alkaline pre-treatment (BP) in order to enhance the protein contents of the bagasse. The product obtained in this stage of the process is an excellent maintenance food; however, it is a protein supplemented. In this technology maralfalfa (*Pennisetum sp*) and fresh cane was included, both processed in a blade mill with sieves rotating at 300 rpm. Also, mycelial biomass from the production of transglutaminase has been incorporated, including the addition of a premix of minerals.

2.2 Chemical analysis

The samples of the aliment were grounded in a hammer mill with a 1 mm. mesh and triply analyzed for dry matter (DM, 945.15), ash (967.05), crude protein (CP, Kjeldahl N x 6.25, 990.03), crude fiber (962.09), ether extract (945.16) in accordance with AOAC (1990). The acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to Mertens, 2002 and AOAC, 1990 (973.187), and Van Soest *et al*. (1991).

2.3 Confocal laser microscopy (MCBL)

Experiments were run were NaOH alkaline treatment conditions and the supplementation of honey-urea in the same concentrations used in the manufacture of aliments were simulated. The NaOH was used in a concentration of 2.0 M in order to observe the changes suffered by the fibers.

2.4 Electronic microscopic scanning

Bagasse morphology was analyzed with electronic microscopic scanning before and after pre-treatments.

Sample imaging was carried out using Quanta 3DFEG (SEM/FIB) electronic scanning microscopes. Performed under vacuum conditions (10 to 130 Pa) at 150KV because the samples are an organic compound at a work distance of 10 mm (Wd).

Images were taken in the three control points, untreated bagasse (BI), after alkaline pre-treatment (BPA) and the final aliment named pre-digested supplemented bagasse (BPS).

3. Results

Chemical composition

The proximal composition of the bagasse and the formulated foods is shown in Table 1. The lignin content decreased in alkaline treated bagasse BP, and on the contrary, the protein content increased, corroborating in that sense that this bagasse can be applied as food for cattle.

The protein content of the BP1 formulation (with traditional supplements) was 12.43 g/100 g dry matter. The formulation supplemented with *Guazima ulmifolia* contained 12.56 g / 100 g of dry matter; however, it was determined that the best BP3 formulation was 18.8 g protein/100 g dry matter.

In the proximal analysis of the BP1 diet, low result was obtained in the protein content. In addition in the BP2 formulation a similar content of protein was found.

Scanning laser confocal microscopy

In image 2b the fiber is partially cover by residual material attributed to lignin residues. In contrast, the core is visible as a fragile and fragmented structure containing piths, which are seen as small pores connecting neighboring cells on the surface of the walls.

The images obtained with this technique are presented in Figure 2. Figure 2b is a comparative image of alkaline post-treated bagasse where the fragmentation of the fiber matter can be noted as a result of the reaction to the alkaline agent. The des-lignification of the fiber is notable due to the fluorescence of the lignin compared against the

image in 2a which corresponds to the untreated whole bagasse.

With scanning laser confocal microscopy an experiment in vitro was performed, a NaOH alkaline treatment was simulated under the same conditions as those used to prepare the base; in addition, the treatment using the honey-urea solution was applied to stop the reaction.

In Figure 2 a sequence of images of such experiment can be observed, images taken in lapses of 60 seconds between each, were the first image corresponds to the initial bagasse, images 1 thru 9 belonging to the alkaline treatment; and image 11 to the applying of the honey-urea salt solution; in images 1 thru 10 the increase of fluorescence can be observed as the rupture of the lignin bonding's are exposed due to the adding of the latter solution.

On account of the use of this technique it can be assured the breaking of the lignin bondages, as observable in the photographic sequence, it can be seen clearly the increase in fluorescence due to the action of the alkaline agent against the lignin bondage. As well as proven the action of the honey- urea in stopping the reaction of the alkaline agent, as it is observable that after it being applied the fluorescence slowly diminishes. 11 Image in the photographic sequence.

Electron microscopy scanning

Non-alkaline treated sample of whole bagasse was prepared so that the structural changes could be observed. In figure 3, thus images are viewable, in the 1a and 3c parenchymal tissue (core) is observable perfectly stratified with a spongy appearance.

This tissue is why in the cellulose and paper industry as well as in the wood agglomerate industry its removal is required due to its non-fiber characteristics and its great absorption properties, which affect the final product quality. In image 3b it is observable a string of fiber and its traversal dimension; also, lignin residues are observable in the form of incrustations in the fiber string. Micrographic image of a desmoplastic channel is visible, associated to protein adhesion.

Conclusions

The alkaline pre-treatment based on a 12.5% solution of NaOH with a retention time of 10 minutes allows a pre-digestion of the sugar cane bagasse fiber component, the scanning laser confocal microscopy morphological studies display changes and ruptures in the texture of the fiber, in the same manner, in the bromatological analysis it is observed a 12% lignin decrease attributed to the delignification by the NaOH

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Table 1. Chemical composition of bagasse, vinasse and silages used in the feeding experiment (% on DM basis)

Item	BP	BP1	BP2
Dry matter	92.5	91.2	
Crude protein	4.2	6.1	18.8
Ether extract	1.7	1.8	
Ash	4	7.8	
FC	80.7	63.89	25.36
FDA	63.89	50.22	
Acid detergent lignin (ADL)	25.36	13.23	
Potasium (K)		1.31	2
Sodium (Na)		1.48	1.16
Magnesium (Mg)		0.12	0.26
Calcium (Ca)		0.52	1.15
Phosphorus (P)		0.11	0.10
Gross energy (kcal/kg)			2.2

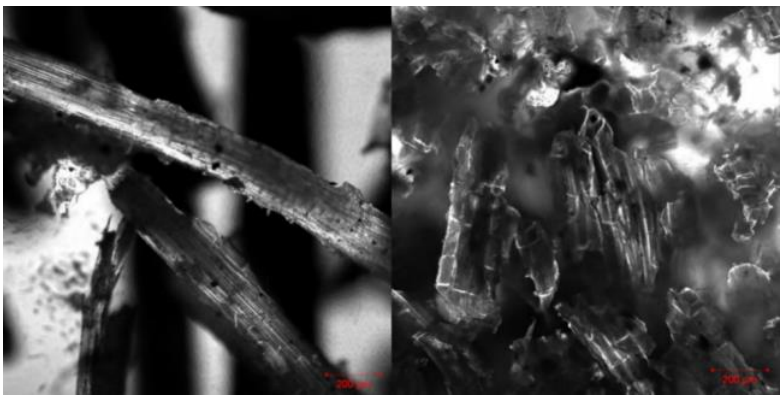

Figure 1. Scanning laser confocal microscopy photomicrographs. (a) Whole bagasse (WB); (b) alkali treated bagasse (BPCA). Photos were done at the Nanoscience Center IPN.

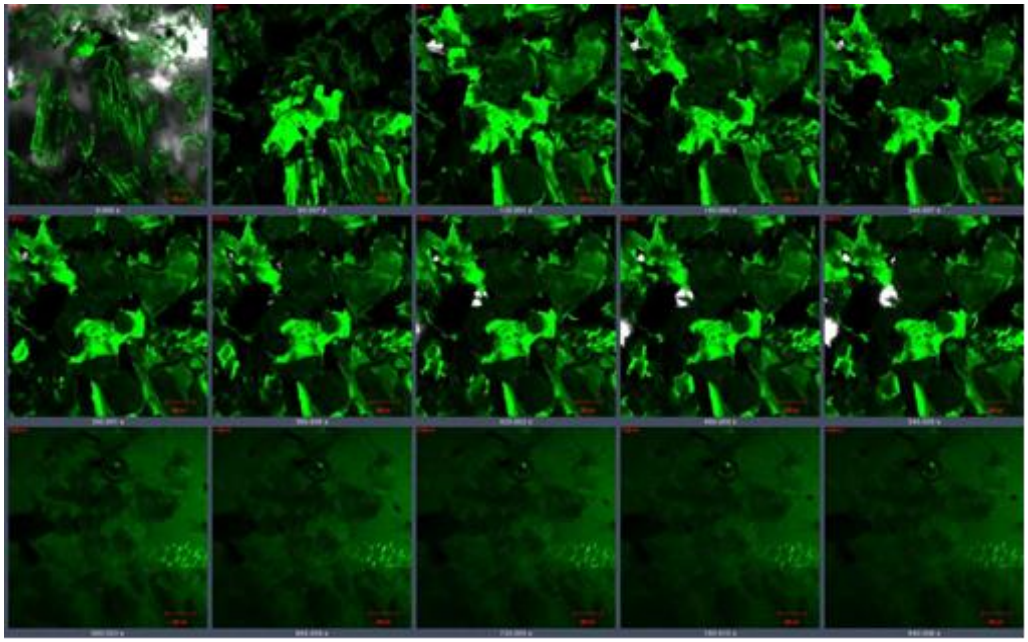

Figure 2. Experiment *in vitro* of Alkali pretreatment in sugar cane bagasse by Scanning laser confocal microscopy

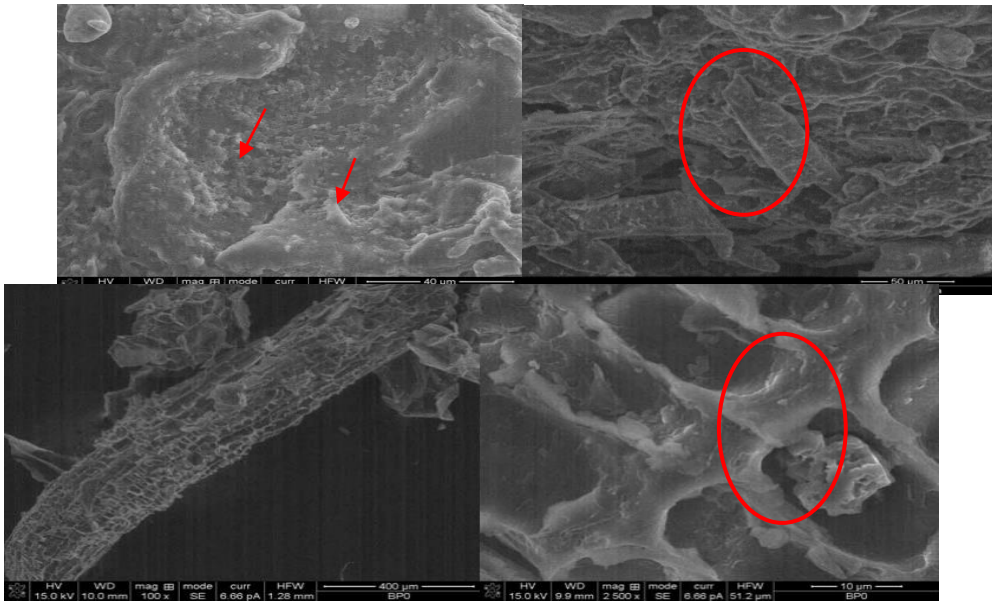

Figure 3. (a), (b), (c) y (d) General view of the sample showing fibers and pith (d) amplifications on the fiber and small fraction of pith. Alkali predigests bagasse (APB), (a) desmoplastic channel, (b) Fiber with lignin residue.

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