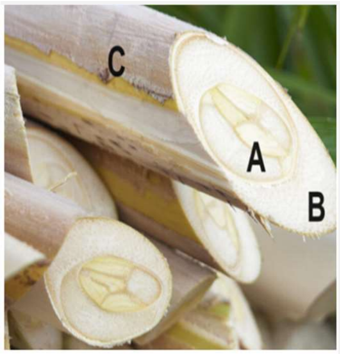
Alkaline peroxide pretreatment improves the enzymatic saccharification of peach palm (*Bactris gasipaes* Kunth) wastes

ORAL/POSTER
Ph.D. Student: Y/N
Journal: NONE

J.R. dos Santos Filho[1], *V.A. de Oliveira Jr*[1], *C. V. Helm*[2], *A. Bracht*[1], *R.M.Peralta*[1]. [1]*Universidade Estadual de Maringá, Maringá, Brazil. E-mail: rmperalta@uem.br; Embrapa-Colombo, Colombo, Brazil. E-mail: cristiane.helm@embrapa.br*



Peach palm. A: edible part; B and C: peach palm waste

Peach palm (*Bactris gasipaes* Kunth) is a palm tree native to the Amazon region and its planting has recently expanded to the Southeastern and Southern Brazilian states. It has been estimated that nearly 84% of the total palm weight is accounted for by lignocellulosic wastes. The objective of this study was to evaluate AHP as pre-treatment to improve the subsequent enzymatic hydrolysis of the cellulose from palm peach lignocellulosic wastes. The sugar yields from AHP pretreated peach palm wastes are 6-fold higher (331.10 ± 5.11 mg/g) than not pretreated ones (55.23 ± 0,51 mg/g). Hence, it can be a promising technique for the pretreatment of peach palm wastes aiming at producing bioethanol and other useful compounds that can be generated by fermentation processes.

Introduction

Extensive research has been conducted for adding value to the plant polysaccharides cellulose and hemicellulose from which valuable fine chemicals and biofuels can be obtained after saccharification and fermentation. To overcome the problems caused by biomass recalcitrance and improve saccharification the lignocellulose biomasses are submitted to several kinds of pretreatments aiming at removing lignin and disrupting the cellulosic crystalline structure. Among these methods, alkaline hydrogen peroxide (AHP) treatment is notable for its effectiveness [1]. *Bactris gasipaes* Kunth is a palm tree native to the Amazon region whose wastes contain 84% of lignocellulosic material. Transforming this important biomass into marketable products would be an intelligent and promising way of dealing with otherwise useless waste. Studies on this respect are lacking, however [2,3], and the objective of this study was to evaluate AHP as pre-treatment in order to improve the subsequent enzymatic hydrolysis of the cellulose from palm peach lignocellulosic wastes.

Material and Methods

Lignocellulosic wastes: The peach palm (*B. gasipaes*) waste was obtained from Embrapa Florestas, Colombo, PR. Sugarcane bagasse was obtained from Usina Santa Terezinha, Iguatemi, PR. The wastes were dried in the sunlight, milled to give a particle size of 1–2 mm thickness. For elimination of total soluble solids, 10 g of each dry material plus 100 mL of distilled water were transferred to a 500 mL Erlenmeyer flask and maintained at 40 °C for 1 h under agitation of 100 rpm. The materials were filtered in a sintered glass crucible and dried in an oven (105 °C) until constant mass. The extractive contents were expressed on a dry basis.

Alkaline peroxide pretreatment: 5 g of powdered peach palm waste or sugarcane bagasse was mixed with 10 mL hydrogen peroxide at 2 and 4%. NaOH (6 M) was added to obtain pH 11.6. After 3 h stirring (130 rpm) and subsequent filtering the insoluble materials were thoroughly washed with water and dried until constant weight.

Saccharification of untreated and AHP pretreated fibers: both materials were subjected to hydrolysis using cellulase (SAE0020-Sigma-Aldrich). The hydrolysis of 0.5 g was accomplished in 50 mmol/L citrate buffer, pH 5.0, containing 10 U/mL cellulase. The reaction time was 48 h at 40 °C, under agitation. Samples were withdrawn and filtered under vacuum. The released reducing sugars were quantified using the 3,5 dinitrosalicylic acid (DNS) method with absorbance measurements at 540 nm [4].

Scanning electron microscopy: A Shimadzu SS-550 Superscan was used for performing scanning electron microscopy (SEM). For the imaging procedures, the samples were sputter coated with gold layers.

Results and Discussion

The extractives of peach palm waste were around 45.40 ± 3,10 g%. This value was similar to that one obtained in a previous work and higher than those found with other lignocellulosic residues [3]. The sugar cane bagasse used in this work presented total soluble solids contents of 2%. The AHP pre-treatment was carried out with the residues from which the total soluble solids were removed with water at room temperature. Non-treated and AHP treated *B. gasipaes* waste and sugarcane bagasse were submitted to enzymatic hydrolysis for 48 h. In all experiments, the same initial amounts of the lignocellulosic materials were incubated with the same amount of enzyme under identical conditions. The results of the experiments in which the enzymatic production of total reducing sugars was measured are shown in Table 1. After 48 h of enzymatic saccharification, the reducing sugars produced from peach palm residues were 6 times higher than those produced from non-pretreated peach palm wastes (maximum 331.10 ± 5.11 mg/g). The reducing sugars obtained from the hydrolysis of AHP pretreated sugarcane bagasse were 2-3-fold higher than those obtained from untreated sugarcane bagasse (maximum 166.80 ± 2.04 mg/g). Unlike sugarcane bagasse, which has been widely explored to obtain reducing sugars for subsequent fermentation and production of diverse products [1,5], peach palm residues have been little explored. In a recent work, biological pre-treatment using the fungus *Pleurotus ostreatus* proved to be efficient, increasing hydrolysis by 6.6-fold in relation to the non-pretreated residue [3].

Table 1. Enzymatic hydrolysis of *B. gasipaes* waste and sugarcane bagasse with and without AHP pretreatment*.

Samples	**Reducing sugar (mg/g)**
Peach palm waste	
Peach palm untreated	55.23 ± 0.51[a]
Peach palm with AHP pretreatment (2%, 3 h)	331.10 ± 5.11[b]
Peach palm with AHP pretreatment (4%, 3 h)	304.92 ± 1.28[c]
Sugar cane bagasse (SCB)	
SCB untreated	53.79 ± 1.02[a]
SCB with AHP pretreatment (2%, 3 h)	166.80 ± 2.04[b]
SCB with AHP pretreatment (4%, 3h)	114.08 ± 1.02[c]
Microcrystaline cellulose	769.81 ± 2.55

*Data are means plus mean standard errors of 2 experiments. Means in each group with different letters are statistically different (ANOVA, p≤0.05)

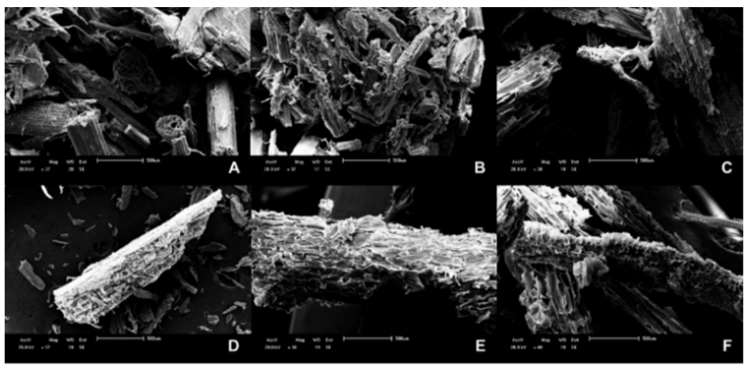


Figure 1. Scanning electron microscopy of the peach palm waste (A, B, C) and sugarcane bagasse (D, E, F). A and D: no pretreatment; B and E: AHP pretreatment 2%, 3h; C and F: AHP pretreatment 4%, 3 h **(**images bars = 500 µm; 37-40 X).

Scanning electronic microscopy of the ligno-cellulosic fibers was performed to verify the structural changes caused by the alkaline AHP pretreatment (Fig. 1). The non-pretreated samples exhibited rigid and highly ordered fibrils (Fig. 1A and 1D). After AHP pretreatment the fibers appear less ordered, with detachment of the fibers, cell wall collapse and with the formation of pores on the cell wall surfaces (Fig. 1B, 1C, 1E, 1F**)**. Such microscopic alterations in the fibers have already been described for other kinds of treatments and have been generally considered to result from lignin removal.

Conclusions

The sugar yields from AHP pretreated peach palm wastes are 6-fold higher than those from not pretreated ones. Hence, it can be a promising technique for the pretreatment of peach palm wastes aiming at producing bioethanol and other useful compounds that can be generated by fermentation processes.

Acknowledgements

The authors acknowledge the financial support from CAPES, CNPq, Fundação Araucária and UEM.

References

[1] E.D. Dutra, F.A. Santos, B.R.A. Alencar, A.L.S. Reis, R.F.R. de Souza, K.A.S. Aquino, M.A.Morais Jr, R.S.C. Menezes. Biomass Conversion and Bio-refinary 8(2018)225

[2] K.C. Spacki, R.C.G. Corrêa,T.M. Uber, L.Barros, I,C,F.R. Ferreira, R.A. Peralta, R.F. Peralta-Muniz-Moreira, C.V. Helm, E.A. de Lima, A. Bracht, R.M. Peralta. Plants 11(2022)3175

[3] K.C. Spacki, D,M.P. Novi, V.A. de Oliveira-Junior, D.C. Durigon, F.C. Fraga, L.F.O. dos Santos, C.V. Helm, E.A. de Lima, R.A. Peralta, R.F. Peralta-Muniz-Moreira, R.C.G. Corrêa, A. Bracht, R.M. Peralta. Plants 12(2023)2824

[4] G.L. Miller. Analytical Chemistry 31(1959)426

[5] S.C. Rabelo, R,R. Rabelo, R. Andrade, A.C. Maciel Filho. Fuel 136(2014)349