REVISTA BRASILEIRA DE ENERGIAS RENOVÁVEIS

EVALUATION OF ALKALINE PRETREATMENT ON THE ENZYMATIC HYDROLYSIS OF CARNAUBA STRAW RESIDUE[1]

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[1] Presented at Biomass and Bioenergy Conference (BBC Brazil) of UFSCar: April 10th to 12th of 2018 – Sorocaba-SP, Brazil

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Abstract

Carnauba (*Copernicia prunifera*) straw residue generated from production of its wax is rich in cellulose, thus showing a potential use in the production of second generation ethanol. However, the high lignin and hemicellulose load associated with cellulose makes it difficult the enzymatic attack, thus having the need of an adequate pretreatment of this material. The objective of this study was to optimize the enzymatic hydrolysis of carnauba straw residue, focusing on the alkaline biomass pretreatment. Therefore, NaOH solutions at concentrations of 1.0% (w/v) (PA1), 2.0% (w/v) (PA2), 3.0% (w/v) (PA3) and 4.0% (w/v) (PA4) were used. The chemical and physical characterization of natural and pre-treated carnauba straw were according to the NREL, and DRX and FTIR performed analyzes. The materials chemical characterization showed that all the used pretreatments were able to remove a significant amount of lignin and hemicellulose, which can improve the enzymes access, favoring the increase of cellulose conversion. In relation to DRX analysis an increase in crystallinity index happens reaching up to 55.15% after the pretreatment PA4, which may be associated to the removal of hemicellulose and amorphous lignin, related to cellulose. After a period of 96 hours of enzymatic hydrolysis, the PA4 pretreated residue showed the best performance with a cellulosic conversion of 78%. Spite of a slightly lower performance of the residue that presented higher cellulose conversion, the pretreated material PA2 is an alternative to reduce costs in the cellulosic ethanol production.

Keywords: Carnauba, Pretreatment, Enzymatic hydrolysis.

AVALIAÇÃO DO PRÉ-TRATAMENTO ALCALINO NA HIDRÓLISE ENZIMÁTICA DA RESÍDUO DE PALHA DE CARNAÚBA

Resumo

O resíduo da palha de carnaúba (*Copernicia prunifera*) gerado da produção da sua cera é rica em celulose, mostrando um potencial do seu uso na produção de etanol de segunda geração. Entretanto, a alta carga de lignina e hemicelulose associadas à celulose dificultam o ataque enzimático, sendo necessário um pré-tratamento adequado deste material. O objetivo deste trabalho foi otimizar a hidrólise enzimática do resíduo de palha de carnaúba, com foco no pré-tratamento alcalino da biomassa. Portanto, foram utilizados soluções de NaOH nas concentrações de 1,0% (p/v) (PA1), 2,0% (p/v) (PA2), 3,0% (p/v) (PA3) e 4,0% (p/v) (PA4). A caracterização química e física da palha de carnaúba natural e pré-tratadas foram avaliadas segundo o NREL e pelas análises de DRX e FTIR. A caracterização química dos materiais mostrou que todos os pré-tratamentos utilizados foram capazes de remover uma quantidade significativa de lignina e hemicelulose, o que pode melhorar o acesso das enzimas, favorecendo o aumento da conversão da celulose. Em relação à análise de DRX, foi observado um aumento no índice de cristalinidade até 55,15% após o pré-tratamento PA4, podendo estar associado à remoção de hemicelulose e lignina amorfa, relacionada à celulose. Após um período de 96 horas de hidrólise enzimática, o resíduo pré-tratado com PA4 apresentou o melhor desempenho com uma conversão celulósica de 78%. Apesar de um desempenho ligeiramente inferior a este último resíduo, o material pré-tratado PA2 destacou-se como uma alternativa para reduzir custos na produção de etanol celulósico.

Palavras-chave: Carnauba, Pré-tratamento, Hidrólise enzimática.

INTRODUCTION

Carnauba (*Copernicia prunifera*) is a typical palm tree from the Brazilian Northeast. It is associated to a range of economic activities for the manufacture of numerous artisan and industrial products, but the main economic activity using it is the wax extraction. In this case, the straw is collected from the palm tree pruning, not harming the plant. Spite of being considered a good example of natural resources use, it should be highlighted that during the wax production, in order to produce 7.8 kg of wax powder 1,000 units of straw are required, leading to an equivalent generation of agroindustrial residue (CARVALHO & GOMES, 2008; CONAB, 2011). The residue of carnauba straw is rich in cellulose, being an alternative carbon source in biotechnological processes, such as the production of second generation ethanol (2G).

The production of cellulosic ethanol or 2G requires hydrolysis process of the biomass for the conversion of cellulose into fermentable sugars. Enzymatic hydrolysis is the key to a viable ethanol production from long-term lignocellulosic substrates. It operates under mild conditions and has potential for high yield and lower maintenance cost compared to acid or alkaline hydrolysis (KUHAD; SINGH; ERICKSSON, 1997). The pretreatment reduces biomass recalcitrance and enhances the cellulase activities, and is also necessary to make the polyssacharydes more accessible to enzymatic hydrolysis (ARAÚJO *et al.,* 2017). Furthermore, the high cost and low yield to cellulase are factors that limit technological developments for conversion of lignocellulose to ethanol (SUKUMARAN *et al.,* 2009).

Lignocellulose pretreatment leads to structure modification of biomass components, which are related to lignin, cellulose and hemicellulose thus resulting in the reduction of crystallinity degree, and favors adequate conditions to the material to hydrolysis (KARIMI & TAHERZADEH, 2016). Among the applied methods, the use of bases in this process has been used for a long time since it operates under milder conditions compared to acid pretreatment, which can reduce equipment maintenance costs due to corrosion. The major reactions during alkaline pretreatment include dissolution of lignin and hemicellulose, and de-esterification (saponification) of intermolecular ester bonds (KIM; LEE; KIM, 2015).

In this study, the enzymatic hydrolysis process of the carnauba straw residue was evaluated, based on the variation of the reagent concentration used in the alkaline pretreatment of the biomass. The influence of the pretreatments was evaluated based on glucose production and cellulose conversion to glucose.

MATERIALS AND METHODS

The carnauba straw residue was donated by "Carnaúba Viva" a non-governmental organization (NGO), located in Assu (Rio Grande do Norte, Brazil). The biomass was collected after wax extraction. The residue was washed and dried at 50 °C for 24 hours. Thereafter, the material was milled and sieved at 20 mesh and finally stored in plastic containers at room temperature.

The alkaline pretreatment was performed from a mixture of 20% (w/v) of the natural carnauba straw residue with sodium hydroxide solutions. The biomass pretreatments were carried out using NaOH solutions at concentrations of 1.0% (w/v) (PA1), 2.0% (w/v) (PA2), 3.0% (w/v) (PA3) and 4.0% (w/v) (PA4). The mixture was heated at 121 ° C for 30 minutes. Thereafter, solid residue was batch washed with 1.5 L portions of water until the wash water reached a pH close to 7.0. The pretreated material was dried at 50 ° C for 24 hours and stored (SILVA *et al*., 2018).

The chemical characterization of natural and pretreated residue was performed according to the National Renewable Energy Laboratory (NREL) methodology (SLUITER *et al.*, 2008), determining the moisture content, extractives, ash, cellulose, lignin and hemicellulose. In addition, the residues were subjected to X-ray analysis (DRX) as well as analysis of functional groups by Fourier transform infrared spectroscopy (FTIR).

The effect of different concentrations of NaOH used in the pretreatment of the carnauba straw residue on the enzymatic hydrolysis of natural and pretreated materials was investigated. For this purpose, a solid loading of 5% (w/v) and the commercial enzyme extract Celluclast 1.5L (Cellulase of *Trichoderma reesei* ATCC 26921) were used. The enzyme loads applied in the tests were: 20 FPU/g of bagasse, 20 CBU/g of bagasse and 10 FXU/g of bagasse. The duration of the hydrolysis process was 96 hours and the cellulosic conversion values were determined.

RESULTS AND DISCUSSION

After the pretreatments were carried out, the yield and quantity of washes required to regulate the biomass pH were determined, as it can be seen in Table 1.

Table 1 - Yield and amount of washes performed in pretreatments.

Pretreatment	Yield (%)	Number of washes
PA1	64.20	21
PA2	48.07	20
PA3	44.97	23
PA4	37.69	36

It can be observed that the residue obtained after the PA4 pretreatment showed a yield of approximately 38% that is much lower compared to the other pretreated residues, besides requiring a greater amount of washes to regularize the desired pH. The pretreatment that obtained the highest yield was PA1, as it could be expected since the NaOH concentration was lower than the others thus it leads to less removal of the components associated to the cellulose, being confirmed after the biomass characterization.

The biomass characterization was performed for natural and pretreated materials, as it can be seen in Table 2.

Table 2 – Composition of natural and pretreated carnauba straw residue.

Component	**Unit**	**Pretreatment**				
		Natural	**PA1**	**PA2**	**PA3**	**PA4**
Ash	%	10.29 ± 0.53	9.48 ± 0.12	6.35 ± 0.15	6.41 ± 0.06	3.03 ± 0.03
Extractives	%	11.39 ± 0.48	1.46 ± 0.32	0.56 ± 0.04	1.00 ± 0.01	1.25 ± 0.42
Cellulose	%	21.59 ± 0.13	29.69 ± 1.80	38.48 ± 0.66	38.15 ± 0.18	46.63 ± 1.14
Lignin	%	33.92 ± 0.57	26.79 ± 1.39	17.92 ± 1.56	24.63 ± 0.06	23.48 ± 0.70
Hemicellulose	%	15.97 ± 0.74	17.00 ± 1.29	20.16 ± 0.61	19.94 ± 0.64	21.85 ± 0.67

Carnauba straw is a potential material for the production of cellulosic ethanol in view of its composition, especially in relation to cellulose having a proportion of 21.5%.

However, a high amount of lignin is present in the material, about 34%. Thus, it should be removed since it reduces the enzymes action during the enzymatic hydrolysis process. When analyzing the proportion of cellulose, it can be seen that the use of the pretreatments increased the content of this polysaccharide. When compared the pretreatments that the solutions of 2% NaOH and 3% NaOH were applied can be seen that the cellulose values were practically the same, being slightly above 38%. For the pretreated carnauba straw residue with solution of 4% NaOH, the pretreated residue had a higher proportion of cellulose about 47%.

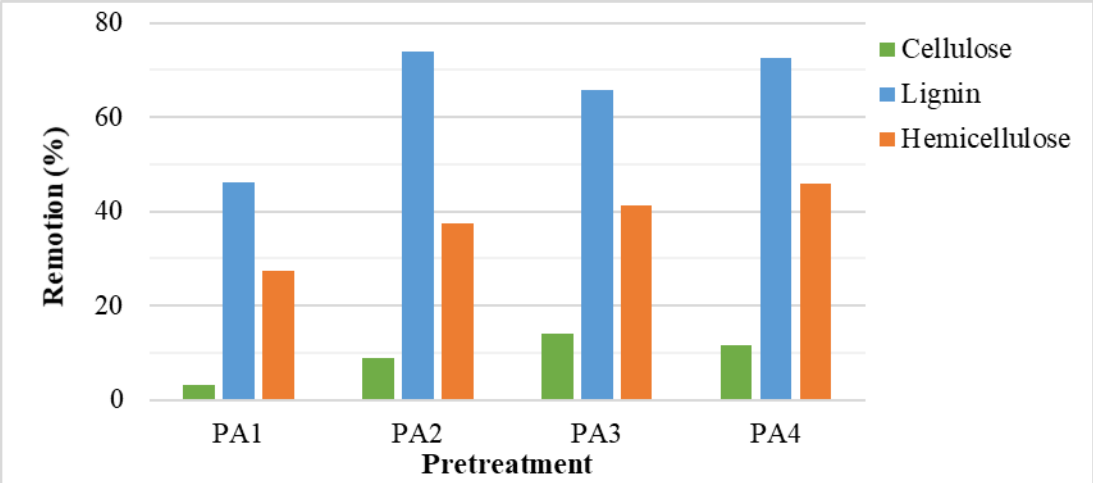

Figure 1 - Removal efficiency of cellulose, hemicellulose and lignin after alkaline pretreatments.

In Figure 1, it can be observed the degree of cellulose removal, lignin and hemicellulose after each pretreatment. In relation to lignin, the alkaline pretreatment was efficient in the removal of this component, to reduce its fraction in the composition of carnauba straw residues even at lower concentration. In addition, an increase in hemicellulose contents is observed in the pretreated materials, but it does not mean that the polymer structure was not degraded during the pretreatment.

DRX analysis allowed the estimation of the crystallinity index (CI) that is concerned to the ratio between the amorphous and crystalline regions of the fiber. The CIs were 41.89%, 48.93%, 50.75%, 53.97% and 55.15%, respectively for the natural and pretreated residues PA1, PA2, PA3 and PA4. Therefore, an increase in the significant CI is observed after the alkaline pretreatments. This can be related, among other factors, to the fact that the change in crystallinity is associated primarily with the removal of amorphous lignin and hemicellulose and, consequently, the increase in crystalline cellulose content, and not necessarily the structural change of cellulose (KIM; LEE; KIM, 2015).

The FTIR analysis aims to detect changes in the chemical structures and functional groups of the components of the lignocellulosic biomass. The spectra provided a qualitative analysis of the constitution of the studied materials. The more noticeable changes were observed in the spectra of the pretreated residues PA1 and PA3, where there is a greater difference in relation to the natural biomass. The results indicated that the applied pretreatments were able to remove a significant amount of lignin and hemicellulose, which may improve the accessibility of the enzymes favoring a greater conversion of the cellulose.

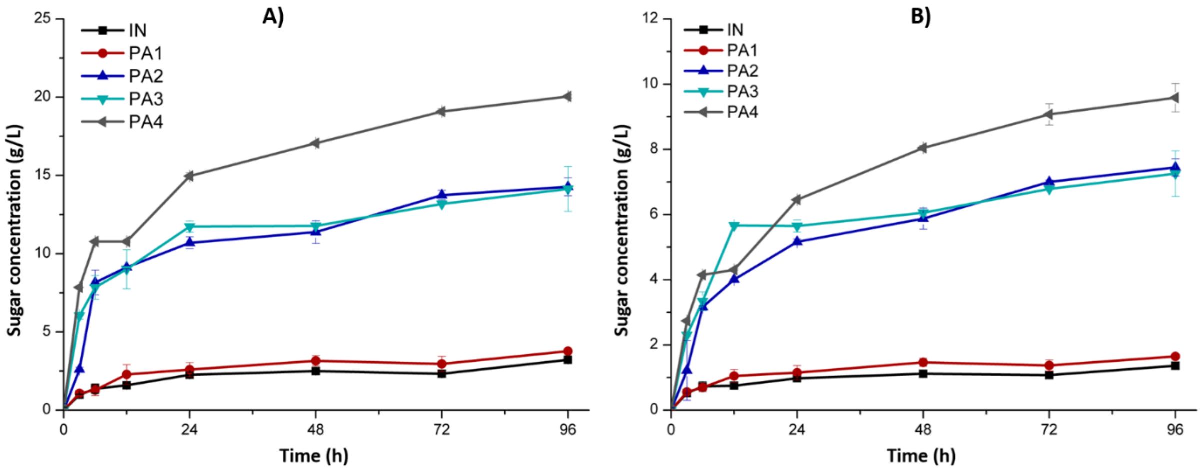

Figure 2 – Sugar concentration (g/L) produced during enzymatic hydrolysis of natural (IN) and pretreated biomass. (A) Glucose and (B) Xylose.

The natural (IN) and pretreated lignocellulosic materials were subjected to enzymatic hydrolysis. In Figure 2, the production of sugars glucose and xylose can be seen during the hydrolysis process. Regarding the glucose production, the PA4 residue showed higher concentrations of this sugar after 96 hours of enzymatic hydrolysis. The pretreated material PA2 and PA3 obtained very similar performances regarding the release of this monomer. In fact, as expected due to the similarity in the cellulose content and the proximity in the index crystallinity observed in the chemical and physical characterization. For the tests in which the used carnauba straw residue was the natural and using the PA1 also presented similar performances, indicating that PA1 pretreatment was not able to expose the cellulose effectively to the action of the enzymes.

Following the same behavior of glucose production, the pretreated PA4 residue showed the highest capacity to release xylose, whereas the IN and pretreated PA1 performance were not good. Studies indicate that the high yield of xylose is correlated with the increase of xylan degradation present in hemicellulose, which in turn increases the accessibility of cellulose to cellulase, increasing the release glucose (PHITSUWAN; SAKKA; RATANAKHANOKCHAI, 2016).

Figure 3 illustrates the percentage of cellulosic conversion of the untreated and pretreated carnauba straw residue. The lowest values of cellulosic conversion were observed to natural residue and that pretreated with PA1. The pretreatment that favored the conversion was PA4, converting approximately 78% of the cellulose present in the biomass into glucose.

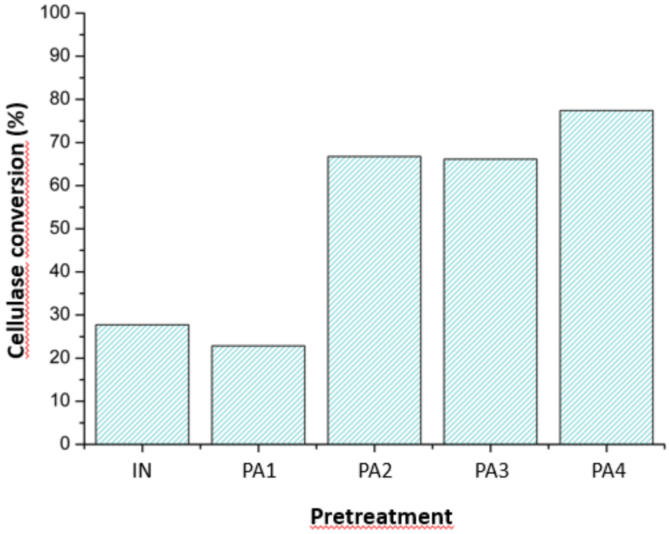

Figure 3 – Cellulosic conversion (%) after 96 h of enzymatic hydrolysis of the carnauba straw residue natural (IN) and using the pretreatments PA1, PA2, PA3 and PA4.

In relation to the enzymatic hydrolysis of the pretreated residue, a rapid increase in glucose and xylose concentration was observed during the first 6 hours of incubation. The pretreatment of the residue with 4% NaOH solution (w/v) (PA4) favored a greater exposure of the cellulose to enzymes, leading to a greater conversion of this polysaccharide. The residue pretreated with PA2 showed a conversion about 11% lower than the pretreated with PA4, which suggests a reduction in the reagent consumption for the pretreatment (half) and the volume of water to wash the material after this process.

CONCLUSION

The alkaline pretreatment was able to remove mainly lignin. The pretreated residue with 4% NaOH (w/v) showed the best performance to convert cellulose to glucose with a conversion of approximately 78%. However, the pretreatment of the biomass with NaOH 2% (w/v) stands out as an alternative to reduce costs in the production of cellulosic ethanol, using half the mass of sodium hydroxide and leading to a lower effluent generation. It can be confirmed after an economic evaluation of the costs of the glucose production process from carnauba straw residue.

ACKNOWLEDGMENT

The authors thank RENORBIO, CAPES and CNPq for the financial support. We thank our colleagues at Laboratório de Engenharia Química – UFRN for the assistance at the research.

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