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Effect of different acid pretreatments in sugarcane bagasse and the generation of inhibitors

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ABSTRACT

The efficiency of biomass enzymatic hydrolysis depends on the pretreatment methods applied, the initial feedstock composition and the enzyme cocktail used to release sugars, used for subsequent fermentation. In this study, sugarcane bagasse was pretreated with 0.5%, 2% and 4% concentrations of sulfuric acid. The biomass composition after this process was investigated to evaluate the influence of the severity of the pretreatment on the release of sugars and other compounds which are known to be inhibitors, such as aliphatic acids (levulinic, acetic and formic) and furan derivatives. Despite a reduction of almost 80% of the hemicellulose fraction with the pretreatment with 4%, the xylose release was close to the one in the pretreatment with 0.5% of acid. The concentration of inhibitors in however, increased five times, and can affect saccharification and fermentation.

Keywords: pretreatment, acid, sugarcane bagasse, inhibitors

INTRODUCTION

Emerging applications in industrial biotechnology are multiplying, mainly due to the use of enzymes in biofuel production and waste treatment of lignocellulosic matrices (Mansour et al., 2016). Lignocellulosic biomass is the world's most abundant renewable feedstock (Jarvis, 2003), and has the potential to be converted to a number of different fuels and chemicals (Jorgensen et al., 2007). Production of ethanol from lignocellulosic biomass contains three major processes, including pretreatment, hydrolysis, and fermentation.

The pretreatment process is required to break down the biomass structure and increase the accessibility of the enzymes, enhancing the bio-digestibility of the wastes (Taherzadeh et al., 2008, Mirahmad et al., 2010). This step allows changing the structure of the lignocelluloses, as well as increasing the surface area and porosity of biomass; modifying and removing the lignin, removing the hemicelluloses, and reducing of the cellulose crystallinity (Zhang et al., 2009). A wide variety of pretreatment methods have been studied; however these methods are typically specific to the biomass and enzymes employed (Xu et al., 2014).

Dilute acid pretreatments are normally used to degrade the hemicellulosic fraction, and common due to the fact that the used acids are commonly available (Maitan-Alfenas et al., 2015a). The disadvantage of acid pretreatments is the formation of furan and short chain aliphatic acid derivatives, which are considered inhibitors in microbial fermentation (Hendriks et al., 2009; Kumar et al., 2009; Liu et al., 2009).

The generation of by-products from the pretreatment is strongly dependent on the feedstock and the pretreatment method. The substances include phenolic compounds, aliphatic acids, furan aldehydes or other fermentation products (Jonsson et al., 2013).

Most biofuels are produced from first generation substrates including sugarcane, corn, sugar beet, etc. that directly compete with food production. For this reason, more attention has been given to the development of biofuels from agricultural residues such as corn stover, wheat straw, rice straw and sugarcane bagasse. First generation biofuels are produced from simple vegetal components including sucrose and starch, while second generation biofuel production requires the conversion of lignocellulosic biomass into simple sugars (Maitan-Alfenas et al., 2015b).

Few studies evaluate the effect of different acid concentration in the pretreatment. In this study, 0.5%, 2% and 4 % concentrations of sulfuric acid are tested. The aim of this work was to investigate the influence of these methods on the hydrolysis of sugarcane bagasse, and also to compare the formation of inhibitors for the fermentation, such as formic, acetic and levulinic acids, and 5-(hydroxymethyl-)-2-furaldehyde or hydroxymethylfurfural (HMF).

MATERIAL AND METHODS

Sugarcane bagasse was washed and dried in an oven at 70°C, until reaching a constant mass, after which it was milled (particle size less < 1 mm) and subject to acid pretreatments, with 0.5%, 2% and 4% concentrations of sulfuric acid. They were used to pretreat the milled sugarcane bagasse samples at a solid loading of 10% (w/v). The process was performed in an autoclave at 120°C for 60 minutes. Pretreated materials were separated into solid and liquid fractions using a Buchner funnel fitted with filter paper.

In order to determine the bagass composition before and after the pretreatment, 3 g of milled samples were extracted with 95% ethanol for 6 hours in a Soxhlet apparatus. Extracted samples were hydrolyzed with 72% (w/w) sulfuric acid at 30°C for 1 hour, as described by Ferraz et al. (2000). Solids were dried to a constant weight at 105°C, from which the insoluble lignin content was determined. Soluble lignin in the filtrate was determined by UV spectroscopy at 205 nm. An absorptivity value of 105 g/L cm was used to calculate the amount of acid-soluble lignin present in the hydrolysate.

The liquid fraction was analyzed in High-performance liquid chromatography (HPLC) on a system consisting of a Aminex HPX-87H column, with the mobile phase flow rate of 0.4 mL/min and temperature of 60 °C for sugars (glucose and xylose), and flow rate of 0.6 mL/min and temperature of 30 °C for aliphatic acids (formic, acetic and levulinic) and HMF (Ximenes et al., 2010, adapted).

RESULTS AND DISCUSSION

After acid pretreatment, the sugarcane bagasse was filtered and the solid fraction was dried for moisture content determination and compositional analysis (Table 1). This pretreatment was able to remove a significant part of the hemicellulose fraction, more strongly as more severe was the acid concentration. For the lignin content, these pretreatments did not result in a discrete change in the amount.

Treatment with 4% of sulfuric acid released almost 80% of the hemicellulose portion, whereas the concentration of xylose did not significantly increase the liquid portion. Both pretreatments presented xylose as the main sugar detected in the liquid fraction. Canilha et al. (2011) found the highest bagasse solubilization when the pretreatment was performed with 2.5% (w/v) sulfuric acid at 150°C for 30 minutes, which led to extensive hemicellulose removal (41.7%), but the amount of xylose recovered in the liquid fraction did not exceed in 57.6% the maximum theoretical value.

Table 1- Compositional analysis of the raw the pretreated sugarcane bagasse (SCB)

Sample	Solid fraction (%)				Liquid fraction (g/L)	
	Lignin	Cellulose	Hemicellulose	Total	Xylose	Glucose
SCB	23.91±0.07	41.25±0.18	27.62±0.50	93±0.56	-	-
SCB acid 0.5%	26.95±0.31	46.76±0.42	17.82±0.26	92±0.75	19.23±1.45	1.57±0.09
SCB acid 2%	33.70±0.41	49.20±0.07	6.76±0.12	90±0.31	20.62±0.66	2.09±0.06
SCB acid 4%	36.11±0.41	50.87±0.82	5.21±0.03	92±0.79	22.09±1.25	2.69±0.13

Figure 1 shows the inhibitors formed during the pretreatment. Under the acidified conditions, the pentoses and uronic acids resulting from hemicellulose hydrolysis undergo dehydration, with formation of 2-furaldeyde, while the hexoses are dehydrated to 5-hydroxymethyl-2-furaldehyde (Jonsson et al., 2016). Under severe pretreatment conditions, such as long reaction time and high temperature and acid concentration, HMF is more easily degraded to levulinic and formic acids (Fengel et al. 1989). Acetic acid, on the other hand, is not a sugar degradation product, but a result of the hydrolysis of the acetyl groups of hemicellulose.

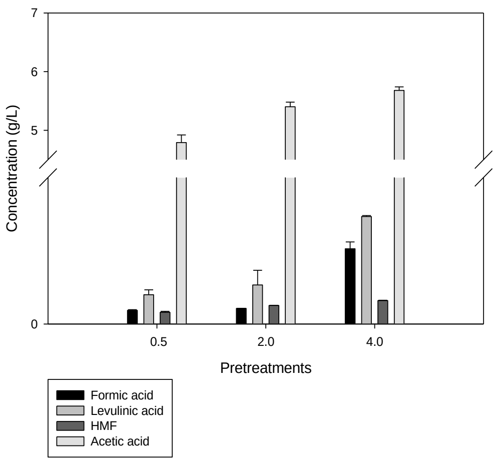

Figure 1 – Concentration of inhibitors generated during the pretreatment at different sulfuric acid concentration

The data show that in the pretreatment of 4% of H_2SO_4, the increases were 550%, 400% and 200% for the formic acid, levulinic acid and HMF, respectively, when compared with the pretreatment of 0.5% of dilute acid. The large amount of these components affects the saccharification and fermentation process, because it inhibits the enzymes.

Aguilar et al. (2002) hydrolyzed the sugarcane bagasse with 2% of H_2SO_4, at 122°C for 24.1 minutes and obtained 3.65 g/L of acetic acid. Cheng et al. (2008) worked at 1.25% of acid for 2 minutes and the amount of acid obtained was 4 g/L. The treatment used in this work is considered more severe, but, in comparison to the previously quoted, releases less inhibitors.

CONCLUSIONS

Pretreatment is a fundamental step in the conversion of lignocellulose to fermentable sugars, due to the crystallinity of cellulose. Despite the higher hemicellulose solubility having been achieved in the pretreatment with 4% concentration of sulfuric acid, the free sugars for fermentation did not show significant difference, but the inhibitors increased at a considerable rate, which can be studied for knowledge of their influence on further steps.

REFERENCES

Aguilar R, Ramirez JÁ, Garrote G, Vazquez M.2002. Kinetic study of acid hydrolysis of sugarcane bagasse. J Food Eng 55:309-318.
Canilha L, Santos VTO, Rocha GJM, Almeida e Silva JB, Guilietti M, Silva SS, Felipe MGA, Ferraz A, Milagres A, Carvalho W.2011. A study on the pretreatment of a sugarcane bagasse sample with dilute sulfuric acid. J Ind Microbiol Biotechnol 38:1467-1475.
Cheng KK, Cai, BY, Zhang JA, Ling HZ, Zhou YJ, Ge JP, Xu JM.2008. Sugarcane bagasses hemicellulose hydrolysate for ethanol production by acid recovery process. Biochem Eng J 38:105-109.
FengelD, Wegener G.1989. Wood Chemistry, Ultrastructure, Reactions. Walter de Gruyter, Berlin.
Ferraz A, Baeza J, Rodriguez J, Freer J.2000. Estimating the chemical composition of biodegraded pine and eucalyptus wood by DRIFT spectroscopy and multivariate analysis. Bioresour Technol 74:201-212.
Hendriks ATWM, Zeeman G.2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioreour Technol 100:10-18.
Jarvis M.2003. Chemistry: cellulose stacks up. Nature 426:611-612.
Jonsson LJ, Martin C.2016. Pretreatment of lignocelluloses: Formation of inhibitory by-products and strategies for minimizing their effects. Bioresour Technol 199:103-112.
Jonsson L, Alriksson B, Nilvebrant N-O.2013. Bioconversion of lignocelluloses: innibitors and detoxification. Biotechnology for Biofuels 1:6-16.
Jorgensen H, Kristensen JB, Felby C.2007. Enzymatic convertion of lignocellulose into fermentable sugars: challenges and opportunities. Biofuels Bioprod Biorefin 1:119-134.
Kumar P, Barret DM, Delwiche MJ, Stroeve, P.2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res 48:3713-3729.
Liu ZLL, Song MZ.2009. Genomic adaptation of *Saccharomyces cerevisiae* to innhibitors for lignocellulosic biomass conversion to ethanol. Appl Mycol 8:136-155.
Maitan-Alfenas GP, Visser EM, Alfenas RF, Nogueira BRG, Campos GG, Milagres AF, de Vries RP, Guimaraes VM.2015a. The influence of pretreatment methods on saccharification of sugarcane bagasse by na enzyme extract from *Chrysoporthe cubensis* and commercial cocktails: a comparative study. Bioresour Technol 192:670-676.
Maitan-Alfenas GP, Visser EM, Guimaraes VM.2015b. Enzymatic hydrolysis of lignocellulosic biomass: converting food waste in valuable products. Current Opinion in Food Science 1:44-49.
Mansour AA, Da Costa A, Arnaud T, Lu-Chau TA, Maria Fdz-Polanco, Moreira MT, Cacho Rivero JA.2016. Review of lignocellulolytic enzyme activity analyses and scale-down to microplate-based assays. Talanta 150:629-637.
Mirah madi K, Kabir MM, Jeihanipour A, Karimi K, Taherzadeh MJ.2010. Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production.2010. Bioresources 5(2):928-938.
Taherzadeh MJ, Karimi K.2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int J Mol Sci 9:1621-1651.
Ximenes E, Kim Y, Mosier N, Dien B, Ladisch M.2010. Inhibition of cellulases by phenols. Enzyme and Microbial Technology 46:170-176.
Xu Z, Huang, F.2014. Pretreatment methods for bioethanol production. Appl Biochem Biotechnol 174:43-62.
Zhang YH, Berson E, Sarkanen S, Dale BE.2009. Pretreatment and biomass recalcitrance: fundamentals and progress. Appl Biochem Biotechnol 153:80-83.