The use of thermochemical pretreatment to enhance the anaerobic biodegradability of sugarcane bagasse

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Abstract
This study aimed to evaluate the effect of physical and thermochemical pretreatments on the sugarcane bagasse biodegradability. After milling, the sugarcane bagasse was pretreated with diluted-hydrochloric acid or sodium hydroxide, at temperature of 120°C. The parameters, reaction time and concentration of the alkali were evaluated for optimization of pre-treatments using a statistical experimental design. After pretreatment, anaerobic biodegradability tests were performed using the material hydrolysed by the set of parameters that presented the best removal efficiency of lignin (alkali pretreatment). The test was also carried out using the milled bagasse and the hydrolysed material after acid pretreatment. In these tests, an inoculum consisting of a mixture of several anaerobic sludges was used. The results showed that when the diluted-acid pretreatment was used, an efficiency of 63.2% of hydrolysis of the cellulosic material was found with 60min of reaction time and 1.3M hydrochloric acid. For the alkaline hydrolysis, 102min reaction and 0.32M NaOH was the best set of parameters for removal of lignin. Using these parameters, the bagasse biodegradability increased from 61% to 64% using the alkali pretreatment, whereas it decreased to 52% using acid pretreatment. However, pretreatment can be useful for systems operated at lower retention time, i.e. eight days.

Keywords
Anaerobic digestion; lignocellulosic waste; lignin; fermentable sugars; hydrolysis.

INTRODUCTION

The lignocellulosic biomass originated from agro-industrial waste are renewable resources available in large quantities, and represent an important source of raw material that can be used in chemical and biotechnological processes, including energy production (Moraes, 2008). The production of sugarcane in Brazil for the year 2015 is estimated in $715x10^6$ ton/year. Considering that each ton of cane sugar generates 0.135 tons of bagasse, the amount of this waste will be $96.5x10^6$ tons. The net calorific value (NCV) of sugarcane bagasse is approximately 8.0 MJ/ton, therefore it would be possible to generate 772.200 GJ/year of energy in 2015 using this biomass (Brasil, 2007).

Lignocellulosic biomass is very resistant to biodegradation because of its inherent properties such as cellulose crystallinity, accessible surface area, lignin and hemicellulose structure, degree of polymerization of the cellulose, and degree of acetylation of hemicelluloses (Hendriks and Zeeman, 2009). To improve aerobic and anaerobic degradation of this material, it is frequently necessary pre-treatments for modifying those properties in order to increase the hydrolysis rate. Each pre-treatment has its own effect on cellulose, hemicellulose and lignin (the three main components of

lignocellulosic biomass). Generally, the physical pretreatment aims to increase the specific area of the biomass and decrease the crystallinity; whereas the chemical pre-treatments aim to remove lignin (using alkali) or to hydrolyse the cellulose and hemicellulose (using acids) into fermentable sugars. The characteristics of each lignocellulosic material, as well as the uses of the by-products will determine the appropriate method for (i) increasing production of reagents that promote the enzymatic attack of cellulosic fibres, (ii) preventing the formation of potential inhibitors of fermentation organisms, (iii) minimizing costs of energy and consumables, and (vi), minimizing waste (Taherzadeh and Karim, 2008).

During the acid hydrolysis, the cellulose will be degraded into glucose, and hemicellulose into xylose, arabinose, glucose, mannose and galactose. Such fermentable sugars have numerous applications, for instance, on the production of biogas, ethanol and xylitol (Rodrigues, 2007). Pretreatment with dilute acid, between 2 and 10M, can be performed either with short reaction time (in the range 5 to 10 min) and high temperatures (from 140 to 220°C) or with long reaction time (between 30 and 300 min) at lower temperatures (between 30 and 120°C). The use of concentrated acid (30 and 70%) is an alternative for decreasing the reaction time and temperature (Taherzadeh and Karim, 2008). However, this kind of pretreatment is rarely used due to the corrosion and toxicity problems (Sun and Cheng, 2002). According to the later authors, the hydrolysis of hemicellulose is more efficient using batch process at temperatures lower than 160°C and mass of substrate by volume of acid solution ratio (w/v) between 10 and 40%. On the other hand, the hydrolysis of cellulose is improved at higher temperatures and lower w/v ratio.

Depending on the severity of the pretreatment, the sugars formed in acid hydrolysis can be further degraded to toxic products such as furfural and 5-hydroxymethylfurfural (HMF). Furfural is exclusively produced from the lignocellulosic biomass by the dehydration of the pentoses with sulphuric acid or hydrochloric acid, whereas the HMF is formed during the decomposition of the hexoses (Taherzadeh and Karim, 2008). Several researchers have reported the inhibition of ethanol fermentation process by furfural and 5-HMF. However, it seems that these compounds can be anaerobically metabolized by methanogens. According to Boopathy (2009), *Methanococcus sp*. was not inhibited by furfural at a concentration of up to 15 mM, and could convert furfural into furfuryl alcohol. Sulfate-reducing bacteria metabolize furfural and furfuryl alcohol to produce acetic acid, which can eventually be converted into methane and CO_2 by acetoclastic methanogens (Belay et al., 1997).

The processes that use alkalis at moderate conditions of temperature and pressure promote "swelling" of biomass due to solvation and saponification of the material, which increase the specific surface and porosity, and decrease the cellulose crystallinity (Sun and Cheng, 2002). Moreover, the alkali promotes the breaking of ester bindings between hemicellulose and lignin, which is toxic to the fermentative microorganisms, and facilitates its removal. The pretreatment is carried out at temperature frim 30 to 130°C and reaction time in the range of 10 min to 18 h. Generally the concentration is in the range of 0.05 to 0.15 grams of alkali per gram of biomass (Vázquez et al., 2007; Moldes et al., 2002; Parajó et al., 1996).

This study aimed to define the appropriate operating conditions (reaction time and concentration of catalysts) of the thermochemical pretreatment using alkali (NaOH), as well as to evaluate the anaerobic biodegradability and biochemical methane potential (BMP) of sugarcane bagasse after three different pretreatments: (i) milling and sieving, (ii) milling and sieving followed by diluted acid (HCl) soaking at 120°C; and (iii) or milling and sieving followed by alkali (NaOH) soaking at 120°C. The physical pretreatment aimed the increasing on surface contact and avoid interference of the particle size profile on the biodegradability tests. The goal of the alkali pretreatment was the removal of lignin, whereas the goal of acid pretreatment was the production of fermentable sugars.

MATERIALS AND METHODS

The sugarcane bagasse was obtained in an ethanol plant located in the State of Pernambuco, Brazil. The main characteristics are: moisture content of 11.2%, cellulose 32.3%, hemicellulose 28.6%,

lignin 21.3% and ash and extractives 5.6%. Before thermochemical pretreatment, the sugarcane bagasse was milled in knife mill (Mill Willy, model Star FT 80) and sieved to 0.3 mm.

Acid pretreatment was carried out using diluted hydrochloric acid (HCl) according to procedures described by Bustos et al. (2002) and Herrera et al. (2003), i.e. at HCl concentration of 1.3M, reaction time of 60 minutes, temperature of 120°C and mass/volume ratio 1/10 (w/v). All experiments were performed in duplicate. The sugars generated in the dilute-hydrochloric acid pretreatment were determined by using DNS (di-nitro salicylic acid) method described by Miller (1995), which gives results of fermentable sugars based on Total Reducing Groups (TGR). The conversion rates of the cellulose and hemicellulose into sugars, and the hydrolysis efficiency of the diluted-acid pretreatment were calculated according to equations described by Cassales (2010).

The alkali pretreatment was evaluated by determining the concentration of soluble lignin in the liquid phase of the hydrolysate, using the method described by Rocha (2000). The results in terms of absorbance are indicatives of the lignin in the liquid phase, which were removed from the bagasse fibre. Different sets of reaction time and concentration of alkali (NaOH) were evaluated using a statistical experimental design, comprised of a factorial 2^2 with levels +1 and -1, 3 central points (level zero), and 4 star-points (-1.41 and +1.41), resulting in 11 experiments for each kind of pretreatment (Box and Wilson, 1951). The experiments were performed randomly and experimental error was obtained through the mean and standard deviation of the central points. The use of factorial design and statistical analysis allowed expressing the process yield in terms of lignin removal in terms of absorbance in a spectrophotometer at 280nm. The results were plotted to generate a response surface, whose equation was used to calculate the parameters that maximize the dependent variable (lignin concentration in the liquid phase of the hydrolysate, in terms of absorbance). The independent variables were reaction time and concentration of NaOH. Table 1 shows the statistical parameters used.

Table 1. Variables of the factorial, central composite rotary design used in this investigation.

Variable	**Coded values**				
	- 1,41	**- 1**	**0**	**+ 1**	**+ 1,41**
Reaction Time (min)	18	30	60	90	102
[NaOH] (M)	0.32	0.53	1.07	1.60	1.80

The anaerobic biodegradability tests were carried out in 0.3L serum bottles (with a working volume of 0.2L), which were filled with inoculum (approximately 21,5gVS/L), sugarcane bagasse (1.5gCOD/L), distilled water, and pH buffer (2.5g/L of sodium bicarbonate). Both nutrients and trace elements were added to the bottles to prevent deficiency during the test. The following nutrients (g/L) were added: NH_4Cl (0.28), K_2HPO_4 (0.25), $MgSO_4.7H_2O$ (0.10), $CaCl_2.2H_2O$ (0.01), and $CaCO_3$ (0.60). For trace elements solution (1mL/L) the following substances (mg/L) were added: $FeCl_2.4H_2O$ (2000), H_3BO_3 (50), $ZnCl_2$ (50), $CuCl_2.2H_2O$ (38), $MnCl_2.4H_2O$ (500), (NH_4) $6Mo_7O_{24}.4H_2O$ (50), $AlCl_3.6H_2O$ (90), and $CoCl_2.6H_2O$ (2000). The tests were performed during 30 days at 35±1ºC using shaking conditions of approximately 120rpm. Methane production was monitored daily during the test by using a Mariotte bottle filled with an acid solution (25gNaCl/L, and addition of H_2SO_4 until pH 2). The methane content in biogas was determined by gas chromatography. The inoculum consisted of a mixture of anaerobic sludges withdrawn from a full-scale UASB reactor of a brewery, two lab-scale reactor treating glycerine and banana pseudostem liquid, and a lab-scale CSTR used for digestion of cashew bagasse. All physical-chemical determinations followed the methods described in APHA (2005). All experiments were performed in triplicate. Maximum biodegradability, in percentage, meaning the maximum percentage of substrate COD that is converted to methane, was calculated according to El-Mashad et al. (2004). Biochemical methane potential (BMP) was calculated according to Pabón-Pereira (2009).

RESULTS AND DISCUSSION

Diluted acid pretreatment

The holocellulose hydrolysis resulted in 38.2g/L of fermentable sugar, reaching 62.83% of conversion efficiency. As this is considered a mild treatment, severity index of about 2.36 (Heitz et al., 1991), it is possible that most of the sugars formed was originated from hemicellulose. These results are consistent with those reported by Bustos et al (2002), who obtained maximum sugar concentration of 29.7g/L (128°C, HCl 0.65M, 51.1 min) from the hydrolysis of sugarcane bagasse (38% cellulose, 26.16% hemicellulose and 23.9% lignin). Herrera et al. (2003) in their study with sorghum straw (35% cellulose, 24% hemicellulose, 25% lignin) achieved up to 20g/L of fermentable sugars, using hydrochloric acid 1.95M, at 122°C, during 70min.

Acid pretreatment also resulted in 349mg/L of furfural, and 51.7mg/L of HMF (5-Hydroxymethylfurfural). Depending on the severity, the pentoses (mainly xylose) derived from the hydrolysis of hemicellulose can be degraded, generating furfural (Boopathy, 2009). According to the later researcher, the concentration of furfural and HMF is below the limit for inhibition of the methanogenic activity.

Alkali pretreatment

Figure 1 shows the experimental results of the alkali pretreatment, with the values of absorbance (Abs) as a function of sodium hydroxide concentration ([NaOH]) and reaction time (T). The absorbance values give an indication of the lignin content in the liquid phase of the hydrolysate, and represent the lignin that was removed from the bagasse fibre. The response surface was also expressed in Equation 3. It can be observed in Figure 1 that increasing the alkali concentration, there is an improvement of the lignin removal capacity. However, the analysis of variance demonstrate that such an effect is not significant (p=0.05). On the other hand, the reaction time had both linear and quadratic effects are significantly positive. This is an indication that increasing the reaction time leads to an exponential increase on the removal of lignin without an optimum point within the studied range of values. Therefore, the economic impact will be the limiting factor for the maximum reaction time.

$$Abs = 0.269 + 0.057 \times T + 0.079 \times T^2 - 0.014 \times [NaOH] + 0.042 \times [NaOH]^2 - 0.034 \times T \times [NaOH] \quad \text{(Eq.3)}$$

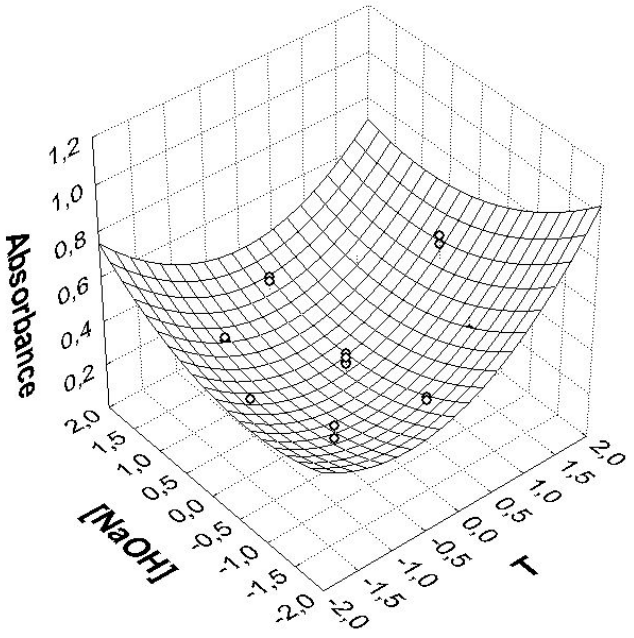

Figure 1. Results of the alkali pretreatment of sugarcane bagasse. Axis "x" and "y" are plotted using coded values, as described in Table 1.

Based on these results, the lowest alkali concentration (0.32M), and the longest reaction time (102 min) were used for maximise the lignin removal. It is possible that a longer reaction time and the

higher concentration of alkali would result in this higher lignin recovery. For instance, Xu et al. (2006) pretreated the bagasse with 1M of NaOH, at 40°C during 18h, and recovered 74% of the lignin content. The lower efficiency is also related with the type of lignocellulosic material as well as the physical pretreatment used before the alkali hydrolysis, as the size of particles affect the chemical step of the process (Pabón-Pereira, 2009). This is probably the case of Silverstein et al. (2007), who hydrolysed cotton stalks by applying an alkali pretreatment using NaOH with concentration of 1.07M, at 120°C during 90 min, achieving 65% in terms of lignin removal.

Methanogenic activity, anaerobic biodegradability and biochemical methane potential

The results of anaerobic biodegradability of the hydrolysates produced during the acid and alkali pretreatments are shown in Figure 2, together with results of the biodegradability of sugarcane bagasse after physical pretreatment (milling and sieving) and of the pure glucose that was used as control.

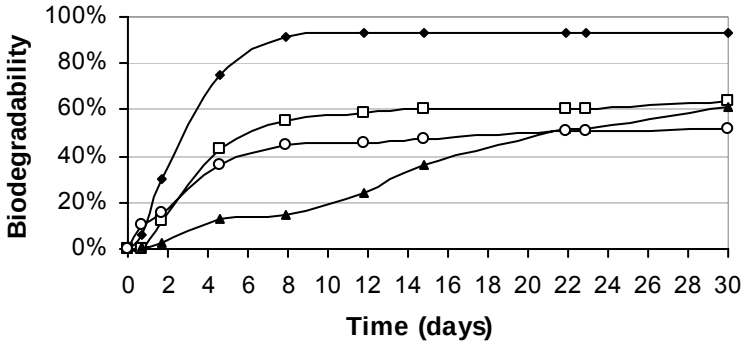

Figure 2. Results of the anaerobic biodegradability assays. ◆ control, batch reactor fed with glucose; □ batch reactor fed with alkali pretreated bagasse; ▲ batch reactor fed milled bagasse; O batch reactor fed with acid pretreated bagasse.

In this investigation, the lignin content of the bagasse was partially removed during alkali pretreatment. Therefore, it is expected that the remained lignin would be the cause of the low biodegradability. On the other hand, the hydroxyl ions cause disruption of intermolecular hydrogen bonds between cellulose and hemicelluloses, liberating hemicellulosic hydrolysate (mainly pentoses) into solution (Xu et al., 2006), which would improve the anaerobic digestion as a whole. In the case of sugarcane bagasse, lignin concentration is relatively high (21.3%), which may have affected negatively the methanogenic activity and anaerobic biodegradability. However, lignin is not the solely factor that affects biodegradability, but also the crystallinity degree as well as the accessible surface area and hemicellulose structure (Pabón-Pereira, 2009; Hendriks and Zeeman, 2009).

The methanisation rate of milled bagasse was 13mLCH_4/gVS.d, while for the hydrochloric-acid hydrolysate was 38mlCH_4/gVS.d. This result indicates that the acid pretreatment released material that is readily degradable by methanogenic archaea, for example fermentable sugars (Rodrigues, 2007). The methanisation rate of the milled bagasse pretreated with NaOH was 29mLCH_4/gSV.d, showing that removal of lignin and swelling of the fibres caused a positive impact on the digestion of lignocellulosic material (Sun and Cheng, 2002). This result was lower than that obtained with acid hydrolysate because this alkali pretreatment is appropriate for removing lignin and not for efficient holocellulose hydrolysis.

The biodegradability of bagasse that was only physically pretreated by milling was 61%, while the biodegradability bagasse that was pretreated with milling and acid hydrolysis was 52% and bagasse pretreated with milling and alkaline hydrolysis was 64%. As discussed before, the alkali caused swelling of holocellulose, making it more accessible to microbial attack. On the other hand, the acid hydrolysis released sugars that were quickly methanised, leaving a portion of cellulose bonded with lignin, which hampered the anaerobic digestion. After 30 days, there was no considerable difference between the biodegradability of the bagasse using or not the alkali pretreatment. However, after eight days, the most of the methanisation has already occurred for the bagasse pretreated with alkali

or acid, 56% and 45% respectively. Whereas, merely 15% of biodegradability was found for the milled bagasse. Therefore, pretreatment can be useful for systems operated at lower retention time.

The BMP values are consistent with the results of biodegradability (Pabón-Pereira, 2009). The BMP of the milled bagasse was 228LCH_4/kgVS, very similar to the BMP of the bagasse hydrolysed with HCl (193LCH_4/kgVS). The bagasse hydrolysed with NaOH resulted in the highest BMP (321LCH_4/kgVS).

CONCLUSIONS

The acid pretreatment (HCl 1.3M, reaction time of 60min, and temperature of 120°C) converted 62.8% of the holocellulose into 38.2g/L of fermentable sugar. However, this process apparently caused a decrease in the biodegradability of the milled sugarcane bagasse after 30 days of anaerobic digestion. Probably the remaining lignin in the hydrolysate hampered was still enough to hamper the anaerobic digestion.

Within the studied experimental conditions for the alkali hydrolysis (temperature of 120°C, NaOH concentration varying from 0.32 to 1.8M, and reaction time ranging from 18 to 102 min), it was found that increasing alkali concentration caused no significant effect, and that increasing the reaction time leads to an increase on the lignin removal. Therefore the lowest alkali concentration and the longest reaction time should be used for maximisation of the lignin removal of the sugarcane bagasse.

The biodegradability of bagasse that was pretreated with (i) milling, (ii) milling followed by acid hydrolysis and (iii) milling and alkali hydrolysis were 61%, 52% and 64%, respectively. The BMP of the aforementioned material were 228, 193 and 321LCH_4/kgVS, respectively.

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