Chemical Engineering Journal 248 (2014) 363–372

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

The use of thermochemical pretreatments to improve the anaerobic biodegradability and biochemical methane potential of the sugarcane bagasse

A.G. Costa [a], G.C. Pinheiro [a], F.G.C. Pinheiro [b], A.B. Dos Santos [c], S.T. Santaella [d], R.C. Leitão [e,*]

[a] Department of Chemistry and Environment, Federal Institute of Education, Science and Technology of Ceará, Brazil
[b] Department of Organic and Inorganic Chemistry, Federal University of Ceará, Brazil
[c] Department of Hydraulic and Environmental Engineering, Federal University of Ceará, Brazil
[d] Institute of Marine Science, Federal University of Ceará, Brazil
[e] Brazilian Agricultural Research Corporation, Tropical Agroindustry National Centre, Brazil

HIGHLIGHTS

- Enhancing anaerobic biodegradability of sugarcane bagasse using pretreatments.
- Delignification for improving biochemical methane potential of sugarcane bagasse.
- Production of renewable energy via anaerobic digestion of sugarcane bagasse.
- Sugarcane bagasse pretreatment optimisation by using response surface methodology.

ARTICLE INFO

Article history:
Received 25 October 2013
Received in revised form 10 March 2014
Accepted 16 March 2014
Available online 26 March 2014

Keywords:
Anaerobic biodegradability
Hydrolysis
Lignocellulosic waste
Pretreatment
Sugar and ethanol industry

ABSTRACT

Lignocellulosic material can be used as biomass for power generation via biogas if it is pretreated to improve the anaerobic hydrolysis step, by either solubilising the hemicellulose (total reducing groups, TRG) or removing lignin (Lig), with consequent exposition of the cellulose fibre to anaerobic degradation. We evaluated the effects of acid, alkaline, and hydrothermal pretreatments on sugarcane bagasse to increase its anaerobic biodegradability and biochemical methane potential (BMP). The highest sugar production (31.14 g TRG/g substrate) was achieved with the acid pretreatment in 6.4 min at 138 °C, with a HCl concentration of 0.63 M, and the highest lignin removal (23.24 g Lig/g substrate) was found with the alkaline pretreatment after 47 min at 184 °C and a NaOH concentration of 0.8 M. However, the best values of BMP (197.5 L CH_4/kg substrate) and anaerobic biodegradability (27.4%) were achieved by the hydrothermal pretreatment after 10 min at 200 °C, which was sufficient to generate power of 6.8 MJ/kg substrate. The results showed that the methane derived from the anaerobic digestion of these hydrolysates produced less energy than the direct burning of the dry bagasse. Thus, the recovered lignin, with its high added-value, may be used to improve environmental sustainability and profitability of the process. In this case, the alkaline pretreatment extracted 80.2% of the lignin present in the bagasse, and the hydrolysate could generate 313.4 L CH_4/kg substrate.

1. Introduction

Sugarcane bagasse is the lignocellulosic by-product generated in the sugar and ethanol industry during sugarcane juice extraction. Bagasse is commonly used as a fuel in boilers that produce low pressure steam [1]. However, not all produced bagasse is used and the surplus that remains leads to environmental and storage problems [2]. The total sugarcane production in Brazil for the 2012–13 harvesting period is estimated to be 596.6×10^6 t [3],

Abbreviations: COD, chemical oxygen demand; DNS, 3,5-dinitrosalicylic acid; HMF, 5-hydroxymethylfurfural; HPLC, high performance liquid chromatography; LHV, lower heating value; Lig, lignin; BMP, biochemical methane potential; SMA, specific methanogenic activity; TRG, total reducing groups; VS, volatile solids.

* Corresponding author. Address: Brazilian Agricultural Research Corporation, Tropical Agroindustry National Centre, Rua Dra. Sara Mesquita, 2270, 60511-110 Fortaleza, CE, Brazil. Tel.: +55 (85) 3391 7343.
E-mail address: renato.leitao@embrapa.br (R.C. Leitão).

http://dx.doi.org/10.1016/j.cej.2014.03.060
1385-8947/

with the bagasse-generation rate of approximately 0.135 t/t sugarcane [4]. Therefore, Brazil will generate approximately 80.5×10^6 t of bagasse by the end of year 2013. The conversion of the bagasse generated during the above mentioned period would result in the production of up to 32×10^9 m^3 biogas. Considering that biogas contains 60% methane and has a lower heating value (LHV) of 34 450 kJ/m^3 [5], this lignocellulosic by-product could generate theoretically 1.1×10^9 GJ of energy.

Sugarcane bagasse fibre consists of cellulose (25–47%), hemicellulose (20–35%), and lignin (15–35%) [2,6,7]. Cellulose and hemicellulose can be converted into methane via anaerobic fermentation, producing energy and increasing the energy potential of the sugar and ethanol industry. However, to maximise energy production by anaerobic digestion, it is necessary to consider the limiting step of the process, hydrolysis [8]. In general, during the anaerobic digestion of lignocellulosic material, the complex organic polymer components initially undergo hydrolysis, via enzymatic decomposition, into monomers such as sugars and organic acids. However, the cellulose and hemicellulose fractions in the lignocellulosic material are surrounded by lignin, which acts as a physical barrier and hinders anaerobic degradation. In this case, a pretreatment method may remove the lignin fraction. Furthermore, the hemicellulose fraction itself acts as a physical barrier to the enzymatic attack of the cellulose. In this case, pretreatment must solubilise the hemicellulose fraction into sugars, allowing the hydrolysis of the cellulose and consequently increasing anaerobic biodegradation [9].

Different factors affect the biodegradability of lignocellulosic materials: cellulose crystallinity, accessible surface to enzymes, structure and distribution of lignin [10–12]. Inter- and intramolecular hydrogen bonds maintain the crystalline regions and make the cellulose resistant to acid, alkaline, or enzymatic hydrolysis. Furthermore, the crystalline regions make cellulose water insoluble, which hinders biodegradation [13]. The resistance to enzymatic degradation of the lignocellulosic material is also influenced by the lignin content and distribution. This hydrophobic polymer forms an interlaced network [14], which decreases the accessible surface and prevents the cellulolytic enzymes attack [15,16], slowing or preventing the anaerobic hydrolysis [17]. Therefore, the delignification process can improve the anaerobic biodegradation [9,10] and may represent a good prospect for lignin recovery which is a high value-added by-product.

The most-used physical pretreatment is milling, which decreases the crystallinity and degree of polymerisation of the cellulose [10]. However, depending on the structure and composition of the biomass (cellulose, hemicellulose and lignin content), as well as on the use of the pretreated material (ethanol, biogas or lignin production), it is necessary a further treatment, which includes: (i) acid hydrolysis (using dilute or concentrated acid), in which part of the cellulose and hemicellulose is converted into fermentable sugars [9]; (ii) hydrothermal hydrolysis, wherein the hemicellulose is solubilised and produces acetic acid that acts as a catalyst for the reaction [18], with further reduction of the polymerisation degree that leads to an increase in sugar yield [19]; (iii) alkaline hydrolysis, typically used in lignocellulosic materials with high lignin content [20–22], that promotes the lignin solubilisation, improves the reactivity of the remaining polysaccharides, removes acetyl groups and various uronic acid substitutions of the hemicellulose [23], swells the material, increases the porosity, and consequently increases the surface accessibility for exoenzymes [2,24]; and (iv) enzymatic hydrolysis, catalysed by a complex composed of various enzymes such as cellulase, hemicellulase, β-glucosidase, xylanase, arabinase, and pectinase [25–27]. The latter method is often used in conjunction with other types of pretreatments to reduce the physical barriers presented by lignin prior to the enzymatic attack.

Several other advanced pretreatment methods for delignification or sugar production from sugarcane bagasse have been investigated: addition of subcritical or supercritical CO_2 to form weak acid catalyst with water [28,29]; ultrasonic cavitation for increasing accessible surface for enzyme attack [30–32]; microwave-based heating for increasing the reaction temperature [33,34]; organosolv delignification [35,36]; lignin degradation by ozone and singlet oxygen produced by plasma [37,38], ammonia fibre expansion for increasing accessible surface and cleavage of the ether bonds of lignin [39,40]; disruption of the inter- and intramolecular hydrogen bonds of cellulose by ionic liquid [41,42]. However, most of the researches on pretreatment of sugarcane bagasse focus on 2nd generation ethanol, which is mainly based on the fermentation of hexoses by *Sacaromices cerevisiae*. On the other hand, the methanogenic consortia can hydrolyse cellulose and hemicellulose, producing sugars and organic acids that will be further converted into methane. Therefore, the pretreatment can be less aggressive or severe, with less input of energy and chemicals, just enough to increase the extracted lignin, to decrease crystallinity and to increase surface accessibility.

This study evaluated several pretreatments applied to sugarcane bagasse to maximise its anaerobic biodegradability, and hence, its biochemical methane potential (BMP). Prior to the anaerobic digestion assays, the bagasse was subjected to hydrothermal pretreatment, acid hydrolysis with dilute hydrochloric acid, or alkaline hydrolysis with sodium hydroxide solution. Each pretreatment was optimised based on temperature, reaction time, and catalyst concentration by applying multivariate factorial design and response surface methodology.

2. Material and methods

2.1. Sugarcane bagasse source

The sugarcane bagasse was provided by an ethanol plant located in Pernambuco, Brazil. Because of its high moisture content, the by-product material was frozen at −20 °C and then lyophilised, milled in a knife mill, sieved (18 mesh, 1 mm), homogenised, and stored at room temperature. Prior to carrying out the three pretreatments, the cellulose, hemicellulose, and lignin contents of the bagasse were characterised (38.7% cellulose, 33.0% hemicellulose, 25.7% lignin, and 2.6% ash and extractives).

2.2. Experimental design and statistical analysis

All hydrolysis assays were performed using 2^2 and 2^3 multivariate experimental designs (two levels and two or three independent variables, depending on the pretreatment), with the central point in triplicate (level 0) and four or six star-points (when necessary), as shown in Table 1. Concentrations of acid or base, reaction time, reaction temperature, and, in the case of hydrothermal pretreatment, the bagasse-mass-to-catalyst-solution-volume ratio, were the independent variables. The P_{TRG} and P_{Lig} were the dependent variables.

The interrelationship between dependent and independent variables was calculated using linear, quadratic and interaction effects. The equations of the response surface were calculated by multiple linear regressions using the least squares methodology. Statgraphics® Centurion XV (StatPoint, USA) was used for statistical analysis and response surface modelling.

2.3. Hydrolysis assays

The hydrolysis assays were conducted in 500 mL high-pressure reactors (Berghof, model BR-300). In all the tests, the ratio of the

Table 1
Factors and levels of several pretreatments applied to sugarcane bagasse.

Factors	Levels				
	$-\alpha$	−1	0	+1	$+\alpha$
Hydrothermal hydrolysis					
Reaction time (min)		10	20	30	
Temperature (°C)		150	175	200	
Acid hydrolysis					
[HCl] (M)	0.63	0.9	1.3	1.7	1.97
Reaction time (min)	6.4	20	40	60	73.6
Temperature (°C)	103.2	110	120	130	136.8
Alkaline hydrolysis					
[NaOH] (M)	0.8	1.0	1.3	1.6	1.8
Reaction time (min)	13	20	30	40	47
Temperature (°C)	116	130	150	160	184

α = 1.424 for hydrothermal hydrolysis. α = 1.682 for acid and alkaline hydrolysis.

bagasse mass to the catalyst solution volume (m/v) was 10%. The hydrothermal pretreatment was evaluated based on sugar solubilisation, in terms of specific TRG production (P_{TRG}), according to different sets of temperatures (T = 150–200 °C) and reaction times (t = 10–30 min) [7,43,44]. Acid hydrolysis with dilute HCl was also evaluated based on the P_{TRG}; the independent variables were T (103–137 °C), acid concentration ([HCl] = 0.63–1.97 M), and t (6.4–73.6 min) [6,45,46]. Alkaline hydrolysis with NaOH was evaluated based on the specific lignin production (P_{Lig}), with different sets of T (116–184 °C), alkali concentration ([$NaOH$] = 0.80–1.80 M), and t (13–47 min).

2.4. Sample preparation

After hydrolysis, the liquid fraction was characterised in terms of sugars (total reducing groups, TRG), total and dissolved chemical oxygen demand (COD), furfural, and 5-hydroxymethylfurfural (HMF) concentrations (for the hydrothermal and acid pretreatments), and lignin concentration (for the alkaline pretreatment). From the best results of the different pretreatments, the solid fractions of the hydrolysates were characterised for total solids, total COD, and lignin content (for the alkaline hydrolysis).

2.5. Anaerobic biodegradability and biochemical methane potential assays

The hydrolysates from the best results of each pretreatment were evaluated in terms of biodegradability and BMP. After the acid and hydrothermal pretreatments, the hydrolysates were neutralised with 2 M NaOH and characterised in terms of COD. A sample of the whole hydrolysate, including the solid and liquid fractions, was used as substrate for each biodegradability test. After alkaline pretreatments, the loose lignin was removed from the hydrolysates. The solid fractions were washed with 200 mL NaOH at the same concentration as that used in the pretreatment, washed with distilled water until neutral conditions, and then characterised in terms of COD. For the alkali pretreatment, only the solid fractions were used as substrate for the biodegradability tests.

The methodology for assessing the anaerobic biodegradability was based on specific methanogenic activity (SMA) assays [47]. The tests were carried out in 0.3 L serum bottles (0.2 L working volume), which were filled with the inoculum (approximately 1.4 g of volatile solids (VS)/L), substrate (1.5 g COD/L), distilled water, and pH buffer (1.0 g/L $NaHCO_3$). Nutrients and trace elements were added to prevent deficiencies during the test (g/L): NH_4Cl (0.28), K_2HPO_4 (0.25), $MgSO_4{\cdot}7H_2O$ (0.10), $CaCl_2{\cdot}2H_2O$ (0.01), and $CaCO_3$ (0.60); and solutions of trace elements (1 mL/L) containing the following substances (mg/L): $FeCl_2{\cdot}4H_2O$ (2000), H_3BO_3 (50), $ZnCl_2$ (50), $CuCl_2{\cdot}2H_2O$ (38), $MnCl_2{\cdot}4H_2O$ (500), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (50), $AlCl_3{\cdot}6H_2O$ (90), and $CoCl_2{\cdot}6H_2O$ (2000). The tests were performed over 30 days at 35 ± 1 °C, under shaking conditions (approximately 120 rpm).

Control flasks (without substrate) were used to monitor the biogas produced by endogenous respiration. The inoculum consisted of a mixture in equal proportions of rumen liquid from goats and sludges withdrawn from three anaerobic sludge blanket reactors treating (i) sewage, (ii) brewery effluent, and (iii) glycerol with nutrients. The assays were performed in a Micro-Oxymax anaerobic respirometer (Columbus Instruments, USA), in which the biogas production was monitored automatically via pressure sensors, and methane was analysed by an infrared sensor. All physical–chemical determinations followed the methods described in Standard Methods for the Examination of Water and Wastewater [48]. All experiments were performed in triplicate.

2.6. Calculations

The maximum biodegradability (%), which is the maximum percentage of substrate COD that is converted to methane, was calculated according to Eq. (1) [49], where $Bio(\%)$ is the sample biodegradability (%); $COD^{30}_{CH4\text{-}substrate}$ is the total methane volume produced in the flask containing the substrate in terms of COD (g), considering 0.395 L CH_4/g COD at 35 °C and 1 atm; $COD^{30}_{CH4\text{-}control}$ is the total volume of methane produced by the control flask, in terms of COD (g); and $COD^{0}_{T\text{-}substrate}$ is the initial quantity of substrate in terms of COD (g) added to each reactor. The BMP was calculated based on the cumulative methane production at the end of the biodegradability test and the mass of the substrate used in the experiment, according to Eq. (2) [49], where BMP is the methane production potential (L CH_4/kg substrate); $V^{30}_{CH4\text{-}substrate}$ is the cumulative methane volume produced after 30 days in the flask containing the substrate (L CH_4); $V^{30}_{CH4\text{-}control}$ is the methane volume produced by the control flask after 30 days (L CH_4); and $M^{0}_{substrate}$ is the initial mass of the substrate in the bottle (kg substrate).

$$Bio(\%) = \frac{\left(COD^{30}_{CH4\text{-}substrate} - COD^{30}_{CH4\text{-}control}\right)}{COD^{0}_{T\text{-}substrate}} \times 100 \quad (1)$$

$$BMP = \frac{\left(V^{30}_{CH4\text{-}substrate} - V^{30}_{CH4\text{-}control}\right)}{M^{0}_{substrate}} \quad (2)$$

The hydrolysis efficiency of anaerobic digestion was calculated based on the production of methane and dissolved organic compounds. The latter were determined in terms of dissolved COD. The hydrolysis efficiency was calculated as shown in Eq. (3) [50], where $H(\%)$ is the anaerobic hydrolysis efficiency (%); $COD^{30}_{Diss\text{-}substrate}$ is the mass of the dissolved organic compounds in the flask containing the substrate after 30 days in terms of COD (g); $COD^{30}_{Diss\text{-}control}$ is

$$H(\%) = \frac{\left[\left(COD^{30}_{CH4\text{-}substrate} - COD^{30}_{CH4\text{-}control}\right) + \left(COD^{30}_{Diss\text{-}substrate} - COD^{30}_{Diss\text{-}control}\right) - COD^{0}_{Diss\text{-}substrate}\right]}{COD^{0}_{T\text{-}substrate}} \times 100 \quad (3)$$

the mass of the dissolved organic compounds in the control flask after 30 days in terms of COD (g); and $COD^{0}_{Diss\text{-}substrate}$ is the initial mass of the substrate in terms of dissolved COD (g).

The hydrolysis efficiencies of the hydrothermal and acid pretreatments were assessed based on the TRG production. The calculations were performed based on the percentage of cellulose and hemicellulose contained in the dry bagasse (before hydrolysis), considering that all the holocellulose content would be converted into TRG. Eqs. (4) and (5) were used to calculate the mass of TRG in the hydrolysate and the maximum achievable TRG, respectively, assuming complete holocellulose hydrolysis [51]. Here, M_{TRG} is the TRG mass in the hydrolysate (g); [*TRG*] is the TRG concentration in the hydrolysate (g/L); V_{Hid} is the hydrolysate volume after the experiment (L); M_{TRG_Max} is the total mass of sugars in the dry bagasse based on TRG (g); *%Cell* is the cellulose percentage in the dry bagasse; *%Hem* is the hemicellulose percentage in the dry bagasse; *FCC* is the conversion factor for cellulose (0.9); *FPC* is the factor for estimating cellulose loss during hydrolysis (1.055); *FCH* is the conversion factor for hemicellulose (0.88); *FPH* is the factor for estimating hemicellulose loss during hydrolysis (1.155); and m_{dry} is the bagasse mass used in the hydrolysis assays, based on dry matter (g).

$$M_{TRG} = [TRG] \times V_{Hid} \tag{4}$$

$$M_{TRG\_Max} = \left(\frac{\%Cell}{FCC \times FPC \times 100} + \frac{\%Hem}{FCH \times FPH \times 100}\right) \times m_{dry} \tag{5}$$

Hydrolysis efficiency of the pretreatments in terms of TRG was calculated using Eq. (6). The P_{TRG}, based on dry matter, was calculated according to Eq. (7), where η_{TRG} is the holocellulose fraction conversion efficiency (cellulose + hemicellulose) of the dry bagasse into TRG (%), and P_{TRG} is the specific TRG production based on dry matter (g TRG/g bagasse) [52].

$$\eta_{TRG} = \frac{M_{TRG}}{M_{TRG\_Max}} \tag{6}$$

$$P_{TRG} = \frac{M_{TRG}}{m_{dry}} \tag{7}$$

The hydrolysis efficiency of the alkali pretreatments was assessed based on the lignin extraction. The calculation was performed based on the lignin percentage contained in the dry bagasse (before hydrolysis), which was determined according to the modified method based on the TAPPI standards [53,54]. Eqs. (8) and (9) were used to calculate the mass of lignin in the hydrolysate and the maximum achievable lignin, assuming complete solubilisation, where M_{Lig_Hid} is the lignin mass in the hydrolysate (g); [*Lig*] is the lignin concentration in the hydrolysate (g/L), which was determined spectrophotometrically as described by Rocha [55]; M_{Lig_Sample} is the total mass of lignin in the dry bagasse (g); *%Lig* is the percentage of lignin contained in the dry bagasse (before hydrolysis); and V_{Hid} and m_{dry} were previously defined.

$$M_{Lig\_Hid} = [Lig] \times V_{Hid} \tag{8}$$

$$M_{Lig\_Sample} = \%Lig \times m_{dry} \tag{9}$$

In the spectrophotometric method used for lignin determination, the lignin solubilisation efficiency, η_{Lig} (%), was calculated using Eq. (10) [52]. P_{Lig} is the specific lignin production based on dry matter (g Lig/g bagasse), calculated using Eq. (11).

$$\eta_{Lig} = \frac{M_{Lig\_Hid}}{M_{Lig\_Sample}} \tag{10}$$

$$P_{Lig} = \frac{M_{Lig\_Hid}}{m_{dry}} \tag{11}$$

The lignin removal efficiency, η_{Lig} (%), for the best result of the alkaline pretreatment was also calculated using Eqs. (12) and (13) [52], where % Lig_{Hid_CF} is the lignin percentage in the hydrolysate based on the lignin mass in the dry bagasse (%); $\%Lig_{Hid}$ is the lignin percentage in the hydrolysate based on the mass of hydrolysed bagasse; $\%Hol_{CF}$ is the percentage of holocellulose in the dry bagasse (%); and % Lig_{CF} is the percentage of lignin in the dry bagasse (%).

$$\%Lig_{Hid\_CF} = \frac{\%Lig_{Hid} \times \%Hol_{CF}}{(1 - \%Lig_{Hid})} \tag{12}$$

$$\eta_{Lig} = \frac{\%Lig_{CF} - \%Lig_{Hid\_CF}}{\%Lig_{CF}} \tag{13}$$

The hydrolysis yield ($\%y_{Hid}$) represents the percentage of the dry fibre that was recovered after hydrolysis. Here, $\%y_{Hid}$ was calculated based on the ratio of the mass of the bagasse and the mass of the hydrolysed fibre, both considering dry matter, according to Eq. (14) [52], where m_{Hid_dry} is the hydrolysed fibre mass after hydrolysis assays, based on dry matter (g).

$$\%y_{Hid} = \frac{m_{dry}}{m_{Hid\_dry}} \tag{14}$$

2.7. Analytic methods

Total solids, moisture, and COD were determined according to the Standard Methods for the Examination of Water and Wastewater [48]. Ash, extractives, holocellulose (cellulose and hemicellulose) and lignin were determined according to modified methods based on the TAPPI T211 om-02, T412 om-02, T204 cm-97, T222 om-02, and T203 cm-09 standards [53,54]. The soluble lignin content evaluated after alkaline hydrolysis was determined using a spectrophotometric method [55]. The method TAPPI 222 om-02 was used for more accurate determination of the lignin concentration, which was necessary to calculate the lignin removal efficiency of the best result of the alkaline pretreatment. The total sugars, in terms of TRG, were determined using the DNS (3,5-dinitrosalicylic acid) method [56]. Furfural and HMF concentrations were determined by high performance liquid chromatography (HPLC) using a Zorbax SB C-18 column (Agilent) at 25 °C, UV/vis detection at 276 nm, and acetonitrile/water (2:8 v/v) with 1% acetic acid as the eluent at a flow rate of 0.7 mL/min. The injected sample volume was 20 μL. Samples were pre-filtered through a cellulose acetate membrane ME25 with a porosity of 0.45 μm and diameter of 13 mm.

3. Results and discussion

3.1. Hydrothermal pretreatment

The results of hydrothermal hydrolysis were determined based on the P_{TRG}. The values of the dependent (P_{TRG}) and independent (*T*, *t*, and m/v) variables, and the results for [TRG], η_{TRG}, HMF, and furfural are shown in Table 2.

The results of the statistical analysis of the hydrothermal pretreatment, with the effects and significant levels of *t* and *T* on P_{TRG}, are presented in Table 3. The independent variable *T* showed a significant positive linear effect ($p < 0.05$), which was an indication that sugar production increased with increasing temperature. However, the variable *t* and the interaction between *T* and *t* had no significant effect ($p > 0.05$). Thus, it can be inferred that, to increase the solubility of the sugars, it is sufficient to increase the temperature while maintaining the shortest reaction time. This is consistent with the results shown in Table 2: the best results in

Table 2
Effects of hydrothermal hydrolysis on sugarcane bagasse.

Assay	T (°C)	t (min)	[TRG] (g/L)	P_{TRG} (gTRG/gBag)	η_{TRG} (%)	HMF (g/L)	Furfural (g/L)
1	150	10	0.89	0.009	1.48	0.0041	0.00
2	200	10	12.51	0.135	21.97	0.0016	0.01
3	150	30	1.94	0.020	3.26	0.0010	0.00
4	200	30	12.15	0.127	20.68	0.0040	0.58
5	175	20	6.02	0.065	10.65	0.0022	0.00
6	175	20	5.40	0.057	9.35	0.0014	0.00
7	175	20	4.90	0.051	8.38	0.0053	0.00
OC	200	10	12.25	0.129	20.99	0.0013	0.01

OC – Optimal condition.

Table 3
Estimated effects, standard errors, levels of significance (p at 95% confidence level), and model coefficients for P_{TRG} after hydrothermal pretreatment of sugarcane bagasse, according to 2^2 experimental design presented in Table 1.

Factor	Effect	Standard error	p	Coefficient
Constant	0.0663	0.003	0.000	−0.409464
A: T – temperature (linear)	0.1165	0.007	0.000	0.00271
B: t – reaction time (linear)	0.0015	0.007	0.8303	0.0034
AB	−0.0095	0.007	0.1747	−0.000019

terms of TRG, η_{TRG}, and P_{TRG} were obtained in assay 2, in which a higher temperature and a shorter reaction time were used. The high sugar solubilisation under these conditions may be associated with an increased concentration of acetic acid, originating from acetyl group cleavage that results from the more severe conditions imposed at 200 °C [57].

A new assay using the conditions that maximised the production of sugars (200 °C, 10 min) was carried out, resulting in a P_{TRG} of 0.129 g/g bagasse, which confirmed the first trial. The hydrolysis efficiency was 21%, which probably corresponds to the hemicellulose fraction, because the hydrothermal pretreatment mainly solubilised that component of the lignocellulosic material [7,9]. The concentrations of furfural and HMF were 0.0013 and 0.01 g/L, respectively. Recent studies have shown that the methanogenic archaea, e.g. *Methanococcus* sp., were inhibited only at furfural concentrations exceeding 1.44 g/L [58], and that the hydrogen-producing bacteria, e.g. *Thermoanaerobacterium thermosaccharolyticum* W16, were not affected at 0.5 g/L concentrations of either furfural or HMF [59]. It is possible that these by-products of sugar degradation do not permanently inhibit the anaerobic metabolism; rather, they may cause a delay in the process and, in furfural and HMF concentrations of up to 1 g/L, an increase in the methane yield is expected [60].

These results are consistent with findings from other studies, in which the conditions for maximum sugars solubility were 2 min at 200 °C [44] and 27 min at 193 °C [43]. Studies with sugarcane bagasse pretreated hydrothermally at 150–190 °C over 15–240 min showed that higher temperatures (190 °C) and an m/v of 5% resulted in the increased solubilisation of sugars from the hemicellulose fraction (53% w/w) at a reaction time of 2 h [7]. However, the authors found that longer reaction times caused degradation of the sugars into furfural and HMF.

Considering that all the solubilised sugars in this research were from hemicellulose hydrolysis, one can calculate that the process achieved 61% efficiency for the hemicellulose fraction. This result was slightly higher than that found by Boussarsar and co-authors [7], but the reaction time was 10 min. This higher performance at shorter reaction time was likely associated with the higher mass-to-volume ratio (10%), which promoted an increment of acetic acid production from acetyl group cleavage. Acetic acid is the catalyst for hydrolysis.

3.2. Acid hydrolysis

The values of the dependent (P_{TRG}) and independent (T, t, and [HCl]) variables, and the results for [TRG], η_{TRG}, HMF, and furfural are shown in Table 4. The estimated effects, according to the significance level (p), representing the solubilisation of sugars from the holocellulosic fraction of the sugarcane bagasse are shown in the Pareto chart in Fig. 1, wherein all variables resulted in significant and negative effects on P_{TRG}. This indicates that the smaller the values of the independent variables, the greater the production of sugars. On the other hand, the quadratic effects of all the variables were positive, which meant that there were points of minimum P_{TRG} within the value ranges of the variables. The effect of the interaction between T and [HCl] was significantly negative, indicating that one variable should be reduced while increasing the other to maximise P_{TRG}. Therefore, it seems that an increase in temperature and a simultaneous reduction in the acid concentration would maximise the solubilisation of the sugars. The effect of variable t was significantly negative, which means that the reaction time increase causes a decrease in the sugar production. Based on the effects of the several parameters and their interactions, one can infer that the operational condition that maximise the solubilisation of sugars within the studies range is the highest temperature, the lowest acid concentration and the shortest reaction time.

Although the coefficient of determination ($R^2 = 0.58$) obtained in the regression analysis was quite low, the variables and their interactions strongly affected P_{TRG}. Therefore, Eq. (15) can represent the behaviour of P_{TRG} as a function of t, T, and [HCl], as shown by the surface response in Fig. 2. The optimal conditions for acid hydrolysis can be calculated based on the derivative of this equation, resulting in a reaction time of 6.4 min, a temperature of 136 °C, and an acid concentration of 0.63 M. Under these conditions, the model result for P_{TRG} was 0.38 g/g bagasse. A new assay using such conditions was carried out to compare the experimental and calculated results.

$$P_{TRG} = 1.87 - 0.00135xt - 0.024 \times T - 0.197 \times [HCl] + 0.000028 \times t^2 + 0.0001 \times T^2 - 0.0019 \times T \times [HCl] + 1.13 \times [HCl]^2 \quad (15)$$

The experimental value found for P_{TRG} was 0.344 g/g bagasse (Table 4), which was the same as that predicted by the model at a confidence interval of 95%. The hydrolysis efficiency of the holocellulosic fraction was approximately 56%. The results found in this study were similar to those obtained by Bustos and co-authors [6], who examined the acid pretreatment of milled sugarcane bagasse

Table 4
Effects of acid hydrolysis on sugarcane bagasse.

Assay	T (°C)	t (min)	[HCl] (g/L)	[TRG] (g/L)	P_{TRG} (gTRG/gFibre)	η_{TRG} (%)	HMF (g/L)	Furfural (g/L)
1	110	20	0.9	23.04	0.253	41.26	0.003	0.00
2	110	60	0.9	21.79	0.235	38.24	0.004	0.58
3	130	20	0.9	19.57	0.213	34.76	0.001	0.00
4	130	60	1.7	14.63	0.158	25.82	0.003	0.99
5	110	20	1.7	19.62	0.212	34.62	0.001	0.80
6	110	60	1.7	15.24	0.167	27.17	0.001	1.32
7	130	20	1.7	10.47	0.117	19.09	0.003	1.71
8	130	60	1.3	7.93	0.084	13.68	0.002	1.82
9	120	40	1.3	13.40	0.146	23.75	0.001	0.55
10	120	40	1.3	13.30	0.147	24.01	0.002	0.00
11	120	40	1.3	12.44	0.139	22.59	0.001	0.00
12	120	6.4	1.3	21.17	0.232	37.87	0.001	0.00
13	120	73.6	1.3	13.89	0.150	24.38	0.003	0.00
14	103.2	40	1.3	11.75	0.127	20.76	0.001	0.00
15	136.8	40	0.63	24.19	0.254	41.43	0.006	0.78
16	120	40	1.97	26.27	0.272	44.39	0.003	1.12
17	120	40	0.63	15.17	0.166	27.10	0.001	0.80
OC_M	136	6.4	0.63		0.377			
OC_E	136	6.4	0.63	31.14	0.344	55.99	0.31	0.33

OC_M – optimal condition (model); OC_E – optimal condition (experimental).

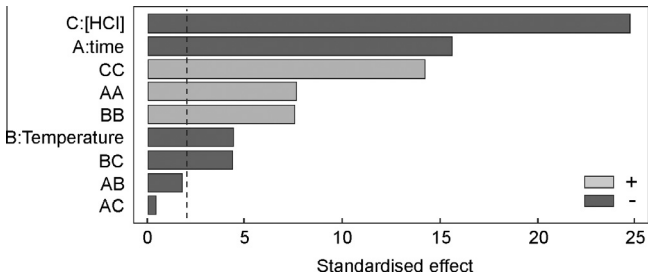

Fig. 1. Pareto diagram of acid hydrolysis assays, showing effects of t, T, and [HCl] on P_{TRG}.

and found that the maximum hydrolysis efficiency was achieved at the lowest acid concentration (0.9 M HCl) and the highest temperature (128 °C), with a reaction time of 51.1 min.

When a higher temperature or longer reaction time is applied, the monosaccharides derived from this reaction undergo further hydrolysis, accelerating the formation of furfural and HMF. Therefore, the degradation of monosaccharides should be avoided to improve the TRG yield [11,61]. In this research, the HMF and furfural concentrations obtained under the optimum conditions were lower than the methanogen-inhibitory levels reported in the literature [58–60].

The solubilisation of the sugars in the sugarcane fibre by acid hydrolysis using other acids has also been investigated [62–69], and results similar to these findings were obtained. However, the experimental conditions that maximised solubilisation were different because the reactive potential of each acid was different. For example, Aguilar and co-authors [63] found a 90% hydrolysis efficiency for the hemicellulose fraction using 2% H_2SO_4 at 122 °C for 24 min. A shorter reaction time was necessary when using H_2SO_4 in place of HCl, possibly because H_2SO_4 is a stronger acid. The acid type is also one of the factors that influences the hydrolysis of lignocellulosic material [70]. However, if the objective of the hydrolysis is to produce methane, H_2SO_4 must be avoided, because the SO_4^{2-} released in the process would be consumed by the sulphate-reducing bacteria to produce H_2S rather than methane [71].

3.3. Alkaline hydrolysis

The values of the dependent (P_{Lig}) and independent (T, t and [$NaOH$]) variables, as well as the results for [Lig] are shown in Table 5. Fig. 3 shows the Pareto chart, representing the extraction of lignin according to the level of significance. The independent

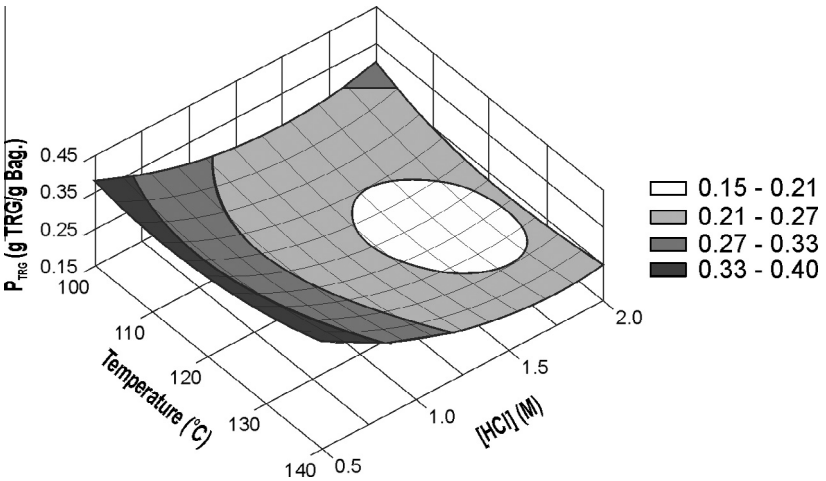

Fig. 2. Response surface plotted using Eq. (15), which describes specific production of sugars as a function of T and [HCl] (t = 6.4 min).

Table 5
Effects of alkaline hydrolysis on sugarcane bagasse.

Assay	T (°C)	t (min)	[NaOH] (M)	[Lig] (g/L)	P_{Lig} (gLig/gBag)
1	130	20	1.00	23.24	0.26
2	170	20	1.00	24.50	0.27
3	130	40	1.00	22.28	0.24
4	170	40	1.00	25.13	0.28
5	130	20	1.60	24.38	0.27
6	170	20	1.60	24.79	0.28
7	130	40	1.60	20.82	0.24
8	170	40	1.60	23.54	0.27
9	150	30	1.30	22.70	0.25
10	150	30	1.30	23.50	0.26
11	150	30	1.30	23.20	0.25
12	116	30	1.30	19.81	0.22
13	184	30	1.30	22.49	0.24
14	150	13	1.30	21.44	0.24
15	150	47	1.30	23.12	0.25
16	150	30	0.80	25.88	0.28
17	150	30	1.80	22.70	0.25
OC_M	184	47	0.80		0.3
OC_E	184	47	0.80	23.24	0.26

OC_M – optimal condition (model); OC_E – optimal condition (experimental).

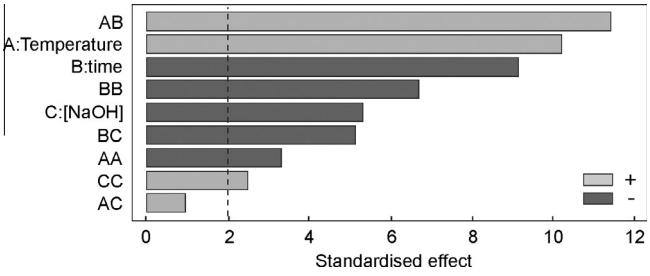

Fig. 3. Pareto diagram of alkaline hydrolysis assays, showing effects of t, T, and [$NaOH$] on P_{Lig}.

variable T had a positive and linear effect on P_{LIG}, and the variables t and [$NaOH$] showed negative and linear effects, indicating that increasing the temperature and reducing the reaction time and alkali concentration favoured lignin extraction. The variable T showed a negative quadratic effect, indicating that there is a maximum P_{LIG}, and therefore, an optimal reaction temperature within the range of the studied values. According to Pan and co-authors [72], an increase in the reaction temperature leads to a decrease in the degree of polymerisation, i.e. an increasing cleavage rate of the ether bonds that cause solubilisation of lignin fragments. On the other hand, the decrease in the lignin content when high values of T (high severity factor) are applied occurs because of its degradation to phenolic compounds such as 4-hydroxybenzoic acid that originate from the rupture of ester bonds that link the hydroxyl groups of cinnamic alcohol, and because of syringaldehyde and syringic acid that originate from the degradation of syringyl propane units [73].

The regression analysis of the data in Table 5 generated Eq. (16) with $R^2 = 0.7$, which is a statistical model of P_{Lig} as a function of t, T, and [$NaOH$]. Based on this equation, the experimental conditions that maximised the extraction of lignin were estimated from the derivative as a function of t, T, and [$NaOH$], resulting in a reaction time of 47 min, a temperature of 184 °C, and an alkali concentration of 0.80 M. Under these conditions, the result of the model for the production of lignin (P_{LIG}) was 0.3 g Lig/g bagasse.

$$P_{Lig} = 0.288 + 0.0006 \times T - 0.004 \times t - 0.049 \times [NaOH] - 0.000008 \times T^2 + 0.00006 \times T \times t - 0.00006 \times t^2 - 0.002 \times t \times [NaOH] + 0.0225 \times [NaOH]^2 \quad (16)$$

Fig. 4 shows the response surface that best represents the distribution of the set of values of t, T, [$NaOH$], and P_{Lig} in space. The experimental value found for P_{Lig} was 0.26 g/g bagasse, which was 80.2% of the total lignin in the fibre, according to Eq. (13). There was a weight loss of 55.7% of the total fibre weight, which largely corresponded to the hemicellulose and lignin that were removed. Kim and Han [20] used an alkaline pretreatment (0.53–2.13 M NaOH, 60–90 min, and 60–100 °C) to solubilise the glucose fraction of rice straw. The maximum glucose yield (254.6 g/kg biomass) was obtained using 1.33 M NaOH after 60 min at 80 °C. The authors could remove 48.3% of the lignin, and found that 59.1% of the total weight was lost. Comparing our results with those of Kim and Han [20], it is clear that the temperature was mainly responsible for the increased lignin removal efficiency. The catalytical cleavage of the ether bonds between lignin and hemicellulose only occurs at high temperatures [72].

3.4. Anaerobic biodegradability and biochemical methane potential

The results of the biodegradability and BMP assays of the hydrolysates produced by the various pretreatments (hydrothermal, acid, and alkaline) are presented in Table 6. The highest anaerobic biodegradability was achieved with alkaline pretreatment, which extracted approximately 80% of the lignin content of the bagasse. This is because lignin acts as a mechanical barrier and is responsible for the integrity, structural rigidity, impermeability, and adhesion of cellulose and hemicellulose, increasing its resistance to microbial attack. Therefore, the delignification process improves the rate and extent of hydrolysis and, consequently, the hydrolysis step of anaerobic digestion [9]. However, the alkaline hydrolysis also hydrolysed part of the holocellulosic fraction, and the sugars that constitute hemicellulose remained dissolved in the liquid phase of the hydrolysate. Thus, the process of washing the fibre to remove the lignin also removes sugars that could be anaerobically digested, reducing the BMP.

According to the results shown in Table 6, the alkaline pretreatment produced a fibre that was easier to hydrolyse anaerobically ($H\%$ = 40%), and therefore, more biodegradable (Bio = 44.4%) and with the highest BMP (313.4 L CH_4/kg substrate). This is because most of the lignin content was solubilised [57], and the fibre became swollen, with increased porosity and accessible surface to the anaerobic hydrolytical enzymes and acids [2,24]. However, only part of the lignocellulosic material ($\%y_{Hid}$ = 44.3%) was used for anaerobic digestion, being the other part solubilised and washed out for removing the lignin. When all the initial mass of the material used for hydrolysis was considered, it was found that the recalculation of biodegradability and BMP resulted in lower values of 19.7% and 138.8 L CH_4/kg substrate, respectively.

The results show that the lignin is a hindrance to hydrolysis, because the value of anaerobic hydrolysis ($H\%$) of the dry bagasse was only 4.4%. According to Soto and co-authors [47], alkaline pretreatment produced a fibre with lower lignin content and with increased surface accessibility for exoenzymes, which improved the anaerobic biodegradability.

Considering that methane has a lower heating value (LHV) of 34 450 kJ/m^3 [7], the best power yield that can be obtained by anaerobic digestion of the sugarcane bagasse is 7.1 MJ/kg dry bagasse. On the other hand, the dry sugarcane bagasse has an LHV of 7.2 MJ/kg [74], indicating that, if the only goal for the bagasse is energy production, then the use of pretreatments is not economically feasible. On the other hand, there is the possibility of using the extracted lignin in the chemical industry [75].

The lignin can be used in the manufacture of pesticides, phenolic resins, additives for paints and varnishes, as an agent to improve the viscosity of the mud from drilling oil wells, as an agglomerating agent or flocculant in wastewater treatment, as a conditioning

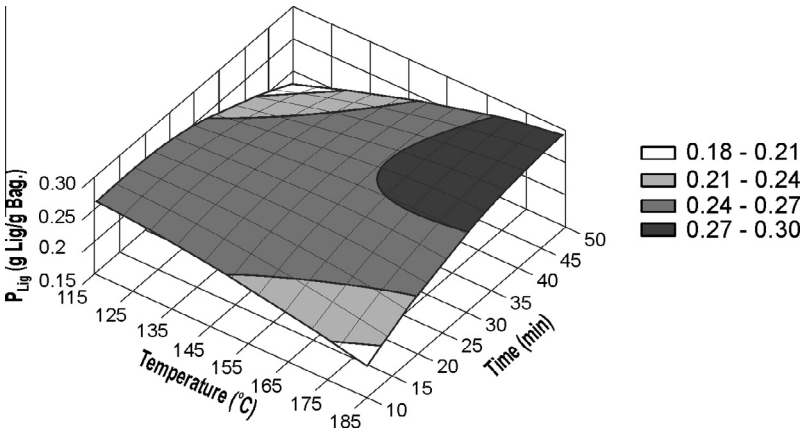

Fig. 4. Response surface plotted using Eq. (16), which describes specific production of lignin as a function of T and t ([$NaOH$] = 0.8 M).

Table 6
Anaerobic biodegradability and BMP assays of the hydrolysates produced by the various pretreatments (hydrothermal, acid, and alkaline).

Pretreatment	$\%y_{Hid}$	H(%)	$Bio(\%)_{Hid}$[a]	$Bio(\%)_{Total}$[b]	BMP_{Hid}[a] (LCH_4/kgSubst)	BMP_{Total}[b] (LCH_4/kgSubst)
Control[c]	100.0	4.0	4.4	4.4	35.6	35.6
$NaOH_{Fibre}$	44.3	40.0	44.4	19.7	313.4	138.8
HCl	85.6	0.2	19.2	16.4	142.7	122.2
Hydrothermal	99.5	14.8	27.6	27.4	198.5	197.5

Obs: For HCl and hydrothermal pretreatments the hydrolysate included the solid and liquid fractions. For NaOH pretreatment, the methane only corresponded to the solid fraction.
[a] Anaerobic biodegradability and BMP based on dry fibre after hydrolysis.
[b] Anaerobic biodegradability and BMP based on dry fibre before hydrolysis.
[c] Dry milled fibre without pretreatment was used in the assays as control.

additive for improving soil, as an agent for the slow release of nitrogen in the soil, as well as other applications [76–81]. In this case, the hydrolysed material will generate 313.4 L CH_4/kg substrate, which will enable energy production of 11.2 MJ/kg substrate, plus the value added by the lignin use.

The results of the acid pretreatment presented in Table 6 show that the hydrolysis efficiency of the anaerobic digestion was very low (0.2%). This occurred largely because most of the hemicellulose fraction was solubilised during the pretreatment, leaving only the most recalcitrant material. This may be associated with the remaining lignin that hampers the enzymatic attack of the fibre and/or the crystallinity of the cellulose fraction [82–84]. According to Pedersen and Meyer [85], acid hydrolysis partially solubilises the lignin, which may be adsorbed on the fibre surface, providing an increased barrier against enzymatic attack. Moreover, phenolic compounds released during lignin degradation are potential inhibitors (aromatic acids, catechol, 4-hydroxybenzaldehyde and vanillin) [60,86,87]. The hydrolysate derived from hydrothermal pretreatment had a higher anaerobic hydrolysis efficiency (14.8%) because this method did not completely solubilise the hemicellulose fraction, which was subsequently degraded via anaerobic digestion.

Nonetheless, we believe that some other options for the pretreated bagasse to produce biogas and energy such as combined cycles and fuel cells must also be considered as well as to do a deep economical and life cycle analysis to better explain the potentialities of the bagasse-pretreatment-biogas-energy route.

4. Conclusions

Based on the initial mass of the sugarcane bagasse, the highest sugar production (31.14 g TRG/g substrate) was achieved with the acid pretreatment in 6.4 min at 138 °C, with a HCl concentration of 0.63 M, and the highest lignin removal (23.24 g Lig/g substrate) was found with the alkaline pretreatment after 47 min at 184 °C and a NaOH concentration of 0.8 M. However, the highest anaerobic biodegradability (27.4%) and BMP (197.5 L CH_4/kg substrate) were achieved using the hydrothermal pretreatment after 10 min at 200 °C, which was sufficient to generate power of 6.8 MJ/kg substrate. The results showed that the methane derived from the anaerobic digestion of these hydrolysates produced less energy than the direct burning of the dry bagasse. Thus, the economic viability of this process may depend on the use of other by-products. It seems that alkaline hydrolysis (t = 47 min, T = 184 °C and [NaOH] = 0.8 M) becomes a promising alternative because it ensures the extraction of up to 0.26 g/g bagasse, which was 80.2% of the total lignin in the fibre, and produces 313.4 L CH_4/kg of the hydrolysed bagasse.

Acknowledgements

This work was carried out with the support of the Brazilian Agricultural Research Corporation (Embrapa), through funds of Call 01/2009 PAC-Embrapa-Macroprograma 2. The Ceará State Foundation for the Support of Scientific and Technological Development (FUNCAP) and the Federal Institute of Ceará State (IFCE) are also acknowledged for scholarships.

References

[1] M.O. Dias, T.L. Junqueira, C.E.V. Rossell, R. Maciel Filho, A. Bonomi, Integrated versus stand-alone second generation ethanol production from sugarcane bagasse and trash, Bioresour. Technol. 103 (2012) 152–161.
[2] J.X. Sun, X.F. Sun, H. Zhao, R.C. Sun, Isolation and characterization of cellulose from sugarcane bagasse, Polym. Degrad. Stab. 84 (2004) 331–339.
[3] CONAB – Companhia Nacional de Abastecimento. Acompanhamento de safra brasileira: grãos (Monitoring of the Brazilian harvest: grains). Brasília, Brazil, 2012. (in Portuguese).

[4] Brazil, Plano Nacional de Energia 2030 (Brazilian National Power Plan 2030). Mines and Energy Ministry. Brasília, Brazil, 2007. (in Portuguese).
[5] R.W. Smith, S.C. Gülen, in: Natural gas power. Fossil Energy, Springer, New York, 2013, pp. 527–595.
[6] G. Bustos, J.A. Ramírez, G. Garrote, M. Vázquez, Modeling of the hydrolysis of sugar cane bagasse with hydrochloric acid, Appl. Biochem. Biotechnol. 104 (2003) 51–68.
[7] H. Boussarsar, B. Rogé, M. Mathlouthi, Optimization of sugarcane bagasse conversion by hydrothermal treatment for the recovery of xylose, Bioresour. Technol. 100 (2009) 6537–6542.
[8] I. Angelidaki, W. Sanders, Assessment of the anaerobic biodegradability of macropollutants, Rev. Environ. Sci. Biotechnol. 3 (2004) 117–129.
[9] M.J. Taherzadeh, K. Karimi, Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review, Int. J. Mol. Sci. 9 (2008) 1621–1651.
[10] C.E. Wyman, Ethanol production from lignocellulosic biomass: review, in: C.E. Wyman (Ed.), Handbook on Bioethanol, Production and Utilization, Taylor & Francis, Washington, DC, 1996.
[11] A.T.W.M. Hendriks, G. Zeeman, Pretreatments to enhance the digestibility of lignocellulosic biomass, Bioresour. Technol. 100 (2009) 10–18.
[12] T.V. Fernandes, G.J. Klaasse Bos, G. Zeeman, J.P.M. Sanders, J.B. Van Lier, Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass, Bioresourc. Technol. 100 (2009) 2575–2579.
[13] P.G. Kiipper. Estudo da pré-hidrólise ácida do bagaço de cana-de-açúcar e fermentação alcoólica do mosto de xilose por Pachysolen tannophilus (Study on pre-hydrolysis of the sugarcane bagasse and ethanolic fermentation of the xylose by Pachysolen tannophilus. Master dissertation, Sao Paulo State University, Brazil, 2009, p. 100. (in Portuguese).
[14] R. Hatfield, R.S. Fukushima, Can lignin be accurately measured?, Crop Sci 45 (2005) 832–839.
[15] H. Song, W.P. Clarke, L.L. Blackall, Concurrent microscopic observations and activity measurements of cellulose hydrolyzing and methanogenic populations during the batch anaerobic digestion of crystalline cellulose, Biotechnol. Bioeng. 91 (2005) 369–378.
[16] P.J. Weimer, J.M. Lopez-Guisa, A.D. French, Effect of cellulose fine structure on kinetics of its digestion by mixed ruminal microorganisms in vitro, Appl. Environ. Microbiol. 56 (1990) 2421–2429.
[17] C. Gallert, J. Winter, Bacterial metabolism in wastewater treatment systems, Biotechnol. Set, Second Ed. (1999) 17–53.
[18] N. Mosier, C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple, M. Ladisch, Features of promising technologies for pretreatment of lignocellulosic biomass, Bioresour. Technol. 96 (2005) 673–686.
[19] F. Hu, A.J. Ragauskas, Hydrothermal pretreatment of switchgrass, Ind. Eng. Chim. Res. 50 (2011) 4225–4230.
[20] I. Kim, J.-I. Han, Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology, Biomass Bioenergy 4 (2012) 210–217.
[21] R. Velmurugan, K. Muthukumar, Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: optimization through response surface methodology, Bioresour. Technol. 112 (2012) 293–299.
[22] A. Duque, Optimization of integrated alkaline – extrusion pretreatment of barley straw for sugar production by enzymatic hydrolysis, Process Biochem. (2013) 1–7.
[23] L.L.G. Fuentes, S.C. Rabelo, R.M. Filho, A.C. Costa, Kinetics of lime pretreatment of sugarcane to enhance enzymatic hydrolysis, Appl. Biochem. Biotechnol. 163 (2011) 612–625.
[24] Y.H.P. Zhang, L.R. Lynd, Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems, Biotechnol. Bioeng. 88 (2004) 797–824.
[25] M.P. Vásquez, J.N.C. da Silva, M.B. de Souza Jr., N. Pereira Jr., Enzymatic hydrolysis optimization to ethanol production by simultaneous saccharification and fermentation, Appl. Biochem. Biotechnol. 12 (2007) 137–140.
[26] D.S. Santos, A.C. Camelo, K.C. Rodrigues, L.C. Carlos, N. Pereira Jr., Ethanol production from sugarcane bagasse by zymomonas mobilis using simultaneous saccharification and fermentation (SSF) process, Appl. Biochem. Biotechnol. 161 (2010) 93–105.
[27] P. Binod, R. Sindhu, R.R. Singhania, S. Vikram, L. Devi, S. Nagalakshmi, N. Kurien, R.K. Sukumaran, A. Pandey, Bioethanol production from rice straw: an overview, Bioresour. Technol. 101 (2010) 4767–4774.
[28] H.D. Zhang, S.B. Wu, Subcritical CO_2 pretreatment of sugarcane bagasse and its enzymatic hydrolysis for sugar production, Bioresourc. Technol. 149 (2013) 546–550.
[29] T. Benazzi, S. Calgaroto, C. Dalla Rosa, J.V. Oliveira, M.A. Mazutti, Hydrolysis of sugarcane bagasse using supercritical carbon dioxide to obtain fermentable sugars, J. Chem. Technol. Biotechnol. 88 (2013) 1766–1768.
[30] P.B. Subhedar, P.R. Gogate, Intensification of enzymatic hydrolysis of lignocellulose using ultrasound for efficient bioethanol production: a review, Ind. Eng. Chem. Res. 52 (2013) 11816–11828.
[31] T. Benazzi, S. Calgaroto, V. Astolfi, C. Dalla Rosa, J.V. Oliveira, M.A. Mazutti, Pretreatment of sugarcane bagasse using supercritical carbon dioxide combined with ultrasound to improve the enzymatic hydrolysis, Enzyme Microb. Technol. 52 (4–5) (2013) 247–250.
[32] Y.-L. Xi, W.-Y. Dai, R. Xu, J.-H. Zhang, K.-Q. Chen, M. Jiang, P. Wei, P.-K. Ouyang, Ultrasonic pretreatment and acid hydrolysis of sugarcane bagasse for succinic acid production using actinobacillus succinogenes bioprocess, Biosystems Eng. 36 (2013) 1779–1785.
[33] K.-H. Tseng, Y.-F. Shiao, R.-F. Chang, Y.-T. Yeh, Optimization of microwave-based heating of cellulosic biomass using taguchi method, Materials 6 (2013) 3404–3419.
[34] M. Ahi, M. Azin, S.A. Shojaosadati, E. Vasheghani-Farahani, M. Nosrati, Optimization of sugarcane bagasse hydrolysis by microwave-assisted pretreatment for bioethanol production, Chem. Eng. Technol. 36 (2013) 1997–2005.
[35] Z. Zhang, O'H.M. Ian, W.O.S. Doherty, Pretreatment of sugarcane bagasse by acidified aqueous polyol solutions, Cellulose 20 (6) (2013) 3179–3190.
[36] X. Zhao, D. Liu, Kinetic modeling and mechanisms of acid-catalyzed delignification of sugarcane bagasse by aqueous acetic acid, Bioenergy Res. 6 (2013) 436–447.
[37] J.A. Souza-Correa, C. Oliveira, L.D. Wolf, V.M. Nascimento, G.J.M. Rocha, J. Amorim, Atmospheric pressure plasma pretreatment of sugarcane bagasse: the influence of moisture in the ozonation process, Appl. Biochem. Biotechnol. 171 (2013) 104–116.
[38] J. Amorim, C. Oliveira, J.A. Souza-Correa, et al., Treatment of sugarcane bagasse lignin employing atmospheric pressure microplasma jet in argon, Plasma Processes Polym. 10 (2013) 670–678.
[39] M. Kim, D.F. Day, Enhancement of the enzymatic digestibility and ethanol production from sugarcane bagasse by moderate temperature-dilute ammonia treatment, Appl. Biochem. Biotechnol. 171 (2013) 1108–1117.
[40] Q. Yu, X. Zhuang, Z. Yuan, W. Qi, W. Wang, Q. Wang, X. Tan, Pretreatment of sugarcane bagasse with liquid hot water and aqueous ammonia, Bioresour. Technol. 144 (2013) 210–215.
[41] J. Bian, F. Peng, X.-P. Peng, X. Xiao, P. Peng, F. Xu, R.-C. Sun, Effect of [Emim]Ac pretreatment on the structure and enzymatic hydrolysis of sugarcane bagasse cellulose, Carbohydr. Polym. 100 (2014) 211–217.
[42] L.-Q. Jiang, Z. Fang, X.-K. Li, J. Luo, S.-P. Fan, Combination of dilute acid and ionic liquid pretreatments of sugarcane bagasse for glucose by enzymatic hydrolysis, Process Biochem. 48 (2013) 1942–1946.
[43] M.J. Díaz, C. Cara, E. Ruiz, I. Romero, M. Moya, E. Castro, Hydrothermal pre-treatment of rapeseed straw, Bioresour. Technol. 101 (2010) 2428–2435.
[44] M. Laser, D. Schulman, S.G. Allen, J. Lichwa, M.J. Antal Jr., L.R. Lynd, A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol, Bioresour. Technol. 81 (2002) 33–44.
[45] S. Gámez, J.J. González-Cabriales, J.A. Ramírez, G. Garrote, M. Vázquez, Study of the hydrolysis of sugar cane bagasse using phosphoric acid, J. Food Eng. 74 (2006) 78–88.
[46] A. Rodríguez-Chong, J.A. Ramírez, G.J. Garrote, M. Vázquez, Hydrolysis of sugarcane bagasse using nitric acid: a kinetic assessment, J. Food Eng. 61 (2004) 143–152.
[47] M. Soto, R. Méndez, J.M. Lema, Methanogenic and non-methanogenic activity tests. Theorical basis and experimental setup, Water Res. 27 (1993) 1361–1376.
[48] APHA, Standard Methods for Examination of Water and Wastewater. 21st ed. American Public Health Association, 2005.
[49] C.P. Pabón-Pereira, Anaerobic digestion in sustainable biomass chains. PhD thesis, Wageningen University, Netherlands, 2009, p. 262.
[50] G. Zeeman, Mesophilic and psychrophilic digestion of liquid manure. PhD thesis, Wageningen University, Netherlands, 1991.
[51] T.J. Irick, K. West, H.H. Brownell, W. Schwald, J.N. Saddler, Comparison of colorimetric and HPLC techniques for quantitating the carbohydrate components of steam-treated wood, Appl. Biochem. Biotechnol. 17 (1988) 137–149.
[52] A.G. Costa, G.C. Pinheiro, F.G.C. Pinheiro, A.B. Dos Santos, S.T. Santaella, R.C. Leitão, Pretreatment strategies to improve anaerobic biodegradability and methane production potential of the palm oil mesocarp fibre, Chem. Eng. J. 230 (2013) 158–165.
[53] J.P.S. Morais, M. F. Rosa, J.M. Marconcini. Procedimentos para análise lignocelulósica (Procedures for analysis of lignocellulose). Documentos 236. Embrapa Algodão. Brazil, 2010, (in Portuguese).
[54] T. Yokoyama, J.F. Kadla, H.M. Chang, Microanalytical method for the characterization of fibre components and morphology of woody plants, J. Agric. Food Chem. 50 (2002) 1040–1044.
[55] G.J.M. Rocha, Deslignificação de bagaço de cana de açúcar assistida por oxigênio (Oxygen delignification of sugarcane bagasse). Ph.D. thesis, São Paulo University, Brazil, 2000, (in Portuguese).
[56] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (1959) 426–428.
[57] E.V. Canettieri, Obtenção dos parâmetros e estudo cinético da hidrólise ácida dos resíduos florestais de eucalipto (Parameters selection and kinetic study of acid hydrolysis of eucalyptus forest residues). PhD thesis, Sao Paulo State University, Brazil, 2004, (in Portuguese).
[58] R. Boopathy, Anaerobic biotransformation of furfural to furfuryl alcohol by a methanogenic archaebacterium, Int. Biodet. Biodegrad. 63 (2009) 1070–1072.
[59] G.-L. Cao, N.-Q. Ren, A.-J. Wang, W.-Q. Guo, J.-F. Xu, B.-F. Liu, Effect of lignocellulose-derived inhibitors on growth and hydrogen production by Thermoanaerobacterium thermosaccharolyticum W16, Int. J. Hydrogen Energy 35 (24) (2010) 13475–13480.
[60] A. Barakat, F. Monlau, J.-P. Steyer, H. Carrere, Effect of lignin-derived and furan compounds found in lignocellulosic hydrolysates on biomethane production, Bioresour. Technol. 104 (2012) 90–99.

[61] Y. Sun, J. Cheng, Hydrolysis of lignocellulosic materials for ethanol production: a review, Bioresour. Technol. 83 (2002) 1–11.
[62] B.P. Lavarack, G.J. Griffin, D. Rodman, The acid hydrolysis of sugarcane bagasse hemicellulose to produce xylose, arabinose, glucose and other products, Biomass Bioenergy 23 (2002) 367–380.
[63] R. Aguilar, J.A. Ramírez, G. Garrote, M. Vázquez, Kinetic study of the acid hydrolysis of sugar cane bagasse, J. Food Eng. 55 (4) (2002) 309–318.
[64] A. Rodríguez-Chong, J.A. Ramírez, G. Garrote, M. Vázquez, Hydrolysis of sugar cane bagasse using nitric acid: a kinetic assessment, J. Food Eng. 61 (2004) 143–152.
[65] S. Gámez, J.J. González-Cabriales, J.A. Ramıréz, G. Garrote, M. Vázquez, Study of the hydrolysis of sugar cane bagasse using phosphoric acid, J. Food Eng. 74 (2006) 78–88.
[66] X.B. Zhao, L. Wang, D.H. Liu, Effect of several factors on peracetic acid pretreatment of sugarcane bagasse for enzymatic hydrolysis, J. Chem. Technol. Biotechnol. 82 (2007) 1115–1121.
[67] X.B. Zhao, L. Wang, D.H. Liu, Peracetic acid pretreatment of sugarcane bagasse for enzymatic hydrolysis: a continued work, J. Chem. Technol. Biotechnol. 83 (2008) 950–956.
[68] X.B. Zhao, F. Peng, K.K. Cheng, D.H. Liu, Enhancement of the enzymatic digestibility of sugarcane bagasse by alkali peracetic acid pretreatment, Enzym. Microb. Technol. 44 (2009) 156–164.
[69] X.B. Zhao, R. Wua, D. Liu, Production of pulp, ethanol and lignin from sugar cane bagasse by alkali–peracetic acid delignification, Biomass Bioenergy 35 (2011) 2874–2882.
[70] E.V. Canettieri, G.J.de M. Rocha, J.A. Carvalho Jr., J.B.A. de Silva, Optimization of acid hydrolysis from the hemicellulosic fraction of Eucalyptus grandis residue using response surface methodology, Bioresour. Technol. 98 (2007) 422–428.
[71] J.W. Abram, D.B. Nedwell, Inhibition of methanogenesis by sulphate reducing bacteria competing for transferred hydrogen, Arch. Microbiol. 117 (1978) 89–92.
[72] X. Pan, D. Xie, N. Gilkes, D.J. Gregg, N.J. Saddler, Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content, Appl. Biochem. Biotechnol. 121–124 (2005) 1069–1079.
[73] L.J. Jönsson, E. Palmqvist, N.O. Nilvebrant, B. Hahn-Hägerdal, Detoxification of wood hydrolysates with laccase and peroxidase from white-rot fungus Trametes versicolor, Appl. Microbiol. Biotechnol. 49 (1998) 691–697.
[74] D.T. Pedroso, R.C. Aiello, Gasification of biomass for electricity generation in the Cuban context, Rev. Ciênc. Exatas 11 (2005) 55–57.
[75] C. Vila, V. Santos, J.C. Parajó, Recovery of lignin and furfural from acetic acid–water–HCl pulping liquors, Bioresour. Technol. 90 (2003) 339–344.
[76] D.M. Silva, D.C. Musial, C. Altmeyer, S.A. Valentini. Obtenção de derivado de celulose a partir do bagaço de cana-de-açúcar com potencial aplicação nas indústrias farmacêutica e cosmética (Obtaining cellulose derivative from sugarcane bagasse with potential application in the pharmaceutical and cosmetic industries). Rev. de Ciências Farmacêuticas Básica e Aplicada, 32 (2011) 41–45 (in Portuguse).
[77] S. Camarero, P. Bocchini, G.C. Galletti, E.M. Martinez, Pyrolysis-gas chromatography/mass spectrometry analysis of phenolic and etherified units in natural and industrial lignins, Rapid Commun. Mass Spectrom. 13 (1999) 630–636.
[78] A.S. Pimenta, B.R. Vital, F.Y. Fujiwara, Alcatrão ou creosoto de eucalipto na produção de adesivos fenólicos para colagem (Tar or creosote from eucalyptus in the production of phenolic adhesives, Química Nova 20 (1997) 365–371 (in Portuguese).
[79] H.F. García, J.F. Martín, J.J. Rodríguez, Posibilidades de aprovechamiento de la lignina en la industria química (Possibilities of utilization of lignin in chemical industry), Ingeniería Química (1984) 249–254 (in Spanish).
[80] P. Benar. Ligninas acetosolv e formacell de eucalipto e de bagaço de cana: isolamento, fracionamento, caracterização e uso como componente de resinas fenolicas do tipo resol (Acetosolv and formacell lignins from eucalyptus and sugarcane bagasse: isolation, fractionation, characterization and use as a component of the resol-type phenolic resins). Ph.D. Thesis, Campinas University, Brazil, 1996, (in Portuguese).
[81] J.M. Harkin. Lignin and its uses. Research note. U.S. Department of Agriculture, Forest Service Forest Products Laboratory, Madison, Wis., EUA, 1969.
[82] P. Kumar, D.M. Barrett, M.J. Delwiche, P. Stroeve, Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production, Ind. Eng. Chem. Res. 48 (2009) 3713–3729.
[83] B.S. Donohoe, S.R. Decker, M.P. Tucker, M.E. Himmel, T.B. Vinzant, Visualizing lignin coalescence and migration through maize cell walls following thermochemical pretreatment, Biotechnol. Bioeng. 101 (2008) 913–925.
[84] J.B. Kristensen, L.G. Thygesen, C. Felby, H. Jørgensen, T. Elder, Cell-wall structural changes in wheat straw pretreated for bioethanol production, Biotechnol. Biofuels 5 (2008) 1–9.
[85] M. Pedersen, A.S. Meyer, Lignocellulose pretreatment severity–relating pH to biomatrix opening: review, New Biotechnol. 27 (2010) 739–750.
[86] M.P. Garciá-Aparicio, I. Ballesteros, A. González, J.M. Oliva, M. Ballesteros, M.J. Negro, Effect of inhibitors released during steam explosion pretreatment of barley straw on enzymatic hydrolysis, Appl. Biochem. Biotechnol. 129 (2006) 278–288.
[87] G. Panagiotou, L. Olsson, Effect of compounds released during pretreatment of wheat straw on microbial growth and enzymatic hydrolysis rates, Biotechnol. Bioeng. 96 (2006) 250–258.