Bioresource Technology 200 (2016) 137–146

Contents lists available at ScienceDirect

Bioresource Technology

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

Optimization of sugarcane bagasse autohydrolysis for methane production from hemicellulose hydrolyzates in a biorefinery concept

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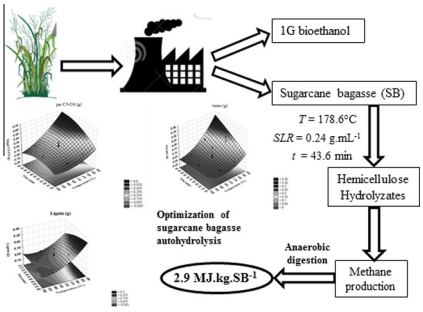
HIGHLIGHTS

- SB autohydrolysis was optimized by experimental design to produce methane.
- Milder AH condition (178.6 °C; 43.6 min; *SLR* = 0.24) was the best for CH_4 production.
- CH_4 production was 1.56 Nm^3 CH_4 kg TOC^{-1} which could potentially generate 2.9 MJ kg SB^{-1}.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 September 2015
Received in revised form 2 October 2015
Accepted 3 October 2015
Available online 10 October 2015

Keywords:
Sugarcane bagasse
Autohydrolysis pretreatment
Anaerobic digestion
Biomethane
Hemicellulose hydrolyzate

ABSTRACT

This study aimed to optimize through design of experiments, the process variables (temperature – *T*, time – *t* and solid-to-liquid ratio – *SLR*) for sugarcane bagasse (SB) autohydrolysis (AH) to obtain hemicellulose hydrolyzates (HH) prone to anaerobic digestion (AD) and biochemical methane production (BMP). The results indicated that severe AH conditions, which lead to maximum hemicelluloses dissolution and sugar content in the HH, were not the best for BMP, probably due to the accumulation of toxic/recalcitrant compounds (furans and lignin). Mild AH conditions (170 °C, 35 min and *SLR* = 0.33) led to the highest BMP (0.79 Nm^3 kg TOC^{-1}), which was confirmed by the desirability tool. HH produced by AH carried out at the desired condition DC2 (178.6 °C, 43.6 min and *SLR* = 0.24) showed the lowest accumulation of inhibitory compounds and volatile fatty acids (VFA) and highest BMP (1.56 Nm^3 kg TOC^{-1}). The modified Gompertz model best fit the experimental data and led to a maximum methane production rate (*R*) of 2.6 mmol CH_4 d^{-1} in the best condition.

1. Introduction

All around the world, a great quantity of energy comes from fossil fuels. It is known that such resources are non-renewable and that their quantity at the Earth's crust remains uncertain. The world's oil reserves require good management to ensure its longevity. In addition, geopolitical risks from dependence on oil by politically unstable countries, and major commitments made to the environment have drawn greater attention to the use alternative energy sources. Resultingly, the use of renewable sources for energy production has been strongly encouraged worldwide (Cherubini and Jungmeier, 2009).

The most common source of renewable fuel produced worldwide is bioethanol, which is usually obtained from corn (starch),

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http://dx.doi.org/10.1016/j.biortech.2015.10.003
0960-8524/

sugar beet (sucrose), and sugarcane (sucrose). In this context, Brazil has occupied a prominent place along with the United States regarding the production of bioethanol from sugarcane juice and cornstarch (Soccol et al., 2010).

Other sources of renewable raw materials widely studied for conversion into biofuel and biogas are the lignocellulose residues. The production of liquid or gaseous biofuels and value-added byproducts from wasted lignocellulosic biomass have highly favorable environmental aspects (Sanchez and Cardona, 2008). Every year, large quantities of lignocellulosic wastes accumulate worldwide due to the agricultural and agro-forestry production. Often, the allocation of these materials is not entirely noble, represents a loss of their energy production potential, besides posing environmental issues. In this context, Brazil assumes a prominent position, when considering the volume of agricultural and agro-forestry production in the country and the consequent generation of lignocellulose residues. Such residual biomasses are available in a reasonably clean form and in large amounts (Pandey et al., 2000).

Among the agricultural wastes generated in large amounts in Brazilian territory, one should highlight that sugarcane bagasse (SB), is a waste generated during the production of first generation (1G) bioethanol in sugarcane mills. It is estimated that the sugarcane crop during 2015/2016 season has reached 663 million tons (CONAB, 2015). Considering that approximately 250–280 kg of SB is generated by 1.0 ton of processed sugarcane, it is proposed that the Brazilian sugar and alcohol sector has reached 166–186 million tons of bagasse during 2015/2016 season (Rocha et al., 2015).

Currently, in Brazil, the majority of SB is used to meet the energy demands in alcohol/sugar plants. It is estimated that the amount of energy produced by its burning to produce steam exceeds by twofold, the amount of energy required for sugar and alcohol production (Costa et al., 2014). Therefore, part of the residual SB is not energetically harnessed. A complete use of this SB is extremely desirable from both economically and environmentally points of view; and, its chemical composition makes it an interesting industrial raw material of strategic economic importance.

The peculiar characteristics of SB, particularly the large amount of carbohydrates in the form of hemicelluloses and cellulose, have encouraged research on bioconversion processes of this material for the production of second generation (2G) bioethanol, biogas (CH_4 and H_2) and other bioproducts (Batalha et al., 2015; Costa et al., 2014). In fact, some authors do not consider SB a byproduct or residue of the sugar and alcohol industry, but instead as a high value co-product, which can be used as a raw material for the production of biofuels and bioproducts (Pandey et al., 2000).

Generally speaking, lignocellulosic biomass is a natural, cheap and abundant source that can be exploited for the production of biofuels and bioproducts. SB, particularly, is mainly composed of cellulose (30–45%), hemicelluloses (25–30%) and lignin (25–30%), which account for about 90% of its dry weight (Badshah et al., 2012). However, the complex interactions between these three main components (cellulose, hemicelluloses and lignin) in the cell wall propose the main challenge for the biotechnological use of lignocellulose residues such as sugarcane bagasse (Sun, 2004). As a result, the development of processes for the conversion of such residues into biofuels should involve an integrated optimization of the type of lignocellulose biomass, chemical characterization and pretreatment needs (Gouveia et al., 2009).

Generally, the methods for lignocellulosic biomass pretreatments can be grouped into different categories: physical, physico-chemical, biological and chemical. The hydrothermal pretreatment, also known as autohydrolysis (AH) pretreatment, has been studied by different researchers (Batalha et al., 2015; Santucci et al., 2015) as a step prior to enzymatic hydrolysis of SB to increase enzymes accessibility and thereby cellulose conversion to C-6 sugar (glucose). Consequently, this could be further used as a substrate for 2G bioethanol production via yeast fermentation.

During AH pretreatment, water molecules penetrate the biomass, promoting cellulose hydration, hemicelluloses dissolution, and slight lignin removal. AH pretreatment is highly effective in increasing the accessibility and susceptibility of cellulose surface area, thereby enhancing its biodegradability and enzymatic conversion. AH pretreatment has the potential of improving monomeric sugars extraction, pentoses recovery and cellulose degradability, with the advantage of producing a hydrolyzate with lower concentration of toxic compounds, such as 2-furfuraldehyde (FF) and 5-hydroxymethyl-2-furfuraldehyde (HMF), when compared to acidic or steam explosion pretreatments (Zheng et al., 2014).

Moreover, AH pretreatment of SB leads to the production of a hemicellulose hydrolyzate rich in pentose sugars (mainly xylose and arabinose), which cannot be easily converted into bioethanol via biochemical processes (Kaparaju et al., 2009). The use of hemicellulose hydrolyzate for the production of 2G bioethanol normally requires the development of genetically modified microorganisms that could thrive on pentose. A process that enables the application of such microorganisms on an industrial scale, however, has not yet been developed (Rabelo et al., 2011).

Another alternative to harvest the energy of hemicellulose hydrolyzates is the production of biomethane via anaerobic digestion (AD). Such an alternative may be part of an energy-viable and sustainable solution for integrating 1G and 2G bioethanol production processes in the sugar and alcohol industries. The biomethane could be an additional source of energy to partially replace sugarcane bagasse as a fuel in cogeneration systems. Furthermore, the use of biomethane produced from anaerobic digestion of hemicellulose hydrolyzates opens the possibility of integral use of the SB in the lignocellulose biorefinery. The remaining cellulose-rich solid fraction generated after hemicellulose extraction could be further used for 2G bioethanol production.

Most of the studies available in the literature consider the whole use of pretreated biomass, i.e. solid and liquid fractions, to produce CH_4 and evaluate the effects of pretreatment in the digestibility of SB when compared to raw SB. The use of whole pretreated material for methane production hinders the rational and efficient use of all biomass fractions, such as the destination of the cellulose-rich solid fraction for 2G bioethanol production. Therefore, this study aimed to evaluate the AH pretreatment of SB in order to obtain hemicellulose hydrolyzates adequate for biomethane production via classic anaerobic digestion. In addition, the present study tests the hypothesis that the optimization of AH conditions cannot be solely based on the sugar content in the hydrolysates if methane production is the main goal.

Special attention has been given to the use of high values of *SLR*, which were evaluated aiming to decrease the water consumption in the AH pretreatment. The biomethane production via AD of hemicellulose hydrolyzates was also intensively studied and optimized through a Doehlert experimental design and desirability tool of Statistica® software. The potential for power generation using biomethane in a biorefinery concept using the best scenario found in this study was also estimated.

2. Methods

2.1. Chemicals

Cyclohexane and ethanol (99.5%) were purchased from Synth (Brazil). Sulfuric acid (95–98%) was purchased from Synth (Brazil). The chromatography–grade standards cellobiose, D-glucose, D-xylose, L-arabinose, acetic acid, formic acid, propionic, isobutyric,

butyric, valeric, isovaleric, 5-hydroxymethyl-2-furfuraldehyde (HMF), and 2-furfuraldehyde (FF) were purchased from Sigma–Aldrich (Brazil).

2.2. Sugarcane bagasse preparation

SB was provided by Jatiboca Sugar and Ethanol Plant (Ponte Nova, MG, Brazil), and was generated in the 2012/2013 harvest. Whole SB was subjected to a washing process employing distilled water at 70 °C for a period of 1 h under mechanical stirring. This step was intended to remove the residual sugars from the sugarcane grinding process. After this procedure the SB was stored at temperatures below 0 °C until processing.

2.3. Analytical methods for characterization of SB and hemicellulose hydrolyzates

An oven dried sample of SB was ground to pass through a 0.40 mm (40 mesh) screen for the compositional tests. A SB sample was then used to determine the ash (inorganic) content according to the method "Ash in wood, pulp, paper and paperboard", TAPPI T211 om-02. The quantitative determination of SB extractives was made by Soxhlet extraction using cyclohexane/ethanol (1:1, v/v), in agreement with the method "Solvent Extractives of Wood and Pulp", TAPPI T204 cm-07. An extractive-free SB sample was used to determine the acid-insoluble lignin content, in accordance with the test method "Acid-insoluble lignin in wood and pulp", TAPPI T222 om-02. In its turn, the determination of acid-soluble lignin was made in accordance with the test method "Determination of acid-insoluble lignin in Biomass", NREL LAP-004.

The concentration of sugars (cellobiose, glucose, xylose and arabinose), acids (acetic and formic acids) and sugar degradation products (FF and HMF) in the hydrolyzate, resulting from the determination of acid-insoluble lignin in SB, was used to estimate the contents of cellulose and hemicelluloses in SB. In addition, such analyses were also used to characterize the hydrolyzates generated by treating the SB under different AH pretreatment conditions. The coefficients used to convert sugars, acids and sugar degradation products into cellulose and hemicelluloses were those reported by Gurgel et al. (2014). The concentration of sugars and sugar degradation products was determined by high performance liquid chromatography (HPLC) in accordance with the test method "Determination of carbohydrates in biomass by high performance liquid chromatography", NREL LAP-002. A Shimadzu HPLC system was used, equipped with a refractive index detector (Shimadzu RID-6A) and an Aminex HPX 87H column (300 × 7.8 mm Bio-Rad) kept at 45 °C. The chromatographic conditions used for separating the analytes involved the use of sulfuric acid (0.5 mmol L^{-1}) as an eluent at a flow rate of 0.6 mL min^{-1}.

The content of organic acids (formic, acetic, propionic, isobutyric, butyric, valeric and isovaleric), FF and HMF were also determined using the same chromatographic conditions described above. However, the detection of these analytes was performed in a UV–Vis detector (Shimadzu SPD-10AV) at the wavelength of 210 nm for acids and 274 nm for the FF and HMF.

The concentration of total organic carbon (TOC) in the hydrolyzates from AH pretreatment of SB was determined using a TOC-L CPH/CPN Shimadzu equipment. Moreover, the xylooligomers (XOS) present in the hydrolyzates were determined as described by NREL LAP-015 by means of an acidic treatment of a hydrolyzate sample (hydrolyzate-to-water ratio of 1:10, 4 wt.% H_2SO_4 at 121 °C for 1 h). The resulting products (sugars, acids, FF and HMF) were quantified according to the methodology described before. The content of XOS was then estimated from the difference in the concentration of monomeric sugars and sugar degradation products found in the hydrolyzates before and after the post-hydrolysis (acidic treatment).

2.4. Experimental design and statistical analysis

The conditions for AH pretreatment of SB were optimized by a Doehlert experimental design (DED). The independent variables evaluated were temperature (T, °C), time (t, min) and the solid-to-liquid ratio (SLR, g mL^{-1}). The dependent variables monitored during the study were the concentration of C5–C6 sugars ($Y1$ – glucose, xylose and arabinose), furans ($Y2$ – FF plus HMF) and lignin ($Y3$ – SL) in the hemicellulose hydrolyzates. The levels assumed for each independent variable are depicted in Supplementary Table 1, whereas the experimental conditions employed for each AH pretreatment are presented in Table 1. Statistical analyses were performed with a 95% significance level, and a response surface was constructed for the three dependent variables (DV) using Eq. (1). The values of all coefficients depicted in Eq. (1) are shown in Supplementary Table 2.

$$DV = b_0 + b_1(SLR) + b_2(T) + b_3(t) + b_{11}\left(SLR^2\right) + b_{22}\left(T^2\right) + b_{33}\left(t^2\right) + b_{12}(SLR \times T) + b_{13}(SLR \times t) + b_{23}(T \times t) \quad (1)$$

After analyzing the results obtained by DED, a 'desirability tool' was used to assess the relationship between the model outputs and the features of dependent variables wanted to obtain a hydrolyzate with a greater biomethane production capacity. The desired conditions used in the 'desirability tool' are shown in Table 2. Both the experimental design and the 'desirability tool' were run using the Statistica® software (version 10.0).

2.5. Autohydrolysis (AH) pretreatment

AH pretreatment experiments of SB were carried out in a tubular reactor made of stainless steel (316 L) (190 mm of height × 58 mm internal diameter × 76 mm external diameter) with a capacity of 195 cm^3. The water content of SB was taken into account only in the mass balances, and the reactor was loaded with 30.0 g of SB, on dry-weight basis, and heated in a 25 L thermostatically controlled bath containing glycerin as a heating fluid. The

Table 1
Experimental conditions used for optimizing the AH pretreatment of SB and hydrolyzate chemical composition after AH pretreatment.

Experiment	SLR (g mL^{-1})	T (°C)	t (min)	C5–C6 sugars (g)	Furans[a] (g)	SL[b] (g)	HR (%)[c]
1	0.333	170	35	0.195	0.060	0.09	57.01
2	0.278	185	35	0.387	0.170	0.18	69.09
3	0.278	175	55	0.328	0.144	0.19	69.67
4	0.111	170	35	0.322	0.173	0.16	46.96
5	0.167	155	35	0.138	0.041	0.01	13.63
6	0.167	165	15	0.042	0.020	0.01	5.83
7	0.278	155	35	0.113	0.025	0.01	9.19
8	0.278	165	15	0.056	0.023	0.00	8.62
9	0.167	185	35	0.516	0.222	0.29	82.90
10	0.222	180	15	0.119	0.045	0.06	42.97
11	0.222	175	55	0.325	0.152	0.04	62.95
12	0.222	160	55	0.136	0.035	0.02	54.90
13	0.222	170	35	0.235	0.069	0.06	41.38
14	0.222	170	35	0.240	0.071	0.05	47.37
15	0.222	170	35	0.285	0.080	0.05	51.71
16	0.222	170	35	0.181	0.051	0.05	54.58
17	0.222	170	35	0.175	0.027	0.06	66.61
18	0.222	170	35	0.227	0.034	0.06	62.71
19	0.222	170	35	0.223	0.037	0.05	58.83

[a] Content of FF plus HMF.
[b] Soluble lignin.
[c] Hemicelluloses removal (HR), as calculated using Eq. (2).

Table 2
AH pretreatment conditions of SB and AH hydrolyzate composition outputted by the desirability tool.

Condition	Desired composition[a] (input values)			AH pretreatment conditions (output values)			Hydrolyzate composition (g)		
	C5–C6 sugars	Furans	SL	*SLR* (g mL^{-1})	*T* (°C)	*t* (min)	C5–C6 sugars	Furans	SL
DC1	1	1	1	0.14286	185.0 (±1.0)	55.00	1.07	0.33	2.02
DC2	1	0	0	0.23809	178.6 (±1.0)	43.57	0.57	0.08	0.49
DC3	1	1	0	0.19047	178.6 (±1.0)	55.00	0.94	0.27	1.44
DC4	1	0	1	0.25397	182.9 (±1.0)	40.71	0.87	0.24	1.47

[a] Coded levels: 0 – minimum and 1 – maximum.

temperatures set were programmed and monitored with the aid of a thermocouple. After the AH pretreatment, the reactor was immediately cooled in an ice bath and opened. Then, the pretreated SB was dewatered in a hydraulic press by applying a pressure of 9 tons (SOLAB®, model SL-10). The hemicellulose syrup (hydrolyzate) recovered after pressing the pretreated SB was collected, stored at temperatures below 0 °C, and characterized in terms of monosaccharides (glucose, xylose and arabinose), organic acids (acetic and formic acids), sugar degradation products (FF and HMF), XOS, SL and TOC, as previously described.

After the characterization, portions of the hydrolyzates were submitted to anaerobic biodegradability tests to determine their biochemical methane potentials (BMP). The pretreated SB (solid fraction) was dried (without washing after pressing) in a forced air circulation oven at 85 °C for 24 h, transferred to a desiccator to cool, and then weighed. The weight loss (*WL*) was gravimetrically determined, and the hemicelluloses removal (*HR*) was calculated using Eq. (2).

$$HR/\% = \left\{\frac{HC_{SB} - [HC_{AH-SB}(Y_{AH-SB}/100)]}{HC_{SB}}\right\} \times 100 \quad (2)$$

where *HR* is hemicelluloses removal (%), $HC_{AH\text{-}SB}$ and HC_{SB} are hemicellulose contents in SB after and before AH pretreatment, respectively, and $Y_{AH\text{-}SB}$ (%) is the yield of AH pretreatment ($Y = 100 - WL$).

2.6. Anaerobic biodegradability

Batch anaerobic digestion tests were carried out in triplicate to assess the BMP of hemicellulose hydrolyzates obtained from AH pretreatment of SB. A control test, without substrate, was also included in the tests to check the methanogenic activity of the inoculum. All experiments were carried out at mesophilic conditions in glass bottles of 275 mL that were placed in a thermostatic orbital shaker (35.1 ± 0.3 °C) at 200 rpm. The liquid volume of each glass bottle was 150 mL, leaving a headspace volume of 125 mL.

The anaerobic inoculum used for the BMP tests was taken from a pilot-scale mesophilic anaerobic digester that had been feeding with raw sewage. The inoculum was pre-incubated for four days (35 °C) in order to offset the methane production from the biodegradation of any residual organic matter.

A sample of hydrolyzate (15.0 mL) was added to the glass bottles containing varied volumes of inoculum, depending on the TOC concentration in each hydrolyzate sample, to keep a food-to-microorganism ratio of 0.4 g TOC g VSS^{-1}. In each experiment a solution containing sodium bicarbonate (pH buffer) and micro- and macronutrients was added. The concentration of these compounds varied and was determined for each experiment in order to maintain a minimum COD:N:P ratio of 350:5:1, as used by Baêta et al. (2013).

The biogas production was monitored until stabilization, and the gas composition was measured by gas chromatography (Shimadzu GC, model 2014/TCD). All values of BMP (Nm^3 kg TOC^{-1}) are presented under standard temperature and pressure conditions (STP: 273 K; 101,315 Pa) as defined by IUPAC (International Union of Pure Applied Chemistry).

2.7. Modeling the experimental data

A mathematical model, the modified Gompertz model, was used to model the experimental data obtained from the BMP tests. The maximum accumulated methane production (AMP) was obtained by nonlinear sigmoidal regression of the modified Gompertz model (Lay, 2000) as follows in Eq. (3):

$$\mathrm{AMP} = P\exp\left\{-\exp\left[\frac{Re}{P}(\lambda - t) + 1\right]\right\} \quad (3)$$

where *P* is the methane production potential (mmol CH_4), *R* is the maximum methane production rate (mmol CH_4 h^{-1}), λ is the lag phase time (d) and *e* is exp(1).

3. Results and discussion

3.1. Characterization of sugarcane bagasse

The chemical composition of SB used in this study averaged 44.16% cellulose, 23.61% hemicelluloses, 25.79% lignin, 2.22% extractives and 1.45% ash (inorganics). This composition is in good agreement with that reported by Vallejos et al. (2012).

3.2. Autohydrolysis pretreatment of sugarcane bagasse

The optimization of AH pretreatment of SB was carried out using the content of C5–C6 sugars (*Y*1), furans (*Y*2) and SL (*Y*3) in the hydrolyzates as response variables. These variables were chosen due to the fact that there are direct relationships between them and the methane production via anaerobic digestion of the hydrolyzates. Since this optimization aimed to produce an easily fermentable hydrolyzate that would lead to an increased methane production, it was important to define an autohydrolysis condition that allowed an effective release of C5–C6 sugars altogether with a small generation of toxic/recalcitrant compounds such as furans and SL. The statistical significant effects of the independent variables (*T*, *t* and *SLR*) of AH pretreatment of SB on the dependent variables *Y*1, *Y*2 and *Y*3 can be seen in Supplementary Fig. 1.

The Pareto's diagram (Supplementary Fig. 1) shows that the variable temperature (*T*), as well as its squared effect (T^2), along with the variable time (*t*), had a significant positive effect on the dependent variable content of C5–C6 sugars. It is possible to notice that the standardized value of the temperature effect is the most pronounced (9.064) when compared to the effects obtained by the other independent variables ($t = 5.920$; $T^2 = 2.367$). One possible explanation for this is the influence of these variables on the pH of the reactional medium, more specifically on the concentration of acetic acid. As pointed out by Costa et al. (2014), the acetic acid released by the cleavage of acetyl groups can act as a catalyst for the hemicellulose hydrolysis reaction, thereby contributing to the increased release of sugars. As can be later shown, this

hypothesis was confirmed by Supplementary Fig. 2, which shows the acetic acid concentration in the hydrolyzates obtained after distinct AH pretreatment assays.

Given these results, it can be inferred that the increase in temperature combined with an increased pretreatment time, contributes for the hydrolysis of hemicelluloses, consequently leading to an increased release of C5 sugars (xylose and arabinose). Furthermore, the increased contact time combined with the higher temperature releases a higher amount of C6 sugars (glucose) from cellulose, which are concurrent with the results found by other authors such as Vallejos et al. (2012). In addition to the effects of T and t, it is also possible to notice that the SLR has a negative effect on the concentration of C5–C6 sugars, whereas for SLR^2 the opposite occurs. This can be explained by the fact that water is needed for the hydrolysis reactions of hemicelluloses to occur efficiently; this also leads to a concomitant release of XOS, monosaccharides (xylose and arabinose) and acetic acid.

When assessing the effect of independent variables on the levels of furans, Supplementary Fig. 1 shows that the greatest effects were related to the variables T (9.9616) and t (6.1942). These results are similar to those observed for C5–C6 sugars, in the way that an increase in temperature and time, positively contributes to higher levels in the hydrolyzate. The effect of varying the SLR on furan production was significant and had a negative effect, implying that the use of higher amounts of water during the AH pretreatment can contribute to higher levels of sugar degradation products in the hydrolyzate. Thus, it is clear that higher values of SLR during SB pretreatment can generate a hydrolyzate with lower levels of compounds (furans) considered toxic to anaerobic microorganisms, thereby rendering a hemicellulose hydrolyzate with a greater potential for methane production.

However, as smaller SLR values increase the sugar content in hemicellulose hydrolyzates, the optimization of this variable during the pretreatment by AH should seek the best compromise between water savings, increased generation of C5–C6 sugars, and lower production of sugar degradation compounds, potential inhibitors of the anaerobic digestion. The analysis of the effect of this variable is very important due to the commitment that must be held by AH process in a lignocellulose biorefinery: the lowest water consumption, and least generation of waste as possible. Moreover, the few studies that sought to evaluate the effect of SLR in the AH pretreatment of lignocellulose biomass presented no clear conclusions on the effect of this variable (Jacobsen and Wyman, 2002).

When the system's behavior in relation to SL content in the hydrolyzate is considered, it can be seen that the variable T and its quadratic term (T^2) have a positive effect on lignin dissolution. At high temperatures and acidic pH values, chemical bonds in lignin such as α–O–4 and β–O–4 breakdown, allowing the condensation reactions and modification of lignin in the plant cell. However, some fragments of lignin that did not undergo condensation reactions are released in the hydrolyzate. The presence of higher concentrations of SL fragments in the hydrolyzate can cause toxicity to methanogenic microorganisms. Alvarez et al. (1991) reported that low molar mass lignin fragments caused major toxicity effects against growth of methanogenic microorganisms.

Supplementary Fig. 2 shows that assay 9 (T = 185 °C and t = 35 min) led to a higher concentration of acetic acid in the hydrolyzate, and a higher amount (0.516 g) of C5–C6 sugars. This confirms the hypothesis presented by other authors (Costa et al., 2014; Vallejos et al., 2012) that hemicellulose hydrolysis into C5–C6 sugars depends on the accumulation of acetic acid, which can be produced in higher amounts at high temperatures and/or contact times, thereby acting as a catalyst for hydrolysis of ester and ether bonds in hemicelluloses. As confirmed in Table 1, the highest hemicellulose removal (83%) was obtained during assay 9.

In assay 2, which was also carried out at 185 °C and 35 min of contact time, led to the second highest accumulation of acetic acid and production of C5–C6 sugars (0.387 g), with a hemicellulose conversion of ~69%. This thereby confirms the direct relationship between the variables T and t and contents of acetic acid and C5–C6 sugars during AH pretreatment of SB. This is in good agreement with other authors as Boussarsar et al. (2009), who showed that maximum sugar accumulation occurred at high temperatures (190 °C) and contact times (15–240 min).

The response surface graphs (Fig. 1a–c) generated by quadratic models that resulted from the Doehlert planning when fixing the SLR variable at the center point (0.222), show how the independent variables T and t positively influence the levels of C5–C6 sugars, furans and SL.

Analyzing the response surface graphs, it is noticed that the AH conditions of SB that led to higher C5–C6 sugars release also led to higher accumulation of furans and SL in the hydrolyzate. Since such compounds are considered toxic to the methanogenic microorganisms (low growth rate effect), it is evident that AH optimization cannot be performed considering only the hydrolyzate C5–C6 levels or the total amount or reducing sugars as proposed by different research groups (Costa et al., 2014). Such an optimization can further impede the methane production due to the accumulation of inhibiting compounds in the hydrolyzate. Given the above conclusion, it is clear that a thorough evaluation of the SB hydrolyzate must be made, and this can be done by carrying on BMP tests, as described next.

3.3. Anaerobic biodegradability tests

BMP tests were carried out with all 19 hydrolyzates obtained with Doehlert planning, in an attempt to evaluate which AH conditions of SB would be better for methane production through anaerobic digestion. Fig. 1f shows that the BMP test carried out with the hydrolyzate obtained from pretreatment assay 9 (SLR = 0.167; T = 185 °C; t = 35 min), which was expected to have the highest methane potential considering its level of C5–C6 sugars (see Table 1), was actually the second best regarding the methane production, with a BMP value of 0.615 Nm^3 kg TOC^{-1}. The highest methane production (0.789 Nm^3 kg TOC^{-1}) was observed with the hydrolyzate generated during pretreatment assay 1 (SLR = 0.333; T = 170 °C; t = 35 min), which contained 2.6 times lesser C5–C6 sugars, and was a result of poor hemicellulose conversion (of only 57%, see Table 1).

These results are important to demonstrate that the pretreatment condition that leads to the hemicellulose conversion and higher sugar content in the hydrolyzate is not necessarily the best for methane production, contrary to what is normally employed by different researchers (Costa et al., 2014).

The BMP results presented in this study proved the hypothesis that was previously mentioned, that using only the variable C5–C6 sugars (or reducing sugars) as a response of AH pretreatment of SB, is flawed when the resulting hydrolyzate is to be submitted to anaerobic digestion. In addition to the sugar content, one should also observe the amount of recalcitrant and toxic compounds, such as SL and furans, which are concomitantly produced during AH pretreatment of SB. Table 1 shows that the concentrations of furans and SL in the hydrolyzate generated by pretreatment assay 1 were 3.7 and 3.2 times lower than those observed in assay 9; and, this seemed to be crucial for the higher methane production obtained with this hydrolyzate.

Recent studies have indicated that methanogenic *archaeas* can be severely inhibited in environments with high amounts of furans such as FF (Boopathy, 2009), thereby hampering methanogenesis. Therefore, it can be inferred that AH pretreatment conditions of SB with lower severity, although implying in lesser amounts of

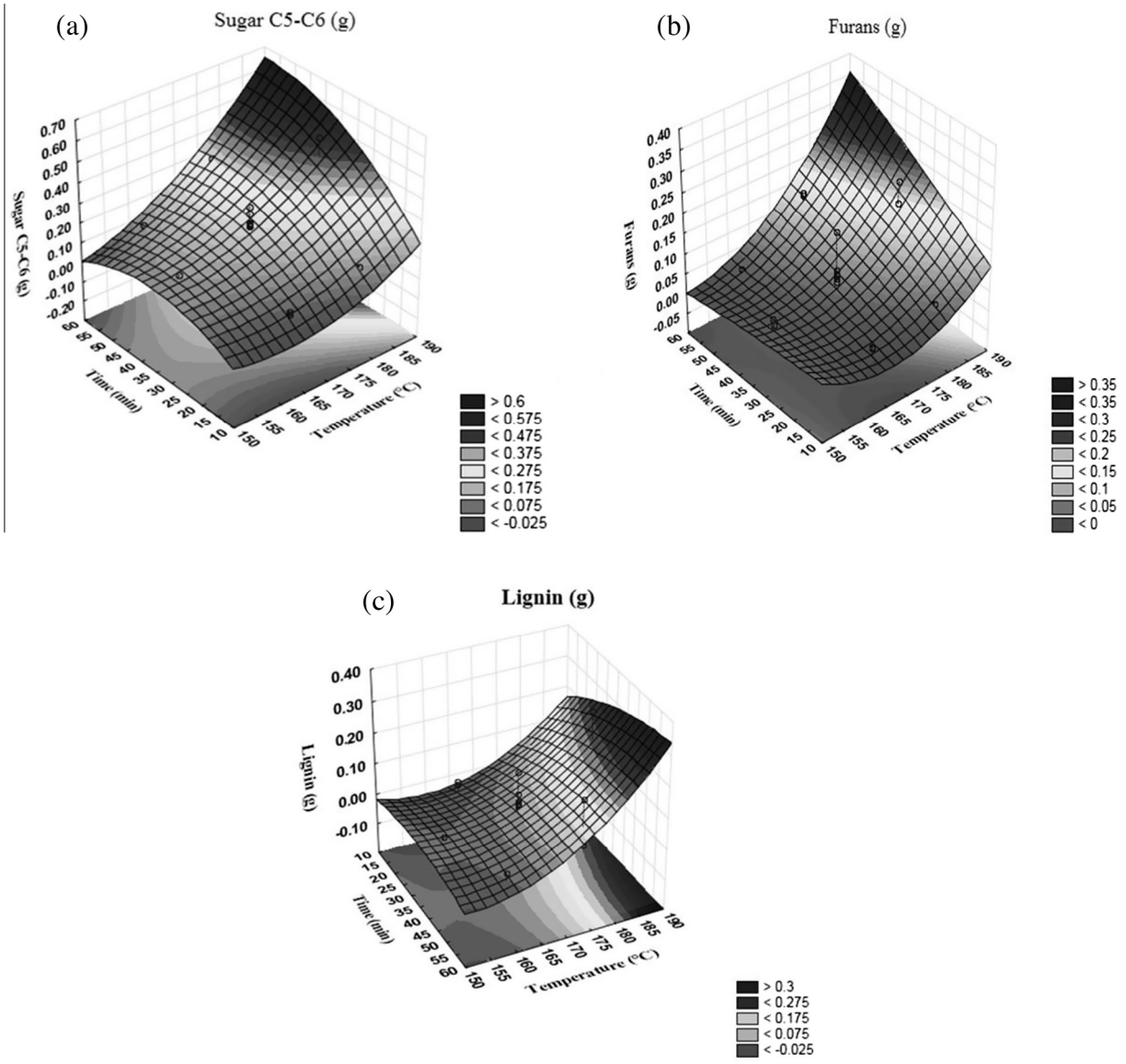

Fig. 1. Response surface graphs (a, b and c) generated from the quadratic model describing the levels of C5–C6 sugars, furans and SL and response surface graphs (d) and contour lines (e) for conversion of organic matter in the hydrolyzates (1–19) (kg of TOC per Nm^{-3} of methane) as function of temperature and time and (f) BMP for the hydrolyzates obtained after AH pretreatment of SB according to Doehlert experimental design.

C5–C6 sugars in the hydrolyzates, should be preferred if methane production is the aim.

Seeking to find a relationship between biomass conversion (into methane) and the AH dependent variables T and t, a nonlinear (second-order polynomial) regression analysis was carried out using the methane production capacity (kg TOC Nm^3 CH_4^{-1}) as a response. This relationship would show which experimental condition (T and t) led to the higher specific methane production, i.e., methane accumulation in relation to the incubated mass of substrate.

Eq. (4) describes the behavior of the response variable methane production capacity (kg TOC Nm^3 CH_4^{-1}) in relation to the temperature and time during AH pretreatment of SB, as per the response surface graphs and contour lines presented in Fig. 1d and e. The polynomial model resulted in a correlation value (R^2) of 0.84, implying that the model was able to satisfactorily predict 84% of the response variable in function of T and t. Considering the complexity of the system studied here and comparing this value with those found by other authors, one can say that the adjustment was pretty good. For instance, Ferreira et al. (2013) tried to find a relationship between methane production and the values of T and t used in the AH pretreatment of wheat straw and found a correlation coefficient (R^2) of only 0.57.

$$\text{kg TOC}\left(\text{Nm}^3\ \text{CH}_4\right)^{-1} = 916.5695 - 31.6044t - 3.2075T + 0.0840t^2 - 0.0074T^2 + 0.1427tT \quad (4)$$

Assessing Fig. 1d, it can be seen that the lowest values of the response variable methane production capacity (kg TOC Nm^3 CH_4^{-1}), were observed for temperatures near 175 °C and contact times in the range of 40–45 min. These values are close to those observed in pretreatment assay 1 of Doehlert planning (Supplementary Table 1), which despite having resulted in only moderate hemicellulose conversion and low C5–C6 sugars content in the hydrolyzate, led to the highest BMP values. This strengthens the hypothesis that the AH conditions that produces high hemicellulose hydrolysis and sugar release, as inferred by Doehlert designs having the chemical composition of hydrolyzate as response

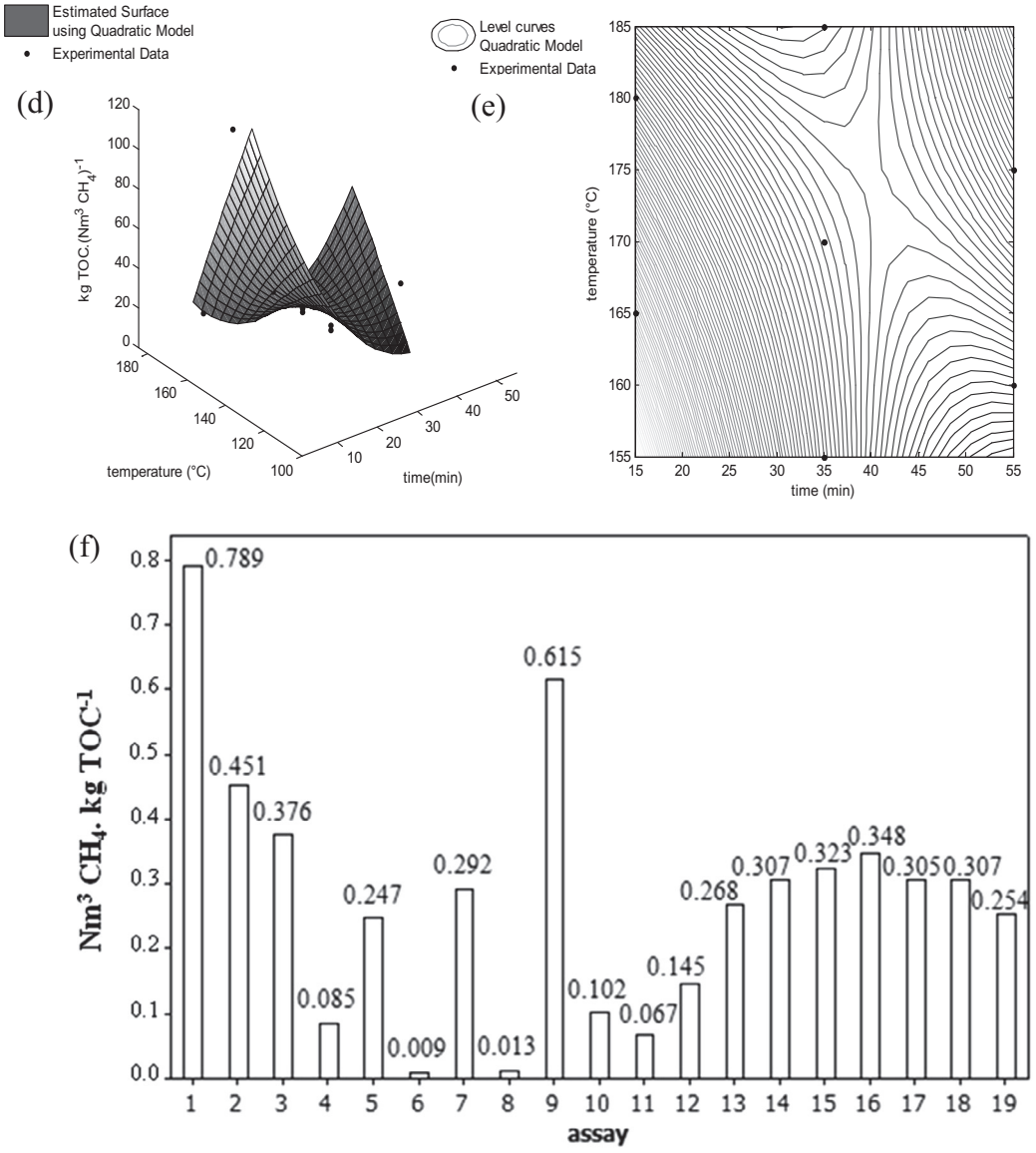

Fig. 1 (*continued*)

variable, are not necessarily the best for methane production. The results presented in Fig. 1d and e also suggest that intermediate values of T (165–175 °C) and t (35–45 min) should be sought to obtain a hydrolyzate containing lower levels of furans and SL, and thereby prone to methane production via anaerobic digestion.

3.4. Desirability tool and BMP assays

As the data presented before showed the sugar (C5–C6) content in the hydrolyzate was not a good variable to estimate its potential for anaerobic digestion, the statistical tool 'desirability' was used to determine what hydrolyzate characteristics are needed to maximize methane production. Desirable conditions were evaluated considering the levels of sugars (C5–C6), SL and furans (FF + HMF) in the hydrolyzate, as described in Table 2, and the output data of statistical tool for the AH conditions of SB as well as the expected hydrolyzate chemical composition (see Table 2). BMP experiments with the hydrolyzates obtained under the conditions from DC1 to DC4 would then allow the evaluation of the influence of furans and SL, as well as their combination, on methane production.

Table 2 shows that the desirability condition DC1 resulted in a hydrolyzate with the highest level of sugars, furans and SL. This scenario was set to verify the negative influence of the toxic/recalcitrant compounds FF, HMF and SL produced during severe AH conditions on methane production. As devised by Table 2, DC1 hydrolyzate would have the highest levels of sugars (1.07 g), furans (0.33 g) and SL (2.02 g) when compared to the other desired conditions (Table 2). On the other hand, the condition DC2 was devised to yield a hydrolyzate with the highest amount of sugars and the least of toxic/recalcitrant compounds, thereby creating the most favorable conditions for methane production. As seen in Table 2, the hydrolyzate generated under such AH conditions would have moderate levels of sugars (0.57 g), the lowest amount of furans (0.08 g), and SL (0.49 g); this was so due to the mild AH conditions employed. On its turn, the AH conditions DC3 and DC4 were set to verify the individual effect of furans and SL on methane production during the anaerobic digestion of a hydrolyzate loaded with sugars. Once more, the output data of desirability tool was consistent with the input coded values, and the hydrolyzate produced under the conditions DC3 and DC4 would have consistent levels of furans and SL (Table 2).

Table 3 shows the measured concentrations of C5–C6 sugars (glucose, xylose, arabinose), furans (FF and HMF) and SL in the hydrolyzates generated according to the four desirability conditions. As seen in Table 3, the concentration of C5–C6 sugars, furans and SL are in good agreement with those predicted by desirability tool.

The BMP values resulting from the anaerobic digestion of the four hydrolyzates obtained in the desired conditions (DC1, DC2, DC3 and DC4), shows that the methane production was higher for the DC2 condition (1.56 ± 0.11 Nm^3 kg TOC^{-1}) when compared to the others (DC1 = 0.74 ± 0.08 Nm^3 kg TOC^{-1}, DC3 = 0.48 ± 0.04 Nm^3 kg TOC^{-1} and DC4 = 0.35 ± 0.07 Nm^3 kg TOC^{-1}). These results confirm the hypothesis that lower concentrations of furans and SL contribute to increase the methanogenic activity. These results also confirm and validate the model proposed by Eq. (4), which predicts that the highest methane production from hydrolyzate is expected to be observed when the AH pretreatment of SB is performed in the temperature and time ranges of 170–180 °C and 40–45 min, respectively (Fig. 1d and e).

According to some authors as Santucci et al. (2015), the AH pretreatment of SB carried out at temperatures near to 170 °C are promising to generate hydrolyzates containing high amounts of XOS and monomeric sugars, as well as low concentration of sugar degradation byproducts, i.e. furans. Moreover, the *SLR* value employed in this study (0.24) is higher when compared to other studies reported in the literature (e.g. *SLR* = 0.10 used by Batalha et al. (2015)) and highly desired since it implies using lower amounts of water, encompassing both environmental and economic advantages. In addition, lower water content during AH pretreatment implies that autoclave reactors were being fed with higher amount of solids, thereby maximizing the quantity of SB per run.

Besides leading to a higher methane production, the hydrolyzate generated by DC2 condition exhibited the highest TOC removal efficiency (93.13%) when compared to DC1 (76.24%), DC3 (67.14%) and DC4 (52.55%) conditions. This indicates that DC2 hydrolyzate had better biodegradability; a possible explanation for this may be related to its higher concentration of arabinose. Some authors as Desai and Rao (2010) stated that some anaerobic and facultative bacteria prefer utilizing arabinose as substrate rather than xylose. These authors also reported that such microorganisms, when fed with mixtures of C-5 sugars (xylose and arabinose), tend to alter their metabolism as the presence of arabinose in the medium cause a catabolite repression for xylose assimilation. This may explain the increased production of methane from the DC2 hydrolyzate since it had the highest amount (309.4 mg L^{-1}) of arabinose (Table 3).

A hypothesis to explain the higher levels of arabinose in the hydrolyzate generated by DC2 condition is the relatively low severity (T = 178.6 °C and t = 43.6 min) employed during this pretreatment. It is well-known that extreme conditions of temperature and time, as those imposed on DC1 and DC4 experiments, are able to solubilize a large amount of C-5 sugars (xylose and arabinose) that are constituents of hemicelluloses. However, under more severe conditions both arabinose and xylose are degraded into furfural (FF), a well-known toxic compound to methanogenic microorganisms.

The C-5 sugars levels in the distinct hydrolyzates (Table 3) imply that the harshest AH conditions (DC1 and DC4) led to the production of hydrolyzates containing less arabinose and more FF. The highest levels of FF in these conditions are due to a higher conversion rate of arabinose into FF, prompted by the lowest activation energy involved in the degradation reaction of arabinose to FF when compared to that of xylose (Jacobsen and Wyman, 2002; Lavarack et al., 2002; Zhao et al., 2012).

The ease conversion of arabinose into FF can also be explained by the sugar position in the hemicelluloses chain. Arabinose is a substituent of the hemicelluloses chain, and therefore, is more easily hydrolyzed as compared to xylose, the major constituent of hemicelluloses chain. The easier solubilization of arabinose during AH pretreatment causes this sugar molecule to remain longer times in the bulk solution under the action of temperature and pH, which favors its degradation into FF and provides the hydrolyzate a higher toxicity. It is noteworthy that in addition to the FF generated from arabinose degradation there is also the contribution of FF coming from xylose. Therefore, the more severe AH conditions impair a further anaerobic digestion of the hydrolyzate due to the greater release of FF (a toxic furan) and the decrease in arabinose, a sugar considered of easy degradation by anaerobic microorganisms.

The lower toxicity observed for the DC2 hydrolyzate when compared to the others (DC1, DC3 and DC4) can be confirmed when evaluating the accumulation of volatile fatty acids (VFA) at the end of the BMP test. It is known that the VFA accumulation in anaerobic systems can occur due to a microbial growth imbalance caused by stressful conditions, such as nutritional deficiency and toxicity. Such conditions can lead to thermodynamic and kinetic constraints in the system compromising the conversion of organic matter into methane. Fig. 2e shows that the concentrations of all monitored VFA at the end of the BMP tests were lower for the DC2 hydrolyzate when compared to the others. This clearly shows that the more severe conditions (higher T or t) that normally lead to higher hemicelluloses dissolution during AH pretreatment of SB are not necessarily the most appropriate if the hydrolyzate is to be further submitted to anaerobic digestion and methane production.

Further evidence that the hemicellulose hydrolyzate generated by the AH condition DC2 showed the best biodegradability and the maximum production rate of methane (R) estimated by the modified Gompertz model following its adjustment to the BMP experimental data (Fig. 2a–d). It is possible to note that the model was capable of reproducing the methane production with very good fittings with values of correlation coefficients (R^2) higher than 0.98 for four AH conditions.

The R-value for the hydrolyzate generated by DC2 condition (2.59 mmol CH_4 d^{-1}) was higher than the values found for DC1 (0.88 mmol CH_4 d^{-1}), DC3 (0.33 mmol CH_4 d^{-1}) and DC4 (0.36 mmol CH_4 d^{-1}) conditions. This is an indication that the hemicellulose hydrolyzate generated by DC2 condition (which had lower furan and SL contents) resulted in lower kinetic imbalance during its anaerobic digestion, which was transformed into faster methane production.

Considering that CH_4 has an inferior calorific power of 34,450 kJ Nm^{-3} (Boussarsar et al., 2009), that the BMP for DC2 hydrolyzate was 1.56 Nm^3 kg TOC^{-1}, and that TOC dissolution during AH of SB was 0.0539 kg TOC kg SB^{-1} (on dry-weight basis), it is

Table 3
Hydrolyzate characteristics obtained by the desirability conditions at the beginning of BMP assays.

Experiment	TOC (mg L^{-1})	*HR* (%)	Glu (mg L^{-1})	Xyl (mg L^{-1})	Ara (mg L^{-1})	FA (mg L^{-1})	HAc (mg L^{-1})	HMF (mg L^{-1})	FF (mg L^{-1})	SL (mg L^{-1})
DC1	2911.67	81.07	8.55	454.19	191.36	248.34	301.21	45.08	158.17	1239.26
DC2	1797.50	48.99	6.81	312.60	309.43	222.57	270.42	23.15	68.12	545.46
DC3	2322.00	77.40	5.74	628.50	239.10	187.12	324.62	44.71	136.42	976.92
DC4	2945.00	73.90	8.34	542.39	115.43	250.77	419.74	68.08	201.61	1380.00

Glu – glucose; Xyl – xylose; Ara – arabinose; FA – formic acid; HAc – acetic acid; HMF – 5-hydroxymetil-2-furfuraldehyde; FF – 2-furfuraldehyde and SL – soluble lignin.

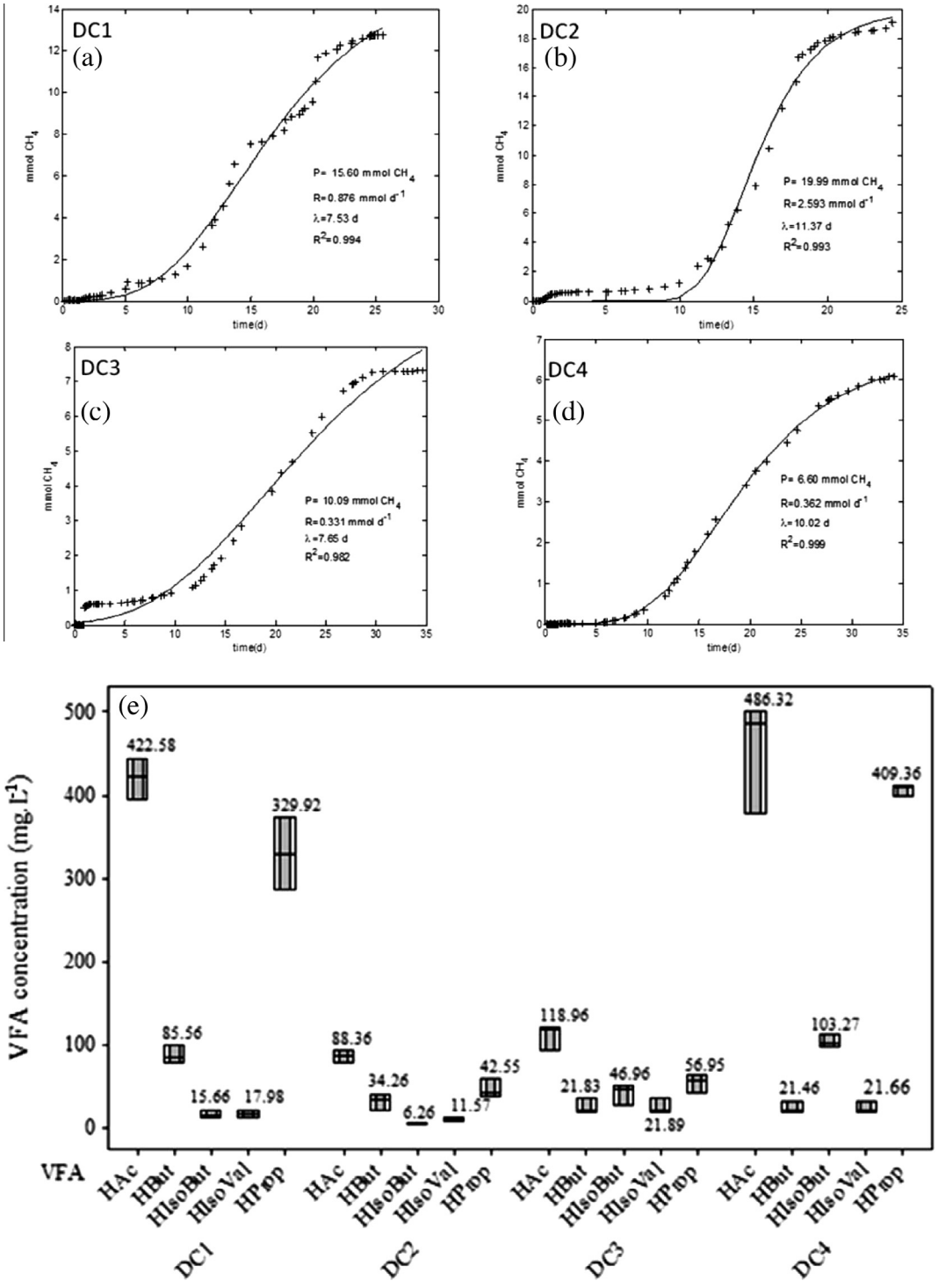

Fig. 2. Fitting (full line) experimental data (+) BMP to Gompertz model (a–d) and VFA concentration accumulated (HAc = acetic acid; HBut = butyric acid; HIsoBut = isobutyric acid; HIsoval = isovaleric acid; HProp = propionic acid) (e) for the hydrolyzates generated by AH pretreatment of SB under the desirability conditions (DC1–DC4).

reckoned that the anaerobic digestion of the hydrolyzate generated by AH of SB at mild conditions ($T \sim 179$ °C; $t \sim 44$ min and $SLR \sim 0.2$) is capable of generating 2.896 MJ kg SB^{-1}. This value is higher than that estimated (1.87 MJ kg SB^{-1}) by other researchers as Rabelo et al., 2011, who used a hydrolyzate generated by pretreating the SB with an alkaline hydrogen peroxide solution.

Costa et al. (2014) estimated a power generation of 7.1 MJ kg SB^{-1} via methane production from a mixture of solid (residual pretreated bagasse) and liquid (hydrolyzate) fractions obtained after alkaline pretreatment of SB. Although this value is nearly 2.5 times greater than that estimated in this study, it should be pointed out that the amount of energy estimated in this study for DC2 condition refers to anaerobic digestion of the soluble fraction only (hemicellulose hydrolyzate). The residual solid fraction, obtained after the AH pretreatment of SB, may be further subjected to delignification process for removing lignin; and, the resulting cellulose rich fraction can be submitted to enzymatic hydrolysis aiming the production of 2G bioethanol.

The use of hemicellulose hydrolyzates for methane production and power generation can pave the way for a sustainable integration of the 1G and 2G bioethanol production, as demonstrated by some authors (Dias et al., 2013; Moraes et al., 2015). It is known that the presence of hemicelluloses during the enzymatic hydrolysis may worsen the conversion yields (Batalha et al., 2015), and therefore, its previous solubilization by AH is beneficial from a process and economical point of view. Moreover, the microorganisms responsible for fermenting sugars to ethanol are not able to use C-5 sugars such as xylose and arabinose, which are the major components of hemicelluloses (Kaparaju et al., 2009). Therefore, the anaerobic digestion of the hemicellulose hydrolyzate creates a new energetic vector (methane), which might help to close the energetic balance in a lignocellulose biorefinery.

The strategic biomethane generation from the hemicelluloses fraction of SB also avoids the necessity of its combustion to produce power and steam, since the biogas could supply this energetic demand. This would spare the pretreated bagasse, a fraction rich in cellulose and lignin, to be further used to obtain 2G bioethanol and/or recover lignin derived value-added compounds.

During the integration of the 1G and 2G ethanol plants, it is also possible to use the vinasse generated during ethanol production for co-digestion with the hemicellulose hydrolyzate. Such action is interesting from an application standpoint since the use of vinasse for biogas production is currently compromised by the high levels of hydrogen sulfide (H_2S) normally formed in the digesters. The concomitant anaerobic digestion of hemicellulose hydrolyzate and vinasse would allow the dilution of sulfate since this anion is not normally present in the SB hydrolyzates, thereby decreasing H_2S in the biogas and improving its quality.

The co-digestion of vinasse and hemicellulose hydrolyzate could also be benefit the methanogenic activity in the digesters if one considers the dilution of toxic compounds, such as FF and HMF, which are normally present in SB hydrolyzates and absent in the vinasse. Another important issue that might boost biogas production in co-digestion is the exploitation of essential nutrients, such as N, P and K, which are present in high concentration in the vinasse and normally scarce in the hydrolyzates. Despite the advantages, Moraes et al. (2015) highlight there are quite a few papers in the literature that aimed at evaluating the possibility of biogas production from combined digestion of vinasse and hemicellulose hydrolyzates.

4. Conclusions

The AH of SB showed that severe conditions (185 °C; 55 min) and lower *SLR* (0.14) led to HH with low BMP (0.74 Nm^3 kg TOC^{-1}), and that milder conditions (178.6 °C; 43.6 min) and *SLR* (0.23) led to a higher BMP (1.56 Nm^3 kg TOC^{-1}). The rate of CH_4 estimated by the Gompertz model was 2.6 mmol d^{-1} for the best desired condition, which was equivalent to 2.9 MJ kg SB^{-1}. The anaerobic digestion of HH contributes to close the energetic balance in a sugarcane biorefinery, thereby sparing the SB for more noble uses such as 2G bioethanol production and value-added compounds recovery.

Acknowledgements

The authors would like to thank the Brazilian National Research Council (CNPq) and the Minas Gerais State Research Funding Agency (FAPEMIG) for their financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2015.10.003.

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