



# Anaerobic digestion of hemicellulose hydrolysate produced after hydrothermal pretreatment of sugarcane bagasse in UASB reactor



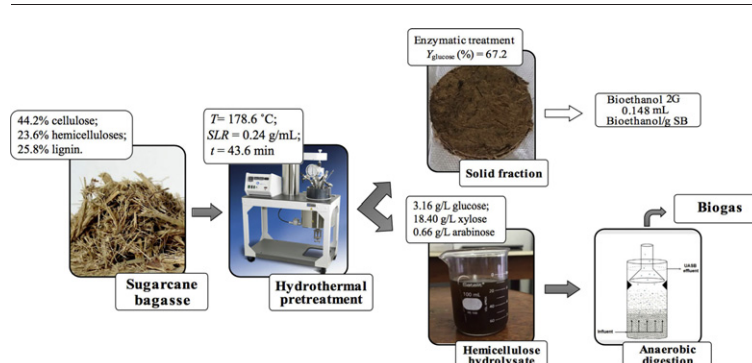
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## HIGHLIGHTS

- Hemicelluloses were obtained after hydrothermal pretreatment of sugarcane bagasse.
- Anaerobic digestion of hemicellulose hydrolysate was assessed in a UASB reactor.
- Hydrolysate dilution and OLR were varied for studying the process performance.
- The highest COD removal (86%) was obtained at an OLR of 2.4 g COD/L·d.
- Methane yield was estimated at 270 L CH<sub>4</sub>/kg COD during the best process condition.

## GRAPHICAL ABSTRACT



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## ABSTRACT

In the context of a sugarcane biorefinery, sugarcane bagasse produced may be pretreated generating a solid and liquid fraction. The solid fraction may be used for 2G bioethanol production, while the liquid fraction may be used to produce biogas through anaerobic digestion. The aim of this study consisted in evaluating the anaerobic digestion performance of hemicellulose hydrolysate produced after hydrothermal pretreatment of sugarcane bagasse. For this, hydrothermal pretreatment was assessed in a continuous upflow anaerobic sludge blanket (UASB) reactor operated at a hydraulic retention time (HRT) of 18.4 h. Process performance was investigated by varying the dilution of sugarcane bagasse hydrolysate with a solution containing xylose and the inlet organic loading rate (OLR). Experimental data showed that an increase in the proportion of hydrolysate in the feed resulted in better process performance for steps using 50% and 100% of real substrate. The best performance condition was achieved when increasing the organic loading rate (OLR) from 1.2 to 2.4 g COD/L·d, with an organic matter removal of 85.7%. During this period, the methane yield estimated by the COD removal would be 270 L CH<sub>4</sub>/kg COD. Nonetheless, when further increasing the OLR to 4.8 g COD/L·d, the COD removal decreased to 74%, together with an increase in effluent concentrations of VFA (0.80 g COD/L) and furans (115.3 mg/L), which might have inhibited the process performance. On the whole, the results showed that anaerobic digestion of sugarcane bagasse hydrolysate was feasible and may improve the net energy generation in a bioethanol plant, while enabling utilization of the surplus sugarcane bagasse in a sustainable manner.

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## 1. Introduction

Agro-industrial waste is the most abundant biomaterial produced worldwide, although generally it is used below its potential in terms of bioenergy (Cardona et al., 2010). In this context, Brazil assumes a prominent position considering the volume of agricultural and agroforestry production and the consequent generation of lignocellulosic waste (Soccol et al., 2010). For instance, sugarcane alcohol industry leads to the generation of a by-product rich in carbohydrate, named sugarcane bagasse, available in a clean form and in large amounts (Karp et al., 2013). In fact, according to Brazil's National Supply Company (CONAB, 2015), the production of sugarcane in the country reached 663 million tons in the 2015/2016 harvest season. Nowadays, part of this biomass is used for supplying the energy requirement for the first-generation (1G) bioethanol plant as a fuel in boilers for steam generation. However, the steam produced exceeds by two-fold the energy demand (Costa et al., 2014). Thus, not all of the sugarcane bagasse biomass is used and the excess entails environmental and storage issues. In this context, research on bioconversion processes for second-generation (2G) bioethanol, biogas and other bioproducts has been investigated in the last decades (Costa et al., 2014).

Up to date, most studies have evaluated the pretreatment and processing of the whole biomass; however, the separation of solid (i.e. cellulose and lignin) and liquid (i.e. hemicelluloses) fractions may enhance bioenergy yields. Therefore, this separation expands possibilities for the complete use of sugarcane bagasse in a lignocellulosic biorefinery concept. In this scenario, the cellulose-rich fraction generated after hemicelluloses extraction can be used for 2G bioethanol, while the liquid or hydrolysed hemicelluloses fraction can generate biogas (Baêta et al., 2016a). The present study specifically focuses on methane production from the liquid hydrolysate produced from sugarcane bagasse, as outlined in Fig. 1.

In terms of bioenergy, biogas production through anaerobic digestion is a well-known process, applied in many waste treatment full-scale facilities, with low operation cost and energy requirements. Nonetheless, sugarcane bagasse is composed mainly of cellulose, hemicelluloses and lignin, which are organized in a complex arrangement. This characteristic limits the hydrolysis step of the anaerobic digestion

process, since organic matter content has low bioaccessibility and biodegradability, hampering microbial attack in the reactor. Therefore, to achieve higher degradation rate and biogas production, lignocellulosic wastes require a pretreatment step before undergoing anaerobic digestion (Monlau et al., 2013). The pretreatment modifies the size, structure and chemical composition of biomass (Nissila et al., 2014). In addition, its effectiveness is related to lignin removal, hemicelluloses solubilisation, cellulose crystallinity reduction and increase in the surface area of the material (Liming and Xueliang, 2004). This preliminary step also justifies the separation of solid and liquid fractions. Among the pretreatment techniques, thermal pretreatment has advantages, such as lower energy requirement and lower formation of toxic and inhibitory compounds when compared to mechanical and chemical methods (Carrere et al., 2010).

Based on the aforementioned, this study intends to investigate the anaerobic digestion performance of hemicellulose hydrolysate generated from hydrothermal pretreatment of sugarcane bagasse. For this, a continuous upflow anaerobic sludge blanket (UASB) reactor was operated during 168 days in several periods by varying the proportion of sugarcane bagasse hydrolysate and the digester organic loading rate (OLR).

## 2. Material and methods

### 2.1. Sugarcane bagasse

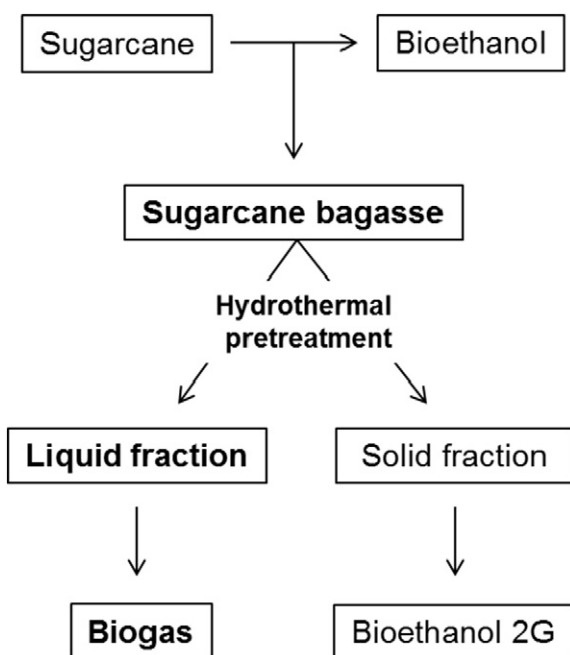
The sugarcane bagasse used in this study was provided by Jatiboca Sugar and Ethanol Plant harvest of 2013/2014 (Ponte Nova, MG, Brazil). The biomass was ground and sifted (10 mesh; 1.7 mm) in the sugarcane plant and stored in the laboratory facilities at room temperature before pretreatment. Sugarcane bagasse composition in dry-weight basis was of 44.2% cellulose, 23.6% hemicelluloses, 25.8% lignin, 2.2% extractives and 1.4% ash. In terms of C5 sugars, which are the main composition of hemicellulose, the composition was 0.126 g xylose/g SB and 0.013 g arabinose/g SB. The amount of sugar was estimated in dry-weight basis from the concentration of xylose and arabinose determined in accordance with the test method NREL LAP-002.

### 2.2. Hydrothermal pretreatment

Hydrothermal pretreatment condition was selected by a previous biochemical methane potential (BMP) test in which temperature, exposure time and solid-to-liquid ratio (SLR) were optimised through a Doehlert experimental design. Following, a desirability tool was applied for selecting the pretreatment condition by combining a high concentration of soluble sugars (C5 and C6) and a low formation of toxic (furfural (FF) and 5-hydroxymethyl-2-furfural (HMF)) and recalcitrant (soluble lignin) compounds. The previous study indicated that the optimal trial was the one pretreated at 0.24 g/mL of SLR, 178.6 °C and 43.6 min. For this condition, hemicellulose hydrolysate produced attained 93% of total organic carbon (TOC) removal and 1.56 NL CH<sub>4</sub>/g TOC in BMP tests (Baêta et al., 2016a).

For this study, 120 g of bagasse, on dry-weight basis, was pretreated in a 2 L Parr reactor (model 4848). Subsequently, pretreated bagasse was dewatered in a hydraulic press (SOLAB, model SL-10) at a pressure of 9 tons. The weight loss during the hydrothermal pretreatment was gravimetrically determined and the value observed was in average 30.2%. The solid fraction generated after the pretreatment represented a composition of 58.9% of cellulose, 17.2% of hemicellulose and 24.6% of lignin. Therefore, the removal values of cellulose, hemicellulose and lignin were 6.8%, 49.0% and 6.3%, respectively.

Finally, 450 mL of hemicellulose hydrolysate was obtained at a concentration of 88.16 g COD/L. The hydrolysate was stored at 0 °C and thawed 3-times a week for preparation of the reactor influent feed. To this aim, sugarcane bagasse was pretreated 20 times. The hydrolysate was analysed by means of pH, carbon oxygen demand (COD), total



**Fig. 1.** Summarised diagram of sugarcane biorefinery, where, the focus of this work, sugarcane bagasse pretreatment and subsequent conversion of liquid fraction into biogas, is highlighted in bold letters.

organic carbon (TOC), organic acids, sugars, sugar degradation products (FF and HMF). The oligomers were estimated from the difference between the concentration of monomeric sugars and the sugar degradation products found in the hydrolysate before and after the pretreatment. The average characteristics of the produced liquid fraction are summarised in Table 1.

### 2.3. Anaerobic digestion in UASB reactor

The lab-scale UASB reactor had 2.3 L of useful volume and it was operated at a HRT of 18.4 h in a continuous mode fed at 0.125 L/h during the whole period. The reactor had 500 mm of height and three different cross sections with diameters of 20 mm, 50 mm and 100 mm. The HRT was selected based on a previous study investigating the methane production of oat straw hydrolysate in a UASB reactor, which reported an optimal OLR ranging from 0.5 to 11.5 g COD/L·d and 24 h HRT (Gomez-Tovar et al., 2012).

For the start-up, the reactor was inoculated with 5.75 g VSS of anaerobic granular sludge (24.79 g VSS/L) provided from a full-scale wastewater treatment plant near Belo Horizonte, MG, Brazil (CePTS/UFMG/Copasa). The inoculum was pre-incubated for four days (35 °C) in order to offset the methane production from the endogenous biodegradation of any residual organic matter. The reactor temperature was not controlled and remained at ambient condition (20–30 °C). The reactor pH was monitored daily and was kept near neutrality by addition of NaHCO<sub>3</sub> (1.5 mol/L) or HCl (1.5 mol/L) solutions in the inlet when necessary.

The reactor was operated during 168 days, from October 15th, 2015 until March 31st, 2016 in six different steps. The different steps of operation are described as follows: I) the preliminary period, known as stabilization step, in which the reactor was fed with 100% of xylose and 1.4 g COD/L·d of OLR; II) fed with 50% of xylose and 50% of sugarcane bagasse hydrolysate and 1.4 g COD/L·d of OLR; III) fed with 25% of xylose and 75% of sugarcane bagasse hydrolysate and 1.2 g COD/L·d of OLR; IV) fed with 100% of sugarcane bagasse hydrolysate and 1.3 g COD/L·d of OLR; V) fed with 100% of sugarcane bagasse hydrolysate and 2.4 g COD/L·d of OLR; and VI) fed with 100% of sugarcane bagasse hydrolysate and 4.8 g COD/L·d of OLR. The conditions are summarised in Table 2.

Feeding solution was prepared for a total volume of 6 L with xylose (in step I) and/or sugarcane bagasse hydrolysate (in steps II, III, IV, V and VI), tap water and nutrient solution, containing sodium bicarbonate buffer, micro- and macronutrients. Even when the reactor was fed with hemicellulose hydrolysate a dilution was performed with tap water for maintaining the desired OLR in each period. The values of dilution factor applied were of 24 and 48 times depending on the OLR values.

The nutrient solution was composed by NH<sub>4</sub>Cl (2.224 g/L), (NH<sub>4</sub>)<sub>2</sub>H<sub>2</sub>PO<sub>4</sub> (0.265 g/L), (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> (89.0 mg/L), MgCl<sub>2</sub> (0.5 g/L), CaCl<sub>2</sub>·2H<sub>2</sub>O (0.378 g/L), NaHCO<sub>3</sub> (5.0 g/L), FeCl<sub>3</sub>·6H<sub>2</sub>O (10.0 mg/L), ZnCl<sub>2</sub> (0.26 mg/L), MnCl<sub>2</sub>·4H<sub>2</sub>O (2.5 mg/L), (NH<sub>4</sub>)<sub>6</sub>MO<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O

**Table 1**

Characterisation of sugarcane bagasse hydrolysate used in the UASB reactor operation (± standard deviation).

Parameter	Value
pH	3.14 (±0.04)
TOC (g/L)	31.44 (±1.27)
COD (g/L)	88.16 (±3.88)
Glucose (g/L)	3.16 (±0.21)
Xylose (g/L)	18.40 (±1.13)
Arabinose (g/L)	0.66 (±0.06)
Formic acid (g/L)	6.58 (±0.15)
Acetic acid (g/L)	10.98 (±1.10)
HMF (g/L)	7.82 (±0.97)
FF (g/L)	3.93 (±0.41)
Oligomers (g/L)	2.12 (±0.33)

**Table 2**

Anaerobic digestion conditions during the different steps in the UASB reactor.

Step	I	II	III	IV	V	VI
Days	1–56	57–69	70–92	93–119	120–144	145–168
Xylose (%)	100	50	25	0	0	0
Sugarcane bagasse hydrolysate (%)	0	50	75	100	100	100
OLR (g COD/L·d)	1.2	1.2	1.2	1.2	2.4	4.8

(3.2 mg/L), AlCl<sub>3</sub>·6H<sub>2</sub>O (0.26 mg/L), CoCl<sub>2</sub>·6H<sub>2</sub>O (10.0 mg/L), NiCl<sub>2</sub>·6H<sub>2</sub>O (26.0 mg/L), H<sub>3</sub>BO<sub>3</sub> (6.0 mg/L), CuCl<sub>2</sub>·2H<sub>2</sub>O (16.0 mg/L) and HCl (1.0 mL/L). The concentration of the nutrients was determined in order to maintain a minimum COD:N:P ratio of 350:5:1 (Baêta et al., 2013). The inlet feed was prepared for attaining an OLR of 1.2, 2.4 and 4.8 g COD/L·d, depending on the conditions set for each period.

### 2.4. Analytical methods

Parameters from the reactor influent and effluent were analysed in triplicates by means of pH, COD, VFA, sugars, sugar degradation products (FF and HMF) and organic acids. pH was measured daily with a portable pH-meter (Metrohm, model 827). COD was determined 3-times a week according to the Standard Methods (APHA/AWWA/WEF, 2005).

The concentrations of sugars (glucose, xylose and arabinose), sugar degradation products (HMF and FF) and VFAs (formic, acetic, propionic, isobutyric, butyric, valeric and isovaleric acids) were analysed once a week through high performance liquid chromatography (HPLC), in accordance with the methods from the National Renewable Energy Laboratory LAP-002. For the determination of sugars content, a Shimadzu HPLC system, equipped with a refractory index detector (Shimadzu RID-6A) and an Aminex HPX 87H column (300 × 7.8 mm Bio-Rad) kept at 55 °C was used. For separating the analytes, sulphuric acid (5 mmol/L) was used as an eluent at a flow rate of 0.6 mL/min. The content of organic acids and sugar degradation products were also determined using the same chromatographic conditions previously described, but detection of these compounds was performed in a UV-Vis detector (Shimadzu SPD-10AV) at the wavelength of 210 nm for the organic acids and 274 nm for furans. VFA concentration was expressed as COD, according to Eq. (1) (Aquino and Stuckey, 2004).

$$\text{VFA (mg COD/L)} = (0.35 * [\text{FA}]) + (1.07 * [\text{AA}]) + (1.50 [\text{PA}]) + (1.82 * [\text{BA} + \text{IBA}]) + (2.04 * [\text{VA} + \text{IVA}]) \quad (1)$$

where [ ] is concentration; FA is formic acid; AA is acetic acid; PA is propionic acid; BA is butyric acid; IBA is isobutyric acid; VA is valeric acid; and IVA is isovaleric acid.

### 2.5. Statistical analysis

The statistical significance of experimental results was evaluated by the ANOVA and Tukey tests, with a significance level ( $\alpha$ ) of 5%, using Minitab software.

## 3. Results and discussion

### 3.1. Hemicellulose hydrolysate characterisation

The average composition of the hemicellulose hydrolysate obtained after hydrothermal pretreatment is summarised in Table 1. The low pH of the hydrolysate (3.14) is probably due to production of endogenous organic acids (i.e. acetic and formic acids) during the hydrothermal pretreatment. Xylose was the sugar found in the greatest amount (18.40 g/L), representing over 80% from the total amount of sugars analysed. Glucose and arabinose were also identified, however at lower concentrations (3.16 and 0.66 g/L, respectively). As previously

reported, xylose is the principal monosaccharide constituent of hemicelluloses (Barakat et al., 2012), which supports the fact that hydrothermal pretreatment of sugarcane bagasse leads to the production of a liquid fraction rich in hemicelluloses content. The higher concentration of xylose in respect to glucose in the pretreatment step relies on the fact that the structure of the hemicelluloses was more easily hydrolyzed than the cellulose during hydrothermal pretreatments (Antunes et al., 2014).

Moreover, the hydrothermal pretreatment conditions used (178.6 °C; 0.24 g/mL SLR, 43.6 min) led to partial degradation of pentoses and hexoses, generating HMF and FF, respectively. The furan derivatives were detected at concentrations of 7.82 ( $\pm$  0.97) g HMF/L and 3.93 ( $\pm$  0.41) g FF/L.

The cellulose solid fraction obtained after pretreatment may be used for 2G ethanol production. In this scenario, it is possible to estimate the amount of 2G bioethanol that could be generated from the solid fraction considering the weight loss (30.2%), the enzymatic hydrolysis efficiency (67.2%), a yield of 0.42 g bioethanol/g glucose during the yeast fermentation (Rabelo et al., 2011) and an ethanol density of 789 g/L. Thus, the ethanol production from the solid fraction would be 0.148 L bioethanol/g SB. In this estimation, the enzymatic hydrolysis efficiency was defined using the methodology and commercial enzymes cocktail described by Gurgel et al. (2014).

### 3.2. Anaerobic digestion in UASB reactor

#### 3.2.1. COD removal

The parameters evaluated during the different steps are summarised in Table 3. The process performance was monitored by analysing pH, COD removal and VFA accumulation. Moreover, methane production was estimated from the COD removal, discounting 10% assimilated for microbial growth (Tchobanoglous et al., 2003), as represented in Eq. (2).

$$\text{Methane yield (mL CH}_4\text{/g COD}_{\text{fed}}) = \frac{350 \text{ mL CH}_4\text{/g COD}_{\text{removed}} * 0.9 \text{ g COD}_{\text{removed}}}{\text{g COD}_{\text{fed}}} \quad (2)$$

As can be seen in Fig. 2, in the preliminary stage, the reactor was fed with a solution of xylose, since it is a readily biodegradable monosaccharide and the main sugar composing the hemicellulose hydrolysate. This strategy was adopted to aid the microbial acclimation, since the direct use of this substrate could lead to VFA accumulation and methanogenic inhibition. As can be seen, during the stabilization step (I), average COD removal was 36.1%, representing a methane yield of 113.72 L CH<sub>4</sub>/kg COD. Subsequently, the hydrolysate was introduced little by little (from 50 to 100%) to the influent feeding of the UASB reactor through different dilutions.

According to the results obtained, the replacement of xylose to sugarcane bagasse hydrolysate increased COD removal. This behaviour can be observed when comparing the steps with higher xylose solution percentage in influent (I: 100% and II: 50%) to steps with lower percentage of xylose solution (III: 25% and IV: 0%). During the latter ones, the reactor reached values of COD efficiency removal greater than 60%, while the previous periods achieved values lower than 40%. In respect to the methane yield, values higher than 200 L CH<sub>4</sub>/kg COD would be produced after adding 50 or 100% of hemicellulose hydrolysate. This

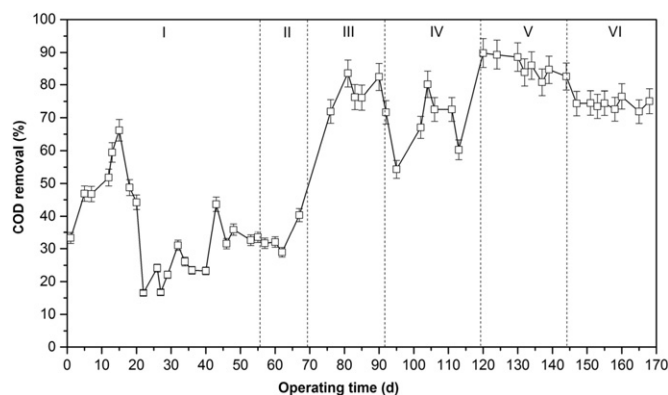


Fig. 2. COD removal (□) obtained during the operating time of the reactor.

indicates that the substrate was biodegraded and did not inhibit the anaerobic microbial activity at this OLR. A possible explanation for the improved performance when the reactor was fed with larger amount of hydrolysate is that the same capable of being biodegraded and converted to methane. These other compounds were mainly acetic acid, formic acid and oligomers (Table 1). Indeed, microorganisms generally prefer to degrade other sugar compounds than xylose (Gong et al., 1999).

In order to study the effect of the OLR increase, in periods IV, V and VI the reactor was fed with 100% hemicellulose hydrolysate at 1.2, 2.4 and 4.8 g COD/L·d, respectively. The statistical analysis carried out showed that the COD effluent concentrations in those periods analysed were significantly different, indicating that the highest removal values were obtained for period V (85.3%), which corresponds to a methane yield of 270 L CH<sub>4</sub>/kg COD. Nonetheless, even after OLR increase up to 4.8 g COD/L·d, COD removal was maintained over 70%.

The result obtained in our study is in accordance with those previously reported in the literature. For instance, hemicellulose hydrolysate produced from thermal pretreatment of sugarcane bagasse at 200 °C for 10 min achieved 200 L CH<sub>4</sub>/kg COD (Costa et al., 2014). Other pretreatments using alkaline agents were also assessed for residual stillage of sugarcane bagasse at 80 °C for 2 h and 0.5 mol·L<sup>-1</sup> NaOH, attaining 307 L CH<sub>4</sub>/kg COD (Liu et al., 2015). On the other hand, lower methane yields were obtained for liquid fraction after thermo-chemical pretreatment of sugarcane bagasse with calcium hydroxide at 90 °C, i.e. 167 mL CH<sub>4</sub>/g COD (Rabelo et al., 2011). The same strategy of separating liquid and solid fractions after pretreatment was also adopted for other biomasses. For instance, the hemicellulose hydrolysate produced after hydrothermal pretreatment of wheat straw at 180 °C for 15 min followed by 190 °C for 5 min reached a methane yield of 340 mL CH<sub>4</sub>/g COD and COD removal of 50–80% for an OLR of 6.97 g COD/L·d (Kaparaju et al., 2009). Nevertheless, all studies mentioned were carried out in batch tests and experiments in continuous reactors, such as this one, is crucial for attaining more credible values.

#### 3.2.2. VFA production

VFA was analysed throughout the operation periods for identifying possible overloading (Figs. 3 and 4). Organic acids are sub-products of the first stages of anaerobic digestion; however the accumulation of these intermediate compounds may limit methanogenesis due to

Table 3

Mean values ( $\pm$  standard deviation) of the anaerobic digestion process performance of sugarcane bagasse hydrolysate in UASB reactor.

Step	I	II	III	IV	V	VI
pH	6.7 ( $\pm$ 0.2)	6.8 ( $\pm$ 0.2)	7.1 ( $\pm$ 0.2)	7.1 ( $\pm$ 0.2)	7.2 ( $\pm$ 0.3)	7.4 ( $\pm$ 0.2)
COD removal (%)	36.1 ( $\pm$ 13.5)	33.2 ( $\pm$ 4.3)	77.0 ( $\pm$ 4.6)	67.8 ( $\pm$ 8.5)	85.7 ( $\pm$ 3.1)	74.1 ( $\pm$ 1.3)
COD <sub>effluent</sub> (g/L)	0.67 ( $\pm$ 0.15)	0.71 ( $\pm$ 0.01)	0.20 ( $\pm$ 0.04)	0.31 ( $\pm$ 0.10)	0.26 ( $\pm$ 0.05)	0.96 ( $\pm$ 0.03)
VFA (g COD/L)	0.60 ( $\pm$ 0.03)	0.58 ( $\pm$ 0.02)	0.14 ( $\pm$ 0.01)	0.18 ( $\pm$ 0.01)	0.18 ( $\pm$ 0.05)	0.80 ( $\pm$ 0.04)
Estimated methane yield (L CH <sub>4</sub> /kg COD) <sup>a</sup>	113.72	104.58	242.55	213.57	269.96	233.42

<sup>a</sup> Note: Methane yield was estimated from the COD removal according to Eq. (2).

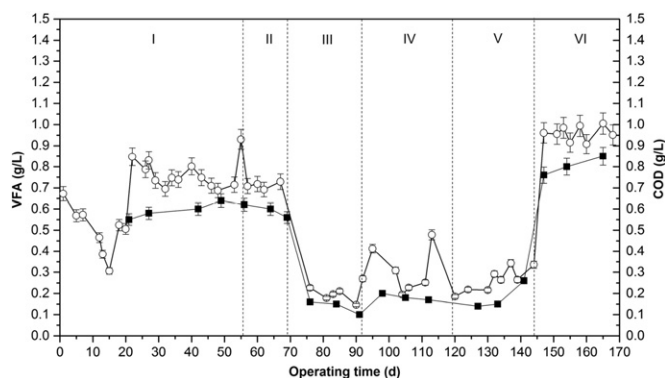


Fig. 3. VFA (■) and COD concentration in the outlet (○) samples of the UASB reactor.

thermodynamic inhibitions (Aquino and Chernicharo, 2005). The VFA and COD<sub>effluent</sub> curves (Fig. 3) showed the same trend throughout the period of operation of the UASB reactor, and by analysing the periods in which the reactor was fed with hydrolysate, the VFA varied from 58% (step IV) to 83% (step VI) in terms of COD. As can be seen, in the first periods (I and II), VFA outlet showed average values of 0.60 g COD/L. This result was probably related to the acclimatisation of microorganisms to the substrate. Afterwards, VFA values decreased and were maintained at 0.10–0.25 g COD/L, suggesting process stability. Finally, in period VI, VFA increased up to 0.80 g COD/L. This increment was associated with the increase of the OLR and led to a decrease of the COD efficiency removal (74.1%). These findings indicated that the reactor was stable when increasing the OLR from 1.2 to 2.4 g COD/L·d, although may have had a negative impact when further incrementing the OLR to 4.8 g COD/L·d. The same trend may be observed for acetic acid concentration, which is the main precursor for biogas production (Fig. 4).

### 3.2.3. FF and HMF production

The hemicellulose hydrolysate may contain derivatives of furans (FF and HMF) and phenol compounds that are known to be toxic to microorganisms at high concentrations (Torry-Smith et al., 2003; Barakat et al., 2012). These compounds are generated during the acid-catalysed degradation of hexoses and pentoses, as well as phenolic compounds from lignin hydrolysis. In fact, the analysis of concentration of sugars and sugar degradation products in the UASB reactor showed that easily degradable compounds such as C5 and C6 sugars were completely used by microorganisms and converted into biogas or new cells in all operating phases. However the furans were partially removed in steps V and VI (Table 4). In step V, 20.5% of HMF and 11.5% of FF were removed in the

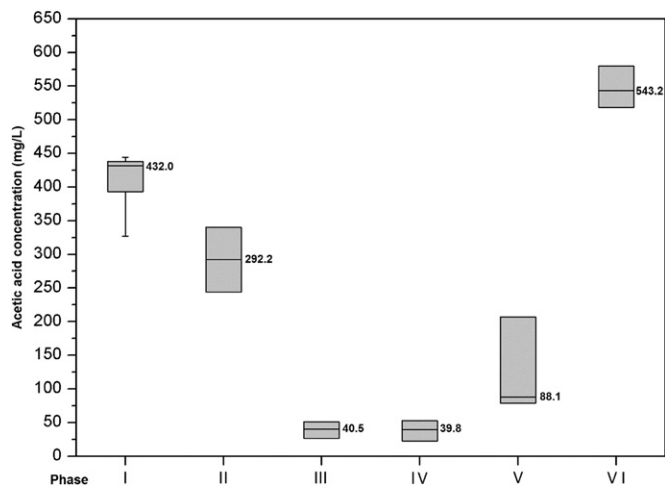


Fig. 4. Accumulation of acetic acid in the UASB reactor for each operating phase.

Table 4

Concentrations of HMF and FF in affluent and effluent solutions of the reactor.

Compound	Step	Affluent concentration (mg/L)	Effluent concentration (mg/L)
HMF	I	–	–
	II	<LOD	<LOD
	III	27.2	<LOD
	IV	72.3	<LOD
	V	124.6	25.5
	VI	222.7	75.5
FF	I	–	–
	II	<LOD	<LOD
	III	16.2	<LOD
	IV	70.6	<LOD
	V	114.9	13.2
	VI	194.3	39.8

<LOD: lower than the limit of detection.

UASB. In period VI, these values were 33.9% and 20.5% for HMF and FF, respectively. According to the results obtained, the concentrations of furans (FF + HMF) in the effluent of the reactor in period VI (115.3 mg/L), in which the highest OLR was assessed (4.8 g COD/L·d), probably led to the inhibition of metabolic activity of the microorganism community. This was supported by the lowest values of methane yield (29.2 mL CH<sub>4</sub>/g COD) and highest VFA concentration (0.80 g COD/L) in this period (Table 3). Although it is known that furans may inhibit the metabolic activity of anaerobic microorganisms, a threshold concentration is not yet clear (Monlau et al., 2014). This is mainly due to the different operation conditions and bagasse characteristics in each study. Moreover, there are no studies on the anaerobic digestion of sugarcane bagasse hydrolysate so far. For bagasse fermentation for bioethanol production, 5 g furans/L decreased the ethanol yield by 13% and growth rates dropped to zero (Palmqvist, 1998). One way to avert the high HMF and FF in the influent would be to dilute the hemicellulose hydrolysate with other sugarcane production wastewaters, such as vinasse. Another possibility would be to perform the anaerobic digestion in two stages (acidogenesis followed by methanogenesis), which has shown to enhance methane production from hemicellulose hydrolysates (Baêta et al., 2016b).

## 4. Conclusions

The present study aimed at evaluating the anaerobic digestion of the hemicellulose hydrolysate produced after thermal pretreatment of sugarcane bagasse in a UASB reactor. The results obtained indicated that a lower dilution of sugarcane bagasse hydrolysate in the feed resulted in better process performance. In fact, better process performance was attained with 100% of hemicellulose hydrolysate in the inlet feed. Furthermore, when increasing the organic loading rate (OLR) from 1.2 to 2.4 g COD/L·d, organic matter removal increased (85.7% COD removal). During this period, the methane yield estimated by the COD removal would be 270 L CH<sub>4</sub>/kg COD. Overall, the findings indicate that the application of anaerobic technology for degradation of sugarcane bagasse hydrolysate and generation of biogas in UASB reactor was feasible. This process could contribute to the generation of energy in a bioethanol industry, as this technology expands the possibilities of full use of sugarcane bagasse in a lignocellulosic biorefinery concept.

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