BIOHYDROGEN PRODUCTION FROM CASSAVA WASTEWATER AND DOMESTIC SEWAGE IN AN ANAEROBIC FLUIDIZED BED REACTOR

L.M.B. VILELA, W.V. MACÊDO and E.L.C. AMORIM

Technology Center, Federal University of Alagoas; Av. Lourival Melo Mota, s/n; Cidade Universitária; 57072-900; Maceió/AL; Brazil; eduardo.lucena@ctec.ufal.br

***Abstract*— This study addresses hydrogen production in two anaerobic fluidized bed reactors (AFBRs) that were fed with cassava wastewater and domestic sewage. The reactors were operated at hydraulic retention times of 4-3 h (AFBR 1) and 4 h (AFBR 2). The maximum hydrogen yields (HY) and the maximum hydrogen production rate (HPR) were, respectively, 0.93 mol H_2.mol glucose^{-1} and 1.69 L.h^{-1}.L^{-1} (AFBR 1) for the AFBR 1, and 2.03 mol H_2.mol glucose^{-1} and 0.95 L.h^{-1}.L^{-1} for AFBR 2. The hydrogen content ranged from 2.25 to 23.25% (AFBR 1) and from 7 to 50.75% (AFBR 2). The hydrogen content increased significantly when the amount of domestic sewage decreased, that is, higher concentrations of cassava wastewater in the substrate favors the hydrogen production. Acetic acid was the predominant metabolite that was soluble in both reactors, indicating that the hydrogen production rate and the hydrogen yield made the acetic acid pathway predominant. The association between cassava wastewater and domestic sewage favored biohydrogen production satisfactorily.**

***Keywords*— Biohydrogen production; cassava wastewater; domestic sewage; anaerobic fluidized bed reactor; ethanol; acetic acid.**

I. INTRODUCTION

Alternative energy sources have received considerable attention from investigators in recent decades, given the scarcity of fossil fuels and the negative environmental impacts associated with their use. Among these sources, biofuels are noted for being eco-efficient, biodegradable and produced from a variety of renewable resources (Puppan, 2002).

Among the different biofuels, hydrogen is considered to be an important candidate because it has a high energy yield (122 kJ.g^{-1}), which is 2.75 times greater than hydrocarbon fuels, and its combustion produces water (Kapdan and Kargi, 2006). Furthermore, it can be produced from a wide variety of substrates such as wastewater from organic waste.

Several methods can be used for hydrogen production. Alternative methods for biological hydrogen generation include bio-photolysis, photo fermentation and organic production from the anaerobic fermentation of organic waste (Kothari *et al*., 2012).

Cassava wastewater is a liquid that results from manioc flour processing. The potential for pollution from cassava wastewater comes from its high concentration of organic matter. This effluent is rich in carbohydrates, with 20-50 g.L^{-1} of chemical oxygen demand (COD), which makes it an important pollutant (Oliveira *et al.*, 2001). Another relevant characteristic that favors the strong negative environmental impact of cassava wastewater is the presence of cyanide at concentrations ranging from 75 to 1000 mg cyanide per kg (Piva, 1987). According to Cappelletti *et al.* (2011), Brazil is one of the largest global producers of cassava, and its production of cassava flour and starch gives rise to about 7m³ wastewater per kg of processed cassava roots.

Glucose is also a substrate that is commonly used as the primary carbon source in fermentation, and it has been used in other research on complex substrates (Prakasram *et al*., 2009) to optimize hydrogen production. An important consideration in hydrogen production from dark fermentation is the fermentation type. Another wastewater that is produced on a large scale by the global population and causes concern because of its high pollution load is domestic sewage. According to Metcalf and Eddy (2003), the average value of COD for untreated domestic sewage is 500 mg.L^{-1} with concentration variations of 250-1000 mg COD.L^{-1}.

Therefore, there is a need to use domestic sewage and industrial effluents as substrates for different forms of biofuel generation. The anaerobic fluidized bed reactor (AFBR) is notable among treatment systems, and it has been used to a significant extent. An AFBR with an attached biofilm has been widely used as a biological treatment system for wastewater with a high efficiency and low hydraulic retention time (HRT) (Zhang *et al*., 2007). Although AFBRs possess favorable characteristics for the production of gaseous products such as hydrogen, they have been less frequently used for hydrogen dark fermentation. AFBR studies have demonstrated conversion efficiencies between 70 and 99.5% when producing hydrogen (Zhang *et al*., 2007; Zhu *et al*., 2009; Barros *et al*., 2010; Reis *et al.*, 2011; Amorim *et al.*, 2012).

Thus, the aim of this study was to use cassava wastewater in association with domestic sewage, which was used as a diluent for hydrogen production in an anaerobic fluidized bed reactor operated under a progressive increase in the organic loading rate.

II. METHODS

A. Inoculum and fermentation medium

The inoculum used in this experiment was obtained from a facultative pond sludge derived from swine

wastewater treatment. The sludge was subjected to a treatment as Maintinguer *et al*. (2008). The medium used for H_2 fermentation contained cassava wastewater (CW), domestic sewage (DS) and the following nutrients (mg.L-1): CH_4N_2O, 125; $NiSO_4{\cdot}6H_2O$, 1; $FeSO_4{\cdot}7H_2O$, 5; $FeCl_3{\cdot}6H_2O$, 0.5; $CaCl_2{\cdot}6H_2O$, 47; $CoCl_2{\cdot}2H_2O$, 0.08; SeO_2, 0.07; KH_2PO_4, 85; $KHPO_4$, 21.7; and $Na_2HPO_4{\cdot}2H_2O$, 33.4 (Amorim *et al*., 2012).

The cassava wastewater used was produced by a local factory in Alagoas, Brazil. The primary characteristics of the cassava wastewater were as follows: pH 4.31, total solids 8.39 $g.L^{-1}$, volatile solids 4.52 $g.L^{-1}$, total carbohydrates 25.61 $g.L^{-1}$, chemical oxygen demand (COD) 43.54 g $COD.L^{-1}$, and total nitrogen 2.62 $g.L^{-1}$.

It was used the cassava wastewater with a COD of 4 g $COD.L^{-1}$ because this concentration could induce the production of acetic acid and butyric acid, favoring hydrogen production according to Amorim *et al.* (2012).

The primary characteristics of the domestic sewage were as follows: pH 7.06, total solids 0.132 $g.L^{-1}$, volatile solids 0.118 $g.L^{-1}$, chemical oxygen demand (COD) 0.148 g $COD.L^{-1}$, ammonia nitrogen 0.034 $g.L^{-1}$, and chloride 0.074 $g.L^{-1}$.

B. Support material

Particles of expanded clay (2.8–3.35mm) were used as support material for biomass immobilization. The material had an apparent density of approximately 1.06 g/cm^3 and a porosity of 23% (Amorim *et al.*, 2012).

C. Anaerobic fluidized bed reactors (AFBRs)

The primary body of AFBR 1 was an acrylic tubular section with a 5.3 cm internal diameter and a height of 190 cm. The reactor had a total volume of 4,192 cm^3, a work volume of 3,200 cm^3 and the height of the static bed of the support material was 90 cm (1.230 kg of expanded clay) (Amorim *et al.*, 2009). The primary body of AFBR 2 was an acrylic tubular section with a 4.0 cm internal diameter and a height of 90 cm. The reactor had a total volume of 1,200 cm^3 and a work volume of 900 cm^3, and the height of the static bed of support material was 30 cm (400 g of expanded clay).

D. AFBR operating conditions

The AFBRs were fed with cassava wastewater, domestic sewage and 10% v/v solution of heat-treated sludge. The operation temperature was ambient (31 ± 1.5 °C). For the AFBR 1 system, the total liquid flow rate Q was maintained at 128 $L.h^{-1}$ (bed expansion = 30%) (Amorim *et al*, 2009). For the AFBR 2 system, the total liquid flow rate Q was maintained at 73 $L.h^{-1}$ (bed expansion = 30%). This flow rate produced a superficial velocity that was 1.30 times greater than the minimum fluidization velocity. The bioreactors were initially operated in batch mode for 48 h to activate the H_2-producing sludge, after which it was switched to continuous mode with a designated hydraulic retention time (HRT) of 4 h.

After reaching steady-state operation based on a constant volumetric H_2-production rate within a 5-10% variation, the HRT was decreased from 4 h to 3 h (only in AFBR 1 in phase F75M3h). The AFBR 1 and AFBR 2 reactors were operated for 72 and 55 days, respectively. The composition of the soluble metabolites (volatile fatty acids and alcohol) produced during H_2 fermentation was monitored as a function of time. The AFBR 1 and AFBR 2 reactors were operated at effluent pH values of 5.01±0.41 and 4.19±0.33, respectively. A gas meter (Type TG1; Ritter Inc., Germany) was used to measure the amount of gaseous product generation (Amorim *et al*, 2009).

Table 1. Operational phases of AFBR 1

Phases	HRT (h)	Vol. CW (%)	Vol. DS (%)	Glucose ($g.L^{-1}$)	Vol. Water (%)	COD_{inf} ($mg.L^{-1}$)	Applied OLR ($kg\ COD.m^{-3}.d^{-1}$)
P10M3G	4	10	90	3	0	4638	23
P40M2G	4	40	60	2	0	7892	36
P50M1G	4	50	50	1	0	8266	42
P50M	4	50	50	0	0	7200	36
P75M	4	75	25	0	0	10800	49
P75M3h	3	75	0	0	25	10800	44

CW: Cassava wastewater; DS: Domestic sewage.

Table 2. Operational phases of AFBR 2

Phases	HRT (h)	Vol. CW (%)	Vol. DS (%)	Glucose ($g.L^{-1}$)	COD_{inf} ($mg.L^{-1}$)	Applied OLR ($kg\ COD.m^{-3}.d^{-1}$)
P10M10G	4	10	90	10	12100	24
P25M5G	4	25	75	5	8930	37
P50M2G	4	40	60	4	9332	49
P100M	4	75	25	0	14400	71

This study was divided into six phases in AFBR 1 and five phases in AFBR 2. The operational phases of the reactors were named according to the percentage of cassava wastewater, domestic sewage and glucose at each stage. For example, the P10M3G phase contained 10% cassava wastewater, 3 g $glucose.L^{-1}$ and 90% domestic sewage. The P75M3h phase (of AFBR 1) contained 75% cassava wastewater, 25% water and HRT at 3h. The phases of AFBRs 1 and 2 are shown in Tables 1 and 2.

E. Chemical analysis

The volatile fatty acid (VFA), alcohol concentrations, and the biogas hydrogen content were measured with a gas chromatograph equipped with a flame-ionization detector (FID) and a Supelcowax 10 column (30 m high × 0.25 mm i.d. × 0.25 µm film thickness), a thermal conductivity detector (TCD), argon as the carrier gas, and the column was packed with a Supelco Carboxen 1010 Plot (30 m×0.53 mm i.d.) (Maintinguer *et al*., 2008).

The pH, COD and carbohydrate concentrations were measured according to the procedures described in the Standard Methods (APHA, 1998).

III. RESULTS AND DISCUSSION

A. The effects of the wastewater composition on hydrogen production

Figure 1 shows the variations in the hydrogen production rate (HPR), hydrogen yield (HY) and organic loading rate (OLR) for each phase in AFBR 1.

This study showed an increase in the HY from 0.56 to 0.93 mol $H_2.mol\ glucose^{-1}$ when the OLR was increased from 36 kg $COD.m^{-3}.d^{-1}$ (P40M2G) to 42 kg $COD.m^{-3}.d^{-1}$ (P50M1G). However, this behavior was not observed in the other phases when the OLR also in-

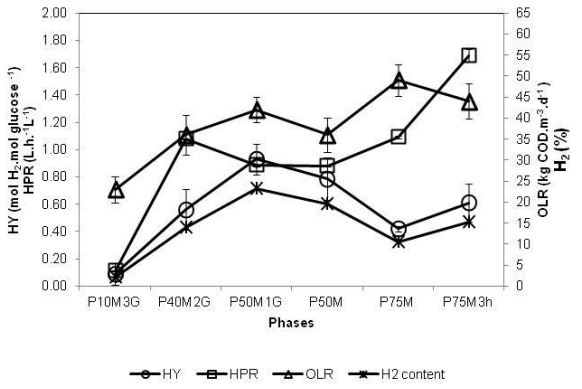

Figure 1. HPR, HY, and OLD in AFBR 1.

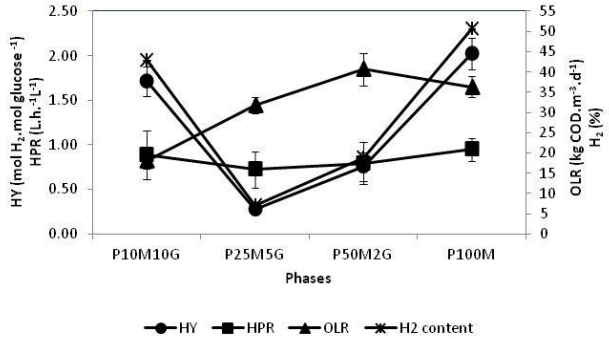

Figure 2. HPR, HY, and OLR in AFBR 2.

creased. The HPR also behaved in the same way as in previous studies, in that it was observed to increase when the OLR was increased from 36 kg COD.m^{-3}.d^{-1} (1.07 L.h^{-1}.L^{-1}) to 44 kg COD.m^{-3}.d^{-1} (1.69 L.h^{-1}.L^{-1}).

In AFBR 1, the hydrogen content ranged from 2.25% (P10M3G) to 23.25% (P50M1G). Similar results were described by Rosa *et al*. (2014), who obtained hydrogen contents between 16.25% and 26.25% from glucose fermentation in an anaerobic fluidized bed (AFBRs), hydrogen production rates of 0.96 L.h^{-1}.L^{-1} (AFBR 1) and 1.92 L.h^{-1}.L^{-1} (AFBR 2) and hydrogen yields from 0.24 mol H_2.mol glucose^{-1} (AFBR 1) and 0.44 mol H_2.mol glucose^{-1} (AFBR 2).

Figure 2 shows the variation in the HPR, HY and OLR for each AFBR 2 phase. According to Fig. 2, the HPR remained almost constant (0.85-0.95 L.h^{-1}.L^{-1}) with the increasing OLR.

The HY decreased when the OLR was increased from 18.04 kg COD.m^{-3}.d^{-1}, phase P10M10G, (1.71 mol H_2.mol glucose^{-1}) to 31.86 kg COD.m^{-3}.d^{-1}, phase P25M5G, (0.28 mol H_2.mol glucose^{-1}). An increased HY was subsequently observed when the OLR was increased to 36.35 kg COD.m^{-3}.d^{-1} and phase P100M (2.03 mol H_2.mol glucose^{-1}).

According to other studies, OLR can interfere with the hydrogen yield, since studies such as those by van Ginkel and Logan (2005), Amorim *et al*. (2009) and Barros *et al*. (2010) found a linear correlation between the HY and OLR. Van Ginkel *et al*. (2001) emphasized that the hydrogen yield production is essentially a function of the capacity of microorganisms to produce H2 from organic material. These results suggested that changes in the microorganismal metabolism occurred when the OLR was modified.

Ren *et al*. (2006) observed a decline in the hydrogen yield when the OLR reached 68.21 kg COD.m^{-3}.d^{-1} because of the accumulation of volatile fatty acids in the reactor. All these studies used synthetic substrate to feed the reactor, thus providing a better handle on the applied OLR. However, the current research involved a real substrate (cassava wastewater), which exhibited variations in the COD and OLR during reactor operations.

In AFBR 2, the hydrogen content ranged from 7% (P25M5G) to 50.75% (P100M). These results corroborate those of Santos *et al*. (2014), who obtained similar results when operating an AFBR with a mixture of sugar cane vinasse and glucose, with different HRTs (8, 6, 4, 2 and 1 h); however, an HRT of 4 h yielded a hydrogen content that reached 50.8% when using a ratio of 33% glucose to 67% sugar cane vinasse.

Reis and Silva (2011) used two AFBRs with synthetic wastewater containing glucose as the primary carbon source (5000 mg.L^{-1}) at different fluidization velocities and different HRTs; an HRT of 6 h had a hydrogen content of 43.03% and 40.60% in the reactors with fluidization velocities of 1.24 and 1.88 cm.s-1, respectively. When the HRT was changed to 2 h, the hydrogen content decreased to 36.04% (to a reactor with 1.24 cm.s^{-1}).

B. Soluble product composition

Hydrogen production is usually accompanied by the formation of these metabolites such as VFAs (volatile fatty acids) and a solvent (eg ethanol). Therefore, the distribution of VFA concentrations, solvents and their fractions are useful indicators for monitoring hydrogen and solvent production (Sivagurunathan *et al*., 2014).

The presence of acetic, propionic, and butyric acids in addition to ethanol was observed during reactor operations. Table 3 shows the distribution of soluble microbial products (SMP) for reactors.

Acetic acid was the most common SMP during the experiment in both reactors (9.79 to 17.72 mM for AFBR 1 and 15.22 to 18.75 mM for AFBR 2).

Acetic acid production was observed during all phases in both reactors; however, this compound was more predominant in the P75M phase of the AFBR 1 (42.8% SMP). In AFBR 2, this predominance occurred during the P10M10G phase (46.5% SMP).

The production of acetic acid indicates that a greater quantity of SMPs favor hydrogen production because hydrogen production occurs when these products are generated (Eq. 1).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$
$$G = -215.69 kJ / mol$$

Cappelletti *et al*. (2011) also used cassava as a substrate to evaluate the effects of varying cassava wastewater concentrations. The authors conducted a series of batch tests at concentrations of 30, 15, 10, 7.5 and 5 g COD.L^{-1}. Acetic acid concentrations were obtained at 0.01 g.L^{-1} (0.17 mM) with 5 g COD.L^{-1}and 0.63 g.L^{-1} (10.5 mM) with 10 g COD.L^{-1}.

Table 3. Producing soluble metabolites for hydrogen production in the reactors.

Reactors	Phases	HAc (mM) SMP (%)	HBu (mM) SMP (%)	HPr (mM) SMP (%)	EtOH (mM) SMP (%)	HAc/HBu	pH
AFBR 1	P10M3G	9.79±0.89 40.4±0.52	3.49±0.32 14.4±0.02	5.17±1.12 21.4±0.21	5.30±1.68 21.9±0.56	2.8±0.46	4.47±0.33
	P40M2G	17.02±1.76 51.0±2.61	5.34±0.39 16.0±0.90	6.08±0.60 18.2±0.77	4.49±1.71 13.3±4.23	3.2±0.11	5.47±0.17
	P50M1G	16.59±0.67 51.4±0.21	4.16±0.24 12.9±0.09	5.81±1.43 18.0±0.84	5.25±1.43 16.3±0.24	4.0±0.56	5.52±0.11
	P50M	14.92±0.56 45.1±4.89	5.23±0.29 15.8±1.98	5.14±1.08 15.5±1.56	7.35±1.56 22.2±0.43	2.9±0.24	4.85±0.24
	P75M	17.72±0.83 42.8±1.57	7.50±0.80 18.2±2.12	6.57±0.47 15.9±0.98	9.04±0.04 21.9±0.32	2.4±0.36	4.86±0.59
	P75M3h	15.79±0.31 45.5±2.10	4.63±0.19 13.3±0.91	6.49±0.84 18.6±1.92	7.42±0.58 21.3±1.11	3.4±0.08	4.87±0.21
AFBR 2	P10M10G	18.75±4.59 46.5±4.8	5.02±0.60 12.6±0.0	11.99±1.33 30.0±1.0	3.78±0.80 9.69±3.38	3.7±0.5	4.01±0.27
	P25M5G	15.22±1.87 48.9±4.0	4.11±0.21 13.2±4.0	9.59±0.41 30.8±5.0	1.74±0.40 5.6±0.02	3.7±0.4	3.87±0.20
	P50M2G	16.32±2.08 47.9±0.4	4.73±0.67 13.9±0.4	10.11±1.83 29.6±0.0	2.43±0.09 7.2±0.79	3.5±0.1	4.00±0.24
	P100M	18.33±1.34 37.9±10.4	5.92±1.63 11.8±0.0	10.52±1.24 21.6±0.0	15.53±4.36 27.8±0.17	3.2±0.6	4.47±0.13

Notes: HAc = acetate, HBu = butyrate, HPr = propionate, EtOH = ethanol, TVFA = total volatile fatty acids, TVFA = HAc + HBu + HPr, SMP = TVFA + EtOH, HAc/SMP = molar acetate-to-SMP ratio, HBu/SMP = molar butyrate-to-SMP ratio, HPr/SMP = molar propionate-to-SMP ratio, and EtOH/SMP = molar ethanol-to-SMP ratio.

Ethanol was also an abundant SMP, and its concentration varied from 4.49 to 9.04 mM (AFBR 1) and 1.74 mM to 15.53 mM (AFBR 2). The presence of ethanol in all phases may be indicative of hydrogen production throughout the metabolic pathway that produces ethanol concomitant to hydrogen as reported by Zhu *et al*., (2009).

Rosa *et al*. (2014) found that ethanol fermentation was dominant in his study, and he employed a co-fermentation of a cheese whey and glucose mixture. The authors noted that it was possible to achieve the simultaneous production of hydrogen and ethanol at high yields of 1.7 mmol H_2 g^{-1} COD and 3.45 mmol EtOH g^{-1} COD, respectively.

Wang *et al*. (2009) reported that ethanol was the predominant soluble metabolite in batch tests when using glucose as the substrate. When the ammonia concentrations were 0.02 and 0.2 g $N.L^{-1}$, the ethanol contents were 88.2% and 87.1%, respectively.

Salerno *et al*. (2006) pointed out that the hydrogen yield in the batch reactor is not affected by an increased ammonia concentration, but the hydrogen production rate can be reduced.

This finding was also observed during the phases of both reactors, because the hydrogen production rate was below 1 $L.h^{-1}.L^{-1}$, with the exception of the F75M3h phase in AFBR 1 (1.69 $L.h^{-1}.L^{-1}$).

Sterling *et al*. (2001) found that adding 600, 1500 and 3000 mg $N.L^{-1}$ initially resulted in the reduction of hydrogen in the biogas. After 24 h, the hydrogen concetration increased in the 600 to 1500 mg $N.L^{-1}$ concentrations, but the hydrogen production was inhibited when 3000 mg $N.L^{-1}$ was added.

The third most abundant SMP was propionic acid. Its concentration ranged from 5.14 to 6.57 mM (AFBR 1) and 9.36 to 11.99 mM (AFBR 2). The absence of propionate during reactor operation is associated with improved hydrogen yields because propionate production consumes 2 mol of hydrogen for every 2 mol of propionate that is produced.

There was a concomitant production of acetic acid and propionic acid during all phases in both reactors, but principally in AFBR 2. This finding may have adversely affected the hydrogen production. In AFBR 2, the propionic acid pathway may have prevailed over the acetic acid pathway, leaving only hydrogen production by pathways for ethanol and butyric acid, respectively.
The largest production of butyric acid in the AFBR 1 was in the F75M phase (7.50 mM), corresponding to 18.2% of the SMP. AFBR 2 exhibited a higher production of butyric acid (11.8% of the SMP) in the P100M phase (5.92 mM).

Kim *et al*. (2004) employed a semi-continuous reactor, and they concluded that the production of butyric acid is favored when the pH is about 4.5-5.0.

In AFBR 1, the pH at the maximum production phase was 4.86 (7.50 mM) and in AFBR 2 it was 4.47 (5.92 mM), which corresponds to the findings presented above.

Other studies in which conditions similar to the ones in this experiment also indicated similarities in relation to the SMPs. However, the SMP distribution does not

always behave similarly in these studies (Barros *et al*., 2010; Reis *et al*., 2011; Amorim *et al*., 2009; Shida *et al*., 2009).

Shida *et al*. (2009) used glucose as a substrate to achieve an acetic acid production of 0.30 $g.L^{-1}$ to 0.49 $g.L^{-1}$ (5 to 8.17 mM) by reducing the 8 h HRT to 2 h. Butyric acid production reached its lowest value (0.35 $g.L^{-1}$ or 3.98 mM) at an HRT of 8 hours and its highest value (0.55 $g.L^{-1}$ or 6.25 mM) at an HRT of 2 h. The highest ethanol yield (0.097 $g.L^{-1}$ or 2.11 mM) occurred at an HRT of 6 h.

Amorim *et al*. (2009) used glucose as a substrate, and they also observed that the production of acetic acid varied between 3.76 mm (HRT 6 h) and 8.87 mM (HRT 2 h). The authors also obtained a butyric acid production of 4.66 mM (HRT 8 h) and 6.60 mM (HRT 2 h). Ethanol production was about 1.16 mM (HRT 1 h) and 2.14 mM (HRT 4 h). However, propionic acid had not been observed in any of the stages, which can be attributed to the specific operating conditions of the reactor and the fact that the authors used a synthetic substrate (glucose) under better controlled conditions.

In hydrodynamic conditions similar to those employed in this study (with an upflow velocity of 1.24 $cm.s^{-1}$), Reis and Silva (2011) observed that acetic and butyric acid production ranged between 0.27 and 0.78 $g.L^{-1}$ (4.5 to 13 mM) and between 0.084 and 0.40 $g.L^{-1}$ (4.55 and 0.95 mM), respectively. The lowest and highest values corresponded to HRTs of 1 h and 4 h, respectively. The increased production of propionic acid (0.6 $g.L^{-1}$ or 8.11 mM) occurred at an HRT of 4 h.

In this study, heat treating the inoculum may have contributed significantly to the selection of hydrogen-producing bacteria and to the inactivation of hydrogen consumers and harvest endospore-forming anaerobic bacteria such as Clostridium sp. and Bacillus sp. (Maintinguer *et al*., 2008), which positively affected hydrogen production.

These results showed that the metabolite concentrations are influenced by changes in the percentage of domestic sewage that are mixed into the wastewater. However, the differences found in this study and others demonstrate the need to control the occurrence of acidogenic populations and to prevent contamination from other non-hydrogen producing microorganisms, because they may lead to competition for the substrate in the system.

C. COD removal and carbon balance

In AFBR 1, the influent COD ranged from 7,200 mg $COD.L^{-1}$ (F50M) to 10,800 mg $COD.L^{-1}$ (F75M3h). The effluent COD varied from 3,942.3 mg $COD.L^{-1}$ (F10M3G) to 6,674 mg $COD.L^{-1}$ (F75M). The COD removal efficiency varied between 9.13 and 39.71%.

In AFBR 2, the influent COD ranged from 8,930 mg $COD.L^{-1}$ (F25M5G) to 14,400 mg $COD.L^{-1}$ (F100M). The effluent COD varied from 5,383.3 mg $COD.L^{-1}$ (F25M5G) to 13,085 mg $COD.L^{-1}$ (F100M). The COD removal efficiency varied between 15 and 38.39%.

Table 4. Theoretical COD values of soluble metabolites, biomass COD, and effluent COD as measured in the AFBRs.

Reactors	Phases	COD_t Acetic acid	COD_t Butyric acid	COD_t Propionic acid	COD_t Ethanol	COD_t Glucose	COD_t Biomass	COD_t Total	COD_m Effluent	COD_m - COD_t Total
AFBR 1	P10M 3G	626.77	558.47	579.47	508.35	1175.95	429.76	3878.77	3942.30	63.53
	P40M 2G	1089.76	854.43	681.11	430.94	807.69	408.27	4272.20	6075.30	1803.10
	P50M 1G	1061.77	665.47	651.40	503.77	2299.34	494.22	5675.98	5721.13	45.16
	P50M	955.13	837.11	575.83	705.25	860.98	496.91	4431.22	4674.00	242.78
	P75M	1134.31	1200.53	736.42	867.97	1318.46	435.13	5692.83	6674.41	981.58
	P75M 3h	1011.16	740.09	684.45	712.58	1536.26	385.44	5069.99	6653.78	1583.78
AFBR 2	F10M 10G	1200.65	803.22	1343.75	363.13	5483.52	439.31	439.31	10126.96	493.38
	F25M 5G	974.38	658.06	1074.39	167.41	2136.50	298.15	298.15	5383.58	74.68
	F50M 2G	1044.89	756.39	1133.07	233.57	1995.30	623.15	623.15	7830.55	2044.18
	F100M	1173.60	946.32	1178.15	1490.83	2898.04	648.67	648.67	13085.61	4750.01

Notes: t is calculated from the measurements of glucose, VFA, ethanol, and total suspended solids (TSS); m is determined in the COD analysis.

The effluent COD of the AFBRs consisted of unconsumed glucose, soluble metabolites, e.g., organic acids, solvents, and other intermediates, and the biomass detached from the support medium; each of their partial contributions is shown in Table 4.

The total theoretical COD is calculated from the sum of the theoretical COD of biomass, the theoretical COD of remaining glucose, and the theoretical COD of metabolites produced during the experiment (Eq. 2).

$$COC_{TotalTheoretical} = COC_{GlucoseTheoretical} + COC_{BiomassTheoretical} + COC_{MetabolitesTheoretical} \quad (2)$$

The smaller and larger differences obtained by Shida *et al*. (2009) that were measured between the effluent COD and total theoretical COD using glucose as the substrate with 2000 mg $COD.L^{-1}$ were 248.31 mg $COD.L^{-1}$ and 764.20 mg $COD.L^{-1}$, respectively.

Amorim *et al*. (2009) employed 4,000 mg glucose L^{-1}, and they found differences between 25 mg $COD.L^{-1}$ and 1,259 mg $COD.L^{-1}$. Reis and Silva (2011) used 5,000 mg glucose L^{-1}because the substrate showed a greater difference (1,602 mg $COD.L^{-1}$) when applying fluid dynamic conditions similar to those employed in this study (with fluidization velocities of 1.24 $cm.s^{-1}$). The authors of these studies attribute this difference to the likely presence of other metabolites to be detected by performing chromatographic analyses, such as lactic acid and formic acid (Reis *et al*., 2011; Amorim *et al*., 2009; Shida *et al*., 2009).

IV. CONCLUSIONS

Hydrogen production in anaerobic fluidized bed reactors also occurred concomitantly with ethanol production.

The hydrogen content ranged from 2.25 to 23.25% (AFBR 1) and from 7 to 50.75% (AFBR 2). The presence of methane gas was detected in all phases of the study. The hydrogen content increased significantly when the amount of domestic sewage decreased, that is, when the increased amount of effluent was present in the carbohydrate mixture.

Acetic acid was the predominant metabolite that was soluble in both reactors, indicating that the hydrogen production rate and the hydrogen yield made the acetic acid pathway predominant.

The association between cassava wastewater and domestic sewage favored biohydrogen production in a satisfactory manner, with the combination of this

wastewater with glucose supplementation, which is essential for hydrogen production.

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