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BIOHYDROGEN PRODUCTION FROM CASSAVA WASTEWATER IN AN ANAEROBIC FLUIDIZED BED REACTOR

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Abstract - The effect of hydraulic retention time (HRT) and organic loading rate (OLR) on biological hydrogen production was assessed using an anaerobic fluidized bed reactor fed with cassava wastewater. The HRT of this reactor ranged from 8 to 1 h (28 to 161 kg COD/m^3-d). The inoculum was obtained from a facultative pond sludge derived from swine wastewater treatment. The effluent pH was approximately 5.00, while the influent chemical oxygen demand (COD) measured 4000 mg COD/L. The hydrogen yield production increased from 0.13 to 1.91 mol H_2/mol glucose as the HRT decreased from 8 to 2 h. The hydrogen production rate significantly increased from 0.20 to 2.04 L/h/L when the HRT decreased from 8 to 1 h. The main soluble metabolites were ethanol (1.87-100%), acetic acid (0.00-84.80%), butyric acid (0.00-66.78%) and propionic acid (0.00-50.14%). Overall, we conclude that the best hydrogen yield production was obtained at an HRT of 2 h.

Keywords: Anaerobic fluidized bed reactor; Dark fermentation; Cassava wastewater; Hydrogen production; Organic loading rate.

INTRODUCTION

Global energy demand is rising to meet societies' needs in the modern world, with oil and its derivatives representing the most frequently used energy sources today. However, the overuse of fossil fuels could result in the depletion of this energy source and negative environmental impacts caused by gases generated during its combustion (Kapdan and Kargi, 2006).

Due to pressing environmental issues and increasing energy demand, the search for alternative – i.e., renewable and less polluting – energy sources has gained prominence. Hydrogen is an alternative source of energy that could replace petroleum-based fuels because water and energy are the only byproducts of its combustion with oxygen (Van Ginkel *et al*., 2001). Moreover, hydrogen is the most abundant chemical element in the universe, comprising almost three-quarters of the universe's entire mass (Das and Verziroglu, 2001). Although abundant, hydrogen is often found combined with other elements in the natural environment (Das and Verziroglu, 2001), and a range of different processes are required to isolate it.

The main methods of producing hydrogen employ fossil fuels, water and biological processes. However, hydrogen production from fossil fuels by steam reforming, or from water by electrolysis and thermochemical decomposition, may not be environmentally friendly and can also be uneconomical (Amorim *et al*., 2009).

The fermentative biological process is a means to produce hydrogen sustainably because it can use various types of carbohydrate-rich industrial and domestic waste as its substrate, thereby mitigating problems caused by the inappropriate disposal of

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*To whom correspondence should be addressed these materials (Reis and Silva, 2011). The use of agro-industrial residues in biological hydrogen production has also been investigated for the same reason (Luo *et al*., 2010a; Cappelletti *et al*., 2011).

Cassava is a typical Brazilian agricultural product that is widely used in the production of flour and starch (Cappelletti *et al*., 2011; Wosiacki and Cereda, 2002). In some of Brazil's northeastern states, cassava is cultivated in small plantations by traditional farming means. Cassava is processed either by hand in small flourmills and cake factories or by mechanized units. During the production of flour, cassava undergoes a pressing process, and the wastewater derived from the pressing is called *cassava wastewater*.

Cassava wastewater has high energy content – approximately 20-40 g of carbohydrate per liter – with high potential for pollution if improperly discarded into the environment. However, the energy potential of cassava wastewater makes it very attractive as a substrate for hydrogen production, and its use in this process would reduce the environmental impact of disposing it without prior treatment (Cappelletti *et al*., 2011).

Some studies using cassava wastewater as substrate have already been conducted, and their results indicate that its use in hydrogen production is economically viable (Luo *et al*., 2010a; Cappelletti *et al*., 2011; Wosiacki and Cereda, 2002; Cheng *et al*., 2011; O-Thong *et al*., 2011; Luo *et al*., 2010b). However, these studies all used batch reactors in their experiments. Working from these researchers' achievements, the main purpose of this study is to produce hydrogen from cassava wastewater in an anaerobic fluidized bed reactor (AFBR). The employment of the AFBR is justified by its many positive features, such as the accumulation of large amounts of biomass attached to the support medium, high organic loading rates (OLR), low hydraulic retention times (HRT), and good mixing characteristics, which optimize mass transfer between the substrate and microorganisms. In addition, AFBR allows high-speed stirring in the liquid phase, which favors hydrogen release at this stage (Wu *et al*., 2003; Lin *et al*., 2006; Zhang *et al*., 2007).

Hydrogen production in an AFBR has been studied by some researchers, who were successful in evaluating this reactor's usefulness (Amorim *et al*., 2009; Reis and Silva, 2011; Shida *et al*., 2009; Barros *et al*., 2010). However, the production of bio-hydrogen from actual substrate, such as cassava wastewater, in an AFBR has not yet been evaluated. These prior studies concluded that some aspects influencing hydrogen production should be further explored, especially the use of actual substrate. Therefore, the aim of this investigation was to study an AFBR using cassava wastewater for hydrogen production.

EXPERIMENTAL PROCEDURE

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 10 min of heat treatment at 90 ºC to inactivate the H_2 consumers and harvest endospore-forming anaerobic bacteria (Maintinguer *et al*., 2008).

The medium used for H_2 fermentation contained cassava wastewater and the following nutrients (mg/L): 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*., 2009).

The cassava wastewater used here was produced by a manioc flour factory in Taquarana, Alagoas, Brazil. The main characteristics of the cassava wastewater were as follows: pH 5.53, total solids 4.45 g/L, volatile solids 3.4 g/L, total carbohydrate 37.54 g/L, chemical oxygen demand (COD) 66.19 g COD/L, total nitrogen 1.26 g/L. After it had been collected, the cassava wastewater was stored at 5 °C before usage. We chose to use the cassava wastewater with COD of 4 g COD/L, because, according to Amorim *et al*. (2012), this concentration might favor the production of acetic acid and butyric acid (route that favors hydrogen production), whereas higher substrate concentrations would favor the production of solvents. Furthermore, Cappelletti *et al*. (2011) concluded that a lower cassava wastewater COD produces a large amount of hydrogen.

Support Material

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

Reactor

Figure 1 shows a schematic of the AFBR system used in this study. The main body of the reactor was an acrylic tubular section with a 5.3 cm internal diameter and a height of 190 cm. The reactor has a total volume of 4,192 cm^3, and the height of the static bed of support material was 90 cm (2,104 g of expanded clay) (Amorim *et al*., 2009).

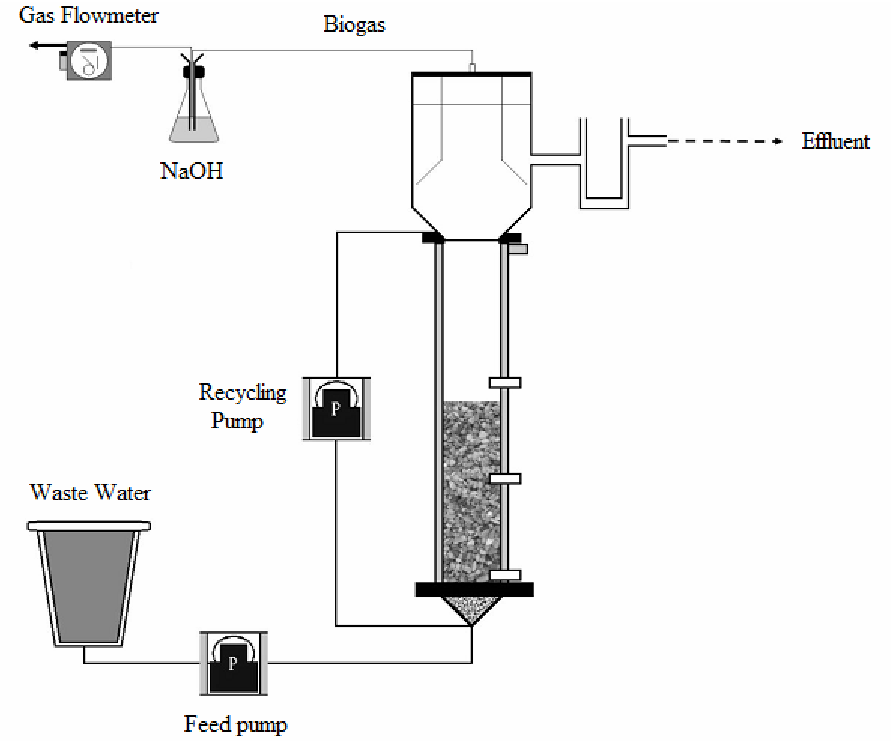

Figure 1: Schematic of the anaerobic fluidized bed reactor.

Operating Conditions of the AFBR

The AFBR was fed with cassava wastewater at 4,000 mg COD/L and 10% v/v of heat-treated sludge. The operation temperature was ambient (28 ± 2 °C). For the AFBR system, the total liquid flow rate, Q, was controlled at 128 L/h (bed expansion = 30%) (Amorim *et al*., 2009). This flow rate produced a superficial velocity 1.30 times greater than the minimum fluidization velocity. The bioreactor was initially operated in batch mode for 48 h to activate the H_2-producing sludge, after which it was switched to a continuous mode with a designated hydraulic retention time of 8 h.

After reaching steady-state operation based on a constant volumetric H_2-production rate within a variation of 5-10% for 10-15 days, the HRT was decreased progressively from 8 h to 1 h. The reactor was operated for 164 days, and the compositions of the soluble metabolites (volatile fatty acids and alcohol) produced during H_2 fermentation were monitored as functions of time. The reactor was operated at an effluent pH of 5.00±0.48. A gas–liquid separator was used at the effluent outlet to collect gaseous and soluble products separately. A gas meter (Type TG1; Ritter Inc., Germany) was used to measure the amount of gaseous products generated (Amorim *et al*., 2009).

Chemical Analysis

The volatile fatty acid (VFA) and alcohol concentrations were measured using a gas chromatograph (GC-2010, Shimadzu, Tokyo, Japan) equipped with a flame-ionization detector (FID), a COMBI-PAL headspace autosampler (AOC model 5000) and a Zebron ZB-WAX column (30 m long × 0.25 mm i.d. × 0.25 μm film thickness) (Maintinguer *et al*., 2008). The biogas hydrogen content was measured using a gas chromatograph (GC-2010, Shimadzu, Japan) with a thermal conductivity detector (TCD) with argon as the carrier gas, and the column was packed with Supelco Carboxen 1010 Plot (30 m × 0.53 mm i.d.) (Maintinguer *et al*., 2008).

The pH and chemical oxygen demand (COD) were measured according to the procedures described in the Standard Methods (1998). The carbohydrate concentrations of the reactor's influent and effluent were measured according to Dubois *et al.* (1956).

RESULTS AND DISCUSSION

Effect of HRT and OLR on Hydrogen Production

Figure 2 shows the variation in the hydrogen production rate (HPR), hydrogen yield (HY) and organic loading rate (OLR) as a function of the hydraulic retention time (HRT).

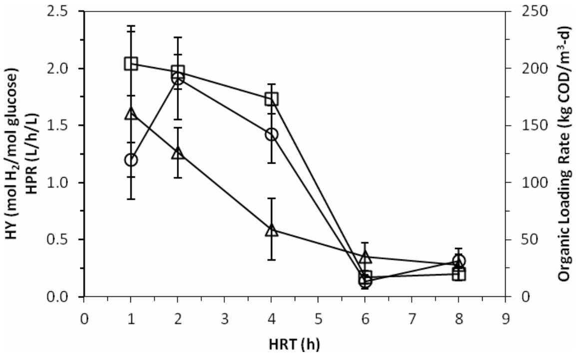

Figure 2: Effect of HRT and OLR (Δ) on the performance of AFBR H_2 production rates and yields. HY (○) is the H_2 yield (the amount (mol) of H_2 formed)/(the amount (mol) of glucose consumed); HPR (□) is the H_2 production rate.

As in other studies (Amorim *et al.*, 2009; Reis and Silva, 2011; Shida *et al.*, 2009; Barros *et al.*, 2010), this study showed an increase in HPR from 0.20 to 2.04 L/h/L when the HRT was reduced from 8 h to 1 h by increasing the OLR from 28 kg COD/m^3-d to 161 kg COD/m^3-d, respectively. The HY also behaved in the same way as in previous studies, in that it was observed to increase when the HRT was reduced from 8 h (0.31 mol H_2/mol glucose) to 2 h (1.91 mol H_2/mol glucose) or when the OLR was increased from 28 kg COD/m^3-d to 126 kg COD/m^3-d. A decrease in the HY was subsequently observed when the HRT was reduced to 1 h (1.20 mol H_2/mol glucose). This finding was also observed in previous studies employing an AFBR (Amorim *et al.*, 2009; Reis and Silva, 2011; Shida *et al.*, 2009; Barros *et al.*, 2010), and such behavior may be attributed to overloads caused by a high OLR (161 kg COD/m^3-d) or kinetic limitations (Amorim *et al.*, 2009).

Shida *et al.* (2009) found that reducing the HRT from 8 h to 1 h resulted in an increase in HPR, which reached its highest value (1.28 L/h/L) at an HRT of 1 h. The HY increased when the HRT was reduced from 8 h to 2 h, i.e., from 1.84 mol H_2/mol glucose to 2.29 mol H_2/mol glucose, respectively. By reducing the HRT to 1 h, the HY decreased to 2.10 mol H_2/mol glucose.

Amorim *et al.* (2009) obtained an increase in HPR from 0.08 L/h/L to 0.97 L/h/L when the HRT was reduced from 8 h to 1 h (the OLR increased from 15.7 kg COD/m^3-d to 116.6 kg COD/m^3-d). Additionally, the HY increased from 1.41 mol H_2/mol glucose to 2.49 mol H_2/mol glucose when the HRT was reduced from 8 h to 2 h (the OLR from 15.7 kg COD/m^3-d to 66.5 kg COD/m^3-d) and decreased to 2.41 mol H_2/mol glucose when the HRT was reduced to 1 h (116.6 kg COD/m^3-d).

Barros *et al.* (2010) observed the same behavior when using expanded clay as the support material in an AFBR. The HY values ranged from 1.51 mol H_2/mol glucose to 2.59 mol H_2/mol glucose when the HRT varied between 8 h and 2 h and decreased when the HRT was reduced to 1 h (1.84 mol H_2/mol glucose). The highest HPR (1.21 L/h/L) was also obtained at an HRT of 1 h.

Reis and Silva (2011) observed a similar behavior; their HPR_{max} was 1.22 L/h/L at an HRT of 1 h. In addition, the HY ranged from 1.53 mol H_2/mol glucose to 2.55 mol H_2/mol glucose when the HRT was reduced from 8 h to 2 h and decreased to 1.87 mol H_2/mol glucose at an HRT of 1 h.

Figure 3 shows the variation in the H_2 content as a function of the HRT. As in other studies (Amorim *et al.*, 2009; Reis and Silva, 2011; Shida *et al.*, 2009; Barros *et al.*, 2010), this study showed that the H_2 content increased significantly from 8% to 48% when the HRT was decreased from 8 to 2 h. After decreasing the HRT from 2 h to 1 h, the H_2 content decreased to 30%. No methane was found in the biogas.

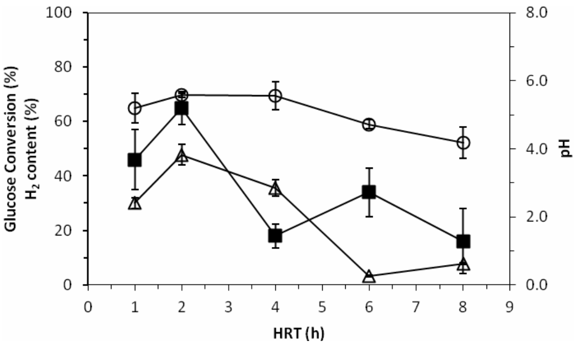

Figure 3: Effect of HRT on the performance of the AFBR in terms of glucose conversion (■), pH (○) and H_2 content (Δ). Glucose conversion is (the amount of glucose utilized)/(the amount (mol) of glucose fed into the reactor).

Composition of Soluble Products

In addition to ethanol, the presence of acetic, propionic, and butyric acids was observed during the AFBR operation. Table 1 shows the distribution of soluble microbial products (SMP) as a function of HRT. Ethanol was the most common metabolite during reactor operation (1.87% to 100% of the SMP), with its concentration ranging between 23.57 mg/L (0.51 mM) and 30.46 mg/L (0.66 mM) when the HRT was reduced from 8 h to 6 h or when the OLR increased from 28 kg COD/m^3-d to 35 kg COD/m^3-d, and it presented an average production of 25.87 mg/L (0.56 mM), indicating an insignificant change in the production of this metabolite throughout AFBR operation.

Dabrock *et al.* (1992) found that ethanol production is minimally influenced by changes in pH. In addition, according to the authors, solvent was produced at low concentrations (2 mM to 5 mM) at pH values ranging between 4.8 and 7. The pH variation in this study was similar to that observed by Dabrock *et al.* (1992) (4.18 to 5.58) and may not have had an impact on ethanol production.

The presence of ethanol in all phases may be indicative of hydrogen production through the metabolic pathway that generates both ethanol and hydrogen (Eq. (1)), as reported by Zhu *et al.* (2009).

$$C_6H_{12}O_6 + H_2O \rightarrow C_2H_5OH + CH_3COOH \\ +2H_2 + 2CO_2 \quad \Delta G = -201\,kJ/mol \tag{1}$$

Propionic acid was the second most common metabolite during the experiment (0.00% to 50.14% of the SMP). This behavior can be explained by the fact that the metabolic pathway that generates this acid is the most favored (as ΔG of metabolic pathways) of all pathways (Eq. (2)).

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH \\ +2H_2O \quad \Delta G = -358\,kJ/mol \tag{2}$$

The highest production of propionic acid was observed at an HRT of 2 h or OLR of 126 kg COD/m^3-d (280.98 mg/L or 3.8 mM). However, this production level did not prevent hydrogen production, which also increased at an HRT of 2 h. This result may be attributed to the high production of acetic acid at this HRT (1450 mg/L or 24.17 mM), corresponding to 84.80% of the SMP, the metabolic pathway which is the best in terms of hydrogen yield (HY) (Eq. (3)). Nonetheless, when an electron flow balance of the metabolic pathways is performed, it is possible to verify that hydrogen production is not significantly affected by simultaneous acetic acid production.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 \\ +4H_2 \quad \Delta G = -215{,}69\,kJ/mol \tag{3}$$

Ren *et al.* (2007) observed that a pH of approximately 5.0 did not provide a stable condition and that microbial populations resulting from the three acidogenic fermentations could coexist at this pH.

During the phase corresponding to an HRT of 2 h, the pH was approximately 5.58. Thus, the production of propionic and acetic acids observed during this phase may have also been influenced by the pH.

Acetic acid production was also observed at an HRT of 4 h or OLR of 59 kg COD/m^3-d (80.0 mg/L or 1.33 mM), corresponding to 55.66% of the SMP. Compared to the previous phase, the pH increased from 4.72 (HRT of 6 h) to 5.56 (HRT of 4 h), which may have created the necessary conditions for the initiation of acetic acid production (1.33 mM). In addition, there was a small reduction in the production of propionic acid (0.51 mm at HRT of 4 h) compared to that of the previous phase (0.67 mM at HRT of 6 h or OLR of 35 kg COD/m^3-d), thereby indicating possible competition between these two metabolic pathways.

In contrast, acetic acid production was not observed at HRTs of 1 h, 6 h, and 8 h or at OLR of 161, 35 and 28 kg COD/m^3-d, respectively.

Table 1: Production of soluble metabolites for hydrogen production in the AFBR.

HRT (h)	HAc (mM)	HBu (mM)	HPr (mM)	EtOH (mM)	HAc/SMP %	HBu/SMP %	HPr/SMP %	EtOH/SMP %	TVFA (mM)	SMP (mM)
8	0 ± 0	0 ± 0	0 ± 0	0.51 ± 0.05	0	0	0	100.00	0	0.51
6	0 ± 0	0 ± 0	0.67 ± 0.02	0.66 ± 0.01	0	0	50.14	49.86	0.67	1.33
4	1.33 ± 0.08	0 ± 0	0.51 ± 0.03	0.55 ± 0.03	55.66	0	21.27	23.08	1.84	2.40
2	24.17 ± 0.60	0 ± 0	3.80 ± 0.19	0.53 ± 0.04	84.80	0	13.32	1.87	27.96	28.50
1	0 ± 0	3.04 ± 0.11	0.96 ± 0.04	0.55 ± 0.05	0	66.78	21.13	12.09	4.00	4.56

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, EtOH/SMP = molar ethanol-to-SMP ratio.

When the HRT was reduced from 2 h to 1 h or when the OLR was increased from 28 kg COD/m^3-d to 161 kg COD/m^3-d, the acetic acid metabolic pathway was replaced with the butyric acid pathway (Table 1). The butyric acid production at an HRT of 1 h was 267.71 mg/L (3.04 mM), corresponding to 66.78% of the SMP.

Kim *et al*. (2004), using a semi-continuous reactor, concluded that butyric acid production is favored when the pH is approximately 4.5-5. However, when the reactor is operated at a long enough HRT to grow methanogenic microorganisms, methanogenic activity cannot be completely inhibited by acidic conditions (pH of approximately 4.5).

The presence of butyric acid only at an HRT of 1 h (OLR of 161 kg COD/m^3-d) may be correlated to the pH value (5.19). This value was close to that obtained by Kim *et al*. (2004), which provided better conditions for the production of this metabolite.

Table 1 shows that the production of SMP tended to increase when the HRT was reduced from 8 h to 2 h or when the OLR was increased from 28 kg COD/m^3-d to 126 kg COD/m^3-d. The SMP values dropped significantly when the HRT was reduced to 1 h (OLR of 161 kg COD/m^3-d) (Table 1). This phenomenon was also observed by Amorim *et al.* (2009). According to the authors, total volatile fatty acids (TVFA) contributed the most to the SMP, which may indicate that the fermentative production of hydrogen that occurred in their AFBR resulted mainly from acidogenic metabolism. Furthermore, such behavior may be attributed to overloads caused by a high OLR (161 kg COD/m^3-d) or kinetic limitations (Amorim *et al*., 2009).

Several other studies, using conditions analogous to those employed during this experiment, also indicate similarities regarding the SMP. However, SMP distribution did not always behave in a similar manner in these studies (Amorim *et al*., 2009; Reis and Silva, 2011; Shida *et al*., 2009; Barros *et al*., 2010).

Sreethawong *et al.* (2010), using cassava wastewater in an anaerobic sequencing batch reactor, produced acetic acid ranging from 0.15 g/L (2.5 mM) to 0.87 g/L (14.5 mM). The authors also observed the presence of butyric acid, the concentration of which ranged from 0.53 g/L (6.2 mM) to 3.73 g/L (42.39 mM). Ethanol was produced in small concentrations (less than 0.01 g/L or 0.22 mM), whereas the propionic acid concentrations ranged from 0.073 g/L (0.99 mM) to 0.36 g/L (4.86 mM).

Cappelletti *et al*. (2011) also used cassava wastewater as substrate, and the authors evaluated the effect of varying the cassava wastewater concentration. They carried out a series of batch tests at concentrations of 30, 15, 10, 7.5 and 5 g COD/L and obtained acetic acid concentrations varying from 0.01 g/L (0.17 mM), by applying 5 g COD/L, to 0.63 g/L (10.5 mM), by applying 10 g COD/L.

The high concentration of acetic acid found in this study at an HRT of 2 h (1450 mg/L or 24,17 mM) coincides with the largest HY (1.91 mol H_2/mol glucose). As in previous studies (Amorim *et al*., 2009; Reis and Silva, 2011; Shida *et al*., 2009), the best AFBR performance in this study was found at an HRT of 2 h. Despite using a synthetic substrate, these studies (Amorim *et al*., 2009; Reis and Silva, 2011; Shida *et al*., 2009) also indicated the presence of metabolites related to this research.

Shida *et al*. (2009), using glucose as the substrate, obtained acetic acid production ranging from 0.30 g/L to 0.49 g/L, or 5 mM to 8.17 mM, by reducing the HRT from 8 h to 2 h. Butyric acid production reached its lowest value (0.35 g/L or 3.98 mM) at an HRT of 8 h and its highest value (0.55 g/L or 6.25 mM) at an HRT of 2 h. The highest ethanol yield (0.097 g/L or 2.11 mM) occurred at an HRT of 6 h.

Amorim *et al*. (2009) also used glucose as a substrate and observed that acetic acid production ranged between 3.76 mM (HRT of 6 h) and 8.87 mM (HRT of 2 h). The authors also obtained characteristic butyric acid production: 4.66 mM at an HRT of 8 h and 6.60 mM at an HRT of 2 h. Ethanol production was approximately 1.16 mM at an HRT of 1 h and 2.14 mM at an HRT of 4 h. On the other hand, propionic acid was not observed in any of the phases, which may be attributed to the specific reactor's operating conditions and the fact that the authors used a synthetic substrate (glucose) under better-controlled conditions.

Under fluid-dynamic conditions similar to those used in this study (upflow velocities of 1.24 cm/s), Reis and Silva (2011) observed that the production of acetic and butyric acid ranged between 0.27 g/L and 0.78 g/L (i.e., 4.5 and 13 mM) and between 0.084 g/L and 0.40 g/L (i.e., 0.95 mM and 4.55 mM), respectively, with the lowest and highest values corresponding to HRTs of 1 h and 4 h, respectively. The highest production of propionic acid (0.60 g/L or 8.11 mM) occurred at an HRT of 4 h, and ethanol production ranged from 0.92 g/L to 1.38 g/L (20 mM to 30 mM) at HRTs of 6 h and 2 h, respectively.

According to the literature, altering some operational parameters, may contribute to the carriage of methanogenic archaea in reactors and the heat treatment of the inoculum, which may result in an increased hydrogen yield.

According to Chen *et al*. (2001), the entrainment of these microorganisms is partly explained by the fact that the highest specific growth rate of methanogenic archaea ($\mu_{max} = 0.0167\ h^{-1}$) is significantly lower than that of acidogenic microorganisms ($\mu_{max} = 0.083\ h^{-1}$). This finding may indicate that methanogens cannot reproduce or remain in equilibrium under these conditions, causing their removal from the reactor.

Furthermore, the heat treatment of the inoculum contributes to the selection of hydrogen-producing bacteria by preventing the growth of hydrogen-consuming methanogens (Maintinguer *et al.*, 2008).

In this study, the SMP results show that concentrations of individual acids are influenced by HRT reduction. Nonetheless, the difference found in this study and the other studies discussed demonstrates the need to control the occurrence of acidogenic populations and prevent contamination from other non-hydrogen-producing microorganisms because they could lead to competition for substrate in the system.

COD Removal and Carbon Balance

Figure 4 shows the COD variation as a function of the HRT. The COD removal was observed to range between 12% and 36%, which is in agreement with the acidogenic phase in anaerobic digestion (Shida *et al.*, 2009).

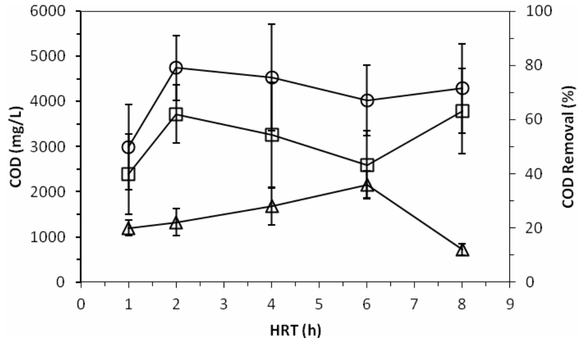

Figure 4: Variation of influent COD (○), effluent (□) and COD removed (Δ) as a function of HRT.

The influent COD ranged from 2.990 mg COD/L (HRT of 1 h) to 4.745 mg COD/L (HRT of 2 h). The effluent COD ranged from 2.388 mg COD/L (HRT of 1 h) to 3.788 mg COD/L (HRT of 8 h). The average influent COD was approximately 4.115 mg COD/L, and the average effluent COD was 3.149 mg COD/L, with an average removal efficiency of 24%.

Table 2 shows the difference between the effluent COD measured analytically and the total theoretical COD estimated from the analyzed components. 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 (Equation (4)), according to Shida *et al.* (2009).

$$\begin{aligned} COD_{Total\ Theoretical} &= COD_{Glucose\ Theoretical} \\ &+ COD_{Biomass\ Theoretical} \\ &+ COD_{Metabolites\ Theoretical} \end{aligned} \tag{4}$$

The difference between analytically measured COD and total theoretical COD based on the produced soluble metabolites (Equation (5)) may have a positive result due to the occurrence of other soluble metabolites not identified through chromatographic analysis (Amorim *et al.*, 2009).

$$\begin{aligned} COD_{Others} &= COD_{Mensured} \\ &- \left(COD_{Products} + COD_{Glucose}\right) \end{aligned} \tag{5}$$

Table 2 shows that the difference between the effluent COD measured analytically and the total theoretical COD ranged from 0 mg COD/L (HRT of 8 h) to 810 mg COD/L (HRT of 4 h). This difference between the measured effluent COD and the total theoretical COD was also found in other studies (Amorim *et al.*, 2009; Reis and Silva, 2011; Shida *et al.*, 2009) using an AFBR for hydrogen production.

Table 2: Average of the theoretical COD of the metabolites and biomass, and measured effluent COD.

HRT (h)	$COD_{t,Glucose}$ (mg/L)	$COD_{t,Acetic\ acid}$ (mg/L)	$COD_{t,Butyric\ acid}$ (mg/L)	$COD_{t,Propionic\ acid}$ (mg/L)	$COD_{t,Biomass}$ (mg/L)	$COD_{t,Ethanol}$ (mg/L)	COD_t Total (mg/L)	$COD_{m,Effluent}$ (mg/L)	COD_m -COD_t Total (mg/L)
8	3388	-	-	-	351	49	3788	3788	-
6	1929	-	-	75	364	64	2432	2594	162
4	2068	85	-	57	183	53	2446	3256	810
2	1279	1546	-	425	372	51	3674	3717	43
1	746	-	487	108	451	53	1845	2388	543

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

The smallest and largest differences obtained by Shida *et al*. (2009), using glucose as substrate at 2.000 mg COD/L, were 248.31 mg COD/L and 764.20 mg COD/L, respectively. Amorim *et al*. (2009), using glucose at 4.000 mg COD/L, found differences varying from 25 mg COD/L to 1,259 mg COD/L, whereas Reis and Silva (2011), using 5.000 mg COD/L of glucose as substrate, obtained the largest difference (1.602 mg COD/L) by applying fluiddynamic conditions similar to those employed in this study (with upflow velocities of 1.24 cm/s).

The authors of these studies attribute this difference to the likely presence of other metabolites undetected by the chromatographic analysis performed, such as lactic acid and formic acid (Amorim *et al*., 2009; Reis and Silva, 2011; Shida *et al*., 2009).

A small amount of hydrogen production (0.20 L/h/L) and a small increase in the HY (0.31 mol H_2/mol glucose) was observed in this experiment during the first phase of AFBR operation (corresponding to an HRT of 8 h). Only ethanol production (0.51 mM) was observed during this phase, which may indicate that hydrogen production was accomplished via the ethanol metabolic pathway. Moreover, the lowest pH value (approximately 4.18) observed during all AFBR experimentation was measured at the same time.

Van Ginkel *et al*. (2001) concluded that the optimal value for hydrogen production should be between 5.5 and 6.0. Some studies evaluating the use of cassava wastewater as a substrate obtained significant hydrogen production when the initial pH was between approximately 5.5 and 7.0 (Luo *et al*., 2010a; Cappelletti *et al*., 2011; Luo *et al*., 2010b; Zhu *et al*., 2009), but none of them used an AFBR. Comparing pH values found in the literature, the low HPR and HY values obtained at an HRT of 8 h may have been influenced by the pH in the experiment, which was approximately 4.18.

During the second phase of the experiment (corresponding to an HRT of 6 h), there was an insignificant decrease in HPR and HY to 0.17 L/h/L and 0.13 mol H_2/mol glucose, respectively. This finding may be attributed to the production of propionic acid (0.67 mM) observed during this phase, which is related to a fermentative route detrimental to hydrogen production (Eq. (2)) because its production requires the consumption of 2 moles of this gas. In addition to propionic acid production, the difference between the measured effluent COD and the total theoretical COD was 162 mg COD/L (HRT of 6 h), a difference that may be related to the production of other metabolites not identified by the chromatographic analysis performed, which could have influenced hydrogen production routes.

When an HRT of 4 h was applied, the HPR and HY increased to 1.73 L/h/L and 1.42 mol H_2/mol glucose, respectively. This increase may have been caused by the increase in pH, which reached 5.56 (Luo *et al*., 2010a; Cappelletti *et al*., 2011; Luo *et al*., 2010b; Zhu *et al*., 2009) and may have favored the production of acetic acid (1.33 mM), which is the metabolite that bolsters hydrogen production the most (Eq. (5)). This phase displayed the largest difference between the measured effluent COD and the total theoretical COD (810 mg COD/L), which suggests the existence of other routes favoring hydrogen production and, in turn, justifies the increase in its production.

An HRT of 2 h gave the highest HPR and HY values of 1.92 L/h/L and 1.91 mol H_2/mol glucose, respectively. This stage was also the stage in which the highest production of acetic acid occurred (24.17 mM), which is most likely the preferred metabolic pathway because the production of propionic acid and ethanol was 3.80 mM and 0.53 mM, respectively. Furthermore, the small difference between the measured effluent COD and the total theoretical COD may indicate that an insignificant amount of metabolites produced went undetected, and for this reason, the metabolic pathway of acetic acid was favored. The highest pH of approximately 5.58 was also observed during this phase, which fell within the optimum pH range for hydrogen production established by Van Ginkel *et al*. (2001) and also within the pH range observed in other studies using cassava wastewater to produce hydrogen through fermentation (Luo *et al*., 2010a; Cappelletti *et al*., 2011; Luo *et al*., 2010b; Zhu *et al*., 2009).

In addition, Figure 3 (glucose conversion efficiency as a function of HRT) shows that the highest glucose conversion efficiency (approximately 65%) was obtained at an HRT of 2 h, which could have promoted higher acetic acid production and, consequently, a higher hydrogen yield.

In the phase corresponding to an HRT of 1 h, the HPR displayed a slight increase to 2.04 L/h/L, whereas the HY decreased to 1.20 mol H_2/mol glucose. Additionally, during this phase, propionic acid production decreased from 3.80 to 0.96 mM when the HRT was reduced from 2 h to 1 h. A decrease in the production of propionic acid may be considered beneficial to hydrogen production because this gas is consumed during propionic acid production (Eq. (7)). Butyric acid production was only observed during this phase; 3.04 mM of this metabolite was obtained.

The production of this acid may have had an impact on HPR growth because the production of this metabolite is advantageous to hydrogen production (Eq. (6)).

The difference between the measured effluent COD and the theoretical total COD at an HRT of 1 h was 543 mg COD/L. Other unidentified metabolites might have been produced that may have also influenced the results found during this phase.

The HY reduction observed in the phase corresponding to an HRT of 1 h may also be ascribed to diminished glucose conversion efficiency (46%). Moreover, this HY reduction may be caused by overloading due to a high OLR applied to the reactor (161 kg COD/m³-d) or kinetic limitations (Amorim *et al.*, 2009).

The fact that ethanol production varied insignificantly throughout the experiment (minimum 0.51 mM and maximum 0.66 mM) indicates that the HRT had little impact on the distribution of this metabolite.

CONCLUSIONS

The results of this experiment indicate that the use of a substrate (cassava wastewater) in an anaerobic fluidized bed reactor for hydrogen production performs satisfactorily.

Both the HY and the HPR significantly increased when the HRT was reduced, as shown by the maximum values of 2.04 L/h/L (HRT of 1 h and OLR of 161 kg COD/m^3-d) and 1.91 mol H_2/mol glucose (HRT of 2 h and OLR of 126 kg COD/m^3-d), respectively.

The H_2 content in biogas increased when the HRT was reduced and its highest value (48%) was obtained in the reactor operated at a HRT of 2 h and OLR of 126 kg COD/m^3-d.

The soluble metabolites present during the operation of the reactor were acetic acid, butyric acid, propionic acid and ethanol. The predominance of propionic acid during the 8 h HRT (early phase of operations) was reflected in the low hydrogen production at this stage. This finding can be attributed to the adaptation phase of the reactor.

The high acetic acid production at an HRT of 2 h coincides with the largest HY, which may be attributed to the greater deflection of the electron flow route for acetic acid production at an HRT of 2 h.

The absence of butyric acid production in the majority of the experiments differs from the findings of previous studies (Amorim *et al.*, 2009; Reis and Silva, 2011; Shida *et al.*, 2009). However, these studies used a synthetic substrate. Because the substrate used in this research was real wastewater (cassava wastewater), it may have had a different behavior than that of synthetic substrates, which can vary in their composition and physico-chemical influence on hydrogen production.

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NOMENCLATURE

Abbreviations

AFBR	Anaerobic fluidized bed reactor	
ΔG	Gibbs free energy	kJ/mol
Q	Total liquid flow rate	L/h
COD	Chemical oxygen demand	mg COD/L
FID	Flame ionization detector	
HRT	Hydraulic retention time	h
HPR	Hydrogen production rate	L/h/L
HY	Hydrogen yield	mol H_2/mol glucose
HAc	Acetic acid concentration	mM
HBu	Butyric acid concentration	mM
HPr	Propionic acid concentration	mM
EtOH	Ethanol concentration	mM
OLR	Organic loading rate	kg COD/m^3d
TCD	Thermal conductivity detector	
SMP	Soluble microbial products	mM
VFA	Volatile fatty acids	mM
VSS	Volatile suspended solids	mg/L
TVFA	Total volatile fatty acids	mM
μ_{max}	Maximum specific growth rate	h^{-1}

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