HYDROGEN PRODUCTION FROM CASSAVA PROCESSING WASTEWATER IN AN 
ANAEROBIC FIXED BED REACTOR WITH BAMBOO AS A SUPPORT MATERIAL


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KARINA Q. DE CARVALHO⁴, SIMONE D. GOMES⁵

ABSTRACT: Attempting to associate waste treatment to the production of clean and renewable energy, this research sought to evaluate the biological production of hydrogen using wastewater from the cassava starch treatment industry, generated during the processes of extraction and purification of starch. This experiment was carried out in a continuous anaerobic reactor with a working volume of 3L, with bamboo stems as the support medium. The system was operated at a temperature of 36°C, an initial pH of 6.0 and under variations of organic load. The highest rate of hydrogen production, of 1.1 Ld⁻¹.L⁻¹, was obtained with application of an organic loading rate of 35 g.L⁻¹.d⁻¹, in terms of total sugar content and hydraulic retention time of 3h, with a prevalence of butyric and acetic acids as final products of the fermentation process. Low C/N ratios contributed to the excessive growth of the biomass, causing a reduction of up to 35% in hydrogen production, low percentages of H₂ and high concentrations of CO₂ in the biogas.

KEYWORDS: fixed bed reactor, agroindustrial wastes, fermentation processes.

INTRODUCTION

A source of efficient and clean energy, hydrogen has been proposed as a promising replacement for fossil fuels. Over the last decade, biological processes have shown the potential to generate hydrogen from organic waste, allying together the generation of energy, stabilisation of organic matter and reduction of costs with the treatment. Among the processes for hydrogen production, fermentation is able to directly convert organic compounds from wastewaters into hydrogen.
Hydrogen gas (PERERA et al., 2012). Approximately, 1 m$^3$ of wastewater from starch production may produce 10 m$^3$ of biogas (THANWISED et al., 2012) with percentages of up to 58% of hydrogen (CHEN et al., 2009).

On fermentation, the complex organic matter is converted into simpler compounds such as hydrogen, carbon dioxide, free sugars, volatile organic acids and alcohols (McCARTY, 1964). Bacteria of the Clostridium sp. species are widely used in the fermentative production of hydrogen. These microorganisms are strictly anaerobic, are often found in microbial associations and are considered effective producers of hydrogen in organic substrates, particularly carbohydrates (SINHA & PANDEY, 2011). Theoretically, starting with glucose, it is possible to obtain 4 moles of H$_2$.mol$^{-1}$ glucose (498 mL H$_2$.g glucose$^{-1}$) and 2 moles of H$_2$.mol$^{-1}$ glucose (249 mL H$_2$.g glucose$^{-1}$) when acetic and butyric acids are the final products of fermentation (GUO et al., 2010). The stoichiometric yields are presented in eqs. (1) and (2) as follows:

$$\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH (Acetic)} + 2\text{CO}_2 + 4\text{H}_2 \tag{1}$$
$$\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH (Butyric)} + 2\text{CO}_2 + 2\text{H}_2 \tag{2}$$

Cassava-processing wastewater is a feedstock that is abundant, readily available, cheap, with a high concentration of carbohydrates and highly biodegradable (GUO et al., 2010); it is characterized as a potential substrate for the fermentative production of hydrogen, which ensures the sustainability of the process. This application has the advantage of adding value of a clean energy source to a highly pollutant wastewater (CAPPELLETTI et al., 2011). Rich in carbohydrates, the wastewater of starch production contains nitrogen and phosphorus, among other nutrients (RIBAS et al., 2010). The composition of the residue is a key issue in the choice of substrate used in the production of hydrogen, as the costs of sources of carbon and nitrogen are high in implementing this technology for an industrial plant, representing around 40% of the total cost of fermentative hydrogen production (WANG et al., 2008).

Therefore, this study sought to evaluate the production of hydrogen from cassava-processing wastewater in a continuous anaerobic fixed bed reactor, submitted to variations of influent organic loading rate.

MATERIAL AND METHODS

Experimental procedures were carried out at the Laboratory of Biological Reactors, Laboratory of Environmental Sanitation, and also at the Laboratory for Agricultural and Environmental Analysis, at the State University of Western Paraná, Cascavel Campus, in the city of Cascavel, in the state of Paraná, Brazil.

This study employed an upflow anaerobic fixed bed reactor made of 5 mm of thick transparent plexiglass. The apparatus was built 75 cm in height, 8 cm in inner diameter and with 3 L of working volume (PEIXOTO et al., 2011). The reactor was divided into three compartments (input of the influent, fixed bed and effluent output), which were separated by a stainless steel mesh. The reactor was fed using a peristaltic dosing pump connected to a tank of 10 L that stored the substrate at a temperature of 7°C. At the top of the reactor, the gasmeter and the effluent output were set up. The reactor was placed in a heated chamber at a temperature of 36°C. The schematic description of the reactor is presented in Figure 1.
As the support media, 12 bamboo stems were used, vertically arranged, which were 40 cm in length and had a porosity of 74% (KUNZLER et al., 2013). The sludge used as inoculum was collected from bamboo pieces, used as support media in a pilot scale anaerobic reactor (total volume of 32,000 L) by treating effluent from starch production. To suppress the activities of hydrogen-consuming bacteria, the sludge was pre-treated by boiling at 95°C for 15 min. (SREETHAWONG et al., 2010).

The cassava-processing wastewater used in the reactor feeding, was collected in the municipality of Toledo, State of Paraná, Brazil. The effluent collects on top of the water from the starch extraction and purification processes and on the water used for the washing of cassava roots. A summary of the results obtained in the characterization of cassava-processing wastewater is shown in Table 1.

TABLE 1. Characterization of cassava-processing wastewater.

<table>
<thead>
<tr>
<th>Samples</th>
<th>pH</th>
<th>COD (mg L⁻¹)</th>
<th>T. Sugar (mg L⁻¹)</th>
<th>TKN (mg L⁻¹)</th>
<th>TS (mg L⁻¹)</th>
<th>TVS (mg L⁻¹)</th>
<th>TVS/TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.22</td>
<td>10737</td>
<td>4683</td>
<td>293</td>
<td>7183</td>
<td>5089</td>
<td>0.71</td>
</tr>
<tr>
<td>2</td>
<td>4.38</td>
<td>11029</td>
<td>2544</td>
<td>279</td>
<td>7084</td>
<td>5968</td>
<td>0.84</td>
</tr>
<tr>
<td>3</td>
<td>5.33</td>
<td>10936</td>
<td>4350</td>
<td>286</td>
<td>8750</td>
<td>7946</td>
<td>0.91</td>
</tr>
<tr>
<td>4</td>
<td>4.90</td>
<td>11800</td>
<td>2789</td>
<td>307</td>
<td>7562</td>
<td>6584</td>
<td>0.87</td>
</tr>
<tr>
<td>5</td>
<td>4.62</td>
<td>11643</td>
<td>3373</td>
<td>249</td>
<td>8625</td>
<td>7075</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Each sample corresponds to a batch of cassava processing wastewater; pH – Hydrogenionic Potential; COD – Chemical Oxygen Demand; T. Sugar – Total Sugars; TKN – Total Kjeldahl Nitrogen; TS – Total Solids; TVS – Total Volatile Solids.

The reactor was inoculated with 300 mL of anaerobic sludge with 25 g TVS L⁻¹ and was initially operated in a batch mode for 48 h to activate the hydrogen-producing biomass (SHIDA et al., 2009). The continuous operation was carried out in 6 stages, with an initial pH of 6.0, theoretical hydraulic retention times (HRT) of 4 h and 3 h and organic loading rates (OLR) varying from 15 to 35 g L⁻¹ d⁻¹, based on the total sugar content of the wastewater (Table 2). The variation of organic loading rate from 15 to 35 g L⁻¹ d⁻¹ occurred due to the difference in the composition of the cassava-processing wastewater, which was used without any dilutions. This variability is closely related to the origin of the cassava plant, age of the tubercl e, storage period and to the type of process used (CAMPOS et al., 2006).
TABLE 2. Operational conditions of the upflow anaerobic fixed bed reactor.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Operation Period (d)</th>
<th>Samples</th>
<th>C/N</th>
<th>HRT (h)</th>
<th>Q (L.d⁻¹)</th>
<th>Influent concentration (mg.L⁻¹)</th>
<th>OLR (g.L⁻¹.d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>5</td>
<td>37</td>
<td>4</td>
<td>18</td>
<td>4683</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>5</td>
<td>40</td>
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<td>18</td>
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<td>38</td>
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<td>24</td>
<td>2789</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>5</td>
<td>47</td>
<td>3</td>
<td>24</td>
<td>3373</td>
<td>27</td>
</tr>
</tbody>
</table>

*a* The same batch of cassava processing wastewater were utilized in stages 3 and 4; *b* Number of samples used to calculate the mean values and standard deviation; *c* Flow rate; *d* Influent concentration in terms of total sugars; HRT – Hydraulic retention time; C/N – carbon (C) and nitrogen (N) ratio in terms of COD and TKN; OLR – Organic loading rate based on the working volume of 3.0 L.

Samples of the reactor effluent were characterized by determining total sugars concentrations with a colorimetric method (DUBOIS et al. 1956); Chemical Oxygen Demand (COD), pH and volatile suspended solids (VSS), according to the Standards Methods for the Examination of Water and Wastewater (APHA, 2005). Volatile fatty acid concentrations were measured using a high performance liquid chromatograph (HPLC Shimadzu Prominance) equipped with an Aminex® HPX-87H column. The composition of the biogas (carbon dioxide, hydrogen and methane) was determined by gas chromatography (GC 2010 Shimadzu) with a Carboxen® 1010 Plot capillary column. The volume of biogas produced was quantified based on the methodology of CAPPELLETTI et al. (2011).

RESULTS AND DISCUSSION

Table 3 shows the values of pH, efficiency in COD removal, sugars consumption and total concentration of volatile organic acids for operational stages with different applied organic loading rates.

TABLE 3. Average values of the parameters in the effluent monitored during the experiment for each organic loading rate (OLR).

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>OLR (g.L⁻¹.d⁻¹)</th>
<th>pH</th>
<th>COD Effluent (mg.L⁻¹)</th>
<th>COD Removal (%)</th>
<th>Sugar Conversion (%)</th>
<th>VFA (mg.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>28</td>
<td>4.7±0.48</td>
<td>7147±434</td>
<td>32±1.2</td>
<td>86±6.1</td>
<td>2538±1418</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>5.4±0.23</td>
<td>6780±632</td>
<td>38.5±5.7</td>
<td>91±2.4</td>
<td>1949±558</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>5.0±0.20</td>
<td>8547±1076</td>
<td>21±10</td>
<td>91±3.2</td>
<td>2116±333</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>4.9±0.16</td>
<td>8692±1193</td>
<td>19±11</td>
<td>93±2.0</td>
<td>1698±938</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>5.3±0.37</td>
<td>6798±1378</td>
<td>39±12</td>
<td>84±11.4</td>
<td>2483±675</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>5.9±0.33</td>
<td>7495±1083</td>
<td>32±9.8</td>
<td>93±4.3</td>
<td>2549±1420</td>
</tr>
</tbody>
</table>

*a* Mean ± standard deviation; OLR – Organic loading rate; COD – Chemical oxygen demand; VFA – volatile fatty acids (lactic, acetic, propionic and butyric acids).

The effluent pH varied from 4.7±0.48 to 5.9±0.33, i.e., within the recommended range of 4.5 to 6.0 for hydrogen production in starch-based substrates by LEE et al. (2008). The application of high OLR, of 28 and 35 g.L⁻¹.d⁻¹, contributed to the pH dropping to average values of 4.7 and 4.9, respectively. Under operational conditions of organic overload the accumulation of H₂, CO₂ and volatile organic acids may reduce pH values (WANG & WAN, 2009) and reductions to a pH below 4.5 can change the metabolic pathway, leading to the production of solvents.
(VIJAYARAGHAVAN & AHMAD, 2006). Apart from the organic loading rates, higher initial pH values (6.0) may make the environment favourable to the rapid formation of acids and as a result, may reduce the pH of the environment (KHALAL et al., 2004). For OLR values of 15; 26 and 22 g.L\(^{-1}.d\(^{-1}\), pH was kept around 5.0, favourable for the production of acetic and butyric acids – the main fermentation products in pH values varying from 5.0 to 5.5; the formation of propionic acid requires pH between 5.5 and 6.0 (LI et al., 2007).

COD removal efficiency varied between 19±11 to 39±12%. Highest percentages of 32±1.2; 38.5±5.7; 39±12.3 and 32±9.8% were observed with application of the OLR of 28; 15; 22 and 27 g.L\(^{-1}.d\(^{-1}\), respectively. Lowest volume of biogas production of 1.1; 1.8; 2.2 and 2.5 L.d\(^{-1}.L\(^{-1}\) were observed under these conditions, as well as the highest concentrations of volatile fatty acids (Table 3). In reactors with dominance of acidogenic bacteria, COD is removed through cyto genesis and gas releases (mainly H\(_2\) and CO\(_2\)), while a significant amount is converted into intermediate products, such as acids and solvents that remain in the system (THANWISED et al., 2012).

The COD removal efficiencies remained under the values expected in fermentative systems for hydrogen production in reactors fed with cassava-producing wastewater at between 20% and 50% (SREETHAWONG et al., 2010; THANWISED et al., 2012). The removal efficiencies may have been influenced by the low C/N ratio in wastewater. O-THONG et al. (2008) demonstrated that lower COD removal efficiencies could be related to lower C/N ratios. These authors reported COD removal efficiencies of 56; 28 e 20% in C/N ratios of 70, 95 and 45. According to BABU et al. (2009), an improvement in the efficiency of substrate removal can be attributed to the effective mass transfer between bacteria and wastewater in biofilm configuration, due to a uniform distribution of substrate in the reactor. Therefore, the excess nitrogen can change the microbial metabolism of the cell growth, leading to an accumulation of biomass in the bed and damaging the flow rate in the reactor, thus decreasing COD removal.

Average of sugar conversion efficiency was 90% for the experimental period. With a variation of 4% in the consumption of total sugar, it was observed that the reduction of the HRT from 4 to 3h did not affect the degradation of carbohydrates because consumption remained between 84% and 93%. High rates of substrate conversion may be attributed to cellular immobilization, which guarantees solids retention for longer periods and greater resistance to hydraulic shock loads (SHIDA et al., 2009; KESKIN et al., 2012). This affirmation was also verified by BARROS et al. (2010), who observed efficiency of glucose conversion of between 82% and 96% in a reactor that had expanded clay as support media and operated with HRT of 4h. On the other hand, ARROJ et al. (2008) obtained 68% sugar removal efficiency in a reactor without immobilized biomass and the same HRT. The ability to retain a large amount of biomass in biofilm allowed for high rates of substrate conversion at lower HRT conditions.

The average concentration of VSS at the effluent was 2426±1577 mg.L\(^{-1}\), which was higher than the 1500 mg.L\(^{-1}\) obtained by PERNÁ et al. (2013) in a continuous system with immobilized biomass, while treating cheese whey in an OLR of 37 g.L\(^{-1}.d\(^{-1}\). From the OLR of 15 g.L\(^{-1}.d\(^{-1}\), it was possible to observe an accumulation of biomass both on and in between the bamboo stems. As the support media was vertically arranged, the flow of gas and liquid were assisted in the reactor, probably due to the formation of preferential paths. This increase in biogas production is compatible with the increase and greater variation of solids concentration in the effluent, due to the dragging of suspended particles by the release of biogas bubbles.

The increase in the thickness of biofilm and the application of high organic loading rates can contribute to the reduction of the intensity of fixation of microorganisms in the support media, which promotes the fragmentation of biomass and increases the concentration of solids in the reactor effluent (SHOW et al., 2010).

The main metabolites formed during the fermentation process were lactic, acetic, propionic and butyric acids, with average concentrations of 427±905; 600±232; 342±193 e 881±384 mg.L\(^{-1}\), respectively. The concentration of lactic, acetic, propionic and butyric acids generated through fermentation are presented in Figure 2.
High concentrations of lactic acid, of 990±1540 mg L\(^{-1}\) and 1480±2093, were observed for the OLR of 27 and 28 g L\(^{-1}\).d\(^{-1}\). The accumulation of this metabolite is usually observed in systems operated with low hydraulic retention times and could be associated with the consumption of acetic and propionic acids (ANTONOPoulos et al., 2011; JO et al., 2008). According to Figure 2, when the lactic acid concentration decreases there is an increase in the concentration of other acids. Considering that the consumption of lactic and acetic acid at a ratio of 1:2, 1 mole of H\(_2\), 2 moles of CO\(_2\) and 1.5 moles of butyric acid could be produced under conditions of average concentrations of lactic acid, which is an improvement in the production of hydrogen that can occur (MATSUMOTO & NISHIMURA, 2007).

However, higher concentrations of lactic acid can inhibit the activities of hydrogen-producing bacteria, resulting in longer periods of lag phase and reductions in hydrogen production (KIM et al., 2012). This behaviour could have affected the performance of the reactor during the application of the OLR of 28 g L\(^{-1}\).d\(^{-1}\), as the volumes of biogas (1.1±0.87 L d\(^{-1}\).L\(^{-1}\)) and hydrogen (0.2±0.15 LH\(_2\).d\(^{-1}\).L\(^{-1}\)) were observed to decrease and the yield (0.15±0.10 LH\(_2\).g\(^{-1}\) sugar) was minimised.

Propionic acid was observed throughout the experimental period at average concentrations lower than those in the other metabolites were. Its absence is associated with the improvement of hydrogen production, since propionic acid consumes 2 moles of hydrogen for every 2 moles of propionate produced (SHIDA et al., 2009). The highest concentrations of propionic acid of 419±69 and 406±220 mg L\(^{-1}\) coincide with the lowest volumes of hydrogen of 0.37±0.09 and 0.23±0.13 L.d\(^{-1}\).L\(^{-1}\), respectively, corresponding to the OLR of 15 and 22 g L\(^{-1}\).d\(^{-1}\).

The maximum hydrogen production of 1.0±0.48 and 1.1±0.68 L.d\(^{-1}\).L\(^{-1}\) and hydrogen yields of 0.75±0.35 and 0.84±0.50 L.g\(^{-1}\) of sugar were observed when there was a predominance of butyric acid (1134±360 and 871±515 mg L\(^{-1}\)) over the acetic acid (519±85 and 406±217 mg L\(^{-1}\)), for OLR of 26 and 35 g L\(^{-1}\).d\(^{-1}\), respectively. The results obtained in this study are compatible to those verified by WANG et al. (2008), SREETHAWONG et al. (2010) and ANTONOPoulos et al. (2011) that confirmed butyric acid to be the main metabolite in hydrogen-production systems.

The fermentation process produced a mixture of gases containing H\(_2\) and CO\(_2\), with the possibility of the mixture containing lesser amounts of CH\(_4\), CO and H\(_2\)S (LEVIN et al., 2004). The biogas consisted mainly of CO\(_2\) and H\(_2\), and the presence of methane was not detected. Despite the variation in volume, the percentage of hydrogen in the biogas during the application of the OLR of 15; 26; 28 and 35 g L\(^{-1}\).d\(^{-1}\) had few variations, ranging from 18.6% to 22%; values lower than 15% were observed for the OLR of 22 and 27 g L\(^{-1}\).d\(^{-1}\). The composition of the biogas was similar to the results noted by AMORIN et al. (2009) and THANWISED et al. (2012), who obtained percentages of hydrogen of 25% and 11.65% in HRT of 4 and 3 h, using sucrose and cassava-processing wastewater as substrates, respectively.

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**FIGURE 2.** Production of soluble metabolites for the hydrogen production under different operating conditions in the anaerobic fixed bed reactor.

![Graph showing soluble metabolites production](image-url)
Figure 3 presents the percentages of \( \text{H}_2 \) and \( \text{CO}_2 \) in the biogas and the biogas and hydrogen production rates.

![Figure 3](image)

**FIGURE 3.** a) Biogas composition under different operating conditions in the anaerobic fixed bed reactor; b) Hydrogen-production rate under different operating conditions in the anaerobic fixed bed reactor.

The maximum biogas production of \( 4.6 \pm 2.26 \text{ L.d}^{-1}.\text{L}^{-1} \) and \( 4.85 \pm 2.17 \text{ L.d}^{-1}.\text{L}^{-1} \), hydrogen production of \( 1.0 \pm 0.48 \text{ and } 1.1 \pm 0.68 \text{ L.d}^{-1}.\text{L}^{-1} \) and hydrogen yield of \( 0.75 \pm 0.35 \) and \( 0.84 \pm 0.50 \text{ L.g}^{-1} \) of sugar were observed with application of \( \text{OLR} \) of 26 and 35 g.L^{-1}.d^{-1} in HRT of 4 and 3 h, respectively. On application of these loads, the same batch of wastewater was used, with higher concentration of total sugars (4350 mg.L^{-1}) and total volatile solids (7946 mg.L^{-1}). The similarity of the results obtained shows, in this specific case, the influence of the composition of the substrate on the production of biogas and hydrogen, rather than the \( \text{OLR} \) and HRT parameters.

The results obtained are compatible with the studies of PERNA et al. (2013) that obtained a hydrogen production rate of \( 1.2 \text{ L.d}^{-1}.\text{L}^{-1} \) in an upflow anaerobic packed bed reactor, fed with cheese whey and operated with an organic loading rate of \( 37 \text{ g.L}^{-1}.\text{d}^{-1} \).

Figure 3 b depicts the behaviour of the system during the experiment. The production of biogas and hydrogen starts in a discreet way as from an \( \text{OLR} \) of 28 g.L^{-1}.d^{-1}, achieving the peak at \( 35 \text{ g.L}^{-1}.\text{d}^{-1} \), decreasing at 22 g.L^{-1}.d^{-1} and rising once again at 27 g.L^{-1}.d^{-1}; however, the consumption of sugars remains constant. LIMA & ZAIAT (2012) observed that the instability in the generation of gas could be related to the development of hydrogen-consuming microorganisms that use alternative routes for the production of acetic acid, while consuming \( \text{H}_2 \) and \( \text{CO}_2 \). Bacteria from the genus Clostridium, of the species \( C. ljungdahlii \), are able to grow heterotrophically, converting simple sugars into acetate and autotrophically producing acetic acid from \( \text{H}_2 \) and \( \text{CO}_2 \) (TANNER et al., 1993).

PEIXOTO et al. (2011) evaluated two fixed-bed reactors operated with HRT of 0.5h in relation to the addition of nutrients. In the reactor without addition of nutrients (C/N=250), the hydrogen production rate was \( 0.4 \text{ L.h}^{-1}.\text{L}^{-1} \), while in the reactor with supplemental nutrients (C/N =57) this production rate was reduced by 50% (\( 0.2 \text{ L.h}^{-1}.\text{L}^{-1} \)). With the reduction of void spaces in the support medium, caused by the excess of biomass, levels of \( \text{CO}_2 \) of over 90% were recorded in the biogas, as well as an increase in the concentration of propionic acid and the inhibition of hydrogen production - possibly caused by changes in the metabolic pathway of the microorganisms.

On the other hand, JO et al. (2008) reported hydrogen content of 50% in the biogas and sugar conversion efficiency of up to 97.4% in a continuous system with 97% of void spaces and using polyurethane foam as the support media. Suspended cell growth is favoured in highly porous beds, increasing the production of hydrogen due to higher generation rates than those found in the biofilm (LEE et al., 2003).
On average, the cassava-processing wastewater presented low C/N ratios ranging from 37 to 47. The low gas production rate, low hydrogen percentage and high concentrations of CO₂ in the biogas indicates that excessive nitrogen concentration can shifts the microbial metabolism to cell growth rather than to gas production (SRRETHAWONG et al., 2010). One of the strategies suggested by PERNNA et al. (2013) to ensure stability of production in continuous systems consists of regular removal of biomass from the reactor to avoid accumulation of sludge and problems with clogging in the reactor.

CONCLUSIONS

Cassava-processing wastewater appears to be a promising substrate for the generation of clean energy through fermentation processes with removal efficiencies higher than 80%.

Lower C/N ratios, inherent to the specific substrate composition, caused the growth of biomass and its rapid accumulation within the reactor. Despite the reduced volumes of biogas observed under some conditions, the variation of organic loading rate was effective in selecting the microbial flora of the reactor. No presence of methane was observed in the biogas.

Considering the operational conditions under which the most expressive results were obtained, it is possible to conclude that the composition of the substrate had a greater influence on hydrogen production than HRT and applied organic loading rate.

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