Valorization of Glucose-Based Wastewater Through Production of Hydrogen, Volatile Fatty Acids and Alcohols

Eduardo Lucena Cavalcante de Amorim, Leandro Takano Sader, Lucas Rodrigues Ramos and Edson Luiz Silva

Abstract

The production of hydrogen in an anaerobic fluidized bed reactor (AFBR) was evaluated under different organic loading rates (OLRs) with the addition of 1 g L\(^{-1}\) sodium bicarbonate for pH control. Expanded clay was used as the support material for microbial attachment. Two AFBRs were operated with glucose concentrations of 10 and 25 g L\(^{-1}\) and a hydraulic retention time (HRT) decreasing from 8 to 1 h at a controlled temperature of 30°C. A linear correlation was observed between the hydrogen production rate (HPR) and the OLR, except for the reactor operated with 25 g L\(^{-1}\) glucose. The maximum HPR of 1.58 L h\(^{-1}\) L\(^{-1}\) was obtained with an HRT of 1 h, and the maximum \(\text{H}_2\) yield of 1.32 mol \(\text{H}_2\) mol\(^{-1}\) glucose was obtained with an HRT of 2 h, in the reactor operated with 10 g L\(^{-1}\) glucose.

Keywords: hydrogen production, anaerobic fluidized bed reactor, substrate concentration, hydraulic retention time, organic loading rate

1. Introduction

The acidogenic fermentation of wastewater or biowaste for \(\text{H}_2\) production has attracted great global interest because it is a cheap and simple technology that produces clean energy from renewable sources while reducing pollutants [1, 2].

According to Reddy et al. [3], one of the major drawbacks of using organic wastes is that only 30–40% of the substrate is used to \(\text{H}_2\) production and 60–70% is converted to several other metabolites. However, some metabolites are commercially attractive, such as acetic...
acid, butyric acid, propionic acid, lactic acid, succinic acid, 1,3-propanediol, ethanol, methanol, etc. [4, 5].

H₂ production has been carried out with a variety of organic wastes, in which the source of carbonaceous organic material is based on glucose, sucrose, starch, xylose, cheese-processing wastewater, tapioca-processing wastewater, and sugarcane vinasse [6–9].

The fermentation process for the production of H₂ in anaerobic reactors is greatly influenced by several factors, such as the type of wastewater, the inoculum, the type of reactor, the nutritional requirements, the temperature, and the pH [10–12].

For practical engineering, industrial H₂ production requires continuous or semicontinuous production processes. Several types of reactors have been studied to effectively generate H₂. Reactors for continuous H₂ production include suspended biomass reactors, e.g., continuous stirred tank reactors (CSTRs) [13–15] and anaerobic sequencing bed reactors (ASBRs) [16], and biofilm reactors such as anaerobic packed bed reactors (APBRs) [17] and anaerobic fluidized bed reactors (AFBRs) [6–9, 18]. The advantages and disadvantages of different reactor types vary. Biofilm reactors can overcome the drawbacks of suspended biomass reactors by decoupling the biomass retention time from HRT, thus increasing the biomass concentration in the reactor. The hydraulic mixing regime is usually more turbulent in AFBRs than in APBRs, which improves mass transfer and treatment efficiencies because bed fluidization favors contact between the biofilm and substrate [19–21].

Hydrogen production is a microbial-mediated process dependent on several parameters that can affect the performance. Some of these are the inoculum source, pH, substrate concentration, accessible nutrients, HRT, and temperature [11, 21]. Their control in appropriate range can enrich the microbial community with hydrogen producers, eliminate hydrogen consumers, shift the metabolism to favor hydrogen production, increase substrate conversion efficiency, and increase the overall process potential [1, 10, 11, 21]. The organic loading rate (OLR; influent substrate concentration/HRT) is a parameter that evaluates the simultaneous effects of influent substrate concentrations and HRTs when synthetic or real wastewaters are used to produce H₂ in anaerobic reactors [13–18, 22–26]. Previous studies in our research group observed hydrogen production with glucose concentrations of 2000 mg L⁻¹ [27–29], 4000 mg L⁻¹ [6, 30] and 5000 mg L⁻¹ [31]. Increasing glucose concentration to 10 g L⁻¹ and 25 g L⁻¹ can determine the range where hydrogen-producing acidogenesis shifts to solventogenesis. Therefore, the present study examines the effect of both OLR and alkalinity supplementation on H₂ production in AFBRs with influent glucose concentrations of 10 g L⁻¹ (OLRs of 30–240 kg COD m⁻³ day⁻¹) and 25 g L⁻¹ (OLRs of 75–600 kg COD m⁻³ day⁻¹).

2. Materials and methods

2.1. Anaerobic fluidized bed reactors and feed composition

A schematic diagram of the two identical jacketed AFBRs used in this study is presented in Figure 1. The reactors were constructed with a transparent acrylic tube, within 5.3 cm of...
internal diameter and 190 cm of height, and filled with expanded clay (diameter = 2.8–3.3 mm, density = 1.5 g cm\(^{-3}\)). Each AFBR was equipped with a water jacket that recirculated heated water from a thermostatic bath to maintain the temperature at 30°C. The AFBRs were fed with synthetic wastewater containing glucose (10 and 25 g L\(^{-1}\)) as the main carbon source supplemented with the following nutrients: SeO\(_2\), 0.07 mg L\(^{-1}\); CoCl\(_2\)·2H\(_2\)O, 0.08 mg L\(^{-1}\); FeCl\(_3\)·6H\(_2\)O, 0.5 mg L\(^{-1}\); NiSO\(_4\)·6H\(_2\)O, 1 mg L\(^{-1}\); FeSO\(_4\)·7H\(_2\)O, 5 mg L\(^{-1}\); K\(_2\)HPO\(_4\), 21.7 mg L\(^{-1}\); Na\(_2\)HPO\(_4\)·2H\(_2\)O, 33.4 mg L\(^{-1}\); CaCl\(_2\)·6H\(_2\)O, 47 mg L\(^{-1}\); KH\(_2\)PO\(_4\), 85 mg L\(^{-1}\); and CO(NH\(_2\))\(_2\), 125 mg L\(^{-1}\). In order to control the pH of the reactors at 5.0–5.5, hydrochloric acid (10 M) and sodium bicarbonate (1 g L\(^{-1}\)) were also used [6].

2.2. Heat treatment of inoculum, AFBR setup and operation conditions

The AFBRs were inoculated with sludge from an upflow anaerobic sludge blanket (UASB) reactor treating swine wastewater effluent. The sludge was heat treated for 10 min at 90°C according to the methodology of Kim et al. [25] in order to eliminate hydrogen consumers and select for endospore producers. The reactors were inoculated at a rate of 10% of the sludge feed volume.
The total liquid flow rate into the AFBRs was maintained at 128 L h\(^{-1}\) (expansion = 30\%). This flow rate produced a superficial velocity 1.30 times greater than the minimum fluidization velocity. At first, in order to activate the H\textsubscript{2}-producing biomass, the two AFBRs were operated in batch mode for 48 h while periodically recording the substrate consumption by microorganisms. When the activation period was over, the reactors were operated in continuous mode with an HRT of 8 h, which was then decreased stepwise to 6 h, 4 h, 2 h, and 1 h. The composition of the gaseous products (H\textsubscript{2} and CO\textsubscript{2}) and soluble metabolites (volatile organic acids and alcohols) produced during fermentative H\textsubscript{2} production was monitored as a function of time.

To facilitate discussion of the results and to identify the reactors, each reactor was named according to the influent glucose concentration: the reactor operated with 10 g L\(^{-1}\) glucose was named “R10,” and the reactor operated with 25 g L\(^{-1}\) glucose was named “R25.”

### 2.3. Chemical analyses

The GOD-PAP enzymatic method \cite{32} was used to determine the glucose concentrations. Total solids (TS), volatile suspended solids (VSS), total volatile solids (TVS), and chemical oxygen demand (COD) analyses were performed according to Standard Methods for the Examination of Water and Wastewater \cite{33}.

A gas chromatograph (GC-2010, Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector (TCD) was used to determine the biogas composition. Argon was used as the carrier gas with a Carboxen 1010 PLOT column (30 m long × 0.53 mm internal diameter). A gas chromatograph (GC-2010, Shimadzu, Tokyo, Japan) equipped with a flame ionization detector (FID) was used to determine volatile organic acids and alcohols. The GC used a COMBI-PAL headspace sample introduction system (AOC 5000 model) and HP-INNOWAX column (30 m long × 0.25 mm internal diameter × 0.25 mm film thickness) \cite{32}.

A gas meter (type TG1; Ritter Inc., Germany) was used to measure the amount of H\textsubscript{2} generated.

### 3. Results and discussion

#### 3.1. Effect of OLR on H\textsubscript{2} production

**Figure 2** presents the variation in pH effluent as a function of OLR for the two AFBRs used in this study. The pH remained stable throughout the system operation within the operating range of acidogenic anaerobic systems, i.e., between 3.7 in Barros et al. \cite{6}, 3.4 and 3.6 in R10, and 3.3 and 3.5 in R25. The influent pH remained between 5.2 and 5.9 in Barros et al. \cite{6}, 4.8 and 5.6 in R10, and 5.5 and 5.9 in R25 (**Figure 2**).

**Figure 3** presents the variation in glucose conversion as a function of OLR for the AFBRs used in this study. To estimate glucose consumption during fermentation, glucose levels were measured in the fermentation medium (**Figure 3**). Glucose consumption by microorganisms was recorded
at all OLR intervals in both AFBRs. The data indicate that glucose conversion decreased with the increase of OLR at all concentrations. For reactor R10, when OLR was increased from 30–120 kg COD m\(^{-3}\) day\(^{-1}\), glucose conversion decreased from 57 to 36%, but when OLR increased to 240 kg COD m\(^{-3}\) day\(^{-1}\), glucose conversion increased to 41%. For reactor R25, when OLR increased from 75 to 600 kg COD m\(^{-3}\) day\(^{-1}\), glucose conversion decreased from 36 to 20%.

Figure 2. pH effluent as a function of the OLR for the AFBRs.

Figure 3. Glucose conversion as a function of the OLR for the AFBRs.
Figure 4 presents the variation in the hydrogen production rate (HPR) as a function of OLR for the two AFBRs used in this study. Similar to the results of Barros et al. [6] for an AFBR with expanded clay as the support material, an influent glucose concentration of 4 g L⁻¹, and alkalinity supplementation (values presented in Figure 2), the HPR values for R10 increased linearly from 0.12 to 1.58 L h⁻¹ L⁻¹ when OLR increased from 30 to 240 kg COD m⁻³. By contrast, a linear relationship between HPR and OLR was not observed in R25 for OLR ranging from 75 to 600 kg COD m⁻³. The maximum HPR values were 1.58 and 0.84 L h⁻¹ L⁻¹ for reactors R10 and R25, respectively.

Figure 5 presents the variation in HY as a function of OLR for the two AFBRs used in this study. The HY values increased with increasing OLR in both reactors. For reactor R10, when OLR was increased from 30 to 120 kg COD m⁻³ day⁻¹, HY increased significantly from 0.48 to 1.32 mol H₂ mol⁻¹ glucose, but when OLR increased to 240 kg COD m⁻³ day⁻¹, HY decreased to 1.04 mol H₂ mol⁻¹ glucose. For reactor R25, when OLR increased from 75 to 300 kg COD m⁻³ day⁻¹, the increase in HY was less significant, i.e., from 0.44 to 0.63 mol H₂ mol⁻¹ glucose, but when OLR increased to 600 kg COD m⁻³ day⁻¹, the yield decreased to 0.56 mol H₂ mol⁻¹ glucose.

Figure 6 presents the variation in H₂ content as a function of OLR for the two AFBRs used in this study. In reactors R10 and R25, the behavior of the H₂ content also varied according to changes in OLR. The hydrogen content of the biogas increased with increasing OLR in both reactors, with a higher H₂ content for HRT 1 h (240 and 600 kg COD m⁻³ day⁻¹, respectively). The H₂ content ranged from 8 to 58% for R10 and 10 to 57% for R25.

The glucose conversion, HPR, HY, and H₂ content of the reactors are consistent with the results of several studies conducted using AFBRs [6, 18, 27, 28, 30–32, 34, 35].
Table 1 compares studies that evaluated OLR and HY. Studies that observed a decrease in HY with increasing OLR used an OLR range of 6–833.3 kg COD m$^{-3}$ day$^{-1}$ and reported HYs of 4.26–0.81 mol H$_2$ mol$^{-1}$ substrate. By contrast, studies that observed an increase in HY with increasing OLR worked with an OLR range of 13.5–480 kg COD m$^{-3}$ day$^{-1}$ and reported HYs of 0.94–2.49 mol H$_2$ mol$^{-1}$ substrate.

![Figure 5. HY as a function of the OLR for the AFBRs.](image1)

![Figure 6. H$_2$ content as a function of the OLR for the AFBRs.](image2)
According to Kraemer and Bagley [26], the reason for the variations of \( \text{H}_2 \) yield at lower or higher OLRs is unknown. High OLR values may reduce the production of \( \text{H}_2 \) by (1) increasing inhibition by volatile fatty acids (VFAs) with increasing OLR, (2) decreasing thermodynamic regulation due to lower dissolved \( \text{H}_2 \) concentrations at lower OLRs, (3) affecting acetogenic activity, and (4) increase \( \text{CO}_2 \) inhibition by increasing the concentration of dissolved \( \text{CO}_2 \). Inhibition by VFAs at high OLR values appears to be a valid explanation. The ability of added external VFA to reduce or inhibit the production of \( \text{H}_2 \) in mixed-culture and continuous-flow systems has been studied, and there is consensus that butyrate increases higher inhibition than the acetate [18, 24, 40].

\( \text{H}_2 \) production was also assessed with or without the addition of sodium bicarbonate as an alkalizing agent. The effect of the alkalizing agent on pH was important for controlling the hydrogen content and \( \text{CO}_2 \) in the system. The high \( \text{HY} \) in the absence of a buffering agent can be attributed to the pH range of the reactor and the \( \text{CO}_2 \) concentrations produced at steady bicarbonate concentrations [41–44].

### 3.2. Soluble microbial products

Table 2 presents the distribution of soluble microbial products (SMPs) with increasing glucose concentration and increasing OLRs in the AFBRs. The molar fractions of acetic and butyric acid were the largest by percentage. Barros et al. [6] for an AFBR with expanded clay as the support material, an influent glucose concentration of 4 g L\(^{-1} \), and alkalinity supplementation (values

| Study                     | Substrate                  | OLR (kg m\(^{-3} \) d\(^{-1} \)) | HY (mol H\(_2\) mol\(^{-1} \) substrate) |
|---------------------------|----------------------------|----------------------------------|-------------------------------------------|
|                           |                            | Low                | High          | Low OLR | High OLR |
| Lower OLR improves \( \text{H}_2 \) production | Rice winery                | 168                | 432          | 1.89    | 1.79    |
| Yu et al. [36]            | Glucose                    | 25.6               | 76.8          | 2.20    | 2.00    |
| Van Ginkel and Logan [24] | Glucose                    | 6                  | 24            | 2.80    | 2.20    |
| Kyazzze et al. [15]       | Sucrose                    | 22.4               | 112.2         | 1.65    | 0.81    |
| Lin et al. [38]           | Sucrose                    | 34.7               | 833.3         | 4.26    | 2.31    |
| Davila-Vasquez et al. [39]| Cheese whey                | 54                 | 138.6         | 2.4     | 1.0     |
| Higher OLR improves \( \text{H}_2 \) production | Sucrose                    | 13.5               | 107.9         | 1.69    | 2.49    |
| Lin et al. [18]           | Sucrose                    | 20                 | 160           | 1.34    | 2.17    |
| Zhang et al. [35]         | Glucose                    | 60                 | 480           | 0.94    | 1.19    |
| Shida et al. [27]         | Glucose                    | 6                  | 48            | 1.84    | 2.29    |
| Perna et al. [17]         | Cheese whey                | 22                 | 37            | 0.5     | 0.67    |

**Table 1.** Comparison of the studies that varied the OLR by changing the substrate concentration.
presented in Table 2) observed a descending order of products of acetate (32.99–46.81%), butyrate (37.30–41.49%), ethanol (10.18–22.95%), and propionate (1.26–4.90%). In our reactor R10, the products in descending order were ethanol (45.54–71.54%), acetate (27.11–50.63%), butyrate (2.91–31.03%) and methanol (0.00–14.41%). In reactor R25, the products in descending order were ethanol (48.00–71.54%), acetate (12.05–37.43%), butyrate (01.02–29.09%), and methanol (0.00–14.41%) (Table 2).

Previous studies employing conditions similar to those used in the present study observed the production of similar metabolites, although differences in the distributions of the metabolites were observed [6, 18, 27, 28, 30–32, 34, 35].

The reactors R10 and R25 produced higher amounts of solvents, such as MetOH and EtOH in the R25 reactor. The higher EtOH concentrations observed in R10 and R25 are similar to the results of Wu et al. [34]. However, our recent studies [6, 27, 29] that used the same medium composition, inoculum, and support material have significantly different results. Barros et al. [6] with an influent glucose concentration of 4 g L⁻¹, and alkalinity supplementation, observed ethanol percentages lower than 22.95% at the beginning of the operation and

| Reactor | OLR (kg COD m⁻³ day⁻¹) | HAc (mM) | HBu (mM) | HPr (mM) | EtOH (mM) | MetOH (mM) | TVFA (mM) | TSolv (mM) | HAc/HBu |
|---------|-------------------------|---------|---------|---------|-----------|-----------|----------|----------|---------|
| Barros et al. [6] | 12 | 6.25 | 7.67 | 0.68 | 4.35 | 0 | 14.60 | 4.35 | 0.81 |
| | 16 | 10.00 | 11.08 | 0.34 | 5.43 | 0 | 21.42 | 5.43 | 0.90 |
| | 24 | 12.50 | 11.08 | 0.41 | 2.72 | 0 | 23.98 | 2.72 | 1.13 |
| | 48 | 12.83 | 10.63 | 0.68 | 4.35 | 0 | 24.13 | 4.35 | 1.21 |
| | 96 | 9.06 | 8.35 | 1.01 | 2.28 | 0 | 18.42 | 2.28 | 1.08 |
| R10 | 30 | 10.73 | 0.62 | 0.00 | 9.35 | 0.49 | 11.34 | 9.84 | 17.42 |
| | 40 | 7.23 | 1.57 | 0.00 | 10.62 | 1.44 | 8.80 | 12.06 | 4.62 |
| | 60 | 9.66 | 3.53 | 0.00 | 12.70 | 9.58 | 13.20 | 22.28 | 2.74 |
| | 120 | 6.37 | 5.75 | 0.00 | 10.70 | 0.00 | 12.11 | 10.7 | 1.11 |
| | 240 | 6.65 | 7.61 | 0.00 | 10.27 | 0.00 | 14.27 | 10.27 | 0.87 |
| R25 | 75 | 9.04 | 2.60 | 0.13 | 11.59 | 0.78 | 11.77 | 12.37 | 3.47 |
| | 100 | 17.39 | 2.70 | 1.20 | 21.24 | 4.10 | 21.30 | 25.34 | 6.43 |
| | 150 | 6.64 | 1.11 | 0.00 | 39.42 | 7.94 | 11.70 | 47.36 | 6.01 |
| | 300 | 5.92 | 3.53 | 0.00 | 10.65 | 2.01 | 9.45 | 12.66 | 1.68 |
| | 600 | 4.88 | 6.18 | 0.00 | 10.18 | 0.00 | 11.06 | 10.18 | 0.79 |

Table 2. Effect of glucose concentration and OLR on the SMP distribution in the AFBRs.
subsequently decreased and stabilized to 11%. EtOH production is considered unfavorable for hydrogen metabolite production because no \( \text{H}_2 \) is consumed or produced (Eq. (1)):

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{CO}_2
\]  

(1)

Propionate was only detected during the operation of the reactor containing 25 g L\(^{-1}\), with maximum concentration of 1.20 mM in the OLR of 100 kg COD m\(^{-3}\) day\(^{-1}\). Propionic acid production was not observed in AFBRs with influent glucose concentration of 2 g L\(^{-1}\) [27, 29]. Zhang et al. [35] suggested that the absence of propionic acid may be due to inhibition of the activity of the bacteria that form this acid under low pH conditions; these bacteria may be sensitive to both low HRTs and high OLRs. Moreover, the absence of propionic acid production ensures greater production of hydrogen due to the lower consumption of \( \text{H}_2 \) for forming propionate (Eq. (2)):

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2 \rightarrow \text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O}
\]  

(2)

Both HAc and HBu are soluble metabolites favoring \( \text{H}_2 \) production because these products are generated during \( \text{H}_2 \) production (Eqs. (3) and (4)):

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{CO}_2 + 4\text{H}_2
\]  

(3)

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2
\]  

(4)

Previous studies have observed that \( \text{H}_2 \) production increases with the molar ratio of HAc/HBu [45, 46]. Table 2 presents the variation of the HAc/HBu ratio in R10 and R25. Barros et al. [6] for an influent glucose concentration of 4 g L\(^{-1}\), and alkalinity supplementation, observed the best proportion of soluble metabolites and therefore a higher yield of hydrogen, with molar ratios of HAc/HBu ranging from 0.81 to 1.21 for OLRs varied 12–96 kg COD m\(^{-3}\) day\(^{-1}\), respectively, but decreasing to 1.08 for an OLR of 96 kg COD m\(^{-3}\) day\(^{-1}\). In our R25, similar behavior of Barros et al. [6] were obtained, but in R10 HAc/HBu ratio decreased from 17.42 to 0.87 when the OLRs increased from 30 to 240 kg COD m\(^{-3}\) day\(^{-1}\).

According to Hafez et al. [45], when OLR increased from 6.5 to 103 g COD L\(^{-1}\) day\(^{-1}\), acetate and butyrate were the main liquid products, with trace concentrations of ethanol and no detectable lactate, whereas in the OLR range of 154–206 g COD L\(^{-1}\) day\(^{-1}\), the concentrations of propionate, isovalerate, valerate, and ethanol increased markedly. The steady-state average molar ratios of acetate/butyrate were 2.3, 2.3, 2.0, and 2.2 for OLRs of 6.5, 25.7, 51.4, and 103 g COD L\(^{-1}\) day\(^{-1}\), respectively, but decreased to 1.1 for OLRs of 154 and 206 g COD L\(^{-1}\) day\(^{-1}\).

According to Prakasham et al. [47], at lower substrate conditions with the limitation of substrate concentration, increasing glucose concentration progressively increases \( \text{H}_2 \) production because of effective metabolism and further \( \text{H}_2 \) production process. However, higher concentrations can also negatively impact \( \text{H}_2 \) production. When the \( \text{H}_2 \) yield observed value reduced
because the glucose concentration was above the optimum value, a limited glucose utilization occurred, or a shift in the metabolic pathway from the acidogenic phase to a solventogenic phase took place.

Hydrogen and CO$_2$ were the only gaseous metabolites during all stages of the experiment. NO CH$_4$ was detected in the biogas from either reactor. The combination of heat treatment of the inoculum and operation under acidogenic pH conditions inhibited the methanogenic activity responsible for the consumption of hydrogen in the system. Furthermore, the results in the literature suggest that manipulating some operational parameters such as the HRT contributes to the elimination of methanogenic archaea in the reactors.

According to Chen et al. [48], these microorganisms fail to thrive in part because the maximum specific growth rate of methanogenic archaea ($\mu_{\text{maximum}} = 0.0167$ h$^{-1}$) is significantly lower than that of acidogenic microorganisms ($\mu_{\text{maximum}} = 0.083$ h$^{-1}$). Thus, methanogenic microorganisms are unable to reproduce or remain in equilibrium under these conditions, resulting in their removal from the reactor.

### 3.3. COD removal and carbon balance

The carbon balance in the reactors can be calculated by Eq. (5) according to Gavala et al. [49]. The comparison between measured and calculated COD concentrations for each steady state is also presented. The COD calculations were performed as the following: the products (COD$_{\text{products}}$) and the glucose (COD$_{\text{glucose}}$) COD concentrations were calculated according to Eqs. (5) and (6), respectively. The COD$_{\text{residual}}$ was calculated after subtraction of the sum of the COD$_{\text{products}}$ and COD$_{\text{glucose}}$ from the COD$_{\text{measured}}$ (Eq. (3)). The COD$_{\text{others}}$ corresponds to the non-identified metabolic products during glucose fermentation:

$$
COD_{\text{products}} = a \cdot \left( \frac{\text{mmolHAc}}{\text{mmolHAc}} \right) \cdot 64 \cdot \frac{\text{mgCOD}_{\text{mmolHAc}}}{\text{mgCOD}_{\text{mmolHAc}}} + b \cdot \left( \frac{\text{mmolHBu}}{\text{mmolHBu}} \right) \cdot 160 \cdot \frac{\text{mgCOD}_{\text{mmolHBu}}}{\text{mgCOD}_{\text{mmolHBu}}} \\
+ c \cdot \left( \frac{\text{mmolHPr}}{\text{mmolHPr}} \right) \cdot 112 \cdot \frac{\text{mgCOD}_{\text{mmolHPr}}}{\text{mgCOD}_{\text{mmolHPr}}} + d \cdot \left( \frac{\text{mmolMetOH}}{\text{mmolMetOH}} \right) \cdot 48 \cdot \frac{\text{mgCOD}_{\text{mmolMetOH}}}{\text{mgCOD}_{\text{mmolMetOH}}} \\
+ e \cdot \left( \frac{\text{mmolEtOH}}{\text{mmolEtOH}} \right) \cdot 96 \cdot \frac{\text{mgCOD}_{\text{mmolEtOH}}}{\text{mgCOD}_{\text{mmolEtOH}}} 
$$

where a, b, c, d, and e are the measured concentrations of the acetic acid, butyric acid, propionic acid, methanol, and ethanol, respectively.

$$
COD_{\text{glucose}} = f \cdot \left( \frac{\text{mg Glucose}}{\text{mg Glucose}} \right) \frac{192 \text{ mg COD}}{180 \text{ mg}}
$$

where $f$ is the measured concentration of glucose.

The difference between COD$_{\text{measured}}$ and COD based on SMP may be attributed to the presence of other soluble metabolites that were not detected, e.g., lactic acid and formic acid, because the chromatographic method of headspace extraction used in this study only detects alcohols and volatile acids.
This difference was calculated based on Eq. (7):

\[
COD_{\text{others}} = COD_{\text{measured}} - (COD_{\text{products}} + COD_{\text{glucose}})
\]  

(7)

Table 3 presents influent and effluent COD values and standard deviations as well as efficiencies for all reactors. Influent COD represents glucose added to the wastewater and carbonaceous matter present in urea. Effluent COD corresponds to the carbonaceous matter in the effluent that was oxidized. Carbonaceous matter present in the effluent consists of non-consumed glucose; soluble metabolites, e.g., organic acids, solvents, and other intermediary compounds; and biomass detached from the support medium.

| OLR (kg COD m\(^{-3}\) day\(^{-1}\)) | Influent COD (mg L\(^{-1}\)) | Effluent COD (mg L\(^{-1}\)) | COD removal (%) |
|----------------------------------------|-----------------------------|-----------------------------|-----------------|
| Barros et al. [6]                      |                             |                             |                 |
| 12                                     | 4216 ± 210                  | 3788 ± 153                  | 10 ± 6          |
| 16                                     | 4140 ± 206                  | 3349 ± 146                  | 19 ± 9          |
| 24                                     | 4139 ± 270                  | 3718 ± 165                  | 10 ± 4          |
| 48                                     | 4487 ± 220                  | 3805 ± 191                  | 15 ± 2          |
| 96                                     | 4312 ± 226                  | 3680 ± 136                  | 15 ± 4          |
| R10                                    |                             |                             |                 |
| 30                                     | 11,298 ± 954                | 8617 ± 457                  | 24 ± 5          |
| 40                                     | 10,439 ± 843                | 9056 ± 419                  | 13 ± 6          |
| 60                                     | 10,693 ± 977                | 8639 ± 433                  | 19 ± 3          |
| 120                                    | 10,175 ± 799                | 8589 ± 447                  | 16 ± 2          |
| 240                                    | 10,969 ± 901                | 8705 ± 512                  | 21 ± 2          |
| R25                                    |                             |                             |                 |
| 75                                     | 26,126 ± 1024               | 20,202 ± 978                | 23 ± 3          |
| 100                                     | 26,447 ± 1201               | 22,352 ± 883                | 15 ± 2          |
| 150                                     | 27,285 ± 1392               | 22,207 ± 791                | 19 ± 2          |
| 300                                     | 26,116 ± 1273               | 23,502 ± 943                | 10 ± 1          |
| 600                                     | 28,216 ± 1321               | 25,242 ± 967                | 11 ± 2          |

Table 3. Influent COD, effluent COD, and COD removal in AFBRs.

The theoretical effluent COD was calculated based on stoichiometric relationships for oxidation of glucose, acetic acid, butyric acid, propionic acid, biomass, ethanol, and methanol to estimate the carbon balance. Theoretical COD values for the remaining glucose, soluble metabolites, and biomass as well as the difference between the theoretical total COD and the COD measured for all reactors are presented in Table 4.

In the reactor operated by Barros et al. [6], this difference varied between 12 and 350 mg L\(^{-1}\), which corresponded to a variation of 0.34 and 9.19%. The reactor R10 showed a difference ranging from 91 to 301 mg L\(^{-1}\) (variation of 1.05 and 3.28%), whereas in the reactor R25, the difference varied between 17 and 1026 mg L\(^{-1}\) (variation of 0.07 and 4.62%). Those differences
may be accredited to the presence of other metabolites such as lactic acid and formic acid that were not detected, probably due to the chromatographic method performed (headspace extraction), considering that this method can only detect volatile acids and alcohols.

The largest variation between COD measured in the effluent and the theoretical COD (corresponding to glucose, soluble metabolites, and biomass in the effluent) was 9.19% based on the results obtained from the carbon balance. However, according to Standard Methods [33], the determination of metabolites and COD produces errors of close to 10%. For that reason, this variation may be attributed to the margin of error of the determination methods used.

4. Conclusions

Satisfactory performance for H₂ production was observed in the anaerobic fluidized bed reactor containing 10 g L⁻¹ glucose. However, in the reactor containing 25 g L⁻¹ glucose, the yield was limited.

The HPR had a linear increase with OLR, with the exception of reactor operated with 25 g L⁻¹ glucose. The maximum HPR was 1.58 L h⁻¹ L⁻¹ obtained in the reactor with 10 g L⁻¹ glucose for

| Reactor | OLR (kg COD m⁻³ day⁻¹) | HRT (h) | COD_t, glucose (mg L⁻¹) | COD_t, acetate (mg L⁻¹) | COD_t, butyrate (mg L⁻¹) | COD_t, propionate (mg L⁻¹) | COD_t, biomass (mg L⁻¹) | COD_t, ethanol (mg L⁻¹) | COD_t, methanol (mg L⁻¹) | COD_total (mg L⁻¹) | COD_measured (mg L⁻¹) | COD_others (mg L⁻¹) |
|---------|------------------------|---------|-------------------------|------------------------|--------------------------|--------------------------|--------------------------|-------------------------|-------------------------|---------------------|----------------------|---------------------|
| Barros et al. [6] | 12 | 8 | 946 | 245 | 1382 | 0 | 192 | 90 | 24 | 3405 | 3788 | 39 |
| | 16 | 6 | 475 | 192 | 1000 | 0 | 157 | 203 | 105 | 3157 | 3349 | 32 |
| | 24 | 4 | 901 | 320 | 1563 | 0 | 161 | 215 | 0 | 3432 | 3719 | 12 |
| | 48 | 2 | 666 | 320 | 1763 | 0 | 155 | 629 | 0 | 3455 | 3805 | 350 |
| | 96 | 1 | 1394 | 235 | 964 | 0 | 181 | 573 | 0 | 3556 | 3680 | 124 |
| R10 | 30 | 8 | 4514 | 757 | 645 | 0 | 148 | 1540 | 940 | 8545 | 8617 | 159 |
| | 40 | 6 | 5807 | 438 | 705 | 0 | 157 | 457 | 564 | 8129 | 9056 | 104 |
| | 60 | 4 | 6935 | 291 | 551 | 0 | 140 | 631 | 0 | 8548 | 8639 | 91 |
| | 120 | 2 | 6659 | 364 | 858 | 0 | 134 | 585 | 0 | 8600 | 8589 | 254 |
| | 240 | 1 | 6639 | 294 | 699 | 0 | 168 | 959 | 104 | 8862 | 8705 | 301 |
| R25 | 75 | 8 | 17,177 | 1210 | 271 | 47 | 148 | 2178 | 144 | 21,174 | 20,202 | 1026 |
| | 100 | 6 | 16,590 | 769 | 330 | 0 | 145 | 4825 | 760 | 23,419 | 22,352 | 486 |
| | 150 | 4 | 19,454 | 452 | 425 | 0 | 141 | 1692 | 275 | 22,439 | 22,207 | 107 |
| | 300 | 2 | 21,122 | 373 | 636 | 0 | 134 | 1360 | 96 | 23,722 | 23,502 | 17 |
| | 600 | 1 | 22,996 | 269 | 751 | 0 | 168 | 1023 | 0 | 25,206 | 25,242 | 35 |

Table 4. Theoretical COD values of soluble metabolites, biomass COD, and effluent COD measured in AFBRs.
OLR of 240 kg COD m\(^{-3}\) day\(^{-1}\) (HRT = 1 h). The maximum HY was 1.32 mol H\(_2\) mol\(^{-1}\) glucose obtained in the reactor with 10 g L\(^{-1}\) glucose for HRT 2 h (OLR = 240 kg COD m\(^{-3}\) day\(^{-1}\)).

The H\(_2\) production with addition of sodium bicarbonate was important to control the pH and CO\(_2\) system. The reactors operated at high glucose concentrations (10 and 25 g L\(^{-1}\)) showed higher proportions of solvents.

**Author details**

Eduardo Lucena Cavalcante de Amorim\(^{1,2}\), Leandro Takano Sader\(^3\), Lucas Rodrigues Ramos\(^3\) and Edson Luiz Silva\(*\)

\*Address all correspondence to: edsilva@ufscar.br

1 Technology Center, Federal University of Alagoas, Maceió, AL, Brazil
2 Department of Hydraulics and Sanitation, University of São Paulo, São Carlos, SP, Brazil
3 Department of Chemical Engineering, Federal University of São Carlos, São Carlos, SP, Brazil

**References**

[1] Bartacek J, Zabranska J, Lens PNL. Developments and constraints in fermentative hydrogen production. Biofuels, Bioproducts Biorefining. 2007;1:201–214. DOI: 10.1002/bbb.

[2] Mohan SV. Harnessing of biohydrogen from wastewater treatment using mixed fermentative consortia: Process evaluation towards optimization. International Journal of Hydrogen Energy. 2009;34:7460–7474. DOI: 10.1016/j.ijhydene.2009.05.062.

[3] Reddy MV, Amulya K, Rohit MV, Sarma PN, Mohan SV. Valorization of fatty acid waste for bioplastics production using Bacillus tequilensis: Integration with dark-fermentative hydrogen production process. International Journal of Hydrogen Energy. 2014;39:7616–7626. DOI: 10.1016/j.ijhydene.2013.09.157.

[4] Sarma SJ, Brar SK, Bihan YL, Buelna G. Liquid waste from bio-hydrogen production—a commercially attractive alternative for phosphate solubilizing bio-fertilizer. International Journal of Hydrogen Energy. 2013;38:8704–8707. DOI: 10.1016/j.ijhydene.2013.05.032

[5] Sarma SJ, Pachapur V, Brar SK, Bihan YL, Buelna G. Hydrogen biorefinery: Potential utilization of the liquid waste from fermentative hydrogen production. Renewable and Sustainable Energy Reviews. 2015;50:942–951. DOI: 10.1016/j.rser.2015.04.191.

[6] Barros AR, Amorim ELC, Reis CM, Shida GM, Silva EL. Biohydrogen production in anaerobic fluidized bed reactors: Effect of support material and hydraulic retention time. International Journal of Hydrogen Energy. 2010;35:3379–3388. DOI: 10.1016/j.ijhydene.2010.01.108.
[7] Rosa PRF, Santos SC, Silva EL. Different ratios of carbon sources in the fermentation of cheese whey and glucose as substrates for hydrogen production and ethanol production in continuous reactors. International Journal of Hydrogen Energy. 2014;39:1288–1296. DOI: 10.1016/j.ijhydene.2013.11.011.

[8] Rosa PRF, Santos SC, Sakamoto IK, Varesche MBA, Silva EL. The effects of seed sludge and hydraulic retention time on the production of hydrogen from cassava processing wastewater and glucose mixture in an anaerobic fluidized bed reactor. International Journal of Hydrogen Energy. 2014;39:13118–13127. DOI: 10.1016/j.ijhydene.2014.06.152.

[9] Santos SC, Rosa PRF, Sakamoto IK, Varesche MBA, Silva EL. Organic loading rate impact on biohydrogen production and microbial communities at anaerobic fluidized thermophilic bed reactors treating sugarcane stillage. Bioresource Technology. 2014;159:55–63. DOI: 10.1016/j.biortech.2014.02.051.

[10] Li CL, Fang HHP. Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. Critical Reviews in Environmental Science and Technology. 2007;37:1–39. DOI: 10.1080/10643380600729071.

[11] Wang J, Wan E. Factors influencing fermentative hydrogen production: A review. International Journal of Hydrogen Energy. 2009;34:799–811. DOI: 10.1016/j.ijhydene.2008.11.015.

[12] Beckers L, Masset J, Hamilton C, Delvigne F, Toye D, Crine M, Thonart P, Hiligsmann S. Investigation of the links between mass transfer conditions, dissolved hydrogen concentration and biohydrogen production by the pure strain Clostridium butyricum CWBI1009. Biochemical Engineering Journal. 2015;98:18–28. DOI: 10.1016/j.bej.2015.01.008.

[13] Chen CC, Chen HP, Wu JH, Lin CY. Fermentative hydrogen production at high sulfate concentration. International Journal of Hydrogen Energy. 2008;33:1573–1578. DOI: 10.1016/j.ijhydene.2007.09.042.

[14] Shen L, Bagley DM, Liss SN. Effect of organic loading rate on fermentative hydrogen production from continuous stirred tank and membrane bioreactors. International Journal of Hydrogen Energy. 2009;34:3689–3696. DOI: 10.1016/j.ijhydene.2009.03.006.

[15] Kyazze G, Martinez-Perez N, Dinsdale R, Premier GC, Hawkes FR, Guwy AJ, Hawkes DL. Influence of substrate concentration on the stability and yield of continuous biohydrogen production. Biotechnology and Bioengineering. 2006;93:971–979. DOI: 10.1002/bit.20802.

[16] Sreethawong T, Chatsiriwatana S, Rangsunvigit P, Chavadej S. Hydrogen production from cassava wastewater using anaerobic sequencing batch reactor: Effect of operational parameters, COD:N ratio, and organic acid composition. International Journal of Hydrogen Energy. 2010;35:4092–4102. DOI: 10.1016/j.ijhydene.2010.02.030.

[17] Perna V, Castelló E, Wenzel J, Zampol C, Fontes Lima DM, Borzacconi L, Varesche MB, Zaiat M, Etchebehere C. Hydrogen production in an upflow anaerobic packed bed reactor
used to treat cheese whey. International Journal of Hydrogen Energy. 2013;38:54–62. DOI: 10.1016/j.ijhydene.2012.10.022.

[18] Lin CN, Wu SY, Chang, JS. Fermentative hydrogen production with a draft tube fluidized bed reactor containing silicone-gel-immobilized anaerobic sludge. International Journal of Hydrogen Energy. 2006;31:2200–2210. DOI: 10.1016/j.ijhydene.2006.05.012.

[19] Jung KW, Kim DH, Kim SH, Shin HS. Bioreactor design for continuous dark fermentative hydrogen production. Bioresource Technology. 2011;102:8612–8620. DOI: 10.1016/j.biortech.2011.03.056.

[20] Show K, Lee D, Chang J. Bioreactor and process design for biohydrogen production. Bioresource Technology. 2011;102:8524–8533. DOI: 10.1016/j.biortech.2011.04.055.

[21] Barca C, Soric A, Ranava D, Giudici-Orticoni MT, Ferrasse JH. Anaerobic biofilm reactors for dark fermentative hydrogen production from wastewater: A review. Bioresource Technology. 2015;185:386–398. DOI: 10.1016/j.biortech.2015.02.063.

[22] Mohammadi P, Ibrahim S, Annuar MSM, Ghafari S, Vikineswary S, Zinatizadeh AA. Influences of environmental and operational factors on dark fermentative hydrogen production: A review. Clean—Soil, Air, Water. 2012;40:1297–1305. DOI: 10.1002/clen.201100007.

[23] Tawfik A, Salem A. The effect of organic loading rate on bio-hydrogen production from pre-treated rice straw waste via mesophilic up-flow anaerobic reactor. Bioresource Technology. 2012;107:186–190. DOI: 10.1016/j.biortech.2011.11.086.

[24] Van Ginkel SW, Logan BE. Inhibition of biohydrogen production by undissociated acetic and butyric acids. Environmental Science and Technology. 2005;39:9351–9356. DOI: 10.1021/es0510515.

[25] Kim S, Han S, Shin H. Effect of substrate concentration on hydrogen production and 16S rDNA-based analysis of the microbial community in a continuous fermenter. Process Biochemistry. 2006;41:199–2007. DOI: 10.1016/j.procbio.2005.06.013.

[26] Kraemer JT, Bagley DM. Improving the yield from fermentative hydrogen production. Biotechnology Letters. 2007;29:685–695. DOI: 10.1007/s10529-006-9299-9.

[27] Shida GM, Barros AR, Reis CM, Amorim ELC, Damianovic MHRZ, Silva EL. Long-term stability of hydrogen and organic acids production in an anaerobic fluidized-bed reactor using heat treated anaerobic sludge inoculum. International Journal of Hydrogen Energy. 2009;34:3679–3688. DOI: 10.1016/j.ijhydene.2009.02.076.

[28] Shida GM, Sader LT, Amorim ELC, Sakamoto IK, Maintinguer SI, Saavedra NK, Varesche MBA, Silva EL. Performance and composition of bacterial communities in anaerobic fluidized bed reactors for hydrogen production: Effects of organic loading rate and alkalinity. International Journal of Hydrogen Energy. 2012;37:16925–16934. DOI: 10.1016/j.ijhydene.2012.08.140.
[29] Amorim ELC, Barros AR, Damianovic MHRZ, Silva EL. Anaerobic fluidized bed reactor with expanded clay as support for hydrogen production through dark fermentation of glucose. International Journal of Hydrogen Energy. 2009;34:783–790. DOI: 10.1016/j.ijhydene.2008.11.007.

[30] Barros AR, Silva EL. Hydrogen and ethanol production in anaerobic fluidized bed reactors: Performance evaluation for three support materials under different operating conditions. Biochemical Engineering Journal. 2012;61:59–65. DOI: 10.1016/j.bej.2011.12.002.

[31] Reis CM, Silva EL. Effect of upflow velocity and hydraulic retention time in anaerobic fluidized-bed reactor used for hydrogen production. Chemical Engineering Journal. 2011;172:28–36. DOI: 10.1016/j.cej.2011.05.009.

[32] Amorim ELC, Sader LT, Silva EL. Effect of substrate concentration on dark fermentation hydrogen production using an anaerobic fluidized bed reactor. Applied Biochemistry and Biotechnology. 2012;166:1248–1263. DOI: 10.1007/s12010-011-9511-9.

[33] Greenberg AE, Clesceri LS, Eaton AD, editors. Standard methods for the examination for water and wastewater. 20th ed. Washington, DC, USA: APHA, WEF, AWWA; 1998. 1496 p.

[34] Wu SY, Lin CN, Chang JS, Lee KS, Lin PJ. Hydrogen production with immobilized sewage sludge in three-phase fluidized-bed bioreactor. Biotechnology Progress. 2003;19:828–832. DOI: 10.1021/bp0201354.

[35] Zhang ZP, Tay JH, Show KY, Yan R, Liang DT, Lee DJ, Jiang WJ. Biohydrogen production in a granular activated carbon anaerobic fluidized bed reactor. International Journal of Hydrogen Energy. 2007;32:185–191. DOI: 10.1016/j.ijhydene.2006.08.017.

[36] Yu HQ, Zhu ZH, Hu WR, Zhang HS. Hydrogen production from rice winery wastewater in an upflow anaerobic reactor by using mixed anaerobic cultures. International Journal of Hydrogen Energy. 2002;27:1359–1365. DOI: 10.1016/s0360-3199(2)00073-3.

[37] Van Ginkel SW, Logan BE. Increased biological hydrogen production with reduced organic loading. Water Research. 2005;39:3819–3826. DOI: 10.1016/j.watres.2005.07.021.

[38] Lin CN, Wu SY, Chang JS, Chang JS. Biohydrogen production in a three-phase fluidized bed bioreactor using sewage sludge immobilized by ethylene-vinyl acetate copolymer. Bioresource Technology. 2009;100:3298–3301. DOI: 10.1016/j.biortech.2009.02.027.

[39] Davila-Vasquez G, Cota-Navarro CB, Rosales-Colunga LM. Continuous biohydrogen production using cheese whey: Improving the hydrogen production rate. International Journal of Hydrogen Energy. 2009;34:4296–4304. DOI: 10.1016/j.ijhydene.2009.02.063.

[40] Gao S, Wang B, Zhu LL, Han W, Chen H, Li YF. Effect of organic loading rate on fermentative hydrogen production in CSTR. Advanced Marerials Research. 2011;156–157:732–736. DOI: 10.4028/www.scientific.net/AMR.
[41] Leite JAC, Fernandes BS, Pozzi E, Barboza M, Zaiat M. Application of an anaerobic packed-bed bioreactor for the production of hydrogen and organic acids. International Journal of Hydrogen Energy. 2008;33:579–586. DOI: 10.1016/j.ijhydene.2007.10.009.

[42] Valdez-Vazquez I, Poggi-Varaldo HM. Alkalinity and high total solids affecting H\textsubscript{2} production from organic solid waste by anaerobic consortia. International Journal of Hydrogen Energy. 2009;34:3639–3646. DOI: 10.1016/j.ijhydene.2009.02.039.

[43] Choi J, Ahn Y. Biohydrogen fermentation from sucrose and piggery waste with high levels of bicarbonate alkalinity. Energies. 2015;8:1716–1729. DOI: 10.3390/en8031716.

[44] Silva AJ, Pozzi E, Foresti E, Zaiat M. The influence of the buffering capacity on the production of organic acids and alcohols from wastewater in anaerobic reactor. Applied Biochemistry and Biotechnology. 2015;175:2258–2265. DOI: 10.1007/s12010-014-1424-y.

[45] Hafez H, Nakhla G, El Naggar MH, Elbeshbishy E, Baghchehsaraee B. Effect of organic loading rate on a novel hydrogen bioreactor. International Journal of Hydrogen Energy. 2010;35:81–92. DOI: 10.1016/j.ijhydene.2009.10.051.

[46] Wang JL, Wan W. The effect of substrate concentration on biohydrogen production by using kinetic models. Science in China Series B-Chemistry. 2008;51:1110–1117. DOI: 10.1007/s11426-008-0104-6.

[47] Prakasham RS, Brahmaiah P, Satish T, SambasivaRao KRS. Fermentative biohydrogen production by mixed anaerobic consortia: Impact of glucose to xylose ratio. International Journal of Hydrogen Energy. 2010;34:9354–9361. DOI: 10.1016/j.ijhydene.2009.09.104.

[48] Chen CC, Lin CY, Chang JS. Kinetics of hydrogen production with continuous anaerobic cultures utilizing sucrose as the limiting substrate. Applied Microbiology and Biotechnology. 2001;57:56–64. DOI: 10.1007/s002530100747.

[49] Gavala HN, Skiadas IV, Ahring BK. Biological hydrogen production in suspended and attached growth anaerobic reactor systems. International Journal of Hydrogen Energy. 2006;31:1164–1175. DOI: 10.1016/j.ijhydene.2005.09.009.