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Chapter 12

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

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Additional information is available at the end of the chapter

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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 H\(_2\) yield of 1.32 mol 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 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 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\(_2\), 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].

![Figure 1. Schematic description of the AFBR.](http://dx.doi.org/10.5772/67101)

### 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 \(\text{H}_2\)-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 (\(\text{H}_2\) and \(\text{CO}_2\)) and soluble metabolites (volatile organic acids and alcohols) produced during fermentative \(\text{H}_2\) 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 [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 [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 \(\times\) 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 \(\times\) 0.25 mm internal diameter \(\times\) 0.25 mm film thickness) [32].

A gas meter (type TG1; Ritter Inc., Germany) was used to measure the amount of \(\text{H}_2\) generated.

### 3. Results and discussion

#### 3.1. Effect of OLR on \(\text{H}_2\) 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. [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. [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 4. HPR as a function of the OLR for the AFBRs.

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 H$_2$ mol$^{-1}$ glucose, but when OLR increased to 240 kg COD m$^{-3}$ day$^{-1}$, HY decreased to 1.04 mol H$_2$ 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 H$_2$ mol$^{-1}$ glucose, but when OLR increased to 600 kg COD m$^{-3}$ day$^{-1}$, the yield decreased to 0.56 mol H$_2$ mol$^{-1}$ glucose.

Figure 6 presents the variation in H$_2$ content as a function of OLR for the two AFBRs used in this study. In reactors R10 and R25, the behavior of the H$_2$ content also varied according to changes in OLR. The hydrogen content of the biogas increased with increasing OLR in both reactors, with a higher H$_2$ content for HRT 1 h (240 and 600 kg COD m$^{-3}$ day$^{-1}$, respectively). The H$_2$ content ranged from 8 to 58% for R10 and 10 to 57% for R25.

The glucose conversion, HPR, HY, and H$_2$ 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 H₂ 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 H₂ mol⁻¹ substrate.

Figure 5. HY as a function of the OLR for the AFBRs.

Figure 6. H₂ content as a function of the OLR for the AFBRs.
According to Kraemer and Bagley [26], the reason for the variations of H\(_2\) yield at lower or higher OLRs is unknown. High OLR values may reduce the production of H\(_2\) by (1) increasing inhibition by volatile fatty acids (VFAs) with increasing OLR, (2) decreasing thermodynamic regulation due to lower dissolved H\(_2\) concentrations at lower OLRs, (3) affecting acetogenic activity, and (4) increase CO\(_2\) inhibition by increasing the concentration of dissolved 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 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].

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 CO\(_2\) in the system. The high HY in the absence of a buffering agent can be attributed to the pH range of the reactor and the CO\(_2\) concentrations produced at steady bicarbonate concentrations [41–44].

### 3.2. Soluble microbial products

Table 2 presents the distribution of soluble microbial products (SMP’s) 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...
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

<table>
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<th>Reactor</th>
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<th>HBu (mM)</th>
<th>HPr (mM)</th>
<th>EtOH (mM)</th>
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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 H\textsubscript{2} is consumed or produced (Eq. (1)):

\[
C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} \rightarrow 2 \text{CH}_3\text{CH}_2\text{OH} + 2 \text{CO}_2 \tag{1}
\]

Propionate was only detected during the operation of the reactor containing 25 g L\textsuperscript{-1}, with maximum concentration of 1.20 mM in the OLR of 100 kg COD m\textsuperscript{3} day\textsuperscript{-1}. Propionic acid production was not observed in AFBRs with influent glucose concentration of 2 g L\textsuperscript{-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 H\textsubscript{2} for forming propionate (Eq. (2)):

\[
C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} + 2 \text{H}_2 \rightarrow \text{CH}_3\text{CH}_2\text{COOH} + 2 \text{H}_2\text{O} \tag{2}
\]

Both HAc and HBu are soluble metabolites favoring H\textsubscript{2} production because these products are generated during H\textsubscript{2} production (Eqs. (3) and (4)):

\[
C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} + 2 \text{H}_2 \rightarrow 2 \text{CH}_3\text{COOH} + 2 \text{CO}_2 + 4 \text{H}_2 \tag{3}
\]

\[
C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2 \text{CO}_2 + 2 \text{H}_2 \tag{4}
\]

Previous studies have observed that H\textsubscript{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\textsuperscript{-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\textsuperscript{3} day\textsuperscript{-1}, respectively, but decreasing to 1.08 for an OLR of 96 kg COD m\textsuperscript{3} day\textsuperscript{-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\textsuperscript{3} day\textsuperscript{-1}.

According to Hafez et al. [45], when OLR increased from 6.5 to 103 g COD L\textsuperscript{-1} day\textsuperscript{-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\textsuperscript{-1} day\textsuperscript{-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\textsuperscript{-1} day\textsuperscript{-1}, respectively, but decreased to 1.1 for OLRs of 154 and 206 g COD L\textsuperscript{-1} day\textsuperscript{-1}.

According to Prakasham et al. [47], at lower substrate conditions with the limitation of substrate concentration, increasing glucose concentration progressively increases H\textsubscript{2} production because of effective metabolism and further H\textsubscript{2} production process. However, higher concentrations can also negatively impact H\textsubscript{2} production. When the H\textsubscript{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:

$$
\text{COD}_{\text{products}} = a \cdot \left( \frac{\text{mmolHAc}}{1} \right) \cdot 64 \cdot \frac{\text{mgCOD}}{\text{mmolHAc}} + b \cdot \left( \frac{\text{mmolHBu}}{1} \right) \cdot 160 \cdot \frac{\text{mgCOD}}{\text{mmolHBu}} + c \cdot \left( \frac{\text{mmolHPr}}{1} \right) \cdot 112 \cdot \frac{\text{mgCOD}}{\text{mmolHPr}} + d \cdot \left( \frac{\text{mmolMetOH}}{1} \right) \cdot 48 \cdot \frac{\text{mgCOD}}{\text{mmolMetOH}} + e \cdot \left( \frac{\text{mmolEtOH}}{1} \right) \cdot 96 \cdot \frac{\text{mgCOD}}{\text{mmolEtOH}}
$$

(5)

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

$$
\text{COD}_{\text{glucose}} = f \cdot \left( \frac{\text{mg Glucose}}{1} \right) \cdot 192 \cdot \frac{\text{mg COD}}{180 \text{ mg}}
$$

(6)

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.

<table>
<thead>
<tr>
<th>OLR (kg COD m⁻³ day⁻¹)</th>
<th>Influent COD (mg L⁻¹)</th>
<th>Effluent COD (mg L⁻¹)</th>
<th>COD removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barros et al. [6]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>4216 ± 210</td>
<td>3788 ± 153</td>
<td>10 ± 6</td>
</tr>
<tr>
<td>16</td>
<td>4140 ± 206</td>
<td>3349 ± 146</td>
<td>19 ± 9</td>
</tr>
<tr>
<td>24</td>
<td>4139 ± 270</td>
<td>3718 ± 165</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>48</td>
<td>4487 ± 220</td>
<td>3805 ± 191</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>96</td>
<td>4312 ± 226</td>
<td>3680 ± 136</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>R10</td>
<td>30</td>
<td>11,298 ± 954</td>
<td>8617 ± 457</td>
</tr>
<tr>
<td>40</td>
<td>10,439 ± 843</td>
<td>9056 ± 419</td>
<td>13 ± 6</td>
</tr>
<tr>
<td>60</td>
<td>10,693 ± 977</td>
<td>8639 ± 433</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>120</td>
<td>10,175 ± 799</td>
<td>8589 ± 447</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>240</td>
<td>10,969 ± 901</td>
<td>8705 ± 512</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>R25</td>
<td>75</td>
<td>26,126 ± 1024</td>
<td>20,202 ± 978</td>
</tr>
<tr>
<td>100</td>
<td>26,447 ± 1201</td>
<td>22,355 ± 883</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>150</td>
<td>27,285 ± 1392</td>
<td>22,207 ± 791</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>300</td>
<td>26,116 ± 1273</td>
<td>23,502 ± 943</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>600</td>
<td>28,216 ± 1321</td>
<td>25,242 ± 967</td>
<td>11 ± 2</td>
</tr>
</tbody>
</table>

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 \( \text{H}_2 \) 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

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

| Reactor | OLR (kg COD m\(^{-3} \) day\(^{-1} \)) | HRT (h) | COD\(_{\text{glucose}}\) (mg L\(^{-1} \)) | COD\(_{\text{acetate}}\) (mg L\(^{-1} \)) | COD\(_{\text{butyrate}}\) (mg L\(^{-1} \)) | COD\(_{\text{propionate}}\) (mg L\(^{-1} \)) | COD\(_{\text{biomass}}\) (mg L\(^{-1} \)) | COD\(_{\text{ethanol}}\) (mg L\(^{-1} \)) | COD\(_{\text{methanol}}\) (mg L\(^{-1} \)) | COD\(_{\text{total}}\) (mg L\(^{-1} \)) | COD\(_{\text{measured}}\) (mg L\(^{-1} \)) | COD\(_{\text{others}}\) (mg L\(^{-1} \)) |
|--------|---------------------------------|--------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Barros et al. [6] | | | 12 | 8 | 946 | 245 | 1382 | 0 | 192 | 90 | 24 | 3405 | 3788 | 39 |
| | | | 16 | 6 | 479 | 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 | 9036 | 104 |
| | | | 60 | 4 | 6935 | 291 | 531 | 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 | 22,332 | 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.

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References


