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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

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acid, butyric acid, propionic acid, lactic acid, succinic acid, 1,3-propanediol, ethanol, methanol, etc. [4, 5].

H_2 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 H_2 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 H_2 production requires continuous or semicontinuous production processes. Several types of reactors have been studied to effectively generate H_2. Reactors for continuous H_2 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 H_2 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 H_2 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

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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}; $CoCl_2 \cdot 2H_2O$, 0.08 mg L^{-1}; $FeCl_3 \cdot 6H_2O$, 0.5 mg L^{-1}; $NiSO_4 \cdot 6H_2O$, 1 mg L^{-1}; $FeSO_4 \cdot 7H_2O$, 5 mg L^{-1}; K_2HPO_4, 21.7 mg L^{-1}; $Na_2HPO_4 \cdot 2H_2O$, 33.4 mg L^{-1}: $CaCl_2 \cdot 6H_2O$, 47 mg L^{-1}; KH_2PO_4, 85 mg L^{-1}; and $CO(NH_2)_2N_2O$, 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].

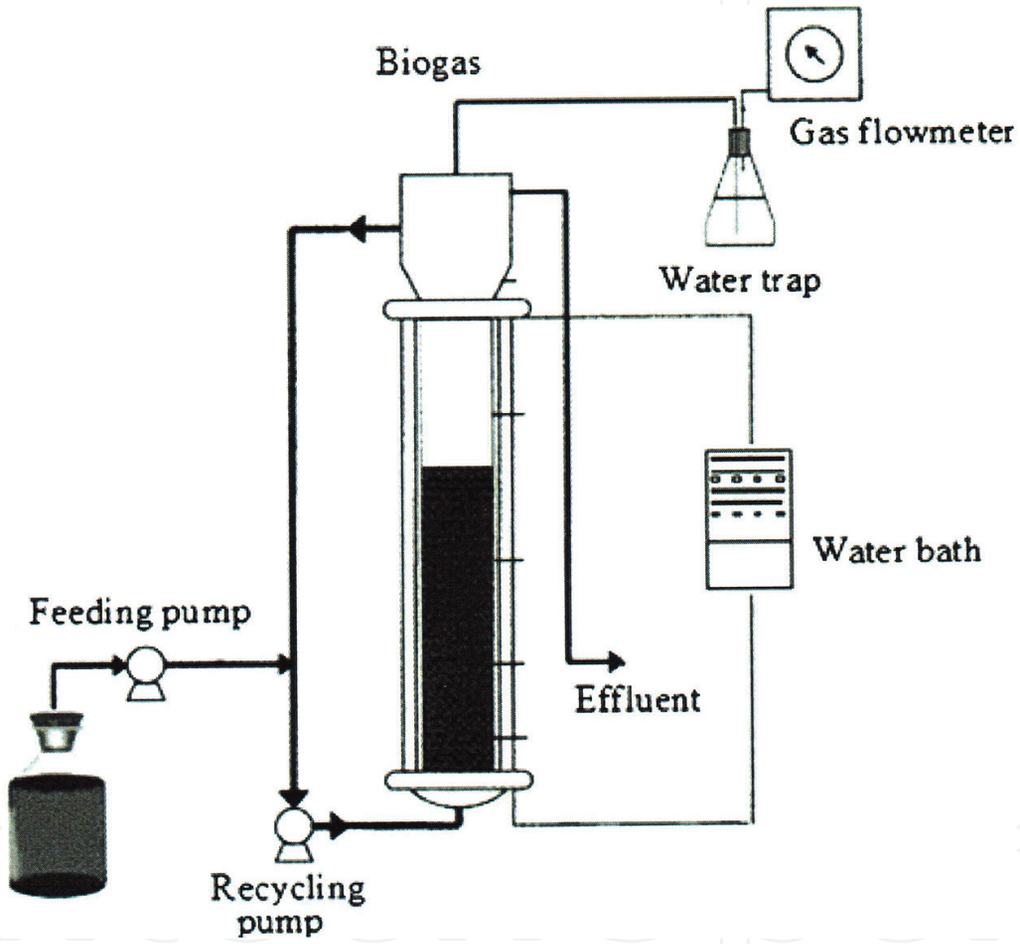

Figure 1. Schematic description of the AFBR.

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_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_2 and CO_2) and soluble metabolites (volatile organic acids and alcohols) produced during fermentative 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 × 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) [32].

A gas meter (type TG1; Ritter Inc., Germany) was used to measure the amount of H_2 generated.

3. Results and discussion

3.1. Effect of OLR on 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

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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%.

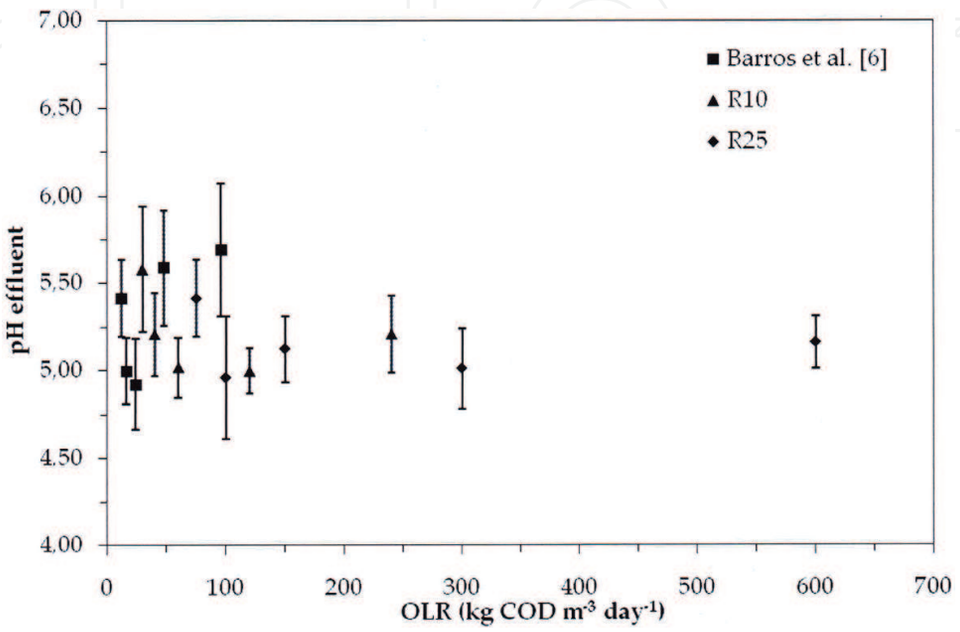

Figure 2. pH effluent as a function of the OLR for the AFBRs.

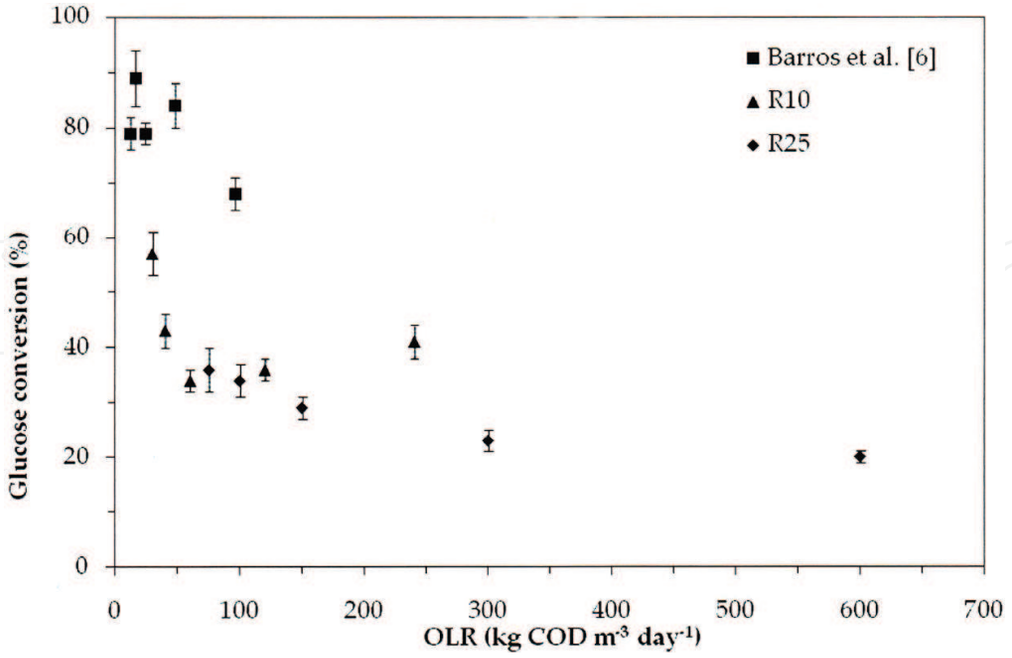

Figure 3. Glucose conversion as a function of the OLR for the AFBRs.

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.

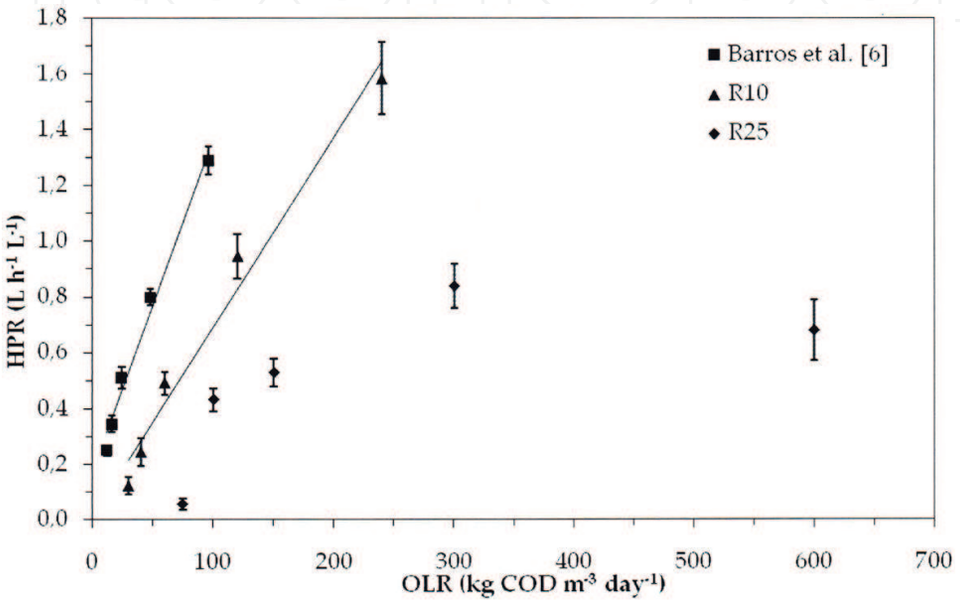

Figure 4. HPR as a function of the OLR for the AFBRs.

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].

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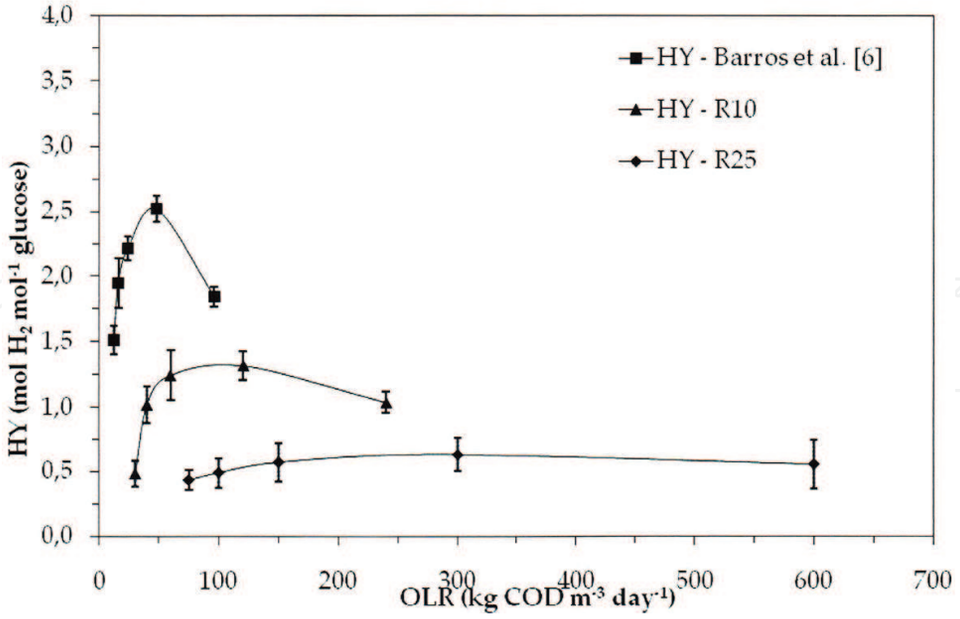

Figure 5. HY as a function of the OLR for the AFBRs.

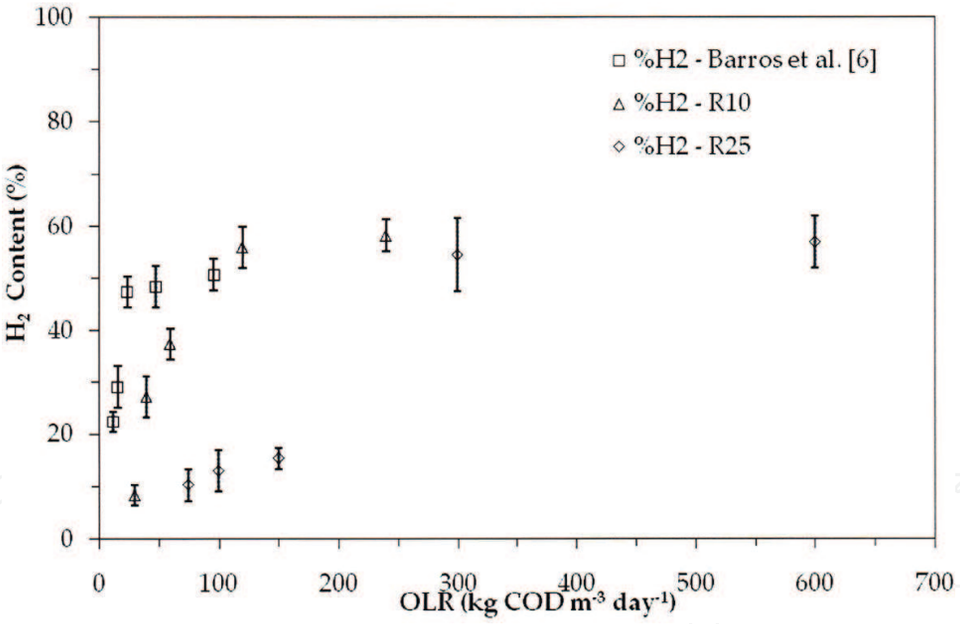

Figure 6. H_2 content as a function of the OLR for the AFBRs.

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.

Study	Substrate	OLR (kg m^{-3} d^{-1})		HY (mol H_2 mol^{-1} substrate)	
		Low	High	Low OLR	High OLR
Lower OLR improves H_2 production					
Yu et al. [36]	Rice winery	168	432	1.89	1.79
Van Ginkel and Logan [24]	Glucose	25.6	76.8	2.20	2.00
Van Ginkel and Logan [37]	Glucose	6	24	2.80	2.20
Kyazze 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 H_2 production					
Lin et al. [18]	Sucrose	13.5	107.9	1.69	2.49
	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

Adapted from Kraemer and Bagley [26].

Table 1. Comparison of the studies that varied the OLR by changing the substrate concentration.

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 (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

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**).

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

HAc acetate, HBu butyrate, HPr propionate, EtOH ethanol, MetOH methanol, TVFA total volatile fatty acids, TVFA HAc + HBu + HPr, SMP TVFA + EtOH + MetOH, HAc/SMP molar acetate-to-SMP ratio, HBu/SMP molar butyrate-to-SMP ratio, HPr/SMP molar propionate-to-SMP ratio, EtOH/SMP molar ethanol-to-SMP ratio, MetOH/SMP molar methanol-to-SMP ratio, HAc/HBu molar acetate-to-butyrate ratio

Table 2. Effect of glucose concentration and OLR on the SMP distribution in the AFBRs.

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

subsequently decreased and stabilized to 11%. EtOH production is considered unfavorable for hydrogen metabolite production because no H_2 is consumed or produced (Eq. (1)):

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (1)$$

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 H_2 for forming propionate (Eq. (2)):

$$C_6H_{12}O_6 + 2H_2 \rightarrow CH_3CH_2COOH + 2H_2O \quad (2)$$

Both HAc and HBu are soluble metabolites favoring H_2 production because these products are generated during H_2 production (Eqs. (3) and (4)):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (3)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (4)$$

Previous studies have observed that 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 H_2 production because of effective metabolism and further H_2 production process. However, higher concentrations can also negatively impact H_2 production. When the 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 ($\mu_{maximum} = 0.0167\ h^{-1}$) is significantly lower than that of acidogenic microorganisms ($\mu_{maximum} = 0.083\ h^{-1}$). 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 ($COD_{products}$) and the glucose ($COD_{glucose}$) COD concentrations were calculated according to Eqs. (5) and (6), respectively. The $COD_{residual}$ was calculated after subtraction of the sum of the $COD_{products}$ and $COD_{glucose}$ from the $COD_{measured}$ (Eq. (3)).The COD_{others} corresponds to the non-identified metabolic products during glucose fermentation:

$$COD_{products} = a \cdot \left(\frac{mmolHAc}{1}\right) \cdot 64\frac{mgCOD}{mmolHAc} + b \cdot \left(\frac{mmolHBu}{1}\right) \cdot 160\frac{mgCOD}{mmolHBu}\left(\frac{mmolHAc}{1}\right)$$

$$+ c \cdot \left(\frac{mmolHPr}{1}\right) \cdot 112\frac{mgCOD}{mmolPr} + d \cdot \left(\frac{mmolMetOH}{1}\right) \cdot 48\frac{mgCOD}{mmolMetOH}$$

$$+ e \cdot \left(\frac{mmolEtOH}{1}\right) \cdot 96\frac{mgCOD}{mmolEtOH} \quad (5)$$

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

$$COD_{glucose} = f \cdot \left(\frac{mg\ Glucose}{1}\right)\frac{192\ mg\ COD}{180\ mg} \quad (6)$$

where f is the measured concentration of glucose.

The difference between $COD_{measured}$ 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):

$$COD_{others} = COD_{measured} - \left(COD_{products} + COD_{glucose}\right) \quad (7)$$

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.

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})	$COD_{t,total}$ (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.

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 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

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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