

Biohydrogen production from soft drink industry wastewater in an anaerobic fluidized bed reactor

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Abstract

The wastewater from carbonated soft drinks production was used as substrate in an anaerobic fluidized bed reactor (AFBR) to evaluate the production of biohydrogen as a renewable energy. The hydraulic retention time (HRT) ranged from 8 to 0.5 hours (7.92 to 137.09 kg COD m⁻³ day⁻¹) throughout the experiment and expanded clay was used as support material for biomass adhesion. The average composition of hydrogen in the biogas under the conditions of this experiment was 34%. The maximum hydrogen yield (HY) and the maximum hydrogen production rate (HPR) was 5.87 mol H₂/mol substrate and 2.74 L H₂ h⁻¹ L⁻¹, respectively, obtained in the HRT of 0.5 hour. Acetic acid was the predominant soluble metabolite detected (88%). Propionic, butyric and caproic acids were quantified with low production (7%, 4% and 1% of soluble metabolites production (SMP)). The anaerobic fluidized bed reactor optimized the average of hydrogen yield by 17% in relation to packed-bed reactors, in a HRT of 0.5 h. The natural fermentation process and operating conditions were favorable to the inhibition of hydrogen-consuming organisms, such as methanogenic archaeas.

Key words: anaerobic digestion, anaerobic fluidized bed reactor, biogas, bio-hydrogen, natural fermentation

INTRODUCTION

Hydrogen is considered a clean fuel due to its combustion with oxygen, which generates only water and energy as sub-products. Its energy potential equals approximately three times the energy obtained by gasoline and double that of methane combustion as 1 kg of hydrogen generate the same amount of energy as 2.8 kg of gasoline or 2.1 kg of methane (Chen *et al.* 2008; Ball & Wietschel 2009). In 2025, it is expected that the contribution of hydrogen to the energy market will reach 10% of the total energy demand (Argun *et al.* 2008). In addition to its applicability as an energy source, hydrogen is used as raw material for manufacturing electronic devices, chemical products, hydrogenated fats, fertilizers, oils in the food industry, rocket fuel, refrigeration fluid, steel, desulphurization, and refining gasoline (IEA 2015; Li *et al.* 2015).

However, hydrogen production is currently obtained mainly from fossil fuels, which contributes to CO₂ emissions (Kapdan & Kargi 2006; Ball & Wietschel 2009; Li *et al.* 2015). Even though it is less efficient than large-scale production technologies, decentralized hydrogen production is a feasible choice for market acceptance as it reduces the transportation cost and the needed infrastructure for the processes (IEA 2015). Biological processes are beneficial for hydrogen production as a renewable energy due to its low polluting impact, reduced demand for sophisticated technologies, and for enabling the use of organic residues as feedstock, which allows the association of wastewater treatment with the generation of profitable sub-products from anaerobic digestion (Sá *et al.* 2014).

Brazil is the third largest producer of soft drinks, with an annual consumption of approximately 69 liters per person (Lima & Afonso 2009; Menda 2011). This wastewater generated from carbonated soft drinks production is characterized by its high organic load (with a chemical oxygen demand (COD) of 777 to 8,000 mgO₂ L⁻¹) and alkaline pH (Temps & Pawlowsky 2000; Wang *et al.* 2005; Silva Filho 2009), which is suitable for biological treatment. Some studies have evaluated the treatment of soft drink industries compared to the production of biogas by anaerobic digestion (Weber 2006; Peixoto *et al.* 2011). Peixoto *et al.* (2011) used this effluent as a substrate for generating biohydrogen (BioH₂) in an anaerobic fixed bed reactor with and without the addition of macro and micronutrients. The authors achieved the highest hydrogen yield (HY) of 3.5 mol H₂/mol sucrose with no addition of macro and micronutrients. Weber (2006) also studied this substrate in a fluidized bed reactor to evaluate organic matter removal and biogas production. The authors obtained 1.61 L CH₄ gCOD⁻¹ day⁻¹ in the biogas production and organic matter removal of 84.17 ± 9.87%. In their research, the studied organic load ranged from 0.09 kgCOD m⁻³ day⁻¹ to 4.00 kgCOD m⁻³ day⁻¹. Despite the fair amount of studies concerning anaerobic digestion of agroindustrial waste, the lack of operational stability in biological processes still prevents it from being widely practiced in the market (Dupla *et al.* 2004). Hallenbeck *et al.* (2012) reviewed recent progress obtained in the wastewater treatment field in order to increase hydrogen yields through physiological manipulation, metabolic engineering, and the use of two-phase systems. Several factors affect the performance and stability of BioH₂ production through anaerobic fermentation, including: pH, temperature, substrate composition, inoculum, inorganic nutrients, support material for biomass adhesion, and hydraulic retention time (HRT). As the effect of these factors is known, it is possible to minimize the inhibitions to the process by applying several control strategies (Naik *et al.* 2014; Ratti *et al.* 2015). The reactor model can also interfere with the anaerobic digestion process. The anaerobic fluidized bed reactor (AFBR) corresponds to a high rate system with adhered microbial growth. In the fluidized bed reactor, the maximum contact between the liquid and the support material minimizes the formation of preferential channels and gas retention as the shear stress optimizes the biological film thickness. Also, in this reactor model, the diffusional resistance of the liquid through the biofilm is minimal due to the movement of the particles and velocity of the liquid (Hickey & Owers 1981). The production of hydrogen in AFBR has been explored in the treatment of synthetic effluents (Barros *et al.* 2011; Amorim *et al.* 2012) and in the treatment of real agroindustrial, which can be considered a feasible process in the production of hydrogen with energy recovery from residues as substrates.

In this context, this study evaluated the performance of an AFBR in the production of BioH₂ using synthetic soft-drink wastewater.

METHODS

Soft-drink wastewater

The synthetic wastewater composition was based on measurements proposed by Peixoto *et al.* (2011), which were: tap water (97.85%), carbonated soft drink (1.97%), sodium hypochlorite in a concentration of 20% (0.09%), alkaline detergent (MERCK Extran MA01) (0.07%), and lubricating agent (0.01%). The wastewater used in this experiment had the following characteristics (mg L⁻¹): BOD of 1,099.3, COD of 2,396.8 ± 406.0, total nitrogen of 7.7, total phosphorus of 0.55, oils and fats of 68, and pH 9.5. To adjust the pH to the optimal range for acidogenesis, hydrochloric acid (10 mol L⁻¹) and sodium bicarbonate (1 g L⁻¹) were added. It was not necessary to add nutrients based on the nitrogen and phosphorous concentrations.

Inoculum

The inoculation was based on the process described by Peixoto *et al.* (2011) via natural fermentation of the synthetic wastewater, in which 10 L of the solution was exposed to the atmosphere for three days at room temperature. After the fermentation period, the wastewater was pumped into the reactor until it was completely full. The reactor was maintained in recirculation for three consecutive days (inoculation period) in which the reactor was not fed.

Anaerobic fluidized bed reactor – AFBR

The AFBR was operated in a continuous flow and without temperature control. The temperature variation was 31 ± 1 °C. Temperature control was not necessary due to the favorable climate in the northeast of Brazil (Cecchi *et al.* 1993). Expanded clay (CINEXPAN – 3222) in a particle size range of 2.8–3.35 mm and density of 1.5 g cm^{-3} was used as support material in the reactor's bed for microbial adhesion. The theoretical fluidization velocity applied to this system was 1.62 cm s^{-1} , which is 1.3-fold the minimum velocity (1.24 cm s^{-1}) according to Amorim *et al.* (2012).

The reactor was made of acrylic with a total volume of $1,250 \text{ cm}^3$ and a working volume of 880 cm^3 . It was filled up with the support material up to a height of 30 cm from the base of the reactor. Figure 1 shows a schematic of the AFBR that was used.

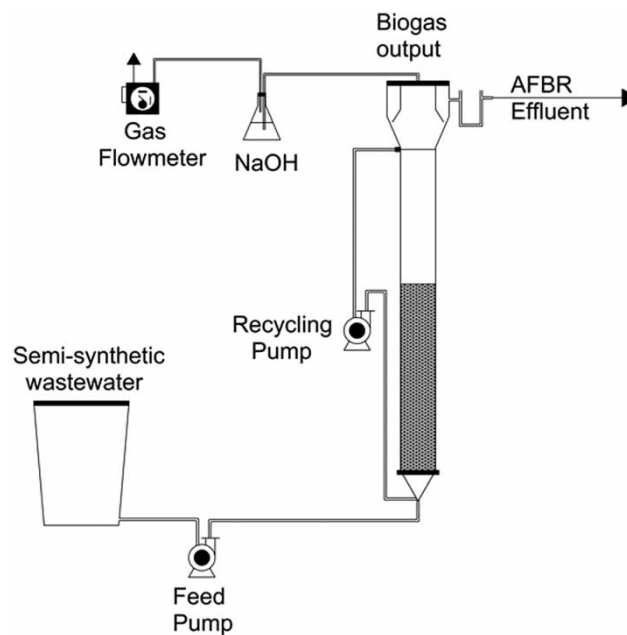


Figure 1 | Schematic of the Anaerobic Fluidized Bed Reactor used in this research.

Analytical methods

Total fixed and volatile suspended solids, $\text{BOD}_{5,20}$, COD, phosphorus, pH, temperature, total nitrogen (N-Total), nitrite (N-NO_2) and nitrate (N-NO_3) were measured according to APHA (2012). Carbohydrates were measured by colorimetry at wavelength 492 nm (Dubois *et al.* 1956). The hydrogen production rate (HPR) was measured using a Milligascounter gas meter (TG1 Ritter Inc., Germany). The composition of the biogas (hydrogen, carbon dioxide, and methane) was determined by gas chromatography (Shimadzu GC-2010 Plus) equipped with a thermal conductivity detector (TCD) (230 °C), a Carboxen #1010 PLOT column (30 m \times 0.53 mm), and argon as the carrier gas

(Maintinguer *et al.* 2008). The assessment of organic acids and ethanol was performed by gas chromatography using a Shimadzu GC-2010 Plus chromatograph equipped with a Flame Ionization Detector (FID Flame Ionization Detector) and SUPELCO WAX 10 column (30 m × 0.25 mm × 0.25 μm) with hydrogen as the carrier gas (Maintinguer *et al.* 2008).

RESULTS AND DISCUSSION

Hydrogen production

Table 1 shows the average carbohydrate concentration and COD in the influent and effluent of the reactor, as well as the removal efficiency, the HPR and HY for each experimental phase. The theoretical HRT in which the reactor best performed for carbohydrate consumption were 8 h and 2 h (36% and 33%, respectively). Sugars degradation decreased markedly with the change in the HRT from 2 h to 1 h (from 33% to 25%). Peixoto *et al.* (2011) obtained in the HRT of 0.5 a carbohydrate removal efficiency superior to 30%. For Amorim *et al.* (2009), high conversion rates of glucose (approximately 90%) in a stable system were obtained. However, the authors used glucose as substrate, which may have favored the maintenance of the operational conditions and, consequently, the process stability.

Table 1 | Carbohydrate concentration, chemical oxygen demand (COD), hydrogen production rate (HPR) and yield (HY) in each operational phase

Operational phase	HRT (h)	Carbohydrate			COD			HPR (L H ₂ h ⁻¹ L ⁻¹)	HY (mol H ₂ mol substrato ⁻¹)
		Influent (mg L ⁻¹)	Effluent (mg L ⁻¹)	Efficiency (%)	Influent (mg L ⁻¹)	Effluent (mg L ⁻¹)	Efficiency (%)		
1	8	2,187 ± 297	1,406 ± 190	36	2,480 ± 283	1,847 ± 143	26	0.063 ± 0.032	0.94 ± 0.00
3	4	2,217 ± 532	1,702 ± 512	23	2,893 ± 520	2,271 ± 308	21	0.183 ± 0.071	1.79 ± 0.45
4	2	2,490 ± 383	1,672 ± 525	33	3,143 ± 259	2,611 ± 269	17	0.170 ± 0.083	3.84 ± 0.00
5	1	2,672 ± 194	2,002 ± 159	25	3,260 ± 401	2,667 ± 74	18	0.804 ± 0.185	2.90 ± 1.04
6	0.5	2,457 ± 360	1,902 ± 310	23	3,129 ± 139	2,658 ± 135	15	1.857 ± 0.524	3.87 ± 1.39

The temporal variation of the HPR and HY are presented in Figure 2. The HPR was stable starting on day 69, reaching a maximum volumetric production on day 79 (2.74 L H₂ h⁻¹ L⁻¹). From day 69 to day 127 (operational stability), the average HPR was 2.02 ± 0.5 L H₂ h⁻¹ L⁻¹. Amorim *et al.* (2014) obtained an average HPR of 2.04 L H₂ h⁻¹ L⁻¹ using cassava wastewater in an AFBR to produce

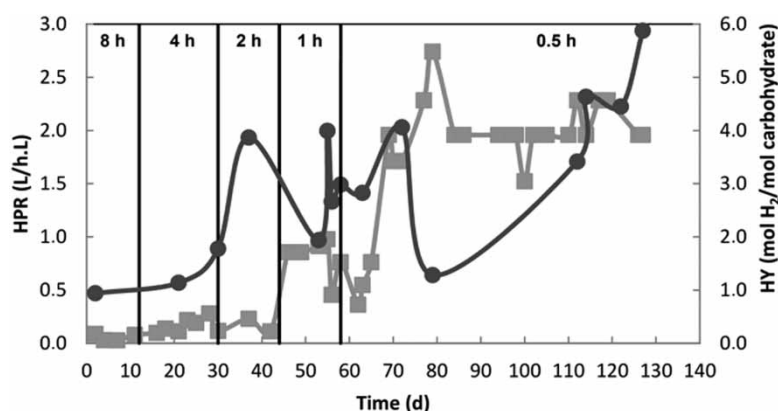
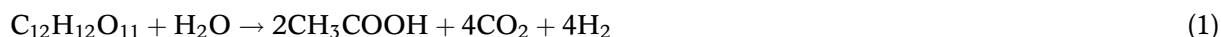


Figure 2 | Temporal variation of hydrogen volumetric production rate (HPR) (■) and hydrogen yield (HY) (●).

BioH₂ in the HRT of 1 h, which is similar to the production obtained in this study. However, Peixoto *et al.* (2011) operating an anaerobic packed-bed reactor also using soft-drink industry wastewater obtained an average HPR of 0.41 L H₂ h⁻¹ L⁻¹, which is inferior to the production rate observed in this study and and by Amorim *et al.* (2014). This may indicate an enhanced performance regarding the HPR when using fluidized bed reactors compared to packed-bed reactors.

The maximum HY obtained during the HRT of 0.5 h was 5.87 mol H₂/mol carbohydrates (day 127) with a theoretical carbohydrate removal efficiency of 73% (considering sucrose as the major carbohydrate source and the acetic acid route – Equation (1)).



The maximum HY observed in this study (5.87 mol H₂/mol carbohydrates, day 127) was greater the HY obtained by Peixoto *et al.* (2011) (3.35 mol H₂/mol sucrose) with a conversion efficiency of 42% in the HRT of 0.5 h using the same residue of this study as substrate in a packed-bed reactor. Chen & Lin (2003) obtained a HY of 4.52 mol H₂/mol sucrose in the HRT of 8 h using sucrose-based synthetic effluent as a substrate in continuous flow stirred tank reactor. Thus, the wastewater generated in soft-drink industries can be considered a suitable substrate for hydrogen production in biological processes. Furthermore, the use of AFBR optimized the average HY in 17% in comparison to the one obtained in packed-bed reactors in a HRT of 0.5 h.

The natural fermentation process and the operational conditions were considered efficient as no methane was detected in the biogas in any of the experimental phases. In the HRT of 0.5 h, hydrogen gas contributed with 34 ± 9% of the biogas generated, which is almost twice as much as the amount produced in the study of Peixoto *et al.* (2011) using similar wastewater. These authors reported an average of 19% and a maximum of approximately 25% of hydrogen in the biogas composition. Amorim *et al.* (2009) operating an AFBR fed with glucose-based substrate with concentration of 2,000 mg L⁻¹ of glucose in a HRT of 1 hour reached 35% of hydrogen in the biogas composition, which is similar to this study.

pH

Figure 3 shows the variation of the influent and effluent pH during the experimental phases.

In the first four phases (HRT de 8, 4, 2 e 1 h), the pH was adjusted to the optimum range for hydrogen production, which is between 4.5 and 6 (Kim *et al.* 2004). These adjustments caused instability in

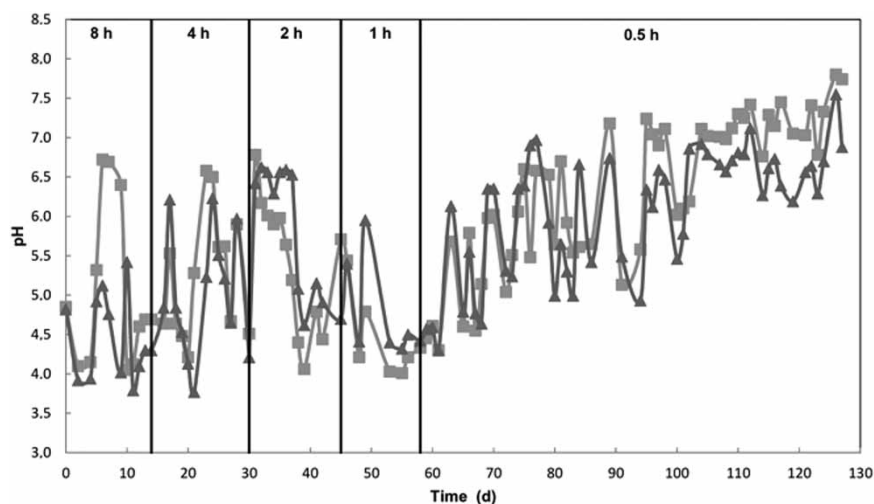


Figure 3 | Variation of the influent (■) and effluent (▲) pH.

the hydrogen production in the first four operational phases. In Phase 5 (HRT of 0.5 h), the influent and effluent pH variation were 6.33 ± 0.95 and 6.08 ± 0.80 , respectively. In the HRT of 0.5 h, the pH control positively affected the hydrogen production ($\text{HPR} = 2.02 \pm 0.5 \text{ L H}_2 \text{ h}^{-1} \text{ L}^{-1}$, and $\text{HY} = 3.87 \text{ mol H}_2 \text{ mol-sucrose}^{-1}$). According to Antonopoulou *et al.* (2008), the pH range between 4 and 5 favors the production of propionic acid, whose metabolic route consumes hydrogen, while the range close to neutrality (between 6 and 7) favors the acetic and butyric acid routes, in which hydrogen is produced during their formation (Equations (1) and (2)). Thus, the pH during the HRT of 0.5 h (6.08 ± 0.80) was favorable to the metabolic routes of acetic and butyric acids and, consequently, to the production of hydrogen.



Soluble metabolites production – SMP

Table 2 shows the metabolites production quantified throughout the experiment. Acetic acid was the predominant metabolite produced ($\geq 83\%$ of SMP) and propionic acid was detected in all phases, representing 3 to 11% of the total SMP. The absence of ethanol contributed to the BioH_2 production, as this route may consume hydrogen (Fernandes *et al.* 2013). The higher production of hydrogen is associated with the low conversion of alcohols (ethanol and butanol). According to Fernandes *et al.* (2013), the production of alcohols consumes substrate that could be used in the production of acids that generate hydrogen during their formation, such as acetic and butyric acids. The presence of caproic acid was minimal ($\leq 2\%$ of SMP) and may have occurred by the conversion of hydrogen and acetic acid (Equation (3)).



Table 2 | Soluble metabolites distribution

HRT (h)	HAc		HPr		HBu		HCa	
	mM	%	mM	%	mM	%	mM	%
8	2.14	83	0.28	11	0.11	4	0.06	2
4	2.59	84	0.30	10	0.12	4	0.07	2
2	8.75	95	0.29	3	0.12	1	0.08	1
1	5.42	92	0.29	5	0.12	2	0.07	1
0.5	5.80	85	0.53	8	0.42	6	0.07	1

HAc: Acetic Acid, HPr: Propionic Acid, HBu: Butyric Acid, HCa: Caproic Acid.

Peixoto *et al.* (2011) obtained a different acid distribution than the one in this work. The authors operated a packed-bed reactor fed with soft-drink wastewater producing essentially acetic, butyric and propionic acids (29%, 29% and 26% of SMP, respectively). In addition, the authors still observed citric, formic, isovaleric, and lactic acid in smaller quantities ($\leq 5\%$ of SMP). The authors state that the production of other metabolites that do not favor the production of hydrogen may have occurred due to the presence of heterogeneous populations of microorganisms in the reactor. Carbon balance is presented in Table 3 in terms of COD to confirm the accuracy of the experimental data. An average accuracy of 93% was observed when comparing the measured effluent COD and the total theoretical COD. For the 0.5 h HRT, the consistency between the data was 96%, indicating the data reliability.

Table 3 | COD balance based on the influent COD and the soluble metabolites in the effluent

HRT (h)	COD _{t,Carbohydrate} (mg/L)	COD _{t,HAc} (mg/L)	COD _{t,HPr} (mg/L)	COD _{t,HBU} (mg/L)	COD _{t,HCa} (mg/L)	COD _{t>Total} (mg/L)	COD _{eff} (mg/L)	COD _{eff} - COD _{t>Total} (mg/L)	COD removal (%)
8	1,499	137	32	18	14	1,699	1,847	148	92
4	1,815	166	34	20	17	2,051	2,271	220	90
2	1,782	560	33	18	21	2,415	2,611	196	92
1	2,134	347	33	19	19	2,551	2,667	116	96
0.5	2,028	371	59	67	18	2,544	2,658	115	96

COD_{eff}: effluent COD; COD_{t,total}: HAc: Acetic Acid; HPr: Propionic acid; HBU: Butyric acid; HCa: Caproic acid.

CONCLUSIONS

In this experiment, the performance of an AFBR to produce biohydrogen from synthetic soft-drink industry wastewater was evaluated. This residue showed great potential as a substrate for hydrogen production with a maximum HPR and yield of 2.74 L H₂ h⁻¹ L⁻¹ and 5.87 mol H₂ mol-substrate⁻¹, respectively. The average HY in the AFBR was optimized in the HRT of 0.5 h with an increase of 17% when compared to the HY obtained for the same operational conditions in a packed-bed reactor. In the first four phases (adaptation stage), adjustments were made to the influent pH that allowed the maintenance of the effluent pH within the optimum range indicated for hydrogen production. These pH variations may have influenced the instability of the process at these phases (1 to 4). Acetic acid was predominant at all phases (88% of SMP) and propionic, butyric and caproic acids were also quantified (7%, 4% and 1% of SMP, respectively). The natural fermentation inoculation process and operating conditions were favorable to the inhibition of hydrogen-consuming organisms, such as methanogenic archaeas. The average composition of hydrogen in the biogas under the conditions of this experiment was 34%.

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