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Evaluation of feeding strategies in upflow anaerobic sludge bed reactor for hydrogenogenesis at psychrophilic temperature

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ABSTRACT

The present work evaluated the biohydrogen production from a 0.4 L upflow anaerobic sludge blanket reactor type (UASB) operating at psychrophilic temperature (21 ±2 °C) at different feeding strategies varying hydraulic retention times (HRT) and sucrose concentration in the feeding. First strategy (24h/31c) fed semi-continuously 31 $g_{sucrose}$ L^{-1} at 24 h HRT; second strategy (12h/19c) fed semi-continuously 19 $g_{sucrose}$ L^{-1} at 12 h HRT; third strategy (4h/8c) fed continuously 8.3 $g_{sucrose}$ L^{-1} at 4 h HRT.

After 70 days of operation, the UASB accumulated 65.44 L H_2. The average HY for the whole operation during the three strategies was 62.6 NmL H_2 $g_{sucrose}^{-1}$, and average hydrogen content was 69.04%. In general terms, the best operation strategy was 12h/19c since it presented good set of results, the best HY (70.6 NmL H_2 $g_{sucrose}^{-1}$) and a comparable hydrogen production rate (2.6 L (L d)$^{-1}$) to that obtained in 4h/8c strategy (3.17 L (L d)$^{-1}$). The average gross energy potential rate from the 12h/19c strategy was 46.21 kJ (L d)$^{-1}$, whereas energy heating losses were circumvented due to operation at psychrophilic regime. Indeed, psychrophilic or room temperatures should be broadly regarded as an effective alternative towards net energy gains in biohydrogen production.

Keywords: bioenergy, dark fermentation, energy balance, room temperature.

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1. Introduction

The worldwide energy production is mainly derived from fossil fuels such as oil, coal and natural gas, comprising activities such as exploration, exploitation, refining, transportation and even combustion, which affect negatively the environment and human health. The conventional stocks of fossil fuels will decrement overtime, whereas energy demand will continue raising as population and consumption habits increase, following urbanization and modernization [1,2]. In order to expand the energy sources and to reduce fossil fuels related problems (e.g. air pollution and greenhouse gas emissions), renewable alternatives are under development [3] such as solar, geothermal, ocean and biomass energies [4]. Bioenergy is produced from biomass through thermochemical, thermal and biotechnological processes for its use in industry, power, and transport sectors [5].

Biohydrogen may be considered a nitrogen- and sulfur-free biofuel when produced from ideal conditions dark fermentation (DF). In practice, biological CO_2 is produced as consequence of microbial anaerobic respiration, hydrogen sulfide may be produced by action of sulfate-reducing bacteria (SRB) if sulfate is present in concentrations over 3 g-SO_3^{2-} L^{-1} [6], and NOx emissions increase when hydrogen is combusted along with gasoline, methane or methanol in spark-ignited combustion engines [7]. Still, hydrogen produced from natural gas, oil, coal gasification and water electrolysis, require high energy consumption, concomitantly causing air pollution and global warming [8].

Various fermentation systems have been developed for hydrogen production, such as the batch, semi-continuous and continuous, for either one or multiple stages. Depending on substrate characteristics the bioreactor type may be considerably different. For insoluble substrates the suspended biomass bioreactors —continuous stirred tank reactor (CSTR), upflow anaerobic sludge bioreactor (UASB), and fluidized bed bioreactors— are a preferred option; whereas for soluble substrates the immobilized biomass reactors (fixed bed bioreactor and biotrickling filter) are better designs. For instance, UASB is appraised to be a good option for treating organic fraction of municipal solid waste and synthetic wastewater, as demonstrated in the work by Alzate-Gaviria et al. [9] who demonstrated that an UASB bioreactor outperformed a packed bed reactor treating such substrates. When using soluble substrates (e.g. cheese whey, sucrose), it has been determined that CSTR is not the most appropriate design, these reactors often have washing of microorganisms caused by a cell growth rate lower than the rate at which the microorganisms leave the digester [10]. Even though biotrickling filters may be used to treat soluble and insoluble matter, insoluble matter may cause clogging problems [11]. On the other hand, agroindustrial wastes have been treated by anaerobic solid substrate fermentation [12] using the systems designed by Poggi-Varaldo and Oleszkiewicz [13], which have been also successfully adapted to semi-continuous acidogenic operation for hydrogen production and to intermittently vented batch operation [14]. Other important alternative for insoluble biomass is the anaerobic sequencing batch reactor (ASBR), since this system is regarded as the batch type of the UASB.

The UASB perform depends on an adequate formation of microbial flocules. If inoculum is not in granular form, promoting its formation can be a difficult and time-consuming task. Start-up periods of 150-300 d are commonly reported in literature [15]. Liu et al. [16] explored

the retention of anaerobic digester sludges using either activated carbon or carbon nanotubes, finding that the latter prevented cell washout more effectively. However, many researchers prefer to use already formed granules from brewery wastewater treatment UASB reactors [17,18]. At laboratory scale, hydrogen production in continuous mode is explored to increase stability, organic matter removal, energy efficiency and yields [19,20]. Important variables used to control and to increase the hydrogen yields and production rates are inoculum pretreatment [21], pH influence [22], effect of hydraulic retention time (HRT) [23], organic loading rate (OLR), carbon and sludge sources [24,25]. Determining the optimal HRT and OLR could maximize the biogas and hydrogen production [26], and thus it has been of interest to design strategies to evaluate its influence on hydrogen production. For instance, medium and long-term operation works usually begin with 48 or 24 h HRT [27,28]; thereupon HRT is reduced by a pair-number factor, mostly of 4 or 6 h [23,24,29]. Moreover, changes between continuous and discontinues modes have also been evaluated [30,31]. Final HRT for soluble substrates –such as monosaccharides– are 8-12 h [32], although some improvements have allowed to reduce it to 4 h not without metabolic unbalance risk [15,23].

It is well known the effect of temperature in hydrogenogenic performance. According to bacterial metabolism, three temperature intervals are observed even in hydrogen production, psychrophilic (<25 ºC), mesophilic (30-40 ºC), thermophilic (50-60 ºC), among which the most used have been mesophilic and thermophilic [33]. However, it should be considered that psychrophilic metabolism at 18-25 ºC has reduced energy expenses compared to its counterparts, making it an attractive alternative that if improved could produce net energy gains to the systems [34]. Therefore, the aim of this study was to explore the biohydrogen

production in a bench-scale UASB reactor operating at psychrophilic temperature through different feeding strategies (i.e. varying hydraulic retention times and sucrose concentration in the feeding); moreover, an energetic evaluation in terms of gross energetic potential rate and energetic heating losses was performed at the best conditions and compared to literature.

2. Materials and Methods

2.1. Inoculum

Methanogenic granules were obtained from brewery industry anaerobic sludges (Obregón, Sonora, Mexico) and stored at 4 °C. Before use, granules were rinsed with sterilized isotonic solution and heat-treated at 90 °C for 1 h in a water bath to inhibit methane producing microflora and to enrich the hydrogen-producing heat-shock resistant microbes [35]. The inoculum was characterized in terms of pH, humidity, total solids (TS), volatile solids (VS) and ashes (Table 1). Humidity, TS, VS and ashes were carried out in triplicate using standard techniques [36].

PLEASE INSERT TABLE 1 HERE

2.2. Experimental system and procedure

A plexiglass 0.4 L working volume UASB reactor (0.056 m diameter, 0.23 m height) was fed with mineral medium containing sucrose as carbon source with a peristaltic pump as shown in Fig. 1. The UASB was installed on a laboratory bench and operated at psychrophilic temperature (21 ± 2 ºC). The initial amounts of inoculum, mineral medium and phosphate

buffer solution were 77 g_{wb}, 40 mL and 283 mL, respectively; consequently, the concentration of the inoculum in terms of VS was 4155 mg L^{-1}. The mineral medium composition was [37] (mg L^{-1}): $ZnCl_2$, 75; $MgCl_2 \cdot 6H_2O$, 100; $MnCl_2 \cdot 4H_2O$, 10.77; $FeCl_3 \cdot 6H_2O$, 25.97; $CuCl_2 \cdot 2H_2O$, 3.41; and $NiCl_2 \cdot 6H_2O$, 101.25. A phosphate buffer (pH = 7.28) was prepared using 30 g K_2HPO_4 L^{-1} and 20 g KH_2PO_4 L^{-1}. The feeding and recirculation operations were performed through a peristaltic pump (323, Watson Marlow, England). Recirculation was carried out at the lowest attainable speed (30 rpm, 0.1 L h^{-1}).

PLEASE INSERT FIGURE-1 HERE

Three different feeding strategies were applied varying HRT and sucrose concentration to evaluate biohydrogen production: first strategy (24h/31c) was a 24 h HRT regime fed daily with 400 mL at 31 $g_{sucrose}$ L^{-1}, second strategy (12h/19c) was a 12 h HRT regime fed daily with 800 mL at 19 $g_{sucrose}$ L^{-1}; third strategy (4h/8c) was a 4 h HRT continuous regime fed with 2400 mL d^{-1} at 8.3 $g_{sucrose}$ L^{-1}. The corresponding OLR for each strategy were 31, 38 and 49.8 g (L d)$^{-1}$. For the 24h/31c and 12h/19c strategies, due to specifications of the peristaltic pump, feeding was performed daily during 4 and 8 h of operation, respectively. The feeding was prepared mixing 40 mL mineral medium and 160 mL phosphate buffer, completing total volume with distilled water if needed. After completing the feeding, the reactors contents were homogenized by recirculation during 5 min at maximum speed of the peristaltic pump.

2.3. Analytical methods

The pH was determined with a pH-meter (PC18, Conductronic, Mexico), whereas biogas production was measured by hydraulic displacement (3 L column) method everyday using NaCl saturated and pH 3.0 acidified (sulfuric acid) solution to inhibit microbial growth and to avoid CO_2 dissolution. Hydrogen composition was measured by absorption in NaOH (4N) and gas chromatography. The biogas and hydrogen volumes were normalized to 273.15 K and 101.3 KPa. The gas chromatograph (GC-15A, Shimadzu Corporation, Kioto, Japan) was equipped with thermal conductivity detector and a Porapak N column. The oven, injector and detector temperatures were 35, 150 and 150 °C, respectively. Helium was used as carrier gas. The total volatile fatty acids (TVFA) concentration was determined by Anderson et al. [38] titration method. VFA profile of the liquid phase was determined by a gas chromatograph (Thermo Scientific Trace 1310) equipped with a capillary column (Nukol, 30 m x 0.25mm, Fused silica, Supelco, USA) and flame ionized detector (FID). For both the injector and the detector the temperature was set at 250 °C. The oven was heated at 120 °C for 1 min, followed by heating to 140°C at 20 °C min^{-1} and kept at this temperature for 1 min. Finally, the oven was heated from 14 °C to 240 °C at heating rate of 40 °C min^{-1}. Previously, the samples were centrifuged (10,000 rpm, 5 min) and filtrated (0.45 μm). Sucrose concentration was determined by total sugars method with sulfuric acid and phenol compared against a calibration curve obtained using dextrose [39].

2.4. Calculations

Concentration of total sugars during feeding for each HRT were correlated through linear regression and expressed through Eq. 1.

$$Y = b_0 + b_1 X \qquad (1)$$

Where *Y* denotes the total sugars (g L^{-1}), *X* the time during feeding (h), b_0 and b_1 are the linear regression coefficients.

For the energy evaluation two variables were defined: energy heating losses (*EHL*) and gross energetic potential rate (EPR_{gross}). According to Eq. 2, *EHL* –expressed as kJ $(L\ d)^{-1}$– is an intensive property equivalent to the energy consumed for heating a liter of influent to the continuous reactor during one average day of operation, *Cp* is the specific heat at constant pressure for the medium considered, which for practical reasons was stated to be the same as water (4.184 kJ $(kg\ ºC)^{-1}$), ΔT is the difference of temperature between operation temperature and a reference temperature (21 ºC), HRT is the hydraulic retention time expressed in days, ρ is the density of the influent (considered the same as water at 20 ºC, 0.9982 kg L^{-1}).

$$EHL = \frac{Cp\Delta T}{HRT} * \rho \qquad (2)$$

According to Eq. 3, EPR_{gross} –expressed as kJ $(L\ d)^{-1}$– is the energy potential of hydrogen produced from a liter of operation volume of the continuous reactor in one average day [25,40]. Eq. 3 was calculated as the product of the hydrogen production rate (*HPR*, in NL H_2 $(L\ d)^{-1}$), the higher heating value of hydrogen (HHV_{H2} = 282.8 kJ mol^{-1}) and the factor for converitng NL H_2 to mol H_2 (1 mol H_2 is equivalent to 22.4 L H_2 according to ideal gas law for 273.15 K and 1 atm).

$$EPR = HPR * HHV_{H2} * \frac{1\ mol_{H2}}{22.4\ L} \qquad (3)$$

3. Results and discussion

The UASB reactor was operated for 70 days at room temperature (21 ± 2 ºC) at different HRT of 24, 12 and 4 h, and sugar concentrations of 12.4, 15.2 and 19.92 $g_{sucrose}$ d^{-1}, respectively. The best operation conditions were 12h/19c in terms of hydrogen yield (HY, 70.6 ± 15.31 NmL H_2 $g_{sucrose}^{-1}$) and 4h/8c in terms of hydrogen production (HPR_{avg} = 1270 ± 177 NmL H_2 d^{-1}, HPR_{max} = 1552 NmL H_2 d^{-1}), whereas the lowest results corresponded to strategy 24h/31c. The intervals of yield in each strategy were 0-35.5 NmL H_2 $g_{sucrose}^{-1}$, 36.0-97.8 NmL H_2 $g_{sucrose}^{-1}$ and 50.3-77.6 NmL H_2 $g_{sucrose}^{-1}$ for 24h/31c, 12h/19c and 4h/8c, respectively. The average yield during the whole operation was 62.6 ± 20.5 NmL H_2 $g_{sucrose}^{-1}$ (0.88 mol H_2 $mol_{sucrose}^{-1}$) and average hydrogen content was 69.04 ± 16.65%. The total volume of accumulated biogas after the 70 days operation was 100.83 NL (65.44 NL H_2).

In Fig. 2a the performance of the UASB reactor in terms of daily hydrogen production is observed, whereas the pH and TVFA variations during the operation of the UASB are found in Fig. 2b. The pH in the reactor was recorded twice a day: the first previous to its daily feeding (pH_r) and the second after it was completed and its contents were homogenized (pH_{adj}).

The first strategy (24h/31c) began after a heat-shock pretreatment of the microbial granules, which were previously acclimated to sucrose during 20 d [24]. It can be seen that despite previous acclimatization to the substrate, the reactor presented a three-day lag phase, most likely due to germination of the spore-forming microbes, which may take from 60 minutes when fermentation occurs at proper pH, temperature, nutrients condition, to several days when operation is performed at not optimal germination conditions [32,41].

Another possibility is the adaptation phenomena of biomass to psychrophilic temperature. Anaerobic bacteria may adapt easily to low temperatures, and high-rate anaerobic treatment has been achieved at psychrophilic conditions [42]. Afterwards, the hydrogen production during 24h/31c raised up to 382.8 NmL H_2 d^{-1} at the end of this strategy. It is noteworthy that pH varied before and after feedings (Fig. 2): pH_r was 5.8-5.3 whereas pH_{adj} was 6.5-6.3. This was indicative of a fermentative activity that was in accordance to high TVFAs contents; the TVFAs exhaust the buffering capacity of the phosphates buffer (alkalinity), which is 6.71-7.71 according to Hendersson-Hasselbach equation. Indeed, adequate pH for hydrogen production has been reported in the range 4.5 to 7, depending on substrate, operation temperature, inoculum and reactor type [15]. The TVFA concentration showed slight variations according to the lag-phase presented in the beginning of the operation; the minimum TVFA concentration was 5800 mg L^{-1} at day 6, and from that day on the concentration of VFA increased continuously. The variations in hydrogen production during days 5 to 11 were lower than 15%, indicating that the reactor achieved the pseudo steady-state. According to Sivagurunathan et al. [43] such variations in time are considered to be acceptable before attempting a change in feed strategy, or a change in HRT and sucrose concentration as in our case. Hereinafter the second strategy 12h/19c was applied.

At the beginning of 12h/19c the biogas production was constantly increasing showing a six cycles wave-like performance (at 19, 25, 32, 40, 45 and 55 h) reaching a highest of 1466 NmL H_2 d^{-1} at day 47. At this point HY reached its maximum value: 97.8 Nm H_2 $g_{sucrose}^{-1}$. Afterwards, production diminished to a minimum of 920 NmL H_2 d^{-1} (day 53), following a new oscillation. This reduction in hydrogen production was corresponded by a reduction in the pH_{adj}, which fell from 6.5 to 5.5. TVFA profile presented a slightly increasingly oscillations

during this strategy. Indeed, 89% of the operation in 12h/19c production fluctuated between 800-1470 NmL H_2 d^{-1}. The average molar hydrogen yield in this strategy was 0.685 mol H_2 mol_{hexose}^{-1}. which was a value much below than the theoretical maximum from concomitant acetic and butyric fermentation of 3 mol H_2 mol_{hexose}^{-1}, or 6 mol H_2 $mol_{sucrose}^{-1}$, considering that half a mol of hexose undergoes acetic fermentation whereas the other half mol undergoes butyric fermentation. When low hydrogen yields are obtained it could be ascribed to part of the substrate being used to microbial growth and maintenance [44], or more likely to a fermentative deviation [14]; for instance, at lower pH values the abundance of lactic acid bacteria is acknowledged, which compete with the hydrogenogenic microorganisms for the carbon source and even alter the redox balance. In our case it is noteworthy that the most abundant metabolite was butyric acid (5.25-7.03 g L^{-1}), which was in accordance with lower hydrogen yields (theoretical HY is 2 mol H_2 mol_{hexose}^{-1} in butyric fermentation, against 4 mol H_2 mol_{hexose}^{-1} in acetic fermentation). Indeed, our HY was in the interval of reported In Table 3 (0.5-1.47 mol H_2 mol_{hexose}^{-1}).

In strategy 4h/8c, biogas production ranged from 1006 to 1550 NmL H_2 d^{-1}, the highest production attained during the whole operation of the UASB reactor, which occurred at day 65. Due to the continuous operation followed in this strategy, no difference could be represented as pH_r and pH_{adj} in Fig. 2, therefore only one measurement of the pH was performed. The pH presented decreasing values from 5.5 down to a minimum of 4.9; concomitantly, TVFA increased up to the maximum for the whole operation (7500 mg L^{-1}). Chang and Lin [24] suggested this effect to be expectable considering that as HRT is reduced, acid fermentation is fostered causing an accumulation in VFA that consequently

decreases alkalinity and pH. In their work, this led to TVFA of 1,083 mg L^{-1}, yield of 100 mL H_2 ($g_{sucrose}$ d)$^{-1}$ and hydrogen composition of ca. 20% at a HRT of 4 h.

The pH effects in hydrogen production are undeniable yet there is no consensus on stablishing an optimal fixed value. Our highest HPR 3625 and 3750 NL H_2 (L d)$^{-1}$ were found at days 45 and 65, with corresponding pH of 5.15 and 5.1. In contrast, Mu et al. [45] studied the role of pH in the fermentative H_2 production from an acidogenic granule-based reactor, finding that at pH 4.2 their HPR_{max} and HY_{max} were 3.48 L H_2 (L d)$^{-1}$ and 1.61 mol H_2 mol_{hexose}^{-1}, respectively. However, operating at low pH is risky since iron containing hydrogenases may be affected [46].

PLEASE INSERT FIGURE-2 HERE

Some authors have assayed low HRT, such as Chang and Lin [24] or Si et al. [23]. Chang and Lin [24] in a UASB fed with sucrose (20 g L^{-1}) found that hydrogen production depended greatly on the HRT changes evaluated from 24 to 4 h. They reported that at 24 and 4 h HRT the hydrogen production was drastically affected, whereas at 8 h HRT they obtained their HPR rate (6.84 L (L d)$^{-1}$) with an average hydrogen content of 32% (v/v). Si et al. [23] in a UASB reactor using glucose as carbon source and carbon nanotubes for supporting the biomass, also found low hydrogen production at 24 h HRT, whereas at 8 and 4 h they achieved the highest productions (4.38 L (L d)$^{-1}$).

In Fig. 3 it is shown the total sugars consumption at HRT 24, 12, and 4 h during their first 10 h after feeding as a depiction of microbial activity. For 24 and 12 h HRT the sucrose concentration was increasing over time pointing to an accumulation substrate. For 4 h HRT, the profile shown in this figure was obtained after beginning the strategy 4h/8c, showing that

sucrose concentration continuously decreased (a deficit of substrate), an indicative of a higher consumption rate of the sugars. According to Eker et al. [47] at low initial sugars concentration (3.84 to 18.9 g L^{-1}) its consumption is faster. The initial sugar concentration at 4, 12 and 24 h HRT was 8.3, 19 and 37 $g_{sucrose}$ L^{-1}. The profiles of each strategy were fitted to a linear regression model, showing that 24 and 4 h HRT had a characteristic linear performance (R^2 = 0.96 and 0.87, respectively), whereas at 12 h HRT deviated from linear behavior (R^2 = 0.6).

PLEASE INSERT FIGURE 3 HERE

Our average results in terms of HY, molar hydrogen yield (MHY), HPR and metabolites found are listed in Table 2; the best results for HY and MHY were at 12h/19c. Even though the 4h/8c strategy had the best average HPR, the average of the strategy 12h/19c included a period of 10 days where the HPR was beginning to rise after the low results from 24h/31c, which lowered the average value.

In Table 3, the best results from our 12h/19c strategy are shown along with selected results from literature works using continuous reactors, mainly UASB, and soluble substrates such as sucrose, glucose or cheese whey as substrate. The best HPR were reported to be either at 6 or 8 h HRT, whereas at 12 h HRT also good results were obtained. For instance, Si et al. [23] reported that at 12 and 4 h HRT their HPR were 2.88 and 5.28 NL H_2 (L d)$^{-1}$, respectively. The highest HPR from 12h/19c (3.66 NL H_2 (L d)$^{-1}$) was comparable to those from literature (2.35 – 4 NL H_2 (L d)$^{-1}$) at similar HRT than ours. Regarding HY, it varied in the range 0.5 – 1.47 mol H_2 mol_{hexose}^{-1} in mesophilic, thermophilic and hyperthermophilic regimes (35, 38, 55 or 70 °C). Our HY (0.685 mol H_2 mol_{hexose}^{-1}) obtained at psychrophilic temperature was close to the minimal value of the precedent

ranges and no real stabilization of HY was obtained during the experimental sequence. Differences may be ascribed mainly to operation temperature and inoculum type.

When comparing the results of 12h/19c against reports at psychrophilic and ambient temperatures (Table 3), it is worth noticing that our HPR was higher. The closest to ours was that obtained by Moureira et al. [48] in a packed bed reactor using 2 g COD L^{-1} sucrose as substrate. In contrast, the highest HY (0.918 mol H_2 mol_{hexose}^{-1}) in similar experiments was that reported by Muñoz-Páez et al. [25] using an anaerobic fluidized bed reactor, compared the hydrogen production from sucrose at 8 g $(L\ d)^{-1}$ using anaerobic fluidized bed reactor (AFBR) at ambient temperature (<30 °C). When comparing their own results against mesophilic operation, they found that HPR at ambient temperature was nearly 2.2-fold (920 NmL H_2 $(l_{bed}\ d)^{-1}$) than mesophilic operation. Other substrate that has shown good results was cheese whey at 20 g COD $(L\ d)^{-1}$, that Carrillo-Reyes et al. [49] used in UASB reactors, obtaining HPR 1.41 NL H_2 $(L\ d)^{-1}$ and HY of 0.56 mol H_2 mol_{hexose}^{-1} at 24 h HRT. Among the reasons of our highest results may dwell in the differences of sludge pretreatment, HRT used and reactor configuration [9,50]. For instance, Carrillo-Reyes et al. [49] disaggregated and heat-treated an anaerobic granular sludge; according to Show et al. [51], hydrogen production by granular sludge exhibits very high HPR at low HRT (up to ten-fold) than disaggregated sludge.

Selected results from Table 3 were used for calculating the *EHL* (Eq. 2) and the EPR_{gross} (Eq. 3); the results are shown in Fig. 4. It was observed that all the works using temperature for maintaining mesophilic, thermophilic or hyperthermophilic regimes presented higher *EHL* than EPR_{gross}, except for those reports using psychrophilic or room temperature regimes despite not having the highest EPR_{gross}. According to Eq. 3, the *EHL* rises as temperatures

of operation increases and HRT decreases, which was particularly evident for the works of Tähti et al. [52], Braga et al. [53] and Yu and Mu. [54], who applied high temperatures (70, 55 and 38 °C, respectively) and short HRT (5, 2 and 6 h, respectively). This remarks the importance that if biohydrogen production systems are to be applied at industrial scale, it is not only important to maximize the yields and production rates, but also to decrease energy expenses. In this sense, the psychrophilic systems may have a real opportunity of development despite the lower hydrogen production when compared to mesophilic, thermophilic or hyperthermophilic systems. Perera et al. [55] arrived to similar observations than ours, arguing that the improvement in hydrogen production at temperatures above 25 °C is not defensible as net energy gains were negative and declining as temperature increased. Moreover, other strategies to increase the energetic gains of psychrophilic biohydrogen production would be by its coupling to other bioenergy processes such as microbial fuel cells, photofermentation and anaerobic digestion, among others [56–59].

PLEASE INSERT TABLE 2 HERE

PLEASE INSERT TABLE 3 HERE

PLEASE INSERT FIGURE 4 HERE

4. Conclusion

Semi-continuous and continuous feeding of sucrose in an UASB reactor operated at psychrophilic temperature achieved considerably hydrogen generation and accumulation of TVFAs. The best operation strategy was 12h/19c since it presented comparable results to those in literature that were obtained at mesophilic temperatures and similar HRT. Using HRT of 24 h gave low hydrogen production, whereas 4 h HRT presented higher production

at the expense of lower hydrogen yields, which is undesirable when pure substances such as sucrose or glucose are used as substrate.

It is noteworthy that biohydrogen systems operating at psychrophilic or ambient temperature would not require energy expenditures for the heating of the influents, which would thus present an opportunity for achieving net energy gains.

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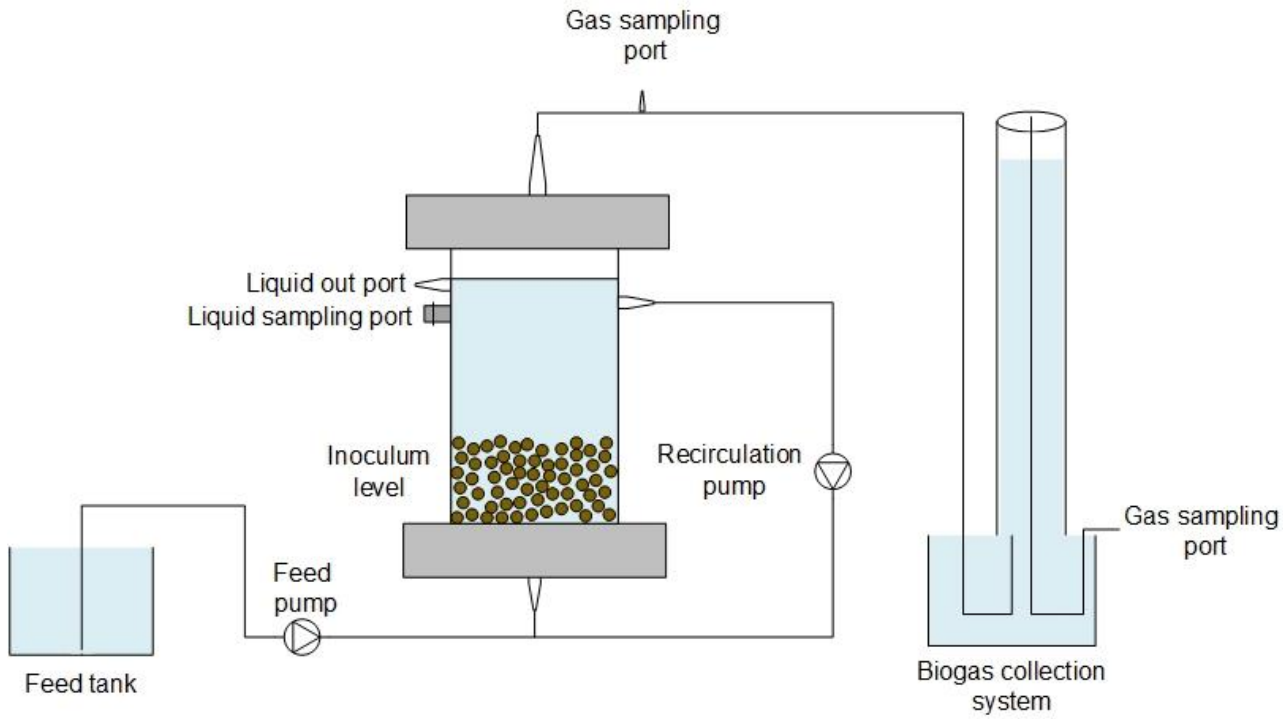

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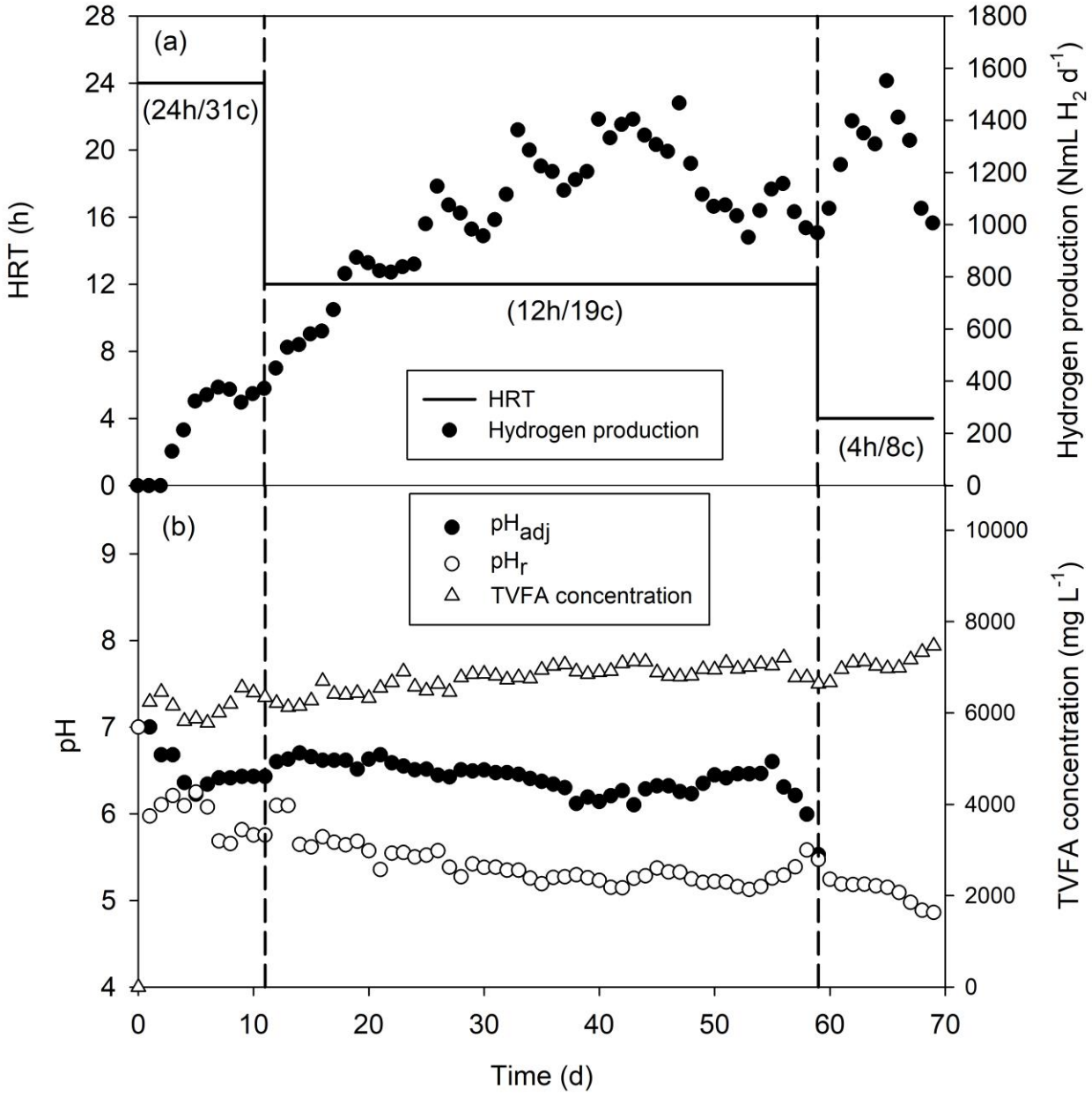

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594 Fig. 2. UASB reactor performance (A) HRT and normalized hydrogen production versus time, (B) pH and TVFAs versus time. 24h/31c operated at 24 h HRT fed with 31 $g_{sucrose}$ L^{-1}; 12h/19c

596 operated at 12 h HRT fed with 19 $g_{sucrose}$ L^{-1}; 4h/8c operated at 4 h HRT fed with 8.3 $g_{sucrose}$ L^{-1}.

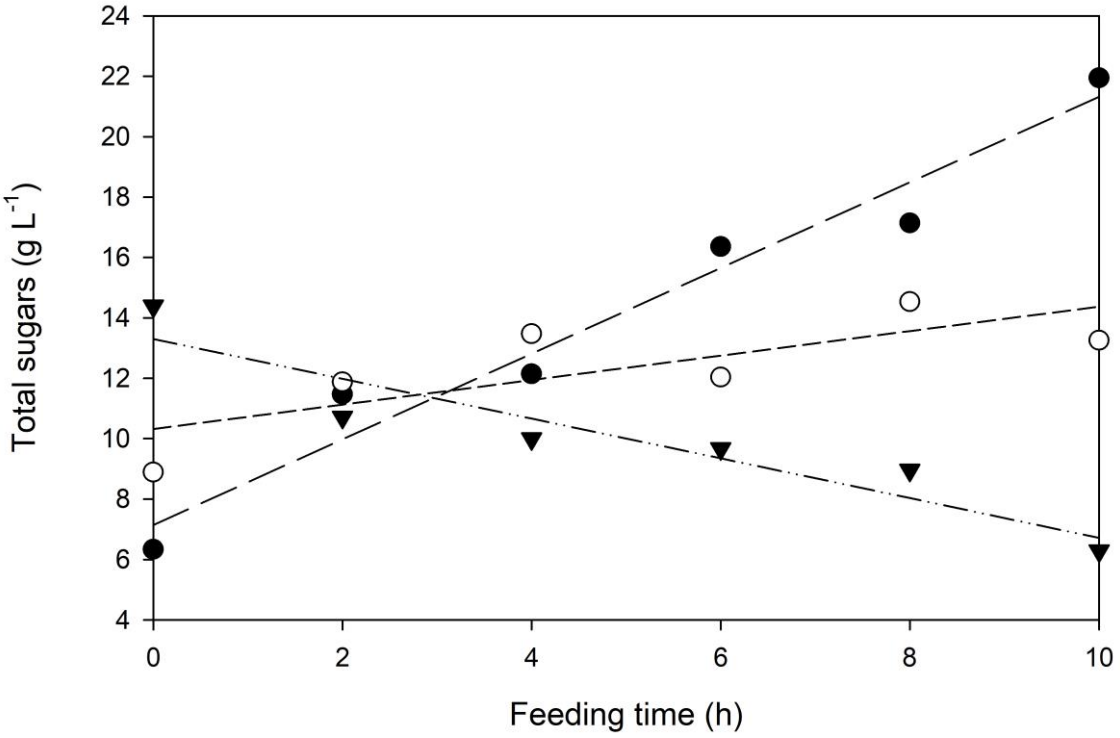

Fig. 3. Influence of HRT on total sugar concentration in function on time. Linear regression curves were represented by dashed lines. (●) 24h/31c, $Y = 7.14 + 1.41X$, $R^2 = 0.96$; (○) 12h/19c, $Y = 10.32 + 0.40X$, $R^2 = 0.60$; (▼) 4h/8c, $Y = 13.29 - 0.66X$, $R^2 = 0.87$.

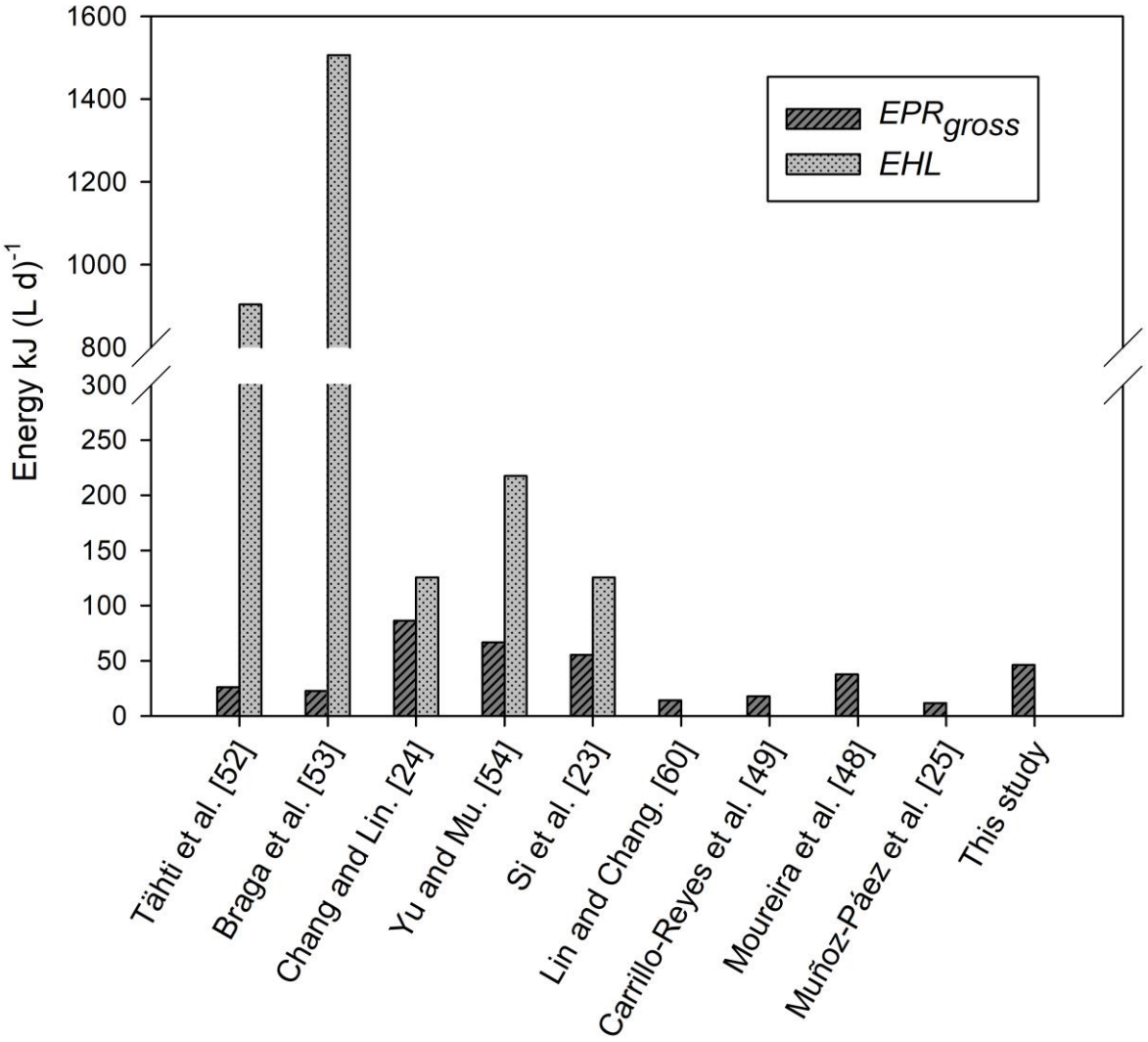

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Table 1. Characteristics of inoculum.

Parameter	
pH	6.66 ± 0.10
Humidity ($\%_{wb}$)	93.80 ± 0.07
Total solids ($\%_{wb}$)	6.20 ± 0.07
Volatile solids ($\%_{db}$)	34.82 ± 3.59
Ash ($\%_{db}$)	65.18 ± 3.59

Notes: wb, wet basis; db, dry basis

Table 2. Average results and soluble metabolites produced by different strategies.

Strategies	**HY (NmL H_2 $g_{sucrose}^{-1}$)**	**MHY (mol H_2 $mol_{sucrose}^{-1}$)**	**HPR (NL H_2 (L d)$^{-1}$)**	**Soluble metabolites (g L^{-1})**					
				EtOH	HAc	HPr	HBu	SM	TVFA
24h/31c	23.9	0.18	0.67	0.47	1.65	0.02	4.89	7.03	6.18
12h/19c	69.1	0.51	2.60	0.13	1.41	0.03	3.68	5.25	6.81
4h/8c	63.5	0.48	3.17	0.26	1.55	0.06	4.67	6.54	7.08

Notes: HY, hydrogen yield; MHY, molar hydrogen yield; HPR, hydrogen production rate; EtOH, ethanol; HAc, acetic acid; HPr, propionic acid; HBu, butyric acid; SM, soluble metabolites (EtOH+HAc+HPr+HBu).

Table 3. Biohydrogen production in continuous reactors operating at different temperatures: psycrophilic (< 25°C), mesophilic, thermophilic and hyperthermophilic conditions.

Substrate (concentration/OLR)	**Inoculum**	**Reactor type**	**HRT/ T_{op}**	**HY/ $[H_2]_{avg}$ in biogas**	**HPR**	**Ref**
Glucose (5 g COD L^{-1}/ 25.1 g COD $(L\ d)^{-1}$)	Granular sludge	UASB	5 h 70 °C	0.73 mol H_2 mol_{hexose}^{-1} 51%	2.07 NL H_2 $(L\ d)^{-1}$	[52]
Sucrose (1.8 g COD L^{-1}/ 24 g COD $(L\ d)^{-1}$)	Granular sludge	UASB	2 h 55 °C	0.865 mol H_2 mol_{hexose}^{-1} 51.49%	1.8 NL H_2 $(L\ d)^{-1}$	[53]
Sucrose (20 g COD L^{-1}/ 60 g COD $(L\ d)^{-1}$)	Sewage sludge	UASB	8 h 35 ºC	0.75 mol H_2 mol_{hexose}^{-1} 42.4%	6.84 NL H_2 $(L\ d)^{-1}$	[24]
(20 g COD L^{-1}/ 40 g COD $(L\ d)^{-1}$)			12 h 35 ºC	0.81 mol H_2 mol_{hexose}^{-1} NR	4 NL H_2 $(L\ d)^{-1}$	
Sucrose (10.67 g COD L^{-1}/ 42.68 g COD $(L\ d)^{-1}$)	Anaerobic sludge	UASB	6 h 38 ºC	0.5 mol H_2 mol_{hexose}^{-1} NR	5.28 NL H_2 $(L\ d)^{-1}$	[54]
(10.67 g COD L^{-1}/ 25.61 g COD $(L\ d)^{-1}$)			10 h 38 ºC	0.9 mol H_2 mol_{hexose}^{-1} NR	2.88 NL H_2 $(L\ d)^{-1}$	
Glucose (8 g L^{-1} / 24 g $(L\ d)^{-1}$)	Anaerobic sludge supported on carbon nanotubes	UASB	8 h 35 ºC	1.47 mol H_2 mol_{hexose}^{-1} 46%	4.38 NL H_2 $(L\ d)^{-1}$	[23]
(8 g L^{-1} / 16 g $(L\ d)^{-1}$)			12 h 35 ºC	1.45 mol H_2 mol_{hexose}^{-1} 38%	3 NL H_2 $(L\ d)^{-1}$	
Glucose (NR/ 12.46 g $(L\ d)^{-1}$)	Sewage sludge	Chemostat-type anaerobic reactor	1.5 [a] 24-28 °C	0.68 mol H_2 mol_{hexose}^{-1} 45.1%	1.13 NL H_2 $(L\ d)^{-1}$	[60]
Cheese whey (NR/ 20 g COD $(L\ d)^{-1}$)	Anaerobic granular sludge	UASB	24 h 22-25 °C	0.56 [b] mol H_2 mol_{hexose}^{-1} 76%	1.41 NL H_2 $(L\ d)^{-1}$	[49]

Sucrose (2 g COD L^{-1}/ NR)	Natural fermentation innoculation	PBR[c]	2 h 25 °C	0.7 mol H_2 mol_{hexose}^{-1} 75.6%	2.99 NL H_2 $(L\ d)^{-1}$	[48]
Sucrose (8 g L^{-1}/ NR)	Methanogenic digestates	AFBR[d]	24 h 30 °C	0.918 mol H_2 mol_{hexose}^{-1} 56%	0.92 NL H_2 $(L\ d)^{-1}$	[25]
Sucrose (19 g L^{-1}/ 38 g $(L\ d)^{-1}$)	Brewery industry anaerobic sludge	UASB	12 h 21 ± 2 °C	0.685 mol H_2 mol_{hexose}^{-1} 69.04%	3.66 NL H_2 $(L\ d)^{-1}$	This study

Notes: [a]solid retention time (HRT equivalent 28.8 h); [b] calculated as hexose equivalent; PBR, packed bed reactor; AFBR, anaerobic fluidized bed reactors; $[H_2]_{avg}$, hydrogen content in biogas; COD, chemical oxygen demand; HPR, hydrogen production rate; HRT, hydraulic retention time; HY, hydrogen yield; NR, not reported; Top, temperature of operation.

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