# Biohydrogen Production in Fluidized Bed Bioreactors: Room Temperature vs 35°C

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ABSTRACT

Hydrogen is considered a versatile and clean bio-fuel and research on biological production of $H_2$ has boosted in the last years. Two key issues on biohydrogen production from organic substrates in submerged fermentation are *(i)* type of bioreactor, and *(ii)* temperature of operation. Anaerobic fluidized bed reactors (AFBR) exhibit attractive features for the production of $H_2$ although their application for this purpose is scarce. On the other hand, use of ambient temperature of operation could help to decrease the use of energy to maintain the temperature at higher levels; yet little is known on dark fermentation in the psychrophilic range. Thus, the aim of this work was to compare the $H_2$ production in a lab scale AFBRs at two levels of operational temperature: ambient temperature (A) and 35 °C (M) and two organic volumetric loading rates $B_v$: 5 and 8 g sucrose /(L d), with a constant hydraulic residence time of 1 day.

The increase of $B_v$ from 5 to 8 g sucrose/L day had a positive effect on the performance of our anaerobic fluidized bed reactors; the $H_2$ productivity increased 2100 and 1684% for AFBR-A and AFBR-M, respectively. With a $B_v$ of 8 g sucrose/L the average performance of AFBR-A was superior to that of AFBR-M: 1.8 times for the $H_2$ concentration in the biogas (54% and 31% respectively) and 2.1 times for the $H_2$ productivity (1330 and 580 $mLH_2$/(Lbed day), respectively). The volatile organic acids (VOA) contributed to most of the soluble microbial products at both temperatures with ratios VOA/SOLV of 18.99 and 1.68 for A and M, respectively (ratio of total volatile organic acid/total solvent products). In conclusion, the $H_2$ production in an AFBR-A at ambient temperature showed encouraging results for $H_2$ production in submerged fermentation of moderate concentration of sucrose.

Key words: anaerobic fluidized bed bioreactor, biohydrogen, dark fermentation, sucrose.

## 1. Introduction

In the last 10 years, interest on biohydrogen has increased exponentially [1]. Hydrogen is one of the most promising fuel candidates: *(i)* it is a versatile, safe, renewable, environmentally compatible and economic fuel [2], *(ii)* it is more secure to manage than domestic natural gas [3], *(iii)* its combustion in automobiles is 50% more efficient than

gasoline, and *(iv)* it possesses an energy content per unit mass of 142 KJ/g o 61,000 Btu/lb [3] that is 2.7 times greater than that contained in certain hydrocarbon [4].

There are several technologies to generate $H_2$; they can be classified as biological, chemical and electrochemical. Chemical technologies include steam reforming, electrolysis and partial oxidation process [5]. On the other hand the biological production processes can be classified into three categories: bio-photolysis, photo-fermentation and dark fermentation [6-8]. We focus here on biological process because they are environmentally friendly and consume less energy. Particularly, $H_2$ production by dark fermentation exhibits several advantages: *(i)* many fermentative bacteria are capable of high hydrogen generation rate, *(ii)* $H_2$ is produced throughout the day and night at a constant rate since it does not depend on energy provided by sunlight, and *(iii)* bioreactors have a relatively small footprint when solid substrate dark fermentation is chosen [9-13].

Sustainability of biohydrogen production depends on the attainability of renewable substrate and the establishment of fermentation conditions that augment both the rate and the yield of hydrogen production [14]. There are factors that determine optimal hydrogen production, such as: *(i)* pH, *(ii)* temperature, *(iii)* concentration of electron donor, *(iv)* type of the reactor, *(v)* alkalinity, *(vi)* nutritional history of the cells, *(vii)* method of inoculum enrichment and method of methanogenesis inhibition used, and *(viii)* type of inocula, among others [15-19]. The thermal regime of operation can affect the growth rate and metabolic activity of microorganisms [20]. This fermentation processes can be conducted at mesophilic (25–40 ◦C), thermophilic (40–65 ◦C), extreme thermophilic (65–80 ◦C), or hyperthermophilic (>80 ◦C) temperatures [21]. Selection of the temperature of incubation should take into account that the heat energy needed to conserve higher fermentation temperatures can diminish the net energy gain of biogas production [22, 23], therefore it is interesting to study the production of hydrogen at ambient temperature (without heating).

Several studies of hydrogen production have been conducted in continuous stirred tank reactors. The continuous stirred tank reactor is one of the most used because of its simple construction, easy operation and effective homogeneous mixing. However, the hydraulic retention time (HRT) controls microbial growth and therefore the HRT must be greater than the maximum rate of growth of microorganisms, higher speeds because the dilution caused loss of microorganisms [24]. To avoid this inconvenient, several mechanisms and devices have been incorporated to bioreactors in order to retain the active microbial biomass, usually by immobilization. In this way, both growth and concentration of microorganisms are essentially independent of the HRT, and high cell concentrations of biomass can be achieved inside the bioreactors [25-27].

Recent studies have found that favorable immobilized-cell anaerobic hydrogen production systems include anaerobic fluidized bed reactors (AFBR; [28]). Tbe AFBR consists of a tall column containing an inert support or granules that

are held in suspension due to the drag force of the upward flow where the microorganisms are retained in the form of biofilm [29,30] and has been widely used as a system for treating wastewater, characterized by high efficiency and the use of low hydraulic retention time [31,32]. In this type of reactor, the accumulation of biogas and deposition of particles take place in different compartments located in the column [33]

Among the advantages of the AFBR we find the following: *(i)* at speeds high mass transfer, *(ii)* it has the ability to control and optimize the thickness of the biofilm by attrition and special devices, *(iii)* the biomass support can be chosen for applications specific, *(iv)* the treated effluent recirculation means it has excellent hydraulic pattern that prevents short circuits and dead zones [34, 35]. Even though AFBR exhibits positive features for the production of biogases such as $H_2$ it has been used primarily for the treatment of wastewater [27,32,36]. Some materials that have been used for $H_2$ production are activated carbon [28], zeolite [37] and expanded clay [38].
To the best of our knowledge, there are no studies for $H_2$ production by AFBR at low temperatures. Therefore, this work was aimed to evaluate the $H_2$ production in an anaerobic fluidized bed reactor using two incubation temperatures: ambient temperature and 35 °C.

**2. Experimental**

*Bioreactors*. The laboratory-scale AFBRs consisted of glass columns of 4.5 cm internal diameter, 185 cm length and 3 L of working volume; loaded with 1 L of granular activated carbon (1-2 mm diameter) colonized by an anaerobic consortium. The hydraulic residence time was 1 day (fluidized bed volume basis).

*Experimental design*. The experimental design examined the effect of two factors on $H_2$ production in AFBR, *i.e.* two temperatures of operation: 35ºC (AFBR-M) and ambient temperature (AFBR-A), and two volumetric loading rates ($B_v$, 5 and 8 g sucrose/(L d).) All AFBRs were operated at 1 d HRT

*Inocula*. AFBRs were seeded with digestates from methanogenic substrate anaerobic digesters degrading sucrose. Those digesters were operated at mesophilic conditions. Before loading into the reactor the digestates were pre-treated by heat-shock (90ºC, 1h)

*Substrate*. The organic carbon source was either 5 or 8 (g/L) of sucrose. The reactors were fed with a synthetic wastewater with the following composition ($mgL^{-1}$; [28, 39]): $CH_4N_2O$ (125); $NiSO_4*6H_2O$ (1); $FeSO_4*7H_20$ (5); $FeCl_3*6H_2O$ (0.5); $CoCl_2*2H_2O$ (0.08); $CaCl_2*6H_2O$ (47); $SeO_2$ (0.07); $KH_2PO_4$ (85); $K_2HPO_4$ (21.7); $Na_2HPO_4*2H_2O$ (33.4); $NaHCO_3$ (1 g/L). Either 5 or 8 g of sucrose per liter were added, depending on the operation $B_v$.

*Analyses*. The main monitoring parameters were hydrogen production, pH and soluble metabolites. Hydrogen and methane contents in biogas were determined by gas chromatography [13] in a Gow-Mac chromatograph model 350 fitted with a thermal conductivity detector (TCD) and Molecular Sieve 5A packed column (injector, detector and column temperatures were 25, 100 and 25 ºC, respectively). Argon was the carrier gas. Soluble metabolites (volatile organic acids, lactic acid, and solvents) were determined in the effluent that was filtered through a glass-membrane filter and an aliquot of the filtrate was injected in a gas chromatography Varian Star 3400 equipped with a FID for metabolite concentration determination. The injector and detector temperatures were set at 250ºC. Nitrogen was used as a carrier gas with a 20 mL/min flow rate. The oven temperature was programmed as follows: 60 ºC for 2 min, increasing to 140 ºC at 5ºC/min, and then kept constant at 140ºC for another 6 min. A 50 m 0.32 mm internal diameter fused silica capillary column coated with 0.2 mm CP-Wax 57 CB was used.

**3. Results and discussion**

*3.1 Effect of increase the volumetric loading rate*

Overall, there was a significant performance improvement of bioreactors at the highest $B_v$. In the first period of operation at $B_v$ of 5 g sucrose/(L d) low concentration of $H_2$ in biogas and $H_2$ productivity was observed (Figure 1a, d, Table 1). After 20 days the $B_v$ was changed to 8 g sucrose/L day; the $H_2$ in biogas increased by 5.2 and 6.3 times in AFBR-A and AFBR-M, respectively, whereas the $H_2$ productivity was enhance by 22 and 33 times in AFBR-A and AFBR-M, respectively.

In the first 8 days of the second period $H_2$ production was no observed in AFBR-M, on the other hand methane production was registered(Figure 1 a & b). This was consistent with reported hydrogen consumption by methanogenesis [40]. Therefore the presence of $CH_4$ could indicate that heat treatment was not sufficient to eliminate the methanogenic microorganisms in the inocula, particularly in the AFBR-M [28]. It is known that heat treatment of the inocula is intended to eliminate non-sporulating, hydrogen-consuming microorganisms such as methanogenic archaea [41].

After 8 day in the second period we removed bicarbonate in the synthetic wastewater in order to allow the pH diminish naturally. Once the pH dropped down to 4.5 the concentration of $CH_4$ in the biogas decreased (Figure 1 b & c). This was in agreement with other studies where it was observed that the combination of heat-treatment of inocula and lower pH value repress the methane-forming microorganisms [16, 40].

Acetic acid (HAc), Butyric acid (HBu), Propionic acid (HPr), Ethanol (EtOH) and Butanol (BuOH) were the soluble microbial products (SMP) detected in this fermentation (Table 1). The volatile organic acids (VOA) contributed to most of the SMP at both temperatures and both Bv with high ratios TVOA/SOLV (TVOA/SOLV: total volatile organic acid/solvents products) in most cases. Values of TVOA/SOLV were of 55 and 19 for the ambient

temperature reactors operated at $B_v$=5 and 8 g/(Ld), respectively, and 72, and 1.2 in mesophilic reactors run at $B_v$=5 and 8 g/(Ld) . The TVOA/SOLV ratio in the first period was almost 3 (AFBR-A) and 24 (AFBR-M) times higher than in the second period.

This could be due to pH values below 4.5 (*ca.* 10 days) in the second period which is known to be associated to solventogenesis and decreased $H_2$ harvesting [42-44].

The presence of HPr in the first period was higher than that in the second period in both reactors and at both temperatures. The may be due to the low pH 4.5 at increased $B_v$ of 8 g sucr /(L d) since it is known that low pH could inhibit propionic production [25,28, 37] (Zhang et al. ,2007, Koskinen et al. 2007, and Barros et al. 2010). In the first period de HPr/SMP was higher in AFBR-M and the propionate fermentation is related with low production of $H_2$ [37] Koskinen et al., 2007) and it can be associated with the low $H_2$ productivity observed.

*3.1 Effect of temperature of incubation*

In general, operation at ambient temperature was associated to better bioreactor performance than in mesophilic regime of operation (Table 1). The average $H_2$ concentration in the biogas in AFBR-A in the 2 $^{nd}$ period was 1.8 times superior than that of AFBR-M (56 and 32% $H_2$, respectively; Table 1). Similarly the $H_2$ productivity in AFBR-A was 2.1 times superior than that of AFBR-M (1232 and 589 $NmLH_2$/(Lbed d), respectively; Table 1). In the second period, the average value of 56% of $H_2$ concentration in biogas from the AFBR-A was in the range of 40–60% reported in other studies with AFB-A (Table 2).

Our results agree with findings of [45] that operated well mixed batch reactors at 22 °C and 37 ° C. They observed that the $H_2$ production was 1.3 times superior in a reactor operated at 22ºC. This result could be attributed to the gradual changes in pH induced by slower kinetics at lower temperature; indeed lower temperatures cater more time for hydrogen producing bacteria to adjust to pH dynamics in unbuffered reactors [46]. Methane was observed in the biogas of AFBR-M in both periods of operation (Figure 1 b), in contrast no $CH_4$ was detected in AFB-A. Since both bioreactors received the same inocula, the fact that methane production was more noticeably in the AFBR-M, could be related to its operation temperature 35 ° C which is reported to be the optimum for methanogenic microorganisms [47].

The $TVOA/SOLV_{AFBR-M}$ was 11.3 lower than $TVOA/SOLV_{AFBR-A,}$ in the second period; the higher contribution of solvents (ethanol and butanol) in the AFBR-M may indicate that the fermentation was being diverted to a solvent production which is consistent with the low production of $H_2$ obtained. Interestingly, at similar pH values in both

bioreactors the solvent production was not observed in AFBR-A. This could be attributed to the fact that a low temperature allows more time to adapt to changes in pH as we mention above [46].

Furthermore the main solvent generated in AFBR-M was ethanol (36%) and the ethanol-type fermentation is related with low production of $H_2$ [37, 48, 49].

In the AFBR-A the major byproduct was butyric acid (59 %). this was in agreement with literature reports of butyrate production in the pH range of 4.0-4.5 [50]. The A/B (acetic to butyric acid ratio, on COD basis) has been apply as a surrogate indication of $H_2$ production in acidogenic systems [37, 51]. In general a higher A/H ratio higher than 0.80 indicates a higher H2 yields, likely with hydrogen production from hexoses with acetogenesis. Values lower than 0.8 are likely associated with hydrogen production from hexoses with butyrogenesis [52]. In our work, A/B ratios were close to 0.8 or lower (Table 1) and this mith indicate that the carbon flow was directed into products another than acetate, HBu for AFBR-A and EtOH for AFBR-M (Table 1; [53]).

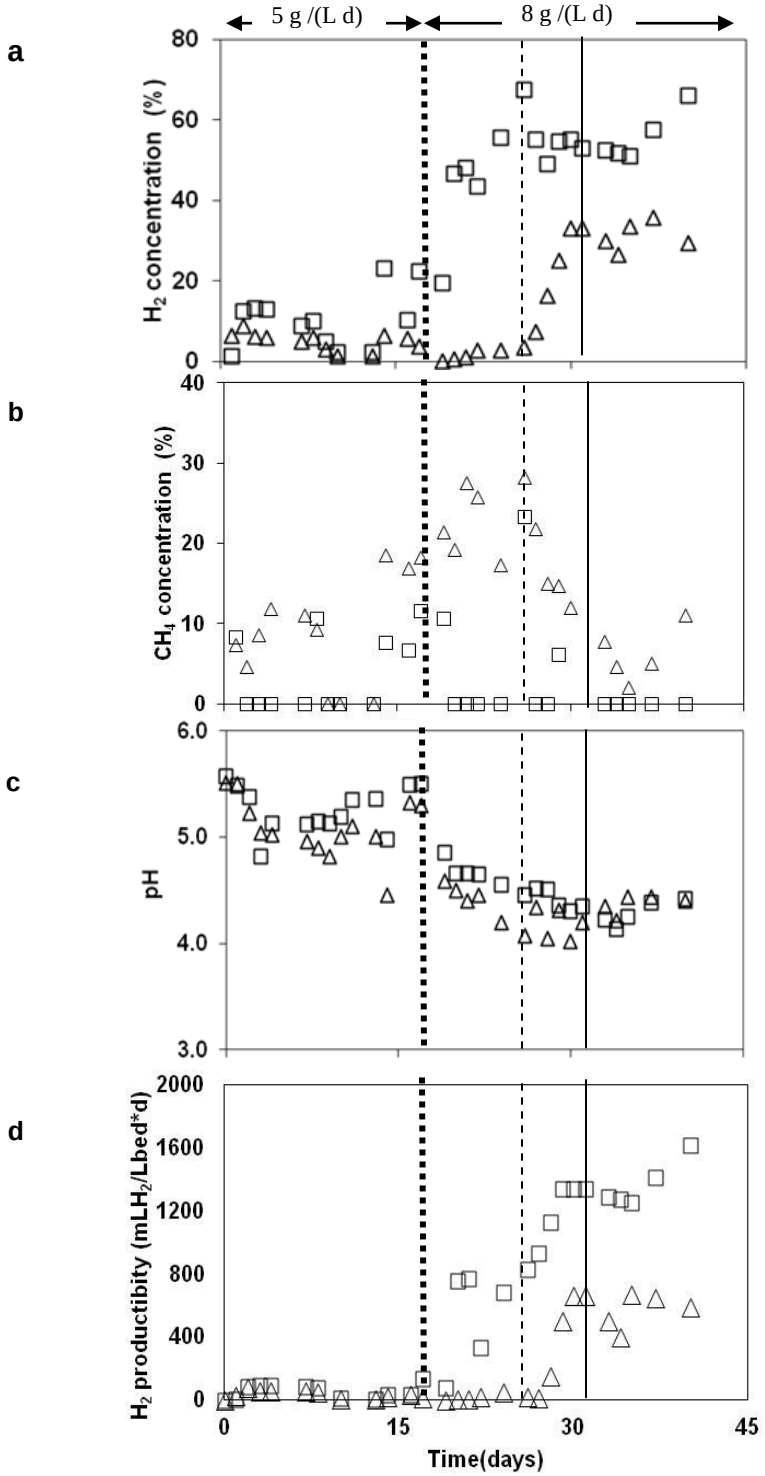


Figure 1. Time courses of: (a) $H_2$ concentration in biogas; (b) $CH_4$ concentration in biogas; (c) pH and $H_2$ productivity □: AFBR-A; Δ: AFBR-M. Note:bold dotted line indicates the change of Bv=5 g sucrose/L to 8 g sucrose/L. Lines in Bv= 8 g sucrose/L indicate the beginning of the steady state: solid line AFBR-A and dotted line AFBR-M

Table 1. Average performance of AFBR-A and AFBR-M.

| Reactor / Parameter | Bv = 5 g sucr/(L.d) | | Bv = 8 g sucr/(L.d) | |
|---|---|---|---|---|
| | **AFBR-A** | **AFBR-M** | **AFBR-A*** | **AFBR-M*** |
| pH | 5.22 ± 0.26 | 5.04 ± 4.20 | 4.35 ± 0.13 | 4.29 ± 0.16 |
| $H_2$ concentration (%) | 10.6 ± 6.9 | 5.0 ± 2.2 | 56.0 ± 6.2 | 31.6 ± 3.1 |
| Biogas production ($mLH_2$/ day) | 791 ± 410 | 57 ± 44 | 2222 ± 449 | 1900 ± 191 |
| $H_2$ productivity ($mLH_2$/$L_{bed}$ day) | 56 ± 44 | 33 ± 26 | 1232.9 ± 241 | 589 ± 103 |
| Soluble microbial products (mg COD/L) | | | | |
| Acetic acid | 1101.2 ± 238.8 | 951. 2 ± 110.3 | 957.1 ± 236.0 | 1172.7 ± 196.0 |
| Propionic acid | 782.5 ± 180.1 | 1024.9 ± 325.4 | 318.1 ± 32.8 | 341.8 ± 69.0 |
| Butiric acid | 838. 1 ± 164.5 | 1403.8 ± 372.9 | 2097.1 ± 427.3 | 1382.5 ± 321.0 |
| Lactic acid | ND | ND | ND | ND |
| Acetone | ND | ND | ND | ND |
| Methanol | ND | ND | ND | ND |
| Ethanol | 106. 0 ± 71.6 | 53.1 ± 27.5 | 88.3 ± 17.9 | 1654.7 ± 49.8 |
| Butanol | 31. 1 ± 15.3 | ND | 89.2 ± 10.5 | 68.3 ± 7.0 |
| EtOH/SMP (%) | 0.8 ± 1.3 | 1.7 ± 0.9 | 2 ± 0 | 36 ± 4 |
| BuOH/SMP (%) | 1.1 0.3 | ------- | 3 ± 0 | 1 ± 0 |
| HAc/SMP (%) | 39.9 ± 2.7 | 43.0 ± 14.0 | 27 ± 3 | 25 ± 1 |
| HPr/SMP (%) | 28.1 ± 2.1 | 40.2 ± 9.7 | 9 ± 2 | 7 ± 0 |
| HBu/SMP (%) | 30.1 ± 2.7 | 45.0 ± 11.0 | 59 ± 2 | 30 ± 3 |
| A/B | 1.3 ± 0.2 | 0.8 ± 0.1 | 0.5 ± 0.1 | 0.9 ± 0.1 |
| TVOA (mg COD/L) | 2721. 7 ± 311.8 | 2594.9 ± 818.2 | 3372 ± 650 | 2897 ± 59 |
| SMP (mg COD/L) | 2771.2 ± 277.2 | 2630. 3 ± 849.0 | 3550 ± 676 | 4621 ± 643 |
| TVOA/SOLV | 55 | 72 | 19 | 1.2 |

*The average data was obtained under steady-state conditions: AFBR-R: day 8 to 22 and AFBR-M: day 15 to 22. A/B: acetic to butyric acid ratio. EtOH: ethanol; BuOH: butanol; HAc: acetate; HPr: propionate; HBu: butyrate; TVOA: total volatile organic acids= HAc+HPr+HBu; SMP: soluble microbial products=TVOA+EtOH+BuOH. Based in COD/L

Table 2. Hydrogen production using anaerobic fluidized bed reactor

| **Inocula** | **Conditions** | **Support material** | **Substrate concentration (mg/L)** | **Y' Hydrogen Pseudoyield ($molH_2$/Kg substrate fed) $H_2$ % in biogas** | **Soluble microbial products** | **Ref.** |
|---|---|---|---|---|---|---|
| Anaerobic sludge hs (105°C, 45 minutes) | HRT:0.5-4 h pH:4.0 T:37° C | Activated carbon | Glucose (1000) | 6.44 @ HRT of 0.5–2 h. 57-61% | Acetic acid Butyric acid Propionic acid Ethanol | 1 |
| Anaerobic sludge hs (90°C, 10 min) | HRT:8-1 h pH: 3.7-4.1 T:30°C Lote:48 h | Expanded Clay | Glucose (2000) | 13.39 @ HRT of 3.7-4.1h 20-30% | Acetic acid Butyric acid Ethanol | 2 |
| Sludge hs (90°C 10 min followed by ice cooling to 25°C) | HRT: 8-1 pH: 3.8 30°C Lote:48 h | Expanded Clay | Glucose (2000) | 12.72 @ HRT of 2 h 21.8-37.6% | Acetic acid Butyric acid Ethanol | 3 |
| Anaerobic sludge hs (90°C 10 min) | HRT:8-1 h pH: 5.5 T:30°C | Expanded clay<br><br>Polystyrene | Glucose (4000) | 14.00@ HRT of 2 h 50%<br>10.6 @ HRT of 2h 40%<br><br>16-47% | Acetic acid Butyric acid Propionic acid Ethanol | 4 |
| Anaerobic sludge hs (90°c, 10 min) | HRT: 8-1 h pH: 5.5 T: 30°C | Ground Tire<br><br>Pet | Glucose (4000) | 11.94@ HRT of 1 h<br><br>52-96%<br>10.39@ HRT of 1 h | Acetic acid, Butiric acid Lactic acid Ethanol | 5 |
| Methanogenic sludge hs (90ºC, 1 h) | HRT:1 day pH: 4.5-5.0 T: 30 ºC ambient | Activated carbon | Sucrose (8000) | 3.59 @ ambient temperature@ HRT of 1 day<br><br>55.96%<br>1.65@ HRT of 1 day @ 35ºC 1.68% | Acetic acid, Butiric acid Propionic acid Ethanol Butanol | 6 |

Notes: hs: heat-shock, HRT: hydraulic retention time; References: 1. [28]; 2.[24] ; 3.[38]; 4.[25], 5. [54] ; 6. This study

## 4. Conclusion

-The increase of $B_v$ from 5 to 8 g sucrose/L day had a positive effect on the performance of our anaerobic fluidized bed reactors; the $H_2$ productivity increased 2100 and 1684% for AFBR-A and AFBR-M, respectively.

-The combination of heat-treatment of inocula and lower pH value avoided the methane production in the second period of operation at $B_v$ of 8 g sucr/(L d).

-In the second period of operation at Bv = 8 g sucr/(L d) the performance of the reactor at ambient temperature was outstandingly superior to that at mesophilic regime; the $H_2$ concentration and the $H_2$ productivity in the ambient reactor were nearly two- fold of the values in the mesophilic reactors

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**Notation**

| | |
|---|---|
| $B_v$ | volumetric loading rate |
| COD | chemical oxygen demand, (mg/L) |
| HRT | hydraulic retention time, (day) |
| HAc | acetic acid concentration, (mgCOD/L) |
| HBu | butyric acid concentration, (mgCOD/L) |
| HLac | lactic acid |
| HPr | propionic acid concentrarion, ( mgCOD/L) |

EtOH ethanol concentration, (mgCOD/L)
BuOH butanol concentration (mgCOD/L)
SMP soluble microbial products, (mgCOD/L)
TVOA total volatile organic acids, (mgCOD/L)
VOA volatile organic acids
Y' hydrogen pseudo yield, ($molH_2$/mol $substrate_{fed}$ or $molH_2$/Kg substrate fed)