

Article

## Biological Hydrogen Production from Corn-Syrup Waste Using a Novel System

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**Abstract:** The reported patent-pending system comprises a novel biohydrogen reactor with a gravity settler for decoupling of SRT from HRT. The biohydrogenator was operated for 100 days at 37 °C, hydraulic retention time 8 h and solids retention time ranging from 2.2–2.5 days. The feed was a corn-syrup waste generated as a byproduct from an industrial facility for bioethanol production located in southwestern Ontario, Canada. The system was initially started up with a synthetic feed containing glucose at concentration of 8 g/L and other essential inorganics. Anaerobically-digested sludge from the St. Mary's wastewater treatment plant (St. Mary, Ontario, Canada) was used as the seed, and was heat treated at 70 °C for 30 min to inhibit methanogens. After 10 days, when the hydrogen production was steady, the corn-syrup waste was introduced to the system. Glucose was the main constituent in the corn-syrup; its concentration was varied over a period of 90 days from 8 to 25 g/L. The change in glucose concentration was used to study the impact of variable organic loading on the stability of hydrogen production in the biohydrogenator. Hydrogen production rate increased from 10 L H<sub>2</sub>/L d to 34 L H<sub>2</sub>/L d with the increase of organic loading rate (OLR) from 26 to 81 gCOD/L d, while a maximum hydrogen yield of 430 mL H<sub>2</sub>/gCOD was achieved in the system with an overall average of 385 mL H<sub>2</sub>/gCOD.

**Keywords:** biohydrogenator; corn-syrup; organic loading rate

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## 1. Introduction

Hydrogen does not contribute to the greenhouse effect and has a high energy yield of 142 kJ/g, 2.75 times more than that of any hydrocarbon [1]. Biological hydrogen production is potentially regarded as one of the most promising alternatives for sustainable green energy production, despite the feasibility of hydrogen production through water electrophoresis and chemical cracking of hydrocarbons. Among different biological processes for hydrogen production, dark fermentation is the most attractive one because of its potential of direct use of wastewater streams and organic wastes and its higher rate of hydrogen production in comparison with photo-fermentative processes.

The conversion of organic waste into hydrogen is attractive both from pollution control and energy recovery points of view. However, only a few studies have been conducted for hydrogen production from real wastewater due to challenges associated with inhibition and microbial shifts. Table 1 shows the process conditions and system performances of selected literature studies using wastewaters from rice winery [2], noodle [3], sugar [4,5], sugar beet [6] and molasses manufacturing [7], food processing [8], and filtered leachate of municipal solid wastes [9]. The highest hydrogen yield of 321 mL H<sub>2</sub>/gCOD was demonstrated by Ueno *et al.* [4] for the treatment of sugar factory wastewater in a continuous stirred tank reactor (CSTR) with 63% glucose conversion efficiency. With the exception of the two batch studies with soil microorganisms as seed [7,8], most of the studies achieved around 200 mL H<sub>2</sub>/gCOD. Furthermore, Table 1 does not show any advantages of packed bed reactors (PBR) over continuously stirred tank reactors (CSTR) in terms of hydrogen production.

The maximum specific growth rate ( $\mu_{\max}$ ) for mixed cultures of hydrogen producing bacteria of 0.333 h<sup>-1</sup> [10] corresponds to a minimum solids retention time (SRT<sub>min</sub>) of 3.0 h and thus CSTRs operated for hydrogen production are characterized by hydraulic retention time (HRT) of 3–8 h. However, high dilution rates result in a marked decrease in biomass content in the reactor due to severe cell washout and system failure ensues [11]. Although, fill and draw (fed-batch) reactors have been used for hydrogen production, they invariably suffered from inconsistent hydrogen production [12] and methane production [13]. In order to overcome biomass washout in hydrogen reactors, decoupling of SRT from HRT in hydrogen bioreactors has been achieved primarily by using biofilms on several media including synthetic plastic media and treated anaerobic granular sludge [14], activated carbon, expanded clay and loofah sponge [15], glass beads [16] and membranes [17]. Problems with the development of methanogenic biofilms on the carrier media adversely impact process stability, which is critical for sustained hydrogen production. Moreover, membranes have not shown many advantages in terms of volumetric hydrogen yield and are also prone to fouling in such a reductive environment. An extensive literature search using Scifinder Scholar has revealed that the concept of using a clarifier for decoupling SRT from HRT has not been explored. Thus, in this innovative research, the use of a clarifier after a hydrogen reactor (Biohydrogenator) [18] for decoupling SRT from HRT through sludge recirculation has been investigated for the first time using corn syrup wastewater. Moreover, the paper will focus on the performance of the biohydrogenator under variable organic loadings, highlighting the various mechanisms contributing to hydrogen production.

**Table 1.** Process and performance parameters for actual wastewaters.

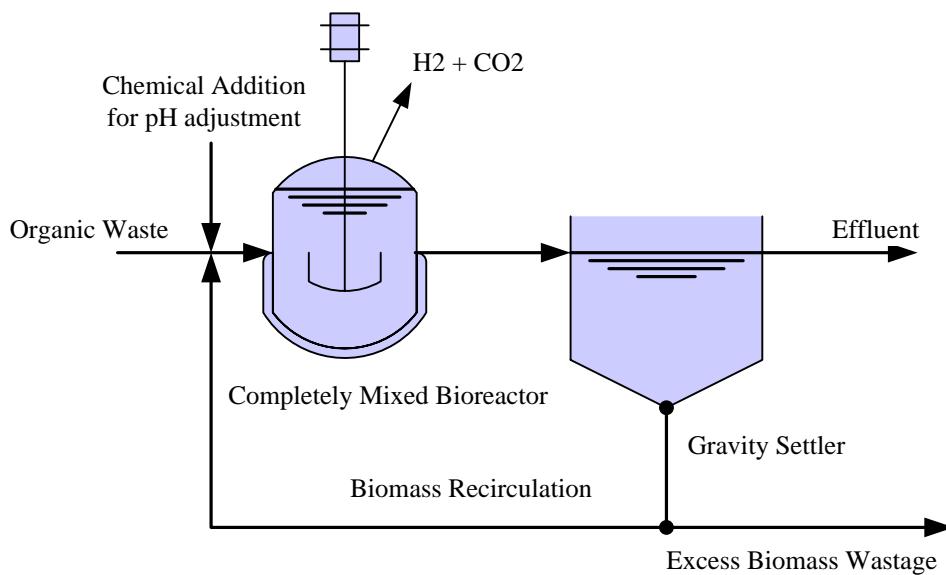
Feedstock	Reactor type	Seed sludge	pH	Temperature (°C)	HR T (h)	Hydrogen Content (%)	Yield (mL H <sub>2</sub> /gCOD)	Maximum volumetric rate (L H <sub>2</sub> /L d)	Ref.
Sugar factory wastewater	CSTR	Compost	6.8	60	12	64	321	4.8	[4]
Wastewater containing sugar and ethyl alcohol	PBR	ADS	6.0–6.5	37	8	60	-	1.8	[5]
Molasses	Batch	Soil	6.0	26	-	-	102	-	[7]
Noodle manufacturing wastewater	CSTR	ADS	5.2	35	18	-	187	-	[3]
Rice winery wastewater	PBR	AS	5.5	55	2	61	272	3.8	[2]
Filtered leachate of waste biosolids	Batch	Waste biosolids	6.7–6.9	35	-	-	184	-	[9]
Sugar beet wastewater	CSTR	ADS	5.2	32	15	57	216	3.0	[6]
Food processing wastewater	Batch	Soil	4.0–6.4	23	-	60	100	3	[8]

Notes: CSTR, continuous stirred tank reactor; PBR, packed-bed reactor; ADS, anaerobic digested sludge; AS, acclimated sludge.

## 2. Experimental Section

### 2.1. Systems setup and operation

The biohydrogenator was operated for 100 days at 37 °C (Figure 1). A summary of the operational conditions is shown in Table 2. The system comprised a CSTR for biological hydrogen production with a 5 L working volume, followed by an 8 L gravity settler. The system effluent was monitored every two days for total chemical oxygen demand (TCOD), soluble COD, volatile fatty acids (VFA), ethanol, lactate, glucose, volatile suspended solids (VSS), total suspended solids (TSS) and daily for biogas composition including hydrogen, methane and nitrogen. Samples were filtered through a 0.45 micron filter paper (Whatman, 7141-104, Japan) prior to measurement of VFAs, ethanol, lactate, and glucose.

**Figure 1.** Experimental setup for the biohydrogenator.**Table 2.** Operational conditions of the biohydrogenator.

Phase	HRT (h)	SRT (d)	Sludge wastage (L/d)	Waste sludge biomass VSS (g/L)	OLR (gCOD/L d)	pH
1	8	2.3	0.6	28	26	5.5–6.5
2	8	2.5	0.4	25	52	5.5–6.5
3	8	2.2	No wastage	-	81	5.5–6.5

## 2.2. Inocula and media composition

Anaerobically-digested sludge from the St. Mary's wastewater treatment plant (St. Mary, Ontario, Canada) was used as the seed. In order to enrich hydrogen producing bacteria, the seed sludge was heat treated at 70 °C for 30 min. The system was seeded with 5 L of sludge and started up in a continuous mode with the feed containing 8 g/L glucose. Solids retention time was controlled by sludge wastage from the clarifier. The feed contained sufficient inorganics (mg/L): NaHCO<sub>3</sub>, 4000; CaCl<sub>2</sub>, 140; MgCl<sub>2</sub> 6H<sub>2</sub>O, 160; NH<sub>4</sub>HCO<sub>3</sub>, 600; MgSO<sub>4</sub> 7H<sub>2</sub>O, 160; urea, 500; Na<sub>2</sub>CO<sub>3</sub>, 124; KHCO<sub>3</sub>, 156; K<sub>2</sub>HPO<sub>4</sub>, 15; trace mineral solution, 500; H<sub>3</sub>PO<sub>4</sub>, 250. After 10 days, when the system reached steady state, the feed was switched to corn-syrup waste generated as a byproduct from an industrial facility for bioethanol production located in southwestern Ontario, Canada. The corn-syrup was characterized by TSS of 400–500 g/L; SCOD of 100–350 g/L; glucose of 80–300 g/L and a pH of 3.5–4.5. The experimental period consists of three consecutive phases of 30 days each. The waste was diluted to approximately 1:10 according to the desired OLR for each phase of the experiment (see Table 2). The organic loading rate was increased in a stepwise fashion from 26 kg COD/m<sup>3</sup> d in phase 1 to 52 and 81 kg COD/m<sup>3</sup> d in phases 2 and 3, respectively. Based on the influent flow rate of 15 L/d, the bioreactor HRT was maintained constant at 8 h in all three phases of operation while the SRT varied narrowly from 2.2 to 2.5 days due to variations in system effluent VSS. The SRT was

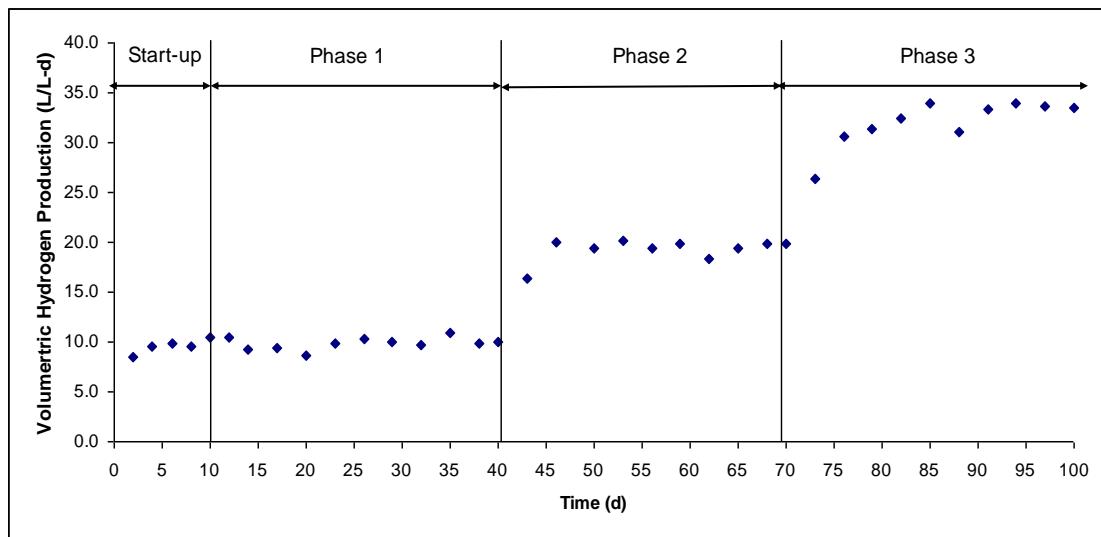
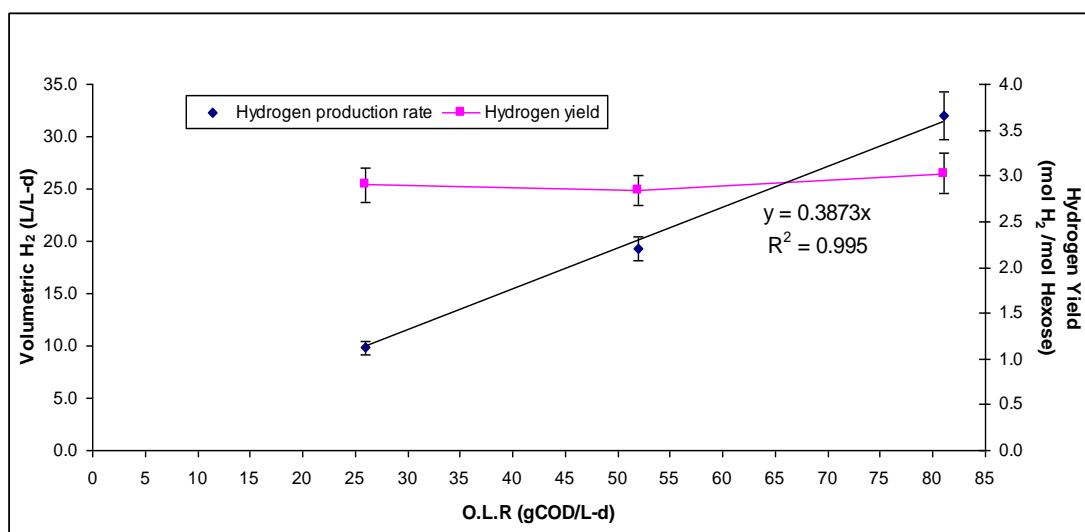
estimated according to the amount of VSS (g) in the hydrogen bioreactor (excluding biomass in the clarifier) divided by the summation of the amount of VSS (g/d) leaving the system in both the clarifier liquid effluent and waste sludge.

### 2.3. Analytical methods

Biogas composition including hydrogen, methane and nitrogen was determined by a gas chromatograph (Model 310, SRI Instruments, Torrance, CA, USA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 6 ft × 1/8 in.). Argon was used as carrier gas at a flow rate of 30 mL/min. The temperatures of the column and the TCD detector were 90 and 105 °C, respectively. The concentrations of volatile fatty acids (VFAs) were analyzed using a gas chromatograph (Varian 8500, Varian Inc., Toronto, Canada) with a flame ionization detector (FID) equipped with a fused silica column (30 m × 0.32 mm). Helium was used as carrier gas at a flow rate of 5 mL/min. The temperatures of the column and detector were 110 and 250 °C, respectively. Lactic acid concentrations were measured using a high-performance liquid chromatography system (1200 series, Agilent Technologies) equipped with Aminex HPX-87H ion exclusion column (300 mm × 7.8 mm i.d.; BIO-RAD), and a UV-detector at 210 nm. The column temperature was adjusted to 30 °C. The same instrument with a refractive index detector (RID) was used to measure the concentrations of glucose. The temperature of the RID detector was set to 35 °C. The amount of volatile suspended solids (VSS) and chemical oxygen demand (COD) were measured according to standard methods [19].

## 3. Results and Discussion

Figure 2 shows the diurnal variation of volumetric hydrogen production rate for the biohydrogenator, with the steady-state data summarized in Table 3. After 10 days of operation on the synthetic glucose solution, when the hydrogen production rate stabilized at  $9.6 \pm 0.7$  L H<sub>2</sub>/L d, the feed was switched to corn syrup wastewater as phase 1 of operation. An organic loading rate of 26 gCOD/L d was maintained for 30 days. As apparent from Figure 1, the change in the feed from synthetic to real waste at the same OLR did not adversely impact hydrogen production, corroborating both the success of the acclimatization phase and the lack of inhibitors in the corn syrup. The system steadily produced hydrogen at a rate of  $9.8 \pm 0.6$  L H<sub>2</sub>/L d. The average hydrogen content in the biogas was  $68 \pm 4\%$ , with carbon dioxide as the balance. At the end of phase 1 the organic loading was doubled to 52 gCOD/L d. The hydrogen production rate increased gradually to 20 L H<sub>2</sub>/L d over a period of 6 days, with the average hydrogen production rate and hydrogen content of the biogas phase 2 of  $19.3 \pm 1.1$  L H<sub>2</sub>/L d and  $60 \pm 4\%$ , respectively. For the last 30 days of operation (phase 3), the organic loading rate was increased to 81 gCOD/L d. After 14 days of operation the system reached a maximum hydrogen rate of 34 L H<sub>2</sub>/L d. In phase 3, the average hydrogen production rate and hydrogen content of the biogas produced were  $32 \pm 2.3$  L H<sub>2</sub>/L d and  $62 \pm 3\%$ , respectively. As depicted in Figure 3, hydrogen production rate increased from 10 to 34 L H<sub>2</sub>/L d with the increase of OLR from 26 to 81 gCOD/L d.

**Figure 2.** Temporal variation of volumetric hydrogen production rate.**Figure 3.** Relationship between hydrogen production rate and biomass yield versus OLR.

The linear relationship between hydrogen production rate and organic loading rate evident from Figure 3 emphasizes the lack of substrate inhibition as well as other inhibitors in the corn syrup at OLR as high as 81 gCOD/L d. The highest hydrogen yield achieved throughout the experimental period was 3.2 mol H<sub>2</sub>/mol hexose. As shown in Figure 4, the maximum hydrogen yield calculated as (mL H<sub>2</sub>/gCOD converted) was 430 mL/gCOD higher than 321 mL/gCOD which was reported by Ueno *et al.* [4]. It must be asserted that as evident from Table 3, the influent glucose was essentially completely removed in all three phases of the study. Assuming a maximum conversion of 544 mL H<sub>2</sub>/g hexose at 25 °C [20], the average conversion efficiency of glucose to hydrogen was 73% in the three phases.

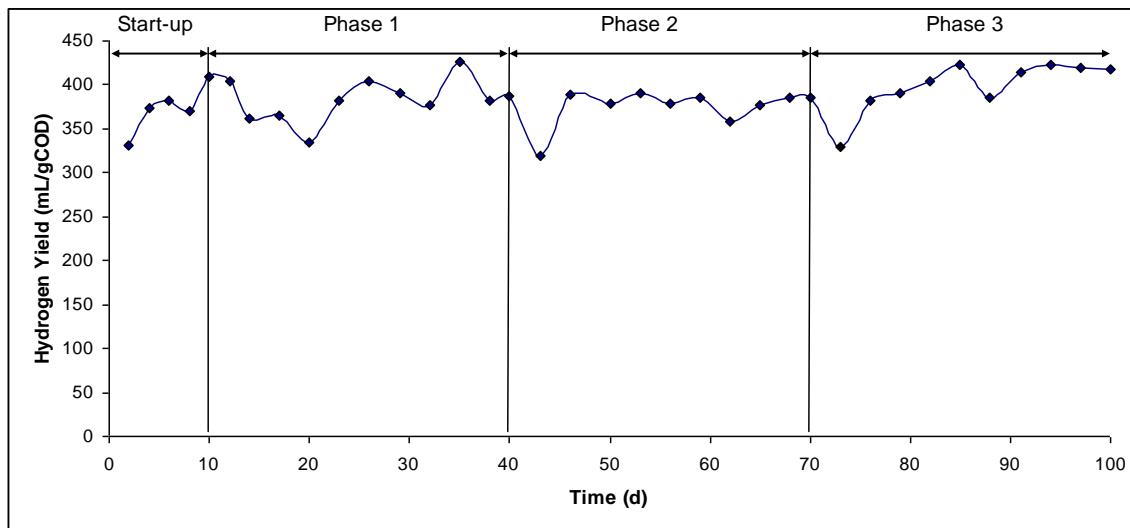
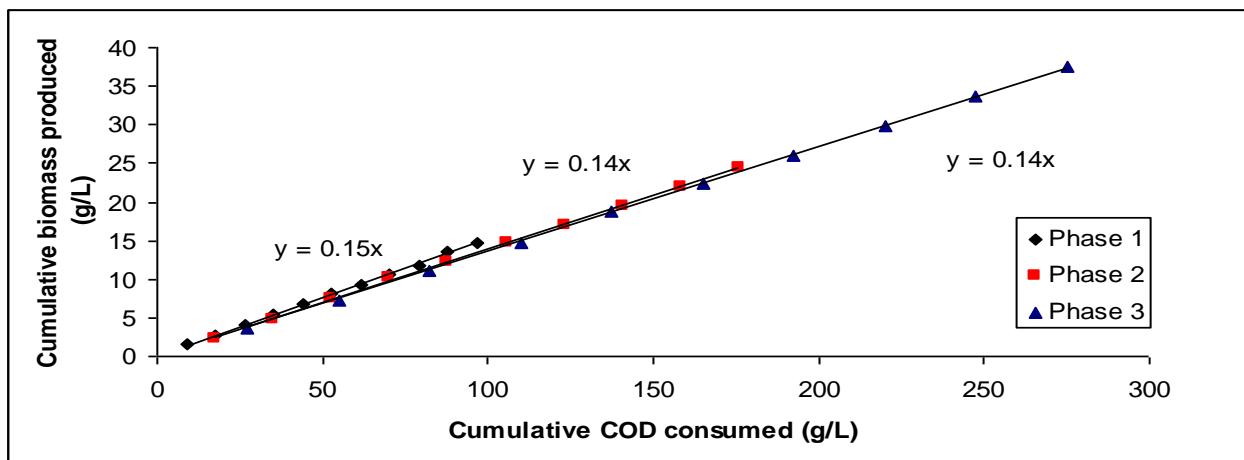
**Figure 4.** Volumetric hydrogen yield profile.

Figure 5 shows the biomass yields for the three phases, calculated based on the data of each phase neglecting the start-up period as the slope of the cumulative biomass produced versus the cumulative COD consumed. It should be noted that biomass production incorporated both the temporal changes in bioreactor mixed liquid volatiles suspended solids (MLVSS) and the solids leaving in the clarifier liquid effluent. The observed biomass yield in phase 1 was 0.15 g VSS/gCOD and 0.14 g VSS/gCOD for phases 2 and 3. Furthermore, using the steady state averages from Table 3 the calculated biomass yields for phases 1 to 3 exactly matched those illustrated in Figure 3. Using Equation 1 below and the biomass yield reported in the literature for hydrogen producers of 0.1 g VSS/g glucose [8]:

$$X_v = X_a + X_i = X_{HP} + X_{nHP} + X_i = \theta_c \cdot Y_{tHP} \cdot OLR \cdot \eta + X_{nHP} + X_i \quad (1)$$

where  $X_v$  is the total biomass,  $X_a$  is the active microbial population in the reactor which in this case is consisted of the biomass of hydrogen producers ( $X_{HP}$ ) and biomass of non-hydrogen producers ( $X_{nHP}$ ),  $X_i$  is the inert remains of microorganisms in the reactor,  $\theta_c$  is solid retention time,  $Y_{tHP}$  is true yield of hydrogen producers, OLR is the organic loading rate and  $\eta$  is the substrate conversion efficiency. It was estimated from Equation 1 that the non-hydrogen producing bacteria constituted 20%, 16% and 12% of the measured bioreactor VSS in phase 1, phases 2 and 3, respectively. The low fraction of non-hydrogen producing bacteria coupled with the decrease of non-hydrogen producing bacteria with time proves that the clarifier selectively enriches the hydrogen producers and minimizes the growth of other competitors that decrease the hydrogen yield, which are washed out in the clarifier effluent. Denaturing gradient gel electrophoresis (DGGE) analysis revealed the predominance of the high hydrogen producers *Klebsiella pneumonia*, *Clostridium pasteurianum* and *Clostridium acetobutylicum*.

To evaluate the settling characteristics of the biomass, both zone settling velocity (ZSV) and sludge volume index (SVI) were performed on a weekly basis throughout the three phases. The ZSV ranged from 5.4 to 7.8 m/h (130–187 m/d) with an average of  $160 \pm 14$  m/d and SVI from 60 to 90 mL/g (average  $SVI = 80 \pm 12$  mL/g). The settleability of the hydrogen producers was considered to be superior to activated sludge since SVI of 100 mL/g and ZSV of 100 m/d are considered typical for good settling activated sludge.

**Figure 5.** Biomass yield.

COD reduction efficiencies in phases 1, 2 and 3 were 28%, 31% and 32%, respectively compared to the theoretical value of 33% and 12% calculated from Equations 2 and 3, respectively [21]. The COD mass balance for the three phases, computed considering the influent and effluent CODs, and the equivalent CODs for both gas and biomass is shown in Table 3. The closure of COD mass balances at 109–113% validates the reliability of the data.

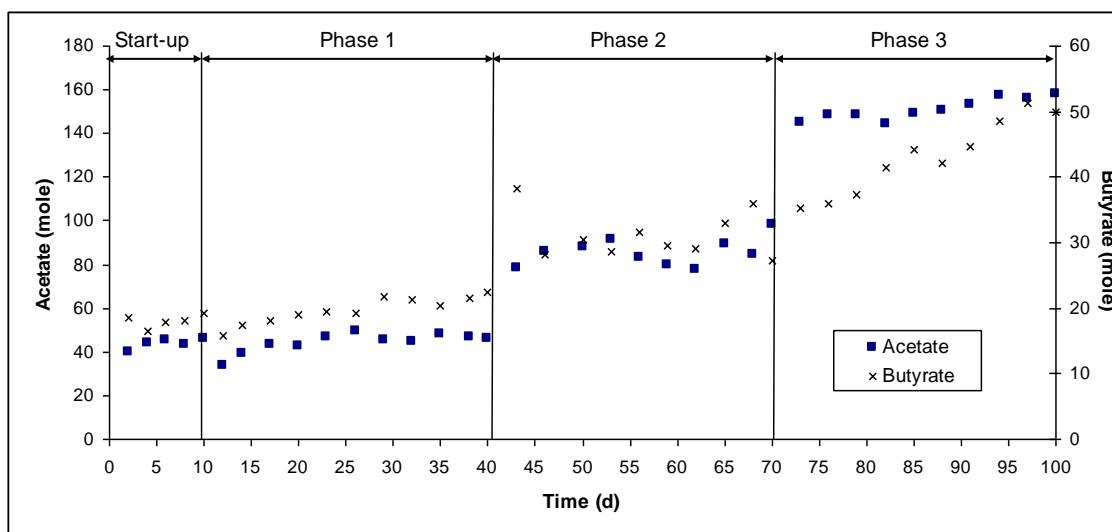
**Table 3.** Metabolites and COD mass balance.

	Phase 1	Phase 2	Phase 3
<b>VSS (mg/L)</b>	$17226 \pm 2496$	$23540 \pm 1997$	$25287 \pm 1879$
<b>VSS out (mg/L)</b>	$1343 \pm 208$	$2446 \pm 228$	$3747 \pm 705$
<b>SCOD out (mg/L)</b>	$6125 \pm 399$	$11753 \pm 787$	$18189 \pm 1335$
<b>Acetate (mg/L)</b>	$2647 \pm 274$	$5139 \pm 385$	$9060 \pm 1352$
<b>Propionate (mg/L)</b>	$36 \pm 16$	$159 \pm 24$	$87 \pm 62$
<b>Isobutyrate (mg/L)</b>	0	0	0
<b>Butyrate (mg/L)</b>	$1730 \pm 178$	$2747 \pm 315$	$3793 \pm 671$
<b>Isovalerate (mg/L)</b>	$3 \pm 3$	$50 \pm 18$	$63 \pm 25$
<b>Valerate (mg/L)</b>	0	0	0
<b>Ethanol (mg/L)</b>	$23 \pm 9$	$78 \pm 22$	$77 \pm 31$
<b>Lactate (mg/L)</b>	0	0	0
<b>VFA (mgCOD/L)</b>	$6087 \pm 579$	$11001 \pm 529$	$17015 \pm 2781$
<b>Glucose Out (mg/L)</b>	0	0	0
<b>Hydrogen Gas (L/d)</b>	$49 \pm 3$	$96 \pm 6$	$160 \pm 12$
<b>Hydrogen Gas (gCOD/d) *</b>	$31 \pm 2$	$61 \pm 4$	$101 \pm 7.5$
<b>COD balance (%) **</b>	$112 \pm 6$	$109 \pm 5$	$113 \pm 7$

\* Based on 8 g COD/g H<sub>2</sub>. \*\* Sample of calculation phase 1: COD balance (%) =  $(1343 \times 1.42 \times 15/1000 + 31 + 6125 \times 15 / 1000)/(135 \times 100)$

The distribution of metabolites formed during biological hydrogen production is often a crucial signal in assessing the efficiency of hydrogen-producing cultures [22]. The composition of the VFAs produced during the three phases is shown in Table 3. It must be asserted that the concentrations of soluble metabolites i.e. acetate, butyrate, propionate, isovalerate, and ethanol in the corn syrup were less than 1% than those measured in the reactor and shown in Table 3, and thus were neglected in the calculation of production rates. The VFA analysis revealed high concentrations of acetate and butyrate, while propionate, isovalerate, ethanol were present in minor amounts, and no detection of lactate, implying that the acid-forming pathway dominated the metabolic electron flow. Figure 6 shows the number of moles for acetate and butyrate throughout the experimental period. The steady-state average molar ratios of acetate/butyrate were 2.2, 2.8 and 3.5 for phases 1, 2 and 3, respectively. The theoretical hydrogen yield from hexose with acetate formation is 4 mol H<sub>2</sub> / mol hexose, which is twice as high as that of butyrate formation, 2 mol H<sub>2</sub>/mol hexose. Previous studies indicate that the hydrogen yield increases with the molar ratio of acetate/butyrate [22,23]. According to the measured concentrations of acetate and butyrate and using Equations 2 and 3, the contribution of the two pathways was estimated. In phases 1 and 2, 68% and 32% of the hydrogen produced were through the acetate and butyrate pathways, respectively. While, in phase 3 approximately 78% and 22% of the hydrogen yield through the acetate and butyrate pathways, respectively. The increased contribution of the high hydrogen producing acetate pathway in phase 3 relative to phase 1 is consistent with the aforementioned increase in molar acetate/butyrate ratio.

**Figure 6.** Temporal variation of molar acetate and butyrate production.



#### 4. Summary and Conclusions

The overall results of this experiment show that biological hydrogen production from corn-syrup using heat pre-treated anaerobically digested sludge can be achieved in the biohydrogenator. The hydrogen production rate was a function of the organic loading rate; it increased from 10 to 34 L H<sub>2</sub>/L d with the increase of OLR from 26 to 81 gCOD/L d. No inhibition of hydrogen production was observed at loadings as high as 81 gCOD/L d. The highest hydrogen yield achieved throughout the experimental period was 3.2 mol H<sub>2</sub>/mol hexose corresponds to 430 mL H<sub>2</sub>/gCOD and an overall

average glucose to hydrogen conversion efficiency of 73%. The relatively high hydrogen yield was verified by the high molar ratio of acetate to butyrate and the COD mass balance. The contribution of the high hydrogen producing acetate pathway increased from 68% of the overall hydrogen at 26 gCOD/L d to 78% at 81 gCOD/L d. Furthermore, the fraction of non-hydrogen producing bacteria in the reactor biomass decreased from 20% at 26 gCOD/L d to only 12% at 81 gCOD/L d. The decoupling of SRT from HRT in biohydrogen production systems facilitated by the superior sludge settling characteristics of hydrogen producers, evaluated in this work, validated the promise of using a gravity settler after a CSTR to maintain high biomass retention in the system and decrease biomass washout, thus improving hydrogen yield and sustainability of hydrogen production, rendering the system as a competitor for biological hydrogen production from waste.

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