A Novel Biological Hydrogen Production System: Impact of Organic Loading

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A Novel Biological Hydrogen Production System: Impact of Organic Loading

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Abstract
The patent-pending system comprises a novel biohydrogen reactor with a gravity settler for decoupling of SRT from HRT. Two biohydrogenators were operated for 220 days at 37 °C, hydraulic retention time 8 h and solids retention time ranged from 1.4 to 2 days under four different glucose concentrations of 2, 8, 16, 32, 48 and 64 g/L, corresponding to organic loading rates of 6.5-206 kg COD/m³-d, and started up using anaerobically-digested sludge from the St. Marys wastewater treatment plant (St.Mary, Ontario, Canada) as the seed. The system steadily produced hydrogen with no methane. A maximum hydrogen yield of 3.1 mol H₂ /mol glucose was achieved in the system for all the organic loading rates with an average of 2.8mol H₂ /mol glucose. Acetate and butyrate were the main effluent liquid products at concentrations ranging from 640-7400 mg/L and 400-4600 mg/l, respectively, with no lactate detection. Microbial community analysis using denaturing gradient gel electrophoresis (DGGE) confirmed the absence of lactate producing bacteria Lactobacillus fermentum and other non-hydrogen producing species, and the predominance of various Clostridium species. Biomass concentrations in the biohydrogenators were steady, during the runs, varying from 1500 mg/L at the OLR of 6.5 kg COD/m³-d to 14000 mg/L at the 104 kg COD/m³-d, thus emphasizing the potential of this novel system for sustained stable hydrogen production and prevention of biomass washout.

1 Introduction
Hydrogen production from renewable substrates can reduce reliance on fossil fuels. It produces only water upon combustion, thus is considered as a clean energy source that can help mitigate pollution and global warming [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 electrolysis and chemical cracking of hydrocarbons [2]. 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 [3].

Organic loading rate (OLR) is an important parameter in studying hydrogen bioreactors. In order to optimize a system for hydrogen production, it is essential to define either a range of the organic loading rates that the system can handle effectively, or an optimal organic loading rate for a maximum hydrogen yield. In the literature, there is no clear relationship between the hydrogen yield and the organic loading rate. In some cases higher OLRs decreased the hydrogen yield [4] whereas in some others higher OLRs increased the hydrogen yield [5]. For waste activated sludge as a seed material, it appears that increasing
the OLR within the 40-160 gCOD/L-d increased hydrogen yield to an optimum of 1.6 mol H₂/mol glucose at an OLR of 120 gCOD/L-d [6], whereas hydrogen yield decreased with increasing OLR for both anaerobically digested sludge [7] and soil microorganisms [4]. Although lower molar H₂ yields at higher OLRs have been attributed to the inhibitory effect of higher H₂ partial pressures in the growth medium [4, 8], variations in the composition of bacterial communities that become established at different OLRs [9] may be a major reason for lower yields. Hydrogen yield with digester sludge at an OLR of 45 gCOD/L-d was 1.3 mol H₂/mol glucose [7] as compared with 0.9 mol H₂/mol glucose with waste activated sludge [6]. Moreover, comparing the biomass concentration in two studies with continuously stirred tank reactors (CSTRs) utilizing agricultural soil as the seed and glucose as a substrate under approximately same OLRs, Van Ginkel and Logan [4] achieved much higher hydrogen yield (2.2 mol/mol) at a biomass concentration of 8 g/L compared to Zhang et al. [10] who reported 0.72 mol H₂/mol hexose with 0.9 g/L biomass. Oh et al. [11] achieved a hydrogen yield of 0.4 mol/mol at a biomass concentration of 2.2 g/L in a CSTR and Wu et al. [6] using a CSTR seeded with silicone-immobilized sludge realized a hydrogen yield of 1.6 mol/mol at 3.5 g/L of biomass compared to a hydrogen yield of 2.1 mol/mol achieved by Zhang et al. [5] at a similar OLR with a higher biomass concentration (4.6 g/L). It is thus clear that the higher biomass concentration in the reactors improved the hydrogen yield, which in essence shows that one of the key factors affecting the stability of hydrogen producing systems is maintaining higher biomass concentrations in the system. In addition, the low hydrogen yield and system failure was attributed to low concentrations of biomass due to washout [4].

This main objectives of this paper focuses primarily on the investigation of the effect of organic loading rate on the performance of a novel integrated biohydrogen reactor clarifier system (IBRCS) [12] and to specify an optimal range for organic loading rate that maximizes hydrogen yield. The premise of the IBRCS is decoupling of hydraulic retention time (HRT) from solids retention time (SRT), which has been demonstrated in a previous work. [13]

2 Materials and Methods

Systems set up and operations

Two lab-scale systems were operated at 37 °C for 220 days (Figure 1), at six different organic loading rates ranging from 6.5 gCOD/L-d to 206 gCOD/L-d. Two integrated biohydrogen reactor clarifier systems (IBRCSs) comprised a continuously stirred reactor (CSTR) for biological hydrogen production (5 L working volume), followed by an uncovered gravity settler (volume 8 L) i.e. open to atmosphere for the decoupling of solids retention time (SRT) from the hydraulic retention time (HRT). Details of the operational conditions for the six runs are listed in Table 1. In order to enrich hydrogen producing bacteria, the sludges were heat treated at 70 °C for 30 minutes. Following the completion of each run and the attainment of steady-state conditions, the systems were cleaned and inoculated with pre-treated sludges. OLR-1 and 2 were run simultaneously, followed by OLR-3 and 4, and lastly OLR-5 and 6. The systems were monitored for total chemical oxygen demand (TCOD), soluble COD, volatile fatty acids (VFA), ethanol, lactate, glucose, volatile suspended solids (VSS), total suspended solids (TSS) and biogas composition including hydrogen, methane
and nitrogen. The quantity of produced biogas was recorded daily using a wet tip gas meter (Rebel wet-tip gas meter company, Nashville, TN, USA).

![Diagram of experimental setup for integrated biohydrogen reactor clarifier system]

**Figure 1:** Experimental Setup for the integrated biohydrogen reactor clarifier system.

**Inocula and media compositions**

Anaerobically-digested sludge from the St. Marys wastewater treatment plant (St. Marys, Ontario, Canada) was used as the seed. The two systems operated in parallel at the same time under two different OLRs for a total of six OLRs (three consecutive runs). The systems were seeded with 5 liters of sludge and started up in a continuous mode with the feed containing glucose at different concentrations as highlighted in Table 1. The same startup technique was repeated for the three runs. It must be emphasized that there was no sludge wastage from the clarifier throughout the operation, and the values of SRTs presented in Table 1 represent the average ± standard deviation (SD) during steady state operation. It is noteworthy that the reactors operation was consistent over time and accordingly the average SRT with SD of less than 10% of the mean SRT is representative of the overall SRT during the run. As expected, the clarifier effluent VSS was substantially lower than the reactor VSS and remained unchanged during steady-state operation. The feed contained sufficient inorganics (mg/L): NaHCO₃, 2000-16000; CaCl₂, 140; MgCl₂·6H₂O, 160; NH₄HCO₃, 600; MgSO₄·7H₂O, 160; urea, 500-2000; Na₂CO₃, 124-300; KHCO₃, 156; K₂HPO₄, 15-20; trace mineral solution, 500; H₃PO₄, 250-1500.
Table 1: Operational conditions.

<table>
<thead>
<tr>
<th>OLR</th>
<th>Glucose (g/L)</th>
<th>HRT (h)</th>
<th>SRT (h)</th>
<th>OLR (gCOD/L-d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>2</td>
<td>8</td>
<td>50 ± 5</td>
<td>6.5</td>
</tr>
<tr>
<td>-2</td>
<td>8</td>
<td>8</td>
<td>45 ± 4</td>
<td>25.7</td>
</tr>
<tr>
<td>-3</td>
<td>16</td>
<td>8</td>
<td>45 ± 6</td>
<td>51.4</td>
</tr>
<tr>
<td>-4</td>
<td>32</td>
<td>8</td>
<td>42 ± 6</td>
<td>103</td>
</tr>
<tr>
<td>-5</td>
<td>48</td>
<td>8</td>
<td>27 ± 3</td>
<td>154</td>
</tr>
<tr>
<td>-6</td>
<td>64</td>
<td>8</td>
<td>26 ± 2</td>
<td>206</td>
</tr>
</tbody>
</table>

Note: Values represent average ± standard deviation

3 Analytical Methods

The biogas composition including hydrogen, methane, and nitrogen was determined by a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 6 ft X 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 (30m × 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 [14]. Particle size distribution was determined by Malvern Mastersizer 2000 (version 5.22) laser beam diffraction granulometer.

4 Results and Discussion

Figures 2 (a and b) shows the diurnal variation of hydrogen production rate and yield (based on the amount of glucose converted). Although steady-state was observed in all runs after 3-7 days of startup, the systems were kept in operation at steady state for 55-75 days. The systems showed stable hydrogen production during the experimental period. The coefficient of variation (calculated as standard deviation divided by the average) for hydrogen production rate and yield in all runs was approximately less than 10%. The two integrated biohydrogen reactor clarifier system (IBRCS) were operated at OLRs of 6.5 gCOD/L-d and 25.7 gCOD/L-d for 55 days in steady state. The hydrogen production rate averaged 12 L/d and 48 L/d for OLR-1 and OLR-2, respectively. The two IBRCSs were then restarted and tested under OLRs of 51.4 gCOD/L-d and 103 gCOD/L-d. The operational period was
extended for a period of 75 days. The hydrogen production rate increased to 97 L/d and 179 L/d for OLR-3 and OLR-4, respectively. The glucose conversion in the system under the three OLRs was almost 100 % and decreased to approximately 95 % at OLR-4.

![Graph of hydrogen production rate](image1)

(a)

![Graph of hydrogen yield](image2)

(b)

Figure 2: Diurnal variation for: a) hydrogen production rate, b) hydrogen yield

Figure 3 depicts the steady-state volumetric hydrogen production and molar yields, calculated based on the data of the last 55 days for OLR-1 and 2, and 75 days for OLR 3 to 6. As illustrated in Figure 3a linear increase in the hydrogen production rate with the increase of the OLR was observed up to 103 gCOD/L-d. On the other hand, the hydrogen yield of 2.8 mol H₂/ mol glucose was almost constant during the same range of OLRs. To determine the optimum OLR that maximizes hydrogen production, the systems were restarted under an OLRs of 154 gCOD/L-d and 206 gCOD/L-d. The average hydrogen yields after 75 days of steady state operation were 1.2 mol H₂/ mol glucose and 1.1 mol H₂/ mol glucose for OLR-5 and OLR-6, respectively. The increase in OLR not only decreased the hydrogen yield but also the hydrogen production rate dropped to approximately 65 L/d (13 L/L/d). The hydrogen content in the biogas was around 72 % in OLR-1 and 2, 66 % in OLR-3 and 4, and 42 % in
OLR-5 and 6 with the balance in all cases CO₂. It is apparent from Figure 3 that the maximum OLR at the system HRT of 8 h in terms of hydrogen production is 103 gCOD/L-d.

![Figure 3: Relationship between hydrogen production rate and hydrogen yield versus OLR.](image)

The biomass concentration in the reactor is an important operational parameter that affects both system stability and hydrogen yield. The average concentration of VSS in the biohydrogen reactor increased ten-fold from 1.5 g/L to 15.7 g/L with the increase in OLR from 6.5 gCOD/L-d to 103 gCOD/L-d. In OLR-5 and OLR-6 the concentrations of VSS were 18.4 g/L and 17 g/L, respectively. Using steady-state data for both VSS (g/L) and hydrogen production rate (L/d) at each OLR, the biomass specific hydrogen production rate was calculated. During the first four OLRs i.e. 6.5 to 103 gCOD/L-d, the average biomass hydrogen production rate was $2.1 \pm 0.3 \text{ L H}_2/\text{gVSS-d}$ and the average food to microorganisms' ratio (F/M) was $5.7 \pm 0.9 \text{ gCOD/gVSS-d}$. When the OLR was increased to 154 gCOD/L-d and 206 gCOD/L-d the biomass specific hydrogen production rate dropped to $0.7 \text{ L H}_2/\text{gVSS-d}$ with average F/M ratios of 8.5 and 12.1 gCOD/gVSS-d, respectively.

**Microbial community analysis**

The DGGE profiles of the 16S rDNA gene fragments at four organic loading rates are demonstrated in Table 2. Comparing the results from OLR-1 and OLR-2, revealed that the relatively higher OLR-2 (25.7 gCOD/L-d) resulted in a significant increase in microbial diversity. *Clostridium acetobutyricum* (band A), *Klebsiella pneumonia* (band B) and uncultured bacterium (DQ464539.1) (band F) were the only observed bands at OLR-1. *Clostridium acetobutyricum*, *Klebsiella pneumonia* are well known hydrogen producers that have been frequently used for hydrogen production [19,20] or detected as active microorganisms in mixed cultures of hydrogen producing bioreactors [21,22]. The uncultured bacterium DQ464539.1 (band F) had also been reported in an acidophilic ethanol-H₂-coproducing system. At OLR-2 another hydrogen producers including *Clostridium butyricum* (band C), a *Clostridium acetobutyricum* affiliated strain (band D) and *Clostridium*
Pasteurianum (band E) were detected. High yields of hydrogen have been reported in the literature with Clostridium butyricum and Clostridium pasteurianum [23,24]. Band G which was available only at OLR-2 identified as an uncultured bacterium (DQ414811.1). This band was 97% similar to a strain which had been reported in a hydrogen production bioreactor by Koskinen et al. [25]. Increasing the OLR to OLR-5 and OLR-6 resulted in formation of different microbial community in the reactors. Although most of the hydrogen producers were also present at OLR-5 and OLR-6 some new bands also appeared with increasing the OLR. Two of these bands which were identified were Lactococcus sp. (band H) and Pseudomonas sp. (band I). Clostridium acetobutyricum (band A) was absent at both OLR-5 and OLR-6. It should be noted that some of the DGGE bands were not identified due to presence of a lot of bands in a small area could also be related to hydrogen producers. The increase in the microbial diversity with the increase in the OLR from 6.5 to 25.7 gCOD/L-d is in agreement with the findings of Luo et al. [9], while at the extremely high OLR-5 and OLR-6 clear microbial shifts only were identified.

<table>
<thead>
<tr>
<th>Affiliation (accession no.)</th>
<th>Bands</th>
<th>Similarity (%)</th>
<th>OLR-1</th>
<th>OLR-2</th>
<th>OLR-5</th>
<th>OLR-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium acetobutyricum (FM994940.1)</td>
<td>A</td>
<td>99</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumonia (GQ214541.1)</td>
<td>B</td>
<td>100</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Clostridium butyricum (DQ831124.1)</td>
<td>C</td>
<td>99</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Clostridium acetobutyricum (FM994940.1)</td>
<td>D</td>
<td>95</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Clostridium pasteurianum (GQ214541.1)</td>
<td>E</td>
<td>99</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncultured bacterium (DQ464539.1)</td>
<td>F</td>
<td>96</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Uncultured bacterium (DQ414811.1)</td>
<td>G</td>
<td>97</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactococcus sp. (YM05004.1)</td>
<td>H</td>
<td>95</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas sp. (AJ846267.1)</td>
<td>I</td>
<td>96</td>
<td>×</td>
<td>×</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Conclusions

Based on the findings of this study within the range of OLRs investigated (6.5 to 206 gCOD/L-d) and at HRT of 8 h and SRT of 1-2 d, the following conclusions can be drawn:

- The optimum volumetric hydrogen production rate occurred at an OLR of 103 gCOD/L-d
- Molar hydrogen yield remained relatively stable at 2.8 mol H₂/mol glucose at OLRs in the range of 6.5 to 103 gCOD/L-d, but declined rapidly thereafter to 1.1 mol H₂/mol glucose.
- Molar hydrogen yield correlated linearly with the acetate-to-butyrate molar ratios, and was mostly around 1 and 2.8 mol H₂/mol glucose at acetate-to-butyrate ratios ranging from 0.8 to 1.3 and 2 to 3, respectively.
- Glucose conversion decreased drastically from 99% at OLRs of 6.5 to 103 gCOD/L-d, to only 56% and 40% at OLRs of 154 and 206 gCOD/L-d, not due to acetate.
inhibition, but primarily due to residual glucose concentrations of 21000 and 38000 mg/L.

- Microbial community analysis on OLR-1 and OLR-2 showed the predominance of hydrogen producers such as *Clostridium acetobutyricum*, *Klebsiella pneumonia*, *Clostridium butyricum*, and *Clostridium pasteurianum*. While at extremely high OLRs of 154 and 206 gCOD/L·d, a microbial shift was clearly evident due to the coexistence of the non-hydrogen producers such as *Lactococcus* sp. and *Pseudomonas* sp.

**References**

Acknowledgments


