Biological hydrogen production by Clostridium acetobutylicum in an unsaturated flow reactor

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ABSTRACT

A mesophilic unsaturated flow (trickle bed) reactor was designed and tested for H\textsubscript{2} production via fermentation of glucose. The reactor consisted of a column packed with glass beads and inoculated with a pure culture (Clostridium acetobutylicum ATCC 824). A defined medium containing glucose was fed at a flow rate of 1.6 mL/min (0.096 L/h) into the capped reactor, producing a hydraulic retention time of 2.1 min. Gas-phase H\textsubscript{2} concentrations were constant, averaging 74\%\textpm3\% for all conditions tested. H\textsubscript{2} production rates increased from 89 to 220 mL/h L of reactor when influent glucose concentrations were varied from 1.0 to 10.5 g/L. Specific H\textsubscript{2} production rate ranged from 680 to 1270 mL/g glucose per liter of reactor (total volume). The H\textsubscript{2} yield was 15–27\%, based on a theoretical limit by fermentation of 4 moles of H\textsubscript{2} from 1 mole of glucose. The major fermentation by-products in the liquid effluent were acetate and butyrate. The reactor rapidly (within 60–72 h) became clogged with biomass, requiring manual cleaning of the system. In order to make long-term operation of the reactor feasible, biofilm accumulation in the reactor will need to be controlled through some process such as backwashing. These tests using an unsaturated flow reactor demonstrate the feasibility of the process to produce high H\textsubscript{2} gas concentrations in a trickle-bed type of reactor. A likely application of this reactor technology could be H\textsubscript{2} gas recovery from pre-treatment of high carbohydrate-containing wastewaters.

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1. Introduction

H\textsubscript{2} is being considered for widespread use as a green energy carrier in the future primarily for transportation. The advantage of using H\textsubscript{2} is that the only by-product of reacting H\textsubscript{2} with oxygen is water, and no carbon dioxide or other greenhouse gases are produced. Most H\textsubscript{2} is currently produced from nonrenewable sources such as oil, natural gas, and coal (Benemann, 1996; Van Ooteghem et al., 2002). H\textsubscript{2} can also be produced from renewable sources such as biomass, but yields are low. If H\textsubscript{2} conversion efficiency could reach 60–80\%, based upon a maximum theoretical conversion of 12 mol H\textsubscript{2}/mol hexose, H\textsubscript{2} production from wastewater could have great potential for economical near-term H\textsubscript{2} production from renewable resources (Benemann, 1996). There are no known fermentation pathways that can achieve a conversion efficiency of greater than 4 mol H\textsubscript{2}/mol hexose (Thauer et al., 1977). However, it has recently been discovered that H\textsubscript{2} can be produced from a fermentation end product (acetate) by modifying a microbial fuel cell by applying a small potential to that generated by the bacteria (Liu et al., 2005b). High H\textsubscript{2} yields in this new process would still require a
pre-fermentation stage so that H₂ could be recovered in a separate process from the fermentation of the sugars. Various types of bioreactors have been used for H₂ production, including batch (Van Ginkel et al., 2001; Logan et al., 2002; Oh et al., 2003), fed-batch (Chin et al., 2003), continuous-flow stirred tank (Fang and Liu, 2002; Hussy et al., 2003), saturated packed-bed column reactors (Rachman et al., 1998; Yokoi et al., 1997; Chang et al., 2002; Palazzi et al., 2002; Lee et al., 2003), and upflow granulated reactors (Liu and Fang, 2003). In batch tests, it has been found that continuous release of the biogas from the system can increase H₂ yields by as much as 40% (Logan et al., 2002). Typical conversion efficiencies using continuous-flow reactors are 1.9–2.4 mol H₂/mol glucose (Lay, 2001; Ueno et al., 2001a; Fang and Liu, 2002). In one study, continuous H₂ production achieved a yield of 2.7 mol H₂/glucose using a mixed culture of *Clostridium butyricum* and *Enterobacter aerogenes* (Yokoi et al., 2002). Biogas transfer out of the liquid phase is limited in aqueous reactors without intensive stirring (Rachman et al., 1998). While stirred reactors facilitate gas release from the liquid phase, continuous stirring of the reactor consumes considerable electric power. The energy needed for mixing in anaerobic wastewater treatment reactors ranges from 85 to 105 kW/1000 m³ (Grady et al., 1999). In aerobic wastewater treatment systems, the large energy input needed for wastewater aeration can be avoided by using an unsaturated flow, or trickling filter, type of reactor. In this system, wastewater is applied over a packing medium at a rate that creates a thin fluid film over the biofilm growing on the medium. This trickle-bed reactor operation promotes high rates of gas transfer into the biofilm due to the thin fluid film and the high gas diffusivity of oxygen (Logan, 1993). We reasoned that this unsaturated flow condition could similarly facilitate rapid H₂ gas evolution out of the biofilm under anaerobic conditions. Unsaturated flow reactors have recently been tested for biological H₂ production under thermophilic conditions (Oh et al., 2004), but so far no reactors have been tested under mesophilic conditions. We therefore tested the feasibility of this concept for H₂ production using a laboratory-scale reactor and a pure culture of a H₂-producing bacterium, *Clostridium acetobutylicum* ATCC 824, under mesophilic conditions. While maintaining a pure culture for a waste stream would not be likely, it is well known that Clostridia are predominant H₂-producing bacteria. The use of a well-characterized pure culture was therefore helpful for testing the concept of this new type of trickling filter reactor. Our results with this unsaturated flow reactor are then compared to the performance of stirred tank reactors on the basis of H₂ production per unit volume of the reactor.

2. Methods

2.1. Medium and culture conditions

*C. acetobutylicum* ATCC 824 was stored at −80 °C. Cells were grown anaerobically on glucose (1 g/L) in a defined medium (pH = 6.2) containing (g/L): NH₄Cl, 0.2; KH₂PO₄, 1.8; K₂HPO₄, 2.40; MgSO₄·7H₂O, 0.1; FeCl₃, 0.02; CaCl₂, 0.01; Na₂BO₇·1H₂O, 0.011; ZnCl₂, 0.015; CoCl₂·6H₂O, 0.01; CuCl₂·2H₂O, 0.015; MnCl₂·4H₂O, 0.01. Cells used to inoculate the reactor were harvested during late-log growth based on optical density at 600 nm (OD₆₀₀) of ca. 0.2.

2.2. Reactor design and operation

The unsaturated flow (trickle bed) column reactor (Fig. 1) used for H₂ production studies was developed by modifying the gas flow of an anaerobic biofilm reactor developed to transfer H₂ from the gas phase into a biofilm (Logan and LaPoint, 2002). Instead of a configuration where gas was injected into the reactor, the gas line was modified to allow gas to be continuously released from the reactor. The main chamber of

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**Fig. 1** – (A) Photograph and (B) schematic of the unsaturated flow reactor.
The reactor (25 cm long, 2.5 cm inside diameter; total volume of 0.123 L) was packed with autoclaved glass beads (3 mm diameter; 1200 m²/m³ calculated projected surface area) supported by a stainless-steel mesh. Water was pumped (Masterflex 7523-30, Cole Palmer Corp.) into the top of the reactor where it dripped onto the reactor packing. The gas flow through the port on the top of the reactor was monitored using a respirometer system (Challenge Environmental Systems AER-200 respirometer, Fayetteville, AR). The liquid effluent line was connected directly to a sealed Zip-Loc bag (1.5 L) containing sodium chloride (150 g) to halt biological activity in the collection system.

The reactor was operated at 30 °C in a constant temperature room. Oxygen was initially removed from the column by purging with nitrogen gas for 30 s. The reactor was then inoculated with cells (70 mL, OD₆₀₀ = 0.2) and operated in fed-batch mode (two complete cycles) until gas production was observed. At this time, the reactor was switched to a continuous liquid flow mode. Nitrogen-sparged sterile medium containing glucose was fed at a flow rate of 1.6 mL/min into the top of the reactor. Headspace gas samples (0.25 mL) were periodically taken using a gas-tight syringe. Effluent samples were taken from the effluent line at a point before the flow entered the collection bag. After collection, aqueous (influent and effluent) samples were immediately centrifuged for 1 min (8000 g) and the supernatant was stored at 4 °C for subsequent analysis. Steady-state operation was indicated by constant gas production and a stable headspace H₂ concentration, and verified by measurements demonstrating constant effluent glucose and volatile acids concentrations measured four times (over at least 24 h).

Four different glucose concentrations (1.0, 3.3, 4.5, and 10.5 g/L) were used in defined medium for the influent flow. These influent glucose concentrations were chosen based on sugar concentrations representative of food processing wastewaters (Van Ginkel and Logan, 2005) and concentrations used in previous H₂ production tests (Mizuno et al., 2000; Fang and Liu, 2002; Iyer et al., 2004). In order to demonstrate reproducibility of the results, H₂ production rates were measured in three trials at an influent glucose concentration of 3.3 g/L (tests A, B, and C). The reactor was cleaned and re-inoculated for two of these experiments (3.3 g/L, tests A and C), and for an influent glucose concentration of 4.5 and 10 g/L. Otherwise, the reactor feed was changed to a new glucose concentration after purging the gas phase with nitrogen gas. In some tests (as noted), excess biomass was removed from the reactor via a method meant to simulate reactor backwashing. First, fresh medium was pumped through the effluent line into the reactor. Then, the beads were gently mixed in the column using an ethanol-wiped spatula to remove excess biofilm. The fluid was then drained from the reactor, purged with nitrogen gas, and the reactor re-started with fresh medium.

### 2.3. Calculations

The volumetric H₂ production rate, \( R_{\text{HV}} \) (mL H₂/h), was calculated from the total gas production rate and the concentration of H₂ in the headspace. The molar H₂ production rate, \( R_{\text{HM}} \) (mmol H₂/h/L), was calculated using the ideal gas law as 

\[
R_{\text{HM}} = \frac{R_{\text{HV}}}{RT},
\]

where \( R = 0.0821 \text{ (L atm)/(mol K)} \) and \( T = 303 \text{ K} \). Conversion efficiency was calculated as 

\[
\text{Conversion efficiency} = \frac{R_{\text{HM}}}{4Rc} \times 100%.
\]

The specific H₂ production rate was calculated as 

\[
S = 1000 R_{\text{HV}}/(180 Rc V),
\]

where \( V \) is the reactor volume.

The maximum theoretical production of H₂ and CO₂ were calculated using measured acetic and butyric acid concentrations assuming (Muller, 2001) the following stoichiometry:

\[
C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2
\]

\[
C_6H_{12}O_6 \rightarrow CH_3(CH_2)COOH + 2H_2 + 2CO_2
\]

### Table 1 – Summary of H₂ production rates and conversion efficiencies (based upon a maximum production of 4 moles of H₂ from every mole of glucose)

<table>
<thead>
<tr>
<th>System conditions</th>
<th>Influent glucose concentration, mM (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>58.4 (10.5) 25.0 (4.5) 18.3 (3.3°) 5.6 (1.0)</td>
</tr>
<tr>
<td>Effluent glucose concentration (mmol/L)</td>
<td>45.7 ± 1.7 9.8 ± 1.2 4.2 ± 0.5 0.1 ± 0.0</td>
</tr>
<tr>
<td>Consumed glucose (mmol/L)</td>
<td>12.7 ± 1.7 15.2 ± 1.2 14.1 ± 0.5 5.5 ± 0.0</td>
</tr>
<tr>
<td>Glucose loading rate (g/h/L of reactor)</td>
<td>8.3 3.5 2.6 0.7</td>
</tr>
<tr>
<td>Glucose consumption rate (R₇) (mmol/L)</td>
<td>1.2 ± 0.2 1.5 ± 0.1 1.4 ± 0.1 0.5 ± 0.0</td>
</tr>
<tr>
<td>Biogas production rate (mL/h)</td>
<td>37 ± 0 31 ± 1 27 ± 1 16 ± 0</td>
</tr>
<tr>
<td>H₂ (%)</td>
<td>74 ± 4 79 ± 2 74 ± 2 70 ± 1</td>
</tr>
<tr>
<td>Effluent pH</td>
<td>4.9 5.6 5.6 5.6</td>
</tr>
<tr>
<td>Volumetric H₂ production rate, ( R_{\text{HV}} ) (mL/h/L of reactor)</td>
<td>220 ± 8 203 ± 8 163 ± 8 89 ± 8</td>
</tr>
<tr>
<td>Molar H₂ production rate, ( R_{\text{HM}} ) (mmol/L of reactor)</td>
<td>8.9 ± 0.8 8.1 ± 0.8 6.5 ± 0.0 3.3 ± 0.0</td>
</tr>
<tr>
<td>Conversion efficiency (%)</td>
<td>22 17 15 8</td>
</tr>
<tr>
<td>Specific H₂ production (mL H₂/L g glucose)</td>
<td>1270 760 680 1040</td>
</tr>
</tbody>
</table>

° From experiment A.

Based on a flow rate of 0.096 L/h.

Based on 4 mol H₂/mmol glucose.

Conversion efficiency for 3 g/L A and B; for 3.3 g/L (C), the conversion efficiency was 19% due to the increased gas production rate.
2.4. Determination of hydraulic retention time

The reactor’s hydraulic retention time (HRT) was determined by spiking the feed line with a concentrated KCl solution (16 g/L), and measuring the conductivity of the reactor effluent (YSI 600XL; YSI Incorporated; Yellow Springs, OH, USA). The retention time was determined by using the front half of the tracer curve with the mean of the distribution defined as the HRT, as previously described (Min et al., 2004; see also Miller and Logan, 2000). The trailing edge of the tracer curve is not used because the method requires an assumption of a normal distribution of data, which is not obtained in a biofilm system due to slow diffusion of the tracer out of the biofilm (Logan, 1999).

2.5. Analytical procedures

H₂ was measured using a gas-tight syringe (0.25 mL injection volume) and a gas chromatograph (SRI instruments, Torrence, CA) equipped with a thermal conductivity detector and a molecular sieve column (Alltech 5A 80/100) with nitrogen gas as the carrier gas. Carbon dioxide and methane were similarly analyzed except that a different column was used (Alltech Porapak Q 80/100) with helium as the carrier gas. Glucose was measured by using a phenol-sulfuric method for reducing sugars (Dubois et al., 1956). Acetate, butyrate, propionate, and ethanol were determined by gas chromatography (Agilent 6890N) and a fused-silica capillary column (DB-FFAP 30 m × 0.32 mm × 0.5 μm) with injector and flame ionization detector temperatures of 250 °C, with helium as a carrier gas at flow rates of 3.5 mL/min (60 °C) to 1.5 mL/min (240 °C). The oven temperature was programmed as follows: 60–120 °C increased at 20 °C/min, then 240 °C at 30 °C/min, and then steady at 240 °C for 3 min.

3. Results and discussion

At all glucose loading rates, there was a high rate of biogas production (Fig. 2). The rate of gas production increased from 16±0 to 37±1 mL/h when the glucose loading rate was increased over the range of 0.7–8.3 g/h L of reactor (Table 1). Lag times for H₂ production varied primarily as a result of glucose feed concentration. At 10 g/L, the effluent pH was 4.9 but it was 5.6 at the other glucose concentrations. Only when the feed bottle was changed to a new glucose concentration, or the reactor was cleaned to remove excess biomass, lag times were only 6–12 h. Gas production rates were reproducible when the reactor inoculation conditions were the same, as shown by the similar gas production rates (28.9±0.6 for 3.3 g/L (A) vs. 35.0±1 mL/h for 3.3 g/L (B); ± S.E. of the slope shown in Fig. 2). Cleaning the column (i.e. removing excess biomass) increased the H₂ production rate from 20±1 (A) to 26±1 mL/h (C). Thus, column cleaning is essential for maximizing H₂ production. In a full-scale system, biomass can be cleaned by backwashing the medium (Min et al., 2004).

Steady-state headspace H₂ concentrations in the gas phase were all in the range of 70–79% and did not show a trend with glucose concentration. The average H₂ headspace concentrations were 74±3%, 79±2%, 74±2%, and 70±1% for glucose concentrations of 10.5, 4.5, 3.3, and 1.0 g/L, respectively (Fig. 3), or overall averaged 74±3%. The effluent pH varied as a function of glucose feed concentration. At 10 g/L, the effluent pH was 4.9 but it was 5.6 at the other glucose concentrations. The reactor’s retention time was determined to be 2.1 min based on a salt tracer test in the presence of the biofilm (Fig. 4).

The conversion efficiencies for producing H₂ from glucose ranged from 15% to 22%, based on a theoretical stoichiometry of 4 moles H₂ from 1 mole of glucose (Table 1). Cleaning the column (3.3 g/L glucose concentration) increased the conversion efficiency from 15% (A) to 19% (C). These conversion efficiencies are slightly less than those of 24% and 23% reported in batch studies (Logan et al., 2002; Oh et al., 2003), and much less than 68% (Yokoi et al., 2002) and 48–55% (Ueno et al., 2001b; Fang and Liu, 2002; Chin et al., 2003; Hussy et al., 2003) achieved in stirred reactors (fed-batch or continuous flow).
Acetate and butyrate were the main soluble products of H₂ production (Fig. 5), with acetate reaching higher concentrations than butyrate (Table 2). Total propionic acid and ethanol concentrations were less than 0.4 mM. Theoretical CO₂ and H₂ yields were calculated from volatile acid concentrations measured in the reactor effluent and from Eqs. (1) and (2). The carbon recovery ranged from 80% to 94% based on volatile acid production, with the balance assumed to have been converted to biomass. The predicted conversion efficiencies of H₂ production from glucose (60–72%) on the basis of these measured volatile acid concentrations, however, were much higher than the actual measured H₂ conversion efficiencies (15–27%) based on H₂s production. This difference could be due to several factors, including biological factors such as the loss of H₂ via homoacetogenesis (Oh et al., 2003), and physical factors such as the loss of biogas in the collection bag. No methane, however, was detected in the gas throughout the tests, indicating that there was no loss of H₂ via methanogenesis.

3.1. Comparison of H₂ production based on reactor volume

Reactor efficiency is often examined on the basis of the production of the product (H₂) normalized to the total reactor volume. Based on the H₂ production rate of 27.2 mL/h for an influent glucose concentration of 10 g/L, the H₂ production rate achieved here was 8.9 mmol H₂/(L h). This is similar to values obtained in other studies using CSTRs of 8.8 mL H₂/(L h) (Iyer et al., 2004) and 7.9 mL H₂/(L h) (Mizuno et al., 2000) (Table 3). However, the H₂ production rate in the unsaturated flow reactor is higher than that reported in another study of 2.52 mmol H₂/(L h) (Fang and Liu, 2002), and lower than that reported for a saturated reactor of 13.6 mmol H₂/(L h) (Yokoi et al., 1997). This suggests that on the basis of reactor volume, the trickle-bed reactor was as effective as a CSTR in terms of overall H₂ production normalized to the reactor volume. The H₂ yield for an

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**Table 2 – Summary of volatile fatty acids production**

<table>
<thead>
<tr>
<th>System conditions</th>
<th>Influent glucose concentration, mM (g/L)</th>
<th>58.4 (10.5)</th>
<th>25.0 (4.5)</th>
<th>18.3 (3.3)</th>
<th>5.6 (1.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumed glucose, R_G (mmol/h)</td>
<td>1.2 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>0.5 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Consumed carbon (mmol/h)</td>
<td>7.3 ± 1.0</td>
<td>8.8 ± 0.7</td>
<td>8.1 ± 0.3</td>
<td>3.0 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Acetate (mmol/h)</td>
<td>1.2 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Butyrate (mmol/h)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Predicted CO₂ (mmol/h)</td>
<td>2.3 ± 0.4</td>
<td>2.5 ± 0.3</td>
<td>2.5 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Carbon recovered in acetate, butyrate, and CO₂ (mmol/h)</td>
<td>6.9 ± 1.1</td>
<td>7.6 ± 1.0</td>
<td>7.5 ± 0.4</td>
<td>2.4 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Carbon recovery (%)</td>
<td>94</td>
<td>87</td>
<td>92</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Predicted H₂ (mmol/h)</td>
<td>3.5 ± 0.5</td>
<td>4.1 ± 0.4</td>
<td>3.8 ± 0.2</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Predicted conversion efficiency (%)</td>
<td>72</td>
<td>69</td>
<td>70</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Observed conversion efficiency (%)</td>
<td>22</td>
<td>17</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

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* From experiment A.
** Based on Eqs. (1) and (2), assuming the production of 1 mole of CO₂ with the production of 1 mole of acetate, and 2 moles of CO₂ with the production of 1 mole of butyrate.
† Based on Eqs. (1) and (2), assuming the production of 2 moles of H₂ with the production of 1 mole of acetate or butyrate.
‡ Predicted conversion efficiency calculated as (predicted H₂)/(4R_G) × 100%.
influent glucose concentration of 10 g/L was comparable
to 0.9 mol H2/mol glucose reported in a CSTR (Mizuno et al., 2000),
but was lower than 1.6–2.3 mol H2/mol glucose
reported in the other studies using CSTR or saturated
flow reactors (Yokoi et al., 1997; Fang and Liu, 2002; Iyer et al.,
2004). Longer HRT, as a result of larger size reactors, will likely
be needed for achieving higher H2 yields with an unsaturated
flow reactor.

3.2 Implications of these results for H2 production using
an unsaturated flow reactor

The high H2 gas concentrations (70–79%) and H2 production
rates (normalized by reactor volume) obtained here using
unsaturated flow reactors demonstrate the feasibility of using
this type of reactor for biological H2 production under
mesophilic conditions. The main advantage of the unsatu-
rated flow reactor compared to a stirred reactor is that the
energy cost of stirring the system can be avoided. In addition,
the H2 concentrations achieved here appear to be similar or
slightly larger than values achieved in stirred reactors (Iyer et al., 1997; Fang and Liu, 2002; Iyer et al.,
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be needed for achieving higher H2 yields with an unsaturated
flow reactor.

Table 3 – Comparison of H2 production rates in the trickle-bed reactor with those reported using a CSTR or fixed-bed reactor

<table>
<thead>
<tr>
<th>Comparison Items</th>
<th>Trickle-bed reactor</th>
<th>Saturated fixed-bed reactor</th>
<th>CSTR</th>
<th>CSTR</th>
<th>CSTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose loading rate (g/L of reactor/h)</td>
<td>8.3</td>
<td>5.0</td>
<td>1.2</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Reactor retention time (min)</td>
<td>2.1 min</td>
<td>2 h</td>
<td>8.5 h</td>
<td>6 h</td>
<td>10 h</td>
</tr>
<tr>
<td>Volumetric H2 production rate (mL/h)</td>
<td>27.2</td>
<td>25.5</td>
<td>457</td>
<td>192</td>
<td>436</td>
</tr>
<tr>
<td>Molar H2 production rate (mmol/h)</td>
<td>1.09</td>
<td>1.02</td>
<td>18.06</td>
<td>7.57</td>
<td>17.53</td>
</tr>
<tr>
<td>Reactor working volume (L)</td>
<td>0.123</td>
<td>0.075</td>
<td>2.3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Normalized H2 production rate (mmol H2/L)</td>
<td>8.9</td>
<td>13.6</td>
<td>7.9</td>
<td>2.5</td>
<td>8.8</td>
</tr>
<tr>
<td>H2 yield (mol/mol glucose)</td>
<td>0.9</td>
<td>2.3</td>
<td>0.9*</td>
<td>2.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* No nitrogen sparging.

influent glucose concentration of 10 g/L was comparable
to 0.9 mol H2/mol glucose reported in a CSTR (Mizuno et al., 2000),
but was lower than 1.6–2.3 mol H2/mol glucose
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energy cost of stirring the system can be avoided. In addition,
the H2 concentrations achieved here appear to be similar or
slightly larger than values achieved in stirred reactors (Iyer et al., 1997; Fang and Liu, 2002) (Table 3).
Reactor clogging due to the biomass build-up was not
specifically examined here, but scale-up of the process would
require regular backwashing to control biofilm growth (Min et al., 2004). The energy needed for this step would need to be
considered in the overall energy balance.

It is envisioned that an unsaturated flow reactor of the type
developed here would be most effectively used as a pre-
treatment method for wastewaters containing high concen-
trations of carbohydrates. These reactors will need to be
much taller than the laboratory-scale reactor examined here
in order to provide sufficient HRTs to fully remove the sugars
and produce H2. Even then, there will be soluble organic
matter remaining in solution that will require further
treatment. Wastewater leaving this reactor could therefore
either be further treated with a conventional aerobic process
such as activated sludge, an anaerobic process such as
anaerobic digestion, or a new type of microbial fuel process
that produces electricity or H2 (Liu et al., 2005a, b). Wastewater
treatment process trains optimized for H2 production have so
far not been extensively tested at large scale, and, thus,
additional research is needed to prove the stability and
usefulness of this technology for wastewater treatment.

Acknowledgment

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