Measurement of H_2 consumption and its role in continuous fermentative hydrogen production

J. T. Kraemer and D. M. Bagley

ABSTRACT

To maximise the yield from fermentative H_2 production, H_2 consumption must be minimised. This work demonstrated for the first time that H_2 consumption exists in an established continuous-flow biohydrogen system. The rate of H_2 consumption was found to be related to the concentration of CO_2, with H_2 consumption inhibited at both low and high CO_2. N_2 sparging of the continuous reactor at 31 mL/min/L-liquid increased the H_2 yield from 1.31 to 1.87 mol H_2/mol glucose, but did not significantly change the *in-situ* rate of H_2 consumption (0.07–0.09 mM/h). Assuming sparging completely inhibited H_2 consumption, it could only account for 2–11% of the H_2 yield increase during sparging, based on H_2 consumption rates measured in the reactor and in vials. Therefore, H_2 consumption may be of minor concern for continuous biohydrogen systems.

Key words | acetogenesis, activity test, carbon dioxide, fermentation, hydrogen, sparging

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INTRODUCTION

Renewable energy sources are currently receiving a lot of interest because of global climate change. Anaerobic digestion is a natural microbial degradation process which can convert organic wastes into renewable energy in the form of hydrogen and methane gases. H_2 is appealing because it does not release CO_2 during its utilization (although CO_2 is released during its formation).

H_2 is produced during the fermentation step of anaerobic digestion. Considerable research has been devoted to fermentative H_2 production recently and the current state of knowledge has been reviewed (Hawkes *et al.* 2007; Kraemer & Bagley 2007). To be successful, these systems must promote conditions conducive to H_2 production while minimising H_2-consumption. Methanogenesis is the most prevalent route of H_2 consumption in anaerobic systems. Methanogens can be killed by heat treatment of the inoculum, but current evidence indicates this practice may not maximise the H_2 yield (Kraemer & Bagley 2007). Instead, operation of a continuous reactor at low pH (~5.5) and an appropriate retention time (~6–12 h) can wash out the methanogens (Kraemer & Bagley 2007). However, there are other routes of H_2 consumption in anaerobic systems and these must be investigated in order to maximise H_2 yields.

Homoacetogenesis is another H_2-consuming pathway that was observed in a batch biohydrogen system by Oh *et al.* (2003) for both heat-treated and non-heat-treated inocula. This work was followed up by Park *et al.* (2005) who observed that acetogenesis and concurrent H_2 removal in a heat-treated batch culture was prevented when the headspace CO_2 was removed using a KOH trap. These observations have lead to the proposition that N_2 sparging in a continuous-flow system might decrease the dissolved concentrations of H_2 and CO_2 sufficiently to decrease the substrate available to acetogens (Hussy *et al.* 2003; Park *et al.* 2005; Kim *et al.* 2006; Kraemer & Bagley 2006).

Although H_2 consumption has been observed in batch culture, what is still unknown is whether H_2 consumption exists in a continuous-flow biohydrogen system. If H_2 consumption does exist, what is its rate and to what extent does it decrease the H_2 yield? And does N_2 sparging decrease H_2 consumption as proposed in the literature?

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The objective of this work was to answer these fundamental questions.

METHODS

Reactor operation

A completely-mixed reactor (2 L liquid) was inoculated with methanogenic sludge (no heat treatment) and operated at 25°C, 10 h hydraulic retention time, pH 5.5, and mineral oil as antifoamant. The details of the reactor have been previously described (Kraemer & Bagley 2006). The substrate was glucose at 10 g/L supplemented with the nutrient solution of Kraemer & Bagley (2006). Nitrogen gas (99.999% pure, Praxair) was sparged into the reactor using a fritted-cylinder dispersion tube (#39533-12C, Corning Inc.). The off-gas was measured continuously using a FLOCELL® bubble meter (Challenge Environmental Systems, Inc.) without sparging or a differential pressure flowmeter (#32446-27, Cole-Parmer Instrument Co.) during sparging. The reactor was operated without sparging for 23 days before the work described here.

Two experimental runs of 14 days each were performed, with the first run using N_2 sparging at 31 mL/min/L-liquid while the second run did not use sparging. Analytical measurements were taken about every other day starting on the 3rd day of each run. Activity tests (described below) were performed during each run.

Analytical measurements

Headspace and dissolved gas concentrations were measured according to Kraemer & Bagley (2006). Gas samples were taken using a SampleLock syringe (Hamilton Co.). Total and volatile suspended solids (TSS, VSS) were measured according to *Standard Methods for the Examination of Water and Wastewater* (1998).

Glucose was analyzed enzymatically using 25 μL of centrifuged sludge supernatant (20,000 *g*, 4°C, 20 min) and 4.0 mL glucose hexokinase reagent (Infinity, Thermo Electron Corp.) dispensed at 4°C. The liquid was vortexed for 1 s, incubated at 37°C for 3 min, and absorbance measured at 340 nm.

Acetone, alcohols (ethanol, 1-propanol, 1-butanol), and volatile fatty acids (acetic, propionic, *i*-butyric, *n*-butyric, *i*-valeric, *n*-valeric) were measured by static headspace-gas chromatography according to Cruwys *et al.* (2002). Modifications were made to accommodate analysis of acetone and alcohols, including the use of 2-methyl-1-butanol as alcohol internal standard (100 μL at 2,000 mg/L) and using a temperature program of 45°C for 1.8 min, 45°C/min to 140°C, and 10°C/min to 166°C. Other differences were: needle temperature 95°C, Zebron FFAP column (Phenomenex), one distilled water wash blank between samples, and use of ordinary least-squares regression.

Measurement of H_2 consumption

H_2-consuming activity was measured either using 22 mL headspace vials ("in-vial") or directly in the reactor ("in-reactor"). For "in-vial" tests, the vials were capped with butyl rubber septa and aluminum crimp caps (Supelco) and purged with a 90% N_2/10% CO_2 gas mixture (Praxair). Then 10 mL of reactor sludge, obtained with a gastight syringe, was injected while venting the vial headspace to maintain ~1 atm pressure. After the addition of 0.5 mL H_2 gas (99.999%, Praxair) and 0.5 mL CH_4 gas as internal standard (99.97%, Praxair), the vials were kept horizontal and mixed at 125 rpm in an orbital shaker (New Brunswick Scientific) at 20°C. The vial headspace gas compositions were measured approximately every 30 min either immediately or after 16–18 h depending on the purpose of the test. For some tests, 2nd and 3rd feedings of 0.3–0.5 mL H_2 were performed and the headspace measured 4–5 times after each feeding. For "in-reactor" H_2 consumption tests, the liquid flows and N_2 sparging (if in use) were stopped and dissolved and headspace H_2 and CO_2 concentrations were measured every 15–20 min.

RESULTS & DISCUSSION

The performance of the continuous-flow biohydrogen reactor with and without sparging is summarized in Table 1. Sparging significantly increased the H_2 yield, decreased the dissolved and headspace H_2 and CO_2 concentrations, and increased the amount of acetate produced. There was no significant change in the n-butyrate

Table 1 | Performance of the fermentative hydrogen production bioreactor with and without N_2 sparging at 31 mL/min/L-liquid. Values are average ± 95% confidence intervals (CI)

	No Sparging	With Sparging
H_2 yield, mol H_2/mol glucose converted	1.31 ± 0.04	1.87 ± 0.05
Headspace gas composition, mol%		
H_2	58.8 ± 0.7	9.7 ± 0.5
CO_2	41.2 ± 0.7	7.2 ± 0.3
Dissolved gas concentration, mM		
H_2	0.72 ± 0.04	0.36 ± 0.03
CO_2	5.5 ± 0.2	0.75 ± 0.06
VSS, mg/L	125 ± 46	47 ± 5
Soluble Constituents, mg/L		
Acetate	1,143 ± 60	1,327 ± 67
n-butyrate	2,235 ± 131	2,322 ± 221

concentration with sparging. Regardless of sparging, the glucose conversion efficiency was 100% and the other compounds measured were <90 mg/L.

Figure 1 shows that sludge taken from an established continuous-flow fermentative hydrogen production bioreactor and placed in an activity test vial will consume H_2. Initially, the headspace H_2 concentration increased, which can be attributed to continued H_2 production by the sludge and mass transfer of H_2 out of solution because the initial vial headspace H_2 concentration (2.4 nmol/μL) was lower than that in the liquid (~3.5 nmol/μL with sparging, ~22 nmol/μL without sparging). H_2 consumption was observable after 4 hours, and this lag time appeared to be similar regardless of whether the sludge came from a sparged or un-sparged reactor.

In another test, repeated additions of H_2 to the activity test vials were also consumed (Figure 2 – closed circles). Furthermore, the rate at which H_2 was consumed increased with each feeding (i.e. increased with time). In Figure 2, the rates of H_2 consumption were 0.33, 0.56, and 0.61 mM/h for the 1st, 2nd and 3rd feedings, respectively. This indicated growth of H_2-consuming organisms.

H_2 consumption could be inhibited by removing CO_2 (Figure 2 – open triangles). After the first H_2 feeding, duplicate vials identical to those above had their headspaces purged with N_2 to remove CO_2 and H_2 and CH_4 (internal standard) were added again. As shown in Figure 2 (open triangles), H_2 was not consumed without CO_2 whereas it was consumed in the vials that still had CO_2 (Figure 2 – closed circles). Park *et al.* (2005) were able to prevent H_2 consumption in batch bottles by removing CO_2 using a KOH trap. This work demonstrates that sludge from a continuous-flow system also consumes H_2 and that, like Park *et al.* (2005), it can be prevented through CO_2 removal.

Additional in-vial activity tests were conducted using several different headspace CO_2 concentrations in the same manner as for Figure 2. The effect of CO_2 concentration on the rate of H_2 consumption is summarised in Figure 3. The rate of H_2 consumption decreased as the CO_2 concentration decreased, with a sharp decline as CO_2 approached zero. Thus, intermediate headspace CO_2 concentrations (1–6 mM) decreased the rate of H_2 consumption without

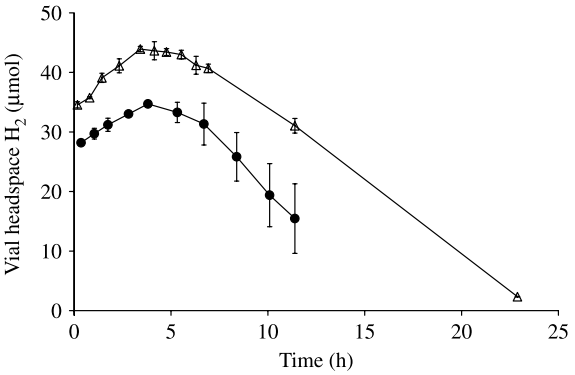

Figure 1 | In-vial H_2 consumption by sludge taken from a continuous fermentative H_2 bioreactor that was sparged with N_2 at 31 mL/min/L (△) or un-sparged (●). Error bars show the range.

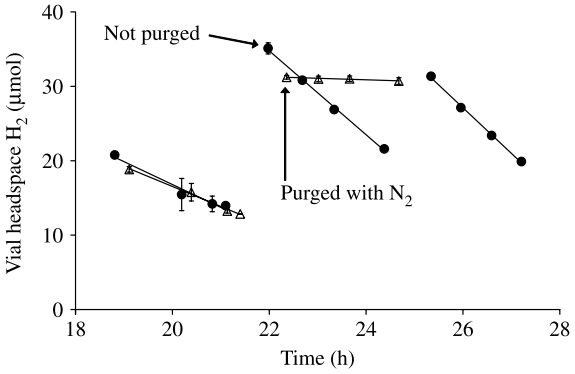

Figure 2 | H_2 consumption by sludge from a sparged biohydrogen reactor. Vials were identical for the 1st feeding, then either purged with N_2 ($CO_2 = 0.3$ mM, △) or unchanged ($CO_2 = 3.8$ mM, ●).

completely inhibiting it. Interestingly, a high CO_2 concentration (27 mM) also decreased H_2 consumption. High CO_2 concentrations are known to be inhibitory (Dixon & Kell 1989) and in biohydrogen systems high CO_2 concentrations in batch bottles have decreased cell growth and H_2 production (Wang *et al.* 2007). While sparging a continuous biohydrogen system with CO_2, Kim *et al.* (2006) observed a decrease in microbial diversity (including acetogens) and decreased glucose utilization, although the H_2 produced per glucose converted was higher than for N_2 sparging.

A possible mechanism for H_2 consumption in the absence of methanogenesis is homoacetogenesis:

$$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O \quad (1)$$

In this study, changes in the acetate concentration during activity tests were undetectable because of the high background acetate from the reactor (Table 1). For example, the acetate concentration in an activity vial could increase by up to 60 mg/L, which is only a 5% change relative to the initial acetate concentration and the same order of magnitude as the reactor variability (Table 1). Park *et al.* (2005) were able to detect changes in acetate because their acetate concentrations were lower (425 mg/L) and expected a larger acetate increase of 235 mg/L.

Figure 4 shows the H_2 consumption tests conducted directly in the reactor. Dissolved H_2 started to decrease immediately after the liquid flows and N_2 sparging were stopped. There were two rates of H_2 removal: a higher initial rate and a slower secondary rate. The initial rates were 0.3 and 1.3 mM/h for sparged and unsparged sludge,

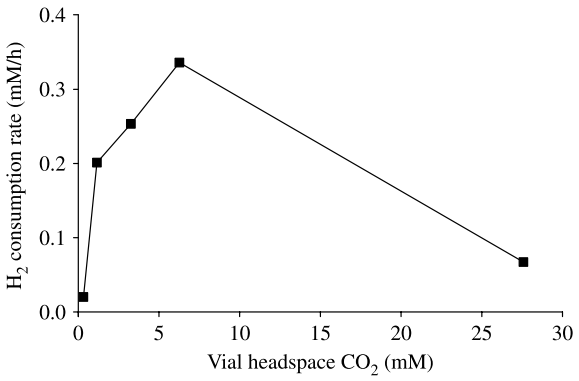

Figure 3 | In-vial H_2 consumption rate as a function of vial headspace CO_2 concentration. H_2 was consumed by sludge taken from an unsparged continuous-flow biohydrogen reactor.

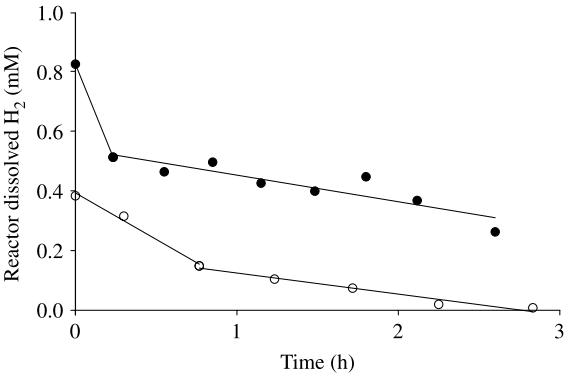

Figure 4 | In-reactor dissolved H_2 removal in a fermentative H_2-producing sludge immediately after stopping all liquid flows and sparging (if in use). Reactor with sparging (○) and without (●).

respectively, although there were not enough data for a statistical analysis. The initial rate included both H_2 consumption as well as H_2 mass-transfer out of solution: initially, the liquid was supersaturated with respect to the headspace, and during this initial period the reactor headspace H_2 concentration increased (not shown). During the second period of H_2 removal, the rates were 0.071 ± 0.006 and 0.09 ± 0.01 mM/h for sparged and unsparged sludge, respectively. Thus, for both periods of H_2 removal sparging may have decreased H_2 consumption.

However, the overall impact of H_2 consumption appears to be small. The H_2 production rates with and without sparging were 10.4 and 7.3 mM/h, respectively, so H_2 consumption for the secondary in-reactor rate was $<2\%$ of the H_2 production rate regardless of sparging. And the highest invial H_2 consumption rate was 0.34 mM/h (Figure 3), which was $<5\%$ of the H_2 production rate. Assuming sparging completely inhibited H_2 consumption, it could account for an H_2 yield of 0.01 and 0.06 mol H_2/mol glucose converted based on the in-reactor and in-vial consumption rates, respectively, whereas the H_2 yield actually increased by 0.56 mol H_2/mol glucose converted (Table 1). Consequently, depending on which rate of H_2 consumption is used, preventing H_2 consumption could account for at most 2–11% of the observed H_2 yield increase.

CONCLUSIONS

This work demonstrated that H_2 consumption exists in an established continuous-flow fermentative H_2 production

system. H_2 consumption decreased as CO_2 decreased, and was inhibited at both low and high CO_2 concentrations. N_2 sparging may have decreased the in-reactor rate of H_2 consumption, although even if sparging did inhibit the highest observed rates of H_2 consumption, it would account for $<11\%$ of the observed increase in the H_2 yield during sparging. Therefore, H_2 consumption in continuous fermentative hydrogen production may be of minor concern.

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