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Inhibition of Biohydrogen Production by Undissociated Acetic and Butyric Acids

STEVEN VAN GINKEL AND BRUCE E. LOGAN*

Department of Civil and Environmental Engineering, 212 Sackett Building, Penn State University, University Park, Pennsylvania 16801

Glucose fermentation to hydrogen results in the production of acetic and butyric acids. The inhibitory effect of these acids on hydrogen yield was examined by either adding these acids into the feed of continuous flow reactors (external acids), or by increasing glucose concentrations to increase the concentrations of acids produced by the bacteria (self-produced). Acids added to the feed at a concentration of 25 mM decreased H_2 yields by 13% (acetic) and 22% (butyric), and 60 mM (pH 5.0) of either acid decreased H_2 production by >93% (undissociated acid concentrations). H_2 yields were constant at 2.0 ± 0.2 mol H_2/mol glucose for an influent glucose concentration of 10−30 g/L. At 40 g glucose/L, H_2 yields decreased to 1.6 ± 0.1 mol H_2/mol glucose, and a switch to solventogenesis occurred. A total undissociated acid concentration of 19 mM (self-produced acids) was found to be a threshold concentration for significantly decreasing H_2 yields and initiating solventogenesis. Hydrogen yields were inhibited more by self-produced acids (produced at high glucose feed concentrations) than by similar concentrations of externally added acids (lower glucose feed concentrations). These results show the reason hydrogen yields can be maximized by using lower glucose feed concentrations is that the concentrations of self-produced volatile acids (particularly butyric acid) are minimized.

Introduction

One way to make renewable biohydrogen production costs competitive with hydrogen production from fossil fuels is to produce H_2 using inexpensive substrates in reactors that have low capital costs. Economical biohydrogen production can be accomplished using high-strength wastestreams such as food processing wastewaters in reactors operating at short hydraulic retention times (*1*−*3*). However, cultures fed high sugar concentrations are susceptible to product inhibition. The products are primarily H_2 and CO_2 gases and acetic and butyric acids. Many authors have studied the effects of these acids on solvent production in the traditional acetone−butanol−ethanol (ABE) fermentation (*4*−*6*). It has been shown that hydrogen fermentation changes to a solvent-forming reaction once the undissociated acid concentration reaches a critical threshold, but the threshold cannot be well predicted as it varies substantially over the range of ∼2−30 mM (undissociated acid concentration) (*5*−*9*). The undissociated form of the acid is known to be the most important factor that causes the switch from H_2 production to solvent production, although the total acid concentration also affects solution ionic strength (*5*−*10*). Inhibition is caused by nonpolar undissociated acids being able to cross the cell membrane at a low pH that then dissociate in the cell at the higher internal pH releasing a proton inside the cell (*10*). The uptake of protons in this way uncouples the proton motive force, which causes an increase in maintenance energy requirements to maintain the intracellular pH near neutrality (*10*). The uptake of acid also causes a decrease in the available coenzyme A and phosphate pools which decreases the flux of glucose through glycolysis (*4*). A detailed description of the mechanisms of undissociated acid inhibition can be found in Jones and Woods (*10*).

A major factor affecting H_2 or solvent production is the pH. The optimum pH for H_2 production is 5.5, while the optimum pH for solvent production is ∼ 4.5 (*10*−*12*). The pH can affect the form of the acids produced during hydrogen production. The concentrations of the undissociated forms of acetic or butryic acid are greater at a pH of 4.5 than at pH 5.5 and thus higher amounts of the undissociated form are present at the lower pH to cause inhibition. Undissociated acid concentrations can also be increased by increasing the substrate concentration from which the acids are produced. The effect of the undissociated acid on the bacteria therefore sets an upper limit on the influent substrate concentration, as a function of pH, for hydrogen production.

The concentration of undissociated acids that inhibits hydrogen production has not been specifically studied, although it can be inferred from the literature that acid concentrations that promote solventogenesis inhibit hydrogen production (*4*−*10*). Due to a wide range in concentrations reported to promote solventogenesis (2−30 mM), however, the concentrations that inhibit hydrogen production using a heat-treated soil innoculum cannot be predicted based on the literature. In this study, we therefore determined acid concentrations that caused a significant decrease in H_2 yields using a continuous flow reactor system. Acetic and butyric acids were varied by either adding them directly to the feed at low glucose feed concentrations, or by increasing the glucose feed concentration to increase the acids produced by the bacteria as fermentation end products.

Experimental Section

Startup and Feeding. Two identical stirred fermentors (2-L New Brunswick BioFlo, Edison, NJ) operated in continuous flow mode were used in these experiments. Reactors were cleaned, filled to 2 L with distilled water, autoclaved for 1 h, and then sparged with N_2 gas for approximately thirty minutes before inoculation. Each reactor was inoculated with 10 g of baked and sieved agricultural soil. The soil was baked for 2 h at 100 °C to select for spore-forming, H_2-producing bacteria (*11*), a process which has been found to result in a microbial community primarily composed of clostridial species (*13*). Hydrogen production from this inoculum source has been shown to be consistent even when stored (−80 °C) over several months (*3*, *11*, *14*). All reactors were operated at 30 °C. During startup, L-cysteine (1 g/L) was added to the reactor when it was operated in batch mode to remove dissolved oxygen. Nutrients were added as described previously (*14*). Feed bottles (10 L) were continuously sparged with N_2 gas and mixed (∼250 rpm) to achieve anaerobic conditions in the feed bottle.

Reactor Operation. Several conditions were systematically employed to determine the effect of undissociated acid concentrations on H_2 yields. The influent glucose concentra-

* Corresponding author e-mail: blogan@psu.edu; phone: 814-863-7908; fax: 814-863-7304.

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TABLE 1. H_2 Yields and Aqueous Product Concentrations at Varying pH, Influent Undissociated Acid, and NaCl Concentrations (mM; Values ± SD for $n = 3$ Samples over Multiple HRTs)

			Aqueous Products							
				HAc (mM)		HBu (mM)		H_2		
condition[a]		redox potential (mV)	glucose (mg/L)	total	undiss.	total	undiss.	(%)[b]	L/hr	yield[c]
pH	4.5	−370	695 ± 115	8 ± 0.2	5 ± 0.1	13 ± 0.5	9 ± 0.3	61	0.23	1.8 ± 0.1
	5.0	*d*	503 ± 27	9 ± 1	3 ± 0.2	11 ± 0.6	4 ± 0.2	61	0.20	1.9 ± 0.1
	5.5[a]	−385	433 ± 16	11 ± 0.3	2 ± 0.1	13 ± 0.6	2 ± 0.1	64	0.29	2.5 ± 0.1
	6.0	−350	155 ± 7	8 ± 1	0.4 ± 0.0	14 ± 1.1	1 ± 0.1	63	0.24	2.0 ± 0.0
NaCl	162	*d*	640 ± 12	8 ± 2	1 ± 0.3	12 ± 0.3	2 ± 0.1	63	0.28	2.3 ± 0.1
HAc	15	−393	678 ± 30	98 ± 22	15 ± 3	14 ± 3	2 ± 0.6	62	0.30	2.4 ± 0.1
	25	−403	540 ± 33	165 ± 5	25 ± 1	15 ± 1	3 ± 0.1	62	0.20	2.2 ± 0.1
HBu	2.5	*d*	581 ± 15	11 ± 7	2 ± 1.0	29 ± 3	5 ± 0.4	65	0.30	2.5 ± 0.1
	5	*d*	848 ± 8	9 ± 1	1 ± 0.1	38 ± 1	6 ± 0.1	63	0.22	2.2 ± 0.2
	25	−334	261 ± 9	10 ± 0.2	1 ± 0.0	158 ± 2	27 ± 0.3	64	0.23	2.0 ± 0.1
HAc & HBu	5, 20	−420	424 ± 6	42 ± 1	6 ± 0.1	128 ± 2	22 ± 0.3	62	0.25	2.1 ± 0.1
	12.5, 12.5	−380	383 ± 8	100 ± 6	15 ± 1	94 ± 4	16 ± 1	62	0.25	1.9 ± 0.3
HAc, pH 5.0	60	*d*	>1200	138 ± 6	50 ± 2	4 ± 1	1 ± 0.0	<10	<0.02	<0.2
HBu, pH 5.0	60			16 ± 1	6 ± 0.2	160 ± 2	63 ± 1			

[a] The base condition is pH 5.5, 5 g COD/L glucose, no added NaCl, and no added acid. [b] Percentage of hydrogen gas in headspace. [c] mol H_2/mol gluc. [d] Not recorded.

tion was kept at a concentration of 5 g COD/L, except as noted, to minimize the concentration of self-produced acids compared to the amount of acid added to the feed (see below). Fermentors were inoculated separately at each new test condition. Each reactor was operated in batch mode until the redox potential dropped below ~-200 mV and some gas was produced. Reactors were then operated at a 10-h hydraulic retention time (HRT) until steady-state conditions (based on H_2 gas production) were obtained. Once steady hydrogen production was observed, by monitoring gas production and the percentage of H_2 in the headspace, three gas and liquid samples were taken one HRT apart. It was shown in a previous study (*3*) that steady state conditions are reproducible if the reactor is re-initialized at the same starting condition with the soil inoculum. Therefore, only single reactor tests were conducted. Multiple measurements were made over several HRTs in order to establish steady-state operation.

In the first set of tests, the reactor pH was decreased to increase the proportion of self-produced volatile acids present in the undissociated form. The pH was controlled automatically by the New Brunswick reactor control module. Reactors were operated at pHs of 4.5, 5.0, 5.5, and 6.0 at a 10-h hydraulic retention time (HRT) (Table 1).

In the second set of tests, acetic (HAc) and butyric (HBu) acids were added (sodium salt form) to the influent individually and together to raise the undissociated acid concentration in the reactor. The control for these experiments was the reactor operated at pH 5.5 (5 g glucose/L) with no added acid. Reactors were operated at a 10-h HRT at added undissociated acid concentrations (pH 5.5) of 15 and 25 mM HAc; 2.5, 5.0, and 25 mM HBu, and 5:20 and 12.5:12.5 mM HAc/mM HBu, respectively. In two additional experiments, acetic or butyric acid were added separately to the feed at a lower pH of 5.0 to produce an undissociated acid concentration of 60 mM. Lowering of the pH from 5.5 to 5.0, while maintaining the same feed concentration, increased the undissociated acid concentration from 25 to 60 mM.

In the third set of tests, the influent glucose concentration was raised to increase self-produced undissociated acid concentrations in the reactor. Reactors were operated at pH 5.5 at glucose concentrations of 10, 20, 30, 40, and 50 g/L at 2.5- and 10-h HRTs.

In a fourth test, the effect of sodium on hydrogen yields was tested to determine if there was any adverse effect of adding acids as sodium salts. NaCl was added at a concentration of 162 mM. This concentration is equal to the highest sodium concentration added when butyrate was added as a sodium salt (25 mM undissociated, pH 5.5). All other reactor conditions remained unchanged during this experiment.

Analytical. Once steady hydrogen production was observed, by monitoring gas production and the percentage of H_2 in the headspace, 3 liquid samples were taken 1 HRT apart. The liquid samples were analyzed for residual glucose concentration, biomass concentration, volatile fatty acids, and solvents. CO_2 and N_2 gas percentages in the reactor headspace were also measured. Glucose and gas measurements were conducted in triplicate and single acid/solvent samples were taken at each sampling time. H_2 gas flow rate measurements were taken at least 5 times per sampling time for a total of 15 measurements per HRT. Glucose was measured using the phenol sulfuric acid assay (*15*) with glucose standards in the range of 10−70 mg/L.

The biogas flowrate was measured using a bubble meter (100-mL volume, Agilent, Palo Alto, CA). Hydrogen percentage in the headspace of vessels was sampled using a gas-tight syringe (0.5-mL injection volume, Hamilton, Reno, NV) and measured using a gas chromatograph (GC; model 310, SRI Instruments, Torrence, CA) equipped with a thermal conductivity detector and a molecular sieve column (Alltech Molesieve 5A, Deerfield, IL) with nitrogen as the carrier gas. Nitrogen and carbon dioxide percentages were measured using a gas chromatograph (SRI Instruments) equipped with a Porapak Q column with helium as the carrier gas. Gas samples were compared to 4 standard volumes of pure gas injections.

Acetic, propionic, and butyric acids, ethanol, propanol, butanol, and acetone in the liquid phase were measured using a gas chromatograph (Agilent, 6890) equipped with a flame ionization detector and a fused-silica capillary column (DB-FFAP 30 m × 0.32 mm × 0.5 μm). Samples were filtered through a 0.2-μm pore-diameter membrane (Whatman, Florham Park, NJ) and acidified using formic acid (0.65 M). The temperature of the GC column was started at 60 °C, increased at 20 °C/min to 120 °C, and then increased at 30 °C/min to a final temperature of 240 °C for another 3 min. The temperatures of injector and detector were both 250 °C. Helium was used as the carrier gas at a constant pressure of 103 kPa. Liquid samples were refrigerated before analysis and were compared to 5−7 standards.

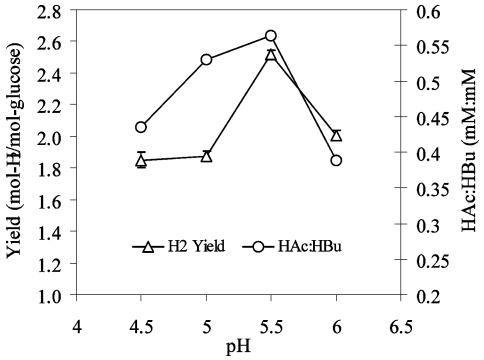

FIGURE 1. H_2 yield and ratio of acetic to butyric acid (HAc/HBu) as a function of pH (5 g glucose/L, 10-h HRT). (Values ± SD for $n = 3$ samples over multiple HRTs).

Glucose loading rate was calculated as $CV/(\text{HRT})$, where C is the influent glucose concentration and V is the reactor volume. On the basis of the total acid concentration added or measured in the reactor contents by gas chromatography, the undissociated acid concentration was calculated using

$$\text{pH} = \text{p}K_a + \log \frac{A^-}{\text{HA}} \quad (1)$$

where A^- and HA are the dissociated and undissociated acid concentrations, and the pK_a is 4.76 for acetic acid and 4.81 for butyric acid. The total acid concentration (HA + A^-) is determined by GC analysis. Statistical analyses were performed using the data analysis/regression functions on MS Excel 1997.

Results

H_2 Yields as a Function of pH. The reactor pH was varied in order to identify an optimum pH for the inoculum and also to establish a base case for other results. The H_2 yield over a pH range of 4.5−6.0 peaked at pH 5.5 at 2.5 ± 0.1 mol H_2/mol glucose (Figure 1). H_2 yields decreased to 2.0 ± 0.0 mol H_2/mol glucose when the pH was increased further to pH 6.0. The H_2 in the headspace remained constant over this pH range at 63 ± 2% (Table 1). Acetic acid concentrations and the HAc/HBu ratio were highest at pH 5.5 (Figure 1). Residual glucose concentrations decreased with increasing pH (Table 1). A carbon balance accounted for 94 ± 2% of the carbon. This carbon balance required an assumption of a bacterial yield of 0.2 g biomass/g glucose derived on the basis of energetic calculations (*3*, *16*). At a pH of 5.5, undissociated acetic and butyric acid concentrations were both 2.0 mM, respectively, while at pH 4.5 the undissociated acetic and butyric acid concentrations were 5 and 9 mM, respectively (Table 1). A switch to solventogenesis was not observed in this pH range of 4.5−6.0 even though the total concentration of undissociated acids reached 15 mM at pH 4.5. The low glucose concentration in the reactor may have been a factor as it has been noted that excess glucose is necessary to initiate solventogenesis (*10*).

H_2 Yields as a Function of Added Acetic and Butyric Acids at pH 5.5. The undissociated acid concentrations calculated at pH 4.5 (<15 mM) were the highest concentrations obtained at a glucose concentration of 5 g COD/L at the pH range tested. A metabolic switch to solventogenesis was not observed. Acetic and butyric acids were therefore added to the feed (2.5 to 25 mM undissociated) to determine the inhibitory effects of these acids on hydrogen production. When acetic and butyric acids were added to the feed, total undissociated acids (slope = −0.0154, $p = 0.05$), and to a lesser extent, undissociated butyric acid (slope = −0.0154, $p = 0.06$) in the reactor were observed to decrease H_2 yields

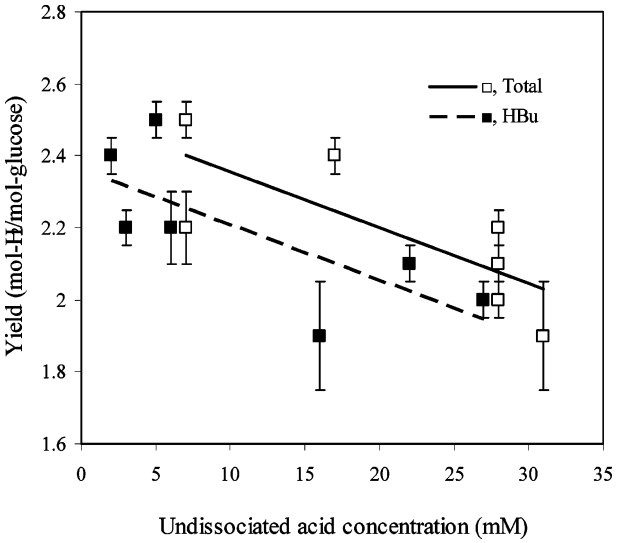

FIGURE 2. H_2 yield as a function of the total concentration of undissociated acetic and butyric acids (□) (slope = −0.0154, $p = 0.05$, $y_{int} = 2.51$, $R^2 = 0.58$) or butyric acid (■) (slope = −0.0154, $p = 0.06$, $y_{int} = 2.36$, $R^2 = 0.54$) concentrations in the reactor. (Values ± SD for $n = 3$ samples over multiple HRTs).

(Figure 2). Undissociated acetic acid had little effect on H_2 yields ($p = 0.89$). H_2 yields decreased from 2.5 ± 0.1 to 2.0 ± 0.1 mol H_2/mol glucose as the influent undissociated butyric acid concentration was increased from 2.5 to 25 mM while H_2 yields decreased to 2.2 ± 0.1 mol H_2/mol glucose at an undissociated acetic acid concentration of 25 mM (Table 1). When both acetic (12.5 mM) and butyric acid (12.5 mM) were added to the influent feed, H_2 yields decreased to 1.9 ± 0.3 mol H_2/mol glucose (Table 1). Solvent production did not occur under these test conditions. There was little difference in the H_2 in the headspace for all of these test conditions, with H_2 averaging 63 ± 1% (Table 1). Effluent glucose concentrations averaged 530 ± 200 mg/L (>88% glucose removal). A carbon balance accounted for 95 ± 5% of the carbon (assuming a bacterial yield of 0.2 g biomass/g glucose, as noted above).

When sodium was added at a concentration of 162 mM, the H_2 yield decreased slightly by 8% to 2.3 ± 0.1 mol H_2/mol glucose (Table 1). The H_2 concentration in the headspace remained at 63%, while the residual glucose concentration increased to 640 ± 12 mg/L. Total acetic and butyric acid concentrations were 8 ± 2 and 12 ± 0.3 mM, respectively. The small effect on hydrogen production produced by the addition of sodium indicates that sodium alone would not account for the relatively larger effects of acetic and butyric acids on hydrogen production.

H_2 Yields as a Function of Added Acetic and Butyric Acids at pH 5.0. There was a significant decrease in H_2 production when acids were added to the feed at pH 5.5, yet the effect was much less, as measured by H_2 production and solvent production, than that observed by other researchers (*5*−*9*) at lower pHs. To increase the undissociated acid concentration and inhibit the cultures further while keeping the total acid concentration the same, the pH was decreased to 5.0 which increased the undissociated acetic and butyric acid concentrations from 25 to 50 mM or more. As a result, there was near complete H_2 production inhibition. The hydrogen production rate was low (<2 mL/min) and the undissociated acetic and butyric acid concentrations were 50 ± 2 and 63 ± 1 mM, respectively (Table 1). Residual glucose concentrations were >1200 mg/L and no solvents were detected as soluble products.

H_2 Production as a Function of Glucose Concentration. To extrapolate the effect of added acid on H_2 yields to

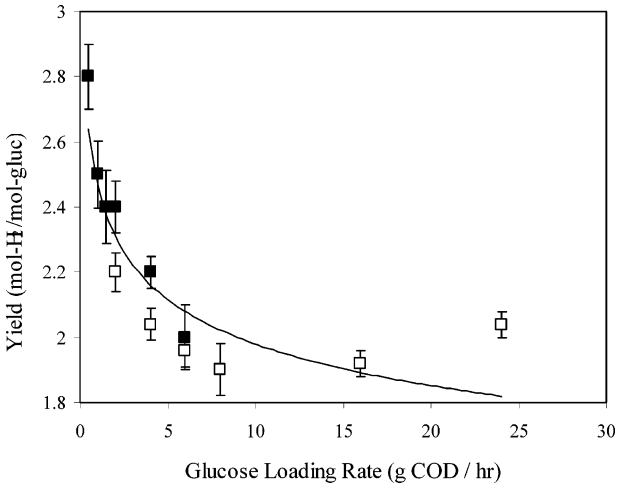

FIGURE 3. Hydrogen yields as a function of glucose concentration loading rate ($p = 0.047$ for the slope, $R^2 = 0.79$). For comparison, H_2 yields observed at the lower glucose concentrations of 2.5−7.5 g COD/L (■ 0.5−6.0 g COD/hr) are also shown. (Values ± SD for $n = 3$ samples over multiple HRTs).

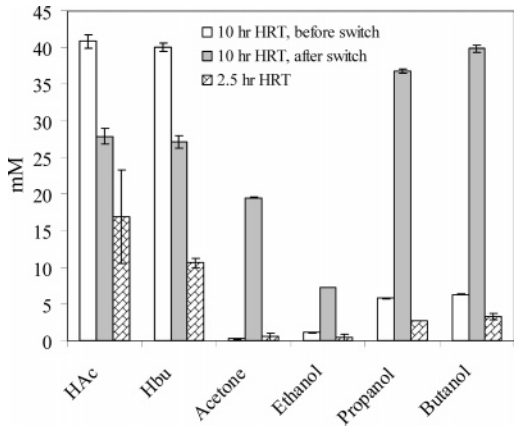

FIGURE 4. Switch from acidogenesis to solventogenesis is shown by the elevated concentrations of acetone and alcohols and a decrease in acetic (HAc) and butyric (HBu) acid concentrations (glucose feed concentration of 40 g/L). (Values ± SD for $n = 3$ samples over multiple HRTs).

conditions where these acids are produced as a result of glucose fermentation (self-produced), the glucose concentration was increased from 10 to 50 g/L at pH 5.5. At glucose concentrations from 10 to 30 g/L (2.5- and 10-h HRT), the H_2 yield remained fairly constant at 2.0 ± 0.2 mol H_2/mol glucose, but the yield decreased to 1.6 ± 0.1 when the glucose concentration was increased to 40 g/L (Table 1). H_2 yields observed in previous experiments (*3*) at lower glucose concentrations (2.35, 4.7, or 7.0 g/L) and at the same HRTs as the present study (2.5 or 10 h) are compared to the higher glucose concentrations examined in the present study (10, 20, 30, 40 g/L) that produced glucose loading rates of 0.5−24 g glucose/hr (Figure 3). H_2 yields decreased with an increase in glucose loading rate. At a glucose concentration of 40 g/L (10-h HRT), a "pseudo steady state" condition appeared to be achieved because after consistent hydrogen production for over 10 HRTs, there was a switch to solvent production as shown by a decrease in acetic and butyric acids, and an increase in acetone, ethanol, propanol, and butanol production (Figure 4). Solvents produced at glucose concentrations from 10 to 30 g/L accounted for less than 10% of the soluble products, while at 40 g glucose/L 62% of the soluble products were solvents (mass basis). Steady-state operation could not be achieved for reactor conditions of 40 g glucose/L (2.5- h HRT) or 50 g glucose/L (2.5- and 10-h HRT). Under these conditions of glucose concentrations (10−40 g/L), a carbon balance accounted for 95 ± 4% of the influent carbon.

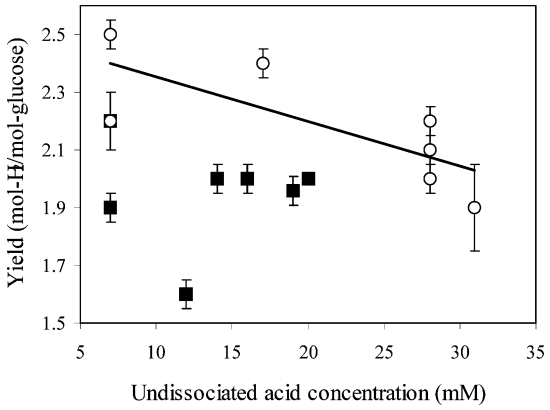

FIGURE 5. Comparison of the effect of externally added acids (○, data taken from Table 1, all tests with added acid at pH 5.5) and self-produced acids (■, data taken from Table 2) on H_2 yields. The regression line is based on results from the externally added acid tests (○, $p = 0.05$ for the slope, $R^2 = 0.58$). (Values ± SD for $n = 3$ samples over multiple HRTs).

By comparing the yields obtained at the concentrations of acids produced at the glucose concentrations of 10−40 g/L with yields obtained at 5 g COD/L when acids were added to the feed, differences between the effect of self-produced and externally added acid can be observed (Figure 5). Although the externally added acids were higher in concentration than the self-produced acids, H_2 yields were lower when the acids were self-produced ($p = 0.003$, *t*-test, paired two sample means).

Discussion

Butyric acid was observed to decrease H_2 production more than acetic acid (added separately at equimolar concentrations) although the addition of both acids simultaneously in the reactor feed at equimolar concentrations caused the greatest decrease in H_2 yields. There is a reduction in hydrogen yield due to the total undissociated acid concentration, but as shown in Figure 2 the undissociated butyric acid concentration accounted for most of this reduction. This finding on the effect of butyric acid for hydrogen production is in agreement with earlier solvent-based studies. For example, Ezeji et al. (*17*) showed that butyric acid was more toxic than acetic acid in ABE fermentation, while Husemann and Papoutsakis (*18*) found that the undissociated acetic acid concentration did not correlate with the initiation of solventogenesis but undissociated butyric acid did. There is no general agreement on why butyric acid is more toxic than acetic acid, but likely it is a consequence of NAD^+ regeneration. The production of acetate allows the regeneration of NAD^+ only through H_2 production, while the production of butyrate regenerates NAD^+ through both H_2 production and the reduction of butryl phosphate. If the butyrate production pathway becomes constricted by excess butyrate, and if H_2 production is unfavorable due to high concentrations of dissolved hydrogen, the only electron sink left is through solvent production which does not result in any H_2 production. In contrast, if the acetate production pathway is constricted by excess acetate or high concentrations of dissolved hydrogen, the bacteria can still regenerate NAD^+ through butyrate production. Therefore, butyric acid may be more inhibitory than acetic acid when the liquid is saturated with H_2 since availability of electron sinks is reduced.

H_2 production decreased by >93% (pH 5.0) when the acetic or butyric undissociated acid concentration was 60 mM.

TABLE 2. H_2 Yields and Aqueous Product Concentrations at Varying Glucose Concentrations and HRTs (pH 5.5)[a]

		Aqueous Products								
		glucose (g/L)		HAc (mM)		HBu (mM)		H_2		
HRT	redox (mV)	influent[b]	effluent	total	undiss.	total	undiss.	(%)[c]	L/hr	yield[d]
10	−410	10	0.8 ± 0.1	18 ± 1	3 ± 0.1	24 ± 1	4 ± 0.1	60	0.52	2.2 ± 0.2
2.5	−400	10	0.6 ± 0.0	21 ± 1	3 ± 0.1	23 ± 1	4 ± 0.1	60	1.42	1.9 ± 0.1
10	−295	20	0.8 ± 0.1	37 ± 0.4	6 ± 0.1	49 ± 12	10 ± 0.0	57	0.96	2.0 ± 0.1
2.5	*e*	20	0.7 ± 0.1	30 ± 2	5 ± 0.3	51 ± 2	9 ± 0.4	57	3.04	2.0 ± 0.1
10	−360	30	1.9 ± 0.0	42 ± 5	6 ± 0.7	74 ± 6	13 ± 1.0	56	1.37	2.0 ± 0.1
2.5	*e*	30	1.8 ± 0.3	43 ± 5	7 ± 0.7	78 ± 6	13 ± 1.0	57	5.5	2.0 ± 0.0
10	−220	40[f]	2.6 ± 0.0	40 ± 7	6 ± 1.1	35 ± 3	6 ± 0.6	46	1.5	1.6 ± 0.1

[a] Values ± SD for $n = 3$ samples over multiple HRTs. [b] g COD/L. [c] Percentage of hydrogen gas in headspace. [d] mol H_2/mol gluc. [e] Not recorded. [f] Results based on the pseudo-state condition observed before switch to solventogenesis.

Van den Heuvel et al. (*19*) determined critical inhibitory concentrations of undissociated butyric acid of 50 mM, and undissociated acid concentrations in this range are known to greatly increase the cell maintenance energy requirements and deplete the ATP reserves necessary for a metabolic switch to solventogenesis (*20*). H_2 yields were constant at influent glucose concentrations of 10−30 g/L. At higher influent glucose concentrations (40 g/L, HRT = 10 h) there was a switch to solventogenesis. This suggests an upper limit for H_2 production of an influent glucose concentrations of 30 g/L, or an undissociated butyric acid concentration of >13 mM (total undissociated concentration of >19 mM; Table 2). This apparent threshold value for the initiation of solventogenesis compares well to undissociated butyrate concentration of 13 mM reported by others (*5*, *8*). The "pseudo steady state" observed here for an influent glucose concentration of 40 g/L, based on a rise in the concentrations of alcohols and solvents, agrees with that reported by Clarke et al. (*21*).

These findings on the role of undissociated acids on hydrogen yields, while similar to those reported in other studies for their effect on initiating solventogenesis, do not eliminate a possible role for dissociated acids on hydrogen yields at high glucose concentrations (40 g/L). A high concentration of dissociated acids will increase the ionic strength, which can result in cell lysis (*22*). In a batch study by Zheng and Yu (*23*), it was found that a total butyric acid concentration of 25 g/L (pH = 6.0) decreased H_2 yields to 0.32 mol H_2/mol glucose. This total butyric acid concentration is nearly twice as high as the concentration used in the present continuous flow study (13.9 g/L) where 2.0 mol H_2/mol glucose was the observed yield. If the dissociated form of the acid was the main cause of inhibition in the study by Zheng and Yu (*23*), the dissociated forms of acetic and butyric acids could also have been responsible for the reduction in H_2 yields observed at the influent glucose concentration of 40 g/L.

Acknowledgments

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