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Influence of Hydrogen Sulfide on the Growth and Metabolism of *Butyribacterium methylotrophicum* and *Clostridium acetobutylicum*

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ABSTRACT

Studies were conducted to determine the effect of hydrogen sulfide (H_2S) on the growth and metabolism of *Butyribacterium methylotrophicum* and *Clostridium acetobutylicum* during batch culture. Hydrogen sulfide concentrations in the range of those present in typical synthesis gas mixtures were examined. Growth rates and product compositions were unaffected by total liquid-phase sulfide concentrations ranging from 0.0 to 2.0 mmol/L for *B. methylotrophicum* and 0.0 to 0.9 mmol/L for *C. acetobutylicum*. An analysis of the effect of H_2S equilibrium partitioning, media pH, and gas-liquid equilibration rates on this system is presented.

Index Entries: Hydrogen sulfide; *Butyribacterium methylotrophicum; Clostridium acetobutylicum;* tolerance; synthesis gas.

INTRODUCTION

Biological conversion of synthesis gas can potentially serve as an alternative to existing chemical processing routes for production of liquid fuels and chemicals. A two-stage, continuous fermentation process has been proposed previously (*1*), in which the CO strain of *B. methylotrophicum* converts the CO present in the synthesis gas to acetic and butyric

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acids in the first stage. These acids, along with the original H_2 present in the synthesis gas, are then used as feedstocks for a second, solventogenic fermentation stage, where the acids are converted to a mixture of butanol, ethanol, and acetone by *C. acetobutylicum*. This approach is radically different from the traditional fermentation of carbohydrates to acetone, butanol, and ethanol with *C. acetobutylicum*, because the primary carbon source for both fermentation stages is the CO present in the synthesis gas, and not a complex carbohydrate or fermentable sugar feedstock.

The use of coal as a resource for synthesis gas production has several advantages, such as its high volatile matter content and relative domestic abundance (2). However, the major limitation with most US coals is an inherently high sulfur content, which upon gasification produces potent catalyst poisons, such as H_2S and carbonyl sulfide (COS) gases. These sulfide species require energy-intensive separation procedures that can add significantly to the production costs of catalytic processes based on coal-derived synthesis gas (3). An attractive advantage of biological synthesis gas conversion is that intensive sulfur gas removal and synthesis gas upgrading may not be required. With microorganisms, such as *B. methylotrophicum* and *C. acetobutylicum*, the 0.5–2 vol% sulfur gases will pass through the process as inert components. Because the CO and H_2 will be removed from the CO gas stream, a reduced volume of gas having a higher H_2S concentration will exit the process. Sulfur removal costs will thus be reduced, because the mass-transfer driving force will be greater, whereas the volume to be processed will be less. Furthermore, *B. methylotrophicum* does not require a strict CO/H_2 ratio, and thus, gas shift operations would potentially be unnecessary.

In order to quantify the sulfur tolerance of *B. methylotrophicum* and *C. acetobutylicum*, the growth and product-formation properties of both organisms in the presence of sulfide were measured. These results are necessary to evaluate the potential economic benefits of the synthesis gas bioconversion process.

METHODS

Microorganisms and Culture Conditions

Stock cultures of the CO strain of *B. methylotrophicum* were maintained in our laboratory in 152-mL sealed, anaerobic serum bottles. These bottles contained approx 50 mL of a phosphate-buffered medium and were initially charged with a 100% CO gas head space at 2 atm total pressure, set by gas regulator. This medium, described in detail elsewhere (4), contains inorganic salts, vitamins, trace minerals, a phosphate buffer, and 0.1% yeast extract. Cells were transferred to fresh bottles every 2–3 wk. The cultures were grown at 37°C in the dark on a shaking platform rotating at 100 rpm, and mid-log phase samples were used as inocula for all experiments.

A mutant strain of *C. acetobutylicum*, previously developed in our laboratory, was used during this study. This strain was maintained on a maltodextrin/corn steep liquor/corn gluten medium in a two-stage chemostat culture using two Multigen fermenters (New Brunswick Scientific Co., Edison, NJ) in series. Working volumes were 390 mL and 700 mL for stage one and stage two, respectively. Dilution rates for stage one and stage two were 0.22 h^{-1} and 0.12 h^{-1}, respectively. Culture pH was controlled only in stage one at a value of 5.2, by addition of 3*N* NaOH. The temperature of both stages was maintained at 35°C.

Experimental Conditions

For experiments using *B. methylotrophicum*, varying sulfide concentrations were generated by directly adding H_2S gas or by addition of a stock Na_2S solution to 152 mL anaerobic bottles containing the phosphate-buffered media with a CO gas head space. Initial equilibrium concentrations of the total liquid sulfide species were calculated for each experiment. Control experiments were always conducted with the standard total sulfide concentration of 0.083 g/L (based on the preequilibration concentration). All bottle experiments were conducted in duplicate. Experiments were initiated by the addition of a 2% v/v mid-log phase *B. methylotrophicum* stock culture. Samples for cell density, pH, CO gas, CO_2 gas, and all liquid-phase products were taken on a daily basis.

For *C. acetobutylicum*, the studies were conducted in either 58-mL sealed, anaerobic serum bottles or in 28-mL sealed, anaerobic culture tubes under nitrogen (N_2) head spaces. H_2S gas or a stock Na_2S solution was added to the bottles or tubes by syringe in order to achieve the desired concentrations. For determinations of growth-phase tolerance, 10 mL of a synthetic medium (*5*) was used per culture tube, and the inoculum source was 0.2 mL (2% v/v) of cells from the stage-one fermenter described above. This synthetic medium consists mostly of salts, phosphate buffers, asparagine, and 0.3% yeast extract (*5*). For determination of stationary-phase tolerance, 10 mL of stage-two effluent described above were transferred to the 58-mL vials, and the corresponding amounts of sulfide species were then added.

Analytical Methods

Both gas and liquid samples were removed from the culture bottles using sterile, N_2-flushed syringes. Cell growth was measured by determining OD at 660 nm on a Sequoia-Turner Model 340 spectrophotometer (Sequoia-Turner Corporation, Mountain View, CA). Cell samples were observed daily for viability, morphology, and contamination. Samples with an OD>0.6 were diluted 10-fold with distilled water. Cell mass determinations for *B. methylotrophicum* were made using a previously derived dry wt vs OD calibration curve (*6*). Molar values for *B. methylotrophicum*, in units of carbon-moles cells, were determined using an empirical

mol wt derived previously (*6*). Concentrations of soluble fermentation products were determined on a Hewlett-Packard 5890A Gas Chromatograph in tandem with a 3392A auto-sampler and 3392A integrator (Hewlett-Packard Co., Avon, PA), equipped with a 1.2-m Chromosorb 101 80/100 mesh column and a flame-ionization detector. The N_2 carrier gas flow rate was 30 mL/min. Operating temperatures were 190, 220 and 250°C for the column, injector, and detector, respectively. CO, CO_2, and H_2S gases were analyzed by gas chromatography in a GowMac Series 580 Gas Chromatograph (GowMac Instrument Co., Bridgewater, NJ) using a 1.8-m Carbosphere 80/100 mesh column and a thermal conductivity detector. Column, injector, and detector temperatures were 100, 115, and 150°C, respectively. A helium carrier gas was used with a flow rate of 50 mL/min.

RESULTS

Equilibrium Partitioning and Dissociation Chemistry of Sulfide

The liquid-phase concentration of H_2S in equilibrium with a given H_2S partial pressure (pH_2S) can be estimated accurately for pressures < 2 atm using Henry's law (*7*):

$$[H_2S] = (K_H)(pH_2S) \quad (1)$$

Values for the Henry's law constant (K_H) in water published in the International Critical Tables and Perry's Chemical Engineer's Handbook have been found to be incorrect by several orders of magnitude (*7*). However, correct values were estimated using a published correlation (*7*) and were used for calculations made during this study.

In aqueous solution, H_2S acts as a diprotic acid, as shown below:

$$\begin{array}{ll} H_2S \; <=\!=\!=> \; H^+ \; HS^- & pK_{a1} = 7.04 \\ HS^- \; <=\!=\!=> \; H^+S^{2-} & pK_{a2} = 12.0 \end{array} \quad (2)$$

The ratios of the various sulfide species may be calculated using the above p*K*a values (*8*) and the Henderson-Hasselbach equation:

$$pH = pK_{a1} + \log[(HS^-)/(H_2S)] \qquad pH = pK_{a2} + \log[(S^{2-})/(HS^-)] \quad (3)$$

Because both *B. methylotrophicum* and *C. acetobutylicum* operate in a pH range of 5.2–7.0, the concentration of S^{2-} will be negligibly small. Based on these relationships, the following equation was developed to give *C*, the total equilibrium sulfide concentration (including all species) in the liquid phase following the initial loading of *n* moles of sulfide:

$$C = \{ n[1 + 10^{(pH - pK_a)}] / V_G/K_HRT + V_L[1 + 10^{(pH - pK_a)}] \} \quad (4)$$

Table 1
Experimental pH Profiles Showing Dynamics of H_2S Dissolution

Time, min	pH of Sample		
	2% H_2S	5% H_2S	10% H_2S
0	6.43	6.46	6.51
2	5.52	5.42	5.24
15	5.50	5.41	5.20
30	5.56	5.43	5.19
45	5.65	5.43	5.26
60	5.71	5.54	5.42
90	5.76	5.55	5.44
120	5.80	ND[a]	5.44

[a]ND denotes "not determined."

where V_G and V_L are the volumes of the gas and liquid phases, respectively. The absolute temperature is represented as T, and the gas constant as R. This equation is valid regardless of the form of sulfide added (e.g., H_2S or Na_2S). At the low pressures involved in these experiments, the number of moles of H_2S added can be estimated using the ideal gas law with the appropriate volume of gas added. The liquid-phase concentrations of individual species may also be calculated, as shown below:

$$\begin{aligned} [H_2S] &= C/[1 + 10^{(pH - pK_a)}] \\ HS^- &= C - (H_2S) \\ pH_2S &= (H_2S)/K_H \end{aligned} \quad (5)$$

The equations used to calculate sulfide concentrations in the gas and liquid phases are based on the assumption of phase equilibrium. To determine whether this assumption was reasonable during the sulfur-tolerance studies, a batch experiment was conducted to estimate the time required for equilibrium to occur after the addition of H_2S to gas head spaces in bottles containing distilled water. This experiment was performed in pressure bottles, which were inverted several times after addition of H_2S gas. Since the H_2S partitions into the liquid phase and dissociates, the pH of the distilled water would be expected to decrease. Equilibrium was assumed to have occurred when the pH stopped decreasing. The results for this experiment are shown in Table 1, for duplicate bottles initially containing 2, 5, and 10% H_2S gas in the head space (by vol). For all of the concentrations tested, the pH ceased decreasing after 15 min, with over 90% of the pH change occurring during the first 2 min. This is a rapid change relative to the 2-h duration of the growth experiments with the sealed-bottle systems. Therefore, the assumption of phase equilibrium was deemed valid for the sulfur-tolerance experiments described below.

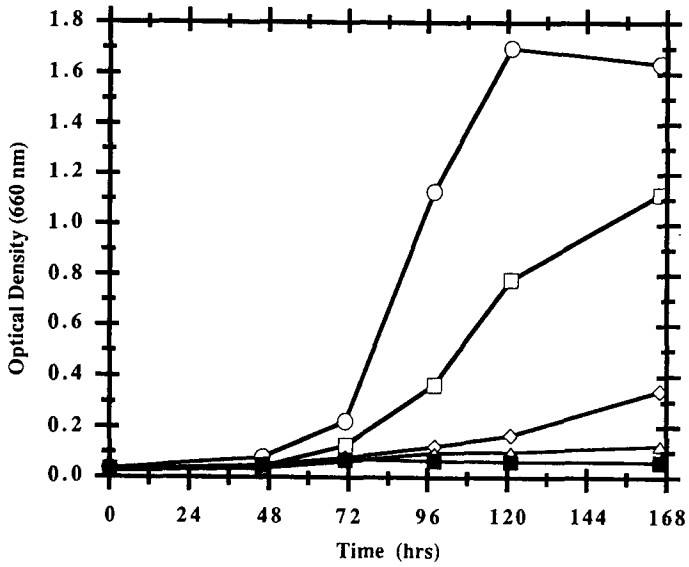

Fig. 1. Growth of *Butyribacterium methylotrophicum* CO strain on carbon monoxide in the presence of H_2S gas. Legend symbols: (○) control; (□) 0.9% H_2S; (◇) 1.2% H_2S; (△) 1.5% H_2S; (■) 2.6% H_2S.

Sulfur Tolerance of *B. methylotrophicum*

The sulfur tolerance of *B. methylotrophicum* grown on CO gas was investigated from the standpoint of total liquid-phase sulfide concentration. This organism requires a medium-reducing agent to maintain anaerobic conditions, and in the phosphate-buffered medium, Na_2S is typically used during batch culture (*6*). The standard amount of sulfide initially added to the liquid is 0.083 g/L, which corresponds to an equilibrium liquid-phase sulfide concentration of 1.7 mmol/L. This value is an amount equivalent to contacting a 102-mL 3.2% H_2S gas phase with a nonreduced liquid medium vol of 50 mL, which are the standard experimental conditions. The equilibrium H_2S gas-phase concentration arising from the control addition would be 1.13%. Thus, this organism is inherently tolerant to sulfide species.

Using an initial liquid-phase sulfide concentration of 1.7 mmol/L as a control value, three distinct sulfide-tolerance experiments were conducted: the first using the control amount of Na_2S plus addition of increasing amounts of H_2S gas, the second using increased amounts of Na_2S relative to the control, and the third using decreased amounts of Na_2S relative to the control. These experiments were all conducted at an initial pH of 7.0. Figures 1 and 2 show the growth and acetate production time-course profiles for batch growth of *B. methylotrophicum* during the first experiment. The values of percent H_2S gas indicate initial equilibrium gas-phase concentrations. The excess sulfide severely inhibited both cell growth and acetate production in the 3.2, 5.2, and 8.0% runs. Molar and specific yield values for acetate production during this experiment are shown in Fig. 3.

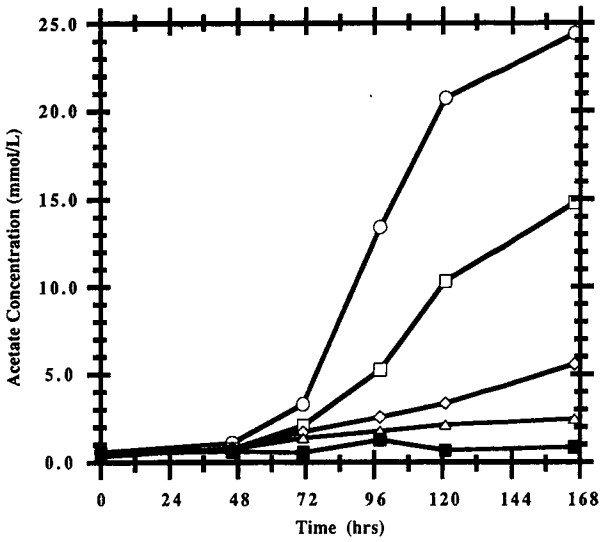

Fig. 2. Acetate production by *Butyribacterium methylotrophicum* CO strain grown on carbon monoxide in the presence of H_2S gas. Legend symbols: (○) control; (□) 0.9% H_2S; (◇) 1.2% H_2S; (△) 1.5% H_2S; (■) 2.6% H_2S.

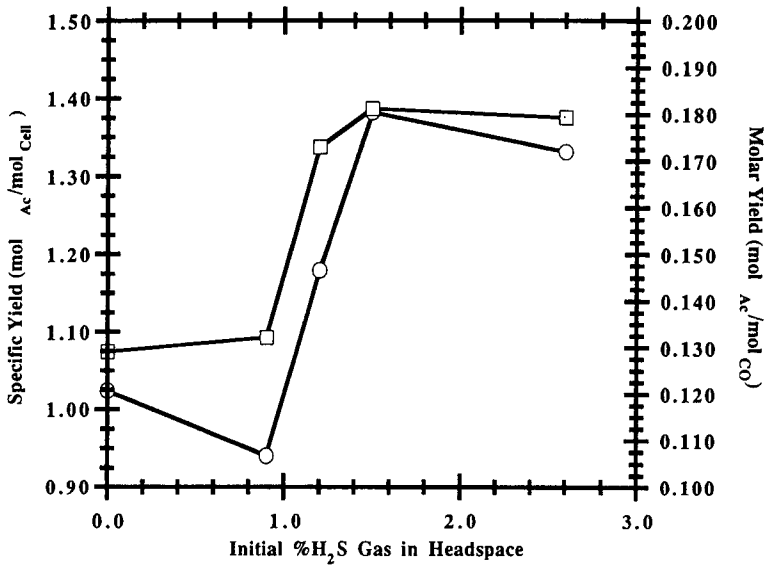

Fig. 3. Acetate yields of *Butyribacterium methylotrophicum* CO strain grown on carbon monoxide in the presence of H_2S gas. Legend symbols: (○) specific yield; (□) molar yield.

Since the initial equilibrium sulfide concentrations increased and the resulting cell growth rates decreased, the specific and molar yields for acetate increased by approx 50%.

For the second experiments, the time-course profiles for cell density are shown in Figs. 4 and 5. The initial liquid-sulfide concentrations shown in these figures are preequilibrium values. At concentrations below the

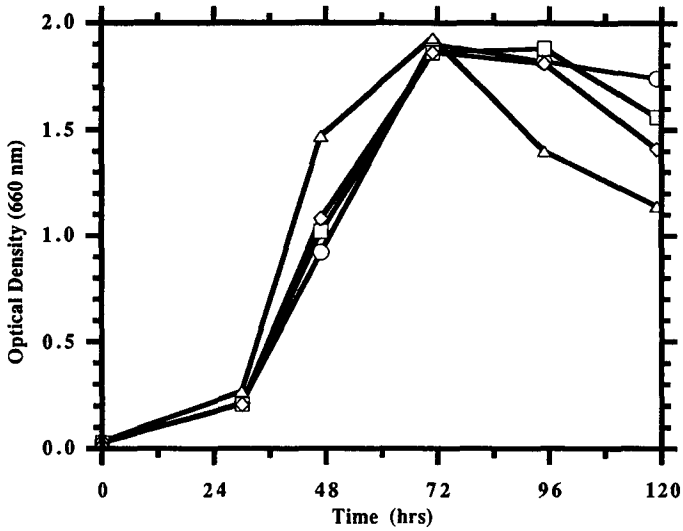

Fig. 4. Growth of *Butyribacterium methylotrophicum* CO strain on carbon monoxide as a function of initial liquid-sulfide concentration. Legend symbols: (○) 0.083 g/L; (□) 0.063 g/L; (◇) 0.043 g/L; (△) 0.023 g/L.

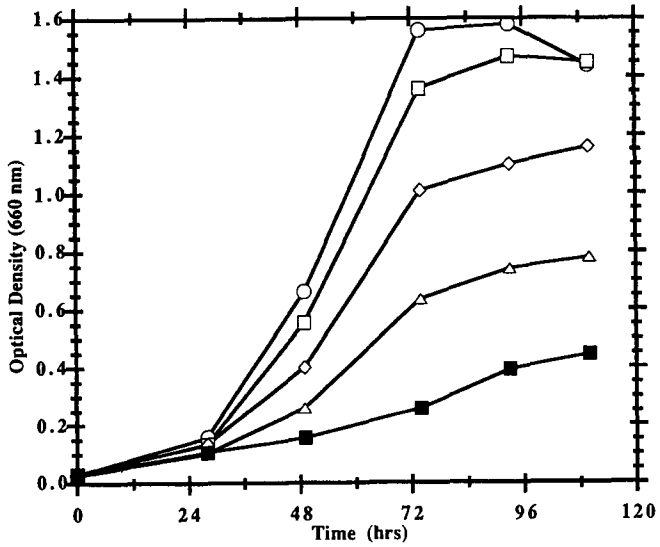

Fig. 5. Growth of *Butyribacterium methylotrophicum* CO strain on carbon monoxide as a function of initial liquid-sulfide concentration. Legend symbols: (○) 0.083 g/L; (□) 0.100 g/L; (◇) 0.147 g/L; (△) 0.200 g/L; (■) 0.254 g/L.

control (0.083 g/L) value, shown in Fig. 4, there was no significant effect on cell growth, except that the lowest concentration tested did show a slight increase in the growth rate. At concentrations above the control, shown in Fig. 5, the growth rate and corresponding cell densities decreased with increasing sulfide concentration. The data for acetate, and minor butyrate, formation during these experiments paralleled the trends for cell density and cell growth, and are therefore not shown. However, the specific and molar yields of acetate and butyrate were significantly in-

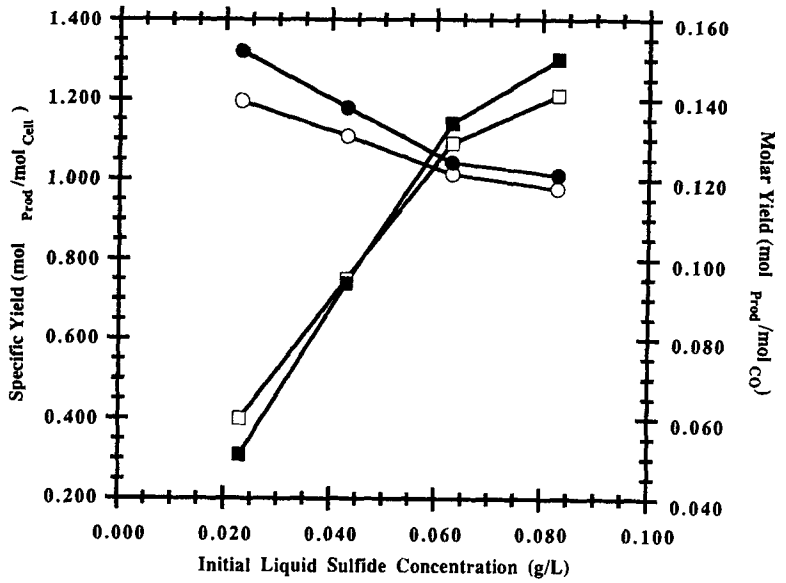

Fig. 6. Acid yields of *Butyribacterium methylotrophicum* CO strain on carbon monoxide as a function of initial liquid-sulfide concentration. Legend symbols: (○) specific yield acetate; (□) specific yield butyrate (×10); (●) molar yield acetate; (■) molar yield butyrate (×10).

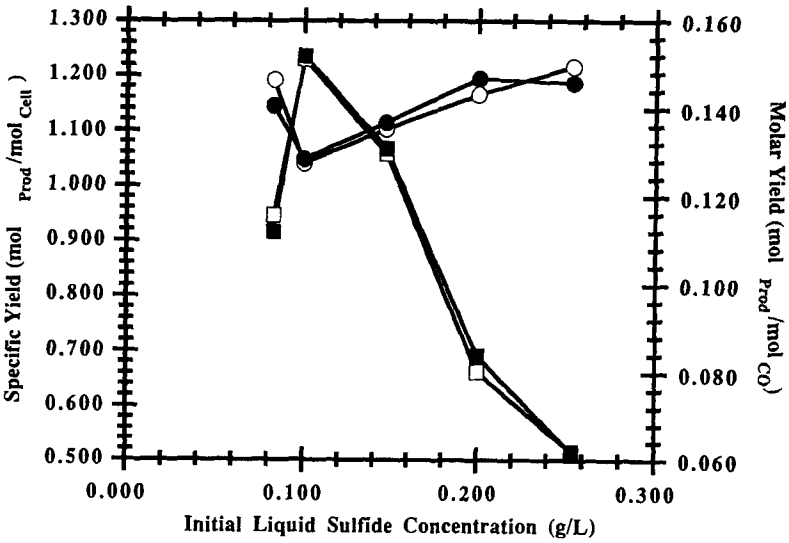

Fig. 7. Acid yields of *Butyribacterium methylotrophicum* CO strain on carbon monoxide as a function of initial liquid-sulfide concentration. Legend symbols: (○) specific yield acetate; (□) specific yield butyrate (×10); (●) molar yield acetate; (■) molar yield butyrate (×10).

fluenced by the concentration of sulfide, and are shown in Figs. 6 and 7. The two experiments are shown in separate figures because of the separate control runs for each experiment. As the sulfide concentration increased, the acetate yields initially decreased, reached a minimum, and then increased. The butyrate yields exhibited the opposite trend.

Table 2
Effect of Liquid-Phase Sulfide Concentration on Cell Growth of *B. methylotrophicum* Grown on CO Gas at an Initial pH 0f 7.0

Total sulfide species in liquid phase, mmol/L	Equilibrium gas-phase H_2S concentration, mol %	% Maximum OD obtained, % control
.479	0.32%	~102%
.890	0.59%	~100%
1.301	0.86%	~100%
1.711	1.13%	100%
2.053	1.36%	~ 93%
3.012	1.99%	~ 72%
4.107	2.71%	~ 48%
5.203	3.32%	~ 27%

Table 3
Influence of Liquid-Phase Sulfide Species from H_2S on the Performance of *C. acetobutylicum*

	Total liquid-phase sulfide species, mmol/L										
	0.0 h			0.24 h		0.49 h		0.73 h		0.97 h	
Parameters	0	24	48	24	48	24	48	24	48	24	48
Butanol (g/L)	6.6	18.2	18.8	18.3	18.9	18.3	18.8	17.6	18.1	17.7	18.0
Total solvents (g/L)	10.1	26.7	27.7	26.8	27.9	26.7	27.7	25.7	26.4	25.8	26.2
Butyrate (g/L)	2.8	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.2
Substrate consumption (g/L)	46.6	79.6	79.8	79.4	79.7	79.7	79.9	77.7	78.3	77.1	77.4

A summary of the results that used Na_2S as the sulfide source is shown in Table 2, which relates the total initial, equilibrium, liquid-phase sulfide concentration to the relative cell growth and corresponding gas-phase H_2S concentrations. Initial equilibrium gas-phase concentrations approaching 1.4% did not significantly inhibit cell growth. Reduced rates of growth were observed between 1.4 and 3.3%, and concentrations above 3.3% were toxic to *B. methylotrophicum*.

Sulfur Tolerance of *C. acetobutylicum*

The tolerance of *C. acetobutylicum* to H_2S gas was first investigated with solventogenic-phase cells. These cells were batch incubated in vials to which H_2S gas was added. The results shown in Table 3, based on the initial equilibrium liquid-phase sulfide species concentration, indicate no significant inhibition of solvent production at the concentrations examined, of which the highest value was 0.9 mmol/L. Butanol concentrations in the

Table 4
Influence of Liquid-Phase Sulfide Species from H_2S on the Growth[a] of *C. acetobutylicum*

	Culture optical density				
	Total liquid-phase sulfide species, mmol/L				
Time, h	0.0	0.24	0.49	0.73	0.97
0	0.95	0.92	0.90	0.92	0.95
4	1.20	1.15	1.25	1.25	1.10
6	1.85	1.75	1.90	1.85	1.65
8	2.65	2.60	2.75	2.65	2.40
10	3.50	3.45	3.55	3.45	3.30

[a]Stage-one inoculum used.

Table 5
Influence of Liquid-Phase Sulfide Species from Na_2S on the Performance of *C. acetobutylicum*

	Total liquid-phase sulfide species, mmol/L										
	0.0 h			0.3 h		0.6 h		0.9 h		1.2 h	
Parameters	0	24	48	24	48	24	48	24	48	24	48
Butanol (g/L)	8.0	18.2	18.8	18.5	18.9	18.1	18.7	17.5	17.9	14.6	14.8
Total solvents (g/L)	11.9	27.0	27.6	26.9	27.5	26.4	27.0	25.3	26.1	20.9	22.1
Butyrate (g/L)	2.7	0.3	0.4	0.4	0.4	0.5	0.5	0.7	0.8	1.2	1.3
Substrate consumption (g/L)	50.2	79.3	79.9	78.7	79.4	78.0	78.3	76.8	77.3	67.9	68.3

range of 18 g/L were achieved irrespective of the added H_2S gas. The batch fermentations were completed in all cases in approx 24 h, and only slight residual butyrate was detected. To investigate the tolerance of *C. acetobutylicum* to H_2S gas during the growth phase, cells were inoculated in culture tubes and grown on synthetic media in the presence of H_2S gas. The results from this experiment are shown in Table 4. The cell densities were not significantly affected by the presence of H_2S gas up to a concentration of 0.97 mmol/L, where some slight growth inhibition was observed.

In order to further investigate the tolerance of *C. acetobutylicum* to sulfide species, increased sulfide concentrations were generated using Na_2S as the sulfur source, and the same experiments were repeated. The results using Na_2S are shown in Table 5 for the solventogenic-phase cells and in Table 6 for the growing cells. Inhibition of solvent production (Table 5) was observed at initial liquid-sulfide concentrations of 0.9 mmol/L and above, with corresponding reduction in butyrate consumption and substrate consumption. Cell growth (Table 6) was also inhibited at the same concentration levels, with reductions in the cell density observed at concentrations of 0.9 mmol/L and above.

Table 6
Influence of Liquid-Phase Sulfide Species from Na_2S on the Growth[a] of *C. acetobutylicum*

	Culture optical density					
	Total liquid-phase sulfide species, mmol/L					
Time, h	0.0	0.3	0.6	0.9	1.2	1.5
0	1.10	1.05	1.05	1.00	1.05	1.05
4	2.35	2.35	2.30	2.20	1.65	1.45
6	3.25	3.30	3.30	2.80	2.45	2.00
8	4.10	4.15	4.05	3.30	2.95	2.50
10	4.85	4.90	4.75	3.90	3.50	2.90

[a]Stage-one inoculum used.

DISCUSSION

Experimental results for sulfide tolerance for both organisms have been obtained with both H_2S gas and Na_2S serving as the sulfur sources. These experiments have determined a practical concentration limit for liquid-phase sulfide species during cell growth and metabolism.

The growth results for *B. methylotrophicum* were based upon the initial liquid concentration of sulfide species that is typically used as a reducing agent in the phosphate-buffered media (1.7 mmol/L). The experiment with excess addition of H_2S gas to the control media showed severe inhibition of cell growth in the presence of $> 2\%$ H_2S gas. The specific mechanism of this inhibition is not clear, but an effect on the molar and specific yields is apparent from the data presented in Fig. 3, where high sulfide concentrations appear to favor acetate formation. This result is most likely a cellular response to the toxic effects of high sulfide concentrations, a response that may require more metabolic energy and, thus, would favor acetate production at the expense of cell mass production.

The data obtained using Na_2S as the sulfur source show that the initial, preequilibrium control concentration of 0.083 g/L total sulfide is on the border between tolerance and inhibition, with lower concentrations having no or little effect on cell growth (Fig. 4) and with higher concentrations leading to inhibition of cell growth (Fig. 5). The specific and molar yields of acetate and butyrate, plotted in Figs. 6 and 7, are affected by sulfide concentration in a complicated manner. At low sulfide concentrations, the acetate yields are higher, decrease to a minimum at an intermediate concentration, and then increase again as the sulfide concentration is increased further. The butyrate specific and molar yields exhibit the opposite trend. Currently, these trends cannot be adequately explained.

Data from batch incubations of *C. acetobutylicum* in the presence of H_2S gas indicate no significant inhibition of either growth or butanol production up to a concentration of approx 0.9 mmol/L sulfide (Tables 3 and 4). This threshold concentration is somewhat less than that observed for *B. methylotrophicum* and corresponds to an equilibrium H_2S gas concentration of approx 0.6% assuming an initial pH of 7.0. This value is an intermediate range of a typical synthesis gas mixture of 0.5–2% sulfur gases. However, the standard operating pH of this organism is between 5.0–6.0. Using an initial pH of 6.0 and 5.0, the equilibrium H_2S gas concentrations would be 1.1 and 1.2%, respectively. The lower pH equilibrium values are thus conducive to the organism from the standpoint of sulfide tolerance. Using Na_2S, the experimental range was expanded up to liquid-phase sulfide concentrations of 1.5 mmol/L. These data, shown in Tables 5 and 6, confirm that at concentrations above 0.9 mmol/L inhibition occurs during both growth and product formation in *C. acetobutylicum.* Moreover, the overall substrate consumption was significantly reduced at a concentration of 1.2 mmol/L sulfide. The corresponding gas-phase mole fractions (mol%) for a 0.9 mmol/L liquid-phase sulfide concentration are 0.6, 1.1, and 1.2% H_2S gas at pH values of 7.0, 6.0, and 5.0, respectively. Thus, at the standard operating pH, *C. acetobutylicum* is tolerant to sulfides at intermediate concentration levels compared to what is typically observed initially in a synthesis gas mixture.

These data thus establish a practical limit for growth of *B. methylotrophicum* and *C. acetobutylicum* in the presence of sulfide species. It has been previously shown that Na_2S addition to *C. acetobutylicum* cultures results in a metabolic shift, resulting in enhanced butyrate production (*9*). The addition of Na_2S to *Thermoanaerobacter ethanolicus* cultures has also been shown to inhibit cell growth at high concentrations (*10*). These findings are consistent with our findings for *B. methylotrophicum* and *C. acetobutylicum.* For *B. methylotrophicum,* liquid-phase sulfide concentrations above 3.0 mmol/L significantly reduce cell growth and product formation. For *C. acetobutylicum,* the tolerance concentration is approximately half of that for *B. methylotrophicum* at 1.0–1.2 mmol/L. Since the equilibrium partial pressures of H_2S gas above these liquid concentrations are in the range of 1–2%, with proper process design, the influence of H_2S on the growth and metabolism of *B. methylotrophicum* and *C. acetobutylicum* should not create any problems during coal-derived synthesis gas conversion.

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