Bioreactor Development for Biological Hydrogen Production

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Abstract

The water-gas shift reaction, in which carbon monoxide is oxidized to carbon dioxide while simultaneously water is reduced to hydrogen, is used to produce hydrogen from synthesis gas. A unique photosynthetic bacterium, *Rubrivivax gelatinosus* CBS, can perform this reaction at ambient temperatures. A biologically-based water-gas shift process may provide an attractive alternative to conventional thermal technology. Experiments were performed to measure the specific carbon monoxide uptake and hydrogen production rates of the bacterium, and to investigate the effect of carbon monoxide concentration and reactor pressure on the volumetric productivity of trickle bed bioreactors. The apparent rate constant (a measure of reactor productivity) was unaffected by inlet carbon monoxide concentrations over the range 8%-99%. Increasing the reactor pressure from ambient to approximately 80 psia increased the reactor productivity. No increase in productivity above 80 psia was seen.

Introduction & Background

The biologically-mediated water-gas shift reaction, in which carbon monoxide is oxidized to carbon dioxide while simultaneously water is reduced to hydrogen, may be a cost-effective technology for the production of hydrogen from synthesis gas. NREL researchers have isolated a number of photosynthetic bacteria that can perform this reaction (Weaver 1980). The overall stoichiometry of this reversible reaction is:

$$CO + H_2O \xleftrightarrow{K_{EQ}} CO_2 + H_2 \qquad (1)$$

Few organisms that can perform reaction 1 have been reported (Uffen et al 1976, Bott et al. 1986, Jung 1999a, Jung 1999b). One significant advantage to using bacteria to perform the water-gas shift reaction is their ability to operate at ambient temperature. Because the reaction occurs at ambient temperature, the reaction is not equilibrium-limited (at 25°C, K_{EQ}~5x10^4). The advantages of low operating temperature and lack of equilibrium limitation make the biological shift reaction a promising alternative to conventional shift technologies. However, much work needs to be done to develop this promising technology. Our research focuses on using well-defined bioreactor geometries to collect quantitative reactor performance data that can be used for the rational scale-up of these novel bioreactors.

This work describes experiments to determine the specific shift activity of the microorganism and the effect of carbon monoxide concentration and pressure on this shift activity. The specific shift activity of the microorganism serves as an absolute upper limit on reactor productivity: the specific activity of the microorganism multiplied by the microbial mass in a bioreactor is the maximum possible volumetric productivity of that reactor. The effects of pressure and inlet carbon monoxide on reactor productivity are important to understand because these two process parameters are likely to vary widely in different commercial applications. We performed batch experiments to measure the specific shift activity of the microorganism, and continuous flow experiments to investigate CO concentration and pressure effects.

Experimental

The microorganism used in this work was *Rubrivivax gelatinosus* CBS, isolated from the natural environment by NREL researchers (Maness and Weaver 1994). The minimal culture media (M-1 basal) had the following composition (amounts are for 1 L of final media preparation): basal salts (120 mg $MgSO_4 \cdot 7H_2O$, 75 mg $CaCl_2 \cdot 2H_2O$, 11.8 mg $FeSO_4 \cdot 7H_2O$, 20 mg EDTA); trace elements (2.8 mg H_3BO_3, 1.6 mg $MnSO_4 \cdot H_2O$, 0.75 mg $Na_2MoO_4 \cdot 2H_2O$, 0.24 mg $ZnSO_4 \cdot 7H_2O$, 0.04 mg $Cu(NO_3)_2 \cdot 3H_2O$, 0.8 mg $CoCl_2 \cdot 6H_2O$, 0.8 mg $NiCl_2 \cdot 6H_2O$); phosphates (1.2 g KH_2PO_4, 1.8 g K_2HPO_4); vitamins (1.0 mg thiamine HCl, 15 ug biotin, 1.0 mg nicotinic acid, 10 ug B-12, 0.1 mg p-aminobenzoic acid); and ammonia (1.5 g NH_4Cl). In addition, malate (5 g/L) and yeast extract (0.5 g/L) were added as carbon sources. The media was prepared using deionized water and stock solutions of basal salts, trace elements, vitamins, buffers, ammonia, and D,L-malic acid. Yeast extract was added as a powder immediately prior to sterilization. The stock solutions were in turn prepared using stock chemicals from various manufacturers, which were used as received.

Bioreactor Experiments

The reactor design used in the continuous reactor experiments was a trickle bed bioreactor (TBR), shown schematically in Figure 1. This reactor has been described previously in detail (Wolfrum 2002). In a TBR, the gas moves through the reactor in single-pass mode, while the liquid is continuously recirculated using a peristaltic pump. A reactor support material (typically called a packing) is placed in the reactor to enhance mass transfer between the gas and the liquid phase, and often to provide a porous surface on which the microorganisms can grow. For the experiments described here, nonporous glass beads (6mm diameter) were used as the reactor support, and the TBRs were operated in counter-current flow: the gas moved upward through the reactor, while the liquid moved downward.

The ambient-pressure experiments investigating the effect of variable carbon monoxide concentration were performed using a TBR with an empty bed volume of approximately 1 L. The variable pressure experiments were performed using a TBR with an empty bed volume of approximately 250 mL. A single-stage pressure regulator on the gas outlet stream controlled the operating pressure of the pressurized bioreactor, and a high-pressure peristaltic pump recirculated the media through the bioreactor. Other than these two modifications, the pressurized bioreactor operated identically to the ambient pressure bioreactor.

Pure cultures of *Rx. gelatinosus CBS* were grown and periodically subcultured under sterile conditions using 20-mL screw-top test tubes and 200-mL serum flasks. These vessels were kept under incandescent illumination until used to inoculate the TBR assemblies. The microorganisms were not exposed to carbon monoxide during the growth/subculture process.

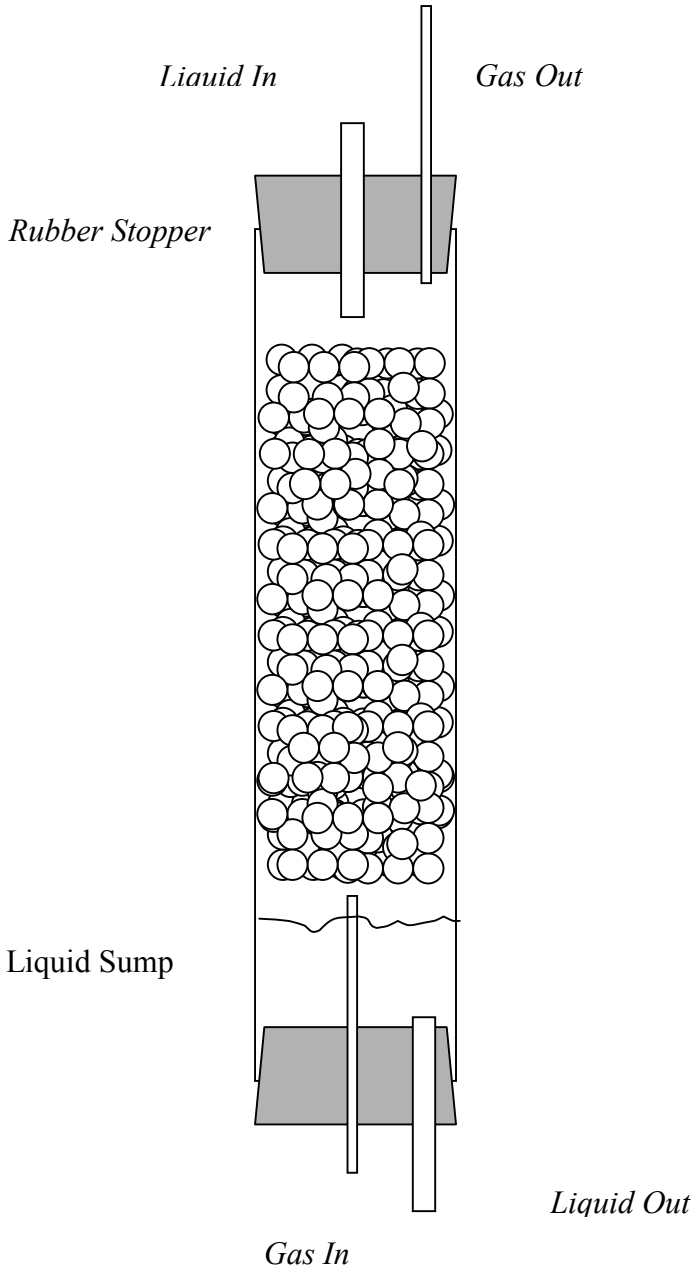

Figure 1. Schematic Diagram of countercurrent Trickle Bed Bioreactors (TBRs) used in the present work.

The reactor, including the external liquid recirculation loop, was assembled, autoclave sterilized, and allowed to cool. The assembly was then installed in a canopy hood, and the gas inlet fitting connected to the source gas (20% CO, 5% CH_4 as tracer gas, balance N_2). The reactor was then rinsed with sterile M-1 media while gas flow was initiated. After several reactor volumes of gas were allowed to flow through the reactor, the reactor was drained of any remaining media and inoculated with one serum flask of *Rx. gelatinosus* CBS. Default gas and liquid flowrates

were established (200mL/min liquid recirculation rate, 25 accm gas flowrate) and the reactor sump was illuminated with a 65W incandescent lamp for several days. CO uptake (and concomitant H_2 production) was induced within approximately 48 hours. Once H_2 production reached a steady state, the lamp was turned off and the reactor loosely covered with black cloth. The operating conditions of the reactor (gas and liquid flowrates) were periodically adjusted, and the outlet gas composition was monitored over time, using a portable gas chromatograph (Agilent Inc. P200). The reactor typically required 12 hours or more to reach steady state after a change in operating conditions. These steady state values were recorded, and then the operating conditions were changed. Periodically, ~10-20mL aliquots of sterile M-1 media were added to the reactor to replenish the liquid removed for pH and cell density measurements. No effect on CO shift rate was seen as a result of these media additions.

As mentioned above, the inlet gas stream contained 5% CH_4 as an inert tracer to compensate for changes in the volumetric gas flowrate across the reactor. The water gas shift reaction causes an increase in the volumetric gas flow rate, since 2 moles of gas (H_2, CO_2) are produced for every mole of CO consumed (water is supplied by the media). This volume change would bias CO outlet concentrations low, since CO would not only be consumed by the microorganisms but also diluted by additional gas flowrate. Similarly, outlet hydrogen concentrations would be biased low due to dilution. Since methane is neither consumed nor produced in the reaction, its molar flowrate is constant. Thus, any change in methane concentration must correspond to a change in the overall gas flowrate. This correction factor was applied to all outlet concentration measurements.

Shake Flask Experiments

The batch shake flask experiments used a bacterial culture grown overnight in M-1 medium supplemented with malic acid and yeast extract. Carbon monoxide (20% CO, balance N_2) was continuously bubbled through the culture. The culture was continuously illuminated using standard 100W flood lamps. By varying the distance between the light source and the culture bottle, we could control the growth rate of the culture, and therefore the resulting optical density of the culture the following day.

After growing overnight, the culture was diluted into (nominal) 150mL bromobutyl septum-capped media bottles (Wheaton Scientific) to different final cell densities. The total liquid phase volume was kept constant at 50mL, and the headspace volume was 108mL. The optical density (measured at 660nm) of the cultures used in the experiments reported here was in the range OD_{660} = 0.02-0.50, corresponding to cell culture densities in the range 0.02-0.52 g/ L (1 OD_{660} = 1.03 g/L). Dithiothreitol (DTT) was added to ensure anaerobic conditions. The bottles were then purged with the 20% CO gas mixture for several minutes, and then placed in an environmental shaker operated at 150 rpm and 30°C. The bottles were shaken in the dark for two hours, repurged with the CO gas mixture, and then returned to the shaker. The headspace gas composition was periodically measured over the next two hours using gas chromatography (Agilent 5890 Series II). We found that the initial two-hour CO-incubation period was helpful to provide repeatable rate measurements during the second two-hour period.

Results and Discussion

Effect of Reactor Pressure

Figure 2 shows the apparent rate constant k_{app} (a measure of reactor productivity) as a function of bioreactor operating pressure. As the system pressure is increased, we see an increase in the apparent rate constant up to approximately 80 psia, after which no further increase in reactor productivity occurs. We believe this was due largely to a decrease in the pH of the bioreac-

tor media due to increased CO_2 solubility. While we had no way to measure the pH of the media *in-situ*, our external pH measurements, taken in open 5-mL disposable spectrometer cuvettes, showed a steady increase in pH over time, consistent with CO_2 outgassing from the media. It is likely that the actual pH of the media in the bioreactor became too acidic for the bacteria to perform the CO shift reaction. Recent laboratory experiments (not shown) show a pH optimum for the CO shift reaction of approximately 7.0, with significantly depressed activity below 6.0.

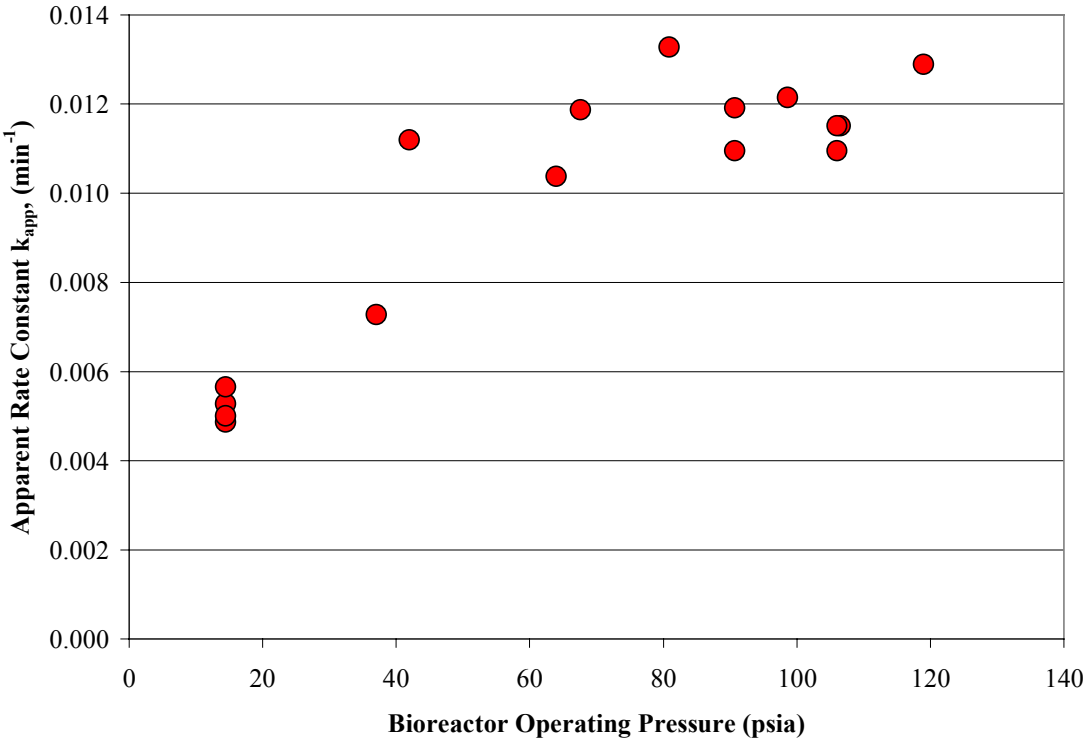

Figure 2. The effect of elevated operating pressure on the apparent bioshift rate constant k_{app}. At modest pressures, a significant increase in rate with pressure is seen. This effect appears to diminish at approximately 80 psia.

Effect of Inlet CO Concentration

As described above, in typical reactor experiments we use a premixed tank containing 20% carbon monoxide, 5% CH_4, and 75% nitrogen. However, in commercial applications, the CO concentration could conceivably vary over a wide range. We performed a series of experiments in a 1-L bioreactor to test the effect of CO concentration on reactor performance, and the results are shown in Figure 3. The curve in Figure 3 is a model fit using a previously developed reactor model (Wolfrum 2002). The data in Figure 3 cover the range from CO inlet concentrations from 8% to 99%. The model appears to be in reasonable agreement with the experimental data, except at low CO concentrations. However, we believe the data at low concentration (triangle symbols) are unreliable, since the repeatability of our analytical equipment (Agilent P200 gas chromatograph) is approximately 2%, and a 25% conversion of a 10% CO stream results in a CO concentration change across the reactor of only 2.5%. Thus, at very low inlet CO concentrations, the measured concentration change was close to the repeatability of our GC. At higher inlet CO concentrations, the change in CO concentration is larger, and the conversion data subsequently more reliable. The data in Figure 3 clearly indicate that the biologically-

mediated CO-shift reaction (Eq 1) is robust over a very wide range of inlet carbon monoxide concentrations; no poisoning of the microorganism occurred at high inlet CO concentrations.

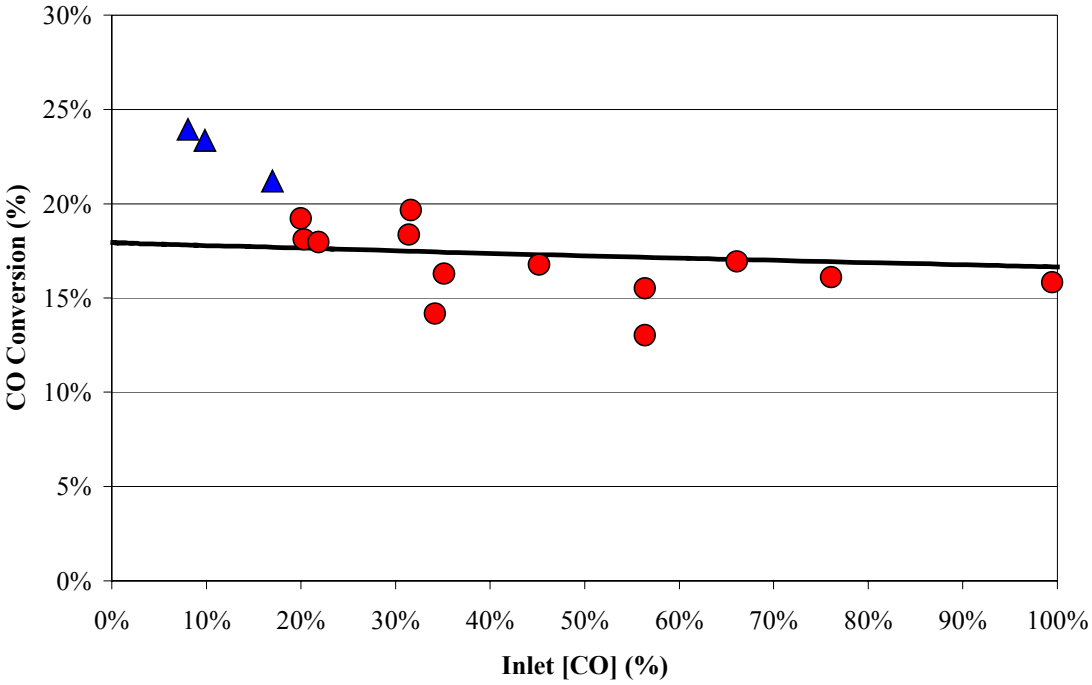

Figure 3. Effect of inlet CO concentration on CO conversion in a 1-L Bioreactor. Curve is model prediction (see text).

Measurement of Specific H_2 Production Rate

The measurement of the specific rate of substrate uptake is in theory very simple: supply a substrate to a bacterial culture, and measure the amount of substrate in the culture over time. The specific nutrient uptake rate is the rate of change of nutrient divided by the mass of the bacteria in the culture. For a metabolite, the specific production rate is the rate of change of metabolite present, also divided by the mass of bacteria in the culture. However, difficulties arise when the substrate or metabolite is a gas.

As a specific example, consider a closed media bottle partially filled with a culture of *Rx. gelatinousus* CBS. The headspace over the media contains a specific concentration of carbon monoxide. In order to be metabolized by the bacterial culture, the CO must first be transferred into the liquid phase. We wish to measure the rate of CO uptake by the bacterial culture by measuring the rate of change of CO in the headspace, since this measurement is conveniently performed using gas chromatography. If we divide the overall CO uptake rate by the total cell mass in the culture, we calculate the specific rate of CO uptake:

specific rate [mol/min/g] = overall CO uptake rate [mol/min] / total cell mass [g]

This relation is true only if no mass transfer limitations exist, since only in the absence of mass transfer limitations is the CO uptake rate directly related to the total mass of cells. For example, if the measured rate was completely controlled by mass transfer, then increasing the cell mass by a factor of 10 would have no effect on the measured overall CO uptake rate. To determine the specific uptake rate, we first rearrange the above equation to give:

overall CO uptake rate [mol/min] = specific rate [mol/min/g] X cell mass [g]

This equation clearly shows that in the absence of mass transfer limitations, increasing the cell density in the bacterial culture should increase the overall CO uptake rate in a linear fashion; doubling the cell density should double the overall CO uptake rate. The slope of a best-fit line of overall CO uptake rates vs. cell mass gives the specific rate (again, in the absence of mass transfer limitations). At very high values of cell mass, the overall CO uptake rate will not increase linearly with cell mass. This allows a graphical determination of mass transfer limitation; only rate data with a linear correlation between overall CO uptake and cell mass is free of mass transfer limitations.

We measured the overall rate of CO uptake in media bottles at various cell densities, and plotted the overall rate (both H_2 production and CO uptake) vs. cell mass to calculate specific rates. Agitating the media bottle increases the rate of transfer of CO gas into the liquid phase, which allows higher overall rates (and higher culture densities) prior to the onset of mass transfer limitations. We used an orbital shaker to provide this agitation.

Figure 4 shows the results of a typical experiment with *Rx. gelatinosus* CBS. Over the course of 2 hours, the CO originally present in the bottle headspace is metabolized and H_2 is produced. The rate of CO uptake and H_2 production is calculated from a linear regression analysis of the linear portion of the data (in this Figure, the data up to 1:40). Each experiment produced a single CO uptake and H_2 production rate data point.

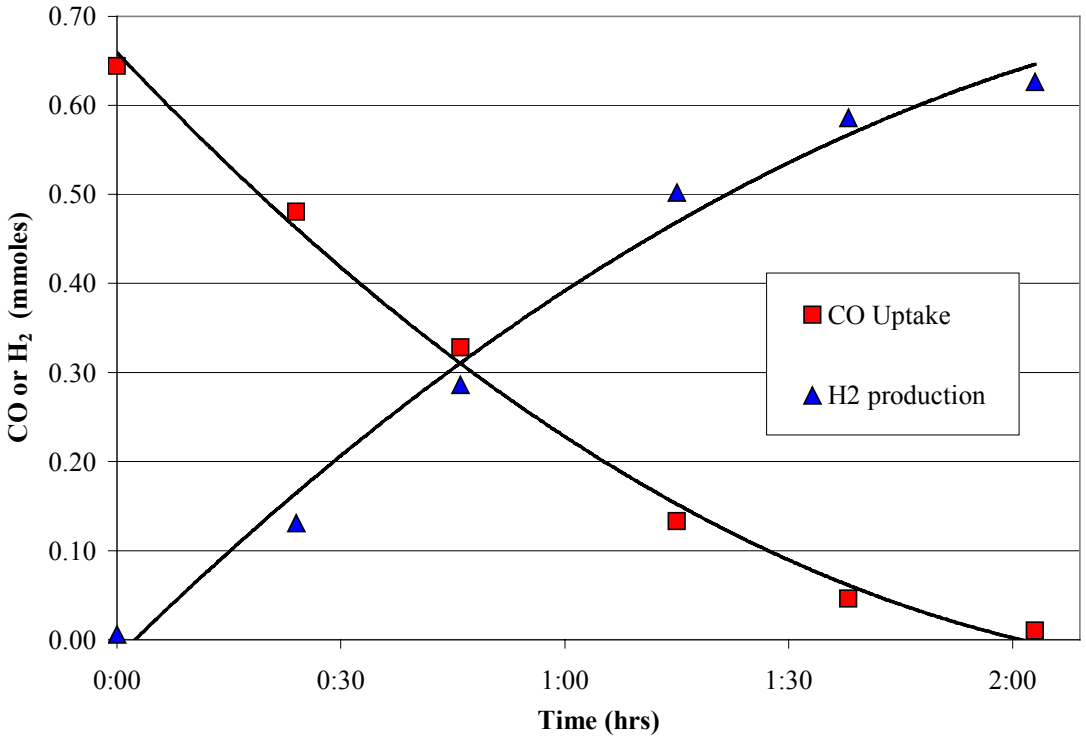

Figure 4. Hydrogen Production and Carbon Monoxide Uptake vs. Time for a 50-mL cell culture containing 0.011 g of the photosynthetic bacterium *Rhodocyclus gelatinosus* CBS. The rate is calculated from the linear portion of these curves (see text).

Figure 5 shows a plot of hydrogen production and carbon monoxide uptake rate vs. the total cell mass for all experiments. When the total cell mass is less than approximately 0.012 g, there is a linear relationship between cell mass and H_2 production and CO uptake. At higher cell mass values, the CO uptake and H_2 production values do not appear to increase linearly with cell mass. This is an indication of bulk (gas-liquid) mass transfer limitations. Figure 6 is a subset of the data in Figure 5 consisting of the linear region of the curves. These data were used to calculate the specific CO uptake rate and H_2 production rate. Figure 7 shows the rate of CO uptake vs. the rate of H_2, again from the linear portion of the curve in Figure 5.

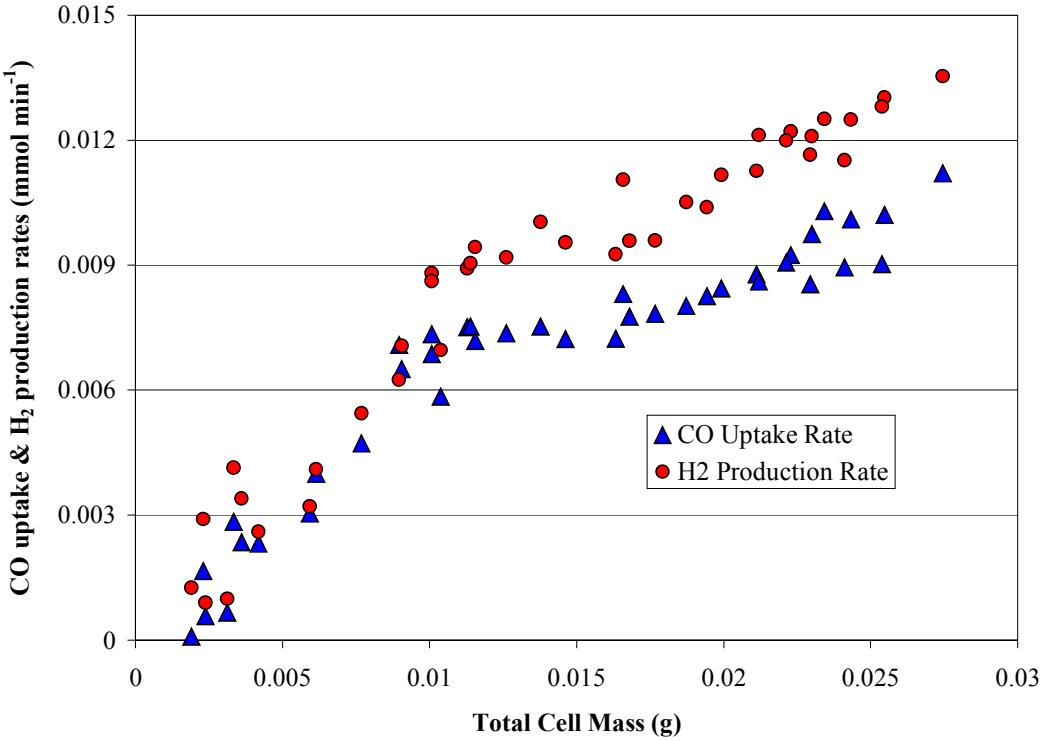

Figure 5. Hydrogen Production and Carbon Monoxide Uptake Rates vs. Total Cell Mass for the photosynthetic bacterium *Rhodocyclus gelatinosus CBS*.

The data in Figures 5-7 are summarized in the following table.

Parameter	Value	Units
Specific CO uptake rate	0.73 ± 0.10	mmol/min/g
Specific H_2 production rate	0.80 ± 0.13	mmol min/g
H_2/CO ratio	1.07 ± 0.14	--

The specific rate of H_2 production appears to be slightly higher than the specific CO uptake rate (0.80 vs. 0.73 mmol min^{-1} g^{-1}), although theoretical stoichiometry predicts that they should be equal. However, at the 95% confidence level, the two parameters are equal, as indicated by the overlap of each parameter's confidence interval. This is supported by the value of the H_2/CO ratio (1.07 ± 0.14), which cannot be distinguished from unity at the 95% confidence level.

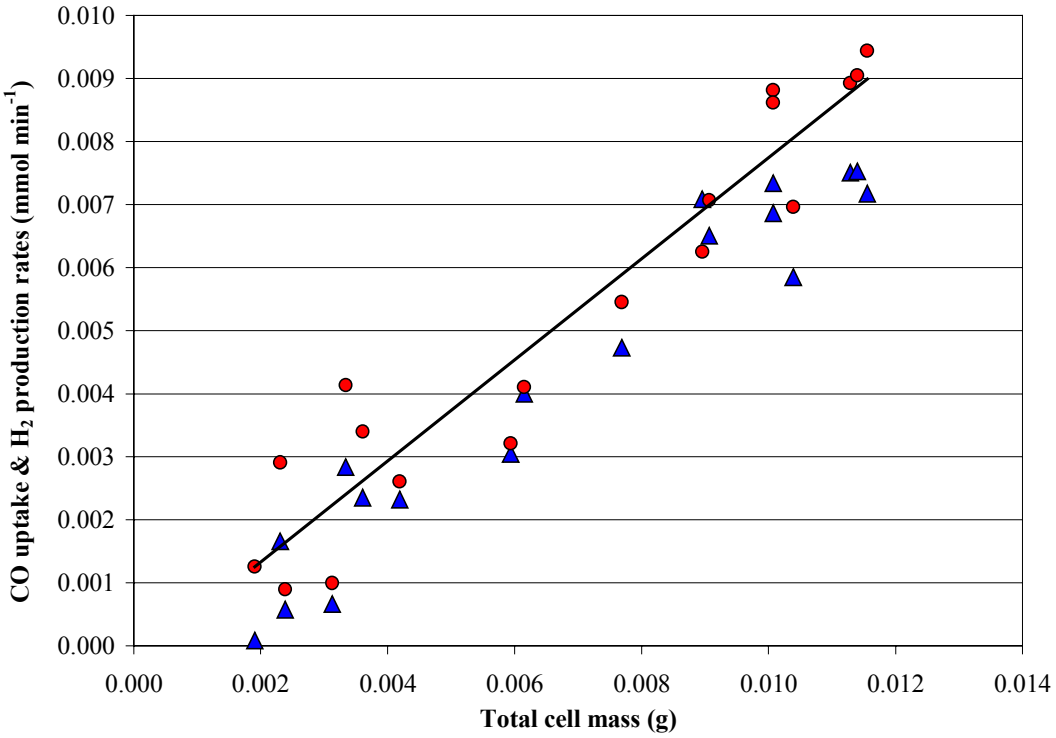

Figure 6. The linear region of the data in Figure 5. A linear correlation between cell dry weight and H_2 production rate corresponds to the lack of mass transfer limitations (see text)

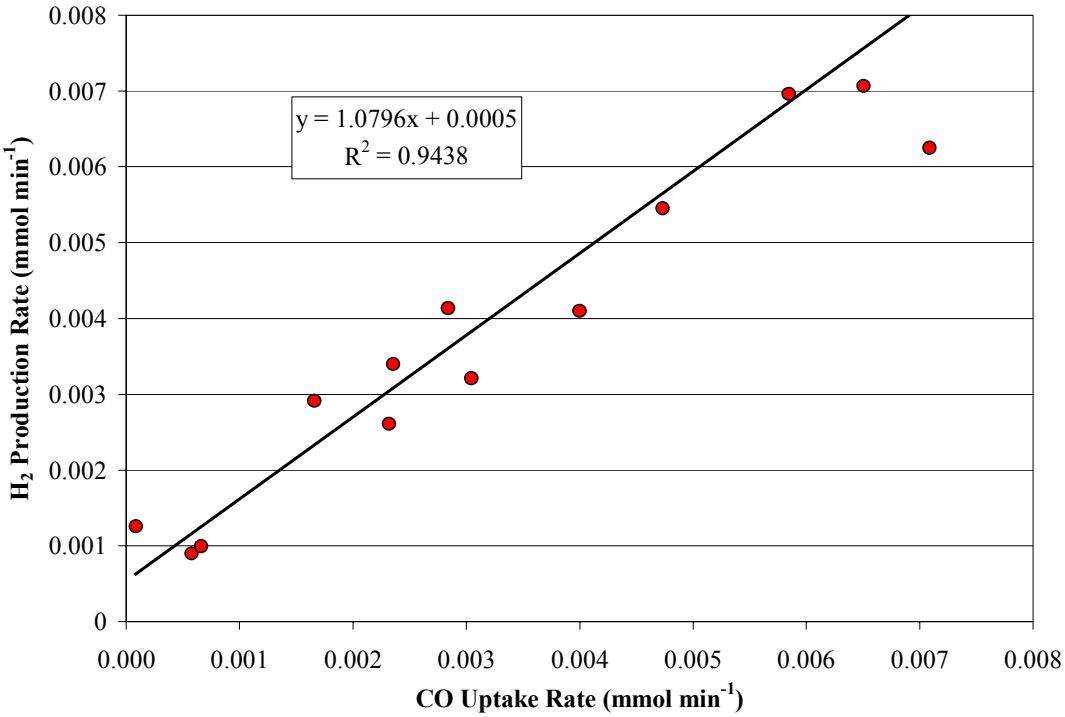

Figure 7. Hydrogen production vs. CO uptake for R. gelatinosus CBS. At the 95% confidence level, the slope of the best-fit line is not distinguishable from unity (see text).

Conclusions

We performed experiments to examine the effect of operating pressure and inlet CO concentration on the shift rate in a trickle bed bioreactor (TBR) and to measure the specific activity of *Rhodocyclus gelatinosus* CBS. The bioreactor conversion efficiency did not change appreciably when the inlet carbon monoxide concentration was varied over the range 8-99% CO (v/v%). Increasing the system pressure increased the bioreactor productivity up to approximately 80 psia, after which no increase in productivity was seen. The specific CO uptake rate was activity 0.73 ± 0.10 mmol/min/g, while the specific H_2 production rate was 0.80 ± 0.13 mmol/min/g. These two rates cannot be distinguished from each other at the 95% confidence level.

Acknowledgments

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