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Evaluation of Mass-Transfer and Kinetic Parameters for *Rhodospirillum rubrum* in a Continuous Stirred Tank Reactor

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ABSTRACT

The photosynthetic bacterium *Rhodospirillum rubrum* has been evaluated for its ability to produce hydrogen from carbon monoxide and water in a continuous stirred tank reactor according to the water-gas shift reaction. An assessment of mass-transfer parameters and reaction kinetics was made for this sparingly soluble substrate system. Experiments were conducted in a nonsteady-state fashion with continuous liquid and gas flow, which allowed for separation of the mass-transfer and kinetic-limited regions. Based on the data obtained, mass-transfer coefficients for the system were determined, and a mathematical expression for the reaction kinetics was formulated. The results showed that the hydrogen production was inhibited by elevated levels of dissolved carbon monoxide in the liquid.

Index Entries: *Rhodospirillum rubrum;* CSTR; carbon monoxide; mass transfer; kinetics

NOMENCLATURE

C	Concentration	mol/L
G	Volumetric gas flow rate	L/h
H	Henry's law constant for CO	L atm/mol
K_La	Overall mass-transfer coefficient	h^{-1}

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p	Partial pressure	atm
q	Specific CO uptake rate by cells	mol/g h
r	Volumetric CO mass-transfer/uptake rate	mol/h L
R	Ideal gas law constant	L atm/mol °K
t	Time	h
T	Absolute temperature	°K
V	Volume	L
X	Cell concentration	g/L

Superscripts and Subscripts

*	Equilibrium value
Ar	Argon, inert tracer gas
CO	carbon monoxide
G	Gas phase
I	Inlet conditions
L	Liquid phase

INTRODUCTION

The photosynthetic nonsulfur purple bacterium *Rhodospirillum rubrum* is one of many microorganisms that have been shown to produce H_2 from a variety of organic substrates, including malate, succinate, fumarate, and pyruvate (*1*). Recent studies (*2,3*) in batch and continuous culture have also demonstrated the production of H_2 by *R. rubrum* utilizing the water-gas shift reaction as described in Eq. (1).

$$CO + H_2O \rightleftharpoons CO_2 + H_2 \quad (1)$$

The water-gas shift reaction is important in shifting CO-rich synthesis gas to H_2-rich gas if chemicals production is desired, since syngas is generally deficient in H_2.

The biological catalysis of the above reaction has also been documented for *Rhodopseudomonas gelatinosa* (*4*), and was briefly mentioned by Dashekvicz and Uffen (*5*) for *R. rubrum*. The conversion is carried out with high efficiency by *R. rubrum*, since the carbon source for growth must be supplied by an additional compound, such as acetate. The use of a growth-limiting substrate (acetate) in the liquid phase is advantageous when studying gas/liquid mass transfer, since the growth is not limited by the gas-phase substrate. The maximum cell concentration may thus be independently controlled from mass transfer by, e.g., changing medium composition, and the rate of growth for photosynthetic bacteria may be controlled by light supply. Consequently, the system involving CO conversion by the bacterium *R. rubrum* differs from aerobic fermentation systems in that growth is not limited by mass transfer.

The purpose of this study was to determine the CO conversion kinetics by *R. rubrum* in a continuous stirred tank reactor and to illustrate the potential for using *R. rubrum* as a model system for the mass-transfer determinations. The experiments were conducted in a fashion that allowed for separation of mass-transfer and kinetic aspects of the fermentation.

MATERIALS AND METHODS

Rhodospirillum rubrum, ATCC 25903, was obtained from the American Type Collection, Rockville, MD. The composition and preparation of the basal medium, as well as the analytical methods for gas-phase composition and cell concentration, have previously been described by Cowger et al. (2).

EQUIPMENT AND PROCEDURES

The continuous stirred-tank reactor used was a New Brunswick Scientific (Edison, NJ) BioFlo II fermenter equipped with temperature, pH, and agitation control. The liquid working vol was 1250 mL, and the overhead gas vol was 350 mL. Illumination necessary for growth was supplied by two tungsten lights (40 W) directed toward the glass fermentation jar. Experiments were carried out at 30°C and pH 7. The feed gas used was a mixture of H_2, Ar, CO, and CO_2 (20/15/55/10%), and was continuously fed to the reactor. Liquid feed was also supplied on a continuous basis. A schematic of the equipment setup is shown in Fig. 1.

Typically experiments were started with constant agitation rate and gas flow rate. During an experimental run, the liquid flow rate was reduced from a high to a low setting. This procedure induced an increase in cell concentration and CO conversion with time as the culture strived to reach a steady state for growth limited by medium composition, liquid flow rate, and illumination. The conversion of CO was monitored, and experiments were considered complete when the gas conversion leveled off. The liquid flow rate was then increased, and the cell concentration allowed to decrease to a low level before starting a new experiment.

THEORY

An overall nonsteady-state CO balance over the CSTR may be written as:

$$[(p^I_{Ar}\, G^I / RT)]\,\{[(p^I_{CO} / p^I_{Ar})] - [(p^G_{CO} / p^G_{Ar})]\} - [(V_G / RT)]\,(dp^G_{CO} / dt) = r_{CO}\, V_L \qquad (2)$$

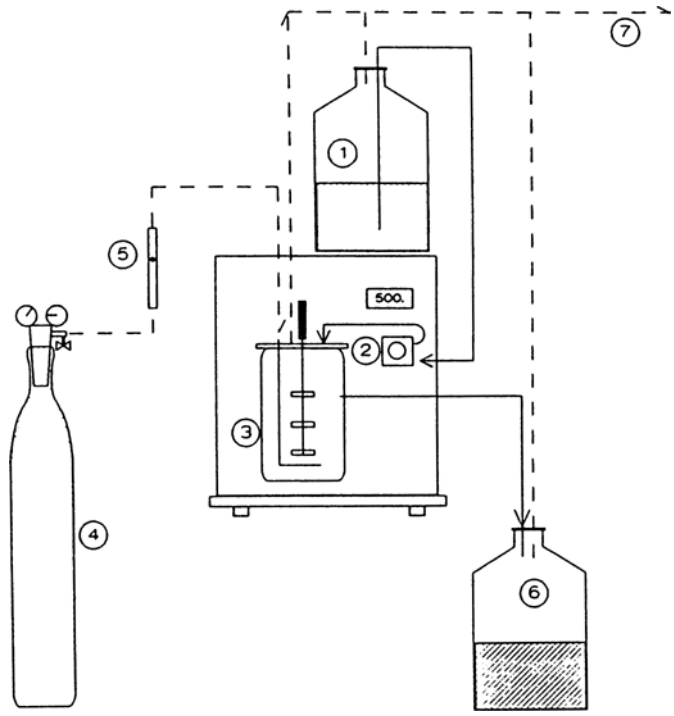

Fig. 1. Schematic setup of the reactor system. Legend: 1—feed reservoir; 2—feed pump; 3—fermenter; 4—gas supply tank; 5—rotameter; 6—effluent reservoir; 7—exit gas.

where r_{CO} is the volumetric CO mass-transfer/uptake rate as defined in Eq. (3).

$$r_{CO} = K_L a(C_L^* - C_L) = [(K_L a / H)](p_{CO}^G - p_{CO}^*) = Xq(p_{CO}^*) \quad (3)$$

The function $q(p_{CO}^*)$ in the above equation describes the microbial kinetics, and may be as simple as a Monod-type expression or may include additional terms.

Based on gas-phase analysis, r_{CO} may be calculated from Eq. (2) for the entire fermentation. As the cell concentration increases with time, the system moves from a kinetic-limited condition to a mass-transfer-limited state. At this point, the CO conversion (and r_{CO}) becomes constant, even though the cell concentration still increases. The mass-transfer coefficient can now be calculated using Eq. (3) with the assumption that $p_{CO}^* << p_{CO}^G$. It is important to realize that the growth of *R. rubrum* is not limited by the CO supply, but by liquid constituents, liquid flow rate, and illumination. The same equation may then be used to estimate the dissolved CO (p_{CO}^*) for the earlier part of the fermentation. Finally, with the calculated values or r_{CO} and p_{CO}^*, and the measured values of X, Eq. (3) may again be used to evaluate the kinetic function, $q(p_{CO}^*)$.

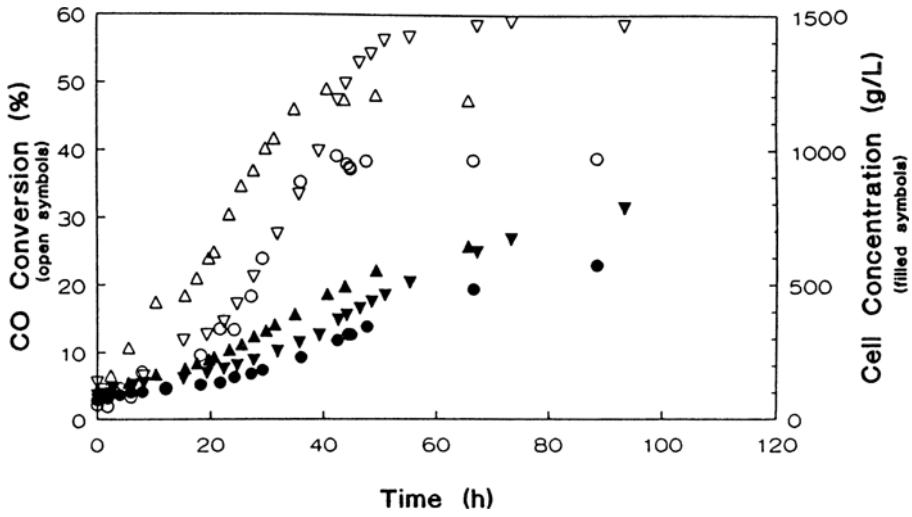

Fig. 2. Cell concentration and CO conversion profiles in the CSTR for agitation rates of 300, 500, and 700 rpm. Agitation rate ○ ● 300 rpm, △ ▲ 500 rpm, ▽ ▼ 700 rpm.

RESULTS AND DISCUSSION

Experiments were conducted using five different agitation rates (300–700 rpm in 100-rpm increments). Gas composition and cell concentrations were measured. In Fig. 2, representative CO conversion and cell concentration profiles for three of the five experiments are displayed. As is noted in the figure, an increase in the agitation rate resulted in a higher value for the maximum conversion at a given agitation rate. A maximum CO conversion of 38% was obtained using an agitation rate of 300 rpm compared to a 58% CO conversion at 700 rpm. The maximum CO conversion, indicated by the plateau obtained for the CO conversion profiles (*see* Fig. 2), corresponds to CO mass-transfer-limiting conditions in the fermenter. It is clear that mass-transfer-limiting conditions are in effect during this time, since the CO conversion leveled off even though cell concentration continued to increase. Thus, the cell concentration in the fermenter was not limited by CO transport, but by the factors described above. By the same token, the maximum CO conversion was not limited by cell concentration, but by agitation rate, gas flow rate, and gas inlet composition.

Based on the gas-phase analysis, the volumetric CO transfer rate (r_{CO}) was calculated according to Eq. (2) for the mass-transfer-limited region of the fermentation (time > 45–60 h). The overall mass-transfer coefficients were then calculated using Eq. (3) and the assumption that $p^*_{CO} << p^G_{CO}$.

Table 1
Mass-Transfer Coefficients Calculated
at Final Conversion Values for Various Agitation Rates

Agitation rate, rpm	p^G_{CO}, atm	r_{CO} mol/h L	K_La, h^{-1}
300	0.2804	$3.731 \cdot 10^{-3}$	14.9
400	0.2307	$4.431 \cdot 10^{-3}$	21.5
500	0.2134	$4.337 \cdot 10^{-3}$	22.8
600	0.2142	$4.549 \cdot 10^{-3}$	23.8
700	0.1679	$5.322 \cdot 10^{-3}$	35.5

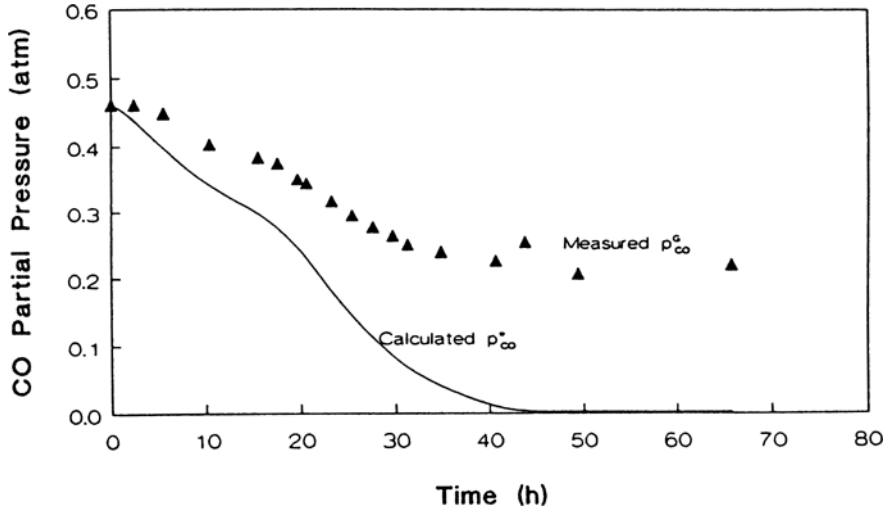

Fig. 3. Measured CO partial pressure and calculated dissolved CO tension in the CSTR for an agitation rate of 500 rpm.

The estimated K_La values ranged from 15 to 35 h^{-1} for the agitation rate range of 300–700 rpm (*see* Table 1). For agitation rates between 400–600 rpm, only small changes in the K_La values were observed. The most likely explanation for this result is that the position of the impellers relative to the liquid level and the effect of vortex mixing are of great importance. Visual inspection showed a clear change in flow patterns between 300 and 400 rpm, and 600 and 700 rpm.

By using the calculated values or r_{CO} (from Eq. [2]) and the estimated values of K_La (*see* Table 1), the dissolved CO concentration (p^*_{CO}) was calculated for the initial (and assumingly kinetic-limited) part of the fermentation using Eq. (3). A representative result is shown in Fig. 3 for an agitation rate of 500 rpm. As is noted, p^*_{CO} was approximately zero after 45 h, corresponding to mass-transfer-limited operation. In addition to calculating p^*_{CO}, the values for the specific CO uptake rate (q), equal to

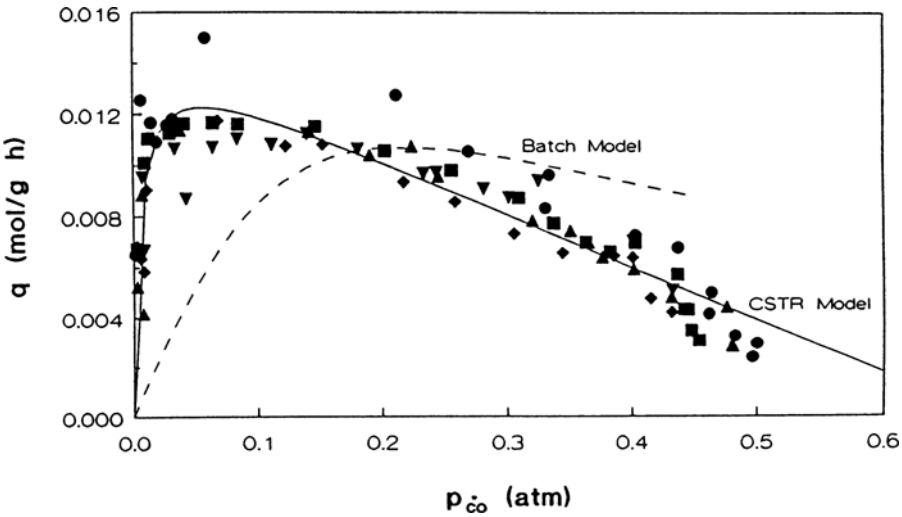

Fig. 4. Measured specific uptake rate as a function of calculated dissolved CO tension for agitation rates of 300–700 rpm. Agitation rate: ● 300 rpm, ▲ 400 rpm, ▼ 500 rpm, ◆ 600 rpm, □ 700 rpm.

r_{CO}/X, were also calculated (*see* Fig. 4). Starting at low values of p^*_{CO}, the data indicated that the specific CO uptake rate increased sharply with increasing values of p^*_{CO}. Above a dissolved CO pressure of 0.05 atm, the specific uptake rate decreased almost linearly with increasing CO partial pressure. It is evident from the result that CO has an inhibitory effect on the CO uptake (*see* Fig. 4). Thus, an attempt was made to fit the data to two inhibition models based on the Monod equation, one commonly used for substrate inhibition and another mostly used to account for product inhibition.

$$q = q_m \cdot p^*_{CO} / [K_p + p^*_{CO} + (p^*_{CO})^2/W] \quad (4)$$

$$q = [q_m \cdot p^*_{CO} / (K_p + p^*_{CO})] [1 - (p^*_{CO} / p_m)] \quad (5)$$

Equation (4) corresponds to Andrews' modification of the Monod relationship to include substrate inhibition (*6*), and Eq. (5) is the Monod expression, modified with a linear inhibition term. Equation (4) has recently been used to describe CO uptake rate by *R. rubrum* in batch culture (*3*).

Nonlinear regression analysis was used to obtain the parameters in Eqs. (4) and (5). The result of this analysis indicated that Eq. (4) was unsuitable for the data, since the regression predicted negative values for at least one parameter. This was considered inconsistent with development of Eq. (4). The solid curve in Fig. 4 is the best fit determined according to Eq. (5) and corresponds to the expression:

$$q = [0.0146 \cdot p^*_{CO} / (0.0053 + p^*_{CO})] [1 - (p^*_{CO} / 0.68)] \quad (6)$$

This equation is very sensitive to the independent variable p^*_{CO}. For low values of p^*_{CO}, the part of the equation corresponding to the Monod model is dominating, whereas for higher values of p^*_{CO}, the linear inhibition term is more prevalent. It may be predicted from the equation that CO uptake is completely inhibited by CO partial pressures of 0.68 atm and above.

The dashed curve in the same figure was generated based on previous results obtained in batch culture (3) using similar experimental conditions. The CO uptake was found to be described by:

$$q = 0.055 \cdot p^*_{CO} / [0.45 + p^*_{CO} + (p^*_{CO})^2/0.106] \quad (7)$$

As is noted in Fig. 4, the correlation previously found for batch data does not fit the data obtained in the CSTR experiments. The CO uptake rate in batch culture was less inhibited by dissolved CO, and q approached zero as p^*_{CO} approached infinity. However, both models predict a maximum specific CO uptake rate of approx 0.011–0.012 mol/g,h. The difference in batch vs continuous data may possibly be explained by CO acclimation. Batch experiments were typically started with CO partial pressures up to 1.6 atm. A long lag phase was always present at these pressures, but the cultures grew eventually. All experiments conducted in the CSTR were started at a maximum CO partial pressure of 0.5 atm, since equipment limitations prevented high-pressure operation in the CSTR. If higher initial CO partial pressures had been used in the CSTR studies, perhaps the results in the CSTR and in batch culture would have been similar.

The hydrogen yield was constant throughout the CSTR experiments at 0.88 mol/mol. This value is 88% of the theoretical value obtained from the stoichiometry of Eq. (1). In batch culture, the yield was estimated to 0.87 mol/mol.

In comparing the rate of H_2 production by *R. rubrum* with H_2 production by other organisms, it is seen that *R. rubrum* has a specific H_2 production rate (equal to 87–88% of the CO uptake rate) of 0.0035–0.01 mol/g,h over an equilibrium partial pressure range of 0.05–0.5 atm. In a review article, Vignais et al. (7) reported maximum H_2 production rates by a variety of photosynthetic bacteria on various substrates. The highest reported H_2 production rate was 0.010 mol/g,h for *Rhodopseudomonas capsulata*, strain B10, grown on lactate in nitrogen-limited culture. Bott et al. (8) reported an H_2 production rate of 0.0024–0.0036 mol/g,h for *Methanosarcina barkeri* grown on 2.5–10% CO at pH 7, and 37°C in the presence of propyl iodide and 2-bromoethanesulfonate.

CONCLUSIONS

Overall mass-transfer coefficients for CO have been determined in a continuous stirred-tank reactor at agitation rates of 300–700 rpm using a biological system with the photosynthetic bacterium *R. rubrum*. A non-

steady-state approach was employed in order to separate mass-transfer and kinetic-limited regions of the fermentation. As a result, a kinetic model could be developed for specific CO uptake by the culture, including the apparent CO inhibition. The maximum specific CO uptake rate found matched the earlier results obtained in batch culture and by other investigators. CO inhibition was more predominant in CSTR culture than in batch culture, perhaps because of CO acclimation.

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