

# Hydrogen Production from Synthesis Gas Using the Photosynthetic Bacterium *Rhodospirillum rubrum*

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Production of biological hydrogen by anaerobic photosynthetic bacteria, specifically *Rhodospirillum rubrum*, from synthesis gas was successfully conducted at ambient temperature and pressure. The influence of initial acetate concentration as the substrate for microbial growth was investigated in a batch system. Series of experiments were conducted using serum bottles as bioreactor. The agitation rate and light intensity were adjusted at 200 rpm and 1,000 lux, respectively. The concentration of acetate as carbon source was varied from 0.5 to 3.0 g/l. It was observed that the increase in concentration of the carbon source from 2.5 to 3 g/l resulted in the decrease both in the growth of the microorganism and in hydrogen production rate. Experimental results showed that the optimum acetate concentration would be from 1 to 2 g/l. The resulting data also showed that in 1–2 g/l acetate, highest hydrogen formation and cell concentration were obtained. Additional acetate in the initial culture medium inhibited the growth of *R. rubrum*. An inverse relationship between acetate concentration and initial cell growth was observed. This article presents a method to calculate the mass transfer coefficient for gaseous substrates and the process parameters involved in a gas and liquid fermentation system. The procedure had been defined by experimental data for the bioconversion of CO to CO<sub>2</sub>, while H<sub>2</sub>O is converted into hydrogen. Hence, a biologically-based water–gas shift reaction provided an attractive alternative improvement for renewable resources to achieve higher hydrogen production.

**Keywords:** Anaerobic bacteria, batch culture, coefficient, CO uptake rate, mass transfer, photobiological hydrogen, and *Rhodospirillum rubrum*.

## INTRODUCTION

One of the most interesting alternative fuel is hydrogen. The use of hydrogen, however, as clean fuel in the automotive industry is still at its infancy

and, thus, limited mostly to experimental models and testing vehicles. Yet the potential of hydrogen as a unique source of energy remains promising. The trend towards environmental sustainability and the development of renewable resources had

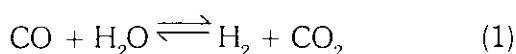
significantly increased interests in the recovery of fermentation products, such as organic acids and synthesis gas (Hoek et al. 2003, Feik et al. 2000). Thus, in order to lower the impact on environment and to search for an economical alternative to fossil fuels, a changeover to other energy sources originating from renewable resources may be an attractive strategy.

In synthesis gas fermentation, anaerobic bacteria are used to convert synthesis gas into biohydrogen and bioethanol. Bioresource Engineering Incorporation has developed synthesis gas fermentation technology which is used to convert cellulosic wastes to bioethanol (Schoert and Song 2002). Hydrogen production from synthesis gas required the optimization of several parameters, such as types of carbon source, light intensity, pH of the media, temperature, supplementary nutrients, amount of carbon source needed, and mass transfer phenomena (Najafpour et al. 2002).

Indeed, microorganisms are used to convert synthesis gas into fuels. Photosynthetic bacteria are able to produce hydrogen either from synthesis gas or organic compounds. Blue-green algae, *Cyanobacteria*, in the presence of sunlight are able to decompose water to produce hydrogen and oxygen. Photosynthetic bacteria are also able to catalyze the water-gas shift reaction or utilize organic compounds to produce hydrogen. Hydrogen production, however, is restricted only to light energy (Ghirardi et al. 2000, Miyake et al. 1999).

*Water-gas shift reaction* is a reaction where CO is oxidized to CO<sub>2</sub>, while simultaneously water is reduced to hydrogen. The advantage of using microorganisms to produce hydrogen from synthesis gas is that the water-gas shift reaction is performed at ambient temperature. This is a significant advantage since the reaction is not equilibrium-limited.

The dimensionless equilibrium constant is quite large ( $K_{eq, 25^{\circ}C} = 5 \times 10^4$ ), which means the reaction may easily proceed for hydrogen production (Wolfrum et al. 2002, Maness et al. 1999):



Microorganisms are known to carry out a similar reaction. There are many potent hydrogen-producing microorganisms reported in literatures such as *Rhodobacter* sp., *Rhodopseudomonas gelatina*, *Clostridium butyricum*, and *Rhodospirillum rubrum*. These, however, are strictly anaerobic bacteria. *Methanosarcina barkeri* and methanogenic bacteria (MB), such as *Methanosaeta concilii*, are also capable of producing hydrogen (Jung et al. 2002, Zhu et al. 2001, Valentine and Blanton 2000, Nakada et al. 1999). It was reported that *Rhodopseudomonas* sp. and *R. palutris* were able to grow on acetate, malate, butyrate, and lactate. *Rhodopseudomonas* sp. showed the ability for higher hydrogen production on acetate. Also, it was mentioned that lower acetate concentration and lower light intensity were required to give high hydrogen production (Barbosa et al. 2001).

In this study, the microorganism *R. rubrum* was used as biocatalyst for its highly specific CO uptake rate and high conversion yield closer to the theoretical value (Jung et al. 2002).

The experiment was carried out at an agitation rate of 200 rpm and a light intensity of 1,000 lux at various acetate concentrations. These acetate concentrations, however, were limited to a defined range. Serum bottles were chosen as the bioreactor for the batch experiments.

The main aim of this research was to define the optimum substrate concentration for the maximum growth of *R. rubrum* with the hydrogen production yield implemented for continuous large-scale production.

## MATERIALS AND METHODS

### Microorganisms

*Rhodospirillum rubrum* ATCC 25903, obtained from American Type Culture Collections VA, was used in this study. For the batch studies, the microorganism was hydrated and grown on malic acid incubated at 30°C in a sealed stoppered-serum bottle (163 ml). A 50-ml medium was transferred into each serum bottle under nitrogen gas. The bottles with liquid media were sterilized at 121°C for 15 min. After autoclave, the serum bottles were purged with synthetic gas

(55% CO, 20% H<sub>2</sub>, 15% Ar, and 10% CO<sub>2</sub>). Argon was selected as internal standard for gas analysis. The media were inoculated with a 5% (v/v) of seed culture.

The composition of the media for one liter were as follows:

| Media (1 L)  |   |
|--|---|
| Acetate  | 0, 0.5, 1, 1.5, 2, 2.5, and 3 g neutralized with NaOH at pH 6.9 |
| Yeast Extract  | 1 g   |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>                                    | 1.25 g  |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O   | 0.2 g   |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O   | 0.07 g  |
| Ferric Citrate   | 0.01 g  |
| EDTA   | 0.02 g  |
| KH <sub>2</sub> PO <sub>4</sub>  | 0.6 g   |
| K <sub>2</sub> HPO <sub>4</sub>  | 0.9 g   |
| Trace metal solution   | 1 ml  |
| ZnSO <sub>4</sub> ·7H <sub>2</sub> O   | 0.01 g  |
| MgSO <sub>4</sub> ·H <sub>2</sub> O  | 0.02 g  |
| H <sub>3</sub> BO <sub>3</sub>   | 0.01 g  |
| Ferric Citrate   | 3 g   |
| CuSO <sub>4</sub> ·5H <sub>2</sub> O   | 0.01 g  |
| EDTA   | 0.5 g   |
| (NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·2H <sub>2</sub> O | 0.02 g  |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O   | 0.2 g   |
| Vitamin B solution   | 7.5 ml  |
| Nicotinamide   | 0.2 g   |
| Thiamine HCl   | 0.4 g   |
| Nicotinic acid   | 0.2 g   |
| Biotin   | 0.008 g   |
| Added distilled water  | to 1 L  |

**Analytical methods**

A gastight syringe (Hamilton CO., Reno, Nevada) was used to take gas samples of 200 ml from the gas phase from each serum bottle at 12-h intervals. Gas Chromatograph (Perkin Elmer, Autosystem XL), equipped with thermal conductivity detector (TCD) and PC with software (Total Chrom) was used to analyze the gas samples. The column used was Carboxen 1000, 100/120 mesh (Supelco).

Temperature programming was implemented for gas analysis in GC. During the experiments, the column temperature was initially maintained at 40°C, after 3.5 min, the oven temperature increased at a rate of 20°C/min until it reached 180°C. The detector and injector temperatures were 200 and 150 °C, respectively. The flow rate for carrier gas (He) was set at 30 ml/min.

The acetate concentration in the media was measured using a gas chromatography (Hewlett Packard, USA) equipped with a flame ionization detector (FID). The GC column was 2.0 m in length and 0.2 cm in ID, 80/120 mesh, Carbowax B-DA/4% Carbowax 20M (Supelco, USA). The oven temperature was 175°C, while the detector and injector temperatures were both at 225°C. The carrier gas was nitrogen (Sitt Tatt, Malaysia) with a flow rate of 25 ml/min. An internal standard method was used for liquid sample analysis with a defined 1-propanol concentration.

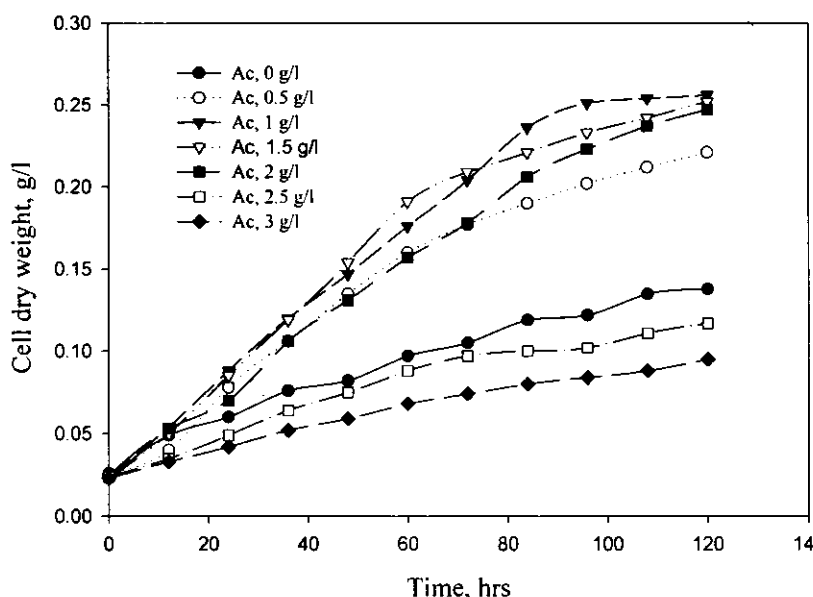


Figure 1. Cell Concentration of *R. rubrum* at Different Initial Acetate Concentrations

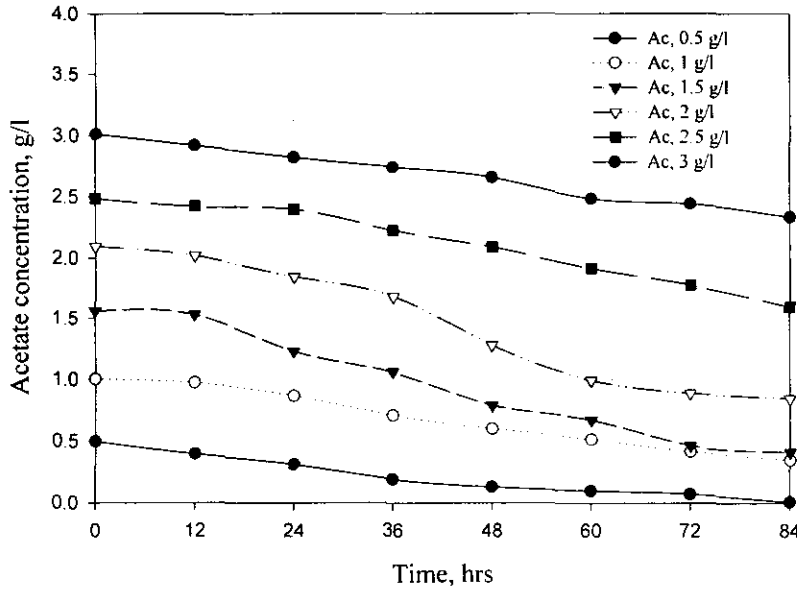


Figure 2. Consumption of Acetate by *R. rubrum*

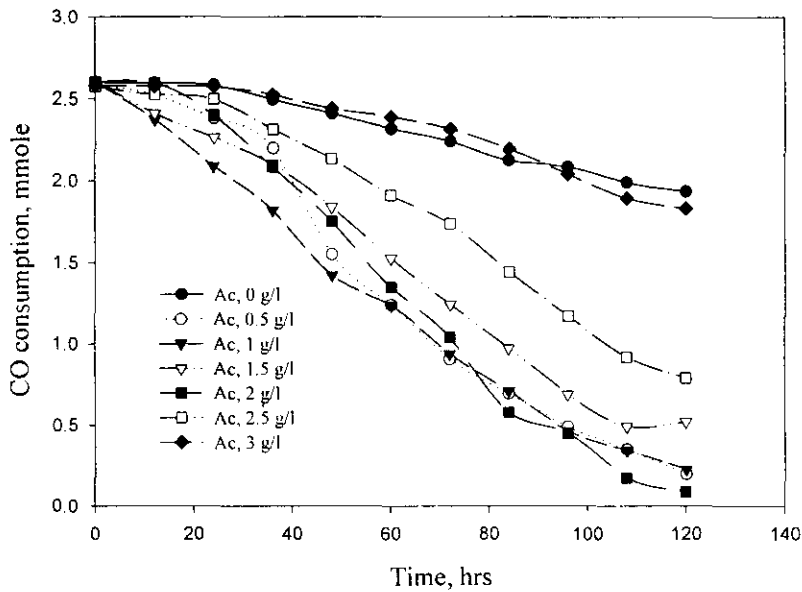


Figure 3. CO Consumption Process in the Presence of a Biocatalyst

The cell concentration of *R. rubrum* was determined by measuring the optical cell density using a spectrophotometer (Cecil 1000) and comparing the collected data with a calibration curve. Each serum bottle was filled with 50 ml of fresh media and inoculated with 2.5 ml of the microorganism grown in a 20h seed culture. The liquid sampling time was done at 12h time intervals. The serum bottles were shaken on an orbital shaker (B Braun) at 200 rpm. The light was supplied by a tungsten lamp (60W) to the serum bottles at 1,000 lux as measured by a lux meter (Sper Scientific, Taiwan).

## RESULTS AND DISCUSSION

In the anaerobic production of hydrogen, high substrate concentration may inhibit the growth of microorganisms. To avoid substrate inhibition phenomena for hydrogen-producing bacteria, the influence of initial acetate concentration on the growth of *R. rubrum* was investigated.

Figure 1 shows the effects of various initial acetate concentrations on cell optical density, from 0.5 to 3 g/l with an increment of 0.5 g/l. The cell densities for 2.5 and 3 g/l acetates were at the lowest level. The minimum cell optical density was

shown in the absence of acetate as a blank. The trend of cell growth with high acetate concentration (2.5, 3 g/l) and the blank were about the same. Substrate inhibition can be seen at this condition. The cell densities for 0.5, 1, 1.5, and 2 g/l showed full growth, with the maximum cell population obtained at 1 and 1.5 g/l acetates. The cell growth curves for suitable acetate concentration of 1 to 2 g/l were about the same shape with the cell density of 0.27 g/l.

The reduction of initial acetate concentration during the course of hydrogen production from synthesis gas is shown in Figure 2. The bacterial populations were affected by the different initial acetate concentrations (0.5 to 3 g/l). The amount of acetate gradually decreased while the cells were in the exponential growth phase. The maximum acetate utilization was obtained with 1.5 g/l acetate in the trend of acetate used at very high and very low substrate concentrations were about the same. The sharp reduction started for 1.5 g/l acetate in the culture media after 24 hours of incubation. However, the trend of acetate used at high concentrations (2.5 and 3 g/l) was about the same. Although at low acetate concentration the organic substrate was fully utilized, at high substrate concentration the rate of acetate utilization dropped by about 30 to 40 %. The

most desired acetate concentration for batch culture were from 1 to 2 g/l, with acetate conversion of 50 to 70%.

Figure 3 shows the reduction of CO oxidized by biocatalysts. The mole fraction of CO in the gas mixture was computed based on gas analysis and the concentration of argon responded as an internal standard. The decrease in moles of CO in the gas phase agreed with the decrease of acetate consumption and cell population in the culture media. The oxidation of CO to CO<sub>2</sub> in the gas phase was observed to be at 2 g/l acetate. This observation means that CO utilization in the gas phase and the acetate consumption in the media are in good agreement. The minimum level of CO oxidation was observed in the absence of acetate. Also, at 3 g/l acetate, the substrate inhibitory effect reflected on the CO oxidation process wherein the cell was retarded due to high substrate concentration. The CO consumption was 0.3 mmole at 1, 1.5, and 2 g/l acetates.

Hydrogen production is shown in Figure 4. The minimum level of hydrogen production was related to zero acetate concentration as carbon source in the liquid media. Low cell density also resulted in poor hydrogen production. The maximum hydrogen production was devoted to the maximum cell density broth with 1 and 1.5 g/l acetates. As

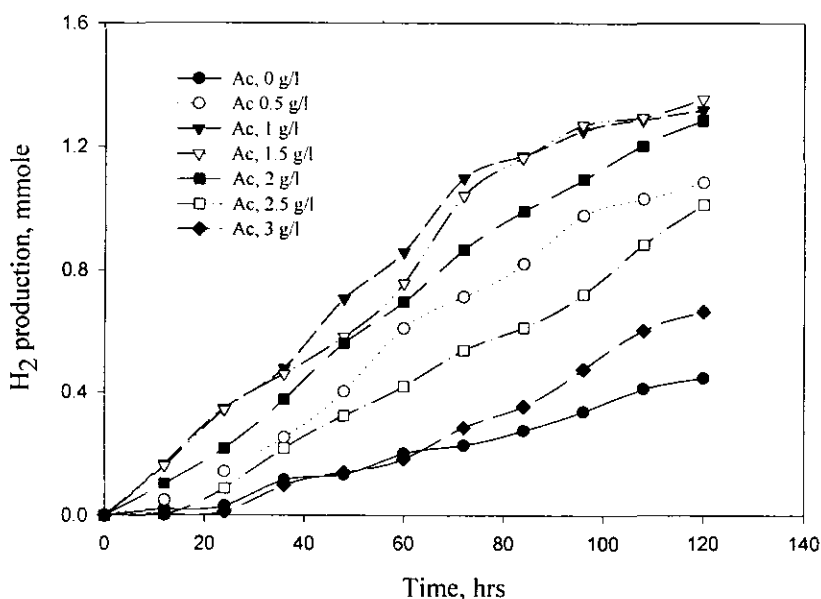


Figure 4. Hydrogen Production by *R. rubrum* at Different Initial Acetate Concentrations

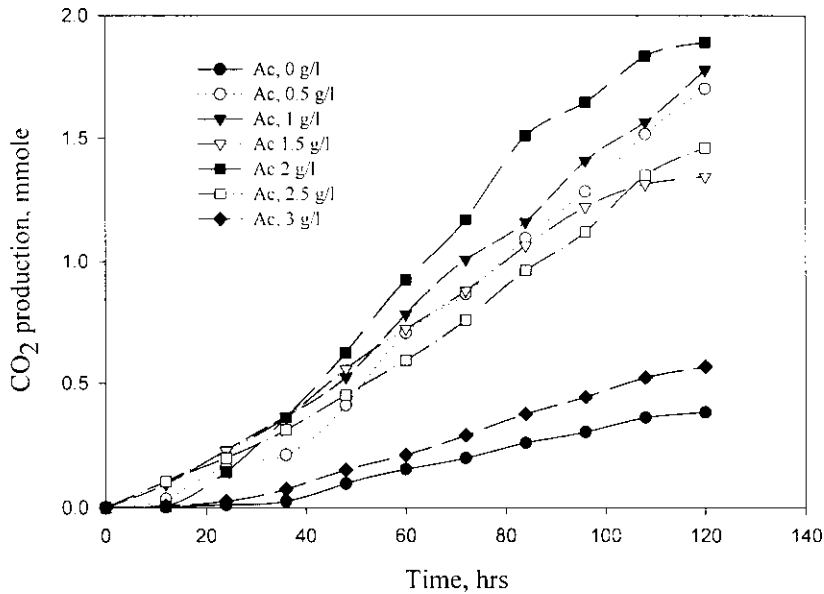


Figure 5.  $\text{CO}_2$  Formation Resulting from Water-Gas Shift Reaction

the acetate concentration increased from 2.5 to 3 g/l, the hydrogen production level dropped by 35%.

Figure 5 illustrates the  $\text{CO}_2$  content of the synthesis gas after the CO molecules were oxidized. The pattern for  $\text{CO}_2$ -formation followed about the same trend as that of biohydrogen production. The decrease in the partial pressure of  $\text{CO}_2$  coincided mostly with the substrate inhibition similar to that shown in hydrogen production at 3 g/l acetate. This was due to the effect of acetate inhibition on the microbial population. The  $\text{CO}_2$  level in the absence of acetate was almost the same as 3 g/l acetate. It was also shown that sufficient optical density of *R. rubrum* had a strong effect to catalyze the water-gas shift reaction in the presence of 1 to 2 g/l initial acetate concentration.

#### Mass transfer coefficient calculation

The mass transfer in a gas-and-liquid system is affected by the agitation rate and the gaseous substrate concentration gradient. The agitated bioreactor may absorb the gaseous feed by the liquid media. The mixture of gases can only diffuse into the liquid phase from the interface. Therefore, the rate of CO transferred from gas phase to liquid phase is expressed in terms of the mole

of gaseous substrates through liquid phase, as follows:

$$-\frac{1}{V_L} \frac{dn_{\text{CO}}}{dt} = \frac{K_L a}{H} (P_{\text{CO,gas}} - P_{\text{CO,liquid}}) \quad (2)$$

where  $V_L$  is the volume of the bioreactor,  $n_{\text{CO}}$  is the number of moles of CO,  $K_L a$  is the volumetric overall mass transfer coefficient,  $H$  is the Henry's law constant (1.116 l.atm/mole CO), while  $P_{\text{CO,gas}}$  and  $P_{\text{CO,liquid}}$  are the partial pressures of CO in gas and liquid phases, respectively.

The actual carbon monoxide consumption rate may vary from point to point and from time to time. It is a justified assumption for CO concentration in liquid phase to be nearly zero and the reaction is considered as fast reaction. In a special case, the reaction rate is not controlled by chemical kinetics since it is not the controlling resistance. This is usually the case when chemical reaction occurs and the value of  $P_{\text{CO,liquid}}$  is other than zero. Multiplying the left hand side of Eq. (2) by  $\left(\frac{dx}{dx}\right)$ , gives:

$$-\frac{1}{V_L} \frac{dn_{\text{CO}}}{dx} \frac{dx}{dt} = \frac{K_L a}{H} (P_{\text{CO,gas}} - P_{\text{CO,liquid}}) \quad (3)$$

The simplest model describes exponential cell growth was introduced as it was predicted by

Malthus (Baily and Ollis 1986). By describing the specific growth rate, which is related to the cell concentration with respect to time:

$$\mu = \frac{1}{x} \frac{dx}{dt} \quad (4)$$

where  $\mu$  and  $x$  are specific growth rates in h<sup>-1</sup> and cell density in g/l, respectively. Assume the dependency of the specific growth rate on substrate and CO utilization. Thus, substituting Eq. (4) into Eq. (3) yields,

$$-\frac{\mu x}{V_L} \frac{dn_{CO}}{dx} = \frac{K_L a}{H} (P_{CO,gas} - P_{CO,liquid}) \quad (5)$$

Yield of product may be defined as the cell mass produced per mole of CO consumed.

$$Y_{x/CO} = -\frac{dx}{dC_{CO}} = -\frac{dx}{\frac{dn_{CO}}{V_L}} \quad (6)$$

where  $Y_{x/CO}$  is the yield coefficient in g cell.mole<sup>-1</sup> carbon monoxide. Substituting Eq. (6) into Eq. (5) resulted in:

$$\frac{\mu x}{Y_{x/CO}} = \frac{K_L a}{H} (P_{CO,gas} - P_{CO,liquid}) \quad (7)$$

where  $(P_{CO,gas} - P_{CO,liquid})$  is the amount of CO uptake by the bacteria.

Thus, by plotting  $\frac{\mu x}{Y_{x/CO}}$  versus the driving force,  $(P_{CO,gas} - P_{CO,liquid})$ , the mass transfer coefficients can be obtained by calculating the slopes of the respective lines. Figure 6 shows the effect of initial acetate concentration on  $K_L a$  values. The experimental data well fitted with the projected model.

The effect of acetate concentration on volumetric overall mass transfer coefficients is shown in Figure 7. The lowest value of mass transfer coefficient was at 3 g/l acetate where substrate inhibition was affected. The maximum  $K_L a$  value was obtained with 1.5 g/l of initial acetate concentration. Eq. (7) was used to evaluate  $P_{CO,liquid}$  in the liquid phase. It is well known that CO utilization depends on specific growth rate. Therefore, the relationship between utilization of CO and specific growth rate follows the Monod rate equation. Then,

$$\mu = \frac{\mu_m P_{CO,gas}}{K_p + P_{CO,gas}} \quad (8)$$

where  $K_p$  is the Monod constant, atm. Substituting Eq. (8) into Eq. (7) gives:

$$\frac{\mu_m x}{Y_{x/CO} K_p + P_{CO,gas}} = \frac{K_L a}{H} (P_{CO,gas} - P_{CO,liquid}) \quad (9)$$

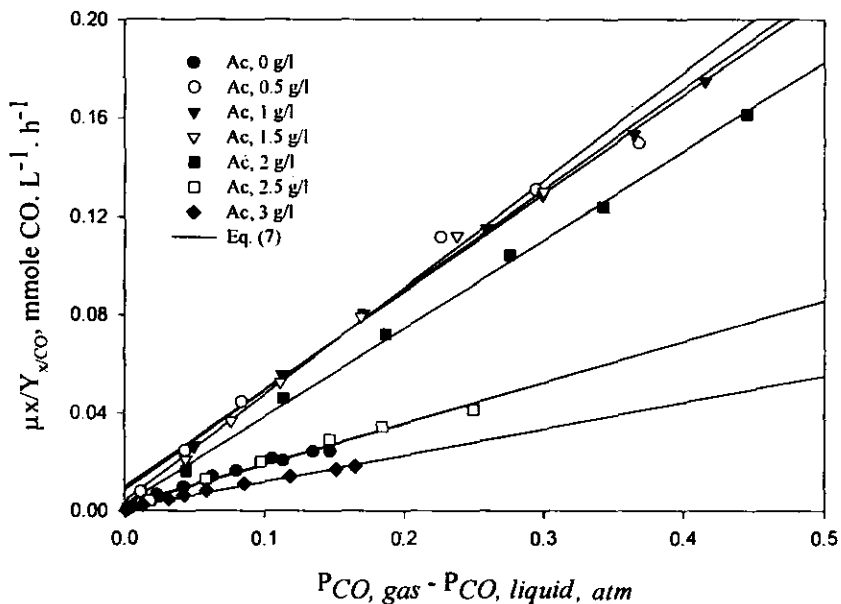


Figure 6. Mass Transfer Coefficients at Different Acetate Concentrations

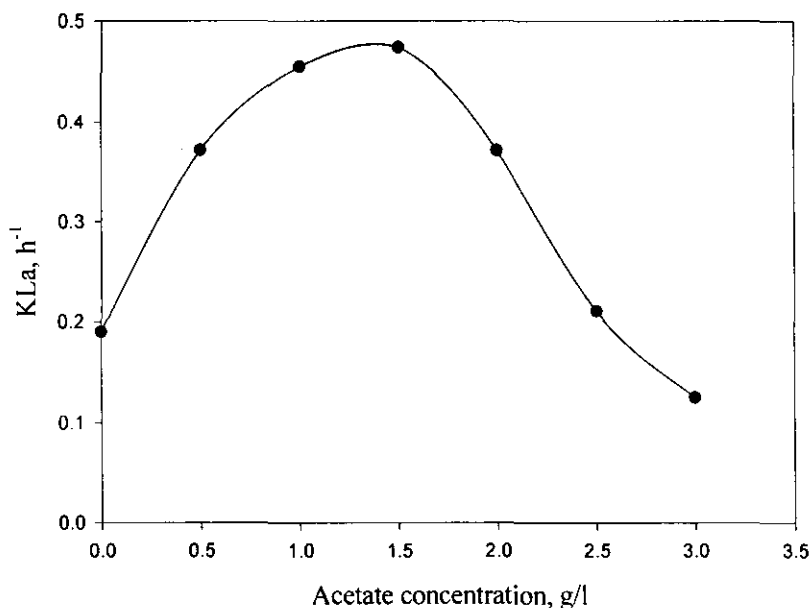


Figure 7. Effect of Acetate Concentration on Mass Transfer Coefficient

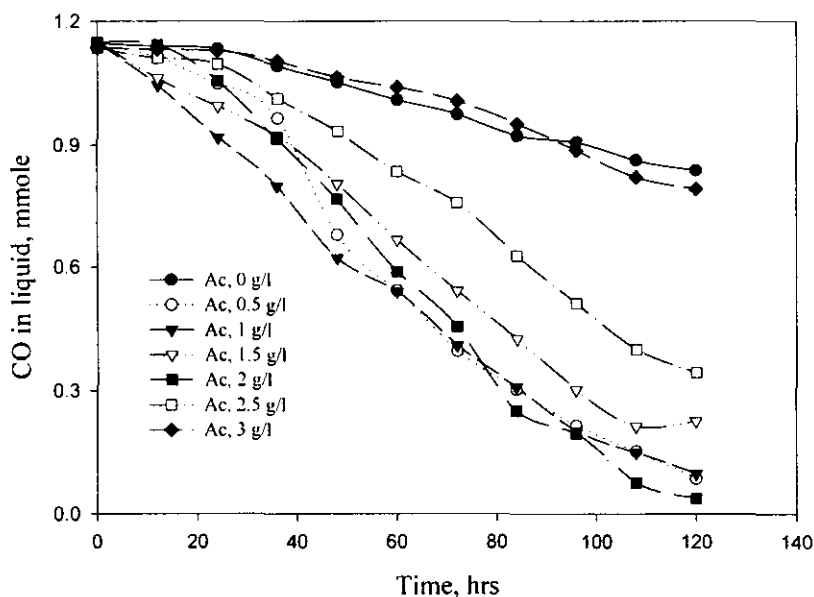


Figure 8. CO in the Liquid Phase at Various Acetate Concentrations

It was assumed that the value of  $P_{CO,gas}$  was greater than the value of  $P_{CO,liquid}$ . In fact, this case may happen in a bioreactor. To define the partial pressure of CO in liquid phase, Eq. (9) was solved for:

$$P_{CO,liquid} = P_{CO,gas} \left[ 1 - \frac{\mu_m x}{Y_{X/CO} \frac{K_L a}{H} (K_p + P_{CO,gas})} \right] \quad (10)$$

Eq. (10) was used to calculate the partial pressure of CO in the liquid phase. Figure 8 shows

the partial pressure of CO at different initial acetate concentrations during the fermentation of synthesis gas for biohydrogen production.

### CONCLUSIONS

Two types of biocatalysts were applied in order to carry out the full bioconversion of synthesis gas to biohydrogen and bioethanol. *R. rubrum*, with suitable initial acetate concentration, has shown to be an effective biocatalyst for hydrogen production from synthesis gas with high CO-uptake rate.



The effect of acetate concentrations of more than 2 g/l and malate has been the subject of Najafpour et al. (2002). In the present study, the effect of initial acetate concentration on mass transfer coefficient was successfully accomplished with defined values for  $K_L a$ . The procedure described in this paper is applicable for other microorganisms, such as *Clostridium ljungdahlii*, to utilize  $H_2/CO$  and  $H_2/CO_2$  for the bioconversion of synthesis gas to liquid fuel, such as bioethanol and acetic acid.

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**NOMENCLATURE**

|                  |  |
|------------------|--|
| $H$              | Henry's constant, <i>atm.l / mole</i>                            |
| $K_L a$          | volumetric overall mass transfer coefficient, $h^{-1}$           |
| $K_p$            | Monod constant, <i>atm.</i>                                      |
| $P_{CO, gas}$    | partial pressure of carbon monoxide in the gas phase, <i>atm</i> |
| $P_{CO, liquid}$ | partial pressure of carbon monoxide in the gas phase, <i>atm</i> |
| $n_{CO}$         | number of moles of carbon monoxide, <i>mmole</i>                 |
| $t$              | time, <i>h</i>   |
| $V_L$            | volume of bioreactor, <i>l</i>                                   |
| $x$              | cell dry weight concentration, <i>g/l</i>                        |
| $Y_{x/CO}$       | growth yield coefficient, <i>g cell produced/ mole CO uptake</i> |
| $\mu$            | specific growth rate, $h^{-1}$                                   |
| $\mu_m$          | maximum specific growth rate, $h^{-1}$                           |

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