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Evaluation of Hydrogen and Carbon Monoxide Mass Transfer and a Correlation between Myoglobin-Protein Bioassay and Gas Chromatography Method for Carbon Monoxide Determination

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Abstract

Syngas fermentation to biofuel is often plagued by mass transfer limitations of sparingly soluble gases, namely carbon monoxide (CO) and hydrogen (H₂), in the aqueous phase. In this study, the volumetric mass transfer coefficients (k_La) for H₂ and CO were examined in a gas-lift reactor coupled with a 20- μ m bulb diffuser. Further, a correlation between myoglobin-protein bioassay (liquid samples) and the head space gas analysis via gas chromatography (GC) for CO was developed for the same reactor configuration. The highest k_La values of 97.2 and 129.6 1/h were observed for H₂ and CO, respectively at gas flow rates of 5.0 L/min. The k_La values determined using GC equipped with a thermal conductivity detector (GC-TCD) and myoglobin-protein bioassay methods for different CO gas flow rates were highly correlated with a correlation (R^2) factors of 0.99 (without microorganisms) and 0.987 (with *Clostridium carboxidivorans* culture media). This study confirms that the myoglobin protein bioassay, which is a much simpler, faster and cheaper method compared well with GC analysis, can be used as a reliable method of determining the volumetric mass transfer coefficient of CO in syngas fermentation studies.

Keywords: Biofuel, gas chromatography, gas-liquid mass transfer, myoglobin (Mb)-protein bioassay, syngas fermentation, volumetric mass transfer coefficient.

1. Introduction

Synthesis gas (syngas), a gas mixture primarily consisting of CO and H₂, is a major building block for biofuel and valuable biochemical production. Syngas can be produced from the gasification of several reduced carbon-rich feedstocks such as coal, oil shale, tar sand, and lignocellulosic biomass¹⁻³. Syngas can be converted into biofuels through two major pathways, namely the Fischer-Tropsch (FT) process (using metal catalysts) and syngas fermentation by microbial catalysts⁴. Syngas fermentation via biocatalysts (such as *Clostridium ljungdahlii*, *Clostridium autoethanogenum*, and *Clostridium carboxidivorans*) offers several advantages over the FT process, including the elimination of the need of expensive metal catalysts, a higher specificity of the biocatalysts, an independence of the H₂:CO ratio for bioconversion, the operation of bioreactors at ambient conditions, and the elimination of issues concerning noble metal poisoning⁴⁻⁷.

The major challenge of syngas fermentation is overcoming the gas-liquid mass transfer limitation of CO and H₂ in the aqueous phase⁸. Different approaches such as high gas and liquid flow rates, large specific gas-liquid interfacial areas, increased pressure, different reactor configurations⁹, innovative impeller designs¹⁰, modified fluid flow patterns¹¹, varying mixing times and speeds¹², and the use of microbubble dispersers¹³ have been examined to enhance gas solubility in the liquid phase. This approach, however, is not economically attractive due to high energy costs and this may also affect the sensitive microorganisms in the culture media¹⁴. In order to achieve energy efficient mass transfer, alternative bioreactor configurations such as trickling beds, airlift reactors and membrane bioreactors have been investigated for syngas fermentation^{5,9}. The gas-liquid volumetric mass transfer coefficient (k_{La}) which represents the hydrodynamic conditions in a reactor can be used as a reliable parameter to examine the

effective mass transfer rate. Munasinghe and Khanal reported k_La values for CO, ranging from 0.4 to 91 1/h for eight different reactor configurations including a submerged composite hollow fiber membrane (CHFM) reactor⁹.

The efficacy of syngas fermentation is evaluated primarily based on the mass transfer rate of CO into the aqueous phase. All syngas fermentation studies reported so far employ Henry's law and the CO partial pressure in the head space to indirectly calculate the dissolved CO in the aqueous phase. Such an approach is not only tedious, but also time consuming and expensive. In this study, we employed a new method known as myoglobin (Mb)-protein bioassay for the direct determination of CO in aqueous samples. The Mb-protein bioassay is a much simpler, faster and cheaper method than gas chromatography (GC) analyses currently used for the determination of CO concentrations in the aqueous phase^{9, 15, 16}.

The current study focused on determining k_La values for H₂ using the headspace gas analysis (GC) method and to correlate the k_La values for CO obtained using the myoglobin-protein bioassay and the GC method. In addition, we also developed a correlation between Mb-protein bioassay (liquid samples) and the head space gas analysis by GC with a thermal conductivity detector (GC-TCD) for k_La values using CO gas in a gas-lift reactor coupled with a 20- μ m bulb diffuser. The gas-lift reactor coupled with a bulb diffuser was selected in this study since this bioreactor configuration resulted in the highest volumetric mass transfer coefficient among eight different reactor configurations previously tested⁹. In this case, the correlation was tested for both tap water (without microbial culture) and with *Clostridium carboxidivorans* culture media. To our knowledge, this is the first study that experimentally confirms that the Mb-protein bioassay is an accurate, reliable and simpler method compared to the GC-TCD method for the aqueous phase CO determination in syngas-to-biofuel conversions.

2. Materials and Methods

2.1 Experimental set-up

Experiments were carried out in a gas-lift reactor coupled with a 20- μm bulb diffuser. A schematic diagram of the experimental set-up is shown in Figure 1. The experiments were conducted using tap water ($25\pm 1^\circ\text{C}$) as the aqueous medium with a working volume of 3.0 L. Ultra high purity ($> 99.99\%$) CO and H₂ (Airgas, GasPro, Honolulu, HI) gases were used in the experiments, and the flow rates were controlled by a 150-mm rotameter (Omega, FL-3000, Stamford, CT) specifically calibrated for CO and H₂ gases. Water circulation was maintained using a digital peristaltic pump (Masterflex L/S 7523-60, Vernon Hills, IL) at a constant flow rate of 0.5 L/min. Hard tubing (Tygon, Acron, OH) with a 10 mm external diameter was used to connect the recirculation ports. Liquid samples were withdrawn through a three way sampling port with a septum, located 120 mm away from the reactor outlet, using a 10- μL gastight high performance syringe (Hamilton Gastight1701, Reno, NV). It was assumed that there was homogeneous mixing within the aqueous phase in the reactor, and this was further supported by the dissolved gas samples obtained at different locations of the reactor. The gas samples were collected from a gas sampling port in the off-gas line, using a gastight high performance syringe (Hamilton Gastight1701, Reno, NV). The bottled gas outlet pressure (inlet pressure to the reactor) was maintained at 0.68 atm(g) for both H₂ and CO gases. The experiments were repeated for gas flow rates ranging from 1.0 to 5.0 L/min. Three trials were conducted for each operating condition to minimize experimental errors.

2.2 Microbial culture media

Clostridium carboxidivorans (P7) (ATCC, BAA 624) was utilized in the syngas fermentation experiments. The bacterium was grown under strict anaerobic conditions in a medium containing 30 mL of mineral stock solution, 10 mL of trace metal solution, 10 mL of vitamin stock solution, 0.5 g yeast extract, 5 g of morpholinoethanesulfonic acid (MES), and 10 mL of 4% cystein-sulfide solution. Resazurin solution (0.1%) was added as the redox indicator. The composition of the minerals, trace metals and the vitamin stock solutions were previously described elsewhere ¹⁷. The batch fermentation experiments were carried out in a series of 250-mL serum bottles with a liquid media of 110 mL. The serum bottles were placed in an incubator shaker (Excella E25, New Brunswick Scientific, NJ) and the temperature was maintained at 37 °C and the stirring rate was 150 rpm. Bottled-gases of H₂ (20%), CO (13-41%), CO₂ (12%) and N₂ (27-45%) were used to fill the head space of the serum bottles. Gas and liquid samples were obtained after 6 days from each fermentation bottle to determine the CO concentration in both gas and liquid phases.

2.3 Determination of dissolved hydrogen concentration

H₂ gas samples were collected from a three-way gas sampling port at an interval of 25 s for two and a half minutes. The first gas sample was collected at 8 s after the introduction of H₂ gas into the reactor. Once the gas sample was collected, it injected into a 20 mL sealed vial. The vial contained water with similar gas phase to liquid phase ratio as the reactor. Then the gas and the liquid mixed well using a vortex mixer for 1 min and allowed 1 h to equilibrate the gas and the liquid phases. A gas sample from the head space was then taken and analyzed for gas composition in the gaseous phase using a gas chromatography (GC) (Perkin Elmer, Auto system, Waltham, MA) with a 40/60 mesh carboxen 1000 column (Supelco Inc., Bellefonte, PA)

connected to a thermal conductivity detector (TCD) with helium (He) as the carrier gas. The carrier gas flow rate was maintained at 20 mL/min. The TCD was operated at 45°C for 5 min, after which the temperature was ramped up to 225°C at a rate of 20°C/min. The head space gas concentration was then converted to the aqueous phase concentration by Henry's law (Eq. (1)).

$$K_H = \frac{p}{x} \quad (1)$$

Where, K_H is the Henry's law constant (atm), p is the partial pressure of gas above the aqueous phase (atm) and x is the mole fraction of gas in the solution (unitless)¹⁸. Henry's law constants used for H₂ and CO in this analysis were 7.09 x10⁴ and 5.82 x10⁴atm (at 25°C and 1 atm), respectively¹⁹.

2.4 Determination of dissolved carbon monoxide concentration

Carbon monoxide concentration in the aqueous phase was determined using two techniques, Mb-protein bioassay (liquid samples) and GC-TCD (gas samples) method. In order to compare the k_{La} values, both liquid and gas samples were taken simultaneously during each experimental run for different CO flow rates.

2.4.1 Myoglobin (Mb)-protein bioassay

The carbon monoxide concentration in the liquid samples was measured by myoglobin (Mb)-protein bioassay. The detailed procedure can be found elsewhere^{9, 10, 15, 16}, but is discussed briefly here. Myoglobin was obtained from a horse heart as a lyophilized powder (Sigma-Aldrich, Saint Louis, MO) with a purity of > 90%. Liquid samples with unknown CO concentrations were prepared in a series of 1.5-mL-semi-micro polystyrene disposable cuvettes (Fisher scientific, Pittsburgh, PA) by adding 1 mL of 0.1 M potassium phosphate (KH₂PO₄) buffer solution at pH 7.0. Small amounts of sodium dithionite (Na₂S₂O₄) were then added to the

cuvette to scavenge for dissolved oxygen (DO) in the solution. The Mb solution was prepared by dissolving a weighted amount of Mb in a known volume of 0.1 M KH_2PO_4 buffer. Finally, 10- μL of CO free, CO saturated or the unknown CO concentration sample was added and scanned using a spectrophotometer (DR 5000, HACH, Loveland, CO) in the wavelength range of 400 to 700 nm. The highest absorption peak for carboxymyoglobin (MbCO) occurs at a wavelength of 423 nm. The data file generated in the spectrophotometer was uploaded to the SpectraSolve (Ames Photonics, Hurst, TX) software for further analysis. During the analysis, the resulting spectra from unknown samples were fitted between the CO-saturated and CO-free absorption profiles. The percentage similarity of each profile with the CO-saturated baseline was obtained by the SpectraSolve software. Actual CO concentrations of the samples were then determined using the following equation (Eq. (2)).

$$\text{CO concentration in aqueous phase} = (C_p)(SS) \left(\frac{V_t}{V_s} \right) \quad (2)$$

Where, C_p is the protein concentration (μM), SS is the percentage of CO saturated spectra obtained from SpectraSolve (%), V_t is the total volume of the sample in the cuvette (μL), and V_s is the sample volume in the cuvette (μL). The protein concentration was determined using the Beer-Lambert law (Eq. (3)).

$$C_p = \frac{A}{l\epsilon_a} \quad (3)$$

Where, A is the absorption value, l is the cell path length (cm), and ϵ_a is the molar absorptivity (for Mb, $\epsilon_a = 188 \text{ 1}/\mu\text{M}\cdot\text{cm}$)²⁰. The average analysis time to determine the CO concentration in a liquid sample using Mb-protein method was around 2 min.

2.4.2 Gas chromatography (GC) method

The head space CO gas samples were analyzed by gas chromatography as described in the earlier section. The gaseous phase CO data was then used to calculate the aqueous phase CO concentration by Henry's law (Eq. (1)). The dissolved CO concentrations were then used to determine the gas-liquid mass transfer rates over a range of volumetric gas flow rates ($1 \leq Q \leq 5$ L/min). The average time taken to appear the H₂ and CO peaks using the head space gas analysis (GC-TCD) was around 7 min with complete analysis time of around 20 min.

2.5 Determination of $k_L a$ for H₂ and CO

Assuming that the concentration in the liquid phase at the gas-liquid interface is in equilibrium with the gas concentration in the gaseous phase, the volumetric mass transfer coefficient ($k_L a$) in the absence of any microorganisms was determined using the following equation (Eq. (4)).

$$\frac{dc}{dt} = k_L a (C_i - C) \quad (4)$$

Where, C is the gas concentration in the liquid phase (mg/L) at any given time t (s) and C_i is the saturated gas concentration (mg/L). Eq. (4) can be further simplified to (Eq. (5)),

$$\ln\left(\frac{C_i - C_0}{C_i - C}\right) = (k_L a)t \quad (5)$$

Where, C_0 is the initial gas concentration in the liquid phase (mg/L).

2.6 Statistical analysis

Statistical analysis software (SAS, Cary, NC) was used to validate the data obtained during each experimental run. For the analyses, the gas flow rate was chosen to be the independent variable, while the gas concentration in the liquid phase was selected as the dependent variable. Duncan's multiple range test and Bonferroni (Dunn) t-tests were conducted

to check the significance of the results under different experimental conditions and to validate the effects of flow rates and sampling times on k_La .

3. Results and Discussion

3.1 Hydrogen mass transfer

The aqueous phase hydrogen concentrations were determined over a range of volumetric flow rates ($1 \leq Q \leq 5$ L/min) in a gas-lift reactor coupled with a 20-micron bulb diffuser. Figure 2 shows a typical H_2 concentration profile as a function of time for different H_2 flow rates. As expected, the H_2 concentration in the liquid phase increased with increasing H_2 flow rate and sparging time. Further, the concentration profiles with higher H_2 flow rates reached a plateau earlier than that at lower flow rates. The theoretical H_2 saturation concentration in an aqueous phase at 25°C and atmospheric pressure was reported to be around 1.6 mg/L²¹, which is very close to the maximum H_2 concentration (~1.5 mg/L) achieved in this study.

Once the H_2 concentration in the liquid phase was determined, the k_La values for each condition were calculated by fitting the data into Eq. (5). In this case the initial H_2 concentration in the sample was assumed to be zero. Theoretically, the relationship between $\ln [(C_i - C_0)/(C_i - C)]$ and $(k_La) t$ is linear and the slope of the straight line gives the k_La value. Figure 3 shows the k_La values obtained at various flow rates. The correlation factor (R^2) for the plots ranged from 0.91 to 0.98. The highest k_La value of 97.2 1/h was obtained at the highest H_2 flow rate of 5.0 L/min, while the minimum was 16.5 1/h observed at the lowest flow rate of 1.0 L/min.

3.2 Carbon monoxide mass transfer

Carbon monoxide –water mass transfer data was obtained using Mb-protein bioassay and the GC-TCD method. The k_La values acquired from these two methods are shown in Figures 4 and 5. The highest k_La values of 129.6 and 101.5 1/h were obtained from the GC-TCD method and the Mb-protein bioassay, respectively. The k_La values acquired for different CO flow rates using these methods exhibited good correlations with the goodness of fit between 0.97 and 0.99.

The presence of gas bubbles in the extracted sample was the most common experimental error associated with Mb-protein bioassay. Under these conditions, extracted samples showed a slightly higher CO concentration caused by continuous CO diffusion from the bubbles. This was a critical factor, especially at higher CO flow rates, which resulted in higher variations in aqueous CO concentration. By conducting triplicate samples and multiple trials, we attempted to minimize the experimental error.

3.3 Correlation between k_La values for CO obtained from Mb-protein bioassay and GC method

3.3.1 Correlation study without microorganism

All previous studies on syngas fermentation evaluated the fermentation efficacy based on dissolved CO concentrations determined using GC analyses and Henry's law. It is therefore, critically important to develop a correlation between the myoglobin-protein bioassay method and the GC analysis method for determining dissolved CO in the aqueous phase. The correlation between the two methods of determining k_La for CO in the aqueous phase for various flow rates is shown in Figure 6 (a). The correlation factor (R^2) for the variation was 0.99.

3.3.2 Correlation study with *Clostridium carboxidivorans* culture

Ethanol and acetic acid were detected as the major fermentation products during syngas fermentation batch experiments. The maximum ethanol and acetic acid yields were obtained as 75.4 and 98.5 mg/L, respectively.

Liquid and gas samples were obtained simultaneously from each serum bottle for the correlation analysis. Figure 6 (b) shows the variation of the CO concentration obtained from the Mb-protein bioassay and the GC method and interestingly, a similar correlation ($R^2=0.987$) was observed with *C. carboxidivorans* culture media. This means that the k_La values obtain from Mb-protein bioassay were close to the values acquired using GC-TCD method. Thus, the Mb-protein bioassay, which is much simpler, quicker and cheaper method than the GC method can reliably be used to determine the CO concentration in the aqueous phase. Further, according to our estimates, the costs of analysis of CO from Mb-protein bioassay and the GC method were US \$ 0.46 and 1.60 per sample, respectively. The details of cost calculation are presented in Table 1. The cost does not account for the capital cost (analytical equipment), which is much higher for the GC method than the Mb-protein bioassay.

3.4 Statistical analysis

The data obtained was validated using several statistical models in Statistical Analysis Software (SAS). A Duncan's multiple range test and Bonferroni t-test were carried out to determine the significance of the sampling time and flow rates. The α value was selected as 0.05 for all statistical analysis models. During the analysis, it was observed that the selected flow rates and sparging times have a significant effect on the mass transfer coefficient.

Further, the flow rates (1.0 to 5.0 L/min) were ranked according to their significance using Duncan's method. The results showed that the flow rates from 1.0 to 3.0 L/min can be categorized into three different Duncan groups, while the other two flow rates (4.0 and 5.0 L/min) can be grouped into one Duncan group. This means that statistically, there is no significant effect on mass transfer coefficient obtained at gas flow rates of 4.0 and 5.0 L/min.

4. Conclusions

The highest volumetric mass transfer coefficients of 97.2 and 129.6 1/h were obtained for H₂ and CO, respectively, at 5.0 L/min gas flow rate in a gas-lift reactor coupled with a 20- μ m bulb diffuser. The study further reveals a good correlation ($R^2=0.99$ (without microbes) and 0.987(with microbial culture)) between the two CO analysis techniques, namely Mb-protein bioassay and GC-TCD method.

Thus, this study confirms that the Mb-protein bioassay, which is a much simpler, faster and cheaper method than GC analysis, can be used as a reliable method of determining CO concentrations and the k_{La} values in the aqueous phase during syngas fermentation. To our knowledge, this is the first study in which the correlation between the gas-phase CO (GC-TCD) and liquid-phase CO (Mb-protein bioassay) concentration was developed and verified.

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Nomenclature

A :	Absorption value (unitless)
C :	Concentration of CO in water (mg/L)
C_0 :	Initial CO concentration in the liquid phase (mg/L)
C_i :	Saturated CO concentration (mg/L)
C_p :	Myoglobin-protein concentration (μM)
K_H :	Henry's law constant (atm)
$k_L a$:	Volumetric mass transfer coefficient (1/h)
l :	Cell path length (cm)
p :	Partial pressure of gas above the aqueous phase (atm)
Q :	Volumetric gas flow rate (L/min)
R^2 :	Correlation factor (unitless)
SS :	Percentage of CO saturated spectra obtained from SpectraSolve (%)
t :	Time (s)
V_s :	Sample volume in the cuvette (μL)
V_t :	Total volume of the sample in the cuvette (μL)
x :	Mole fraction (unitless)
α :	Statistical significance (unitless)
ϵ_a :	Molar absorptivity ($1/\mu\text{M}\cdot\text{cm}$)

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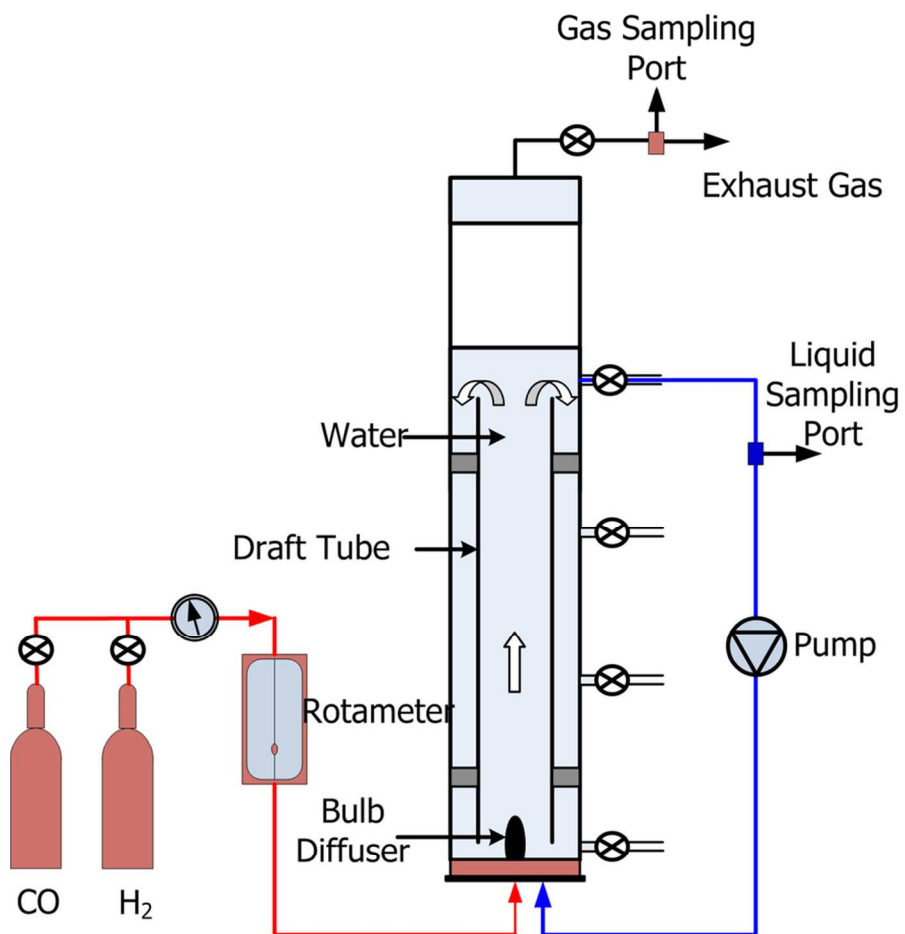
Figure 4. Determination of k_{La} from dissolved CO concentration data using myoglobin (Mb)-protein bioassay.

Figure 5. Determination of k_{La} from dissolved CO concentration data using GC-TCD method.

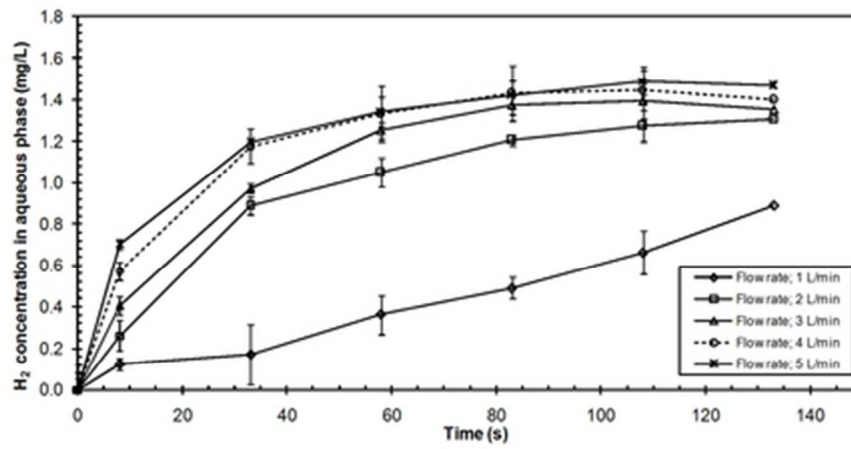
Figure 6. Correlation between CO k_{La} values obtained from myoglobin-protein bioassay and the GC method. (a) without microbes; (b) with *C. carboxidivorans*.

Table 1. Comparison of sample analysis cost between gas chromatography method and myoglobin-protein bioassay.

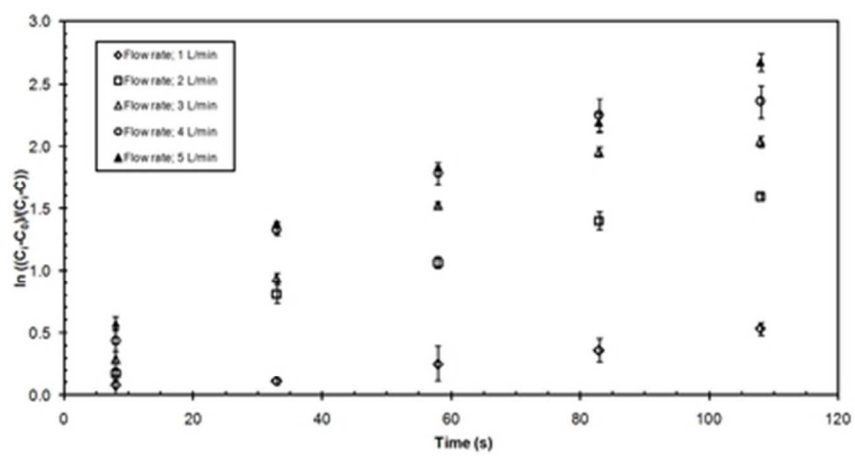
Gas Chromatography method		Myoglobin protein bioassay	
Item	Cost /sample (\$)	Item	Cost /sample (\$)
GC (ware and tare)	0.30	Spectrophotometer (ware and tare)	0.20
Carrier gas	0.77	Cost of myoglobin bioassay	0.06
Gas syringe	0.23	Syringe	0.14
Standard gas for calibration	0.30	Cuvette	0.06
Total	1.60	Total	0.46



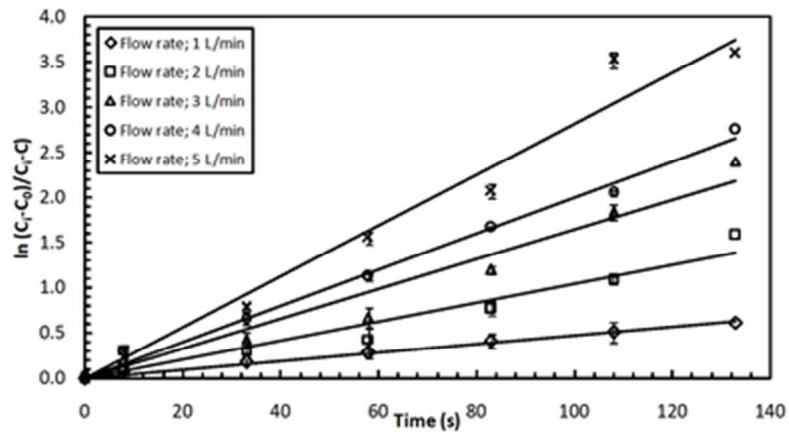
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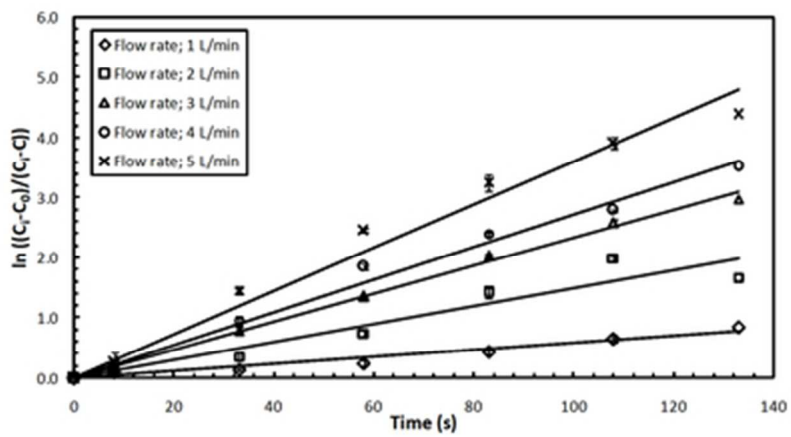
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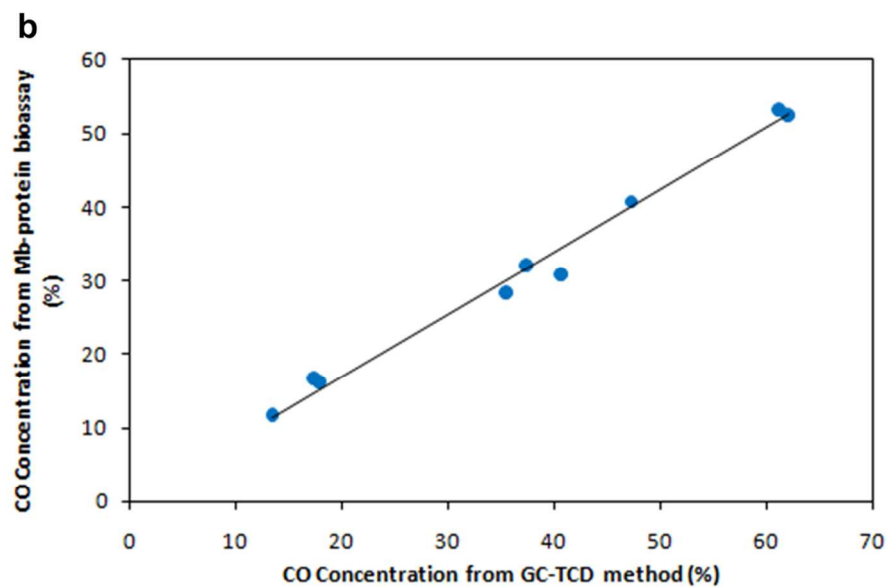
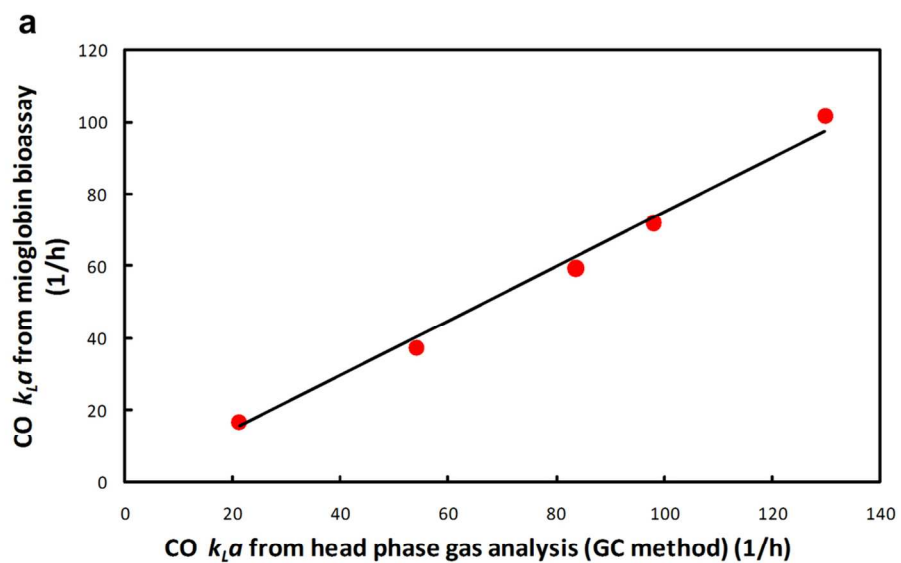
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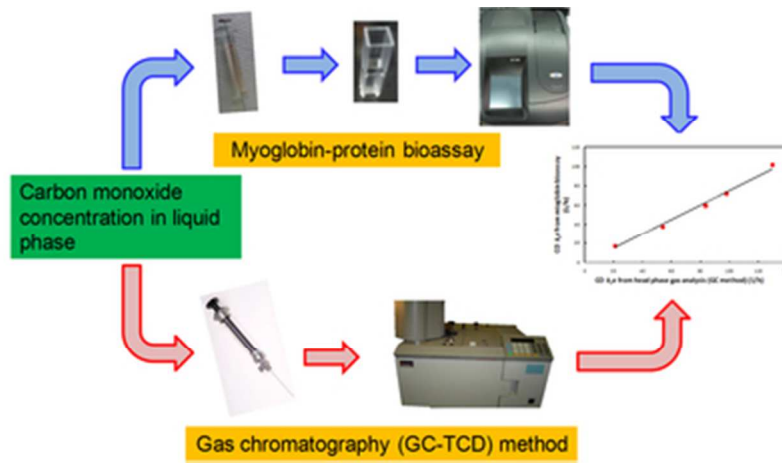


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Myoglobin protein bioassay, which is a much simpler, faster and cheaper method compared well with GC analysis, can be used as a reliable method of determining the volumetric mass transfer coefficient of CO in syngas fermentation studies.



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