PERFORMANCE OF BIOLOGICAL HYDROGEN PRODUCTION PROCESS FROM SYNTHESIS GAS, MASS TRANSFER IN BATCH AND CONTINUOUS BIOREACTORS

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Abstract Biological hydrogen production by anaerobic bacterium, *Rhodospirillum rubrum* was studied in batch and continuous bioreactors using synthesis gas (CO) as substrate. The systems were operated at ambient temperature and pressure. Correlations available in the literature were used to estimate the gas-liquid mass transfer coefficients (K_La) in batch reactor. Based on experimental results for the continuous reactor, new correlation was generated. The results showed that the agitation, gas flow rate and dilution rate were greatly influenced the hydrogen production as well as on K_La. It was found that the K_La of continuous bioreactor was 180 times higher than the mass transfer coefficient reported in batch reactor. It can be considered that the estimation of K_La for the continuous bioreactor may be successful for the large-scale biological hydrogen production.

Key Words Biological Hydrogen, Bioconversion, Fermentation, Mass Transfer Coefficient, Synthesis Gas

چکیده تولید هیدروژن به روش بیولوژیکی به کمک میکروارگانیزمها *Rhodospirillum rubrum* و در شرایط بی هوازی در بیوراکتورهای ناپیوسته و پیوسته از گاز سنتز CO مورد مطالعه قرار گرفت. سیستمهای بیولوژیکی در دما و فشار محیط عمل نموده و ضریب انتقال جرم در بیوراکتورهای ناپیوسته از منابع علمی تخمین زده شد. ولی برای راکتورهای پیوسته از روابط تجربی بدست آمده استفاده گردید. نتایج حاصله نشان می‌دهد که ضریب انتقال جرم به دبی گاز و نرخ رقیق شدن محیط کشت بستگی دارد. میزان K_La در بیوراکتورهای پیوسته ۱۸۰ برابر بیشتر از ضریب انتقال جرم گزارش شده در منابع علمی برای راکتورهای ناپیوسته بوده است. نتایج بدست آمده در بیوراکتورهای پیوسته موفقیت آمیز تلقی شده که انتظار می‌رود برای تولید هیدروژن به روش بیولوژیکی در مقیاس صنعتی بطور موثر مورد استفاده قرار گیرد.

1. INTRODUCTION

Gasification technology has been known for more than 100 years, and its end product, synthesis gas, has been used in the commercial production of fuels and chemicals [1]. It can accommodate a variety of feedstock such as biomass, coal, shredded waste tire etc. This means that most organic wastes can be gasified and converted to synthesis gas, where the major component is carbon monoxide (CO). The synthesis gas cools as it passes through the waste heat boiler, and moves to the gas scrubber to remove dust and ash before being compressed by a compressor. The conventional process of synthetic fuel production utilizing the synthesis gas was pioneered by the Fischer Tropsch synthesis, where the CO was converted to hydrogen via the water-gas shift (WGS) reaction as shown in Equation 1:

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

Depending on the H_2/CO ratio, reaction conditions and catalysts used, different chemicals are synthesized. However, the latter process, the Fischer Tropsch synthol process operating at

temperatures ranging from 220 to 340°C and pressure reaching up to 25 bars [2,3] which may not be considered economical.

Apart from this, photo biological hydrogen generation has received considerable attention due to several advantages offered by some bacteria. Much has been published in the literature on the types of microorganisms capable of producing hydrogen from various organic wastes [4-6]. Furthermore, not many processes were capable of upgrading the synthesis gas (CO) to an H_2-rich gas. Among the famous hydrogen producers studied utilizing CO as substrate were the photosynthetic bacteria *Rhodopseudomonas gelatinosa*, *Rhodospirillum rubrum* [7], *Rubrivivax gelatinosus* [8], *Rhodopseudomonas palustris* and a chemoheterotrophic bacteria *Citrobacter sp. Y19* [9]. The above-mentioned biocatalysts have the potential to carry out the WGS reaction as stated in Equation 1 at ambient temperature and pressure. These bacteria had the key enzyme responsible for the conversion of CO to CO_2, which is carbon monoxide dehydrogenase (CODH) [10]. While the biochemical oxidation of CO has been preformed by the biocatalysts in an anaerobic condition, the oxygen must be provided by the dissociation of water molecule, as a result hydrogen is produced. The use of *R. rub rum* is of interest in this paper and some preliminary studies have been carried out by other groups of researchers [11-14]. A notable feature of *R. rubrum* preferable than other anaerobic bacteria is the ability to tolerate up to 93% of CO in the gas phase [15]. Also, it can stand small amount of oxygen and sulfur compounds often present in the synthesis gas [7]. This study is considered as a continuation of the research where the route to scale up remains a major challenge.

If hydrogen is to be the fuel for the future, it has to be produced renewably and in large scale. To accomplish the large-scale production, a translation factor between two scales is needed. Since this case deals with a sparingly soluble gas, constant mass transfer coefficient (K_La) is a good translation factor. Correct measurement and subsequent estimation of the K_La is a crucial step in designing bioreactors. Furthermore, when a gas such as CO is limiting, the K_La is a useful indicator for process improvement. A comparison was made between two systems, a small batch reactor such as serum bottle and a stirred tank reactor in bench scale, to determine which system offers the greatest potential for practical application.

This investigation was carried out for continuation of studies due to the advantage of cost effective process and ecological production with clean combustion. Besides, fuel is produced from waste materials. It has the ability to compete with not only fossil fuel but with other renewable energy sources such as wind, solar, ocean thermal and wave powers [16].

2. MATERIALS AND METHOD

2.1 Growth Media and Culture Conditions

Rhodospirillum rubrum (ATCC 25903) was used in this study. The bacteria were activated and grown in a broth and the stock culture was stored on semisolid nutrient agar in a slant tube. The growth medium composition in 1 liter solution was as follows: 2.5g Malic acid (Merck) neutralized with NaOH (Merck) at pH 6.9, 1g Yeast Extract (Merck), 1.25g $(NH_4)_2SO_4$ (Merck), 0.2g $MgSO_4.7H_2O$ (Calbiochem), 0.07g $CaCl_2.2H_2O$ (Merck), 0.01g Ferric citrate (Merck), 0.02g EDTA (Sigma), 0.6g KH_2PO_4 (Sigma), 0.9g K_2HPO_4 (Sigma). Trace metal solution (1 ml); 0.01g $ZnSO_4.7H_2O$ (Merck), 0.02g $MgSO_4.H_2O$ (Calbiochem), 0.01g H_3BO_3 (Merck), 3g Ferric citrate (Merck), 0.01g $CuSO_4.5H_2O$ (Merck), 0.5g EDTA (Sigma), 0.02g $(NH_4)_6Mo_7O_{24}.2H_2O$ (Sigma), 0.2g $CaCl_2.2H_2O$ (Merck). B-Vitamin Solution (7.5 ml); 0.2g Nicotinamide (Sigma), 0.4g Thiamine HCl (Sigma), 0.2g nicotinic acid (Sigma) and 0.008g Biotin (Sigma). Distilled water was added to make 1-liter solution. The sterilized media was distributed in serum bottles (Fisher Scientific, UK) under purified nitrogen gas (Air Products, Malaysia) to create anaerobic condition. The bacterium was grown under tungsten light at 500 lux measured by a luxmeter (Sper Scientific, Taiwan). The growth was established by the purple red color of the culture.

2.2. Hydrogen Production Media The media was almost the same with the growth media, except the 2.5g Malic acid was replaced with 1.5g

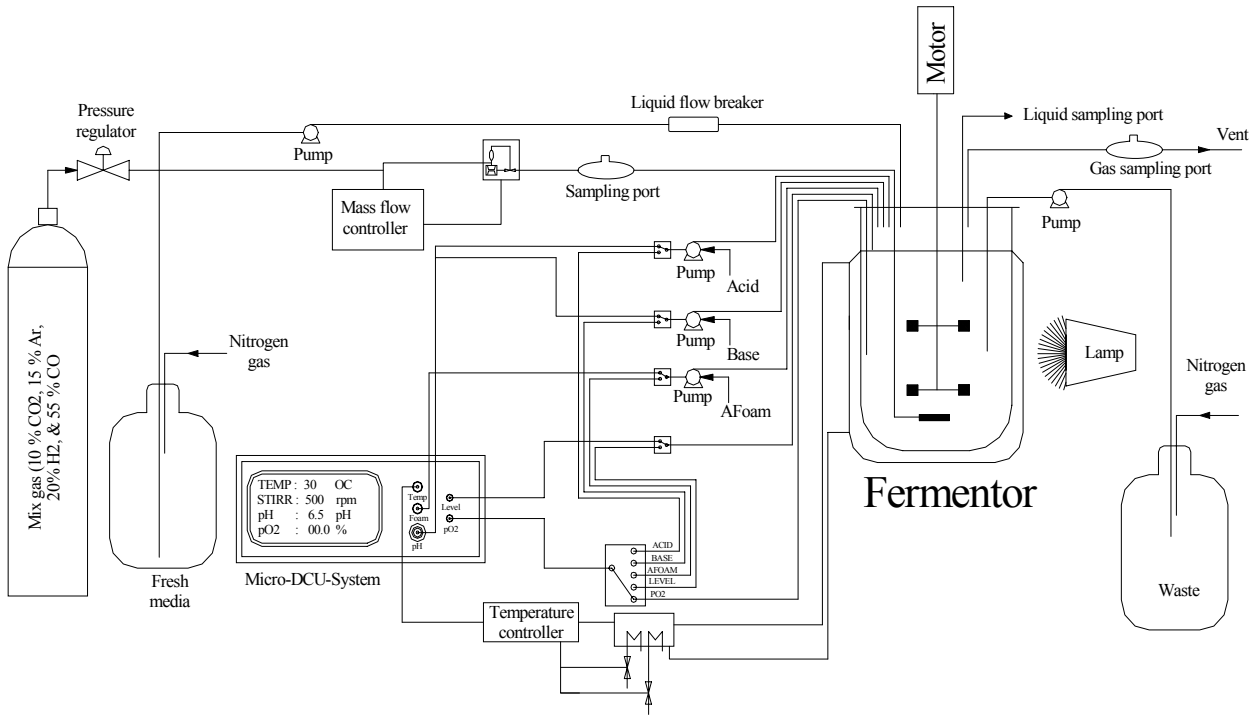

Figure 1. Schematic representation of experimental set up, fermentation vessel and associated units.

Sodium acetate (Sigma). The media pH was adjusted to 6.9. This media was used for both batch and continuous bioreactors.

2.3 Batch Experiments Each set of experiments was carried out in serum bottles, 163 ml. The working volume was 50 ml. Two needles for gas inlet and outlet were injected on the serum bottle's stopper. The serum bottles were purged with mixed gas (Air Products, Malaysia) consisting of 15% H_2, 20% Ar, 55% CO and 10% CO_2 for 30 seconds to push out nitrogen from the system. The gas composition was chosen based on the coal-derived syngas composition [12]. A trace amount of nitrogen remaining in the system was considered not harmful. Argon was the internal standard for gas analysis. The size of inoculum injected was 5% that was equivalent to 2.5 ml. The inoculum was injected from the broth culture into the serum bottles using a sterile syringe. The horizontally stored serum bottles were shaken at the rates of 150 rpm, 200 rpm and 250 rpm using an orbital shaker under tungsten light at 1000 lux.

2.4 Continuous Experiments This experiment was accomplished in a 2-liter fermenter (Biostat A, B Braun, Germany) equipped with pH (Mettler Toledo, Germany), temperature, level and dissolved oxygen sensor (Mettler Toledo, Germany). Gas (15% H_2, 20% Ar, 55% CO and 10% CO_2) and a five times concentrated liquid media were supplied continuously. The overflow was kept in a waste container. The whole system was kept under anaerobic condition. Figure 1 shows the schematic representation of fermenter vessel with the associated set-up and the geometrical dimensions are shown in Figure 2.

Two sets of impellers, the Rushton turbine with a diameter of 5 cm were used. Both impellers were placed 8 cm apart. The vessel was also fitted with four baffles (10 mm). Working volume was 2 liters and 5% from the log phase seed culture was used for inoculation. Two tungsten lamps (40 W) were provided from two sides of the fermenter for light illumination. The temperature was controlled by a water bath (B Braun, Germany) set at 30°C circulated in the fermenter's water jacket. The pH

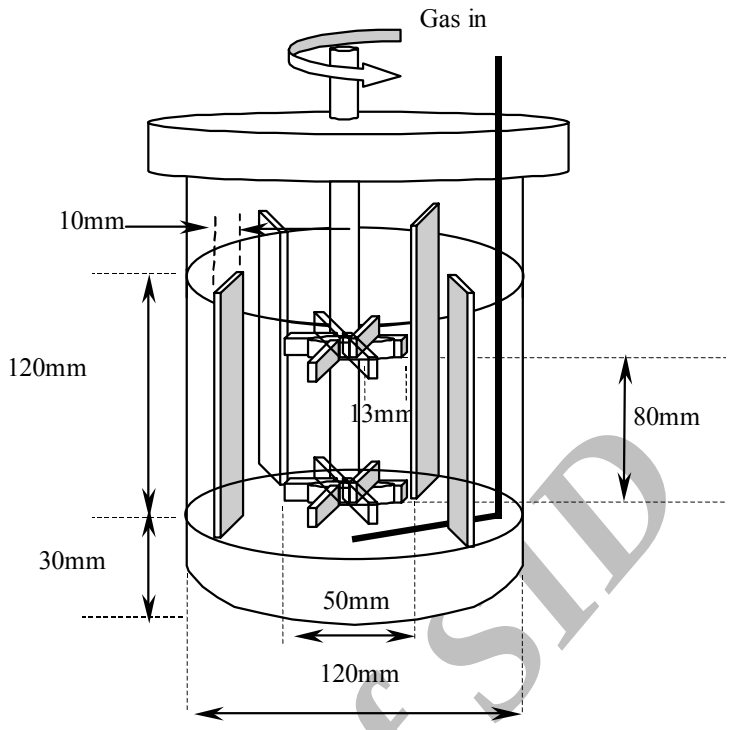

Figure 2. Geometrical dimensions of fermentation vessel

of the media was kept constant throughout the experiments at 6.3 by the addition of acid (1M HCl) and base (1M NaOH). The gas mixture was bubbled through a stainless steel perforated ring sparger with 14 orifices (diameter: 0.5mm). Agitation speed was adjusted in the range of 300 rpm to 800 rpm.

2.5 Analytical Methods Gas samples were collected directly from the reactors with a gas tight syringe (Hamilton, Nevada). Two hundred microliters of gas sample was analyzed by a gas chromatography (Perkin Elmer, Autosystem XL, USA) equipped with a Thermal Conductivity Detector (TCD) and a 15′ x 1/8″ Carboxen-1000 column (Supelco, USA) with 100/120 mesh. The calculation for gas concentration was carried out using a licensed software product, Total Chrom Workstation Version 6.2 (Perkin Elmer, USA). The oven temperature was initially maintained at 40^{o}C for 3.5 minutes and increased at the rate of 30^{o}C/min to 220^{o}C. The detector and injector temperatures were set at 200^{o}C and 150^{o}C, respectively. Helium (Air Products, Malaysia) was supplied as the carrier gas at a flow rate of 30 ml/min.

3. RESULTS AND DISCUSSION

3.1 Batch Mode Operation Batch production of hydrogen was carried out in serum bottles for incubation period of 5 days. The agitation was resulted by orbital shaker that has influenced on mass transfer and was important especially for a process requiring transfer of sparingly soluble gases such as CO. A comparison was made between three shaking frequencies and a static condition on the CO consumption, specific growth rate and hydrogen production. Table 1 shows the tabulated results for the specific growth rate, CO conversion and hydrogen production for the four different agitated and static conditions. The specific growth rate was almost the same between the static and shaking (frequency 150 rpm) media.

TABLE 1. Specific Growth Rate, CO Conversion and Hydrogen Production at Various Shaking Frequencies.

Shaking Frequency (rpm)	Specific growth rate, μ (h^{-1})	CO Conversion (%)	Maximum Hydrogen Production (mmoles)
0 (Blank)	0.06	20.15	0.23
150	0.05	15.36	0.27
200	0.10	100	1.64
250	0.06	86.03	1.91

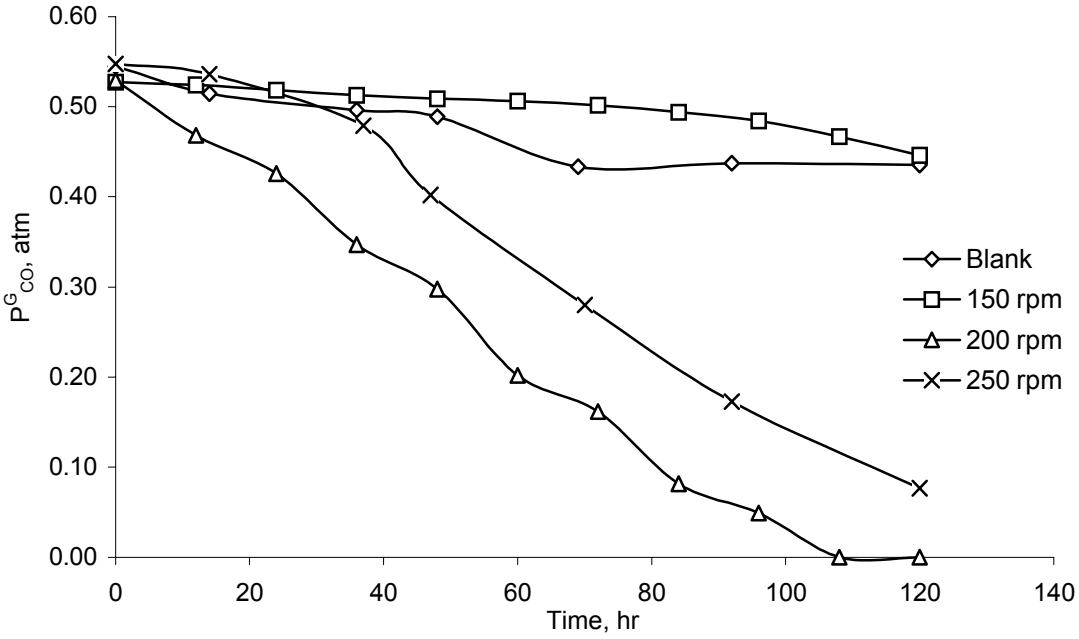

Figure 3. Effect of shaking frequency on CO consumption.

The specific growth rate increased at 200 rpm, but it started to decrease when 250 rpm was employed. The maximum specific growth rate for *R. rub rum* was resulted when the shaker was set at 200 rpm (0.1 h^{-1}). Decrease in cell growth possibly due to the shear forces and turbulent mixing, which could give a negative impact where it can damage the cell wall of the bacteria.

Comparing the specific growth rate between the cultures shaken at 250 rpm with the static culture, both achieved 0.06 h^{-1}. But the CO conversion for the shaken culture was higher. This result confirmed that even though the growth rate was high, but without mixing, the CO cannot be uptake by the cells. In other words, there was minimal mass transfer at static condition, where the CO bubbles were not thoroughly mixed with the microbial culture; therefore, the interfacial area of gas and liquid was limited. As a result, the oxidation of CO was also limited.

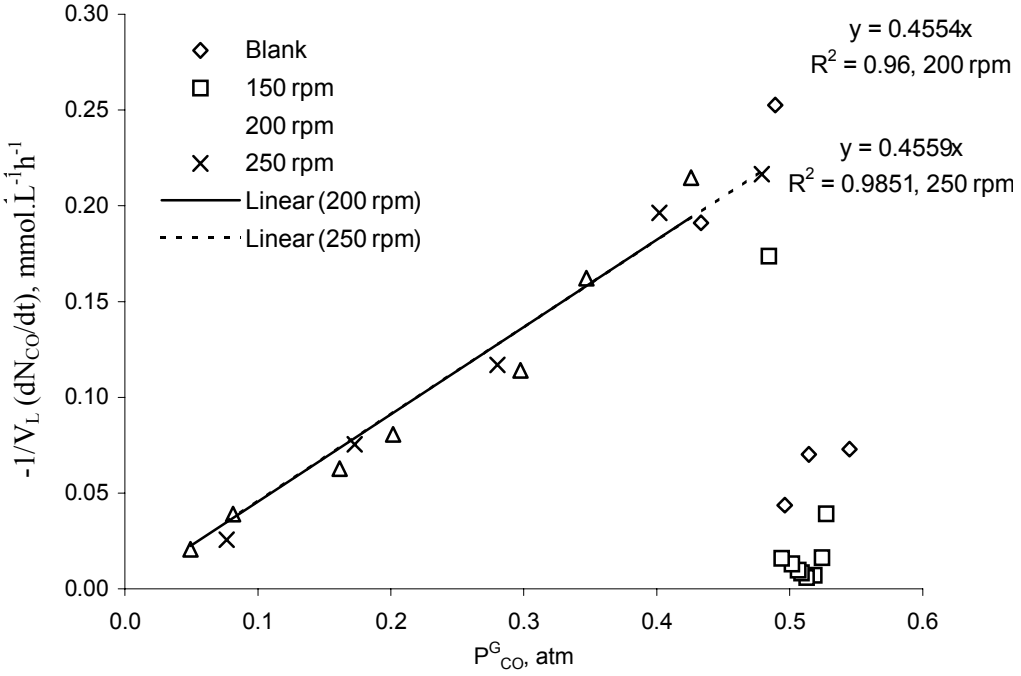

Figure 4. Effect of shaking frequency on K_La.

As expected, for hydrogen production, highest mass transfer with maximum mixing resulted in highest hydrogen production. The hydrogen production of 250 rpm reached the highest level after 120 hrs of incubation. The amount of hydrogen production at 150 rpm and stagnant condition was almost negligible. The high concentration of hydrogen productions was 14.5 and 16.9 mmol/l at 200 and 250 rpm, respectively.

Figure 3 shows the partial pressure of CO at different shaking frequencies. The drop of CO partial pressure indicated the CO consumption by *R. rubrum* in the gas phase. It shows that the CO partial pressure dropped drastically at high revolution rates of the shaker, 200 and 250 rpm, while at 150 rpm and static condition, a very low CO uptake was observed. However, the highest drop in CO partial pressure occurred in the cultures agitated at 200 rpm. When the media was shaken at higher rate, 250 rpm, the CO consumption started to drop. The most probable explanation was that some of the cells might be damaged due to shear and CO uptake was not satisfactory. This was clearly shown by the drop of specific growth rate at 250 rpm. The results from the above were used to validate the K_La. The correlation used for estimating the K_La was described in detail in the cited literature [17], where the final equation is:

$$-\frac{1}{V_L}\frac{dN_{CO}}{dt} = \frac{K_L a}{H}(P_{CO}) \qquad (2)$$

where V_L is the liquid volume, N_{CO} is the moles of CO transported, $K_L a$ is the mass transfer coefficient, H is the Henry's law constant and P_{CO} is the partial pressure of CO.

Employing the correlation in Equation 2, a linear model was obtained from the data resulted from 200 and 250 rpm only, whereas the data at 0 and 150 rpm were non-linear due to almost unchanged CO partial pressure. The stagnant media and low shaking frequency caused this, where insufficient mass transfer occurred. From the slope obtained in Figure 4, the value of K_La at both 200 and 250 rpm were found to be very close with insignificant difference, 0.48 hr^{-1}. Based on the results obtained, 200 rpm was chosen as the optimum shaking frequency for the batch bioconversion since an increase of more than 250 rpm didn't result in any improvement.

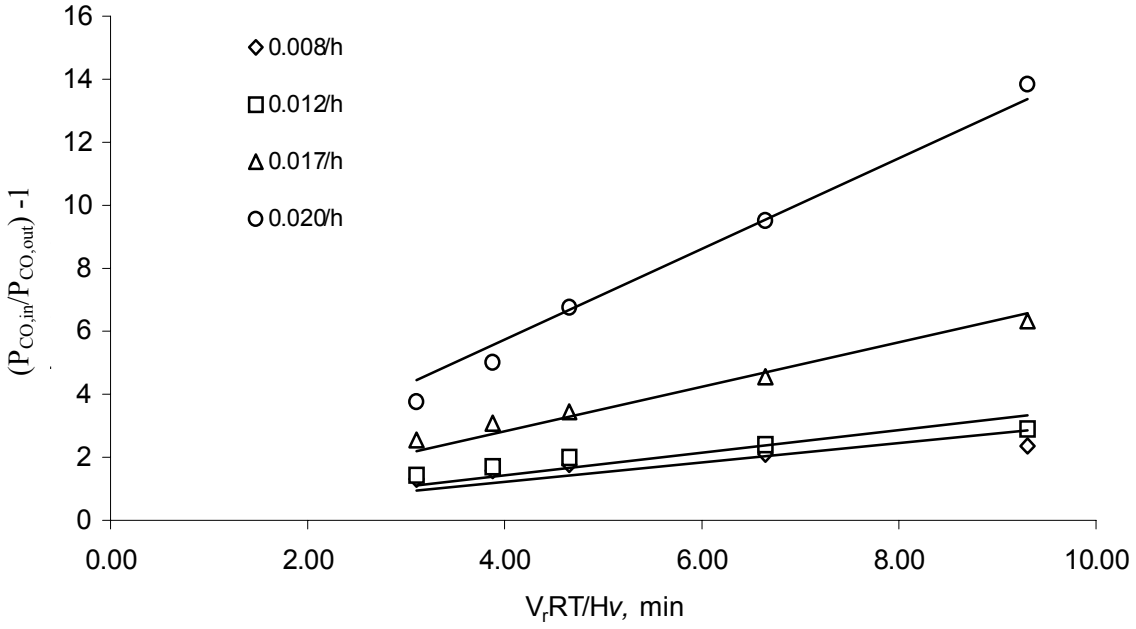

Figure 5. Effect of media dilution rate on K_La.

3.2 Continuous Mode Operation For a system to be commercialized, a continuous operation is necessary. The same media and condition was employed in the continuous system. For the K_La calculation, a correlation was developed based on material balance.

Following the material balance suggested by Levenspiel [18] for agitated tank contactor, it is assumed that the composition of the gases is uniform throughout the fermenter. Thus, the material balance for CO uptake rate and CO transferred from the gas phase has to be equal to the CO involved in the bioconversion process:

$$CO\big|_{\text{transferred from gas phase}} - CO\big|_{\text{uptake rate}} = CO\big|_{\text{consumption in the bioconversion}} \tag{3}$$

$$\frac{F_g}{\pi}\left(P_{CO,in} - P_{CO,out}\right) - \frac{K_L a}{H} P_{CO,out} V_r = \left(-r_{CO}\right) V_r \tag{4}$$

where $K_L a$, F_g and H are volumetric mass transfer coefficient (min^{-1}), molar flow rate of the gas (mole/min), and Henry's constant (atm.L/mole), respectively. The working volume of the reactor is V_r, and π is the total pressure of the gas phase:

$$\pi = P_{H_2} + P_{CO} + P_{CO_2} + P_{Ar} \tag{5}$$

The rate of CO disappearance by the reaction is shown as:

$$-r_{CO} = \frac{1}{V_r}\frac{dP_{CO,out}}{dt} = \frac{1}{V_r}\frac{dP_{CO,out}}{dX}\frac{dX}{dt} \tag{6}$$

where X is the cell density of the microorganism. By multiplying the above equation with V_r:

$$\left(-r_{CO}\right) V_r = \frac{dP_{CO,out}}{dt} = \frac{dP_{CO,out}}{dX}\frac{dX}{dt} \tag{7}$$

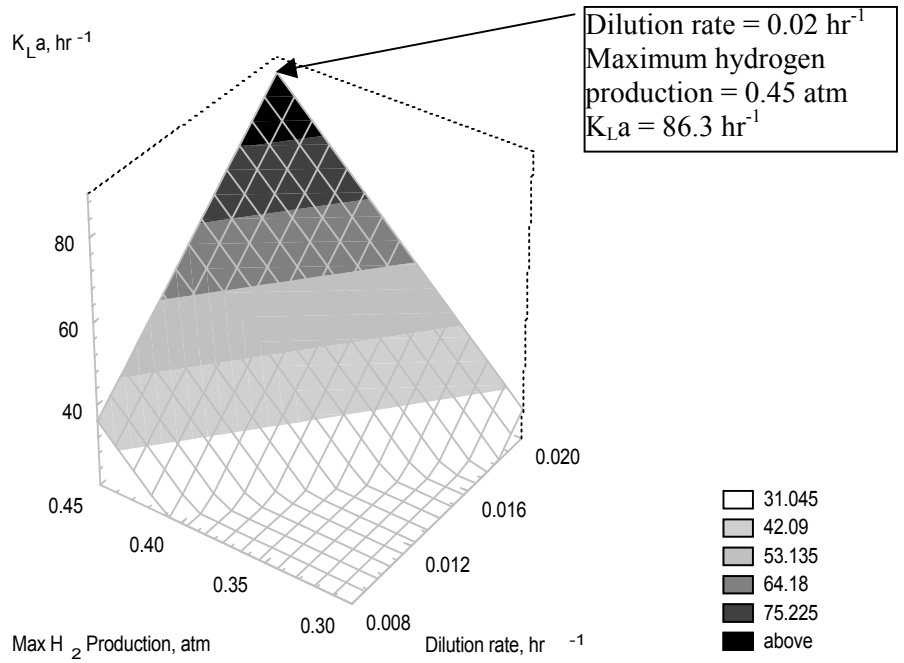

Figure 6. Change in K_La and hydrogen production at various liquid flow rates.

The microbial growth can be described by the Monod equation:

$$\mu X = \frac{dX}{dt} = \frac{\mu_m X P_{CO,out}}{K_M + P_{CO,out}} \quad (8)$$

Substituting this equation into Equation 7 yields:

$$(-r_{CO})V_r = \frac{dP_{CO}}{dX}\frac{\mu_m X P_{CO,out}}{K_M + P_{CO,out}} \quad (9)$$

where, K_M, μ, X and μ_m are Monod constant, specific growth rate, cell density and maximum specific growth rate, respectively.

By replacing the above equation in the mass balance results in:

$$\frac{F_g}{\pi}(P_{CO,in} - P_{CO,out}) - \frac{K_L a}{H} P_{CO,out} V_r = \frac{dP_{CO}}{dX}\frac{\mu_m X P_{CO,out}}{K_M + P_{CO,out}} \quad (10)$$

At steady state, rate of CO transferred from the gas phase, CO uptake and CO disappearance by reaction are equal. Then, both sides of the above equation are divided by $P_{CO,out}$. At steady state, $\frac{dP_{CO}}{dX} = 0$, resulted in

$$\frac{F_g}{\pi}\left(\frac{P_{CO,in}}{P_{CO,out}} - 1\right) = \frac{K_L a}{H} V_r \quad (11)$$

Rearranging the above equation resulted in a linear model with the slope representing mass transfer coefficient as stated below:

$$\left(\frac{P_{CO,in}}{P_{CO,out}} - 1\right) = \frac{K_L a}{H}\frac{V_r \pi}{F_g} \quad (12)$$

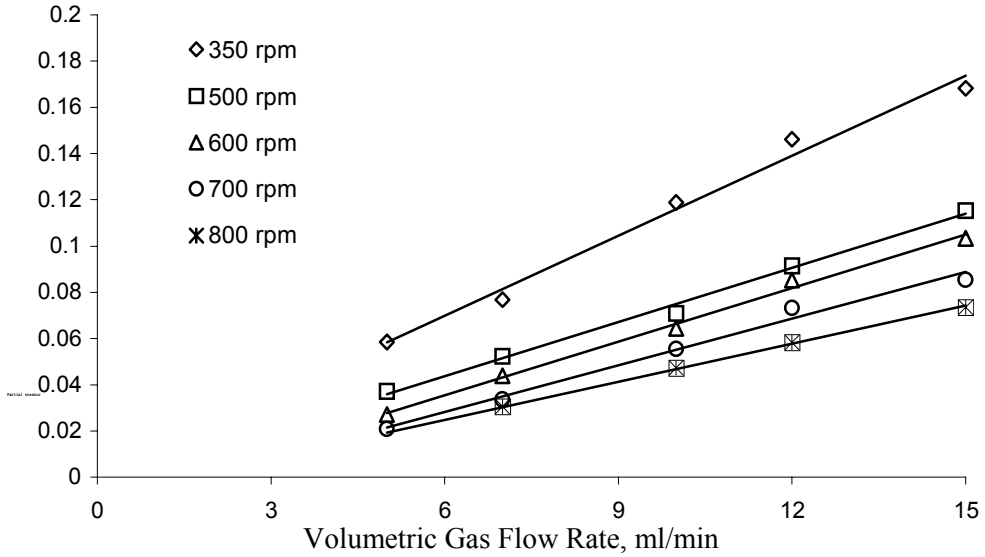

Figure 7. Effect of agitation rate and gas flow rate on CO partial pressure.

Applying ideal gas law to have the correlation in terms of gas pressure:

$$P_g v_g = F_g RT \text{ or } F_g = \frac{P_g v_g}{RT}$$

where subscript g is referring to inert gas

(13)

$$\left(\frac{P_{CO,in}}{P_{CO,out}} - 1\right) = \frac{K_L a}{H} \frac{V_r \pi\, RT}{P_g v_g} = K_L a \frac{V_r RT}{Hv}$$

(14)

By plotting $\left(\frac{P_{CO,in}}{P_{CO,out}} - 1\right)$ versus $\frac{V_r RT}{Hv}$, $K_L a$ can be determined from the slope.

3.3 Determination of $K_L a$ at Different Dilution Rates Effect of dilution rate was studied in order to observe the optimum condition for cell growth and to identify the critical dilution rate, D_c. In addition, it was necessary to know the efficient flow rate, to ensure that the sufficient nutrients needed by *R. rubrum*. The range of dilution rates studied was from 0.008 to 0.023 hr^{-1}. For every dilution rate studied, gas flow rate ranging from 5 to 15 ml/min were employed. The factors being considered in this experiment were the CO consumption in order to calculate $K_L a$, and hydrogen production. From the experiments, 0.023/h of dilution date was resulted in washout phenomena after 3 days of operation. At 0.02/h, the bioreactor was stable, maintaining a constant cell density of 0.29 g/L (result not shown). It was also yielded the highest hydrogen production, 14.5 mmol/hr compared to other dilution rates.

For the $K_L a$ measurement, the calculation was done by using Equation 14. The slope for each line representing the $K_L a$, was calculated. Figure 5 shows a significant improvement on mass transfer coefficient, when employing the high liquid flow rate of 0.65 ml/min (0.02/h) compared to other flow rates.

By increasing the dilution rate, the $K_L a$ was increased. The $K_L a$ was doubled once the media flow rate was increased by 18% (from 0.017 to 0.02 hr^{-1}). This may be related to the cell density when liquid flow rates were varied. It showed that

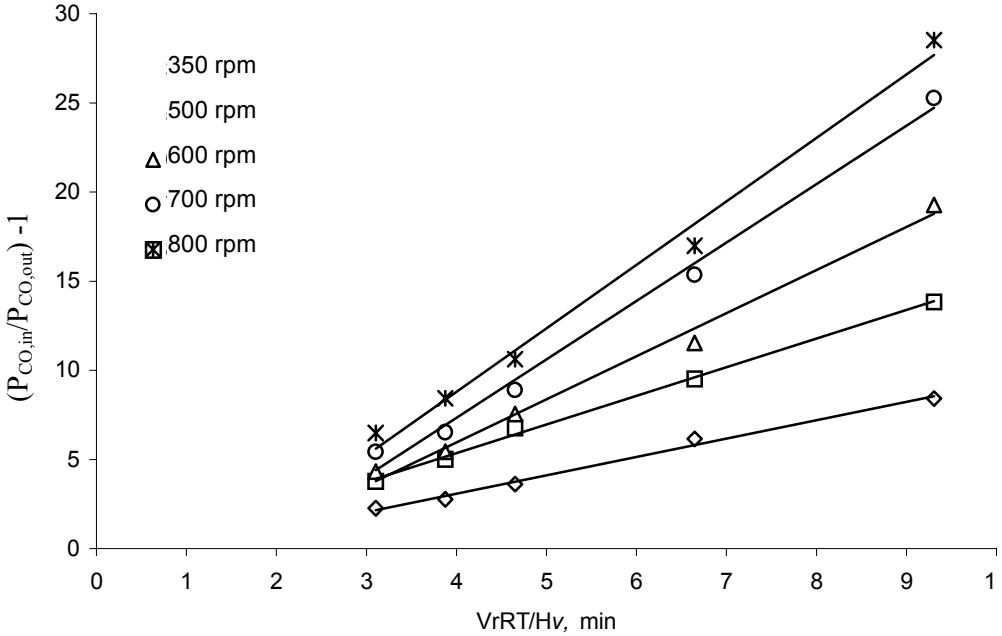

Figure 8. Effect of agitation rate on K_La.

the nutrients in the liquid media have significant effect on cell growth and K_La. Higher flow rate gave enough carbon sources and more longevity and stability to the cells, which resulted in higher CO uptake. To view the overall effect of dilution rate on K_La, and hydrogen production, it is best represented in a 3 dimensional graph as shown in Figure 6. Maximum hydrogen production of 16.3 mmol/hr at $0.02hr^{-1}$ dilution rate and K_La of 86.3 hr^{-1} was obtained.

Increasing the dilution rate also increased the mass transfer coefficient and hydrogen production. It was determined that 0.02 hr^{-1} was the maximum dilution rate, since it gave the maximum hydrogen productivity. For the following experiments, the flow rate of 0.65 ml/min (0.02 hr^{-1}) was implemented due to the stability of cell density and high hydrogen production.

3.4 Determination of K_La at Various Agitation Rates An optimum dilution rate of 0.02 hr^{-1} and constant light intensity, 2700 lux was employed throughout the experiment. Five agitation rates were selected, ranging from 350 to 800 rpm. A sufficiently high impeller speed was used so that flooding of impeller was avoided. The maximum agitation allowed by the fermenter motor was 1000 rpm. Five gas flow rates (5, 7, 10, 12 and 15 ml/min) were employed in order to calculate the K_La. Observations were made on the cell density, hydrogen production and CO uptake. Based on these considerations, a suitable agitation rate was chosen. Figure 7 presents the effect of agitation rate on CO partial pressure at various gas flow rates (5 to 15 ml/min) at ambient condition.

It is clear that by increasing the agitation rate, the partial pressure of CO in the outlet stream was decreased, which indicated that more CO were being consumed by *R. rubrum* and converted to hydrogen. This was due to the fact that the CO bubbles were dispersed in smaller sizes and therefore, surface area becomes larger. The bubbles were also recirculated in the liquid for sufficient time, enough to be uptake by bacteria. In contrast, applying high gas flow rate resulted in less CO uptake by the microorganism. The reason may be due to the ability to uptake CO was easier at lower flow rate. High flow rates tend to cause the CO bubble to leave the liquid phase faster and

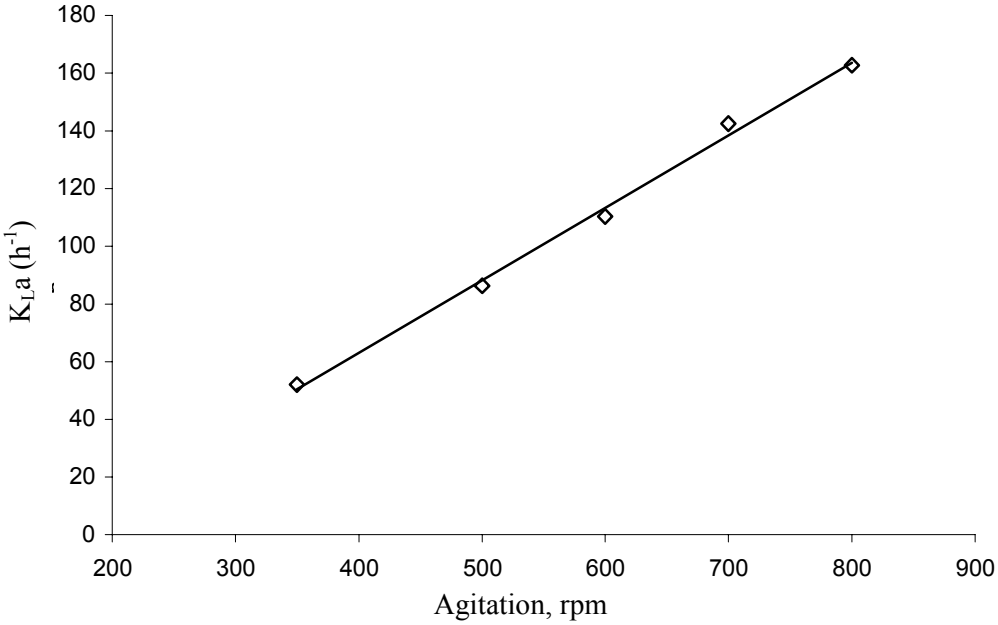

Figure 9. Effect of agitation rates on K_La.

gave less residence time in the liquid.

Mass transfer coefficient at various agitation rates was determined as shown in Figure 8. Referring to the slopes of the lines, the mass transfer coefficient at 350, 500, 600, 700 and 800 rpm were 52.2, 86.8, 110.4, 142.8 and 162.6 hr^{-1}, respectively. The highest mass transfer coefficient was obtained at 800 rpm; 162.6 hr^{-1}. The trend of K_La was a linear increment as the agitation speed was increased as summarized in Figure 9.

With an increment of 100 rpm for agitation, the K_La increased approximately 30%. These data agreed well with the theory that at higher agitation rate, more area is available for CO transfer and also the escape of gas bubbles from the liquid is delayed, thus high K_La is achieved. But in long-term usage, high agitation rate is not preferred due to foaming problem and high shear forces. This foam formation is undesirable because of the adverse effect such as the decrease of working volume and accumulation of reactants or products in the foam. Foaming also increase the chance of contamination especially when the outlet gas filter was wetted. Antifoam has not been used in this study since it may have an inhibiting effect on the growth of microorganisms. It acts by altering the surface tension characteristics that will also affect coalescence behavior of the liquid phase, which in turn will affect the K_La [19]. Unfortunately, the fermenter was not equipped with a mechanical foam breaker. Besides that, excessive shear due to vigorous mixing was known to damage suspended cells, thus leading to loss of viability and disruption of cells [20, 21]. Figure 10 explains the problem faced in hydrogen production when high agitation rates were employed. The data were taken based on constant gas flow rate, 10 ml/min and constant light intensity, 2700 lux.

In steady state and continuous process, the hydrogen production was almost constant for duration of one week, but the production was low at 350 rpm, constant at 8 mmol/hr and increased to 14.5 and 16.3 mmol/hr for higher agitation rate of 500 and 600 rpm, respectively. At very high agitation rate of 700 and 800 rpm, the hydrogen production were very high, reaching 17.4 and 18.1 mmol/hr, respectively for the first 3 days, but tend to decrease the following days. This showed that high agitation rate only gave a temporary excellent result but may not be stable and was not suitable for prolonged application. Moreover, high agitation rate may also lead to high

TABLE 2. Performance Data of Continuous Bioreactor at Various Agitation Rates.

Agitation Rate	350 rpm	500 rpm	600 rpm	700 rpm	800 rpm
Maximum CO Consumption, %	89	93	95	96	97
Maximum H_2 Production, mmol/hr	9.4	16.0	16.6	17.4	18.1
Mass Transfer (K_La), hr^{-1}	52.2	86.4	110.4	142.8	162.6
Reynolds Number*	11472	16389	19667	22946	26223
Power Consumption, W/m^3*	156	455.2	786.7	1250.3	1863.4
Condition	Stable	Stable	Stable	Foaming	Foaming

power consumption. Table 2 summarizes the experimental values from different agitation rates.

It was calculated that all the agitations employed provided turbulent flow to the media. From the data above, the suitable agitation chosen was between 500 to 600 rpm, since both gave a stable condition at a considerably long time. In addition, there was no foaming problem and the K_La values were also quite high. The hydrogen production and CO consumption did not differ much from the higher agitations. The optimum agitation rate chosen was 500 rpm, since the CO consumption and hydrogen production did not vary much with 600 rpm. Even though the K_La for 600 rpm was high, but the hydrogen production was almost the same with 500 rpm. Moreover, the power consumption of 500 rpm was lower. The hydrogen production at 500 rpm managed to be constant at 16 mmol/hr.

Besides the degree of agitation, the magnitude of K_La was also influenced by aeration. Indirectly, gas flow rate effect was observed in other experiments due to the need in calculating K_La. Figure 11 shows the effect of gas flow rate on CO partial pressure at other constant variables; 500 rpm, 0.02 hr^{-1} and 2700 lux.

Based on the results obtained, lower gas flow rate was much more favorable in terms of achieving high CO uptake. Gas flow rate at 5 to 7 ml/min gave the highest CO conversion based on the low CO partial pressure at the gas exit, 0.18 atm. At 10 ml/min, the CO partial pressure was about 0.22 atm. above that of this flow rate. The result showed a small fluctuation in the CO concentration. The CO partial pressure fluctuated between 0.25 to 0.3 atm at 12 ml/min, while at 15 ml/min, the reading of CO concentration fluctuated between 0.35 to 0.4 atm. The CO conversion at high flow rates were very low compared to lower gas flow rates. This fact is clearly shown in Figure 12. The highest CO conversion, 68% was obtained by employing gas flow rate of 5 ml/min, followed by 7 ml/min, 66%. The flow rate of 10, 12 and 15 ml/min each achieved 60, 52 and 34% respectively.

3.5 Performance of Reactors Both the batch and continuous reactors have their own advantages and disadvantages. From one point of view, continuous culture is an alteration from the batch culture, where the continuous supply of fresh media and withdrawn of spent broth and cells occurs simultaneously.

An advantage of the batch reactor is that it is easier for screening and ease of handling. Compared to continuous reactor, accountability on the bubble formation, foam generation and stability for prolong use were absent. It is also a simple and cheap process. The risk of contamination was small. However, a drawback from the batch reactor

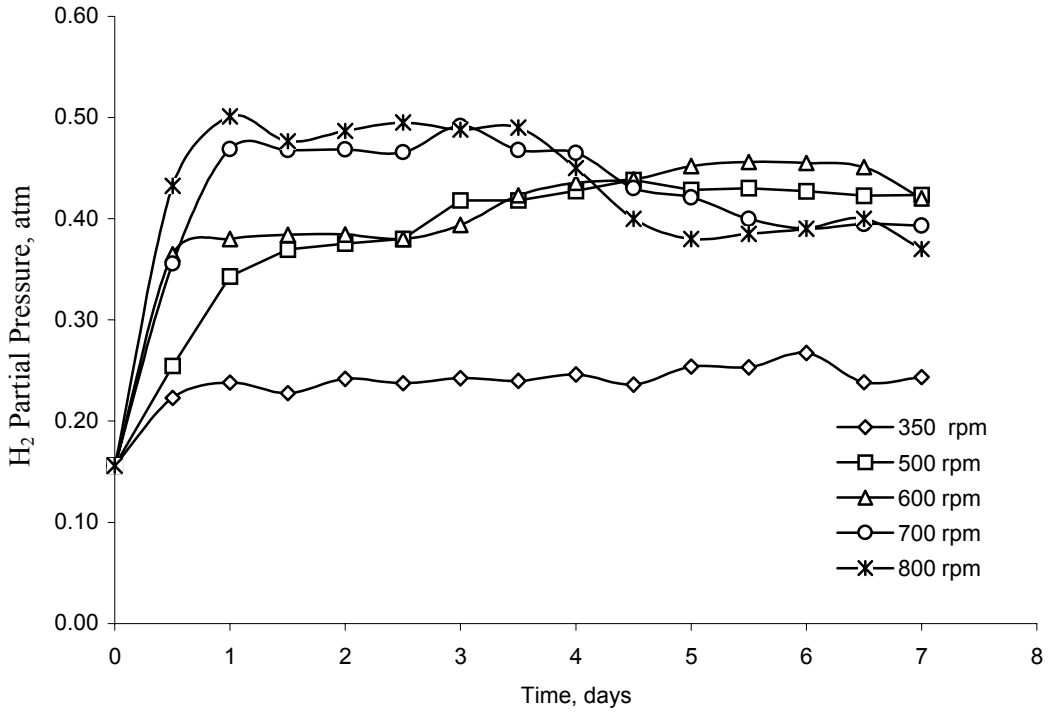

Figure 10. Effect of agitation rate on hydrogen production.

is that some internal parameters such as pH cannot be controlled. Moreover, the mass transfer was very low caused by insufficient mixing.

The advantage of continuous reactors is that it enables the maintenance of steady state where the cell concentration and production doesn't change with time. It helps to study the behavior of the microorganism when certain parameters are changed with respect to time. In all runs, the hydrogen production was found to start after a few hours of operation and CO was consumed nearly 75% within 24 hrs. Compared to the batch reactor, it took 4 days to consume the entire CO. This resulted in a low driving force and the CO uptake rate is considered to be kinetically limited.

4. CONCLUSION

Synthesis gas from biomass can be a cheap resource to produce clean fuel with *R. rubrum* as biocatalyst. The estimated K_La from both reactors were remarkably different. Higher K_La was obtained when continuous reactor was employed. In batch reactor, the highest K_La obtained was only 2.7 hr^{-1}, where in continuous reactor it reached to 162 hr^{-1} at 800 rpm. The K_La at optimum condition for both batch and continuous culture were 0.48 hr^{-1} and 86.4 hr^{-1}, respectively. The K_La was improved about 180 times from batch to continuous culture. Continuous hydrogen production was sustained at 16 mmoles H_2/hr. The content of this paper is hoped to give advances towards practical applications of biological hydrogen production from synthesis gas. It is considered that biological hydrogen production is the most challenging undertaking issue in the field of energy.

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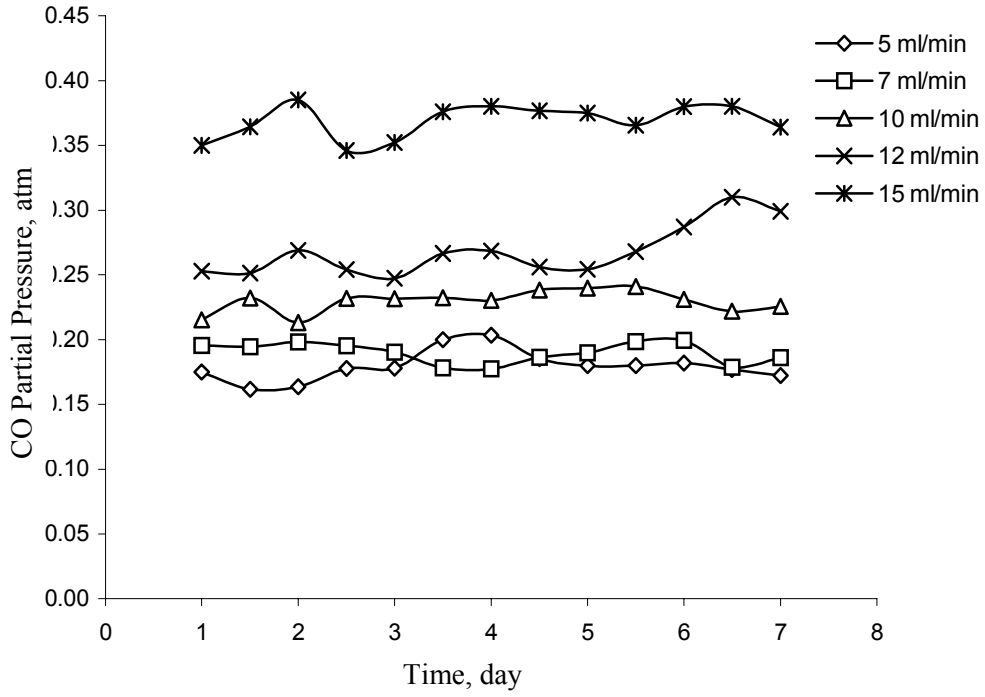

Figure 11. Effect of gas flow rate on CO partial pressure.

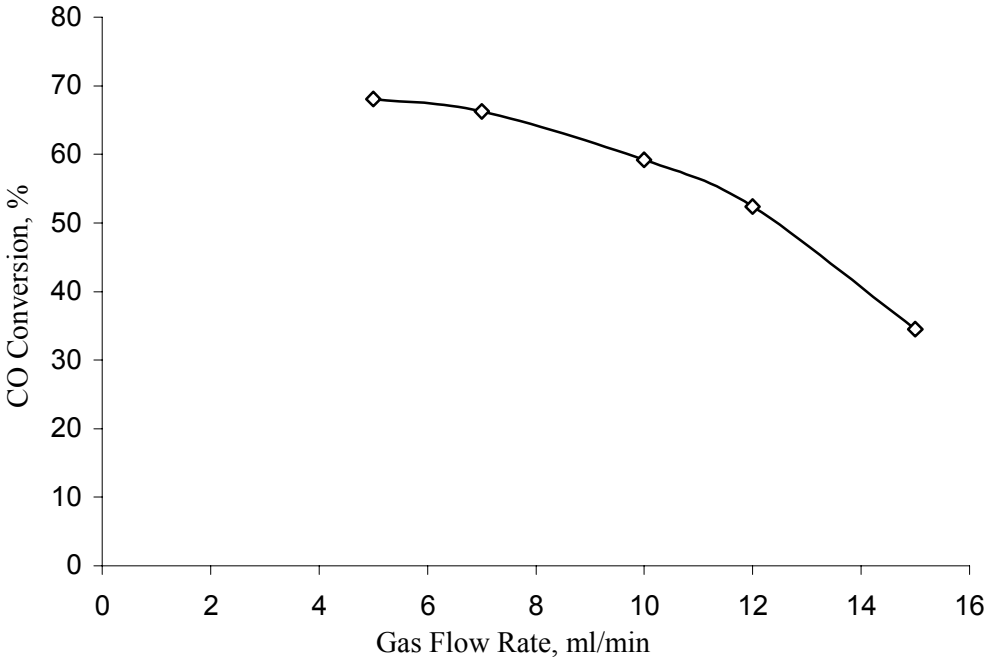

Figure 12. Effect of gas flow rate on CO conversion.

6. NOMENCLATURE

F_g	Molar flow rate of inert gas (mole/min)
H	Henry's law constant (L atm/mole)
$K_L a$	Overall mass transfer coefficient transported (h^{-1})
K_M	Monod constant (mole/L)
$P_{CO,in}$	Partial pressure ratio of CO at inlet atm
$P_{CO,out}$	Partial pressure ratio of CO at outlet atm
R	Ideal gas law constant atm. (L/mole°K)
$-r_A$	Rate of reaction for component A (mole/L.h)
T	Absolute temperature (°K)
t	Time (hr)
V_r	Working volume of reactor (L)
v	Volumetric gas flow rate (ml/min)
X	Cell density (g/L)
π π	Total partial pressure of the gases atm
μ	Specific growth rate h^{-1}
μ_m	Maximum specific growth rat h^{-1}

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