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CONTINUOUS HYDROGEN PRODUCTION VIA FERMENTATION OF SYNTHESIS GAS

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Abstract. *Biological hydrogen production from synthesis gas has been carried out in a continuous stirred tank bioreactor. The photosynthetic anaerobic bacterium,* Rhodospirillum rubrum *was used to oxidize CO and water to* CO_2 *and hydrogen in a CSTR fermenter. The dual-impeller bioreactor equipped with microsparger was used for biohydrogen production via water-gas shift reaction fermentation. At the steady state condition, the cell density was about 1.3 - 1.4 g/L with minimum dilution rate of 0.25 ml/min. The cell density was decreased at higher media flow rate (0.7 ml/min) in which wash out phenomena occurred. A linear kinetic model, Monod type of rate equation and Lineweaver-Burk plot were correlated with the experimental data. The values of* μ_{max} *and* K_s *for* R. rubrum *were 0.0225* h^{-1} *and 0.0135 g/L, respectively. Biomass yield was 0.36 g cell.* g^{-1} *of acetate. In terms of productivity, cell and substrate concentrations, Monod chemostat model was simulated well with the experimental data. The optimum value of dilution rate was 0.018* h^{-1} *(0.43* day^{-1}*). The hydrogen production rate and yield, at constant synthesis gas flow rate of 14ml/min, was 16 mmol* H_2 */ g cell. h and 80 %, respectively.*

Key words: *biohydrogen, Rhodospirillum rubrum, synthesis gas, Monod Chemostat Model, dilution rate*

Introduction

Preview of current researches are shown that, hydrogen is produced by number of processes, such as: electrolysis of water, thermo-catalytic reformation of hydrogen-rich organic compounds, pyrolysis of lignocellulosic biomass, and biological processes [1]. An excellent new area of fuel technology which has great potential to develop for future and practical applications is bio-hydrogen production. The biological route of hydrogen production provides a wide range of approaches to generate hydrogen, which includes direct bio-photolysis, indirect bio-photolysis, photo-fermentation, and dark-fermentation [2, 3, 4 & 5]. Hydrogen is produced via water-gas shift reaction by photosynthesis bacteria. While carbon monoxide is oxidized to carbon dioxide in presence of anaerobic bacteria, hydrogen is released from water gas shift reaction, shown below [6, 7 & 8]:

$$CO_{(g)} + H_2O_{(l)} \rightarrow H_{2(g)} + CO_{2(g)} \quad \triangle G = -20 \text{ kJ/mol} \qquad (1)$$

Many photosynthetic bacteria such as *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, *Rhodocyclus gelatinosus* and *Rhodospirillum rubrum* are able to catalyze the water-gas shift reaction under anaerobic condition at ambient temperature [9, 10 & 11]. However, the obtained results have shown that *Rhodospirillum rubrum* capable of producing hydrogen form synthesis gas with a very yield, close to theoretical value [12].

In this study, the experiments were carried out in a continuous stirred tank bioreactor (CSTR) using *Rhodospirillum rubrum* as biocatalyst to produce biological hydrogen from synthesis gas under anaerobic condition. The main objective of the present research work was to investigate the optimum condition of liquid flow rate and bubble size of inlet gas using microsparger.

Material and Methods

Microorganism. *Rhodospirillum rubrum* ATCC 25903 obtained from the American Type Culture Collection (ATCC, Virginia, USA) was used in this study. The microorganism was grown under anaerobic condition in a ATCC media with two tungsten light (40W) at 30°C. Agitation was applied to enhance microbial cell growth.

Continuous Fermentation. The fermentations were carried out in a 2-liter Biostat A (B Braun, Germany), cylindrical, flat-bottom jacketed vessel with 4 baffles. The diameter of the fermenter was 120 mm. Two sets of six-bladed disc turbine of the Rushton type were installed on one-third and two-third of the height from the bottom of the bioreactor. Gas was bubbled through a microsparger. The fermenter was equipped with a pH probe (Mettler Toledo, Germany), dissolved oxygen probe (Mettler Toledo, Germany) and level sensor. The fermenter was adapted for use under anaerobic conditions with continuous flow of liquid media and gas. Figure 1 shows the schematic diagram of the experimental setup with permanent stainless steel gas line and sterilizable silicon tubes for media in continuous operation.

The fermenter working volume was 2 liters and a 5% inoculum was used to start the experiment and also to eliminate the microbial lag phase. Two tungsten lamps (40 W) were provided from two sides of the fermenter for light illumination at average

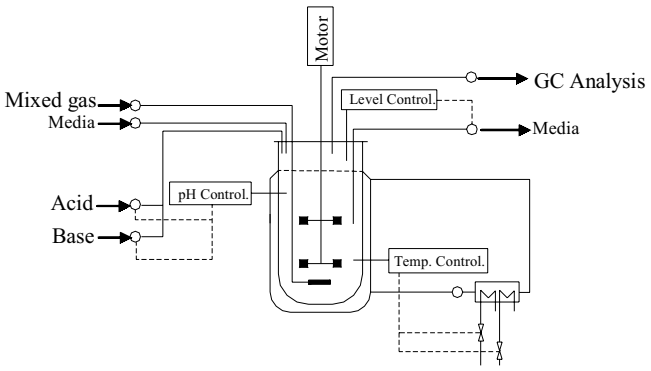

Figure 1. Continuous Stirred Tank, Experimental Setup with instrumentation and controllers

intensity of 4000 lux. The optimum pH (6.5) was controlled by 0.1 molar of acid and base solutions, HCl and NaOH (Merck) using peristaltic pumps. Synthesis gas flow rate (12 ml/min) was adjusted by a digital flow meter (Brooks, Holland). The liquid media flow rates were controlled with an external peristaltic pump (Masterflex, Illinois), in a specified flow rate range of 0.25 – 0.65 ml/min. The level controller was used to control the liquid level and the effluent stream was collected in a waste container. During experiments, agitation rate was fixed at 500 rpm.

Analytical Methods. The gas composition was quantified using a gas chromatograph (Perkin Elmer Autosystem XL, USA) equipped with a thermal conductivity detector (TCD) and a 60/80 Carboxene 1000 (Supelco, USA) column. The oven temperature was initially set at 40°C for 3.5 minutes, and then the temperature was programmed with a rate of 20°C/min till it reaches to 180°C. The detector and injector temperatures were 200 and 150°C, respectively. The flow rate of carrier gas, Helium (Sitt Tatt, Malaysia) was 30 ml/min. Argon gas was used as an internal standard. Calculations for the inlet and outlet gaseous compositions were accomplished by using the GC software, TotalChrom Workstation (Perkin Elmer, USA). To detect the residual acetate concentration in the liquid media, 0.5 ml of liquid sample was filtered (Whitman, 0.45 μm pore size, USA). The supernatant was diluted up to 1ml and acidified with 40 μl of propionic acid 1% as internal standard. A 0.4μl sample was analyzed by gas chromatography (Hewlett Packard 5890 series II, USA) equipped with a flame ionization detector (FID) and a 80/120 Carbopack B-DA/ 4% Carbowax 20 M (Supelco, USA) column. The oven temperature was kept constant at 175°C during the gas analysis. Both, the injector and detector temperatures were 225°C. The flow rate of carrier gas, Nitrogen (Sitt Tatt, Malaysia) was 25 ml/min. To measure the cell concentration of *R. rubrum*, 1ml of cell suspension was diluted up to 10 ml and the cell dry weight was measured by spectrophotometer (Cecil 1000series, UK) with standard cell dry weight calibration curve.

Results and Discussion

Hydrogen production. *Rhodospirillum rubrum,* the purple nonsulfur anaerobic bacteria, which are capable of catalyzing the Fisher-Tropsch type reaction and water-gas shift (WGS) reaction [13]. While, the microorganism required carbon source other than carbon monoxide to propagate, such as acetate, malate, glucose, yeast extract and ammonium [14, 15 and 16]. It has been reported that *R. rubrum* was able to uptake CO with faster growth rate and higher cell density in compare to other hydrogen producing microorganisms [17]. It has also been reported that the initial substrate concentration may inhibit the cell growth of microorganism and prevent hydrogen production [18]. In continuous hydrogen production, 4 g/L acetate was used as source of energy for bacterial growth. Figure 2 shows the cell density and acetate concentration with respect to fermentation time at different liquid media flow rate. There was slightly fluctuation in cell density with the change of media flow rate. The cell density was maintained in the range of 1.2 to 1.4 g/L with media flow of 0.25 to 0.7 ml/min.

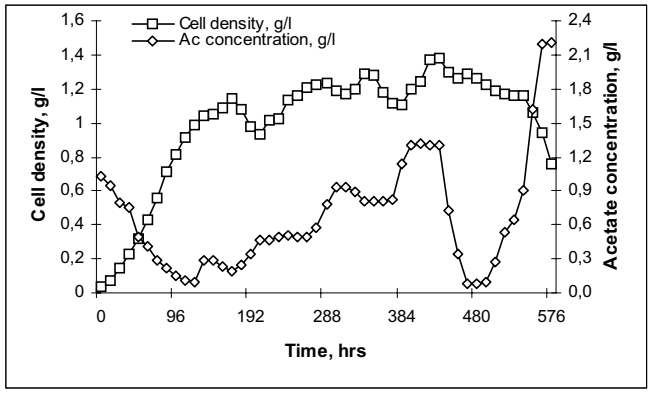

Figure 2. Cell density and acetate concentration with variable media flow rates

The cell density was stable at 1.3 g/L with low media flow rate of 0.25 ml/min and very low substrate concentration while containing sufficient cells in the fermentation broth. High media flow rate (0.7 ml/min) caused the cell density dropped since insufficient retention time caused the acetate was not fully used for cell growth. Therefore the cells were diluted and washed out from the bioreactor. The decrease in cell concentration resulted in an increase of acetate concentration. In this case, the microorganism might be inhibited at higher feed flow rate, high substrate concentration and minerals. The average biomass substrate yield of 0.36 g cell/g substrate was obtained.

The mechanism of hydrogen production was reported that photosynthetic bacteria obtained energy from catabolism of organic substrates that may drive hydrogen production, but not utilizing water as a source of reducing power [19 & 20]. CO is the major component of the synthesis gas, which is produced by gasification of coal or biomass [21]. However, photoheterotrophic microorganisms are capable to grow either in presence or absence of organic sources. They are able to oxidize CO to CO_2 while simultaneously water is reduced to H_2 [22 & 23].

In this study, continuous production of molecular hydrogen by *R. rubrum* was successfully carried out in a 2 liter CSTR with the acetate as electron donor and energy source for growth in continuous hydrogen production and stable duration of 25 days. In the present study, *R. rubrum* was grown on acetate as carbon source of energy for growth in a 2 liter continuous stirred tank bioreactor. Figure 3 shows steady hydrogen production and CO consumption for duration of 25 days. There was system disturbance at the time of media reservoir interchanging as indicated in figure 3. The hydrogen production rate and pro-

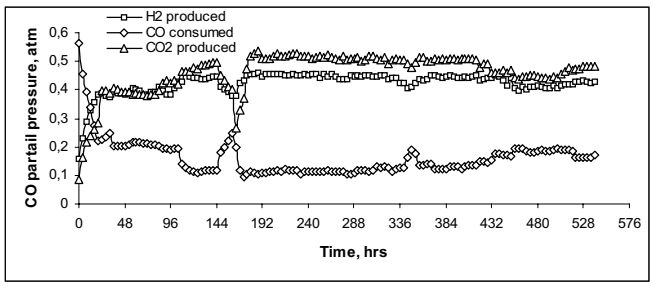

Figure 3. Hydrogen production for duration of 25 days in continuous operation mode

ductivity yield, at fixed synthesis gas flow rate of 14ml/min, were 16 mmol/ g cell. h and 80 %, respectively. The continuous anaerobic hydrogen driven bioreactor by *R. rubrum* was used to produce molecular hydrogen whey consisting lactic acid solution. It was reported that hydrogen productivity was 20 ml/ g cell. h with 65% yield [24]. Production of hydrogen from synthesis gas by *R. rubrum* was also studied by other research scientists in a continuous CSTR. The rate of hydrogen production was reported 3.5-10 mmol/g cell. h with 88% yield. [25].

Monod chemostat model. At steady state operation, it is considered that the volumetric flow rates in and out of bioreactor are the same and no live cells enter to the fermenter as the media stream was fed at sterile condition. Therefore, the common parameter to bioreactors are called, dilution rate

$$D = \frac{v_o}{V} \tag{2}$$

where, D, v_o, and V are dilution rate (h^{-1}), volumetric flow rate (l/ h), and bioreactor volume (l), respectively. The cell growth rate can be determined by Monod equation:

$$\mu = \frac{1}{x}\frac{dx}{dt} = \frac{\mu_{\max} C_s}{K_s + C_s} \tag{3}$$

where, μ, x, t, μ_{max}, C_s, and K_s are specific growth rate (h^{-1}), cell density (g/L), time (h), maximum specific growth rate (h^{-1}), substrate concentration (g/L), and Monod constant (g/L), respectively. At steady state operation with constant cell density the material balance would leads to:

$$\mu = D \tag{4}$$

Examination of equation (4) describes that by controlling the dilution rate can control the specific growth rate. Substituting equation (4) into equation (3) gives:

$$D = \frac{\mu_{\max} C_s}{K_s + C_s} \tag{5}$$

Solving equation (5) for the steady state substrate concentration yields:

$$C_s = \frac{DK_s}{\mu_{\max} - D} \tag{6}$$

It has been observed that the total amount of cell mass formed by the cell growth is proportional to the mass of substrate utilized [26]. Thus, the following ratio defined the called yield coefficient

$$Y_{x/s} = \frac{\Delta x}{\Delta s} \tag{7}$$

As mentioned above, there was no cell live in the fresh media, as the media were sterile. The biomass produced is based on substrate utilized time yield coefficient:

$$x = Y_{x/s}(C_{s_o} - C_s) \tag{8}$$

Substituting equation (6) into equation (8) provides:

$$x = Y_{x/s}\left(C_{s_o} - \frac{DK_s}{\mu_{\max} - D}\right) \tag{9}$$

Multiplying both sides of equation (9) by D gives:

$$Dx = DY_{x/s}\left(C_{s_o} - \frac{DK_s}{\mu_{\max} - D}\right) \tag{10}$$

where, Dx is the rate of cell production per unit volume of reactor or productivity, g/L.t. To investigate the effect of dilution rate, it can be calculated the maximum cell output by differentiating the cell production rate with respect to the dilution rate:

$$\frac{d(Dx)}{dD} = 0 \tag{11}$$

Then, by differentiating equation (11) yields:

$$D_{\max\, output} = \mu_{\max}\left(1 - \sqrt{\frac{K_s}{K_s + C_{s_0}}}\right) \tag{12}$$

Equation (12) shows that, if $C_{s_0} >> K_s$, the value of $D_{max\ output}$ approaches to μ_{max} and nearly is washout.

The growth kinetics parameters such as μ_{max} and K_s values for *R. rubrum* were computed. A linear model can be used by linearization method known as Lineweaver-Burk plot, which follows by the rearrangement of equation 3, it gives:

$$\frac{1}{\mu} = \frac{1}{\mu_{\max}} + \frac{K_s}{\mu_{\max}}\frac{1}{C_s} \tag{13}$$

The final expression that correlates the specific growth rate of substrate for *R. rubrum* is described in equation (14):

$$\mu = \frac{0.0225 C_s}{0.13 + C_s} \tag{14}$$

The plotted data is shown in Figure 4. The obtained data are well fitted with the linear model. The smaller value of K_m is shown that the better progressive rate equation was achieved. The substrate concentration profile and cell density is shown in Figure 5.

The dual-impeller bioreactor of which was equipped with microsparger was selected for kinetic studies and biohydrogen production using *R. rubrum* for the water-gas shift reaction fermentation.

In the case of CSTR for continuous fermentation, high impeller tip velocity was carried out, the gas dispersion and the gas hold-up was increased with the agitation rate. The increase in gas hold-up with gas pressure (P_g) may be explained by the increase in recirculation of the liquid and the bubble size [27].

It was reported that the addition of tensioactive materials tends to decrease bubble size and the effect of impeller is not significant to produce smaller bubble size [28]. Due to the non-coalescing system, in this studies preferred to examine the effect of the reduced bubble size of the gas and the results are compared with the data from literature. Biological hydrogen production from CO by using a new chemohetetrophic bacterium

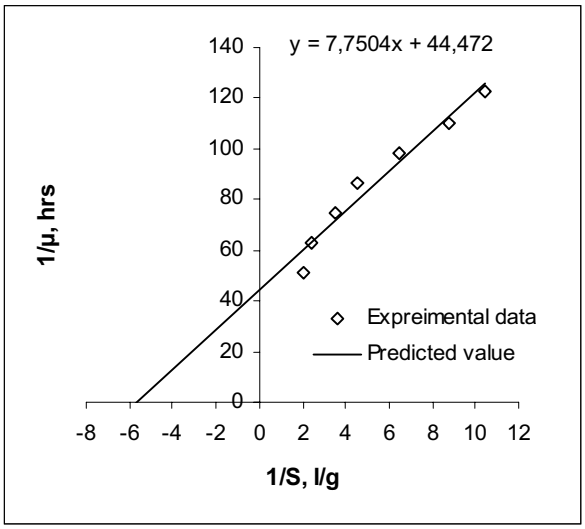

Figure 4. Monod rate equation based on Lineweaver-Burk plot

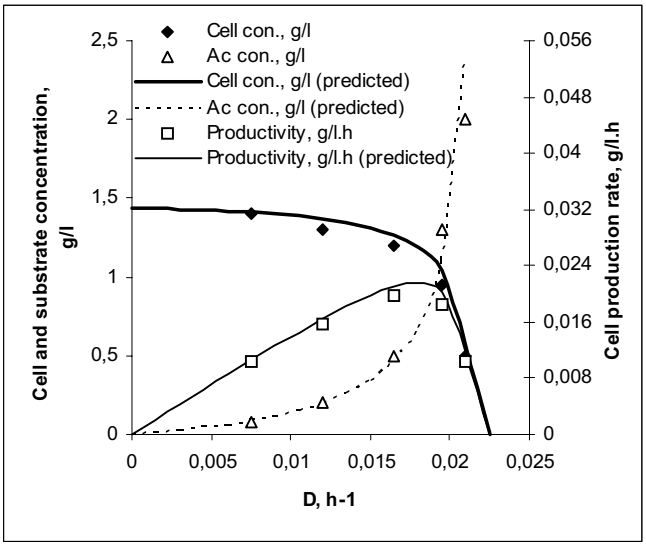

Figure 5. Effect of dilution rate on cell density, substrate concentration, and cell production rate

Citrobacter sp. Y19 was investigated. It was reported that the maximum hydrogen production was 9 mmol/g cell. h [29]. The obtained rate stated above was 16 mmol/g cell. h, that is about 78% higher than the reported data in the literature.

Hydrogen evolution of *R. rubrum* from synthesis gas was also studied at various dilution rate. It was reported that the hydrogen production by a continuous culture of *R. rubrum* on mixture of lactic and glutamic acid was carried out at the dilution rate of $0.0135h^{-1}$ [24]. The dilution rate in the present studies was $0.018h^{-1}$ ($0.43day^{-1}$). The obtained data was compared with reported result in a 1.25L CSTR was $0.67day^{-1}$ [30]. The comparison shows that there was slightly improvement on decreasing retention time. Figure 5 shows the dependence of cell concentration, substrate concentration, and productivity on continuous liquid dilution rate was calculated from the equation (6), (9), and (10) with the parameters stated in table 1.

Table 1. The parameters resulted from Monod Chemostat model

Maximum specific growth rate, h^{-1}	Biomass yield on substrate, $g.g^{-1}$	Substrate constant, g/L	Initial substrate concentration, g/L
0.0225	0.36	0.13	4

The obtained value for the dilution rate was $0.018\ h^{-1}$ was very close and comparable to the computed value from the rate equation (12), $0.0185h^{-1}$.

Conclusion

Hydrogen production as a clean fuel and is one of the most important useful source of energy for future. However, in this study, it has been demonstrated that biohydrogen production by *R. rubrum* was successful from the synthesis gas fermentation by dissipating smaller bubble size of the gas purged in the liquid media. According to the achievement made in this research, the yield of the hydrogen production by microorganism was increased. The obtained results compared with data reported in the literature [25], the hydrogen production rate was increased by 33% with the present research studies.

Nomenclatures

D = dilution rate, h^{-1}
v_o = volumetric flow rate, lh^{-1}
V = volume of bioreactor, l
μ = specific growth rate, h^{-1}
x = cell density, gl^{-1}
t = time, h
μ_{max} = maximum specific growth rate, h^{-1}
C_s = substrate concentration, gl^{-1}
K_s = Monod constant, gl^{-1}
$Y_{x/s}$ = biomass yield coefficient, g cell. g^{-1} substrate

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