
RESEARCH NOTE

DYNAMIC MODEL FOR PRODUCTION OF BIOHYDROGEN VIA WATER-GAS SHIFT REACTION

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Abstract In design of anaerobic bioreactor, rate equation is commonly used. Mathematical model was developed at steady state condition, to project concentration of gaseous substrate and product in biological oxidation of carbon monoxide with water to produce hydrogen and carbon dioxide. The concept of bioconversion was based on transport of CO from gas phase to liquid phase, as the CO consumption was instantaneous and the moles of CO in liquid phase was oxidized to CO₂, and H₂ was liberated from water. The moles of produced H₂ were identical to the moles of CO transported to the fermentation media. The data was experimentally obtained in a continuous stirred tank bioreactor. A photosynthetic bacterium, *Rhodospirillum rubrum*, was used as biocatalyst to facilitate the oxidization of carbon monoxides via water-gas shift reaction. The rate of CO consumption and hydrogen production were projected based on dynamic model at steady state condition. The experimental data were fitted to a few rate models and the best suitable dynamic model for hydrogen production was obtained. The model was used for scale up calculation and dependency of the rate equation and the model to a few process variables were analyzed. The liquid phase medium was supplied for microbial growth with initial concentration of 4 g/l. The media flow rate to the reactor space time (F/V_L) was 0.2 h⁻¹. At the steady state condition, the concentration of acetate was independent of the dilution rate and it was approximated about 1.5 g/l.

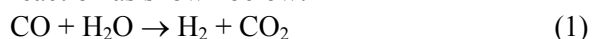
Keywords CO transport Model, Steady State Mass Transfer, Continuous Bioreactor, Photosynthetic Bacteria, Hydrogen, *Rhodospirillum rubrum*.

چکیده در طراحی بیوراکتورهای بی هوازی از معادله سرعت به طور معمول استفاده می شود. مدل ریاضی در شرایط ماندگار که پیش بینی غلظت سوبسترای گازی و فراورده های بیولوژیکی حاصل از واکنش اکسیداسیون گاز منو اکسید کربن با آب، که منجر به تولید هیدروژن و گاز کربنیک گردیده را می نماید. اصول تبدیل بیولوژیکی بر اساس انتقال گاز CO از حالت گاز به مایع بوده، همچنان که گاز منو اکسید کربن، به خودی خود نمی تواند در حضور ارگانیزم مصرف شود، اکسیداسیون CO در فاز مایع صورت می گیرد، در نتیجه هیدروژن و گاز کربنیک از آب آزاد می شوند. مول های گاز CO انتقال یافته به محیط کشت یا مول های هیدروژن تولید برابرند. داده های تجربی که از بیوراکتورهای پیوسته CSTR بدست آمده است، میکروارگانیزم های فتوسنتزی *Rhodospirillum rubrum* را به عنوان بیوکالیست که موجب تسریع واکنش جابجایی آب و گاز با اکسیداسیون منواکسید کربن می گردد. سرعت مصرف گاز CO و تولید هیدروژن، طبق مدل دینامیکی سرعت واکنش در شرایط ماندگار بدست آمده است. سرعت واکنش حاصل برای مقیاس بزرگ قابل استفاده است. وابستگی سرعت واکنش و مدل دینامیکی با چند متغیر فرایندی مورد تجزیه و تحلیل قرار گرفته است. غلظت استات در محیط کشت 4 گرم در لیتر بوده که برای سرعت رقیق شدن 0.2 h⁻¹ و در شرایط ماندگار که غلظت استات به رقیق شدن محیط کشت بستگی نداشته و میزان آن 1/5 گرم در لیتر تعیین گردید.

1. INTRODUCTION

Today, global energy requirements are absolutely depend primarily on fossil oil. The utilization of fossil fuel causing environmental pollution and global climate changes resulted from emission of combusted gases, soot and ash. Hydrogen is known as clean fuel when is combusted water is formed. An alternative source of energy such as biomass has been considered as a suitable alternative fuel for replacement of fossil fuel. Biomass gasification was considered as a potential process to produce synthesis gas [1, 2]. The aim of gasification process was to convert the chemical energy into combustible fuel. The organic wastes are another raw material for gasification process which is converted to synthesis gas, where the major component is carbon monoxide, produced via gasification of nongaseous material such as coal and biomass [3, 4, 5]. The CO is converted to fuels and chemicals through catalytic process such as Fisher-Tropsch synthesis. The processes are operated at elevated operating temperatures and pressures, therefore these processes may not be economically feasible [6, 7].

In conventional process of synthetic fuel production the synthesis gas was pioneered by the Fischer Tropsch synthesis, where the CO was converted to hydrogen via water-gas shift (WGS) reaction as shown below:



The Fischer Tropsch synthetic alcohol process operates at temperatures range of 220 to 340°C and pressure reaching up to 25 bars [1]. Apart from that, photobiological hydrogen generation has received special attention due to several advantages offered by strains of bacteria. There are many potent microorganisms capable of producing hydrogen from various organic wastes [8, 9, 10]. Among the famous hydrogen producers studied utilizing CO as substrate was the photosynthetic bacteria: *Rhodospseudomonas gelatinosa*, *Rhodospirillum rubrum*, *Rubrivivax gelatinosus*, *Rhodospseudomonas palustris* and a chemoheterotrophic bacteria *Citrobacter sp. Y19* [11, 12]. The biocatalysts have the ability to carry out the WGS reaction according to Equation (1) at ambient temperature and pressure. The use of microorganism *R. rubrum* was the main interest of

the present research paper. A notable feature of *R. rubrum* was preferable than any other anaerobic bacteria. *R. rubrum* had the ability to tolerate up to 93% of CO in the gas phase [13]. However, the obtained results have shown that *R. rubrum* was capable of producing hydrogen from synthesis gas with a very high yield, close to theoretical value [14.]

In this study, the experiments were carried out in a continuous stirred tank bioreactor (CSTR) using *R. rubrum* as biocatalyst to produce biological hydrogen from synthesis gas under anaerobic condition. The main objective of the present research work was to use a dynamic model with the suitable kinetic model that fit the experimental data with the proposed model. Projection of substrates and product concentrations was successfully demonstrated by comparing the actual data with the value calculated by the model and to show how the bench scale process was continuously operated for long duration of hydrogen production.

2. 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 basal media with two tungsten light (40W) at 30°C. Agitated vessel was applied to enhance microbial cell growth.

3. 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 Fermentor 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 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 rates in the 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.

4. 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 reach 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 Carbowax 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.

5. RESULTS AND DISCUSSION

Kinetics of Acetate Utilization and biomass production

Rhodospirillum rubrum, the purple nonsulfur anaerobic bacteria, which were capable of catalyzing the water-gas shift reaction. While, the microorganism required carbon source other than carbon monoxide to propagate, such as acetate, ammonium chloride as nitrogen source and trace metal. In continuous hydrogen production, 4 g/l acetate was used as nutrient for bacterial growth. Figure 2 illustrates the overall balance for cell (biomass), acetate concentration, CO concentration and hydrogen production. There was a slightly fluctuation in cell density with the changes of media flow rate (dilution rate, D). 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 (dilution rate of 7.5×10^{-3} to 2.1×10^{-2} h⁻¹). First order kinetic assumption was justified based on progressive growth of microorganism in exponential phase. The dynamic model was used with first order kinetic rate for utilization of acetate. Other rate models were also examined. With the proposed model, the acetate concentration was leaving from the bioreactor vessel was projected based on concept discussed in following paragraph.

A bench scale bioreactor was continuously operated with constant working volume (2L) with sufficient mixing. The cells were cultivated in the fermentation broth on continuous basis, as the acetate was pumped in with fixed dilution rate. As

a result CO in liquid phase was oxidized with water in presence of biocatalysts, hydrogen and CO₂ were formed. Additional assumption was made for the agitated vessel that the biochemical reaction in the broth had uniform composition through out the entire experiment. The mixing was provided by means of disc turbine of the Rushton type impellers and the rising gas bubbles were uniformly distributed with micro-sparger in the entire working volume.

The substrate balance in the fermentation vessel is shown by the following equation derived by mole balance:

$$\begin{aligned} & \text{Moles acetate}|_{in} - \text{Moles acetate}|_{out} + \text{Moles of} \\ & \text{acetate utilized by microorganisms} \\ & = \text{Accumulation} \end{aligned}$$

Acetate balance in the fermentation vessel is written as follows:

$$V \frac{dC_A}{dt} = Q_{A,in} C_{A,in} - Q_{A,out} C_{A,out} - (-r_A)(V) \quad (2)$$

The rate of disappearance of acetate utilized by microorganism, assumed to be first order rate equation:

$$-r_A = k_1 C_A \quad (3)$$

After substitution of rate equation into equation 2 and dividing the balanced equation by fermentation volume (V), resulted:

$$\frac{dC_A}{dt} + k_1 C_A = \frac{Q_{A,in} C_{A,in} - Q_{A,out} C_{A,out}}{V} = D(C_{A,in} - C_A) \quad (4)$$

The substrate balance leads to a non-homogenous ordinary differential equation, may easily be solved with initial condition. By integration and substitution of initial condition with known concentration of acetate at initial condition for the experiment, the differential equation has been resulted concentration profile for acetate with respect to time, given in equation 6:

$$C_A = e^{-(D+k_1)t} \left[\int D C_{A,in} e^{(D+k_1)t} dt + c \right] = \frac{D C_{A,in}}{(D+k_1)} + c e^{-(D+k_1)t} \quad (5)$$

where c is the integration constant, it was computed with defined initial condition $t = 0$, $C_A = C_{A0}$, $c = C_{A0} - \frac{D C_{A,in}}{(D+k_1)}$

$$C_A = C_{A0} e^{-(D+k_1)t} + \frac{D C_{A,in}}{(D+k_1)} \left(1 - e^{-(D+k_1)t} \right) \quad (6)$$

For special case, when $C_{A0} = C_{A,in}$, then equation 6 is reduced to:

$$C_A = \frac{C_{A,in}}{(D+k_1)} [D + k_1 e^{-(D+k_1)t}] \quad (7)$$

With the lowest substrate concentration at maximum time, let us define a new boundary condition. Equation 6 was differentiated with respect to time and then maximized. As a result, the utilization rate constant was determined, that is shown in equation 8. When $t = t_{max}$,

$$\frac{dC_A}{dt} = [-(D+k_1)C_{A0} + D C_{A,in}] e^{-(D+k_1)t_{max}} = 0 \quad (8)$$

Equation 8 was solved for the rate constant and k_1 was defined as following:

$$k_1 = D \left(\frac{C_{A,in}}{C_{A0}} - 1 \right) \quad (9)$$

Given initial acetate and inlet acetate concentrations with maximum dilution rate, the rate constant was calculated based on equation 9 and is linearly illustrated in Figure 3. The experimental data followed and fitted fairly well with the proposed equation for the rate constant.

Figure 4 shows the acetate concentration leaving the bioreactor. The experimental data were fitted with variable media flow rates presented by the culture dilution rates were in the range of 0.0 to 0.025 h⁻¹.

The continuous culture of microbial cells was grown in an open system where the cell concentration was maintained constant while the fresh media was replaced with the old culture at the same rate. The growth rate was determined by the rate of supplied essential nutrients. The relation between dilution rate and cell concentration was expressed by biomass balance. Sterilized media was used; the biomass balance is expressed in the following equations:

Accumulation of Biomass =

Biomass in – Biomass out + Biomass growth

$$\frac{dx}{dt} = -DX + \mu X \quad (10)$$

For the cell growth rate equation, there are number of microbial growth kinetics summarized in Table 1 [15]. Logistic rate model was used. Substituting μ into equation 10 is resulted the following differential equation:

$$\frac{dX}{dt} = -DX + X\mu_m \left(1 - \frac{X}{X_m}\right) \quad (11)$$

The general solution for equation 11 is given in equation 12.

$$X = \frac{X_o e^{-(D-\mu_m)t}}{1 - \left(\frac{\mu_m}{(D-\mu_m)}\right) \left(\frac{X_o}{X_m}\right) \left[1 - e^{-(D-\mu_m)t}\right]} \quad (12)$$

The analysis of data may lead to compare growth rate, μ with the media dilution rate, D. There are three special cases of μ greater than D, μ equal to D and μ less than D. When D is equal to zero, equation 12 is reduced to logistic model, presented by equation 13.

$$X = \frac{X_o e^{\mu_m t}}{1 - \left(\frac{X_o}{X_m}\right) \left[1 - e^{\mu_m t}\right]} \quad (13)$$

Figure 5 presents the biomass concentration generated while acetate was utilized by *R. rubrum*, the hydrogen producer from gaseous substrate. The biomass concentration profile is in increasing trend while the dilution rate was increased. The drastic

changes at about 500 hours of operation were resulted from the system disturbance which was created by the replacement of the fresh media reservoir.

Another case which was considered based on substrate consumption rate at steady state condition, using chain rule resulted the following equation:

$$-\frac{dC_{Ac}}{dt} = -r_A = -\frac{dC_{Ac}}{dX} \frac{dX}{dt} = -\frac{1}{Y_{X/Ac}} \mu X \quad (14)$$

By substitution of equation 14 into equation 2, resulted following rate equation for acetate consumption:

$$\frac{dC_{Ac}}{dt} = D(C_{A,in} - C_{Ac}) - \frac{1}{Y_{X/Ac}} \mu X \quad (15)$$

Both sides of equation 15 were multiplied by $Y_{X/Ac}$ with additional term incorporate with equation 10 yielded the following equation which project substrate concentration:

$$X = (C_{A,in} - C_{Ac}) Y_{X/Ac} + A e^{-Dt} \quad (16)$$

where A is an arbitrary constant in equation 16.

Steady State Monod chemostat model

At steady state operation, it was considered that the volumetric flow rates in and out of bioreactor are the same and no live cells entered to the Fermenter as the media stream was fed at sterile condition. The cell growth rate was defined by Monod equation:

$$\mu = \frac{1}{X} \frac{dx}{dt} = \frac{\mu_{max} C_s}{K_s + C_s} \quad (17)$$

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:

$$\mu = D \quad (18)$$

Examination of equation 18 describes that by controlling the dilution rate can control the specific growth rate. Substituting equation 18 to equation 17 gives:

$$D = \frac{\mu_{\max} C_s}{K_s + C_s} \quad (19)$$

Solving equation (19) for the steady state substrate concentration yields:

$$C_s = \frac{DK_s}{\mu_{\max} - D} \quad (20)$$

It has been observed that the total amount of cell mass formed by the cell growth is proportional to the mass of substrate utilized [15]. Thus, the following ratio defined the called yield coefficient

$$Y_{x/s} = \frac{\Delta X}{\Delta S} \quad (21)$$

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:

$$x = Y_{x/s} (C_{s_0} - C_s) \quad (22)$$

Substituting equation (20) into equation (22) provides:

$$X = Y_{x/s} \left(C_{s_0} - \frac{DK_s}{\mu_{\max} - D} \right) \quad (23)$$

Multiplying both sides of equation (23) by D gives:

$$DX = DY_{x/s} \left(C_{s_0} - \frac{DK_s}{\mu_{\max} - D} \right) \quad (24)$$

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:

$$\frac{d(Dx)}{dD} = 0 \quad (25)$$

Then, by differentiating equation (25) yields:

$$D_{\max \text{ output}} = \mu_{\max} \left(1 - \sqrt{\frac{K_s}{K_s + C_{s_0}}} \right) \quad (26)$$

Equation (26) shows that, if $C_{s_0} \gg K_s$, the value of $D_{\max \text{ output}}$ approaches to μ_{\max} and nearly is washout.

The steady state obtained data are well fitted with the proposed model. For the small value of K_m is shown that a good progressive rate equation was achieved. The substrate concentration profile, rate of productivity and cell density are shown in Figure 6.

6. CONCLUSION

The model was compatible with the experimental biological hydrogen production using the photosynthetic bacterium, *R. rubrum*. This work was considered as successful in bioconversion of the synthesis gas to hydrogen. According to the achievement made in this research, the yield of the hydrogen production by microorganism was 98% of the theoretical value. The hydrogen production rate was increased by 33% in compare to reported data in the literature [11, 12]. 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 14, 0.0185h⁻¹.

7. NOMENCLATURES

D=	dilution rate, h ⁻¹
Q=	mass flow rate, g.h ⁻¹
v _o =	volumetric flow rate, l h ⁻¹
V=	volume of bioreactor, l
S=	substrate concentration, g.l ⁻¹
C _S =	substrate concentration, g.l ⁻¹
B=	proportionality constant

λ =	constant
μ =	specific growth rate, h^{-1}
X =	cell density, $g.l^{-1}$
X_m =	maximum cell density, $g.l^{-1}$
t =	time, h
μ_{max} =	maximum specific growth rate, h^{-1}
C_A =	substrate concentration, $g.l^{-1}$
k_1 =	reaction rate constant, h^{-1}
K_s =	Monod constant, $g.l^{-1}$
$Y_{x/s}$ =	biomass yield coefficient, $g \text{ cell } g^{-1}$ substrate

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