Bioconversion of Waste Gases into Biofuel via Fermentation in a Continuous Stirred Tank Bioreactor

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ABSTRACT

Biological hydrogen production was carried out in a continuous stirred tank bioreactor. A photosynthetic bacterium, Rhodopseudomonas rubrum, was used as biocatalyst to oxidize carbon monoxide in the waste gas generated from biowaste in a fermentation process. The fresh liquid media was supplied for microbial growth which contains sodium acetate as carbon source at initial concentration of 4 g L⁻¹. The optimum media space velocity or the suitable ratio of liquid flow rate to the reactor volume (PVR) was 0.02 h⁻¹. At the steady state condition, the concentration of alcaline was independent of the dilution rate and it was approximately 1.5 g L⁻¹. The average cell dry weight in the fermentation broth was at satisfactory concentration, approximately 3.4 g L⁻¹ with dilution rate at 0.55 ml min⁻¹. The maximum value of K₅₀ and CO conversion were about 58 h⁻¹ and 80%, respectively, with agitation speed at 500 rpm and gas flow rate at 1.4 ml min⁻¹. At this condition, the maximum yield of hydrogen production was 0.92 mmol H₂ mmol⁻¹ CO.

Key words: Continuous Stirred Tank Bioreactor, Biocatalyst, Photosynthetic Bacteria, Water-Gas Shift Reaction, Rhodopseudomonas rubrum

INTRODUCTION

It has been reported that several potential species of microorganisms are able to produce hydrogen from synthesis gas. Also many types of bacteria have been frequently cited to produce hydrogen by utilizing a variety of organic substrates. In general, the four categories of hydrogen producing bacteria are: heterotrophic anaerobes, heterotrophic facultative anaerobes, heterotrophic strict anaerobes and photosynthetic bacteria. However, certain photosynthetic bacteria are able to produce hydrogen from fructose in dark condition (Schultz and Weaver, 1982). The hydrogen production from wastewater was also introduced by many researchers (Zhu et al., 2002; Urgaonkar et al., 2003). In the process of biological hydrogen production, the reactions occur under mild conditions of temperature and pressure with the formation of the specified metabolites. The bacteria are able to produce hydrogen from synthesis gas and carbon monoxide was the major component of the synthesis gas (Klasson et al., 1992a and 1992b; Jung et al., 1999 and 2001; Maness and Weaver, 2000; Zumer and Brodhun, 1976; Najafpour et al., 2003). Among the famous hydrogen producers identified in the literature, the purple non-sulfur bacteria, Rhodopseudomonas gelatinosa, Rhodopseudomonas capsulata, Rhodovulum sulfidophilum (Maness and Weaver, 2002) and Rhodopseudomonas rubrum (Schultz and Weaver, 1982; Cowger et al., 1992; Najafpour et al., 2004) were investigated for biohydrogen production. All of the biological hydrogen production processes are fundamentally dependent upon the presence of hydrogen-producing enzymes. The main enzymes catalyzing these reactions are known as nitrogenase, Fe-hydrogenase and Ni-Fe hydrogenase (Hallenbeck and Beinwein, 2002). These enzymes are extremely sensitive to free oxygen. It was also indicated that the enzymatic activity of hydrogenase was affected by the pH, which was crucial to hydrogen production (Fang and Liu, 2002). Effect of pH on hydrogen production and the pH growth related rate equation were investigated earlier and stated in the literature (Bailey and Ollis, 1986; Najafpour and Younesi 2009).

In the present study, acetate was used as an energy source for cell growth. The cells used as biocatalyst to enhance hydrogen production from synthesis gas (syngas) via water-gas reaction. R. rubrum, a member of the photosynthetic purple non-sulfur (PNHS) bacteria was implemented to produce hydrogen from synthesis gas in order to introduce a clean fuel to protect the environment without air emission.

MATERIALS AND METHODS

Pure culture of Rhodopseudomonas rubrum was obtained from the American Type Culture Collection, ATCC 25633, University Boulevard, Manassas, Virginia, 20110-2209 USA. The cultivation of R. rubrum and media preparation for operation of the batch bioreactor was performed in previous studies (Najafpour et al., 2003). The effect of organic compounds in the media and pH were carried out in previous studies (Najafpour et al., 2004; Najafpour and Younesi, 2003).
Growth Medium
Maltic acid 2.5g neutralized with NaOH at pH 6.9. Yeast Extract 1g. (NH4)2SO4 1.25g. MgSO47H2O 0.2g. CaCl22H2O 0.07g. Ferric Citrate 0.01g. EDTA 0.02g. KH2PO4 0.5g. K2HPO4 0.5g. Trace metal solution (1 ml). ZnSO47H2O 0.01g. MgSO4H2O 0.05g. H2BO3 0.01g. Ferric Citrate 3g. CuSO45H2O 0.01g. EDTA 0.2g. (NH4)MoO42H2O 0.02g. CaCl22H2O 0.2g. B-vitamin Solution (7.5 ml). Nicotinamide 0.2g. Thiamine HCl 0.4g. Nicotinic acid 0.2g. Biotin 0.0016g. with distilled water to 1 liter.

Continuous Eperiment
The experiments were carried out in a 2 liter fermenter, Biostat A. B Braun under anaerobic condition with continuous supply of synthesis gas (55% CO, 20% H2, 15% Ar, 10% CO2) provided by Sirt Tafii (Penang, Malaysia) and liquid media. It was equipped with pH, temperature, dissolved oxygen and level sensor. Working volume of the bioreactor was 2 liters (Figure 1). An inoculum of 5% was used to eliminate the lag phase. Two tungsten lamps (40 W) were provided from two sides of the fermenter to light illumination at average of 1500 lux. The light intensity was measured by a luxmeter (Soper Scientific, Taiwan).

The optimum pH of 6.3 was controlled by adding 0.2M HCl and 0.2M NaOH solutions using peristaltic pumps. SynGas flow rate was adjusted to be constant at 10 ml/min by a digital flow meter (Brooks, USA) which bubbled through a sparger. The liquid media flow rate was controlled by an external peristaltic pump (Cole Parmer, USA) at various flow rates (0.55 ml/min). Liquid effluent flow was controlled by a level controller through the liquid outlet stream into the waste container. Aeration was provided by two sets of turbine impeller kept at 500 rpm throughout the experiment.

Sampling of the outlet gas composition was routinely carried out using a gas tight syringe through a gas trap. Liquid samples for analysis and cell density measurements were withdrawn from the reactor through an immersed tube ending in a septum on top of the fermenter using a sterile syringe. To maintain anaerobic condition, the system was purged with purified nitrogen.

Analytical Methods
The cell concentration was determined by optical density measurement using a spectrophotometer (Ceci 1000 series) at 400 nm. It was then converted to cell dry weight using a calibration curve.

Gas compositions were determined by a gas chromatograph (Perkin Elmer Autosystem XL) equipped with thermal conductivity detector (TCD) and a Carboxypeptidase 1000 (Supelco) column. Oven temperature was initially maintained at 40°C after 3.5 minutes, the temperature was programmed with a rate of 20°C min⁻¹ until it reached 90°C. Detector and injector temperatures were 200 and 107°C, respectively. The flow rate of carrier gas, Helium was 20 ml min⁻¹. Argon was used as an internal standard. Calculations for composition of gases were accomplished using the Total Chroma software.

The acetate in the liquid samples was analyzed by gas chromatography, using HP 5890 Series II column (Hewlett Packard, Avondale, PA, USA) equipped with flame ionization detector (FID) and integrator (HP 3398). The column used was 2 m x 2 mm stainless steel, 80/120 mesh Carbowax 20M, Carbowax 20 P (Supelco, Park, Bellefonte, PA, USA) was used. The oven temperature was maintained at 175°C. The injector and detector temperatures were 200 and 220°C, respectively.

The carrier gas, nitrogen flow rate was set at 20 ml min⁻¹. Propionic acid (1%) was used as internal standard with concentration of 20 μl per ml of sample. The injection sample volume was 0.4 μl.

Mathematical Modelling
Figure 1 shows the schematic diagram of an agitated bioreactor with two sets of impeller for gas-liquid system. The synthetic gas and liquid media solution of sodium acetate, was continuously fed to a 2L bioreactor. The operating parameters, the volumetric gas flow rate and agitation speed were investigated for carbon monoxide (CO) biocconversion. The transport of CO from gas phase to liquid phase was examined in the continuous stirred tank bioreactor. In the agitated vessel, the liquid phase was assumed to be well mixed and the gas phase behaved ideal. Based on above assumptions, an appropriate mass balance for carbon monoxide transferred from gas phase to liquid phase was developed.

\[ \frac{dC_{\text{CO}}}{dt} = K_c \left( C_{\text{GCO}} - C_{\text{LCO}} \right) = K_d \frac{C_{\text{CO}}}{H} - \frac{dC_{\text{CO}}}{dt} = \ldots (1) \]

Figure 1: Schematic diagram of an agitated bioreactor with two sets of impeller for gas-liquid system

Therefore, the equation which describes the transport of carbon monoxide from the gas phase to liquid phase is stated as following.
where \( C_{CO,\text{eq}} \) is carbon monoxide concentration in the gas phase in mol L\(^{-1}\), \( t \) is time in s, \( K_L \Delta r \) is overall volumetric mass transfer coefficient in h\(^{-1}\), \( H \) is Henry’s constant in atm M\(^{-1}\) CO, \( P_{CO,\text{gasm}} \) and \( P_{CO,\text{liquid}} \) are partial pressure of carbon monoxide in gas and liquid phase, respectively, in atmosphere, \( C^* \) is carbon monoxide concentration in the gas phase with equilibrium to the liquid phase, and \( C_{CO,\text{eq}} \) is concentration of carbon monoxide in the liquid phase in mol/l. In a gas system with a mixture of hydrogen (\( P_H_2 \)), argon (\( P_{Ar} \)), carbon monoxide (\( P_{CO} \)), and carbon dioxide (\( P_{CO_2} \)), we shall define total partial pressure of mixed gases as following:

\[
\pi = P_{H_2} + P_{Ar} + P_{CO} + P_{CO_2} \quad \ldots(2)
\]

Then, the equation (1) can be rewritten as follow:

\[
-K_L \Delta r = K_L \pi \left( \frac{\dot{F}_{CO}}{H} - \frac{C_{CO,\text{eq}}}{C^*} \right) \quad \ldots(3)
\]

where, \( \lambda_{CO} \) is mole fraction of CO in the gas phase. The mass balance on the gas phase which includes parameters flow at inlet and outlet of the bioreactor and terms describes the transfer of CO from gas phase within the accumulation term:

\[
\text{Mol of CO}_\text{eq} = \frac{\text{mol of CO} \text{transfer of CO from gas phase}}{\text{accumulation}} \quad \ldots(4)
\]

\[
(C_{CO,\text{gasm}})(\lambda_{CO}) - (C_{CO,\text{eq}})(\lambda_{CO}) = \frac{\dot{F}_{CO}}{H} - \frac{C_{CO,\text{eq}}}{C^*} \quad \ldots(5)
\]

where, \( \dot{F}_{CO} \) and \( \lambda_{CO} \) are volumetric gas flow rate in ml min\(^{-1}\) and \( \dot{F}_L \) is volume of the bioreactor in L. Inserting equation (3) into ideal gas law, \( P_{CO} = C_{CO,\text{RT}} \) yields:

\[
\frac{x}{RT}(y_{CO} - y_{CO,\text{eq}}) - K_L \pi \left( \frac{\dot{F}_{CO}}{H} - \frac{C_{CO,\text{eq}}}{C^*} \right) = \frac{\dot{F}_L y_{CO}}{RT} \Delta r \quad \ldots(6)
\]

At steady state condition, it was assumed that there was no accumulation. Therefore, equation (6) is simplified to:

\[
\frac{x}{RT}(y_{CO} - y_{CO,\text{eq}}) - K_L \Delta r (y_{CO,\text{eq}} - \frac{C_{CO,\text{eq}}}{C^*}) = 0 \quad \ldots(7)
\]

By rearranging equation (7) solve for mass transfer coefficient, gives:

\[
K_L \Delta r = \frac{H (y_{CO} - y_{CO,\text{eq}})}{RT \frac{\dot{F}_L y_{CO}}{RT}} \quad \ldots(8)
\]

In a small bench scale bioreactor, it is assumed that there was no variation of hydrostatic pressure within the vessel, thus \( y_{CO} \) and \( y_{CO,\text{eq}} \) are evaluated at equal hydrostatic pressure. That means the change in volumetric flow rate at inlet and outlet gas is sufficiently small that does not influence the pressure, thus:

\[
K_L \Delta r = \frac{H (y_{CO} - y_{CO,\text{eq}})}{RT \frac{\dot{F}_L y_{CO}}{RT}} \quad \ldots(9)
\]

In the bioreactor, the biocatalyst oxidizes carbon monoxide to carbon dioxide so that dissolved carbon monoxide in the bulk solution was assumed to be zero:

Applying \( \dot{X}_{CO} = 1 - \frac{\dot{X}_{CO}}{\dot{X}_{CO}} \) into equation (9) and assuming that carbon monoxide concentration in the liquid phase was zero. Then, equation (9) was simplified as shown in the following relation:

\[
K_L \Delta r = \frac{H \dot{X}_{CO}}{RT \frac{\dot{F}_L y_{CO}}{RT} (1 - \dot{X}_{CO})} \quad \ldots(10)
\]

The volumetric mass transfer coefficient \( K_L \Delta r \) is an important parameter in an agitation bioreactor. The direct effect of \( K_L \Delta r \) on conversion was expected due to the high agitation speed, resulted high mass transfer and high carbon monoxide conversion. Equation (10) was employed to determine \( K_L \Delta r \) of the CSTR bioreactor.

RESULTS AND DISCUSSION

In our previous studies, R rubrum was grown in a constricted stirred tank bioreactor (CSTR) with various liquid flow rate (LFR), in order to maintain suitable microbial growth and growth, an optimum ratio of F/V \( (0.02 h^{-1}) \) was defined (Hajipour et al., 2003).

Figure 2 shows the relationship between the cell dry weight and substrate utilization in a 2L CSTR. The bioreactor was inoculated with 5% of seed culture grown at exponential phase. The bioreactor was initially operated in semi batch-mixture until the late stage of exponential phase was reached. Fresh liquid media were pumped on third day. The liquid flow rate was at 0.65 ml min\(^{-1}\) \((0.02h^{-1})\). The supplied substrate as energy reserve for cell growth was sodium axelate with initial concentration of 4 g L\(^{-1}\) in
the fresh media. The optimum initial acetate concentration and dilution rate was obtained in previous studies (Najafpour, et al., 2004). The rate of growth decreased at high substrate concentration. This reason was due to effect of ionic strength, osmotic pressure, or organic over-loading. The maximum cell dry weight was about 3.4 g L\(^{-1}\) for dilution rate at 0.55 mL min\(^{-1}\). The substrate consumption was proportional specific growth rate of \(R\) rubrum. At steady state condition, the concentration of acetate was at 1.5 g L\(^{-1}\) and independent of dilution rate. The maximum biomass yield obtained about 0.35 g cell\(^{-1}\) acetate.

![Figure 2: The dependency of cell dry weight concentration on growth-limiting substrate concentrator with dilution rate in a continuous stirred tank bioreactor at steady state condition](image)

Figure 3 shows bioconversion of carbon monoxide to carbon dioxide, while water to hydrogen in the continuous bioreactor. The fermentation vessel was agitated at 150, 300, 450 and 500 rpm. The agitation speed was increased interval 200 hrs in the operation. The trend of increasing hydrogen was developed as the agitation speed increased from 150 to 500 rpm. The hydrogen production rate increased stepwise due to increase of the agitation speed. The maximum hydrogen production rate reached 0.18 mmol H\(_2\) min\(^{-1}\) with agitation speed at 500 rpm and gas flow rate at 14 mL min\(^{-1}\). However, the maximum carbon monoxide consumption rate obtained at 0.22 mmol CO min\(^{-1}\). The maximum yield of hydrogen production was about 0.82 mmol H\(_2\) mmol\(^{-1}\) CO with agitation speed at 500 rpm and gas flow rate at 14 mL min\(^{-1}\).

![Figure 4: Conversion of carbon monoxide versus impeller speed in a continuous stirred tank bioreactor](image)

Figure 4 demonstrates the linear relationship for CO conversion with agitation speeds. The CO conversion increased with the increase of impeller speed. This is due to the reaction occurred between water and gas, higher CO conversion with higher agitation speed resulted high mass transfer. When the gas was introduced into the agitated vessel, at low agitation speed, the gas rises through the impeller region without being effectively dispersed. This phenomenon is known as flooding condition. The reason is due to that an increase in gas flow rate, the total volume of the bioreactor increased. Thus, the power input was required to overcome flooding phenomenon. This means that higher agitation speeds was applied in order to distribute gas into the bioreactor and break-up the bubble into smaller gas bubble size. On the other hand, the gas hold-up was increased due to higher agitation speeds. The maximum CO bioconversion was about 80% with agitation speed at 500 rpm.

The most common type of industrial bioreactor is agitation vessel. These types of bioreactor are used when high rate of mass transfer is needed. A number of parameters in the agitated tank are involved, such as distribution and size of gas bubbles, gas phase hold-up, liquid phase mixing pattern and bubble break up and coalescence. In this study, a microsparger was used to distribute smaller gas bubble size in the bioreactor vessel. The value of \(K_a\) was obtained at various agitation speeds and constant gas flow rate \((u)\) in 14 mL min\(^{-1}\). The constant values of Equation (10) for \(K_a\), calculation of the agitated bioreactor vessel were as followed. The bioreactor was operated at 30\(^\circ\)C and atmospheric pressure (1 atm). The ideal gas law constant was 0.082 atm mol\(^{-1}\) \(\text{K}^{-1}\). The Henry's constant \((\text{KH})\) at this...
temperature was 1.116 atm L mmol$^{-1}$ CO. The working volume of the bioreactor ($V_{b}$) was 2 L.

Figure 5 demonstrates an increasing trend of mass transfer coefficient while the transported carbon monoxide was instantaneously consumed in liquid phase. The experimental data showed that the CO conversions increased as the $K_{a}$ value increased.

![Graph showing mass transfer coefficient $(K_a)$ vs. CO conversion](image)

Figure 6: The mass transfer coefficient obtained for the continuous stirred tank bioreactor at steady state condition

The increase of $K_{a}$ value was expected due to higher agitation speed gives higher CO conversion. This means that the agitation speed influenced both $K_{a}$ and conversion in the stirred tank bioreactor. The fact is that, the $K_{a}$ value changed with agitation speed. It is obvious that the reaction is mass transfer limited when the gas was introduced into the bioreactor. Thus, applying mass transfer limitation condition may increase the CO conversion of the inlet gas flow rate.

CONCLUSION

The common stirred tank bioreactor was used in the bioconversion of synthesis gas to hydrogen. The maximum cell dry weight was about 3.4 g L$^{-1}$ for the dilution rate of 0.05 min$^{-1}$ (FFV = 0.02 h$^{-1}$). The maximum biomass yield achieved at 0.35 g cell g$^{-1}$ acetate at the above condition. The acetate concentration at steady state condition was independent of dilution rate and it reached 1.5 g L$^{-1}$ from the influent of bioreactor. The maximum hydrogen production yield exhibited at 0.82 mmol H$_2$ mmol$^{-1}$ CO.

Nomenclature

- $C_{CO, gas}$ = carbon monoxide concentration in the gas phase, mol L$^{-1}$
- $C_{CO, eq}$ = equilibrium concentration of carbon monoxide, mol L$^{-1}$
- $C_{CO, liquid}$ = concentration of carbon monoxide in the liquid phase, mol L$^{-1}$
- $H$ = Henry’s law constant, atm L mol$^{-1}$
- $K_{a}$ = overall volumetric mass transfer coefficient, h$^{-1}$
- $\pi$ = total system pressure, atm
- $P_{CO, gas}$ = partial pressure of carbon monoxide in gas phase, atm
- $P_{CO, liquid}$ = partial pressure of carbon monoxide in liquid phase, atm
- $P_{H_2}$ = partial pressure of hydrogen in the gas phase, atm
- $V_{CO}$ = mol fraction of CO in gas phase
- $t$ = time, h
- $r_{CO}$ = rate of CO consumption, mol L$^{-1}$ h$^{-1}$
- $r_{flow}$ = volumetric gas flow rate in, mL min$^{-1}$
- $V_{net}$ = volumetric gas flow rate out, mL min$^{-1}$
- $V_{L}$ = volume of the bioreactor, L
- $R$ = gas constant in ideal gas law, 0.082 L atm K$^{-1}$

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REFERENCES


