Effects of VFAs Concentration on Bio-hydrogen Production with *Clostridium Bifermentans 3AT-ma*

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Abstract

This study evaluated that the effects of concentration of VFAs (volatile fatty acids), such as acetate and butyrate, on bio-hydrogen production from high salinity organic substrate with halophilic hydrogen-producing bacteria of *Clostridium bifermentans 3AT-ma*. Glucose medium was used as a substrate. Supplementary acetate and butyrate concentrations were ranged 0 to 500 mM and 0 to 250 mM, respectively. The increased concentration of acetate and butyrate significantly influenced to hydrogen production. At the initial condition, VFAs concentration of 0 g/L, maximum hydrogen was produced. The hydrogen production was decreased as increasing dosage of acetate and butyrate. The inhibitory effect by adding acetate on fermentative hydrogen production was less significant compared to that by butyrate. A modified Gompertz equation could be successfully described the cumulative \(H_2\) production at different VFAs concentrations. In addition, the inhibition by adding VFAs on hydrogen production was described well by a non-competitive inhibition model.

1. Introduction

Global environmental problems and sustainable energy supply are serious concerns today and for the future generations. Hydrogen is highly energy efficient (122kJ/g) excluding contaminations such as CO\(_2\), CO, C\(_n\)H\(_m\), SO\(_x\), NO\(_x\), and ashes during combustion; therefore the hydrogen is considered as a next...
generation energy source [1-2]. The hydrogen production technology can be divided by biological method and physicochemical method. The biological hydrogen production includes photosynthetic hydrolysis and photo-fermentation using the light and anaerobic dark-fermentation [3]. High strength organic waste such as food waste can be used as an efficient substrate for the bio-hydrogen production via dark-fermentation. However, food waste in Korea contains high concentration of salts (2~3%) resulted in inhibiting on bio-hydrogen production. The activity of hydrogen producing bacteria can be affected by toxicity from the salt [4]. In addition, volatile fatty acids (VFAs) as by-products, such as lactate, butyrate, acetate, and formate, made via dark fermentation affect to hydrogen production efficiency [5]. In this study, the effects of concentration of acetate and butyrate on hydrogen production were evaluated using halophilic hydrogen-producing bacteria (HHPB), *Clostridium bifermentans 3AT-ma*.

2. Experimental Methods and Material

2.1. Material and operating conditions

The seed was isolated from anaerobic digested sludge collected from wastewater treatment plant at Ansan City in Korea. The bacteria, *Clostridium bifermentans 3AT-ma*, can be survival under high concentration of NaCl and can produce hydrogen without any inhibition. PYG medium: peptone 5 g/L, trypton 5 g/L, yeast extract 10 g/L, glucose 10 g/L, CaCl₂ 0.008 g/L, MgSO₄·7H₂O 0.016 g/L, K₂HPO₄ 0.04 g/L, KH₂SO₄ 0.04 g/L, NaHCO₃ 0.04 g/L, NaCl 0.08 g/L and 30 g/L of NaCl was used. L-cysteine of 0.25 g/L was supplemented to remove trace oxygen in the medium [6]. Acetate and butyrate concentration examined was ranged from 0 to 500 mM and from 0 to 250 mM, respectively. The pH was adjusted to 7 using 4 M of KOH and 2 M of HCl. 20 mL of the medium was injected into a 120 mL of serum bottle, and the head space was purged with nitrogen gas. Thereafter the serum bottle was sealed and autoclaved. Dark-fermentation under anaerobic condition was performed using a shaking incubator with 125 rpm at 35°C in batch.

2.2. Analysis method

The CO₂ and H₂ gas produced from dark-fermentation was measured by GC (M600D, Younglin Co., Korea) equipped with TCD. The column used was Carboxen-1000 (60/80 mesh; inner diameter=2.1mm), and carrier gas was nitrogen. The temperatures of the oven, injector, and detector were maintained at 150 °C, 150 °C, and 200 °C, respectively. Organic acid was determined by IC (Basic 792, Ion chromatography, Metrohm UK limited, England) with organic acid column (Metrosep), 0.5 mmol/L perchloric acid for eluent, and 10 nmol/L lithium chloride as the suppressor. The optical density (OD₆₆₀) of the microorganism was measured using a UV photometer. Glucose concentration was analyzed by DNS method (3,5-Dinitrosalicylic Acid method) [7].

2.3. Kinetic modeling

Gompertz equation (Eq. 1) was used to interpret the characteristics of hydrogen produced from the batch experiments [1,8,9].
where \( P_h \) is cumulative hydrogen gas (mL); \( P \) is hydrogen volume (mL), \( R_m \) is maximum gas production rate (mL/hr); \( \lambda \) is a lag time to be produced hydrogen (hr).

2.4. Non-competitive inhibition model

Noncompetitive inhibition model (Eq. 2) was used to identify the effects by inhibition of acetate and butyrate on hydrogen production [10,11].

\[
r = r_{\text{max}} \left( 1 - \frac{C}{C_{\text{crit}}} \right)^n \left( \frac{S}{S + K_S} \right)
\]

where \( r \) is the hydrogen production rate (mL/hr/L); \( r_{\text{max}} \) is the maximum hydrogen production rate (mL/hr/L); \( C \) is the added VFAs concentration (mM); \( C_{\text{crit}} \) is the critical VFAs concentration at which \( H_2 \) production ceases (mM); \( K_s \) is the apparent half velocity constant for the substrate (g/L); \( S \) is the substrate concentration (g/L), and \( n \) is the degree of inhibition. When the substrate concentration is unlimited (\( S \gg K_s \)), the Equation 2 can be simplified as following Equation 3.

\[
r = r_{\text{max}} \left( 1 - \frac{C}{C_{\text{crit}}} \right)^n
\]

Each model was simulated using SIGMA PLOT 10.0 (Systat Software Inc., USA).

3. Results and Discussion

3.1. Effects of acetate and butyrate on glucose consumption and cell growth

The glucose consumption, cell growth, and the final pH variation by *Clostridium bifermentans 3AT-ma* were observed at different acetate and butyrate concentrations (Fig. 1). As acetate concentration was increased, the cell growth defined as OD_{660} and glucose consumption rate were decreased. At 0 to 300 mM of acetate, glucose was degraded as much as 75~90%, while the glucose was consumed as 52% with 400 mM of acetate and as 15% with 500 mM of acetate. Over 100 mM of acetate disturbed the cell growth. Adjusted pH 7 for all the experiments was decreased by the accumulated VFAs as by-products produced from the dark-fermentation, and the difference between initial and final value was significant when low concentration of acetate was added [11]. Similar results were also observed at the experiments with butyrate. The glucose was degraded as 87% with 0 mM, 70% with 20 mM, and 50~60% with 50~200 mM of butyrate, respectively. At 250 mM of butyrate, the glucose was consumed as 18% that was conspicuously low rate compared to that by adding 250 mM of acetate (76%). Hence, butyrate was significantly affected to the glucose consumption rather than acetate. The pH was dropped from 7 to 5.5 during the operation, and the pH was slightly increased depending on adding butyrate.
3.2. Hydrogen production by Clostridium bifermentans 3AT-ma

The results of cumulative hydrogen production measured were presented in Fig. 2. Hydrogen was produced as much as 33 mL with each 0 mM of acetate and butyrate. As the acetate and butyrate concentration were increased, the cumulative hydrogen was toward to be decreased. All the experimentally produced cumulative hydrogen data were regressed by Gompertz equation \((r^2=0.99)\). The calculated hydrogen production volume \((P)\) and maximum hydrogen production rate \((R_m)\) were decreased by increasing concentration of the acetate and butyrate, while the lag time \((\lambda)\) was increased. In control condition, the calculated \(P\), \(R_m\), and \(\lambda\) were 33.2 mL, 3.3 mL/hr and 1.2 hr, respectively. Each parameter was presented as 21.9 mL of \(P\), 2.2 mL/hr of \(R_m\), and 8.9 hr of \(\lambda\) with 250 mM of acetate, and as 5.3 mL of \(P\), 0.15 mL/hr of \(R_m\), 69.3 hr of \(\lambda\) with 250 mM of butyrate. Based on these results, it found that the acetate and butyrate were prominently affected to the hydrogen production. In addition, the butyrate was more significantly toxic to the bacteria rather than acetate.

3.3. Effects on final by-products by acetate and butyrate addition

The effects of supplementary injection of acetate and butyrate on final by-products production were observed (Figure 3). Acetate consists of significant portion as one of the major by-products. As the concentration of acetate and butyrate was increased, the net production of the acetate was decreased.
When over 300 mM of acetate was added, acetate was not produced during the operation. The acetate was even less than the initial injection as much as 50 mM, while propionate and butyrate was increased. The reduced acetate was converted to propionate or butyrate. Yi et al. (2008) also reported a similar result that acetate was less than initially injected one at the final point of operation [12]. When butyrate was added as 250 mM, the acetate concentration was significantly decreased, while butyrate concentration was spontaneously increased in this study.

Fig. 3. Effects of acetate (a) and butyrate (b) dose on the distribution of final VFAs products

3.4. Inhibition modeling

The hydrogen production rate was regressed using the noncompetitive inhibition equation (Eq. 3) depending on the acetate and butyrate concentration (Fig. 4). In applied for acetate, the calculated maximum hydrogen production rate \( r_{\text{max}} \) was 58 mL/hr/L; the critical VFAs concentration at which \( \text{H}_2 \) production ceases \( (C_{\text{crit}}) \) was 502.2 mM; and the degree of inhibition \( (n) \) was 0.52. In applied for butyrate, the parameters were calculated as \( r_{\text{max}} \) of 60.1 mL/hr/L, \( C_{\text{crit}} \) of 334.1 mM, and \( n \) of 2.35. Based on these results, butyrate was more significantly affected to the bacteria rather than acetate by causing less \( C_{\text{crit}} \) and large \( n \) value. The similar result was also confirmed in other studies [11,13]. However, the calculated values in each study were dissimilar due to different experimental conditions such as bacteria, pH, temperature, substrate, concentration range examined.

Fig. 4. The relationship between the specific hydrogen production rate and added concentration of acetate (a) and butyrate (b) fitted with inhibition model
4. Conclusion

The effects of acetate and butyrate addition on hydrogen production from saline medium were examined using halophilic hydrogen production bacteria (HHPB), *Clostridium bifermentans 3AT-ma*. Increased acetate and butyrate were resulted in low cell growth rate, substrate consumption rate, and hydrogen production rate. Butyrate was more significantly inhibited to the bacteria rather than acetate. A modified Gompertz equation could be successfully described the cumulative H₂ production at different VFAs concentrations. Also the inhibition by VFAs addition on hydrogen production was described well by a non-competitive inhibition model.

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References


