

# Effect of hydrogen partial pressure control on fermentative hydrogen production from organic wastewater

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**Abstract.** The partial pressure of hydrogen is a significant factor for dark fermentation. The effect of reduced hydrogen partial pressure as well as different operation condition was investigated in this study. The results showed that hydrogen production enhanced when partial pressure reduced. The reduction of hydrogen partial pressure (reduced by 20%, interval=2h) increased the efficiency of hydrogen production by 54%(202.15mL) compared with the control group. The kinetic parameters of hydrogen production show that the maximum hydrogen production ( $P_{\max}$ ) and the maximum hydrogen production rate ( $R_{\max}$ ) were increased by the reduction of hydrogen partial pressure. And the hydrogen production delay time ( $\lambda$ ) decreased. Moreover, with the decrease of hydrogen partial pressure, ethanol content gradually increased, acetic acid / ethanol ratio decreased, total VFAs increased. The compositions of soluble microbial products as well as ecological factors were affected by hydrogen partial pressure.

## 1. Introduction

Hydrogen is one of the most ideal alternatives for fossil fuels [1]. Bio-hydrogen production plays an increasingly important role in the research field of new energy. The technology of anaerobic fermentation hydrogen production based on activated sludge is always one of the most promising international research topics [2]. Dark fermentation has advantages of high hydrogen production, no need for light-energy input and can treat organic waste such as organic wastewater, straw, livestock manure and kitchen garbage at the same time. But the rate of hydrogen production is still not enough for industry application [3].

The partial pressure of hydrogen is one of the significant factors for hydrogen production. High hydrogen partial pressure of the headspace can affect the concentrations of the liquid phase [4]. So controlling hydrogen partial pressure of the headspace is a crucial aspect of dark fermentation hydrogen production. Kim *et al* [5] reported that experimental groups purging with external gases reached higher hydrogen yield and more butyrate production than the control group. Lee *et al* [6] communicated a reduced pressure strategy (130-760 mmHg) via vacuum to enhance the hydrogen production headspace.

In this study, the method of hydrogen partial pressure controlling to further improve the hydrogen production capacity of the fermentation system as well as the capacity of wastewater treatment with lower cost will be discussed.

## 2. Materials and methods



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### 2.1. Wastewater and inoculated sludge

The wastewater is simulated sugar wastewater with brown sugar as substance. N, P and other elements are added for the growth of microbes. The mass ratio of COD, N and P is about 1000:5:1 [7]. The inoculated sludge is taken from a continuous hydrogen production reactor, and the type of fermentation in the reactor is ethanol type fermentation, of which ethanol and acetic accounted for more than 65% of the end products in the liquid phase.

### 2.2. Culture method

The fermentation experiments were conducted in serum bottles with a working volume of 500mL. Inoculated with sterile syringes and inoculated with nitrogen flow for 5 min after inoculation [8]. The sealed serum bottles were placed in a thermostatic air bath oscillating box and fermented at 150 r/min and 35°C at constant temperature for 56 h. The experiments were carried out in parallel with 3 culture bottles, and the average value was obtained. The partial pressure of Hydrogen was decreased via air exhaust by glass syringe and nitrogen purging into the headspace at the same time. Thus the headspace gas pressure was maintained barometric pressure.

### 2.3. Analytical methods

The pH was measured by the PHS-25 acidity meter. COD, VSS and SS were measured by the national standard method. The volume of fermentation gas was measured by a glass syringe after collecting in the gas bags and converted into a standard state (0°C, 101.325 kPa) volume. The modified Gompertz model is used to simulate the cumulative hydrogen production by nonlinear fitting formula (1) [9].

$$H = P_{\max} \times \exp \left\{ -\exp \left[ \frac{R_{\max} \times e}{P_{\max}} (\lambda - t) + 1 \right] \right\} \quad (1)$$

In the formula, H is the cumulative hydrogen production, mL;  $P_{\max}$  is the largest amount of hydrogen production, mL;  $R_{\max}$  is the maximum hydrogen production rate, mL/h;  $\lambda$  is the delay time, h; t is reaction time, h.

The gas products and components were determined by SC- II gas chromatography. The components and content of the liquid end fermented products were determined by GC-122 gas chromatography. The carrier gas was argon gas and the column (2 m stainless steel) was packed with hydrogen flame detector. The temperature at the column was 190°C. The temperature of test room was 240°C. The sample was centrifuged, filtered (0.22  $\mu$ m) and acidized adding 100  $\mu$ L formic acid as pretreatment.

## 3. Results and discussion

### 3.1. Effect of hydrogen partial pressure on the efficiency of hydrogen production

The partial pressure of hydrogen was decreased with a proportion varying from 10% to 40%. Figure 1 is the amount of hydrogen production in the batch fermentation system under different hydrogen partial pressure conditions and different frequency of pressure decreasing. As indicated in figure 1, the hydrogen production in the system of reduced hydrogen partial pressure is greater than that of the control group. Therefore, reducing the hydrogen partial pressure of the headspace can increase the hydrogen production of the system. The best experimental group (cumulative hydrogen production = 202.15 mL) achieves a cumulative hydrogen yield of 54% higher than that of the control group (cumulative hydrogen production = 131.31 mL) without hydrogen partial pressure control. This result is in accordance with the study of Mizuno *et al* [10], in which an enhanced hydrogen yield was achieved from 0.85 mol/mol hexose to 1.43 mol/mol hexose.

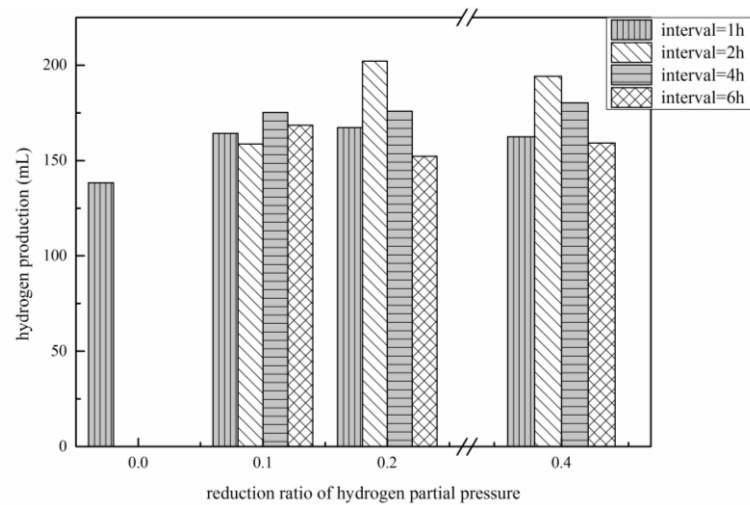


**Figure 1.** Hydrogen production under different hydrogen partial pressure.

In the case of the reducing same amplitude of hydrogen partial pressure, different interval time reached different efficiency. In the system with 10% lower pressure, different frequency differs a little, but in the system which decreased the hydrogen partial pressure by 20% and by 40%, the efficiency under different frequencies measured evidently increased initially and then decreased, so it can draw the optimum pressure frequency corresponding to the interval of 2 h (20% lower) and interval 4 h (40% lower). In terms of reducing the proportion of partial pressure, the best hydrogen production can be obtained by 20% depressurization which reached the optimum hydrogen production.

The hydrogen production kinetics of the fermentation system under different hydrogen partial pressure conditions was analyzed, and the modified Gompertz model was used to fit and analyze the hydrogen production.

**Table 1.** Kinetic parameters under different hydrogen partial pressure conditions.

| Reduction ratio of hydrogen partial pressure | $P_{\max}$<br>(mL) | $R_{\max}/$<br>(mL·h <sup>-1</sup> ) | $\lambda$<br>(h) | $R^2$  |
|----------------------------------------------|--------------------|--------------------------------------|------------------|--------|
| 0                                            | 146.86             | 3.878                                | 4.56             | 0.9943 |
| 10%                                          | 188.92             | 5.138                                | 6.77             | 0.9970 |
| 20%                                          | 156.79             | 3.911                                | 6.25             | 0.9913 |
| 30%                                          | 192.16             | 6.532                                | 10.38            | 0.9957 |
| 40%                                          | 210.92             | 6.149                                | 8.33             | 0.9922 |

From table 1, we can see that the correlation coefficients of each condition are all above 0.99, indicating that in this experiment, the change of cumulated hydrogen production in the reaction process can be described by the improved Gompertz model. The kinetic parameters of hydrogen production obtained from the model fitting can be used as the analysis factor. The hydrogen production characteristic curve and fitting parameters were analyzed. The hydrogen partial pressure increased the maximum hydrogen production ( $P_{\max}$ ) and the maximum hydrogen production rate ( $R_{\max}$ ). The maximum hydrogen production rate and the maximum hydrogen production rate in the test group were all larger than those in the control group. However, the hydrogen delay time ( $\lambda$ ) elongated with reducing hydrogen partial pressure possibly due to system pressure changes call for adaptation of microorganisms, therefore need to extend the delay period.

### 3.2. Effect of hydrogen partial pressure on the soluble microbial products

The content of the end products and the total amount of VFAs (volatile acids) in the liquid phase under different hydrogen was illustrated in figure 2. As showed in figure 2, the total amount of the end product is generally consistent, but the composition has varied. With the reduction of hydrogen partial pressure, the content of ethanol increased and the content of butyric acid and propionic acid decreased. It explained the reason why the hydrogen production of the experimental groups is better than that of the control group, and it is also proved that the end product of the liquid phase changes because of the hydrogen partial pressure control.

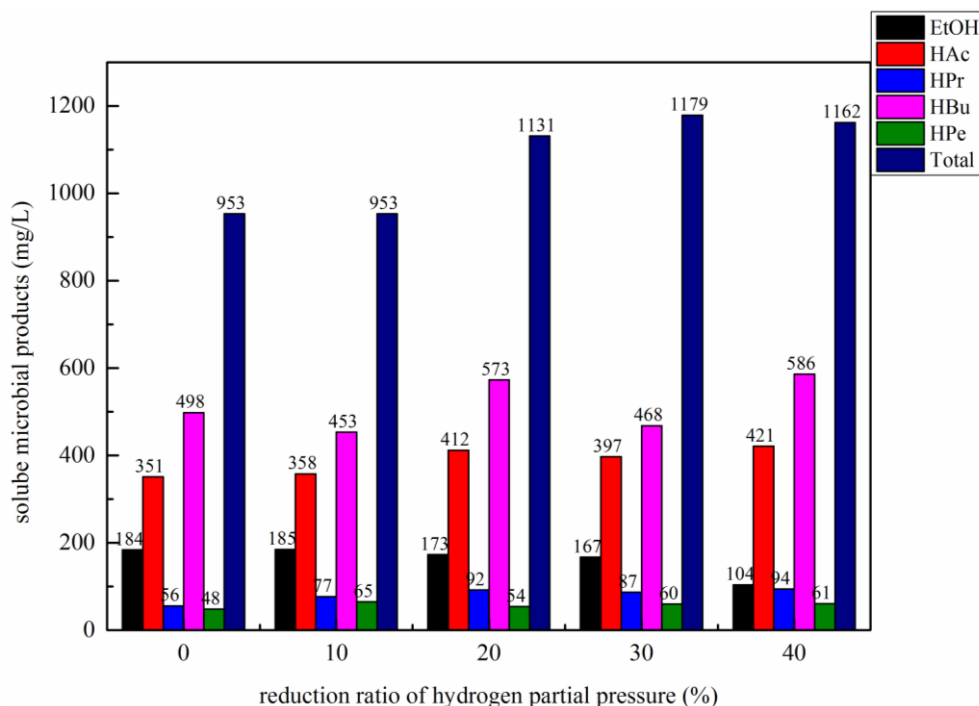


**Figure 2.** The compositions of soluble microbial products.

Ethanol, butyric acid and total volatile acids reached the maximum amount in experimental groups with reduced partial pressure, which is consistent with the efficiency of hydrogen production (showed in figure 1). With the decrease of hydrogen partial pressure, ethanol content gradually increased, acetic acid / ethanol ratio decreased, total VFAs increased. Lower hydrogen partial pressure promoted conversion of mixed-acid fermentation to ethanol type fermentation (increased ethanol content in liquid products), thus enhanced hydrogen production rate. Because of the substrate consumption, gas production decreased over time, but the system with reducing hydrogen partial pressure is more productive compared to the control system in hydrogen production. Hydrogen pressure affects the reaction equilibrium, the equilibrium shifts towards hydrogen producing reaction, promotes the effect of hydrogen production. And because of the increased content of total volatile acid, the hydrogen consuming bacteria (HCB) hydrogen metabolisms were inhibited. The hydrogen partial pressure control can affect the liquid metabolites of the mixed-acid type fermentation, so it can also be used as one of the means to regulate the type of hydrogen production.

Propionic acid has adverse effects on hydrogen production and methane production. The anaerobic oxidation of propionic acid is the most difficult among all short chain fatty acids. Therefore, propionic acid is easily accumulated in anaerobic system, which can result in the decrease of anaerobic reactor efficiency and operational stability [11]. It is important to improve the degradation rate of propionic acid in the fermentation system and avoid the accumulation of propionic acid. As figure 2 illustrated, the dependency ratio of propionic acid / acetic acid to the hydrogen partial pressure is very low and has no significant effect. This result further explained that the effect of hydrogen partial pressure on

propionic acid is not directly affected but is caused by two mechanisms of physiological and ecological conditions. This is in accordance with the study of Ren and so on [12].

### 3.3. Effect of hydrogen partial pressure on the ecological factors

#### ● pH

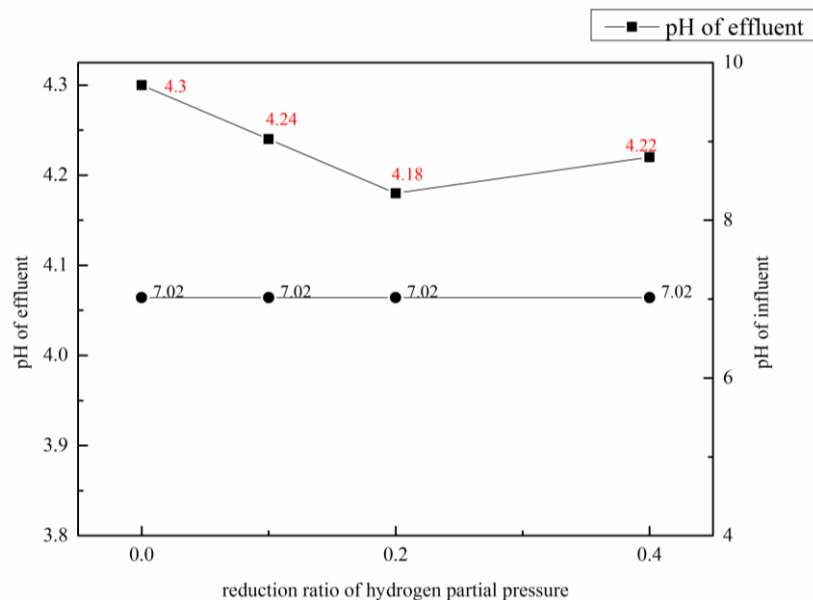
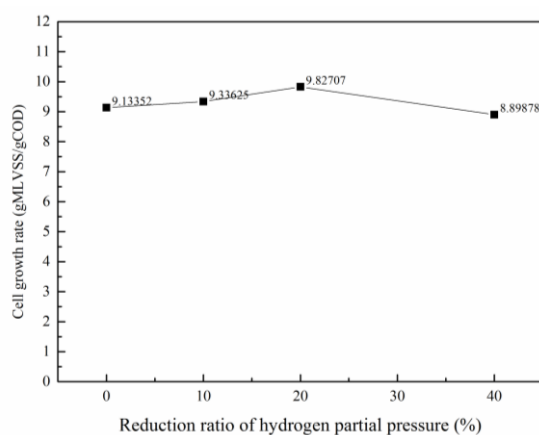


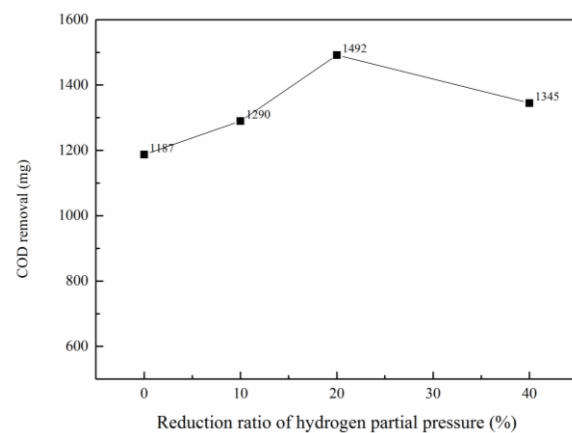
**Figure 3.** pH of fermentation systems under hydrogen partial pressure.

The pH of inflow and effluent was showed in figure 3. The pH of inflow was kept under the same condition; the effluent pH fluctuated between 4.18–4.3. In experimental group with the hydrogen partial pressure decreasing by 20%, pH is the minimum value, consistent with the maximum volatile acid yield. The appropriate pH for butyric acid, mixed acid fermentation and ethanol fermentation is 5.5 to 6.5, 5 to 5.5 and 4.5 to 5 respectively [13].

#### ● Substrate and biomass



(a)



(b)

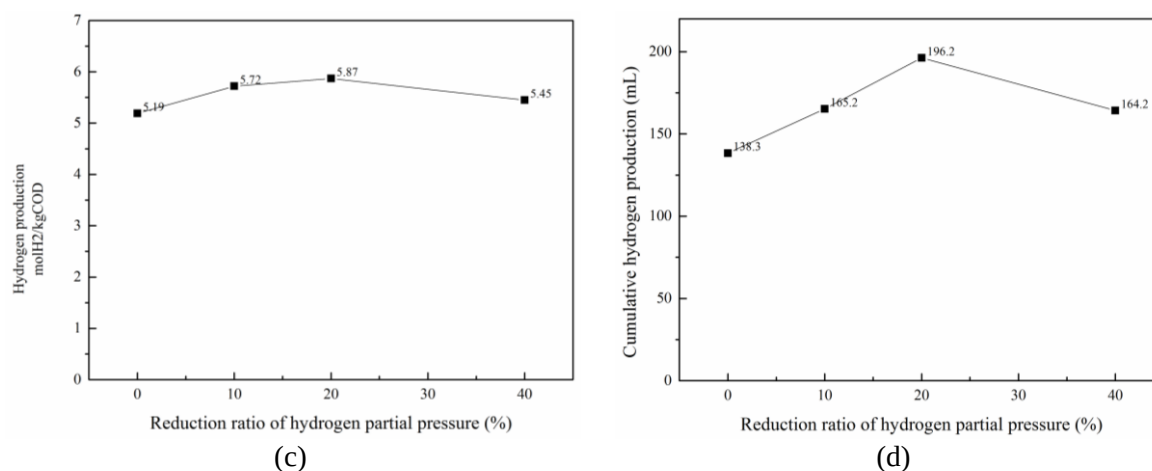


**Figure 4.** Effects of different hydrogen partial pressure on hydrogen conversion and bacterial growth (a) Cumulative hydrogen production, (b) COD removal, (c) Hydrogen production (molH<sub>2</sub>/kgCOD) and (d) Cell growth rate (gMLVSS/gCOD).

Figure 4 shows cumulative hydrogen production, COD removal, Hydrogen production and Cell growth rate with different reduction ratio of hydrogen partial pressure. The hydrogen partial pressure affects the organic matter removal rate and cell growth rate. Under the optimal hydrogen partial pressure, the cumulative hydrogen yield and hydrogen conversion rate are the highest (As showed in figure 4(a)), and the COD removal is also the largest (as showed in figure 4(b)). It shows that the fermentation bacteria use more substrates, so the NADH and the energy are also more, which is beneficial to the positive progress of hydrogen production. However, as showed in figure 4(d), the cell growth rate was less than that of the control when the ratio of hydrogen partial pressure decreased by 40%. So the increase of hydrogen production was the result of cell growth and cell activities.

#### 4. Conclusions

The proposed method of hydrogen partial pressure reduction led to increase in the H<sub>2</sub> production performance—higher hydrogen production rate ( $P_{\max}$ ), higher hydrogen production rate ( $R_{\max}$ ) and shorter delay time ( $\lambda$ ). The reduction of hydrogen partial pressure (reduced by 20%, interval=2 h) increased the efficiency of hydrogen production significantly (202.15 mL) by 54% compared with the control group (131.31 mL). Moreover, the compositions of soluble microbial products as well as ecological factors were observed changed by lower hydrogen partial pressure. The end products of the liquid phase changed because of the hydrogen partial pressure control.

Therefore, the proposed strategy of reduced partial pressure fermentation seems to be practical in improving H<sub>2</sub> production. There are different types of ecological niches participating in anaerobic digestion process. Since varieties of microbes are involved in the mixed culture fermentation, the ecological and biological analysis of the fermentation culture still requires further investigation.

#### Acknowledgments

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