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# Optimizing Fermentation Conditions for bioH2 Production with Clostridium Butyricum CGS2 Using Statistical Experimental Design

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**Abstract**

As the global temperature keeps rising, the demand for reliable and effective energy alternatives is increasingly urgent. Among the developing alternative energy resources, hydrogen is recognized as a clean and recyclable energy carrier and is considered one of the major energy sources in the future. Hydrogen fermentation is a non-pollutant way of producing $H_2$. Among fermentative $H_2$ production processes, the $H_2$ production rate by dark fermentation is higher than photo fermentation, thereby having higher viability for commercial applications. In this study, an indigenous isolate *Clostridium butyricum* CGS2 able to convert sugar (such as glucose, fructose, sucrose and xylose) into hydrogen was used the bacterial $H_2$ producer. Using sucrose as the carbon source in a batch process, *C. butyricum* CGS2 gave a maximum $H_2$ production rate ($v_{H2}$) and $H_2$ yield ($Y_{H2}$) of 262.2 ml/h/l and 2.26 mol $H_2$/mol sucrose, respectively. Response surface methodology (RSM) was employed to identify the optimal conditions for hydrogen production of *C. butyricum* CGS2 using sucrose concentration, temperature and pH as the primary operation parameters. With a performance index of $Y_{H2}$, the optimum condition predicted from RSM analysis was: pH, 5.2; temperature, 35.1 °C; sucrose concentration, 22.5 g COD/l. Under this condition, the hydrogen content in the biogas was 58.5%, □$H_2$ was 0.54 l/h/l, total hydrogen production was 7.2 l, and $Y_{H2}$ was 2.91 mol $H_2$/mol sucrose. On the other hand, when □$H_2$ was used as the performance index, the optimum condition was: pH, 5.36; temperature, 35.1°C; sucrose concentration, 26.1 g COD/l. This condition gave a hydrogen content of 63.3%, a $Y_{H2}$ of 3.26 mol $H_2$/mol sucrose, a total hydrogen production of 10.5 l, and a □$H_2$ of 0.50 l/h/l. The validity of RSM predictions was confirmed by additional experiments, suggesting that using RSM design could attain an optimal culture condition for *C. butyricum* CGS2 to enhance its hydrogen production performance.

## 1 Introduction

As biomass energy becomes one of the major global energy alternatives, many research efforts have been devoted to converting inexpensive waste biomass feedstock (e.g., agricultural wastes) into bioenergy, such as ethanol, biodiesel, and hydrogen (Tsai et al., 2007 [1]; Vrije et al., 2002 [2]). Although bioethanol and biodiesel are currently the major targets of biomass energy, hydrogen is still considered the ultimate solution of clean and recyclable energy carrier in a long term (Kapdan and Kargi, 2006 [3]). Biomass feedstock contains a large amount of cellulosic materials, such as cellulose, hemicellulose, and lignin

(Chandrakant and Bisaria, 1998 [4]). Among those three major components, cellulose and hemicellulose are much easier to degrade biologically, thereby being more economically viable for energy conversion (Chandrakant and Bisaria, 1998 [4]). Direct fermentation of raw cellulosic feedstock is usually inefficient because cellulose and hemicellulose are not readily assimilable to most energy-producing bacteria (for instance, yeast or $H_2$-producing acidogenic bacteria). Thus, it seems to be more feasible to use a two-stage biomass energy producing process, in which cellulosic materials are first hydrolyzed via physico-chemical or biological means, followed by a fermentative energy conversion step (Chandrakant and Bisaria, 1998 [4]).

In anaerobic digestion of organic substrates, the acidogenic process, producing hydrogen and volatile fatty acids as major products, is considered an efficient and promising way of producing clean $H_2$ energy (Levin et al. 2004 [5]). Most effective fermentative $H_2$ producers belong to anaerobic acid-forming bacteria (such as *Clostridium* sp.) (Levin et al., 2004 [5]). In our recent work, several highly efficient bioH2-producing processes were developed using mixed-cultures (Lee et al., 2003 [6]). Bacterial community structure analysis revealed that the sludge contained several *Clostridium* species (e.g., *C. butyricum* and *C. pasteurianum*) (Lo et al. 2008 [7]), which are known effective $H_2$ producers from organic substrates (esp. carbohydrates). It is thus of great value to isolate and characterize effective $H_2$-producing pure strains from the aforementioned sludge for the potential use in maintaining or improving $H_2$ production performance of mixed-culture systems via bioaugmentation strategies.

## 2 Materials and Methods

### 2.1 Microorganism and medium

Hydrogen-producing bacterial strain *Clostridium butyricum* CGS2 was isolated from effluent sludge of a continuous dark fermentation bioreactor able to produce $H_2$ from synthetic wastewater containing sucrose (20 g COD $l^{-1}$) or xylose (20-40 g COD $l^{-1}$) as the sole carbon source (Lo et al. 2008 [7]). The detailed procedures for strain isolation and identification were described in our recent work (Lo et al., 2008 [7]). The 16S rRNA gene sequence of *C. butyricum* CGS2 used in this study has been deposited in the NCBI nucleotide sequence database with an accession number of AY540106. The pure strain was pre-cultured under anaerobic conditions (Lo et al., 2008 [7]) on the medium consisted of (g $l^{-1}$): sucrose, 17.8; $NH_4HCO_3$, 6.72; $NaHCO_3$, 5.24; $K_2HPO_4$, 0.125; $MgCl_2.6H_2O$, 0.1; $MnSO_4.6H_2O$, 0.015; $FeSO_4.7H_2O$, 0.025; $CuSO_4.5H_2O$, 0.005; $CoCl_2.5H_2O$, $1.25\times10^{-4}$.

### 2.2 Fermentation medium and condition for bioH2 production

The medium for dark $H_2$ fermentation with the pure cultures was (g/l): sucrose, 17.8 (adjustable); $NH_4HCO_3$, 6.72; $NaHCO_3$, 5.24; $K_2HPO_4$, 0.125; $MgCl_2.6H_2O$, 0.1; $MnSO_4.6H_2O$, 0.015; $FeSO_4.7H_2O$, 0.025; $CuSO_4.5H_2O$, 0.005; $CoCl_2.5H_2O$, $1.25\times10^{-4}$. The culture temperature and pH was 37 °C and 7.5, respectively. Response surface methodology (RSM) was employed to identify the optimal conditions for hydrogen production of *C. butyricum* CGS2 using sucrose concentration, temperature and pH as the primary operation parameters. During the course of fermentation, cell concentration, pH, residual carbon

substrate concentration, and production of biogas and soluble metabolites were monitored with respect to culture time.

## 3 Results and Discussion

**Table 1: Dark-$H_2$ production performance of *C. butyricum* CGS2 at different sucrose concentration.**

| Sucrose conc. | $H_2$ content | Conversion | Vmax,$H_2$ | Model simulation | | | |
|---|---|---|---|---|---|---|---|
| (mg COD/l) | (%) | (%) | (ml) | Hmax(ml) | Rmax(ml/h) | λ(h) | $R^2$ |
| 5000 | 33 | 100 | 32 | 33.7 | 5.77 | 13.3 | 0.996 |
| 10000 | 44 | 97 | 51 | 53.5 | 5.97 | 18.3 | 0.999 |
| 20000 | 47 | 45 | 5 | 104.9 | 28.0 | 19.8 | 0.999 |
| 30000 | 48 | 41 | 82 | 83.3 | 20.1 | 22.9 | 0.999 |

### Effect of sucrose concentration on the $H_2$ production performance

Using different sucrose concentration to investigate dark-$H_2$ production performance of *Clostridium butyricum* CGS2. Table 1 shows the performance of dark-$H_2$ production in temperature of 37°C and initial pH of 7.5. When sucrose concentration was increased, $H_2$ content was increased from 33% to 48%. Model simulation analysis by modified Gompertz equation (Eqn. 1) shows that using sucrose concentration of 20000 mg COD/l resulted in maximum $H_2$ production rate ($R_{max}$) of 28.0 ml/h (Table 1). The lag time was similar (λ=13-22 h) for all sucrose concentration examined (Table 1). Table 2 show the soluble metabolites production during fermentative $H_2$ production of *C. butyricum* CGS2. The main soluble metabolites were butyrate and acetate. When sucrose concentration was increased, the butyrate concentration, acetate concentration and total volatile fatty acids concentrations were increased. The results shows the high $H_2$ yield and high $H_2$ production was get, the butyrate concentration, acetate concentration and total volatile fatty acids concentrations were increased.

$$H = H_{\max} \exp\{-\exp[\frac{R_{\max,H_2} \times e}{H_{\max}}(\lambda - t) + 1]\} \quad (1)$$

**Table 2: Production of soluble metabolites during fermentative $H_2$ production of *C. butyricum* CGS2**

| Carbon source | | Soluble metabolite (mg COD/l) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | EtOH | HAc | HPr | HBu | HVa | TVFA | SMP |
| Sucrose | 5000 | 545 | 361 | 12 | 2586 | 16 | 2975 | 3520 |
| | 10000 | 624 | 790 | 79 | 5451 | 34 | 6354 | 6978 |
| | 20000 | 422 | 1441 | 92 | 6255 | 96 | 7884 | 8305 |
| | 30000 | 505 | 1442 | 151 | 6289 | 115 | 7997 | 8502 |

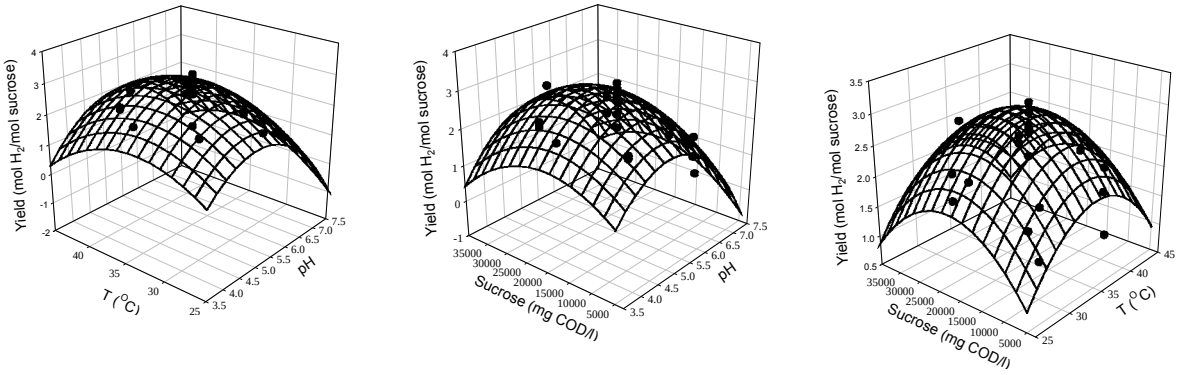


**Figure 1: Response surface methodology (RSM) for optimal condition (sucrose concentration, temperature and pH).**

## Response surface methodology (RSM) was employed to identify the optimal conditions for hydrogen production of C. butyricum CGS2 using sucrose concentration, temperature and pH

Optimal condition (sucrose concentration, temperature and pH) was identified with response surface methodology (RSM). Figure 1 show the results of response surface methodology for optimal condition (sucrose concentration, temperature and pH). With a performance index of $Y_{H2}$, the optimum condition predicted from RSM analysis was: pH, 5.2; temperature, 35.1 °C; sucrose concentration, 22.5 g COD/l. Under this condition, the hydrogen content in the biogas was 58.5%, $\square H_2$ was 0.54 l/h/l, total hydrogen production was 7.2 l, and $Y_{H2}$ was 2.91 mol $H_2$/mol sucrose. On the other hand, when $\square H_2$ was used as the performance index, the optimum condition was: pH, 5.36; temperature, 35.1°C; sucrose concentration, 26.1 g COD/l. This condition gave a hydrogen content of 63.3%, a $Y_{H2}$ of 3.26 mol $H_2$/mol sucrose, a total hydrogen production of 10.5 l, and a $\square H_2$ of 0.50 l/h/l. The validity of RSM predictions was confirmed by additional experiments, suggesting that using RSM design could attain an optimal culture condition for *C. butyricum* CGS2 to enhance its hydrogen production performance.

## References

[1] Tsai, W.T., Lin, C.C. and Yeh, C.W. (2007) An analysis of biodiesel fuel from waste edible oil in Taiwan. Renewable and Sustainable Energy Reviews **11**(5), 838-857.

[2] Vrije, T.d., Haas, G.G.d., Tan, G.B., Keijsers, E.R.P. and Claassen, P.A.M. (2002) Pretreatment of *Miscanthus* for hydrogen production by *Thermotoga elfii*. Int. J. Hydrogen Energy **27**(11-12), 1381-1390.

[3] Kapdan, I.K. and Kargi, F. (2006) Biohydrogen production from waste materials. Enzyme Microb. Technol. **38**(5), 569-582.

[4] Chandrakant, P. and Bisaria, V.S. (1998) Simultaneous bioconversion of cellulose and hemicellulose to ethanol. Crit. Rev. Biotechnol. **18**(4), 295-331.

[5] Levin, D.B., Pitt, L. and Love, M. (2004) Biohydrogen production: prospects and limitations to practical application. Int. J. Hydrog. Energy **29**(2), 173-185.

[6] Lee, K.S., Lo, Y.S., Lo, Y.C., Lin, P.J. and Chang, J.S. (2003) $H_2$ production with anaerobic sludge using activated-carbon supported packed-bed bioreactors. Biotechnology Letters **25**, 133-138.

[7] Lo, Y.C., Chen, W.M., Hung, C.H., Chen, S.D., Chang, J.S., 2008. Dark $H_2$ fermentation from sucrose and xylose using $H_2$-producing indigenous bacteria: Feasibility and kinetic studies. Water Res. 42, 827-842.