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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; $CoCl_2.5H_2O$, 1.25×10^{-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; $CoCl_2.5H_2O$, 1.25×10^{-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

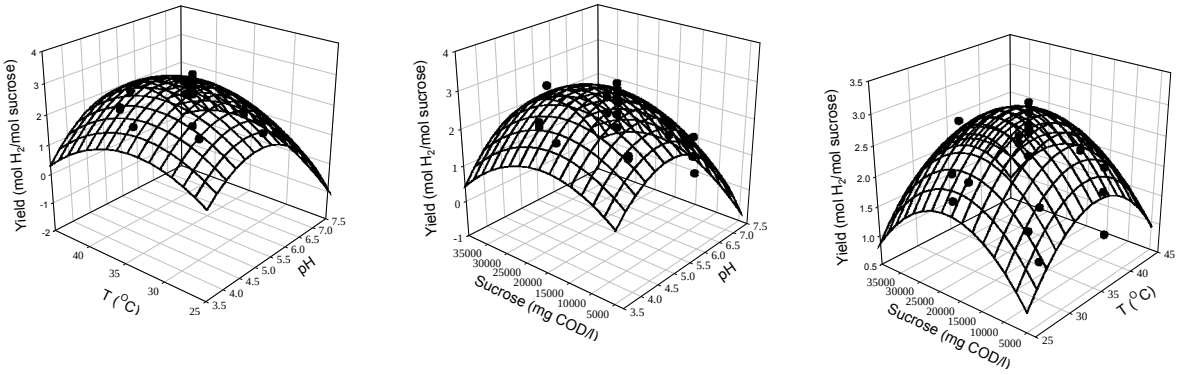

Figure 1: Response surface methodology (RSM) for optimal condition (sucrose concentration, temperature and pH).

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.

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