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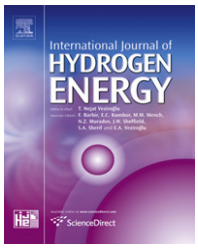

# Optimization of key variables for the enhanced production of hydrogen by *Ethanoligenens harbinense* W1 using response surface methodology

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ABSTRACT

The optimization of process conditions for the production of hydrogen by *Ethanoligenens harbinense* W1 was investigated using response surface methodology (RSM). Three parameters namely inoculum to substrate ratio, initial pH value and temperature were chosen as variables. The adequately high $R^2$ value (99.4%) indicated the statistical significance of the model. The optimum process conditions for hydrogen production rate were determined by analyzing the response surface three-dimension surface plot and contour plot and by solving the regression model equation with Design Expert software. The central composite design (CCD) was used to optimize the process conditions, which showed that an inoculum to substrate ratio of 14%, initial pH value of 4.32 and the experimental temperature of 34.97 °C were the best conditions. Under the optimized conditions, the maximum specific hydrogen production rate (SHPR) was 35.74 mL/g-CDW.h based on cell dry weight. The results were further verified by triplicate experiments. The batch reactors were operated under an optimized condition of the inoculum to substrate ratio of 14%, initial pH value of 4.3 and the experimental temperature of 35 °C. The maximum SHPR was estimated at 35.57 mL/g-CDW.h, which further verified the practicability of this optimum strategy.


## 1. Introduction

Biological hydrogen production rate by fermentation is an effective way to solve our energy crisis. Thus, hydrogen technology is receiving considerable attention due to the unique properties of hydrogen such as clean, renewable, and high heat value. In the past decade, researches on the characteristics and cultivation conditions of the pure hydrogen producing bacteria have been reported extensively [1–4]. As reported previously, pH value has affected much on anaerobic hydrogen production rate. The optimal pH value for maximum hydrogen production rate varied from 4.0 to 5.5 according to different kinds of hydrogen producing bacteria cultivated [5–9]. Other studies also showed significant effects of parameters on bacteria cultivation and target production generation, such as ratio of inoculum to substrate, cultivation temperature at mesophilic conditions [10,11]. However, most studies were focused on the "one factor at a time" approach. Only a few studies use Response Surface Methodology (RSM) for the optimization study of hydrogen production [12–15]. Response Surface Methodology (RSM) using central composite design (CCD) which involves full factorial search by examining simultaneous, systematic and efficient

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variation of important components was applied to model the hydrogen production rate process, identify possible interactions, higher order effects and determine the optimum operational conditions [16]. As extensively shown in the literature [10], the inoculum to substrate ratio, initial pH value control and temperature were significant in successfully cultivating biological hydrogen production bacteria. However, the mutual effects of these factors were not well studied to achieve enhanced hydrogen production performance.

Consequently, in the present study, the effects of ratio of inoculum to substrate, initial pH value and cultivation temperature were evaluated using the CCD approach, and a suitable medium using response surface methodology was also employed for maximizing specific hydrogen production rate by the hydrogen producing bacteria *Ethanoligenens harbinense* W1.

## 2. Materials and methods

### 2.1. Microorganism and basal fermentation media

Hydrogen producing strain *E. harbinense* W1, was obtained from State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, China which was stored at 4 °C and sub-cultured monthly with glucose-based fresh medium at 35 ± 0.5 °C.

The media composition (g/L): glucose, 15; polypepton, 4; beef extract, 2; yeast extract, 1; NaCl, 2; $K_2HPO_4$, 1.5; $MgCl_2 \cdot 6H_2O$, 0.6; $FeSO_4 \cdot 7H_2O$, 0.2; L-cysteine, 0.5; was used as the basal medium. 10 mL trace element solution and 10 mL vitamin solution were also added to each 1 L of the basal medium to maintain the necessary nutrient requirement of the cells. The trace element solution (g/L) and the composition of vitamin solution (g/L) was described in the previous work [12].

### 2.2. Batch reactor and experimental procedure

All batch experiments were carried out in 150 mL Erlenmeyer flasks reactors described before [17]. The batch reactors were contained of the basal medium and the prepared bacteria of 10 mL to make a final media volume of 100 mL. The batch reactors were run for 48 h at 120 rpm to provide release of biogas and make better mass transfer between substrate and microorganisms. The batch reactor was flushed with nitrogen gas before the startup to guarantee the anaerobic environment [18].

The reactor was wrapped by electrothermal wire to keep a consistent operating temperature of the designed value (21.6 °C, 25 °C, 30 °C, 35 °C and 38.4 °C) with a precision of ±0.1 °C. The initial pH control was adjusted by adding 0.1 mol/L HCl solution or 0.1 mol/L NaOH solution.

### 2.3. Analytical methods

The $H_2$ was analyzed by an SP-2305 gas chromatograph (Lunan Instrument Factory, Shandong, China) equipped with a thermal conductivity detector and a 2-m stainless column packed with Porapak TDS-01 (60/80 mesh). The operational procedure was described before [12,19]. The liquid samples were centrifuged at 4000 rpm for 15 min, and then the supernatants were filtered through 0.45 μg cellulose acetate membranes for analysis of the volatile fatty acids (VFAs) and ethanol. The concentrations of the VFAs and ethanol were determined using a model GC-122 gas chromatograph (Shanghai Analytical Apparatus Corporation, China) with a flame ionization detector and a 2-m stainless (5-mm inside diameter) column packed with Porapak GDX-103 (60/80 mesh). The operational temperatures of the injection port, the column and the detector were 220, 190 and 220 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 50 mL/min [19]. Glucose concentration was determined by the method described before [20]. pH value was determined by standard methods [21].

### 2.4. Experimental design and statistical analysis

A three-level-three-factor central composite design (CCD) obtained by using the "Design Expert" software (Version 7.1.4.0, Stat-Ease Inc., Minneapolis, USA) statistical package was used to find out the effect on hydrogen production rate by most effective independent variables, including ratio of inoculum to substrate, pH and temperature according to literature experiences [11,22]. The specific hydrogen production rate was taken as the response. The levels of factors used for optimization are presented in Table 1.

Since the factorial was full, the total number of experiments with three variables and six central points was calculated as 20 ($2^k + 2k + 6$), and the distance from the central points was calculated as 1.68 ($\alpha = 2^{k/4}$), where $k$ is the number of independent variables. In the regression equation, the test variables were coded according to the equation

$$X_1 = (U_i - U_i^0)/\Delta U_i \quad (1)$$

where, $X_1$ is the independent variable coded value, $U_i$ is the independent variable real value, $U_i^0$ is the independent variable real value on the center point.

**Table 1 – Levels of factors used for optimization of specific hydrogen production rate.**

| Variable | Label | Level | | | | |
|---|---|---|---|---|---|---|
| | | $-1.682(-\alpha)$ | $-1$ | 0 | 1 | 1.682 $(+\alpha)$ |
| $X_1$ | Ratio (volume ratio of inoculum to substrate) | 3.27 | 6 | 10 | 14 | 16.73 |
| $X_2$ | Initial pH value | 3.89 | 4.30 | 4.90 | 5.50 | 5.91 |
| $X_3$ | Temperature | 21.59 | 25 | 30 | 35 | 38.41 |