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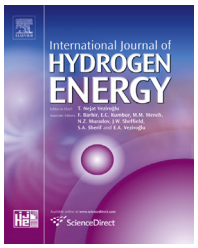

Optimization of volatile fatty acids concentration for photofermentative hydrogen production by a consortium

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ABSTRACT

The optimum concentration of volatile fatty acids that maximized the hydrogen production by photobacteria was determined using Box–Behnken and Central Composite designs. A photofermentative hydrogen-producing consortium, obtained from a bioelectrochemical system, was used as inoculum. *Rhodopseudomonas palustris* was identified in the consortium. Experiments were conducted in anaerobic batch reactors at 30–35 °C and illuminated by a constant radiation of 5000 lux. Hydrogen production decreased when propionic acid is higher than 715 mg L^{-1}. Ammonia formation was responsible for the pH increase. A maximum hydrogen production rate of 6.1 mL d^{-1} $L_{culture}^{-1}$ h^{-1} and a maximal amount of hydrogen produced of 108.8 mmol $L_{culture}^{-1}$ were obtained. Optimal concentrations of acetic, propionic and butyric acids were 1200 mg L^{-1}, 715 mg L^{-1} and 1571 mg L^{-1}, respectively.

Introduction

At present, fossil fuels are considered the main source of energy supply. However, because of their growing demand and limited availability, an alternative source of energy is needed [1]. Hydrogen is one of the most promising fuels of the future due to its high energy content (122 kJ g^{-1}), which is 2.72 times than that for gasoline [2,3]. In addition, H_2 is an important energy carrier and can be used in fuel cells for the generation of electricity [4,5]. However, hydrogen gas is not readily available in nature as are fossil fuels and natural gas but it can be produced from renewable materials, such as biomass [2] and water [6]. The biological production of hydrogen is environmentally friendly. In particular, this process can use a variety of organic substrates as carbon sources, including wastes [7]. Therefore, there exists a double benefit: waste reduction and energy production.

The combination of photosynthetic bacteria with fermentative bacteria can enhance the amount of hydrogen produced by the system, and at the same time, there is a reduction of residual organic matter [5]. Photosynthetic non-sulfur (PNS) bacteria have the ability to convert volatile fatty acids (VFA) into H_2 and CO_2 under anoxygenic conditions [5,8]. PNS bacteria also have the ability to use carbon sources, such as glucose, sucrose, and succinate, rather than VFA for H_2

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production [9–11]. The most widely known PNS bacteria that are used in photofermentative H_2 production are *Rhodobacter sphaeroides O.U001*, *Rhodobacter capsulatus*, *R. sphaeroides-RV*, *Rhodobacter sulfidophilus*, *Rhodopseudomonas palustris* and *Rhodospirillum rubrum* [12]. Both hydrogenase and nitrogenase enzymes have been detected in PNS bacteria [5,13]. Nitrogenase is the main enzyme that is responsible for molecular H_2 production under anoxygenic conditions [13,14]. Photofermentation culture is very promising because of the purity of the generated biogas (H_2: 80%) [15]. However, the potential of different substrates must be considered, as in the case when the dark fermentation is to be coupled to the photofermentation process, where acetate, propionate and butyrate are generated [16]. Also, it can be interesting to evaluate the use of a consortium instead of a pure culture because sterile conditions are unnecessary, rendering the operation of a reactor easier to control. In addition, a microbial consortium can exhibit a better robustness against inhibitors that can be produced by variations in the substrate fed to the system.

The fractional factorial design provides an alternative when the number of runs for a full factorial design is too large to be practicable [17,18]. Taguchi design, Plackett–Burman design, central composite design and Box–Behnken design are fractional factorial designs that have been frequently used for fermentative hydrogen production processes, providing a practical alternative in the study of factors [19]. In the present study, Box–Behnken design and Central Composite design (full factorial design) were used to evaluate the effect of a mixture of acetic, propionic and butyric acids on the hydrogen production rate using a consortium of photobacteria. Then, the optimum concentration of volatile fatty acids for maximum hydrogen production was found by applying response surface methodology.

Experimental

Inoculating and enriching the microbial consortium

The photofermentative hydrogen-producing consortium was obtained scratching the anode of a bio-electrochemical system used to produce hydrogen [20]. The use of this inoculum was suggested by the presence of *R. palustris* in microbial fuel cells [21]. The inoculum plus 150 mL of medium were placed in 250-mL glass bottles. The culture medium was supplemented with acetate (2.46 g L^{-1}), butyrate (3.30 g L^{-1}) and sodium glutamate (0.37 g L^{-1}). Then, argon gas was purged in the headspace to ensure anaerobic conditions. The initial pH was adjusted to 6.8 with 1 N NaOH. The bottle was incubated at 30–35 °C, illuminated by four fluorescent lamps (20 W) and four tungsten lamps (60 W). After growing for 100 h, the biomass with the purple non-sulfur photofermentative bacteria was harvested and a new cultivation cycle was started. The procedure was repeated until enough biomass was produced.

Operation of the batch reactors

The influence of initial concentration of VFA mixtures was evaluated on the photofermentative hydrogen production by using a consortium previously grown.

For these experiments, 100 mg of VSS L^{-1} of adapted biomass and 100 mL of basal medium and the required amount of VFAs were used. The bottles were sparged with argon for a period of 30 s to remove dissolved oxygen and to create an oxygen-free microenvironment prior to closing with rubber septum. All of the bottles were placed on a shaker plate at 100 rpm, 30–35 °C and illuminated under a constant radiation of 5000 lux. This light intensity was already reported as the optimal for photofermentative hydrogen production [7,15,22,23]. The basal medium [24] was composed of 0.75 g L^{-1} K_2HPO_4, 0.85 g L^{-1} KH_2PO_4, 0.2 g L^{-1} $MgSO_4$, 11.78 mg L^{-1} $FeSO_4 \cdot 7H_2O$, 2.8 mg L^{-1} H_3BO_3, 0.75 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 0.24 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 2.1 mg L^{-1} $MnSO_4 \cdot 4H_2O$, 0.04 mg L^{-1} $CuCl_2 \cdot 2H_2O$, 0.75 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 2.0 mg L^{-1} EDTA-Na, 3.78 mg L^{-1} B1 vitamin and 3.57 mg L^{-1} biotin. For this stage, the C/N ratio was 16.

Box–Behnken design

Box–Behnken design (BBD) is a three-level fractional factorial design that was developed by Box and Behnken. This design can be thought of as a combination of a two-level factorial design with an incomplete block design. In each block, a certain number of factors are put through all combinations for the factorial design, while the other factors are kept at central levels. BBD provides an economical alternative to the central composite design (CCD) because it has fewer factor levels than does the CCD and does not contain extremely high or extremely low levels. For that reason, in a first set of experiments, a BBD matrix was used to investigate the effect of VFAs concentration on photofermentative hydrogen production. The designated variables were the acetic acid concentration (X_1), butyric acid concentration (X_2) and propionic acid concentration (X_3) on hydrogen production. The factors X_1, X_2 and X_3 were considered independent variable, and the maximal amount of hydrogen produced (H_{max}) and maximum hydrogen production rate (R_{max}) were the response (dependent variable). The low, middle and high levels of each variable were coded as −1, 0 and +1, respectively [25]. The levels for the volatile fatty acids concentrations were selected based on a literature review [26–29]. The coded and actual values are given in Table 1.

The following second-order polynomial equation was used to study the effects of the variables to the response.

$$H_{max}, R_{max} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

Table 1 – Experimental range, level and code of independent variables for the optimization of hydrogen production for the Box–Behnken design.

Independent variables	Symbol coded	Range and levels		
		−1	0	1
Acetic acid concentration (mg L^{-1})	X_1	125	1235	2345
Butyric acid concentration (mg L^{-1})	X_2	50	160	270
Propionic acid concentration (mg L^{-1})	X_3	175	672.5	1170