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Optimization of H_2 photo-fermentation from benzoate by *Rhodopseudomonas palustris* using a desirability function approach

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ABSTRACT

The operational conditions of H_2 production from benzoate by *Rhodopseudomonas palustris* was optimized in batch experiments by using the response surface methodology (RSM) and a desirability function approach. The estimated best H_2 production performance, i.e., maximum substrate-to-hydrogen conversion efficiency of 0.61 and light conversion efficiency of 3.20%, was achieved at: benzoate concentration 9.97 mM, NH_4Cl concentration 0.21 g/L and pH 6.76. These parameters were employed for running a continuous H_2 production bioreactor, for which the optimal hydraulic retention time was found to be 6 d. Under a light–dark cycle, *R. palustris* survived and maintained their H_2 production activity in the continuous operating system. The results demonstrate that it is possible to operate an outdoor photobioreacror for continuous H_2 production by utilizing aromatic compound as substrate. This works offer implications for guiding the design and operation of more energy–productive processes for treatment of aromatic compound-containing wastewater treatment.

1. Introduction

Aromatic compounds present a major class of environmental recalcitrant pollutants [1]. Biological degradation of such compounds, as a low-cost and environmental-benign approach, has been under intensive research in the past decades [2,3]. Especially, the process of anaerobic degradation of aromatic compounds with concurrent H_2 production has attracted great interests recently. Although anaerobic H_2 fermentation from carbohydrate-rich wastewater is already approaching practical implementation [4], however, recovering H_2 from aromatic wastewater is still very difficult due to its toxic and recalcitrant nature. In particular, some degradation intermediates are even more toxic than their parent compounds, thereby severely limiting the biodegradation efficiency and hamper a H_2 production [5]. As a countermeasure, some bacteria with good resistance and specific degradation ability are usually adopted to further degrade the intermediates and even to simultaneously produce H_2. For example, a pure photosynthetic bacterium, *Rhodopseudomonas palustris*, has been found to efficiently convert aromatic acids to H_2 with the highest yields reaching 45% of the maximal theoretical stoichiometric amount [6].

Since anaerobic metabolisms of many aromatic compounds, such as aniline, p-cresol and toluene phenylacetate,

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can lead to the formation of benzoyl-CoA [2,7], a two-step bioconversion process can thus be readily established to efficiently and completely remove a wide range of aromatics and produce H_2 simultaneously. In such a process, the aromatic compounds are anaerobically degraded to form benzoate (an aromatic acid with the simplest structure), which can then be further degraded by some specific bacteria, e.g., photosynthetic bacteria (PSB), to produce H_2 [8,9]. However, such a photo-fermentation process can be influenced by a number of environmental and operating factors such as inoculum type, substrate compositions, cell concentration, light intensity, carbon to nitrogen ratio, temperature, and pH etc. [10]. Thus, an optimization of these factors is essential to improve benzoate degradation and H_2 production efficiencies [11,12].

One useful tool for process optimization is response surface methodology (RSM) [13], which allows determination of the optimal conditions for multiple influential factors at limited number of experiments [14]. RSM has been successfully used to optimize the batch operation of H_2 photo-fermentation processes [15]. However, up to date there is no report regarding the optimization of such benzoate-based H_2 photo-fermentation process, and its continuous operation.

This work aims to evaluate the effects of benzoate concentration, pH and NH_4Cl concentration on H_2 production by *R. palustris*, and to find the optimal conditions using RSM and a desirability function approach. Since continuous-flow processes are usually preferred to achieve a stable H_2 production and treatment performance [16], the calculated optimal conditions were experimentally validated in a continuous H_2 production system at different hydraulic retention times (HRTs). In addition, the effect of light–dark cycle on H_2 production was also investigated, which offered implication for outdoor H_2 production systems.

2. Materials and methods

2.1. Inocula and pre-culture

R. palustris was grown in a modified aSy medium, which was composed of a basal medium, supplemented with: yeast extract 1 g/L; $(NH_4)_2SO_4$ 1.25 g/L; sodium succinate 1.0 g/L. The detailed composition of basal medium can be found elsewhere [13]. The culture was grown anaerobically in 300-mL glass reactors with rubber-stopper at 30 ± 1 °C, pH of 6.8 and light intensity of 5 W/m^2.

2.2. Batch experiments

The bacterial cells were harvested in their exponential phase, and were transferred into the H_2 production medium, which contained (in per liter) KH_2PO_4 1.0 g; $MgSO_4 \cdot$0.2 g; $CaCl_2 \cdot 2H_2O$ 0.05 g. Benzoate was used as the sole carbon source with the concentrations varied from 4 to 12 mM. The pH varied within 6.0–8.0 and the concentration range of NH_4Cl (as the sole nitrogen source) was 0.1–0.3 g/L. The light intensity was adjusted to 50 W/m^2. The other conditions were the same as the pre-culture conditions. Each experiment was conducted in duplicate.

2.3. Experimental design

A three-level, three-parameter (benzoate concentration, pH and NH_4Cl concentration) experimental design of Box–Behnken method was used to evaluate the optimum H_2 production condition (Table 1). The experiments were performed at random order. The Y, including substrate-to-hydrogen conversion efficiency (SHCE) and light conversion efficiency (LCE), is a function of independent variables X_1, X_2 and X_3, which are benzoate concentration, NH_4Cl concentration and pH, respectively. Regression analysis was performed with the data obtained. The response function is a second-order polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j}^{3} \beta_{ij} X_i X_j \tag{1}$$

where Y is the response, β_0 is the constant coefficient, β_i,β_{ii} andβ_{ij} are the coefficients estimated by the model. They represent the liner, quadratic and cross-product effects of the X_1, X_2 and X_3 respectively.

The parameters of the response equation and analysis of variance (ANOVA) were evaluated using Origin 7.5 (OriginLab Inc., USA) and MATLAB 6.5 (Mathworks Inc., USA), respectively. However, for multi-response, a desirability function approach can be used to transformed several response variables into a desirability function, which can be optimized by univariate techniques. A modified desirability approach, proposed by Dirringer and Suich [17] is defined as:

$$D = \left(d_1^{w_1} d_2^{w_2} d_3^{w_3} d_4^{w_4} d_n^{w_n}\right)^{1/\sum w_i} \tag{2}$$

where w_i is a response weight, D is the overall desirability, and d_i is an individual response desirability. Here, D belongs to weighted composite desirability, whose definition and function are described in the Desirability method [18,19]. One-sided transform of d_i is used in the study and given by:

$$d_i = \begin{cases} 0 & Y_i \le Y_{i-min} \\ \left[\dfrac{Y_i - Y_{i-min}}{Y_{i-max} - Y_{i-min}}\right]^r & Y_{i-min} < Y_i < Y_{i-max} \\ 1 & Y_i \ge Y_{i-max} \end{cases} \tag{3}$$

where Y_i is the response values, $Y_{i\text{-}min}$ is the minimum acceptable value for response i, $Y_{i\text{-}max}$ is the maximum acceptable value for response *i*, and *r* is a weight used to determine scale of desirability and equals to 1 in this work.

2.4. Continuous H_2 production

To validate the calculated optimizing results, continuous H_2 production system was operated by employing the predicted optimum conditions of benzoate concentration, pH and NH_4Cl concentration from the batch experiments. After inoculation, the 300-mL reactors were flushed with argon, and maintained for 240 h before applying continuous feeding. The reactors were illuminated by using two tungsten lamps to give a light intensity of 50 W/m^2. The other conditions were the same as the pre-culture conditions. The effects of HRT on H_2 production performance were investigated. The HRT varied from 7.5 d to 4.0 d.