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Continuous Photo-hydrogen Production from Acetate Using *Rhodopseudomonas Palustris* WP 3-5

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Abstract

Phototropic hydrogen production using components of dark fermentation metabolites (i.e. acetate) is an ideal biohydrogen production route since it enables the highest yield possible (i.e., 12 mol H_2/mol hexose) and could also reduce COD content in the dark fermentation effluent to mitigate the environmental burden. In this work, a purple nonsulfur photosynthetic bacterial isolate *Rhodopseudomonas palustris* WP3-5 was utilized to produce H_2. To assess the performance of the photo-H_2 production system, the continuous photobioreactor was operated at a hydraulic retention time (HRT) of 48 h under different acetate concentrations. The optimal H_2 production efficiency occurred when the acetate concentration was 3000 mg COD/l, leading to an overall H_2 production rate and a H_2 yield of 20.4 ml/h/l and 0.98 mol H_2/mol acetate, respectively. In all tests, the acetate conversion was nearly constant at 87.0±3.1%. Next, the preferable HRT leading to the best H_2 production performance was identified. The results show that at an acetate concentration of 3000 mg COD/L, H_2 production rate increased with a decrease in HRT, giving the best performance at HRT=12 h with an overall H_2 production rate of 42.05 ml/h/l and a H_2 yield of 1.39 mol H_2/mol acetate. Operation under the aforementioned optimal substrate concentration and HRT, the overall H_2 production rate could be enhanced two-fold higher when compared with that obtained from preliminary tests. In addition, the HRT setting was found to play a key role in influencing the performance of continuous H_2 production by *R. palustris* WP3-5. The proposed continuous cultures seem to be a favourable choice of bioreactor strategy, possessing the potential to achieve higher H_2 production efficiency with an excellent stability.

1 Introduction

Hydrogen is a clean energy since combustion of H_2 produces only water without greenhouse gases. Hydrogen can also be directly utilized by hydrogen fuel cell to generate electricity at very high efficiency [1], thereby being considered a promising alternative energy carrier of the future. To this end, developing hydrogen production technology leading to a sufficient and sustainable H_2 supply is highly demanded [2]. Biological H_2 production considered as the most environmentally friendly route of producing H2 [3], thereby fulfilling the goals of recycling of renewable resources and clean energy production [4]. Phototrophic H_2 production has the advantage of high theoretical substrate conversion efficiency and

minerialization of organic substrates (e.g., organic acids), thereby being considered a critical step in the integrated fermentative hydrogen production [5-8]. This study was to identify the best carbon substrate (acetate) concentration since the organic loading often plays a critical role in affecting the kinetics of catabolism. Meanwhile, the stability of the continuous H_2 production system using different HRT concentration was also assessed to improve the performance of phototrophic H_2 production by *R. palustris* WP3-5.

2 Materials and Methods

2.1 Bacterial strain and cultivation medium

The *Rhodopseudomonas palustris* WP3-5 isolated from a swine wastewater treatment plant located in central Taiwan [9] was used for phototrophic H_2 production. The culture medium was using 2000 mg COD/l sodium acetate as a sole carbon substrate. The cells were cultivated at 32^{o}C anaerobically for 48 h under a light intensity of approximately 50 W/m^2 (illuminated by tungsten filament lamp). The initial pH value of medium prior to incubation was adjusted to 7.0-7.1. Argon gas was used to create an anaerobic condition.

2.2 Fabrication and operation of photobioreactor

The photobioreactor was a 1.0-liter glass-made vessel illuminated with external light sources (100 W tungsten filament lamp). The total light intensity for each illumination system was adjusted to ca. 95 W/m^2. After the reactor apparatus was sterilized by autoclave, cells of *R. palustris* WP3-5 were inoculated into the reactor with a 10% inoculum. All the fermentation was operated at pH 7.1 with an agitation rate of 200 rpm. A gas meter (Type TG1; Ritter Inc., Germany) was used to measure the amount of gas products generated and the gas volumes were calibrated to 25 ^{o}C and 760 mmHg. Gas samples were taken from sampling port by gas syringe at desired time intervals to measure the gas composition. The liquid sample was also collected from the sealed glass vessel with respect to time to determine cell concentration, pH and residual acetate concentration.

3 Results and Discussion

3.1 Effect of sodium acetate concentration on the photo-hydrogen production performance

Literature shows that the sodium acetate concentration is critical factors affecting the fermentation kinetics of biohydrogen production [10]. Therefore, in this study, acetate was used as the sole carbon source for phototropic H_2 production. The effect of acetate concentration on photo-H_2 production performance was examined in continuous cultures containing different initial acetate concentrations (sodium acetate = 1000–4000 mg COD/l). The results are indicated in Table 1. The volumetric H_2 production rate and specific H_2 production rate appeared to increase with increasing acetate concentration from 1000 to 4000 mg COD/l in all tests. This trend is quite reasonable as higher acetate (carbon substrate) loading usually leads to higher H_2 production. The optimal H_2 production efficiency occurred when the acetate concentration was 3000 mg COD/l, gave the best overall H_2

production rate and H_2 yield of 20.4 ml/h/l and 0.98 mol H_2/mol acetate, respectively. Meanwhile, the acetate conversion was nearly constant at 87.0±3.1%. Therefore, the acetate concentration of 3000 mg COD/l could give the relatively higher overall H_2 production rate and H_2 yield than other sodium acetate concentration used.

3.2 Effect of HRT on the photo-hydrogen production performance

To assess the applicability of the phototrophic H_2 production system, continuous culture by using different HRT was performed for a prolonged period of time under the optimal conditions in the present work (i.e., sodium acetate concentration = 3000 mg COD/l, light source = TL, light intensity = 95 W/m^2). As shown in Table 2, at 48 h HRT, the continuous culture gave a volumetric H_2 production rate of 20.4 ml/l/h. When the HRT was decreased to 12 h, the volumetric H_2 production rate was rapidly increased from 20.4 ml/l/h to 42.05 ml/l/h. Meanwhile, the cell concentration reached a nearly constant value of 3.11 g/l. The acetate conversion efficiency was obviously decreased with a decrease in HRT. These results clearly indicate that HRT was an important effecting factor for the photo-H_2 production of continuous operation by *R. palustris* WP3-5. This suggests that continuous cultures seem to be a more favorable choice of bioreactor strategy, having the potential to achieve higher H_2 production. Meanwhile, the H_2 content in biogas was nearly constant at 81.2±2.9% at 12 h HRT, indicating easier separation in downstream processing to obtain purified H_2 products for fuel cell applications.

Table 1: Photo-H_2 production performance at different sodium acetate concentration (HRT=48 h).

CH_3COOH (mg COD/L)	H_2 yield (mol H_2/mol actate)	Volumetric H_2 production rate (mL/h/L)	Specific H_2 production rate (mL/h/g cell)	Acetate conversion (%)
1000	0.86	6.86	2.65	98
2000	1.15	17.49	5.18	99
3000	0.98	20.40	5.46	87
4000	1.06	22.65	6.51	67

Table 2: Photo-H_2 production performance at different HRT (sodium acetate concentration = 3000 mg COD/L).

HRT (h)	H_2 yield (mol H_2/mol actate)	Volumetric H_2 production rate (mL/h/L)	Specific H_2 production rate (mL/h/g cell)	Acetate conversion (%)
48	0.98	20.40	5.46	87
36	1.46	28.85	7.37	62
24	1.55	35.33	7.90	48
12	1.39	42.05*	9.50	31

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