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Fermentative hydrogen production from acetate using *Rhodobacter sphaeroides* RV

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ABSTRACT

The study of photosynthetic hydrogen production by using *Rhodobacter sphaeroides* RV from acetate was described. We investigated the effects of light source (fluorescent, halogen and tungsten lamps), light intensity (1200–6000 lux), inoculum quantity (OD_{660} 0.212–OD_{660} 1.082) and initial pH (4.0–10.0) on biohydrogen production. The results indicated that the hydrogen production for halogen and tungsten lamps was better than it for fluorescent lamp as light source. The best light intensity of hydrogen production was 3600 lux for tungsten lamp as light source. Inoculum quantity experiments indicated that the higher hydrogen production volume and hydrogen conversion rate were obtained at initial OD_{660} of 0.931. The effect of initial pH on hydrogen production indicated that the maximum hydrogen yield reached to 653.2 mmol H_2/mol acetate at initial pH 7.0.

1. Introduction

Hydrogen gas has been and remains of attracting much attention as its cleanness, renewable character, and the highest gravimetric energy density (122 kJ g^{-1}) [1–3]. As compared to the conventional chemical and electrolytical routes to produce hydrogen [4,5], biological hydrogen production provides a promising way because of its environmentally harmless [6,7]. Dark fermentation has been extensively studied because of their high efficiency and their potential to use renewable sources of biomass for hydrogen production [8,9]. However, its bottleneck is lower hydrogen yield as high effluent short-chain organic acid which can continue to pollute the environment [10–12]. Photosynthetic hydrogen production is a theoretically perfect process with transforming solar energy into hydrogen by photosynthetic bacteria (12 mol H_2/mol glucose). Photosynthetic bacteria can utilize organic substrates derived from wastewater to produce hydrogen [13], such as sugar industry wastewater [14,15], olive mill wastewater [16] and dairy industry wastewater [17]. However, the actual hydrogen yields are much lower than the theoretical maximum value.

Promoting the hydrogen production yield of photosynthetic bacteria is one of the challenging issues presently faced by researchers. Some researchers have studied the effects of several parameters, such as carbon source, nitrogen source, C/N ratio, temperature, light intensity and nutrient medium [18–23] on photosynthetic hydrogen production. However, high hydrogen yield for the production of hydrogen has not been obtained. The photosynthetic bacteria contain light-harvesting system, which converts light energy to chemical energy [24,25]. Light source is crucial because all energy required for hydrogen production and electron transport is derived from the light energy [26]. In addition, only part of spectrum can be utilized by photosynthetic bacteria. The energy conversion efficiency from light to hydrogen varies under

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different light sources [27]. pH is another important factor influencing photosynthetic hydrogen production. The proper pH plays an important role in the hydrogen-producing process due to its influences on bacterial activity [28,29] and metabolism pathways [30,31].

Among the photosynthetic bacteria, *Rhodobacter sphaeroides* has been extensively studied. In previous work, we have evaluated the effects of carbon sources on photosynthetic hydrogen production [32]. In this study, the effects of light source, light intensity, inoculum and initial pH on biohydrogen production were investigated using *R. sphaeroides* RV. This work was aimed to optimize hydrogen-producing factors and improve hydrogen production yield. The results of this study are expected to provide favorable reference toward the application of photosynthetic hydrogen production.

2. Materials and methods

2.1. Photosynthetic bacteria and growth medium

Rb. sphaeroides RV was purchased from Institute of Microbiology, Chinese Academy of Sciences. The strains were grown in basic medium, which was composed of a basal mineral medium (1 L of basal mineral medium was composed of K_2HPO_4, 600 mg; KH_2PO_4, 500 mg; NaCl, 400 mg; $CaCl_2\cdots 2H_2O$, 50 mg; $MgCl_2\cdots 6H_2O$, 200 mg; $FeSO_4\cdots 7H_2O$, 5 mg; $ZnCl_2$, 7 mg; $CuSO_4\cdots 5H_2O$, 2.4 mg; H_3BO_3, 16 mg; $MnSO_4\cdots 4H_2O$, 10 mg; $NiCl_2\cdots 6H_2O$, 2 mg; $CoCl_2\cdots 6H_2O$, 2 mg; $NaMoO_4\cdots 2H_2O$, 4 mg; EDTA-2Na, 0.4 mg), vitamins (1.4 mg/L vitamin H, 0.5 mg/L vitamin B1, 3.0 mg/L nicotinamide), 0.3 g/L yeast extract, 1.25 g/L $(NH_4)_2SO_4$, 1.0 g/L glucose and 6.84 g/L sodium succinate. The strains were cultivated anaerobically for 24 h under photosynthetic condition of 4000 lux at 32 °C (light-grown cells) and used as inoculum. When required, an inoculum culture was concentrated by centrifugation at 4000 rpm for 10 min.

2.2. Experimental method in photo-fermentation

Hydrogen-production batch experiments were performed in 320 or 40 mL glass column photobioreactor. Fig. S1 schematically describes the photobioreactor (Supplementary materials). The temperature was maintained at 32 °C. The basic mineral mediums, sodium glutamate (0.67 g/L) and sodium acetate (1.45 g/L) were used as the medium for photosynthetic hydrogen production. The basic mineral mediums were the same as Section 2.1. Initial pH was adjusted with 4% (w/v) sodium hydroxide or 4% (w/v) hydrochloric acid. Argon gas was used to create anaerobic conditions. The gas volume was calibrated to 25 °C and 760 mm Hg. Each experimental condition was carried out in triplicate. All chemicals used in the experiments were of AR grade.

2.3. Chemical analysis

The hydrogen content was determined by a gas chromatograph (Techcomp. Co., China, 7890II) equipped with a thermal conductivity detector (TCD) and a 2-m stainless steel column packed with Porapak Q (80–100 mesh). The operating temperatures of the injection port, the oven and the detector were set at 70, 50 and 70 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 mL/min. At each time interval, the total volume of biogas production was measured by a plunger displacement method using appropriately sized glass syringes [33]. The cumulative hydrogen volume was calculated by the following equation [34]:

$$V_{H,i} = V_{H,i-1} + C_{H,i}\left(V_{G,i} - V_{G,i-1}\right) + V_{H,0}\left(C_{H,i} - C_{H,i-1}\right)$$

where $V_{H,i}$ and $V_{H,i-1}$ are cumulative hydrogen volumes at the current (i) and previous ($i-1$) time intervals, $V_{G,i}$ and $V_{G,i-1}$ are the total biogas volumes in the current (i) and previous ($i-1$) time intervals, $C_{H,i}$ and $C_{H,i-1}$ are the fraction of hydrogen in the headspace of the bottle at the current (i) and previous ($i-1$) intervals and $V_{H,0}$ is the total volume of headspace in the bottle. Detection of the alcohols and volatile fatty acids (VFAs, C2-C6) were measured by a gas chromatograph (SHIMADZU Co., Japan, GC 14B) using a flame ionization detector (FID) and a 2-m glass column packed with Unisole F-200 (30–60 mesh). The temperatures of the injection port, the oven and the detector were set at 200, 165 and 200 °C, respectively. The carrier gas was argon at a flow rate of 30 mL/min. Light intensity was measured with TES-1332 luminometer (Shenzhen Langpu Electronic Tech. Co. Ltd). Cell mass concentration was determined spectrophotometrically as optical density at 660 nm (OD_{660}).

2.4. Model analysis

The cumulative hydrogen production in the batch experiments followed the modified Gompertz equation [35]:

$$H = P\exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\}$$

where H is the cumulative hydrogen production (mL), P is hydrogen production potential (mL), R_m is the maximum hydrogen production rate (mL/h), e is 2.71828, λ is the lag-phase time (h), and t is the incubation time (h). The corresponding values of P, R_m and λ for each batch were estimated using Origin 7.5, which is a scientific graphing and data analysis software.

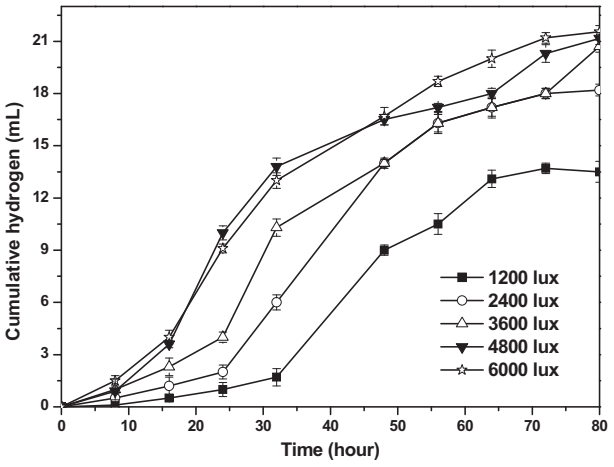

Fig. 1 – Effect of light intensity on cumulative hydrogen production.