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Process Biochemistry 41 (2006) 2041–2049

Process Biochemistry

www.elsevier.com/locate/procbio

Enhancing phototropic hydrogen production by solid-carrier assisted fermentation and internal optical-fiber illumination

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Received 1 March 2006; received in revised form 27 April 2006; accepted 3 May 2006

Abstract

Three strategies were applied to promote the phototrphic H_2 production of an indigenous purple nonsulfur bacterium *Rhodopseudomonas palustris* WP3-5 using acetate as the sole carbon substrate. First, a small amount of solid carriers (e.g., activated carbon, silica gel, and clay) was supplemented to fermentation broth to stimulate cell growth and H_2 production. Second, the acetate concentration leading to optimal production of H_2 was identified. Finally, an innovative optical-fiber illuminating system was designed to facilitate the efficiency of the photobioreactor. The results show that addition of clay and silica gel was effective in promoting H_2 production, resulting in 67.2–50.9% and 37.2–32.5% increases in H_2 production rate (υ_{H_2}) and H_2 yield (Y_{H_2}), respectively. For clay-supplemented batch cultures, the optimal acetate concentration was 1000 mg COD/l, leading to a υ_{H_2} and Y_{H_2} value of 28.5 ml/h/l and 2.97 mol H_2/mol acetate, respectively. Moreover, combination of internal optical-fiber illumination system, clay addition, and optimal acetate concentration further elevated the υ_{H_2} and Y_{H_2} to a maximum level of 43.8 ml/h/l and 3.63 mol H_2/mol acetate, respectively. These values are considerably higher than most reported results from relevant studies. Meanwhile, the results of continuous cultures operated at 36 h HRT (hydraulic retention time) show that the high phototrophic H_2 production efficiency was stably maintained for over 17 days with a steady-state υ_{H_2} and Y_{H_2} of 44.0 ml/h/l and 3.57 mol H_2/mol acetate, respectively.

Keywords: Phototrophic hydrogen production; *Rhodopseudomonas palustris*; Photosynthetic bacteria; Photobioreactor; Optical fiber; Solid carriers

1. Introduction

The world has recognized the pivotal role that H_2 may play in future energy supply. To make the concept of ''hydrogen economy'' [1] a reality, innovation of technologies leading to sustainable, sufficient, and economically feasible production of H_2 is extremely critical. Biological production of H_2 is considered the most environmentally friendly route of producing H_2. In particular, hydrogen production through light-dependent or independent fermentative conversion of organic substrates is of great interest, due to its dual function of waste reduction and clean energy production [2–4]. Dark fermentation involving mainly acidogenic bacteria (such as *Clostridium* sp.) produces H_2 while converting organic substrates into volatile fatty acids and alcohols [3], which could be further mineralized by photosynthetic bacteria (e.g., purple nonsulfur bacteria), producing more H_2 at the expense of photo energy [2,3]. Therefore, combination of dark and photo fermentation seems to be an ideal biohydrogen producing model leading to the highest theoretical H_2 yield possible [5,6]. However, the economical feasibility of phototrophic H_2 production appears to be limited by the poor H_2 production rate of the photosynthetic bacteria, arising primarily from the low cell growth rate and the inefficient light energy utilization [3,7]. Thus, improving phototrophic H_2 production rate would be a substantial step towards development of a successful H_2 production process that integrates dark and photo fermentation.

In this study, three strategies from biochemical and bioreactor engineering perspectives were applied to improve the performance of phototrophic H_2 production with an indigenous purple nonsulfur photosynthetic bacterium *Rhodopseudomonas palustris* WP3-5 isolated from a wastewater treatment plant located in central Taiwan [8]. The first approach was to supplement selected porous solid carriers (such as activated carbon, silica gel, and clay) into fermentation broth to examine for the effect of carriers on phototrophic H_2 production. These carriers were shown to be effective in stimulating cell growth and production of target metabolites

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1359-5113/$ – see front matter
doi:10.1016/j.procbio.2006.05.005

(e.g., H_2 and biosurfactant) in recent studies [9–12]. It is thought that the carriers can provide more surface attachment sites, enhancing biofilm formation [13] and granular sludge formation [9,14]. The solid carriers may also provide buffer capacity for extreme conditions such as high organic loadings, pH shock, etc. [13,15]. Moreover, similar to the function of immobilized cells, the carriers could enhance cell retention for continuous cultures, thereby avoiding wash-out of cells while operating at a high dilution rate (or a low hydraulic retention time) [16,17]. It has been reported that using immobilized *R. palustris* or cells of other photosynthetic bacteria could improve photo-hydrogen production [18–22]. However, for this photo-energy-dependent fermentation system, surface attachment seems to be a more reasonable cell immobilization approach than cell entrapment, because the efficiency of photo energy utilization is a crucial issue.

The second strategy for performance improvement was to identify the best carbon substrate (acetate) concentration since the organic loading often plays a critical role in affecting the kinetics of catabolism. Acetate was selected as the carbon source because it is a major soluble metabolite from dark H_2 fermentation, making it a perfect connection between dark and photo fermentation. In addition, effort was made on increasing the availability and utilization efficiency of light energy. The idea was to add an internal illumination system by direct insertion of optical fibers into the bioreactors. The effect of this innovative photobioreactor design on H_2 production was investigated on batch and continuous modes. The objective of this work was to assess the effectiveness and feasibility of using the three proposed strategies (namely, carrier addition, carbon substrate optimization, and internal optical-fiber illumination) to improve the performance of phototrophic H_2 production by *R. palustris* WP3-5.

2. Materials and methods

2.1. Microorganism and medium

The phototrophic H_2 producer used in this study was *R. palustris* WP3-5, which was isolated from a wastewater treatment plant in central Taiwan [8]. The culture medium for the strain consisted of (in g/l) acetate, 1000–4000 (mg COD/l; COD denotes chemical oxygen demand); glutamic acid, 0.4; K_2HPO_4, 0.5; KH_2PO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.2; NaCl, 0.4; $CaCl_2{\cdot}2H_2O$, 0.05; yeast extract, 0.2; iron citrate solution (1.0 g/l), 5 ml/l; trace element solution, 1 ml. The trace element solution contained (in mg/l) $ZnCl_2$, 70; $MnCl_2{\cdot}4\,H_2O$, 100; H_3BO_3, 60; $CoCl_2{\cdot}6H_2O$, 200; $CuCl_2{\cdot}2H_2O$, 20; $NiCl_2{\cdot}6\,H_2O$, 20; $NaMoO_4{\cdot}2H_2O$, 40; HCl (25%), 1 ml/l. For continuous cultures, the feeding medium contained an acetate concentration of 1.0 g/l. The anaerobic fermentation was carried out at 32 °C and a light intensity of ca. 95 W/m^2 with the illumination of tungsten filament lamps. The initial pH of the medium was adjusted to 7.1 by 0.1N NaOH.

2.2. Solid carriers

Three types of carriers were added into the fermentation culture to promote phototrophic H_2 production. These carriers are activated carbon (AC), clay, and silica gel. The AC obtained from China Carbon, Inc. (Taipei, Taiwan) was of cylindrical shape with a diameter of 3–4 mm and a height of 9 mm. The clay carriers (expanded clay) obtained from Taihort Inc. (Taipei, Taiwan) were spherical particles with an average diameter of 5 mm. Silica gel was obtained from Hsuan Chun Industrial Co., Tainan, Taiwan). The carriers were sterilized prior to use.

2.3. Setup and operation of photobioreactor

The photobioreactor (PBR) was a 1-l glass-made vessel equipped with side-light optical fiber (SLOF) and external light sources (100 W tungsten filament lamps or 100 W halogen lamp) (Fig. 1). The side-light optical fiber (SLOF) protected in a glass tube was immersed into the liquid medium inside the photobioreactor. Since the SLOF did not have direct contact with the medium, there was no need to autoclave the optical fibers prior to use. The SLOF was composed of a polymethyl methacrylate (PMMA) core coated with fluorinated alkyl methacrylate copolymer (diameter: 11 mm, length: 25 cm) obtained from Baycom Optic-Electronic Co. (Hsin-Chu, Taiwan). The protective cladding was removed by mechanical polishing, allowing direct light emission from the PMMA core (i.e., a side-light optical fiber). One of the two fiber-ends on which the light is incident was also polished to attain maximum light emission. Prior to installation inside the photobioreactor, the SLOF was physically polished until the desired light intensity and uniform light distribution were obtained. The PBR was illuminated with single or multiple light sources (e.g., optical fiber, tungsten filament lamp, halogen lamp), while the total light intensity for each illumination system was kept at ca. 95 W/m^2. After autoclave sterilization of the bioreactor, *R. palustris* WP3-5 cells were inoculated (10% inoculum) into the reactor containing 800 ml of the culture medium mentioned earlier.

Both batch and continuous cultures were performed at 32 °C, pH 7.1, and 100 rpm agitation with a working volume of 800 ml. At the beginning of fermentation, three types of carriers (activated carbon, clay and silica gel) were added into the bioreactors at a weight to volume (w/v) ratio of 2%. This carrier loading (i.e., w/v ratio) was found to be suitable for carrier-assisted fermentation according to our recent findings [12]. 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 °C and 760 mmHg. Gas samples were taken by gas syringe at desired time intervals to measure the gas composition. The liquid sample was also collected from the reactor as a function of time to determine cell concentration, pH and residual acetate concentration. For batch cultures, time-course data of cumulative H_2 production were simulated by modified Gompertz equation (Eq. (1)) [23,24] to determine the kinetic parameters of photo-H_2 production:

$$H = H_{\max} \exp\left\{ -\exp\left[\frac{R_{\max} e}{H_{\max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

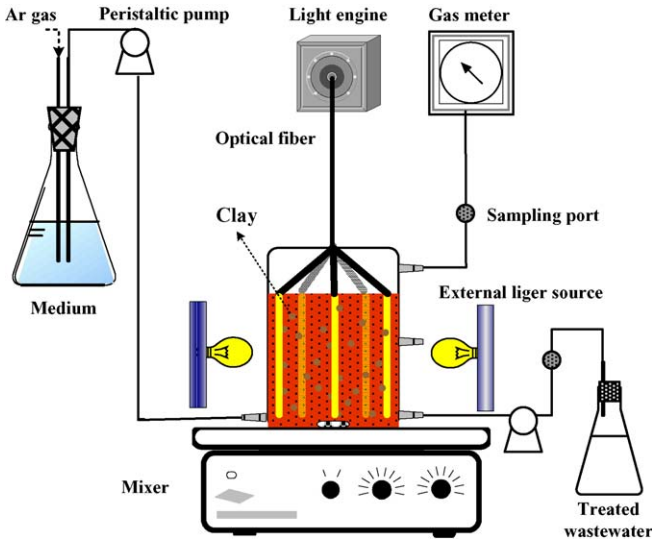

Fig. 1. Schematic description of the photobioreactor system using clay as the carrier illuminated with internal (optical fiber) and external (halogen and/or tungsten filament lamp) light sources.