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Bioconversion characteristics of *Rhodopseudomonas palustris* CQK 01 entrapped in a photobioreactor for hydrogen production

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HIGHLIGHTS

- The conversion efficiencies during H_2 production were evaluated.
- The system at 590 nm could engage in metabolism of energy storage for H_2 production.
- The system was of higher energy recovery capacity to the special monochromatic light.
- The total energy efficiency was far lower than the light conversion efficiency.

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ABSTRACT

The performance of the entrapped-cell photobioreactor during H_2 production was assessed by using glucose as substrate in a continuous operation mode. The maximal hydrogen production rate and light conversion efficiency, 2.61 mmol/L/h and 82.3%, were obtained at a HRT of 11.4 h, an substrate loading rate of 4.2 mmol/h and an illumination of 590 nm and 6000 lux, the corresponding hydrogen yield and total energy efficiency were 0.62 mmol H_2/(mmol glucose) and 4.8%, respectively. The results indicate the H_2 production system illuminated at 590 nm wavelength engaged in energy storage for H_2 production due to more ATP synthesized in primary reaction center, and was of higher energy recovery capacity. Furthermore, the total energy efficiency was far lower than the corresponding light conversion efficiency due to intermediates production.

1. Introduction

Carbon dioxide emissions owing to extensive use of fossil fuels make our planet face the problem of global warming. In response to this crisis, finding carbon-free or carbon-neutral alternative energy sources have become a pressing need in most nations' agenda (Azbar et al., 2009). Hydrogen (H_2) is the only carbon-free fuel and has been recognized as the ideal choice as it has the highest energy density among the known gaseous fuels and water is the only combustion product, eliminating greenhouse gas emission, acid rain formation and ozone depletion (Dursun and Tepe, 2005). H_2 production is one of the key technical issues in the wide use of hydrogen. Traditional methods for H_2 production such as electrolysis of water, coal gasification and steam reforming of hydrocarbons are usually energy intensive and environmentally unfriendly (Zabut et al., 2006). Therefore, the sustainable energy development urgently demands an energy-saving and clean H_2 production technology. Biological H_2 production technology is one of the ideal solutions to this problem for operating at ambient temperature and pressure, which can lower the energy consumption, capital cost and pollutant discharge (Hafez et al., 2009; Meher and Das, 2008). Bio-H_2 production technologies can be divided into many types in terms of their biological processes. Among them, photosynthetic bacteria (PSB) such as *Rhodopseudomonas palustris* are the most promising microbial system due to its high conversion yield of substrate, ability to absorb wide spectrum of light and lack of Photosystem II which automatically eliminates the difficulties associated with O_2 inhibition to H_2 production (Jamil et al., 2009). Moreover, PSB can utilize waste organic substrates as sole carbon sources (electron donors) for high purity H_2 production, thus, it can give the functions of both the organic wastes treatment and clean fuel production (Das and Veziroglu, 2001; Kovács et al., 2006).

Bioreactor is the key component in the bio-H_2 production system. According to the cell status in reactors, bioreactors basically can fall into two types: suspended-cell bioreactors and immobilized-cell bioreactors. Compared with suspended-cell systems,

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the immobilized-cell system using physical or biological immobilization approaches can effectively enhance biomass retention to overcome cells washout, significantly improving its stability and reusability (Jo et al., 2008; Roeselers et al., 2008; Chang et al., 2002). Among these immobilized-cell techniques, the cell entrapment in porous gels is one of the promising techniques for bio-H_2 production due to being inexpensive and easy to handle (Wu et al., 2006). This gel entrapment allows H_2-producing bacteria in the reactors to be enriched, and creates a local anaerobic environment that is well suitable for oxygen-sensitively fermentative H_2 production (Gavala et al., 2006). Besides, since it can be continuously and stably operated at a low hydraulic retention time, H_2 production rate can be improved by elevating organic loading rates. This immobilized photosynthetic H_2-producing system had successfully been utilized in experiments (Chen et al., 2011; Tian et al., 2009).

Performance of the immobilized-cell bioreactor for H_2 production is affected by multi operation parameters. Organic substrate is one of the important factors. It provides reductant for H_2 evolution by photo-fermentation, that is, H_2 production by PSB requires electron donor from organic substrate to develop the photochemical reactions (Gfeller and Gibbs, 1984; Melnicki et al., 2008). Some researchers had investigated the effects of type and concentration of substrate on H_2 evolution by PSB (Keskin et al., 2011; Wang et al., 2011; Laurinavichene et al., 2010). The efficiency of substrate conversion to H_2 can be reflected by hydrogen yield, which varies among different PSB species, or even among different strains of the same species due to the change of the metabolic pathways in accordance with the conditions of nutrients, culture pH or concentrations of metabolites (Tao et al., 2008). Furthermore, light absorption by the photosynthetic apparatus is essential for H_2 generation since the photosynthesis pigment molecules do show different utilization efficiencies for the different wavelength lights (Argun and Kargi, 2010). Therefore, it is necessary to use suitable light sources that provide sufficient light energy with needed wavelengths for a H_2-producing photobioreactor. Currently, researchers have paid more attention to the light conversion efficiency during H_2 production. The largest light conversion efficiency (LCE) of *Rhodobacter* existing in low light intensity and low nitrogen environment with a pH of 6.7–7.5 was obtained (Lee et al., 2007), and the LCE decreased at a high light intensity (Wakayama and Miyake, 2002). Theoretically, part of the produced H_2 energy should derived from the consumed substrate. Thus, the chemical energy in the consumed substrate also needs to be considered to evaluate the efficiency of total energy recovery in the H_2 production process. However, research on this work hasn't almost been done.

In the present work, a photobioreactor, packed with entrapped-PSB gel granules for H_2 production, was operated in a continuous mode. The strain used in this research was isolated from urban sewage sludge and identified as *R. palustris*. Monochromatic LED lights were chosen as external light source in the present study. The present work mainly focused on the effects of hydraulic retention time, organic loading rate, light wavelength and illumination intensity on H_2 production behaviors and energy conversion efficiencies of *R. palustris* CQK 01 in an immobilized-cell bioreactor.

2. Methods

2.1. Microorganism

This H_2-producing PSB strain was isolated from the urban sewage sludge of Chongqing City (China) and enriched with synthetic medium using glucose as sole carbon source in an anaerobic serum bottle at 30 °C, under an illumination intensity of 3000 lux from a tungsten filament lamp. After enrichment cultures for 4 times, the inoculum of the culture was spread on agar solid plates by using inoculating needle and incubated for about 72 h at 3000 lux illumination intensity. The single colony growing fast was then selected and re-streaked 3 times to purify the objective strain. Next, the PCR amplification of 16S rDNA gene of the pure culture was processed using a pair of bacterial universal primers (5′-AGAGTTTGATC CTGGCTC-3′, 5′-GGTTACCTTGTTACGACTT-3′). The amplified 16S rDNA genes were then cloned with a T-vector and sequenced. According to the taxonomy from the Bergey's Manual of Determinative Bacteriology (the 8th edition) and comparison with the corresponding standard strain's 16s rDNA gene sequence in NCBI (The National Center for Biotechnology Information, USA), the obtained strain was identified as *R. palustris* and named as *R. palustris* CQK 01.

For preculture, the pure culture was inoculated in 100 mL synthetic medium containing (g/L): $K_2HPO_4{\cdot}3H_2O$, 1.006; KH_2PO_4, 0.554; $MgSO_4{\cdot}7H_2O$, 0.20; $FeSO_4{\cdot}7H_2O$, 0.042; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.0010; $ZnSO_4{\cdot}7H_2O$, 0.0010; NaCl, 0.20; $CaCl_2$, 0.010; $CO(NH_2)_2$, 1.35; $C_6H_{12}O_6{\cdot}H_2O$, 4.024. Moreover, 1.0 g yeast extract and 1 mL growth factors solution (biotin, 1.0 g/L; pyridoxine hydrochloride, 1.0 g/L; riboflavin, 1.0 g/L; thiamine hydrochloride, 1.0 g/L; nicotinic acid, 1.0 g/L) were added to 1 L synthetic medium to improve growth conditions. The initial pH value of the culture medium was adjusted to 7.0 by 0.1 mol/L NaOH solution before autoclave sterilization. Hundred milliliters inoculated medium was grown anaerobically at 28 °C for 72 h with 3000 lux illumination intensity from a tungsten filament lamp. Argon gas was introduced to create anaerobic conditions before cultivation.

2.2. Amplification and entrapment of PSB cells

Thirty milliliters inoculum from the 72-h preculture was added to 3 L synthetic culture medium containing 8.04 g glucose/L and the culture was one-sidedly illuminated by LED lamps (red light) with an illumination intensity of approximate 5000 lux. Argon was used to create an anaerobic environment. After 62 h (exponential phase) of inoculation, precipitate from the culture was first removed by centrifugation at 2250 g for 5 min, and cells were harvested by centrifugation at 20300 g for 10 min, washed twice with deionized water, then, suspended in 50 mL of 0.1 mmol/L phosphate buffer and used as inoculum for the preparation of entrapped-cell granules.

The entrapped-cell matrix components consisted of polyvinyl alcohol (PVA, with 1750 ± 50° of polymerization), sodium alginate and carrageenan powder. These entrapped-cell particles were prepared as description in the reference (Wang et al., 2010).

2.3. Operation of a packed-bed bioreactor for H_2 photo-production

Continuous bio-H_2 production by a photobioreactor filled with entrapped-cell particles was performed in an anaerobic mode. The flat panel photobioreactor (100 × 40 × 200 mm) with a working volume of 800 mL was made of transparent polymethylmethacrylate (5 mm thickness). The front surface of the bioreactor was exposed to incident light, while other walls were covered by polyurethane foam for thermal insulation. External illumination with a radiation area of 200 mm^2 was, respectively provided by four sets of LED lamps with main wavelengths of 630, 590, 520 and 470 nm and the illumination intensity was adjusted from 2000 to 8000 lux by adjusting the distance between the LED lamps and the bioreactor. Prior to the experiment, the test system was sterilized by autoclave except the photobioreactor body which was sterilized by formalin vapor, and the interior of the test system was cleaned 3 times by sterile water. Synthetic medium solution in a fluid reservoir, containing glucose as the sole carbon source and the other components that were the same as those described in