Bioresource Technology 101 (2010) 5315–5324

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Formation and hydrogen production of photosynthetic bacterial biofilm under various illumination conditions

Qiang Liao *, Ye-Jun Wang, Yong-Zhong Wang, Xun Zhu, Xin Tian, Jun Li

Institute of Engineering Thermophysics, Chongqing University, Chongqing 400030, China

ARTICLE INFO

Article history:
Received 14 December 2009
Received in revised form 30 January 2010
Accepted 3 February 2010
Available online 11 March 2010

Keywords:
Hydrogen production
Light conversion
Biofilm
Illumination

ABSTRACT

The application of immobilized-cell technology in photobioreactor for hydrogen production could offer improvements in photo-hydrogen production rate and light utilization efficiency. Indigenous *Rhodopseudomonas palustris* CQK 01 was attached to the surface of a cover glass slide in a flat-panel photobioreactor, to form biofilm under illumination with a range of intensities and wavelengths. The morphology and structure of mature photosynthetic bacterial (PSB) biofilm were determined to elucidate the relationship between biofilm formation and hydrogen production performance. The effects of operation conditions on hydrogen production performance of the biofilms formed under various illumination conditions were experimentally investigated. The results showed that illumination wavelength and intensity substantially influenced the morphology and structure of the biofilm, and the hydrogen production performance of mature biofilm varied significantly with the illumination conditions that were used for biofilm formation. Biofilm formed under 590 nm and 5000 lx illumination showed the highest hydrogen production performance.

1. Introduction

Hydrogen is universally considered as one of the most promising alternatives to fossil fuels, for its high caloric value and efficient burning or use in fuel cells. Conventional physicochemical methods for hydrogen production are energy intensive and not always environmentally friendly. Biological hydrogen production is recognized as one of the prospective options for massive hydrogen production due to low production costs, less pollutant discharge and smaller energy consumption (Kotay and Das, 2008). Furthermore, these processes can use many organic substrates as carbon sources (Manish and Banerjee, 2008), so that they offer the dual function of waste biodegradation and hydrogen production (Chen et al., 2006).

Bio-hydrogen can be produced from the dark fermentation of carbohydrate rich wastes, and photo-fermentation of organic acid-rich wastewaters. The photo-fermentation process is frequently used to produce hydrogen because of the high purity of the hydrogen produced, high theoretical substrate conversion efficiency, relatively small quantity of byproducts and no O_2-evolving activity which causes O_2 inactivation in green algae hydrogen production systems (Fascetti and Todini, 1995; Lee et al., 2002; Shi and Yu, 2005). The light absorption spectrum of PSB (400–950 nm) is wider than that of green algae (400–700 nm) (Akkerman et al., 2002), and PSB can produce hydrogen from the mineralization of organic acids such as acetic acid and butyric acid, which are predominant metabolites from dark fermentation in the hydrogen production processes.

Previous work focused mainly on investigation of the effects of operating conditions such as illumination condition (Kim et al., 2006), type and concentration of substrate (Barbosa et al., 2001; Ren et al., 2008), initial cell density and pH (Kosourov et al., 2003, 2002) on hydrogen production of PSB in suspended or batched culture. Recently it has been generally acknowledged that the low hydrogen production rate of PSB, which is primarily attributed to low cell growth rate and inefficient light energy utilization (Chen et al., 2006; Zhu et al., 1999), is the major bottleneck in industrial application of the photo-fermentation process. Thus, research on improving the hydrogen production rate and light conversion efficiency of photobioreactor is attracting more extensive attention. It has been found that the former can be achieved by increasing biomass retention per unit volume of the reactor, and the latter is affected mainly by the structure of bioreactor and the light source that is used (Chen et al., 2006; Wakayama and Miyake, 2002).

Generally, the immobilization processes of hydrogen-producing cultures should be an effective approach to increase biomass retention in bioreactors (Tian et al., 2009). Recent studies of immobilized-cell dark fermentation hydrogen production show that relatively high unit volumetric production rates are found in immobilized-cell anaerobic systems, as a consequence of increased biomass retention (Zhang et al., 2008). The hydrogen production

* Corresponding author.
E-mail address: lqzx@cqu.edu.cn (Q. Liao).

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.02.019

rate of these immobilized-cell systems achieved peak values of 6.6 l/l/h in a granular reactor (Zhang et al., 2008), 7.3 l/l/h in a self-flocculated anaerobic granular reactor (Lee et al., 2004) and 1.01 l/l/h in a thermal trickling biofilter (Oh et al., 2004). These immobilization technologies, based mainly on the granulation process or biofilm attachment process (Kim et al., 2005; Zhang et al., 2008), are also the most popular techniques for hydrogen production because of their increased biomass retention and improved tolerance to inhibitors, compared with suspended-cell systems (Bagai and Madamwar, 1998; Chang and Lin, 2004; Kumar and Das, 2001; Wu et al., 2006).

Unfortunately, those studies were focused mainly on dark fermentation, and only a few studies aimed at immobilized-cell technologies for photo-hydrogen production have been reported to date. Chen and Chang (2006) supplemented fermentation broth with clay and silica gel to investigate phototrophic hydrogen production, and found 67.2–50.9% and 37.2–32.5% increases in hydrogen production rate and yield. Fiβler et al. (1995) used immobilized cells of *Rhodopseudomonas palustris* in polymeric materials to obtain a doubled hydrogen production rate relative to that from liquid cultures. Zhu et al. (1999) entrapped anoxygenic phototrophic bacteria of *Rhodopseudomonas sphaeroides* in cationic polymer/agar gels to prevent the inhibitory effect of NH_4^+ and improve photo-hydrogen production. Tian et al. (2009) reported that immobilization of *R. palustris* in a polyvinyl alcohol (PVA)-boric acid gel granule could remarkably facilitate hydrogen production. Compared with granulation immobilization for photo-hydrogen fermentation systems, biofilm attachment technology avoids the limitation to light penetration resulting from the dense packing of immobilized PSB granules, enhancing hydrogen production because the efficiency of light energy utilization is crucial in the design of the photo-hydrogen fermentation bioreactor (Chen et al., 2006; Tsygankov et al., 1994). However, to our knowledge studies on photo-hydrogen fermentation bioreactors with biofilm attachment are very sparse except for the work of Tian et al. (2010) on immobilized cells of *R. palustris* on the surface of packed glass beads to form biofilm, and effects of various parameters on photo-hydrogen production performance.

In the present study, biofilm of an indigenous purple non-sulfur photoheterotrophic bacterium, *R. palustris* CQK 01, was obtained using immobilization cell technology of surface adsorption on a cover glass slide under various illumination intensities and wavelengths. The objectives of the present study were to assess the effects of illumination on biofilm formation and structure, and then further investigate photo-hydrogen production performance for different biofilms in the photobioreactor under various illumination wavelengths and intensities, flow rates and substrate concentrations.

2. Methods

2.1. Microorganism and cultivation

An indigenous photosynthetic bacterium designated *R. palustris* CQK 01 for phototrophic hydrogen production was isolated from local municipal sewage sludge. The strain was grown in a synthetic medium consisting of (g/l) $K_2HPO_4{\cdot}3H_2O$, 1.0060; $MgSO_4{\cdot}7H_2O$, 0.2000; KH_2PO_4, 0.5440; $FeSO_4{\cdot}7H_2O$, 0.0417; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.0010; NaCl, 0.2000; $CaCl_2$, 0.0100; $CO(NH_2)_2$, 1.6770; $ZnSO_4{\cdot}7H_2O$, 0.0010; yeast extract, 1.0000; $C_5H_8NNaO_4{\cdot}H_2O$, 0.5000, growth factors solution (comprising biotin 1 g/l, pyridoxine hydrochloride 1 g/l, riboflavin 1 g/l, thiamine hydrochloride 1 g/l and nicotinic acid 1 g/l), 1 ml/l. For pre-culture, the carbon source was a glucose solution with initial concentration 9.9000 g/l. The cells were anaerobically cultivated at 30 °C under illumination from a light-emitting diode (LED) with illumination intensity approximately 4000 lx. The initial pH of the medium prior to incubation was adjusted to 7.0 with 0.1 M NaOH. Argon was used to create anaerobic atmosphere. Enriched photosynthetic bacteria were initially used as inoculums for start-up of the bioreactor.

2.2. Experimental setup and operation

The experimental apparatus, illustrated in Fig. 1, consisted of a medium container, a peristaltic pump, a flat-panel photobioreactor, a liquid/biogas separator and a collection bottle. Two types of flat-panel photobioreactors were fabricated to satisfy the specific requirements of the biofilm formation experiments and the hydrogen production experiments. For the biofilm formation experiments the flat-panel bioreactor was made of polymethyl methacrylate (PMMA) with a cover glass slide (24 mm × 60 mm × 0.17 mm), which offered a solid carrier surface for the adsorption and growth of bacteria to facilitate measurement of biofilm thickness, dry weight and microstructure. The flow cross-section and working volume of bioreactor were 18 mm × 10 mm and 9000 mm^3, respectively. For the hydrogen production experiments, the cover glass slide of the flat-panel photobioreactor was enlarged to 72 mm × 100 mm × 3 mm to increase hydrogen production rate so as to attain higher accuracy of measurement of hydrogen production. Correspondingly, the flow cross-section and the working volume of the flat-panel photobioreactor were increased to 72 mm × 10 mm and 72,000 mm^3, respectively. A liquid distributor, composed of two perforated plates holding a packed bed of glass beads 4 mm in diameter, was installed at the entrance of the bioreactor to maintain uniform flow distribution in the bioreactor. The liquid and biogas were drained from the effluent on the top side of the photobioreactor to facilitate eduction of the biogas that was produced. Silicone rubber hose was chosen for the supply pipes in the experimental system, and all bare surfaces of the photobioreactor and supply pipes, except the irradiation surface of photobioreactor, were covered with aluminum foil.

The substrate medium solution was fed by peristaltic pump (BT01-YZ1515, China); the flow rate was adjusted by changing the pump speed. The temperature of the substrate medium solution in the photobioreactor was maintained at approximately 30 °C during the experiments. An external light source was mounted above the cover glass slide of the photobioreactor. Four monochromatic LED lamps, emitting at 630, 590, 520 and 470 nm were used as light sources, and the illumination intensity was adjusted from 1000 to 8000 lx by varying the distance between the LED and the photobioreactor. To maintain anaerobic conditions, the photobioreactor and silicone hoses were filled with substrate solution and the top of the medium container was purged with argon. The experiments were conducted in two stages: the start-up stage and steady-state operation. During the start-up stage of the bioreactor, the medium was recycled to avoid loss of activated PSB cells in the circulated liquid. The PSB cells were immobilized on the cover glass slide to form biofilm under a range of illumination conditions, at specific influent substrate solution concentration and flow rate, to study the effect of illumination conditions on biofilm formation. About 250 ml of substrate solution was replaced by fresh sterilized nutrient medium every 24 h until steady-state operation was reached. The steady-state experiments were conducted in the open loop system where the biogas produced in the bioreactor was collected in the collection bottle by the water displacement method, to determine the hydrogen production rate. The hydrogen production rate of the bioreactor with a specific biofilm was measured under various illumination conditions, as well as influent substrate solution concentrations and flow rates, to study the effect of PSB biofilm formation and operation conditions on the hydrogen production performance.