Appl Biochem Biotechnol (2008) 145:79–86
DOI 10.1007/s12010-007-8032-z

Bioreactors for H_2 Production by Purple Nonsulfur Bacteria

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Received: 11 May 2007 / Accepted: 27 August 2007 /
Published online: 13 September 2007

Abstract Two types of laboratory-scale bioreactors were designed for H_2 production by purple nonsulfur bacteria. The bioreactors employed a unique type of hydrogenase activity found in some photosynthetic bacteria that functions in darkness to shift CO (and H_2O) into H_2 (and CO_2). The mass transport of gaseous CO into an aqueous bacterial suspension was the rate-limiting step and the main challenge for bioreactor design. Hollow-fiber and bubble-train bioreactors employing immobilized and free-living bacteria have proven effective for enhancing the mass transfer of CO. The hollow-fiber bioreactor was designed so that both a growth medium and CO (10% in N_2) passed from the inside of the fibers to the outside within the bioreactor. Bacteria were immobilized on the outer surface of the hollow fibers. Hydrogen production from CO at an average rate of 125 ml g cdw^{-1} h^{-1} (maximum rate of 700 ml g cdw^{-1} h^{-1}) was observed for more than 8 months. The bubble-train bioreactor was built using polyvinyl chloride (PVC) tubing, wound helically on a vertical cylindrical supporting structure. Small bubbles containing CO were injected continuously through a needle/septum connection from the gas reservoir (20% CO). Up to 140 ml g cdw^{-1} h^{-1} of H_2 production activity was observed using this bioreactor for more than 10 days.

Keywords Biohydrogen · Purple bacteria · Bioreactor · Water–gas shift reaction · Hollow fibers

Introduction

Hydrogen (H_2) is considered a fuel with low environmental impact as its combustion product (water) is non-polluting. Hydrogen is a renewable energy carrier; it can be produced from water again. The conventional industrial methods for H_2 production are costly and the problem has been to find a cheaper way to produce hydrogen. Biological H_2

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production by microbial species has a number of advantages and it could be a cost-effective alternative to the current industrial methods of H_2 production. Some microscopic bacteria produce H_2 using water as the source of electrons and sunlight as the energy source. Microbiological H_2 production based on sunlight and water is very attractive for practical applications. It holds the promise of generating a renewable fuel as a result of the availability of large amounts of solar light and water and the ability of H_2 to produce water again.

A unique type of H_2-producing activity was found in a strain of nonsulfur purple photosynthetic bacterium by Uffen [1]. Cultures of Uffen's strain in a complex organic medium carry out a water–gas shift reaction in darkness to produce H_2 according to the equation:

$$CO + H_2O \rightarrow CO_2 + H_2$$

Since the time of Uffen's discovery, numerous strains of photosynthetic bacteria, including *Rubrivivax gelatinosus* CBS, have been isolated at the National Renewable Energy Laboratory (NREL) in Colorado, USA. These bacteria utilize CO in light and in darkness and do not require complex organic substrates [2, 3]. In addition, these strains will quantitatively shift the CO component of synthesis gas (*e.g.*, from thermally gasified biomass) into H_2. During the shift reaction, the bacterial cells produce hydrogen from water as verified by experiments with ^3H_2O [4]. However, purple bacteria do not use light energy directly to produce H_2 from water during the shift reaction.

Data collected at NREL suggest that the water–gas shift reaction is far more rapid than the rate at which CO can be supplied to the bacterial culture [5]. Mass transport of gaseous CO into an aqueous bacterial suspension is thus the rate-limiting step in the process of shift reaction and was the main challenge during bioreactor design for the current study. In this study, two types of simple laboratory-scale hollow-fiber and bubble-train bioreactors employing immobilized and free-living *Rubrivivax gelatinosus* CBS were analyzed. The bioreactor with immobilized cells was built around bundles of hollow fibers composed of semipermeable polymeric membranes. Salts and gases can freely diffuse through these membranes. Bacterial cells, because of their larger size, cannot pass through the membrane. Bacterial cells that can grow on hollow fibers are called immobilized cells. Immobilization leads to an increase/stabilization of H_2 production for several months or more [6]. Little is known about the mechanisms that induce changes in H_2-producing activity when cells are immobilized. Many microorganisms exist naturally in an immobilized-like state, either on a surface of soil particles or in symbiosis with other organisms. The second type of a bioreactor (bubble-train bioreactor) was constructed out of transparent polyvinyl chloride (PVC) tubing.

Materials and Methods

Bacterial Culture

Before inoculation into bioreactors *Rubrivivax gelatinosus* CBS was grown in closed 250-mL volume flasks using basic basal medium without yeast extract [7] plus 10% CO, shaken and illuminated with incandescent lamps (35 W m^{-2}). Cell dry weight was determined by trapping the bacteria on Whatman # 114 filter paper and drying the cell suspensions at 90°C to a constant weight.

Bioreactors

Two types of bioreactors were designed and constructed. The first one (Fig. 1) consisted of:

1. A 250×33 mm AM-4OM-SD cartridge with hydrophilic cuprammonium rayon hollow fibers (Asahi Medical Co., Ltd, Japan). The total surface area of the 180-μm-diameter hollow fibers was 0.8 m^2, and the cartridge volume outside the fibers was 48 mL.
2. A H_2 measurement port, where H_2 and CO_2 were vented from the bioreactor cartridge.
3. A CO gas cylinder (10% in N_2). CO flow rates were in the range of 0.05–0.5 mL/min.
4. A peristaltic pump (Masterflex, Cole-Palmer Instrument, Niles, IL) for adding the basal medium caused by evaporation from the bioreactor cartridge (one time per week).
5. A medium reservoir
6. A pH controller for automatically adjusting the pH of the medium to 6.8 caused by CO_2 build-up.

The bioreactor was designed such that both the basal growth medium and the CO passed from the inside to the outside of the fibers within the column. A bacterial cell inoculum (0.65 g/L) readily adsorbed to the outer surface of the hollow fibers, and the column was maintained in the darkness.

The second bioreactor was a 0.5-L (liquid volume), 0.8-m-tall device as diagrammed in Fig. 2. This bioreactor was constructed from:

1. A 9.8-m transparent PVC tube (Tygon, Akron, OH), with a 6.3 mm inner diameter, wound helically on a vertical cylindrical supporting structure.
2. A pump (Masterflex, Cole-Palmer Instrument) for circulating (pumping speed 15 mL min^{-1}) the bacterial suspension (0.36 mg cdw mL^{-1}).
3. A needle injector for 20% CO in N_2 (2 mL min^{-1}).
4. A 300-mL gas reservoir.

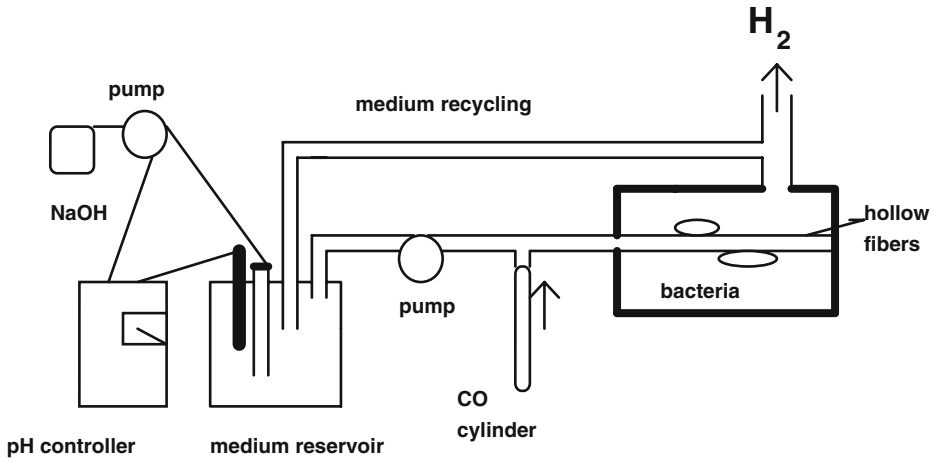

Fig. 1 Schematic diagram of a hollow-fiber bioreactor for continuous shifting of CO into H_2 by *Rubrivivax gelatinosus* CBS

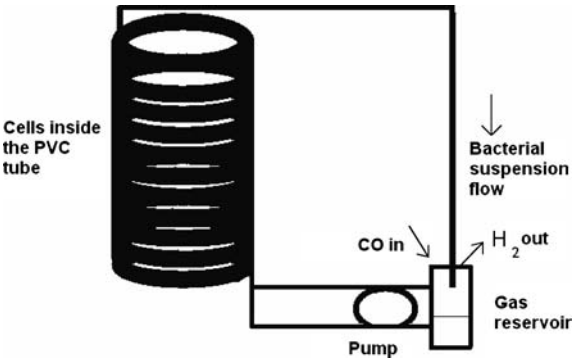

Fig. 2 Schematic diagram of a helical PVC tubular bioreactor to shift CO into H_2 by *Rubrivivax gelatinosus* CBS

The bioreactor was designed so that small bubbles containing CO were injected continuously through a needle/septum connection from the gas reservoir (initially 20% CO). The bubbles rose with the pumped medium (3.5-min transit time) from the bottom of the bioreactor to the top. The high surface area of the bubble train promoted enhanced mass transport of gaseous CO into the aqueous bacterial suspension. To keep the pH of the medium from dropping as a result of carbonic acid build-up, the gas phase of the bioreactor was degassed with N_2 once daily, after which CO (20% in a N_2 balance) was reinjected into the system. The bioreactor was covered with a black cloth to prevent photosynthetic H_2 consumption from exposure to ambient light according to the reaction: $2H_2 + CO \rightarrow (CH_2O)_n + H_2O$.

The bioreactor was inoculated with bacterial culture at cell density of 0.65 g/L.

The bioreactors did not require sterilization because CO is either toxic to, or will not support the growth of, most potential invading organisms. Both bioreactors were maintained at room temperature (25–26°C).

Measurement of Hydrogen Production

Hydrogen production rates were measured using a Varian Model 3700 gas chromatograph (Walnut Creek, CA) equipped with a molecular sieve 5A column and a thermal conductivity detector. Argon was used as the carrier gas.

Scanning Electron Microscopy

Immobilized bacterial cells were examined using the freeze-drying method. Cells were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 (containing 6% sucrose wt/vol) for 24 h at 25°C and then washed in the same buffer containing 12% sucrose (wt/vol). The specimens were freeze-dried. Dried specimens were coated with gold and examined in a model JSM 25 S scanning microscope (Jeol Ltd., Japan) at accelerating voltage of 10 kV.

Results

H_2 Production by the Hollow-Fiber Bioreactor

Before construction of the hollow-fiber bioreactor, the adhesion of bacterial cells to hollow fibers was studied in batch cultures. Cells were immobilized on 2-cm-long pieces of

hydrophilic cuprammonium rayon hollow fibers. The degree of immobilization of cells was assessed by examining the attachment of the cells using scanning electron microscopy (Fig. 3). Bacterial cells almost entirely covered the hollow-fiber surfaces. Because of this high degree of immobilization, a cuprammonium rayon hollow-fiber cartridge was selected for construction of the bioreactor.

Hydrogen production in hollow-fiber bioreactor by *Rubrivivax gelatinosus* CBS from CO at an average rate of 125 ml g cdw^{-1} h^{-1} (maximum rate of 700 ml g cdw^{-1} h^{-1}) was observed for more than 8 months (Fig. 4). No detectable remaining levels of CO (<18 ppm) in the effluent gas were observed during this period.

H_2 Production by the Bubble-Train Bioreactor

Continuous H_2 production by *Rubrivivax gelatinosus* CBS at rates up to 140 ml g cdw^{-1} h^{-1} was observed in a bubble-train bioreactor for more than 10 days (Fig. 5). Rates of H_2 production were low at first, probably because of the exposure of the bacterial culture to O_2 during bacterial transfer to the bioreactor. Once more favorable anaerobic conditions were established for the bacteria in the bioreactor, rates of H_2 production started to increase. At the higher rates, 2 h was sufficient to shift all of the added CO in the reservoir gas phase into H_2. No detectable level of CO remained in the gas phase (less than 18 ppm). The bulk of the added CO was shifted during the first hour after feeding. Repetitive batch feeding of CO (the gas phase was changed once a day and reestablished with 20% CO in N_2) maintained the culture in a highly active state.

Hydrogen production as a function of the distance from the gas injection port within the bubble-train bioreactor was studied in a separate experiment as well. For this experiment, the PVC tube used within the bioreactor was much longer (50 m long versus the original 9.8 m). Rubber stoppers were inserted at regular intervals along the bioreactor tube. Samples of the gas phase were taken and H_2 production and CO utilization was determined. The results of this study are shown in Fig. 6. The highest H_2 production rate (as well as the CO consumption rate) was observed within 10 m from the CO injection port. Apparently, a long PVC is not necessary for the bioreactor construction, at least not from the point of H_2 production and CO consumption.

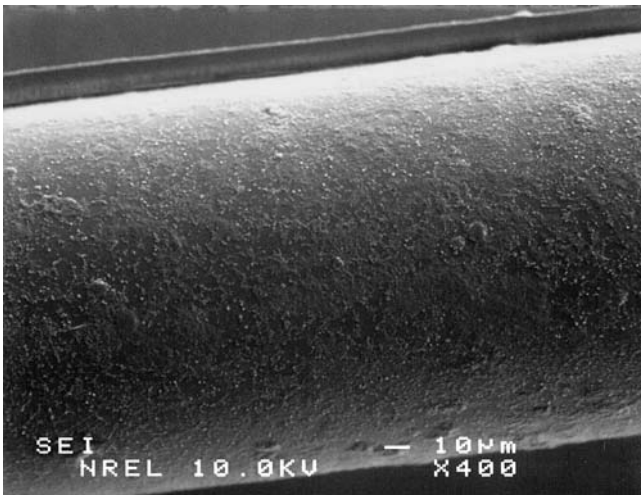

Fig. 3 Scanning electron microscopy of *Rubrivivax gelatinosus* CBS immobilized on cuprammonium rayon hollow fiber (AM-4OM-SD)

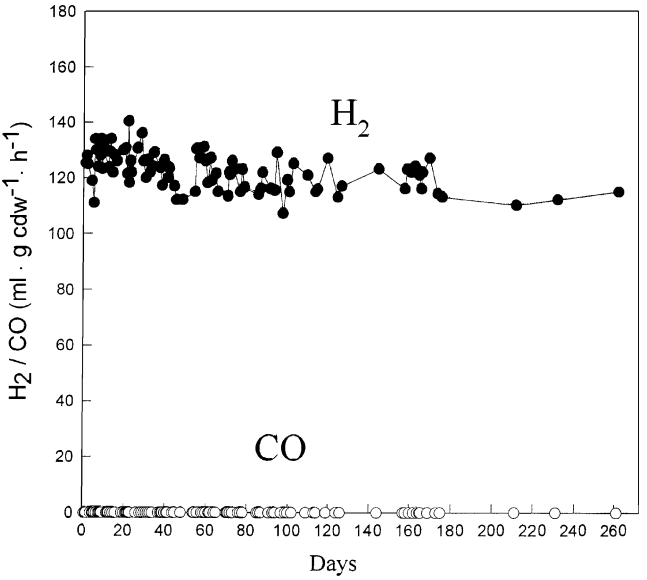

Fig. 4 Continuous shifting of CO into H_2 by *Rubrivivax gelatinosus* CBS in a hollow-fiber bioreactor

Discussion

In this study, two types of bioreactors were tested for H_2 production by purple nonsulfur bacterium using a shift reaction. The main idea was to improve mass transport of gaseous CO into an aqueous bacterial suspension. A simple method of using hollow fiber membrane technology to enhance mass transfer of CO has proven effective, but is likely too expensive for commercial applications at the present time. Different types of membrane-based

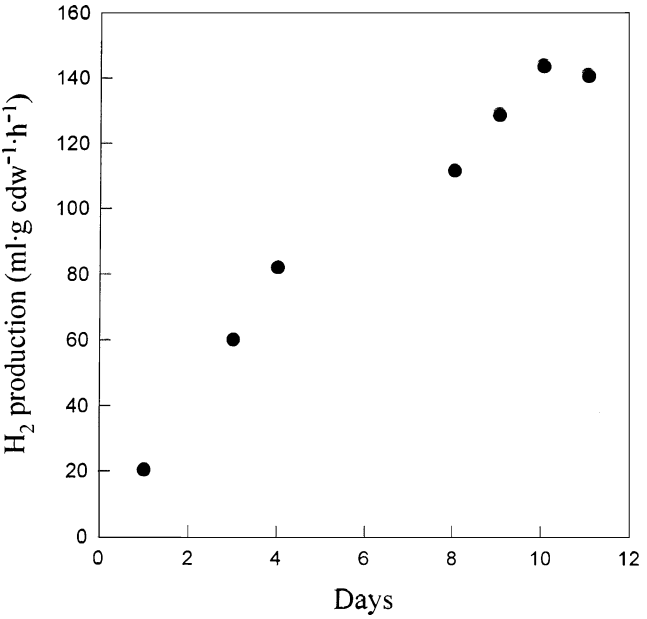

Fig. 5 Shifting of CO into H_2 by *Rubrivivax gelatinosus* CBS in the PVC tubular bioreactor

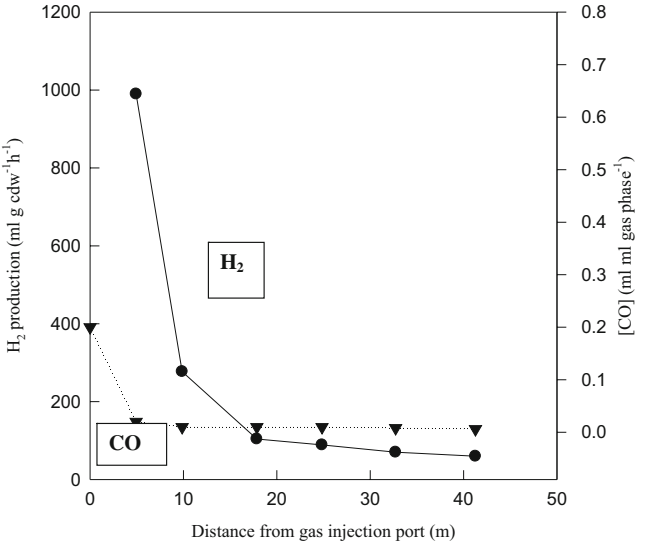

Fig. 6 Shifting of CO into H_2 by *Rubrivivax gelatinosus* CBS as a function of distance from CO injection port in the PVC tubular bioreactor

bioreactors such as a spiral sheet membrane bioreactor can be scaled up using a prototype already available for filtration applications with cartridges produced by Amicon, Inc (Beverly, MA). The price for that type of membrane bioreactors could be more affordable. The cost of the bubble-train bioreactor described here is significantly less than that of any type of membranous bioreactors or any type of bioreactor that uses immobilized cells. This bioreactor exhibited superior mass transfer rates as well.

The gaseous product from both bioreactors, enriched in H_2 (20% H_2) and devoid of any remaining CO, was sufficiently clean for direct injection into a H_2 fuel cell. In fact, the effluent gas from the hollow-fiber bioreactor has been directly injected into small millivolt fuel cells and shown capable of generating enough electricity to power small motors and lamps. No negative effect on the fuel cells was noted.

Carbon monoxide for the water–gas shift reaction may derive from a synthesis gas (predominantly CO) obtained by a thermochemical gasification of biomass (*e.g.*, wood waste). Growth and H_2 production using this synthesis gas was recently reported for another purple nonsulfur bacterium *Rhodospirillum rubrum* [8].

Carbon monoxide for the shift reaction may also come from other microorganisms. A number of bacteria are able to degrade different molecules such as chlorophyll with the release of CO [9].

Acknowledgements This work was supported by the U.S. Department of Energy Hydrogen Program. The authors thank Asahi Medical Co., Ltd. (Japan) for supplying the hollow fibers.

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