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Spiral Tubular Bioreactors for Hydrogen Production by Photosynthetic Microorganisms

Design and Operation

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ABSTRACT

Spiral tubular bioreactors were constructed out of transparent PVC tubing for H_2 production applications. Both a cyanobacterial *Anabaena variabilis* mutant that lacks uptake hydrogenase activity and the photosynthetic bacterium *Rhodobacter* sp. CBS were tested in the bioreactors. Continuous H_2 photoproduction at an average rate of 19 mL · min^{-2} · h^{-1} was observed using the *A. variabilis* mutant under an air atmosphere (without argon sparging or application of a partial vacuum). The cyanobacterial photobioreactor was run continuously for over one month with an average efficiency of light energy conversion to H_2 of 1.4%. Another H_2-producing approach employed a unique type of activity found in a strain of photosynthetic bacteria that shifts CO (and H_2O) into H_2 (and CO_2) in darkness. Continuous dark H_2 production by *Rhodobacter* sp. CBS from CO (in anticipation of using synthesis gas as the future substrate) at rates up to 140 mL · g cdw^{-1} · h^{-1} was observed in a bubble-train bioreactor for more than 10 d.

Index Entries: Hydrogen; bioreactors; *Anabaena variabilis*; water-gas shift reaction; *Rhodobacter*.

INTRODUCTION

Hydrogen is considered to be an environmentally desirable fuel because it can be produced from renewable resources, and its combustion product (water) is nonpolluting. Several biological approaches are being used to produce H_2 either from water and solar energy or from biomass (*1,2*). The main challenge here is to design simple, efficient bioreactors that

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consume as little energy as possible. In recent years, several groups have studied the efficacy of tubular bioreactors for cultivation of photosynthetic micro-organisms *(3–5)*. In comparison to open ponds or tank reactors, these tubular bioreactors have the following advantages:

1. A high surface area to culture volume ratio, allowing photosynthetic micro-organisms to absorb light energy more effectively;
2. Better gas mass transfer rates into liquid media; and
3. Low mixing energy requirements.

In the present study, two simple polyvinyl chloride (PVC) tubular bioreactors vertically spiraled to facilitate continuous H_2 production by photosynthetic micro-organisms were analyzed.

The first type of bioreactor incorporated a cyanobacterial mutant of *Anabaena variabilis*. Hydrogen was photoevolved from water and released from solution at atmospheric pressure, under conditions where the organisms were continuously exposed to ambient levels of O_2. This was possible because of the use of the *A. variabilis* PK84 mutant obtained from Prof. S. V. Shestakov (Moscow State University) that lacks uptake hydrogenase activity *(6)*. The second type of bioreactor utilized a unique type of H_2-producing activity originally found in a strain of photosynthetic bacteria by Uffen *(7)*. Fermentative dark cultures of this strain in complex media with CO carried out a water-gas shift reaction to produce H_2 according to the reaction, $CO + H_2O \rightarrow CO_2 + H_2$. Numerous strains of photosynthetic bacteria, including *Rhodobacter* sp. CBS, have been isolated at the National Renewable Energy Laboratory that utilize CO in the light as well as in darkness and do not require complex organic substrates *(8)*. These strains quantitatively shift the CO component of synthesis gas (e.g., from thermally gasified biomass) into H_2. However, mass transport of gaseous CO into an aqueous bacterial suspension is the rate-limiting step in the process and was the main concern in bioreactor design for the current study.

MATERIALS AND METHODS

Microbial Cultures

Prior to inoculation into bioreactors, *A. variabilis* PK84 was grown with shaking as a batch culture in the medium of Allen and Arnon *(9)* without combined nitrogen. Continuous light was provided by cool white fluorescent lamps (3.0 $W \cdot m^{-2}$). Before inoculation into the bioreactor, *Rhodobacter* sp. CBS was cultivated as a batch culture in closed bottles on basal medium *(10)* plus 10% CO with shaking and illuminated with incandescent lamps (35 $W \cdot m^{-2}$). Cell dry weights were determined by trapping the cyanobacteria or bacteria on Whatman # 114 filter paper and drying the cell suspensions at 90°C to constant weight.

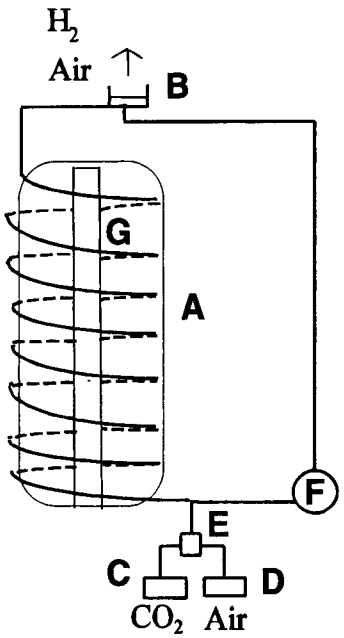

Fig. 1. Schematic diagram of a helical PVC tubular photobioreactor for H_2 production by an *A. variabilis* mutant. **A**, PVC tubing, **B**, H_2 measurement port; **C**, CO_2 gas cylinder; **D**, an air input line; **E**, rotameter; **F**, pump; **G**, lamp.

Bioreactors

Two types of bioreactors were designed and constructed. The first was a 2 L (total volume), 0.4 m high photobioreactor for cyanobacteria as shown in Fig. 1. The bioreactor consisted of:

1. A 42 m transparent PVC (Nalgene, Rochester, NY), 7.9 mm internal diameter tube wound helically on a vertical transparent cylindrical supporting structure;
2. A H_2 measurement port, where H_2 and air are vented from the cyanobacterial suspension (gas flow rate 46 mL· min^{-1});
3. A CO_2 gas cylinder;
4. An air input line;
5. A gas proportioning rotameter (Omega, Stamford, CT), which was used to measure and mix CO_2 and air;
6. A peristaltic pump (Masterflex, Cole-Palmer Instrument, Niles, IL) for circulating (35 mL · min^{-1}) the cyanobacterial suspension. Cell concentration for the cyanobacterial bioreactor during inoculation was 0.65 mg cdw · mL^{-1} and increased because of cyanobacterial growth during the bioreactor operation. It was difficult to measure

accurately the cell biomass during the bioreactor operation because of cell adhesion to the bioreactor walls; and

7. A 33 W cool white fluorescent lamp.

The bioreactor suspension was bubbled with a mixture of CO_2 (about 5%) and air through a needle/septum connection at the base of the photobioreactor to supply the cells with a carbon source and remove H_2. The inner cylindrical surface of the bioreactor (0.22 m^2) was illuminated continuously with fluorescent light (average irradiance 3.0 $W \cdot m^{-2}$). Light irradiance was measured using a radiometer (Model 65A, Yellow Spring Instruments, Yellow Springs, OH) at different points on the inner surface of the cyanobacterial photobioreactor.

The second bioreactor was a 0.5 L (liquid volume), 0.8 m high device for the dark bacterial production of H_2 from CO (and H_2O) as diagrammed in Fig. 2. The bioreactor was constructed from:

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

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 in N_2). The bubbles rose with the pumped medium from the bottom of the bioreactor to the top (3.5 min transit time). The high surface area of the bubble train promoted enhanced mass transport of gaseous CO into the aqueous bacterial suspension. In order to keep the medium pH from dropping due to bicarbonate build-up, the gas phase of the reservoir was degassed with N_2 once every day, and then 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: $2H_2 + CO_2 \rightarrow (CH_2O)_n + H_2O$.

The cyanobacterial bioreactor was sterilized with a 5% sodium hypochlorite solution and washed with sterile distilled water several times before inoculation. The photosynthetic bacterial bioreactor did not require sterilization because CO is either toxic to, or will not support growth of, most potential invading organisms. Both bioreactors were maintained at room temperature (23–24°C).

Hydrogen Production

Hydrogen production rates were measured using a Varian Model 3700 gas chromatograph (Walnut Creek, CA) equipped with a molecular

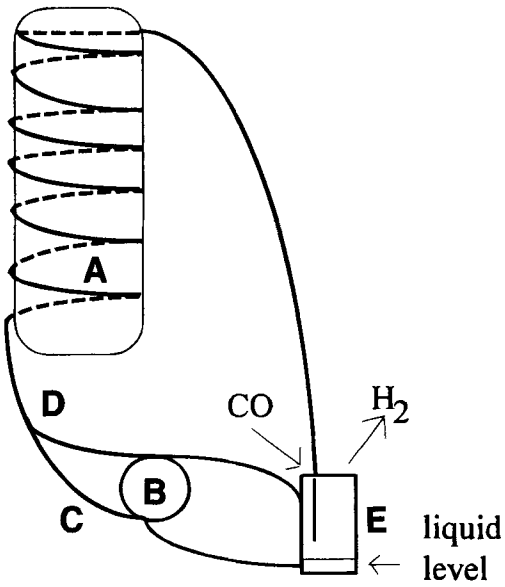

Fig. 2. Schematic diagram of a helical PVC tubular bioreactor to shift CO into H_2 by *Rhodobacter* sp. CBS. **A**, PVC tubing; **B**, pump; **C**, bacterial suspension entrance; **D**, CO injector; **E**, gas reservoir.

sieve 5A column and a thermal conductivity detector. Argon was used as the carrier gas. Light energy conversion efficiencies (to H_2) in the cyanobacterial photobioreactor were calculated as follows:

$$\text{Efficiency (\%)} = \frac{H_2 \text{ production rate} \times H_2 \text{ energy content}}{\text{Incident Light Irradiance}} \times 100\% \quad (1)$$

The heat of H_2O formation, (241,000 J · mol^{-1}) was used as the energy content of the H_2 produced.

RESULTS

Operation of the Photobioreactor for H_2 Production by *A. variabilis*

H_2 production by the *A. variabilis* mutant is shown in Fig. 3. Initially, H_2 production increased as the cyanobacterial culture grew and then decreased as the cyanobacteria aged. H_2 production was observed for about one month during the period that the culture was most active. The percentage of H_2 in the effluent gas varied from 0.03 to 1%. After 25 d under H_2-producing conditions, the cyanobacterial culture appeared to turn more green in color from its natural blue-green appearance. This change in color coincided with the loss of H_2 evolution activity, which in turn was probably a result of nutrient limitation. The CO_2 was consumed during the bioreactor run (<1% at the exit port). Cell adhesion to the bioreactor walls was also observed. Control exper-

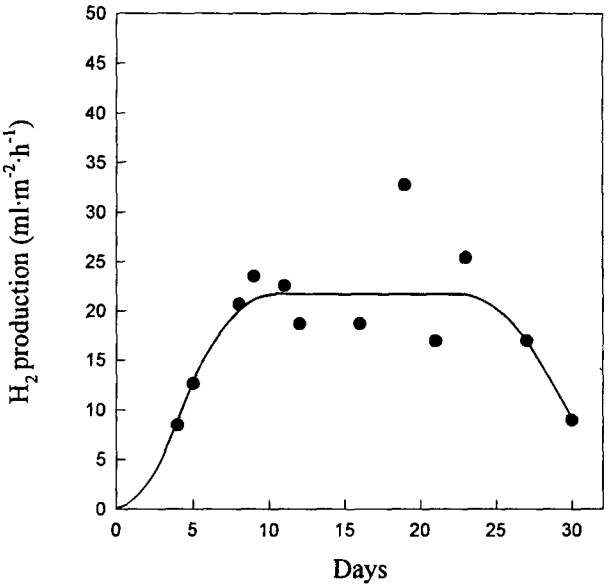

Fig. 3. H_2 production by an *A. variabilis* mutant in the PVC tubular photobioreactor.

iments, after removal of all suspended nonbound cells, indicated that the adsorbed cells produce H_2. These adsorbed cells could be removed easily by scouring the PVC tubing with pressurized air from the bottom end *(11)*.

The efficiency of light energy conversion to H_2 in the photobioreactor was calculated using Equation 1. The average rate of H_2 production over 30 d of the run (Fig. 3) was 18.9 mL · m^{-2} · h^{-1}, which is equal to 6.29 · 10^{-4} mol · m^{-2} · h^{-1} or 1.74 · 10^{-7} mol · m^{-2} · s^{-1}. Using the aforementioned information, we calculate an average efficiency of

$$\frac{1.74 \cdot 10^{-7}\ \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \times 24 \times 10^{5}\ \text{J} \cdot \text{mol}^{-1}}{3\ \text{J} \cdot \text{m}^{-2} \cdot \text{s}^{-1}} \times 100\% = 1.4\% \qquad (2)$$

Operation of the Bioreactor for H_2 Production by *Rhodobacter* sp. CBS

Continuous H_2 production from CO at rates up to 140 mL H_2 · g cdw^{-1} · h^{-1} was observed in a bubble-train bioreactor for more than 10 d (Fig. 4). 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. Then, under more favorable anaerobic conditions 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

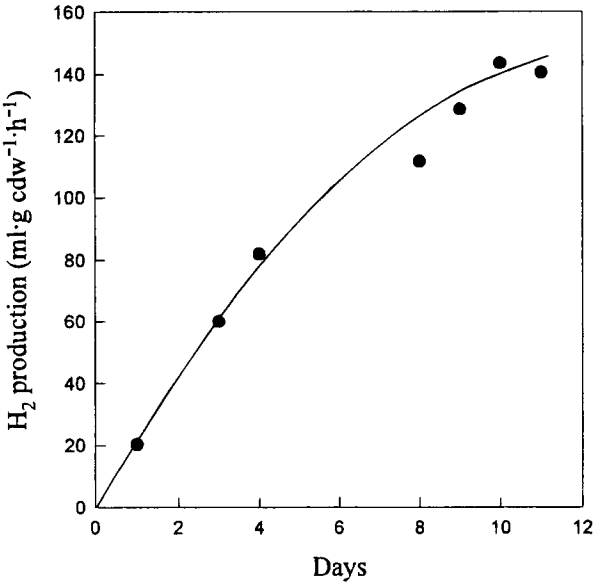

Fig. 4. Shifting of CO into H_2 by *Rhodobacter* sp. CBS in the PVC tubular dark bioreactor.

state. The product gas, containing up to 20% H_2 in N_2 and devoid of any remaining CO, was sufficiently clean for direct injection into a H_2 fuel cell.

DISCUSSION

In this preliminary study, helical bioreactors made of transparent PVC tubing were employed for the first time to examine H_2 production by two distinct types of micro-organisms. It is shown that it is possible to produce H_2 using this type of simple, low-cost bioreactor. In the case of the photobioreactor employing the *A. variabilis* mutant, the important new result was that H_2 could be produced from water under ambient conditions. Previous work employed an argon sparging system (90% argon with CO_2 and N_2) to produce H_2 from a tubular (glass) bioreactor *(12)*, a system inherently more complex. The second bioreactor system (bubble-train bioreactor) used *Rhodobacter* sp. CBS to produce H_2 from CO (in anticipation of using synthesis gas as substrate). Prolonged movement of small bubbles of CO through this bioreactor increased the contact time between the bacterial suspension and CO, which enhanced mass transport of the gas into the aqueous bacterial suspension. The bioreactor is now being modified by adding more PVC tubing so that the bulk of the CO will be shifted in a single pass of entrained bubbles.

Our results with cyanobacterial photobioreactors suggest that to achieve long-term operation and steady-state H_2 production levels, it may be necessary to supply fresh medium periodically and remove the old cells. By doing this, the authors expect to achieve H_2 production for periods as

long as 9 mo. They have operated continuous, H_2-producing bioreactors for such periods of time with the same microbial cells immobilized on hollow-fiber arrays *(13)*. However, the cost of tubular PVC bioreactors is significantly less than that of hollow-fiber bioreactors. Development of a computerized bioreactor system will also help to optimize H_2 production by careful regulation of the gas supply and control of pH *(14)*. To improve the energy balance of the bioreactor system, air-lift designs will save on energy consumption by pumps. Also, to further improve the economics, H_2 production can be combined with the synthesis of secondary products, such as commodity chemicals or animal feed.

ACKNOWLEDGMENTS

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