Pergamon

PII: S0360-3199(96)00134-6

Int. J. Hydrogen Energy, Vol. 22, No. 5, pp. 521–524, 1997

0360-3199/97 $17.00+0.00

PHOTOPRODUCTION OF HYDROGEN BY CYANOBACTERIA UNDER PARTIAL VACUUM IN BATCH CULTURE OR IN A PHOTOBIOREACTOR

S. A. MARKOV,* A. D. THOMAS,† M. J. BAZIN and D. O. HALL

Division of Life Sciences and † Computing Centre, King's College London, University of London, Campden Hill Road, London W8 7AH, U.K.

(*Received for publication* 18 *June* 1996)

Abstract—Optimal conditions for H_2 photoproduction by the cyanobacterium *Anabaena variabilis* were investigated in batch experiments. Increasing light intensities during the incubation of cyanobacterial cells led to increased H_2 production which was accompanied by suppression of O_2 evolution. The batch experimental results were subsequently applied to the use of cyanobacteria in a computer-controlled photobioreactor for H_2 production. Some devices associated with the photobioreactor such as the pH electrode, CO_2 cylinder and air line via proportional control gas flow valves and the vacuum and peristaltic pumps were connected via an interface card to an IBM computer. BBCBASIC (86) plus was the chosen language to write a program to control the photobioreactor. A two-phase H_2 production system is suggested as being feasible for practical demonstration. In the first phase the cyanobacterial cells photosynthesize under light intensities of 45–55 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and establish conditions for subsequent H_2 production. In the second phase the cells produce H_2 under light intensities of 170–180 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Photoproduction of H_2 at rates of about 12.5 ml $H_2 \cdot gcdw^{-1} \cdot h^{-1}$ was observed.

INTRODUCTION

Considerable research has been done on the utilization of solar energy for H_2 photoproduction by photobiological methods [1, 2]. One suitable candidate for the development of biological environmentally acceptable H_2 production is cyanobacteria [3, 4]. Cyanobacteria are unique in their ability to produce H_2 using water as their ultimate electron substrate, CO_2 and N_2 from air and solar energy as an energy source. Cells of cyanobacteria can evolve H_2 in reactions catalyzed by nitrogenase and/or hydrogenase in light.

Cyanobacteria produce O_2 during photosynthesis as well. The aim was to obtain maximum yields of H_2 production with minimal O_2 evolution using batch cyanobacterial culture. The results were subsequently applied to the use of cyanobacteria in a computer-controlled photobioreactor for H_2 production.

EXPERIMENTAL

Chemicals

All chemicals used were of the highest commercial purity obtained from BDH Merck Ltd, U.K. CO_2 was commercial purity from BOC (U.K.).

* Address for correspondence: National Renewable Energy Laboratory, 1617 Cole Boulevard, Golden, CO, 80401, U.S.A.

Batch culture

Anabaena variabilis (Kützing) Bornet & Flahault 1403/4B from CCAP (Culture Collection of Algae and Protozoa, Freshwater Biological Association, Ambleside, U.K.) was grown in the medium of Allen and Arnon [5] at 28°C without combined nitrogen. Continuous light was provided by cool white fluorescent lamps (15 $\mu mol \cdot s^{-1} \cdot m^{-2}$ irradiance on the surface of the culture) in an orbital incubator with shaking (140 rpm) and 1.7% CO_2.

Computer-controlled photobioreactor

The photobioreactor consisted of a 300 ml culture vessel (Fig. 1). The vessel was connected to a 10 l medium reservoir and a biomass receiver bottle to constantly feed in medium and take out culture at a defined flow rate (3 ml per hour) using a peristaltic pump (LKB, Sweden).

The photobioreactor was maintained at 25°C and illuminated continuously with a warm fluorescent lamp that surrounded the vessel (45–55 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at the surface of the vessel). Light intensity measurements were made using a LI-189 Quantum/Radiometer/Photometer (LI-COR, Inc., U.S.A.). The photobioreactor suspension was bubbled with a mixture of CO_2 (about 5%) and air (5 $ml \cdot min^{-1}$ CO_2 flow rate).

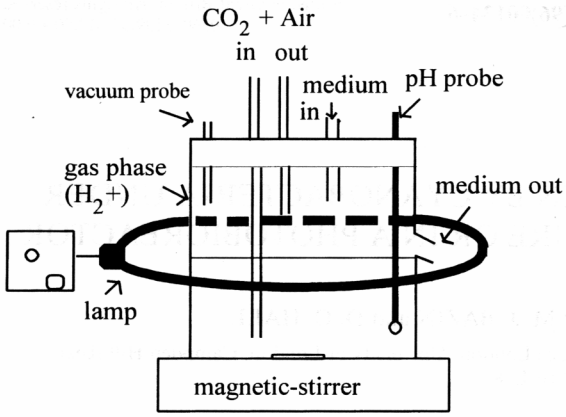

Fig. 1. Schematic diagram of the photobioreactor.

The photobioreactor control system

The photobioreactor is controlled by an IBM-compatible PC (Dan Technology 286 AT, U.K.) fitted with a specially-designed interface card, together with the associated control software package. Using this system, we were able to control all of the devices in the bioreactor as well as measure and store various parameters existing within the photobioreactor such as: control of a peristaltic pump; control of air and CO_2 gas supplies and the vacuum supply; measuring and displaying on-screen the concentration, gas flow rate and pressure for the gases in the system; measuring and displaying various other parameters such as pH and vacuum; storing the data for future analysis. In addition, facilities are provided for the calibration of the various transducers and sensors employed and for configuring the way in which the system as a whole operates. A block diagram of the system appears in Fig. 2. At the heart of the system lies the computer which is linked to the photobioreactor system via the interface card. The interface card was designed and constructed at King's College London. The peristaltic pump in the photobioreactor is connected directly

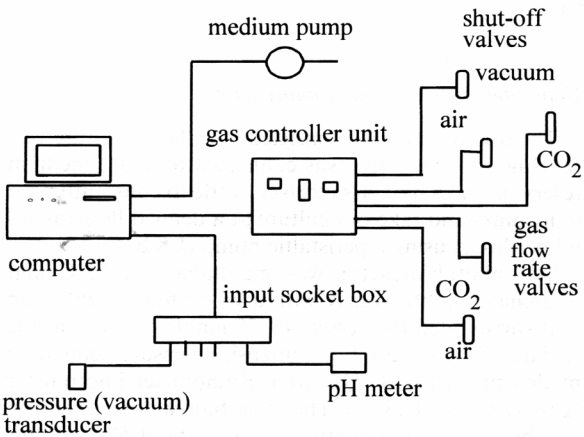

Fig. 2. Block diagram of the computer control of the photobioreactor.

to the interface card. Also linked to the interface card is the gas controller unit, a stand-alone controller providing power and control for the gas shut-off valves (Platon Flowbits Ltd, U.K.) and flow control valves (MKS Instruments, Inc., U.S.A.) in the photobioreactor. The gas controller unit was designed and constructed at King's College London. The transducer measuring gas pressure and vacuum in the photobioreactor is connected to the input socket box/conditioning unit which in turn feeds the interface card. Additional laboratory instrumentation for measuring pH, etc. (as temperature and H_2 concentrations are not applicable for this study) also feeds the interface card via the input socket box.

The program controlling the system was written in BBCBASIC (86) plus, a version of the well-known BBC Micro BASIC written for the IBM PC and compatible computers, providing good screen display, graphics and file-handling capabilities suited to this application. The choice of this programming environment was largely influenced by the fact that many of those working in the academic community are familiar with this version of BASIC and future development of the software could easily be undertaken by others.

Hydrogen production

1. *Batch culture.* Three millilitres of cell suspension was added to 15 ml glass vials fitted with Suba Seal rubber stoppers. Prior to incubation the vials were vacuum degassed (270–300 torr). The degree of vacuum (the pressure of gas remaining in a partial vacuum) was measured with a manometer. Samples were incubated for 5 hours in a shaking water bath at 25°C and illuminated with tungsten lamps.

2. *Photobioreactor.* Every 6 days the photobioreactor vessel was degassed using a vacuum pump (270–300 torr) for 15 min and then illuminated for 5 hours with a xenon lamp (170–180 $\mu mol \cdot s^{-1} \cdot m^{-2}$ irradiance at the surface of the vessel). The degree of vacuum was measured with a transducer (Sensym., U.K.) connected to the computer.

Hydrogen production was measured using a Taylor Servomex gas chromatograph (Crowborough, U.K.) fitted with a Porapak Q column, heated to 55°C, and a microcatharometer equipped with a thermal conductivity detector, with nitrogen as carrier gas; the hydrogen peak was recorded 20 s after injection.

The results of the experiments were expressed in ml instead of moles because of the experimental conditions (partial vacuum, etc).

Oxygen evolution

Oxygen evolution was measured using the gas chromatograph as described for hydrogen production.

Cell dry weights

Cell dry weights (cdw) were determined by trapping the cyanobacteria on Whatman #114 filter paper and drying the cell suspensions at 90°C to constant weight.

RESULTS

H_2 production in batch culture

Hydrogen was produced by cyanobacterial cells under different light intensities after 5 hours incubation in vials when amounts of H_2 had been measured. Increasing light intensities led to increased H_2 production which was accompanied by suppression of O_2 evolution (Fig. 3). Studies conducted by a number of authors have shown that exposure of cyanobacteria, algae, and higher plants to high-intensity light reversibly suppresses the oxygen-evolving system of photosynthesis [6]. Our results demonstrated that photoinhibition of O_2 evolution in cyanobacteria can be associated with stimulation of H_2 production.

No significant differences in H_2 photoproduction over a range of pHs between 7 and 10 was observed (Fig. 4). H_2 production did, however, decrease at acid pHs.

H_2 production in a computer-controlled photobioreactor

The batch experiments results with different light intensities were applied to establish conditions for a computer-controlled photobioreactor for H_2 production.

The photobioreactor was operated under light intensities of 45–55 $\mu mol \cdot s^{-1} \cdot m^{-2}$ to promote photosynthesis (O_2 evolution). Optimum CO_2 concentrations (5%) for H_2 production as we found before [4] and pH (7.5) were maintained by the computer. Every six days a photobioreactor vessel was sealed and incubated under partial vacuum and under light intensities of 170–180 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and then H_2 production was measured. The vacuum pressure was monitored by the computer. No CO_2 or fresh medium were added at this time. Steady state H_2 production by *A. variabilis* was observed over a one month period but not completed (Fig. 5).

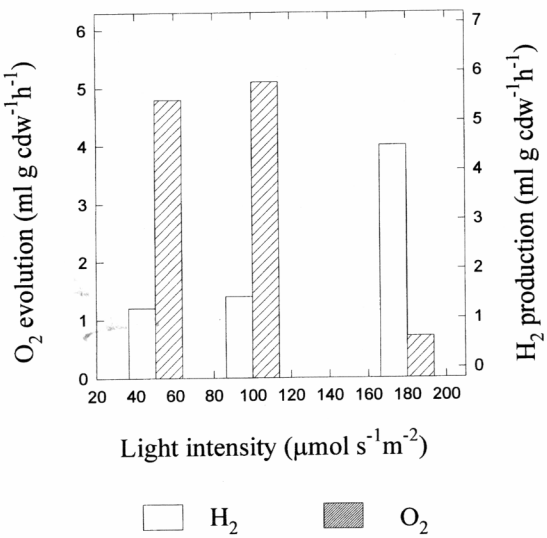

Fig. 3. H_2 and O_2 evolution by *A. variabilis* as a function of light intensity under partial vacuum.

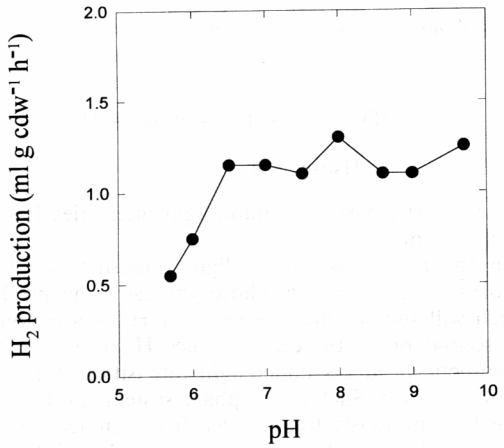

Fig. 4. H_2 photoproduction by *A. variabilis* in vials in buffer (standard concentration) as a function of pH.

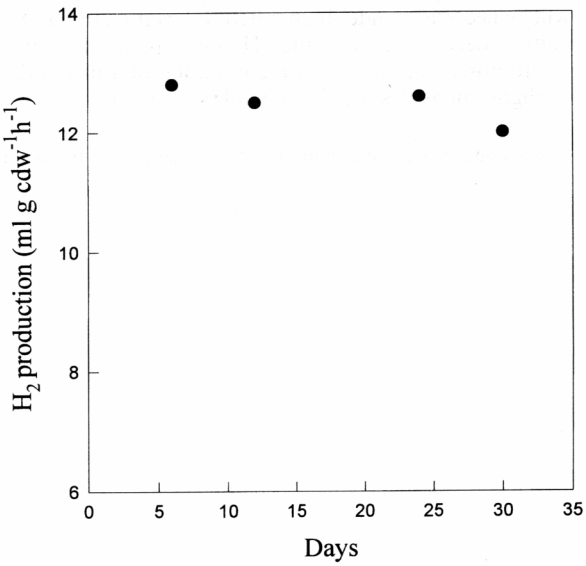

Fig. 5. H_2 photoproduction by *A. variabilis* in the computer-controlled photobioreactor.

DISCUSSION

The differing optimum for H_2 production and O_2 evolution (photosynthesis) under different light intensities found in batch experiments enables us to select a two-phase photobioreactor system for practical demonstration.

First phase (photosynthesis)

$$\begin{array}{ccccc} O_2 & & & & CO_2 \\ \uparrow & & & & \downarrow \\ H_2O & \rightarrow & PSII & \rightarrow & PSI \rightarrow [CHO] \end{array}$$

Phase criteria: ambient conditions, light intensities 45–55 $\mu mol \cdot s^{-1} \cdot m^{-2}$.

Second phase (H_2 photoproduction)

$$\begin{array}{l} CO_2 \\ \uparrow \\ [CHO] \rightarrow PSI \rightarrow Fd \rightarrow N_2ase \rightarrow H_2 \\ \downarrow \\ H_2ase \rightarrow H_2 \end{array}$$

Phase criteria: partial vacuum, light intensities 170–180 $\mu mol \cdot s^{-1} \cdot m^{-2}$.

In the first phase under light intensities of 45–55 $\mu mol \cdot s^{-1} \cdot m^{-2}$ the cells photosynthesize the products which will subsequently be used for H_2 production. In the second phase the cells produce H_2 using the photosynthetic products under light intensities of 170–180 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Such a two-phase system can be maintained continuously for months. It is significant that it was possible to produce H_2 under higher light intensities than is normal for outdoor conditions. The relationship between H_2 production and light intensity is dependent on the density of culture, gas phase, etc. Additional work will be necessary under higher light intensities and higher culture densities to optimize H_2 photoproduction in a photobioreactor in outdoor conditions of full midday sunlight intensities (i.e. 2000 $\mu mol \cdot s^{-1} \cdot m^{-2}$).

Acknowledgements—We thank the New Energy and Industrial Technology Development Organization and Research Institute of Innovative Technology for the Earth (Japan) for financial support.

REFERENCES

1. Miyake, J. and Kawamura, S., Efficiency of light energy conversion to hydrogen by photosynthetic bacterium *Rhodobacter sphaeroides*. *International Journal of Hydrogen Energy*, 1987, **12,** 147–149.
2. Serebriakova, L., Zorin, N. and Lindblad, P., Reversible hydrogenase in *Anabaena variabilis* ATCC 29413. *Archives of Microbiology*, 1994, **161,** 140–144.
3. Benemann, J. R. and Weare, N. M., Hydrogen evolution by nitrogen-fixing *Anabaena cylindrica* cultures. *Science*, 1974, **184,** 174–175.
4. Markov, S. A., Bazin, M. J. and Hall, D. O., Hydrogen photoproduction and carbon dioxide uptake by immobilized *Anabaena variabilis* in a hollow-fiber photobioreactor. *Enzyme and Microbial Technology*, 1995, **17,** 306–310.
5. Allen, M. B. and Arnon, D. I., Studies on nitrogen fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica. Lemn. Plant Physiology*, 1955, **30,** 366–372.
6. Samuelsson, G., Lönnenborg, A., Gustafsson, P. and Öquist, G., The susceptibility of photosynthesis to photoinhibition and the capacity of recovery on high and low light grown cyanobacteria, *Anacystis nidulans*. *Plant Physiology*, 1987, **83,** 438–441.