HYDROGEN EVOLUTION FROM IMMOBILIZED CULTURES OF THE CYANOBACTERIUM *ANABAENA CYLINDRICA* B629

Grant R. LAMBERT, Arlene DADAY and Geoffrey D. SMITH
Department of Biochemistry, Faculty of Science, The Australian National University, Canberra, ACT 2600, Australia

Received 6 March 1979

1. Introduction

The production of hydrogen from water using a biological catalyst and sunlight as an energy source (biophotolysis) is a potential substitute for fossil fuels [1–3]. The heterocystous cyanobacteria have been widely studied in this respect since they were first demonstrated to simultaneously produce hydrogen and oxygen [4,5]. However, to establish a technically and economically viable system a number of requirements must be fulfilled [6,7].

A major problem for the use of cyanobacteria or algae is the requirement for continuous agitation to keep cultures uniformly suspended, since without it the organisms settle out and form clumps which exhibit no hydrogen formation [6]. While agitation of cultures is readily achieved in the laboratory, on a larger scale it presents a more formidable and expensive undertaking. A further problem which arises as a result of agitation is filament breakage and structural degeneration of vegetative cells, both of which are associated with cessation of biophotolysis in liquid cultures [6].

In this communication we report a procedure whereby filamentous cyanobacteria can be uniformly suspended as a mixture with small glass beads without any need for continuous agitation. Hydrogen and oxygen production was measured for extended periods and rates of formation were compared with those from shaken and unshaken suspensions without glass beads. The rates of hydrogen and oxygen formed in immobilized cultures are shown to be comparable with those in shaken suspensions.

2. Materials and methods

2.1. *Algae and their growth*

Anabaena cylindrica (strain B629) was obtained from the Culture Collection of Algae, University of Texas at Austin, and grown as in [8,9]. Cultures were harvested by settling and their dry weights determined as in [8,9].

2.2. *Incubations and analyses*

Algal suspensions (5 ml) were pipetted into flasks. Immobilization of the cyanobacteria in glass beads (80 mesh, for gas chromatography, British Drug Houses Ltd, Poole) was achieved by pipetting 2 ml algal suspension into a flask and adding 8.6 g glass beads very slowly while gently shaking the flask. Subsequent agitation of these flasks was carefully avoided. The distribution of algal filaments between the beads was uniform, as judged visually, and remained so indefinitely (fig.1). After addition of the glass beads, the suspension was ~5 ml total vol. Each assay was done from triplicate flasks. Gas atmospheres were made up, sampled and analyzed by procedures based on those in [8,9]. Oxygen was measured gas chromatographically, simultaneously with hydrogen. Reasonable accuracy was obtained by comparing it with the nitrogen peaks; knowing the O_2/N_2 ratio for air enabled the oxygen produced photosynthetically to be distinguished from that which leaked into syringes during transfer. This procedure could only be used for those flasks containing argon as the principal gas.

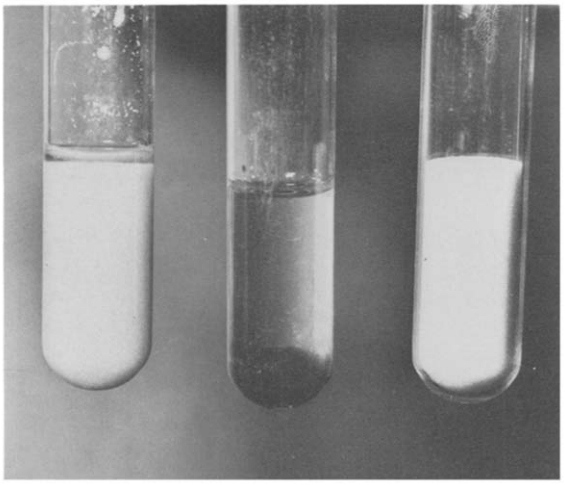

Fig.1. (Left) *A. cylindrica* suspended in glass beads. (Centre) Clumped organisms as observed in the absence of agitation without glass beads. (Right) Glass beads without suspended cyanobacteria.

3. Results

Hydrogen and oxygen evolution was measured over an extended period from *A. cylindrica* which was either immobilized in glass beads, shaken in liquid medium or unshaken in liquid medium (fig.2). In the last case the organisms were clumped at the bottom of the solution. With the immobilized culture, hydrogen evolution initially exhibited a lag period of $\leqslant$10 h; no such lag was evident with the shaken liquid cultures. The lag period was expected in view of the glass beads inhibiting diffusion of dissolved gases and the lack of shaking. Over longer time periods this lag was insignificant. The gaseous environment above the culture was analyzed and renewed every 2–4 days. It can be seen from fig.2 that over a period of several weeks hydrogen and oxygen evolution by unshaken immobilized cultures was comparable with that from shaken liquid cultures, whereas evolution from unshaken liquid cultures was much reduced by comparison.

Hydrogen evolution was also measured from unshaken cultures immobilized in glass beads with gas phases of air or N_2 supplemented with CO (0.2%) and C_2H_2 (5%). The results are shown in fig.3. Although aerobically incubated cultures showed reduced

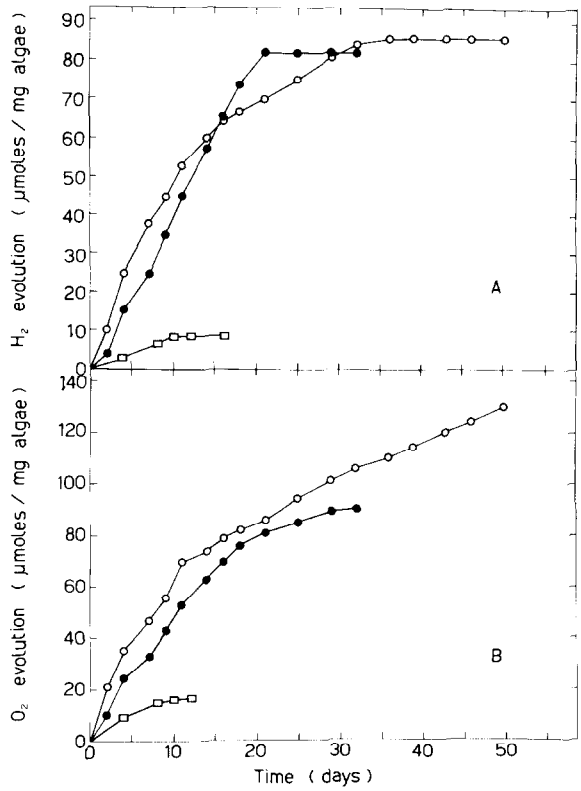

Fig.2. Hydrogen (A) and oxygen (B) evolution by unshaken cultures immobilized in glass beads (○), shaken liquid cultures (●) and unshaken liquid cultures (□). In each case the organisms were incubated beneath an initial gas atmosphere of argon/4% CO_2, renewed every 2–4 days. Flasks without glass beads contained 4.7 mg dry wt bacteria; those with beads contained 1.9 mg.

hydrogen formation, those incubated in dinitrogen were comparable with the cultures in argon. It is of interest that whereas hydrogen production by cultures incubated beneath argon ceased completely by day 30 (fig.3), cultures incubated beneath N_2 continued to produce the gas at ~5% of the initial rate even after day 57 of incubation (this is not readily evident with the scale used in fig.3).

4. Discussion

Immobilization is now a widely used process in the study and application of enzymes, including hydro-

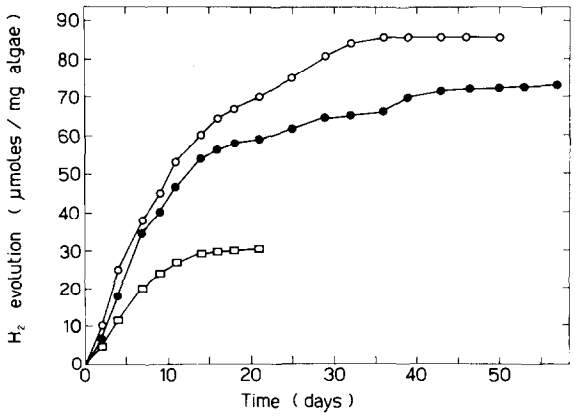

Fig.3. Hydrogen evolution by unshaken cultures immobilized in glass beads and incubated beneath gaseous atmospheres of argon/4% CO_2 (○), nitrogen/0.2% CO/5% C_2H_2/4% CO_2 (●) or air/0.2% CO/5% C_2H_2/4% CO_2 (□). Bacterial concentrations were as for fig.2.

genases [10,11] and whole bacterial cells, including hydrogen producing organisms [12]. Its application to intact photosynthetic microorganisms for hydrogen formation has not, however, been previously reported.

A potential limitation of immobilization for such organisms is the problem of light penetration through the supporting matrix. As seen in fig.2 this does not appear to have been a siginificant problem in the present work, although it may be with deeper suspensions. It is interesting that no chemical crosslinking or encapsulation of the organisms was required as is usually required for immobilization. Presumably the cyanobacteria themselves stick to the beads; the large surface area provided by the beads would thus ensure a uniform distribution of organisms. We have not systematically investigated the relationship between organism concentration and uniform distribution but preliminary experiments showed that much higher concentrations than used herein could be uniformly suspended, although a point was reached where hydrogen evolution was no longer proportional to the concentration of cyanobacteria.

As seen in fig.2 some hydrogen is evolved from clumped, settled organisms although previously it had been noted that such clumped organisms produced no gas [6]. The small amount produced herein is presumably explained by the shallow (~1 cm) solution in these experiments, leading to less severe clumping. Nevertheless, much more gas was formed in shaken cultures or those immobilized in glass beads, both being comparable. Oxygen evolution continued at a reduced rate after hydrogen evolution had ceased in each case (fig.2). That hydrogen formation ceased at about the same time in immobilized and shaken cultures (fig.2) suggests that the cessation is not significantly due to structural degeneration caused by shaking, but to the metabolic degeneration which would inevitably occur over a significant non-growth period.

With regard to the possible application of cyanobacteria in biosolar energy conversion, obviation of the need for constant agitation has obvious advantages. Small glass beads provide an excellent supporting matrix in that they are translucent, resistant to microbial degradation and not too large to allow settling of the filaments between units. It should be possible to actually grow the organisms within the lattice after initial suspension since the only requirement for shaking during growth is to maintain an even suspension [13]. The only provision is that the thickness of the lattice is not sufficient to effectively inhibit the transfer of required gases, particularly N_2 and CO_2 from the atmosphere above. It is of interest that hydrogen evolution beneath a gas phase predominantly comprised of nitrogen is also possible for immobilized cultures (fig.3), this gas presumably being more readily available than argon.

Immobilized cyanobacteria may also prove useful in laboratory studies, although implementation in short-term metabolic experiments is limited by the slowness of phase transfer of gases to and from solution. The procedure may have potential for photosynthetic bacteria immobilized in glass columns, permitting continuous addition of dissolved substrates and removal of products.

Acknowledgement

This work was supported by a grant (D2-76/15666) from the Australian Research Grants Committee.

References

[1] Buvet, R., Allen, M.-J. and Massué, J.-P., eds (1977) in: Living Systems as Energy Converters, Elsevier/North-Holland Biomedical Press, Amsterdam, New York.

[2] Mitsui, A., Miyachi, S., San Pietro, A. and Tamura, S., eds (1977) in: Biological Solar Energy Conversion, Academic Press, London, New York.
[3] Schlegel, H. G. and Barnea, J., eds (1976) in: Microbial Energy Conversion, Pergamon Press, New York, Oxford.
[4] Benemann, J. R. and Weare, N. M. (1974) Science 184, 174–175.
[5] Oshchepkov, V. P., Nikitina, A. A., Gusev, M. V. and Krasnovskii, A. A. (1974) Dokl. Akad. Nauk SSSR 213, 557–560.
[6] Weissman, J. C. and Benemann, J. R. (1977) Appl. Environ. Microbiol. 33, 123–131.
[7] Jeffries, T. W., Timourian, H. and Ward, R. L. (1978) Appl. Environ. Microbiol. 35, 704–710.
[8] Daday, A., Platz, R. A. and Smith, G. D. (1977) Appl. Environ. Microbiol. 34, 478–483.
[9] Lambert, G. R. and Smith, G. D. (1977) FEBS Lett. 83, 159–162.
[10] Lappi, D. A., Stolzenbach, F. E., Kaplan, N. O. and Kamen, M. D. (1976) Biochem. Biophys. Res. Commun. 69, 878–884.
[11] Berenson, J. A. and Benemann, J. R. (1977) FEBS Lett. 76, 105–107.
[12] Karube, I., Matsunaga, T., Tsuru, S. and Suzuki, S. (1976) Biochim. Biophys. Acta 444, 338–343.
[13] Fogg, G. E., Stewart, W. D. P., Fay, P. and Walsby, A. E. (1973) in: The Blue–Green Algae, Academic Press, London, New York.