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A NEW TYPE OF IMMOBILIZED-CELL PHOTOBIOREACTOR WITH INTERNAL ILLUMINATION BY OPTICAL FIBRES

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SUMMARY

A prototype of an immobilized-cell photobioreactor based on a composite agar layer/microporous membrane structure is described. This photobioreactor has been tested for hydrogen-gas production using viable cells of *Rhodospirillum rubrum* and a phosphate buffer supplemented with malate and glutamate as nutrient medium. The major problem was the high diffusional resistance of the immobilized-cell layer at high cell population. The device has been patented and might be readily applied to other light-dependent bioreactions having more short-term economic interest than hydrogen photoproduction.

INTRODUCTION

Increasing attention has recently been paid to the biotechnological potentialities of solar energy bioconversion by free or immobilized photosynthetic biocatalysts. In addition to the mass cultivation of algal biomass, the production of high-value biomolecules by photosynthetic eukaryotic cells and the production of molecular hydrogen from industrial wastes by photosynthetic organisms have been more particularly investigated. However, relatively few studies have been devoted to photobioreactors themselves.

In a previous study (Mignot *et al.*, 1987), we presented composite biocatalytic structures composed of an immobilized-cell agar layer bounded by a microporous membrane filter which is placed at the interface between the gel and the broth containing the substrate(s) of microbial metabolism: the microporous membrane prevents the leakage of organisms into the nutrient medium during incubation of the gel structure. Furthermore, the metabolic activity of viable cells of *Escherichia coli* confined inside these structures remained stable during extended periods of time. These are interesting properties for operating the composite structures in bioreactors: we used them for entrapment of denitrifying bacteria to remove nitrates from water (Lemoine *et al.*, 1988), and, more recently, we have shown that this immobilization procedure can be applied to hydrogen photoproduction by photosynthetic

bacteria (Planchard *et al.*, 1989). In the latter study, the tests for hydrogen photoproduction were performed in a laboratory reactor using a planar composite structure illuminated by a standard tungsten incandescent bulb. In the present paper, we describe a photobioreactor of original design based on a tubular composite structure and we test this device for hydrogen photoproduction by entrapped cells of *R. rubrum*.

MATERIALS AND METHODS

Bacterial strain and culture conditions

A strain of *Rhodospirillum rubrum* (strain No. 7061 from the Pasteur Institute Collection, Paris, France) was used. Bacteria were incubated at 30°C, anaerobically (argon bubbling) and under light (illuminance: c. 20 klx), in 1-dm^3 flasks containing a mixture (1/1 vol./vol.) of Columbia broth (Diagnostic Pasteur, Marnes La Coquette, France) and Ormerod medium (Carr, 1969). After incubation of the flasks for 24 h, a bacterial suspension with high cell density (c. 2.6×10^{10} organisms cm^{-3}, i.e 52.5 mg dry weight cm^{-3}) was obtained by sedimentation.

Biophotoreactor design

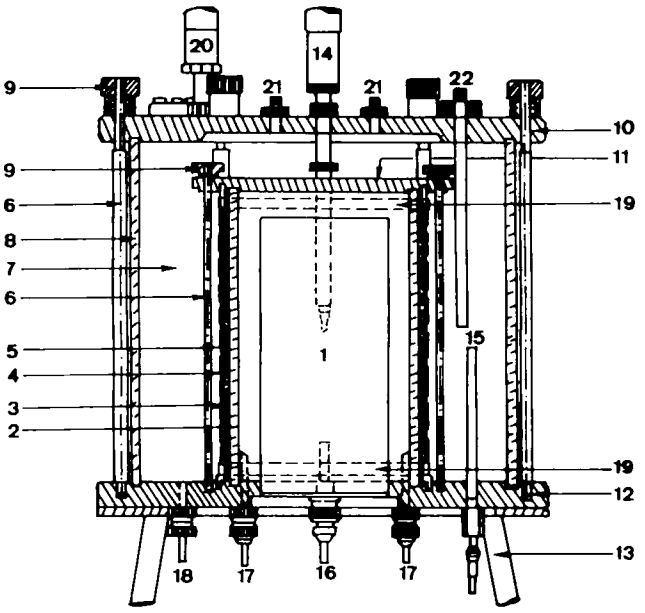

Figure 1. Detailed diagram of the immobilized-cell photobioreactor.
1, optical-fibre barrel (see Fig. 2); 2, inner glass cylinder; 3, immobilized-cell agar layer; 4, outer cylindrical stainless-steel grid; 5, microporous membrane; 6, assembling pin; 7, tank; 8, reactor wall; 9, nut; 10, reactor cap; 11, stainless-steel plate; 12, bottom plate; 13, bracket; 14, pH electrode; 15, temperature probe; 16, heating resistance; 17, nutrient medium inlet; 18, nutrient medium outlet; 19, rubber band; 20, safety-valve; 21, gas outlet; 22, argon inlet.

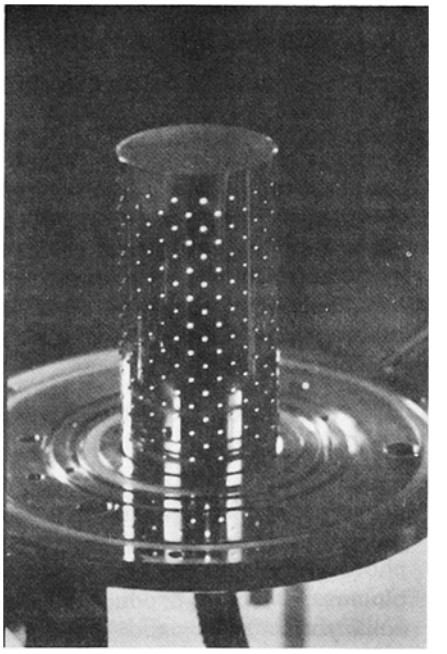

Figure 2. The optical-fibre illumination device
A bundle of optical fibres (360 here) is introduced into a hollow stainless-steel barrel, the outer wall of which is drilled. The holes are regularly distributed at the barrel surface. Each optical fibre ends at a hole. The optical-fibre barrel is shown fastened to the bottom plate of the reactor.

Fig. 1 shows a detailed diagram of the prototype of an immobilized-cell photobioreactor constructed with industrial support (Biosystem International, Petit-Quevilly, France). It consists of a cylindrical glass vessel closed by two stainless-steel circular plates. An internal cylindrical structure is fastened hermetically to the bottom plate above a cylindical opening made in the center of the plate. This structure comprises an inner light-transparent cylinder and an outer cylindrical stainless steel grid to which a microporous membrane filter is fastened. The space between the two envelopes contains the immobilized-cell gel. The composite biocatalytic structure is closed hermetically by a stainless-steel plate. A barrel of optical fibres (Fig. 2), inserted inside the inner glass cylinder, ensures the illumination of the gel layer.

Fabrication of the immobilized-cell layer

The glass cylinder and the cylindrical stainless steel grid, removed from the reactor vessel, were fastened hermetically to a metal support. The outer grid was covered with an aluminium sheet. The device was sterilized by autoclaving and kept under sterile atmosphere in a thermostatted microbiological safety cabinet.

A mass of 18 g of agar (Sigma) was dissolved in 700 cm^3 of distilled water by heating the suspension to 100°C. Then the solution was cooled to 40°C in the microbiological safety cabinet and mixed with 100 cm^3 of dense *R. rubrum* culture obtained as described above. A volume of 550 cm^3 of the biocatalytic mixture was poured into the cavity delimited by the inner glass cylinder and the cylindrical stainless-steel grid rendered impervious by the aluminium sheet. The cabinet temperature was lowered to 30°C. Gel hardening was achieved after about 15 min. The biocatalytic agar layer obtained had the following characteristics: agar content, 2.2 % wt./vol.; height, 190 mm; thickness, 6 mm; outer surface, 955 cm^2; cell content, 6.5×10^8 organisms cm^{-3} (i.e. 1.3 mg d.wt. cm^{-3}).

Pre-treatment of the agar layer

After removal of the aluminium sheet, the biocatalytic structure with its support was incubated for 24 h at 30°C, anaerobically and under light (20 klx), in a mixture of Columbia broth and Ormerod medium (1/1 vol./vol.) agitated by sterile argon bubbling. Then the structure was washed by being left for 24 h in sterile distilled water.

Arrangement of the composite gel/membrane structure

The biocatalytic structure was fastened to the bottom plate of the reactor vessel, the different elements of which had been previously autoclaved and stored in the microbiological safety cabinet. A microporous membrane (Millipore, type Durapore, porosity: 0.45 μm), shaped into a cylinder of suitable dimensions, was slipped down around the external grid of the structure; two strong rubber bands were placed at the foot and at the top of the structure to strengthen the adhesion of the membrane to the grid. Finally, the outer envelope and the stainless steel cover of the reactor were positioned.

Experimental setup for hydrogen photoproduction

The immobilized-cell photobioreactor was filled with 10 dm^3 of nutrient medium, consisting of a phosphate buffer (10.5 g K_2HPO_4, 3.5 g KH_2PO_4 per liter of distilled water) supplemented with different amounts of DL-malate and L-glutamate as carbon and nitrogen sources, respectively (pH adjusted to 7.0 with KOH). Then the nutrient medium was deoxygenated by strong bubbling of

sterile argon for 1 h and continuously circulated in closed circuit using a peristaltic pump (flow rate: 1 dm^3 min^{-1}). The broth was thermostatted at 30°C by a Jumbo Tran thermic regulation device. The immobilized-cell layer was illuminated by the barrel of optical fibres (illuminance: 10 klx) connected to a 150-W light generator (model Lux 150S, Fort, Dourdan, France). The flow of gas evolved was measured by a Brooks model 5850 TR mass flow controller connected to a Linear D X-time potentiometric recorder; its composition was determined by gas chromatography (Intersmat model IGC 120 ML chromatograph equipped with a Porapack Q column, 100-120 mesh; gas vector: nitrogen).

RESULTS

The kinetics of hydrogen production by the tubular immobilized-cell structure were quite similar to those recorded previously using a small laboratory reactor equipped with a planar biocatalytic layer (Planchard *et al.*, 1989): after a lag period with no H_2 production, a rapid rise in the H_2 evolved occurred, followed by an end period during which the H_2-producing activity progressively disappeared. The value of the main parameters describing schematically these kinetics, i.e. the total amount of H_2 produced and the maximum and average production rates, increased with both the substrate concentration and the light intensity. As reported previously (Planchard *et al*, 1989), no noticeable H_2 production could be detected when the amount of malate provided to the nutrient medium was lower than c. 60 mM, whatever the light intensity.

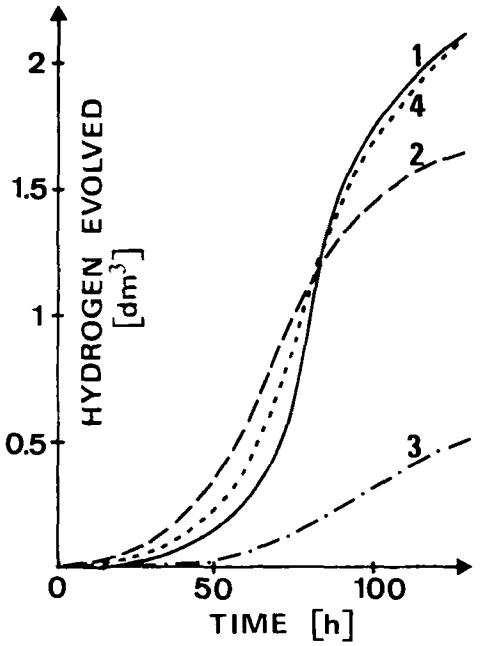

Figure 3. Successive kinetics of H_2 production by the same biocatalytic structure.

Between two assays, the nutrient medium (271 mM malate and 34 mM glutamate) was emptied, the reactor vessel was filled with sterile distilled water and washed for 6 h, circulation pump switched on. Then the rinsing water was replaced by fresh nutrient broth. A 48-h *in situ* incubation step of the biocatalytic structure in Ormerod medium was added before the fourth assay for H_2 production.

Fig. 3 shows three successive kinetics of H_2 production by the same biocatalytic structure incubated in the optimum conditions previously determined: light intensity of 10 klx, C/N ratio of 8. The overall H_2-evolving activity decreased as a function of the total duration of incubation, which shows that the nutrient broth was unable to support this activity over several days. Such decrease with time of the H_2-producing activity had already been observed using synthetic waste water instead of phosphate buffer as the salt medium (Planchard *et al.*, 1989): the H_2-evolving activity of the immobilized-cell agar layer could be regenerated by incubation in a rich

nutrient broth (under light and in anaerobic conditions). We investigated the possibility of regenerating the H_2-evolving activity *in situ*, i.e. without removing the composite structure from the reactor vessel: the activity level of a freshly-prepared structure could be recovered by incubation in Ormerod medium for 48 h (Fig. 3).

DISCUSSION AND CONCLUSION

The purpose of this paper is to present a new type of immobilized-cell photobioreactor based on a tubular gel layer/microporous membrane structure with an inner optical-fibre illumination device. The use of optical fibres to guide light from an external source (e.g. solar light) to the reactor medium is a suitable means for suppressing inhibiting thermic effects at the photosynthetic biocatalyst level. The arrangement of the tubular immobilized-cell layer around the cylindrical optical-fibre device ensures the biocatalytic layer both an homogeneous illumination and an active surface of suitable dimensions. While polymeric beads offer an exchange surface much larger than blocks or layers, their effectively active surface is noticeably decreased by light shading when they are packed into columns (von Felten *et al.*, 1985) or planar reactors (Hirayama *et al.*, 1986) illuminated by external light sources.

The versatility of the prototype adds to these significant advantages offered by its general arrangement and illumination system. For example, it is possible to adapt mechanical stirring or air lift; when the circulation pump is used, an additional cylindrical wall can be placed around the biocatalytic structure to ensure a laminar flow of medium at the level of the microporous membrane. In addition, the immobilization procedure can be applied to various photosynthetic materials, whole cells or organelles. When the immobilized biocatalysts do not proliferate or grow only slowly inside the gel layer, the tubular biocatalytic structure can be deprived of its outer microporous membrane.

The testing of this device for hydrogen photoproduction has given results which are quite consistent with those obtained previously using small laboratory reactors (Planchard *et al.*, 1989), already discussed in terms of H_2-production efficiency by referring to other published studies. As concerns the prototype itself, it was particularly interesting from a practical viewpoint to demonstrate the possibility of regenerating the activity of immobilized cells *in situ*, ensuring a long-term conservation of this activity without removing the biocatalytic structure from the reactor vessel. The problem of the diffusional resistance of the composite structure was confirmed. This drawback might be noticeably reduced by using microporous membranes with increased permeability to the substrate(s) and product(s), which would still prevent the crossing of bacteria, e.g. inorganic membranes with honeycomb structure, less tortuous than polymeric membranes. We have shown, however (Mignot *et al.*, in preparation), that the diffusional resistance of the immobilized-cell agar layer alone is far from being negligible, more especially when the cell content of the gel is high; moreover, it depends on the cellular shape of immobilized organisms.

To conclude, the prototype and its illumination device have been patented recently (Junter *et al.*, 1989). Since hydrogen photoproduction remains a long-term challenge and is no longer an urgent problem, the industrial future of this photobioreactor will very probably involve other photoreactions of greater economic interest, e.g. the production of high-value biomolecules by immobilized micro-algae (Gudin and Thépenier, 1986) and plant cells

(Brodelius, 1988). In the near future, we will focus on these promising extensions - which justify the modelling and optimization of the reactor.

ACKNOWLEDGEMENTS

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