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PRODUCTION OF HYDROGEN USING IMMOBILIZED *Rhodospirillium rubrum*[1]

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A standard method for assaying *Rhodospirillium rubrum* for hydrogen production with malate rather than pyruvate was developed. It was also found that *R. rubrum* could produce H_2 over extended periods of time when immobilized.

INTRODUCTION

Since the advent of the "oil crisis," there has been an increasing interest in energy production by methods other than petroleum. One of the potential methods could be the use of biological systems capable of producing energy in the form of hydrogen gas.

This report describes our initial attempts to stabilize a microorganism capable of oxidizing an organic compound in the presence of light, producing molecular hydrogen (H_2).

The bacterium chosen for this study was *Rhodospirillium rubrum*, which is a nonsulfur, purple bacterium. It is known that maximal yields of H_2 are observed with resting cells in quantities closely approximating those predicted on the basis of complete conversion of the organic compound to $H_2 + CO_2$ (1). With malate, the reaction is as follows:

$$C_4H_6O_5 + 3\,H_2O \rightarrow 4\,CO_2 + 6\,H_2$$

Overall, the effect on the photometabolism of the organic substrate by H_2-producing *R. rubrum* is to divert carbon from the dissimilatory anaerobic citric acid cycle to assimilatory pathways.

Our major goal in this study was to determine whether it was possible to immobilize *R. rubrum* and use malate as substrate to produce H_2 in a continuous process.

[1]This report is dedicated to Professor G. Manecke, one of the pioneers in the area of solid-phase biochemistry, in honor of his 60th birthday.

MATERIALS AND METHODS

Rhodospirillium rubrum grown in chemically defined medium (2) was stored in the spent media at 4°C until use. All batch experiments were carried out in a glove box filled with argon. After preparation, the flasks were stoppered and incubated 60–95 min on a mechanical shaker with constant illumination. Illumination was provided by two rows of 100-W standard incandescent lightbulbs. In the case of the shaker flasks, the lights were placed under a large Lucite block into which the small flasks were placed. Aliquots of gas (usually 0.2–1.0 ml) were removed from the flask and assayed for H_2, N_2, and O_2 production on a Tracor 550 Gas Chromatograph using an 8 ft × 1/8 in. column of molecular sieve 5A.

Determination of K_m of R. rubrum for Malate. A suspension of *R. rubrum* in 0.05 M Tris, 0.001 M MgCl at pH 7.6 containing 600 mg wet w/ml was prepared. Aliquots of 1 ml of the *R. rubrum* suspension were added to 1.0 ml 0.1–0.001 M malate solution, and incubated in 5-ml flasks with constant illumination.

Determination of Acitivity of R. rubrum. Several samples of *R. rubrum* were assayed in 0.01 M malate at cell concentrations ranging from 20 to

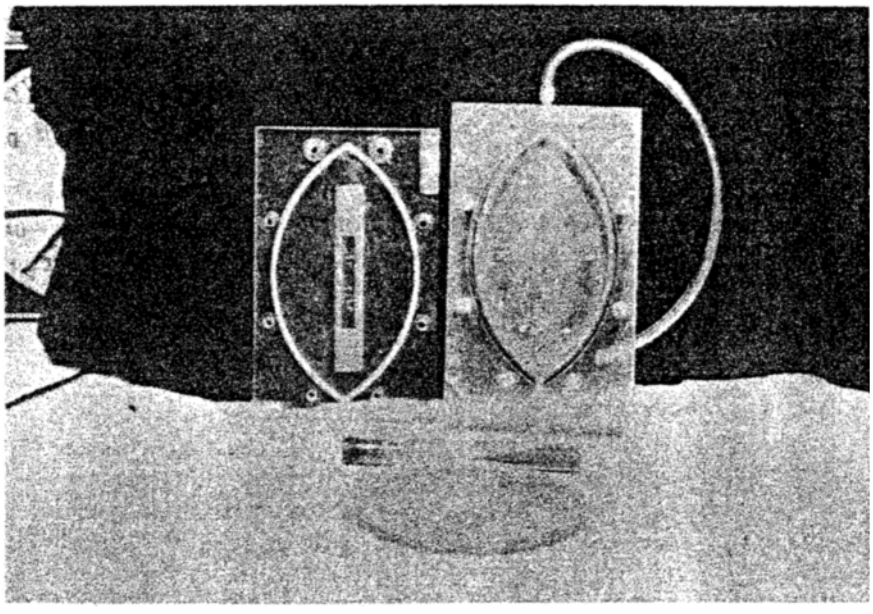

FIG. 1. Photograph of the reactor used for the continuous operation of the *R. rubrum*. The two halves fit together, sealing the solid block with the bacteria within the reactor.

300 mg wet w/ml as previously described. The H_2 production vs. time was determined for each cell concentration.

Immobilization of R. rubrum. R. rubrum, 4 g wet w, was added to 15.0 ml 5% solution of Noble Agar. This solution was spread evenly on both sides of a plastic slab composed of a series of thin plastic layers (Fig. 1). The slab was put into an airtight system and 0.01 M malate, continually purged with argon, was passed through the system in an upward flow at 8.0 ml/h. The system was immersed in a water bath maintained at 18–19°C. The gas produced was channeled to a double-ended glass tube that was kept airtight by septums. The malate solution was pulled through the collector with a syringe, displacing any gas with substrate. Collection of the produced gas displaced the liquid. A sample was taken after the gas and the malate equilibrated, and then a constant flow rate was reestablished. Illumination was provided by a rack of 7 100-W standard incandescent bulbs on each side of the reactor.

Assay of Immobilized R. rubrum. A volume of 0.75 ml of gas was withdrawn from the collector. Of this amount, 0.5 ml was injected into the Tracor 550 Gas Chromatograph. The quantity of H_2 produced (μl) was determined from a standard curve prepared with pure H_2. The percentage of H_2 in the 0.5-ml sample was then determined. The final quantity of H_2 was expressed in μl H_2 produced/min at a constant flow rate of 8 ml/h.

RESULTS

Kinetics of Hydrogen Production from Malate

It was found that *R. rubrum* would produce reasonable quantities of H_2 at concentrations above 120 mg wet w/ml. Additional experiments indicated that the most efficient combination of variables and a standard method for assaying was as follows: 1 ml *R. rubrum* (300–500 mg wet w/ml), 1.0 ml 0.01 M malate, and constant illumination for 90 min, which yielded 3.5–5.7 μl H_2 in a 0.5-ml sample taken from a 5-ml flask. These conditions were determined from the K_m studies (Fig. 2).

Immobilized R. rubrum

The results from a typical reactor are given in Fig. 3. The reactor operated for 150 h, producing a maximum of 22.01 μl H_2/min. The total H_2 produced during the first $t_{1/2}$ of operation was 1.09×10^5 μl. One reactor reached a high of 45 μl H_2/min. This reactor contained a higher quantity of bacteria and gave an initial increase in activity before exponential decay was observed.

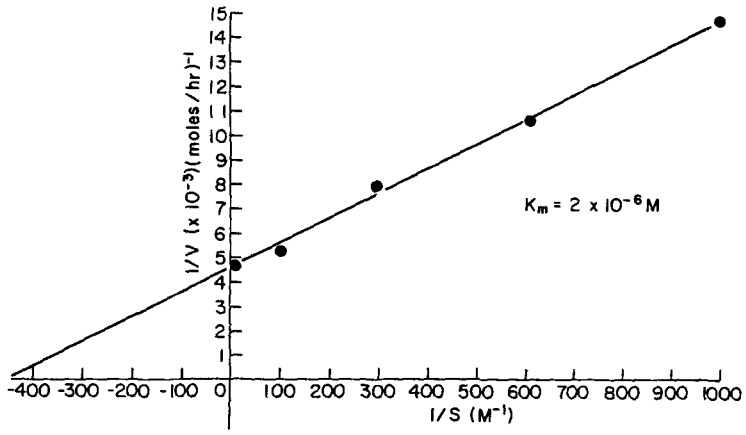

FIG. 2. K_m results using malate as substrate with *R. rubrum* for the production of H_2 gas.

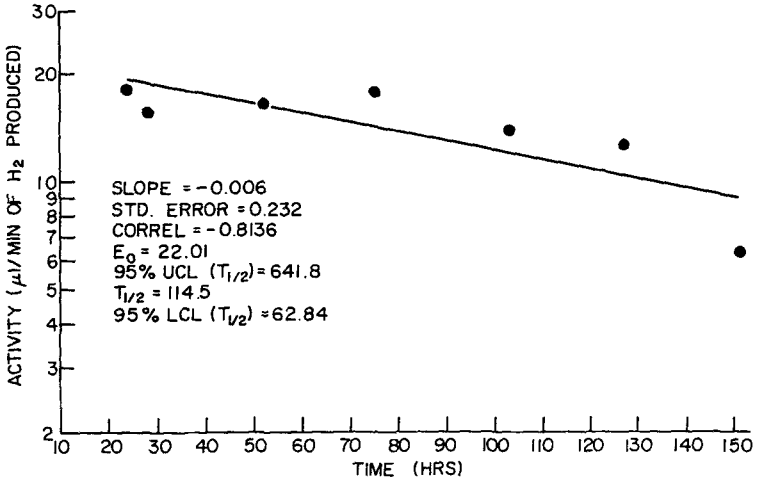

FIG. 3. Activity vs. operation time for immobilized *R. rubrum*. The substrate was 0.01 M malate in 0.05 M Tris, 0.001 M $MgCl_2$, pH 7.6. UCL and LCL represent 95% confidence limits. The correlation gives the relationship of the experimental data to the log-linear curve.

DISCUSSION

It was found that active preparations were obtained only if handled under anaerobic conditions. Some difficulty was encountered in preparing identical preparations for the reactor particularly in regard to the thickness of the agar layer. This difficulty resulted in variations in reaction rates due to diffusion, and in apparent half-lives. In a few cases, half-lives as low as 35 h were observed. However, in these cases, we believe that O_2 contamination played a major role in the observed results.

It is obvious, however, that whole cells of *R. rubrum* can be immobilized in an active form, and that they will produce H_2 on a continuous basis.

As an academic exercise, some simplified calculations as to the cost of producing H_2 using *R. rubrum* based on the initial substrate being glucose, rather than malate, were made. The calculations include only glucose cost. The data conclude that as a potential producer of H_2, *R. rubrum* cannot be used economically on a substrate having a cost of even 10¢/lb at this time.

Cost of hydrogen from glucose

$C_6H_{12}O_6$ mol.wt. 180.2: 6 mol H_2/mol glucose

$$\frac{(6 \text{ lb-mol } H_2/\text{lb-mol glucose}) \quad (359 \text{ ft}^3 H_2/\text{lb-mol } H_2)}{(180.2 \text{ lb/mol glucose})}$$

$$= 12.0 \text{ ft}^3 H_2/\text{lb glucose}$$

@10¢/lb glucose

$$\frac{1000 \text{ ft}^3 \times 10¢/\text{lb glucose}}{12 \text{ ft}^3/\text{lb glucose}} = \$8.33/1000 \text{ ft}^3$$

The heating capacity of H_2 is about $\frac{1}{3}$ that of natural gas vol/vol. Thus the price is equivalent to $\$25/1000 \text{ ft}^3$ natural gas based on raw materials only. A cubic foot of H_2 contains approximately 1000 BTUH; the cost of H_2 is therefore \$25/1 MM BTUH. Present fuel for heating runs between \$1.50 and \$2.50/1 MM BTUH. The cost of H_2 is thus at least 1 order of magnitude high. It is possible that in some specific situations, such costs could be acceptable.

It is always possible that less expensive sources of substrate may become available. Glucose from cellulose may be a potential fuel. Another potential substrate could come via a biophotolytic oxidation of water.

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