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Application of Immobilized Hydrogenase to H_2 Storage in Concentrated Solutions of Methyl Viologen

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Abstract

It has been found that immobilized cells of *C. pasteurianum* possessing hydrogenase activity efficiently catalyze reversible reduction of concentrated (up to 0.5*M*) solutions of methyl viologen with H_2. A 0.5*M* aqueous solution of methyl viologen dissolves 240 times as much H_2 as pure water under the same pressure of hydrogen. The experimentally obtained levels of methyl viologen reduction and H_2 evolution are in satisfactory agreement with theoretical calculations. The potential of concentrated solutions of methyl viologen containing immobilized hydrogenase as a H_2 storage medium is discussed.

Index Entries: Hydrogenase, catalysis of H_2 storage; immobilized whole cells, in H_2 storage; hydrogen storage, hydrogenase catalysis of; enzymatic energy storage; solar energy, and H_2 storage; methyl viologen, and H_2 storage.

Introduction

Hydrogen has the potential of being a versatile and efficient energy carrier and therefore is considered by many as the fuel of the future (*1*). Numerous methods of hydrogen production from water, in particular using solar energy, have been developed (*2*). It appears today that the success of the "hydrogen economy" will greatly depend on the availability of technically and economically feasible systems for storing (and transporting) hydrogen (*3*). A number of approaches to H_2 storage have been suggested, e.g., storage of gaseous, liquid, or solid hydrogen and stor-

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age of H_2 in chemicals (*2, 4*). Unfortunately, all of these possible solutions suffer from serious drawbacks in terms of cost, efficiency, and safety. Thus alternative methods are highly desirable.

We have been interested in the use of enzymes for hydrogen storage. For example, we have recently proposed a coimmobilized bienzymic system employing hydrogenase and formate dehydrogenase for H_2 storage in the form of formate (*5*). The aforementioned bienzymic system reversibly converts H_2 and CO_2 into formate. Aqueous solutions of the latter are stable and can be easily stored and transported. When it is needed, the same immobilized bienzymic system can be used to produce hydrogen back from formate. The major bottleneck of this system seems to be a strong inhibition of the enzymatic activity by high concentrations of bicarbonate (CO_2) and formate that limits the charge–discharge rate of the system.

An optimal carrier for hydrogen storage should be cheap, readily available and safe. Water immediately comes to mind as an ideal candidate. Unfortunately, the solubility of H_2 in water is prohibitively low—only about 0.8 m*M* (at $p_{H_2} = 1$ atm and 20°C). However, it is known that some compounds can stoichiometrically and reversibly bind H_2 (in the presence of a suitable catalyst). If such a compound is reasonably soluble in water, its concentrated aqueous solutions would be an efficient hydrogen storage medium. This represents the direction of our approach.

In the present work we have employed methyl viologen (1,1′-dimethyl-4,4′-bipyridinium) to enhance the effective solubility of H_2 in water. Methyl viologen (also known as paraquat) is widely used as a herbicide (*6*) and hence is relatively inexpensive and available in industrial quantities. Solubility of methyl viologen in water at 25°C can exceed 1 *M* (depending on the nature of the counterion). In the presence of either the enzyme hydrogenase or noble metal catalysts, methyl viologen can be reversibly reduced with molecular hydrogen. The use of the enzyme seems to be advantageous here, since noble metals (e.g., platinum), in addition to the reversible reduction, can also catalyze subsequent irreversible side-reactions (*7*).

We report herein our data on application of immobilized bacterial cells of *Clostridium pasteurianum* containing hydrogenase activity for H_2 storage in concentrated aqueous solutions of methyl viologen.

Materials and Methods

Cells of *Clostridium pasteurianum* W5 were grown as described by Nakos and Mortenson (*8*). Methyl viologen (diCl) and kappa-carrageenan were obtained from Sigma; sodium dithionite was purchased from the Fisher Scientific Co. All other chemicals used were of analytical grade.

Immobilization of whole *C. pasteurianum* cells in 3.3% kappa-carrageenan gel followed the method previously described by us for *A. eutrophus* (*9, 10*) with only the exception that all operations were conducted under argon.

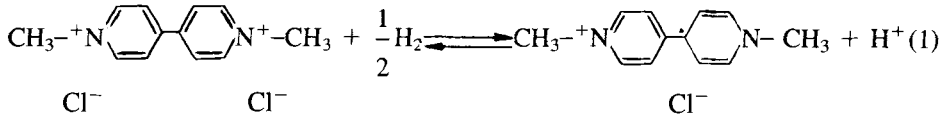
The time course of immobilized hydrogenase-catalyzed reduction of methyl viologen with H_2 was followed spectrophotometrically. Forty-five mL of a methyl

viologen solution in 1*M* Tris·HCl buffer were placed in an Erlenmeyer flask. Then 5 g of immobilized *C. pasteurianum* were added and the flask was sealed with a serum stopper. After that, it was evacuated, filled with H_2, and shaken at room temperature. At certain time intervals, aliquots of the solution from the flask were withdrawn by a syringe and diluted 100-fold with deoxygenated Tris·HCl (pH 8.0) buffer, followed by the measurement of the optical density of the latter at 780 nm ($\epsilon_{780} = 1.12 \times 10^3\ M^{-1}cm^{-1}$ as experimentally determined by us).

The time course of immobilized hydrogenase-catalyzed H_2 evolution from reduced methyl viologen was followed manometrically. Forty-five mL of a reduced methyl viologen solution in 1*M* Tris·HCl buffer (obtained either as described above or by reduction with sodium dithionite) containing 5 g of immobilized *C. pasteurianum* were placed in an Erlenmeyer flask under argon, and the flask was sealed with a serum stopper. Then it was put on an evacuated manifold connected to a mercury manometer. Since the volume of the system is known by measuring the pressure change in the system (arising from the enzymatic H_2 evolution from reduced methyl viologen), one can calculate the amount of H_2 produced.

Results and Discussion

The objective of our work was to assess the feasibility of the processes shown in Eq. (1), catalyzed by immobilized hydrogenase, for reversible H_2 storage.



The ability of the enzyme hydrogenase to catalyze both the forward and reverse reactions in Eq. (1) is well established (*11, 12*), but these reactions have been always carried out at millimolar or micromolar concentrations of methyl viologen. However, from the standpoint of H_2 storage, it is imperative that concentrated solutions of methyl viologen be used. Below we both analyze the equilibrium of Eq. (1) theoretically and present our experimental results on enzymatic H_2 storage in 0.1—1.0 *M* solutions of methyl viologen.

Theoretical Considerations

At pH 7.0 and 30°C the redox potential for methyl viologen is −0.445 V (*13*). Therefore the equilibrium constant for Eq. (1) equals:

$$K = \frac{[H_2]^{1/2}[MV^{2+}]}{[H^+][MV^{+\cdot}]} = 3.6 \times 10^7 \qquad (2)$$

TABLE 1
Fractions of the Reduced Form of Methyl Viologen ($MV^{+\cdot}$) at Equilibrium [Eq. (1)] at Different pHs Calculated on the Basis of Eq. (2)[a]

pH	Fraction of $MV^{+\cdot}$, %
6.0	3
7.0	22
7.5	47
8.0	74

[a] $[H_2]$ = 1 atm.

where MV^{2+} and $MV^{+\cdot}$ are the oxidized and reduced forms of methyl viologen, respectively. Using Eq. (2), one can calculate the fractions of reduced and oxidized methyl viologen at $[H_2]$ = 1 atm and different pHs. The corresponding data are shown in Table 1. As one can see, at pH < 7 most methyl viologen under equilibrium with H_2 (1 atm) will exist in the oxidized form, while at pH > 8 the reduced form will be predominant.

If an aqueous solution of the reduced methyl viologen containing hydrogenase is placed under vacuum, the equilibrium in Eq. (1) will be shifted to the left and the system will evolve hydrogen. If the H_2 produced is constantly removed (e.g., evacuated), i.e., $[H_2]$ = 0 is maintained, all the hydrogen accumulated in the solution will be eventually released.

Hydrogen Uptake

We have studied the effect of methyl viologen concentration on reduction of the dye with H_2 catalyzed by immobilized *C. pasteurianum* cells. Importantly, no appreciable substrate inhibition was observed up to 0.5*M* methyl viologen. Typical results are presented in Fig. 1.

The final concentrations of reduced methyl viologen at pH 7.0 and 8.0 are in a reasonable agreement with the calculations in Table 1 (taking into account the difference between our conditions and those in ref. *13*). Hence, even in concentrated solutions of methyl viologen, the thermodynamic equilibrium can be nearly reached in the presence of the enzyme.

At pH 8.0 and $[H_2]$ = 1 atm, about 75% of methyl viologen can be reduced. If the initial concentration of the dye is 0.5*M*, these 75% correspond to about 0.38*M*. In accordance with Eq. (1), each molecule of methyl viologen binds 0.5 molecules of H_2. Therefore, a 0.5*M* methyl viologen solution can in fact "dissolve" 0.19*M* of H_2 (pH 8.0, $[H_2]$ = 1 atm). That is a more than *200-fold* increase in the concentration of dissolved H_2 in comparison to pure water.

Hydrogen Evolution

The second part of the H_2 storage cycle is the enzymatic evolution of the gas from reduced methyl viologen. Figure 2 shows the time course of immobilized *C. pasteurianum* cells-catalyzed H_2 production from 0.1*M* reduced methyl viologen

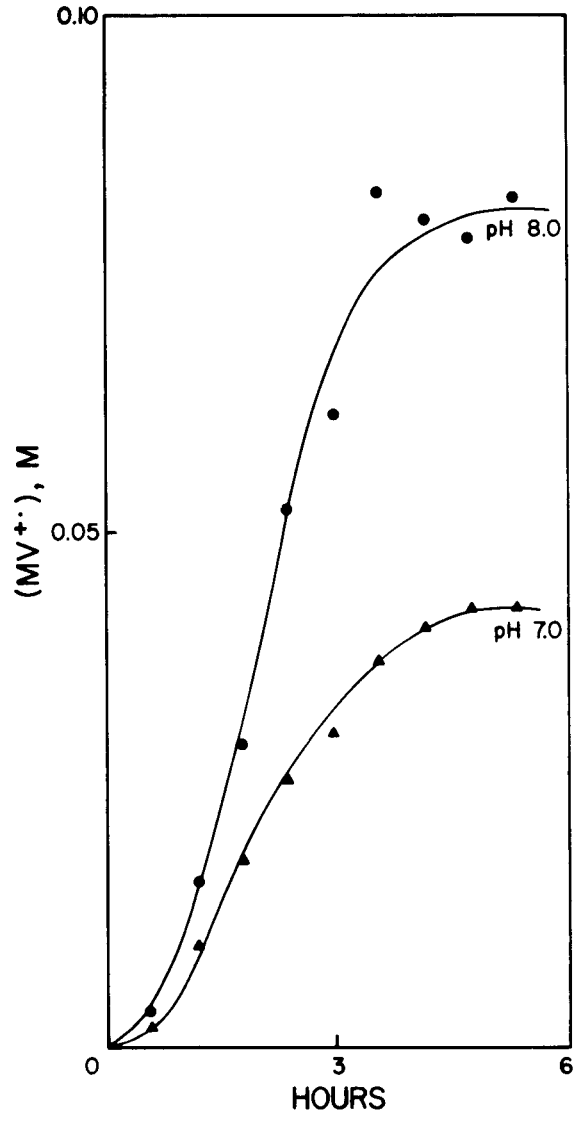


Fig. 1. Reduction of methyl viologen with H_2, catalyzed by immobilized cells of *C. pasteurianum*. Conditions: 0.1*M* methyl viologen in 1*M* Tris·HCl buffer, $[H_2]$ = 1 atm; for other conditions, see Materials and Methods.

in a closed system at different pHs. Since the system is closed (i.e., H_2 produced is not removed following initial evacuation), when the equilibrium concentration of hydrogen is produced, no more H_2 is evolved. The experimentally obtained saturation levels are in fair agreement with theoretical calculations (see Table 1). Qualitatively, the amount of H_2 evolved increases upon a decrease in pH (increase in $[H^+]$), as one would expect from Eq. (1).

As was pointed out above, if the hydrogen produced is constantly removed from the system, the entire amount of it can be released; that is, the H_2 storage system will be completely discharged.

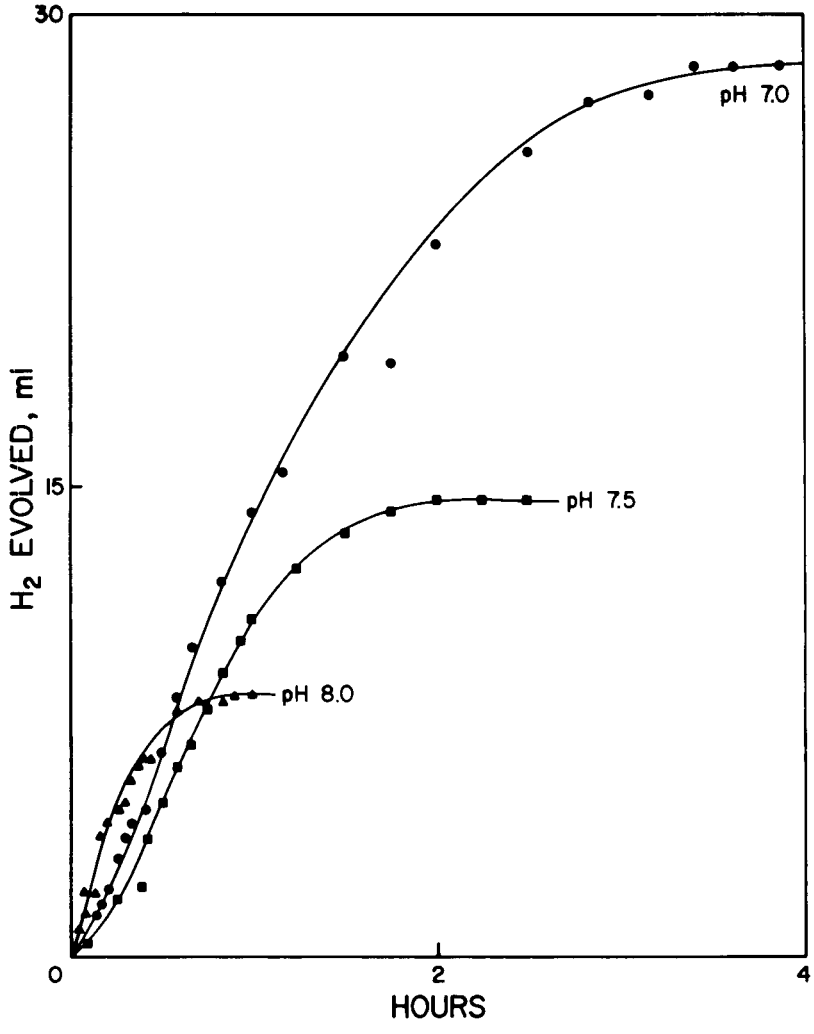


Fig. 2. H_2 evolution from reduced methyl viologen catalyzed by immobilized cells of *C. pasteurianum*. Conditions: 0.1*M* reduced methyl viologen in 1*M* Tris · HCl buffer, 45 mL solution in a closed system; for other conditions, see Materials and Methods.

Concluding Remarks

We have shown in this study that concentrated solutions of methyl viologen can be used for hydrogen storage. Both the charge (methyl viologen reduction) and discharge (H_2 evolution from reduced methyl viologen) of the system can be efficiently catalyzed by immobilized *C. pasteurianum* cells. Hydrogenase activity of the cells is quite stable in the absence of O_2 (e.g., under H_2), in particular in the presence of methyl viologen, which stabilizes the enzyme (*14*).

The H_2 storage process can be envisioned as follows. The hydrogen to be stored will be bubbled through a solution of methyl viologen containing immobilized hydrogenase. When a near-to-equilibrium amount of H_2 is taken up, the system

will be closed and stored as long as needed under 1 atm pressure of hydrogen. If immobilized hydrogenase is removed (e.g., filtered out), the reduced methyl viologen solution can be readily pumped through pipes. One of the shortcomings of the system is its O_2 sensitivity: oxygen oxidizes reduced methyl viologen. Fortunately, we have found a way to overcome this obstacle. A solution of reduced methyl viologen kept under hydrogen was frozen, and then exposed to air at −20°C for several days. It was then thawed and used to enzymatically evolve H_2 under vacuum. It turned out that the contact of the frozen solution with air had no appreciable detrimental effect on hydrogen production (probably because there is only a very slow diffusion of atmospheric O_2 into ice).

Although the hydrogen storage capacity of 0.5*M* methyl viologen is much lower than that of iron–titanium hydride, today's method of choice for H_2 storage [0.04 vs 1.75% (*4*)], the former has some advantageous features over the latter, e.g., it can be piped, it is not flammable, no considerable volume change occurs during the charge–discharge cycle, and so on. It is quite possible that hydrogen carriers other than methyl viologen can be developed that will be significantly more soluble in water. In this case the H_2 storage system, the prototype of which is described in this paper, will find practical applications.

Acknowledgment

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