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PHOTOBIOCATALYSIS: HYDROGEN EVOLUTION USING A SEMICONDUCTOR COUPLED WITH PHOTOSYNTHETIC BACTERIA

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Abstract—Photobiocatalytic production of hydrogen in the presence of Bi_2O_3 semiconductor, methyl viologen (MV^{2+}) as an electron mediator and three different bacteria (*Rhodopseudomonas capsulata*, *Rhodospirillum rubrum* and *Escherichia coli*) as hydrogen evolution enzyme catalysts has been carried out in different environments. Addition of intact bacterial cells is found to increase the hydrogen production efficiency. It has been suggested that the nitrogenase enzymes of the bacterial cells catalyze the hydrogen evolution process rather than the hydrogenase enzymes of the bacteria. The presence of carbohydrates (fructose, dextrose and starch) and organic acids (oxalic acid, EDTA and ascorbic acid) as electron donors with the above system further enhances the hydrogen production efficiency due to the reaction of photogenerated valence band holes of the semiconductor with these substrates, thereby preventing the $e^- - h^+$ recombination. The effects of loading the semiconductor with RuO_2 and Rh_2O_3 and addition of divalent metal ions, Ca^{2+}, Mg^{2+} and Mn^{2+} to the system towards hydrogen production efficiency were also studied and discussed.

INTRODUCTION

Photobiocatalytic hydrogen production is a "biophotolysis" by which hydrogen gas from water or aqueous solution is generated by coupling photocatalytic and photobiological methods [1]. Coupling of semiconductors with bacterial hydrogenase and production of hydrogen were successfully achieved [1]. In our laboratory, we have already carried out photocatalytic hydrogen production using semiconductor particulate systems [2]. Methyl viologen was used as an electron relay and transition metals such as Pt and Rh were used as hydrogen evolution catalysts. Nikandrov *et al.* [1] coupled isolated bacterial hydrogenase enzyme as hydrogen evolution catalyst with inorganic semiconductor and investigated the photoinduced electron-transfer from the semiconductor to the enzyme through methyl viologen as an electron mediator. They have also studied a system [3] in which the particles of inorganic semiconductor catalysts were coupled to hydrogenase-containing *Clostridium butyricum* bacterial cells, and the proposed mechanism involves the hydrogenase enzyme of the bacteria in the hydrogen evolution process.

In this article, we report the possibility of using the intact cells of three different bacteria, *Rhodopseudomonas capsulata*, *Rhodospirillum rubrum* and *Escherichia coli*, for the photoproduction of hydrogen in the presence of Bi_2O_3 as an inorganic semiconductor, MV^{2+} as an electron mediator and some organic substrates as electron donors.

EXPERIMENTAL

All the chemicals used were of the purest research grades available. The bacterial cells were gift samples (see Acknowledgements) which were grown under standard photosynthetic conditions as reported [4]. Naked and transition metal oxide loaded samples of Bi_2O_3 semiconductor photocatalysts were prepared as per our earlier report [5]. In all the experiments, 80 mg of the catalyst powders were dispersed in 80 ml of the solution containing 1.0×10^{-4} mol dm^{-3} MV^{2+}, 1 ml of intact bacterial cells and 0.04 mol dm^{-3} of the electron donor. The contents were kept in a pyrex glass photoreactor, thermostatted at 25°C and irradiated with a 250 W tungsten–halogen lamp. The emitted light before entering the reaction vessel was passed through u.v. and i.r. filters. The reaction medium and the apparatus were flushed with argon for 30 min before irradiation. The evolved gaseous products were passed through 50% KOH to remove CO_2 (if formed due to the presence of electron donors) and alkaline pyrogallol solution at 0–5°C to remove oxygen (if formed due to water oxidation by valence band holes) and the rest of the gas was collected in a water manometer and was detected as hydrogen by gas chromatography (CIC, India) using molecular sieves (5 Å column) and argon as the carrier gas.

RESULTS AND DISCUSSION

Bi_2O_3 ($E_g = 2.8$ eV) has been used in our experiments since it absorbs the visible radiation of the solar spectrum notably. It is evident from an early report [4] that $MV^{\cdot+}$, formed due to the reduction of MV^{2+} by the conduction band electrons of the semiconductor, is capable of penetrating the bacterial cell membrane. Figure 1 shows hydrogen production with different systems. It is seen that the addition of bacterial cells to the system containing inorganic photocatalyst and MV^{2+} as an electron mediator

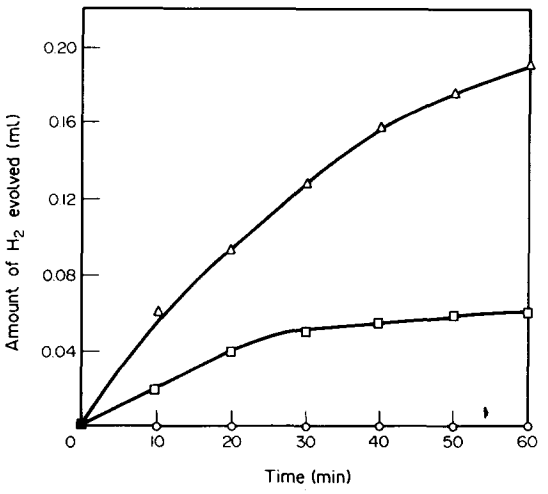


Fig. 1. Photobiocatalytic hydrogen production by using the Bi_2O_3 semiconductor (80 mg), *R. capsulata* (1 ml) and methyl viologen (1×10^{-4} mol dm^{-3}): O → Bi_2O_3/H_2O; □ → $Bi_2O_3/MV^{2+}/H_2O$; Δ → Bi_2O_3/*R. capsulata*.

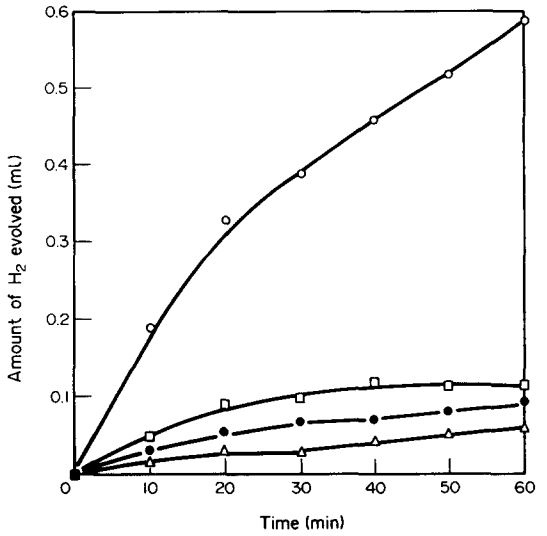
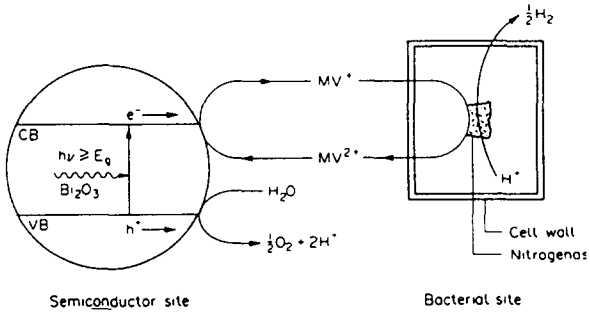


Fig. 2. Hydrogen evolution by using Bi_2O_3 semiconductor (80 mg) and different bacterial cells (1 ml); $[MV^{2+}] = 1 \times 10^{-4}$ mol dm^{-3}: O → Bi_2O_3/MV^{2+}/starch/*R. capsulata*; □ → Bi_2O_3/MV^{2+}/*R. rubrum*; ● → Bi_2O_3/MV^{2+}/starch; Δ → Bi_2O_3/MV^{2+}/starch/*E. coli*.

notably enhances the hydrogen production efficiency. The reason for this is that the $MV^{\cdot+}$, after penetrating the bacterial cell wall, releases an electron at the enzymatic site and comes out of the cell. The specific reduction of H^+ to molecular hydrogen is catalyzed at this enzymatic center. Nikandrov *et al.* [1] have suggested that hydrogenase is an enzyme catalyst for hydrogen evolution. But on referring to the reports [6–9] on photobiological production of hydrogen, it has been suggested that nitrogenase activity is responsible for the evolution of hydrogen. In general, two classes of enzymes, hydrogenase (iron sulfur proteins) and nitrogenase (protein complexes containing iron, sulfur and molybdenum) are closely associated with the hydrogen evolving actively in photosynthetic bacteria. Even though the primary function of each enzyme is quite different (nitrogenase functions in biological nitrogen fixation and hydrogenase catalyzes hydrogen uptake or consuming reactions), under certain conditions both can catalyze H_2 production. However, evolution of hydrogen in photosynthetic bacteria is now known to be a function of nitrogenase rather than hydrogenase [6]. In the absence of nitrogen or NH_4^+, nitrogenase enhances the reduction of H^+ to H_2, whereas the main function of hydrogenase is to catalyze hydrogen uptake reactions. It is also proved that in the case of *R. capsulata*, mutants defective in nitrogenase activity do not evolve hydrogen [6, 10]. To understand the actual mechanism, we have chosen three different bacteria, namely, *R. capsulata*, *R. rubrum* and *E. coli*. Among these bacteria, the first two possess both the hydrogenase and nitrogenase activities, whereas the last one has only hydrogenase activity. From Fig. 2, it is understood that the activity of *E. coli* is almost nil towards the hydrogen evolution process, supporting the idea that the assistance of nitrogenase activity is necessary for photoproduction of hydrogen as in the case of mutant *R. capsulata* reported earlier [6, 10]. Hence it may be confirmed that nitrogenase activity catalyzes the photoproduction of hydrogen. In the absence of any other electron donor, water can behave as a reducing agent provided the photogenerated holes of the semiconductor are capable of oxidizing water to molecular oxygen. Scheme 1 is given for the



Scheme 1. Photobiocatalytic hydrogen production involving Bi_2O_3 semiconductor coupled with bacterial nitrogenase.

biophotolysis of water in the presence of a semiconductor, electron mediator and bacterial cells. Due to absorption of light energy, $E \geq E_g$ (band-gap energy), electrons are promoted from the valence band to the conduction band of the semiconductor (e_{cb}^-) leaving positively charged holes in the valence band (h_{vb}^+). The e_{cb}^- reduces MV^{2+} to $MV^{\cdot+}$ which penetrates the cell wall and reaches the nitrogenase–hydrogenase enzymatic site. The enzyme catalyzes the hydrogen production,

$$MV^{\cdot+} + H^+ \xrightarrow{\text{enzyme}} MV^{2+} + \tfrac{1}{2}H_2.$$

The MV^{2+} formed enters into the recycling process. In the presence of any electron donor, the h_{vb}^+ oxidizes the electron donor depending upon the nature of the donor molecules. The addition of an electron donor (reducing

agent) to the above system is found to enhance the hydrogen production efficiency further. This is mainly due to the assistance of the electron donors, minimizing the recombination of the photogenerated electron and hole by reacting with h^+_{vb}, thereby facilitating the availability of e^-_{cb} for the reduction of MV^{2+}. Such an effect by oxalic acid on WO_3 semiconductor photocatalysis has already been reported [11]. In the present investigation, different carbohydrates and organic acids as electron donors have been used to find the effect of their presence on hydrogen production and the results are given in Table 1. It is found that carbohydrates possess better efficiency than acids, which is in good agreement with the fact that carbohydrates are better reducing agents than acids.

Previous reports [12, 13] on semiconductor photocatalysis proved that loading of transition metal oxides on the semiconductor particles enhances the photocatalytic efficiency of the semiconductor in two ways:

(i) it sensitizes the semiconductor, thereby increasing the absorption of the semiconductor in the visible region of the solar spectrum [12];

(ii) it can assist in electron−hole separation or prevent recombination [13].

We have reported that RuO_2 enhances the $e^- - h^+$ separation up to a certain concentration limit, and beyond that it behaves as a $e^- - h^+$ recombination center [13]. From Fig. 3, it is clear that RuO_2 enhances the hydrogen production efficiency of the system up to a certain limit beyond which the efficiency decreases, which is similar to the previous observation [13]; Sakata and his co-workers [14] have also reported similar behavior with RuO_2/TiO_2. Loading of Bi_2O_3 with Rh_2O_3 is also found to enhance the activity, and the efficiency is found to be better than that loaded with RuO_2 (Fig. 4). This observation is also in accordance with the earlier studies that among different transition metals, Rh was the best as an electron transferring agent from the conduction band of the semiconductor to the electrolyte [15].

Addition of divalent metal ions to the system, semiconductor/MV^{2+}/donor/bacteria, is found to increase the hydrogen production efficiency of the system (Table 2). It

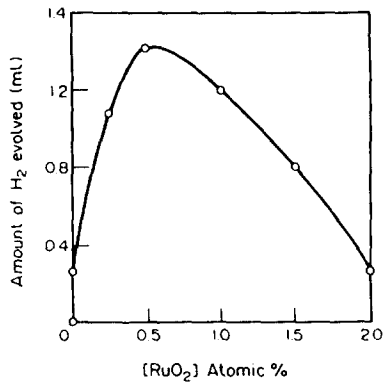


Fig. 3. Photobiocatalytic hydrogen production using RuO_2-loaded Bi_2O_3 (80 mg) and *R. capsulata* (1 ml) in the presence of MV^{2+} (1×10^{-4} mol dm^{-3}); [RuO_2] variation.

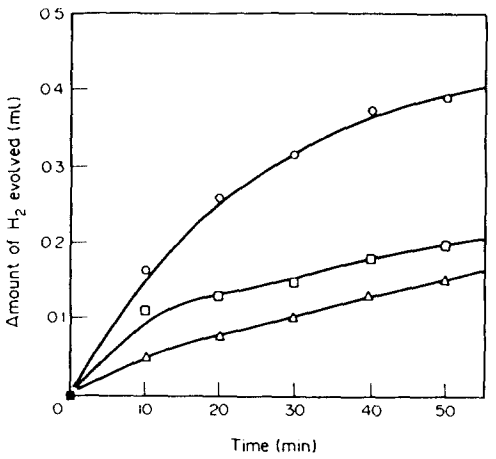


Fig. 4. Photobiocatalytic hydrogen evolution in the system RuO_2-loaded Bi_2O_3, Rh_2O_3 loaded Bi_2O_3, *R. capsulata* and MV^{2+} (1×10^{-4} mol dm^{-3}); △ → Bi_2O_3/MV^{2+}/EDTA/*R. capsulata*; □ → 0.5 mol% RuO_2-Bi_2O_3/MV^{2+}/EDTA/*R. capsulata*; ○ → 0.5 mol% Rh_2O_3-Bi_2O_3/MV^{2+}/EDTA/*R. capsulata*.

Table 1. Effect of the electron donor on photobiocatalytic hydrogen production

Electron donor	Amount of H_2 evolved (ml/h)
Oxalic acid	0.26
Ascorbic acid	0.26
EDTA	0.26
Fructose	0.52
Dextrose	0.39
Starch	0.59

Amount of Bi_2O_3 = 80 mg; total volume = 80 ml; [MV^{2+}] = 1×10^{-4} mol dm^{-3}; volume of *R. capsulata* = 1 ml; [electron donor] = 4×10^{-2} mol dm^{-3}.

Table 2. Effect of the added divalent metal ions on photobiocatalytic hydrogen production

Metal ion	Amount of H_2 evolved (ml/h)
No metal ion	0.26
Ca^{2+}	0.33
Mg^{2+}	0.66
Mn^{2+}	1.32

Amount of Bi_2O_3 = 80 mg; total volume = 80 ml; [MV^{2+}] = 1×10^{-4} mol dm^{-3}; [EDTA] = 4×10^{-2} mol dm^{-3}; volume of *R. capsulata* = 2 ml; [M^{2+}] = 1×10^{-3} mol dm^{-3}.

is known that proteins have a tendency to bind with metal ions, and this might alter the efficiency of the proteins towards a particular reaction. Addition of Mg^{2+}, Mn^{2+} and Ca^{2+} to the reaction system in the present investigation exhibited the following trend of hydrogen production efficiency: $Ca^{2+} < Mg^{2+} < Mn^{2+}$ (Table 2). The maximum efficiency observed with Mn^{2+} agrees with the report of Wilberg [16] that Mn^{2+} is a potent activator of enzymes.

Thus, the present investigation explores the possibility of using photobiocatalytic systems involving semiconductors as the source of electrons generated by light energy and bacterial enzymes as the hydrogen evolution catalysts; both are being coupled with an electron mediator in the presence and absence of electron donors, metal oxides as $e^- - h^+$ separating agents and divalent metal ions to increase the enzymatic activity.

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