Photoanodic and cathodic role of anodized tubular titania in light-sensitized enzymatic hydrogen production

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ABSTRACT

An anodized tubular titania (TiO_2) electrode (ATTE) is prepared and utilized as both a photoanode and a cathode in a photoelectrochemical system designed to split water into hydrogen (for use in fuel cells) with the assistance of a hydrogenase enzyme and an external bias of 1.5 V. In particular, the cathodic ATTE acts as a substrate for the immobilization of the enzyme due to its large surface area that results from the tubular oxides. The optimum molar concentration of KOH in anode and cathode compartments is 1.0 M and the optimum amount of enzyme for the cathode is ca. 3.66 units per geometrical unit area (1 cm × 1 cm) of the cathodic ATTE. After exposure to air for three weeks, the enzyme shows a hydrogen evolution rate that is 85.8% of that of an argon-purged enzyme. The rate of hydrogen evolution is increased from ca. 65 (in a slurry system) to more than 140 μmol cm^{-2} h^{-1}, even after eliminating the electron relay (methyl viologen) and costly platinum counter electrode.

1. Introduction

Clean production of fuels by photocatalysis has been an attractive issue since early 1970s when Honda and Fujishima [1] made pioneering efforts to produce hydrogen by splitting water with solar light concentrated on UV-absorbing materials such as titania (TiO_2). Recently, there has been increasing interest in hydrogen to supplement and ultimately replace fossil fuels for the production of energy, especially via fuel cells. In addition, the emission of greenhouse gases also attracts much attention, due to the mounting problem of global warming. Therefore, photoelectrochemical (PEC) water splitting into hydrogen and oxygen is promising, because the light-driven electrolysis of water using a semiconductor photoanode is a non-polluting, waste free, renewable method.

The efficiency of the photoelectrochemical production of hydrogen is determined by a combination of factors such as imperfections in the crystalline structure, the bulk and surface properties of the semiconductor photoanode, its resistance to corrosion in electrolytes, and its ability to drive the water-splitting reaction [2]. Hence, in order to raise the efficiency of the PEC conversion of solar energy, it is essential to design an energetically coordinated, stably operated, and economically feasible cell configuration. On the

other hand, the photocatalytic process has been criticized as being uneconomical compared with other hydrogen production systems, due to its inherently low efficiency, the need for an appropriate light source, and high overall energy costs [3]. Consequently, many recent publications and reviews have addressed the research and development of the photoelectrochemical production of hydrogen [4,5], semiconductor particulate systems [3], TiO_2 photocatalysts [6], and other material-related issues [4,7].

Compared with other photocatalysts, TiO_2 is much more promising, as it is stable, non-corrosive, environmentally friendly, abundant and cost-effective [6]. The functional properties of TiO_2 for solar hydrogen production have been thoroughly reviewed by Nowotny et al. [8]. Over the last decade, photoanodes covered with TiO_2-based films have been prepared using techniques such as anodization [9–14] and sputtering [15]. Photoanodes covered with TiO_2-based films prepared by anodization have attracted particular interest as stable, light-sensitizing photoanodes and cathodes for the enzymatic production of hydrogen driven by light [16–18].

The light-sensitized enzymatic (LSE) system is a way of producing hydrogen by coupling an inorganic semiconductor with enzymes in a photoelectrochemical configuration. The system uses the intrinsic proton reduction ability of immobilized hydrogenase enzyme on a cathode in tandem with an anodic compartment in which electron donors, such as water, undergo oxidative reaction on a light-sensitized photoanode. The generated electrons are separated and moved to the cathodic compartment through an external

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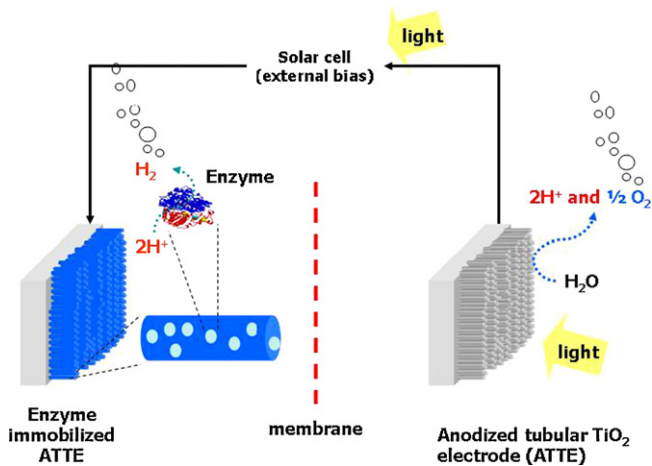E-mail address: hkjoo@kier.re.kr (H. Joo).

Fig. 1. Schematic view of system used for light-sensitized enzymatic production of hydrogen with anodized tubular TiO_2 photoanode, solar cell, and NF membrane.

circuit powered by a solar cell and used to reduce protons to hydrogen on the active sites of the enzymes. The oxidized ions move to the cathodic compartment through a nanofiltration (NF) membrane. The proposed LSE system replaces the in-vitro biological system used for hydrogen production, in which complex electron transport systems, known as main barriers, exist such as photosystems (PSs) II and I, and ferredoxin (FD).

The present work aims to optimize the system by enhancing the rate of hydrogen evolution while maintaining its economic feasibility. The role of the anodized tubular TiO_2 as a photoanode and cathode in the light-sensitized enzymatic production of hydrogen is investigated and improvement of the system is demonstrated.

2. Experimental

2.1. Sample preparation

All chemicals were used without further purification. Titanium foils (0.25 mm thick, 99.6% pure, Goodfellow, England) were cut into pieces (2 cm × 2 cm) for anodization. Potentiostatic anodization was performed at 20 V for 45 min in a two-electrode electrochemical cell that used a platinum counter electrode. The electrolyte was 0.5 vol% hydrofluoric (HF) acid (48–51%, DC Chemical), and was magnetically agitated at 5 °C. The samples were then annealed in an oxygen atmosphere (400 ml min^{-1}) at 350–850 °C for 5 h. This electrode is called an 'anodized tubular TiO_2 electrode' or 'ATTE'. Detailed explanation of the role of each component of the anodizing solution is given in [9–13].

The area of the illuminated working electrode (photoanode) and the cathode for enzyme immobilization was 1 cm × 1 cm. An electrical contact was made to the back of the titanium foil after scribing out the oxide layer. Then, an electrically insulated copper wire was attached by means of silver paste and non-conductive epoxy resin.

Potassium hydroxide solutions with different molar concentrations (0.4, 0.7, 1.0 and 2.0 M) were prepared from KOH pellets (99.99%, Sigma–Aldrich, USA) and used as the electrolyte in both compartments. Dithiothreitol (DTT, 99% Aldrich) and $MgCl_2$ (99.99%, Aldrich) were used as stabilizers before the enzyme was immobilized. DTT is effective in sample buffers for reducing protein disulfide bonds and can also be used for reducing the disulfide bridges of the cross-linker. It has been reported [19] that disulfide bonds, cross-linked using thiolation reagents, can
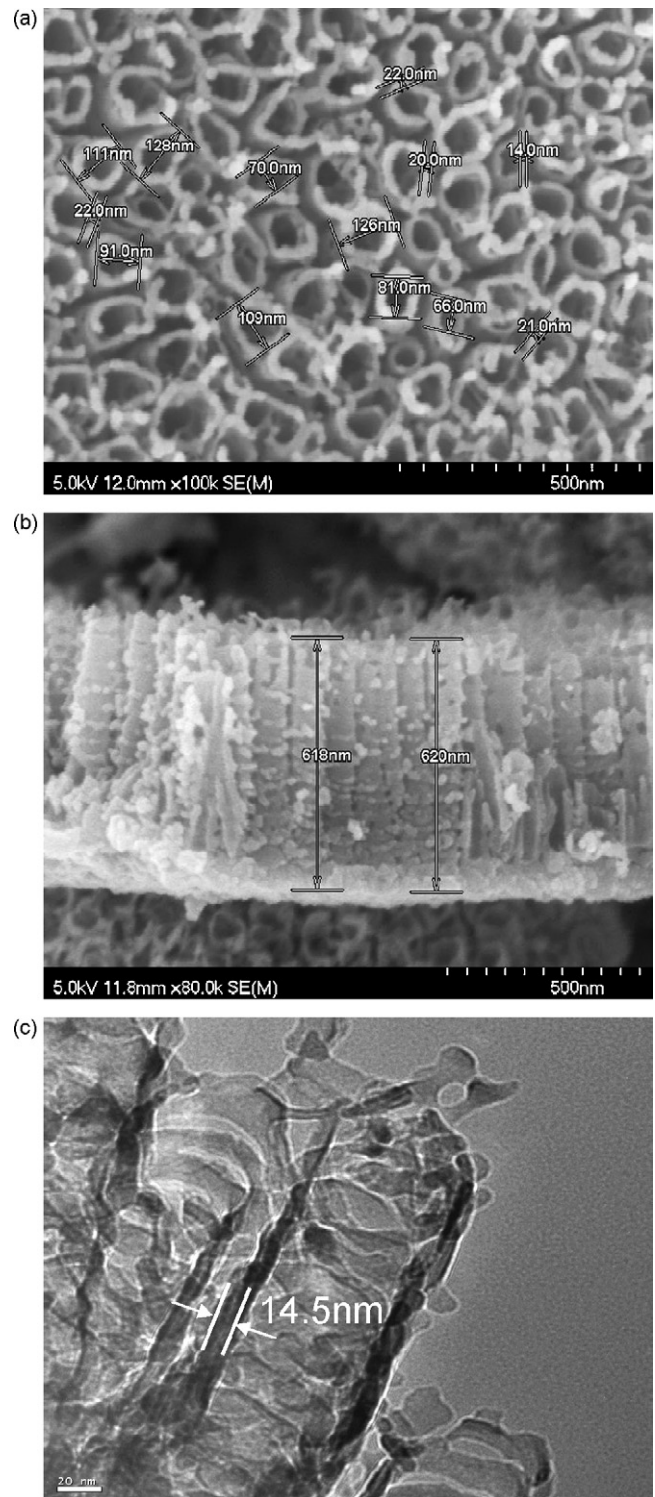

Fig. 2. Scanning electron micrographs of selected anodized tubular TiO_2 electrodes anodized (in 0.5% hydrofluoric (HF) solution at 5 °C, annealed at 650 °C for 5 h; (a): top view, (b): side view, (c) HR-TEM image of peeled-off tubes).

be cleaved by reduction with DTT, an excellent reagent for maintaining SH groups in the reduced state, in order to introduce sulfhydryl groups. Purified hydrogenase (from *Pyrococcus furiosus, 'Pfu'* hereafter) was obtained from Prof. Adams at the University of Georgia, who was the first to isolat *Pfu*. This enzyme with a molar mass of 150 kDa is known to be remarkably resistant
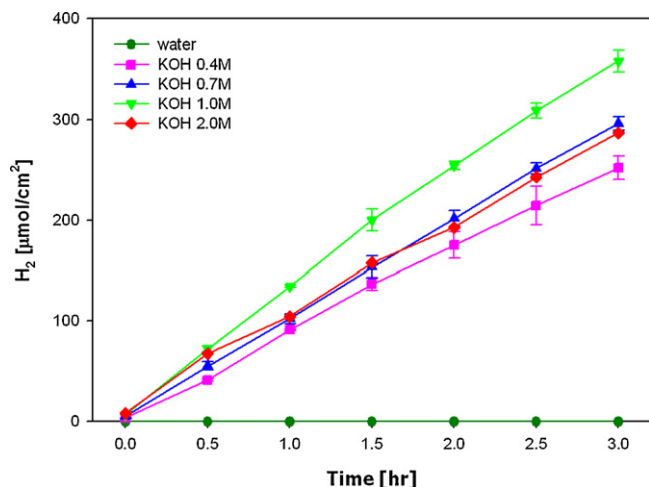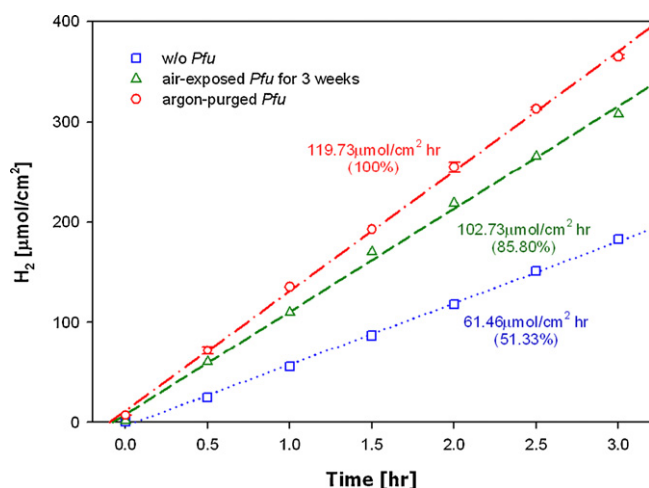
Fig. 3. Time-course profile of hydrogen evolution with different mole concentrations of KOH as electrolyte (external bias of 1.5 V from solar cell, *Pfu* (10.98 units) immobilized on ATTE, ca. 74 ± 3.4 mW cm^{-2}).

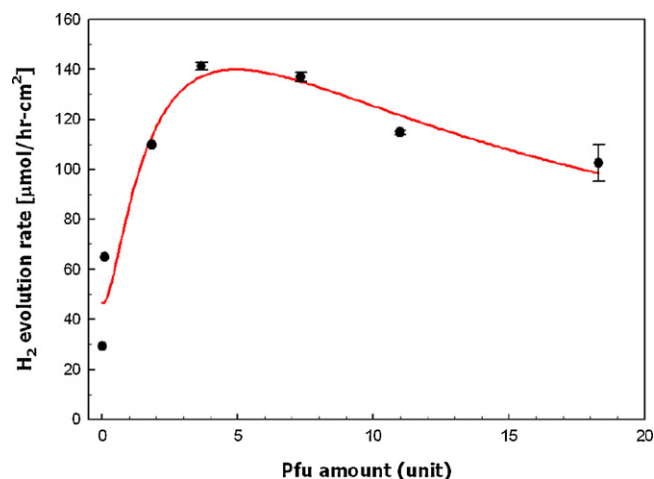**Fig. 5.** Effect of the amount of Pfu enzyme (used for immobilization on photoanode) on H$_2$ evolution rates external bias of 1.5 V from solar cell, ca. 74 ± 3.4 mW cm^{-2}.

to inactivation by heat and chemical reagents [20]. The activity assay of *Pfu* (21,834 unit cm^{-3}) was much higher than that of *Clostridium butyricum* (1442 unit cm^{-3}) and *Thiocapsa roseopersicina* (1704 unit cm^{-3}), as determined with Tris–HCl (50 mM, pH 8.5, 50 °C, absorbance at 570 nm). The specific activity (unit mg^{-1}, Bio-rad protein assay, absorbance at 750 nm) of *Pfu* was two to three times higher than that of the latter hydrogenases. One unit of hydrogenase activity catalyzes the production of 1 μmol of H$_2$ per min. For immobilization of the enzyme, the ATTE was immersed for 12 h in 50 mM Tris–HCl buffer solution (pH 7.5 at 4 °C, Trizma base, minimum 99.9% titration and 2M HCl, Sigma) containing 3.6–18.3 units of enzyme. Then, electropolymerization of polypyrrole was carried out on the ATTE in 2.0 mM of pyrrole monomer (purity > 98%, Sigma) at 0–0.8 V vs. Pt counter electrode for 20 min.

2.2. Apparatus and analysis

The experiments were conducted in a two-compartment reactor, as shown in Fig. 1. The cylindrical-shaped anodic compartment had a volume of 80 ml (headspace volume ca. 55 ml) and con-

tained an aqueous solution of KOH for water oxidation. The cathodic compartment was a water-jacketted, cylindrical-shaped cell (80 ml, headspace volume ca. 55 ml) that was filled with KOH solution and sealed with a silicone rubber gasket. Prior to the reaction, the reactor was de-aerated with argon for 30 min to remove the oxygen in the water and headspace, unless otherwise noted. The two terminals from a solar panel (10 cm × 10 cm, crystalline silicon) were attached to the photoanodic ATTE and the cathodic ATTE, respectively, in order to apply an external voltage bias to the system. An NF membrane (molecular weight cut-off, MWCO = 200) made of polyamide (SU-210, Toray, Japan) was inserted between the two cells to allow proton transport. Chronoamperometry (CA), cyclic voltammetry (CV) and open-circuit voltage (OCV) measurements were performed with a potentiostat (G300 w/PHE200 software, GAMRY Instruments Electrochemistry, PA, USA). A platinum mesh electrode served as the counter electrode (CE) and Ag|AgCl (saturated in 3.0 M KCl) as the reference electrode (RE). Polymerization of the pyrrole was performed for the immobilization of the enzyme using the ATTE as the CE. The light source was a 1000 W xenon lamp (Oriel, USA) and was passed through a 10-cm IR water filter. The irradiated light intensity was ca. 74 ± 3.4 mW cm^{-2} (at 350–450 nm, measured with a portable radiometer: UM-10 with UM-400 from Minolta Co., Japan), but the light intensity absorbed by the sample appeared to be slightly lower than the irradiated
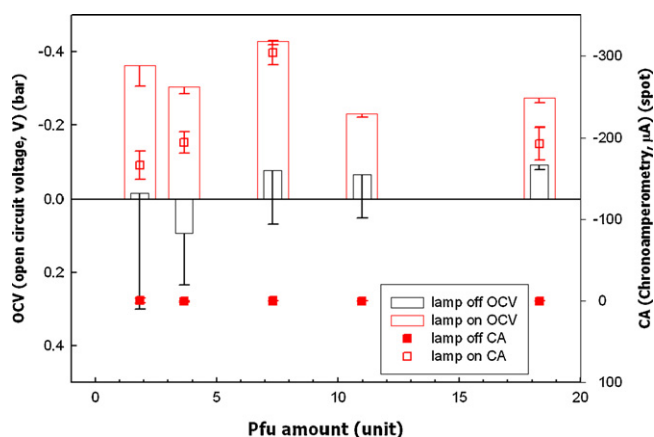

Fig. 4. Effect of pretreatment of enzyme, when immobilized, on hydrogen evolution (external bias of 1.5 V from a solar cell, *Pfu* (10.98 units) immobilized on ATTE, ca. 74 ± 3.4 mW cm^{-2}).

Fig. 6. Effect of amount of Pfu enzyme (used for immobilization on the photoanode) on electrical properties such as open-circuit voltage and chronoamperometry measurements (external bias of 1.5 V from solar cell, ca. 74 ± 3.4 mW cm^{-2}).
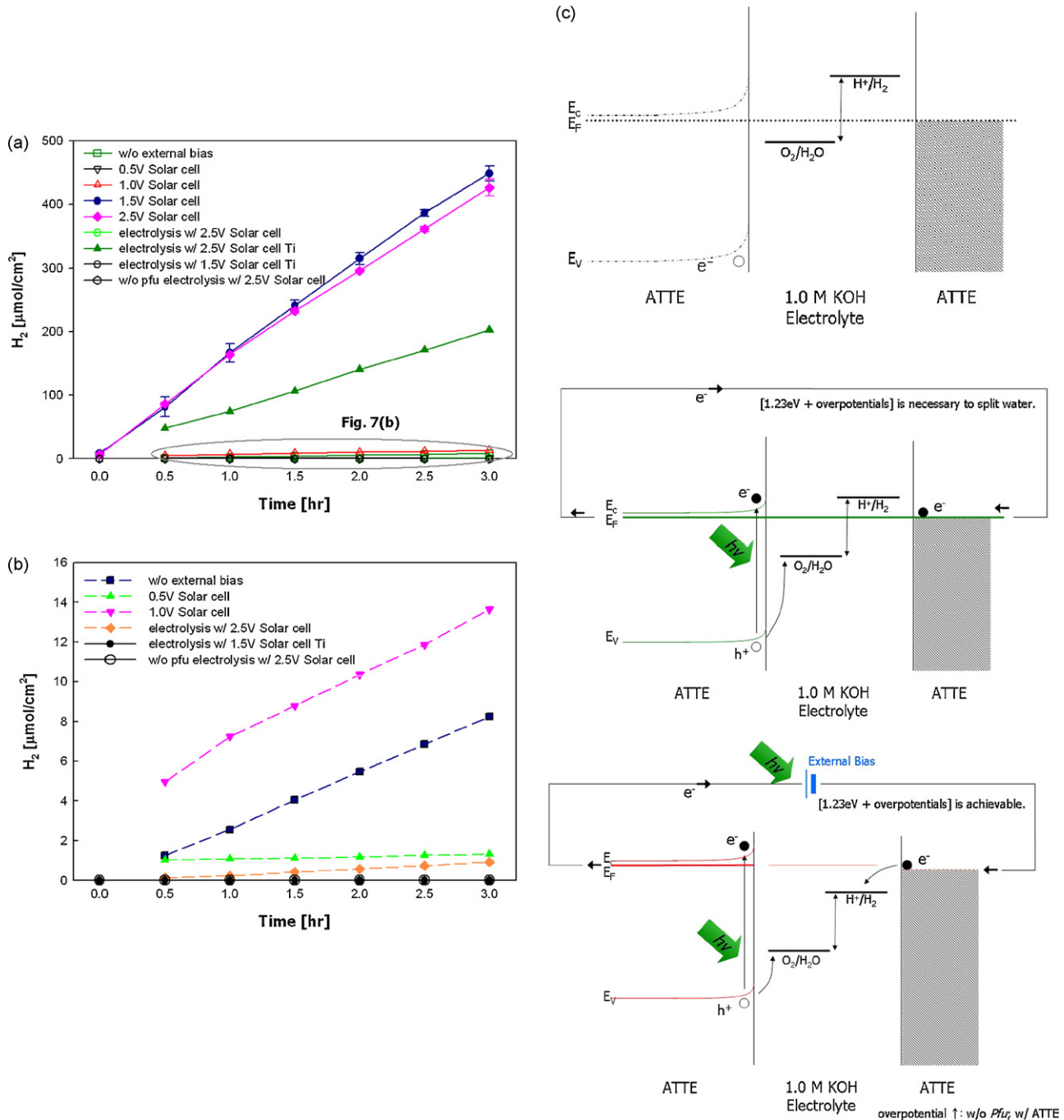
Fig. 7. Time-course profiles of hydrogen evolution under different reaction conditions with 1.0 M KOH electrolyte. 'Ti' in (a) and (b) refers to the raw Ti foil without anodization, and schematic representations with energy diagrams are shown in (c), *Pfu* (3.66 units) immobilized on ATTE, ca. 74 ± 3.4 mW cm^{-2}).

intensity, due to the loss by reflection and absorption at the reactor window and electrolyte. The structure and morphology were investigated with a scanning election microscope (SEM/EDAX, Hitachi S-4700) and a field emission transmission electron microscope (FE-TEM, Tecnai F30 S-Twin, FEI, Netherlands). The crystal phase and size were determined by X-ray diffraction (XRD, Miniflex, Rigaku; $k = 0.89$, $\lambda = 0.15418$ for Cu Kα X-ray, 30 kV, 15 mA). The hydrogen and oxygen produced were analyzed by a gas chromatograph with a thermal conductivity detector (TCD at 260 °C, oven at 40 °C). The column used in the system was a 5A molecular sieve (Supelco, USA).

3. Results and discussion

In our previous studies, the morphology of the prepared samples varied noticeably according to the electrolyte, applied voltage and bath temperature, The findings showed the feasibility of using an ATTE made from Ti foil with a thickness of 0.25 mm anodized at an applied voltage of 20 V in 0.5 vol% hydrofluoric acid and annealed at a temperature of 450–650 °C [17,18]. Selected SEM images of the ATTE applied in this study (anodized at 20 V for 45 min in the electrolyte at 5 °C, then annealed at 650 °C for 5 h) are shown in Fig. 2, where the TiO$_2$ tubes with a wall thickness of 14–20 nm and a length
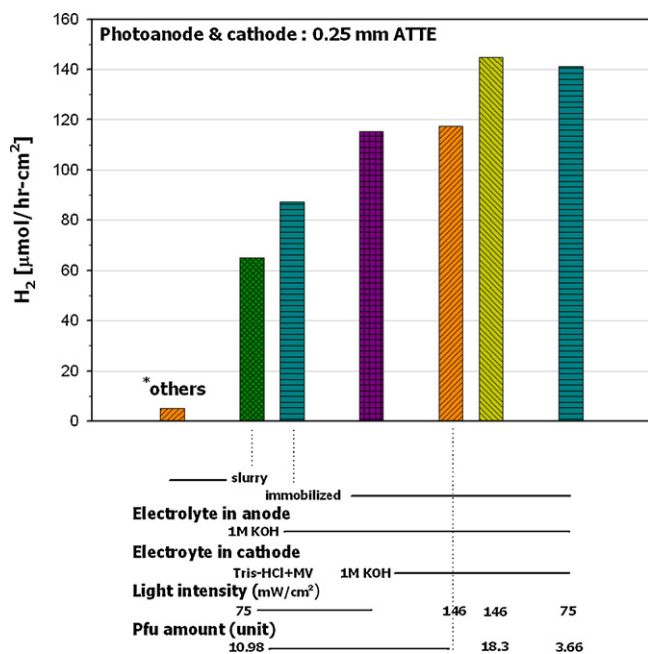
Fig. 8. Trends of H$_2$ production rate for different system configurations (ATTEs anodized at 20 V/45 min in 0.5 vol% HF at 5 °C, then annealed at 650 °C for 5 h; 'others' denotes the cases in Fig. 7(b)).

of ca. 620 nm have a periodic ring structure (Fig. 2(c)). The thickness of the wall varies considerably depending on the electrolyte. This is mainly due to the variation of the wet chemical etching rate, and the mixed electrolyte produced incomplete or cracked tubes. Porous structures are formed during the anodization through two processes: field-enhanced oxidation and field-enhanced oxide dissolution. Furthermore, solid-state sintering is likely to take place at elevated temperatures, which leads to grain growth, densification and, ultimately, the complete collapse of the structure. These changes are more remarkable during the phase transformations that often accompany bond-breaking and enhanced mass transport [9,10]. In the diffraction patterns, all of the as-deposited samples are found to be amorphous, while heat-treatment in dry O$_2$ ambient supports the formation of the anatase phase up to 650 °C and the rutile phase from 475 °C [17].

Unlike in our previous studies [16–18], the enzyme was immobilized on the cathodic ATTE. This eliminates the methyl viologen used as an electron relay and the buffer solution in the cathodic compartment, and thereby achieves better contact between the enzyme and the cathode. Since immobilization of the enzyme does not require any stabilizer, tests for the replacement of the buffer with KOH solution were performed. The time-coursed profile of hydrogen evolution is shown in Fig. 3, using different mole concentrations of KOH as the electrolyte and 10.98 units of *Pfu* for the enzyme-immobilized ATTE, along with an external bias of 1.5 V from a solar cell. The amount of *Pfu* chosen, 10.98 units, was found to be optimum in a system of equivalent scale, in which the enzyme was dispersed in the cathodic compartment. The variation of the H$_2$ evolution rate with the concentration of KOH is in the order of 1.0 M > 0.7 M ≥ 2.0 M > 0.4 M. As the concentration of OH$^-$ ions increases, electrical conductivity increases to some extent, until the amount of OH$^-$ ions exceeds a certain threshold, above which the OH$^-$ ions can prevent diffusion of the produced protons and produce H$_2$O$_2$, which absorbs photon energy. Hence, there must be an optimum concentration of electrolyte and in our system this is 1.0 M.

The aerobic exposure of the enzyme before its use reduces the hydrogen evolution rate by a factor of ca. 0.858. As a reference, the hydrogen evolution rate is checked in the absence of the enzyme. The result is about a half of that obtained with the argon-purged enzyme (Fig. 4). This directly demonstrates the role of the enzyme and indicates that the photoelectrochemical cell with ATTEs can still split water to generate hydrogen with the assistance of an external bias and photocurrent. The enzyme contains a large subunit (NiFe, ca. 60 kDa) and small sub-units (Fe-S, ca. 30 kDa) and these clusters are active sites for electron relay. The active sites seem to be deactivated by air only very slowly and this is supported by the fact that the prepared samples can be used for half an year with only a slight loss of activity. Meanwhile, ultraviolet irradiation causes a drastic loss in enzyme activity.

The optimized amount of *Pfu* for immobilization was investigated using different amounts of the enzyme. The geometrical area (1 cm × 1 cm) of the cathodic ATTE was fixed throughout the study. Previously, the optimum amount of the enzyme was found to be 10.98 units for dispersion in a cathodic compartment (< 70 μmol cm^{-2} h^{-1}) in a slurry system). When 10.98 units of *Pfu* are immobilized, however, an H$_2$ evolution rate of ca. 115 μmol cm^{-2} h^{-1} is observed and an even higher rate occurs when 3.66 units of *Pfu* are immobilized (ca. 140 μmol cm^{-2} h^{-1}), as shown in Fig. 5. The higher evolution rate with the lower amount of *Pfu* is due to more efficient contact between with the enzyme and the cathode than that obtained in the slurry system with methyl viologen. The existence of the optimum point may be due to the dual role of the enzyme in the reduction of protons and the oxidation of hydrogen. Excessive *Pfu* could act as an oxidase of hydrogen to protons.

The trend of the H$_2$ evolution rate is similarly correlated with the photocurrent measured by chronoamperometry shown in Fig. 6, even though the optimum point is slightly shifted to 7.32 units. This change may be due to the disparity in the measuring method as follows. Chronoamperometry was performed with the enzyme-immobilized electrode biased by a potentiostat, not by a solar cell and a photovoltage which were used when hydrogen evolution was measured. In the latter case, therefore, ionic transport may be enhanced and the electrical barrier may be reduced, so that a lower amount of enzyme could be necessary to give peak activity. The profiles of the two measurements, on the other hand, follow the same trend, as shown in Fig. 6, because the movement of photo-generated electrons and the oxidation by holes are intrinsic characteristics of the system with the same electrodes, electrolytes, and light intensity. In this work, it can also be demonstrated that more than 73% of the generated current is converted into hydrogen.

Fig 7(a) shows the time-course profiles of hydrogen evolution under different reaction conditions with 1.0 M KOH electrolyte and 3.66 units of Pfu immobilized on the cathodic ATTE; 'Ti' in the legend denotes the raw Ti foil before anodization and 'electrolysis' means irradiation only on the solar cell. An applied external bias of at least 1.5 V and band bending along with the upward shift of the energy level in the anodic ATTE caused by irradiation is sufficient for light-sensitized enzymatic hydrogen production. In the absence of the excited electrons in the anodic ATTE, an external bias of 2.5 V with a bare Ti foil as an anode can also split water to generate hydrogen at half the rate of that with excited electrons. Nevertheless, the other conditions are not adequate to evolve a significant amount of hydrogen, as shown in detail in Fig. 7(b). As described above, in the absence of the enzyme, the evolution rate becomes slower (Fig. 4). In addition, no or only a trace of hydrogen is produced when no or a lower external bias (0.5 and 1.0 V) is applied or electrolysis is carried out with the anodic ATTE or with a Ti foil with a bias of 1.5 V from the solar cell. Fig. 7(c) shows a schematic representation with energy diagrams of the system used in this study. Once

the semi-conductive photoanode absorbs light, electrons move towards the bulk, while holes move towards the surface and are injected into the electrolyte (top and middle of Fig. 7(c)). Hence, the band bending is reduced and the Fermi level shifts upward. At this time, the energy level of water oxidation (O_2/H_2O) should be above the valence band (VB, E_v) of the photoanode and that of the proton reduction (H^+/H_2) should be below the Fermi level (E_f) of the metal cathode required to split water. If this is not the case (i.e., H^+/H_2 is above the Fermi level), water splitting does not occur. When an external bias is applied, the resulting further shift of the Fermi level enhances the ability of the electrons to reduce protons (bottom of Fig. 7(c)).

Fig. 8 illustrates the improvement in the performance of the system. Compared with the slurry system, the hydrogen evolution rate is increased from ca. 65 to more than $140 \mu \, mol \, cm^{-2} \, h^{-1}$ with a much simpler configuration and lower amount of *Pfu*. We are currently reformulating the photoanode into a visible light-absorbing ATTE and a prototype cell is being designed and fabricated in a flat shape for possible application outdoors.

4. Conclusions

By replacing the electron transport systems in a biological process with a photoanode, the present work aims to optimize the system and enhance the rate of hydrogen evolution, while maintaining its economic feasibility. The possible role of the ATTE as a photoanode and cathode has been investigated and the rate of hydrogen evolution is demonstrated to be drastically increased from ca. 65 to more than $140 \mu mol \, cm^{-2} \, h^{-1}$, even after eliminating the electron relay (methyl viologen) and costly platinum counter electrode. This successful trial is encouraging the design and fabricates a flat-shape reactor for potential outdoor use.

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