

Light-sensitized Enzymatic Hydrogen Evolution in Photoelectrochemical Configuration

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ABSTRACT: This study introduces the photoelectrochemical (PEC) system for enzymatic hydrogen evolution by a light-sensitized photoanode and addresses the role of anodized tubular TiO₂ electrodes (ATTE) both as a photoanode and a cathode in the system. The enzyme is immobilized on the cathodic ATTE increasing the rate of hydrogen evolution from 65 to 140 $\mu\text{mol}/(\text{cm}^2 \times \text{hr})$. The enzyme exposed to air for even 3 weeks showed a hydrogen evolution rate that was 85.8% of that of the argon-purged enzyme. Further research has kept on designing a flat type reactor, enlarged electrode and the optimization of immobilized enzyme and red-shifted light absorption of the photoanode.

Keywords: water splitting, photoanode, solar, hydrogen

1. INTRODUCTION

There has been increasing interest in hydrogen, which has the potential to supplement and ultimately replace fossil fuels for the production of energy. In addition, the emission of greenhouse gases also attracts much attention, due to the 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 the 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 [1]. 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.

Compared with other photocatalysts, TiO₂ is much more promising, as it is stable, non-corrosive, environmentally friendly, abundant and cost effective [2]. The functional properties of TiO₂ for solar hydrogen production were thoroughly summarized in a previous report [3]. Over the last decade, photoanodes covered with TiO₂-based films have been prepared using techniques such as anodization [4] and sputtering [5]. Photoanodes covered with TiO₂-based films prepared by anodization have attracted our particular interest as

stable, light-sensitizing photoanodes and cathodes for the enzymatic production of hydrogen driven by light.

The present work aims to put the preliminary results together and address recent progresses and the direction of further research; flat type reactor, enlarged electrodes, enzyme immobilization and red-shifted light absorption of the photoanode.

2. EXPERIMENTAL

2.1 Sample Preparation

The potentiostatic anodization was performed at 20 V for 45 min in a two-electrode electrochemical cell ($\approx 5^\circ\text{C}$) connected to a dc power supply using a platinum counter electrode with magnetic agitation mainly in 0.5 vol% hydrofluoric (HF) acid, after which the sample was annealed in an oxygen atmosphere (400 ml/min) at 650 for 5hr. This electrode is called an anodized tubular TiO_2 electrode or "ATTE". An electrical contact was made on the backside of the titanium foil after scribing out the oxide layer. Then, an electrically insulated copper wire was attached to the backside using silver paste and nonconductive epoxy resin.

Purified hydrogenase (from *Pyrococcus furiosus*, 'Pfu' hereafter) was purchased from Prof. Adams at the University of Georgia. For the immobilization of the enzyme, the ATTE was immersed for 12hrs in 50 mM Tris-HCl buffer solution (pH 7.5 at 4°C , Trizma base, minimum 99.9% titration and 2N HCl) containing 3.6 ~ 18.3 units of the enzyme. Then, the electropolymerization of polypyrrole was carried out on the ATTE in 2.0 mM of pyrrole monomer (purity > 98%) at 0 to 0.8V vs. Pt counter electrode for 20 min.

2.2 Apparatus and Analysis

The experiments were conducted in a two-compartment reactor (anodic and cathodic parts), separated by a nanofiltration (NF) membrane (polyamide, MWCO=200)

and a solar cell (10cm $\times$ 10cm, crystalline silicon). KOH was a choice of electrolyte and the two terminals from a solar cell panel were attached to the photoanodic ATTE and the cathodic ATTE, respectively, in order to apply an external bias to the system.

The light source used was a 1000 W xenon lamp, which was filtered through a 10-cm IR water filter. A potentiostat and a gas chromatograph were used for system analysis while TEM, SEM/EDAX, confocal laser scanning microscope and XRD were for sample analysis.

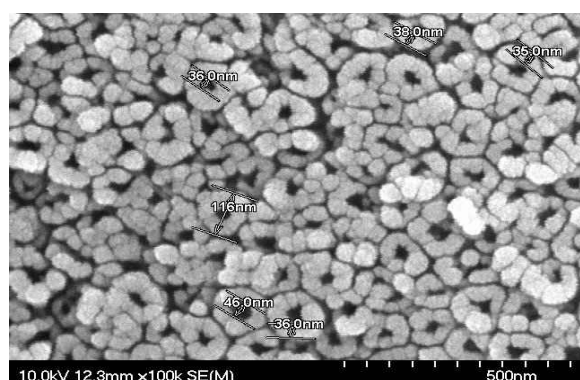
3. RESULTS AND DISCUSSION

3.1 ATTE characteristics

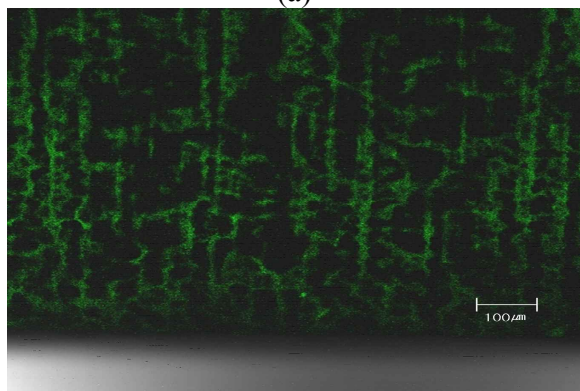
Clean TiO_2 tubes were evident (Fig. 1(a)) on the prepared ATTE. The morphology of the porous film with the TiO_2 tubes was strongly affected by the electrolyte (concentration and pH) and anodization/annealing temperature. The HR-TEM image of tubes peeled off from the sample clearly presents a lateral view of the nanotubes with a wall thickness of ca. 14.5 nm and periodic ring structure, as well as a crystal fringe at the wall. All of the as-deposited samples were found to be amorphous, while heat-treatment in dry O_2 ambient supported the formation of anatase phase up to 650°C and rutile phase from 475°C . In addition, complete transformation to rutile phase occurred at a temperature close to 750°C .

A confocal microscopic image of the enzyme immobilized on the cathodic ATTE was shown in Figure 1(b). Before the image was taken, fluorescent labelling with N-hydroxysuccinimide (NHS)-ester had been conducted, followed by dialysis to exchange buffers and to remove the unused dye. The NHS-Fluorescein labelling reagent forms a crosslink between the NHS-ester on the dye and primary amines (*Pfu* in this study) on the protein that results in the formation of a stable, covalent amide bond. The optimized amount of enzyme was 3.66 units for the

area of 1cm^2 of the ATTE, resulting in $140\ \mu\text{mol}/(\text{cm}^2 \times \text{hr})$. After that, the rate gradually decreased to $105\ \mu\text{mol}/(\text{cm}^2 \times \text{hr})$. Lack of enzyme directly represents less active site to reduce protons, while excess enzyme reversibly activates hydrogen oxidation back to protons. As a comparison, the amount of *Pfu* of 10.98 units was previously found to be optimum in the equivalent scale enzyme-dispersed system.



(a)



(b)

Figure 1: SEM image of the bare ATTE (a) and confocal image of the enzyme-immobilized ATTE (b).

3.2 PEC configuration

Blank tests were performed as follows. With the presence of the enzyme, only the solar cell was irradiated, resulting in no noticeable hydrogen detected. In our system, at least 2.5V of external bias via the solar cell with raw Ti cathode (not an anodized TiO_2) was needed to produce hydrogen. With the ATTE irradiated, at least 1.5V of external bias was critical to evolve the hydrogen. Without enzyme, even with the

irradiation over the ATTE, produced amount of hydrogen was remarkably reduced.

The optimum molar concentration of KOH in anode and cathode compartments is 1.0M. The variation of the H_2 evolution rate with the concentration of KOH was in the order of $1.0\text{ M} > 0.7\text{ M} \geq 2.0\text{ M} > 0.4\text{ M}$. 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_2O_2 , which absorbs photon energy. The enzyme exposed to air for 3 weeks shows a hydrogen evolution rate that was 85.8% of that of the argon-purged enzyme. The rate of hydrogen evolution is increased from ca. 65 (in a slurry system) to more than $140\ \mu\text{mol}/(\text{cm}^2 \times \text{hr})$, even after eliminating the electron relay (methyl viologen) and costly platinum counter electrode.

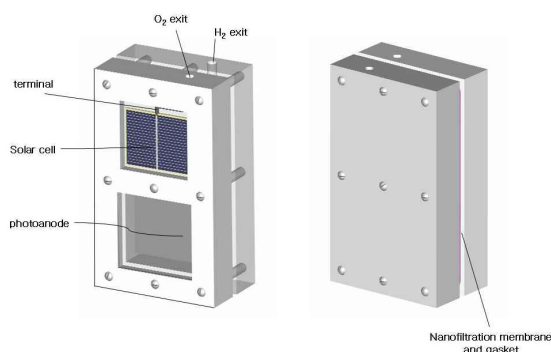


Figure 2: Prototype reactor for enzymatic hydrogen evolution by a light-sensitized photoanode.

3.3 Energetic representation

Once the semi-conductive photoanode absorbs light, electrons move towards the bulk, while holes move towards the surface and are injected into the electrolyte. Hence, the band bending is reduced and the Fermi level shifts upward. At this time, the energy level of water oxidation ($\text{O}_2/\text{H}_2\text{O}$) 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 ((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.

3.3 System reforming

Each part of the system has been thoroughly examined to improve efficiency as well as engineering and economics. First, the ATTE was considered to have abilities of absorbing visible range of the light and more photons. For these aspects, it has been studied that lengthening the anodized TiO_2 tubes and doping of non-metal such as N and F. The change in UV/Vis absorbance is shown in Figure 3. Nitrogen-doped ATTE clearly demonstrated the red-shifted absorbance peaked at 510 nm (yellowish color). The observed red-shift may not increase the photocatalytic activity in a powdery system due to increasing rate of the charge pairs even though the reformed TiO_2 tubes absorb more photons and produce more charge pairs. However, in a photoelectrochemical system when external bias is applied, generated charge pairs are intentionally separated and hence participated in more redox reactions respectively.

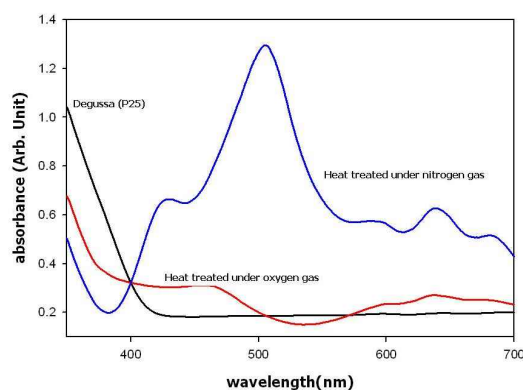


Figure 3: UV/Vis spectra of anodized TiO_2 anodes under different atmosphere.

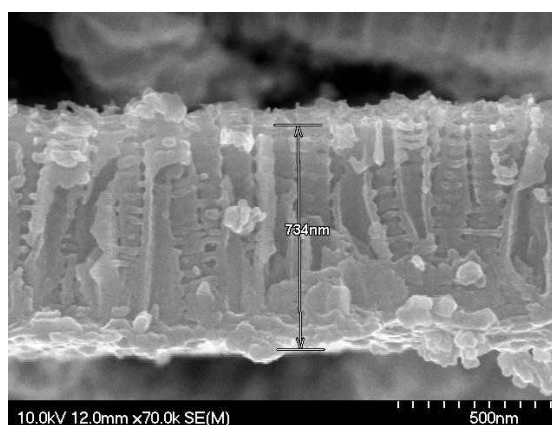
The other approach has been applied to dope fluorine content in the ATTE by means of thermal treating after anodization without washing. In this way, fluorine content in the

electrolyte could be automatically inserted into the TiO_2 matrix when annealed. Noticeable difference in F content was obtained with EDAX analysis (Table 1).

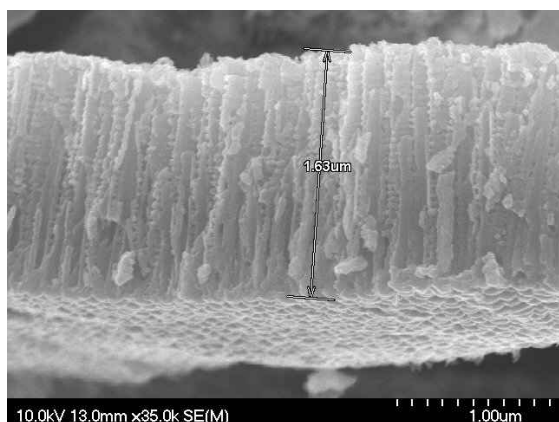
Like previously mentioned, the morphology of the ATTE is strongly affected by a kind of electrolyte used [6]. Since we have found that characteristic properties of TiO_2 such as wall thickness and diameter of the tubes were not critical for system efficiency, we have tried to lengthen the tubes (Figure 4). Lengthened TiO_2 tubes, to some extent, increase the amount of light-absorbing and enzyme-fixing semiconductor material, resulting in higher system efficiency.

Table I: Fluorine content in the ATTE by washing after anodization.

Element	Weight (%)	
	Without Washing	washed
O	32.51	28.92
F	-	6.48
Ti	67.49	64.60
Total	100	100



(a)



(b)

Figure 4: SEM images of ATTEs with different lengths in HF (a) and organic acid (b).

4. CONCLUSION

By substituting the electron transport system in a biological process with a photoanode and enzyme-immobilized cathode, the present work successfully initiated the system for water-splitting and enhanced the rate of hydrogen evolution, while obtaining its simplicity. Possible direction for improvement was suggested. The flat-shape reactor was firstly introduced for potential outdoor use.

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