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Microcontact Imprinting of Algae on Poly(ethylene-*co*-vinyl alcohol) for Biofuel Cells

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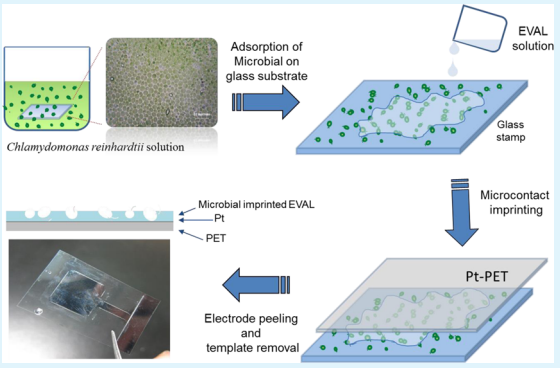
Supporting Information

ABSTRACT: Hydrogen can be produced using microorganisms (e.g., bacteria and algae); algal production has the additional ecological benefit of carbon dioxide fixation. The conversion of hydrogen to electricity via fuel cells may be more efficient compared to other energy sources of electricity. However, the anode of biofuel cells requires the immobilization of microorganisms or enzymes. In this work, poly(ethylene-*co*-vinyl alcohol) (EVAL), was coated on the electrode, and green algae was microcontact imprinted onto the EVAL film. The readsorption of algae onto algae-imprinted EVAL thin films was compared to determine the ethylene content that gave highest imprinting effectiveness and algal binding. Scanning electron microscopy and fluorescence spectrometry were employed to characterize the surface morphology, recognition capacity, and reusability of the algae-imprinted cavities. The recognition of an individual algal cell by binding to the imprinted cavities was directly observed by video microscopy. Finally, the power and current density of the algal biofuel cell using the algae-imprinted EVAL-coated electrode were measured at about 2-fold higher than electrode sputtered platinum on poly(ethylene terephthalate).



KEYWORDS: *algae, microcontact imprinting, poly(ethylene-co-vinyl alcohol), biofuel cells*

1. INTRODUCTION

Energy prices are spiraling upward: the price of crude oil reached a record high of US $147 per barrel in 2008, and have soared again recently. Biological sources of energy, such as corn, have attracted great interest as alternatives to fossil fuels. However, many researchers have argued that using corn as an alternate energy source still produces substantial carbon dioxide, which is associated with climate change, and that it will increase food prices, having an especially adverse impact in poor countries. Therefore, several microorganisms such as green algae, cyanobacteria, and photosynthetic bacteria have been evaluated for potential use in generating hydrogen,[1] which can be used to generate electricity in fuel cells.

The roadmap of algae biofuels technology in the U.S. has been elucidated since 2008 (http://www.orau.gov/algae2008/resources.htm). Most studies are focused on either strain screening and mutation, or the scale up of the algal culture. Many researchers have also tried to immobilize bacteria on electrodes in microbial fuel cells (MFC),[2−4] an approach which has been reviewed by Franks and Nevin[5] and Pant et al.[6] Recently, miniaturization and energy output from algae-based microbial fuel cells were reviewed by Wang et al.[7] and Velasquez-Orta et al.[8]

Cell imprinting technology provides the capability to recognize and bind imprinted cells, either via cell shape or via recognition and binding of cell surface biomolecules. Dickert and Hayden adopted yeast as a template in surface sol−gel imprinting.[9] The growth stage measurement of yeast was then used to test for contamination or for food quality control. This group also developed the imprinting and sensing of microorganisms (such as yeast[10] and picornaviruses[11]), using quartz crystal microbalance (QCM) mass sensing. Recently, Zare's group developed cellular treatment with formaldehyde, glutaraldehyde, or a combination of the two leads to markedly improved capture selectivity.[12]

Our earlier work demonstrated the recognition of proteins by exploiting microcontact imprinting with a monomer mixture via conventional polymerization[13] and with poly(ethylene-*co*-vinyl alcohol) via solvent evaporation.[14] As an imprinting material, poly(ethylene-*co*-vinyl alcohol) has been adapted for use with different transducers (e.g. electrochemical,[15] optical,[16,17] magnetic,[18] and gravimetric[19]). In this study, *Chlamydomonas Reinhardtii* was used as the template, and microcontact imprinting was performed on a sputter-coated platinum electrode. Quantitative readsorption measurements were used to screen different ethylene contents for the starting polymer, in

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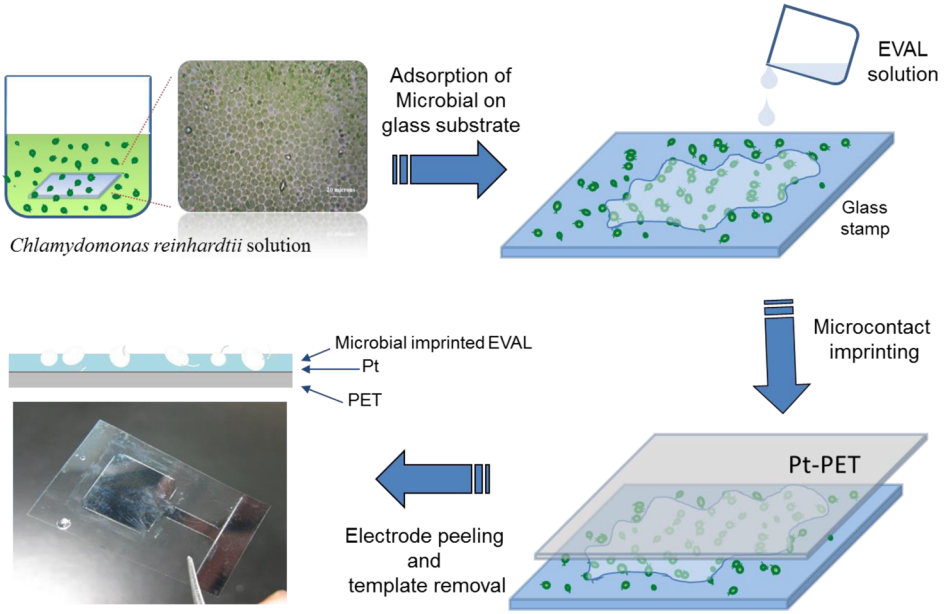
Scheme 1. Preparation of Microcontact Algae-Imprinted EVAL-Coated Pt-PET Electrode for the Biofuel Cells



order to determine which composition gave highest algal binding. A fuel cell was then assembled using the immobilized anode electrode, Nafion film, and a platinum cathode to measure the voltage output of the fuel cell and to evaluate the hydrogen production performance.

2. MATERIALS AND METHODS

2.1. Reagents. *Chlamydomonas reinhardtii* was a generous gift by Professor Chung-Kuang Lu at National Museum of Marine Biology and Aquarium (Pingtung, Taiwan). Poly(ethylene-*co*-vinyl alcohol)s (EVALs) containing ethylene of 27, 32, 38, and 44 mol % were purchased from Scientific Polymer Products (Ontario, NY). Dimethyl sulfoxide (DMSO), sodium dodecyl sulfate (SDS) and potassium hydrogen phosphate were from J. T. Baker (ACS grade, NJ). Nafion PFSA Membrane N117 was from DuPont Fuel Cells (Wilmington, DE). Proteose pertone was from Fluka Biochemika (Buchs, Switzerland). Sodium chloride and magnesium sulphate heptalydrate were from Sigma-Aldrich Co. (St. Louis, MO). Calcium chloride dihydrate and Potassium dihydrogen phosphate were from Riedel-deHaën Co. (Germany). Sodium nitrate was from Wako Pure Chemical Ltd (Osaka, Japan). The culture medium for *Chlamydomonas reinhardtii* contained 2.94 mM sodium nitrate, 0.17 mM calcium chloride dehydrate, 0.30 mM magnesium sulphate heptalydrate, 0.43 mM potassium hydrogen phosphate, 1.29 mM potassium dihydrogen phosphate, 0.43 mM sodium chloride, and 0.1% of proteose pertone. All chemicals were used as received unless otherwise mentioned.

2.2. Microcontact Imprinting of Algae-Imprinted Polymeric Thin Films. A poly(ethylene terephthalate) (PET) thin film was cut into the size of 2.5 × 3.0 cm^2, cleaned with detergent, and then dried in an oven at 60 °C. The PET thin film was sputtered with platinum at 10 mA for 300 s with an ion sputter coater (Hitachi E-1045) to form a Pt-PET electrode. Although electrodes have been made from carbon paper,[20] carbon cloth,[21,22] carbon mesh,[23] graphite rods,[24] stainless steel plate,[25] and platinum mesh,[26] we selected a sputter-coated platinum electrode because the electrode area can be well-defined by sputtering on a planar plastic thin film for the microcontact imprinting. The synthesis of algae-imprinted EVAL thin film by microcontact imprinting[13,14] included three steps (as shown in scheme 1). (1) A glass slide (1.3 × 1.3 cm^2) was washed with isopropanol, deionized water, ethanol, and deionized water in 55 °C for 30 min under sonication. This glass slide was then placed in a 2 mL 1 × 10^6 cell/mL algae solution for 30 min, dried under very gentle nitrogen blowing. The algae absorbed glass slide was used as the algae stamp; (2) the EVAL solution (EVAL/DMSO = 1.0 wt %) was cast onto an algae stamp, and the Pt-PET electrode was placed on the EVAL-coated algae stamp and then dried in an oven for 3 h to evaporate DMSO; (3) the algae-imprinted EVAL Pt-PET electrode was peeled off and washed with deionized water and PBS 3 times and 10 min each time. All membranes were equilibrated with phosphate buffered saline (PBS) overnight before use.

2.3. Characterization of Algae-Imprinted Polymeric Thin Films. The adsorption to the algae- and non-imprinted polymer films were examined by immersion into 2 mL algae solution (1 × 10^7 cells/mL) for 60 min, and then measuring the algae concentration in the solution with a fluorescence spectrophotometer (F-7000, Hitachi Co., Japan) with excitation wavelength of 485 nm and emission wavelength of 685 nm, respectively. The algae cell numbers can be calibrated with fluorescence intensity. Algae- and non-imprinted polymers were freeze-dried before examination by a scanning electron microscope (Hitachi S4700, Hitachi High-Technologies Co., Tokyo, Japan).

2.4. Performance Measurement of the Algae Fuel Cell. Before assembling the fuel cells, all parts were sterilized in an autoclave and irradiated under UV in a laminar flow hood. A 3.0 × 3.0 cm^2 Nafion 117 film was used as the proton exchange membrane (PEM). After adding 250 mL of PBS to the cathode cell, algae culture medium (without the addition of magnesium sulphate heptalydrate) was added to the anode cell. The anode cell was then purged with nitrogen gas to enhance hydrogen production. Finally, a platinum foil (2.0 × 2.0 cm^2) and the algae-imprinted EVAL-coated Pt-PET electrode were used as the cathode and anode, respectively. The voltage output was monitored with a power source measurement device (U-2722A, Agilent, Santa Clara, CA) under zero driving current. A potentiostat (model 608-1A, CH instruments Inc., Austin, TX) was employed to measure the current output by amperomertic i−t curve. The initial voltages were decreased from the maximum voltage output by −0.05 V/step. The polarization and power curves were the plots of current vs. voltage and power output, respectively. Power density ($P = VI/A$) was calculated from the measured voltage (V), current ($I = V/R$), and surface area of the anode electrode (A).[27]

3. RESULTS AND DISCUSSION

Microcontact imprinting improves the adsorption of algae, compared to non-imprinted films. Figure 1 shows the adsorption of algae onto algae-imprinted EVAL thin films, for different mole percentages of ethylene. Imprinting effectiveness (ratio of binding on Algae-imprinted (AIP) to non-imprinted

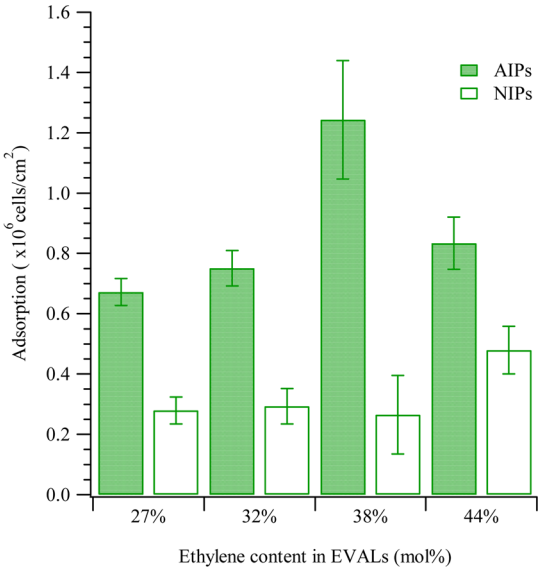


Figure 1. (a) Adsorption of algae on algae- and nonimprinted EVALs containing different mol % of ethylene. Algae were rebound from a solution concentration of 1×10^7 cells/mL for 60 min, and the binding was assayed as described in the experimental section 2.3. The adsorption on Pt-PET electrodes was $3.13 \pm 0.55 \times 10^5$ algae cells/cm^2.

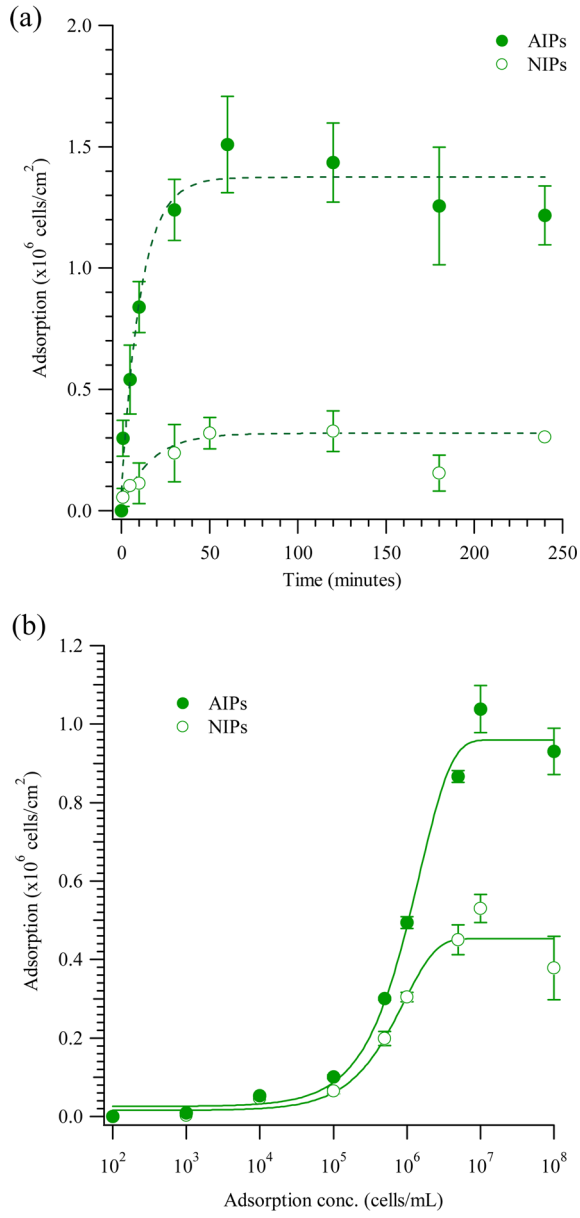


Figure 2. (a) Time course of rebinding of algae on algae-imprinted and nonimprinted EVAL thin films with 38 mol % ethylene. (b) Saturated adsorption with different algae solution concentrations, on 38 mol % ethylene imprinted and nonimprinted EVAL films.

polymers (NIP)) was lowest for 44 mol % of ethylene in EVAL, but highest for the 38 mol %, which gave a ratio of 4.8. Further decreases in ethylene content decreased specific adsorption but not the non-specific adsorption, resulting in decreased effectiveness. Adsorption was always less than 5×10^5 algae cells/cm^2 on nonimprinted EVAL thin films and $3.13 \pm 0.55 \times 10^5$ algae cells/cm^2 on Pt-PET electrodes. Most importantly for the intended application, the highest total binding of algae was obtained with 38 mol % ethylene EVAL, and this composition was selected for fuel cell studies.

The adsorption of algae on the EVAL (38 mol % ethylene) thin films was found to saturate after around 50 mins of binding, shown in Figure 2a. An apparent reduction in adsorption at long times may be an artifact caused by algal growth in the medium, as absorption was subtractively measured as depletion from the medium. In addition, the binding of adsorbed algae is not irreversible; the adsorbed algae may leave the imprinted cavities as showed in the multimedia file in the Supporting Information.

Adsorption was also measured as a function of algae concentration, as shown in Figure 2b. The imprinting stamp is made by adsorbing algal cells to a 1 cm^2 glass slide; assuming the algae cell size of about 10 μm in diameter, a saturated substrate would contain about 10^6 algal cells, and we should expect this to be the maximal binding to the AIP. This was found to be true, as shown in Figure 2b. In contrast, the nonimprinted polymer showed much less binding. Interestingly, the affinity of the binding sites was about the same for imprinted and nonimprinted polymers, as reflected in the solution concentration that resulting in half the sites being bound. The readsorption results were fitting with the Freundlich isotherm and gave 1.29 mL/cm^2 and 1.19 for the Freundlich adsorption constant and exponent.

In Figure 3, the scanning electron microscope (SEM) image displays the imprinting of algae on the poly(ethylene-*co*-vinyl alcohol) thin film, showing that the size of algae-imprinted cavity on the EVAL is around 5−6 μm. Submicrometer cavities (ca. 200 nm) are also observed in the image. In the Supporting Information, the video clip shows the recognition of an alga to the algae-imprinted EVAL thin film. It took about 50s to complete an alga recognition including about 10 micrometer migrations in the first 45 s and the last 5 s for the rotation to form the complementary shape recognition. The surface element analysis (carbon, oxygen, and phosphorus atomic %) of three locations in Figure 3b: an alga on AIPs (green spot) are 76.7, 21.67, and 1.32; NIPs (light blue spot) are 75.67, 24.12, and 0.00; AIPs (deep blue spot) are 79.90, 19.66, and 0.00.

To test the power output, we employed algae concentration 1×10^7 cells/mL in the algal biofuel cells. As shown in Figure 4a, the preliminary analysis of output voltage when using algae- and non-imprinted EVAL on Pt-PET electrode indicates that initial output open circuit voltages are 0.63 ± 0.02, 0.50 ± 0.02,

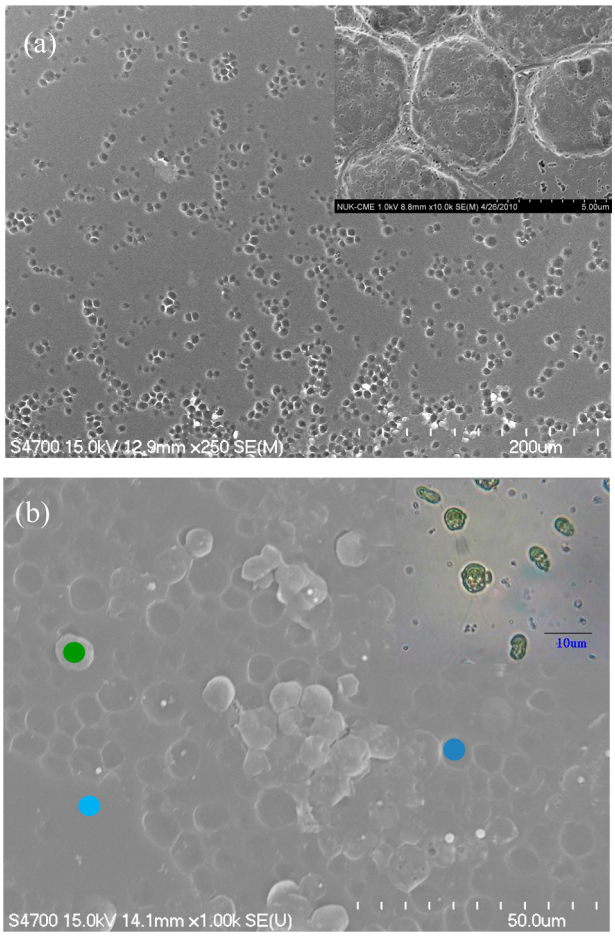


Figure 3. SEM images of the algae-imprinted EVAL (containing 38 mol % of ethylene) thin films (a) after and (b) before the algae removal. The surface element analysis (carbon, oxygen, and phosphorus atomic %) of three locations in b: alga on AIPs (green spot) are 76.7, 21.67 and 1.32; NIPs (light blue spot) are 75.67, 24.12 and 0.00; AIPs (deep blue spot) are 79.90, 19.66, and 0.00. The optical image of algae is shown in the inset of b.

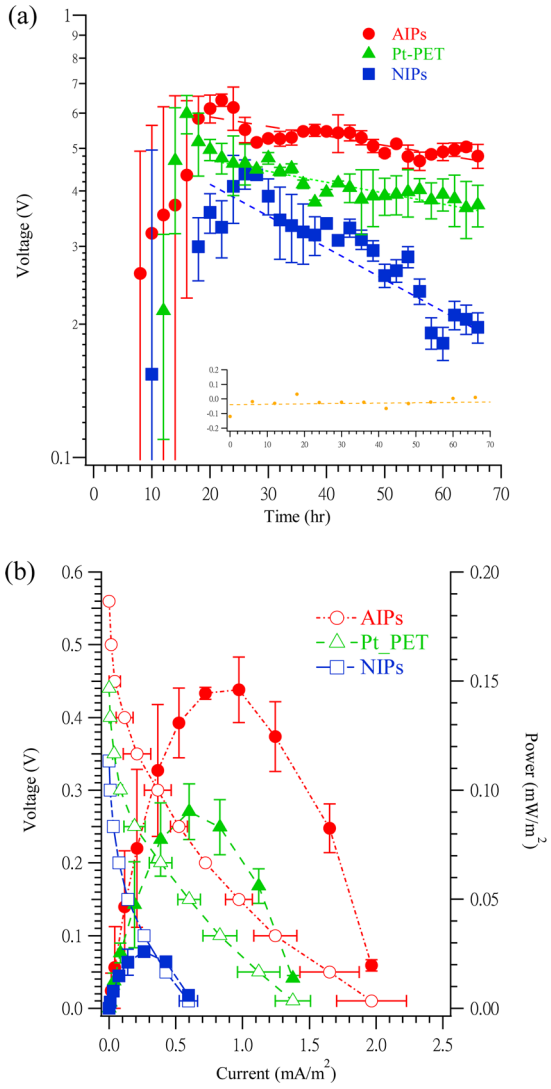


Figure 4. (a) Time course measurements of the open circuit voltage (OCV) of algae fuel cells using algae-, nonimprinted EVAL-coated, and bare Pt-PET electrodes as the anode. The inset indicates no algae in biofuel cells with Pt-PET electrodes. (b) Polarization behavior of the algae fuel cells. The blank without algae could be found in the original point.

and 0.51 ± 0.02 V, respectively. The higher voltage output of algae-imprinted EVAL electrode also indicates that electron transfer efficiency from algae exceeds that of using a bare and NIP-coated electrodes. The decreased rates with time are around 2.3 ± 0.3 to 3.7 ± 0.4 mV/h. The polarization behavior of the algae fuel cells measured is shown in Figure 4b. The output voltages are deceased with increasing the loading current densities. The output power density is about 0.15 ± 0.02 mW/m^2 when the current density is 0.97 ± 0.10 mA/m^2 when using the algae-imprinted EVAL coated Pt-PET electrode, which is about 2-fold and 5-fold of that using bare and NIP-coated Pt-PET electrodes. This may attribute the higher metabolites oxidation of algae.[28] A blank experiment without the addition of algae was also performed, giving an output voltage of −0.028 ± 0.033 V, shown in the inset of Figure 4a. The control voltage is so low as to be obscured by the original point in Figure 4b.

The power output voltage in this study is very similar to that associated with the production of hydrogen.[29] Following a lag phase of ∼20 h in Figure 4a, the output voltage dramatically increased to a maximum. Late log growth phase and stationary phase of algae may cause the slightly decreased of hydrogen gas concentrations.[29] Therefore, the electrochemical performance appears to be a good proxy for hydrogen production.

Lastly, the reusability of the AIPs was studied in Figure 5. Rebinding capacity measurements in Figure 5a were carried out at room temperature for 60 min. For the measurements shown, a *Chlamydomonas reinhardtii* solution was added to algae-imprinted EVAL thin films; the solution was measured; the algae was removed (with 0.1 wt % SDS solution and DI water on a orbital shaker), and the cycle was repeated 10 iterations showed only a drop of 21.6% in binding. Using the same washing protocols, AIPs coated electrodes were employed in the biofuel cells. The relative output voltage compared to first time usage is shown in Figure 5b; the output voltage decreased only around 10%, which may indicate algae were promoted to produce hydrogen after being adsorbed on the AIPs even if their binding capacity was decreased.

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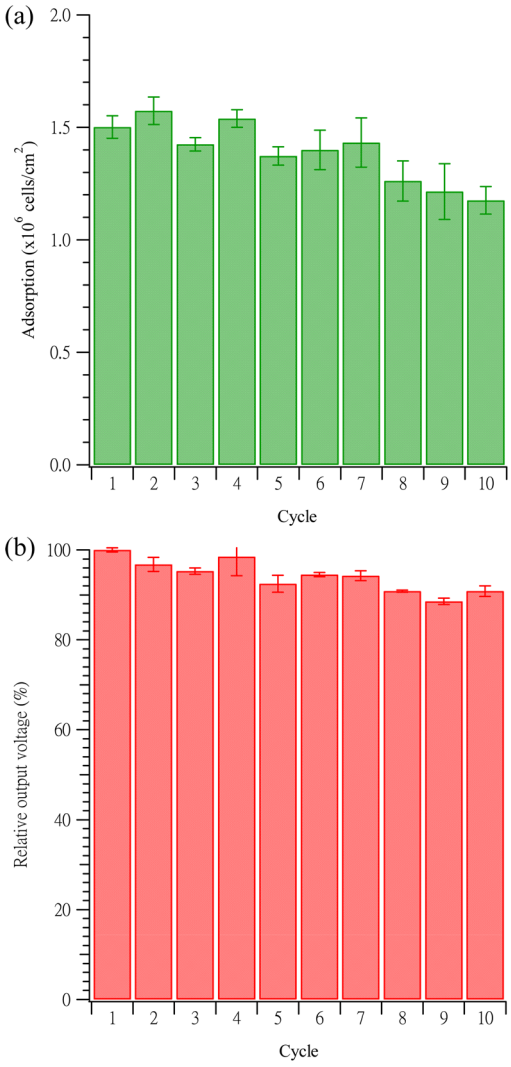

Figure 5. Reusability of the algae-imprinted (38 mol % ethylene) EVAL thin films by (a) rebinding capacity and (b) relative output voltage measurements. The standard deviation is based on at least three individual experiments.

4. CONCLUSIONS

Using algae as a green energy source not only reduces cost, but also reduces carbon dioxide. This work showed that EVAL with 38 mol % ethylene had the highest recognition capability for algae and possessed good potential for use in a modified fuel cell electrode. The performance comparison between the algae-imprinted EVAL coated and bare Pt-PET electrodes indicated that the algae-imprinted EVAL electrode had a higher output voltage. Finally, the algae concentration of 1×10^7 cells/mL not only gave the higher voltage output of the algal biofuel cells but also saturated the surface of the microcontact algae-imprinted EVAL thin films.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Video clip showing the recognition of an alga to the algae-imprinted EVAL thin film (mp4). This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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