A new approach for in situ cyclic voltammetry of a microbial fuel cell biofilm without using a potentiostat

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Abstract

Electrochemically active bacteria in a microbial fuel cell (MFC) usually exist as a biofilm attached to an electrode surface. Conventional cyclic voltammetry using potentiostat is considered as a powerful and reliable method to study electrochemical behavior of MFC biofilm. In this paper, we introduce a new approach to evaluate redox behavior of an electro-active MFC biofilm without using a potentiostat. Analogous to a conventional cyclic voltammetry study, we controlled the biofilm-electrode potential by computer-feedback controlling the external resistance of an operating MFC. In this way, the MFC can still operate as a “fuel cell” without being “interrupted” by an external device (i.e. potentiostat) that normally does not belong to the system. Relationship between current and biofilm-electrode potential was obtained and showed agreement with a potentiostat-controlled method under similar experimental conditions. The method could be added to our technical repertoire for analysis of bacterial mediator involved in the exocellular electron transfer of a MFC-biofilm, and it could potentially serve as a practical process monitoring method for MFC operation. The application of computer-control components should be further explored to facilitate control, diagnosis as well as optimization of MFC processes.

Keywords: Anodophilic bacteria; Biofilm; Computer-controlled bioprocess; Mediators; Redox

Introduction

In light of the increasing demand of renewable and sustainable energy, microbial fuel cell (MFC) technology becomes one of the most rapidly developing environmental biotechnologies [1], [2] and [3]. Yet, the technology is still in its infancy and needs further research to improve understanding and performance of the process. MFC is a bacteria-catalyzed electrochemical device, in which a bacterial biofilm on the anode transfers electrons from the oxidation of an organic substrate to the anode enabling a current flow from anode to cathode. The presence of a suitable resistive load in the external circuit enables the production of electrical power.

Arguably the most interesting phenomenon in MFC electron flow is the transfer of electrons from the bacteria to the anode surface. Although different mechanisms have been proposed, the most widely accepted view is that electron mediators are produced by the bacteria for this transfer. Bacterial mediators (also termed electron shuttles or electron carriers) have also been found in other microbial reactions involving an insoluble solid electron acceptor such as ferric iron or elemental sulfur [4] and [5]. After the bacteria have reduced an oxidized mediator as their terminal electron acceptor, it will then “shuttle” the electrons to the electron accepting anode where it becomes reoxidized, and becomes available to the bacteria again [6], [7] and [8].

The redox property of the bacterial mediators can be seen as critical for the effective operation of the MFC. In the literature, redox properties of mediators were commonly studied by using cyclic voltammetry (CV) method. This method is considered as a standard technique for studying redox behavior of solutions [6], [9], [10], [11], [12] and [13].

In CV, the potential of a working electrode is scanned forward and backward at a predefined rate (commonly ranges from 5 to 100 mV s^{-1}). The presence of any mediators is detected from the fact

that a current is produced during the cyclic oxidation and reduction of the mediator, provided that the mediator can accept (/ donate) electrons from (/ to) the working electrode. The mid potential of the mediator can then be obtained from the current-voltage plot (i.e. cyclic voltammogram) [9] and [14].

Using conventional CV (i.e. using potentiostat to control a half cell potential) to evaluate electrochemical behavior of an electrode-associated biofilm has several advantages. For examples: (i) the target electrode processes (e.g. biofilm-anode) can be studied separate from the opposing electrode (i.e. cathode) reaction and separate from other side effects such as ion transfer processes across the separating membrane; (ii) the potential can be varied in a wide range, even exceeding the potential range of a chemical cathode.

However, in conventional CV the MFC has to be disconnected from the external power user (i.e. external resistance) in order to connect with a potentiostat. From a practical viewpoint, this may lead to an operation down time of the process during which the MFC can no longer generate power. In the present study, we proposed a new method of controlling the MFC external resistance to perform cyclic voltammetric analysis of an anode biofilm. In this way, the MFC can still operate as a “fuel cell” without being “interrupted” by an external device (i.e. potentiostat) that normally does not belong to the system. Further, the optimal external resistance of a MFC at which the MFC delivers the highest power may also be defined or maintained by using the proposed method.

It is the aim of this paper to develop a practical approach of performing CV analysis for MFC process without using a potentiostat. This method will supplement the well established conventional CV method of evaluating redox properties of an electrode-associated biofilm. We hypothesized that CV of a MFC biofilm could be done by feedback-controlling the external resistance of an electricity producing MFC without the need of a potentiostat. In this paper we report our tests in verifying this

hypothesis. This means no additional equipment is needed other than the computer-controlled MFC itself. Our MFC was set-up and operated as described in our previous work [15].

Experimental

Underlying principle of the proposed method

Under close-circuit operation of an active MFC (i.e. electrons are allowed to flow readily from the biofilm anode to the cathode), there is a continuous reduction and reoxidation of the mediator involved in the electron transfer from the bacteria and the anode. The reduction reaction can be realized by using a larger external resistance, resulting in an accumulation of reduced mediator and hence charging up the biofilm-anode (i.e. decreased electrode potential). While the oxidation reaction is facilitated by using a smaller external resistance, resulting in faster anode discharge (i.e. increased electrode potential). Similar to the theory of traditional CV our proposed method aims to recognize mediators by their capacity to donate or accept electrons when the electrode potential is continuously shifted (scanned). However, instead of using an external reducing power source (i.e. potentiostat), our proposed method relies solely on the bacteria to reduce their self-produced mediators using electrons obtained from their substrate oxidation. In this case the electrode serves only as the final electron acceptor. This method can selectively detect redox species involved exclusively in the bacterial electron transfer chain, and therefore it has the potential to offer more specific information compared to traditional CV in evaluating redox properties of a MFC biofilm.

Microbial fuel cell

A two-chamber MFC made of transparent Perspex was used in the present study. Fig. 1 shows the schematic of the reactor and its peripheral settings. The two chambers were of equal volume and dimension (316 mL (14 cm × 12 cm × 1.88 cm)). They were physically separated by a cation-

selective membrane (CMI-7000, Membrane International Inc.) with a surface area of 168 cm^2. Conductive graphite granules (El Carb 100, Graphite Sales, Inc., Chagrin Falls, OH, U.S.A., granules 2–6 mm diameter) were used as both the anode and cathode electrodes, which decreased the internal liquid volume from 316 to 120 mL. This internal void anolyte volume was used to calculate the volumetric current and power densities of the MFC. The biofilm in the anode chamber was originated from an activated sludge collected from a local wastewater treatment plant. The growth medium (anolyte) composed of (mg L^{-1}): NH_4Cl 125, $NaHCO_3$ 125, $MgSO_4 7H_2O$ 51, $CaCl_2$ $2H_2O$ 300, $FeSO_4$ $7H_2O$ 6.25, and 1.25 mL L^{-1} of trace element solution, which contained (g L^{-1}): ethylene-diamine tetra-acetic acid (EDTA) 15, $ZnSO_4$ $7H_2O$ 0.43, $CoCl_2$ $6H_2O$ 0.24, $MnCl_2$ $4H_2O$ 0.99, $CuSO_4$ $5H_2O$ 0.25, $NaMoO_4$ $2H_2O$ 0.22, $NiCl_2$ $6H_2O$ 0.19, $NaSeO_4$ $10H_2O$ 0.21, H_3BO_4 0.014, and $NaWO_4 2H_2O$ 0.050. The medium was complemented with 50 mM phosphate buffer to maintain a constant pH of 7. Yeast extract solution was periodically added (ca. every 3 to 5 days) to the medium (50 mg L^{-1} final concentration) as bacterial growth supplement. Air-saturated solution of potassium ferricyanide (100 mM), $K_3Fe(CN)_6$ (Sigma-Aldrich, Inc., purity ca. 99%) complemented with 100 mM phosphate buffer was used as the catholyte. Detail operation and monitoring of the MFC during the initial start-up period were described in our previous paper [15].

Cyclic voltammetry by feedback-controlling the external resistance

The biofilm-electrode potential was controlled by selecting appropriate external resistance of the MFC. In initial experiments, the external resistance of the MFC was continuously changed in a linear fashion by using an eight channel computer controlled relay board (Ocean Controls, Victoria, Australia;www.oceancontrols.com.au) (Fig. 2A). The relay was directly controlled by the parallel port output (8 bits) from the computer. The slow rate linear sweeping of external resistance (0.69 Ω min^{-1}) had reproducibly yielded the so-called polarization and power density curves which are commonly used to assess MFC performance [10] (Fig. 2B). Furthermore, this constant resistance sweeping technique was validated in our previous study to establish the relationship between the

current and the anodic potential (Fig. 2C), from which the affinity of an electrochemically active biofilm for the electrode potential could be determined [15].

In practice, the external resistance can be controlled in a number of ways, including the use of an electronically adjustable resistor. In our experiments the switching of 8 individual resistors via the relay board allowed up to 256 different resistance values, which was sufficient for adequate control of the biofilm-electrode potential. This improved way of changing the resistors allowed a relatively linear scan of potential over time (Fig. 3A and B). This was accomplished as follows: a computer program was designed using LabVIEW™ programming software (Version 7.1) to allow the operation of the MFC at a constant electrode (here anode) potential setpoint. To maintain the electrode potential setpoint the external resistance was controlled (see above) by a simple feedback loop in the program, which enabled not only the operation to a desired potential setpoint but also the programmed stepwise shift of the potential. This could allow a linear potential change over time (here we used 0.1 mV s^{-1}), as it is known in CV (i.e. upward and downward scans represent shifts of potential toward a less and a more negative values, respectively) (Fig. 3B). It should be noted that these experiments were carried out with a fully developed biofilm that was saturated with organic substrate supply (here acetate, about 10 mM) which served as a relatively constant source of reducing power in place of a potentiostat. The ultra low scan rate of 0.1 mV s^{-1} was selected to allow sufficient time for the biofilm (microbially-reduced mediator) to interact with the oxidizing electrode. As a result, the method enabled the detection of mediators that were not only located within the boundary layer of the electrode-solution interface, but also mediators far away from the electrode (i.e. confined within the biofilm or inside the bacterial cells). Using this method with scan rates that are normally used in traditional CV, no significant peaks/valleys were detected (data not shown).

Cyclic voltammetry using a three-electrode potentiostat

In the present study, the new method was validated by comparing with results obtained from a potentiostat-controlled method. In this Control experiment, the potential of the same biofilm-electrode was regulated by using a three-electrode scanning potentiostat (Model no.362, EG&G, Princeton Applied Research, Instruments Pty. Ltd.). The working, counter and reference electrodes of the potentiostat were connected to the anode, cathode and the silver-silver chloride reference electrode of the acetate-saturated MFC, respectively. The electrode potential was changed at a similar scan rate of 0.1 mV s^{-1} to allow reasonable comparison with the new method. To assure quality of the results, all experimental data (obtained with both the new method and the Control method) were validated for reproducibility by running for at least three repetitive cycles in each experimental testing.

Results and discussion

Feedback controlling the external resistance of a MFC enables cyclic voltammetry of MFC biofilm

The current obtained during the scans of biofilm-electrode potential was recorded and showed characteristic and reproducible patterns (Fig. 3C). In theory, decreasing electrode potentials will thermodynamically impede the electron flow from the bacteria to the electrode and hence resulting in lower currents. However, depending on whether the potential was gradually increased or decreased, different current lines were obtained (Fig. 3B and C). This is indicative of charging and discharging of bio-electrochemically active compounds. To visualize more clearly the redox behavior of the electrochemically active biofilm the current was plotted as a function of electrode potential (i.e. cyclic voltammogram).

To test the hypothesis of the present study, the new method was verified by comparing with results obtained from a potentiostat-controlled method. Fig. 4 indicates that both computer-feedback- and potentiostat-controlled experiments revealed similar results: the upward shift of electrode potential from about − 380 to − 200 mV vs Ag/AgCl gave significantly higher currents compared to currents obtained from the downward shift in the same potential range. While at potentials outside this range, the resultant currents recorded from both upward and downward scans were virtually identical. Many repeat experiments verified this to be a reproducible phenomenon. These reproducible equilibrium currents (i.e. bacterial activity) were independent on the direction of potential scans and indicated that bio-electrochemically active species were not active (charging/ discharging) at these potentials (Fig. 4).

The drop in current when the potential decreased from about − 200 to − 330 mV vs Ag/AgCl suggests one of two possibilities:

1) Bacteria became less active at lowered electrode potentials;
2) The electrons released by the bacteria were accepted and stored by a “pool” of alternative electron acceptor (i.e. oxidized mediators) instead of the electrode (capacitance from mediator).

Since a further decrease in potential from − 330 to − 380 vs Ag/AgCl resulted in increasing currents again, the first possibility seems unlikely (Fig. 4). The observed current “valley” in both experiments was likely caused by the redox reactions of the mediator. This is supported by the fact that during upwards shifts of potentials there was no “valley”. From the cyclic voltammograms obtained in these modified CV experiments (Fig. 4), one could obtain the mid potential of the mediator from the potential at which the upward and downward curves are most widely apart (i.e.

highest capacitance). In our acetate fed MFC biofilm, such potential value was about − 330 mV vs Ag/AgCl.

It has to be noted that as MFC is a bio-electrochemical system, the anode associated biofilm can grow and develop over time. Hence, over hours of operation during the CV experiments, it is expected that the current output obtained at even the same electrode potentials would change. This explains the difference between the current-potential curves in Fig. 4. Nevertheless, our results suggested that both the proposed and the conventional CV methods indicated a same redox behavior of the same biofilm.

However, it is speculative to characterize the nature of a redox active species involved in the anodic electron transfer in a MFC (i.e. whether it is purely membrane associated such as cytochrom c or nanowire (i.e. direct electron transfer) or soluble (i.e. indirect electron transfer) solely based on results obtained from both the proposed and the conventional CV techniques. According to earlier findings [7] and [16], bacterial soluble mediators appear to be confined mainly within the biofilm rather than in the bulk medium. This property was due to an electrostatic interaction between the soluble mediator and the anode [7]. Therefore, CV of a biofilm-anode can not be used as a stand-alone method to ascertain the physiochemical nature of the mediator involved in the electron transfer process.

At this stage, we concluded that the new method demonstrated a good controllability of the biofilm electrode potential, and it showed a similar capacity to the conventional potentiostat-controlled method in evaluating in situ electrochemical properties of a MFC biofilm. Further, provided that the electron donor is not limiting in the system, evaluation of the electro-active biofilm in a MFC can be conducted without the need of a potentiostat. To our knowledge, this is the first report in the literature to control the biofilm-electrode potential by feedback controlling the external resistance of a MFC in a

way similar to a CV analysis. Redox information about the mediator(s) involved in the bacterial electron transfer as well as the affinity of the electro-active biofilm on the electrode potential (i.e. values of critical electrode potential and half-saturation electrode potential as defined in [15] and [17]) could be obtained with minimal disturbance of the bioprocess.

Limitations and future outlook

The established technique has two major limitations that are needed to be addressed in future studies.

1) It requires the presence of bacterial substrate. In MFC, as the electrons flow exclusively in one direction starting from the substrate → bacteria → bacterial mediator(s) → anode → external resistor → cathode → terminal electron acceptor, sufficient substrate (electron donor) must be provided to the biofilm during the analysis. While in the absence of substrate, there is no reducing power to reduce the involved mediator(s) and hence the electrode. Perhaps, such criterion could be further explored for investigating specific electron transfer processes with different substrates.

2) It requires an efficient cathodic reaction. The cathodic reduction process should not be limiting over the electrode potential range in which bioelectrocatalysis of the bacterial mediators is taking place. In the present study, the use of ferricyanide solution enabled a stable and strong cathodic reduction, allowing the effective control of anodic potential by using the proposed method. However, it is expected that with the continuous improvement of MFC cathodic process, such limitation could be alleviated.

Apart from the application as discussed above, the established technique may also be useful to gain other important insights for MFC process control. For instance, selection of external resistor is considered as critical for optimization of MFC process. Maximal and sustainable power output can only be achieved when the MFC is running with an appropriate external resistance. While most

studies reported thus far have only arbitrarily selected the external resistance to operate their MFCs, and very often the power outputs of their systems were reported by using an inappropriate external resistor [18]. By feedback controlling the external resistance of the MFC using either current/ power generation as the reference parameter, we could in theory operate the MFC at its optimal current/ power production point. Overall, it is expected that more sophisticated and intelligent application of computer control components will be emerged in the future to deal with optimization and diagnosis of such dynamic bio-electrochemical process.

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Figure 1. Schematic of the computer-feedback controlled microbial fuel cell used in the present study

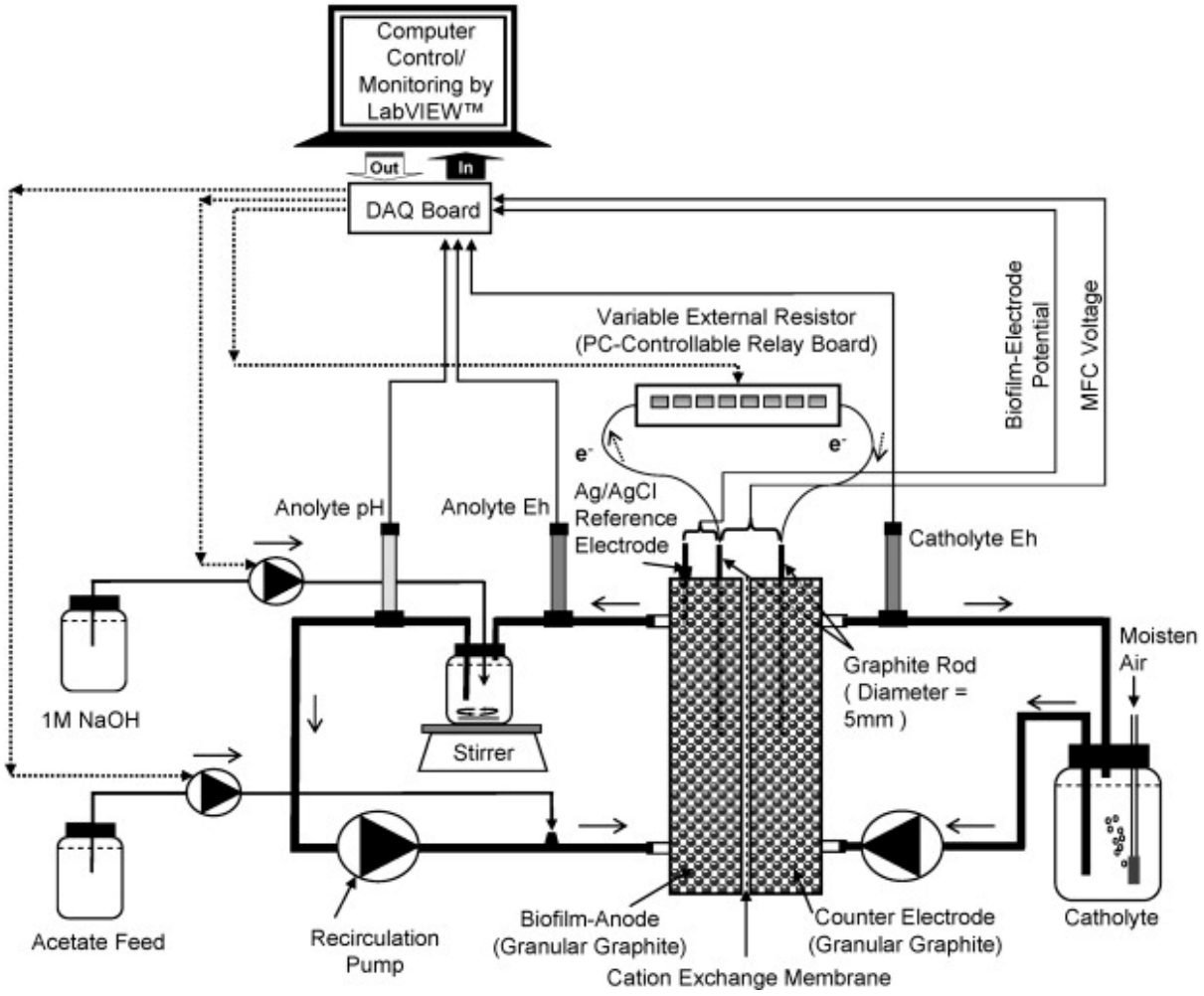

Figure 2. Constant sweeping of the external resistance of the computer-feedback controlled acetate-saturated microbial fuel cell (0.69 Ω min^{-1}). (A) Resistance vs. time; (B) polarization and power density curves; (C) current vs. electrode potential. Note: ↑ or ↓ R represents increasing or decreasing sweep of external resistance, respectively.

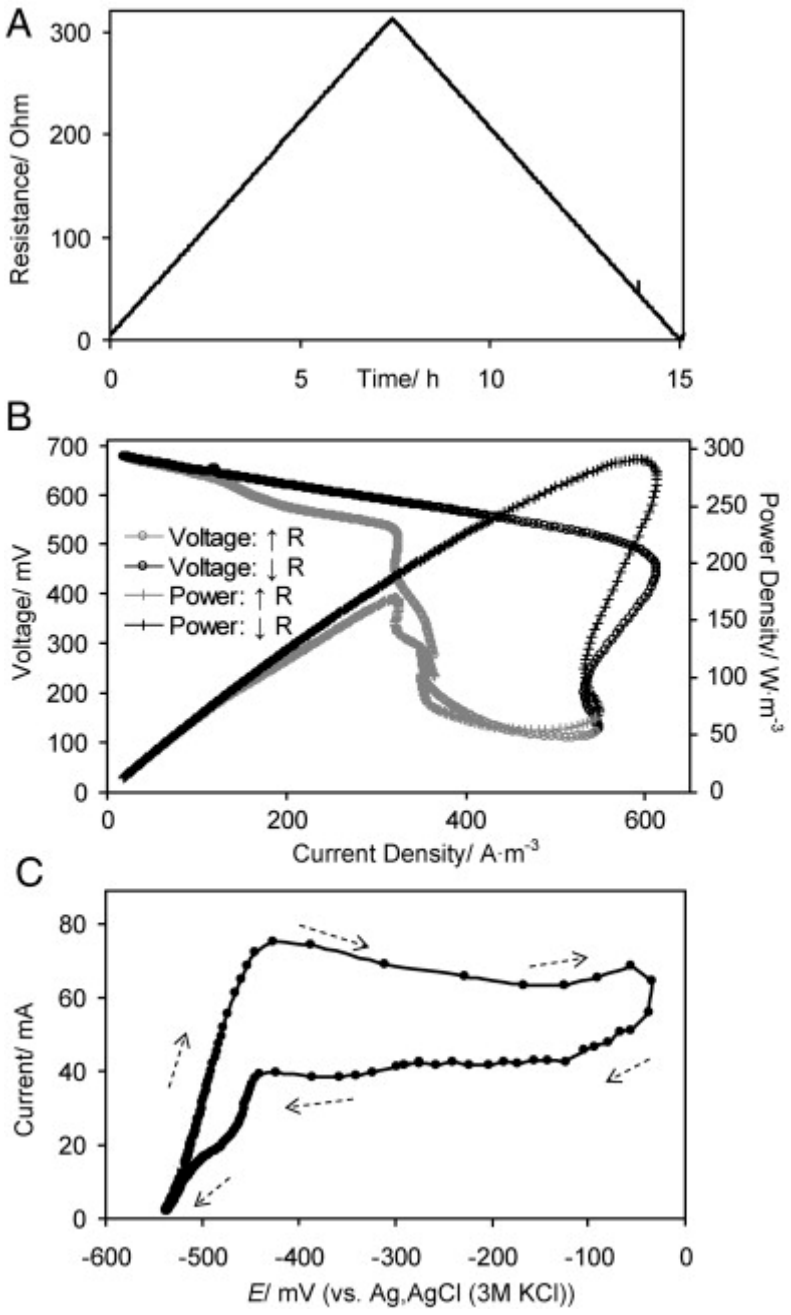

Figure 3. Time profiles of (A) external resistance; (B) electrode potential; and (C) current during the computer-feedback control of the external resistance of an acetate-saturated microbial fuel cell. Electrode potential scan rate was 0.1 mV s^{-1}.

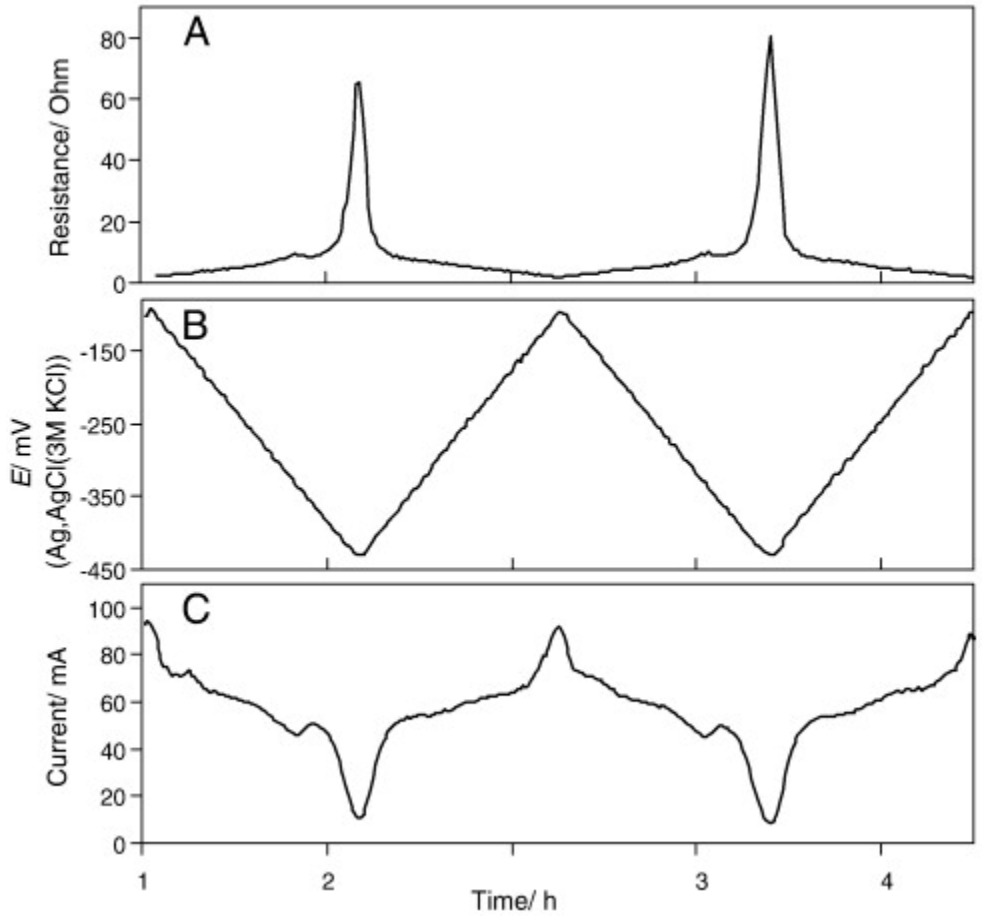

Figure 4. Comparison between the current-potential plots (cyclic voltammograms) obtained by (a) computer-feedback controlling the external resistance and (b) by using a potentiostat in an acetate-saturated microbial fuel cell. Scan rates of the electrode potential in both methods were 0.1 $mV \cdot s^{-1}$; dotted arrows indicate the direction of the potential scan; the difference between the currents obtained from the two methods was due to the different times of the two experiments.