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A New Insight into Potential Regulation on Growth and Power Generation of *Geobacter sulfurreducens* in Microbial Fuel Cells Based on Energy Viewpoint

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The anode potential in microbial fuel cells (MFCs) defines the possible metabolic energy gain (PMEG) for the bacteria growth. This study focused on the mechanism behind anode potential controlling microbial growth and power generation in MFCs from an energy perspective. Four sets of MFCs were operated with varied conditions: three with different applied anode potential (−160, 0, and 400 mV vs standard hydrogen electrode (SHE)) and one with an external resistor (500 Ω). A model strain *Geobacter sulfurreducens* was used here. The evolution of biomass was measured and its quantitative relationship with PMEG was analyzed. Linear voltammetry and cyclic voltammetry were also carried out. Results indicated a notable gain in biomass and power density when anode potential increased from −160 to 0 mV. However, no gain in biomass and power generation was detected when anode potential further increased to 400 mV. At anode potential of 0 mV and below, *G. sulfurreducens* extracted a significant portion of PMEG for growth, while utilization of PMEG significantly decreased at 400 mV. Furthermore, the anode potential has a minor influence on individual *G. sulfurreducens* cell activity, and the maximum power density of MFC proportionate to biomass.

Introduction

Microbial fuel cells (MFCs) are promising technology that oxidize organic and inorganic matters and generate current using bacteria as the catalysts (*1*). However presently, commercial utilization is infeasible due to low yields of power generation. In the anode chamber of MFCs, the biocatalytic activity of exoelectrogens is crucial in converting chemical energy to electrical energy (*2*). To improve power generation, it is essential to investigate and optimize the factors influencing growth and activity of exoelectrogens. Previous literature states that microorganisms harvest some of the energy for their own surviving and reproduction by electron transfer from substrate to anode surface (hence, the loss of electrical energy in a MFC) (*3*). The anode potential in MFCs controls the theoretical energy gain for microorganisms, which closely relates to their growth rate (*2*). From a thermodynamic perspective, the possible metabolic energy gain for the bacteria under standard biological conditions can be calculated using the following equation (*1*):

$$\Delta G^{0\prime} = -nF(E^{0'}_{\text{substrate}} - E_{\text{anode}}) \quad (1)$$

where $\Delta G^{0'}$ (J/mol) denotes the change of Gibbs free energy at pH 7, n is the number of electrons involved, F is the Faraday constant (9.64853 × 10^4 C/mol), and $E^{0'}_{\text{substrate}}$ (V) and E_{anode} (V) denotes the standard biological potential of the substrate and the anode potential, respectively. The equation shows that the higher the difference between the anode potential and the redox potential of the substrate, the higher the possible metabolic energy gains for the bacteria. In the anode compartment of MFCs, electrons are liberated from the substrate and passed down an electron transfer chain where energy is finally released to sustain the growth of bacteria. The electrons are then transferred to the anode from a point on the electron transfer chain which can be referred to as microbial terminal electron donors (MTED), for example an outer-membrane protein (*3*). For bacteria with a direct electron transfer mechanism, the energy come from the flow of electrons from substrate to MTED can be fully used by bacteria for growth, hereby expressed as Gibbs free energy $\Delta G^{0'}_{\text{bio}}$ at pH 7.

$$\Delta G^{0\prime}_{\text{bio}} = -nF(E^{0'}_{\text{substrate}} - E^{0'}_{\text{MTED}}) \quad (2)$$

While the potential energy between MTED and the anode surface, which is equal to $\Delta G^{0'} - \Delta G^{0'}_{\text{bio}}$ in theory, is lost (negligible in $\Delta G^{0'}$ in some cases). Therefore, the growth and reproduction rate of bacteria are basically determined by the potential of MTED.

A number of previous studies have reported the influence of anode potential on bacterial activity and growth in MFCs (*2*), along with anode potential regulation being utilized as a way to accelerate start-up and enhance power generation of MFCs (*4*−*6*). Finkelstein et al. (*4*) investigated the colonization of a sediment MFC by mineral reducing microorganisms with three different anode potentials (−58, 103, and 618 mV vs Ag/AgCl). It was found that a more positive applied potential resulted in a larger and earlier production of maximum current and that the microorganisms adapt their electron transferring system to a level just below the anode potential. Dumas et al. (*5*) reported that a more positive anode potential (0.20 V vs Ag/AgCl) favors startup when the MFC was inoculated with *Geobacter sulfurreducens*. Aelterman et al. (*2*) operated three reactors fed with acetate continuously at a poised anode potential of 0, −200, and −400 mV vs Ag/AgCl and investigated the resulting bacterial activity. The results indicated that anode potential had no influence on the startup time of the three reactors; however, an optimal anode potential of −200 mV vs Ag/AgCl exists, regulating the activity and growth of bacteria to sustain an enhanced current and power generation. Wang et al. (*6*) found the maximal current output increased from 0.42 to 3 mA when the anode potential was poised at 200 mV vs Ag/AgCl during start-up period. Torres et al. (*7*) placed multiple anodes with different applied potentials inside the same microbial electrolysis cell with a mixed inoculum. They found that anode-respiring bacteria which are similar to *G. sulfurreducens* predominated on anodes poised at more negative potentials, whereas it made up a smaller proportion of the community at the most positive potential (+0.37 V vs SHE).

It can be seen that conflicting results were reported by different researchers. It is important to note that few studies were made on the influence of anode potential on MTED

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and that the above studies lack quantitative analysis on the relationship between biomass production and energy gain for bacteria under different anode potentials. In addition, the prevailing use of microbial mixed cultures in previous MFC research makes an exact quantification of biomass production and activity of per unit biomass extremely difficult. It is clear that further research is needed to better understand the rate of $\Delta G^{0'}$ utilization for bacterial growth under different anode potentials.

To explore the mechanism of anode potential on bacterial growth and activity, we operated three sets of MFCs, each at a different applied anode potential. In addition, another set of MFCs was operated under an external resistance of 500 Ω for comparison. Under different anode potentials, the relationship between possible metabolic energy gain for the bacteria and biomass during startup of the MFCs was quantitatively analyzed from an energy perspective. Furthermore, the effect of anode potential on biocatalytic activities of bacteria and power generation was also studied. *G. sulfurreducens* is a model strain which has been most intensively studied to date. Therefore, it will be used for this study for convenient mechanisms analysis and to avoid any interference with mixed culture in biomass measurements.

Materials and Methods

Reactor Construction and Operation. Two-botttle "H" type MFCs were constructed as previously described (*8*) with the bottles separated by a cation membrane (CMI7000, Membranes International Inc., U.S.). Both anode and cathode chambers were initially sealed with rubber stoppers, and stirred slowly using magnetic stir bars at 60 rpm. Carbon paper (Toray, Japan) was used as the anode (4.0 ×2.5 cm) and graphite felt (Sanye, China) was used as the cathode (projected surface area of 8 cm^2), with graphite electrodes connected to the circuit using graphite rods. A saturated calomel electrode (SCE, 0.242 V vs standard hydrogen electrode (SHE), Leici, China) was fitted through the rubber stopper of anode chamber and used as a reference electrode.

G. sulfurreducens PCA was purchased from Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH and routinely cultured under anaerobic conditions with acetate (20 mM) acting as the electron donor and fumarate (40 mM) acting as the electron acceptor. The anode chamber was filled with medium and inoculated using 15 mL stationary-phase cultures of *G. sulfurreducens* suspension with an optical density at 600 nm of 0.590 ± 0.011. The medium (pH 7.0) contained (per liter): 4.4 g KH_2PO_4, 3.4 g $K_2HPO_4 \cdot 3H_2O$, 1.64 g CH_3COONa, 1.5 g NH_4Cl, 0.1 g $MgCl_2 \cdot 6H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.1 g KCl, and 10 mL of trace mineral metals solution. The cathode chamber was filled with 50 mM $K_3Fe(CN)_6$ solution in 100 mM KH_2PO_4 buffer (pH 7.0). Four sets of MFCs were operated at a poised anode potential of −160 mV (P_{-160}), 0 mV (P_0), or 400 mV (P_{400}) (vs SHE) and a fixed load of 500 Ω (R_{500}), respectively. For each startup condition, a set of five identical MFCs were inoculated and operated simultaneously; the MFCs were stopped one by one every 0.5 day to dynamically analyze the changes of biomass production and activity of *G. sulfurreducens* during the startup period. Biomass and electrochemical analyses were then performed after the open circuit potential was stable. In this study, each MFC operated with a control reactor identical in every aspect with the exception of being operated on an open circuit. All MFCs were operated at a temperature of 30.0 ± 0.5 °C, and each experiment was repeated two times.

Analyses and Calculations. When the reactor was operated at a selected anode potential, the anode potential was continuously poised using a potentiostat (MSTAT T8000, Arbin, U.S.) and the resulting current was recorded every 1 s. When the reactor was connected to the external resistance with 500 Ω, a data acquisition system (DAQ2213, ADLINK, Beijing, China) was used to record the voltage output and anode potential every 1 s. Let t denote a certain time point during the startup period. Then the possible metabolic energy gain for the bacteria (PMEG, expressed in units of J) during the time interval [0, t] can be calculated with the current and anode potential data. In this study, MFCs were operated under standard biological conditions (pH 7.0), so the $\Delta G^{0'}$ in eq 1 is a measure of PMEG when 1 mol CH_3COONa is decomposed by *G. sulfurreducens*. Suppose that k mol CH_3COONa were oxidized by *G. sulfurreducens* during the time interval [0, t], then

$$\mathrm{PMEG} = k\mathrm{nF}(E_{\mathrm{anode}} - E^{0'}_{\mathrm{substrate}}) \tag{3}$$

Let I_t(A) denote the current at time t(s), the charge transferred during the time interval [0, t], expressed in Coulomb (C), can be calculated by $\int_0^t I_t dt$, which equals to knF. As a result, a modified version of eq 3 with the nF replaced by $(1)/(k)\int_0^t I_t \mathrm{d}t$ was used to calculate PMEG:

$$\mathrm{PMEG} = \int_0^t (E_{\mathrm{anode}} - E^{0'}_{\mathrm{substrate}}) I_t \mathrm{d}t \tag{4}$$

As the current and anode potential were recorded every 1 s, the approximation of PMEG can be obtained as follows:

$$\mathrm{PMEG} \approx \sum_0^t (E_{\mathrm{anode}} - E^{0'}_{\mathrm{substrate}}) I_t \times 1(\mathrm{s}) \tag{5}$$

The standard biological potential of CO_2/acetate −0.29 V (pH 7.0) was selected as the $E^{0'}_{\mathrm{substrate}}$ in this calculation (*9*).

Slow rate linear voltammetry (0.5 mV s^{-1}) was used to determine the maximum power density in MFC as previously outlined (*10*). The power density was normalized to the volume of total anodic compartment. To obtain the anode resistance and limiting current, galvanodynamic linear sweep voltammetry (10 μA s^{-1}) were carried out in three electrode arrangement using the potentiostat (*11*). The anode resistance was calculated from the slope of the linear portion of the "potential∼current" curve. The limiting current was equal to the corresponding current at the inflection point of the "potential∼current" curve. Cyclic voltammetry (CV) was carried out at 5 mV s^{-1} scan rate from −0.50 to 0.50 V vs SCE using CHI 660A electrochemical working station (Chenhua, Shanghai, China) with a three-electrode arrangement. In a galvanodynamic linear sweep voltammetry and CV test, the working electrode was the anode and the reference electrode was a SCE.

The biomass of *G. sulfurreducens* was determined using phospholipid analysis as described by Aelterman et al. (*2*) The absorbance at 610 nm was then read using a spectrophotometer (DR 5000, Hach, U.S.) and the biomass concentration was expressed as the mass of phosphorus per square centimeter of carbon paper. To eliminate the interference of passive adsorption resulted from continuous stir in anode chamber, all biomass data shown in this paper were calculated by subtracting that of corresponding control reactor from the original biomass data.

Results

Current and Biomass Generation during Startup Phase. Changes in current and biomass for each startup conditions are shown in Figure 1A and B, respectively. For all conditions, the most notable trend is a gradual increase over time for both current and biomass values. P_0, P_{400}, and R_{500} produced a maximum current of 2.05, 2.07, and 0.94 mA, respectively, at about day 3. However, P_{-160} produced a maximum current of 1.32 mA near the end of day 4. Additional observation shows that P_0 and P_{400} produced similar values in current

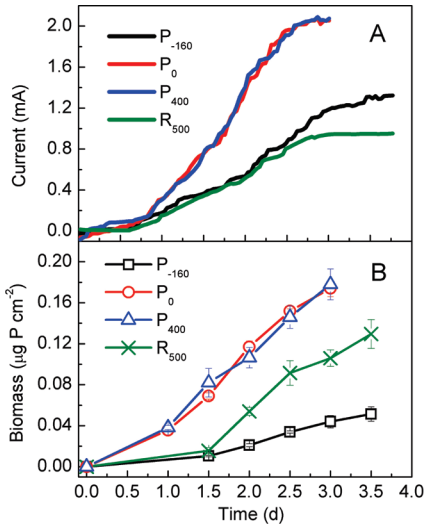

FIGURE 1. Electricity generation (A) coupled to biomass production (B) time profiles for four groups of MFCs operated at selected anode potentials of −160 mV (P_{-160}), 0 mV (P_0), and 400 mV (P_{400}) (vs SHE) and a fixed external resistance of 500 Ω (R_{500}) during startup period.

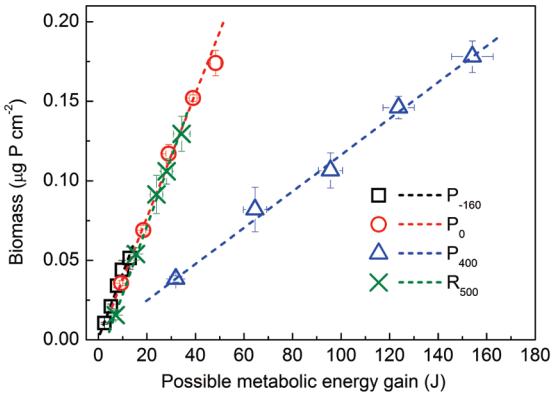

FIGURE 2. Biomass production as a function of the possible metabolic energy gained for the bacteria under different applied potential and external resistance.

and biomass during startup, much higher than those of P_{-160} and R_{500}. Therefore, the MFC at higher applied anode potential can produce more current and biomass during startup, but only up to a certain limit; the anode potential had no influence on current and biomass at 0 mV and above. This is consistent with the findings of Aelterman et al. who reported a lower concentration in biomass at low anode potential (*2*). Dumas et al. (*5*) investigated the effect of anode potential regulation on *G. sulfurreducens* biofilm formation and growth on stainless steel anodes. They also found the electrode polarized at higher potential exhibited higher currents. Another note in Figure 1 is that P_{-160} produced a higher current than R_{500} for most of the time, while R_{500} provided much higher biomass production than P_{-160}. The above phenomenon will be interpreted in further discussion on the correlation between energy gain and growth in the following section.

Correlation between Biomass Production and the Possible Metabolic Energy Gained for Bacteria Growth. In order to explore the essence of anode potential affecting biomass production, the biomass at different stages of startup were plotted against the PMEG in Figure 2. Results show a 0.99 linear correlation with biomass increasing as a function of PMEG for *G. sulfurreducens*. Note that the data for P_{-160}, P_0, and R_{500} were almost collinear, while P_{400} had a lower slope. This indicates similar percent utilization of PMEG for biomass growth at applied anode potentials of 0 mV and below, but

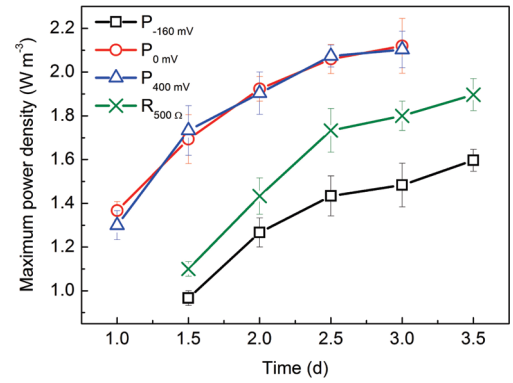

FIGURE 3. Development of maximum power density as a function of operation time.

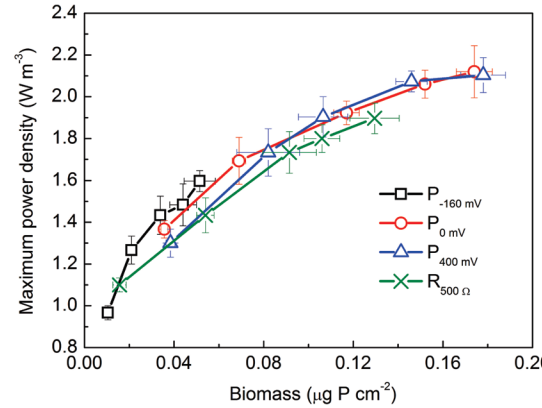

FIGURE 4. Development of maximum power density as a function of biomass.

a much lower utilization of the same PMEG at 400 mV. It also can be seen that the anode potential of R_{500} decreased gradually from 500 mV to −130 mV (vs SHE) during startup, resulting in a portion of the PMEG at the potential range of 0 mV and above probably being unavailable for *G. sulfurreducens*. However, this portion make up only 13% of the PMEG at day 3.5 (SI Figure 1S), and can therefore be neglected.

Effect of Anode Potential on Power Generation. The evolution of maximum power density with changes of operation time is depicted in Figure 3. The results show an enhancement in power generation when the applied anode potential increased from −160 to 0 mV followed by no obvious enhancement when further increased to 400 mV. P_0 and P_{400} produced much higher maximum power densities than R_{500} during startup period. Note that these curves mirror the biomass results shown in Figure 1B, which suggests a correlation between the biomass and the power generation for the MFCs operating in their respective conditions. To illustrate such correlation, a plot of the maximum power density as a function of biomass is shown in Figure 4. It can be seen that a positive correlation was found between the maximum power density and the biomass in all startup conditions. In addition, all data showed colinearity, indicating biomass as the main factor influencing power generation. We know that the power density is dependent on the dual factors of biomass and biocatalytic activity of *G. sulfurreducens* cells. Therefore, the effect of anode potential on biocatalytic activity was investigated in the following section.

Effect of Anode Potential on Biocatalytic Activity of *G. sulfurreducens*. Maximum current generation or anode resistance is usually used to measure biocatalytic activity of anode biofilm. In order to evaluate the effects of anode potential on biocatalytic activity per unit of *G. sulfurreducens* cells, the maximum current and anode resistances (as performed through galvanodynamic experiments) were both

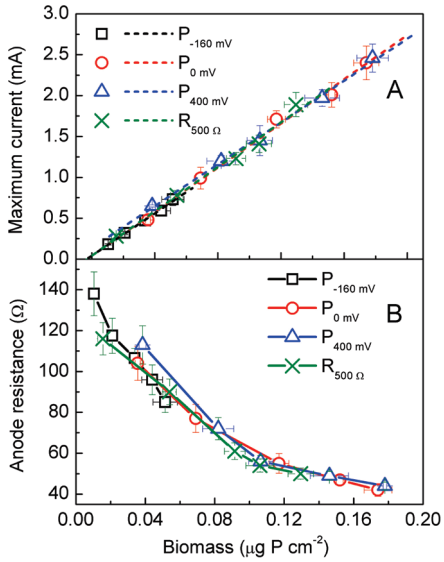

FIGURE 5. Development of maximum current (A) and anode resistance (B) as a function of biomass during startup period.

plotted against biomass as shown in Figure 5A and B, respectively. Figure 5A shows maximum current increasing as a linear function of biomass with a correlation coefficient of more than 0.99 for all the data points obtained in the four startup conditions. Generally, the limiting current produced through galvanodynamic experiments usually resulted from the limiting of substrate supply. However, in this investigation the *G. sulfurreducens* biofilm was still in its infancy with very limited biomass, allowing sufficient migration of substrate and electrons in biofilm. As a result, it was the biomass rather than the mass transfer that limited current further increase, and this limiting current reflected the maximum current generation capability of biofim. In accordance the maximum current, the anode resistance gradually decreased as biomass increased, with all the data points distributed along the same curve (Figure 5B). As we know, any difference in maximum current generation or internal resistance between MFCs of identical configuration was resulted from the differences in both biomass and activity of the *G. sulfurreducens* cells. Since biomass was the only factor in determining maximum current generation and anode resistance in this study, we can conclude that the biocatalytic activity of *G. sulfurreducens* cells was hardly affected by anode potential.

CV analysis is widely used in MFC research: the position of oxidation−reduction peaks indicated redox potential of electron transfer components of bacteria, and the size indicated electrochemical activity of colony (*12*). To determine the influence of applied anode potential on electron transfer structures and electrochemical activity, CV was performed on anode after startup. Figure 6 shows a section of CV curve in a potential range of −0.20 to 0.05 V (vs SHE). It can be seen that P_0 and P_{400} gave larger redox peaks than other MFCs, which is consistent with the biomass data. In addition, similar oxidization peaks at −0.09 V and reduction peaks at −0.12 V were observed for the four startup conditions investigated. This demonstrates that the electron transfer components of *G. sulfurreducens* were not affected by applied anode potential. These CV results are similar to those obtained by Srikanth et al. (*13*) who investigated the electrochemical characterization of *G. sulfurreducens* cells immobilized on graphite and observed the midpoint potentials of oxidation−reduction peaks centered at −0.15 V (vs SHE).

Discussion

It is known that the growth rate of microorganisms is positively proportional to the energy gained for its growth

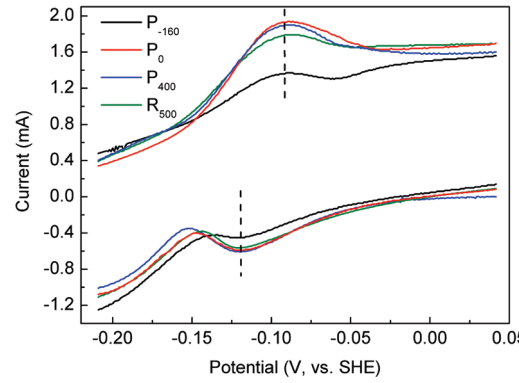

FIGURE 6. CV analysis of the anode after startup period. The vertical dotted lines indicate the position of oxidation and reduction peaks.

(*2*). Mentioned previously, the PMEG was defined by the anode potential. Therefore the essential role of anode potential lies in its regulation on energy gained for bacterial growth. The results in this study showed that the utilization rate of PMEG was not consistent with increasing applied anode potential. We can infer that only the energy liberated through electron transfer from substrates to MTED ($\Delta G^{0'}_{bio}$) can be obtained by *G. sulfurreducens*. The actual energy gain is defined by the potential of MTED, which is restricted to a value lower than anode potential. Consequently, the actual energy gain deceased with decline of anode potential when it is less than or equal to 0 mV. Within this anode potential range, we can also deduce that a significant portion of PMEG was extracted by *G. sulfurreducens* for growth, and the potential energy loss between MTED and anode potential ($\Delta G^{0'} - \Delta G^{0'}_{bio}$, as mentioned previously) was negligible. That is to say, the potential of MTED was adapted to a level just below the anode potential when the applied anode potential vary in the range of −160 to 0 mV. This interpretation follows with that of Finkelstein et al., in which the electrode-reducing microbial consortia self-regulate the potential of their operative terminal reductases to a value that is only slightly more reducing (35−55 mV depending on current) than the potential applied to the underlying electrode (*4*). In addition, a similar interpretation could be given for the results reported by Wang et al. (*6*). It is proposed that this behavior ensures adequate electrode reduction kinetics while maximizing potential energy conserved by the colony from oxidation of acetate (*4*). It also appears that a more positive anode potential imparts a greater stimulus for colonization. However, when the anode potential increased to 400 from 0 mV, the biomass production almost remained unchanged. This result suggests an upper limit of the potential of MTED, which was close to 0 mV for *G. sulfurreducens*.

The self-regulation mechanism of the potential of MTED can be interpreted by means of the direct electron transfer mechanism of *G. sulfurreducens*. The direct electron transfer requires that the respiratory chain transfer electrons from the inside of the cell to its outside, terminating in an electron donor that allows the electron to transfer to the anode (*3*). The respiratory chain consists of a series of electron-transport components which were arranged by order from low potential to high potential. When the applied anode potential vary in the range of −160 to −0 mV, to maximize its energy gain, the electron transferred to the anode through an electron-transport component with the redox potential compliant with the electrode potential. It has been reported that some outmembrane cytochrome, such as PpcA, OmcB, OmsC, OmsE, and OmsF, can function as MTED (*14*−*18*). When the applied anode potential is higher than the respiratory chain terminal potential, the electron still transferred to the anode through this terminal of the respiratory chain. The deduction that

the upper limit potential of MTED was close to 0 mV is align with that of Kim et al. (*19*−*21*), where they studied the electrochemical activity of various bacterial strains by means of cyclic voltammetry and an identical redox potential (with a mean value of 0 V vs SHE) was detected. The authors ascribed the redox activity to electron mediation via outer membrane cytochromes. Further, microscopical testing must be made in order to accurately determine the upper limit of the potential in the electron-transport component.

Although the potential of MTED was restricted by anode potential, the analysis of maximum current production, maximum power density and CV after the removal of the applied potential indicated that the biocatalytic activity of *G. sulfurreducens* was slightly influenced by anode potential. The results are explained by the electron transfer routes tending to be uniform for all the *G. sulfurreducens* cells in the absence of applied anode potential control. Consequently, the electron respiratory chain of *G. sulfurreducens* reflects adaptability to different anode potentials, and its structure and function were unaffected. Aelterman et al. (*2*) reported that the anode potential can affect the activity of the bacteria, and that a higher activity was obtained at an optimal anode potential of −200 mV vs Ag/AgCl. Torres et al. (*7*) used wastewater-activated sludge as inoculum and found the two electrodes at the lowest potentials (−0.15, −0.09 V vs SHE) showed a faster biofilm growth and produced the highest current densities. This may be due to a mixed culture being inoculated and its community structure being optimized at this potential. Therefore, anode regulation on community structure is necessary when mixed culture is applied in future research.

Mentioned previously, the maximum power density has a good positive correlation with the biomass of *G. sulfurreducens*. During steady-state, however, the balance between the growth and death rates of *G. sulfurreducens* results in a constant biomass. Thus the maximum power density is also constant. It should be pointed out that anode potential is still a key factor in deciding power generation during steady-state. The variation of anode potential has an effect on the energy gain for *G. sulfurreducens* and therefore breaks the balance between the growth and death rates. Finally, the maximum power density changes with the biomass. Based on the conclusion obtained in this research, properly increasing anode potential promotes *G. sulfurreducens* growth and therefore accelerates the startup of MFC. In addition, a relatively lower internal resistance can also obtained at the end of startup period. During steady-state, a proper anode potential (for example, connecting an external resistance similar with the internal resistance) is also preferred to keep a high biomass and therefore a high power density.

Acknowledgments

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Supporting Information Available

Calculation of the percentages of unavailable portion of PMEG when the MFC was operated at a fixed load of 500 Ω (R_{500}). This material is available free of charge via the Internet at http://pubs.acs.org.

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