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Impact of intermittent polarization on electrode-respiring *Geobacter sulfurreducens* biofilms

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HIGHLIGHTS

- Response of *G. sulfurreducens* biofilms to intermittent polarization was observed.
- Higher current produced by intermittent polarization with shorter pulse width.
- Increased current output by intermittent and constant polarization analyzed by EIS.
- Intermittent polarization increased current output but lowered columbic efficiency.

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ABSTRACT

In practice, electrochemically active bacteria such as *Geobacter sulfurreducens* do not generally have uninterrupted access to a polarized electrode to transfer electrons. This is because many strategies for harvesting energy from these bacteria to power electrical devices rely on intermittent operation. Thus, understanding how electrochemically active bacteria respond to intermittent polarization can improve future energy-harvesting technologies. The goal of this work is to determine the change in electron transfer rates of electrode-respiring *G. sulfurreducens* biofilms exposed to conditions of intermittent polarization. Intermittent polarization is simulated using cycles of polarization and open circuit of varying durations, or pulse widths. Compared with an 18-day baseline current, an elevated current response is observed for pulse widths less than 5 min. The elevated current response reaches a maximum of 1.9 mA for a 0.5-min pulse width. Impedance analysis used to separate resistive and capacitive behavior suggests that intermittent polarization results in an increase in total resistance at pulse widths greater than 5 min. Changes in biofilm capacitive behavior are found to be negligible.

1. Introduction

Bioelectrochemical systems (BES), including a wide range of biologically catalyzed electrochemical processes, are an emerging technology which could be a potential solution to energy deficiency and waste water treatment problems. In BES, microbial biofilms transfer electrons released through metabolic activities to electrodes, where they grow, using the electrodes as electron acceptors. Current production, or the electron transfer rate, indicating bacterial metabolism, undoubtedly plays a critical role in energy recovery.

A BES, like many other energy devices, requires an energy harvesting strategy to become useful [1–4]. Typically, the strategy involves a power management system that controls the BES energy output to maximize energy recovery. This is accomplished using voltage control that electrically disconnects and isolates the BES from power draw. When the BES cell voltage discharges to a cutoff voltage, the BES is disconnected. As the BES cell voltage recharges because of microbial activity, the BES is reconnected. The result of this voltage control is intermittent polarization spaced between transitional periods of open circuit. The consequence of this type of power management design has been explored previously. Dewan et al. [5] observed that the maximum power intermittently harvested from an MFC connected with an ultracapacitor to store energy temporarily was 111% higher than that of a cell operated in continuous mode. It was also demonstrated that, compared with the intermittent charging mode, an alternate charging and discharging mode led to a 22–32% increase in average current [6]. In small-scale MFCs, an intermittent loading strategy was used to improve power generation, and 78% more power was produced than under continuous loading [3].

Microbial biofilms in BES and in other physiological environments

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are conditioned to react to external changes in their environment. This is notable in the different types of biofilms observed in biofilm reactors. In the case of *Geobacter sulfurreduces* biofilms, the biofilm thickness is influenced by the electrode type, electron donor availability, operation mode (fed-batch, batch, or continuous) and operation duration. Interestingly, the thickness of these biofilms varies with the anode (or working electrode) material, from 3 to 18 μm with carbon cloth, to *c.*50 μm with graphite sticks [7], to 120–160 μm with polished "fingered" graphite [8], and to ~200 μm with glassy carbon [9,10]. Thicker biofilms, on one hand, mean more biomass, or cells contributing to electron producing and transferring, but they are not conducive to obtaining a desirable mass diffusion coefficient or substrate availability for cells at the bottom of biofilms, as demonstrated previously [9,11]. Thus, it is beneficial to use strategies that maintain mass transport in the interior of biofilms that is as favorable as that in the exterior, providing more electron donors and improving bacterial activity. For example, it was reported that stopping polarization increased effective diffusivity in *G. sulferredu*cens biofilms [11]. Cycling polarization on and off could work like a pump to improve convectional mass transfer in biofilm. This could be a practical way to increase acetate delivery to the bottom of biofilms and increase current when the electrode becomes polarized to accept electrons again.

The capacity of a biofilm to hold charge may also play an important role in how it reacts to intermittent polarization. The capacitance of *G. sulfurreducens* biofilms is well-known [12–15]; it is about 740 μF under non-turnover conditions [12], or around 620 μF/cm^2 normalized by the effective surface area of the electrodes, which is comparable to that of synthetic supercapacitors [14]. As for the capacitive behavior of biofilms, charge could be accumulated by the reduction of *c*-type cytochromes in biofilms when the system is in the absence of an electron acceptor (e.g. an intermittently polarized working electrode), and a transient current, several-fold higher than the steady state current, would be observed when the electrode is polarized again. Thus, to some extent, the cytochromes work as electron sinks to bridge the cell metabolism and electron acceptor. It has been clearly demonstrated in the literature that it is theoretically feasible to improve the electron transfer rate through periodically polarizing the electrode, i.e., operating in intermittent mode. However, we currently have limited knowledge of whether the overall electron transfer rates for *G. sulfurreducens* biofilms could increase with intermittent electrode polarization.

The goal of this work was to determine the change in electron transfer rates of electrode-respiring *G. sulfurreducens* biofilms exposed to conditions of intermittent polarization (IP). IP was simulated using cycles of polarization and open circuit of varying durations, or pulse widths. We expected that with IP, higher current would be produced because of increased capacitance and improved electron donor diffusion to the bottom of the electrode [11]. We used a three-electrode BES to grow electrode-respiring *G. sulfurreducens* biofilms continuously. The *G. sulfurreducens* biofilms were continuously polarized and, when needed, polarization was controlled in intermittent mode. Cyclic voltammetry (CV) was used to determine the behavior of the biofilms. Electrochemical impedance spectroscopy (EIS) was used to determine charge transfer resistances and capacitance, which provided important information for explaining how current generation changes with IP. Finally, we calculated columbic efficiencies under a variety of operating conditions.

2. Materials and methods

2.1. Bioelectrochemical reactor

A cylindrical bioelectrochemical reactor was constructed to grow *G. sulfurreduces* biofilm (Fig. 1) on polarized electrodes.

The working volume was 145 mL. A glassy carbon plate (SPI-Glas ™ grade 11: 25.4 mm × 25.4 mm × 2 mm) was used as the working electrode. The geometric area of the working electrode was 6.45 cm^2 because only one face of the plate was exposed to the solution. We used silicone rubber sealant (available at any local hardware store) to exclude the solution from the rear face as well as the edges. The counter electrode was a graphite rod (Sigma-Aldrich, catalog #496545) with a diameter of 6 mm, and the reference electrode was an Ag/AgCl reference. Norprene tubing (Cole-Parmer, catalog #EW-06404-14 and #EW-06404-16) was used to feed in the influent and to discharge the waste streams. Gas filters (0.2 μm) were used both at the gas inlet, supplying a mixture of N_2 and CO_2 (80%/20%), and at the gas outlet. The whole reactor except the reference electrode was autoclaved at 121 °C for 20 min, and the reference electrode was sterilized with 70% (v/v) ethanol and then rinsed with autoclaved DI water before being inserted into the reactor.

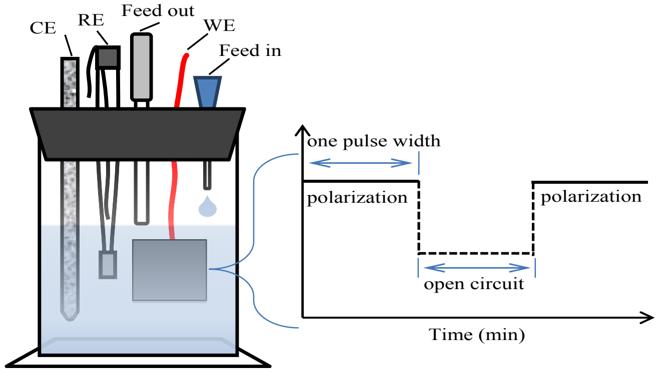

Fig. 1. Cylindrical bioelectrochemical reactor used to grow *G. sulfurreducens* biofilms on a glassy carbon electrode. Biofilms (not shown) grew on the working electrode. Gas in/out are not shown. WE: working electrode; CE: counter electrode. The diagram on the right shows one cycle of intermittent polarization. WE was periodically polarized at 0.3 $V_{Ag/AgCl}$.

2.2. Inoculum and medium

An inoculum of *G. sulfurreducens* strain PCA (ATCC 51573) was prepared using the Hungate technique as previously described [10]. The medium contained: sodium acetate, 1.64 g/L (20 mM); salts solution (100X), 10 mL/L; Wolfe's vitamin solution (100X), 10 mL/L; and modified Wolfe's mineral solution (100X), 10 mL/L. The components of the salts solution, vitamin solution and mineral solution are listed in Table S1, Table S2 and Table S3, respectively. The medium pH was adjusted to 6.8, and then 2 g/L of sodium bicarbonate was added as a buffer. Finally, the medium was autoclaved at 121 °C for 20 min and sparged with a mixture gas of N_2 and CO_2 (80%/20%) for 24 h.

2.3. Start-up of reactor

For start-up, the working electrode was continuously polarized at 0.3 $V_{Ag/AgCl}$ using an Interface 1000 potentiostat (Gamry Instruments, Warminster, PA). Initially, the reactor ran in batch mode. After one batch, the reactor was switched to continuous-fed mode, and the same bulk acetate concentration was maintained to eliminate its effect on current production. The flow rate of the inlet was 10.8 mL/h, resulting in a hydraulic retention time of 13.4 h. Finally, a steady current of 1.25 mA was obtained over 3 days, and then the reactor was ready for IP experiments. All experiments were performed in an incubator set to a temperature of 30 °C.

2.4. Intermittent polarization

IP was performed using the same potentiostat as was used for biofilm growth. Cycles of IP were tested at decreasing pulse widths. For example, 15 min of polarization was followed by 15 min of open circuit (no polarization), which represents a 15-min pulse width as shown in

Fig. 1. With a duty cycle of 0.5, we also tested pulse widths of 10, 5, 2, 1, and 0.5 min. Each pulse width was tested for 24 h. Following the 24-hr IP test, another 24 h of continuous polarization (CP) was added as a buffer for the transition between pulse width tests. We tested each pulse width twice. Finally, the reactor was polarized continuously for 18 days to determine the long-term impact of IP.

2.5. Electrochemical analysis

The total charge (Q_{total}) transferred was calculated using the following equation (1):

$$Q_{total} = \int_0^T i\,dt \tag{1}$$

where i is the current (A), t is the period of polarization, and T (seconds) is the total time of polarization performed. The total times for IP and CP were 12 h and 24 h, respectively. Average current was calculated by dividing total charge by total time for polarization, which excluded time intervals when the system was at open circuit in IP.

At the end of each condition, CV was collected, scanning from −0.7 V to 0.4 $V_{Ag/AgCl}$ at a scan rate of 10 mV per second. Also, EIS was performed, with a perturbation amplitude of 5 mV_{RMS}, and the frequency was varied from 1 MHz to 100 mHz. Square wave voltammetry was conducted to obtain a DC voltage for the EIS test. To fit the EIS data to a model, an equivalent electrical circuit as previously described was used [12,16,17]. CV and EIS data were collected using the same potentiostat as mentioned above. Echem Analyst (Gamry Instruments, Warminster, PA) was used to fit EIS data to the equivalent circuit using the simplex method. The total resistance of the system is composed of solution resistance (R1), biofilm resistance (R2), interfacial resistance (R3), and two constant phase elements (CPE) that model the total capacitive response of the biofilm. These are the biofilm CPE and the interfacial CPE, each of which has a corresponding CPE coefficient (Q) and exponential factor (α). These are the interfacial Q and the biofilm Q and the corresponding interfacial α and biofilm α. Statistical analysis of biofilm resistance, interfacial resistance, and Q was conducted using one-way ANOVA.

2.6. Columbic efficiency

Columbic efficiency (CE) was calculated using the equation: $CE = C_{Ex}/C_{Th}$, where C_{Ex} is the charge produced, calculated by integrating the current over the duration of polarization (12 and 24 h for intermittent polarization and continuous polarization, respectively); C_{Th} is the charge theoretically released through acetate oxidation by *G. sulfurreducens* biofilms, calculated as $C_{Th} = F{*}b{*}\,\Delta S\,{*}\dot{V}{*}t$, where F is Faraday's constant (96485 C/mol-e^-); b = 8, the number of moles of electrons produced per moles of acetate; t = 24 h, the duration of each experiment; $\dot{V} = 3 \times 10^{-6}$ L/sec (10.8 mL/h), the volumetric flow rate; and ΔS is the difference in acetate concentration between inlet and outlet. Acetate concentration was analyzed using HPLC (Agilent HPLC 1100 series, Agilent Technologies, CA, USA), following procedures described elsewhere [10].

3. Results and discussion

3.1. Current production

Mature *G. sulfurreducens* biofilms were used to determine the change in electron transfer rates of electrode-respiring *G. sulfurreducens* biofilms exposed to conditions of intermittent polarization (IP). The biofilms tested here maintained a constant current output for at least three days before IP was initiated. The current output of an entire sequence of IP testing is shown in Fig. S1. These results are summarized in Fig. 2A, presenting the average current observed in IP and CP modes. Fig. 2B showed the ratios of total charges transferred between IP and CP at different pulse widths. Theoretically, the ratios should be 0.5 as the duty cycle (0.5). They are actually higher than 0.5 and finally increased to 0.61 ± 0.01, which further demonstrate that IP could improve current production.

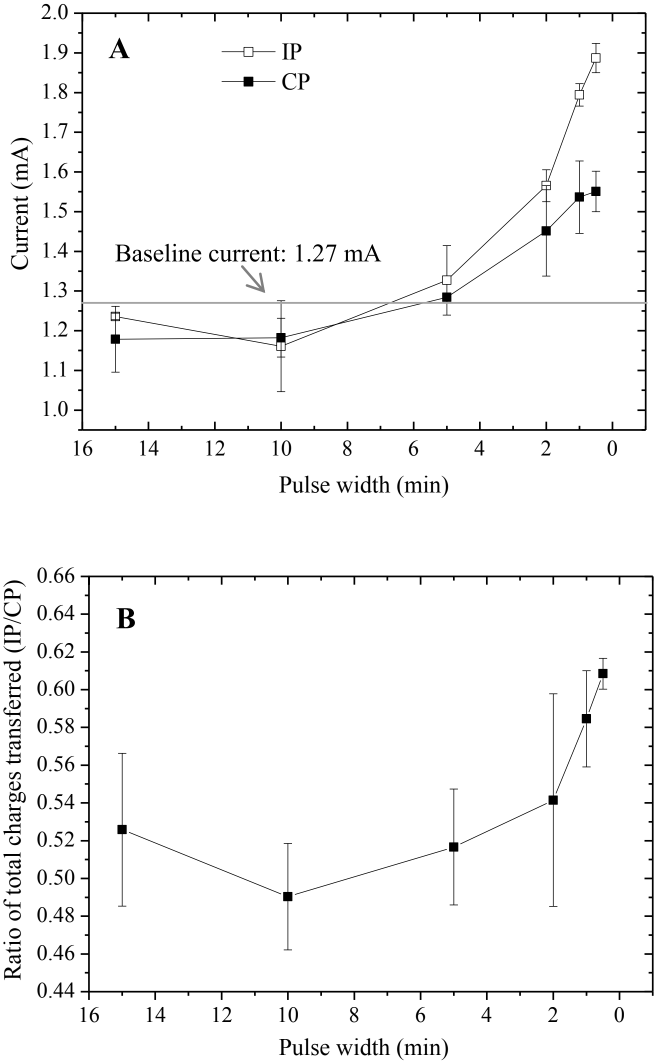

Fig. 2. Average current generated (A) and ratios of total charges transferred (B) over 24 h operation in IP and CP modes (n = 2). The gray line is the baseline current of 1.27 mA obtained over 18 days of constant polarization.

For average current, it was found that current production increased in IP and CP modes, and that IP further improved current production compared with CP in the same group. Specifically, when normalized by baseline current, 48.7 ± 2.9% more current was produced under IP with a pulse width of 0.5 min. Whereas only 22.2 ± 4.0% more current was produced under CP with a pulse width of 0.5 min, (Fig. S2). Also, the ratios of current generated under IP to that under CP in the same experimental groups suggest a gradually increased current output in IP mode within the conditions explored, and that finally up to 21.7 ± 1.6% more current was generated than in CP mode (0.5 min). In summary, shorter pulse widths led to higher current outputs. Similarly, higher current output was ascribed to the lower switching time between the charging and the discharging state of MFCs inoculated with mixed culture [4]. Moreover, the duty cycle ($t_{on}/t_{on} + t_{off}$) of IP in this work was 0.5 min, and its optimization could further enhance current generation. For example, maximum performance was reported with duty cycles between 0.75 and 0.95 for an MFC system [18].

The difference between IP and CP in Fig. 2 appears as the "pulse"-

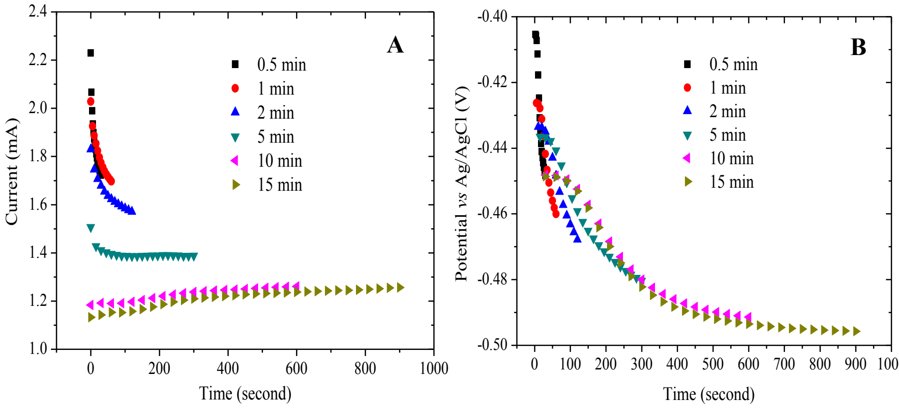

Fig. 3. (A) Current and (B) OCP profiles over one cycle of intermittent polarization performed with a variety of pulse widths. Current was observed during polarization, and OCP was observed during periods of open circuit.

like response in Fig. S1 that increases with decreasing pulse width. These long-term responses to IP are not short-term capacitive charge–discharge, as the CVs obtained at the end of each of these steps disprove this possibility (Fig. S3). Instead, the CVs show that the biofilm temporarily respired at a faster rate, which could be suppressed by a 24-hr CP interval immediately afterwards. Furthermore, the 18-day CP baseline response after IP indicates that IP made no overall permanent change in the biofilm respiration rate. This observation alone suggests that IP does not facilitate more growth in mature biofilm.

For IP experiments, more information could be obtained from current and open circuit potential (OCP) profiles. It should be noted that for clarity, only the current and OCP profile of the last cycle in each pulse width was selected as representative. For 15-min and 10-min groups, current gradually increased and almost reached a plateau when the system was polarized again after a period of non-polarization, while we observed different outcomes for the 5-min, 2-min, 1-min and 0.5-min groups (Fig. 3A). It was observed that the starting potentials during the OCP phase varied with pulse width, becoming less negative with a narrower pulse width. All OCP profiles trended more negative and finally arrived at stable potentials if the system remained non-polarized for long periods (15 min and 10 min, Fig. 3B). Generally, a longer interruption is expected to yield a higher current jump, because a longer interruption means more chance for capacitive biofilms and the interface between electrode and biofilms to store electrons that would be transferred to the electrode when it is available as an electron acceptor later. It has been demonstrated that the longer the disconnection, the more charge accumulates in biofilms of *S. oneidensis* MR-1 and *G. sulfurreducens* [15,19]. However, Gardel et al. [20] argued that in a mixed-culture sediment MFC, a shorter switching interval would lead to more charge accumulation because of less time spent in the plateau region. The capability of charge storage of a capacitive biofilm is fixed. On the other hand, the open circuit means an interruption to the biofilm's respiration. Therefore, it is expected to have a balance between "charge storage" (positive effect) and "reduced bacterial activity" (negative effect) for IP. In this work, the decreased current generation observed for wide pulse widths (15 min, 10 min) could be ascribed to the relatively long interruption to the biofilm's activity.

Narrowing the pulse width led to higher current production, as seen in Figs. 2 and 3A. It was demonstrated that both charge accumulation and discharge were accomplished during the first several seconds of polarization and open circuit mode (Fig. 3). This indicates that the current output could be efficiently enhanced using a narrower pulse width, avoiding the plateau region [15,19,20]. In other words, accumulating more charge through a longer disconnection is not necessary for obtaining a higher current when the circuit is closed again. In addition, as shown in Fig. 3A, the current does not reach a steady state with a narrow pulse width. In the OCP portion of the cycle, the biofilms do not reach the fully reduced condition. Therefore, for a narrow pulse cycle, nutrients are allowed to diffuse in while the biofilm extracellular mediators act as intermediate electron acceptors. With a wide pulse width, current is lower because biofilm activity is depressed by the lack of an electron acceptor. This is evident from the fully reduced OCP values. For example, OCP observed for a 15-min pulse width arrived at $-0.495\ \mathrm{V_{Ag/AgCl}}$. In addition to the pseudocapacitance of redox mediators, there must be other important factors improving current production which can be interpreted by means of EIS analysis.

3.2. EIS analysis

EIS was used to quantify the difference in total resistance between IP and CP modes. Total resistance comprises of contributions from the solution resistance (R1), biofilm resistance (R2), and interfacial resistance (R3). As shown in Fig. 4, total resistance increased with increasing pulse width, which likely contributed to the decrease in current production. Compared to CP, lower total resistances were observed in the of 2-min, 1-min and 0.5-min groups. This was expected to be one of the reasons for current production in IP mode. Therefore, decreased resistance resulting from the IP strategy is also an explanation for the improved current production.

R1, R2 and R3 were individually estimated to ascertain their individual contributions to the total resistance. First, R1, the solution resistance, remained constant at 20 Ω, because the same defined

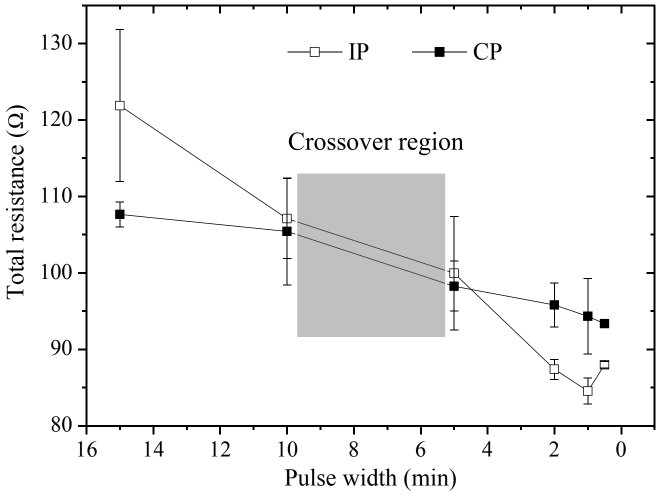

Fig. 4. Total resistance in IP and CP modes, calculated from the fittings of EIS (n = 2). Total resistance is the sum of solution resistance (R1), biofilm resistance (R2), and interfacial resistance (R3).

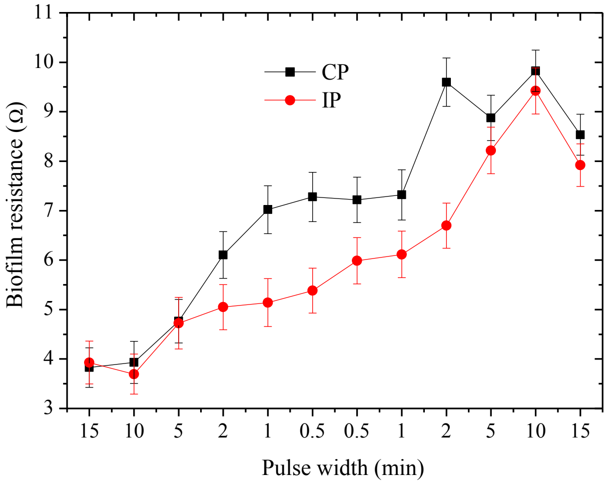

Fig. 5. Biofilm resistances for each pulse width throughout the experiments, presented as mean ± standard deviation (n = 2), estimated from fitting the equivalent circuit to the biofilm impedance spectra.

medium solution was used throughout the experiments. This also served as a consistency check of our EIS model fit and parameter estimation.

The biofilm resistance (R2) gradually increased from 3.7 ± 0.4 Ω to 9.8 ± 0.4 Ω (Fig. 5). The increase in R2 with increased pulse width may be due to thicker biofilms causing an acetate limitation [11,21]. Increased thickness could increase biofilm resistance through an increased electron transfer distance. In general, when operating conditions were switched periodically, from IP to CP, a wave-shaped resistance profile was obtained and smaller biofilm resistances were obtained at the end of IP experiments than at the end of CP experiments in the same group (Fig. 5). Also, slightly decreased biofilm resistances were observed under IP conditions, indicating that biofilm activity partially recovered with an IP strategy. Thus, the reduced biofilm resistance could be one reason for the higher current collected under IP conditions (Fig. 2). On the other hand, compared with the solution resistance and interfacial resistance of this system (Fig. 6), the biofilm resistance is very low, which could be attributed to the high conductivity of *G. sulfurreducens* biofilms [22]. In general, IP reduced biofilm resistance and thus improved current and charge transfer. Currently we don't know the fundamental reasons behind this. It is possible that the IP mode of operation promotes cytochrome or nanowire production compared to the CP mode.

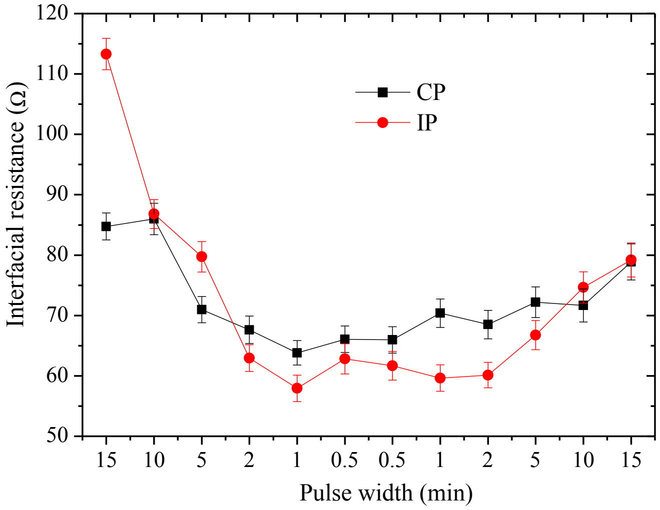

Fig. 6. Interfacial resistances for each pulse width throughout the experiments, presented as mean ± standard deviation (n = 2), estimated from fitting the equivalent circuit to the biofilm impedance spectra.

The interfacial resistance (R3) first decreased and then gradually increased to the initial level (Fig. 6). The average interfacial resistances decreased in both IP and CP modes from the 15-min group to the 0.5-min group. Also, interfacial resistances in IP mode were lower than those in CP mode for the last three groups (2-min, 1-min and 0.5-min), while the opposite results were observed for the first three groups, suggesting that a relatively narrow pulse width leads to lower interfacial resistance, which is another reason for the higher current production with the IP strategy (Fig. 2, Fig. S1). Furthermore, using one-way ANOVA, interfacial resistances in IP mode and CP mode were found to be significantly different ($P < 0.05$), suggesting the IP mode changed interfacial resistance. Recent studies found that increased interfacial electron transfer resistance lowered power density [23,24] and that the localization of phenazines, redox mediators for extracellular electron transfer, at the electrode surface lowered interfacial resistance and facilitated electron transfer in an MFC inoculated with *Pseudomonas aeruginosa* [24]. For *G. sulfurreducens* biofilms, the reduced interfacial resistance observed in this work is probably due to the denser biofilms near the bottom (or electrode surface) [11,12] and increased OmcZ, which is an essential *c*-type cytochrome. It has been found that deletion of the gene for OmcZ dramatically increases resistance for electron transfer from cells to electrode [25,26]. Decreased interfacial resistance in both IP and CP modes is expected to increase current production.

It has been demonstrated that capacitive biofilms can store electrons through the reduction of redox mediators when the electrode is nonpolarized. In practical terms, this effect is realized when the open circuit potential decreases to more negative potentials. These stored electrons can be immediately transferred to electrodes once the electrode is polarized again. Thus, the capacitive response of the biofilm under IP is expected to be an important source of power generation. The capacitive response is estimated using a CPE element with two fit parameters, the CPE coefficient (Q) and an exponential factor (α). Similar to the total resistance, the total capacitive response is composed of a biofilm and an interfacial capacitance. Fig. S4 shows the effect of pulse width on the biofilm and interfacial Q as well as on the biofilm and interfacial α. Consistent with previous results, the interfacial Q was around 500 times higher than the biofilm Q for both IP and CP conditions. Biofilm and interfacial α did not vary with pulse width and held a consistent value of ~0.8, which indicates that CPEs modeled capacitive behavior. As expected, biofilm Q did not change with IP or CP experiments because pregrown biofilm was used for the tests ($P < 0.05$, one-way ANOVA); this is consistent with previously published results [12]. Interestingly, it was observed that interfacial Q remained stable in CP mode but increased by 33.8% finally in IP mode, suggesting that IP mode changed interfacial Q especially for the last four pulse widths (5 min, 2 min, 1 min and 0.5 min). Therefore, the increased interfacial capacitance is also a positive factor for the improved current production in IP because more electrons can be stored when the system is disconnected and higher current is observed when the system is connected again.

3.3. Columbic efficiency

In this work, we also quantified columbic efficiency to determine whether the increased current and lower total resistance affected the columbic efficiency of the biofilms. Specifically, columbic efficiencies first decreased to 26.6 ± 2.6% and 38.7 ± 1.6% in IP and CP, respectively, for the 10-min group. Afterwards, both increased for the 10-min to the 1-min groups, and finally slightly decreased again for the 0.5-min group (Fig. 7). Similarly, slightly higher columbic efficiencies were observed in CP mode. This is mostly attributed to the half duration (12 h) of polarization for IP mode, in contrast to the 24 h of polarization for CP mode, and to fewer total coulombs being generated in IP mode

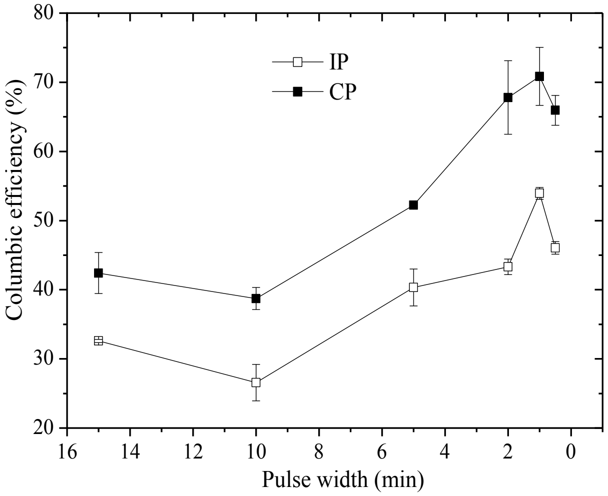

Fig. 7. Columbic efficiencies under IP and CP conditions (n = 2).

even though a higher current was produced (Fig. 2). Interestingly, the changing patterns of columbic efficiencies are similar to those of average current outputs (Fig. 2).

Overall, it was suggested that *G. sulfurreducens* biofilms can continue oxidizing and releasing electrons even when polarization interruptions are conducted (open circuit), but slower than under polarized conditions. Thus, electrons produced during the period of open circuit were stored in the *G. sulfurreducens* biofilm and transferred to the working electrode once the electrode was polarized again, leading to a jump in current. In short, the IP mode produces higher current while the CP mode enables BES to reach higher CEs. Also, the narrower pulse width had a positive effect on the CEs (Fig. 7), because it had the advantage of allowing fresh nutrient to diffuse into the biofilm, maintaining favorable biofilm activity. Conversely, the wider pulse width reduced biofilm activity and more electrons were shunted to cell maintenance, which resulted in low CE.

In addition, heterogeneity of biofilms will result in different reduction rates of electroactive species (e.g., acetate), suggesting that denser biofilms near the electrode will represent a faster reduction rate [11,21]. Furthermore, for *G. sulfurreducens* biofilms with a depth of ~200 μm, the diffusion coefficient decreases with depth, which suggests that inner biofilms are more inclined to suffer from acetate-limiting conditions [10,11]. Recently, Atci et al. [9] directly demonstrated that acetate concentration decreased with the depth of *G. sulfurreducens* biofilms, resulting in acetate limitation for cells near the bottom when the circuit was on, while there was no difference in acetate concentration between biofilms and bulk solution when the circuit was off. Therefore, IP may alleviate the acetate diffusion limitation in mature biofilm and allow a high initial current for each new repolarization cycle [5]. However, acetate limitation may result in less effective biofilm, especially in the inner part of biofilms [21]. Therefore, different acetate availabilities between the IP and CP modes is a possible explanation for the difference in current production.

Our IP results indicate that biofilm operates in an inefficient manner when exposed to CP. This suggests that the maximum electron transfer rate is slightly inhibitory. In terms of cell behavior, this is attributed to the overdraw of nutrients within the biofilm and difficulty with which nutrients can penetrate the interior of the biofilm. In the case of conductive biofilms, the balance will likely fall between the rate of discharge of the capacitance and the rate of diffusion of nutrient into the biofilm. At the same time, the metabolic rate of the biofilm will also respond to these changes. Such a balance suggests an optimal pulse width exists for maximum current production. This is therefore a three-part challenge to optimizing an energy harvesting strategy.

4. Conclusions

In this work, we discovered that there was an optimal pulse width during IP that maximized electron transfer rates. Our results revealed that both the IP and CP modes increased current in comparison with baseline current, and IP even further enhanced current outputs. EIS analysis suggested that for IP, reduced interfacial resistance and increased capacitive ability led to increased higher current, while for CP, the increased current output is caused by a decreased interfacial resistance. Furthermore, lower biofilm resistance and higher interfacial capacitance in IP contributed to the further increased current production in IP over CP. It should be noted that there was no difference in solution resistances, presenting no effect on current production. Columbic efficiencies were higher in CP than in IP. Thus, while IP improves energy harvesting, CP improves columbic efficiencies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpowsour.2018.10.053.

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