Flow-based deacidification of *Geobacter sulfurreducens* biofilms depends on nutrient conditions: A microfluidic bioelectrochemical study

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**Abstract**

Biofilms from *Geobacter sulfurreducens* are promising materials for new bioelectrochemical systems. To improve the performance of such systems, limitations related to biofilm acidification should be addressed. This work examines a long-held assumption that liquid flow can deacidify biofilm pH by enhancing molecular mass transport in the biofilm subdomain. A microfluidic electrochemical system was used to measure changes to biofilm pH in situ while accurately modulating hydrodynamic conditions under turnover, nutrient-limited and starvation conditions. We discovered that increased flow rates could indeed mitigate biofilm acidification, but not under turnover concentrations, which are the predominant conditions used in research studies. This effect is demonstrated with the observation that relative increases to bio-current under increased flow rates were stronger for experiments conducted under nutrient-limited concentrations compared to turnover concentrations. This could potentially open the way for a solution to poor performance of some bioelectrochemical systems at low concentrations.

**Introduction**

Biofilms from electro-active bacteria are a topic of intensive research as new biocatalysts for green chemistry with applications in waste remediation, energy production, and synthesis of chemical compounds and nanomaterials. For electrode-adhered electro-active bacteria such as *Geobacter sulfurreducens*, substrate oxidation occurs in parallel with electron conduction through an extracellular bioconductive network. *G. sulfurreducens* are among the most efficient electroactive species due to their direct electron transfer mechanism via conductive appendages and between redox groups within the densely packed biofilm. This process is coupled with proton production to maintain electroneutrality, which can cause acidification in the biofilm due to reduced diffusivity. In turn, this acidification can limit bacterial metabolism and system performance. For example, biofilm acidification has been shown to reduce power output in microbial fuel cells (MFCs) and to limit the efficiencies of other bioelectrochemical systems.

In biofilms from *G. sulfurreducens*, complete metabolic inhibition can occur at pH values lower than 5. Therefore, strategies that can reduce biofilm acidity in bio-electrochemical systems are an important goal. It has been proposed that flow through systems might deliver performance improvements due to deacidification related to enhanced mass transfer of either acidic byproducts out of the biofilm or deprotonated buffer molecules into the biofilm. Similar claims have been made for bulk MFCs in the presence of stir-bar agitation and for variable-speed rotating-disk electrode measurements. However, to date, the hypothesis that flow can reduce biofilm acidity remains unproven experimentally. Moreover, even if flow can cause biofilm deacidification, its effect on current production compared with that of flux-enhanced nutrient availability should be explored. To address this question, strict control over flow and chemical concentrations should be implemented while conducting *in situ* measurement of pH changes.

The field of microfluidics has introduced new research opportunities to the life sciences and biotechnology due to its ability to precisely control culture conditions without exposure to ambient conditions. Specifically, flow rate and related hydrodynamic properties (shear stress, flow velocity, nutrient flux) can be manipulated over a large dynamic range while limiting the consumption of nutrient solutions and extending the experimental duration. Combined with the ability to rapidly adjust concentrations and other physiochemical parameters, complex multi-step experimental sequences can be devised. However, *in situ* measurements of changing pH within microchannels are not straightforward. Microfluidic designs that include embedded miniature pH electrodes have been demonstrated for measurement of liquid analytes but challenges include the fragility of typical porous glass materials used in these designs, mismatches in geometry with channel walls, and the likelihood of

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Supporting information for this article is given via a link at the end of the document.
disruptions to local flow conditions. Moreover, long-term growth around the non-conductive tip of an embedded pH probe are not be representative of a typical electrode-adhered biofilm. pH-sensitive fluorophores have been used in characterisation of biofilms in MFCs, but they have only been demonstrated for short-term measurements following biofilm maturation, likely due to problems associated with biocompatibility. Recently, nanoparticles capable of sensitive metal-enhanced florescence of embedded pH-sensitive fluorophores have been exploited for microfluidic studies of intact lactic acid-producing oral biofilms. Covalent attachment of the nanoparticles to the biointerface prevented washout, and fluorophore confinement in a silica shell enhanced photo-stability, sensitivity and isolation from the biological environment, but in the present application this approach would require nanoparticle attachment to the electrode surface, which could interfere with electron transfer. Potentiostatic techniques, on the other hand, are very promising for this application. First, the use of such techniques, generally, for in situ electrochemical measurements in microfluidic channels is growing due to straightforward approaches to electrode miniaturization and integration, as well as a diversity of techniques that can be applied with the same experimental setup. Second, the existing working electrode (WE) could also act as the sensing surface, thereby avoiding the need for foreign chemical probe molecules or additional sensors. In bulk liquid cells, cyclic voltammetry (CV) has been demonstrated as an effective technique for observing pH-induced shifts in the formal potential \( E_r (V) \) (arithmetic average of the cathodic and anodic redox peaks) for proton-involved redox processes (Eq. 1),

\[
[H_2Red] \rightleftharpoons [Ox] + ne^- + mH^+
\]

where \([H_2Red]\) and \([Ox]\) are the respective concentrations of the initial redox species in its reduced and oxidized forms, with \( n \) and \( m \) denoting the number of electrons and protons involved, respectively. In metabolically active Geobacter spp. biofilms, redox changes to cytochrome c are coupled to other bioelectrochemical steps in bacterial respiration. Changes to \( E_\text{R} \) of cytochrome c groups involved in the electron transfer chain can be an indicator of the biofilm pH (pH\text{E}) if they are coupled to H\text{m} production steps, such as in Eq. 1. It is important to note that this technique is sensitive to net \( n \) over the entire electroactive biofilm rather than local pH gradients within different layers. In general, the dependence of \( E_r \) on pH for the redox reaction in Eq. 1 is given by the Nernst equation or its simplified form (Eq. 2) when \( m = n \):

\[
E_r = E^0 - (0.059 \times \text{pH})
\]

where \( E^0 \) (V) is the cytochrome c redox potential at pH = 0. Therefore, pH changes (\( \Delta \text{pH} \)) can be quantitatively tracked by monitoring \( \Delta E_r \). In the present case, Eq. 2 indicates that \( E_r \) increases represent biofilm acidification and vice versa. Under hydrodynamic conditions, the combined effects of flow-controlled exchange of nutrient, buffer and acidic byproduct molecules on the changes to biofilm pH (\( \Delta \text{pH} \)) can be studied as a function of the flow rate of a typical acetate (Ac)-based nutrient solution. We note that although the flux of ions might affect, somewhat, the redox reaction kinetics, they do not affect \( E_r \).

**Results and Discussion**

To better understand how the bulk flow of a typical Ac-based nutrient solution affects the pH of a G. sulfurreducens biofilm, a three-electrode microfluidic system was used. The reader is directed to previous work that discusses the fabrication protocol to insert the electrodes flush against the microchannel wall in a similar device used for electrochemical impedance spectroscopy of non-electroactive biofilms. Figure 1a shows the device schematic, including placement of electrodes and direction of liquid flow. Figure 1b shows an electron microscopy image of the orientation of a mature biofilm on the WE following an experiment. The flow rate and acetate concentrations ([Ac]) were controlled via programmable syringe pumps and upstream valves. To verify the stability of the gold pseudo reference electrode (RE) throughout the experiment, we collected cyclic voltammograms for a redox solution consisting of 10 mM potassium ferriyanide. The redox molecules were added to the same nutrient medium used in the remainder of the experiments to ensure that ions and support molecules were present during the test. The position of the redox peaks in the CV curves were unchanged during the two-week experiment, indicating the stability of the gold RE. Changes to the flow rate also did not affect the position of the redox peaks (\( \Delta E_r = 0 \)), as expected, because proton concentration was not changed as part of the redox process involving ferriyanide (Figure S2). As discussed earlier, cytochrome c proteins in an inactive biofilm also had a value of \( \Delta E_r = 0 \) during flow rate cycling which again was expected because metabolic inactivity resulted in no proton production. In addition, changes to [Ac] did not affect RE performance. To ensure that the experimental conditions did not affect the microfluidic three-electrode flow cell performance, the RE was placed at the furthest upstream point such that it was always contacted by fresh nutrient solution. Byproducts from half-cell reactions at the WE and the counter electrode (CE) were carried downstream and did not contaminate the liquid environment around the RE because the upstream diffusion of H\text{m} was slower than the downstream convection (see Table S1 for mass transfer calculation results). The electrode configuration which placed the RE upstream were it could be exposed to a constant flux of fresh nutrient media has been shown previously as a method for achieving stable performance from an Au pseudo RE for non-electroactive bacteria and their biofilms. For electroactive biofilms used here, stability was enhanced because G. sulfurreducens bacteria could not complete the Krebs cycles via electrode respiration at the RE because no current is permitted to flow through it. As a result, the RE remained completely free from bacterial contamination. This observation was verified by SEM and resonance Raman spectroscopy following the experiments, as shown previously. Thus, under regular experimental conditions (Q = 0.2 mL h\textsuperscript{-1}, [Ac] = 10 mM), the measured \( E_r \) for cytochrome c was stable. We note that a different
The electrode arrangement may be required if the experiment involves changes to liquid phase chemistry during the experiment. For example, a three-electrode microfluidic microbial cell was recently demonstrated with an Ag/AgCl RE under a constant co-flow of electrolyte solution and the applied chemical changes being applied only to the WE, enabling excellent, long-term measurement stability of G. sulfurreducens biofilms. However, care would have to be taken to ensure that the co-flow interface is stable during large changes to flow rate as we do here.

Inoculation of the microfluidic device was conducted by injection of the fumarate-containing G. sulfurreducens inoculum solution through the connective tubing using a 10 mL gastight glass syringe. The role of fumarate as a soluble electron acceptor allowed the bacteria to survive in planktonic form. Inoculum flowed for 3 h at Q = 0.5 mL·h⁻¹ while the WE was held at 410 mV vs. Au, approximately 0 mV vs. Ag/AgCl (see Figure S1). This potential was sufficient to oxidize cytochrome c, thus enabling electrode respiration. Subsequently, the source was changed to a 50 mL gastight syringe containing fumarate-free nutrient medium ([Ac] = 10 mM) solution flowing at Q=0.2 mL·h⁻¹, without changing the potential at the WE. In the absence of dissolved fumarate, bacterial growth continued exclusively on the WE while the current was monitored via chronoamperometry (CA). Figure 2a is a typical dataset showing current increase collected using CA during the initial biofilm growth. We note from separate experiments that cycling between Q = 0.2 and 1 mL·h⁻¹ at turnover concentrations ([Ac]ᵣ = 10 mM) reproduced previous observations that variations in hydrodynamic conditions could affect the current output (Figure 1C).

After observing the expected response of the G. sulfurreducens biofilm in the microfluidic electrochemical flow cell, our next goal was to verify that changes in Q could only affect $E_\text{f}$ for metabolically active biofilms. First, after an initial growth period of approximately 160 h, we changed the nutrient concentration to [Ac] = 0 mM from [Ac]ᵣ (while maintaining Q = 0.2 mL·h⁻¹) and the current began to drop (Figure 2a, asterisk region). In separate experiments, the rate at which the current dropped was related to Q (data not shown), indicating a flow-dependent mechanism for the elimination of nutrient reserves trapped in the biofilm. This could indicate mass transfer enhancement by convection through the biofilm subdomain, especially due to the head-on approach of the liquid. After 6 h at Q = 0.2 mL·h⁻¹, the current had decreased to nearly 15% of its pre-starvation value. We then began to acquire slow-scan cyclic voltammograms (CV; in Figure 2a) to observe the effect of Q on redox potentials. A baseline subtraction protocol was applied to identify the cytochrome c formal redox potential. Two redox centres were identified with formal potentials of $E_\text{f1} = -25$ mV and $E_\text{f2} = 55$ mV vs. Au. Based on calibration measurements (Figure S1), these values matched

![Figure 1](image-url)

**Figure 1.** (a) Schematic of a three-electrode glass-sealed microfluidic flow cell with dimensions 2 mm width, 400 µm height and 30 mm length. The system consists of graphite working (WE) and counter-electrodes (CE) and a gold pseudo-reference electrode (RE). (b) Cross-sectional view of the microchannel with the sealing glass on top (purple) and an electrode on the bottom. Inset (below) shows an SEM image of the G. sulfurreducens biofilm at the downstream edge of the WE acquired after the end of an experiment. (c) Changes to current outputs (I) for biofilms subjected to solutions with [Ac] = 10 mM during modulations of their flow rates to between Q = 0.2 and Q = 1 mL·h⁻¹ (green and red arrows, respectively, shown for the first flow cycle).
the reported potentials for outer membrane cytochrome c groups (OmcB and OmcZ) and periplasmic cytochrome c (PpcA), with values of $E_{f1} = -435 \text{ mV}$ and $E_{f2} = -355 \text{ mV}$ vs. Ag/AgCl, respectively. The separation of reduction and oxidation peaks for $E_{f1}$ and $E_{f2}$ produced values of 64 and 56 mV, respectively, demonstrating fully reversible electrochemical processes. Cycling Q during this time interval resulted in reversible changes to $E_f$ (Figure 2b). According to theory, changes to $E_f$ should occur if the redox reaction involves a change to $[H^+]$, according to Eq. 1. This implies that the biofilm maintained some metabolic activity, even under starvation conditions. G. sulfurreducens biofilms are known to retain a certain amount of metabolic activity for similar durations following a switch to starvation conditions, due to limited nutrient reserves, so temporary sensitivity of $E_f$ to Q was not surprising. Exposure to $[Ac] = 0 \text{ mM}$ for another 16 h resulted in true biofilm starvation, including complete loss of metabolic activity and CV measurements (CV in Figure 2a) of $E_f$ which were flow-independent (Figure 2c). We concluded that flow-based changes to molecular mass transfer between the biofilm and the liquid phase could not cause changes to $E_f$ unless the biofilm was metabolically active and $H^+$ production was coupled with the cytochrome c redox process as a part of the overall biofilm electron transfer chain. This result supports the previous observation that changes to hydrodynamic conditions did not affect measurements for an inactive G. sulfurreducens biofilm. See the Supporting Information for additional discussion of the differences between true starvation and temporarily reduced activity following either a switch to nutrient-free conditions or to nutrient-limited conditions. Finally, the system was returned to $[Ac]_0, ([Ac] = 10 \text{ mM}$), and the current slowly recovered to pre-starvation levels after 24 h, and continued growing after that (Fig. 2a).
changing between Q = 0.2 (blue and black) and Q = 1 mL·h⁻¹ (red). The inset shows baseline subtraction acquired at Q = 0.2 mL·h⁻¹ to clarify the position of the electrochemical redox potentials. Green and orange arrows point to redox peaks associated with the first and second redox centers (E₁ and E₂), respectively. The positions of E₁ and E₂ (green and orange dots, respectively) were calculated using the arithmetic mean of the reduction and oxidation electrochemical potentials of each redox center. (c) Non-responsive formal potential under same flow rates as in (a) after continuation of starvation conditions for another 16 h (CV₂). In both (b) and (c), CVs were collected with scan rate (1 mV·s⁻¹) after a flow stabilization period of 15 min.

The next goal was to quantify the flow-based changes to E₁ from active biolms under a typical nutrient solution with [Ac]₀ and nutrient-limited Ac concentrations ([Ac]₁₈ = 0.3 mM). First cyclic voltammograms obtained under [Ac]₀ displayed a sigmoidal shape (Figure 3a), which is typical due to the large catalytic current. As expected, a first-derivative plot showed a single redox centre at E₁ = 30 mV vs. Au (corresponding to -385 mV vs. Ag/AgCl), which was the average of E₁ and E₂ from above. Second, increasing the flow rate from Q = 0.2 mL·h⁻¹ to Q = 1 mL·h⁻¹ resulted in a small increase in the limiting current, but E₁ remained constant (Figure 3a, inset). We continued to increase Q until a slight but statistically significant shift to lower potentials could be detected (ΔE₁ = -18 mV) at Q = 5 mL·h⁻¹. According to Eq. 1, the shift to more negative potentials indicated biofilm deacidification. Third, the nutrient conditions were switched from [Ac]₀ to [Ac]₁₈ using an upstream valve while maintaining the flow rate at Q = 0.2 mL·h⁻¹. We acquired CVs after the system stabilized under the new nutrient conditions. As shown in Figure 3b, under [Ac]₁₈, E₁ was easily reduced even with modest increases to Q, indicating increases in biofilm pH (pHₐ). For example, changes as great as -125 mV at Q = 2 mL·h⁻¹ were observed. The shift in E₁ was reversible, returning to higher values when Q was reduced, thus indicating a return to relatively acidic conditions.

According to Eq. 2, changes to E₁ under different values of Q could form the basis for study of the hydrodynamic effects on changes to pHₐ (ΔpHₐ). To verify the experimental changes to E₁ for biolms used here with the known solution pH (pHₐ), we followed the typical procedure reported in the literature. Briefly, a 130 h biolm grown under [Ac]₀ was subsequently exposed to an Ac-free medium in a standard bulk electrochemical cell. After reaching 10% of the original current, the medium was switched to another Ac-free medium with pHₐ values set at 6, 7, and 8, and cyclic voltammograms were acquired. Under these conditions, the low current generated by the relatively young biolm should have significantly limited the production and accumulation of H⁺ in the biolm, as reported previously. Therefore, pHₐ and pHave were expected to be nearly the same. However, due to the temporary activity retained by the biolm in the [Ac] = 0 mM solution, as discussed above, E₁ still maintained pHₐ dependency. As is typical for such calibration experiments, these were performed in a bulk (static) electrochemical cell to accommodate a Ag/AgCl probe, thereby maintaining a consistent reference as the solution-phase pH was adjusted. See the Supporting Information (Section 1) for additional details on the electrochemical setup used in calibration measurements.

Figure 4a shows the CV curves which shifted to lower potential with reduction of the imposed pHₐ in the range of 6 to 8. From
repeated experiments, the average $E_r$ was obtained for each pH value, and a calibration plot was constructed. As shown in the inset of Figure 4a, we observed a linear relationship between $E_r$ and pH, as expected from Eq. 2. A fitting algorithm produced a slope of -52.5 ± 1.4 mV/pH unit. This value compares well with the predicted value of -59 mV/pH unit from the Nernst equation (ie. Eq. 2) and the previous values measured using the same technique.\(^{33,24}\) This result also reinforces our observations above that the electrochemical process was reversible. The slope of the calibration curve could be universally applied to accurately predict the changes in the net biofilm pH ($\Delta p$H\(_b\)) based on $\Delta E$ values, independent of the choice of RE. We note that it has recently been shown that good correlation can be achieved between $E_r$ of cytochrome c and net pH\(_b\) of a G. sulfurreducens biofilm in standard nutrient solution.\(^{26}\) However, $\Delta p$H\(_b\) was analysed in this work instead of pH\(_b\) to avoid possible complications arising from small measured offsets in the applied potentials with use of different gold REs from device to device or the possibility of slight differences between pH\(_b\) and pH\(_s\) during the calibration step described above.

Next, using $Q = 0.2$ mL·h\(^{-1}\) as a lower limit, $\Delta p$H\(_b\) was determined from measured $E_r$ values at different flow rates. Each experiment was repeated on separate days and consisted of the acquisition of three sequential CVs after flow stabilization. The $E_r$ values from the second and third CVs from all three experiments were averaged together. Thus, each data point and its respective error bar (Figure 4b) was result of a total of 6 measurements of $E_r$ acquired over three separate days. The results acquired matched other preliminary measurements that were conducted on separate biofilms. Figure 4b shows the effect on $\Delta p$H\(_b\) from Q cycling under turnover and nutrient-limited conditions. Under turnover conditions, pH\(_b\) was nearly unchanged over a wide range of flow rates, likely because the kinetics of acidic byproduct production were too fast to be washed out or neutralized by buffer molecules, even at elevated flow rates. In fact, increased flow rates would have actually increased the nutrient flux through the channel ($J = Q \times [Ac]$) and the related nutrient availability, thus further increasing the $H^+$ production rate in parallel with higher measured electrical currents. On the contrary, increased flow rates did efficiently influence the pH\(_b\), when the liquid medium was nutrient-limited. This was likely because the lower rate of $H^+$ production could more easily be washed out or neutralized by the higher influx buffer molecules. We note that the $\Delta p$H\(_b\) of nearly 1.5 at high flow rate nearly matches the difference in pH measured previously between the nutrient solution and the mean pH\(_c\).\(^{25}\) Thus, we tentatively conclude that a high flow rate of a nutrient-limited solution can almost completely de-acidify the biofilm.

Figure 5 summarizes the flow-based factors that might have caused the observed increase in current ($\Delta I$) for different nutrient solution concentrations. For $[Ac]_{lim}$, positive values of $\Delta I$ upon changing the flow rates (eg. Figure S5) from low ($Q_1$) to high ($Q_2$) were not accompanied by obvious changes to biofilm pH ($\Delta p$H\(_b\) = 0). Therefore, in this case, $\Delta I$ was due to increases to bacterial activity resulting from biofilm deacidification but was instead likely due to increases in Ac flux through the microchannel only ($\Delta J = (Q_2 - Q_1) \times [Ac]_{lim}$). However, cycling the flow under $[Ac]_{lim}$ conditions would have caused changes to both the nutrient availability via increases to Ac flux through the microchannel ($\Delta J = (Q_2 - Q_1) \times [Ac]_{lim} > 0$) and the biofilm deacidification ($\Delta p$H\(_b\) > 0). This dual effect could explain why $\Delta I$ values from Q cycling of nutrient-limited solutions were so similar to those obtained under turnover concentrations, despite having [Ac] and Ac flux that were nearly 30 times less (Figure S5). In fact, taken as a fraction of I at the slow flow rate ($I_1$), the $\Delta I/I_1$ was much more than double for nutrient-limited conditions compared turnover conditions (see Section 7 in Supporting information). Therefore, we predict that bioelectrochemical systems based on G. sulfurreducens and potentially other electroactive biofilms, could disproportionately
benefit from operation under hydrodynamic conditions when nutrient concentrations are low. This could potentially open the way for a solution to poor performance of some bioelectrochemical systems at low concentrations.40

Conclusion

This work examined the role of bulk liquid hydrodynamic conditions and nutrient concentrations on changes to pH within electrode-adhered G. sulfurreducens biofilms. A three-electrode microfluidic flow system was used to accurately modulate the flow rates while conducting cyclic voltammetry measurements in situ. By following the pH-sensitive changes to the formal potential of cytochrome c, it was discovered that changes in hydrodynamic conditions could affect the acidity of G. sulfurreducens biofilms. However, flow rates were only effective in modulating acidity under nutrient-limited concentration conditions or temporarily, before the biofilm became completely inactive, after exposure to starvation conditions. Thus, previous assumptions that pH changes in electroactive biofilms could have contributed to higher current production when subjected to hydrodynamic conditions should be re-evaluated. Future studies should focus on the role of biofilm thickness, density and structural heterogeneity in preventing changes to pH with by elevated flow rates of nutrient solutions at turnover concentrations.

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Summary of the effects of hydrodynamic cycling on ΔpH and proposed factors (Italics) leading to a higher electrical current from electroactive biofilms in a microchannel. Deacidification is a general term that includes wash out of acidic byproducts and increased neutralization by buffer molecules from the nutrient solution. The biofilm is shown protruding into the cross-flow stream from a continuous segment of the microchannel wall consisting of an embedded working electrode and the surrounding PDMS (cross-hatched and orange, respectively).

Experimental Section

1. **Bacterial preparation**

Frozen samples of Geobacter sulfurreducens (strain PCA, ATCC 51573) were cultured in an anaerobic glove box for 7 days and subcultured at least 2 times prior to injection into the electrochemical device. The glove box environment consisted of 10% H₂ and 10% CO₂ balanced with N₂. The G. sulfurreducens sub-culturing medium contained the following amounts per litre in distilled water: 1.5 g NH₄Cl, 0.6 g NaH₂PO₄, 0.1 g KCl, 2.5 g NaHCO₃, 0.82 g CH₃COONa (10 mM), 8 g Na₂C₄H₂O₄ (40 mM), 10 mL vitamin supplement ATCC® MD-VS™, and 10 mL trace mineral supplement ATCC® MD-TMS™. With the exception of sodium fumarate and vitamin/trace mineral supplements, all chemical compounds were dissolved in distilled water and autoclaved at 110 °C and 20 psi for 20 min. The G. sulfurreducens medium was transferred to the glove box and the filtered sterilized sodium fumarate and vitamin/trace mineral supplements were added. The anaerobic nutrient medium was ready for use after overnight incubation in an anaerobic glove box. The pH of the medium was adjusted to 7 prior to sub-culturing in the glove box. For electrochemical growth of G. sulfurreducens in electrochemical cells, sodium fumarate and vitamins were excluded, and the pH was adjusted to 7.5. Sub-cultures that were transferred more than 8 times were discarded.40

2. **Device fabrication and anaerobic environment**

We followed a recent report that demonstrated the use of embedded electrodes at the bottom of a polydimethylsiloxane
(PDMS) microchannel such that the transition to the electrode was seamless.29 The device consisted of graphite WE and CE and a gold (Au) RE. Two graphite strips (GraphiteStore.com Inc., USA) with dimensions of 3 mm × 20 mm were embedded and used as the WE and CE. The surface area exposed to the solution for both electrodes after fabrication was 3 mm × 2 mm. The gold-coated polystyrene RE was fabricated via electroless deposition. The electrode was subsequently cut into 3 × 20 mm strips and embedded into the microfluidic device in the same manner as in the WE and CE. A mixture of liquid polydimethylsiloxane (PDMS) and cross-linking agent Sylgard184 (Dow Corning, Canada) (10:1) was poured over the mould (FlowJEM Inc., Toronto, Canada) while the electrodes were placed on features of the mould with dimensions of 30 mm (L) × 2 mm (w) × 0.4 mm (h). After curing for 4 h at 70 °C, the device with embedded electrodes was removed from the mould. Cleaning and sterilization of the channel with a 70% ethanol solution and of the electrodes with 1 M HCl were conducted before sealing. The microfluidic electrochemical cell was sealed with a microscope slide by exposure to air plasma (PCD-001 Harrick Plasma, Ithaca, NY, USA). Because the microfluidic device was fabricated in PDMS, which is known to be porous to small molecules including O2, an anaerobic enclosure (McIntosh and File's, 28029 Sigma-Aldrich) filled with anaerobic gas (20% CO2 and 80% N2) was used with a controlled temperature of 22 ± 1 °C (Figure S3). The device was placed in the enclosure, and sterile perfluoroalkoxy connective tubing (PFA tube 1/16, Hamilton Inc., Canada) and electrical connections were attached via airtight feedthroughs in the enclosure cap. To minimize gas diffusion through the connective tubing outside of the enclosure, a layer of gas-impermeable tape (Lolite 249 Anaerobic Blue Threadlocker Tape, Medium Strength, Henkel Corp., Mississauga, Canada) was applied to the tubing, which was subsequently covered by epoxy glue. Electrical leads were fixed to the graphite WE and CE via alligator clips, and the gold RE was connected by solder with a protective coating of epoxy to physically stabilize the connection. Electrical leads were fixed to the graphite WE and CE via alligator clips and the gold RE was connected by solder with a protective coating of epoxy to physically stabilize the connection. Scanning electron microscopy (SEM) was performed after the experiment was completed to observe the bacteria attached to the WE (Figure S6). Before the electrode was removed from the microfluidic device, a fixation solution (2.5% glutaraldehyde in phosphate buffer) was flowed through the channel (Q = 0.5 mL·h−1) for 2 h while the device remained under anaerobic conditions. The device was subsequently removed from the anaerobic enclosure and cut open to reveal and remove the working electrode. The biofilm-coated electrode was left exposed to the same fixation solution in a bath overnight. The next day, the electrode was transferred to a solution with 1% osmium tetroxide for 1.5 h and rinsed in phosphate buffer. Finally, the sample was sequentially dehydrated in 50, 75, 95 and 100% aqueous ethanol solutions for 15 min each, followed by drying at room temperature overnight. Prior to image capture, a thin layer of gold was sputtered on the biofilms and electrode (Model: Nanotech, SEM REP 2). The images were collected with a JEOL JSM-6360 LV electron microscope.

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Conflict of interest

The authors declare no competing financial interest.

Keywords: Geobacter sulfurreducess • bioelectrochemical systems • microfluidics • deacidification • hydrodynamic voltammetry

3. Electrochemical measurements

Electrochemical measurements included chronocapacitometry (CA) and cyclic voltammetry (CV) performed using a commercially available potentiostat (Volta Lab PST006, Radiometer analytical, USA) controlled by the relevant software (Volta Master 4, Hach Radiometer Analytical, USA). Analysis was conducted using Origin Pro 2015.

4. SEM Imaging
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