Multivariate landscapes constructed by Bayesian estimation over five hundred microbial electrochemical time profiles

Highlights
- A high-throughput bioelectrochemical device parallelly runs 1920 chronoamperometry
- SD remained less than 10%, with 96 standalone electrochemical cells
- Bayesian estimation was applied to 576 time profiles of microbial current production
- High performance of riboflavin at negative potential was mechanically validated

Authors
Waheed Miran, Wenyuan Huang, Xizi Long, Gaku Imamura, Akihiro Okamoto

Correspondence
okamoto.akihiro@nims.go.jp

In brief
Data science is an innovative approach to optimizing complex biological power generation operating conditions. However, technology capable of delivering large amounts of high-quality training data is essential for its practical use. Here, we developed an electrochemical device that achieves more than hundreds of times higher output than conventional ones for constructing a high-quality database. The data-driven discovery of a high-performance electron transfer mechanism under unexplored conditions verifies the effectiveness of our approach to integrating data science and microbial electrochemistry.
Multivariate landscapes constructed by Bayesian estimation over five hundred microbial electrochemical time profiles

Waheed Miran,1,2 Wenyuan Huang,1,3 Xizi Long,1 Gaku Imamura,1,4 and Akihiro Okamoto1,3,5,*

1International Center for Materials Nanoarchitectonics, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan
2School of Chemical and Materials Engineering, National University of Sciences and Technology, Islamabad 44000, Pakistan
3Graduate School of Chemical Sciences and Engineering, Hokkaido University, North 13 West 8, Kita-ku, Sapporo, Hokkaido 060-8628, Japan
4Graduate School of Information Science and Technology, Osaka University, 1-2 Yamadaoka, Suita, Osaka 565-0871, Japan
5Lead contact
*Correspondence: okamoto.akihiro@nims.go.jp
https://doi.org/10.1016/j.patter.2022.100610

SUMMARY

Data science emerges as a promising approach for studying and optimizing complex multivariable phenomena, such as the interaction between microorganisms and electrodes. However, there have been limited reports on a bioelectrochemical system that can produce a reliable database until date. Herein, we developed a high-throughput platform with low deviation to apply two-dimensional (2D) Bayesian estimation for electrode potential and redox-active additive concentration to optimize microbial current production (Ic). A 96-channel potentiostat represents <10% SD for maximum Ic. 576 time-Ic profiles were obtained in 120 different electrolyte and potentiostatic conditions with two model electrogenic bacteria, Shewanella and Geobacter. Acquisition functions showed the highest performance per concentration for riboflavin over a wide potential range in Shewanella. The underlying mechanism was validated by electrochemical analysis with mutant strains lacking outer-membrane redox enzymes. We anticipate that the combination of data science and high-throughput electrochemistry will greatly accelerate a breakthrough for bioelectrochemical technologies.

INTRODUCTION

Electroactive bacteria that perform extracellular electron transfer (EET) to/from electrodes show great potential for applications in the fields of energy and environmental sustainability, including power generation from wastewater, bioremediation, chemical production, and amperometric biosensors.1,2 With increasing demand for commercialization,3 various strategies are being
adopted to improve and optimize the performance of bioelectrochemical systems (BESs), including reactor configurations and varying operating conditions, electrode material development, and additives based on fundamental EET mechanisms.4–9 However, understanding the complexity of BESs, which includes elucidation of the interactions between different impactful parameters (Figure 1A) and control of microbial electrochemical catalysis, requires breakthroughs at the scientific and engineering levels. Data science shows potential for capturing the landscape of such complex systems from limited databases. However, the effective use of data science in BESs has been a challenge, as they require a massive dataset with defined parameters and high reproducibility, referred to as a “high-quality database.” The less controlled experimental aspects, while obtaining large data from manual experiments, often cause serious reproducibility concerns, which restrict the consensual knowledge gain. Hence, a high-quality database is the core area for applying data science to experimental studies.10–13 However, although significant development has been achieved in high-throughput BESs, there are no reports on a system that can achieve enough reproducibility while simultaneously controlling the following three parameters for each reactor: potential, electrolyte, and microorganism, which are critical for BES performance.14–19 Furthermore, appropriate data processing algorithms for dealing with a large volume of current-time profile data have not been established yet.18 Therefore, even in the most recent studies, the use of data science for the number of datasets used for training the model are very limited, at nine.20–22

RESULTS AND DISCUSSION

To evaluate the variation in the electrochemical signal from each well in a high-throughput system, the same sample conditions and an analysis platform for massive time-current profile data.

In this study, we developed a high-throughput potentiostat system with 96-well plates with silk-screen-printed electrodes that was applied to landscape the redox mediator concentration and electrode potential constructed from 576 time profiles of microbial current production (Figures 1B and 1C). One of the most important controllable factors for BES performance is the type and concentration of the redox mediator.23 External redox mediators play a vital role in enhancing the electron transfer between bacteria and the electrode.24,25 The performance of mediators may be considerably different depending on their redox potential, diffusion constant of shuttling electron mediators, and ability to bind with bacterial membrane enzymes.24,27 Furthermore, the electrode potential can modify global gene regulation and metabolic pathways in electrogenic bacteria. Therefore, microbial current production may be beyond our physicochemical understanding of the EET mechanism, which is suitable for application in data science. We used Shewanella oneidensis MR-1 to compare the impact of variables, different mediators (riboflavin [RF], flavin mononucleotide [FMN], 2-hydroxy-1,4-naphthoquinone [HNQ], anthraquinone-1,5-disulfonate [AQDS]), the concentration of mediators (1–100 μM), and poised potentials (+200 to −300 mV versus Ag/AgCl). In addition, the potential and RF concentration dependency were examined using Geobacter sulfurreducens PCA. Flavins are known to strongly enhance current generation in S. oneidensis MR-1 and G. sulfurreducens PCA, model EET bacteria, as a bound cofactor by forming an intermediate semi-reduced state.26,27,28 In contrast, HNQ and AQDS indirectly shuttle electrons between bacteria and the electrode.

The basis for combining data science and microbial electrochemistry can provide a series of methods, not only as a solid basis for optimizing and enhancing BES performance but also for identifying critical parameters for biotic and abiotic complex systems.
were measured in a 96-well three-electrode screen-printed electrochemical plate with our custom-made potentiostat, capable of measuring 20 units in parallel. *S. oneidensis* MR-1 cells were filled into all the wells at an optical density (OD) of 0.5 in an electrolyte of 200 μL, and the electrode potential was then poised at +200 mV (versus Ag/AgCl) in the anaerobic chamber at 30°C. A total of 95 out of 96 wells showed stable current generation without any significant noise (Figure 2A), indicating that manufacturing the custom potentiostat did not noticeably limit the number of effective channels. Each well showed almost identical time-current (*I*-*T*) profiles, where the current production started with a steep rise and then gradually saturated. Given that scarce current was produced in the absence of lactate as the sole electron donor (Figure 2A), the current production (*Iₚ*) in each well was attributable to the EET process associated with metabolic lactate oxidation. Furthermore, the *Iₚ* value and *I*-*T* profile were almost identical to those of the well-established commercially available potentiostat (VMP3; Biologic) (Figure S1), suggesting that potentiostatic conditions were achieved using our custom-made potentiostat. At the end of the current measurement, the working electrode (WE) surface was covered by MR-1 cells (Figure 2B), similar to conventional BES reactors. For *S. oneidensis* MR-1, an increase in *Iₚ* with an increase in mediator concentration was observed for all redox mediators at each electrode potential (Figure 3). For the positively poised electrode at +200 mV, flavins and HQN showed relatively large currents at low (1–10 μM) and high concentrations (100 μM), respectively. This is consistent with the difference between the bound cofactor and electron shuttling mechanism reported previously. AQDS showed the least current enhancement among the four redox mediators. A more negative electrode potential (−100 to −300 mV) resulted in a lower *Iₚ* (*Iₚ-* for all tested mediators and concentrations. Meanwhile, the impact of the decrease was substantial in the case of HQN, as shown in Figure 3; a reduction in current by seven times was observed when the poised potential was decreased gradually from +200 to −300 mV. However, the *Iₚ* decrease was only observed from −200 to −300 mV in RF and FMN, suggesting that bound cofactors and redox shuttles have different potential dependencies on the *Iₚ* performance.

To analyze such differences in potential dependency among these redox mediators in detail, we estimated the enhancement factor and its efficiency against the concentration of redox mediators (Figure 4). In some cases, *Iₚ* did not reach its peak during the electrochemical assay (Figure S3C). Therefore, we evaluated the performance in terms of the current enhancement factor using the maximum slope achieved in each case. The current enhancement factor was calculated using the following relation:

\[
\text{EF} (\alpha) = \frac{S_{\text{max}} - S_{\text{max, ref}}}{S_{\text{max, ref}}},
\]

where EF is the current enhancement factor, *Sₘₐₓ* is the maximum slope for each tested case with different mediators and poised

---

**Figure 2. Low deviation current profiles in 96-well screen-printed electrochemical plate**

(A) Catalytic current profiles of microbial lactate oxidation by *S. oneidensis* MR-1 in 96-well screen-printed electrochemical plate poised at +200 mV (versus Ag/AgCl).

(B) *S. oneidensis* MR-1 attached on the electrode surface observed by fluorescence microscopy. The fluorophores SYTO 9 and propidium iodide were used for assessing the vitality. The excitation/emission wavelengths used were 480/500 nm and 490/635 nm for SYTO 9 and PI, respectively. The arrows indicate the bacterial cells. Scale bar is 5 μm.

(C and D) Statistical parameters for maximum current production, slope, and curvature. Box in (C) represents mean ± SD (n = 95). See also Figure S1.
potentials, and $S_{\text{max,ref}}$ is the maximum slope of the reference cells without mediators at different poised potentials. The EF was then normalized to the added mediator’s concentration to present the efficiency of each mediator molecule to enhance EET using the relation:

$$\text{Additives performance} = \frac{\text{EF}}{C};$$  \hspace{1cm} (Equation 2)

where $C$ is the concentration of mediators for each tested case. To plot additive performance against electron potential or mediator concentration, we conducted Bayesian estimation. Bayesian statistics were employed to explore the most efficient conditions, which were determined by the balance between additive addition and current enhancement. The basic idea of optimizing a function $f(x)$ is to determine $x$ that maximizes $f(x)$. In Bayesian optimization, a regression model based on a Gaussian process (GP) is built for $f(x)$ from a dataset of observed $x$ and $f(x)$. In this study, a two-dimensional (2D) vector composed of the poised voltage and the concentration of the mediator, and $f(x)$ is the additive performance. In this study, scikit-learn library (i.e., sklearn GP regressor) was used to build a GP model, and a combination of three types of kernels (i.e., radial basis function, constant, and white kernels) was employed as the GP kernel. From the mean and SD of the GP posterior predictive at $x$ ($\mu(x)$ and $\sigma(x)$, respectively), the most probable $x$ that might give the maximum value is estimated on the basis of an acquisition function. We used expected improvement (EI) as the acquisition function, which is defined as the following formula:

$$\text{EI}(x) = \begin{cases} (\mu(x) - f(x)_{\text{max}} - \xi)\Phi(Z) + \sigma(x)\phi(Z), & \sigma(x) > 0 \\ 0, & \sigma(x) = 0 \end{cases},$$  \hspace{1cm} (Equation 3)

where

$$Z = \frac{\mu(x) - f(x)_{\text{max}} - \xi}{\sigma(x)}.$$  \hspace{1cm} (Equation 4)

Here, $\Phi(Z)$ and $\phi(Z)$ are the cumulative distribution function and the probability density function of $Z$, respectively. The parameter $\xi$ was introduced to tune the degree of trade-off between exploration and exploitation. In this study, $\xi$ was set at 0.01 of the SD of a dataset.

The EF profiles showed that, at lower mediator concentrations, RF and FMN exhibited higher current enhancement than HNQ and AQDS (Figure 4). For flavins, the EFs did not significantly vary with electrode potential at all concentrations (Figure 4A). High EF was observed with HNQ and AQDS,
specifically when concentration and potential were high and positive, respectively (Figure 4B). These results demonstrate that the EFs show the overall transition in the I-T profile under different conditions, as shown in Figure 3. The GP models and corresponding EIs are depicted for additive performance as 2D heatmaps, as shown in Figures 4B and 4C, with the optimized conditions for each mediator marked in these panels. The GP models effectively integrate the data into a 2D landscape, and the estimated condition for the peak performance is consistent with raw data, except for the highest performance of HNQ at 1 μM concentration, which may be associated with the amplification of error from the low current range. As shown in Figure 4C, a low (<10 μM) concentration of RF was estimated for additive peak performance, and the peak potential ranged from +200 to −100 mV. In contrast, the peak performance was localized at positive potentials from +150 to 0 mV and at HNQ concentrations greater than 50 μM. FMN and AQDS showed similar tendencies to RF and HNQ, respectively (Figures 4B and 4C).

The potential dependency of EI at peak performance concentration for each mediator showed that the EI functions of RF and FMN had one large peak and a shoulder, while those of HNQ and AQDS had one peak (Figure S4), suggesting that the two redox reactions are involved in Ic in the presence of flavins. Given that the redox potentials of bound RF and FMN, HNQ, and AQDS are closely located, the peak and the shoulder at around −100 mV are most likely assignable to their redox reaction with the electrode surface. Meanwhile, the main peak of flavins observed at −50 to 0 mV may also be attributable to the bound flavin cofactors in the outer membrane cytochrome (OMC). Bound flavin cofactors in OMCs mediate the single-electron redox reaction to form a semiquinone state (Sq); therefore, there are two types of redox reactions, oxidized (Ox)/Sq and Sq/hydroquinone (Hq). While the Sq/Hq coupling redox reaction was reported to mediate electron uptake from the electrode surface, the Sq/Hq reaction may mediate anodic Ic at a negative electrode potential, more so than the Ox/Sq coupling reaction in MR-1.

Figure 4. Additive’s performance dependency on concentration and electrode potential for redox mediators (A) Current enhancement for RF, FMN, HNQ, and AQDS additive concentration and poised electrode potentials, where enhancement factor was calculated using maximum slope against base current without any additives. Data are represented as mean ± SEM (n = 4). (B) Gaussian process regression model for the RF, FMN, HNQ, and AQDS additive performance against additive concentration and poised electrode potentials. (C) Heatmaps for the expected improvements in RF, FMN, HNQ, and AQDS additive performance against additive concentration and poised electrode potentials. See also Figure S4.
To examine the origin of \( I_c \) enhancement at the negative electrode potential in the presence of flavins, we electrochemically analyzed the presence of Sq/Hq redox coupling using differential pulse voltammetry (DPV) during potential poising at \(-200 \text{ mV}\) at various RF concentrations (Figures 5 and S5). We observed peaks at \(-460 \) and \(-30 \text{ mV}\) versus Ag/AgCl, and the peak current increased with increasing RF concentration (Figure 5A). Plots of \( I_c \) and the peak currents at \(-460 \text{ mV}\) showed a linear correlation passing through the origin point (Figure S5B), suggesting that both peaks are assignable to RF and that the redox signal at \(-460 \text{ mV}\) attributable to RF mediates \( I_c \) under \(-200 \text{ mV}\) incubation condition. Accordingly, the linear voltammetry measurement showed the onset potential of linear sweep voltammetry (LSV) started from around \(-0.6 \text{ V}\), consistent with the onset of the RF peak at \(-460 \text{ mV}\) (Figure 5C). When an excessive amount of RF was added to detect the DPV signal of the free form, an additional peak at \(-430 \text{ mV}\) was detected. The half-peak width \( (E_{w1/2}) \) for the signals at \(-460 \) and \(-30 \text{ mV}\) was approximately \(130 \text{ mV} \), and the \( E_{w1/2} \) for the \(-430 \text{ mV}\) peak was approximately \(50 \text{ mV} \), which is consistent with the one- and two-electron flavin redox processes, suggesting that \(-460, -430, -30 \text{ mV}\) are Sq/Hq, Ox/Hq, and Ox/Sq redox reactions, respectively. This assignment is in accordance with the relative location potential for each redox couple, that is, the two-electron Ox/Hq peak is between the two redox peaks for the single-electron redox reaction, and the observation that \(-460 \) and \(-30 \text{ mV}\) peak currents both increased with the added flavin concentration. Given that the Sq/Hq redox couple was stabilized in OMCs under cathodic electrode conditions in MR-1,\(^{36}\) we used a mutant strain lacking OMCs (\( \triangle \text{omcAll} \)). The impact of gene deletion resulted in slight \( I_c \) enhancement with increasing RF concentration. A clear decrease in the LSV and DPV signals was attributable to electron flow mediated by Sq/Hq (Figures 5C and 5D). These results further demonstrate that the Sq/Hq redox couple is the main mechanism for \( I_c \) enhancement under negative electrode poising conditions.

In contrast, such tolerance for the negative electrode potential was not observed in \( G. \) \textit{sulfurreducens}, which is also capable of using RF as a redox cofactor in \( c \)-type cytochromes.\(^{36} \) \( I_c \) was measured under the same conditions as \( S. \) \textit{oneidensis} MR-1, except for the electrolyte medium and concentration range of RF because the dissociation constant of RF for binding OMCs was 100 times lower than that in \( S. \) \textit{oneidensis} MR-1.\(^{36} \) The effect of RF addition was not significant, most likely because the RF secreted or contained in the medium was sufficiently higher than the \( K_d \) value. Meanwhile, \( I_c \) was considerably suppressed at more negative electrode potential than \(-0.1 \text{ V}\) (Figure 3). Assuming that the low \( I_c \) at a negative potential is caused by the dissociation of RF from OMCs, the interaction of OMCs with the negatively poised electrode may change the binding affinity of RF to OMCs. These results suggest that while the \( I_c \) capability of \( G. \) \textit{sulfurreducens} is higher than that of \( S. \) \textit{oneidensis} MR-1 under positively poised conditions, \( S. \) \textit{oneidensis} MR-1 is advantageous for sustainability under conditions of anodic potential fluctuation with varying wastewater conditions. In real wastewater treatment systems, the redox potential substantially changes depending on the wastewater oxidation-reduction reactions.\(^{37} \) For instance, the redox potential of wastewater increases in the presence of strong oxidizing agents such as hydrogen peroxide or decreases in the presence of strong reducing agents such as sodium bisulfite.\(^{38} \) The biological oxidation-reduction reactions such as nitrification, denitrification, biological phosphorus removal, and the removal of biological oxygen demand (carbon- and hydrogen-containing compounds) also dictate the redox potential conditions. Most of these processes occur in the range of \(-300 \text{ to +200 mV}\), varying from anaerobic to aerobic systems.\(^{39,40} \) In this respect, the tolerance to negative electrode potential...
in MR-1 is important, as among the bacteria that power BESs, S. oneidensis MR-1 species are widely studied for bioremediation and environmental energy recovery, owing to their robust growth in both aerobic and anaerobic environments within a wide range of redox potentials.\textsuperscript{40,41} However, the potential range advantage of S. oneidensis MR-1 has not been highlighted. This study, which captured the landscape of varying electrode potentials, not only identified the optimum additive conditions against the range of potentials but also helped elucidate the mechanism in EET. This will help real wastewater systems to control the addition of current enhancement agents in varying redox potentials for the maximum performance of BESs.

Conclusions
We demonstrated the utility of applying data science to complex bacteria and electrode interactions by successfully developing a high-throughput and low-deviation electrochemical platform. 2D landscapes of electrode potential and additive concentration generated from Bayesian estimation were primarily consistent with the bound and diffusible mechanisms in redox mediators, verifying that the quality of the database is sufficient to apply data science analysis. Furthermore, the uninvestigated region of electrode potential and mediator concentration showed an electron transfer mechanism via a bound flavin cofactor with a negatively poised electrode surface. This finding validates the database quality and provides the first example of an EET mechanism revealed by data science, highlighting the importance for the fundamental understanding of BES. By further combining it with the other data science model, our Bayesian estimation model may enable complex BES models to improve the performance of practical systems.\textsuperscript{42} Owing to the flexibility of the electrolyte, potential poising, and electrode material, the present method can be extended to many other applications in electromicrobiology—microbial fuel cells and microbial electrolysisis, or metabolic activity sensor technologies for antibiotic drug discovery.\textsuperscript{39,44}

EXPERIMENTAL PROCEDURES

Resource availability
Lead contact
The lead contact is Akihiro Okamoto (okamoto.akihiro@nims.go.jp). He can be contacted for information relevant to the paper and requests for code and data.

Materials availability
This work did not produce any physical materials.

Data and code availability
All data reported in this paper will be shared by the lead contact upon request. Original codes have been deposited at Zenodo under https://doi.org/10.5281/zenodo.7050972 and are publicly available. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Materials and methods
S. oneidensis MR-1 and G. sulfurreducens PCA cultivation
S. oneidensis MR-1 was cultured in 10 mL LB medium at 30 °C for 20 h under aerobic conditions by picking a single colony from the LB solid medium plate. The bacteria were washed twice with defined medium (DM) with a composition of 2.5 g NaHCO\textsubscript{3}, 0.08 g CaCl\textsubscript{2}, 1 g NH\textsubscript{4}Cl, 0.2 g MgCl\textsubscript{2}, 10 g NaCl, 7.2 g HEPE, and 0.5 g yeast extract in 1 L ultrapure water. The cells were centrifuged at 7,200 \( \times \) g for 5 min, and the supernatant was removed. Cell pellets obtained after centrifugation were resuspended in 10 mL DM medium with 10 mM lactate (DML). Microbial cells were precultured in DML for 4 h under anaerobic conditions and then washed. Finally, the cell OD at 600 nm (OD\textsubscript{600nm}) was adjusted to 0.5, using DML as the final concentration in the screen-printed electrochemical cells. \textit{somc}\textit{All} that referred to deletion of all outer membrane multimeme cytochrome gene homologs was constructed by deleting the genes SO1778 to SO1782, SO2931, and SO1659 from S. oneidensis MR-1 as described earlier.\textsuperscript{45,46}

\textit{G. sulfurreducens} PCA freeze stock was used for inoculation of the culture in anaerobic PSN medium\textsuperscript{26} supplied with 20 mM acetate and 80 mM fumarate. Cells were were grown in an anaerobic bottle at 30 °C for 3–4 days. Finally, the suspended cells were centrifuged (10 min at 5,000 \( \times \) g) and washed with the PSN medium for electrochemical measurements.

Experimental plan
Screen-printed electrochemical array formed by 96 three-electrode electrochemical cells (DRP\textsubscript{x11L} (U100), Metrohm, DropSens, Tokyo, Japan) was utilized for this study. The electrochemical array was fixed at the bottom of a standard microtiter ELISA plate with 96 wells. Plastic substrate (L7.4 cm \( \times \) W11 cm \( \times \) H0.5 mm) was used as the base for screen printing the three electrodes. The screen-printed carbon (surface area: 7.07 mm\textsuperscript{2} for each well) was used as the WE. Also, for each cell, screen-printed carbon and Ag/AgCl were used as the auxiliary (counter electrode [CE]) and RE, respectively. The backside of the plates was printed with gold-plated contact paths where 96 \( \times \) 3 contacts were present, corresponding to the independent WE, CE, and RE printed for each well. Each well had a standard volume capacity of around 300–400 \( \mu \)L, and a working volume of 200 \( \mu \)L was used. After the anolyte addition, all plates were sealed with sterile aluminum seals. Since multichannel 96-well systems were used where effective volume of each cell was much less (~200 \( \mu \)L), it was ensured that no turbulence affected the operation by adding the medium, bacterial cells, and mediators at the beginning of experiments. Nevertheless, to probe the mechanism, control cells were included in the experiments where no exogeneous small-molecule mediators were added, and hence rational analysis for elucidation of mechanism can be ensured.

In the scheme of current-time profile experiments, first, single potential amperometry measurements with our custom potentiostat using 96-well plates were compared with a commercial potentiostat (VMP3; Biologic Science Instruments, Seyssinet-Pariset, France) using screen-printed electrodes with the same electrode material and working volume as the 96-well plates. The variation in the data for 96-well plate was analyzed for the maximum \( I_c \), slope, and curvature. For the multivariable impact study, five 96-well electrochemical plates were selected for S. oneidensis MR-1 and one plate for \textit{G. sulfurreducens} PCA (Figure S2). For S. oneidensis MR-1, the first plate was checked by poising a single potential (versus Ag/AgCl) for two rows each, that is, the first two rows were poised at +200 mV, followed by
two rows at −100, −200, and −300 mV without the addition of any external mediator (Figure S2A). Four plates were tested by posing a single potential per plate with varying mediator concentrations (Figure S2B). DM (pH 7.8) containing lactate and the desired concentrations of flavin analogs and quinones (1–100 μM), and MR-1 cell suspensions with OD600nm = 0.5. PLATEMASTER P220 (Gilson, Middleton, WI, USA) was used for pipetting volumes of 2–220 μL for high-throughput manual pipetting of 96 wells. For G. sulfurreducens PCA, a single plate was used to study 24 conditions (Figure S2C). This includes four poised potential conditions (+200, −100, −200, and −300 mV) and six RF mediator concentrations (0, 100, 250, 500, 1,000, and 5,000 nM). All the experiments were performed in an anaerobic chamber filled with 100% N2. The chamber was also intermittently filled with H2 gas mix (5% or less) that reacted with a palladium catalyst to remove O2 by forming a H2O molecule. The temperature was maintained at 30 °C during the measurements.

At the end of bioelectrochemical experiment, S. oneidensis MR-1 attached on the electrode surface were observed by fluorescence microscopy. The fluorophores SYTO 9 and propidium iodide (PI) were used for assessing the vitality, the excitation/emission wavelengths used were 480/500 nm and 490/635 nm for SYTO 9 and PI, respectively. Fluorescein isothiocyanate (FITC) and Texas Red (TxB) were used as filters.

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.patter.2022.100610.

ACKNOWLEDGMENTS
The financial support for this work was provided by a Grant-in-Aid for Research from the Japan Society for Promotion of Science KAKENHI grant nos. 20H05590 and 22H02265) and the Japan Agency for Medical Research and Development (19gm6010002h0004 and 21he0322002j0002). This work was also supported by JST, PRESTO grant number JPMJPR19H1, Japan, and by JAEA Nuclear Energy S&T and Human Resource Development Project through concentrating wisdom grant number JPJA20P2033393.

AUTHOR CONTRIBUTIONS
Conceptualization, W.M. and A.O.; methodology, W.M., W.H., and A.O.; formal analysis, W.M., G.I., and A.O.; investigation, W.M., W.H., X.L., and A.O.; writing – original draft, W.M., W.H., X.L., G.I., and A.O.; writing – review & editing, G.I. and A.O.; funding acquisition, A.O.; resources, A.O.; supervision, A.O.

DECLARATION OF INTERESTS
A.O. filed a patent application for the high-throughput device.

REFERENCES
1. Logan, B.E., Rossi, R., Ragab, A., and Saikaly, P.E. (2019). Electroactive microorganisms in bioelectrochemical systems. Nat. Rev. Microbiol. 17, 307–319. https://doi.org/10.1038/s41579-019-0173-x.
2. Shi, L., Dong, H., Reguera, G., Beyenal, H., Lu, A., Liu, J., Yu, H.-Q., and Fredrickson, J.K. (2016). Extracellular electron transfer mechanisms between microorganisms and minerals. Nat. Rev. Microbiol. 14, 651–662. https://doi.org/10.1038/nrmicro.2016.93.
3. Ziara, R.M.M., Dvorak, B.I., and Subbiah, J. (2018). Chapter 7 - sustainable waste-to-energy technologies: bioelectrochemical systems. In Sustainable Food Waste-to-Energy Systems, T.A. Trabold and C.W. Babbitt, eds. (Academic Press), pp. 111–140.
4. Liang, P., Wang, H., Xia, X., Huang, X., Ma, Y., and Cao, X. (2011). Carbon nanotube powders as electrode modifier to enhance the activity of anodic biofilm in microbial fuel cells. Biosensors and Bioelectronics 26, 3000–3004. https://doi.org/10.1016/j.bios.2010.12.002.
5. Watson, V.J., Salto, T., Hickner, M.A., and Logan, B.E. (2011). Polymer coatings as separator layers for microbial fuel cell cathodes. Journal of Power Sources 196, 3009–3014. https://doi.org/10.1016/j.jpowsour.2010.11.105.
6. Jiang, D., Li, X., Raymond, D., Mooradain, J., and Li, B. (2010). Power recovery with multi-anode/cathode microbial fuel cells suitable for future large-scale applications. International Journal of Hydrogen Energy 35, 8683–8689. https://doi.org/10.1016/j.ijhydene.2010.04.136.
7. Logan, B.E. (2010). Scaling up microbial fuel cells and other bio-electrochemical systems. Appl. Microbiol. Biotechnol. 85, 1665–1671. https://doi.org/10.1007/s00253-009-2378-9.
8. Gadkari, S., Fontmorin, J.-M., Yu, E., and Sadhukhan, J. (2020). Influence of temperature and other system parameters on microbial fuel cell performance: numerical and experimental investigation. Chem. Eng. J. 388, 124176.
9. Chen, S., Patil, S.A., Brown, R.K., and Schröder, U. (2019). Strategies for optimizing the power output of microbial fuel cells: Transitioning from fundamental studies to practical implementation. Applied Energy 233, 15–28. https://doi.org/10.1016/j.apenergy.2018.10.015.
10. Ahmadi, M., Ziatdinov, M., Zhou, Y., Lass, E.A., and Kalinin, S.V. (2021). Machine learning for high-throughput experimental exploration of metal halide perovskites. Joule 5, 2797–2822. https://doi.org/10.1016/j.joule.2021.10.001.
11. An, N.G., Kim, J.Y., and Vak, D. (2021). Machine learning-assisted development of organic photovoltaics via high-throughput in situ formulation. Energy Environ. Sci. 14, 3438–3448. https://doi.org/10.1039/D1EE00641J.
12. Zhong, M., Tran, K., Min, Y., Wang, C., Wang, Z., Dinh, C.-T., De Lunea, P., Yu, Z., Rasoulis, A.S., Brodersen, P., et al. (2020). Accelerated discovery of CO2 electrocatalysts using active machine learning. Nature 587, 178–183. https://doi.org/10.1038/s41586-020-2242-8.
13. Du, X., Lüer, L., Heumueller, T., Wagner, J., Berger, C., Osterrieder, T., Wortmann, J., Langner, S., Vongsay, U., Bertrand, M., et al. (2021). Elucidating the full potential of OPV materials utilizing a high-throughput robot-based platform and machine learning. Joule 5, 495–506. https://doi.org/10.1016/j.joule.2020.12.013.
14. Molderz, T.R., Zhang, X., Rabaey, K., and Verhelst, M. (2019). A current-driven six-channel potentiostat for rapid performance characterization of microbial electrolysis cells. IEEE Trans. Instrum. Meas. 68, 4694–4702. https://doi.org/10.1109/TIM.2019.2888049.
15. Vergani, M., Carminati, M., Ferrari, G., Landini, E., Caviglia, C., Heiskanen, A., Comminges, C., Zör, K., Sabourin, D., Dufva, M., et al. (2012). Multichannel biopotentiostat integrated with a microfluidic platform for electrochemical real-time monitoring of cell cultures. IEEE Trans. Biomed. Circuits Syst. 6, 498–507. https://doi.org/10.1109/TBCAS.2012.2187783.
16. Tahernia, M., Mohammadifar, M., Gao, Y., Panmanee, W., Hassett, D.J., and Choi, S. (2020). A 96-well high-throughput, rapid-screening platform
of extracellular electron transfer in microbial fuel cells. Bioens. Bioelectron. 162, 112259.

17. Call, D.F., and Logan, B.E. (2011). A method for high throughput bio-electrochemical research based on small scale microbial electrolysis cells. Bioens. Bioelectron. 26, 4526–4531. https://doi.org/10.1016/j.biolei.2011.05.014.

18. Molderez, T.R., Prévotau, A., Ceyssens, F., Verhelst, M., and Rabaey, K. (2021). A chip-based 128-channel potentiosat for high-throughput studies of bioelectrochemical systems: optimal electrode potentials for anodic biofilms. Bioens. Bioelectron. 174, 112813.

19. Szulwinski, L., Ehlich, J., Goryanin, I., and Pasternak, G. (2021). High-throughput 96-well bioelectrochemical platform for screening of electro-active microbial consortia. Chemical Engineering Journal 371692. https://doi.org/10.1016/j.cej.2021.131692.

20. Tsopanias, M.-A., You, J., Wallis, L., Greenman, J., and Ieropoulos, I. (2019). Artificial neural network simulating microbial fuel cells with different membrane materials and electrode configurations. Journal of Power Sources 436, 226832. https://doi.org/10.1016/j.jpowsour.2019.226832.

21. Lesnik, K.L., and Liu, H. (2017). Predicting microbial fuel cell biofilm communities and bioelectrore performance using artificial neural networks. Environ. Sci. Technol. 51, 10881–10892. https://doi.org/10.1021/acs.est.7b01413.

22. Zhang, X., Li, X., Zhao, X., Li, Y., and Ieropoulos, I.A. (2019). Evaluation of artificial neural network algorithms for predicting the effect of the urine flow rate on the power performance of microbial fuel cells. RSC Adv. 213, 118806–118823. https://doi.org/10.1039/C9RA03605A.

23. Zhang, X., Li, X., Zhao, X., and Li, Y. (2019). Factors affecting the efficiency of a bioelectrochemical system: a review. RSC Advances 9, 19748–19761. https://doi.org/10.1039/C9RA03605A.

24. Martinez, C.M., and Alvarez, L.H. (2018). Application of redox mediators in bioelectrochemical systems. Biotechnol. Adv. 36, 1412–1423.

25. Kumar, A., Hsu, L.-H.H., Kavanagh, P., Barrière, F., Lens, P.N.L., Lapinssonnière, L., Lienhard V, J.H., Schroeder, U., Jiang, X., and Leech, D. (2017). The ins and outs of microorganism–electrode electron transfer reactions. Nat. Rev. Chem. 1, 9024. https://doi.org/10.1039/s41570-017-0024.

26. Okamoto, A., Saito, K., Inoue, K., Naito, K.H., Hashimoto, K., and Nakamura, R. (2014). Uptake of self-secreted flavins as bound cofactors for extracellular electron transfer in Geobacter species. Energy Environ. Sci. 7, 1357–1361.

27. Okamoto, A., Hashimoto, K., Nealon, K.H., and Nakamura, R. (2014). Uptake of self-secreted flavins as bound cofactors for extracellular electron transfer in microbial fuel cells. Biosens. Bioelectron. 59, 1–9. https://doi.org/10.1016/j.bios.2014.01.010.

28. Okamoto, A., Nakamura, R., Nealon, K.H., and Hashimoto, K. (2014). Bound flavin model suggests similar electron-transfer mechanisms in Shewanella and Geobacter. ChemElectroChem 1, 1808–1812. https://doi.org/10.1002/celc.201402151.

29. Patil, S.A., Górecki, K., Hägerhall, C., and Gorton, L. (2013). Cisplatin-induced elongation of Shewanella oneidensis MR-1 cells improves microbe-electrode interactions for use in microbial fuel cells. Energy Environ. Sci. 6, 2626–2630. https://doi.org/10.1039/C3EE41974F.

30. Zhao, C.-e., Wu, J., Kjelleberg, S., Loo, J.S.C. and Zhang, Q. (2015). Employing a flexible and low-cost polypropylene nanotube membrane as an anode to enhance current generation in microbial fuel cells. Small 11, 3440–3443.

31. Riba, J., Gleichmann, T., Zimmermann, S., Zengerle, R., and Kol typically produce and deposit single bacterial cells from heterogeneous samples for clonal culturing. Sci. Rep. 6, 32837. https://doi.org/10.1038/srep32837.

32. Matsui, T.S., Wu, H., and Deguchi, S. (2018). Deformable 96-well culture plate compatible with high-throughput screening platforms. PLoS One 13, e0203448. https://doi.org/10.1371/journal.pone.0203448.

33. Hirose, A., Kasai, T., Aoki, M., Umemura, T., Watanabe, K., and Kouzuma, A. (2018). Electrochemically active bacteria sense electrode potentials for regulating catalytic pathways. Nat. Commun. 9, 1083. https://doi.org/10.1038/s41467-018-03416-4.

34. Shi, Z., Zachara, J.M., Shi, L., Wang, Z., Moore, D.A., Kennedy, D.W., and Fredrickson, J.K. (2012). Redox reactions of reduced flavin mononucleotide (FMN), riboflavin (RBF), and anthraquinone-2, 6-disulfonate (AQDS) with ferricydite and lepidocrocite. Environ. Sci. Technol. 46, 11644–11652. https://doi.org/10.1021/es301544b.

35. Wu, Y., Liu, T., Li, X., and Li, F. (2014). Exogenous electron shuttle-mediated extracellular electron transfer of Shewanella putrefaciens 200: electrochemical parameters and thermodynamics. Environ. Sci. Technol. 48, 9306–9314. https://doi.org/10.1021/es5017312.

36. Okamoto, A., Hashimoto, K., and Nealon, K.H. (2014). Flavin redox bifurcation as a mechanism for controlling the direction of electron flow during extracellular electron transfer. Angew. Chem. Int. Ed. Engl. 53, 10988–10991.

37. Li, B., and Bishop, P.L. (2004). Oxidation-reduction potential changes in aeration tanks and microprofiles of activated sludge floc in medium- and low-strength wastewaters. Water Environ. Res. 76, 394–403. https://doi.org/10.2175/106143004x151662.

38. Higgins, P. (2008). ORP Management in Wastewater as an Indicator of Process Efficiency (YSI). ysi.com/media/pdfs/A567-ORP-Management-in-Wastewater-as-an-Indicator-of-Process-Efficiency.pdf.

39. Goncharuk, V.V., Bagri, V.A., Mel’nik, L.A., Chebotareva, R.D., and Bask翰, S.Y. (2010). The use of redox potential in water treatment processes. J. Water Chem. Technol. 32, 1–9. https://doi.org/10.3103/S1063455X10010017.

40. Lovley, D.R. (2006). Bug juice: harvesting electricity with microorganisms. Nat. Rev. Microbiol. 4, 497–508. https://doi.org/10.1038/nrmicro1442.

41. Verma, J., Kumar, D., Singh, N., Katti, S.S., and Shah, Y.T. (2021). Electrocipers and microbial fuel cells for bioremediation and bioenergy production: a review. Environ. Chem. Lett. 19, 2091–2126. https://doi.org/10.1007/s10311-021-01199-7.

42. Shahriari, B., Swersky, K., Wang, Z., Adams, R.P., and de Freitas, N. (2015). Probing the evolution of optimal configurations. Proc. IEEE 103, 148–175. https://doi.org/10.1109/JPROC.2015.2494218.

43. Naradus, D., Guionet, A., Miran, W., and Okamoto, A. (2020). Microbial current production from Streptococcus mutans correlates with biofilm metabolic activity. Biosens. Bioelectron. 162, 112396.

44. Miran, W., Naradus, D., and Okamoto, A. (2021). Pathogeneses and the importance of in-situ metabolic activity monitoring and drug assessment in biofilms. iScience 4, 102088. https://doi.org/10.1016/j.isci.2021.102088.

45. Bücking, C., Popp, F., Kerzenmacher, S., and Gascher, J. (2010). Influence and specificity of Shewanella oneidensis outer membrane cytadymes in the reduction of soluble and solid-phase terminal electron acceptors. FEMS Microbiol. Lett. 306, 144–151. https://doi.org/10.1111/j.1574-6968.2010.01949.x.

46. Rowe, A.R., Rajeev, P., Jain, A., Pirbadian, S., Okamoto, A., Gralnick, J.A., et al. (2018). Tracking Electron Uptake from a Cathode into Shewanella oneidensis. mBio 9, e02203–e02217. https://doi.org/10.1128/mBio.02203-17.