



Modeling, design guidelines, and detection limits of self-powered enzymatic biofuel cell-based sensors[☆]

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ABSTRACT

Enzymatic biofuel cell (EBFC)-based self-powered biochemical sensors obviate the need for external power sources thus enabling device miniaturization. While recent efforts driven by experimentalists illustrate the potential of EBFC-based sensors for real-time monitoring of physiologically relevant biochemicals, a robust mathematical model that quantifies the contributions of sensor components and empowers experimentalists to predict sensor performance is missing. In this paper, we provide an elegant yet simple equivalent circuit model that captures the complex, three-dimensional interplay among coupled catalytic redox reactions occurring in an EBFC-based sensor and predicts its output signal with high correlations to experimental observations. The model explains the trade-off among chemical design parameters such as the surface density of enzymes, various reaction constants as well as electrical parameters in the Butler–Volmer relationship. The model shows that the linear dynamic range and sensitivity of the EBFC-based sensor can be independently fine-tuned by changing the surface density of enzymes and electron mediators at the anode and by enhancing reductant concentrations at the cathode. The mathematical model derived in this work can be easily adapted to understand a wide range of two-electrode systems, including sensors, fuel cells, and energy storage devices.

1. Introduction

Recent advances in materials engineering, biotechnology, and electronics have laid the foundations for the development of new classes of miniaturized biochemical sensors (Gao et al., 2016; Heikenfeld et al., 2019; Jin et al., 2016; Song et al., 2017; Zhao et al., 2019; Zeng et al., 2020a,b) that offer performance similar to conventional, laboratory-based, benchtop analytical instruments. Of particular interest is the rise of enzymatic biofuel cell (EBFC)-based biochemical sensors as self-powered systems (Willner and Katz, 2009; Zhou et al., 2012; Huang et al., 2020; Yu et al., 2018) for their applications in energy-limited settings including wearable technology (Bandodkar et al., 2017; Hickey et al., 2016; Jia et al., 2013). In contrast to traditional amperometric sensors (Claussen et al., 2011, 2012; Jin and Alam, 2019; Jin et al., 2016; Zhou et al., 2014; Zhu et al., 2016) (Fig. 1(a) and (b)) that require bulky external power sources and complex electrical designs and components, their EBFC-based counterparts (Cinquin et al., 2010; Cooney et al., 2008; Fraiwan et al., 2013; Halámková et al., 2012; Minter et al., 2007; Zebda et al., 2011) (Fig. 1(c)) rely on spontaneous

redox reactions thus circumventing the need for external energy source and complex electronics. These attributes allow several orders of device miniaturization essential for the realization of new sensor platforms, for example, tissue-integrated biochemical sensors in the form of epidermal (Jia et al., 2013; Kim et al., 2011) and ocular systems (Badugu et al., 2004; Mitsubayashi and Arakawa, 2016; Park et al., 2018; Yao et al., 2012).

The growing interest in wearable and field deployable miniaturized sensors indicate imminent widespread exploration of EBFC-based sensors as self-powered alternatives (Chen et al., 2019; Jeerapan et al., 2019; Yeknami et al., 2018) to conventional approaches. While the literature points to extensive experimental research in developing these sensors, the field of analytical modeling of these unconventional sensors remain understudied. Such analytical models are crucial for the success of the EBFC-based sensors as these can help researchers fathom complex inter-related redox reactions occurring within the EBFC-based sensors and provide valuable guidelines to develop systems with desired performance. Unfortunately, traditional

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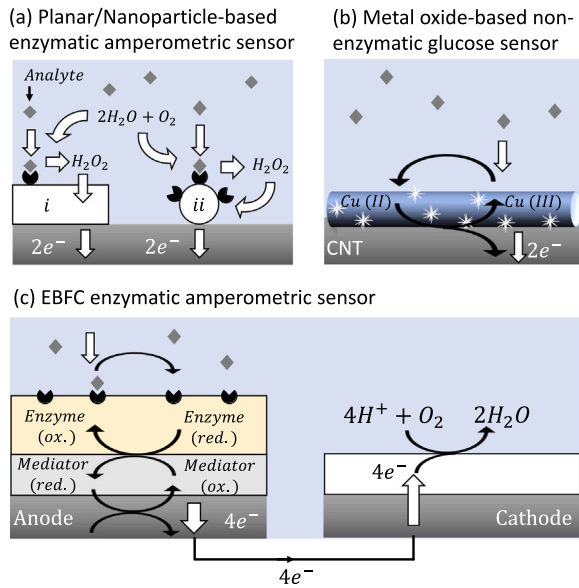


Fig. 1. Schematic illustrations of the working mechanism of (a) (i) Planar/(ii) Nanoparticle-based enzymatic glucose/lactate sensor. GOx/LOx (Y-shaped black molecules) serve as the catalytic enzyme and H_2O_2 works as an electron mediator (b) Metal oxide-based non-enzymatic glucose sensor. (c) EBFC-based amperometric enzymatic glucose/lactate sensor. The reaction details are shown in Fig. 3(a).

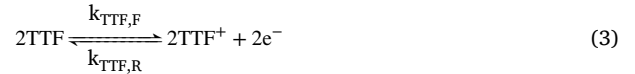
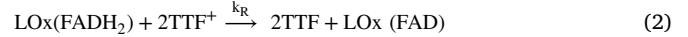
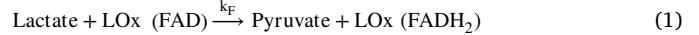
theoretical papers in the field of EBFCs focus on optimizing the performance of EBFC as an energy source (Choi, 2015; Cosnier et al., 2018; Escalona-Villalpando et al., 2019) (e.g. increase output power density, reduce charging time, suppress self-heating and parasitic reaction, etc.). Song et al. illustrate a Michaelis–Menten kinetics derived 3-D modeling and numerical simulation framework for EBFC interdigitated microelectrode arrays with the goal of improving energy density and output power (Song et al., 2014). To assess charging time and self-heating, Chan et al. introduce a dynamic model of anode function in EBFC (Chan et al., 2012). The model includes sophisticated anode reactions and analyze their impact on the battery charging potential, power density and, dynamic response. However, the performance metrics of an EBFC biosensor differ significantly from an EBFC designed as an energy source (Falk et al., 2013; Xiao et al., 2018) and hence cannot be directly applied to EBFC-based sensors. For example, unlike an energy source, a biosensor must be: (a) as small as possible for ease of integration, (b) the cell must always operate at physiologically relevant and time-variable analyte concentrations, (c) the linearity of response is a critical factor for calibration and any long-term drift in the conversion efficiency can mislead clinical diagnosis, (d) the cathode-reaction due to oxygen starvation is often a concern that is seldom encountered in EBFCs used as an energy source, (e) signal-to-noise ratio is a critical parameter to define selectivity and the limits of detection, and (f) the quasi-static evolution of signal need not be described by fast charge–discharge models and self-heating is not a concern.

The goal of this paper is to develop a comprehensive theoretical model (based on physical, chemical, and geometrical parameters) to transparently correlate the analyte concentration to the amperometric current so that one can optimize for the specific metric of EBFC-based biosensor. We use sophisticated numerical modeling plus simple analytical formulas to capture the essential physics, which agree closely with the sensor experimental measurements. We apply an equivalent circuit method to self-consistently include the impact of both anode and cathode. Our work would help theorists to understand the contributions of EBFC-based sensor design parameters and empower experimentalists to predict sensor performance.

2. Model for EBFC-based biosensors

2.1. Working principle of EBFC-based biosensors

Our model is based on a typical EBFC-based lactate biosensor as recently described in Bandodkar et al. (2019). The working mechanism of such a system is the same as the EBFC enzymatic amperometric sensor shown in Fig. 1(c) wherein the lactate concentration dependent EBFC current output is transformed into a voltage signal using an external resistor for wireless data acquisition. Here the lactate present in the sample initiates a two-cycle synergistic redox reaction at the anode: The first cycle involves the enzymatic oxidation of lactate by the enzyme, specifically, the lactate oxidase (LOx), where polymerized flavin adenine dinucleotide (FAD) serves as the substrate for charge transfer. The second cycle involves electron transfer reaction mediated by the mediator: tetrathiafulvalene (TTF). The anode reaction is summarized as follows:



Initially, the lactate molecules diffuse from the bulk solution towards the anode. Once they are captured by the LOx(FAD) enzyme, the enzymatic reaction in Eq. (1) occurs. Electrons are transferred from the lactate molecule through the two redox pairs (LOx and TTF in Eqs. (1) and (2), respectively). Subsequently, the electrons are extracted from the TTF by the voltage dependent reaction in Eq. (3) and collected by the CNT electrode substrate.

The cross-coupling and complexity of enzymatic and mediator reactions at the anode influences the linearity, detection limit, and time-dependent evolution of sensor performance. Unlike traditional amperometric sensors, however, in the EBFC-based sensors the cathodic reaction is also important, see Fig. 1(c). The electrons generated at the anode transfer through an external resistance and reach the cathode where dissolved O_2 and protons (slightly acidic environment for sweat sensor application) gains the electrons and reduces to H_2O as the following:



Unlike in an EBFC used as an energy source, the oxygen starvation in the cathode reaction plays an important role in dictating the linearity and detection limit of EBFC sensors. Moreover, Eqs. (3) and (4) are voltage dependent reactions, and we will see later in Section 2.4 that their reactant-dependent voltage-partitioning have a dramatic effect on sensor response. In the next section, we are going to introduce mathematical models to describe the anodic and cathodic reactions.

To prevent mass crossover between anode and cathode, layers of chitosan and PVC are coated on the anode to reduce leaching of the TTF from the anode to cathode. On the other hand, the fast reaction kinetics and high concentration of TTF and other reagents compared to that of the O_2 at the anode ensure that the anodic reaction is dominated by TTF rather than O_2 . Therefore, the effect of mass crossover between the cathode and anode should be negligible.

2.2. 3D transient modeling of EBFC-based biosensor

As described in the previous section, the lactate molecules diffuse in the bulk biofluid solution and react with the enzyme immobilized onto the anode. The diffusion and reaction of lactate molecules is subject to the differential equation as:

$$\frac{d[\text{C}_3\text{H}_6\text{O}_3]}{dt} = D_L \nabla^2 [\text{C}_3\text{H}_6\text{O}_3] - k_F [\text{C}_3\text{H}_6\text{O}_3] [\text{LOx (FAD)}] \quad (5)$$

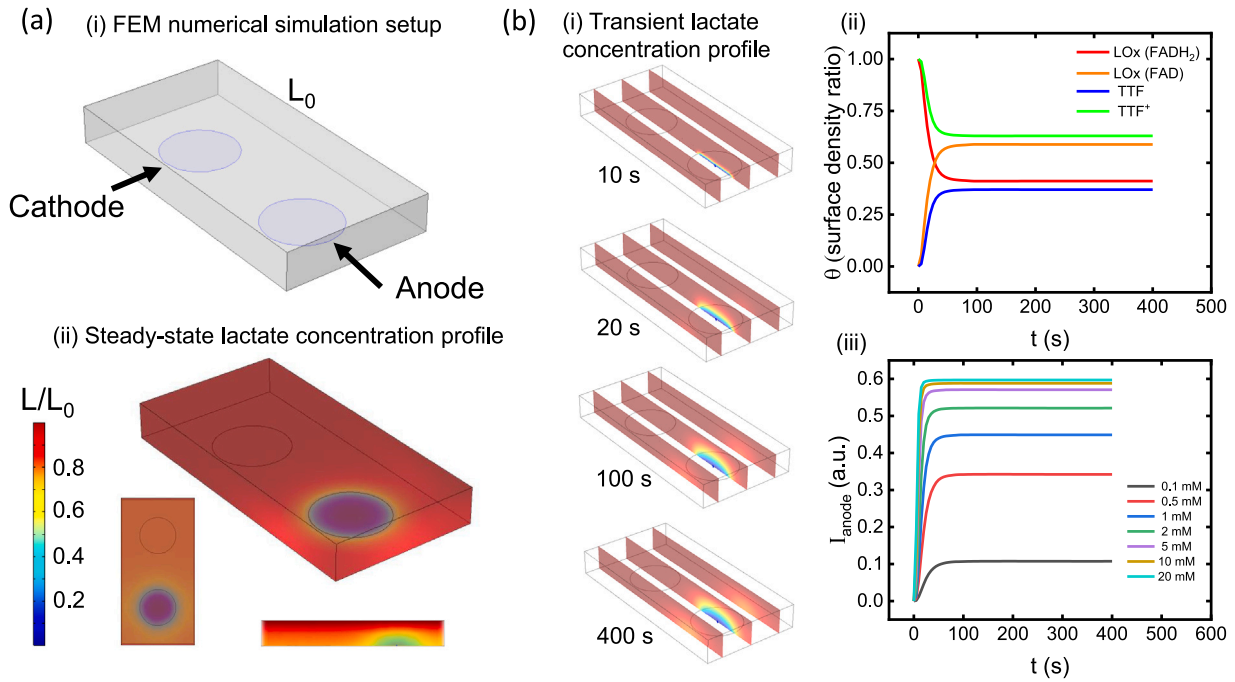


Fig. 2. 3D Finite Element Numerical simulation. (a) (i) Simulation setup: A planar disk-like cathode/anode at the bottom of the simulation domain. (ii) Steady-state lactate concentration profile. (b) The transient response of EBFC-based sensor (i) Time sequence plot of lactate concentration. (ii) The surface densities of the intermediate reactants. (iii) The transient output voltage from numerical simulation.

where D_L is the diffusivity of the lactate molecule in the bulk solution. k_F is the forward oxidation reaction constant in Eq. (1), and Eqs. (6) to (9) describe the reaction fluxes for the analytes in the following two-cycle redox reactions:

$$\frac{d[\text{LOx}(\text{FADH}_2)]}{dt} = D_Q \nabla^2 [\text{LOx}(\text{FADH}_2)] + k_F [\text{C}_3\text{H}_6\text{O}_3] [\text{LOx}(\text{FAD})] - k_R [\text{LOx}(\text{FADH}_2)] [\text{TTF}^+] \quad (6)$$

$$\frac{d[\text{LOx}(\text{FAD})]}{dt} = D_P \nabla^2 [\text{LOx}(\text{FAD})] - k_F [\text{C}_3\text{H}_6\text{O}_3] [\text{LOx}(\text{FAD})] + k_R [\text{LOx}(\text{FADH}_2)] [\text{TTF}^+] \quad (7)$$

$$\frac{d[\text{TTF}]}{dt} = D_M \nabla^2 [\text{TTF}] + k_R [\text{LOx}(\text{FADH}_2)] [\text{TTF}^+] - k_{\text{TTF},F} [\text{TTF}] + k_{\text{TTF},R} [\text{TTF}^+] \quad (8)$$

$$\frac{d[\text{TTF}^+]}{dt} = D_N \nabla^2 [\text{TTF}^+] - k_R [\text{LOx}(\text{FADH}_2)] [\text{TTF}^+] + k_{\text{TTF},F} [\text{TTF}] - k_{\text{TTF},R} [\text{TTF}^+] \quad (9)$$

Here, D_Q , D_P , D_M , and D_Q are the diffusion coefficient for enzyme LOx (LOx (FAD)-LOx(FADH₂)), and the electron transfer mediator TTF-TTF⁺ respectively. The two paired reactants (i) LOx (FAD) and LOx (FADH₂) and (ii) TTF and TTF⁺ follow the mass conservation relation as:

$$[\text{LOx}(\text{FADH}_2)] + [\text{LOx}(\text{FAD})] = R_0 \quad (10)$$

$$[\text{TTF}] + [\text{TTF}^+] = K_0 \quad (11)$$

where R_0 and K_0 are the total concentrations of enzyme and electron transfer mediator on the anode surface. On the cathode side, the oxygen reduction reaction follows similar flux and mass conservation equations:

$$\frac{d[\text{O}_2]}{dt} = D_H \nabla^2 [\text{O}_2] + k_{\text{O}_2,R} [\text{H}_2\text{O}]_{\text{Cathode}} - k_{\text{O}_2,F} [\text{O}_2] [\text{H}^+] \quad (12)$$

$$\frac{d[\text{H}_2\text{O}]_{\text{Cathode}}}{dt} = k_{\text{O}_2,F} [\text{O}_2] [\text{H}^+] - k_{\text{O}_2,R} [\text{H}_2\text{O}]_{\text{Cathode}} \quad (13)$$

$$[\text{O}_2] + [\text{H}_2\text{O}]_{\text{Cathode}} = O_0 \quad (14)$$

where D_H is the diffusivity of O₂, $k_{\text{O}_2,F}$ and $k_{\text{O}_2,R}$ are the voltage dependent reaction constants from Eq. (4). O_0 is the total dissolved

oxygen element concentration (in the form of O₂ or H₂O_{Cathode}) near the cathode. Generation of one 1 mol of H₂O_{Cathode} consumes 2 moles of electrons, which requires a consumption of 2 moles of TTF from the anode. Therefore, an additional electron balance equation between the anode and the cathode should be included as:

$$2 [\text{TTF}] = [\text{H}_2\text{O}]_{\text{Cathode}} \quad (15)$$

Note that the reaction constants from Eqs. (5) to (9) and Eqs. (12), (13) depend on the electrode potential that follow the exponential relationship defined by the Butler–Volmer equations as:

$$k_{\text{TTF},F} = k_{0,A} \cdot \exp((E_A - E_{0,A})/\beta_{\text{Ox},A}) \quad (16)$$

$$k_{\text{TTF},R} = k_{0,A} \cdot \exp(-(E_A - E_{0,A})/\beta_{\text{Red},A}) \quad (17)$$

$$k_{\text{O}_2,F} = k_{0,C} \cdot \exp((E_C - E_{0,C})/\beta_{\text{Ox},C}) \quad (18)$$

$$k_{\text{O}_2,R} = k_{0,C} \cdot \exp(-(E_C - E_{0,C})/\beta_{\text{Red},C}) \quad (19)$$

where $k_{0,A}$ and $k_{0,C}$ are the reaction constant prefactor, E_A and E_C are the anode/cathode potential. $E_{0,A}$ and $E_{0,C}$ are the anode/cathode equilibrium potential, while $\beta_{\text{Ox},A}$, $\beta_{\text{Red},A}$, $\beta_{\text{Ox},C}$, $\beta_{\text{Red},C}$ are the Tafel slopes of the oxidation/reduction reactions on the anode and cathode respectively. Next, we will solve for Eqs. (5) to (19) both numerically and analytically.

Based on the specific lactate sensor design parameters reported in Bandodkar et al. (2019), Eqs. (5) to (19) are solved numerically in a 3D coupled diffusion–reaction Finite Element Method (FEM) solver in COMSOL. The numerical simulation of the geometrical structure is shown in Fig. 2(a)(i). The planar disk-like anode and cathode with radius of r are located at the bottom of the unit cell. The anode is coated with two layers of redox reactants (LOx layer on top of the TTF layer). The reaction flux on the circular electrode surface is set to be $J_{\text{rec}} = k_F [\text{C}_3\text{H}_6\text{O}_3] [\text{LOx}(\text{FAD})]$. The size of the unit cell (w: 4 mm, l: 8 mm, h: 1 mm) represents a droplet of 0.032 mL biofuel sample solution. The bulk lactate concentration L_0 is held fixed at the top surface of the unit cell. We chose reflective boundary condition on the side surface of the unit cell. Preliminary studies reveal that increasing the size of the unit cell does not affect the results significantly.

The numerical solution in Fig. 2(a)(ii) shows the lactate concentration profile in the bulk cell in steady state. The lactate concentration depletes near the anode due to the lactate redox reaction. For the transient response, a time sequence of lactate bulk concentration is shown in Fig. 2(b)(i). The time dependent surface density ratio of LOx (FAD), LOx (FADH₂), TTF, and TTF⁺ are shown in Fig. 2(b)(ii). After the transient phase lasting several tenth of seconds, the surface density of each species saturates to a constant value determined by the lactate concentration. The total anode current generated from lactate reactions can be calculated by integrating the electron flux over the entire anode follows:

$$I_{anode} = qN_A \int J_{e-} dS \quad (20)$$

where N_A is the Avogadro constant and J_{e-} is the electron generation flux. In Fig. 2(b)(iii), we sweep the lactate bulk concentration L and record the anode current I_{anode} . The corresponding steady state response (current vs. concentration) shows that I_{anode} saturates to a constant value at high lactate concentration. The 3-D numerical model is an important contribution of this research because it can serve as a design tool for EBFC of arbitrary geometrical configuration and reaction rate constants.

2.3. Quasi-steady state analytical model: Anodic reaction limited EBFC-based sensor response

A key insight from the numerical simulation is that diffusion limits do not play an important role in the range of analyte concentration of interest and the ultra-fast time-response itself is not of significant interest for relatively slow varying physiological signals (e.g. lactate concentration in biofluids). We can therefore neglect the diffusion delay terms from Eqs. (5) to (19). In addition, we approximate the Butler–Volmer reaction constant in Eqs. (16) and (17) as $k_{TTF,F} = k_p$ and $k_{TTF,R} = 0$ since the exponential voltage-dependence makes $k_{TTF,F} \gg k_{TTF,R}$. Remarkably, with these modest simplifications, Eqs. (6) to (9) transforms into a coupled system of rate equation amenable to analytical simulation. The goal is to write simple but generalized equations suitable for EBFC-based biosensors to assist in design optimization and to identify system limits. The model results are validated against numerical simulation and experimental results to ensure that approach is justified.

$$\frac{dQ}{dt} = k_F LP - k_R QN \quad (21)$$

$$\frac{dP}{dt} = k_R QN - k_F LP \quad (22)$$

$$\frac{dM}{dt} = k_R QN - k_p M \quad (23)$$

$$\frac{dN}{dt} = k_p M - k_R QN \quad (24)$$

P , Q , N , and M represent the surface density of LOx (FAD), LOx (FADH₂), TTF, and TTF⁺ since the diffusion of the chemicals across different layers on the electrode is ignored. R_0 and K_0 from Eqs. (10) and (11) also reduce to the total surface density of LOx(FAD), LOx (FADH₂), TTF, and TTF⁺ respectively. We can solve Eqs. (21) to (24) analytically in a normalized form by rewriting the parameters: $L^* = L/L_0$ (L_0 is chosen to be the upper limit of the lactate detection concentration), $P^* = P/R_0$, $Q^* = Q/R_0$, $M^* = M/K_0$, $N^* = N/K_0$, $t = \frac{t}{t_0} = \frac{t}{1/k_R K_0}$. In the steady state, we can set the reaction flux in Eqs. (21) to (24) equal to zero. Including the mass conservation Eqs. (10) to (11), the normalized form of the system of equations can be simplified as the following:

$$\frac{dQ^*}{dt^*} = -\frac{dP^*}{dt^*} = \alpha L^* P^* - Q^* N^* = 0 \quad (25)$$

$$\frac{dN^*}{dt^*} = \frac{\alpha}{\beta} QN - \beta M^* = 0 \quad (26)$$

$$P^* + Q^* = 1 \quad (27)$$

$$M^* + N^* = 1 \quad (28)$$

where $\alpha = \frac{k_F L_0}{k_R K_0}$ and $\beta = \frac{k_p}{k_R K_0}$ are unitless parameters derived from the combination of different sensor parameters.

By solving for Eqs. (25) to (28) analytically, we can express the anodic current in steady state as the following:

$$I_{anode} = A \cdot q \cdot N_A \cdot k_p K_0 \cdot M^* = AqN_A \cdot k_p K_0 \frac{1}{2} \left(\Delta - \sqrt{\Delta^2 - 4\sigma} \right) \quad (29)$$

where A is the effective area of the sensor electrode, $\Delta = 1 + \frac{\alpha}{\beta} L^* + \alpha L^*$, $\sigma = \frac{\alpha}{\beta} L^*$. With $\frac{4\sigma}{\Delta^2} \ll 1$, Eq. (29) can be further approximated as the following:

$$I_{anode} \approx A \cdot q \cdot N_A \cdot \frac{k_p K_0}{1 + \beta} \cdot \frac{L^*}{L^* + \frac{\beta}{(\beta+1)\alpha}} \quad (30)$$

This expression for EBFC-based sensor response has the same form as the Michaelis–Menten equation where the term $\frac{\beta}{(\beta+1)\alpha}$ in the denominator determines the output linear dynamic range while the pre-factor $AqN_A \cdot \frac{k_p K_0}{1 + \beta}$ dictates the sensitivity of the EBFC sensor.

Next, we calculate the output voltage signal measured across the external resistor. In an ideal case where the cathode can accept any number of electrons generated by the anode, i.e. there is enough dissolved O₂ at the cathode in the system, the overall EBFC output current is only limited by the anode reaction. The output voltage can be simply expressed as:

$$\psi_{total} = R \cdot I_{anode} + \psi_0 \quad (31)$$

where R is the external resistance, ψ_0 is the constant background signal generated by the parallel secondary reaction. Eqs. (30) and (31) define the ultimate performance limit of EBFC-based biosensors.

2.4. Cathode-reaction limited (self-consistent) response

In practice, however, the anode-limited performance limit is seldom achieved: Fig. 3(b) shows a cyclic-voltammetry experimental measurement result. In this measurement, we isolate the anodic reaction by utilizing the anode as a working electrode in a conventional three electrode system comprising of a platinum wire-based counter electrode and an Ag/AgCl reference electrode. We found that the linear dynamic range extends up to 50 mM which is higher than that offered by the EBFC-based sensor. This interesting phenomenon is due to the oxygen redox reaction at the cathode of the EBFC-based sensor becomes the rate limiting factor due to the small amount of dissolved oxygen in the biofluid. In this section, we include the contributions from the coupled anodic and cathodic reactions shown in Fig. 3(a) to generate a more accurate analytical model that resembles the real-life scenarios.

To include the effect of oxygen starvation, we solve the voltage dependent Butler–Volmer equations self-consistently as an equivalent circuit shown in Fig. 3(c). Since the redox reaction current I is proportional to the reaction constants, the I-V characteristics of the voltage dependent redox reactions at both anode and cathode can be viewed as an ideal diode in series with a constant voltage source (the value equals to the redox equilibrium potential E_0). The forward oxidation and reverse reduction reactions on both electrodes are equivalent to two of such diode pairs connected in parallel but in opposite direction. By applying this model to solve for the circuit equilibrium point where $I_{anode} = I_{cathode}$, we take care of all the voltage dependent reaction constants for both anode and cathode in a self-consistent way. No approximations (e.g. $k_{TTF,F} = k_p$, $k_{TTF,R} = 0$ in the previous section) are made in this condition. The sensor output voltage would be the exact solution of the voltage drop across the external resistor R in the equivalent circuit.

The voltage-drops driving anode and cathode reactions must be solved self-consistently. There is no explicit analytical expression for the self-consistent Butler–Volmer equivalent circuit in equilibrium. Here, we use MATLAB equation solver to find the implicit solution of

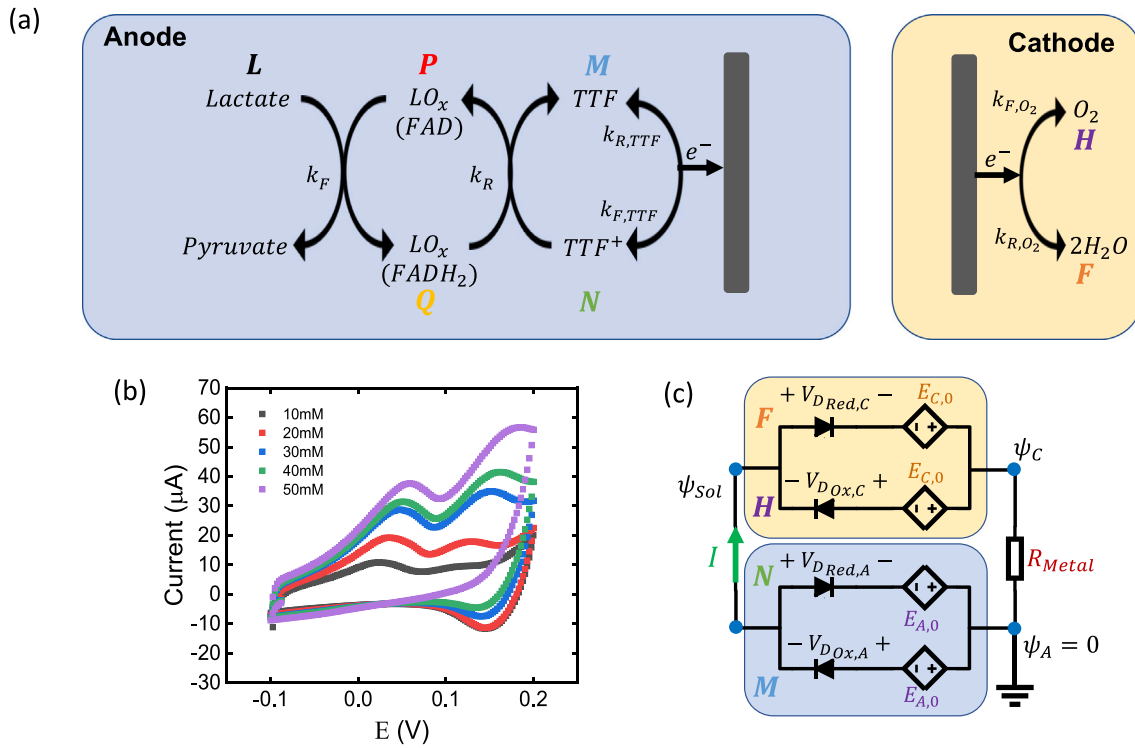


Fig. 3. (a) Detection mechanism of EBFC-based lactate sensor. (b) C-V measurement of anode against commercial Ag/AgCl reference electrode and platinum wire-based counter electrode. In this 3-terminal idealized setup, the anode can respond at least up to 50 mM of lactate concentration, much higher than the 20mM response observed in 2-terminal EBFC-based sensor shown in Fig. 4(a), indicating that EBFC-sensor is limited by cathode reaction. (c) Illustration of the equivalent circuit model.

the following system of equations plus the mass conservation Eqs. (14) and (15):

$$I_{anode} = I_{Ox,A} - I_{Red,A}$$

$$= qN_A k_{0,A} \left[\exp\left(-\frac{\psi_{Sol} + E_{A,0}}{\beta_{Ox,A}}\right) M - \exp\left(\frac{\psi_{Sol} + E_{A,0}}{\beta_{Red,A}}\right) N \right] \quad (32)$$

$$I_{cathode} = I_{Red,C} - I_{Ox,C}$$

$$= qN_A k_{0,C} \left[\exp\left(\frac{\psi_{Sol} + E_{C,0} - I_{total} \cdot R_{Metal}}{\beta_{Red,C}}\right) H - \exp\left(\frac{I_{total} \cdot R_{Metal} - \psi_{Sol} - E_{C,0}}{\beta_{Ox,C}}\right) F \right] \quad (33)$$

$$I_{total} = I_{anode} = I_{cathode} = qN_A \cdot k_F LP = qN_A \cdot k_R QN \quad (34)$$

where $I_{Ox,A}$, $I_{Red,A}$, $I_{Ox,C}$, $I_{Red,C}$ are the oxidation and reduction current at anode and cathode respectively. ψ_{Sol} is the electrical potential in the solution. As shown in Fig. 3(c), M and N are the surface densities of TTF and TTF⁺ on the anode. H and F are the concentrations of O₂ and H₂O_{Cathode} near the cathode. They follow the mass balance and the electron balance equations from Eqs. (11), (14) and (15) as: $M + N = R_0$, $H + F = O_0$, and $2N = F$.

Fig. 4(a) shows the implicit solution of output voltage ψ_c of the Eqs. (14), (15), (32) to (34) at different lactate concentrations. It also shows the non-linear property of the output signal where the ψ_c saturates at high lactate concentration.

3. Experimental validation of the analytical results

Next, we validate our model against both the steady-state and transient response of the experimental measurement data from Bandodkar et al. (2019). The EBFC-based lactate sensor design consists of circularly cut carbon nanotube (CNT) paper as the anode. The CNT paper

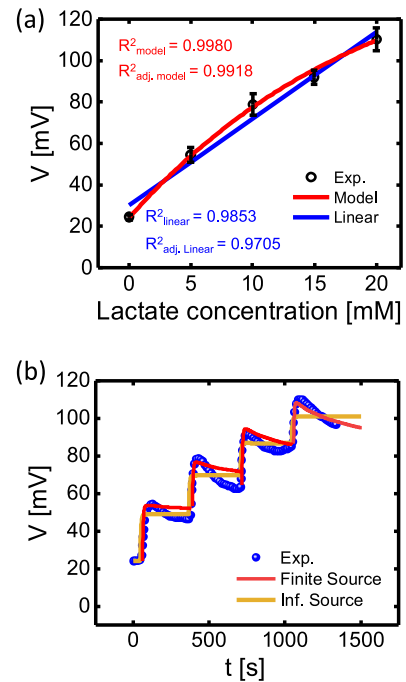


Fig. 4. (a) Steady-state response from experiment vs. model vs. linear fitting. (b) Transient response of EBFC-based lactate sensor: experiment (blue dots) vs. model (red line: finite source setup, yellow line: infinite source setup). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

provides a conductive and high-surface area substrate to immobilize the oxidase enzyme and TTF for shuttling electrons from enzyme to CNT conducting paper.

Table 1
Fitting parameters for analytical model.

Fitting parameters		Known parameters	
$\alpha = \frac{k_F L_0}{k_R K_0}$	1.378	r	1 mm
$\beta = \frac{k_p}{k_R R_0}$	4.91	R	50 k Ω
$k_p \cdot K_0$	6.22×10^{-5} mol/s/m ²	A	πr^2

3.1. Steady-state response

Fig. 4(a) shows how our model interprets the steady-state lactate experimental data. Since the average concentration of lactate in sweat is around 14 mM, the *in-vitro* measurement take place in phosphate buffer (pH 7.0) solution with lactate concentration ranges from 0 mM to 20 mM with a 5 mM increment. By fitting the unknown parameters (α , β , k_p , K_0 .) summarized in Table 1, our model as described in Section 2.4 captures the experimental data with high fidelity. At $c_L = 0$ mM, we find the constant background signal ψ_0 generated by the parallel reaction to be 22 mV. Our model perfectly captures the non-linear response of the EBFC-based lactate sensor. Fig. 4(a) shows a comparison between the traditional linear fitting vs. our model. The adjusted R^2 value increases from 0.97 to 0.99 and the root-mean square error reduce from 3.36 to 1.25.

3.2. Transient response

Fig. 4(b) shows the transient measurement data of the EBFC-based lactate sensor. The measurement starts from 0 mM lactate solution with a sequential 5 mM lactate concentration increment up to 20 mM. For each concentration, the measurement lasts ~ 300 s until the output voltage signal is stabilized. Here, we apply our numerical model to describe the sensor transient behavior. We keep fitting parameter (α , β , and γ) the same as the steady state model and increase the lactate concentration every 5 mM at $t = 45, 365, 706$, and 1043 s. For the infinite source case (yellow solid line in Fig. 4(b)), we use a constant lactate concentration at the top boundary as described in the numerical simulation section. When the sample concentration increases by 5 mM, transient voltage response jumps up and saturates to the steady-state value. In the experimental data, however, the voltage signal initially jumps up to a larger value, and then decays slowly to a stable value. This interesting phenomenon is due to the finite lactate source in the sampling solution. The initial large jump corresponds to a large amount of lactate molecules instantly being introduced and mixed in the sample solution. The mixing adds additional convection causing the signal overshoot. Then the mixed solution reaches the steady state slowly with consumption of the lactate molecules by the EBFC-based lactate redox reactions in Eqs. (1) and (2). In a new numerical simulation setup, we include the effects of finite lactate source by setting a constant initial lactate concentration in bulk solution. The simulation result shown in Fig. 4(b) (red solid line) follows the trend of the signal decay.

4. Design principles for EBFC

In the previous section, we have interpreted the experimental results of the steady-state and transient response of a EBFC-based lactate sensor. In this section, we are going to explore the effect of some critical design parameters of the sensor. Based on our model prediction, we will provide design guidelines for improving the sensor performance.

For the EBFC-based sensor experimentalist, there are two important parameters that can be easily tuned during the sensor fabrication process: LOx surface density R_0 and TTF surface density K_0 on the working electrode. In addition, the dissolved oxygen level O_0 near the cathode is another important limiting factor for the biofuel sensor system.

4.1. Effect of enzyme surface density

Fig. 5(a) shows the effect of enzyme (LOx) surface density R_0 on the enzyme-functionalized electrode of the biofuel cell-based sensor. In our analytical model, we increase R_0 from 5×10^{-7} mol/m² to 3×10^{-6} mol/m² while keeping the other parameters the same. We find that an increase in the enzyme concentration R_0 increases the sensitivity in the linear dynamic region, but the non-linear curve saturates at a lower lactate concentration. Therefore, there is a trade-off between the linear dynamic range and sensitivity when tuning the enzyme surface density.

This interesting phenomenon has been validated by control experiment. Fig. 5(b) shows the characterization results of three different EBFC-based lactate sensors (dots) prepared by immobilizing 4 μ g, 40 μ g, and 80 μ g of LOx. We calibrate our model to fit the experimental data by choosing LOx surface density R_0 the correlates to the amount of the immobilized LOx enzyme. Despite some small discrepancy at relatively low quantities (4 μ g) of LOx loading, the results from the model correlates well with that from the experiments, thus illustrating the capabilities of the model to predict sensor performance.

4.2. Effect of electron transfer mediator surface density

In Fig. 5(c), we analyze the importance of TTF surface density K_0 in Eq. (11). We find that unlike the effect of R_0 , increasing K_0 does not control the sensitivity of the EBFC output signal in the linear dynamic range. Instead, it increases the width of this linear dynamic range. Hence, experimentalists can manipulate this electron transfer mediator (TTF) density on their EBFC anode design to tune the linear dynamic range of the output signal without affecting the sensitivity. This conclusion is purely derived from the model. We would suggest systematic design of experiment as a future work to validate our theoretical predictions.

4.3. Limiting effect of the cathode reaction

In Section 4.1, we study the effect of enzyme surface density and electron transfer mediator surface density of the EBFC output response by assuming infinite oxygen supply from the cathode side from the self-consistent equivalent circuit model. Our model could also illustrate the effect of O_2 starvation on the cathode. The dashed lines in Fig. 5(d) shows that when dissolved oxygen is insufficient, an increase in the dissolved O_2 near the cathode scales up the output signal at all lactate concentrations. The model predicts that the limiting effect of the cathodic reaction vanishes at dissolved O_2 concentrations above $\sim 10^{-5}$ mM. Therefore, the dashed lines calculated from our equivalent circuit model approach the red solid line calculated from the simple single-anode analytical model where we assume that the redox reaction on the cathode is not the rate limiting factor. This is a unique feature of the EBFC-based sensor wherein the type of cathode redox reactions as well as the reactants should be carefully chosen in order to reduce the impact of the cathode. Our equivalent circuit model provides a quantitative option for inspecting this interesting cathodic reaction.

To eliminate this oxygen-deficit limitation, several novel cathode designs have been reported in the literature. Jeeran et al. use polychlorotrifluoroethylene (PCTFE), an oxygen-rich cathode material, to provide internal oxygen supply for EBFC cathode reduction reaction (Jeeran et al., 2018). Yu et al. demonstrate a new solid-state Ag_2O/Ag cathode design which makes it possible for EBFC to operate under anaerobic condition (Yu et al., 2016). Our model can easily adapt to quantitatively characterize the performance of such innovative EBFC cathode by calibrating the cathodic reaction constants as well as the concentration of different reactants in Eqs. (5) to (19), and (32) to (34).

Last but not the least, other geometrical sensor design parameters, electrode radius r for instance, could also scale the amperometric response of the EBFC-based sensor. We have discussed the sensor electrode geometrical factors in details in our previous published papers (Jin and Alam, 2019; Jin et al., 2016).

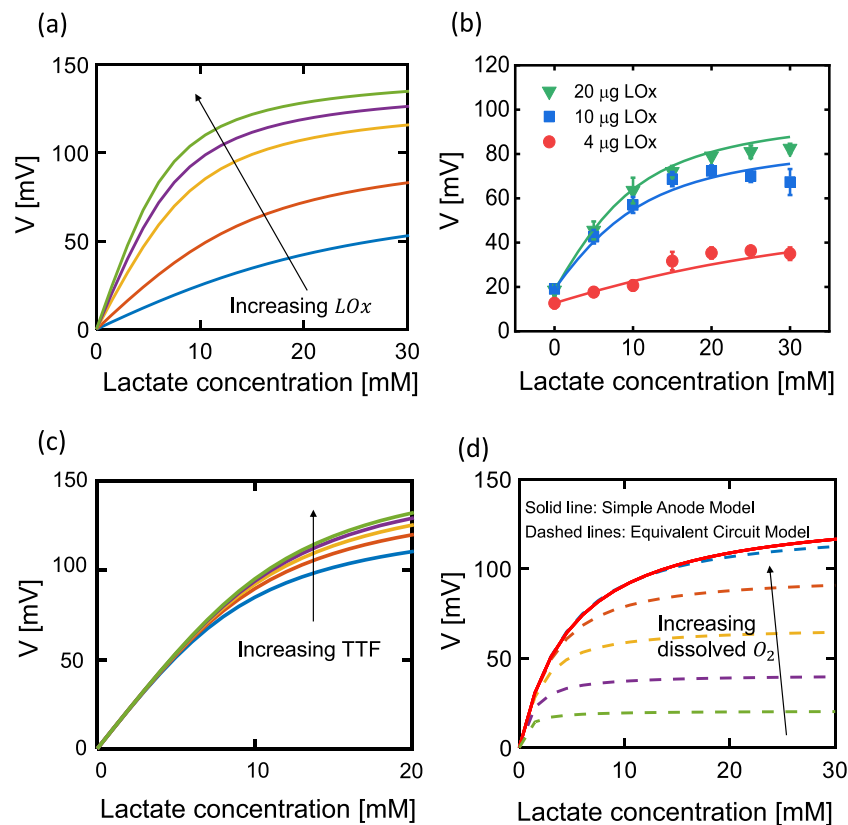


Fig. 5. (a) The simulated parametric response of biofuel sensors. Potentiometric response of biofuel lactate sensor with different TTF surface density. (b) Experimental validation of the effect of surface density of LOx. (LOx layer is prepared by 1 mg/ml, 10 mg/ml, 20 mg/ml of LOx load solution) (c). Potentiometric response of EBFC lactate sensor with sweeping LOx surface density. (d) The impact of reactant concentration (dissolved oxygen) at the cathode of EBFC.

5. Conclusions

To summarize, we address the fundamental impact of different design parameters of EBFC-based sensors with sophisticated numerical modeling plus simple analytical formulas capturing the essential physics. We apply a self-consistent equivalent circuit method to couple the reactions for both the anode and cathode. Our model agrees closely with the experimental measurement data. We show that:

1. The *sensitivity* of the EBFC-based sensor can be enhanced by increasing the surface density of enzymatic layer (LOx). The electron mediator (TTF) does not affect the sensitivity.
2. The *linear dynamic range* of the steady state response is controlled by both the enzyme and the electron mediator, but in an opposite way. Increasing the surface density of oxidoreductase enzyme would shrink the linear region while an increase in the surface density of the electron mediator layer would enlarge it.
3. The redox reaction on the cathode is another limiting factor for the sensitivity of EBFC-based biosensor. In the specific case of lactate EBFC sensor, the *oxygen starvation* effect is critical and should be carefully handled for real-life applications.

Our self-consistent equivalent circuit method for Butler-Volmer relationship could be applied to help optimize other two-electrode amperometric redox reaction systems such as fuel cell battery design, metal corrosion problems in new energy storage systems (Asadpour et al., 2019).

CRediT authorship contribution statement

Xin Jin: Conceptualization of this study, Methodology, Theoretical derivation, Model fitting. **Amay J. Bandodkar:** Design and lead the experiment. **Marco Fratus:** Derivation of the general n-number reaction

loop model, the details can be obtained from the corresponding author.

Reza Asadpour: Conceived the idea of self-consistent equivalent circuit model. **John A. Rogers:** Supervised the experimental design of the EBFC-sensor design. **Muhammad A. Alam:** Supervised the theoretical analysis and correspondence between theory and experiments..

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Material and method

Sensor fabrication: The EBFC fabrication process includes first depositing 20 nm Ti followed by 100 nm Au on 75 μm thick polyimide (PI) sheet (Argon Inc., CA, USA) via electron beam evaporation. Subsequently, the Au-coated PI sheet is laser patterned using a UV laser (LPKF U4, Germany) to obtain the current collector, circular current collector, serpentine interconnects, and contact pads from this sheet. Separately, anode functionalization process is carried out which includes using a mechanical punch to excise a circular pad (diameter: 2 mm) of CNT paper (Thin Film BA-01-145; NanoTechLabs, NC, USA) followed by applying 2 μl of 0.1M TTF (Sigma Aldrich, MO, USA) solution prepared in acetone/ethanol (1:9 v/v) and allowing it to dry for 1 h at ambient conditions. Next, 4 μl of LOx (Toyobo USA Inc, NY) is deposited onto the TTF-coated CNT paper. Subsequently,

2 μl of 1 wt% chitosan (CAS Number 9012-76-4; Sigma Aldrich, MO, USA) suspension prepared in 0.1M acetic acid is drop casted onto the enzyme-functionalized CNT pad. Thereafter, the pad is dip coated using the chitosan solution for 10 s. The pad is then dip coated using a 3 wt% PVC (Selectophore[®] grade, Sigma Aldrich, MO, USA) for 10 s. At every step the pad is air dried for 1 h at ambient conditions. Finally, the pad is bonded to the circular current collector using a silver epoxy and left at ambient conditions for 2 h for room temperature curing of the epoxy. The cathode for the lactate sensor resulted from drop casting 15 μl of 10 mg/ml platinum black (Sigma Aldrich, MO, USA) suspension prepared in deionized water, followed by applying 1 μl of Nafion[®] 117 solution (Sigma Aldrich, MO, USA), onto the cathode designated gold current collector. Storing the sensors at 4 °C for at least 1 week before use allowed the chitosan and PVC membranes to stabilize.

Appendix B. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.bios.2020.112493>.

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