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Functional modeling of electrochemical whole-cell biosensors

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ABSTRACT

The response modeling of whole-cell biochip represents the link between cellular biology and transducer output, allowing better system engineering. It provides the mathematical background for signal and noise modeling, performance prediction and data analysis. Here we describe an analytical model for whole-cell biosensors with electrochemical detection for single use, test and dispose applications. In this system the electrochemical signal is generated by the oxidation of the by-products of the reaction between an external substrate and the enzyme alkaline phosphatase. The enzyme expression can be either normal or enhanced due to the response of the biological cell to an external excitation. The electrochemical oxidation current is measured as a function of time. The model is based on the electrochemical reaction rate equations; an analytical solution is presented, compared to data and discussed.

1. Introduction

Over the last few years, microchips' footprint decreased and their complexity increased, due to advances in micro- and nano-fabrication and cross-disciplinary micro and nano system technology. Biochips may integrate a variety of biological components on a chip; in a subset of this field, microbial cells are integrated into micro-environmental systems and their reactions to the tested samples are monitored on-chip [1–4]. In these bio-micro-electro-mechanical-systems (bio-MEMS) sensors, live microbial cells are part of the mechanism that converts a chemical, physical or a biological signal into an electrical one [5]. Microbial sensors can be genetically engineered [6] to detect very complex series of reactions that can exist only in an intact, functioning cell [7]. Microbial biosensors have been proposed for usage in diverse applications including monitoring glucose [8], microbial growth rate [9] and response to biocides [10]. They are also used for environmental monitoring including the detection of toxicity, genotoxicity, and the presence of specific groups of chemicals [11–13].

Whole-cell biosensors can be integrated onto analytical devices made up of a combination of a specific microbial cell that recognizes an analyte and a transducer that translates the biorecognition event into an electrical signal. The electrical component and circuit modeling is an inherent part of such system's design and evaluation; however, the modeling of the biological system, as part of a "system on a chip" is still at its infancy. Electrochemical [14] and optical [15] whole-cell biosensors have recently been numerically modeled to elucidate the influence of different parameters of the biosensor, e.g. geometry and cell concentration, on its performance. Furthermore, an analytical mathematical model was developed to monitor the electrical signal generated by optical whole-cell biosensor [16–18]. Although various models had been developed to monitor enzymatic electrochemical biosensors [19–22], analytical model describing the signals generated by amperometric whole-cell biosensors has not been reported.

In this paper, we modeled the bioelectrochemical signal generated by amperometric whole-cell biosensor by solving the system differential rate equations that are based on the enzymatic kinetics developed by Michaleis and Menten [23]. The model validity was tested by monitoring the functional response of a recently developed electrochemical whole-cell biosensor for water toxicity analysis [12]. This model is also essential in the process of understanding and verification of the biodetection functionality. By directly relating the analyte concentration to the signal generated

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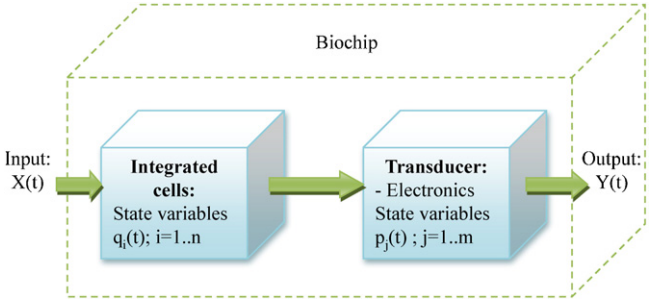

Fig. 1. Whole-cell biosensor block diagram.

by the biosensor, the influence of other associated parameters (such as analyte diffusion coefficient or promoter activity) can be studied and optimized. Moreover, the proposed model can be beneficial in planning other bacterial based biosensors for different applications.

2. Theory

The operating principle of biochips harboring whole-cell biosensors is their response to the presence of external stimulations. These input signals can be any chemical or physical stimulation that causes protein expression that can be detected later. Whole-cell biosensors can operate either as sensors responding to some external excitation (e.g. toxicity, heat) or as differential sensors monitoring changes in the behavior of cells. The schematic block diagram of a whole-cell biosensor is shown in Fig. 1.

The biological response can be modeled using a set of state equations linking a set of internal variables $q_i(t)$ that are related to the reporter protein expression. The biological response function is assumed to be determined by a small number of rate limiting equations, thus simplifying the system. Typically, the signal generation mechanism is non-linear and is the result of a simultaneous response of a large number of cells integrated on the microchip. In this model, we assume identical cells that generating the signal in parallel independently. Those assumptions simplify the system helping us to understand the basic phenomena and derive simple output/input relations. Such approach can be extended to a more elaborated model taking into consideration other effects, both biological and physical. For example, we can take into consideration the effect of the boundaries on the microbes, the analyte transport effect and the interaction between the microbes themselves. In this paper we present a model for the signal; such model can be used for noise modeling yielding expression for the signal to noise estimation.

The response of the biological system depends on various state variables, which are defined by the vector $\{q_i\}$, $i = 1 \ldots n$, where q_i is the concentration of the ith component in the system. The set of the system state equations can be written in a very general form as follows:

$$\dot{q}_i = f(x(t), q_i, \dot{q}_i, t) \tag{1}$$

where i is the index of the ith component that is being taken into consideration. Those state equations are derived from a series of rate equation describing the interdependence between the variables and their change rate. Note that we assume that the rate of change of the variables depends on the variable themselves as well as their rate of change. The functions are non-linear since they may involve some complex interactions involving mass-action laws between the components. In case of electrochemical output where a single protein is generated in response to the excitation signal the state equation decides it change as a function of the other system variables. That protein reacts with a substrate generating products diffusing toward the anode where they can be partly oxidized. The oxidation process affects the system current yielding a measurable signal. Typically, this kind of sensors operates in an amperometric mode where a fixed bias is applied and the current is measured as a function of time.

In the model that is described here for whole-cell electrochemical bio-sensing the following assumptions are postulated: (1) the reporting protein (e.g. enzyme) is generated in response to an external induction and (2) the signal generation is in response to the reporting enzyme activity. We assume that all the responses are immediate and neglect the inherent time delay that is caused by other effects, e.g. transport phenomena, steric rearrangement, etc. We divide the process into two parts: protein expression and protein–enzyme interaction. The first part is modeled assuming that the biological expression rate depends on the concentration of the exciting compound. The rate of the second part depends on the concentration of the expressed enzyme and the substrate and also on the nature of the interaction of the enzyme – substrate which is typically described by the Michaelis–Menten equation. Note, that in the model described in this work we neglect the transport effects assuming they mainly contribute to the transient effect during the early stages of sensing. Nonetheless, transport should be included once we want to deal with the response right after the excitation or the application of the substrate. Therefore, we construct two coupled systems: (1) the reactions that determine the rate of the electro-active by-products generation and (2) the reaction that generates the signal from the by-products.

The enzyme production is initially modeled by the promoter activity in the live cell [14,16,17,24].

$$P_r = \mu \cdot G_0 \cdot C_{Tox} \tag{2}$$

where P_r [M] is the promoter's products concentration, μ [ml bacteria^{-1}] is the promoter production constant, G_0 [bacteria ml^{-1}] is the concentration of the bacterial cells in the sample, and C_{Tox} [M] is the concentration of the toxic material.

We assume that the promoter's product generation is linearly proportional to the bacteria concentration and the toxicant concentration [14,17,24]. At high bacteria concentration or at high toxicant concentration this relation may be different [16,24].

Since the promoter is the sole generator of the responding enzyme concentration we assume that the generation of the enzyme is proportional to the promoter concentration:

$$\frac{dE_T}{dt} = \alpha \cdot P_r \tag{3}$$

where E_T [M] is the total enzyme concentration in the system and α [s^{-1}] is the enzyme production rate constant. Combining expressions (1) and (2) we assume that the total enzyme concentration is resulted by Eqs. (2) and (3):

$$\frac{dE_T}{dt} = k_0 \cdot C_{Tox} \tag{4}$$

where k_0 [s^{-1}] is a rate constant that comprised of the product of α, μ, and G_0. Note that this expression is correct as long as the bacteria concentration and the toxic material concentrations are not too high.

The enzyme can appear in its free state, E, or in its captured complex state ES, therefore the total concentration of the enzyme is given by the following equation:

$$E_T = E + ES \tag{5}$$

The enzyme-substrate indication is modeled by the Michaelis–Menten kinetics [23]. The substrate – enzyme interaction is given as:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} P + E \tag{6}$$