Computational Analysis of Mechanisms Governing the Sensitivity and Efficiency of Enzyme-Based Biosensors and Bioreactors

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Introduction

Enzymes play a crucial role in analytical biosensing systems due to their ability to specifically recognize analytes (substrates) and catalyze their conversion into products that can be readily detected using conventional analytical methods, such as electrochemical, optical, and other techniques [1]. In such systems, enzymes are primarily used in immobilized forms as biosensors or bioreactors. Enzyme-based biosensors and bioreactors are widely used in various fields, including medical and clinical diagnostics, environmental monitoring, as well as industrial and biotechnological processes [2, 3].

Computational modeling of enzyme-based biosensors and bioreactors enables the simulation of biosensor responses and bioreactor yields under both steady-state and transient conditions. The simulations consider biosensors and bioreactors with complex geometries and kinetic schemes that describe the action of biocatalysts. Mathematical and computational tools are widely used to optimize existing biochemical systems and to develop novel ones [4].

The aim of this work was to investigate the influence of partitioning and diffusion limitations on the efficiency of enzyme-based bioreactors and biosensors using a three-layer model incorporating different schemes of enzyme kinetics [5-7].

Mathematical model

The changes in substrate S and product P concentrations **in the enzyme layer** over time are governed by reaction-diffusion equations ($0 \le x \le a_1$, $t > 0$),

$$\frac{\partial S_1}{\partial t} = D_{S_1}\frac{\partial^2 S_1}{\partial x^2} - V(S_1)\,,$$

$$\frac{\partial P_1}{\partial t} = D_{P_1}\frac{\partial^2 P_1}{\partial x^2} + V(S_1)\,, \quad x \in (0, a_1),\ t > 0\,,$$

where $V(S_1)$ is the rate of biochemical reaction:

$$V(S_1) = \frac{V_{\max} S_1}{K_M(1 + S_1/K_I') + S_1(1 + S_1/K_I)}$$

Only the mass transport by diffusion of both compounds takes place in the **other two layers** ($t > 0$),

$$\frac{\partial S_i}{\partial t} = D_{S_i}\frac{\partial^2 S_i}{\partial x^2},$$

$$\frac{\partial P_i}{\partial t} = D_{P_i}\frac{\partial^2 P_i}{\partial x^2},\ x \in (a_{i-1}, a_i),\ t > 0,\ i = 2,3$$

where $S_i(x, t)$ and $P_i(x, t)$ are the concentrations of the substrate and product, D_{Si} and D_{Pi} are the diffusion coefficients, $V_{\max}$ is the maximal enzymatic rate, K_M is the Michaelis constant, and K_I and K'_I are the inhibition constants.

The governing equations, along with the appropriate initial and boundary conditions, form an initial boundary value problem that serves as the mathematical model of the biosensor or bioreactor.

The conditions between the concentrations involve the formal partition coefficients.

Computational simulation

Transient system action was numerically investigated using the finite difference technique.

A semi-implicit linear finite difference scheme has been built, and the resulting system of algebraic equations was solved using the Thomas algorithm.

The simulator has been programmed in Java.

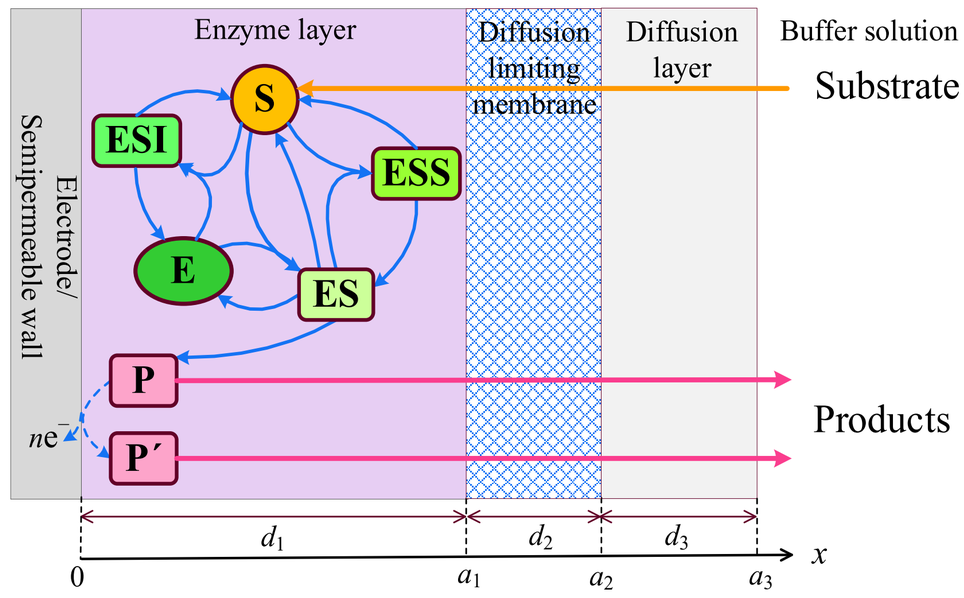
Analytical steady-state solutions were obtained for first- and zero-order reaction rates.

The simulation results were visualized using Origin.

Schematic representation of the treated system

The treated system consists of three relatively thin layers:

- an enzyme-loaded membrane applied to the surface of an electrode (in the case of a biosensor) or an impermeable wall of a bioreactor,
- a diffusion-limiting (semipermeable) membrane, and
- an outer diffusion layer.



Main characteristics

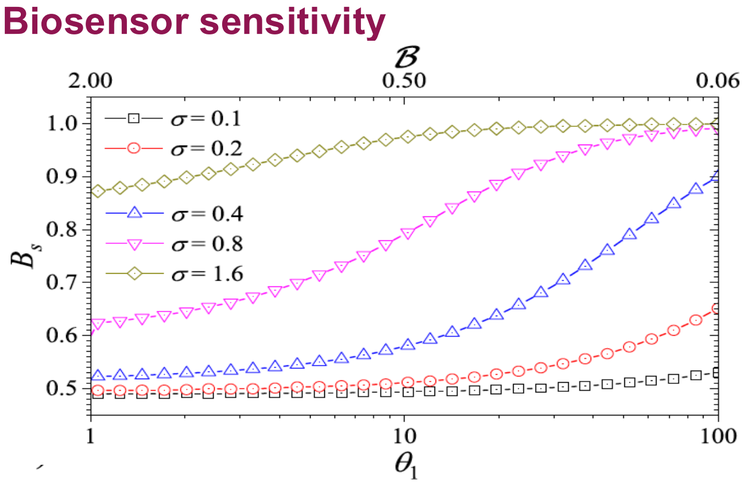
Biosensor output current: $I(t) = n_e F D_{P_1} \left.\frac{\partial P_1}{\partial x}\right|_{x=0}$,

Steady-state current: $I_{\mathrm{ss}} = \lim_{t\to\infty} I(t)$,

Biosensor sensitivity: $B_s(S_0) = \frac{\mathrm{d}I_{ss}(S_0)}{\mathrm{d}S_0} \times \frac{S_0}{I_{ss}(S_0)}$,

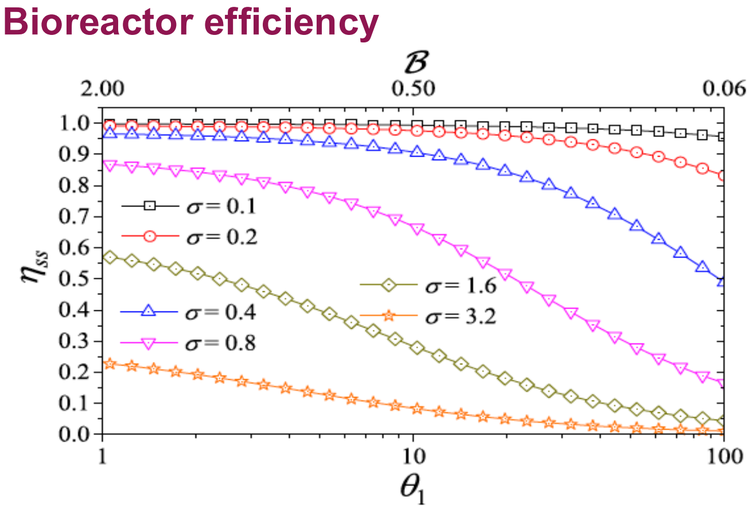
Effectiveness: $\eta(t) = \frac{\int_0^{a_1} V(S_1)\,\mathrm{d}x}{\int_0^{a_1} V(S_0)\,\mathrm{d}x} = \frac{C(t)}{a_1 V(S_0)}$,

Biosensor sensitivity



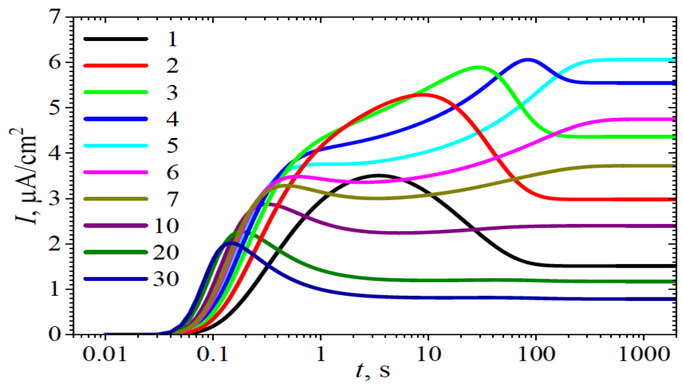
Sensitivity B_s vs. partition coefficient θ_1 and Biot number B at 5 values of the diffusion module $\sigma^2 = V_{\max}a_1^2/(K_M D_{S1})$ $S_0 = K_M$, $K_I = K'_I = \infty$ in amperometric injection analysis by biosensor [5].

Bioreactor efficiency



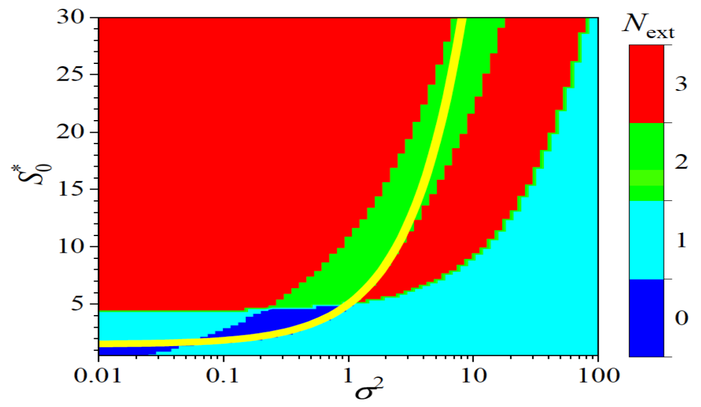
Steady-state effectiveness η_{ss} vs. partition coefficient θ_1 and Biot number B at 6 values of the diffusion module σ^2, $S_0 = K_M$, $K_I = K'_I = \infty$ in potentiometric injection analysis and in a bioreactor [6].

Dynamics of biosensor current



$I(t)$ at ten values of $S_0^* = S_0/K_M$; $V_{\max} = 100$ μM/s, $\theta = 0.75$, $K_I = K_M$, $K'_I = \infty$ in amperometric batch analysis [7].

Number of extrema



Number of extrema in $I(t)$ *vs. diffusion module* $\sigma^2 = V_{\max}a_1^2/(K_M D_{S1})$ *and* S_0/K_M; $K_I = K_M$, $K'_I = \infty$, *in amperometric batch analysis* [7].

Conclusions

Computational modelling provides a framework for analysing the effects of enzymatic kinetics, diffusion limitations, and partitioning on the characteristics of biotechnological devices, and it **enables optimization** of their design parameters for industrial applications.

Under steady-state conditions, the **three-layer** system **can be reduced to** an equivalent **two-layer** model by introducing effective diffusion coefficients and Biot numbers.

At high substrate concentrations and in the presence of external diffusion limitation, the transient **response** of an amperometric biosensor exhibiting uncompetitive substrate inhibition may **follow a five-phase pattern** ($N_{\mathrm{ext}} = 3$).

Oscillations in the transient biosensor response should be taken into consideration when using the biosensor calibration curve **for industrial applications**.

References

1. H. Bisswanger, Enzyme Kinetics: Principles and Methods, 2nd ed., Wiley-Blackwell, 2008.
2. B.D. Malhotra, C.M. Pandey, Biosensors: Fundamentals and Applications, Smithers Rapra, 2017.
3. P.M. Doran, Bioprocess Engineering Principles, 2nd edn., Academic Press, 2013.
4. R. Baronas, F. Ivanauskas, J. Kulys. Mathematical Modeling of Biosensors, 2nd ed. Springer, 2021.
5. R. Baronas. Nonlinear effects of partitioning and diffusion-limiting phenomena on the response and sensitivity of three-layer amperometric biosensors. Electrochim. Acta, 2024, 478, 143830.
6. R. Baronas. Nonlinear effects of partitioning and diffusion limitation on the efficiency of three-layer enzyme bioreactors and potentiometric biosensors. J. Electroanal. Chem., 2024, 974, 118698.
7. R. Baronas. Non-monotonic effect of substrate inhibition in conjunction with diffusion limitation on the response of amperometric biosensors. Biosensors, 2025, 15, 441.