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ORIGINAL PAPER

Degradation of substrate and/or product: mathematical modeling of biosensor action

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Abstract Mathematical model for evaluation of the multilayer heterogeneous biocatalytic system has been elaborated. The model consists of nonlinear system of partial differential equations with initial values and boundary conditions. An algorithm for computing the numerical solution of the mathematical model has been applied. Two cases: when product diffuses out of the biosensor and when the outer membrane is impermeable for product (product is trapped inside the biosensor) have been dealt with by adjusting boundary conditions in the mathematical model. Profiles of the impact of the substrate and product degradation rates on the biosensor response have been constructed in both cases. Value of the degradation impact has been analyzed as a function of the outer membrane thickness. The initial substrate concentration also affects influence of the degradation rates on the biosensor response. Analytical formulae, defining approximate values of relationships between the degradation rates and the outer membrane thickness or the initial substrate concentration, have been obtained. These formulae can be employed for monitoring of the biosensor response.

Keywords Biosensor modeling · Enzyme electrode · Substrate degradation · Product degradation · Michaelis–Menten kinetics

1 Introduction

As an analytical instrument, biosensors have found wide application in medicine, environment, and food-quality control [1,2]. Applications of biosensors in medicine and industry require automated algorithms for monitoring biosensor action. Biosensors constructed for practical applications usually contain several operational and

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protecting layers. Action of such biosensors is result of processes in each layer, interacted in some complicated, nonlinear way. A number of attempts have been made to propose mathematical models of the action of biosensors [3–8]. Mathematical modeling of a multilayer heterogeneous analytical system should not be understood as an outcome or summation of separate models, defined in corresponding layers. Mathematical analysis must deal with entire multilayer biosensor as one inseparable system. Proposed mathematical modeling of the biosensor action cannot describe full mechanism of the biosensor action; however, it can define a range of importance of biosensor parameters and their weights in the response formation.

Stability of the biosensor action presents a crucial quality of an analytical system. A lot of external and internal factors can influence the response, and thereby the stability of biosensor. In our previous paper [9], we have described mathematical model of the electrochemical biosensor and have evaluated influence of thickness (of membranes and enzymatic layer), diffusion parameters and pH on the response of the biosensor. In this work we will describe impact of unstable substrate and product on the action of the enzyme-based electrochemical biosensor, finding out how it depends on different parameters of the biosensor. Very often substrate or product to be determined is consumed by extraneous enzymes, microorganisms, spontaneous decomposition or other side reactions. How it will influence on the response of the biosensor? How diffusion parameters and thickness of biosensor membranes will affect this influence? These dependences can be very useful for the constructors of reliable biosensors selecting limiting thickness and diffusion parameters of separate layers of the biosensor targeting to minimize influence of the creep processes inside the biosensor. For estimation of the influence of creep processes we are going to propose a mathematical algorithm and biosensor response correcting formulae. Evaluation of creep processes in biosensors will predict limiting conditions of the biosensor application and improve the reliability of the biosensors that is absolutely necessary implementing biosensors in the automated monitoring processes in industry, environment control and medicine.

Recent publications on biosensor research are mainly oriented on novel matrices and more and more complicated construction of the biosensor. On the other hand, a number of new useful tools are applied to verify parameters and surface of the biosensors. For example, polyaniline/carbon nanotubes biosensor matrix electrochemical parameters have been characterized by scanning electrochemical microscopy [10]. Very often atomic force microscopy is used to characterize surface of an electrode [11]. All of these tools can be applied to get additional information to be included into the algorithm of the validation of biosensor action and can be a source to complement elaborated formulae of the correction of the biosensor response.

2 Model

2.1 Biosensor

As a model device, an electrochemical biosensor is dealt with (Fig. 1) [12]. The biosensor consists of flat electrochemical electrode. On the surface of the electrode a

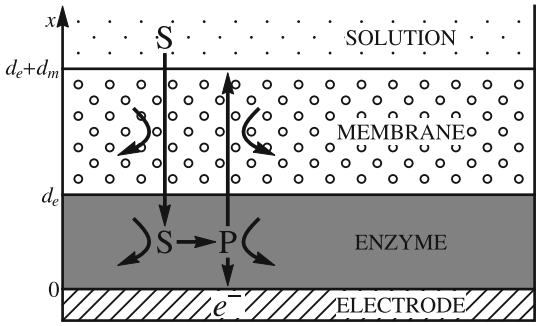

Fig. 1 General scheme of electrochemical biosensor. Mathematical variable x denotes distance to the electrode

thin layer of enzyme is adsorbed or a layer of polymer containing immobilized enzyme is deposited. Enzyme catalyzes conversion of substrate (S), which is our target, to product (P), which is electrochemically active and can be detected on the electrode:

$$S \overset{\text{ENZYME}}{\longrightarrow} P$$
$$P \overset{\text{ELECTRODE}}{\longrightarrow} Q \quad +/- \quad n_e\, e^- (\text{electrons}).$$

Current of the electrode defines the response of the biosensor. The same principal scheme can be applied to optical, electromagnetic and a number of other biosensors.

Enzyme containing layer is characterized by thickness (d_e) and diffusion coefficients for substrate (D_{S_e}) and product (D_{P_e}).

Enzymatic layer is covered by protecting inert membrane. This membrane can be polymer membrane, possessing electrical charge, or neutral. In some cases the role of this membrane can play thin layer of the unmixed solvent on the surface of the enzyme containing layer. This outer membrane can be characterized by thickness (d_m) and diffusion coefficients for substrate (D_{S_m}) and product (D_{P_m}).

2.2 Mathematical model

For simplicity, action of the enzyme will be expressed as a Michaelis–Menten process. It means that we accept conditions, when concentration of the product P inside the enzyme containing membrane will be lower than concentration of the substrate S. In the steady-state conditions this requirement can be realized when the rate of P consumption on the electrode surface will be very fast.

2.2.1 Differential equations

Mathematically, both functions $S = S(x, t)$ (substrate concentration) and $P = P(x, t)$ (product concentration) depend on coordinate variable x (distance to the biosensor electrode; values $0 < x < d_e$ correspond to the enzyme layer while points inside the membrane are defined by $d_e < x < d_e + d_m$, see Fig. 1) and time variable $t \geqslant 0$.

Let us assume, that the rate of the substrate and the product *degradation* is expressed by a first order reaction with rate constants correspondingly C_1 and C_2. Then, for $0 < x < d_e + d_m$, $t > 0$, substrate and product kinetics is governed by *nonlinear* reaction-diffusion equations

$$\frac{\partial S}{\partial t} = \frac{\partial}{\partial x}\left(D_S(x)\frac{\partial S}{\partial x}\right) - C_1 S - \alpha(x)\frac{V_{max}\, S}{K_M + S}, \tag{1}$$

$$\frac{\partial P}{\partial t} = \frac{\partial}{\partial x}\left(D_P(x)\frac{\partial P}{\partial x}\right) - C_2 P + \alpha(x)\frac{V_{max}\, S}{K_M + S}, \tag{2}$$

here

$$D_S(x) = \begin{cases} D_{S_e}, & 0 < x \leqslant d_e, \\ D_{S_m}, & d_e < x < d_e + d_m, \end{cases} \tag{3}$$

$$D_P(x) = \begin{cases} D_{P_e}, & 0 < x \leqslant d_e, \\ D_{P_m}, & d_e < x < d_e + d_m, \end{cases} \tag{4}$$

$$\alpha(x) = \begin{cases} 1, & 0 < x \leqslant d_e, \\ 0, & d_e < x < d_e + d_m, \end{cases} \tag{5}$$

the parameter V_{max} is the maximum rate of the reaction in the enzyme layer and K_M represents the Michaelis constant. Note that no enzymatic process takes place inside the biosensor outer membrane, therefore, for $d_e < x < d_e + d_m$ Eqs. (1), (2) become linear ($\alpha(x) \equiv 0$).

2.2.2 Biosensor parameters

As a model electrode—glucose oxidase immobilized on Pt electrode has been applied. Current of hydrogen peroxide electrochemical oxidation has been recorded. In this case two-electron process ($n_e = 2$) takes part. In numerical experiments activity of immobilized glucose oxidase has been accepted to be $V_{max} = 0.3\,\text{mmol m}^{-3}\,\text{s}^{-1}$, that is about three times lower than activity of native enzyme, taking into account that under immbolization process enzyme can loss 2/3 of the initial activity. K_M of glucose oxidase from *Aspergillus niger* is $0.23\,\text{mol m}^{-3}$ and it has been assumed that during the immobilization procedure this parameter is not influenced. Numerical experiments have been performed at $S_0 = 0.07\ \text{mol m}^{-3}$ (concentration of substrate in buffer solution) as default. It is approximately 3.3 times lower than K_M, i.e., biosensor operates in linear diapason of substrate.

Layer of immobilized enzyme has been covered with cellulose or acetylated cellulose film (outer membrane). It is typical biosensor reported in many papers. Such biosensor has been designed and response curves have been experimentally recorded (curves not shown).

Thickness of enzymatic layer (d_e) has been chosen 9 μm. Thickness of outer membrane (d_m) has been chosen 10 μm. Diffusion coefficients have been adjusted in accor-

dance with experimental biosensor response curves: $D_{S_e} = 22\,\mu\text{m}^2\ \text{s}^{-1}$, $D_{P_e} = 20\,\mu\text{m}^2\ \text{s}^{-1}$, $D_{S_m} = 7\,\mu\text{m}^2\ \text{s}^{-1}$, $D_{P_m} = 6\,\mu\text{m}^2\ \text{s}^{-1}$.

2.2.3 Initial values

Suppose, that we immerse the biosensor into buffer solution of substrate. The concentration of substrate in the solution is S_0, and remains stable during all process time. At the beginning ($t = 0$) there is no substrate ($S = 0$), nor product ($P = 0$) inside the enzymatic layer and the outer membrane:

$$S(x,0) = \begin{cases} 0, & 0 \leqslant x < d_e + d_m, \\ S_0, & x = d_e + d_m, \end{cases} \tag{6}$$

$$P(x,0) = 0, \quad 0 \leqslant x \leqslant d_e + d_m. \tag{7}$$

2.2.4 Boundary conditions

Substrate is electrochemically inactive substance. The rate of electrochemical conversion of product is very fast in compare with enzymatic reaction rate. Hence, on the biosensor electrode ($x = 0$) boundary conditions apply (for $t > 0$):

$$\left.\frac{\partial S}{\partial x}\right|_{x=0} = 0, \qquad P(0,t) = 0, \qquad t > 0. \tag{8}$$

Another boundary condition defines S values at the biosensor border ($x = d_e + d_m$) with buffer solution:

$$S\,(d_e + d_m, t) = S_0, \qquad t > 0. \tag{9}$$

In this study we are going to investigate two different options. The first one assumes situation when product diffuses out of the biosensor:

$$P\,(d_e + d_m, t) = 0, \qquad t > 0. \tag{10}$$

Alternatively, we can assume that the outer membrane is not permeable for product P. In this case P is trapped inside the biosensor. However, substrate permeability is the same as in the first case (10). Such a situation can happen when both the product and the outer membrane are charged. This implies the boundary condition

$$\left.\frac{\partial P}{\partial x}\right|_{x=d_e+d_m} = 0, \qquad t > 0. \tag{11}$$

2.3 Numerical algorithm

The mathematical model (1), (2) presents a nonlinear system of partial differential equations with the initial values (6), (7) and the boundary conditions (8)–(10)

or (8), (9), (11). Analytical (exact) solutions to nonlinear differential problems can be found in exceptional cases, only. In our case, since exact solution is unknown, we have employed numerical modeling, based on finite difference approximation [8,13].

A non-uniform (finer in the neighbourhoods of the juncture points $x = 0$, $x = d_e$ and $x = d_e + d_m$; in these bordering regions gradients of the concentration of compounds under investigation are of largest magnitude, see Fig. 1) mesh Ω_h has been applied to partition the interval $0 \leqslant x \leqslant d_e + d_m$. Also, due to a sudden jump (at $x = d_e + d_m$) in the initial values (6), a semi-uniform (finer at the starting point $t = 0$) mesh ω_τ has been introduced for discretization of time variable $t \geqslant 0$.

The differential model has been approximated by the Crank–Nicolson method (a second-order implicit finite difference scheme) [8,13]. For each discrete time layer $t_k \in \omega_\tau$, the resulting nonlinear system of algebraic equations can be solved iterating by the nonlinear part and using the Thomas algorithm (also known as the tridiagonal matrix algorithm) [13] for the linear part.

Both meshes Ω_h and ω_τ can be characterized by values of their maximal steps h_{max} and τ_{max}, respectively. To find optimal setup (compromising accuracy and time of computations), we have experimented with different choices of h_{max} and τ_{max}. The results of numerical experiments presented in this study have been obtained with the values $h_{max} = (d_e + d_m)/442$ and $\tau_{max} = 0.01$ s.

2.4 Biosensor response

As a response of the electrochemical biosensor, a steady-state diffusion current density (I) is considered:

$$I = \lim_{t \to \infty} i(t), \qquad i(t) = n_e \, F \, D_{P_e} \left. \frac{\partial P}{\partial x} \right|_{x=0}, \tag{12}$$

here $i(t)$ denotes time-dependent current density, n_e—number of electrons, participating in electrochemical conversion of product molecule, and F is the Faraday constant.

To estimate the value of I numerically we propose the following empirical formula:

$$I \approx i(t^*), \quad t^* = \min_{t_k \in \omega_\tau} \left(t_k : \frac{i'(t_k)}{\max\limits_{t_m \leqslant t_k} i'(t_m)} < \delta \right), \tag{13}$$

with $\delta = 10^{-3}$ and requiring that $i'(t_k) > 0$, $t_k \in \omega_\tau$ (that is, the current density must be monotonically increasing, while saturating; otherwise I is undefined).

The saturation criterion (13) searches for the discrete time moment t^* when slope (derivative) of the increasing function $i(t)$ decreases $1/\delta = 1{,}000$ times, compared to maximum slope.

Derivatives in the expressions (12) and (13) have been computed from discrete values of functions, employing parabolic interpolation.

3 Results and discussions

3.1 Degradation impact on biosensor response

Substrate and product degradation is controlled by the parameters $C_1 \geqslant 0$ and $C_2 \geqslant 0$ in Eqs. (1), (2). Hence, if all other coefficients in the mathematical model are fixed, the biosensor response I appears as a function $I = I(C_1, C_2)$. To reveal impact of both quantities C_1 and C_2 on the response I, we have computed and portrayed (see Fig. 2) the dependency of the relative decay

$$\Delta I = \left(1 - \frac{I(C_1, C_2)}{I(0,0)}\right) \cdot 100\,\%. \tag{14}$$

in two-dimensional coordinate plane (C_1, C_2).

In Fig. 2, points in:

- a *white region* correspond to the values of C_1 and C_2 such that $0\,\% \leqslant \Delta I \leqslant 1\,\%$;
- a *criss-crossed region on a white background*—such that $1\,\% \leqslant \Delta I \leqslant 2\,\%$;
- a *light grey region*—such that $2\,\% \leqslant \Delta I \leqslant 3\,\%$;
- a *criss-crossed region on a light grey background*—such that $3\,\% \leqslant \Delta I \leqslant 4\,\%$,

and so on.

It should be noted that ΔI isolines in (C_1, C_2) coordinate plane are nearly linear (see Fig. 2).

This analysis allows us to conclude that the response of the biosensor is more sensitive to the degradation process of the substrate (compared to the degradation of the product). For example, in the case of boundary conditions (8), (9), (10) (Fig. 2, upper illustration), the value $\Delta I = 5\,\%$ may be reached by the rate $C_1 = 3.22\,\mathrm{s}^{-1} \cdot 10^{-3}$ (while $C_2 = 0\,\mathrm{s}^{-1} \cdot 10^{-3}$) or by the rate $C_2 = 33.5\,\mathrm{s}^{-1} \cdot 10^{-3}$ (while $C_1 = 0\,\mathrm{s}^{-1} \cdot 10^{-3}$). That is, the same value of the response decay would be achieved with the substrate degradation (assumed the product does not degrade) rate approximately 10 times lesser than the product degradation (assumed the substrate does not degrade) rate. 5 % fluctuations of the biosensor response is usually acceptability limit in practical (food, environmental and medicine) applications. We will use this limiting characteristics in further numerical studies.

In the considered biosensor we have assumed that the outer membrane and the electrochemically active product do not interact. However, in other types of biosensors recordable product of the enzymatic process as well as of the outer membrane can be charged. If the charges are opposite, it will lead to the extraction of the charged product from the enzymatic layer and, thereby, the influence of the product degradation rate becomes even less significant. If both charges are of the same sign, the product is trapped inside the biosensor (the case of boundary conditions (8), (9), (11), see Fig. 2,

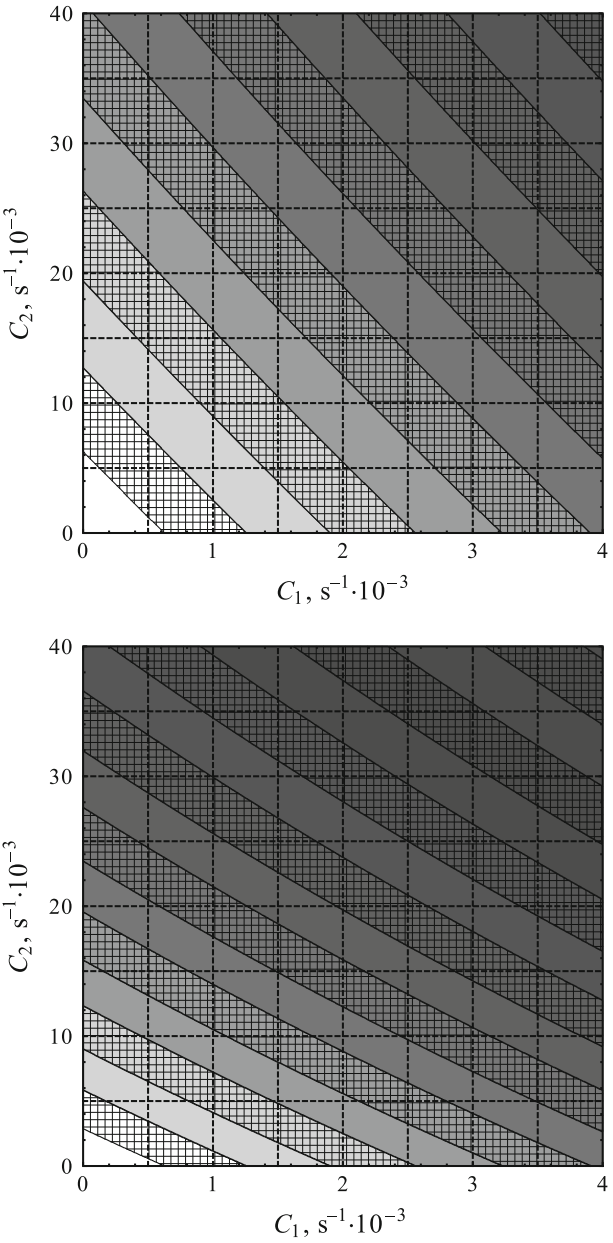

Fig. 2 Relative decay ΔI of biosensor response versus degradation rates C_1 and C_2. *Upper illustration:* the case when the product diffuses out of the biosensor [boundary conditions (8), (9), (10)]. *Lower illustration:* the case when the outer membrane is impermeable for the product [boundary conditions (8), (9), (11)]

lower illustration), the impact of the substrate degradation is the same as in the previous case, but the biosensor becomes more sensitive to the rate of the product degradation. In this case, the value $\Delta I = 5\,\%$ may be reached by the rate $C_2 = 15.9\,s^{-1} \cdot 10^{-3}$.

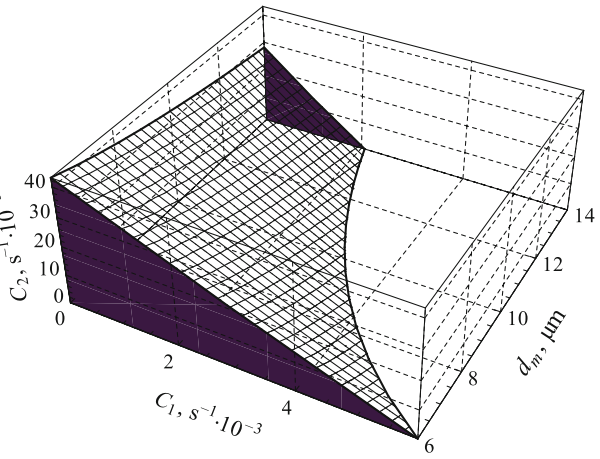

Fig. 3 Dependency of the area Ψ (displayed in *dark gray* background) on the outer membrane thickness d_m. The case when the product diffuses out of the biosensor [boundary conditions (8), (9), (10)]

3.2 Influence of outer membrane thickness

In the analyzed model of the biosensor we have assumed that the thickness of the outer membrane is constant. However, in real situations this parameter may vary. Cells and proteins from biological media can adsorb on surfaces of the outer membrane, "gluing" the outer membrane, hence increasing the thickness of the outer membrane. Also, the thickness of the outer membrane can be affected by pressure fluctuations of the bulk. pH fluctuations can impact the swelling properties etc. How would the varying thickness of the outer membrane influence the impact of the degradation process to the biosensor response?

Following the definition (14) and Fig. 2, let us define the area in (C_1, C_2) coordinate plane, limited by ΔI 5 % isoline:

$$\Psi = \{(C_1 \geqslant 0, C_2 \geqslant 0) : \Delta I \leqslant 5\,\%\}\,. \tag{15}$$

The area Ψ (displayed in dark gray background) and its dependency on the outer membrane thickness d_m is presented in Fig. 3. It should be pointed out that when the thickness of outer membrane increases, the influence of the degradation processes increases as well.

3.2.1 Analytical formulae of $\Delta I = 5\,\%$

Also, it is worth mentioning that for all values $6\,\mu\text{m} \leqslant d_m \leqslant 14\mu\text{m}$, and the values of all other model coefficients listed in Sect. 2.2.2, the ΔI 5 % isoline (defined by the equation $\Delta I = 5\,\%$) remains nearly linear. By employing least squares fitting, we have obtained *the analytical* approximation of the curve $\Delta I = 5\,\%$:

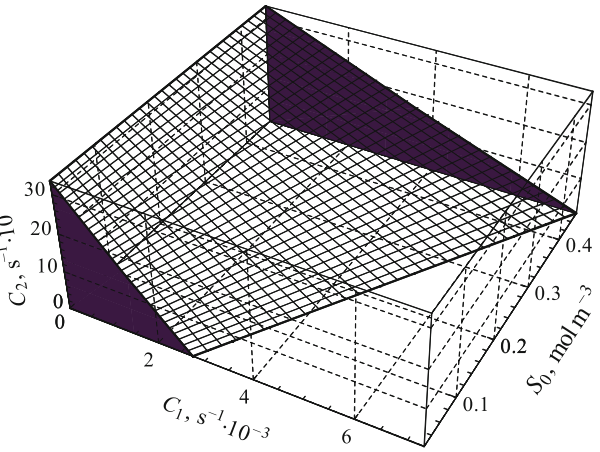

Fig. 4 Dependency of the area Ψ (displayed in *dark gray* background) on the substrate concentration S_0 in buffer solution. The case when the product diffuses out of the biosensor [boundary conditions (8), (9), (10)]

$$C_2 = K(d_m)\, C_1 + M(d_m)\,, \quad C_1 \geqslant 0, \quad C_2 \geqslant 0, \tag{16}$$

$$K(d_m) = -0.792\, \frac{d_m}{\mu\mathrm{m}} - 2.49, \tag{17}$$

$$M(d_m) = \frac{-0.583\, \dfrac{d_m}{\mu\mathrm{m}} + 28.3 + \dfrac{110\mu\mathrm{m}}{d_m}}{10^3}\ \mathrm{s}^{-1}. \tag{18}$$

3.3 Influence of substrate concentration

Analogously as in Sect. 3.2, we have analyzed the impact of different values of the substrate concentration S_0 (in buffer solution) on the area Ψ [defined by (15)]. Results (with fixed values of all other model coefficients, except S_0) are presented in Fig. 4.

3.3.1 Analytical formulae of $\Delta I = 5\,\%$

Least squares fitting yields *the analytical* formulae of the curve $\Delta I = 5\,\%$:

$$C_2 = k(S_0)\, C_1 + m(S_0)\,, \quad C_1 \geqslant 0, \quad C_2 \geqslant 0, \tag{19}$$

$$k(S_0) = -\frac{33.5}{10.5\, S_0\, \mathrm{mol}^{-1}\, \mathrm{m}^3 + 2.49}, \tag{20}$$

$$m(S_0) \equiv 0.0335\ \mathrm{s}^{-1}. \tag{21}$$

As can be expected, with increase of substrate concentration, the substrate degradation rate (needed for the limiting 5 % level) increases linearly (see Fig. 4).

Analogous (to Sects. 3.2 and 3.3) analysis can be done for the case with the outer membrane impermeable for product [boundary conditions (8), (9), (11)].

The expressions (16)–(18) and (19)–(21) define relationships between the product and substrate degradation rates and fundamental parameters of detectoring system like the outer membrane thickness, the input parameter (the substrate concentration). These findings can be included into a biosensor monitoring algorithm, as correction of allowed limit of the biosensor response.

Also we have analyzed situation with different V_{max}. This analysis shows that activity of the biocatalyser does not influence the impact of the degradation processes on the biosensor response.

4 Conclusions

When the product diffuses out of the biosensor, the response is more sensitive to the degradation process of the substrate (compared to the degradation of the product). That is, the same value of the response decay would be achieved with the substrate degradation (assumed the product does not degrade) rate approximately 10 times lesser than the product degradation.

If the outer membrane is impermeable for the product (the product is trapped inside the biosensor), the impact of the substrate degradation is the same as in the previous case, but the biosensor becomes more sensitive to the rate of the product degradation.

When the thickness of the outer membrane increases, the degradation processes become more influential. With increase of substrate concentration, the substrate degradation rate (needed for the same value of the response decay) increases linearly. The activity of the biocatalyser does not influence the impact of the degradation processes on the biosensor response.

Analytical formulae, relating the degradation rates and the outer membrane thickness or the initial substrate concentration have been obtained. These findings can be included into a biosensor monitoring algorithm, as correction of allowed limit of the biosensor response.

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