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INHIBITOR MULTIENZYME BIOSENSOR SYSTEM INDYNAMICMODE – PHOSPHATE MEASUREMENT

ABSTRACT:
In this paper a multienzyme inhibitor system is investigated. A hybrid inhibitor biosensor for measuring concentration of phosphate is used. Enzyme kinetic of Michaelis-Menten and ping-pong kinetics is accepted. Partial differential equations of that complex system are solved numerically and are received concentration profiles of five reagents. The influence of starting concentration of inhibitor is investigated and influence of reaction rate constant of inhibitor.

KEYWORDS:
mathematical modeling, inhibitor biosensor, simulations, phosphate

INTRODUCTION

Biosensors are analytical devices which tightly combine biorecognition elements and physical transducer for detection of the target compounds. Biosensors useful serve ecological purposes by enabling precision pollutant control [1, 2, 3]. In practice the most important are biosensors that identify water conditions [4, 5, 6, 7, 8] and to a lesser extent air [9, 10] and soil condition [11]. Two main water pollutant are phosphates and fluorides. For determination of phosphate and fluoride ions enzyme, microbial and multienzyme biosensors can be used. Multienzyme biosensors however are very complex devices.

DESCRIPTION OF THE MATHEMATICAL MODEL

The starting concentrations of substrate, co-substrate and inhibitor in the research medium are denoted with S0,C0, I0. The concentration profiles for substrate S(x), co-substrate C(x) and inhibitor I(x) are formed in the active membrane. In this paper a hybrid biosensor with two enzymes acid phosphatase (AP) and glucoseoxidase (GOD) is used for the investigation.
Operation principle of the hybrid biosensor is based on the given biochemical reaction:

$$\text{glucose}-6-\text{phosphate}+H_2O \xrightarrow{AP} \text{glucose}+HPO_4^{2-} \quad (1)$$

$$\text{glucose}+O_2 \xrightarrow{GOD} \text{gluconolactone}+H_2O_2$$

Under the activity of the enzyme acid phosphatase the glucose-6-phosphate is hydrolyzed to glucose and inorganic phosphate. In the second reaction the oxygen present oxidizes the obtained glucose. The amount of hydrogen peroxide being produced is measured electrochemically. In the presence of phosphate the hydrogen peroxide is produced at a slower rate. This happens because of the inhibitory effect of those element have on the catalytic activity of the acid phosphatase. As a result the glucose production is decreased which leads to more production of H2O2. As the AP is inhibited from the phosphate the substance can be identified with a biosensor according to its ability to support the formation.
The reactions above can be present with following successive enzyme reactions with competitive inhibition:

$$S \xrightarrow{E_1} P_1 + P_2$$
$$I \xrightarrow{E_1} EI \qquad (2)$$
$$P_1 + C \xrightarrow{E_2} P_3+P_4$$

AP is the first enzyme, let denote its reaction velocity with V_1, GOD is the second enzyme let denote its reaction velocity with V_2; P_1 - glucose, first product; P_2 - second product, not informative; S - glucose-6-phosphate, substrate; I -(KH_2PO_4) measured inhibitor, C - oxygen, co-substrate; P_3 - product H_2O_2 and P_4 - galactonic acid.
We admit that indicatory electrode has symmetrical geometry and assume that diffusion is one-dimensional in space and is described with second Fick's law than we can write the system of equations for those bi-substrate sensitive amperometric system.

$$\frac{\partial S}{\partial t} = Ds\frac{\partial^2 S}{\partial x^2} - \frac{V_1 S}{Ks\left[1+\frac{I}{k_I}\right]+S}$$

$$\frac{\partial I}{\partial t} = Ds\frac{\partial^2 S}{\partial x^2} - \frac{V_1 S}{Ks\left[1+\frac{I}{k_I}\right]+S} \qquad (3)$$

$$\frac{\partial C}{\partial t} = Dc\frac{\partial^2 C}{\partial x^2} - \frac{V_2}{1+\frac{Kp_1}{P_1}+\frac{Kc}{C}}$$

$$\frac{\partial P_1}{\partial t} = Dp_1\frac{\partial^2 P_1}{\partial x^2} + \frac{V_1 S}{Ks\left[1+\frac{I}{k_I}\right]+S} - \frac{V_2}{1+\frac{Kp_1}{P_1}+\frac{Kc}{C}}$$

$$\frac{\partial P_3}{\partial t} = Dp_3\frac{\partial^2 P_3}{\partial x^2} + \frac{V_2}{1+\frac{Kp_1}{P_1}+\frac{Kc}{C}}$$

where: Ds, Dc, Dp_1, Dp_2 and Dp_3 are diffusion coefficients for substrate, co-substrate, product 1 and product 3, Ks - reaction constant for substrate, Ki - reaction constant for inhibitor, Kc - reaction constant for co-substrate, Kp_1 - reaction constant for product 1, Kp_3 - reaction constant for product 3. The output current is proportional to gradient of H_2O_2 concentration at the electrode surface

$$I = nFAD_{P_3}\frac{\partial P_3}{\partial x}\Big|_{x=d} \text{ ,[A]} \qquad (4)$$

where: n is the number of electrons taking part in electrochemical reaction, F is the Faraday's number, A is the electrode surface [m^2].

Let we denote x = 0 for the bulk/membrane interface and x = d for the electrode surface. The action in biosensor starts when some quality of substrate is appears into biological recognition element - active membrane. The initial conditions are:

t = 0 S(x,0) = So I(x,0) = Io C(x,0) = Co

P_1(x,0) = 0 P_3(x,0) = 0 (5)

Limiting conditions are:

x = 0 S(0,t) = So I(0,t)= Io

C(0,t) = Co P_1(0,t) = 0 P_3(0,t) = 0 (6)

The substrate, and co-substrate didn't react with the electrode, oxygen and glucose fully exhausted and medium is well stirred and it remain constant at the electrode surface, then the limiting conditions are:

x = d

$$\frac{\partial S}{\partial x}\Big|_{x=d} = 0, \quad C(d,t) = 0 \quad P_1(d,t) = 0$$

$$\frac{\partial P_1}{\partial x}\Big|_{x=d} = 0,$$

P_3(d,t) = 0 (7)

RESULTS AND DISCUSSIONS

For solving system (4) of non-linear partial differential equations (PDE) we use Matlab solver pdepe. It use both finite difference and finite element methods as described in [12]. pdepe solve initial-boundary value problems for system of parabolic-elliptic PDEs in the one space variable x and time t. The ordinary differential equations resulting from discretization in space are integrated to obtain approximate solutions at times specified in a time vector. Time vector specifying the points at which a solution is requested for every value in distance vector. The pdepe function returns values of the solution on a mesh provided in a distance vector. Distance vector specifying the points at which a numerical solution is requested for every value in time vector.

Concentration profiles of substrate, co-substrate, inhibitor, product 1 and product 3

Because oxygen is consumed during enzymatic conversion output current of biosensor is descending function. Parameters used for simulations are

n = 2 , So =100 mM, Co = 0,25 mM, Io = changed, Po1 = 0,0mM , Po3 = 0,0 mM

F = 96,5A.s / mmol - Faraday's number

A = 7,85.10-7 m2 - diameter of cathode is 1mm

K_s = 80 mM - reaction rate constant for substrate

K_c = 0,5 mM - reaction rate constant for oxygen

K_i = 0,1 mM, K_{P_1} = 100 mM - reaction rate constant for inhibitor and products 1

D_S=2,50. 10^{-10} m^2/s, D_C=2,5. 10^{-11} m^2/s, D_I=2,50. 10^{-9} m^2/s, D_{P1}=2,5.10^{-10} m^2/s, D_{P3}=2,5.10^{-10} m^2/s,

d = 60 μm, Vm1 = 1 mM/s , Vm2 = 20 mM/s ,

At fig.1, 2, 3, 4, and 5 in three dimensional size are given concentration profiles of substrate S(x,t), inhibitor I(x,t), co-substrate C(x,t), product 1 P_1(x,t), product 3 P3(x,t) in active membrane with thickness d = 60μm for the time t = 8s, for values of reaction velocities V_1 = 1 mM/s and V_2 = 20 mM/s. The value of inhibitor is Io=0.0 mM and the value of substrate is So=100 mM.

Figure 7 shows the output current I which is proportional to the concentration of the oxygen. It is seen that oxygen is consumed very rapidly for the case starting concentration Io =0, because there is no inhibitor in the research medium. Hydrogen peroxide (product P3) has value about 0.25 because the oxygen is almost exhausted. The velocity of changing of concentration of co - substrate depends of presence of the inhibitor (eq.3), because now there is no inhibitor oxygen is consumed very rapidly- fig.4.

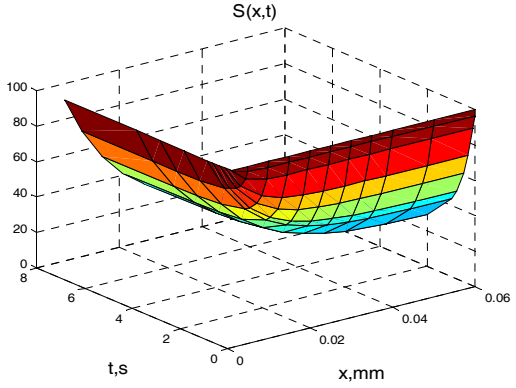

Fig. 2. Concentration profile of substrate. Io = 0 mM

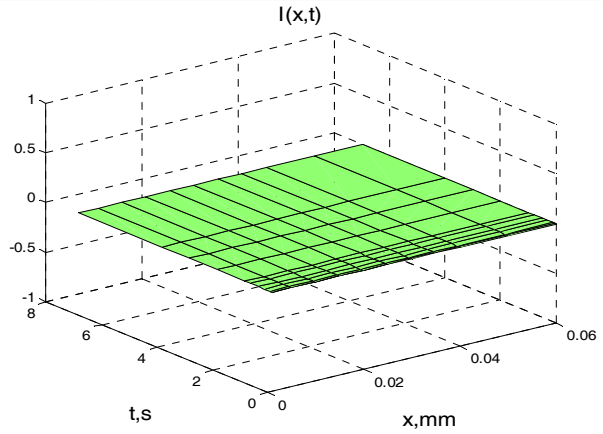

Fig. 3. Concentration profile of inhibitor.Io = 0 mM

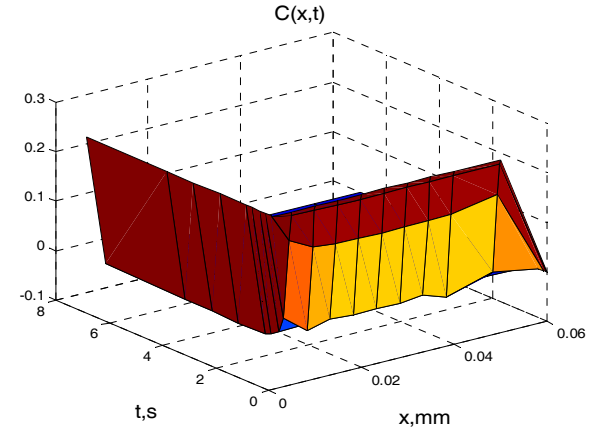

Fig. 4. Concentration profile of co-substrate. Io = 0 mM

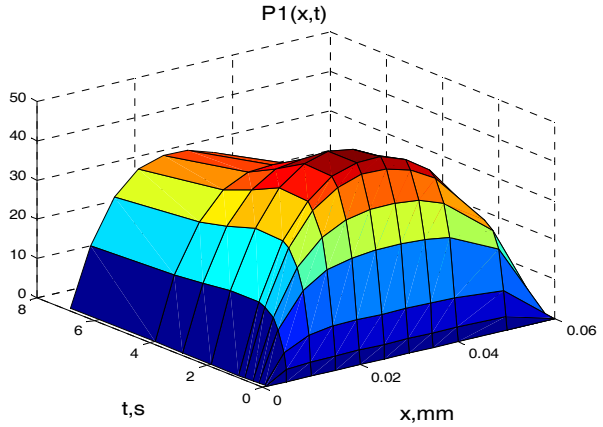

Fig. 5. Concentration profile of Product 1.Io = 0 mM

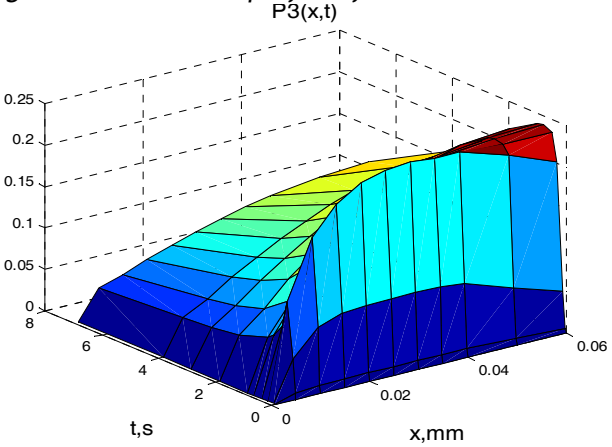

Fig. 6. Concentration profile of Product 3. Io = 0 mM

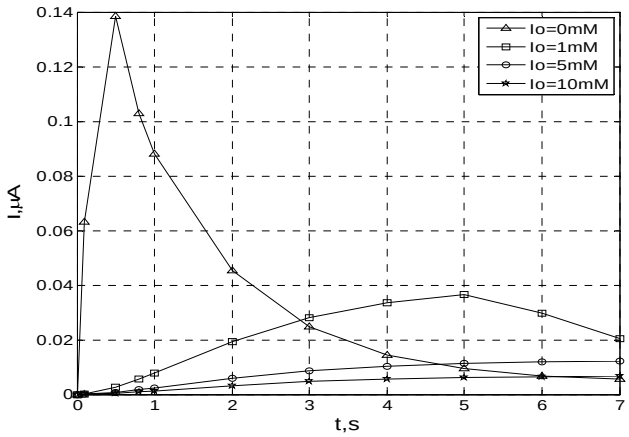

Fig. 7. Output current of the biosensor

The investigated biosensor is co-substrate sensitive and because of that it is important to analyze of changing of c0-substrate C and inhibitor I. At the next pictures are given the dependence of the output current of the biosensor and concentration profiles of substrate, co - substrate, inhibitor and products for the values of I = 1.0 mM.

At fig.8, 9, 10, 11, and 12 are given concentration profiles of substrate S(x,t), inhibitor I(x,t), co-substrate C(x,t), product 1 P_1(x,t), product 3 P3(x,t) for the starting value of inhibitor Io = 5.0 mM.

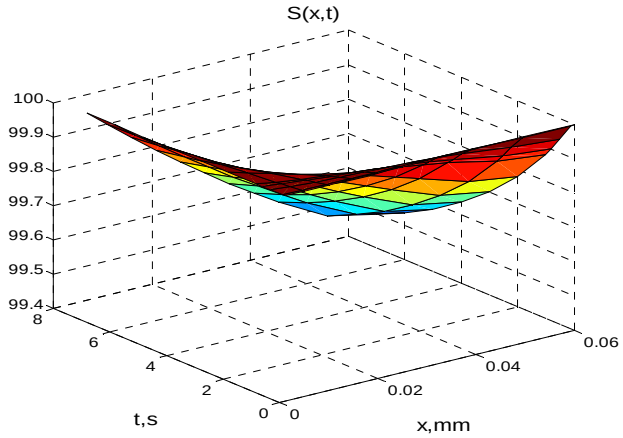

Fig. 8. Concentration profile of substrate. Io = 5 mM

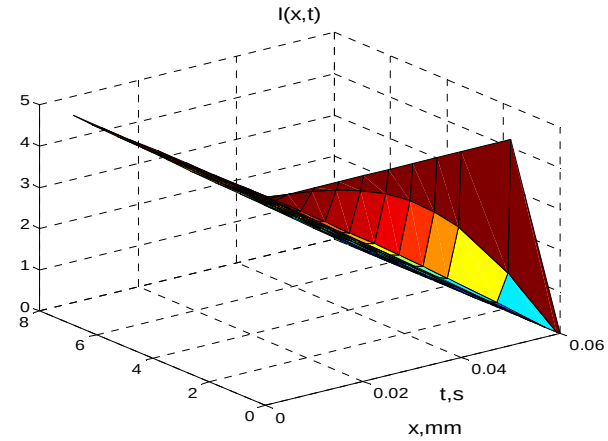

Fig. 9. Concentration profile of inhibitor. Io = 15mM

It is seen clearly how the inhibitor effects over the all reagents. Substrate decreasing very little - from 100mM to 98mM, for the difference at figure 2 where

the decreasing is from 100mM to 20 mM when there is missing inhibitor in the medium. Consuming of the oxygen is less, product 3 formation is increase (fig.12) with the time for the difference at fig.6 where is poorly.

At fig. 7 is given the transient process of the output current for the four values of starting concentration of inhibitor Io = 0, 1, 5 and 10 mM. For the bigger starting concentration of Io the value of steady state of the current is increasing (this is the value fo rthe time biggre thet 7 s), but it is seen that the dependancy is non linear. At fig. 13 it is seen more precise, value of Io are -0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 mM.

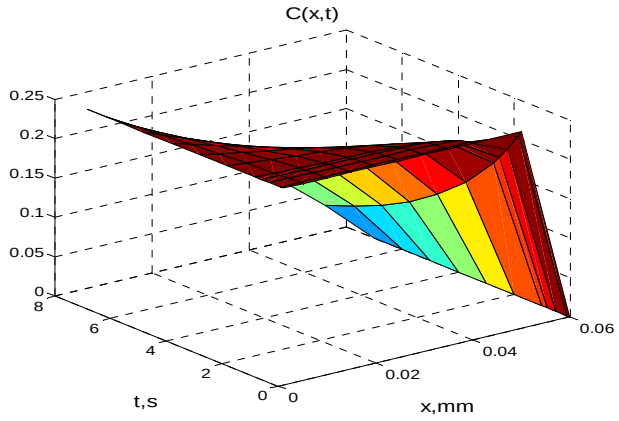

Fig. 10. Concentration profile of co-substrate. Io = 5 mM

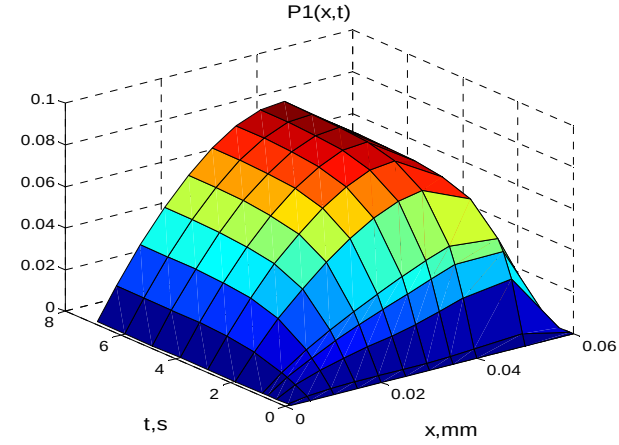

Fig. 11. Concentration profile of Product 1. Io = 5 mM

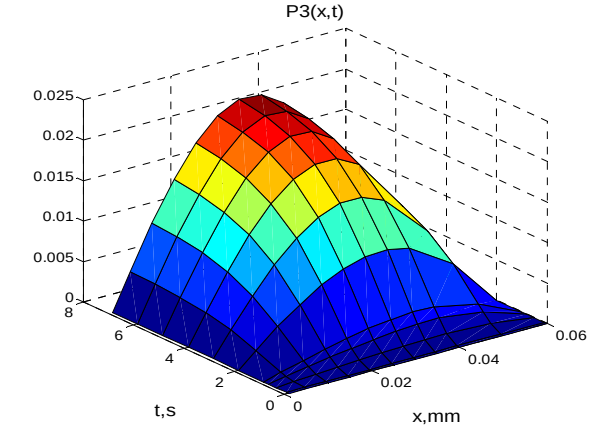

Fig. 12. Concentration profile of Product 3. Io = 5 mM

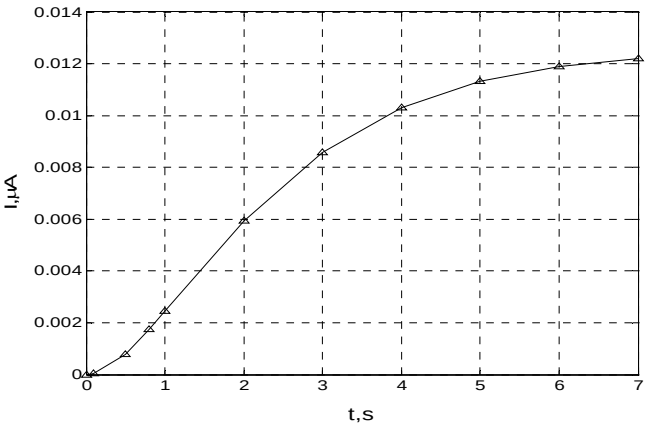

Fig. 13. Output current of the biosensor. Io = 5 mM

At fig. 14 is invstigated the influence of reaction rate constante for inhibitor Ki - 0,05 0,1 0,5 1 2 5 mM at the constante starting concentration of Io = 5mM over substrate concentration profile S (x,t) for x=d. With increasing the Ki substrate consentration in active membrane is decreasing.

At fig. 15 is invstigated the influence of reaction rate constante for inhibitor Ki - 0,05 0,1 0,5 1 2 5 mM for the constante starting concentration of Io = 5mM over the output current. It is seen that transient proceses for the output current strongly depend from Ki. With increasing the reaction rate constante for inhibitor transient process of the current loosing it first order system form.

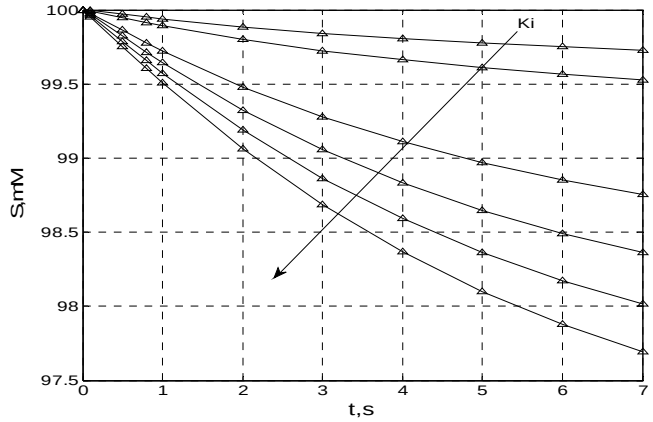

Fig.14.Influence of reaction rate constante over substrate concentration

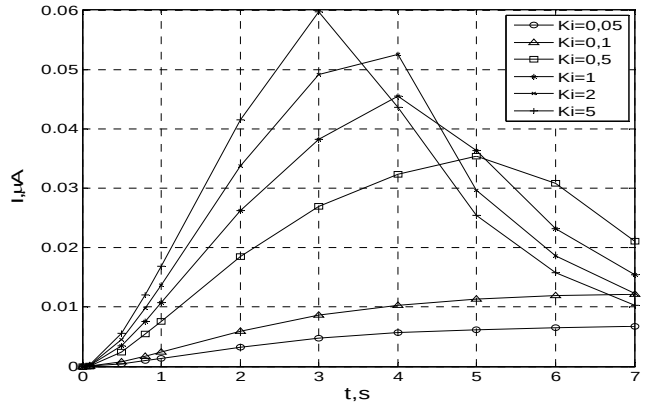

Fig. 15. Influence of reaction rate constante over output current

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CONCLUSION

In the paper is investigated the influence of inhibitor starting concentration over biosensor output current for the hybrid biosensor with two enzymes - acid phosphatase and glucoseoxidase in the dynamic mode. Partial differential equations of that complex system are solved numerical and received concentration profiles of five reagents. In the future it will be investigated the influence of enzymes rate over biosensor response and some technical parameters.

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