



## INHIBITOR MULTIENZYME BIOSENSOR SYSTEM IN DYNAMIC MODE – FLUORIDE MEASUREMENT

<sup>1</sup> Vania RANGELOVA, <sup>2</sup> Antonia PANDELOVA, <sup>2</sup> Nikolay STOYANOV\*

<sup>1</sup> Technical University Sofia – Branch Plovdiv, BULGARIA

<sup>2</sup> Technical University Sofia, BULGARIA

### ABSTRACT

In this paper a multienzyme inhibitor system is investigated. A hybrid inhibitor biosensor for measuring concentration of fluoride is considered. Enzyme kinetic of Michaelis-Menten and ping-pong kinetic are 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.

### KEY WORDS:

mathematical modeling, inhibitor biosensor, simulations.

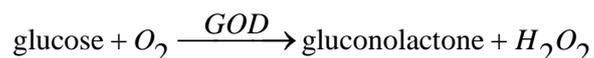
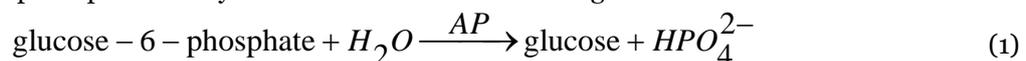
### 1. INTRODUCTION

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. Their role in the measurement systems have not analog in the measurement systems, because their actions show very big selectivity and high sensitivity, still didn't reached of nonbiological measurement devices. That mean combination of biological and nonbiological elements give new opportunities of measurement technique at all. Usage of informative (programmable, intelligent) terminals [12] or intelligent sensors are contributed for that. Mathematical modeling is the way to understand behavior of such complex devices and help using them in more-widely areas.

### 2. DESCRIPTION OF THE MATHEMATICAL MODEL

The starting concentrations of substrate, co-substrate and inhibitor in the research medium are denoted with  $S_0, C_0, I_0$ . 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 [13] 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:

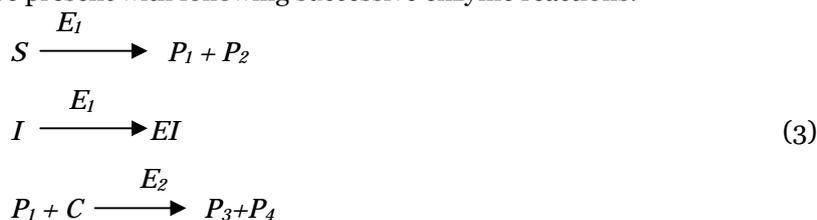


Under the activity of the enzyme acid phosphatase the glucose-6-phosphate is hydrolyzed to glucose and inorganic phosphate. In the second reaction the oxygen present oxidizes the obtained glucose. The amount of oxygen being used is measured electrochemically. In the presence of fluoride the oxygen is consumed at a slower rate. This happens because of the inhibitory effect these elements have on the catalytic activity of the acid phosphatase



As a result the glucose production is decreased which leads to less consumption of oxygen. As the AP is inhibited from the fluoride the substance can be identified with a biosensor according to its ability to obstruct the formation.

The reactions above can be present with following successive enzyme reactions:



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;  $I$  – NaF 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

$$\begin{aligned}
 \frac{\partial S}{\partial t} &= D_s \frac{\partial^2 S}{\partial x^2} - \frac{V_1 S}{K_s \left[ 1 + \frac{I}{k_I} \right] + S}; \quad \frac{\partial I}{\partial t} = D_s \frac{\partial^2 S}{\partial x^2} - \frac{V_1 S}{K_s \left[ 1 + \frac{I}{k_I} \right] + S}; \quad \frac{\partial C}{\partial t} = D_c \frac{\partial^2 C}{\partial x^2} - \frac{V_2}{1 + \frac{K_{p_1}}{P_1} + \frac{K_c}{C}} \\
 \frac{\partial P_1}{\partial t} &= D_{p_1} \frac{\partial^2 P_1}{\partial x^2} + \frac{V_1 S}{K_s \left[ 1 + \frac{I}{k_I} \right] + S} - \frac{V_2}{1 + \frac{K_{p_1}}{P_1} + \frac{K_c}{C}}; \quad \frac{\partial P_3}{\partial t} = D_{p_3} \frac{\partial^2 P_3}{\partial x^2} + \frac{V_2}{1 + \frac{K_{p_1}}{P_1} + \frac{K_c}{C}}
 \end{aligned} \tag{4}$$

where:  $D_s$ ,  $D_c$ ,  $D_{p_1}$ ,  $D_{p_2}$  and  $D_{p_3}$  are diffusion coefficients for substrate, co-substrate, product 1 and product 3,  $K_s$  - reaction constant for substrate,  $k_I$  - reaction constant for inhibitor,  $K_c$  - reaction constant for co-substrate,  $K_{p_1}$ - reaction constant for product 1,  $K_{p_3}$  - reaction constant for product 3.

The output current is proportional to gradient of  $H_2O_2$  (product 3) concentration at the electrode surface

$$I = nFAD_c \left. \frac{\partial C}{\partial x} \right|_{x=d}, [A] \tag{5}$$

where:  $n=4$  is the number of electrons taking part in electrochemical reaction,  $F = 96485$  C/mol 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:

$$t = 0 \quad S(x,0) = S_0 \quad I(x,0) = I_0 \quad C(x,0) = C_0 \quad P_1(x,0) = 0 \quad P_3(x,0) = 0$$

Limiting conditions are:

$$x = 0 \quad S(0,t) = S_0 \quad I(0,t) = I_0 \quad C(0,t) = C_0 \quad P_1(0,t) = 0 \quad P_3(0,t) = 0$$

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

$$x = d, \quad \left. \frac{\partial S}{\partial x} \right|_{x=d} = 0, \quad C(d,t) = 0 \quad P_1(d,t) = 0 \quad \left. \frac{\partial P_1}{\partial x} \right|_{x=d} = 0, \quad \left. \frac{\partial P_3}{\partial x} \right|_{x=d} = 0$$

### 3. 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 [14]. *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 & product 3

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

$n = 4$ ,  $S_0 = 100$  mM,  $C_0 = 0,25$  mM,  $I_0 =$  changed,  $P_{01} = 0,0$ mM,  $P_{03} = 0,0$  mM

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

$A = 7,85 \cdot 10^{-7}$  m<sup>2</sup> - 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,  $K_{p1} = 100$  mM - reaction rate constant for inhibitor and products 1

$D_s = 2,50 \cdot 10^{-10}$  m<sup>2</sup>/s,  $D_c = 2,5 \cdot 10^{-11}$  m<sup>2</sup>/s,  $D_{i1} = 2,50 \cdot 10^{-9}$  m<sup>2</sup>/s,  $D_{p1} = 2,5 \cdot 10^{-10}$  m<sup>2</sup>/s,  $D_{p3} = 2,5 \cdot 10^{-10}$  m<sup>2</sup>/s,

$d = 60$  μm

$V_{m1} = 1$  mM/s,  $V_{m2} = 20$  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  $P_3(x,t)$  in active membrane with thickness  $d = 60$ μm for the time  $t = 8$ s, for values of reaction velocities  $V_1 = 1$  mM/s and  $V_2 = 20$  mM/s. The value of inhibitor is 0.0 mM and the value of substrate is 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  $I_0 = 0$ , because there is no inhibitor in the research medium. Hydrogen peroxide (product  $P_3$ ) 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.

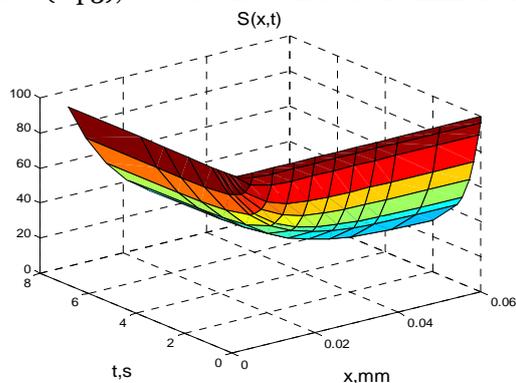


Fig. 2. Concentration profile of substrate.  $I_0 = 0$  mM..

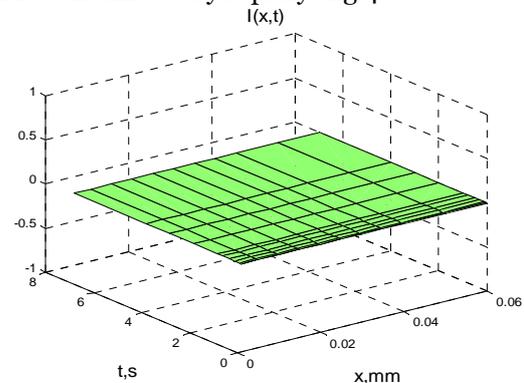


Fig. 3. Concentration profile of inhibitor.  $I_0 = 0$  mM

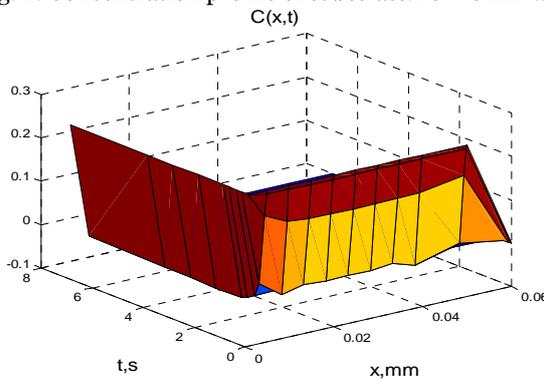


Fig. 4. Concentration profile of co-substrate.  $I_0 = 0$  mM.

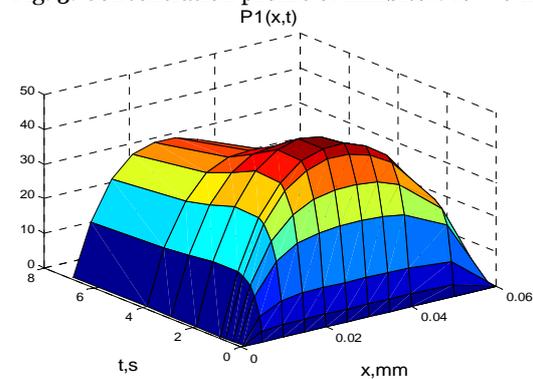


Fig. 5. Concentration profile of Product 1.  $I_0 = 0$  mM

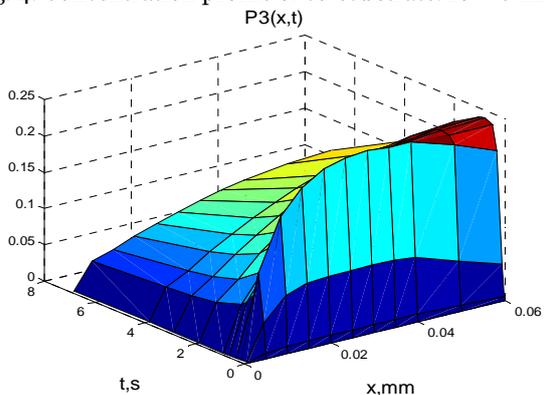


Fig. 6. Concentration profile of Product 3  $I_0 = 0$  mM.

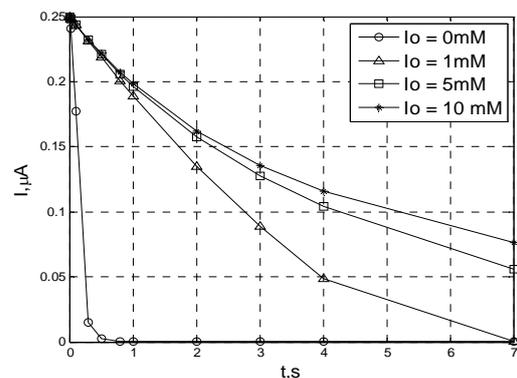


Fig. 7. Output current of the biosensor.

The investigated biosensor is co-substrate sensitive and because of that it is important the analyze of changing of co-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  $I = 1.0 \text{ mM}$ .

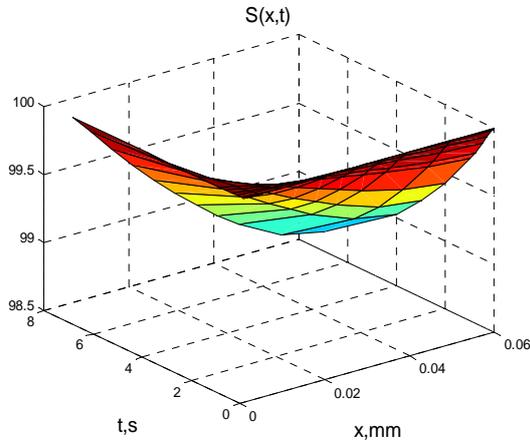


Fig. 8. Concentration profile of substrate.  $I_0 = 1 \text{ mM}$ .

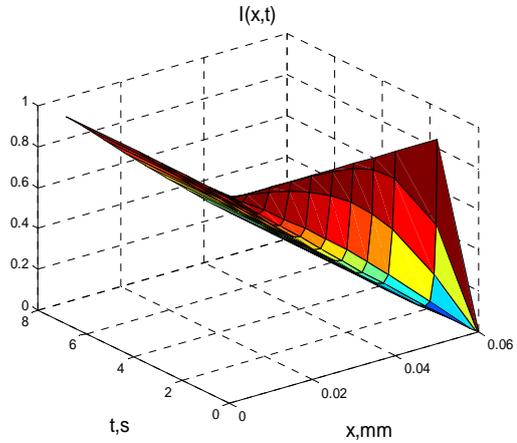


Fig. 9. Concentration profile of inhibitor.  $I_0 = 1 \text{ mM}$

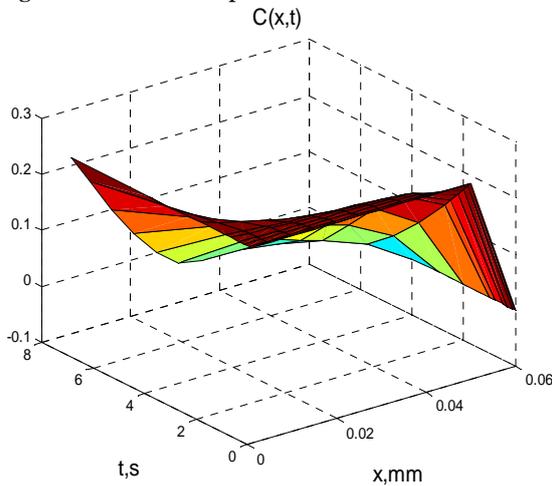


Fig. 10. Concentration profile of co-substrate.  $I_0 = 1 \text{ mM}$ .

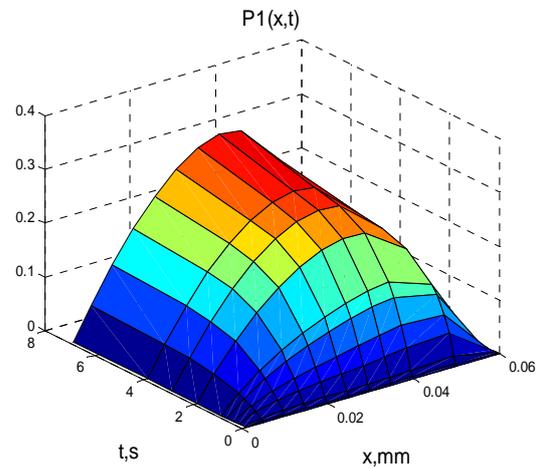


Fig. 11. Concentration profile of Product 1.  $I_0 = 1 \text{ mM}$

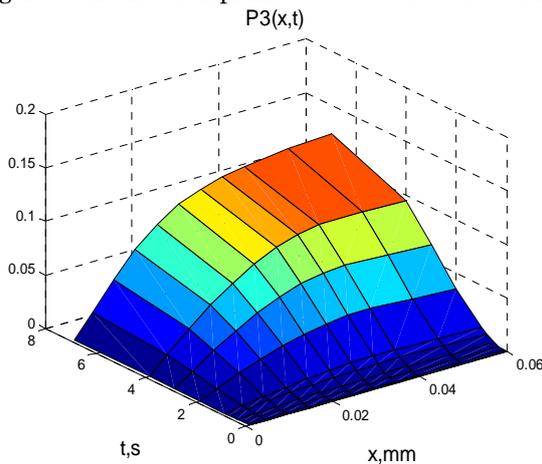


Fig. 12. Concentration profile of Product 1.  $I_0 = 1 \text{ mM}$

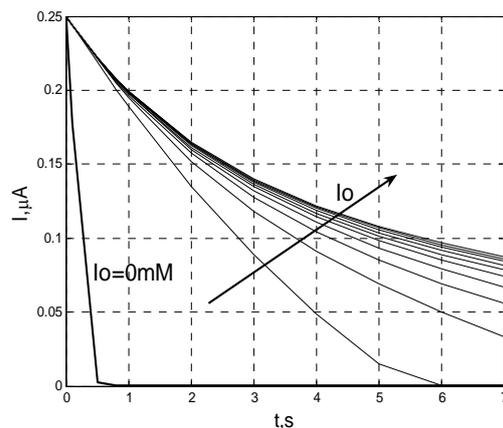


Fig. 13. Output current of the biosensor

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  $P_3(x,t)$  for the starting value of inhibitor  $I_0 = 1.0 \text{ mM}$ . It is seen clearly how the inhibitor effects over the all reagents. Substrate decreasing very little – from  $100 \text{ mM}$  to  $98 \text{ mM}$ , for the difference at figure 2 where the decreasing is from  $100 \text{ mM}$  to  $20 \text{ mM}$  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  $I_0 = 0, 1, 5$  and  $10$  mM. For the bigger starting concentration of  $I_0$  the value of steady state of the current is increasing ( this is the value for the time bigger than 7 s), but it is seen that the dependency is non linear. At fig. 13 it is seen more precise, value of  $I_0$  are  $-0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19$  mM.

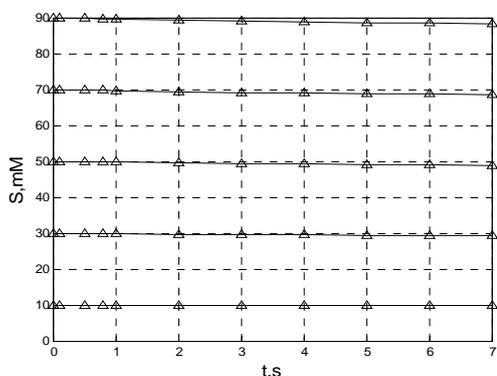


Figure 14.

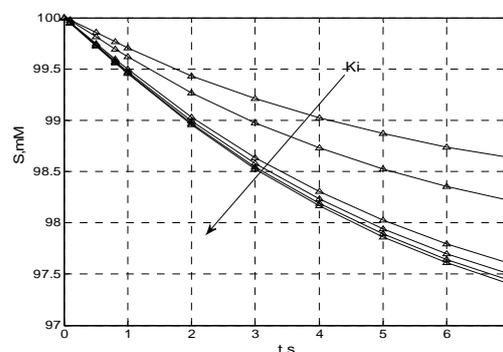


Figure 15.

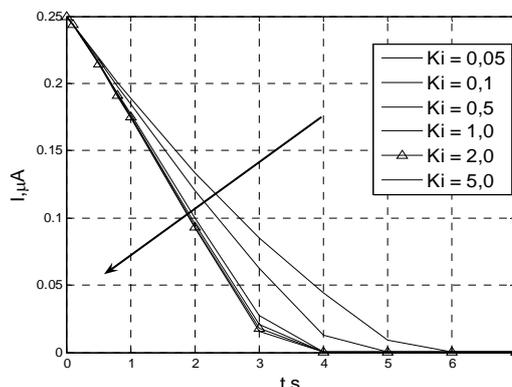


Figure 16.

At fig. 14 is investigated the influence of starting concentration of substrate  $S_0 = 10, 30, 50, 70, 90$  mM for the constant value of starting concentration of inhibitor  $I_0 = 0,5$  mM over the substrate concentration profile  $S(x,t)$  for  $x=d$ . the influence is insignificant.

At fig. 15 is investigated the influence of reaction rate constant for inhibitor  $K_i = 0,05, 0,1, 0,5, 1, 2, 5$  mM at the constant starting concentration of  $I_0 = 0,5$  mM over substrate concentration profile  $S(x,t)$  for  $x=d$ . With increasing the  $K_i$  substrate concentration in active membrane is decreasing.

At fig. 16 is investigated the influence of reaction rate constant for inhibitor  $K_i = 0,05, 0,1, 0,5, 1, 2, 5$  mM for the constant starting concentration of  $I_0 = 0,5$  mM over the output current. It is seen that current reached the steady state value more fast, that mean smaller transient process.

#### 4. 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.

#### REFERENCES

- [1.] GU M. B., MITCHELL R. J., KIM B. C., *Whole-cell-based biosensor for environmental biomonitoring and application*, *Adv. Biochem. Eng. Biotechnol.* 87, 269-305, 2004.
- [2.] RESHETILOVA, *Microbial, Enzymatic, and Immune Biosensors for Ecological Monitoring and Control of Biotechnological Processes*, *Applied Biochemistry and Microbiology*, 41, 442-449, 2005.

- [3.] D'SOUZA S. F., JHA S. K., KUMAR J., *Environmental Biosensors, Environ. Biotech.* 4, 54-59, 2005.
- [4.] LEI Y., P. MULCHANDANI, W. MULCHANDANI, A. MULCHANDANI, *Biosensor for Direct Determination Fenitrothion and EPN Using Recombinant Pseudomonas putida JS444 with Expressed Organophosphorous Hydrolase. 2. Modified Carbon Paste Electrode. Applied Biochemistry and Biotechnology*, 136, 243-250, 2007.
- [5.] PROLL G., TSCHMELAK J., GAUGLITZ G., 2005, *Fully automated biosensors for water analysis, Anal Bioanal Chem*, 381, 61–63, 2005.
- [6.] CAMPANELLA L., CUBADDA F., SAMMARTINO M.P., SAONCELLA A. *An algal biosensor for the monitoring of water toxicity in estuarine environments, Water Research*, 35, 69-76, 2001.
- [7.] DURRIEU C., CHOUTEAU C., BARTHET L., CHOVELON J. M., TRAN-MINH C., *A Bi-enzymatic Whole-Cell Algal Biosensor for Monitoring Waste Water Pollutants, Analytical Letters*, 37, 1589-1599, 2004.
- [8.] RODRIGUEZ B. B., *Urease-glutamic dehydrogenase biosensor for screening heavy metals in water and soil samples*, 380, 284-292, 2004.
- [9.] SANDSTROM K. J. M., NEWMAN J., SUNESESSON A. L., LEVIN J. O., TURNER A. P. F., *Amperometric biosensor for formic acid in air, Sens&Actuators B*, 70, 182-187, 2000.
- [10.] LANYONA Y. H., IBTISAM G. M., TOTHILLA E., MASCINI M., *Benzene analysis in workplace air using an FIA-based bacterial biosensor, Biosen&Bioelectron.* 20, 2089-2096, 2005.
- [11.] GUM B., CHANG S. T., *Soil biosensor for the detection of PAH toxicity using an immobilized recombinant bacterium and a biosurfactant, Biosen&Bioelectron.* 16, 667-674, 2001.
- [12.] ELENKOVA., *Periferial terminals in the computing systems, Technical University – Sofia*, 1997.
- [13.] PANDELOVA A., S. PETROVA, A. NEYKOV., *Experimental research of a hybrid biosensor for ecological purposes, Biotechnology and Biotechnological equipment*, 17, pp. 187-193, 2003.
- [14.] SKEEL, R.D., BERZINS, M., *A method for the spatial discretization of parabolic equations in one space variable. SIAM J. Scient. Stat. Comput.* 11, 1–32, 1990.



**ANNALS OF FACULTY ENGINEERING HUNEDOARA  
– INTERNATIONAL JOURNAL OF ENGINEERING**

copyright © University Politehnica Timisoara,

Faculty of Engineering Hunedoara,

5, Revolutiei, 331128, Hunedoara,

ROMANIA

<http://annals.fih.upt.ro>