Numerical analysis of initial amount of substrate and biomass in substrate inhibition process

JUAN CARLOS BELTRÁN-PRIETO[1], LONG HUYNH BACH SON NGUYEN[2]

[1]Faculty of Applied Informatics,
Tomas Bata University in Zlín, nám.
T. G. Masaryka 5555, 760 01 Zlín,
CZECH REPUBLIC

[2]Faculty of Chemical and Environmental Engineering,
Lac Hong University,
No. 10, Huynhvannghe Street, Bienhoa, Dongnai Province,
VIETNAM
prieto@utb.cz

Abstract: - The process of inhibition of enzymes is important because it serves as a fundamental control mechanism in many biological systems and allows the regulation of metabolic pathways. In fact, many medications act by inhibiting specific enzymes in the brain or in body tissues. Therefore, the understanding of enzymatic inhibition mechanism is, essential. Inhibitors are frequently used as tools for the study of the mechanism of the enzymes themselves. In the present paper we have studied the process of substrate inhibition by developing a mathematical model that allow us to understand the influence of initial amount of substrate and initial biomass concentration on maximum growth rate value. Numerical analysis using Matlab software was performed to model this inhibition process.

Key-Words: - substrate concentration, inhibition, numerical analysis, simulation, enzyme kinetics, yield, maximum growth rate

1 Introduction

Enzymes are proteins that catalyze chemical reactions and allow an increment in the rate of reaction in several orders of magnitude. Enzymes are highly select and specialized in the catalysis unique chemical reactions with specific substrates [1]. However, after genome-scale model it was found that in *Escherichia coli,* 65% of the reactions that occur in the metabolism are catalyzed for 37% of the enzymes, which have several active sites and recognize several substrates. Therefore, the majority of enzymes in *E.coli* are specific but great amount of the metabolic reactions are carried out by non-specific enzymes. There are several differences between specialist and generalist enzymes. The former are essential, have a higher metabolic flux and require regulation of the activity [2]. Enzymes can participate in reactions in living cells under certain conditions very different from those in experimental systems, such as in vitro kinetic cases. Some enzymes, especially those that catalyze the reactions in the central routes, are present in high concentrations (e.g. exoquinase, which is present in the muscle can have a concentration higher than 100 μM while enzymes that catalyze the biosynthesis of coenzymes, are probably present in concentrations

as low as 0.01 μM. Accordingly, the concentration in the cytoplasm of several metabolites that act as substrates has been measured and in many cases the reported concentration is between between 5 and 500 μM. In many cases, if not most, the concentration of the substrate in the intact cells is not enough to saturate the enzyme; in fact, in some of them, the substrate concentration is not much higher than the concentration of the enzyme. It is clear that the enzymes, in the intact cell, do not necessarily exhibit the classical kinetic behavior of Michaelis-Menten [3], which assumes that the concentration of the enzyme is negligibly small compared to the concentration of the substrate. The quantitative analysis of the kinetics of the enzymes in the intact cell is a field of enzymology that has just started and is of utmost importance for the understanding of the biological regulation of enzymatic activity. It is common to test the enzymes using optimum pH conditions, and substrate concentration above the saturation level, such that the initial rate of reaction is zero order with respect to the substrate. Under these conditions, the initial rate of the reaction is only proportional to the concentration of the enzyme. In the case of enzymes that require the cooperation of cofactors, (e.g metal

ions or coenzymes) these must also be added in concentrations that are higher than saturation, so that the true factor limiting the speed in the system is the concentration of the enzyme [4].

Biochemical reactions are generally performed using cells or living organisms which in the presence of specific nutrients are able to grow and perform biochemical operations. Research using living cells for the production of new compounds, secondary metabolites and high value added products (i.e. biopharmaceuticals, antibiotics, proteins, and chemicals used in industry) is a recent trend in microbiology and biotechnology. For this purpose, different forms of microorganism (algae, bacteria or fungi) and cells (human, vegetable or animal) are widely used [5]. The commercial preparation of several products of fermentation process uses various species of microorganism (e.g. bacteria, yeast and fungi). These fermentative processes are normally autocatalytic since the microorganism not only catalyzes the reaction but also grows and reproduces in the medium broth. These types of reactions generally have inhibition by product [6]. Several reactor configurations are used to carry out the fermentation process. One of the most common uses the continuous reactor. This type of process can be considered as an open system in which biomass and substrate are continuously added to the bioreactor and simultaneously the same volume of fermentation medium is eliminated. The most used continuous reactor to carry out this type of reactions is the continuous stirred tank reactor. Selection of the proper reactor is a very important part in the design of the biotechnology process because it provides the environmental conditions necessary to carry out the culture [7].

Microorganisms are able to grow either by increasing the population number or the cell density after consuming the nutrients of the medium under optimum conditions of temperature, pH, turbulent regime, and concentration of particular elements and compounds found in the culture medium [8]. In the dynamic models proposed for the biochemical reactions, the concentration of biomass, substrate and product are dependent variables of the time that allow the evaluation of the reaction yield [9]. The modeling of this process requires assuming the absence of intracellular reactions, and considering the biomass as homogenous and in a steady environment. This leads to a balance growth state. Under these considerations, it has been observed that the growth velocity of microorganisms is proportional to the existing population, that is $dx/dt = \mu x$, where μ is the specific growth rate, x is the cell concentration and t represents the time

[10]. The yield ($Y_{X/S}$) is the ratio of product to reactant consumed [11]. It is important to note that the specific growth rate does not remains constant during the process as it generally depends on the concentration of nutrients [12]. The concentration of substrate is important because it allows the growth of the microorganism or to increase the synthesis of products. However, excessive concentration can be detrimental due to inhibition or poisoning effects. Several mathematical models have been proposed aiming to describe the process of growth kinetics [13]. Some common models generally used were proposed by Monod, Haldane, Tessier, Andrews, Moser, Aiba, and Contois to fit the values of experimental data reported [14-17]. The inhibition of the growth of the microorganism by the presence of some substance -which can be a substrate or a product- in a certain amount, has been considered by many authors, but the most common expression of inhibition by substrate, is the one proposed by Wayman and Tseng in 1976. These authors considered that there is a critical concentration of substrate, below which there is no growth inhibition. Very often, statistical analysis are performed to analyze the statistical difference between the corresponding models tested according to the fitness to experimental data using common methods such as the coefficient of determination, the root mean square error, bias or accuracy factors among other statistical methods [18].

In the present paper we aim to study the Haldane model. This model is used to describe the inhibitory behavior of microorganisms or cells, which can occur at specific values of substrate concentration. Haldane model has been used to fit experimental data of several kinetic models, i.e. process in anaerobic reactors that describes sulfate reduction process considering different concentrations of sulfate, biomass and sulfide [19], phenol degradation in batch operations by means of description of the influence of concentration of an inhibition substrate on specific growth rate and the proposal of analytical expressions that correlates the biomass and substrate using homotopy perturbation methods [20].

2 Description and solution of the system under study

Haldane kinetic model is represented by equation (1)

$$\mu = \frac{\mu_{\max} S}{Ks + S + \frac{S^2}{Ki}} \qquad (1)$$

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where μ_{max} is the maximum growth rate, Ki is the inhibitory constant, S is the substrate concentration, Ks is the half saturation constant [1]. As we can observe, from this equation we can obtain information about the rate of growth and the concentration of substrate that cause inhibition[2]. However, from this equation there is no correlation to the initial substrate concentration (S_0). This can be approached by including the biomass yield factor equation (2), which represents the proportion of biomass that has been produced to the amount of substrate consumed

$$Y_{\frac{X}{S}} = \frac{X\text{-}Xo}{So\text{-}S} \qquad (2)$$

where $Y_{X/S}$ represents the yield, and X_0 and X are the initial and final concentration of biomass respectively. From equation (2) we can obtain (3). Then, substitution of (3) into (1) leads to (4), which can be also represented as in expression (5) after solving the square of the trinomial.

$$S = \frac{SoY_{\frac{X}{S}} + Xo - X}{Y_{\frac{X}{S}}} \qquad (3)$$

$$\mu = \frac{\mu_{max} \frac{SoY_{\frac{X}{S}} + Xo - X}{Y_{\frac{X}{S}}}}{Ks + \frac{SoY_{\frac{X}{S}} + Xo - X}{Y_{\frac{X}{S}}} + \frac{\left(\frac{SoY_{\frac{X}{S}} + Xo - X}{Y_{\frac{X}{S}}}\right)^2}{Ki}} \qquad (4)$$

$$\mu = \frac{\mu_{max} \left(\frac{SoY_{\frac{X}{S}} + Xo - X}{Y_{\frac{X}{S}}}\right)}{Ks + \left(\frac{SoY_{\frac{X}{S}} + Xo - X}{Y_{\frac{X}{S}}}\right) + \left[\left(\frac{So^2 Y_{\frac{X}{S}}^2 + 2So Y_{\frac{X}{S}} Xo - 2So Y_{\frac{X}{S}} X - 2XXo + Xo^2 + X^2}{Ki Y_{\frac{X}{S}}^2}\right)\right]} \qquad (5)$$

After simplification of the denominator, it follows that:

$$\mu = \frac{\mu_{max} \left(\frac{SoY_{\frac{X}{S}} + Xo - X}{Y_{\frac{X}{S}}}\right)}{\frac{b}{Ki Y_{\frac{X}{S}}^2}} \qquad (6)$$

where

$b = (KsY_{\frac{X}{S}}^2 Ki + KiSoY_{\frac{X}{S}}^2 + KiXoY_{\frac{X}{S}} + So^2 Y_{\frac{X}{S}}^2 + Xo^2 + 2SoY_{\frac{X}{S}} Xo - KiXY_{\frac{X}{S}} - 2SoY_{\frac{X}{S}}X - 2XXo + X^2)$.

$$\mu = \frac{\mu_{max} (SoY_{\frac{X}{S}} + Xo - X)Y_{\frac{X}{S}}^2 Ki}{b * Y_{\frac{X}{S}}} \qquad (7)$$

$$\mu = \frac{\mu_{max} (SoY_{\frac{X}{S}} + Xo - X)Y_{\frac{X}{S}} Ki}{c} \qquad (8)$$

where $c = KsY_{\frac{X}{S}}^2 Ki + KiSoY_{\frac{X}{S}}^2 + KiXoY_{\frac{X}{S}} + So^2 Y_{\frac{X}{S}}^2 + Xo^2 + 2SoY_{\frac{X}{S}} Xo + X(X - KiY_{\frac{X}{S}} - 2SoY_{\frac{X}{S}} \text{-}2Xo)$

We can substitute the value of μ in the definition of growth velocity to have equation (9)

$$\frac{dx}{dt} = \frac{X\mu_{max} \left[SoY_{\frac{X}{S}} + Xo\text{-}X\right] Y_{\frac{X}{S}} Ki}{c} \qquad (9)$$

Then, it follows that

$$\mu_{max} dt = \frac{(c)dx}{Y_{\frac{X}{S}} Ki \left[SoY_{\frac{X}{S}} + Xo\text{-}X\right] X} \qquad (10)$$

and after performing integration in the limits (X) and (Xo) we observe that we can separate the terms in the second integral

$$\int_0^t \mu_{max} dt = d + e \qquad (11)$$

where

$$d = \int_{xo}^{x} \frac{\left(KsY_{\frac{X}{S}}^2 Ki + KiSoY_{\frac{X}{S}}^2 + KiXoY_{\frac{X}{S}} + So^2 Y_{\frac{X}{S}}^2 + Xo^2 + 2SoY_{\frac{X}{S}} Xo\right) dx}{Y_{\frac{X}{S}} Ki \left(SoY_{\frac{X}{S}} + Xo - X\right) X}$$

$$e = \int_{xo}^{x} \frac{X\left(X - KiY_{\frac{X}{S}} - SoY_{\frac{X}{S}} \text{-}2Xo\right) dx}{Y_{\frac{X}{S}} Ki \left(SoY_{\frac{X}{S}} + Xo - X\right) X}$$

Considering that $A/SoY_{\frac{X}{S}} + Xo - X + B/X = 1$, then it follows that $AX + B[SoY_{\frac{X}{S}} + Xo - X] = 1$ and then it can be also expressed as $X(A - B) + B(SoY_{\frac{X}{S}} + Xo) = 1$. Therefore $A - B = 0$. As a result, $B = A = \frac{1}{SoY_{\frac{X}{S}} + Xo - X}$. We can then proceed to perform the integration as described next in (12) to (14)

$$\mu_{max} t = \left(\frac{KsY_{\frac{X}{S}}^2 Ki + KiSo Y_{\frac{X}{S}}^2 + KiXoY_{\frac{X}{S}} + So^2 Y_{\frac{X}{S}}^2 + Xo^2 + 2So Y_{\frac{X}{S}} Xo}{Y_{\frac{X}{S}} Ki}\right)$$

$$\int_{xo}^{x} \frac{dx}{\left(SoY_{\frac{X}{S}} + Xo - X\right)X} + \int_{xo}^{x} \frac{\left(X - KiY_{\frac{X}{S}} - 2So Y_{\frac{X}{S}} \text{-}2Xo\right) dx}{\left(SoY_{\frac{X}{S}} + Xo - X\right)} \qquad (12)$$

$$\mu_{max} t = \left(\frac{KsY_{\frac{X}{S}}^2 Ki + KiSoY_{\frac{X}{S}}^2 + KiXoY_{\frac{X}{S}} + So^2 Y_{\frac{X}{S}}^2 + Xo^2 + 2SoY_{\frac{X}{S}} Xo}{Y_{\frac{X}{S}} Ki}\right)$$

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$$\int_{xo}^{x}\frac{dx}{\left(SoY_{\frac{X}{S}}+Xo-X\right)\left(SoY_{\frac{X}{S}}+Xo\right)}+\int_{xo}^{x}\frac{dx}{X\left(SoY_{\frac{X}{S}}+Xo\right)}+$$

$$\int_{xo}^{x}\frac{\left(X-KiY_{\frac{X}{S}}-2SoY_{\frac{X}{S}}-2Xo\right)dx}{\left(SoY_{\frac{X}{S}}+Xo-X\right)} \qquad (13)$$

$$\mu_{\max}t=\frac{KsY_{\frac{X}{S}}^{2}Ki+KiSoY_{\frac{X}{S}}^{2}+KiXoY_{\frac{X}{S}}+So^{2}Y_{\frac{X}{S}}^{2}+Xo^{2}+2SoY_{\frac{X}{S}}Xo}{Y_{\frac{X}{S}}Ki\left(SoY_{\frac{X}{S}}+Xo\right)}$$

$$\left(\ln\left|\frac{SoY_{\frac{X}{S}}}{SoY_{\frac{X}{S}}+Xo-X}\right|+\ln\left|\frac{X}{Xo}\right|\right)+$$

$$\int_{xo}^{x}\frac{\left(X-KiY_{\frac{X}{S}}-2SoY_{\frac{X}{S}}-2Xo\right)dx}{SoY_{\frac{X}{S}}+Xo-X} \qquad (14)$$

Inserting the result of the integrals, we finally obtain (15)

$$\mu_{\max}t=\frac{KsY_{\frac{X}{S}}^{2}Ki+KiSoY_{\frac{X}{S}}^{2}+KiXoY_{\frac{X}{S}}+So^{2}Y_{\frac{X}{S}}^{2}+Xo^{2}+2SoY_{\frac{X}{S}}Xo}{Y_{\frac{X}{S}}Ki\left(SoY_{\frac{X}{S}}+Xo\right)}$$

$$\left(\ln\left|\frac{SoY_{\frac{X}{S}}}{SoY_{\frac{X}{S}}+Xo-X}\right|+\ln\left|\frac{X}{Xo}\right|\right)-SoY_{\frac{X}{S}}-$$

$$Xo\left(ln\left|\frac{SoY_{\frac{X}{S}}+Xo-X}{SoY_{\frac{X}{S}}}\right|\right)+(Xo-X)+\left(KiY_{\frac{X}{S}}+\right.$$

$$\left.2SoY_{\frac{X}{S}}+2Xo\right)\left(\ln\left|\frac{SoY_{\frac{X}{S}}+Xo-X}{SoY_{\frac{X}{S}}}\right|\right) \qquad (15)$$

Expression (15) is useful to understand the direct correlation of several parameters, namely the maximum growth rate (μ_{max}), the limiting nutrient concentration at which the specific growth is half its maximum value (Ks), the inhibitory constant (K_i), the yield factor ($Y_{X/S}$) at particular values of biomass (X), the initial concentration of substrate (S_0) and the initial amount of biomass required (X_0) in time (t)

3 Numerical simulation

For the purpose of performing numerical simulation, we take into consideration values of inhibition constant=214.5 mg/L, inhibition by substrate=18.3 mg/L, Yield of biomass per substrate=0.63. We studied an initial concentration of substrate and biomass in the range between 0 mg/L and 2 mg/L as described in Fig.1. Accordingly, the influence of biomass concentration and initial substrate amount on substrate concentration for

two different of yield values achieved (Y=5.63; b)Y=0.8) are presented in Fig.2. Numerical simulation was performed using Matlab software. We can observe that for this particular numerical case, the maximum growth rate is obtained at lower values of initial substrate concentration. Accordingly, the equation can be further used to analyze the effect of time in the maximum growth rate achieved.
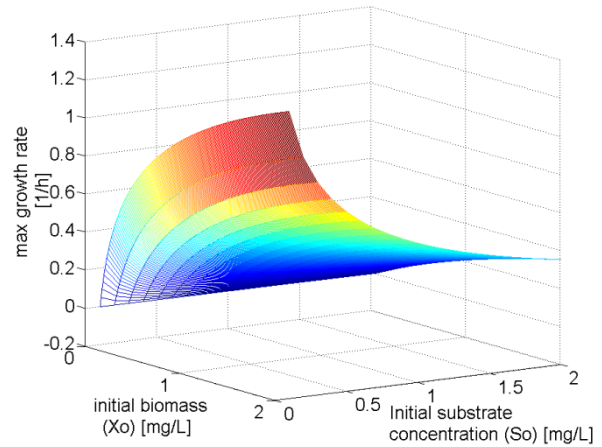

Fig.1. Numerical simulation. Influence of initial substrate concentration and biomass on maximum growth rate
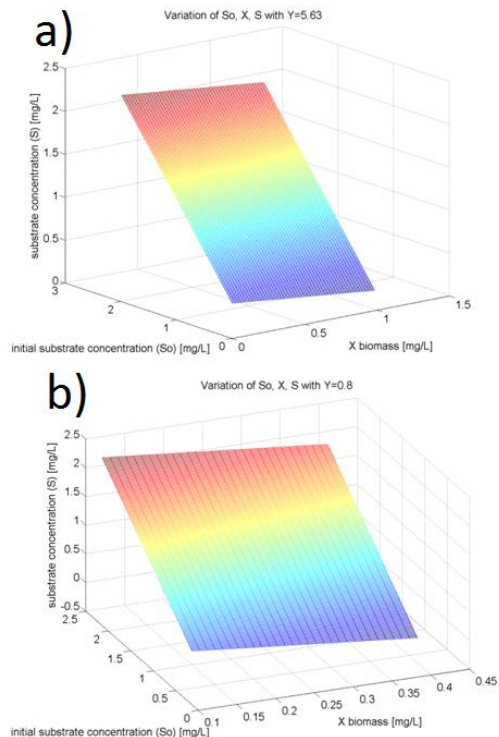

Fig.2. Influence of biomass concentration and initial substrate amount on substrate concentration for two different of Yield values achieved a) Y=5.63; b)Y=0.8

4 Conclusion

The use of mathematical models in chemical process and biochemical engineering is important because it helps in the understanding of the system. It can be used as tools for the simulation of continuous fermentative processes, in order to obtain detailed information on the behavior of the reactor at any period of time. When a reactor is used, we can obtain information about the variation of substrate and product concentration in time and also the requirements of substrate in the feed to change parameters of composition of desired components. Accurate and deep understanding of these parameters can guide to adjust enzymatic processes or the growth of microorganisms on specific substrates and to improve the system by means of prediction and to propose an operation range. Investigations in biochemical reactions have resulted in a high number of different mathematical models that describe microbial growth. The most famous of them is the expression proposed by Monod. Although each of these models can be described by a flexible equation, in general of three parameters, the lack of consistency with the experimental data has led to the development of alternative mathematical models for fitting the growth and kinetics, such as those proposed by Teissier, Moser, Haldane, Andrews and Noack, Aiba, Webb, Yano and Koga and Hinshelwood, among others. Substrate inhibition is one of the common mechanisms of regulation of the enzymatic activity of the cell. Generally at low concentrations, the binding of a substrate molecule with the enzyme in one of its active sites apparently increases the affinity of the enzyme for other substrate molecules, increasing the rate of the catalyzed reaction until reaching a maximum substrate concentration. When this critical concentration is exceeded, the speed of the reaction decreases. In some cases the excess of substrate influences the osmotic pressure and the consequent dehydration of the cell.

Acknowledgements

References:

[1] R. K. Murray, *Harper's Illustrated Biochemistry.* McGraw-Hill Medical, 2012.

[2] Y. Taniguchi et al., Quantifying E. coli Proteome and Transcriptome with Single-Molecule Sensitivity in Single Cells, *Science* Vol. 329, No. 5991, 2010, pp. 533–538.

[3] J. J. Heijnen and R. Kleerebezem, Bioenergetics of Microbial Growth, in *Encyclopedia of Industrial Biotechnology*, Hoboken, NJ, USA: John Wiley & Sons, Inc., 2010, pp. 1–66.

[4] J. W. Finley and J. M. DeMan, *Vitamins, in Principles of Food Chemistry*, Springer International Publishing, 2018, pp. 365–395.

[5] K.V. Ramana, J.R. Xavier, and R.K. Sharma, *Pharmaceutical. Biotechnology Current Research* Vol. 1, No. 1. 2017, pp. 1-10.

[6] M. Lieberman, A. D. Marks, and A. Peet, *Marks' basic medical biochemistry : a clinical approach*, Wolters Kluwer Health/Lippincott Williams & Wilkins, 2013.

[7] Y. Chisti and M. Moo-Young, *Bioreactors, Encyclopedia of Physical Science and Technology*, Academic Press, 2003 pp. 247–271.

[8] F. Garcia-Ochoa and E. Gomez, *Biotechnology Advances*. Vol. 27, No. 2, 2009, pp. 153-176.

[9] M. de. Cuyper and J. W. M. Bulte, *Physics and chemistry basis of biotechnology.* New York: Kluwer Academic Publishers, 2001.

[10] G. D. Najafpour, *Biochemical engineering and biotechnology*. New York: Elsevier, 2007.

[11] N.S. Panikov, *Microbial Growth Kinetics*, Springer, Netherlands,1995, pp. 378

[12] K. Kovárová-Kovar and T. Egli, *Microbiology and Molecular Biology Reviews,* Vol. 62, No. 3, 1998, pp. 646-666.

[13] M. Matsuda and T. Sugawara, Evaluation of Several Mathematical Models for Fitting the Growth and Kinetics of the Catechol-degrading Candida parapsilosis: Part 2, *Journal of Environmental Bioremediation and Toxicology*, Vol. 2, No. 2, 2017, pp. 53–57.

[14] J.E. Bailey and D.F. Ollis, *Biochemical Engineering Fundamentals*, Tata McGraw-Hill, New Delhi, 2010.

[15] Y. Tan, Z.-X. Wang, and K.C. Marshall, Modeling substrate inhibition of microbial growth, *Biotechnology and Bioengineering*. Vl. 52, No. 5. 2000, pp. 602-608.

[16] M.S.M. Annuar, I.K.P. Tan, S. Ibrahim, and K.B. Ramachandran, A kinetic model for growth and biosynthesis of medium-chain-length poly-(3-hydroxyalkanoates) in

[17] pseudomonas putida, *Brazilian Journal of Chemical Engineering*, Vol. 25, No. 2, 2008, pp. 217-228.

[18] A.K. Jana, *Chemical Process Modelling and Computer Simulation*, PHI Learning, New Delhi, 2001, pp 90-116.

[19] D. C. Montgomery, *Design and Analysis of Experiments*, Mexico: Wiley, 2004.

[20] F.A. Cuevas-Ortiz, M.I. Neria-González, and R. Aguilar-López, Nonlinear observer for switched systems: application to a batch bioreactor *Revista Mexicana de Ingeniería Química* Vol. 14, No. 1, 2015, pp. 137-147.

[21] R. Sathya, M. Rasi, and L. Rajendran, Non-linear analysis of Haldane kinetic model in phenol degradation in batch operations *Kinetics and Catalysis*. Vol. 56, No. 2, 2015, pp. 141-146.

[22] T. E. Creighton, *Encyclopedia of Molecular Biology*. John Wiley, 1999.

[23] D. Tazdait, T. Tazdait, N. Abdi, H. Grib, H. Lounici, A. Pauss, N. Mameri, Comparison of different models of substrate inhibition in aerobic batch biodegradation of malathion, *Turkish Journal of Engineering & Environmental Sciences*, Vol. 2013, No. 37, 2013, pp. 221–230