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A Proposal for Reversible Enzymatic Inhibition Applied to the Michaelis-Menten Model in the Transient State

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***Abstract*—Enzymatic processes that obey the classic Michaelis-Menten kinetic model were studied in the light of various proposals that aim to describe reversible inhibition. The proposed inhibition models were compared using a generic process in which the kinetic constants received unit values and the numeric value of the substrate concentration was greater than ten (10) times the numerical value of the enzyme concentration. For each proposed inhibition model, numerical solutions were obtained from a nonlinear system of ordinary differential equations, which produced the results shown in graph, revealing the variation in the enzyme and enzyme complexes and the variation in the substrate and reaction products. A model was developed in which the performance indicated a behavior pattern similar to that seen in the classic Michaelis-Menten model, in which the reaction complex is formed rapidly and, during the process, decays towards zero. In the proposed new model, the inhibitory effect starts at zero and, during the process, tends towards the initial nominal value of the enzyme concentration. Such responses were shown to be valid for distinct enzyme concentration values and process times, demonstrating robustness. The proposed model was applied to enzymatic hydrolysis, providing a fit with mass conservation regarding the product concentration responses upon completion of the process.**

Keywords—enzymatic catalysis. kinetic model. Michaelis-Menten model.

I. INTRODUCTION

More than a century after the publication of the work on the enzyme kinetics of sucrose inversion, written by Leonor Michaelis and Maud Leonora Menten, the inhibition mechanism in reversible enzymatic catalysis reactions that obey the classic Michaelis-Menten model remains to be adequately clarified.

The mathematical modeling for kinetics applied to most enzymatic reactions sets out from the Michaelis-Menten hypothesis, which describes the reaction rate in the condition where the substrate concentration is higher than the enzyme concentration and the sum of the enzyme concentrations and enzyme complexes formed remains constant throughout the processing time [1].

The classic Michaelis-Menten model, however, does not contemplate the inhibition process, although the vast majority of enzymatic reactions do not convert 100% of the substrate, indicating the existence of a point at which, for a given condition, the process is interrupted [2].

The inhibitors are typically characterized based on the assumption of equilibrium, in the steady-state, between the enzymatic complex reaction and the substrate. Such an assumption would only be valid for very inefficient enzymes [3].

This paper adopts the strategy of defining a pictorial process where the nominal value of the substrate concentration is higher than ten (10) times the nominal value of the enzyme concentration. The numerical solution of the nonlinear systems of ordinary differential equations (ODEs) was obtained using a routine developed in Matlab®, applying the 4th order Runge-Kutta method.

II. THE CLASSIC MODEL IN THE LITERATURE

To evaluate the different inhibition perspectives, it is first necessary to adopt an approach in relation to classic Michaelis-Menten kinetics applied to a generic catalysis process, as described in Scheme 1.

Scheme 1 – The classic Michaelis-Menten kinetic model

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

Where k_1, k_{-1} and k_2 are the kinetic constants of the reactions. The other components are: the enzyme concentration, E; substrate concentration, S; the concentration of the enzyme substrate complex, ES; the product concentration, P.

In the mathematical modeling of the above reactions, the variations in the substrate, the enzyme, the product and the enzymatic complex are obtained as follows:

$$\frac{dE}{dt} = k_{-1}ES - k_1(E \cdot S) + k_2ES \quad (1)$$

$$\frac{dES}{dt} = k_1(E \cdot S) - k_{-1}ES - k_2ES \quad (2)$$

$$\frac{dS}{dt} = k_{-1}ES - k_1(E \cdot S) \quad (3)$$

$$\frac{dP}{dt} = k_2ES \quad (4)$$

In the solution of the nonlinear system ordinary differential equations described above, the primary requirement is the strict observance of the Law of Mass

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Conservation for the enzyme and the complex, through the following expression:

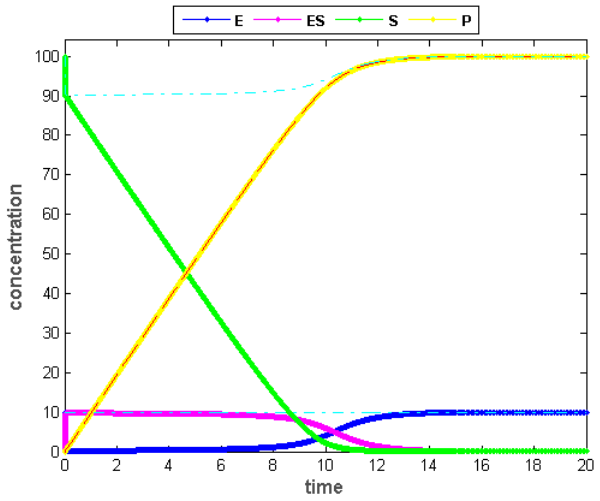
$$\frac{dE}{dt} + \frac{dES}{dt} = \frac{d}{dt}(E + ES) = 0 \qquad (5)$$

The numerical solution of the model is applied to the suggested pictorial process, as shown in Figure 1.

The graph in Figure 1 shows the variation of the enzyme and the enzyme complex, with, in the lower dotted line, the sum of the contributions of these variables. Figure 1 also shows the variation of the substrate and product, with, in the upper dotted line, the sum of the contribution of these two components.

It is important to note that the lower dotted line always remains at the nominal value of the added enzyme, which is consistent with the Law of Mass Conservation.

Figure 1. Response for the kinetic model of Scheme 1



In the upper dotted line in Figure 1, there is a drop at the beginning and a subsequent convergence to the nominal value of the added substrate. This initial drop is linked to the relationship between the enzyme and substrate and is equal to the ratio between those components. In the example shown, the ratio is equal to 10/100, i.e., it represents an initial 10% drop in the sum of the substrate and product, at the start of the process, tending towards the initial value of the substrate concentration during processing.

The reaction complex ES is formed immediately at the beginning of the reaction and, later, decreases from an initial value very close to the nominal enzyme value towards zero. During a significant part of the process time, the variation in the concentration of the complex is small, suggesting the presence of an 'quasi-stationary state' in this time segment of the process may be considered, i.e.:

$$\frac{dES}{dt} \cong 0 \qquad (6)$$

As a rule, the studies available in the literature are concerned with the resolution of the inhibition step based on the assumed steady-state or 'quasi-stationary' state, suggesting an assumed equilibrium between the formation and dissociation of the enzyme substrate [4, 5, 6, 7].

III. PRODUCT INHIBITION

One way to represent the reversible inhibition process in enzyme-catalyzed hydrolysis is to consider that the inhibitor is a product of its own reaction. In this proposal a new enzyme complex appears, , which represents the complex formed by the enzyme and the product and would be the reversible inhibition complex in this model, as proposed by van Boekel [8].

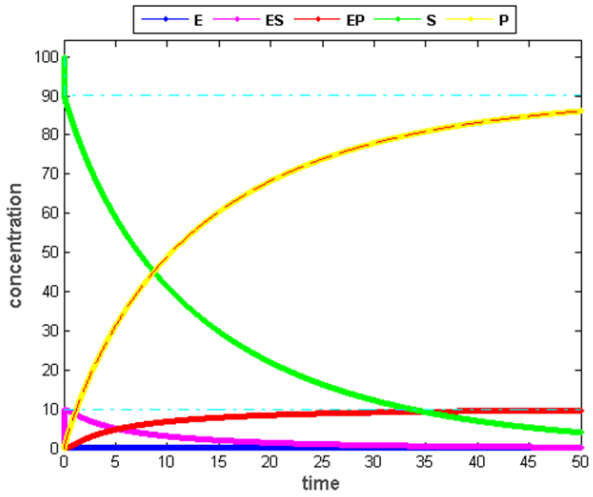
Scheme 2 – Michaelis-Menten model with product inhibition

$$E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_2} E + P \underset{k_{-3}}{\overset{k_3}{\longleftrightarrow}} EP$$

Source: [8]

Figure 2 shows the numerical results for the pictorial process of the model with product inhibition, with all the unitary constants. The process time has been extended to better represent the variation in the model.

Figure 2. Response of the Scheme 2 kinetic model



In the graph in Figure 2, no convergence of the product towards the initial value of the substrate can be seen at the end of processing, as found in the classic Michaelis-Menten model.

In the model with product inhibition, as proposed by van Boekel [8], there is an error percentage (E_f (%)) approximately equal to the enzyme substrate ratio (ESR), at the end of the process, namely:

$$ESR = \frac{E}{S} \qquad \rightarrow \qquad E_f(\%) \cong ESR * 100 \qquad (7)$$

This situation is seen in the graph in Figure 3, which shows the behavior of van Boekel's model [8] with extension of the process time to allow for the consumption of the entire substrate.

At the bottom of the graph in Figure 3, the ES complex can be seen to be instantly formed and undego, throughout the process, a reduction towards zero, exactly as expected for an enzyme catalysis that obeys the Michaelis-Menten model.

The EP complex, on the contrary, grows from zero and tends towards the initial value of the enzyme. The free

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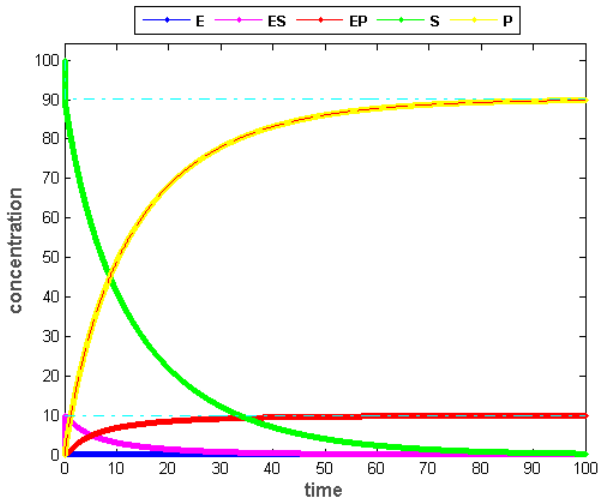
enzyme (*E*) instantly drops to zero and remains close to that value throughout processing, resulting in:

$$\frac{dEP}{dt} \cong -\frac{dES}{dt} \qquad (8)$$

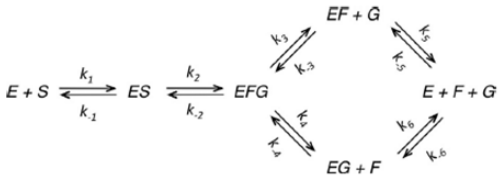
Figure 3. van Boekel's model with extended process time



The above expression suggests that a suitable representation of the inhibition of a reversible enzymatic process would include a decrease in the complex reaction (*ES*) roughly equivalent to the increase in the inhibition complex.

Specifically for the enzymatic hydrolysis of sucrose, Johnson and Goody [1] proposed a structure for the hypothesis of product inhibition, in which *S* represents sucrose, *G* is glucose, *F* is fructose, *EG* is the enzyme glucose complex and *EF* represents the fructose enzyme complex.

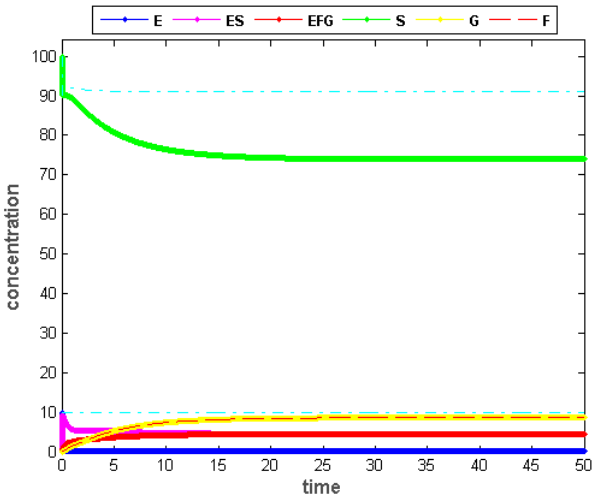
Scheme 3 - Model of sucrose inversion with product inhibition



Source: [9]

The representation for the said process is presented in the paper from Johnson [9], as shown in Scheme 3, where the formation of *EFG*, a novel intermediate compound, is postulated.

Figure 4. Response of the Scheme 3 kinetic model



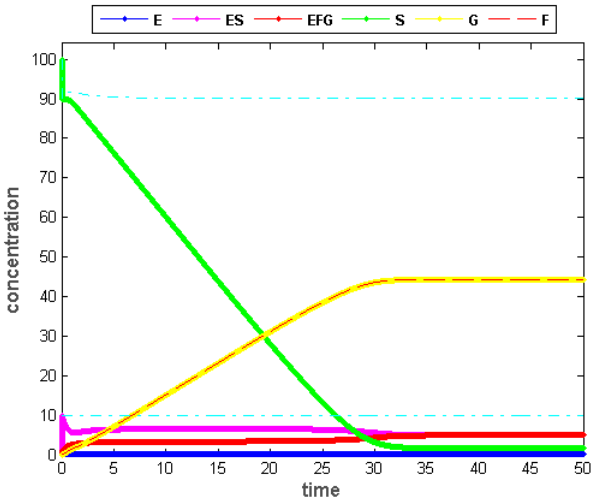
The *EFG* complex proposed in the study by Johnson [9] represents product inhibition in a similar way to the *EP* complex in the representation suggested in van Boekel [8].

Figure 4 shows the result of the pictorial process for Johnson's [9] model, with the same conditions as above.

The response from the Johnson's [9] model maintains the behavior observed in the van Boekel's [8] model with regard the error associated with the numerical value of the percentage of the enzyme-substrate ratio.

The upper dotted line in Figure 5, where the initial kinetic reaction constant value ($k_1 = 1200$) was added and the other values were maintained equal to the representation in Figure 4, shows the error trend in Johnson's model [9].

Figure 5. Response from Johnson's model with $k_1 = 1200$



Recently, Martins and Oliveira [10] proposed a new way of representing the product inhibition for the hydrolysis of disaccharides.

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Scheme 4 – Model of enzymatic hydrolysis of disaccharides

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} EP_I + P_{II} \qquad \text{(initial reaction step)}$$

$$EP_I \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E + P_I \qquad \text{(hydrolysis step)}$$

$$EP_I + P_{II} \underset{k_{-i}}{\overset{k_i}{\rightleftarrows}} EI \underset{k_i}{\overset{k_{-ii}}{\rightleftarrows}} E + P_I + P_{II} \qquad \text{(inhibition step)}$$

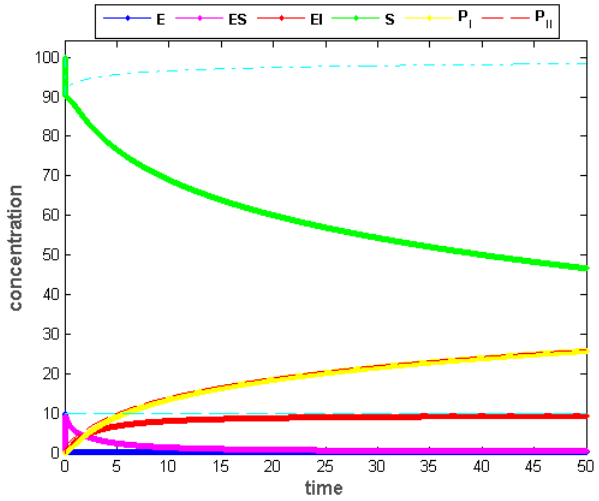
Source: [10]

Figure 6 shows the numerical results for the generic procedure of the Martins and Oliveira model [10].

The graph in Figure 6 shows the expected behavior of a reaction process in which a substrate is divided into two products of equal molecular weight.

There is fluctuation at the beginning of the reaction, showing the P_{II} concentration is slightly higher than that of P_I, but, during the process, a tendency towards the approximation of the concentrations of both products can be seen.

Figure 6. Response for Martins and Oliveira's model

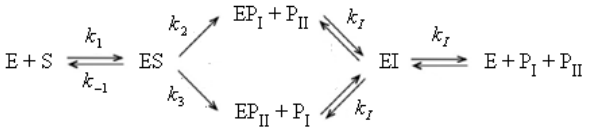


IV. A PROPOSED NEW MODEL

The new model sets out from the premise presented in Johnson's paper [9], in which a substrate, S ,is converted into two (2) products of equal molecular mass (P_I and P_{II}), arising from the formation of two intermediate reaction complexes, EP_I and EP_{II}.

The proposed model assumes a product inhibition mechanism to express the final step of catalysis as presented in the paper from Martins and Oliveira [10], postulating that the inhibition effect occurs both on the intermediate reaction complexes and on the free enzyme, forming an inhibition complex, EI.

Scheme 6 - Proposal for inhibition in this paper



(reaction and inhibition)

$$EP_I \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} E + P_I \qquad (P_I \text{ hydrolysis})$$

$$EP_{II} \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} E + P_{II} \qquad (P_{II} \text{ hydrolysis})$$

Figure 7 shows the numerical results for the generic process in the present paper.

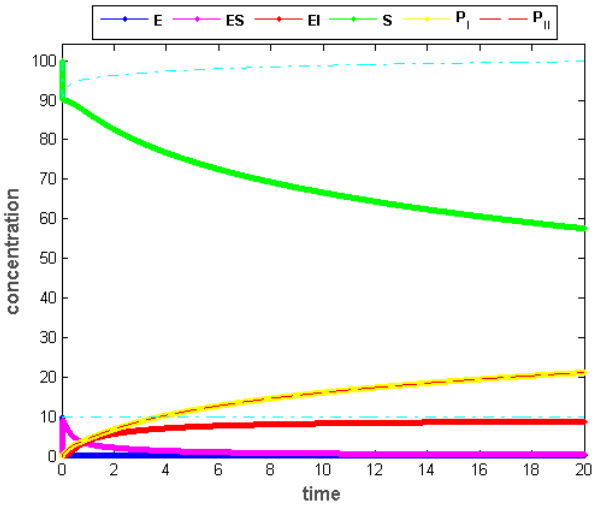
Unlike the results obtained by Martins and Oliveira [10], who found fluctuation at the beginning of the reaction, with a P_{II} concentration slightly higher than that of P_I, the present study shows both products attained similar concentration levels throughout the reaction.

The model's mass conservation (*MCC*) throughout the process was calculated using the following expression, where y_i is the concentration value of each carbohydrate over time and V represents the different carbohydrates in the system.

The error value at the end of the process (E_f (%)), as it is not linked to the enzyme substrate ratio (*ESR*) was calculated by comparing the initial numerical value of the substrate (S_0) with the conservation of mass at the end of the process.

$$MMC = \sum_{i=1}^{V}(y_i) \qquad E_f(\%) = \left(\frac{S_0 - \sum_{i=1}^{V}(y_i(n+1))}{S_0} \right) * 100 \qquad (09)$$

Figure 7. Response of the process for the model in this paper



The solution for the ordinary differential equations was obtained using the 4th order Runge-Kutta method and a step equal to 0.0001.

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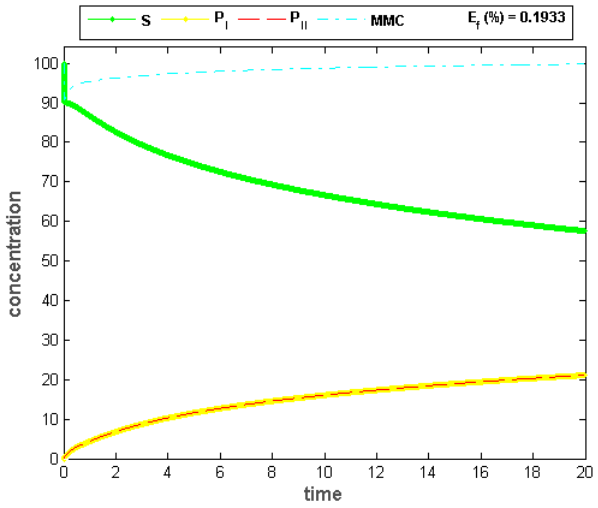
Figure 8. Variation in the substrate and the products to the present model



To check the error associated with the proposed model and visualize the initial fluctuation in the product concentration, Figure 8 only shows the result with the variation in the carbohydrates throughout the generic process, maintaining the percentage of the enzyme substrate ratio equal to 10% ($S = 100$ and $E = 10$).

It can be seen that the model error calculated at the end of the process shown in Figure 8 was 0.1933% and, therefore, is unrelated to the percentage of the enzyme substrate ratio, as in the case of inhibition model presented in Johnson's study [9].

Figure 9 shows the result for the present model obtained while maintaining the parameters for substrate concentration, enzyme concentration and process time, with adjustment of the kinetic constants to achieve an error close to zero.

The numerical values of the kinetic constants in Figure 9 were:

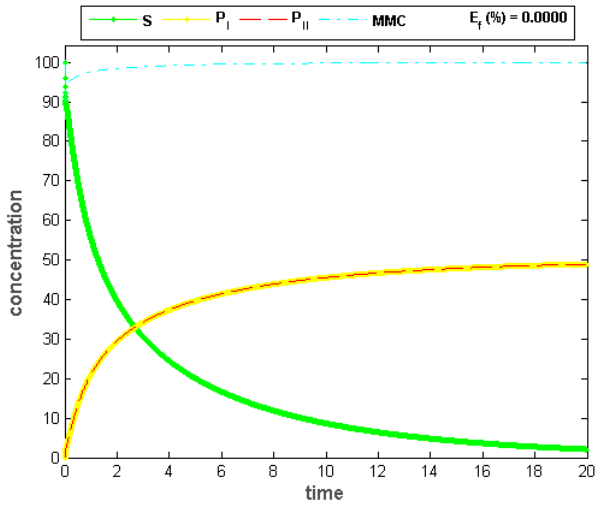
a) $k_1 = 50.992$

b) $k_3 = k_2 = k_{-1} = 6.995$

c) $k_4 = k_5 = k_{-4} = k_{-5} = 6.231$

d) $k_I = 10.0$

Figure 9. Response of the present model with an increase in substrate consumption



The response of the reaction products shown in Figure 9 conforms to the theoretical behavior expected in such enzymatic processes, with catalysis with first order kinetics at the beginning of the process and zero order at the end of process.

V. CONCLUSION

In the present study, during enzymatic reaction, represented by Michaelis-Menten's classic kinetic model, the behavior of the inhibition step is described by applying proposed inhibition effects induced by increasing the product concentration, in numerical solution of a generic process.

The proposed model presented herein was applied to an enzymatic process which generated two (2) reaction products, both with the same molecular mass, as in the example of enzymatic hydrolysis.

Unlike the models in the literature whose representation of product inhibition offer a model error linked to the percentage of the enzyme-substrate ratio, the model proposed in the present study provides a result that minimizes the percentage error of the model, in which it is possible obtain mass conservation for the sum of the substrate and the products following the proper adjustment of the numerical values for the kinetic constants.

The model proved robust in relation to the variation in the enzyme-substrate ratio as well as the variation in processing time.

The adoption of this approach towards the inhibition effect in the construction of new models should be differentiated and applied to each specific situation, in the case of enzymatic catalysis that generate different reaction products.

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