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Michaelis-Menten Kinetics in Transient State: Proposal for Reversible Inhibition Model and its Application on Enzymatic Hydrolysis of Disaccharides

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ABSTRACT
The enzymatic processes according Michaelis-Menten kinetics have been studied from various approaches to describe the inhibition state. Proposals for inhibition were compared from a generic process, where kinetic constants have received unitary values, and the numeric value of the concentration of substrate was ten (10) times higher than the numerical value of the concentration of enzyme. For each inhibition model proposed, numerical solutions were obtained from nonlinear system of ordinary differential equations, generating results presents by graphs showing the variation of the enzyme and enzyme complexes, also the variation of substrate and product of the reaction. Also, was designed a model with performance, indicating similar behavior to that seen in the Michaelis-Menten kinetics, where complex of reaction is rapidly formed and throughout the process, tends to decay to zero. Thus, in this new proposed model, the effect of inhibition starts at zero and, throughout the process, tends to the nominal value of the initial enzyme concentration. Such responses have proved to be valid for different values of enzyme concentration and process time, showing robustness. The proposed model was applied to the hydrolysis of disaccharides, providing a setting with conservation of mass of the model at the end of the process regarding the responses of the carbohydrate concentration.
Keywords **-** enzymatic catalysis, kinetic model, Michaelis-Menten kinetics.

I. INTRODUCTION

More than 100 years after the publication of the emblematic work on enzyme kinetics of the inversion of sucrose disaccharide, by Leonor Michaelis and Muad Leonora Menten, the inhibition mechanism of the enzymatic catalysis processes on Michaelis-Menten kinetics is still not sufficiently clarified. In mathematical modeling for kinetics applied to most of enzymatic reactions, starts from 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 enzyme and enzyme complexes concentrations formed will remain constant throughout the processing time [1].

A major question regarding the process of enzyme-catalyzed reaction refers to the condition in which the inhibiting stage develops. The Michaelis-Menten kinetics does not contemplate the inhibition, even though the vast majority of enzymatic processes do not provide a 100% of substrate conversion, indicating that there is a point in which, for a given condition, the process is discontinued [2].

The literature on biochemistry shows four (4) possible mechanisms of reversible inhibition of enzymatic processes, known as: competitive; non-competitive; mixed and uncompetitive [3].

Also, solutions for general problem of steady-state enzyme kinetics have been limited in helping researchers, given that the generated models do not adequately fit the experimental observations. Inhibitors are traditionally characterized on assumption equilibrium in steady state, between the enzyme complex and the substrate reaction. According Fange et al. [4], this assumption would be valid only for very inefficient enzymes.

Therefore, this study aims to assess the inhibition during the process, represented by Michaelis-Menten kinetics also it suggests new representations for catalysis in mathematical models on transient state representation.

For that, it is applied a pictorial process for defining, where substrate concentration value is ten (10) times higher than the nominal value of enzyme concentration. The numerical solution of nonlinear systems of ordinary differential equations (ODE) was obtained by elaborating a routine in Matlab® with the method of Runge-Kutta 4th order.

II. MICHAELIS-MENTEN KINETICS AND INHIBITTION MODELS

For evaluating the different perspectives of inhibition, it is necessary, firstly, a careful approach of Michaelis-Menten kinetics applied to a generic process of catalysis, as described in Scheme 1.

Scheme 1 - Michaelis-Menten kinetics

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

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

In mathematical modeling of preceding reactions, variations of substrate, enzyme, product and enzyme complex are obtained as follows:

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

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

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

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

For solution of the nonlinear system of ordinary differential equations described, the main requirement concerns the strict observance of the Law of Conservation of Mass for the enzyme and complex, by means the expression below:

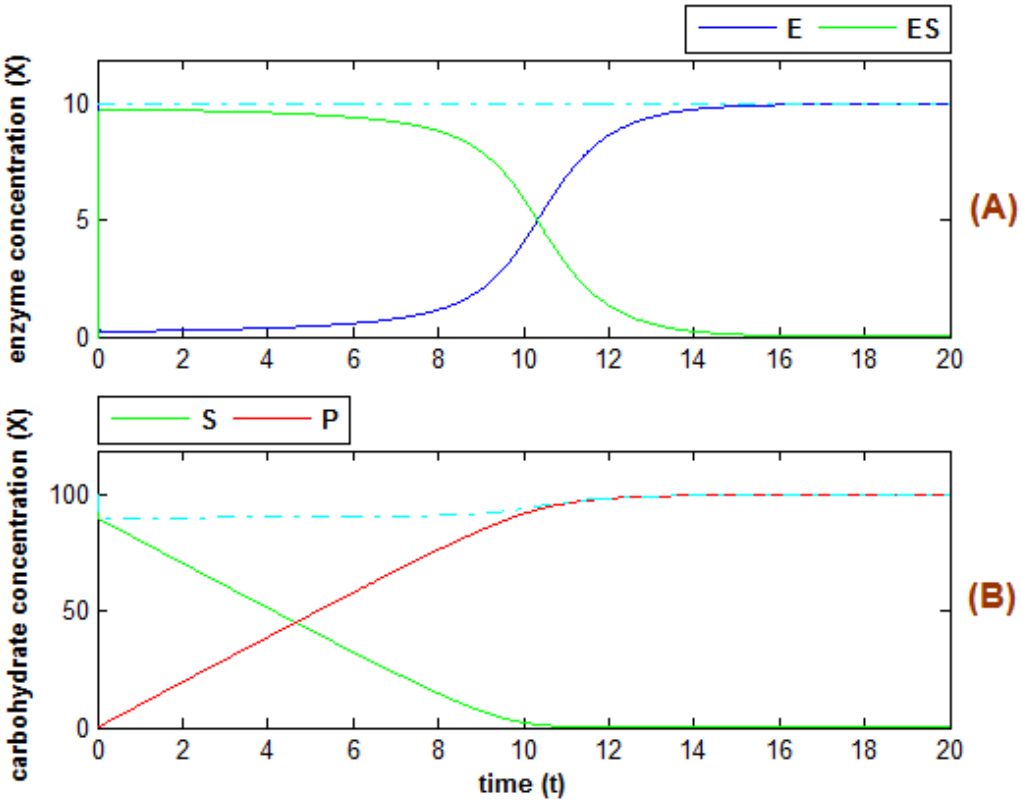
$$\frac{dE}{dt} + \frac{dES}{dt} = \frac{d}{dt}(E + ES) = 0 \quad (5)$$

In this model, the numerical solution is applied to the suggested pictorial process - shown in Figure 1a - with carbohydrate concentration and enzyme hypothetical unit (X) representing a relation between mass and volume, also with process time on hypothetical unit (t).

Figure 1a shows the variation of the enzyme and enzyme complex, and, on dotted line, the sum of contributions of these variables. Plus, Figure 1b shows the variation of substrate and product, and on dotted line, the sum of the contribution of these two components.

It is noteworthy, that on dotted line in Figure 1a the nominal value of the added enzyme remains, which is consistent with the Law of Conservation of Mass. And, on dotted line in Figure 1b it is possible to observe decay, in early stages, and eventually convergence to the nominal value of added substrate.

Figure 1 - Response to the kinetic model of Scheme 1



This initial decay is linked to the relation between enzyme and substrate and it is equal to the ratio between these components.

In the example shown, the fraction is equal to 10/100, i.e., it represents an initial decay of 10% in the substrate and product sum, (compared with the process beginning), tending to the initial value of substrate concentration during the process.

The complex reaction *ES* is formed immediately at the beginning of the reaction, and after that, decreases from an initial value, very close to the enzyme nominal value, to zero. During a significant part of the process, the variation of the complex concentration is small, what can indicate the possibility of considering this state as 'quasi-stationary', i.e.:

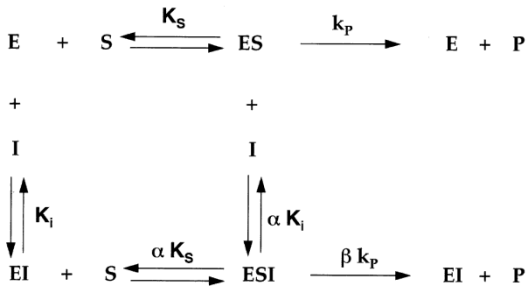
$$\frac{dES}{dt} \cong 0 \qquad (6)$$

Furthermore, available current studies, usually, approaches the inhibition stage from this assumption: stationary or 'quasi-stationary' state, pointing an assumed balance between formation and dissociation of the enzyme substrate complex [5, 6, 7, 8].

A generic representation of the various possibilities to enzyme inhibition inversion is shown in Scheme 2, as proposed (modified) for Botts and Morales [9].

In this proposal two new enzymatic complexes are shown: *EI* representing inhibition complex and *ESI* representing the intermediate inhibition complex.

Scheme 2 - Michaelis-Menten kinetics Mechanism of Inhibition



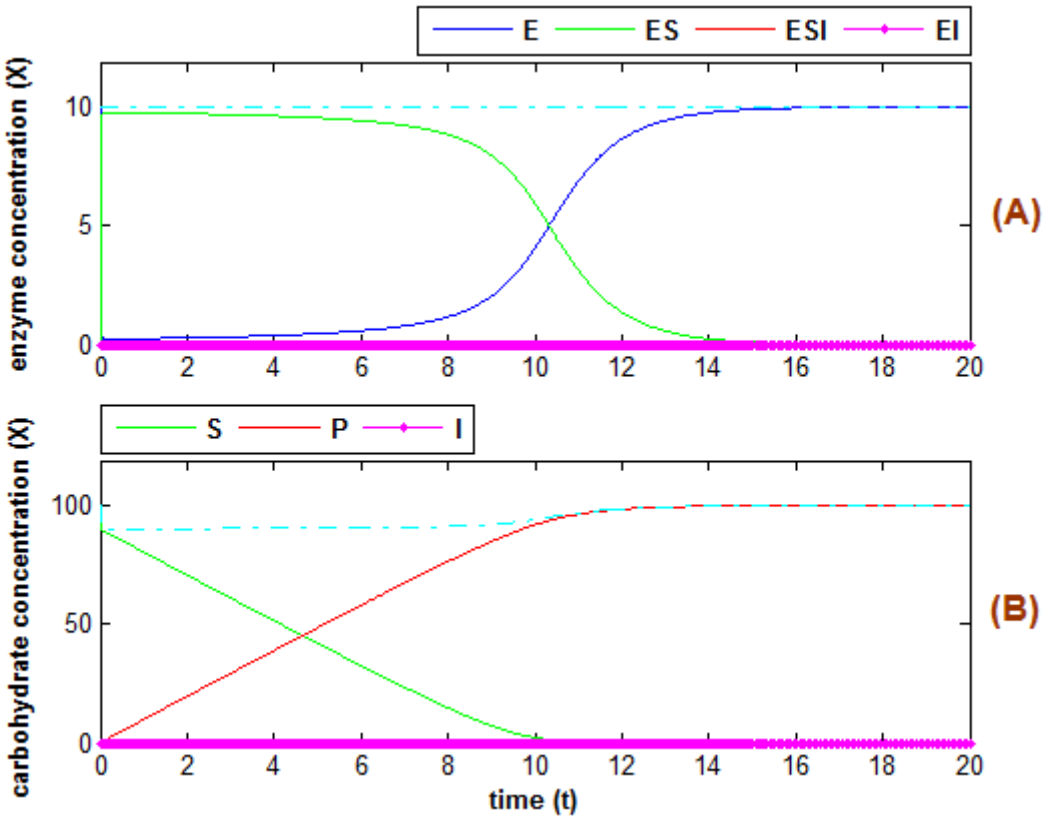
Source: [10]

In this general mechanism, it is also shown a substance *I*, which will be responsible for inhibition.

In the Scheme 2, the coefficient α sets the ratio between the competitive and uncompetitive inhibition, and β is a multiplication factor of the kinetics' constant rate.

The representation for ODEs system related to the general mechanism of Scheme 2 is shown below, and the Figure 2 shows a numerical solution of the system, from the pictorial model, and unit values for α and β.

Figure 2 - Response to the generic mechanism of Scheme 2



$$\frac{dE}{dt} = k_S ES - k_S(E \cdot S) + k_p ES + k_i(EI \cdot S) - k_i(E \cdot S \cdot I) \quad (7)$$

$$\frac{dES}{dt} = k_S(E \cdot S) - k_S ES - k_p ES + \alpha k_i ESI - \alpha k_i(ES \cdot I) \quad (8)$$

$$\frac{dESI}{dt} = \alpha k_i(ES \cdot I) - \alpha k_i ESI + \alpha k_S(EI \cdot S) - \alpha k_S ESI - \beta k_p ESI \quad (9)$$

$$\frac{dEI}{dt} = k_i(E \cdot S \cdot I) - k_i(EI \cdot S) + \alpha k_S ESI - \alpha k_S(EI \cdot S) + \beta k_p ESI \quad (10)$$

$$\frac{dS}{dt} = k_S ES - k_S(E \cdot S) + \alpha k_S ESI - \alpha k_S(EI \cdot S) \quad (11)$$

$$\frac{dP}{dt} = k_p ES + \beta k_p ESI \quad (12)$$

$$\frac{dI}{dt} = k_i(EI \cdot S) - k_i(E \cdot S \cdot I) + \alpha k_i ESI - \alpha k_i(ES \cdot I) \quad (13)$$

In this response, it is observed that the behavior of the enzyme and its complex is similar Michaelis-Menten kinetics without inhibition.

In the Figure 2a the response indicates that *ESI* and *EI* complexes, remains equal to zero over the entire process time. Accordingly, in Figure 2b, the concentration of the inhibiting substance, also will remain equal to zero.

Additionally, the observed response - in generic mechanism - will be repeated if, individually studied proposals for competitive, noncompetitive, mixed inhibition or uncompetitive.

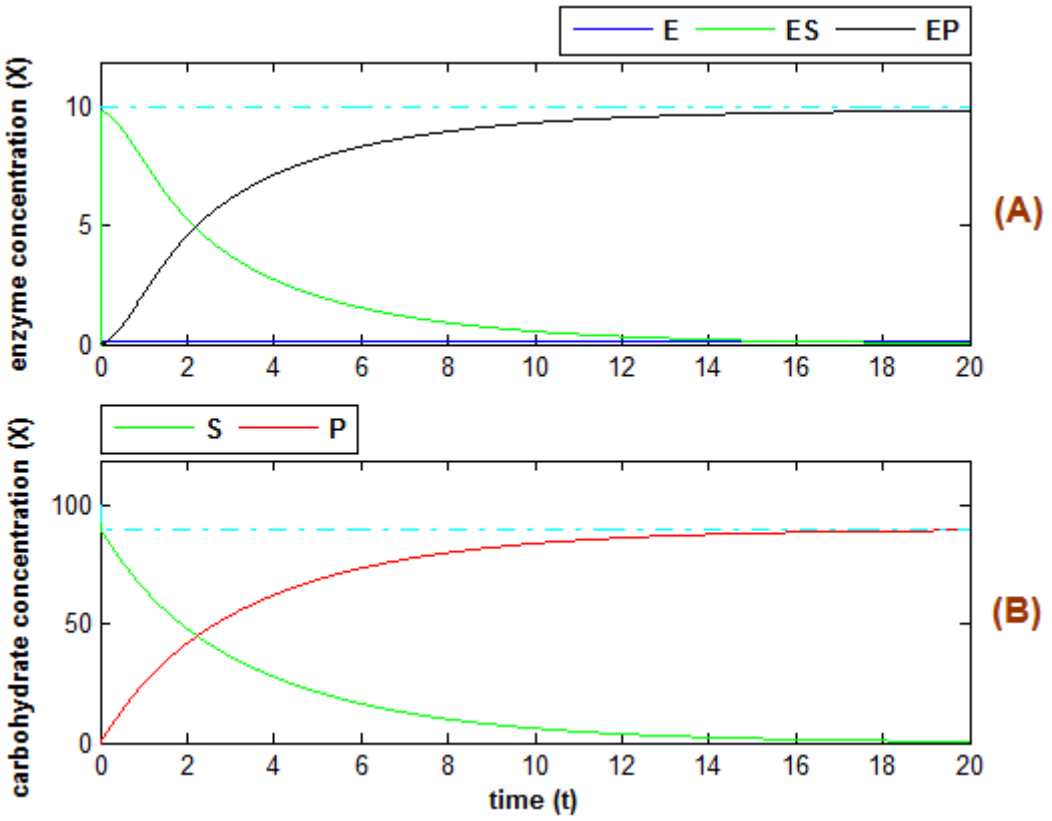
Furthermore, another way to represent the process of inhibition is considering the inhibitor as a product of the reaction, as shown in Scheme 3. Figure 3 shows results for process with product inhibition, where: $k_1 = k_2 = 3$, and the other constant unitary.

Scheme 3 - Michaelis-Menten kinetics with product inhibition

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

Source: [11]

Figure 3 Response kinetic model of Scheme 3



$$\frac{dE}{dt} = k_{-1}ES - k_1(E \cdot S) + k_2ES + k_{-3}EP - k_3(E \cdot P) \quad (14)$$

$$\frac{dES}{dt} = k_1(E \cdot S) - k_{-1}ES - k_2ES \quad (15)$$

$$\frac{dEP}{dt} = k_3(E \cdot P) - k_{-3}EP \quad (16)$$

$$\frac{dS}{dt} = k_{-1}ES - k_1(E \cdot S) \quad (17)$$

$$\frac{dP}{dt} = k_2ES + k_{-3}EP - k_3(E \cdot P) \quad (18)$$

The result shown in Figure 3b may be observed that there is no convergence of the product to the initial value of the substrate at the end of the process, as observed in Michaelis-Menten kinetics with inhibition - Scheme 2.

In the model with product inhibition, proposed in Scheme 3, a percentage error, at the end of the process occurs (E_f (%)) and it is equivalent to enzyme-substrate ratio (*ESR*), i.e.:

$$ESR = \frac{E}{S} \rightarrow E_f(\%) \cong ESR * 100 \quad (19)$$

In the Figure 3a, it is observed that *ES* is instantly formed, and during the process, it decays, tending to zero. The complex *EP*, on the other hand, increases from zero and tends to the initial value of enzyme.

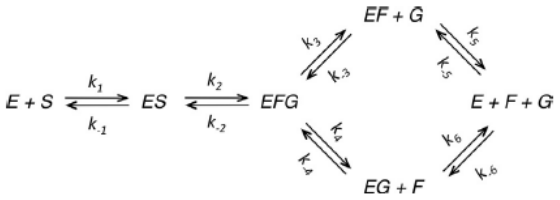
The free enzyme (*E*) instantly decays to zero and remains near that value throughout the process, resulting in:

$$\frac{dEP}{dt} \cong -\frac{dES}{dt} \quad (20)$$

Specifically, for the hydrolysis of sucrose by the enzyme invertase - firstly studied and published in 1913 by Michaelis and Menten - recently, Johnson and Goody [1] - have proposed a framework for the product inhibition hypothesis, where *S* is sucrose, *G* is glucose, *F* is fructose, *EG* is the glucose-enzyme complex and *EF* represents the fructose-enzyme complex.

Representation for such process is shown by Johnson [12], according Scheme 4.

Scheme 4 - Model of sucrose inversion with inhibition by products



Source: [12]

Figure 4 shows the result of the pictorial process according Johnson [12] model in the same conditions, for the system of ordinary differential equations - on model of Scheme 4, as shown below:

$$\frac{dE}{dt} = k_{-1}ES - k_1(E \cdot S) + k_5(EF \cdot G) - k_{-5}(E \cdot F \cdot G) + k_6(EG \cdot F) - k_{-6}(E \cdot F \cdot G) \quad (21)$$

$$\frac{dEG}{dt} = k_4EFG - k_{-4}(EG \cdot F) + k_{-6}(E \cdot F \cdot G) - k_6(EG \cdot F) \quad (22)$$

$$\frac{dEF}{dt} = k_3EFG - k_{-3}(EF \cdot G) + k_{-5}(E \cdot F \cdot G) - k_5(EF \cdot G) \quad (23)$$

$$\frac{dES}{dt} = k_1(E \cdot S) - k_{-1}ES + k_{-2}EFG - k_2ES \quad (24)$$

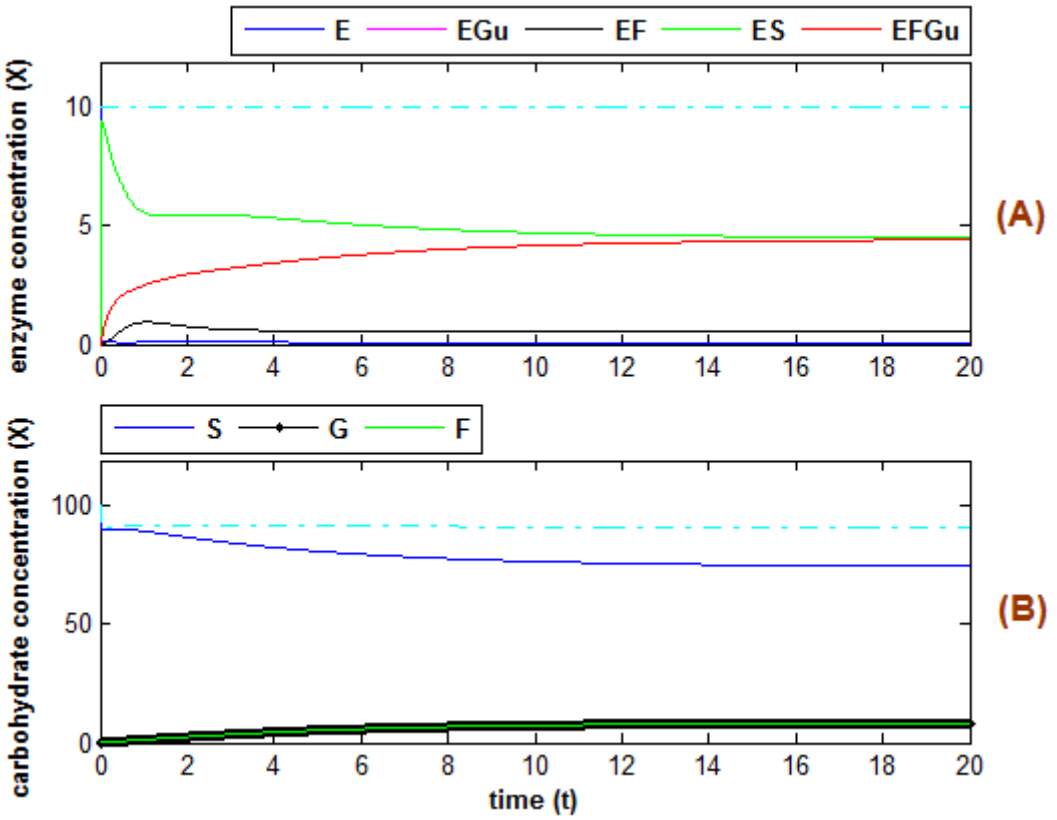
$$\frac{dEFG}{dt} = k_2ES - k_{-2}EFG + k_{-3}(EF \cdot G) - k_3EFG + k_{-4}(EG \cdot F) - k_4EFG \quad (25)$$

$$\frac{dS}{dt} = k_{-1}ES - k_1(E \cdot S) \quad (26)$$

$$\frac{dF}{dt} = k_4EFG - k_{-4}(EG \cdot F) + 0{,}5(k_{-6} - k_{-5})(E \cdot F \cdot G) + 0{,}5k_5(EF \cdot G) - 0{,}5k_6(EG \cdot F) \quad (27)$$

$$\frac{dG}{dt} = k_3EFG - k_{-3}(EF \cdot G) + 0{,}5(k_{-5} - k_{-6})(E \cdot F \cdot G) + 0{,}5k_6(EG \cdot F) - 0{,}5k_5(EF \cdot G) \quad (28)$$

Figure 4 - Response of kinetic model on Scheme 4



It is possible to note in Figure 4b the response in which the range of the product indicates the tendency for obtaining the same numerical values for concentrations of glucose and fructose. In Figure 4a, it is shown the complex reaction (*ES*) slowly tending to zero, while complex of inhibition (*EI*) tends even slower to the numerical value of the initial substrate concentration.

The response of Johnson [12] model, however, keeps the behavior observed in the van Boekel [11] model, which refers to the error associated with the numerical value of the ratio percentage of the enzyme substrate.

III. PROPOSAL FOR A NEW MODEL

In order to design a model aiming to represent the effect of inhibition on Michaelis-Menten kinetics it is necessary, firstly, to establish some assumptions:

a. Continuously, and throughout the process time, the model should strictly follow the Law of Conservation of Mass, regarding to the variation of enzyme and its complexes sum;
b. The model should converge the substrate and product sum to the nominal value of the added substrate, in a high enough time and with the lowest error (as possible) to ensure stability to the process;
c. To ensure the process stability, the model must be sufficiently robust, so, allowing variation on the ratio enzyme - substrate, in the longest possible time;
d. The model should facilitate a performance of reaction complex (enzyme substrate complex) with a high rate at the beginning of reaction, and decreasing overtime, tending to zero at the end of the process;
e. The model should represent the effect of inhibition from a enzyme complex which can act on the complex reaction, thus indicating its relation with other components of the enzymatic catalysis;
f. In case of more than one enzymatic complex and more than one product of reaction, its contributes should be considered for the purpose of inhibition;
g. The model should provide an initial formation of the inhibition complex equal to zero, increasing overtime and tending to the initial value of the enzyme at the end of the process.

As follow, it is applied the aforementioned premises to a process of enzymatic reaction, in which a generic substrate, *S* is converted to two (2) products with equal molecular weight, P_I and P_{II} typical representation of hydrolysis of disaccharides. Scheme 5 presents the proposal such model.

In this proposal, it is both formed a reaction complex with the substrate (*ES*) as a complex with one of the products (EP_I) also, the representation of inhibition complex (*EI*).

Scheme 5 - Proposed model for the inhibition of enzymatic hydrolysis of disaccharides

$$E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_2} EP_I + P_{II} \quad \text{(A)}$$

$$EP_I \underset{k_{-3}}{\overset{k_3}{\longleftrightarrow}} E + P_I \quad \text{(B)}$$

$$EP_I + P_{II} \underset{k_{-i}}{\overset{k_i}{\longleftrightarrow}} EI \underset{k_{ii}}{\overset{k_{-ii}}{\longleftrightarrow}} E + P_I + P_{II} \quad \text{(C)}$$

In the Scheme 5 the stage (A) is the initial reaction, according to Michaelis-Menten kinetic.

The stage (B) represented the hydrolysis process and the stage (C) represented the inhibition process.

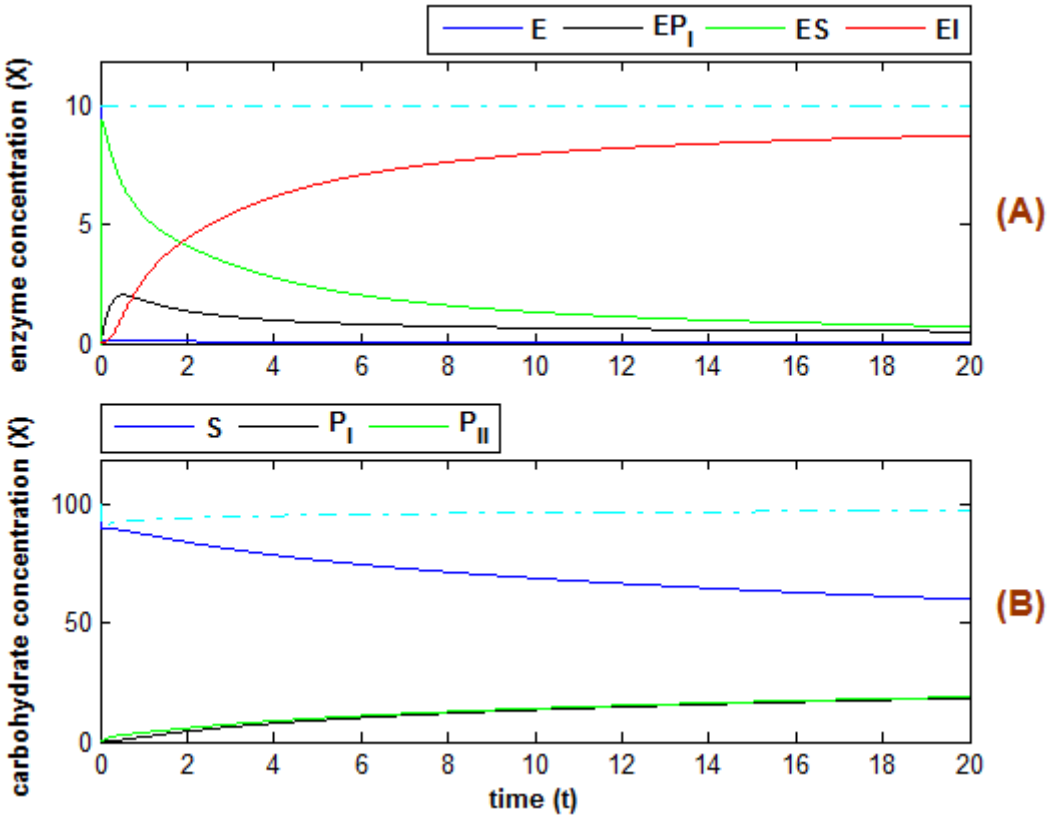
The proposed model assumes the mechanism of inhibition by the product to express the catalysis stage, asserting that inhibition effect occurs on the reaction complex (*ES*) forming a complex of inhibition (*EI*).

The complex of inhibition is related to the free enzyme (*E*), with enzyme-product complex (EP_I) and with the hydrolysis products (P_I and P_{II}), respecting the stoichiometry of the reaction.

This is a competitive type of inhibition, by both reaction products and uncompetitive inhibition by reaction product that has no affinity to form a complex with the enzyme (in this case P_{II}).

Thus, the model designs a nonlinear system with seven (7) ordinary differential equations and nine (9) kinetic constants. Figure 5 shows the numerical results for the generic process.

Figure 5 Response of the generic process for the enzymatic hydrolysis of disaccharides



$$\frac{dE}{dt} = k_{-1}ES - k_1(E \cdot S) + k_3 EP_I - k_{-3}(E \cdot P_I) + k_{-ii}EI - k_{ii}\left(E \cdot P_I \cdot P_{II}\right) \quad (29)$$

$$\frac{dEP_I}{dt} = k_2 ES + k_{-3}(E \cdot P_I) - k_3 EP_I + k_{-i}EI - k_i(EP_I \cdot P_{II}) \quad (30)$$

$$\frac{dES}{dt} = k_1(E \cdot S) - k_{-1}ES - k_2ES \tag{31}$$

$$\frac{dEI}{dt} = k_{ii}(E \cdot P_I \cdot P_{II}) - k_{-ii}EI + k_i(EP_I \cdot P_{II}) - k_iEI \tag{32}$$

$$\frac{dS}{dt} = k_{-1}ES - k_1(E \cdot S) \tag{33}$$

$$\frac{dP_I}{dt} = k_3EP_I - k_{-3}(E \cdot P_I) + 0{,}5 \cdot k_{-ii}EI - 0{,}5 \cdot k_{ii}(E \cdot P_I \cdot P_{II}) \tag{34}$$

$$\frac{dP_{II}}{dt} = k_2ES + 0{,}5 \cdot k_{-ii}EI - 0{,}5 \cdot k_{ii}(E \cdot P_I \cdot P_{II}) + k_{-i}EI - k_i(EP_I \cdot P_{II}) \tag{35}$$

The Figure 5b presents the expected behavior for a reaction process in which a substrate is divided into two products of equal molecular mass.

At the beginning of the reaction there is a fluctuation, showing a concentration of P_{II} slightly higher than the P_I but throughout the process was observed a tendency of obtaining approximate concentrations of both products.

Figure 5a shows the trend of increasing complex of inhibition and the consequent reduction of the reaction complex, with an initial fluctuation of the enzyme-product complex.

This fluctuation on enzyme-product complex variation is associated, apparently, to the difference in the initial fluctuation of products concentration in the graph (Figure 5b).

IV. RESULTS AND DISCUSSION

The conservation of mass of the model (*CMM*) - throughout the process - was calculated according the following expression, where y_i is the value of each carbohydrate concentration overtime and V is the different carbohydrate in the system.

The value of the error - at the end of the process (E_f) - for not being linked to the enzyme substrate ratio (*ESR*) was calculated by comparing the numerical value of the initial substrate (S_0) with the conservation of mass at the endpoint of the process.

$$CMM = \sum_{i=1}^{V}(y_i); \quad E_f = S_0 - \sum_{i=1}^{V}(y_i(n+1)) \tag{36}$$

Figure 6 shows the result for the model proposal in this work only to substrate and products variation.

Figure 6 - Response for variation of substrate and products

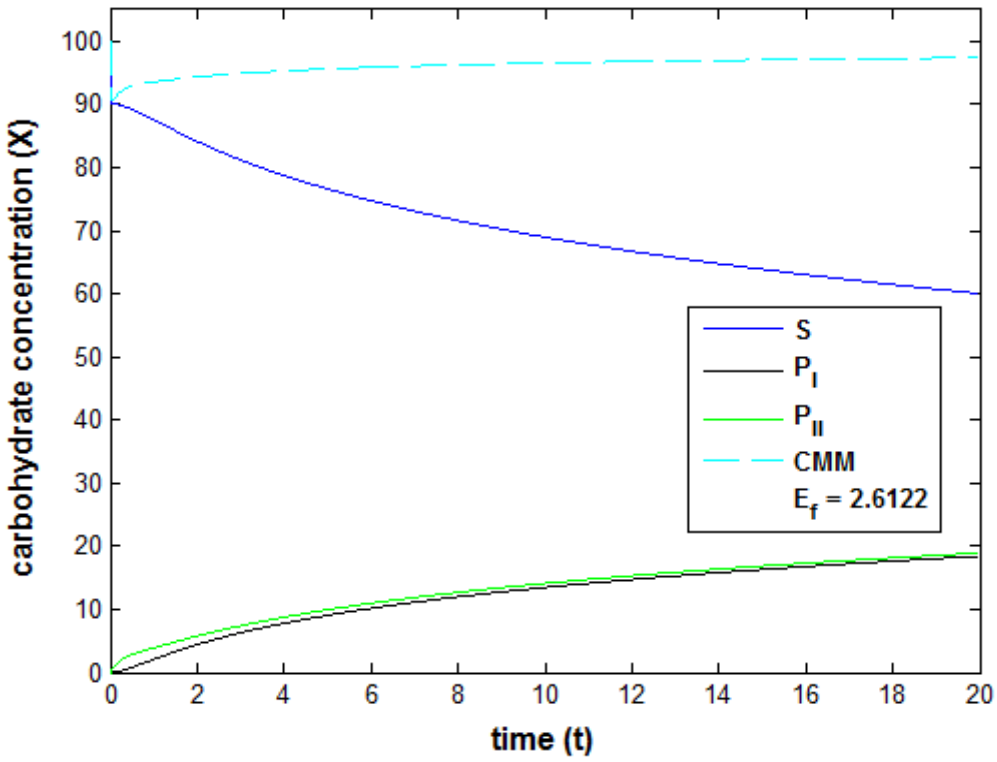


Figure 7 - Response of hydrolysis of disaccharides with increment in time

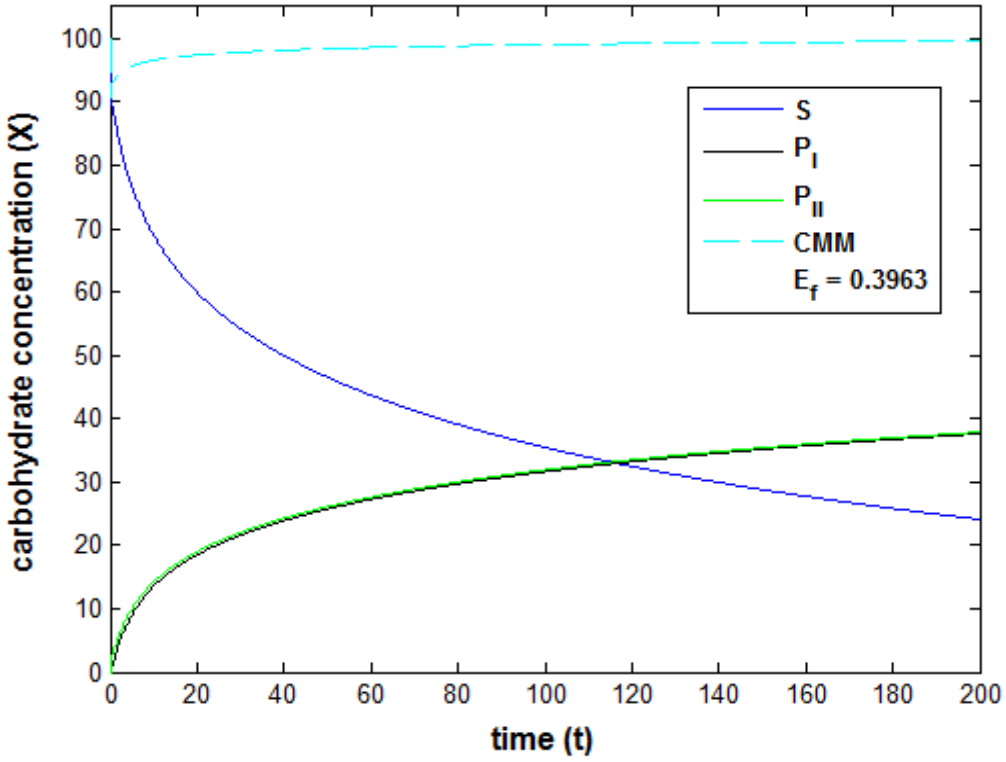


Figure 7 shows the result, for the model designed in this work, when the numerical value of the process time is increased ten times, maintaining other parameters equal to those in Figure 6.

Additionally, it is observed that the sum of carbohydrate, at the end of the process in Figure 7, have a significantly lower error compared to the results obtained in the error of Figure 6.

In order to check the error associated with the proposed model, and view the initial fluctuation in concentration of product, Figure 6 and Figure 7 shows the result only by changing the carbohydrate along the generic process, keeping the enzyme substrate ratio (ESR) equal to 0.1 (S = 100 and E = 10).

Thus, it is possible to observe that the error, calculated at the end of the process, was 2.6122 and 0.3963, respectively to Figure 6 and Figure 7, and therefore, not related to the percentage ratio of enzyme-substrate, such as the inhibition presented by Johnson [12].

In the graph of Figure 7, it is seen a trend for error decay overtime, which allows us to infer the existence of a set of numerical values for the kinetic constants, which provide an error close to zero in a given process time.

Thus, the situation indicates that the model, despite a proposal of product inhibition, differs from the behavior observed in Johnson [12], which was designed to represent the process of hydrolysis of sucrose disaccharides.

Figure 8 shows the numerical results for the generic process S = 100, E = 10 and t = 20, foreseeing the conservation of mass of carbohydrates at the end of process.

This response is shown the result with complete substrate conversion, keeping the same process time conditions and concentrations of substrate and enzyme shown in Figure 6. The numerical values of the kinetic constants, in Figure 8, were k_1 = 165, k_2 = k_{-1} = 70, other constants with numerical values equal to ten (10).

The response, in Figure 8, shows - for the reaction products, the theoretical behavior expected for such enzymatic processes, with first order catalysis - kinetics in the beginning of the process, and at the end of the process, zero-order, i.e., it can be described (the reaction velocity) (v) as:

$$v = -\frac{dS}{dt} = \frac{dP}{dt} \quad \rightarrow v = -\frac{dS}{dt} = \frac{dP_I}{dt} + \frac{dP_{II}}{dt} \tag{37}$$

Thus, it can be described as follow:

$$-\left[k_{-1}ES - k_1(E \cdot S)\right] = k_3 EP_I - k_{-3}(E \cdot P_I) + k_2 ES + k_{-ii} EI - k_{ii}(E \cdot P_I \cdot P_{II}) + k_{-i} EI - k_i(EP_I \cdot P_{II})$$

$$ES = \frac{1}{k_{-1} + k_2}\left[k_1(E \cdot S) + k_{-3}(E \cdot P_I) - k_3 EP_I + k_i(EP_I \cdot P_{II}) + k_{ii}(E \cdot P_I \cdot P_{II}) - (k_{-i} + k_{-ii})EI\right] \tag{38}$$

The Michaelis-Menten constant (k_M), widely known, was applied to the equation *ES*:

$$k_M = \frac{k_{-1} + k_2}{k_1} \tag{39}$$

$$ES = \frac{(E \cdot S)}{k_M} + \frac{1}{k_{-1} + k_2}\left[k_{-3}(E \cdot P_I) - k_3 EP_I + k_i(EP_I \cdot P_{II}) + k_{ii}(E \cdot P_I \cdot P_{II}) - (k_{-i} + k_{-ii})EI\right] \tag{40}$$

Hereafter, is postulated the existence of one kinetic constant for hydrolysis (k_H) in step, assuming equal numerical values for the rate of this process stage, and also a single constant in the inhibition stage (k_I) assuming equal constant numerical values for the effect of inhibition as follows:

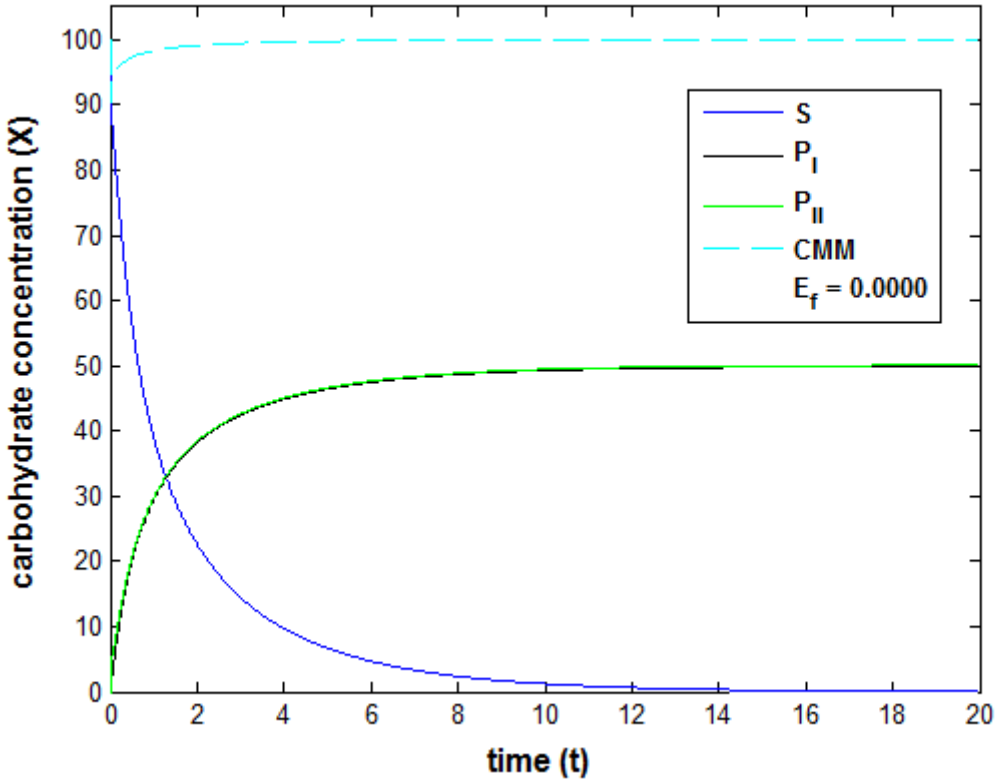
$$k_3 = k_{-3} = k_h \quad \rightarrow \quad k_H = \frac{k_{-1} + k_2}{k_h} \tag{41}$$

$$k_i = k_{-i} = k_{ii} = k_{-ii} = k_{in} \quad \rightarrow \quad k_I = \frac{k_{-1} + k_2}{k_{in}} \tag{42}$$

By applying the expressions of the hydrolysis and inhibition constant, in the equation representing *ES*, we have:

$$ES = \frac{(E \cdot S)}{k_M} + \frac{\left[(E \cdot P_I) - EP_I\right]}{k_H} + \frac{\left[(EP_I \cdot P_{II}) + (E \cdot P_I \cdot P_{II}) - 2EI\right]}{k_I} \tag{43}$$

Figure 8 - Response for hydrolysis of disaccharides with full conversion



Finally, it was assessed if the model is sufficiently robust to the situation in which both enzyme substrate ratio (ESR) and process time are modified.

Thus, the example of enzymatic hydrolysis of sucrose by invertase, originally studied by Michaelis and Menten is considered. Scheme 6 shows the model for the reaction shown in Figure 9, assuming that there is affinity of the enzyme to form a complex with glucose exclusively.

Figure 9 shows the response to the process condition in which the numerical value of the substrate concentration was equal to 50 and the numerical value of the concentration of the enzyme was equal to 0.42. The numerical value of the process time was equal to 330.

Scheme 6 - Proposed model for the inhibition of enzymatic hydrolysis of sucrose

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} EG + F \qquad \text{(Initial stage of reaction)}$$

$$EG \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E + G \qquad \text{(Hydrolysis stage)}$$

$$EG + F \underset{k_{-i}}{\overset{k_i}{\rightleftarrows}} EI \underset{k_{ii}}{\overset{k_{-ii}}{\rightleftarrows}} E + G + F \qquad \text{(Inhibition stage)}$$

Figure 9 - Response of enzymatic hydrolysis of sucrose

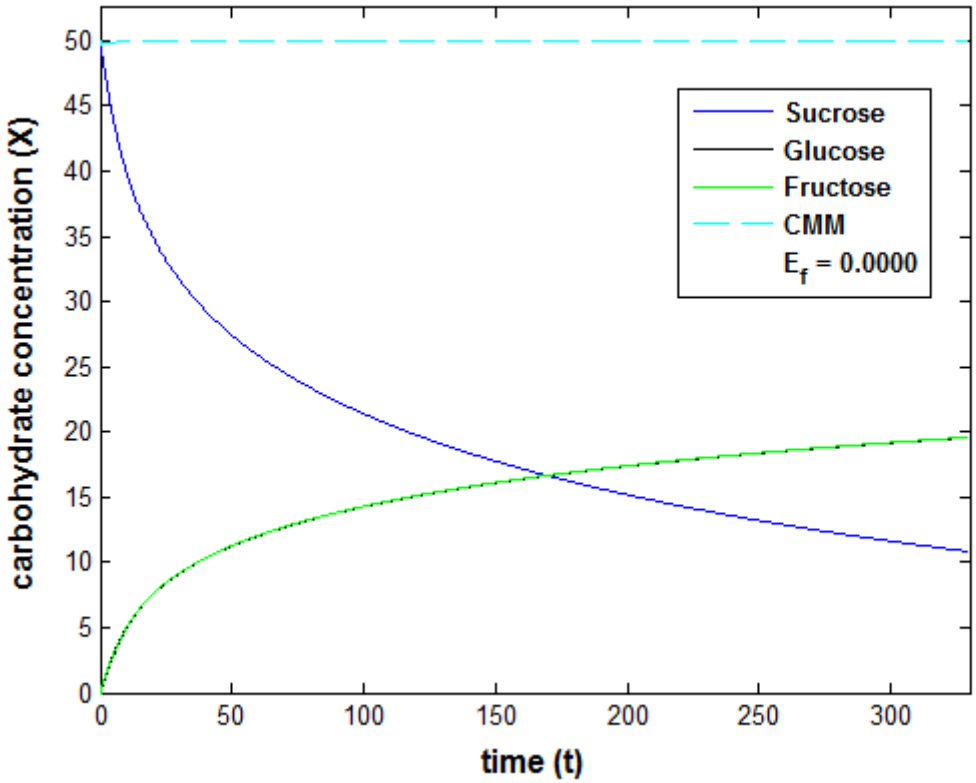


Figure 9 shows the response to the process condition in which the numerical value of the substrate concentration was equal to 50 and the numerical value of the concentration of the enzyme was equal to 0.42.

The numerical value of the process time was equal to 330. In this model, the inhibition effect occurs from the increased concentrations of the products, competitive inhibition for glucose and fructose and uncompetitive inhibition for fructose.

The enzyme substrate ratio (*ESR*) was equal to 0.0084, considering that, in previous ratios, was 0.1.

Numerical values of the kinetic constants, for the adjustment shown in Figure 9 are: k_1 = 5.50; k_{-1} = 5.32; k_2 = 3.87; $k_3 = k_{-3}$ = 1.06; and $k_i = k_{-i} = k_{ii} = k_{-ii}$ = 50.

The response on Figure 9 shows that reducing the amount of enzyme-substrate ratio (ESR) it tends to minimize fluctuations previously observed at the initial stage of products reaction, as regards the behavior of the model in this model.

For Scheme 6, the equation representing variation of the complex reaction is shown as:

$$ES = \frac{(E \cdot S)}{k_M} + \frac{\left[(E \cdot G) - EG\right]}{k_H} + \frac{\left[(EG \cdot F) + (E \cdot G \cdot F) - 2EI\right]}{k_I} \qquad (44)$$

Also, numerical values for the adjustment shown in Figure 9, in relation to the kinetic constants of hydrolysis of inhibition by Michaelis-Menten were: $k_M = 1.67$; $k_H = 8.67$ and $k_I = 0.18$.

V. CONCLUSION

In this work, the inhibition stage of enzymatic reaction, represented by Michaelis-Menten kinetics, had its behavior described, by applying the proposed inhibition - increasing the concentration of the product in numerical solution of a generic process.

The new model was applied on the enzymatic process that generated two (2) reaction products, both with equal molecular mass - in the case of hydrolysis of disaccharides.

Unlike the current models which present inhibition by product, and provide an error linked to the percentage of the enzyme-substrate fraction; the aim of this work, combining competitive and uncompetitive inhibition, was allow to reach a result that minimizes the percentage error, and could provide conservation of mass to the sum of the substrate and products - after proper adjustment of the numerical values of the kinetic constants. Moreover, the model was robust concerning the variation ratio of the enzyme-substrate, also during the time process variation.

Concluding, application of this approach - inhibition effect in the construction of new models - should be differentiated and applied to each specific situation, especially in cases of enzymatic catalysis that generate different reaction products.

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