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Mathematical Model for the Conversion of Lactose and Synthesis of Galacto-Oligosaccharides (GOS) with Simultaneous Reversible Inhibition by Glucose and Galactose

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***Abstract* — This study evaluated mathematical models for the conversion of lactose and synthesis of galacto-oligosaccharides (GOS) from the β-galactosidase enzyme by comparing proposals found in the literature that presented solutions without enzyme inhibition and those with reversible inhibition by both galactose and by glucose, to develop and propose a new model based on Michaelis-Menten kinetics. Comparisons were made of generic processes in which the numerical value of the substrate concentration was ten (10) times greater than the numerical value of the enzyme concentration and the kinetic constants were, initially, attributed a single numerical unit value. Thereafter, situations in which there were altered kinetic rates and variation in the numerical values of the enzyme concentration were evaluated. The proposed new model represents a reversible inhibition process in which glucose and galactose act as inhibitory substances. The performance of the proposed general inhibition mechanism was compared to models available in the literature. The results indicated the performance of the new kinetic model was robust. The model obtained was compared to experimental results and provided R^2 values between 0.9085 and 0.9926. Upon completion of the process, the experimental values were adjusted in order to provide for conservation of mass, with respect to the carbohydrates.**

Keywords—enzyme kinetics; lactose conversion; mathematical modeling; galacto-oligosaccharides synthesis.

I. INTRODUCTION

In the mathematical modeling of lactose conversion, in a β-galactosidase enzyme-based process of hydrolysis and transgalactosylation, Michaelis-Menten kinetics were adopted. The latter describe the enzymatic reaction rate in the condition where the substrate concentration is higher than the enzyme concentration and the sum of the concentrations of enzyme and enzymatic complexes formed remain constant throughout the processing time [1]-[4].

In most studies that present mathematical models of lactose conversion and galacto-oligosaccharides (GOS) synthesis, the inhibition step is intentionally omitted for the solution of the model. Some authors show the inhibition step in their models, but exclude this step when seeking the solution of ordinary differential equations, arguing that the inhibition stage can be ignored [1]-[5].

The classical Michaelis-Menten model does not contemplate inhibition, even though the vast majority of enzymatic processes do not provide 100% conversion of the substrate, indicating that there is a point at which, for a given condition, the process is interrupted [6].

As a rule, the studies in the literature deal with the resolution of the inhibition step by assuming a steady or 'quasi-stationary' state suggesting an assumed balance between the formation and dissociation of the complex formed by the enzyme and the substrate [7]-[10].

The solutions to the general problem of steady-state enzyme kinetics have been of limited use to researchers, given that the models generated do not adequately fit the experimental observations. Inhibitors are typically characterized based on an assumed equilibrium, in the steady state, between the reactive enzymatic complex and the substrate. According to Fange et al.[11], such an assumption would only be valid for very inefficient enzymes.

This paper proposes to evaluate the different possibilities of reversible inhibition in the process of lactose hydrolysis and transgalactosylation of GOS, from the perspective established in various models available in the literature, and seeks to suggest a new model applicable for the transient state. To achieve that goal, a pictorial process was selected where the numerical value of the substrate concentration is greater than ten (10) times the enzyme concentration. Kinetic constants in this generic process initially assumed the unit value.

The numerical solution of the nonlinear systems of ordinary differential equations (ODEs) was obtained based on a routine developed in Matlab®, using the 4th order Runge-Kutta method.

The responses are presented in figures with two distinct graphs that separately show the variation of the enzyme and enzymatic complexes and the variation of the substrate and products.

II. REVIEW OF THE MODELS IN THE LITERATURE

In the specific case of the conversion of lactose and transgalactosylation of GOS, the reference work in the literature is that of Kim et al. [2], which proposes a kinetic model without inhibition. In this proposal, *E* represents the enzyme concentration, *GOS* represents the concentration of galacto-oligosaccharides, *L* the lactose concentration, *Ga* the galactose concentration and *Gu* the glucose concentration. The kinetic constants are: k_1, k_{-1}, k_2, k_3, k_{-3}, k_4 and k_{-4}.

The enzyme complexes are represented by *EL*, the substrate enzyme complex and by *EGa*, the galactose enzyme complex.

The great ease in solution of the Michaelis-Menten system, as proposed by Kim et al. [2], shown in Scheme 1, can be explained by the absence of an inhibition step. This suggests all the substrate is converted to product in a given time, provided it fulfills the basic premise of the model, namely that the enzyme concentration is lower than the concentration of the substrate.

Scheme 1 – Model for lactose conversion without inhibition

$$E + L \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EL \xrightarrow{k_2} EGa + Gu \qquad \text{(initial reaction step)}$$

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

$$EGa + L \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} E + GOS \qquad \text{(GOS formation step)}$$

Source: [2]

Figure 1 shows the numerical solution to the generic process, with the substrate and enzyme concentrations having a hypothetical unit (X), which represents a relationship between mass and volume, and the process time having a hypothetical unit (t).

Figure 1 – Response according to the model from [2]

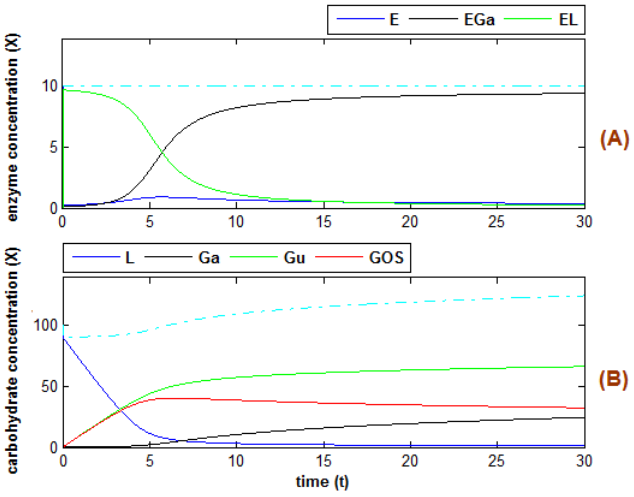


Figure 1 shows two graphs representing the solution of the representative ODEs in the model from Kim et al. [2]. The graph in Figure 1-A shows the variation of the enzyme and enzyme complexes, and, the dotted line, the mass conservation for these variables. The graph in Figure 1-B shows the variation of the substrate and products, and the dotted line indicates the sum of the contribution of these variables over time.

In Figure 1-B, the numerical value of glucose can be seen to quickly exceed half the initial value of the substrate, and the dotted line indicates that there is no mass conservation for the sum of the substrate and products throughout the process.

It is important to note in that Figure 1-A the dotted line constantly remains at the nominal value of the added enzyme, which is consistent with the Law of Conservation of Mass, namely:

$$\frac{dE}{dt} + \frac{dEL}{dt} + \frac{dEGa}{dt} = \frac{d}{dt}(E + EL + EGa) = 0 \qquad (1)$$

In their work, Rodríguez-Fernández et al. [5] developed a model for GOS synthesis from lactulose with inhibition by galactose.

Scheme 2 shows the model adapted to the situation in which the substrate is lactose.

Scheme 2 – Model with inhibition by galactose

$$E + L \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EL \xrightarrow{k_2} EGa + Gu \qquad \text{(initial reaction step)}$$

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

$$EGa + Ga \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} E + DI \qquad \text{(DI formation step)}$$

$$EGa + L \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} E + TRI \qquad \text{(TRI formation step)}$$

$$E + Ga \underset{k_{-i}}{\overset{k_i}{\rightleftarrows}} EI \qquad \text{(inhibition step)}$$

Source: adapted from [5]

The only difference in relation to the model from Rodríguez-Fernández et al. [5] is the inclusion of glucose as a product of hydrolysis of the substrate instead of fructose.

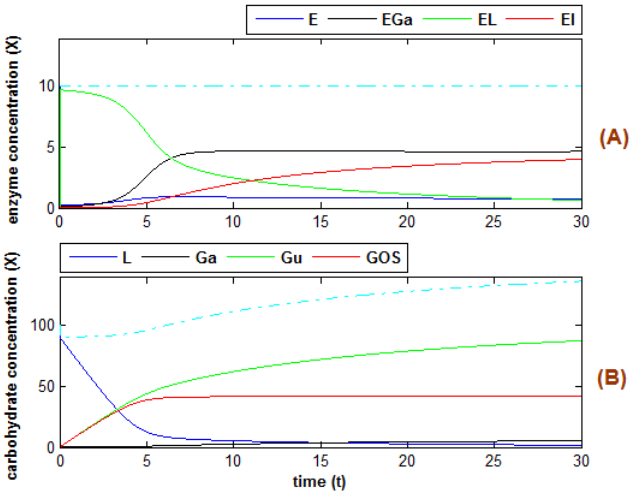
The *DI* compound represents a galactosyl-galactose disaccharide. The *TRI* compound represents a trisaccharide, formed by lactose transgalactosylation and *EI* represents the inhibition complex. The concentration of galacto-oligosaccharides is represented, in this model, as the sum of disaccharides (*DI*) and trisaccharides (*TRI*).

Figure 2 shows the numerical solution of the generic process. Note that there is a trend towards the continuous increase of glucose, shown in the graph of Figure 2-B. In this model, at the limit, glucose would steadily increase until

it reaches the nominal value of the substrate concentration at the start of the reaction.

In the solution presented in the work of Rodríguez-Fernández et al. [5], the authors considered the inhibition negligible when comparing the proposed model to the experimental results.

Figure 2 – Response according to the model adapted from [5]



More recently, Palai et al. [12] proposed a new model with inhibition by glucose, as shown in Scheme 3.

Scheme 3 – Model with inhibition by glucose

$$E + L \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} EL \xrightarrow{k_2} EGa + Gu \qquad \text{(initial reaction step)}$$

$$EGa \underset{k_{-3}}{\overset{k_3}{\longleftrightarrow}} E + Ga \qquad \text{(hydrolysis step)}$$

$$EGa + L \underset{k_{-4}}{\overset{k_4}{\longleftrightarrow}} E + GOS \qquad \text{(GOS formation step)}$$

$$E + Gu \underset{k_{-i}}{\overset{k_i}{\longleftrightarrow}} EI \qquad \text{(inhibition step)}$$

Source: [12]

Figure 3 – Response according to the model from [12]

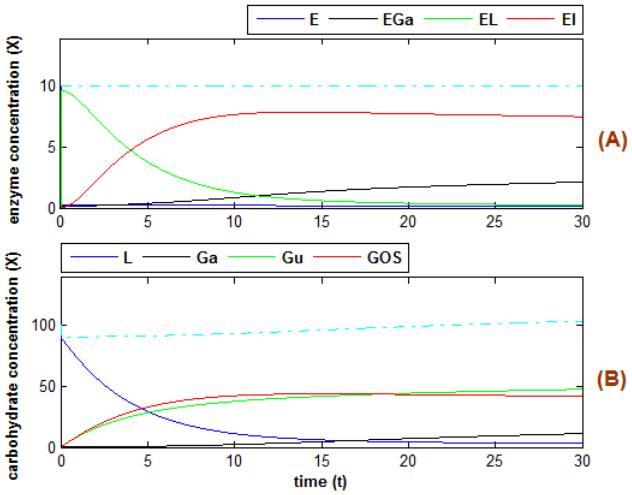


Figure 3 shows the numerical solution of the proposed pictorial process. In Figure 3-A the *EI* complex can be seen to grow, and at the end of processing, its numerical value decreases, whereas *EGa* grows at the end of the process and *EL* continuously decreases.

Figure 3-B shows an initial tendency towards GOS formation and, after a certain time, hydrolysis of the galacto-oligosaccharides. The formation effect and subsequent hydrolysis of GOS is cited from experimental observations in the literature [13]-[14].

Figure 3-A indicates that the inhibition complex grows up to a certain point, apparently associated with the *GOS* growth curve, after which point, the *EGa* complex can be seen to grow while the *EI* complex shrinks.

III. PROPOSITION OF A NEW MODEL

Prior to developing the new model to express lactose conversion and GOS synthesis, based on Michaelis-Menten kinetics, it was necessary to establish some assumptions:

- ✓ The model should strictly conform to the Law of Mass Conservation as regards the variation of the sum of the enzyme and its complexes continuously throughout the processing time.
- ✓ The model should converge the sum of carbohydrates to the nominal value of the substrate added, within a sufficiently long period of time and with the smallest possible error, to ensure the stability of the process.
- ✓ The model should be sufficiently robust to allow variation in the enzyme-substrate relation over sufficient time to ensure the stability of the process.
- ✓ The model should provide a similar reaction complex performance to that found in Michaelis-Menten kinetics, with a high formation rate at the beginning of the process and a reduction in concentration over time, tending towards zero at the end of process.
- ✓ The model should provide an initial enzymatic-complex-inhibition formation with a value of zero, with growth over time and tending to the initial value of the enzyme towards the end of the process.

The proposed model assumes that the inhibition occurs as an effect of both the increases in the concentration of glucose and the increased concentration of galactose.

The model proposed in this paper assumes the inhibition mechanism by the product to express the final step of the catalysis, postulating that the inhibitory effect occurs in the reaction complex (*EL*) forming an inhibition complex (*EI*).

In the inhibition step, the model has two (2) effects: a competitive-type inhibitory effect, where the galactose (*Ga*) and glucose (*Gu*) products compete for the active site of the enzyme (*E*); and by contrast, a non-competitive-type inhibitory effect, where glucose (*Gu*) induces inhibition of the galactose enzyme complex (*EGa*). There was a generic expression for the trisaccharides (*TRI*), which may be formed either by the transgalactosylation of lactose (*L*) or of galactosyl galactose (*DI*).

The expression of the reversible inhibition step follows the model suggested by Martins and Oliveira [15].

Scheme 4 – Model with inhibition by glucose and galactose

$$E + L \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} EL \xrightarrow{k_2} EGa + Gu \qquad \text{(initial reaction step)}$$

$$EGa \underset{k_{-3}}{\overset{k_3}{\longleftrightarrow}} E + Ga \qquad \text{(hydrolysis step)}$$

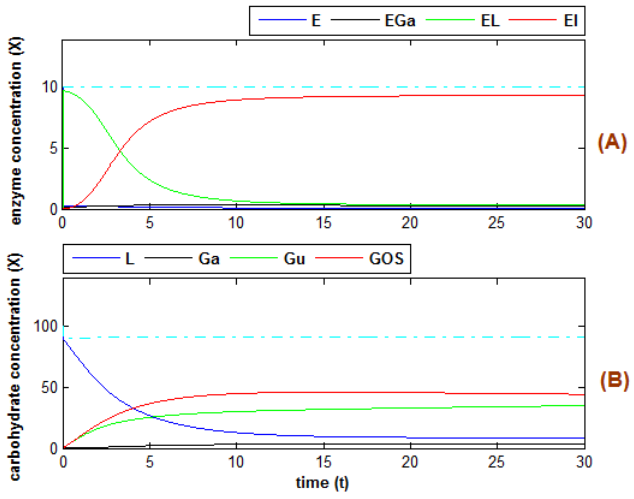
$$EGa + Ga \underset{k_{-4}}{\overset{k_4}{\longleftrightarrow}} E + DI \qquad \text{(DI formation step)}$$

$$EGa + DI \underset{k_{-5}}{\overset{k_5}{\longleftrightarrow}} E + TRI \underset{k_6}{\overset{k_{-6}}{\longleftrightarrow}} EGa + L \qquad \text{(TRI formation step)}$$

$$EGa + Gu \underset{k_{-i}}{\overset{k_i}{\longleftrightarrow}} EI \underset{k_{ii}}{\overset{k_{-ii}}{\longleftrightarrow}} E + Ga + Gu \qquad \text{(inhibition step)}$$

Figure 4 shows the result of the numerical solution of the present study model with the same conditions as above.

Figure 4 – Response according the model proposed herein



IV. RESULTS AND DISCUSSION

The nonlinear system that models the enzymatic reaction proposed in this paper is highly complex. There are nine (9) ordinary differential equations (ODEs) and fifteen (15) kinetic constants.

The graph in Figure 4-A shows the variation of the enzyme and enzyme complexes, with the *EI* tending to grow until reaching the nominal value of the initial substrate and the *EL* tending to shrink, approaching zero, while maintaining the other components very close to zero.

The model strictly conforms to the Law of Mass Conservation for the enzyme and enzyme complexes, i.e.:

$$\frac{dE}{dt} + \frac{dEL}{dt} + \frac{dEGa}{dt} + \frac{dEI}{dt} = \frac{d}{dt}(E + EL + EGa + EI) = 0 \qquad (2)$$

It can also be seen that, in the graph shown in Figure 4-A, both the free enzyme (*E*) and the enzyme galactose complex (*EGa*) have variations close to zero throughout the process that is:

$$\frac{dE}{dt} \cong 0 \quad \frac{dEGa}{dt} \cong 0 \qquad (3)$$

This situation indicates that for the model presented in this paper, the increase in the inhibitory complex (*EI*) is, throughout the processing time, approximately equal to the decrease in the reaction complex (*EL*), which is typical for a product inhibitory process [16], that is:

$$\frac{dEI}{dt} \cong -\frac{dEL}{dt} \qquad (4)$$

The conservation of mass of the model (*CMM*) and the percentage of error upon completion of the process (E_f *(%)*) were calculated using the following expressions, where L_0 is the initial concentration of the substrate, y_i represents the concentration values of each variable over time and y_i *(n+1)* represents the value of the concentration of each variable upon completion of process.

$$CMM = \sum_{i=1}^{V} (y_i) \qquad (5)$$

$$E_f(\%) = \left[1 - \frac{\sum_{i=1}^{V} (y_i(n+1))}{L_0}\right] * 100 \qquad (6)$$

Finally, the model was evaluated by comparing with experimental results. For the goodness-of-fit of the model, the square root of the sum of the errors is defined (*RMSE*), where y_i, *exp (t)* are the experimental results in time and y_i *(t, n)* are the results of the model for the carbohydrates in this process, as in equation below [17]-[18].

$$RMSE = \sqrt{\frac{\sum_{i=1}^{V}\sum_{t=1}^{M} (y_{i,\exp}(t) - y_i(t,n))^2}{n+1}} \qquad (7)$$

In the study by Lisboa [19], the commercial enzyme Lactozym® 3000L (Novozymes, Denmark) was used in a galacto-oligosaccharides synthesis process, with lactose as substrate.

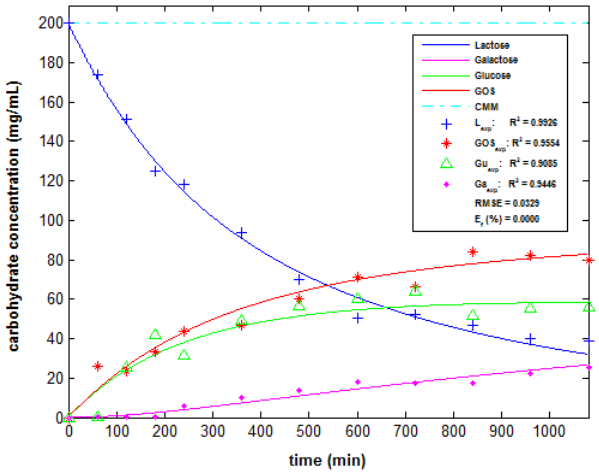
To begin, 125 mL of lactose solution in phosphate buffer, with an enzyme concentration of 10 U/mL was used in a process carried out at a temperature of 30°C. The initial substrate concentration was of 200 and 400 mg/mL.

In order to apply the initial conditions of the process to the mathematical model in this paper, the enzymatic activity was converted to mass enzyme concentration, based on the composition of the enzymatic extract, corresponding to 0.112 mg/mL.

Figure 5 shows the response of condition 01, where the initial substrate concentration was 200 mg/mL and the total processing time was 1.080 min with an enzyme concentration of 0.112 mg/mL.

In the response in Figure 5, the conservation of mass of model (*CMM*) is maintained throughout the processing, indicating the model is robust.

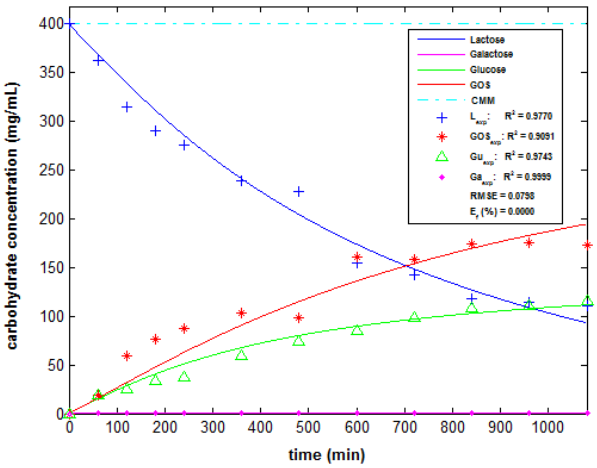
Figure 5 – Result of the model for the goodness-of-fit to the experimental values (condition 01)



The R^2 values for the substrate and products, in Figure 5, are greater than 90% indicating a relevant correlation between the experimental results and the model's response.

Figure 6 shows the response of condition 02, with 400 mg/mL of lactose at the start of the reaction and a total processing time of 1.080 min. Table 1 shows the values of the kinetic constants for the goodness-of-fit to the experimental conditions in both processes.

Figure 6 – Result of the model for the goodness-of-fit to the experimental values (condition 02)



The goodness-of-fit obtained for condition 01 (Figure 5) showed a better approximation between the model and experimental values when compared with condition 02 (Figure 6), as shown by the *RMSE* results being equal to 0.0329 and 0.0798 respectively.

The closer the value of the square root of the sum of the errors (*RMSE*) is to zero, the better the goodness-of-fit of the model to the experimental data will be.

In Figure 5, the best goodness-of-fits to the experimental values can be seen for lactose and GOS, with R^2 equal to 0.9926 and 0.9554, respectively. By contrast, Figure 6 showed the best goodness-of-fits to the experimental values of glucose and galactose with R^2 equal to 0.9743 and 0.9799, respectively.

With regard to the numerical values of the kinetic constants obtained following the adjustment to the experimental results found this study and shown in Table 1, there was a variation of between 1.10^{-3} and 3.043, which indicates a significantly smaller interval than those observed in other studies in the literature.

Table 1. Values of the kinetic constants for adjustment to the experimental conditions

Kinetic constant	Condition 01	Condition 02
k_1 (mL.min.mg^{-1})	1.317	0.462
k_{-1} (min^{-1})	0.552	0.801
k_2 (mL.min.mg^{-1})	2.329	2.309
k_3 (mL.min.mg^{-1})	0.069	0.700
k_{-3} (min^{-1})	0.001	1.254
k_4 (mL.min.mg^{-1})	0.001	0.931
k_{-4} (min^{-1})	0.001	0.502
k_5 (mL.min.mg^{-1})	0.008	0.539
k_{-5} (min^{-1})	1.769	0.991
k_6 (mL.min.mg^{-1})	0.744	1.593
k_{-6} (min^{-1})	0.898	0.001
k_7 (mL.min.mg^{-1})	1.715	1.002
k_{-7} (min^{-1})	1.010	1.016
k_{11} (mL.min.mg^{-1})	0.058	3.043
k_{-11} (min^{-1})	1.034	0.589

This is the case regarding the study from Palai and Bhattacharya [20], which began with an initial substrate concentration of 100 to 150 g/L and an initial enzyme concentration of 6 and 12 kU/L, respectively, when conducting lactose conversion evaluated at a reaction period of 30 h.

The kinetic constants in that study varied from numerical values in the range of $6.6.10^{-7}$ to $1.8.10^{3}$. The experimental results in the work of Palai and Bhattacharya [20] were obtained using commercial Biolacta® FN5 β-galactosidase, in a process carried out with an immobilized enzyme. Similarly in the present study a commercial enzyme, Lactozym® 3000L was also used in a free-medium process.

The study by Rodríguez-Fernández et al. [5] investigated the formation of GOS from lactulose using the same commercial enzyme used in the present study, in which the reaction was studied at a temperature of 40°C.

The values of the kinetic constants ranged between $8.3.10^{-1}$ and $1.2.10^{2}$, in a model where the inhibitory effect was not considered for the solution of nonlinear equation system.

In the study by Metelkin et al. [21], which analyzed the conversion of lactose from β-galactosidase obtained from *Escherichia coli*, the numerical values of the kinetic constants varied between 20 and 4.10^{4} in a model where the inhibition was neglected.

In this study, all the adjustments of the model to the experimental results were conducted to minimize the error at the end of the process and so that the sum of the values of substrate and products throughout the process would tend towards the numerical value of the initial substrate concentration. In the study by Rodriguez-Fernandez et al. [5], the model error at completion of the process was less than 10% in a procedure carried out using the same commercial enzyme used in this study.

V. CONCLUSION

The conversion of the lactose and the galacto-oligosaccharides (GOS) synthesis were evaluated based on the elaboration of a new mathematical model designed to represent the kinetics of the reaction in an enzymatic process with inhibition by glucose and galactose. The new proposed model was compared to models available in the literature without inhibition, with inhibition by galactose and with inhibition by glucose.

The performance of this new means of representing the enzyme kinetics occurring in lactose conversion and GOS synthesis was robust with regard to the model error as well as the variation in the enzyme/substrate ratio and the processing time.

The proposed model is represented by a system with nine (9) ordinary differential equations and with fifteen (15) kinetic constant that can assume different numerical values, which hinders the goodness-of-fit of the model to the experimental results. However, the adjustment of the model to the experimental data may provide for the conservation of mass of the substrate and product at the end of the processing time.

The goodness-of-fit of the model for lactose showed R^2 varying between 0.9770 and 0.9926. In the case of the GOS, the variation was between 0.9091 and 0.9554. For glucose it was between 0.9085 and 0.9743 and, finally, for galactose, the R^2 for the goodness-of-fit was between 0.9446 and 0.9799.

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