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Research article

Modeling of lactose enzymatic hydrolysis using Monte Carlo method

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ABSTRACT

Background: Mathematical modeling is useful in the analysis, prediction, and optimization of an enzymatic process. Unlike the conventional modeling methods, Monte Carlo method has special advantages in providing representations of the molecule's spatial distribution. However, thus far, Monte Carlo modeling of enzymatic system is namely based on unimolecular basis, not suitable for practical applications. In this research, Monte Carlo modeling is performed for enzymatic hydrolysis of lactose for the purpose of real-time applications.
Results: The enzyme hydrolysis of lactose, which is conformed to Michaelis–Menten kinetics, is modeled using the Monte Carlo modeling method, and the simulation results prove that the model predicts the reaction kinetics very well.
Conclusions: Monte Carlo modeling method can be used to model enzymatic reactions in a simple way for real-time applications.
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1. Introduction

Enzymatic reaction has many advantages and is important in biomanufacturing [1]. β-Galactosidase is widely used in food industry in removing lactose from milk product and in manufacturing galactose products [2]. Galactose produced from lactose hydrolysis can be used as the substrate for the biosynthesis of rare sugars, for example, tagatose. Application of immobilized β-galactosidase in lactose hydrolysis is cost-effective for repeated use of the enzyme. Bioprocess modeling is useful in process analysis, prediction, and optimization, which can save time, cost, and efforts in process development and optimization, and has made quite much successes in real-time applications [3,4,5,6,7]. In general, enzyme kinetics models can be categorized into three major groups (deterministic, empirical, and stochastic) according to the implemented methodology [7]. Among them, the stochastic Monte Carlo modeling method has special advantages in providing representations of the spatial distribution of the molecules during the reaction, thereby allowing direct imaging of the molecule repartition on the lattice of the reaction system [8]. Thus far, most of the reports on Monte Carlo modeling of the enzymatic system are based on unimolecular basis and used for modeling tiny scale, for example, inside a cell [9,10,11,12,13], or for mechanism study [14]. In this research, Monte Carlo modeling is performed for enzymatic lactose hydrolysis in a system consisting of immobilized β-galactosidase and substrate on a 3D lattice, and this model is simple and practical for predicting the time course of the enzyme reaction process.

2. Materials and methods

β-Galactosidase (10000 U/g) is purchased from Zhongnuo Biotechnology Development Jiangsu Co., Ltd., China. . Silica gel of 40–80 mesh is purchased from Qingdao Yonghai . Silica Gel Co., Ltd., China. The silica gel is aminated for β-galactosidase immobilization, which is carried out as follows. The silica gel is repeatedly washed using deionized water and immersed into an activation solution containing 0.8 mol/L NaOH:. dimethyl sulfoxide (DMSO):epoxy chloropropane in the ratio of 7:10:8, shaken in water bath at 40°C, 170 rpm for 2.5 h, and then washed using deionized water until pH neutral. The above-activated . silica gel is immersed into 17.5% ammonia solution, shaken in water bath at 30°C, 160 rpm overnight for amination. Then, the aminated . silica gel is washed using deionized water until pH neutral. β-Galactosidase immobilization is carried out as follows. Five

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grams of aminated . silica gel is added to 50 ml of 2% glutaraldehyde solution, shaken in water bath at 30°C, 160 rpm for 3 h, and washed repeatedly using deionized water; then, it is put into an enzyme solution containing 50 mL of pH 6.0 buffer and 50 mL of β-galactosidase solution, shaken at 30°C, 160 rpm overnight, washed using pH 6.0 buffer, and stored at 4°C for usage. Activity of the immobilized β-galactosidase is measured as reported previously [15]. One unit of enzyme activity is defined as the enzyme amount producing 1 μmol of glucose per minute in lactose hydrolysis. Enzyme activity recovery of the immobilization method is approximately 60%. Enzymatic lactose hydrolysis is performed in a flask containing lactose of 100 g/L and immobilized β-galactosidase (12 U/ml) at pH 5 in a water bath at 55°C, shaken at 160 rpm. Lactose hydrolysis is calculated from glucose production. Glucose concentration is measured using a 10 μm Carbomix Pb-NP column and HPLC equipped with a refractive index detector. Pure water is used as the mobile phase with a flow rate of 0.5 mL/min.

Python 2.0 is used in software programming, and the software program runs on an IBM-compatible personal computer with Windows 8.0. Differential equation is solved by using Runge-Kutta method in solving Michaelis–Menten model.

2.1. Mathematical modeling

The Monte Carlo algorithm is used in simulating the enzymatic lactose hydrolysis process. Lactose is hydrolyzed, thereby producing galactose and glucose as shown in [Equation 1].

$$S \xrightarrow[E]{k} P_1 + P_2 \quad (1)$$

where S indicates lactose, P_1 indicates galactose, P_2 indicates glucose, k indicates reaction rate, and E indicates enzyme.

A 3D lattice is made for simulating the reaction space, and the particles representing immobilized enzyme and substrates, respectively, are randomly dispersed in the 3D lattice. All the particles will move randomly to new vacant positions in the next time interval. When substrate particles enter into the neighboring enzyme particles, which are the six positions before, behind, left, right, up, and down the enzyme particles, they will have the probability of P_k for combination with the enzyme forming the enzyme–substrate complex and taking reaction afterwards. The maximum amount of substrate catalyzed in one time interval is limited by the maximum reaction rate r_{max}.

To decrease the computation task and make the model practical for modeling pilot-scale or large-scale bioreaction system, the substrate and enzyme particles are not based on unimolecular basis. In this case, the space of the 3D lattice is defined 1 L for simplification. One substrate particle in the 3D lattice represents one unit of substrate (equal to the initial substrate concentration/substrate particle number) other than one substrate molecule, and total particles of substrate in the 3D lattice is equal to the substrate concentration in the unit of grams per litter. Similarly, the number of enzyme particles in the 3D space is not necessarily equal to the number of immobilized enzyme particles, and total particles of enzyme in the 3D lattice is equal to the total enzyme activities added in one litter of the reaction broth.

Table 1
The parameter values used in the model.

Monte Carlo model		
N_1	200	(*0.5 g/L)
N_2	70	(*171.4 U)
L	30	(*1/3 cm)
r_{max}	2	(*0.5 g/L/min)
P_k	0.3	(−)
Michaelis–Menten model		
V_{max}	4.35	(g/L/min)
k_S	72.5	(g/L)

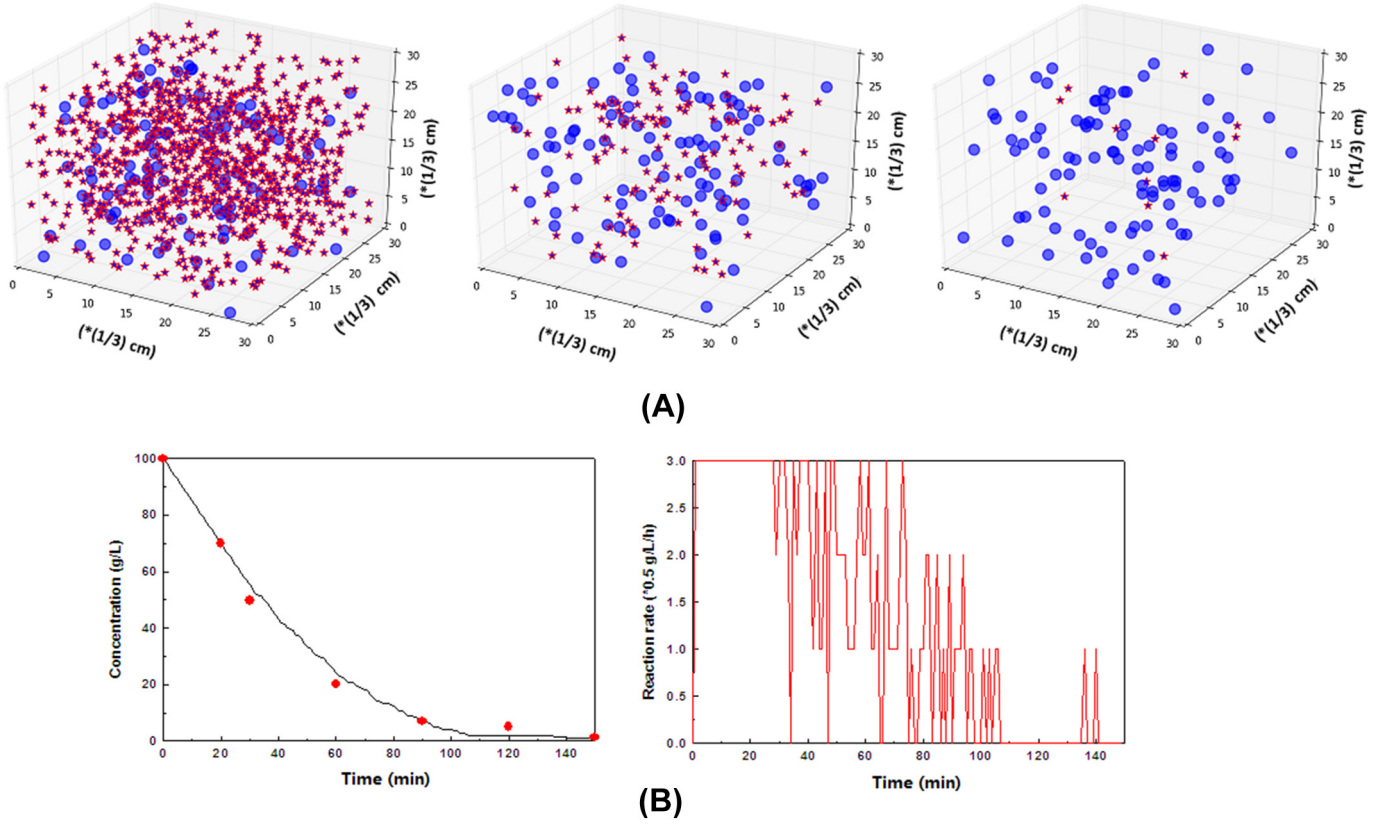

Fig. 1. Lactose hydrolysis process prediction using the Monte Carlo model shown by (A) 3D lattice and (B) time course of substrate concentration and reaction rate. A, Star, substrates; circle, enzyme; figures from the left to right are calculated at 3, 75, and 150 min, respectively, of the simulation.

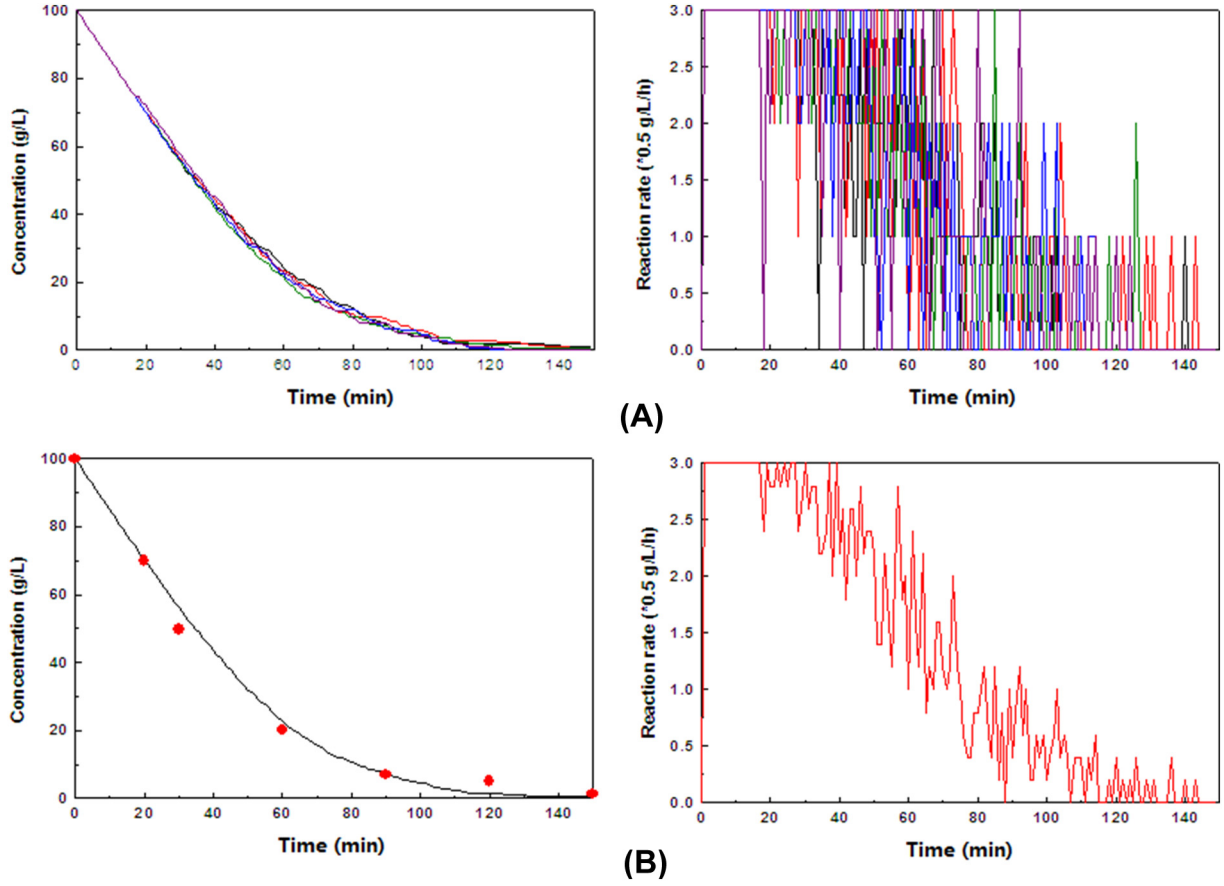

Fig. 2. Lactose hydrolysis process prediction using the Monte Carlo model with (A) five rounds of repeats, and (B) the average of the five repeats.

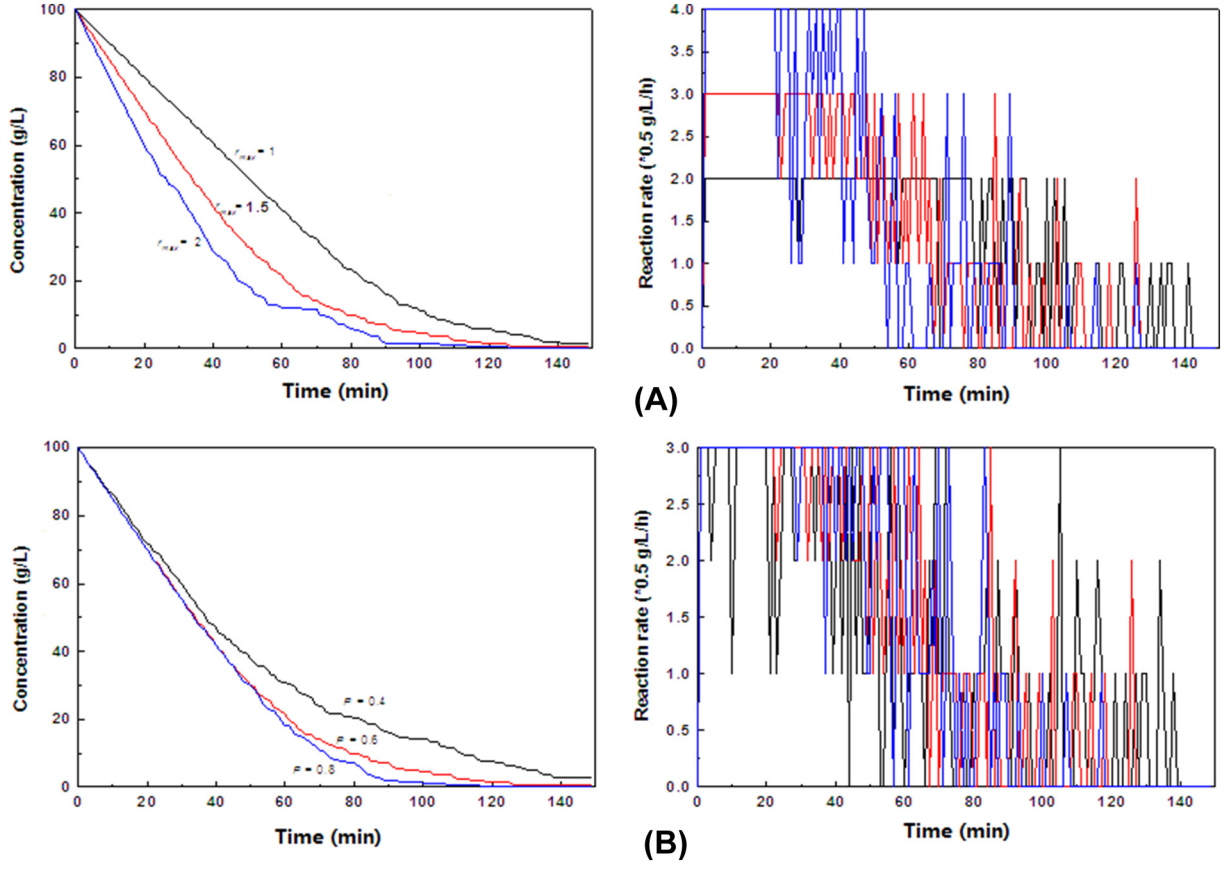

Fig. 3. Monte Carlo model predictions at (A) different reaction rates and (B) different substrate–enzyme combination probabilities.

3. Results and discussion

3.1. Modeling and simulation of lactose enzymatic hydrolysis using Monte Carlo method

The lactose hydrolysis process, which involves immobilized β-galactosidase, is modeled and simulated using the Monte Carlo modeling method, and the results are shown in Fig. 1. As shown in Fig. 1A, the substrate in the 3D lattice is decreasing with increase in reaction time. The Monte Carlo model predicts the lactose enzymatic hydrolysis process quite well as shown in Fig. 1B.

To get a good fit of the prediction of Monte Carlo model with the experimental data in the above simulation, the two model parameters of r_{max} and P_k are optimized by using genetic algorithm (GA), an optimization method imitating the biology evolution process invented by Holland [16,17], to minimize the error between the model prediction and the experimental data. In using GA, two spans around the initial values of r_{max} and P_k, respectively, are given. Then, GA will search the two parameter values within above two spans by minimizing the model prediction error. The initial value of r_{max} can be approximately obtained by the total enzyme activity added to the enzyme reaction system. The initial value of P_k is unknown; hence, a larger span from 0.4 to 1 is given ($0 \le P_k \le 1$). The GA optimization method is described in detail in the section after the next. The parameter values optimized by GA and the other predetermined parameter values of the Monte Carlo model are listed in Table 1.

The Monte Carlo model works in a random way. To show the repeatability of this method, five rounds of predictions are made, and the results are shown in Fig. 2. It shows that the five repeats do not variate quite much as shown in Fig. 2A, and the average of the five rounds fits perfectly with the aimed data shown in Fig. 2B. The fitting results indicate that the Monte Carlo modeling method is capable of modeling the enzymatic reaction process.

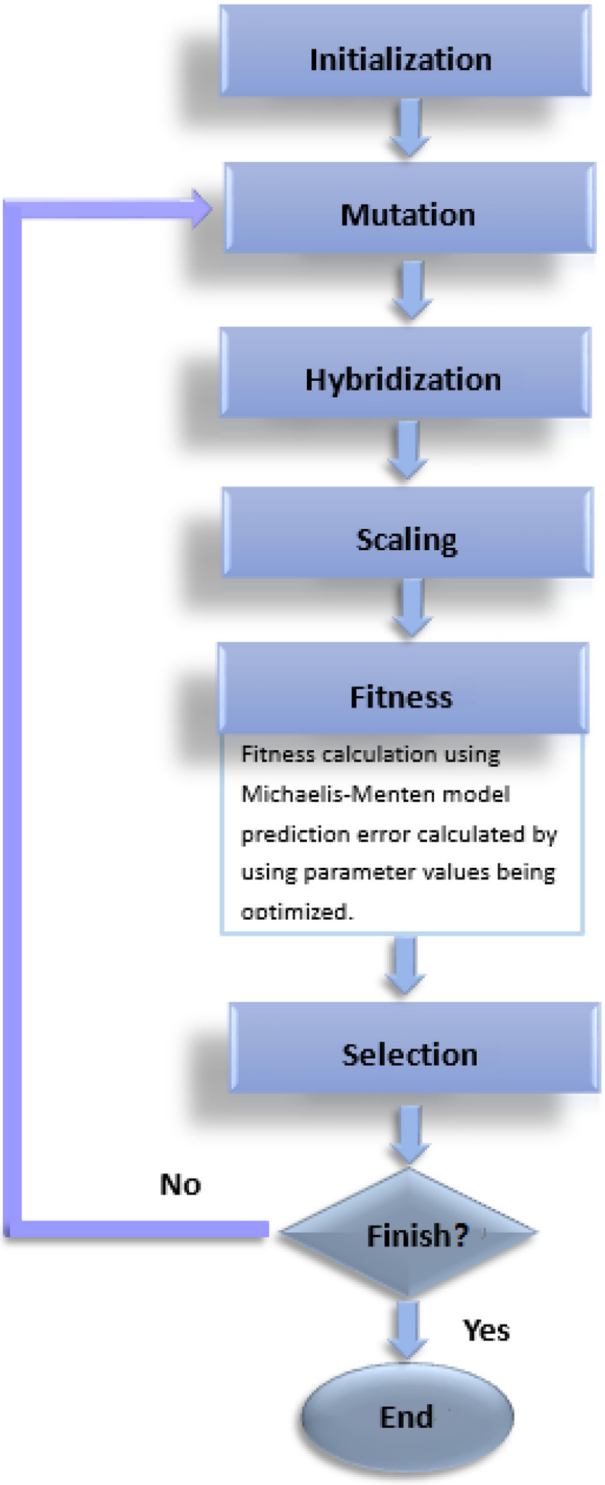

Fig. 4. Calculation diagram of genetic algorithm for model parameter optimization.

3.2. Effects of parameter values on Monte Carlo model

The effects of parameter values on Monte Carlo model are investigated. The effects on changing r_{max} are shown in Fig. 3A. r_{max} is similar to V_{max} of Michaelis–Menten equation but different in that r_{max} is easily reached in the Monte Carlo model, while V_{max} of Michaelis–Menten equation can only be reached when substrate concentration tends to the maximum value. r_{max} can be approximately calculated using [Equation 2].

$$r_{\max} = \frac{V_{\max} \cdot S_0}{k_S + S_0} \tag{2}$$

where V_{max} is the maximum enzyme reaction rate, S_0 is the initial substrate concentration, and k_s is the substrate affinity coefficient. The effects on changing P_r are shown in Fig. 3B. P_r is the probability of substrate binding with the enzyme, which is related to k_s in Michaelis–Menten equation, but in a reverse relation, the larger the P_r, the smaller is the k_s. The parameter of P_r is related to the nature of the enzyme, while the parameter of r_{max} is related to both the nature of the enzyme and the amount of the enzyme added.

In addition to the above two model parameters, the other parameters such as the number of substrate particles, N_1; the number of enzyme particles, N_2; the axis lengths of the 3D lattice, L, which are related to the reaction system, also have influences on the model performance. In this work, one unit of N_1 is defined as equal to 0.5 g/L of substrate, and then the initial substrate concentration of 100 g/L is equal to 200 particles of N_1. The value of 70 is used for N_2, which is approximately one-third of N_1. The lengths of the three axes of the 3D lattice are the same as that of L with the value of 30. The volume or space of the 3D lattice can be arbitrarily determined to be one unit, 1 L, for example, for the ease of computation. The values of N_1, N_2, and L are correlated to determine the density of the particles in the 3D lattice, which will affect the binding chance of the substrate with the enzyme and affect the performance of the calculation in the last. These parameter values are determined in arbitrary as a compromise between acceptable computation task and model performance and then checked and modified by trial and error.

The simplification to make substrate and enzyme particles represent unit amounts of substrate and enzyme, respectively but not molecules of them is reasonable, as the molecular thermodynamic movement is

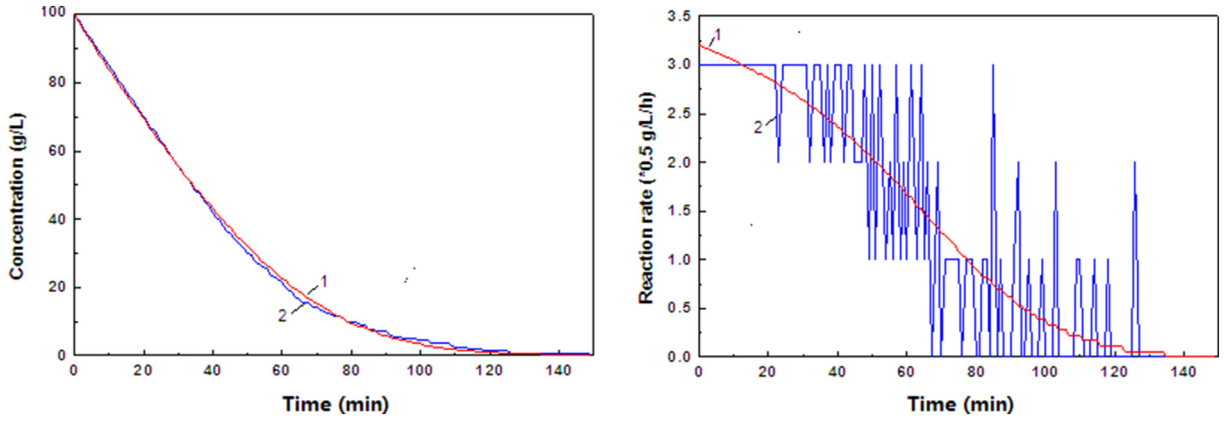

Fig. 5. Comparison of the Michaelis–Menten model with the Monte Carlo model. 1, Michaelis–Menten model; 2, Monte Carlo model.

neglected and only fluid mechanics is considered in modeling the enzyme reaction system. The characteristics of the effects of N_1, N_2, and L on the performance of the system model can be used in modeling and simulation of bioreaction in not well-mixed bioreactor, in which case fluid mechanics can be first used to calculate the uneven distribution of N_1 and N_2 in the subspaces of the bioreactor.

3.3. Modeling the Monte Carlo model output by using Michaelis–Menten model

The Michaelis–Menten model is most widely used in modeling enzyme reaction. Modeling the Monte Carlo model output using the Michaelis–Menten model is useful in the comparison and analysis of the reaction kinetics. In doing this, the output of the Monte Carlo model is fitted using the Michaelis–Menten model by using [Equation 3] and [Equation 4], using GA in optimization of the model parameter values of V_{max} and k_s.

$$v = \frac{V_{\max} \cdot S}{k_S + S} \tag{3}$$

where v is the enzyme reaction rate, V_{max} is the maximum enzyme reaction rate, S is the substrate concentration, and k_s is the substrate affinity coefficient. The mass balance equation is as follows:

$$\frac{dS}{dt} = -v \tag{4}$$

GA is used in the optimization of parameter values. In GA, a population containing n individuals (or chromosomes) are first initialized, which is constituted by $m \times k$ bits of binary digits coding for k genes. Each gene coding one parameter value and one individual coding all model parameter values being optimized. Then, the population undergoes mutation, hybridization, and selection operations in each generation in a cyclic way. During the cycling, the individuals with higher fitness (small model prediction error) have higher probability to be selected, and the average fitness of this population will increase during the evolution process (rounds of calculation). Finally, the individual with the highest fitness is selected, which codes the optimized parameter values. The fitness is defined by the reciprocal of the sum of squared model prediction errors. The scaling procedure transforms the integers coded by the genes into floats within the parameter value ranges. The calculation ends when the maximum calculation rounds is reached. The calculation diagram is shown in Fig. 4.

In the calculation, the initial value of V_{max} can be largely estimated using [Equation 1]. Then, a relatively small range around this value is used in search of optimized V_{max} value by using GA. The initial value of k_S is unknown; hence, a larger range of search span is set for k_S value. The simulation results using the optimized parameter values are shown in Fig. 5, and the results are in the acceptable range. The fact that the Monte Carlo model output can be well fitted by using the Michaelis–Menten model is reasonable, as the Monte Carlo model is developed according to the Michaelis–Menten kinetics as described in the section of Mathematical modeling. The results indicate that the Monte Carlo model is suitable for modeling enzyme reaction kinetics. The Monte Carlo model can be another choice in addition to the most widely used deterministic models in enzyme kinetics modeling [18]. A new approach in enzymatic reaction process modeling is the application of Boltzmann entropy equation [19,20]. Boltzmann entropy equation involves fundamental laws of nature, and the reaction tends to end as the entropy tends to the maximum. Even if, in some sophisticated cases, calculation of each component involved in the reactions in a closed system is difficult in applying this method, it is still worth to be further investigated.

4. Conclusion

In this study, the enzymatic lactose hydrolysis process is modeled and simulated using the Monte Carlo model, and the model can predict the reaction process accurately. On the other hand, the output of the Monte Carlo model can be well fitted by using the Michaelis–Menten model, which is helpful in the comparison and analysis of the reaction kinetics. The results indicate that the Monte Carlo model is suitable for application in enzyme kinetic modeling.

Conflict of interest statement

The authors of the manuscript entitled “Modeling of Lactose Enzymatic Hydrolysis using Monte Carlo Method” declare that there's no financial/personal interest or belief that could affect our objectivity on above research work.

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