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Numerical Analysis of the Reaction-diffusion Equation for Soluble Starch and Dextrin as Substrates of Immobilized Amyloglucosidase in a Porous Support by Using Least Square Method

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Abstract

Substrates' concentration profile was studied in a porous matrix containing immobilized amyloglucosidase for glucose production. This analysis was performed by using an analytical method called Least Square Method, and the results were compared with numerical solution. Effects of effective diffusivity, Michael's constant, maximum reaction rate and initial substrate concentration were studied on Soluble Starch and Dextrin concentration in the spherical support. The outcomes revealed that Least Square Method has an excellent agreement with numerical solution, and in the center of support, substrate concentration is minimum. Increasing of effective diffusivity and Michael's constant reduced the Soluble Starch and Dextrin profile gradient.

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1. Introduction

In the past decades, while conventional chemical processes have been developed for production and purification of products, utilization of enzymes has been one of the alternative methodologies to achieve a higher efficiency and safety [1-4]. Enzymes are immobilized within or on the supports' structure by using various methods. Use of these biocatalysts as industrial catalysts has been interested, and properties of immobilized enzymes (for example, kinetic properties) have been described by a variety of methods. Generally, reports have mostly concentrated on enzyme derivation methods, practical means to use immobilized enzymes efficiently, and investigation of the kinetic behavior of immobilized enzymes [5-7]. Enzymes immobilization can be broadly classified as physical and chemical methods. In physical methods, weak interaction exists between the enzyme and the support molecules; however, in chemical methods, covalent bonds are formed between the enzyme and the artificial support [8, 9]. When pretreated support contacts with the enzyme solution, the enzyme molecules diffuse into the support particles and afterward adsorb or bind chemically to the internal surfaces of the support. As a consequence of enzyme-support interaction, the distribution of the enzyme in the support is generally nonuniform, and restricted diffusion phenomena can complicate the substrate diffusion within the support particles [10-12].

The kinetic properties of immobilized enzymes such as Michael's constant and effective diffusivity are

greatly affected by temperature, as it has a great influence on the pore structure and microenvironment of the support matrices [13].

When immobilized enzymes follow a Michaelis-Menten equation, the effect of intraparticle diffusion in membranes on the kinetic behavior of immobilized enzymes has been investigated in terms of effectiveness factor, and an approximate equation is obtained for effectiveness factor [14]. For understanding the immobilization process, mathematical models have been formulated for single enzyme immobilization in porous supports. The model recognized that immobilized enzyme profile within the catalyst pellets is nonuniform [15, 16]. In the packed-bed reactors, including immobilized enzymes in spherical supports, which follow Michaelis-Menten kinetics, internal diffusion effects are presented by a design equation, which is explainable in irreversible and competitive product inhibition kinetics. By this equation, the substrate profiles are calculated, and the dependence of the effectiveness factor along the bioreactor length are evaluated and the theoretically predicted values are examined to fit well with the experimentally measured results [17]. Substrate mass transfer parameters including effective diffusivity and overall external mass transfer coefficient are estimated by a simple optimization methodology for immobilized enzyme systems, and the governing differential equation, which follows Michaelis-Menten mechanism, is solved using a numerical solution [18]. Enzyme is immobilized on the internal pore surfaces of a porous matrix, and thus the substrate diffuses through the pores, and reacts with the immobilized enzyme. A mathematical model is presented to explore the influences of various parameters on the distribution of immobilized enzyme and the amount of enzyme loaded in the porous matrix. This model which includes the quasi-steady-state approximation for diffusion into the support particles is solved numerically for different values of parameters [19].

When substrate balance is written for immobilized enzyme on the internal surface of a porous spherical support, the derived nonlinear equation can be numerically solved using related boundary conditions to determine the substrate profile in the support [20]. The nonlinear diffusion equation in steady state conditions states that reactions in constrained enzyme solutions are of interest in biotechnology engineering applications. Exact analytical solutions do not exist for nonlinear differential equations in most cases. A general procedure is demonstrated for solving numerically for the substrate concentration profile determination utilizing the transformation method [21].

The main aim of this paper is to solve mass balance differential equation for substrate diffusion in immobilized enzyme in spherical support using an analytical method. Least square method was used as an analytical solution method, and effects of various parameters such as effective diffusion coefficient, Michaela's constant, maximum reaction rate are demonstrated on soluble starch and dextrin concentration as a substrate for glucose production. Since the use of analytical methods for dissolution of nonlinear differential equations remains unnoticed, the main advantages of this study is comparing analytical solution results with the numerical solution, which confirms the high accuracy of the Least Square Method (LSM).

2. Numerical and applied methods

2.1. Description of the problem

Enzymes are frequently used on porous supports in order to contain the enzyme, and allow continued catalytic activity. While some of the activity relative to the free enzyme is lost, the remaining catalytic activity can be utilized in diverse reactors by protection of the enzyme on the support media.

When enzymes are immobilized on the internal surface of a porous spherical support, the substrate diffuses thorough the pathway among the pores, and reacts with the immobilized enzyme. Assume that enzymes are uniformly distributed in a spherical porous matrix; the reaction kinetics follows Michaelis-Menten kinetic, and there is no partitioning of the substrates between the interior and exterior of the porous matrix and external diffusion limitation is negligible (Figure 1). Assuming steady state condition:

$$J_s = k_L (S_0 - S) = \frac{V_m S}{K_m + S} \qquad \text{Eq. 1}$$

Michaelis-Menten equation is defined in moles per unit time per unit area as:

$$J_s = D_e \left(\frac{d^2 S}{dr^2} + \frac{2}{r}\frac{dS}{dr}\right) = \frac{V_m S}{K_m + S} \qquad \text{Eq. 2}$$

Assuming that there is no external diffusion limitation, Michaelis-Menten equation is defined in moles per unit time per unit volume, so:

$$\frac{d^2 \bar{S}}{d\bar{r}^2} + \frac{2}{\bar{r}}\frac{d\bar{S}}{d\bar{r}} = \frac{\phi^2 \bar{S}}{1 + \bar{S}/\beta} \qquad \text{Eq. 3}$$

$$\bar{S} = \frac{S}{S_0} \quad \bar{r} = \frac{r}{R} \quad \beta = \frac{K_m}{S_0} \quad \phi = R\sqrt{\frac{V_m}{D_e K_m}} \qquad \text{Eq. 4}$$

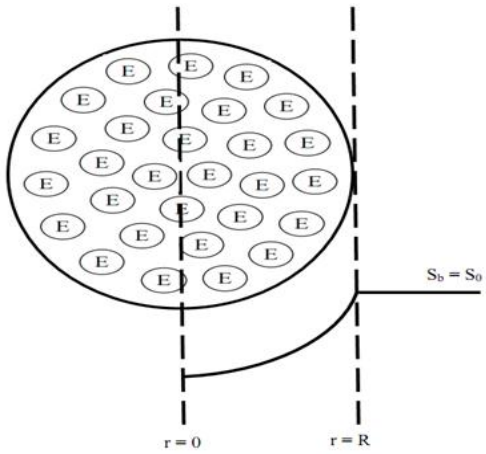

Figure 1. Schematic of the problem (substrate concentration profile in immobilized enzyme in a spherical porous matrix).

where, is the dimensionless substrate concentration, S_0 is the bulk substrate concentration, is the dimensionless radius, K_m is the Michael's constant, V_m is maximum reaction rate, and D_e is effectiveness diffusion coefficient. The appropriate boundary conditions are:

$$\bar{r}=1 \;:\; \bar{S}=1 \qquad \text{Eq. 5}$$

$$\bar{r}=0 \;:\; d\bar{S}/d\bar{r}=0 \qquad \text{Eq. 6}$$

Most of researchers used numerical method to solve this equation; however, in this paper, analytical method is used to solve this equation. Generally, for clear explanation and solving this problem, we used the numerical values presented in Table 1 [18,22,23].

Table 1 includes numerical values for glucose production from soluble starch and dextrin as a substrate by using immobilized Amyloglucosidase in the Honeycomb Ceramic Slab and Porous Spherical Glass Beads reactors.

2.2. Applied method

There is an approximation method to solve ordinary differential equations (LSM). Consider the following differential equation:

$$D\left(u\left(x\right)\right)=p\left(x\right) \qquad \text{Eq. 7}$$

Let consider the function u an approximation of, which is a linear combination of trial functions:

$$u\cong\tilde{u}=\sum_{i=1}^{n}c_i\varphi_i \qquad \text{Eq. 8}$$

By substituting this into the differential equation, an error or residual will exist:

$$R\left(x\right)=D\left(\tilde{u}\left(x\right)\right)-p\left(x\right)=0 \qquad \text{Eq. 9}$$

The notion in LSM is to force the residual to zero, so:

$$\int_X R(x)W_i\left(X\right)dx=0,\quad i=1,2,...,n \qquad \text{Eq. 10}$$

Where, is weight function, and n is the number of unknown constants in. The result is a system of n algebraic equations for obtaining the unknown constants. If the continuous summation of all of the squared residuals is minimized, the rationale behind the LSM's name can be seen:

$$S=\int_X R(x)R(x)dx=\int_X R^2\left(x\right)dx \qquad \text{Eq. 11}$$

For obtaining the minimum of the function, the derivatives of with respect to all constants must be zero:

$$\frac{\partial S}{\partial c_i}=2\int_X R\left(x\right)\frac{\partial R}{\partial c_i}dx=0\quad,\qquad i=1,2,...,n \qquad \text{Eq. 12}$$

By comparing Eqs. (8) and (12), the weight functions are obtained as:

$$W_i=2(\partial R/\partial c_i) \qquad \text{Eq. 13}$$

Hatami and Ganji have presented some advantages of LSM comparing to other numerical methods [24,25]. Here, we apply the LSM on the present problem. We should first choose a trial function. Since the trial function must satisfy the boundary conditions (Eq. (5) and (6)), so it will be assumed as:

$$S(r)=1+c_1(1-r^2)+c_2(1-r^3)+c_3(1-r^4)+c_5(1-r^6) \qquad \text{Eq. 14}$$

By combining the above equation with Eq. (3), residual function will be found, and via substituting the residual function into Eq. (12), a system of equation with five equations will appear. Also by solving this set of equations, coefficients $c_1,\ldots,c_5$ will be obtained. The analytical solution of the problem is in the following form for φ=1, β=1:

$$S(r)=0.191+0.0798r^2)+0.0r^3+0.0011r^4-0.000001\mathrm{r}^5-0.000001\mathrm{r}^6 \qquad \text{Eq. 15}$$

To validate our solution and to find the accuracy of the method, we compared the results of the LSM and numerical solution in Table 2. The numerical solution is performed by using the algebra package Maple 15.0 to solve the present case. The package uses a fourth–fifth order Runge-Kutta–Fehlberg procedure for solving the nonlinear boundary value problem (BVP). The algorithm is proved to be precise and accurate in solving a wide range of mathematical and engineering problems. As shown in Table 2, the results of LSM have an excellent accuracy, and order of the error is about 10^{-6} to 10^{-5}.

3. Results and discussion

In this study, LSM was applied as an analytical method for determining soluble starch and dextrin concentration profile in immobilized Amyloglucosidase in spherical support at various conditions. Comparison of dimensionless concentration profile of soluble starch and dextrin for glucose production is shown in Figure 2 by using the presented K_m, V_m and D_e in Table 1 when the initial substrate concentration is equal to 50 mol m^{-3} (S_0=50 mol m^{-3}).

Table 1. The numerical value of model parameters.

Enzyme	Product	Bioreactor	Substrate	R(m)	D_e $(\frac{m^2}{s})$	K_m $(\frac{mol}{m^3})$	V_m $(\frac{mol}{sm^3})$	S_0 $(\frac{mol}{m^3})$
Amylo-glucosidase	Glucose	SBR	Soluble Starch	1.6×10^{-4}	3.67×10^{-12}	0.73	0.07	10, 50, 100, 150 &
Amylo-glucosidase	Glucose	RDBR	Dextrin	1.6×10^{-4}	53.0×10^{-12}	1.25	2.4	10, 50, 100, 150 &

Table 2. Comparison of the LSM and numerical solution results for $\phi = 1, \beta = 1$.

Radius	LSM (Eq. 15)	Numerical solution	Error
r=0	0.919109	0.919095	1.42E-5
r=0.1	0.919908	0.919914	6.20E-6
r=0.2	0.922304	0.922294	1.00E-5
r=0.3	0.926302	0.926289	1.29E-5
r=0.4	0.931908	0.931897	1.13E-5
r=0.5	0.939132	0.939122	9.95E-6
r=0.6	0.947985	0.947975	9.62E-6
r=0.7	0.958480	0.958473	6.90E-6
r=0.8	0.970635	0.970627	7.48E-6
r=0.9	0.984468	0.984456	1.18E-5
r=1	1.0	1.0	0.0

As regards to Figure 2, it is obvious that soluble starch and dextrin concentrations are zero in the center of the spherical support, and the soluble starch available in the bulk media diffuses more than dextrin into the spherical support; thus, the dextrin concentration gradient is more than that of soluble starch. In other words, in specified radius of porous support, concentration of soluble starch is more than dextrin concentration.

Regarding that effective diffusion coefficient is the function of media condition such as temperature, Equation (3) shows that concentration profile is a function of S_0, K_m, V_m and D_e. In constant value of initial concentration, enzymatic reaction rate is a function of K_m and V_m, and by reducing K_m or increasing V_m, the rate of enzymatic reaction increases, and consequently, the substrate's concentration reduces in various layers of the spherical support.

Effect of the soluble starch's effective diffusivity coefficient (D_e) on substrate concentration profile is shown in Figure 3a when:

$K_m^S = 0.73\ \text{mol m}^{-3}, V_m^S = 0.07\ \text{mol m}^{-3}\text{s}^{-1}$ and $S_0 = 200\ \text{mol m}^{-3}$. With effective diffusivity increasing, substrate diffuses further and further in the interior layers of support and thus substrate profile gradient decreases when D_e increases from $0.5 \times 3.67 \times 10^{-12}$ *to* $2 \times 3.67 \times 10^{-12}$. In Figure 3b, the effect of D_e^D on dextrin concentration profile is demonstrated when

$K_m^S = 1.25\ \text{mol m}^{-3}, V_m^S = 2.40\ \text{mol m}^{-3}\text{s}^{-1}$ and $S_0 = 200\ \text{mol m}^{-3}$.

Diffusion coefficient reduction of dextrin increases the difference of substrate concentration between the bulk medium and the center of immobilized enzyme support due to increasing of mass transport resistance through the immobilized enzyme support matrices so that the substrate concentration equals zero in the center of the support when $D_e^D = 0.5 \times 53.00 \times 10^{-12}\ \text{m}^2\ \text{s}^{-1}$ and r= 0.7×10^{-4} m (see Figure 3b).

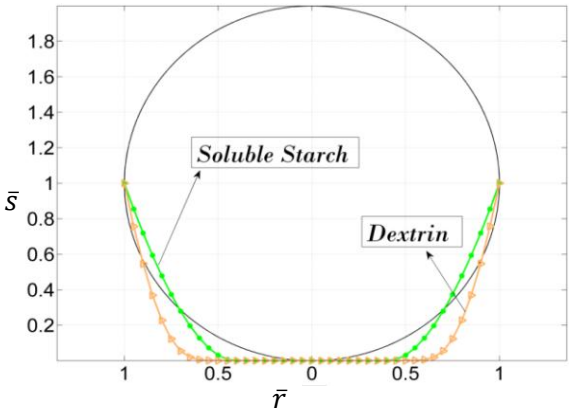

Figure 2. Comparison of soluble starch and dextrin concentration profile in the spherical support.

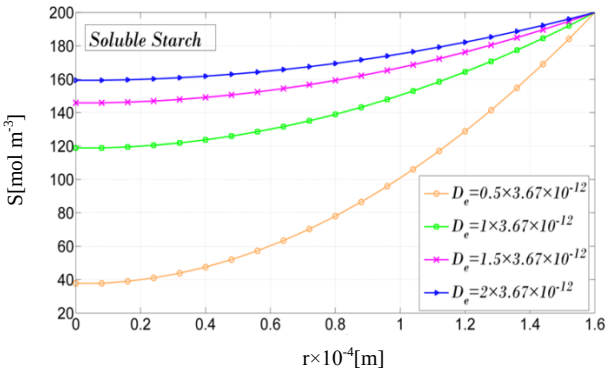

Figure 3a. Effect of D_e^s on substrate concentration profile for immobilized amyloglucosidase in the support.

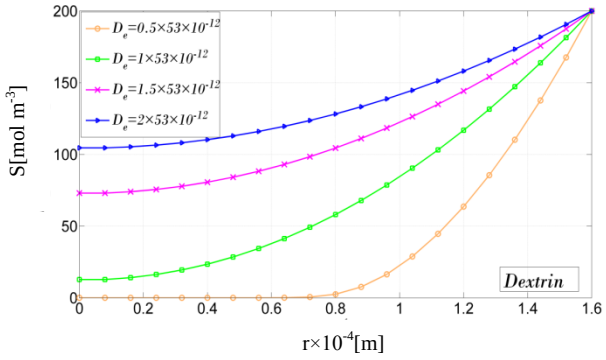

Figure 3b. Effect of D^D_e on substrate concentration profile for immobilized amyloglucosidase in the support.

The Michaelis-Menten constant is the substrate concentration, which is at half-maximum in the reaction rate. The value of K_m is related to the substrate and enzyme, as well as conditions such as pH and temperature [26]. Effects of K_m have been show on soluble starch and dextrin concentration as substrate in Figures 4a and 4b, when ($S_0 = 10\ \frac{\text{mol}}{m^3}$) and the numerical values given in Table 1 are used for V_m and D_e. A low value of K_m suggests that the enzyme has a high affinity to react with the substrate; so, the substrate's concentration should be decreased with reduction of K_m.

Since V_m is dependent on concentration of enzyme not to substrate, so effect of maximum reaction rate on substrate profile is demonstrated in Figures 5a and 5b when S_0=10 mol m^{-3}. With increasing of V_m value, soluble starch concentration reduces in the porous support and thus the substrate profile gradient increases. Dextrin concentration profile in various values of maximum reaction rate is shown in Figure 5b. In the center of the support, substrate's concentration is zero, and the procedure of substrate profile gradient is similar to that of soluble starch with variation of V_m.

Based on Fick's law, by increasing the initial concentration of substrate in the bulk medium, concentration profile gradient increases between the center of the support and the bulk medium, and so the substrate diffuses into the support more quickly [27]. As seen in Figures 6a and 6b, initial substrate concentration is effective on the soluble starch and dextrin concentration profile. The numerical values (Table 1) are used for evaluating the effects of initial substrate. Substrate concentration approaches to 0 in half radius of the support when S_0=50 mol m^{-3}, and increasing of the initial concentration increases the substrate concentration in the layers of matrices.

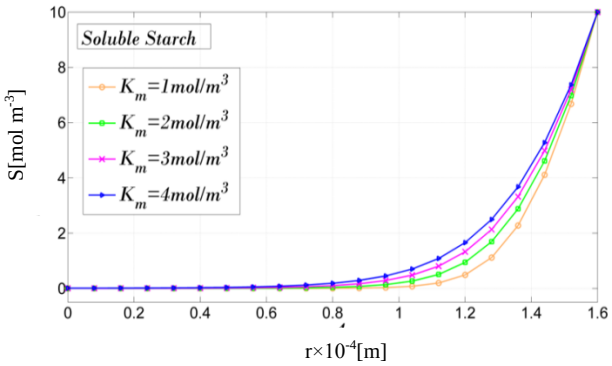

Figure 4a. Effect of K_m on soluble starch concentration profile for immobilized amyloglucosidase in the honeycomb ceramic slab.

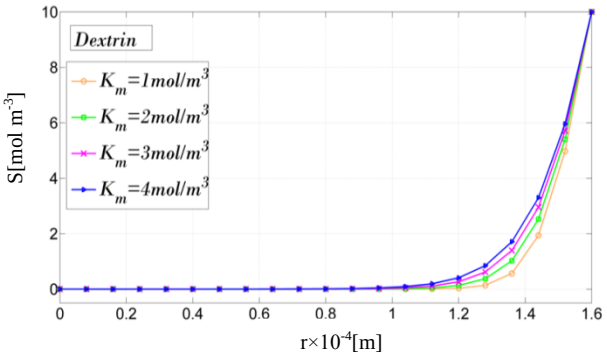

Figure 4b. Effect of K_m on dextrin concentration profile for immobilized amyloglucosidase in the porous spherical glass beads.

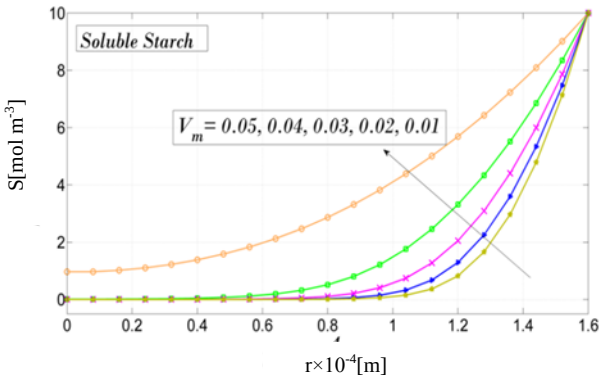

Figure 5a. Effect of maximum reaction rate *(V_m)* on the soluble starch concentration profile.

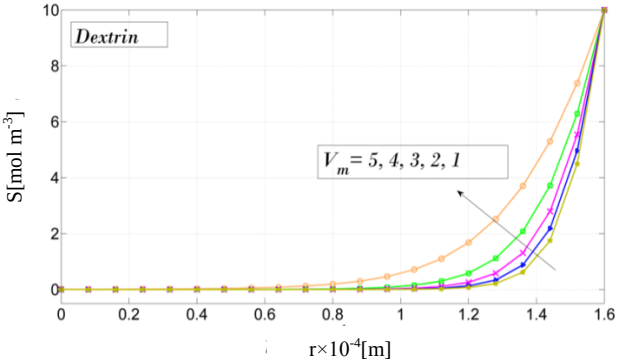

Figure 5b. Effect of maximum reaction rate *(V_m)* on the dextrin concentration profile.

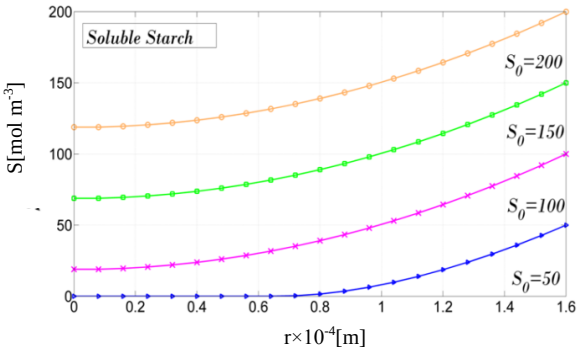

Figure 6a. Effect of initial substrate concentration *(S_0)* on the soluble starch concentration profile.

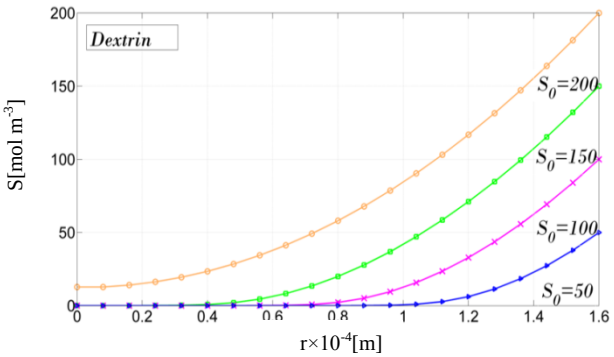

Figure 6b. Effect of initial substrate concentration *(S_0)* on the dextrin concentration profile.

4. Conclusion

Biocatalysis is an important tool for synthesis of chemicals. It mostly uses occurring enzymes or micro-organisms as catalysts. Due to mass balance, differential equation is non-linear in quasi-steady state for immobilized enzyme in spherical porous matrix. A significant challenge is analytical solution of this equation. In this study, the effects of various parameters such as effective diffusivity coefficient of substrates, maximum reaction rate, Michael's constant and initial concentration of substrate were studied on soluble starch and dextrin profile for glucose production using amyloglucosidase and analysis by LSM as analytical approach. In spherical support of immobilized enzyme, with approaching to the center of porous matrix, the substrate's concentration reduces and its gradient is function of media and substrate-enzyme properties. Substrate concentration gradient reduces by increasing the effective diffusivity and Michael's constant, but reducing the maximum reaction rate decreases the slope of substrate profile within the spherical support matrix. By increasing the initial concentration of substrate in the bulk solution, the concentration of soluble starch and dextrin increases in various layers of the spherical support. The results further showed that LSM and numerical solution had an excellent agreement.

5. Acknowledgments

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6. Conflict of interest

The authors agree to submit the article in the Journal of Applied Food Biotechnology and there is no conflict of interest.

Table 3. Unified nomenclature for LSM analysis.

D_e	effective diffusivity of substrate	S_0	initial substrate concentration
D_e^D	effective diffusivity of dextrin	$\overline{S}$	dimensionless substrate concentration
D_e^s	effective diffusivity of soluble starch	V_m	maximum reaction rate
K_m	Michael's constant	V_m^D	maximum reaction rate of dextrin
K_m^D	Michael's constant for dextrin	V_m^S	maximum reaction rate of soluble starch
K_m^s	Michael's constant for soluble starch	*W(X)*	weight function
		SBR	Stirred Batch Reactor
r	radius	RDBR	Recycling Differential Batch Reactor
R	radius of support	**Greek symbols**	
$\overline{r}$	dimensionless radius	φ	thiele modulus
S	substrate concentration	β	dimensionless Michael's constant

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