Research Article

Theoretical Analysis of an Amperometric Biosensor Based on Parallel Substrates Conversion

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The behaviour of an amperometric biosensor based on parallel substrates conversion for steady-state condition has been discussed. This analysis contains a nonlinear term related to enzyme kinetics. Simple and closed form of analytical expressions of concentrations and of biosensor current is derived. This model was originally reported by Vytautas Aseris and his team (2012). Concentrations of substrate and product are expressed in terms of single dimensionless parameter. A new approach to Homotopy perturbation method (HPM) is employed to solve the system of nonlinear reaction diffusion equations. Furthermore, in this work, the numerical solution of the problem is also reported using Matlab program. The analytical results are compared with the numerical results. The analytical result provided is reliable and efficient to understand the behavior of the system.

1. Introduction

A biosensor is an analytical device, used for the detection of an analyte, which combines a biological component with a physiochemical detector [1]. Analyzed substrate is biochemically converted to a product when the biosensor operation is being undertaken. In most of the cases, biosensor response is directly proportional to the concentration of the reaction product [2]. The amperometric biosensors have proved to be reliable in various systems with applications in the field of medicine, food technology, and the environmental industry [3, 4]. The understanding of the kinetic peculiarities of biosensors is of crucial importance for their design. The mathematical modeling is rather widely used to improve the efficiency of the biosensors design and to optimize their configuration [5–8]. Since 1970s, various mathematical models of biosensors have been developed and used successfully to study and optimize analytical characteristics of biosensors [9–14]. Mathematical modeling of two-enzyme biosensors has been started in 1980s with the modeling of an amperometric monolayer enzyme electrode with two coimmobilized enzymes [15, 16]. Later, nonlinear mathematical models have been developed for amperometric two-enzyme biosensors with different enzymes [17, 18].

The numerical method of solving partial differential equations is to make calculation at all intervals of substrates

concentration and at different diffusion and enzymatic reaction rates. The nonstationary diffusion equations [19], containing a nonlinear term related to the enzymatic reaction, are carried out using the implicit difference scheme [20]. In recent years, analytical solutions are reported for various types of biosensors [21–27].

To the best of our knowledge, there is no simple analytical expression for steady-state concentrations and current of amperometric-based biosensor utilizing parallel substrates conversion for all values of parameters. In this paper, we present the simple and closed form of the analytical expressions for the concentrations of substrate and of product using new Homotopy perturbation method (HPM) [28]. The current density was determined corresponding to all possible values of the parameters.

2. Mathematical Formulation of the Problem and Analysis

The reaction scheme employing two parallel substrates (S_1 and S_2), two enzymes (E_1 and E_2), and two reaction products (P_1 and P_2) in biosensor may be as follows:

First phase of the biosensor action contains hydrogen peroxide (S_1) only. The biosensor response reaches the steady-state at the end of the first phase. The second phase of the biosensor action begins at the second substrate (S_2) poured into the solution. The relative changes between the responses in both phases are measured as the final response of the biosensor. The schematic diagram of the system is provided in Figure 1. The above reaction in the enzymatic layer with the one-dimensional-in-space diffusion, described by Fick's law, leads to the following nonlinear transient reaction-diffusion equations [29]:

$$\begin{aligned} D_{S_{1,e}} \frac{\partial^2 S_{1,e}}{\partial x^2} - k_1 e_1 S_{1,e} - \frac{k_{21} k_{22} e_2 S_{1,e} S_{2,e}}{k_{21} S_{1,e} + k_{22} S_{2,e}} &= 0, \\ D_{S_{2,e}} \frac{\partial^2 S_{2,e}}{\partial x^2} - \frac{k_{21} k_{22} e_2 S_{1,e} S_{2,e}}{k_{21} S_{1,e} + k_{22} S_{2,e}} &= 0, \\ D_{P_{1,e}} \frac{\partial^2 P_{1,e}}{\partial x^2} + \frac{k_1 e_1 S_{1,e}}{2} &= 0, \\ D_{P_{2,e}} \frac{\partial^2 P_{2,e}}{\partial x^2} + \frac{k_{21} k_{22} e_2 S_{1,e} S_{2,e}}{k_{21} S_{1,e} + k_{22} S_{2,e}} &= 0, \end{aligned} \quad (2)$$

where x denotes space, $S_{1,e}$, $S_{2,e}$ are the molar concentration of the substrates, and $P_{1,e}$, $P_{2,e}$ are the molar concentration of products. $D_{S_{1,e}}$, $D_{S_{2,e}}$, $D_{P_{1,e}}$, and $D_{P_{2,e}}$ are the corresponding diffusion coefficients and k_1 , k_{21} , and k_{22} are the reaction rate constants. Graphical representation of the products $P_{1,e}$, $P_{2,e}$ and substrates $S_{1,e}$, $S_{2,e}$, with steady-state boundary conditions employed in the simulated multilayered biosensor is provided in Figure 2. The mass transport by diffusion of the substrates and products takes place outside the enzyme layer. The diffusion layer is treated as the Nernst diffusion layer. The boundary conditions for the above system of nonlinear equation are as follows:

$$\begin{aligned} D_{C_e} \frac{\partial c_e}{\partial x} \Big|_{x=0} &= 0, \quad P_{1,e}(x=0) = 0, \quad c = S_1, S_2, P_2 \\ S_{i,e}(x = d_e + d_d) &= S_{i0}, \quad P_{i,e}(x = d_e + d_d) = 0, \quad i = 1, 2, \end{aligned} \quad (3)$$

where d_e and d_d denote the thickness of the enzyme and diffusion layer. For the steady-state condition, the following sets of dimensionless variables are introduced:

$$\begin{aligned} S_{1N} &= \frac{S_{1,e}}{S_{10}}, \quad S_{2N} = \frac{S_{2,e}}{S_{20}}, \\ P_{1N} &= \frac{P_{1,e}}{S_{10}}, \quad P_{2N} = \frac{P_{2,e}}{S_{10}}, \\ y &= \frac{x}{d}, \quad \gamma_{S1} = \frac{d^2 k_1 e_1}{D_{S_{1,e}}}, \quad \gamma_{S2} = \frac{d^2 k_{22} e_2}{D_{S_{1,e}}}, \\ \gamma_{S3} &= \frac{d^2 k_{22} e_2}{D_{S_{2,e}}}, \quad \gamma_{P1} = \frac{d^2 k_1 e_1}{D_{P_{1,e}}}, \quad \gamma_{P2} = \frac{d^2 k_1 e_1}{D_{P_{2,e}}}, \\ \alpha &= \frac{S_{10}}{S_{20}}, \quad \beta = \frac{k_{22}}{k_{21}}, \end{aligned} \quad (4)$$

FIGURE 1: Schematic representation of biosensor utilising parallel substrate conversion with enzymatic layer (of thickness d_e) and the diffusional layer (of thickness d_d) the bioelectrode functioning in the presence of hydrogen peroxide (S_1) or catalyses (S_2). E_1 and E_2 represent the catalase and peroxidase activities. P_1 and P_2 are products.

where $d = d_e + d_d$. The governing nonlinear reaction diffusion equations (2) are expressed in the following steady-state nondimensional form:

$$\frac{d^2 S_{1N}}{dy^2} = \gamma_{S1} S_{1N} + \gamma_{S2} \frac{S_{1N} S_{2N}}{\alpha S_{1N} + \beta S_{2N}}, \quad (5)$$

$$\frac{d^2 S_{2N}}{dy^2} = \gamma_{S3} \frac{S_{1N} S_{2N}}{\alpha S_{1N} + \beta S_{2N}}, \quad (6)$$

$$\frac{d^2 P_{1N}}{dy^2} = -\gamma_{P1} \frac{S_{1N}}{2}, \quad (7)$$

$$\frac{d^2 P_{2N}}{dy^2} = -\gamma_{P2} \frac{S_{1N} S_{2N}}{\alpha S_{1N} + \beta S_{2N}}, \quad (8)$$

where S_{1N} and S_{2N} represent the dimensionless substrate concentrations, P_{1N} and P_{2N} represent the dimensionless product concentrations, and y represents the dimensionless distance. The reaction/diffusion parameters of substrates and products are considered to be γ_{S1} , γ_{S2} , γ_{S3} , γ_{P1} , and γ_{P2} . The boundary conditions are represented as follows:

$$\begin{aligned} \frac{dS_{1N}}{dy} = \frac{dS_{2N}}{dy} = \frac{dP_{2N}}{dy} &= 0, \quad P_{1N} = 0 \quad \text{when } y = 0, \\ S_{1N} = S_{2N} &= 1, \quad P_{1N} = P_{2N} = 0 \quad \text{when } y = 1. \end{aligned} \quad (9)$$

The dimensionless current density is given by

$$\psi = \frac{i_s d}{n_e F D_{P_{1,e}} A S_{10}} = \frac{dP_{1N}}{dy} \Big|_{y=0}. \quad (10)$$

3. A New Approach to Homotopy Perturbation Method and Its Advantages

The Homotopy perturbation method is first introduced to solve nonlinear wave equations. The advantage of the method is that it does not need a small parameter in the system, leading to wide application in nonlinear wave equations [30]. Recently, many authors have used HPM to various

FIGURE 2: Graphical representation of steady-state boundary conditions of the products $P_{1,e}$, $P_{2,e}$ and substrates $S_{1,e}$, $S_{2,e}$, for multilayered biosensor. Here, $x = 0$ is taken at the surface of the electrode and $x = d_e + d_d$ is taken as the surface of the diffusional layer.

problems and reported the efficiency of the HPM for handling nonlinear engineering problems [31–34]. This method is a combination of Homotopy in topology and classic perturbation techniques. He used the HPM to solve the Light hill equation [35]; this wide variety of applications shows the power of the HPM to solve functional equations. The HPM has uniqueness in its applicability, accuracy, and efficiency. Recently, a new approach to HPM is introduced to solve the nonlinear problem, in which one will get better simple approximate solution in the zeroth iteration [28].

4. Analytical Expression for the Concentration of Substrate and Product Using New Homotopy Perturbation Method

In this paper, a new approach to Homotopy perturbation method is used (Appendix A) to solve the nonlinear differential equations (5)–(8). Using this method, the analytical expression of the two substrate concentrations (S_{1N} and S_{2N}) and two product concentrations (P_{1N} and P_{2N}) can be obtained as follows:

$$S_{1N}(y) = \frac{\cosh(Ay)}{\cosh(A)}, \quad (11)$$

$$S_{2N}(y) = \frac{\cosh(By)}{\cosh(B)}, \quad (12)$$

$$P_{1N}(y) = \frac{\gamma_{P1} [1 - \cosh(Ay) + y \cosh(A) - y]}{2A^2 \cosh(A)} \quad (13)$$

$$P_{2N}(y) = \frac{\gamma_{P2}}{\gamma_{S3}} \left[1 - \frac{\cosh(By)}{\cosh(B)} \right], \quad (14)$$

where

$$A = \sqrt{\frac{\gamma_{S1}(\alpha + \beta) + \gamma_{S2}}{\alpha + \beta}}, \quad B = \sqrt{\frac{\gamma_{S3}}{\alpha + \beta}}. \quad (15)$$

Equations (11)–(14) satisfy the boundary conditions (9). These equations represent the simple and closed form of analytical expressions of concentrations of substrate and product for all possible values of the parameters. Using (6) and (8) with boundary conditions (9), the following relation is also obtained:

$$P_{2N}(y) = \frac{\gamma_{P2}}{\gamma_{S3}} [1 - S_{2N}(y)]. \quad (16)$$

The analytical expression of the current using (10) is as follows:

$$\psi = \frac{id}{n_e F A D_{P_{1,e}} S_{10}} = \frac{\gamma_{P1} [\cosh(A) - 1]}{2A^2 \cosh(A)}. \quad (17)$$

When $A \rightarrow 0$, the above expression can be simplified as $\psi = \gamma_{P1}/4$.

4.1. Limiting Case. The mathematical as well as the corresponding computational models of the biosensor were evaluated. Assume that zero concentration of the second substrate ($S_{20} = 0$) leads to reducing the governing equations (5)–(8) to the following equations:

$$\frac{d^2 S_{1N}}{dy^2} = \gamma_{S1} S_{1N}, \quad (18)$$

$$\frac{d^2 P_{1N}}{dy^2} = -\gamma_{P1} \frac{S_{1N}}{2}.$$

The steady-state solution of the above equations for the boundary condition (9) is given by

$$S_{1,N} = \frac{\cosh(\sqrt{\gamma_{S1}} y)}{\cosh(\sqrt{\gamma_{S1}})},$$

$$P_{1N}(y) = \frac{\gamma_{P1} [1 - \cosh(\sqrt{\gamma_{S1}} y) + y \cosh(\sqrt{\gamma_{S1}}) - y]}{2\gamma_{S1} \cosh(\sqrt{\gamma_{S1}})}. \quad (19)$$

The dimensionless steady-state current using (10) is given by

$$\psi = \frac{id}{n_e F A D_{P_{1,e}} S_{10}} = \frac{\gamma_{P1} [\cosh(\sqrt{\gamma_{S1}}) - 1]}{2A^2 \cosh(\sqrt{\gamma_{S1}})}. \quad (20)$$

Equation (20) is identical to (17), when $S_{20} = 0$ (i.e., $\alpha = \infty$) in (17).

5. Comparison of Analytical Expressions with Experimental Work and Numerical Simulation

To show the efficiency, the steady-state result (11)–(14) of the reaction diffusion equations is compared with the numerical solution of (5)–(8). The numerical solutions are found using pdepe (finite element method) in Matlab [36]. This Matlab program [37] is also given in Appendix B. The comparison is shown in Figures 3–6 and it gives satisfactory agreement.

FIGURE 3: Normalized substrates and products concentration versus dimensionless space y were computed using (11)–(14) for various values of reaction/diffusion parameters and for some fixed values of the other existing parameters. The key to the plot: (—) (11)–(14) and (•••) and (+++) numerical simulation of (5)–(8).

FIGURE 4: Normalized substrate concentration S_{1N} versus dimensionless space y was computed using (11) for various values of reaction/diffusion parameter γ_{S1} , dimensionless parameter α and for some fixed values of the parameters γ_{S1} , γ_{S2} , α , β . The key to the plot: (—) (11) and (•••) numerical simulation of (5).

FIGURE 5: Normalized substrate concentration S_{2N} versus dimensionless space y was computed using (12) for various values of reaction/diffusion parameter γ_{S3} , dimensionless parameter α and for some fixed values of the parameters $\alpha, \beta, \gamma_{S3}$. The key to the plot: (—) (12) and (•••) numerical simulation of (6).

6. Discussion

Equations (11)–(14) are the new simple and closed approximate analytical expressions for the dimensionless substrate concentrations (S_{1N} and S_{2N}) and product concentrations (P_{1N} and P_{2N}) in terms of A or B . The analytical results (11)–(14) are compared with numerical solution, which is provided in Figures 3–7. Figure 3 shows the plots of all the concentration versus dimensionless distance y .

The kinetic response of a biosensor depends on the concentration species. The concentration depends on the following factors: γ_{S1} , γ_{S2} , γ_{S3} , γ_{P1} , and γ_{P2} . The diffusion parameters γ_{S1} and γ_{S3} represent the ratio of the characteristic time of the enzymatic reaction to that of concentration diffusion. These parameters can be varied by changing the thickness of the enzyme or diffusional layer. These parameters describe the relative importance of diffusion and reaction in both layers. When γ_{S1} or γ_{S3} is small, the kinetics is dominant

resistance; the uptake of concentration species is kinetically controlled. Under these conditions, the concentration species across both the layers is essentially uniform. When the diffusion parameter γ_{S1} or γ_{S3} is large, diffusion limitations are the principal determining factor.

Figures 4 and 5 show the dimensionless concentration of substrates (S_{1N} and S_{2N}) versus dimensionless distance y . Figures 3 and 4(a) and 4(b) are plotted for various values of diffusion parameters γ_{S1} and γ_{S3} for some fixed values of α, β and γ_{S2} . From these figures, it is clear that the concentration of substrates S_{1N} and S_{2N} decreases when the value of γ_{S1} and γ_{S3} increases, respectively. The concentration is approximately equal to one when $\gamma_{S1} \leq 0.01$ and $\gamma_{S3} \leq 0.01$. Figures 4(c) and 5(c) are plotted for various values of dimensionless parameter α for some fixed values of other existing parameters. From these figures, it is clear that concentration increases for large values of α . Figures 4(d) and 5(d) are plotted for various values of A and B , respectively. It

FIGURE 6: Normalized product concentration P_{1N} versus dimensionless space y was computed using (13) for various values of reaction/diffusion parameter γ_{P1} , γ_{S1} and for some fixed values of the parameters γ_{S1} , γ_{S2} , γ_{P1} , α , β . The key to the plot: (—) (13) and (•••) numerical simulation of (7).

FIGURE 7: Normalized product concentration P_{2N} versus dimensionless space y was computed using (14) for various values of reaction/diffusion parameter γ_{P2} , dimensionless parameter α and for some fixed values of the parameters γ_{P1} , γ_{S3} , α , β . The key to the plot: (—) (14) and (•••) numerical simulation of (8).

is evident that the concentration is uniform, when $\gamma_{S1}(\alpha + \beta) + \gamma_{S2} < (0.01)(\alpha + \beta)$ (or $A < 0.1$) and $\gamma_{S3} < (0.01)(\alpha + \beta)$ (or $B < 0.1$).

Figures 6(a)–6(f) show the dimensionless product concentration profile P_{1N} versus dimensionless distance y using (13). Figures 6(a) and 6(b) are plotted for all various values of γ_{P1} for some fixed values of α , β , γ_{S1} and γ_{S2} . Thus, it is concluded that there is a simultaneous increase in the values of the concentration P_{1N} as well as in γ_{P1} . Figure 6(c) is plotted for various values of α and the fixed value of γ_{S1} , γ_{S2} , γ_{P1} , and β . It shows that the concentration is always minimum for all values of α . Concentration versus distance is plotted in Figures 6(d) and 6(e) for various values of γ_{S1} and for some fixed values of other existing parameters. Thus, it is concluded that the concentration is always minimum and also there is a simultaneous decrease in the values of the concentration P_{1N} as the value of γ_{S1} increases. Figure 6(f) is plotted for various values of A , and from these graphs, it is inferred that concentration is minimum and uniform when $A \geq 0$.

Figures 7(a)–7(d) are plotted for the dimensionless product concentration P_{2N} as ordinate versus dimensionless space y as abscissa using (14). From Figures 7(a) and 7(b), it is evident that the concentration of P_{2N} increases as the diffusivity parameter γ_{P2} increases. The concentration is approximately equal to zero, that is, ($P_{2N} \approx 0$) when $\gamma_{P2} \leq 0.1$ for all possible values of the parameters α , β and γ_{S3} . From Figure 7(c), it is inferred that concentration P_{2N} decreases, when the value of α increases and $P_{2N} \approx 0$ when $\alpha \geq 10$. Figure 7(d) is plotted for various values of B ; it is evident that the concentration increases as the value of B increases. The concentration increases, when $\gamma_{S3} > (0.01)(\alpha + \beta)$ or $B > 0.1$.

Figures 8(a)–8(e) are plotted using (17), which shows the dynamics of normalized steady-state current ψ versus dimensionless parameters γ_{P1} , $(\alpha + \beta)$, γ_{S1} , γ_{S2} , and A , respectively. From Figure 8(a), it is noted that, for small values of γ_{S1} ($\gamma_{S1} \leq 0.1$) and for any values of α and β , the value of the current is significantly high. The current grows notably faster at higher values of γ_{S1} but in the case of $\gamma_{S1} \geq 10$ and $\alpha = \beta =$

FIGURE 8: Dimensionless current ψ versus dimensionless parameters γ_{P1} , $(\alpha + \beta)$, γ_{S1} , γ_{S2} and A for some fixed values of the other parameters using (17).

0.1, the current does not increase. Figure 8(b) is plotted for various values of γ_{P1} and for some fixed values of γ_{S1} and γ_{S2} . It is observed that the current increases rapidly and reaches the steady-state when $(\alpha + \beta) \geq 0.5$. From Figures 8(c) and 8(d), it is evident that the current is uniformly decreasing for large values of γ_{P1} , that is, $(\gamma_{P1} \geq 5)$, and constant elsewhere. Figure 8(e) is plotted for various values of the reaction/diffusion parameter γ_{P1} as abscissa and normalized steady-state current ψ as ordinate. It is observed that the current increases as the value of γ_{P1} increases. Also current decreases for all values of the reaction/diffusion parameter γ_{P1} and reaches zero when $A \geq 10$.

7. Conclusion

Mathematical model, describing the steady-state response of an amperometric biosensor based on parallel substrate conversion (catalase-peroxidase biochemical reactions), is presented. A nonlinear time independent coupled system of partial differential equations has been solved analytically using a new Homotopy perturbation method. Analytical expressions for substrate and product concentrations as function of single dimensionless parameter A or B are derived. The optimum value of A or B is also reported. The analytical expressions provided are much simpler and more efficient to understand and to predict the behavior of the system. Our analytical solution for substrates and products are compared with numerical solution providing consistent results. Concentration of substrates, products, and current depends upon reaction/diffusion parameters γ_{S1} , γ_{S2} , γ_{S3} , γ_{P1} , and γ_{P2} . The response of the steady-state current with respect to all parameters is discussed. For all different concentrations, the analytical results matched well with the simulated result. The close matching of simulated result and the analytical data shows that our proposed solution is able to simulate the dynamic performance of sensing system using the parameters. The analytical results provided here are useful in sensor design, optimization, and prediction of electrode response.

Appendices

A. Approximate Analytical Solution of the Nonlinear Equations (5)–(8) Using the Boundary Conditions (9) by New Homotopy Perturbation Method

In this appendix, we indicated how to find the solution of (5)–(8). We first construct the Homotopy as follows:

$$(1-p) \left[\frac{d^2 S_{1N}}{dy^2} - \gamma_{S1} S_{1N} - \gamma_{S2} \frac{S_{1N} S_{2N}(1)}{\alpha S_{1N}(1) + \beta S_{2N}(1)} \right] + p \left[\alpha S_{1N} \frac{d^2 S_{1N}}{dy^2} + \beta S_{2N} \frac{d^2 S_{1N}}{dy^2} - \alpha \gamma_{S1} S_{1N}^2 - \beta \gamma_{S1} S_{1N} S_{2N} - \gamma_{S2} S_{1N} S_{2N} \right] = 0, \quad (A.1)$$

$$(1-p) \left[\frac{d^2 S_{2N}}{dy^2} - \gamma_{S3} \frac{S_{1N}(1) S_{2N}}{\alpha S_{1N}(1) + \beta S_{2N}(1)} \right] + p \left[\alpha S_{1N} \frac{d^2 S_{2N}}{dy^2} + \beta S_{2N} \frac{d^2 S_{2N}}{dy^2} - \gamma_{S3} S_{1N} S_{2N} \right] = 0, \quad (A.2)$$

$$(1-p) \left[\frac{d^2 P_{1N}}{dy^2} - \frac{\gamma_{P1} S_{1N}}{2} \right] + p \left[\frac{d^2 P_{1N}}{dy^2} - \frac{\gamma_{P1} S_{1N}}{2} \right] = 0, \quad (A.3)$$

$$(1-p) \left[\frac{d^2 P_{2N}}{dy^2} - \gamma_{P2} \frac{S_{1N}(1) S_{2N}}{\alpha S_{1N}(1) + \beta S_{2N}(1)} \right] + p \left[\alpha S_{1N} \frac{d^2 P_{2N}}{dy^2} + \beta S_{2N} \frac{d^2 P_{2N}}{dy^2} - \gamma_{P2} S_{1N} S_{2N} \right] = 0. \quad (A.4)$$

The boundary conditions are as follows:

$$\begin{aligned} \text{when } y = 0; \quad \frac{dS_{1N}}{dy} = \frac{dS_{2N}}{dy} = \frac{dP_{2N}}{dy} = 0; \quad P_{1N} = 0, \\ \text{when } y = 1; \quad S_{1N} = S_{2N} = 1, \quad P_{1N} = P_{2N} = 0. \end{aligned} \quad (A.5)$$

The approximate analytical solutions of (A.1) to (A.4) are given by

$$\begin{aligned} S_{1N} &= S_{1N0} + p S_{1N1} + p^2 S_{1N2} + \dots \\ S_{2N} &= S_{2N0} + p S_{2N1} + p^2 S_{2N2} + \dots \\ P_{1N} &= P_{1N0} + p P_{1N1} + p^2 P_{1N2} + \dots \\ P_{2N} &= P_{2N0} + p P_{2N1} + p^2 P_{2N2} + \dots \end{aligned} \quad (A.6)$$

Substituting (A.6) into (A.1)–(A.4) and arranging the coefficients of powers p ,

$$\begin{aligned} p^0: \quad \frac{d^2 S_{1N0}}{dy^2} - \left(\gamma_{S1} + \frac{\gamma_{S2}}{\alpha + \beta} \right) S_{1N0} &= 0, \\ p^0: \quad \frac{d^2 S_{2N0}}{dy^2} - \left(\frac{\gamma_{S3}}{\alpha + \beta} \right) S_{2N0} &= 0, \\ p^0: \quad \frac{d^2 P_{1N}}{dy^2} + \frac{\gamma_{P1}}{2} S_{1N0} &= 0, \\ p^0: \quad \frac{d^2 P_{2N}}{dy^2} - \left(\frac{\gamma_{S3}}{\alpha + \beta} \right) S_{2N0} &= 0. \end{aligned} \quad (A.7)$$

Solving (A.7) for the boundary conditions (A.5), we can find the following results:

$$\begin{aligned} S_{1N0}(y) &= \frac{\cosh \left(\sqrt{(\gamma_{S1}(\alpha + \beta) + \gamma_{S2}) / (\alpha + \beta)} y \right)}{\cosh \left(\sqrt{(\gamma_{S1}(\alpha + \beta) + \gamma_{S2}) / (\alpha + \beta)} \right)}, \\ S_{2N0}(y) &= \frac{\cosh \left(\sqrt{\gamma_{S3} / (\alpha + \beta)} y \right)}{\cosh \left(\sqrt{\gamma_{S3} / (\alpha + \beta)} \right)}, \end{aligned}$$

```

function pdex9
m = 0;
x = linspace(0,1);
t = linspace(0,10000000000);

sol = pdepe(m,@pdex4pde,@pdex4ic,@pdex4bc,x,t);

u1 = sol(:,:,1);
u2 = sol(:,:,2);
u3 = sol(:,:,3);
u4 = sol(:,:,4);

%-----
figure
plot(x,u1(end,:))
title(' u1(x,t) ')
xlabel(' Distance x ')
ylabel(' u1(x,1) ')
%-----
figure
plot(x,u2(end,:))
title(' u2(x,t) ')
xlabel(' Distance x ')
ylabel(' u2(x,2) ')
% -----
figure
plot(x,u3(end,:))
title(' u3(x,t) ')
xlabel(' Distance x ')
ylabel(' u3(x,3) ')
%-----
figure
plot(x,u4(end,:))
title(' u4(x,t) ')
xlabel(' Distance x ')
ylabel(' u4(x,4) ')
%-----
function [c,f,s] = pdex4pde(x,t,u,DuDx)
c = [0; 0; 0; 0];
f = [1; 1; 1; 1].* DuDx;
r1 = 0.1; r2 = 0.1; r3 = 0.1; r4 = 0.1; r5 = 0.1; a = 0.1; b = 0.1;
F1 = - r1 * u(1) - (r2 * u(1) * u(2))/(a * u(1)+u(2) * b);
F2 = - (r3 * u(1) * u(2))/(a * u(1)+u(2) * b);
F3 = ((r4/2) * u(1));
F4 = (r5 * u(1) * u(2))/(a * u(1)+u(2) * b);
s = [F1; F2; F3; F4];
% -----
function u0 = pdex4ic(x)                                     %create a initial conditions
u0 = [1; 1; 0; 0];
% -----
function [pl,ql,pr,qr]=pdex4bc(xl,ul,xr,ur,t)               %create a boundary conditions
pl = [0; 0; ul(3); 0];
ql = [1; 1; 0; 1];
pr = [ur(1)-1; ur(2)-1; ur(3)-0; ur(4)-0];
qr = [0; 0; 0; 0];

```

ALGORITHM 1: Matlab program to find the numerical solution of (5)–(8).

$$\begin{aligned}
P_{1N0}(y) &= \left[\gamma_{P1}(\alpha + \beta) \left(-\cosh \left(\sqrt{\frac{\gamma_{S1}(\alpha + \beta) + \gamma_{S2}}{\alpha + \beta}} y \right) \right. \right. \\
&\quad \left. \left. + y \cosh \left(\sqrt{\frac{\gamma_{S1}(\alpha + \beta) + \gamma_{S2}}{\alpha + \beta}} \right) \right. \right. \\
&\quad \left. \left. - y + 1 \right) \right. \\
&\quad \left. \times \left(2(\gamma_{S1}(\alpha + \beta) + \gamma_{S2}) \right. \right. \\
&\quad \left. \left. \times \cosh \left(\sqrt{\frac{\gamma_{S1}(\alpha + \beta) + \gamma_{S2}}{\alpha + \beta}} \right) \right)^{-1} \right], \\
P_{2N0}(y) &= -\frac{\cosh \left(\sqrt{\gamma_{S3}/(\alpha + \beta)} y \right) \gamma_{P2}}{\cosh \left(\sqrt{\gamma_{S3}/(\alpha + \beta)} \right) \gamma_{S3}} + \frac{\gamma_{P2}}{\gamma_{S3}}.
\end{aligned} \tag{A.8}$$

Since it is a new approach to HPM, we need not to find further iterations. We consider S_{1N0} , S_{2N0} , P_{1N0} , and P_{2N0} as the better approximate solutions. According to the HPM, we can conclude that

$$\begin{aligned}
S_{1N}(y) &= \lim_{p \rightarrow 1} S_{1N}(y) = S_{1N0} + S_{1N1} + \dots \approx S_{1N0}(y), \\
S_{2N}(y) &= \lim_{p \rightarrow 1} S_{2N}(y) = S_{2N0} + S_{2N1} + \dots \approx S_{2N0}(y), \\
P_{1N}(y) &= \lim_{p \rightarrow 1} P_{1N}(y) = P_{1N0} + P_{1N1} + P_{1N2} + \dots \\
&\approx P_{1N0}(y), \\
P_{2N}(y) &= \lim_{p \rightarrow 1} P_{2N}(y) = P_{2N0} + P_{2N1} + P_{2N2} + \dots \\
&\approx P_{2N0}(y).
\end{aligned} \tag{A.9}$$

After putting (A.8) into (A.9), respectively, the final results can be described in (11) to (14) in the text.

B. Matlab Program to Find the Numerical Solution of (5)–(8)

See Algorithm 1.

Nomenclature

$S_{1,e}, S_{2,e}$:	Substrate concentrations (mol cm ⁻³)
e_1, e_2 :	Enzyme concentrations (mol cm ⁻³)
$P_{1,e}, P_{2,e}$:	Product concentrations (mol cm ⁻³)
$D_{S_{1,e}}, D_{S_{2,e}}, D_{P_{1,e}}, D_{P_{2,e}}$:	Diffusion coefficients (cm ² sec ⁻¹)
k_1, k_{21}, k_{22} :	Reaction rate constant (mol cm ⁻³)
d_e, d_d :	Thickness of the enzyme and diffusion layer (cm)
S_{10}, S_{20} :	Bulk concentrations (mol cm ⁻³)

x : Space (cm)
 i : Current (Ampere).

Dimensionless Parameters (Greek Symbols)

S_{1N}, S_{2N} :	Dimensionless substrate concentrations
P_{1N}, P_{2N} :	Dimensionless product concentrations
$\gamma_{S1}, \gamma_{S2}, \gamma_{S3}, \gamma_{P1}, \gamma_{P2}$:	Dimensionless reaction/diffusion parameters
α, β :	Dimensionless parameters
y :	Dimensionless space
$A = \frac{\sqrt{(\gamma_{S1}(\alpha + \beta) + \gamma_{S2})/(\alpha + \beta)}}{\sqrt{\gamma_{S3}/(\alpha + \beta)}}$:	Dimensionless parameter
$B = \sqrt{\gamma_{S3}/(\alpha + \beta)}$:	Dimensionless parameter.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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