MODELING AND SIMULATION OF POROUS MULTI LAYER MICROBIOREACTORS

Linas Petkevičius, Romas Baronas
Institute of Computer Science
Vilnius University
LT-08303, Didlaukio 47, Vilnius, Lithuania
E-mail: linas.petkevicius@mif.vu.lt

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ABSTRACT
The paper presents a non-linear mathematical model of the batch stirred tank reactor with spherical catalyst porous particles as microreactors. The mathematical model when microreactors are covered with an outer protective layer is investigated. The model is based on a system of reaction-diffusion equations, containing a non-linear term related to the Michaelis-Menten kinetics, and involves four regions: the enzyme microreactor where the enzyme reaction as well as mass transport by diffusion take place, a protective layer and the Nernst layer with diffusion limiting regions, where only the mass transport by diffusion takes place, and a convective region, where the analyte concentration is maintained constant. The influence on process duration and process efficiency of the diffusion modulus, the Biot number and inverse adsorption capacity has been numerically investigated. The simulation results showed that protective layer allow to control product emission as well as allow easier control the target concentration. The digital simulation was carried out using the finite difference technique.

INTRODUCTION

Nowadays, a lot of research in application of using capsules become common. Capsules, immobilized enzyme reactor models, have evolved significantly with wide range of applications in food industry (Dubey et al, 2016), waste cleaning (Wong et al, 2016), bacteria cells immobilization (Konti et al, 2016). Even more, increasing interest in using capsules as targeted drug delivery systems (Siepmann et al, 2012).

The properties of capsules depend on the application. For the chemical processes in industry the fast mass convection might be necessary in such case various system optimizations take place (Rossetti, 2018; Baronas et al, 2016). On the other hand in drug release applications, the control of the drug dose released in the human body, allow to increase therapeutic efficacy and significantly reduce side effects. In drug release is common to have slower reactions which does not over exceed the some targeted drug concentration.

In multiple recent works (Kaoui et al, 2018; Carr and Pontrelli, 2018) the simplified models, which includes only mass diffusion take place was published on drug release from multi-layer capsules. However, in practice most of the processes depend of enzyme-catalyzed reactions where multiple processes take place (Baronas et al, 2019, 2018).

In this work we analyzing the behavior of system product (ex. drug) release to outer layer, and how protection layer of capsules might allow to control targeted concentration flux. The protection layers are common in order to control the systems behavior (Kaoui et al, 2018; Kim et al, 2015).

It is worth to mention that, in practice, different layers are from different materials so the porosity of levels should be consider especialy in drug release modelling (Pontrelli and De Monte, 2010). More importantly the porosity is commonly used in the biochemical engineering (Do and Greenfield, 1983; Doran, 2013).

We consider an array of identical spherical microreactors placed in a CSTR shown in Figure 1 (Doran, 1995), where area $\Omega_m$ denotes a microreactor, $\Omega_p$ denotes protection layer, $\Omega_d$ denotes surrounding diffusion shell and $\Omega_b$ is a convective (bulk) region.

The goal of this work was to investigate the dependencies of the protection layer diffusion limitations on the yield of the reaction product as well as system efficiency, modelled by reaction-diffusion equations, containing a non-linear term related to Michaelis-Menten kinetics (Villadsen et al, 2011; Baronas et al, 2010). Due to a strong non-linearity of the reaction term, the computer simulation was carried out using the finite difference technique (Britz and Strutwolf, 2016).
MATHEMATICAL MODEL

We consider an array of identical spherical microreactors placed in a continuous ideally stirred-tank reactor (Doran, 1995). Assuming a uniform distribution of the microreactors in the tank and a relatively great distance between adjacent microreactors and fact that microreactor volume $V_m$ is significantly smaller than tank volume $V$, the spherical unit cell was modelled by an enzyme-loaded microreactor, protection layer and a surrounding diffusion shell.

Assuming the steady-state approximation, the concentration of the intermediate complex (ES) does not change and may be neglected when modelling the biochemical behaviour of the microreactor (Doran, 1995; Gutfreund, 1995; Segel and Slemrod, 1989). In the resulting scheme, the substrate (S) is enzymatically converted to the product (P),

\[ S \xrightarrow{E} P. \quad (1) \]

Governing equations

Assuming the symmetrical geometry of the microreactor (region $\Omega_m$) with the one-dimensional-in-space diffusion, described by Fick’s second law, and assuming the steady-state for a system (1) lead to the following governing equations of the reaction-diffusion type (0 < $r < R_0$):

\[
\frac{\partial S_m}{\partial t} = D_{S,m} \Delta S_m - \frac{V_{\text{max}} S_m}{K_M + S_m}, \quad (2a) \\
\frac{\partial P_m}{\partial t} = D_{P,m} \Delta P_m + \frac{V_{\text{max}} S_m}{K_M + S_m}, \quad t > 0, \quad (2b)
\]

where $S_m = S_m(r,t)$, $P_m = P_m(r,t)$ is the concentration of the substrate in the microreactor, and concentration of the product, respectively, $r$ and $t$ stand for the space and time, respectively. $\Delta = (1/r^2)\partial/\partial r(r^2 \partial/\partial r)$ is the Laplace operator in spherical coordinates, $R_0$ is the radius of the MR and $D_{S,m}, D_{P,m}$ is the effective diffusion coefficient of substrate and product, respectively (Bartlett, 2008).

Assuming particle is coated with protective film (Kim et al., 2015; Kaoui et al., 2018). No reaction takes in protective layer ($R_0 < r < R_1$). It’s common to assume that a protective diffusion shell adjacent to the microreactor surface remains at a constant thickness $h_1 = R_1 - R_0$,

\[
\frac{\partial S_p}{\partial t} = D_{S,p} \Delta S_p, \quad (3a) \\
\frac{\partial P_p}{\partial t} = D_{P,p} \Delta P_p, \quad t > 0, \quad (3b)
\]

where $S_p = S_p(r,t)$, $P_p = P_p(r,t)$ is the concentration of the substrate and product, respectively, in the protection shell, and $D_{S,p}, D_{P,p}$ is the corresponding diffusion coefficients.

No reaction takes place outside the particle ($r > R_1$). Applying the Nernst approach (Villadsen et al., 2011; Bartlett, 2008), a thin diffusion shell adjacent to the protective layer surface remains at a constant thickness $h_2 = R_2 - R_1$,

\[
\frac{\partial S_d}{\partial t} = D_{S,d} \Delta S_d, \quad t > 0, \quad (4a) \\
\frac{\partial P_d}{\partial t} = D_{P,d} \Delta P_d
\]

where $S_d = S_d(r,t)$, $P_d = P_d(r,t)$ is the concentration of the substrate in the diffusion shell, and $D_{S,d}, D_{P,d}$ is the corresponding diffusion coefficients.

The thickness $h_2$ of the diffusion shell depends upon the nature and intensity of the stirring of the buffer solution and do not influence different particles (Kasche et al., 1979). The less intense stirring corresponds to the thicker diffusion shell (greater $h_2$) (Villadsen et al., 2011; Bartlett, 2008).

The substrate is assumed to be uniformly distributed throughout the outside of the diffusion shell and its concentration depends only on time (Do and Greenfield, 1983; Sagiv, 2001). The rate at which substrate leaves the particle of volume $4\pi (R_2^3 - R_1^3)/3$ is always equal to that at which it enters the diffusion shell over the surface of the area $4\pi R_2^2$,\n
\[
\frac{dS_b}{dt} = -\frac{1}{q} D_{S,d} \frac{\partial S_d}{\partial r} \bigg|_{r=R_2}, \quad (5a) \\
\frac{dP_b}{dt} = -\frac{1}{q} D_{P,d} \frac{\partial P_d}{\partial r} \bigg|_{r=R_2}, \quad t > 0, \quad (5b)
\]

where $S_b = S_b(t)$, $P_b = P_b(t)$ is the substrate and product concentrations, respectively, in the bulk (convective shell, $R_2 \leq r \leq R_3$), $q$ is the ratio of the volume of the convective enclosure ($R_1 \leq r \leq R_2$) to the area of the outer surface of the diffusion shell ($r = R_2$),

\[
q = \frac{4\pi (r_2^3 - r_1^3)/3}{4\pi r_2^2} = \frac{r_2^3 - r_1^3}{3r_1^2}. \quad (6)
\]

The value $1/q$ can be also considered as the adsorption capacity of the microreactor (Bidabehere et al., 2006; Bidabehere and Sedran, 2015). $h_3 = R_2 - R_1$ is the thickness of the convective shell.

Initial and boundary conditions

The process starts ($t = 0$) when substrate is placed into the solution,

\[
S_m(r,0) = 0, \quad P_m(r,0) = 0, \quad 0 \leq r \leq R_0, \quad (7a) \\
S_p(r,0) = 0, \quad P_p(r,0) = 0, \quad R_0 \leq r \leq R_1, \quad (7b) \\
S_d(r,0) = S_0, \quad P_d(r,0) = 0, \quad R_1 \leq r \leq R_2, \quad (7c) \\
S_b(0) = S_0, \quad P_b(0) = 0 \quad (7d)
\]

where $S_0$ is the initial concentration of the substrate in the bulk solution.

Due to assumption about the symmetrical geometry, the zero-flux boundary condition is defined on the centre of the spherical microreactor ($t > 0$),

\[
D_{S,m} \frac{\partial S_m}{\partial r} \bigg|_{r=0} = 0, \quad D_{P,m} \frac{\partial P_m}{\partial r} \bigg|_{r=0} = 0. \quad (8)
\]
The stream of the substrate and product through the protection shell is assumed to be equal to the stream entering the microreactor surface. The formal partition coefficient \( \psi \) is used in the matching conditions to describe the specificity in the concentration distribution of the substrate between two neighboring regions \( t > 0 \) (Godongwana, 2016; Vos et al., 1990),

\[
D_{S,m} \frac{\partial S_m}{\partial r} \bigg|_{r=R_0} = D_{S,p} \frac{\partial S_p}{\partial r} \bigg|_{r=R_0}, \quad (9a)
\]

\[
D_{P,m} \frac{\partial P_m}{\partial r} \bigg|_{r=R_0} = D_{P,p} \frac{\partial P_p}{\partial r} \bigg|_{r=R_0}, \quad (9b)
\]

\[
S_m(R_0, t) = \psi_1 S_p(R_0, t), \quad (9c)
\]

\[
P_m(R_0, t) = \psi_1 P_p(R_0, t). \quad (9d)
\]

The dimensionless partition coefficient \( \psi_1 \) is from interval \([0, 1]\) as the averaged concentration of the substrate in the microreactor becomes lower than the concentration in the protection layer due to porosity of the materials. The partition coefficient is common in drug release capsules (Pontrelli and De Monte, 2010; Ozturk et al, 1990).

The formal partition coefficient \( \psi_2 \) is used in the matching conditions to describe the specificity in the concentration distribution of the substrate between two neighboring regions of protection and diffusion layers \( t > 0 \) (Pontrelli and De Monte, 2010; Godongwana, 2016; Vos et al, 1990),

\[
D_{S,p} \frac{\partial S_p}{\partial r} \bigg|_{r=R_1} = D_{S,m} \frac{\partial S_m}{\partial r} \bigg|_{r=R_1}, \quad (10a)
\]

\[
D_{P,p} \frac{\partial P_p}{\partial r} \bigg|_{r=R_1} = D_{P,m} \frac{\partial P_m}{\partial r} \bigg|_{r=R_1}, \quad (10b)
\]

\[
S_m(R_0, t) = \psi_2 S_p(R_0, t), \quad (10c)
\]

\[
P_m(R_0, t) = \psi_2 P_p(R_0, t). \quad (10d)
\]

The dimensionless partition coefficient \( \psi_2 \) is also from interval \([0, 1]\) as the averaged concentration of the substrate in the protection layer becomes lower than the concentration in the bulk solution due to the insoluble microreactor carrier.

The coefficient \( \psi \) can also be considered as the porosity of the MR and defined as the ratio of the porous volume to the total volume of the microreactor (Coche-Guerente et al, 2001; Velkovsky et al, 2011; Ozturk et al, 1990).

The boundary condition (10) and the governing equation (5) are only specific to the batch mode of the bioreactor operation (BSTR) (Baronas et al, 2018).

**Microbioreactor Characteristics**

In many industrial processes, especially in the production of low-value-added products like biopesticides, bio-fertilizers, bio-surfactants etc. (Wong et al, 2016), as well as in drug release from capsules (Kaoui et al, 2018; Carr and Pontrelli, 2018), it is important to continuously improve the yield and/or productivity (Villadsen et al, 2011) as well as effectiveness.

The yield of the desired product on the substrate is one of the most important criteria for design and optimization of bioreactors or capsules. The economic feasibility of the process is expressed by the yield factor as the ratio of product formation rate and the substrate uptake rate (Doran, 1995; Villadsen et al, 2011).

The bioreactor construction is efficient enough when the product emission rate is relatively large with given substrate amount used. The product emission rate \( \bar{E}_{P,O}(t) \) can be calculated by an integration of the product flux over the outer surface of the diffusion shell (Villadsen et al, 2011),

\[
\bar{E}_{P,O}(t) = - \int_0^{2\pi} \int_0^\pi D_{P,d} \frac{\partial P_p(r, t)}{\partial r} \bigg|_{r=R_2} R_2^2 \sin(\theta) d\theta d\phi.
\]

The average consumption \( \bar{C}_S \) of the substrate over the whole microreactor can be calculated as follows:

\[
\bar{C}_S(t) = \int_0^{R_0} \int_0^{2\pi} \int_0^\pi \frac{V_m S_0(r, t)}{K_M + S_0(r, t)} \sin(\theta) d\theta d\phi \, dr
\]

\[
= \frac{4}{3} \pi \int_0^{R_0} \frac{V_m S_0(r, t)}{K_M + S_0(r, t)} r^2 \, dr.
\]

The yield factor \( \gamma_t \) for the microreactor system, as well as for the entire system can be defined by the ratio of the product emission rate to the substrate consumption rate \( t > 0 \),

\[
\gamma_t = \frac{\bar{E}_{P,O}(t)}{\bar{C}_S(t)}.
\]

The yield factor is equal to unity \( (\gamma_t = 1) \) when whole the consumed substrate is converted to the product and the whole the product eluted into the bulk (convective region \( \Omega_b \)). The microbioreactor is absolutely inefficient \( (\gamma_t = 0) \), if no product falls into the bulk.

The effectiveness factors characterise the interaction between action in microbioreactor in porous catalytic particle and microreactors when particles solid fuel (Belfiore, 2003) and often used in the biochemical engineering (Do and Greenfield, 1983; Doran, 2013). The effectiveness factors are usually defined for the stationary mode of biocatalytic systems (Belfiore, 2003; Doran, 2013; Harriott, 2003). The effectiveness factor \( \eta_t \) can be calculated (Vos et al, 1990):

\[
\eta(t) = \frac{3(K_M + S_0(t))}{V_m S_0(r, t)} \int_0^{R_0} \frac{V_m S_0(r, t)}{K_M + S_0(r, t)} R_2^2 \, dr.
\]

The Biot number is another widely used dimensionless parameter that compares the external and internal mass transfer resistances (AL-Muftah and Abu-Reesh, 2005; Baronas et al, 2018),

\[
\beta = \frac{D_{r1}}{D_m (r_1 - r_0)} = \frac{\alpha R_1}{R_1 - R_0}.
\]
the Biot number increases the effect of the external diffusion becomes less important.

The dimensionless factor $\sigma^2$ is known as the dimensionless diffusion module or Damköhler number or Thiele modulus (Doran, 2013; Davis and Davis, 2003),

$$\sigma^2 = \frac{V_{\text{max}} R_d}{K_M D_m}. \quad (16)$$

If $\sigma \ll 1$, then enzyme kinetics controls the microreactor action, and the action is under diffusion control when $\sigma \gg 1$.

**DIGITAL SIMULATION OF EXPERIMENTS**

The governing equations (2)-(10) contain multiple non-linear parts in boundary value problem so general analytical solution is unknown, hence the numerical model was constructed and solved using finite difference technique (Britz and Strutwolf, 2016).

An explicit scheme of finite difference was used, the scheme contained on a uniform discrete grid in polar coordinates with 128 points in space direction (Baronas et al, 2010). The simulator has been programmed by the authors in C++ language (Vetterling, 2002).

In all the numerical experiments, the following values of the model parameters were kept constant (Baronas et al, 2019; Doran, 2013; Andrés, 2008; Davis and Davis, 2003):

$$D_d = 600 \mu m^2/s, \; D_m = 200 \mu m^2/s, \; K_M = 100 \mu M, \; R_0 = 225 \mu m, \; \psi_1 = 0.5, \; \psi_2 = 0.5. \quad (17)$$

Fig. 2 shows the dynamics of the substrate concentration and the transient effectiveness factors calculated for the protection shell thickness $h_1$ of 50 $\mu m$ ($R_1 = 275 \mu m$), calculated for the diffusion shell thickness $h_1$ of 20 $\mu m$ ($R_2 = 295 \mu m$), the convective shell thickness $h_2$ of 200 $\mu m$ ($R_3 = 295 \mu m$), the maximal enzymatic rate $V_{\text{max}}$ of 10 $\mu M/s$ and the moderate initial concentration $S_0 = K_M = 100 \mu M$.

**RESULTS AND DISCUSSION**

To investigate the effects of the protection layer of the microreactor, the reactor action was simulated and the yield factor was calculated for very different values of the Biot number $\beta$, the Thiele module $\sigma$, protective layer $h_1 = R_1 - R_0$ thickness and bulk thickness $h_3 = R_3 - R_2$.

**Impact of the substrate concentration Biot number and diffusion module**

To investigate the dependence of the effectiveness as well as yield factor $\gamma$ on the Biot number $\beta$, the factor $\gamma_l$ and effectiveness $\eta_l$ was calculated at different values of the Thiele module $\sigma$ in a range of $[10^{-1}, 10^1]$, changing the Biot number in a range of $[10^{-2}, 10^2]$, changing dimensionless protective layer in range $h_1 = [0.1, 1]$ and changing dimensionless bulk layer in range $h_1 = [0.25, 2.25]$. Figure 3 shows the effectiveness $\eta_l$ as an increasing function of the Biot number $\beta$. However, the high effectiveness can be achieved only with large $\beta > 1$ values. For small values of Biot number $\beta < 0.1$ the impact on effectiveness can be assumed negligible. On can be see in Figure 3 that product will not be produced to bulk within reasonable (less than 1 day) time under $\beta < 1$. Figure 4 demonstrates that at stationary time the yield, practically, does not depend on Biot number. Similar results
was found in continues (open system) mode microreactors (Petkevicius and Baronas, 2017). However, the times when product reach the bulk solution is significantly different and might be differ by a magnitude of two orders. And the stationary value is reached at order of $10^4(s)$ for small $\beta$ values, while at order of $10^4$ for large $\beta$ values. The reason why no product is produced for low diffusion layer because of strict limitations in protective layer. Figure 5 shows that effectiveness is monotonically decreasing function of diffusion module $\sigma$. It’s also important that even at twice as efficient configuration the system, can produce the product to bulk rather quickly. This effect demonstrate the ability to create the fast as well as slow product emission to bulk, to given application. It can be seen in Figure 6 that the yield factor $\gamma$, practically, does not depend on $\sigma$ and approaches steady-state non-zero value. In continues (open system) mode the limiting value is zero. The stationary value is achieved approximately at order of $10^4(s)$. The fact that yield does not, practically, depend on diffusion module $\sigma$ means that there is no necessity to be worry about too big product concentrations stream to bulk solution. Figure 7 shows the non-linear effects on effectiveness on changes of protective layer thickness. First the effectiveness $\eta_o$ increases as thickness $h_1$ is decreasing. In this case the limit value $h_1 = 0$ be equivalent of no protective layer. The steady effectiveness value is reached at similar times. But the quantity of the product concentration in bulk is higher at thin protection layer. Figure 8 presents the dependence of the yield factor on the thickness of the Nernst diffusion layer. Values of $\gamma$ were calculated changing the dimensionless thickness $\tilde{h}_1$. The reactor behavior at larger values of $\tilde{h}_1$ is not so important due to low product emission at very low rate.

As one can see in Figure 8, the $\gamma_1$ is a increasing function of $\tilde{h}_1$, which confirms a hypothesis about the importance of the protective layer for the reactors productivity control (Davis and Davis, 2003). The linear dependency from $\tilde{h}_1$ can be achieved at stationary time. This demonstrates that, in order to control the targeted product concentration in bulk solution, the protective layer selection is crucial. The Figure 9 demonstrates that number of particles, which is equivalent of number of particles as well as at which substrate volume at given interval, practically, does not impact the particle effectiveness. The particles effectiveness is reached at similar time and does not vary a lot at after steady-state. Figure 10 shows that product yield increase as increase the bulk layer thickness. It’s also worth to mention that most of the product can be produced to bulk with in less than 5 minutes. Which, might be crucial for time consuming applications.
CONCLUSIONS

The mathematical model (2)-(10) of the porous multi-layer microbioreactor have been formulated and numerical simulation based parameters analysis was investigated.

The thickness of the protective layer noticeably effects the reaction product emission (Figures 7, 8). The yield especially decreases linearly when the protective layer thickness is decreasing. This property be-

comes important when the size of microbioreactors used in industrial applications continuously reduces, while the protective layer remains the same, which is often assumed neglected. For other parameters the yield reaches steady-state and, practically, does not depend on $\beta,\sigma,h_3$ (see Figures 4, 6, 10). But the time steady-state is reached might be different by magnitude of two orders see Figure 4.

The effectiveness of the system increases with increasing with increasing the Biot number (Figure 4). However, an increase in the substrate concentration becomes ineffective when the enzyme reaction approaches the zero order kinetics $\sigma < 1$ (Figure 5), where can be achieved high system efficiency.

The models of multi layer microreactors with the Michaelis-Menten kinetics equation may be useful in the design phase of a bioreactors as well as drug release capsules by providing information about the product yield times at which microbioreactors may be success-

fully operated. Also such models may provide insight into which materials may be used as protection layer to the bioreactors manufacture in order to achieve highly efficient system.

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LINAS PETKEVICIUS is a Ph.D. student at Institute of Computer Science of Vilnius University. He graduated Vilnius University in 2015. His research interests are focused on computational modeling and simulation of biochemical processes as well as image analysis and deep learning.

ROMAS BARONAS was born in 1959 in Kybartai, Lithuania. He enrolled at the Vilnius University, where he studied applied mathematics and received his Ph.D. degree. Now he is a professor and serves as the head of the institute of Computer Science at Vilnius University. His research interests are in database systems, software engineering, and computational modeling of nonlinear phenomena in life sciences.