# Analysis of Mass-Transfer Limitations of Immobilized Cellulase

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**Introduction**

Enzyme immobilization offers a number of advantages over enzymes in suspension. Immobilization permits the reuse of the enzyme, improves environment for catalyst activity, and reduces denaturation. Figure 1 depicts a typical immobilized particle.

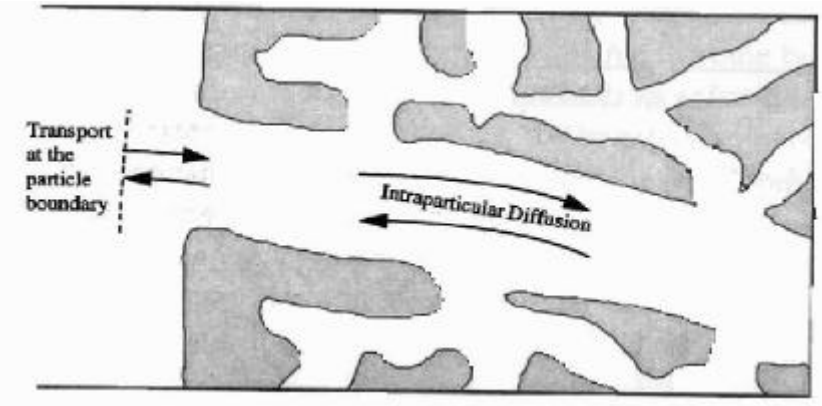



**Figure 1.** Cross-sectional diagram of a porous immobilized particle [10].

The kinetic behavior of immobilized enzymes may differ from those of soluble enzymes due to the following effects: 1) Conformational effects 2) Electrostatic and partitioning effects and 3) Diffusion/mass-transfer effects [10]. Since the observed reaction rate is influence by exchange of mass between the particle interior and its surroundings, it will be the focus throughout the rest of this communication.

The observed kinetics of an immobilized enzyme may not be controlled solely by interactions between the enzyme and substrate. Instead, it may be restricted to some extent by the rate of substrate diffusion to surface of the particle, and/or by the rate of substrate diffusion through the pores of the support. The internal support usually consists of inert polymeric or inorganic matrices.

The major objectives of this work are as follows:

(1) Derive an analytic solution that takes into account first-order reaction, intraparticle diffusion resistance, and internal and external mass transfer resistance with $K_M = 0.5$, determined experimentally for a cellulase-cellulose reaction network.
(2) Analyze the effect of Michaelis-Menton kinetics and intraparticle diffusion resistance on the observed rate of reaction using the finite difference method.
(3) Provide evidence that a steady state assumption is valid for Michaelis-Menton and first-order kinetics and intraparticle diffusion resistance.
(4) Show how the Thiele Modulus and Biot Number affect concentration profiles at steady state Michaelis-Menton and first-order kinetics.

**Mathematical Formulation, Results, and Discussion**

The following reaction network,

[cellulase] + [cellulose] ⇄ [cellulase*cellulose] → [cellobiose] + [cellulase]

yields a mathematical expression for the rate of reaction of cellulose given by eq. 1 [7].

$$-\frac{dc_s}{dt} = \frac{V_M * c_s}{K_M + c_s} \tag{1}$$

For the above reaction network, the following assumptions have been made:

(1) The enzymes are considered to be uniformly distributed inside the spherical particle.

(2) The process is isothermal, intraparticle diffusion follows Fick's first law, and the corresponding effective diffusivities remain invariable.

(3) The reaction network obeys Irreversible Michaelis-Menton kinetics and there is no product inhibition

Under these hypotheses, the continuity equation for the overall process of diffusion and reaction for a substrate (i.e. cellulose) can be written in dimensionless form as follows [5].

$$\frac{\partial \bar{c}_s}{\partial \bar{t}} = \frac{\partial^2 \bar{c}_s}{\partial \bar{r}^2} + \frac{2}{\bar{r}}\frac{\partial \bar{r}}{\partial \bar{c}_s} - \frac{j^2 \bar{c}_s}{\bar{K}_M + \bar{c}_s} \tag{2}$$

**Internal Resistance**

$$\underline{B.C.'s}$$

$$\left.\frac{d\bar{c}_s}{d\bar{r}}\right|_{r=0} = 0 \tag{3}$$

$$\bar{c}_s\big|_{\bar{r}=1} = 1$$

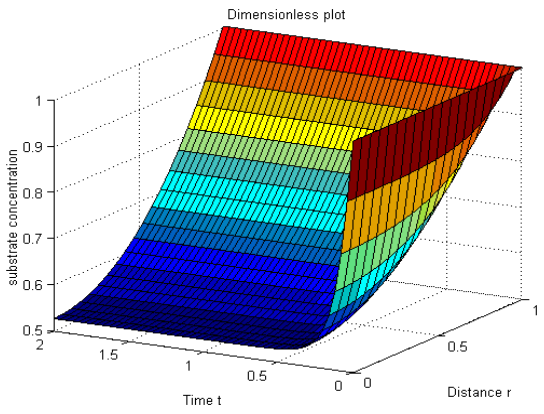
**Internal & External Resistance**

$$\underline{B.C.'s}$$

$$\left.\frac{d\bar{c}_s}{d\bar{r}}\right|_{r=0} = 0 \tag{4}$$

$$Bi\left(\bar{c} - \bar{c}_s\big|_{r=1}\right) = \frac{d\bar{c}_s}{d\bar{r}}$$

A solution to eq. 2 was determined with Matlab via numerical solution with appropriate boundary conditions for intraparticle diffusion resistance; zero flux at center of particle and surface concentration is equal to the bulk concentration. Transient concentration profiles generated with Matlab are show below.



Steady state is approached almost instantaneously (0.5 sec) when compared to normal reaction times (3-day). This evidence supports the validity of a steady state assumption for Michaelis-Menton kinetics.

In certain cases, restriction of the rate expression (eq. 1) to low substrate concentrations (i.e. $c_s \ll K_M$) is an acceptable condition for the investigation of enzyme kinetics [1]. The enzyme kinetics can be simplified to a pseudo-first order expression, which leads to the continuity equation expressed in dimensionless form as follows.

$$\frac{\partial \bar{c}_s}{\partial \bar{t}} = \frac{\partial^2 \bar{c}_s}{\partial \bar{r}^2} + \frac{2}{\bar{r}} \frac{\partial \bar{r}}{\partial \bar{c}_s} - j^2 \bar{c}_s \tag{5}$$

A solution to eq. 5 was determined with Matlab via numerical solution with appropriate boundary conditions (eq. 3) for intraparticle diffusion resistance. Transient concentration profiles generated with Matlab are show below.

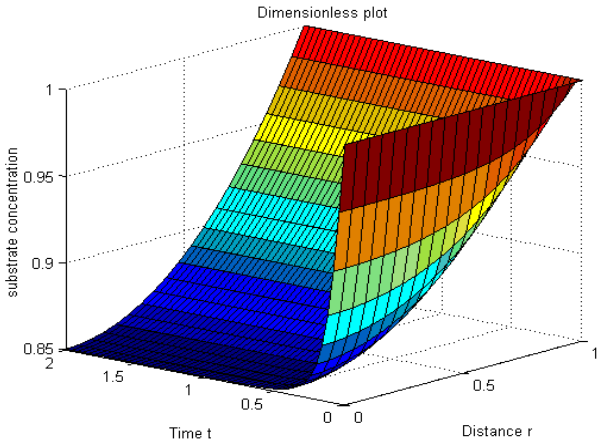



Figure 3

Steady state is approached almost instantaneously (0.5 sec) when compared to normal reaction times (3-day).

This evidence confirms the validity of a steady state assumption for low substrate concentrations (i.e. first order kinetics) and Michaelis-Menton kinetics. In light of this information, the remainder of this communication will focus on the steady state diffusion and reaction problem.

Using the steady state assumption and Nonlinear Michaelis-Menton kinetics, the continuity equation reduces to the following dimensionless form [5].

$$\frac{d^2 \bar{c}_s}{d \bar{r}^2} + \frac{2}{\bar{r}} \frac{d \bar{r}}{d \bar{c}_s} = \frac{j^2 \bar{c}_s}{\bar{K}_M + \bar{c}_s} \tag{6}$$

A solution to eq. 6 was determined via finite difference method with appropriate boundary conditions for intraparticle diffusion resistance.

Concentration profiles generated with the numerical solution to eq. 6 for different Thiele moduli are show below.

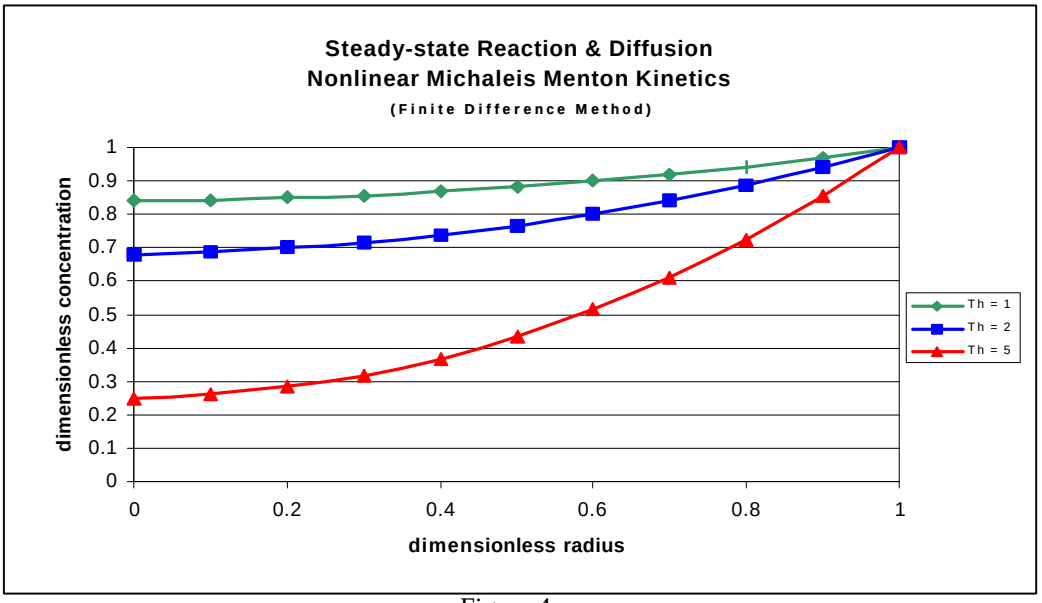



Figure 4

A mathematical expression for the Thiele modulus, the square root of the ratio of the two time scales, is given below.

$$j = R\left(\frac{V_{\max}}{K_M D_{eff}}\right)^{1/2} \tag{7}$$

The two time scales are the one associated with the intrinsic chemical-reaction rate (numerator) and the rate of diffusion through the particle (denominator). The Thiele modulus is a measure of whether the process is reaction controlled (low φ) or diffusion controlled (high φ). As is evident from figure 4, lower Thiele numbers, which are equivalent to lower resistance to mass transfer when compared with resistance to chemical reaction, yield shallower intraparticle concentration gradients, thus higher values at the center of the particle, i.e. r = 0. This has been shown by others conducting similar investigation into concentration profiles as a function of Thiele modulus (1,2,4,10).

Using the steady state assumption and first order kinetics (i.e. $c_s \ll K_M$), the continuity equation reduces to the following dimensionless form.

$$\frac{d^2\bar{c}_s}{d\bar{r}^2} + \frac{2}{\bar{r}}\frac{d\bar{r}}{d\bar{c}_s} = j^2\bar{c}_s \tag{8}$$

An analytical solution to eq. 8 for the dimensionless concentration and effectiveness factor was determined with appropriate boundary conditions for intraparticle diffusion resistance (eq. 3), and is given on the following page [10].

$$\bar{c}_s = \frac{\sinh\left(j * \bar{r}\right)}{\bar{r} * \sinh\left(j\right)}$$

$$h = \frac{3}{j}\left(\frac{1}{\tanh\left(j\right)} - \frac{1}{j}\right) \tag{9}$$

An analytical solution to eq. 8 for the dimensionless concentration and effectiveness factor was determined with appropriate boundary conditions for internal and external mass transfer resistance (eq. 4), and is given below [5].

$$\bar{c}_s = \frac{Bi * \bar{c} * \sinh\left(j * \bar{r}\right)}{\bar{r} * \left[j\ \cosh\left(j\right) - \sinh\left(j\right) + Bi * \sinh\left(j\right)\right]}$$

$$h_G = \frac{\frac{3}{j}\left[\frac{1}{\tanh\left(j\right)} - \frac{1}{j}\right]}{\left[\frac{j}{Bi}\left(\frac{1}{\tanh\left(j\right)} - \frac{1}{j}\right) + 1\right]} \tag{10}$$

Concentration profiles from eq. 9 (internal resistance only) and eq. 10 (internal and external resistance) for a variety of Biot numbers were generated and presented below.

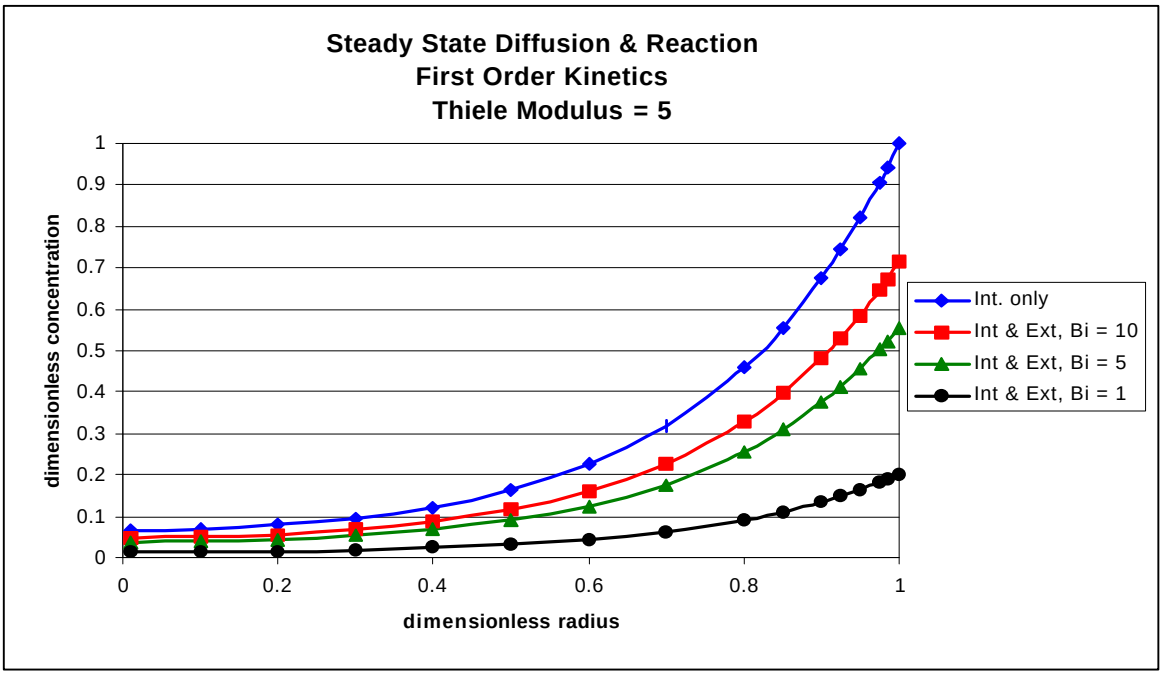



**Figure 5**

Figure 5 indicates that irrespective of mass transfer resistance(s); the concentration profiles demonstrate the classical concave shape facing upward. As expected, the profile for intraparticle resistance only shows a concentration equal to unity at the surface of the particle, thus satisfying the boundary condition. The boundary condition for negligible external transfer resistance corresponds to the case when Bi infinity. The Biot number ($Bi = kg*R/D_{eff}$) is a dimensionless parameter that expresses the relative extent of external and internal diffusional resistances. Investigating figure 5 shows that higher Biot numbers correspond to higher concentrations at the surface of the particle (i.e. r = 1). This trend was anticipated, because higher Biot numbers are associated with lower external mass transfer resistances, so resistance to molecular transport within the bead becomes dominant.

It is an important objective to determine the effectiveness of the immobilized enzyme. An effectiveness factor ($\eta$) was introduced, which is defined in eq. 9 as the ratio of the observed reaction rate and the rate in the absence of intraparticle concentration gradient. In eq. 10, the global effectiveness factor ($\eta_G$) is defined as the ratio of the reaction rate with internal mass transfer resistances ($\eta$) and the reaction rate with external mass transfer resistances through the boundary layer. A plot of $\eta$ as a function of $\varphi$ for the steady state reaction and diffusion problem with first order kinetics is shown in Figure 6.

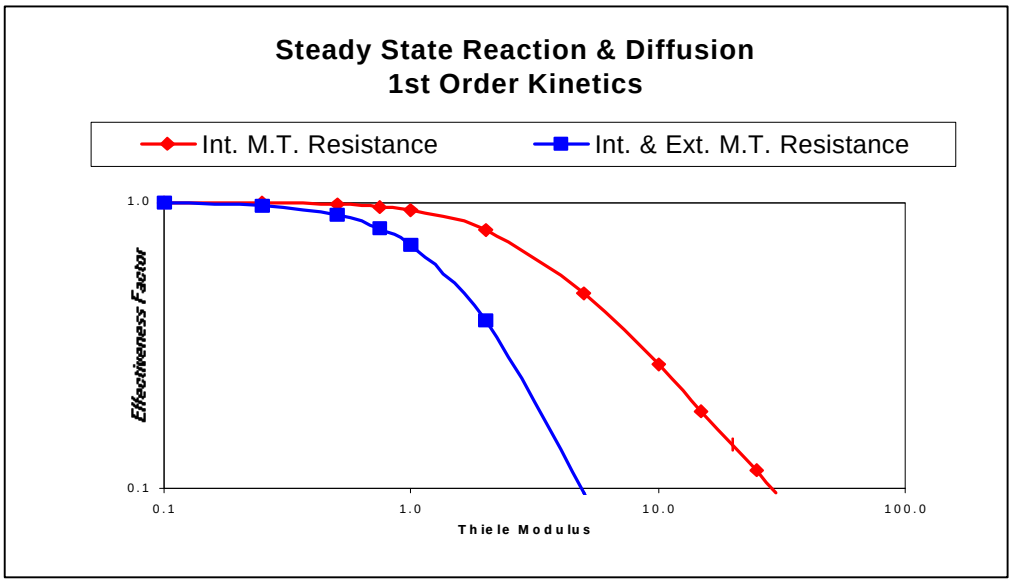



**Figure 6**

Figure 6 indicates the effectiveness factor approaches unity as the Thiele modulus approaches zero. This trend was expected because as the Thiele modulus approaches zero, the process becomes reaction controlled, observed rate of reaction equals the intrinsic rate of reaction (i.e. $\eta = 1$), and the resistance to mass transfer is no longer a factor. This approach was used to study kinetics properties of invertase immobilized on a bead surface [2,3]. In the other extreme, as the Thiele modulus approaches infinity, the limitations due to diffusion become dominant. Both cases are limiting/extreme cases and are rarely seen in actual porous particles.

The concentration profiles for Michaelis-Menton kinetics determined via finite difference method and first order kinetics ($c_s \ll K_M$) via analytical solution under the same conditions were of interest. The comparison was put in graphical form and presented in Figure 7.

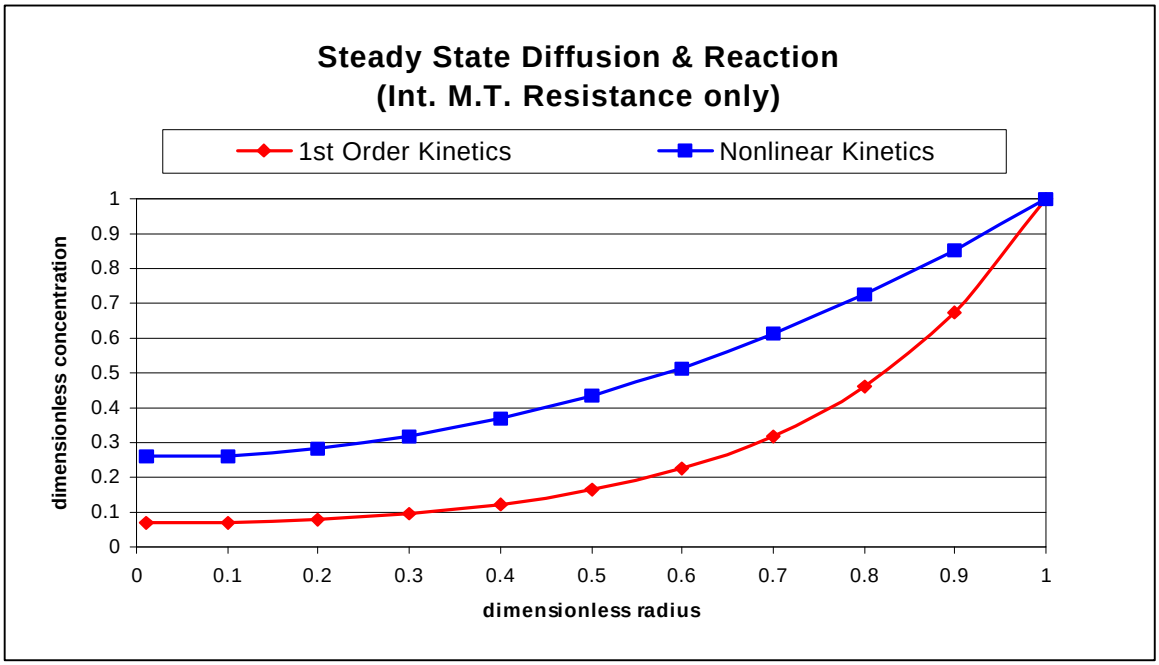



**Figure 7**

Figure 7 indicates a difference in concentration profiles for first order and Michaelis-Menton kinetics. This means that care should be taken when the assumption of low substrate concentration is made. The cellulose-cellulase reaction network experimentally obeys the Michaelis-Menton rate expression and should be modeled accordingly. Certain immobilized enzyme systems will correlate with first order kinetics; however, Michaelis-Menton kinetics is the preferred choice when describing general enzyme reactivity.

**References**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