On the Numerical Computation of Enzyme Kinetic Parameters [1]

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Abstract

We consider the enzyme kinetic reaction scheme originally proposed by V. Henri of single enzyme-substrate dynamics where two fractions of the enzyme—free and bound—are involved. Henri's scheme involves four concentrations and three rate constants and via the mass action law it is translated into a system of four ODEs. In two case studies we demonstrate how the rate constants can be computed whenever time course experimental data are available. The obtained results are compared with analogous results implied by the classical Michaelis-Menten model. Our approach focuses on the uncertainties in the experimental data, as well as on the use of contemporary computational tools such as CAS *Mathematica*.

Keywords: enzyme kinetics, biomass-substrate-product dynamics, computation of rate constants, ODE systems, uncertainties, interval methods, verification methods, CAS Mathematica

1 Introduction

We study mathematically and computationally time course experimental data for the dynamics of fermentation processes related to waste-water denitrification [2], [19], paying special attention to the measurement errors involved. We describe and motivate our modelling approach applying the biochemical reaction scheme of the simple enzyme-substrate dynamics where two fractions of the enzyme (free and bound) are involved. Our approach is also applied to a set of available experimental data for the dynamics of acetylcholine hydrolysis by acetylcholinesterase [29].

We focus on contemporary computational tools that are available for dealing directly with time course experimental data, so that there is no need to make use of approximate models. In particular, we demonstrate some *Mathematica* tools allowing for the estimation of the rate parameters of the ODE system by means of appropriate fitting of the system solutions to available time course experimental measurement data.

2 Enzyme kinetic basic models

2.1 Henri-Michaelis-Menten reaction scheme

Scientists studying enzymatic processes by the end of the 19th century have initially tried to explain the dynamics of the substrate uptake during fermentation by means of the simple chemical catalist reaction scheme:

$$S + E \xrightarrow{k} P + E, \tag{1}$$
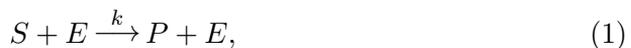
wherein S is the substrate, E is the enzyme and P is the product. Applying the mass action law, the above kinetic scheme leads to the following differential equation for the substrate concentration $s = [S]$:

$$\frac{ds}{dt} = -kes. \tag{2}$$

Assuming that the concentration $e = [E]$ of the enzyme is (nearly) constant the above differential equation leads to a solution for the concentration s of the substrate which is an exponential decay. Such a solution sometimes deviates from the experimental data e.g. when the enzyme concentration is much smaller than the substrate one. In such situations the substrate

s changes almost linearly (with constant rate) for the most part of the fermentation process, cf. Fig.1. However, in other situations the substrate experimental curves may be closer to the solution of (2).
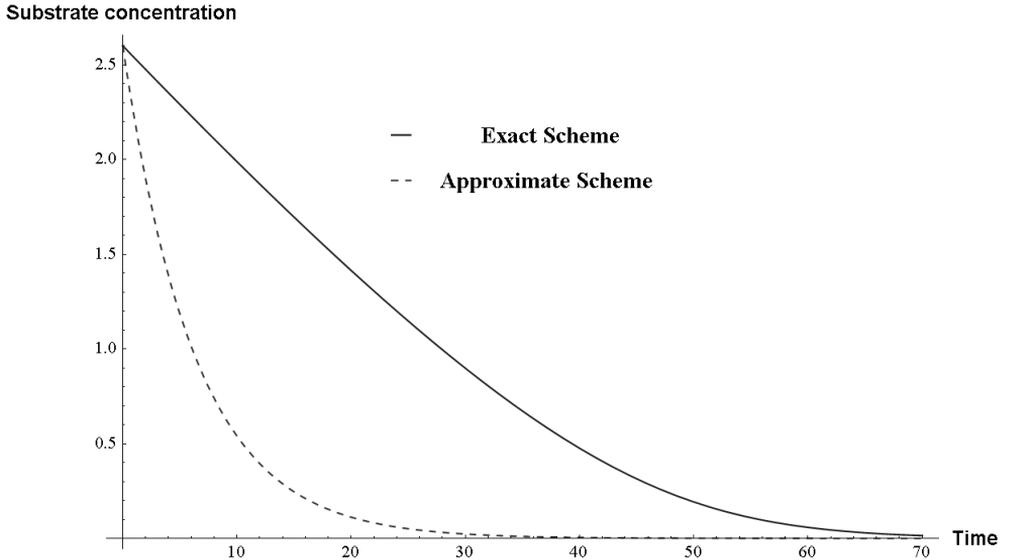

Fig 1

Figure 1: Substrate dynamics according to the approximate scheme (1) and the exact scheme (3). The rate constants of (3) are $k_1 = 2.62, k_{-1} = 0.1, k_2 = 1.25$, initial conditions: $s_0 = 2.6, e_0 = 0.06$; the parameters of (1) can be evaluated to $K_m = (k_{-1} + k_2)/k_1 = 0.51526, V_{max} = k_2 e_0 = 0.075$

The observed discrepancy between the experimental data and expected theoretical solution based on the kinetic scheme (1) has lead Victor Henri [11]–[14], [27] to the following more involved reaction scheme:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} SE \overset{k_2}{\longrightarrow} P + E. \tag{3}$$
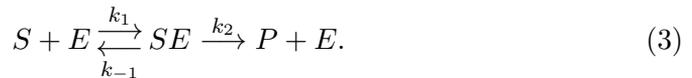
Reaction scheme (3) describes the reaction mechanism between an enzyme E with a single active site and a substrate S, forming reversibly an enzyme-substrate complex SE, which then yields irreversibly product P. Henri's

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reaction scheme (3) says that during the transition of the substrate S into product P the enzyme E bounds the substrate into a complex SE having different properties than the free enzyme and thus necessarily considered as a separate substance.

2.2   Michaelis-Menten equation

Applying the mass action law to Henri's reaction scheme (3) one obtains a general dynamical system, which under the so-called quasi-steady-state assumption [18], [20], [21], [22] leads to the following reaction equation for the substrate rate ds/dt:

$$\frac{ds}{dt} = -\frac{V_{max}s}{K_m + s}.$$ (4)

The quasi-steady-state assumption is applied whenever the ratio [E]/[S] is small so that the fermentation process has a considerably long time interval during which the concentration [ES] of the bound enzyme is constant [28].

In their seminal paper Michaelis and Menten discussed in detail Henri's reaction scheme and equation (4) which became known as the Michaelis-Menten equation (MM-equation) [17], see also [15]. In addition Michaelis and Menten discussed at length the meaning of the rate constants in Henri's reaction scheme and proposed a protocol for the practical calculation of the constant K_m in (4). The constant K_m is known as Michaelis constant (for the history of these investigations see [5], [26].

The MM-equation (4) can be written in the form

$$\frac{ds}{dt} = -\frac{V_{max}s}{K_m + s} = -\frac{V_{max}}{K_m/s + 1},$$

showing that for large values of s the right-hand side is close to the constant $-V_{max}$; hence the uptake rate is almost constant (zero-order kinetic).

Michaelis-Menten equation (4) is simple, can be easily used by non-mathematicians; the protocol for calculation of the Michaelis constant K_m suggested in [17] has been later modified [16] and is still used in practice.

However, the MM-equation (4) gives good approximation only under certain conditions [9], [28]. Thus the condition $e_0 << s_0$ assures good approximation and is ubiquitous for many fermentation processes, but is

not present e.g. in living cells [25]. Next we propose methods and tools for the computation of the Michaelis constant based on the general system of differential equations induced by Henri's reaction scheme (3).
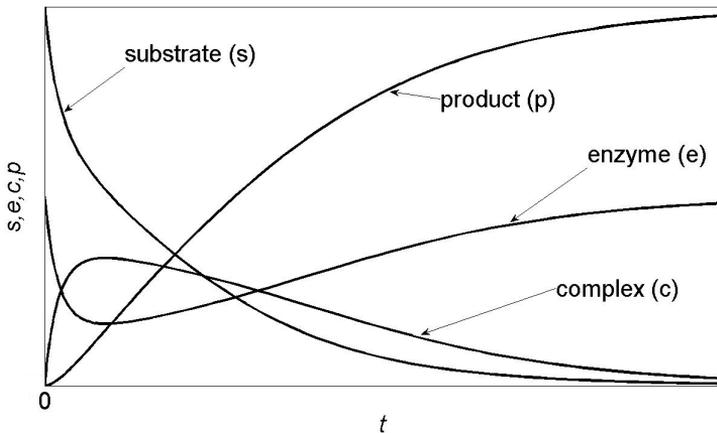
2.3 Enzyme kinetics induced by Henri's reaction scheme

Figure 2: Graphics of the solutions of system (5)

Denote the concentrations $s = [S]$, $e = [E]$, $c = [SE]$, $p = [P]$. Applying the Mass Action Law to Henri's reaction scheme (3) we obtain the general ODEs system:

$$
\begin{aligned}
\frac{ds}{dt} &= -k_1 es + k_{-1} c, & \frac{de}{dt} &= -k_1 es + (k_{-1} + k_2)c, \\
\frac{dc}{dt} &= k_1 es - (k_{-1} + k_2)c, & \frac{dp}{dt} &= k_2 c,
\end{aligned}
\tag{5}
$$

to be further briefly denoted as HMM-system in tribute to the pioneering work of Henri [11]–[14] and Michaelis and Menten [17].

Remark. Note that the Mass Action Law applied to any reaction scheme induces an ODE system in an unique way. For example, in the case of system (5) the first equation for s says that the rate of change of the concentration $[S]$ is made up of a loss rate proportional to $se = [S][E]$ and a gain rate proportional to $c = [SE]$.

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If the rate constants k's are known, then the HMM-system (5) can be treated as an initial ODE problem with initial conditions $s(0) = s_0 > 0$, $e(0) = e_0 > 0$, $c(0) = 0$, $p(0) = 0$. However, in practice these constants are not known and have to be found. The contemporary approach to this task is to consider the rate constants as parameters in the HMM-system (5) and to compute them by fitting the solutions of the system to available time course experimental data, a problem to be considered in the next section.

The graphics of the solutions of the HMM-system (5) for a particular set of initial values and rate constants are presented in Fig. 2.

Substrate concentration
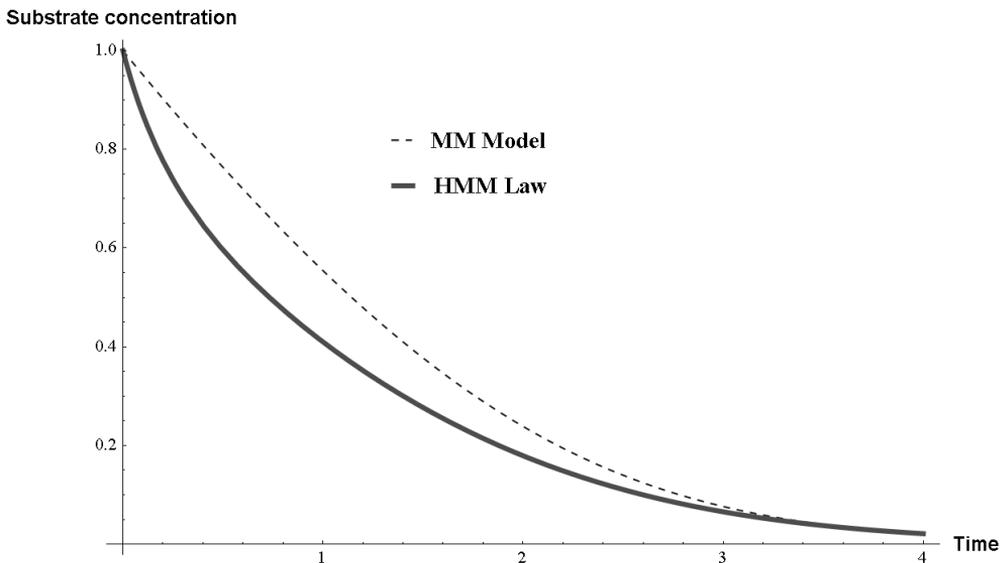
-- MM Model

— HMM Law

Figure 3: Graphics of the substrate dynamics according to MM-model (4) and HMM-system (5). The rate constants of (5) are $k_1 = 2.62, k_{-1} = 0.1, k_2 = 1.25$, initial conditions: $s_0 = 1, e_0 = 1.5$; the parameters of (4) can be evaluated to $K_m = (k_{-1} + k_2)/k_1 = 0.51526, V_{max} = k_2 e_0 = 1.875$

In Fig.3 the substrate uptake s is presented in two different ways. The two graphics present the approximate solution for s to MM-model (4): $ds/dt = -V_{max}s/(K_m + s)$ as well as the "true" solution of the HMM-system (5).

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In order to correctly compare the two solutions one has to establish certain consistency relations between the parameters in the MM-equation and the HMM-system. The presented solutions in Fig. 3 make use of the familiar relations:

$$\begin{aligned} V_{max} &= k_2 e_0 \\ K_m &= (k_{-1} + k_2)/k_1, \end{aligned} \qquad (6)$$

induced by the derivation of the MM-equation from the HMM-system using the quasi-steady-state assumption, cf. e.g [18].

The following numerical computations show how different the approximate substrate concentration solution s to the MM-equation may look like depending on the value of the ratio of the initial values of the substrate (s_0) and the enzyme (e_0).

2.4 Numerical computations of enzyme kinetic models with various values for the ratio e_0/s_0

In this subsection we present the computational results of three numerical examples for the comparison of the substrate dynamics of the two models (4), (5) for different values of the ratio e_0/s_0. The values of the initial conditions and the values of the rate constants $k_i, i = -1, 1, 2$ in (5) are chosen to be the same for all three examples. The initial values and the rate parameters in (4) are consistent with those in (5); the relations (6): $V_{max} = k_2 e_0, K_m = (k_{-1} + k_2)/k_1$ have been used.

For completeness we also include the graphics of the solution of simple decay equation (2). The rate constant k in the decay equation is chosen to be equal to k_1 and the enzyme concentration e in the right-hand side is fixed as $e = e_0$.

Example 1. The first numerical example shows how close the solutions for the substrate to the MM-equation and the HMM-system can be when e_0/s_0 is "small" (Figure 4).

The values of the parameters used in models (4), (5) are as follows: initial conditions are $s_0 = 2.6, e_0 = 0.06$; everywhere below $c_0 = 0, p_0 = 0$. The values of the rate parameters used in (5) are: $k_1 = 2.62, k_{-1} = 0.1, k_2 = 1.25$. The values of the parameters in (4) are $V_{max} = k_2 e_0 = 0.075, K_m = (k_{-1} + k_2)/k_1 = 0.51526$.
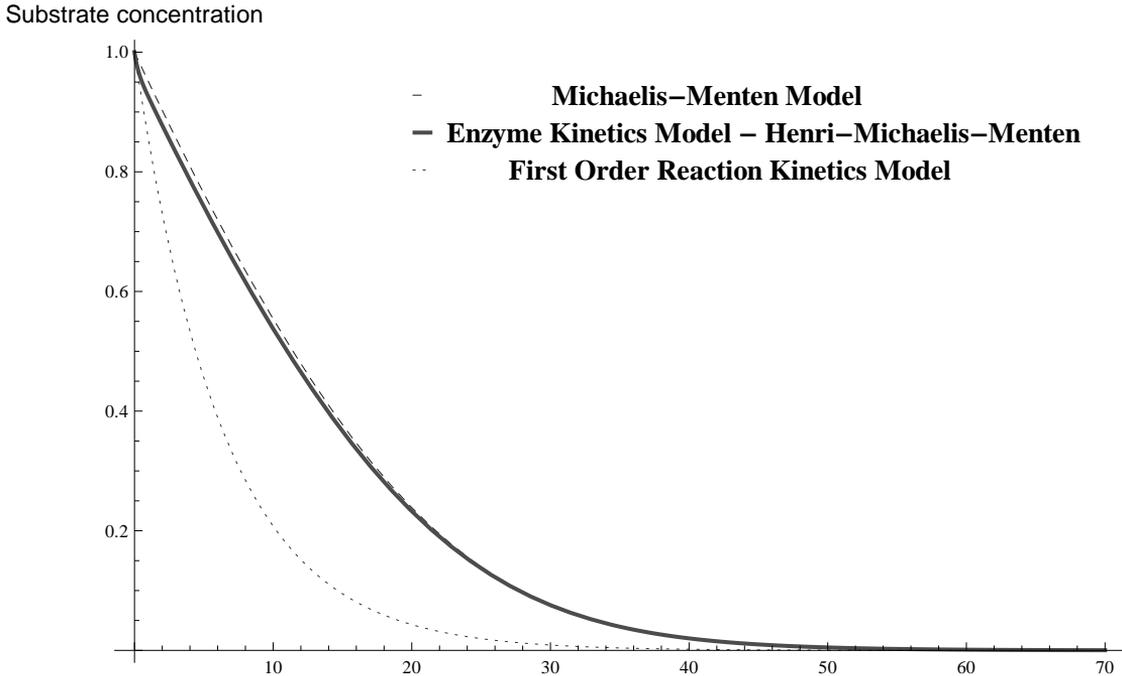
Figure 4: The substrate solutions of (4), (5) for $e_0 << s_0$

From this numerical example we conclude that when the condition $e_0 << s_0$ holds then the MM-model (4) can be a good approximation of the "true" HMM-model (5). The form of the solutions for the substrate concentrations suggest the hypothesis that whenever the condition $e_0 << s_0$ holds then the uniform distance between the two solutions is of the order of the ratio $\epsilon = e_0/s_0$. As we know the MM-equation has been derived from the HMM-system under the quasi-steady-state assumption involving the condition ϵ close to zero.

Note that in this example the Michaelis constant K_m used for the computation of the approximate MM-solution is derived from the coefficients k_i in the exact HMM-model. This means that both models describe the process dynamics using equivalent rate constants and, since we consider the HMM-model to be true, this implies the approximate model is also valid

under the assumption $e_0 << s_0$. Our next two numerical examples aim to demonstrate what happens whenever this assumption does not hold.

Example 2. Our second numerical example shows how the substrate solutions start to deviate when s_0 and e_0 are close to each other (Figure 5).
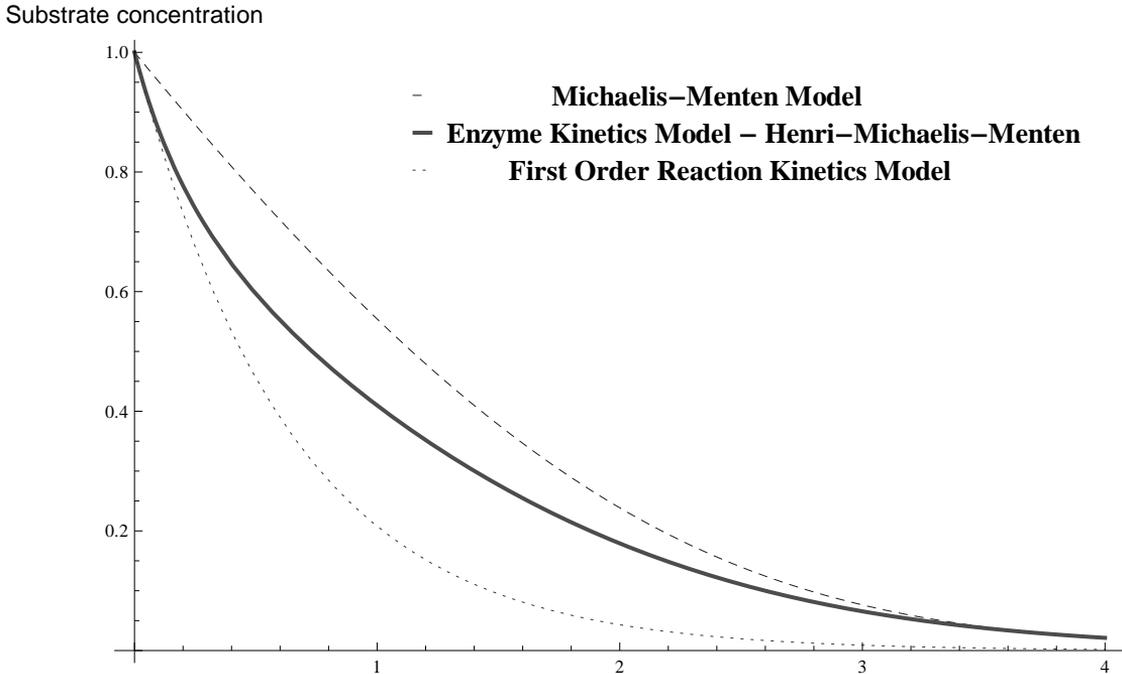
Substrate concentration

Figure 5: The substrate solutions of (4), (5) for $e_0 \sim s_0$.

The values of the parameters used for this example are as follows: $s_0 = 1, e_0 = 0.6, k_1 = 2.62, k_{-1} = 0.1, k_2 = 1.25$. As before, using using (6) we obtain: $V_{max} = k_2 e_0 = 0.75, K_m (k_{-1} + k_2)/k_1 = 0.51526$.

Example 3. In this numerical example $e_0 > s_0$. The values of the parameters used for the given solution are as follows: initial values $s_0 = 1, e_0 = 1.5$; rate constants $k_1 = 2.62, k_{-1} = 0.1, k_2 = 1.25$. Calculated as above, we have $V_{max} = k_2 e_0 = 1.875, K_m (k_{-1} + k_2)/k_1 = 0.51526$.

One can observe that the HMM-solution for the substrate concentration

is even closer to the exponential decay solution (Figure 6).
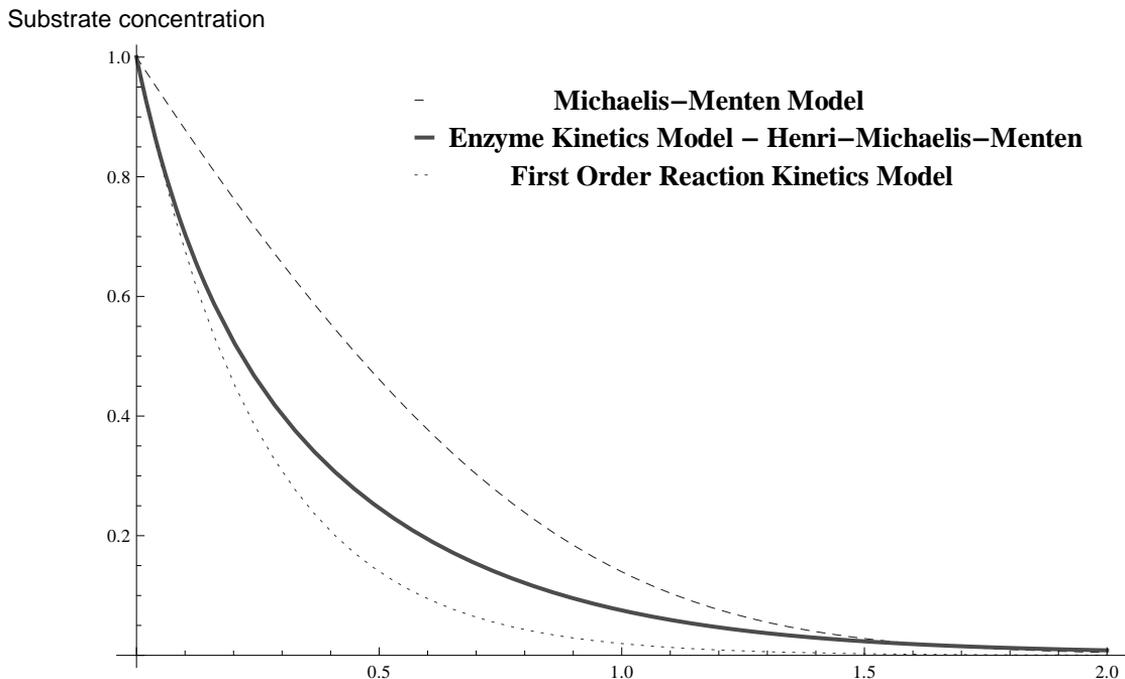
Substrate concentration

Figure 6: The substrate solutions of (4), (5) for $e_0 > s_0$

Examples 2 and 3 clearly show that the MM-model's solutions are far from those of the exact HMM-system despite taking care of the consistency of the parameters used in (4) and (5). Such a discrepancy between the two solutions can be expected as the condition ϵ close to zero used for the derivation of the approximate MM-model has been violated.

In order to study the dynamics of the fermentation processes, we next focus on the HMM-system. Our goal is to obtain a good fit of the HMM-system to available time course experimental data keeping in mind the measurement errors contained in the data. The model parameters obtained from the fit of the experimental data are then compared to rate constants from the literature corresponding to the same physical processes. This

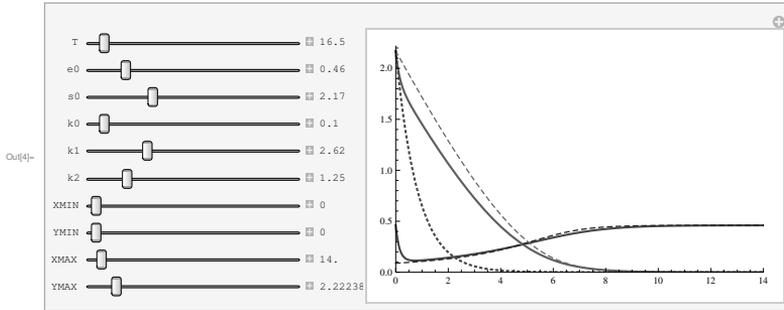allows us to also verify the correctness of the dynamics suggested by the model.

Figure 7: An example of the usage of dynamical numeric solution of the systems and their graphical representation. The plots are prepared using CAS *Mathematica*

3 Computation of the rate parameters of the HMM-system using time course experimental data

3.1 Fitting the HMM-system against experimental data of acetylcholine hydrolysis

The following plots demonstrate the process of model-fitting against experimental data using the HMM system. The physical process we use as an example is the acetylcholine hydrolysis by acetylcholinesterase [29]. Experimental data has been obtained for the substrate, product and enzyme concentrations over the time course of the experiment. We take into account the measurement errors in the data and they are displayed as concentration intervals for each point in the plots.

The experimental data has been obtained using the rate constants from the Results section (Hydrolysis of ACH + ACHE2, HXA method) of [29] in the following way - the solutions of (5) were computed with the given rate constants. We evaluated the solutions $s(t), e(t), p(t)$ for a number of time points t_i and we added a certain amount of noise to them in accordance to the standard deviations given in [29].

Our computational problem can be formulated as follows. Given time course experimental data (together with measurement errors) for the substrate, enzyme and product concentrations find values for the parameters k_{-1}, k_1, k_2 and initial values E_0, s_0 such that the solution of the HMM system (5) fit well against the experimental data and possibly fit into the measurement intervals. Our procedure for solving this problem passes in two stages: first we find an initial rough "guess" for the parameter values, then we consecutively improve the parameter set, resp. the solutions, using some optimization possibilities of the numerical computing environment *MATLAB*. More precisely, we've used *MATLAB*'s *lsqnonlin* procedure (optimization algorithm defaults to Trust-Region-Reflective Algorithm), where the minimized function 1) computes the enzyme kinetics model solutions (using *ode23* or *ode23s* solvers) for a given set of rate constants and initial conditions (optimization procedure parameters), 2) evaluates the solutions for the time points t_i corresponding to the observations and 3) subtracts the experimental data from them.

The initial parameter "guess"-values are obtained using the *Manipulate* and *NDSolve* functions of CAS *Mathematica* in order to get a rough fit of the experimental data, Fig. 7. These values are used only as an initial guess in the optimization procedure. The solution of the ODEs HMM-system (5) with initial guess parameters is shown on Figure 8. The values of the parameters used for the given solution are as follows: $k_1 = 25000\text{M}^{-1}s^{-1}, k_{-1} = 0s^{-1}, k_2 = 10s^{-1}; s_0 = 2.5 * 10^{-3}\text{M}, e_0 = 5.4 * 10^{-8}\text{M}$.

The results of the optimization procedure we applied using the initial parameters can be seen on Figure 9 and Figure 10. Figure 9 represents the slow process [4] (the synthesis of products; the process is governed by the rate constant k_2 and it's the focus of the Michaelis-Menten approximation), while Figure 10 represents the fast process (described by enzymes binding to substrate in order to form a complex; the process is governed by the rate constants k_1, k_{-1}).

The values of the parameters used for the given solution are as follows: $k_1 = 16847\text{M}^{-1}s^{-1}, k_{-1} = 7s^{-1}, k_2 = 12s^{-1}; s_0 = 2.5 * 10^{-3}\text{M}, e_0 = 5.4 * 10^{-8}\text{M}$. Using the parameter set we have found to solve the HMM-system we get solutions that fit nicely the experimental data we started with.

The Michaelis constant can be derived from the optimal parameters in the HMM-system, using that $K_m = (k_{-1} + k_2)/k_1$, which gives us $K_m =$
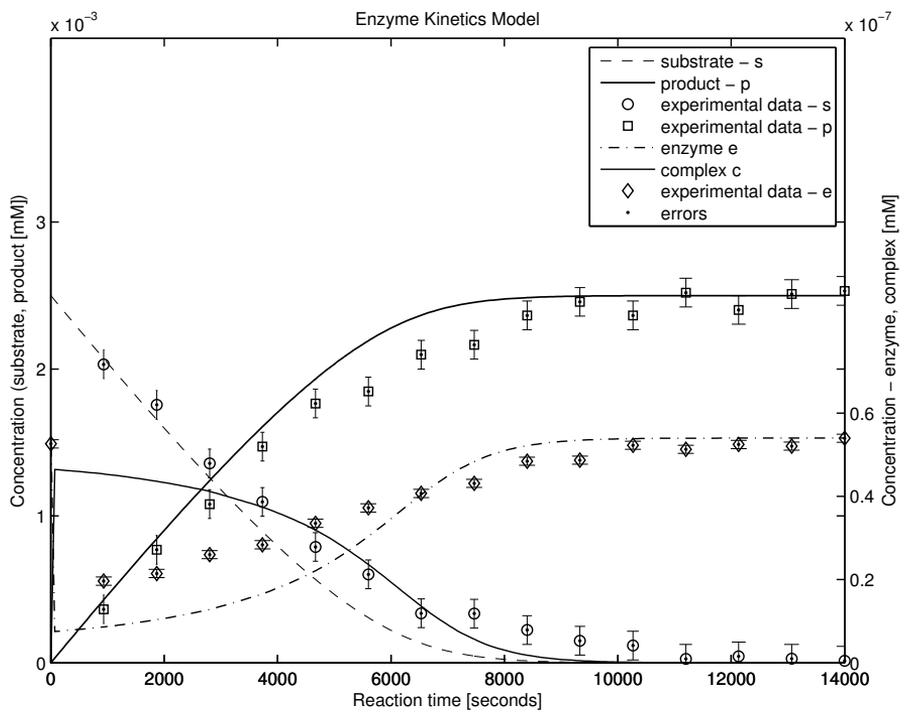
Figure 8: The numerical solution of the HMM-system with an initial parameter "guess" set of the unknown parameters
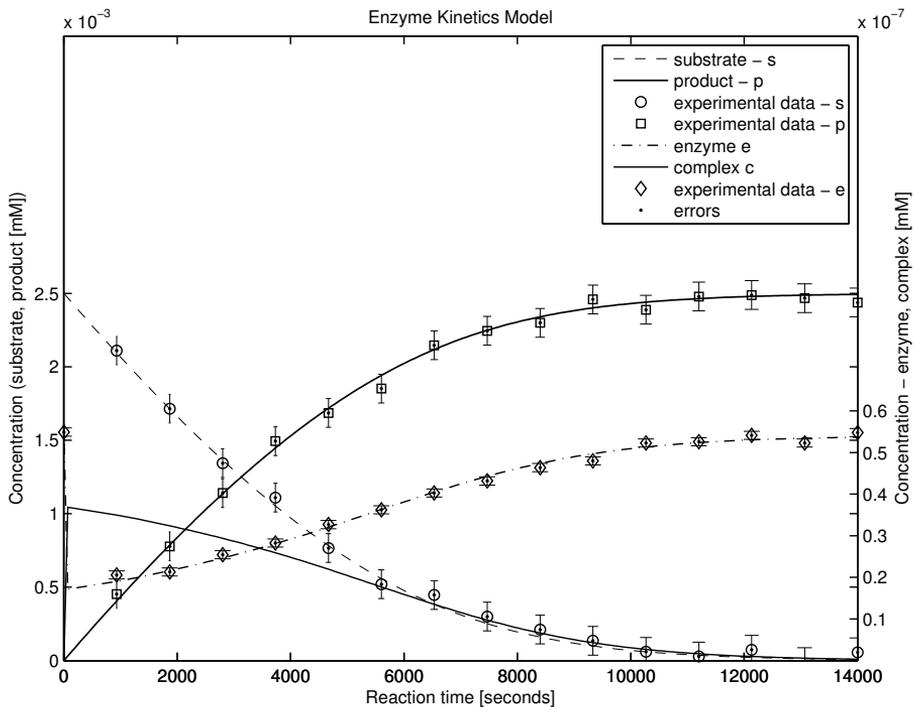
Figure 9: The numerical solution of the HMM-system (5) after the fitting of the model

0.00112M. This value is close to known values of Michaelis constant from other sources ([6], [10]). We can conclude that the model estimates correctly the behavior of the underlying biochemical process.

The MM-model (4) on the other hand provides very different results for the same experimental data (Figure 11). Although the obtained parameters ($V_{max} = 7.12 * 10^{-7}$Ms^{-1}; $K_m = 2.193 * 10^{-3}$M) are relatively close to the real ones, the solutions do not pass through the intervals of the data. As in 2.4, Example 2 and Example 3, the approximate model's solutions deviate from the real ones even though its parameters may be close to the exact model's rate constants. This can easily be explained by the fact that the complex c has more complicated dynamics (see Figure 9 for the dynamics of c and e) than what the MM-model can estimate. A rather good fit using
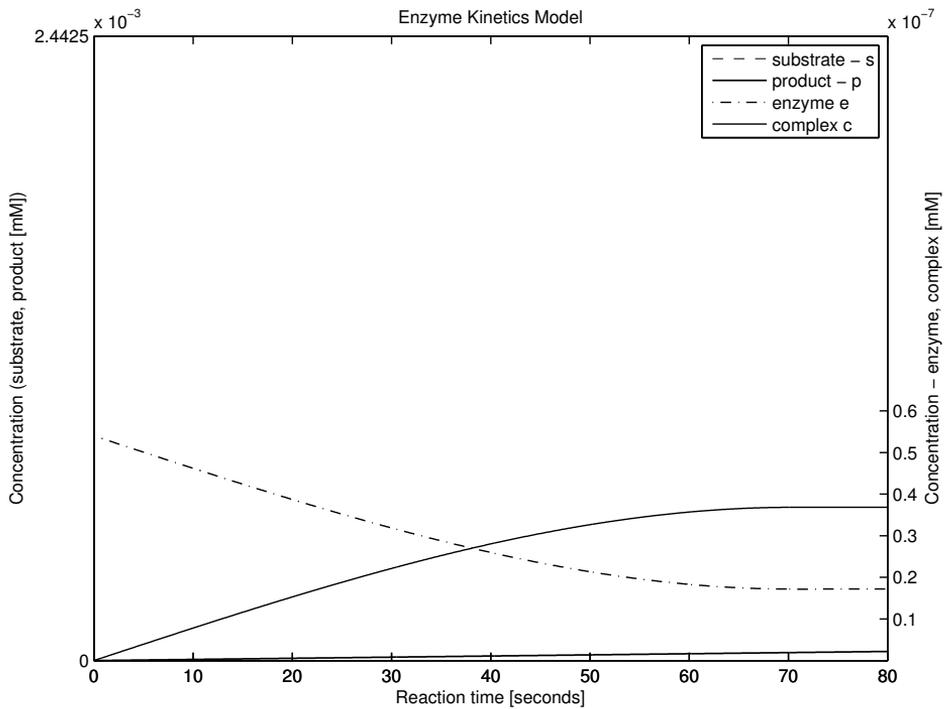
Figure 10: The numerical solution of the "fast" reaction. The solutions of the substrate and the product are barely visible on the graphic due to their limited change from the initial values.

the same approximate model (4) can be achieved by omitting the first observation data point of the enzyme (Figure 12). The values of the model parameters ($V_{max} = 6.6 * 10^{-7}\mathrm{M}s^{-1}$; $K_m = 1.26 * 10^{-3}\mathrm{M}$) are even closer to those found using the HMM model. In general, we cannot expect data points related to the "faster" reaction (taking place during the t_c timescale) to be approximated well with the MM-model because it is entirely focused on the "slower" reaction and the solutions we get cannot possibly follow the dynamics during both timescales.
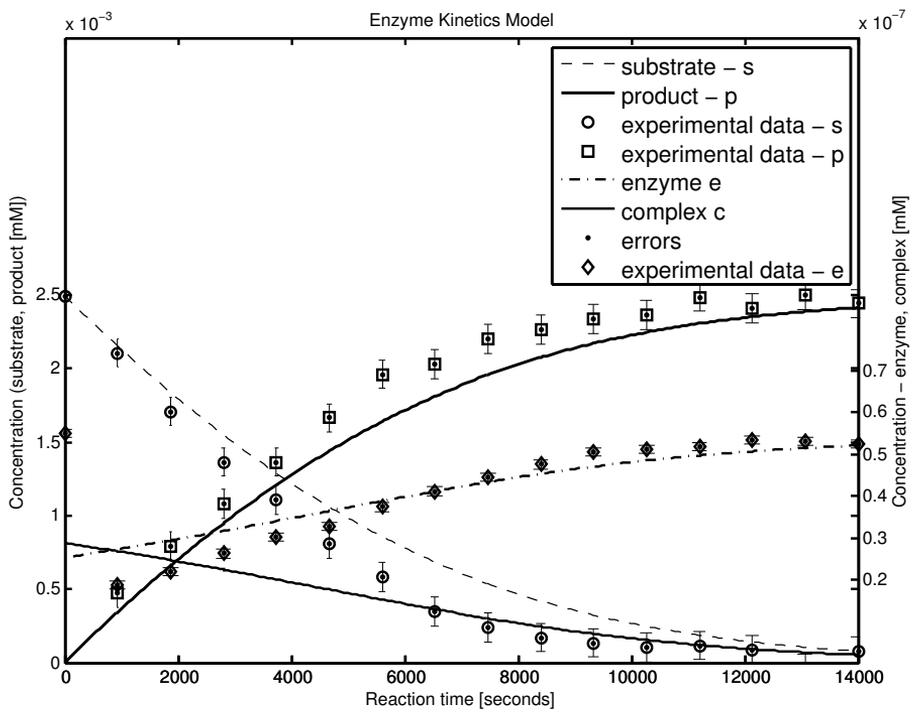
Figure 11: The numerical solution of the MM-model after fitting it to the experimental data
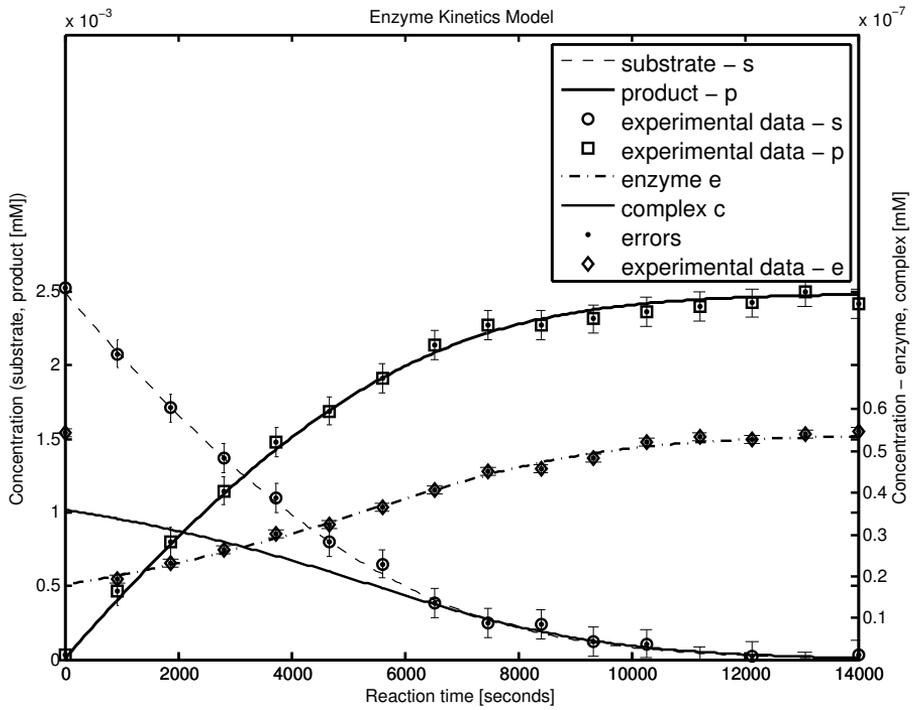
Figure 12: The numerical solution of the MM-model after fitting it to the experimental data. The first experimental data point for the enzyme has been omitted from the optimization.

3.2 Fitting the model against experimental data of biochemical nitrate reduction

Our modeling approach has been applied to experimental data of biochemical nitrate reduction [19], which consists of observations for the substrate variable. The measurement errors are in the range 5% to 7%. The solutions of the HMM-system and the MM-model are compared in regard to the wellness of the fit they provide for the experimental data as well as the correctness of the obtained rate constants in the context of the underlying process dynamics.
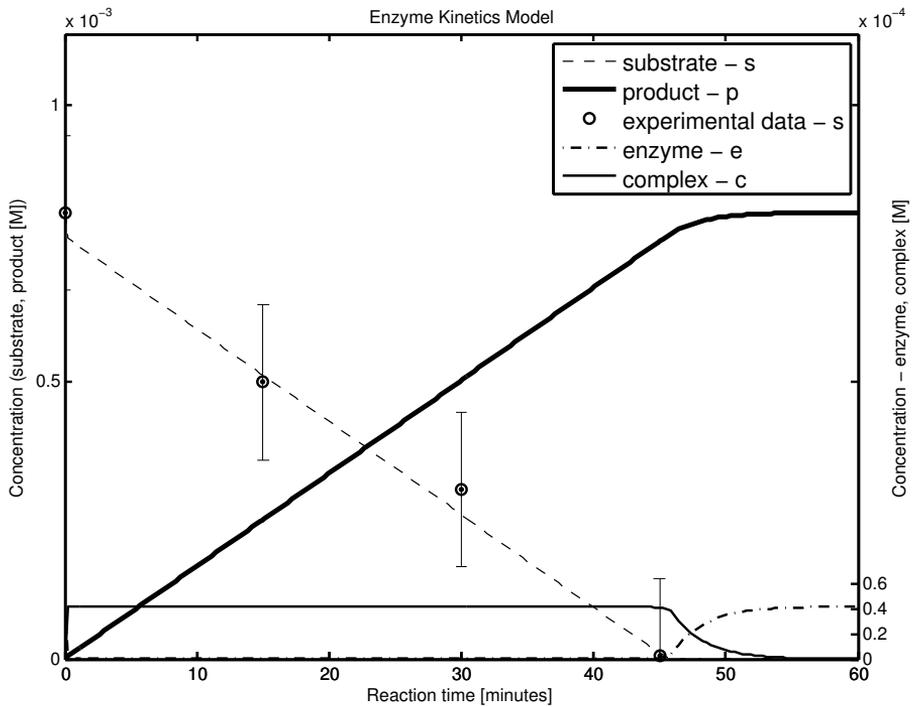

Figure 13: The numerical solution of the HMM-system after fitting the model to the experimental data

The values of the optimized HMM-system parameters are: $k_1 = 10^6$ M^{-1} min^{-1}, $k_{-1} = 0 min^{-1}$, $k_2 = 0.4 min^{-1}$; $s_0 = 0.8 m$M, $e_0 =$

$0.042m$M. The numerical solutions for the substrate, enzyme, complex and product are displayed on Figure 13. The Michaelis constant derived from the above mentioned parameter values is $K_m = 0.4\mu$M, and $V_{max} = 0.0168$ mM min^{-1}.
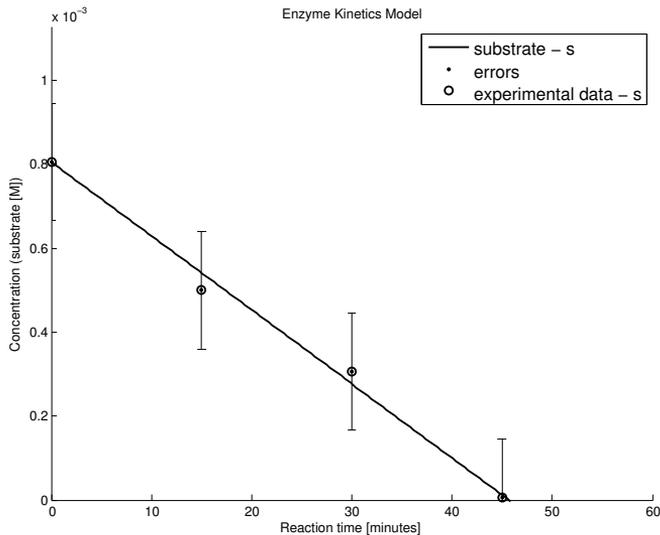

Figure 14: Nitrate reduction fit according to the MM-model

The result of the MM-model fitting is displayed on Figure 14. The values of the optimized parameters are: $V_{max} = 0.017658$ mM $min^{-1}; K_m = 0.1\mu$M.

As we can see from Figure 13 the dynamics of the process in this case study is in compliance with the assumptions of the MM-model, namely that the complex c and the enzyme e are in equilibrium. The obtained parameters from both the HMM-system and the MM-model are close to each other. Although the number of experimental data points for the case study is very limited, we may still conclude that both models under consideration can be suitable for studying the underlying process.

In another paper [19] the value of K_m was estimated as $0.04m$M.

4 Concluding remarks

The present paper is devoted to computational experiments for the classic Henri-Michaelis-Menten enzyme kinetic scheme and the approximate Michaelis-Menten model, derived under the quasi-steady-state assumption. A comparison of the results from the two models with experimental data is also shown. The given examples demonstrate the advantages of the exact model in comparison to the approximate models. The topic has been thoroughly analyzed by other authors [31] in terms of the magnitude of the error of the approximate models and the conditions under which they are adequate. In this paper we present an approach of numerical analysis applying the model proposed by V. Henri, while using contemporary software tools.

There are many benefits of using the Henri's reaction scheme when studying biological processes. Even complex systems (e.g. metabolic networks) with many reactants or consisting of several interconnected Michaelis-Menten reactions can be modeled accurately in contrast to simpler models where many approximations may contradict with the behavior of the whole system. Working with models that follow directly from physical/chemical laws allows for deeper, more serious analysis of the process due to the level of detail they provide over the reactions that take part in it. For example, all rate constants k_i have been obtained for the considered models part of our case studies which could be essential to subsequent analysis. Furthermore, the parameters of the approximate model can also be obtained from these rate constants, allowing us to validate our results against known values of the classic Michaelis constant.

Such approximate models may have been used with more care in the past since they are computationally intensive when it comes to numerical experiments, but this should hardly be considered an issue nowadays. There are also very rich software products which provide powerful tools for the numerical experiments and analysis of complex physical processes.

A topic worth further analysis as a potential future direction would be whether we could fit a time series using Calder's sigma-isocline [30] instead of the QSSA.

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