A GENERAL MODEL OF REACTION KINETICS IN BIOLOGICAL SYSTEMS

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Abstract

Dynamic mathematical models in biotechnology require, besides the information about the stoichiometry of the biological reaction system, knowledge about the reaction kinetics. Modulation phenomena like limitation, inhibition and activation occur in different forms of competition with the key enzymes responsible for the respective metabolic reaction steps. The identification of *a priori* unknown reaction kinetics is often a critical task due to the non-linearity and (over-)parameterization of the model equations introduced to account for all the possible modulation phenomena. The contribution of this paper is to propose a general formulation of reaction kinetics, as an extension of the Michaelis-Menten kinetics, which allows limitation/activation and inhibition effects to be described with a reduced number of parameters. The versality of the new model structure is demonstrated with application examples.

1 Introduction

The dynamic model of a perfectly stirred tank bioreactor is usually derived from mass balances, which lead to a differential equation system for the concentration vector $\vec{c} \in \mathbb{R}^n$:

$$\frac{\mathrm{d}\vec{c}(t)}{\mathrm{d}t} = K\vec{r}\left(\vec{c}(t)\right) - \vec{c}(t)\, D(t) + \vec{u}(t)\,; \quad \vec{c}(t_0) = \vec{c}_0. \quad (1)$$

The matrix $K \in \mathbb{R}^{n\times m}$ contains the information on the stoichiometry of the reaction system and is usually time-invariant. The vector $\vec{r} \in \mathbb{R}^m$ contains the reaction rates of each individual reaction and is usually a non-linear vector function of the concentrations. $D \in \mathbb{R}$ is the renewal (or dilution) rate and $\vec{u} \in \mathbb{R}^n$ contains the reactor input conditions.

There is a large variety of mathematical descriptions of the reaction kinetics available in the literature, most of them adapted heuristically to a specific phenomenon and often similar one to each other. A rather extensive list of models is given in [3].

Mathematical modelling of biological reaction systems is a difficult task, when little *a priori* knowledge about the stoichiometric coefficients and the kinetics is available. A systematic approach is therefore necessary to find the best model structure and the best values of the model parameters with respect to some imposed criterion. For instance, in terms of model identification, the optimal structure is characterised by minimal correlations between parameters and maximal identifiability properties. In terms of state estimation and control, however, simplicity and (non-)linearity play important roles.

A model identification strategy has recently been proposed by Bogaerts [5], which – under certain conditions – decouples the estimation of stoichiometry and kinetics within a macroscopic modelling approach. Therefore, both model components can be identified independently of each other. However, the formulation of the kinetic model used by Bogaerts [5] departs from the commonly accepted models (exponential functions instead of rational models) and does not allow all the modulation phenomena to be parcimoniously represented.

Following the above-mentionned identification strategy, this contribution addresses the problem of deriving a general formulation of the reaction kinetic model, which would be in agreement with widely-used classical expressions, and which would allow the description of the main limitation/activation and inhibition effects with a minimum number of parameters.

This paper is organised as follows: In section 2, some commonly used models are introduced and discussed. A general modelling approach for limited and inhibited reaction kinetics is proposed in section 3 and its properties are discussed. The potential of the new formulation is illustrated by means of two examples in section 4. Section 5 concludes this paper.

2 Classical Models

The most famous model is certainly the phenomenologically based approach of Monod [7], which was found for the growth of bacteria cultures on a single substrate:

$$\mathrm{S} \xrightarrow{\mu x} \mathrm{X}. \quad (2)$$

It is characterised by a maximum (specific) growth rate $\mu_{\max} \in \mathbb{R}$

$$\mu(s) = \mu_{\max} \frac{s}{s+K}, \tag{3}$$

which is reached with an increasing substrate concentration $s \in \mathbb{R}$ of the limiting substrate according to the constant $K \in \mathbb{R}$, often called half-saturation constant, because $\mu(K) = \frac{1}{2}\mu_{\max}$. Monod's equation (3) is structurally identical to Michaelis-Menten kinetics [2] derived for enzymatically catalysed reactions:

$$\mathrm{S} + \mathrm{E} \rightleftharpoons \mathrm{ES} \xrightarrow{q} \mathrm{P} + \mathrm{E} \tag{4}$$

assuming reaction equilibrium in the first enzymatic step and a rate-limiting irreversible reaction in the second step:

$$q(s) = q_{\max} \frac{s}{s+K_{\mathrm{M}}}. \tag{5}$$

Following the same line of thought, expressions for more complex cases can be derived as shown in table 1.

The presence of modulators (or effectors) also influence the reaction rate. Inhibitors (or negative effectors) form an inactive complex with at least one of the substrates or intermediates in the reaction chain. For the enzymatic system (4), there are already three different patterns leading to the respective equations shown in table 2.

inhibition type	enzymatic reaction path	kinetic expression
competitive	$\mathrm{E} + \mathrm{I} \rightleftharpoons \mathrm{EI}$	$q = \frac{q_{\max} s/K_{\mathrm{S}}}{1+s/K_{\mathrm{S}}+i/K_{\mathrm{I}}}$
uncompetitive	$\mathrm{ES} + \mathrm{I} \rightleftharpoons \mathrm{ESI}$	$q = \frac{q_{\max} s/K_{\mathrm{S}}}{1+s/K_{\mathrm{S}}(1+i/K_{\mathrm{I}})}$
non-competitive	$\mathrm{E} + \mathrm{I} \rightleftharpoons \mathrm{EI}$ $\mathrm{ES} + \mathrm{I} \rightleftharpoons \mathrm{ESI}$ $\mathrm{EI} + \mathrm{S} \rightleftharpoons \mathrm{ESI}$	$q = \frac{q_{\max} s/K_{\mathrm{S}}}{(1+s/K_{\mathrm{S}})(1+i/K_{\mathrm{I}})}$

Table 2: Different types of reversible inhibition of enzymatic reactions (4).

As a special case, the non-competitive reversible autoinhibition, i.e. $\mathrm{I} \equiv \mathrm{S}$, leads to the well-known Haldane equation [1]

$$q = q_{\max} \frac{\frac{s}{K_{\mathrm{S}}}}{\left(1+\frac{s}{K_{\mathrm{S}}}\right)} \cdot \frac{1}{\left(1+\frac{s}{K_{\mathrm{I}}}\right)} = \frac{q_{\max}}{1+\frac{s}{K_1}+\frac{K_2}{s}}, \tag{6}$$

which is often applied to global reactions such as growth of biomass on an inhibitory substrate.

Many other laws have been reported in the literature for simple systems, especially single substrate – single biomass – single product systems, most of them found heuristically in order to describe a specific phenomenon. The choice among them is often a question of taste, since many of them are rather similar. There is therefore no real justification for the preference of one kinetic model over the others. Anyway, Monod's law is the most widely used kinetic expression due to its simplicity and its physical and phenomenological background.

For more complex reaction systems with little *a priori* qualitative knowledge on the exact enzymatic interaction on the individual reactions, one is often content to write the kinetic expression as the product of individual phenomena such as limitation, activation and inhibition:

$$q = q_{\max} \prod_j \alpha_j\left(c_{i_j}\right) \tag{7}$$

with $0 \leq \alpha_j \leq 1 \, \forall j$. This expression usually represents an extension of the Michaelis-Menten kinetics with modulations of non-competitive character.

For example, Batt and Kompala [4] have used the approach (7) for the compartmental modelling of hybridoma cells with

$$\alpha_j = \frac{c_{i_j}}{c_{i_j}+K_j} \text{ for limitation and} \tag{8}$$

$$\alpha_j = \frac{K_j}{c_{i_j}+K_j} \text{ for inhibition.} \tag{9}$$

If no *a priori* knowledge is available about the structure, i.e. if a systematic modelling approach is necessary for the selection of the reaction kinetics, a rather general formulation could therefore be written

$$q = q_{\max} \prod_{i=1}^{n} \frac{c_i}{c_i+K_{\mathrm{lim},i}} \cdot \frac{K_{\mathrm{inh},i}}{c_i+K_{\mathrm{inh},i}}, \tag{10}$$

which results in $2n+1$ model parameters for each considered reaction. This obvious overparametrisation of the kinetic expression will certainly lead to identifiability problems. Moreover, due to the physical meaning of the modulation constants K_{lim} and K_{inh}, which are reasonable for positive values only, the identification method has to handle (lower bound) constraints. Finally, if the reaction rate q is insensitive to a component i, the solution for its modulation constants is $K_{\mathrm{lim},i} \equiv 0$ and $K_{\mathrm{inh},i} \equiv \infty$, which could cause problems to the optimisation algorithm.

3 On a General Kinetic Model

For the systematic identification of a model with *a priori* unknown kinetics, it is necessary to build up a model structure capable of representing the most common biological phenomena with the fewest parameters. This almost always results in a compromise between the model generality and the level of description (parametrisation) that can be recovered in the model identification step.

A general kinetic model should at least be able to reproduce the two major tendencies of modulation:

- the positive effect (limitation, activation) of a component on the reaction rate, i.e. q is monotonically increasing with c_i;
- the negative effect (inhibition) of a component on the reaction rate, i.e. q is monotonically decreasing with c_i.

The invariance of q with respect to c_i should also be contained as a special case.

case	enzymatic reaction path	kinetic function
reversible reaction	$\mathrm{E}+\mathrm{S} \rightleftharpoons \mathrm{ES} \rightleftharpoons \mathrm{E}+\mathrm{P}$	$q\left(s,p\right)=\frac{q_{\mathrm{S,max}}s/K_{\mathrm{S}}-q_{\mathrm{P,max}}p/K_{\mathrm{P}}}{1+s/K_{\mathrm{S}}+p/K_{\mathrm{P}}}$
two-substrate reaction	$\mathrm{E}+\mathrm{S}_1+\mathrm{S}_2 \rightleftharpoons \mathrm{ES}_1+\mathrm{S}_2 \rightleftharpoons \mathrm{ES}_1\mathrm{S}_2$ $\mathrm{E}+\mathrm{S}_1+\mathrm{S}_2 \rightleftharpoons \mathrm{ES}_2+\mathrm{S}_1 \rightleftharpoons \mathrm{ES}_1\mathrm{S}_2$ $\mathrm{ES}_1\mathrm{S}_2 \xrightarrow{q} \mathrm{E}+\mathrm{P}$	$q\left(s_1,s_2\right)=\frac{q_{\max}}{1+\frac{K_{21}}{s_1}+\frac{K_{12}}{s_2}+\frac{1}{2}\frac{K_2K_{21}+K_1K_{12}}{s_1s_2}}$
multiple reactions on one enzyme	$\mathrm{E}+\mathrm{S}_i \rightleftharpoons \mathrm{ES}_i \xrightarrow{q_i} \mathrm{E}+\mathrm{P}_i$	$q_i\left(s_1,\ldots,s_n\right)=\frac{q_{i,\max}s_i/K_i}{1+\sum_{j=1}^{n}s_j/K_j}$

Table 1: Some expressions for enzymatically catalysed reactions [2].

3.1 Formulation

As the model of Monod is the most-widely accepted in biotechnology, the following model structure is inspired from this classical law:

$$q_j\left(c_1,\ldots,c_n\right)=q_{j,\max}\prod_{i=1}^{n}\alpha_{ij}\left(c_i\right) \tag{11}$$

with

$$\alpha_{ij}\left(c_i\right)=\begin{cases}\frac{c_i}{c_i+K_{ij}^{*2}} & \text{if } K_{ij}>0;\\ \frac{1}{1+K_{ij}^{*2}c_i} & \text{otherwise.}\end{cases} \tag{12}$$

3.2 Structure

It is obvious that equation (12) is eqivalent to a Michaelis-Menten-like expression for positive K_{ij}^* with the Michaelis constant $K_{\mathrm{M}}=K_{ij}^{*2}$. Negative K_{ij}^* result in a non-competitive inhibition term with $K_{\mathrm{I}}=K_{ij}^{*-2}$. For $K_{ij}^*\equiv 0$, the influence of the respective component on the reaction kinetics vanishes: $\alpha\left(c_i\right)\equiv 1$.

3.3 Number of Parameters

The advantage of the proposed kinetic model is the unbounded range for the modulation constants K_{ij}^*, whereas the modulation constants in the classical laws are often constrained to be positive. The representation of the two modulation effects by one parameter reduces consequently the number of kinetic parameters to $n+1$ per reaction, which is particularly beneficial to the model identification procedure.

3.4 Physical Constraints

Some physical constraints have to be imposed in order to ensure that a component i, which is consumed in the reaction j, cannot be further consumed, when its concentration vanishes. Let ν_{ij} be the respective stoichiometric coefficient, the modulation constant K_{ij}^* has to fulfill

$$\nu_{ij}<0 \quad\wedge\quad K_{ij}^*>0 \quad\Rightarrow\quad q_j\left(c_i=0\right)=0 \tag{13}$$

as a sufficient condition to guarantee $c_i\left(t\right)\geq 0\ \forall t\ \forall c_{i,0}$.

Consequently, a substrate cannot have an inhibitory effect in this approach. Although this is a possible scenario, our approach for systematic kinetic modelling does not consider this case, which is often rather difficult to detect through experimental data due to the non-injectivity of the resulting reaction rate with respect to the concentration of the auto-inhibiting component. The use of a non-injective function would make the identification problem more delicate.

3.5 Continuity and Differentiability

The modulation function $\alpha_{ij}\left(c_i,K_{ij}^*\right)$ in (12) is differentiable almost everywhere in the admitted range

$$\mathcal{Q}_{ij}=\left\{c_i\in\mathbb{R},K_{ij}^*\in\mathbb{R}|c_i\geq 0\right\}, \tag{14}$$

especially at the transition $K_{ij}^*=0$:

$$\frac{\partial\alpha_{ij}}{\partial K_{ij}^*}=\begin{cases}\frac{-2c_iK_{ij}^*}{\left(c_i+K_{ij}^{*2}\right)^2}, & \text{if } K_{ij}^*>0,\\ \frac{-2c_iK_{ij}^*}{\left(1+K_{ij}^{*2}c_i\right)^2}, & \text{otherwise,}\end{cases} \tag{15}$$

which is particularly important for gradient-based optimisation algorithms used to estimate of the kinetic model parameters:

$$\lim_{K_{ij}^*\to 0-}\frac{\partial\alpha_{ij}}{\partial K_{ij}^*}=\lim_{K_{ij}^*\to 0+}\frac{\partial\alpha_{ij}}{\partial K_{ij}^*}=\left.\frac{\partial\alpha_{ij}}{\partial K_{ij}^*}\right|_{K_{ij}^*=0}=0. \tag{16}$$

Only $(c_i,K_{ij}^*)=(0,0)$ is a discontinuity point. However, if the initial concentration of component i is non-zero, the solution for c_i of the system equation (1) will always remain non-zero, i.e.

$$c_{i,0}>0 \quad\wedge\quad q_j\left(c_i=0\right)=0 \quad\wedge\quad \left.\frac{\partial q_j}{\partial K_{ij}^*}\right|_{c_i=0}<\infty\ \forall j \Rightarrow \quad c_i\left(t\right)> \tag{17}$$

and the critical point (0,0) will never be reached.

Crossing the critical point $K_{ij}^*=0$ for $c_{ij}\equiv 0$ in the course of parameter estimation can be interpreted as a binary decision: is the respective component limiting or not? And therefore is the reaction rate affected by the zero concentration ($\alpha_{ij}=0$) or not ($\alpha_{ij}=1$)?

3.6 Model Sensitivities

The differential equation for the output sensitivity with respect to the kinetic parameter vector $\vec{p}_q\left(q_{\max},K^*\right)$ is derived from

equation (1):

$$\frac{\mathrm{d}}{\mathrm{d}t}\left\{\frac{\partial\vec{c}(t,\vec{p}_q)}{\partial\vec{p}_q}\right\} = K\left(\frac{\partial q\left(\vec{c}(t),\vec{p}_q\right)}{\partial\vec{p}_q}+\frac{\partial q\left(\vec{c}(t,\vec{p}_q),\vec{p}_q\right)}{\partial\vec{c}}\frac{\partial\vec{c}(t,\vec{p}_q)}{\partial\vec{p}_q}\right). \tag{18}$$

The respective partial derivatives are calculated as follows:

$$\frac{\partial q_j}{\partial q_{j,\max}} = \prod_{i=1}^{n}\alpha_{ij}\left(c_i,K_{ij}^*\right) \tag{19}$$

$$\frac{\partial q_j}{\partial K_{ij}^*} = q_{j,\max}\prod_{k=1,\ k\neq i}^{n}\alpha_{kj}\left(c_k,K_{kj}^*\right)\frac{\partial\alpha_{ij}\left(c_i,K_{ij}^*\right)}{\partial K_{ij}^*} \tag{20}$$

$$\frac{\partial q_j}{\partial c_i} = q_{j,\max}\prod_{k=1,\ k\neq i}^{n}\alpha_{ij}\left(c_k,K_{kj}^*\right)\frac{\partial\alpha_{ij}\left(c_i,K_{ij}^*\right)}{\partial c_i} \tag{21}$$

with

$$\frac{\partial\alpha_{ij}\left(c_i,K_{ij}^*\right)}{\partial c_i} = \begin{cases}\frac{K_{ij}^{*2}}{\left(c_i+K_{ij}^{*2}\right)^2}, & \text{if } K_{ij}^*>0,\\ \frac{-K_{ij}^{*2}}{\left(1+K_{ij}^{*2}c_i\right)^2}, & \text{otherwise,}\end{cases} \tag{22}$$

and $\frac{\partial\alpha_{ij}}{\partial K_{ij}^*}$ according to (15).

3.7 Physical Units

The unit of K_{ij}^* depends on the respective case in (12) and changes therefore in the following manner:

$$[K^*] = \begin{cases}[c]^{1/2}, & \text{if } K^*>0,\\ [c]^{-1/2}, & \text{otherwise.}\end{cases} \tag{23}$$

4 Examples

The potentials and features of the proposed kinetic model are first illustrated by means of a small ideal-case example. Consider a reaction system with $n=3$ components, – one substrate (S), one biomass (X) and one product (P), – and $m=1$ reaction, – the biomass growth, – according to the following stoichiometry:

$$\frac{1}{Y_{\mathrm{S}}}\mathrm{S}\xrightarrow{\mu}\mathrm{X}+\frac{1}{Y_{\mathrm{P}}}\mathrm{P} \tag{24}$$

with known yield coefficients Y_{S} and Y_{P}.

The reference dynamics is given by the extended Monod law for the specific growth rate:

$$\mu=\mu_{\max}\frac{s}{s+K_{\mathrm{S}}}\cdot\frac{K_{\mathrm{I}}}{p+K_{\mathrm{I}}}. \tag{25}$$

The resulting system of ordinary differential equations is therefore written:

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix}x\\s\\p\end{bmatrix} = \begin{bmatrix}1\\-\frac{1}{Y_{\mathrm{S}}}\\\frac{1}{Y_{\mathrm{P}}}\end{bmatrix}\mu(s,p)\,x+\left(\begin{bmatrix}0\\s_{\mathrm{in}}\\0\end{bmatrix}-\begin{bmatrix}x\\s\\p\end{bmatrix}\right)D. \tag{26}$$

Assuming that the reaction kinetics is completely unknown, the general kinetic model (11) and (12) is applied here for $\bar{\mu}$, and its $m(n+1)=4$ parameters

$$\vec{p}^{\mathrm{T}} = \begin{bmatrix}\bar{\mu}_{\max} & K_{\mathrm{X}}^* & K_{\mathrm{S}}^* & K_{\mathrm{P}}^*\end{bmatrix} \tag{27}$$

are calculated uniquely by the set of equation given in table 3.

$\bar{\mu}_{\max}$	$=\mu_{\max}$
K_{X}^*	$=0$
K_{S}^*	$=K_{\mathrm{S}}^{1/2}$
K_{P}^*	$=-K_{\mathrm{I}}^{-1/2}$

Table 3: Kinetic parameters of the general model for ideal example.

As a second example, consider again the reaction scheme (24) the kinetic expression

$$\mu=\mu_{\max}\frac{s}{s+K_{\mathrm{S}}\left(1+\frac{p}{K_{\mathrm{I}}}\right)}. \tag{28}$$

featuring a competitive inhibition by the product, which does not exactly fit the proposed general reaction kinetic structure (11) and (12).

Since the kinetic model structures are not equivalent, the parameters have to be estimated by minimising the following cost function:

$$f\left(\vec{p}\right)=\frac{1}{t_{\mathrm{e}}}\sum_{i=1}^{n}\int_{t_0}^{t_{\mathrm{e}}}\left(c_i(\tau)-\bar{c}_i\left(\tau,\vec{p}\right)\right)^2\mathrm{d}\tau, \tag{29}$$

i.e. a least-square criterion corresponding to the ideal case of continuous error-free concentration measurements.

The model parameters chosen for the reference system (24,28) are given in table 4.

parameter	value
Y_{S}	$1/2$
Y_{P}	$1/3$
$\mu_{\max}$	1
K_{S}	2
K_{I}	3

Table 4: Numerical values of the reference model parameters – Example 2 (non-ideal case).

The reference system (24,28) is simulated for the experimental conditions (initial conditions, inlet substrate concentration and dilution rate) described in table 5.

The cost function (29) is minimised in order to determine the four model parameters $\mu_{\max}$, K_{X}^*, K_{S}^*, K_{P}^*. Their values are given in table 6 together with their interpretation in terms of modulation.

The cost function value at the optimum is $f\left(\vec{p}_{q,\mathrm{opt}}\right)=0.0100$. This value represents a measure of the error made with the

$$
\begin{aligned}
x\,(0) &= 1 \\
s\,(0) &= 10 \\
p\,(0) &= 0.1 \\
s_{\mathrm{in}} &= 10 \\
D\,(t) &= \begin{cases} 0; & 0 \le t \le 10 \\ 0.05\,(t-10)\,; & 10 < t \le 20 \\ 0.5; & 20 < t \le 30 \end{cases}
\end{aligned}
$$

Table 5: Operational parameters for the reference system – Example 2 (non-ideal case).

parameter	value	interpretation	
$\bar{\mu}_{\max}$	2.0772	—	
K^*_{X}	1.1565	limitation	$K_{\mathrm{lim,X}} = 1.3374$
K^*_{S}	1.9500	limitation	$K_{\mathrm{lim,S}} = 3.8025$
K^*_{P}	−0.5219	inhibition	$K_{\mathrm{inh,P}} = 3.6716$

Table 6: Non-ideal case example: general kinetic model parameter values and their interpretation.

model structure. It approaches zero in the case of a total equivalence of the kinetic structures, as in the previous ideal case example and can be interpreted as the summed mean variance σ^2 of all the considered components. The identified model has therefore a summed mean standard deviation of $\sigma = 0.1$, which is relatively low compared to the orders of magnitudes of the concentrations.

This result is confirmed by the graphical comparison of the model and the reference systems in figures 1 and 2. The

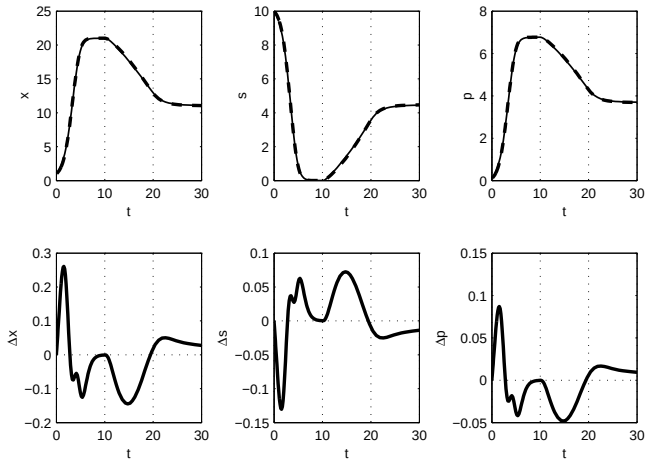

Figure 1: Non-ideal case example: General model identified on structurally different kinetic model. Upper row: comparison system with general kinetic model (dashed) vs. reference system (solid). Lower row: deviation of model system from reference system $\Delta\vec{c}(t) = \vec{c}(t) - \vec{\bar{c}}(t, \vec{p}_q)$.

deviations from the reference model are in a range of about one percent for all the concentrations, and the maximum deviation of the reaction rates is around ten percent at the beginning

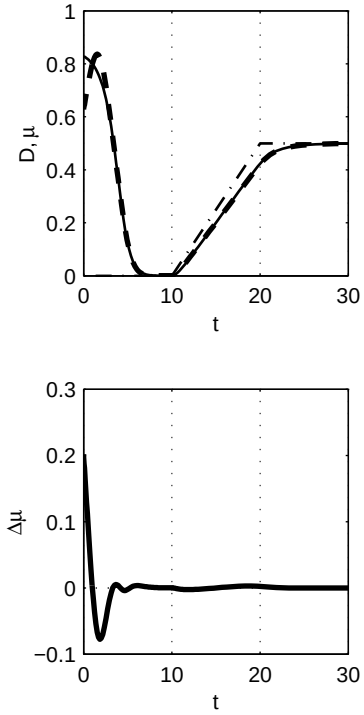

Figure 2: Non-ideal case example: General model identified on structurally different kinetic model. Top: comparison general model (dashed) vs. reference model reaction rate (solid) Bottom: deviation of general from reference model reaction rate $\Delta\mu\,(t) = \mu\,(\vec{c}(t)) - \bar{\mu}\,\big(\vec{\bar{c}}(t, \vec{p}_q)\big)$.

of the experiment, where the model mismatch becomes apparent. Nevertheless, the integration smoothes these deviations, and the impact on the concentration trajectories becomes negligibly small, as time evolves.

Due to the different model structures, the identified values of the general model parameters differ from their "corresponding" reference values (cf. table 4) Though, the parameters in table 6 show the same tendencies as their reference counterparts: The product P is inhibiting with a "non-competitive" inhibition constant of 3.67, which is in the same range of values as the "competitive" inhibition constant. Also, the limiting effect of the substrate S is correctly identified, although the modulation constant has a higher value than the reference parameter. Additionally, biomass X is found to be limiting for the specific growth rate, however with a relatively low Michaelis constant compared to the biomass concentrations of the experiment. This limiting effect does therefore not play an important role in this specific experiment and could be neglected, i.e. set to zero for similar experiments.

It is remarkable that the identified maximum specific growth rate is about twice the reference value as is the limitation constant of the substrate. These multiplicative factors compensate each other at low substrate concentrations s, when the modulation constant K^{*2}_{S} in the general kinetic formulation becomes dominant in the denominator of α_{S}, i.e. when the kinetics fol-

lows approximatively a first-order law.

This already indicates the limitation of the proposed approach. If some *a priori* knowledge about the kinetic model structure is available, this information can of course be used in order to derive a physically relevant model equation. However, if no knowledge about this structure is available, one has to try a modelling approach, which describes the biological behaviour in a sufficiently general way and estimate the corresponding model parameters with an appropriate model identification procedure based on informative experimental data.

In this connection, the proposed model allows a parcimonious representation of important modulation effects, e.g. limitation, activation and inhibition, as well as a distinct physical interpretation of each model parameter. The sensititivities of the model output with respect to the kinetic parameters can be easily exploited for further model reduction and simplification.

5 Conclusions

A systematic approach is needed for the identification of mathematical models in biotechnology. If possible, it is recommended to separate specific sets of parameters, as for example stoichiometric coefficients and kinetic parameters, and to identify each set individually. For macroscopic modelling approaches, such a separation is possible at least approximatively for systems with a smaller number of reactions than components [6, 5]. The unknown reaction rates $\vec{r}$ in (1) are then eliminated by a linear combination of the differential equations in excess, i.e. through a linear transformation into a lower-dimension state space, in which the influence of the reaction kinetics is eliminated.

The second step of such an identification procedure is therefore dedicated to the identification of the underlying reaction kinetics, which is often the most delicate task, since the non-linear kinetic functions are often not known exacty. In such cases, it is indispensable to choose a kinetic model structure with a relatively low number of unknown parameters in order to avoid identifiability problems, but with a sufficiently high potential to reproduce the majority of biological kinetic phenomena, such as inhibition and limitation.

A kinetic model representing a reasonable compromise is proposed in this paper. It is based on an extension of the Michealis-Menten kinetics and is capable reproducing limitation/activation and (non-competitive) inhibition effects. It includes one parameter per reaction for scaling the maximum reaction rate and n parameters per reaction characterising the modulation effect of each component. Its limited number of kinetic parameters allows identifiability problems to be alleviated. Two application examples, – one ideal case and one non-ideal case, in which the kinetic model structures are different, – show the versatility of the proposed approach.

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