A New Generic Mass Balance Model with Multi-Layer Perceptron-Based Kinetics and Stoichiometry

Ph. Bogaerts*

** 3BIO-BioControl, Université libre de Bruxelles, Av. F.-D. Roosevelt 50 C.P. 165/61, B-1050 Brussels, Belgium (e-mail: philippe.bogaerts@ulb.be).*

Abstract: This paper proposes a new generic mass balance model that allows simulating biological cultures in bioreactors at a macroscopic scale. A multi-layer perceptron (MLP) describes the kinetic and stoichiometric parts of the model with one input layer (made of the concentrations of the different components, as well as their inverse TReLU – Thresholded Rectified Linear Unit – transforms), one hidden layer (each neuron output corresponding to one specific reaction rate and being activated by a reciprocal function 1 / x) and one output layer (each neuron output being the sum of all the reaction contributions of a specific component to its mass balance). The parameters to be identified are split into two subsets: one for the kinetic parameters (weights on the links between input layer and hidden layer) and one for the stoichiometric parameters (weights on the links between hidden layer and output layer). This MLP structure exhibits several advantages, among which its versatility, the biological interpretation of the parameters, and an easy and efficient first estimation of the kinetic and stoichiometric parameters based on measurements of the component concentrations and estimations of their time derivatives. The first parameter estimation can subsequently be used for model reduction and as initial guess for a final nonlinear parameter estimation of the set of ODEs describing the mass balances. The performances of the new generic model are illustrated with a simulated case study.

Keywords: mass balance model, kinetic model, kinetic parameters, stoichiometric parameters, multi-layer perceptron, biological culture, bioprocess, parameter identification.

1. INTRODUCTION

Mass balance models at a macroscopic scale are abundantly used for predicting concentration time profiles of the main extracellular components (e.g., substrates, metabolites, products of interest), as well as the biomass, in biological cultures performed in bioreactors (Bastin and Dochain, 1990; Mairet and Bernard, 2019). Lots of specific kinetic model structures have been introduced, accounting for different effects (e.g., activation, saturation, inhibition) of some components in some reactions. As the choice of specific model structures consists of a key issue, generic model structures have been introduced. For example, S-systems were based on power laws (Savageau, 1979), with the drawback that a given component cannot exhibit a double effect of activation and inhibition in a given reaction (as it can be done with a Haldane law). Their equivalence with generalized Volterra-systems has also been studied (Voit and Savageau, 1982). Several macroscopic modeling approaches for describing mammalian cell growth and metabolism are reviewed in Ben Yahia et al. (2015). Given the difficulties linked to kinetic parameter estimation, and especially the risks of overparameterization and/or convergence to local minima when minimizing the identification cost function, some generic methods were developed aiming at efficient parameter estimation. Haag et al. (2005) proposed a general formalism based on an extension of Monod kinetics (but with the drawback, already mentioned above, concerning the impossibility to represent a double effect of activation and inhibition). Another general formalism coupled power laws for activation effects and negative exponentials for inhibition effects (Bogaerts et al., 1999; Grosfils et al., 2007), with the advantage that both effects could be simultaneously defined, that the kinetic model structure could be rigorously linearized w.r.t. the parameters and that it could be easily translated in classical extended Monod formalisms (Richelle and Bogaerts, 2015). The linearization requires however the possibility to identify the stoichiometric parameters independently of the kinetics (Bogaerts et al., 2003, Bernard and Bastin, 2005), which is unfortunately not possible in many cases. Other approaches were based on Artificial Neural Networks – ANNs – (Chen et al., 2000; Vande Wouwer et al., 2004), which benefit from a large flexibility to describe nonlinearities associated to the reaction kinetics, but which are limited to black-box descriptions in which the biological interpretation of the kinetic parameters is lacking. Instead of considering generic kinetic model structures, Mailier and Vande Wouwer (2012) proposed a bank of candidate kinetic model structures among which the choice is made by some decision algorithm. Recently, Reinforcement Learning was used to tackle the case of history-dependent kinetic systems (Mowbray et al., 2023).

There is still a need for generic kinetic model structures that i) are flexible enough to describe the basic phenomena (activation and inhibition) by any component in any reaction, and, ii) allow an efficient parameter estimation, especially in terms of convergence to a global minimum instead of local minima. For reaching these goals, a new formalism is proposed

in this paper and consists in lumping all the reaction terms included in a system of mass balances in a Multi-Layer Perceptron (MLP). This latter is not a classical black-box ANN without biological interpretation. It is made of three (input – hidden – output) layers, some neurons of which containing nonlinear activation functions without parameters to be identified. All the parameters to be estimated appear only as weights on the links between, on the one hand, input and hidden layers for the kinetic parameters, and, on the other hand, hidden and output layers for the stoichiometric parameters. Three different categories of kinetic parameters account for kinetic constants, activation parameters and inhibition parameters. Inputs and outputs of the MLP simply consist of, respectively, the component concentrations (or the inverse of their TReLU transform) and their (estimated) time derivatives. This allows an efficient first identification of the kinetic and stoichiometric parameters which consists in a supervised learning of the weights included in this MLP. This result can then be used for model reduction and as an initial guess in a final nonlinear identification step that uses the system of ODEs describing the mass balances.

This new generic model, and especially the part corresponding to the MLP, is described in Section 2. The methodology is illustrated with a simulated case study in Section 3. Finally, Section 4 draws some conclusions and perspectives.

2. GENERIC MASS BALANCE MODEL

2.1 Reaction scheme and mass balances

We consider here a reaction scheme that describes the main reactions occurring at a macroscopic scale within a biological culture of microorganisms (e.g., bacteria, yeasts) or animal cells, that takes place in a (batch, or continuous, or fed-batch) bioreactor. Let *m* be the number of reactions and *n* the number of components involved in the scheme (e.g., biomass, substrates, metabolites). Let $c \in \mathbb{R}^n$ be the vector of all the component concentrations, one of them being $X \in \mathbb{R}$ the biomass concentration. Let $\varphi \in \mathbb{R}^m$ be the vector of the reaction rates, structured in the usual form $\varphi = \mu\, X$ where $\mu \in \mathbb{R}^m$ is the vector of specific reaction rates. Even though not explicitly mentioned, all these variables depend on time *t*. The mass balance model is then written under the classical form

$$\frac{dc}{dt} = K\,\mu\,X - \frac{F}{V}\,c + \frac{F}{V}\,c_{in} \tag{1}$$

where $K \in \mathbb{R}^{n\times m}$ is the stoichiometric matrix (or matrix of macroscopic yield coefficients), $c_{in} \in \mathbb{R}^n$ is the vector of input concentrations and, regarding the flow rate $F \in \mathbb{R}$, $F = 0$ with a constant volume $V \in \mathbb{R}$ (batch mode), or $F = F_{in} = F_{out} \neq 0$ with a constant volume *V* (chemostat), or $F = F_{in} \neq 0$ with $F_{out} = 0$ and a volume *V* such that $\frac{dV}{dt} = F$ (fed-batch mode).

2.2 Multi-layer perceptron-based kinetics and stoichiometry

The specific reaction term $K\,\mu$ in (1) is represented with a multi-layer perceptron (MLP) given in Fig. 1. The input layer builds the signals that will be used as activation (respectively, inhibition) factors thanks to green (respectively, red) neurons. The outputs of inhibition (red) neurons simply correspond to the concentrations of the different components c_i ($i \in [1,n]$). The outputs of activation (green) neurons correspond to inverse Thresholded Rectified Linear Units (TReLU) of the component concentrations, $1/\mathrm{TReLU}(c_i)$, where

$$\mathrm{TReLU}(c_i) = \max(c_i, \delta) \tag{2}$$

with $\delta > 0$, an arbitrarily low threshold.

Based on the activation and inhibition factors, the hidden layer builds the specific reaction rates

$$\mu_j = \frac{1}{\alpha_j + \sum_{i=1}^{n}\left(\frac{\gamma_{ij}}{\mathrm{TReLU}(c_i)} + \beta_{ij}\,c_i\right)} \tag{3}$$

with $j \in [1,m]$ and $\alpha_j \geq 0$ the bias. This latter plays an equivalent role of a kinetic constant. It allows, e.g., defining a constant specific rate $\mu_j = 1/\alpha_j$ if $\beta_{ij} = \gamma_{ij} = 0\ \forall i$. The inhibition effect of c_k on μ_j when $\beta_{kj} > 0$ is obvious in this reciprocal function. The activation effect of c_k on μ_j when $\gamma_{kj} > 0$ comes from

$$\lim_{c_k \to 0} \mu_j = \frac{\delta}{\delta\alpha_j + \delta \sum_{\substack{i=1 \\ i\neq k}}^{n}\left(\frac{\gamma_{ij}}{\mathrm{TReLU}(c_i)} + \beta_{ij}\,c_i\right) + \gamma_{kj}} \tag{4}$$

which tends to 0 if δ tends to 0 and $\gamma_{kj} > 0$, while it tends to a nonzero value $\left(1/\left(\alpha_j + \sum_{\substack{i=1 \\ i\neq k}}^{n}\left(\frac{\gamma_{ij}}{\mathrm{TReLU}(c_i)} + \beta_{ij}\,c_i\right)\right)\right)$ if δ tends to 0 and $\gamma_{kj} = 0$.

Note that if a specific rate μ_j only depends on a single component concentration c_i (e.g., a specific growth rate depending on a single substrate concentration), then the kinetic model (3) becomes rigorously equivalent to a Haldane kinetic law when $\alpha_j > 0$ (and for $c_i > \delta$):

$$\mu_j = \frac{1}{\alpha_j}\frac{c_i}{\frac{\gamma_{ij}}{\alpha_j} + c_i + \frac{\beta_{ij}}{\alpha_j}c_i^2} = \mu_{max}\frac{c_i}{K + c_i + \frac{c_i^2}{K_i}} \tag{5}$$

with $\mu_{max} = 1/\alpha_j$, $K = \gamma_{ij}/\alpha_j$ and $K_i = \alpha_j/\beta_{ij}$. This rigorous equivalence doesn't hold anymore in case $\alpha_j = 0$ which leads to

$$\mu_j = \frac{c_i}{\gamma_{ij} + \beta_{ij}c_i^2} \tag{6}$$

or in case of a specific rate depending on two or more component concentrations.

Finaly, the output layer builds the reaction term for the mass balance of each component, i.e., $\sum_{j=1}^{m} k_{ij}\,\mu_j$ with $i \in [1,n]$.

The main advantages of this MLP are that

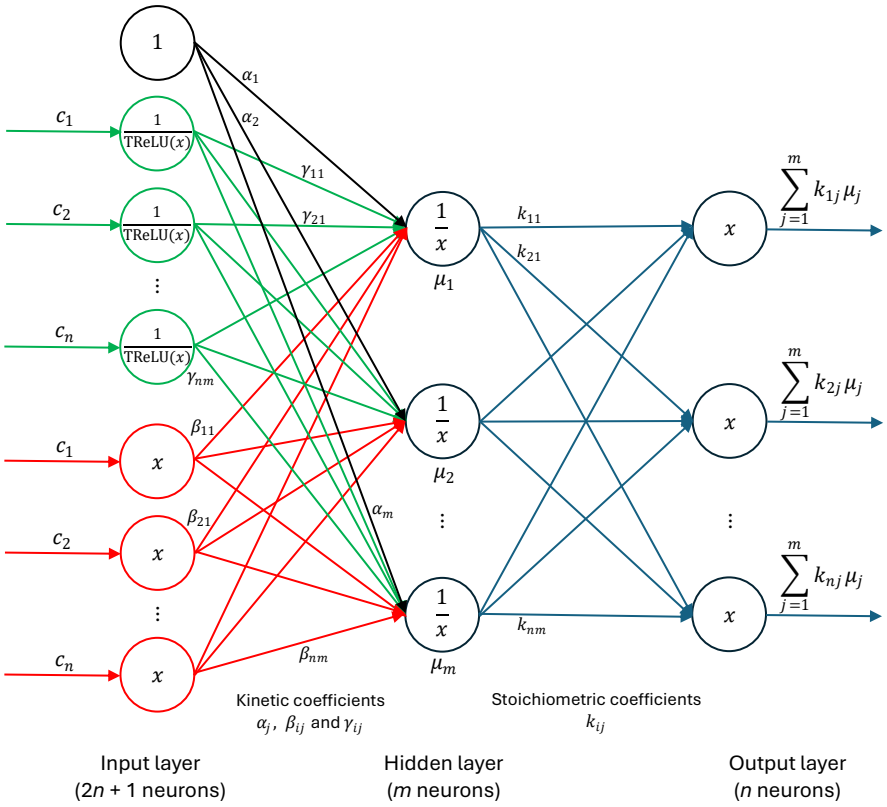

Fig. 1. MLP representing the specific reaction term $K\,\mu$ in the mass balance model (1).

1) the parameters appear in two sets of linear weights: the kinetic coefficients α_j, β_{ij} and γ_{ij} between the input and hidden layers, and the stoichiometric coefficients k_{ij} between the hidden and output layers;

2) the nonlinearities are confined within some of the neurons (the activation green neurons of the input layer and the neurons of the hidden layer) and do not include any parameter to estimate;

3) inputs and outputs of the MLP are available: its inputs consist in (discrete measurements of) the component concentrations $c_i(t_k)$ $(i \in [1,n])$, while its outputs $y_{meas,i}(t_k)$ can be deduced from (1) and estimates $\hat{\dot{c}}_i$ of the time derivatives of c_i:

$$y_{meas,i}(t_k) = \left(\hat{\dot{c}}_i(t_k) + \frac{F(t_k)}{V(t_k)}\,c_i(t_k) - \frac{F(t_k)}{V(t_k)}\,c_{i,in}(t_k)\right)/X(t_k). \quad (7)$$

Given these advantages, a first estimation of the unknown kinetic and stoichiometric parameters can be efficiently obtained on the basis of the MLP and the measurements/estimations of its inputs/outputs, e.g., using a backpropagation algorithm in the MLP supervised training, or any other optimization algorithm for minimizing a least squares criterion written as

$$J_{LS}(\vartheta) = \sum_{k=1}^{N}\sum_{i=1}^{n} w_i(t_k)\left(y_{meas,i}(t_k) - y_i(t_k,\vartheta)\right)^2 \quad (8)$$

where N is the total number of measurement times, $w_i(t_k)$ are user-defined weights accounting for the measurement accuracy, $y_{meas,i}(t_k)$ is given by (7), $y_i(t_k,\vartheta)$ by

$$y_i(t_k,\vartheta) = \sum_{j=1}^{m} k_{ij}\,\mu_j(t_k) \quad (9)$$

with $\mu_j(t_k)$ from (3), and $\vartheta \in \mathbb{R}^{(3n+1)m}$ is the vector of the parameters to identify:

$$\vartheta^T = [k_{ij} \quad \alpha_j \quad \beta_{ij} \quad \gamma_{ij}] \quad i \in [1,n], j \in [1,m] \quad (10)$$

under some equality and/or inequality constraints (see below).

A final classical nonlinear estimation of the parameters can then be implemented based on the set of nonlinear ODEs (1) and the minimization of a least squares cost function, starting with the results $\hat{\vartheta}_1$ which minimized $J_{LS}(\vartheta)$ in (8). Note that parameters close to 0 in $\hat{\vartheta}_1$, indicate possible model reduction as will be illustrated in the case study of Section 3. The solution $\hat{\vartheta}$ of this final problem would minimize the least squares criterion (8) but with

$$y_{meas,i}(t_k) = c_{meas,i}(t_k) \quad (11)$$

which only involves the concentration measurements $c_{meas,i}$ (without the estimation of their time derivatives), and

$$y_i(t_k,\vartheta) = c_i(t_k,\vartheta) \quad (12)$$

obtained by integrating (1) with an ODE solver and using the set of parameters in ϑ.

The number of reactions *m* can be determined, e.g., based on a Principal Component Analysis as proposed by Bernard and Bastin (2005). The model is fully generic in the sense that it can detect

- the involvement of any component in any reaction, which corresponds to $k_{ij} \neq 0$, with $k_{ij} < 0$ if the *i*-th component is consumed in the *j*-th reaction and $k_{ij} > 0$ if it is produced;

- the activation effect of any component within any reaction rate, which corresponds to $\gamma_{ij} > 0$ for the *i*-th component in the *j*-th reaction;

- the inhibition effect of any component within any reaction rate, which corresponds to $\beta_{ij} > 0$ for the *i*-th component in the *j*-th reaction.

Note that some additional information can be provided, if available, by adding linear (equality or inequality) constraints on some parameters, e.g.,

- normalizing the stoichiometry of a given reaction with respect to a given component: $k_{ij} = -1$ (if consumed) or $k_{ij} = 1$ (if produced);

- constraining the consumption (respectively, production) of a given component in a given reaction: $k_{ij} < 0$ (respectively, $k_{ij} > 0$);

- cancelling any activation (respectively, inhibition) effect of a given component in a given reaction: $\gamma_{ij} = 0$ (respectively, $\beta_{ij} = 0$).

3. SIMULATED CASE STUDY

To illustrate the use of the new MLP-based mass balance model, we consider the simulated case study proposed in Pimentel et al. (2024). The reaction scheme is made of 3 reactions accounting, respectively, for substrate oxidation (13), substrate overflow (14) and biomass death (15):

$$k_{31}G + k_{41}Gn \xrightarrow{\varphi_1} X_v + k_{61}P \tag{13}$$

$$k_{32}G + k_{42}Gn \xrightarrow{\varphi_2} X_v + k_{52}L \tag{14}$$

$$X_v \xrightarrow{\varphi_3} X_d + k_{63}P \tag{15}$$

where X_v, X_d, G, Gn, L and P stand, respectively, for viable biomass, dead biomass, glucose, glutamine, lactate and the product of interest (i.e., monoclonal antibodies), representing the components as well as their concentrations. k_{ij} are the stoichiometric coefficients and φ_j are the reaction rates. The system of mass balances (1) becomes here

$$\frac{d}{dt}\begin{bmatrix} X_v \\ X_d \\ G \\ Gn \\ L \\ P \end{bmatrix} = \begin{bmatrix} 1 & 1 & -1 \\ 0 & 0 & 1 \\ k_{31} & k_{32} & 0 \\ k_{41} & k_{42} & 0 \\ 0 & k_{52} & 0 \\ k_{61} & 0 & k_{63} \end{bmatrix} \begin{bmatrix} \varphi_1 \\ \varphi_2 \\ \varphi_3 \end{bmatrix}. \tag{16}$$

The reaction rates are structured as

$$\varphi_j = \mu_j X_v \tag{17}$$

where μ_j are the specific reaction rates describing overflow metabolism (μ_1 and μ_2) and cell death (μ_3):

$$\mu_1 = \min(\mu_G, \mu_{Gmax}) \tag{18}$$

$$\mu_2 = \max(0, \mu_G - \mu_{Gmax}) \tag{19}$$

$$\mu_3 = \mu_{dmax} \frac{K_{Gd}}{K_{Gd}+G} \frac{K_{Gnd}}{K_{Gnd}+Gn} \tag{20}$$

with

$$\mu_G = \mu_{max1} \frac{Gn}{K_{Gn}+Gn} \tag{21}$$

$$\mu_{Gmax} = \mu_{max2}. \tag{22}$$

Parameter values and initial conditions of the state variables can be found in Table 1 (taken from Pimentel et al. (2024)). We use the same simulated dataset proposed by the authors, i.e., one batch experiment lasting $7d$, with discrete measurements of the 6 state variables every $0.1d$ and a Gaussian measurement noise with zero mean and a relative standard deviation equal to 0.5%.

Table 1. Simulation parameters (from Table 2 in Pimentel et al. (2024)).

Parameters	**Values**	**Parameters**	**Values**
μ_{max1}	0.484 d^{-1}	k_{52}	23.9
μ_{max2}	0.319 d^{-1}	k_{61}	43.5
μ_{dmax}	0.866 d^{-1}	k_{63}	14.2
K_{Gn}	0.0089 g/L	$X_v(0)$	0.100 cells/mL
K_{Gd}	1.58 g/L	$X_d(0)$	0.0151 cells/mL
K_{Gnd}	1.33 g/L	$G(0)$	5.99 g/L
k_{31}	3.12	$Gn(0)$	0.303 g/L
k_{32}	15.2	$L(0)$	0.360 g/L
k_{41}	0.624	$P(0)$	6.53 µg/mL
k_{42}	1.22		

A first parameter estimation leading to $\hat{\vartheta}_1$ (ϑ being defined as in (10)) is obtained by minimizing a least squares criterion (8), involving (7) and (9), and using a trust-region-reflective algorithm (function LSQNONLIN in MATLAB R.2024b). The lower bound $\delta = 10^{-4}$ is used in the TReLU function (2). Lower and upper bounds are used to define equality and

inequality constraints defined, for each reaction, in the corresponding 2nd (respectively, 1st) line in Table 2 (respectively, Table 3). Results of this first estimation $\hat{\vartheta}_1$ can be found, for each reaction, in the corresponding 3rd (respectively, 2nd) line in Table 2 (respectively, Table 3). Values of the stoichiometric coefficients in the original model are given, for each reaction, in the corresponding 1st line in Table 2. Original values of the parameters are not provided in Table 3 given that the kinetic model is structurally different in the original model and in the MLP-based model. It is worth noting that the original values of the stoichiometric coefficients are systematically recovered with a very good accuracy. The model complexity regarding its stoichiometric part is the same as in the original model, with 7 nonzero estimated parameters, this result being obtained without any use of pruning or sparsity approach.

A final estimation $\hat{\vartheta}$ is obtained by minimizing a least squares criterion (8), involving this time (11) and (12), using the same trust-region-reflective algorithm, with $\hat{\vartheta}_1$ as initial guess. A model reduction is performed when computing $\hat{\vartheta}$ from the initial guess $\hat{\vartheta}_1$, given that 8 parameters close to 0 in $\hat{\vartheta}_1$ are constrained to 0 in the estimation of $\hat{\vartheta}$ (corresponding to the bold values in Tables 2 and 3). The results are given, for each reaction, in the corresponding 4th (respectively, 3rd) line in Table 2 (respectively, Table 3). The final estimates $\hat{\vartheta}$ keep close to their first estimates in $\hat{\vartheta}_1$, illustrating that the essential of the stoichiometric and kinetic coefficients has been captured just based on the MLP and the measurements/estimates of its inputs/outputs. It is worth noting that the inhibition effects of glucose and glutamine on the original specific death rate (20) are clearly detected via the nonzero values of β_{G3} and β_{Gn3} in Table 3. Model validation is proposed in Fig. 2, which exhibits the ability of the MLP-based model to accurately reproduce the data. Fig. 3 shows that the MLP-based kinetics are close to the original kinetic models, although they are based on drastically different model structures as they do not contain the min/max nonlinearities involved in (18)-(19) to account for the overflow metabolism. The model complexity, regarding its kinetic part, has been increased from 6 parameters (in the original model) to 10 nonzero estimated parameters (in the new model). The total number of parameters has increased from 13 to 17, with the recovery of all stoichiometric parameters (see Table 2) and of the essential features of the kinetic rates (see Fig. 3).

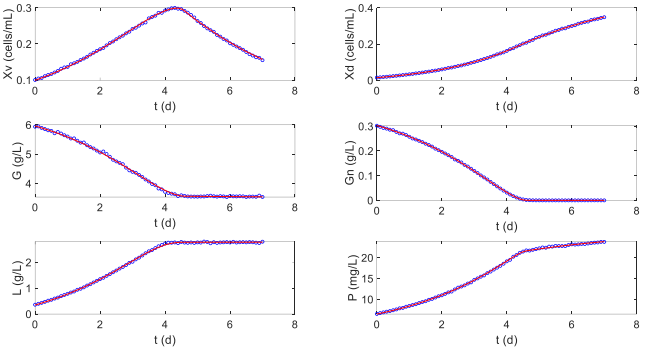

Fig. 2. Model validation (blue circles: simulated measurements; red curves: MLP-based model simulation, based on (3), (16) and (17)).

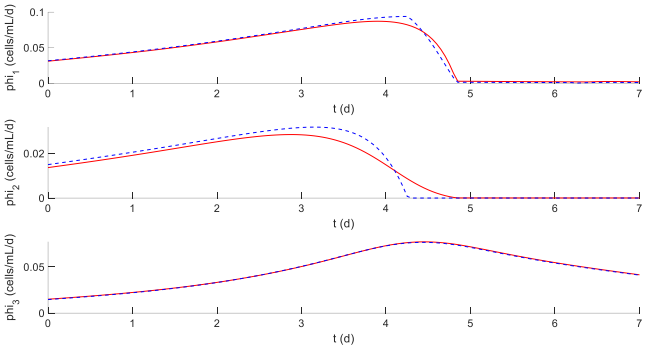

Fig. 3. Reaction rate estimation based on smoothing splines of the measured component concentrations (blue dotted curves: original model reaction rates, based on (17)-(22); red curves: MLP-based model reaction rates, based on (3) and (17)).

4. CONCLUSIONS

The MLP that we propose to describe kinetics and stoichiometry in a generic mass balance model includes biologically interpretable parameters which only consist in its links between input and hidden layers (for the kinetic parameters) and between hidden and output layers (for the stoichiometric parameters). Nonlinearities appear in (some of) the neurons and without parameters to be identified. Given that inputs and outputs of the MLP are available via measurements of the component concentrations and estimations of their time derivatives, a first estimation of the kinetic and stoichiometric parameters can easily be obtained and subsequently used i) for a model reduction and ii) as initial guess for a final nonlinear estimation of the parameters in this reduced model. Versatility, biological interpretation and efficient two-step parameter estimation are the main strengths of this new model.

Future works could focus on other simulated and real case studies, on the sensitivity of the parameter estimation w.r.t. measurement noise and / or w.r.t. the choice of the tuning parameter δ in the TReLU function, on the adaptability of the approach to more complex macroscopic systems with larger scales, on the potential addition of some pruning strategy and on the way to exploit this specific MLP-based model structure for building state observers and/or closed-loop controllers.

Table 2. Identified stoichiometric coefficients (for each reaction: original values in line 1, constraints in line 2, first estimation $\hat{\vartheta}_1$ in line 3, final estimation $\hat{\vartheta}$ in line 4). Bolded values correspond to equality constraints deduced from $\hat{\vartheta}_1$ and added for the final estimation.

k_{Xv1}	k_{Xd1}	k_{G1}	k_{Gn1}	k_{L1}	k_{P1}
1	0	-3.12	-0.624	0	43.5
1	0	[-10^2,0]	[-10^2,0]	[0,10^2]	[0,10^2]
1	0	-2.81	-0.610	0.000	42.5
1	0	-3.30	-0.653	**0**	42.1
k_{Xv2}	k_{Xd2}	k_{G2}	k_{Gn2}	k_{L2}	k_{P2}
1	0	-15.2	-1.22	23.9	0
1	0	[-10^2,0]	[-10^2,0]	[0,10^2]	0
1	0	-16.1	-1.27	24.4	0
1	0	-15.3	-1.19	26.1	0
k_{Xv3}	k_{Xd3}	k_{G3}	k_{Gn3}	k_{L3}	k_{P3}
-1	1	0	0	0	14.2
-1	1	0	0	[0,10^2]	[0,10^2]
-1	1	0	0	0.000	15.2
-1	1	0	0	**0**	14.2

Table 3. Identified kinetic coefficients (for each reaction: constraints in line 1, first estimation $\hat{\vartheta}_1$ in line 2, final estimation $\hat{\vartheta}$ in line 3). Bolded values correspond to equality constraints deduced from $\hat{\vartheta}_1$ and added for the final estimation.

α_1	γ_{G1}	γ_{Gn1}	γ_{L1}	β_{G1}	β_{Gn1}	β_{L1}
[0,10^2]	[0,10^2]	[0,10^2]	0	[0,10^2]	[0,10^2]	[0,10^2]
1.6861	0.0000	0.0238	0	0.2182	0.0000	0.1045
1.4852	**0**	0.0091	0	0.2366	**0**	0.1866
α_2	γ_{G2}	γ_{Gn2}	γ_{L2}	β_{G2}	β_{Gn2}	β_{L2}
[0,10^2]	[0,10^2]	[0,10^2]	0	[0,10^2]	[0,10^2]	[0,10^2]
0.0000	0.0000	0.5435	0	0.0000	16.8958	0.6264
0	**0**	0.5461	0	**0**	16.9559	0.9177
α_3	γ_{G3}	γ_{Gn3}	γ_{L3}	β_{G3}	β_{Gn3}	β_{L3}
[0,10^2]	0	0	0	[0,10^2]	[0,10^2]	[0,10^2]
0.8869	0	0	0	0.8035	2.9863	0.0000
0.8912	0	0	0	0.7970	3.2146	**0**

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