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Kinetic Modeling and Parameter Estimation in a Tower Bioreactor for Bioethanol Production

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Abstract In this work, a systematic method to support the building of bioprocess models through the use of different optimization techniques is presented. The method was applied to a tower bioreactor for bioethanol production with immobilized cells of *Saccharomyces cerevisiae*. Specifically, a step-by-step procedure to the estimation problem is proposed. As the first step, the potential of global searching of real-coded genetic algorithm (RGA) was applied for simultaneous estimation of the parameters. Subsequently, the most significant parameters were identified using the Placket–Burman (PB) design. Finally, the quasi-Newton algorithm (QN) was used for optimization of the most significant parameters, near the global optimum region, as the initial values were already determined by the RGA global-searching algorithm. The results have shown that the performance of the estimation procedure applied in a deterministic detailed model to describe the experimental data is improved using the proposed method (RGA–PB–QN) in comparison with a model whose parameters were only optimized by RGA.

Keywords Ethanol fermentation · Parameter estimation · Modeling · Optimization techniques · Artificial intelligence

Introduction

Bioethanol (ethanol from biomass) is nowadays the largest fermentation product obtained from sugar cane. In fact, bioethanol seems to be the most promising alternative energy source to be used as a fuel, either alone or as mixture in gasoline. Besides, from bioethanol, many chemicals products may be produced, making the sugar cane-based feedstock process

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to be very interesting from the environmental point of view and an economic attractive raw material to chemicals production. For instance, it is possible to achieve high-quality acetaldehyde, acetic acid, ethyl acetate, and ethylene and from them a huge amount of chemicals, including polymers. Although the bioethanol production is running for several years, improvements are required to increase process performance. A suitable route is to improve the fermentation process, including the investigation of an alternative process as a tower with immobilized microorganisms, to obtain bioethanol and use ethanol chemistry to obtain others chemicals.

There are many minor industrial problems associated with the ethanol fermentation processes to be solved nowadays, when optimal operation is a target. Among them, there is the lack of process robustness in the presence of fluctuations in operational conditions, which leads to changes in the kinetic behavior, with impact on yield, productivity, and conversion. These changes are very common in ethanol plants, where they occur not only because of the variations in the quality of the raw material but also because of variations of dominant microorganisms in the process.

The lack of robustness can be corrected by adjustments in the operational and control parameters of the process when fluctuations occur. To accomplish this, it is important that a mathematical model be available to aid in the decision making, mainly when the difficulties of monitoring the key process variables are taken into account. Care has to be taken with the values of the model parameters, especially the kinetic ones so that reliable predictions can be made.

Compared with growing studies of microorganism populations, few improvements on the development and application of structured deterministic models for product formation have appeared. They are essential to develop more advanced operation strategies as well as to develop control and optimization algorithms.

Estimation of kinetic parameters of deterministic models is usually complex, mainly because of nonlinearities, great number of parameters, and interactions among them. In biochemical engineering, the most classical method involves the mathematical estimation of model parameters based on the minimization of some cost function built up with the parameters to be estimated. Several kinetic models have been proposed for the alcoholic fermentation process [1–3]. Many techniques are available for minimizing the error of estimation, generally methods based on gradient search, such as the quasi-Newton algorithm (QN). At this point, it is important to bear in mind that when detailed structured models are considered, the number of the parameters to be identified and also the interactions among them increase significantly.

Artificial intelligence, such as Genetic Algorithms (GAs), covers a wide range of techniques and tools that facilitate decision making. It is often as powerful and effective as gradient search methods in many engineering applications but with some advantages related to independence of the initial guess to achieve the solution. These methods have already been successfully applied in the optimization and control of bioprocesses for more than 20 years [4]. GAs have been successfully utilized for kinetic parameter estimation in biotechnological processes [5–7].

In this work, the proposed optimization procedure is based on the combination of different optimization techniques, to know: real-coded GA (RGA), Placket–Burman (PB) design, and QN. The approach is applicable when the structure of a kinetic model has been set up and the kinetic parameters should be estimated.

The parameter estimation methodology was demonstrated on an alternative dynamic structured model [8], adapted from Rotboll and Jorgensen [9] to simulate a tower bioreactor for ethanol production by immobilized *Saccharomyces cerevisiae*. The model contains 34

kinetic parameters and nine parameters (K_E, K_{E2}, F_1, F_2, F_3, F_5, F_6, k_d, C), related to the glycolitic and respiratory (tricarboxylic acid [TCA]) paths, which were re-estimated.

Methodology

This section describes a parameter estimation methodology in three steps, which was developed to estimate kinetic parameters in structured models. First, a RGA was applied for simultaneous estimation of the parameters, and secondly, the most significant parameters were identified using the PB design. Finally, the QN was used for optimization of the most significant parameters, near to the global optimum region, as the initial values were already determined by the RGA global searching algorithm.

Case Study: Kinetic Model for a Structured Detailed Mechanistic Model

Process Description

The case study is based on a tower-type bioreactor that uses *S. cerevisiae* immobilized in pellets with 4% of citric pectin, for production of bioethanol. The bioreactor is divided in four stages with gas separators between them to prevent the CO_2 accumulation during the fermentation process because the CO_2 release may eventually result in a drop in the fermentation yield. The experiments were performed at 30 °C, pH 4.0, initial substrate concentration of 161.4 g/L, feed flow rate of 40 mL/h, and residence time of 6.12 h. After 40 h of operation, the system has reached a steady state. A diagram of the system is shown in Fig. 1.

The developed deterministic detailed model based on the work of Stremel [8] for the dynamic simulation of this system, shown in the Appendix, also includes terms for the inhibition by ethanol, substrate, and saturation by the cells inside the pellets. Stremel [8] also investigated all the parameters and their effects to identify the most significant but did not use a methodology to optimize simultaneously the parameters of the model.

Optimization by Real-coded Genetic Algorithm

The GAs used was, basically, the FORTRAN RGA developed by Yedder [10], with some modifications. In the proposed strategy, the chromosome or individual are vectors where each real value (gene) stands for each one of the unknown parameters in the kinetic model. The stopping criterion is selected when the maximum number of fixed generations is reached. The RGA parameters were adjusted to minimize the number of generations (iterations) required to reach a satisfactory fitness (objective function) value by minimizing Eq. 1, which reflects a good agreement between the measured concentration and the concentration computed by the model.

$$E(\theta) = \sum_{n=1}^{\mathrm{np}} \left[\frac{(S_n - \mathrm{Se}_n)^2}{\mathrm{Se}_{\max}^2} + \frac{(P_n - \mathrm{Pe}_n)^2}{\mathrm{Pe}_{\max}^2} \right] = \sum_{n=1}^{\mathrm{np}} \varepsilon_n(\theta) \tag{1}$$

In this equation, Se_n and Pe_n are the measured concentrations of substrate and ethanol at the sampling time n. S_n and P_n are the concentrations computed by the model at the sampling time n. $\mathrm{Se}_{\max}$ and $\mathrm{Pe}_{\max}$ are the maximum measured concentrations and the term np is number of sampling points. In this equation, $\varepsilon_n(\theta)$ is the error in the output because of the nth sample.

Some basic information on the GA is reported in Table 1.

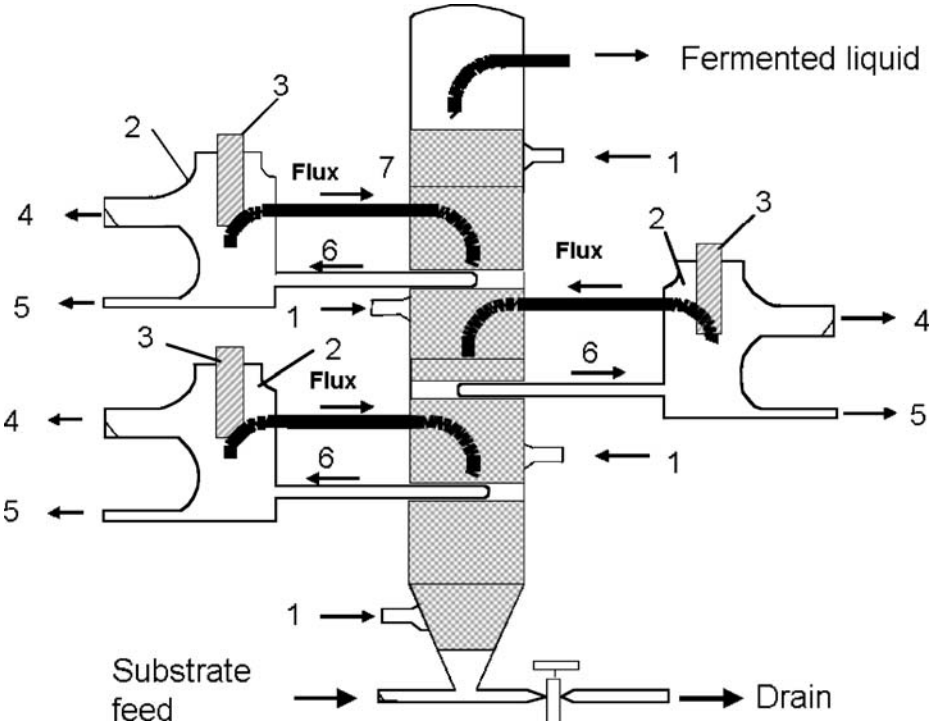

Fig. 1 Tower bioreactor. *1*, Inlet and outlet of the pellets; *2*, gas–liquid separator; *3*, inductive sensor; *4*, CO_2 outlet; *5*, evacuation of sample; *6*, fermented liquid with CO_2 flow; *7*, fermented liquid without CO_2 flow

Identification of Significant Parameters

This step consists of applying the PB design to identify the kinetic parameters that are significant on the optimization problem. The PB design is a partial factorial method that allows the testing of multiple independent process variables within a single experiment. The influence of 43 parameters on Eq. 1 was investigated using the methodology of Plackett and Burman [11]. Four variables were designated as "dummy" variables because no change is made to them, but they are used to give an estimate of the standard error for each factor. Each parameter (factor) is tested at two levels, a high (+) and a low (−) level, which will be determined after the optimization by RGA. The PB design contains a total of 47 trials.

Table 1 Main technical features used by the real-coded genetic algorithm.

Option chosen—RGA	Parameters	Parameter values
Niching, elitism, barycentric crossover, nonuniform mutation	Individual length (number of parameters in the model)	43
	Population size	10
	Crossover probability	0.9
	Mutation probability	0.03

Final Optimization by Quasi-Newton Algorithm

A simultaneous estimation of the kinetic parameters selected in the previous step is performed using the QN, whereas all other parameters remained fixed in the global optimum region, calculated by the RGA. The FORTRAN IMSL routine DBCONF was

Table 2 Optimized parameters by real-coded genetic algorithm.

Parameter	Parameter values optimized by RGA
k_1	0.469
k_2	0.262
k_3	8.112
k_4	2.467E−03
k_5	1418.132
k_6	1.150
k_8	0.377
k_9	2.883E−02
k_{10}	2.275E−03
s_1	1.213E−02
s_2	4.920E−04
s_3	8.072E−02
s_4	1.00E−06
s_5	0.358
s_6	1.801E−02
s_8	2.016E−02
s_9	1.0E−06
s_{1e}	0.147
s_{5e}	5.770E−02
s_{8e}	1.978E−03
s_{9e}	9.558
s_{10e}	168.26
k_{1e}	29.262
k_{4e}	1.264
k_{8e}	3.615E−03
k_{10e}	0.892
K_E	5.092E−02
K_{E2}	6.038E−02
k_{1i}	6.435
m_2	3.477
m_{2e}	3.476
k_{4i}	9919.791
k_{5i}	1981.616
k_{5r}	0.303
k_{9i}	2716.006
k_{10i}	504.813
k_d	7.910E−04
F_1	2.249
F_2	1.276
F_3	0.420
F_5	2.362
F_6	1.212
C	1.098

used for this purpose. The straightforward idea is to implement the optimization problem as a nonlinear programming problem that can be written as:

$$\text{Minimize Eq. 1}$$
$$\text{Subject to } l_p \leq x_p \leq u_p, p = 1, ..., 5$$

where x_p is the parameters. The l_p and u_p are specified lower and upper bounds on the parameters, with $l_p \leq u_p$.

Table 3 Effect estimate on Eq. 1 from results of Plackett–Burman design.

Factor	Effect	SE	t (4) value	P value	−95%	95%
Mean	0.081860*	0.000503*	162.6241*	0.000000*	0.080462*	0.083258*
k_1	0.000571	0.001007	0.5673	0.600833	−0.002224	0.003366
k_2	0.000514	0.001007	0.5107	0.636455	−0.002281	0.003309
k_3	0.000470	0.001007	0.4669	0.664869	−0.002325	0.003265
k_4	−0.000473	0.001007	−0.4696	0.663097	−0.003268	0.002322
k_5	0.000766	0.001007	0.7604	0.489370	−0.002030	0.003561
k_6	−0.012757*	0.001007*	−12.6718*	0.000223*	−0.015552*	−0.009962*
k_8	0.006145*	0.001007*	6.1039*	0.003645*	0.003350*	0.008940*
k_9	−0.001227	0.001007	−1.2185	0.289984	−0.004022	0.001568
k_{10}	0.001798	0.001007	1.7855	0.148728	−0.000998	0.004593
s_1	0.000386	0.001007	0.3830	0.721203	−0.002410	0.003181
s_2	0.000459	0.001007	0.4557	0.672248	−0.002336	0.003254
s_3	−0.000939	0.001007	−0.9324	0.403912	−0.003734	0.001856
s_4	0.000494	0.001007	0.4910	0.649146	−0.002301	0.003289
s_5	−0.000230	0.001007	−0.2285	0.830464	−0.003025	0.002565
s_6	−0.000306	0.001007	−0.3036	0.776569	−0.003101	0.002489
s_8	−0.000521	0.001007	−0.5174	0.632173	−0.003316	0.002274
s_9	0.000187	0.001007	0.1855	0.861863	−0.002608	0.002982
s_{1e}	−0.000262	0.001007	−0.2601	0.807626	−0.003057	0.002533
s_{5e}	−0.001247	0.001007	−1.2391	0.283046	−0.004043	0.001548
s_{8e}	0.000423	0.001007	0.4202	0.695930	−0.002372	0.003218
s_{9e}	−0.000205	0.001007	−0.2038	0.848458	−0.003000	0.002590
s_{10e}	−0.001174	0.001007	−1.1664	0.308253	−0.003969	0.001621
k_{1e}	−0.002425	0.001007	−2.4092	0.073619	−0.005221	0.000370
k_{4e}	−0.000807	0.001007	−0.8013	0.467855	−0.003602	0.001988
k_{8e}	0.001162	0.001007	1.1543	0.312646	−0.001633	0.003957
k_{10e}	0.000644	0.001007	0.6396	0.557227	−0.002151	0.003439
K_E	0.016200*	0.001007*	16.0913*	0.000087*	0.013405*	0.018995*
K_{E2}	0.007588*	0.001007*	7.5369*	0.001660*	0.004793*	0.010383*
k_{1i}	0.001114	0.001007	1.1065	0.330574	−0.001681	0.003909
m_2	0.000628	0.001007	0.6235	0.566748	−0.002167	0.003423
m_{2e}	0.000541	0.001007	0.5371	0.619683	−0.002254	0.003336
k_{4i}	−0.000171	0.001007	−0.1696	0.873557	−0.002966	0.002624
k_{5i}	0.000715	0.001007	0.7101	0.516848	−0.002080	0.003510
k_{5r}	0.000108	0.001007	0.1071	0.919866	−0.002687	0.002903
k_{9i}	−0.000721	0.001007	−0.7166	0.513234	−0.003517	0.002074
k_{10i}	−0.000685	0.001007	−0.6808	0.533368	−0.003481	0.002110
k_d	0.000506	0.001007	0.5027	0.641591	−0.002289	0.003301
F_1	−0.024644*	0.001007*	−24.4788*	0.000017*	−0.027439*	−0.021849*
F_2	0.022353*	0.001007*	22.2032*	0.000024*	0.019558*	0.025148*
F_3	0.023282*	0.001007*	23.1265*	0.000021*	0.020487*	0.026077*
F_5	0.021074*	0.001007*	20.9330*	0.000031*	0.018279*	0.023869*
F_6	−0.002826*	0.001007*	−2.8074*	0.048445*	−0.005621*	−0.000031*
C	−0.000778	0.001007	−0.7726	0.482874	−0.003573	0.002017

* Significant for a 95% confidence level.

Table 4 Re-estimated parameters by quasi-Newton.

Parameter	Parameter values optimized by QN
K_E	5.099E−02
F_1	2.244
F_2	1.276
F_3	0.435
F_5	2.353

Results

The optimized parameters by RGA are shown in Table 2.

By considering the values optimized by RGA as central point in a PB design, the effects for all parameters on Eq. 1 (response), for a 95% confidence level, were calculated. The effects of the parameters are given in Table 3.

A total of five most significant parameters (shown in Table 4), i.e., greater effects on error, Eq. 1, were re-estimated by QN, whereas all other parameters remained fixed in the global optimum region, in the values set by the RGA. Under such optimal model, the computed profiles for ethanol, *P*, and substrate, *S*, are shown by the dashed lines in Figs. 2 and 3, respectively.

The residual standard deviation (RSD) [12], Eq. 2, written as a percentage of the average of the experimental values, was the measurement used for characterizing the quality of the prediction of the model.

$$RSD(\%) = \frac{\left(\frac{1}{\mathrm{np}}\left(\sum_{p=1}^{\mathrm{np}}\left(d_p - x_p\right)^2\right)^{0.5}\right)}{\overline{d}_p} \times 100 \tag{2}$$

where x_p and d_p are, respectively, the value predicted by the mathematical model and experimental value, $\overline{d}_p$ is the average of the experimental values, and *np* is the number of experimental points.

The RSD (%) for the model optimized by only RGA and the methodology proposed in this work (RGA–PB–QN) are shown in Table 5. The results obtained using the methodology RGA–PB–QN were better than the results using only RGA. It can be seen that the deviations are 8.6 and 2.3% for concentrations of substrate and ethanol, respectively. In bioprocess

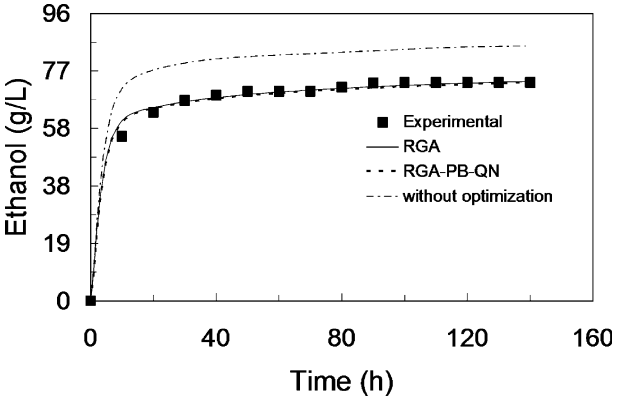

Fig. 2 Experimental (ethanol, *squares*) and modeling (model optimized by using only RGA [*solid line*]; model optimized by using RGA-PB-QN [*dashed line*]; model without optimization [*point-dash line*]) results

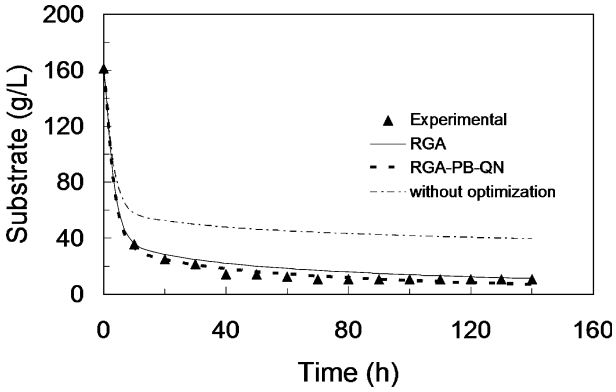

Fig. 3 Experimental (substrate, *triangles*) and modeling (model optimized by using only RGA (*solid line*); model optimized by using RGA-PB-QN [*dashed line*]; model without optimization [*point-dash line*]) results

engineering, values of RSD (%) below 10% can be considered acceptable [12]. Thus, the estimated model was able to fit experimental observations satisfactorily.

Concluding Remarks

A structured detailed mechanistic model to simulate a tower bioreactor for bioethanol production with immobilized *S. cerevisiae* was studied and coupled with an optimization procedure. This computer-aided process engineering tool allows to identify kinetic parameters as well as to explore different operational strategies.

The major problem with structured models is their large number of parameters, which makes the estimation procedure a difficult task. The use of deterministic optimization methods, such as QN, to estimate a large number of parameters (in the studied case 43) usually leads to lack of convergence. On the other hand, GAs are well suited to large-scale problems but have the drawback of slow convergence. In this work, it is proposed an estimation methodology in four steps that can be used always that a re-estimation of parameters is necessary.

The first step is to calculate the parameters in the model using a RGA. The second step is to identify the most significant of the 43 parameters using the PB design, and finally, the most significant (in the studied case five parameters) are optimized using a QN, which converges much more quickly than RGA to the optimal.

The results have shown that the performance of the model to describe the experimental data (measured as RSD (%)) is improved using the proposed methodology in comparison with a model whose parameters were only optimized by RGA.

Finally, it should be noted that the proposed methodology can be applied in many other parameter estimation problems and can be used as a reliable tool for optimizing other types of biotechnological processes.

Table 5 Residual standard deviation, RSD (%), used to characterize the prediction quality of the model.

Output variable	RSD (%)	
	RGA	RGA–PB–QN
Ethanol (g/L)	2.4	2.3
Substrate (g/L)	25.7	8.6

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Appendix

A.1

The generic dynamic model for the bioparticle, where the kinetic expressions are adapted of Rotboll and Jorgensen [9] is given by Eq. 3. In the model, [ ] represents a generic concentration. Substrate conversion in the pellet through the reactions of the Embden–Meyerhof–Parnas pathway forms intermediates such as acetaldehyde [A] and pyruvate [P] that are reduced to ethanol [E] or oxidized in the steps of the TCA pathway.

$$\frac{\partial[\,]}{\partial t} = \alpha_{[\,]}\Delta^2_{r[\,]} \pm \left[\sum_{i,j} F_i R_j X_s\right] \exp\left(-K_E E_f\right) X_{[T]} \tag{3}$$

where

$$R_j = f(v_k,\ [S],\ [A],\ [P],\ [E],\ [X_s],\ [X_r],\ [X_{\mathrm{fe}}]) \tag{4}$$

where i=1...6 (related to Eq. 3); j=1...7 (related to Eqs. 3 and 4); k=1...34 (related to Eq. 4).

The first and second terms of Eq. 3 represent the diffusion through the particle and fluid reaction rate, respectively. The signal (±) shows if the substance is being consumed (−) or formed (+). The subscripts of the reaction rates R_j (h^{-1}) are related to the different subscripts of the factors (F_i). F_1, F_2, F_3, F_5, and F_6 are parameters to be adjusted for the glycolytic and respiratory pathways (see Table 6). The synthetic composition $X_{[S]}$ (g/g dry mass basis) is responsible for the metabolic synthesis, fermentation, and respiration.

The generic Eq. 5 is valid for the variables that do not spread outside of the pellets, where $X_{[s]}$, $X_{[p]}$, and $X_{[r]}$ (g/g dry-mass basis) are synthetic, structural, and enzymatic components of the respiratory pathway, respectively, and $X_{[\mathrm{fe}]}$ is the fermentative component.

$$\frac{\partial X_{[\,]}}{\partial t} = \left[\sum_i R_i\right] X_{[S]} - \left[\sum_k R_k X_{[S]}\right] X_{[\,]} \tag{5}$$

Table 6 Reaction rates and factors for Eq. 3.

Substance [ ]	F_i	R_j	Signal (±)	$F_i R_j$
Glucose [S]	F_1, F_6	R_1, R_6	−	F_1R_1, F_6R_6
Ethanol [E]	F_5, $-F_4$	R_5, R_7	+	F_5R_5, $-F_4R_7$
Acetaldehyde [A]	F_3, $-F_4$	R_3, R_4, R_5, R_7	+	F_3R_3, $-F_4R_4$, $-F_4R_5$, $-F_4R_7$
Pyruvate [P]	F_2, $-F_4$	R_1, R_2, R_3	+	F_2R_1, $-F_4R_2$, $-F_4R_3$

Table 7 Reaction rates and factors for equation 5 and 7.

Components	R_i	R_k
$X_{[s]}$	R_6, R_7	R_6, R_7
$X_{[p]}$	R_8	R_6, R_7
$X_{[r]}$	R_9	R_6, R_7
$X_{[fe]}$	R_{10}	R_6, R_7
$X_{[T]}$	–	R_6, R_7

The reaction rates of the components (g/g h), as function of the parameters v_l and variables [ ] are:

$$R_i = f(v_l,\ [S],\ [A],\ [P],\ [E],\ [X_r]) \tag{6}$$

Equation 7 represents the concentration of total cell mass

$$\frac{\partial X_{[T]}}{\partial t} = \left[\sum_k R_k X_{[S]}\right] X_{[T]} FI_X - k_d X_{[T]} \tag{7}$$

The reaction rates R_k (see Table 7) as function of the parameters v_m and variables [ ] are:

$$R_k = f(v_m,\ [S],\ [A],\ [P],\ [E],\ [X_r]) \tag{8}$$

where i=6...10 (related to Eq. 5); k=6, 7 (related to Eq. 5 and 7), l=12...14; 21...34 (related to Eq. 6); m=12...14; 21...22 (related to Eq. 8).The number of parameters v is 34. The other constants considered are: K_E, K_{E2}, F_1, F_2, F_3, F_5, F_6, k_d, and C.

A simplification of the glycolytic and respiratory routes considered in the deterministic model to represent bioethanol synthesis is shown below by stoichiometric expressions. More details of the kinetics can be found in Stremel [8].

$$\begin{aligned}
&F_1[S] \xrightarrow{R_1} F_2[P]\\
&F_6[S] \xrightarrow{R_6} 0.732X_{[S]} \xrightarrow{0.732R_6X_{[S]}} X_{[T]}\\
&F_4[P] + O_2 \xrightarrow{R_2} TCA + CO_2\\
&F_4[P] \xrightarrow{R_3} F_3[A]\\
&F_4[A] + O_2 + X_{[r]} \xrightarrow{R_4} TCA + X_{[r]} + CO_2\\
&F_4[A] + X_{[fe]} \xrightarrow{R_5} F_5[E] + X_{[fe]} + CO_2\\
&F_4[A] \xrightarrow{R_7} 0.850X_{[S]} \xrightarrow{0.850R_7X_{[S]}} X_{[T]}\\
&X_{[S]} \xrightarrow{R_8} X_{[P]} \xrightarrow{(0.732R_6 + 0.850R_7)X_{[P]}} X_{[T]}\\
&X_{[S]} \xrightarrow{R_9} X_{[r]} \xrightarrow{(0.732R_6 + 0.850R_7)X_{[r]}} X_{[T]}\\
&X_{[S]} \xrightarrow{R_{10}} X_{[fe]} \xrightarrow{(0.732R_6 + 0.850R_7)X_{[fe]}} X_{[T]}\\
&X_{[T]} \xrightarrow{k_d} X_{[nv]}
\end{aligned} \tag{9}$$

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