Towards a quantitative modeling of the synthesis of the pectates lyases, essential virulence factors in *Dickeya dadantii*

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1. Model

1.1. Chemical interaction description and mass action modeling

The following Tables and simplifications provide a complete overview on all model equations.

To describe the dynamics of the network, we define all the chemical interactions occurring between molecules of the network. Table 1 summarizes the reaction types

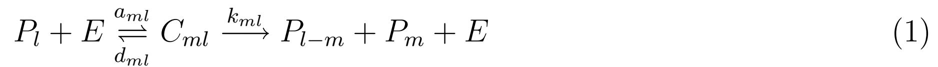
Table 1. List of reactions occurring between molecules involved in the dynamics of the simplified regulatory network.

Reactions	Reagents	Products	rate	units
Transcription	Pr-*Pel*	Pr-*Pel* + mRNA-*Pel*	k_3	min^{-1}
Transcription	Pr-*KdgR*	Pr-*KdgR* + mRNA-*KdgR*	k_4	min^{-1}
Translation	mRNA-*Pel*	Pel + mRNA-*Pel*	η_1	min^{-1}
Translation	mRNA-*KdgR*	KdgR + mRNA-*KdgR*	η_2	min^{-1}
Dimerisation	2 KdgR	$KdgR_2$	a_2	$\mu M.min^{-1}$
Dissociation	$KdgR_2$	2 KdgR	d_2	min^{-1}
Repression	Pr-*Pel* + $KdgR_2$	Pr-*Pel*.$KdgR_2$	a_6	$\mu M.min^{-1}$
Repression dissoc	Pr-*Pel*.$KdgR_2$	Pr-*Pel* + $KdgR_2$	d_6	min^{-1}
Transport	UGA(product of degradation)	KDG	γ	min^{-1}
Complex formation	$KdgR_2$ + 2 KDG	$KdgR_2.KDG_2$	a_3	$\mu M^{-2}.min^{-1}$
Complex dissoc	$KdgR_2.KDG_2$	$KdgR_2$ + 2 KDG	d_3	min^{-1}
Phosphorylation	KDG	KDGp	k_5	min^{-1}
Degradation	mRNA-*Pel*	∅	γ_1	min^{-1}
Degradation	mRNA-*KdgR*	∅	γ_2	min^{-1}
Degradation	Pel	∅	α_1	min^{-1}
Degradation	KdgR	∅	α_2	min^{-1}
Degradation	$KdgR_2$	∅	$\alpha_2^{'}$	min^{-1}
Degradation	$KdgR_2.KDG_2$	∅	$\alpha_2^{''}$	min^{-1}
Degradation	Pr-*Pel*.$KdgR_2$	∅	$\alpha_2^{'''}$	min^{-1}
Degradation	Pr-*KdgR*.$KdgR_2$	∅	$\alpha_2^{''''}$	min^{-1}

occurring between molecules, except for the intermediary molecules involved between the PGA and the UGA in the metabolic module of the network (confined in the box with continuous line in Fig. 2). Table 2 summarizes the dynamical equations for each molecules involved in the regulatory network. These dynamical equations are built by applying the mass-action principle to each reaction of Table 1.

1.2. Modeling the enzymatic degradation of PGA

The metabolic module describes the degradation of PGA, the formation of KDG and its inductive effect catalyzed by Pel enzymes [1, 2]. This directed enzymatic degradation of Pectin into KDG, is assumed to follow Michaelis Menten kinetics. In order to model this enzymatic degradation, lets consider a polymer P_l (of molecular weight $l\mathcal{P}$) which can be degraded by and enzyme E. One will suppose that the enzyme can bind and cut any sites of the polymer. For a polymer P_l (constituted of l monomers), $l-1$ complexes can be formed between the polymer and the enzyme. These considerations lead to the following chemical equations:



$$P_l + E \underset{d_{ml}}{\overset{a_{ml}}{\rightleftharpoons}} C_{ml} \xrightarrow{k_{ml}} P_{l-m} + P_m + E \qquad (1)$$

with $l = 2, ..., L$ and $m = l+1, ..., L$; where C_{ml} is the $E.P_l$ complex with the enzyme fixed on the $(m, m+1)$ dimer. L is the maximal polymer length.

The conservation law on the total enzyme gives:

$$E_T = E + \sum_{l=2}^{L} \sum_{m=1}^{l-1} C_{ml} \qquad (2)$$

Table 2. List of variables and dynamic equation of each molecule used to model the dynamics of the regulatory network. Dynamical equations are built by applying the mass-action principle to each reaction.

Molecules	Concentration	Equation of dynamics
Total Pel	x	$x = x^* + \sum_{l=2}^{L} \sum_{m=1}^{l-1} C_{ml}$
Total KdgR	y	$y = y^* + 2y_d + 2q_1 + 2c_2$
Total Pr-*Pel*	P_1	$P_1 = P_1^* + q_1$
Total Pr-*KdgR*	P_2	
KDG	w	$\frac{dw}{dt} = \gamma(1-\rho)z - (k_5 + \mu(t))w - a_3 w^{*2} y_d + d_3 c_2$
"Free" Pr-*Pel*	P_1^*	$\frac{dP_1^*}{dt} = -a_6 P_1^* y_d + d_6 q_1 + (\alpha_2^{'''} + \mu(t))q_1$
"Free" Pel	x^*	$\frac{dx^*}{dt} = \eta_1 \left(\frac{\rho}{1-\rho}\right) m_1 - \alpha_1 x^* - \sum_{l=2}^{L} \sum_{m=1}^{l-1} (a_{ml} s_l x^* - (d_{ml} + k_{ml}) C_{ml})$
"Free" KdgR	y^*	$\frac{dy^*}{dt} = \eta_2 m_2 - (\alpha_2 + \mu(t))y^* - 2(a_2 y^{*2} + d_2 y_d)$
mRNA-*Pel*	m_1	$\frac{dm_1}{dt} = k_3 P_1^* - \gamma_1 m_1$
mRNA-*KdgR*	m_2	$\frac{dm_2}{dt} = k_4 P_2 - \gamma_2 m_2$
PGA.Pel	c_1	$\frac{dc_1}{dt} = a_1 s x^* - (d_1 + k_1) c_1$
$KdgR_2$.KDG	c_2	$\frac{dc_2}{dt} = a_3 w^{*2} y_d - d_3 c_2 - (\alpha_2^{''} + \mu(t)) c_2$
Pr-*Pel*.$KdgR_2$	q_1	$\frac{dq_1}{dt} = a_6 P_1^* y_d - d_6 q_1 - (\alpha_2^{'''} + \mu(t)) q_1$
$KdgR_2$	y_d	$\frac{dy_d}{dt} = a_2 y^{*2} - d_2 y_d - a_3 w^{*2} y_d + d_3 c_2 - a_6 P_1^* y_d + d_6 q_1 - (\alpha_2^{'} + \mu(t)) y_d$

this is a sum of several contributions: C_{ml} contains enzyme which is captured by the enzymatic degradation and E is the free available enzyme. In our network, E corresponds to the Pel enzyme. An analog interpretation holds for the total Pel and KdgR conservation equations (See Table 2). The degradation dynamics of the molecular concentration (s_l) of polymer P_l (taking into account the fact that the final product of degradation (UGA) is then imported in the intracellular milieu) is given by :

$$\frac{ds_l}{dt} = 2 \sum_{j=l+1}^{L} k_{lj} C_{lj} - \sum_{m=1}^{l-1} (d_{ml} C_{ml} - a_{ml} E s_l) \tag{3}$$

$$\frac{ds_1}{dt} = 2 \sum_{j=2}^{L} k_{1j} C_{1j} - \gamma \rho s_1 \tag{4}$$

$$\frac{dC_{ml}}{dt} = a_{ml} E P_l - d_{ml} C_{ml} - k_{ml} C_{ml} \tag{5}$$

with $l = 2, ...L$

1.3. Modeling the biomass growth

In order to take into account the biomass growth during experiment, one introduces a specific growth rate $\mu(t) = \frac{\dot{\rho}}{\rho}$. $\rho = \frac{v_b}{V_e}$ represents the bacteria volume fraction, v_b is the total bacteria volume and V_e the extracellular volume. This growth rate function depends on the amount of substrate introduced and on the initial number of inoculated bacteria, N_0. The logistic function is used for the bacteria volume fraction growth description:

$$\frac{d\rho}{dt} = \sigma \rho \left(1 - \frac{\rho}{\rho_s}\right) \tag{6}$$

where σ is the bacteria growth rate and ρ_s is the maximum bacteria volume fraction. The initial bacteria volume fraction is given by $\rho_0 = \frac{N_0 v_0}{V_e}$, where $v_0 \simeq 1(\mu m)^3$ is the volume of one cell.

In order to determine the dependence of these parameters on the initial bacteria N_0 and the initial substrate $[PGA]_0$, for several experiments with varying amounts of substrate, the parameters of the logistic function are obtained by fitting the biomass growth with the logistic function. Therefore, as shown on Figs. 1 using a linear regression the dependence of these parameters on the initial amount of PGA introduced can be derived:

$$\rho_s = (1.71[PGA]_0 + 1.75) \times 10^{-3} \tag{7}$$

$$\sigma = 0.56[PGA]_0 + 0.59 \tag{8}$$

Therefore, knowing the initial amount of substrate and the initial number of bacteria, the bacteria growth equation can be solved.

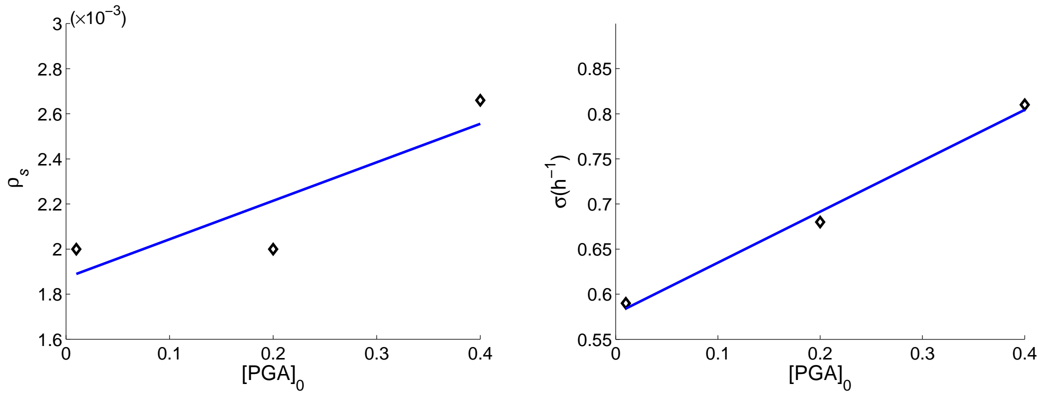


Figure 1. Determination of the variation of bacteria growth parameters ρ_s and σ as a function of the initial amount of substrate (PGA) introduced. The black points are obtained from three sample experiments with different amount of initially introduced PGA (0.01%, 0.2% and 0.4% w/v) by fitting the A_{600} with the logistic function. The blue line corresponds to the linear regression function that allows to obtain the variation parameters ρ_s and σ as a function of the initial amount of substrate.

2. Model reduction using QSSA

2.1. Case of a polymer of length $L > 2$

In this Section, one will describe how the complete model presented in Table 2 is simplified using QSSA. Considering the fact that the dynamics of transcripts are very fast compared with the one of proteins, one can apply the fast and slow variables separation. Therefore, by distinguishing two concentration vectors $B = (x, y, s_l, w, z,)$ and $R = (x^*, y^*, P_1^*, m_1, m_2, q_1, c_1, c_2, y_d, C_{ml})$ respectively for the slow and the fast variables, the complete model in Table 2 can be rewritten in the abstract form:

$$\frac{dB}{dt} = F(B, R) \tag{9}$$

$$\frac{dR}{dt} = G(B, R) \tag{10}$$

Where $F(R, B)$ and $G(R, B)$ are the right-hand side of differential equations in Table 2. In this new set of variables, the time-scales of the dynamics are well separated. F contains only the terms describing slow variables dynamics, and G fast variables dynamics. Thus the standard QSSA for the R variables can be applied allowing to reduce the analysis of the dynamics to the slow variables B given by:

$$\frac{dx}{dt} = \eta_1\Big(\frac{\rho}{1-\rho}\Big)m_1 - \alpha_1 x^* \tag{11}$$

$$\frac{dy}{dt} = \eta_2 m_2 - (\alpha_2 + \mu(t))y \tag{12}$$

$$\frac{ds_l}{dt} = 2\sum_{j=l+1}^{L} k_{lj}C_{lj} - \sum_{m=1}^{l-1} k_{ml}C_{ml} \tag{13}$$

$$\frac{ds_1}{dt} = 2\sum_{j=2}^{L} k_{1j}C_{1j} - \gamma\rho s_1 \tag{14}$$

$$\frac{dw}{dt} = \gamma(1-\rho)s_1 - (k_5 + \mu(t))w \tag{15}$$

with $l = 2, ..., L$ and the following constraints obtained for $G = 0$:

$$k_3P_1^* - \gamma_1 m_1 = 0 \tag{16}$$

$$k_4P_2 - \gamma_2 m_2 = 0 \tag{17}$$

$$a_2y^{*2} - d_2y_d = 0 \tag{18}$$

$$a_3w^{*2}y_d - d_3c_2 = 0 \tag{19}$$

$$a_6P_1^*y_d - d_6q_1 = 0 \tag{20}$$

$$a_{ml}P_lx^* - d_{ml}C_{ml} - k_{ml}C_{ml} = 0 \tag{21}$$

Solving constraints, one gets the following expression of species with fast dynamics at equilibrium as function of w, y_d, P_1 and P_2 only (with P_1 and P_2 constants) :

$$P_1^* = \frac{d_6}{d_6 + a_6y_d}P_1$$

$$m_1 = \frac{k_3}{\gamma_1}P_1^*$$

$$m_2 = \frac{k_4}{\gamma_2}P_2$$

$$y_d = \frac{a_2}{d_2}y^{*2}$$

$$q_1 = \frac{a_6P_1}{d_6 + a_6y_d}y_d$$

$$c_2 = \frac{a_3}{d_3}w^2y_d$$

The free Pel (x^*) and KdgR (y^*) have not yet been determined. In order to determine x^*, one will consider here that the kinetic rates a_{ml}, d_{ml} and k_{ml} describing chemical reactions (1) does not depend on m and l, due to the fact that the considered interaction is specific (i.e depends only on the interaction of located particles, an enzyme and a site on the polymer). So in order to simplify, one takes $a_1 = a_{ml}, d_1 = d_{ml}$ et $k_1 = k_{ml}$. Using this hypothesis, the Michaelis and Menten constant K_{ml} for each chemical reaction described in equation (1) are identical and equal to:

$$K_m = \frac{d_1 + k_1}{a_1}$$

Let us notice that K_m is in mM. The constraint equation (21) and the conservation law on total Pel give:

$$x = x^* \left(1 + \sum_{l=2}^{L} (l-1) \frac{s_l}{K_m}\right)$$

and

$$\sum_{m=1}^{l-1} C_{ml} = \frac{s_l}{K_m + \sum_{j=2}^{L} (j-1) s_j} x$$

For the determination of y^*, one assumes that the free KdgR concentration (y^*) can be negligible compared to the total KdgR (y), therefore, one have the following approximation:

$$y \simeq 2y_d + 2q_1 + 2c_2$$

one can have a relation between y, w and, y_d by replacing q_1 and c_2 by their expression:

$$y = 2y_d \left[1 + \frac{a_3}{d_3} w^2 + \frac{a_6 P_1}{d_6 + a_6 y_d}\right]$$

Substituting these expressions into equations (11)-(15), and defining some effective parameters, one has

$$\frac{dx}{dt} = \beta_1 \left(\frac{\rho}{1-\rho}\right) \left(\frac{1}{1 + y_d/K_{d6}}\right) - \alpha_1 \frac{K_m}{K_m + \sum_{j=2}^{L} (j-1) s_j} x \quad (22)$$

$$\frac{dy}{dt} = \beta_2 - (\alpha_2 + \mu(t)) y \quad (23)$$

$$\frac{ds_l}{dt} = \frac{k_1 x}{K_m + \sum_{j=2}^{L} (j-1) s_j} \left(\sum_{m=l+1}^{L} 2s_m - (l-1) s_l\right) \quad (24)$$

$$\frac{ds_1}{dt} = \frac{k_1 x}{K_m + \sum_{j=2}^{L} (j-1) s_j} \left(\sum_{m=l+1}^{L} 2s_m\right) - \gamma \frac{v_b}{V_e} s_1 \quad (25)$$

$$\frac{dw}{dt} = \gamma (1-\rho) s_1 - (k_5 + \mu(t)) w \quad (26)$$

with $l = 2, ...L$

where

$$y = 2y_d \left[1 + \left(\frac{w}{K_{d3}}\right)^2 + \frac{P_1}{K_{d6} + y_d}\right]$$

with $K_{d3}^2 = \frac{d_3}{a_3}$ and $K_{d6} = \frac{d_6}{a_6}$. K_{di} correspond to dissociation constants.

From a mathematical point of view, the model can be further simplified by remarking that

$$\frac{y/2 - P_1}{1 + \frac{w^2}{K_{d3}^2}} \leq y_d \leq \frac{y/2}{1 + \frac{w^2}{K_{d3}^2}}$$

At the equilibrium, $y = \frac{\beta_2}{\alpha_2} \simeq 2.02 muM$ and $P_1 = 1.44 \times 10^{-3} \mu M$, so $y/2 <<< P_1$. Therefore one has:

$$y \simeq 2y_d \left[1 + \left(\frac{w}{K_{d3}} \right)^2 \right] \tag{27}$$

The volume fraction $\left(\frac{v_b}{V_e - v_b} \right)$ takes into account the dilution effects due to the export process through the bacteria membrane.

2.2. Simple case $L = 2$

In the case where $L = 2$, one has:

$$\frac{dx}{dt} = \beta_1 \Big(\frac{\rho}{1-\rho} \Big) \frac{1}{1 + y_d/K_{d6}} - \alpha_1 \frac{K_m}{K_m + s_2} x \tag{28}$$

$$\frac{dy}{dt} = \beta_2 - (\alpha_2 + \mu(t)) y \tag{29}$$

$$\frac{ds_2}{dt} = -k_1 \frac{s_2}{K_m + s_2} x \tag{30}$$

$$\frac{ds_1}{dt} = 2k_1 \frac{s_2}{K_m + s_2} x - \gamma \rho s_1 \tag{31}$$

$$\frac{dw}{dt} = \gamma (1-\rho) s_1 - (k_5 + \mu(t)) w \tag{32}$$

where

$$y_d = \frac{y}{2} \Big[1 + \Big(\frac{w}{K_{d3}} \Big)^2 \Big]^{-1} \tag{33}$$

with $\frac{\mathcal{P}_{KDG}}{\mathcal{P}} \simeq 1$ as $\mathcal{P}_{KDG} = 178 g/mol$, $k_1 = \varepsilon k_{cat}$ in h^{-1}.

3. Steady states analysis and initial conditions

The analysis of equations (22) to (27) allows to deduce the following steady states:

$$y_{eq} = \frac{\beta_2}{\alpha_2} \tag{34}$$

$$x_{eq} = \left(\frac{\beta_1}{\alpha_1} \right) \left(\frac{v_b}{V_e - v_b} \right) \left(\frac{1}{1 + y_{eq}/2K_{d6}} \right) \tag{35}$$

$$w_{eq} = 0 \tag{36}$$

$$s_{l.eq} = 0 \tag{37}$$

Equations (34) to (36) will be consider for simulations as initial conditions for the corresponding species. At the beginning of the experiments, a certain mass amount of

PGA is added in the media. In the following, one will explain how the corresponding molecular concentration of PGA is determined.

The molecular weight of a monomer of galacturonate is $\mathcal{P} = 176 g/mol$. The length of PGA polymers lies between 142 and 284 probably with a Gaussian distribution centered on a median value of approximately 210. The mass concentration (M_l) of polymer P_l is given by:

$$M_l = l\, s_l\, \mathcal{P}$$

The mass conservation is:

$$\sum_{l=1}^{L} M_l = M_{tot} \tag{38}$$

where M_{tot} is the total mass of PGA introduces at the beginning of the experiment. Due to the length distribution of polymer, one will consider here a distribution profile depending on the length of polymer to describe initial conditions. Therefore, one can consider:

$$s_l(0) = s_{tot}\, p_l$$

where $s_{tot} = \sum_{l=1}^{L} s_l(0)$ is the total molar concentration of polymer and p_l the probability to find a polymer of length l at $t = 0$. The average polymer length is given by:

$$\bar{l} = \sum_{l=1}^{L} l p_l \tag{39}$$

For instance, for a Gaussian distribution centered around $\bar{l}$ with variance σ^2, one would have:

$$s_l(0) = s_{tot}\, \frac{1}{Z(\sigma)} e^{-(l-\bar{l})^2/2\sigma^2}$$

with $Z(\sigma)$ the normalization factor. Using the mass conservation law (38) the initial concentration of polymer s_l is given by:

$$s_l(0) = \frac{M_{tot}}{\bar{l}\mathcal{P}} \tag{40}$$

3.1. Determining K_m via the measure of the mass-characterized Michealis-Menten constant.

The products of degradation of PGA by the pectate lyases are unsaturated oligo-galacturonates (UGA). In order to quantify the enzymatic activity one measures the absorbtion at 230 nm of the unsaturated cleaved links of PGA. Let us denote by U the total molar quantity of unsaturated poly- and oligo-galacturontates. We can then estimate U by computing:

$$U = \sum_{l=1}^{L} s_l$$

Then, using the kinetic equations for s_l introduced above, one deduces that –in an enzyme assay– the rate of product formation is given by:

$$\frac{dU}{dt} = k_{cat} E_T \frac{\sum_l (l-1) s_l}{K_m + \sum_l (l-1) s_l} \tag{41}$$

where E_T is the Pel enzyme present in the reactive medium. We wish to express the kinetic equation of U in terms of the total mass of PGA polymer, denoted by M_{tot}. This can be done by using eqs. (38) and (39). The final expression for $\frac{dU}{dt}$ can be written in the form:

$$\frac{dU}{dt} = k_{cat} E_T \frac{M_{tot}}{K_M + M_{tot}} \tag{42}$$

where $K_M = K_m \mathcal{P}$ is the mass-characterized Michaelis Menten constant. Let us remark that in deducing eq.(42), we have done the approximation $(1-1/\bar{l}) \sim 1$, which is justified because the mean length polymer $\bar{l}$ is of the order 200. Therefore, by measuring the rate of formation of UGA in function of M_{tot} one retrieves the classical Michaelis Menten law (an hyperbolic curve) from which one can deduce K_M as the mass of PGA that yields the half-maximum reaction velocity. Such a curve has been drawn on Fig.5 of the main text and the value of $K_M = 1.2$ g/l was determined. So $K_m = K_M/\mathcal{P} = 6.8$ mM, as reported on Table 1 of the article.

In a low substrate regime, eq.(41) can by simplified as:

$$\frac{dU}{dt} \sim \frac{k_{cat} E_T}{K_m} \left(s_2 + 2 s_3 + \cdots + (L-1) s_L \right) \tag{43}$$

This latter expression is used in the simulations in order to follow the decrease of PGA whose measurements have been reported on Figs.(6)-(7) of the main text. Indeed, to monitor the PGA degradation an indirect strategy was used, as described in the Experimental Procedures of the main text. Each point of the PGA curves (Figs.(6)-(7)) corresponds to taking a sample from the culture medium and measuring the product rate dU/dt obtained with a fixed amount of Pels.

4. parameters fitting

In this section, we explain how unknown parameters are obtained using numerical simulations tools. Unknown parameters of the model are: ε, β_1, γ, K_{d6} and k_5. First of all, using literatures, parameters are constraints in biological interval values. In Ref [2], values of the dissociation constant K_{d6} for KdgR-operator of pel genes were measured and found typically between 10^{-4} and $10^{-2} \mu M$. ε is the ratio of the rate constant k_{cat} of the enzymatic Pel reactions in the culture medium and in the reactional medium. The k_{cat} of the 5 major Pel enzymes were measured in purified conditions, as reported in Ref. [5], giving an average value of $6 \times 10^4 min^{-1}$. It appears that it should be slightly smaller for the culture medium, so $\epsilon \leq 1$. The parameter β_1 is a sensitive parameter of the model. A small variation of β_1 induces a large variation on the time delay and on the maximal level of Pel synthesis. Parameters β_1, γ and k_5 are varied a priori over a large range, between 10^{-3} and 10^3 h^{-1}.

An iterative simple algorithm minimizing the quadratic error function

$$\chi^2 = \sum_{i=1}^{N} [x_{pred}(i,a) - x_{exp}(i)]^2 + \sum_{i=1}^{N} [s_{pred}(i,a) - s_{exp}(i)]^2$$

is computed. N is the number of data of the experiment, x_{exp} and s_{exp} are experimental Pel activity and PGA respectively. x_{pred} and s_{pred} are the predicted theoretical evolution of Pel activity and PGA using the model with a given set of the unknown parameters a. These theoretical values are obtained by solving the system equations (28) to (33) for a given set of the vector parameter a.

First, by a random search of about 10^5 iterations between parameters interval values, the two best sets of parameters given the minimal value of χ^2 are retained. Let us note that after several simulations, the two best sets of parameters are always very close to each other, suggesting the presence of a local minimum. Therefore, a systematic sweeping is made around the set of values by successive small variations of parameters and reevaluation of χ^2 until the minimum is reached. Finally, we pick as best parameters the set of parameter vector a that minimize χ^2. It is observed after several simulations that the set of unknown parameters obtained in the restricted region may be unique.

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