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ANALYSE DE SENSIBILITE D'UN MODEL DE CHELATION DE FER APPLIQUÉE Á UNE SOUCHE PHYTOBÉNÉFIQUE DE *PSEUDOMONAS FLUORESCENS*

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Résumé

Description du sujet : L'analyse de la sensibilité locale (SA) a pour but d'orienter les recherches en mettant l'accent sur les paramètres qui contribuent le plus à la propagation de l'incertitude.
Objectifs : Le but de la présente étude est d'évaluer un modèle basé sur une description phénoménologique quantifiée du niveau de population et de dynamique des ressources (fer) en termes de sa capacité à capturer des caractéristiques de croissance typiques et identifier les facteurs clés (entrées du modèle) qui contribuent le plus à la variation des sorties du modèle.
Méthodes : Une analyse de sensibilité variant un facteur à la fois ainsi que le calcul des sensibilités locales logarithmiques utilisant un ensemble de 10.000 vecteurs aléatoires dans lesquels chaque paramètres respectif du model est échantillonnée indépendamment ont été effectué afin de classer les sensibilités des paramètres par rapport aux variables d'états du modèle étudié.
Résultats : Les résultats de l'analyse de sensibilité ont permis de capturer les caractéristiques cinétiques essentielles de toutes les variables d'état (correspondance numérique et conceptuelle), ainsi qu'elle a permis de classer les paramètres du modèle en fonction de leur importance relative sur le modèle.
Conclusion : Les résultats de l'analyse de sensibilité indiquent quels composants du système doivent être mesurés avec plus de précision et de fiabilité, afin d'obtenir de meilleures prévisions, mais aussi ceux qui doivent être optimisés pour de meilleurs rendements
Mots clés: *Pseudomonas fluorescens*; Analyse de sensibilité locale; milieu appauvri en fer.

SENSITIVITY ANALYSIS OF A CHELATING PROCESS MODEL APPLIED TO A PHYTOBENEFICAL *PSEUDOMONAS FLUORESCENS*.

Abstract

Description of the subject: Local sensitivity analysis (SA) is mathematical modelling technique, which is widely used to ascertain the response of a simulation model to changes in its input parameters by focusing on the parameters that contribute the most to the uncertainty of the model response.
Objective: The aim of the present study is to assess a chelating process model, based on a qualitative-phenomenological description on the level of population and resource dynamics in term of its ability to capture typical growth features and pinpoint key factors (model inputs) that contribute most to the variation of model outputs.
Methods: A one factor at a time approach along with logarithmic local sensitivities using a set of 10.000 random parameter sets in which individual parameters were sampled independently, was carried out in order to asses and rank parameter sensitivities toward models states variables.
Results: Sensitivity analysis results allowed to capture essential kinetic features by successfully predicting key features of all state variables (numerical and conceptual correspondence), and pinpoint and rank models parameters according to their relative importance toward models state variables.
Conclusion: The results of the sensitivity analysis indicate which components of the system need to be measured more precisely and reliably, in order to obtain better predictions but also those that need to be optimized for better yields.
keywords: *Pseudomonas fluorescens*; Local sensitivity analysis; Iron depleted media.

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INTRODUCTION

Soil-borne, phytobenefic bacteria are organisms that are well adapted to the constraints of their biological and physioco-chemical environment [1]; these bacteria are known by several generic names, including biological control agents (BCAs), plant growth promoting rhizobacteria (PGPR) or biopesticides. Genera, such as, Pseudomonas, are well described Gram-negative microorganisms that are known as colonizers of the rhizosphere [2, 3] and the phyllosphere [4, 5]. In addition, It has been demonstrated that Pseudomonads can be used to control plant pathogens because of their catabolic versatility which releases a remarkable diversity of exoproducts with antimicrobial, metal chelating (sidérophores i.e. pyoverdine), lytic, and phytohormonal activity. Indeed, Pseudomonads strains, do not involve a systematic use of antibiotics compounds such as some other biocontrol strains that exhibit direct antagonistic activities against phytopathogens. Then, they have the additional advantage that they do not raise the concern of resistance development [2, 6-8].

Based on the aforementioned characteristic features, some species of the Pseudomonas genus can be considered to be serious potential candidates for bioproducts development in substitution to chemical introns use. In this way, current efforts to isolate, characterize and select the best bacterial antagonists to control phytopathogens worldwide are continuously reported in the literature [6, 8-15]. In parallel, predictive modelling techniques that rely on mathematical models are used to cope with the classical approach limitations and select the best antagonist strains by the way of laboratory-based measurements of simple variables that are put in models to indirectly deduce and predict the value of growth and yield parameters that cannot be directly measured. Thus, mathematical modelling offers the possibility to explore a large number of scenarios without resorting to time-consuming field or laboratory-based experiments.

Among various biological modelling techniques, sensitivity analysis (SA) is a widely used method that ascertains the response of a simulation model to changes in its input parameters [9]. In practice, SA is not only applied to examine the importance of input parameters but also it is used in model development process as it allows to elucidate the impact of different model structures and assumptions; orientate parameter estimation and experimental design [10]; prepare for model parameterization and direct research priorities by focusing on the parameters that contribute the most to uncertainty of the model response [11, 12]. By this way, the most sensitive model input parameters and their corresponding poorly known biological processes are the potential targets for further experimental analysis for each specific case.

In this study, we conduct a local sensitivity analysis on a phenomenological lag phase model describing the dynamics of a phytobenifical *Pseudomonas fluorescence* strain, toward its various parameters. The model involves a number of state variables and parameters that are related to growth activity, substrate availability and the amount of released secondary metabolites (pyoverdine) in the culture broth media which is triggered when the concentration of iron get low in the system. For this purpose, we have adapted the initial conditions and stimuli to our own laboratory conditions and assumed the same model structure as the one proposed by Fgaier *et al.* [13].

MATERIAL AND METHODS

1- Growth Measurement and siderophore assay

Pseudomonas fluorescens, an autochthonous strain isolated from palm date rhizosphere in southern Algeria (Ghardaia) was used in the study [14, 15]. Bacterial cultures were grown 40 h at 25°C with continious shaking at 200 rpm in a 500 ml Erlenmeyer flask containing 150 ml of King's-B medium, the whole was maintained at pH 7. For growth and pyoverdine measurements 2 mL samples were taken at time zero, and then at 1-hour intervals all over the experimental period [16].

Both growth and siderophore content were determined according to the method of Meyer and abdallah [16]. Bacterial growth was calculated turbidimetrically at 600 nm. For the estimation of the quantity of secreted siderophore into the culture medium, this was determined by removing bacterial cells (Solid phase) by centrifugation at 6000 rpm during 20 mn and measuring the absorbance of the supernatant at 400 nm in a Shimadzu spectrophotometer device.

2- Numerical Experiments

2.1. Presentation of the mathematical model

A lag phase model consisting of a non-autonomous system of five nonlinear ordinary differential equations built by Fgaier *et al.* [13] on the basis of the well-known Baranyi's model was used in this experiment [17]. This model assumes that only a fraction α of the whole bacterial population (N), contributes to the growth process when introduced into a new environment, while the remaining cells adapt their physiological state to the new conditions and constraints. The bacterial population is characterized by two variables, population size (N) and its lumped physiological state, expressed in terms of a function α (t). The chelator (pyoverdine) is described by its optical density (P). Bacterial growth depends on iron availability in the medium and is conceptually represented in the model by two terms giving: the freely dissolved iron (S), and bound by chelator molecules (Q).

In this model, coefficients Yn, μ, $k, S^{\infty}, \sigma, \beta, \delta$, ,v are all positive values and represent for:

Yn , the growth yield constants, commonly referred to as the substrate-to-biomass yield factor, used to convert between cell growth rate and substrate utilization; μ, the specific growth rate as a function of substrate concentration; k, the value of the concentration of nutrients S where the specific growth rate μS has half its maximum value (half-growth concentration rate); S^{∞}, iron concentration triggering secondary metabolite synthesis (pyoverdine); σ, coefficient related to the amount of chelated iron; β, coefficient related to the amount of freely available iron; δ, is a coefficients related to chelation speed rate; v, recovery rate of the PGPR population; α(t), function of physiological adaptation state given by the term dα/dt in the model [13].

2.2. Local sensitivity analysis

In this study, we have performed a one factor at time sensitivity analysis which measures the sensitivities that reflect the magnitude of relative change in a model's output variable induced by a local (+/- 10 & 20%) relative change in a model's parameter X (model inputs). This procedure is done by varying only one input variable while keeping the rest constant to their nominal values.

In addition, we have calculated the logarithmic local sensitivities, S_{ij} (t), in the vicinity of the default parameter set, at time moments t. [18]:

$$S_{ij} = \partial Log X_i\,(t) / \partial Log p_j = (dX_i/X_i) / (dp_j/p_j)$$

Here, Xi (t) is the model's variable order and pj is the model's jth parameter (of the model's 8 main parameters). Sensitivities are calculated for each of the 41 evenly spaced time points that discretize the total of 1.5-day simulation interval into 1-h subintervals (i.e., hour 0, hour 1, and so forth).

In this section, 10,000 random parameter vectors in which each individual parameters is sampled independently from intervals, permitting up to 2-fold deviations (up or down) from the corresponding optimal default values X^0, are generated and ranked by using their absolute values. For vector generation, a Matlab function (LHSDESIGN) performing a Latin Hypercube Sampling method was used [19].

RESULTS

1. Model sensitivity analysis

The parameterisation of the governing system of differential equations was set according to real data entering results obtained from laboratory-based measurements of biomass and pyoverdine content. One at time, sensitivity analysis results are represented in (Fig. 1).

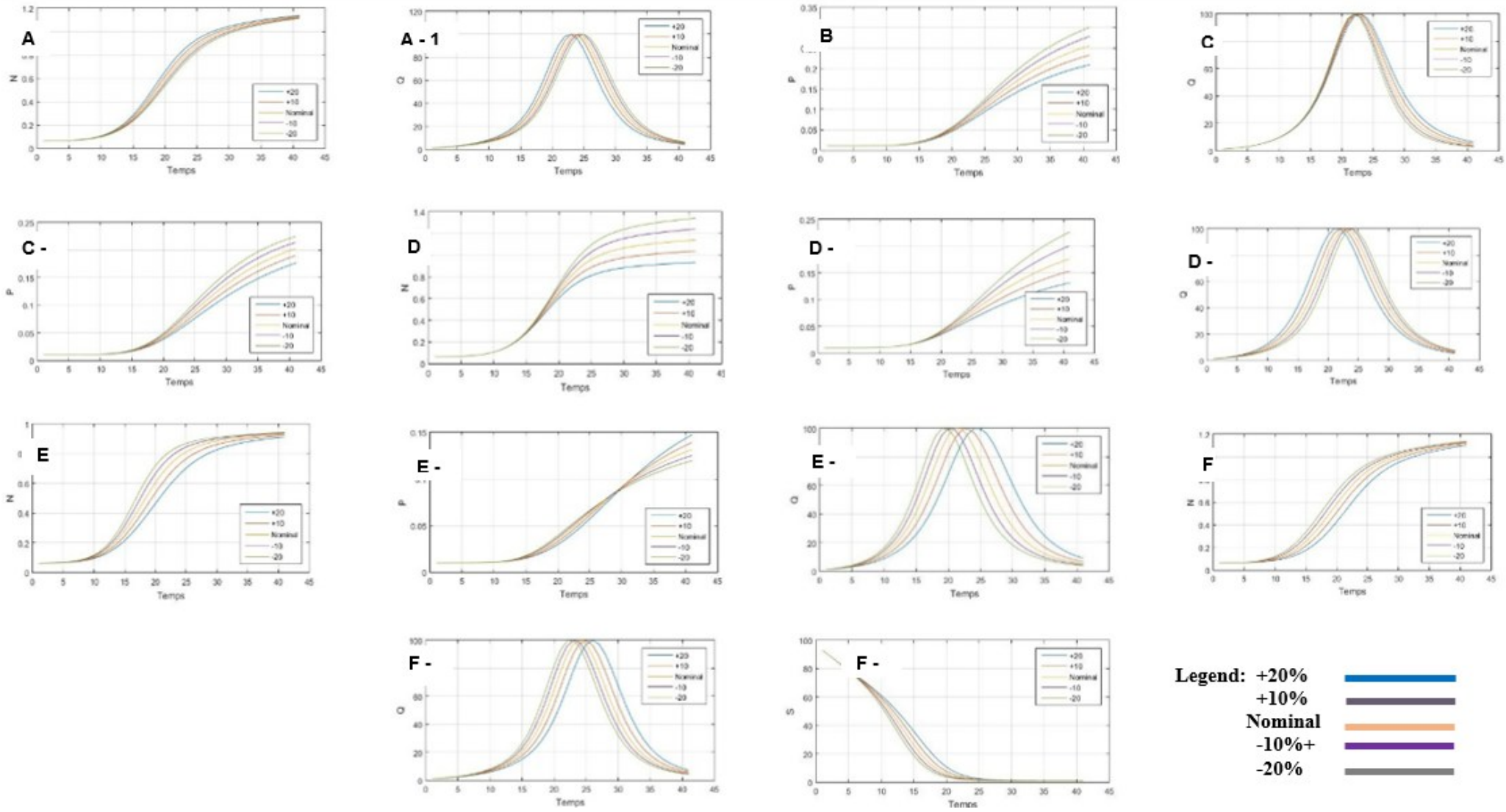


Figure 1: Single parameter variation plots for the effect of k (A; A-1); Beta (B); Sigma (C; C-1); Yn (D; D-1; D-2); mu (E; E-1; E-2); v (F; F-1; F-2), on model's solution.

For models dynamical behaviour, simulations results show that the bacterial population density (N) pass through a lag phase during which the model account for microbial cells adaptation to their new environment and become entirely healthy by rewiring of regulatory networks via natural selection of horizontal gene transfers, gene duplication, deletion, readjustment of kinetic parameters, and myriad other genetic reorganizational events. This phase is followed by an exponential growth phase where they multiply actively and produce pyoverdine (P) that diffuse in the culture broth (i.e. Fig.1. D, D-1, E). Ultimately, when iron becomes limited in the medium, bacterial population transits to a stationary phase during which the maximum population density is reached (Fig.1. D, E). In parallel, depletion of the freely available iron (S) passes through different phases (Fig.1. F-2), which are first slow but then accelerates as the population passes the initial phase of adaptation. The drop in free iron (S) induces an increase in the chelated iron (Q) which eventually drops to the state of total depletion as well (Fig.1. E-2, F-1).

Sensitivity analysis results show that single parameter variation of Beta, Sigma, Delta and S^{∞}, indicate little or no effect on the on the two biomass state variable (N) and the amount of free iron (S). However, the latter show a decreasing effect on the pyoverdine slope curve (Fig. 1 (B; C-1)), since we observe different levels of synthesis speed rate giving rise to a variable amount of chelating molecules over time if their optimum values are lowered. In addition and unlike to the rest of above-mentioned parameters that had no effect on the chelated iron profiles (Q). The parameter Sigma showed a moderate effect that was expressed in terms of chelation rate of the freely available iron molecules (Fig. 1 (C)) occurring during the last part of the exponential growth stage and is associated with a fall in the number of chelating molecules over time until reaching the state of total exhaustion.

Moreover, an inverse relationship is observed between the bacterial biomass production status variable and the siderophore synthesis speed rate, since the increase in the nominal value of the parameter (Mu) is associated with an increase in pyoverdine content that exhibits a slight but delayed decline in the amount of biomass produced (Fig. 1 (E, E1)). In parallel, the increase in the nominal value of the parameter (Mu) results in a chelation delay of the freely available iron of about 5 to 6 hours, leading to a less rapid depletion of the latter in the system (Figure. 1 (E2)).

Furthermore, compared to the effect of (v), (Mu) has exhibited a much more pronounced effect on the slope of all state variables and on the speed at which the stationary equilibrium phase is reached for the variables microbial biomass (N) and pyoverdine content (P).

2. State variables robustness analysis

To test the robustness of models parameters, the logarithmic sensitivities of the 10,000 randomly selected parameter sets for each of the considered 41 simulation time points and each of the 4 state variables are calculated. The occurrence frequencies of each of the parameters for which a given variable demonstrated the highest, second-highest, and/or third-highest sensitivity with respect to the most important (4) rate perturbations are calculated and plotted in the figure 2, for three representative points.

In response to the modulation of each one of the constitutive model parameters, a dynamic evolution of the sensitivity of each variable is observed for all ranks, this change leads to a progressive shift of the sensitivity profiles of one or more sensitive variables to the benefit of others (Fig. 2).

Our model investigations show that the state variables (S), (Q) and (P) (in descending order of magnitude), are the most sensitive with significant relative changes in their values in response to the variation of each of the parameters listed above (Figure. 2).

In our analysis, the variables (S) and (Q), accounting for the bioavailability of the substrate are the most important with respectively 52.80% and 29.60% occurrence frequency over the 10,000 studied vectors. (P) and (N) follows these parameters, with respectively 12.29% and 5.30% occurrence frequencies.

Overall, the state variable (S) can be considered as the most sensitive indicator of local changes for the two parameters (μ) and (Yn).

This result is not surprising since (Yn) and (μ) are both parameters related to bacterial growth which are both depending on the amount of freely available iron in the medium.

Thus, there is a positive relationship between iron availability and microbial growth in function of the amount of chelates in the medium.

Paradoxically, some parameters had the same cumulative effect while having opposite dynamic behaviour. Consequently, the following parameter pairs (μ, k) and (β, S^{∞}) that had roughly the same cumulative effects and the same ranks associated with closely identical sensitivity profiles (Fig. 2).

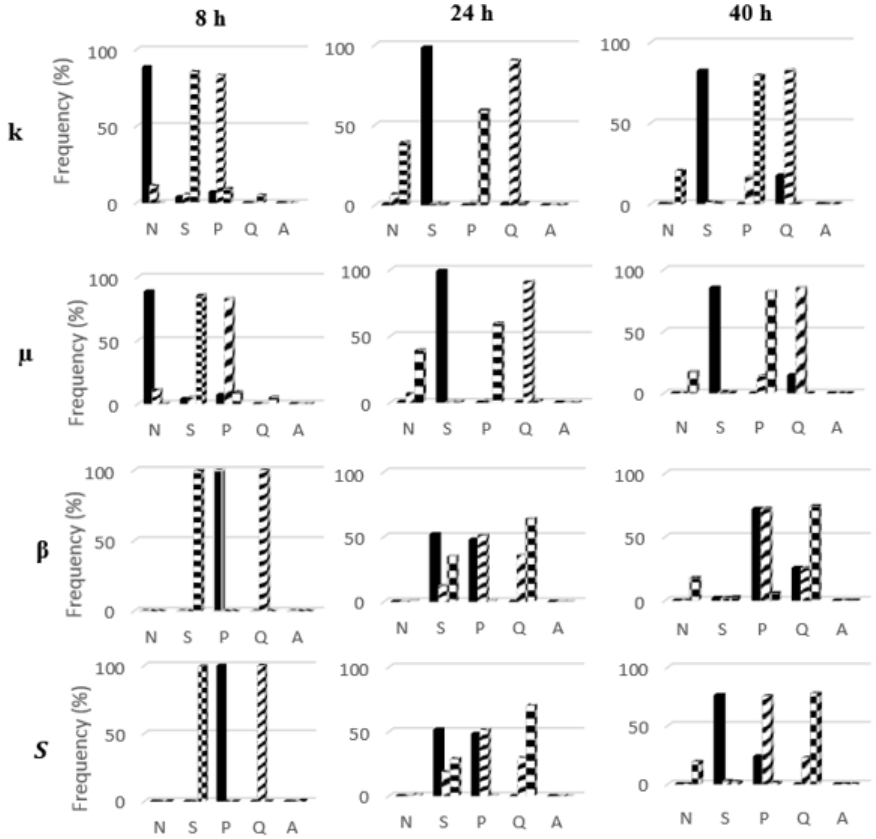
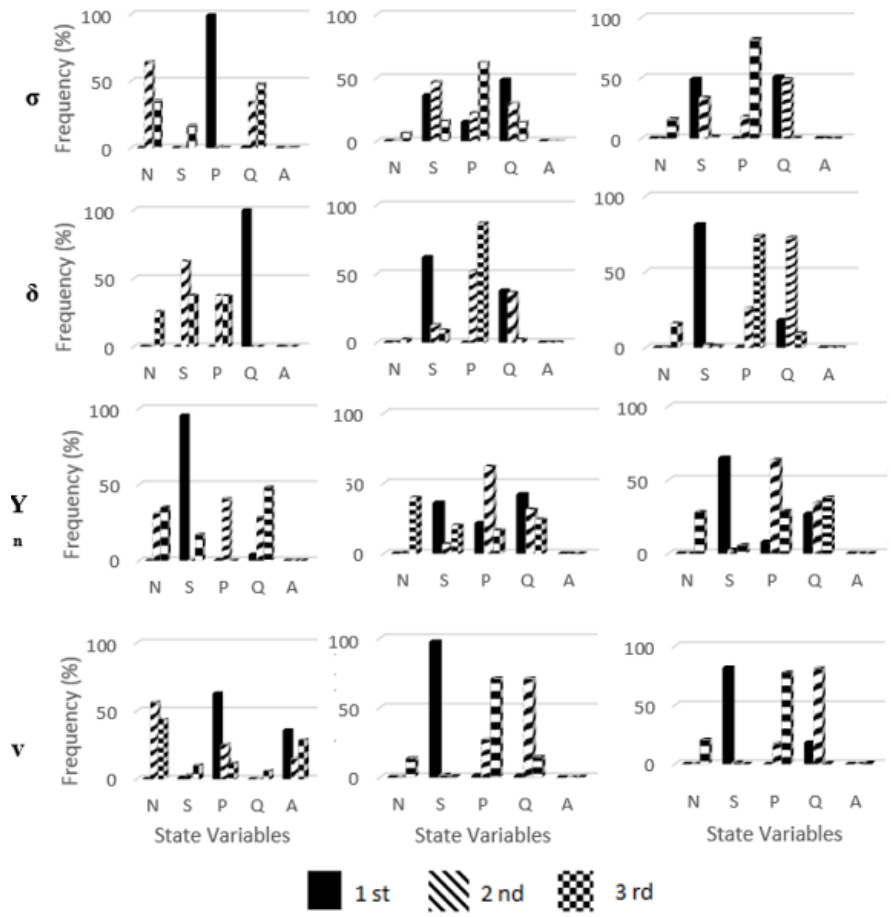


Figure 2. Sensitivity analysis of the first (Solid bars), second (Striped bars) and third (Bars with squares) most important variable that exhibited the large relative change in response to parameter modulation for five representative simulation time points.



Following Figure 2. Sensitivity analysis of the first (Solid bars), second (Striped bars) and third (Bars with squares) most important variable that exhibited the large relative change in response to parameter modulation for five representative simulation time points.

DISCUSSION

The computational model captured essential kinetic features by successfully predicting key features of all state variables (such as the overall curve shape, peak and resolution time), as well as it gave predictions for iron dynamic over time for whom no experimental data were available to compare (Figure.1. A-1, D-2, E-2, F-1).

For the one at time sensitivity analysis, all model parameters showed a deviation typically less than 10%. This can be considered as a very satisfactory result since these parameters describe complex biological systems involving a very large number of reactions taking place between the microorganism and their direct environment. These observations suggest a possible use of the different sensitive parameters that can be easily measured in the local environment of the culture media, as predictive indicators of the three essential variables of the model, which are the microbial growth rate, pyoverdine production rate and level of bioavailable iron [9].

Globally, the results of the sensitivity analysis indicate which components of the system need to be measured more precisely and reliably, in order to obtain better predictions but also those that need to be optimized for better yields. In addition, and in a context of large-scale production, the parameters (Yn) and (μ) are the key factor to be optimized, if an increase in the microbial biomass is desired in the first place.

Concerning the real-time monitoring of the iron bioavailability, the parameter (δ) must be tuned since it gives a direct relationship between the quantity of freely available and chelates iron and conditions the iron chelation process. Along with the parameter (σ) which gives a partial relationship between the two quoted state variables, which are

function of the amount of pyoverdine content in the system. Thus, they must be taken into account since this one conditions the process of iron release in the cytosol of bacterial entities [20].

Overall, sensitivity analysis of the model parameters emphasized the importance given to the bacterial growth parameters over those associated with substrate use [20]. Changes in biomass concentrations following variation in the maximum specific growth rate (μ) and (Yn), followed by (ν) and (k) in order of decreasing effect magnitude are greater than those obtained from parameters associated with iron bioavailability and use of the substrate represented by the parameters (δ) and (σ). For the rest of the parameters, the sensitivity was rather weak for (k, beta and S^{∞}).

for the sake of model simplification, the insensitive parameters must be set to their respective default value or simply deleted or substituted by other parameters, which are more decisive for the description of the phenomenon of interest. However, it must be kept in mind that the more the number of parameters is increased, the greater the complexity of the model will be, leading to a decrease in our ability to test this model in a critical and objective way [11, 21].

in our study, the great sensitivity of the studied system to the variation of the parameters (Yn, μ and ν), representing respectively (the growth yield constants, the specific growth rate and the physiological state of the population) is translated biologically by the management of the flow of matter and energy that makes the microorganism maximizes the production of the elements necessary for growth when the energy is abundant. Thus, at the beginning of growth, the conversion efficiency of the substrate into biomass is high, but the energy yield is low. Then, when the energy is limiting, the microbial population modifies the composition of the environment like in the case of the overflow metabolism activation (lack of carbon source), or when the production and excretion of secondary metabolites towards the external environment causes a drop in the status of the pH, that modifies the physicochemical properties of the surrounding fluid, in this specific case, all the reactions are conducted with maximum energy efficiency, sometimes at the detriment of the conversion efficiency of the substrate [22; 23]. This is all more valid than the present experiment was done in Erlenmeyer flasks containing 250 ml of media, where the drop in the pH level can occur quickly.

Finally, we should be cautious about the precision of sensitivity analysis results, especially when the model's parameters are not identifiable (parameters of the model that could not be directly measured experimentally) or if one focus on single parameters variation and do not take into account possible linear or non-linear interactions amongst parameters.

CONCLUSION

In this study, we used a computational modelling approach to gain mechanistic insights into a lag-phase growth model describing the bacterial evolution and pyoverdine production in an iron-deficient media. First, our system of differential equation–based model successfully predicted key kinetic features of all state variables and gave predictions for iron dynamic over time for which no experimental data were available to compare with. Second, local sensitivity analysis allowed us to cover a large range of the parameter space and gain insight about the relative importance of input parameters together with the output sensitivity to the most important parameters considered by the model.

Finally, and even if the concept of local sensitivity analysis is simple, and is effective if the localized sensitivity is of interest, no absolute good sensitivity analysis method for all biological models exist because most of the methods have their pros and cons.

Thus, if information on the overall effect of an input factor on the model is needed, the limitation of this derivative-based method is evident, as it cannot represent the overall sensitivity index for most numerical models that involve nonlinear relationships and strong interactions.

REFERENCES

[1]. Parke J. (1991). Root colonization by indigenous and introduced microorganisms. The rhizosphere and plant growth: Springer. p. 33-42.

[2]. Walsh UF., Morrissey JP & O'Gara F. (2001). Pseudomonas for biocontrol of phytopathogens: from functional genomics to commercial exploitation. Current Opinion in Biotechnology;12. p.289-95.

[3]. Weller DM. (2007). Pseudomonas biocontrol agents of soilborne pathogens: looking back over 30 years. Phytopathology;97. p.250-6.

[4]. Hirano SS & Upper CD. (2000). Bacteria in the Leaf Ecosystem with Emphasis onPseudomonas syringae—a Pathogen, Ice Nucleus, and Epiphyte. Microbiology and molecular biology reviews;64. p.624-53.

[5]. Krimm U., Abanda-Nkpwatt D., Schwab W & Schreiber L. (2005). Epiphytic microorganisms on strawberry plants (Fragaria ananassa cv. Elsanta): identification of bacterial isolates and analysis of their interaction with leaf surfaces. FEMS Microbiology Ecology;53. p.483-92.

[6]. Johansson PM & Wright SA. (2003). Low-temperature isolation of disease-suppressive bacteria and characterization of a distinctive group of pseudomonads. Applied and environmental microbiology;69. p.6464-74.

[7]. O Callaghan M., Swaminathan J., Lottmann J., Wright D & Jackson T. (2006). Seed coating with biocontrol strain Pseudomonas fluorescens F113. New Zealand Plant Protection;59. p.80-5.

[8]. Someya N., Tsuchiya K., Yoshida T., Noguchi M & Sawada H. (2007). Encapsulation of cabbage seeds in alginate polymer containing the biocontrol bacterium Pseudomonas fluorescens strain LRB3W1 for the control of cabbage soilborne diseases. Seed Science and Technology;35. p.371-9.

[9]. Banga JR & Balsa-Canto E. (2008). Parameter estimation and optimal experimental design. Essays Biochem;45. p.195-210.

[10]. Bentele M., Lavrik I., Ulrich M., Stösser S., Heermann D., Kalthoff H, et al. (2004). Mathematical modeling reveals threshold mechanism in CD95-induced apoptosis. The Journal of cell biology;166. p.839-51.

[11]. Raue A., Kreutz C., Maiwald T., Klingmuller U & Timmer J. (2011). Addressing parameter identifiability by model-based experimentation. IET systems biology;5. p.120-30.

[12]. Saltelli A., Tarantola S & Campolongo F. (2000). Sensitivity analysis as an ingredient of modeling. Statistical Science. p.377-95.

[13]. Fgaier H., Feher B., McKellar RC & Eberl HJ. (2008). Predictive modeling of siderphore production by *Pseudomonas fluorescens* under iron limitation. J Theor Biol;251. p.348-62.

[14]. Benchabane M. (2005). Caractérisation des éffets d'antagonisme microbien et de promotion de la croissance végétale de souches de pseudomonas spp. fluorescents p. 232-68.

[15]. Toua D., Benchabane M., Bensaid F & Bakour R. (2013). Evaluation of Pseudomonas fluorescens for the biocontrol of fusarium wilt in tomato and flax. African Journal of Microbiology Research;7. p.5449-58.

[16]. Meyer Ja &Abdallah M. (1978). The fluorescent pigment of *Pseudomonas fluorescens*: biosynthesis, purification and physicochemical properties. Microbiology +;107. p.319-28.

[17]. Baranyi J & Roberts TA. (1994). A dynamic approach to predicting bacterial growth in food. International journal of food microbiology;23. p.277-94.

[18]. Mitrophanov AY., Churchward G & Borodovsky M. (2007). Control of *Streptococcus pyogenes* virulence: Modeling of the CovR/S signal transduction system. J Theor Biol;246. p.113-28.

[19]. Nagaraja S., Wallqvist A., Reifman J & Mitrophanov AY. (2014). Computational approach to characterize causative factors and molecular indicators of chronic wound inflammation. The Journal of Immunology;192. p.1824-34.

[20]. Berdja R., Abderrahmane O & Benchabane M. (2018). Parameter optimization of pyoverdine content and growth kinetics on Pseudomonas fluorescens pf-10 strain in iron deficient liquid state media. African Journal of Microbiology Research;12. p.127-35.

[21]. Scott EM., Rattray EA., Prosser J., Killham K., Glover L., Lynch J, et al. (1995). A mathematical model for dispersal of bacterial inoculants colonizing the wheat rhizosphere. Soil Biology and Biochemistry;27. p.1307-18.

[22]. Amar P. (2013). Contributions à l'étude de la dynamique des systèmes biologiques et aux systèmes de calcul en biologie synthétique: Université Paris Sud-Paris XI.p-232-59.

[23]. Bylund F., Collet E., Enfors S-O & Larsson G. (1998). Substrate gradient formation in the large-scale bioreactor lowers cell yield and increases by-product formation. Bioprocess Engineering;18. p.171-80.