



# Twelve quick tips for designing sound dynamical models for bioprocesses

Francis Mairet, Olivier Bernard

## ► To cite this version:

Francis Mairet, Olivier Bernard. Twelve quick tips for designing sound dynamical models for bioprocesses. PLoS Computational Biology, Public Library of Science, 2019, 15 (8), pp.e1007222. 10.1371/journal.pcbi.1007222 . hal-02421763

**HAL Id: hal-02421763**

**<https://hal.inria.fr/hal-02421763>**

Submitted on 20 Dec 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Twelve quick tips for designing sound dynamical models for bioprocesses

Francis MAIRET <sup>1</sup>, Olivier BERNARD<sup>2,3,4,\*</sup>,

**1** Ifremer, Physiology and Biotechnology of Algae laboratory, rue de l'Île d'Yeu, 44311 Nantes, France

**2** Côte d'Azur University, INRIA, BIOCORE, BP93, 06902 Sophia-Antipolis Cedex, France

**3** Sorbonne University, CNRS, LOV, 06230 Villefranche-sur-mer, France

**4** ENERSENSE, Department of Energy and Process Engineering, NTNU, 7491 Trondheim, Norway

\* olivier.bernard@inria.fr

Because of the inherent complexity of bioprocesses, mathematical models are more and more used for process design, control and optimization etc... These models are generally based on a set of biochemical reactions. Model equations are then derived from mass balance, coupled to empirical kinetics. Biological models are nonlinear and represent processes, which by essence are dynamic and adaptive. The temptation to embed most of the biology is high, with the risk that calibration would not be significant anymore. The most important task for a modeler is thus to ensure a balance between model complexity and ease of use. Since a model should be tailored to the objectives which will depend on applications and environment, a universal model representing any possible situation is probably not the best option.

Here are twelve tips to develop your own bioprocess model. For more details on bioprocess modelling, the readers could refer to [1]. More tips concerning computational aspects can be found in [2, 3].

## Tip 1: Define your objective and the application context

14

Years of high school learning about how to set-up mechanistic models based on the fundamental  $F = m.a$  relationship of mechanics, or on the Ohm law have corrupted our minds. It took centuries to identify the corpus of laws supporting today's physical models. Fig 1 recalls that, previously, there used to be some "less accurate" predictive models that have been forgotten. At present, models in these fields, even if empirical, are excellent approximations and -at least for those we studied at school- always ended-up in rather simple, often linear and mathematically tractable models. The complexity of biological systems requires a more open viewpoint, where different models of the same process can be useful and complementary. Therefore, before writing equations, one must first clearly define the model objective. The model can be designed for numerous reasons, among which prediction of future evolution, understanding of the process behaviour, estimation of unmeasured variables or fluxes, operator training, detection and diagnosis of failures, optimization and control.

15

16

17

18

19

20

21

22

23

24

25

26

27

## Tip 2: Adapt your modelling framework with your objective, your knowledge and your data set

28

29

When developing a model, it is crucial to keep in mind the objectives of the model and the framework for its application. A model targeting the understanding of some metabolic processes inherently requires the user to embark on the details of the cell metabolism [5,6]. Predicting the impact of meteorology on outdoor microalgal processes means that light and temperature must be included somewhere in the model. A model for on-line control can be more straightforward (often because it will benefit from on-line information on process state). So, keeping in mind the model objective, one has to choose which variables to include, but also the type of model: deterministic versus stochastic, homogeneous versus heterogeneous (in terms of space or phenotype). The available data set or data that can be provided by the experimental set-up will also constrain the choice of model complexity.

30

31

32

33

34

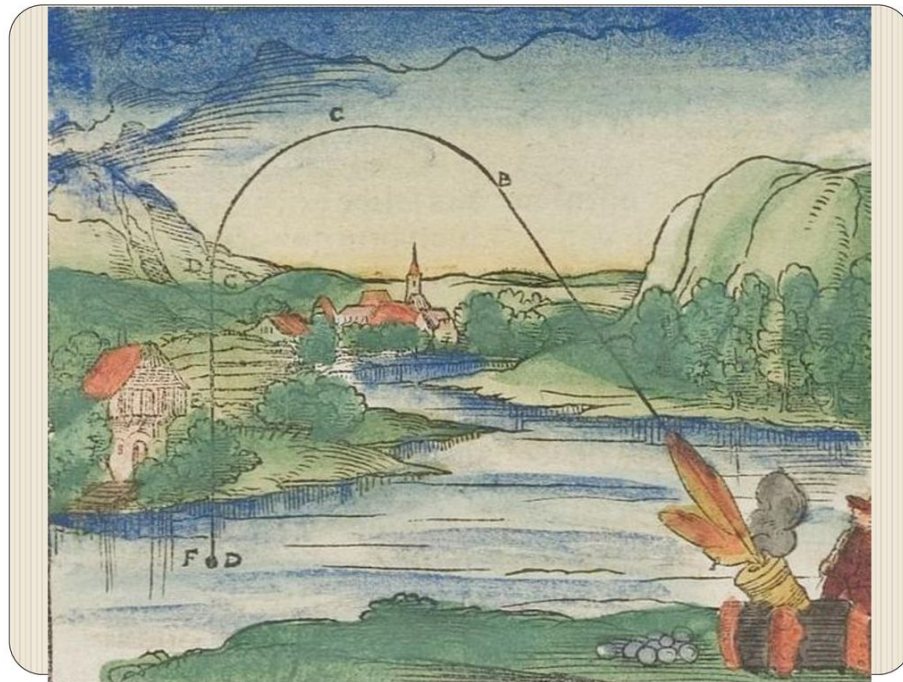
35

36

37

38

39



**Fig. 1.** Medieval theory of the canon ball trajectory, from Walther Hermann Ryff (1547) [4]. The canon ball trajectory was an assemblage of circular arcs and segments. Models in physics are now excellent approximations, but they have sometimes been improved during century-long periods. In biology, we are still at the dawn of model development.

Parameters should be calibrated at some point, or at least reasonably determined from 40  
the experimental information. Model complexity can first be measured by the number of 41  
state variables (variables with dynamics) together with the number of parameters and stay 42  
compatible with the objectives and data. 43

### **Tip 3: Take care with dimensions, intensive and extensive 44 properties 45**

This tip seems very basic, but, in our opinion, it is worth emphasising. The dimension of 46  
the model equation should be checked. Particular care should be taken between intensive 47  
and extensive variables [7]. This is particularly true when dealing with a metabolic model. 48  
A metabolite concentration could be expressed per unit of culture volume or intracellular 49  
volume. The concentration dynamics should then include the dilution by the reactor feeding 50

or by cellular growth, respectively. Moreover, the kinetics of intracellular reactions should depend on intracellular concentrations, not culture concentrations. In several studies, it remains unclear.

#### **Tip 4: Do not assume gas concentrations equilibrate with atmosphere**

Assuming gas concentrations equilibrate with the atmosphere is a common mistake. If we measure the dissolved  $CO_2$  concentration in a glass of water in equilibrium with the atmosphere, it will be proportional to  $P_{CO_2}$ , the  $CO_2$  partial pressure at the interface (*i.e.* in the air):  $[CO_2] = K_h P_{CO_2}$  where  $K_h$  is Henry's constant at the considered temperature and salinity. At steady state, there is no more gas exchange between the atmosphere and liquid phase.

If algae are developing in the glass, the  $CO_2$  concentration will be lower, because the algae permanently consume it. As a consequence, there is a permanent flux of  $CO_2$  from air to water, with a flow rate

$$Q_{CO_2} = K_L a (CO_2 - K_h P_{CO_2})$$

which will balance the consumption of  $CO_2$  by the algae. Now the concentration of  $CO_2$  is lower than  $K_h P_{CO_2}$ , its natural equilibrium value without algae.

#### **Tip 5: Check the mathematical soundness of your model**

A mathematical analysis of your model may help to detect potential errors, limitations and drawbacks in model design, and to better apprehend the process. Whenever possible, one should check mass conservations, check the boundedness of the variables (in particular their positivity), and study the asymptotic behaviour of the model. This last point could be, for some models, particularly challenging. It is essential to keep in mind that nonlinear dynamical models are complex mathematical objects with potentially weird behaviours,

including limit cycles, chaos or abrupt change in behaviours after bifurcation when one 71  
of the model parameters has been slightly modified [8]. Mathematicians spend months 72  
trying to understand and prove the behaviour of systems of low dimension, *e.g.* with "only" 73  
three state variables. The mathematical complexity is breath-taking when considering 74  
standard bioprocess models. Often, the properties of these models are hardly suspected, 75  
and Pandora's box stays closed. Even the number of equilibria that can be produced is 76  
rarely discussed. Adding new features or including more realism into a model extends the 77  
risk of unexpected model behaviours. 78

The objective is to determine whether the trajectories of your system converge towards an 79  
equilibrium (a global equilibrium, or different equilibria depending on the initial conditions), 80  
if they present sustained oscillations (limit cycle) or even show a chaotic behaviour. These 81  
properties should be in line with the behaviour of your bioprocess, otherwise the model 82  
should be revised. 83

## Tip 6: Be aware of structural identifiability 84

Most of the parameters in physical modelling have a clear meaning and can be directly 85  
measured on the process. Also, physical models are often linear. The theory of linear 86  
systems and their identification has received much attention, indirect identification of a 87  
tenth of parameters can be accurately carried out by modern algorithms [9,10]. For the 88  
biological systems, which are in turn nonlinear and described by rough approximations, 89  
more modesty is required. 90

Theoretical identifiability of the parameters is a complex mathematical property [11], 91  
which is often characterized by cryptic (but accurate) mathematical formulations. In a 92  
nutshell, this theoretical mathematical property states that a parameter value can be 93  
uniquely determined by (nonlinear) combinations of measurements and their derivatives 94  
(with respect to time) at any order. More simply, a unique set of parameters can produce a 95  
given model output. With non-linear models, it is possible that two sets of parameters can 96  
produce exactly the same results. To illustrate the non-identifiability pathology, we present 97

in Table 1 two illustrative astonishing examples for trivial models. 98

The first example is unfortunately not so rare. It consists in representing an inhibition 99  
kinetics (from substrate  $S$ ) with a product of Monod and a hyperbolic inhibition term. A 100  
numerical example is given in Table 1 (Example 1), where two parameter sets produce 101  
exactly the same values. Parameters here are only locally structurally identifiable. 102

The second example in Table 1 uses a trivial logistic equation ( $x$  is the biomass) modified 103  
to deal with mortality rate (which is obviously a very bad idea). Here, an infinity of 104  
parameters provide the same biomass dynamics, they are structurally not identifiable. 105

These two examples also demonstrate that it is useless to attribute a biological meaning 106  
to a non-identifiable parameter. In the first case, what was, in turn, the inhibition constant: 107  
 $K_i$  or  $K_s$ ? In the second example, is  $K$  the carrying capacity of the medium? 108

Perhaps more problematic when using an automatic algorithm for parameter identifica- 109  
tion, non-identifiable parameters will kill any approach. Especially if it is a global approach, 110  
any optimisation algorithm will oscillate between several of the possible solutions, or average 111  
them, and often will never converge. 112

In general, assessing identifiability for complex dynamical models is very challenging. 113  
This is a reason why modellers must refrain from embedding too many processes into a 114  
model, and privilege lower complexity models when only a limited set of measurements is 115  
available for validation. 116

## Tip 7: Double check numerical implementation 117

If your model has been implemented only once, then it probably contains at least three 118  
mistakes. We know this is not true for you, but it is for most of the people. So if the model 119  
was right, after a rapid change in one of the equations for testing the effect of one factor, it 120  
would become wrong because eventually the test is not removed. There are strict coding 121  
rules and use of validation tests [12], but they are rarely respected for model development 122  
because the model implementation is generally not carried out by computer scientists. Also, 123  
the way models are implemented can highly differ, and some computer languages may be 124

**Table 1.** Analysis of two simple examples with identifiability issues.

	Parameter set #1	Parameter set #2	False claim parameter meaning	Function
<b>Example 1:</b>	substrate uptake with inhibition			$\phi(S) = \bar{\mu} \frac{S}{S+K_s} \frac{K_i}{S+K_i}$
Numerical values	$\bar{\mu} = 2,$ $K_i = 1,$ $K_s = 2.$	$\bar{\mu} = 1,$ $K_i = 2,$ $K_s = 1.$	max. growth rate inhibition constant affinity constant	$\phi(S) = 2 \frac{S}{(S+1)(S+2)}$
General case	$\bar{\mu},$ $K_i,$ $K_s.$	$\bar{\mu} \frac{K_i}{K_s},$ $K_s,$ $K_i.$	max. growth rate inhibition constant affinity constant	$\phi(S) = \bar{\mu} \frac{S}{S+K_s} \frac{K_i}{S+K_i}$
<b>Example 2:</b>	logistic growth with mortality			$\dot{x} = \bar{\mu}(1 - \frac{x}{K})x - Rx$
Numerical values	$\bar{\mu} = 2,$ $K = 1,$ $R = 1.$	$\bar{\mu} = 3,$ $K = 1.5,$ $R = 2.$	max. growth rate carrying capacity mortality rate	$\dot{x} = (1 - 2x)x$
General case	$\bar{\mu},$ $K,$ $R.$	$\bar{\mu} + \theta,$ $K \frac{\bar{\mu} + \theta}{\bar{\mu}},$ $R + \theta.$	max. growth rate carrying capacity mortality rate	$\dot{x} = \bar{\mu}(1 - \frac{x}{K})x - Rx$

In Example 1, two different parameter sets produce the same value of the function  $\phi(S)$ . In Example 2, an infinite number of parameter sets can produce the same dynamics  $\dot{x}$  for an arbitrary value of  $\theta$ . The parameters meaning (as often claimed) does then not make any sense.



more difficult to cross check. Excel<sup>®</sup> is an excellent tool for displaying data and for simple 125  
computations, but it is not an appropriate tool for simulating complex models since it is 126  
almost impossible to cross-check implementation. Some graphical languages also have these 127  
drawbacks when a connection to a wrong node can corrupt the result while being almost 128  
impossible to detect. 129

One way of reducing the risk of error is a double implementation, with two different 130  
computer programmers and two different languages. This has been the case for the models 131  
used in wastewater treatment, ADM1 for anaerobic digestion [13] and ASM1 for activated 132  
sludge [14]. The first comparison between different implementations revealed to be quite 133  
quaint. Also, simple case studies must help to check simple theoretical properties (positivity 134  
of variables, mass conservation, etc...) that must be respected. 135

## **Tip 8: Pay attention to practical identifiability** 136

The cost criterion to be optimised (typically the sum of squared errors) is generally non- 137  
convex, and many local minima perturb parameter identification. In practice, it is often 138  
not possible to get an accurate estimate of parameters from the data sets. The most 139  
efficient algorithms are generally limited to three parameters to be determined per measured 140  
quantity (assuming a reasonable sampling over time). The weird consequence is that fitting 141  
a model to a set of data is generally possible, but that does not mean that the estimated 142  
parameters are reasonable. Whenever a parameter has a clear meaning, the validity of the 143  
identified value must always be checked, and bounds can be added during the identification 144  
process. Multiple algorithm initialisations are also strongly recommended. Collecting 145  
informative data is also key for practical identifiability, which means data corresponding 146  
to high sensitivities of the model outputs with respect to parameter variations (cf. Fisher 147  
information matrix [9]). As a matter of illustration, it is not possible to estimate a parameter 148  
related to growth inhibition if substrate concentration is always too low to trigger inhibition. 149

Finally, a literature review is an essential resource for parameter values, in particular 150  
for algorithm initialisation. Nonetheless, exotic chimaera can appear when picking up 151

parameters from different papers!

152

## **Tip 9: apply the "divide and conquer" strategy to identify**

153

### **your parameters**

154

Do not try to get all your parameters at once, through a never converging optimization

155

algorithm and rather identify subsets of parameters. In many cases, after simple algebraic

156

manipulations some parts of the model can lead to relationships between some measured

157

quantities and eventually provide some combinations of the parameters. For example, the

158

pseudo-stoichiometry can often be identified independently of the reaction rates after some

159

straightforward transformations [15]. Some working modes do considerably simplify the

160

model, and are often an opportunity to extract such relationships. For example, during a

161

phase when nutrients are nonlimiting, the Michaelis-Menten kinetics can be replaced by

162

constants. Similarly, if different equilibria can be observed for various inputs, they would

163

probably lead to very interesting relationships between some of the model parameters [16].

164

## **Tip 10: determine parameter and model uncertainties**

165

Assessing measurement uncertainty propagation is of utmost importance to assess model

166

accuracy. This first means that the experimental data must be associated to the variance of

167

their measurement error. There are different strategies to compute not only the parameter

168

values but also their confidence intervals. This is straightforward when parameters are

169

deduced from linear relationship, but it can also be estimated in a more complex case

170

thanks to the covariance matrix of parametric errors [9]. The strong scientific added value

171

is that the simulation scheme will predict not only outputs but also the confidence intervals

172

derived from the covariance matrix of prediction errors, or from Monte Carlo simulations.

173

## Tip 11: Validate the model with data not used for identification 174

### tion 175

When observing the vast diversity in bioprocess models, only a few of them have been 176  
appropriately validated. First, because it is not possible to validate a model, a model 177  
can only be discarded when it is not compliant with experimental records [17]. However, 178  
assuming a relaxed use of the "validation" term, it would mean that the model has been 179  
proven accurate for a large variety of cases. In particular for cases significantly different 180  
from the learning data set (data that has been used for the calibration). This ideal situation 181  
is very difficult to meet in practice, and most of the time the validation datasets only differ 182  
by some initial conditions, or by a single different forcing variable. If the model has enough 183  
parameters, it can probably fit a calibration dataset nicely with only a few points. However, 184  
it will exhibit abysmal performances for cross-validation. For larger calibration data sets, 185  
the fit will probably less successfully highlight the quality of the model, but prediction 186  
capacity might be highly enhanced. The plot will not look that nice, but the model will 187  
definitely be more powerful and relevant. 188

Claiming that the model is valid is, therefore, an act of faith, and a very weak scientific 189  
assertion. As running experiments takes time and is money consuming, the number of 190  
experiments is by essence limited. As consequence, it becomes clear that the conditions for 191  
which the model has been validated must be clearly stated. Knowing the "model validation 192  
domain" will in itself be precious for future model use. Also, providing data sets for which 193  
the model did not do its job is intrinsically useful, although rarely done. 194

Often, the question is instead to choose the best model among a few candidates. A more 195  
complex model, with more parameters, will mechanically better fit the data. However, that 196  
does not mean it is more correct, it just means it is more flexible. The Akaike criterion [18] 197  
is a good option to compare the performance of two models of different levels of complexity. 198  
However, the only real criterion to assess the predictive power of a model, and therefore 199  
to compare model performances is cross-validation, assessing the model with data which 200  
were not used for calibration (and data whose dynamics are significantly different from 201

the calibration data set). Additionally, the candidate models can even be used to find the experimental conditions that will allow to differentiate them better [17].

Finally, models can include the effects of different factors which often have been studied separately. The models then gather these effects classically by multiplying the different terms or using Liebig's law of minimum. Validation experiments could be the last chance to test possible interactions between these factors and find the best way to combine their effects in the model.

## Tip 12: Share codes, tips, tools, and model limitations

More and more journals require this, and it is to be welcomed. Providing your model - with all the files necessary to reproduce your simulations (including parameter values, initial conditions etc...) - will favour its dissemination within the scientific community. Your model would thus be further validated with new data sets. Additionally, it promotes error checking, helps the reader if some model details in the manuscript are unclear, and removes any suspicion of fraud.

More generally, what makes the success and the efficiency of a model, is not limited to the biology it embeds and to the realism of its predictions. A model is inexorably associated with a set of tools to calibrate it, estimate which are the most sensitive parameters, optimise a criterion, determine the input which maximizes productivity etc... The associated toolbox to make the model applicable and efficient is probably at least as necessary as the model itself. Great models can have complex structures or behaviours, which eventually make their use more tricky. For example, the outstanding Geider model [19] is in turn rather challenging to calibrate, and specific methods dedicated to its calibration are needed [20]. Even simpler models, such as the Hinshelwood model [21] for temperature, advantageously predicts a mortality rate [22], but calibrating this model often turns into a nightmare [23]. Keeping two different modelling approaches can significantly help in this case, by using the toolbox of one of the models to manage the other one. Typically, using a temperature response model from [24] as a gauging device makes the calibration of Hinshelwood's model

much less painful. Providing all these kinds of information on your model should promote 229  
its adoption by the community. 230

## Conclusion 231

Modelling in biology is a question of choices and trade-offs. The striking difference between 232  
two different modellers is often the choice in model complexity. Extensive tests, using 233  
cross-validation datasets or based on Akaike criteria may reveal that one model has a 234  
better prediction capability than the other, but in other circumstances, it might be the 235  
opposite. Our culture has contributed to hatch the illusion of a unique and universal model 236  
behind nature. However, even if this idea were right, we are far from having discovered it. 237  
Also, always trying to run after such universal representation of nature, inexorably leads to 238  
models whose complexities do not match the available measurements and our capability 239  
to validate the model. So, why should we keep a unique model? Why not use a series 240  
of models of increasing complexity? Surrogate models consist of a simplified version of 241  
a simulator, which is easier to handle mathematically, resulting in more straightforward 242  
use for optimisation or control. The surrogate model can be derived and calibrated from 243  
the most complex model, but the opposite is also true. A simplified model, with limited 244  
accuracy, can provide bounds for a more detailed model. Also, a complicated model can 245  
be simplified into different sub-models depending on the environment and the limiting 246  
factor (nutrients, light or temperature). Working with a set of coherent models should 247  
not necessarily increase difficulty, it creates a consistent framework that can prove to be 248  
very useful for different purposes, from model calibration and process optimisation, up to 249  
advanced control. 250

## Acknowledgements 251

The authors are grateful to Jacob J. Lamb and J. Ras for their help with improving the 252  
English. O. Bernard is thankful to ENERSENSE for welcoming him during his sabbatical. 253

## References

1. Dochain D. Automatic control of bioprocesses. John Wiley & Sons; 2013. 254
2. Sandve GK, Nekrutenko A, Taylor J, Hovig E. Ten simple rules for reproducible 255  
computational research. PLoS computational biology. 2013;9(10):e1003285. 256  
257
3. Osborne JM, Bernabeu MO, Bruna M, Calderhead B, Cooper J, Dalchau N, et al. 258  
Ten simple rules for effective computational research. PLoS Computational Biology. 259  
2014;10(3):e1003506. 260
4. Hermann Ryff W. Architectur. Springer Verlag; 1997. 261
5. Baroukh C, Muñoz-Tamayo R, Bernard O, Steyer JP. Mathematical modeling of 262  
unicellular microalgae and cyanobacteria metabolism for biofuel production. Current 263  
opinion in biotechnology. 2015;33:198–205. 264
6. Baroukh C, Turon V, Bernard O. Dynamic metabolic modeling of heterotrophic 265  
and mixotrophic microalgal growth on fermentative wastes. PLOS Computational 266  
Biology. 2017;13(6):e1005590. 267
7. Fredrickson A. Formulation of structured growth models. Biotechnology and bioengi- 268  
neering. 1976;18(10):1481–1486. 269
8. Strogatz SH. Nonlinear dynamics and chaos: with applications to physics, biology, 270  
chemistry, and engineering. CRC Press; 2018. 271
9. Walter E, Pronzato L. Identification of parametric models from experimental data. 272  
Springer Verlag; 1997. 273
10. Ljung L. System identification. In: Signal analysis and prediction. Springer; 1998. p. 274  
163–173. 275
11. Walter E. Identifiability of parametric models. Elsevier; 2014. 276
12. Duvall PM, Matyas S, Glover A. Continuous integration: improving software quality 277  
and reducing risk. Pearson Education; 2007. 278

13. Batstone D, Keller J, Angelidaki RI, Kalyuzhnyi SV, Pavlostathis SG, Rozzi A, 279  
et al. The iwa anaerobic digestion model no.1 (adm1). *Water Science Technology.* 280  
2002;45(10):65–73. 281
14. Henze M, Gujer W, Mino T, Van Loosdrecht M. *Activated sludge models ASM1,* 282  
*ASM2, ASM2d and ASM3.* IWA publishing; 2000. 283
15. Bernard O, Bastin G. On the estimation of the pseudo-stoichiometric matrix for 284  
macroscopic mass balance modelling of biotechnological processes. *Mathematical* 285  
*biosciences.* 2005;193(1):51–77. 286
16. Bernard O, Hadj-Sadok Z, Dochain D, Genovesi A, Steyer JP. Dynamical model 287  
development and parameter identification for an anaerobic wastewater treatment 288  
process. *Biotechnology and bioengineering.* 2001;75(4):424–438. 289
17. Vatcheva I, De Jong H, Bernard O, Mars NJ. Experiment selection for the discrim- 290  
ination of semi-quantitative models of dynamical systems. *Artificial Intelligence.* 291  
2006;170(4-5):472–506. 292
18. Burnham KP, Anderson DR. Multimodel inference: understanding AIC and BIC in 293  
model selection. *Sociological methods & research.* 2004;33(2):261–304. 294
19. Geider RJ, MacIntyre HL, Kana TM. A dynamic regulatory model of phytoplanktonic 295  
acclimation to light, nutrients, and temperature. *Limnol Oceanogr.* 1998;43:679–694. 296
20. Smith SL, Yamanaka Y. Quantitative comparison of photoacclimation models for 297  
marine phytoplankton. *ecological modelling.* 2007;201(3-4):547–552. 298
21. Hinshelwood CN. *Chemical kinetics of the bacterial cell.* Oxford At The Clarendon 299  
Press; London; 1946. 300
22. Serra-Maia R, Bernard O, Gonçalves A, Bensalem S, Lopes F. Influence of temperature 301  
on *Chlorella vulgaris* growth and mortality rates in a photobioreactor. *Algal research.* 302  
2016;18:352–359. 303

23. Grimaud GM, Mairet F, Sciandra A, Bernard O. Modeling the temperature effect 304  
on the specific growth rate of phytoplankton: a review. *Reviews in Environmental* 305  
*Science and Bio/Technology*. 2017;16(4):625–645. 306
24. Bernard O, Remond B. Validation of a simple model accounting for light and 307  
temperature effect on microalgae growth. *Bioresource technology*. 2012;123:520–527. 308  
doi:10.1016/j.biortech.2012.07.022. 309