



Optimal strategies for estimating the parameters of the Baranyi-Ratkowsky model: from (optimal) experiment design to model fitting methods

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ABSTRACT

The Baranyi growth model combined with the Ratkowsky secondary model (Baranyi-Ratkowsky hereafter) has demonstrated their reliability for describing microbial growth as a function of temperature. However, these models are based on empirical parameters that must be estimated from data, so robustness depends on the experimental design and the model fitting approach. This study applies a rigorous statistical analysis based on Information Theory and numerical simulations to provide clear guidelines on the best model fitting approaches and experimental designs for the Baranyi-Ratkowsky model.

First, the study concludes that one-step fitting approaches result in lower parameter dispersion than two-steps approaches (for the simulated conditions: 44 %, 85 % and 96 % lower for b , $\log C_0$ and T_{min} , respectively, no reduction for $\log N_{max}$ and $\log N_0$). Numerical simulations demonstrate that, unlike for two-steps methods, the error of regression from the one-step approach is a realistic estimate of parameter uncertainty/variability, strengthening the case for this method. This motivates restricting the calculation of Optimal Experiment Designs (OEDs) for this approach only. The study clearly demonstrates that the experimental design has a clear impact on parameter dispersion. However, OEDs tend to be impractical, as they focus on conditions that require long experimental runs. Accordingly, a penalty term is introduced in OED definition, resulting in two strategies: to lower the number of experiments at lower temperatures, and to terminate the experiments at lower temperatures before reaching stationary phase. Based on this result, we propose a staggered experimental design that is updated recursively (storage temperature and position of time points) until convergence. Considering that isothermal experiments are still the gold standard in the field, future studies could greatly benefit from these suggestions by minimizing the experimental effort needed to obtain robust estimates for the Baranyi-Ratkowsky model.

1. Introduction

Mathematical models for the growth of bacterial populations are a keystone of modern food science, being an important part of shelf life estimation, precision fermentation or risk assessment (Balsa-Canto et al., 2020; Lucero-Mejía et al., 2025; Possas et al., 2021; Rodríguez-Caturla et al., 2023; Sánchez-Martín et al., 2025). The methodologies for the development of growth models are defined by predictive microbiology, which most often follows a two-steps approach (Perez-Rodríguez &

Valero, 2012). First, primary models describe how microbial concentration varies through time. Primary models have parameters that depend on the environmental conditions (e.g., the specific growth rate depends on temperature), a relationship that is described by secondary models (Whiting & Buchanan, 1993).

The Baranyi growth model is probably the most popular primary model for microbial growth. This model is based on dynamic hypotheses that extend the classic first order kinetics to account for the lag and stationary phases often observed in microbial populations (Baranyi &

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Roberts, 1994). The Baranyi growth model is often combined with the Ratkowsky secondary growth model (Ratkowsky et al., 1982) to describe the effect of the storage temperature on the maximum specific growth rate (μ_{max}) for suboptimal temperatures. The combination of both models is called the Baranyi-Ratkowsky model hereafter.

Like any empirical model, the Baranyi-Ratkowsky model has unknown model parameters that must be estimated from experimental data, usually consisting of a compilation of individual experiments performed at constant temperature conditions. Although the use of dynamic experiments (where each experiment explores more than one temperature) has been suggested (Huang, 2017a, 2020), this methodology is rather niche, probably due to experimental limitations and higher mathematical complexity. Instead, the Baranyi-Ratkowsky models is most often fitted to data obtained under isothermal conditions, as acknowledged in ISO 23691.

Therefore, the goal of this study is to identify parameter estimation strategies for the Baranyi-Ratkowsky model from isothermal data. This is understood as those that provide the lowest parameter uncertainty with the least experimental effort. Therefore, the study is based on the assumption that model fitting approaches can be a source of parameter uncertainty. A variety of approaches currently co-exist in the field, with the most common one probably being two-steps estimation. First, primary models are fitted independently to the data obtained at each temperature. Then, on a second step, secondary models are fitted to the estimates of the primary model (the lag phase duration, λ , and the specific growth rate, μ). Alternatively, primary and secondary models can be estimated directly from the microbial concentrations observed using nonlinear regression (Dolan et al., 2007; Huang, 2017b). Although this increases the mathematical complexity of parameter estimation, it is generally acknowledged to be more statistically robust (Cattani et al., 2016; Fernández et al., 1999).

This study uses a broader definition of “fitting approach” that considers not just the fitting algorithm but also the experimental design. This is motivated by previous studies showing that parameter uncertainty can be reduced through more efficient designs. Those studies often used model-based Optimal Experiment Designs (OED) (Balsacanto et al., 2008) to identify the most informative experimental conditions for a given model. This methodology is based on general assumptions, so it applies to most dynamic models (Villaverde et al., 2021), including growth models from predictive microbiology (Akkermans et al., 2018; Bernaerts et al., 2000; Grijspeerd & Vanrolleghem, 1999; Guillén et al., 2024; Río et al., 2024).

Despite its broad application, no previous study has calculated OEDs for the Baranyi-Ratkowsky model. This could be due to the optimization of this model adding additional complexity, as the design space is two-dimensional (combination of time and temperature), whereas the optimization of primary models (sampling time), secondary models (storage temperature) or dynamic experiments (temperature levels) is one-dimensional.

Hence, this study calculates OEDs for the Baranyi-Ratkowsky model. Please note that the calculation of OEDs for the one-step and two-steps methods require a largely different mathematical approach. Therefore, the study first compares the one-step and two-step approaches, to justify the restriction of the calculation of OEDs to a single fitting method. Then, the robustness of the different experimental designs (optimal or not) is based on a simulated datasets (Garre et al., 2019). This has the advantage of generating thousands of growth experiments that cannot be distinguished from actual experimental data, providing a generally more robust analysis of the statistical properties of the models than one based on (perhaps cherry-picked) limited experimental data.

2. Materials and methods

2.1. Model fitting approaches

2.1.1. Conventional (two-step) model fitting approach

The present study compares three different model fitting approaches. In the “conventional” approach, the Baranyi primary model (Baranyi & Roberts, 1994) is first fitted to the data obtained at each isothermal temperature. The algebraic solution of this model for constant temperature is shown in Eq. (1), showing that this model predicts a sigmoidal growth curve parameterized by four parameters: N_0 (the initial microbial concentration), N_{max} (the maximum microbial concentration), μ_{max} (the maximum specific growth rate during the exponential phase) and λ (the lag phase duration).

$$\ln N = \ln N_0 + \mu_{max} A(t) - \ln \left(1 + \frac{e^{\mu_{max} A(t)} - 1}{e^{\ln N_{max} - \ln N_0}} \right) \quad (1)$$

$$A(t) = t - \lambda + \frac{1}{\mu_{max}} \ln(1 - e^{-\mu_{max} t} + e^{-\mu_{max}(t-\lambda)})$$

Once primary models have been fitted (one per temperature), secondary models describe the impact of temperature changes in either μ_{max} or λ . The relationship between μ_{max} and temperature (T) was described using the sub-optimal Ratkowsky model (Ratkowsky et al., 1982), which assumes a linear relationship between T and $\sqrt{\mu_{max}}$ with slope b (Eq. 2). This model also introduces a theoretical minimum temperature for growth (T_{min}).

$$\sqrt{\mu_{max}} = b(T - T_{min}); T > T_{min} \quad (2)$$

$$\sqrt{\mu_{max}} = 0; \text{ otherwise}$$

Based on previous knowledge on the multiplication $\lambda \cdot \mu$ remaining constant between replications of the same experiment (Amézquita et al., 2005; Augustin et al., 2000; Jaloustre et al., 2011), a recent study concluded that the only valid secondary model for λ would be an inverse-square root relation (Garre et al., 2025). This translates into the secondary model shown in Eq. (3), where a_λ and b_λ are the intercept and the slope of the regression line.

$$\frac{1}{\sqrt{\lambda}} = a_\lambda + b_\lambda T \quad (3)$$

Therefore, the conventional approach includes 6 parameters: N_0 , N_{max} , b , T_{min} , a_λ and b_λ . To improve identifiability, parameters N_0 and N_{max} have been log-transformed for model fitting.

2.1.2. Two-step fitting considering coupling between secondary models

The study by Garre et al. (2025), besides identifying the inverse-square root relation for λ also identified a link between both secondary models. Namely, the secondary model for λ can be written as shown in Eq. (4). Note that parameter T_{min} appears in both secondary models (Eqs. 2 and 4). Furthermore, coefficient B is defined as per Eq. (5), which also includes parameter b from the Ratkowsky model (Eq. 2). Therefore, the secondary model for λ only introduces a single parameter (C_0 ; related to the hypotheses for the lag phase in the Baranyi model).

$$\frac{1}{\sqrt{\lambda}} = \frac{1}{\sqrt{B}}(T - T_{min}) \quad (4)$$

$$B = \frac{\ln\left(1 + \frac{1}{C_0}\right)}{b^2} \quad (5)$$

Hence, this approach (“two-steps” hereafter) is equivalent on its first step to the conventional one, as it starts by fitting primary models to the data obtained at each temperature. Then, instead of fitting independent secondary models for λ and μ_{max} , it fits both secondary models (Eqs. 2 and 5) at the same time by nonlinear regression. This results in a

reduction of one parameter with respect to the conventional approach, being defined by N_0 , N_{max} , b , T_{min} and C_0 . For identifiability reasons, parameter C_0 has been log-transformed for fitting, as well as N_0 and N_{max} .

2.1.3. One-step fitting approach

It is generally regarded that one-step fitting methods, where secondary models are estimated directly from the microbial concentrations (i.e., without fitting the primary models in a separate step) are more robust (Fernández et al., 1999). Therefore, eqs. (1), (2), (4) and (5) were combined into Eq. (6). This allows the five model parameters (N_0 , N_{max} , b , T_{min} and C_0) to be estimated directly from the values of N observed for different combinations of t and T using non-linear regression (Garre et al., 2023). For consistency with the other methods, parameters C_0 , N_0 and N_{max} were log-transformed for identifiability.

$$\ln N = \ln N_0 + \mu_{max} A(t) - \ln \left(1 + \frac{e^{\mu_{max} A(t)} - 1}{e^{\ln N_{max} - \ln N_0}} \right) \quad (6)$$

$$A(t) = t - \lambda + \frac{1}{\mu_{max}} \ln(1 - e^{-\mu_{max} t} + e^{-\mu_{max}(t-\lambda)})$$

$$\sqrt{\mu_{max}} = b(T - T_{min})$$

$$\frac{1}{\sqrt{\lambda}} = \frac{1}{\sqrt{B}}(T - T_{min})$$

$$B = \frac{\ln \left(1 + \frac{1}{C_0} \right)}{b^2}$$

2.2. Optimal experiment design (OED)

2.2.1. Calculation of local sensitivity functions

Local sensitivity functions with respect to some parameter p_i (s_{pi}) are defined as the partial derivative of the response (y ; the log-microbial concentration in this case) with respect to each model parameter (Eq. 7). For the Baranyi-Ratkowsky model, they are a function of the storage time (t) and temperature (T).

$$s_{pi} = \frac{\partial y}{\partial p_i}(t, T) \quad (7)$$

The values of s_p were estimated by finite differences using an approach analogous to the one implemented in the *FME* package (Soetaert & Petzoldt, 2010). For any value of t and T , the ideal response is calculated using the Baranyi-Ratkowsky model ($y(t_j, T_k; p)$). Then, a small perturbation is introduced in the parameter p_i ($\Delta p_i = p_i \cdot 10^{-8}$) and microbial concentration is again calculated according to the Baranyi-Ratkowsky model ($y(t_j, T_k; p + \Delta p_i)$). Then, the value of the local sensitivity function for parameter p at (t_j, T_k) can be approximated by the difference in the microbial concentration divided by the magnitude of the perturbation (Eq. 8).

$$s_p(t_j, T_k) \approx \frac{y(t_j, T_k; p + \Delta p_i) - y(t_j, T_k; p)}{\Delta p_i} \quad (8)$$

2.2.2. Determination of D-optimal experiment designs

OEDs were calculated based on the Fisher Information Matrix (*FIM*). Under simplifying hypotheses (Balsa-Canto et al., 2008), the *FIM* for a given experimental design can be calculated from the local sensitivity functions evaluated at the sampling points (Eq. 9). In this equation, (t_j, T_k) represent each of the n sampling conditions. The term $s_{\theta}(t_j, T_k)$ is the vector of local sensitivities ($s_{pi}(t_j, T_k)$) calculated for each of the unknown model parameters. Finally, Q is a weight matrix, which Backspace[""] was defined as the identify matrix in this study.

$$FIM = \sum_{i=1}^n (s_{\theta}(t_j, T_k))^T \cdot Q \cdot (s_{\theta}(t_j, T_k)) \quad (9)$$

Different criteria are available to maximize the *FIM*. Here we focus on the D-criterion, which implies finding the combination of $(t_j, T_k)_n$ that maximizes the determinant of the *FIM* (Eq. 10). This is equivalent to minimizing the volume of the confidence ellipsoid of the model parameters (de Aguiar et al., 1995).

$$\max_{(t_j, T_k)_n} \det(FIM) \quad (10)$$

Using the standard definition of the OED, each sampling condition (t_j, T_k) would be independent. This could result in optimal configurations that test a large number of temperature values, something impractical because it would require inoculations of different matrixes that would be stored at different temperatures. Instead, reducing the number of temperatures tested is often desirable.

Therefore, the elements of (t_j, T_k) were rewritten as shown in Eq. 11. This formulation defines N individual growth experiments and n time points per experiment. Accordingly, every time point i within an experiment j ($t_{j,i}$) share the same temperature T_j .

$$(T_1, t_{1,1}, t_{1,2}, \dots, t_{1,n}), (T_2, t_{2,1}, t_{2,2}, \dots, t_{2,n}), \dots, (T_N, t_{N,1}, t_{N,2}, \dots, t_{N,n}) \quad (11)$$

An additional issue for the definition of the optimization problem is the bounds for the experimental conditions. The temperature range was defined between 6 and 37 °C (reasonable for vegetative bacteria). However, this introduces complexity in the definition of bounds for the maximum experimental duration, as a complete growth curve often requires 24 h at 37 °C but several weeks at 6 °C. On preliminary calculations, we defined an overall upper bound of 24,000 h and that strategy failed to converge, most likely due to experiments at high temperatures being on stationary phase (where local sensitivity functions are flat) through most of the design space. Therefore, the optimization problem was reformulated, writing it in terms of the expected microbial concentration without stationary phase ($y_{bound,i}$) defined in Eq. 12.

$$y_{bound,i} = \ln N_0 + (t - \lambda(T_i)) \cdot \mu_{max}(T_i) \quad (12)$$

Hence, bounds were defined directly on $y_{bound,i}$, resulting on adaptive upper bounds for t_i depending on the temperature of the experiment. Namely, an upper bound of 12 log CFU/g was defined, resulting in a maximum duration equivalent to the one required to reach a concentration 4 logs above $\log N_{max}$ if there was no stationary phase. A lower bound of -2 log CFU/g was used (as a lower bound of 0 log CFU/g would introduce a lower limit at $t_i = \lambda$). Then, for the calculation of the *FIM*, conditions with $t_i < 0$ were set as zero. This results in the optimization problem shown in Eq. (13).

$$\max_{(y_i, T_i)} \det(FIM) \quad (13)$$

$$6^\circ\text{C} \leq T_i \leq 37^\circ\text{C}; \forall i$$

$$-2 \log \text{CFU/g} \leq y_{bound,i} \leq 12 \log \text{CFU/g}; \forall i$$

2.2.3. Determination of D-optimal experiment designs with penalty

D-optimal experiments, although optimal from the point of view of information theory, might be impractical. Particularly for microbial growth, they tend to favor experiments with an excessively long duration. Hence, we followed an approach similar to Guillén et al. (2024), introducing a penalty term in the optimization problem (Eq. 14), scaled by a weight coefficient (ϕ).

$$\max_{(t_j, T_j)} \det(FIM) + \phi \cdot P(t, T) \quad (14)$$

The penalty term is defined as the sum of the durations of each of the N experiments in the design (Eq. 15). Note that this introduces a small deviation with respect to Guillén et al. (2024), as that study focused on secondary growth models, so each experiment was independent. Here, we looked at both primary and secondary models. Therefore, each experiment at the same temperature included several time points. Accordingly, the duration of each experiment is defined by the highest time point within the design (Eq. 15).

$$P(t, T) = \sum_{i=1}^N \max(t_i, T_i) \quad (15)$$

2.3. In-silico simulation of growth experiments

To evaluate the statistical properties of the different fitting strategies (fitting approaches or experimental designs), an artificial dataset of growth experiments was generated by numerical simulation, following a methodology adapted from a previous study (Garre et al., 2019). The numerical method assumes that the Baranyi-Ratkowsky model describes the “true” response of the microbial population, and that the experimental error (accounting for variability and uncertainty) introduces an uncorrelated random error of mean zero and known variance on the observed log-microbial concentration. The approach can be summarized in the following steps:

For i in 1 to $n_{\text{experiments}}$:

1. For each temperature included in the design:
 - a. Calculate the value of μ_{\max} and λ according to the secondary models (Eqs. 2 to 5).
 - b. Calculate the microbial concentration at each time point in the design ($\log N_{\text{ideal},i}$) based on the Baranyi primary model (Eq. 1).
 - c. Duplicate the ideal values according to the number of replicates.
 - d. Calculate the experimental error at each time point (ε_i) by taking random samples from a normal distribution with mean zero and variance $\sigma_{\log N}^2$.
 - e. Calculate the “observation” as $\log N_{\text{obs},i} = \log N_{\text{ideal},i} + \varepsilon_i$

As a demonstration, the following model parameters were used: $\log N_0 = 2 \log \text{CFU/g}$; $\log N_{\max} = 8 \log \text{CFU/g}$; $b = 0.04 \text{ h}^{-1}$; $\log C_0 = -4$; $T_{\min} = 5^\circ \text{C}$. These values are based on the parameters estimated by Garre et al. (2025), although C_0 was reduced to have a more noticeable lag phase. The study was repeated for different parameter values, reaching the same conclusions from a qualitative point of view.

Supp. Fig. 1 illustrates the type of data generated for each iteration. Independent researchers (experienced in growth modelling and from other institutions) were unable to distinguish between simulated and actual experimental data, therefore it was considered that those simulations were representative of true scenarios. The main advantage of this approach is that they allow the generation of a homogeneous dataset of thousands of experiments, something that is rarely feasible by other approaches, such as (systematic) literature review.

2.4. Computer implementation

Calculations were implemented in R version 4.2.3 (R Core Team, 2022) and are available from the GitHub page of one of the co-authors (<https://github.com/albgarre/robust-baranyi-ratkowsky>). For both the conventional and two-steps approach, primary models were fitted by nonlinear regression (Bates & Watts, 2007) using the functions included in *biogrowth* (Garre et al., 2023). For the conventional approach, the secondary models were fitted by nonlinear regression using the functions included in R. The algorithm for model fitting considering coupling (both one-step and two-steps) were implemented in version 1.1 of *biogrowth* (Garre et al., 2023) in the function *fit_coupled_growth*. This function uses nonlinear regression by the Levenberg-Marquardt algorithm

supported by the functions included in *FME* (Soetaert & Petzoldt, 2010).

The optimization problems defining the OED and OED+penalty were solved by the Enhanced Scatter Search algorithm (Egea et al., 2009), using the implementation included in the MEIGO package (Egea et al., 2014). The number of function evaluations was determined by checking that the objective function had converged. Then, a local refinement by the DHC algorithm was applied.

The weight coefficient, ϕ , for the penalty function was defined iteratively. First, it was checked that a value of $\phi = 0$ resulted in D-optimal designs. Then, this parameter was increased until the aggregated time of the optimal solution was close to the one of the uniform design. This resulted in values of $5 \cdot 10^6$, $1 \cdot 10^7$, $5 \cdot 10^7$ and $2 \cdot 10^8$ for designs with 6, 8, 10 and 12 experiments, respectively.

3. Results and discussion

3.1. Comparison between model fitting strategies

This study compares three different strategies for estimating the parameters of the Baranyi-Ratkowsky model from a set of isothermal experiments performed at different temperatures. The first approach might be the gold standard in the field, where primary models are independently fitted to the data obtained under each temperature. This provides a table of primary model parameters ($\log N_0$; $\log N_{\max}$; μ and λ) for each temperature. On a second step, secondary models are fitted to describe how temperature changes affect μ and λ , providing estimates for T_{\min} ; b ; a_λ ; b_λ . The second approach follows the recommendations by Garre et al. (2025), considering the link between the secondary models for μ and λ . Accordingly, the secondary models would be described by three parameters (T_{\min} ; b ; C_0) instead of four like in the conventional approach. In addition, a third approach considered in this study fits both the primary and secondary models in a single step using nonlinear regression, also considering the coupling of the secondary models.

Based on numerical simulations, we can conclude that the model fitting strategy affects the robustness of the parameter estimates, in line with previous studies (Cattani et al., 2016; Dolan et al., 2007; Fernández et al., 1999; Huang, 2020; Huang, 2017a). Fig. 1 illustrates the dispersion of the parameter estimates as a function of the number of experiments (i.e., number of temperature levels included in the design) for the three strategies, with Supp. Table 1 including summary indexes. Please note that the number of model parameters differs between methods (e. g., the conventional method does not fit C_0), and therefore the number of boxes differs between facets.

As expected, increasing the number of experiments reduces the dispersion of parameter estimates, with the conventional and two-step methods being comparable in terms of dispersion. Although the method that includes the coupling between secondary models results in lower dispersion for b and T_{\min} , this could be a numerical artefact, as the numerical simulations include such link. The results show how the one-step method would be more robust than two-step approaches. Particularly, this method has the same precision as two-steps methods for parameters $\log N_{\max}$ and $\log N_0$, whereas parameters b , $\log C_0$ and T_{\min} have lower dispersion (44 %, 85 % and 96 % lower, respectively). This is reasonable, as parameters $\log N_{\max}$ and $\log N_0$ are linked to primary models, whereas the other three are part of the secondary model definition.

The lower dispersion of the parameters obtained using the one-step approach is of great relevance for building growth models. The differences in parameter estimates obtained between independent experiments can be attributed to two different sources: variability and uncertainty. Predictive models must reflect the former, as it is an inherent part of the microbial response, whereas uncertainty should be minimized (Nauta, 2000). Currently, this is done by gathering additional data and/or improving the experimental protocols to reduce experimental error. However, the results presented in Fig. 1 clearly illustrate that parameter uncertainty can be reduced by just using more robust

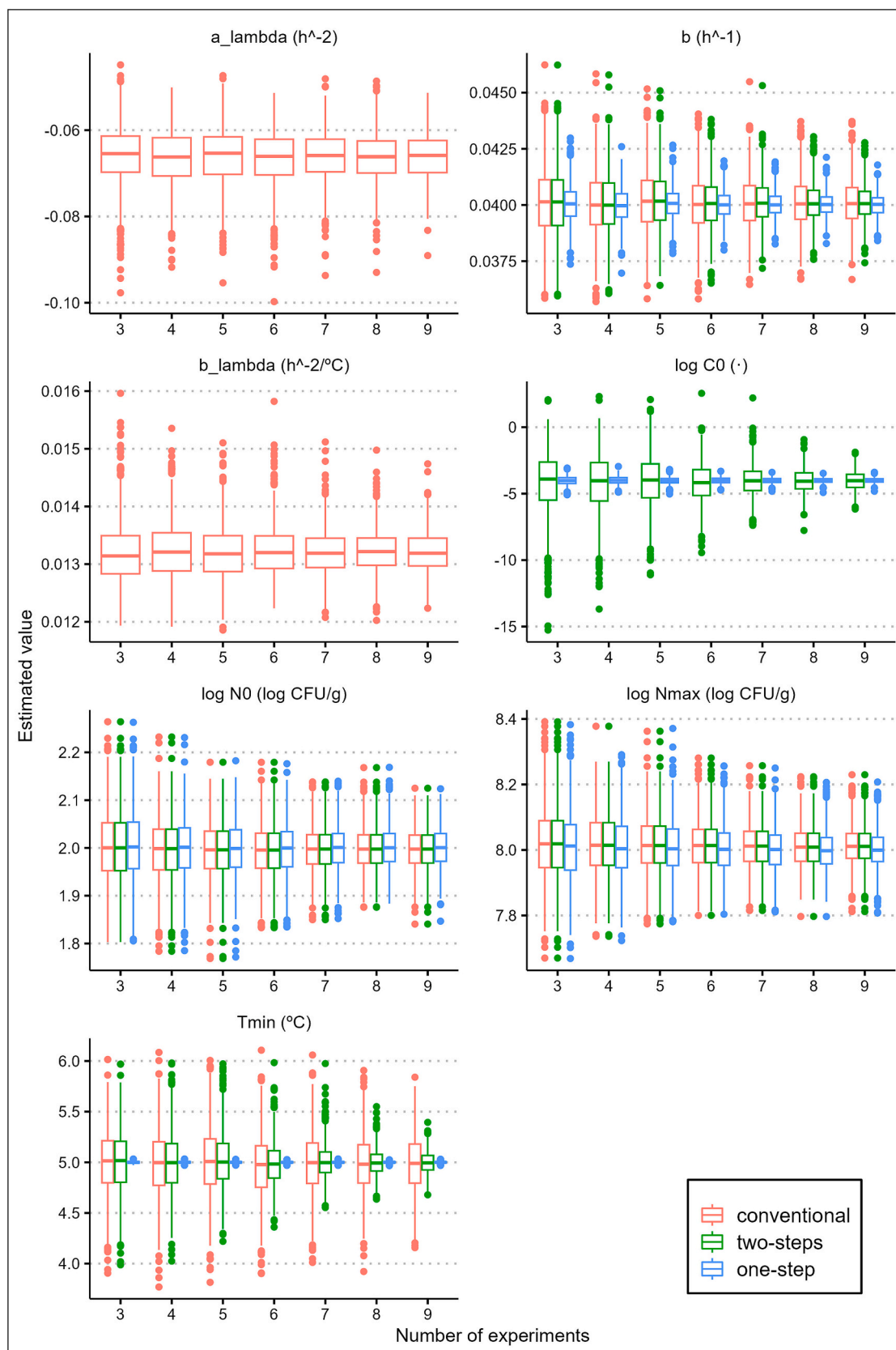


Fig. 1. Illustration of the dispersion in parameter estimates (1000 simulated experiments per condition) as a function of the number of experiments (i.e., number of different temperatures tested) and the model fitting approach.

model fitting approaches (please note that the different methods are fitted to the same simulated datasets). Therefore, this results in a quantitative support to the general knowledge in the field about one-step methods being more robust. Nonetheless, it must be underlined that this conclusion is based on artificial data that was simulated considering that the hypotheses of the Baranyi-Ratkowsky model are true (including the coupling between both secondary models). Although there is scientific evidence to support that assumption (e.g., the product $\lambda \cdot \mu$ or “work to be done” being constant between experiments), independent empirical validation is still needed.

As mentioned above, variability and uncertainty are of high relevance for predictive microbiology (den Besten et al., 2017). Therefore, the ability to quantify them is an important aspect when assessing the robustness of a parameter estimation method (Garre, Pielat, et al., 2022). Here we compare two possible approaches for uncertainty/variability estimation. The first one is to repeat the experiment several times, obtaining several values of the parameter estimates. Then, variability/uncertainty can be estimated from the standard deviation of the model parameters. The second possibility consist in using the standard error of regression to estimate the variance-covariance of the model parameters, using this matrix to represent variability/uncertainty (e.g., see (Bates & Watts, 2007) for a detailed description of the calculations).

Fig. 2 summarizes the uncertainty estimates obtained for each parameter as a function of the number of experiments. In every case, parameter standard deviation decreases as the number of experiments increases. This is reasonable, as additional data is expected to reduce parameter uncertainty. The error of regression of the one-step method (Fig. 2C) is very close to the standard deviation of most parameters, with

the only exception being T_{min} . Nevertheless, the standard deviation of this parameter shows erratic behavior which is most likely due to poor convergence. This implies that the standard error of regression for the one-step method should be considered as a reliable method for estimating parameter variability/uncertainty. In fact, it should be accounted to be more reliable than the standard deviation of parameter estimates, as it appears to be more statistically robust (Fig. 2C implies that a lower number of simulated experiments is required for convergence).

On the other hand, the standard error of regression for both the conventional and two-step fitting methods is far from the actual parameter variability/uncertainty (Fig. 2A and B). This is most likely due to two-step methods using only the parameter estimates of primary models. Hence, parameter uncertainty in the primary model is not accounted when fitting the secondary models, resulting in unreliable estimates of parameter uncertainty/variability. In fact, the standard error of regression for two-step methods is an entirely unrealistic estimator of parameter variability and uncertainty. Instead of decreasing for an increased number of experiments, this parameter remains mostly constant. This is most likely due to the low statistical power of this estimate (e.g., the standard error from three temperatures is calculated on a single degree of freedom). Hence, the standard error of regression should generally be avoided as an estimate of variability/uncertainty in two-step methods.

Parameter correlation is another common issue of empirical methods that can depend on the fitting approach. Supp. Figs. 2–4 include the parameter correlation estimated from the 1000 simulated experiments according to each fitting method. Please note that $\log N_0$ and $\log N_{max}$

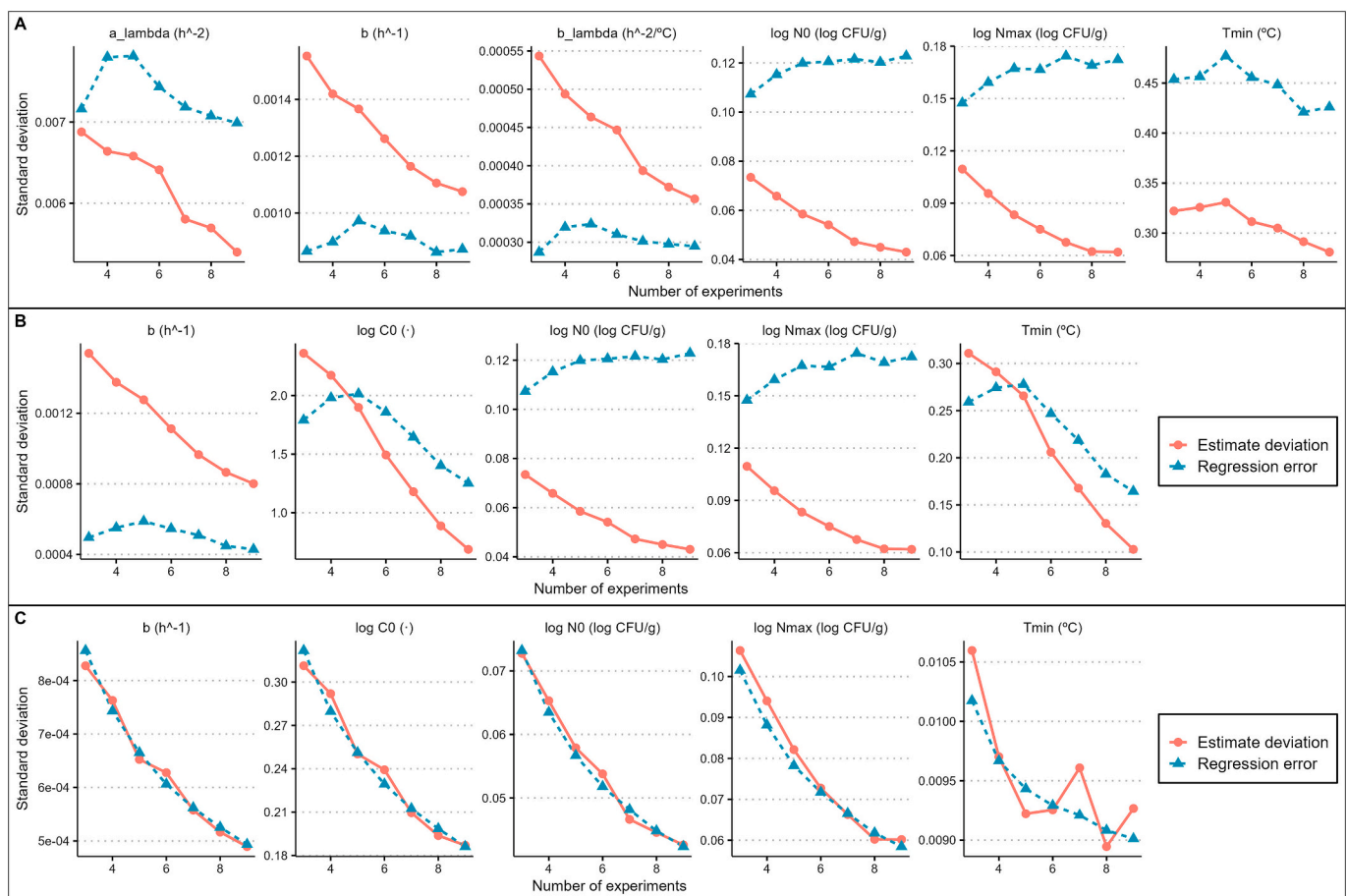


Fig. 2. Estimates of parameter uncertainty for models fitted using the conventional approach (A), using a two-steps approach accounting for coupling (B) and using a one-step approach (C). Uncertainty is calculated as the standard deviation of the parameter value estimated from 1000 simulated experiments or the median of the regression error.

have been excluded from the figures, as they have low correlations with the other parameters. The conventional two-steps approach results in high parameter correlation (supp. Fig. 2), as is well known for the Baranyi-Ratkowsky model (Guillén et al., 2024; Rosso et al., 1993). Introducing the coupling between both secondary models does not resolve these identifiability issues (supp. Fig. 3). In fact, parameters $\log C_0$ and T_{min} have almost-perfect correlation.

The one-step fitting approach does result in a reduction in parameter correlation for parameter T_{min} , which has practically zero correlation with b and $\log C_0$. This would increase the robustness of the estimates for this parameter, especially in terms of uncertainty estimation. Moreover, this would indicate that the correlation between those parameters is a statistical artefact rather than a biological constraint, as it is dependent on the fitting approach. On the other hand, the simulations still show high parameter correlation between b and $\log C_0$, although values are comparable to those obtained from the conventional two-steps approach. Hence, the one-step model fitting approach should also be favored to the conventional and two-steps methods based on arguments related to parameter autocorrelation.

3.2. Comparison between experimental designs

3.2.1. Calculation of optimal experiment designs

The numerical results from the previous section demonstrate that one-step methods are statistically more robust than the two-step ones for fitting the Baranyi-Ratkowsky model. Therefore, considering that one-step and two-steps methods require independent OED definitions, this section focuses on the impact of different experimental designs when fitting the models using the one-step method.

Local sensitivity functions are a useful way to qualitatively assess the amount of information provided by an experimental design. In principle, samples located at highest absolute values of the local sensitivity functions contribute more towards parameter estimation than those located in lower absolute local sensitivities (Soetaert & Petzoldt, 2010). As is common in nonlinear models, the local sensitivity functions vary largely between model parameters of the Baranyi-Ratkowsky model (supp. Fig. 5). This implies that some areas of the design space are more informative than others when estimating each parameter. As a preliminary assessment, the most informative areas for $\log N_0$ and $\log N_{max}$ take place during the lag and stationary phases, in line with previous findings (Grijpsperdt & Vanrolleghem, 1999). On the other hand, the

most informative areas for T_{min} and b coincide at the transition between exponential and stationary phase, whereas the maximum for $\log C_0$ is located at the middle of the exponential growth phase.

One of the most innovative aspects of this research with respect to previous studies on OED for the Baranyi-Ratkowsky model (Grijpsperdt & Vanrolleghem, 1999; Guillén et al., 2024; del Río et al., 2024) is the consideration of a two-dimensional design space that accounts for both the storage time (primary model) and the storage temperature (secondary models). Accordingly, the OED must calculate local sensitivity functions in two dimensions. The trend observed for isothermal conditions remains when temperature changes, with the maximum of the local sensitivity functions occurring at the same relative places within the growth curve (supp. Fig. 6). It is of high importance that, under the assumptions made, the value of the maximum local sensitivity does not depend on temperature for parameters b , $\log C_0$, $\log N_0$ and $\log N_{max}$. This implies that temperature does not have a high influence on the estimation of these parameters. The only exception is parameter T_{min} , whose maximum local sensitivity increases for lower temperatures. Hence, lower temperatures are favored when it comes to estimating this parameter.

OEDs were first calculated by direct optimization of the determinant of the FIM (D-optimal design). As illustrated in Fig. 3, the OED is focused on the minimum (6 °C) and maximum (37 °C) temperatures of the design space, with the same number of experiments at each temperature. This is in line with previous studies following a similar methodology, where D-optimal designs for linear (or quasi-linear) secondary models focused on the extremes (Guillén et al., 2024; Peñalver-Soto et al., 2019; del Río et al., 2024) because these points have the highest leverage. Within each temperature, the sampling times concentrate in four areas: the beginning of the lag phase, the beginning of the exponential phase, the end of the exponential phase, and the stationary phase. This is also a common result for OEDs, which tend to focus every sample in the most informative areas (Garre et al., 2018; Grijpsperdt & Vanrolleghem, 1999; Río et al., 2024). The position of the sampling points is similar to those identified previously for the Baranyi primary model (Grijpsperdt & Vanrolleghem, 1999), being close to the optima of the local sensitivity functions (supp. Fig. 5–6). The calculations were repeated for a different number of experiments (i.e., number of temperatures), obtaining similar configurations (not shown).

Although the results of the OED are optimal from the point of view of information theory, the focus on low temperatures might be impractical.

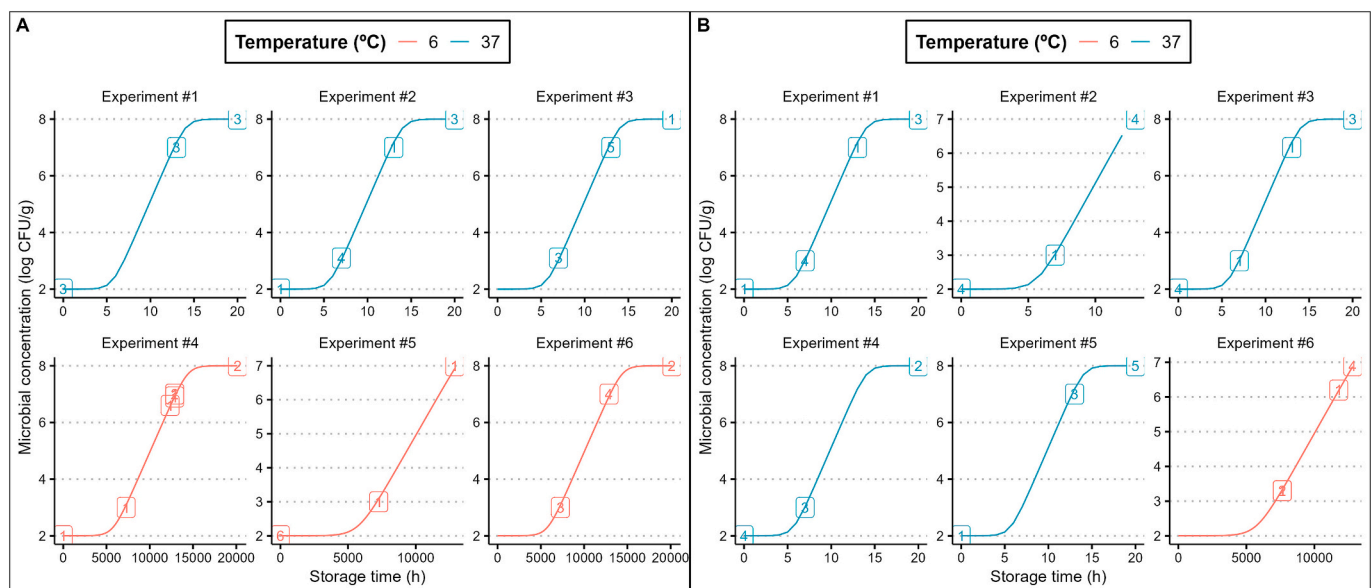


Fig. 3. Configuration for the OED (A) and OED + penalty (B) for a design with six different experiments. The color represents the temperature of the experiment, with the squares indicating the position of the optimal sampling times. The number within the square represents the number of samples to take at that time.

As shown in Fig. 3, experiments at 6 °C would require a duration of 20,000 h (833.3 days), whereas experiments at 37 °C would be completed in 20 h. Such long experiments involve additional challenges that are unaccounted for by the OED, as already discussed at length elsewhere (Guillén et al., 2024). For this reason, the OED was recalculated including a penalty function to reduce the duration of the experiments.

The resulting design is illustrated in Fig. 3B (similar configurations were calculated for a different number of experiments; not shown). By comparing the design against the original one, we can identify that the introduction of the penalty term in the optimization problem results in two strategies to reduce the aggregated duration of the experiment. First, the experimental design now favors the higher treatment temperature (37 °C), including a single experiment at 6 °C (D-optimal solutions had the same number of experiments at each extreme temperature). This strategy could be anticipated, as these temperatures are selected due to their high leverage on the secondary models for both μ and λ , therefore it is reasonable to favor one leverage point when a penalty function is implemented.

The second strategy identified by the OED + penalty optimization is more “creative”. Besides reducing the number of experiments at 6 °C, the experimental design does not build a whole growth curve at that temperature. Instead, the experiment is terminated before the transition from exponential to stationary phase. This is due to sampling points in

the stationary phase providing mostly information of $\log N_{max}$. As the experiments at 37 °C already includes enough information to estimate this parameter reliably (and it was assumed that $\log N_{max}$ was temperature-independent), the optimization algorithm omits sampling points in the stationary phase for the experiment at 6 °C, cutting the duration of that experiment almost in half. As the experiment at 6 °C requires by far the longest time, this results in a dramatic reduction in the aggregated duration of the experimental design (supp. Fig. 7).

3.2.2. Precision of the (optimal) experimental designs

Fig. 4 compares the precision of each experimental design, expressed as the expected relative error of each model parameter based on the standard error of regression (as demonstrated in section 3.1, this index is representative of parameter variability/uncertainty), with numerical values included in supp. Table 2. The results for the uniform design are as expected, with the number of growth experiments steadily increasing the precision of the parameter estimates. Nonetheless, it is worth noting that the rate of increase is parameter-dependent. Particularly, increasing the number of experiments has a relatively low impact on T_{min} . This can be related to the local sensitivity functions (supp. Fig. 5–6), that have a maximum for T_{min} at the lowest temperature. As including additional experiments in a uniform design only “fills up” intermediate points, their contribution to the estimation of T_{min} is only minor, compared to the other parameters whose maximum local sensitivity is not largely

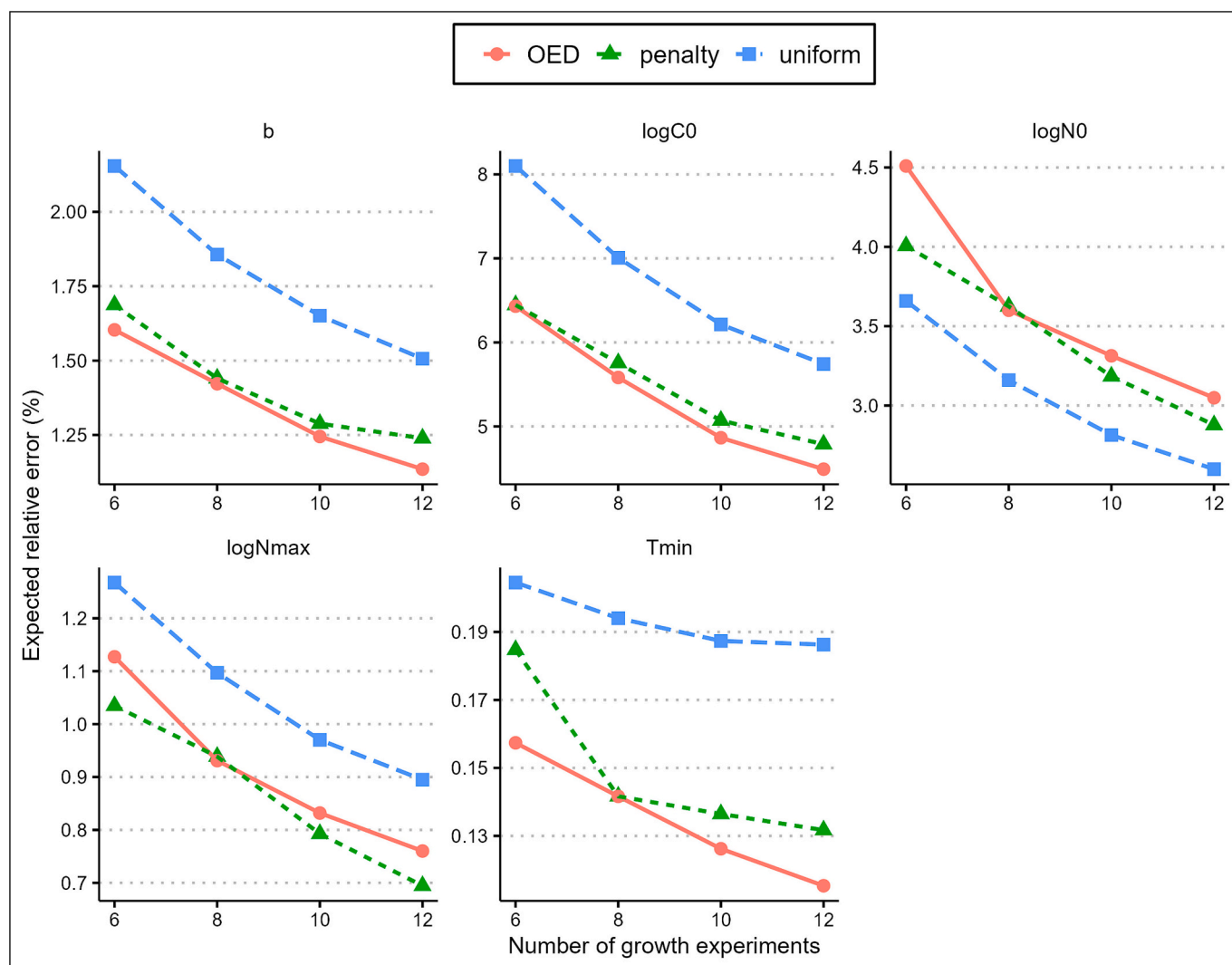


Fig. 4. Precision of each experimental design (measured as the expected relative error of 500 MC simulations for each model parameter) as a function of the number of growth experiments (i.e., potentially different temperatures) in the design.

affected by temperature.

These numerical results are in line with expert knowledge in the field, which underlines the need to perform experiments close to the growth limit for a reliable estimation of T_{min} (Pinon et al., 2004). Nonetheless, the numerical simulations presented here provide a theoretical basis for that recommendation, including quantitative estimates. More interestingly, the approach presented here provides clear suggestions on how to make growth experiments more efficient. The optimal solution calculated by OED results in an increase in the precision of most parameters (26 % reduction in standard error for b ; 21 % for $\log C_0$; 23 % for T_{min}). However, the OED actually reduces the precision of the $\log N_0$ estimate (24 % increase in standard error). This result is typical for D-optimal designs where the optimality criterion minimizes the volume of the confidence ellipsoids (Balsa-Canto et al., 2008). This can lead to situations where the overall parameter uncertainty is reduced while increasing the uncertainty of one particular parameter estimate (Guillén et al., 2024). Nonetheless, $\log N_0$ might be the less critical parameter when estimating growth curves (it is often closely controlled by the experimenter), making a reduction in its precision practically irrelevant.

Despite its reduction in parameter uncertainty, the OED requires an aggregated experiment duration much larger than the uniform one (supp. Fig. 7), making it impractical. On the other hand, the aggregated duration of the OED + penalty is comparable to the uniform design (even being shorter for the case of six experiments) due to the focus on the highest temperature (37 °C). As illustrated in Fig. 4, this has little impact on the robustness of most parameter estimates with respect to the OED. The only parameter with a noticeable lower precision is T_{min} . Note that, in this case, the precision has a sharp “jump” between six and eight experiments. This is due to the OED + penalty configuration having one experiment at 6 °C for six experiments and two between eight and twelve experiments (Fig. 3).

Despite the OED + penalty having higher parameter uncertainty for T_{min} than the OED, the OED + penalty design provides substantially more robust parameter estimates compared to the uniform design (besides the lower precision in $\log N_0$ already discussed). Therefore, considering that the aggregated time of the uniform and OED + penalty designs are comparable (supp. Fig. 7), it can be concluded that the OED + penalty approach provides an efficient method for the definition of experimental designs for the Baranyi-Ratkowsky growth model.

3.3. Practical recommendations based on the numerical results

Scientists often see standard errors as a nuance parameter that must be minimized. However, that view is not entirely applicable to predictive microbiology, where the standard error of the microbial concentration observed experimentally is partly a reflection of the inherent variability of the microbial response. This includes biological sources of variability, such as within-strain and between-strain variability (Aryani et al., 2015; Aspidou & Koutsoumanis, 2020; Koyama et al., 2025), as well as other sources such as the impact of the variability in the composition of food batches (Verheyen et al., 2019). The final goal of predictive microbiology is predicting the actual microbial response within the food supply chain (Ross et al., 2014). Therefore, as variability is an integral part of the response, models should not be judged just by their ability to predict the expected response, but also by their ability to describe variability.

A main challenge is posed by the fact that standard errors are not just the result of variability. Experimental error (understood not just as mistakes, but every type of technical limitation/simplification) is unavoidable in empirical studies (Box et al., 2005). Accordingly, the variation observed in microbial growth experiments is a combination of inherent variability and experimental errors (often called “uncertainty” in the field to clearly separate it from variability). The challenge of predictive microbiology is thus to reduce the contribution of uncertainty, so the standard error of regression mostly represents variability (Garre, Zwietering, & van Boekel, 2022). This is most often done by

increasing the amount and/of quality of experimental data. This study demonstrates that there is an alternative approach for reducing uncertainty: combining more informative experimental designs with robust model fitting strategies.

This study clearly demonstrates that one-step fitting methods are superior to two-step ones from a statistical standpoint because this approach reduces dispersion in parameter estimates. This result is aligned with the conclusions of previous studies (Cattani et al., 2016; Dolan et al., 2007; Huang, 2020), advancing the state of the art by demonstrating that the standard error of regression for one-step models accurately represents the variability/uncertainty of parameter estimates (unlike two-steps approaches). This result had not been reported previously and presents an important step forward towards improving variability and uncertainty estimation for the microbial response.

Nonetheless, one-step methods also have limitations with respect to two-step ones. One-step methods do not explicitly check for the validity of secondary models (i.e., eqs. 2 and 4) because secondary models are fitted directly from $\log N$. This increases the risk of inadvertently fitting a secondary model that is not suitable for the microbial response, something that is less likely in two-step approaches where secondary models must be defined independently (Georgalis et al., 2023). We consider that this check is important, as one cannot ensure the validity of the Baranyi or Ratkowsky models due to their empirical nature (Le Marc et al., 2002). Hence, our recommendation is to first explore the data using a two-step approach. Once the validity of the primary and secondary models has been ensured, the models should be fitted again using the one-step method considering the link between the secondary models for μ and λ , resulting in five parameter estimates (Garre et al., 2025). These values should be reported as “the true model”, including their standard error of regression as estimates of variability/uncertainty.

In terms of experimental design, OEDs make two clear recommendations: (1) to select extreme temperatures (one close to T_{min} and one close to T_{opt}) and (2) to use sampling times near the transition between growth phases (lag/exponential; exponential/stationary), as well as at $t = 0$ and in the stationary phase. However, two main challenges remain. The first one is that experiments at the lowest temperatures require extremely long times. The OED + penalty showed that the total experimental time can be dramatically reduced by two strategies: (1) lowering the number of experiments at the lowest temperature and (2) taking only samples during the lag and exponential phase for the experiments at lower temperatures. The second challenge is related to the position of the transition between phases, as this is not known beforehand (if we did know, experiments would not be required). Therefore, an iterative approach, where the experimental design is refined as more data is available, is still required (Vilas et al., 2018).

Considering these challenges, as well as the OED recommendations, we propose a staggered experimental design that is recursively updated over three weeks for fitting the Baranyi-Ratkowsky model. As illustrated in Fig. 5 for a simulated dataset, on Week #0 samples would be incubated at four different temperatures: optimal growth conditions (e.g., 37 °C), one medium-high condition (e.g., 21 °C), one medium-low condition (e.g., 15 °C) and one close to the growth limit (e.g., 7 °C). After one week, the experiments at 37 and 21 °C would have produced a complete growth curve. Although the experiment at 15 °C might not have reached the stationary growth phase, the OED + penalty showed that this data can already be highly informative. Therefore, after one week, there would be enough information to fit a preliminary Baranyi-Ratkowsky model, with the experiment at 21 °C being used to verify the secondary models for μ and λ .

The OED identified that sampling times near the transition between growth phases are more informative. This was not considered in the experiments at Week #0, which used a uniformly distributed sampling scheme for convenience. Nonetheless, the preliminary models obtained from this data can be used to further refine the sampling scheme. Accordingly, a second repetition of the experiments at 15 and 37 °C would be performed on Week #2, focusing on the transition areas. In

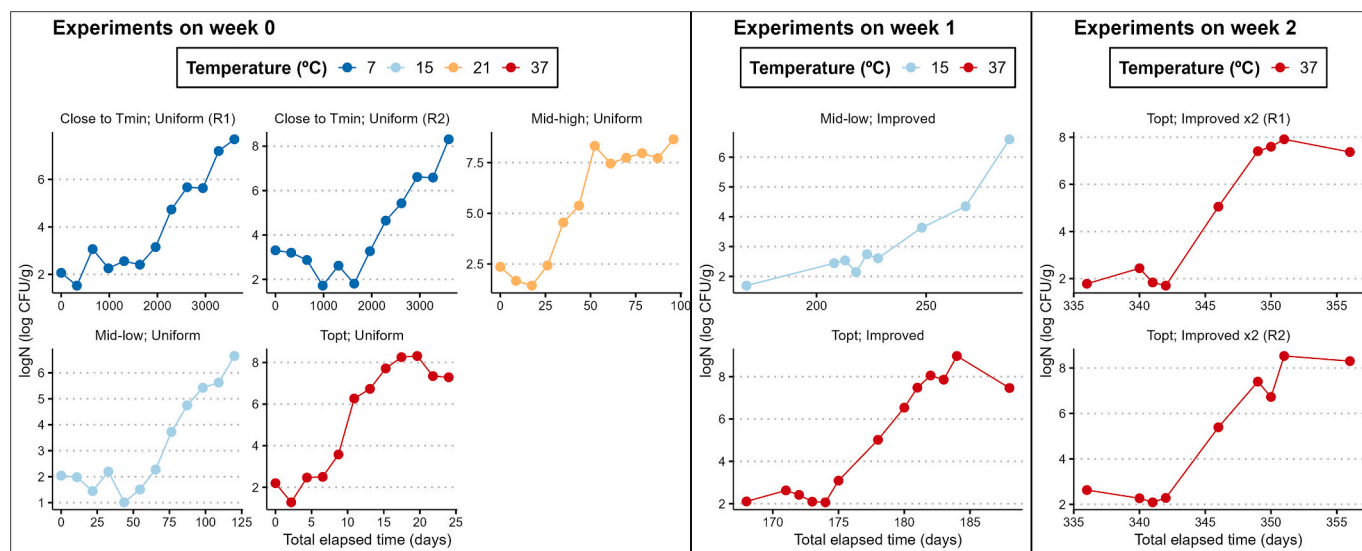


Fig. 5. Illustration of the proposed staggered experimental design, where the sampling scheme is iteratively improved over three weeks based on OED recommendations.

this second experimental batch, no experiment would be performed at 21 °C, as this data point is mostly used for verification of the secondary model. By the end of Week #2, the experiments at 15 °C would be completed (as the stationary phase is not required), together with the one at 37 °C. The results of the latter would be used to further refine the position of the time points for the last two replicates of this experiment on Week #3. Hence, after three weeks, the biological replicates of the experiments at 15 °C (2 replicates), 21 °C (1 replicate) and 37 °C (4 replicates) would be completed. Even if the experiment at 7 °C still remains in the lag phase (i.e., it does not provide information), the partial dataset obtained already provides preliminary information on the model parameters.

Fig. 6 illustrates the evolution of the parameter estimates and their

uncertainties as a function of the number of weeks for this experimental setting using a simulated data set. After 2 weeks, the model has already converged for parameters $\log N_0$, $\log N_{max}$ and b . This is reasonable, as the two microbial concentrations are parameters for the primary model and b is the slope of the Ratkowsky model (and there are experiments at several temperatures). However, the estimate of T_{min} and $\log C_0$ are highly uncertain, due to these parameters being related to the effect at low temperatures. As the weeks progress, the estimates of these parameters are improved, as data points from the experiment at 7 °C become available. This iterative approach provides a useful way to define the total duration of the experiment, as the one where the estimates of T_{min} and $\log C_0$ (as well as their standard errors) have converged. This provides a more robust indication than traditional

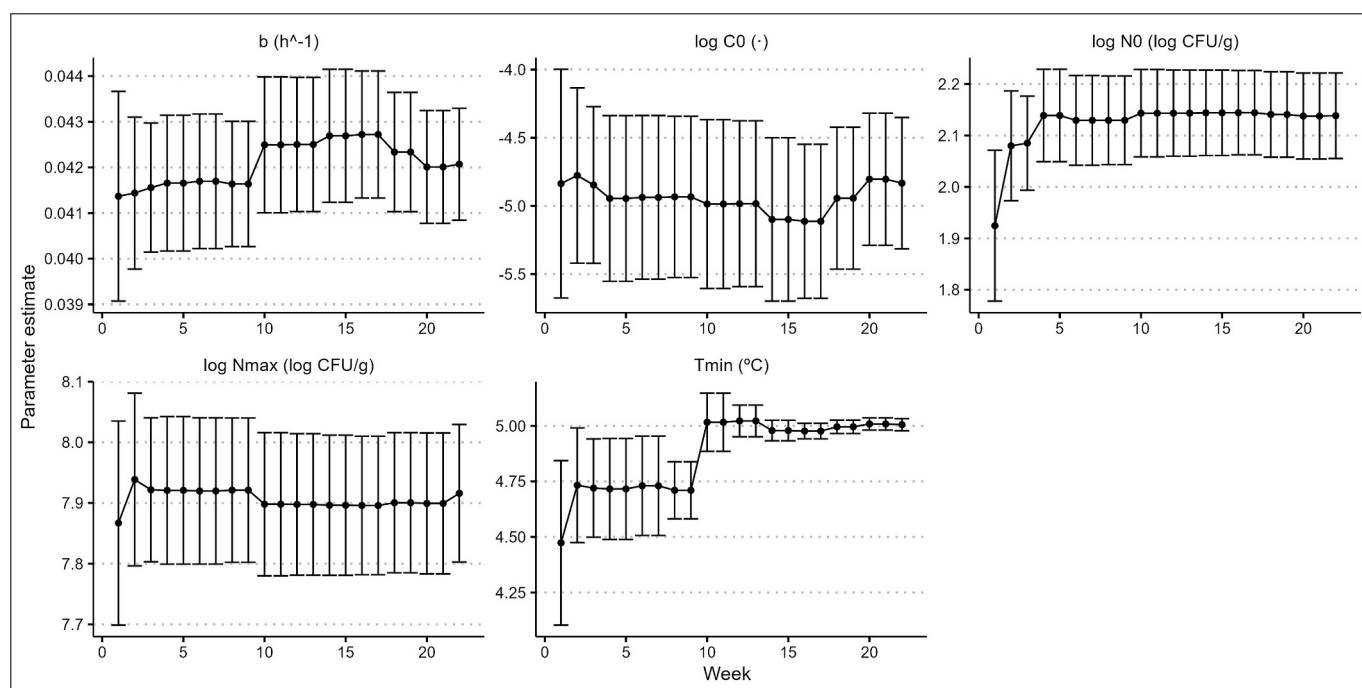


Fig. 6. Illustration using simulated data of the changes in parameter estimates (dots) and standard errors (bars) for the Baranyi-Ratkowsky model using the staggered experimental design in Fig. 5. The x-axis illustrates the improvement of the models as more data is available through the weeks.

approaches, where the experimental duration would be defined beforehand and there is no information on convergence.

3.4. Limitations and future work

As mentioned in the introduction, this study focused on model fitting from isothermal growth experiments, as this remains the most common experimental approach in the field. However, primary and secondary models can also be estimated by dynamic fitting from data obtained under varying temperature conditions (Garre et al., 2023; Huang, 2017a). This approach is motivated by the assumption that dynamic conditions would present a more efficient exploration of the design space, as each experiment combines several temperatures rather than one. However, it also requires more complex experimental and statistical methods, so its application is mostly restricted to research, as evidenced by ISO 23691 including only recommendations for model fitting from isothermal experiments. Therefore, this study was limited to the reference methodology because its main goal was to provide guidelines to a broad audience.

Besides the limitations mentioned above about the practicality of OEDs, this study also has limitations related to model assumptions. The first assumption that differs from the conventional approach is the introduction of an inverse square root secondary model for λ that is linked to the secondary model for μ (Eq. 4). Nevertheless, this is a direct implication of the hypotheses of the Baranyi and Ratkowsky models (Garre et al., 2025). Therefore, it cannot be questioned without challenging the basic assumptions of those models.

On the other hand, the hypotheses regarding N_{max} can indeed be questioned. The modelling approach introduced here assumes that this parameter is independent from temperature. This must be seen as a required simplification, as there is evidence for microbial concentration at the stationary phase being affected by the environmental conditions (Rees et al., 1995). However, one-step fitting requires the definition of secondary models for every model parameter (or the assumption that they are constant). As there are no broadly accepted secondary models for the relationship between N_{max} and temperature, considering this parameter to be constant was a required simplification. At this point, it must be noted that this simplification is very common in the field. For instance, dynamic fitting methods mentioned above do not account for any history effect when modelling N_{max} (i.e., they fit the Baranyi model in differential form without any modification). Accordingly, we believe this simplification to be reasonable considering the state of the art. Even in the worst case where N_{max} had a strong temperature-dependence, estimates of this parameter have very little influence on the estimates for b , T_{min} or C_0 , due to their low parameter correlation. As these parameters are often more relevant for QMRA or shelf life estimation, the practical impact of deviations from this assumption would be mostly minor.

A possible criticism to the guidelines provided in section 3.3 is their rigidity. However, they should be seen as guides, not as rules. They intend to translate the results of the OED into practical recommendations to obtain experimental designs that are as informative as possible with the lowest experimental load. For that reason, it suggests an iterative approach to allow the flexibility to update the experimental design as data becomes available and models are updated. In this sense, replicates at various conditions should be tested for outliers, repeating experiments when required (rather than waiting until the experiment at the lowest temperature is completed). Also, temperature values (7, 15, 21 and 37 °C) were suggested based on the typical biokinetic range of most bacteria and their industrial relevance. Nevertheless, it should be adapted when studying species that deviate from this range (e.g., *Campylobacter* spp.) or when other temperature values are of particular relevance. It could also be interesting to include additional intermediate temperatures, especially in cases where there are doubts regarding the validity of the Ratkowsky model.

Another possible challenge for the application of this approach is the

requirement of more complex statistical methods. However, the availability of Open Access packages (Garre et al., 2023) and web applications (<https://foodlab-upct.shinyapps.io/biogrowth4/>) that already implement these approaches practically makes the calculations trivial (Possas et al., 2022). Another limitation is the requirement of initial guesses for the model parameters to design the OED. Nonetheless, this limitation also applies to the selection of time points and dilutions for conventional methods. In any case, the scientific literature/historical data often provides reasonable guesses, which can be updated iteratively (Vilas et al., 2018) leveraging the staggered experimental design.

Finally, this study puts the focus on experimental designs for parameter estimation. It is generally acknowledged in the field that those parameters should be taken with care, so an external validation under conditions as close to industry as possible should always be performed (Mejlholm et al., 2010; Oscar, 2005; Tarlak & Pérez-Rodríguez, 2021). This involves additional challenges, especially considering that dynamic conditions might result in microbial responses that cannot be observed under isothermal conditions (Antolinos et al., 2012; Georgalis et al., 2022). Therefore, following the guidelines proposed here for model fitting does not exempt models for independent validation.

4. Conclusions

It is broadly accepted that uncertainty can be reduced by gathering more and/or better data. This study provides clear recommendations on how to get “better data”, underlining that using more informative experimental designs can improve parameter estimation (without requiring additional data points). Namely, for the Baranyi-Ratkowsky model, extreme temperatures should be favored (for practicality, especially those close to optimal growth conditions) and time points close to the transition between growth phases. This strategy should be combined with one-step fitting approaches, using the standard errors of regression as estimates of variability/uncertainty.

Being able to reduce uncertainty by using better designs and algorithms is of great importance for the field, as it does not require any modification to experimental (“wet”) approaches. The only requirement is the use of more complex numerical (“dry”) methods. Considering that Open Access software applications already implement these methods, this limitation is very minor compared to its potential to improve the overall robustness of predictive microbiology models.

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CRedit authorship contribution statement

Alberto Garre: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Conceptualization. **Theocharia Tsagkaropoulou:** Writing – review & editing, Visualization, Investigation. **Silvia Guillén:** Writing – review & editing, Visualization, Methodology, Conceptualization. **Kimón Andreas G. Karatzas:** Writing – review & editing, Validation, Supervision, Conceptualization. **Alfredo Palop:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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