International Journal of Food Microbiology 413 (2024) 110604

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Optimal experimental design (OED) for the growth rate of microbial populations. Are they really more "optimal" than uniform designs?

Silvia Guillén [a,b], Aricia Possas [c], Antonio Valero [c], Alberto Garre [a,*]

[a] Department of Agronomical Engineering & Institute of Plant Biotechnology, Universidad Politécnica de Cartagena, Murcia, Paseo Alfonso XIII, 48, 30203, Spain
[b] Departamento de Producción Animal y Ciencia de los Alimentos, Instituto Agroalimentario de Aragón - IA2 - (Universidad de Zaragoza-CITA), Zaragoza, Spain
[c] Departamento de Bromatología y Tecnología de los Alimentos, UIC Zoonosis y Enfermedades Emergentes ENZOEM, ceiA3, Universidad de Córdoba, Campus Rabanales, 14014 Córdoba, Spain

ARTICLE INFO

Keywords:
Parameter estimation
Predictive microbiology
Simulations
Penalty criterion

ABSTRACT

Secondary growth models from predictive microbiology can describe how the growth rate of microbial populations varies with environmental conditions. Because these models are built based on time and resource consuming experiments, model-based Optimal Experimental Design (OED) can be of interest to reduce the experimental load. In this study, we identify optimal experimental designs for two common models (full Ratkowsky and Cardinal Parameters Model (CPM)) for a different number of experiments (10–30). Calculations are also done fixing one or more model parameters, observing that this decision strongly affects the layout of the OED. Using in silico experiments, we conclude that OEDs are more informative than conventional (equidistant) designs with the same number of experiments. However, OEDs cluster the experiments near the growth limits (X_{min} and X_{max}) resulting in impractical designs with aggregated experimental runs ~10 times longer than conventional designs. To mitigate this, we propose a novel optimality criterion (i.e., the objective function) that accounts for the aggregated time. The novel criterion provides a reduction in parameter uncertainty with respect to the conventional design, without an increase in the experimental load. These results underline that an OED is only based on information theory (Fisher information), so the results can be impractical when actual experimental limitations are considered. The study also emphasizes that most OED schemes identify where to measure, but do not give an indication on how many experiments should be made. In this sense, numerical simulations can estimate the parameter uncertainty that would be obtained for a particular experimental design (OED or not). These results and methodologies (available in Open Code) can guide the design of future experiments for the development of secondary growth models.

1. Introduction

The ability to predict the growth of microbial populations using mathematical models is of great interest in food microbiology. To mention just a few examples, microbial growth is the basis of many food fermentations (van Rijswijck et al., 2019) and shelf-life estimation (García et al., 2015; Koutsoumanis et al., 2021). However, the molecular mechanisms that define microbial growth are highly complex and not yet fully understood (Notebaart et al., 2018), making the development of mechanistic models impossible in most industrial applications. Consequently, applied studies are based on empirical models fitted to data obtained under laboratory conditions mimicking production, distribution and storage conditions as closely as possible (Pinon et al., 2004). The cost of these experiments can be substantial, requiring specific laboratory equipment and consumables, trained personnel and long running times (especially near the growth limits of microorganisms).

Model-based Optimal Experimental Design (OED) can be an effective way to reduce the experimental load required for estimating the parameters of growth models. It is a general methodology that uses results from information theory (Fisher information) to identify the most informative sampling conditions for a given model (Banga and Balsa-Canto, 2008). Several studies have used this methodology to rationalize experimental designs for parameter estimation in predictive microbiology, for modelling inactivation (Cunha et al., 1997, 1998; Frías et al., 1998; Garre et al., 2018b, 2019; Peñalver-Soto et al., 2019; Van Derlinden et al., 2010) or growth (Grijspeerdt and Vanrolleghem,

* Corresponding author.
E-mail address: alberto.garre@upct.es (A. Garre).

https://doi.org/10.1016/j.ijfoodmicro.2024.110604
Received 20 June 2023; Received in revised form 29 November 2023; Accepted 21 January 2024
Available online 27 January 2024

1999; Longhi et al., 2017; Mertens et al., 2012; Van Derlinden et al., 2013, 2008; Van Derlinden and Van Impe, 2012; Versyck et al., 1997).

One main limitation of model-based OED is that the solution is calculated considering only statistical aspects (namely, the local sensitivity functions and the properties of the Fisher Information Matrix). This can result in designs that are impractical when considering practical experimental limitations. For instance, Garre et al. (2018a,b) obtained optimal designs for dynamic microbial inactivation with a distance between time points too small to be applied in the laboratory. Consequently, these authors modified the OED approach introducing a penalty function. A second important limitation of OEDs is that they identify the most informative areas of the design space (e.g., the most informative experiments or sampling points). However, this method is generally not able to indicate how many experiments in total are required to reach a minimum level of precision for the parameter estimates (e.g., standard error).

In this study, we critically analyse the OED methodology for the estimation of parameters of secondary growth models that describe the relationship between the storage temperature and the growth rate during the exponential phase. We expand upon previous studies with a similar scope (Mertens et al., 2012; Van Derlinden et al., 2013) by calculating the OEDs for a different number of growth experiments and applying a numerical simulation scheme to predict the parameter uncertainty of each experimental design. On top of that, we also consider the feasibility of the designs, combining Fisher information and practical knowledge to compare them against a conventional design where sampling conditions are uniformly distributed (uniform design). The calculations were done for two secondary growth models commonly used to describe microbial growth in predictive microbiology: the Cardinal Parameter Model (CPM; also called Cardinal Temperature Model with Inflection) (Rosso et al., 1995) and the full Ratkowsky model (Ratkowsky et al., 1983).

2. Materials and methods

2.1. Secondary models for microbial growth

The CPM describes the relationship between the growth rate during the exponential growth phase (μ; considered in log CFU/h [where "log" represents the decimal logarithm] although the conclusions are also valid when this parameter is defined in terms of the natural logarithm) and an environmental factor, X (e.g., temperature), as shown in Eq. (1) using four parameters. It assumes that below a minimum value of X (X_{min}), the microorganism is not able to grow. In a similar way, according to this model there is no growth when X is greater than X_{max}. This model also introduces the value of X where the growth rate is maximum, X_{opt}, with the maximum growth rate being defined by μ_{opt}. Besides the so-called cardinal parameters (X_{min}, X_{opt} and X_{max}), the CPM includes parameter n, often called the "order of the model", that describes the curvature of the relationship. This parameter is most often not directly estimated from the data, being assigned an integer value instead.

$$\begin{aligned}
&\mu(X) = 0; X \le X_{min}\\
&\mu(X) = 0; X \ge X_{max}\\
&\mu(X) = \mu_{opt}\frac{(X - X_{max})(X - X_{min})^{n}}{(X_{opt} - X_{min})^{n-1}\left((X_{opt} - X_{min})(X - X_{opt}) - (X_{opt} - X_{max})\left((n-1)X_{opt} + X_{min} - nX\right)\right)}; X \in (X_{min}, X_{max})
\end{aligned} \tag{1}$$

Eq. (2) defines the full Ratkowsky model. This model also introduces the maximum and minimum values of X allowing growth (X_{max}; X_{min}). However, instead of defining X_{opt} and μ_{opt}, it introduces parameters b and c that describe the slope of the relationship between $\mu(X)$ and X for values above or below X_{opt}. Besides convenience, this model is written in terms of $\sqrt{\mu}$ because it is often accepted that this transformation stabilizes the variance (McMeekin et al., 2013).

$$\sqrt{\mu}(X) = b(X - X_{min})\left(1 - e^{c(X - X_{max})}\right) \tag{2}$$

Note that, in this model, the value of X_{opt} can be calculated by the identity shown in Eq. (3), where W_n is the Lambert W function (Garre et al., 2023).

$$X_{opt} = \frac{W_n(e^{-cX_{min}+cX_{max}+1}) + cX_{min} - 1}{c} \tag{3}$$

2.2. Optimal Experimental Design based on the Fisher Information Matrix

Model-based Optimal Experimental Designs (OEDs) were calculated based on the Fisher Information Matrix (*FIM*), following a similar approach to previous studies in the field (e.g. Balsa-Canto et al., 2008; Garre et al., 2018b; Mertens et al., 2012). For statistical aspects regarding the methods, the reader is referred to those references and the citations therein.

The *FIM* can be calculated for an experimental design with k sampling points as shown in Eq. (4), where $\frac{\partial y}{\partial p}(X_i)$ represents the vector of local sensitivity functions with respect to parameter p evaluated at X_i. The symbol Q represents a weight matrix (in this study, and most often, an identity matrix).

$$FIM = \sum_{i=1}^{k}\left[\left(\frac{\partial y}{\partial p}(X_i)\right)^{T} \cdot Q \cdot \left(\frac{\partial y}{\partial p}(X_i)\right)\right] \tag{4}$$

The calculation of the OED is based on the optimization of a measure of the *FIM* with respect to some factors of the experimental design (location of the sampling points, temperature etc.). In this study, we optimize the temperatures of the growth experiments using two common optimality criteria: D-optimality and modified E-optimality. D-optimality consists in the maximization of the determinant of the *FIM* (Eq. (5)). Considering det(FIM) is proportional to the confidence hyperellipsoids of the model parameters, this criterion is equivalent to minimizing their volume.

$$\max_{X_i} det(FIM) \tag{5}$$

On the other hand, the modified E-optimality criterion minimizes the ratio between the maximum and minimum eigenvalues (ρ) of the *FIM* (Eq. (6)). It is equivalent to the minimization of the ratio between the maximum and minimum axis of the confidence ellipsoids (i.e. the optimum would be a sphere).

$$\min_{X_i} \frac{\rho_{max}(FIM)}{\rho_{min}(FIM)} \quad (6)$$

As shown in the results section below, the application of these criteria can result in designs optimal from the point of view of information theory but requiring impractically long experimental runs. For that reason, we introduce in this study a novel *OED + penalty* criterion, where we include a penalty term on the E-optimality (Eq. (7)).

$$\min_{X_i} \frac{\rho_{max}(FIM)}{\rho_{min}(FIM)} + \psi \cdot P(X_i) \quad (7)$$

The penalty term ($P(X_i)$) is defined as the total aggregated time of the experiments. As shown in Eq. (8), we assumed that the time required for each experiment is calculated as the time to increase the population size in six log-units (considering only the exponential phase) with a growth rate given by the secondary model (Eqs. (1) or (2), depending on the secondary model studied). The weight of the penalty term is defined by the multiplicative scaling factor, ψ (which accounts for the different units of P and the objective function of E-optimality). Then, a value of $\psi = 0$ would result in the E-optimality criterion and higher values of ψ would result in experimental designs with shorter aggregated times.

$$P(X_i) = \sum_{i=1}^{k} 6/\mu(X_i) \quad (8)$$

2.3. In-silico simulations to predict the error of parameter estimates

Although the OED identifies the most informative combination of storage temperatures for the growth experiments, it cannot predict the precision of the parameter estimates (e.g., their standard errors). For this reason, we used *in-silico* experiments to estimate the precision of the parameters for different designs, adapting a previous numerical algorithm (Garre et al., 2019). It can be divided in the following steps:

For i in 1 to $n_{experiments}$

1. Define the experimental design (i.e., the temperatures included in the experimental design)
2. For each temperature in the design (each value of X), calculate the ideal value of $\sqrt{\mu}_{ideal}$ according to the secondary model (substituting in Eqs. (1) or (2) the nominal values of the secondary model parameters).
3. Duplicate the ideal values according to the number of repetitions of the experiment.
4. Calculate the experimental error (ε) as a random value from a normal distribution with mean zero and standard deviation $\sigma_{\sqrt{\mu}}$ (defined as an input parameter).
5. Calculate the "observation" as $\sqrt{\mu_{obs}} = \sqrt{\mu_{ideal}} + \varepsilon$.
6. Fit the secondary model to the "observed" data generated in step 5.

This algorithm generates an array of models (i.e., their model parameters) of length $n_{experiments}$. The distribution of the parameter estimates and their standard errors can be used to analyse how the experimental error and the experimental designs affect the precision of the parameter estimates and their uncertainty (Garre et al., 2019). In this study, we analysed the distribution of the parameter estimates to assess the accuracy of the estimators, as well as the presence of any parameter correlation that is an artifact of the experimental error. Furthermore, we used the median of the coefficient of variation (standard error divided by estimated value) as an estimator of the parameter uncertainty for a given experimental design.

The convergence of these measures depends on the value of $n_{experiments}$. It was defined by repeating the simulations for different values of this factor until the quantities of relevance converged (i.e., there were no relevant differences if the calculations were repeated). This resulted in 8000 simulations. The seed of the pseudo-random number generator was set before performing the calculations reported here for reproducibility.

2.4. Computer implementation and numerical methods

All the calculations were implemented in R version 4.2.3 (R Core Team, 2016). Local sensitivity functions were calculated using the *sensFun* function from **FME** (Soetaert and Petzoldt, 2010), which uses finite differences. Model fitting was done using *modFit* from the same package, based on nonlinear regression using the Levenberg-Marquardt algorithm.

The optimization problem for the calculation of the OED was resolved using the scatter search optimization algorithm (Egea et al., 2010) implemented in the R package **MEIGO** (Egea et al., 2014). To avoid singularities in the calculations, the upper and lower bounds for the experimental temperature were fixed to 1 °C above and below X_{min} and X_{max}, respectively.

The model-based OED approach followed in this study requires the definition of "nominal values" for the parameter estimates. The values reported by Nunes Silva et al. (2020) for *Listeria monocytogenes* were used for the CPM ($X_{min} = -1.425$ °C; $X_{opt} = 38.17$ °C; $X_{max} = 44.36$ °C; $\mu_{opt} = 0.976$ log CFU/h; $n = 2$). For the full Ratkowsky model, we used the same values of X_{min} and X_{opt} and modified b and c to obtain values ($b = 0.027$ log CFU/h/°C; $c = 0.4$ 1/°C) that resulted in a similar secondary model. This was checked by plotting both secondary models (supp. Fig. 1) and checking the value of X_{opt} using Eq. (3). The *in-silico* experiments were done considering two repetitions of each experiment. The calculations were repeated for different values of the model parameters, obtaining similar results. The code is available from the GitHub page of one of the co-authors (https://github.com/albgarre/OED_secondary).

The coefficient weighting the penalty function for the OED + penalty method (ψ) was defined iteratively. The OED was first calculated setting $\psi = 0$, checking that the result was equivalent to the one calculated using the modified E-criterion. Then, the calculations were repeated increasing ψ, until the aggregated time of the experimental design was lower than the one of the uniform (conventional, equidistant) experimental design. This resulted in values of $\psi = 10$ and $\psi = 50$ for the CPM and full Ratkowsky models, respectively. This difference between both models is reasonable. As illustrated in Eq. (8), this coefficient weights the aggregated time against the amount of information according to the *E*-criterion. The latter is dependent on the model equation, so different models should need different weightings. Furthermore, the amount of information according to the E-criterion also depends on the parameter values. Hence, one cannot ensure that the values used here (10,50) will be suitable for other studies. Instead, the value of the weighting coefficient should be determined for each study using a method like the one used here.

3. Results and discussion

3.1. Optimal Experimental Designs (OEDs) for different optimality criteria

The OEDs calculated for the full Ratkowsky and CPM are illustrated in Figs. 1 and 2. In these figures, the y-axis represents the number of growth experiments included in the experimental design (from 10 to 30), whereas the x-axis indicates the optimal combination of growth temperatures according to the experimental design. Regardless of the optimality criteria and the number of sampling temperatures, the OED algorithm "clusters" the growth experiments at a few temperatures. Namely, depending on the condition, they are grouped in two to four temperatures. This concentration of the design in a few, very informative, conditions is a common result of model-based OED (Grijspeerdt and Vanrolleghem, 1999; Peñalver-Soto et al., 2019; Van Derlinden et al., 2008).

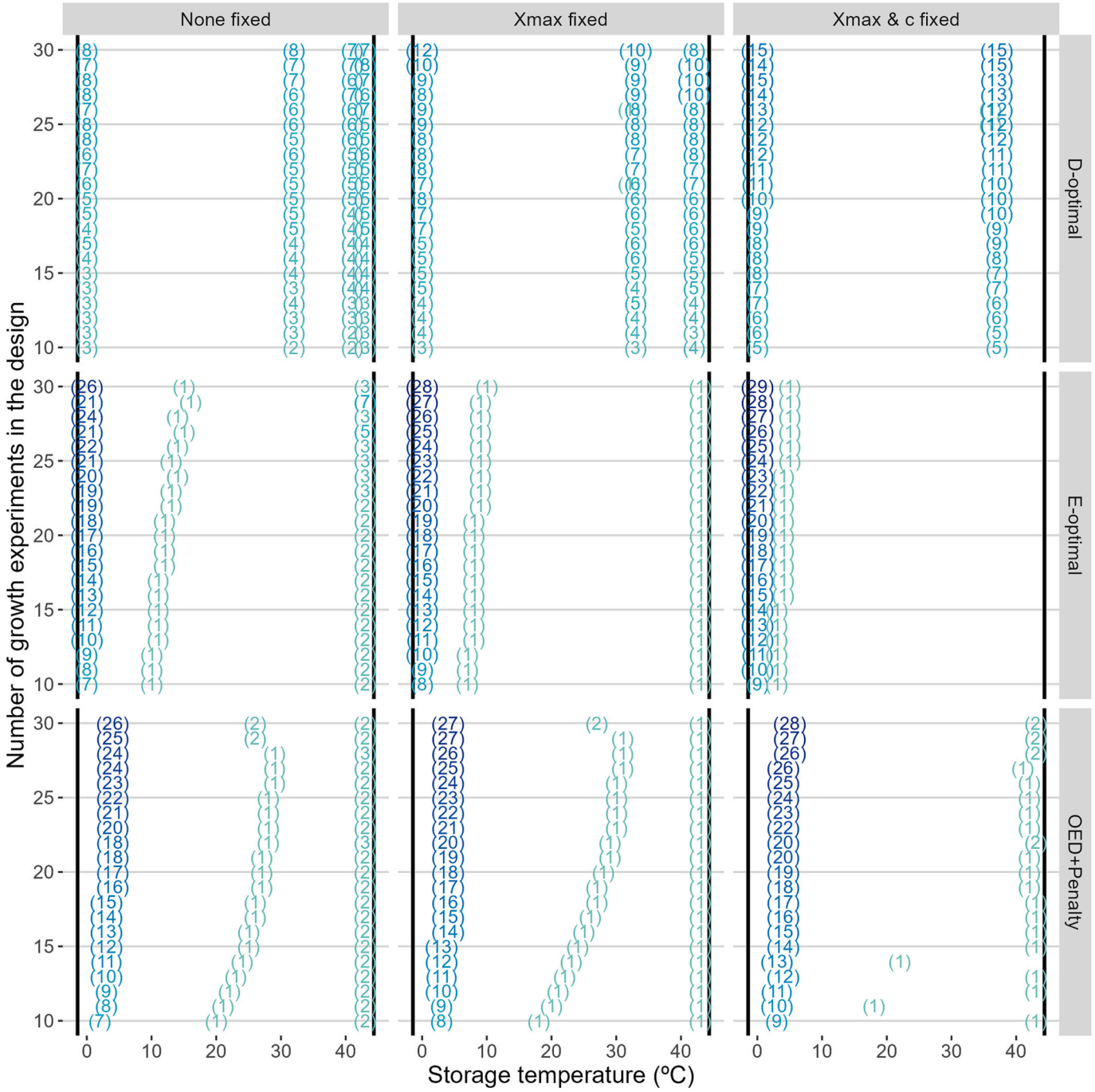

Fig. 1. Illustration of the experimental design for the full Ratkowsky model ($b = 0.027$ log CFU/h/°C; $c = 0.4$ 1/°C; $X_{min} = -1.425$ °C; $X_{max} = 44.36$ °C) according to the D-optimality criteria, the E-optimality criteria and the OED with the penalty function. The numbers within brackets indicate the number of samples at the given point, with darker labels indicating more experiments at a particular temperature. The designs were calculated fitting every model parameter, fixing X_{max} and fixing both X_{max} and c (subplots). The vertical, black lines illustrate the values of X_{max} and X_{min}.

Fig. 1 depicts the results for the full Ratkowsky model, showing that there are several similarities between the OED calculated based on the D-optimality criterion and the modified E-criterion. The most striking result is that the design is focused on temperatures close to X_{min} and X_{max}. This reflects the shape of the local sensitivity functions for the model parameters, which are practically flat except for the areas close to these limits (illustrated in Supp. Fig. 2). Nonetheless, there are also differences between the designs calculated using either criterion. The modified E-criterion includes more growth experiments close to X_{min} than to X_{max}, with only a single experiment at an intermediate temperature. On the other hand, the D-criterion results in more balanced designs with almost the same number of experiments at each temperature included in the design. Furthermore, the location of the intermediate temperature varies depending on the experimental design (~10 °C for the modified E, ~30 °C for D).

Fig. 2 illustrates the OED scheme for the CPM. The results have several similarities with the ones calculated for the full Ratkowsky model (Fig. 1). Regardless of the optimality criteria, the OED also groups the growth experiments at temperatures close to X_{min} and X_{max}, with just a few experiments at intermediate temperatures. However, unlike for the full Ratkowsky model, the OED for the CPM uses two intermediate temperatures. The first one is located near the optimum of the local

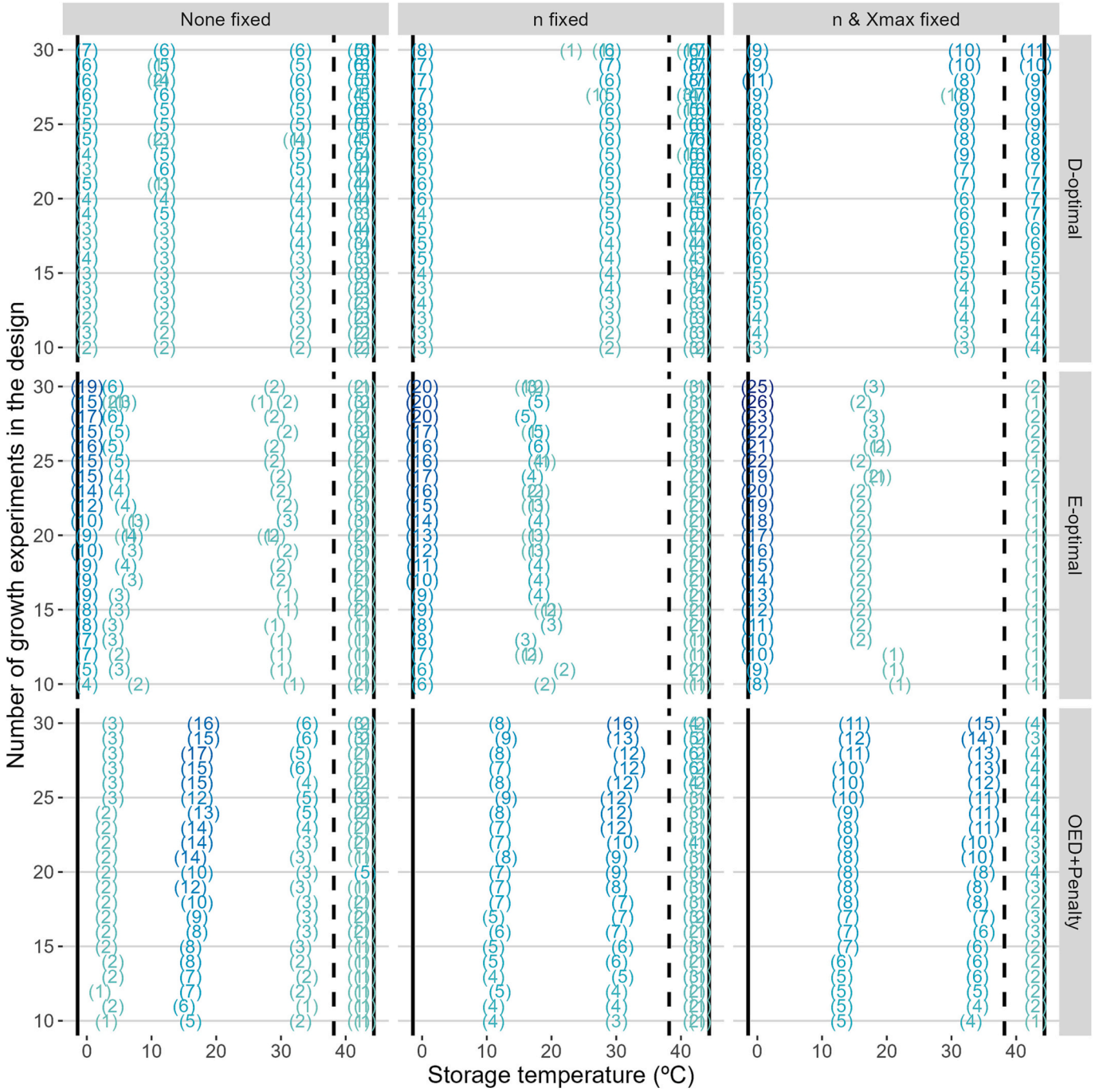

Fig. 2. Illustration of the experimental design for the CPM ($X_{min} = -1.425$ °C; $X_{opt} = 38.17$ °C; $X_{max} = 44.36$ °C; $\mu_{opt} = 0.976$ log CFU/h; $n = 2$) according to the D-optimality criteria, the E-optimality criteria and the OED + penalty. The numbers within brackets indicate the number of samples at the given point. The designs were calculated fitting every model parameter, fixing n and fixing both n and X_{max} (subplot6s). The vertical, black lines illustrate the values of X_{max} and X_{min}, whereas the vertical, dashed line represents the value of X_{opt}.

sensitivity function for parameter n (supp. Fig. 3), whereas the second one is located at the sub-optimal temperature range, close to X_{opt}. Moreover, in a similar way as in the full Ratkowsky model, the OED based on the E-optimality criterion tilts the design towards X_{min}, whereas the D-optimal criterion results in a more balanced design for the CPM.

Although these experimental designs calculated for the full Ratkowsky and CPM models are optimal from the point of view of information theory (Fisher information), there are several known issues with this approach. They are mainly because the solution of a model-based OED is only valid if the model is "true". In this case, that means that the growth of the microbial population can be described perfectly by the model equation (full Ratkowsky or CPM model), that the model parameters are correct (i.e., the nominal parameter values describe the bacterial response without any uncertainty) and that the variance behaves according to the model hypotheses (in this case, that $\sqrt{\mu}$ is homoscedastic). This is problematic because these hypotheses are hard to verify without actually doing the experimental work. These problems are aggravated by the tendency of OEDs to group sampling points in a few locations. This makes validating these hypotheses practically impossible when a design is based solely on the results on a model-based OED. For that reason, it is often recommended to combine an OED with other types of designs to ensure that the microbial response does not

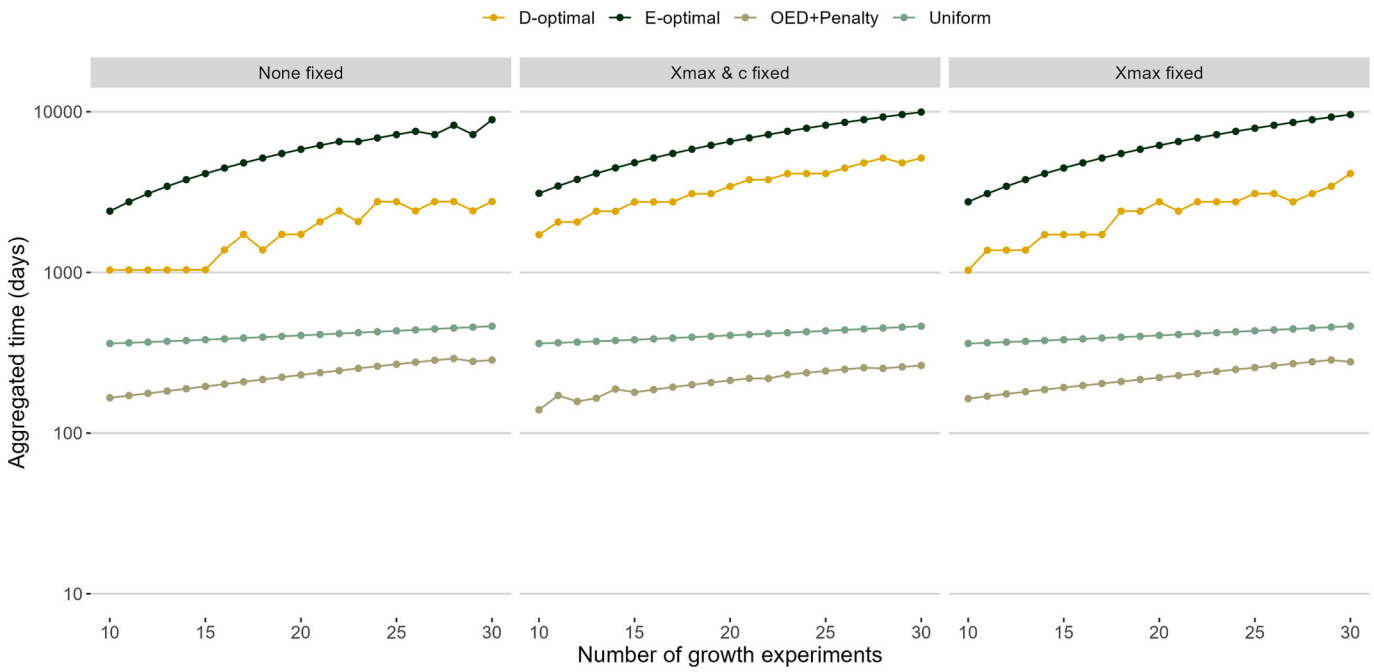

Fig. 3. Aggregated experimental time as a function of the number of growth experiments included in the design for the full Ratkowsky model for four different types of experimental designs: D-optimal OED, E-optimal OED, OED with penalty term and uniform design (colors). The designs were calculated fitting every parameter, fixing X_{max} and fixing both X_{max} and c (subplots).

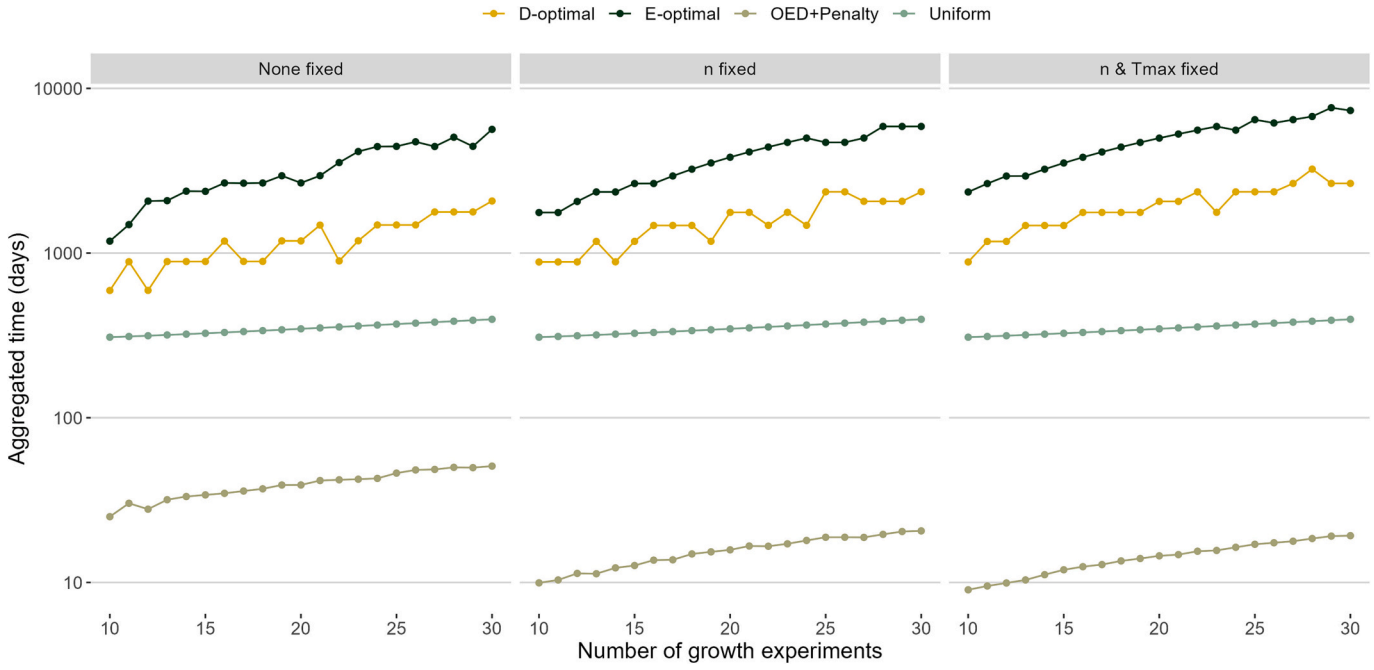

Fig. 4. Aggregated experimental time as a function of the number of temperatures included in the design for the CPM for three different experimental designs: D-optimal OED, E-optimal OED, OED with penalty term and uniform design (colors). The designs were calculated fitting every parameter, fixing n and fixing both n and X_{max}.

deviate largely from the model hypotheses (Peñalver-Soto et al., 2019).

Apart from these well-known limitations of OEDs, we also observe an additional problem that may make this type of experiment impractical for secondary growth models. As already mentioned, the OEDs cluster the conditions of the growth experiments near X_{min} and X_{max}. Considering that microbial growth in the vicinity of these temperatures is very slow (i.e., several weeks are required to reach stationary growth phase), this results in extremely long experimental runs. This is illustrated in Figs. 3 and 4, where the aggregated time of the experimental design is shown (i.e., the sum of the time required for each individual experiment). Although this is not a realistic representation of the total time required for the experiment (repetitions can be done in parallel), it serves as an assessment of the experimental load of the experimental design. For the full Ratkowsky model (Fig. 3), the uniform experimental design has an aggregated time slightly higher than 300 days when the design comprises between 10 and 30 experiments. This value is one

order of magnitude lower than the ones corresponding to the *E*-optimal (~3000 days) and D-optimal (~1000 days) designs. The E-optimal design has shown to need the most resources since the aggregated time steadily increased up to ~10,000 days. Similar results are observed for the CPM (Fig. 4).

This result is very relevant because the main purpose for an OED is to obtain similar precision in the parameter estimates with a reduced experimental effort. Although the design schemes are more informative based on the FIM, this statistical index only considers how much information is provided by the experiments; it does not account for other nuances of the experimental design. As illustrated in Figs. 3 and 4, when the experimental load is accounted for (represented as the aggregated time), the main principle of the OED may no longer be true, with the OED actually requiring more experimental effort than conventional (suboptimal) designs.

Due to the impracticality of the OEDs calculated (the excessive aggregated time), this study proposes a modified OED criterion that introduces a penalty term on the calculation, balancing amount of information and experimental load. Figs. 1 and 2 illustrate the experimental design resulting from this optimality criterion for the two secondary growth models considered. The criterion is derived from E-optimality, so it has some similarities with it, such as clustering the experimental design at a few temperatures. Nevertheless, it also shifts the sampling temperatures away from X_{min}, towards conditions more favourable for microbial growth (i.e. with higher μ), also including additional experiments at intermediate temperatures. This results in a very substantial reduction of the aggregated experimental time, especially for the CPM model. Indeed, as illustrated in Figs. 3 and 4, the aggregated experimental time is one to two orders of magnitude lower than for the conventional OEDs (~100 days for the full Ratkowsky model, ~50 days for the CPM).

On the other hand, our modified OED still suffers from some of the common limitations for model-based OEDs. The design clusters the growth conditions in a few locations (Figs. 1 & 2), making it practically impossible to test the suitability of the secondary model (model equation, nominal parameter values, and variance model) used to calculate the OED. Consequently, although the modified OED mitigates the issues of an excessive aggregated experimental time, it is advisable to combine it with other methods (e.g., uniform design) to test the suitability of the model, as generally recommended for model based OED (Peñalver-Soto et al., 2019).

3.2. Optimal Experimental Design for a reduced parameter space (fixing parameters)

The OED calculations in the previous section assumed that every model parameter was estimated from the data. However, this is often not the case when fitting secondary growth models (Muramatsu et al., 2019). A typical example is the order of the CPM (parameter n), which is often considered known (typically, 1 or 2). Alternatively, many studies are focused in sub-optimal conditions (e.g. temperatures below X_{opt}), so X_{max} (and also c in the full Ratkowsky model) would be a relatively unimportant parameter that could be fixed to an approximate value.

This reduction in the dimension of the parameter space has a strong influence in the layout of the OED for both models. In the case of the full Ratkowsky model (Fig. 1), fixing X_{max} does not have a big impact on the position of the sampling points, but it changes the distribution of the growth experiments. Namely, it reduces the number of experiments at temperatures close to X_{max}, increasing the number of experiments at temperatures close to X_{min}. If both X_{max} and c are fixed, the layout of the OED is modified both qualitatively and quantitatively. In this case, the temperatures included in the experimental design are reduced to two. Furthermore, the layout of the experiment is focused on low temperatures, close to X_{min}.

Fixing one or more parameters before model fitting also affects the OED for the CPM (Fig. 2). Fixing n removes one of the intermediate sampling temperature located close to the optimum for the local sensitivity function with respect to n. This emphasizes the association between the local sensitivity functions and the OED. It also shifts the location of the other intermediate temperature, bringing it closer to X_{opt}. Fixing X_{max}, as well as n, further influences the experimental design. Although the location of the sampling points does not vary much, the design is more focused on temperatures below X_{opt}, increasing the number of growth experiments on this area at the expense of temperatures above X_{opt}.

These results demonstrate that fixing one or more model parameters has a very strong influence in the statistical properties of a model. Fixing X_{max} and c in the full Ratkowsky model practically defines the shape of the secondary model for temperatures above the maximum temperature for growth. As a result, experiments at high temperatures provide little information on the values of the unknown model parameters, so they are avoided by the OED method. A similar situation takes place when n and X_{max} are fixed in the CPM model. This result is in line with previous studies that emphasized the risks associated with fixing model parameters before model fitting (Schmidt et al., 2019). Although this approach can facilitate the convergence of the fitting algorithm, it also changes the statistical properties of the model, so a model with fixed parameters should be considered as a novel model. This implies that the hypotheses that justify that a parameter is known (i.e., it has no uncertainty) should always be enunciated and justified in detail.

3.3. Correlations between parameter estimates as artifacts of the experimental error

Fig. 5 shows a pairs plot of the parameter estimates obtained for the full Ratkowsky model in 8000 numerical simulations for the uniform experimental design. It shows a clear correlation between parameters X_{max} and c, as well as between X_{min} and b. Although this information could be partly inferred by a correlation analysis of the local sensitivity functions (supp. Figs. 2 & 3), that type of analysis provides only limited information when compared to Monte Carlo analysis. For instance, it cannot identify bimodality in the parameter estimates, whether the correlations are nonlinear and higher order correlations (i.e., more than two parameters being related). This result had already been reported by other authors (Baranyi et al., 2017; Rosso et al., 1993), although its causes had not been clearly enunciated. Both studies pointed at a possible biological link between both parameters, with Baranyi et al. (2017) stating that "It is vital to see that Eq. (6) [a linear relationship between b and T_{min}] describes a *biological* relationship and not a regression-related correlation between the b and T_{min} parameters." In this study, we have observed this correlation between parameters b and X_{min} (as well as between c and X_{max}) by simulating a dataset that only considers a random error on $\sqrt{\mu}$ normally distributed with mean zero. That is, an empirical artifact without any biological relationship. Considering that experimental error is unavoidable in empirical studies and that parameter identifiability issues cannot be erased when fitting a model, it is highly unlikely that the correlations observed empirically are just a reflection of a biological link. Instead, they would (at least partly) be a "regression-related" artifact caused by structural identifiability issues (parameter correlation) of the full Ratkowsky model.

Regarding the CPM, Fig. 6 illustrates this correlation for a uniform experimental design fitting the model after fixing the parameter n. The numerical simulations also show parameter correlation, although the magnitude of the correlation is lower than for the full Ratkowsky model. This result is in agreement with Rosso et al. (1993), who observed higher correlations between the parameters of the full Ratkowsky model than for the CPM. This is an additional argument against the hypothesis that the relationship between b and X_{min} (or c and T_{max}) is the reflection of a biological mechanism. Parameters b of the full Ratkowsky model and μ_{opt} of the CPM are closely related (Equation (3) of this manuscript, as derived in Garre et al., 2023). Hence, if the cause of this relationship was

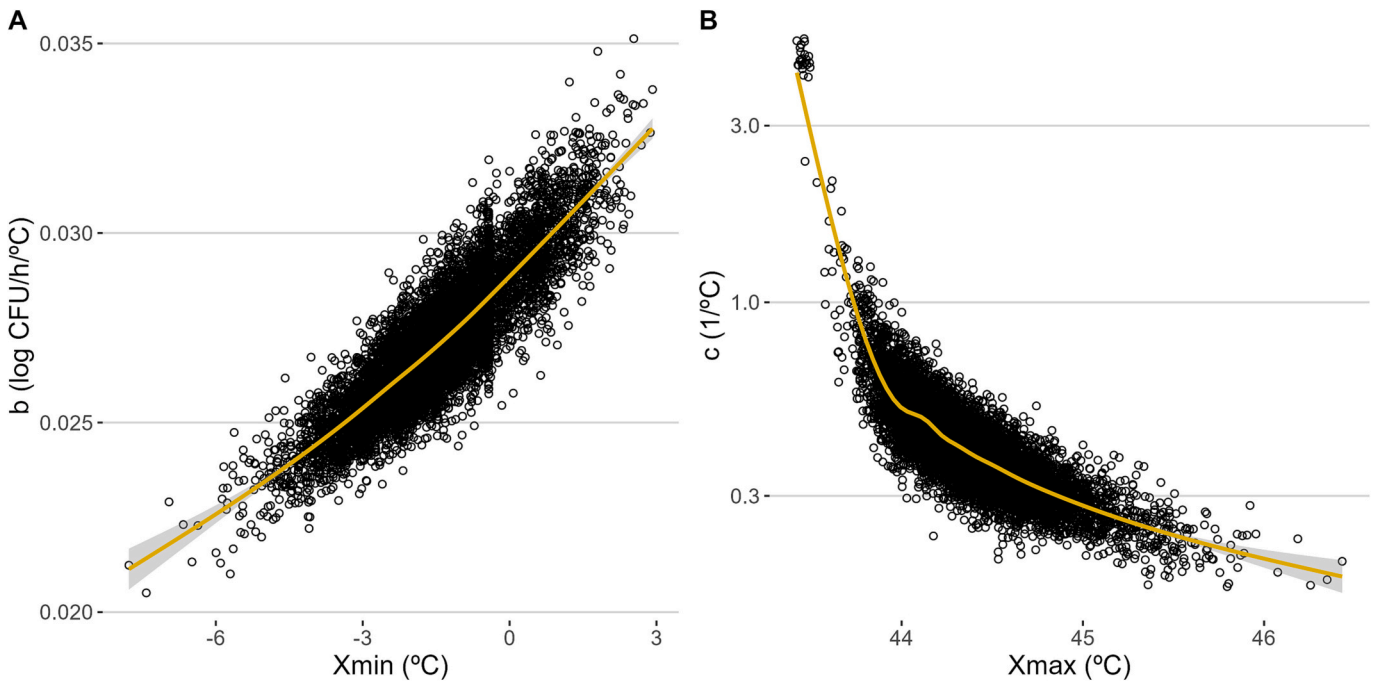

Fig. 5. Pairs plot for parameters X_{min} and b; Spearman correlation of 0.85 (A) and X_{max} and c; Spearman correlation of −0.82 (B) of the full Ratkowsky model with a uniform design with 12 growth experiments for 500 simulations. The yellow line represents a trend line calculated using local regression. Similar results were obtained for designs with a different number of experiments (not shown).

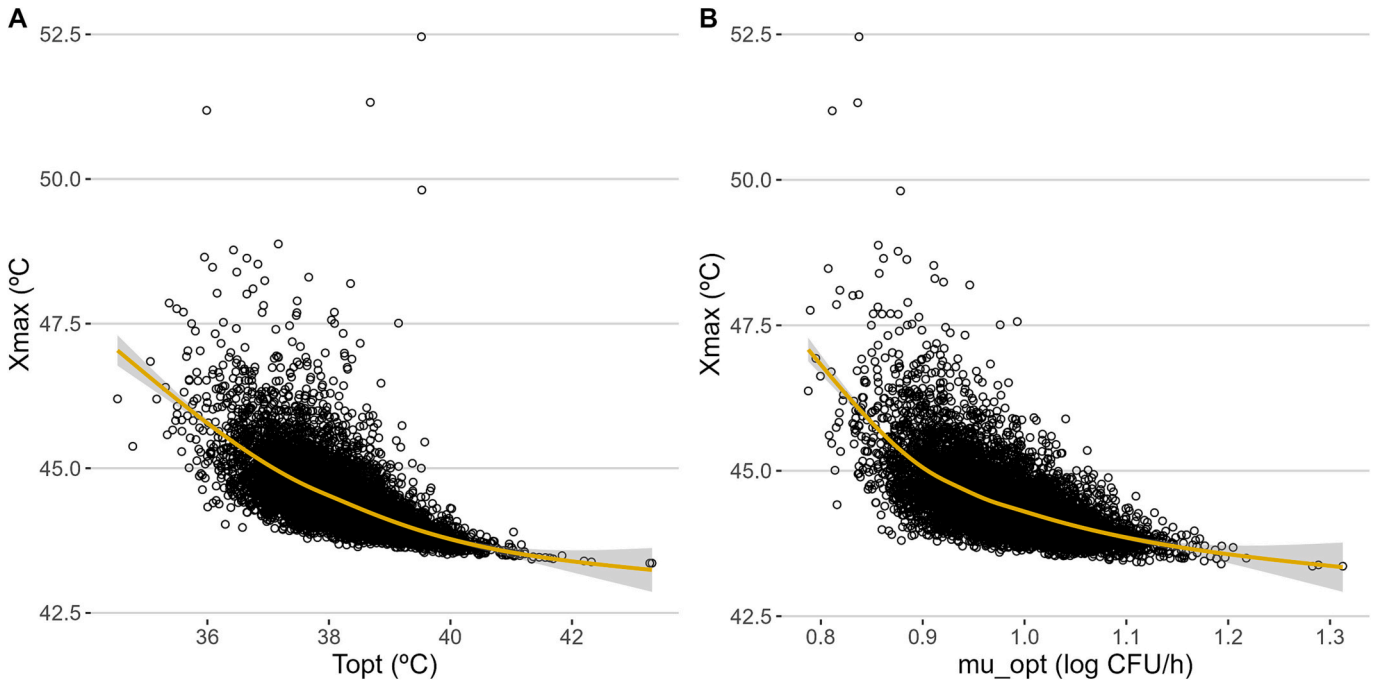

Fig. 6. Pairs plot for parameters X_{opt} and X_{max} (A), Spearman correlation of −0.72; X_{max} and μ_{opt} (B), Spearman correlation of −0.64; of the CPM when parameter n is fixed with a uniform design with 20 data points for 8000 simulated experiments. The yellow line represents a trend line calculated using local regression. Similar results were obtained for different designs (not shown).

biological, one would expect a similar one between μ_{opt} and X_{min}. The calculations were repeated considering n as a parameter to estimate from the data. The results (supp. Fig. 4) also show a high correlation between parameters n and X_{min}, and n and X_{opt}. Furthermore, estimating n from the data also results in a slight increase in the correlation between X_{max} and X_{opt}.

These differences in the correlations between model parameters also have implications for the inclusion of parameter uncertainty in growth predictions in the context of microbial risk assessment or shelf-life estimation. Currently, the most common approach is to define probability distributions for the model parameters and estimate the distribution of the output by forward uncertainty propagation (Akkermans et al., 2018; Garre et al., 2017). However, these distributions are often defined independently, without considering parameter correlation. As observed

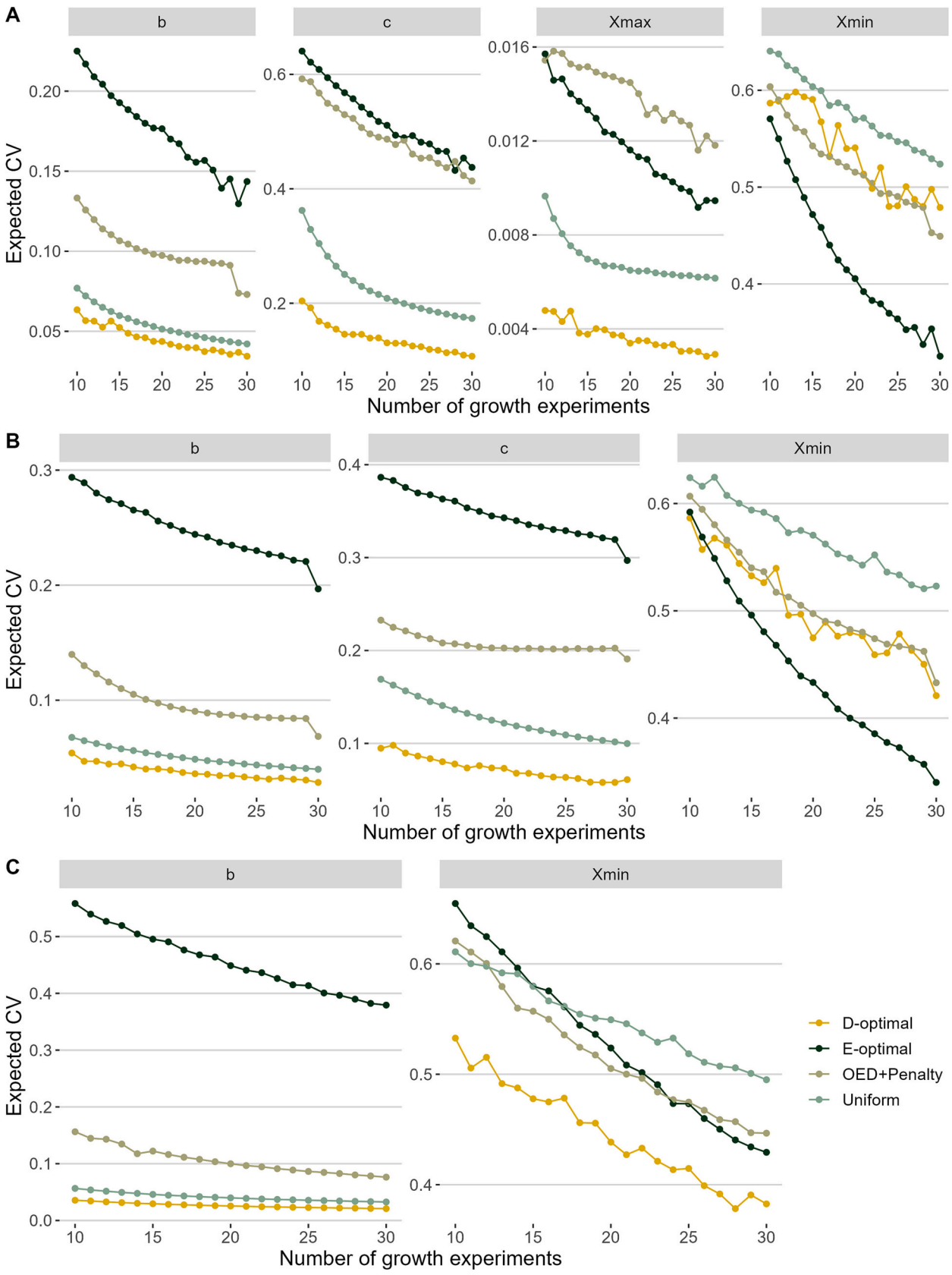

Fig. 7. Predicted precision of the parameter estimates for the full Ratkowsky model (median of 8000 MC simulations) as a function of number of growth experiments included in the design for four different types of experimental design (uniform, D-optimal, E-optimal and OED + penalty). The designs were calculated fitting every model parameter (A), fixing X_{max} (B) and fixing both T_{max} and c (C). Calculations were made assuming a standard deviation for the observed value of $\sqrt{\mu}$ of 0.08 log CFU/h and two independent replicates.

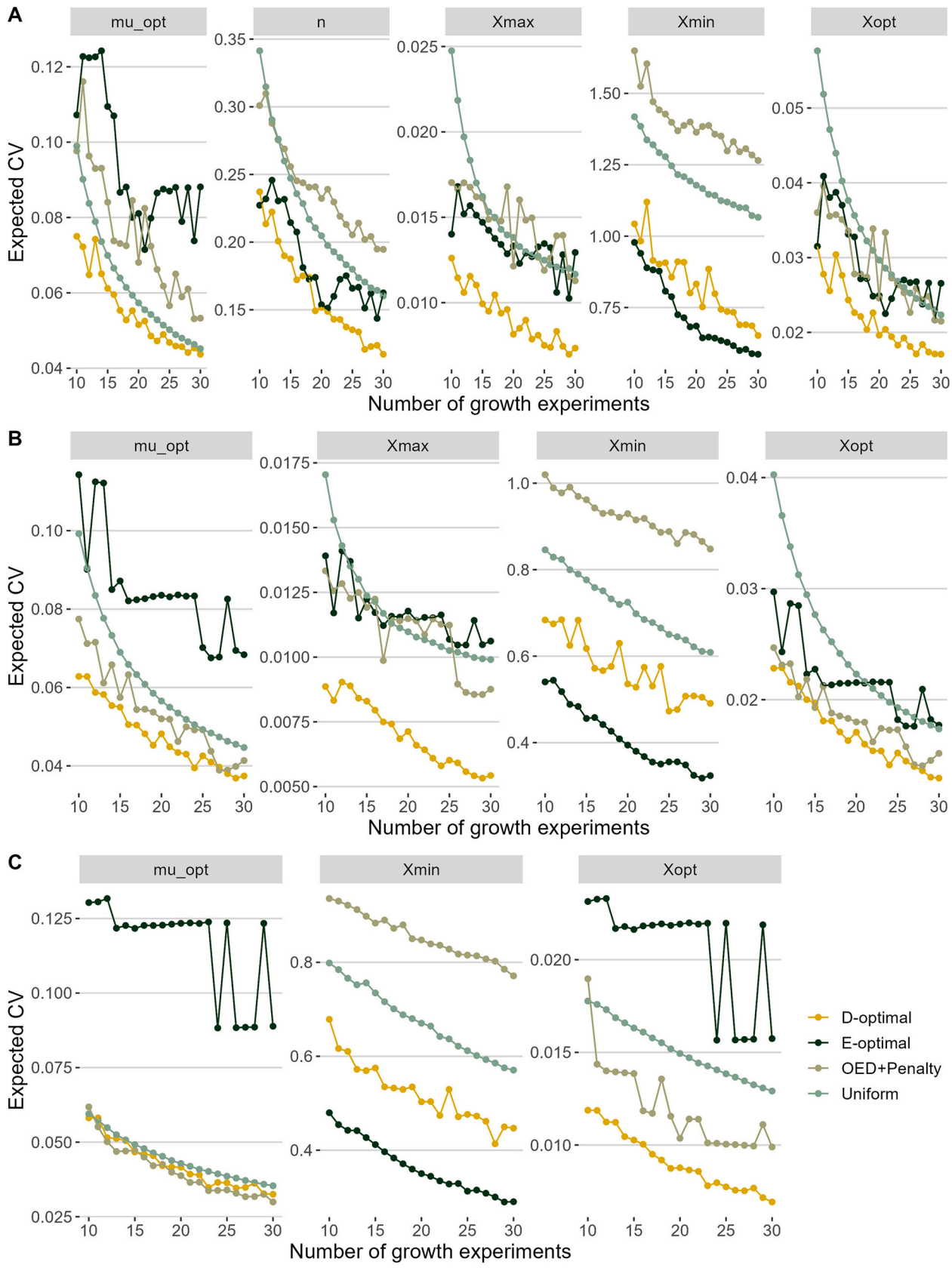

Fig. 8. Predicted precision of the parameter estimates for the CPM (median of 500 MC simulations) as a function of number of experiments (i.e. number of temperatures) included in the design for a uniform design (■), *D*-optimal design (■), *E*-optimal design (■) and OED + penalty (■). The designs were calculated fitting every model parameter (A), fixing n (B) and fixing both n and T_{max} (C). Calculations were made assuming a standard error for the observed value of $\sqrt{\mu}$ of 0.08 log CFU/h and two independent replicates.

here, these correlations are most likely a reflection of structural identifiability, so parameter uncertainty should be defined using multivariate normal distributions (or similar multivariate distributions). Furthermore, these correlations would also be model dependent, so correlations estimated using a particular model (e.g., CPM) should be considered as generally not applicable to other models (e.g., Ratkowsky).

3.4. Predicting the uncertainty of the parameter estimates

The application of OED in the previous sections has allowed the identification of the most informative growth conditions for different parameters of the design (number of growth experiments, type of OED and fixing model parameters). However, the OED methodology used there does not provide much information about the uncertainty of the parameter estimates for a given design. Hence, we used in silico experiments to complement the OED, providing further information to aid in experimental design. This allows an estimation of the uncertainty (represented as the coefficient of variation; CV) of each parameter for different experimental designs. The results are illustrated in Fig. 7 for the full Ratkowsky model and in Fig. 8 for the CPM.

As expected, an increase in the number of growth experiments (x-axis) generally reduces parameter uncertainty of every model parameter for the uniform experimental design. However, the magnitude of the reduction in uncertainty is parameter-dependent and it also varies between models and experimental designs. The results show a "law of diminishing returns" in this relationship, with the uncertainty reduction seeming to converge towards a horizontal asymptote. This is especially evident for parameter X_{max} of the full Ratkowsky model when every model parameter is fitted (Fig. 7A). This result, which was also observed for microbial inactivation models (Garre et al., 2018a), points out that parameter uncertainty cannot be reduced entirely when there is some noise in the data, especially for nonlinear models.

The results also show that some model parameters are easier to identify than others. For instance, parameter X_{max} is easier to estimate than b in the full Ratkowsky model (Fig. 7A). Although this can be assessed by comparing the local sensitivity functions, it can only be concluded quantitatively using numerical simulations (or other quantitative approaches). This result is directly applicable for experimental design, which often tries to obtain the parameter estimates with a maximum uncertainty. As shown in Fig. 7, reducing the CV of parameter X_{min} below the 50 % is practically impossible with a uniform experimental design. This result is also reasonable, because the nominal value of this parameter is $X_{min} = -1.425$ °C, so a 50 % CV is equal to a standard error of 0.7 °C (a temperature that can hardly be measured experimentally).

Although this information could be inferred from the analysis of local sensitivity functions (e.g., defining a minimum value for the local sensitivity for a given parameter), the conclusions of that type of analysis would be quite limited. First, parameter identifiability is closely linked to the experimental design (e.g., Fig. 7). Therefore, a parameter that is theoretically identifiable based on its local sensitivities may be practically non-identifiable for some experimental designs. Furthermore, analyses based on local sensitivities are often based on arbitrary thresholds (e.g., 10 %), so they do not consider how much precision is desirable for each parameter (i.e., how "critical" it is) or the precision of the experimental technique (parameters with 10 % sensitivity may be identifiable or not depending on the error of the experimental method).

Regarding the OEDs, the D-optimal design has lower CV than the uniform design in every situation. This is not the case for the *E*-optimal OED, where some model parameters have higher uncertainty than for the uniform design, whereas others have much lower uncertainty (even lower than for the D-optimal OED). This reflects the different objective functions used by both criteria. The D-optimal OED aims at minimizing the volume of the confidence ellipsoids, resulting in a "homogeneous" reduction of parameter uncertainty with respect to the uniform design. The modified E-criterion, on the other hand, tries to generate confidence ellipsoids that are as close to spheres as possible (i.e., every model parameter having the same uncertainty and no correlation). Consequently, the uncertainty of the parameters with poor identifiability might be reduced at the expense of an increase in the uncertainty of the more identifiable parameters. This result can be clearly visualized for the full Ratkowsky model when X_{max} is fixed (Fig. 7B). The modified E-criterion results in the lowest uncertainty among all the designs tested for the parameter with the poorest identifiability X_{min} and the highest uncertainty for the other two parameters (b and c). As a result, the three parameters have a similar CV, resulting in almost spherical confidence ellipsoids.

Another result that can be considered striking is that the trend lines for the OEDs illustrated in Figs. 7 and 8 have some "noise", whereas the ones for the uniform experimental design are uniform. This is likely due to the principles of the model-based OED methodology. As illustrated in Figs. 1 and 2, the OEDs algorithm identifies between two and four discrete temperatures as the most informative ones. Then, adding one experiment to the design introduces an additional experiment in one of these discrete locations. Because the local sensitivity functions for the secondary models studied (CPM and full Ratkowsky) are zero in a relatively large temperature range (e.g., X_{max} is practically zero for temperatures below X_{opt}; supp. Fig. 2), adding an additional data point results in a large reduction in the uncertainty of one parameter but not for the rest. This is not the case for the uniform experimental design, where additional experiments are added homogeneously along the temperature range, so parameter uncertainty is reduced gradually for every parameter (except in particular situations; see Garre et al., 2019).

As a final remark regarding these results, Figs. 7 and 8 illustrate that fixing some model parameters reduces the uncertainty of the parameters estimated from the data, especially for the uniform experimental design. Although this reduction in parameter uncertainty can be seen as an argument supporting fixing model parameters, this must be done with care. Due to the existence of correlations between the model parameters (Figs. 6 and 7), fixing some model parameters can introduce a bias in the ones that are fitted (Schmidt et al., 2019). Hence, as a general rule, model parameters should be fixed only when they are not relevant for the data (e.g., X_{max} for data with temperatures much lower than X_{opt}) or when there is a very strong biological basis to support this decision.

It is worth emphasizing that the results in Figs. 7 and 8 are a rough estimation of the precision of an experimental design based on very simple hypotheses regarding the noise of experimental data. Namely, we assume that residuals with respect to $\sqrt{\mu}$ will be independent draws from normal distributions with mean zero and constant variance. In this study, we have used a value of $\sigma_{\sqrt{\mu}}$=0.08 $(\log \text{CFU/h})^{1/2}$ based on literature data. However, this value will depend on several aspects of the experimental design, such as the type of experiment (e.g., plate count vs absorbance-based methods), the experimental design (e.g., number of time points) and internal sources of variability (e.g., combination of biological and/or technical replicates, variability in the media). For this same reason, we advise against the definition of a "universal standard" for this standard deviation, as it would be unreasonable to expect the same precision for a growth experiment on a liquid laboratory media (easy to control; low variability) than in a food product such as a fermented sausage (many uncontrollable sources of error; high variability). Consequently, this value should be reconsidered for each case study.

Furthermore, the hypothesis of independent errors with constant variance is also questionable. For instance, experiments at temperatures close to the growth boundary are harder to control, so it is likely that they have larger errors than experiments near optimal growth conditions. This result is not simple to describe reasonably in mathematical terms, so it is not considered in the simulations. Finally, due to random chance, different replications of the exact same experiment will result in different experimental results (Garre et al., 2021). This implies that independent repetitions of an experimental design will always result in different parameter estimates and parameter uncertainties. Therefore, it is impossible to predict the parameter uncertainty that will be estimated

in a particular repetition of an experiment. Despite these limitations, this methodology based on in silico simulations provides and estimation of the parameter uncertainty that will be obtained before doing any experimental work. This is a valuable source of information that can aid rationalizing experimental design, as will be illustrated in the next section.

3.5. Application of the OED results to practical cases

The diagrams illustrated in Figs. 7 and 8 provide a convenient support for the design of growth experiments to characterize secondary growth models. The diagrams show the variation of the CV as a function of the number of growth experiments for each experimental design. The CVs have been selected because they are unitless, so they facilitate the comparison between model parameters. Therefore, they are used here to illustrate how numerical simulations can guide experimental design, although there are some situations where alternative statistical indexes (e.g., absolute error of the parameter) may be more informative than CVs.

As an illustration, let's assume that we aim at estimating the parameters of the CPM model for the growth of *L. monocytogenes* as a function of temperature. Traditionally, the number of growth experiments is often based on previous experience or data from the literature, an approach that is highly uncertain and prone to bias. The numerical results of this investigation open the gate for an innovative approach for experimental design, enabling the definition of a target uncertainty for a model parameter. For instance, we will define as a target for the experimental design having a 6 % CV for μ_{opt}. As is often the case in predictive microbiology, we assume that the order of the model is known, so the design is based on Fig. 8B. The left hand side panel shows that a uniform design with 19 growth experiments would provide the target parameter uncertainty. It is expected that this design would result in a CV < 1.25 % for X_{max}, <75 % for X_{min} and <2.5 % for X_{opt}. The diagram also shows that it is unlikely to attain this precision for an *E*-optimal design, and that the use of a D-optimal design would reduce the number of growth experiments to 12. However, due to the location of most experiments at the vicinity of X_{min} (Fig. 2), the OED with 12 experiments would have a greater elapsed duration (~10 times longer) than the uniform design with 19 experiments (Fig. 4). Then, it would be a question for the experimenter to decide if these experiments provide enough information, if the target parameter uncertainty should be revised or if the number of replicates (Figs. 7 and 8 show the expected CV for 2 replicates) should be modified.

On the light of these results, it can be questioned whether optimal experimental designs are still optimal from outside the narrow perspective of information theory. OEDs are calculated based only on the properties of the *FIM*, so they do not account for empirical limitations not included in the local sensitivity functions. These empirical limitations are often hard to quantify and are dependent on the experimental protocol. For instance, in the case of microbial inactivation under dynamic conditions, the main experimental limitation not included in *FIM* is the impossibility to sample time points that are too close (Garre et al., 2018b). In that particular case, the OED was still advantageous even when considering this limitation, with an OED with 10 sampling points having similar precision as a uniform design with 40 time points (Garre et al., 2019). However, this does not seem to be the case for secondary growth models, where OEDs are optimal from a theoretical point of view, but may not be optimal from a practical perspective. This is due to the OED focusing on temperatures close to the growth limits of the microorganism, where growth occurs at a very slow rate requiring excessively long experimental runs. To enhance this aspect, this study defines a modified OED criterion that includes a penalty term in the calculation. This criterion balances the information of the temperatures included in the design with the experimental load, resulting in an aggregate time reduction of up to 10 times with respect to the uniform design (Figs. 3 and 4).

However, it should be noted that the modified OED criterion proposed here also has some drawbacks. Because it is a modification of the modified E-criterion, it results in an increase in the uncertainty of some parameters with respect to the uniform design (Figs. 7 and 8). Furthermore, although it is not as extreme as the OED, it still focuses the experiments at temperatures relatively close to the growth limits; and these conditions present additional challenges that are not easy to quantify in mathematical terms. The longer the experiment, the more likely it is to face challenges that could compromise the validity and reliability of the results obtained, such as maintaining the environmental conditions (temperature, pH etc.), avoiding contamination, and ensuring the stability of the experimental system. Further, the resolution of analytical methods should increase because at these limiting conditions there could be injured cells which may not grow in selective media, thus adding another source of uncertainty (Arvaniti and Skandamis, 2022). Hence, it is important to carefully consider the balance between time and experimental quality in the design of an optimal experiment, and to choose an experimental design that adequately balances both factors.

4. Conclusions

The computational methodology presented in this article can be a valuable tool to support the design of experiments for the estimation of parameters of secondary growth models. Local sensitivity functions can be used as a first step to provide qualitative information on the experimental design, but the definition of an OED requires the optimization of some measure of the *FIM*. Even then, OEDs do not directly provide an estimate of the expected uncertainty of a particular model parameter, requiring an additional step involving numerical simulations (or equivalent methods).

Our calculations show that OEDs for secondary growth models depends on the secondary model (full Ratkowsky or CPM), the optimality criterion (D or modified E), whether model parameters were fixed, and the number of growth experiments in the design. Our results also show that it may be questioned whether an OED is really justified for secondary growth models, since they focus on growth temperatures close to the growth limits. Therefore, OEDs require significantly longer experimental runs, making it necessary to weigh the potential benefits of the OED against the cost and feasibility of such a time-consuming experimental design. This motivated the derivation of a novel optimality criterion that combines information theory (Fisher information) with practical feasibility. The inclusion of a penalty term in the E-optimality criterion may be helpful to balance the number of experiments across the temperature range, reducing the aggregated time of analysis and the uncertainty of those less identifiable model parameters. These conclusions, as well as the computational methodology developed here, can inform future studies aiming at characterizing the growth of populations, helping rationalize the experimental design.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2024.110604.

CRediT authorship contribution statement

Silvia Guillén: Formal analysis, Investigation, Validation, Writing – review & editing. **Aricia Possas:** Conceptualization, Formal analysis, Validation, Writing – review & editing. **Antonio Valero:** Conceptualization, Project administration, Supervision, Writing – review & editing. **Alberto Garre:** Conceptualization, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing.

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.

Data availability

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

Acknowledgments

This Research was partly funded with project PID2020-116318RB-C32 by the Spanish Ministry of Science and Research, MCIN/AEI/10.13039/501100011033, as well as by the AGROALNEXT programme and was supported by MCIN with funding from European Union Next-GenerationEU (PRTR-C17.I1) and by Fundación Séneca with funding from Comunidad Autónoma Región de Murcia (CARM). Alberto Garre acknowledges being funded by a Maria Zambrano Scholarship and by a Ramon y Cajal Fellowship (RYC-2021-034612-I). Silvia Guillen was supported by a Margarita Salas scholarship. The authors wish to thank the Spanish Government (Ministry of Science and Innovation, research project PID2019-108420RB-C31-ASEQURA).

References

Akkermans, S., Nimmegeers, P., Van Impe, J.F., 2018. A tutorial on uncertainty propagation techniques for predictive microbiology models: A critical analysis of state-of-the-art techniques. Int. J. Food Microbiol. 282, 1–8. https://doi.org/10.1016/j.ijfoodmicro.2018.05.027.

Arvaniti, M., Skandamis, P.N., 2022. Defining bacterial heterogeneity and dormancy with the parallel use of single-cell and population level approaches. Curr. Opin. Food Sci. 44, 100808. https://doi.org/10.1016/j.cofs.2022.100808.

Balsa-Canto, E., Alonso, A., Banga, J., 2008. Computing optimal dynamic experiments for model calibration in predictive microbiology. J. Food Process Eng. 31, 186–206. https://doi.org/10.1111/j.1745-4530.2007.00147.x.

Banga, J.R., Balsa-Canto, E., 2008. Parameter estimation and optimal experimental design. Essays Biochem. 45, 195–210. https://doi.org/10.1042/bse0450195.

Baranyi, J., Buss da Silva, N., Ellouze, M., 2017. Rethinking tertiary models: relationships between growth parameters of *Bacillus cereus* strains. Front. Microbiol. 8, 1890. https://doi.org/10.3389/fmicb.2017.01890.

Cunha, L.M., Oliveira, F.A.R., Brandão, T.R.S., Oliveira, J.C., 1997. Optimal experimental design for estimating the kinetic parameters of the Bigelow model. J. Food Eng. 33, 111–128. https://doi.org/10.1016/S0260-8774(97)00047-2.

Cunha, L.M., Oliveira, F.A.R., Oliveira, J.C., 1998. Optimal experimental design for estimating the kinetic parameters of processes described by the Weibull probability distribution function. J. Food Eng. 37, 175–191. https://doi.org/10.1016/S0260-8774(98)00085-5.

Egea, J.A., Martí, R., Banga, J.R., 2010. An evolutionary method for complex-process optimization. Comput. Oper. Res. 37, 315–324. https://doi.org/10.1016/j.cor.2009.05.003.

Egea, J.A., Henriques, D., Cokelaer, T., Villaverde, A.F., MacNamara, A., Danciu, D.-P., Banga, J.R., Saez-Rodriguez, J., 2014. MEIGO: an open-source software suite based on metaheuristics for global optimization in systems biology and bioinformatics. BMC Bioinform. 15, 136. https://doi.org/10.1186/1471-2105-15-136.

Frías, J.M., Oliveira, J.C., Cunha, L.M., Oliveira, F.A., 1998. Application of D-optimal design for determination of the influence of water content on the thermal degradation kinetics of ascorbic acid at low water contents. J. Food Eng. 38, 69–85. https://doi.org/10.1016/S0260-8774(98)00099-5.

García, M.R., Vilas, C., Herrera, J.R., Bernárdez, M., Balsa-Canto, E., Alonso, A.A., 2015. Quality and shelf-life prediction for retail fresh hake (*Merluccius merluccius*). Int. J. Food Microbiol. 208, 65–74. https://doi.org/10.1016/j.ijfoodmicro.2015.05.012.

Garre, A., Fernández, P.S., Lindqvist, R., Egea, J.A., 2017. Bioinactivation: software for modelling dynamic microbial inactivation. Food Res. Int. 93, 66–74. https://doi.org/10.1016/j.foodres.2017.01.012.

Garre, A., Egea, J.A., Iguaz, A., Palop, A., Fernandez, P.S., 2018a. Relevance of the induced stress resistance when identifying the critical microorganism for microbial risk assessment. Front. Microbiol. 9 https://doi.org/10.3389/fmicb.2018.01663.

Garre, A., González-Tejedor, G., Peñalver-Soto, J.L., Fernández, P.S., Egea, J.A., 2018b. Optimal characterization of thermal microbial inactivation simulating non-isothermal processes. Food Res. Int. 107, 267–274. https://doi.org/10.1016/j.foodres.2018.02.040.

Garre, A., Peñalver-Soto, J.L., Esnoz, A., Iguaz, A., Fernandez, P.S., Egea, J.A., 2019. On the use of *in-silico* simulations to support experimental design: a case study in microbial inactivation of foods. PLoS ONE 14. https://doi.org/10.1371/journal.pone.0220683.

Garre, A., den Besten, H.M.W., Fernandez, P.S., Zwietering, M.H., 2021. Not just variability and uncertainty; the relevance of chance for the survival of microbial cells to stress. Trends Food Sci. Technol. 118, 799–807. https://doi.org/10.1016/j.tifs.2021.10.033.

Garre, A., Koomen, J., Besten, H.M.W.den, Zwietering, M.H., 2023. Modeling population growth in R with the biogrowth package. J. Stat. Softw. 107, 1–51. https://doi.org/10.18637/jss.v107.i01.

Grijspeerdt, K., Vanrolleghem, P., 1999. Estimating the parameters of the Baranyi model for bacterial growth. Food Microbiol. 16, 593–605. https://doi.org/10.1006/fmic.1999.0285.

Koutsoumanis, K., Tsaloumi, S., Aspridou, Z., Tassou, C., Gougouli, M., 2021. Application of Quantitative Microbiological Risk Assessment (QMRA) to food spoilage: principles and methodology. Trends Food Sci. Technol. 114, 189–197. https://doi.org/10.1016/j.tifs.2021.05.011.

Longhi, D.A., Martins, W.F., da Silva, N.B., Carciofi, B.A.M., de Aragão, G.M.F., Laurindo, J.B., 2017. Optimal experimental design for improving the estimation of growth parameters of *Lactobacillus viridescens* from data under non-isothermal conditions. Int. J. Food Microbiol. 240, 57–62. https://doi.org/10.1016/j.ijfoodmicro.2016.06.042.

McMeekin, T., Olley, J., Ratkowsky, D., Corkrey, R., Ross, T., 2013. Predictive microbiology theory and application: is it all about rates? Food Control 29, 290–299. https://doi.org/10.1016/j.foodcont.2012.06.001.

Mertens, L., Van, Derlinden, Van Impe, J.F., 2012. Comparing experimental design schemes in predictive food microbiology: optimal parameter estimation of secondary models. J. Food Eng. 112, 119–133. https://doi.org/10.1016/j.jfoodeng.2012.03.018.

Muramatsu, Y., Dolan, K.D., Mishra, D.K., 2019. Factors influencing estimation of thermal inactivation parameters in low-moisture foods using a test cell. J. Food Eng. 262, 100–108. https://doi.org/10.1016/j.jfoodeng.2019.05.003.

Notebaart, R.A., Kintses, B., Feist, A.M., Papp, B., 2018. Underground metabolism: network-level perspective and biotechnological potential. Curr. Opin. Biotechnol. Food Biotechnol. Plant Biotechnol. 49, 108–114. https://doi.org/10.1016/j.copbio.2017.07.015.

Nunes Silva, B., Cadavez, V., Teixeira, J.A., Ellouze, M., Gonzales-Barron, U., 2020. Cardinal parameter meta-regression models describing *Listeria monocytogenes* growth in broth. Food Res. Int. 136, 109476 https://doi.org/10.1016/j.foodres.2020.109476.

Peñalver-Soto, J.L., Garre, A., Esnoz, A., Fernández, P.S., Egea, J.A., 2019. Guidelines for the design of (optimal) isothermal inactivation experiments. Food Res. Int. 126, 108714 https://doi.org/10.1016/j.foodres.2019.108714.

Pinon, A., Zwietering, M., Perrier, L., Membré, J.-M., Leporq, B., Mettler, E., Thuault, D., Coroller, L., Stahl, V., Vialette, M., 2004. Development and validation of experimental protocols for use of cardinal models for prediction of microorganism growth in food products. Appl. Environ. Microbiol. 70, 1081. https://doi.org/10.1128/AEM.70.2.1081-1087.2004.

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N., Chandler, R.E., 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J. Bacteriol. 154, 1222–1226.

Rosso, L., Lobry, J.R., Flandrois, J.P., 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. J. Theor. Biol. 162, 447–463. https://doi.org/10.1006/jtbi.1993.1099.

Rosso, L., Lobry, J.R., Bajard, S., Flandrois, J.P., 1995. Convenient model to describe the combined effects of temperature and pH on microbial growth. Appl. Environ. Microbiol. 61, 610–616.

Schmidt, P.J., Emelko, M.B., Thompson, M.E., 2019. Recognizing structural nonidentifiability: when experiments do not provide information about important parameters and misleading models can still have great fit. Risk Anal., risa.13386 https://doi.org/10.1111/risa.13386.

Soetaert, K., Petzoldt, T., 2010. Inverse modelling, sensitivity and Monte Carlo analysis in *R* using package FME. J. Stat. Softw. 33 https://doi.org/10.18637/jss.v033.i03.

Van Derlinden, E., Van Impe, J.F., 2012. Modeling growth rates as a function of temperature: model performance evaluation with focus on the suboptimal temperature range. Int. J. Food Microbiol. 158, 73–78. https://doi.org/10.1016/j.ijfoodmicro.2012.05.015.

Van Derlinden, E., Bernaerts, K., Van Impe, J.F., 2008. Accurate estimation of cardinal growth temperatures of *Escherichia coli* from optimal dynamic experiments. Int. J. Food Microbiol. 128, 89–100. https://doi.org/10.1016/j.ijfoodmicro.2008.07.007.

van Derlinden, E., Balsa-Canto, Impe, Van, 2010. (Optimal) experiment design for microbial inactivation. In: Valdramidis, V.P., Van Impe, J.F.M. (Eds.), Progress on Quantitative Approaches of Thermal Food Processing, Advances in Food Safety and Food Microbiology. Nova Publishers, pp. 67–98.

Van Derlinden, E., Mertens, L., Van Impe, J.F., 2013. The impact of experiment design on the parameter estimation of cardinal parameter models in predictive microbiology. Food Control 29, 300–308. https://doi.org/10.1016/j.foodcont.2012.06.018.

Van Rijswijck, I.M.H., van Mastrigt, O., Pijffers, G., Wolkers-Rooijackers, J.C.M., Abee, T., Zwietering, M.H., Smid, E.J., 2019. Dynamic modelling of brewers' yeast and *Cyberlindnera fabianii* co-culture behaviour for steering fermentation performance. Food Microbiol. 83, 113–121. https://doi.org/10.1016/j.fm.2019.04.010.

Versyck, K.J., Claes, J.E., Impe, J.F.V., 1997. Practical identification of unstructured growth kinetics by application of optimal experimental design. Biotechnol. Prog. 13, 524–531. https://doi.org/10.1021/bp970080j.