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The Most Probable Curve method - A robust approach to estimate kinetic models from low plate count data resulting in reduced uncertainty

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ABSTRACT

A novel method is proposed for fitting microbial inactivation models to data on liquid media: the Most Probable Curve (MPC) method. It is a multilevel model that makes a separation between the "true" microbial concentration according to the model, the "actual" concentration in the media considering chance, and the actual counts on the plate. It is based on the assumptions that stress resistance is homogeneous within a microbial population, and that there is no aggregation of microbial cells. Under these assumptions, the number of colonies in/on a plate follows a Poisson distribution with expected value depending on the proposed kinetic model, the number of dilutions and the plated volume.

The novel method is compared against (non)linear regression based on a normal likelihood distribution (traditional method), Poisson regression and gamma-Poisson regression using data on the inactivation of *Listeria monocytogenes*. The conclusion is that the traditional method has limitations when the data includes plates with low (or zero) cell counts, which can be mitigated using more complex (discrete) likelihoods. However, Poisson regression uses an unrealistic likelihood function, making it unsuitable for survivor curves with several log-reductions. Gamma-Poisson regression uses a more realistic likelihood function, even though it is based mostly on empirical hypotheses. We conclude that the MPC method can be used reliably, especially when the data includes plates with low or zero counts. Furthermore, it generates a more realistic description of uncertainty, integrating the contribution of the plating error and reducing the uncertainty of the primary model parameters. Consequently, although it increases modelling complexity, the MPC method can be of great interest in predictive microbiology, especially in studies focused on variability analysis.

1. Introduction

Microbial inactivation is one of the main elements of food safety control systems. Considering that most foodborne pathogens are ubiquitous in the environment and are able to survive in processing environments (e.g., in biofilms), ingredients used for making food products cannot be guaranteed to be free of pathogenic microorganisms, nor that these products will not be recontaminated during production (Guillén et al., 2021). The application of processing treatments to reduce the potential microbial load in the product is, therefore, often essential for food safety, as a way to reduce the microbial concentration to an appropriate level of protection (Gorris, 2005).

Although a variety of alternative technologies has been applied during the last decades, the application of high temperatures (thermal treatments) remains the most common technology in food production (Peng et al., 2017). While being effective at inactivating foodborne pathogens, the application of thermal treatments can also have a negative impact on the sensorial and/or nutritional quality of food products (van Boekel et al., 2020). Furthermore, their application in industrial settings can consume a high amount of energy and have a relevant environmental impact. Consequently, it is of interest for food industries, and for society in general, to apply minimal treatments to achieve the desired microbial inactivation with minimal negative impacts.

One of the applications of predictive models is their use to support process design (Allende et al., 2022). In the case of microbial inactivation, these models predict the reduction in the microbial population as a

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function of treatment time and process parameters; e.g., temperature (Perez-Rodriguez and Valero, 2012). This requires the description of a highly complex system, as microbial inactivation is ultimately governed by a complex combination of molecular reactions that are not yet fully understood, as well as thermodynamics (Smelt and Brul, 2014). Consequently, the inactivation models used for process design, shelf life estimation and microbial risk assessment are, to some extent at least, empirical models, which model parameters must be estimated from experimental data.

The most common method to estimate model parameters from data in predictive microbiology is the application of ordinary least-squares (OLS) from a frequentist perspective. However, this approach has some limitations for microbial inactivation. Experimental protocols for building inactivation models often require several log-reductions in the microbial concentration (in the order of 6 or 7 logs (National Advisory Committee on microbiological criteria for foods, 2010)), so it is not possible to directly observe the microbial concentrations in media. Instead, microbial concentration is estimated by colony counting in/on Petri dishes after an adequate number of serial dilutions. Observations obtained by this method can be an issue for parameter estimation based on OLS, when the dataset includes plates with low colony count and/or zero count especially towards higher log-reductions (Chik et al., 2018; Duarte et al., 2015; Garcés-Vega and Marks, 2014; Garre et al., 2019). For this reason, guidelines often recommend discarding plates with <30 colonies. However, this recommendation may be too restrictive in some cases, and the study may need the information contained in those plates with low colony count (or even zero count).

In this article, we propose a novel method to fit microbial inactivation models from experimental data obtained in liquid systems. The main motivation is to also use information contained in plates with low colony count and/or zero counts for parameter estimation without introducing a bias in the parameter estimates. The method is based on similar statistical hypotheses regarding the distribution of the number of colonies to those underlying the Most Probable Number method (MPN), commonly used in microbiology (Alexander, 1965). These are extended including stochastic hypotheses about inactivation in liquid media during the treatment previously described by us (Garre et al., 2019, 2021) and implemented as a multilevel Bayesian model (Garre et al., 2020). The MPN method is used to estimate the microbial concentration of one sample. Here we used the same basis to determine the most probable curve through data at various timepoints, both at higher and lower concentrations. Due to this inspiration, the method is named the *Most Probable Curve* (MPC) method. The statistical hypotheses of the method are derived in Section 2.2.4 below.

The novel method is compared against traditional regression fitted to the logarithm of the microbial counts (based on normal distributions, equivalent to OLS), which can be considered as the reference approach in the field. Furthermore, it is also compared against several other methods based on variations of generalized linear models that have been suggested during the last years (Hiura et al., 2021). The comparison is based both on a critical inspection of the statistical hypotheses of each method (i.e., their likelihood functions), and on the analysis of an already published dataset on the inactivation of *Listeria monocytogenes* (Aryani et al., 2015). This dataset includes curves showing both linear and non-linear inactivation kinetics, allowing the evaluation of the methods for different microbial responses.

Another reason to select this dataset is its inclusion of independent biological replicates for each bacterial strain. This allows the analysis of variability in microbial inactivation, defined as the reflection of inherent sources of variation (such as genetic differences between cells) that cannot be reduced by gathering more or better data (Nauta, 2000). In spite of its relevance for food safety (Aspridou and Koutsoumanis, 2020), variability can be hard to include in predictive models, requiring advanced statistical methods. In this article, we also show how the multilevel nature of the MPC method allows the inclusion of variability following a methodology already used in a previous study for a different type of model (Garre et al., 2020).

2. Materials and methods

2.1. Datasets used to compare the methods

The data on inactivation of *Listeria monocytogenes* published by Aryani et al. (2015) was used to compare the different methods studied in the present article. This study followed standard methods from food microbiology to perform thermal treatments under isothermal conditions on 20 different strains of *L. monocytogenes*. Thermal treatments were performed under isothermal conditions using flasks filled with brain heart infusion (BHI) immersed in a water bath.

Although the study by Aryani et al. (2015) included 20 different *L. monocytogenes* strains and three different temperatures, for the scope of the current study the data of only two strains was used: FBR16 and FBR14 at 55 °C. They were selected because a visual exploration shows that the response of FBR16 is close to log-linearity whereas FBR14 had clearly nonlinear survivor curves.

The usual response variable in microbial inactivation models is the microbial concentration (N_{obs}; often in CFU/ml), where N_{obs} is calculated from plate counts after correcting for the number of dilutions (Eq. (1)). However, in the MPC method the response variable is the number of colonies in/on a plate (N_{plate}). Then, the volume of liquid menstruum plated (V_{plate}) and the number of decimal dilutions (d) are included in the likelihood estimation. Therefore, the MPC method does not require pre-processing of the data using Eq. (1) before model fitting. Instead, this calculation is implicitly included in the method.

$$N_{obs} = N_{plate}\frac{1}{V_{plate}}10^{d} \quad (1)$$

2.2. Modelling approaches compared in the study

2.2.1. Traditional method - (Bayesian) OLS

Model fitting by OLS can be written equivalently in a Bayesian framework as illustrated in Eq. (2). Although this model is most often fitted using frequentist statistics, here we use an equivalent Bayesian formulation to ease comparison with the other methods tested.

$$log_{10}N_{obs} \sim \text{Normal}(y(t), \sigma_{obs}) \quad (2)$$

According to this model, the variation in the logarithm of the observed microbial concentration ($\log_{10} N_{obs}$) follows a normal distribution with constant standard deviation (σ_{obs}) (i.e. N_{obs} follows a log-normal distribution). The expected value is supposed to vary with time (t) according to a function $y(t)$; i.e., the primary inactivation model. In this article, we will use two primary models, one for each particular strain. Note that, because this method is defined for the logarithm of the microbial concentration, it cannot include plates without colonies (the logarithm of zero is minus infinity).

Because the survivor curves of strain FBR16 do not deviate strongly from linearity, we used a Bigelow (log-linear) primary model. This model is shown in Eq. (3), where N_0 is the initial microbial count and D is the D-value (treatment time to reduce the microbial count with one log).

$$y(t) = logN_0 - t/D \quad (3)$$

On the other hand, the survivor curves of strain FBR14 clearly deviated from log-linearity. Consequently, the Mafart inactivation model (Mafart et al., 2002), shown in Eq. (4), was used to describe the inactivation for this microbial strain. In this model, the curvature is described by parameter β. Values of this parameter lower than one indicate an upward curvature, whereas values higher than one result in the opposite. Parameter δ (δ-value) has a similar interpretation to the D-value, being equal to the treatment time required for the first log-reduction in the microbial count.

$$y(t) = logN_0 - (t/\delta)^{\beta} \quad (4)$$

2.2.2. Poisson regression

As formulated by Hiura et al. (2021), Poisson regression for microbial inactivation can be defined in Bayesian notation as shown in Eq. (5).

$$N_{obs} \sim \text{Poisson}(Y(t)) \quad (5)$$

This model differs from the traditional method in the use of a different likelihood function. Instead of assuming that the logarithm of the microbial concentration ($\log_{10} N_{obs}$) follows a normal distribution, this model assumes that the microbial count (N_{obs}) follows a Poisson distribution. Therefore, this model is defined for discrete variables (number of colonies; e.g. as CFU/ml) and can account for plates with zero count, unlike the traditional method that is defined on continuous ones (microbial concentration; e.g. as log CFU/ml). It is also worth noting that the normal distribution has one more parameter than the Poisson distribution: the Poisson distribution imposes that the variance equals the expected value, whereas the normal distribution uses the variance (σ^2) as an additional parameter. As a result, the normal distribution is more "flexible" than the Poisson distribution.

In the Poisson model, the expected value of the microbial count is given by function $Y(t)$ (note the use of a different notation to indicate that this function is in units of CFU/ml, not log CFU/ml). In a similar way as for the traditional method, a linear (Bigelow model; Eq. (6)) and a nonlinear (Mafart model; Eq. (7)) primary model were used to analyse the experimental data.

$$Y(t) = N_0 \cdot 10^{-t/D} \quad (6)$$

$$Y(t) = N_0 \cdot 10^{-(t/\delta)^{\beta}} \quad (7)$$

2.2.3. Gamma-Poisson regression

As mentioned above, the Poisson distribution imposes that the variance (often called dispersion in statistical literature) equals the mean. Experimental data often deviates from this hypothesis; for instance, the term "overdispersion" is used when the variance of the data is greater than its mean. This can be accounted for by introducing additional parameters or model hypotheses that account for this (over) dispersion.

One common approach is to assume that the expected value of the distribution is not fixed but follows a gamma distribution. This assumption results in the Gamma-Poisson or, equivalently, the negative binomial distribution (Vose, 2008), as shown in Eq. (8). In this article, we use the alternative parameterization of the negative binomial distribution (*neg_binomial_2* in *Stan*, described below). This parameterization separates between the expected value of the distribution and its overdispersion (indicated by parameter ϕ in Eq. (8)) with respect to the Poisson distribution. Therefore, its formulation is more convenient for describing microbial inactivation using primary models from predictive microbiology.

$$N_{obs} \sim \text{NegBinomial}(Y(t), \phi) \quad (8)$$

In a similar way as for the Poisson regression model, the expected value of the microbial count is described by the linear or nonlinear primary inactivation model, $Y(t)$, defined in Eqs. (6) and (7). Note that, as well as the Poisson regression model, this model is defined for discrete random variables (N_{obs}), so it can account for plates with zero colonies.

The overdispersion is introduced in this model by parameter $\phi \in (0, +\infty)$. The effect of this parameter on the variance is shown in Eq. (9). For large values of ϕ, the Gamma-Poisson model is equivalent to the Poisson distribution (no overdispersion), whereas lower values indicate increasingly larger overdispersion.

$$\text{Var}(N_{obs}) = Y(t) + \frac{Y(t)^2}{\phi} \quad (9)$$

Note that the Gamma-Poisson model cannot include underdispersion. Nonetheless, this is not relevant for our case studies, as the results of microbial inactivation experiments rarely show underdispersion with respect to the Poisson distribution.

2.2.4. Most Probable Curve method

The Most Probable Curve (MPC) method is defined based on stochastic, mechanistic hypotheses regarding microbial inactivation, as well as the sampling error due to serial dilutions and plating, previously derived by Garre et al. (2019) and Garre et al. (2021), based on real sampling deviations like pipetting error, but mainly determined by the probability distribution of having a certain number of cells in the final volume. Under the assumption that stress resistance in microbial populations is homogeneous (i.e., every individual cell has the same probability to be inactivated) and the number of cells in the media at the beginning of the experiment follows a Poisson distribution, the number of microbial cells in the heating menstruum (N_m) follows a Poisson distribution, as formulated in Eq. (10). Note that, as described by Garre et al. (2021), this variation does not fit within the usual definitions of variability and uncertainty often used in microbial risk assessment. Consequently, they recommended calling it "chance".

$$N_m \sim \text{Pois}(Y(t)) \quad (10)$$

As mentioned above, the expected value ($Y(t)$) is supposed to follow either log-linear (Eq. (6)) or nonlinear (Eq. (7)) kinetics for the present case study (note that it could be any other primary inactivation model depending on the microbial response).

It is worth highlighting the differences between Eq. (10) and the Poisson regression model (Eq. (5)). In Poisson regression, the likelihood function is directly related to the observed microbial count. The MPC method extends the Poisson regression by introducing the fact that the microbial count in the media (N_m) cannot be measured directly. Instead, it is approximated based on the number of colonies identified in a given volume (V_{plate}) in a petri dish (N_{plate}), in most cases, after a number of decimal dilutions (d). As described in Garre et al. (2019), under the hypothesis that the cell positions are independent (e.g., there is no aggregation), the number of cells in a Petri plate conditional to N_m would follow a binomial distribution (Eq. (11)) of size N_m and probability $V_{plate} \cdot 0.1 \cdot d$.

$$N_{plate} | N_m \sim \text{Binomial}\left(N_m, V_{plate} \cdot 0.1^d\right) \quad (11)$$

Considering that N_m follows a Poisson distribution (Eq. (10)), the number of colonies in a plate would follow a Poisson distribution (Eq. (12)).

$$N_{plate} \sim \text{Pois}\left(Y(t) \cdot V_{plate} \cdot 0.1^d\right) \quad (12)$$

Therefore, the MPC method is a type of multilevel model (Garre et al., 2020) that defines a hierarchical relationship between the uncertainty of the true concentration in the media (the primary model; Eqs. (6)–(7)), the actual microbial count in the media accounting for chance (N_m; Eq. (10)) and the measured microbial concentration based on the number of colonies in a plate (combination of Eqs. (12) and (1)). Note that, because it is defined for N_{plate}, the MPC method can account for plates with zero count.

Regarding function $Y(t)$, as commented above, the same two primary models were used in the case study: the Bigelow model (Eq. (6)) and the Mafart model (Eq. (7)). From a biological point of view, the curvature of the Mafart model can be explained by two different phenomena. One is based on the hypothesis that the resistance is heterogeneous among the microbial cells (Peleg and Cole, 1998). An equivalent one for isothermal conditions is that the stress resistance of cells is homogeneous within the population but dynamic. That implies that the cells have some physiological response to the treatment that affects their stress resistance, either increasing it (upward curvature) or decreasing it (downward curvature). For consistency with the hypothesis of the Most Probable

Curve method, we will use in this article the second hypothesis (i.e., a homogeneous, dynamic resistance).

2.3. Introduction of variability in the MPC method using multilevel models

The variability in the results of a microbial inactivation experiment is the result of a combination of various sources (Garre et al., 2021). Some of them, like the error associated with the plating method, are spurious and should not be included in predictions. However, other sources, such as biological variability, are very relevant in food science and should, ideally, be included in model predictions (Zwietering et al., 2021). As defined in the previous section, the MPC method only accounts for the sampling error associated with the serial dilution and plating method. Nevertheless, its multilevel nature allows extending it to include additional sources of variability and uncertainty, so these are quantified and can be incorporated in model predictions.

As an illustration, we introduce the effect of within-strain variability in the case studies, using an approach similar to the one by Garre et al. (2020). Instead of assuming that the parameters of the primary model (N_0, D, δ, β) are fixed among all the repetitions, they are assumed as a vector of length 3 (reflecting the number of independent biological replicates in this particular dataset, the length will obviously vary between case studies). Then, it is assumed that the logarithm of the elements of each of these vectors follows a normal distribution with unknown mean and variance (Eqs. (13)–(16)).

$$log N_0 \sim \text{Normal}\left(\overline{log N_0}, \sigma_{\text{logN0}}\right) \tag{13}$$

$$log D \sim \text{Normal}\left(\overline{log D}, \sigma_{\text{logD}}\right) \tag{14}$$

$$log \delta \sim \text{Normal}\left(\overline{log \delta}, \sigma_{\text{log}\delta}\right) \tag{15}$$

$$log \beta \sim \text{Normal}\left(\overline{log \beta}, \sigma_{\text{log}\beta}\right) \tag{16}$$

2.4. Model fitting and computational methods

The models were fitted following Bayesian regression using the Hamiltonian Monte Carlo method based on the No-U-Turn sampler (NUTS) included in *Stan* (Carpenter et al., 2017). The convergence of the Markov Chains was checked using standard visualisations (Brooks, 2011), as well as evaluating the $\widehat{R}$ statistic (McElreath, 2016).

For every model, we used a weakly informative prior distribution for every model parameter. For the initial microbial concentration (N_0), this was a log-normal prior with expected value 8 log CFU/ml and standard deviation 2 log CFU/ml. Regarding the kinetic parameters of single-level models, for the D-value and the δ-value log-normal distributions were used with expected values 0 log min (i.e., in log scale) and standard deviation 2 log min. In the case of the curvature parameter of the Mafart model, β, we use a log-normal distribution with expected value 0 (in log scale; i.e., $\beta = 1$, equivalent to the Bigelow model) and standard deviation of 1 (also in log scale).

For fitting by the traditional method, we used an exponential prior for σ_{obs} with expected value of 1 log CFU/ml. To parameter ϕ of the Gamma-Poisson model, a Gamma prior was assigned with parameters $k = 1$ and $\theta = 1$. Moreover, we introduced a lower bound in the *Stan* code to avoid $\theta = 0$, as this results in a singular likelihood (infinite variance according to Eq. (9)). Regarding the additional parameters of the multilevel model, we use weakly informative normal priors for the grand means ($\overline{log N_0} \sim \text{N}(8, 2)$; $\overline{log D} \sim \text{N}(0, 2)$; $\overline{log \delta} \sim \text{N}(0, 2)$; $\overline{log \beta} \sim \text{N}(0, 1)$) and weakly informative exponential priors for the standard deviations ($\sigma_{\text{log}N_0} \sim \text{Exp}(2)$; $\sigma_{\text{log}D} \sim \text{Exp}(1)$; $\sigma_{\text{log}\delta} \sim \text{Exp}(1)$; $\sigma_{\text{log}\beta} \sim \text{Exp}(1)$). In every case, the calculations were repeated for different prior distributions without observing any relevant impact on the results (not shown).

The calculations were implemented in R version 3.6.3 (R Core Team, 2016) using the *rstan* package version 2.21.2 (Stan Development Team, 2019). Credible intervals (CI) were estimated based on the quantiles of the Monte Carlo simulations. Unless otherwise stated, every CI is calculated for a 90 % confidence level. The convergence of the predictions was evaluated repeating the calculations for an increased number of iterations, without observing any relevant difference. Moreover, calculations were repeated for several seeds of the pseudo-random number generator, without observing any impact in the results. The code and data are available in the GitHub page of one of the authors (https://github.com/albgarre/MostProbableCurve).

3. Results and discussion

3.1. Model fitting without variability

3.1.1. Linear inactivation - L. monocytogenes strain FBR16

Fig. 1 illustrates the fit of each one of the models tested for the inactivation of *L. monocytogenes* FBR16. These models account for different types of uncertainty. The coloured ribbons in Fig. 1A–C (which may look like a line due to their very small width) reflect the uncertainty of the primary model in estimating the expected value (the mean). This can be seen as a reflection of the uncertainty in the estimates of D and log N_0 (quantified by the standard errors reported in Table 1), that represent an "ideal" microbial concentration in the media (Eq. (3) or Eq. (6), depending on the model). The ribbons depicted by dashed lines introduce the remaining variation that is not included in the uncertainty of the primary model in estimating the mean, and that reflects the dispersion of the observations. In the models, this additional dispersion is quantified by the variance parameters (σ or ϕ). Note that the plot for the MPC method (Fig. 1D) includes an additional ribbon. This reflects the fact that this model introduces an additional level between the ideal microbial concentration in the media (Eq. (6)), the actual concentration in the media (Eq. (10)), and the observed concentration based on plate count (Eq. (12)). Note that this separation between different levels of uncertainty is relatively simple in the (multilevel) Bayesian modelling approach, and is one of its main advantages with respect to frequentist statistics (van Boekel, 2020).

The model obtained by the traditional method (i.e., based on a normal likelihood for the residuals) (Fig. 1A) behaves as expected, being able to represent the general trend in the data. Both credible and prediction intervals have regular width (a reflection of the hypothesis of constant variance), and the interval for the observations cover most of the data points used to build the model.

On the other hand, the fit of the Poisson regression model (Fig. 1B) is much poorer than the one of the traditional model. The fitted model clearly deviates from the general trend of the data points, systematically fitting expected microbial counts higher than the observed ones. This is most likely due to the likelihood function of this model. The Poisson distribution imposes a relationship between the mean and the variance, reducing the flexibility of the distribution by one parameter. As a result, regardless of the values of the model parameters, the shape of the credible interval of the observations will always have the "trumpet" shape (i.e., very narrow for high expected counts, wider for low expected counts) illustrated in Fig. 1B. The variance in the observations clearly deviates from this shape defined by the Poisson likelihood. Although the variance is higher at the end of the experiment, this increment is much smaller than predicted by the Poisson likelihood.

This type of deviation with respect to the underlying statistical model assumptions is a common source of bias in model fitting. A typical example is fitting a regression model by OLS to data with severe heteroskedasticity (i.e., non-constant variance) (Bates and Watts, 2007). In a similar way, fitting the Poisson regression model to data whose variance does not follow the distribution expected by a Poisson likelihood results in biased model fits, as illustrated in Fig. 1B. This is further confirmed in Table 1, where the values of the parameter estimates for each model are reported. The Poisson regression model estimates a D-value of 27.0 min, much higher than the value estimated by the

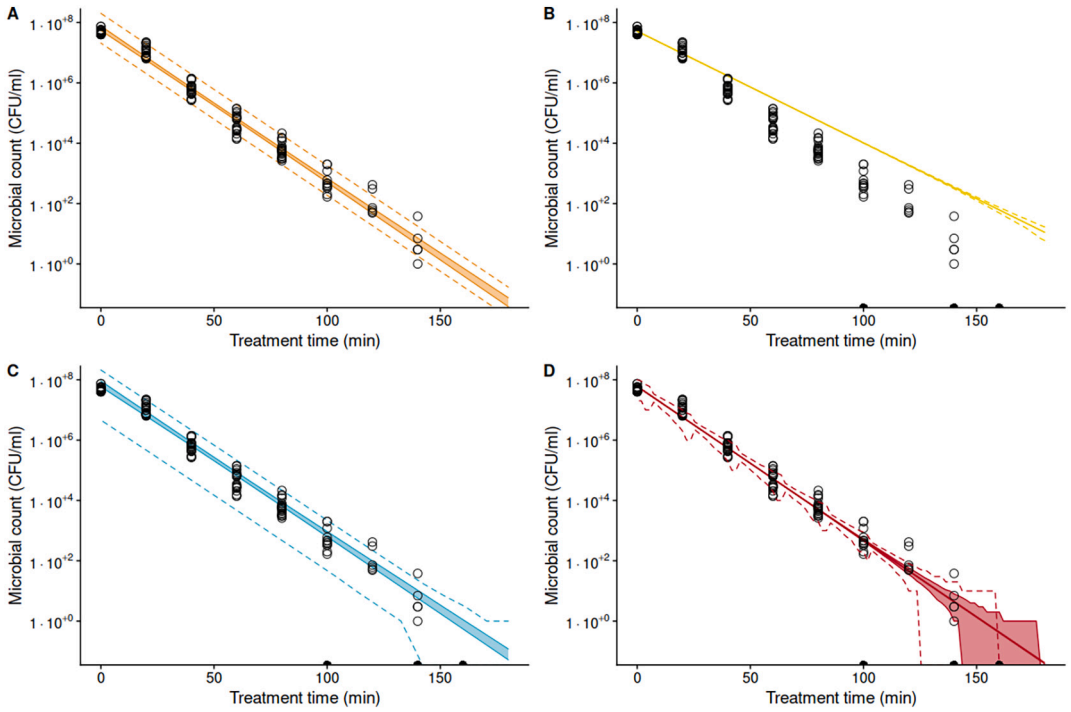

Fig. 1. Comparison of the models fitted to the inactivation data of *L. monocytogenes* FBR16 (o). The coloured ribbon shows the uncertainty of the primary model (90 % credible interval). The dashed line illustrates the uncertainty of the observations according to each model (90 % credible interval). The panels show the fit of each model: (A) Traditional regression using the normal likelihood for log *N*, (B) Poisson regression, (C) Gamma-Poisson regression, (D) Most Probable Curve (MPC). The latter has an additional coloured ribbon because this model introduces a separation between the primary model ($Y(t)$), the microbial concentration in the media (N_m) and the observations (N_{plate}). The additional ribbon illustrates the 90 % credible interval of N_m. Half circles on the x-axis represent plates with zero colonies (not included in panel A because they cannot be considered by the traditional method). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Model parameters estimated from the isothermal inactivation data on *L. monocytogenes* FBR16.

Model	Parameter	Estimate	Standard error[a]	90 % credible interval
Traditional	log N_0 (log CFU/ml)	7.81	0.04	[7.72, 7.89]
	D (min)	19.9	0.26	[19.4, 20.9]
	σ (log CFU/ml)	0.30	0.02	[0.26, 0.34]
Poisson	log N_0 (log CFU/ml)	7.71	0.00	[7.71, 7.71]
	D (min)	27.0	0.00	[27.0, 27.0]
Gamma-Poisson	log N_0 (log CFU/ml)	7.86	0.06	[7.75, 7.98]
	D (min)	20.1	0.34	[19.4, 20.8]
	ϕ (·)	1.09	0.12	[0.87, 1.35]
MPC	log N_0 (log CFU/ml)	7.78	0.01	[7.77, 7.79]
	D (min)	19.6	0.03	[19.6, 19.7]

[a] For kinetic parameters, the fit is done in log-scale, but the parameters are reported in their natural scale here. Hence, their standard error should not be interpreted as the one of a normal distribution.

traditional approach (19.9 min). An additional reason to suspect this model fit were problems during model fitting of this model with *Stan*. Unlike for the other models tested, the fit had to be repeated several times due to lack of convergence of the Markov chain. This is a common indicator of poor model hypotheses (McElreath, 2016).

The flexibility introduced by the overdispersion parameter of the Gamma-Poisson regression model ($\phi = 1.09 \pm 0.12$) mitigates many of the issues of the Poisson regression. As illustrated in Fig. 1C, the fitted model correctly described the overall trend of the observations. The Gamma-Poisson model practically fits the same primary inactivation model as the traditional approach (CI for D of [19.4, 20.9] min for the traditional; [19.4, 20.8] min for Gamma-Poisson), resulting in equivalent uncertainty in the primary model (coloured ribbon in Fig. 1C). Nevertheless, there are noticeable differences in the CI of the observed microbial counts between the Gamma-Poisson model and the traditional approach. Whereas the traditional approach calculates a CI with constant width, the Gamma-Poisson CI of the model widens at the end of the experiment, when the expected microbial count is lower than 1 log CFU/ml. Furthermore, the CI of the Gamma-Poisson model is asymmetrical, with its upper bound being similar to the traditional approach but with a tail towards lower microbial concentrations. Both effects are a reflection of the different likelihood function (Eq. (2); Eq. (8)). The Gamma-Poisson model uses a negative binomial distribution, an asymmetrical distribution defined for discrete random variables. As a logical result, the credible intervals for the observations are also asymmetrical. Furthermore, it has been demonstrated based on numerical simulations that discrete microbial counts result in an increased variance for low microbial concentrations (Garre et al., 2019, 2021). This is conveniently reflected in the Gamma-Poisson model due to the use of a discrete likelihood function.

The MPC method estimates similar D-values as the traditional approach, although with lower uncertainty (CI for D of [19.4, 20.9] min for the traditional; [19.6, 19.7] min for the MPC). As a result, the uncertainty of the primary model estimated by the MPC method (dark ribbon in Fig. 1D) has a similar expected value than for the traditional model, but a lower uncertainty with a very narrow band that may be mistaken for a line in Fig. 1D.

The reduction in the uncertainty of the parameters of the primary model is possible because the MPC method uses a multilevel approach that separates between three levels: (1) the ideal concentration according to the primary model ($Y(t)$; Eq. (6)), (2) the concentration in the media (N_{media}; Eq. (10)), and (3) the number of colonies that are

observed on/in a plate (N_{plate}; Eq. (12)). This separation is illustrated in Fig. 1D, where the MPC method has one additional coloured ribbon with respect to the other three methods. The additional interval represents the variation of N_{media} with respect to the concentration predicted by the primary model due to the effect of chance. As already described by Garre et al. (2021), this effect is negligible for large microbial concentrations. However, when the number of cells is low (approximately <100 CFU/ml) it becomes relevant, increasing the variance of the microbial concentration.

It must be highlighted that N_{media} cannot be observed directly. Instead, the experimental observations (the dots in Fig. 1) represent the microbial concentrations calculated based on the number of colonies on/in a plate using Eq. (1). Three of the methods tested (traditional method, Poisson regression, Gamma-Poisson regression) disregard this fact, calculating the likelihoods directly from the microbial concentrations (log-transformed or not) resulting from Eq. (1). Then, the sampling error of the serial dilution and plating method is integrated in the overall uncertainty (σ in the traditional approach, ϕ in the Gamma-Poisson), or disregarded (Poisson regression).

This is not the case for the MPC method, which makes a precise account of the uncertainty associated with the serial dilution and plating method. This can be done because the number of dilutions and the plated volume for each individual data point are retained for the likelihood calculation (Eq. (12)). Note that the MPC method does not need introducing an additional parameter, because these effects are described using mechanistic assumptions (i.e., chance and the sampling error of the plating) that are generally valid as long as their basic hypotheses hold (i.e., homogeneous resistance and no cell aggregation). This makes it different from the Gamma-Poisson model, which introduces an empirical relationship quantified by an additional parameter (Eq. (9)). In the MPC method, the unexplained uncertainty is partly included in the standard error of the parameter estimates. For instance, the standard error of log N_0 has a similar influence in the uncertainty of the model predictions as the overall variance (σ) of the traditional method.

The dashed interval in Fig. 1D illustrates the credible interval of the observed microbial concentrations according to the model fitted using the MPC method. The shape of this interval is extremely different from the one calculated using the other three methods, with its width increasing and decreasing through the treatment time, and largely increasing by the end of the experiment. The reason for this is that the variance of N_{plate} depends on the number of dilutions and the plated volume (Eq. (12)). Consequently, the sharp reductions in width of the CI correspond to time points where the number of dilutions is reduced by one. As a result, this interval is not directly comparable to the experimental data because it includes data from different dilutions for the same time point (e.g. $t = 80$ min includes data for dilution 0 and dilution −1). Another interesting aspect of the CI for the observations is that its upper limit reaches zero approximately at $t = 155$ min, earlier than the interval for the actual concentration in the media considering chance (N_m, approximately at $t = 125$ min). This is an illustration of the sampling error: when the microbial concentration is very low, the microorganism will not be detected in most plates (Duarte et al., 2015; Zwietering et al., 2021).

Fig. 2 provides a conceptual illustration of the impact of the plated volume and the number of dilutions in the variance of the observations. It is clear from this plot that the variance of the observations is strongly dependent on the experimental design, as previously illustrated based on numerical simulations (Garre et al., 2019). A comparison of Fig. 2A–B

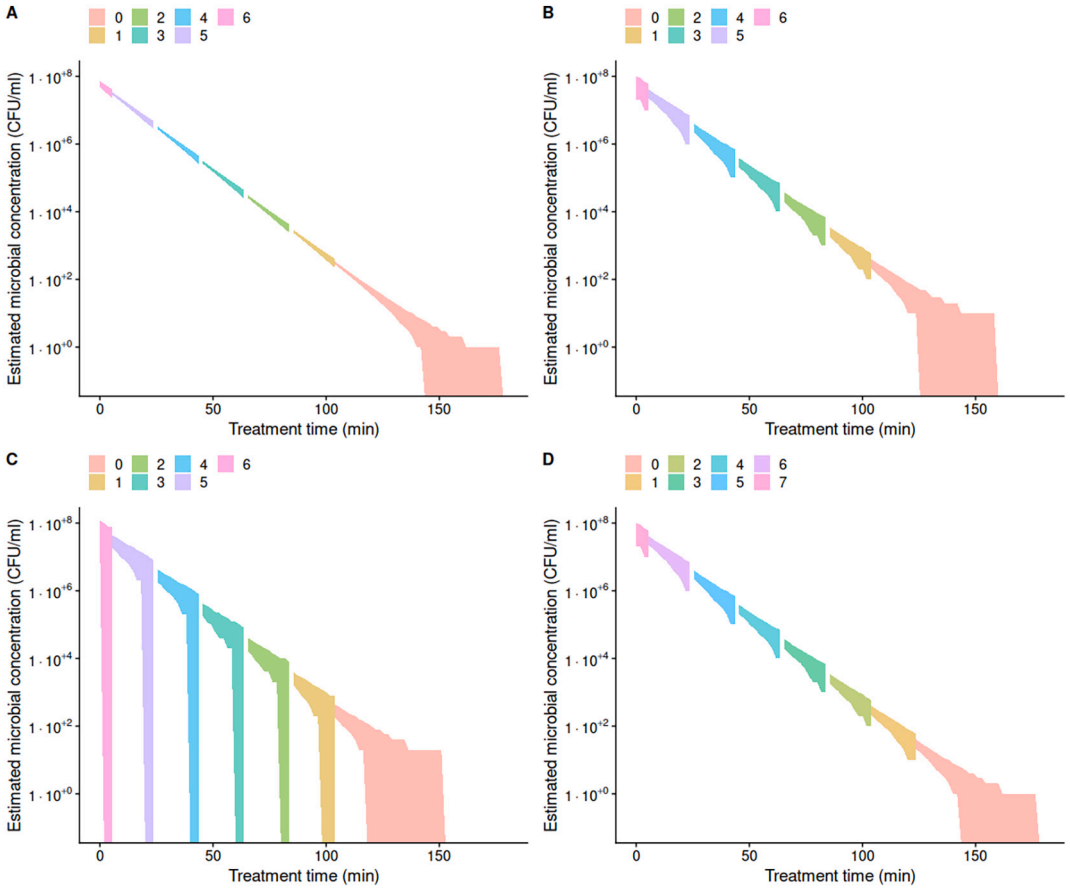

Fig. 2. Credible intervals of the microbial concentration estimated based on the plate counts accounting for the sampling error of the plating method for *L. monocytogenes* FBR16. The results are based on 1000 MC simulations for 4 different experimental designs: (A) $V_{plate} = 1$ ml; (B) $V_{plate} = 0.1$ ml; (C) $V_{plate} = 0.05$ ml; (D) $V_{plate} = 1$ ml with an increase in the number of dilutions. The number of serial dilutions before plating is illustrated by the colours of the shaded areas.

shows that a reduction in the plated volume from 1 ml to 0.1 ml results in a very relevant increase in uncertainty. Reducing the plated volume even more, to 0.05 ml (Fig. 2C), can potentially result in misleading empirical results. With this experimental design, the possibility of having plates without any colony is higher than 5 % for most time points, even at the beginning of the experiment. Therefore, it would be possible to obtain survivor curves where the microorganism is not observed for intermediate time points and "resuscitate" in posterior points. This resuscitation effect would not be the result of any biological mechanism; it would be an artefact caused by the sampling error of the serial dilution and plating method. This result emphasises the relevance of the plated volume and the number of dilutions for the design of microbial inactivation experiments.

Fig. 2 also illustrates that an alternative approach to reduce the plating variability that comes from the number of serial dilutions before plating is by modifying the number of serial dilutions before plating. As depicted in Fig. 2D, an increase in the number of dilutions significantly increases the variance of the observations. Furthermore, this plot also highlights that the variance is not constant. Because it is the reflection of an underlying Poisson distribution (Eq. (12)) that imposes a relationship between the expected value and the variance, it widens with the treatment time until it is reduced sharply when the next dilution is used. This result supports the recommendations that discourage the combination of microbial concentrations (in log CFU/ml or CFU/ml) calculated from different dilutions for the same time point when using the traditional model fitting approach (Garcés-Vega and Marks, 2014; Jarvis, 2008). Nonetheless, the MPC method accounts for the effect of the number of dilutions, so it can be used to fit models to data that combine different dilutions for the same time point.

The effect of the probability distribution illustrated in Fig. 2 is also relevant for optimal experiment design. Previous studies have increased the information extracted in the experiment by optimising the position of the sampling points or changing the temperature profile (Garre et al., 2018; Mertens et al., 2012; Peñalver-Soto et al., 2019; Telen et al., 2012; van Derlinden et al., 2010). However, all these studies were based on the hypothesis of normality of residuals with constant variance. As illustrated in Fig. 2, for an ideal scenario that considers only the sampling error of the dilutions, this hypothesis can be violated in greater or lower measure depending on the experimental design. Furthermore, the plated volume and the number of serial dilutions have a very strong influence on the variance of the observations. Therefore, they are suitable targets for optimal experiment design, in combination with the location of the time points and the type of inactivation treatment.

In conclusion, the introduction of mechanistic hypotheses regarding the sampling error of the plating method using a multilevel approach in the MPC method results in a significant reduction of the unexplained variance for linear microbial inactivation with respect to traditional approaches. It is worth highlighting that the credible interval relevant for microbial risk assessment, shelf life estimation or process design is the one referred to the microbial concentration in the product, not the one of the observations. In other words, the sampling error of the plating occurs in the laboratory, but not in the food chain. Hence, the MPC method can reduce the parameter uncertainty of the model in the case of log-linear inactivation. Although the Poisson regression and gamma-Poisson regressions are also based on the Poisson distribution, they do not make a distinction between the microbial concentration in the product and the one observed in the plate. Therefore, they do not introduce this reduction in uncertainty.

3.1.2. *Non-linear inactivation - L. monocytogenes strain FBR14*

The inactivation of *L. monocytogenes* FBR14 showed clear deviations with respect to non-linearity. Therefore, unlike for strain FBR16, the data for this strain was analysed using the Mafart inactivation model (Eq. (3); Eq. (7)). Fig. 3 compares the credible and prediction intervals

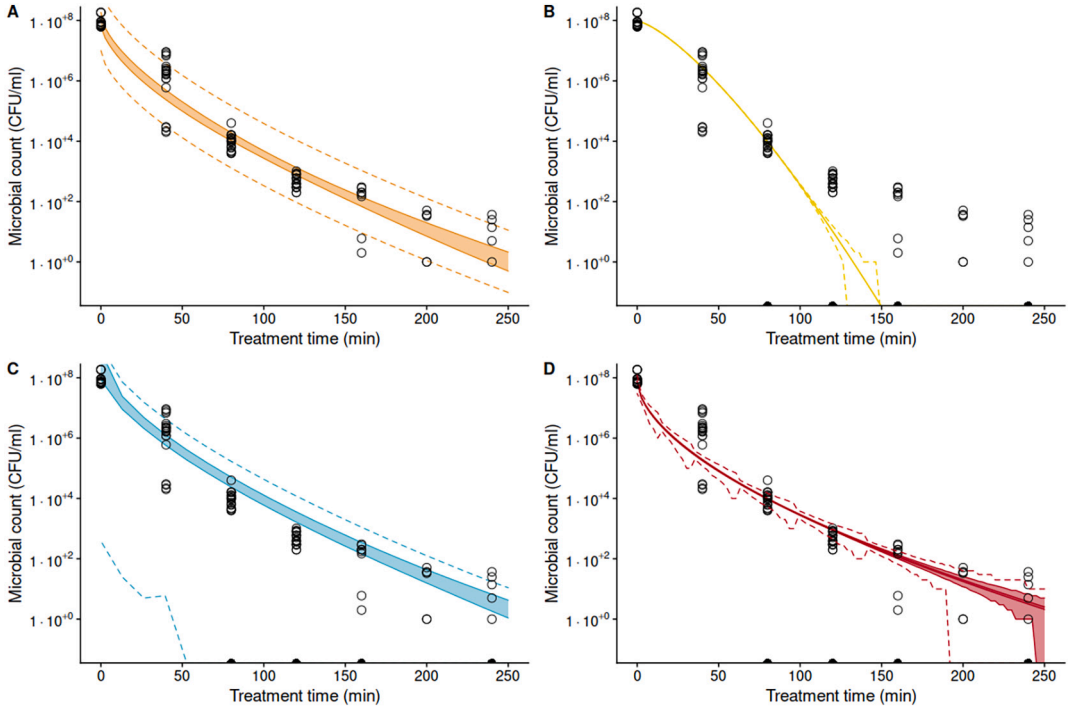

Fig. 3. Comparison of the models fitted to the inactivation data of *L. monocytogenes* FBR14 (o). The coloured ribbon shows the uncertainty of the primary model (90 % credible interval). The dashed line illustrates the uncertainty of the observations according to each model (90 % credible interval). The panels show the fit of each model: (A) Traditional regression using the normal likelihood, (B) Poisson regression, (C) Gamma-Poisson regression, (D) Most Probable Curve (MPC). The latter has an additional coloured ribbon because this model introduces a separation between the primary model ($Y(t)$), the microbial concentration in the media (N_m) and the observations (N_{plate}). The additional ribbon illustrates the 90 % credible interval of N_m. Half circles on the x-axis represent plates with zero colonies (not included in panel A because they cannot be considered by the traditional method). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

calculated for each of the four methods used in this study against the data used for model fitting, and Table 2 reports the parameter estimates. From a qualitative point of view, the results have similarities with those observed for strain FBR16 (Fig. 1). The traditional approach (Fig. 3A) correctly describes the general trend of the microbial counts during the treatment. Furthermore, as expected, this approach calculates a credible interval with constant width (in the y-direction) that envelops most of the observations.

As depicted in Fig. 3B, the Poisson regression model strongly deviates with respect to the general trend of the observations, even more severely than for strain FBR16 (Fig. 1B). Again, this is most likely due to the unrealistic likelihood used in this modelling approach. The variance in the data at the beginning of the experiment is much larger than predicted by this model (especially for $t = 40$ min). As a result, the Poisson regression model has unrealistic confidence intervals (Fig. 3B) and parameter values ($\delta = 29.1$ min; $\beta = 1.37$) that largely deviate with respect to those estimated for the other methods. Also in this case, the fitting algorithm had convergence issues when fitting this model, further highlighting the unsuitability of this model for this data. Note that, in spite of being unsuitable to describe the experimental data, the parameter estimates have very low standard deviation. This emphasises that the goodness of the fit should not be validated solely based on statistical indexes (such as the standard errors of the model parameters), and that it requires a more holistic approach (Dayal, 2015).

As well as for strain FBR16, the introduction of overdispersion in the Gamma-Poisson model largely mitigates the issues of the Poisson regression model. This model estimates a similar primary model than the traditional method ($\delta = [5.79, 16.0]$ min, $\beta = [0.57, 0.75]$ for Gamma-Poisson; $\delta = [6.5, 12.9]$ min, $\beta = [0.57, 0.70]$ for the traditional method) reflected in similar CI for the primary inactivation model (coloured ribbon in Fig. 3A and C). However, there are obvious differences between the CI of the observations. As well as for the linear case, the Gamma-Poisson model predicts an asymmetric CI, with an upper limit similar to the one of the traditional method and a much wider lower limit. This difference is more clear in this case due to the larger value of the overdispersion parameter ($\phi = 0.21$ for FBR14; $\phi = 1.09$ for FBR16), which can be interpreted as the model for FBR14 having more unexplained variation (higher uncertainty) than the one for FBR16.

One possible reason for this increase in uncertainty is the experimental design. The data for strain FBR14 combines at $t = 40$ min data obtained after 4 serial dilutions with data after 2 serial dilutions. As discussed above and illustrated in Fig. 2, the number of dilutions is a very significant factor for the variance of the observations. The traditional regression method explains differences between the expectation of the primary model and the observations using an overall error term (σ). However, the Gamma-Poisson model utilises a more complex relationship between the expected value and the variance through parameter ϕ that could be more sensitive to deviations in the data. Although this hypothesis should be further validated, this result emphasises the recommendation to avoid (when possible) the combination in the same time point of microbial concentrations calculated for different dilutions because these observations come from different distributions when the model fitting approach uses a likelihood function that does not consider the number of dilutions.

On the other hand, the MPC method does include the number of dilutions and the plated volume for parameter estimation, so it is more robust for experimental designs combining several dilutions. As well as for strain FBR16, the MPC method is able to describe the general trend of the observations, with narrower credible intervals than the traditional methods (Fig. 3D). This is again reflected in parameter estimates with lower uncertainty ($\delta = [7.3, 8.0]$ min, $\beta = [0.57, 0.59]$ for MPC; $\delta = [6.5, 12.9]$ min, $\beta = [0.57, 0.70]$ for the traditional). Similarly as for the linear case (Fig. 1D), this is due to part of the total uncertainty being assigned to the sampling error of the plating method (Supp. Fig. 1). Note that, although for this dataset the model has been fitted using a non-linear model (Eq. (7)), the MPC model formulated in such a way does not make any assumption regarding variability. Therefore, the reduction in the uncertainty in the concentration in the food product is due to a reduction of the unexplained uncertainty (Supp. Fig. 1), not due to the inclusion of variability. This lack of variability in the model can explain the relatively poor description of the data at $t = 40$ min. In the next section, we illustrate how this can be accounted for by augmenting the MPC method including biological variability.

Table 2
Model parameters estimated from the isothermal inactivation data of *L. monocytogenes* FBR14.

Model	Parameter	Estimate	Standard error[a]	90 % credible interval
Traditional	log N_0 (log CFU/ml)	8.04	0.14	[7.77, 8.31]
	δ (min)	9.5	1.6	[6.5, 12.9]
	β (·)	0.64	0.03	[0.57, 0.70]
	σ (log CFU/ml)	0.63	0.04	[0.55, 0.72]
Poisson	log N_0 (log CFU/ml)	7.98	0.00	[7.98, 7.98]
	δ (min)	29.1	0.00	[29.1, 29.1]
	β (·)	1.37	0.00	[1.37, 1.37]
Gamma-Poisson	log N_0 (log CFU/ml)	8.33	0.26	[7.9, 9.0]
	δ (min)	10.8	2.59	[5.79, 16.0]
	β (·)	0.66	0.04	[0.57, 0.75]
	ϕ (·)	0.21	0.02	[0.16, 0.26]
MPC	log N_0 (log CFU/ml)	7.88	0.01	[7.86, 7.89]
	δ (min)	7.64	0.18	[7.29, 7.98]
	β (·)	0.58	0.00	[0.57, 0.59]

[a] For parameter δ and β, the fit is done in log-scale, but the parameters are reported in their natural scale here. Hence, their standard error should not be interpreted as the one of a normal distribution.

3.2. MPC method with variability

3.2.1. Linear inactivation with variability - L. monocytogenes strain FBR16

The MPC method can be seen as an extension of the MPN method to estimate the parameters of inactivation models instead of just microbial concentrations at a single time point, by including the effect of the sampling error of the serial dilution and plating method. However, numerous sources of variability and uncertainty affect the variance of microbial inactivation. The MPC method, being a multilevel method, has the advantage that it can be extended to account for additional sources of variability and/or uncertainty (Garre et al., 2020). As an example, it was extended to include within-strain variability in the heat resistance of *L. monocytogenes* FBR 16.

Fig. 4 illustrates the model fitted using a multilevel model where the kinetic parameters (N_0 and D-value) vary between three independent biological replicates. The parameter estimates are included in Supp. Table 1. As expected, based on the goodness of fit of the single-level model, the multilevel-MPC method is able to describe the general trend of the data, showing slightly different D-values for each biological replicate. Again, the credible interval of the primary model is extremely low for the reasons explained above: the assignment of part of the total uncertainty to the sampling error of the plating method.

Although the plot shows three different fitted models (one per biological replicate), the multilevel approach assumes that the model parameters for each replicate are drawn from an underlying distribution (in this case, a log-normal distribution; Eq. (13)). Therefore, the three models illustrated in Fig. 4 are three replications of a unique model that includes a description of variability. This allows both an estimation of the impact of variability, as well as making predictions for biological replicates of *L. monocytogenes* FBR16 not observed in the laboratory.

Fig. 5A illustrates the estimated probability density function for the D-value of *L. monocytogenes* FBR16. This plot illustrates that, although the MPC method estimates a relatively small variability ($\sigma_{\log D} = 0.07$

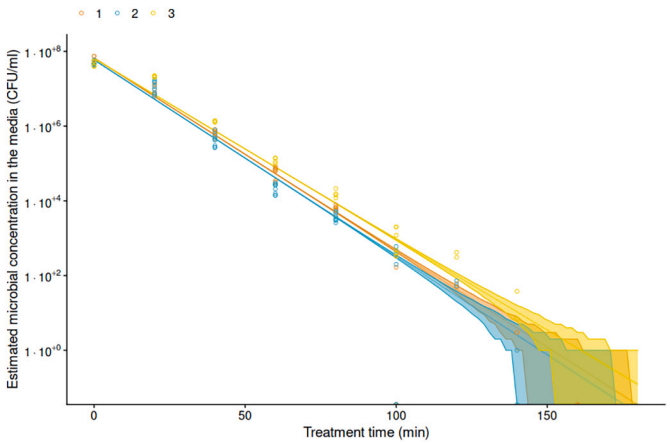

Fig. 4. Inactivation model fitted using the MPC method with within-strain variability to the isothermal inactivation data of *L. monocytogenes* FBR16 (o). The points were obtained from three independent bacterial cultures and are coloured accordingly. The dark ribbons represent the uncertainty of the primary model ($Y(t)$; 90 % credible intervals). The lighter ribbons represent the uncertainty associated to the microbial concentration in the media (N_{media}; 90 % credible intervals). They data points and ribbons are coloured according to the biological replicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

log min), this estimate is poorly characterised and has large uncertainty (CI of [0.01, 0.33] log min). This is an outcome of the amount of information included in the experimental data used to estimate this source of variability. Although it includes a reasonable number of data points per curve, it only includes three independent biological replicates. As a result, each individual replicate can be characterised with accuracy (Fig. 4), but the within-strain variability of the *D*-value can only be characterised with poor precision (i.e., with high uncertainty).

The high uncertainty in the variability estimates is reflected in the model predictions when variability is included. As illustrated in Fig. 5B, the estimated credible interval of the prediction mostly consists of uncertainty. This result can be surprising, when compared with the extremely low uncertainty of the model predictions for each individual biological replicate (Fig. 4) or for the complete data as a whole (Fig. 1D). Nonetheless, it is a logical reflection of the information included in the experimental data that allows to analyse each individual repetition accurately, but does not contain enough information to predict the variability of future repetitions.

This result emphasises the strong link that exists between statistical methods for model fitting and experimental design. The use of more complex statistical methods can improve how the parameters of empirical models (and their distributions) reflect the information included in the experimental data. However, these models can never include information beyond what is included in the data. As an illustration, inactivation experiments in predictive microbiology are often designed considering two different dimensions mirroring the division of the modelling procedure in primary and secondary models. The first dimension is related to the intensity of the inactivation agents (e.g., the number and range of treatment temperatures), and should be informative enough to describe the secondary model. The second dimension is the number and position of the time points, which should be adequate to describe the primary inactivation model (e.g., when there are shoulders in the data). In order to build reliable primary and secondary models, the experimental design at both levels must be adequate (Peñalver-Soto et al., 2019).

Including variability in inactivation models adds an additional dimension to the experimental design, which must be informative enough to estimate this attribute of the microbial population. Consequently, following the same logic, experimental studies aiming at the characterization of variability should account for a sufficiently large

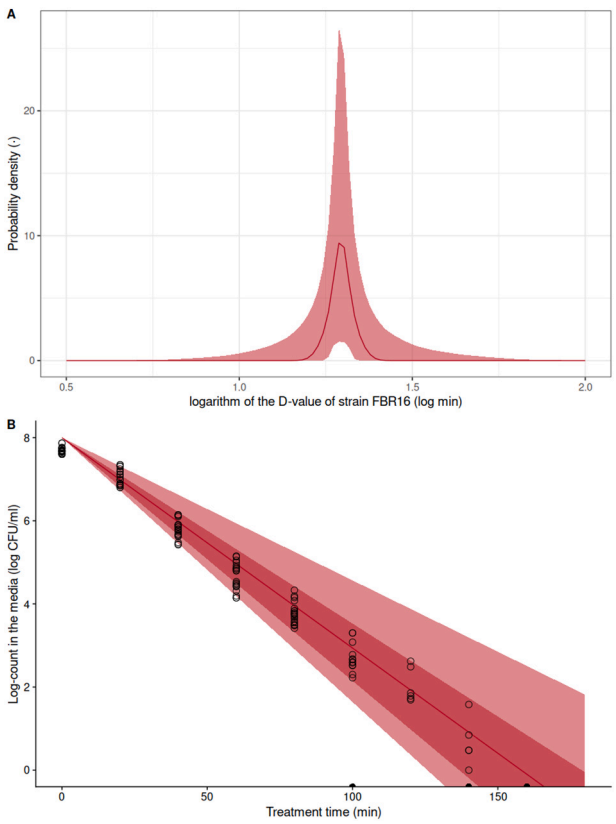

Fig. 5. (A) Probability density function of the variability of the *D*-value within strain FBR16. The solid line represents the expected value and the shaded area the 90 % credible interval (calculated using MC simulations with 1000 simulations). (B) Credible interval of the predicted microbial count of *L. monocytogenes* FBR16 in the media during an inactivation treatment at 55 °C for a fixed initial concentration of 8 log CFU/ml accounting for within-strain variability and the uncertainty associated to the estimate of variability (calculated using 100 MC simulations in the variability dimension and 10·100 MC simulations in the uncertainty dimension). The experimental data used to build the model is included for reference (o), although the interval does not include the effect of the experimental error.

number of conditions. In other words, they should include a sufficiently large number of "truly" new experiments (e.g., using independent biological cultures). Otherwise, although advanced data analysis tools (optimal experimental design, Bayesian statistics) can reduce the amount of experimental information required, it is impossible to obtain realistic estimates for variability if the data does not include enough information on variability.

3.2.2. *Non-linear inactivation with variability - L. monocytogenes strain FBR14*

Fig. 6 compares the models fitted by the MPC method including within-strain variability using a multilevel approach for *L. monocytogenes* FBR14. The values of the parameter estimates are reported in Supp. Table 2. As well as in the previous cases, the fitted model is able to describe the overall trend of the experimental data for each individual biological replicate. Of special interest is the curve fitted for the second repetition (yellow in Fig. 6). When the data is plotted on log-scale, it seems that the data for this repetition has a tail at concentrations slightly lower than 1 log CFU/ml. Indeed, fitting a model by the traditional approach to this repetition independently results in a survivor curve with upwards curvature ($\beta = 0.75$). However, a closer inspection of the data elucidates that this tail corresponds to observations with <6 colonies per plate, lower than the lower threshold often recommended (~30 colonies). It has been previously demonstrated that, under the

hypothesis of no aggregation of microbial cells, the error of the sampling can induce this type of tail when plates with low colony counts are used for model fitting (Garre et al., 2019). Furthermore, there are several plates without any colonies (half points on the x-axis) at $t = 80$ and $t = 120$ min that cannot be included in the traditional approach. Consequently, there are reasons to question whether this tail is a biological mechanism or an experimental artefact.

The traditional model for fitting inactivation models cannot account for this nuance, and will always find the best fitting line disregarding the number of colonies in the plate (i.e., it directly considers the concentration estimated by Eq. (1)). The MPC method, on the other hand, considers in the calculation of the likelihood the number of colonies observed, the number of dilutions and the plated volume. Consequently, it can account for both possibilities (curvature of the primary model, or plating error) and fit the most probable one. In this particular case, based on the available information and according to the model likelihood, the MPC method determines that it is more likely that this observed tail is an artefact of the plating. Nevertheless, it is always important to corroborate the results of complex statistical methods with expert knowledge. Although this approach introduces some hypotheses to consider the error of the plating in parameter estimation, these hypotheses are based on a "correct" procedure and idealizations. If there was any kind of experimental mistake (e.g., mislabelling of plates), or some biological mechanism (e.g., cell aggregation), it would not be compensated by the complex hypotheses of this method.

The large variation between the models fitted to each biological replicate is reflected in the estimates of variability for *L. monocytogenes* FBR14, as illustrated in Fig. 7. For this strain, we have used the Mafart primary inactivation model, where the curvature direction of the survivor curve is described by parameter β. When $\beta > 1$, the survivor curves have a downward curvature, whereas $\beta < 1$ results in the opposite. As illustrated in Fig. 7B, the variability distribution estimated for this parameter includes values both greater and smaller than $\beta = 1$ ($\overline{log\beta} = -0.2$; $\sigma_{log\beta} = 0.4$). An interpretation of this result is that, according to the experimental data and considering the likelihood function, the multilevel-MPC model could not conclude whether survivor curves for independent cultures not included in the experiment would have upward or downward curvature.

The estimates of variability also have large uncertainties (Fig. 7). This is not the result of some limitation of the modelling approach. Indeed, when the MPC method is used to describe the data as a whole (Fig. 3D) or for each repetition independently (Fig. 6), the method is able to estimate the primary model with lower uncertainty than the other methods. Furthermore, when the multilevel modelling approach is supplied with inactivation data from a large number of strains, it can estimate the variability with precision (Garre et al., 2020). Instead, this uncertainty is due to the amount of information included in the data (lack of information, in this case) because, as already mentioned for strain FBR16, variability is estimated based only on three experiments. In the case of strain FBR14, this lack of information is enlarged due to the larger discrepancies between the individual repetitions. This emphasises that every estimate of variability will have some uncertainty, and that it is important to include this uncertainty in model predictions. Although variability is often parameterized as a variance, it is a model parameter estimated from empirical data. Therefore, as any other parameter, its parameter uncertainty should be quantified using standard errors, confidence intervals, prediction intervals or similar statistical quantities.

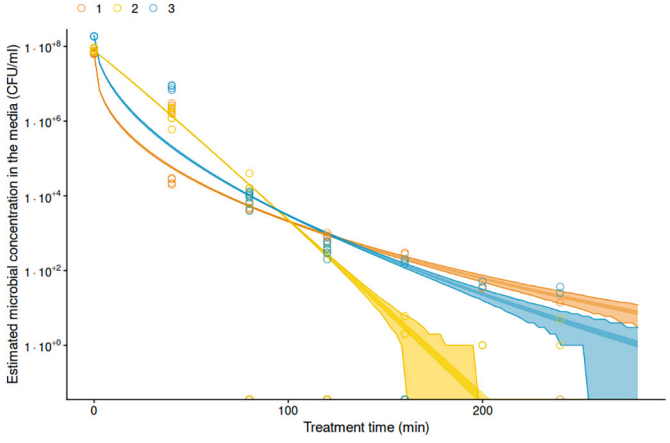

Fig. 6. Comparison of the data on the inactivation of *L. monocytogenes* FBR14 used to build the models (o) and the credible intervals of the models fitted at the 90 % level (ribbons). The points were obtained from three independent bacterial cultures and are coloured accordingly. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

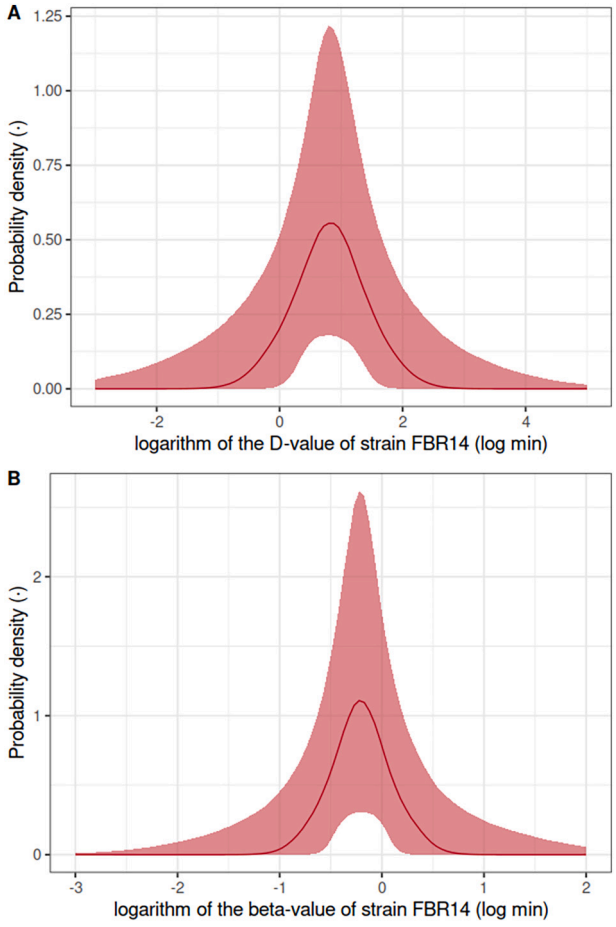

Fig. 7. Probability density function of the variability of the δ-value (A) and the β-value (B) within strain FBR14. The solid line represents the expected value and the shaded area the 90 % credible interval (calculated using MC simulations with 1000 simulations).

3.3. Final recommendations based on the comparison between the methods

The development of novel methods to estimate more accurate models from experimental data has received some attention during the last years (Chik et al., 2018; Duarte et al., 2015; Garre et al., 2020; Hiura et al., 2021; van Boekel, 2020, 2021). However, when developing such methods, it is important to reflect on the limitations of the reference method that the novel method must improve. In the case of microbial inactivation in liquid products, the reference method is undoubtedly

OLS regression using a frequentist approach. This approach assumes that the residuals follow a normal distribution with mean zero and constant variance (Eq. (2)). Microbial inactivation experiments often deviate from these hypotheses mainly for three reasons. The first one is that the normal distribution is defined for continuous values, whereas microbial concentrations are estimated based on the number of colonies counted on a Petri dish. Therefore, this empirical approach can only calculate a discrete set of microbial concentrations (although these discrete numbers do not have to be integers). If the expected number of colonies in the plate is high, this limitation is practically irrelevant. However, if the expected number of colonies is low (in the order of tens), the method can only estimate a limited range of values (Garre et al., 2019), resulting in a large deviation with respect to the hypothesis of normality of regression.

The second reason that can make experimental data on microbial inactivation deviate from the normality hypothesis is the fact that (under the simplifying assumptions described in Section 2.2.4) the number of colonies in a plate follows a Poisson distribution. If the expected number of colonies in a plate is high (in the order of hundreds), the probability mass function of the Poisson distribution is similar to the probability density function of a normal distribution. However, if the expected number of colonies in the plate is low (in the order of tens), the Poisson distribution has a right hand tail (Garre et al., 2019). As shown in Eq. (11), the expected value of the number of colonies on the plate depends both on the number of dilutions and the plated volume. This can result in observations with non-constant variance (Fig. 2; Supp. Fig. 1), introducing an additional deviation with respect to the model hypotheses. On top of this, the third deviation with respect to the assumptions of regression is the autocorrelation between the observations of each experiment, introducing a violation of the independence assumption of the residuals (van Boekel, 2021).

Consequently, the sampling error of the plating method introduces some violations of the stochastic hypotheses of regression. Nonetheless, the relevance of this deviation will depend on the experimental design. If the experimental protocol follows common recommendations, including only plates with >30 colonies (Jarvis, 2008), there should not be any relevant violation of the normality hypotheses (due to the sampling error) and the traditional method could be used safely. However, this is not always attainable in practical situations. The first step in experimental protocols is the preparation of a bacterial culture, which will seldom reach a concentration higher than 8 log CFU/ml. Next, experimental protocols often require at least one dilution before carrying out the experiments, so the initial microbial concentrations are often between 6 and 7 log CFU/ml. Considering that process design often targets a number of reductions in the order of 6 log-reductions, the concentration at the end of the experiment would be between 0 and 1 log CFU/ml. According to Eq. (12), in order to expect 100 colonies in a plate, a microbial concentration of 1 log CFU/ml would require pouring 10 ml (0.01 l) of liquid media in a Petri dish, a fully unpractical volume in most experimental settings. A concentration of 0 log CFU/ml would require an outrageous volume of 100 ml (0.1 l). As a result, microbial inactivation experiments often need to consider plates with low colony counts (in the order of tens), especially at the end of the experiment.

Considering that low plate counts can introduce a relevant violation on the hypotheses of regression, the development of novel methods to introduce this information robustly for model fitting can be of great interest. In this article, we have analysed three methods for this: Poisson regression (Eq. (5)), gamma-Poisson regression (Eq. (8)) and the MPC method (Eq. (11)), a novel approach proposed within this article. Our results show that the Poisson regression method has several limitations for microbial inactivation that generally discourage its application. Although it can account for discrete microbial counts, this method proposes an unrealistic likelihood function that results in biased parameter estimates. The reason for this deviation is that, although the microbial concentration during an inactivation treatment follows a Poisson distribution under some simplifying hypotheses (Garre et al., 2021), it is impossible to directly observe the microbial concentration in the media. Instead, we have to use as a surrogate of the number of colonies in a Petri dish after a given number of dilutions. Although (under simplifying assumptions (Garre et al., 2019)) the sampling error of the plating does not change the type of distribution, it does change its expected value, which depends both on the number of dilutions and the plated volume (Eq. (12)). As evidenced in Fig. 3, this results in a variance in the experimental observations that deviates severely with respect to the expectation of the Poisson regression (illustrated in Fig. 2B). Moreover, in Supp. Document 1, we replicate the fit of the data reported by Hiura et al. (2021), reaching the same conclusions. Therefore, the Poisson regression method would only be valid for data without any dilution, something extremely rare in microbial inactivation experiments.

In this article, we propose the MPC method for fitting microbial inactivation models in liquid media. It can be seen as an improvement upon Poisson regression that accounts for the sampling error of the plating and serial dilution, resulting in a more realistic likelihood function. Therefore, it is a robust method to include plates with low numbers of colonies in inactivation models, as well as for experimental designs that combine different dilutions (or plated volumes) for the same time point. A second advantage of the MPC method is that it separates between the "ideal" microbial concentration according to the primary model ($Y(t)$), the "actual" microbial concentration in the media considering the effect of chance (N_{media}), and the observed microbial count on/in a plate (N_{plate}). This can result in a large reduction in the uncertainty of the primary model parameters (Figs. 1, 3; Tables 1–2). It is worth highlighting that this uncertainty reduction is "for free", in the sense that it is not based on the introduction of additional model parameters (e.g., ϕ in Gamma-Poisson). It is based on the introduction of equations describing the sampling error of the plating method based on mechanistic hypotheses (as derived by Garre et al. (2019)). Although it has been used here to fit only primary models, its extension for fitting also secondary models in a one-step approach is trivial (Stan code available in https://github.com/albgarre/MostProbableCurve/blob/main/MPC_one_step.stan), and is also easily applicable to microbial growth.

The identification of different sources of uncertainty and variability, their quantification and their separation has received plenty of interest by the scientific community in recent years (den Besten et al., 2018; Garre et al., 2020; Jaloustre et al., 2012; Koyama et al., 2019). Nevertheless, it is important to reflect on the types of uncertainties included in a model, and whether they are of relevance for the case studied. In this sense, the uncertainty introduced by the sampling error of the plating is unique to the experimental setting. In the food chain, the consumer intakes every microbial cell in a serving, not a sample taken after a number of dilutions. Therefore, this source of uncertainty should, in general, not be part of a predictive model for MRA or shelf life estimation. Considering that the MPC method is able to separate its contribution, this method poses a clear advantage with respect to the other methods that include a single source of variance. Nonetheless, microbial inactivation is affected by numerous sources of variability and uncertainty. In this sense, the MPC method, being a multilevel model, has the advantage that it can be extended to include additional sources of variability and/or uncertainty.

Nonetheless, it is worth highlighting that the application of the MPC method as defined in Eq. (11) does not introduce any variability in the model. Variability is often defined as an inherent source of variance that is part of the system, so it cannot be reduced by gathering better or more information (Nauta, 2000). As illustrated in Fig. 2, the contribution of the plating method can be strongly modified using different experimental designs. Therefore, it is a source of uncertainty. Furthermore, even if the underlying inactivation model is non-linear (Eq. (7)), this equation does not introduce any sort of explicit hypotheses regarding variability. There are two hypotheses to explain the type of curvature observed in Weibullian inactivation models: a heterogeneous microbial

population with static resistance or a homogeneous population with dynamic resistance. Assuming a heterogeneous population would deviate from the hypothesis introduced by Garre et al. (2021) to justify the Poisson distribution within the heating media. Therefore, the MPC method is based on a homogeneous population; i.e., it does not include any sort of variability by default. In order to include variability, one would need to extend the multilevel model with explicit hypotheses on how the kinetic parameters vary within different levels (for instance, as illustrated in Sections 2.3 and 3.2).

In spite of its advantages with respect to other methods, the MPC method also has some limitations. The first one is the use of a different type of input. Instead of using the microbial concentration (e.g., log CFU/ml) calculated by Eq. (1) for different time points, it uses the number of colonies observed in a plate (CFU), the number of serial dilutions and the plated volume for each observation. This changes the way data should be stored and organised, and is a first hurdle (albeit minor) for the use of this method. A second (more relevant) limitation is that it uses a complex likelihood function (Eq. (12)). Although the model could potentially be fitted using frequentist statistics, it would require the application of advanced numerical algorithms, especially for the estimation of confidence intervals (Vilas et al., 2018). For that reason, it is advisable to fit the MPC model using Bayesian statistics. This introduces an additional complexity in terms of model definition, fitting, validation, interpretation and communication that should not be underestimated (Garre et al., 2020).

A third relevant limitation of the MPC approach is model validation. There are plenty of guidelines and recommendations for evaluating the goodness of the fit of regression models (Bates and Watts, 2007). However, most of these statistical tests and graphical checks are based on the hypotheses that the residuals follow a normal distribution with constant variance. Hence, they are not generally applicable for the MPC method because this method uses a different likelihood function (nor are they applicable for Poisson regression or Gamma-Poisson regression). This is a main limitation of the MPC method because it does not include a specific parameter to quantify the unexplained variance (such as σ in the traditional method or ϕ in Gamma-Poisson regression). Consequently, in a similar way as the Most Probable Number method, the robustness of the estimates by MPC is dependent on the underlying model hypotheses (described in Section 2.2.4) being true.

For this reason, the development of reliable methods to validate the goodness of the fit of this modelling approaches with non-normal likelihoods are a topic of great relevance for the application of methods with non-normal likelihoods, especially those lacking an error term. In this sense, the Bayesian approach has large potential due to its focus not only on fitting (also called "retrodiction" (McElreath, 2016)), but also on predictive capacity of the models. Therefore, methods like posterior predictive checks, WAIC (widely applicable information criterion), WBIC (Widely Applicable Bayesian Information Criterion) and loo-cv (leave-one-out-cross-validation, including Pareto smoothed importance sampling) could potentially be used to evaluate the goodness of this type of model (Gelman et al., 2014; Vehtari et al., 2017).

Considering the limitations of the MPC method and those of the traditional method summarised above, we recommend using the MPC method when the data contains a large number of plates with low counts ($<$30 colonies) or with zero colonies. If this is not the case, the standard regression method should return similar parameter estimates without the added complexity. The Gamma-Poisson method could be used as a convenience solution that mixes some advantages and disadvantages of both the traditional and MPC method. According to our results, Poisson regression has strong limitations for the analysis of microbial inactivation data, and its use should be discouraged against methods with more realistic likelihood functions.

Some of these limitations of the MPC method could be mitigated through the development of user-friendly (web) applications that allow using this advanced method without the need to implement its calculations (Possas et al., 2022). A first step is the development of R packages such as *rethinking* (McElreath, 2016) or *brms* (Bürkner, 2017), which simplify the definition of Bayesian models. Nonetheless, it is worth highlighting that a cook-and-look approach can be dangerous. In other words, it is necessary that researchers are aware of the underlying (statistical) hypotheses of models. In this sense, the Bayesian approach can be seen as an advantage because it "forces" researchers to communicate these assumptions explicitly (van Boekel, 2020).

4. Conclusions

This article presents a new method to fit microbial inactivation models from data on liquid media: the Most Probable Curve (MPC) method. It is a multilevel model based on two hypotheses: homogeneous stress resistance within the microbial population, and the lack of aggregation of microbial cells. Under these assumptions, it can be concluded based on mechanistic arguments that the number of colonies in/on a plate follows a Poisson distribution with expected value depending on the kinetic parameters, the number of dilutions and the plated volume.

A comparison between the MPC method, traditional regression, Poisson regression and Gamma-Poisson regression highlights that the traditional method loses robustness when the inactivation data includes a large number of plates with low or zero counts. In those cases, the Gamma-Poisson and the MPC methods can improve the robustness of the estimates. Despite the fact that the low and zero count data contain less robust information, the information is accounted for statistically correct in contrary to the traditional method. Poisson regression defines a likelihood function that seems unrealistic for high microbial concentrations, so its application is discouraged.

An advantage of the MPC with respect to the other methods tested is a separation between the uncertainty of the primary model, the effect of chance and the uncertainty associated with the sampling error of the plating method. This results in a significant reduction of the prediction uncertainty for microbial risk assessment and shelf life estimation. On the other hand, the MPC method involves additional complexity in model definition, implementation, validation, and communication. Based on our results, we conclude that the MPC method can be of great interest for parameter estimation in predictive microbiology, especially from data that include low plate counts. Furthermore, its hypotheses can be used to support (optimal) experiment design, including the effect of the number of dilutions and the plated volume on the variance of the experimental observations. Consequently, we anticipate that the method will be of great interest for predictive microbiologists, especially in studies focused on variability analysis.

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Declaration of competing interest

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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Data availability

Data will be made available on request.

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References

Alexander, M., 1965. Most-probable-number method for microbial populations. In: Methods of Soil Analysis. John Wiley & Sons, Ltd, pp. 1467–1472. https://doi.org/10.2134/agronmonogr9.2.c49.

Allende, A., Bover-Cid, S., Fernández, P.S., 2022. Challenges and opportunities related to the use of innovative modelling approaches and tools for microbiological food safety management. Curr. Opin. Food Sci. 100839 https://doi.org/10.1016/j.cofs.2022.100839.

Aryani, D.C., den Besten, H.M.W., Hazeleger, W.C., Zwietering, M.H., 2015. Quantifying variability on thermal resistance of listeria monocytogenes. Int. J. Food Microbiol. 193, 130–138. https://doi.org/10.1016/j.ijfoodmicro.2014.10.021.

Aspridou, Z., Koutsoumanis, K., 2020. Variability in microbial inactivation: from deterministic Bigelow model to probability distribution of single cell inactivation times. Food Res. Int. 137, 109579 https://doi.org/10.1016/j.foodres.2020.109579.

Bates, D.M., Watts, D.G., 2007. Nonlinear Regression Analysis and Its Applications, 1 edition. Wiley-Interscience.

Brooks, S., 2011. Handbook of Markov Chain Monte Carlo. https://doi.org/10.1201/b10905.

Bürkner, P.-C., 2017. Brms: an R package for Bayesian multilevel models using Stan. J. Stat. Softw. 80 (1), 1–28. https://doi.org/10.18637/jss.v080.i01.

Carpenter, B., Gelman, A., Hoffman, M.D., Lee, D., Goodrich, B., Betancourt, M., Brubaker, M., Guo, J., Li, P., Riddell, A., 2017. Stan: a probabilistic programming language. J. Stat. Softw. 76 (1), 1–32. https://doi.org/10.18637/jss.v076.i01.

Chik, A.H.S., Schmidt, P.J., Emelko, M.B., 2018. Learning something from nothing: the critical importance of rethinking microbial non-detects. Front. Microbiol. 9, 2304. https://doi.org/10.3389/fmicb.2018.02304.

Dayal, V., 2015. Anscombe's quartet: graphs can reveal. In: Dayal, V. (Ed.), An Introduction to R for Quantitative Economics: Graphing, Simulating and Computing. Springer, India, pp. 59–63. https://doi.org/10.1007/978-81-322-2340-5_9.

den Besten, H.M.W., Wells-Bennik, M.H.J., Zwietering, M.H., 2018. Natural diversity in heat resistance of bacteria and bacterial spores: impact on food safety and quality. Annu. Rev. Food Sci. Technol. 9 (1), 383–410. https://doi.org/10.1146/annurev-food-030117-012808.

Duarte, A.S.R., Stockmarr, A., Nauta, M.J., 2015. Fitting a distribution to microbial counts: making sense of zeroes. Int. J. Food Microbiol. 196, 40–50. https://doi.org/10.1016/j.ijfoodmicro.2014.11.023.

Garcés-Vega, F., Marks, B.P., 2014. Use of simulation tools to illustrate the effect of data management practices for low and negative plate counts on the estimated parameters of microbial reduction models. J. Food Prot. 77 (8), 1372–1379. https://doi.org/10.4315/0362-028X.JFP-13-462.

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., Egea, J.A., Esnoz, A., Palop, A., Fernandez, P.S., 2019. Tail or artefact? Illustration of the impact that uncertainty of the serial dilution and cell enumeration methods has on microbial inactivation. Food Res. Int. 119, 76–83. https://doi.org/10.1016/j.foodres.2019.01.059.

Garre, A., González-Tejedor, G., Peñalver-Soto, J.L., Fernández, P.S., Egea, J.A., 2018. 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., Zwietering, M.H., den Besten, H.M.W., 2020. Multilevel modelling as a tool to include variability and uncertainty in quantitative microbiology and risk assessment. Thermal inactivation of listeria monocytogenes as proof of concept. Food Res. Int. 137, 109374 https://doi.org/10.1016/j.foodres.2020.109374.

Gelman, A., Hwang, J., Vehtari, A., 2014. Understanding predictive information criteria for Bayesian models. Stat. Comput. 24 (6), 997–1016. https://doi.org/10.1007/s11222-013-9416-2.

Gorris, L.G.M., 2005. Food safety objective: an integral part of food chain management. Food Control 16 (9), 801–809.

Guillén, S., Nadal, L., Álvarez, I., Mañas, P., Cebrián, G., 2021. Impact of the resistance responses to stress conditions encountered in food and food processing environments on the virulence and growth fitness of non-typhoidal salmonellae. Foods 10 (3), 617. https://doi.org/10.3390/foods10030617.

Hiura, S., Abe, H., Koyama, K., Koseki, S., 2021. Bayesian generalized linear model for simulating bacterial inactivation/growth considering variability and uncertainty. Front. Microbiol. 12, 1492. https://doi.org/10.3389/fmicb.2021.674364.

Jaloustre, S., Guillier, L., Morelli, E., Noël, V., Delignette-Muller, M.L., 2012. Modeling of Clostridium perfringens vegetative cell inactivation in beef-in-sauce products: a meta-analysis using mixed linear models. Int. J. Food Microbiol. 154 (1), 44–51. https://doi.org/10.1016/j.ijfoodmicro.2011.12.013.

Jarvis, B., 2008. Statistical Aspects of the Microbiological Examination of Foods. Elsevier Academic Press.

Koyama, K., Aspridou, Z., Koseki, S., Koutsoumanis, K., 2019. Describing uncertainty in salmonella thermal inactivation using Bayesian statistical modeling. Front. Microbiol. 10, 2239. https://doi.org/10.3389/fmicb.2019.02239.

Mafart, P., Couvert, O., Gaillard, S., Leguerinel, I., 2002. On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. Int. J. Food Microbiol. 72 (1–2), 107–113. https://doi.org/10.1016/S0168-1605(01)00624-9.

McElreath, R., 2016. Statistical Rethinking: A Bayesian Course With Examples in R and Stan. CRC Press/Taylor & Francis Group.

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 (3), 119–133. https://doi.org/10.1016/j.jfoodeng.2012.03.018.

Nauta, M.J., 2000. Separation of uncertainty and variability in quantitative microbial risk assessment models. Int. J. Food Microbiol. 57 (1), 9–18.

National Advisory Committee on microbiological criteria for foods, 2010. Parameters for determining inoculated pack/challenge study protocols. J. Food Prot. 73 (1), 140–202.

Peleg, M., Cole, M.B., 1998. Reinterpretation of microbial survival curves. Crit. Rev. Food Sci. Nutr. 38 (5), 353–380. https://doi.org/10.1080/10408699891274246.

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.

Peng, J., Tang, J., Barrett, D.M., Sablani, S.S., Anderson, N., Powers, J.R., 2017. Thermal pasteurization of ready-to-eat foods and vegetables: critical factors for process design and effects on quality. Crit. Rev. Food Sci. Nutr. 57 (14), 2970–2995. https://doi.org/10.1080/10408398.2015.1082126.

Perez-Rodriguez, F., Valero, A., 2012. Predictive Microbiology in Foods. Springer.

Possas, A., Valero, A., Pérez-Rodríguez, F., 2022. New software solutions for microbiological food safety assessment and management. Curr. Opin. Food Sci. 44, 100814 https://doi.org/10.1016/j.cofs.2022.100814.

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.R-project.org/.

Smelt, J.P.P.M., Brul, S., 2014. Thermal inactivation of microorganisms. Crit. Rev. Food Sci. Nutr. 54 (10), 1371–1385. https://doi.org/10.1080/10408398.2011.637645.

Stan Development Team, 2019. RStan: The R Interface to Stan. http://mc-stan.org/.

Telen, D., Logist, F., Van Derlinden, E., Tack, I., Van Impe, J., 2012. Optimal experiment design for dynamic bioprocesses: a multi-objective approach. Chem. Eng. Sci. 78, 82–97. https://doi.org/10.1016/j.ces.2012.05.002.

van Boekel, M.A.J.S., 2020. On the pros and cons of bayesian kinetic modeling in food science. Trends Food Sci. Technol. 99, 181–193. https://doi.org/10.1016/j.tifs.2020.02.027.

van Boekel, M.A.J.S., 2021. To pool or not to pool: that is the question in microbial kinetics. Int. J. Food Microbiol. 354, 109283 https://doi.org/10.1016/j.ijfoodmicro.2021.109283.

van Boekel, M.A., ter Steeg, P.F., Dahoe, A.E., 2020. Co-optimization of safety, quality and legislation: opening Pandora's box? Curr. Opin. Food Sci. 35, 65–71. https://doi.org/10.1016/j.cofs.2020.02.001.

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. Nova Publishers, pp. 67–98. https://www.novapublishers.com/catalog/product_info.php?products_id=29798.

Vehtari, A., Gelman, A., Gabry, J., 2017. Practical bayesian model evaluation using leave-one-out cross-validation and WAIC. Stat. Comput. 27 (5), 1413–1432. https://doi.org/10.1007/s11222-016-9696-4.

Vilas, C., Arias-Mendez, A., Garcia, M.R., Alonso, A.A., Balsa-Canto, E., 2018. Toward predictive food process models: a protocol for parameter estimation. Crit. Rev. Food Sci. Nutr. 58 (3), 436–449. https://doi.org/10.1080/10408398.2016.1186591.

Vose, D., 2008. Risk Analysis—A Quantitative Guide, 3rd ed. Wiley.

Zwietering, M.H., Garre, A., Wiedmann, M., Buchanan, R.L., 2021. All food processes have a residual risk, some are small, some very small and some are extremely small: zero risk does not exist. Curr. Opin. Food Sci. 39, 83–92. https://doi.org/10.1016/j.cofs.2020.12.017.