Food Control 29 (2013) 401–408

Contents lists available at SciVerse ScienceDirect

Food Control

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

Parameter estimation for dynamic microbial inactivation: which model, which precision?

K.D. Dolan [a,b,*], V.P. Valdramidis [c], D.K. Mishra [a,d]

[a] Department of Biosystems & Agricultural Engineering, Michigan State University, East Lansing, MI 48824, USA
[b] Department of Food Science & Human Nutrition, Michigan State University, East Lansing, MI 48824, USA
[c] Department of Food Studies & Environmental Health, Faculty of Health Sciences, University of Malta, Msida 2080, Malta
[d] Néstle Nutrition, Fremont, MI, USA

ARTICLE INFO

Article history:
Received 2 January 2012
Received in revised form
22 April 2012
Accepted 16 May 2012

Keywords:
Microbial inactivation modeling
Parameter estimation
Non-isothermal
Reference temperature
Sublethal
Sequential estimation

ABSTRACT

Ordinary least squares (OLS) one-step regression and the sequential procedure were applied to estimate the dynamic thermal microbial inactivation parameters of *Escherichia coli* K12 using the differential form of five different models. The best-performing models based on their statistical assessment were, in order: Geeraerd et al. sublethal (7 parameters), Geeraerd et al. stress adaptive (7 parameters); reduced Geeraerd et al. (6 parameters), Weibull (6 parameters), and the first-order model (5 parameters) all integrated with the secondary Bigelow model. The statistics used to evaluate the models were: lowest AIC_c, minimum root mean square error (RMSE); distribution of residuals; asymptotic relative errors of parameters; scaled sensitivity coefficients; and sequential estimation. RMSE for the first-order model was more than twice that for Geeraerd et al. sublethal model, showing that the first-order model was inappropriate for these data. The optimum reference temperature (T_{ref}) for the secondary model (Bigelow type) was interpolated by estimating all other parameters for different fixed T_{ref} values, and choosing T_{ref} that minimized the correlation coefficient between $AsymD_{ref}$ and z. The advantage of finding the optimum T_{ref} was that it minimized the relative error for $AsymD_{ref}$. Scaled sensitivity coefficients of the Geeraerd et al. sublethal model revealed that a) none of the parameters was linearly correlated with others, and b) that the most easily estimated parameters were the three initial microbial concentrations $\log N(0)$, followed by $AsymD_{ref}$, z, $\log C_c(0)$, and sublethal β. The sequential method was also applied to estimate updated parameter values by successively adding each data point. Sequential results showed that each parameter reached a constant after ~2.5 log reductions. These results show that a) parameters may be affected by rate of heating, and b) dynamic microbial inactivation parameters can be estimated accurately and precisely, directly from few experiments, potentially eliminating the need to apply isothermal parameters to dynamic industrial processes.

1. Introduction

Transposition of results obtained from static to dynamic conditions has shown that adjustment of the initial mathematical structure is required (Bernaerts, Servaes, Kooyman, Versyck, & Van Impe, 2002; Dolan, 2003). A similar study has illustrated that inactivation model equations and their associated parameter values obtained under static acid stress conditions cannot be used directly for predicting inactivation under dynamic conditions limiting the value and reliability of the developed mathematical tools (Janssen et al., 2008). Dolan (2003) and Valdramidis, Geeraerd, Bernaerts, and Van Impe (2008) have also highlighted that even if the results are excellent by the use of isothermal inactivation parameters one does not know the actual values of non-isothermal estimates. These observations pinpoint the importance of further studying parameter identification techniques under dynamic conditions representative of a realistic (processing) environment.

Several microbial studies revealed that microbial adaptations are evident at different types of stressful environments (e.g., Skandamis, Stopforth, Yoon, Kendall, & Sofos, 2009; Valdramidis, Geeraerd, & Van Impe, 2007; Velliou et al., 2011). These environments have an impact on the physiological state of the microorganisms and can result in an increase of their microbial resistance or a change to their adaptation time. Recent investigators tried to

* Corresponding author. Department of Food Science & Human Nutrition, Michigan State University, 135 Trout Food Science Building, East Lansing, MI 48824, USA. Tel.: +1 517 355 8474x119; fax: +1 517 353 8963.
E-mail addresses: dolank@msu.edu (K.D. Dolan), vasilis.valdramdis@um.edu.mt (V.P. Valdramidis), Dharmendra.Mishra@rd.nestle.com (D.K. Mishra).

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doi:10.1016/j.foodcont.2012.05.042

Table 1
Statistical ordinary least squares (OLS) results for all models ($n = 96$). Parameters were estimated for all the heating rates simultaneously.

Model	# of parameters	SSE ($\log_{10}$(cfu/mL))^2	RMSE ($\log_{10}$(cfu/mL))	Optimum T_{ref} (°C)	AIC_c
1) First-order	5	17.18	0.435	57.983	−152.2
2) Weibull	6	7.63	0.291	58.681	−227.8
3) Geeraerd et al.	6	4.40	0.221	58.117	−280.6
4) Geeraerd et al. with $k(<phys>)$	7	3.66	0.203	63.246	−296.0
5) Geeraerd et al. with τ	7	3.46	0.197	62.177	−301.3

deal with these phenomena by adjusting or extending widely used mathematical structures. In the field of microbial inactivation modeling some examples include the development of (i) a model building block related to the microbial physiology ($k<$phys$>$) (Valdramidis et al., 2007), (ii) a factor describing the sublethal thermal history (Stasiewicz, Marks, Orta-Ramirez, & Smith, 2008), (iii) an extension of the Weibullian-log logistic model by the introduction of a logistic adaptation factor (Corradini & Peleg, 2009).

Combining knowledge coming from parameter identification techniques applied under dynamic conditions and the model structure properties proposed to handle the microbial physiological state is a challenging issue. The objectives of this work were: (1) to demonstrate that non-isothermal microbial inactivation kinetic model parameters could be accurately and precisely estimated using one-step nonlinear regression following an ordinary least squares and a sequential approach; and (2) to determine based on statistical indices the best-performing out of five differential models some of which can account for the microbial sublethal/induced thermal resistance. This paper comes out of a presentation (Dolan, Valdramidis, & Mishra, 2011).

2. Materials and methods

2.1. Experimental

Previously published data (Valdramidis et al., 2008) were used. Briefly, liquid samples of 100 μL with initial *Escherichia coli* K12 microbial concentration $\sim 10^9$ cfu/mL were heated at three different rates (fast: 1.64, intermediate: 0.43, or slow: 0.15 °C/min), in duplicate, from 49.5 °C to 60 °C over a total experimental time from 11 to 60 min.

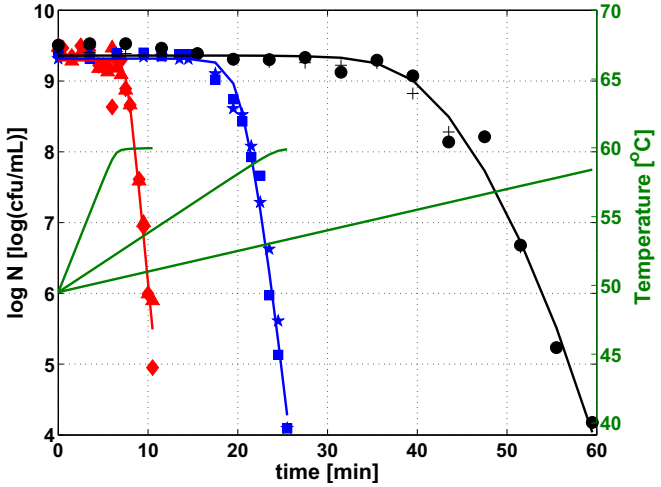

Fig. 1. Observed (markers) and fitted (lines) values for Geeraerd et al. with sublethal (model #5) ($n = 96$).

2.2. Calculation

2.2.1. Models

Five different types of models were used for this study: (1) a first-order (parameters were $\log_{10}N(0)_1$, $\log_{10}N(0)_2$, $\log_{10}N(0)_3$, D_{ref}, and z), (2) a Weibull (refer to its differential form; parameters were $\log_{10}N(0)_1$, $\log_{10}N(0)_2$, $\log_{10}N(0)_3$, δ, z, and p) (Albert and Mafart, 2005) (3) the reduced model of Geeraerd, Herremans, & Van Impe (2000) not incorporating the so called tailing effect (hereafter called the "Geeraerd et al. model"; parameters were $\log_{10}N(0)_1$, $\log_{10}N(0)_2$, $\log_{10}N(0)_3$, AsymD_{ref}, and z), (4) the Geeraerd et al. model with a stress adaptive rate term (additional parameter k_1), and (5) the Geeraerd et al. model with a sublethal integral term (same parameters as #3, plus additional parameter β). The Geeraerd et al. model reads as follows:

$$\frac{dN(t)}{dt} = k \cdot N(t) \Rightarrow \frac{d\log N(t)}{dt} = -\frac{1}{\ln 10} \cdot k \quad (1)$$

$$\frac{d\log C_c(t)}{dt} = -\frac{1}{\ln 10} \cdot k_{max} \quad (2)$$

where k is

$$k = k_{max} \cdot \left(\frac{1}{1 + 10^{\log C_c}} \right) \quad (3)$$

and k_{max} is given by the Bigelow model

$$k_{max} = \frac{\ln 10}{\text{Asym}D_{ref}} \cdot \exp\left(\frac{\ln 10}{z} \left(T - T_{ref} \right) \right) \quad (4)$$

N is the microbial cell density cfu/mL, C_c is related to the physiological state of cells [−], k_{max} is the specific inactivation rate [1/min], AsymD_{ref} is the asymptotic decimal reduction time at reference temperature T_{ref}, and z is the degrees Celsius temperature change causing a 10-fold change in AsymD_{ref}.

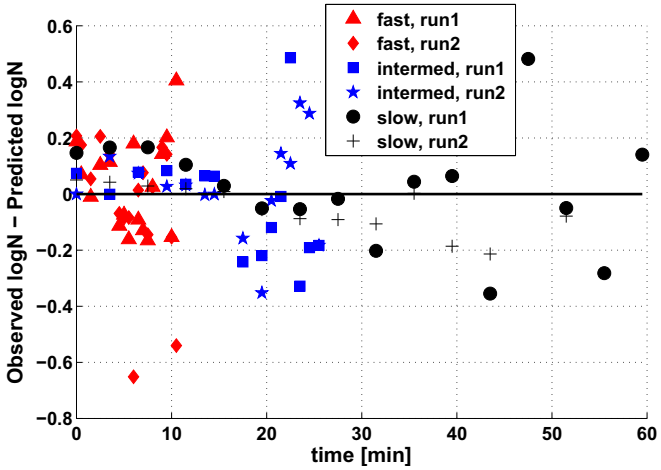

Fig. 2. Residual scatter plot for Geeraerd et al. with sublethal (model #5). Data markers for the six runs are identical to those in Fig. 1.