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A suitable model of microbial growth

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A microbial growth model with three interpretable parameters was introduced in this study. This model, which was only applicable up to the early stationary phase of the microbial growth, was used to describe the published isothermal growth curves of *Listeria monocytogenes* in milk at 1.5 - 16°C and temperature dependence of its parameters was fitted by ad hoc empirical secondary models. Published isothermal growth curves of *Pseudomonas* spp. in poultry at 2 - 20°C were also fitted with this model but in this case one of the parameters in this model was fixed to reduce the number of parameters. This reduced model produced almost the same goodness-of- fit as the full model. Although more studies should be carried out to evaluate the applicability of the proposed model under dynamic temperature conditions, this study has shown that microbial growth data -up to early stationary phase- could be successfully described by this model.

Key words: Growth curves, *Listeria monocytogenes*, *Pseudomonas* spp., mathematical models, predictive microbiology.

INTRODUCTION

Modeling the microbial growth in food is a basic tool for the prediction of food safety and shelf-life in the food chain (McMeekin et al., 1993) and throughout the years numerous growth models have been published. In general, the growth of a homogeneous microbial population can be described by a curve with three phases if the death phase is excluded: a lag phase (adaptation period of microbial cells to their new environment) followed by an exponential growth phase (multiplication of cells exponentially) and finally a stationary phase (reaching to the maximum population density) (Isabelle et al., 2006).

The continuous logistic equation proposed by Verhulst (1838) has been a most illustrative model of organism's growth dynamics in a habitat of finite sources. One can find the improved versions of the logistic model in literature (Fujikawa et al., 2004). Gompertz model [originally proposed by Gompertz (1825)] and its modified versions (Zwietering et al., 1990; Smith and Schaffner, 2004) were widely used to describe the sigmoid growth curves. The Baranyi and Roberts model (1994), which was derived from the logistic function, is also one of the widely used models to describe isothermal and non-isothermal growth curves of microorganisms today. There are also some other, recently proposed, growth models (Van Impe et al., 2005; Poschet et al., 2005).

While all the mentioned models whether in their original or modified forms can be used to fit isothermal growth data, none of them can be considered as unique. Moreover, "as many sigmoid growth curves can be described by mathematical expressions having only three adjustable parameters, the fit of above models (since they have at least four adjustable parameters; depending on how you define the terms) should not come as a surprise" (Corradini and Peleg, 2005). The aim of this study was to introduce a new empirical equation with clearly interpretable parameters to be used to describe isothermal microbial growth.

MATERIALS AND METHODS

Theory

Equation (1) is proposed to describe the microbial growth in different media. This equation was used to describe the plant growth by Yin et al. (2003). In that study the mathematical derivation of Equation (1) from a differential form was also shown.

$$\log_{10}\frac{N(t)}{N_0} = A\left(1+\frac{t_A - t}{t_A - t_m}\right)\frac{t}{t_A}\exp\left(\frac{t_A - t}{t_A - t_m}\right) \quad (1)$$

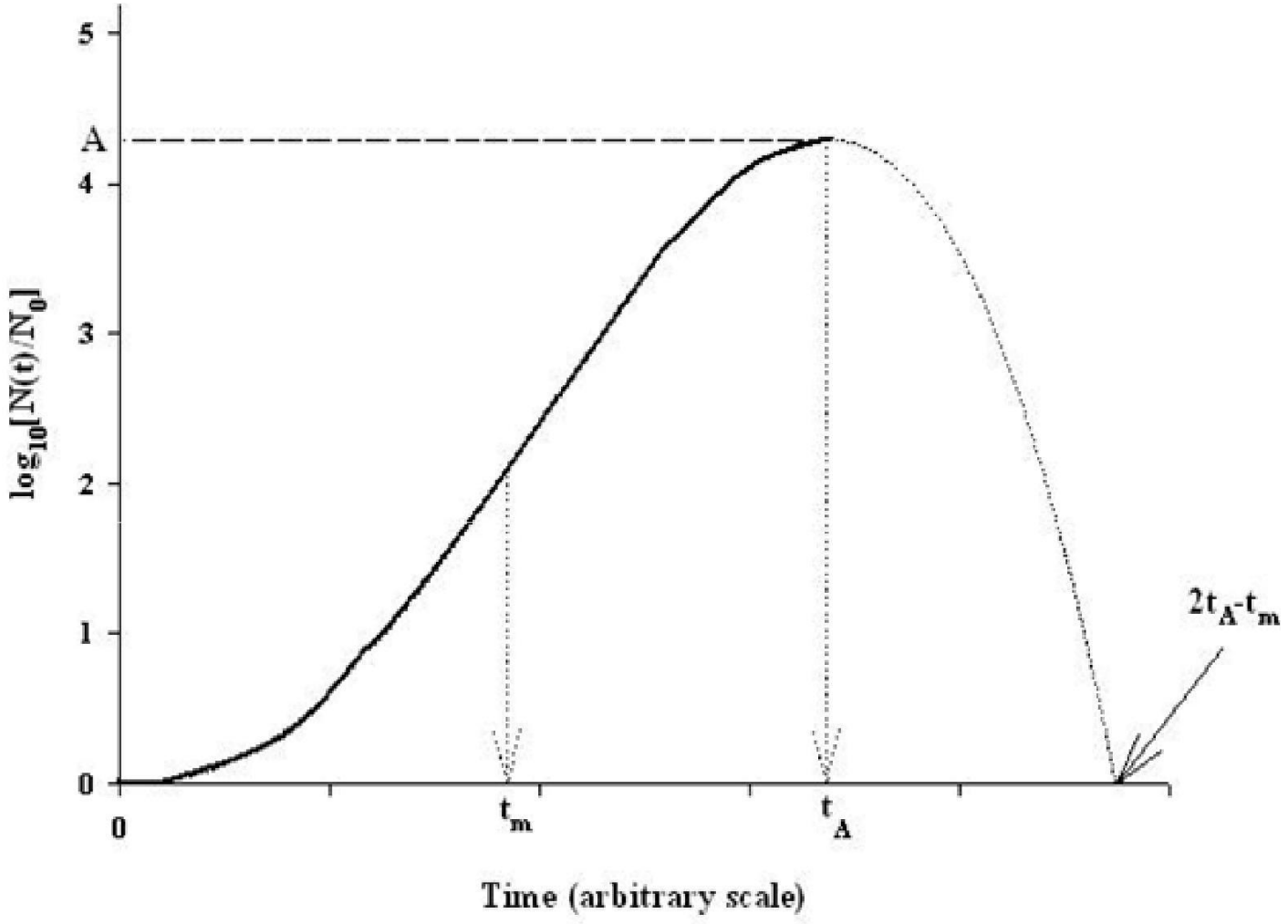


Figure 1. Demonstration of Equation (1) and its interpretable parameters (A, t_A, t_m) to describe the growth curve up to early stationary phase (bold lines). Dotted lines indicate the continuation of the Equation (1) after t_A. Adapted from Ref. (12).

Where $N(t)$ and N_0 are the momentary and initial number of the population, respectively. A is the asymptotic value which is reached at time t_A and t_m is the time at which maximum growth rate is achieved (Figure 1).

According to this model, when t = 0, $\log_{10}[N(t)/N_0]$ = 0 that is, $N(t)= N_0$. It is also very easy to estimate the initial values of the parameters of Equation (1) for nonlinear regression just by looking at the raw data; however, Equation (1) can only be used for growth curves up to early stationary phase because Equation (1) still produces unimodal curve if t_A is exceeded (Figure 1). Nevertheless, this will suffice as there is often little interest in modeling the stationary phase (McKellar and Lu, 2004).

Application of the model to published data on the isothermal growth of *Listeria monocytogenes* in milk and *Pseudomonas* spp. in poultry.

The growth data found in literature (Xanthiakos et al., 2006 and Gospavic et al., 2008 respectively) were scanned and digitized using a software program WinDIG 2.5 (written by Mr. Dominique Lovy, Geneva, Switzerland). The original data were in the form of $\log_{10}N(t)$ versus time, were converted into $\log_{10}[N(t)/N_0]$ versus time. Then, these were subjected to nonlinear regression using Equation (1). Both regression and plotting of the results were carried out with SigmaPlot 2000 Version 6.00 (Chicago, IL, USA). The goodness-of-fit of the model was assessed using adjusted determination coefficient (R^2_{adj}) and mean square error (MSE) values.

RESULTS AND DISCUSSION

Published isothermal growth data of *L. monocytogenes* in milk at 1.5, 4, 8, 12 and 16°C (Xanthiakos et al., 2006) fitted with Equation (1) as a model is shown in Figure 2. R^2_{adj} and MSE values given in Table 1 indicated that Equation (1) produced reasonable fits for *L. monocytogenes* in milk at various temperatures. Admittedly, Equation (1) did not produce good fits for the published literature growth data that had long stationary phase period (Figure 1) – results not shown.

Temperature dependence of parameters of Equation (1) was described by ad hoc empirical secondary models

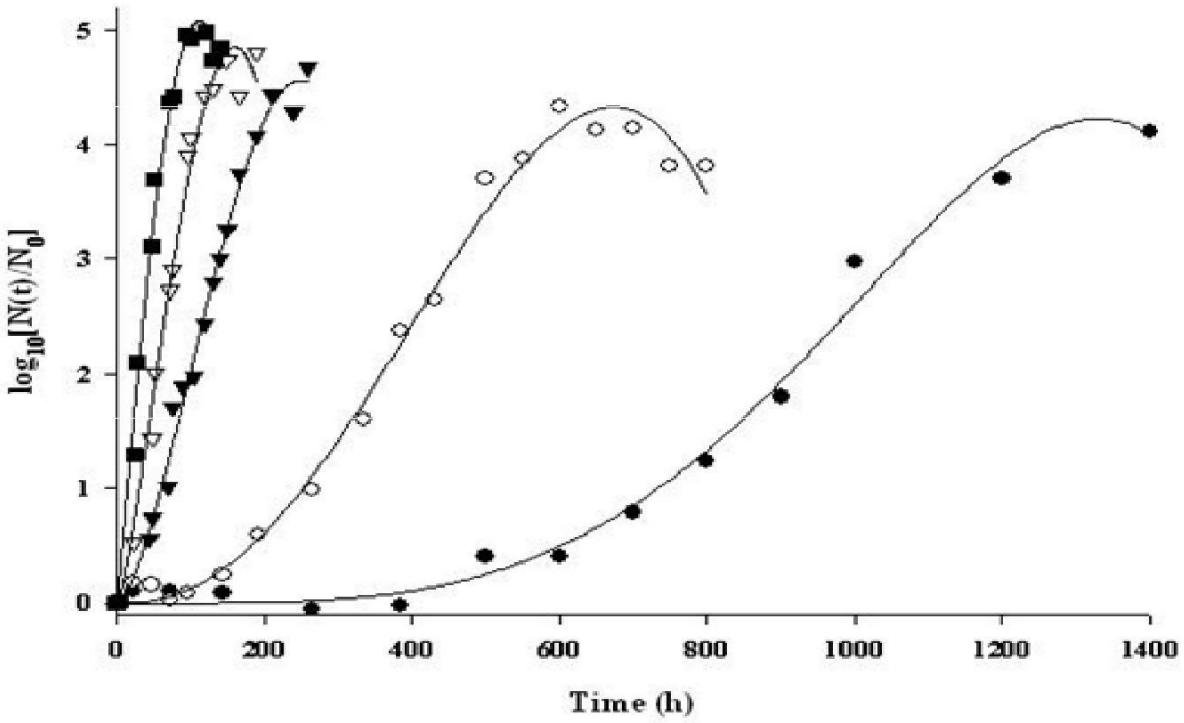


Figure 2. Published isothermal growth data of *L. monocytogenes* in milk at 1.5°C (closed circle), 4°C (open circle), 8°C (reversed closed triangle), 12°C (reversed open triangle) and 16°C (closed square) fitted with Equation (1) (solid lines). Original data are from Ref. (14).

Table 1. Goodness-of-fit of Equation (1) for published isothermal growth data of *L. monocytogenes* (Ref. 14) in milk at 1.5, 4, 8, 12 and 16 °C.

T (°C)	Equation (1)	
	R^2_{adj} [a]	MSE [b]
1.5	0.988	0.03
4	0.989	0.03
8	0.988	0.03
12	0.983	0.05
16	0.990	0.04

[a] Adjusted regression coefficient; [b] mean square error.

(Figure 3): A is linearly increasing with temperature while the temperature dependency of t_A and t_m could be successfully described by exponential decay function.

Solid lines in Figure 4 indicates that published isothermal growth data of *Pseudomonas* spp. in poultry at 2, 4, 10, 15 and 20°C (Gospavic et al., 2008) were fitted with Equation (1). Since the parameter A does not depend on temperature for *Pseudomonas* spp. (Figure 5) this parameter was fixed (Equation (2)) and the re-gression was repeated with two adjustable parameters (t_A and t_m). Dashed lines in Figure 4 indicated that data were fitted with Equation (1) with fixed A value (A_{fixed} = 5.89) that is,

Equation (2):

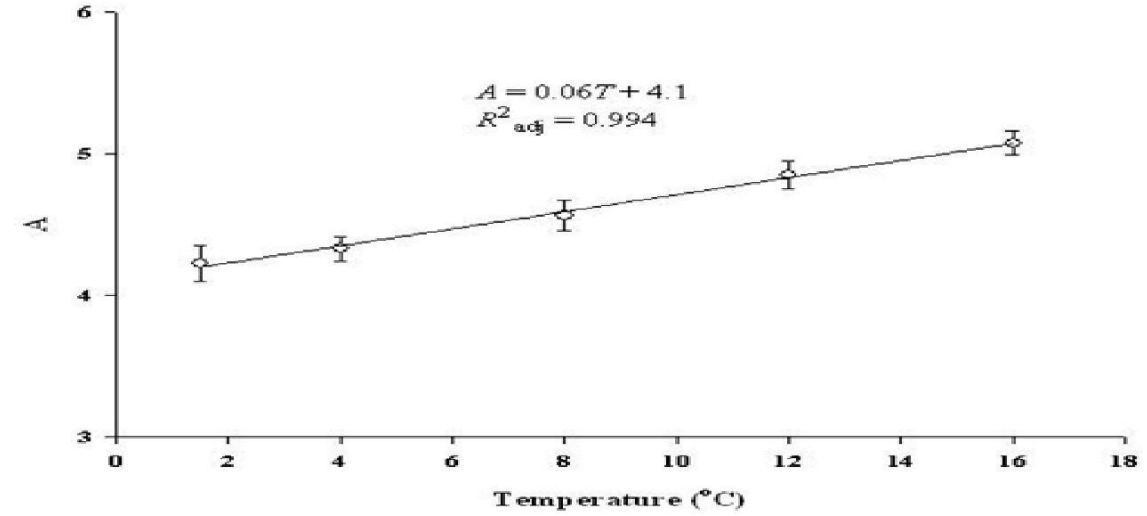
$$\log_{10}\frac{N(t)}{N_0} = A_{fixed} + \frac{t_A - t}{t_A - t_m}\frac{t}{t_A}$$

(2)

Table 2 shows the R^2_{adj} and MSE values for both full [Equation (1)] and reduced [Equation (2)] models. It is clear that reduced model produced almost the same fit and goodness- of-fit was as good as the full model. It should also be noted that since the number of data in each kinetic was not equal, each kinetic have not the same weight on A_{fixed} evaluation. Therefore, A_{fixed} value was evaluated by estimating the process on the whole set of data (Couvert et al., 2005).

Whether it is survival or growth modeling, it may be possible to reduce the number of parameters of model by using fixed value of one or two parameters of the original model with a slight loss of goodness-of-fit. The concept is not new; for example, in literature it is possible to find the usage of the fixed shape parameter of the Weibull model (Couvert et al., 2005; Corradini et al., 2005; Buzrul, 2007; Fernández et al., 2007). Recently, Avsaroglu et al. (2007) and Buzrul et al. (2007) reduced the parameters (from four to two) of empirical models for the hypochlorite and

(a)



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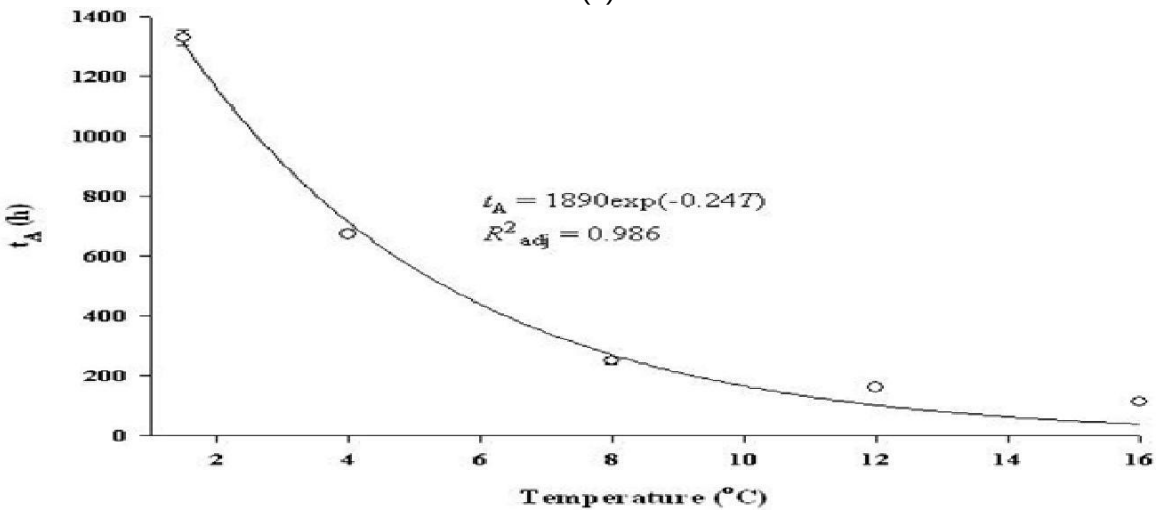

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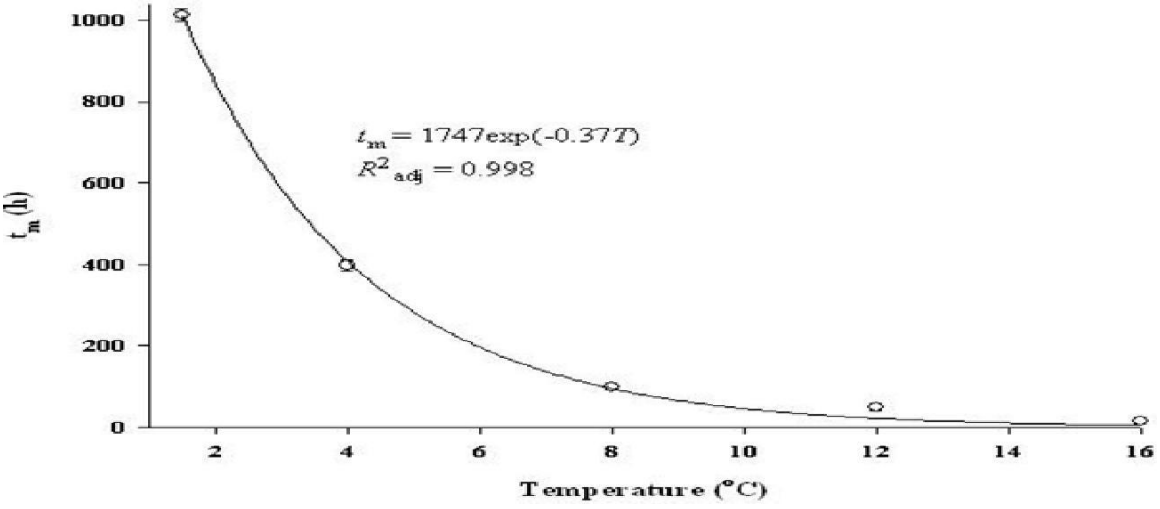

Figure 3. Temperature dependence (secondary modeling) of the parameters (a) A; (b) t_A and (c) t_m obtained from the fitting of Equation (1) for *L. monocytogenes*. Error bars represent standard error values.

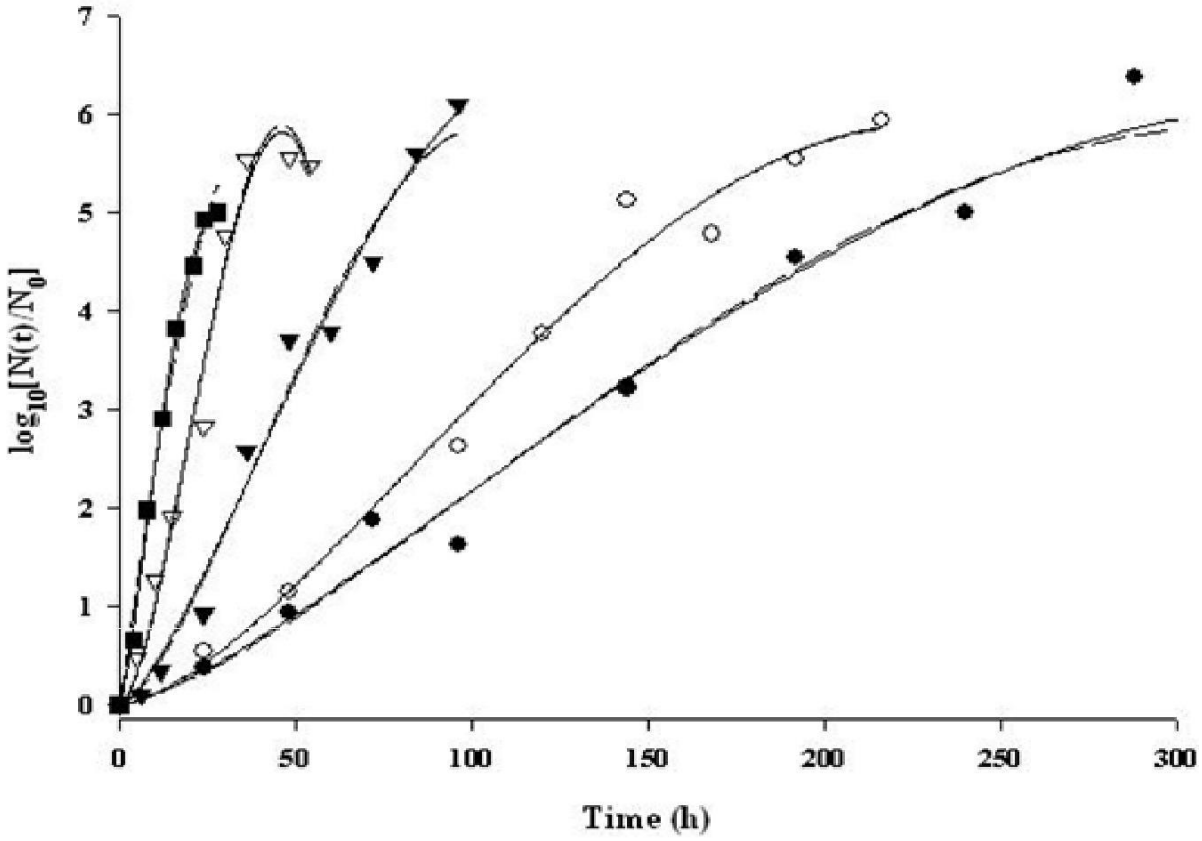

Figure 4. Published isothermal growth data of *Pseudomonas* spp. in poultry at 2°C (closed circle), 4°C (open circle), 10°C (reversed closed triangle), 15°C (reversed open triangle) and 20 °C (closed square) fitted with Equation (1) (solid lines) and with Equation (2) (dashed lines). Original data are from Ref. (15)

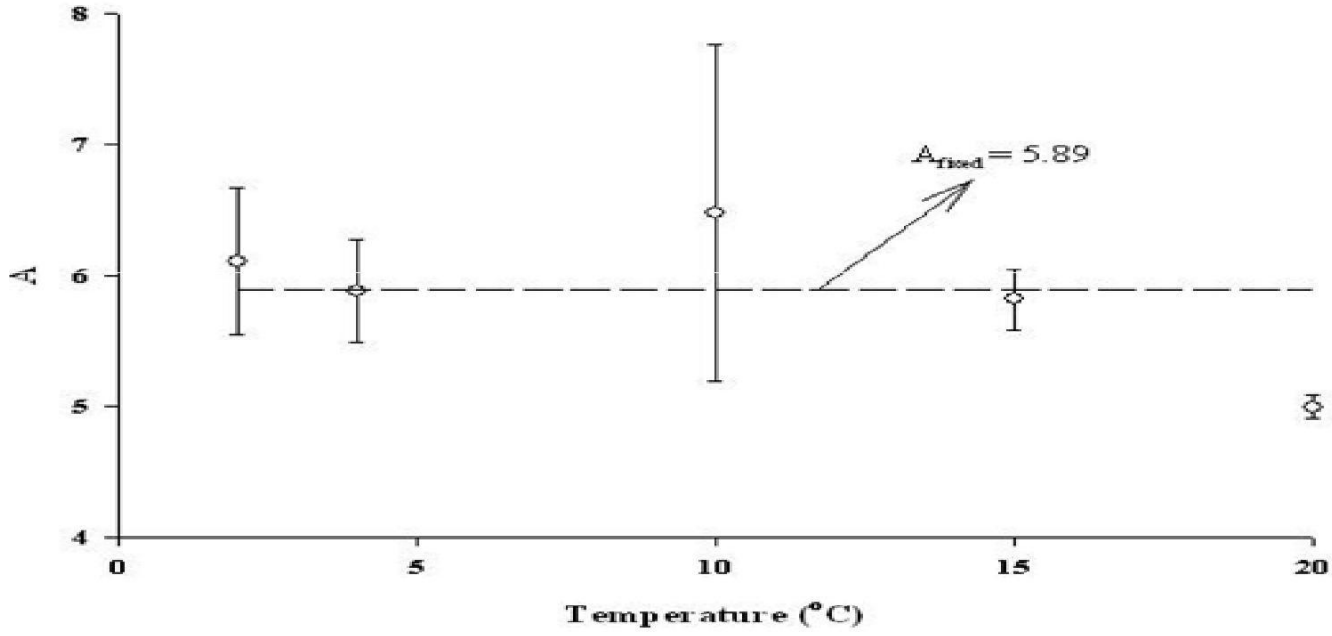

Figure 5. The parameter A ± standard error values obtained from the fitting of Equation (1) for published isothermal growth data of *Pseudomonas* spp. in poultry. Dashed line indicates the fixed value of the parameter A (A_{fixed} = 5.89) to be used for Equation (2).

Table 2. Goodness-of-fit of the full [Equation (1)] and the reduced [Equation (2)] models for published isothermal growth data of *Pseudomonas* spp. (Ref. 15) in poultry at 2, 4, 10, 15 and 20 °C.

T (°C)	R^2_{adj} [a]		MSE [b]	
	Equation (1)	Equation (2)	Equation (1)	Equation (2)
2	0.977	0.979	0.13	0.11
4	0.981	0.983	0.10	0.09
10	0.978	0.980	0.12	0.11
15	0.976	0.979	0.13	0.11
20	0.997	0.989	0.01	0.04

[a] Adjusted regression coefficient; [b] mean square error.

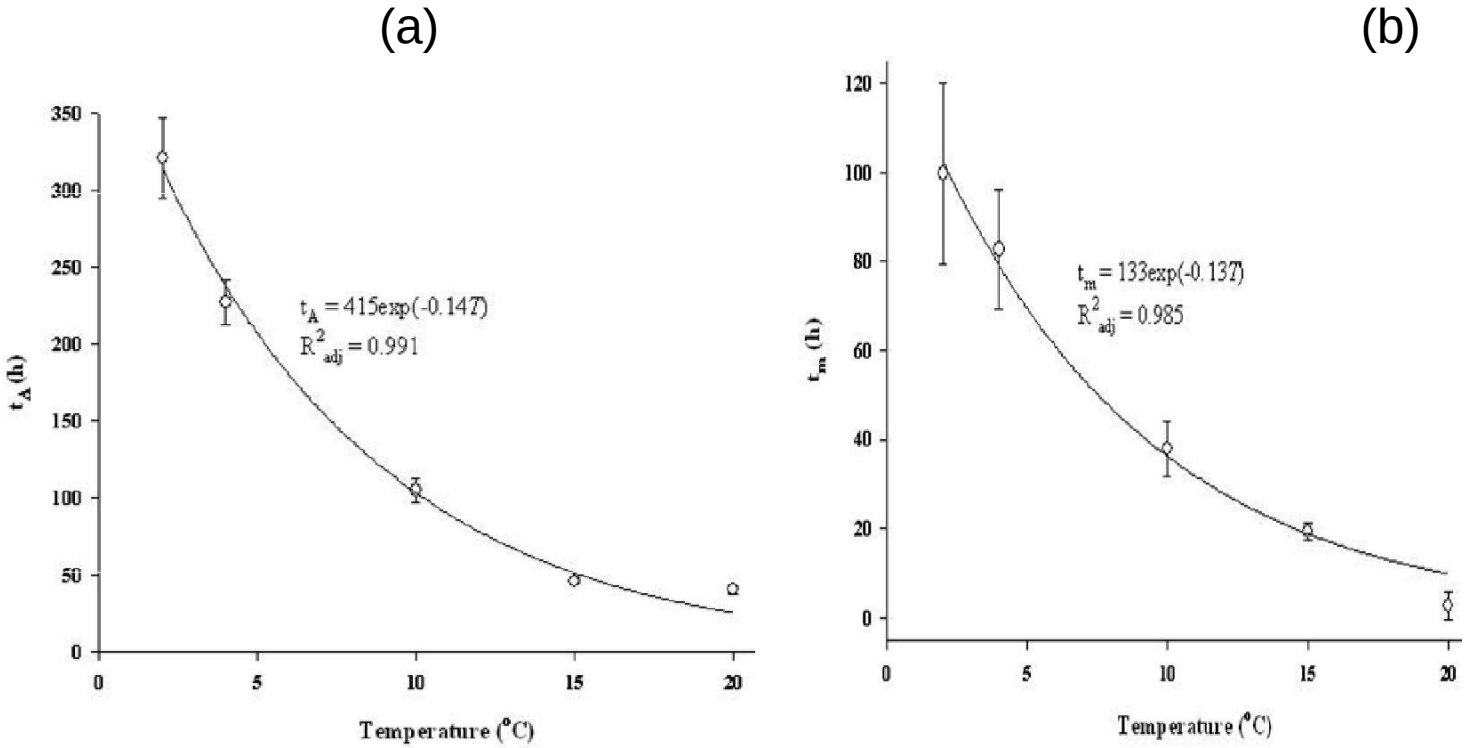

Figure 6. Temperature dependence (secondary modeling) of the parameters (a) t_A and (b) t_m obtained from the fitting of Equation (2) for *Pseudomonas* spp. Error bars represent standard error values.

isopropanol inactivation of lactococcal bacteriophages, respectively. Corradini and Peleg (2005) also fixed asymptotic value (just as in this study) of the modified logistic equation.

Fixing the parameter *A* increased the robustness of the model since the standard errors or confidence intervals of the adjustable parameters (t_A and t_m) are lowered when compared with the full model – results not shown. Therefore, fixing one or more parameters in a model (and then repeating the regression with the fixed parameters) would be useful when warranted by the results.

Secondary models for t_A and t_m are shown in Figure 6.

The same equations (exponential decay) could be used and once again reasonable fits could be obtained. Nevertheless it should be noted that secondary model descriptions should be limited to the experimental ranges.

In principle, it may be possible to use the proposed model to describe the isothermal growth data of micro-organisms. The model presented here has three meaningful parameters but in some cases parameters could be reduced from three to two. It should also be noted that this model is only applicable up to early stationary phase. More studies should be done to check the applicability of the proposed model under dynamic temperature conditions

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