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Modeling of Bacterial Growth as a Function of Temperature

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The temperature of chilled foods is a very important variable for microbial safety in a production and distribution chain. To predict the number of organisms as a function of temperature and time, it is essential to model the lag time, specific growth rate, and asymptote (growth yield) as a function of temperature. The objective of this research was to determine the suitability and usefulness of different models, either available from the literature or newly developed. The models were compared by using an *F* test, by which the lack of fit of the models was compared with the measuring error. From the results, a hyperbolic model was selected for the description of the lag time as a function of temperature. Modified forms of the Ratkowsky model were selected as the most suitable model for both the growth rate and the asymptote as a function of temperature. The selected models could be used to predict experimentally determined numbers of organisms as a function of temperature and time.

Predictive modeling is a promising field in food microbiology. Models are used to describe the behavior of microorganisms at different physical and chemical conditions, such as temperature, pH, and water activity. They can be used to predict microbial safety or shelf life of products, to find critical points in the process, and to optimize production and distribution chains. A major factor determining the specific growth rate of microorganisms in chilled foods is temperature. Various models have been proposed to describe this relationship. Spencer and Baines (16) proposed a linear dependency of the rate of microbial spoilage of fish on temperature. This relationship was shown to be valid only at temperatures below 6°C (8). Therefore, Olley and Ratkowsky (8) proposed an Arrhenius (2)-type equation. This equation could predict results up to 15°C. However during cooling, freezing, heating, or thawing, regions in the product can have a temperature far above 15°C, and therefore a wider growth-temperature range is important. Schoolfield et al. (13) proposed a nonlinear Arrhenius type of model on a biological basis, describing the specific growth rate as a function of temperature over the whole biokinetic temperature range. Further empirical models were proposed by Ratkowsky et al. (10, 11), i.e., the square root model, describing the specific growth rate up to 15°C, and the expanded square root model, describing the growth rate over the whole biokinetic temperature range. A model which is only seldom used is the model of Hinshelwood (7), although it is a simple model with a biological basis. Adair et al. (1) modeled the growth rate and the inverse of the lag time using the Ratkowsky and Schoolfield models and concluded that the Schoolfield model gives the best predictions.

The literature provides us with a number of models. However, a systematic approach to determine the most suitable model is lacking. The objective of this research was to determine the suitability and usefulness of the different models by systematic and statistical analysis of a large amount of experimental data.

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THEORY

Description of experimental bacterial growth data. The growth curve is defined as the logarithm of the relative population size [$y = \ln (N/N_0)$] as a function of time (t). For bacteria, the growth rate shows a lag phase that is followed by an exponential phase, and finally it shows a decreasing growth rate down to zero resulting in a maximum value of the number of organisms. A growth model with three parameters can describe this growth curve (18): the maximum specific growth rate μ_m, which is defined as the tangent in the inflection point; the lag time λ, which is defined as the t-axis intercept of this tangent; and the asymptote A, which is the maximal value reached. The three parameters are determined from growth data by describing them by the Gompertz model (6). Therefore, the Gompertz model (6), with parameters a, b, and c, was rewritten (18) to include A, μ_m, and λ [$e = \exp(1)$].

Modified Gompertz:

$$y = A\exp\left\{-\exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

Growth-temperature relations. A number of growth-temperature relations are compared. Included are models from the literature as well as modified forms. The models are all written with the growth rate as a function of temperature. Transformation of the growth rate (square root, logarithm) was not executed, as others tend to do (1, 4, 9, 17), to fit all data in the same way. Using transformations on data results in a different weighting of different numerical values. Using the minimum residual sum of squares (RSS) criterion, one has to take into account that a transformation changes the distribution of errors at different numerical values. If regression without weighting is used, the measuring error must be normally distributed with the same standard deviation at all different T values.

The growth rate used is the μ_m found with the modified Gompertz model.

(i) Square root model of Ratkowsky et al. (11). This model does not have a biological basis. It is based on the observation that at lower temperatures the square root of the specific growth rate is linear with temperature (11):

$$\mu_m = [b_1(T - T_{min})]^2 \text{ (Ratkowsky 1)} \quad (2)$$

where b is a Ratkowsky parameter ($°C^{-1}\ h^{-0.5}$), and T_{min} is the minimum temperature at which growth is observed (°C). The subscript 1 relates to Ratkowsky 1.

(ii) Expanded square root model of Ratkowsky et al. (10). To describe the growth rate around the optimum and the maximum temperatures, Ratkowsky et al. (10) expanded their equation:

$$\mu_m = \left(b_2(T - T_{min}) \cdot \{1 - \exp[c_2(T - T_{max})]\}\right)^2 \text{ (Ratkowsky 2)} \quad (3)$$

where c is a Ratkowsky parameter ($°C^{-1}$), and T_{max} is the maximum temperature at which growth is observed (°C). The subscript 2 relates to Ratkowsky 2.

(iii) Modified Ratkowsky model (Ratkowsky 3). At temperatures above T_{max}, equation 3 predicts positive values of the growth rate; therefore, this model cannot be used above T_{max}. We modified the Ratkowsky model so that the decline of μ_m toward T_{max} is described by an exponential function and not by the square of an exponential function, so that extrapolation above the maximum growth temperature T_{max} predicts no positive values of the growth rate:

$$\mu_m = [b_3(T - T_{min})]^2 \cdot \{1 - \exp[c_3(T - T_{max})]\} \text{ (Ratkowsky 3)} \quad (4)$$

The subscript 3 relates to Ratkowsky 3.

(iv) Model of Schoolfield et al. (13). The Schoolfield model is based on the model of Sharpe et al. (14, 15), which has the following assumptions. (i) The total amount of all compounds in the cell is constant (balanced growth), and only one enzyme reaction is rate controlling. The rate-controlling enzyme is reversibly denatured at very low and at very high temperatures. (ii) The total amount of rate-controlling enzyme per cell is constant. (iii) The reaction rate of the rate-controlling enzyme reaction is zero order. (iv) The enzyme reaction and both the high- and low-temperature inactivation show an Arrhenius type of temperature dependency. This results in the following equation:

$$\mu_m = \frac{k_a \exp\left(\frac{-E_a}{RT}\right)}{1 + k_l \exp\left(\frac{-E_l}{RT}\right) + k_h \exp\left(\frac{-E_h}{RT}\right)} \quad (5)$$

where the subscript a relates to the controlling enzyme reaction, the subscript h relates to high-temperature inactivation, and the subscript l relates to low-temperature inactivation. $k_a(h^{-1})$, $k_l(-)$, and $k_h(-)$ are frequency factors, E is the activation energy ($J \cdot mol^{-1}$), R is the gas constant ($J \cdot K^{-1} \cdot mol^{-1}$), and T is the temperature (K).

In equation 5, the parameters are strongly correlated. Schoolfield et al. (13) modified the equation to diminish the correlation:

$$\mu_m = \frac{\mu_{25}\frac{T}{298}\exp\left[\frac{H_a}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{H_l}{R}\left(\frac{1}{T_l} - \frac{1}{T}\right)\right] + \exp\left[\frac{H_h}{R}\left(\frac{1}{T_h} - \frac{1}{T}\right)\right]} \quad (6)$$

where μ_{25} is the growth rate at 25°C (h^{-1}), T_l is the temperature (K) at which the enzyme is 50% inactivated due to low temperature, H is the enthalpy of activation ($J \cdot mol^{-1}$), and T_h is the temperature (K) at which the enzyme is 50% inactivated due to high temperature.

(v) Hinshelwood model (7). The Hinshelwood model is based on the following assumptions. (i) The total amount of all compounds in the cell is constant (balanced growth), and only one enzyme reaction is rate controlling. (ii) The product of this enzyme reaction is a heat-sensitive essential biomolecule which is irreversibly denatured at high temperatures. Both the enzyme reaction and the high-temperature denaturation show an Arrhenius type of temperature dependency and are zero order. This results in the following equation:

$$\mu_m = k_1 \cdot \exp\left(-\frac{E_1}{RT}\right) - k_2 \cdot \exp\left(-\frac{E_2}{RT}\right) \quad (7)$$

where k_1 and k_2 are frequency actors (h^{-1}) and E_1 and E_2 are the activation energies ($J \cdot mol^{-1}$) of the enzyme reaction and the high-temperature denaturation, respectively.

(vi) General model. There is also a model (general model) that uses the mean values of the measured data. At every temperature, this model gives the mean value of the data at that temperature. This model is of the type "at temperature q the growth rate is z" and is therefore not useful for interpolation.

At one temperature T_i (with $i = 1$ to $i = 1.18$), m growth rates are measured (duplicates, triplicates . . .). In our case, m is not the same value at different temperatures. Then the model for the best prediction of a growth rate at a certain temperature can be proposed, that is, defined as the mean value $\bar{\mu}_m(i)$ of the measured growth rates at that temperature. This model is called the general model:

$$\bar{\mu}_m(i) = \sum_{j=1}^{m} \frac{\mu_m(i, j)}{m} \quad (8)$$

with $\mu_m(i, j)$ being the j^{th} growth rate at T_i and $\bar{\mu}_m(i)$ being the mean growth rate at T_i.

Asymptote-temperature relations. For the asymptote data, no extensive literature exists as it did for the growth rate. For the asymptote data, a number of models were tested, including the Hinshelwood (equation 7), Ratkowsky 2 (equation 3), Ratkowsky 3 (equation 4), and Schoolfield (equation 6) models. These equations can be regarded as empirical fit models only. Since the asymptote did not show a strong dependency on temperature at the lower temperature range, a second modified Ratkowsky model is proposed (Ratkowsky 4):

$$A = b_4 \{1 - \exp[c_4(T - T_{A_{max}})]\} \quad (9)$$

where b_4 is the final level reached at low temperatures and $T_{A_{max}}$ is the maximum temperature at which growth is observed (°C).

Lag time-temperature relations. Adair et al. (1) modeled the inverse of the lag time data with growth models (Ratkowsky, Schoolfield). By taking the inverse of the data, numerical values of the lag time that are $\gg 1$ will approach zero after the transformation and are therefore weighted less. Transforming the model predictions back to lag times then results in large prediction errors ($0 \approx 1/8{,}000 \approx 1/800$ but $\infty \neq 8{,}000 \neq 800$). For this reason, we did not use the inverse of the data, but the inverse of the growth rate equations are used as empirical models, to fit the data.

In this study, the lag time data showed a large measuring error at high numerical values (the standard deviation was proportional to the mean value). To limit this influence, a

TABLE 1. Comparison of the models describing the growth rate

Model[a]	No. of parameters	df	RSS_2	f	F
$\mu_m = 0$	0	38	20.793	139.5	2.1
$\mu_m = a$	1	37	5.994	41.74	2.2
$\mu_m = aT$	1	37	3.744	25.63	2.2
$\mu_m = aT + b$	2	36	3.738	27.19	2.2
Hinshelwood	4	34	0.248	**0.729**	2.2
Ratkowsky 3	4	34	**0.211**	**0.409**	2.2
Ratkowsky 2	4	34	0.220	**0.483**	2.2
Schoolfield	6	32	0.215	**0.516**	2.3
General	18	20 (df_1)	0.164 (RSS_1)		
$\mu_m = \mu_m(i, j)$	38	0	0.000		

[a] μ_m is the growth rate to be modeled; $\mu_m(i, j)$ is the j^{th} growth rate at T_i; a and b are regression coefficients; T is the temperature.

logarithmic transformation is used on the experimental data and on the model equations. In conclusion, the transformed lag time data are then modeled by using the logarithm of the inverse of the growth rate models. For instance, for the Ratkowsky 2 model (equation 3), we fitted:

$$\ln(\lambda) = \ln\left[\left(b_2(T - T_{min}) \cdot \{1 - \exp[c_2(T - T_{max})]\}\right)^{-2}\right] \quad (10)$$

which is the same equation as equation 3, except for that the model is inverted and the natural logarithm of the model is taken.

The results of Adair et al. (1) and Gill et al. (5) show a hyperbolic behavior of the lag time and the temperature; therefore, a hyperbolic equation is also used:

$$\ln(\lambda) = \frac{p}{(T - q)} \quad (11)$$

The parameter q is the temperature at which the lag time is infinite (no growth). The parameter p is a measure for the decrease of the lag time when the temperature is increased.

Comparison of the models. The models are compared statistically with the use of an F ratio test. With the general model (equation 8), the measuring error is estimated by determining the deviation of the measured values from the mean value at one temperature. The sum of squares of the deviations between the data and the general model is calculated (RSS_1):

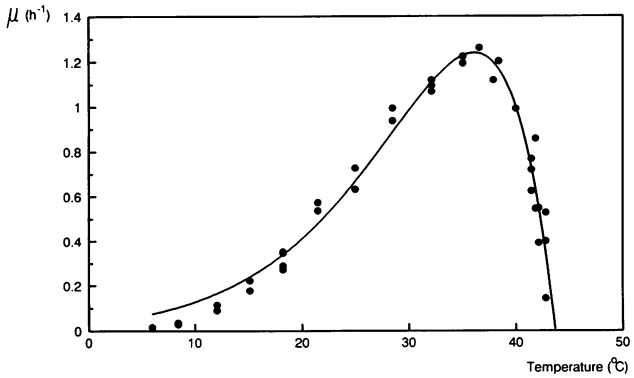

FIG. 1. Growth rates modeled with the Hinshelwood model. Symbols: ●, estimated growth rate values; ——, the model.

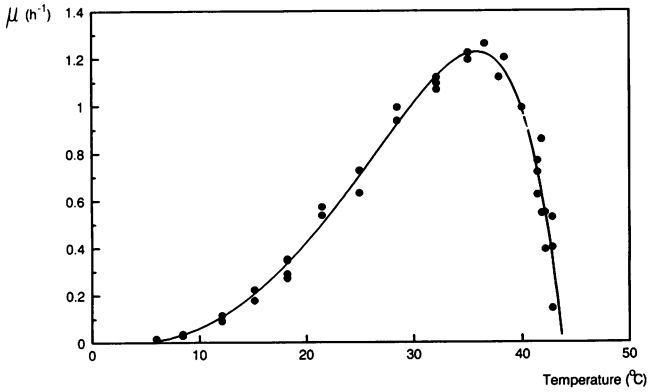

FIG. 2. Growth rates modeled with the Ratkowsky 3 model. Symbols: ●, estimated growth rate values; ——, the model.

$$RSS_1 = \sum_{i=1}^{k} \sum_{j=1}^{m} [\mu_m(i, j) - \bar{\mu}_m(i)]^2$$

(general model) (12)

with $\mu_m(i, j)$ being the j^{th} growth rate at T_i and $\bar{\mu}_m(i)$ being the mean growth rate at T_i.

The sum of squares of the deviations between the data and a given growth temperature model is calculated (RSS_2) as:

$$RSS_2 = \sum_{i=1}^{k} \sum_{j=1}^{m} [\mu_m(i, j) - \hat{\mu}_m(i)]^2$$

(growth-temperature model) (13)

with $\hat{\mu}_m(i)$ being the model prediction at temperature T_i.

RSS_2 will always be larger than or equal to RSS_1. The RSS_2 of the growth-temperature model used is built up from both the measuring error and the lack of fit; therefore, the difference between the RSS_2 of the model and RSS_1 (the measuring error) is calculated as an estimation of the lack of fit. If the lack of fit ($RSS_2 - RSS_1$) is much smaller than the measuring error (RSS_1), the model is adequate. If the lack of fit is much larger than the measuring error, the model is not adequate. This comparison between the lack of fit and the measuring error can be quantified statistically by the f testing value:

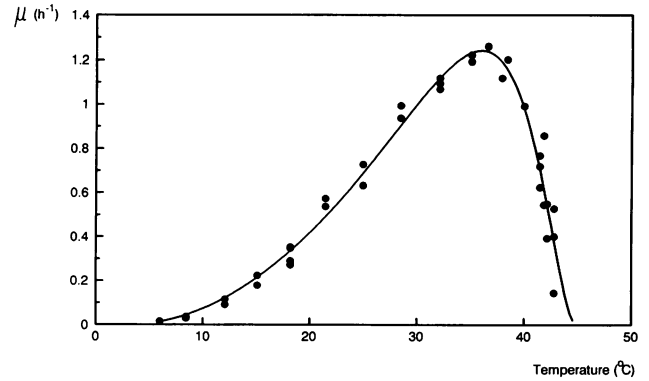

FIG. 3. Growth rates modeled with the Ratkowsky 2 model. Symbols: ●, estimated growth rate values; ——, the model.

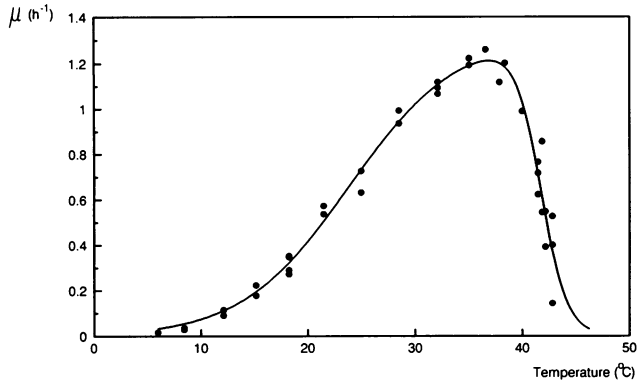

FIG. 4. Growth rates modeled with the Schoolfield model. Symbols: ●, estimated growth rate values; ——, the model.

$$f = \frac{(RSS_2 - RSS_1)/(df_2 - df_1)}{RSS_1/df_1}$$

$$\text{tested against } F^{df_2 - df_1}_{df_1} \tag{14}$$

where df_1 is the number of degrees of freedom from the general model that equals the total number of datum points minus the number of different temperatures measured (38 − 18 = 20). df_2 is the number of the degrees of freedom from the growth-temperature model that equals the number of datum points minus the number of parameters.

These statistics are not valid for nonlinear models but at least give an indication about the suitability of the models, since even for nonlinear models, the variance ratio shown above is approximately F distributed when the sample size is large (12). This analysis is an approximation at best, and this procedure should be considered as an informal process, rather than a rigorous statistical analysis, because of the use of nonlinear models (12).

MATERIALS AND METHODS

Microbial experiments. In 38 experiments at 18 different temperatures, *Lactobacillus plantarum* (American Type Culture Collection determined; no ATCC number) was cultivated in MRS medium (Difco Laboratories). The culture was stored frozen (−16°C). The bacteria were cultivated twice at 30°C, first for 24 h and second for 16 h. Growth was monitored by using 20-ml tubes, each containing 10 ml of medium and inoculated with the test organism to reach a target initial titer of 5×10^5 CFU/ml. The test tubes were incubated statically at different temperatures from 6°C up to 43°C as follows (temperatures in °C and number of experiments in parentheses): 6.0 (1); 8.5 (2); 12.1 (2); 15.2 (2); 18.2 (5); 21.5 (2); 25.0 (2); 28.5 (2); 32.1 (3); 35.1 (3); 36.6 (1); 37.9 (1); 38.4 (1); 40.0 (1); 41.5 (3); 41.9 (2); 42.2 (2); 42.8 (3). At appropriate time intervals (depending on temperature), the inoculated cultures were vortexed and samples of 0.1 ml were removed for serial dilution in peptone saline solution (1 g of Bacto-Peptone [Difco], 8.5 g of NaCl [Merck p.a.] per liter). Bacterial numbers were determined with a pour plate (MRS medium with 12 g of agar [Agar Technical Oxoid Ltd.] per liter). The pour plates were incubated for 48 h at 30°C before counting.

Fitting of the data. The modified Gompertz equation (equation 1) was fitted to the data of the 38 growth curves by nonlinear regression with a Marquardt algorithm (18). This resulted in estimates for the specific growth rate, lag time, and asymptote of these 38 different growth curves. The model equations were also fitted to these data by nonlinear regression. Confidence intervals are based on the variance-covariance matrix of the parameters, calculated with the Jacobian matrix.

Selection of the models. First the models were compared

TABLE 2. Results of the Hinshelwood parameter estimation

Parameter	Estimate	95% Confidence interval
k_1 (h^{-1})	1.249E+21[a]	−1.903E+24 to 1.906E+24
E_1 (kJ)	107.2	0.7415 to 213.7
k_2 (h^{-1})	1.319E+21	−1.903E+24 to 1.906E+24
E_2 (kJ)	107.4	0.6361 to 214.1

[a] Where E + 21 means $\times 10^{21}$.

TABLE 3. Correlation matrix for Table 2 parameters

Parameter	k_1	E_1	k_2	E_2
k_1	1.000	0.998	0.99999	−0.998
E_1	0.998	1.000	0.998	−0.993
k_2	0.99999	0.998	1.000	−0.998
E_2	−0.998	−0.993	−0.998	1.000

TABLE 4. Results of the Schoolfield parameter estimation

Parameter	Estimate	95% Confidence interval
μ_{25} (h^{-1})	1.42	−0.0576 to 2.91
H_a (kJ)	−5.43	−59.8 to 49.0
H_l (kJ)	−141.1	−182.2 to −100.1
T_l (K)	297.7	286.0 to 309.3
H_h (kJ)	687.9	402.1 to 973.7
T_h (K)	314.7	314.0 to 315.3

TABLE 5. Correlation matrix for Table 4 parameters

Parameter	μ_{25}	H_a	H_l	T_l	H_h	T_h
μ_{25}	1.000	−0.990	0.610	0.997	0.436	0.711
H_a	−0.990	1.000	−0.512	−0.981	−0.506	−0.791
H_l	0.610	−0.512	1.000	0.642	−0.011	0.051
T_l	0.997	−0.981	0.642	1.000	0.417	0.682
H_h	0.436	−0.506	−0.011	0.417	1.000	0.721
T_h	0.711	−0.791	0.051	0.682	0.721	1.000

TABLE 6. Results of the Ratkowsky 2 parameter estimation

Parameter	Estimate	95% Confidence interval
b_2	0.0377	0.0321 to 0.0433
T_{min}	2.82	−0.223 to 5.86
c_2	0.250	0.173 to 0.326
T_{max}	44.9	44.2 to 45.5

TABLE 7. Correlation matrix of Table 6 parameters

Parameter	b_2	T_{min}	c_2	T_{max}
b_2	1.000	0.963	−0.824	0.628
T_{min}	0.963	1.000	−0.687	0.499
c_2	−0.824	−0.687	1.000	−0.922
T_{max}	0.628	0.499	−0.922	1.000

TABLE 8. Results of the Ratkowsky 3 parameter estimation

Parameter	Estimate	95% Confidence interval
b_3	0.0410	0.0335 to 0.0485
T_{min}	3.99	0.881 to 7.11
c_3	0.161	0.0940 to 0.228
T_{max}	43.7	43.4 to 44.1

TABLE 9. Correlation matrix for Table 8 parameters

Parameter	b_3	T_{min}	c_3	T_{max}
b_3	1.000	0.960	−0.910	0.591
T_{min}	0.960	1.000	−0.783	0.466
c_3	−0.910	−0.783	1.000	−0.804
T_{max}	0.591	0.466	−0.804	1.000

statistically by using the F test. This gave all the models that are statistically accepted, and then other criteria could be used to choose the best model. First the models with the fewest number of parameters were selected. From this subset of models, the model with the lowest RSS_2 was selected.

If one of the statistically accepted models is based on biological principles and the parameter estimates are confident and have an acceptable value, the biological relevance of this model is discussed.

RESULTS AND DISCUSSION

Growth-temperature relations. The specific growth rates as function of temperature (38 measurements) are described by using different models. The RSS_2 values and the f testing values of the different growth temperature relations are shown in Table 1. Additionally, some simpler models are given such as "the growth rate is zero at all temperatures" ($\mu = 0$); "the growth rate is constant at all temperatures" ($\mu = a$); "the growth rate is linearly dependent on temperature" ($\mu = aT + b$). It is clear from Table 1 that the RSS_2 value decreases with an increasing number of parameters. The general model with 18 parameters exactly predicts the mean values of the measured data. This model is of the type "at temperature q the growth rate is z" and is therefore not useful for interpolation. This comparison clearly shows what can be achieved with modeling: reduction of data to a limited number of parameters, more specifically to a model that is accepted statistically with as few parameters as possible.

TABLE 10. Comparison of the models describing the asymptote

Model[a]	No. of parameters	df	RSS_2	f	F
$A = 0$	0	38	2,378	183	2.1
$A = a$	1	37	128	9.37	2.2
$A = aT$	1	37	587	47.1	2.2
$A = aT + b$	2	36	111	8.41	2.2
Hinshelwood	4	34	178	16.4	2.2
Ratkowsky 2	4	34	28.7	**1.43**	2.2
Ratkowsky 3	4	34	28.3	**1.39**	2.2
Ratkowsky 4	**3**	35	31.3	**1.58**	2.2
Schoolfield	6	32	20.4	**0.711**	2.3
General	18	20 (df_1)	14.3 (RSS_1)		
$A = A(i, j)$	38	0	0.0		

[a] A is the asymptote to be modeled; $A(i, j)$ is the j^{th} asymptote at T_i; a and b are regression coefficients; T is the temperature.

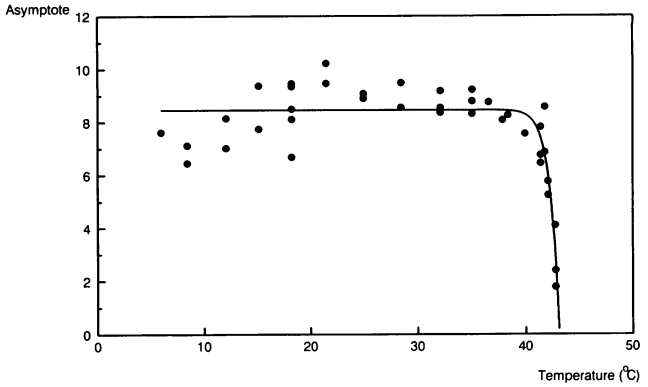

FIG. 5. Asymptote data modeled with the Ratkowsky 4 model. Symbols: ●, estimated asymptote values;——, the model.

Some investigators (1) only compare the RSS_2 of models and decide which model is the best by determining which model gives the lowest RSS_2. From Table 1 it can be seen that there are always models with a lower RSS_2, even one with $RSS_2 = RSS_1$. But these models have so many parameters that the aim of modeling, reducing the data to a statistically accepted model with a limited number of parameters, is not achieved. The lack of fit is probably a more stringent test of model adequacy.

From the curvature of the datum points in Fig. 1, it can be easily seen that the data are not well described by a constant value or a straight line. Indeed, for the first four models the f testing value is much larger than the F table value, and therefore these models are rejected. From Table 1 it can be concluded that the Hinshelwood (four parameters), Ratkowsky 2 (four parameters), Ratkowsky 3 (four parameters), and Schoolfield (six parameters) models are accepted statistically, because the f testing value is lower than the F value. In Fig. 1 to 4, where for these four models the predicted and measured values are shown, it can be seen that these models describe the curvature of the growth rate-temperature relation. As these four models are all accepted statistically, other criteria can be used to choose the best model.

Among the four-parameter models, the Hinshelwood model is based on a fundamental model (Arrhenius). In the Hinshelwood model, the parameters are strongly correlated (Table 3). A value of 1 for two parameters in the correlation matrix means that these two parameters are totally correlated with each other. Parameters that are strongly correlated (>0.999) are difficult to estimate, because a change in one parameter will be compensated for by a change in a correlated parameter, and numerous iterations are necessary. Moreover, the confidence intervals of such parameters are very large (Table 2). A second problem with the Hinshelwood model is that the estimates for the activation energies E_1 and E_2 are almost the same value (107.2 and 107.4 kJ). This results in a subtraction of two large values to calculate the growth rate. Reparameterization of the model, however, can possibly reduce these problems. Normally, the activa-

TABLE 11. Results of the Ratkowsky 4 parameter estimation

Parameter	Estimate	95% Confidence interval
b_4	8.46	8.09 to 8.82
c_4	1.25	0.709 to 1.78
$T_{A_{max}}$	43.1	42.9 to 43.4

TABLE 12. Comparison of the models describing the lag time

Model[a]	No. of parameters	df	RSS_2	f	F
$\ln(\lambda) = 0$	0	38	127	17.6	2.1
$\ln(\lambda) = a$	1	37	58.6	7.97	2.2
$\ln(\lambda) = aT$	1	37	95.6	13.7	2.2
$\ln(\lambda) = aT + b$	2	36	29.1	3.57	2.2
Hyperbola	**2**	36	**9.71**	**0.357**	2.2
$(\text{Ratkowsky 1})^{-1}$	**2**	36	18.3	**1.60**	2.2
$(\text{Ratkowsky 2})^{-1}$	4	34	9.14	**0.238**	2.2
$(\text{Ratkowsky 3})^{-1}$	4	34	9.21	**0.221**	2.2
General	18	20 (df_1)	7.55 (RSS_1)		
$\ln(\lambda) = \ln[\lambda(i, j)]$	38	0	0.0		

[a] λ is the lag time to be modeled; $\lambda(i, j)$ is the j^{th} lag time at T_i, a and b are regression coefficients; T is the temperature.

tion energy for an enzyme-catalyzed reaction is 10 to 80 kJ, and for a denaturation reaction it is 400 to 1,200 kJ (3). Neither estimated activation energy is within these intervals. This means that the fitting of the Hinshelwood relation to the data results in an estimation of unrealistic activation energy values. This makes the biological background of the model discussable. A third problem with the Hinshelwood model is that the predictions of the growth rate at low temperatures are too high. Growth rates at low temperatures are especially important during chilled food storage. Concluding all these aspects, this model can be regarded as not appropriate.

Schoolfield et al. (13) reparameterized their model to overcome the correlation problem, and as can be seen in Table 5, they were successful. The parameter H_a should be the enthalpy of activation of the reaction that is catalyzed by the rate-controlling enzyme. A negative value, however, was found. However, part of the confidence interval covers realistic values (Table 4). The other parameters also show realistic values. Therefore, the biological background of the Schoolfield model can exist. However, often the six parameters of the Schoolfield model are used as fitting parameters instead of estimates of biologically relevant parameters. Only with a very large data set can this model be used to estimate the biological parameters. Even with 38 datum points, the confidence intervals of the parameters are too large (Table 4).

As can be seen in Tables 7 and 9, the correlation matrices of the Ratkowsky 2 and Ratkowsky 3 models show no

TABLE 13. Results of the hyperbolic parameter estimation

Parameter	Estimate	95% Confidence interval
p	23.9	19.1 to 28.7
q	2.28	1.19 to 3.37

nondiagonal values of >0.999, so in these models the parameters can be estimated easily.

Statistical evaluation of the models shows that the Hinshelwood, Ratkowsky 2, Ratkowsky 3, and Schoolfield models all describe the growth rate data sufficiently. Therefore, an appropriate model can be chosen on the basis of other grounds. The models with the lowest number of parameters (the four-parameter models) were chosen. The Ratkowsky 3 equation has the lowest RSS_2 of all four-parameter models. Therefore, the Ratkowsky 3 model appeared to be the most suitable to describe the specific growth rate as function of temperature. The RSS_2 of the Ratkowsky 3 model is even smaller than the RSS_2 of the Schoolfield model, although the Schoolfield model has two more parameters.

The Ratkowsky 3 equation shows an exponential drop of the growth rate at high temperatures and shows no positive values of the growth rate at temperatures above the maximum growth temperature.

Asymptote-temperature relations. The asymptote value as a function of temperature was analyzed with various models (Table 10). The asymptote data did not differ much in the lower temperature range, and therefore a model with a constant asymptote in the lower temperature range was also taken into account (Ratkowsky 4). The first four models can be rejected on basis of the F test. In this case, the Hinshelwood model is rejected also. None of the other models can be rejected on the basis of statistics. While the Ratkowsky 4 model is not rejected, there is no statistical evidence that there is an effect of temperature on the asymptote in the lower temperature range. Although it seems that the asymptote value increases with increasing temperature (Fig. 5), the measuring error is too large to discriminate statistically. It is possible that with more datum points or data with a smaller standard deviation the effect of temperature on the asymptote in the lower temperature range can be shown. Yet, since for the measured datum points the Ratkowsky 4 model was accepted statistically and had the lowest number of parameters (from the models which are accepted), this model was

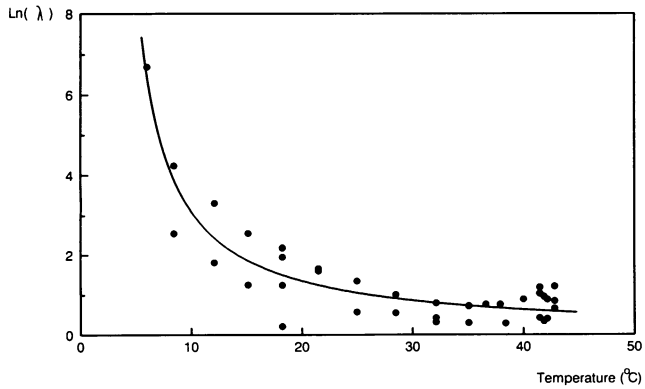

FIG. 6. Lag time data modeled with a hyperbola model. Symbols: ●, estimated lag time values; ——, the model.

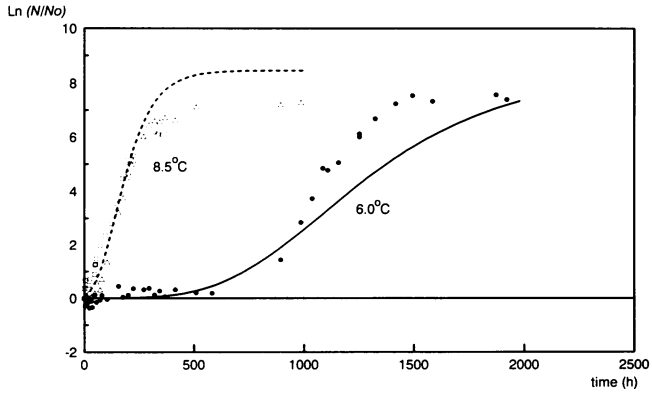

FIG. 7. Growth data and total model at 6.0°C (●) and 8.5°C (□, △). Different symbols indicate different duplicates.

TABLE 14. Parameters for models shown by equation 4, equation 9, and equation 11[a]

Growth rate (μ_m) (equation 4): $[b_3(T - T_{min})]^2\{1 - \exp[c_3 (T - T_{max})]\}$		Asymptote (A) (equation 9): $b_4\{1 - \exp[c_4 (T - T_{A_{max}})]\}$		Lag time (λ) (equation 11): $\ln(\lambda) = \frac{p}{(T - q)}$	
Parameter	Estimate	Parameter	Estimate	Parameter	Estimate
b_3	0.0410	b_4	8.46	p	23.9
T_{min}	3.99	c_4	1.25	q	2.28
c_3	0.161	$T_{A_{max}}$	43.1		
T_{max}	43.7				

[a] $y = A\exp\left\{-\exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\}$. For the parameter b_4, 21.58 − ln (N_0) must be used if another inoculation level is used.

selected (Fig. 5). The parameter estimates of this model are shown in Table 11.

In these experiments, the same inoculation level (5×10^5 organisms per ml) was always used. If it is assumed that the final absolute number of organisms N_∞ is constant (and therefore not dependent on the inoculum level), the asymptote is dependent on the inoculum level as:

$$b_4 = A = \ln(N_\infty/N_0) \quad (15)$$

$$\ln(N_\infty) = b_4 + \ln N_0 = 8.46 + \ln(5E5) = 8.46 + 13.12 = 21.58 \quad (16)$$

The parameter b_4 (the final level reached at lower temperatures) must be used [$b_4 + \ln(5E5) - \ln(N_0) = 21.58 - \ln(N_0)$] if another inoculation level is used.

Lag time-temperature relation. To fit the lag time, a logarithmic transformation was used, because the data showed a larger measuring error at high numerical values (the standard deviation was proportional to the mean value). After the transformation, the distribution of measuring errors at different temperatures was almost the same. Adair et al. (1) fitted the logarithm of the inverse lag time data with the Schoolfield model and the square root of the inverse of the lag time data with the Ratkowsky model. After transforming the model predictions back to lag times, they calculated the RSS_2 between their measured data and the model predictions and they found, for instance, RSS_{Rat} = 16,186 and RSS_{School} = 683. If they would have fitted the logarithm of the lag time data with the logarithm of the inverse of the Ratkowsky model (as it is proposed in this report; equation 10) and transformed back to lag time, they would have found RSS_{Rat} = 632. Note that the fitting to the models is done with different models but that the calculation of the RSS values is done comparing lag time data (without transformation) with model data. This is a striking example to show the importance of the choice of the transformation before fitting.

The logarithm of the lag time as a function of temperature was described with different models (Table 12). In this case, the first four models were rejected again. All other models were accepted. The models with the lowest number of parameters had to be selected. These were the models with two parameters that are accepted statistically (Ratkowsky 1 and a hyperbola). Between these latter two models, the hyperbola model had the lowest RSS_2, and therefore this model was selected (Fig. 6). The parameter estimates are given in Table 13.

Growth curve-temperature relation. The different models can now be integrated. Using equation 4, equation 9, and equation 11 and the estimated parameters for these models (Table 14), the growth rate, asymptote, and lag time at every desired temperature can be calculated, and using equation 1, a growth curve at that temperature can be described.

If the measured growth data are compared with the model predictions, the resulting model can be evaluated (Fig. 7 to 10). The model describes the data adequately. The growth rate at 6°C and the asymptote at 8.5°C are not very well estimated. The measured growth rate at 6°C is a very small value (0.0164 h^{-1}) and is estimated by the model as 0.00675 h^{-1}. The lag time at 6°C is estimated well, which results in a

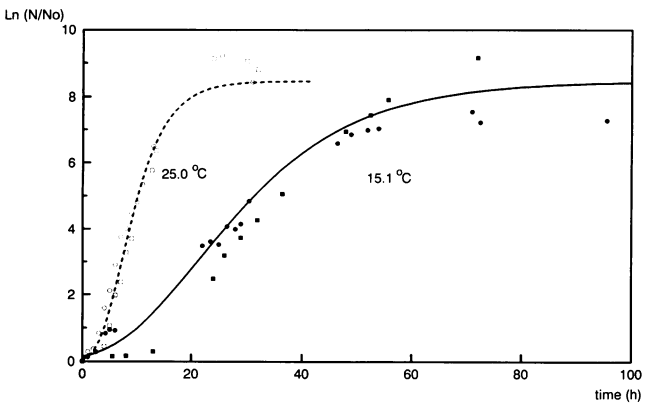

FIG. 8. Growth data and total model at 15.1°C (■, ●) and 25.0°C (□, ○). Different symbols indicate different duplicates.

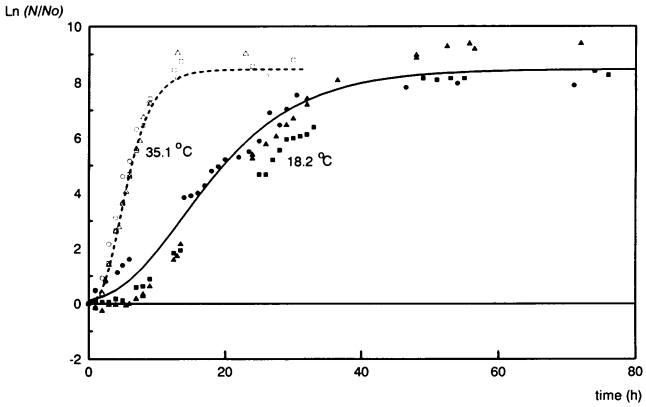

FIG. 9. Growth data and total model at 18.2°C (■, ●, ▲) and 35.1°C (□, ○, △). Different symbols indicate different duplicates.

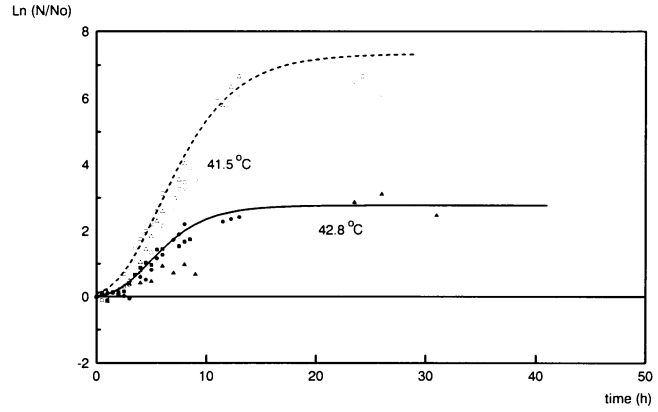

FIG. 10. Growth data and total model at 41.5°C (□, ○, △) and 42.8°C (■, ●, ▲). Different symbols indicate different duplicates.

reasonable prediction of the dynamic behavior over a long period (almost 3 months). The asymptote at 8.5°C is not very well estimated. The reason can be found in the fact that the model prediction at 8.5°C in Fig. 5 is greater than the datum points. All the other predictions (also at the temperatures not presented here) agreed very well with the measured values. The model prediction is usually in between the duplicate or triplicate observations.

Conclusions. We now have a model describing the growth curve of *L. plantarum* in MRS medium including lag time, growth rate, and asymptotic value. In these studies, a simple medium was chosen to collect a large number of datum points as it was the objective of this study to distinguish between models. With the model proposed here, growth over the whole relevant temperature range can be predicted. In practical situations other media will be used and the parameter values will have to be determined for that situation. Often a much smaller number of datum points will be collected. This indicates again the importance of a small number of parameters, because the solutions are more stable and the estimates of the parameters have a larger number of degrees of freedom using a model with a smaller number of parameters. In our case (38 experiments, 18 temperatures), models with a small number of parameters are selected. But normally growth rates are measured at far less different temperatures, so models with more parameters will not be relevant. Since the models are not rejected with a large amount of data (38 growth curves at 18 different temperatures), it is not advisable to use models with a larger number of parameters with many fewer datum points.

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