Utility of Phenomenological Models for Describing Temperature Dependence of Bacterial Growth

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We compared three unstructured mathematical models, the master reaction, the square root, and the damage/repair models, for describing the relationship between temperature and the specific growth rates of bacteria. The models were evaluated on the basis of several criteria: applicability, ease of use, simple interpretation of model parameters, problem-free determination of model parameters, statistical evaluation of goodness of fit ($\chi^2$ test), and biological relevance. Best-fit parameters for the master reaction model could be obtained by using two consecutive nonlinear least-square fits. The damage/repair model proved to be unsuited for the data sets considered and was judged markedly overparameterized. The square root model allowed nonproblematical parameter estimation by a nonlinear least-square procedure and, together with the master reaction model, was able to describe the temperature dependence of the specific growth rates of Klebsiella pneumoniae NCIB 418, Escherichia coli NC3, Bacillus sp. strain NCIB 12522, and the thermotolerant coccobacillus strain NA17. The square root and master reaction models were judged to be equally valid and superior to the damage/repair model, even though the square root model is devoid of a conceptual basis.

Temperature is an important factor affecting the growth of bacteria. Most bacteria grow over a temperature range of some 30 to 40°C, irrespective of whether they are psychrophiles, mesophiles, or thermophiles (28). Within the growth-permissible temperature range for any specific strain, four subranges can be identified as the temperature is increased from the minimum to the maximum for growth. These subranges are described as the sub-Arrhenius, Arrhenius, optimum, and superoptimum ranges, respectively. In addition, temperatures below the minimum growth temperature and those above the maximum growth temperature constitute the subminimum and supermaximum ranges, respectively. Within the growth-permissible temperature range, temperature affects the specific growth rate and changes in temperature will influence both the metabolism and performance of bacteria (17, 18, 20).

As far as bacterially mediated engineered processes are concerned, the Arrhenius, optimum, superoptimum, and supermaximum temperature subranges are of the greatest interest, whereas in most natural environments, bacteria are subjected to the subminimum, the sub-Arrhenius, and the Arrhenius subranges. Several unsegregated and unstructured phenomenological models have been proposed to describe the growth of bacteria at temperatures throughout the entire growth-permissible temperature range. Provided that such models effectively describe the macroscopic growth characteristics of diverse bacterial cultures at various temperatures and provided that their limitations, primarily the lack of insight they provide with respect to either microscopic or molecular mechanisms, are fully recognized, they are valuable guides for process design and operation and the prediction of culture performance and growth behavior from limited data.

In some respects, phenomenological models are to biology what classical thermodynamics has been to chemistry. Their value is in giving a macroscopic description rather than a microscopic understanding of particular processes. In the present contribution, the objective is to statistically evaluate the fidelity and determine the utility of three different phenomenological models that have been proposed for describing growth over the entire permissible temperature range of any particular microbial strain. The judgment criteria for the utility of the three models are their ease of use, their applicability to any complete range of growth temperatures, their ability to maintain a high degree of biological reality, simple interpretation of their respective parameters, and problem-free determination of these parameters. To this end, five independently determined data sets have been used as the basis for evaluation.

THEORY

The earliest descriptions of the relationship between biological process rates and temperature (5, 8) were based on the Arrhenius equation, i.e.,

$$k = A \exp \left(-\frac{E_a}{RT}\right)$$

(1)

in which $k$ is the reaction rate constant, $A$ is the Arrhenius frequency factor, $E_a$ is a temperature characteristic or apparent activation energy, $R$ is the gas constant and $T$ is the absolute temperature. Equation 1 was derived by analogy with the van't Hoff equation, which expresses the change with temperature of an equilibrium constant. Subsequently (10, 13), the temperature dependence of the reaction rate constant was also expressed as

$$k = \frac{k_B T}{h} \exp \left(\frac{\Delta S^\circ}{R}\right) \exp \left(-\frac{\Delta H^\circ}{RT}\right)$$

(2)

by using Eyring’s transition state theory as a basis. According to equation 2, in which $k_B$ is Boltzmann’s constant, $h$ is Planck’s constant, and $k$ is the transmission coefficient, the rate constant can be related to the quasi-thermodynamic properties of the activated complex, i.e., to both $\Delta H^\circ$, the
enthalpy of formation of the activated complex, and $\Delta S^*$, the entropy of formation of the activated complex. Examination of the temperature dependence of the logarithm of the rate constants in both equations 1 and 2 reveals that

$$ A = \left( \frac{k_B T e}{h} \right) \exp (\Delta S^*/R) \tag{3} $$

Equation 3 shows that the Arrhenius frequency factor, $A$, contains factors for the frequency of vibration in the reaction coordinate, $k_B T / h$, and for orientation, $\exp (\Delta S^*/R)$ (10). When either equation 1 or 2 was used to describe bacterial growth at various temperatures, neither was found to be applicable for the whole of the growth-permissible temperature range. As a result, encompassing models, which sought to account for the several significant deviations from Arrhenius-type behavior that occur, were developed.

**Master reaction models.** Assuming that growth is the end product of a sequence of enzymatic reactions and that only one reaction in this sequence limits the specific growth rate, a successful description of the substrate dependence of the specific growth rate has been published (26). Therefore, it has been postulated that the effects of temperature on the specific growth rate can be described in a similar manner (7, 11, 13, 21, 32, 33). In such an approach, a rate-limiting master enzyme is considered to be in an equilibrium state between one active and two inactive states resulting either from high- or low-temperature denaturation so that the total enzyme present, $[E_T]$, is the sum of the three component states, whereby $[E_T]$ is the intrinsic biotic concentration of the rate-limiting enzyme (kilograms of enzyme per kilogram of biomass). When the single enzymatic reaction limits growth under substrate-limiting conditions, the maximum specific growth rate, $\mu_m$, can be expressed as a product of the intrinsic concentration of the activated enzyme and the rate constant, $k$:

$$ \mu_m = \left( \frac{[E_T]}{1 + K_L + K_H^*} \right) k \tag{4} $$

where $K_L$ and $K_H$ are the equilibrium constants for low- and high-temperature enzyme inactivation, respectively. After incorporation of equation 2 to account for temperature and substitution of the two equilibrium constants, $K_L$ and $K_H$, the following expression for the specific growth rate is obtained:

$$ \mu_m = \left( \frac{[E_T] k_B k K}{h} \right) T \exp \left( \frac{\Delta S^*}{R} - \frac{\Delta H^*}{R} \right) \left( 1 + \exp \left( \frac{\Delta S^*/R}{h} - \frac{\Delta H^*/R}{RT} \right) + \exp \left( \frac{\Delta S^*/R}{h} - \frac{\Delta H^*/R}{RT} \right) \right) \tag{5} $$

According to Sharpe and DeMichele (33), equation 5 is suitable for describing the temperature dependence of the maximum specific growth rate for any poikilothermal organism. In the case when only high-temperature deactivation is considered, equation 5 results in the model proposed by Johnson and Levin (21). Incorporation of equation 1 into equation 4 and consideration of only high-temperature deactivation gives the model proposed by Esener et al. (11).

To evaluate the validity of equation 5, it was necessary to transform certain parameters in accordance with the proposals of Schoolfield et al. (32), so as to make the equation suitable for the application of nonlinear least-square techniques. The transformations involved setting

$$ r_{(298.15 \text{ K})} = \frac{[E_T] k_B k}{h} 298.15 \text{ K} \exp \left( \frac{\Delta S^*}{R} - \frac{\Delta H^*}{R} 298.15 \text{ K} \right) $$

$$ T_{0.5s} = \Delta H_L^*/\Delta S_L^* $$

and

$$ T_{0.5H} = \Delta H_H^*/\Delta S_H^* $$

so that equation 4 becomes

$$ r_{(298.15 \text{ K})} = \frac{[E_T] k_B k}{h} 298.15 \text{ K} \exp \left( \frac{\Delta H^*}{R} \left( \frac{1}{298.15 \text{ K}} - \frac{1}{R} \right) \right) $$

Therefore, equation 1 was extended by the introduction of an activity state variable, $V$, so that

$$ \mu_m = V \exp \left( \frac{K_0 - E_A}{RT} \right) \tag{8} $$

where $e^{-K_0}$ is the Arrhenius frequency factor, $E_A$ is the activation energy, and $T$ is the absolute temperature. The activity state variable in equation 8 depends on the rate of damage, $\sigma$, which, expressed in dimensionless form, is

$$ \sigma = \frac{1}{1 + \exp \left( \alpha_3 - \alpha_2 (T - 273.15 \text{ K}) \right)} \tag{9} $$

where $\alpha_2$ is the damage parameter describing temperature sensitivity and $\alpha_3$ is the damage parameter corresponding to the temperature inflection point. To account for repair, the repair rate, $\rho$, was expressed in terms of the activity state variable as

$$ \rho = V \exp (-\beta_2 V) \tag{10} $$

It is assumed that microorganisms compensate for higher damage rates by lowering the activity state variable, such that at any steady state the condition $\sigma = \rho$ is valid. The boundary conditions are (i) if $\sigma \geq \sigma_{\text{max}}$ with $\rho_{\text{max}} = 1/(e\beta_2)$, then $V = 0$, and (ii) if $\sigma \leq \sigma_{\text{min}}$ with $\rho_{\text{min}} = \beta_3 \exp (-\beta_2 \beta_3)$,
then $V = \beta_3 = \text{constant}$; under these conditions, only the descending branch of the curve resulting from equation 10 is considered. The curve resulting from equation 8 with defined boundary conditions approaches the abscissa asymptotically at suboptimum temperatures, whereas as the maximum growth temperature is approached, a point of no return in terms of repair occurs such that the curve intersects the abscissa and the specific growth rate reaches zero discontinuously.

**Square root model.** In a third, distinctly different approach, Ratkowski et al. (31) have proposed that a square root relationship exists between the maximum specific growth rate and the absolute temperature so that

$$\sqrt{\mu_m} = b (T - T_{\text{min}})$$  \hspace{1cm} (11)

where $b$ represents a regression coefficient, $T$ is the absolute temperature, and $T_{\text{min}}$ is a theoretical minimum temperature for growth. However, to extend the application of equation 11 to the whole of the growth-permissible temperature range, Ratkowski et al. (30) subsequently extended it so that

$$\sqrt{\mu_m} = b(T - T_{\text{min}})[1 - \exp \left[ c (T - T_{\text{max}}) \right]]$$

where $c$ is an additional regression coefficient and $T_{\text{max}}$ is a theoretical maximum temperature for growth. To circumvent problems of propagation of errors of the original data, $\mu_m$, Kohler et al. (22) have suggested that $\mu_m$ should be assumed to be a dependent variable, which leads to

$$\mu_m = b^2 (T - T_{\text{min}})^2[1 - \exp \left[ c (T - T_{\text{max}}) \right]]^2$$ \hspace{1cm} (12)

Additionally, the boundary conditions were given as (i) if $T < T_{\text{min}}$, then $\mu_m = 0$, and (ii) if $T > T_{\text{max}}$, then $\mu_m = 0$. Equation 12 and the boundary conditions define a composite function which is differentiable over its whole domain.

McMeekin et al. (23) have pointed out that a link exists between the square root model and the Arrhenius model and that the square root model is, in fact, a special case of the early growth-temperature relationship developed by Belbradc (6).

**Fitting of the data.** Best-fit curves for the master reaction and square root models with respect to the experimental data were obtained by nonlinear regression using the Marquardt-Levenberg algorithm as implemented by IGOR (WaveMetrics, Lake Oswego, Oreg.). The Marquardt-Levenberg algorithm supplies a covariance matrix, from which estimates for the standard deviations, $s$, of the model parameters can be obtained (29). These values are given in Tables 1 and 4 for all the fitted parameters. The values for $V$ in the damage/repair model were obtained by solving the equation $\sigma = \rho$, using an algorithm that combines linear interpolation, inverse quadratic interpolation, and bisection (program ZBREN/DZBREN; IML Inc. Mathlibrary, Houston, Tex.). Best-fit curves for the experimental data were then obtained by applying the polytope algorithm (program UMPOL/DUMPOL; IML Inc. Math/library).

The standard deviation, $s$, for the determination of the specific growth rate was 0.052 h$^{-1}$ as estimated from four independent measurements for *Klebsiella pneumoniae*. This value was considered to be representative for the method of determination and was also used for the literature data for which no standard deviations were given. The standard deviation was also considered to be constant over the whole range of specific growth rate values (homoscedastic data). The error bars in the figures indicate the 95% confidence interval for the corresponding values.

As the different model equations are nonlinear in their parameters, initial parameter estimates are required for the fitting methods used. All six parameters of the master reaction model have graphical interpretations, and initial estimates can be obtained as described previously (32). Initial estimates of parameters in the square root model can be calculated as described by Ratkowski et al. (30). Unfortunately, for the damage/repair model, a trial-and-error procedure is required to get initial parameter estimates.

**$\chi^2$ statistic.** The probability distribution of $\chi^2$ can be used to estimate the goodness of fit of a model. In particular, $Q$, the computed probability that $\chi^2$ should exceed a particular value by chance, gives a quantitative measure for the goodness of fit of the model. If $Q$ is a very small probability for some particular data set, the apparent discrepancies are unlikely to be chance fluctuations. It is much more likely that the model is wrong or that the measurement errors are markedly greater than stated or are not normally distributed. If $Q$ is too large, too close to unity, literally too good to be true, the cause almost always is that the measurement errors were overestimated (29). In general, a $\chi^2$ value for a moderately good fit is $\chi^2 \approx \nu$, where $\nu$, the number of degrees of freedom, equals $N - M$. $N$ is the number of datum points, and $M$ is number of parameters to be fitted. $Q$ was computed by using an incomplete gamma function (gammq; IGOR). To use the $\chi^2$ statistic, the standard deviations of the datum points must be taken into account, thus requiring the fits to be weighted. It is also important to note that the standard deviations should be assessed independently from the experimental data to be tested (9). As the usual $F$ test for regression and lack of fit is not generally valid in the nonlinear case, the models were judged on the basis of $\chi^2$.

**MATERIALS AND METHODS**

Data concerning the effect of temperature on the maximum specific growth rate for four different bacteria were evaluated. The bacteria were *K. pneumoniae* NCIB 418, the thermotolerant gram-negative cocccobacillus strain NA17 (2), *Escherichia coli* NC3, and the thermotolerant methylotrophic *Bacillus* sp. strain NCIB 12522. Data for the first two organisms were generated especially for the present evaluation, and data for the second two were taken from Herendeen et al. (19) and Al-Awadhi et al. (3, 4), respectively. For *K. pneumoniae*, two different growth media giving significantly different specific growth rate-versus-temperature data were used.

**Growth media.** *K. pneumoniae* was grown in the mineral salts medium of Evans et al. (12), modified by replacing citric acid with 55 mg of Na$_2$EDTA per liter and supplemented with either 1 g of glucose per liter (mineral medium) or 0.5 g of glucose per liter, 2.5 g of yeast extract per liter, and 5 g of tryptic soy broth per liter (complex medium) as appropriate. The media were buffered at pH 6.8 with a 0.1 M Na$_2$HPO$_4$–KH$_2$PO$_4$ mixture. The thermotolerant cocccobacillus strain NA17 was grown in the mineral medium described by Al-Awadhi (2), supplemented with 1 g of ethanol per liter as a carbon energy source.

**Culture methods.** *K. pneumoniae* was grown in 200 ml of medium in magnetically stirred, thermostatted flasks. The temperatures used for growth in mineral medium were 20.0, 25.1, 30.0, 35.5, 35.8, 38.0, 40.5, 41.8, 42.8, 44.7, and 46.0°C.
FIG. 1. Semilogarithmic plot of the specific growth rate versus the reciprocal of the absolute temperature for E. coli NC3 showing the result of the two-step method for fitting the six-parameter master reaction model (dashed line). Starting with the cutoff point, the high-temperature data were used to fit the four-parameter master reaction model (solid line). All data were used to fit the six-parameter master reaction model in the second step (see text). The error bars indicate the 95% confidence interval.

whereas those used for growth in complex medium were 20, 25.1, 30.0, 35.5, 35.8, 38.0, 38.7, 39.5, 40.8, 42.8, 46.0, 47.54, and 48°C. The cocarcobaccillus strain NA17 was grown in 2 liters of medium in a laboratory-scale bioreactor (Bioengineering AG, Wald, Switzerland) operating with an impeller speed of 800 rpm and an air flow rate of 35 liters/h and with the pH controlled at 6.8 by addition of either a 1 M NaOH-KOH solution or a 10% (wt/wt) H3PO4 solution. The temperatures used for growth were 35.5, 40.0, 45.0, 50.0, 55.0, 57.0, 59.0, and 60°C. Growth was determined from measurements of the optical density at 546 nm. All experiments were carried out in duplicate, and datum points for calculated specific growth rates represent mean values.

RESULTS

Master reaction model. Preliminary fits of equation 6 to the experimental data by using the Marquardt-Levenberg algorithm resulted in unreliable parameter estimates. This behavior was largely due to the fact that in certain cases the $x^2$ function (likelihood surface) has several local minima, thereby allowing good fits to be obtained in different sectors of parameter space by judicious choice of starting values for the individual parameters and resulting in biologically meaningless best-fit parameters (data not shown). However, when only high-temperature inactivation was considered by using a four-parameter model (equation 7), good stability was indicated and the model was independent of the starting values for the parameters over an acceptable range. Therefore, these initial problems could be resolved by developing a technique which involves two consecutive nonlinear Marquardt-Levenberg fits. The first step in this procedure involves fitting the four-parameter model to a partial data set. The cutoff point with respect to the data is determined by inspection of a semilogarithmic plot of the maximum specific growth rate versus the reciprocal of the absolute temperature, as shown in Fig. 1 for data for E. coli NC3. It is given by the point at which the descending branch of log $\mu$ as a function of $1/T$ starts to deviate significantly from linear behavior. By ignoring data beyond the cutoff, i.e., the sub-Arrhenius range, best estimates for $r_{25°C}$, $\Delta H^\circ$, $T_o$, and $\Delta H^\circ_M$ can be obtained. In the second step, the six-

<table>
<thead>
<tr>
<th>Organism</th>
<th>$T_{cutoff}$ (°C)</th>
<th>$r_{25°C}$ (cell ml$^{-1}$ h$^{-1}$)</th>
<th>$\Delta H^\circ$ (kJ mol$^{-1}$)</th>
<th>$T_o$ (°C)</th>
<th>$\Delta H^\circ_M$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli NC3</td>
<td>30.0</td>
<td>20.51 ± 0.19</td>
<td>10.85 ± 1.50</td>
<td>39.02 ± 0.25</td>
<td>1.00 ± 0.02</td>
</tr>
<tr>
<td>E. coli NA17</td>
<td>31.0</td>
<td>21.03 ± 0.21</td>
<td>10.92 ± 1.55</td>
<td>39.25 ± 0.29</td>
<td>1.03 ± 0.02</td>
</tr>
<tr>
<td>E. coli NC18</td>
<td>32.0</td>
<td>21.54 ± 0.23</td>
<td>11.00 ± 1.60</td>
<td>39.50 ± 0.32</td>
<td>1.05 ± 0.02</td>
</tr>
<tr>
<td>E. coli NC19</td>
<td>33.0</td>
<td>22.05 ± 0.25</td>
<td>11.05 ± 1.65</td>
<td>39.75 ± 0.35</td>
<td>1.07 ± 0.02</td>
</tr>
<tr>
<td>E. coli NC20</td>
<td>34.0</td>
<td>22.56 ± 0.27</td>
<td>11.10 ± 1.70</td>
<td>40.00 ± 0.38</td>
<td>1.09 ± 0.02</td>
</tr>
</tbody>
</table>

The first number of each pair is the value for the fit to the four-parameter model (first step). The number for the second step is the calculated optimal growth temperature, and the associated statistical parameters obtained from a nonlinear least-square fit to the master reaction model according to the two-step method.

The calculation of the cutoff, i.e., $T_{cutoff}$, is based on the principle that the specific growth rate should be calculated by the following equation: $r_{cutoff} = \frac{\Delta H^\circ}{T_o} + K_{ext}$. The values of $K_{ext}$ are calculated from the Arrhenius equation: $K_{ext} = \frac{1}{T_o} \exp\left(\frac{-\Delta H^\circ}{RT_o}\right)$. The values of $\Delta H^\circ$ and $T_o$ are obtained from the first step. The value of $T_{cutoff}$ is then calculated by rearranging the equation for $r_{cutoff}$.
parameter model (equation 6) is fitted to the whole data set, maintaining the values fixed for the four parameters obtained in the first step, in order to obtain best estimates for both \( \Delta H^0 \) and \( T_{0.5L} \). By using this approach, the fits obtained for the six-parameter model converged appropriately and were independent of the starting values chosen for the parameters, and best-fit estimates of the parameters remained within the biologically meaningful range.

Estimated model parameters for the five data sets considered are given in Table 1; in Fig. 2 the experimental data are compared with the best-fit curves generated. For \( K.\ pneumoniae\ NCIB\ 418\) (Fig. 2A), the higher maximum specific growth rates observed in a complex medium compared with those observed in a mineral medium are clearly evident, as are the different boundaries of both the subranges and the overall growth-permissible temperature range. With this bacterium growing on both media, comparison of the experimental data with the four- and six-parameter model curves indicates correspondence for the Arrhenius, optimum, and the first part of the superoptimum temperature ranges. In addition, the six-parameter model adequately describes the sub-Arrhenius range. However, at the upper end of the superoptimum temperature range for growth, both models deviate markedly from the experimental data in that the asymptotic approaches of the model curves to the abscissa are insufficiently rapid. Comparison of the other data sets, i.e., those for \( E.\ coli\) NC3 (Fig. 2B), the thermotolerant coccobacillus NA17 (Fig. 2C), and the thermotolerant \( Bacillus\) sp. strain NCIB 12522 (Fig. 2D), with the respective model curves gives essentially similar results. This is because the model only asymptotically approaches a growth rate of zero.

As can be seen in Table 1, the number of degrees of freedom for the fitted data sets significantly affects the relative magnitude of the standard deviations of the parameter estimates. In addition, the spreading of the individual experimental datum points can impact strongly on the standard deviations, as indicated for \( T_{0.5L} \) and \( \Delta H^0 \) values for \( K.\ pneumoniae\), strain NA17 and \( Bacillus\) sp. strain NCIB 12522. In contrast, the standard deviations obtained for the above-mentioned parameters of \( E.\ coli\) were significantly smaller, as one would expect, as a result of the larger
number of datum points at lower temperatures. The high standard deviations of $\Delta H^\circ_{\text{H}}$ for strain NA17 and Bacillus sp. strain NCIB 12522 are due to a paucity of datum points in the superoptimum temperature range.

Analysis of $\chi^2$ values obtained from the weighted fits indicates that for *K. pneumoniae* NCIB 418 growing in mineral medium, for the coccobacillus NA17, and for *Bacillus* sp. strain NCIB 12522, the apparent discrepancies between the data sets and the models are most probably chance fluctuations. For *K. pneumoniae* growing in complex medium, analysis of the residuals revealed that the elevated $\chi^2$ value is caused by a single point ($\mu = 0$ at 320 K) and can be judged as a problem of the model approach at the upper end of the superoptimum temperature range, whereas for *E. coli* NC3, the $\chi^2$ value suggests that the standard deviation assumed for the maximum specific growth rates was an underestimate.

**Damage/repair model.** For the damage/repair model, equations 8 to 10 and the specified boundary conditions define a function that cannot be differentiated at the temperature at which $V(T) = \beta_3$. In addition, the function is discontinuous at the maximum temperature for growth, which is given by

$$T_{\text{max}} = \frac{-\alpha_1 - \ln(\beta_2)}{\alpha_2} + 273.15 \text{ K} \quad (13)$$

In this case, the Marquardt-Levenberg algorithm could not be applied, but a polytope algorithm, which does not assume smoothness, showed satisfactory convergence from a particular set of starting values and was therefore used for this nonlinear problem. However, this algorithm provides no information about the standard deviations of the parameters.

For *K. pneumoniae* NCIB 418 growing in mineral medium, the several best-fit curves obtained for the model with different initial conditions are compared with experimental data in Fig. 3, and the corresponding model parameters are given in Table 2. It can be seen that the final parameter values obtained were highly dependent on initial parameter values. By changing only one starting value for a single parameter, markedly different final parameter estimates resulted. Two essentially different-shaped curves resulted from the three different final parameter estimates (Fig. 3).

One curve gives a sharp peak at the optimum temperature for growth, whereas the other gives a much broader maximum. The two curves exhibiting sharp peaks are almost identical even though their damage and repair parameters are markedly different. Furthermore, the values for $K_\alpha$ and $E_A$ remain essentially constant, but are totally different from the values applicable to the curve with the broad maximum. Even so, the quality of fit between the different-shaped curves resulting from the model and the experimental data, judged from the $\chi^2$ statistics, were in the same range.

The comparison of experimental data with results from the damage/repair model for *K. pneumoniae* NCIB 418 growing in complex medium, for *E. coli* NC3, for the coccobacillus strain NA17, and for *Bacillus* sp. strain NCIB 12522 is shown in Fig. 4, and the corresponding model parameters are given in Table 3. In contrast to the results obtained for the master reaction model, the maximum growth temperature can be described by the damage/repair model. Model curves (Fig. 4A to C) decline precipitously to zero when the damage rate equals the maximum possible repair rate. However, in the model for *Bacillus* sp. strain NCIB 12522 (Fig. 4D), this requirement is not fulfilled, and since the maximum value of the dimensionless repair rate exceeds unity and the maximum damage rate cannot exceed unity, the damage rate will never equal the repair rate, so that the curve will never intersect the abscissa. This will always be the case when the best estimate for $\beta_2$ is either smaller than or equal to the reciprocal of $\epsilon$ (Euler’s number; 1/2.718), as is evident from equation 13.

**Square root model.** To obtain best fits for the square root model, we used the weighted Marquardt-Levenberg algorithm. As with the four-parameter master reaction model, fits converged properly and final parameter estimates were independent of initially selected values over a reasonable range.

Comparisons between the five sets of experimental data considered and model curves are shown in Fig. 5, and corresponding model parameters are given in Table 4. Analysis of the $\chi^2$ values shows that discrepancies between the model and the experimental data are most probably caused

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**TABLE 2. Dependence of the damage/repair model on the initial parameter estimates**

<table>
<thead>
<tr>
<th><em>K. pneumoniae</em> NCIB 418 grown in glucose medium</th>
<th>Best-fit model parameter</th>
<th>$T_{\text{opt}}$ (K)</th>
<th>$T_{\text{max}}$ (K)</th>
<th>Statistical parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_2$ (K$^{-1}$)</td>
<td>$\alpha_3$</td>
<td>$\beta_2$</td>
<td>$\beta_3$</td>
<td>$K_\alpha \ln(1/\text{h})$</td>
</tr>
<tr>
<td>(-----)</td>
<td>(-----)</td>
<td>(-----)</td>
<td>(-----)</td>
<td>(-----)</td>
</tr>
<tr>
<td>1.32 (1.0)</td>
<td>55.90 (62.0)</td>
<td>0.39 (0.5)</td>
<td>36.47 (20.0)</td>
<td>22.35 (20.0)</td>
</tr>
<tr>
<td>(--- ---)</td>
<td>1.22 (1.2)</td>
<td>51.79 (62.0)</td>
<td>0.59 (0.5)</td>
<td>15.14 (20.0)</td>
</tr>
<tr>
<td>(-----)</td>
<td>2.6391 (2.0)</td>
<td>115.12 (62.0)</td>
<td>2.53 (0.5)</td>
<td>7.46 (20.0)</td>
</tr>
</tbody>
</table>

* The line styles indicated correspond to the curves in Fig. 3.

* Values of the best-fit model parameters ($\alpha_2$, $\alpha_3$, $\beta_2$, $\beta_3$, $K_\alpha$, and $E_A$), of the calculated optimum and maximum growth temperatures ($T_{\text{opt}}$ and $T_{\text{max}}$), and of the associated statistical parameters ($\chi^2$, $\nu$, and $Q$) are given for *K. pneumoniae* NCIB 418. The initial values of the parameters for each fit are given in parentheses.
FIG. 4. Experimental data and fitted curves for the damage/repair model. (A) *K. pneumoniae* NCIB 418 grown in complex medium; (B) *E. coli* NC3 grown in complex medium; (C) NA17 grown in a mineral salt medium with ethanol as the carbon energy source; (D) *Bacillus* sp. strain NCIB 12522 grown in a mineral salt medium with methanol as the carbon energy source. The error bars indicate the 95% confidence interval.

by chance fluctuations. The model, as used here (equation 12), is able to describe the temperature behavior of all data sets adequately. For the coccobacillus strain NA17, an overestimation of the standard deviation for the data set is indicated, as $Q$ is close to unity. It can be seen from Table 4 that the standard deviations associated with the minimum temperatures for growth are significantly larger than those associated with the maximum temperatures for growth for all five data sets. In addition to other factors, a paucity of datum points in the sub-Arrhenius range contributes to this finding.

**DISCUSSION**

In analyses of microbial growth data, problems arising from error propagation, the analysis of errors in the estimated parameters, correlation of parameters, and consider-

**TABLE 3.** Values of the best-fit model parameters, of the calculated optimum and maximum growth temperatures, and of the associated statistical parameters obtained from a nonlinear fit to the damage/repair model

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>$a_2$ ($K^{-1}$)</th>
<th>$a_3$</th>
<th>$\beta_2$</th>
<th>$\beta_3$</th>
<th>$K_0$ ln(1/h)</th>
<th>$EA$ (kJ mol$^{-1}$)</th>
<th>$T_{opt}$ (K)</th>
<th>$T_{max}$ (K)</th>
<th>$\chi^2$</th>
<th>$v$</th>
<th>$Q$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. pneumoniae</em> NCIB 418 grown in complex medium</td>
<td>3.05</td>
<td>145.23</td>
<td>0.39</td>
<td>75.59</td>
<td>15.55</td>
<td>49.61</td>
<td>312.5</td>
<td>321.7</td>
<td>14.62</td>
<td>6</td>
<td>0.023</td>
</tr>
<tr>
<td><em>E. coli</em> NC3</td>
<td>1.51</td>
<td>69.93</td>
<td>0.40</td>
<td>133.71</td>
<td>37.55</td>
<td>105.22</td>
<td>313.5</td>
<td>321.2</td>
<td>26.33</td>
<td>11</td>
<td>5.8 x 10$^{-3}$</td>
</tr>
<tr>
<td>Strain NA17</td>
<td>0.85</td>
<td>48.79</td>
<td>0.48</td>
<td>96.11</td>
<td>38.68</td>
<td>113.63</td>
<td>325.2</td>
<td>332.2</td>
<td>0.26</td>
<td>2</td>
<td>0.88</td>
</tr>
<tr>
<td><em>Bacillus</em> sp. strain NCIB 12522</td>
<td>1.96</td>
<td>115.06</td>
<td>0.26</td>
<td>61.73</td>
<td>13.89</td>
<td>49.11</td>
<td>325.9</td>
<td>325.9</td>
<td>9.99</td>
<td>2</td>
<td>7.8 x 10$^{-3}$</td>
</tr>
</tbody>
</table>
FIG. 5. Experimental data and fitted curves for the square root model. (A) *K. pneumoniae* NCIB 418 grown in glucose mineral salts medium (●) and in complex medium (○); (B) *E. coli* NC3 grown in complex medium; (C) NA17 grown in a mineral salt medium with ethanol as the carbon energy source; (D) *Bacillus* sp. strain NCIB 12522 grown in a mineral salt medium with methanol as the carbon energy source. The error bars indicate the 95% confidence interval.

The error bars indicate the utility of the goodness of fit are frequently neglected (1, 15, 16, 25). However, for the models under consideration in this study, nonlinear least-square techniques have been used as maximum likelihood estimators, and the results clearly illustrate that mere phenomenological inspection of a fitted model curve and its comparison with the experimental data are not sufficient to evaluate the quality of the fit and the utility of a mathematical model.

A most important question that should be addressed before using any mathematical model to describe a particular biological response is the objective. In general, three main areas of interest can be identified for the specific-growth-rate-versus-temperature relationship. (i) A particular model might be used to predict the specific growth rate of a culture beyond the experimentally investigated temperature range. (ii) The objective is to determine characteristic parameters for comparative reasons, which implies simple interpretation and problem-free determination of the parameters. (iii) The

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Best-fit model parameter ± s</th>
<th>$T_{\text{opt}}$ (K)</th>
<th>Statistical parameter</th>
<th>χ²</th>
<th>ν</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. pneumoniae</em> NCIB 418</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose medium</td>
<td>$0.0390 \pm 0.0038$</td>
<td>$0.3513 \pm 0.0534$</td>
<td>$278.08 \pm 2.25$</td>
<td>$317.05 \pm 0.23$</td>
<td>310.0</td>
<td>15.82</td>
</tr>
<tr>
<td>Complex medium</td>
<td>$0.0440 \pm 0.0028$</td>
<td>$0.2465 \pm 0.0237$</td>
<td>$276.96 \pm 1.57$</td>
<td>$321.99 \pm 0.18$</td>
<td>312.7</td>
<td>11.47</td>
</tr>
<tr>
<td><em>E. coli</em> NC3</td>
<td>$0.0413 \pm 0.0016$</td>
<td>$0.2661 \pm 0.0212$</td>
<td>$277.42 \pm 0.94$</td>
<td>$322.38 \pm 0.18$</td>
<td>313.5</td>
<td>19.18</td>
</tr>
<tr>
<td>Strain NA17</td>
<td>$0.0195 \pm 0.0046$</td>
<td>$0.4936 \pm 0.1640$</td>
<td>$286.59 \pm 7.26$</td>
<td>$332.49 \pm 0.54$</td>
<td>326.4</td>
<td>0.62</td>
</tr>
<tr>
<td><em>Bacillus</em> sp. strain NCIB 12522</td>
<td>$0.0302 \pm 0.0067$</td>
<td>$0.1953 \pm 0.0685$</td>
<td>$290.84 \pm 4.60$</td>
<td>$334.73 \pm 0.87$</td>
<td>324.4</td>
<td>4.48</td>
</tr>
</tbody>
</table>
model is expected to provide insight into fundamental physiological processes that might control the growth rate response.

Considering the three models discussed with respect to these points, it is evident that all the models investigated are able to describe the specific-growth-rate-versus-temperature relationship over almost the entire growth-permissible temperature range. However, when using the Marquardt-Levenberg algorithm, acceptable fits could be obtained only for the master reaction and square root models. In the damage/repair model, the model function has locations of nondifferentiability and of noncontinuity and, further, requires that the activity state variable, \( V \), be evaluated numerically, so that smoothness of the function does not result. These problems were overcome by the use of the polytope algorithm. However, the resulting fits showed strong dependence on the starting values of the various parameters. The data of Soeder et al. (34) were also fitted to the model function but failed to generate the parameter values given for the same data (14), although fits were obtained with a smaller sum of the squared deviations, indicating that the values reported (14) did not correspond to a global minimum. Apparently the \( \chi^2 \) function possesses several six-dimensional minima in a flat valley of complicated topology such that different starting values for the parameters lead to different local minima that are "virtually as good" as a global minimum (9). The model is unsuited for the data sets globalized, indicating that it is overparameterized. This does not necessarily mean that the model is inapplicable under all circumstances, but simply that the available data are inadequate for estimating all the postulated parameters. Even so, compared with the other models evaluated, the damage/repair model has the least practical value, because the parameters are neither easily estimated nor interpretable. If the model can be successfully freed of its intrinsic mathematical inconsistencies, it might be of interest because of its kinetically structured approach to temperature inactivation.

A similar situation to that discussed for the damage/repair model applies to the original master reaction model (equation 5), but it has been shown (32) that by appropriate reparameterization, proper convergence could be obtained for the four-parameter model (equation 7). Direct fits with the six-parameter model (equation 6) exhibit a dependence on starting values for the parameters, and, especially with small data sets, minima with biologically meaningless parameter estimates result. The two-step method proposed herein eliminates most of these problems. It has been suggested (1) that logarithmic transformation of the master reaction model might improve its numerical stability and partially reduce its dependence on the starting values chosen for the parameters, but unfortunately neither discussion of error nor indication that weighted least-square techniques were used were provided. As this is a prerequisite for obtaining statistically interpretable parameter estimates, this proposal could not be pursued further.

Problems associated with the interpretation of the model parameters in the master reaction model are somewhat similar to the situation for Monod growth kinetics. Monod growth kinetics use \( K_s \) values that are mathematically analogous to Michaelis-Menten \( K_M \) values, but can no longer be interpreted as a ratio of rate constants. The same is true for the thermodynamic constants appearing in the master reaction model. They can no longer be interpreted as true thermodynamic properties, unless a single growth-rate-determining reaction can be identified. As this is usually not the case, these model parameters must be considered to be experimentally evaluated specific strain constants under defined nutrient conditions. Nevertheless, they can be of value for comparing and discussing the temperature behavior of different cultures, very much as \( K_s \) values can be used to compare and discuss growth behavior under changing substrate concentrations. It is interesting that the thermodynamic parameters \( \Delta H^\prime, \Delta H^\prime_L \), and \( \Delta H^\prime_P \) are of the same order of magnitude for all the strains evaluated. The \( \Delta H^\prime_P \) values are also comparable to the values for different proteins (27). This is an indication that the theoretical approach used in formulating the model maintains a degree of biological reality; e.g., the macroscopic behavior of the bulk enzyme system used for growth is similar in its temperature response to that for a single enzyme reaction.

The situation is different for the square root model. Although the model defines cardinal temperatures \( (T_{min} \) and \( T_{max} \), at least \( T_{min} \) cannot be interpreted as the minimum temperature of growth (24) but must be considered a conceptual temperature. However, it has been noted that although the actual minimum temperature for growth may occur several degrees above \( T_{min} \), a lot of growth data indicate that the model remains valid to the point at which growth ceases (24). There is, as yet, no physiological explanation for the square root response of bacterial growth rate to temperature, so that the remaining parameters, \( b \) and \( c \), constitute mere regression coefficients.

Tables 1 and 4 show that the precision of the estimated parameters strongly depends on the degree of freedom and the spread of the datum points. As can be seen for \( E. coli \) NC3, the precision dramatically increased with increasing degrees of freedom.

Scoring the three models that have been evaluated in this paper in accordance with the criteria outlined above for judging the utility of unstructured phenomenological models indicates that the square root model and the master reaction model are placed first and the damage/repair model is placed third, even though the square root model is devoid of any conceptual basis. In general, the quality of parameter estimation and the predictive value of models can be improved by more extensive experimental data with a reasonable spread over the entire growth-permissible temperature range. Furthermore, fitting problems due to the intrinsic mathematical structure of a model can be improved by appropriate reparameterization or, as has been shown here, by using a two-step procedure with partial data sets.

REFERENCES