Convenient Model To Describe the Combined Effects of Temperature and pH on Microbial Growth

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A new model in which the maximum microbial specific growth rate (μ_{max}) is described as a function of pH and temperature is presented. The seven parameters of this model are the three cardinal pH parameters (the pH below which no growth occurs, the pH above which no growth occurs, and the pH at which the μ_{max} is optimal), the three cardinal temperature parameters (the temperature below which no growth occurs, the temperature above which no growth occurs, and the temperature at which the μ_{max} is optimal), and the specific growth rate at the optimum temperature and optimum pH. The model is a combination of the cardinal temperature model with inflection and the cardinal pH model (CPM). The CPM was compared with the models of Wijtzes et al. and Zwietering et al. by using previously published data sets. The models were compared on the basis of the usual criteria (simplicity, biological significance and minimum number of parameters, applicability, quality of fit, minimum structural correlations, and ease of initial parameter estimation), and our results justified the choice of the CPM. Our combined model was constructed by using the hypothesis that the temperature and pH effects on the μ_{max} are independent. An analysis of this new model with an *Escherichia coli* O157:H7 data set showed that there was a good correspondence between observed and calculated μ_{max} values. The potential and convenience of the model are discussed.

In recent years, interest in developing mathematical models to describe the growth of microorganisms has increased, especially in the fields of medicine and food science. The advantage of such models is that they can be used to simulate the effects of different environmental conditions on growth kinetics. Temperature and pH are the major environmental factors that affect growth which are studied most because of their importance in fundamental research (taxonomy, microbial metabolism) and their practical importance (control of bioprocesses in biotechnology and safe handling of goods, especially in the agriculture and food industries).

Models of microbial growth usually describe variation in the maximum specific growth rate (μ_{max}), which is a reflection of metabolic activity. Several authors have proposed models to describe the combined effects of temperature and pH on μ_{max}. Adams et al. (1) modified the model of Ratkowsky et al. (12) to obtain a combined model which describes growth at temperature and pH values below the optimal values. Wijtzes et al. (15) have proposed a combined growth model for the whole range of pH values at which growth occurs and for suboptimal temperatures. Finally, Zwietering et al. (17) have proposed a combined model for all growth temperatures and pH values, in which nine parameters were defined.

None of these models respects all of the prescribed quality criteria for descriptive models. Some parameters have no obvious biological significance; this makes using the models difficult. In addition, the models were constructed by using the model of Ratkowsky et al. (11), which has the mathematical form $F(x) = f(x)e^{g(x)}$; this form often induces important structural correlations between parameters and increases parameter estimation problems (9, 13).

In this paper we describe a new model in which we tried to avoid these problems.

MATERIALS AND METHODS

Models. We studied two different previously published models. The first model was the combined model of Wijtzes et al. (15), which takes into account temperature and pH as control factors,

$$\mu_{max} = b_1\{(\text{pH} - \text{pH}_{min})[1 - e^{c_1(\text{pH} - \text{pH}_{max})}](T - T_{min})\}^2 \tag{1}$$

where pH_{max} is the pH above which no growth occurs, pH_{min} is the pH below which no growth occurs, T (in degrees Celsius) is the temperature, T_{min} is the temperature below which no growth occurs, and b_1 (hour^{-1} degrees Celsius^{-2}) and c_1 (dimensionless) are biologically meaningless parameters.

This model was used with a constant temperature value to describe the effect of pH on the μ_{max} (the model of Ratkowsky et al. applied to pH):

$$\mu_{max} = b_2\{(\text{pH} - \text{pH}_{min})[1 - e^{c_1(\text{pH} - \text{pH}_{max})}]\}^2 \tag{1'}$$

where b_2 (hour^{-1} degrees Celsius^{-2}) is a biologically meaningless parameter.

The second model which we used was the complete model of Zwietering et al. (17):

$$\mu_{max} = \mu_{opt} \, \gamma(\text{pH}) \, \gamma(T) \tag{2}$$

with

$$\gamma(\text{pH}) = \left\{ \frac{(\text{pH} - \text{pH}_{min})[1 - e^{c_2(\text{pH} - \text{pH}_{max})}]}{(\text{pH}_{opt} - \text{pH}_{min})[1 - e^{c_2(\text{pH}_{opt} - \text{pH}_{max})}]} \right\}^2 \tag{2.1}$$

$$\gamma(T) = \left\{ \frac{(T - T_{min})[1 - e^{c_3(T - T_{max})}]}{(T_{opt} - T_{min})[1 - e^{c_3(T_{opt} - T_{max})}]} \right\}^2 \tag{2.2}$$

where pH_{opt} is the pH at which the μ_{max} is optimal, T_{opt} is the temperature at which the μ_{max} is optimal, T_{max} is the temperature above which no growth occurs, μ_{opt} (hour^{-1}) is the μ_{max} under optimal conditions (pH_{opt}, T_{opt}), and c_2 (dimensionless) and c_3 (degrees Celsius^{-1}) are regression coefficients.

This complete model was also used to describe the effect of pH on the μ_{max} at a constant temperature:

$$\mu_{max} = \mu_{opt} \, \gamma(\text{pH}) \tag{2'}$$

where μ_{opt} is the μ_{max} determined at pH_{opt}.

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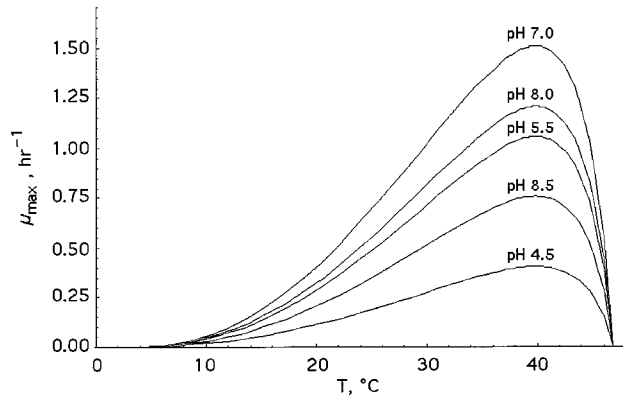
FIG. 1. Influence of temperature and pH on the CTPM. The following parameter values were chosen: T_{min}, 5°C; T_{opt}, 40°C; T_{max}, 47°C; pH_{min}, 4; pH_{opt}, 7; pH_{max}, 9; μ_{opt}, 1.5 h^{-1}.

In addition, Zwietering et al. have shown that c_2 and c_3 verify the following two equations (17):

$$1 - (c_2\, pH_{opt} - c_2\, pH_{min} + 1)\, e^{c_2(pH_{opt} - pH_{max})} = 0 \quad (3)$$

$$1 - (c_3\, T_{opt} - c_3\, T_{min} + 1)\, e^{c_3(T_{opt} - T_{max})} = 0 \quad (4)$$

Both of the models described above were compared with a new combined model by taking into account pH and temperature as control factors.

As suggested by previous experimental observations (1) and proposed by Zwietering et al. (17), temperature and pH seem to have independent effects on μ_{max}, as shown in Fig. 1. This hypothesis is biologically simple and can be expressed by the following equation, simulations of which are shown in Fig. 1:

$$\mu_{max}\,(T, pH) = CTPM(T, pH) = \mu_{opt}\,\tau(T)\,\rho(pH) \quad (5)$$

where $\tau(T)$ is a function of temperature only and $\rho(pH)$ is a function of pH only. For $\tau(T)$, the parameters used are T_{min}, T_{max}, and T_{opt}. For $\rho(pH)$, the parameters used are pH_{min}, pH_{max}, and pH_{opt}.

This formula was chosen because of its mathematical simplicity, and in this formula the number of parameters is reduced to the barest minimum (i.e., seven including μ_{opt}). This new model is called the cardinal temperature and pH model (CTPM).

The change in the μ_{max} as a function of temperature alone was previously described by a cardinal temperature model with inflection (13). A previous analysis of this model by Rosso et al. (13) showed that in contrast to the models of Hinshelwood (7), Ratkowsky et al. (11), and Zwietering et al. (16), there was no structural correlation between parameters (results obtained for 47 data sets and especially for a 217-point data set); this model also demonstrated the simple biological significance of all parameters and exhibited a good quality of fit. The cardinal temperature model with inflection is defined as follows:

$$\mu_{max} = \begin{cases} T < T_{min}, & 0.0 \\ T_{min} < T < T_{max}, & \mu_{opt}\,\tau(T) \\ T > T_{max}, & 0.0 \end{cases}$$

$\tau(T)$

$$= \frac{(T - T_{max})\,(T - T_{min})^2}{(T_{opt} - T_{min})\,[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]} \quad (6)$$

This equation can be written $\mu_{max}\,(T) = \mu_{opt}\,\tau(T)$.

The change in μ_{max} as a function of pH alone involves three cardinal pHs, pH_{min}, pH_{opt}, and pH_{max}. This change can be described by a cardinal pH model (CPM), as follows:

$$\mu_{max} = \begin{cases} pH < pH_{min}, & 0.0 \\ pH_{min} < pH < pH_{max}, & \mu_{opt}\,\rho(pH) \\ pH > pH_{max}, & 0.0 \end{cases}$$

$$\rho(pH) = \frac{(pH - pH_{min})\,(pH - pH_{max})}{(pH - pH_{min})(pH - pH_{max}) - (pH - pH_{opt})^2} \quad (7)$$

The CPM is a simplification of a previously published model in which there is no inflection point between pH_{min} and pH_{opt} (9) and for which no structural correlation between parameters can be demonstrated.
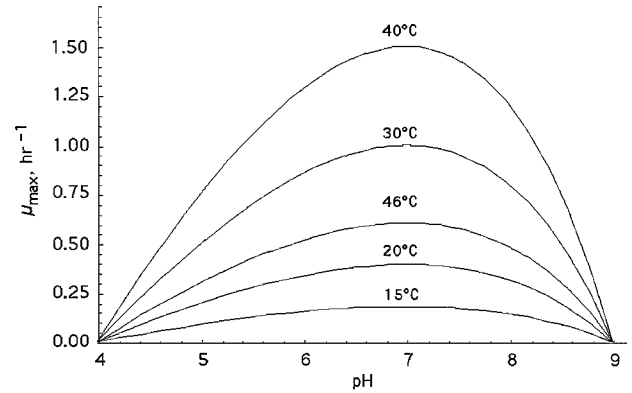
Data. All data used in this study are typical of data that could be obtained in practice.

(i) Data for CPM validation. The CPM (equation 7) was compared with the model of Wijtzes et al. (equation 1′) and the model of Zwietering et al. (equation 2′) by using nine different data sets obtained from previously published studies performed in different fields of research; the data used were data for *Propionibacterium acnes* (14) from medicine, data for *Listeria monocytogenes* (10) and *Brucella melitensis* (5) from food safety studies, and data for *Butyrivibrio fibrisolvens* (8), *Megasphaera elsdenii* (14), *Streptococcus bovis* (8, 14), and *Selenomonas ruminantium* subsp. *lactilytica* (14) from ecological studies.

(ii) Data for CTPM validation. The CTPM (equation 5) and the complete model of Zwietering et al. (equation 2) were studied by using an *Escherichia coli* O157:H7 data set previously published by Buchanan and Klawitter (4). The data, which were obtained from a study in which a good experimental design was used, contained 34 μ_{max} values that were estimated by the Gompertz function as described by Gibson et al. (6); in this study the authors used aerobic conditions, temperatures of 5 to 42°C, pH values ranging from 4.5 to 8.5, and a salt concentration of 0.5% (wt/vol).

Data processing. (i) Model fit. The ordinary least-squares criterion was used to fit the models to the data. The sum of the squared residuals (SSR) was defined as follows:

$$SSR = \sum_{i=1}^{n} [\mu_{max_i}\,observed - \mu_{max_i}\,calculated]^2 \quad (8)$$

where n is the number of data points.

The smaller the SSR, the better the fit. The minimum SSR values (SSR_{min}) were computed with double precision by using calls to IMSL 1.1 subroutine DUMINF (IMSL, Inc., Houston, Tex.), a derivative-free modification (3) of the Levenberg-Marquardt algorithm. Starting values for parameters were chosen directly from the graphic representations of the data for the CPM and CTPM. For the models of Wijtzes et al. and Zwietering et al., when a parameter was biologically meaningless and did not have any direct graphic counterpart, its starting value was chosen on the basis of the results of an

TABLE 1. SSR_{min} values obtained with the CPM and equation 1′ for nine previously published data sets

Data set	SSR_{min}	
	CPM	Equation 1′
Butyrivibrio fibrisolvens	0.03536	—[a]
Brucella melitensis	0.00021	0.00031
Listeria monocytogenes	0.01017	0.02465
Megasphaera elsdenii	0.00338	0.01116
Propionibacterium acnes	0.00073	0.00183
Streptococcus bovis set a	0.12694	0.14625
Streptococcus bovis set b	0.01134	0.07513
Selenomonas ruminantium set a	0.00002	0.00383
Selenomonas ruminantium set b	0.01504	0.09777

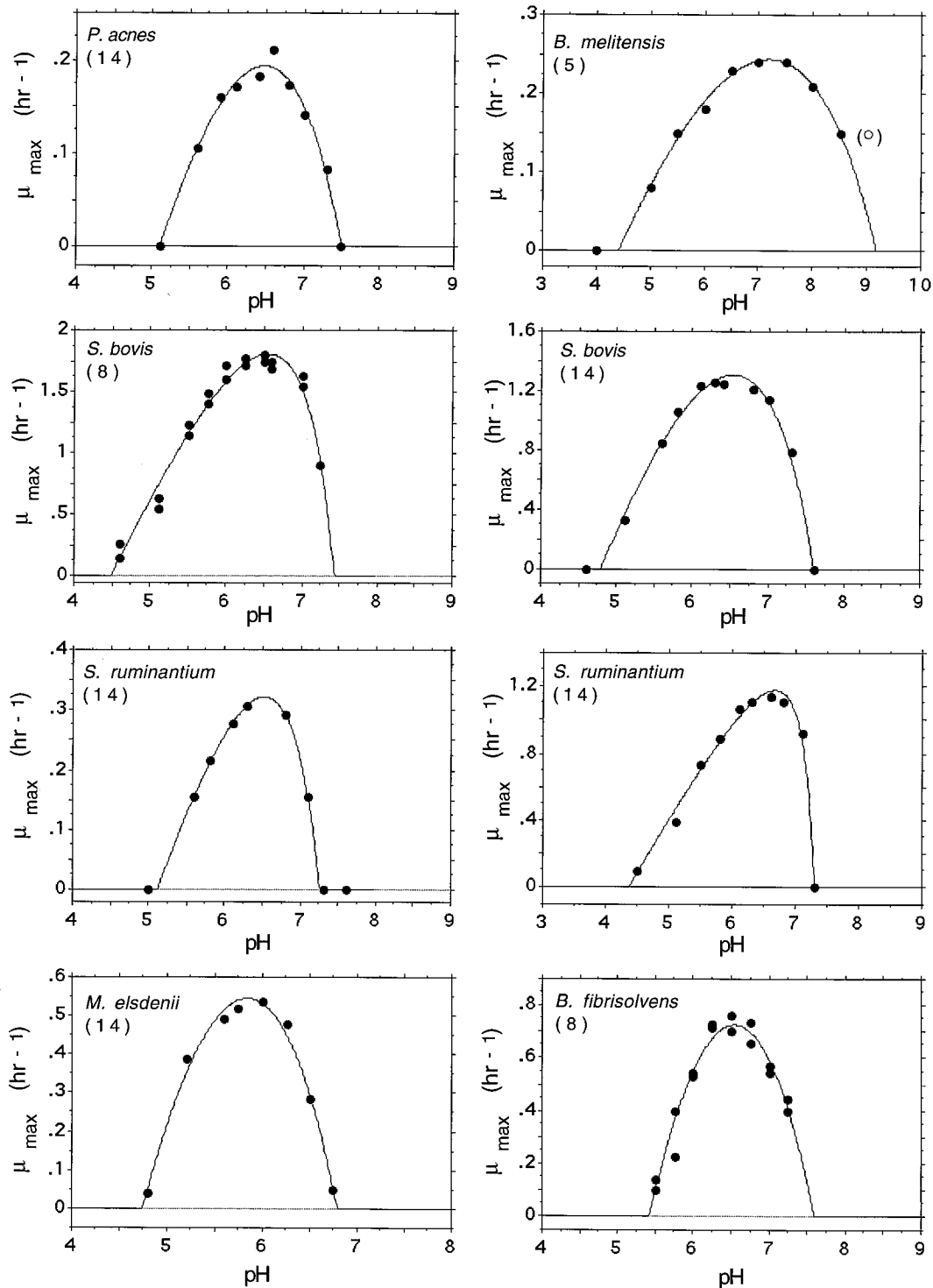[a] —, No convergence was observed after 50,000 iterations.

FIG. 2. Fit of the CPM with eight different previously published data sets. The numbers in parentheses are reference numbers. The open circle on the *Brucella melitensis* graph is considered an outlier.

empirical trial (equations 1 and 2) or was computed by iteratively solving equations 3 and 4.

(ii) Parameter confidence limits. Confidence regions ($\alpha = 0.05$) for parameter values were defined as described by Beale (2) and were determined by a previously described method (9), with some modifications. This method involves systematic random sampling in the parameter space of points whose SSR value is less than the threshold value given in Beale's theory. All of these points are projected in each of the parameter planes, which materializes the confidence region. This method minimizes underestimating the parameter confidence limits, as is the case with standard approximate marginal confidence limits.

RESULTS AND DISCUSSION

Validation of CPM and comparison with equations 1' and 2'. We encountered some difficulties with equations 1' and 2'
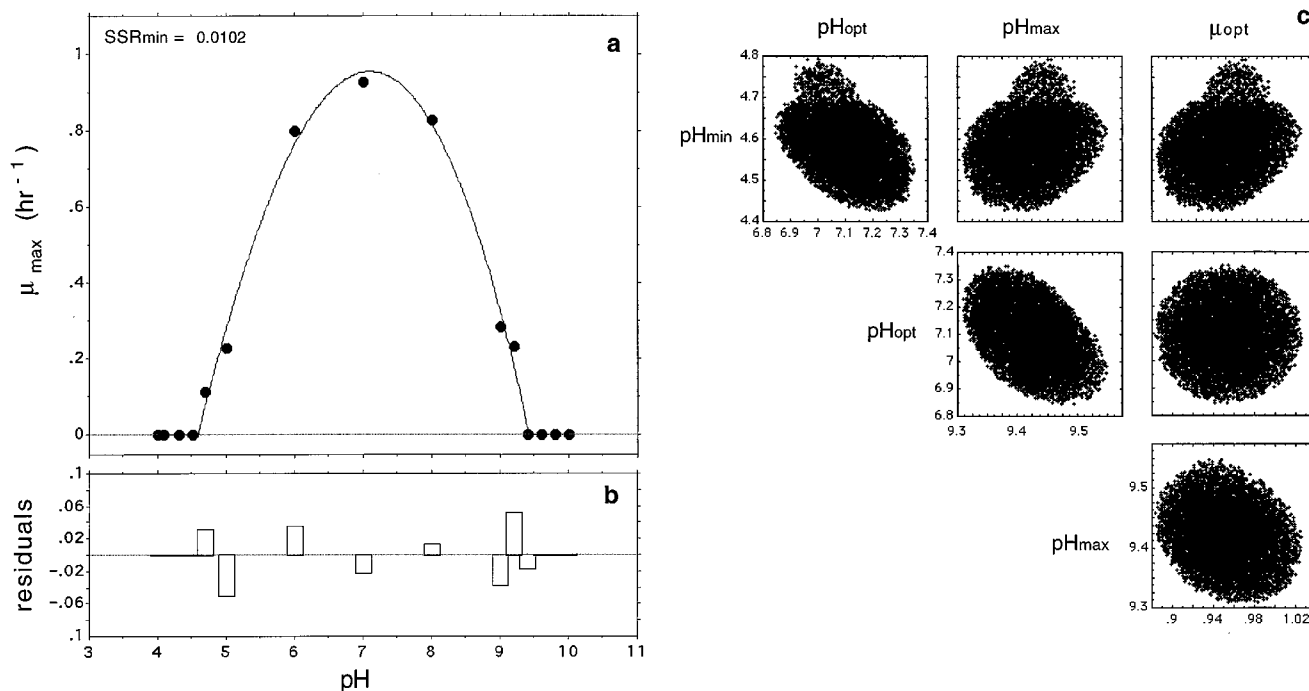
FIG. 3. (a) Fit of the CPM with the data set of Petran and Zottola. (b) Residual plot (observed-calculated). (c) Confidence regions of parameter value estimates. The regions (10,000 points were computed) look bilobed, which could materialize the presence of a local minimum of the SSR criterion.

during computations. The lack of biological or simple mathematical significance of some parameters (b_1, c_1, and c_2) made estimating their initial values difficult. Moreover, the convergence procedure had to be repeated several times with different initial parameter values and with more than 100 iterations. The convergence toward SSR_{min} was impossible with equation 1′ when the *Butyrivibrio fibrisolvens* data set was used even after 50,000 iterations.

In contrast to equations 1′ and 2′, the CPM gave immediate convergence with less than 100 iterations thanks to the simple initial parameter value estimates. Figures 2 and 3 show the fit of the CPM with the nine data sets.

Equations 1′ and 2′ gave the same SSR_{min} values in all cases. This indicates that there was overparameterization in equation

2′; the addition of one parameter was not rewarded with an improvement in the fit. Thus, equation 2′ was not used for the comparison with the CPM.

Table 1 shows the SSR_{min} values obtained for the CPM and equation 1′ when the data sets were used. These two models have the same number of parameters, and hence, they can be compared on the basis of their SSR_{min} values. The CPM gave the smallest SSR_{min} values at all times. All of the results showed that the CPM was always more appropriate than equations 1′ and 2′. An analysis of a plot of the residual values corroborated the difference in quality of fit between the CPM and the two other models because there was no nonrandom pattern and no obvious heterosedasticity.

A more thorough comparison based on the parameter confidence regions was performed for the three models. Figures 3 and 4 show the results of this complete comparison when the *L. monocytogenes* data set was used. The confidence regions ($\alpha = 0.05$) revealed a strong structural correlation between the parameters of equations 1′ and 2′ when this data set was used. The major correlations observed were correlations between b_2 and c_1 for equation 1′ and correlations between μ_{opt} and pH_{opt} for equation 2′ (Fig. 4). These structural correlations resulted in confidence limits for the parameter values that were large or even unlimited (Table 2), thus suggesting that there was serious overparameterization of the models.

The serious structural correlations between parameters in the models of Wijtzes et al. and Zwietering et al. which we observed explain in part the difficulty in obtaining a stable SSR_{min} value during computations.

Unlike equations 1′ and 2′, the CPM exhibits no structural correlations between parameters and allows the simple and accurate estimation of parameter values and their confidence limits (Table 2). This finding is consistent with previously published results (9). For example, the widths of the confidence intervals for estimated pH_{min} and pH_{max} values are less when
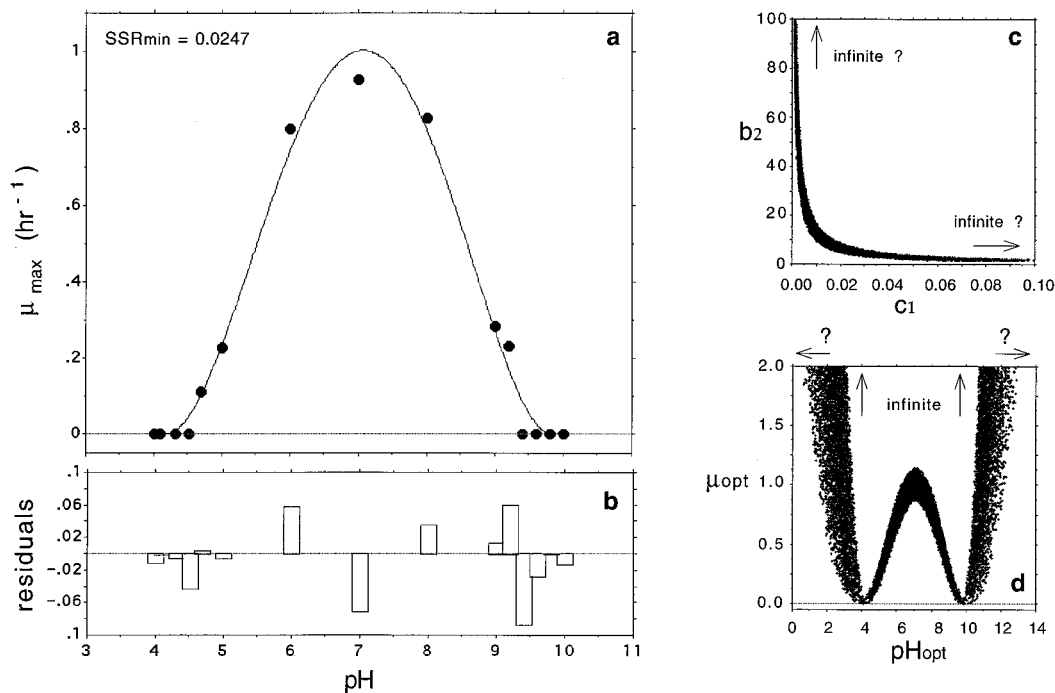
TABLE 2. Estimated parameter values for the CPM and equations 1′ and 2′ for the *L. monocytogenes* data set

Model	Parameter	Estimated value
CPM	pH_{min}	4.6 (4.42, 4.8)[a]
	pH_{opt}	7.1 (6.85, 7.35)
	pH_{max}	9.4 (9.3, 9.55)
	μ_{opt}	0.95 h^{-1} (0.88, 1.02)
Equation 1′	pH_{min}	4.2 (3.85, 4.4)
	pH_{max}	9.8 (9.62, 10.1)
	b_2	4.01 (0.0, ∞)
	c_1	0.032 (0.0, ∞)
Equation 2′	pH_{min}	4.2 (3.61, 4.44)
	pH_{opt}	7.0 ($-\infty$, ∞)
	pH_{max}	9.8 (9.60, 10.32)
	μ_{opt}	1.0 h^{-1} (0.0, ∞)
	c_2	0.032 (-0.213, 0.355)

[a] The values in parentheses are the 95% confidence limits.

FIG. 4. (a) Fit of equations 1′ and 2′ with the data set of Petran and Zottola (the two models gave the same fit). (b) Plot of the residuals (observed-calculated). (c and d) Two examples of the high correlations observed with the models between two of the parameters. (c) Correlation between b_2 and c_1 for equation 1′ (10,000 points were computed). (d) Correlation between pH_{opt} and μ_{opt} for equation 2′ (20,000 points were computed). The extents of the correlations seem to be infinite.

the CPM is used (0.38 and 0.25, respectively) than when equation 1′ (0.55 and 0.48, respectively) or equation 2′ (0.83 and 0.72, respectively) is used.

Hence, our preliminary analysis showed that the CPM is more convenient to use than the two other models because (i)

the biological interpretability of all of its parameters allows simple parameter starting values to be set, and (ii) the lack of structural correlation between parameters allows the determination of optimal parameter values rapidly and facilitates the determination of parameter confidence limits. Moreover, as the data sets used in this study are typical, we think that these results are of general interest.

Description of the combined effect of temperature and pH on μ_{max}. With the properties of the cardinal temperature model with inflection and the CPM established, we decided to evaluate the descriptive power of the combined form of these two models (CTPM) and compare it with the descriptive power of the full model of Zwietering et al. (equation 2). The CTPM and the model of Zwietering et al. (equation 2) were studied by using the *E. coli* O157:H7 data set. The initial values for bio-
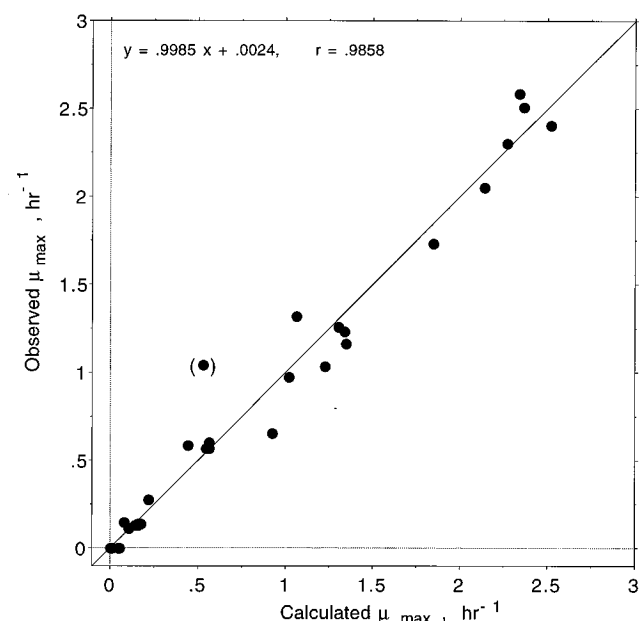

FIG. 5. μ_{max} values calculated with the CTPM versus observed μ_{max} values for *E. coli* O157:H7. The equation and the line were obtained by linear regression (r is the correlation coefficient). The experimental value in parentheses observed at 28°C and pH 4.5 seems to be an outlier.

TABLE 3. Estimated parameter values for the CTPM and the complete model of Zwietering et al. (equation 2) for the *E. coli* O157:H7 data set

Parameter	Estimated value for[a]:	
	CTPM	Equation 2
pH_{min}	3.88	3.26
pH_{opt}	7.20	14.07
pH_{max}	12.17	25.01
T_{min}	3.06°C	2.62°C
T_{opt}	41.10°C	37.71°C
T_{max}	45.06°C	49.23°C
μ_{opt}	2.635 h^{-1}	0.5036 h^{-1}
c_2		0.3106
c_3		−0.2537°C^{-1}

[a] For the CTPM the SSR_{min} was 0.65278 and the $\hat{\sigma}^2_{residual}$ was 0.0242. For equation 2 the SSR_{min} was 0.66393 and the $\hat{\sigma}^2_{residual}$ was 0.0266.

logically meaningful parameters were estimated from the observed data set, as follows: T_{min}, 4°C; T_{opt}, 40°C; T_{max}, 45°C; pH_{min}, 4; pH_{opt}, 7; pH_{max}, 10; and μ_{opt}, 2.42 h^{-1}. The initial values of meaningless parameters c_2 and c_3 in equation 2 were estimated by iteratively solving equations 3 and 4 (c_2, -1.25×10^{-4}; c_3, 0.6342°C^{-1}).

After less than 100 iterations, the fit obtained with the CTPM revealed that there was good correspondence between the observed and calculated values, except for the point at 28°C and pH 4.5, which was probably an outlier (Fig. 5). The estimated values of the seven parameters are shown in Table 3. The CTPM and equation 2 do not use the same number of parameters, and, moreover, the models are apparently not nested. A comparison of the fit of the data with the two models was made by using the estimated residual variance ($\hat{\sigma}^2_{residual}$), which is a criterion that takes into account differences in the number of parameters (degrees of freedom); the smaller the $\hat{\sigma}^2_{residual}$, the more appropriate the model for the data set. The estimated $\hat{\sigma}^2_{residual}$ was calculated as follows:

$$\hat{\sigma}^2_{residual} = SSR_{min}/(n - p) \tag{9}$$

where n is the number of points and p is the number of parameters. For the CTPM the SSR_{min} was 0.65278, and the $\hat{\sigma}^2_{residual}$ for 27 degrees of freedom ($n - p$) was 0.0242.

The good quality of fit could be also quantified by analyzing the linear regression between the observed μ_{max} values and calculated values. This regression gave the following equation, whose correlation coefficient (r) was 0.9858:

$$\mu_{max_{observed}} = 0.9985\ \mu_{max_{calculated}} + 0.0024 \tag{10}$$

The Student t test ($\alpha = 0.05$) showed that equation 10 is not significantly different from the equation $y = x$ ($t_{slope} = 0.051$; $t_{constant} = 0.072$).

The residual plot analysis corroborated the good quality of fit of the CTPM (Fig. 6a). No obvious heterosedasticity was observed, and the residual values seemed to be fairly randomly distributed. A plot of residual quantiles versus standard normal quantiles (Fig. 6b) showed that all of the points except the point at 28°C and pH 4.5 fell on a line. The errors seemed to be normally distributed, so the choice of SSR as a convergence criterion was reasonable. In addition, the residual analysis confirmed that the point at 28°C and pH 4.5 may be considered an outlier because it lies above the general tendency in the normal quantile-quantile plot.

The fit of the model of Zwietering et al. (equation 2) with the *E. coli* O157:H7 data set needed 50 times more iterations than the fit of the CTPM, and the results obtained with the model of Zwietering et al. gave a less satisfactory quality of fit than the results obtained with the CTPM (SSR_{min}, 0.6639). Moreover, this fit was computed with nine parameters instead of seven, and the $\hat{\sigma}^2_{residual}$ was greater than the $\hat{\sigma}^2_{residual}$ obtained with the CTPM ($\hat{\sigma}^2_{residual}$ for 25 degrees of freedom, 0.0266). Some of the estimated parameter values (Table 3) were biologically aberrant (pH_{opt}, 14.07; pH_{max}, 25.0), and the estimated μ_{opt} was very different from the observed values (estimated μ_{opt}, 0.5036 h^{-1}); this was probably due to the strong structural correlation between parameters observed previously for its pH partial form (equation 2′).

The CTPM, as well as its pH-reduced form (CPM), seems to be more convenient to use than the full model of Zwietering et al. The simple biological meaning of the parameters and the absence of structural correlation result in easy convergence and parameter estimates consistent with biological observations even if the number of points is small. In fact, the more
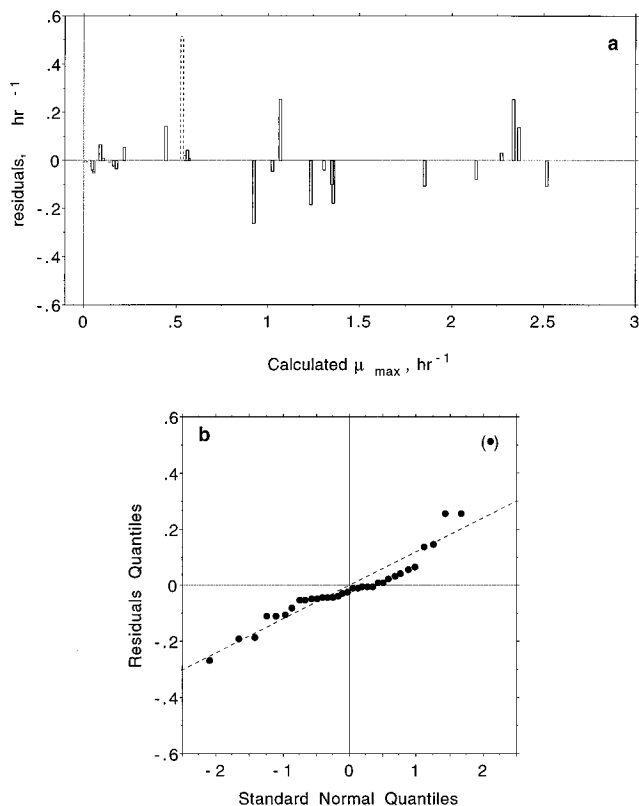

FIG. 6. Analysis of residuals for the fit of the CTPM with the *E. coli* O157:H7 data set. (a) Residuals (observed-calculated) versus calculated μ_{max} values. (b) Normal quantile-quantile plot for the residuals. The point at 28°C and pH 4.5 may be considered an outlier because it produces an important residual error (dashed line in panel a) and is outside (point in parentheses in panel b) and is the linear pattern (dashed line in panel b) observed for the other points.

structurally correlated the parameters, the greater the number of experimental points for satisfactory convergence should be.

Conclusions. The results of the comparison between the CTPM and the full model of Zwietering et al. (equation 2) highlighted the problems associated with model overparameterization. In this case, the structural correlation induced a loss of parameter identifiability and meaning from a mathematical and biological standpoint, which is an illustration of William of Ockham's precept "non sunt multiplicanda entia praeter necessitatem" (entities are not to be multiplied beyond necessity).

In this paper we emphasize that it is necessary to build and test models on the basis of several criteria, including simple biological meaning and minimum number of parameters, applicability, quality of fit, minimum structural correlations, and ease of initial parameter estimation. Taken together, these criteria not only make a model "well-conditioned" but also make it convenient to use for biologists. Hence, the CTPM may be used with a minimum knowledge of strain and medium characteristics. Its predictive ability can therefore be tested. In this light, an organism-medium database such as the one described by Zwietering et al. (17) would be very useful for obtaining the necessary information.

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