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Modelling Bacterial Growth of *Lactobacillus curvatus* as a Function of Acidity and Temperature

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Models that describe the effect of acidity, temperature, and the combined effect of these variables on the growth parameters of *Lactobacillus curvatus* are developed and validated. Growth parameters (lag time, specific growth rate, and maximum population density) were calculated from growth data at different temperature-acidity combinations. Experiments were set up to assess the quantitative effects of temperature and acidity on the growth parameters rather than for parameter estimation solely. The effect of acidity is monitored at several constant temperature values. Models are set up and fitted to the data. The same procedure is used at constant acidity values to model the effect of temperature. For lag time, specific growth rate, and maximum population density, the effect of temperature could be multiplied with the effect of acidity to obtain combinatory models that describe the effect of both controlling factors on the growth parameters. Lag time measurements showed large deviations, and therefore the lag time models developed can only be used to estimate the order of magnitude of lag time.

The quality and shelf life of foods are often determined by the growth of microorganisms. Microbial growth is dependent on intrinsic factors, such as water activity and acidity, and extrinsic factors, such as temperature and oxygen availability. In recent years, the interest in developing mathematical models to describe the growth of microorganisms as a function of controlling factors has increased. Much work on the combined effects of several controlling factors on bacterial growth was carried out; see, for example, papers by Buchanan (2), McMeekin et al. (3), and Sutherland et al. (9). The mathematical models can be used to predict the change in quality of a food over time and can therefore be applied to estimate the shelf life of foods. The models can help to decrease the required amount of costly and time-consuming challenge tests, may help to design more efficient ways of challenge testing, and finally can be used for distribution chain optimization. Another important feature of models is the acquisition of improved knowledge of the factors that determine food quality.

The combination of models for growth as a function of more than one controlling factor has been carried out by, for instance, McMeekin et al. (3), Adams et al. (1), and Wijtzes et al. (10). McMeekin et al. (3) suggested the multiplication of the effect of temperature on specific growth rate with the effect of water activity. Adams et al. (1) did the same for acidity and temperature. Wijtzes et al. (10) multiplied the effect of acidity, water activity, and temperature.

The authors mentioned above assume that the effect of one controlling factor does not influence the effect of other factors. Multiplication of the effects of temperature and acidity implies that neither the minimum nor the maximum pH at which growth takes place is a function of temperature. Minimum and maximum temperature are also supposed to be independent of acidity. In this paper, the validity of the above assumptions will be evaluated and the models will be verified.

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This paper provides for a structured way to model combinations of effects of various controlling factors. By using both microbiological expertise and practical modelling techniques, the mathematical relations between the controlling factors temperature and acidity (pH) and specific growth rate, lag phase, and maximum population density will be derived. For this purpose, an extensive data set is collected.

MODEL DEVELOPMENT

Theoretical growth curves. Zwietering et al. (12) derived a mathematical relation, based on the Gompertz model for the increase of a population over time, that relates population size over time to specific growth rate, lag time, and asymptotic level of organisms. The natural logarithm of the maximum number of microorganisms (N_{max}) is defined as the sum of the natural logarithm of the estimated initial number (N_0) and estimated asymptotic value (A) from the reparameterized Gompertz equation. Since direct estimates of $\ln(N_{max})$ were required for this data analysis, the reparameterized Gompertz equation was rewritten as:

$$ln\,(N_t) = \ln\,(N_0) + \{\ln\,(N_{max}) - \ln\,(N_0)\}$$
$$\exp\left\{-\exp\left[\frac{\mu_m e}{\{\ln\,(N_{max}) - \ln\,(N_0)\}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where t is time (in hours), N_t is the number of microorganisms at time t (in CFU per milliliter), N_0 is the asymptotic number of microorganisms at time zero (in CFU per milliliter), N_{max} is the maximum number of microorganisms (in CFU per milliliter), μ_m is the maximum specific growth rate (per hour), and λ is the lag time (in hours).

Temperature effect on specific growth rate. Bélehrádek-type models, as described below, were suggested for the first time in predictive microbiology by Ratkowsky et al. (7). The Bélehrádek-type model known as the Ratkowsky equation describes the relation between specific growth rate and suboptimal temperature for various microorganisms as:

$$\mu_m = b_2(T - T_{min,2})^2 \quad (2)$$

where b_2 ($h^{-1}\,°C^{-2}$) and $T_{min,2}$ (°C) are regression coefficients, $T_{min,2}$ representing the theoretical minimum temperature for growth; index 2 indicates the number of the equation. This model has the advantage of containing an easily interpretable parameter, T_{min}, the extrapolated temperature at which a microorganism cannot grow. All other models in this paper will contain as much of this type of parameter as possible, since these parameters may have biological meaning and can be interpreted easily.

Acidity effect on specific growth rate. Two models will be set up that may describe specific growth rate as a function of pH. It is well known that below the minimum pH and above the maximum pH, microorganisms stop growing and even die. At the optimum pH, the organisms grow fastest. This behavior can be described mathematically by using, for instance, a parabolic equation:

$$\mu_m = -b_3(\mathrm{pH} - \mathrm{pH}_{min,3})(\mathrm{pH} - \mathrm{pH}_{max,3}) \tag{3}$$

where b_3 (h^{-1}) is a regression coefficient and $\mathrm{pH}_{min,3}$ and $\mathrm{pH}_{max,3}$ are the theoretical minimum and maximum pH for growth, respectively.

Another function that may be used to describe the mentioned curvature is based on the expanded Ratkowsky equation for temperature and specific growth rate (6). From the proposed equation, the square root is omitted, to result in equation 4:

$$\mu_m = b_4(\mathrm{pH} - \mathrm{pH}_{min,4})\{1 - \exp[c_4(\mathrm{pH} - \mathrm{pH}_{max,4})]\} \tag{4}$$

where c_4 is a new regression coefficient. The difference between equations 3 and 4 is the possibility of adjusting the location of the optimum pH. If the optimum pH is exactly in the middle between pH_{min} and pH_{max}, equation 3 can be used; if not, equation 4 applies. This can be verified by assessing the confidence interval and value of c_4. If the confidence interval overlaps zero, the curvature of equation 4, statistically, cannot be distinguished from symmetrical (see appendix). In that case, equation 3 is favored, since it has one parameter less and describes the curvature as well as equation 4 does.

Temperature effect on lag time. From the observation that $\mu * \lambda$ versus μ is uncorrelated, Zwietering et al. (11) proposed to use the inverse of equation 2 to model the length of the lag phase as a function of temperature:

$$\lambda = \frac{1}{b_5(T - T_{min,5})^2} \tag{5}$$

Acidity effect on lag time. Here again, the reciprocal of the equation for specific growth rate as a function of pH is used. Since two different relations may apply, two lag phase models are proposed:

$$\lambda = \frac{-1}{b_6(\mathrm{pH} - \mathrm{pH}_{min,6})(\mathrm{pH} - \mathrm{pH}_{max,6})} \tag{6}$$

$$\lambda = \frac{1}{b_7(\mathrm{pH} - \mathrm{pH}_{min,7})\{1 - \exp[c_7(\mathrm{pH} - \mathrm{pH}_{max,7})]\}} \tag{7}$$

where all the parameters are as described above.

Temperature effect on maximum population density. A model to relate the maximum number of microorganisms (N_{max}) to temperature is proposed by Zwietering et al. (11) as:

$$\ln(N_{max}) = a_8\frac{(T - T_{min,8})(T - T_{max,8})}{(T - T_{sub\ min,8})(T - T_{sup\ max,8})} \tag{8}$$

where a_8 is a regression coefficient, $T_{sub\ min,8}$ (°C) is a temperature just below the theoretical minimum temperature for growth, $T_{sup\ max,8}$ (°C) is a temperature just above the theoretical maximum temperature for growth, and all other parameters are as defined above.

In this paper, the effect of suboptimal temperature is modelled, and therefore the part of equation 8 that describes the temperature effect near the maximum can be omitted, resulting in equation 9, where the parameters are as defined above:

$$\ln(N_{max}) = a_9\frac{(T - T_{min,9})}{(T - T_{sub\ min,9})} \tag{9}$$

If N_{max} values do not decrease with decreasing temperature, a constant value for N_{max} may describe the data correctly. Therefore, another equation is proposed:

$$\ln(N_{max}) = c_{10}. \tag{10}$$

Acidity effect on maximum population density. Similar N_{max} models for acidity can be suggested. pH is substituted for temperature to result in:

$$\ln(N_{max}) = a_{11}\frac{(\mathrm{pH} - \mathrm{pH}_{min,11})(\mathrm{pH} - \mathrm{pH}_{max,11})}{(\mathrm{pH} - \mathrm{pH}_{sub\ min,11})(\mathrm{pH} - \mathrm{pH}_{sup\ max,11})} \tag{11}$$

The maximum number of microorganisms can also be supposed to be constant over the entire acidity range:

$$\ln(N_{max}) = c_{12}. \tag{12}$$

Combining acidity and temperature models for specific growth rate. If the effects of acidity and temperature can be multiplied, the combinatory effect on specific growth rate may have either of two forms. If the parabolic relationship between acidity and specific growth rate is preferred, equation 13 will be used:

$$\mu_m = -b_{13}(T - T_{min,13})^2(\mathrm{pH} - \mathrm{pH}_{min,13})(\mathrm{pH} - \mathrm{pH}_{max,13}) \tag{13}$$

If the Ratkowsky-like curvature for pH is preferred, equation 14 may apply:

$$\mu_m = b_{14}(T - T_{min,14})^2(\mathrm{pH} - \mathrm{pH}_{min,14})\{1 - \exp[c_{14}(\mathrm{pH} - \mathrm{pH}_{max,14})]\} \tag{14}$$

Combining acidity and temperature models for lag time. The model for the combinatory effect of temperature and pH on the length of the lag phase is dependent on the model developed for specific growth rate. Therefore, specific growth rate models will be ratified first, and lag phase models will result from these.

Combining acidity and temperature models for maximum population density. For each controlling factor, the most appropriate model will be chosen. Then, after checking the validity of the multiplication of the effect of temperature and acidity on the maximum number of organisms, the product of the two best models will be chosen.

MATERIALS AND METHODS

Organism. A pure culture of *Lactobacillus curvatus*, a typical spoilage organism of different types of meat and meat products (4, 5), was used as a model organism.

Microbial experiments. At eight different suboptimal temperatures between 6 and 31°C and various preset acidities between previously determined minimum and maximum pH values, 87 growth curves were determined. *L. curvatus* was grown in MRS broth (Difco Laboratories). After sterilizing the broth (121°C, 20 min), pHs were set by using 2 N HCl (Merck) or 2 N NaOH (Merck) diluted in sterile demiwater. The pH was measured with an electrode (Schott Geräte, N 5900 A) that was disinfected with 70% alcohol (Merck, distilled water).

Before the experiments, a frozen culture (−18°C) of *L. curvatus* was cultivated

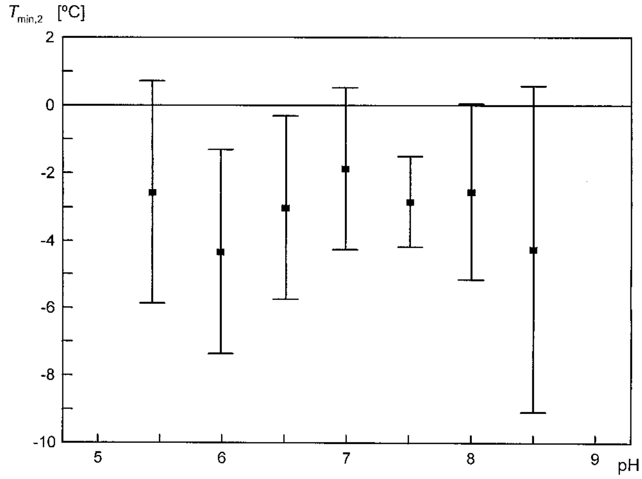

FIG. 1. $T_{min,2}$ as a function of acidity.

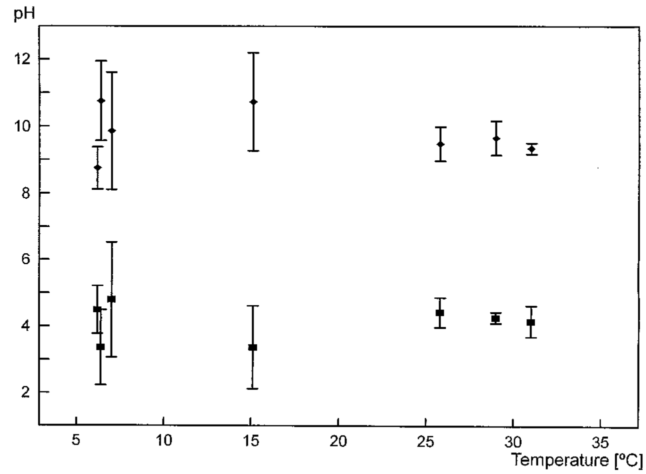

FIG. 3. $pH_{min,3}$ (■) and $pH_{max,3}$ (◆) as a function of temperature.

twice at 30°C, first for 24 h and then for 16 h. To prepare for the growth experiment, the last preculture was diluted to reach an initial level in the broth of 10^3 CFU/ml. The experiments were carried out in 50-ml tubes containing MRS broth at preset pHs and incubated at a static, constant temperature.

Acidity was not controlled during the experiments. Acidity values remain constant in the range where growth rate and lag time are estimated. In the region where the growth curve bends towards the asymptote, pH drops until a value of 4.26 is reached in the asymptote. If the asymptote is reached, the glucose concentration drops to 0 mol/liter.

The need to incubate plates anaerobically (anaerobic jar with BBL-Gaspack; Becton Dickinson and Company) was investigated previous to the growth experiments. The colony counts did not vary by more than a factor of 3 between 3 and 5 days of incubation, and growth rate, lag time, and asymptote fits to the Gompertz equation indicated overlapping 95% confidence intervals for each parameter. Since no significant differences were found between anaerobic and aerobic methods, for convenience, the aerobic incubation method was chosen.

At appropriate time intervals, a 0.1- or 1-ml sample was taken aseptically from the tube, and the time was noted. The number of microorganisms was determined in two ways. Part of the data set was acquired by the pour plate method, and part was acquired by using a spiral plate device (Spiral Systems Inc., model D). Growth rate, lag time, and asymptote fits to the Gompertz equation indicated overlapping 95% confidence intervals for each parameter, and therefore no significant differences were found between the two counting methods. All plates were incubated aerobically for at least 3 days at 30°C. The organisms were counted by the appropriate method. The plates in the extreme growth conditions group were incubated for 5 days and recounted. The colony counts did not vary by more than a factor of 3 between 3 and 5 days of incubation, and growth rate, lag time, and asymptote fits to the Gompertz equation indicated overlapping 95% confidence intervals for each parameter. Therefore, no significant differences in the number of colonies were found after 3 and 5 days of incubation, even under extreme growth conditions.

Clustering acidity data. Since the experiments were set up at constant temperatures and the pH was set at approximately the required value, a variation in pH values can be expected. The pH used was the calculated mean pH of a class. The deviation from the mean of the class was never more than 0.10 pH unit, so the deviation from the mean is about as large as the measuring error.

Statistical methods. All regressions were carried out with standard statistical software (SAS). A Marquardt algorithm was used for nonlinear regression (8). Confidence intervals were calculated by using the estimated asymptotic covariance matrix and the Student t value.

The counts from the experiments at one set temperature and pH value were fitted to the rewritten Gompertz equation (equation 1).

The dependent variables were related to the controlling factors without transforming the data first. Upon regression, the appropriate distribution of errors was used to fit the equations to the data. For specific growth rate and lag time data, a variance analysis showed that a gamma distribution of the dependent variable is most appropriate. A gamma distribution corresponds to taking the square root on both sides of the equation. For ln (N_0) and ln (N_{max}) data, a Gaussian distributed error was found, and therefore no further transformation was required.

Lack-of-fit test. Model predictions were plotted against the observed values by using the appropriate transformation method. The residuals, the deviation from the diagonal, should be distributed homogeneously throughout the range of observed values, and the values should not be too far away from the diagonal. F ratio tests provide a less subjective model validation (12). The deviations of the model predictions were tested against the mean of the measured values at

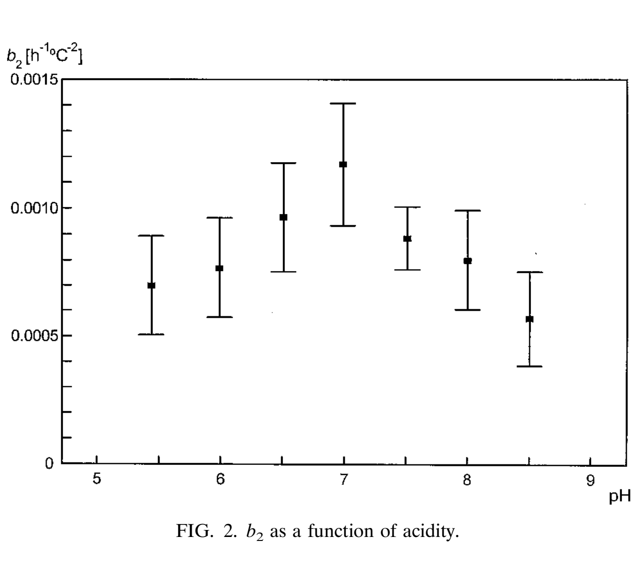

FIG. 2. b_2 as a function of acidity.

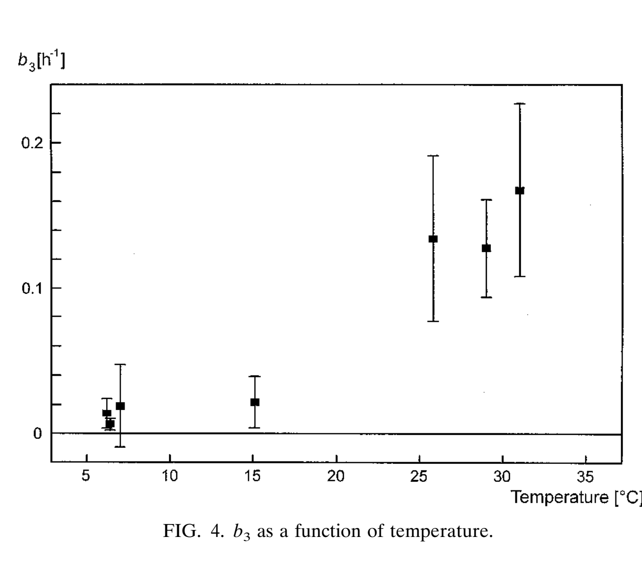

FIG. 4. b_3 as a function of temperature.

TABLE 1. Estimated parameters from equation 4 and their 95% confidence intervals

T (°C)	$pH_{min,4}$		$pH_{max,4}$		b_4 (h^{-1})		c_4	
	Fitted value	±95%[a]	Fitted value	±95%	Fitted value	±95%	Fitted value	±95%
6.2	3.07	5.99	8.34	6.80	-2.68×10^{-3}	4.81×10^{-2}	−0.36	18.05
6.4	4.28	1.33	14.08	32.22	1.95×10^{-2}	5.10×10^{-2}	1.33	4.60
7.0	4.79	7.71	9.86	8.95	3.56	4.15×10^{3}	5.32×10^{-3}	6.26
15.1	3.36	4.41	10.73	4.88	11.75	1.07×10^{5}	1.84×10^{-3}	1.68
25.8	4.39	1.54	9.45	1.77	3.93	1.45×10^{2}	3.58×10^{-2}	1.44
29.0	4.24	0.30	9.49	1.19	1.85	11.88	8.14×10^{-2}	0.61
31.0	4.14	2.04	9.52	1.35	11.70	1.01×10^{3}	1.46×10^{-2}	1.29

[a] The 95% confidence interval around the fitted value (e.g., a fitted value of 3.07 and a 95% value of 5.99 means the 95% confidence interval equals {−2.92, 9.06}).

clustered temperature-acidity combinations. The α value for the F ratio test was 0.05. The latter method was used to assess the statistical acceptability of a model.

Modelling procedure. Modelling of the data was carried out in several stages. First, temperature equations were fitted at constant pH values; then, pH equations were fitted to the data at constant temperatures. Then all estimated parameters from the models will be plotted against the other controlling factor to observe any trends. If trends are observed in the parameters that are supposed to be constant (pH_{min}, pH_{max}, T_{min}, etc.), the conclusion may be drawn that combinatory effects do exist and extension of the models is necessary.

RESULTS AND DISCUSSION

Modelling the effect of temperature on specific growth rate. The growth rate data at constant acidity values are fitted with equation 2. The results from these fits are given in Fig. 1 and 2. In Fig. 1, $T_{min,2}$ is shown as a function of acidity. As can be seen, there is no significant trend in the data. The slope of the straight line fitted through the datum points of T_{min} was not significantly different from zero, and therefore, T_{min} can be supposed to be constant over the measured pH range. The mean $T_{min,2}$ resulting from the data in Fig. 1 equals −3.06°C.

The results from the estimations of b_2 are given in Fig. 2. The trend in the curve of Fig. 2 is a parabolic one, and therefore the effect of acidity on growth rate is reflected in the values for b_2.

Modelling the effect of acidity on specific growth rate. The data set covers specific growth rate over the entire pH range for growth. Equation 3 is fitted to the data at various constant temperatures for which at least six datum points have been gathered, resulting in a minimum number of degrees of freedom of 3. In Fig. 3, the parameters $pH_{min,3}$ and $pH_{max,3}$ and their 95% confidence intervals are shown as a function of temperature. From these data, it follows that neither $pH_{min,3}$ nor $pH_{max,3}$ shows a trend as a function of temperature.

In Fig. 4, the value of b_3 is displayed as a function of temperature; a quadratic curve can be drawn through the datum points. The conclusion may be drawn that only parameter b_3 is a function of temperature. Parameter b_3 as a function of temperature shows the same behavior as specific growth rate as a function of temperature.

Equation 4 is fitted to the growth rate data at the same constant temperatures as equation 3. In Table 1, the resulting minimum and maximum pH, b_4, c_4, and their 95% confidence intervals at different temperatures are given. Again, no trend can be observed in $pH_{min,4}$ and $pH_{max,4}$. Therefore, the entire temperature effect must be present in the either of the two regression parameters b_4 and c_4. The values of c_4 are always close to zero, and the confidence intervals overlap zero, resulting in a close to symmetrical curve. The conclusion can therefore be drawn that, at least in this particular case, the parabolic equation for acidity (equation 3) can be used to best describe the measured specific growth rates. The large confidence interval values around b_4 and c_4 are an indication of overparameterization of the model and are explained in the appendix.

In conclusion, the fits of both equations 3 and 4 to the data support the conclusion that pH_{min} and pH_{max} are independent of temperature.

Combining the effect of temperature and acidity on specific growth rate. Figure 3 shows that $pH_{min,3}$ and $pH_{max,3}$ are independent of temperature, and Fig. 1 indicates that $T_{min,2}$ is independent of acidity. Furthermore, Fig. 4 and 2 show an apparent parabolic relationship between b_2 and temperature as well as a parabolic relationship between b_3 and acidity. The suggested multiplication of the effects of temperature and acidity on specific growth rate is therefore allowed.

Since the parabolic equation (equation 3) for the relation between specific growth rate and pH is favored, the combined model for the relation between specific growth rate and temperature and pH should be equation 13. The results from the fit are given in Table 2.

The square root of measured specific growth rate was plotted against the square root of the fits of the overall model for specific growth rate as a function of temperature and acidity (equation 13), and no trend was found. The calculated f value equals 1.05, whereas the reference 95% right-hand γ point F value equals 1.726, and therefore the model is statistically accepted.

The trend is described well at all temperatures. As an example, in Fig. 5, the measured data and the fit of the model are given at three temperatures (6, 15, and 29°C).

Modelling the effect of temperature on lag time. The effects of temperature and pH on the length of the lag phase may be modelled as the reciprocal of the models for specific growth rate. However, at constant acidity values, a trend is visible in the $\mu * \lambda$ versus μ graph. At constant pH values, lag time curves display hyperbolic behavior, as can be seen in Fig. 6, and therefore a hyperbolic type of model equation is required. Different hyperbolic equations were fitted to the data. It proved that, at all acidity values, equation 5 had the highest correlation coefficient and can therefore best be fitted to the data.

Parameter b_5 should be a function of acidity. As can be seen in Fig. 7, the relation between b_5 and acidity shows a parabolic

TABLE 2. Results of fit for overall model for specific growth rate based on equation 13

Parameter	Estimated value	95% Confidence interval
b_{13} (h^{-1} °C^{-2})	1.263×10^{-4}	1.052×10^{-4}, 1.474×10^{-4}
$T_{min,13}$ (°C)	−3.27	−4.48, −2.06
$pH_{min,13}$	4.26	4.13, 4.38
$pH_{max,13}$	9.77	9.53, 10.01

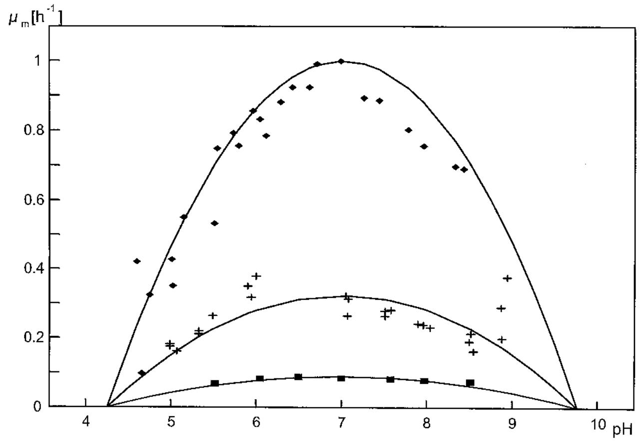

FIG. 5. Measured and fitted specific growth rates at 6°C (■), 15°C (+), and 29°C (◆).

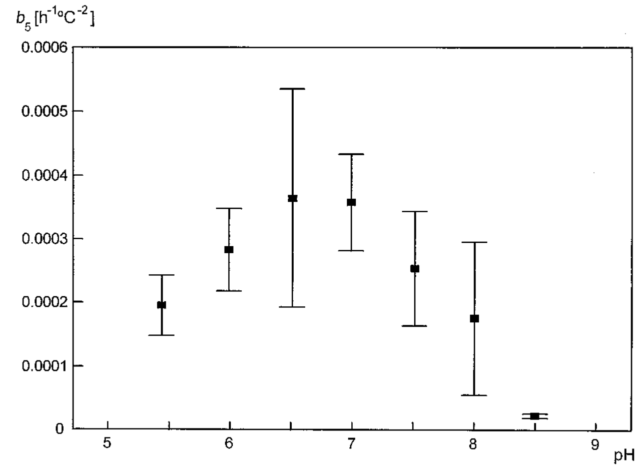

FIG. 7. b_5 as a function of acidity.

curvature, and therefore the relation between acidity and lag time at constant temperatures can be modelled as such.

Modelling the effect of acidity on lag time. The lag time model as a function of temperature indicated that the pH effect took the form of an inverse parabola (equation 6). At constant temperature values, $\mu * \lambda$ versus μ data were correlated again, invalidating the proposed inverse growth rate model. To retain the characteristic hyperbolic curvature, an exponent was introduced over equation 6.

To assess the value of the newly introduced exponent, an overall model had to be set up. The overall model describes the effects of both temperature and acidity on lag time, assuming that the effect of temperature and pH can be multiplied. This can be preliminarily concluded from the modelling of the temperature effect on lag time, although it still has to be proven. The exponent in this equation was then fitted along with the other regression coefficient.

The value for the exponent resulting from the overall model equals 2.14, and the confidence interval around this value ranges from 1.34 to 2.94. By analogy with equation 5, the value for the exponent over the acidity effect is set to 2.

At constant temperatures, the newly developed model is fitted with constant parameters $pH_{min,6}$ and $pH_{max,6}$ taken from Table 2, and the exponent is set to 2. Upon regression of the new equation, the values of the regression coefficients b_6 were estimated and are shown in Fig. 8. In all cases the trend in the data was described well. Again, the regression coefficient b_6 takes the parabolic form of a temperature curve.

Combining the effect of temperature and acidity on lag time. For lag time, it can also be concluded that the effects of temperature and acidity can be multiplied. This results in an overall model, as given in equation 15:

$$\lambda = \frac{1}{b_{15}\{(pH - pH_{min,13})(pH - pH_{max,13})(T - T_{min,13})\}^2} \quad (15)$$

where $pH_{min,13}$, $pH_{max,13}$, and $T_{min,13}$ are fixed, being the fitted values from the overall specific growth rate model (see Table 2), resulting in a one-parameter model. The regression parameter b_{15} is found to equal 4.18×10^{-6} h^{-1} °C^{-2}, with a confidence interval value of $\pm 1.19 \times 10^{-6}$ h^{-1} °C^{-2}.

Validation of the overall lag time model. The reproducibility of the measured lag times is poor, and therefore, it is hard to model the effect of lag time. In the fitted versus observed plot, a trend is visible, and by using the F ratio test, the model predictions are rejected ($f = 6.909$; $F = 1.716$); therefore, the conclusion can be drawn that the overall model does not describe the data adequately. The model, however, can be used to

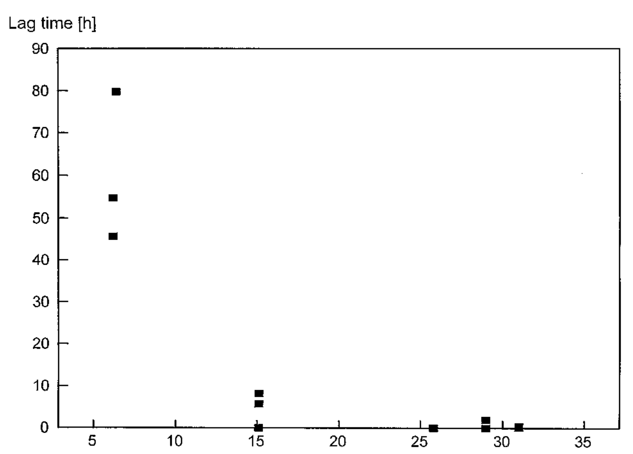

FIG. 6. Lag time as a function of temperature at pH 5.45.

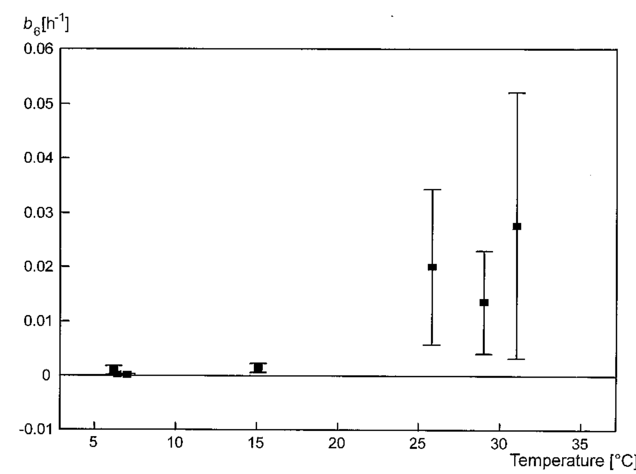

FIG. 8. b_6 as a function of temperature.

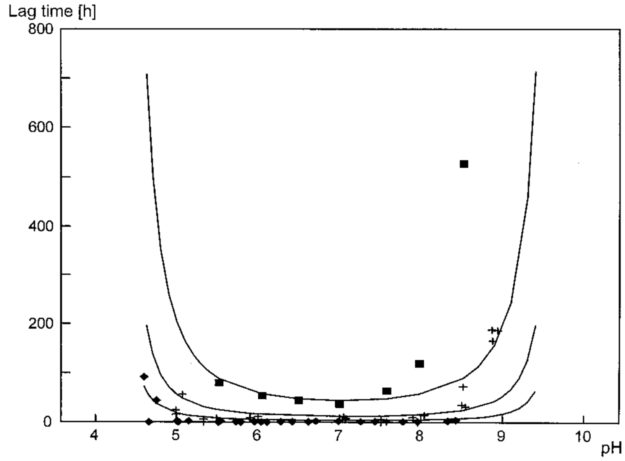

FIG. 9. Measured and fitted lag times at 6°C (■), 15°C (+), and 29°C (◆).

obtain an estimate of the order of magnitude of lag time but not more than that. In Fig. 9, lag time data and model predictions at 6, 15, and 29°C are given. As can be seen, the overall trend is described well, but in individual cases, the model predictions can be quite far off, especially near the pH extremes. This can be an indication that pH_{min} and pH_{max} should not be fixed but fitted along with the other parameters of the model. Figure 10 shows the results when pH_{min} and pH_{max} have newly fitted values of 3.39 ± 1.15 and 9.23 ± 0.11, respectively, whereas b_{15} equals $4.00 \times 10^{-6} \pm 3.42 \times 10^{-6}$ h^{-1} $°C^{-2}$. The significantly different values for minimum and maximum pH compared with the fitted values of the growth rate models may indicate that the mechanisms that control growth rate are different from those controlling lag time. The calculated f value was 3.799, whereas the reference F value was 1.720. Therefore, this model is also rejected statistically, but the predictions come closer than the model with fixed pH_{min} and pH_{max} taken from the growth rate model.

Modelling maximum population density. Four models were proposed to describe the effect of temperature and acidity on maximum population density. Here again, the effects of temperature and acidity were modelled separately first and then combined into one model. At constant temperatures, the maximum population density data did not deviate significantly from a constant value, and therefore equation 10 was used.

TABLE 3. Results of fit of the overall model for the natural logarithm of the maximum population density based on equation 11

Parameter	Estimated value	95% Confidence interval
a_{11}	22.87	22.30, 23.44
$pH_{sub\ min,11}$	4.14	4.12, 4.17
$pH_{sup\ max,11}$	9.80	9.76, 9.83

Near the extremes for acidity, i.e., the minimum and maximum pH, the maximum population density data become smaller, and therefore equation 11 was chosen to model this effect. The minimum and maximum pH were fixed at their fitted specific growth rate values, at the appropriate temperature. $pH_{sub\ min,11}$ and $pH_{sup\ max,11}$ were independent of temperature.

The overall model to describe the effect of both temperature and acidity on the height of the asymptote is therefore the product of equations 10 and 11, which makes it similar to equation 11.

Upon fitting this equation, the values for $pH_{min,11}$ and $pH_{max,11}$ were fixed again, now at their fitted values resulting from the overall model for specific growth rate (Table 2). The results from this fit can be found in Table 3.

Validation of the overall maximum population density model. In Fig. 11, the predictions of the overall model for maximum population density are given along with the datum points; the trend is described well. However, assessing the fit-versus-observed plot, the data did not appear uncorrelated. The F ratio test agrees with the latter, since f equals 3.47 against a reference F value of 1.72. Although the deviation from the required F value is small, the model is rejected statistically.

Conclusions. At suboptimal temperatures, the parabolic relationship between specific growth rate and temperature was found to apply. Over the entire pH range, the parabolic relationship between acidity and specific growth rate proved to be the most adequate model to describe the data. The combination of the parabolic model for acidity and temperature by multiplication into one resulted in a good model to describe the effect of temperature and acidity on specific growth rate.

The proposed models for lag time as a function of temperature described the trend well. The acidity effect was accounted for in the regression coefficient. Therefore, the effect of acidity could be multiplied with the effect of temperature.

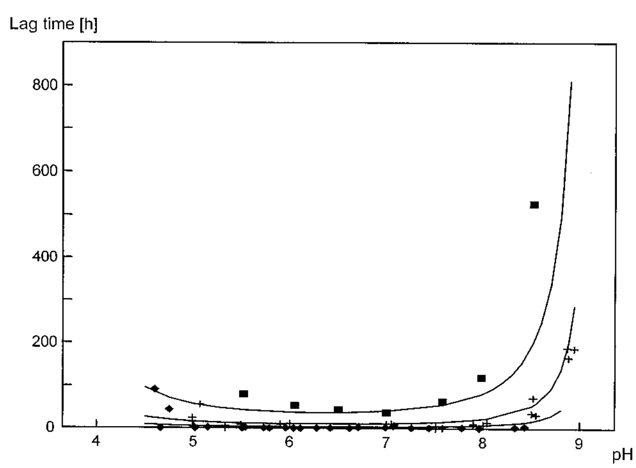

FIG. 10. Measured and fitted lag times at 6°C (■), 15°C (+), and 29°C (◆); fitted pH_{min} and pH_{max}.

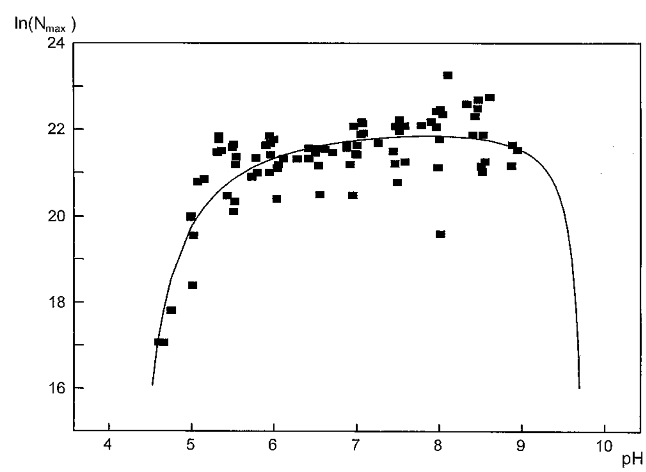

FIG. 11. Maximum population density as a function of acidity.

The model for lag time as a function of acidity needed alteration; a power was introduced and a new equation was set up. From the new equation, the effect of temperature could be multiplied with the effect of acidity. The overall model was rejected by the F ratio test.

The proposed models for maximum population density required no alteration on the basis of the measured data set. The overall model for maximum population density data predicts the trend well, although the model is statistically rejected.

A preliminary idea of the type of curve expected in growth data helps in modelling the effects of controlling factors on the growth parameters. It is useful to set up a hypothetical model before carrying out experiments, since from interpreting this model, it is possible to assess the range of controlling factors over which it is necessary to take enough measurements to verify the model.

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APPENDIX

The derivation of symmetry in the Ratkowsky equation (6),

$$\sqrt{\mu} = b(T - T_{\min})\{1 - \exp[c(T - T_{\max})]\} \quad \text{(A1)}$$

can be rewritten as

$$y = b(x - x_{\min})\{1 - \exp[c(x - x_{\max})]\} \quad \text{(A2)}$$

The first derivative of this equation can be written as

$$\frac{dy}{dx} = b\{1 - [c(x - x_{\min}) + 1]\exp[c(x - x_{\max})]\} \quad \text{(A3)}$$

At the optimum, the first derivative equals zero, and if b doesn't equal zero, then

$$[c(x_{\text{opt}} - x_{\min}) + 1]\exp[c(x_{\text{opt}} - x_{\max})] = 1. \quad \text{(A4)}$$

One of the properties of this type of symmetrical curve is that the optimum lies exactly in the middle between the minimum and the maximum; therefore

$$x_{\text{opt}} = \frac{x_{\min} + x_{\max}}{2}. \quad \text{(A5)}$$

Substituting A5 into equation A4 results in

$$\left[\frac{c}{2}(x_{\max} - x_{\min}) + 1\right]\exp\left[\frac{c}{2}(x_{\min} - x_{\max})\right] = 1 \quad \text{(A6)}$$

Rewriting $\frac{c}{2}(x_{\max} - x_{\min})$ as Ω, the above equation becomes

$$[\Omega + 1]\exp[-\Omega] = 1. \quad \text{(A7)}$$

This equality only holds if Ω equals zero. Since $x_{\min}$ and $x_{\max}$ have different, fixed values, c has to become zero. However, if c equals zero, y and μ also equal zero (see equations A1 and A2). Finding the optimum using this method is defined as making x approach x_{opt}, and therefore c approaches zero instead of being zero.

Another property of symmetrical curves is that for every distance from the optimum x value θ ($x_{\text{opt}} + \theta$, $x_{\text{opt}} - \theta$), the resulting y values of the function have to be equal. So

$$b(x_{\text{opt}} - x_{\min} - \theta)\{1 - \exp[c(x_{\text{opt}} - x_{\max} - \theta)]\} \quad \text{(A8)}$$

has to be equal to

$$b(x_{\text{opt}} - x_{\min} + \theta)\{1 - \exp[c(x_{\text{opt}} - x_{\max} + \theta)]\} \quad \text{(A9)}$$

Rewriting $(x_{\text{opt}} - x_{\min} - \theta)$ as ν and $(x_{\text{opt}} - x_{\min} + \theta)$ as κ, the required equality can be written as

$$\nu\{1 - \exp[c\nu]\} = \kappa\{1 - \exp[c\kappa]\} \quad \text{(A10)}$$

which is similar to

$$\nu - \kappa = \nu\exp[c\nu] - \kappa\exp[c\kappa] \quad \text{(A11)}$$

From the foregoing, it was concluded that $c = 0$. In that case, the equality holds.

Fitting data. The closer c comes to zero, the closer y will be to zero. To compensate for this effect, b has to increase. Fitting this equation to nearly symmetrical acidity data will result in highly correlated b and c values and will give large asymptotic confidence intervals around b and c.

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