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Model for the Combined Effects of Temperature, pH, and Sodium Lactate on Growth Rates of *Listeria innocua* in Broth and Bologna-Type Sausages

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A modified Monod equation was successfully applied to describe the maximum specific growth rate of *Listeria innocua* in a broth model in the presence of various concentrations of sodium lactate or NaCl. The combined effects of temperature and pH were assessed by translating the parameters of the modified Monod equation (μ_m, α, and p') as functions of pH and/or temperature. As a result, the area in which the growth rate could be predicted was extended to include as a variable not only the salt concentration but also pH and temperature. The number of parameters needed to describe the experimental data was thereby reduced from 48 to 4 (NaCl) and from 42 to 5 (sodium lactate). The decline in the goodness of fit that accompanied the reduction in the number of parameters was within statistically acceptable ranges. The resulting model was compared with a polynomial fit, and it was proposed that the former was more suitable for the purpose of this study. The broth model for sodium lactate was evaluated with Bologna-type sausages. Because of the "worst-case" design of the broth model, it was necessary to reestimate one or all parameters to obtain a good description of the growth rate of *L. innocua* in the meat product. However, the simplicity of the model and the practical usefulness of its parameters offer considerable prospects for its use in predictive microbiology.

Sodium lactate is used in meat products as a natural ingredient. In the past few years, we have been concerned with the microbial aspects of this application. Besides investigating the boundaries between growth and no growth of microorganisms in a model system (broth) with microorganisms that are significant for meat products (2), a model was developed that could accurately predict the growth rate of a model organism (*Listeria innocua*) under various conditions (pH, temperature, lactate concentration) in the area that allowed for growth of this organism up to the MIC of sodium lactate (3). In this way, one can not only discriminate between growth and no growth, but also estimate the time span in which significant microbial growth might occur under certain conditions. The inhibition of microbial growth by sodium lactate was compared with that of NaCl, also a common ingredient in meat products. Sodium lactate could replace part of the NaCl to produce less salty products with a longer shelf life.

The maximum specific growth rate (μ) was modelled as a function of the concentration of sodium lactate or NaCl present in the broth. This resulted in a modified Monod equation with three parameters, each combination of pH and temperature having its own set of parameters. This model accurately described the effect of both NaCl and sodium lactate on the growth rate (3). The disadvantage of such a model was that it was not possible to apply it at temperatures and pH values different from those used to collect the experimental data. In the present study, the temperature and pH were included in the model to solve this problem. An advantage of doing so is that the number of parameters needed to describe the experimental data is greatly reduced.

Because the model was developed for studying the effect of sodium lactate on microbial growth in meat products, its suitability for predicting growth of the model organism in a Bologna-type sausage was evaluated as well. Although the results for both NaCl and sodium lactate models are presented, the emphasis is on the latter, since our research is focused on the antimicrobial activity of sodium lactate.

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MATERIALS AND METHODS

Organism. *L. innocua* DSM 20649 (type strain) was used in these experiments.

Data collection. Growth experiments had been performed previously in a peptone-yeast extract broth at 4, 10, 20, and 30°C and pH 5.5, 6.0, 6.5, and 7.0 in the presence of different concentrations of sodium lactate (103 curves) or NaCl (112 curves) (3). This data set (215 growth curves) contained 45 replicate experiments under 20 different conditions. These data were used to determine the variance of the measured growth rate data and to estimate the measurement error.

Analysis of variance. The variance of the maximum specific growth rate (μ) was expressed as a function of the mean value of this variable under a particular condition, and the correlation coefficient was determined by performing a linear regression of the variance data. Student's *t* test was used to determine if the correlation was significant (5).

Fitting. The parameters of the modified Monod equation (3) were first plotted as a function of temperature and pH. Depending on the curvature, a constant linear or quadratic behavior was assumed. A first estimate of the parameters was made by linear regression of the modified Monod parameters as a function of temperature and pH with the help of a commercial spreadsheet program. The equations were incorporated into the modified Monod model. This model was further optimized for describing the original growth rate data, with the help of a nonlinear fitting routine, based on a Marquardt algorithm (7). The resulting model was compared with the fit of a polynomial equation to the experimental data. Finally, the suitability of the model for predicting the growth rate of *L. innocua* in a meat product was demonstrated by using Bologna-type sausage.

Bologna-type sausage experiments. Bologna-type sausage containing 2% (wt/wt) NaCl and 120 μg of nitrite per g was supplied with different amounts of sodium lactate (0, 1, 2, 3, and 4% [wt/wt] of a 60% sodium lactate syrup; PURAC, Gorinchem, The Netherlands). Three different batches were prepared, one in which the pH (6.2) was not adjusted, one in which the pH was lowered to 5.8 with 2 N HCl, and one in which the pH was increased to 6.6 with 2 N KOH. This resulted in 15 different kinds of sausage. The meat was put into cans (about 1,800 g per can), pasteurized in a water bath (160 min at 78°C), and stored at −20°C until required.

After being thawed at room temperature, 5,400 g of each kind of sausage was cut into smaller pieces and inoculated with a suspension of *L. innocua* to a final

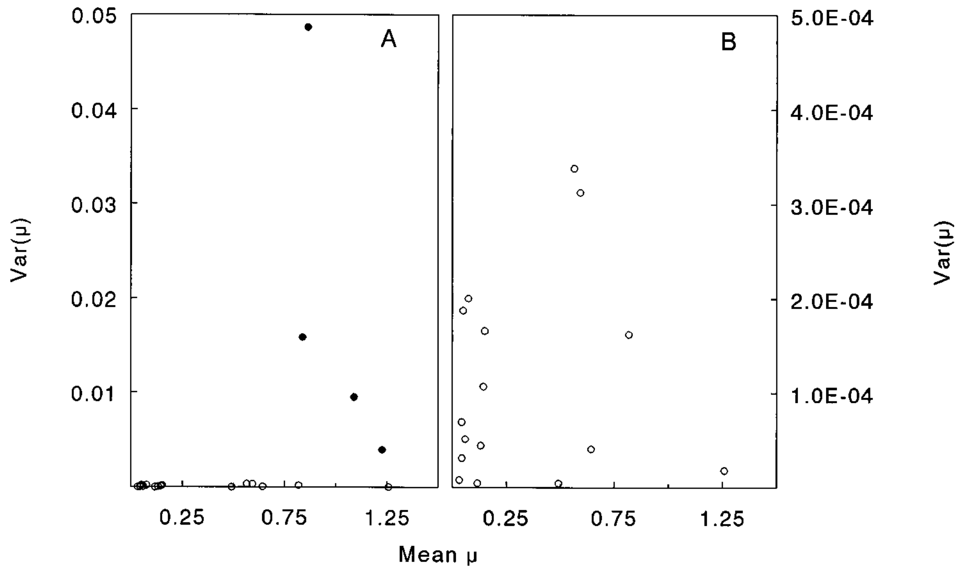

FIG. 1. Variance of maximum specific growth rates (μ) plotted against the mean of μ for all replicate measurements (A) and for all replicate measurements minus four (B).

level of about 10^4 bacteria per g. The bacteria were dispersed throughout the meat by cutting it for 75 s in a sterile cutter. The minced meat was then divided into portions of 40 g, which were put into plastic pouches with an oxygen permeability of 1.5×10^{-11} m · Pa^{-1} · day^{-1} at 20°C, flattened, and vacuum packed. These packages were stored at either 7, 10, 15, or 20°C. Growth of *L. innocua* was monitored by plate counting at intervals. For each sample, a new package was opened. The modified Gompertz equation (7) was used to fit the resulting growth curves, and the calculated growth rates were used for validation of the modified Monod model.

Model comparison. The models were validated statistically with the use of the *F*-ratio test as described by Zwietering et al. (5). The measurement error was estimated from replicate experiments, by calculating the sum of squares (RSS_{ME}) of the deviation of the measured values from the mean value of the growth rate (μ) under specific growth conditions. For each model, the residual sum of squares (RSS) of all μ data was calculated as $RSS_{model} = \Sigma(\mu_{predicted} - \mu_{measured})^2$. Then, the lack of fit ($RSS_{model} - RSS_{ME}$) was compared with the measurement error. This comparison between the lack of fit and the measurement error can be quantified statistically by the f testing value (5).

RESULTS AND DISCUSSION

Analysis of variance. The t value that was obtained from the regression analysis of the plot of the variance of μ as a function of the mean value of μ under a particular condition (Fig. 1A) was 1.70, and the 95% critical t value for 18 degrees of freedom (DF) is 2.10. This supports the assumption, based on visual inspection of the variance data, that no correlation exists between the variance of μ and the mean value of μ. There is also a theoretical basis for this assumption. The larger the value of μ, the larger the 95% confidence interval of the estimated value supplied by the modified Gompertz model (data not shown). On the other hand, the closer the salt concentration gets to the MIC ($\mu = 0$), the larger the variation between the values of replicate measurements. Therefore, high variance values could possibly arise for both high and low μ values. The relatively high variances of the data of four experimental conditions (black dots) shown in Fig. 1A correspond to measurements at 536 and 625 mM sodium lactate (pH, 6.5; T, 30°C) and 893 mM NaCl (pH, 6; T, 30°C) and to measurements in the absence of salt (pH, 6.5; T, 30°C). If these points are left out (Fig. 1B), there is still no correlation between the variance of μ and the mean value ($t = 0.456$). For this reason, no transformation was applied to the growth rate data.

Modified Monod equation. The model that was proposed previously (model 1) (3) describes μ as a function of the concentration of sodium lactate or NaCl present in the growth medium as:

$$\mu = \mu_m \times \frac{\alpha\,(p' - p)}{p'\,(\alpha - p)} \text{ for } p \leq p'$$

where p is the salt concentration (millimolar), μ_m is the specific growth rate at $p = 0$, p' is the MIC, and α is a shape parameter. If $p > p'$, then $\mu = 0$. This equation contains three parameters that have different values for each combination of pH and temperature at which growth experiments were performed. When sodium lactate was present in the growth medium, growth was very poor at pH 5.5, and therefore not enough data were obtained at 4 and 30°C to determine the parameter values under these conditions. The entire experimental range therefore included 48 parameters [4(T) × 4(pH) × 3(parameters)] to describe the NaCl data and 42 {[(4 × 4) − 2] × 3} to describe the sodium lactate data. The actual values of the parameters are given in Table 1 (sodium lactate) and Table 2 (NaCl).

This model was validated statistically (see Table 3) with the use of the F-ratio test as described by Zwietering et al. (5). The measurement error was estimated with the use of replicate experiments as:

$$MS_{ME} = \frac{RSS_{ME}}{DF_{ME}} = \frac{0.123}{45 - 20} = \frac{0.123}{25} = 0.00493$$

This value can be compared with the variance as found by Zwietering et al. (6). The F test shows that the values are not significantly different:

$$MS_{ME} = \frac{RSS_{ME}}{DF_{ME}} = \frac{0.164}{20} = 0.00820$$

$$f = \frac{0.00820}{0.00493} = 1.66$$

$$F(20,25) = 2.01$$

The lack of fit of the model was compared with the measurement error. The results are presented in Table 3. The

TABLE 1. Values of parameters for the modified Monod model (model 1) that best described the maximum specific growth rate of *L. innocua* as a function of sodium lactate concentration in peptone-yeast extract broth

pH	Temp. (°C)	μ_m	95% CI[a] for μ_m	α	95% CI for α	p'	95% CI for p'
5.5	4	—[b]		—		—	
6.0	4	0.0395	0.0390	313	257	291	20.9
6.5	4	0.0498	0.00547	939	267	624	28.7
7.0	4	0.0499	0.0132	990	252	892	40.0
5.5	10	0.114	NA[c]	367	NA	217	NA
6.0	10	0.123	0.00725	580	34.6	486	6.17
6.5	10	0.145	0.0197	1,250	397	869	49.3
7.0	10	0.154	0.0186	1,231	172	1,067	29.6
5.5	20	0.490	NA	340	NA	217	NA
6.0	20	0.537	0.0556	1,082	444	589	33.1
6.5	20	0.614	0.0142	1,497	97.9	968	10.4
7.0	20	0.643	0.0464	1,875	385	1,267	47.3
5.5	30	—		—		—	
6.0	30	1.15	0.0352	731	52.5	485	5.54
6.5	30	1.20	0.108	1,283	237	970	48.0
7.0	30	1.26	0.0953	1,508	197	1,164	27.5

[a] 95% CI, 95% confidence interval.
[b] —, parameters could not be determined because of lack of data.
[c] NA, not applicable (the number of available data [three] was the same as the number of parameters).

models for both sodium lactate and NaCl were accepted on the basis of the F test.

The disadvantage of model 1 is that μ cannot be predicted under conditions where temperature and/or pH values are different from those used for the data collection. Furthermore, a large number of parameters are needed. Therefore, the parameters (μ_m, α, and p') were defined as a function of temperature. For sodium lactate, both temperature and pH were included as a variable, since the MIC (p') of this salt was strongly dependent on the pH of the growth medium (3), which is also observed from Table 1. Table 2 shows that the influence of pH on p' is not significant when NaCl is used as the growth-inhibitory substance.

TABLE 2. Values of parameters for the modified Monod model (model 1) that best described the maximum specific growth rate of *L. innocua* as a function of NaCl concentration in peptone-yeast extract broth

pH	Temp. (°C)	μ_m	95% CI[a] for μ_m	α	95% CI for α	p'	95% CI for p'
5.5	4	0.0375	0.00512	1,193	47.4	1,161	7.07
6.0	4	0.0462	0.00622	1,210	58.7	1,161	10.3
6.5	4	0.0400	0.00319	1,317	45.2	1,250	8.38
7.0	4	0.0596	0.00147	1,365	23.2	1,250	3.85
5.5	10	0.119	0.00405	1,845	71.2	1,607	12.5
6.0	10	0.141	0.00728	2,432	263	1,782	34.7
6.5	10	0.159	0.0148	3,061	984	1,784	88.5
7.0	10	0.153	0.0181	2,783	952	1,781	97.6
5.5	20	0.553	0.111	2,289	777	1,784	113
6.0	20	0.609	0.0710	3,259	1,222	1,957	116
6.5	20	0.634	0.0900	3,589	2,091	1,951	156
7.0	20	0.661	0.0466	4,020	1,416	1,952	82.8
5.5	30	1.23	0.407	1,881	1,008	1,606	116
6.0	30	1.71	0.539	3,299	3,638	1,687	174
6.5	30	1.48	0.220	2,588	979	1,682	92.0
7.0	30	1.34	0.235	2,459	1,022	1,690	116

[a] 95% CI, 95% confidence interval.

TABLE 3. Statistical analysis of the modified Monod model (model 1) used for predicting the maximum specific growth rate of *L. innocua* in peptone-yeast extract broth in the presence of NaCl and sodium lactate[a]

Model	nd	np	DF	RSS	MS	f	F
Model 1 NaCl	112	48	64	0.419	0.00654		
LOF			39	0.296	0.00759	1.54	1.88
Model 1 NaL	103	42	59	0.168	0.00284		
LOF			34	0.045	0.00132	0.268	1.90
Measurement error		20	25	0.123	0.00493		

[a] Abbreviations: nd, number of μ data; np, number of parameters; LOF, lack of fit; NaL, sodium lactate; DF, degrees of freedom; RSS, residual sum of squares; MS, mean square; f, $MS_{LOF}/MS_{meas.err.}$; F, F table value (95% confidence).

Parameter update—model 2. Plotting of the derived parameters (μ_m, α, and p') of model 1 as a function of temperature or pH showed either constant, linear, or quadratic behavior that was translated into equations 1 to 6, below. Subsequently, parameter values (b_1 to d_8) were determined by linear regression of the μ, α, and p' data for sodium lactate (42 parameters) and μ, α, and p' data for NaCl (48 parameters) to yield model 2.

For sodium lactate, the equations are as follows:

$$\mu_m = b_1^2 \times (T - b_2)^2 \tag{1}$$

(Equation 1 is according to Ratkowsky et al. [4].)

$$\alpha = b_3 \times T^2 + b_4 \times T + b_5 \times \text{pH}^2 + b_6 \times \text{pH} + b_7 \tag{2}$$

$$p' = b_8 \times T^2 + b_9 \times T + b_{10} \times \text{pH}^2 + b_{11} \times \text{pH} + b_{12} \tag{3}$$

For NaCl, the equations are as follows:

$$\mu_m = d_1^2 \times (T - d_2)^2 \tag{4}$$

(Equation 4 is according to Ratkowsky et al. [4].)

$$\alpha = d_3 \times T^2 + d_4 \times T + d_5 \tag{5}$$

$$p' = d_6 \times T^2 + d_7 \times T + d_8 \tag{6}$$

The estimated parameter values of model 2 are given in Tables 4 and 5 for sodium lactate and NaCl, respectively.

Parameter update—model 3. A more accurate parameter update was carried out with the help of the computerized nonlinear fitting procedure, fitting the original μ data (103 datum points for sodium lactate and 112 for NaCl) at various values of pH, T, and salt concentration, to calculate optimum values for b_1, etc. (model 3 [Tables 4 and 5]). The parameter estimates obtained for model 2 were used as initial values. Calculation of the confidence intervals of the parameters showed that this procedure allowed a further reduction in the number of parameters (some parameters were no longer significant; i.e., 0 was in the 95% confidence interval). After each parameter elimination, the nonlinear fitting procedure was repeated to update the remaining parameter values and to check whether the resulting model was not statistically rejected.

With sodium lactate, α was not necessarily dependent on temperature or pH but p' obviously was related to the pH of

TABLE 4. Parameter estimates for the various models used to predict the maximum specific growth rate of *L. innocua* in peptone-yeast extract broth in the presence of sodium lactate

Parameter	Value of parameter in[a]: Model 2 (np = 12)	Model 3 (np = 12)	Model 3b (np = 5)	Equation no.[b]	Value of parameter in model 4 (np = 9)[a]	Equation no.[b]
b_1	0.0349	0.0342	0.0361	1	−26.1	7
b_2	−1.38	−2.10	−0.927	1	0.296	7
b_3	−2.53	−0.205	0	2	−0.0101	7
b_4	104	−13.6	0	2	6.60	7
b_5	−347	559	0	2	−0.00328	7
b_6	5,192	−6,670	0	2	−0.486	7
b_7	−18,671	21,325	1,335	2	−0.00953	7
b_8	−1.11	−0.895	0	3	0.00133	7
b_9	47.4	35.0	0	3	0.0000157	7
b_{10}	−85.5	−35.4	0	3	0	7
b_{11}	1,724	1,135	606	3		
b_{12}	−7,134	−5,288	−3,066	3		

[a] Model 2, equations 1 to 3 fitted to μ, α and p'; model 3, equations 1 to 3 incorporated in model 1 and fitted to the growth rate data; model 3b, model 3 with exclusion of nonsignificant parameters; model 4, polynomial equation (equation 7). np, number of parameters.
[b] This number refers to the equation in the text in which the parameter is used.

the growth medium, so that the initial number of 12 parameters (equations 1 to 3) was reduced to 5 (b_1, b_2, b_7, b_{11}, and b_{12}) (model 3b [Table 4]). With NaCl, α and p' were not necessarily dependent on temperature, so that the initial number of 8 parameters (equations 4 to 6) could be reduced to 4 (d_1, d_2, d_5, and d_8) (model 3b [Table 5]). Model 1 shows that p' and α do not play a role in determining μ when $p = 0$, so that the models for sodium lactate and NaCl are basically the same under this condition (equations 1 and 4). The values of b_1 and b_2 (Table 4) therefore should be equal to the values of d_1 and d_2, respectively (Table 5). Indeed, the 95% confidence intervals showed overlap in all cases (data not shown).

Polynomial fit. Also, a polynomial fit (equations 7 and 8) was applied to the experimental data (model 4 [Tables 4 and 5]). Nonsignificant terms were excluded, resulting in the following equations:

$$\mu_{\mathrm{NaL}} = \exp(b_1 + b_2 \times T + b_3 \times p + b_4 \times \mathrm{pH} + b_5 \times T^2 + b_6 \times \mathrm{pH}^2 + b_7 \times \mathrm{pH} \times T + b_8 \times \mathrm{pH} \times p + b_9 \times T \times p + b_{10} \times p^2) \quad (7)$$

$$\mu_{\mathrm{NaCl}} = \exp(d_1 + d_2 \times T + d_3 \times p + d_4 \times T^2 + d_5 \times p^2) \quad (8)$$

MICs could not be incorporated into the polynomial fit, because the regression analysis was performed after a logarithmic transformation of the μ data (ln μ), so those conditions under which $\mu = 0$ were not taken into account. The logarithmic transformation was necessary to obtain sufficient accuracy. When regression was performed on the raw data (no transformation) or when the MICs were taken into account by substitution of $\mu = 0$ by $\mu = 10^{-8}$ or 10^{-3}, more parameters were necessary to obtain a sufficiently low RSS. The RSS was calculated on the basis of the raw μ data.

Comparison of the models. The statistical validation of the various models mentioned above is shown in Table 6 (sodium lactate) and Table 7 (NaCl). The various models were compared with the measurement error. The *f* value was used to discriminate between the number of parameters needed to describe the experimental data for sodium lactate and NaCl.

Figure 2 shows that the prediction from model 3 is less accurate than the prediction from model 1. However, the decline in the goodness of fit is accompanied by a large reduction in the number of parameters needed (Tables 6 and 7), whereas the area in which predictions are valid is enlarged from combinations of four temperatures with four pH values to the whole range of values for these variables that are between the

TABLE 5. Parameter estimates for the various models used to predict the maximum specific growth rate of *L. innocua* in peptone-yeast extract broth in the presence of NaCl

Parameter	Value of parameter in[a]: Model 2 (np = 8)	Model 3 (np = 8)	Model 3b (np = 4)	Equation no.	Value of parameter in model 4[a] (np = 5)	Equation no.[b]
d_1	0.0385	0.0385	0.0389	4	−4.08	8
d_2	−0.714	−0.173	−0.0692	4	0.240	8
d_3	−7.77	−5.69	0	5	0.000576	8
d_4	313	217	0	5	−0.00331	8
d_5	155	497	2,145	5	−0.000000826	8
d_6	−2.90	−2.27	0	6		
d_7	115	88.3	0	6		
d_8	822	934	1,629	6		

[a] Model 2, equations 4 to 6 fitted to μ, α, and p'; model 3, equations 4 to 6 incorporated in model 1 and fitted to the growth rate data; model 3b, model 3 with exclusion of nonsignificant parameters; model 4, polynomial equation (equation 8). np, number of parameters.
[b] This number refers to the equation in the text in which the parameter is used.

TABLE 6. Statistical analysis of the different models used for predicting the maximum specific growth rate of *L. innocua* in peptone-yeast extract broth in the presence of sodium lactate[a]

Model	np	DF	RSS	MS	f	F
Model 1	42	59	0.168	0.00284		
LOF 1		34	0.0447	0.00131	0.267	$F(34,25) = 1.90$
Model 2	12	89	0.639	0.00718		
LOF 2		64	0.516	0.00806	1.63	$F(64,25) = 1.82$
Model 3	12	91	0.241	0.00265		
LOF 3		66	0.118	0.00179	0.363	$F(66,25) = 1.81$
Model 3b	5	98	0.630	0.00643		
LOF 3b		73	0.507	0.00695	1.41	$F(73,25) = 1.80$
Model 4	9	79	0.458	0.00580		
LOF 4		54	0.335	0.00620	1.26	$F(54,25) = 1.83$
Measurement error	20	25	0.123	0.00493		

[a] Data are based on 103 growth curves. Abbreviations: np, number of parameters; LOF, lack of fit; DF, degrees of freedom; RSS, residual sum of squares; MS, mean square; f, $MS_{LOF}/MS_{meas.err.}$; F, F table value (95% confidence).

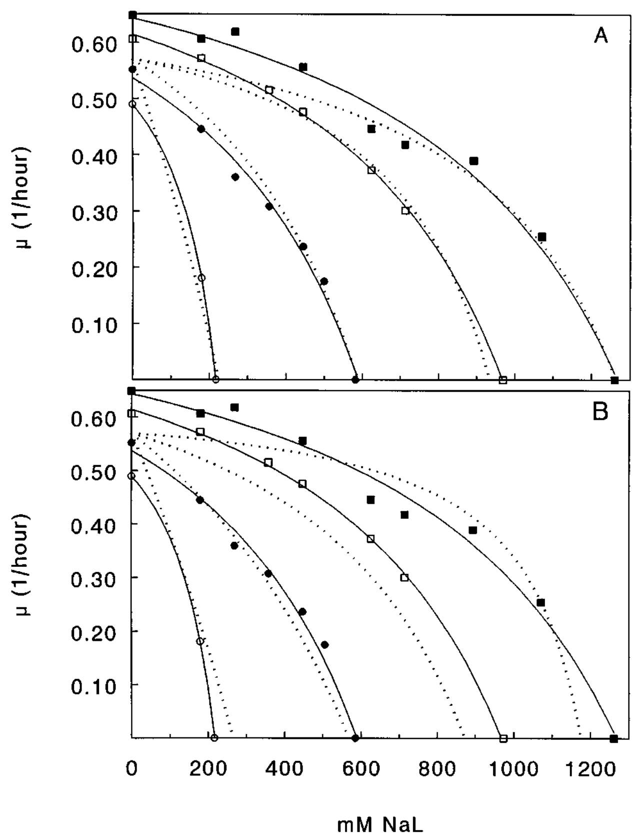

FIG. 2. Maximum specific growth rate (μ) of *L. innocua* in peptone-yeast extract broth in the presence of sodium lactate (NaL) at 20°C; comparison between the fit of model 1 (solid lines) and model 3 (dotted lines) (A) or model 3b (dotted lines) (B) to the experimental data (symbols) at pH 5.5 (○), pH 6.0 (●), pH 6.5 (□), and pH 7.0 (■).

extremities used for datum collection. Model 3 has one serious disadvantage compared with model 1. This is that the predictions of μ at 4°C (Fig. 3A) are relatively poor compared with those at 30°C (Fig. 3B). Since the model was fitted to the data by ordinary (i.e., unweighted) least squares, there is a tendency for the higher growth rates (i.e., those at high temperatures) to be more influential in determining the least-squares line than the data at low temperatures.

It may therefore be questioned whether a polynomial model is more appropriate. Table 6 shows that for predicting μ from the sodium lactate data, at least nine parameters are needed. The resulting model is significantly less accurate than model 1, even if 10 parameters are included (data not shown). For NaCl, the data are described by a polynomial equation with five parameters in a manner that is statistically acceptable (Table 7), so that is comparable to model 3b. However, the polynomial model had a poor ability to predict μ at salt concentrations close to and at the MIC (Fig. 4), which might be because MIC data cannot be included in the polynomial model. Since we are especially interested in the conditions under which no or only very slow growth is observed, in a situation in which lactate significantly reduced the growth rate, the polynomial equation is less applicable.

Notably, the parameters in the polynomial equations have no practical meaning, whereas p' and μ_m in the modified Monod model have. Both p' and μ_m are easily determined. For this reason, it is more convenient to check the parameters from the modified Monod model; consequently, inaccurate predictions can be easily identified. All these arguments are in favor of the use of the modified Monod model.

In conclusion, the maximum specific growth rate of *L. innocua* was satisfactorily described with a minimum number of parameters by model 3b, which is obtained by translating the relevant parameters in equations 1 to 3 (sodium lactate) and equations 4 to 6 (sodium chloride) into the formula for model 1. By doing so, the following equation is obtained for sodium lactate:

$$\mu = b_1^2 \times (T - b_2)^2 \times \frac{b_7 \times [(b_{11} \times \text{pH} + b_{12}) - p]}{(b_{11} \times \text{pH} + b_{12})(b_7 - p)}$$

Then the parameters are substituted by the values in Table 4 (model 3b, five parameters):

$$\mu = 0.0361^2 \times (T + 0.927)^2 \times \frac{1{,}335 \times [(606\ \text{pH} - 3{,}066) - p]}{(606 \times \text{pH} - 3{,}066)(1{,}335 - p)}$$

TABLE 7. Statistical analysis of the different models used for predicting the maximum specific growth rate of *L. innocua* in peptone-yeast extract broth in the presence of NaCl[a]

Model	np	DF	RSS	MS	f	F
Model 1	48	64	0.419	0.00654		
LOF 1		39	0.296	0.00758	1.54	$F(39,25) = 1.87$
Model 2	8	104	0.715	0.00687		
LOF 2		79	0.592	0.00749	1.52	$F(79,25) = 1.80$
Model 3	8	104	0.564	0.00542		
LOF 3		79	0.441	0.00558	1.13	$F(79,25) = 1.80$
Model 3b	4	108	0.625	0.00579		
LOF 3b		83	0.502	0.00605	1.23	$F(83,25) = 1.79$
Model 4	5	91	0.528	0.00580		
LOF 4		66	0.405	0.00613	1.24	$F(66,25) = 1.81$
Measurement error	20	25	0.123	0.00493		

[a] Data are based on 112 growth curves. Abbreviations: np, number of parameters; LOF, lack of fit; DF, degrees of freedom; RSS, residual sum of squares; MS, mean square; f, $MS_{LOF}/MS_{meas.err.}$; F, F table value (95% confidence).

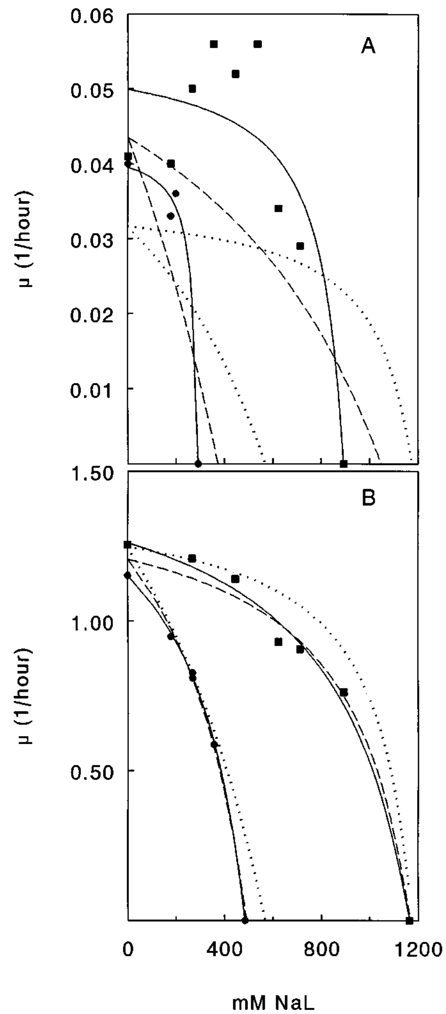

FIG. 3. Maximum specific growth rate (μ) of *L. innocua* in peptone-yeast extract broth in the presence of sodium lactate (NaL); comparison between the fit of model 1 (solid line), model 3 (dashed line), and model 3b (dotted line) to the experimental data (symbols) at 4°C (A) and 30°C (B). The experimental data refer to pH 6.0 (●) and pH 7.0 (■).

When the same procedure is followed for NaCl (model 3b, parameters d_1, d_2, d_5, and d_8 [Table 5]), the maximum specific growth rate in the presence of NaCl is described as follows:

$$\mu = 0.0389^2 \times (T + 0.0692)^2 \times \frac{2{,}145 \times (1{,}629 - p)}{1{,}629 \times (2{,}145 - p)}$$

Validation of the model for sodium lactate in a meat product. The growth rate of *L. innocua* as predicted by the broth model was far higher than that observed in the Bologna-type sausages that were formulated with different amounts of sodium lactate (Fig. 5). The broth model was designed to mimic worst-case circumstances, and this could explain the observed deviation between the data and the predicted values. The sausage contained not only sodium lactate as a growth-inhibitory substance but also nitrite (120 μg/g) and NaCl (2%, wt/wt). Furthermore, the broth model was related to aerobic conditions whereas growth in the sausage took place anaerobically. *L. innocua* is a facultatively anaerobic microorganism, and in

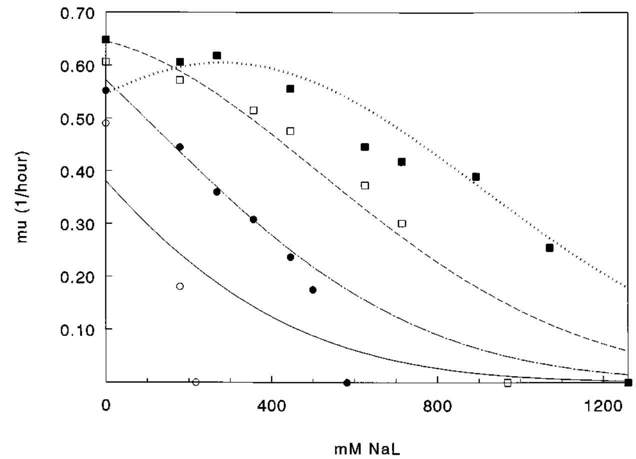

FIG. 4. Maximum specific growth rate (μ) of *L. innocua* in peptone-yeast extract broth in the presence of sodium lactate (NaL) at 20°C; fit of the polynomial model with nine parameters (lines) to the experimental data (symbols) at pH 5.5 (solid line, ○), pH 6.0 (dash-dot line, ●), pH 6.5 (dashed line, □), and pH 7.0 (dotted line, ■).

our experience it preferred aerobic to anaerobic conditions for growth, although the effect of oxygen on growth of the genus *Listeria* in meat (products) as described in the literature is confusing (1).

If no mutual interactions of these factors exist and if the effects are not correlated with pH and T, the observed deviation can be simply adjusted by reestimating the value for μ_m (Fig. 6A). The resulting RSS of 0.218 gave an MS of 0.00545. Compared with the measurement error of the broth experiments, this results in an f value of 1.105 [$F(11,25) = 2.20$]. The measurement error in the experiments with the sausage is expected to be larger than that in the broth experiments.

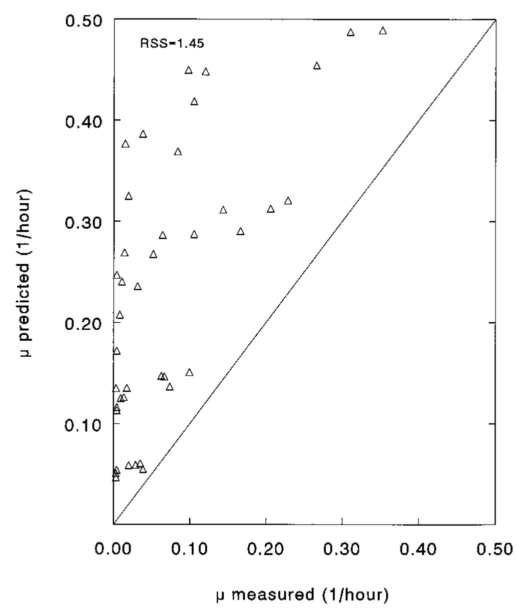

FIG. 5. Model 3b for sodium lactate, developed on the basis of experiments with broth, applied to predict the maximum specific growth rate of *L. innocua* in a Bologna-type sausage. Predicted values for μ as a function of the measured values (symbols) are shown. The line represents the ideal situation, in which the predicted values are exactly the same as the measured values.

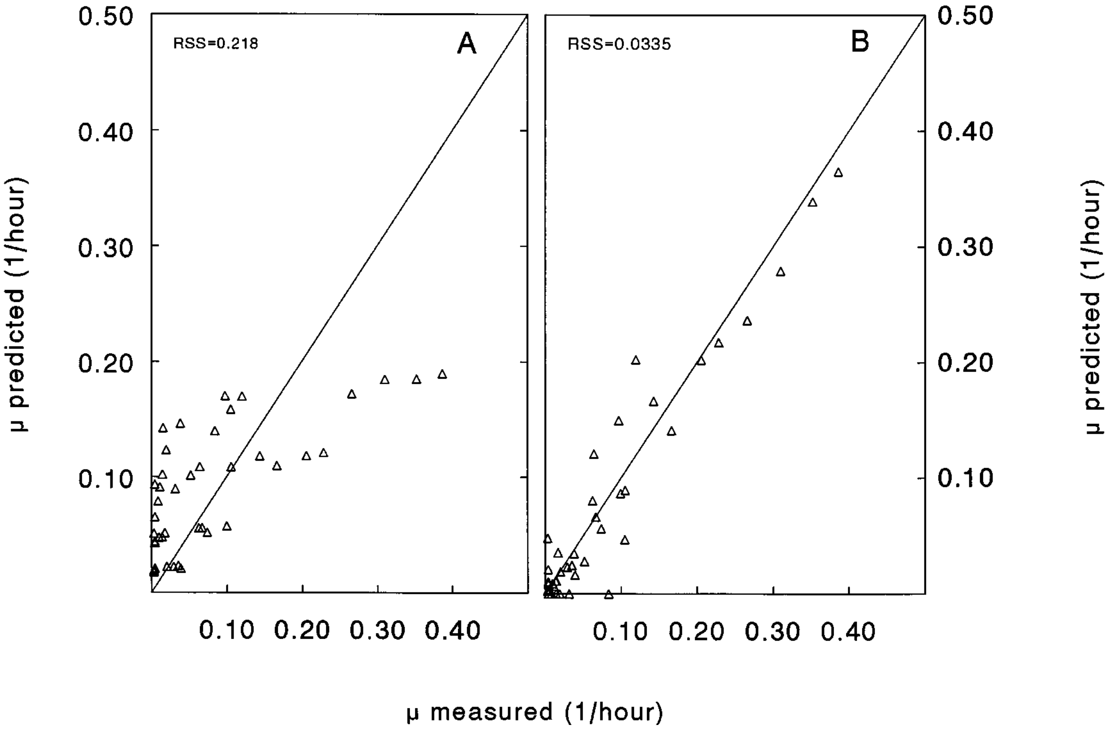

FIG. 6. Model 3b for sodium lactate, developed on the basis of experiments with broth, used to predict the maximum specific growth rate of *L. innocua* in a Bologna-type sausage after parameter b_1 has been reestimated ($b_1 = 0.0222$) (A) and after all parameters have been reestimated ($b_1 = 0.0380$, $b_2 = 1.47$, $b_7 = 3{,}000$, $b_{11} = 418$, $b_{12} = -2{,}283$) (B). The line represents the ideal situation in which the predicted values are exactly the same as the measured values.

Therefore, it can be concluded that the model described the data in a manner that was statistically acceptable after μ_m was adjusted.

However, careful comparison of predicted and measured values showed that at high measured values of μ, the predicted value was clearly too low (Fig. 6A). Therefore the other parameters of the model were also reestimated by performing nonlinear regression of the μ data obtained in growth experiments in the sausage (41 growth curves). The result is shown in Fig. 6B. Inevitably, the increased accuracy of the model after reestimation of the parameters is accompanied by an increase in the number of cases in which the growth rate is underestimated. This is possibly caused by regular statistical inaccuracy.

More data as well as replicate experiments are necessary to evaluate the usefulness of the model presented in this paper for predicting microbial growth in other meat products. As was shown, the model can be used even when growth conditions differ from those on which the model is based, after μ_m is reestimated. Depending on the degree of agreement between model predictions and measured values and the demands that are made on the accuracy of the model predictions, the modeller has to decide whether it is necessary to collect more experimental data. Overall, the simplicity of the model, in combination with the practical usefulness of its parameters, offers considerable prospects for its use in the field of predictive microbiology.

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