Modeling of the Microbial Quality of Food

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Modeling of the Microbial Quality of Food

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ABSTRACT

Zwietering, M.H. (1993) Modeling of the Microbial Quality of Food. Ph.D. thesis, Agricultural University Wageningen (152 pp., English and Dutch summaries)

Keywords: Modeling, microbial growth, temperature, growth curves, model validation, temperature shifts, decision support systems (DSS).

In this thesis it is shown that predictive modeling is a promising tool in food research, to be used to optimize food chains. Various models are developed and validated to be used to describe microbial growth in foods.

A tool is developed to discriminate between different models and to restrict the number of parameters in models. Models to describe a growth curve and to describe the effect of temperature, and the effect of shifts in temperatures, are developed and validated with a large amount of experimental data.

Furthermore, a procedure is developed to couple quantitative and qualitative information in a structured manner. Simple procedures to make (preliminary) shelf-life predictions are given, as are procedures to extend these (simple) models.

The most important advantage of modeling is that insight is gained in the progression of microbial growth within a product chain. Furthermore, these models are shown to be essential to calculate quality changes with the use of decision support systems (DSS).

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

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STELLINGEN

1. Smith (1985) doet ten onrechte uitspraken over temperatuurrichtlijnen in slachthuizen, omdat hij warmte-indringing in het vlees niet in beschouwing neemt.

   Smith M.G. (1985). The generation time, lag time, and minimum temperature of growth of coliform organisms on meat, and the implications for codes of practice in abattoirs. J. Hyg. Camb. **94**: 289-300.

2. Barreto et al. (1991) verklaren hun resultaten met een 'apparent lag time', veroorzaakt door een levensvatbaarheid lager dan 100%. Dezelfde resultaten kunnen ook verklaard worden met een normale lag-fase en 100% levensvatbaarheid.

   Barreto M.T.O., E.P. Melo, J.S. Almeida, A.M.R.B. Xavier, and M.J.T. Carrondo (1991). A kinetic method for calculating the viability of lactic starter cultures. Appl. Microbiol. Biotechnol. **34**:648-652.

3. De modellen gepresenteerd door Beal en Corrieu (1991) voorspellen onder andere een rechtlijnig verband tussen pH en de maximale specifieke groeisnelheid van *Lactobacillus bulgaricus*. Het desalniettemin vermelden van een optimale pH voor deze groeisnelheid wijst erop dat de auteurs een computerprogramma gebruiken dat extrapolaties niet toestaat.

   Beal C., and G. Corrieu (1991). Influence of pH, temperature, and inoculum composition on mixed cultures of *Streptococcus thermophilus* 404 and *Lactobacillus bulgaricus* 398. Biotechnol. Bioeng. **38**:90-98.

4. Het gebruik van vijfendertig parameters om het effect van vier variabelen te beschrijven in een derde-ordepolynoom resulteert niet in een vergroot inzicht en kan bovendien in gevaarlijke voorspellingen resulteren.

   Palumbo S.A., A.C. Williams, R.L. Buchanan, and J.G. Phillips (1991). Model for the aerobic growth of *Aeromonas hydrophila* K144. J. Food Protection **54**:429-435.

5. Een neuraal netwerk is vaak een eufemisme voor fitten met teveel parameters.

   bijvoorbeeld: Linko P., K. Uemura, Y.H. Zhu, and T. Eerikäinen (1992). Application of neural network models in fuzzy extrusion control. Food and Bioproducts Processing **70**:131-137

6. Modellering is essentieel voor het gestructureerd verzamelen van voldoende gegevens.

7. De krant is een van de meest bederfelijke produkten.

8. Combinatie van Just In Time (JIT) met Murphy's Law resulteert in Just Too Late (JTL) en ontevreden klanten.

9. Bij het berekenen van kengetallen voor kwaliteit van onderzoek door auteurschappen van wetenschappelijke publikaties op te tellen, wordt vaak vergeten te delen door het aantal auteurs.

10. Bij het gebruik van het begrip % dient de noemer duidelijk gedefinieerd te zijn.

11. Door het gebruik van spellingscontroleprogrammatuur worden steeds meer woorden, die in het Nederlands aan elkaar geschreven moeten worden, niet meer aan elkaar geschreven.

12. Drieduizend jaar voor onze jaartelling ondervonden de oude Egyptenaren dat een zuiver beeldschrift tekort schiet om alles op te tekenen. Nu, 5000 jaar later, blijkt opnieuw dat pictogrammen minder begrijpelijk zijn dan hun ontwerpers zich voorstelden.

    K. Th. Zauzich (1980). Hieroglyphen ohne Geheimnis. P. von Zabern, Mainz am Rhein.
    Microsoft Windows version 3.1 © 1985-1992 Microsoft Corp.

13. Als een regering haar volk probeert te overtuigen van de veiligheid van kernenergie dient zij de toekomstige centrales in de buurt van de regeringsgebouwen te plannen.

14. Het aan de buitenkant aanbranden van vlees bij het barbecuen heeft als voordeel dat de (actieve) kool al gelijk met de, aan de binnenkant overlevende pathogenen, geconsumeerd wordt.

Stellingen behorende bij het proefschrift "Modeling of the Microbial Quality of Food"

M.H. Zwietering
Wageningen, 29 September 1993

CONTENTS

CHAPTER 1

INTRODUCTION:

MODELING MICROBIAL QUALITY OF FOOD

FOOD QUALITY

Definition. Food quality can be defined as the sum of the characteristics of a food that determine the satisfaction of the consumer and compliance to legal standards. Thus, food quality is a combination of numerous factors, such as organoleptic properties (e.g., texture, taste, flavour, smell, colour), nutritional value (e.g., caloric content, fatty acid composition), shelf life (e.g., microbial number), and safety conditions (e.g., presence of pathogens, toxins, hormones). Some of these (e.g., microbial numbers) can be relatively easily quantified, while others are very difficult to assess (e.g., taste). Food quality thus cannot be quantified in every detail, and overall quantification depends strongly on the priority among quality determining aspects. To determine total food quality, quality indicators are needed and must be weighted, since their relative importance depends on product, trends, producer, and market.

Significance. Food quality attracts ever more attention and prediction of the rate of quality loss is important for the following reasons:

- The food market is subject to saturation in most cases, therefore, quality becomes more important than quantity.
- There are new quality attributes which are highly appreciated by the modern consumer (in contrast to traditional quality demands). Consumers show an increasing interest in convenience foods with the appearance and taste of fresh products and in food quality

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Food Control 4:89-96

aspects such as flavour and (assumed) health aspects (e.g., nutritional value, fatty acid quantity and composition, energy content, salt concentration, presence of additives such as preservatives).

- Consumers are willing to pay a higher price for quality.
- Manufacturers want to deliver constant quality products at the lowest costs.
- Many products have a limited shelf life. Production and storage conditions affect quality very strongly; therefore, production and distribution are often critical. From the past there are many dried, salted, frozen, and sterilised products, while nowadays chilled and intermediate-moisture foods are becoming more important.
- The shelf life of a product determines the distribution regime: daily delivery for perishable products such as fresh milk, bread, fresh vegetables, and fresh meat, or less frequently as for salads, margarine, etc.
- Due to more open borders in the European Community (EC), the distribution routes have become longer, and therefore, there is a need for an increased shelf life.
- In some areas there is a rather rapid product development (changes in product formulation). Consequently, it will be useful to make an estimation of the shelf life during product development.
- Formulation of products may be different in different countries or regions, because of legal requirements or regional food preferences. Therefore, it would be useful to know the effect of different compositions on the shelf life, to avoid each formulation requiring a laborious shelf-life test.

New procedures are being developed to meet these quality demands, such as new technologies (e.g., microwave heating, ultrahigh temperature (UHT) processes, modified atmosphere packaging, supercooling, irradiation), and new strategies (e.g., logistics and modeling).

Quality loss along a chain. Quality loss can result from microbial, chemical, enzymatic, or physical reactions. Various factors influence quality loss, such as the composition of the product. A product without lipids, for example, cannot show lipid oxidation; iron (Fe^{3+}) acts as catalysing agent for vitamin C degradation (2). Other factors influencing quality loss are processing and storage conditions (temperature, time, packaging material, gas atmosphere, machinery).

Thus, product quality is determined by the composition and quality of the raw materials, and by the process and storage conditions. Quality is often measured during production and distribution by taking samples of a product somewhere along the chain (Fig. 1). This can give valuable information about long-term quality changes and bottlenecks in the line. This, however, gives little information about the separate effect of particular process steps in the production and distribution chain on the total quality. It is important to get insight in the progression of the deterioration reactions, i.e. the kinetics of spoilage in each step in the process. Quality loss can be determined by a single processing step, but can also result from partial losses at several stages of the production or distribution. Without insight it is impossible to optimize the process, and to evaluate changes in costs in relation to changes in quality. It is therefore useful to examine the whole chain of food products from raw materials to consumption (Fig. 1).

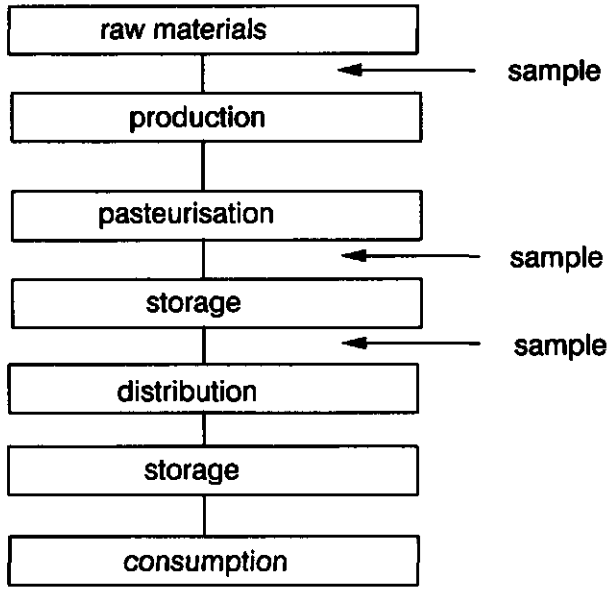

FIG. 1. Example for a pathway of a food product from raw materials to consumer.

Modeling can be a useful tool to get insights into the importance of factors in any part of the production and distribution chain, and is based on quantitative predictions of the rate of spoilage. Such modeling allows prediction of the quality or shelf life of products, detection of critical points in the production and distribution process, and optimization of production

and distribution chains by combining cost models with the spoilage models. Moreover, by making the models and by evaluating the predictions, insight in the relevant processes can be obtained.

PREDICTIVE MODELING

Significance. Predictive modeling is a promising methodology in food research, to be used to optimize food chains. Models are used to describe deterioration under different physical or chemical conditions such as temperature (T), pH, and water activity (a_w). First, deterioration reactions have to be modeled and the models must be validated with quantitative data. The model parameters can then be estimated. These models and model parameters will only be valid for the product and the conditions for which the data are collected. However, in some cases the model parameters and/or the model will also hold for similar products and conditions.

In some cases, certain deterioration reactions can be excluded. For example, if the a_w of a product is lower than 0.6, microbial growth can be excluded and if the a_w is lower than 0.2, Maillard browning activity can be excluded (2). If the physical and chemical conditions of the product allow a specific deterioration reaction, an estimate can be made of the kinetics of the reaction. For example *Escherichia coli* can grow between pH 4.4 and 9.0 (4). If the pH of a product is 4.6, *Escherichia coli* can still grow in that product, but not very fast (and slower than at pH=5). To predict the kinetics of the various deterioration processes more quantitatively, models describing the effects of different conditions are essential. Several models are known to predict deterioration reactions. Examples are given by Ratkowsky et al. (3) and Zwietering et al. (5) to describe the effect of temperature on microbial growth. For the effect of pH a parabolic behaviour can be assumed, for instance. The Arrhenius model (1) can be used for the temperature effect on chemical and physical processes. The resulting quantitative estimation can be used to predict the shelf life.

For the validation of the models numerous measurements are needed. However, once the model is validated, predictions can be made with none, or only very few, experiments, and insight into the process is gained.

It is often useful to search for literature models and literature parameters for comparable products and microorganisms (Fig. 2). With such models and parameters preliminary predictions can be made and insight can be gained. This can also be very useful for

experimental design. From the discrepancy between these predictions and actual data, strategies for improvement may be derived, such as better models and/or better parameter estimates.

Especially microbial risks have increased due to the trends mentioned before. From the past there were many dried, salted, frozen, sterilised products. So in the past growth limits (a_w, salt, T) and inactivation kinetics (during sterilization) were of importance. Growth was not possible, since the organisms were absent (sterilisation) or the conditions were changed so that growth was not possible (dried, salted, frozen). Nowadays chilled and intermediate-moisture foods are becoming more important. Then, growth kinetics and the effect of shelf life increasing factors (e.g., T, a_w, pH) on the kinetics are of larger importance. Moreover, the modern consumer often wants a lower salt concentration, and absence of additives such as preservatives. This emphasises the need to determine the effect of combinations of factors that are of importance for the growth of micro-organisms.

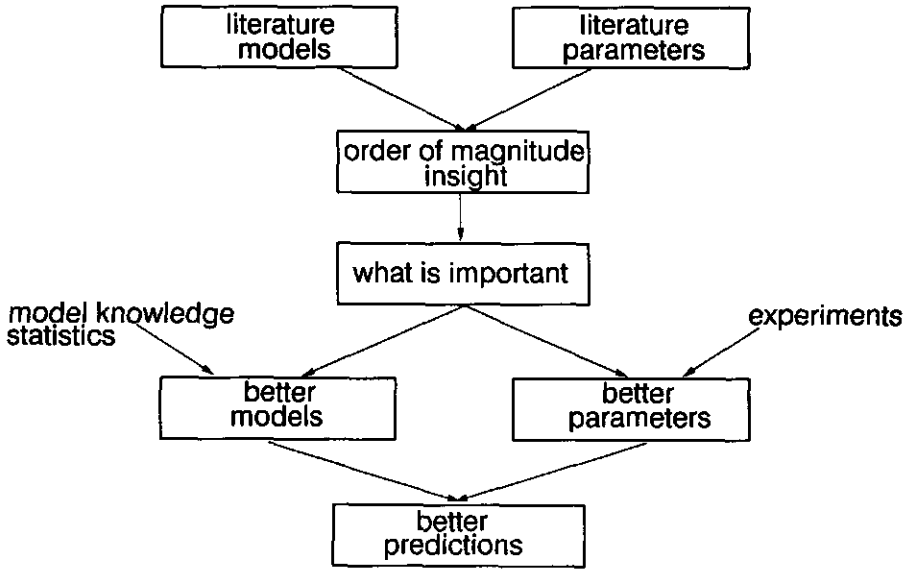

FIG. 2. Schematic representation of a procedure to make kinetic predictions of food deterioration processes.

Values and needs in predictive modeling. The most important needs in predictive modeling are:

- A good procedure to describe growth curves of microorganisms.
- Tools to discriminate between different models.
- Models that are validated with a large amount of experimental data.
- Models for shifts in variables (these are scarce).
- Protocols to extend quantitative models with qualitative knowledge.
- Models to describe the effect of additional variables (only a few variables have been investigated, such as T, a_w, pH).
- Integrated models for the combined effect of multiple factors, and determination of interaction terms.

Objectives of modeling. The value of a model is strongly dependent on the objective for which the model is used. For instance the control of a certain variable (y) by a control variable (u), (e.g., pH control of a fermentor) often only requires a simple model (on-line feedback). Only a global description of the dynamic behaviour of y as function of u is sufficient, to obtain the right value for the control signal. Subsequently, y is measured again and if the value is not correct the control procedure will continue, resulting finally in the desired value. On the other hand, if one wants to predict the death rate of *Clostridium botulinum* spores in a sterilisation procedure, the global dynamic behaviour will often not be sufficient, and a much more accurate model is necessary, since there is no feed back (or only off-line). In other cases, the aim can be to understand more mechanistically what happens during sterilisation. A more mechanistic model is then necessary, which must be validated with experimental data. In some cases this model is only used to test a hypothesis and it does not need to be very accurate in predicting.

Warning note. Modeling techniques can be very useful. However, with models and also with computer programs nonsense can be generated. It is, therefore, of great importance that modelers combine knowledge in food science, informatics, and mathematics, and use the expertise of all these disciplines to detect possible errors. Computer programs and mathematical equations may yield predictions which deviate enormously from reality. Therefore, a food scientist must be sceptical about predictions. An example of this can be shown with the gravity acceleration (g=9.81 m/s^2 in The Netherlands) and Newtons laws. With only the gravity acceleration, the position and velocity of a falling stone and of a falling ashtray can be predicted. A falling leaf however gives totally different results, since the air

resistance and air flow phenomena are of large influence, so the gravitational acceleration, the air resistance, and the direction of the air flow are of importance, which makes the problem more complex. So the acceleration will be equal to the gravity acceleration in some cases, but will markedly deviate in other cases. Also the model will be valid in certain ranges only, since Newtons laws are not valid if velocities approach light velocity.

CONCLUSIONS

Modelling can be an important tool to predict the shelf life of products, to optimize production and distribution chains, and to gain insight about important variables that determine product deterioration. Predictive models, kinetic data, expertise, logistics, and simulation and optimization routines can be combined to support decisions in production and distribution, and product development. This can help to determine possible spoilage organisms, and changes in growth rates of organisms can be estimated when the physical properties are changed.

OBJECTIVE OF THIS THESIS

In this work a procedure to compare different models will be examined. With this procedure a model will be selected to describe a bacterial growth curve and to estimate the specific growth rate, lag time and asymptote from growth data by examining a large amount of growth curves. Furthermore, models for the effect of temperature on the growth rate will be compared, with a large amount of growth rates at different temperatures. Models for the effect of temperature on the lag time and the asymptote will be selected. Furthermore, the effects of shifts in temperature will be examined. A larger system to collect modeling results, parameter values and expertise will be built in order to combine quantitative and qualitative information. This will be developed as a decision support system.

OUTLINE OF THIS THESIS

In Chapter 2 of this thesis a comparison is made between different models that describe bacterial numbers as a function of time. The models are reparameterized so that they contain biologically meaningful parameters. A procedure to estimate growth parameters from a set of data is given. In Chapter 3 the method as described in Chapter 2 is compared with other methods, as described in literature. In Chapter 4 different growth/temperature models are

compared. In Chapter 5 the correct variance-stabilising transformations are determined with a large amount of data and the models of Chapter 4 are validated and updated. In Chapter 6 the effect of temperature steps on bacterial growth is evaluated. In Chapter 7 the first steps are taken to build a knowledge-based system, which can help to detect possible spoilage organisms and which can estimate growth parameters. In Chapter 8 (general discussion) the models are discussed and some examples are given. Possibilities to combine the models with models for heat transfer and logistics are given. These combined models can be used in simulation programs, that predict the outgrowth of bacteria as function of time and location inside the product, in food chains, with different storage temperatures. The result of this work is evaluated, and future expansions are proposed.

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CHAPTER 2

MODELING OF THE BACTERIAL GROWTH CURVE

ABSTRACT

Several sigmoidal functions (logistic, Gompertz, Richards, Schnute, and Stannard) were compared to describe a bacterial growth curve. They were compared statistically by using the model of Schnute, which is a comprehensive model, encompassing all other models. The *t* test and the *F* test were used. With the *t* test, confidence intervals for parameters can be calculated and can be used to distinguish between models. In the *F* test, the lack of fit of the models is compared with the measuring error. Moreover, the models were compared with respect to their ease of use. All sigmoidal functions were modified so that they contained biologically relevant parameters. The models of Richards, Schnute, and Stannard appeared to be basically the same equation. In the cases tested, the modified Gompertz equation was statistically sufficient to describe the growth data of *Lactobacillus plantarum* and was easy to use.

INTRODUCTION

Predictive modeling is a promising field of food microbiology. Models are used to describe the behavior of microorganisms under different physical or chemical conditions such as temperature, pH, and water activity. These models allow the prediction of microbial safety or shelf life of products, the detection of critical parts of the production and distribution process, and the optimization of production and distribution chains. In order to build these models, growth has to be measured and modeled. Bacterial growth often shows a phase in which the specific growth rate starts at a value of zero and then accelerates to a maximal value (μ_m) in a certain period of time, resulting in a lag time (λ). In addition, growth curves contain a final phase in which the rate decreases and finally reaches zero, so that an asymptote

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Appl. Environ. Microb. 56:1875-1881

(*A*) is reached. When the growth curve is defined as the logarithm of the number of organisms plotted against time, these growth rate changes result in a sigmoidal curve (Fig. 1), with a lag phase just after $t = 0$ followed by an exponential phase and then by a stationary phase.

Growth curves are found in a wide range of disciplines, such as fishery research, crop science, and biology. Most living matter grows with successive lag, growth, and asymptotic phases; examples of quantities that follow such growth curves are the length or mass of a human, a potato, or a fish and the extent of a population of fish or microorganisms. In addition, these sigmoidal curves are used in medical science for dose-mortality relations.

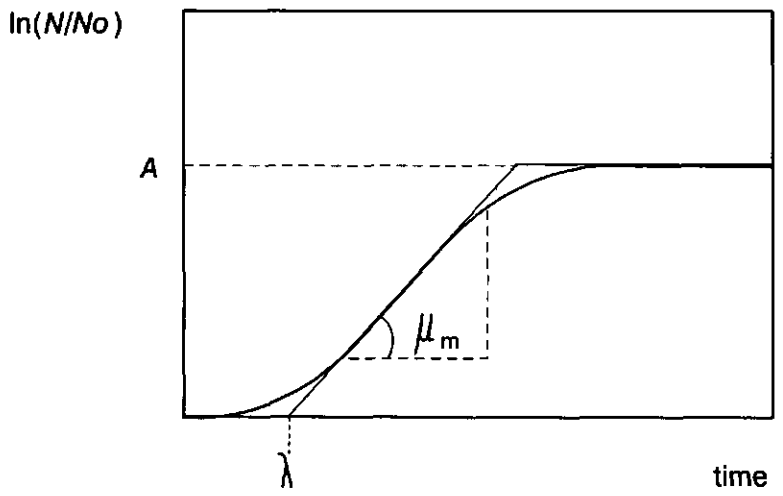

FIG. 1. A growth curve (A = Asymptotic level, μ_m = maximum specific growth rate, λ = lag time, N = number of organisms, N_o = number at time 0).

To describe such a curve and to reduce measured data to a limited number of interesting parameters, investigators need adequate models. A number of growth models are found in the literature, such as the models of Gompertz (7), Richards (14), Stannard et al. (17), Schnute (16), and the logistic model and others (15). These models describe only the number of organisms and do not include the consumption of substrate as a model based on the Monod equation would do. The substrate level is not of interest in our application, as we assume that there is sufficient substrate to reach intolerable numbers of organisms.

Besides the lag period and the asymptotic value, another valuable parameter of the growth curve is the maximum specific growth rate (μ_m). Since the logarithm of the number is used, μ_m is given by the slope of the line when the organisms grow exponentially. Usually this parameter is estimated by deciding subjectively which part of the curve is approximately linear and then determining the slope of this curve section, eventually by linear regression (Table 1). A better method is to describe the entire set of data with a growth model and then estimate μ_m, λ, and A from the model. Some authors indeed use growth models to describe their data (Table 1). These models describe the number of organisms (N) or the logarithm of the number of organisms [$\log(N)$] as a function of time.

TABLE 1. Some growth models used in the literature

Author(s)	Modeling	Model(s)
Adair et al. (1)	$\log(N)$	Linear regression
Bratchell et al. (2)	$\log(N)$	Gompertz
Broughall et al. (3)	$\log(N)$	Linear regression
Einarsson and Eriksson (4)	$\log(N)$	Logistic, polynomial
Gibson et al. (5)	$\log(N)$	Logistic, Gompertz
Gibson et al. (6)	$\log(N)$	Gompertz
Griffiths and Phillips (8)	$\log(N)$?	Stannard
Jason (9)	N	Logistic
Mackey and Kerridge (10)	N	Gompertz
Phillips and Griffiths (12)	$\log(N)$?	Stannard
Stannard et al. (17)	$\log(N)$?	Stannard

The motivation for the decision to use a given model is usually not stated. Only Gibson et al. (5) found better results by a fitting procedure with the Gompertz model when they compared that model with the logistic model. A large number of models as given in Table 1 are used, all more or less complicated and with different numbers of parameters. It can be expected that a difference in the results of the models exists for our application. Besides, the models are not written in terms of growth rate, lag time, and asymptotic value, which makes interpretation of the parameter values difficult.

The objective of this work is to evaluate similarities and differences between the models and to deal with the question of which model(s) can be used, on the basis of statistical reasoning. The models are rewritten in such a way that they contain parameters that are microbiologically relevant.

THEORY

Description of the bacterial growth curve. Since bacteria grow exponentially, it is often useful to plot the logarithm of the relative population size [$y = \ln(N/N_o)$] against time (Fig. 1). The three phases of the growth curve can be described by three parameters: the maximum specific growth rate, μ_m, is defined as the tangent in the inflection point; the lag time, λ, is defined as the x-axis intercept of this tangent; and the asymptote [$A = \ln(N_\infty/N_o)$] is the maximal value reached. Curves may show a decline. This kind of behavior is called the death phase and is not considered in this chapter.

TABLE 2. Models used an their modified forms

Equation ($y=$)	Modified equation[a] ($y=$)
Logistic: $\dfrac{\alpha}{[1+\exp(b-cx)]}$	$\dfrac{A}{\left\{1+\exp\left[\frac{4\mu_m}{A}(\lambda-t)+2\right]\right\}}$
Gompertz: $\alpha\exp[-\exp(b-cx)]$	$A\exp\left\{-\exp\left[\dfrac{\mu_m \cdot e}{A}(\lambda-t)+1\right]\right\}$
Richards: $\alpha\{1+\upsilon\exp[k(\tau-x)]\}^{-1/\upsilon}$	$A\left\{1+\upsilon\exp(1+\upsilon)\exp\left[\dfrac{\mu_m}{A}(1+\upsilon)^{\left(1+\frac{1}{\upsilon}\right)}(\lambda-t)\right]\right\}^{-1/\upsilon}$
Stannard: $\alpha\left\{1+\exp\left[-\dfrac{(l+kx)}{p}\right]\right\}^{-p}$	$A\left\{1+\upsilon\exp(1+\upsilon)\exp\left[\dfrac{\mu_m}{A}(1+\upsilon)^{\left(1+\frac{1}{\upsilon}\right)}(\lambda-t)\right]\right\}^{-1/\upsilon}$
Schnute: $\left\{y_1^b+(y_2^b-y_1^b)\dfrac{1-\exp[-a(t-\tau_1)]}{1-\exp[-a(\tau_2-\tau_1)]}\right\}^{1/b}$	$\left(\mu_m\dfrac{1-b}{\alpha}\right)\left[\dfrac{1-b\exp(a\lambda+1-b-at)}{1-b}\right]^{1/b}$

[a] $e=\exp(1)$; υ = shape parameter.

Reparameterization of the growth models. Most of the equations describing a sigmoidal growth curve contain mathematical parameters ($\alpha, b, c, \ldots$) rather than parameters with a biological meaning (A, μ_m, and λ). It is difficult to estimate start values for the parameters if they have no biological meaning. Moreover, it is difficult to calculate the 95% confidence intervals for the biological parameters if they are not estimated directly in the equation but have to be calculated from the mathematical parameters. Therefore, all the growth models were rewritten to substitute the mathematical parameters with A, μ_m, and

λ. This was done by deriving an expression of the biological parameters as a function of the parameters of the basic function and then substituting them in the formula. As an example, we show here the modification of the Gompertz equation, which is written as:

$$y = a \cdot \exp[-\exp(b - ct)] \tag{1}$$

To obtain the inflection point of the curve, the second derivative of the function with respect to t is calculated:

$$\frac{dy}{dt} = ac \cdot \exp[-\exp(b - ct)] \cdot \exp(b - ct) \tag{2}$$

$$\frac{d^2y}{dt^2} = ac^2 \exp[-\exp(b - ct)] \cdot \exp(b - ct) \cdot [\exp(b - ct) - 1] \tag{3}$$

At the inflection point, where $t = t_i$, the second derivative is equal to zero:

$$\frac{d^2y}{dt^2} = 0 \quad \rightarrow \quad t_i = b/c \tag{4}$$

Now an expression for the maximum specific growth rate can be derived by calculating the first derivative at the inflection point.

$$\mu_m = \left(\frac{dy}{dt}\right)_{t_i} = \frac{ac}{e} \tag{5}$$

The parameter c in the Gompertz equation can be substituted for by $c = \mu_m e/a$.
The description of the tangent line through the inflection point is:

$$y = \mu_m \cdot t + \frac{a}{e} - \mu_m t_i \tag{6}$$

The lag time is defined as the t-axis intercept of the tangent through the inflection point:

$$0 = \mu_m \cdot \lambda + \frac{a}{e} - \mu_m t_i \tag{7}$$

Using equations 4, 5, and 7 yields:

$$\lambda = \frac{(b-1)}{c} \tag{8}$$

The parameter b in the Gompertz equation can be substituted for by:

$$b = \frac{\mu_m e}{a}\lambda + 1 \tag{9}$$

The asymptotic value is reached for t approaching infinity:

$$t \to \infty: \qquad y \to a \qquad \Rightarrow \quad A = a \tag{10}$$

The parameter a in the Gompertz equation can be substituted for by A, yielding the modified Gompertz equation:

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\} \tag{11}$$

The models with four parameters also contain a shape parameter (ν). Table 2 shows the results for all equations used in this chapter.

TABLE 3. Selection of models based on Schnute (16)

Values of a and b	Model	No. of parameters
$a > 0, b = 0$	Gompertz	3
$a > 0, b < 0$	Richards	4
$a > 0, b = -1$	Logistic	3
$a = 0, b = 1$	Linear	2
$a = 0, b = 0.5$	Quadratic	2
$a = 0, b = 0$	tth power	2
$a < 0, b = 1$	Exponential	3

The modified Stannard equation appears to be the same as the modified Richards equation. The parameters *a* and *b* in the Schnute equation are retained in the modified Schnute equation because they may be used for model selection (Table 3). However, substitution of *a* and *b* in the Schnute equation would result in the modified Richards equation.

Broughall et al. (3) used the Verhulst differential equation (resulting in a logistic curve) at times greater than the lag time and used $N = N_o$ if the time was smaller than the lag time. This relation has no smooth transition from a lag phase to a growth phase. Since all our growth data show such a smooth transition this model was not considered.

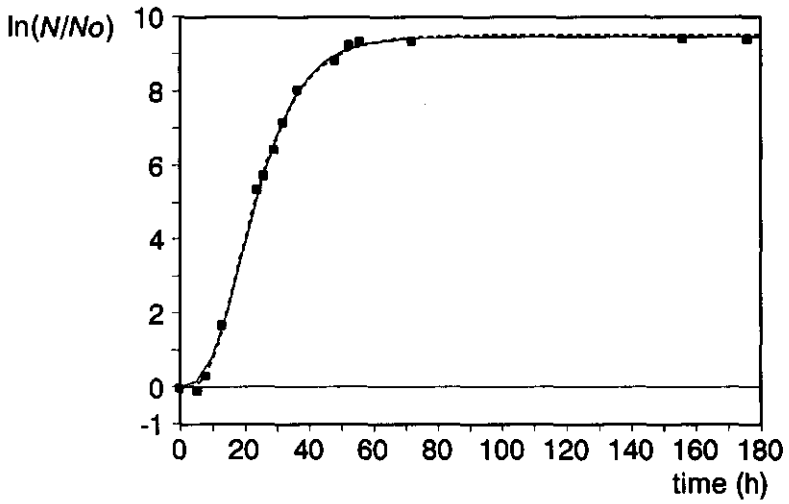

FIG. 2. Growth curve of *L. plantarum* at 18.2°C fitted with the Gompertz (———) and Richards (--------) models.

Fitting of the data. The nonlinear equations were fitted to growth data by nonlinear regression with a Marquardt algorithm (11,13). This is a search method to minimize the sum of the squares of the differences between the predicted and measured values. The program automatically calculates starting values by searching for the steepest ascent of the curve between four datum points (estimation of μ_m) , by intersecting this line with the x axis (estimation of λ), and by taking the final datum point as estimation for the asymptote (A). The algorithm then calculates the set of parameters with the lowest residual sum of squares (RSS) and their 95% confidence intervals.

Model comparison. One way to discriminate among models is to compare them statistically. In that case, the RSS alone does not give enough information because different models can have a different number of parameters. Models with a greater number of parameters usually give a lower RSS. A better method is to determine whether it is worthwhile to use more parameters to lower the RSS. Therefore, data fits obtained by using the various models were compared statistically by the use of the t test and the F ratio test.

t test. First, the data were fitted by the Schnute model and parameters a and b were evaluated. The Schnute model is a comprehensive model; it encompasses all of the other simpler models. This is shown in Table 3, in which values of the Schnute parameters a and b are given, leading to one of the other models. The 95% confidence intervals of the different parameters were calculated with the value given by the Student t test. If, for instance, a value of zero is in the 95% confidence interval of b (and $a > 0$), the Gompertz model is suitable (Table 3).

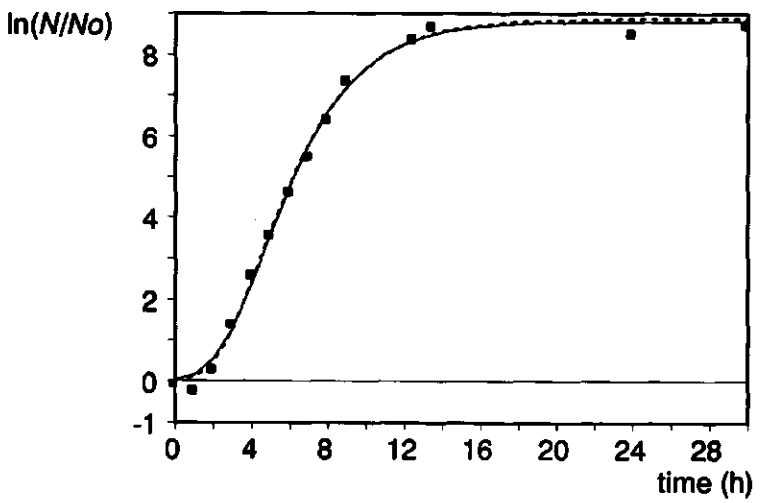

FIG. 3. Growth curve of *L. plantarum* at 35.0°C fitted with the Gompertz (———) and Richards (--------) models.

F test. The logistic, Gompertz, Richards, and Schnute models were used to fit the data, and the RSS was calculated. Under the assumption that the four-parameter Schnute model exactly predicts the number of organisms, the RSS of the Schnute model was taken as an estimate of the measuring error. Whether a three-parameter model would be sufficient to

describe the data could then be validated with an *F* test. In this test, the difference between the RSS values for the three- and four-parameter models was compared to the RSS of the four-parameter model. The difference in RSS of the three- and the four-parameter models is the profit we get from adding one parameter. If this profit is much smaller than the measuring error, as determined from the four-parameter model, adding the extra parameter is not worthwhile, as it would not be observable. If, however, this profit is much greater than the measuring error, it is worthwhile to add the extra parameter. The following is then calculated:

$$f = \frac{(RSS_2 - RSS_1)/(DF_2 - DF_1)}{RSS_1/DF_1} \quad \text{tested against} \quad F_{DF_1}^{DF_2-DF_1} \tag{12}$$

where RSS_1 is the RSS from the Schnute model, RSS_2 is the RSS from the three-parameter model, DF_1 is the number of degrees of freedom from the Schnute model and equals the number of datum points (npoints) - 4, and DF_2 is the number of degrees of freedom from the three-parameter model and equals npoints - 3. Note that $DF_2 - DF_1 = 1$, so the *F* test becomes:

$$f = \frac{RSS_2 - RSS_1}{RSS_1/DF_1} \quad \text{tested against} \quad F_{DF_1}^{1} \tag{13}$$

If the models were linear in their parameters, this *f* value would be *F*-distributed under the assumption that the four-parameter model is correct. Even for nonlinear models, the variance ratio shown above is approximately *F*-distributed when the sample size is large (16). This analysis is an approximation at best, and this procedure should be considered an informal process, rather than a rigorous statistical analysis, because of the use of nonlinear models (16). In some boundary cases, the Student *t* test and the *F* test can therefore give contradictory results.

MATERIALS AND METHODS

In 40 experiments, *Lactobacillus plantarum* (American Type Culture Collection [ATCC]-identified; no ATCC number) was cultivated in MRS medium (Difco Laboratories) at different temperatures. Growth was measured with plate counts on pour plates (MRS medium with 12 g of agar [Agar technical; Oxoid Ltd.] per liter). The inoculation level was 0.01% (about $5 \cdot 10^5$ organisms).

Growth data of *Candida parapsilosis*, *Pseudomonas putida*, *Enterobacter agglomerans*, a *Nocardia* sp., *Salmonella heidelberg*, *Staphylococcus aureus*, and *Listeria monocytogenes* were kindly provided by J.P.P.M. Smelt, C.J.M. Winkelmolen, P. Breeuwer, and F.G.C.T. Sommerdijk.

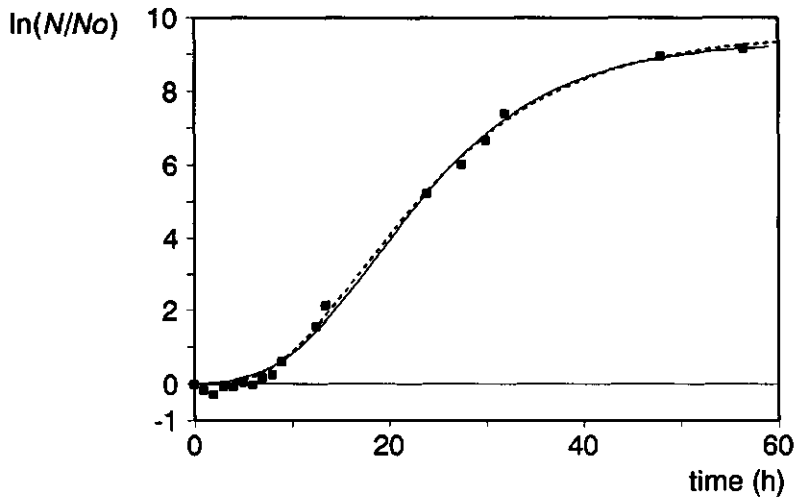

FIG. 4. Growth curve of *L. plantarum* at 18.0°C fitted with the Gompertz (———) and Richards (--------) models.

RESULTS

All the models visually gave reasonably good fits of the data (Fig. 2 and 3, for example). In some cases, the Schnute and Richards models gave some problems with the fitting because the parameter estimates came in an area where the function predicted such a large value that an overflow error resulted. The Gompertz and logistic models never gave problems with fitting. In all cases, the RSS values for the Richards and Schnute models were the same, which was expected because the models are basically the same.

Plate count data for *L. plantarum*. In Table 4, the results of the parameter estimation for 40 sets of data are reported. In this table, the temperature at which the experiment was conducted is given. With the use of the Student t test value, the 95% confidence intervals for the parameters a and b were calculated. The lower 95% confidence limit of the parameter a is given in Table 4 to determine whether $a = 0$ is within the confidence interval. Furthermore,

TABLE 4. Statistical-analytical data for 40 growth curves of *L. plantarum*

T (°C)	a_{min}[ab]	b_{min}[abc]	b_{max}[ab]	f[d]		F	RSS		
				Gom	Log	table	Gom	Log[f]	Richards
6.0	0.003	-0.988	0.994	**0.001**	**0.078**	4.13	1.70	1.70	1.70
6.1	-0.0006	**-1.18**	1.98	**0.194**	**1.49**	4.75	0.282	0.312	0.277
8.3	0.011	-0.113	0.435	**1.91**	53.6	4.21	0.707	1.97	0.660
8.6	0.008	-0.748	0.470	**0.204**	**4.21**	4.33	1.09	1.30	1.08
12.0	0.024	-0.247	0.548	**0.577**	18.4	4.36	1.13	2.11	1.10
12.2	0.023	-0.337	0.672	**4.10**	40.6	4.97	0.424	1.56	0.300
15.1	0.049	-0.786	0.289	**0.875**	5.03	4.45	1.06	1.30	1.00
15.2	0.049	-0.096	0.501	**1.80**	46.2	4.84	0.375	1.67	0.322
18.0	0.065	-0.086	0.540	7.84	49.9	4.60	0.611	2.02	0.391
18.2	0.059	**-4.23**	0.275	**1.45**	**0.485**	4.75	1.39	**1.29**	1.24
18.2	0.076	-0.058	0.471	**3.78**	60.4	4.84	0.233	1.12	0.173
18.2	0.068	-0.192	0.350	**0.301**	30.0	4.31	0.516	1.20	0.509
18.6	-0.607	**-4.72**	6.39	**1.99**	**6.35**	6.60	0.155	0.252	0.111
21.5	-0.322	**-2.04**	2.89	**0.277**	**2.50**	5.99	0.486	0.658	0.464
21.5	0.092	-0.167	0.614	**1.05**	19.2	4.75	0.648	1.55	0.596
25.0	0.116	-0.317	0.786	**3.10**	23.8	4.97	0.800	2.07	0.611
25.0	0.082	-0.147	0.970	**2.44**	16.8	4.84	1.07	2.21	0.876
28.4	0.259	-0.676	0.201	**1.69**	10.0	4.84	0.430	0.714	0.373
28.6	0.252	-0.352	0.183	**0.481**	34.5	4.84	0.210	0.833	0.201
32.0	0.257	**-1.06**	0.432	**1.06**	**2.71**	4.97	0.883	1.01	0.798
32.0	0.260	-0.933	0.367	**1.33**	**4.85**	5.59	0.334	0.475	0.281
32.4	0.272	-0.276	0.328	**0.035**	32.4	4.84	0.241	0.948	0.240
34.9	0.301	-0.544	0.421	**0.099**	13.3	5.59	0.200	0.571	0.197
35.0	0.255	-0.314	0.623	**0.510**	17.0	4.97	0.482	1.24	0.459
35.3	0.257	-0.320	0.455	**0.136**	21.1	4.75	0.471	1.28	0.466
36.6	0.262	-0.207	0.635	**1.08**	22.2	4.67	0.722	1.81	0.667
37.9	0.245	-0.299	0.676	**0.604**	15.4	4.60	0.880	1.77	0.844
38.4	0.264	-0.870	0.487	**0.469**	**5.11**	5.99	0.229	0.394	0.213
40.0	0.247	**-1.73**	0.391	**2.65**	**0.382**	4.84	1.17	**0.974**	0.941
41.4	0.151	**-1.26**	0.713	**0.416**	**1.65**	5.12	0.668	0.756	0.638
41.5	0.043	-0.220	0.855	5.18	29.1	4.75	0.331	0.792	0.231
41.5	0.070	-0.403	1.26	**1.06**	6.45	4.75	1.57	2.22	1.44
41.8	-0.278	-0.641	1.69	**0.899**	**4.76**	6.60	0.209	0.346	0.178
41.9	-1.01	**-4.50**	5.40	**0.464**	**1.49**	7.72	0.844	1.04	0.757
42.1	0.156	-0.152	0.726	**3.31**	26.8	4.67	0.400	0.975	0.319
42.2	0.014	-0.355	1.46	**1.93**	6.15	4.67	1.47	1.89	1.28
42.6	-0.101	-0.443	2.44	**3.20**	**6.11**	6.60	0.633	0.858	0.386
42.8	-7.82	**-52.1**	46.0	**1.79**	**0.395**	5.12	0.633	**0.551**	0.528
42.8	0.315	**-1.09**	0.824	**0.083**	**2.63**	4.75	0.141	0.171	0.140
42.8	-0.061	**-2.20**	1.61	**0.084**	**0.527**	5.12	0.105	0.110	0.104

[a] a and b are Schnute parameters; [b] min and max are 95% confidence limits; [c] Boldface data indicate acceptance of logistic model with t test; [d] Boldface data indicate acceptance of given model with F test; [f] Boldface data indicate that RSS with Gompertz model is greater than RSS with logistic model.

the 95% confidence limits for b are given. Comparing the confidence intervals in Table 4 for b with Table 3 results in Table 5. In Table 3, we can see that if $b = 0$, the Schnute model changes into the Gompertz model, so we accepted the Gompertz model if the value of zero

was within the 95% confidence interval of b. This was true in all cases (Table 4), so the Gompertz model, although a three-parameter model, was accepted in all cases by the t test. Furthermore, the f-testing values for the Gompertz and the logistic models and the F table values are given in Table 4. With the use of the F test, the difference between the RSS values for the three- and four-parameter models was compared to the RSS of the four-parameter model. For the Gompertz model, the f-testing value was lower than the F table value in all but two cases (Table 4).

TABLE 5. Determination of models for *L. plantarum* based on the method of Schnute (16)

Values of a and b	Model	No. (% of total)[a] of results accepted with:	
		t test	F test
$a > 0, b = 0$	Gompertz	40 (100)	38(95)
$a > 0, b < 0$	Richards	40 (100)	
$a > 0, b = -1$	Logistic	11 (28)	17(43)
$a = 0, b = 1$	Linear	8 (20)	
$a = 0, b = 0.5$	Quadratic	8 (20)	
$a = 0, b = 0$	tth power	8 (20)	
$a < 0, b = 1$	Exponential	8 (20)	

[a] Total number of experiments = 40.

In the cases in which the F test favored the Richards model over the Gompertz model (Fig. 4 and 5), the differences between the two models were still very small. The logistic model, however, was accepted by the t test only 11 times (out of 40) and by the F test 17 times (Table 4).

In addition, the RSS values of the Gompertz, logistic, and Richards models are given in Table 4. The RSS values for the four-parameter models were always lower than the RSS values for the three-parameter models. In only three cases, the logistic model gave a lower RSS value than the Gompertz model (Table 4; Fig. 6), but in these cases the Gompertz model still fitted the data acceptably.

In Fig. 7, the confidence intervals for the parameter b (Schnute model with the t test) are shown. In this graph, it can be seen that the value of zero (Table 3, Gompertz model) was always within the confidence interval; however, the value of -1 (Table 3, logistic model) was much less frequently within the confidence interval (only 11 times).

In Fig. 8, the results from the F test for the Gompertz model are shown. The squares represent the f-testing values, and the pluses represent the critical F table values (95%

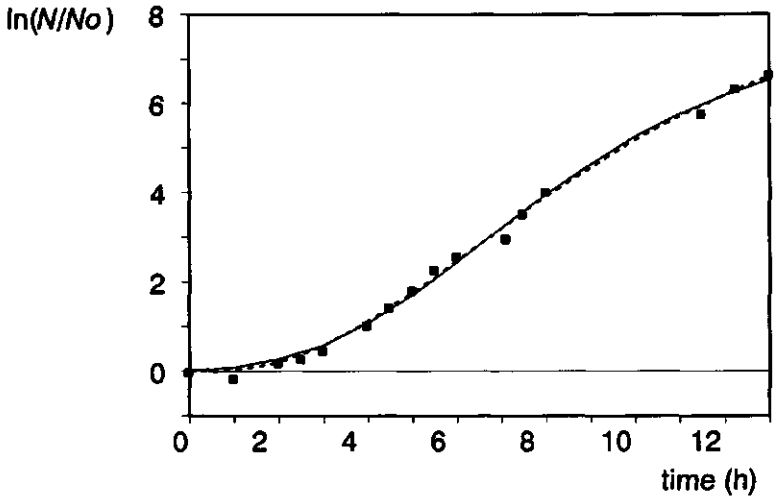

FIG. 5. Growth curve of *L. plantarum* at 41.5°C fitted with the Gompertz (———) and Richards (--------) models.

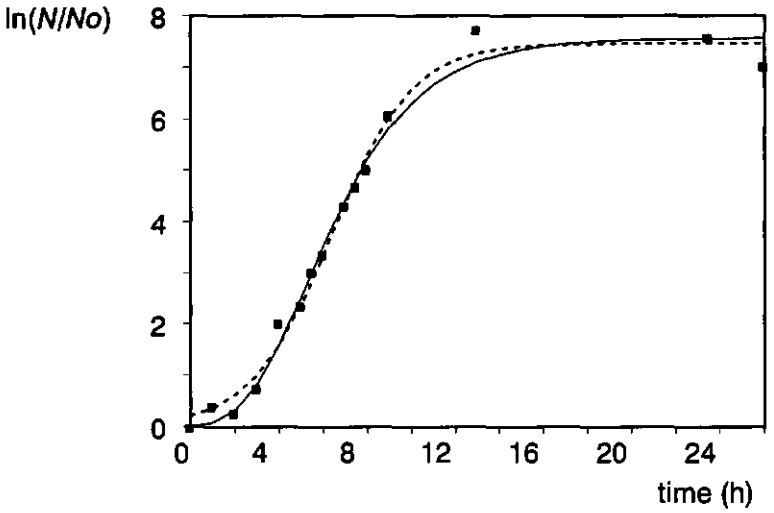

FIG. 6. Growth curve of *L. plantarum* at 40.0°C fitted with the Gompertz (———) and logistic (--------) models.

confidence). If the f-testing value was smaller than the F table value, the three-parameter model was accepted. In this graph, it can be seen that the Gompertz model was rejected only 2 times out of 40 (5%). This 5% rejection level may be expected with a 95% confidence level.

TABLE 6. Statistical-analytical data for 27 growth curves of organisms other than *L. plantarum*

Organism	a_{min} [ab]	b_{min} [abc]	b_{max} [ab]	f [d]		F	RSS		
				Gom	Log	table	Gom	Log[f]	Rich
Candida parapsilosis	0.038	**-1.60**	0.761	**1.71**	**1.11**	10.1	0.114	**0.100**	0.073
C. parapsilosis	0.136	**-1.12**	0.154	**6.67**	**4.86**	10.1	0.086	**0.070**	0.027
C. parapsilosis	0.039	**-1.60**	1.16	**0.071**	**1.07**	10.1	0.102	0.135	0.100
C. parapsilosis	0.117	-0.673	0.751	**0.557**	21.1	10.1	0.030	0.205	0.025
C. parapsilosis	-0.071	**-3.68**	1.70	**3.22**	**0.000**	10.1	0.362	**0.175**	0.175
Pseudomonas putida	0.050	**-2.42**	-0.513	18.7	**1.08**	4.17	4.47	**2.86**	2.76
P. putida	0.027	0.114	1.12	**3.84**	15.3	4.17	6.42	8.60	5.70
P. putida	0.059	-0.169	0.701	**1.25**	16.2	4.13	3.98	5.66	3.83
P. putida	0.037	-0.174	1.54	6.31	17.5	4.16	11.9	15.4	9.85
Enterobacter agglomerans	0.002	0.141	1.10	15.2	41.5	4.14	13.4	20.7	9.19
E. agglomerans	0.015	0.412	0.802	26.7	111	4.17	4.87	12.1	2.58
E. agglomerans	0.020	0.240	0.793	9.26	48.8	4.13	5.07	9.70	3.98
E. agglomerans	0.025	0.432	1.01	17.7	59.9	4.15	7.80	14.4	5.03
Nocardia sp.	0.072	**-2.08**	1.29	**0.540**	**1.21**	5.12	0.176	0.188	0.166
Salmonella heidelberg	-3.18	**-10.9**	12.9	**0.720**	**1.43**	161	1.23	1.74	0.717
Staphylococcus aureus	0.178	-0.759	1.82	**0.843**	**3.86**	6.61	0.526	0.798	0.450
S. aureus	0.009	**-3.73**	5.39	15.2	42.5	6.61	0.543	1.28	0.134
S. aureus	-2.13	**-3.39**	5.39	**1.25**	**1.73**	6.61	1.87	2.01	1.49
S. aureus	-3.60	**-5.07**	7.07	**1.28**	**1.56**	6.61	0.886	0.926	0.706
S. aureus	-0.529	**-2.11**	4.09	**2.86**	**4.66**	6.61	4.54	5.58	2.89
S. aureus	-0.315	**-4.67**	6.44	**1.51**	**3.31**	6.61	0.807	1.03	0.620
S. aureus	0.062	0.235	1.77	10.8	21.6	10.1	1.12	1.99	0.243
S. aureus	0.094	-0.056	2.06	14.4	29.6	10.1	0.873	1.63	0.151
S. aureus	0.452	0.747	1.25	**3.44**	**5.31**	10.1	0.273	0.353	0.127
S. aureus	0.066	-0.512	1.87	**2.62**	**8.59**	10.1	0.628	1.29	0.335
S. aureus	-0.060	**-1.66**	1.81	**0.094**	**3.20**	18.5	0.031	0.078	0.030
Listeria monocytogenes	-16.5	**-171**	172	**0.000**	**0.136**	18.5	0.007	0.007	0.007

[a] a and b are Schnute parameters; [b] min and max are 95% confidence limits; [c] Boldface data indicate acceptance of logistic model with t test; [d] Boldface data indicate acceptance of given model with F test; [f] Boldface data indicate that RSS with Gompertz model is greater than RSS with logistic model.

Plate count data for other organisms. While it could be that only *L. plantarum* growth data are described well by the Gompertz model, the same comparison of models was carried out with growth data from other microorganisms (Tables 6 and 7). With these data, the Gompertz model was accepted in 70% of the cases by the t test (b=0 within the confidence

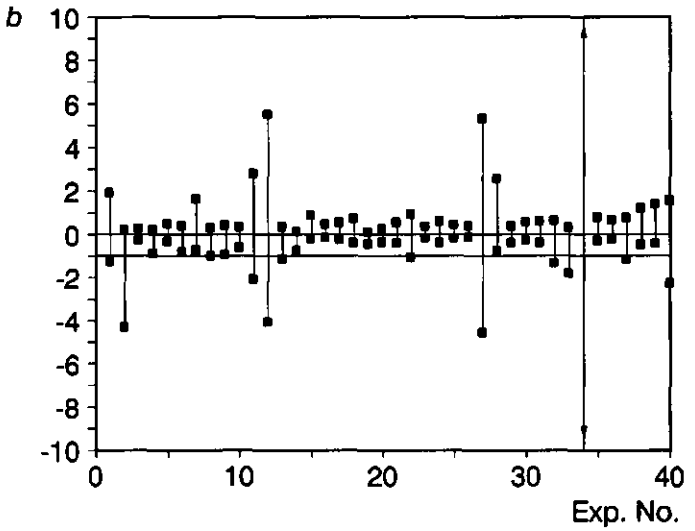

FIG. 7. *b* confidence intervals of *L. plantarum* growth data fitted with the Schnute model (16). Exp. No., Experiment number.

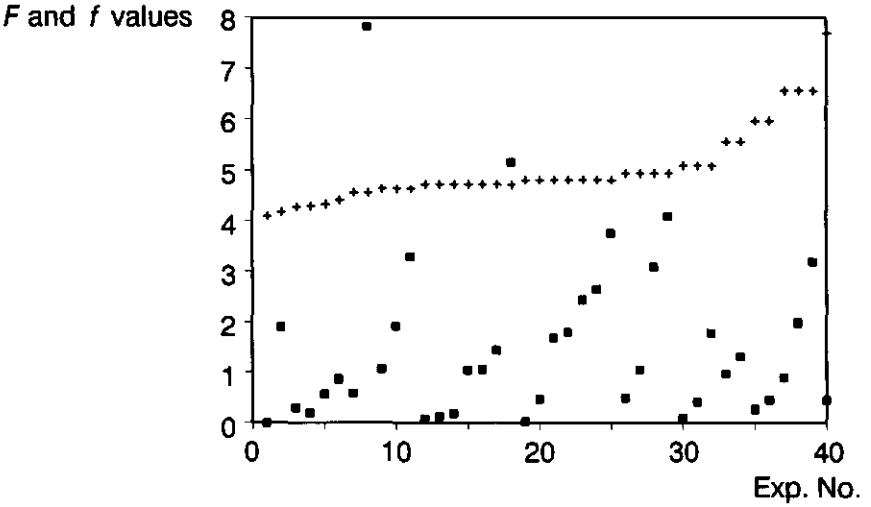

FIG. 8. Results of an *F* test of *L. plantarum* growth data. The Gompertz and Richards models are compared. ▪ *f* value, + *F* value.

interval) and in 67% of the cases by the F test. The logistic model was accepted in 52% of the cases by the t test (b = -1 within the confidence interval) and in 59% of the cases by the F test.

TABLE 7. Determination of models for organisms other than *L. plantarum* based on the method of Schnute (16)

Values of a and b	Model	No. (% of total)[a] of results accepted with:	
		t test	F test
$a > 0, b = 0$	Gompertz	19(70)	18(67)
$a > 0, b < 0$	Richards	20(74)	
$a > 0, b = -1$	Logistic	14(52)	16(59)
$a = 0, b = 1$	Linear	8(30)	
$a = 0, b = 0.5$	Quadratic	8(30)	
$a = 0, b = 0$	tth power	8(30)	
$a < 0, b = 1$	Exponential	8(30)	

[a] Total number of experiments = 27.

DISCUSSION

In order to build models to describe the growth of microorganisms in food, it is necessary to measure growth curves. To reduce the measured data to interesting parameters such as the growth rate, it is recommended that the data be described with a model instead of by using linear regression over a subset of the data. Sigmoidal models to describe the growth data can be constructed with three or four parameters.

We compared several models statistically and found that, for *L. plantarum*, the Gompertz model was accepted in all cases by the t test and was accepted in 95% of the cases by the F ratio test; therefore, the Gompertz model can be regarded as sufficient to describe the growth curves of *L. plantarum*. The logistic model, however, seems not to be sufficient to describe the data. It was accepted in 28% of the cases by the t test and in 43% of the cases by the F test with *L. plantarum*.

With the data of other microorganisms, the Gompertz model was accepted in 70% of the cases. With the data of the other organisms, the logistic model was accepted in 52% of the cases with the t test and in 59% of the cases with the F test. Linear, quadratic, tth-power, and exponential models were accepted in very few cases. Therefore, we can conclude that all growth curves are better fitted with the Gompertz model than with logistic, linear, quadratic, tth-power, and exponential models.

In some cases, the confidence interval of the Schnute parameter b (b_{min}-b_{max}) was very large. In these cases, there were not enough data to describe all three growth phases. Therefore, the confidence level of the resulting parameters is not very high. These sets of data are not very suitable for the estimation of parameters.

In a number of cases, the four-parameter Schnute model was statistically better than the Gompertz model (*P. putida* and *E. agglomerans*). These growth curves contained a very large number of datum points (34 to 38) and with such a large number of datum points the difference in degrees of freedom between three- or four-parameter models is not important (with 34 datum points: 31 degrees of freedom for Gompertz or 30 degrees of freedom for Richards). For the other organisms (*C. parapsilosis* and *S. aureus*), the Gompertz model was accepted in most cases. For the growth curves of the *Nocardia* sp., *Salmonella heidelberg*, and *L. monocytogenes*, the Gompertz model was accepted in all the cases, but only one curve for these organisms was used.

The three-parameter models gave no difficulties in finding the least-square parameters. In almost all the cases, the Gompertz model can be regarded as the best model to describe the growth data. If a three-parameter model is sufficient to describe the data, it is recommended over a four-parameter model because the three-parameter model is simpler and therefore easier to use and because the three-parameter solution is more stable since the parameters are less correlated. Moreover, when a three-parameter model is used, the estimates have more degrees of freedom, which can be important when a growth curve with a small number of measured points is used. Furthermore, it is very important that all three parameters can be given a biological meaning. The fourth parameter in the four-parameter models is a shape parameter and is difficult to explain biologically.

In a number of cases (especially when a large number of datum points are collected), a four-parameter model can be significantly better; therefore, it is recommended that the procedure given in this chapter be carried out with a number of sets of data in order to find out the best model to describe the specific sets of data.

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CHAPTER 3

COMPARISON OF DEFINITIONS OF THE LAG PHASE AND THE EXPONENTIAL PHASE IN BACTERIAL GROWTH

ABSTRACT

Different definitions of the lag time and of the duration of the exponential phase can be used to calculate these quantities from growth models. The conventional definitions were compared with newly proposed definitions. It appeared to be possible to derive values for the lag time and the duration of the exponential phase from the growth models, and differences between the various definitions could be quantified. All the different values can be calculated from the growth parameters μ_m, λ, and A. Therefore, it appeared to be unnecessary to use complicated mathematical equations: simple equations were adequate. For the Gompertz model the conventional definition of the lag time did not differ appreciably from the newly proposed definition. The end-point of the exponential phase and thus the duration of the exponential phase differed considerably for the two definitions. For the logistic model the two definitions lead to considerable differences for all quantities. It is recommended that the conventional definition is used for calculating the lag time. For the duration of the exponential phase it is recommended that the new definition is used. The value can be calculated, however, directly from the conventional growth parameters.

INTRODUCTION

In predictive microbiology, models are used to describe the growth of micro-organisms under different environmental conditions. In order to build these models growth has to be measured and modelled. Bacterial growth often shows a phase in which the growth starts from a zero rate and then accelerates to a maximal value (μ_m) in a certain period of time, resulting in a lag time (λ). In addition, growth curves contain a final phase in which the rate decreases

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and finally reaches zero, so that an asymptotic level (A) is reached. The bacterial growth curve can be described with, e.g. the Gompertz and logistic growth models. Using these models the parameters μ_m, λ, and A can be estimated from growth data (2, 4). The most common way of calculating the duration of the lag time (λ) is extrapolation of the tangent at the inflection point of the growth curve, back to the inoculation level (Fig. 1). After this lag phase the exponential phase sets in. The end of this exponential phase (α) can be defined as the time at which the extrapolation of the tangent in the inflection point reaches the final level (Fig. 1). Buchanan and Cygnarowisz (1) proposed a new definition for calculating the lag time in bacterial growth by deriving the time at which the change of the growth rate is maximal (maximum acceleration of the growth rate). This is given as the time at which the third derivative of the logarithm of the number of organisms with respect to time is zero (Fig. 2). This is an interesting new way of defining the lag time. Moreover, the duration of the exponential phase can be calculated with the same definition, as the difference between the times of maximum acceleration and maximum deceleration, i.e. the time between two zero values of the third derivative.

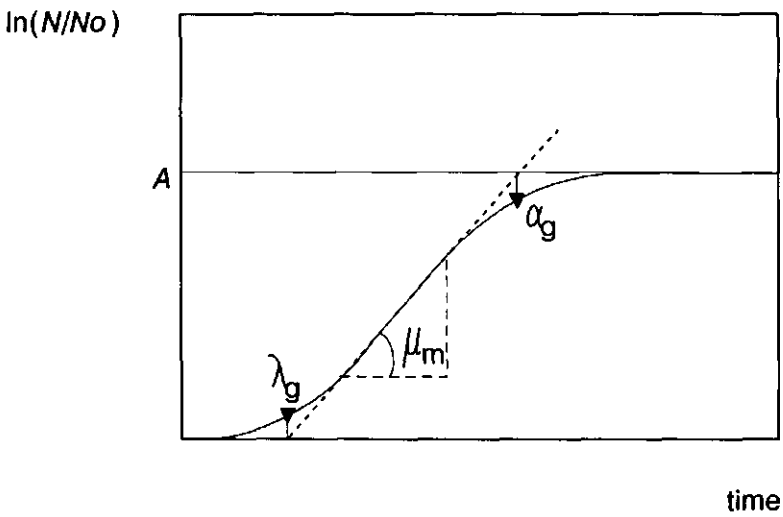

FIG. 1. A growth curve with the parameters μ_m (specific growth rate), λ_g (lag time), α_g (end of exponential phase), and A (asymptote). λ_g is determined as the time where the tangent crosses the starting level, α_g is determined as the time where the tangent crosses the final level.

The determination of the lag phase will be of most interest in the modelling of the growth of food-borne pathogens. Duration of the exponential growth will be more relevant for the modelling of the growth of spoilage organisms, and for defining the growth state of the organisms.

The object of this work was to evaluate differences between different definitions of the lag time, and the duration and the end of the exponential phase.

THEORY

Different definitions of the lag phase. Using a description of the growth curve like the Gompertz or logistics equations, the third derivative can be derived from these equations. Like Buchanan and Cygnarowisz (1) we adopted the form of the Gompertz equation, as used by Gibson et al. (2):

$$y(t) = D + C\exp\{-\exp[-B(t-M)]\} \tag{1}$$

where: $y(t)$ = $\log_{10}$ count at time t, D = log number at $t = -\infty$, C = final log increase in bacterial numbers, M = time at which culture achieves its maximum growth rate (h), B = relative growth rate at time M (1/h), t = time (h).

If we use:
$$\Phi = \exp[-B(t-M)] \tag{2}$$

we get for the Gompertz equation:

$$y(t) = D + C\exp(-\Phi) \tag{3}$$

Using:
$$\frac{d\Phi}{dt} = -B \cdot \Phi \tag{4}$$

the subsequent derivatives may be calculated as:

$$\frac{dy}{dt} = BC\Phi\exp(-\Phi) \tag{5}$$

$$\frac{d^2y}{dt^2} = B^2C\Phi(\Phi-1)\exp(-\Phi) \tag{6}$$

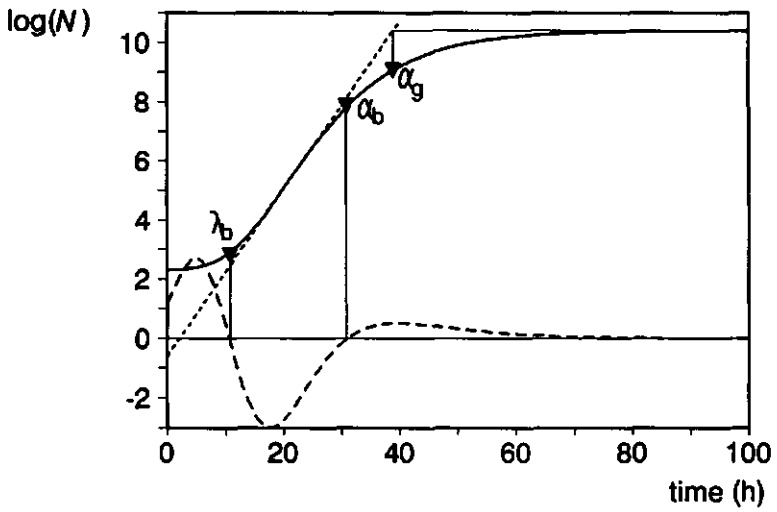

FIG. 2. The Gompertz function (——) with D=2.3, B=0.095, C=8.1, and M=20.8; the third derivative (1000*, — — — —), the newly proposed lag time (λ_b) and end of the exponential phase (α_b). λ_b is determined as the time where the third derivative is zero, α_b is determined as the time where the third derivative is zero for the second time, α_g is determined as the time where the tangent (--------) crosses the final level.

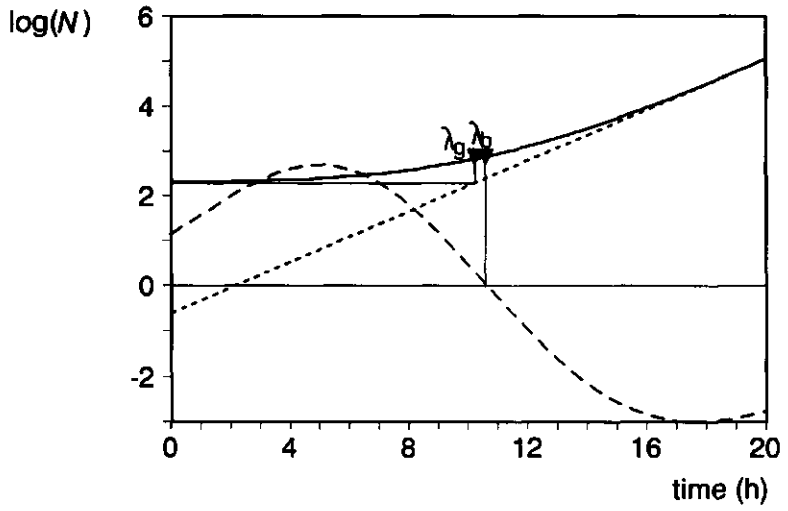

FIG. 3. Beginning of the Gompertz function (——) with D=2.3, B=0.095, C=8.1, and M=20.8. λ_g is determined as the time where the tangent (--------) crosses the starting level, λ_b is determined as the time where the third derivative (1000*, — — — —) is zero.

$$\frac{d^3y}{dt^3} = B^3C\Phi(\Phi^2 - 3\Phi + 1)\exp(-\Phi) \tag{7}$$

The Gompertz equation and its third derivative are shown in Fig. 2. In Fig. 3 the beginning of the Gompertz function is given.

The lag time (λ_b) is now defined as the smallest t-value for which this third derivative is equal to zero (1). The factors Φ and $\exp(-\Phi)$ on the right hand side of equation 7 cannot equal zero. This results in:

$$\exp[-2B(t-M)] - 3\exp[-B(t-M)] + 1 = 0 \tag{8}$$

This can be rewritten as:

$$\left\{\exp[-B(t-M)] - \exp\left[-\frac{B}{2}(t-M)\right] - 1\right\}\left\{\exp[-B(t-M)] + \exp\left[-\frac{B}{2}(t-M)\right] - 1\right\} = 0 \tag{9}$$

Buchanan and Cygnarowisz (1) used this equation to calculate the two zero values of the third derivative of the Gompertz function. They used a root-finding procedure to calculate where one of the two parts of equation 9 is zero. However, equation 8 is a simple quadratic equation, and can thus be solved analytically:

$$\Phi^2 - 3\Phi + 1 = 0 \tag{10}$$

The two solutions are obtained from:

$$\{\exp[-B(t-M)]\}_{1,2} = \frac{3 \pm \sqrt{9-4}}{2} \tag{11}$$

$$t_{1,2} = M - \frac{1}{B}\ln\left(\frac{3 \pm \sqrt{5}}{2}\right) \tag{12}$$

Since the lag time is the root with the smallest time the definition of Buchanan and Cygnarowisz (1) gives the lag time (λ_b) as:

$$\lambda_b = M - \frac{1}{B}\ln\left(\frac{3+\sqrt{5}}{2}\right) = M - \frac{0.96}{B} \tag{13}$$

The conventional way of defining the lag time (λ_g) results in the following equation (2):

$$\lambda_g = M - \frac{1}{B} \tag{14}$$

Equations 13 and 14 show that there is little numerical difference between the two definitions of the lag time.

RESULTS

Comparison of different definitions of the lag phase. The various definitions of the lag time are compared quantitatively in Table 1, by using the parameter values (D, B, C, M) of Buchanan and Cygnarowisz (1) and calculating the values of the different lag times. Each parameter is also doubled and halved to describe the effects in a wider range of parameters.

TABLE 1. Comparison of lag time estimates

Gompertz parameters				Calculated values			
D	B	C	M	μ_m	G.T.	λ_g	λ_b
2.30	0.095	8.10	20.8	0.283	1.063	10.27	10.67
1.15	0.095	8.10	20.8	0.283	1.063	10.27	10.67
4.60	0.095	8.10	20.8	0.283	1.063	10.27	10.67
2.30	0.0475	8.10	20.8	0.142	2.127	-0.253	0.538
2.30	0.190	8.10	20.8	0.566	0.532	15.54	15.74
2.30	0.095	4.05	20.8	0.142	2.127	10.27	10.67
2.30	0.095	16.2	20.8	0.566	0.532	10.27	10.67
2.30	0.095	8.10	41.6	0.283	1.063	31.07	31.47
2.30	0.095	8.10	10.4	0.283	1.063	-0.126	0.269
2.30	0.095	8.10	10.0	0.283	1.063	-0.526	-0.131

D, B, C, M, Gompertz parameters; μ_m, specific growth rate (log count/h), equals BC/e; parameter values of Buchanan and Cygnarowisz (1) are used. Each parameter is also varied to describe the effects in a wider range of parameters; G.T. Generation time (h), equals $\log(2) \cdot e/BC$; λ_g, conventional lag time (h), equals $M - 1/B$; λ_b newly proposed lag time (h), equals $M - 0.96/B$.

As can be seen in Table 1 there are no large numerical differences between the lag times λ_g and λ_b. This can also be seen in Fig. 3, where the λ_g and λ_b are closely together. Since the two definitions show no large differences, it is recommended that the conventional definition (λ_g) is used, as this enables comparison to be made between values from the literature. Moreover, equation 14 is easier to use and to incorporate into the Gompertz model (4), although this could also be done for the newly proposed lag time. Table 1 shows more parameter values resulting in negative values for the lag time, when using the conventional definition (λ_g). This could be an argument in favour of the newly proposed definition. However, in these cases the differences between the two definitions also show small deviations, which will be smaller than the measuring error of determining the lag time. Therefore, in these cases it can be concluded that the lag time will be around zero with both definitions. Equations 13 and 14 show that λ_b and λ_g can be easily recalculated. In conclusion, it is not necessary to use the complicated third derivative numerical procedure if λ is the only quantity of interest.

Buchanan and Cygnarowisz (1) also proposed that the end of the exponential growth phase can be calculated as the point where the third derivative of the growth model becomes zero for the second time (see Fig. 2). We call this the time α_b:

$$\alpha_b = M - \frac{\ln\left(\frac{3-\sqrt{5}}{2}\right)}{B} = M + \frac{0.96}{B} \tag{15}$$

Alternatively, the end of the exponential phase may be defined as the intersection between the tangent in the inflection point of the growth curve and the final level ($D+C$, see Fig. 2). This time α_g can then be calculated with the description of this tangent (tangent method, comparable to conventional definition of λ_g):

$$y = \frac{BC}{e} \cdot (t - M) + D + \frac{C}{e} \tag{16}$$

$$D + C = \frac{BC}{e} \cdot (\alpha_g - M) + D + \frac{C}{e} \tag{17}$$

$$\alpha_g = M + \frac{1.72}{B} \tag{18}$$

Equations 15 and 18 show a significant numerical difference between the two αs. The Gompertz curve is not symmetrical around the inflection point; the curvature at the beginning of the curve is more pronounced than at the end, towards the stationary phase. Therefore, the estimation of α_g gives a higher value than the estimation using the definition of Buchanan and Cygnarowisz (1). This can also be seen in Fig. 2.

ϵ_b, the duration of the exponential phase, can be calculated from equations 15 and 13 as:

$$\epsilon_b = \alpha_b - \lambda_b = -\frac{\ln\left(\frac{3-\sqrt{5}}{2}\right)}{B} + \frac{\ln\left(\frac{3+\sqrt{5}}{2}\right)}{B} = \frac{1.92}{B} \tag{19}$$

and ϵ_g can be calculated from equation 18, and 14 as:

$$\epsilon_g = \alpha_g - \lambda_g = \frac{2.72}{B} \tag{20}$$

It can be seen that there is a large numerical difference between the two definitions of the end of the exponential phase and, with that, also in the duration of the exponential phase. However, $\epsilon_b = 0.71\,\epsilon_g$ and therefore the ϵ values always have the same ratio. Even so, the end-points of the exponential phase α_b and α_g can be calculated if the growth parameters are known. This makes the use of any of the two methods arbitrary. In Fig. 2 it can be seen that for the tangent method, the growth behaviour at the predicted end of the asymptotic phase already differs largely from exponential behaviour.

Comparison of different modifications of the Gompertz equation. Zwietering et al. (4) modified the Gompertz equation, so that the growth parameters μ_m, λ, and A are used instead of mathematical parameters. Since they define a growth curve as $\ln(N/N_{-\infty})$ versus time, the starting value of the logarithmic growth curve is equal to zero. Table 2 gives a conversion table for the various definitions of the Gompertz model, used by Gompertz (3), Gibson et al. (2), and Zwietering et al. (4).

For the modified Gompertz the third derivative can also be calculated (Table 3). With this equation the newly defined lag time and end of the exponential phase can be calculated and compared with the conventional definitions (Table 4). As the equation is written only in a modified form, with the modified Gompertz model of Zwietering et al. (4) we get results comparable with the Gompertz model used by Gibson et al. (2).

TABLE 2. Conversion table of different Gompertz functions

Zwietering et al. (4)	$b = \frac{\mu_m e}{A}\lambda + 1 ; c = \frac{\mu_m e}{A} ; \alpha = A$	Gompertz (3)
$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\}$	$\longleftrightarrow$	$y = \alpha\exp[-\exp(b - ct)]$
	$\lambda = \frac{b-1}{c} ; \mu_m = \frac{Ac}{e} ; A = \alpha$	
Zwietering et al. (4)	$M = \lambda + \frac{A}{\mu_m e} ; B = \frac{\mu_m e}{A} ; D = 0 ; C = A$	Gibson et al. (2)
$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\}$	$\longleftrightarrow$	$y = D + C\exp\{-\exp[-B(t - M)]\}$
	$\lambda = M - \frac{1}{B} ; \mu_m = \frac{CB}{e} ; A = C$	
Gibson et al. (2)	$b = BM ; c = B ; \alpha = C$	Gompertz (3)
$y = D + C\exp\{-\exp[-B(t - M)]\}$	$\longleftrightarrow$	$y = \alpha\exp[-\exp(b - ct)]$
	$B = c ; M = \frac{b}{c} ; D = 0 ; C = \alpha$	

Buchanan and Cygnarowisz (1) proposed that their definition can also be used for other growth models, such as logistic. For the modified logistic equation proposed by Zwietering et al. (4) the third derivative is calculated (Table 3).

For the modified logistic model it can be seen in Table 4 and Fig. 4 that both the lag time and the end of the exponential phase differ using the two different definitions. However, with this equation the value of the lag time proposed by Buchanan and Cygnarowisz (1) can also be calculated analytically from the values found with the modified logistic equation.

The various definitions of the lag time are compared quantitatively in Table 5 for the Gompertz model, by using the parameter values (A, μ_m, and λ_g) and calculating the values of the different lag times. Some of our own growth data of *Lactobacillus plantarum* at different temperatures are given by Zwietering et al. (5).

Some extreme cases are also used in which $A = \ln(10^{10}) = 23$ as a maximum value and $A = 1$ as a minimum value. A maximum value of the growth rate is calculated as a growth rate with a generation time of 20 minutes: $\mu_m = 3\ln(2)$ (1/h). A minimum growth rate is calculated as growth from 1 to 10^{10} organisms during one year: $\mu_m = \frac{23}{24 \cdot 365} = 0.0026$ (1/h).

TABLE 3. Calculation of the third derivative of the modified Gompertz and the modified logistic models

Gompertz equation	Logistic equation:
$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\}$	$y = A\left\{1 + \exp\left[\frac{4\mu_m}{A}(\lambda - t) + 2\right]\right\}^{-1}$
$\Phi = \exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]$	$\Phi = \exp\left[\frac{4\mu_m}{A}(\lambda - t) + 2\right]$
$\frac{d^3y}{dt^3} = \exp(-\Phi)\cdot\frac{(\mu_m e)^3}{A^2}\Phi(\Phi^2 - 3\Phi + 1)$	$\frac{d^3y}{dt^3} = 64\frac{\mu_m^3}{A^2}(1+\Phi)^{-4}\Phi(\Phi^2 - 4\Phi + 1)$
$\Phi^2 - 3\Phi + 1 = 0$	$\Phi^2 - 4\Phi + 1 = 0$
$\lambda_b = \lambda_g + \frac{A}{\mu_m e}\left[1 - \ln\left(\frac{3+\sqrt{5}}{2}\right)\right]$	$\lambda_b = \lambda_g + \frac{A}{4\mu_m}[2 - \ln(2+\sqrt{3})]$
$\alpha_b = \lambda_g + \frac{A}{\mu_m e}\left[1 - \ln\left(\frac{3-\sqrt{5}}{2}\right)\right]$	$\alpha_b = \lambda_g + \frac{A}{4\mu_m}[2 - \ln(2-\sqrt{3})]$

$y = \ln(N/N_-)$; $A = \ln(N_+/N_-)$; μ_m = maximum specific growth rate (1/h); λ = lag time (h); t = time (h).

TABLE 4. Comparison of λ, α, and ϵ using the modified Gompertz and modified logistic model

	Conventional (4)	New definition (1)
Modified Gompertz	λ_g	$\lambda_b = \lambda_g + 0.014\frac{A}{\mu_m}$
	$\alpha_g = \lambda_g + \frac{A}{\mu_m}$	$\alpha_b = \lambda_g + 0.72\frac{A}{\mu_m}$
	$\epsilon_g = \frac{A}{\mu_m}$	$\epsilon_b = 0.71\frac{A}{\mu_m}$
Modified Logistic	λ_g	$\lambda_b = \lambda_g + 0.17\frac{A}{\mu_m}$
	$\alpha_g = \lambda_g + \frac{A}{\mu_m}$	$\alpha_b = \lambda_g + 0.83\frac{A}{\mu_m}$
	$\epsilon_g = \frac{A}{\mu_m}$	$\epsilon_b = 0.66\frac{A}{\mu_m}$

For the lag time a value of zero is chosen as minimum extreme value; this would be found if totally adapted organisms are used. Furthermore, a value of 10 h is arbitrarily chosen for fast-growing organisms, and a value of 1500 h is chosen for slow growth, as maximum extreme values.

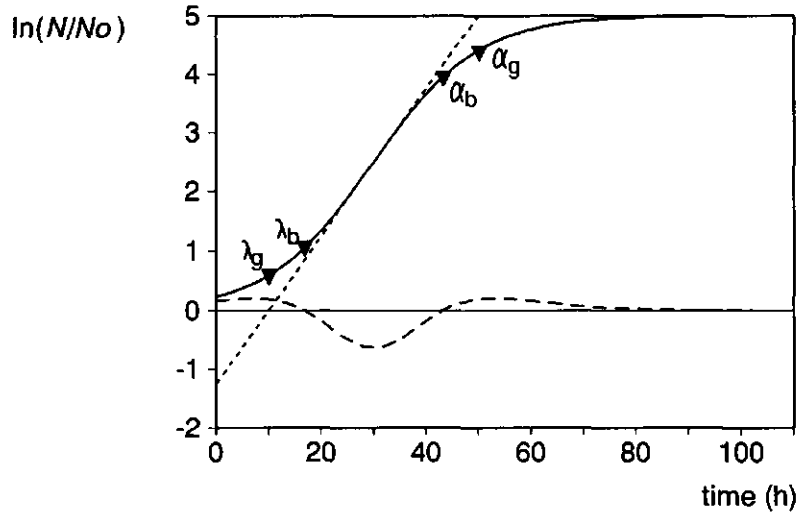

FIG. 4. The modified logistic function (——) with $A=5$, $\mu_m = 0.125$, $\lambda = 10$; the tangent (--------) and the third derivative (1000*, — — — —).

Table 5 shows, for the measured growth data, only small differences between the two definitions of the lag time. Between the αs and also between the ϵs there are significant differences. However, the values of λ_b, α_b, and ϵ_b can all be derived from the growth parameters μ_m, λ_g, and A. For the extreme cases there is also little numerical difference between the two definitions of the lag time, except in the cases of slow growth and zero lag time. The difference between the two definitions, however, is negligible on the time scale of that hypothetical experiment.

DISCUSSION

The newly proposed definition of Buchanan and Cygnarowisz (1) for calculating the duration of the lag time and the exponential phase gives interesting results. For the Gompertz model the duration of the lag phase is almost identical when calculated with the new or the conventional definition, over a large range of parameter values. Therefore, it is recommended

TABLE 5. Comparison λ, α, and ϵ definitions

T (°C)	A	μ_m	λ_g	λ_b	α_g	α_b	ϵ_g	ϵ_b
Data given by Zwietering et al. (5):				Calculated values				
6	7.62	0.016	809.5	816.0	1275	1146	465.5	329.6
15	9.38	0.223	12.88	13.47	54.91	43.23	42.03	29.75
22	9.48	0.538	5.27	5.51	22.88	17.98	17.62	12.47
35	8.81	1.223	2.06	2.16	9.26	7.26	7.20	5.10
40	7.57	0.992	2.44	2.54	10.07	7.94	7.63	5.40
43	4.11	0.145	2.34	2.74	30.79	22.88	28.45	20.14
Hypothetical data:				Calculated values				
	23.0	2.00	0.00	0.16	11.50	8.30	11.50	8.14
	23.0	2.00	10.00	10.16	21.50	18.30	11.50	8.14
	23.0	0.002	0.00	161.0	11500	8303	11500	8142
	23.0	0.002	1500	1661	13000	9803	11500	8142
	1.00	2.00	0.00	0.007	0.500	0.361	0.500	0.35
	1.00	2.00	10.00	10.01	10.50	10.36	0.500	0.35
	1.00	0.002	0.00	7.00	500.0	361.0	500.0	354.0
	1.00	0.002	1500	1507	2000	1861	500.0	354.0

that the conventional definition is used for the lag time, since this is the method used until now and because this method is much simpler. The duration of the exponential phase according to Buchanan and Cygnarowisz (1) differs from the tangent method value. The newly defined length of the exponential phase better covers the part of the growth curve with exponential behaviour (Fig. 2 and 4), and, therefore, seems to give a more realistic value than the tangent definition. Therefore, it is recommended that the end-point and the duration of the exponential phase are calculated with the definition of Buchanan and Cygnarowisz (1). These values can be calculated from the parameters of the modified Gompertz equation. The calculation method used should, therefore, be the simple analytical equations given in this chapter.

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CHAPTER 4

MODELING OF BACTERIAL GROWTH AS A FUNCTION OF TEMPERATURE

ABSTRACT

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

INTRODUCTION

Predictive modeling is a promising field in food microbiology. Models are used to describe the behavior of microorganisms at different physical and chemical conditions, such as temperature, pH, and water activity. They can be used to predict microbial safety or shelf life of products, to find critical points in the process, and to optimize production and distribution chains. A major factor determining the specific growth rate of microorganisms in chilled foods is temperature. Various models have been proposed to describe this relationship. Spencer and Baines (16) proposed a linear dependency of the rate of microbial spoilage of fish on temperature. This relationship was shown to be valid only at temperatures below 6°C (8).

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Modeling of Bacterial Growth as a Function of Temperature
M.H. Zwietering, J.T. de Koos, B.E. Hasenack, J.C. de Wit, K. van 't Riet (1991)
Appl. Environ. Microb. 57:1094-1101

Therefore, Olley and Ratkowsky (8) proposed an Arrhenius-type (2) equation. This equation could predict results up to 15°C. However during cooling, freezing, heating, or thawing, regions in the product can have a temperature far above 15°C, and therefore a wider growth-temperature range is important. Schoolfield et al. (13) proposed a nonlinear Arrhenius type of model on a biological basis, describing the specific growth rate as a function of temperature over the whole biokinetic temperature range. Further empirical models were proposed by Ratkowsky et al. (10,11), i.e., the square root model, describing the specific growth rate up to 15°C, and the expanded square root model, describing the growth rate over the whole biokinetic temperature range. A model which is only seldom used is the model of Hinshelwood (7), although it is a simple model with a biological basis. Adair et al. (1) modeled the growth rate and the inverse of the lag time using the Ratkowsky and Schoolfield models and concluded that the Schoolfield model gives the best predictions.

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

THEORY

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

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

Growth-temperature relations. A number of growth-temperature relations are compared. Included are models from the literature as well as modified forms. The models are all written with the growth rate as a function of temperature (T). Transformation of the growth

rate (square root, logarithm) was not executed, as others tend to do (1,4,9,17), to fit all data in the same way. Using transformations on data results in a different weighting of different numerical values. Using the minimum residual sum of squares (RSS) criterion, one has to take into account that a transformation changes the distribution of errors at different numerical values. If regression without weighting is used, the measuring error must be normally distributed with the same standard deviation at all different T values.

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

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

$$\mu_m = [b_1(T - T_{min1})]^2 \qquad \text{(Ratkowsky 1)} \qquad (2)$$

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

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

$$\mu_m = \left(b_2(T - T_{min2}) \cdot \{1 - \exp[c_2(T - T_{max2})]\} \right)^2 \qquad \text{(Ratkowsky 2)} \qquad (3)$$

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

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

$$\mu_m = [b_3(T - T_{min3})]^2 \cdot \{1 - \exp[c_3(T - T_{max3})]\} \qquad \text{(Ratkowsky 3)} \qquad (4)$$

The subscript 3 relates to Ratkowsky 3.

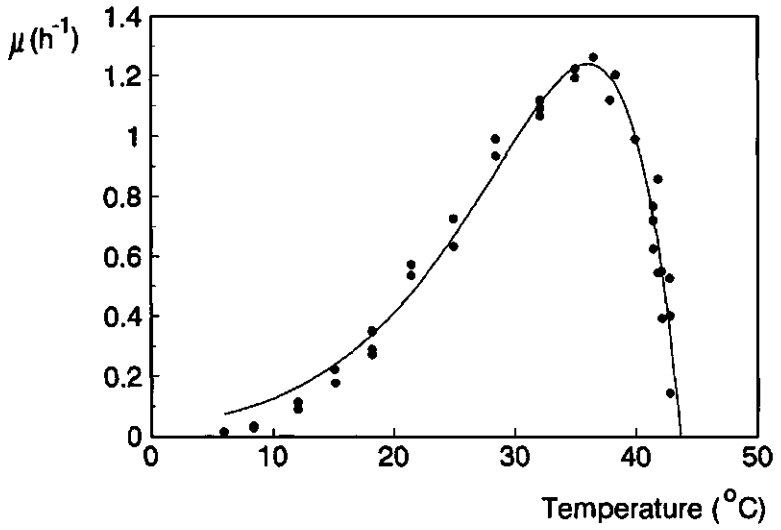

FIG. 1. Growth rates modeled with the Hinshelwood model
(• : estimated growth rate values; ——: model prediction).

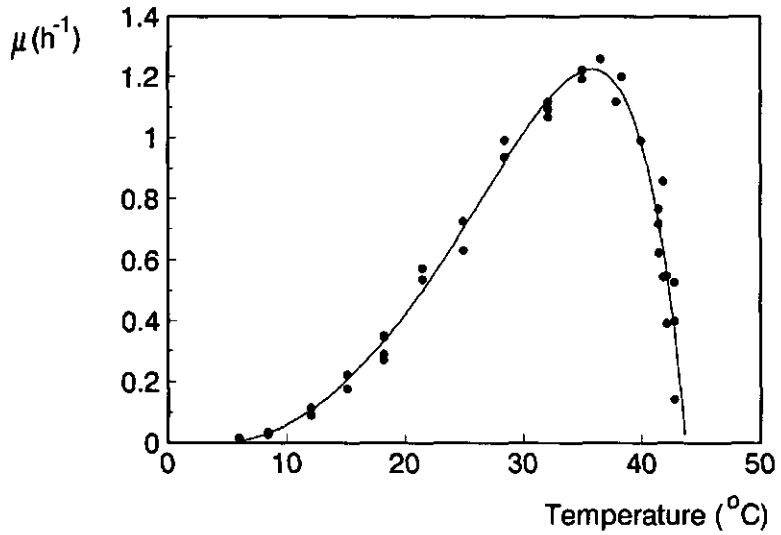

FIG. 2. Growth rates modeled with the Ratkowsky 3 model
(• : estimated growth rate values; ——: model prediction).

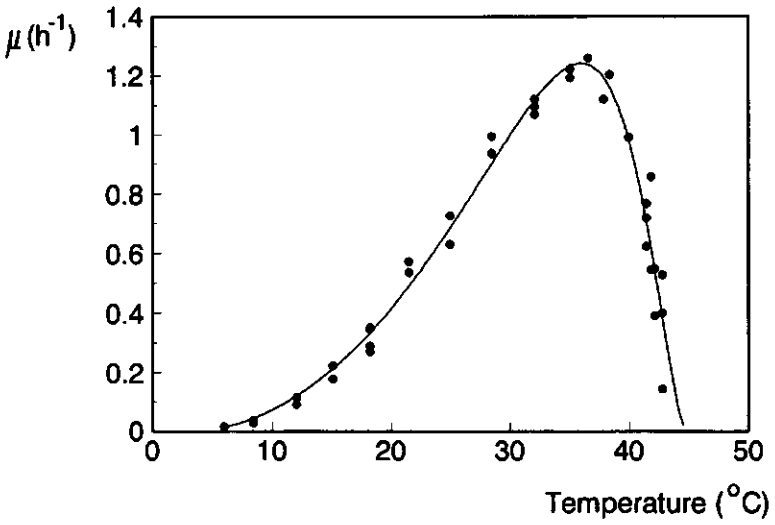

FIG. 3. Growth rates modeled with the Ratkowsky 2 model
(• : estimated growth rate values; ——: model prediction).

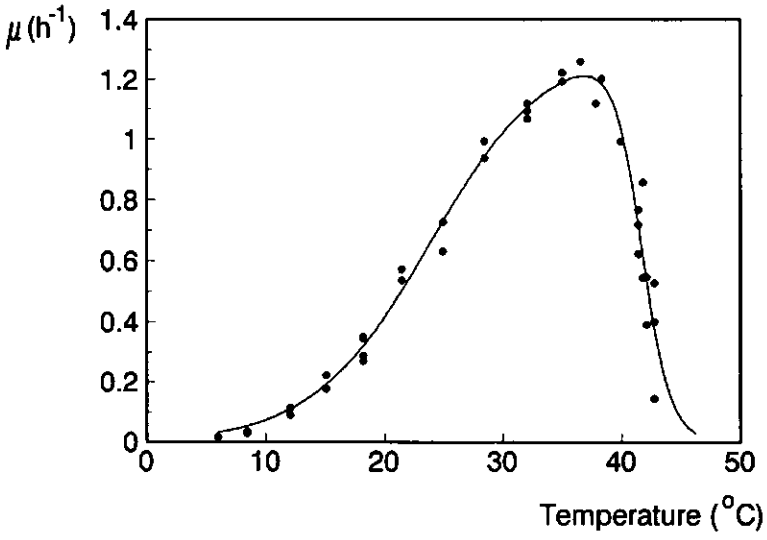

FIG. 4. Growth rates modeled with the Schoolfield model
(• : estimated growth rate values; ——: model prediction).

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

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

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

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

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

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

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

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

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

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

At one temperature T_i (with $i=1$ to $i=18$), m growth rates are measured (duplicates, triplicates...). In our case, m is not the same value at different temperatures. Then the model for the best prediction of a growth rate at a certain temperature can be proposed, that is, defined as the mean value $\bar{\mu}_m(i)$ of the measured growth rates at that temperature. This model is called the general model:

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

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

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

$$A = b_4\{1 - \exp[c_4(T - T_{max4})]\} \tag{9}$$

where b_4 is the final level reached at low temperatures and T_{max4} is the maximum temperature at which growth is observed (°C).

Lag time-temperature relations. Adair et al. (1) modeled the inverse of the lag time data with growth models (Ratkowsky, Schoolfield). By taking the inverse of the data,

numerical values of the lag time that are $\gg 1$ will approach zero after the transformation and are therefore weighted less. Transforming the model predictions back to lag times then results in large prediction errors ($0 \approx 1/8000 \approx 1/800$ but $\infty \neq 8000 \neq 800$). For this reason, we did not use the inverse of the data, but the inverse of the growth rate equations are used as empirical models, to fit the data.

TABLE 1. Comparison of the models describing the growth rate

Model[a]	No. of parameters	DF	RSS_2	f	F
$\mu_m = 0$	0	38	20.793	139.5	2.1
$\mu_m = \alpha$	1	37	5.994	41.74	2.2
$\mu_m = \alpha T$	1	37	3.744	25.63	2.2
$\mu_m = \alpha T + b$	2	36	3.738	27.19	2.2
Hinshelwood	4	34	0.248	**0.729**	2.2
Ratkowsky 3	**4**	**34**	**0.211**	**0.409**	2.2
Ratkowsky 2	4	34	0.220	**0.483**	2.2
Schoolfield	6	32	0.215	**0.516**	2.3
general	18	$20(DF_1)$	$0.164(RSS_1)$		
$\mu_m = \mu_m(i, j)$	38	0	0.000		

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

In this study, the lag time data showed a large measuring error at high numerical values (the standard deviation was proportional to the mean value). To limit this influence, a logarithmic transformation is used on the experimental data and on the model equations. In conclusion, the transformed lag time data are then modeled by using the logarithm of the inverse of the growth rate models. For instance, for the Ratkowsky 2 model (equation 3), we fitted:

$$\ln(\lambda) = \ln\left[\left(b_5(T - T_{min5}) \cdot \{1 - \exp[c_5(T - T_{max5})]\}\right)^{-2}\right] \qquad (10)$$

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

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

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

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

TABLE 2. Results of the Hinshelwood parameter estimation

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

TABLE 3. Correlation matrix for Table 2 parameters

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

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

$$RSS_1 = \sum_{i=1}^{k} \sum_{j=1}^{m} [\mu_m(i,j) - \bar{\mu}_m(i)]^2 \qquad \text{(general model)} \tag{12}$$

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

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

$$RSS_2 = \sum_{i=1}^{k} \sum_{j=1}^{m} [\mu_m(i,j) - \hat{\mu}_m(i)]^2 \qquad \text{(growth-temperature model)} \qquad (13)$$

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

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

$$f = \frac{(RSS_2 - RSS_1)/(DF_2 - DF_1)}{RSS_1/DF_1} \text{ tested against } F_{DF_1}^{DF_2-DF_1} \qquad (14)$$

where DF_1 is the number of degrees of freedom from the general model, that equals the total number of datum points minus the number of different temperatures measured (38-18=20). DF_2 is the number of the degrees of freedom from the growth-temperature model that equals the number of datum points minus the number of parameters.

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

MATERIALS AND METHODS

Microbial experiments. In 38 experiments at 18 different temperatures, *Lactobacillus plantarum* (American Type Culture Collection determined; no ATCC number) was cultivated in MRS medium (Difco Laboratories). The culture was stored frozen (-16°C). The bacteria were cultivated twice at 30°C, first for 24 h and second for 16 h. Growth was monitored by using 20-ml tubes, each containing 10 ml of medium and inoculated with the test organism to

reach a target initial titer of $5 \cdot 10^5$ CFU/ml. The test tubes were incubated statically at different temperatures from 6°C up to 43°C as follows (temperatures in °C and number of experiments in parentheses): 6.0 (1); 8.5 (2); 12.1 (2); 15.2 (2); 18.2 (5); 21.5 (2); 25.0 (2); 28.5 (2); 32.1 (3); 35.1 (3); 36.6 (1); 37.9 (1); 38.4 (1); 40.0 (1); 41.5 (3); 41.9 (2); 42.2 (2); 42.8 (3). At appropriate time intervals (depending on temperature), the inoculated cultures were vortexed and samples of 0.1 ml were removed for serial dilution in peptone saline solution (1 g of Bacto-Peptone [Difco], 8.5 g of NaCl [Merck p.a.] per liter). Bacterial numbers were determined with a pour plate (MRS medium with 12 g of agar [Agar Technical Oxoid Ltd.] per liter). The pour plates were incubated for 48 h at 30°C before counting.

TABLE 4. Results of the Schoolfield parameter estimation

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

TABLE 5. Correlation matrix for Table 4 parameters

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

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

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

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

TABLE 6. Results of the Ratkowsky 2 parameter estimation

Parameter	Estimate	95% Confidence interval	
b_2 :	0.0377	0.0321	0.0433
T_{min2}:	2.82	-0.223	5.86
c_2 :	0.250	0.173	0.326
T_{max2}:	44.9	44.2	45.5

TABLE 7. Correlation matrix of Table 6 parameters

Parameter	b_2	T_{min2}	c_2	T_{max2}
b_2	1.000	0.963	-0.824	0.628
T_{min2}	0.963	1.000	-0.687	0.499
c_2	-0.824	-0.687	1.000	-0.922
T_{max2}	0.628	0.499	-0.922	1.000

RESULTS AND DISCUSSION

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

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

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

Among the four-parameter models, the Hinshelwood model is based on a fundamental model (Arrhenius). In the Hinshelwood model, the parameters are strongly correlated (Table 3). A value of 1 for two parameters in the correlation matrix means that these two parameters are totally correlated with each other. Parameters that are strongly correlated (>0.999) are difficult to estimate, because a change in one parameter will be compensated for by a change in a correlated parameter, and numerous iterations are necessary. Moreover, the confidence intervals of such parameters are very large (Table 2). A second problem with the Hinshelwood model is that the estimates for the activation energies E_1 and E_2 are almost the same value (107.2 and 107.4 kJ). This results in a subtraction of two large values to calculate the growth rate. Reparameterization of the model, however, can possibly reduce these problems. Normally, the activation energy for an enzyme-catalyzed reaction is 10 to 80 kJ, and for a denaturation reaction it is 400 to 1200 kJ (3). Neither estimated activation energy is within these intervals. This means that the fitting of the Hinshelwood relation to the data results in an estimation of unrealistic activation energy values. This makes the biological background of the model discussable. A third problem with the Hinshelwood model is that the predictions of the growth rate at low temperatures are too high. Growth rates at low temperatures are especially important during chilled food storage. Concluding all these aspects, this model can be regarded as not appropriate.

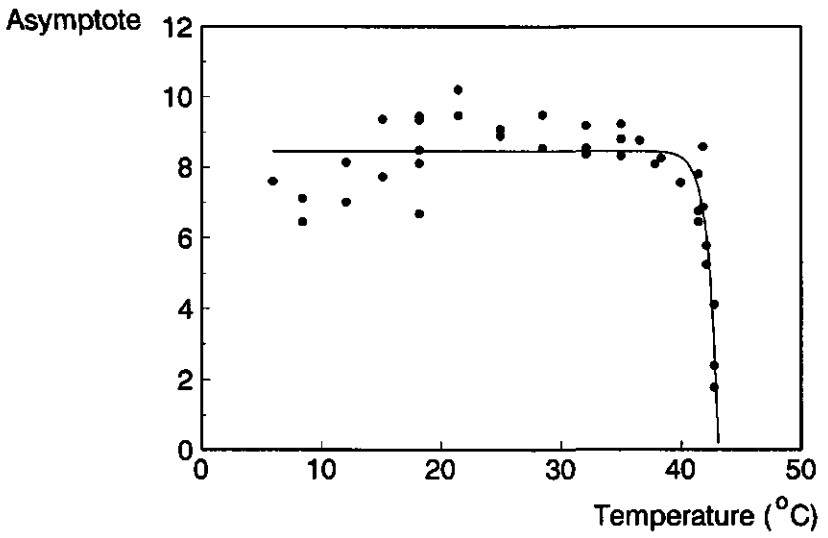

FIG. 5. Asymptote data modeled with the Ratkowsky 4 model
(• : estimated asymptote values; ——: model prediction).

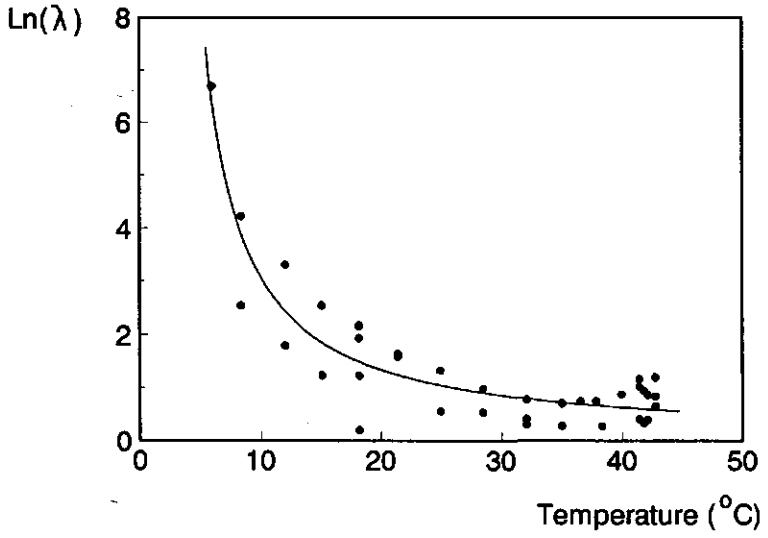

FIG. 6. Lag time data modeled with a hyperbola model
(•: estimated lag time values; ——: model prediction).

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

As can be seen in Tables 7 and 9, the correlation matrices of the Ratkowsky 2 and Ratkowsky 3 models show no nondiagonal values of >0.999, so in these models the parameters can be estimated easily.

TABLE 8. Results of the Ratkowsky 3 parameter estimation

Parameter	Estimate	95% Confidence interval	
b_3 :	0.0410	0.0335	0.0485
T_{min3}:	3.99	0.881	7.11
c_3 :	0.161	0.0940	0.228
T_{max3}:	43.7	43.4	44.1

TABLE 9. Correlation matrix for Table 8 parameters

Parameter	b_3	T_{min3}	c_3	T_{max3}
b_3	1.000	0.960	-0.910	0.591
T_{min3}	0.960	1.000	-0.783	0.466
c_3	-0.910	-0.783	1.000	-0.804
T_{max3}	0.591	0.466	-0.804	1.000

Statistical evaluation of the models shows that the Hinshelwood, Ratkowsky 2, Ratkowsky 3, and Schoolfield models all describe the growth rate data sufficiently. Therefore, an appropriate model can be chosen on the basis of other grounds. The models with the lowest number of parameters (the four-parameter models) were chosen. The Ratkowsky 3 equation has the lowest RSS_2 of all four-parameter models. Therefore, the

Ratkowsky 3 model appeared to be the most suitable to describe the specific growth rate as function of temperature. The RSS_2 of the Ratkowsky 3 model is even smaller than the RSS_2 of the Schoolfield model, although the Schoolfield model has two more parameters.

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

TABLE 10. Comparison of the models describing the asymptote

Model[a]	No. of parameters	DF	RSS_2	f	F
$A = 0$	0	38	2378	183	2.1
$A = a$	1	37	128	9.37	2.2
$A = aT$	1	37	587	47.1	2.2
$A = aT + b$	2	36	111	8.41	2.2
Hinshelwood	4	34	178	16.4	2.2
Ratkowsky 2	4	34	28.7	**1.43**	2.2
Ratkowsky 3	4	34	28.3	**1.39**	2.2
Ratkowsky 4	3	35	31.3	**1.58**	2.2
Schoolfield	6	32	20.4	**0.711**	2.3
General	18	$20(DF_1)$	$14.3(RSS_1)$		
$A = A(i, j)$	38	0	0.0		

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

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

statistically and had the lowest number of parameters (from the models which are accepted), this model was selected (Fig. 5). The parameter estimates of this model are shown in Table 11.

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

$$b_4 = A = \ln(N_\infty / N_o) \tag{15}$$

$$\ln(N_\infty) = b_4 + \ln N_o = 8.46 + \ln(5E5) = 8.46 + 13.12 = 21.58 \tag{16}$$

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

TABLE 11. Results of the Ratkowsky 4 parameter estimation

Parameter	Estimate	95% Confidence interval	
b_4	8.46	8.09	8.82
c_4	1.25	0.709	1.78
T_{max4}	43.1	42.9	43.4

Lagtime-temperature relation. To fit the lag time, a logarithmic transformation was used, because the data showed a larger measuring error at high numerical values (the standard deviation was proportional to the mean value). After the transformation, the distribution of measuring errors at different temperatures was almost the same. Adair et al. (1) fitted the logarithm of the inverse lag time data with the Schoolfield model and the square root of the inverse of the lag time data with the Ratkowsky model. After transforming the model predictions back to lag times, they calculated the RSS_2 between their measured data and the model predictions and they found, for instance, $RSS_{Rat} = 16186$ and $RSS_{School} = 683$. If they would have fitted the logarithm of the lag time data with the logarithm of the inverse of the Ratkowsky model (as it is proposed in this report; equation 10) and transformed back to lag time, they would have found $RSS_{Rat} = 632$. Note that the fitting to the models is done with different models but that the calculation of the RSS values is done comparing lag time data (without transformation) with model data. This is a striking example to show the importance of the choice of the transformation before fitting.

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

TABLE 12. Comparison of the models describing the lag time

Model[a]	No. of parameters	DF	RSS_2	f	F
$\ln(\lambda) = 0$	0	38	127	17.2	2.1
$\ln(\lambda) = a$	1	37	58.6	7.79	2.2
$\ln(\lambda) = aT$	1	37	95.6	13.4	2.2
$\ln(\lambda) = aT + b$	2	36	29.1	3.47	2.2
Hyperbola	**2**	36	**9.70**	**0.325**	2.2
(Ratkowsky 1)$^{-1}$	**2**	36	18.3	**1.71**	2.2
(Ratkowsky 2)$^{-1}$	**4**	34	9.14	**0.267**	2.2
(Ratkowsky 3)$^{-1}$	**4**	34	9.21	**0.282**	2.2
General	18	$20(DF_1)$	$7.70(RSS_1)$		
$\ln(\lambda) = \ln[\lambda(i, j)]$	38	0	0.0		

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

TABLE 13. Results hyperbolic parameter estimation

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

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

If the measured growth data are compared with the model predictions, the resulting model can be evaluated (Fig. 7 to 10). The model describes the data adequately. The growth rate at 6°C and the asymptote at 8.5°C are not very well estimated. The measured growth rate at 6°C is a very small value (0.0164 h^{-1}) and is estimated by the model as 0.00675 h^{-1}. The lag time at 6°C is estimated well, which results in a reasonable prediction of the dynamic behavior over a long period (almost 3 months). The asymptote at 8.5°C is not very well estimated. The reason can be found in the fact that the model prediction at 8.5°C in Fig. 5 is greater than the datum points. All the other predictions (also at the temperatures not presented here) agreed very well with the measured values. The model prediction is usually in between the duplicate or triplicate observations.

TABLE 14. Parameters for models (equation 4), (equation 9) and (equation 11)[a]

Growth rate (μ_m) (equation 4) $[b_3(T-T_{min3})]^2\{1-\exp[c_3(T-T_{max3})]\}$		Asymptote (A) (equation 9) $b_4\{1-\exp[c_4(T-T_{max4})]\}$		Lag time (λ) (equation 11) $\ln(\lambda)=\frac{p}{(T-q)}$	
Parameter	Estimate	Parameter	Estimate	Parameter	Estimate
b_3	0.0410	b_4	8.46	p :	23.9
T_{min3}	3.99	c_4	1.25	q :	2.28
c_3	0.161	T_{max4}	43.1		
T_{max3}	43.7				

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

For the parameter b_4, 21.58-$\ln(N_o)$ must be used if another inoculation level is used.

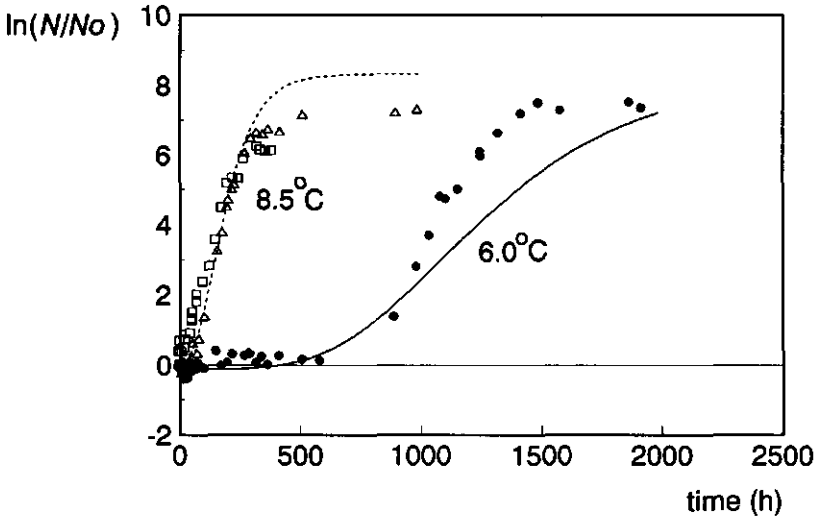

FIG. 7. Growth data and total model at 6.0 (solid symbols) and 8.5°C (open symbols). Different symbols indicate different duplicates.

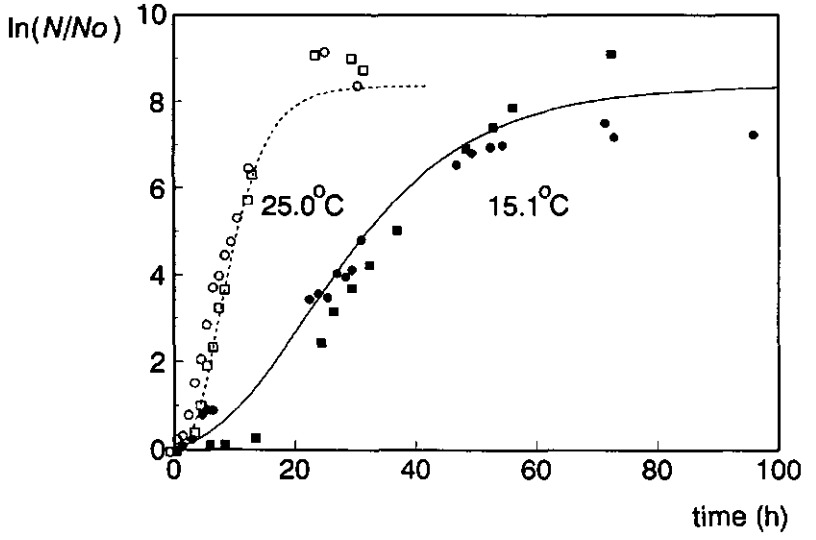

FIG. 8. Growth data and total model at 15.1 (solid symbols) and 25.0°C (open symbols). Different symbols indicate different duplicates.

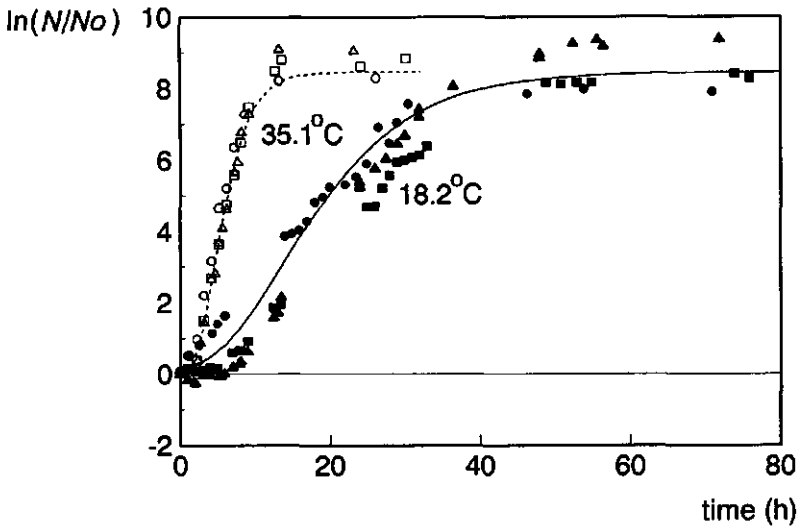

FIG. 9. Growth data and total model at 18.2 (solid symbols) and 35.1 °C (open symbols). Different symbols indicate different duplicates.

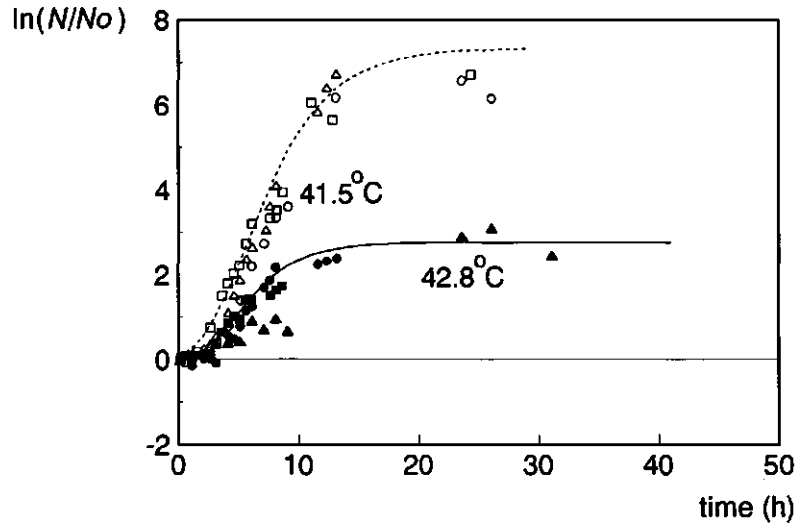

FIG. 10. Growth data and total model at 41.5 (open symbols) and 42.8 °C (solid symbols). Different symbols indicate different duplicates.

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

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CHAPTER 5

EVALUATION OF DATA TRANSFORMATIONS AND VALIDATION OF MODELS FOR THE EFFECT OF TEMPERATURE ON BACTERIAL GROWTH

ABSTRACT

The temperature of chilled foods is an important variable for controlling microbial growth in a production and distribution chain. Therefore, it is essential to model growth as a function of temperature in order to predict the number of organisms as a function of temperature and time. This paper deals with the correct variance-stabilising transformation of the growth parameters A, μ, and λ. This is of eminent importance for the regression analysis of the data. A previously gathered data set is extended with new data. With the total data set (original and new data), an analysis of variance is carried out to determine which transformation should precede fitting. For the asymptote data no, for the growth rate a square root, and for the lag time a logarithmic transformation was found to be appropriate. Model corrections were made and model parameters were estimated using the original data. The models were validated with the new data. The predictions of the models for μ and λ were adequate. The model for A showed a significant deviation, therefore a new model for A is proposed. Finally, the model parameters were updated using the total data set.

INTRODUCTION

Temperature is a major factor determining the progress of many food deterioration reactions. For microbial spoilage the effect of temperature on the specific growth rate and the lag phase is important. Various models to describe the effect of temperature are used (7). Models often are compared only to the data on which the model is fitted (measured versus

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fitted) and are only rarely validated with new data (measured versus predicted). Yet, such validation can provide useful information about the accuracy and predicting value of the models.

The effect of temperature on growth rate is modeled often after a transformation (square root or logarithm). This transformation, however, changes the distribution of errors. Unweighted regression may only be performed if the variance is equally distributed with temperature. Therefore, it is of great importance to determine which type of transformation gives a temperature-independent variance. Ratkowsky (3) used multiple measurements at each temperature to calculate the variance. He advises to use a square-root transformation to stabilize variance for the growth rate [or (generation time)$^{-0.5}$] and a logarithm for the lag phase duration. Alber and Schaffner (1) used the in-experiment error (no replicates) to calculate the variance and recommended to use a logarithmic transformation to stabilize the variance of the growth rate. In our former paper (7) the growth rate and the asymptote were modeled without transformation since the variance seemed to be equally distributed in that particular data set. The lag time was fitted after a logarithmic transformation, since this transformation smoothed the variance.

It will be clear that different opinions do exist up till now. This is largely due to the rather small data sets that were used. In order to estimate the variance at least duplicate measurements at each temperature are needed. With an extensive data set it can be examined with which transformation the variance is stable over a large temperature interval.

THEORY

Description of the experimental bacterial growth rate data. Growth curves are defined as the logarithm of the relative population size [$\ln(N/N_o)$] as a function of time. A sigmoidal growth model (modified Gompertz) with three parameters can describe the growth curve (6), at a given temperature.

$$\ln(N/N_o) = A\exp\left\{-\exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\} \qquad (1)$$

with A = Asymptotic level $\ln(N_\infty/N_o)$, μ_m = maximum specific growth rate (h^{-1}), λ = lag phase duration (h), and $e = \exp(1)$.

Analysis of Variance. The variances of A, μ_m, and λ are calculated at different temperatures using the mean values of the measured data. At one temperature T_i, m_i replicate curves are measured. In our case, m_i does not have the same value at different temperatures. Then the model for the best prediction of the y-value (A, μ, or λ) at a certain temperature can be proposed, that is defined as the mean value $\bar{y}(i)$ of the measured y-values at that temperature. This model is called the general model:

$$\bar{y}(i) = \sum_{j=1}^{m_i} \frac{y(i,j)}{m_i} \tag{2}$$

with $y = A, \mu$, or λ; $y(i,j)$ being the j^{th} y-value at T_i, and $\bar{y}(i)$ being the mean y-value at T_i.

The variance at temperature T_i is calculated at those temperatures for which more than one observation is obtained with:

$$\begin{aligned} RSS_i &= \sum_{j=1}^{m_i} [y(i,j) - \bar{y}(i)]^2 \\ s_i^2 &= \frac{RSS_i}{DF_i} \end{aligned} \tag{3}$$

with RSS_i the Residual Sum of Squares at T_i; DF_i the Degrees of Freedom at T_i (equals m_i-1); and s_i^2 the residual variance at T_i.

According to Ratkowsky (3) the variance can be plotted against the mean value, as well as the variance divided by the mean, the square of the mean, and the cube of the mean, in order to determine the appropriate transformation. If the variance is dependent on the mean, models should be fitted after transforming the data or by using non-normal error assumptions. If the variance divided by the mean shows no correlation a square root transformation is suitable [$var(\sqrt{y_i}) = var(y_i)/4y_i$]. If the variance divided by the square of the mean shows no correlation a logarithmic transformation is suitable to correct for heterogeneity of variance [$var(\ln[y_i]) = var(y_i)/y_i^2$].

As an alternative procedure the variance is calculated after carrying out the transformation (the variance of the transformed data). Then the variance of the untransformed data, the square root and the logarithm of the data are plotted against the mean.

To quantify correlation (for both above mentioned methods) linear regression is carried out and the correlation coefficient is calculated. With a t test it can be examined if there is a correlation:

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}} \tag{4}$$

with t = t-testing value; r = correlation coefficient; n = number of observations.

It should be noted that linear regression is used, although the relations will not be linear. This gives a global indication of the correlation and not an exact value. Visual inspection of the variance data is also crucial.

Growth/temperature relations. The previously proposed models for the effect of temperature on the asymptote (A), specific growth rate (μ_m), and lag time (λ) with parameter values are given in Table 1.

TABLE 1. Parameter values and models for the effect of temperature on the asymptote (A), growth rate (μ_m) and lag time (λ) for *Lactobacillus plantarum* in MRS-medium [from Zwietering et al. (7)]

Asymptote, Ratkowsky 4	b_4	8.46[a]
$A = b_4\{1 - \exp[c_4(T - T_{max4})]\}$	c_4	1.25
	T_{max4}	43.1
Specific growth rate, Ratkowsky 3	b_3	0.0410
$\mu_m = [b_3(T - T_{min3})]^2\{1 - \exp[c_3(T - T_{max3})]\}$	T_{min3}	3.99
	c_3	0.161
	T_{max3}	43.7
Lag time, hyperbola	p	23.9
$\ln(\lambda) = \frac{p}{(T - q)}$	q	2.28

[a] Parameter b_4 depends on the inoculation level according to the equation $b_4 = 21.58 - \ln(N_o)$.

The hyperbolic model for the lag time has the complication that the lag time at higher temperature approaches asymptotically to one (the logarithm of the lag time approaches zero). This is of course an arbitrary value, and it is independent of the unit in which the lag time is expressed. This is an undesirable imperfection of the hyperbolic model. Furthermore, it can

be assumed that the lag phase increases at temperatures higher than optimum, which can also be seen in the data. The hyperbolic model, however, does not show such behaviour. Therefore, the previously proposed reciprocal of the Ratkowsky model (equation 5) is reconsidered, since this model overcomes these two problems. However, this model contains four parameters. This can be overcome by assuming T_{min} and T_{max} values, and eventually also the c value to be equal to the parameters of the equation describing the growth rate.

$$\ln(\lambda) = \ln\left[\left(b_S(T - T_{minS}) \cdot \{1 - \exp[c_S(T - T_{maxS})]\}\right)^{-2}\right] \tag{5}$$

Comparison of the models. The models are validated statistically with the use of the F ratio test. With the general model (equation 2), the measuring error is estimated by determining the deviation of the measured values from the mean value at one temperature. The sum of squares of the deviations between the data and the general model is calculated for all temperatures (RSS_g):

$$RSS_g = \sum_{i=1}^{k} RSS_i = \sum_{i=1}^{k} \sum_{j=1}^{m_i} [y(i,j) - \bar{y}(i)]^2 \qquad \text{(general model)} \tag{6}$$

with $y(i,j)$ being the j^{th} y-value at T_i and $\bar{y}(i)$ being the mean y-value at T_i.

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

$$RSS_m = \sum_{i=1}^{k} \sum_{j=1}^{m_i} [y(i,j) - \hat{y}(i)]^2 \qquad \text{(growth-temperature model)} \tag{7}$$

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

RSS_m will always be larger than RSS_g. The RSS_m of the growth-temperature model consists of both the measuring error and the lack of fit; therefore, the difference between the RSS_m of the model and the RSS_g (the SS due to the measuring error) is calculated as an estimate of the lack of fit. If the mean square of the lack of fit $[(RSS_m\text{-}RSS_g)/(DF_m\text{-}DF_g)]$ is of the same order of magnitude as the mean square of the measuring error (MS_{error}), the model is adequate. This comparison between the lack of fit and the measuring error can be quantified statistically by the f testing value:

$$f = \frac{(RSS_m - RSS_g)/(DF_m - DF_g)}{MS_{error}} \text{ tested against } F^{DF_m - DF_g}_{DF_{error}} \quad (8)$$

where DF_g is the number of degrees of freedom due to the residual variance, that equals the total number of observations minus the number of different temperatures at which is measured; DF_m is the number of the degrees of freedom from the growth-temperature model that equals the number of observations minus the number of parameters. MS_{error} = the mean square of the measuring error with DF_{error} degrees of freedom.

MATERIALS AND METHODS

Microbial experiments. In 60 experiments at 17 different temperatures, *Lactobacillus plantarum* (American Type Culture Collection determined; no ATCC number) was cultivated in MRS medium (Difco Laboratories). The culture was stored frozen (-16°C). The bacteria were cultivated twice at 30°C, for 24 h and for 16 h. Growth was monitored in 20 ml tubes, each containing 10 ml of medium and inoculated with the test organism to reach a target initial titer of $5 \cdot 10^5$ CFU/ml. The test tubes were incubated statically at different temperatures from 6°C up to 40°C as follows (temperatures in °C and number of experiments in parentheses): 6.0 (1); 8.9 (1); 9.8 (7); 10.0 (5); 11.9 (1); 14.0 (1); 14.9 (6); 15.2 (5); 16.7 (3); 18.2 (1); 19.8 (6); 20.2 (7); 24.8 (7); 25.0 (6); 30.0 (1); 34.9 (1); 40.8 (1). At appropriate time intervals (depending on temperature), the inoculated cultures were vortexed and samples of 0.1 ml were removed for serial dilution in peptone saline solution (1 g of Bacto-Peptone [Difco], 8.5 g of NaCl [Merck p.a.] per liter). Bacterial numbers were determined with a pour plate (MRS medium with 12 g of agar [Agar Technical Oxoid Ltd.] per liter). The pour plates were incubated for 48 h at 30°C before counting.

Fitting of the data. The model equations were fitted to the data by nonlinear regression.

RESULTS AND DISCUSSION

Analysis of variance. The previously measured data set [38 growth curves, from Zwietering et al. (7)] was extended with new data (60 growth curves). From this total data set (98 growth curves) only the sub-set of growth parameters up to 35°C (80 growth curves, at 17 different temperatures) was used to determine the variance at different temperatures. This sub-set is used since above the optimum temperature (35°C) the variances are much

larger than below the optimum, since the growth decreases rapidly, resulting in large errors. With this sub-set the variance is analysed to find which transformation: none, a square root one, or a logarithmic one (ln), is necessary. The variance at different temperatures as function of the mean value of the variable is given in Fig. 1, 2, and 3. Furthermore, the variance divided by the mean, divided by the square of the mean, and divided by the cube of the mean are given. For these data the correlation coefficient was determined by performing a linear regression of the variance data. With the *t* test it was determined if correlation was significant. The results are given in Table 2.

Additionally, the data are transformed (with a square-root, a logarithmic, and a reciprocal root transformation) and the variances of the transformed data are calculated. Also with these data a linear regression is performed. These regression data with *t* testing value as well are given in Table 2.

TABLE 2. Results of determination of the correlation coefficient by performing a linear regression of the variance data as function of the mean of the data

A	r^a	t value[b]	Transformation	r^a	t value[b]
var	0.0305	**0.118**	A	0.0305	**0.118**
var/mean	-0.00440	**-0.0171**	$\sqrt{A}$	-0.0178	**-0.0689**
var/mean^2	-0.0442	**-0.171**	$\ln(A)$	-0.0686	**-0.266**
var/mean^3	-0.0900	**-0.350**	$1/\sqrt{A}$	0.123	**0.478**
μ	r^a	t value[b]	Transformation	r^a	t value[b]
var	0.671	3.51	μ	0.672	3.51
var/mean	0.0375	**0.145**	$\sqrt{\mu}$	0.0176	**0.0681**
var/mean^2	-0.357	**-1.48**	$\ln(\mu)$	-0.671	-3.51
var/mean^3	-0.299	**-1.22**	$1/\sqrt{\mu}$	0.848	6.19
λ	r^a	t value[b]	Transformation	r^a	t value[b]
var	0.998	60.9	λ	0.998	60.9
var/mean	0.978	18.1	$\sqrt{\lambda}$	0.975	17.0
var/mean^2	0.146	**0.573**	$\ln(\lambda)$	0.331	**1.36**
var/mean^3	-0.238	**-0.949**	$1/\sqrt{\lambda}$	0.321	**1.31**

[a] correlation coefficient. [b] The 95% critical *t* value for 15 degrees of freedom is 2.13. Bold values indicate no significant correlation.

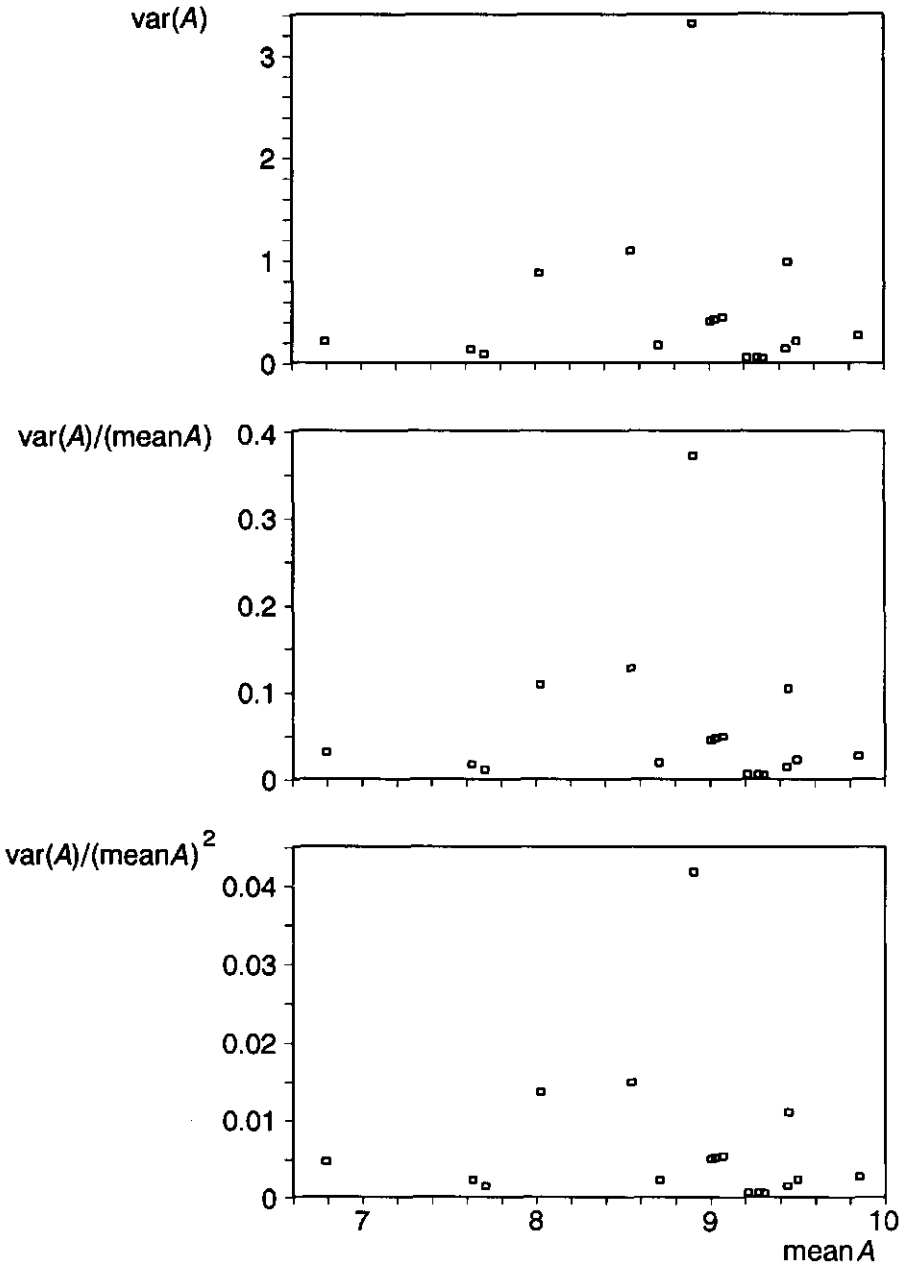

FIG. 1. Variance of A, variance divided by the mean and divided by the square of the mean, plotted against the mean of A.

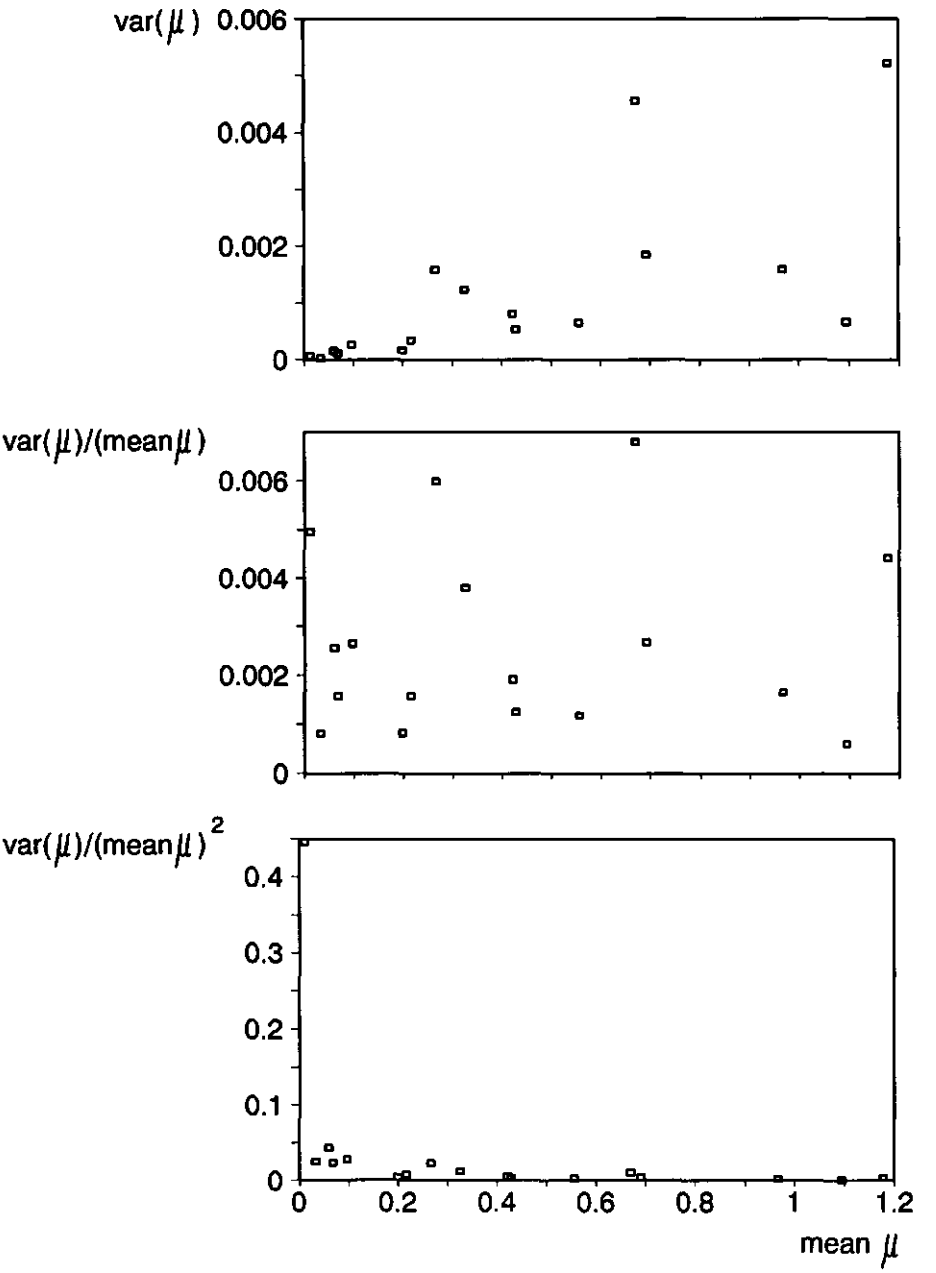

FIG. 2. Variance of μ, variance divided by the mean and divided by the square of the mean, plotted against the mean of μ.

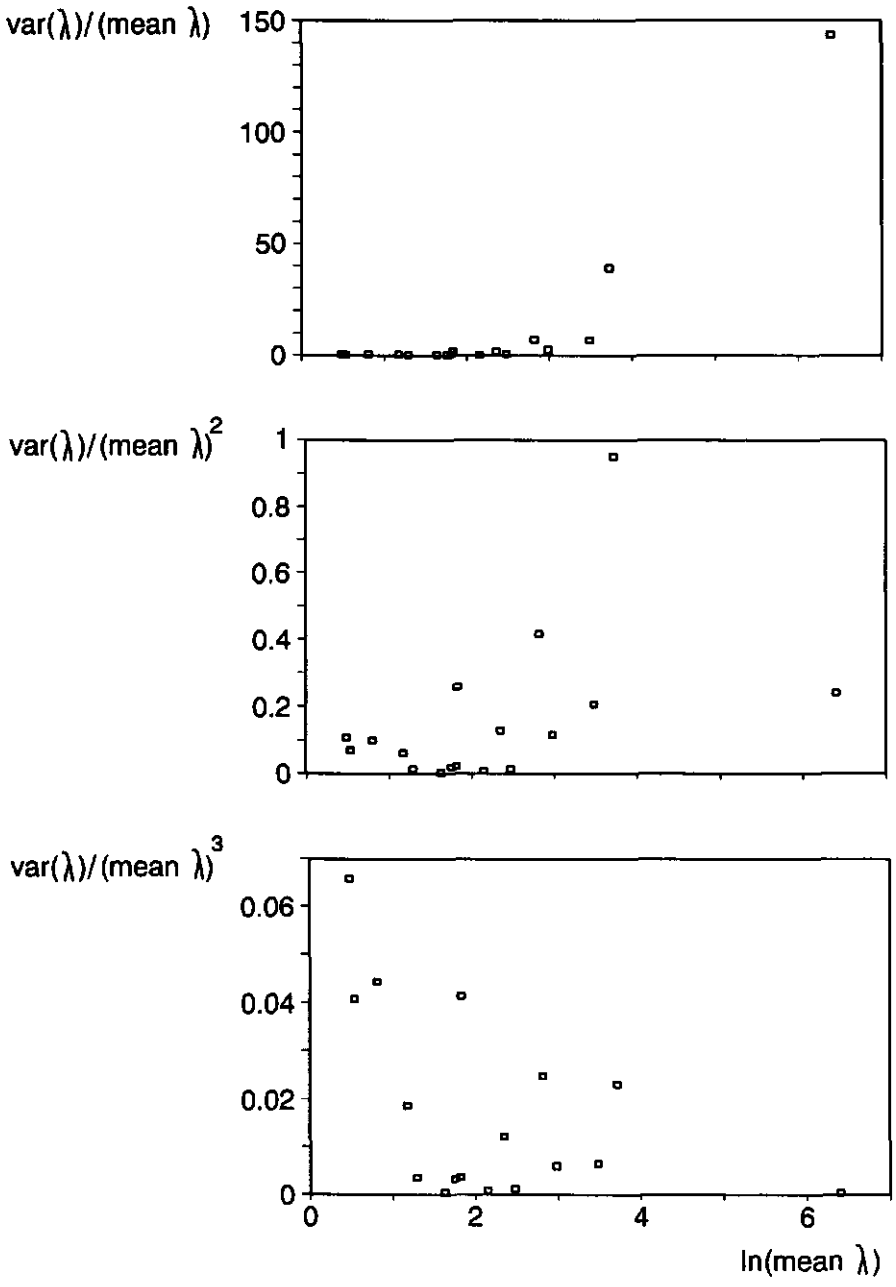

FIG. 3. Variance of λ divided by the mean and divided by the square and the cube of the mean, plotted against logarithm of the mean of λ.

Fig. 1 shows that the variance of the asymptote gives no clear correlation with the mean. This holds also for the variance divided by the mean, divided by the square of the mean, and divided by the cube of the mean. In Table 2 it can be seen that with both methods for all cases no significant correlation is found. Therefore, it can be concluded that transformations do not stabilize the variance and no transformation should be used for the asymptote data.

In Fig. 2 it can be seen that the variance of the growth rate shows a positive correlation with the mean. The variance divided by the mean shows no clear correlation, and the variance divided by the square of the mean shows a negative correlation. In Table 2 it can be seen that the variance of the growth rate gives a significant correlation with the mean. The variance divided by the mean, the variance divided by the square of the mean, and divided by the cube of the mean, show no significant correlation. If the growth rate data are transformed all cases give a significant correlation, except for the square root transformation. Therefore, the square root transformation is chosen to stabilize the variance of the growth rate data.

In Fig. 3 it can be seen that the variance of the lag time divided by the mean shows a positive correlation with the logarithm of the mean (because of the large range of lag-time values a logarithmic transformation is used in these graphs). The variance divided by the square of the mean shows no, and the variance divided by cube of the mean, shows a negative correlation. In Table 2 it can be seen that the variance, and the variance divided by the mean show a significant correlation. If the lag time data are transformed, no transformation and a square root transformation give a significant correlation. The logarithm and reciprocal root transformation give no significant correlation. Concluding, the logarithmic transformation is chosen to stabilize the variance of the lag time data.

TABLE 3. Results of the Ratkowsky parameter estimation for the $\sqrt{\mu}$ data

Parameter	Estimate	95% Confidence interval	
b_2	0.0385	0.0343 to 0.0427	
T_{min2}	3.37	1.60 to 5.13	$\sqrt{\mu_m} = b_2(T - T_{min2})\{1 - \exp[c_2(T - T_{max2})]\}$
c_2	0.256	0.175 to 0.336	
T_{max2}	44.7	44.1 to 45.4	

	No. of parameters	DF	RSS	MS	f	F
Ratkowsky	4	34	0.125	0.00367		
LOF		14	0.023	0.00162	0.317	2.2
General	18	20	0.102	0.00511		

LOF= Lack of Fit; DF= Degrees of Freedom; RSS= Residual Sum of Squares; MS= Mean Square; f= $MS_{LOF}/MS_{general}$; F= F table value (95% confidence).

Model update. Now that with the analysis of variance the correct transformations are found, the previously proposed models (7) can be updated. For the asymptote no transformation was used in our former model development and the analysis of variance shows that transformation is not necessary, therefore the model and model parameters determined from the original data set remain unchanged. As shown by the analysis of variance the square root transformation is the best transformation to stabilize the variance of the growth rate data. Therefore, the model should be fitted to the square root of the data. The results of fitting the previously measured data (7) to the square root relation are given in Table 3. The lack of fit of the model is compared to the measuring error and the square root relation is accepted on basis of the F test.

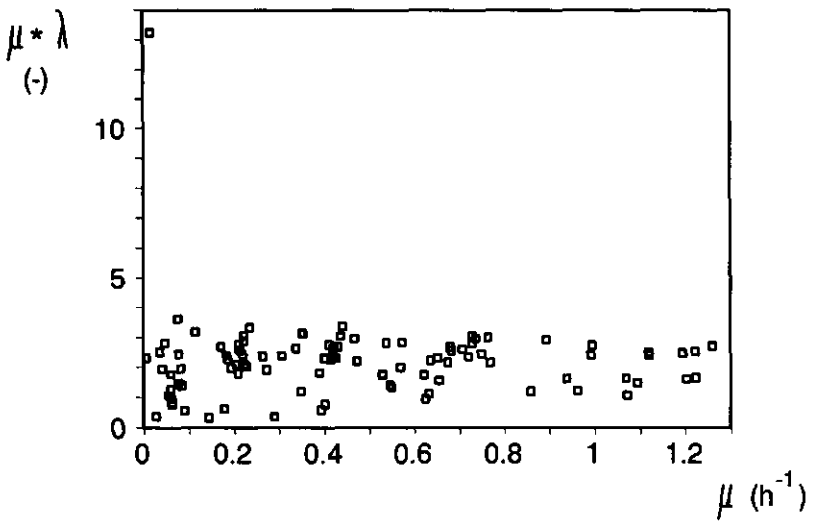

FIG. 4. Product of $\mu * \lambda$ against μ.

For the lag time data the logarithmic transformation is stabilizing the variance, and was already used (7). However, the previously proposed reciprocal of the Ratkowsky model (equation 5) is also tested. If it is assumed that the T_{min} and T_{max} value are equal to the T_{min} and T_{max} value of the equation describing the growth rate, this model also contains two parameters. If also the c-value is fixed, the model contains only one parameter. The results of fitting the previously measured lag time data to the hyperbolic model and reciprocal square root relation are given in Table 4. The reciprocal Ratkowsky model with all parameters fixed, except for b is accepted by the F test. This result indicates that the lag time is reciprocally proportional

TABLE 4. Results of the reciprocal Ratkowsky parameter estimation for the $\ln(\lambda)$ data

Parameter	Estimate	95% Confidence interval		
p	23.9	19.1 to 28.7	$\ln(\lambda) = \frac{p}{(T-q)}$	model 1
q	2.28	1.19 to 3.37		
b_5	0.0274	0.0240 to 0.0308		
T_{min5}	3.37	fixed	$\ln(\lambda) = -2\ln\left(b_5 (T-3.37)\{1-\exp[c_5(T-44.7)]\}\right)$	
c_5	0.373	0.228 to 0.518		
T_{max5}	44.7	fixed	model 2	
b_5	0.0299	0.0268 to 0.0329		
T_{min5}	3.37	fixed	$\ln(\lambda) = -2\ln\left(b_5 (T-3.37)\{1-\exp[0.256(T-44.7)]\}\right)$	
c_5	0.256	fixed		
T_{max5}	44.7	fixed	model 3	

	No. of parameters	DF	RSS	MS	f	F
Model 1	2	36	9.70	0.269		
LOF 1		16	2.00	0.125	0.325	2.2
Model 2	2	36	12.65	0.351		
LOF 2		16	4.95	0.309	0.803	2.2
Model 3	1	37	14.03	0.379		
LOF 3		17	6.33	0.372	0.967	2.2
General	18	20	7.70	0.385		

LOF= Lack of Fit; DF= Degrees of Freedom; RSS= Residual Sum of Squares; MS= Mean Square; f= $MS_{LOF}/MS_{general}$; F= F table value (95% confidence).

to the growth rate. This was already suggested by Simpson et al. (4). Similar results for the T_{min} value for the growth rate and lag time are also given by Chandler and McMeekin (2) and Smith (5). The multiplication of the growth rate and lag time are given in Fig. 4. This graph shows that the growth rate and the lag time are reciprocally proportional (except for one point) over a large range of growth rate values. This model now contains only one parameter and predicts a lag time increase at higher temperature.

The updated models and parameter values, based on the previously measured data (7) are given in Table 5.

TABLE 5. Parameter values and models for the effect of temperature on the asymptote (A), specific growth rate (μ_m) and lag time (λ) for *Lactobacillus plantarum* in MRS-medium

Asymptote, Ratkowsky 4	b_4	8.46
$A = b_4\{1 - \exp[c_4(T - T_{max4})]\}$	c_4	1.25
	T_{max4}	43.1
Specific growth rate, Ratkowsky	b_2	0.0385
$\sqrt{\mu_m} = b_2(T - T_{min2})\{1 - \exp[c_2(T - T_{max2})]\}$	T_{min2}	3.37
	c_2	0.256
	T_{max2}	44.7
Lag time, reciprocal Ratkowsky	b_5	0.0299
$\ln(\lambda) = -2\ln\left(b_5(T - T_{min5})\{1 - \exp[c_5(T - T_{max5})]\}\right)$	T_{min5}	3.37
	c_5	0.256
	T_{max5}	44.7

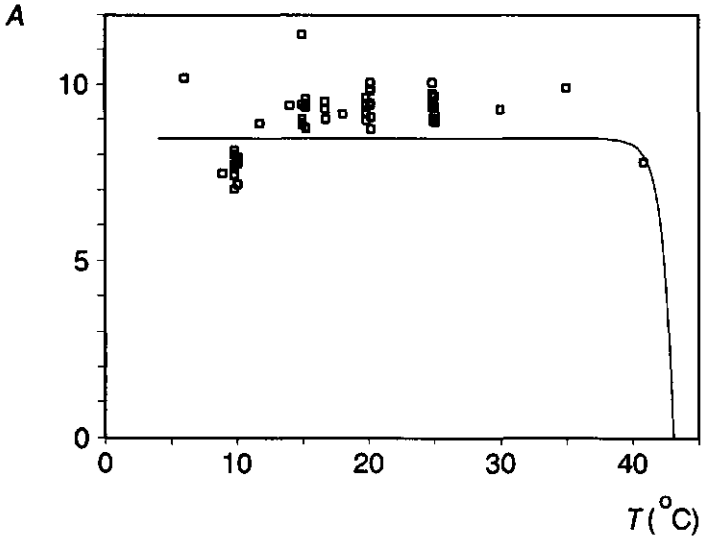

FIG. 5. New asymptote data and model (——) based on previously measured data.

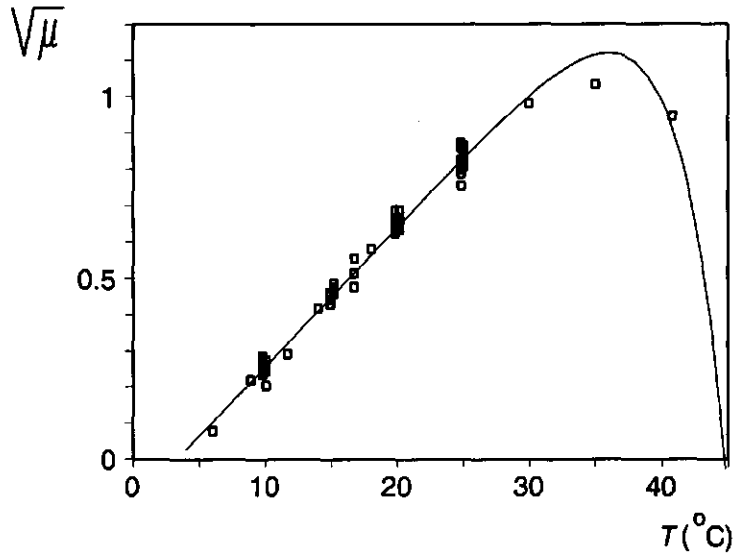

FIG. 6. New growth rate data and model (——) based on previously measured data.

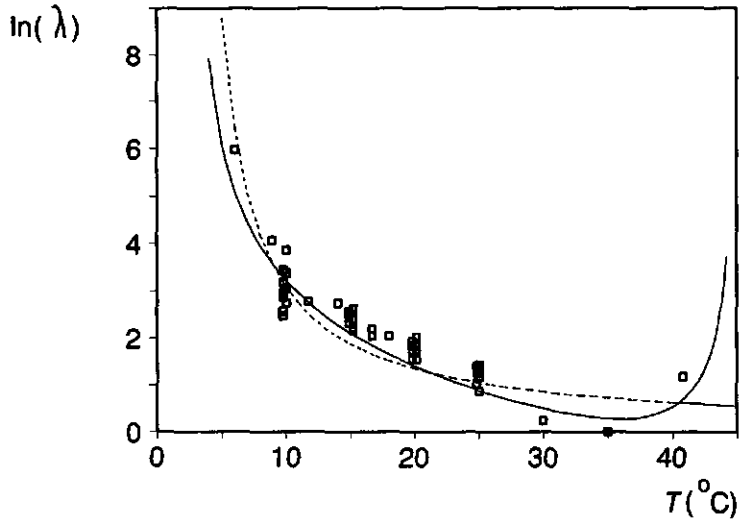

FIG. 7. New lag time data and reciprocal Ratkowsky model (——) and hyperbolic model (- - - - - -) based on previously measured data.

Model validation. Now the updated model can be used to predict the newly measured data (60 growth curves, measured at 17 different temperatures). The newly measured growth parameters are plotted with the predictions in Fig. 5, 6, and 7. The growth rate is transformed with a square root, and the lag time is transformed with a logarithmic (ln) transformation. From these graphs it can be concluded that the data are reasonably well predicted by the earlier developed models. For the growth rate data the residuals show no trend. However, the lag-time data and the asymptote data show some discrepancies (the residuals are not equally well distributed around zero). It should be noted that the new data are all at temperatures below 40°C. Therefore, the models are only validated within the range 6-40°C. Furthermore, it should be noted that the new data are compared with predictions using parameter values determined with other data.

TABLE 6. Result of the *F*-test, comparing new data and model

	A	$\sqrt{\mu_m}$	$\ln(\lambda)$		
RSS_{error}	31.3	0.125	14.0		
DF_{error}	35	34	37		
MS_{error}	0.895	0.00367	0.379		
	Ratkowsky 4	Ratkowsky	Hyperbole	$(Ratk)^{-1}$	
RSS_g (DF_g=43)	9.79	0.0269	2.39	2.39	
RSS_m (DF_m=60)	59.0	0.0425	13.1	10.4	
LOF (DF_{LOF}=17)	49.2	0.0157	10.8	8.00	
MS_{LOF}	2.90	0.000921	0.633	0.470	
f	3.24	0.251	1.67	1.24	$F_{34}^{17} \approx 2.00$

RSS= Residual Sum of Squares; DF= Degrees of Freedom; MS= Mean Square; error= error previous data; RSS_g= RSS of general model (mean of replicates); RSS_m= RSS of model prediction; $(Ratk)^{-1}$= reciprocal Ratkowsky model (equation 5); LOF= Lack Of Fit (RSS_m-RSS_g); f= MS_{LOF}/MS_{error}; F= F table value (95% confidence).

The lack of fit of the models is compared with the measuring error by the *F*-test (Table 6). The mean square of the lack of fit must be tested against the mean square of the measuring error. The measuring error is estimated by calculating the deviation of the first set of data with the growth model. The lack of fit is calculated by subtracting the RSS of the growth

temperature model (RSS_m with DF_m=60-0=60; the number of observations minus the number of parameters) and the RSS due to the residual variance (RSS_g with DF_g=60-17=43; total number of observations minus the number of different temperatures measured).

From this statistical test we can conclude that for the growth rate data and the lag time data the deviation between the model prediction and the data is of the same order as the measuring error. The reciprocal Ratkowsky model had a better predicting ability (in this case) than the hyperbola model.

For the asymptote data, however, there is a significant deviation between the model and the data (this can also be seen globally in Fig. 5).

Parameter update. Now that the model is validated the parameters can be updated using all data together. So finally, the model parameters are updated using all 98 growth curves. The final model parameters are given in Table 7. By comparing the parameters in Table 7 (parameters based on 98 growth curves) and Table 5 (parameters based on 38 growth curves) it can be seen that the update resulted in small changes only. The results are shown graphically in Fig. 8 to 10.

TABLE 7. Parameter values and models for the effect of temperature on the asymptote (A), specific growth rate (μ_m) and lag time (λ) for *Lactobacillus plantarum* in MRS-medium, final parameter values

		Estimate	95% Confidence interval
Asymptote, Ratkowsky 4	b_4	8.83	8.63 to 9.02
$A = b_4\{1 - \exp[c_4(T - T_{max4})]\}$	c_4	1.05	0.679 to 1.43
	T_{max4}	43.2	42.9 to 43.4
Specific growth rate, Ratkowsky	b_2	0.0385	0.0368 to 0.0402
$\sqrt{\mu_m} = b_2(T - T_{min2})\{1 - \exp[c_2(T - T_{max2})]\}$	T_{min2}	3.29	2.63 to 3.95
	c_2	0.247	0.207 to 0.288
	T_{max2}	44.8	44.4 to 45.2
Lag time, reciprocal Ratkowsky	b_5	0.0276	0.0263 to 0.0289
$\ln(\lambda) = -2\ln\left(b_5(T - T_{min5})\{1 - \exp[c_5(T - T_{max5})]\}\right)$	T_{min5}	3.29	
	c_5	0.247	
	T_{max5}	44.8	

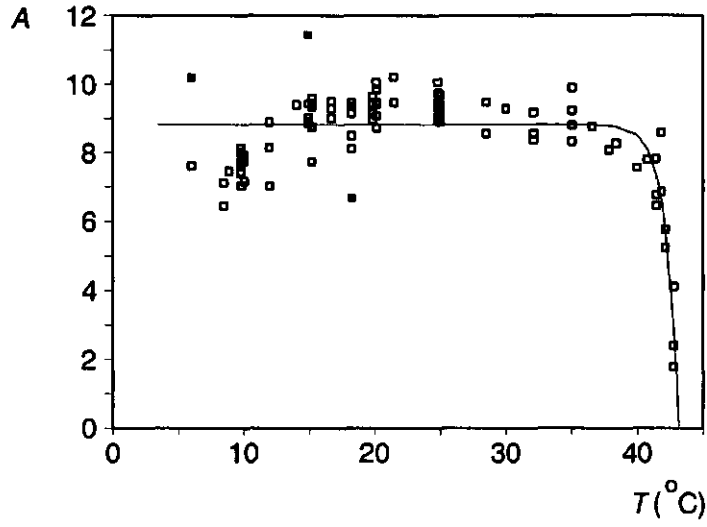

FIG. 8. All asymptote data and updated model (——). Solid blocks are outlayers.

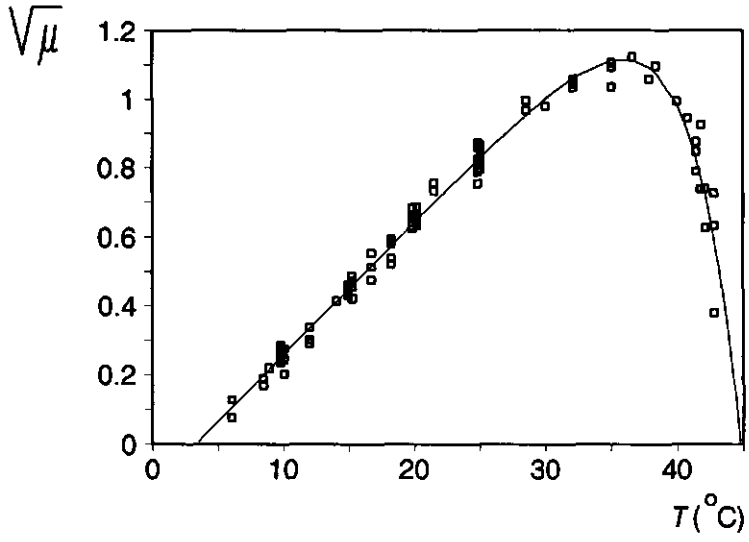

FIG. 9. All growth rate data and updated model (——).

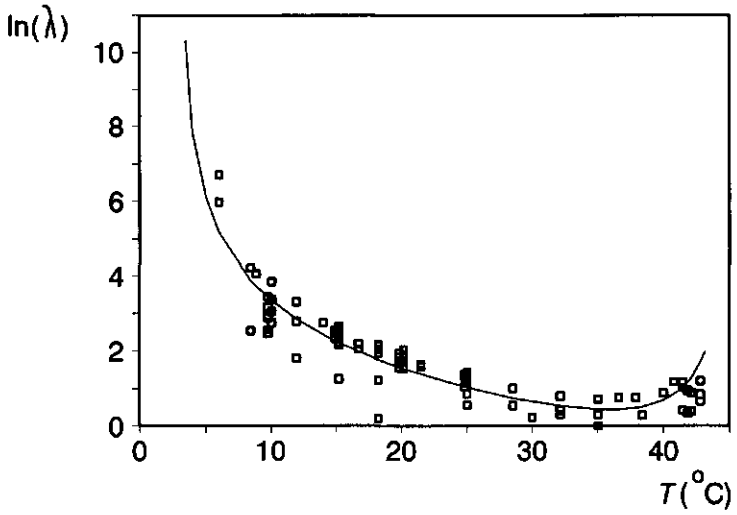

FIG. 10. All lag time data and updated model (——).

Asymptote model. It is shown that there is a significant deviation between the model and the asymptote data (Fig. 5, 8 and Table 6). Therefore, another model is attempted. The following model is proposed:

$$A = a \cdot \frac{(T - T_{min6})(T - T_{max6})}{(T - b_6)(T - c_6)} \tag{9}$$

In this model parameter b_6 must be somewhat lower than T_{min6} and c_6 must be a little higher than T_{max6} (b_6 and c_6 being the temperatures at which the asymptote will reach minus infinity). The results of fitting this equation are given in Table 8. The datum points indicated with a solid box in Fig. 8 are not taken into account, since these data deviate largely. The rejection of three points out of 98 seems justified.

It can be seen in Table 8 that the Ratkowsky 4 model is rejected on basis of the F test and the newly proposed model is accepted. The confidence interval of T_{min} includes the T_{min} value of the growth rate data (Tables 7 and 8), so this value can be fixed. The confidence interval of T_{max} does not include the T_{max} value of the growth rate data. With a fixed T_{min} value the model is also accepted by the F test and this model is shown graphically in Fig. 11. It shows a decrease of the asymptote values (number of cells ultimately produced) at extremes of temperatures. These effects may result from the relative increase of the maintenance energy at low growth rates. If more energy is consumed for maintenance, a lower cell number

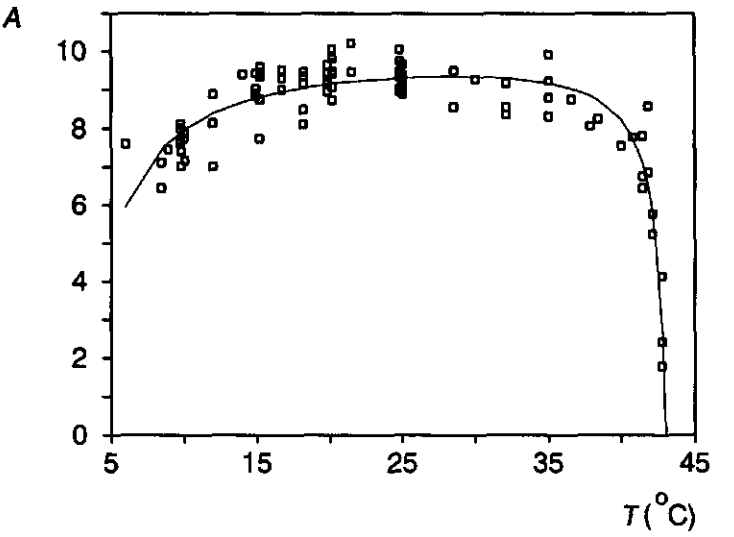

FIG. 11. All asymptote data and new model (——).

will be reached. The decline at low temperatures was mentioned in our previous paper (7), but could not be proven statistically with 38 observations. With the current 95 observations this effect is shown to be statistically significant.

CONCLUSIONS

It has been shown with 80 growth curves at 17 different temperatures, that the asymptote can best be modeled without transformation, the growth rate with a square root transformation, and the lag time with a logarithmic transformation. The choice of the transformation is of eminent importance for the regression analysis of the data.

The previously proposed lag time model has the complication that the lag time at higher temperatures approaches an arbitrary value of one, while at higher temperatures it can be assumed that the lag phase increases. Therefore, the previously proposed reciprocal of the Ratkowsky model (equation 5) seems better. For this reason, the hyperbolic model is replaced by the reciprocal Ratkowsky model.

TABLE 8. Results of the additional asymptote model

Parameter	Estimate	95% Confidence interval	
b_4	8.80	8.62 to 8.99	
c_4	1.06	0.711 to 1.41	$b_4\{1-\exp[c_4(T-T_{max4})]\}$
T_{max4}	43.2	42.9 to 43.4	model 1, Ratkowsky 4
a	10.8	9.83 to 11.7	
T_{min6}	2.20	-1.51 to 5.92	$a\cdot\frac{(T-T_{min6})(T-T_{max6})}{(T-b_6)(T-c_6)}$
T_{max6}	43.1	42.9 to 43.2	
b_6	-0.352	-6.11 to 5.41	model 2
c_6	43.7	43.4 to 44.1	
a	10.5	10.1 to 11.0	
T_{min6}	3.29	fixed	$a\cdot\frac{(T-3.29)(T-T_{max6})}{(T-b_6)(T-c_6)}$
T_{max6}	43.1	42.9 to 43.2	
b_6	1.29	0.770 to 1.82	model 3
c_6	43.7	43.4 to 44.0	

	No. of parameters	DF	RSS	MS	f	F
Model 1	3	92	65.4	0.711		
LOF 1		26	46.1	1.77	6.08	
Model 2	5	90	32.3	0.359		
LOF 2		24	13.0	0.543	1.86	2.0
Model 3	4	91	32.6	0.359		
LOF 3		25	13.4	0.536	1.84	
General	29	66	19.2	0.292		

LOF= Lack of Fit; DF= Degrees of Freedom; RSS= Residual Sum of Squares; MS= Mean Square; f= $MS_{LOF}/MS_{general}$; F= F table value (95% confidence).

The models are validated with new data (60 growth curves at 17 different temperatures). The growth rate data are very well predicted. The reciprocal Ratkowsky model appears to be somewhat better than the hyperbolic model for prediction of the lag phase duration, and has the desired ability to increase at higher temperatures. The asymptote data are reasonably well predicted by the Ratkowsky 4 model, however, at low temperatures there is a significant deviation. Therefore another model (equation 9) is proposed which showed to describe the

behaviour at low temperatures much better. The decline at low temperatures could now be proven statistically with the current 95 observations. For kinetic predictions the lag time and the growth rate are the most important parameters.

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CHAPTER 6

MODELING OF BACTERIAL GROWTH WITH SHIFTS IN TEMPERATURE

ABSTRACT

The temperature of chilled foods is an important variable for the shelf life of a product in a production and distribution chain. To predict the number of organisms as a function of temperature and time, it is essential to model the growth as a function of temperature. The temperature is often not constant in various stages of distribution. The objective of this research was to determine the effect of shifts in temperature. The suitability and usefulness of several models to describe the growth associated with fluctuating temperatures was evaluated. An assumption can be that temperature shifts within the lag phase can be handled by adding relative parts of the lag time to be completed and that temperature shifts within the exponential phase result in no additional lag phase. With these assumptions the kinetic behaviour of temperature shift experiments was reasonably well predicted. Only shifts of temperature around the minimum temperature of growth showed very large deviations compared to the model prediction. Even better results were obtained with the assumption that a temperature shift (within the lag phase as well as within the exponential phase) results in an additional lag phase of one-fourth of the lag time normally found at the temperature after the shift.

INTRODUCTION

Temperature is a major factor determining the kinetics of food deterioration reactions. As microbial spoilage is of major concern the effect of temperature on the specific growth rate of microorganisms is important. Various models to describe the effect of a constant temperature are given by Zwietering et al. (6, 8). However, the temperature is often not

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constant during distribution. It can be assumed for instance, that the microorganisms respond instantaneously to changes in temperature. Another possibility is that the organisms need to adapt to the new temperature, so that they run through a lag phase due to the stress of the temperature shift.

Simpson et al. (5) propose to calculate the lag time during changing temperature conditions, by adding the relative parts of the lag phase. So, if a culture has completed half of the lag time at the first temperature of incubation, and then is transferred to a new temperature, it still has to complete half of the lag time at the new temperature. Langeveld and Cuperus (2) found that bacteria respond immediately to a change in temperature within the exponential phase. Ng et al. (3) and Shaw (4) also carried out experiments with temperature shifts within the exponential phase, and they found that shifts in the moderate temperature region resulted in immediate exponential growth at the growth rate of the new temperature. However, they found that steps to or from low temperatures resulted in an adaptation period. Fu et al. (1) found a significant effect of a temperature step, both for the exponential and the lag phase. Biologically this can be expected, since the cells are 'out of balance' and need to adjust, for instance, their enzyme pool to a new equilibrium.

It can be concluded that the literature gives no consistent procedure to handle temperature shifts. The objective of this research was to determine the effect of shifts in temperature for bacteria that are still within their lag phase and for bacteria that are growing exponentially. With a large data set and a statistical analysis, it will be determined, what procedure can be used to handle temperature changes. Several models are proposed and compared to make kinetic predictions of the bacterial growth taking into account a change in temperature. The suitability and usefulness of these models will be discussed.

THEORY

Description of the experimental bacterial growth data. Growth curves are defined as the logarithm of the relative population size [$y = \ln(N/N_o)$] as a function of time. A growth model with three parameters can describe the growth curve (7). One method to describe a growth curve is to assume no growth within the lag phase as well as within the asymptotic phase, and to assume exponential growth within the exponential phase:

$$\begin{aligned} y &= 0 && t \le \lambda \\ y &= \mu \cdot (t - \lambda) && \lambda < t < A/\mu + \lambda \\ y &= A && t \ge A/\mu + \lambda \end{aligned} \qquad (1)$$

Since bacterial growth curves often show a sigmoidal shape a second method is to describe the data by a sigmoidal function, for example the modified Gompertz equation. The specific growth rate (μ_m), the lag time (λ), and the asymptote (A), can be determined from growth data by fitting them with this modified Gompertz model (7):

$$y = A\exp\left\{-\exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\} \qquad (2)$$

Growth/temperature relations. Models to describe the effect of a constant temperature on the asymptote (A), specific growth rate (μ) and lag time (λ), with parameter values are given in Table 1 (6).

TABLE 1. Parameter values and models for the effect of temperature on the asymptote (A), specific growth rate (μ) and lag time (λ) for *Lactobacillus plantarum* in MRS-medium (6)

Asymptote	a	10.5
	T_{min6}	3.29
$A = a\frac{(T - T_{min6})(T - T_{max6})}{(T - b_6)(T - c_6)}$	T_{max6}	43.1
	b_6	1.29
	c_6	43.7
Specific growth rate, Ratkowsky	b_2	0.0385
	T_{min2}	3.29
$\sqrt{\mu_m} = b_2(T - T_{min2})\{1 - \exp[c_2(T - T_{max2})]\}$	c_2	0.247
	T_{max2}	44.8
Lag time, reciprocal Ratkowsky	b_5	0.0276
	T_{min5}	3.29
$\ln(\lambda) = -2\ln\left(b_5 (T - T_{min5})\{1 - \exp[c_5(T - T_{max5})]\}\right)$	c_5	0.247
	T_{max5}	44.8

For the parameter a, $23.62 - \ln(N_o)$ must be used if another inoculation level is used.

Growth with temperature changes. Cultures are shifted at time t_s from temperature T_1 to T_2. Growth parameters (at constant temperature, determined from Table 1) before the temperature shift have index 1 and after the shift index 2 [$A(T_1) = A_1$; $A(T_2) = A_2$; $\mu(T_1) = \mu_1$; $\mu(T_2) = \mu_2$; $\lambda(T_1) = \lambda_1$; $\lambda(T_2) = \lambda_2$]. The dimensionless time of shift (t_s^*) is defined as t_s divided by λ_1. From the growth data after the shift, a lag time λ_{shift} can be estimated. In order to predict this λ_{shift} several hypotheses can be proposed.

Temperature shift within the lag phase ($t_s \leq \lambda_1$, $t_s^* \leq 1$). If bacteria are subjected to a temperature shift from T_1 to T_2 during their lag phase, they will not have completed their lag period and will still show a lag phase at the new temperature. Three hypotheses are being tested:

i) The effect of the temperature shift results in a new lag phase that is equal to the lag phase normally found at T_2.

$$\lambda_{shift} = \lambda_2 \tag{3}$$

In this case it is assumed that the preincubation at T_1 had no effect, and that the shift disturbs the cells in such a manner, that they start over again their full lag period.

ii) The effect of the temperature shift results in a new lag phase that is equal to the relative part of the lag phase that still has to be completed. So, if for instance one-third of the lag phase is completed during incubation at the first temperature ($t_s = \frac{1}{3}\lambda_1 ; t_s^* = \frac{1}{3}$) still two-third of the lag time has to be completed at the temperature after the shift ($\lambda_{shift} = \frac{2}{3}\lambda_2$). For a general case this can be written as:

$$\lambda_{shift} = \left(1 - \frac{t_s}{\lambda_1}\right) \cdot \lambda_2 = (1 - t_s^*) \cdot \lambda_2 \tag{4}$$

iii) It can also be expected that a temperature shift results in an additional lag phase since the cells are stressed by the temperature shift. Therefore, it is assumed that the effect of a temperature shift results in a new lag phase that is equal to the relative part of the lag phase that still has to be completed plus an additional lag due to the shift in temperature:

$$\lambda_{shift} = (1 - t_s^*) \cdot \lambda_2 + \lambda_x \tag{5}$$

with λ_x the additional lag due to the shift in temperature.

If we now assume that the additional lag due to the shift in temperature is proportional to the lag phase at T_2 ($\lambda_x = \alpha \cdot \lambda_2$) we get:

$$\lambda_{shift} = (1 + \alpha - t_s^*) \cdot \lambda_2 \tag{6}$$

with α the proportionality constant.

Temperature shift within the exponential phase ($t_s > \lambda_1$, $t_s^* > 1$). Again three hypotheses are being tested:

i) The effect of the temperature shift gives such a disturbance that it results in a new lag phase that is equal to the lag phase normally found at T_2.

$$\lambda_{shift} = \lambda_2 \tag{7}$$

ii) If it is assumed that the bacteria show no surplus lag phase due to a change in temperature during the exponential phase, the growth continues immediately with the specific growth rate of T_2.

$$\lambda_{shift} = 0 \tag{8}$$

iii) It can also be assumed that a temperature shift results in an additional lag phase (analogous to equation 5 and 6) since the cells are stressed by the temperature shift:

$$\lambda_{shift} = \lambda_x = \alpha \cdot \lambda_2 \tag{9}$$

with λ_x the additional lag due to the shift in temperature and α the proportionality constant.

Summary of the hypotheses. In all the above mentioned hypothesis, the lag time after the shift (λ_{shift}) is proportional to λ_2. Therefore, a relative lag time can be defined as :

$$\lambda^* = \frac{\lambda_{shift}}{\lambda_2} \tag{10}$$

A summary of the effect of shifts within the lag and exponential phase for the three hypotheses on this λ^* value is given in Table 2. Note that hypothesis 3 is equal to hypothesis 2 with $\alpha = 0$.

TABLE 2. Relative lag time λ^* resulting from the three hypotheses

Hypothesis	Shift within lag phase ($t_s^* \leq 1$)	Shift within exponential phase ($t_s^* > 1$)
1	1	1
2	$1 - t_s^*$	0
3	$1 + \alpha - t_s^*$	α

MATERIALS AND METHODS

Microbial experiments. The culture of *Lactobacillus plantarum* (American Type Culture Collection [ATCC]-determined; no ATCC-number) was stored frozen (-16°C). The bacteria were cultivated twice at 30°C, first for 24 hours, and secondly for 16 hours. The inoculation level from this last preculture into the test-tube was 0.01% (about $5 \cdot 10^5$ organisms) and in some cases was 0.0001% (about $5 \cdot 10^3$ organisms). All incubations were performed in MRS medium (Difco Laboratories) in a temperature gradient incubator (8). Growth was measured with plate counts on pour plates (MRS medium with 12 g of agar [Agar Technical Oxoid Ltd.] per liter).

At each of the temperatures 10, 15, 20, and 25°C, 10 independent growth curves were measured. The combined datum points for each temperature were compared with model predictions, and the resulting residual sum of squares (RSS) was used to estimate the experimental measuring error.

Temperature shifts within the lag phase were carried out in 71 experiments. Temperature shifts within the exponential phase were carried out in 53 experiments.

Temperature shifts. Temperature shifts were carried out by moving tubes from one temperature to another temperature in the gradient incubator. Tubes had an inner diameter of 1.35 cm and were filled with 10 ml medium, resulting in a liquid height of 7 cm. Temperature responses were measured with different temperature steps. After 8 minutes the temperature difference between the tube and the incubator was less than 10% of the step value, after 16 minutes this value was less than 1%.

Comparison of the models. The lag time predicted by the various models was compared to the measured lag time with the use of a t test.

The variance of the measuring error at different temperatures was determined by calculating the residual sum of squares of the data at these temperatures (10, 15, 20, and 25°C, 10 independent growth curves) compared to the model prediction (modified Gompertz function with parameter values from Table 1). The pooled variance (var_{me}) was calculated by dividing the pooled RSS by the total number of datum points (no parameters used).

The growth after the temperature shift was examined by fitting y-data to the modified Gompertz equation (equation 2). For the lag time experiments the y value was used as such, since this represents the growth after the temperature shift as there is no significant change in bacterial numbers during the incubation at temperature T_1 (still within the lag phase). For the

experiments in the exponential phase the growth after the temperature shift is given by $y - y_s$, where y_s was taken as the y value at the time of shift. The time after the shift was used as the time value.

The 95% confidence interval of the lag time was calculated with:

$$\lambda_{min,max} = \hat{\lambda} \pm t\sqrt{V_{\lambda\lambda} \cdot var_{me}} \quad (11)$$

with $\hat{\lambda}$ the estimated lag time value, t the student t value, $V_{\lambda\lambda}$ the diagonal value corresponding to λ in the Jacobian matrix (results from the Marquardt fitting procedure), and var_{me} the variance of the measuring error.

RESULTS AND DISCUSSION

Constant temperature experiments. At the temperatures 10, 15, 20, and 25°C, 10 independent growth curves were measured and the combined datum points for each temperature were compared with model predictions from formerly built and validated models (Fig. 1 and 2, Table 1). Fig. 1 and 2 show that the dynamic behaviour of bacterial growth can be predicted very well. However exact predictions can not be made due to experimental errors. The resulting residual sum of squares (RSS) was used to estimate the experimental error (Table 3).

TABLE 3. RSS of the combined datum points for different temperatures described with model predictions from previously validated models (6)

Temperature	RSS	*n* points	var
10	71.3	191	0.374
15	25.4	151	0.168
20	22.6	170	0.133
25	28.1	134	0.210
Pooled	147.5	646	0.2283 (=var_{me})

RSS= Residual Sum of Squares; *n* points= number of datum points; var= variance (RSS/*n* points).

The variances differ at different temperatures, nevertheless they are pooled to calculate the variance of the measuring error (var_{me}).

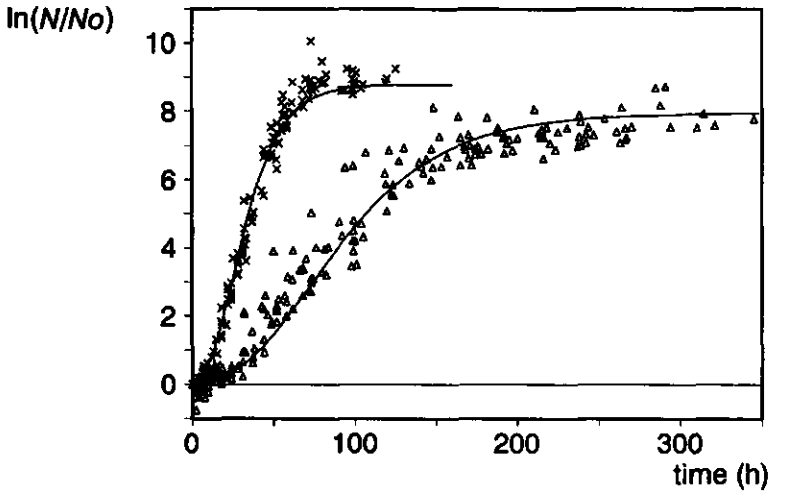

FIG. 1. Growth curves of *L. plantarum* at 10 (▵) and 15°C (×) compared with model prediction (——).

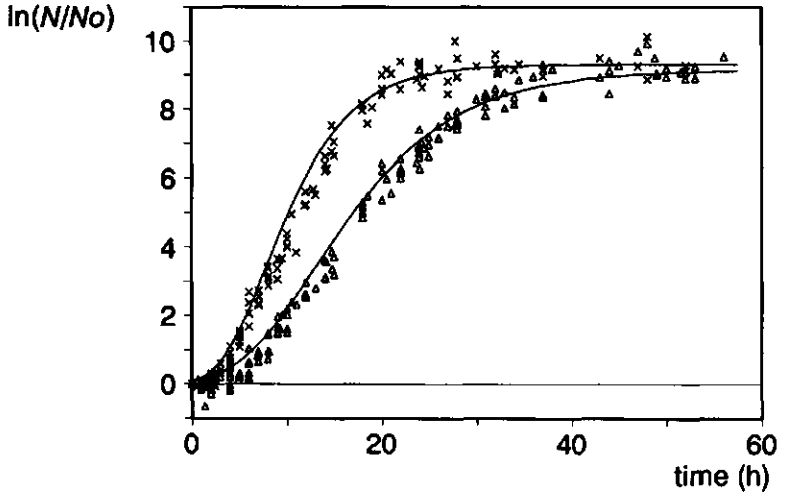

FIG. 2. Growth curves of *L. plantarum* at 20 (▵) and 25°C (×) compared with model prediction (——).

Temperature shift experiments. From the growth curves after the temperature shift (t-t_s), the lag time (λ_{shift}) was estimated. This λ_{shift} value was divided by the lag time normally found at T_2 to get the relative lag time (λ^*). The three hypotheses can be tested by plotting this relative lag time (λ^*) versus t_s^* (Fig. 3, Table 2). Fig. 3 shows that the relative lag time decreases if t_s^* goes from zero to one, and remains constant at higher t_s^* values. Hypothesis 1 assumes that the relative lag time is equal to one in all cases. Fig. 3 shows that the data do not support this assumption. Therefore, hypothesis 1 can be rejected. Furthermore, it can be seen that most of the data are above the line predicted by hypothesis 2 ($\alpha = 0$). This indicates that a temperature shift results in an additional lag phase. It was already mentioned that this could be expected, since the cells will be stressed by the temperature shift.

The α value was estimated by optimising the statistical acceptance of hypothesis 3 and was found to be 0.25, indicating that a temperature shift results in a new extra lag time (λ_x) equal to one-fourth of the lag time normally found at the temperature after the shift (T_2).

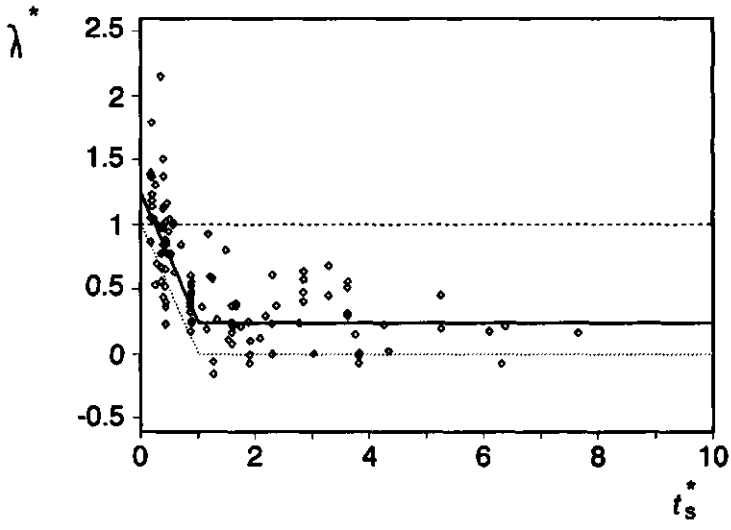

FIG. 3. Relative lag time (∘) plotted versus the relative time of shift, compared with model predictions. (-------- = hypothesis 1; ····· = hypothesis 2; ——— = hypothesis 3 with $\alpha = 0.25$).

The three hypotheses were tested with the t test by calculating the confidence interval of the estimated lag time of the growth data after the shift. For the various model predictions it was tested whether the predictions are within this confidence interval. The experiments with steps within the lag phase are given in Table 4 and the experiments with steps within the exponential phase are given in Table 5.

TABLE 4. Results of temperature shifts within the lag phase

no[a]	T_1	T_2	t_s[b]	n[c]	λ_1	λ_2[d]	t_s^*[f]	λg	min[h]	max[h]	$\alpha = 0$[i]	$\alpha = \frac{1}{4}$[k]
L1	14.9	10.1	2.50	17	9.76	28.36	0.256	15.11	4.31	25.91	**21.10**	28.19
L2	25.1	10.1	1.25	15	2.81	28.36	0.445	11.43	-0.60	23.45	**15.73**	**22.82**
L3	24.8	10.0	1.25	18	2.88	29.21	0.434	6.90	-4.57	18.37	**16.54**	23.84
L4	25.0	9.9	1.25	18	2.83	30.10	0.442	11.09	-0.24	22.43	**16.81**	24.34
L5	24.8	9.7	1.25	21	2.88	32.01	0.434	16.77	6.85	26.69	**18.12**	**26.13**
L6	14.9	10.1	5.00	17	9.76	**28.36**	0.512	21.52	9.88	33.15	**13.84**	**20.93**
L7	25.1	10.1	2.50	15	2.81	28.36	0.891	6.95	-2.99	16.89	**3.09**	**10.18**
L8	24.8	10.0	2.50	18	2.88	29.21	0.868	5.28	-5.32	15.88	**3.87**	**11.17**
L9	25.0	9.9	2.50	18	2.83	30.10	0.883	7.79	-2.52	18.11	**3.52**	**11.04**
L10	24.8	9.7	2.50	21	2.88	32.01	0.868	12.83	3.94	21.71	**4.24**	**12.24**
L11	10.5	15.3	10.0	13	25.30	**9.13**	0.395	8.92	5.00	12.85	**5.52**	**7.80**
L12	9.9	15.1	6.00	12	30.10	**9.44**	0.199	10.80	6.46	15.15	**7.56**	**9.92**
L13	9.9	15.1	6.00	14	30.10	**9.44**	0.199	11.23	7.39	15.06	**7.56**	**9.92**
L14	9.9	15.0	6.00	16	30.10	**9.60**	0.199	11.43	7.65	15.20	**7.69**	**10.09**
L15	9.6	14.9	6.00	17	33.03	**9.76**	0.182	8.45	4.57	12.34	**7.99**	**10.43**
L16	25.1	15.2	1.25	12	2.81	**9.28**	0.445	8.11	4.26	11.97	**5.15**	**7.47**
L17	24.8	15.1	1.25	14	2.88	**9.44**	0.434	8.01	4.23	11.79	**5.34**	**7.70**
L18	25.0	15.0	1.25	17	2.83	**9.60**	0.442	8.22	4.51	11.93	**5.36**	**7.76**
L19	24.8	15.0	1.25	18	2.88	**9.60**	0.434	6.30	2.62	9.98	**5.43**	**7.83**
L20	10.5	15.3	15.0	12	25.30	**9.13**	0.593	9.22	5.18	13.25	**3.72**	**6.00**
L21	9.9	15.1	12.0	11	30.10	**9.44**	0.399	7.43	3.01	11.85	**5.68**	**8.03**
L22	9.9	15.1	12.0	14	30.10	**9.44**	0.399	9.45	5.80	13.09	**5.68**	**8.03**
L23	9.6	15.0	12.0	15	33.03	**9.60**	0.363	6.39	2.55	10.24	**6.11**	**8.51**
L24	9.6	14.9	12.0	16	33.03	9.76	0.363	5.50	1.70	9.30	**6.22**	**8.66**
L25	25.1	15.2	2.50	12	2.81	9.28	0.891	4.47	0.69	8.24	**1.01**	**3.33**
L26	24.8	15.1	2.50	12	2.88	9.44	0.868	4.08	0.50	7.66	**1.25**	**3.61**
L27	25.0	15.0	2.50	16	2.83	9.60	0.883	4.47	0.90	8.04	**1.12**	**3.52**
L28	24.8	15.0	2.50	17	2.88	9.60	0.868	3.55	-0.25	7.34	**1.27**	**3.67**
L29	10.5	20.2	10.0	15	25.30	**4.62**	0.395	3.91	2.32	5.51	**2.79**	**3.95**
L30	9.9	19.9	6.00	14	30.10	**4.78**	0.199	5.06	3.25	6.86	**3.83**	**5.03**
L31	9.9	19.9	6.00	14	30.10	**4.78**	0.199	6.56	4.49	8.63	3.83	**5.03**
L32	9.6	20.0	6.00	16	33.03	**4.73**	0.182	6.58	4.57	8.58	3.87	**5.05**
L33	9.6	19.6	6.00	16	33.03	**4.96**	0.182	4.28	2.46	6.09	**4.06**	**5.30**
L34	14.9	20.3	2.50	13	9.76	**4.56**	0.256	4.64	2.33	6.96	**3.40**	**4.54**
L35	25.1	20.3	1.25	14	2.81	**4.56**	0.445	3.87	1.96	5.77	**2.53**	**3.67**
L36	24.8	19.8	1.25	14	2.88	**4.84**	0.434	4.23	2.33	6.14	**2.74**	**3.95**
L37	25.0	19.8	1.25	13	2.83	**4.84**	0.442	4.96	2.94	6.97	2.70	**3.91**
L38	24.8	19.9	1.25	14	2.88	**4.78**	0.434	4.23	2.35	6.11	**2.71**	**3.91**
L39	10.5	20.2	15.0	12	25.30	**4.62**	0.593	4.64	2.57	6.72	1.88	**3.03**
L40	9.9	19.9	12.0	12	30.10	4.78	0.399	2.12	-0.28	4.52	**2.88**	**4.07**
L41	9.9	19.9	12.0	11	30.10	**4.78**	0.399	5.47	2.52	8.42	**2.88**	**4.07**
L42	9.6	20.0	12.0	13	33.03	**4.73**	0.363	4.63	1.86	7.41	**3.01**	**4.19**
L43	9.6	19.6	12.0	13	33.03	**4.96**	0.363	3.87	1.21	6.53	**3.16**	**4.40**
L44	14.9	20.3	5.00	13	9.76	**4.56**	0.512	4.77	2.08	7.46	**2.23**	**3.37**
L45	25.1	20.3	2.50	13	2.81	4.56	0.891	2.54	0.54	4.54	0.50	**1.64**
L46	24.8	19.8	2.50	13	2.88	4.84	0.868	1.59	-0.24	3.41	**0.64**	**1.85**
L47	25.0	19.8	2.50	13	2.83	4.84	0.883	2.56	0.71	4.41	0.57	**1.78**
L48	24.8	19.9	2.50	15	2.88	4.78	0.868	2.11	0.39	3.84	**0.63**	**1.83**
L49	10.5	25.0	10.0	13	25.30	**2.83**	0.395	3.19	1.96	4.42	1.71	**2.42**
L50	9.9	24.9	6.00	11	30.10	**2.86**	0.199	3.54	2.17	4.91	**2.29**	**3.00**

TABLE 4 continued.

no[a]	T_1	T_2	t_s[b]	n[c]	λ_1	λ_2[d]	t_s*[f]	λ[g]	min[h]	max[h]	$\alpha = 0$[i]	$\alpha = \frac{1}{4}$[k]
L51	9.9	24.9	6.00	14	30.10	**2.86**	0.199	3.94	2.69	5.18	2.29	**3.00**
L52	9.6	25.0	6.00	12	33.03	**2.83**	0.182	3.98	2.66	5.29	2.32	**3.02**
L53	9.6	24.7	6.00	15	33.03	**2.91**	0.182	3.05	1.84	4.27	**2.38**	**3.11**
L54	14.9	25.1	2.50	12	9.76	**2.81**	0.256	3.68	2.46	4.89	2.09	**2.79**
L55	20.2	25.1	1.33	15	4.62	**2.81**	0.288	1.95	1.01	2.88	**2.00**	**2.70**
L56	10.5	25.0	15.0	13	25.30	**2.83**	0.593	1.79	0.43	3.14	**1.15**	**1.86**
L57	9.9	24.9	12.0	10	30.10	**2.86**	0.399	3.94	1.48	6.40	**1.72**	**2.43**
L58	9.9	24.9	12.0	11	30.10	**2.86**	0.399	4.34	1.49	7.18	**1.72**	**2.43**
L59	9.6	25.0	12.0	12	33.03	**2.83**	0.363	6.11	2.14	10.09	1.80	**2.51**
L60	9.6	24.7	12.0	12	33.03	**2.91**	0.363	2.86	0.95	4.77	**1.85**	**2.58**
L61	14.9	25.1	5.00	10	9.76	**2.81**	0.512	2.18	0.63	3.73	**1.37**	**2.07**
L62	20.2	25.1	2.67	13	4.62	**2.81**	0.578	2.80	1.65	3.95	1.18	**1.89**
L63	10.2	30.7	19.5	11	27.55	**1.86**	0.708	1.57	0.82	2.33	0.54	**1.01**
L64	10.2	30.7	24.0	10	27.55	**1.86**	0.871	1.13	0.27	1.99	0.24	**0.70**
L65	4.0	20.0	1873	22	2616	4.73	0.716	22.56	19.83	25.30	1.34	2.52
L66	4.0	35.0	1873	18	2616	1.57	0.716	15.14	14.11	16.16	0.45	0.84
L67	14.0	24.0	5.25	16	11.47	**3.10**	0.458	3.64	1.43	5.85	**1.68**	**2.46**
L68	14.0	24.0	2.25	17	11.47	3.10	0.196	5.58	3.55	7.62	2.49	3.27
L69	14.7	20.0	5.00	16	10.11	**4.73**	0.495	4.48	2.57	6.39	2.39	**3.57**
L70	14.7	20.0	2.50	16	10.11	**4.73**	0.247	4.95	2.94	6.95	**3.56**	**4.74**
L71	20.0	30.0	2.50	8	4.73	**1.94**	0.529	1.50	0.29	2.71	**0.91**	**1.40**

[a]Experimental number, bold code = curve plotted in graph (Fig. 4 to 13);[b]t_s= time of shift (h); [c]n= number of datum points in growth curve; [d] bold value indicates that hypothesis 1 is accepted by t test; [f]$t_s^* = t_s/\lambda_1$; [g] λ = estimated lag time from growth data; [h]min, max = minimum and maximum 95% confidence limit of lag time, 95% confidence intervals are calculated with $t = 1.96$ (at infinite degrees of freedom, the variance of the measuring error was calculated with 646 datum points); [i] bold value indicates that hypothesis 2 is accepted by t test; [k] bold value indicates that hypothesis 3 is accepted by t test.

Table 4 shows that hypothesis 1 and hypothesis 2 are accepted for most of the experiments and hypothesis 3 is accepted for almost all experiments.

In two cases (curves L65 and L66) there were very large deviations between the confidence interval of the measured lag phase duration and the model predictions. In Fig. 4 the data for these temperature shift experiments are plotted. The kinetic behaviour appears to be reasonably well predicted, except for the dying during incubation at 4°C. However, if the time axis of the part of the curve after the temperature shift is extended (Fig. 5), it can be seen that the predicted lag time is much smaller than the actual lag time. In these experiments the first incubation is around the minimal temperature of growth [T_{min} value is 3.29°C (Table 1)]. Incubation at very low temperatures (around or below T_{min}) may be damaging to the cells, resulting in (slow) dying, and increased lag times, when transferred to higher temperatures. More work is needed to quantify these effects. These two experiments will not be taken into account for the following. For no other cases a significant effect could be found resulting from the time of the shift, the first or second incubation temperature, or the size of the step.

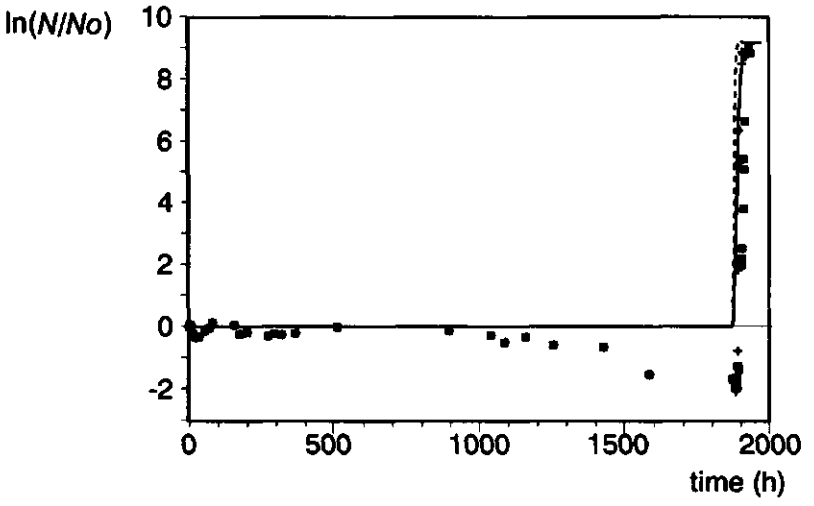

FIG. 4. Growth curve with a shift from 4°C to 20 (L65) and 35°C (L66) at 1873 h compared with model prediction (▪ , ——— = 20°C; + , -------- = 35°C).

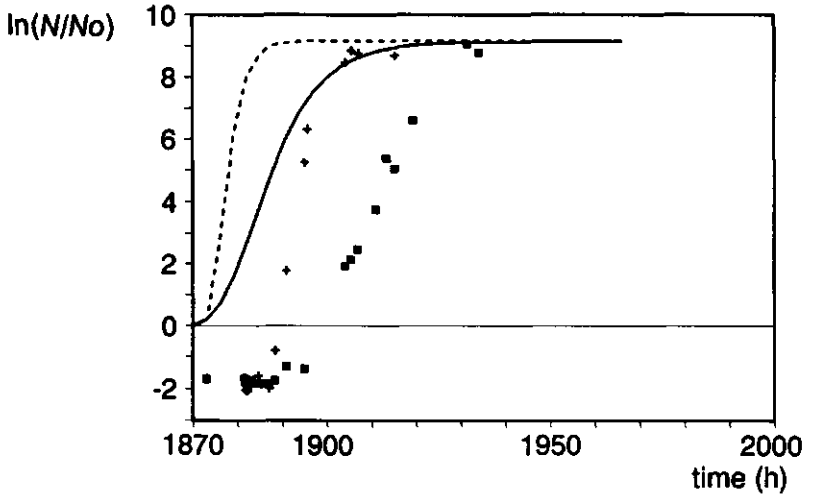

FIG. 5. Growth curve after a shift from 4°C to 20 (L65) and 35°C (L66) at 1873 h compared with model prediction (▪ , ——— = 20°C; + , -------- = 35°C).

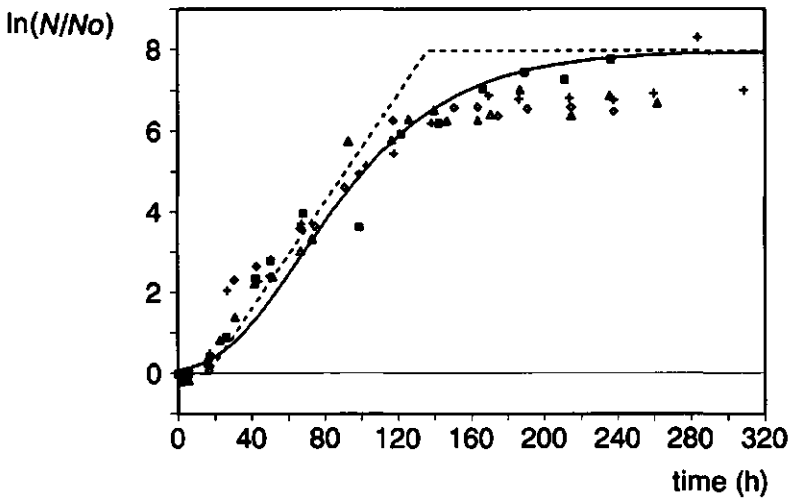

FIG. 6. Growth curves with a shift from 25°C to 10°C at 1.25 h compared with model prediction (——— = Gompertz with hypothesis 3; -------- = linear with hypothesis 2;▪ L2,+ L3,• L4,▲ L5).

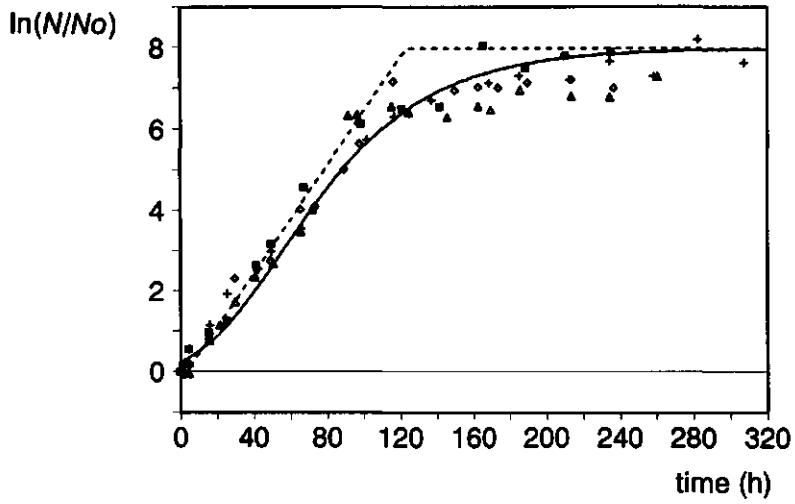

FIG. 7. Growth curves with a shift from 25°C to 10°C at 2.5 h compared with model prediction (——— = Gompertz with hypothesis 3; -------- = linear with hypothesis 2; ▪ L7, + L8, • L9, ▲ L10).

TABLE 5. Results of temperature shifts within the exponential phase

no[a]	T_1	T_2	t_s[b]	n[c]	λ_1	λ_2[d]	t_s^*[f]	λ[g]	min[h]	max[i]	$\alpha = \frac{1}{4}$[k]
E1	8.9	14.2	119	21	41.79	11.06	2.848	4.51	1.39	7.63	**2.76**
E2	8.9	16.5	119	17	41.79	7.55	2.848	4.82	2.33	7.31	1.89
E3	8.9	17.8	119	17	41.79	6.26	2.848	3.00	0.94	5.06	**1.56**
E4	8.9	20.1	119	17	41.79	4.67	2.848	2.70	1.10	4.30	**1.17**
E5	14.0	24.0	72.5	15	11.47	3.10	6.320	-0.22	**-1.77**	1.33	**0.78**
E6	14.0	24.0	70.0	17	11.47	3.10	6.102	0.56	**-0.92**	2.04	**0.78**
E7	14.0	24.0	43.0	6	11.47	3.10	3.748	0.49	**-1.27**	2.25	**0.78**
E8	14.0	24.0	25.0	11	11.47	3.10	2.179	0.92	**-0.26**	2.10	**0.78**
E9	14.0	24.0	20.0	12	11.47	3.10	1.743	0.67	**-0.53**	1.88	**0.78**
E10	14.0	24.0	17.5	14	11.47	3.10	1.525	0.35	**-0.93**	1.62	**0.78**
E11	14.7	20.0	64.5	15	10.11	4.73	6.380	1.04	**-1.10**	3.18	**1.18**
E12	14.7	20.0	43.0	14	10.11	4.73	4.254	1.09	**-0.85**	3.04	**1.18**
E13	14.7	20.0	24.0	14	10.11	4.73	2.374	1.77	**-0.25**	3.80	**1.18**
E14	14.7	20.0	19.0	16	10.11	4.73	1.879	1.19	**-0.31**	2.69	**1.18**
E15	14.7	20.0	16.0	16	10.11	4.73	1.583	1.17	**-0.28**	2.62	**1.18**
E16	14.7	20.0	13.5	17	10.11	4.73	1.335	1.27	**-0.23**	2.78	**1.18**
E17	16.7	10.4	26.5	16	7.32	26.02	3.618	7.75	**-0.86**	16.36	**6.50**
E18	16.7	12.5	26.5	16	7.32	15.51	3.618	4.93	**-0.85**	10.71	**3.88**
E19	16.7	14.6	26.5	15	7.32	10.29	3.618	5.29	0.82	9.76	**2.57**
E20	16.7	18.8	26.5	15	7.32	**5.48**	3.618	3.07	0.28	5.85	**1.37**
E21	18.0	13.9	20.0	21	6.09	11.69	3.284	7.99	4.34	11.64	2.92
E22	18.0	22.1	20.0	17	6.09	3.74	3.284	1.71	0.14	3.27	**0.94**
E23	18.0	13.9	32.0	16	6.09	11.69	5.254	2.36	**-3.17**	7.89	**2.92**
E24	18.0	22.1	32.0	12	6.09	**3.74**	5.254	1.70	**-1.09**	4.50	**0.94**
E25	40.8	25.0	18.0	10	2.35	2.83	7.653	0.47	**-1.68**	2.63	**0.71**
E26	40.8	25.0	3.50	13	2.35	**2.83**	1.488	2.27	0.74	3.80	0.71
E27	9.0	14.0	50.5	15	40.34	**11.47**	1.252	6.69	**-0.07**	13.46	**2.87**
E28	8.0	20.7	70.0	8	59.29	**4.36**	1.181	4.04	1.78	6.30	1.09
E29	11.7	16.6	22.5	14	18.60	**7.43**	1.210	4.44	0.07	8.80	**1.86**
E30	14.2	17.3	18.5	11	11.06	6.71	1.673	2.52	1.13	3.91	**1.68**
E31	14.2	20.5	18.5	12	11.06	4.46	1.673	1.75	0.39	3.11	**1.11**
E32	14.5	24.8	20.0	12	10.47	2.88	1.910	-0.01	**-1.25**	1.23	**0.72**
E33	14.5	30.3	20.0	11	10.47	1.91	1.910	-0.13	**-1.02**	0.76	**0.48**
E34	14.5	34.8	20.0	10	10.47	1.58	1.910	0.17	**-0.58**	0.92	**0.39**
E35	15.3	30.5	19.0	5	9.13	**1.88**	2.082	0.24	**-1.48**	1.97	**0.47**
E36	15.0	25.0	22.0	9	9.60	2.83	2.292	0.67	**-0.47**	1.81	**0.71**
E37	16.6	11.7	22.5	17	7.43	18.60	3.027	0.15	**-9.97**	10.26	**4.65**
E38	16.8	8.0	20.0	15	7.22	59.29	2.772	14.22	**-9.36**	37.79	**14.82**
E39	20.2	8.0	20.0	15	4.62	59.29	4.331	1.69	**-34.30**	37.68	**14.82**
E40	25.0	14.2	4.50	10	2.83	11.06	1.590	4.15	**-0.73**	9.02	**2.76**
E41	25.0	17.3	4.50	10	2.83	6.71	1.590	1.16	**-2.11**	4.43	**1.68**
E42	25.0	18.9	4.50	10	2.83	5.41	1.590	1.22	**-1.28**	3.72	**1.35**
E43	25.0	9.0	6.50	28	2.83	**40.34**	2.296	24.71	8.05	41.37	**10.08**
E44	25.0	11.0	6.50	26	2.83	22.13	2.296	0.06	**-7.48**	7.61	**5.53**
E45	26.0	36.8	3.00	9	2.60	1.57	1.155	0.31	**-0.36**	0.98	**0.39**
E46	30.7	10.1	2.00	9	1.86	28.36	1.075	10.35	1.04	19.65	**7.09**
E47	30.6	10.5	3.00	8	1.87	25.30	1.603	5.47	**-5.36**	16.30	**6.33**
E48	35.0	20.0	2.00	9	1.57	4.73	1.272	-0.71	**-2.21**	0.79	1.18
E49	35.0	20.0	6.00	6	1.57	4.73	3.817	-0.04	**-1.68**	1.60	**1.18**
E50	35.0	25.0	2.50	10	1.57	2.83	1.590	0.24	**-0.90**	1.38	**0.71**
E51	35.0	25.0	6.00	6	1.57	2.83	3.817	0.04	**-1.06**	1.13	**0.71**
E52	35.0	30.0	2.00	11	1.57	1.94	1.272	-0.11	**-0.74**	0.52	**0.49**
E53	35.0	30.0	6.00	6	1.57	1.94	3.817	-0.13	**-1.12**	0.87	**0.49**

[a]Experimental number, bold code = curve plotted in graph (Fig. 10 to 13); [b]t_s = time of shift (h); [c]n = number of datum points in growth curve; [d] bold value indicates that hypothesis 1 is accepted by t test; [f]$t_s^* = t_s/\lambda_1$; [g]λ = estimated lag time from growth data; [h]min = minimum 95% confidence limit of lag time, bold value indicates that $\alpha = 0$ is within the confidence interval (hypothesis 2); [i]max = maximum 95% confidence limit of lag time, 95% confidence intervals are calculated with $t = 1.96$ (at infinite degrees of freedom, the variance of the measuring error was calculated with 646 datum points); [k] bold value indicates that hypothesis 3 is accepted by t test.

Table 5 shows that hypothesis 1 is accepted for only a few experiments. Hypothesis 2 is accepted for most of the experiments and hypothesis 3 is accepted for almost all experiments.

In Table 6 the total number of accepted cases of each hypothesis is given for all curves (except for the curves L65 and L66). Table 6 shows that hypothesis 1 is accepted only in 47% of the cases and does not appear to be appropriate (see also Fig. 3). Hypothesis 2 is accepted in most of the cases (73%) and hypothesis 3 is accepted in almost all cases (93%). Therefore, hypothesis 3 appears to be the most appropriate.

TABLE 6. Acceptance rates of the three hypotheses (% between brackets)

Hypothesis	Lag phase	Exp. phase	Total
1	49 (71)	8 (15)	57 (47)
2	51 (74)	38 (72)	89 (73)
3	65 (94)	48 (91)	113 (93)
Total	69	53	122

Predictions compared with experiments. Now the measured data can be compared to the model predictions. For several temperature shift experiments growth data and model predictions (following hypothesis 3 with $\alpha = 0.25$) are compared. For shifts within the lag phase examples are given in Fig. 6 to 9. The model predictions have been calculated using equations 12 and 13 (see Appendix). Curves L3 and L4 are examples for which hypothesis 3 was rejected.

In Fig. 10 to 13 results of temperature changes at different times are given. For shifts within the exponential phase the model predictions have been calculated with equations 14 and 15 (see Appendix). It can be seen that the predictions agree very well with the measured data. It should be noted that the predictions arise from models based on earlier growth data (6) at constant temperature, so no fitting occurred.

Hypothesis 2 was accepted in many cases (73%) and for simulation purposes this assumption is more convenient, especially if there are many temperature changes during the shelf life of a product or during dynamically changing temperatures. In Fig. 8 and 9 the predictions using this assumption ($\alpha = 0$) are also given. It can be seen that this model also describes the kinetic behaviour of the data well enough for a number of practical applications. Curves L51, L52, and L59 are examples for which hypothesis 2 was rejected, for temperature shifts within the lag phase.

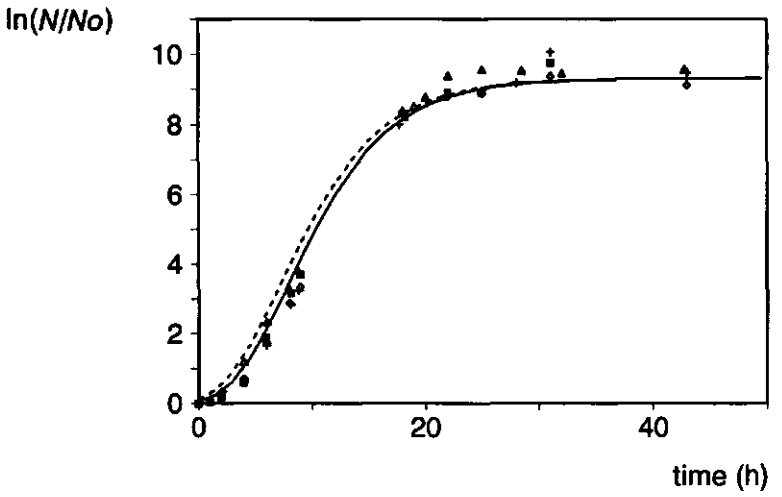

FIG. 8. Growth curves with a shift from 10°C to 25°C at 6 h compared with model prediction (——— = Gompertz with hypothesis 3; -------- = Gompertz with hypothesis 2; ▪ L50, + L51, ◆ L52, ▲ L53).

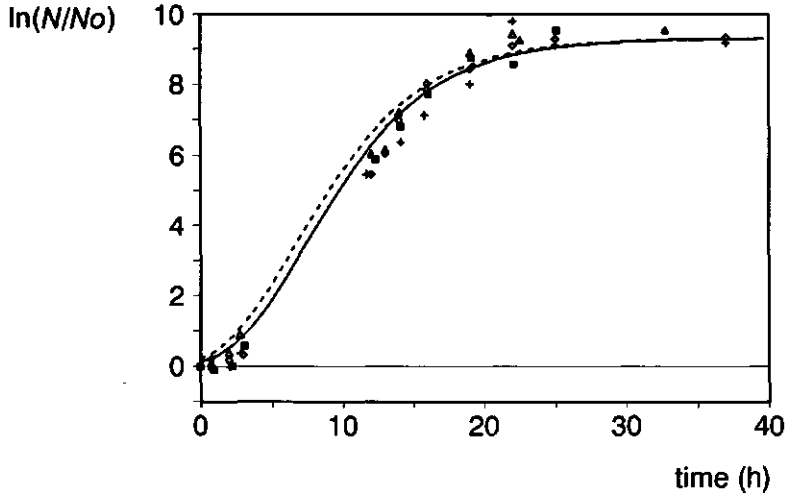

FIG. 9. Growth curves with a shift from 10°C to 25°C at 12 h compared with model prediction (——— = Gompertz with hypothesis 3; -------- = Gompertz with hypothesis 2; ▪ L57, + L58, ◆ L59, ▲ L60).

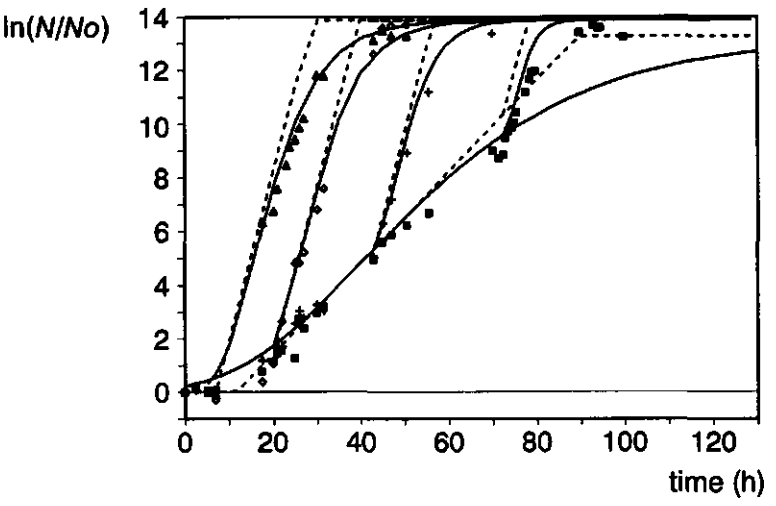

FIG. 10. Growth curves with shifts from 14°C to 24°C compared with model prediction (——— = Gompertz with hypothesis 3; -------- = linear with hypothesis 2; ▪ E5, + E7, ◆ E9, ▲ L67).

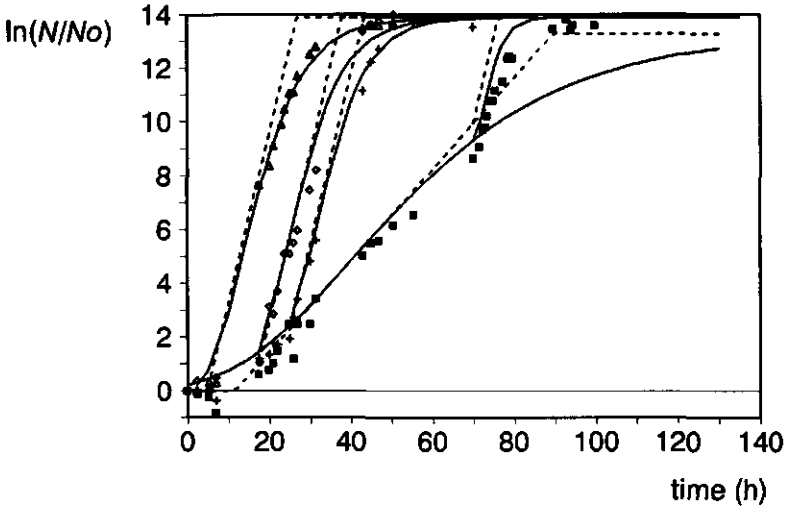

FIG. 11. Growth curves with shifts from 14°C to 24°C compared with model prediction (——— = Gompertz with hypothesis 3; -------- = linear with hypothesis 2; ▪ E6, + E8, ◆ E10, ▲ L68).

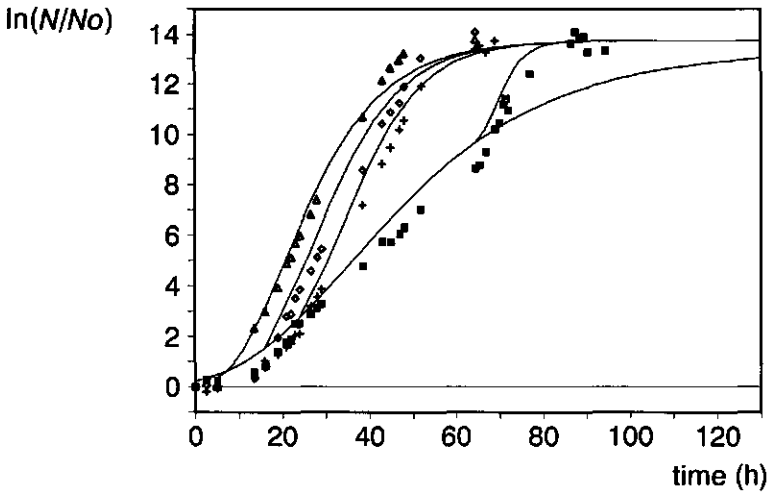

FIG. 12. Growth curves with shifts from 15°C to 20°C compared with model prediction (—— = Gompertz with hypothesis 3; ▪ E11, + E13, ◆E15, ▲ L69).

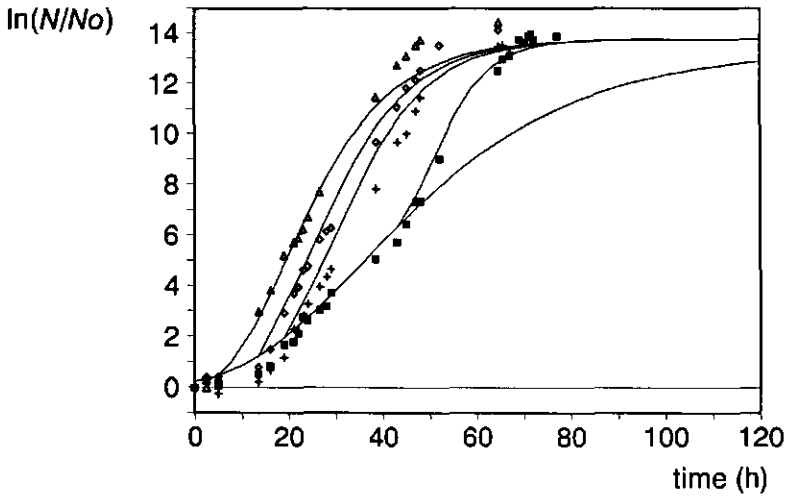

FIG. 13. Growth curves with shifts from 15°C to 20°C compared with model prediction (—— = Gompertz with hypothesis 3; ▪ E12, + E14, ◆ E16, ▲L70).

By using both hypotheses 2, and the linear growth model (equation 1) instead of the modified Gompertz model, the calculations become easier. The predictions of the linear model (with the use of hypothesis 2) are compared with the modified Gompertz model (with hypothesis 3), in Fig. 6, 7, 10 and 11. Curve L68 is an example for which hypothesis 3 was rejected. The linear model gives comparable results to the modified Gompertz model, only where the growth curve bends off towards the asymptote, the linear model fails to describe the data. The asymptotic level [$\ln(N/N_o)$] is dependent strongly on the inoculum level, and shows often large experimental error (6, 8). Moreover, the asymptotic level is often of no real practical importance, since food products are generally spoiled, before the asymptotic level is reached. For a number of applications the global kinetic behaviour will be sufficient and the linear model could be chosen, since this model has the advantage of simplicity.

CONCLUSIONS

It can be assumed that temperature shifts within the lag phase can be handled by adding relative parts of the lag time still to be completed and that temperature shifts within the exponential phase result in no additional lag. These two assumptions were accepted statistically in more than 70% of the experiments. The kinetic behaviour was well predicted using these assumptions. The hypothesis that a temperature shift results in an additional lag phase of one-fourth of the lag time normally found at the second temperature is accepted in more than 90% of the experiments. This observation shows that the bacteria are exposed to stress by a shift in temperature in the lag phase as well as in the exponential phase.

With this knowledge growth curves can be predicted with the modified Gompertz equation. For a number of practical applications the procedure can be simplified by neglecting the additional lag time. Also the linear growth equation predicts data within a certain range and therefore can be preferred when simplicity of the function is preferred.

Shifts of temperature around the minimum temperature of growth showed firstly dying of the cells and secondly very large deviations compared to the model prediction. This observation indicates the need for more experimental work around the minimum temperature of growth.

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APPENDIX: Equations for predicting microbial growth

Prediction of bacterial growth at constant temperature. If the temperature remains constant, the bacterial growth can be predicted by using the modified Gompertz equation (equation 2) and using the growth parameters (A, μ and λ) calculated from Table 1.

Equations for predicting microbial growth with temperature changes.

1) Using hypothesis 3 ($\alpha = 0.25$)

Temperature shift within the lag phase ($t_s \leq \lambda_1$, $t_s^* \leq 1$). The lag time to be completed after the shift within the lag phase can be calculated with equation 6. With $\alpha = 0.25$ this results in:

$$\lambda_{shift} = (1 + \alpha - t_s^*) \cdot \lambda_2 = \left(1.25 - \frac{t_s}{\lambda_1}\right) \cdot \lambda_2 \tag{12}$$

Subsequently, the modified Gompertz equation can be used to calculate the growth at times after the shift in temperature:

$$y = A_2 \exp\left\{-\exp\left[\frac{\mu_2 \cdot e}{A_2}(\lambda_{shift} - [t - t_s]) + 1\right]\right\} \tag{13}$$

Temperature shift within the exponential phase ($t_s > \lambda_1$, $t_s^* > 1$). Before the time of shift, the modified Gompertz equation can be used to describe the bacterial growth and the number of organisms at time of shift [$y_1(t_s) = y_s$] can be predicted:

$$y_1(t) = A_1 \exp\left\{-\exp\left[\frac{\mu_1 \cdot e}{A_1}(\lambda_1 - t) + 1\right]\right\} \qquad 0 \leq t \leq t_s \tag{14}$$

After the shift in temperature growth continues from y_s with an additional lag λ_{shift}. This results in:

$$y_2(t) = y_s + (A_2 - y_s) \exp\left\{-\exp\left[\frac{\mu_2 e}{A_2 - y_s}(\lambda_{shift} - [t - t_s]) + 1\right]\right\} \tag{15}$$

$$t > t_s \qquad y_s = y_1(t_s) \qquad \lambda_{shift} = \alpha \cdot \lambda_2 = 0.25 \cdot \lambda_2$$

2) Using hypothesis 2 ($\alpha = 0$)

Temperature shift within the lag phase ($t_s \leq \lambda_1$, $t_s^* \leq 1$). The lag time to be completed after a shift within the lag phase can be calculated by assuming $\alpha = 0$ in equation 6:

$$\lambda_{shift} = (1 + \alpha - t_s^*) \cdot \lambda_2 = \left(1 - \frac{t_s}{\lambda_1}\right) \cdot \lambda_2 \tag{16}$$

Subsequently, the modified Gompertz equation (equation 13) can be used to calculate the growth at times after the shift in temperature.

Temperature shift within the exponential phase ($t_s > \lambda_1$, $t_s^* > 1$). The number of organisms before the time of shift can be predicted by equation 14. The number of organisms after the time of shift can be predicted by:

$$y_2(t) = y_s + (A_2 - y_s)\exp\left\{-\exp\left[\frac{\mu_2 e}{A_2 - y_s}(-[t - t_s]) + 1\right]\right\} \tag{17}$$

$$t > t_s \qquad y_s = y_1(t_s)$$

3) Using linear growth

It will be even more convenient to use linear growth and hypothesis 2 ($\alpha = 0$).

Temperature shift within the lag phase ($t_s \leq \lambda_1$, $t_s^* \leq 1$). The lag time to be completed after a shift within the lag phase can be calculated by equation 16. After the lag time is completed exponential growth will occur until the asymptote is reached:

$$\begin{aligned} y &= 0 && t - t_s \leq \lambda_{shift} \\ y &= \mu_2 \cdot (t - t_s - \lambda_{shift}) && \lambda_{shift} < t - t_s < A_2/\mu_2 + \lambda_{shift} \\ y &= A_2 && t - t_s \geq A_2/\mu_2 + \lambda_{shift} \end{aligned} \tag{18}$$

Temperature shift within the exponential phase ($t_s > \lambda_1$, $t_s^* > 1$). The number of organisms before the time of shift can be predicted by:

$$\begin{aligned} y &= 0 && t \le \lambda_1 \\ y &= \mu_1 \cdot (t - \lambda_1) && \lambda_1 < t < t_s \end{aligned} \tag{19}$$

and the growth after the time of shift (t_s) results in:

$$\begin{aligned} y &= \mu_1 \cdot (t_s - \lambda_1) + \mu_2 \cdot (t - t_s) && t_s \le t \le \frac{A_2 - \mu_1 \cdot (t_s - \lambda_1)}{\mu_2} + t_s \\ y &= A_2 && t > \frac{A_2 - \mu_1 \cdot (t_s - \lambda_1)}{\mu_2} + t_s \end{aligned} \tag{20}$$

This equation can be expanded very easily to more temperature shifts.

Various temperature shifts. If many temperature shifts occur or during dynamically changing temperature conditions it will be better and easier to use hypothesis 2 ($\alpha = 0$) and linear growth. The modified Gompertz equation is far to complex to be used with many shifts.

Temperature shift within the lag phase. The lag time to be completed after many shifts within the lag phase can be calculated by assuming $\alpha = 0$ in equation 6 and adding all relative parts of the lag time that are completed until one is reached:

$$\Phi = \sum \frac{\Delta t_i}{\lambda_i} \text{ until } \Phi = 1 \tag{21}$$

or for dynamically changing temperatures:

$$\Phi = \int \frac{dt}{\lambda} \text{ until } \Phi = 1 \tag{22}$$

Temperature shift within the exponential phase. As soon as $\Phi = 1$ the exponential phase sets in. The time at which $\Phi = 1$ is defined as t_Φ.

$$\begin{aligned} y &= 0 && t \le t_\Phi \\ y &= \mu_1 \cdot (t - t_\Phi) && t_\Phi < t < t_{s1} \end{aligned} \tag{23}$$

and the growth after the first time of shift (t_{s1}) in the exponential phase results in:

$$y = \mu_1 (t_{s1} - t_\Phi) + \mu_2 (t - t_{s1}) \qquad t_{s1} \le t \le t_{s2} \tag{24}$$

and the growth after the second time of shift (t_{s2}) in the exponential phase results in:

$$\begin{aligned} y &= \mu_1 (t_{s1} - t_\Phi) + \mu_2 (t_{s2} - t_{s1}) + \mu_3 (t - t_{s2}) \\ t_{s2} < t &< \frac{A - \mu_1 (t_{s1} - t_\Phi) - \mu_2 (t_{s2} - t_{s1})}{\mu_3} + t_{s2} \end{aligned} \tag{25}$$

until the asymptote is reached:

$$y = A \qquad t \ge \frac{A - \mu_1 (t_{s1} - t_\Phi) - \mu_2 (t_{s2} - t_{s1})}{\mu_3} + t_{s2} \tag{26}$$

CHAPTER 7

A DECISION SUPPORT SYSTEM FOR PREDICTION OF THE MICROBIAL SPOILAGE IN FOODS

ABSTRACT

A method was developed to combine qualitative and quantitative information to predict possible growth of microorganisms in foods. The pH, water activity, temperature, and oxygen availability of foods are coupled to the growth characteristics of microorganisms. Therefore, a database with characteristics of foods and a database of kinetic parameters of microorganisms were built. In the first database, a tree structure based on physical similarity was built, for the case that information about the characteristics of a particular food is unknown. The product information can be estimated by comparing with similar products at the same level of the tree or the level above. A method is developed to make an estimation of the microbial growth kinetics on the basis of models. This is done by introducing a growth factor, which can be calculated on the basis of readily available data from literature. Since all the information can be altered, the system can give better predictions when more and more accurate information is added.

INTRODUCTION

Food Quality. Food quality can be defined as the sum of the characteristics of a food that determines the satisfaction of the consumer and compliance to legal standards. Thus, food quality is a combination of numerous factors, such as organoleptic properties (e.g., texture, flavor, color), nutritional value (e.g., caloric content, fatty acid composition), and safety conditions (e.g., microbial number, toxins, hormones). Some of these factors (e.g., microbial numbers) can be quantified relatively easily, others (e.g., flavor) are very difficult to assess quantitatively. Food quality thus cannot be quantified in every detail, and overall

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quantification depends strongly on the priority of the different aspects determining the quality. To determine the total food quality, quality indicators are needed and must be weighted, depending on the product, trends, producer, and market.

Food quality is gaining more and more interest for a number of reasons. The food market deals in most cases with satiation; therefore, quality becomes more important than quantity. There are new quality attributes which are highly appreciated by the modern consumer (in contrast to traditional quality demands). Consumers show an increasing interest in convenience foods with the appearance and taste of fresh products and food quality aspects such as flavor and (assumed) health aspects (e.g., nutrition, fatty acid quantity and composition, energy content, salt concentration, additives, such as preservatives).

Prediction of the kinetics of possible quality loss is important for the following reasons. Consumers are willing to pay a higher price for quality. The manufacturer wants to produce constant quality products at the lowest costs. Many products have a limited shelf life. Production and storage conditions affect quality very strongly; therefore, production and distribution are often critical. From the past there are many dried, salted, frozen, and sterilized products, while nowadays chilled and intermediate-moisture foods are becoming more important. The shelf life of a product should match the distribution regime: daily delivery for perishable products such as fresh milk, bread, fresh vegetables, and fresh meat, or less frequently as for salads, margarine, etc. Distribution routes have become longer, and therefore, there is a need for an increase in shelf life. In some areas there is a rather rapid product development (changes in product formulation). Consequently, it will be very useful to make an estimation of the shelf life during product development. Formulation of products may be different in different countries or regions, because of legal requirements or regional food preferences. Therefore, it would be useful to know the effects of different compositions on the shelf life, so that each formulation does not require a laborious shelf-life test.

New techniques are being developed to meet these quality demands, such as new technologies (e.g., microwave, ultrahigh temperature processes, modified atmosphere packaging, irradiation), and new strategies (e.g., logistics, and modeling).

Quality loss. Quality loss can be a result of microbial, chemical, enzymatic, or physical reactions. Different factors influence quality loss, such as the composition of the product, and the processing and storage conditions. Deterioration reactions may occur when the physical variables of the product are within the range of the specific reaction. For quantification of the reaction rate, the kinetics of the reaction should be known. This can be done by models that include the range in which spoilage can occur and the physical variables of the product. As

soon as good models are available and values for the physical variables are estimated, the rate of spoilage processes can be predicted. The resulting quantitative estimation can be compared with quality parameters.

A considerable amount of research has been conducted on the deterioration of food (e.g., 2, 10). This research yielded a large amount of quantitative data. Also, much qualitative information is available. Yet, for a quantitative prediction of the quality of a given food product, there are often not enough data, or the data show too large a measuring error to be used. It is difficult to combine a broad range of information, from qualitative to quantitative. However, it will be very useful to combine all this information in a structured manner, to predict product quality in the best possible way.

The objective of this work is to develop a system in which quantitative data can be combined in a structured manner with qualitative data, quantitative information, and models. Such a system would be a useful tool in product development, predicting possible spoilage and estimating the kinetics of possible deterioration. As soon as more data, knowledge, or new models are gathered, this can be added, resulting in more valuable predictions.

SYSTEM STRUCTURE

The system. A system was developed that determines the possible growth of microorganisms on a certain food product. Numerous factors can influence growth. From the most important factors, pH, water activity (a_w), temperature (T), and oxygen availability are taken into account. These physical properties of the product are compared with the properties of the microorganisms (Fig. 1). The physical variables of various foods are collected in a database (database 1) as are the growth data of microbes (database 2). The data for each microorganism are matched with the physical variables of the food product, by simply determining if the physical variables of the product are within the growth limits of that microorganism ("pattern matching"). For these organisms, an estimate of the growth rate is calculated on the basis of the value of the physical variables. The estimation of growth is carried out by using models that describe growth over the range of physical variables.

The list of organisms that may spoil the product is sorted by growth rate. This list can be altered by applying rules to diminish the number of organisms in the list or to improve the value of the predictions. Some of these rules are dependent on the product, some on the properties of organisms, and some are of general application. In this way the final list of organisms is obtained, which is now based on physical variables of the product, growth parameters of microorganisms, models, and qualitative reasoning.

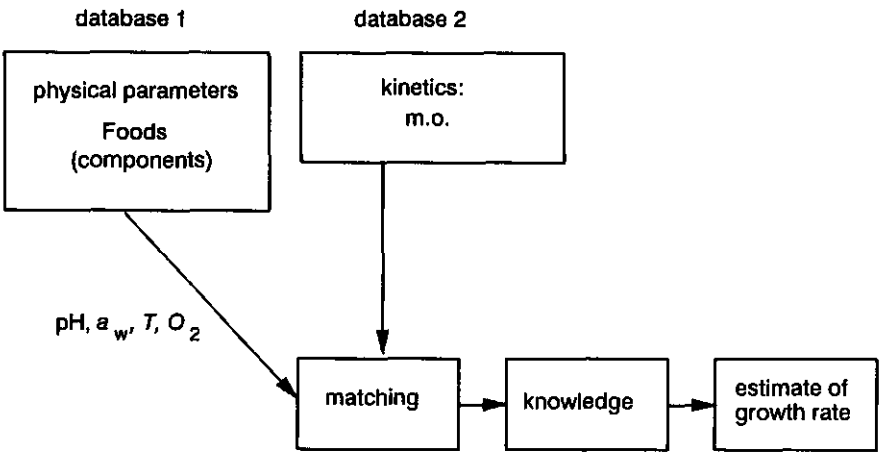

FIG. 1. Structure of the system.

TABLE 1. An example of the information stored in the product database

Name	yogurt
Identification code	S.A.B.A.C
Temperature (T)	5
pH	4.2
a_w	0.990
Oxygen availability	no oxygen

It should be noted that spoilage of a product will only occur if an organism is present and able to grow in that product. Until now it is assumed that all organisms can be present everywhere. This can result in predictions of growth of organisms that are unlikely to be present in such a product. Therefore, knowledge of the presence of organisms on certain products can be included in the information database. Another possibility is that the usual microflora of products is included in the food database. However, organisms that up to now have not caused problems in a product are often not known to be present in a product. If the composition or processing of such a product is changed, these organisms may start to cause problems. Therefore, it is assumed that all organisms can be present everywhere.

Food database. Database 1 (Fig. 1) contains the physical variables of different foods, as shown in Table 1. The physical variables are known only for a limited number of products. The remainder contains name and identification code (I.D.). The I.D. of a product determines the position of the product in the classification tree (Fig. 2). The first letter (S) stands for food, addition of the second for the first subdivision of food [dairy (S.A), bakery (S.D), vegetables (S.F), meat (S.H), etc.]. These groups of products are divided by addition of the third letter of the I.D., etc.

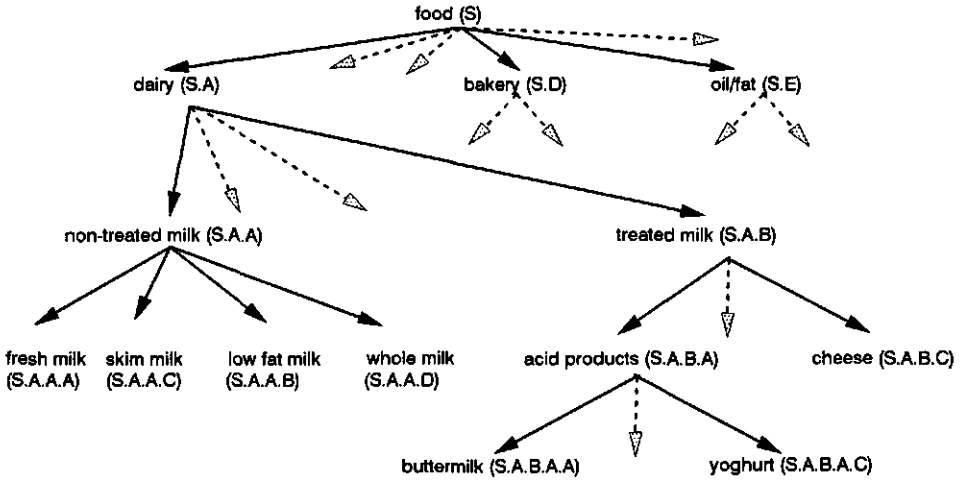

FIG. 2. Structure of database 1, in which foods are sorted by their physical characteristics.

The number of food products is more or less infinite and, as may be expected, not all the physical variables of all different foods are known. Therefore, the database is structured in such a way that the products are sorted with respect to their physical properties so that foods that are grouped together are closely related (Fig. 2). The classification system proposed by Jowitt (4) based on the physical properties of foods is therefore used. In such a way, missing information, even when a product is absent, can be substituted with knowledge from comparable products.

Moreover, the physical variables can always be altered. If comparable products are not found, estimated variables can be entered.

Organism database. A database is built for microorganisms, to contain the information as given in Table 2. The database contains the name of the organism and the growth ranges of the physical variables: oxygen requirement, the minimum and maximum pH, a_w, and temperature. Additionally, the optimum growth rate and the optimum values of pH and temperature are stored to be used in kinetic models. Furthermore, the Gram staining, type, and spore-forming abilities are stored to be used for further selection procedures (qualitative reasoning) or future use. The possibility exists to alter all information.

TABLE 2. An example of the information stored in the organism database

Genus				*Pseudomonas*		
Species				*putida*		
Oxygen necessity				aerobic		
Type (bacterium, yeast, mould)				bacterium		
Gram stain (only for bacteria)				negative		
Spore forming				no		
T:	min	max	opt	-8	43	30
pH:	min	max	opt	4.7	8.5	7.8
a_w:	min	max	opt ≈ max	0.96	1.000	
optimum growth rate (h^{-1})				1		

Selection of organisms that can grow on a particular product. As soon as the physical variables of a product are known, a matching is carried out with the organisms database. All the organisms that can grow on that product on the basis of the physical variables are found by "pattern matching". First, the total list of organisms is reduced to those organisms that can grow at the pH of the product, then at the temperature of the product, and then at the a_w of the product. Lastly, the effect of the availability of oxygen is included. The procedure results in a list of organisms which can grow in a certain product with particular physical variables.

Kinetic models. To estimate the growth rate of organisms at suboptimum conditions for T, a_w, and pH, models have to be used. The growth rate can be estimated using models relating growth at the actual value of a variable to the optimum value and the limits. Each variable that is not at the optimum value can reduce the growth rate. Therefore, a method to combine these effects must be established. This is done by introducing a growth factor:

$$\gamma = \frac{\mu}{\mu_{opt}} \tag{1}$$

with μ the actual growth rate (h^{-1}), μ_{opt} the growth rate (h^{-1}) at optimum conditions, and γ the actual growth factor.

This growth factor is equal to 1 at optimum conditions and between 0 and 1 for all other conditions. Others have shown that the inhibitory effect of temperature and a_w and the effect of temperature and pH can be multiplied (1, 6). It is assumed therefore that the growth factor can be calculated by multiplying all $\gamma(x)$ values, with $\gamma(x)$ defined for each of the variables separately, independent of the value of the other variables:

$$\gamma = \gamma(T) \cdot \gamma(\mathrm{pH}) \cdot \gamma(a_w) \cdot \gamma(O_2) \tag{2}$$

If all variables are at optimum conditions, the growth rate is equal to μ_{opt}. If one of the variables is below the minimum or above the maximum value, this results in one of the γ's to be zero, resulting in a growth rate of zero.

Each $\gamma(x)$ factor can be determined from the database data, in combination with a model for that variable. In the microorganism database, the minimum, optimum, and maximum temperatures for growth of different organisms can be found. If these data are known, the parameter c of the Ratkowsky equation (9) can be calculated:

$$\mu = \left(b\,(T - T_{\min})\{1 - \exp[c(T - T_{\max})]\} \right)^2 \tag{3}$$
$$T_{\min} \le T \le T_{\max}$$

with b, c, T_{min}, T_{max}: Ratkowsky parameters, and T the actual temperature (°C)
If c is known, the growth factor $\gamma(T)$ for each temperature value can be evaluated with:

$$\gamma(T) = \frac{\mu}{\mu_{opt}} = \left(\frac{(T - T_{\min})\{1 - \exp[c(T - T_{\max})]\}}{(T_{opt} - T_{\min})\{1 - \exp[c(T_{opt} - T_{\max})]\}} \right)^2 \tag{4}$$

The value of c can be calculated from the known T_{min}, T_{max}, and T_{opt} as follows. The derivative of equation 3 can be calculated as:

$$\frac{d\mu}{dT} = 2 \cdot b(T - T_{min})\{1 - \exp[c(T - T_{max})]\} \cdot$$
$$b\left\{1 - \exp[c(T - T_{max})] - c \cdot (T - T_{min}) \cdot \exp[c(T - T_{max})]\right\} \quad (5)$$

At $T=T_{opt}$, this derivative is zero. Since b cannot equal zero and T_{opt} cannot equal T_{min} or T_{max}, the first part of the equation cannot equal zero. Therefore, the second part of the equation must be zero:

$$1 - \exp[c(T_{opt} - T_{max})] - c \cdot (T_{opt} - T_{min}) \cdot \exp[c(T_{opt} - T_{max})] = 0 \quad (6)$$

This can be rewritten as:

$$1 - (cT_{opt} - cT_{min} + 1) \cdot \exp[c(T_{opt} - T_{max})] = 0 \quad (7)$$

c can be calculated iteratively from this equation, to be used in equation 4.

The same procedure is carried out for the pH. The same formula from Ratkowsky (9) is used, only T is substituted for pH:

$$\mu = \left(f(pH - pH_{min})\{1 - \exp[g(pH - pH_{max})]\}\right)^2$$
$$pH_{min} \le pH \le pH_{max} \quad (8)$$

with f, g, pH_{min}, pH_{max}: Ratkowsky parameters, and pH the actual pH.

$$\gamma(pH) = \frac{\mu}{\mu_{opt}} = \left(\frac{(pH - pH_{min})\{1 - \exp[g(pH - pH_{max})]\}}{(pH_{opt} - pH_{min})\{1 - \exp[g(pH_{opt} - pH_{max})]\}}\right)^2 \quad (9)$$

with g to be calculated from:

$$1 - (gpH_{opt} - gpH_{min} + 1) \cdot \exp[g(pH_{opt} - pH_{max})] = 0 \quad (10)$$

McMeekin et al. (6) show that the growth rate is linear with a_w at suboptimum water activity levels. For the water activity therefore, a linear relation is assumed:

$$\gamma(a_w) = \frac{a_w - a_{w,\min}}{1 - a_{w,\min}} \qquad (11)$$
$$a_w \geq a_{w,\min}$$

with $a_{w,min}$ minimum water activity, a_w actual water activity.

Oxygen availability is used as a selection parameter ($\gamma(O_2) = 0$ or 1, Table 3). For oxygen availability, this segmentation model is used, since for most microorganisms the growth kinetics as a function of the oxygen availability are not known. The same is true for the oxygen concentration in products. If models and model parameters are known, this can be altered, since the segmentation model as shown in Table 3 is a very rigorous one.

The combination model selected and used here is not yet thoroughly validated. However, it can be used to make kinetic predictions, although the numerical value is indeed a prediction only. Yet, a good estimate is made about the growth rate. Whenever more knowledge is present for models describing the effect of the variables used here, these models can be incorporated. It should be noted that there are no correlation effects assumed between T, a_w, pH, and O_2.

TABLE 3. The effect of the availability of oxygen on $\gamma(O_2)$

	Organism		
	Aerobic	Facultative anaerobic	Anaerobic
Product with oxygen	1	1	0
Product with very little oxygen	0	1	0
Product with no oxygen	0	1	1

There are many more variables determining the growth rate of microorganisms, such as preservatives (sorbic and benzoic acid, alcohol, nitrite). These compounds will often be present in specific products, such as alcohol in alcoholic beverages and nitrite in meat products. The effect of these compounds can be incorporated by applying knowledge rules. This method is better than the use of kinetic models, since at present models and model parameters are not well established for these compounds.

Only if all information is present can exact predictions be made. This is an impossible situation; therefore, every result will always be an approximation.

Addition of qualitative reasoning. Information can be added to the system to decrease the number of possible organisms that can cause problems. Four types of rules are implemented in the system (Table 4); i) relationship between microorganisms and product characteristics (example: In high moisture food and low acid products molds and yeasts will be overgrown by bacteria if the temperature is below 35°C); ii) interaction among microorganisms (example: Antagonists of *Salmonella* spp. are lactic acid bacteria); iii) interaction among microorganisms in combination with the product [example: On meat if either *Pseudomonas* spp., *Moraxella* spp., or *Acinetobacter* spp. is present, all three are likely to be present (7)]; iv) general rules (example: If pasteurization is carried out, only thermoduric organisms will survive).

Before these rules are applied, the user is asked if this rule is applicable, since they can be too stringent sometimes.

TABLE 4. Knowledge rules used in the system

On raw meat, yeasts and molds will be overgrown by bacteria.
On raw fish, yeasts and molds will be overgrown by bacteria.
On products that are comparable to bread, only molds will grow.
On meat, the *Achromobacter*-combination will be present: if *Pseudomonas* spp., or *Moraxella* spp. or *Acinetobacter* spp. is present, all three are likely to be present (7).
On vegetables, only yeasts and molds will grow.
On fruits, only yeasts and molds will grow.
On products with a low water activity, spore-forming microorganisms can be present (no growth).
If bacteria are present, they will overgrow molds in low acid and high moisture products.
If bacteria are present, they will overgrow yeasts in low acid and high moisture products.
If the growth rate of an organism is less than 10% of that of the fastest growing organism on that product, this organism will be overgrown.
If the growth rate of an organism is zero, this organism will be overgrown.
Antagonists of *Clostridium perfringens* : Lactic acid bacteria.
Antagonists of *Salmonella* spp. : Lactic acid bacteria.
Antagonists of *Staphylococcus aureus* : Lactic acid bacteria.

RESULTS AND DISCUSSION

The program was developed using TURBO-Pascal 5.0 (Borland). No expert system shells are used, because a shell that exactly fulfils our needs could not be found. Furthermore,

programming in a second generation language has the advantage that all necessary procedures can be programmed. Then the problem does not need to be fitted into the possibilities of a shell.

The system is started entering the name of a food. The program then searches database 1 for the name of that food, or foods, with a very similar name. Within this list of names, a final selection can be made for the food of interest. The physical variables of this product are displayed when present in the database. If the physical variables of the product are not known, comparable products (if any) are given (Fig. 2). An estimation of the physical variables can be obtained by a selection from this list.

With the physical variables of the food, a matching is carried out with the organisms database. The organisms that can grow on that product considering the physical variables (pH, T, a_w, and oxygen availability) are determined on basis of the growth ranges of the organisms by "pattern matching". This results in a list of microorganisms that can cause spoilage together with the growth factor (equation 2). Now rules can be applied to diminish the number of organisms in the list or to improve the value of the predictions. The knowledge rules that are implemented are given in Table 4.

In total, 845 products are collected in database 1 at present. For 153 of these products, this database contains information as given in Table 1. In total, 20 gram-negative bacteria, 19 gram-positive bacteria, 10 yeast species, and 9 molds are incorporated in database 2 at present. If the optimum growth rate of organisms is not known, for bacteria 1 h^{-1}, for yeasts 0.5 h^{-1}, and for molds 0.1 h^{-1} is assumed.

Open structure. The parameters of the organisms, the physical variables of the products, and the qualitative rules can be altered and expanded. It should be noted, however, that the value of the system is dependent mainly on the quality of the data that are stored. Therefore, changes in the databases can only be made by authorized users.

Output. Two lists of possible spoilage organisms are generated. The first list contains the possible spoilage organisms, based on physical variables. The second list contains the knowledge rules that are applied and the list of spoilage organisms that are likely to spoil the product, after the knowledge rules are applied. As output, not only the final list should be considered, the first list also contains valuable information. (For example, if pasteurization is carried out, the final list will give no thermosensitive organisms. However, if the product is contaminated after pasteurization, the thermosensitive organisms can be of interest).

The possibility exists to remove certain groups of organisms (gram positive, gram negative, all nonspore-forming organisms, molds, and yeasts). This can be valuable for instance if a product is pasteurized (e.g., remove all nonspore-forming organisms).

Example for milk. In the following part, an example for milk stored at refrigeration temperature is given.

Selection of organisms. During storage of milk, a temperature of 5°C can be achieved. The atmosphere is aerobic. In the components database, the following data can be found for milk: pH = 6.6; $a_w = 0.993$. In the organism database, the data of all organisms are examined. For instance, for *Pseudomonas* the data are given in Table 2. Combining this information results in the deduction that *Pseudomonas* can grow in milk stored at 5°C. If this is carried out for all organisms, the system comes up with 11 bacteria, 6 molds, and 6 yeast species (Table 5). All bacteria are reported to grow in milk at 5°C by Gilmour and Rowe (3).

If this exercise was done for skim milk, it may have appeared that the database did not have the physical variables for skim milk. The system would then have searched for a product that is physically comparable to skim milk, which would have been milk (Fig. 2).

Kinetic estimation. For all organisms that can grow on milk, the growth factor will be calculated (equation 2). This will be done as an example for *Pseudomonas*.

For the temperature, equation 7 can be used, using the kinetic data of *Pseudomonas* (Table 2), resulting in:

$$1 - (38c + 1) \cdot \exp(-13c) = 0 \tag{12}$$

This equation can be solved iteratively and results in $c = 0.143$. With equation 4, the $\gamma(T)$ can be calculated at every temperature.

$$\gamma(T) = \left(\frac{(T+8)\{1 - \exp[0.143(T-43)]\}}{38\{1 - \exp[0.143(-13)]\}} \right)^2 = \left(\frac{(T+8)\{1 - \exp[0.143(T-43)]\}}{32.08} \right)^2 \tag{13}$$

For the pH, equation 10 can be used resulting in:

$$1 - (3.1g + 1) \cdot \exp[g(-0.7)] = 0 \tag{14}$$

This equation can be solved iteratively and results in $g=3.55$. With equation 9, the $\gamma(pH)$ can be calculated.

TABLE 5. Prediction of the microorganisms that can grow in milk stored at 5°C (alphabetized)

Lit.	Bacteria
*	*Acinetobacter*
*	*Aeromonas*
*	*Bacillus subtilis*
*	*Brochotrix* spp.*(thermosphacta)*
*	*Enterobacter* spp. + *Enterobacter aerogenes (aerobacter)*
*	*Lactobacillus plantarum*
*	*Listeria* spp.*(monocytogenes)*
*	*Moraxella* spp.
*	*Proteus* spp.
*	*Pseudomonas fluorescens* + *Pseudomonas putida/fragi*
*	*Yersinia enterocolitica*
	Yeasts
	Rodotorula spp.
*	*Candida* spp.*(macedoniensis)*
*	*Debaryomyces* spp.*(hansenii)*
	Hansenula spp. *(anomala)*
	Pichia spp.*(membranaefaciens)*
*	*Torulopsis* spp.*(psychrophilica)* + *Torulopsis candida*
	Molds
	Aspergillus spp.
	Botrytis spp.
	Cladosporium spp.
	Geotrichum candidum (o.lactis)
	Mucor spp.
*	*Penicillium* spp.

* = microorganisms able to grow in milk at 5°C given by (3).

$$\gamma(\mathrm{pH})=\left(\frac{(\mathrm{pH}-4.7)\{1-\exp[3.55(\mathrm{pH}-8.5)]\}}{3.1\{1-\exp[3.55(-0.7)]\}}\right)^2= \\ =\left((\mathrm{pH}-4.7)\frac{\{1-\exp[3.55(\mathrm{pH}-8.5)]\}}{2.842}\right)^2 \tag{15}$$

For the a_w, equation 11 can be used resulting in:

$$\gamma(a_w)=\frac{a_w-0.96}{0.04} \tag{16}$$

For milk, the following growth factors are calculated:

for T=5, $\gamma(T)=0.163$;
for pH=6.6, $\gamma(\mathrm{pH})=0.446$; and
for a_w=0.993, $\gamma(a_w)=0.83$.

$$\gamma=\gamma(T)\cdot\gamma(\mathrm{pH})\cdot\gamma(a_w)=0.060 \\ \mu_{opt}=1\ \mathrm{h}^{-1} \tag{17}$$

Now the growth rate can be estimated with equation 1.

$$\mu=\gamma\cdot\mu_{opt}=0.060 \tag{18}$$

Addition of qualitative reasoning. In the reasoning, the knowledge about the heat treatment of the milk can be added to the system. If no pasteurization is used, we have to deal with the natural contamination of milk. The bacteria will grow much faster than the yeasts and the molds (because milk has a very high water activity and a neutral pH). An estimation of the growth rate can be made on the basis of models, describing the effect of the physical variables on the growth rate. In the example given above, the conditions in milk are not optimum for *Pseudomonas*; therefore, the growth rate will be smaller than the optimum growth rate. It is assumed that the fastest growing organisms will cause problems (10% rule). If this knowledge is used, the system comes up with four species (Table 6), including *Pseudomonas* and *Enterobacter* which are reported to be main spoilers of milk (3, 5, 8). The kinetic predictions are not yet very precise.

If a pasteurization is carried out, only the thermoduric organisms will survive. This results in 1 bacterium (*Bacillus*), 6 yeasts, and 6 molds. As the spores of yeasts and molds are less likely to grow out very fast, it can be concluded that the bacterium *Bacillus* will be the main spoiler. Of course, the product may be contaminated after the heat treatment; therefore, the information that is produced before the effects of the heat treatment is of importance. It must be stressed that all the information, from the beginning to the end, must be studied, as it is always a result of a number of models, i.e., simplification.

TABLE 6. Microorganisms that are predicted to cause spoilage in milk stored at 5°C

Species	Growth rate (h^{-1})	Literature value
Enterobacter	0.322	0.075 (5)
Proteus	0.134	-
Pseudomonas	0.060	0.18 (8)
Brochotrix	0.057	-

Possible expansion of the system. A great deal of information is present on quality loss processes in foods, depending on composition, process variables, and kinetics. It could be useful to develop a system in which this information is combined. The system can be expanded by including chemical, enzymatic, and physical spoilage in the same manner as described. In this way, effects of a large number of changes in the product or process can be evaluated. This can be done for instance for the addition of onions to a salad dressing (microbial, enzymatic, physical). The effect of chemical and microbial spoilage when a heat treatment is carried out at a higher temperature can be evaluated, as can the effect of storage in a modified atmosphere (if the effects of gases are known). For new product development, the possible spoilage reactions, the order of magnitude of these reactions, the approximate shelf life, and distribution temperature can be evaluated. By using more or less complicated models and kinetic parameters, predictions can be made of these deterioration reactions.

CONCLUSIONS

A system was developed which shows a promising potential for product development and shelf-life prediction. Quantitative and qualitative information and predictive models can be combined to predict possible spoilage reactions, with an estimate of their kinetics, on the basis of models. To that purpose, a database was built and filled with physical variables of foods, as was a database with organisms with their growth limits for the same physical

variables. A combined model was built to be able to make a kinetic estimation, on basis of the data in the databases. Furthermore, an information base is built which can be used to add qualitative information concerning products and microorganisms. The system combines all this information. Since it is impossible to collect quantitative data for all possible deterioration reactions on different products, a prediction is made on the basis of the data and knowledge collected in the system until now.

The program can help to determine possible spoilage organisms. It can estimate the change in growth rates of organisms, when the physical properties are changed. It should be noted that the program does not give an exact, complete list of all possible spoilage organisms, since it is based on limited information. The more information that is combined and added to the program, the better the predictions will be.

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CHAPTER 8

CONCLUDING REMARKS:

EXTENDED USE OF PREDICTIVE MODELING

ABSTRACT

The kinetics of food deterioration reactions are important for the optimization of food chains. Different types of models for prediction of deterioration kinetics are briefly discussed. Examples of the use of models are given. Some possibilities for expanding modeling techniques into decision support systems are given. Predictive models, kinetic data, expertise, logistics, and simulation and optimization routines can be combined to support decisions in production, distribution, and product development.

PREDICTIVE MODELING

Values and needs in predictive modeling. In Chapter 1 the most important needs in predictive modeling were mentioned. Some of these needs have been given attention in this thesis:

- A procedure was developed to estimate the maximum specific growth rate, lag time and asymptote out of growth data (see Chapters 2 and 3).
- Formal criteria were proposed to discriminate between models (Chapters 2, 4, 5 and 6).
- A model for the effect of temperature on bacterial growth was validated with a large number of experimental data (Chapters 4 and 5).
- A model was developed to take into account shifts in temperature (Chapter 6); This model was validated with a large number of experimental data.

Part of this chapter was used for the publication:
Some Aspects of Modelling Microbial Quality of Food
M.H. Zwietering, F.M. Rombouts, and K. van 't Riet (1993)
Food Control 4:89-96

- A procedure was developed to link quantitative and qualitative information in a structured manner (Chapter 7).

The work done in this thesis mainly focuses on the effect of temperature, since temperature is a variable that can be easily changed along a chain, without inherent product changes. In general, literature focuses on the variables T, a_w, and pH. To be able to make predictions for different formulations of a product, more work is needed on the effect of different product characteristics, i.e. on following subjects:

- Models to describe the effect of additional variables, e.g. gas atmosphere, presence of organic acids, preservatives.
- Integrated models for the simultaneous effect of different factors, and the determination of interaction terms.

Relevance of modeling. For the validation of the models numerous measurements are needed. However, once the model is validated, predictions can be made with none, or only very few, experiments, and insight into the process is gained. With the square-root model of Ratkowsky et al. (11), microbial product spoilage by *Lactobacillus plantarum* can be predicted if temperature, pH, and a_w are known (Fig. 1). Moreover, an estimate can be made of the effect of changes in the process, for example of uncooled transport. The difference in product quality (e.g., microbial count), and the difference in the shelf-life can be calculated easily (Fig. 1). Furthermore, it can be easily seen (Fig. 1) that quality is mainly lost in this case during storage by the consumer. This insight into kinetics is a great advantage of the modeling procedure compared to fragmentary sampling for quality control.

Types of models. In order to build and/or validate models, large amounts of experimental data must be gathered. Fig. 2 illustrates the procedure to validate a model for the effect of temperature on microbial growth. First, a number of growth curves is measured at different temperatures. These growth data are then analysed with a growth model, e.g. Gompertz (6). In doing so, the values of the kinetic parameters are estimated. Then these parameters of the different growth curves at different temperatures can be used to select a model that describes the effect of temperature on these parameters. In Fig. 2, the expanded square root model of Ratkowsky (10) is given as an example. This model then can be used to predict growth curves at any temperature, and graphs such as Fig. 1 can be calculated. The same procedure can also be used for other variables such as pH and a_w. In this manner, a total model can be developed for microbial spoilage.

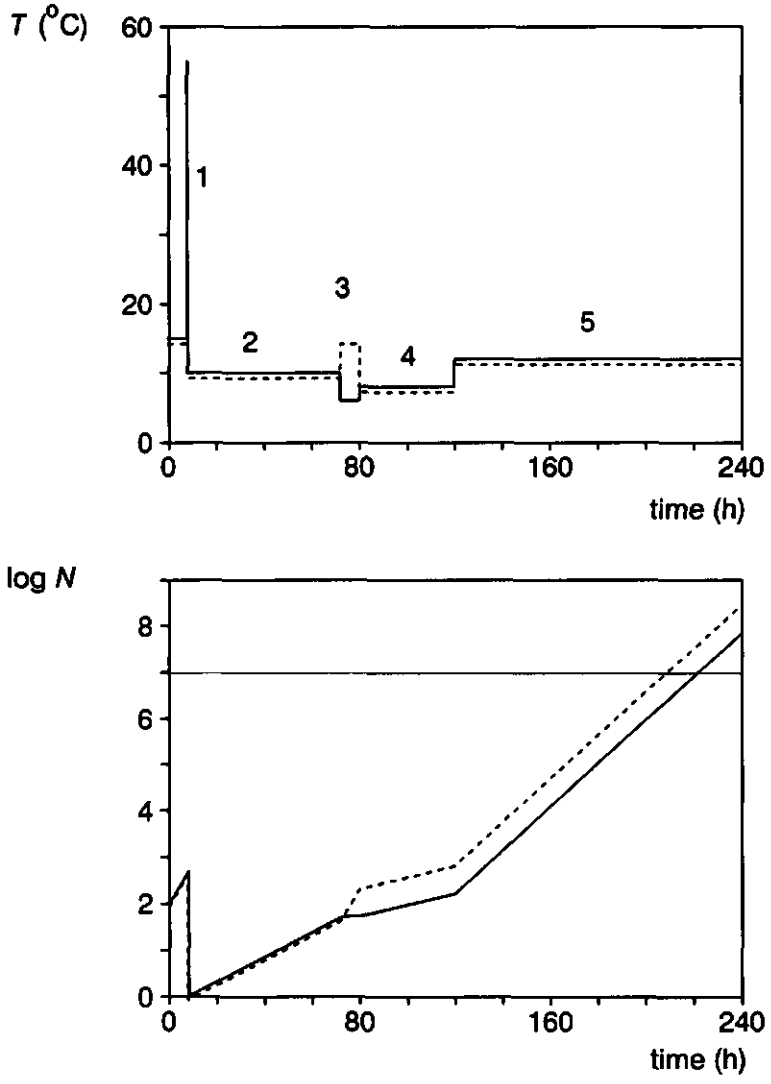


FIG. 1. Temperature history (example) and calculated development of the number of organisms. 1=production and pasteurisation; 2=storage; 3=distribution (cooled and uncooled); 4=retail; 5=consumer storage. ——— cooled transport; -------- uncooled transport; ——— spoilage level, T= temperature, N= number of organisms (cfu/g).

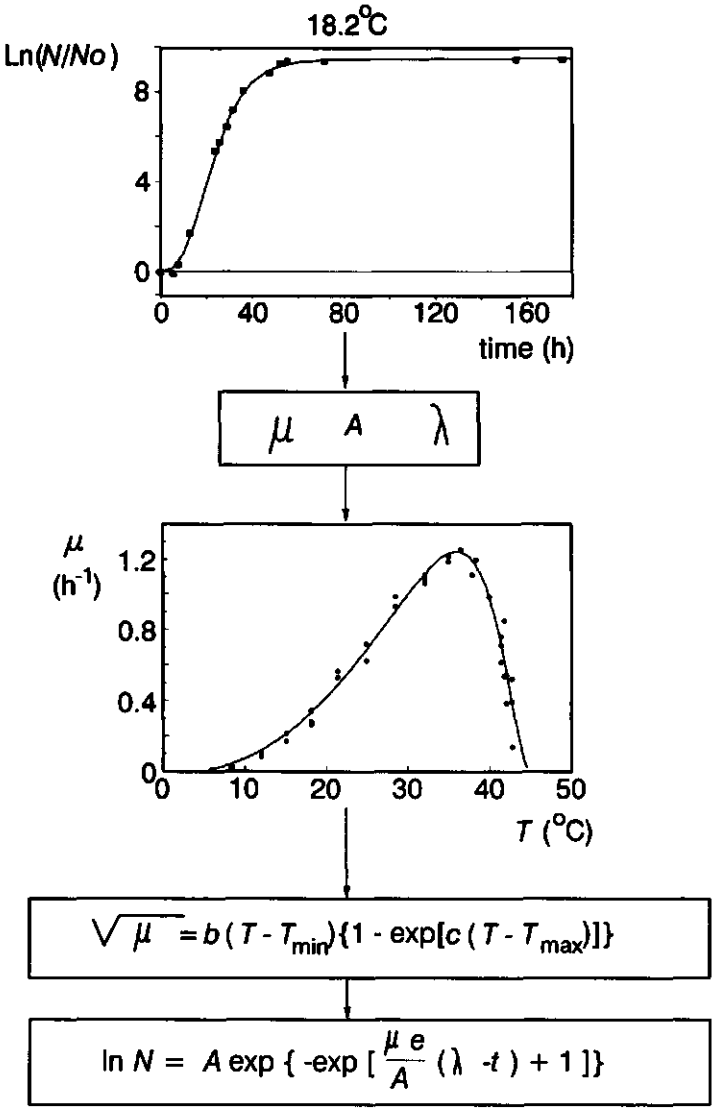


FIG. 2. Pathway of model building for bacterial growth as function of temperature (μ = specific growth rate (h^{-1}), A = asymptotic level, λ = lag time (h), N = Number of organisms (cfu/ml), N_o = number at time zero, e=exp(1), t = time (h), b, c, T_{min}, T_{max} are Ratkowsky parameters).

To describe the effect of physical and chemical conditions on deterioration, many models are already proposed and many new ones can be generated. Selection procedures are therefore needed. It is difficult to discriminate between models. Some advantages and disadvantages of different modeling techniques will be presented.

Polynomial models: Polynomial models have the advantage that they are easy to use (linear regression can be used), very straightforward and no knowledge is needed of the process that is described. The disadvantages are: they do not contribute to knowledge about the mechanisms underlying the process, they often have numerous parameters, because of the numerous terms they can become unclear, problems can be encountered by fitting data that disagree totally with the chosen polynomial function, and they are valid simply and solely within the range of variables of the underlying measurements.

Empirical models: Empirical models (other than polynomials) have the advantage that they can describe any curvature, and often need only few parameters. They do not contribute much to insight, although often more so than polynomial models. Also these models can be used only in a limited interval.

Mechanistic models: Mechanistic models have the advantage that they may increase the insight into the process. They often can be combined more easily with other mechanistic models. On the other hand, they often contain a large number of parameters and also are applicable within a limited range. Often the model does not exactly describe what is happening and discrepancies are tolerated, to keep the advantages of the mechanistic model. In an Arrhenius plot, for instance, it is not unusual for the data to deviate from linearity in one or more temperature regions [The Arrhenius model (1) was given a fundamental basis by Eyring (4, 5)].

Use of models. In this thesis it has been shown that several descriptions of the effect of temperature can be used to predict the growth of *Lactobacillus plantarum*. The temperature is often one of the main variables that determine deterioration kinetics. To get a first quantification of the development of the microbial numbers in a chilled product, several growth curves of the main spoiler(s) have to be measured at different temperatures. The growth parameters can be estimated from the resulting bacterial numbers using the modified Gompertz equation (see Chapter 2). Firstly the effect of temperature (at suboptimum temperatures) on the maximum specific growth rate can be described by the square-root equation of Ratkowsky (11):

$$\sqrt{\mu_m} = b\,(T - T_{min}) \qquad (1)$$

With linear regression, the parameters b and T_{min} can be estimated. At different temperatures the maximum specific growth rate can be calculated from these parameters. If it is assumed that the bacteria are adapted to the environment (no lag time), that substrate levels are sufficient, that metabolic product concentrations are not relevant, and that the initial spoilage level (N_o) is known, then exponential growth can be assumed and the number of bacteria in time can be calculated from:

$$N(t) = N_o \exp(\mu_m \cdot t) \tag{2}$$

With the parameters determined from these experiments we can calculate the microbial quality at different temperatures, at any time during storage. Also the shelf-life (Θ) can be calculated at different temperatures, if the maximum allowed spoilage level (N_θ) is known:

$$\theta = \frac{\ln\left(\frac{N_\theta}{N_o}\right)}{b^2 \cdot (T - T_{min})^2} \tag{3}$$

It is shown in Chapter 6 that in most cases the effect of temperature steps can be neglected (immediate change of growth rate at a change of temperature) and that the exponential growth, preceded by an eventual lag phase gives a reasonable prediction of the development of the microorganisms. The above mentioned procedure can already give valuable results concerning the progression of deterioration.

An example. As stated, a large amount of experimental data is essential to build and validate models. As soon as a model is assumed to be correct it can be used with a much smaller amount of experimental data. If, for instance, a chilled salad is mainly spoiled by *Lactobacillus*, the growth rate of these bacteria is of importance. If the product should remain unchanged, i.e. pH, water content, antimicrobial agents etc. are fixed, the temperature is the variable left to control the spoilage. If equation 1 is used and the growth rate of *Lactobacillus* at three temperatures (say 15°C, 20°C, and 25°C) is measured, the parameters T_{min} and b can be calculated by linear regression, resulting in the following equation for the data given in Table 1:

$$\sqrt{\mu_m} = 0.03(T - 5.25) \tag{4}$$

The data and the regression line are given in Fig. 3. If we assume exponential growth we get:

$$\ln\left(\frac{N}{N_o}\right) = 0.0009(T-5.25)^2 \cdot t \tag{5}$$

TABLE 1. Growth of lactobacilli in a salad (example)

T (°C)	μ_m (h^{-1}) (measured)	Regression parameters	μ_m (h^{-1}) (predicted)
15	0.092	b=0.030	0.086
20	0.177	T_{min}=5.25	0.196
25	0.364		0.351

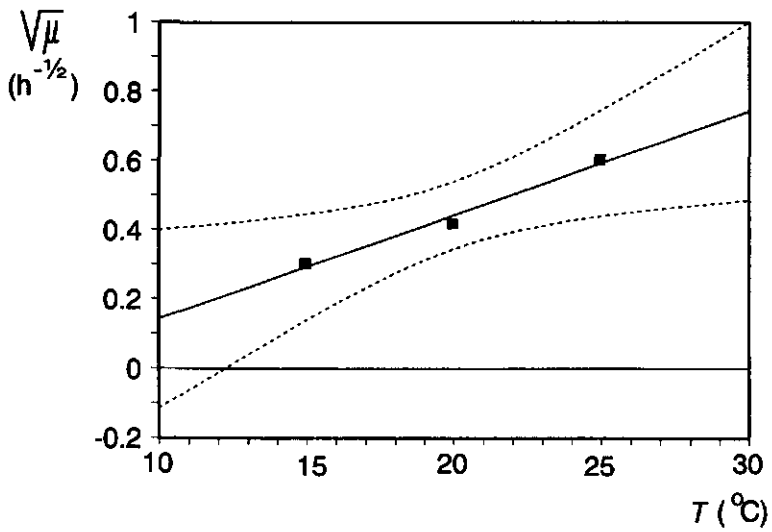


FIG. 3. Ratkowsky plot of the specific growth rate of *Lactobacillus* in a salad (with 90% confidence interval).

With these three experiments equation 5 gives the microbial load at any desired temperature between 15°C and 25°C, at any time during storage, if the initial spoilage level is known. There is also some confidence that the model can be extrapolated to cover a larger

range, i.e. from 10°C up to 30°C. Also the shelf-life (Θ) can be calculated (equation 3) at any desired temperature, if the maximum allowable spoilage level is known (Table 2, Fig. 4). In Fig. 4 we see that the extrapolation to 10°C is rather questionable. Although the extrapolation to 10 °C seems appropriate for the growth rate (Fig. 3), the use of this growth rate to calculate a shelf-life at 10°C (Fig. 4) is statistically seen not acceptable.

TABLE 2. Shelf-life of a salad (example)

Initial spoilage level:	10^2 lactobacilli/g
Maximum spoilage level:	10^5 lactobacilli/g
T (°C)	Shelf-life (h)
15	81
20	35
25	20
10	340 (extrapolation !)
30	13 ,,

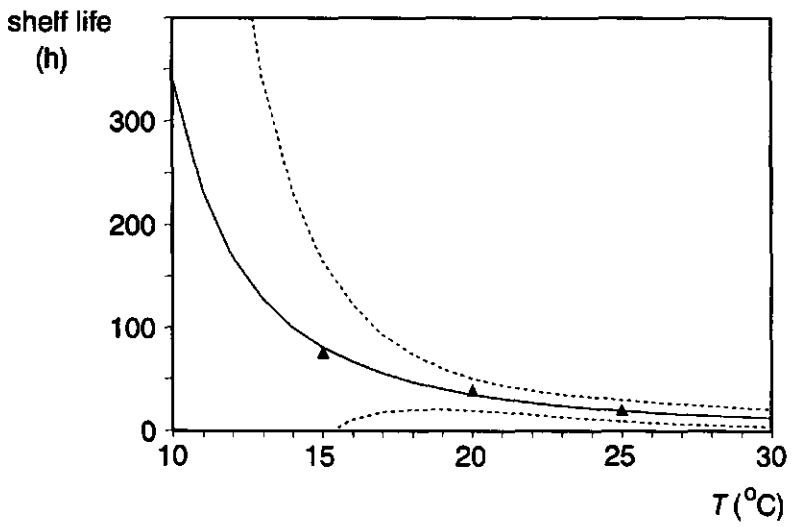


FIG. 4. Predicted shelf-life of a salad as function of temperature (with 90% confidence interval). Line represents shelf-life calculated with predicted growth rate, datum points represent shelf-life calculated with measured growth rate data.

As shown, we can get preliminary insight from only a few experiments, once we have a good model. Effects of temperature and initial spoilage level can be examined. It should be noted, however, that more datum points give more accurate parameter values (with smaller confidence intervals) and thus more accurate predictions. Furthermore, for every case appropriate assumptions must be made.

The square-root relation (equation 1) is validated on par-fried fries and on chicken. Specific growth rates at different temperatures are taken from literature data and from own unpublished data. These measurements were all at suboptimum temperatures, therefore the square-root relation can be used. The square-root of the specific growth rate is plotted versus temperature, and the data are very well described by a straight line (Fig. 5 and 6). The fact that the specific growth rates of both Wieringa and Viering (13) and Michener et al. (8) match very well is very promising for the use of predictive models, since they were obtained in studies using Dutch and American potatoes, in different laboratories (Fig. 5). Furthermore, the total aerobic count is used, and the linear behaviour over a considerable range of temperatures shows that there is no change resulting in a new flora with a totally different kinetic behaviour. Also, the data on chicken (Fig. 6) agree very well. The data of Regez et al. (12) were aerobic counts on chicken skin, our own unpublished data are *Pseudomonas* on chicken skin, and the data of Pooni and Mead (9) and Barnes (2, 3) were *Pseudomonas* in Heart Infusion Broth. These data from different laboratories, with different strains, on different media all agree. The Ratkowsky parameters of these curves are given in Table 3.

TABLE 3. Ratkowsky parameters on fries and chicken

	b ($°C^{-1}h^{-0.5}$)	T_{min} (°C)
Fries	0.0215	-7.36
Chicken	0.0301	-6.04

The main spoilage organism on both products was *Pseudomonas* spp, and both parameter sets are of comparable order of magnitude. These examples demonstrate that with relatively few experiments, or even with literature data only, a simple model can be constructed which can be used for (preliminary) shelf-life prediction and optimization.

If the spoilage level of chicken is $5 \cdot 10^7$ (c.f.u./g) and the initial contamination after production is 10^3 (c.f.u./g) and the storage temperature is 0°C, the shelf-life can be calculated with equation 3 to be 14 days. In the distribution chain, it is difficult to maintain a temperature of 0°C. If a producer has control over the distribution during the first 5 days and keeps the temperature at 0°C, the number of bacteria can be calculated with equation 2 to increase to

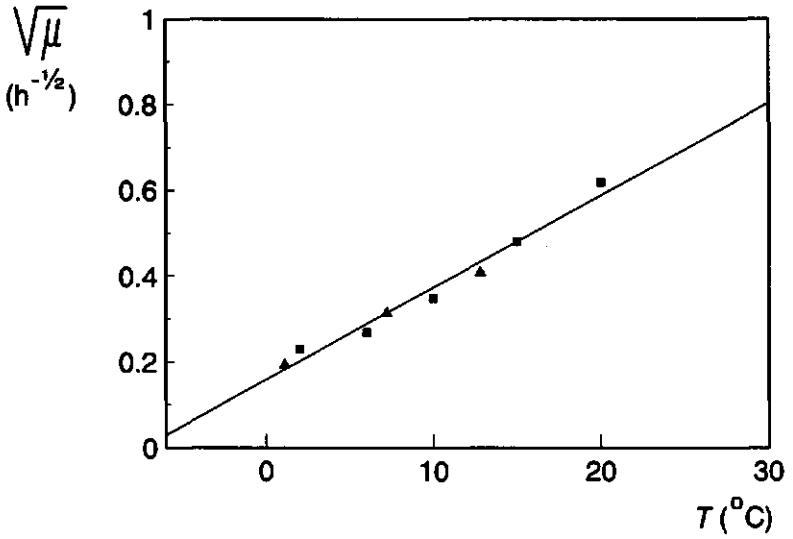


FIG. 5. Ratkowsky plot for aerobes on fries.
■ Wieringa (13), ▲ Michener(8).

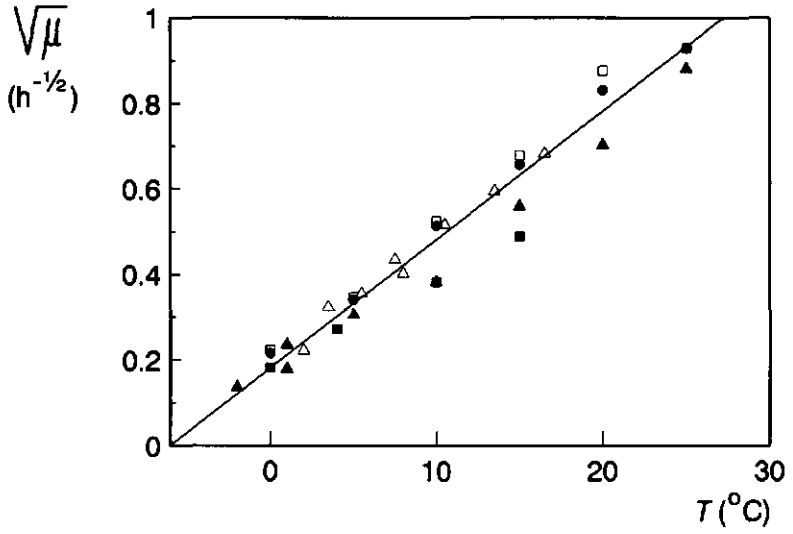


FIG. 6. Ratkowsky plot for *Pseudomonas* on chicken.
■ Regez (12), ▲ Barnes (2,3), □ ● Pooni and Mead (9), △ unpublished data.

$5 \cdot 10^4$. If the product is then kept at 4°C (e.g., during retail display) the remaining shelf-life can be calculated to be 3 days. So the total shelf-life is reduced from 14 days to 8 days and the shelf-life during retail display is reduced from 9 days to 3 days. This can also be shown graphically (Fig. 7). This kind of calculations can be used to show the importance of supercooling to all parties participating in the food chain.

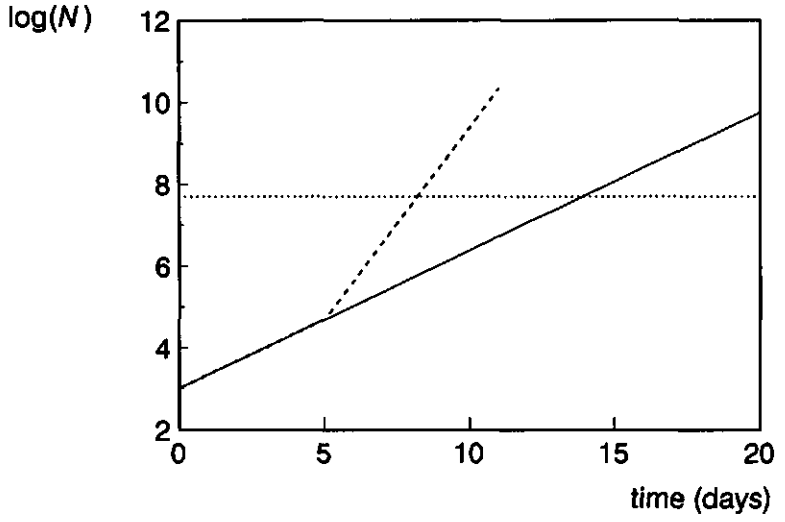


FIG. 7. Spoilage of chicken at 0°C and 5 days at 0°C and the remainder at 4°C (—— 0°C; -------- 5 days 0°C, then 4°C; ····· spoilage level); N in c.f.u./g).

EXPANSION OF THE MODELS

These simple models can be expanded in several ways (resulting in more complex equations):

- Incorporate a lag time and asymptote (See Chapters 4, 5 and 6).
- Use of a sigmoidal growth curve for the predictions (see Chapters 2 and 6).
- Use of the expanded square-root equation (necessary if also temperatures around or above the optimum temperature are of importance) (Chapter 4).
- Incorporate the effect of heat transport (see Heat Transport, below).
- Incorporate logistics (operations research) (see Logistics, below).
- Include other quality reactions, for instance chemical or physical (see Other Deterioration Reactions, below).

Logistics. From the process variables in food production and distribution (e.g., temperature), the growth parameters (A , μ_m , λ) of the organisms can be predicted (see Fig. 8). With these growth parameters the number of organisms with time can be predicted, and feedback can follow, resulting eventually in adjustments in production or distribution (e.g., the temperature during distribution). This cycle of prediction and feedback can be repeated many times.

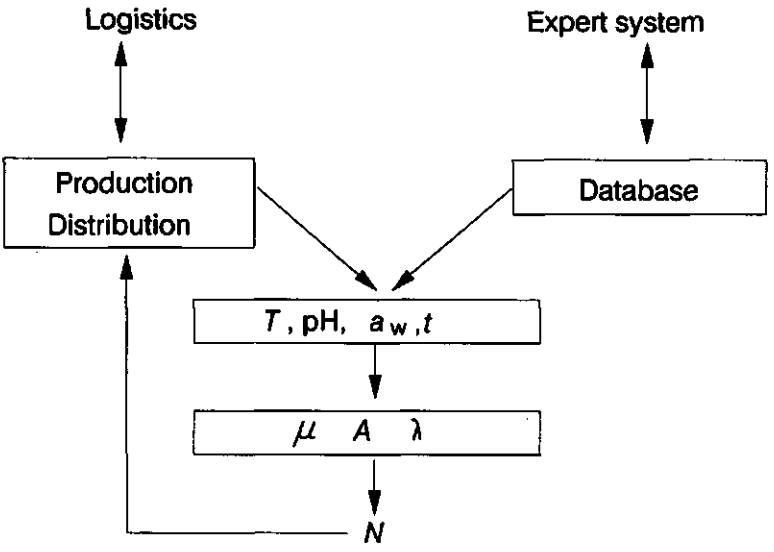


FIG. 8. Optimization cycle of bacterial numbers.

It can also be worthwhile to incorporate logistics into this procedure, so that not only the effect of changes in physical parameters of the process (e.g., temperature during distribution) can be evaluated, but also the effect of changes in duration of the different stages (e.g., duration of processing, holding, transportation, storage, break-down, or switch time). Furthermore, the effect of distributions in several variables can be evaluated, such as distributions in raw-material contamination, storage time in the retail display, temperature, etc.

A preliminary program is developed to calculate costs of cooling in different stages of a cooling chain. The optimum temperatures in a cooling chain can be calculated, combining the square-root model and the maximum allowed bacterial number in a product. Three steps in the distribution chain of milk are chosen as an example (factory storage, distribution, supermarket storage). An overview of the most important contributions to energy costs is

made. The most important contributions to energy costs for cooling are found to be the losses through wall, floor, and roof; losses by opening of the door; and heat to be removed from the product to reach the desired temperature.

With dynamic programming (backwards dynamic programming with fixed grid-points) the optimum temperatures in the three phases are determined, with minimal costs, given a certain quality. This procedure can be used for short term decisions. At present research on this subject is in progress.

Management Tool. The quality functions given in this thesis can also be used for long-term strategic decisions. For instance, the effect of an extra distribution centre, more trucks for transportation, machinery with a lower technical failure chance etc. can be evaluated. For these strategic decisions quality models are essential.

Other deterioration reactions. For other deterioration reactions (chemical, physical) the same procedure can be followed. For chemical spoilage reactions, theory and application are illustrated by Labuza (7). The progression of chemical and physical spoilage indicators can be calculated simultaneously with the bacterial numbers, as a function of the physical parameters. In this manner it can be evaluated which quality reaction determines the shelf-life.

Knowledge-based system. As can be expected, all these procedures require a large number of parameter values, such as the growth parameters of bacteria in different foods, depending on different physical parameters, and the physical parameters of the different foods. Therefore a large number of datum points must be collected and a large number of experiments must be carried out, which can be collected in a database (Fig. 8). For practical purposes it is impossible to have a database with all necessary information; therefore, it could be useful to incorporate some knowledge into the database.

A combination of quantitative and qualitative data is of large importance, since there is already a large quantity of these data. A start is made to develop a method to combine qualitative and quantitative information. If this is done in a structured manner the method can be used to predict product quality in the best possible way, given a limited amount of information. This has been shown to result in a powerful tool to predict possible microbial growth in different foods (see Chapter 7).

Residence Time Distribution. Growth models of microorganisms can be coupled to residence time distributions (RTD) and computational fluid dynamics (CFD) programs to calculate the effect of fluid flow in, for example, storage tanks or pipes. The effect of residence time distribution and stagnant zones can be evaluated. In parts were the product will stay long(er), more growth can occur, and due to mixing with the main stream a continuous contamination occurs.

Heat transport. The above-mentioned square-root model for the temperature dependency of μ_m can be combined with a model for heat conductance in a product. This will be explained by some examples. For the case that heat production can be neglected, Fourier's second law for an infinite slab with thickness 2L, surrounded by fluid of temperature T_f at both sides gives:

$$\frac{\partial T}{\partial t} = a\frac{\partial^2 T}{\partial x^2} \tag{6}$$

with a the thermal diffusivity ($m^2 \cdot s^{-1}$), T the temperature (K), t the time (s), x the location (m).

The boundary conditions are:

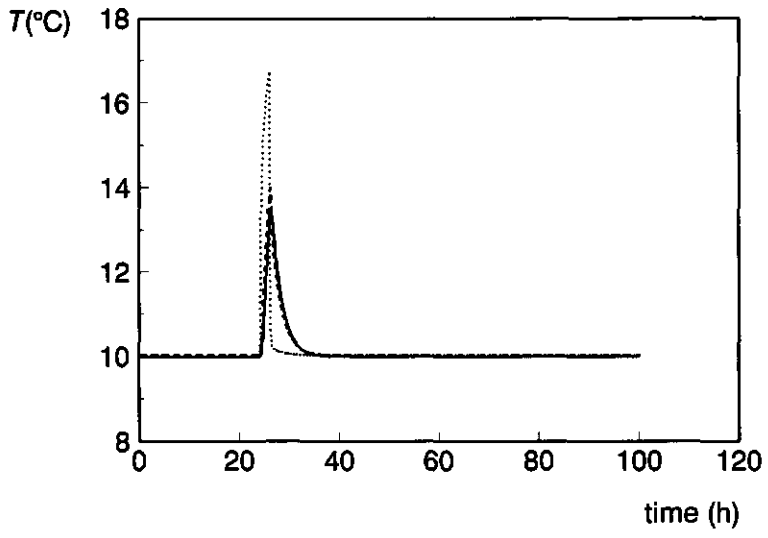
1) Due to the symmetry in the centre:

$$\left(\frac{\partial T}{\partial x}\right)_{x=0} = 0 \tag{7}$$

2) Since the transport through the surface of the slab is equal to the heat transport from the fluid to the slab:

$$\left(-k\frac{\partial T}{\partial x}\right)_{x=L} = \alpha(T_f - T_L) \tag{8}$$

with α the external heat transfer coefficient ($W \cdot m^{-2} \cdot K^{-1}$), and k the thermal conductivity ($W \cdot m^{-1} \cdot K^{-1}$). Equation 6 can be solved numerically by using difference equations.



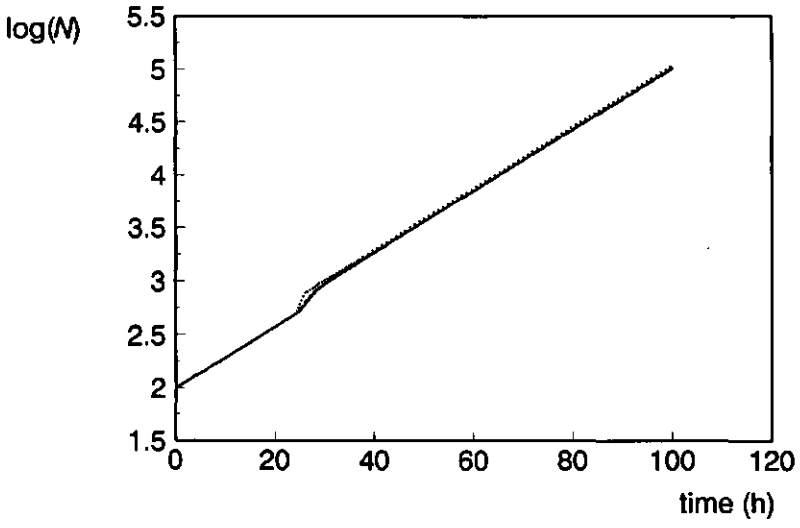


FIG. 9. Temperature history and development of the number of *Lactobacillus*. Simulation 1.
——— centre; -------- intermediate position; ····· surface , T= temperature,
N= number of organisms (cfu/g).

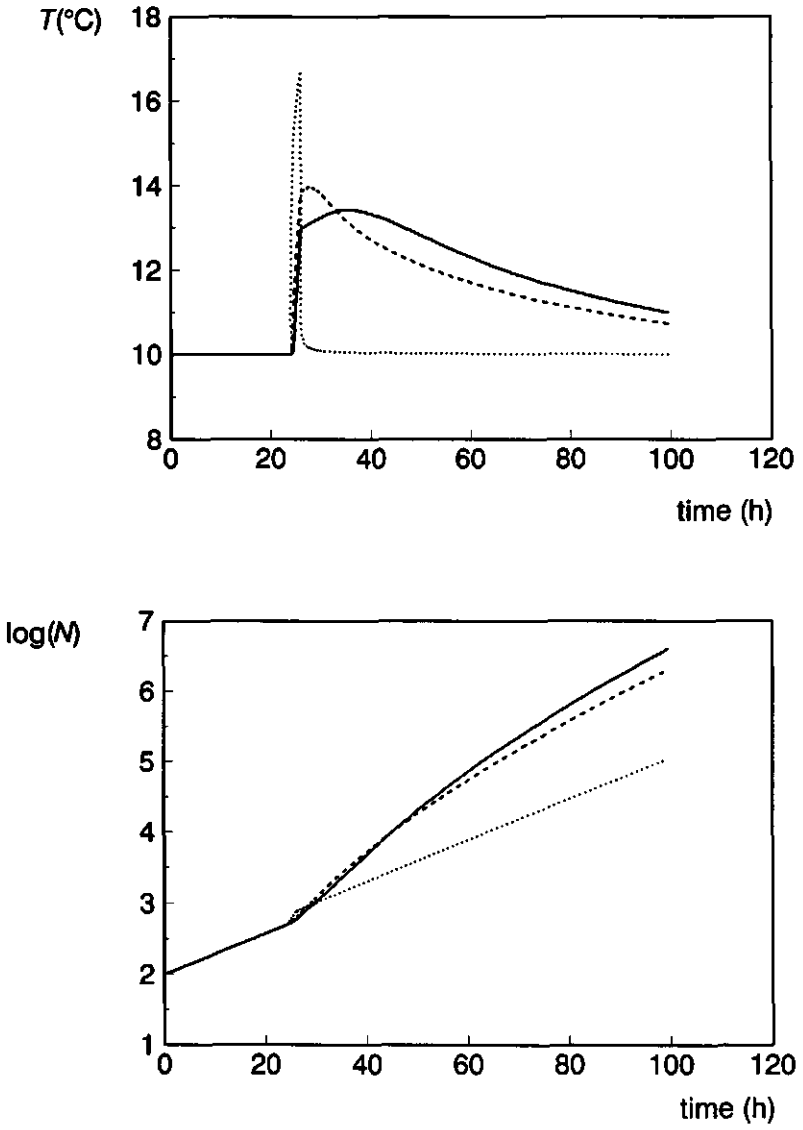


FIG. 10. Temperature history and development of the number of *Lactobacillus*. Simulation 2.
——— centre; -------- intermediate position; ····· surface, T= temperature,
N= number of organisms (cfu/g).

In the program that is developed several successive phases can be entered (e.g., storage raw-materials, production, cooling, product storage), with the temperature of the fluid in that phase, as can the product characteristics. The set of equations then can be solved for every time step and the temperature is calculated as a function of the location inside the product, and as a function of time. Subsequently, the bacterial growth can be calculated, with the square-root model, at any time at any point in the product. In this manner the effect of heating and cooling of the product on the bacterial growth can be evaluated.

An example of a simulation is given below. A product is stored during 24 hours at 10°C, and then enters the 20°C production. The product stays there for 2 hours. After production the product is stored in a cooling cabin with ventilation at 10°C (Table 4, Fig. 9). It is assumed that the organisms are already in their exponential phase (lag time = 0).

For simulation 2 everything is the same as in simulation 1 except that during cooling the product is cooled with 5 product units together, therefore the characteristic radius R is increased by a factor 5 (Fig. 10).

In Fig. 9 and 10 the calculated temperature and the number of microorganisms are given at three different locations in the product; the surface, the centre and in between (intermediate position). In Fig. 9 it can be seen that the fastest temperature increase occurs at the surface of the product, and about 17°C is reached. In the centre of the product and in the intermediate position the heat equalisation goes slower. The effect of the higher temperature during production on the number of micro-organisms is very small, due to the short time span.

TABLE 4. Parameters for Simulation 1

	Time (h)	Phase	T_o(°C)	T_f(°C)	
Process	0-24	storage	10	10	
	24-26	production	10	20	α = 25 ($W \cdot m^{-2} \cdot K^{-1}$)
	26-100	cooling	20	10	α = 400 (convection)
Product	infinite slab				
	characteristic radius		R=0.05 m		
	thermal diffusivity		$a = 0.143 \cdot 10^{-6}$ ($m^2 \cdot s^{-1}$)		
	thermal conductivity		k= 0.60 ($W \cdot m^{-1} \cdot K^{-1}$)		
Organism	*L. plantarum*: N_o=100		b=0.0385 T_{min}=3.29 c=0.247 T_{max}=44.8		

T_o= product temperature when product enters phase, T_f= temperature of fluid in phase.

If the characteristic radius is increased by a factor 5 during cooling (Fig. 10), by joining 5 product units together, the cooling-down goes 25 times slower (proportional to the square of the radius). The cooling-down of the middle and in the intermediate position of the slab goes dramatically slower, which strongly facilitates bacterial growth at these positions. In products with surface contamination only, this will be no real problem; in products with an interior contamination, however, this can result in a serious problem.

Also the growth of an additional organism can be calculated, for instance *Pseudomonas* spp, which is more psychrophilic than *Lactobacillus*. In simulation 3 everything is the same as in simulation 1 except that the growth of *Pseudomonas* spp. (Table 5) is calculated as is the growth of *L. plantarum*.

TABLE 5. Parameters for *Pseudomonas* spp

Pseudomonas spp.:	$N_o=10$	$b=0.03$ $T_{min}=-6.0$	$c=0.157$ $T_{max}=36.0$

In Fig. 11 the growth of *Lactobacillus* ($N_o=100$) and *Pseudomonas* ($N_o=10$) is compared. Although the initial contamination is lower, the psychrophilic organisms will be the main spoiling organisms.

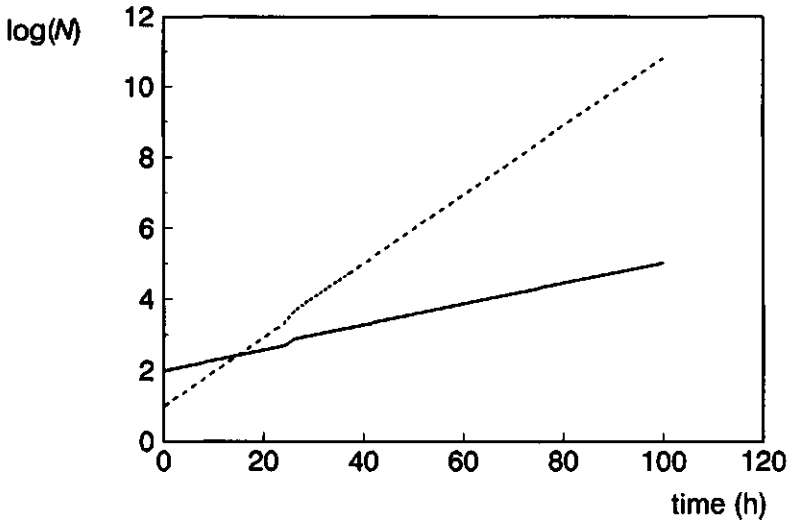


FIG. 11. Development of the number of *Lactobacillus* (——) and *Pseudomonas* (--------) on the surface. Simulation 3. $N=$ number of organisms (cfu/g).

With comparable simulations the effects of numerous changes in production, product, etc. can be evaluated. This can be an important tool for process optimization. The growth of different organisms can be calculated by changing the growth parameters, as can the effect of a change in initial contamination. Furthermore, the effect of different time/temperature histories can be examined, as can the effect of different heating and cooling procedures (forced convection, steam vs. water or air, different characteristic radii).

FINAL CONCLUSION

Predictive modeling has shown to be a promising tool in food research, to be used to optimize food chains. Various models are developed and validated to be used to describe deterioration reactions.

A tool is developed to discriminate between different models and to restrict the number of parameters in models. Models to describe a growth curve and to describe the effect of temperature, and the effect of shifts in temperatures, are developed and validated with a large amount of experimental data.

Furthermore, a procedure is developed to couple quantitative and qualitative information in a structured manner. Simple procedures to make (preliminary) shelf-life predictions are given, as are procedures to extend these (simple) models.

The most important advantage of modeling is that insight is gained in the progression of microbial growth within a product chain. Furthermore, these models are shown to be essential to calculate quality changes with the use of decision support systems (DSS).

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SUMMARY

Growth of spoilage microorganisms in foods receives much attention since there is a trend towards minimally processed foods. In these products growth of microorganisms is possible. Therefore it is useful to know how fast microorganisms grow and which factors determine this growth.

In this thesis different models are described to predict deterioration reactions. Models are simplified descriptions of reality. By making appropriate assumptions, reality can be described with mathematical relations. With these mathematical equations predictions can be made of the progress of a process. This can give insight in these kinetics. These equations can also be used to optimize processes.

Various factors determine the growth of microorganisms like temperature, pH, water activity, gas-atmosphere and the presence of preservatives. Some of these factors not only influence the growth rate of microorganisms but also the properties of the product (pH, water activity, salt concentration, preservatives). A change in storage temperature or gas-atmosphere (within certain limits) only changes the growth rate of the microorganisms. With these two factors it is possible to alter the spoilage rate of a product and thus the shelf life, without changing the product. In this thesis mainly the effect of temperature is investigated.

A tool to discriminate between different models is proposed, leading to restriction of the number of parameters in the models applied. Models are developed to describe a bacterial growth curve. Furthermore, models are developed to describe the effect of temperature, and the effect of shifts in temperature. These models are validated with a large number of experimental data. Simple procedures to make shelf life predictions with these models are given, as are procedures to extend these models.

In order to make shelf-life predictions of products with varying composition, several parameters are needed. For some of these, quantitative information is available, for others only qualitative. Therefore, a procedure is developed to couple quantitative and qualitative information in a structured manner. In this manner an estimate of the shelf life can be made on the basis of parameter values combined with qualitative information, in order to get a prediction as good as possible, on basis of available knowledge.

The most important advantage of modeling is that insight is gained in the kinetics of microbial growth within a product chain. Furthermore, these models are essential to calculate quality changes in decision support systems (DSS).

SAMENVATTING

Groei van bederfveroorzakende micro-organismen in levensmiddelen staat sterk in de belangstelling, aangezien veel produkten momenteel minder conserveringsbewerkingen ondergaan dan voorheen. Door het geheel of gedeeltelijk ontbreken van deze bewerkingen (zoals bijvoorbeeld sterilisatie, zouten, het gebruik van conserveermiddelen), wordt groei van micro-organismen begunstigd. Het is dan nuttig te weten hoe snel de micro-organismen groeien en welke factoren die groei beïnvloeden.

In dit proefschrift worden verschillende modellen beschreven, die microbiologische bederfreacties kunnen beschrijven. Modellen zijn vereenvoudigde voorstellingen van de werkelijkheid. Door geschikt gekozen veronderstellingen te doen kan de werkelijkheid beschreven worden met wiskundige relaties. Met deze wiskundige relaties kunnen dan voorspellingen gedaan worden over het verloop van bepaalde processen. Hierdoor kan inzicht verkregen worden in de kinetiek van die processen. Deze relaties kunnen ook gebruikt worden om processen te optimaliseren.

Verschillende factoren zijn van invloed op de groei van micro-organismen, zoals de temperatuur, de pH, de wateractiviteit, de gasatmosfeer en de aanwezigheid van groeiremmende stoffen. Sommige van deze factoren veranderen behalve de groeisnelheid van de micro-organismen echter ook de aard van het produkt (pH, wateractiviteit, zoutconcentratie, conserveringsmiddelen). Verandering van de bewaartemperatuur of de gasatmosfeer (binnen bepaalde grenzen) verandert alleen de groeisnelheid van de micro-organismen. Bij een gegeven produktsamenstelling is het dus mogelijk de bederfsnelheid en daarmee de houdbaarheid te beïnvloeden, zonder het produkt te veranderen. In dit proefschrift is voornamelijk onderzoek verricht naar het effect van de temperatuur.

Er is een methode opgesteld om tussen verschillende modellen te discrimineren om zo het aantal parameters in de modellen te minimaliseren. Allereerst zijn er modellen ontwikkeld om een groeicurve van micro-organismen te beschrijven. Verder zijn er modellen ontwikkeld om het effect van de temperatuur en het effect van temperatuurstappen te voorspellen. Deze modellen zijn getoetst met een groot aantal experimentele gegevens. Eenvoudige procedures om houdbaarheidsvoorspellingen te doen worden gegeven, evenals procedures om deze modellen uit te breiden.

Om houdbaarheidsvoorspellingen te kunnen doen van allerlei produkten met verschillende samenstelling zijn verschillende parameters nodig. Over enkele hiervan is kwantitatieve kennis beschikbaar, over andere slechts kwalitatieve. Daarom is er een procedure ontwikkeld om kwantitatieve en kwalitatieve gegevens op een gestructureerde manier te koppelen. Op deze manier kan de best haalbare schatting van de houdbaarheid gemaakt worden op basis van parameterwaarden gekoppeld aan kwalitatieve kennis.

Het grootste voordeel van modelleren is dat inzicht kan worden verkregen in de kinetiek van de groei van micro-organismen in produktketens. Verder zijn deze modellen essentieel om kwaliteitsveranderingen te berekenen in beslissings-ondersteunende systemen (BOS).

NAWOORD

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CURRICULUM VITAE

Marcel Zwietering werd op 10 augustus 1963 geboren in Sittard. In 1981 behaalde hij het diploma VWO-B met lof aan de Serviam scholengemeenschap in Sittard. In datzelfde jaar begon hij met zijn studie aan de toenmalige Landbouwhogeschool Wageningen.

In mei 1985 legde hij het kandidaatsexamen in de richting Moleculaire Wetenschappen met lof af. In de doctoraalfase van deze studie deed hij de hoofdvakken Microbiologie en Proceskunde en de bijvakken Wiskunde en Meet- Regel- en Systeemtechniek. Zijn stage-periode bracht hij door bij het Department of Chemical Engineering, MIT, Cambridge, USA, bij professor Cooney. In november 1987 studeerde hij met lof af. Hij werd in 1987 onderscheiden met een Unilever Research Prijs.

Van december 1987 tot en met december 1988 was hij als AIO verbonden aan de sectie Proceskunde van de Landbouwuniversiteit Wageningen. Van januari 1989 tot heden is hij werkzaam als universitair docent bij dezelfde sectie. Van december 1987 tot december 1992 verrichtte hij het onderzoek (mede gefinancierd door Unilever Research) dat leidde tot dit proefschrift.