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Concepts and Tools for Predictive Modeling of Microbial Dynamics

KRISTEL BERNAERTS,[1] ELS DENS,[1] KAREN VEREECKEN,[1] ANNEMIE H. GEERAERD,[1] ARNOUT R. STANDAERT,[1] FRANK DEVLIEGHERE,[2] JOHAN DEBEVERE,[2] AND JAN F. VAN IMPE[1]*

[1]BioTeC—Bioprocess Technology and Control, Department of Chemical Engineering, Katholieke Universiteit Leuven, W. de Croylaan 46, B-3001 Leuven, Belgium; and [2]Laboratory for Food Microbiology and Food Preservation, Department of Food Technology and Nutrition, Ghent University, Coupure Links 653, B-9000 Gent, Belgium

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ABSTRACT

Description of microbial cell (population) behavior as influenced by dynamically changing environmental conditions intrinsically needs dynamic mathematical models. In the past, major effort has been put into the modeling of microbial growth and inactivation within a constant environment (static models). In the early 1990s, differential equation models (dynamic models) were introduced in the field of predictive microbiology. Here, we present a general dynamic model-building concept describing microbial evolution under dynamic conditions. Starting from an elementary model building block, the model structure can be gradually complexified to incorporate increasing numbers of influencing factors. Based on two case studies, the fundamentals of both macroscopic (population) and microscopic (individual) modeling approaches are revisited. These illustrations deal with the modeling of (i) microbial lag under variable temperature conditions and (ii) interspecies microbial interactions mediated by lactic acid production (product inhibition). Current and future research trends should address the need for (i) more specific measurements at the cell and/or population level, (ii) measurements under dynamic conditions, and (iii) more comprehensive (mechanistically inspired) model structures. In the context of quantitative microbial risk assessment, complexity of the mathematical model must be kept under control. An important challenge for the future is determination of a satisfactory trade-off between predictive power and manageability of predictive microbiology models.

Predictive food microbiology involves the quantification of microbial ecology in foods by means of mathematical models *(54).* These models can then be used to predict food safety and shelf life, to develop and implement safety assurance systems in the food industry (e.g., hazard analysis critical control point programs), and to establish exposure studies in the framework of risk assessment *(13, 15, 53, 72).* Whereas challenge testing tends to be the most common policy in the food industry, information on microbial kinetics in food products is being increasingly consolidated into mathematical models, which may significantly reduce the number of challenge tests required to determine, for example, shelf life. In combination with predictive models for heat transfer and other process variables and the initial contamination level, these models are essential building blocks in time-saving simulation studies used to optimize and design processing, distribution, and storage conditions (e.g., temperature-time regimes), which guard food safety and discourage spoilage *(18).*

In the early years of predictive microbiology, strong preference was given to sigmoidal functions that produced an accurate description of growth curves obtained under nonvarying environmental conditions. The most commonly used growth model was the modified Gompertz model *(78).* Microbial inactivation at high temperatures (exhibiting log-linear behavior) could generally be described as a first-order decay reaction *(3).* Effects of environmental conditions on the parameters of these primary models (i.e., evolution of cell number as a function of time) are embedded into secondary models *(71).* Dynamic primary models capable of dealing with realistic time-variable conditions and taking into account the previous history of the food product in a natural way have been introduced beginning in the early 1990s *(7, 63).* In addition to the need for such dynamic models, real food product conditions (e.g., fluctuating environmental conditions, food structure, low numbers of contaminating cells, inimical conditions near the growth–no growth interface) should be taken into account during modeling *(38, 39, 73).*

Here, we outline a consistent, well-founded dynamic modeling strategy in the field of predictive microbiology. Consequently, this article can be viewed as a tutorial (partly) revisiting some general concepts from dynamic bioprocess modeling and illustrating them with examples. A comprehensive reference book on bioprocess modeling in all its facets was written by Roels *(52).* Here, the dynamic model-building approach is first illustrated for modeling simple growth and inactivation behavior. However, accurate modeling of microbial evolution in foods mostly demands for more complex model structures. In this respect, the modeling of microbial lag under time-variable temperature conditions via an individual-based approach and the modeling of interspecies microbial interactions mediated by product inhibition are discussed. The fundamentals of microscopic

* Author for correspondence. Tel: +32-16-32.14.66; Fax: +32-16-32.29.91; E-mail: jan.vanimpe@cit.kuleuven.ac.be.

(individual) and macroscopic (population) modeling also are revisited.

Given the elementary building block for dynamic mathematical models describing microbial dynamics, (mechanistic) knowledge of microbial behavior in foods can be gradually built in to yield a generic model structure describing the microbial dynamics of interest. During this model development process, there is a continuous trade-off between model complexity and manageability. The mathematical model should incorporate sufficient knowledge (mechanistically inspired or extracted from experimental data) to generate accurate predictions, which are indispensable for instilling confidence in predictive microbiology within the food industry. However, these mathematical models must remain user friendly and computationally manageable to ensure their industrial applicability.

The use of mechanistically inspired dynamic models is strongly encouraged. However, the choice between (semi)mechanistic and empirical modeling approaches is not always clear. Empirical (black box) models are derived from experimental data and can be considered as purely curve-fitting models (e.g., polynomials, artificial neural networks), whereas genuine mechanistic (white box) models are a precise mathematical translation of the underlying mechanism (e.g., metabolic models). Given the complexity of biological phenomena and cell metabolisms, semimechanistic (hybrid or gray box) models encompassing (partial) mechanistic knowledge and model elements derived from the experimental data may be of more interest (and more suitable) in the field of predictive microbiology. (Semi)mechanistic models tend to be more robust when used outside the range of conditions for which they have been developed, they contribute to a better understanding of the principles governing the process, and they are more amenable to refinement as knowledge of the system increases *(54)*. Empirical models can only perform well when the model with controlled complexity is identified for extensive and sufficiently rich sets of experimental data (hereby avoiding overfitting) and is applied under the (highly standardized) conditions for which it has been developed. Recently, Geeraerd et al. *(25)* demonstrated that the implementation of prior microbiological knowledge (e.g., based on cardinal values) during identification of empirical models (such as polynomials) may overcome the problem of badly positioned data (e.g., ranges with no data) and/or prohibit overfitting. However, in situations characterized by serious reproducibility problems and experimental noise, the development of sound mechanistic models is impossible and the development of good empirical models is improbable (after *(38)*). The difficulty encountered when attempting to accurately predict biological phenomena (e.g., the microbial lag phase) is often due to the lack of mechanistic knowledge (e.g., the physiological status of the organisms) and of sufficient, reliable and informative experimental data (e.g., data under dynamic conditions).

GENERAL DYNAMIC MODELING METHODOLOGY

Elementary dynamic model building block. The elementary dynamic model building block describing microbial evolution under batch cultivation within an homogeneous environment consists of the following set of differential equations:

$$\frac{dN_i(t)}{dt} = \mu_i[N_i(t), \langle N_j(t)\rangle_{i\neq j}, \langle env(t)\rangle, \langle P(t)\rangle, \langle S(t)\rangle, \langle phys(t)\rangle, \cdots] \cdot N_i(t) \quad (1)$$

where $i, j = 1, 2, \ldots, n$ is the number of microbial species involved (analogous to other equations *(5, 52, 67)*). $N_i(t)$ represents the cell density of species i, and $\mu_i(\cdot)$ [h^{-1}] defines its overall specific evolution rate depending on interaction(s) within and/or between microbial populations (N_i and/or N_j, respectively), physicochemical environmental condition(s) ($\langle$env$\rangle$), microbial metabolite concentration(s) ($\langle P\rangle$), the physiological state(s) of the cells ($\langle$phys$\rangle$), substrate concentration(s) ($\langle S\rangle$), and other factors. The presence or absence of one or multiple influencing factors of each category depends on their relevance in the microbial process under study. Microbial growth is obtained when $\mu_i(\cdot) > 0$, and microbial decay results from $\mu_i(\cdot) < 0$.

All influencing factors may depend on time. For example, temperature may change with time and thus acts as an input when solving the system of differential equations. To describe the time-dependent evolution of metabolite production and the physiological state of the cells, for example, additional coupled differential equations are added to equation 1.

Within structured food systems, equation 1 describes the local dynamic behavior of microorganisms. In these systems, local inputs are needed. For example, local temperatures can be computed using heat transfer models. Microbial dynamics will be influenced by spatially variable substrates and nutrient concentrations, which may become restricted because of diffusion limitations. Diffusion limitations also cause spatial gradients of metabolic products. The need for a valid transport model for microbial cells (i.e., describing spatial colony dynamics) also is evident *(26)*.

Basic elements for modeling growth. When environmental conditions are constant, the microbial growth curve, i.e., the (natural) logarithm of the cell density as a function of time, typically exhibits a sigmoidal shape consisting of three phases: the lag phase, the exponential phase, and the stationary phase (see Fig. 1, top left). First, the population needs to adjust to its new environment. Second, the population attains its maximum specific growth rate characteristic for the specific environment. Although metabolites are produced and substrates consumed during single species growth under constant environmental conditions (thus causing dynamic changes in the environment), a constant maximum specific growth rate (or exponential growth rate) is usually attained for a period. Under laboratory condition involving rich buffered media, effects of metabolites and substrates often are redundant during the exponential phase. However, in real food systems and multispecies systems, the impact of these factors can be important. Third, growth ceases because of, e.g., inhibitory effects of metabolites. Eventually, inactivation results. The course of the overall

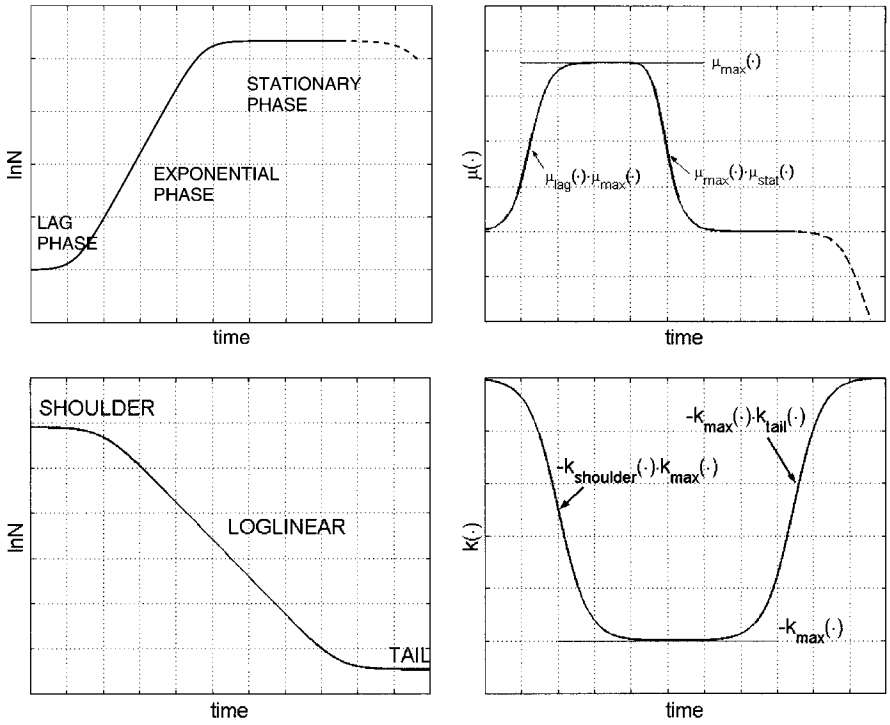

FIGURE 1. *Top: Typical growth curve (solid line) under constant environmental conditions (left) and the corresponding course of the specific growth rate (right). Bottom: Typical inactivation curve under mild constant processing conditions (left) and the corresponding course of the specific inactivation rate (right).*

specific growth rate, $\mu(\cdot)$, as a function of time is depicted in Figure 1 (top right), and three factors having a pronounced effect in each of the growth phases can be defined:

$$\frac{dN(t)}{dt} = \underbrace{\mu_{lag}(\cdot) \cdot \mu_{max}(\cdot) \cdot \mu_{stat}(\cdot)}_{\mu(\cdot)} \cdot N(t) \qquad (2)$$

During the exponential phase, the specific growth rate remains constant at μ_{max}, which is the maximum specific growth rate that can be realized within the actual environment. The dependence on environmental factors such as temperature, pH, and water activity is typically incorporated into secondary models *(76).* When the environmental conditions vary with time, a secondary model is to be integrated in equation 1, replacing μ_{max}. The first factor, $\mu_{lag}(\cdot)$, is introduced to describe the lag behavior and thus needs to reflect the gradual increase of the overall specific growth rate from (approximately) zero to μ_{max}. The third factor, $\mu_{stat}(\cdot)$, induces the gradual decrease of the specific growth rate towards zero, resulting in the stationary phase.

Dynamic models in predictive microbiology have been described *(6, 7, 27, 28, 37, 63, 64).* A well-known dynamic model is the growth model of Baranyi and Roberts *(6)*:

$$\frac{dN_i(t)}{dt} = \left[\frac{Q(t)}{1 + Q(t)}\right] \cdot \mu_{max} \cdot \left[1 - \frac{N(t)}{N_{max}}\right] \cdot N(t)$$

$$\frac{dQ(t)}{dt} = \mu_{max} \cdot Q(t) \qquad (3)$$

Compare the three factors in the right side of the top equation with those in equation 2. The first factor, the so-called adjustment function, describes the gradual adaptation of the population to attain μ_{max}. Here, an additional state variable $Q(t)$ is introduced into the model; thus, $\mu_{lag}[Q(t)]$. This variable denotes the physiological state of the cells that should grow until the adjustment function reaches (approximately) its maximum value of 1. At that point, the exponential phase starts. The initial value of $Q(t)$ and the maximum specific growth rate determines the lag phase duration. Graphically, μ_{max} corresponds to the slope of the log-linear part of the growth curve. From a mathematical point of view, the adjustment function is only exactly equal to 1 at infinity, whereas the inhibition function approximates 1 when $N(t) \ll N_{max}$. However, from a numerical point of view, both factors are 1 during a considerable part of the growth curve. Hence, during the log-linear part, μ_{max} is reached (see Fig. 1, top right). The third factor, the so-called inhibition function, causes the growth rate to decrease asymptotically to zero when the population density reaches its maximum level N_{max}; thus, $\mu_{stat}[N(t), N_{max}]$.

Environmental conditions affecting the outgrowth of microorganisms in food products often change over time. In such cases, predictions of the food safety and the shelf life can be generated by combining a dynamic primary model with a secondary model relating the typical primary parameters with environmental conditions, e.g., $\mu_{max}[\langle env(t)\rangle]$. When combining these models, it is implicitly assumed that the primary parameters, e.g., the maximum specific growth rate, immediately adapt to the actual changing environmental factor(s) and the secondary model. Consequently, delayed responses (lag) induced by (sudden) fluctuations of the surrounding environment cannot be predicted *(10).* The cessation of growth is a response to starvation following exhaustion of nutrients, inhibition by metabolic products, quorum sensing, or combinations of these phenomena *(5, 33).* Inhibition within mixed cultures by, e.g., product formation cannot be consistently described when using the single model parameter N_{max}.

In the subsequent sections, we illustrate how basic model elements for microbial growth (equation 2) can be fine tuned toward the modeling of microbial lag and growth inhibition. Our goal is to develop robust mechanistically inspired models.

Basic elements for modeling inactivation. During mild heat treatment (at constant temperature), microbial inactivation often shows a non–log-linear behavior characterized by a delayed response (shoulder) and a resistant population (tailing) (see Fig. 1, bottom left). According to equation 1, a general model structure reads as follows:

$$\frac{dN(t)}{dt} = \underbrace{-k_{\text{shoulder}}(\cdot)\cdot k_{\max}(\cdot)\cdot k_{\text{tail}}(\cdot)}_{k(\cdot)}\cdot N(t) \qquad (4)$$

To express the specific microbial inactivation rate, the symbol k is commonly used.

Based on the mechanistic insight of the occurrence of the shoulder and tailing phenomena *(1, 14, 44, 45)*, Geeraerd et al. *(24)* established the following functions modeling the shoulder and tailing behavior:

$$\frac{dN(t)}{dt} = -\left[\frac{1}{1 + C_c(t)}\right]\cdot k_{\max}\cdot\left[1 - \frac{N_{\text{res}}}{N(t)}\right]\cdot N(t)$$

$$\frac{dC_c(t)}{dt} = -k_{\max}\cdot C_c(t) \qquad (5)$$

The first factor in the right side of the top equation models the shoulder of the inactivation curve. Before first-order inactivation of the population takes place (at a specific inactivation rate $k_{\max}$), some critical protective component C_c (units per cell) must be inactivated. This inactivation is assumed to occur according to a first-order relationship (bottom equation). The shoulder is obtained by applying a Michaelis-Menten–based adjustment function: $[1 + C_c(t)]^{-1}$; thus, $k_{\text{shoulder}}[C_c(t)]$. Starting at a low value, the adjustment function increases toward 1, and at that point log-linear inactivation is observed. Analogous to the physiological state $Q(t)$ in the dynamic growth model (equation 3), $C_c(t)$ can be interpreted as the physiological state of the population in the context of inactivation. The tailing phenomenon can be explained by some resistant subpopulation N_{res} that is unaffected during the (heat) treatment. This tailing of a residual population N_{res} is here modeled by $(1 - N_{\text{res}}/N(t))$; thus, $k_{\text{tail}}[N(t),N_{\text{res}}]$. This residual subpopulation is not necessarily a constant value but may vary when modeling nonthermal inactivation *(30, 56, 69, 75)* or when subjecting the microbial population to sequences of inactivation treatments *(57)*.

The general model structure (equation 4) and model (equation 5) also encompass classical log-linear inactivation. In equation 5, log-linear inactivation is generated by selecting (after identification from experimental data) a very low value for C_c (0) and N_{res}, implying the absence of a shoulder and a tail, respectively.

INDIVIDUAL-BASED MODELING OF MICROBIAL LAG

From population to cell dynamics. Factors affecting the occurrence and extent of the commonly observed initial (population) lag phase (Fig. 1, top left) can be attributed to the past environment, the new environment, the magnitude of the environmental change, the rate of the environmental change, the growth status (e.g., exponential or stationary) of the inoculated cell culture, and the variability of individual cell lag phases *(60)*. These environmental changes may involve nutritional, chemical, and physical changes. Obviously, environmental fluctuations during exponential growth can also cause lag (i.e., intermediate lag). Large temperature gradients, for example, applied during the exponential growth phase can induce an intermediate lag phase observed as a transient adaptation of the growth rate *(10, 77)*.

Secondary models describing the relation between the (population) lag phase duration and the physicochemical environment are usually based on highly standardized experiments during which cells are grown to their stationary phase under optimal growth conditions before being transferred to the new environment, which is not deliberately varied during subsequent growth. Such mathematical models perform well under the conditions for which they have been developed. However, any deviation within the prehistory of the contaminating population may seriously alter the lag behavior *(29, 70)*.

Discrepancies between model predictions and growth observations under rapidly changing temperature conditions outside the common growth region have been explained by others as intermediate lag phases, which cannot be predicted by the applied models *(8, 31, 42)*. However, Mitchell et al. *(43)*, Bovill et al. *(11)*, and Augustin et al. *(4)*, among others, observed the instantaneous adaptation of exponentially growing cells subjected to sudden temperature changes. In contrast to abrupt temperature variations, moderate temperature changes can be predicted well by common dynamic growth models *(8, 64)*. However, the temperature history effect on the initial lag phase remains difficult to predict properly *(2, 8)*. Within the field of predictive microbiology, extensive and rigorous studies evaluating growth dynamics under dynamic temperature conditions are scarce (there are notable exceptions *(40, 61)*). Sampling points sufficient to distinguish properly between lag and measurement errors are often lacking.

Here, we use the development of a model structure describing microbial lag due to temperature shifts to explain individual-based modeling (IbM) as a microscopic modeling strategy (for an extensive description and evaluation of the presented model, see Dens et al. *(20, 21)*. In contrast to the more traditional population (macroscopic) modeling approach, basic mechanistic knowledge concerning the mechanism causing lag at the cell level is embedded into the model. In particular, the theory of cell division is implemented within an IbM approach to enable the description of lag phases that can be induced by sudden temperature increases.

Principles of IbM. The fundamental unit of bacterial life is the cell, encapsulating action, information storage, processing, and variability. It is therefore appropriate to construct microbial models in terms of the individual cells *(32).* This is the domain of IbM. The basic idea behind this approach is that if it is possible to specify the rules governing the behavior of the cells, then global multicellular behavior can be explained by the interactions between individual cell activities. The rules constituting the model reflect the (presumed) behavior of the individual cells, such as nutrient consumption, biomass growth, cell division, movement, differentiation, communication, maintenance, and death. Because a change in microscopic (individual based) rules may lead to significantly different macroscopic (population) behavior, it might be possible to eliminate impossible mechanisms and investigate the true mechanisms. A very important property of individual-based models is that they easily allow for differences between individuals by using random variables drawn from an experimentally derived statistical distribution *(23, 41).* The introduction of a range of randomness and the consideration of a high number of individuals interacting independently with the environment leads to an adequate representation of reality and to a better understanding of cellular metabolism *(9).* Spatial effects can be relatively easily incorporated. Kreft et al. *(32)* introduced the spatial aspect in their model to reproduce the growth of *Escherichia coli* cells in a colony.

In general, individual-based models incorporating underlying mechanistic knowledge of species dynamics are widely used in ecological applications but are relatively unexplored in the field of predictive microbiology. Model parameters are usually assumed to be deterministic, i.e., have one typical value. When incorporating cell-to-cell variability into population-based models, population-related model parameters are considered to be random or distributed variables *(47).* Individual-based models have an advantage in that the cell-to-cell variability can be incorporated at the cell level, i.e., the level from which variability actually originates. The general concepts of individual-based models and their applicability in the context of predictive microbiology have been discussed by Dens et al. *(20, 21)* and Standaert et al. *(58).*

Implementation of mechanistic insight into an individual-based model. The mechanistic insight into the theory of cell division has been incorporated into an individual-based framework called BacSim, originally developed by Kreft et al. *(32).* In contrast to the general equation 1 describing the evolution of a bacterial population, $N(t)$, biomass growth of the individual cells, $m(t)$, is considered and is assumed to occur exponentially at any time *(27, 55)*:

$$\frac{dm(t)}{dt} = \mu_{max} \cdot m(t) \qquad (6)$$

This expression forms the elementary building block of the proposed individual-based model. Cooper and Helmstetter *(17)* observed that for a constant temperature each round of DNA replication takes a constant amount of time, $C + D$, which encompasses the time required to complete DNA replication (C) and the time needed to establish cell division (D). Based on the findings of Cooper and Helmstetter *(17)* and Schaechter et al. *(55),* Donachie *(22)* concluded that the cell mass at which DNA replication is initiated is always a multiple of a critical cell mass m^*: $2m^* \cdot 2^n$, where $n = 0, 1, 2, \ldots$. Multiple replication rounds can be initiated per cell. Related to the number of concurrent replication rounds initiated per cell, the observed generation time will exceed $C + D$, range between $C + D$ and D, or be equal to D *(21).* From this theory, Donachie *(22)* derived the following relationship for the amount of biomass at cell division, m_d:

$$m_d = 2m^* \cdot \exp[\mu \cdot (C + D)] \qquad (7)$$

where μ is the specific growth rate of the cell biomass (in combination with equation 6, μ represents μ_{max}). Following this equation, the cell mass at division (and thus also the mean cell mass of the population) is proportional to the exponent of the product $\mu \cdot (C + D)$. The number of replication rounds at the time of division can thus be derived from the ratio $m_d/2m^*$.

Based on equation 7 and previous work, a number of hypotheses concerning the effect of dynamic temperatures on the cell division process (and thus the overall specific cell number growth rate) can be formulated.

1. Hypothesis I. The product $\mu \cdot (C + D)$ stays constant for different temperature conditions. This means that temperature variations do not alter the size and chemical composition of the cells, as postulated by Cooper *(16).*
2. Hypothesis II. Trueba et al. *(62)* reported that the average volume of an *E. coli* cell decreases with decreasing temperatures. Consequently, for these observations, the product $\mu \cdot (C + D)$ depends on temperature because the mean cell volume is proportional to m_d.
3. Hypothesis III. A lag in biomass growth of *E. coli* induced by sudden shifts from low to high temperatures was reported by Ng et al. *(46).* The authors assumed that cells growing at low temperatures express some indication of their damaged status, which needs to be repaired before active growth at high temperatures can be achieved. This damaged state can be indicated by a limiting concentration of one or more enzymes. When passing from a low to a high temperature, cells first need to increase the concentration of these limiting enzymes before they can increase their biomass growth rate. A (simplified) mathematical translation of this hypothesis reads as follows:

$$\frac{dm(t)}{dt} = \left\{ \frac{E(t)}{m(t)} \cdot \frac{\mu_{max}[T(t)]}{L} \right\} \cdot m(t) = \mu(\cdot) \cdot m(t)$$

where

$$\frac{dE(t)}{dm(t)} = L \qquad (L = L_1 \text{ or } L_h)$$

and where $E(t)$ is some critical growth factor and L is the rate at which E is synthesized *(46).* This production rate changes according to temperature (in a discrete way), i.e., L_l and L_h are the typical production rates for

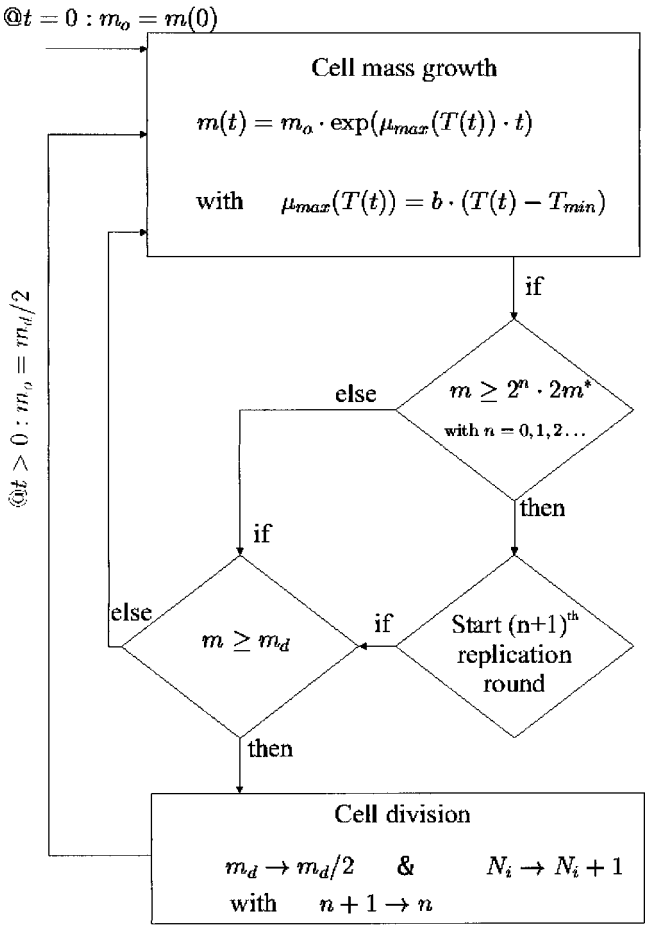

FIGURE 2. *Schematic representation of a single object, i.e., a cell, within the individual-based model developed for microbial lag due to sudden temperature shifts. The scheme describes the growth process of a single cell. The mass of each cell (*m*) grows exponentially following the temperature-dependent maximum specific growth rate: $\mu_{max}[T(t)]$. During cell growth, multiple replication forks (*n*) may be initiated. Whenever cell mass reaches the mass at division (m_d), cell division takes place (i.e., a discrete event). Each daughter cell continues to grow according to this scheme. The population dynamics emerge from the sum of all individual objects.*

low and high temperatures, respectively. For *E. coli* populations, the high-temperature zone ranges from 20 to 37°C, which is the normal physiological range of *E. coli (46).*

Temperature dependence in the suboptimal growth temperature range can be modeled by the square root model of Ratkowsky et al. *(51)*:

$$\sqrt{\mu_{\max}[T(t)]} = b\cdot[T(t) - T_{\min}] \qquad (8)$$

Figure 2 illustrates the basic model structure (corresponding with hypothesis I) within the individual-based model concept. Cell mass growth is described as a continuous process, whereas cell division occurs at discrete time periods whenever a single cell reaches its mass at cell division m_d. The superposition of the single cell dynamics yields a description of the total population dynamics (for more details, see Dens et al. *(20, 21)*).

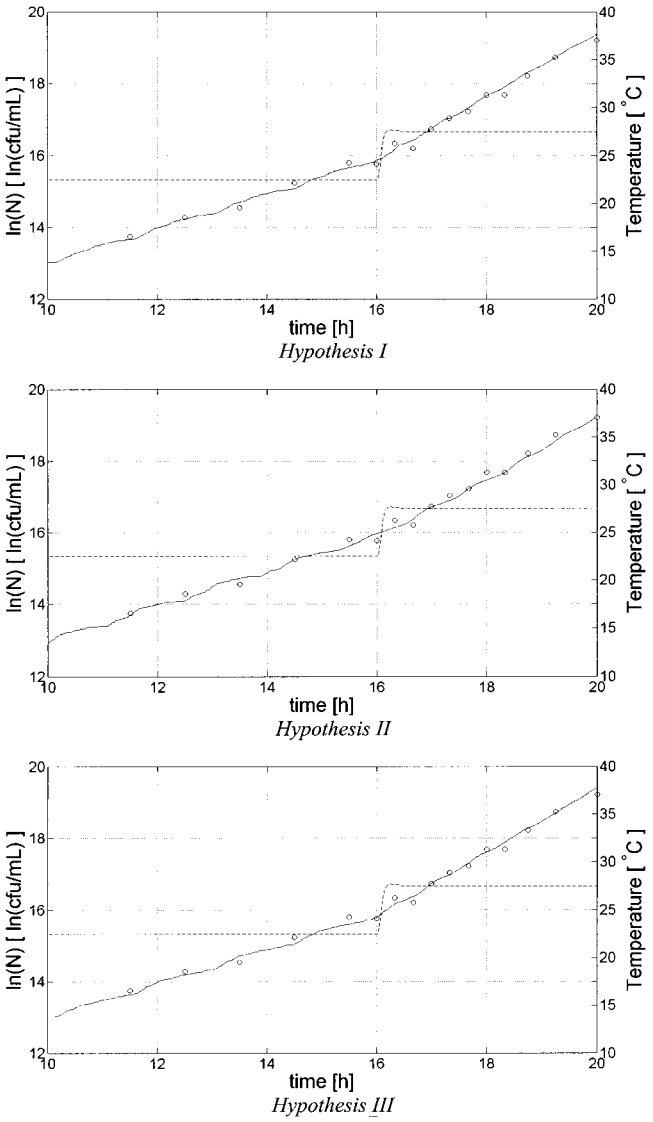

FIGURE 3. *Simulation of the proposed individual-based model with experimental data on* E. coli *(○) submitted to a sudden temperature shift from 22.5 to 27.5°C during exponential growth (Bernaerts et al. (10) and Dens et al. (20)). The solid line represents the model description using the measured temperature profile (dashed line). The initial cell mass,* m*(0),was selected randomly from a normal distribution, and cell division yields two daughter cells with a specific growth rate randomly varied around the mean μ_{max} (see Kreft et al. (32)). Parameters values are from Bernaerts et al. (10) and Dens et al. (20).*

Simulation. As a case study, the effect of abrupt temperature upshifts on the growth of *E. coli* was examined. The experimental data in Figures 3 and 4 depict the effect of small (5°C) and large (20°C) positive temperature shifts, respectively. Full details on generation of the experimental data were reported by Bernaerts et al. *(10).* For each of the temperature shifts, the three hypotheses have been tested. The small temperature increase from 22.5 to 27.5°C did not alter the balanced growth dynamics of the microorganisms (Fig. 3), which supports all three hypotheses. However, cell density data generated during the larger temperature shift from 15°C (low temperature range) to 35°C (high temperature range) indicated a lagged growth response, which sup-

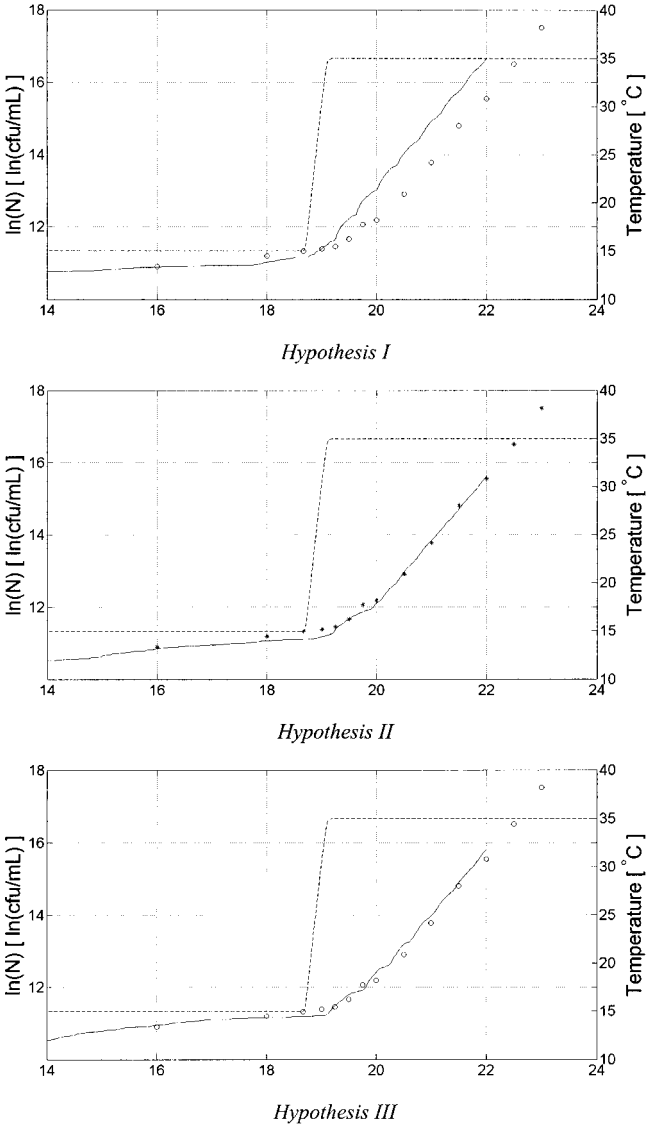

FIGURE 4. *Simulation of the proposed individual-based models with experimental data on* E. coli *(○) submitted to a sudden temperature shift from 15 to 35°C during exponential growth (Bernaerts et al. (10) and Dens et al. (20)). The solid line represents the model description using the measured temperature profile (dashed line). See Figure 3 for details of parameter values.*

ports hypotheses II and III only (see Fig. 4). Based on hypothesis II, the lag phase is the time needed to increase the cell volume to the new critical mass at division. Biomass growth exhibits an immediate rate adjustment whereas cell number shows lag behavior. Based on hypothesis III, the lag phase originates at the level of biomass growth and propagates into the cell number evolution.

Discussion. This example illustrates how cell-related mechanistic knowledge can be built into an individual-based model in a relatively straightforward and easy way. Simulations with the individual-based model can then explain the population dynamics. Three cell mechanisms describing the effect of dynamic temperatures were extracted from the literature. Two of the three hypotheses could describe both a small and a large temperature shift equally well. Given only population density measurements, it was impossible to discriminate between the established models. At this point, additional measurements are needed to further establish the model structure. Such measurements can be, for example, biomass weight, DNA concentration, RNA concentration, or protein concentration. In the field of predictive microbiology, these types of measurements are seldom considered, although they are widely used in general microbiological studies. Furthermore, the precision of these measurements is often significantly lower than that of common viable counts. The lack of (sufficient) measurement accuracy generally hampers the applicability of experimental data during model building. The revised modeling example clearly points out the two-way interaction between model building and data generation. Besides the selection of essential measurements, this two-way interaction embraces the design of informative experiments, i.e., the selection of appropriate (dynamic) input conditions *(10, 35)* or (static) treatment combinations *(19, 50).* A disadvantage of the IbM approach is that the models may become relatively complex and computationally tedious during simulation. However, the obtained mechanistic knowledge can eventually form a sound basis for population-based models, which are more easily managed. At present, the only alternative dynamic model able to describe an intermediate lag phase is the model of Hills and Wright *(28).* A review of dynamic mathematical models describing microbial lag phenomena in general was done by Swinnen et al. *(60).*

MODELING MICROBIAL INTERACTION WITH PRODUCT INHIBITION

The inhibition phenomenon. During the fermentation process of lactic acid bacteria, lactic acid is produced (first biological process). The lactic acid released into the medium will dissociate and lower the pH of the medium (chemical process). Both the concentration of undissociated lactic acid ([LaH]) and the decreased pH ($[H^+]$) have an inhibitory effect on microorganisms (second biological process). The lactic acid production will cause the inhibition of bacterial growth. The cessation of growth observed as the stationary phase can thus be (partially) assigned to a self-induced inhibitory effect (i.e., an intraspecies interaction). This lactic acid production also affects neighboring microorganisms (i.e., an interspecies interaction). Pathogenic bacteria, such as *Yersinia enterocolitica* and *Listeria monocytogenes,* can be very sensitive to this inhibitory compound *(45, 65).* The increasing lactic acid concentration will cause an early termination of their growth processes. For this reason, lactic acid bacteria can be exploited as a natural antimicrobial agent within (fermented) food products or as a protective culture.

We modeled the interaction of lactic acid bacteria (antagonist) with pathogenic bacteria (target). Information was extracted from the studies of Vereecken et al. *(65, 66)* and Vereecken and Van Impe *(68).* The experimental design was such that the production of lactic acid acted as a single inhibitory phenomenon (no substrate or nutrient limitation). This strategy reasonably facilitates the development of a consistent basic model structure that can be subsequently extended to include other relevant interaction effects.

Modeling. In contrast to the empirical formulation in equation 3 where the stationary phase is modeled as a function of $N(t)$ and N_{max}, growth suppression is described in this model in terms of the lactic acid production, which is therefore explicitly incorporated into the model structure. This general expression describing the growth characteristics of both target and antagonist is

$$\frac{dN_i(t)}{dt} = \mu_{lag}(\cdot)\cdot\mu_{max}(\cdot)\cdot\mu_{LaH,H^+}([LaH], [H^+])\cdot N_i(t) \quad (9)$$

Description of the lactic acid production, particularly by the antagonist, requires an additional coupled differential equation:

$$\frac{d[LaH]_{tot,i}(t)}{dt} = \pi(\cdot)\cdot N_i(t)] \quad (10)$$

where $\pi(\cdot)$ is the specific lactic acid production rate of the antagonistic bacterium (i). The $[LaH]_{tot}$ term refers to the total lactic acid concentration, i.e., the sum of the undissociated and dissociated lactic acid concentrations. When multiple lactic acid–producing strains are present, the overall growth rate of each strain will be affected by the sum of $[LaH]_{tot,i}$. To describe the chemical process of lactic acid dissociation in a complex medium, several methods based on traditional chemical laws are available *(68, 74)*. Given a specific growth medium and initial pH, the process of lactic acid dissociation can be fully identified regardless of the microbial evolution model. Both [LaH] and $[H^+]$ vary with time and are determined by the lactic acid–producing strain and the dissociation properties of $[LaH]_{tot}$ in the growth medium. Several inhibitory functions can be proposed for $\mu_{LaH,H}([LaH], [H^+])$ *(66)*. Given a set of model requirements based on the typical metabolic lactic acid profile, Vereecken et al. *(66)* selected the following equation to express the inhibitory effect of undissociated lactic acid and the proton concentration (from Passos et al. *(48)*):

$$\mu_{LaH,H^+} = \left(1 - \frac{[LaH]}{[LaH]_{max}}\right)^{\alpha}\cdot\left(1 - \frac{[H^+]}{[H^+]_{max}}\right)^{\beta}$$
$$\text{when } [LaH] \le [LaH]_{max}$$
$$\text{and } [H^+] \le [H^+]_{max}$$
$$= 0 \quad \text{when } [LaH] > [LaH]_{max}$$
$$\text{or } [H^+] > [H^+]_{max} \quad (11)$$

where $[LaH]_{max}$ is the lactic acid concentration at which growth ceases, $[H^+]_{max}$ is the proton concentration associated with the minimum pH for growth, and α and β are fixed to $1 + \epsilon$ where ϵ is some small positive value (the need for the exponents α and β was explained by Vereecken et al. *(66)*). The inhibition terms have no effect on microbial evolution as long as the undissociated lactic acid and proton concentrations remain well below their inhibitory values. In such cases, both functions are approximately equal to 1. When [LaH] or $[H^+]$ get higher as time proceeds, either function evolves towards zero and growth stagnates. To complete the model structure, the specific lactic acid production rate, $\pi(\cdot)$, must be mathematically modeled. Combining the traditional linear law with the concept of metabolism-inhibiting concentrations, $\pi(\cdot)$ in equation 10, can be represented as follows:

$$\pi(\cdot) = \underbrace{Y_{LaH/N_i}\cdot\mu_i(\cdot)}_{\text{growth}} + \underbrace{m_i([LaH], [H^+])}_{\text{maintenance}} \quad (12)$$

where Y_{LaH/N_i} is the yield coefficient (mmol CFU^{-1}), $\mu(\cdot)$ is the overall specific growth rate (from equation 9), and $m_i([LaH], [H^+])$ is the maintenance coefficient (mmol CFU^{-1} h^{-1}). The first factor presents the growth-related production, whereas the maintenance factor $m_i([LaH], [H^+])$ assures the observed production of $[LaH]_{tot}$ during the (first hours of) the stationary phase. This maintenance-related production is assumed to be constant *(36, 49)* or to cease when some metabolism-inhibiting proton or undissociated lactic acid concentration is reached *(66)*.

The overall model structure made from the coupled differential equations 9 and 10 yields accurate descriptions for monoculture and mixed-culture growth. This model is illustrated with experimental data from *Lactobacillus lactis* and *Listeria innocua* (as a representative for *L. monocytogenes*) in Figure 5. Model parameters are obtained as follows. First, the chemical process is modeled using the approach described by Vereecken et al. *(68)*. Second, the microbial growth process is identified (i.e., equations 9 and 11). Third, the parameters for lactic acid production are determined (equations 10 and 12). Integration of these values within the overall model structure yields the predictions shown in Figure 5.

Discussion. The model building strategy described in this example starts from the identification of main phenomena determining the dynamics of the microbial system. The derived general model structure allows the description of the stationary phase in a natural (mechanistically sound) and consistent way. The mechanistic model structure can easily describe both single species and multiple species dynamics (with lactic acid–mediated interaction or more generally metabolic product–mediated interaction). This example illustrates also how microbial evolution itself creates dynamic environmental conditions.

DISCUSSION

Dynamic mathematical models allow for consistent computation of the impact of different steps associated with the production, distribution, and retailing of a food (characterized by conditions that vary over time) on microbial evolution. The intrinsic properties of microbial evolution such as growth-related metabolic product formation and inhibition can be integrated and predicted.

Examples given here illustrate how we can learn from predictive modeling based on biological and physical concepts. The IbM approach, for instance, is an excellent tool for testing generic cell mechanisms with respect to observed population behavior. However, such a modeling approach with an increased level of detail demands specific measurements at the cell and/or population level.

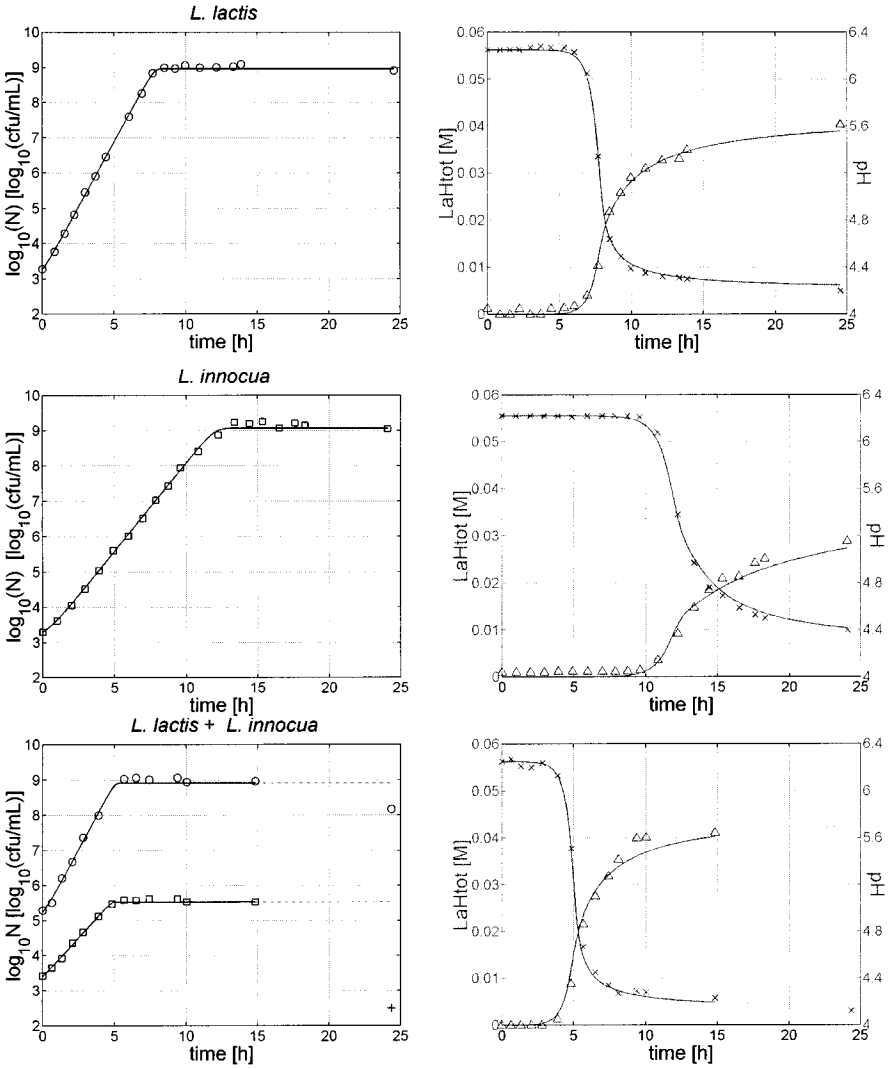

FIGURE 5. *Description of experimental data on* Lactobacillus lactis *(○) and* Listeria innocua *(□) grown in mono- and coculture based on the dynamic model structure (equations 9 and 10) in combination with equations 11 and 12 (data from Vereecken et al. (65)) (*x *refers to cell numbers below detection limit). The total lactic acid concentration* $[LaH]_{tot}$ *(△) and pH (×) are depicted in the right plots. The dissociation kinetics of the applied medium have been computed according to Vereecken et al. (68). The decreased cell density cannot be predicted by the model structure (dashed line).*

To expand the applicability of predictive models, researchers must be encouraged to increase the generality and thus transferability of model structures. For example, the complete model structure established for microbial interaction can describe the individual behavior of lactic acid bacteria and the inhibitory mechanism in the presence of pathogenic or spoilage bacteria. This example also illustrates that cell density measurements are not always sufficient for establishing complex model structures. Components interfering with microbial evolution, such as metabolite formation, should be identified, measured, and built into the model structure. Given this increased experimental understanding of microbial dynamics, we can produce more robust (semi-)mechanistic models of high predictive quality.

In this respect, model builders can learn more from dynamic experimental data. Microbial dynamics under realistically time-varying conditions are not necessarily observable from commonly available static data. For example, the application of time-varying temperature profiles revealed the induction of an intermediate lag phase during the exponential growth of *E. coli.*

When extrapolating model structures established on static experimental data to more realistic dynamic conditions, e.g., a combination of processing steps, model predictions may fail to describe microbial evolution accurately. Stephens et al. *(59)* observed that slow heating rates applied during inactivation of *L. monocytogenes* induced thermotolerance. Predictions using an inactivation model developed based on the results of static experiments (not taking into account heating rate) systematically overestimated the effect of the applied heat treatment. Future research should thus include dynamic model development using dynamic experimental data. In such cases, complementary effects of dynamic conditions or subsequent treatments can be properly incorporated within the model structure. Synergistic effects form the basic principles within the hurdle technology *(34)* often addressed in the food industry.

Overall, model improvement is motivated by increasing predictive accuracy. However, increased modeling accuracy must be balanced with model structure complexity. The purpose for which the model is being developed must always be clearly specified. An important challenge for the future is a satisfactory trade-off between predictive power and manageability of mathematical models: ''When is simple good enough?'' *(12).* Buchanan et al. *(12)* illustrated that a simple three-phase linear model can describe a microbial growth curve under static conditions as well as can

more complex models (e.g., equation 2) if the intended purpose of the model is limited to the characterization of the lag time duration, the exponential growth rate, and the stationary phase cell density. The simplicity of the three-phase linear model, however, implies that the model is not applicable under dynamic conditions. Individual-based models are particularly attractive for exploring and gaining insight into microbiological phenomena. Thes models are manageable when describing the dynamics of relatively low cell numbers; but when applied for the description of population dynamics, their computational complexity hampers their widespread use. In such case, developed individual-based models may be reduced to less complex mechanistically inspired dynamic population models.

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