



## Predictive Modeling of Mixed Microbial Populations in Food Products: Evaluation of Two-species Models

KAREN M. VEREECKEN, ELS J. DENS AND JAN F. VAN IMPE\*

*BioTeC—Bioprocess Technology and Control, Department of Food and Microbial Technology,  
Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Leuven, Belgium*

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Predictive microbiology is an emerging research domain in which biological and mathematical knowledge is combined to develop models for the prediction of microbial proliferation in foods. To provide accurate predictions, models must incorporate essential factors controlling microbial growth. Current models often take into account environmental conditions such as temperature, pH and water activity. One factor which has not been included in many models is the influence of a background microflora, which brings along microbial interactions. The present research explores the potential of autonomous continuous-time/two-species models to describe mixed population growth in foods. A set of four basic requirements, which a model should satisfy to be of use for this particular application, is specified. Further, a number of models originating from research fields outside predictive microbiology, but all dealing with interacting species, are evaluated with respect to the formulated model requirements by means of both graphical and analytical techniques. The analysis reveals that of the investigated models, the classical Lotka–Volterra model for two species in competition and several extensions of this model fulfill three of the four requirements. However, none of the models is in agreement with all requirements. Moreover, from the analytical approach, it is clear that the development of a model satisfying all requirements, within a framework of two autonomous differential equations, is not straightforward. Therefore, a novel prototype model structure, extending the Lotka–Volterra model with two differential equations describing two additional state variables, is proposed to describe mixed microbial populations in foods.

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### 1. Introduction

As food safety is of major concern in modern society, the scientific discipline of predictive microbiology gains more and more interest worldwide. An important research topic in this field is the development of mathematical models able to predict the growth of pathogenic microorganisms in foods. Such models present a valuable tool in risk assessment and HACCP studies

(*Hazard Analysis Critical Control Points*), when combined with a dose–response relationship, i.e. the probability of infection as a function of the pathogen level in a food product (Buchanan & Whiting, 1996). During the last decade, a whole variety of predictive models has been proposed, taking into account growth-influencing factors such as temperature, pH and water activity (see e.g. Zwietering *et al.*, 1990; McMeekin *et al.*, 1993; Whiting *et al.*, 1997).

A factor which has not been included in most of the available models is the presence of a—usually

\* Author to whom correspondence should be addressed.  
E-mail: [jan.vanimpe@agr.kuleuven.ac.be](mailto:jan.vanimpe@agr.kuleuven.ac.be)

heterogeneous—background microflora and associated microbial interactions. Classical predictive models focus on the growth of a *single* species, thus incorporating *intraspecies* but not *interspecies* interactions. Although these models are sufficient for products with a low microbial load (e.g. as a consequence of a sterilization or pasteurization process), the effect of interspecies interactions on pathogenic growth cannot be neglected in those foods in which a considerable number of microorganisms is inherently present. Examples are *fermented food products*, where *starter cultures* are used to achieve some favorable physico-chemical changes in the ingredients. Also, in the case of novel *biological preservation techniques*, *protective cultures* are added to foods in order to inhibit the growth of pathogens (i.e. biocontrol).

The lack of suitable predictive models for mixed microbial growth in spite of the need for such models for a number of industrial applications, is primarily due to the complex nature of interspecies interactions. While single-species growth can mostly be characterized in terms of a lag phase, an exponential growth phase and a stationary phase, no such general outline exists for multiple-species growth. Different types of interactions occur, as is illustrated in a classification scheme proposed by Mossel *et al.* (1995). In this scheme, distinction is made between synergistic (positive) and antagonistic (negative) interactions, and for each type different mechanisms are postulated, such as the production of certain microbial metabolites and microbially effected changes in pH and water activity. With respect to food safety and predictive microbiology, especially the antagonistic interactions are of importance.

This paper describes a three-fold contribution to the field of predictive modeling of mixed microbial growth. At first, a set of model requirements is specified, which a model has to fulfill to be of use for this particular application. These requirements are listed in Section 2. Secondly, a number of existing multiple-species models, originating from research fields outside predictive microbiology, are studied with respect to their relations to the stated model requirements on the basis of both a graphical and a mathematical analysis. An overview of the models together with

some general information on each of them is presented in Section 3, while the analysis itself can be found in Sections 4 and 5. Finally, based on the knowledge resulting from this analysis, a novel prototype model for mixed microbial growth in food products is briefly introduced in Section 6.

## 2. Model Requirements

In this contribution, we concentrate on the following class of autonomous continuous-time/two-species models:

$$\frac{dN_1}{dt} = f(N_1, N_2), \frac{dN_2}{dt} = g(N_1, N_2) \quad (1)$$

with  $N_1$  being the number of individuals of species 1 and  $N_2$  the number of individuals of species 2. In the context of the above-mentioned applications, these two species can be regarded as representing pathogenic organisms on the one hand and starter or protective cultures on the other.

When investigating the potential of new or existing models for a particular application, the specification of an appropriate set of model requirements is a prerequisite. In the field of predictive modeling of mixed microbial growth this is not evident as, in contrast to single-species growth, extensive experimental data sets of mixed culture growth are not available. However, based on the biological interpretation of the model and on a number of studies of microbial interactions in food products, the following four requirements are postulated.

*Model requirement #1.* First of all, model predictions should be realistic under all conditions, i.e. species number should be (i) positive, (ii) bounded, and (iii) if a species is not present, either initially or through extinction resulting from interaction with the other species, the species number should remain zero.

This requirement is adopted in order to exclude those models which belong to the general class (1), but which are not suited to represent living populations inherently characterized by positive and finite species numbers.

*Model requirement #2.* Model solution curves which oscillate as a function of time are not allowed if all environmental conditions are constant.

This second requirement is included, since to the best of authors' knowledge, oscillatory behavior has never been reported in studies on mixed microbial populations in foods under invariant environmental conditions. Note however that oscillations in species numbers resulting from fluctuating environmental conditions are allowed, but are not considered in this paper.

*Model requirement #3.* Starting from non-zero species numbers  $N_1$  and  $N_2$ , the model should be able to describe two different types of evolution, namely (i) evolution to coexistence of the two microbial populations, and (ii) evolution to extinction of one species and survival of the other.

Several examples of these evolution types can be found in the literature. A study on the inhibition of the bacterial pathogen *Listeria monocytogenes* by the lactic acid bacterium *Lactobacillus plantarum* (Ashenafi, 1991) revealed that under certain conditions of pH and inoculum densities, a coculture of both species is obtained, while for other conditions, the viable count of *L. monocytogenes* decreased underneath the detection level, suggesting a complete extinction of the latter species. Similar results are obtained for *L. monocytogenes* in combination with *Lactobacillus bavaricus* (Winkowski *et al.*, 1993) and for *L. monocytogenes* or *Yersinia enterocolitica* with *Lact. plantarum* or *Leuconostoc* spp. (Jeppesen & Huss, 1993). Inactivation of *L. monocytogenes* to levels below the detection limit are also reported by Degnan *et al.* (1992).

*Model requirement #4.* The model should be able to describe a lag phase in the dynamics of both  $N_1$  and  $N_2$ .

From the outset of predictive microbiology, much research effort has been directed to a proper modeling of the lag phase inherent to microbial growth (see e.g. Baranyi & Roberts, 1994). A lag phase is typically seen in microbial growth curves, representing the logarithm of the cell density vs. time, as an initial phase in which the specific growth rate rises from a low towards a more or less constant value. It is generally accepted that the lag is a period during which the cells are adjusting their physiology and biochemistry to exploit the environment in which they find themselves (McMeekin *et al.*, 1993). With respect to food safety, the kinetics of the lag of

pathogenic organisms is of great importance. Different approaches to cope with this factor can be found in Gibson *et al.* (1987), Van Impe *et al.* (1992), Baranyi & Roberts (1994) and Buchanan *et al.* (1997). Observe that, outside the field of predictive microbiology, alternative approaches exist to deal with time lags in biological models (see e.g. MacDonald, 1978).

### 3. Existing Models

Table 1 presents nine autonomous continuous-time/two-species models which will be evaluated for potential application in predictive microbiology. The models originate from different research fields in ecology and are more or less arranged according to increasing complexity. Among them, both well-known and less well-known models are present, but all will contribute to illustrate benefits and/or disadvantages in view of the specified application, as will become clear in further sections.

Unless otherwise specified,  $r_i$  (1/time) indicates the intrinsic rate of growth of species  $i$ ,  $k_i$  (1/time) is a measure for the decay rate of species  $i$ ,  $K_i$  (number) represents the saturation level (or carrying capacity of the environment) for species  $i$  when no other species is present, and  $a_{ij}$  [ $1/(\text{number} \times \text{time})$ ] for models 1 and 2 and (—) for models 3–9] is a coefficient of interaction measuring the effects of species  $j$  on species  $i$ . Parameter values are all positive.

Model 1 is proposed by McLean & Nowak (1992) as an immune system model describing the evolution of a human pathogen  $N_1$  in the presence of immune cells  $N_2$ . In this model,  $N_2$  increases with a rate  $r_2 N_1$  proportional to  $N_1$ .

Model 2, originally formulated by Lotka (1956) and Volterra (1931), is well known in the literature as a predator–prey model. This relation has been thoroughly studied with respect to both mathematical properties (see e.g. Dubois, 1978) and coincidence with real ecological communities (e.g. Bailey & Ollis, 1986).

The same authors (Lotka, 1956; Volterra, 1931) were also the first to formulate model 3, a now widely used relation describing two species in competition. It is based on the logistic theory of population growth and assumes a linear decay in

TABLE 1  
Nine autonomous continuous-time/two-species models ( $i, j = 1, 2; i \neq j$ )

Model	Function	References
1	$\frac{dN_1}{dt} = N_1 (r_1 - \alpha_{12}N_2), \frac{dN_2}{dt} = -k_2N_2 + r_2N_1$	McLean & Nowak (1992)
2	$\frac{dN_1}{dt} = N_1 (r_1 - \alpha_{12}N_2), \frac{dN_2}{dt} = N_2(-k_2 + \alpha_{21}N_1)$	Dubois (1972)
3	$\frac{dN_i}{dt} = \frac{r_iN_i}{K_i} (K_i - N_i - \alpha_{ij}N_j)$	Lotka (1956), Volterra (1931)
4	$\frac{dN_i}{dt} = \frac{r_iN_i}{K_i} (K_i - N_i - \alpha_{ij}N_j - \beta_jN_j^2)$	Ayala <i>et al.</i> (1973)
5	$\frac{dN_i}{dt} = \frac{r_iN_i}{K_i} (K_i - N_i - \alpha_{ij}N_j - \beta_iN_i^2)$	Ayala <i>et al.</i> (1973)
6	$\frac{dN_i}{dt} = \frac{r_iN_i}{K_i} (K_i - N_i - \alpha_{ij}N_j - \delta_j(1 - \exp(-\gamma_jN_j)))$	Ayala <i>et al.</i> (1973)
7	$\frac{dN_i}{dt} = \frac{r_iN_i}{K_i^{\theta_i}} (K_i^{\theta_i} - N_i^{\theta_i} - \alpha_{ij}N_j/K_i^{1-\theta})$	Ayala <i>et al.</i> (1973)
8	$\frac{dN_i}{dt} = \frac{r_iN_i}{\ln(K_i)} (\ln(K_i) - \ln(N_i) - \alpha_{ij} \ln(N_j))$	Coleman & Gomatam (1972)
9	$\frac{dN_1}{dt} = R_1N_1 \left[ \frac{I_{O1}}{N_1 + \beta N_2} + \frac{I_{E1}}{N_1} - \gamma_{11}N_1 - \gamma_{12}N_2 - C_1 \right]$ $\frac{dN_2}{dt} = R_2N_2 \left[ \frac{\beta I_{O2}}{N_1 + \beta N_2} + \frac{I_{E2}}{N_2} - \gamma_{22}N_2 - \gamma_{21}N_1 - C_2 \right]$	Schoener (1976)

growth rate with increasing  $N_1$  and  $N_2$ . Successful applications in ecology were obtained with, e.g. protozoans (Vandermeer, 1969). However, a considerable number of communities seem inadequately described by model 3, amongst which are salamanders (Wilbur, 1972), microcrustaceans (Neill, 1974), lizards (Schoener, 1974) and *Drosophila* (Ayala *et al.*, 1973). For this reason, alternatives to this classical logistic-based competition model have been proposed by Ayala *et al.* (1973) (models 4–7), Coleman & Gomatam (1972) (model 8) and Schoener (1976) (model 9).

Both models 4 and 5 add one term to the Lotka–Volterra model. In model 4, this results in an extra interaction term, but the underlying single-species growth model is the same as for model 3, while in model 5 the single-species dynamics is no longer logistic. The parameter  $K_i$  in

the latter model no longer represents the single-species carrying capacity. (Note however that the concept of carrying capacity does not have to be related to one parameter or a single species, as it actually represents a non-zero population density at which growth ceases or otherwise said a non-trivial solution of  $dN_i/dt = 0$ .) Concerning the interaction terms,  $\alpha_{ij}$  can be interpreted as a measure of competition due to sharing of resources, while parameter  $\beta$  (1/number) represents intraspecific interactions (other than sharing of resources) affecting the growth of the species itself ( $\beta_i$  in model 5) or the other species ( $\beta_j$  in model 4) (Ayala *et al.*, 1973). Like the previous models, model 6 is an extension of model 3 and reduces to the logistic growth model in case of single-species growth. The second interaction term  $\delta_j(1 - \exp(-\gamma_jN_j))$  varies between 0 and  $\delta_j$  and

can be seen as a term representing another kind of interactions than those included in the term  $\alpha_{ij}N_j$  (Ayala *et al.*, 1973). Parameters  $\delta_j$  (number) and  $\gamma_j$  (1/number) have no straightforward biological interpretation.

The last extension of model 3 is model 7. The fourth parameter in this model,  $\theta_i$  (—), changes the function relating growth rate to population size. An important consequence with respect to the underlying single-species model is that a maximum specific growth rate is attained at a density smaller ( $\theta_i < 1$ ) or larger ( $\theta_i > 1$ ) than  $K_i/2$ , where for the original logistic function ( $\theta_i = 1$ ) this maximum is reached at exactly  $K_i/2$  (Ayala *et al.*, 1973). Within a group of 11 candidate models (including models 3–8) tested on an experimental data set of *Drosophila* by Ayala *et al.*, model 7 accounted best for the observed results.

Model 8 was suggested by Coleman & Gomatam (1972) to describe interactions between *Drosophila* species. The interaction term  $N_i \ln(N_j)$  is a generalization of the single-species saturation term  $N_i \ln(N_i)$  originally used by Gompertz (1825) in another context. As for model 4,  $K_i$  does not equal the single-species saturation level anymore. The model can be solved analytically, resulting in a set of static equations with obvious similarities with the original (static) Gompertz model, the basis of the often used modified Gompertz model in predictive microbiology.

Model 9, developed by Schoener (1976), assumes that each species has access to exclusive and common resources, represented by  $I_{Ei}$  and  $I_{Oi}$  (energy/time), respectively. Other parameters in the model are a yield coefficient  $R_i$  (number/energy), intraspecific and inter-specific interaction coefficients  $\gamma_{ii}$  and  $\gamma_{ij}$  [energy/(number<sup>2</sup> × time)] and a maintenance coefficient  $C_i$  [energy/(number × time)].

#### 4. Model Evaluation: A Graphical Approach

In this section, model evaluation is performed on the basis of a graphical analysis. Two main parts can be distinguished.

1. A first part involves a study of the qualitative behavior of the model in the  $N_1N_2$ -phase-

plane; in other words, the evolution of the model for different initial population sizes  $N_{1,0}$  and  $N_{2,0}$ . This implies the construction of the phase portrait and a stability analysis of the critical points.

For a good comprehension of the following text, a brief overview of the basic principles and definitions of this methodology is given. A phase portrait of a system of the general form (1) is a representation of all kinds of trajectories [solution curves of eqn (1)] in the phase plane. Curves, along which  $f$  or  $g$  in eqn (1) are zero, are called zero isoclines. Critical points are points in the phase plane for which both  $f$  and  $g$  equal zero. Depending on the stability of, and the shape of trajectories near a critical point, it is classified as an (un)stable node, an (un)stable focus (or spiral point), a saddle point or a center. Stability and trajectory shapes can be investigated by plotting trajectories from a large number of initial states spread over the phase plane, or by evaluation of the eigenvalues of the Jacobian matrix of the system.

2. A second topic concerns the investigation of the main characteristics of species vs. time curves predicted by the model.

Within the framework of this graphical analysis recipe, the model requirements stated in Section 2 can be reformulated as follows.

- *Model requirement #1*: trajectories leading to (i) negative or (ii) infinite numbers of species 1 or 2 are not allowed, and (iii) trajectories starting on the  $N_1$ - or  $N_2$ -axis may not deviate from this axis. This can be easily verified by means of phase-plane analysis.
- *Model requirement #2*: no centers or spiral points are allowed. As for requirement #1, this can be checked by phase-plane analysis.
- *Model requirement #3*: the model should be able to generate a stable node (i) in the first quadrant, and (ii) on the  $N_1$ - and  $N_2$ -axis. Agreement with this model requirement is thus again completely determined by the model's behavior in the  $N_1N_2$ -plane.
- *Model requirement #4*: the model should be able to generate a lag phase in trajectories representing logarithmically transformed species numbers ( $\ln(N_1)$  and  $\ln(N_2)$ ) as a function

of time. In contrast with the previous requirements, this cannot be deduced from phase portraits. Therefore, time plots need to be constructed.

#### 4.1. TRAJECTORY EVOLUTION IN THE PHASE PLANE

A considerable part of the results can be explained on the basis of the phase portraits of models 1–4 and 8. As can be seen from Figs 1–5, these models give rise to five different types of behavior in the  $N_1N_2$ -plane and the phase por-

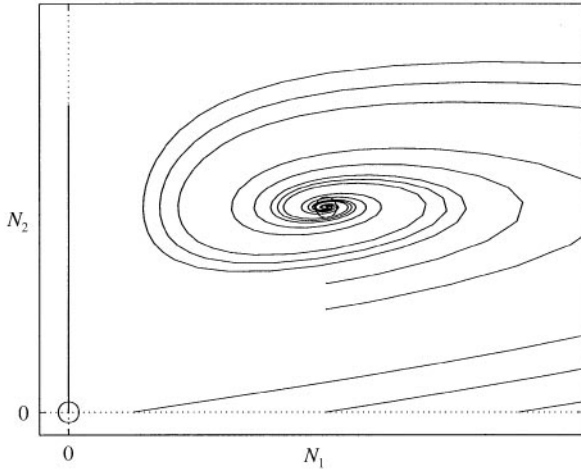


FIG. 1. Phase portrait of model 1 in Table 1: trajectory (—); critical point (○).

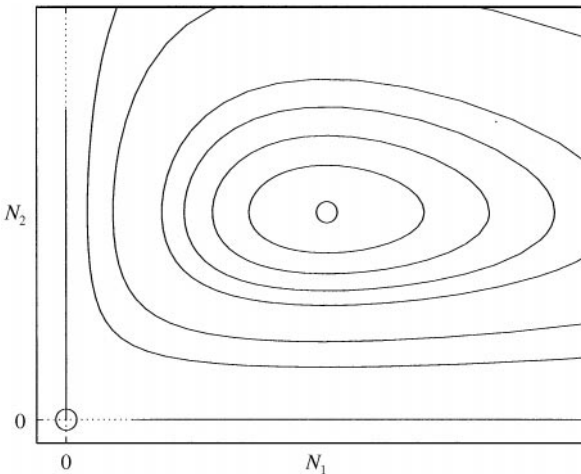


FIG. 2. Phase portrait of model 2 in Table 1: trajectory (—); critical point (○).

traits of the other models are for the main part comparable with one of these types (see below).

Verification of model requirements #1 and #2 is rather straightforward. Concerning the first requirement, trajectories starting on the  $N_1$ -axis deviate from this axis for model 1 (Fig. 1) or lead to infinity for model 2 (Fig. 2). Evolution to infinite species numbers is also possible for model 8 [Fig. 5(b)–(d)]. Consequently, requirement #1 is not satisfied for models 1, 2 and 8. No further violations of this requirement were encountered for the other models (see e.g. Figs 3 and 4). However, trajectories on the axes are impossible for model 9, due to the  $I_{Ei}/N_i$  term. This issue will be addressed further in Section 5.

Because of the presence of (un)damped oscillations, models 1 and 2 are in obvious disagreement with requirement #2, as can be seen in Figs 1 and 2. This oscillatory behavior is not encountered for the other models (see e.g. Figs 3–5).

Another model requirement that can be checked by means of phase-plane analysis is requirement #3. As indicated above, a study of the nature and location of the critical points of a model forms the key to verify if requirement #3 is satisfied. Both models 1 and 2 have two critical points of which one is the origin and the other is lying in the first quadrant. Due to the absence of critical points—or more precisely stable nodes—on the axes, species extinction is not possible. Moreover, evolution to coexistence cannot be described by model 2. The remaining models in Table 1 are of the general form ( $i, j = 1, 2; i \neq j$ )

$$dN_i/dt = \mu_i(N_i, N_j) N_i \quad (2)$$

with  $\mu_i(N_i, N_j)$  being a continuous function, representing the specific evolution rate. As each critical point results from the intersection of a zero isocline of species 1 [ $N_1 = 0$  or  $\mu_1(N_1, N_2) = 0$ ] with a zero isocline of species 2 [ $N_2 = 0$  or  $\mu_2(N_1, N_2) = 0$ ], the following critical points can be distinguished.

- the origin ( $N_1 = 0, N_2 = 0$ ),
- the intersection point(s) (if existing) of  $N_1 = 0$  and  $\mu_2(N_1, N_2) = 0$ , lying on the  $N_2$ -axis,
- the intersection point(s) (if existing) of  $N_2 = 0$  and  $\mu_1(N_1, N_2) = 0$ , lying on the  $N_1$ -axis,

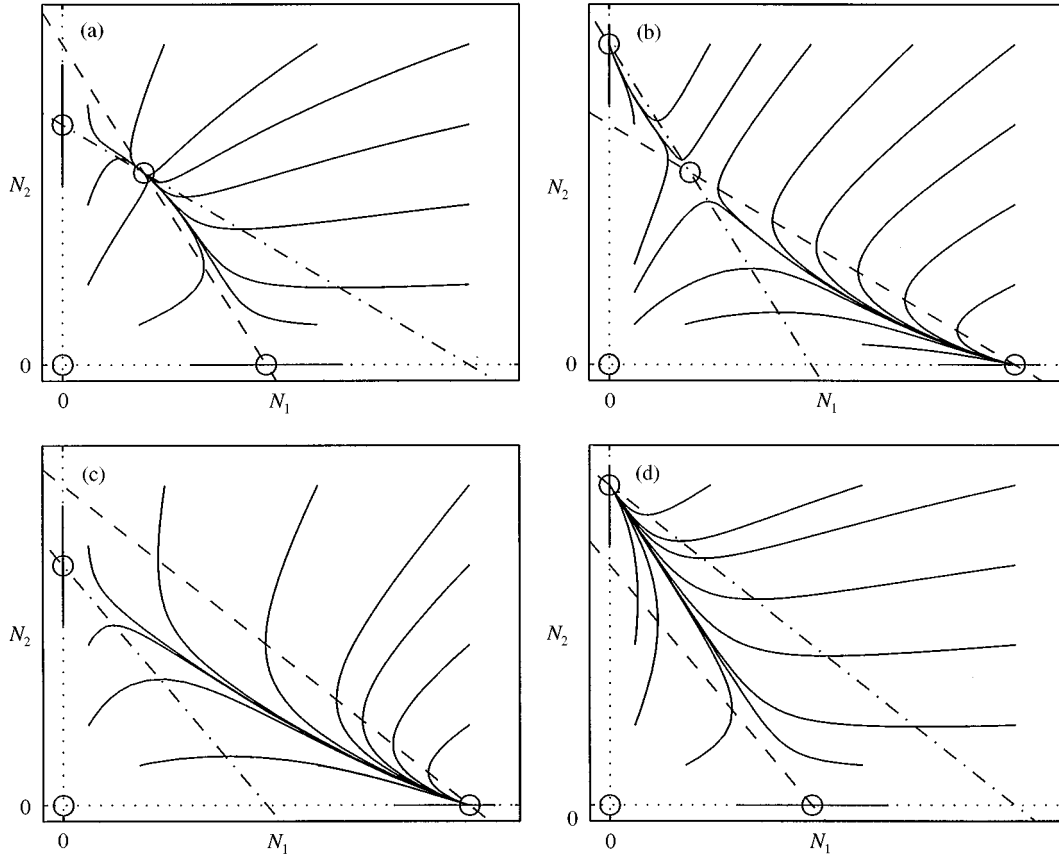


FIG. 3. Phase portraits of model 3 in Table 1 for different values of the model parameters: trajectory (—); critical point (○); zero isocline of species 1 (---); zero isocline of species 2 (·-·-·).

- the intersection point(s) (if existing) of  $\mu_1(N_1, N_2) = 0$  and  $\mu_2(N_1, N_2) = 0$ , which can be found back in the whole phase plane.

As these last curves and associated critical points play a crucial role in the further discussion of the models, the term *zero isocline* (or just *isocline*) will, for convenience, in the following only be used to refer to latter relations.

Model 3 presents the simplest examples of these different intersection points, namely  $(0, 0)$   $(K_1, 0)$  on the  $N_1$ -axis,  $(0, K_2)$  on the  $N_2$ -axis, and  $[(K_1 - \alpha_{12}K_2)/(1 - \alpha_{12}\alpha_{21}), (K_2 - \alpha_{21}K_1)/(1 - \alpha_{12}\alpha_{21})]$ , the intersection point of the zero isoclines. For this model, each parameter set  $(r_1, K_1, \alpha_{12}, r_2, K_2, \alpha_{21})$  qualitatively coincides with one of the four cases depicted in Fig. 3 (in which the origin is always an unstable node): (i) a stable node in the first quadrant surrounded by two

saddle points on the axes [Fig. 3(a)], which means that regardless of the initial population ratio, there is evolution to stable coexistence (except when a species is not present), (ii) a saddle point in the first quadrant in combination with two stable nodes on the axes [Fig. 3(b)], implying the extinction of species 1 or 2 depending on the initial species numbers, (iii) no intersection point in the first quadrant and a node and a saddle point on the  $N_1$ -axis and  $N_2$ -axis, respectively [Fig. 3(c)], resulting in the extinction of species 2, whenever species 1 is present, and, (iv) the opposite of the previous case, resulting in the extinction of species 1, whenever species 2 is present [Fig. 3(d)]. From these four cases, it follows that coexistence as well as species extinction can be described. Consequently, model 3 is in agreement with requirement #3. For certain parameter values, model 4 (Fig. 4) and models 5–7 (results not shown) exhibit phase portraits with strong

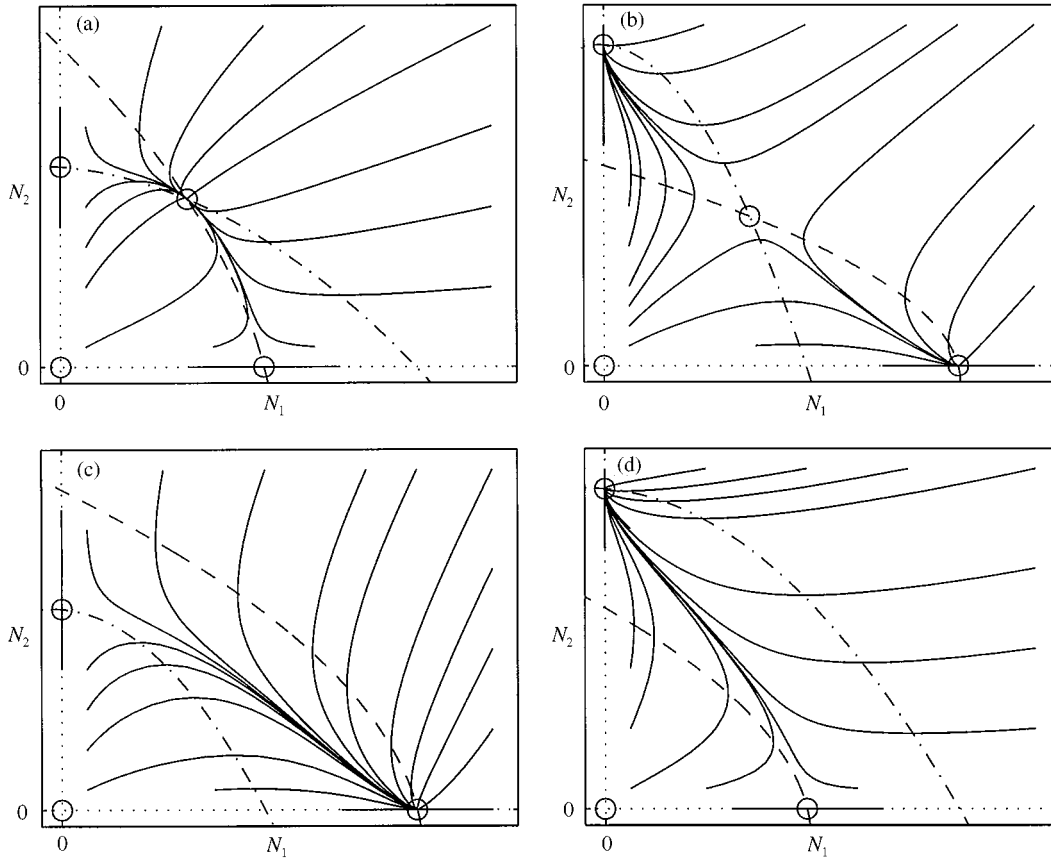


FIG. 4. Phase portraits of model 4 in Table 1 for different values of the model parameters: trajectory (—); critical point (○); zero isocline of species 1 (---); zero isocline of species 2 (-·-·-).

resemblance to those of model 3: each of these models is capable of generating the four cases just described (cf. e.g. the corresponding phase portraits of Figs 3 and 4). The third model requirement is thus also satisfied for models 4–7.

The two last models in Table 1 fulfill requirement #3 only partially. From the phase portraits of model 8 in Fig. 5, it can be seen that a stable node in the first quadrant, and, in consequence, evolution to stable coexistence is possible. However, due to the logarithmic terms in the zero isoclines of this model, no intersection with the axes exists and species extinction cannot be predicted. The same features, i.e. stable coexistence can be described but not species extinction, are also valid for model 9. This will be explained further on.

Several models, which permit stable coexistence and/or species extinction and which can generate (part of) the four types of phase por-

traits described before, are thus available. Among them, model 3 is the least complex with respect to mathematical structure and number of parameters. At this point, the questions arise whether important differences exist between model 3 and the other, more complex models and whether the latter are to be preferred above model 3 or, on the contrary, possess some disadvantageous properties. As indicated before, the behavior of each of the models 3–9 is mainly determined by the shape and location of its zero isoclines. Table 2 lists some major characteristics of these curves, namely the first and second derivatives and the behavior for  $N_1$  approaching  $-\infty$  or  $+\infty$ , from which the following conclusions can be drawn.

1. For  $N_1, N_2 > 0$ , the zero isoclines monotonically decrease in the phase plane. This can be seen from the expressions for  $dN_2/dN_1$ , which are negative for positive species numbers. [The



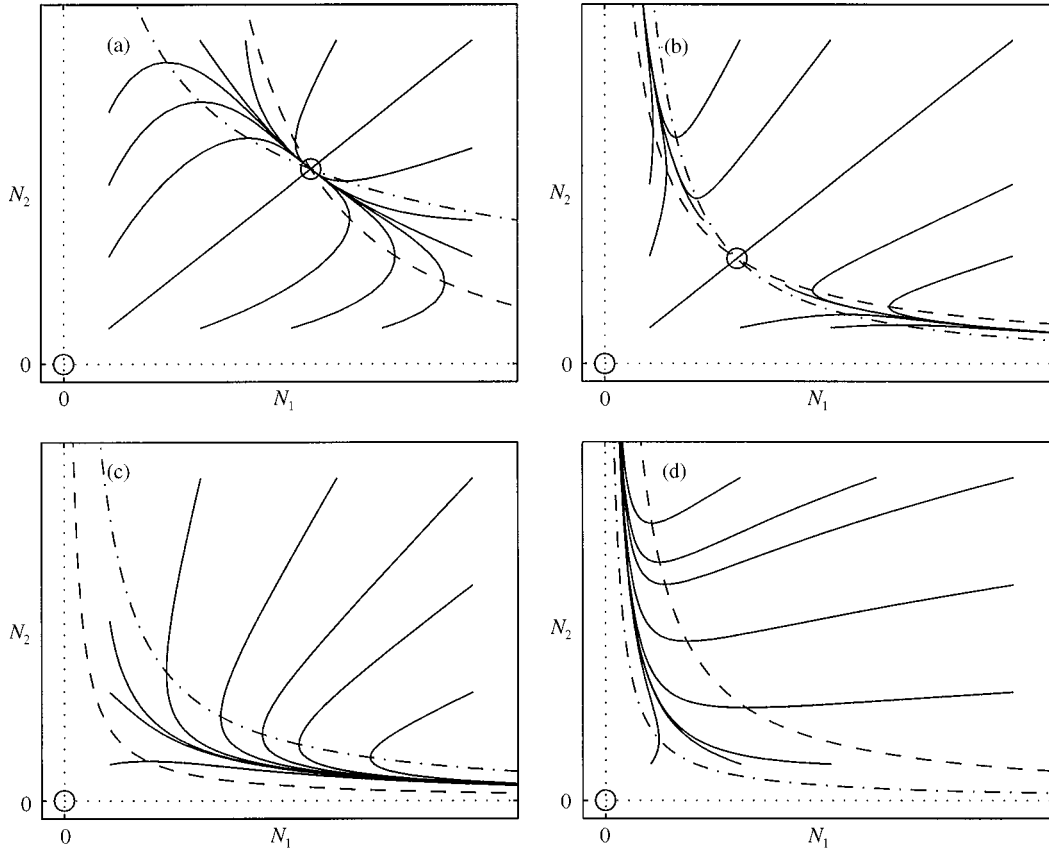


FIG. 5. Phase portraits of model 8 in Table 1 for different values of the model parameters: trajectory (—); critical point (○); zero isocline of species 1 (---); zero isocline of species 2 (·-·-·).

complex expressions for the first derivatives of the zero isoclines of model 9 are omitted in Table 2. In Schoener (1976), it is proved that they are negative for  $N_1, N_2 > 0$ .]

2. For  $N_1, N_2 > 0$ , the zero isoclines are convex ( $d^2N_2/dN_1^2 < 0$ ) for models 4 and 5, and concave ( $d^2N_2/dN_1^2 > 0$ ) for models 6, 8 and 9. [For model 9, this is proved by Schoener (1976).] The concavity of the zero isoclines of model 7 depends on the value of  $\theta_i$ . Convex or concave curves are obtained for  $\theta_i > 1$  or  $\theta_i < 1$ , respectively. The isoclines of model 3 are, of course, straight lines.

3. From the last two columns of Table 2, it can be concluded that the course of the zero isoclines of models 8 and 9 is asymptotically bounded for  $N_1 \rightarrow -\infty$  (or  $N_1 \rightarrow 0$ ) and  $N_1 \rightarrow +\infty$ . For the isoclines of model 6, there is only one true (i.e. linear) asymptote (for  $N_1 \rightarrow -\infty$  or  $N_1 \rightarrow +\infty$ ) but exponential functions approximating the

isocline at the opposite site of the phase plane can be specified.

The concave or convex nature of the zero isoclines of models 4–9 mostly allows different intersection points over the whole phase plane, of which more than one may lie in the first quadrant (depending on parameter values). This is in contrast with model 3, for which the straight isoclines permit at most one intersection point, whether or not for positive species numbers. In comparison with the classical Lotka–Volterra relationship, the possible occurrence of multiple intersection points in the first quadrant alters the model's behavior thoroughly. To illustrate this, Fig. 6 depicts phase portraits of model 4 with two or even three intersection points at positive species numbers. From left to right, a succession node–saddle point or node–saddle point–node is encountered, but the opposite is also possible. This flexibility

TABLE 2  
Mathematical properties of the isoclines of the models in Table 1

Model	Isocline	$dN_2/dN_1$	$d^2N_2/dN_1^2$	$\lim_{N_1 \rightarrow -\infty} N_2$	$\lim_{N_1 \rightarrow +\infty} N_2$
3	$\mu_1 = 0$	$-1/\alpha_{12}$	0	$-\infty$	$+\infty$
	$\mu_2 = 0$	$-\alpha_{21}$	0	$-\infty$	$+\infty$
4	$\mu_1 = 0$	$-1/(\alpha_{12} + 2\beta_2 N_2)$	$-2\beta_2/(\alpha_{12} + 2\beta_2 N_2)^3$	$-\infty$	$+\infty$
	$\mu_2 = 0$	$-(\alpha_{21} + 2\beta_1 N_1)$	$-2\beta_1$	$-\infty$	$+\infty$
5	$\mu_1 = 0$	$-(1/\alpha_{12})(1 + 2\beta_1 N_1)$	$-2\beta_1/\alpha_{12}$	$-\infty$	$+\infty$
	$\mu_2 = 0$	$-\alpha_{21}/(1 + 2\beta_2 N_2)$	$-2\beta_2\alpha_{21}^2/(1 + 2\beta_2 N_2)^3$	$-\infty$	$+\infty$
6	$\mu_1 = 0$	$-1/(\alpha_{12} + \delta_2\gamma_2 \exp(-\gamma_2 N_2))$	$\frac{\delta_2\gamma_2^2 \exp(-\gamma_2 N_2)}{(\alpha_{12} + \delta_2\gamma_2 \exp(-\gamma_2 N_2))^3}$	$\frac{1}{\alpha_{12}}(K_1 - \delta_2 - N_1)$	$\frac{-1}{\gamma_2} \ln((K_1 + \delta_2 + N_1)/\delta_2)$
	$\mu_2 = 0$	$-(\alpha_{21} + \delta_1\gamma_1 \exp(-\gamma_1 N_1))$	$\delta_1\gamma_1^2 \exp(-\gamma_1 N_1)$	$K_2 - \delta_1(1 - \exp(-\gamma_1 N_1))$	$K_2 - \delta_2 - \alpha_{21}N_1$
7	$\mu_1 = 0$	$-(1/\alpha_{12})\theta_1 K_1^{1-\theta_1} N_1^{\theta_1-1}$	$(1/\alpha_{12})\theta_1(1 - \theta_1)K_1^{1-\theta_1} N_1^{\theta_1-2}$	$-\infty$	$+\infty$
	$\mu_2 = 0$	$-\alpha_{21}/(\theta_2 K_2^{1-\theta_2} N_2^{\theta_2-1})$	$(1 - \theta_2)\alpha_{21}^2 N_2/(\theta_2^2 K_2^{2(1-\theta_2)})$	$-\infty$	$+\infty$
8	$\mu_1 = 0$	$-N_2/(\alpha_{12}N_1)$	$N_2(\alpha_{12} + 1)/(\alpha_{12}^2 N_1^2)$	0*	0
	$\mu_2 = 0$	$-N_1/(\alpha_{21}N_2)$	$N_1(\alpha_{21} + 1)/(\alpha_{21}^2 N_2^2)$	0*	0
9	$\mu_1 = 0$	†	†	0*	$-\frac{(C_1 + \gamma_{11}N_1)}{\gamma_{12}} \text{ or } \frac{-N_1}{\beta} \ddagger$
	$\mu_2 = 0$	†	†	$-\frac{(C_2 + \gamma_{22}N_2)}{\gamma_{21}} \text{ or } -\beta N_2 \ddagger$	0

\* $\lim_{N_1 \rightarrow 0}$  instead of  $\lim_{N_1 \rightarrow -\infty}$ .

†See Schoener (1976).

‡Asymptote depending on parameter values—see Schoener (1976).

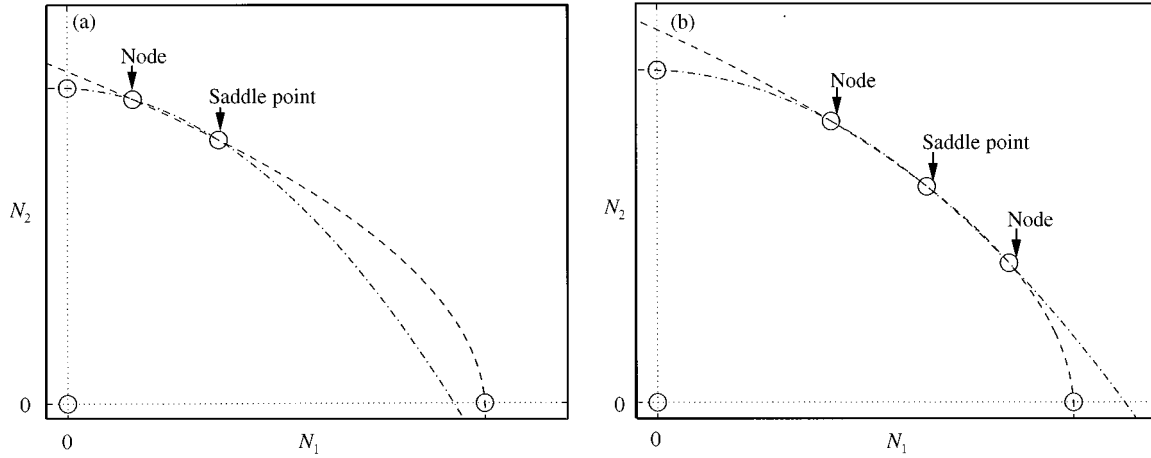


FIG. 6. For certain parameter values, model 4 in Table 1 permits multiple strictly positive critical points. The zero isoclines intersect twice (a) or three times (b) in the first quadrant: critical point ( $\circ$ ); zero isocline of species 1 (---); zero isocline of species 2 (-.-.-).

with respect to number and nature of non-negative critical points results in a larger applicability in comparison with the Lotka–Volterra model.

- For a given set of model parameters, model 4 can describe a system that evolves to stable coexistence or species extinction depending on initial conditions. This can be easily seen from Fig. 6(a) where the saddle point on the right divides the phase plane into two parts. Trajectories starting in the upper part lead to the stable node, while trajectories starting in the lower part evolve to the critical point on the  $N_1$ -axis. This behavior is not possible for model 3. Whenever this model exhibits a stable node for  $N_1, N_2 > 0$ , evolution to coexistence occurs for all non-zero initial conditions.
- Model 4 can describe a system with two different stable coexistence points, since two stable nodes in the first quadrant can be obtained [Fig. 6(b)]. As before, the saddle point divides the phase plane into two regions, with trajectories starting in the upper region leading to the upper node and trajectories starting in the lower region leading to the lower node. For model 3, this is not possible since the straight isoclines allow at most one stable coexistence point.

Besides for model 4, also for models 5, 6, 7 and 9 the isoclines can generate three strictly positive intersection points. (Note that in case of model 7,

three intersections are only possible when both isoclines are either convex or concave.) For model 8, multiple intersections are impossible despite the curved shape of the isoclines (Coleman & Gomatam, 1972). In contrast with models 4, 5 and 7, there are limitations concerning the nature of each critical point in the first quadrant for models 6 and 9. The limits of the isoclines of model 6 (Table 2) reveal that for  $N_1 \rightarrow -\infty$ , the second isocline yields higher  $N_2$ -values than the first, while the inverse is true for  $N_1 \rightarrow +\infty$ . Since both isoclines are monotonically decreasing and no inflection points occur, the total (i.e. over the whole phase plane) number of intersection points must be exactly 1 or 3. An example of both situations, with strictly positive intersections, is shown in Fig. 7. As in case of a stable node the first isocline is (locally) steeper than the second isocline, while for a saddle point the opposite is valid, a single intersection like in Fig. 7(a) is always a saddle point and a three-fold intersection [Fig. 7(b)] is always a succession of a saddle point, a node and a saddle point. Note that when only two intersections are in the first quadrant, the third intersection point belongs to quadrant II or IV and no restriction exists on the sequence of the critical points in quadrant I. A similar reasoning can be built up for model 9. The asymptotes (Table 2) in combination with the monotonous decrease and the absence of inflection points results in a stable node in case of

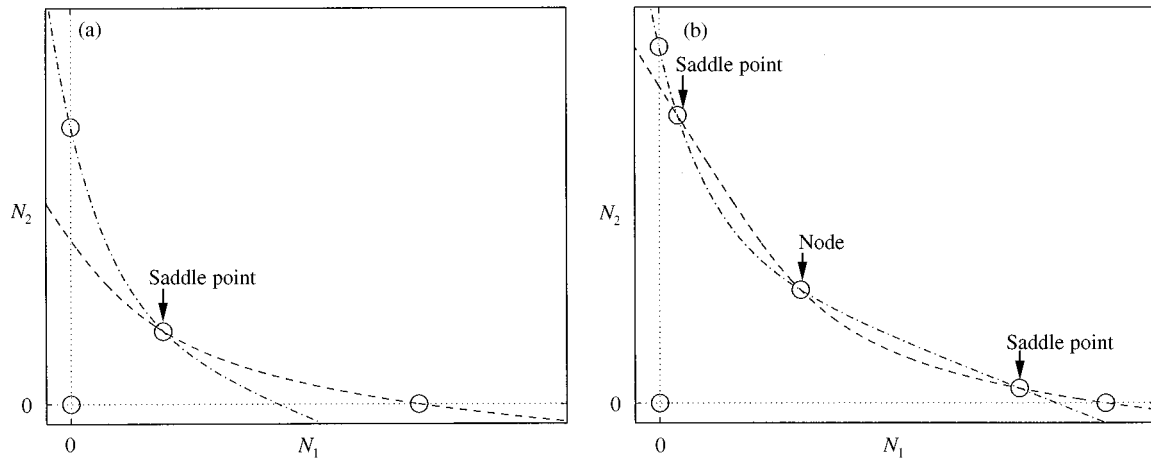


FIG. 7. For certain parameter values, model 6 in Table 1 permits a single (a) or three (b) positive critical points. The zero isoclines intersect once (a) or three times (b) in the first quadrant: critical point (○); zero isocline of species 1 (---); zero isocline of species 2 (·-·-·).

a unique intersection and two nodes separated by a saddle point whenever three critical points occur. Intersection points for this model are always strictly positive since the  $N_2$ -axis is an asymptote for the first isocline and the  $N_1$ -axis for the second one. This implies also that it is not possible to have exactly two critical points with positive  $N_1$  and  $N_2$ . As was already indicated before, model 9 never permits species extinction.

#### 4.2. TRAJECTORY EVOLUTION IN TIME

To verify if the last of the four model requirements is fulfilled, the time dependence of the

species dynamics needs to be considered. Two typical examples of the associated (semilogarithmic) time curves of the phase portrait of model 3 in Fig. 3(a) are depicted in Fig. 8(a) and (b). In case of Fig. 8(a), the initial values for both species numbers are situated below both isoclines in the phase plane ( $dN_1/dt|_{t=0} > 0$  and  $dN_2/dt|_{t=0} > 0$ ). From these curves, which initially increase with constant slope and deflect towards a constant value for  $t \rightarrow \infty$ , it is clear that for neither of the two species a lag phase is present. In case of Fig. 8(b), the initial species numbers lie in between the two isoclines in such a way that

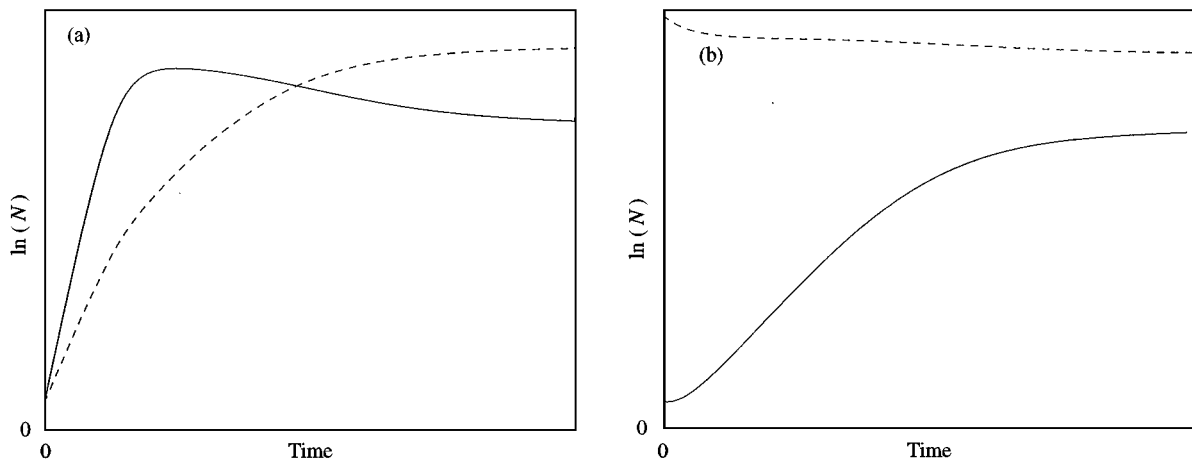


FIG. 8. Two typical semilogarithmic species versus time plots of model 3 in Table 1: species 1 (—); species 2 (---).

$dN_1/dt|_{t=0} > 0$  and  $dN_2/dt|_{t=0} < 0$ , which results in a sigmoidally shaped growth curve for species 1. It can be concluded that for certain initial species values, model 3 can generate a lag phase, while for other initial conditions, lag description is not possible. Consequently, model 3 does not satisfy the fourth model requirement.

Similar time plots are obtained for models 4–9.

### 5. Model Evaluation: an Analytical Approach

In Section 2, four model requirements have been postulated, inspired by available knowledge on mixed microbial population dynamics in foods. In Section 4, the relation between the model requirements and a number of candidate models (introduced in Section 3) has been explored mainly by graphical analysis of model solution curves (i.e. in phase and time plots). For this purpose, the model requirements have been reformulated as a set of properties that can be easily verified on the basis of these plots. Alternatively or complementary to the graphical analysis, a more analytical approach is presented here. This involves a second reformulation of the model requirements in terms of analytical or structural properties of the functions  $f$  and  $g$  in the general model (1).

- *Model requirement #1:* to satisfy this requirement a model must be of the form (2). Indeed, the zero isoclines  $N_1 = 0$  and  $N_2 = 0$  guarantee that no trajectories starting in the first quadrant leave this quadrant and that no trajectories starting on axes deviate from these. Furthermore, to exclude trajectories leading to infinity, additional conditions are needed, namely

$$\lim_{\substack{N_1 \rightarrow +\infty \\ N_2 = 0}} dN_1/dt < 0, \quad \lim_{\substack{N_1 = 0 \\ N_2 \rightarrow +\infty}} dN_2/dt < 0 \quad (3)$$

for bounded trajectories on the axes, and

$$\lim_{\substack{N_1 \rightarrow +\infty \\ N_2 \rightarrow 0}} dN_1/dt < 0, \quad \lim_{\substack{N_1 \rightarrow 0 \\ N_2 \rightarrow +\infty}} dN_2/dt < 0, \quad (4)$$

$$\lim_{\substack{N_1 \rightarrow +\infty \\ N_2 \rightarrow +\infty}} dN_1/dt < 0, \quad \lim_{\substack{N_1 \rightarrow +\infty \\ N_2 \rightarrow +\infty}} dN_2/dt < 0 \quad (5)$$

for bounded trajectories in the first quadrant. [Observe that conditions (3) and (4) coincide for all models but model 8 which is not applicable on the axes.] Since  $N_1$  and  $N_2$  represent positive species numbers, inequalities (3)–(5) can be formulated in terms of  $\mu_1$  and  $\mu_2$  for models of the class (2):

$$\lim_{\substack{N_1 \rightarrow +\infty \\ N_2 = 0}} \mu_1 < 0, \quad \lim_{\substack{N_1 = 0 \\ N_2 \rightarrow +\infty}} \mu_2 < 0, \quad (6)$$

$$\lim_{\substack{N_1 \rightarrow +\infty \\ N_2 \rightarrow 0}} \mu_1 < 0, \quad \lim_{\substack{N_1 \rightarrow 0 \\ N_2 \rightarrow +\infty}} \mu_2 < 0, \quad (7)$$

$$\lim_{\substack{N_1 \rightarrow +\infty \\ N_2 \rightarrow +\infty}} \mu_1 < 0, \quad \lim_{\substack{N_1 \rightarrow +\infty \\ N_2 \rightarrow +\infty}} \mu_2 < 0. \quad (8)$$

Conditions (6)–(8) are fulfilled when the curves  $\mu_1 = 0$  and  $\mu_2 = 0$  intersect both axes at strictly positive species numbers.

- *Model requirement #2:* the absence of centers and focuses requires the eigenvalues of the linearized model in all critical points in the first quadrant to be real. In the appendix, it is proven that for models of the form (2), the eigenvalues are always real for critical points on the axes [see relations (A2–A4)], while for strictly positive critical points the following condition must be satisfied [see relation (15)]:

$$\left( \frac{\partial \mu_1}{\partial N_1} N_1 - \frac{\partial \mu_2}{\partial N_2} N_2 \right)^2 + 4N_1 N_2 \frac{\partial \mu_1}{\partial N_2} \frac{\partial \mu_2}{\partial N_1} \geq 0.$$

- *Model requirement #3:* a number of conditions have to be fulfilled to be in agreement with this requirement. As for the previous requirements, only models belonging to class (2) are considered.

1. Firstly, an intersection of  $\mu_1 = 0$  with the  $N_1$ -axis and of  $\mu_2 = 0$  with the  $N_2$ -axis must be possible, with all corresponding eigenvalues being negative. Considering relations (A.2) and (A.4), a parameter set must exist for which both  $\mu_2$  and  $\partial \mu_1 / \partial N_1$ , evaluated in the former intersection point, are negative and a second—not necessarily

the same—set must exist for which both  $\mu_1$  and  $\partial\mu_2/\partial N_2$ , evaluated in the latter intersection point, are negative.

2. Secondly, a strictly positive intersection of  $\mu_1 = 0$  and  $\mu_2 = 0$ , for which both eigenvalues are real and negative, must be possible. Thus, a parameter set must exist for which expression (15), evaluated in the specified intersection, is negative real.

- *Model requirement #4*: as already indicated in Section 2, the presence of a lag phase has to be checked in  $\ln(N_i)$  vs.  $t$  plots. If we let  $h_i(t)$  represent a solution curve of a model (1) in such a plot [i.e.,  $\ln(N_i) = h_i(t)$ ], then the presence of a lag phase implies an inflection point, where  $h_i$  evolves from an increasing, concave upward function towards an increasing, concave downward function. More precisely, before the inflection point,  $h'_i \geq 0$  and gradually increases, while after the inflection point;  $h'_i > 0$  and starts to decrease. For models of the form (2), the equality between  $\mu_i$  and  $h'_i$  can be easily demonstrated:

$$h'_i = \frac{d \ln(N_i)}{dt} = \frac{1}{N_i} \frac{dN_i}{dt} = \mu_i.$$

Therefore, the course of a lag phase is as follows: starting from the initial species number  $N_{i,0}$ , the function  $\mu_i$  is larger than or equal

to zero and gradually increases, until the inflection point is reached where  $\mu_i$  starts to decrease. To fulfill model requirement #4,  $\mu_i$  must be able to generate the above-sketched course for every non-critical initial state  $(N_{i,0}, N_{j,0})$ , in which  $dN_i/dt \geq 0$ , i.e. before any growth phase. (A lag phase as described in Section 2 is of course not appropriate when  $dN_i/dt < 0$  for  $N_{i,0}$ , which can be interpreted as an initial state exceeding the carrying capacity for  $N_i$ .) This analysis will be illustrated further in Section 6 (and Fig. 9).

Starting from the model class (2), all requirements can thus be reduced to characteristics of  $\mu_1$  and  $\mu_2$ . This facilitates identification of similarities and/or differences between these characteristics. Depending on (i) the specific type of characteristic and (ii) the requirements it is related to, distinction can be made between two categories. A first category of characteristics concerns the zero isoclines  $\mu_1 = 0$  and  $\mu_2 = 0$  and is related to requirements #1 and #3. For these requirements, an intersection of  $\mu_1 = 0$  and  $\mu_2 = 0$  with both axes (at strictly positive species numbers) must always exist, and a strictly positive intersection of the curves must be possible. A second category of characteristics considers  $\mu_1$ ,  $\mu_2$  and their partial derivatives with respect to  $N_1$  and  $N_2$ . In view of requirements #2, #3 and #4, the sign of these functions and/or their derivatives in the initial and equilibrium states is of importance.

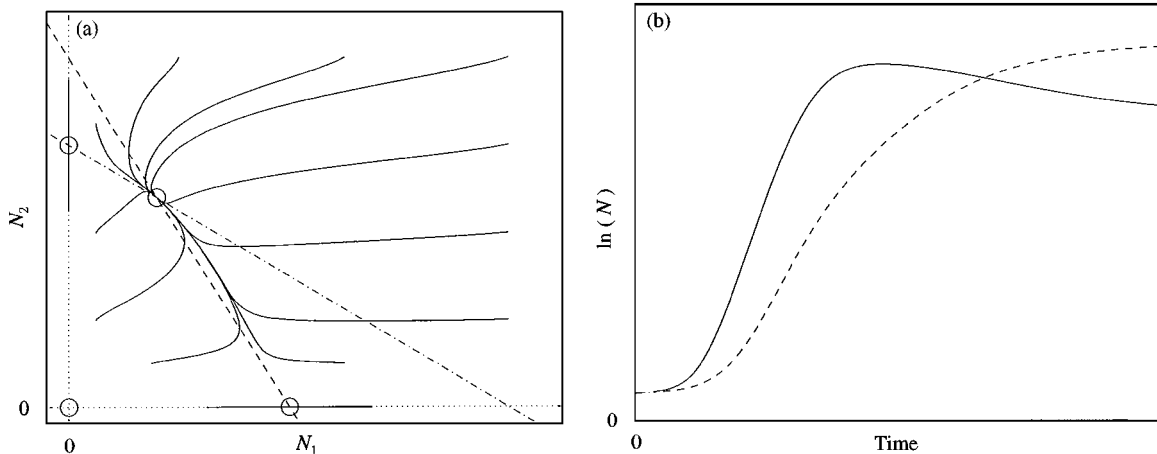


FIG. 9. (a) Phase portrait of model (13) with a stable node in the first quadrant: trajectory (—); critical point (○); zero isocline of species 1 (---); zero isocline of species 2 (·-·-·). (b) A typical semilogarithmic species versus time plot of model (13): species 1 (—); species 2 (---).

TABLE 3

*Model requirements to describe two-species competition, according to Smitalova & Sujan (1991)*

Biological principle	Mathematical description
(a) Both population sizes are nonnegative	$dN_1/dt = \mu_1(N_1, N_2) N_1$ $dN_2/dt = \mu_2(N_1, N_2) N_2$
(b) An increase in the density of one causes a decrease in the growth rate of the other	$\partial\mu_1/\partial N_2 < 0, \partial\mu_2/\partial N_1 < 0$
(c) There exists an intraspecies competition in both populations	$\partial\mu_1/\partial N_1 < 0, \partial\mu_2/\partial N_2 < 0$
(d) Every population has its carrying capacity	$\exists! m, n > 0: \mu_1(m, 0) = 0 \text{ and } \mu_2(0, n) = 0$
(e) The density of a population cannot increase when the density of the other population achieves a certain level	$\exists! r, s > 0: \mu_1(0, r) = 0 \text{ and } \mu_2(s, 0) = 0$
(f) Small population densities of both populations increase	$\mu_1(0, 0) > 0, \mu_2(0, 0) > 0$

The previous analytical formulation of the model requirements makes it possible to compare them with a set of requirements for a general model (1) to describe two populations in competition, suggested by Smitalova & Sujan (1991). As can be seen from Table 3 several similarities with the requirements stated in the current paper exist. Because the two state variables  $N_1$  and  $N_2$  in eqn (1) represent living organisms, Smitalova & Sujan also postulate a model of the form (2). Furthermore, requirements (d) and (e) can be classified into the first category of characteristics described above, while requirements (b), (c) and (f) belong to the second [although the last requirement is in fact redundant, since it can be deduced from requirements (a)–(e)].

Concerning the *first category*, the mathematical descriptions of (d) and (e) in Table 3 correspond literally to two conditions for the two isoclines  $\mu_1 = 0$  and  $\mu_2 = 0$ , which is not surprising as the biological principles (d) and (e) involve a boundedness of the population sizes which can be found back in model requirement #1.

A comparison between all requirements of the *second category* is more complicated. Combination of expression (A.1) with condition (b) reveals that no imaginary eigenvalues are possible if (b) is satisfied. Although oscillations are not considered in the principles summed up in Table 3, centers and focuses are automatically excluded. For requirement #3, no full analog is present in

Table 3. However, most models satisfying conditions (a)–(e) are able to generate a stable node on the axes and/or in the first quadrant. Indeed, the existence of critical points on the  $N_1$ - and  $N_2$ -axis is already guaranteed by condition (d). Further, it can be seen from the expressions for the eigenvalues (A.2) and (A.3) that these critical points are stable nodes whenever  $s < m$  and  $r < n$ , while a stable node in the first quadrant is obtained if the tangent to  $\mu_1 = 0$  in the critical point is steeper than the tangent to  $\mu_2 = 0$  in this point (see before). Consequently, requirement #3 is slightly more comprehensive than condition (d). Finally, no analog of requirement #4 is present in Table 3. Moreover, requirement #4 is totally incompatible with conditions (b) and (c). A model of class (2) in which the functions  $\mu_1$  and  $\mu_2$  decrease with increasing  $N_1$  and  $N_2$  is not able to describe a lag phase in case  $dN_1/dt > 0$  and  $dN_2/dt > 0$ , as this would require a function  $\mu_i$  which is first increasing and afterwards decreasing. From the above, it is clear that the model requirements stated in this research and the conditions proposed by Smitalova & Sujan partially coincide and partially differ. When considering the differences, it should be kept in mind that conditions (a)–(f) are inspired by a number of mechanistic assumptions on competition (the biological principles in Table 3) which are translated into a mathematical description. In contrast, requirements 1–4 do not assume an

TABLE 4  
*Analytical conditions corresponding to stable coexistence or species extinction for the models in Table 1*

Model	Conditions for stable coexistence of species 1 and 2	Conditions for extinction of species 1 and survival of species 2
3	$1 > \frac{K_1}{K_2} \alpha_{21}$ $1 > \frac{K_2}{K_1} \alpha_{12}$	$1 > \frac{K_1}{K_2} \alpha_{21}$ $1 < \frac{K_2}{K_1} \alpha_{12}$
4	$1 > \frac{K_1}{K_2} (\alpha_{21} + \beta_1 K_1)$ $1 > \frac{K_2}{K_1} (\alpha_{12} + \beta_2 K_2)$	$1 > \frac{K_1}{K_2} (\alpha_{21} + \beta_1 K_1)$ $1 < \frac{K_2}{K_1} (\alpha_{12} + \beta_2 K_2)$
5	$1 > \alpha_{21} \left( \frac{\sqrt{1 + 4\beta_1 K_1} - 1}{2\beta_1 K_2} \right)$ $1 > \alpha_{12} \left( \frac{\sqrt{1 + 4\beta_2 K_2} - 1}{2\beta_2 K_1} \right)$	$1 > \alpha_{21} \left( \frac{\sqrt{1 + 4\beta_1 K_1} - 1}{2\beta_1 K_2} \right)$ $1 < \alpha_{12} \left( \frac{\sqrt{1 + 4\beta_2 K_2} - 1}{2\beta_2 K_1} \right)$
6	$1 > \frac{K_1}{K_2} \left( \alpha_{12} + \frac{\beta_1}{K_1} (1 - \exp(-\gamma_1 K_1)) \right)$ $1 > \frac{K_2}{K_1} \left( \alpha_{21} + \frac{\beta_2}{K_2} (1 - \exp(-\gamma_2 K_2)) \right)$	$1 > \frac{K_1}{K_2} \left( \alpha_{12} + \frac{\beta_1}{K_1} (1 - \exp(-\gamma_1 K_1)) \right)$ $1 < \frac{K_2}{K_1} \left( \alpha_{21} + \frac{\beta_2}{K_2} (1 - \exp(-\gamma_2 K_2)) \right)$
7	$1 > \frac{K_1}{K_2} \alpha_{21}$ $1 > \frac{K_2}{K_1} \alpha_{12}$	$1 > \frac{K_1}{K_2} \alpha_{21}$ $1 < \frac{K_2}{K_1} \alpha_{12}$
8	See Coleman & Gomatam (1972)	Not possible
9	See Schoener <i>et al.</i> (1976)	Not possible

interaction mechanism as competition, but are mainly inspired by experimental data on mixed microbial dynamics in foods (see Section 2).

Of course, the mathematical translation of the model requirements does not alter the conclusions drawn based on the graphical analysis in the previous section. However, the analytical approach enables to specify where the models in Table 1 fail from a mathematical point of view. Models 1 and 9 do not belong to model class (2). While this is obvious for model 1, the mathematical expression of model 9 in Table 1 is misleading. Expanding out products in this expression

reveals that not all terms contain  $N_i$  and therefore the model does not fit in the mathematical form (2). For models 2 and 8, the required intersections of the curves  $\mu_1 = 0$  and  $\mu_2 = 0$  with the axes are not present: the zero isoclines of model 2 only intersect one axis and the zero isoclines of model 8 do not intersect either of both axes. The remaining models in Table 1 are in agreement with the first three requirements. However, since the functions  $\mu_i$  in these models satisfy conditions (b) and (c) in Table 3, description of a lag phase is not possible when both  $dN_i/dt|_{t=0} > 0$  and  $dN_j/dt|_{t=0} > 0$ , as in Fig. 8(a) in Section 4. If



$dN_i/dt|_{t=0} > 0$  and  $dN_j/dt|_{t=0} < 0$  (and  $|dN_j/dt|$  large enough), as in case of Fig. 8(b), a lag phase can be seen in the dynamics of species  $i$ .

Next to the analytical evaluation of the models with respect to the model requirements, an assessment of analytical conditions corresponding to stable coexistence or species extinction is useful. An example for the Lotka–Volterra model is presented by Ayala *et al.* (1973). According to these authors, the following inequalities are necessary and sufficient for stable coexistence:

$$\alpha_{12} < K_1/K_2, \quad \alpha_{21} < K_2/K_1. \quad (9)$$

This leads to the following necessary condition:

$$\alpha_{12}\alpha_{21} < 1. \quad (10)$$

The relations can be easily derived considering that a stable node in the first quadrant is surrounded by two saddle points on the axes. Calculation of the eigenvalues of the critical points on the axes yields [see also relations (A.2) and (A.3)]

$$\lambda_1 = -r_1, \quad \lambda_2 = r_2 \left( 1 - \alpha_{21} \frac{K_1}{K_2} \right) \quad (11)$$

for  $(K_1, 0)$  and

$$\lambda_1 = -r_2, \quad \lambda_2 = r_1 \left( 1 - \alpha_{12} \frac{K_2}{K_1} \right) \quad (12)$$

for  $(0, K_2)$ . Since one eigenvalue ( $\lambda_1$ ) is automatically negative for both critical points, requiring the other ( $\lambda_2$ ) to be positive is sufficient, yielding relations (9) and (10). The derivation presented here is easily applicable to the other models, resulting in a series of sufficient conditions for a single positive intersection point to be stable (Table 4). However, it should be stressed that conditions for stability are less important for predictive microbiology purposes than for the research fields the models originate from. Indeed, stable coexistence is often desired in, e.g. ecology, where different species form a stable community, or fermentation technology, where a mixed

culture may accomplish biotransformations which cannot be obtained with an axenic culture. On the contrary, predictive microbiology attempts to identify factors suppressing pathogenic microbial growth underneath an infectious level. In other words, stable coexistence can only be tolerated if the corresponding pathogen density is low enough. On the other hand, pathogen extinction always guarantees a safe product. Letting  $N_1$  be the pathogen and  $N_2$  the antagonist in model 3, pathogen extinction is always obtained when  $(K_1, 0)$  is a saddle point and  $(0, K_2)$  a stable node. Based on relations (11) and (12), the sufficient condition in Table 4 is obtained. Other conditions in Table 4 are derived in the same way, except for models 8 and 9, which cannot describe species extinction. Note that for models 4–7, these relations degrade to necessary conditions: a saddle point on the  $N_1$ -axis with a stable node on the  $N_2$ -axis can be accompanied with two positive intersection points (but not one or three, since it is not possible to have two adjacent nodes or saddle points). In this case, pathogen extinction will only be obtained for certain initial population ratios.

## 6. A Novel Prototype Model

Several models in Table 1 fulfil three of the four requirements stated in Section 2. All models violate the last model requirement. Therefore, a novel prototype model has been developed for which this requirement is satisfied as well. A detailed analysis of this model can be found elsewhere (Dens *et al.*, 1999). In this paper, we limit ourselves to presenting the model's basic principles, together with the evaluation of the model requirements.

The classical Lotka–Volterra model for two species in competition, which is the simplest model in Table 1 that fulfils three of the four requirements, forms a starting point for the development of the new model. From the previous section, it follows that an adaptation of this model to incorporate a lag phase within the model framework (2) (describing two states with two autonomous differential equations) is not straightforward. Therefore, the following set of four autonomous differential equations is

proposed (with  $i, j = 1, 2; i \neq j$  and  $N_i, Q_i > 0$ ):

$$\begin{aligned}\frac{dN_i}{dt} &= \frac{Q_i}{1 + Q_i} \frac{r_i N_i}{K_i} (K_i - N_i - \alpha_{ij} N_j), \\ \frac{dQ_i}{dt} &= r_i Q_i.\end{aligned}\quad (13)$$

The extension with the new state variables  $Q_i(-)$ , increasing according to first-order kinetics, enables the model to deal with a lag phase in a similar manner as the logistic equation is adjusted for lag description by Baranyi & Roberts (1994) in the case of single-species growth. The interpretation of the state  $Q$  in their model as a characteristic of the physiological condition of the microbial species, can be maintained for the states  $Q_i$  in the interaction model (13). It should be noted that the autonomous ( $N_1, N_2, Q_1, Q_2$ ) four-variable system (13) can be reduced to a non-autonomous two-variable system ( $N_1$  and  $N_2$ ). For a discussion on the two ways (autonomous and non-autonomous) of representation of the novel model (13) we refer to Dens *et al.* (1999).

The four model requirements are now all fulfilled. This can be demonstrated both graphically and analytically.

#### 1. Graphical

- *Trajectory evolution in the phase plane:* in Dens *et al.* (1999), a stability analysis of system (13) is performed, revealing that trajectories for this system evolve to the same critical points as for model 3. Consequently, the new model is in agreement with the first three model requirements. An example of a phase portrait, in which the intersection point of the isoclines is a stable node, is shown in Fig. 9(a).
- *Trajectory evolution in time:* plots of  $\ln(N)$  as a function of time demonstrate that the model is now suited to describe a lag phase for one or both species, irrespective of the values of  $dN_1/dt|_{t=0}$  and  $dN_2/dt|_{t=0}$ . An example of a semilogarithmic time plot, in which  $N_{1,0}$  and  $N_{2,0}$  have the same values as in Fig. 8(a) (i.e. initial species numbers are situated below both isoclines in the phase plane), is shown in Fig. 9(b).

#### 2. Analytical

- *Model requirements #1–3:* system (13) can be considered as an extension of eqn (2) with two strictly positive states  $Q_i$ . It is easily seen that trajectory evolution to negative species numbers or trajectory deviation from the axes is impossible for the set of differential eqns (13), as also pointed out for model class (2). The further conditions specified in Section 5 for  $\mu_i$  in (2) can be preserved for the new model (13), taking into account the new expression for the function  $\mu_i$ , which incorporates the state  $Q_i$ :

$$\mu_i = \frac{Q_i}{1 + Q_i} \frac{r_i}{K_i} (K_i - N_i - \alpha_{ij} N_j). \quad (14)$$

It is straightforward to show that all analytical conditions corresponding to the first three requirements are satisfied for eqn (14).

- *Model requirement #4:* whatever the initial value for the state  $Q_i$ , its value increases at a constant rate  $r_i$ . In consequence, for  $t \rightarrow \infty$ , the factor  $Q_i/(1 + Q_i)$  always approaches 1 and the model reduces to the Lotka–Volterra model. Whatever the initial state  $N_{i,0}$  (and associated rate  $dN_i/dt|_{t=0}$ ), incorporation of a lag phase is possible when  $Q_{i,0}$  is low, so that the function  $\mu_i$  evolves from a positive increasing towards a positive decreasing function. In case no lag phase is to be modeled,  $Q_{i,0}$  must be selected high.

Note that the other models in Table 1 can be extended in the same way as model 3, yielding also a model for two species in interaction. For this reason, eqns (13) must only be regarded as a prototype model, satisfying all requirements stated before. Moreover, when experimental data suggest specific types of interactions not included in the new model (e.g. a species influencing the lag time or the intrinsic growth rate  $r$  of another species), a model can be built up starting from the differential eqns (13), incorporating latter interaction types.

## 7. Conclusions

The lack of suitable models in the field of predictive microbiology to describe mixed

population growth, where interspecies interactions need to be incorporated, is considered in this research. The potential of autonomous continuous-time/two-species models (1) is explored. Firstly, four model requirements are formulated, inspired by the biological interpretation of the model and available knowledge from literature. Secondly, a number of existing autonomous continuous-time/two-species models, originating from research fields outside predictive microbiology, are evaluated on the basis of the stated requirements. Both a graphical and an analytical approach is used and for these purposes, the requirements are reformulated twice, as a set of graphical and analytical properties, respectively. From the two types of analysis, it follows that several of the models under investigation are in agreement with the first three requirements, but all violate the last requirement. Finally, a novel prototype model structure for mixed microbial populations is briefly discussed, for which the last model requirement is fulfilled as well. Since the analytical formulation of the model requirements reveals that it is not straightforward to develop a suitable model satisfying all requirements and within a framework of two autonomous differential equations, a set of four autonomous differential equations is proposed. The model is essentially based on the Lotka-Volterra model for two species in competition, which is the simplest of the investigated models satisfying the first three requirements. Future research will concentrate on the experimental validation of the model. Moreover, since the present model focuses on interspecies interactions which result in a decreased carrying capacity for one or both species, possible extensions to incorporate other types of interactions will be investigated.

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## REFERENCES

- ASHENAFI, M. (1991). Growth of *Listeria monocytogenes* in fermenting tempeh made of various beans and its inhibition by *Lactobacillus plantarum*. *Food Microbiol.* **8**, 303–310.
- AYALA, F. J., GILPIN, M. J. & EHRENFIELD, J. G. (1973). Competition between species: theoretical models and experimental results. *Theor. Popul. Biol.* **4**, 331–356.
- BAILEY, J. E. & OLLIS, D. F. (1986). *Biochemical Engineering Fundamentals*, 2 Edn. New York: McGraw-Hill, Inc.
- BARANYI, J. & ROBERTS, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.* **23**, 277–294.
- BUCHANAN, R. L. & WHITING, R. C. (1996). Risk assessment and predictive microbiology. *J. Food Prot.* (Suppl.) 31–36.
- BUCHANAN, R. L., WHITING, R. C. & DAMERT, W. C. (1997). When is simple good enough: a comparison of the Gompertz, Baranyi and three-phase linear models for fitting bacterial growth curves. *Food Microbiol.* **14**, 313–326.
- COLEMAN, T. P. & GOMATAM, J. (1972). Application of a new model of species competition to *Drosophila*. *Nat. New Biol.* **239**, 251–253.
- DEGNAN, A. J., YOUSEF, A. E. & LUCHANSKY, J. B. (1992). Use of *Pediococcus acidilacti* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. *J. Food Prot.* **55**, 98–103.
- DENS, E. J., VERECKEN, K. M. & VAN IMPE, J. F. (1999). A prototype model structure for mixed microbial populations in homogeneous food products. *J. theor. Biol.* **201**, 159–170.
- DUBOIS, D. M. (1978). State-of-the-art in predator-prey systems modeling. In: *State-of-the-Art in Ecological Modelling* (Jørgensen, S. E., ed.), *Proceedings of the ISEM Conference on Ecological Modelling*, Copenhagen, Denmark, 28 August–2 September 1978, pp. 163–217.
- GIBSON, A. M., BRATCHELL, N. & ROBERTS, T. A. (1987). The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *J. Appl. Bacteriol.* **62**, 479–490.
- GOMPERTZ, B. (1825). On the nature of the function expressiveness of the law of human mortality, and on a new mode of determining the value of life contingencies. *Philos. Trans. Roy. Soc. Lond.* **115**, 513–585.
- JEPPESEN, V. F. & HUSS, H. H. (1993). Antagonistic activity of two strains of lactic acid bacteria against *Listeria monocytogenes* and *Yersinia enterocolitica* in a model fish product at 5°C. *Int. J. Food Microbiol.* **179**, 179–186.
- LOTKA, A. J. (1956). *Elements of Mathematical Biology*. New York: Dover.
- MACDONALD, N. (1978). *Time Lags in Biological Models*. Berlin: Springer-Verlag.
- MCLEAN, A. R. & NOWAK, M. A. (1992). Models of interactions between HIV and other pathogens. *J. theor. Biol.* **155**, 69–102.
- MCMEEKIN, T. A., OLLEY, J. N., ROSS, T. & RATKOWSKY, D. A. (1993). *Predictive Microbiology: Theory and Application*. Somerset: Research Studies Press.
- MOSSSEL, D. A. A., CORRY, J. E. L., STRUIJK, C. B. & BAIRD, R. M. (1995). *Essentials of the Microbiology of Foods: A Textbooks for Advanced Studies*. Chichester: John Wiley and Sons.
- NEILL, W. E. (1974). The community matrix and interdependence of the competition coefficients. *Am. Nat.* **108**, 399–408.
- SCHOENER, T. W. (1974). Competition and the form of habitat shift. *Theor. Popul. Biol.* **4**, 56–84.

- SCHOENER, T. W. (1976). Alternatives to Lotka-Volterra competition: models of intermediate complexity. *Theor. Popul. Biol.* **10**, 309–333.
- SMITALOVA, K. & SUJAN, S. (1991). *A Mathematical Treatment of Dynamical Models in Biological Science*. Chichester: Ellis Horwood.
- VANDERMEER, J. H. (1969). The competitive structure of communities: an experimental approach with protozoa. *Ecology* **50**, 362–371.
- VAN IMPE, J. F., NICOLAI, B. M., MARTENS, T., DE BAERDEMAEKER, J. & VANDEWALLE, J. (1992). Dynamic mathematical model to predict microbial growth and inactivation during food processing. *Appl. Environ. Microbiol.* **58**, 2901–2909.
- VOLTERRA, V. (1931). *Leçons sur la Théorie Mathématique de la Lutte pour la Vie*. Paris: Goutliers-Vallard.
- WHITING, R. C., ROBERTS, T. A. HAAS, C. N., ROSE, J. B., GERBA, C. P., CROCKETT, C. S. SCHAFFNER, D. W., LABUZA, T. P., MILLER, A. J. & SMITH, J. L. (1997). Overview: predictive food microbiology: where do we go from here? *Food Technol.* **51**, 81–103.
- WILBUR, H. M. (1972). Competition, predation, and the structure of the *Ambystoma-Rana Sylvatica* community. *Ecology* **53**, 187–189.
- WINKOWSKI, K., CRANDALL, A. D. & MONTVILLE, T. J. (1993). Inhibition of *Listeria monocytogenes* by *Lactobacillus bavaricus* MN in beef systems at refrigeration temperatures. *Appl. Environ. Microbiol.* **59**, 2552–2557.
- ZWIETERING, M. H., JONGENBURGER, I., ROMBOUTS, F. M. & VAN T RIET, K. (1990). Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.* **56**, 1875–1881.

## APPENDIX

In this appendix, the eigenvalues of the Jacobian matrix of the two-species system (2), evaluated for a critical point  $(N_1^*, N_2^*)$ , are calculated. The Jacobian matrix can be written as

$$J = \begin{bmatrix} \frac{\partial f}{\partial N_1} & \frac{\partial f}{\partial N_2} \\ \frac{\partial g}{\partial N_1} & \frac{\partial g}{\partial N_2} \end{bmatrix} = \begin{bmatrix} \mu_1 + N_1 \frac{\partial \mu_1}{\partial N_1} & N_1 \frac{\partial \mu_1}{\partial N_2} \\ N_2 \frac{\partial \mu_2}{\partial N_1} & \mu_2 + N_2 \frac{\partial \mu_2}{\partial N_2} \end{bmatrix}$$

with the corresponding characteristic equation

$$\left( \mu_1 + N_1 \frac{\partial \mu_1}{\partial N_1} - \lambda \right) \left( \mu_2 + N_2 \frac{\partial \mu_2}{\partial N_2} - \lambda \right) - N_1 N_2 \frac{\partial \mu_1}{\partial N_2} \frac{\partial \mu_2}{\partial N_1} = 0$$

The roots of this quadratic equation yield the eigenvalues  $\lambda_1$  and  $\lambda_2$ . Depending on the position in the phase plane, four groups of critical points are distinguished.

1. For a strictly positive critical point ( $\mu_1 = 0$ ,  $\mu_2 = 0$ ),

$$\lambda_{1,2} = \frac{1}{2} \left[ \left( N_1 \frac{\partial \mu_1}{\partial N_1} + N_2 \frac{\partial \mu_2}{\partial N_2} \right) \pm \sqrt{\left( N_1 \frac{\partial \mu_1}{\partial N_1} - N_2 \frac{\partial \mu_2}{\partial N_2} \right)^2 + 4 N_1 N_2 \frac{\partial \mu_1}{\partial N_2} \frac{\partial \mu_2}{\partial N_1}} \right]$$

2. For a critical point on the  $N_1$ -axis ( $\mu_1 = 0$ ,  $N_2 = 0$ ),

$$\lambda_1 = N_1 \frac{\partial \mu_1}{\partial N_1}, \quad \lambda_2 = \mu_2. \quad (\text{A.2})$$

3. For a critical point on the  $N_2$ -axis ( $\mu_2 = 0$ ,  $N_1 = 0$ ),

$$\lambda_1 = N_2 \frac{\partial \mu_2}{\partial N_2}, \quad \lambda_2 = \mu_1. \quad (\text{A.3})$$

4. At the origin ( $N_1 = 0$ ,  $N_2 = 0$ ),

$$\lambda_1 = \mu_1, \quad \lambda_2 = \mu_2. \quad (\text{A.4})$$