

# *S* & *P*-type models: a novel class of predictive microbial growth models

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## Abstract

Food safety and quality are largely influenced by the presence and possible proliferation of pathogenic and spoilage micro-organisms during the *farm-to-fork* path of the product. In order to describe and predict the microbial evolution in foods, mathematical models are developed in the field of predictive microbiology. In this research a novel class of simple yet elegant models to describe single species microbial growth is discussed. In contrast with the currently used logistic-type models, the novel model class explicitly incorporates nutrient exhaustion and/or metabolic waste product effects. As such, these novel model types can be extended in a natural way towards microbial interactions in co-cultures and microbial growth in structured foods.

## Introduction

Food safety and quality are largely influenced by the presence and possible proliferation of pathogenic and spoilage micro-organisms during the *farm-to-fork* path of the product. In order to describe and predict the microbial evolution in foods, mathematical models are developed in the field of predictive microbiology.

Single species microbial growth, whether in a bioreactor or in a (liquid) food product, normally passes three distinct phases. In a first phase, the so-called *lag phase*, the microbial cells adapt to their new environment and do not multiply. The total number of microbial cells remains constant during this phase. During the next phase, the *exponential growth phase*, the microbial cells multiply exponentially. Finally, the microbial cells cease multiplying and their total number remains constant at the maximum population density. This third and final phase is called the stationary phase. A typical growth curve is shown in Figure 1.

Microbial growth can be considered as a self-limiting process principally due to either (i) the *exhaustion of one of the essential nutrients*, or (ii) the *accumulation of metabolic (waste) products* which inhibit growth (Lynch and Poole, 1979). The effect of both phenomena on the maximum population concentration is depicted in Figure 2.

If an increase of the initial substrate concentration results in an increase of the maximum microbial population density attained (as in the left part of the plot), then the limiting factor is the substrate availability. If an increase of the initial substrate concentration (whether a C-source, N-source, essential element or a vitamin) does not affect the maximum microbial

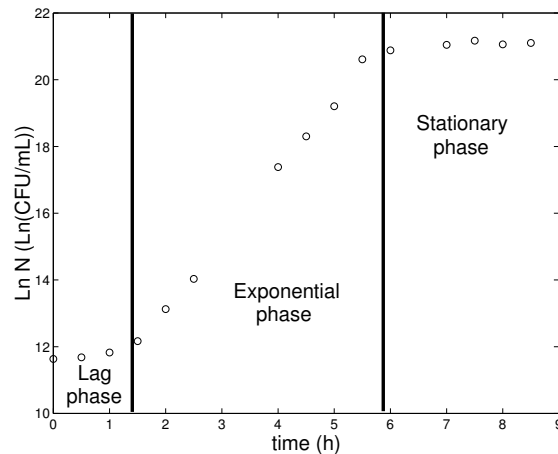


Figure 1: Typical microbial growth curve for single species growth.

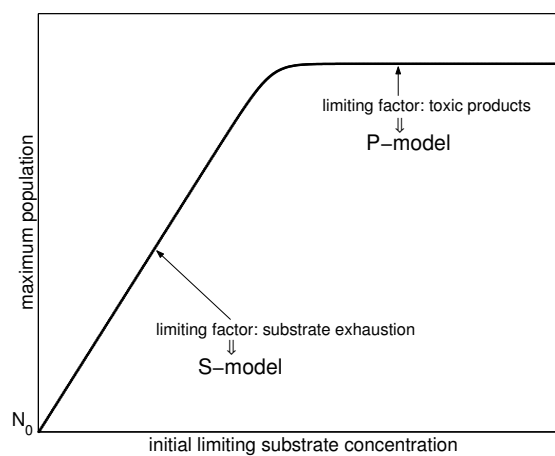


Figure 2: Influence of the initial substrate concentration on maximum microbial load population (adapted from Bailey and Ollis, 1986).

population density, then the limiting factor is the formation of (a) toxic product(s). If there is no substrate initially available, the microorganisms do not grow and the maximum microbial load concentration is equal to the initial microbial load concentration  $N_0$ .

In this research a novel class of microbial growth models is at study. In contrast with the currently used logistic models, e.g., the model of Baranyi and Roberts (1994), the novel model class explicitly incorporates nutrient exhaustion and/or metabolic waste product effects. As such, these novel model types can be extended in a natural way towards microbial interactions in co-cultures and microbial growth in structured foods. Two limiting case studies of the novel model types are presented.

## Model of Baranyi and Roberts

Most models used in predictive microbiology do not *explicitly* consider the higher mentioned microbiological knowledge on the self-limiting growth process. This is illustrated with the nowadays widely used growth model of Baranyi and Roberts (see e.g., McClure et al., 1997; van Gerwen et al., 1998), which is in this research considered as the reference growth model.

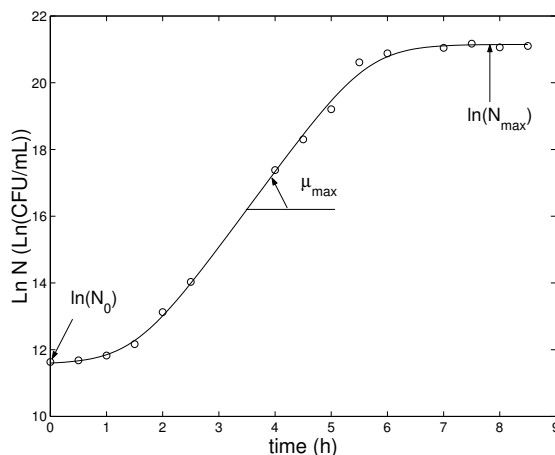


Figure 3: Description of the model of Baranyi and Roberts on an experimental data set of *Escherichia coli* K12 at 35°C in a rich medium (Brain Heart Infusion).  $\ln N_0 = 11.601$ ,  $\ln N_{max} = 21.151$ ,  $\mu_{max} = 2.3045 \text{ h}^{-1}$  and  $\ln Q_0 = -3.4316$ .

## Model description

The implicit formulation valid under dynamic environmental conditions is as follows (Baranyi and Roberts, 1994):

$$\frac{dN(t)}{dt} = \left( \frac{Q(t)}{1 + Q(t)} \right) \cdot \mu_{max} \cdot \left( 1 - \frac{N(t)}{N_{max}} \right) \cdot N(t) \quad \text{with } N(t = 0) = N_0 \quad (1)$$

$$\frac{dQ(t)}{dt} = \mu_{max} \cdot Q(t) \quad \text{with } Q(t = 0) = Q_0 \quad (2)$$

The first differential equation describes the evolution of the microbial load  $N$  [CFU/mL], as illustrated in Figure 3. The first factor in the right hand side of Equation (1) is called the *adjustment function* and describes the lag phase by means of a variable representing the physiological state of the cells  $Q(t)$  [-]. The latter is assumed to be proportional to the concentration of a (hypothetical) critical substance which is the bottle-neck in the growth process. The adjustment function is a strictly monotonically increasing function with values between a very small positive value (e.g.,  $10^{-8}$ ) and one. The second factor expresses the exponential phase, with  $\mu_{max}$  [ $\text{h}^{-1}$ ] the maximum specific growth rate. The third factor describes the transition to the stationary phase and is called the *inhibition function* with the maximum microbial load  $N_{max}$  [CFU/mL]. The inhibition function is a strictly monotonically decreasing function with values between one and zero. Typical inhibition and adjustment functions for a growth curve with lag are presented in Figure 4.

The second differential equation, Equation (2), describes the evolution of  $Q(t)$ , which increases exponentially.

For static environmental conditions (e.g., constant temperature, pH, ...), Equation (1) and (2) can be solved analytically since  $\mu_{max}$  is then a constant with respect to time  $t$ . This results in following analytical solution:

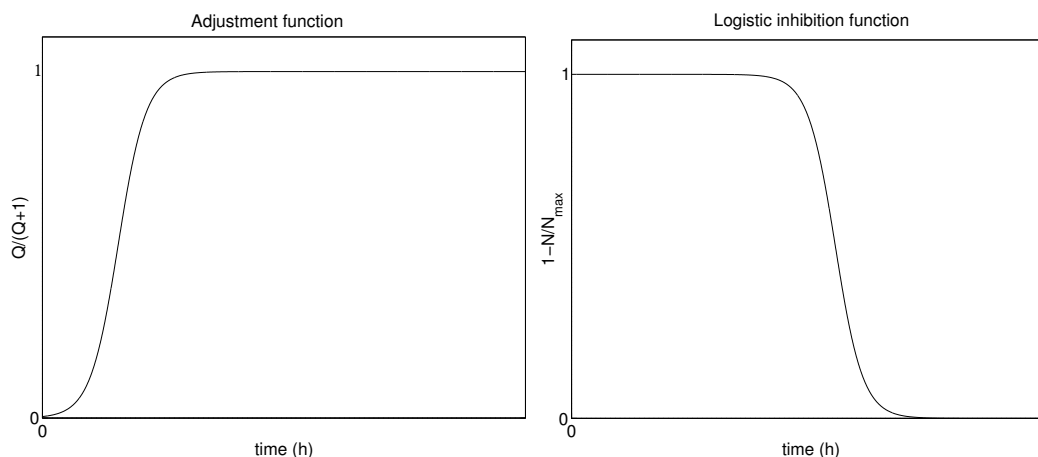


Figure 4: Typical adjustment and inhibition functions.

$$N(t) = \frac{N_0 N_{max} \left( \frac{1 + Q_0 \exp(\mu_{max} t)}{1 + Q_0} \right)}{N_{max} - N_0 + N_0 \left( \frac{1 + Q_0 \exp(\mu_{max} t)}{1 + Q_0} \right)} \quad (3)$$

The initial microbial load  $N(t=0)$ , which can be calculated by replacing  $t$  in Equation (3) by 0, is equal to  $N_0$ . The maximum microbial load  $N(t = \infty)$ , which can be calculated by replacing  $t$  in Equation (3) by  $\infty$ , is equal to  $N_{max}$ .

## Model simulations

The influence of the four model parameters on the microbial growth curve is illustrated in Figure 5. Model simulations were performed while varying only one of the four model parameters and keeping the other three constant. This procedure was repeated for all four model parameters.  $N_0$ ,  $\mu_{max}$  and  $N_{max}$  influence the shape in a transparent way.  $Q_0$  (upper right plot) influences the length of the lag phase: the smaller  $Q_0$ , the longer the lag phase.

## Model evaluation

The model of Baranyi and Roberts (1994) is widespread used because of a number of reasons: (i) it is easy to use, (ii) it is applicable under dynamic environmental conditions, (iii) it has a good fitting capacity, and (iv) most of the model parameters are biologically interpretable. Contrary to the adjustment function, the inhibition function is not mechanistically inspired. Although clearly interpretable, this mathematical abstraction, inherited from the logistic model type, lacks a mechanistic base since it does not encapsulate a reason *why* the microbial population stops growing. In other words, it does not reflect any cause-effect relationship. The model fails in describing more complex yet more realistic situations (e.g., co-cultural growth, growth in structured media). In the case of growth in structured media, the inhibition of growth because of substrate depletion because of hindered migration of the substrate by the (solid) structure of the medium cannot be described by a single model parameter  $N_{max}$  which is not related with the medium structure and the available substrate concentration. In the case of co-cultural growth, the inhibition of growth because of (i) substrate depletion (which is competitively consumed by

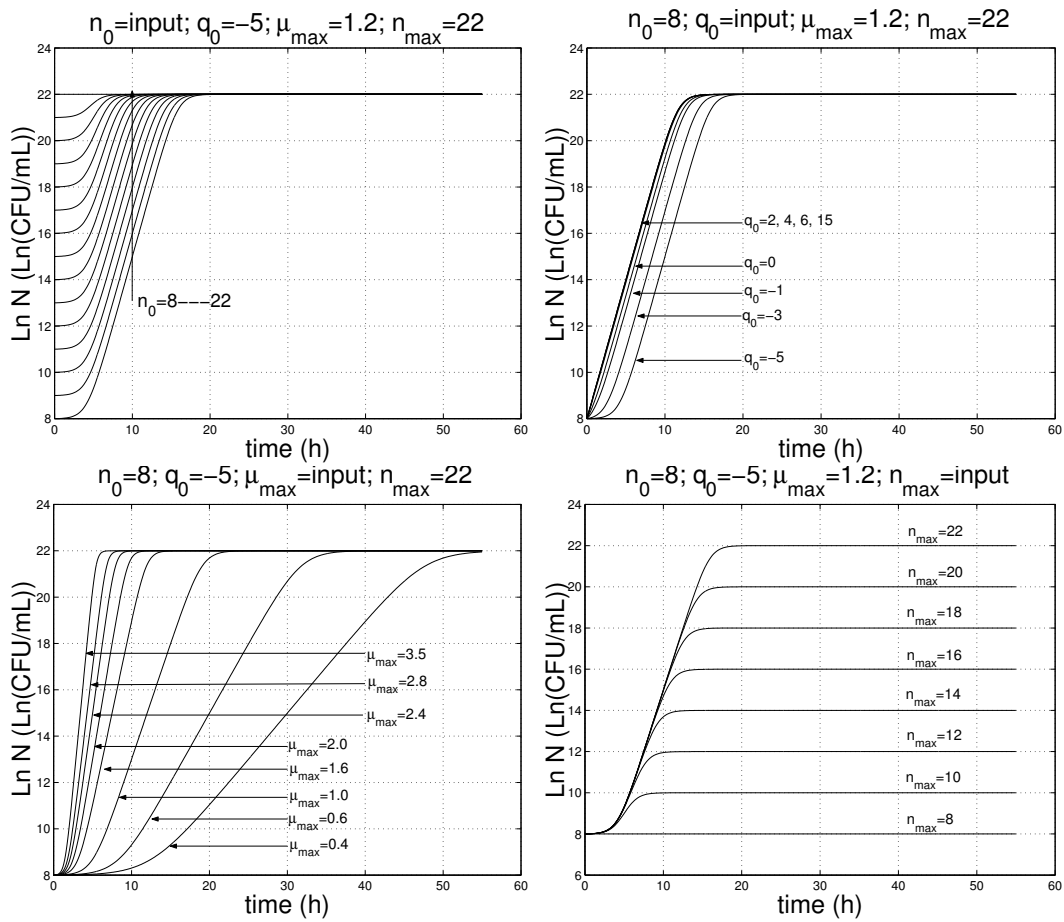


Figure 5: Influence of the Baranyi model parameters on the microbial growth curve.  $n_0 = \ln N_0$ ,  $n_{\max} = \ln N_{\max}$  and  $q_0 = \ln Q_0$ .

all organisms in the medium) and/or (ii) toxic product formation by some organisms cannot be described by means of a single  $N_{max}$  which is not related to the toxic product concentration, the initial concentration of all other organisms in the medium, . . . . In order to overcome the latter, a novel class of microbial growth models with a mechanistically inspired inhibition function was constructed.

## A novel class of predictive growth models

A novel class of mechanistically inspired, modular extendable models was constructed (Van Impe et al., 2005). The global structure of the novel class of simple predictive growth models consists of a general expression for the microbial evolution

$$\frac{dN}{dt} = \mu_Q(Q) \cdot \mu_{max} \cdot \mu_S(S) \cdot \mu_P(P) \cdot N \quad (4)$$

together with the appropriate differential equations for the physiological state  $Q$  [-], the substrate  $S$  [M] and the toxic product  $P$  [M]. The first factor  $\mu_Q(Q)$  accounts for the lag phase and is equal to the adjustment function of the model of Baranyi and Roberts (1994). The second factor describes the exponential growth with maximum specific growth rate  $\mu_{max}$  [1/h]. The third factor  $\mu_S(S)$  describes the influence of the phenomenon of exhaustion of a substrate  $S$  on the microbial evolution as for example a Monod type relationship:

$$\mu_S(S) = \frac{S}{S + K_S} \quad (5)$$

with  $K_S$  [M] the Monod constant. The fourth factor  $\mu_P(P)$  accounts for the inhibition of microbial growth by a toxic product  $P$ . An example is:

$$\mu_P(P) = 1 - \frac{P}{K_P} \quad (6)$$

with  $K_P$  [M] the concentration of the product at which growth ceases.  $1/K_P$  can be considered as the sensitivity of the microbial cells towards  $P$ . This novel class of predictive models is more mechanistically inspired and is easier to extend in comparison with the existing models.

Moreover, it can be seen as the prototype example for mono-culture growth when looking at the *elementary dynamic model building block* describing microbial evolution under batch cultivation within an homogeneous environment (Bernaerts et al., 2004). This elementary model building block consists of the following set of differential equations

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

with  $i, j = 1, 2, \dots, n$  the number of microbial species involved.  $N_i(t)$  represents the cell density of species  $i$  and  $\mu_i(\cdot)$  [ $h^{-1}$ ] defines its *overall specific evolution rate* depending on interactions within/between microbial populations ( $N_i$  or  $N_j$ ), physico-chemical environmental conditions ( $\langle env \rangle$ ), microbial metabolite concentrations ( $\langle P \rangle$ ), substrate concentration(s) ( $\langle S \rangle$ ), the physiological state of the cells ( $\langle phys \rangle$ ), and other factors. Microbial growth is resulting when  $\mu_i(\cdot) > 0$  and microbial decay results from  $\mu_i(\cdot) < 0$ .

Two limiting case studies of the novel class of predictive models are further investigated in detail.

## Inhibition due to substrate exhaustion: a $S(\text{ubstrate})$ -model

In a first case study, the stationary phase is assumed to be solely the result of substrate exhaustion, and not of toxic product inhibition.

### Model description

Mathematically the above mentioned assumption implies that the factor  $\mu_P(P)$  in Equation (4) is assumed to be equal to 1. For this case study it is also assumed that (i) a linear relation is appropriate to describe the influence of substrate depletion on the microbial growth, (ii) there is no substrate consumption for maintenance, (iii) there is no substrate breakdown in the medium, (iv) no additional substrate is added during the growth process, and (v) there is only one limiting substrate. These assumptions result in following 3 differential equations:

$$\frac{dN(t)}{dt} = \left( \frac{Q(t)}{1+Q(t)} \right) \cdot \mu_{max} \cdot S(t) \cdot N(t) \quad \text{with } N(t=0) = N_0 \quad (7)$$

$$\frac{dQ(t)}{dt} = \mu_{max} \cdot Q(t) \quad \text{with } Q(t=0) = Q_0 \quad (8)$$

$$\frac{dS(t)}{dt} = - \left( \frac{Q(t)}{1+Q(t)} \right) \cdot \mu_{max} \cdot \frac{S(t)}{Y_{N/S}} \cdot N(t) \quad \text{with } S(t=0) = S_0 \quad (9)$$

The first equation describes the microbial evolution in time, and consists of the *adjustment function* of the model of Baranyi and Roberts (see Equation (1)) and an inhibition function which is linear in function of the substrate concentration  $S$ . The second equation is equal to the second equation of the model of Baranyi and Roberts (Equation (2)) and describes the exponential evolution of the physiological state. The third equation describes the evolution of the substrate concentration  $S$ . It is assumed that there is only substrate consumption for the growth process, and not for the maintenance process. By consequence, the right hand side of Equation (9) is, except for the minus sign and the yield coefficient  $Y_{N/S}$ , equal to the right hand side of Equation (7). This model has three initial states ( $N_0$ ,  $Q_0$  and  $S_0$ ) and two model parameters ( $\mu_{max}$  and  $Y_{N/S}$ ). Since the inhibition function has to be initially equal to 1,  $S_0$  has to be equal to 1 (and corresponds, as such, to a rescaled substrate concentration). A model description under the assumption that  $S_0$  is equal to 1 on the static experimental data set of *Escherichia coli* K12 is presented in Figure 6. Remark that Figure 3 and Figure 6 are almost identical.

Dividing the first model equation by the third one results in

$$\frac{dN}{dS} = -Y_{N/S} \quad (10)$$

Rearranging and integrating with the appropriate boundaries results in following relation between  $N(t)$  and  $S(t)$

$$N(t) = N_0 + Y_{N/S} \cdot (S_0 - S(t)) = N_0 + Y_{N/S} \cdot (1 - S(t)) \quad (11)$$

The asymptotic microbial load is then equal to  $N_0 + Y_{N/S}$ . For this model a static version can be

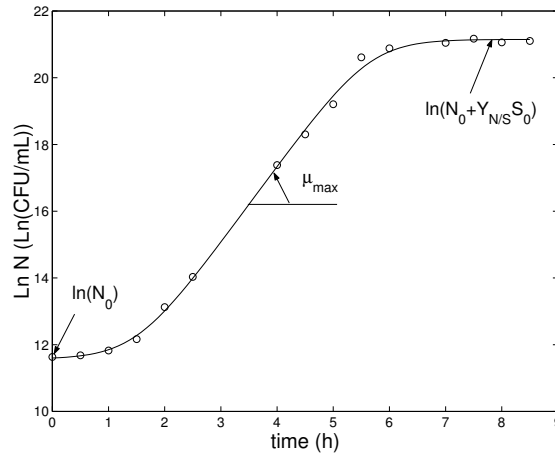


Figure 6: Description of the S-model on an experimental data set of *Escherichia coli* K12 at 35°C in a rich medium (Brain Heart Infusion).  $\ln N_0 = 11.602$ ,  $\ln Y_{N/S} = 21.152$ ,  $\mu_{max} = 2.3030 \text{ h}^{-1}$  and  $\ln Q_0 = -3.4357$ .

derived:

$$N(t) = \frac{N_0(N_0 + Y_{N/S}) \left( \frac{1 + Q_0 \exp(\mu_{max}t)}{1 + Q_0} \right) \frac{N_0 + Y_{N/S}}{Y_{N/S}}}{Y_{N/S} + N_0 \left( \frac{1 + Q_0 \exp(\mu_{max}t)}{1 + Q_0} \right) \frac{N_0 + Y_{N/S}}{Y_{N/S}}} \quad (12)$$

This static model version only slightly differs from the static model version of the model of Baranyi and Roberts. Since  $N_0$  is negligible compared to  $Y_{N/S}$  (using a rescaled substrate concentration), both models can be considered as numerically equal. The initial microbial load  $N(t=0)$ , which is calculated by replacing  $t$  in Equation (12) by 0, is equal to  $N_0$ . The maximum microbial load  $N(t = \infty)$ , which is calculated by replacing  $t$  in Equation (12) by  $\infty$ , is equal to  $N_0 + Y_{N/S}$ .

## Model simulations

A simulation study reveals the influence of the different parameters on the microbial growth curve. In the upper left plot of Figure 7, it is clearly shown that a high initial microbial load  $N_0$  increases the asymptotic microbial load. This is in compliance with the biological knowledge explained in Bailey and Ollis (1986) and experimental data presented in Carlin et al., (1995). The upper right and middle left plots are similar to the corresponding ones of the model of Baranyi and Roberts. In the middle right plot, it can be seen that  $Y_{N/S}$  corresponds to the asymptotic microbial load for high values of  $Y_{N/S}$ , but not for low values. The lower plot illustrates the influence of  $S_0$  (if considered variable) on both the maximum specific growth rate and the asymptotic microbial load which is biologically seen sound. Both influences are also experimentally encountered in Verluyten et al. (2004), when studying the influence of an up to four-fold increase of the initial complex nutrient source (i.e., bacteriological peptone and yeast extract) in standard MRS medium on the resulting growth rate and maximum attainable concentration of *Lactobacillus curvatus*.



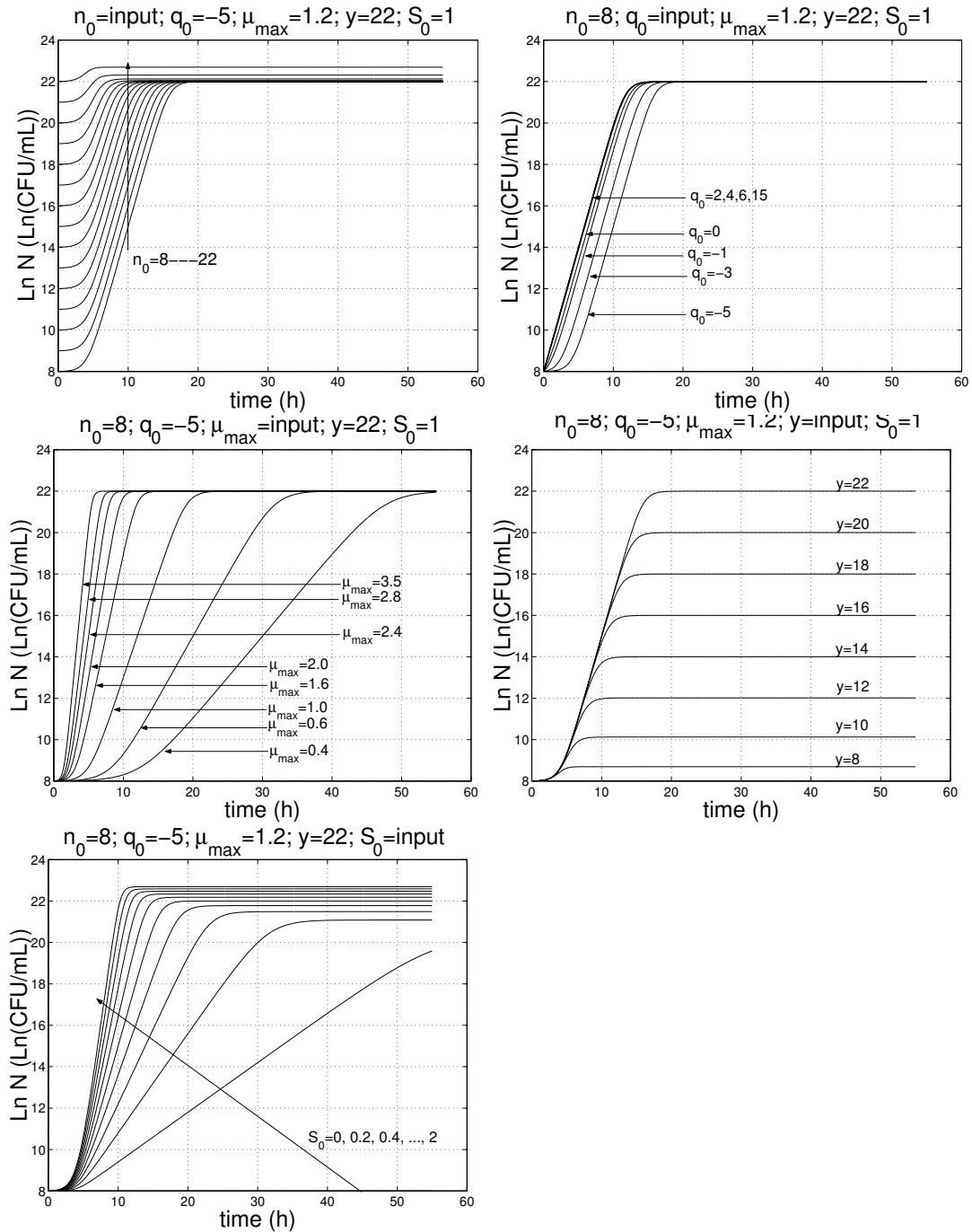


Figure 7: Influence of the different S-model parameters on the microbial growth curve.  $n_0 = \ln N_0$ ,  $y = \ln Y_{N/S}$  and  $q_0 = \ln Q_0$ .

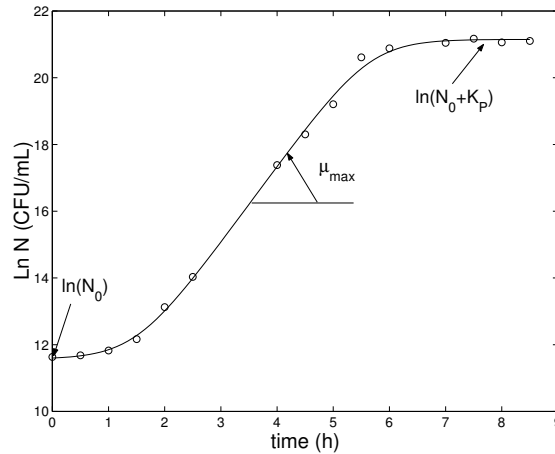


Figure 8: Description of the P-model on an experimental data set of *Escherichia coli* K12 at 35°C in a rich medium (Brain Heart Infusion).  $\ln N_0 = 11.601$ ,  $\ln K_P = 21.151$ ,  $\mu_{max} = 2.2995$  and  $\ln Q_0 = -3.4300$ .

## Inhibition due to product formation: a *P*(roduct)-model

In a second case study the stationary phase is assumed to be solely resulting from toxic product inhibition.

### Model description

Mathematically the above mentioned assumption implies that the factor  $\mu_S(S)$  in Equation (4) is equal to 1. A second assumption is that the yield coefficient for product over microorganisms is equal to 1. It is also assumed that the initial concentration of the toxic product  $P(t = 0)$  is equal to 0. The model consists of the following 3 differential equations:

$$\frac{dN(t)}{dt} = \left( \frac{Q(t)}{1 + Q(t)} \right) \cdot \mu_{max} \cdot \left( 1 - \frac{P(t)}{K_P} \right) \cdot N(t) \quad \text{with } N(t = 0) = N_0 \quad (13)$$

$$\frac{dQ(t)}{dt} = \mu_{max} \cdot Q(t) \quad \text{with } Q(t = 0) = Q_0 \quad (14)$$

$$\frac{dP(t)}{dt} = - \left( \frac{Q(t)}{1 + Q(t)} \right) \cdot \mu_{max} \cdot \left( 1 - \frac{P(t)}{K_P} \right) \cdot N(t) \quad \text{with } S(t = 0) = N_0 \quad (15)$$

The first equation describes the microbial evolution in time, and consists of the *adjustment function* of the model of Baranyi and Roberts (see Equation (1)) and an inhibition function which is linear in function of the toxic product concentration  $P$ . The second equation is equal to the second equation of the model of Baranyi and Roberts (Equation (2)) and describes the exponential evolution of the physiological state. The third equation describes the evolution of the toxic product concentration  $P$ . Equation (15) expresses that there is only growth associated production of the toxic product  $P$ . This model has two initial states ( $N_0$  and  $Q_0$ ) and two model parameters ( $\mu_{max}$  and  $K_P$ ). A model description on the static experimental data set of *Escherichia coli* K12 is presented in Figure 8. Remark that Figure 3, 6 and 8 are almost identical.

Dividing Equation (13) by Equation (15) results in

$$\frac{dN(t)}{dP(t)} = 1 \quad (16)$$

Rearranging and integrating with the appropriate boundaries results in following relation between  $N(t)$  and  $P(t)$

$$N(t) = P(t) + N_0 \quad (17)$$

The asymptotic microbial load  $N_{as}$  is equal to  $N_0 + K_P$ , as the maximum value for  $P$  is equal to  $K_P$ . Substituting all this knowledge in Equation (13) results in:

$$\frac{dN(t)}{dt} = \left( \frac{Q(t)}{1 + Q(t)} \right) \cdot \mu_{max} \cdot \left( 1 - \frac{N(t) - N_0}{N_{as} - N_0} \right) \cdot N(t) \quad (18)$$

After comparison of Equation (18) with Equation (1), it can be concluded that the P-model and the model of Baranyi and Roberts are -again- only slightly different. For this model also a static version can be derived:

$$N(t) = \frac{N_0(N_0 + K_P) \left( \frac{1 + Q_0 \exp(\mu_{max}t)}{1 + Q_0} \right)^{\frac{N_0 + K_P}{K_P}}}{K_P + N_0 \left( \frac{1 + Q_0 \exp(\mu_{max}t)}{1 + Q_0} \right)^{\frac{N_0 + K_P}{K_P}}} \quad (19)$$

Since  $N_0$  is negligible to  $K_P$ , this static model version is also only slightly different from the static model version of the model of Baranyi and Roberts. The initial microbial load  $N(t=0)$ , which can be calculated by replacing  $t$  in Equation (19) by 0, is equal to  $N_0$ . The maximum microbial load  $N(t = \infty)$ , which can be calculated by replacing  $t$  in Equation (19) by  $\infty$ , is equal to  $N_0 + K_P$ .

## Model simulations

A simulation study reveals the influence of the different parameters on the microbial growth curve.

In the upper left plot of Figure 9, it is clearly shown that a high initial microbial load  $N_0$  also increases the asymptotic microbial load. The upper right and lower left plots are similar to the corresponding ones of the model of Baranyi and Roberts. In the lower right plot, it can be seen that  $K_P$  corresponds to the asymptotic microbial load for high values of  $Y_{N/S}$ , for low values the effect of  $N_0$  is visible.

## Further reading

A more elaborated P-model describing the interaction between a target organism, *Listeria innocua*, and a microbial antagonist, the lactic acid bacterium *Lactococcus lactis* is presented in Poschet et al. (2005), based on the combination of research results reported in Vereecken et al. (2001), Vereecken et al. (2002), Vereecken and Van Impe (2002), Vereecken et al. (2003).

A nutrient depletion model was constructed by Leroy and Devuyst (2001) by taking into account three inhibition factors for the growth of a bacteriocin-producing *Lactobacillus sakei* strain: (i) a factor for the effect of sugar concentration (Monod-type), (ii) a factor for the effect of the

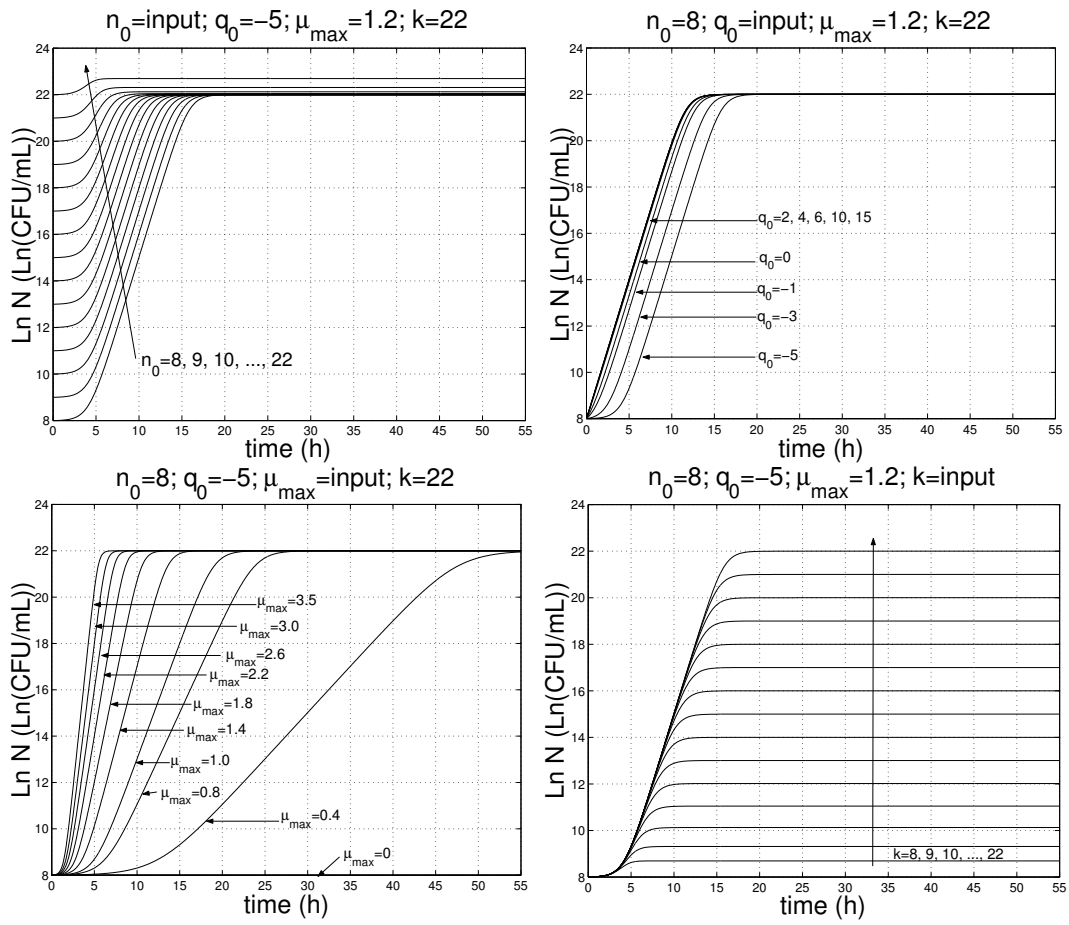


Figure 9: Influence of the different P-model parameters on the microbial growth curve.  $n_0 = \ln N_0$ ,  $k = \ln K_P$  and  $q_0 = \ln Q_0$ .

produced lactic acid (as in Passos et al., 1993 & 1994), and (iii) a factor for the effect of the biomass concentration itself. This last factor imposes that the model has both mechanistic and logistic (non-mechanistic) features and was included because it is difficult to have a clear view on all specific, growth-inhibiting components. Successful applications by extending this nutrient depletion model are presented in Leroy and De Vuyst (2003 & 2005). A similar approach was followed by Tracqui et al. (2005), developing a nutrient-depletion model for the growth and subsequent decay of an endothelial human cell line.

## Conclusions

The main contribution of this paper is the introduction and analysis of a novel class of predictive microbial growth models which reflect the (micro)biological phenomena governing the microbial growth process. This research particularly focuses on the transition from the exponential growth phase to the stationary phase, which is generally assumed to be the result of substrate depletion and/or toxic product inhibition. Two limiting case studies of the novel class of microbial growth models are carefully analyzed and compared to the model of Baranyi and Roberts, the currently most used logistic-type model. Both the so-called P-model and S-model (i) have an equal fitting capacity as the model of Baranyi and Roberts, (ii) are more mechanistically inspired than the logistic type models, and, by consequence, (iii) are easier to extend to more complex situations, and (iv) are applicable to both the macroscopic (i.e., population) as the microscopic (i.e., individual cell) level. More elaborated models underline the need for novel measurements at the level of substrates and metabolites to enable a thorough deployment of this novel class of predictive models.

## Acknowledgements

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