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Application of artificial neural networks as a non-linear modular modeling technique to describe bacterial growth in chilled food products

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Abstract

In many chilled, prepared food products, the effects of temperature, pH and %NaCl on microbial activity interact and this should be taken into account. A grey box model for prediction of microbial growth is developed. The time dependence is modeled by a Gompertz model-based, non-linear differential equation. The influence of temperature, pH and %NaCl reflected in the model parameters is described by using low-complexity, black box artificial neural networks (ANN's). The use of this non-linear modeling technique makes it possible to describe more accurately interacting effects of environmental factors when compared with classical predictive microbiology models. When experimental results on the influence of other environmental factors become available, the ANN models can be extended simply by adding more neurons and/or layers.

Keywords: Predictive microbiology; Bacterial growth; Secondary modeling; Artificial neural networks; Chilled food products

1. Introduction

The increased consumer and catering interest for chilled, prepared food products and food components can be explained by their essential characteristics: freshness, easy to use (convenience), gain of time, easy to stock and choice [see, e.g., Martens (1996)]. In contrast with classical sterilization processes, a trade-off between safety and quality is attained by killing pathogenic vegetative cells by a pasteurization process. The shelf life of this type of product is determined by the evolution of surviving micro-organisms which can spoil the product or possibly even cause pathogenic effects.

The field of predictive microbiology [reviewed by Ross and McMeekin (1994) and Whiting et al. (1997)] aims at developing mathematical models for these non-linear inactivation and growth processes. The non-linearity is inherent to the living microbial population and limits strongly the use of classical linear modeling techniques to describe the evolution

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of micro-organisms as a function of time (primary model).

The main factors influencing the microbial stability of chilled, prepared food products are temperature, pH, and %NaCl. The temperature in particular may vary significantly throughout the complete production and distribution chain. Therefore, to predict accurately the shelf life of this type of product, dynamic mathematical models (i.e., involving differential equations) are needed as primary model [see, e.g., Van Impe et al. (1992) or Baranyi et al. (1993)]. Prototype dynamic models to describe growth and inactivation of a microbial population as a function of time and temperature have been presented in Van Impe et al. (1992), Van Impe et al. (1995) and Baranyi et al. (1996).

2. Material studied, methods, techniques

2.1. Dynamic hybrid growth model

2.1.1. Dynamic model for microbial growth

The Modified Gompertz equation, proposed by Zwietering et al. (1990) is commonly accepted (Garthright, 1991) as a possible static equation to describe the growth of micro-organisms N [cfu/ml] as a function of time t[h] (primary model).

$$\ln\left(\frac{N}{N_0}\right) = A \exp\left[-\exp\left(\frac{\mu_{\max} e}{A}(\lambda - t) + 1\right)\right] \quad (1)$$

This model owns its success to the physical relevance of its parameters and the limited correlation between them. N_0 [cfu/ml] is an approximation to the initial population. λ[h] represents the lag time. The maximum growth rate is given by the parameter $\mu_{\max}$ [1/h]. (However, this parameter does not represent the maximum specific growth rate as mentioned in the literature [see, e.g., Baranyi et al. (1993)] when dealing with the slope of the exponential growth phase.) A [-] represents the asymptotic growth which will be reached at $t \to \infty$ and is equal to the difference between A_0 [which equals $\ln(N_\infty)$] and $\ln(N_0)$.

A dynamic extension of this model is developed by Van Impe et al. (1992) and Van Impe et al. (1995) and makes it possible to describe accurately microbial growth in time-varying environmental conditions, which is important for chilled, prepared food products.

$$\frac{dn}{dt} = c(n - n_0)\ln\left(\frac{a}{n - n_0}\right)$$
$$\text{with } n \triangleq \ln(N),\ a \triangleq A_0 - n_0,\ c \triangleq \frac{\mu_{\max} e}{A_0 - n_0} \quad (2)$$

Throughout this paper, this existing dynamic model will be used as primary growth model.

2.1.2. Incorporation of the influence of environmental factors

By modeling the growth parameters $\mu_{\max}$, λ and A_0 as a function of temperature T, pH and %NaCl (secondary models) a dynamic model as function of time and environmental factors is obtained. In the literature four main model types can be found: Bělehrádek type models, (Modified) Arrhenius type models, Cardinal models and polynomial relationships [see, e.g., Wijtzes et al. (1993); Davey (1994); Rosso et al. (1995) and McClure et al. (1993), respectively].

It is important to clarify the difference between purely additive models, in which it is assumed that the environmental factors act independently from each other, and synergistic models, in which a combined (interacting) effect is described. This point is often discussed in the literature. The general statistical concept of studying additive/interactive effects (see, e.g., Neter et al. (1990), Chapter 7) is illustrated in the paper of Davies (1993), concerning the design of experiments for predictive microbial modeling.

An example of a simple hypothetical additive model, describing the influence of temperature T and pH on the growth rate, could be

$$\sqrt{\mu_{\max}} = a + b \cdot T + c\ \text{pH} \quad (3)$$

in which a, b and c are regression coefficients. The 3-dimensional plot of this model is presented in Fig. 1 (left), which is similar to Fig. 1 in Davies (1993). Indeed, according to Davies (1993), for factors that do not interact, all results lie on a flat but sloping surface. More general (Neter et al., 1990), for non-interacting factors the curvature (not only a straight line) of the dependent variable (e.g., $\sqrt{\mu_{\max}}$) as a

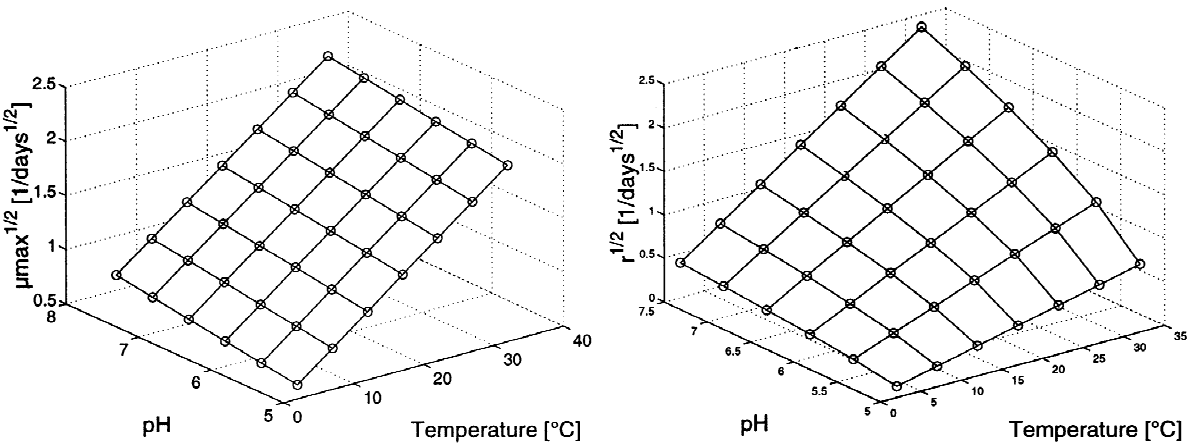

Fig. 1. (left) Representation of an hypothetical additive model. (right) Representation of a synergistic model.

function of 1 independent variable (e.g., T), does not depend on the value of another independent variable (e.g., pH).

On the other hand, for factors that do interact, the curvature of the dependent variable as a function of 1 independent variable, does depend on the value of another independent variable. This can be simulated using the following multiplicative Bělehrádek type model proposed by Adams et al. (1991)

$$\sqrt{r} = d \cdot (\mathrm{pH} - \mathrm{pH}_{\min}) \cdot (T - T_{\min}) = d \cdot (\mathrm{pH}_{\min} \cdot T_{\min} - \mathrm{pH}_{\min} \cdot T - T_{\min} \cdot \mathrm{pH} + \mathrm{pH} \cdot T) \quad (4)$$

with r the growth rate, d a regression coefficient and $\mathrm{pH}_{\min}$ and $T_{\min}$ the conceptual minimum pH and temperature for growth, respectively. The model is presented in Fig. 1 (right), which is similar to Fig. 2 in Davies (1993) describing interacting factors: the results do not lie on a flat surface.

Therefore, it can be concluded that, contrary to discussions often found in the literature, all four model types mentioned previously are capable of describing interacting effects (synergism/antagonism) of environmental factors, with the rare exception of very simple polynomial models [see, e.g., Eq. (3)].

Going beyond this, the adaptation in, e.g., the Bělehrádek type model Eq. (4), to possible synergistic effects comes in at the important point of the conceptual nature of the cardinal values of temperature, pH. . . [Observe, e.g., that $T_{\min}$ is usually 2–3°C lower than the temperature at which growth is actually observed (McMeekin et al., 1993)]. Conceptually, $T_{\min}$ could be identified on a data set describing $\mu_{\max}$ as a function of temperature at optimal pH, while $\mathrm{pH}_{\min}$ could be identified on a data set describing $\mu_{\max}$ as a function of pH at optimal temperature. However, this procedure does not guarantee the validity of the obtained parameter values under more stringent conditions of temperature and pH. To obtain reliable parameter values for d, $T_{\min}$, and $\mathrm{pH}_{\min}$, they have to be identified simultaneously on a data set spanning a wide range of temperature and pH values. (Of course, the values of $T_{\min}$ and $\mathrm{pH}_{\min}$ obtained with the previous conceptual procedure can serve as excellent starting points for the simultaneous identification.) The underlying reason for this is the possibility that when the temperature is suboptimal, the pH range over which an organism can grow becomes more narrow. In that case, the growth rate can be made zero by application of a specific combination of temperature and pH, in which each of these factors separately would not be able to inactivate the growth. In other words, the reduction in growth rate is frequently greater than would be expected if the different influencing factors were assessed separately (Mossel et al., 1995, p. 70) and the effects are not independent/additive but interactive.

The combined use of several preservation methods to halt microbial growth can be motivated by the hurdle concept (Leistner and Rodel, 1976) and a

synergistic effect of the applied hurdles is likely (Leistner, 1996). This is extremely important when considering the increasing interest in chilled, prepared food products, for which the consumer increasingly demands for lower levels of nitrite and/or NaCl and a high nutritional value. Of course, in this case, the temperature is suboptimal and the influence of all other factors is affected by this condition. As such, producers are forced towards the borders of food additives and food preservation methods. Therefore, the need for models capable of describing interacting effects at, especially, low temperatures becomes more and more urgent.

The interacting curvature, capable to be described by Bělehrádek type models, (Modified) Arrhenius type models, and CTMI models, depends merely on the structural properties of the model and, if applicable, the realistic values of biologically meaningful parameters. However, there is no guarantee that these structures are indeed capable of describing all kinds of curvatures.

A polynomial model, the fourth model type mentioned above, is a first prototype model for which it is mathematically proven that the structure is flexible enough to incorporate, e.g., even very strong interactive effects at the growth/no growth interface, usually by including the cross products of different environmental factors. However, a high number of parameters is needed to describe accurately a specific data set. This issue will be addressed further in the next subsection.

Because of the lack of sufficient microbial/biochemical knowledge concerning the growth of micro-organisms, it is not possible at this moment to derive completely mechanistic models and a black box modeling approach is the suitable way to approach the problem. (Observe the fact that all models mentioned above have black box (or empirical) characteristics because no mechanistic deduction of the proposed model structure is known.)

In this paper, low complexity artificial neural networks are investigated on their usefulness in the field of predictive microbiology. An overview of the methodology of artificial neural network modeling can be found in Najjar et al. (1997) (Part I) and Hajmeer et al. (1997) (Part II). As such, only a short introduction is included here, focusing on some theoretical aspects and the specific approach used in this research.

2.1.3. Artificial neural networks as a black box modeling technique

The development of artificial neural networks (ANN's) is inspired by the elementary principle of the human nervous system: an interconnection between neurons leading to a new nerve which causes a weighted, non-linear response. As such, not all the information is amplified in the same way.

Each neuron performs the simple operation of adding a weighted sum (weights w_j) of the incoming input signals p_j, to a bias term (or threshold) β_r and feeding the result to a nonlinear activation (or transfer) function $\sigma(\cdot)$ (e.g., sigmoidal unit or binary threshold unit) which results in the output value y_i of the neuron.

$$y_i = \sigma\left(\sum_{j=1}^{m} (w_j p_j + \beta_r)\right)$$

Different neurons can be connected to a neuron layer and different neuron layers can be placed behind each other forming a complete neural network. Interactions between different inputs p_j can be modeled without specifying them in advance. The choice for this black box modeling approach is based on the following ANN properties.

- Based on the Universal Approximation Property of ANN's (Kolmogorov, 1957) it is stated that neural networks consisting of three layers-the first with input variables, the second using sigmoidal and the third using linear transfer functions-are capable of arbitrarily accurate approximation to an arbitrary function after identification of the model parameters (training of the neural network) [see, e.g., Hornik et al. (1989)]. Note the fact that this property is not proven for other model types available in the literature, e.g., Arrhenius type models, except for the polynomial models. However, the number of parameters needed in polynomial model fitting grows with the number of inputs d as d^M (with M the order of the polynomial function), whereas the number of free parameters grows only linearly (d) or eventually quadratically (d^2) with d the dimension of the input space, for a given number M of hidden units in a neural network model (Bishop, 1995).
- Barron (1993) has studied the way in which the

residual sum-of-squares error (SSE) decreases as the number of parameters in a model is increased. For neural networks, he showed that this error falls with order $O(1/M)$ where M is the number of hidden units in the network, irrespective of the number of input variables. By contrast, the error only decreases as $O(1/M)^{2/d}$, where d is the dimensionality of input space and M the order of the polynomials or any other series expansion in which the coefficients of linear combinations of fixed basis functions are adapted. This efficient scaling with dimensionality implies the advantage of neural network models for cases where $d \geq 3$.

- Due to the black box characteristics, as mentioned above, no *a priori* knowledge about the mechanistics of the processes or their interactions is needed. Rich, i.e., informative experimental data are needed to identify the model structure and the parameters.
- Based on their modular properties, ANN's are very suited to tackle a problem in a structural way, e.g., by first modeling μ_{max} as a function of temperature, and, later on, if necessary, by extending the neural network model structure by incorporating additional neurons and/or layers if the influence of pH, %NaCl, . . . , also needs to be modeled.

This approach is different from the one in Najjar et al. (1997) and Hajmeer et al. (1997). These authors started from an extensive dataset: 66 training data sets describing the influence of T, pH, %NaCl, and $\%NaNO_2$ on M, the time corresponding to the maximum growth rate and B, the relative growth rate at $t=M$. This means that 132 data points were used to identify a large artificial neural network, using 120 parameters. The data originated from Zaika et al. (1994) using the Modified Gompertz equation to describe the growth of *Shigella flexneri*.

However, in food microbiology, data are scarce. Therefore, this paper focusses on extracting as much information as possible out of data sets usually (much) less extensive. In this case, low complexity artificial neural networks must be searched for. (Observe that at least 120 data points are required when using 120 model parameters.) Moreover, good modelling practice prescribes that the number of data points should be (much) higher than the number of parameters. Otherwise, model robustness may be damaged [see, e.g., Delignette-Muller et al. (1995)]. In such a case, so-called overfitting is unavoidable yielding a model with poor interpolative properties.

A specific ANN structure has to learn which output it has to produce for given inputs. The technique of supervised learning [see, e.g., Patterson (1996)] consists of presenting to the ANN a set of inputs with their associated outputs, the target-values. The training of the network starts with an initial value for every parameter (weights and biases) and calculates the output for every set of inputs. The produced output is compared with the target-values and some optimization algorithm is used to minimize the error between the produced output and the target-values by adjusting the parameter values. In the field of non-linear modeling techniques, this implies non-linear optimization of the parameters. Observe that this procedure is not different from classical modelling identification, in spite of another terminology.

All optimization algorithms have to cope with the problem of local minima. In such a case, the algorithm finds a set of parameter values which form a minimum (the gradient of the error function with respect to all parameters is zero), but there exists another parameter set with a lower error (global minimum). Observe that the problem of local minima is inherent to all models which are non-linearly parameterized, and is therefore not specific to ANN models.

In this research, the Quasi-Newton algorithm is applied, known [see, e.g., Bishop (1995)] to be more efficient compared to the Gradient Method used in Najjar et al. (1997) and Hajmeer et al. (1997). Different starting values for the parameters are tested in order to avoid the selection of a local minimum.

A very important issue during mathematical modeling of experimental data is the optimization of the trade off between (i) the model complexity, and (ii) the goodness of fit. More specifically, for the artificial neural networks under investigation, model complexity is determined by the number of layers, the number of neurons per layer, and the associated number of interconnection weights and biases. The error criterion chosen for this study is the residual sum of squares error criterion (SSE). To compare models with a different number of parameters, the MSE (residual mean square error) can be used (although this criterion is strictly speaking only valid for linear models), defined as follows

$$\mathrm{MSE} = \frac{\mathrm{SSE}}{n-p}$$

with $n-p$ the number of degrees of freedom of the model under consideration (n is the number of data points, p is the number of parameters). Observe the fact that a model using too many parameters compared to the number of data points will be rejected when using this criterion.

The complete grey box hybrid model, which will be developed in this paper, is schematically presented in Fig. 2. This model is called grey box because of the combination of black box neural networks and the grey box (mechanistically inspired) dynamic Gompertz equation. (The use of neural networks as primary growth model is theoretically possible, but not investigated in this research.)

The description of the identification and validation data, used to identify appropriate ANN model structures, can be found in the next subsections.

2.2. Modeling the influence of temperature

2.2.1. Identification data

Zwietering et al. (1991) compared different models available in the literature, capable of describing the temperature dependence of the growth parameters and selected the following models.

$$\mu_{\max} = b_1^2(T - T_{\min})^2(1 - \exp[c_1(T - T_{\max})]) \quad (5)$$

$$\ln(\lambda) = p/(T - q) \quad (6)$$

$$A = b_2(1 - \exp[c_2(T - T_{A,\max})]) \quad (7)$$

Because of the constant level of inoculum used in this study, A_0 can be modelled by adding a term $\ln(N_0)$ to Eq. (7). The three models are simulated by using specific parameter values for *Lactobacillus plantarum*. As such, three suitable training sets are obtained, one for each growth parameter. The use of simulation data makes it possible to create an extensive data set. However, it is important to keep in mind that these data are not real experimental data.

2.2.2. Validation: dynamic growth data

The hybrid grey box model, obtained by combination of Eq. (2) and the ANN models to describe the temperature dependence of the growth parameters, will be validated on dynamic growth data of *Lact. plantarum*.[1] The predictions will be compared with those of the model of Van Impe et al. (1992), Van Impe et al. (1995), combining the same primary growth model [Eq. (2)] with Eq. (5) to Eq. (7).

2.2.3. Validation: experimental results of other micro-organisms

To evaluate the modeling capability of the selected neural network models, the neural network model describing the temperature dependence of $\mu_{\max}$ will be validated on a whole set of experimental data (not

[1]These data are obtained from Dr. M. Zwietering, Wageningen Agricultural University, The Netherlands.

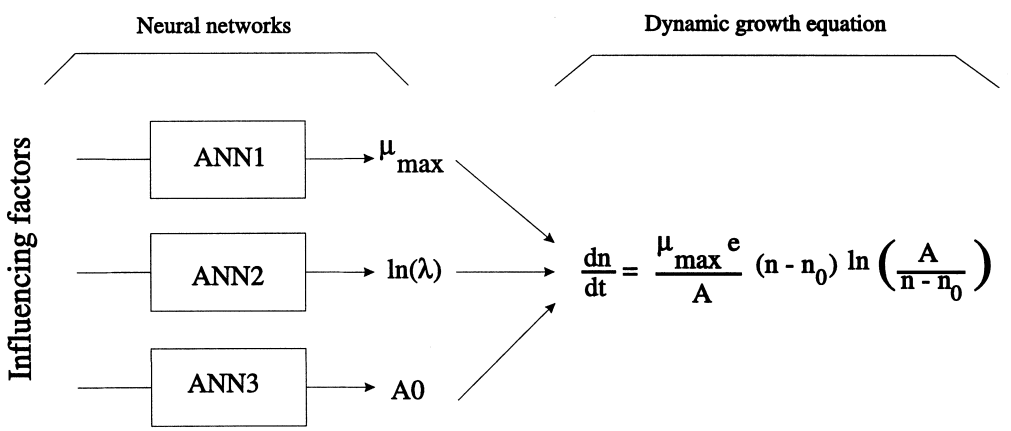

Fig. 2. Grey box hybrid model.

specific for chilled, prepared food products), obtained in the framework of an EU-project.[2]

As such, the structural possibilities of the proposed artificial neural network models can be investigated for a large range of micro-organisms including non-pathogenic ones. The data sets investigated comprise: *Acinetobacter sp.* 2.55, *Acinetobacter sp.* 4.41, *Bacillus stearothermophilus* 238, and *Bacillus subtilis* (Ratkowsky et al., 1983); *Clostridium botulinum* type A and type B (Ohye and Scott, 1953); *Escherichia coli* (Barber, 1908); Psychrophilic *Pseudomonas* 21-3c (Ingraham, 1958); *Pseudomonas syringae* and *Xanthomonas pruni* (Young et al., 1977).

The MSE obtained with the artificial neural network model will be compared with the MSE of the following three modeling approaches (each having four parameters): (i) Eq. (5), used previously to identify an appropriate artificial neural network model structure, (ii) the model of Ratkowsky et al. (1983).

$$\mu_{\max} = b^2(T - T_{\min})^2(1 - \exp[c(T - T_{\max})])^2 \qquad (8)$$

and (iii) the CTMI model (Cardinal Temperature Model with Inflection point) of Rosso et al. (1995)

$$\mu_{\max} = \mu_{\text{opt}}\tau(T) \qquad (9)$$

$$\tau(T) = \begin{cases} T < T_{\min}, & 0 \\ T_{\min} < T < T_{\max}, & \dfrac{(T - T_{\max})(T - T_{\min})^2}{(T_{\text{opt}} - T_{\min})[(T_{\text{opt}} - T_{\min})(T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\max})(T_{\text{opt}} + T_{\min} - 2T)]} \\ T > T_{\max}, & 0 \end{cases}$$

2.3. Modeling the combined influence of temperature and pH

2.3.1. Data for structural identification

For the structural identification of neural network models, capable of describing the combined and eventually interactive influence of temperature and pH on growth parameters, simulation data are used as explained above. In McClure et al. (1993) polynomial relationships are presented which can describe the temperature, pH and %NaCl content dependence of the growth parameters for *Brochothrix thermosphacta* [by combination with the Modified Gompertz Eq. (1) or the dynamic growth model of Baranyi et al. (1993)]. The polynomial relationships are presented in Eq. (10).

$$\ln(g) = p_1 + \begin{bmatrix} p_2 & p_3 & p_4 \end{bmatrix} \begin{bmatrix} T \\ \text{pH} \\ \text{NaCl} \end{bmatrix} + \begin{bmatrix} T & \text{pH} & \text{NaCl} \end{bmatrix} \begin{bmatrix} p_8 & p_5 & p_6 \\ p_5 & p_9 & p_7 \\ p_6 & p_7 & p_{10} \end{bmatrix} \begin{bmatrix} T \\ \text{pH} \\ \text{NaCl} \end{bmatrix} \qquad (10)$$

with g one of the growth parameters ($\mu_{\max}$, A_0 or λ) and $p_1 \ldots, p_{10}$ the 10 parameters for the model. As such, these three models need 30 parameters in total.

By keeping the %NaCl content at a specific, constant value [namely, 0.5% (w/v)], these polynomials can be used to generate a large amount of simulation data as functions of temperature and pH forming the basis of this first identification step.

2.3.2. Complete identification: experimental data of *B. thermosphacta*

An important remark on the previous identification step is that models, derived out of simulation data, may describe more these data than real experimental data. Moreover, the polynomial relationships used in the previous step are developed to incorporate three environmental factors. It is possible that a polynomial model, specifically developed to describe the influence of temperature and pH alone, would be more adapted to the experimental data points. Therefore, it will be evaluated how the selected artificial neural network structures perform when used on the experimental data of *B. thermosphacta*. If these data reveal an interacting effect between temperature and pH, not included in the polynomial description, the artificial neural network model will adapt its parameters to this situation. The data are obtained in the framework of an EU-Project[3] and form the basis for the model in McClure et al. (1993). As such, the

[2]FAIR CT97-3129: PREMIUM: Predictive microbiology of structured foods: development of a unifying modelling framework and application to microbial interactions.

[3]AFRF-CT91-0047: Development of computer-aided process design procedures to improve the quality and safety of products with a limited shelf-life.

derived artificial neural network models will be based on the same data as the polynomial relationships Eq. (10).

2.3.3. Validation data: experimental data of other micro-organisms

The value of the obtained artificial neural network models will be highlighted on experimental data of other micro-organisms, namely *Escherichia coli* 0157:H7 and *Aeromonas hydrophila* K144. The network structure will be retained but the parameters, typical for each micro-organism and its environment, reidentified.

The data from *E. coli* are obtained in the framework of an EU-project[4]. This data set consists of 34 data points, from which one is not used because of an unrealistic high value of one of its components. The data originated from Buchanan and Klawitter (1992) who developed a polynomial model describing the influence of temperature, pH and %NaCl.

A polynomial relationship for *A. hydrophila* is developed by Palumbo et al. (1991), incorporating the influences of temperature, pH, %NaCl and $\%NaNO_2$. Only the data with the lowest, constant levels of %NaCl and $\%NaNO_2$ are retained, yielding a data set with 17 points.

2.4. Modeling the combined influence of temperature, pH and %NaCl

2.4.1. Identification data

As the data set for *B. thermosphacta*, mentioned above, contains 41 growth curves influenced by temperature, pH and %NaCl, these experimental data can be used as such to identify appropriate ANN model structures and associated parameters. The new models will be compared with the original polynomial relationships of McClure et al. (1993) presented in Eq. (10). More specifically, an *F*-test will be conducted (although strictly speaking this is only valid for linear models) making use of the 3 duplicates available in the data set. The duplicates give an idea of the experimental error by construction of a general model [see, e.g., Zwietering et al. (1991)]. This model uses the sample mean $\bar{Y}_j$ of the measured data $Y_{i,j}$ at each combination j of independent factors. The pure error sum of squares is defined as

$$\mathrm{SSPE} = \sum_i \sum_j (Y_{i,j} - \bar{Y}_j)^2$$

Observe the fact that combinations of independent factors where no duplicate is available make no contribution to SSPE. The lack of fit sum of squares is defined as

$$\mathrm{SSLF} = \mathrm{SSE} - \mathrm{SSPE}$$

The test statistic is

$$F = \frac{\mathrm{SSLF}}{c-p} \Big/ \frac{\mathrm{SSPE}}{n-c}$$

with n the total number of data points, p the number of parameters in the artificial neural network or the polynomial model and c the number of different levels of combinations of independent factors. F^* is tested against $F(1-\alpha, c-p, n-c)$.

2.4.2. Application of the grey box model

Upon completion of the previous step, the grey box model depicted in Fig. 2 is established. Its predictive value will be evaluated by application to the experimental growth curves of *B. thermosphacta*.

Note the fact that in the literature often the application of the secondary model (after combination with an appropriate primary model) on the original experimental data is not shown. Considering practical applications, it is important to evaluate the time prediction of the combined primary/secondary model.

3. Results

3.1. Modeling the influence of temperature

3.1.1. Identification data

After an extensive study of different ANN models taking into account the tradeoff between the number of parameters and SSE, the ANN model, presented in Fig. 3, is identified to describe the temperature dependence of μ_{max}. For the temperature dependence of the other two growth parameters, namely, λ and A_0, other ANN model structures are selected. An overview can be found in Table 1. Observe the different transfer functions of the individual neurons.

[4]FAIR CT97-3129: PREMIUM: Predictive microbiology of structured foods: development of a unifying modelling framework and application to microbial interactions.

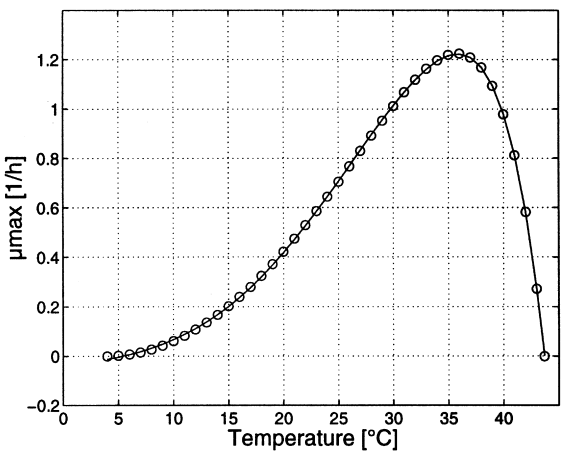

Fig. 3. ANN description (full line) for the simulation data (''o'') of Eq. (5) to describe the temperature dependence of μ_{max}.

3.1.2. Validation: dynamic growth data

Combination of the ANN models, obtained in the previous step, with the dynamic version of the Modified Gompertz equation [Eq. (2)] makes a prediction of the growth of the microbial population possible as a function of time. Some results can be found in Fig. 4. The predictions are compared with those of the model of Van Impe et al. (1992), Van Impe et al. (1995), consisting of Eq. (2) combined with the classical Eq. (5) to Eq. (7).

3.1.3. Validation: experimental results of other micro-organisms

The description of experimental data, different from *Lact. plantarum*, is performed by reidentifying the seven ANN model parameters for μ_{max} to incorporate the characteristics of the micro-organism

Table 1
Overview of the selected ANN models, describing the temperature dependence of μ_{max}, λ and A_0

Growth parameter	Model	Neuron description	# parameters
μ_{max}	ANN 1	$N_1 = \exp[-(W_1 \cdot T + C_1)^2]$ $N_2 = \exp[-(W_2 \cdot T + C_2)^2]$ $\mu_{max} = W_3 \cdot N_1 + W_4 \cdot N_2 + C_3$	7
λ	ANN 2	$N_1 = \exp(W_1 \cdot T)$ $N_2 = \exp(W_2 \cdot T)$ $\ln(\lambda) = W_3 \cdot N_1 + W_4 \cdot N_2$	4
A_0	ANN 3	$A_0 = \tanh(W_1 \cdot T + B_1)$	2

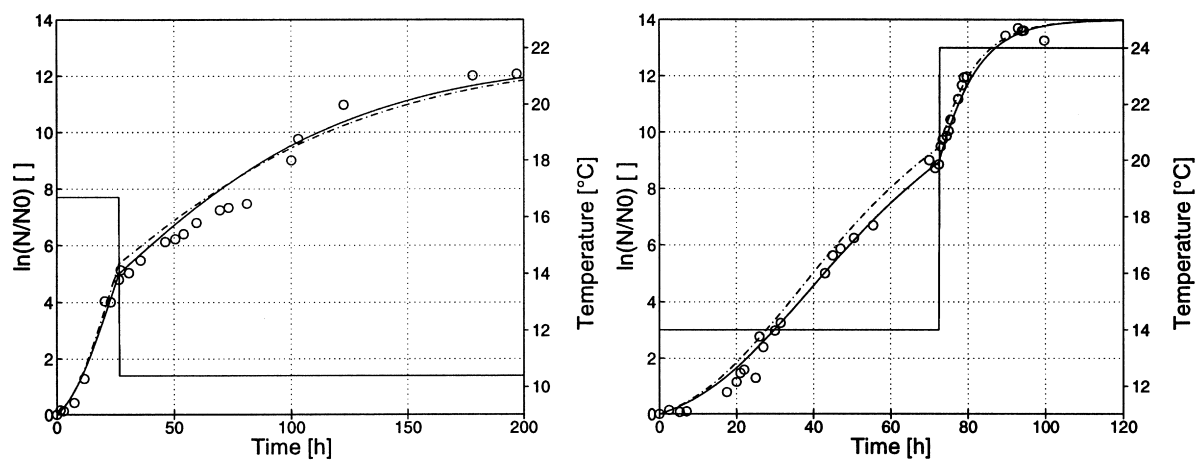

Fig. 4. Growth curves of *Lactobacillus plantarum*, predicted by combination of the Modified Gompertz Equation for growth and the ANN models (full line) or the more classical models (dashed line) for a temperature step.

and its experimental environment (besides temperature). A comparison of the MSE of the ANN and three model approaches, namely Eq. (5), Eq. (8) and Eq. (9) is provided in Table 2. Fig. 5 illustrates the ANN description.

3.2. Modeling the combined influence of temperature and pH

3.2.1. Data for structural identification

Again, an extensive study reveals the structural properties of several ANN models. The ANN models, derived in the previous section, form a guideline for the development of new ANN models because of their modular properties. This is illustrated in Table 3 for the selected ANN model 4a describing the temperature and pH dependence of μ_{max}. The network structure is the same as for temperature alone, upon adding an extra input pH. For μ_{max}, three ANN models are selected: ANN model 4a, 4b and 4c.

3.2.2. Complete identification: experimental data of B. thermosphacta

The structures of the ANN models are used in this second step on the real experimental data. The performance of ANN model 4b to describe the temperature and pH dependence of μ_{max} is illustrated in Fig. 6, together with the residuals (defined as the difference between the data points and the model

Table 2
Comparison between the ANN model description and three more classical models for the temperature dependence of μ_{max}

Micro-organism	MSE ANN model	MSE Eq. (5)	MSE Eq. (8)	MSE Eq. (9)
Acinetobacter sp. 2.55	1.988e−008*	2.769e−008	2.256e−008	3.277e−008
Acinetobacter sp. 4.41	8.182e−009	7.659e−009*	8.032e−009	1.219e−008
Bacillus stearothermophilus 238	1.293e−007*	1.694e−007	2.909e−007	1.375e−007
Bacillus subtilis	0.00114*	0.00182	0.00228	0.00182
Clostridium botulinum type A	0.00024	0.00011*	0.00017	0.00020
Clostridium botulinum type B	0.00027*	0.00148	0.00056	0.00185
Escherichia coli	0.02284	0.03518	0.02588	0.02239*
Psychrophilic *Pseudomonas* 21−3c	0.00073*	0.00186	0.00078	0.00109
Pseudomonas syringae	0.00038	0.00060	0.00035*	0.00075
Xanthomonas pruni	0.00093	0.00072	0.00061*	0.00087

*lowest MSE.

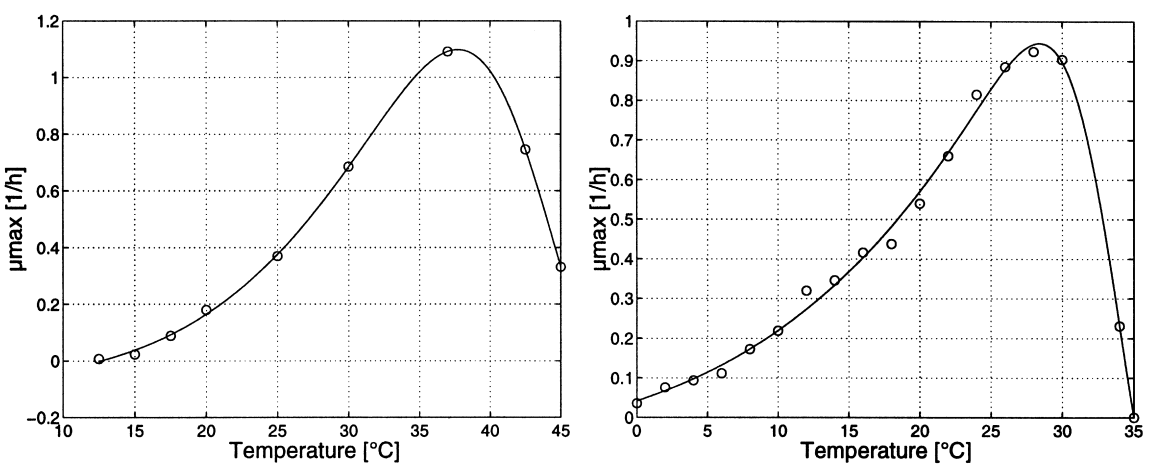

Fig. 5. (left) ANN description (full line) for the temperature dependence of μ_{max} for *Clostridum botulinum* type B. (right): idem for *Pseudomonas* 21−3c.

Table 3
Overview of the selected ANN models, describing the temperature and pH dependence of μ_{max}, λ and A_0

Growth parameter	Model	Neuron description	# parameters
μ_{max}	ANN 4a	$N_1 = \exp[-(W_1 \cdot T + W_2 \cdot \mathrm{pH} + C_1)^2]$ $N_2 = \exp[-(W_3 \cdot T + W_4 \cdot \mathrm{pH} + C_2)^2]$ $\mu_{max} = W_5 \cdot N_1 + W_6 \cdot N_2 + C_3$	9
	ANN 4b	$N_1 = \exp[-(W_1 \cdot T + W_2 \cdot \mathrm{pH} + C_1)^2]$ $N_2 = 0.5 \cdot [\tanh(W_3 \cdot T + W_4 \cdot \mathrm{pH} + C_2) + 1]$ $\mu_{max} = W_5 \cdot N_1 + W_6 \cdot N_2 + C_3$	9
	ANN 4c	$N_1 = 0.5 \cdot [\tanh(W_1 \cdot T + W_2 \cdot \mathrm{pH}) + 1]$ $N_2 = 0.5 \cdot [\tanh(W_3 \cdot T + W_4 \cdot \mathrm{pH}) + 1]$ $\mu_{max} = W_5 \cdot N_1 + W_6 \cdot N_2$	6
λ	ANN 5	$N_1 = \exp(W_1 \cdot \mathrm{pH})$ $N_2 = 0.5 \cdot [\tanh(W_2 \cdot T + W_3 \cdot \mathrm{pH}) + 1]$ $\lambda = W_4 \cdot N_1 + W_5 \cdot N_2$	5
A_0	ANN 6	$N_1 = \exp(W_1 \cdot T + W_2 \cdot \mathrm{pH})$ $N_2 = 0.5 \cdot [\tanh(W_2 \cdot T + W_3 \cdot \mathrm{pH}) + 1]$ $A_0 = W_4 \cdot N_1 + W_5 \cdot N_2$	6

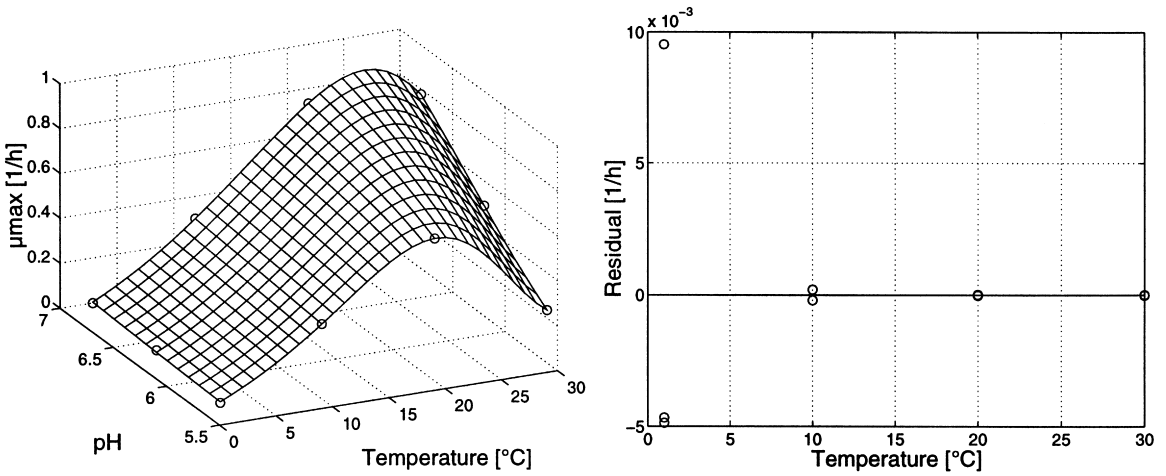

Fig. 6. Description of ANN model 4b (plane) for μ_{max}, and the associated residual values.

description). This residual plot (as others in subsequent figures) visualizes more clearly the 3-dimensional plot with regard to the distance between the data points (''o'') and the ANN model description in Fig. 6, left. The mean value of the residuals is indicated with a straight line and is very close to 0. The residuals should fall within a symmetric horizontal band around 0, displaying no systematic tendencies to be positive or negative. Figs. 7 and 8 present the description of ANN model 5 and 6 for the temperature and pH dependence of λ and A_0, respectively.

3.2.3. Validation data: experimental data of other micro-organisms

The performance of ANN model 4a and 4b is not completely satisfactory on the experimental data set of *E. coli*: some overfitting at combinations of high temperature and low pH is observed, although the overall description is good. However, when fitting the data set of *A. hydrophila* a serious overfitting occurred at the combination of high temperatures and low pH where only a few data points are available (results not shown). Therefore, a simpler ANN model is selected for these data sets, making use of an ANN

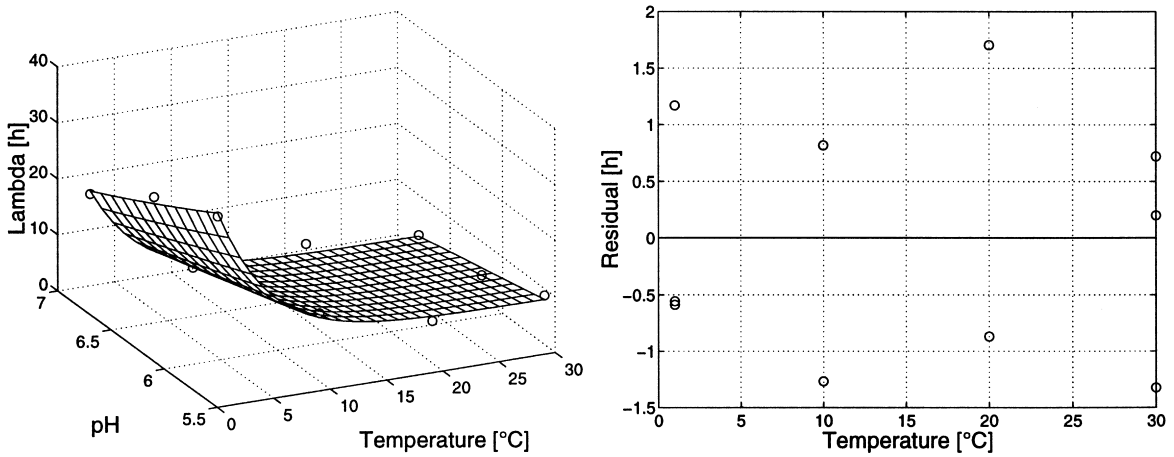

Fig. 7. Description of ANN model 5 for A, and the associated residual values.

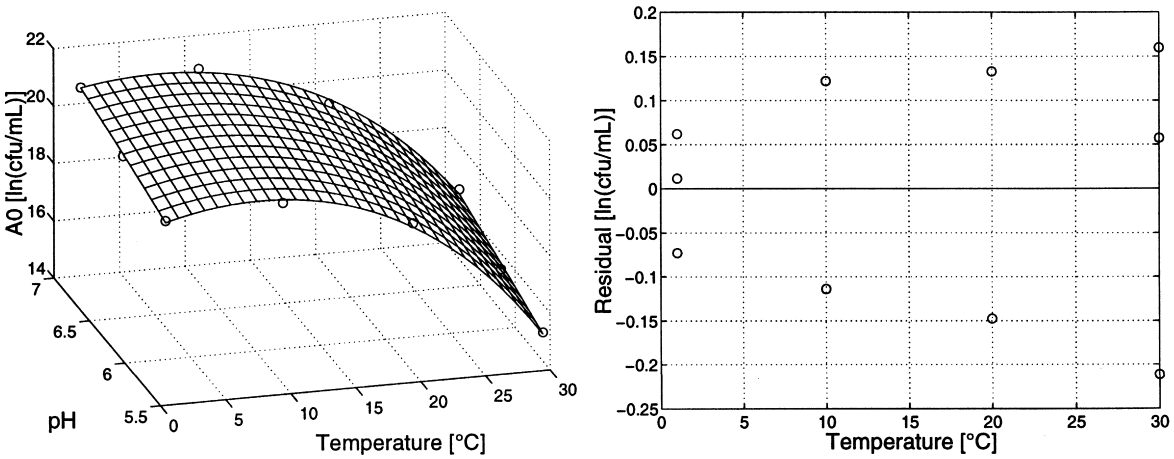

Fig. 8. Description of ANN model 6 for A_0, and the associated residual values.

model structure tested previously but not retained for the data of *B. thermosphacta*. This ANN model 4c is already described in Table 3 and compared with other model types in Table 4. Identification results are presented in Figs. 9 and 10.

3.3. Modeling the combined influence of temperature, pH and %NaCl

3.3.1. Identification data

In this step, no simulation data are used. Instead, the selection of appropriate ANN models is based on 41 growth curves of *B. thermosphacta*. An overview of the results is presented in Table 5. Figs. 11–13 present the ANN models. In these figures, the description of the data points (''o'') using the ANN

Table 4
Comparison of three ANN models and two other models available in the literature on experimental data of *E. coli*

Model	# parameters	SSE	MSE
ANN Model 4a	9	0.233	0.0093
ANN Model 4b	9	0.246	0.0098
ANN Model 4c	6	0.532	0.0197
Zwietering et al., 1991	9	0.664	0.0266
Rosso et al., 1995	7	0.653	0.0242

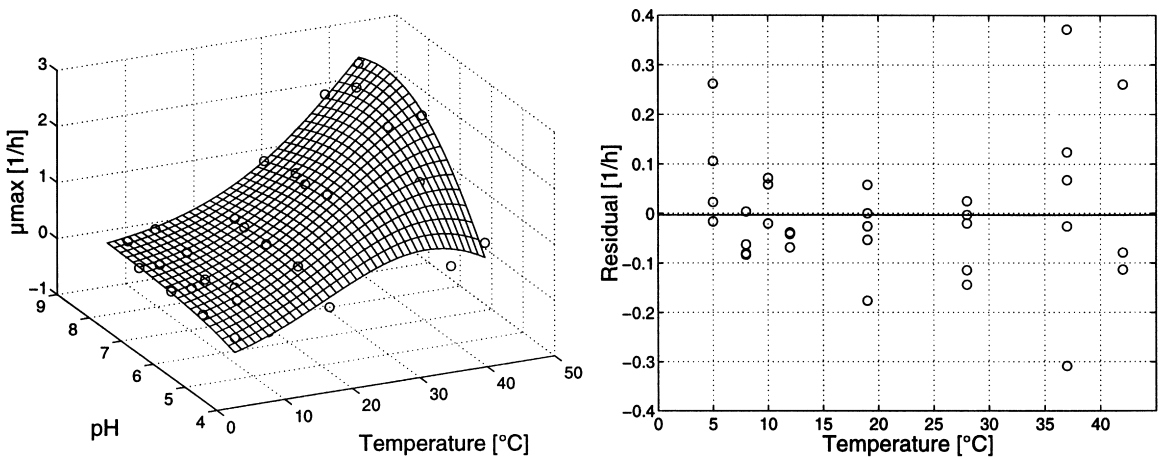

Fig. 9. Performance of ANN model 4c on *E. coli*, and the associated residual values.

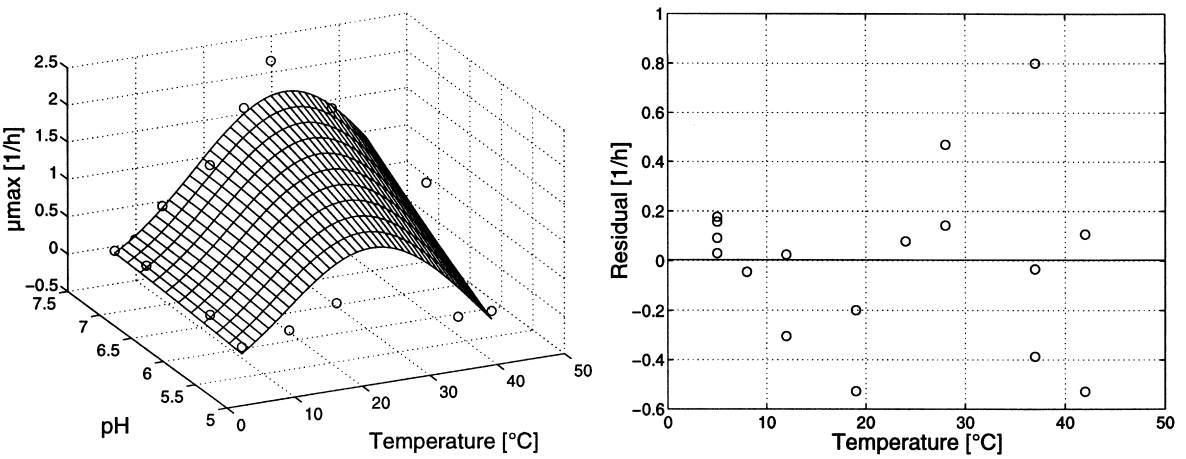

Fig. 10. Performance of ANN model 4c on *A. hydrophila*, and the associated residual values.

Table 5
Overview of the selected ANN models, describing the temperature, pH and %NaCl dependence of μ_{max}, λ and A_0

Growth parameter	Model	Neuron description	# parameters
μ_{max}	ANN 7	$N_1 = \exp[-(W_1 \cdot T + W_2 \cdot \mathrm{pH} + W_3 \cdot \mathrm{NaCl} + C_1)^2]$ $N_2 = \exp[-(W_4 \cdot T + W_5 \cdot \mathrm{pH} + W_6 \cdot \mathrm{NaCl} + C_2)^2]$ $\mu_{max} = W_7 \cdot N_1 + W_8 \cdot N_2 + C_3$	11
λ	ANN 8	$N_1 = \exp(W_1 \cdot T + W_2 \cdot \mathrm{pH} + W_3 \cdot \mathrm{NaCl})$ $N_2 = 0.5 \cdot [\tanh(W_4 \cdot T + W_5 \cdot \mathrm{pH} + W_6 \cdot \mathrm{NaCl}) + 1]$ $\ln(\lambda) = W_7 \cdot N_1 + W_8 \cdot N_2$	8
A_0	ANN 9	$N_1 = \exp(W_1 \cdot T + W_2 \cdot \mathrm{pH} + W_3 \cdot \mathrm{NaCl})$ $N_2 = 0.5 \cdot [\tanh(W_4 \cdot T + W_5 \cdot \mathrm{pH} + W_6 \cdot \mathrm{NaCl}) + 1]$ $A_0 = W_7 \cdot N_1 + W_8 \cdot N_2$	8

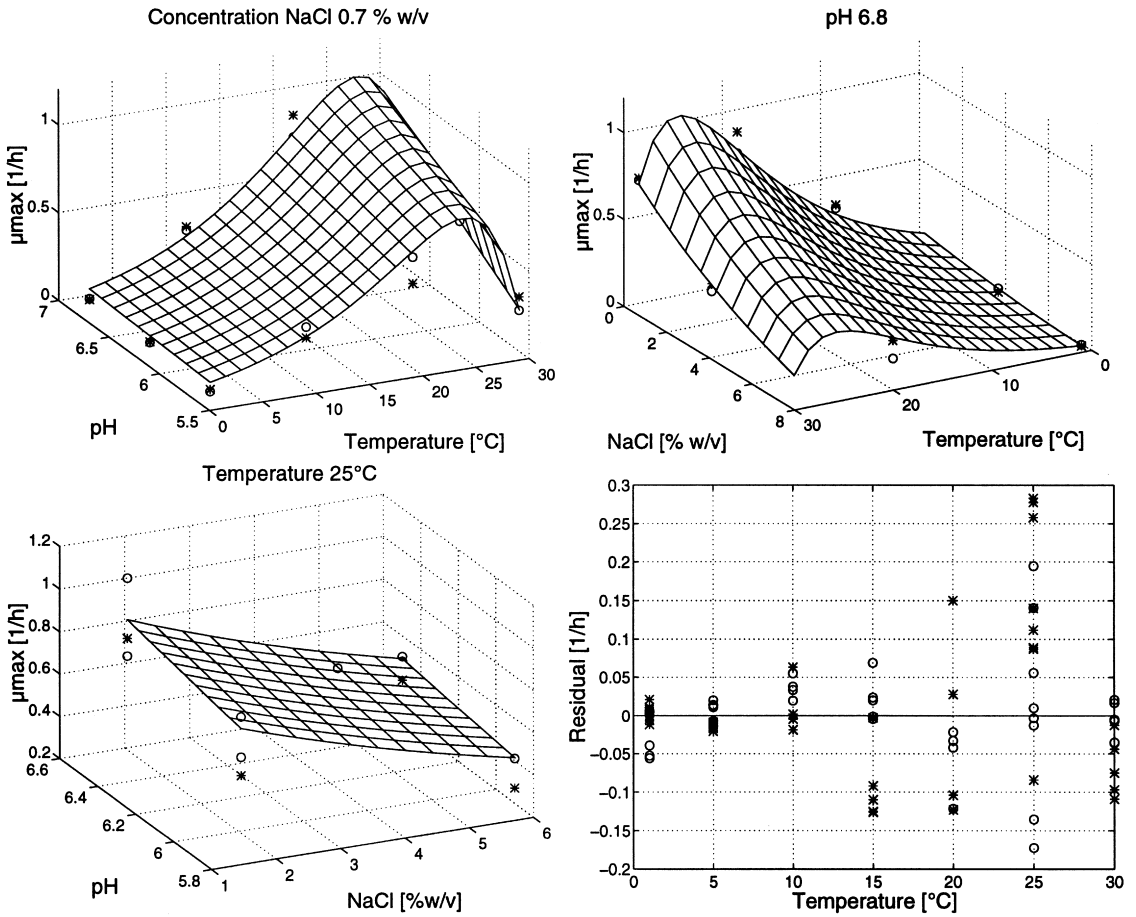

Fig. 11. ANN model 7 (plane) describing μ_{max} (''o'' = data points) as a function of temperature, pH and a_w. * = polynomial description. The associated residual values are also shown.

models is compared with the polynomial description (''*''). Because of the fact that three environmental factors need to be described, one factor is held constant to visualize the results. Concerning the residual plots, the difference between the data points and the ANN model description (''o'') is shown, together with the difference between the data points and the polynomial description (''*'').

The ANN models are compared with the polynomial models in Table 6. The total number of parameters is 27, whereas the polynomial relationships need 30 parameters. The *F*-testing values (not shown), indicate that all 6 models (3 polynomial models and 3 ANN models) are appropriate to describe the experimental results when compared with the experimental error.

3.3.2. Application of the grey box model

The combination of the selected ANN models and the Modified Gompertz equation, as depicted in Fig. 2, makes it possible to visualize the identification results on the experimental data. In Fig. 14, 9 growth curves are shown, namely, those obtained at NaCl equal to 4.125% (w/v). The combination of the Modified Gompertz equation with the polynomial relationships (McClure et al., 1993) is shown in dashed lines.

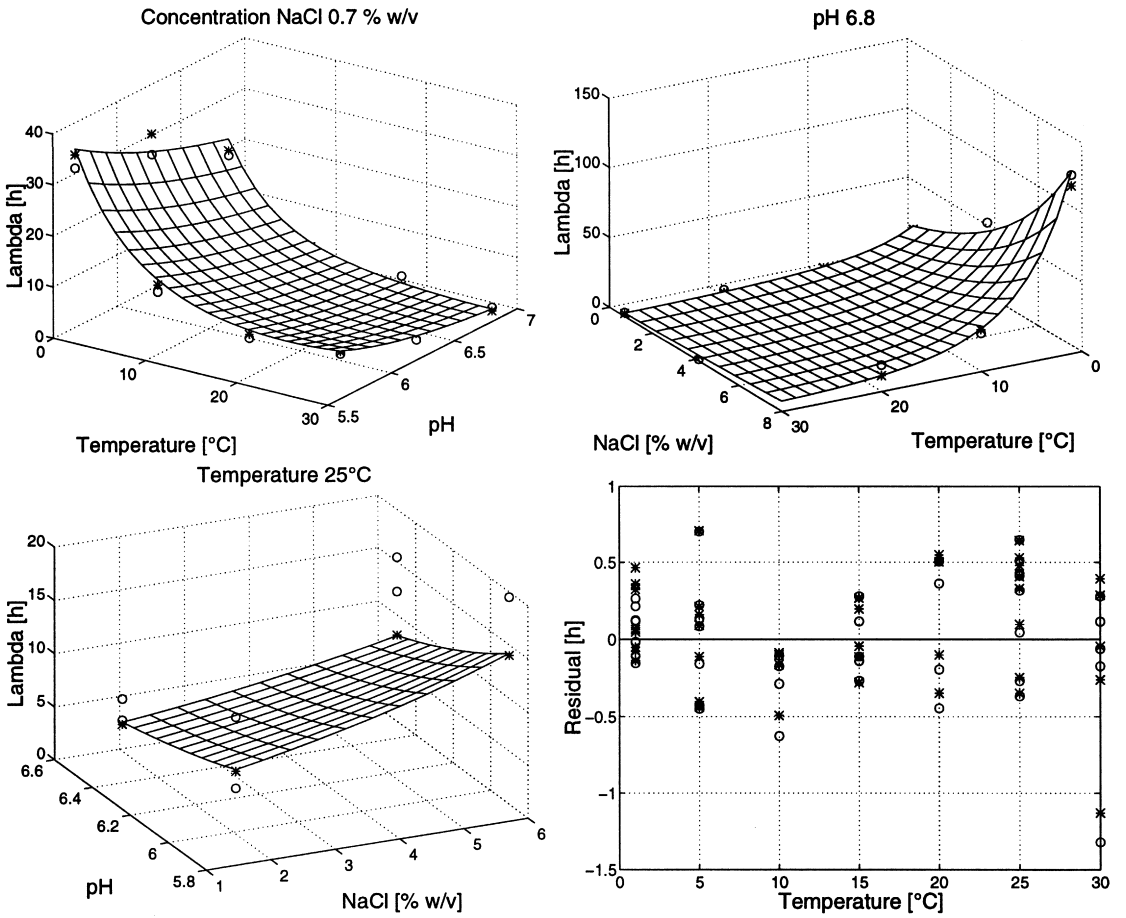

Fig. 12. ANN model 8 (plane) describing λ (“o”=data points) as a function of temperature, pH and a_w. *=polynomial description. The associated residual values are also shown.

4. Discussion

4.1. Modeling the influence of temperature

4.1.1. Identification data

The ANN model, presented in Fig. 3, provides a very accurate description of μ_{max} as a function of temperature. In comparison with more classical models [Eq. (5) to Eq. (7)] used to derive the ANN structures presented in Table 1, the total number of parameters is increased by four (a total of 9 parameters for Eq. (5) to Eq. (7), and a total of 13 parameters for the ANN model descriptions). This illustrates the capabilities of ANN models to be of rather low complexity when only simple models are needed.

4.1.2. Validation: dynamic growth data

The combination of ANN models and the dynamic version of the Modified Gompertz equation (Fig. 4, full line) is compared with the combination of the classical models [Eq. (5) to Eq. (7)] and the same primary model (dashed line). As can be seen, the accuracy of the ANN models is good.

These results have been presented in Geeraerd et al. (1997).

4.1.3. Validation: experimental results of other micro-organisms

The results in Table 2 indicate the modeling capabilities of the selected ANN model for μ_{max}. The use of MSE incorporates the fact that 7 parameters are needed, whereas the other model structures [Eq.

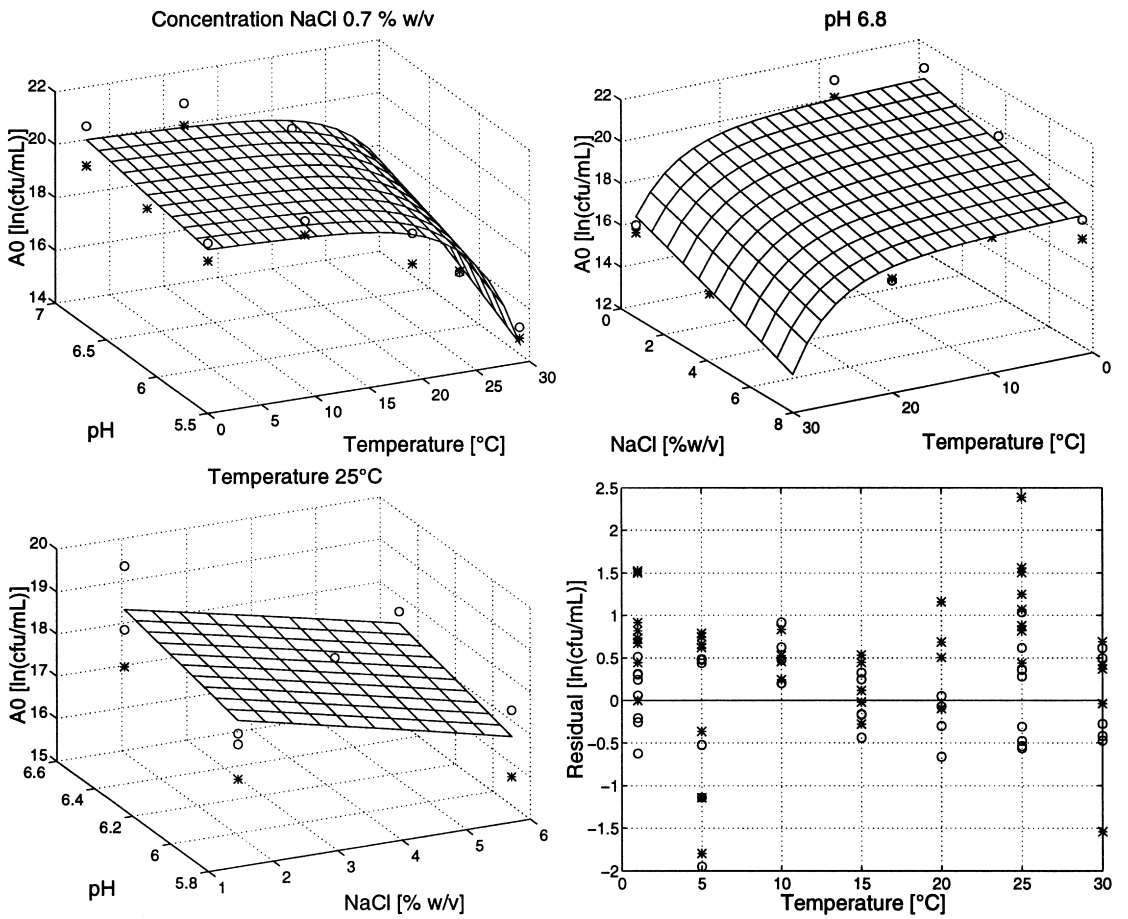

Fig. 13. ANN model 9 (plane) describing A_0 (''o''=data points) as a function of temperature, pH and a_w. *=polynomial description. The associated residual values are also shown.

Table 6
Comparison of ANN models and polynomial models

Growth parameter	Model	# parameters	SSE	MSE
μ_{max}	Polynomial model	10	0.416	0.013
	ANN model 7	11	0.150	0.005
A_0	Polynomial model	10	35.6	1.148
	ANN model 8	8	13.4	0.406
$\ln(\lambda)$	Polynomial model	10	5.59	0.180
	ANN model 9	8	5.71	0.173

(5), Eq. (8) and Eq. (9)] use only 4 parameters. However, the ANN model description has in five out of the ten cases the lowest MSE. In 2 other cases, the ANN model comes at the second place. The results in Fig. 5 show that the description is satisfactory without any overfitting.

It can be concluded that ANN models are good alternatives for more classical models, even in the

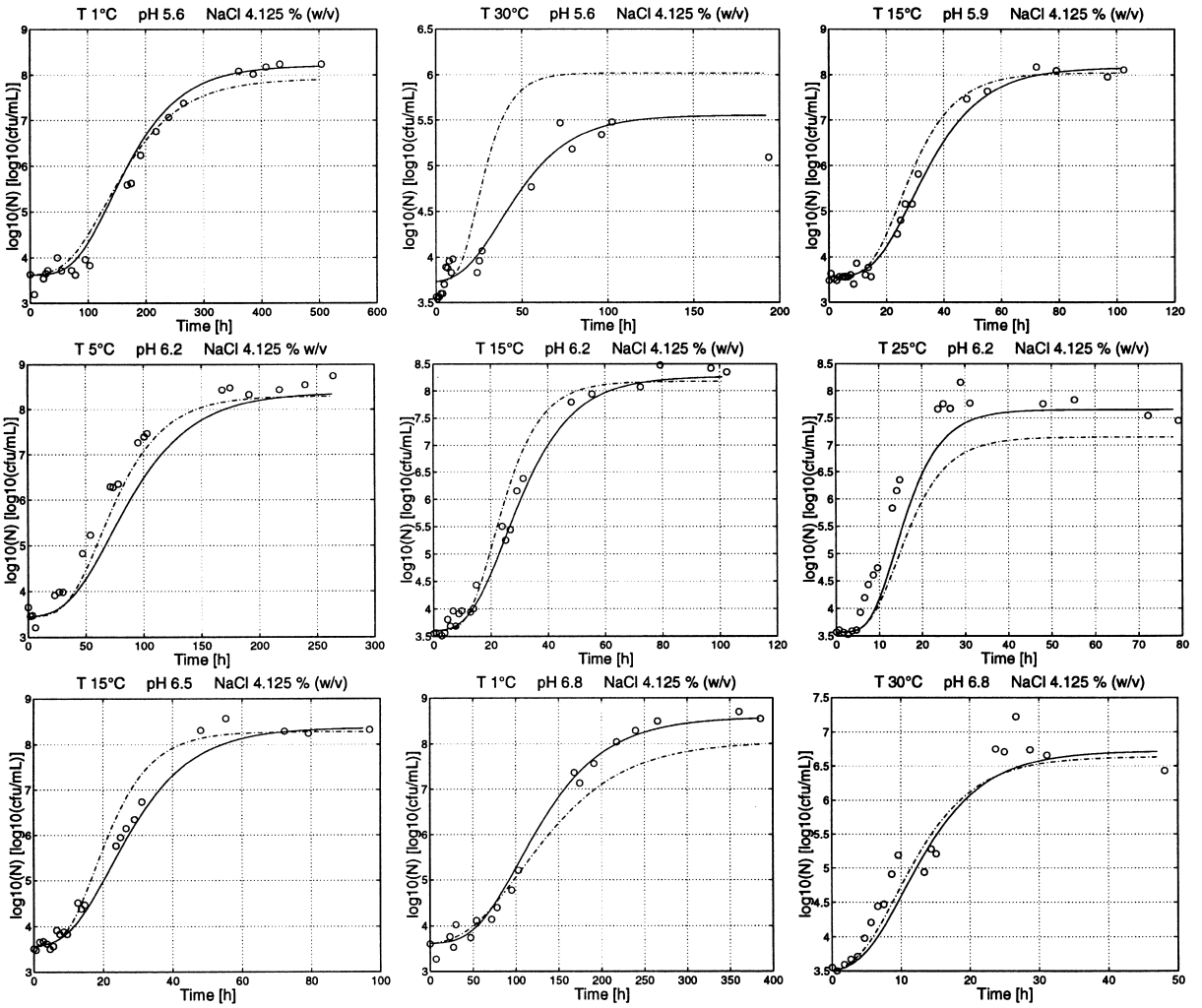

Fig. 14. Growth curves of *B. thermosphacta* at NaCl equal to 4.125% (w/v). Full line: ANN model, dashed line: polynomial model of McClure et al. (1993).

case where the number of inputs d is only one, namely, temperature.

4.2. Modeling the combined influence of temperature and pH

4.2.1. Data for structural identification

For the data of *B. thermosphacta*, two ANN models are selected: ANN model 4a and 4b (Table 3). The modular property of artificial neural network is highlighted by ANN model 4a. Both models perform equally well (the SSE on the simulation data is more or less the same). The total number of parameters for the 3 ANN model descriptions is 20, whereas the polynomial relationships need 18 parameters. In this case the number of inputs d equals 2. It can be observed that the difference in the number of parameters needed in the ANN model description relative to the number of parameters in the polynomial description is less than in the previous section where only temperature was modeled ($20-18$ is less than $13-9$). This illustrates the efficient scaling with dimensionality, a property which becomes clearer as the number of inputs increases.

4.2.2. Complete identification: experimental data of B. thermosphacta

Figs. 6–8 illustrate the satisfactory descriptions of the ANN models following the general trend in the data. This smooth behavior is necessary for the generalization and interpolation properties of the derived model structures.

4.2.3. Validation data: experimental data of other micro-organisms

The performance of ANN model 4c when the parameters are identified on the data set of *E. coli* and *A. hydrophila* is illustrated in Figs. 9 and 10, together with the residual values. The MSE of this model, ANN models 4a and 4b and two more classical models can be found in Table 4. For the ANN models, it is not the one with the lowest MSE that reveals itself to be the best general descriptor. Although the MSE incorporates the number of parameters, it is still possible that a model, selected on the basis of this criterion only, overfits the experimental data. Therefore, the selection of the model must also be based on its visual performance, taking into account that the factors limiting bacterial growth act in a continuous and slowly retarding way. In this case, the simpler ANN model 4c, using only six parameters, is the best one generalizing the overall trend in these experimental data. Concerning *B. thermosphacta*, ANN models 4a and 4b will be investigated further.

4.3. Modeling the combined influence of temperature, pH and %NaCl

4.3.1. Identification data

Figs. 11–13 reveal that the ANN model descriptions are excellent, and illustrate the capability of the ANN models to describe the data points (‘‘o’’) as compared with the polynomial model Eq. (10) (‘‘*’’), especially for μ_{max} and A_0. This is further shown in Table 6 with the (much) lower MSE of the artificial neural network models, and, consequently, the higher F-test values (not shown). In this case the number of inputs d equals 3, and the artificial neural network model description needs less parameters than the polynomial relationships. This could be expected from a theoretical point of view, as explained higher.

4.3.2. Application of the grey box model

In Fig. 14, the nine growth curves shown illustrate the satisfactory time prediction when the selected ANN models (full line) are combined with the Modified Gompertz equation as depicted in Fig. 2.

5. Conclusion

The main conclusions of this research can be stated as follows.

- Artificial neural networks reveal themselves to be a low complexity non-linear modeling technique, capable of describing accurately experimental data in the field of secondary models in predictive microbiology, even when the number of independent variables is less than 3. This justifies the use of this non-linear modeling technique in the common situation of scarce experimental data when compared with the very complex artificial neural network introduced in predictive microbiology by Hajmeer et al. (1997).
- The complexity of the artificial neural network model needed in a specific application can be adapted taking into account the general trend and the number of data points. Modeling expertise, built up to gather these research results, aids qualitatively to postulate appropriate artificial neural network model structures.
- The artificial neural network model describing the growth parameters as a function of temperature, pH and %NaCl is far more accurate than the polynomial relationship available in the literature. This can be explained by the flexible basis functions used in artificial neural network modeling compared to the fixed basis functions of a polynomial or any other linear modeling technique. As such, an accurate description of experimental data points in the region where the growth/no growth interface appears (which occurs when the boundaries of temperature, pH and/or %NaCl are reached) is theoretically founded and, as shown in this paper, practically feasible.

In future, product validation of the derived artificial neural network models is required to complete the modeling cycle (Van Impe et al., 1998). As such,

the importance in relation to chilled food products can be discussed. Moreover, the artificial neural network will be extended towards a possible fourth independent variable, namely nitrite.

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