Artificial neural networks as a tool for incorporating microbial stress adaptations in the quantification of microbial inactivation.

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Résumé
Il est impératif de mesurer les réponses microbiennes adaptées dues aux stress thermiques à l'aide d'une méthodologie précise pour quantifier l'efficacité d'un processus de chaleur. Deux modèles de réseaux de neurones artificiels (ANN) différents sont construits pour étudier l'induction croissante de la résistance thermique du *Escherichia coli* K12 sous un traitement de taux de chauffage décroissants. Dans la première structure de modèle il y a deux vecteurs entrés, le temps t et le taux de la température dT/dt, tandis qu'il est ajouté dans le deuxième cas un troisième vecteur, la charge microbienne retardée d'une unité de temps N_{k-1}. Pour ces deux modèles une minimalle complemente connectée "feedforward" architecture est employée. Elle se compose d'un neurone caché et d'un neurone sortie. Les résultats, basés sur les possibilités de prévision des structures modèles, démontrent par comparaison l'avantage quand une architecture d'ANN avec un retard dans ses entrées est utilisée. L'incorporation des événements passés semble être une entrée essentielle pour prendre en compte la résistance thermique microbienne induite observée.
Mots-clés : Microbiologie prévisionnelle, réseaux de neurones artificiels, résistance thermique induite, destruction microbienne

Abstract
Quantifying microbial adapted responses due to thermal stresses by an accurate methodology is imperative for assessing the efficacy of a heat process. Two different artificial neural network (ANN) models are constructed for studying the increased induction of the heat resistance of *Escherichia coli* K12 under a treatment of decreasing heating rates. In the first model structure there are two input vectors, namely, time t and temperature rate dT/dt, whereas in the second case is also added a third one, namely, the microbial load delayed with one time unit N_{k-1}. For both models a minimal fully-connected feedforward architecture is used consisting of one hidden neuron and one output neuron. Results as based on the prediction capability of the model structures demonstrate the comparative advantage when an ANN architecture with a delay in its inputs is employed. Incorporation of past events seems to be an essential input for taking into account the observed induced microbial heat resistance.
Keywords : predictive microbiology, artificial neural networks, induced heat resistance, microbial inactivation

1 Introduction

The microbial safety of thermally processed foods relies on the inactivation of pathogenic microorganisms during heating. Possible induction of an *increased* microbial heat resistance due to a specific time-temperature history (e.g., slowly increasing temperatures) may lead to unexpectedly unsafe food products (Juneja and Novak, 2003).

Previous studies have shown that the chosen temperature conditions before treatment or during the process conditions could have a significant impact on the microbial viability. Different stresses could determine the nature of the adaptive cell response (Marechal et al., 1999). According to Marechal et al. (1999) the microbial cell may react actively (synthesis of intracellular molecules, commonly known as heat shock proteins or HSP's) or passively (membrane permeability changes) to any external perturbation, in order to prevent the denaturation of the cell integrity or activity. Therefore, it is imperative that the response of bacteria to applications of this kind of

sub-lethal stresses is further evaluated and quantified. Quantification of this phenomena can be performed within the discipline of *Predictive Microbiology*.

When considering mathematical modelling applications in the field of predictive microbiology, in most cases a suitable model has to be selected out of a pre-specified set of candidate models. This is due to the lack of generally applicable structure characterisation techniques for non-linear systems (Van Impe et al., 2001). The careful examination of a priori microbiological knowledge may aid in choosing an appropriate mathematical expression. The microbial kinetic models can be divided (with respect to their structural characteristics) as follows (see, e.g., Ljung (1999)):

1. White box or mechanistic (physical) models are constructed based on theory or underlying mechanisms and are amenable to refinement as knowledge of the system increases (McMeekin et al., 2002).
2. Black box or data-driven models are purely based on experimental data (e.g., polynomial models, artificial neural networks).
3. Grey box or hybrid models lay on the interface of white box and black box models, i.e., combining information from both theory and data and having partly interpretable parameters.

The majority of the microbial inactivation models are lying in the second and third category due to the lack of sufficient microbial/biochemical knowledge concerning the inactivation of microorganisms. This model division as defined in relation to the data-theory richness can be visualised in Figure 1. Observe that in a situation that gets sufficiently complex and there is high data richness, a black box (through learning) might be preferred than performing a large amount of approximations to a white box model (Rumelhart et al., 1994).

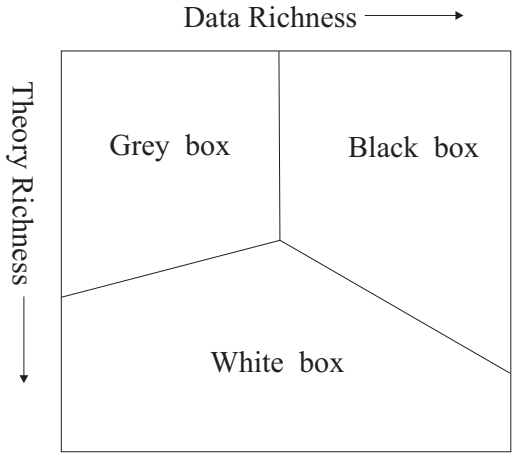

FIG. 1 – *Model division in relation to data and theory richness (adapted from Rumelhart et al. (1994); Basheer and Hajmeer (2000)).*

The non-linear technique of Artificial Neural Networks (ANN) lies in the black box modelling group. This technique has been used for describing accurately the interacting effect of extrinsic/intrinsic factors (e.g., Geeraerd et al. (1998)) on the microbial growth kinetics. Limited studies have been performed for predicting the thermal inactivation of bacteria. Lou and Nakai (2000) proposed an ANN for studying the effects of temperature, pH and a_w on the thermal inactivation rate of *E. coli*. The methodology generated accurate results when compared with other secondary models (Lou and Nakai, 2000). Additionally, the use of ANNs as an integrated primary-secondary inactivation model can contribute in an overall approach for modelling the microbial inactivation dynamics (a similar example but for growth kinetics is the one of Cheroutre-Vialette and Lebert (2000)).

This work focuses on the quantification of the inactivation of *Escherichia coli* K12 under time-temperature conditions responsible for inducing a microbial heat stress. A comparative study on the predictive capability of two different artificial neural network models -differing on their ability to incorporate information from past events- is assessed. Additionally, classical model predictions (using parameters derived from isothermal microbial studies) are also compared with the developed ANN methodologies. In the latter study thermal resistance of the microorganism is quantified under different isothermal conditions by the use of a non-linear sigmoid-like model. Predictions were then performed by assuming that the parameters of the dynamic microbial model describing the microbial resistance remain valid in the studied dynamic environment (Valdramidis et al., 2006).

2 Materials and Methods

2.1 Microbial data

Six different heating regimes of *Escherichia coli* K12 (a surrogate for the food-borne pathogen *Escherichia coli* O157:H7) with heating rates dT/dt of 0.15°C/min (exslowest), 0.20°C/min (slowest), 0.40°C/min (slower), 0.55°C/min (slow), 0.82°C/min (intermediate), and 1.64°C/min (fast) were employed, while the initial and the final temperatures were set to 30 and 55°C for all heating rates (see Valdramidis et al. (2006) for full details on data collection).

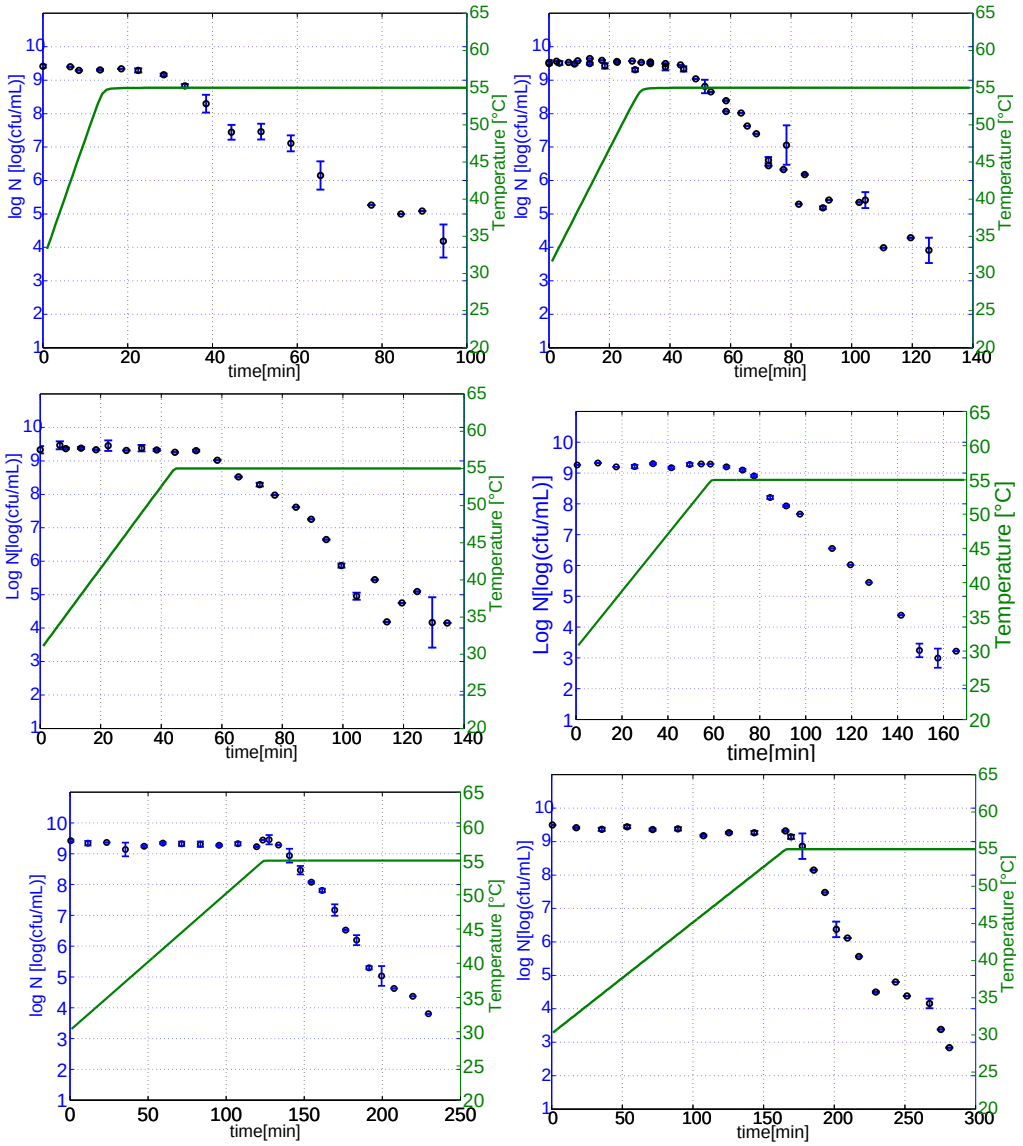

FIG. 2 – *Dynamic microbial inactivation experiments of* E. coli *K12 with corresponding standard deviation (for data where duplicates are available) and temperature measurements. Left top plot: fast (1.65°C/min), right top plot: intermediate (0.82°C/min), left middle plot: slow (0.55°C/min), right middle plot: slower (0.40°C/min), left bottom plot: slowest (0.20°C/min), right bottom plot: exslowest (0.15°C/min).*

The heating rates were estimated by the use of a modified Dabes model, as it was described by Van Impe et al., (1994) and discussed in Valdramidis et al. (2006).

$$T = T_o + T_{diff} \cdot \frac{(t + t_{crit}) - \sqrt{(t + t_{crit})^2 - 4 \cdot (t_{crit} - B) \cdot t}}{2 \cdot (t_{crit} - B)} \tag{1}$$

Parameter T_o [°C] represents the initial temperature, T_{diff} [°C] is the difference between the initial and the final temperature, t_{crit} [min] the time at which the final temperature is reached (i.e., the come-up time) and finally B [min] refers to a parameter which should lie between $0 < B < t_{crit}$ and influences the smoothness of the transition to the final temperature. The estimated heating rates (presented in Figure 2) were calculated as follows : $dT/dt = T_{diff}/t_{crit}$.

2.2 Neural Network Predictor

A fully-connected feedforward (FF) ANN architecture that consists of one hidden neuron and one output neuron is used aiming at the minimisation of ANN structures capable of approximating non-linear functions (see Figure 3). This is in accordance to Cheroutre-Vialette and Lebert (2000) who considered that one hidden layer of neuron(s) seemed to be sufficient to approximate continuous non-linear functions. Additionally, networks with more hidden layers or more neurones did not improve the accuracy of prediction (for this case study) any further. The logistic-sigmoid and the linear transfer function are employed for the hidden and the output neuron, respectively.

Two types of FF ANN are considered for these case studies:

– a standard FF architecture with an input vector that consists of time t and temperature rate dT/dt and an output vector (**it's not a vector, it's a single output value**) of the microbial load N_k and,

– a FF architecture, named FFD, with an additional input (in comparison with the previous type of network), namely the experimentally measured microbial load delayed with one time unit N_{k-1} and the same output vector N_k. Observe that when FFD is trained the N_{k-1} represents the experimentally measured microbial load delayed with one time unit. When the FFD is activated (with the test data), then the output error is propagated through the input-output cycle of the network.

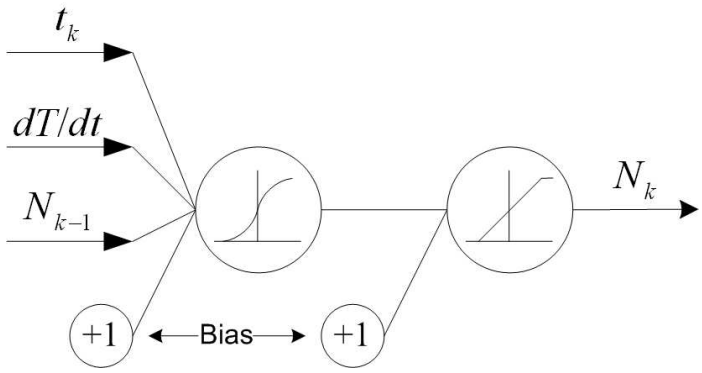

FIG. 3 – *The Arificial Neural Network architecture.*

All input values are linearly normalised into [0, 1] before they are entered into the network. Initial values that lie within [-1, 1] are picked randomly from a uniform distribution for the ANN's connection weights.

The early stopping methodology is used for overfitting-avoidance while Levenberg-Marquardt Hagan and Menhaj (1994) backpropagation is used to train the ANNs. This algorithm appears to be the fastest method for training moderate-sized feedforward ANNs and has given the highest performance training results among other training algorithms employed. The algorithm is terminated either when it converges to a good training mean square error value ($MSE = 1/n \sum_{i=1}^{n} (y_i - \hat{y}_i)^2$), n denotes the number of samples (input-output pairs), y_i is the observed value and $\hat{y}_i$ is the predicted value) or when the MSE on the test data set increases (i.e early stopping) or once a predefined large number of epochs (e.g. 5000 epochs) is completed. Since the performance of the network is highly dependent on the non-deterministic feature of the connection weight initialisation (i.e., random uniform distribution), all backpropagation runs are repeated for 50 times and the run with the lowest MSE on the test set is picked.

The microbial experimental sets are partitioned in training, validation and test sets before being processed according to the suggested ANN structures. The training sets consist of four experimental sets in which the *exslowest* and the *fast* heating regimes of the inactivation experiments (i.e., 0.15°C/min, and 1.64°C/min) are always included. This choice is accomplished in order to test the prediction capability of the developed modelling approaches only by interpolation. By choosing one experimental set for validating and one experimental set for testing the ANN, a total combination of twelve case studies is constructed. The advantages for the selection of the specific transfer functions and the use of early stopping are also investigated.

2.3 Isothermal based modelling

The dynamic sigmoidal-like model of Geeraerd, Herremans and Van Impe (2000) (having characteristics of a gray box model) was also used to describe the microbial inactivation of *Escherichia coli* K12 MG1655 (for the studied heating regimes) as based on isothermal inactivation data (see Valdramidis et al. (2006)). The model has two parameters (k_{max} and N_{res}) and two states (C_c and N):

$$\frac{dN}{dt} = -k_{max} \cdot \left(\frac{1}{1+C_c}\right) \cdot \left(1 - \frac{N_{res}}{N}\right) \cdot N \tag{2}$$

$$\frac{dC_c}{dt} = -k_{max} \cdot C_c \tag{3}$$

Herein, N represents the microbial cell density [cfu/mL], C_c is related to the physiological state of cells [units/cell], k_{max} denotes the specific inactivation rate [1/min] and N_{res} the residual population density [cfu/mL].

The description of k_{max} with respect to temperature is described with the Bigelow model (Bigelow, 1921) :

$$k_{max}(T) = \frac{\ln 10}{\mathrm{Asym}D(T)} = \frac{\ln 10}{\mathrm{Asym}D_{ref}} \cdot \exp\left(\frac{\ln 10}{z} \cdot (T - T_{ref})\right) \tag{4}$$

Herein, $\mathrm{Asym}D_{ref}$ [1/min] denotes the asymptotic decimal reduction time at a reference temperature T_{ref} [°C], and z [°C], the thermal resistance constant, i.e., the number of degrees change of temperature required to achieve a tenfold change in $\mathrm{Asym}D$-value.

The estimated inactivation parameters that have been used for performing microbial predictions at the different studied heating regimes are tabulated in Table 1.

$\mathrm{Asym}D_{56.3^oC}$ [min] $\pm$ SE	z [°C] $\pm$ SE	$\log(C_c(0))$ [-]$\pm$ SE
5.67$\pm$ 0.61	4.11 $\pm$ 0.16	0.82 $\pm$ 0.39

TAB. 1 – *Estimated model parameters of the integrated model (Equations 2, 3 and 4).*

For more details on the isothermal based modelling approach reference is made to Valdramidis et al. (2006).

2.4 Prediction capability

Various statistical indices are suggested in the literature in order to compare competing models. Among these indices Jeyamkondan et al. (2001) referred to graphical plots, mean relative percentage residual, mean absolute relative residual and root mean squared residual. Similarly, Ross (1996) suggested the use of the bias and accuracy factors. In our case study the mean relative percentage residual (MRPR) is considered:

$$MRPR = \frac{1}{n^*} \cdot \sum \frac{(y_i - \hat{y}_i)}{y_i} \cdot 100 \tag{5}$$

The $MRPR$ can be used to derive a measure analogous to the accuracy factor described by Ross (1996). An $MPPR$ value of zero indicates that there is no bias in the predictions. Positive values indicate an under-prediction and negative values an over-prediction (Jeyamkondan et al., 2001). For example if MRPR is -15%, it means that on average, the predicted microbial load was 15% higher than the observed microbial load.

2.5 Software

Programs for simulation, optimization, and fitting were written in $\mathrm{MatLab}^{\circledR}$ Version 6.1 (The MathWorks, Inc.) while Neural Networks Toolbox is also used. The routine e04ucf is used for the minimization of the nonlinear function presented in Section 2.3. This routine originates from the $\mathrm{NAG}^{\circledR}$ (Numerical Algorithms Group) Foundation Toolbox for MatLab 6 (NAG Ltd., Oxford, UK).

3 Results and discussion

The choice of the transfer functions in use as well as the advantage of using an early stopping methodology was studied by evaluating the prediction capability of the ANN structures for all the cases that the test set was the *slowest*.

Case 1	Transfer functions: Sigmoid - Linear			
	Experimental sets		MRPR [%]	MRPR [%]
test	train	validation	FFD	FF
slowest	Exslowest, Slower, Slow, Fast	Int	-4.14	-7.07
	Exslowest, Slower, Int, Fast	Slow	-0.83	-1.13
	Exslowest, Slow, Int, Fast	Slower	0.11	-8.82

Case 2	Transfer functions: Sigmoid - Sigmoid			
	Experimental sets		MRPR [%]	MRPR [%]
test	train	Validation	FFD	FF
slowest	Exslowest, Slower Slow, Fast	Int	3.72	-7.74
	Exslowest, Slower, Int, Fast	Slow	2.66	4.15
	Exslowest, Slow, Int, Fast	Slower	-2.94	-5.79

Case 3	Transfer functions: Sigmoid - Linear			
	Experimental sets		MRPR [%]	MRPR [%]
test	train	No Validation	FFD	FF
slowest	Exslowest, Slower Slow, Fast		6.33	11.43
	Exslowest, Slower, Int, Fast		6.27	7.60
	Exslowest, Slow, Int, Fast		-1.20	8.32
	All		-3.07	12.81

TAB. 2 – *Mean relative percentage residual ($MRPR$) comparison for a set of case studies of different transfer functions and for the use of validation test.*

The results show that early stopping contributes on a better predicting power of the developed ANN structure than in cases that this technique is not used. Overfitting of the ANN is avoided since learning is stopped when the MSE error on the validation set is increased between two training epochs. Additionally, the choice of a sigmoid function followed by a linear transfer function is a justified choice for the experimental studies at hand (see values of $MRPR$). Previous studies in the field are in line with the latest observation (see e.g., Lou and Nakai (2000); Cheroutre-Vialette and Lebert (2000)).

Results, as calculated based on the prediction capability of the models in use show that FFD structure predicts the microbial inactivation of *E. coli* better than the FF approach (Table 3). Moreover, the slower the heating rate of the test data set, the better the FFD prediction in most cases. The comparative study also illustrates that microbial predictions derived from isothermal experimental data resulted in lower prediction capability, i.e., higher $MRPR$, than the ANNs and in continuous underestimation ($MRPR > 0$) of the microbial kinetics (see Table 3 and Figure 4).

Particularly, the isothermal modelling approach gave poor prediction accuracy for all the selected case studies. As it was discussed in Valdramidis et al. (2006) this is due to the induced microbial heat resistance (changes of the physiological state of the cells) which is imposed by the dynamic temperature processing conditions. Cheroutre-Vialette and Lebert (2002) discussed about similar problems associated with variable conditions when differential equations are employed. It is also apparent from these studies that transposition of results obtained from constant conditions to variable conditions may require adjustment of the initial mathematical structures in use.

When the two studied ANN structures are compared with the isothermal based modelling study, an advantage of the FFD methodology is observed for all cases but one. This indicates that the training approach of these two structures seem to be more informative than previously studied approaches. The FFD approach resulted in even more accurate predictions than the FF. Particularly, in the case of the slowest test set, when validation on data originating from conditions inducing pronounced changes in the microbial physiology (i.e., slower set) gave more information on the structure modelling approach (see MSPR for the validation set of slower). This observation is more evident when the test set is the slowest and slower, i.e., sets at which induced resistance is more pronounced (Valdramidis et al., 2006).

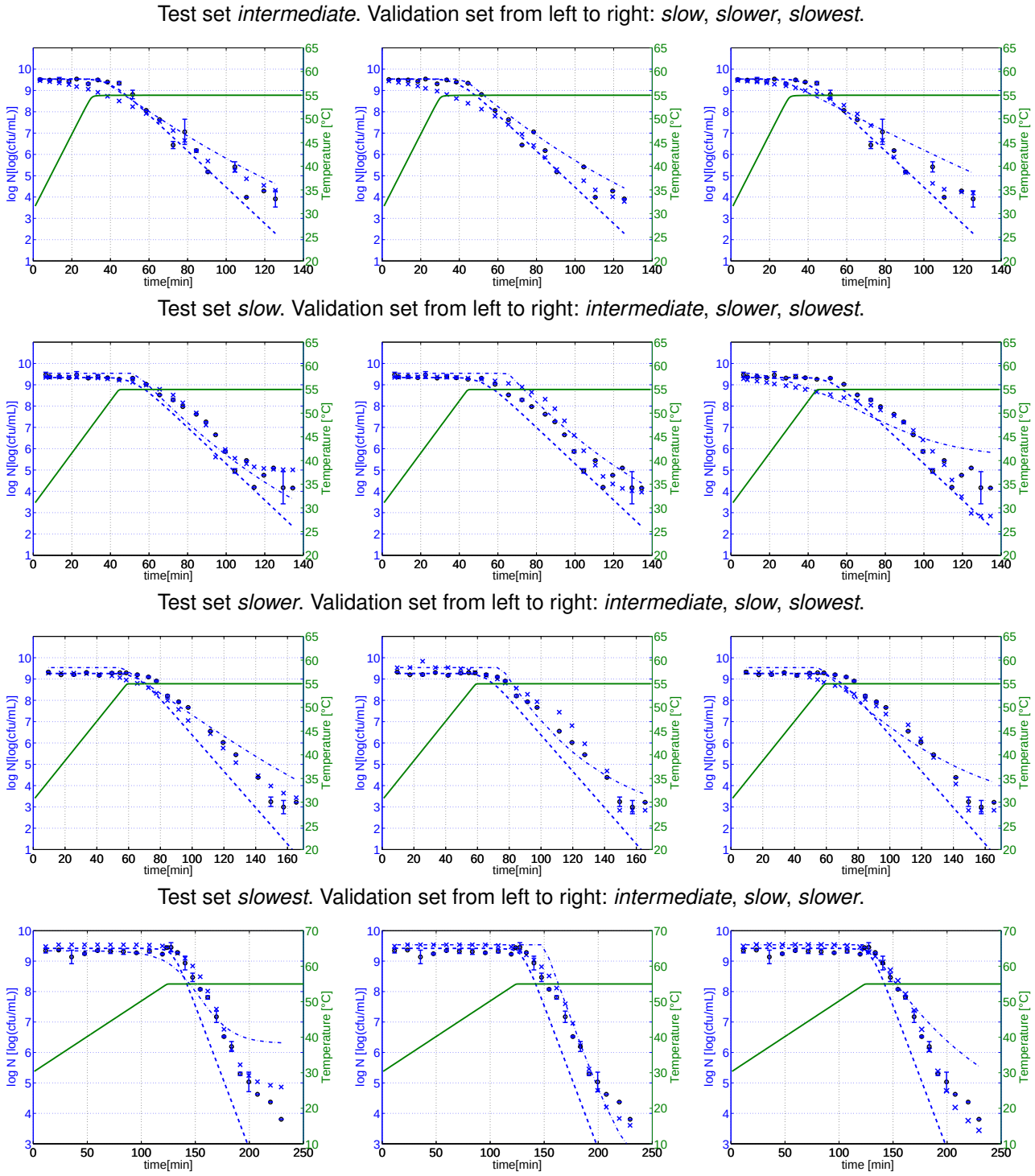

FIG. 4 – *Microbial inactivation experiments ('•') of* E. coli *K12 with their standard deviation and corresponding temperature profile (continuous line) at all the studied heating rates. Dotted lines: predictions with FF approach ('-.'), predictions with FFD approach ('x'), and predictions with approach based on isothermal data ('- -').*

In general, FF architectures with a delay in one of the inputs appear to be more efficient predictors than the FF or the microbial model based on isothermal data for this case study. A better prediction capability of the FFD network than the FF and the isothermal based models, in all the twelve cases (but one), indicates that an input incorporating past events, i.e., N_{k-1}, is sufficient to encompass the microbial stress adaptation of the examined microbe due to the slowly increasing temperatures. This input delay can be considered as a dynamic *a priori microbiological knowledge* suitable for extracting the information considered in the microbial experimental data. Further experimental investigation of these adaptations may require studies focusing on the mechanisms influen-

cing the microbial physiology.

	Experimental sets		FFD	FF	Previous study
Test	Train	Validation	MRPR [%]	MRPR [%]	MRPR [%]
Slowest	**Exslowest**,Slower,Slow,**Fast**	Int	-4.14	-7.07	14.69
	Exslowest, Slower,Int,**Fast**	Slow	-0.85	-1.13	14.69
	Exslowest,Slow,Int,**Fast**	Slower	0.11	-8.82	14.69
Slower	**Exslowest**,Slowest,Slow,**Fast**	Int	-0.12	-8.70	14.60
	Exslowest,Slowest,Int,**Fast**	Slow	-2.22	-3.91	14.60
	Exslowest,Slow,Int,**Fast**	Slowest	3.19	-4.47	14.60
Slow	**Exslowest**,Slowest,Slower,**Fast**	Int	-2.60	2.14	9.64
	Exslowest,Slowest,Int,**Fast**	Slower	-2.18	-6.48	9.64
	Exslowest,Slower,Int,**Fast**	Slowest	5.99	-6.09	9.64
Int	**Exslowest**,Slowest,Slower,**Fast**	Slow	-0.35	-5.31	6.45
	Exslowest,Slowest,Slow,**Fast**	Slower	3.56	-5.44	6.45
	Exslowest,Slower,Slow,**Fast**	Slowest	0.57	-7.57	6.45

TAB. 3 – *Mean relative percentage residual ($MRPR$) comparison for the different modelling approaches (FFD, FF, isothermal study). The bias weight for the hidden layer is included. Note that, for the FFD and FFNN methods the first data point of the test set is not included in the calculation of the $MRPR$.*

The outcome of this study shows that it is important to be taken into account the variations of environmental factors, which can occur during a food processing and induce a stress situation for the microorganims. Recurrent neural networks (RNN) as those developed by Cheroutre-Vialette and Lebert (2002) for describing the growth of *Listeria monocytogenes* can be similarly used for the inactivation kinetics. Nevertheless, the proposed FFD structure demonstrates that a single output delay of one-time unit, where the output error is propagated through the input-output cycle of the network, is adequate for successfully describing the inactivation of *E. coli*. In other words, it appears that the embedded memory of RNNs is not essential for performing the studied predictions. Finally, this study had shown that microbial heat resistance should be further identified and quantified by incorporating available information into differential microbial modelling approaches. These adjustments by the use of additional model building blocks will potentially permit reliable quantification (similar to that achieved by the tested ANN structures) from the widely used in the field of Predictive Microbiology differential equations.

Acknowledgments

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