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A RECURRENT NEURAL NETWOK FOR MODELLING THE GROWTH OF *LISTERIA MONOCYTOGENES* IN DYNAMIC CONDITIONS

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1. INTRODUCTION

Predictive microbiology combined the knowledge of bacterial growth responses over a range of conditions with the power of mathematical modelling to enable predictions of growth. Most mathematical models were elaborated from data coming from growth carried out in constant conditions of environmental factors (Wijtzes et al., 1993; Fernandez et al., 1997). But, food-manufacturing processes can decrease the pH of food, produce organic acids or reduce water activity. These processes are extensively used as mechanisms to prevent microbial growth and to ensure food safety. The objective of this work was to produce a dynamic model that predicts the growth of *Listeria monocytogenes* as a function of fluctuating conditions of acid pH (with acid acetic), alkaline pH (with NaOH) and concentration of NaCl by using a recurrent multilayer neural network (Jones, 1992).

2. MATERIALS AND METHODS

A combination of a factorial design and two central composite designs was used to assess quantitatively the effects and interactions of water activity (1-0.95) and pH (5.6-9.5) variations on the growth of *L. monocytogenes* 14 in a meat broth at two temperatures (10 °C and 20 °C). For each combination, the cells were exposed to the addition of NaCl and acetic acid or NaCl and NaOH at inoculation (= limiting condition) or at the beginning of the exponential phase (= shock condition). At least, for each temperature, fifty experiments were performed according to twenty-five growths in shock condition and twenty-five growths in limiting condition (Figure 1).

The optimum neurone number of the hidden layer was determined by developing several recurrent neural networks (RNNs) of different size of the hidden layer (3 to 10 neurones were tested). A seven neurones hidden layer was determined as the best structure. The sigmoid function $f(x) = 1/(1+\exp(-x))$ was chosen as activation function. The weights of the neural connections, initially chosen randomly, were adjusted by a non-linear optimisation technique: the Quasi-Newtonian formula of Shanno (1970). A learning base (Figure 1) was used to adjust the weights, a testing base to provide over-learning during weight optimisation, and a validation base for validation of results. At least, 60% of experiments were included in the learning and testing bases; the last 40% were in the validation base. In order to ensure the validity of the study, additional growth results with various modes of shock exposure were included into the validation base (Table 1).

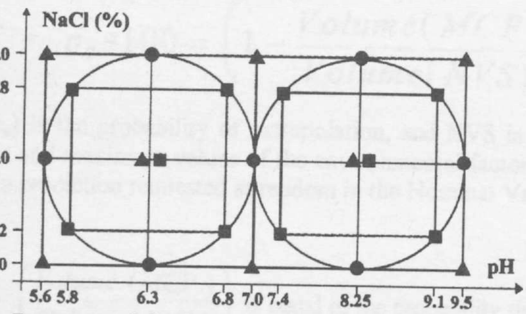

Figure 1: Experimental design showing the combination of osmotic and pH shocks (10°C and 20°C).
▲ *Learning base* – ● *Testing base* – ■ *Validation base*

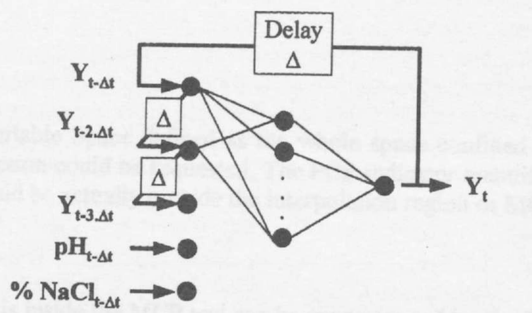

Figure 2 : Schematic structure of the recurrent neural network

Table 1 - Additional experiments at 20°C.

Addition mode	Time to obtain the addition of 8% NaCl
4 steps of 2%	4 hours (1 hour between steps)
continuous	1.8 hour
continuous	7.7 hours

In the present study, a recurrent multilayer structure was used (Figure 2). It contained one input layer, one hidden layer and one output layer:

1. in the input layer, six input parameters: $Y_{t-\Delta t}$, $Y_{t-2.\Delta t}$, $Y_{t-3.\Delta t}$, $T_{t-\Delta t}$, $pH_{t-\Delta t}$ and $NaCl_{t-\Delta t}$ (%)
2. in the output layer, one output parameter : Y_t which represented the predicted response

3. RESULTS

The analysis of the growth predictions in the limiting conditions showed that the RNN represented satisfactorily the experimental data whatever the conditions tested (alkaline-osmotic or acid-osmotic) and the temperature (10°C or 20°C). The RNN was able to predict growth when one of the parameters vary or two parameters vary simultaneously. The different characteristics of the *L. monocytogenes* response, *i.e.* induction of a lag time and growth recovery different to those observed in the new environment, were taken into account by the RNN whatever the combination, alkaline-osmotic or acid-osmotic (Figure 3A). Furthermore, RNN was able to predict the effect of the type of shocks and their combinations. As indicated in the Figure 3B, the growth of *L. monocytogenes* 14 was particularly affected by the combined acid-osmotic shocks (pH 5.6 and 8% NaCl) in exponential phase since no increase of optical density was observed during the experimental period. There was a good agreement between the experimental growth and the prediction. Additional experiments were used to investigate the capacity of the RNN to represent the response of *L. monocytogenes* cells shocked in exponential phase with 8% NaCl added in various modes. The effects of adding 8% NaCl by steps of 2% or continuously during 7.7 hours (*i.e.* four generation times at 20 °C) until the final concentration reached 8% are shown in Figure 3C and Figure 3D respectively. The extrapolation to new experimental conditions (mode of shock exposure) was made with a good agreement by the RNN. It confirmed that the RNN has the capacity to predict growths carried out in different experimental conditions from those used for its elaboration.

4. CONCLUSION

These results obtained in variable conditions showed that neural networks could effectively be used to study the complex effects of fluctuating environmental conditions on microorganism behaviour. Such dynamic model could follow the microbial impact of different steps associated with production; distribution and retailing of a food and so could be an important support to HACCP and food safety systems.

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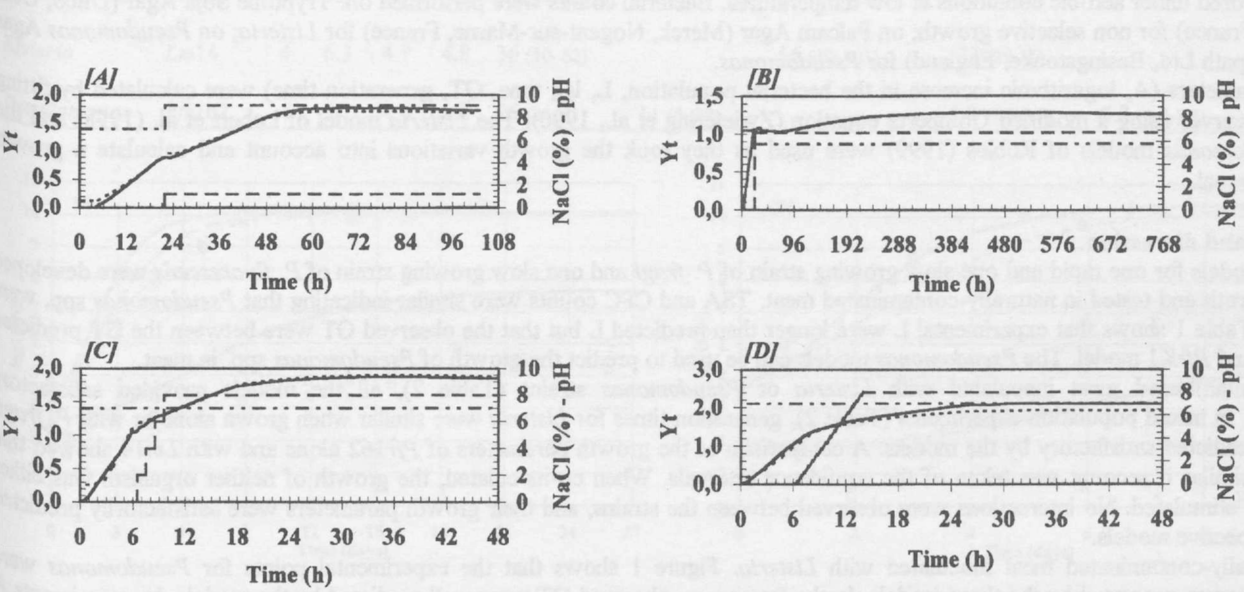

Figure 3: Comparison between experimental (—) and calculated (···) growth curves:
[A] = shock pH 9.1 and 1.2 % NaCl at 10°C -°- [B] = shock pH 5.8 and 6.8 % NaCl at 10°C
[C] = osmotic shock (8 % NaCl) applied by steps of 2 % at 20°C -°- [D] = osmotic shock (8 % NaCl) applied in 7.7 hours at 20°C
% NaCl (— — —) - pH (— · · —)