International Journal of Food Microbiology 240 (2017) 85–96

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Introducing a novel interaction model structure for the combined effect of temperature and pH on the microbial growth rate

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A R T I C L E   I N F O

Article history:
Received 26 February 2016
Received in revised form 27 May 2016
Accepted 11 June 2016
Available online 15 June 2016

Keywords:
Microbial growth rate
Cardinal parameter model
Environmental conditions
Predictive microbiology

A B S T R A C T

Efficient modelling of the microbial growth rate can be performed by combining the effects of individual conditions in a multiplicative way, known as the gamma concept. However, several studies have illustrated that interactions between different effects should be taken into account at stressing environmental conditions to achieve a more accurate description of the growth rate.

In this research, a novel approach for modeling the interactions between the effects of environmental conditions on the microbial growth rate is introduced. As a case study, the effect of temperature and pH on the growth rate of *Escherichia coli* K12 is modeled, based on a set of computer controlled bioreactor experiments performed under static environmental conditions. The models compared in this case study are the gamma model, the model of Augustin and Carlier (2000), the model of Le Marc et al. (2002) and the novel multiplicative interaction model, developed in this paper. This novel model enables the separate identification of interactions between the effects of two (or more) environmental conditions. The comparison of these models focuses on the accuracy, interpretability and compatibility with efficient modeling approaches. Moreover, for the separate effects of temperature and pH, new cardinal parameter model structures are proposed.

The novel interaction model contributes to a generic modeling approach, resulting in predictive models that are (*i*) accurate, (*ii*) easily identifiable with a limited work load, (*iii*) modular, and (*iv*) biologically interpretable.

1. Introduction

Combining different preservation techniques to ensure microbial food safety and stability is a strategy that facilitates the production of foods with high sensory and nutritional quality (Leistner, 2000). However, building predictive models that accurately predict the growth rate at such stressing conditions has been found to be difficult. Membré and Lambert (2008) demonstrated that large deviations exist between predictions of the growth of *Listeria monocytogenes* obtained with different simulation packages when combining a stressing temperature, pH and water activity.

One of the most widely adopted methods to model the combined effect of environmental conditions (such as temperature T and pH) on the microbial specific growth rate relies on the gamma hypothesis (McMeekin et al., 1987; Zwietering et al., 1993). This hypothesis assumes that each of the environmental conditions has an independent effect on the reduction of the growth rate. Models built according to this hypothesis are composed of a multiplication of factors, each of which represents the influence of one of the environmental conditions on the growth rate. If this hypothesis is valid, models for the combined effect of environmental conditions on the growth rate can be built by only studying the separate effects of the environmental conditions.

This makes the gamma hypothesis very attractive because the experimental load required to study the individual effects is much less than the experimental load required to study the combined effect. Many studies also reported a good prediction quality when using the gamma concept (te Giffel and Zwietering, 1999; Pinon et al., 2004; Lambert and Biblas, 2007; Biblas and Lambert, 2008; Leroi et al., 2012; Wijtzes et al., 2001). Additionally, the gamma models are compatible with the cardinal parameter models (Rosso et al., 1995; Ross and McMeekin, 2003), which contain biologically interpretable parameters, making them easy to use.

However, studies focusing on (combinations of) stressing environmental conditions revealed deviations from the gamma hypothesis. Publications in the domain of predictive microbiology often refer to these deviations as interactions. It should be noted that the gamma

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http://dx.doi.org/10.1016/j.ijfoodmicro.2016.06.011
0168-1605/© 2016 Published by Elsevier B.V.

models, by construction, already contain interactions in the conventional sense of *additive interactions*. Indeed, when multiplying out the factors of a gamma model, terms (like T · pH) will be found reflecting the combined effects of environmental conditions on the growth rate. The definition used for interactions in this paper is therefore one of so-called *multiplicative interactions*, i.e., those effects that cannot be found by only studying the separate effects of environmental conditions.

To account for such deviations from the gamma hypothesis, Augustin and Carlier (2000) integrated the calculation of the growth boundaries into a gamma model for *L. monocytogenes* and observed that this improved the prediction quality. Later, Le Marc et al. (2002) developed a factor to describe interactions between the effects of temperature, pH and organic acids on the growth rate of *L. monocytogenes*. This interaction factor was also inspired by the growth/no growth boundaries. Recently, Baka et al. (2013) demonstrated that the gamma concept is inadequate when describing the effect of temperature and pH on the growth rate of *Escherichia coli* K12. This conclusion was drawn by illustrating that the parameters of the secondary model for temperature were dependent on pH. However, no adaptation of the gamma concept was proposed in their research yet.

The initial objective of the current research is to demonstrate the need for secondary models that include multiplicative interactions for the effect of temperature and pH on the maximum specific growth rate. For this purpose, a dedicated experimental design is applied to bioreactor experiments with *E. coli* K12. The second objective, is to compare different model structures in their ability to describe the combined effect of temperature and pH on the growth rate. The considered models are: the gamma model without interactions, the model of Augustin and Carlier (2000), the model of Le Marc et al. (2002) and a novel multiplicative interaction model. In addition, to describe the individual effects of temperature and pH in these models, a set of new cardinal parameter models is developed.

2. Model development

This section discusses the models that were obtained from literature and the new models that were developed. Growth curves were described using the primary model of Baranyi and Roberts (1994). The individual effects of environmental conditions on the maximum specific growth rate were modeled using cardinal parameter models. The advantage of this type of models compared to the square-root-type models (e.g., Ratkowsky et al., 1983) is that these only use biologically interpretable parameters. The parameters of these models represent the growth limits and the optimal conditions. It should be stressed that these parameters are in fact the theoretical growth limits and optimal conditions (McMeekin et al., 2013), which are only equal to the real values if the model describes the exact relationship between the environmental condition and the growth rate.

2.1. Primary model

To describe the evolution of the cell density N [CFU/mL] with time t [h], the widely used primary model of Baranyi and Roberts (1994) was implemented:

$$\frac{dN(t)}{dt} = \frac{Q(t)}{1+Q(t)} \cdot \mu_{max}(T,pH) \cdot \left(1 - \frac{N(t)}{N_{max}(T,pH)}\right) \cdot N(t) \frac{dQ(t)}{dt} = \mu_{max}(T,pH) \cdot Q(t) \tag{1}$$

with $\mu_{max}(T,pH)$ [h^{-1}] the maximum specific growth rate and $N_{max}(T,pH)$ [CFU/mL] the maximum cell density for a specific temperature (T [°C]) and pH [−]. Q(t) [−] is a measure for the physiological state of the cells and serves to describe the lag phase of the growth curve. For computational purposes, N(t) and Q(t) are replaced with their natural logarithms n(t) [ln(CFU/mL)] and q(t) [−], resulting in (Baranyi and Roberts, 1994):

$$\begin{aligned} \frac{dn(t)}{dt} &= \frac{1}{1+\exp(-q(t))} \cdot \mu_{max}(T,pH) \cdot [1 - \exp(n(t) - n_{max}(T,pH))] \\ \frac{dq(t)}{dt} &= \mu_{max}(T,pH). \end{aligned} \tag{2}$$

The initial values of n(t) and q(t) are respectively n_0 and q_0.

2.2. Secondary models for independent effects

2.2.1. Temperature effect

2.2.1.1. CTMI. The individual effect of temperature on the maximum specific growth rate is often described with the Cardinal Temperature Model with Inflection (CTMI, Fig. 1; Rosso et al., 1993). The advantage of this model is that it uses four interpretable parameters. The minimum temperature T_{min} [°C] and the maximum temperature T_{max} [°C] define the range of environmental conditions where growth is possible. The optimum temperature T_{opt} [°C] is the temperature at which the optimum growth rate μ_{opt} [h^{-1}] is reached. These parameters are combined in the following model structure:

$$\begin{aligned} \mu_{max}(T) &= \mu_{opt} \cdot \gamma_T(T) \\ \gamma_T(T) &= \frac{(T - T_{min})^2 \cdot (T - T_{max})}{(T_{opt} - T_{min}) \cdot [(T_{opt} - T_{min}) \cdot (T - T_{opt}) - (T_{opt} - T_{max}) \cdot (T_{opt} + T_{min} - 2T)]} \end{aligned} \tag{3}$$

with $\gamma_T(T)$ the reduction of the growth rate with respect to μ_{opt}, due to a non-optimal temperature.