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Estimation of the temperature dependent growth parameters of *Lactobacillus viridescens* in culture medium with two-step modelling and optimal experimental design approaches

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Abstract

In predictive microbiology, the model parameters has been estimated using the traditional two-step modeling approach (TS), in which primary models are fitted to the microbial growth data and secondary models represent the dependence of model parameters with environmental variables. The optimal experimental design approach (OED) has been used as an alternative to TS, mainly because the improvement of model identifiability and reduction of the experimental workload and costs. The fitting of mathematical model to experimental data in TS is sequential, whereas in OED is simultaneous. *Lactobacillus viridescens* is a lactic acid bacteria that is of great interest to the meat products preservation. The objective of this study was to estimate the growth parameters of *L. viridescens* in culture medium with TS and OED. For TS, the experimental data were obtained in six temperatures; for OED, the data were obtained in four optimal non-isothermal experiments, two experiments with increasing temperatures (ITOED) and two with decreasing temperatures (DTOED). The Baranyi and Roberts, and the Square Root models were used to describe the microbial growth, in which the b and T_{min} parameters (± 95% confidence intervals) were estimated from the experimental data. The parameters obtained for TS were b = 0.0290 (±0.0020) h$^{-0.5}$ °C^{-1} and T_{min} = -1.33 (±1.26) °C, with R^2 = 0.991; for ITOED were b = 0.0314 (±0.0019) h$^{-0.5}$ °C^{-1} and T_{min} = 0.12 (±0.71) °C, with R^2 = 0.995; for DTOED were b = 0.0295 (±0.0019) h$^{-0.5}$ °C^{-1} and T_{min} = -1.57 (±1.05) °C, with R^2 = 0.999. The parameters obtained in the OED approach presented smaller confidence intervals, higher R^2 and less experimental time than the parameters obtained in the traditional TS approach. In this way, it is possible to answer positively that OED approach is feasible and could be widely applied in predictive microbiology.

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1. Introduction

The shelf life of meat and meat products has been linked to the growth of lactic acid bacteria, in which *Lactobacillus* has been indicated as one of the main genus[1,2] and the *Lactobacillus viridescens* has been reported as one of the main specific spoilage organisms[3]. The bacterial growth has been assessed in culture medium, which are more homogeneous and allow getting results easier to interpret than in meat products; furthermore, the growth observed in culture medium provides a conservative estimation of the microbial growth in foods.

The temperature of chilled foods usually varies greatly during transport, retail and at home, and it has great influence on the kinetics of microbial growth in food[4]. The temperature increase causes the decrease of the adaptation time and the increase of the maximum specific growth rate of the spoilage bacteria, which can lead to negative effects, such as the reduction of the food shelf life and the impact on food quality[5].

The microbial growth can be described by mathematical models, in which the parameters are estimated from experimental growth data, whose values depend on the approach used for their estimates. The Two-Step Modelling (TS) approach consists of sequential fitting of the primary model to the experimental growth data under isothermal conditions and the fitting of the secondary model to the primary model parameters[6]. This approach has been the most reported in the literature[7,8,9], despites its high costs, workload and long experimental time. On the other hand, in the Optimal Experimental Design (OED) approach, the simultaneous fitting of primary and secondary models to the experimental data obtained under optimal non-isothermal conditions is done[10]. The optimal non-isothermal experiments are designed taking in account certain constraints of the system under study and the optimization of a certain criteria of the Fisher Information Matrix, that consider the sensitivity of the model responses to the variations in the values of the model parameters[11]. The experimental time and costs can be reduced in the OED, and smaller confidence intervals of the model parameters can be obtained[12].

The objective of this study was to estimate the growth parameters of *L. viridescens* in culture medium by TS and OED, and to compare the experimental time needed and confidence intervals of the model parameters obtained by both approaches.

2. Material and methods

2.1. Microorganism and experimental procedures

The *Lactobacillus viridescens* (CCT 5843 ATCC 12706, Lot 22.07) used in this study was purchased in lyophilized form from the *André Tosello* Foundation of Tropical Cultures (Campinas, Brazil). The strains were rehydrated, grown in MRS – *Lactobacillus* medium (Acumedia Manufactures, Michigan, USA), and stored in Eppendorf tubes with MRS medium containing 20% glycerol at -24 °C until its use. The reactivation of the culture for preparing the inocula was carried out in MRS medium at 30 °C for 18 hours. The experiments were performed in 250 mL Erlenmeyer flasks with 160 mL of MRS medium and approximately 10^3 CFU/mL. The flasks were inserted in incubators (Dist, Florianópolis, Brazil) at different temperature conditions.

The microbial growth under isothermal conditions were evaluated in twelve experiments (4, 8, 12, 16, 20 and 30 °C, in duplicate, with repetition). These experimental data were used to estimate the parameters by the TS approach. The growth under non-isothermal conditions were performed in four experiments, including two experiments with increasing temperatures between plateaus, ITOED (at 4-8-12-16 °C and 12-16-20-25 °C) and two experiments with decreasing temperatures between plateaus, DTOED (at 16-12-8-4 °C and 25-20-16-12-8-4 °C). The times to shift between the temperatures plateaus were optimally designed minimizing the E-modified criteria of the Fisher Information Matrix[11]. All experiments were conducted until the stationary growth phase, and mini data loggers (Testo 174, Lenzkirch, Germany) recorded the temperature every 5 minutes.

2.2. Mathematical models

The Baranyi and Roberts primary model[13], shown in Eq. (1) and (2), was used to describe the microbial growth, and the Square Root secondary model[14], shown in Eq. (3), was used to describe the dependence of μ_{max} parameter with the temperature. In Equations (1), (2) and (3), *y* is the natural logarithm of the cell concentration *N* ($y = \ln(N)$) at time