Modelling of lactic fermentation of carrot slices in salted brines

Modelo de fermentación de rodajas de zanahorias en salmuera

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Increases in suspended biomass and variation in the concentrations of reducing sugars, salt, and lactic acid in brine containing sliced carrots were followed for a period of several days. A tentative unstructured, unsegregated model for the metabolism of suspended *Lactobacillus plantarum* coupled with Fick's second law of diffusion for the transport of solutes within the carrot material was postulated. This general model was fitted by non-linear multiresponse regression analysis to an extensive set of experimental data encompassing several processing temperatures and initial brine concentrations. Maximum specific growth rates decreased with initial sodium chloride (NaCl) concentration and were maximal at ca 30°C leading to a peak concentration of reducing sugars in the brine. The lag phase of microbial growth in the brine is apparently disguised by the lag phase of sugar leaching from the carrots into the brine. Lactic acid was confirmed to be a growth-associated product. Intrinsic diffusivities of the various compounds considered ranged up to ca 10^{-11} m^2/s, and varied with temperature according to the Arrhenius law; the maximum activation energy was at ca 8% (w/v) NaCl. The work developed is useful for the simulation and eventual optimization of pickled carrot manufacture.

Keywords: lactic fermentation, modelling, carrot, brine, pickles

Se ha estudiado el incremento de la biomasa en suspensión y la variación de las concentraciones de azúcares reductores, cloruro sódico y ácido láctico de la salmuera de rodajas de zanahoria a lo largo de varios días. Se ha propuesto un modelo no estructurado y no segregado para el metabolismo del *Lactobacillus plantarum* en suspensión que responde a la segunda ley de difusión de Fick para el transporte de solutos en las rodajas de zanahoria. Los resultados obtenidos con diferentes temperaturas y concentraciones de sal se ajustaron a un modelo general mediante un análisis de regresión no lineal. La velocidad específica máxima de crecimiento disminuye según la concentración inicial de sal y es máxima a la temperatura de alrededor de 30°C; bajo estas condiciones óptimas se obtuvo una concentración pico para los azúcares reductores en la salmuera. El crecimiento microbiano en la salmuera aparentemente viene condicionado por la difusión de los azúcares de rodajas de zanahoria a la salmuera. Se confirmó que el ácido láctico es un producto asociado al crecimiento. Las difusividades intrínsecas de varios de los compuestos considerados se encuentran en valores de aproximadamente 10^{-11} m^2/s y varían con la temperatura de acuerdo con la ley de Arrhenius, con un máximo de la energía de activación localizado aproximadamente para un 8% de sal. Los resultados obtenidos pueden aplicarse a la simulación y eventual optimización de la fabricación de encurtidos de zanahoria.

Palabras clave: fermentación láctica, modelización, zanahoria, salmuera, encurtidos

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INTRODUCTION

Traditionally, brined vegetables are obtained via spontaneous fermentation brought about by their epiphytic microflora. The fermentable sugars in such vegetables, as well as other nutrients, leach from the vegetables into the brines whereas other soluble components, such as products of fermentation, diffuse from the brines into the vegetables (Fleming, 1982; Passos *et al.*, 1994; Nabais *et al.*, 1995; Nabais and Malcata, 1995a, 1995b). Long-term preservation is eventually achieved via the low pH obtained as a result of an increasing concentration of, for example, lactic acid generated *in situ*. Mathematical models applied to the simulation of this form of food processing should in principle provide a rational framework for understanding the microbial ecology of food; therefore, using such models one should be able to predict how a given set of environmental processing conditions will affect the final microbiological profile, and physicochemical characteristics resulting therefrom, both of which are directly related to the final quality of the food.

Although simulation of the growth of, and metabolite production by, microorganisms in homogeneous suspensions is a well established mathematical procedure, modelling of the dynamic behaviour of such systems after the introduction of a second phase that affects the inventory in solutes (by either absorption or leaching) is a much more involved task, which has not generated as extensive a scientific interest. Models of the microbiology of fermentation processes, usually considered as the nucleus of a discipline termed predictive microbiology, are based upon the premises that: (*i*) the responses of populations of microorganisms to environmental factors are reproducible; and (*ii*) by considering environments in terms of identifiable dominating constraints, it is possible to predict the responses of those microorganisms based on past observations (Ross and McMeekin, 1994). In general, two basic approaches can be followed in the design of a microbiological predictive model: the probability approach and the kinetic approach.

Probability modelling consists of the construction of models to predict the likelihood of some event within a given time period. Examples encompass the use of a hyperbolic distribution of probability (Lindroth and Genigeorgis, 1986; Gibson and Roberts, 1989), an exponential distribution of probability (So *et al.*, 1987), or detailed analysis of kinetic experiments based on the assumption of a more complex distribution of observations lying somewhere between the normal and Poisson distributions (Schaffner, 1994). Irrespective of the actual probability distribution selected, the case of a biphasic fermentation has not been tackled to date.

Kinetic models should be able to simulate rates of growth and metabolism as functions of measurable physicochemical parameters, and have met with a considerable degree of industrial success. Examples of such models (which are often based on purely empirical assumptions) include (but are not limited to) attempts to describe: (*i*) the effect of temperature on the growth rates of *Lactobacillus plantarum* (Zwietering *et al.*, 1991), lactobacilli (van Impe *et al.*, 1992), psychrophilic bacteria (Ingraham, 1958), and bacteria sp. (Ratkowsky *et al.*, 1982a,b); (*ii*) the effect of product concentration on the growth rates of *Lactobacillus bulgaricus* (Torrestiana *et al.*, 1994), lactic acid bacteria (Ambrane and Prigent, 1994), and bacteria sp. (Ratkowsky and Ross, 1995); (*iii*) the combined effects of temperature and water activity on the growth rates of bacteria sp. (Broughall *et al.*, 1983; Davey, 1989) and on the growth lag phase of bacteria sp. (Davey, 1991); (*iv*) the combined effects of temperature and pH on the growth rates of bacteria sp. (Rosso *et al.*, 1995); (*v*) the combined effects of temperature, water activity, and pH on the growth rates of bacteria sp. (Broughall and Brown, 1984); (*vi*) the combined effects of sodium chloride (NaCl), pH, and acetic and lactic acids on the growth rate of *Lactobacillus plantarum* (Passos *et al.*, 1993a, 1993b) and on the rate of lactic acid production (Passos *et al.*, 1994) in cucumber juice; (*vii*) the combined effects of pH, water activity and concentration of NaCl on the growth rates of bacteria sp. (Skinner *et al.*, 1994); and (*viii*) the combined effects of pH, water activity, and concentration of sodium nitrite on the growth rates of lactic acid bacteria (Metaxopoulos *et al.*, 1981). Once again, the efforts reported in the literature pertain to suspensions of biomass containing the solutes in soluble or, at most, colloidal form, but not in solid form.

The major purpose of this research effort was to prove that a given unstructured, unsegregated (kinetic) model was adequate for the mathematical description of the metabolism of suspended *Lactobacilus plantarum* (coupled with Fick's second law of diffusion for the transport of solutes, e.g. reducing sugars, lactic acid, and NaCl within the solid carrot material, into or out of, the aqueous suspension) via fitting of such a model by non-linear, multiresponse regression analysis to data encompassing the brine concentrations of the various solutes and the suspended biomass using several temperatures and initial concentrations of NaCl.

MATERIAL AND METHODS

Material

Fresh carrots were randomly bought at local markets (dominating cultivar: Nantes). Sodium chloride, sodium hydroxide, potassium dichromate, copper sulfate, potassium permanganate, silver nitrate, double sodium and potassium tartrate, and anhydrous D(+)-glucose were purchased from Merck (Germany). Dinitrosalicilic acid was obtained from BDH (UK). The inocula of *Lactobacillus plantarum* were a gift from Textel/Lactolabo Marschall (Spain). MRS agar for viability maintenance of *L. plantarum* was purchased from Oxoid (UK). MRS broth for pregrowth procedures and YNB medium with added yeast extract were obtained from Difco (USA).

Equipment

Centrifugation was performed with an Universal Hettich centrifuge (Germany). Titrations and pH measurements were performed with a Titroprocessor Model 682 connected to a Dosimat Model 665 equipped with a combined pH glass electrode No. 60202100, all from Metrohm (Switzerland). Isothermal conditions were achieved using thermostatted water baths Kottermann 3047 from Labortechnik (Germany). Spectrophotometric measurements were performed with a Model 350 spectrophotometer from Pye Unicam (UK). Sterilization was accomplished using a laboratory retort Austester MOD 437 G from Selecta (Spain). Approximate weight determinations were made with a PC 2000 scale from Mettler (Switzerland) and accurate weight determinations were made with an S 2000 analytical balance from Bosch (Germany). A dual sensor thermometer G-90200–00 from Cole Parmer (USA) was used to monitor the temperature of the solutions during calibration of the Titroprocessor. Isothermal chambers B-80 from Memmert (Germany) and Fitoclima 750 E from Aralab (Portugal) were used for the procedures of drying and incubation, respectively.

Methods

Preparation of brines

Brines were prepared at 25 °C using appropriate amounts of NaCl (previously dried overnight at 60 °C) and diluting them to the desired volume with deionized water. The solutions thus prepared were distributed into 1-L glass flasks and sterilized for 15 min.

Preparation of DNS stock solution

The stock solution of dinitrosalycilic acid (DNS) was prepared by dissolving 5 g of dinitrosalicilic acid in 100 ml of a 2 N solution of sodium hydroxide in water at 25 °C. Double sodium and potassium tartrate (150 g) was then added and the solution was diluted to a final volume of 500 ml with deionized water. This stock solution was stored for no longer than 2 weeks below 7 °C in Teflon flasks protected externally with aluminium foil. Prior to use, the stock solution was warmed to room temperature and filtered through regular ash-free filter paper.

Preparation of inocula

After the package containing the lyophilized strain of *Lactobacillus plantarum* was opened, viability was maintained by weekly subculture on MRS agar in Petri dishes and storing at 7 °C. Before each experiment a sample of a colony was taken and incubated for 24 h in MRS medium (50 ml). After this pregrowth stage, the cell suspension was centrifuged at 7000 *g* and washed with an aqueous solution of NaCl (8.5 g/L). The cells recovered were resuspended in sterilized deionized water until the desired concentration of inoculum was obtained (5 g biomass/100 ml of suspension). This inoculum was used to prepare the fermentation media using 1 ml of inoculum/500 ml of fermentation medium (which corresponds roughly to a final optical density of 0.05 at 640 nm). Alternatively, the cells recovered were resuspended in sterilized YNB medium containing 10 g/L glucose and 2.5 g/L yeast extract until the desired concentration of inoculum was obtained (0.02 g of biomass/100 ml of suspension, which corresponds roughly to an optical density of 0.10 at 640 nm); this inoculum was used to prepare the growth calibration curve.

Experimental fermentations

After removal of the tops and bottoms the carrots were washed thoroughly with tap water, submerged for 1.5 min in a 0.2% (w/v) aqueous solution of copper sulfate, and finally submerged for 2.0 min in a 0.2% (w/v) aqueous solution of potassium permanganate. The carrots were then sliced perpendicularly to their longitudinal axis of symmetry into approximately circular pieces 1 cm thick and 3 cm in diameter, and 40 such pieces were then submerged in 500 ml of the appropriate brine equilibrated at the chosen temperature. The brine was then inoculated with 1 ml of the pre-growth inoculum, and gently stirred in a uniform fashion for the fermentation period. All combinations of five processing temperatures (15, 20, 30, 40, and

45 °C) and five initial concentrations of NaCl (0, 5, 7.5, 10, and 15% w/v) were tested in batch fermentation for times up to 2 weeks. Each fermentation batch (containing 500 ml of brine and 40 carrot slices) was sampled once only for biomass, NaCl, reducing sugars, and lactic acid (the independent experimental fermentations set up numbered more than 400 in total).

Assay for biomass

The growth of the strain of *L. plantarum* was followed by reading the optical density at 640 nm (diluting with deionized water, where necessary, to achieve readings in the range 0.1–0.5). The cells were then harvested from the suspension by centrifugation and washing with distilled water and were dried at 60 °C in Petri dishes until a constant weight was achieved (this process typically took ca 48 h). The dry cell weight [DCW (kg/m^3)] was correlated linearly with the optical density of the corresponding cell suspension at 640 nm, OD_{640}, according to the relationship $DCW = 0.2890\ OD_{640} + 0.06053$ ($r^2 = 0.944$).

Assay for NaCl

Aliquots (3 ml) of the brines were periodically taken and centrifuged at 7000 *g*, and 2 ml of the clarified supernatant was titrated with a 0.05 N aqueous solution of silver nitrate using 1 ml of potassium dichromate as an indicator.

Assay for reducing sugars

Aliquots (3 ml) of the brines were periodically taken (from a fermentation batch selected at random at each time), centrifuged at 7000 *g* and 0.5 ml of the clarified supernatant was added to 0.5 ml of the DNS stock solution in vials that were then covered with metal stoppers and boiled in a water bath for 5 min. The vials were immediately cooled down with tap water, and their content diluted with 5 ml of deionized water. Since the basis of this method is the reduction of 3,5-dinitrosalicilic acid to 3-amino-5-nitrosalicilic acid by the carbonyl group of the reducing sugars, the results were converted to glucose equivalents according to a calibration curve prepared daily for the range 0–1 g/L.

Assay for lactic acid

Aliquots (5 ml) of the brines were periodically taken (from a fermentation batch selected at random at each time), centrifuged at 7000 *g* and 2 ml of the clarified supernatant was diluted to 50 ml using deionized water; this was then titrated with a 0.01 N aqueous solution of sodium hydroxide until the end point of pH = 8.2 was obtained and maintained for 99 s. The titre of the blank (prepared in the same way from pure water instead of brine and containing the same amount of carrot material) was deducted accordingly. The results were expressed as lactic acid equivalents; this simplified methodology was justified by the observation (results not shown) that production of acetic acid and ethanol by fermentation (as assayed by HPLC) were negligible.

Mathematical analysis

A basic model was considered in this analysis. Such a model (the most complex to be fitted in our modelling effort) considers most traditional mechanisms for molecular transport of solutes, biomass production and kinetics of substrate utilization and product formation. In order to investigate whether the mathematical complexity of such a basic model (involving 20 adjustable parameters) was required from a statistical point of view, nested simplifications were also tested for adequacy of fit.

The basic model was derived starting from the following assumptions: (*i*) the production of biomass is assumed to follow Monod kinetics with inhibition by reducing sugars, lactic acid and NaCl, and with an initial lag phase; (*ii*) the appearance of reducing sugars in the brine is the result of molecular transport from the carrots, whereas utilization of reducing sugars includes conversion to cell mass, formation of lactic acid, and consumption for maintenance; (*iii*) the formation of lactic acid is assumed to be both growth- and non-growth-associated according to Leudeking–Piret kinetics, whereas disappearance of lactic acid is accounted for by molecular transport into the carrots; (*iv*) the disappearance of NaCl from the brine is due to molecular transport into the carrots; and (*v*) the molecular transport of reducing sugars within the carrot material is assumed to be described by a variable diffusibility because, as claimed by Nabais *et al.* (1995), the inventory of sugars available for diffusion in the intercellular fluid increases with time as carrot cell walls are disrupted, whereas transports of NaCl and lactic acid within the carrot material are assumed to be described by constant diffusibilities (because the inventory of these compounds available for diffusion through the intercellular fluid is essentially constant provided that the brine is well stirred). Under all these assumptions, the balance equations for the various components in solution take the form

$$\frac{dC_X}{dt} = \frac{\mu_{max} K_P K_S C_R C_X}{[1 + \psi \exp(-\xi t)]\,(K_R + C_R)\,(K_P + C_P)\,(K_S + C_S)}$$

At $t = 0$, $C_X = C_{X_0}$ (1)

$$\frac{dC_R}{dt} = -\frac{AD_R}{1 - \varepsilon\,[1 - \exp(-\phi t)]}\left(\frac{\partial \hat{C}_R}{\partial x}\right)_{x=L} - \frac{1}{Y_{X/R}}\frac{dC_x}{dt} - \frac{1}{Y_{P/R}}\frac{dC_P}{dt} - m\,C_X$$

At $t = 0$, $C_R = 0$ (2)

$$\frac{dCP}{dt} = \alpha\frac{dC_X}{dt} + \beta C_X - AD_P\left(\frac{\partial \hat{C}_P}{\partial x}\right)_{x=L}$$

At $t = 0$, $C_P = 0$ (3)

and

$$\frac{dC_S}{dt} = -AD_S\left(\frac{\partial \hat{C}}{\partial x}\right)_{x=L}$$

At $t = 0$, $C_S = C_{S_0}$ (4)

whereas the balance equations for the various components within the carrot material take the form

$$\frac{\partial \hat{C}_R}{\partial t} = \frac{D_R}{1 - \varepsilon\,[1 - \exp(-\phi t)]}\frac{\partial^2 \hat{C}_R}{\partial x^2}$$

At $t = 0$, $0 \leq x \leq L$, $\hat{C}_R = \hat{C}_{R_0}$

At $t \geq 0$, $x = 0$, $\frac{\partial \hat{C}_R}{\partial x} = 0$

At $t \geq 0$, $x = L$, $\hat{C}_R = k_R\,C_R$ (5)

$$\frac{\partial \hat{C}_P}{\partial t} = D_P\frac{\partial^2 \hat{C}_P}{\partial x^2}$$

At $t = 0$, $0 \leq x \leq L$, $\hat{C}_P = 0$

At $t \geq 0$, $x = 0$, $\frac{\partial \hat{C}_P}{\partial x} = 0$

At $t \geq 0$, $x = L$, $\hat{C}_P = k_P\,C_P$ (6)

$$\frac{\partial^2 \hat{C}_S}{\partial x^2} = D_S\frac{\partial \hat{C}_S}{\partial t}$$

At $t = 0$, $0 \leq x \leq L$, $\hat{C}_S = 0$

At $t \geq 0$, $x = 0$, $\frac{\partial \hat{C}_S}{\partial x} = 0$

At $t \geq 0$, $x = L$, $\hat{C}_S = k_S\,C_S$ (7)

where the symbols are all defined in the Nomenclature. Equations (5)–(7) have the form of Fick's second law in a single dimension. This unidimensional assumption is acceptable in view of the small ratio of time scales for diffusion (the order of magnitude of which is given by δ^2/D) in the axial direction ($\delta = 0.5$ cm and $D \sim 10^{11}$ m^2/s) to that in the radial direction ($\delta = 1.5$ cm and $D \sim 10^{11}$ m^2/s) of each slice, i.e. 0.11; this means that molecular transport in the radial direction is approximately ten times slower than in the axial direction and may thus be neglected for modelling purposes. A similar rationale applied as a basis for comparison of diffusion in the axial direction ($D \sim 10^{11}$ m^2/s) to diffusion in the boundary layer ($D \sim 10^{9}$ m^2/s) indicates that in order to raise a mass transfer resistance comparable to that raised by the carrot material, the stagnant layer should have a thickness of 5 cm, which is excessively high in view of the size of the equipment and the dimension of the vortices generated by stirring. Therefore, the boundary condition on the surface of the carrot slice could safely be taken as the bulk brine concentration.

An analytical solution to the above set of differential equations and associated initial and boundary conditions is not possible and one must resort to numerical techniques. In the present situation, the finite difference approach was successfully employed and the corresponding discretization of Equations (1)–(7) is included in the Appendix.

Statistical analysis

Model parameters were estimated by multiresponse non-linear regression to the experimental data (biomass dry weight, concentration of lactic acid, concentration of NaCl, and concentration of reducing sugars in the brine for each set of experimental conditions at each fermentation time) using a General REGression package (GREG: Caracotsios *et al.*, 1985), level 20. At this level, the program performs non-linear regression analysis of the data using finite differences as approximates of the derivatives of the objective function with respect to each parameter and uses as the objective function the minimization of $N \ln\{|\mathbf{R}|\}/2$ where the auxiliary matrix is defined as $\mathbf{R} = \mathbf{Z}^T\mathbf{Z}$ and where $\mathbf{Z}$ is the $M \times N$ matrix of the residuals of each of the M measurements ($M = 4$: biomass dry weight, concentration of lactic acid, concentration of NaCl, and concentration of reducing sugars in the brine) for each of the N experiments (N = number of independent experimental conditions × number of sampling times). The determinant of the residual matrix, $\mathbf{R}$, containing all (unweighted) experimental data rather than the sum of squares of the residuals, $tr(\mathbf{R})$, was selected as an objective function due to the multiresponse nature of our experiments (and

because there was no apparent *a priori* reason to doubt the assumption of a normal distribution of the residuals accompanying any of the measured variables); the use of the logarithm value of |R| was aimed at increasing the numerical efficiency because the actual values of |R| often lead to digital underflow. Given the starting estimates, this regression package expands the objective function as a local quadratic, finds a solution for the feasible minimum of this quadratic expansion in terms of parameter values, and implements a weak line search for a smaller value of the objective function.

RESULTS

From all the curves obtained using the model proposed (after elimination of several parameters for which the null hypothesis was consistently accepted at the 5% level of significance, as discussed below) and using the best estimates of the remaining parameters irrespective of whether or not their 95% confidence intervals encompassed the null hypothesis), those associated with the temperatures of 15 and 30 °C for the five initial NaCl concentrations tested were selected for plotting as Figures 1–10.

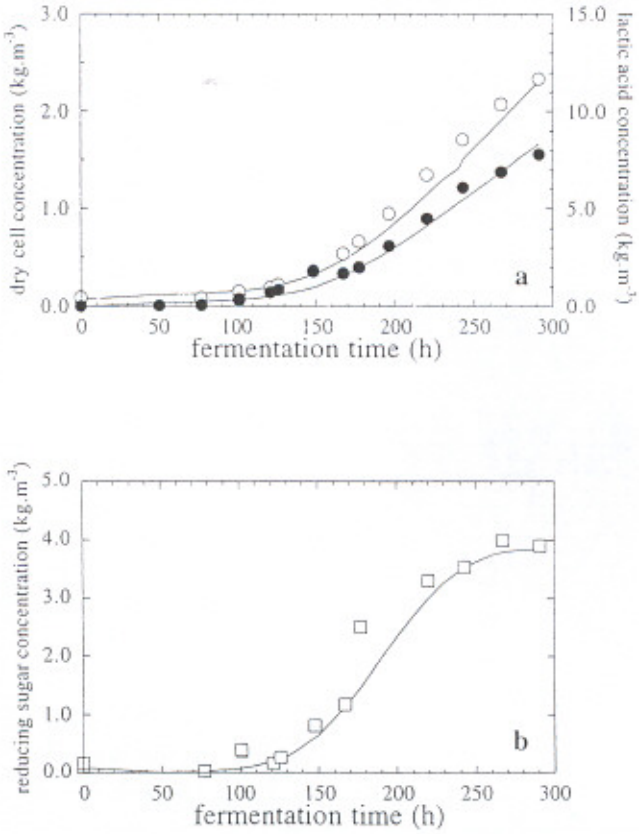


Figure 1. Plot of (a) biomass dry weight (○) and concentration of lactic acid (●), and (b) concentration of reducing sugars (□) for the brine, as a function of time for the initial concentration of 0% sodium chloride and a temperature of 15 °C.

Figura 1. Representación de: (a) el peso en seco de la biomasa (○) y la concentración de ácido láctico (●), y (b) la concentración de azúcares reductores (□) en función del tiempo, para una concentración inicial del 0% de sal y una temperatura de 15 °C.

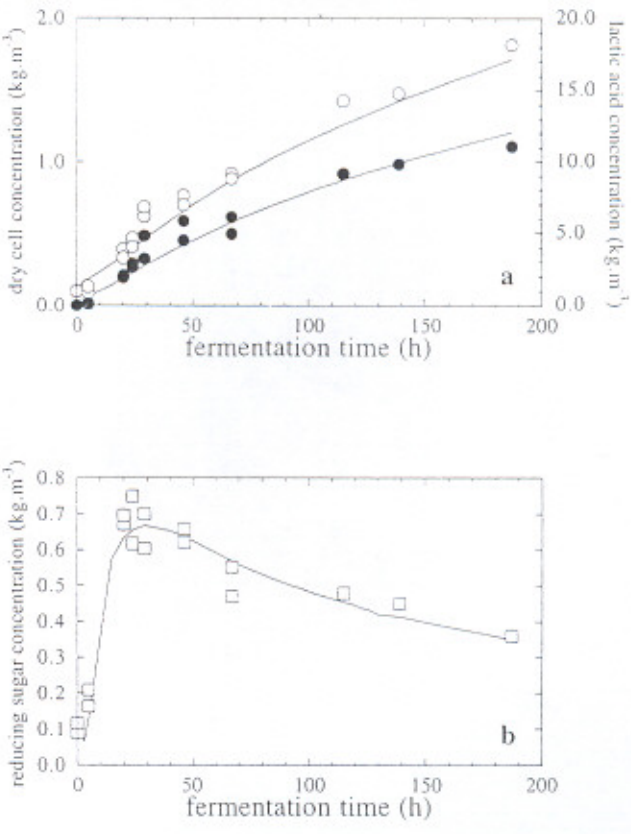


Figure 2. Plot of (a) biomass dry weight (○) and concentration of lactic acid (●), and (b) concentration of reducing sugars (□) for the brine, as a function of the batch time for the initial concentration of 0% sodium chloride and a temperature of 30 °C.

Figura 2. Representación de: (a) el peso en seco de la biomasa (○) y la concentración de ácido láctico (●), y (b) la concentración de azúcares reductores (□) en función del tiempo, para una concentración inicial del 0% de sal y una temperatura de 30 °C.

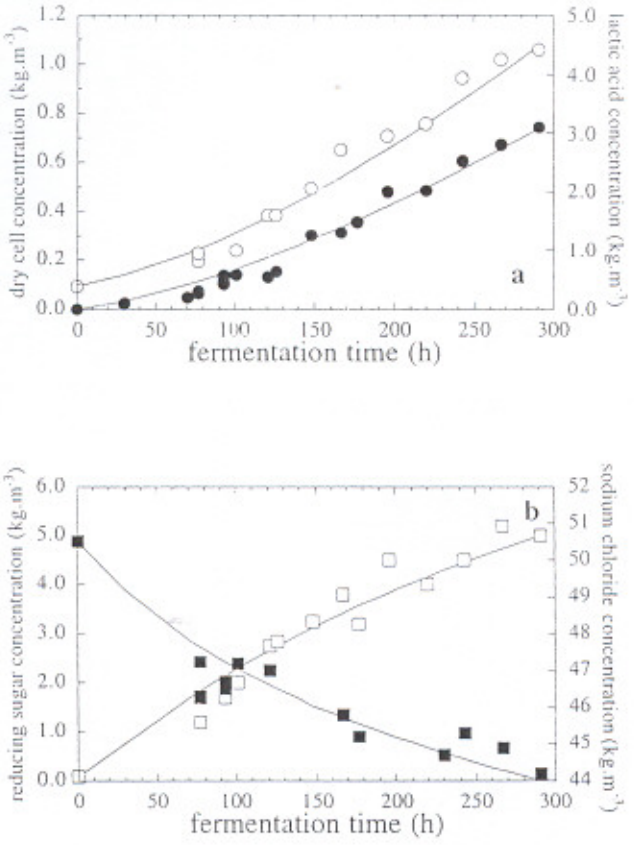


Figure 3. Plot of (a) biomass dry weight (○) and concentration of lactic acid (●), and (b) concentration of reducing sugars (□) and concentration of sodium chloride (■) for the brine, as a function of the batch time for the initial concentration of 5% sodium chloride and a temperature of 15 °C.

Figura 3. Representación de: (a) el peso en seco de la biomasa (○) y la concentración de ácido láctico (●), y (b) la concentración de azúcares reductores (□) y la concentración de cloruro sódico (■), para una concentración inicial de 5% de cloruro sódico y una temperatura de 15 °C.

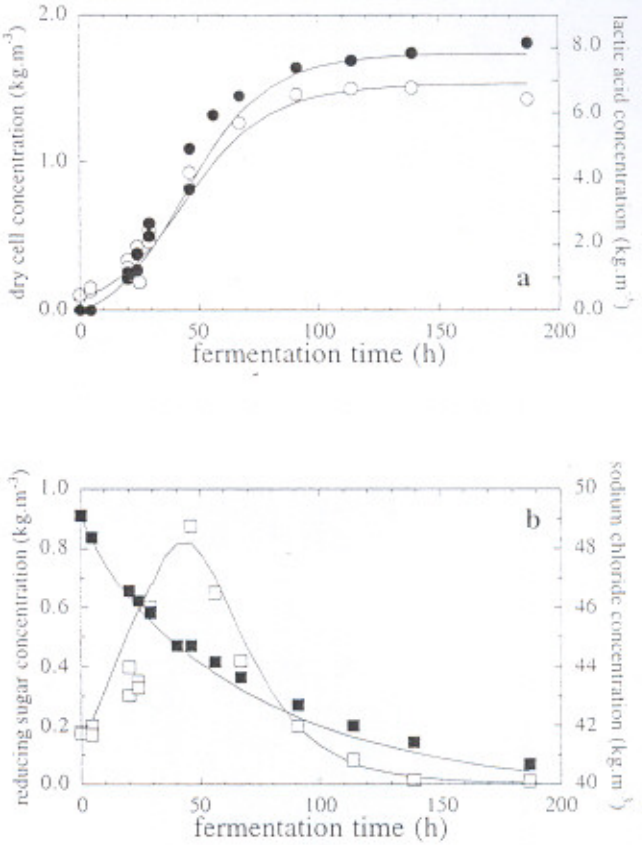
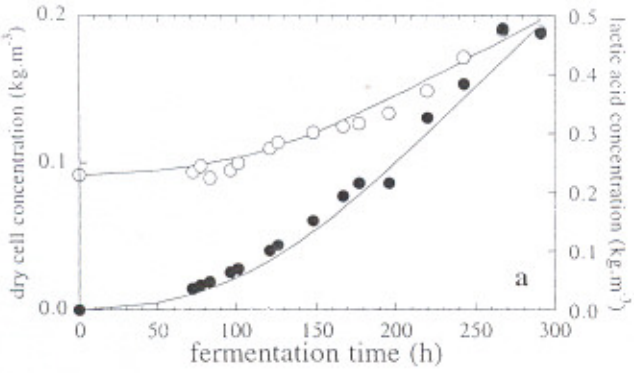


Figure 4. Plot of (a) biomass dry weight (○) and concentration of lactic acid (●), and (b) concentration of reducing sugars (□) and concentration of sodium chloride (■) for the brine, as a function of the batch time for the initial concentration of 5% sodium chloride and a temperature of 30 °C.

Figura 4. Representación de: (a) el peso en seco de la biomasa (○) y la concentración de ácido láctico (●), y (b) la concentración de azúcares reductores (□) y concentración de cloruro sódico (■), para una concentración inicial de 5% de cloruro sódico y una temperatura de 30 °C.

The results of the non-linear, multiresponse regression analysis (as provided by the post-convergence report generated by GREG) are tabulated in Tables 1–5 for the parameters of the full (or basic) model; the values for L and A were obtained by direct measurement and so were not fitted; the estimates of k_R, k_P, and k_S were obtained from independent fits to alternative data on equilibrium partition of each solute between the carrot material and the brine (Nabais *et al.*, 1995).

In order to decide whether a simple nested model (rather than the basic model) fits the data set adequately, we proceeded as in the linear case and used a likelihood ratio test (Draper and Smith, 1981). Because of the spherical normal assumption, this test leads to an assessment of the extra determinant value due to the extra parameters involved in going from the partial (nested) to the full (basic) model (Bates and Watts, 1988). The results of the extra determinant analyses between the full model, given by Equations (1)–(7) with the 20 parameter values given in Tables 1–5, and the partial model that uses the same set of equations but considers only parameters μ_{max}, K_R, K_P, K_S, α, $\hat{C}_{R_0}$, D_R, ε, ϕ, D_P, D_S, $Y_{X/R}$, $Y_{P/R}$, k_R, k_P and k_S



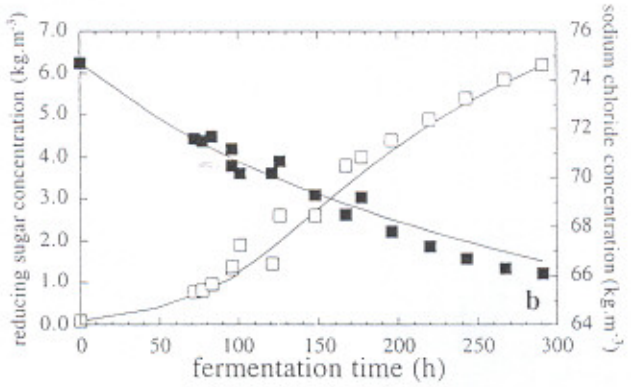
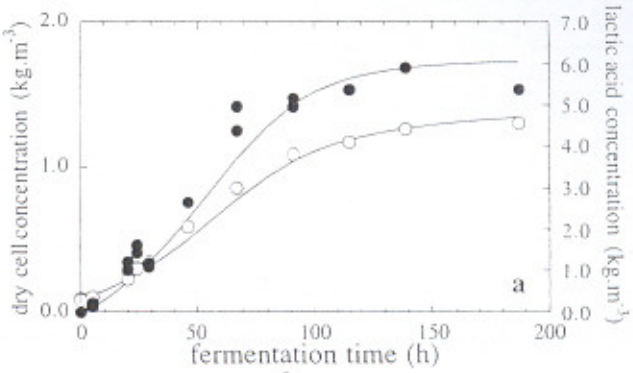


Figure 5. Plot of (a) biomass dry weight (○) and concentration of lactic acid (●), and (b) concentration of reducing sugars (□) and concentration of sodium chloride (■) for the brine, as a function of the batch time for the initial concentration of 7.5% sodium chloride and a temperature of 15 °C.

Figura 5. Representación de: (a) el peso en seco de la biomasa (○) y la concentración de ácido láctico (●), y (b) la concentración de azúcares reductores (□) y concentración de cloruro sódico (■), para una concentración inicial de 7,5% de cloruro sódico y una temperatura de 15 °C.



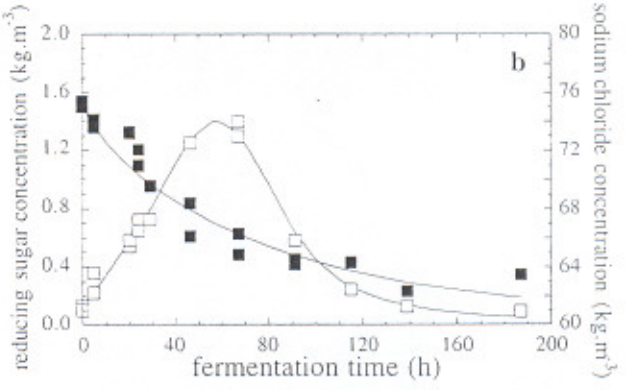


Figure 6. Plot of (a) biomass dry weight (○) and concentration of lactic acid (●), and (b) concentration of reducing sugars (□) and concentration of sodium chloride (■) for the brine, as a function of the batch time for the initial concentration of 7.5% sodium chloride and a temperature of 30 °C.

Figura 6. Representación de: (a) el peso en seco de la biomasa (○) y la concentración de ácido láctico (●), y (b) la concentración de azúcares reductores (□) y concentración de cloruro sódico (■), para una concentración inicial de 7,5% de cloruro sódico y una temperatura de 30 °C.

(i.e. those parameters that could be estimated with a marginal confidence interval not overlapping zero for at least one of the data sets produced), are summarized in Tables 6–10.

DISCUSSION

In attempts to model the kinetics of any process, the experimental layouts should in principle involve as many combinations as possible of values for environmental factors such as temperature, ionic strength, pH, concentration of substrates, ratio of solid to liquid phase, and size of inoculum (in the case of fermentative processes). In the present situation, only the temperature and the ionic strength (via the initial NaCl concentration) were deliberately varied according to a 5×5 factorial design because those are the processing parameters that can, in practice, be more easily manipulated on the industrial level. In fact, control of pH at different levels could have been achieved at the expense of additives with buffering properties, but most of those commonly available are not licensed for use in direct food processing. Furthermore, the substrates in the present situation are reducing sugars, which are contributed by the

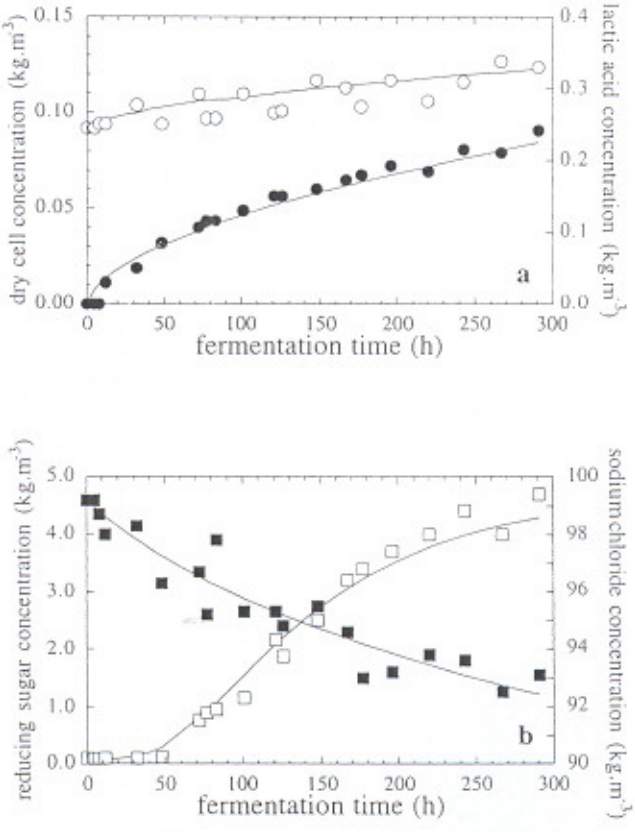


Figure 7. Plot of (a) biomass dry weight (○) and concentration of lactic acid (●), and (b) concentration of reducing sugars (□) and concentration of sodium chloride (■) for the brine, as a function of the batch time for the initial concentration of 10% sodium chloride and a temperature of 15 °C.

Figura 7. Representación de: (a) el peso en seco de la biomasa (○) y la concentración de ácido láctico (●), y (b) la concentración de azúcares reductores (□) y concentración de cloruro sódico (■), para una concentración inicial de 10% de cloruro sódico y una temperatura de 15 °C.

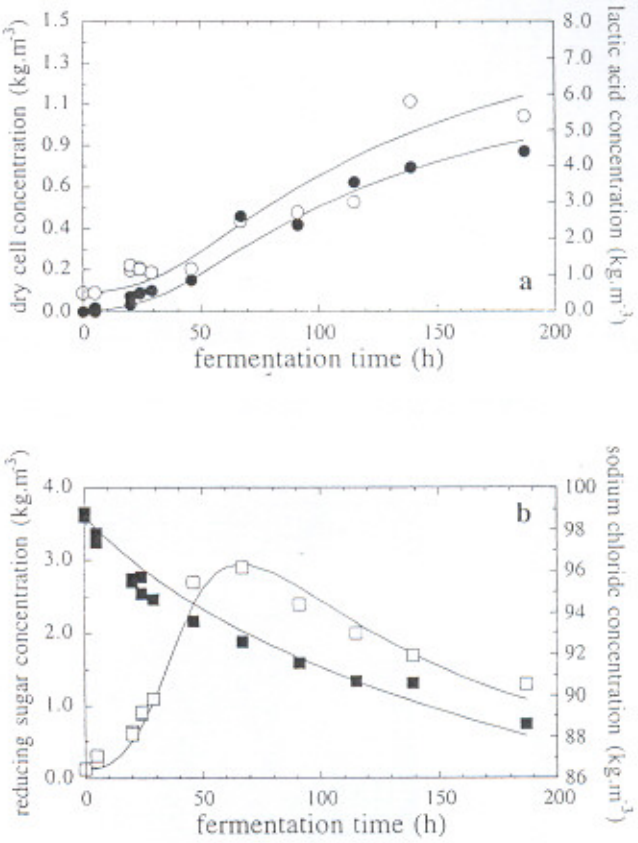


Figure 8. Plot of (a) biomass dry weight (○) and concentration of lactic acid (●), and (b) concentration of reducing sugars (□) and concentration of sodium chloride (■) for the brine, as a function of the batch time for the initial concentration of 10% sodium chloride and a temperature of 30 °C.

Figura 8. Representación de: (a) el peso en seco de la biomasa (○) y la concentración de ácido láctico (●), y (b) la concentración de azúcares reductores (□) y concentración de cloruro sódico (■), para una concentración inicial de 10% de cloruro sódico y una temperatura de 30 °C.

carrot cells themselves and hence possess a qualitative and concentration profile sufficiently ill-defined to justify collective assay by the DNS method (as done in most previous studies of this subject); therefore, deliberate variation of their concentration would have disturbed the system and the extra accuracy that would have been obtained for the model form and statistical significance of the parameters therein would have been compromised by the concomitant loss of physical significance. It should also be borne in mind that addition of sugars is not allowed in any fermentation process for fresh-cut vegetables, e.g. spontaneous fermentations of cabbage and winemaking. However: (*i*) since the initial (grouped) concentration of reducing sugars was left as an adjustable parameter in the non-linear regression fits (via parameter $\hat{C}_{R_0}$ in Tables I–5); (*ii*) since the range covered by this overall concentration was rather large (in the cases where it could be fitted at all); and (*iii*) since the actual values taken by this (fitted) overall concentration do not display biased trends or any sort of correction with the processing temperature or the NaCl concentration in the brine, then there is no apparent reason to doubt the adequacy of its assessment via the experimental layout considered. The ratio of solid phase (carrot slices) to liquid phase

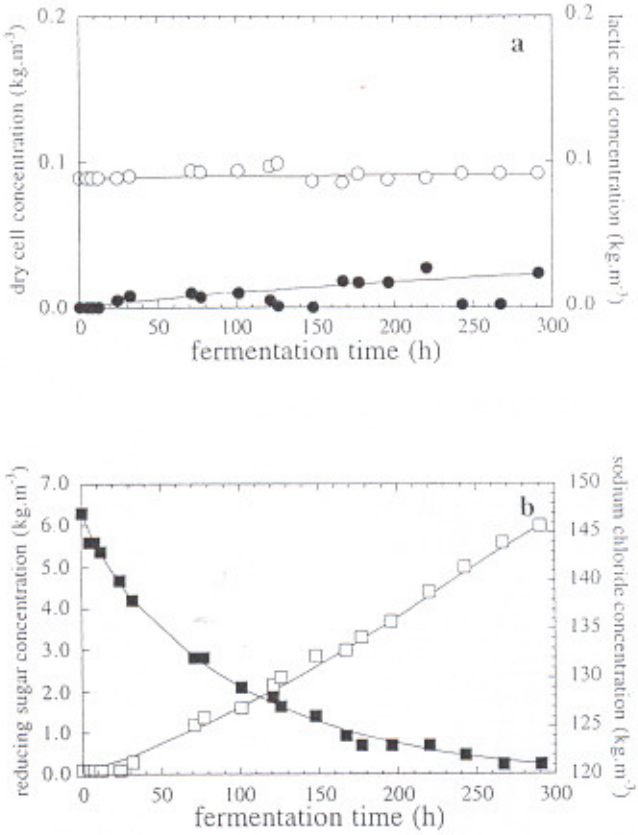


Figure 9. Plot of (a) biomass dry weight (○) and concentration of lactic acid (●), and (b) concentration of reducing sugars (□) and concentration of sodium chloride (■) for the brine, as a function of the batch time for the initial concentration of 15% sodium chloride and a temperature of 15 °C.

Figura 9. Representación de: el peso en seco de la biomasa (○) y la concentración de ácido láctico (●), y (b) la concentración de azúcares reductores (□) y concentración de cloruro sódico (■), para una concentración inicial de 15% de cloruro sódico y una temperatura de 15 °C.

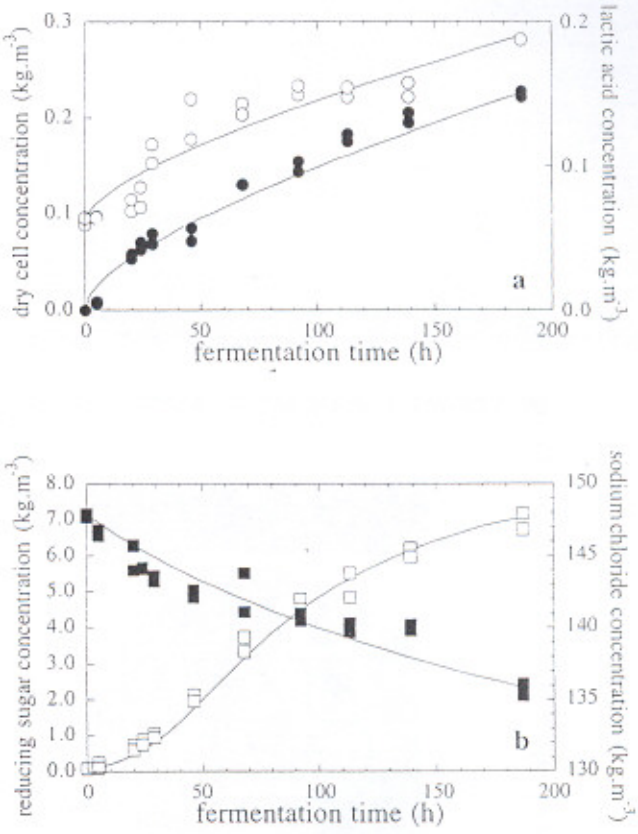


Figure 10. Plot of (a) biomass dry weight (○) and concentration of lactic acid (●), and (b) concentration of reducing sugars (□) and concentration of sodium chloride (■) for the brine, as a function of the batch time for the initial concentration of 15% sodium chloride and a temperature of 30 °C.

Figura 10. Representación de: (a) el peso en seco de la biomasa (○) y la concentración de ácido láctico (●), y la concentración de azúcares reductores (□) y concentración de cloruro sódico (■), para una concentración inicial de 15% de cloruro sódico y una temperatura de 30 °C.

(brine) was set at the value that common practice has dictated to be the most convenient in an industrial context; in fact, too small values for such a ratio constrain leaching of reducing sugars into the liquid phase, whereas too large values lead to excessively low average concentrations of the sugars. Finally, the size of the inoculum was preset at a given value which was known to provide the best overall performance for the fermentation batch in terms of several simultaneous quality factors (Nabais and Malcata, 1995b). It should also be noted that the large fermentation times assayed, the factorial nature of the experimental design, and the limitations of the experimental equipment available raised constraints on the total number of experiments that could be run under different experimental conditions, and so the most reasonable assumption, in view of the reasoning detailed above, was associated with all possible combinations of five levels of two manipulated variables only.

It may be argued that since the number of parameters in the basic model is rather large, attempts to simultaneously fit all such parameters may pose the risk of generating mutual parameter correlations close to unity (i.e., changes in one given parameter could be accounted for by changes in another parameter) and unrealistic parameter values in view of their physicochemical meaning. The alternative, classical

Table 1. Estimates of adjustable parameters in partial model and corresponding 95% marginal inference intervals for data collected with brines containing 0% (w/v) of salt.

Tabla 1. Valores de los parámetros y su intervalo de confianza marginal al 95% obtenidos con el ajuste al modelo parcial de los datos recogidos con la salmuera con un 0% (w/v) de sal.

	Temperature				
Parameter	15 °C	20 °C	30 °C	40 °C	45 °C
μ_{max} (h^{-1})	$(6.47 \pm 2.33) \times 10^{-5}$	$(3.37 \pm 1.28) \times 10^{-4}$	$(3.89 \pm 2.22) \times 10^{-2}$	$(3.81 \pm 1.12) \times 10^{-5}$	$(2.13 \pm 0.260) \times 10^{-3}$
ψ (–)	$4.224 \times 10^{-4} \pm \infty$	$1.0040 \times 10^{-5} \pm \infty$	$1.9680 \times 10^{-3} \pm \infty$	$2.294 \pm \infty$	$9.271 \times 10^{-5} \pm \infty$
ξ (h^{-1})	$7.9201 \times 10^{-4} \pm \infty$	$5.4420 \times 10^{-6} \pm \infty$	$5.9081 \times 10^{-6} \pm \infty$	$6.2900 \times 10^{-6} \pm \infty$	$2.0301 \times 10^{-4} \pm \infty$
K_R ($kg.m^{-3}$)	$(2.46 \pm 1.77) \times 10^{-1}$	$1.12 \times 10^{-1} \pm \infty$	$2.82 \times 10^{-1} \pm \infty$	$(3.30 \pm 5.9) \times 10^{-1}$	$9.5890 \times 10^{-2} \pm \infty$
K_P ($kg.m^{-3}$)	$4.30 \times 10^{-3} \pm \infty$	$(1.512 \pm 0.561) \times 10^{-1}$	$(2.13 \pm 1.52) \times 10^{-3}$	$(3.080 \pm 0.807) \times 10^{-1}$	$6.00 \times 10^{-4} \pm \infty$
K_S ($kg.m^{-3}$)	n.f.	n.f.	n.f.	n.f.	n.f.
α (–)	4.50 ± 0.33	4.29 ± 2.02	7.378 ± 0.550	8.407 ± 0.731	$(1.219 \pm 0.223) \times 10$
β (h^{-1})	$0.0001 \pm \infty$	$-1.584 \pm \infty$	$-0.004 \pm \infty$	$0.0002 \pm \infty$	$0.0006 \pm \infty$
$Y_{X/R}$ (–)	$1.910 \times 10^{-1} \pm \infty$	$(1.12 \pm 0.711) \times 10^{-1}$	$2.5000 \times 10^{-1} \pm \infty$	$2.5000 \times 10^{-1} \pm \infty$	$1.7670 \times 10^{-1} \pm \infty$
$Y_{P/R}$ (–)	$(8.87 \pm 1.91) \times 10^{-1}$	$(5.95 \pm 4.77) \times 10^{-1}$	$0.9990 \pm \infty$	$0.3767 \pm \infty$	$0.1800 \pm \infty$
m (h^{-1})	$1.0000 \times 10^{-30} \pm \infty$	$1.000 \times 10^{-30} \pm \infty$	$4.0000 \times 10^{-30} \pm \infty$	$1.0000 \times 10^{-30} \pm \infty$	$1.0000 \times 10^{-30} \pm \infty$
C_{R_0} ($kg.m^{-3}$)	$(5.77 \pm 4.64) \times 10^{2}$	$90.00 \pm \infty$	$(7.49 \pm 5.25) \times 10$	$77.80 \pm \infty$	48.19 ± 7.26
D_R ($m^2.s^{-1}$)	$1.0 \times 10^{-12} \pm \infty$	$(4.92 \pm 2.03) \times 10^{-13}$	$(3.58 \pm 3.18) \times 10^{-12}$	$(1.757 \pm 0.514) \times 10^{-12}$	$(1.504 \pm 0.412) \times 10^{-11}$
ϵ (–)	$0.0001 \pm \infty$	$0.000 \pm \infty$	0.9890 ± 0.0048	$0.5000 \pm \infty$	$0.9990 \pm \infty$
ϕ (–)	$(1.177 \pm 0.005) \times 10^{-7}$	$(8.61 \pm 2.59) \times 10^{-6}$	$2.2200 \times 10^{-5} \pm \infty$	$1.0001 \times 10^{-8} \pm \infty$	$(1.382 \pm 6.12) \times 10^{-5}$
D_P ($m^2.s^{-1}$)	$(2.75 \pm 0.90) \times 10^{-12}$	$(3.191 \pm 0.900) \times 10^{-12}$	$4.04 \times 10^{-12} \pm \infty$	$6.11 \times 10^{-12} \pm \infty$	$6.42 \times 10^{-12} \pm \infty$
D_S ($m^2.s^{-1}$)	n.f.	n.f.	n.f.	n.f.	n.f.

n.f. = not fitted; k_R = 1.03 (%w/w / %w/v); k_P = 0.89 (%w/w / %w/v).

Table 2. Estimates of adjustable parameters in partial model and corresponding 95% marginal inference intervals for data collected with brines containing 5% (w/v) of salt.

Tabla 2. Valores de los parámetros y su intervalo de confianza marginal al 95% obtenidos con el ajuste al modelo parcial de los datos recogidos con la salmuera con un 5% (w/v) de sal.

	Temperature				
Parameter	15 °C	20 °C	30 °C	40 °C	45 °C
μ_{max} (h^{-1})	$(1.4250 \pm 0.0903) \times 10^{-4}$	$(2.14 \pm 1.07) \times 10^{-2}$	$(2.21 \pm 1.09) \times 10^{-3}$	$(1.68 \pm 1.24) \times 10^{-3}$	$(1.81 \pm 3.68) \times 10^{-4}$
ψ (–)	$1.50 \times 10^{-5} \pm \infty$	$5.47 \times 10^{-4} \pm \infty$	$3.00 \times 10^{-2} \pm \infty$	$3.00 \times 10^{-2} \pm \infty$	$1.01 \times 10^{-3} \pm \infty$
ξ (h^{-1})	$9.61 \times 10^{-5} \pm \infty$	$1.05 \times 10^{-5} \pm \infty$	$3.01 \times 10^{-2} \pm \infty$	$1.01 \times 10^{-2} \pm \infty$	$1.93 \times 10^{-3} \pm \infty$
K_R ($kg.m^{-3}$)	$1.70 \times 10^{-5} \pm \infty$	$1.82 \times 10^{-5} \pm \infty$	$(3.101 \pm 0.712) \times 10^{-1}$	$1.00 \times 10^{-7} \pm \infty$	$1.00 \times 10^{-7} \pm \infty$
K_P ($kg.m^{-3}$)	$(1.112 \pm 0.413) \times 10^{-1}$	$(8.85 \pm 6.04) \times 10^{-1}$	$(6.64 \pm 2.51) \times 10^{-2}$	$(4.44 \pm 2.94) \times 10^{-3}$	$6.86 \times 10^{-3} \pm \infty$
K_S ($kg.m^{-3}$)	$0.180 \pm \infty$	$4.57 \pm \infty$	$6.88 \pm \infty$	$1.05 \pm \infty$	0.1510 ± 0.0179
α (–)	$(3.212 \pm 0.879) \times 10^{-2}$	$(3.671 \pm 0.318) \times 10^{-1}$	6.699 ± 0.168	3.59 ± 1.67	7.93 ± 2.33
β (h^{-1})	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$2.38 \times 10^{-28} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$
$Y_{X/R}$ (–)	$9.99 \times 10^{-1} \pm \infty$	$9.99 \times 10^{-1} \pm \infty$	$2.72 \times 10^{-1} \pm 0.132$	$1.30 \times 10^{-1} \pm \infty$	$5.01 \times 10^{-1} \pm \infty$
$Y_{P/R}$ (–)	$0.999 \pm \infty$	0.101 ± 0.026	$2.00 \times 10^{-1} \pm \infty$	$1.30 \times 10^{-1} \pm \infty$	$1.30 \times 10^{-1} \pm \infty$
m (h^{-1})	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$
C_{R_0} ($kg.m^{-3}$)	$(1.148 \pm 0.776) \times 10^{2}$	$(1.82 \pm 1.57) \times 10^{2}$	45.66 ± 2.50	41.93 ± 5.91	23.12 ± 3.71
D_R ($m^2.s^{-1}$)	$(3.367 \pm 0.317) \times 10^{-13}$	$(2.33 \pm 1.44) \times 10^{-14}$	$(1.08 \pm 1.06) \times 10^{-12}$	$(6.147 \pm 0.875) \times 10^{-12}$	$(2.45 \pm 1.21) \times 10^{-11}$
ϵ (–)	0.999 ± 0.449	0.99905 ± 0.00511	0.985 ± 0.0162	$0.999 \pm \infty$	$0.500 \pm \infty$
ϕ (–)	$(1.487 \pm 1.29) \times 10^{-6}$	$(1.507 \pm 0.134) \times 10^{-5}$	$(3.32 \pm 1.16) \times 10^{-5}$	$(3.318 \pm 0.619) \times 10^{-5}$	$1.00 \times 10^{-16} \pm \infty$
D_P ($m^2.s^{-1}$)	$1.82 \times 10^{-12} \pm \infty$	$3.49 \times 10^{-12} \pm \infty$	$(7.281 \pm 0.258) \times 10^{-12}$	$(1.575 \pm 0.331) \times 10^{-11}$	$1.79 \times 10^{-11} \pm \infty$
D_S ($m^2.s^{-1}$)	$(4.127 \pm 0.664) \times 10^{-12}$	$(6.176 \pm 0.886) \times 10^{-12}$	$(2.056 \pm 0.326) \times 10^{-11}$	$(1.456 \pm 0.481) \times 10^{-11}$	$(3.490 \pm 0.653) \times 10^{-11}$

Note: k_R = 1.03 (%w/w / %w/v); k_P = 0.89 (%w/w / %w/v); k_S = 0.70 (%w/w / %w/v).

Table 3. Estimates of adjustable parameters in partial model and corresponding 95% marginal inference intervals for data collected with brines containing 7.5% (w/v) of salt.

Tabla 3. Valores de los parámetros y su intervalo de confianza marginal al 95% obtenidos con el ajuste al modelo parcial de los datos recogidos con la salmuera con un 7,5% (w/v) de sal.

	Temperature				
Parameter	15 °C	20 °C	30 °C	40 °C	45 °C
μ_{max} (h^{-1})	$(2.53 \pm 1.01) \times 10^{-4}$	$(7.76 \pm 1.40) \times 10^{-4}$	$(1.427 \pm 0.660) \times 10^{-3}$	$(1.599 \pm 0.199) \times 10^{-4}$	$4.32 \times 10^{-4} \pm \infty$
ψ (–)	$1.30 \times 10^{-6} \pm \infty$	$9.03 \times 10^{-5} \pm \infty$	$8.08 \times 10^{-6} \pm \infty$	$2.19 \times 10^{-6} \pm \infty$	$1.04 \times 10^{-6} \pm \infty$
ξ (h^{-1})	$1.09 \times 10^{-5} \pm \infty$	$7.88 \times 10^{-6} \pm \infty$	$3.88 \times 10^{-5} \pm \infty$	$1.79 \times 10^{-3} \pm \infty$	$6.31 \times 10^{-4} \pm \infty$
K_R ($kg.m^{-3}$)	$(6.800 \pm 0.798) \times 10^{-2}$	$1.84 \times 10^{-2} \pm \infty$	$(1.300 \pm 0.447) \times 10^{-1}$	$1.00 \times 10^{-6} \pm \infty$	$4.73 \times 10^{-3} \pm \infty$
K_P ($kg.m^{-3}$)	$2.17 \times 10^{-1} \pm \infty$	$(3.59 \pm 0.99) \times 10^{-1}$	$(2.89 \pm 1.24) \times 10^{-1}$	$(1.693 \pm 0.269) \times 10^{-2}$	$(5.08 \pm 1.93) \times 10^{-3}$
K_S ($kg.m^{-3}$)	$20.0 \pm \infty$	$1.42 \times 10^{3} \pm \infty$	$1.18 \times 10^{4} \pm \infty$	$3.23 \times 10^{4} \pm \infty$	$2.38 \pm \infty$
α (–)	$3.68 \times 10^{-1} \pm \infty$	2.638 ± 0.187	5.966 ± 0.392	$(1.98 \pm 1.18) \times 10$	$5.46 \times 10 \pm \infty$
β (h^{-1})	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$3.50 \times 10^{-30} \pm \infty$	$6.00 \times 10^{-30} \pm \infty$
$Y_{X/R}$ (–)	$5.40 \times 10^{-1} \pm \infty$	$(5.31 \pm 2.17) \times 10^{-1}$	$9.00 \times 10^{-1} \pm \infty$	$9.00 \times 10^{-1} \pm \infty$	$4.79 \times 10^{-1} \pm \infty$
$Y_{P/R}$ (–)	$9.80 \times 10^{-1} \pm \infty$	$9.99 \times 10^{-1} \pm \infty$	$(2.040 \pm 0.150) \times 10^{-1}$	$1.00 \times 10^{-1} \pm \infty$	$6.97 \times 10^{-1} \pm \infty$
m (h^{-1})	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$
C_{R_0} ($kg.m^{-3}$)	$(4.50 \pm 2.93) \times 10$	$(3.363 \pm 0.464) \times 10$	$7.22 \times 10^{2} \pm \infty$	$(2.345 \pm 0.221) \times 10$	$(4.238 \pm 0.229) \times 10$
D_R ($m^2.s^{-1}$)	$1.30 \times 10^{-6} \pm \infty$	$(1.758 \pm 0.887) \times 10^{-13}$	$(4.25 \pm 3.74) \times 10^{-12}$	$(2.66 \pm 1.73) \times 10^{-11}$	$(2.448 \pm 0.430) \times 10^{-11}$
ϵ (–)	$(9.800 \pm 0.173) \times 10^{-1}$	$(9.95 \pm 1.14) \times 10^{-4}$	$(9.333 \pm 0.921) \times 10^{-1}$	$5.00 \times 10^{-1} \pm \infty$	$5.00 \times 10^{-1} \pm \infty$
ϕ (–)	$(9.39 \pm 2.21) \times 10^{-6}$	$(8.085 \pm 0.329) \times 10^{-6}$	$(1.405 \pm 0.111) \times 10^{-5}$	$1.00 \times 10^{-9} \pm \infty$	$1.00 \times 10^{-12} \pm \infty$
D_P ($m^2.s^{-1}$)	$4.49 \times 10^{-12} \pm \infty$	$6.50 \times 10^{-12} \pm \infty$	$7.52 \times 10^{-12} \pm \infty$	$(1.12 \pm 1.02) \times 10^{-11}$	$1.28 \times 10^{-11} \pm \infty$
D_S ($m^2.s^{-1}$)	$(2.900 \pm 0.236) \times 10^{-12}$	$(5.781 \pm 0.791) \times 10^{-12}$	$(1.412 \pm 0.266) \times 10^{-11}$	$(3.421 \pm 0.381) \times 10^{-11}$	$(2.229 \pm 0.631) \times 10^{-11}$

Note: k_R = 1.03 (%w/w / %w/v); k_P = 0.89 (%w/w / %w/v); k_S = 0.70 (%w/w / %w/v).

Table 4. Estimates of adjustable parameters in partial model and corresponding 95% marginal inference intervals for data collected with brines containing 10% (w/v) of salt.

Tabla 4. Valores de los parámetros y su intervalo de confianza marginal al 95% obtenidos con el ajuste al modelo parcial de los datos recogidos con la salmuera con un 10% (w/v) de sal.

Temperature					
Parameter	15 °C	20 °C	30 °C	40 °C	45 °C
μ_{max} (h^{-1})	$6.00 \times 10^{-5} \pm \infty$	$(7.29 \pm 2.46) \times 10^{-5}$	$(1.38 \pm 1.24) \times 10^{-3}$	$1.14 \times 10^{-4} \pm \infty$	$(2.27661 \pm 0.0331) \times 10^{-7}$
ψ (–)	$7.14 \times 10^{-7} \pm \infty$	$1.96 \times 10^{-6} \pm \infty$	$1.80 \times 10^{-7} \pm \infty$	$4.47 \times 10^{-6} \pm \infty$	$7.76 \times 10^{-7} \pm \infty$
ξ (h^{-1})	$6.00 \times 10^{-5} \pm \infty$	$5.31 \times 10^{-5} \pm \infty$	$1.54 \times 10^{-4} \pm \infty$	$3.26 \times 10^{-3} \pm \infty$	$1.47 \times 10^{-4} \pm \infty$
K_R ($kg.m^{-3}$)	$5.90 \times 10^{-5} \pm \infty$	$(2.46 \pm 0.451) \times 10^{-1}$	$1.32 \times 10^{-1} \pm \infty$	$1.19 \times 10^{-2} \pm \infty$	$2.00 \times 10^{-2} \pm \infty$
K_P ($kg.m^{-3}$)	$(1.100 \pm 0.164) \times 10^{-2}$	$(3.48 \pm 1.09) \times 10^{-2}$	$(1.095 \pm 0.361) \times 10^{-1}$	$3.46 \times 10^{-2} \pm \infty$	$(2.00 \pm 1.68) \times 10^{-2}$
K_S ($kg.m^{-3}$)	$5.00 \pm \infty$	$6.95 \times 10^{-2} \pm \infty$	$1.58 \times 10^{4} \pm \infty$	$3.18 \times 10^{-1} \pm \infty$	3.174 ± 0.971
α (–)	1.100 ± 0.155	2.357 ± 0.212	4.994 ± 0.835	$(1.084 \pm 0.627) \times 10^{3}$	$(3.43 \pm 1.14) \times 10^{2}$
β (h^{-1})	1.100 ± 0.155	2.357 ± 0.212	4.994 ± 0.835	$(1.084 \pm 0.627) \times 10^{3}$	$(3.43 \pm 1.14) \times 10^{2}$
$Y_{X/R}$ (–)	$5.00 \times 10^{-1} \pm \infty$	$9.84 \times 10^{-1} \pm \infty$	$2.50 \times 10^{-1} \pm \infty$	$2.29 \times 10^{-1} \pm \infty$	$4.68 \times 10^{-1} \pm \infty$
$Y_{P/R}$ (–)	$9.80 \times 10^{-1} \pm \infty$	$(2.360 \pm 0.694) \times 10^{-1}$	$(6.32 \pm 2.63) \times 10^{-1}$	$(4.78 \pm 2.14) \times 10^{-1}$	$9.95 \times 10^{-1} \pm \infty$
m (h^{-1})	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$
C_{R_0} ($kg.m^{-3}$)	$(2.000 \pm 0.583) \times 10$	$8.92 \times 10 \pm \infty$	$(1.098 \pm 0.100) \times 10^{2}$	$(3.347 \pm 0.773) \times 10^{1}$	$(4.045 \pm 0.236) \times 10^{1}$
D_R ($m^2.s^{-1}$)	$(3.101 \pm 0.869) \times 10^{-13}$	$2.00 \times 10^{-13} \pm \infty$	$(3.897 \pm 0.107) \times 10^{-12}$	$(8.42 \pm 3.26) \times 10^{-12}$	$(3.052 \pm 0.502) \times 10^{-11}$
ϵ (–)	$(9.80 \pm 3.06 \times 10^{-3}$	$(0.9640 \pm 0.0822) \times 10^{-3}$	$(9.886 \pm 0.102) \times 10^{-3}$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$
ϕ (–)	$(1.30 \pm 1.26) \times 10^{-5}$	$(1.403 \pm 0.275) \times 10^{-5}$	$3.51 \times 10^{-5} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$
D_P ($m^2.s^{-1}$)	$3.5 \times 10^{-12} \pm \infty$	$5.55 \times 10^{-12} \pm \infty$	$4.73 \times 10^{-12} \pm \infty$	$(5.72 \pm 3.27) \times 10^{-12}$	$6.30 \times 10^{-12} \pm \infty$
D_S ($m^2.s^{-1}$)	$(3.203 \pm 0.419) \times 10^{-12}$	$(2.645 \pm 0.325) \times 10^{-12}$	$(4.447 \pm 0.628) \times 10^{-12}$	$(9.165 \pm 0.189) \times 10^{-12}$	$(1.1430 \pm 0.0921) \times 10^{-11}$

Note: k_R = 1.03 (%w/w / %w/v); k_P = 0.89 (%w/w / %w/v); k_S = 0.70 (%w/w / %w/v).

Table 5. Estimates of adjustable parameters in partial model and corresponding 95% marginal inference intervals for data collected with brines containing 15% (w/v) of salt.

Tabla 5. Valores de los parámetros y su intervalo de confianza marginal al 95% obtenidos con el ajuste al modelo parcial de los datos recogidos con la salmuera con un 15% (w/v) de sal.

Temperature					
Parameter	15 °C	20 °C	30 °C	40 °C	45 °C
μ_{max} (h^{-1})	$8.00 \times 10^{-8} \pm \infty$	$6.00 \times 10^{-7} \pm \infty$	$(1.153 \pm 0.116) \times 10^{-4}$	$6.00 \times 10^{-9} \pm \infty$	$3.80 \times 10^{-8} \pm \infty$
ψ (–)	$7.70 \times 10^{-7} \pm \infty$	$7.14 \times 10^{-7} \pm \infty$	$(1.153 \pm 0.116) \times 10^{-4}$	$7.14 \times 10^{-10} \pm \infty$	$4.10 \times 10^{-9} \pm \infty$
ξ (h^{-1})	$1.49 \times 10^{-8} \pm \infty$	$1.49 \times 10^{-8} \pm \infty$	$4.54 \times 10^{-9} \pm \infty$	$1.47 \times 10^{-12} \pm \infty$	$2.91 \times 10^{-10} \pm \infty$
K_R ($kg.m^{-3}$)	$5.92 \times 10^{-5} \pm \infty$	$5.90 \times 10^{-5} \pm \infty$	$(1.242 \pm 0.578) \times 10^{-2}$	$5.90 \times 10^{-5} \pm \infty$	$8.30 \times 10^{-2} \pm \infty$
K_P ($kg.m^{-3}$)	$1.10 \times 10^{-2} \pm \infty$	$1.10 \times 10^{-2} \pm \infty$	$(9.599 \pm 0.961) \times 10^{-4}$	$1.10 \times 10^{-2} \pm \infty$	$3.12 \times 10^{-2} \pm \infty$
K_S ($kg.m^{-3}$)	$2.00 \times 10^{2} \pm \infty$	$2.00 \times 10^{2} \pm \infty$	$1.00 \times 10^{7} \pm \infty$	$2.00 \times 10^{2} \pm \infty$	$6.00 \pm \infty$
α (–)	$1.35 \times 10^{-1} \pm \infty$	$3.68 \times 10^{-1} \pm \infty$	$(7.78 \pm 1.11) \times 10^{-1}$	$1.02 \pm \infty$	$2.98 \pm \infty$
β (h^{-1})	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$
$Y_{X/R}$ (–)	$5.00 \times 10^{-1} \pm \infty$	$5.00 \times 10^{-1} \pm \infty$	$5.00 \times 10^{-1} \pm \infty$	$5.00 \times 10^{-1} \pm \infty$	$5.01 \times 10^{-1} \pm \infty$
$Y_{P/R}$ (–)	$9.80 \times 10^{-1} \pm \infty$	$9.80 \times 10^{-1} \pm \infty$	$9.80 \times 10^{-1} \pm \infty$	$9.80 \times 10^{-1} \pm \infty$	$9.80 \times 10^{-1} \pm \infty$
m (h^{-1})	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$	$1.00 \times 10^{-30} \pm \infty$
C_{R_0} ($kg.m^{-3}$)	$(3.01 \pm 1.20) \times 10$	$3.00 \times 10 \pm \infty$	$(3.321 \pm 0.493) \times 10$	$(3.000 \pm 0.379) \times 10$	$(4.500 \pm 0.468) \times 10$
D_R ($m^2.s^{-1}$)	$(8.04 \pm 6.48) \times 10^{-13}$	$(1.000 \pm 0.677) \times 10^{-12}$	$(8.48 \pm 7.55) \times 10^{-13}$	$(7.19 \pm 4.61) \times 10^{-12}$	$(1.920 \pm 0.561) \times 10^{-11}$
ϵ (–)	$(9.80 \pm 1.09) \times 10^{-2}$	$9.80 \times 10^{-1} \pm \infty$	$(9.33 \pm 5.87) \times 10^{-2}$	$9.80 \times 10^{-1} \pm \infty$	$9.90 \times 10^{-1} \pm \infty$
ϕ (–)	$(3.20 \pm 1.03) \times 10^{-6}$	$(3.05 \pm 2.18) \times 10^{-6}$	$(2.008 \pm 0.968) \times 10^{-5}$	$(3.050 \pm 0.347) \times 10^{-6}$	$3.80 \times 10^{-8} \pm \infty$
D_P ($m^2.s^{-1}$)	$5.02 \times 10^{-13} \pm \infty$	$6.20 \times 10^{-13} \pm \infty$	$5.66 \times 10^{-13} \pm \infty$	$5.95 \times 10^{-13} \pm \infty$	$6.10 \times 10^{-13} \pm \infty$
D_S ($m^2.s^{-1}$)	$(2.801 \pm 0.356) \times 10^{-12}$	$(3.300 \pm 0.457) \times 10^{-12}$	$(2.743 \pm 0.303) \times 10^{-12}$	$(2.900 \pm 0.326) \times 10^{-12}$	$(3.750 \pm 0.355) \times 10^{-12}$

Note: k_R = 1.03 (%w/w / %w/v); k_P = 0.89 (%w/w / %w/v); k_S = 0.70 (%w/w / %w/v).

approach of dividing the whole set of parameters into subsets of directly related parameters that are to be estimated via fits to separate sets of experiments was followed with respect to parameters k_R, k_P, and k_S in view of the thermodynamic nature of such equilibrium constants (which must, for actual thermodynamic consistency, take the same fitted value irrespective of the experimental layout); however, a similar approach for the remaining adjustable parameters was deliberately not considered because the interaction effects would not be decoupled (e.g., the inhibition pattern depends on the physiological state of the starter culture, which in turn depends on the sugar inventory in solution, which in turn depends on the rate of diffusion from the carrot, which in turn depends on the sugar inventory in solution via a boundary condition, which in turn depends on the rate of metabolism of sugars by the microorganism, which in turn depends on the inhibition of the microorganisms by soluble sugars) and the physicochemical advantage arising from the use of the one-parameter-at-a-time fitting pattern from the physicochemical point of view would probably be offset by the disadvantage of poor fits associated with imposing a given set of predetermined parameter estimates in attempts to describe our actual experimental situations. In the present analysis, the model forms previously tested for the diffusion behaviour with respect to reducing sugars, NaCl, and lactic acid (Nabais *et al.*, 1995) were considered in the definition of the base model, and the corresponding parameter estimates were used as starting estimates in the overall fitting of the basic model (although GREG was allowed to proceed unconstrained until convergence was achieved). One of the clear advantages of the overall approach considered was identification of parameters that consistently cannot be estimated beyond doubt from any of the 25 independent data sets produced: such parameters were β, ψ, ξ and m. Hence, only 13 parameters could be, at best, clearly fitted given the overall data set generated; although it may be claimed that virtually any type of data set can be fitted with such a high number of parameters, it was the first time that such a concerted attempt has been successfully effected with a fermentation system because (as seen below) most fitted parameters still satisfy reasonable physicochemical constraints. On the other hand, it should be emphasized that, in addition to the 25 independent experimental conditions considered, the evolution throughout fermentation time gave rise to between 13 and 22 independent samples associated with each of the aforementioned experimental conditions, with as many independent physicochemical conditions.

Since our experimental layout included more than one experimental response (biomass concentration, reducing sugar concentration, NaCl concentration, and lactic acid concentration, all measured in the

brine) for each experiment, then information from all measured responses should be used to provide more precise parameter estimation; hence, such information ought to be combined to reflect reasonable assumptions about the behaviour of the disturbance terms in the measurements (Bates and Watts, 1988). The determinant parameter estimation criterion used for our multiresponse data was initially derived by Box and Draper (1965) under the assumptions that the disturbance terms of a given response in different experiments are uncorrelated with one another (which was probably the case since independent experimental batches were sampled at different fermentation times for each combination of NaCl concentration of the brine and processing temperature) but the disturbance terms of different responses in a given experiment have a fixed (unknown) variance–covariance matrix (which was likely the case because different aliquots of the same sample were analytically assayed). A simpler, yet more unrealistic, alternative approach would be to consider that disturbance terms of different responses in the same experiment are not correlated with one another (i.e. all residuals are normally distributed and independent with the same variance); if this assumption were true, then least squares of residuals would be appropriate and the estimation criterion would be to minimize the trace of **R**, *tr*(**R**) (Bates and Watts, 1988). Two major advantages of the determinant criterion employed in this analysis relative to the pseudo-uniresponse counterpart just discussed are: (*i*) the inference regions of the former are usually much smaller than those of the latter; and (*ii*) the former criterion is much more accurate than the latter in discriminating between rival models. Therefore, the statistical selection of the most complex form of the models, one of the objectives of our work, was rather safe and accurate in view of the determinant, multiresponse criterion used. This statement is consubstantiated via computation of the *t*-ratio for each parameter (i.e., the ratio of the parameter confidence interval to the parameter estimate), which is considerably below unity for most parameter estimates. On the other hand, not all parameters in the full model could be estimated; the results shown in Tables 6–10 suggest that partial (or simplified) models containing only those parameters for which clear estimates could be obtained at least once (Tables 1–5) are statistically adequate, i.e. the decrease in the determinant of the residual matrix brought about by changing from the partial form of the model to the full (basic) model is statistically negligible at the 5% confidence level (or, in other words, there is less than 5% probability that such high values for the ratio of the mean determinants are due to pure chance). Finally, in the fits obtained (Figures 1–10) no biased trends of the residuals of the experi-

Table 6. Analyses of extra determinants or the models fitted to data obtained with brines containing 0% (w/v) of salt.

Tabla 6. Análisis del determinante suplemental para los modelos ajustados con los datos de la salmuera con un 0% (w/v) de sal.

Temperature (°C)	Source	Determinant	NDF[a]	Mean determinant[b]	*F*-ratio[c]	*F*-ratio(5%)[d]
15	Extra parameters	2.51×10^{-12}	4	6.28×10^{-13}	0.616	6.26
	Full model	5.11×10^{-12}	5	1.02×10^{-12}		
	Partial model	7.62×10^{-12}	9			
20	Extra parameters	8.1×10^{-19}	4	2.03×10^{-19}	0.0525	6.94
	Full model	7.74×10^{-18}	2	3.87×10^{-18}		
	Partial model	8.55×10^{-18}	6			
30	Extra parameters	7.0×10^{-13}	4	1.75×10^{-13}	1.42	6.04
	Full model	9.8×10^{-13}	8	1.23×10^{-13}		
	Partial model	1.68×10^{-12}	12			
40	Extra parameters	3.6×10^{-5}	4	9.00×10^{-6}	1.59	5.91
	Full model	6.8×10^{-5}	12	5.67×10^{-6}		
	Partial model	1.04×10^{-4}	16			
45	Extra parameters	3.6×10^{-15}	4	9.00×10^{-16}	0.288	5.94
	Full model	3.44×10^{-14}	11	3.13×10^{-15}		
	Partial model	3.80×10^{-14}	15			

[a]NDF = number of experiments – number of parameters fitted, or number of degrees of freedom
[b]Mean determinant = determinant/number of degrees of freedom
[c]*F*-ratio = extra mean determinant/mean determinant of the full model
[d]*F*-value = value of *F*-ratio that would lead to acceptance of null hypothesis at the 5% significance level.

Table 7. Analyses of extra determinants for the models fitted to data obtained with brines containing 5% (w/v) of salt.

Tabla 7. Análisis del determinante suplemental para los modelos ajustados con los datos de la salmuera con un 5% (w/v) de sal.

Temperature (°C)	Source	Determinant	NDF[a]	Mean determinant[b]	*F*-ratio[c]	*F*-ratio(5%)[d]
15	Extra parameters	4.5×10^{-13}	4	1.13×10^{-13}	0.299	6.26
	Full model	1.89×10^{-12}	5	3.78×10^{-13}		
	Partial model	2.34×10^{-12}	9			
20	Extra parameters	1.01×10^{-23}	4	2.53×10^{-24}	0.239	6.94
	Full model	2.11×10^{-23}	2	1.06×10^{-23}		
	Partial model	3.12×10^{-23}	6			
30	Extra parameters	2.68×10^{-13}	4	6.70×10^{-14}	0.852	6.04
	Full model	6.29×10^{-13}	8	7.86×10^{-14}		
	Partial model	8.97×10^{-13}	12			
40	Extra parameters	1×10^{-20}	4	2.50×10^{-21}	0.00529	5.91
	Full model	5.67×10^{-18}	12	4.73×10^{-19}		
	Partial model	5.68×10^{-18}	16			
45	Extra parameters	1.12×10^{-11}	4	2.80×10^{-12}	0.778	5.94
	Full model	3.96×10^{-11}	11	3.60×10^{-12}		
	Partial model	5.08×10^{-11}	15			

[a]NDF = number of experiments – number of parameters fitted, or number of degrees of freedom
[b]Mean determinant = determinant/number of degrees of freedom
[c]*F*-ratio = extra mean determinant/mean determinant of the full model
[d]*F*-value = value of *F*-ratio that would lead to acceptance of null hypothesis at the 5% significance level.

Table 8. Analyses of extra determinants for the models fitted to data obtained with brines containing 7.5% (w/v) of salt.

Tabla 8. Análisis del determinante suplemental para los modelos ajustados a los datos de la salmuera con un 7,5% (w/v) de sal.

Temperature (°C)	Source	Determinant	NDF[a]	Mean determinant[b]	*F*-ratio[c]	*F*-ratio(5%)[d]
15	Extra parameters	2.51×10^{-14}	4	6.28×10^{-15}	0.678	6.26
	Full model	4.63×10^{-14}	5	9.26×10^{-15}		
	Partial model	7.14×10^{-14}	9			
20	Extra parameters	7.5×10^{-11}	4	1.88×10^{-11}	0.145	6.94
	Full model	2.59×10^{-10}	2	1.30×10^{-10}		
	Partial model	3.34×10^{-10}	6			
30	Extra parameters	8.7×10^{-29}	4	2.18×10^{-29}	0.212	6.04
	Full model	8.27×10^{-28}	8	1.03×10^{-28}		
	Partial model	9.14×10^{-28}	12			
40	Extra parameters	7.4×10^{-7}	4	1.85×10^{-7}	0.925	5.91
	Full model	2.40×10^{-6}	12	2.00×10^{-7}		
	Partial model	3.14×10^{-6}	16			
45	Extra parameters	1.13×10^{-61}	4	2.83×10^{-62}	0.983	5.94
	Full model	3.17×10^{-61}	11	2.88×10^{-62}		
	Partial model	4.30×10^{-61}	15			

[a]NDF = number of experiments – number of parameters fitted, or number of degrees of freedom
[b]Mean determinant = determinant/number of degrees of freedom
[c]*F*-ratio = extra mean determinant/mean determinant of the full model
[d]*F*-value = value of *F*-ratio that would lead to acceptance of null hypothesis at the 5% significance level.

Table 9. Analyses of extra determinants for the models fitted to data obtained with brines containing 10% (w/v) of salt.

Tabla 9. Análisis del determinante suplemental para los modelos ajustados a los datos de la salmuera con un 10% (w/v) de sal.

Temperature (°C)	Source	Determinant	NDF[a]	Mean determinant[b]	*F*-ratio[c]	*F*-ratio(5%)[d]
15	Extra parameters	3.02×10^{-22}	4	7.55×10^{-23}	0.712	6.26
	Full model	5.29×10^{-22}	5	1.06×10^{-22}		
	Partial model	8.31×10^{-22}	9			
20	Extra parameters	1.92×10^{-6}	4	4.80×10^{-7}	0.306	6.94
	Full model	3.14×10^{-6}	2	1.57×10^{-6}		
	Partial model	5.06×10^{-6}	6			
30	Extra parameters	1.4×10^{-6}	4	3.50×10^{-7}	0.285	6.04
	Full model	9.8×10^{-6}	8	1.23×10^{-6}		
	Partial model	1.12×10^{-5}	12			
40	Extra parameters	1.45×10^{-12}	4	3.63×10^{-13}	1.78	5.91
	Full model	2.45×10^{-12}	12	2.04×10^{-13}		
	Partial model	3.90×10^{-12}	16			
45	Extra parameters	3.4×10^{-67}	4	8.50×10^{-68}	0.964	5.94
	Full model	9.7×10^{-67}	11	8.82×10^{-68}		
	Partial model	1.31×10^{-66}	15			

[a]NDF = number of experiments – number of parameters fitted, or number of degrees of freedom
[b]Mean determinant = determinant/number of degrees of freedom
[c]*F*-ratio = extra mean determinant/mean determinant of the full model
[d]*F*-value = value of *F*-ratio that would lead to acceptance of null hypothesis at the 5% significance level.

Table 10. Analyses of extra determinants for the models fitted to data obtained with brines containing 15% (w/v) of salt.

Tabla 10. Análisis del determinante suplemental para los modelos ajustados a los datos de la salmuera con un 15% (w/v) de sal.

Temperature (°C)	Source	Determinant	NDF[a]	Mean determinant[b]	*F*-ratio[c]	*F*-ratio(5%)[d]
15	Extra parameters	2.0×10^{-17}	4	5.00×10^{-18}	0.291	6.26
	Full model	8.6×10^{-17}	5	1.72×10^{-17}		
	Partial model	1.06×10^{-16}	9			
20	Extra parameters	1.70×10^{-8}	4	4.25×10^{-9}	0.253	6.94
	Full model	3.36×10^{-8}	2	1.68×10^{-8}		
	Partial model	5.06×10^{-8}	6			
30	Extra parameters	1.10×10^{-9}	4	2.75×10^{-10}	2.15	6.04
	Full model	1.02×10^{-9}	8	1.28×10^{-10}		
	Partial model	2.12×10^{-9}	12			
40	Extra parameters	1.9×10^{-13}	4	4.75×10^{-14}	0.679	5.91
	Full model	8.4×10^{-13}	12	7.00×10^{-14}		
	Partial model	1.03×10^{-12}	16			
45	Extra parameters	1.92×10^{-18}	4	4.80×10^{-19}	0.932	5.94
	Full model	5.66×10^{-18}	11	5.15×10^{-19}		
	Partial model	7.58×10^{-18}	15			

[a]NDF = number of experiments – number of parameters fitted, or number of degrees of freedom
[b]Mean determinant = determinant/number of degrees of freedom
[c]*F*-ratio = extra mean determinant/mean determinant of the full model
[d]*F*-value = value of *F*-ratio that would lead to acceptance of null hypothesis at the 5% significance level.

mental data with respect to the model are apparent, and this observation provides an indication that convergence to the true minimum in the determinant of the residual matrix was achieved.

According to the general form of the model depicted in Equation (1), the maximum specific growth rate, μ_{ap} was assumed to change in time, t, according to $\mu_{ap} = \mu_{max} [1 + \psi \exp(-\xi t)]$, where μ_{max}, ψ and ξ are constants. This empirical form implies that the specific growth rate is minimum at $t = 0$ but increases faster and faster as time elapses up to a maximum value of μ_{max}; hence, both the lag and exponential phases can be easily (and accurately) described. However, inspection of Tables l-5 indicates that parameters ψ and ξ could not be fitted to any data set whatsoever with a finite marginal confidence interval, and the estimates to which convergence was achieved always corresponded to $\psi \exp(-\xi t) << 1$, irrespective of the actual value of t considered in our experiments. Although this observation would statistically suggest absence of a lag phase for microbial growth, it actually results from a disguise of the microbial lag phase by the initial lag phase associated with leaching of sugars (i.e. the microbial nutrients) from the carrot material to the brine solution as required by disruption of the carrot cell wall; it should be emphasized that when temperature increases the microbial lag phase decreases (because implementation of the enzymatic machinery is faster due to higher metabolic rates) and the time required for extensive diffusion to be initiated also decreases (Nabais *et al.*, l995). Decreases in the specific growth rate which occur in the stationary phase are accounted for in the model depicted in Equation (l) by the inhibition term in the denominator associated with the increasing concentration of lactic acid, $(K_p + C_p)$, where K_p is a constant. As can be concluded from Equation (2), the diffusivity of sugars, $D_{R,ap}$, was also considered variable in time, $D_{R,ap} = D_R/(1 - \varepsilon(1 - \exp\{-\phi t\}))$ (where D_R, ε and ϕ are adjustable parameters), instead of the diffusivity of lactic acid or NaCl, which were assumed to be given by parameters D_p and D_S, respectively. The rationale behind these assumptions is derived from the fact that whereas both lactic acid and NaCl can diffuse more or less freely from the brine into the intercellular spaces within the carrot material, a similar diffusion of reducing sugars in the opposite direction is possible only after these bulky, cytoplasmic compounds have crossed the cell membrane/wall barrier (a process that requires the time-consuming preliminary step of bursting the carrot cells). As discussed elsewhere (Nabais *et al.*, 1995), the functional form elected is associated with the assumption that the rate of cell bursting is first order with respect to the amount of intact cells and also first order with respect to the amount of burst cells.

Table l shows that the initial condition associated with the concentration of reducing sugars in the carrot was considered as an adjustable parameter unlike the initial conditions associated with the concentrations of either NaCl or lactic acid. This decision was due to the high variability in the initial mass inventory of the carrots in reducing sugars, which depends heavily on the maturation state of these vegetable when harvested (Nabais and. Malcata, 1995a).

The estimated values for μ_{max} ($6 \times 10^{-9} - 3.9 \times 10^{-2}$/h) are within the range usually reported in the literature (McDonald *et al.*, 1990; Passos *et al.*, 1994). It is interesting to note that μ_{max} goes through a maximum at ca 30 °C for every initial NaCl concentration considered, but these maxima tend to decrease as the initial concentration of NaCl increases (Tables l–5). Although NaCl seems to have a direct inhibitory effect upon the intrinsic growth rate of *L. plantarum* in the heterogeneous system studied, it should he noted that lactic acid is produced as fermentation time elapses and it also exhibits an inhibitory effect; this fact may partially account for (for example) the value of $3.81 \times l0^{-5}$ for μ_{max} at 0% NaCl and 40 °C (see Table l) which leads to a final acid level of ca 7.2 kg/m^3, coupled with the value of 1.68×10^{-3} for μ_{max} at 5% NaCl and the same temperature (see Table 2) which leads to a final acid level of ca 3.75 kg/m^3 .

The production of lactic acid is clearly growth-associated as shown by Figure l0a where the ratio of the rate of increase of biomass concentration to rate of increase of lactic acid concentration remains essentially constant; this observation, which mathematically reads $dC_p/dC_X = \alpha$, is consistent with the form of Equation (3) after setting β equal to zero. Further confirmation of this conclusion is obtained indirectly from the realization that parameter β could not be fitted within finite marginal confidence intervals irrespective of the environmental conditions selected (Tables 1–5). This conclusion is, however, in disagreement to some extent with the conclusions of Passos *et al.* (1994). Since parameter α could be estimated for various temperatures, logarithmic plots of the estimates of α obtained at each initial NaCl concentration versus the reciprocal of the absolute temperature were prepared and yielded activation energies of 3.05, 15.0l, 13.42, 17.43, and 7.97 kJ/mol for brines with 0, 5, 7.5, l0, and 15% NaCl, respectively. These figures indicate that α indeed varies with temperature following an Arrhenius dependence, but there seems to be a maximum for the activation energy at ca 8% NaCl. If the analysis shown in Table 10 is repeated

in a similar fashion for the Arrhenius model vs the assumption that no particular dependency of α on temperature exists using the sum of squares of the residuals instead of the determinant of the residuals (Nabais and Malcata, 1995a), the mean sum of squares for the Arrhenius model is 0.0395 whereas the incremental mean sum of squares is 0.249, which yields an F-ratio of 6.30, which is above the critical value of the F statistic for one and 19 degrees of freedom at the 5% significance level. Hence, the temperature dependence postulated is statistically significant. The Monod constants associated with the reducing sugars and the lactic acid (i.e. K_R and K_P, respectively) could both be fitted in several experimental conditions, unlike constant K_S associated with NaCl, which could only be fitted for data obtained at 45 °C and 5% or 10% NaCl (Tables 1–5). Parameter K_R ranges up to 3×10^{-1} g/L, whereas parameter K_P ranges up to 1.1×10^{-1} g/L.

The intrinsic diffusivity of the reducing sugars within the carrot matrix ranges from 2×10^{-14} to 3×10^{-11} m^2/s, whereas the diffusivity of lactic acid under similar conditions ranges from 1.5×10^{-12} to 7.3×10^{-11}, and that of NaCl ranges from 2.6×10^{-13} to 3.8×10^{-11} (Tables 1–5). The ranges encountered are similar to those previously found (Nabais *et al.*, 1995) when the transport of the same compounds was studied in an independent fashion in the absence of lactic fermentation. It is interesting that the upper limit of such diffusivities is between two and three orders of magnitude below that of a typical diffusivity within a liquid, and such differences might be explained by the complex network nature of the carrot material. Assumption of an Arrhenius dependence of both D_S and D_P on temperature yielded the following estimates for the activation energies: 5.72, 6.87, 4.42, and 0.42 kJ/mol for brines with 5, 7.5, 10, and 15% NaCl, respectively, for the diffusivity of NaCl; and 2.69, 7.01, 3.02, 1.28, and 0.36 kJ/mol for brines with 0, 5, 7.5, 10, and 15% NaCl, respectively, for the diffusivity of acid. These values are small enough for diffusion to be considered as a purely physical process as is the case. Parameters ε and ϕ, which are used in Equation (2) to account for the sigmoidal behaviour of the apparent diffusivity of the reducing sugars with time, are easily fitted for low temperatures, but become indeterminate at high temperatures; this observation, which indicates that the transport of reducing sugars tends to follow a Fickian mechanism as temperature increases, is in agreement with results reported elsewhere (Nabais *et al.*, 1995).

The yield of lactic acid on reducing sugars, $Y_{P/R}$, ranges from 0.1 to 0.999 (Tables 6–10), which are acceptable limits in view of carbon source balances, although those estimates closer to 0.999 are somewhat suspicious and should be regarded mainly as statistical estimates without emphasizing their particular physicochemical meaning. By the same token, the values of 0.112 and 0.999 for the yield of biomass on reducing sugars listed in Table 1 and in Table 3 for 20 °C are reasonable.

Since the reducing sugars appear in the brine as a result of diffusion from the carrot material and are simultaneously consumed in order to permit microbial growth and lactic acid production (but not maintenance, an assertion that derives from the impossibility to fit parameter m as shown by Tables 1–5), it is expected that a maximum in the concentration of reducing sugars in solution may eventually occur. This fact is apparent from inspection of Figures 2b, 4b, 6b and 8b, which represent those experimental conditions that favour microbial growth (i.e. temperature of 30 °C and intermediate initial NaCl concentrations). It is also noteworthy that the time at which such an optimum is observed becomes displaced as the initial NaCl concentration increases (ca 30, 45, 50, and 70 h, respectively), which is consistent with previous discussion on the issue that increasing NaCl concentrations lead to lower maximum growth rates beyond a certain threshold.

The experimental ranges selected for the carrot fermentation batches were rather wide in terms of both the temperature and the NaCl concentration of the brine in order to produce the scientific data necessary to comprehensively characterize the performance of *Lactobacillus plantarum* in the presence of diffusion limitations for transport of solutes within the carrot material. Despite the fact that the more meaningful results from a practical standpoint are those associated with NaCl concentrations below 15% w/v and temperatures in the range 20–30 °C, the aforementioned experimental ranges fulfilled their goal to a large extent in that they allowed fitting of a considerable number of parameters on the 5% level of statistical significance.

NOMENCLATURE

- A Overall specific outer surface area of the slice (m^{-1})
- C Concentration in the brine (kg/m^3)
- C Concentration in the carrot material (kg/m^3)
- D Diffusivity within the carrot material (m^2/s)
- k Partition coefficient between the solution and the carrot material [%(w/w) /%(w/v)]
- K Monod constant (kg/m^3)
- L Half-thickness of the slice (m)

m Maintenance coefficient with respect to reducing sugars (h^{-1})
M Number of measurements in each experiment
N Number of experiments
t Time elapsed after inoculation (h)
x Longitudinal co-ordinate within the carrot slice (m)
Y Yield (dimensionless)
α Leudeking–Piret parameter associated with growth rate (dimensionless)
β Leudekin–Piret parameter associated with actual biomass (h^{-1})
δ Molecular path (m)
ε Initial integrity factor of carrot cells (dimensionless)
ϕ Kinetic constant describing structural decay of carrot material (h^{-1})
ψ Pre-exponential factor associated with the lag phase (dimensionless)
ξ Exponential factor associated with the lag phase (h^{-1})
μ Specific growth rate (h^{-1})

Subscripts

0 initial conditions
ap apparent value
max maximum value
P lactic acid
R reducing sugars
S NaCl
X biomass
X/R biomass on substrate
P/R product on substrate

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APPENDIX

Solution of Equations (1)–(7) was obtained numerically using the one-sided, finite-difference method. For such a method, the discretized form of the model takes the following form:

$$C_X^{(i+1)} = C_X^{(i)} + \frac{\mu_{max} K_P K_S C_R^{(i)} C_X^{(i)} \Delta t}{[1 + \Psi \exp(-\xi i \Delta t)](K_R + C_R^{(i)})(K_P + C_P^{(i)})(K_S + C_S^{(i)})} \tag{A.1i}$$

$$C_X^{(0)} = C_{X_0} \tag{A.1ii}$$

$$C_R^{(i+1)} = C_R^{(i)} - \frac{A}{\Delta x}\left(\frac{D_R}{1 - \varepsilon[1 - \exp(-\phi t)]}\left(\hat{C}_R^{(i,M)} - \hat{C}_R^{(i,M-1)}\right) + \frac{C_X^{(i)} - C_X^{(i-1)}}{Y_{X/R}\,\Delta t} + \frac{C_P^{(i)} - C_P^{(i-1)}}{Y_{P/R}\,\Delta t} + m\, C_X^{(i)}\right)\Delta t \tag{A.2i}$$

$$C_R^{(0)} = 0 \tag{A.2ii}$$

$$C_P^{(i+1)} = C_P^{(i)} + \alpha \frac{C_X^{1(i)} - C_X^{(i-1)}}{\Delta t} + \beta\, C_X^{(i)} - \frac{A\, D_P(\hat{C}_P^{(i,M)} - \hat{C}_P^{(i,M-1)})\,\Delta t}{\Delta x} \tag{A.3i}$$

$$C_P^{(0)} = 0 \tag{A.3ii}$$

$$C_S^{(i+1)} = C_S^{(i)} - \frac{A\, D_S\,(\hat{C}_S^{(i,M)} - \hat{C}_S^{(i,M-1)})\,\Delta t}{\Delta x} \tag{A.4i}$$

$$C_S^{(0)} = C_{S_0} \tag{A.4ii}$$

$$\hat{C}_R^{(i,j)} = \hat{C}_R^{(i-1,j)} + \frac{D_R\,(\hat{C}_R^{(i-1,j-1)} - 2\hat{C}_R^{(i-1,j)} + \hat{C}_R^{(i-1,j+1)})\,\Delta t}{\{1 - \varepsilon[1 - \exp(-\phi\, i\, \Delta t)]\}\,(\Delta x)^2} \tag{A.5i}$$

$$\hat{C}_R^{(0,j)} = \hat{C}_{R_0},\quad 0 \leqslant j \leqslant M \tag{A.5ii}$$

$$\hat{C}_R^{(i,1)} = \hat{C}_R^{(i,0)},\quad 0 \leqslant i \leqslant N \tag{A.5iii}$$

$$\hat{C}_R^{(i,M)} = k_R\,\hat{C}_R^{(i)},\quad 0 \leqslant i \leqslant N \tag{A.5iv}$$

$$\hat{C}_P^{(i,j)} = \hat{C}_P^{(i-1,j)} + \frac{D_P\,(\hat{C}_P^{(i-1,j-1)} - 2\hat{C}_P^{(i-1,j)} + \hat{C}_P^{(i-1,j+1)})\,\Delta t}{(\Delta x)^2} \tag{A.6i}$$

$$\hat{C}_P^{(0,j)} = 0,\quad 0 \leqslant j \leqslant M \tag{A.6ii}$$

$$\hat{C}_P^{(i,1)} = \hat{C}_P^{(i,0)},\quad 0 \leqslant i \leqslant N \tag{A.6iii}$$

$$\hat{C}_P^{(i,M)} = k_P\,\hat{C}_P^{(i)},\quad 0 \leqslant i \leqslant N \tag{A.6iv}$$

$$\hat{C}_S^{(i,j)} = \hat{C}_S^{(i-1,j)} + \frac{D_S\,(\hat{C}_S^{(i-1,j-1)} - 2\hat{C}_S^{(i-1,j)} + \hat{C}_S^{(i-1,j+1)})\,\Delta t}{(\Delta x)^2} \tag{A.7i}$$

$$\hat{C}_S^{(0,j)} = 0,\quad 0 \leqslant j \leqslant M \tag{A.7ii}$$

$$\hat{C}_S^{(i,1)} = \hat{C}_S^{(i,0)},\quad 0 \leqslant i \leqslant N \tag{A.7iii}$$

$$\hat{C}_S^{(i,M)} = k_S\,\hat{C}_S^{(i)},\quad 0 \leqslant i \leqslant N \tag{A.7iv}$$

where the integer variable i takes values in the range $0 \leqslant i \leqslant N = t_f/\Delta t$ (where t_f is the largest time of the experiments and Δt is the desired step increment in the time co-ordinate), the integer variable j takes values in the range $0 \leqslant j \leqslant M = L/\Delta x$ (where Δx is the desired step increment in the spatial coordinate), and where superscripts i and j denote the value at time $i.\Delta t$ and at position $j.\Delta x$, respectively.