

## Article

# Nisin Synthesis, Relationship with the Growth of the Producing Strain, Culture pH and Nitrogen Consumption

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**Abstract:** Nisin, an antibacterial compound produced by *Lactococcus lactis* strains, has been approved by the US Food and Drug Administration to be used as a safe food additive to control the growth of undesirable pathogenic bacteria. Nisin is commonly described as a pH-dependent primary metabolite, since its production depends on growth and culture pH evolution. However, the relationship between bacteriocin synthesis and the consumption of the limiting nutrient has not been described until now. Therefore, this study aimed to develop a competitive four-dimensional Lotka Volterra-like equation to describe the relationships between culture pH, limiting nutrient (total nitrogen: TN) consumption and production of biomass (X) and nisin (BT) in four series of batch fermentation with *L. lactis* CECT 539 in diluted whey (DW)-based media. The developed four-dimensional LV-like equation (with a unique set of parameters) could not be used to describe all cultures belonging to each fermentation series. However, the four-dimensional LV-like equation described accurately each individual culture, providing a good description of the relationships between pH, TN, X and BT, higher values for  $R^2$  and  $F$ -ratios, lower values ( $< 10\%$ ) for the mean relative percentage deviation modulus, with bias and accuracy factor values approximately equal to one.

**Keywords:** nisin; antibacterial molecule; batch fermentation; four-dimensional LV equation; *Lactococcus lactis*.

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## 1. Introduction

Nisin, a bacteriocin produced by *Lactococcus lactis* strains, has a wide antibacterial activity against food spoilage and pathogenic bacteria. For this reason, this biomolecule has been recognized by the US Food and Drug Administration as a natural and safe bio-preservative in food products, being allowed in the USA and several European Union countries. The advantages of using nisin in foods include the reduction in both the thermal treatment and addition of chemicals to food products and increase in their shelf life [1].

For high nisin production at low cost, it is necessary to know the relationship between the main culture variables, that could be elucidated with the use of appropriate mathematical models. This could also allow a proper monitoring and control of these bioprocesses [2,8].

Different mathematical models have been commonly used to describe the kinetics of growth (e.g., Verhulst, Gompertz, Richards, Bertalanffy, Weibull, Monod) and bacteriocin synthesis (e.g. unmodified and modified forms of the Luedeking and Piret model) by lactic acid bacteria (LAB) in batch fermentations [2–7].

An appropriate model for biomass production should consider the main factors affecting growth, including the effect of the time-dependent dynamics of other culture variables (culture pH and the concentrations of nutrients and products) [2,3,5,9,10]. In addition, a model describing nisin synthesis should relate the kinetics of product formation rate to the growth rate and biomass concentration [11], but if another factor (*e.g.* pH or an essential nutrient) produces a specific effect on product synthesis, a term for explaining such effect should be included in the model [5,9,12]. However, models describing the relationships between the dynamics of culture pH, the limiting nutrient consumption and production of biomass and nisin are scarce [5,9,11,12].

In a previous study [8], a three-dimensional Lotka Volterra (LV)-like equation was developed to explain, in the simplest way, the relationships between the kinetics of culture pH, growth and bacteriocin production by different LAB in various culture media. Nevertheless, this simple three-dimensional equation neither accounts for the effect of total nitrogen (TN) consumption (the growth limiting nutrient in these fermentations [5,8]) on the growth and nisin production, nor includes an equation to describe the dynamics of TN consumption for biomass and nisin synthesis.

Therefore, in this study, a four-dimensional equation based on competitive Lotka–Volterra (LV) system assumptions [13] was developed for the first time to give a best description of the nisin production system of *L. lactis* CECT 539 in different batch fermentations in diluted whey (DW) media containing different initial nutrient compositions or adjusted to various pH values [14–16].

The values of the biokinetic parameters for each equation were determined using statistical analysis (non-linear regression). The adequacy and utility of the developed four-dimensional equation were discussed by taking into account the statistical significance of the model parameters, the trends observed in the experimental data and the different culture media used.

## 2. Results and Discussion

### 2.1. Modeling the Batch Nisin Production System in Different Series of Fermentation in DW Media Using a Global Set of Model Parameters

The capability of the four-dimensional LV equation (1)–(4) to describe the batch nisin production system was first assessed by adjusting a unique global set of model parameters (general equation) to the entire set of experimental data in different series of batch cultures. The fermentations were performed in culture media (Tables 1 and 2) prepared with diluted whey (DW) containing different initial: i) concentrations of glucose (DW-G series) [14], ii) concentrations of total sugars and phosphorous (DW-TS-TP series) [15], iii) concentrations of MRS broth nutrients (DW-MRS series) [16], or in DW100 medium adjusted to different initial pH values (DW-pH series) [16].

**Table 1.** Initial concentrations (mean  $\pm$  standard deviations) of total sugars (TS), nitrogen (TN), phosphorous (TP) and proteins (Pr) in culture media prepared with deproteinized diluted whey (DW) and concentrated mussel-processing wastes (CMPW) [14,16].

| Medium | TS (g/L)           | TN (g/L)         | TP (g/L)         | Pr (g/L)          |
|--------|--------------------|------------------|------------------|-------------------|
| DW     | 20.54 $\pm$ 0.514  | 0.45 $\pm$ 0.014 | 0.25 $\pm$ 0.021 | 2.04 $\pm$ 0.083  |
| DW25   | 20.96 $\pm$ 0.021  | 1.20 $\pm$ 0.006 | 0.30 $\pm$ 0.001 | 5.39 $\pm$ 0.025  |
| DW50   | 21.93 $\pm$ 0.014  | 1.96 $\pm$ 0.013 | 0.36 $\pm$ 0.05  | 8.72 $\pm$ 0.034  |
| DW75   | 22.89 $\pm$ 0.016  | 2.72 $\pm$ 0.002 | 0.43 $\pm$ 0.07  | 12.05 $\pm$ 0.019 |
| DW100  | 23.85 $\pm$ 0.010  | 3.49 $\pm$ 0.016 | 0.50 $\pm$ 0.017 | 15.38 $\pm$ 0.031 |
| DW125  | 24.81 $\pm$ 0.023  | 4.25 $\pm$ 0.004 | 0.57 $\pm$ 0.013 | 18.71 $\pm$ 0.026 |
| CMPW   | 101.33 $\pm$ 1.314 | 0.54 $\pm$ 0.024 | 0.06 $\pm$ 0.009 | 3.47 $\pm$ 0.046  |

**Table 2.** Initial concentrations (mean  $\pm$  standard deviations) of TS, TN, TP and Pr in culture media prepared with DW medium mixed with different volumes of CMPW medium and supplemented with  $\text{KH}_2\text{PO}_4$  to give different initial TS and TP concentrations (DW-TS-TP cultures) [15].

| Points                   | Experiment | TS (g/L)           | TP (g/L)          | TN (g/L)          | Pr (g/L)          |
|--------------------------|------------|--------------------|-------------------|-------------------|-------------------|
| Factorial                | 1          | 48.321 $\pm$ 0.001 | 0.589 $\pm$ 0.001 | 0.479 $\pm$ 0.002 | 2.531 $\pm$ 0.018 |
|                          | 2          | 48.321 $\pm$ 0.001 | 0.281 $\pm$ 0.001 | 0.479 $\pm$ 0.002 | 2.531 $\pm$ 0.018 |
|                          | 3          | 25.639 $\pm$ 0.001 | 0.589 $\pm$ 0.001 | 0.453 $\pm$ 0.003 | 2.122 $\pm$ 0.028 |
|                          | 4          | 25.639 $\pm$ 0.001 | 0.281 $\pm$ 0.001 | 0.453 $\pm$ 0.003 | 2.122 $\pm$ 0.028 |
| Axial                    | 5          | 51.352 $\pm$ 0.003 | 0.435 $\pm$ 0.002 | 0.483 $\pm$ 0.001 | 2.583 $\pm$ 0.047 |
|                          | 6          | 22.611 $\pm$ 0.001 | 0.435 $\pm$ 0.002 | 0.450 $\pm$ 0.001 | 2.068 $\pm$ 0.012 |
|                          | 7          | 36.984 $\pm$ 0.002 | 0.631 $\pm$ 0.003 | 0.466 $\pm$ 0.002 | 2.318 $\pm$ 0.026 |
|                          | 8          | 36.984 $\pm$ 0.002 | 0.240 $\pm$ 0.001 | 0.466 $\pm$ 0.002 | 2.318 $\pm$ 0.026 |
| Center (five replicates) | 9-13       | 36.984 $\pm$ 0.002 | 0.435 $\pm$ 0.001 | 0.466 $\pm$ 0.002 | 2.318 $\pm$ 0.026 |

The data (symbols) corresponding to the four series of batch cultures [14–16] and the corresponding predictions (dashed lines) of the developed four-dimensional LV-like equation (1)–(4) are shown in Figures 1–5. The values for the constants and the statistical analysis of each equation in each series of cultures are shown in Table 3.

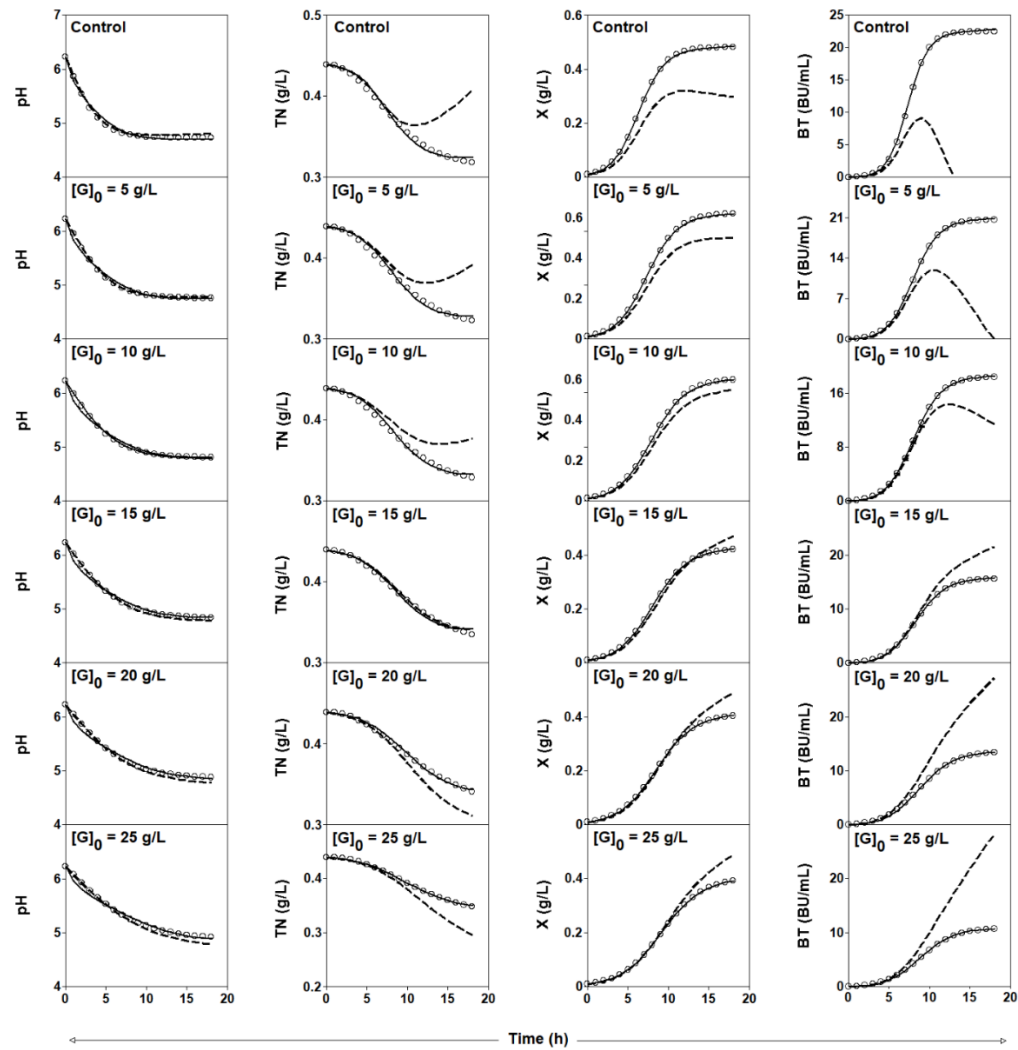


Figure 1. Experimental data (symbols) of culture pH, TN consumption and X and Nis synthesis by *L. lactis* CECT 539 in batch fermentations in DW medium supplemented with different glucose levels ( $G_0$ ). Dashed lines drawn through the experimental data are the predictions of the global four-dimensional LV-like equation (1)–(4) obtained with the parameters shown in Table 3. Solid lines were obtained by adjusting the four-dimensional LV-like equation to the experimental data corresponding to each individual culture (see parameter values in Table 4). Reproduced with permission from Costas et al. [14], Appl. Microbiol. Biotechnol.; published by Springer Nature, 2016.

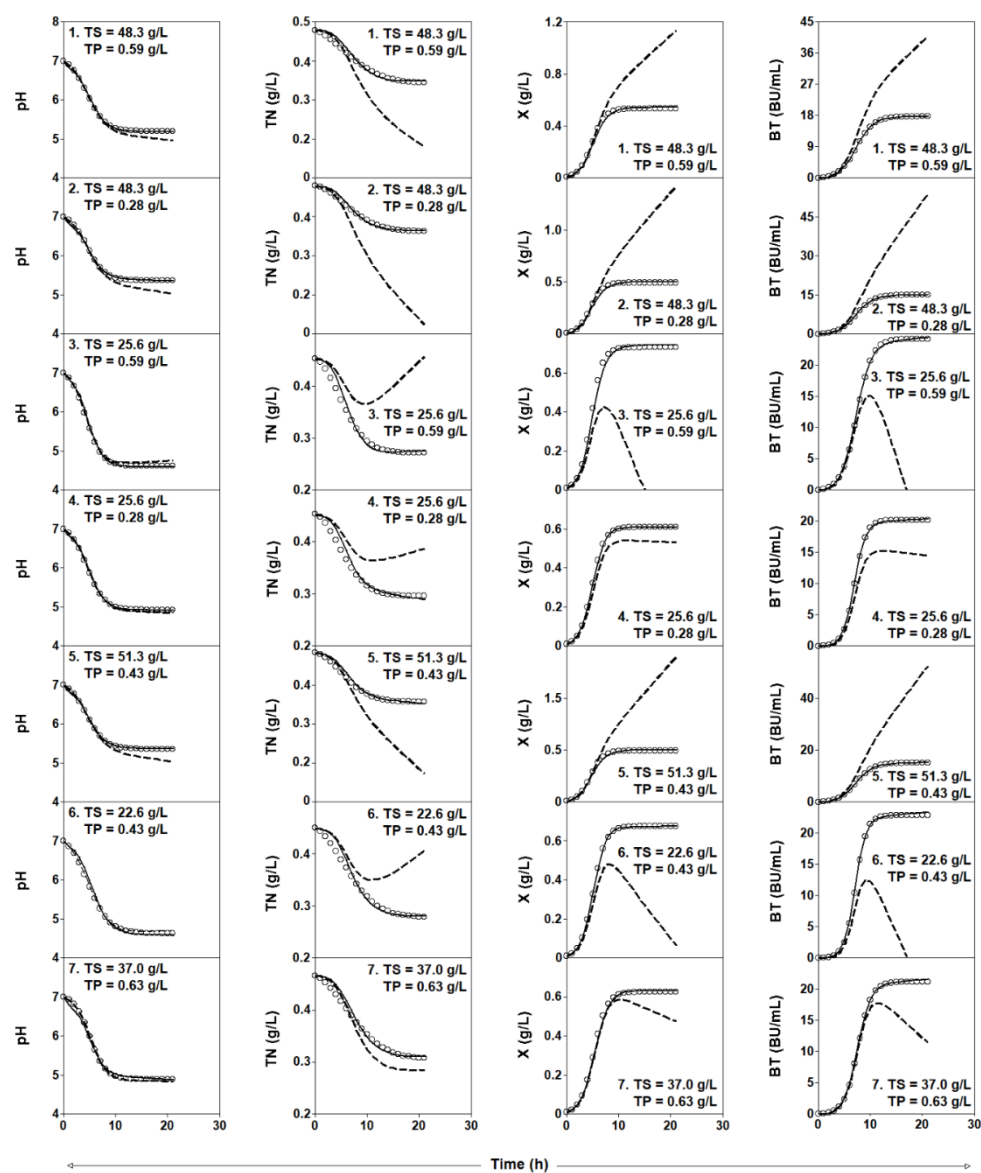


Figure 2. Experimental data (symbols) of culture pH, TN consumption and X and Nis production by *L. lactis* CECT 539 in the first seven batch cultures of the experimental matrix (Table 2), corresponding to the fermentation series DW-TS-TP. Dashed lines drawn through the experimental data are the predictions of the global four-dimensional LV-like equation (1)–(4) obtained with the parameters shown in Table 3. Solid lines were obtained by adjusting the four-dimensional LV-like equation (1)–(4) to the experimental data corresponding to each individual culture (see parameter values in Table 5). Reproduced with permission from Costas et al. [15], 3-Biotech; published by Springer Nature, 2018.

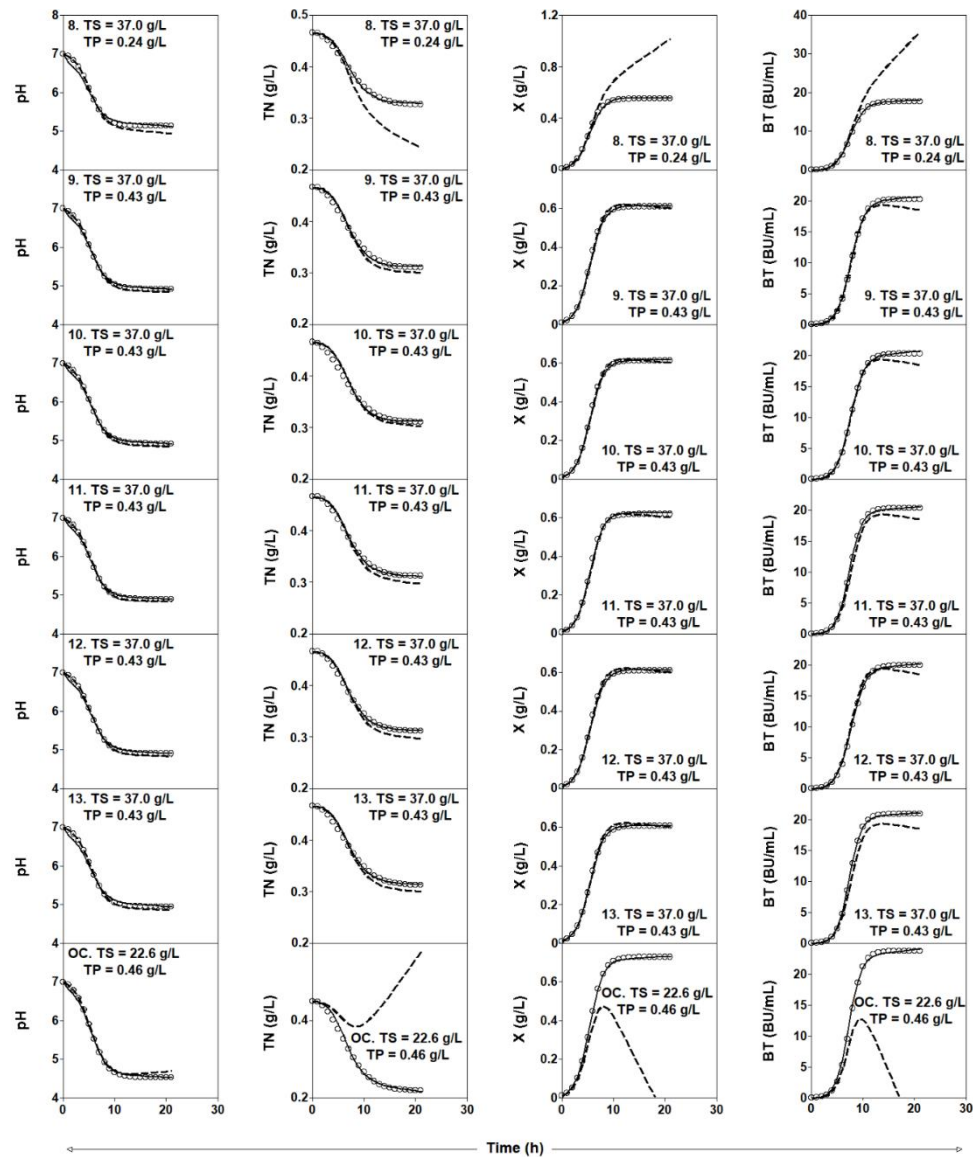


Figure 3. Experimental data (symbols) of culture pH, TN consumption and X and Nis production by *L. lactis* CECT 539 in the last six batch cultures of the experimental matrix (Table 2) and in the optimum conditions (OC), corresponding to the fermentations series DW-TS-TP. Dashed lines drawn through the experimental data are the predictions of the global four-dimensional LV-like equation (1)–(4) obtained with the parameters shown in Table 3. Solid lines were obtained by adjusting the four-dimensional LV-like equation (1)–(4) to the experimental data corresponding to each individual culture (see parameter values in Table 6). Reproduced with permission from Costas et al. [15], 3-Biotech; published by Springer Nature, 2018.

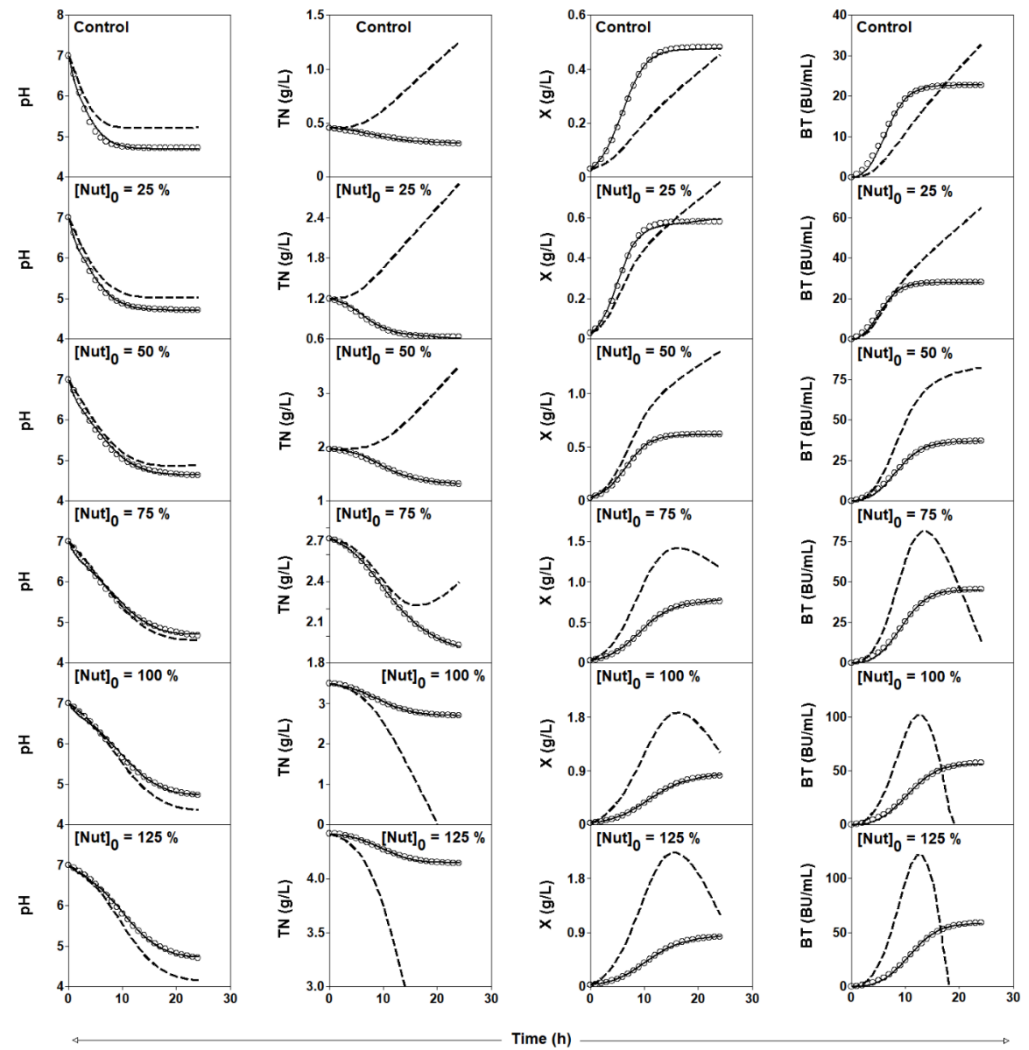


Figure 4. Experimental data (symbols) of culture pH, TN consumption and X and Nis formation by *L. lactis* CECT 539 in batch cultures in DW medium supplemented with 0, 25, 50, 75, 100, and 125% (w/v) of the standard concentrations of the MRS broth nutrients ( $Nut_0$ ) with the exception of glucose and Tween 80. Dashed lines drawn through the experimental data are the predictions of the global four-dimensional LV-like equation (1)–(4) obtained with the parameters shown in Table 3. Solid lines were obtained by adjusting the four-dimensional LV-like equation (1)–(4) to the experimental data corresponding to each individual culture (see parameter values in Table 7).



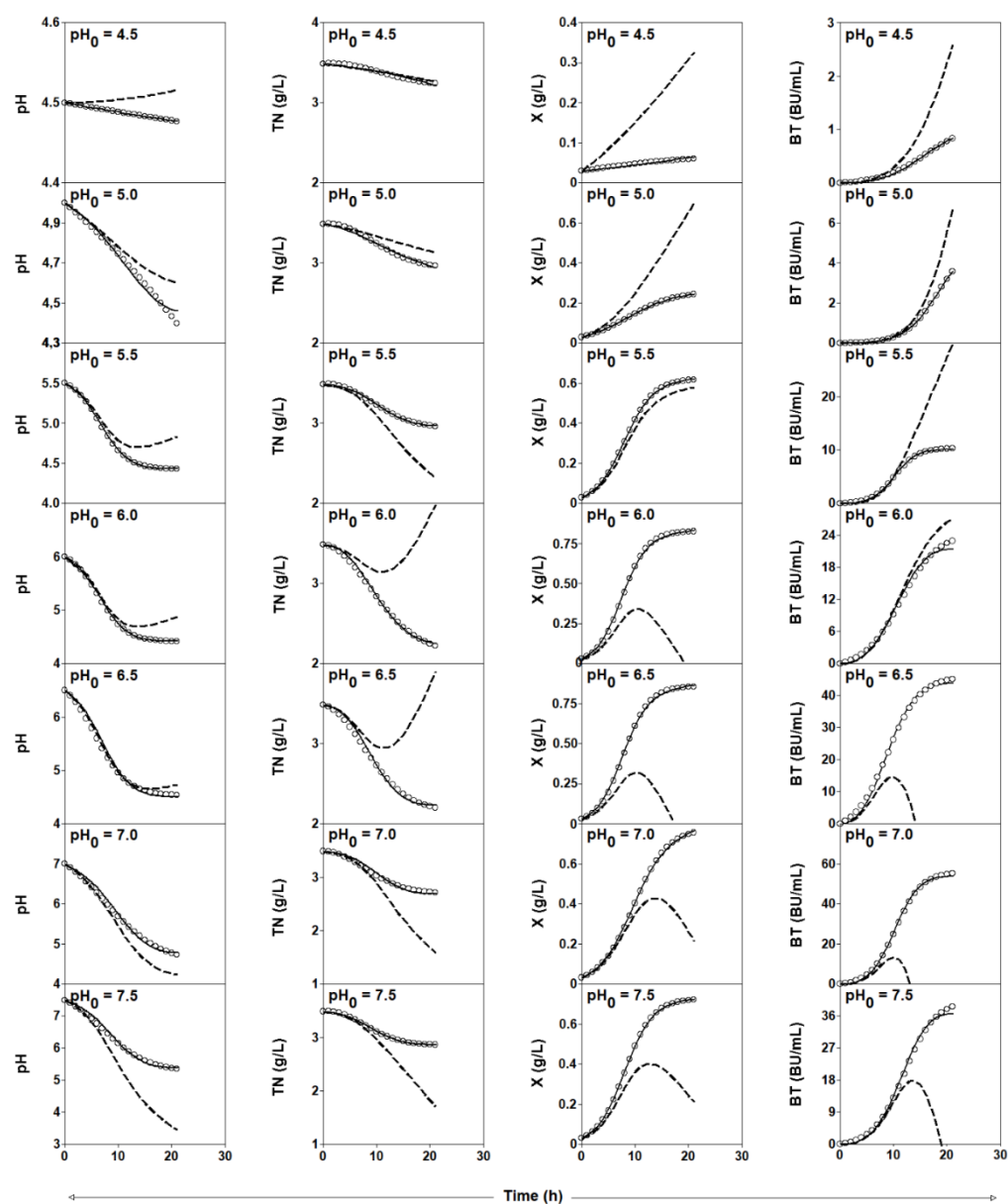


Figure 5. Experimental data (symbols) of culture pH, TN consumption and X and Nis formation by *L. lactis* CECT 539 in batch cultures in DW100 medium adjusted to different initial pH values ( $pH_0$ ). Dashed lines drawn through the experimental data are the predictions of the global four-dimensional LV-like equation (1)–(4) obtained with the parameters shown in Table 3. Solid lines were obtained by adjusting the four-dimensional LV-like equation (1)–(4) to the experimental data corresponding to each individual culture (see parameter values in Table 8).



**Table 3.** Parameter values (as estimates  $\pm$  confidence intervals) calculated with the global set of model parameters of the four-dimensional LV-like equation (1)–(4) to describe the batch nisin production system in the different series of fermentations.

| Parameter         | DW-G series                                            | DW-TS-TP series                       | DW-MRS series                                         | DW-pH series                          |
|-------------------|--------------------------------------------------------|---------------------------------------|-------------------------------------------------------|---------------------------------------|
| $\alpha_{BC}$     | 0.934 $\pm$ 0.006<br>( $P < 0.0001$ )                  | 0.818 $\pm$ 0.014<br>( $P < 0.0001$ ) | 0.726 $\pm$ 0.021<br>( $P < 0.0001$ )                 | 0.722 $\pm$ 0.064<br>( $P < 0.0001$ ) |
| $\alpha_{pH,X}$   | 0.063 $\pm$ 0.002<br>( $P < 0.0001$ )                  | 0.174 $\pm$ 0.010<br>( $P < 0.0001$ ) | 0.188 $\pm$ 0.016<br>( $P < 0.0001$ )                 | 0.182 $\pm$ 0.017<br>( $P < 0.0001$ ) |
| $K_1$             | 4.814 $\pm$ 0.015<br>( $P < 0.0001$ )                  | 4.946 $\pm$ 0.016<br>( $P < 0.0001$ ) | 4.747 $\pm$ 0.022<br>( $P < 0.0001$ )                 | 4.675 $\pm$ 0.050<br>( $P < 0.0001$ ) |
| $R_{pH^2}$        | 0.9957                                                 | 0.9652                                | 0.9435                                                | 0.4953                                |
| RPDM              | 0.5750                                                 | 2.3678                                | 4.6731                                                | 3.9241                                |
| $B_f$             | 0.9977                                                 | 1.0032                                | 1.0069                                                | 0.9858                                |
| $A_f$             | 1.0058                                                 | 1.0230                                | 1.0477                                                | 1.0428                                |
| F-ratio           | 61313.50                                               | 4040.25                               | 6023.08                                               | 70.67                                 |
| P-value           | < 0.0001                                               | < 0.0001                              | < 0.0001                                              | < 0.0001                              |
| $\alpha_{TN,X}$   | 0.296 $\pm$ 0.266<br>( $P = 0.2671$ )                  | 0.239 $\pm$ 0.127<br>( $P = 0.0602$ ) | 0.058 $\pm$ 0.9841<br>( $P = 0.9531$ )                | 0.318 $\pm$ 0.099<br>( $P = 0.0016$ ) |
| $K_2$             | 0.238 $\pm$ 0.0780<br>( $P = 0.0029$ )                 | 0.247 $\pm$ 0.050<br>( $P < 0.0001$ ) | 0.370 $\pm$ 5.531<br>( $P = 0.9468$ )                 | 2.893 $\pm$ 0.084<br>( $P < 0.0001$ ) |
| $\alpha_{TN,BT}$  | 0.009 $\pm$ 0.007<br>( $P = 0.1905$ )                  | 0.005 $\pm$ 0.004<br>( $P = 0.1823$ ) | 0.004 $\pm$ 0.017<br>( $P = 0.8076$ )                 | 0.004 $\pm$ 0.003<br>( $P = 0.1051$ ) |
| $K_3$             | 0.516 $\pm$ 0.177<br>( $P = 0.0043$ )                  | 0.465 $\pm$ 0.087<br>( $P < 0.0001$ ) | 3.540 $\pm$ 6.416<br>( $P = 0.5820$ )                 | 2.264 $\pm$ 0.303<br>( $P < 0.0001$ ) |
| $R_{TN^2}$        | -                                                      | -                                     | -                                                     | -                                     |
| RPDM              | 4.4814                                                 | 16.0026                               | 82.2462                                               | 11.0581                               |
| $B_f$             | 1.0167                                                 | 0.9970                                | -                                                     | 1.0085                                |
| $A_f$             | 1.0444                                                 | 1.1594                                | -                                                     | 1.1109                                |
| F-ratio           | -                                                      | -                                     | -                                                     | -                                     |
| P-value           | 1.0000                                                 | 1.0000                                | 1.0000                                                | 1.0000                                |
| $\alpha_{X,TN}$   | 2.744 $\pm$ 0.626<br>( $P < 0.0001$ )                  | 2.507 $\pm$ 1.922<br>( $P = 0.1933$ ) | 0.356 $\pm$ 0.346<br>( $P = 0.3053$ )                 | 0.099 $\pm$ 0.033<br>( $P = 0.0035$ ) |
| $\alpha^*_{X,BT}$ | 1.960 $\times 10^{-5}$ $\pm$ 0.000<br>( $P < 0.0001$ ) | 0.006 $\pm$ 0.002<br>( $P = 0.0076$ ) | 1.87 $\times 10^{-6}$ $\pm$ 0.048<br>( $P = 0.9997$ ) | 0.001 $\pm$ 0.001<br>( $P = 0.5621$ ) |
| $\alpha_{X,pH}$   | 0.122 $\pm$ 0.038<br>( $P = 0.0016$ )                  | 0.048 $\pm$ 0.093<br>( $P = 0.6075$ ) | 4.81 $\times 10^{-7}$ $\pm$ 0.102<br>( $P = 1.0000$ ) | 0.005 $\pm$ 0.036<br>( $P = 0.8982$ ) |
| $K_4$             | 1.047 $\pm$ 0.180<br>( $P < 0.0001$ )                  | 0.956 $\pm$ 0.463<br>( $P = 0.040$ )  | 0.699 $\pm$ 0.361<br>( $P = 0.0544$ )                 | 0.668 $\pm$ 0.166<br>( $P < 0.0001$ ) |
| $R_X^2$           | 0.8293                                                 | 0.3466                                | -                                                     | -                                     |
| RPDM              | 12.9625                                                | 29.5177                               | 95.0636                                               | 67.2153                               |
| $B_f$             | 0.9058                                                 | -                                     | 1.5467                                                | -                                     |
| $A_f$             | 1.1523                                                 | -                                     | 1.8459                                                | -                                     |
| F-ratio           | 255.04                                                 | 51.28                                 | -                                                     | -                                     |

| <i>P</i> -value   | < 0.0001                              | < 0.0001                              | 1.0000                                | 1.0000                                |
|-------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| $\alpha_{BT,X}$   | 0.002±1.141<br>( <i>P</i> = 0.9988)   | 0.002±0.105<br>( <i>P</i> = 0.9872)   | 0.002±0.374<br>( <i>P</i> = 0.9965)   | 0.002±0.526<br>( <i>P</i> = 0.9976)   |
| $\alpha_{BT,TN}$  | 1.518±5.682<br>( <i>P</i> = 0.7899)   | 2.023±3.279<br>( <i>P</i> = 0.5378)   | 0.495±1.571<br>( <i>P</i> = 0.7529)   | 0.131±0.222<br>( <i>P</i> = 0.5544)   |
| $\alpha^*_{BT,X}$ | 1.518±0.000<br>( <i>P</i> < 0.0001)   | 0.579±13.449<br>( <i>P</i> = 0.9657)  | 0.024±74.506<br>( <i>P</i> = 0.9997)  | 0.008±52.316<br>( <i>P</i> = 0.9999)  |
| $\alpha_{BT,pH}$  | 0.005±12.136<br>( <i>P</i> = 0.9997)  | 0.005±4.822<br>( <i>P</i> = 0.9992)   | 0.005±18.285<br>( <i>P</i> = 0.9998)  | 0.053±6.813<br>( <i>P</i> = 0.9938)   |
| $K_5$             | 17.261±60.981<br>( <i>P</i> = 0.7777) | 20.379±31.475<br>( <i>P</i> = 0.5178) | 37.968±13.674<br>( <i>P</i> = 0.7715) | 25.497±42.752<br>( <i>P</i> = 0.5519) |
| $R_{BT}^2$        | -                                     | -                                     | -                                     | -                                     |
| <i>RPDM</i>       | 43.7888                               | 40.5902                               | 110.5949                              | 60.1353                               |
| $B_f$             | -                                     | -                                     | -                                     | -                                     |
| $A_f$             | -                                     | -                                     | -                                     | -                                     |
| <i>F</i> -ratio   | -                                     | -                                     | -                                     | -                                     |
| <i>P</i> -value   | 1.0000                                | 1.0000                                | 1.0000                                | 1.0000                                |

The parameter value is considered statistically significant if its corresponding *P*-value is lower than 0.05.

Satisfactory results were obtained when the global equation (1) was set to describe the time course of the culture pH in the DW-G series (dashed lines in Figure 1). Thus, the values of  $R_{pH}^2$  (0.9957) and *F*-ratio (61313.50) were considerably higher, the *RPDM* value was lower than 10 and both the  $B_f$  (0.9977) and  $A_f$  (1.0058) values were near one. In addition, statistically significant values (*P* < 0.0001) for both the parameters and global pH equation were obtained (Table 3).

In the case of the DW-TS-TP, DW-MRS and DW-pH series of cultures (Figures 2–5, Table 3), the results were less satisfactory compared with the DW-G cultures (Figure 1, Table 3), considering the lower values obtained for  $R_{pH}^2$  (0.9652, 0.9435, and 0.4953) and *F*-ratios (4040.25, 6023.08, and 70.67). Although the *RPDM* values calculated for the DW-TS-TP, DW-MRS and DW-pH cultures (2.3678, 4.6731, and 3.9241) were lower than 10, and the values of the parameters and equation were statistically significant (*P* < 0.0001), the predictions of the global pH equation (1) were not always consistent with the experimental pH data (Figures 2–5). In fact, with the exception of the DW-G cultures, the predictions of the global pH equation (1) showed a clear deviation from the experimental data for the DW-TS-TP (Figures 2 and 3), DW-MRS (Figure 4) and DW-pH cultures (Figure 5).

The general total nitrogen (*TN*) consumption equation (2) only satisfactorily described the time course of this nutrient in the fermentation in DW medium supplemented with 15 g glucose/L (Figure 1) or 37.0 g of TS and 0.43 g TP per L of medium (cultures 9 to 13 in Figure 3). The *RPDM* value was only lower than 10 in the DW-G cultures (Table 3), but the fit of equation (2) to the experimental *TN* data was found to be statistically non-significant (*P* = 1.0000) in all series of fermentation, as occurred with almost all values of the parameters.

In addition, both the general growth (*X*) equation (3) and nisin (*BT*) production equation (4) unsatisfactorily described the evolution of both variables in the different series of fermentation (Figures 1–5), except the fermentations in DW medium supplemented with 37.0 g of TS and 0.43 g TP per L of medium (cultures 9 to 13 in Figure 3).

Surprisingly, the fit of equation (3) to the experimental biomass data in the series DW-G was statistically significant ( $P < 0.05$ ) and provided statistically significant values ( $P < 0.05$ ) for the values of the parameters (Table 3), with a relatively higher value for  $R^2$  (0.8293) and  $F$ -ratio (255.04). However, the  $RPDM$  value (12.9625) was higher than 10 and the  $B_f$  (0.9058) and  $A_f$  (1.1523) values were relatively far from one. From the statistical viewpoint, the fitting equation (3) to the experimental data of the series DW-TS-TP, DW-MRS and DW-pH did not always provide statistically significant coefficients, being non-significant ( $P = 1.0000$ ) the fitted equation (3) for the DW-MRS and DW-pH cultures (Table 3). In these latter cultures, the  $R^2$  coefficient could not be calculated with the statistical software used.

Concerning the use of equation (4), it can be noted that the fitted equations were found to be non-significant ( $P = 1.0000$ ) for the four series of cultures (Table 3).

These observations suggest that the four-dimensional LV equation could not be used as a general equation to describe the nisin production system in the four series of batch fermentations.

These unsatisfactory results could be related to the different initial compositions of the media used in each series of cultures: DW media supplemented with different initial concentrations of glucose (Figure 1), TS and TP (Figures 2 and 3) and MRS broth nutrients (Figure 4), or adjusted to different initial pH values (Figure 5). So that, the different initial media composition in the first three series of batch fermentations (DW-G, DW-TS-TP, and DW-MRS) modulated the time-course of the culture variables: pH and the concentrations of total nitrogen, biomass and nisin (Figures 1–5). In this way, it is well known that the initial culture conditions affect the evolution of these culture variables (pH, TN, X and BT) in a different way [8,17–19]. For example, the pH drop depends on the presence and interaction between some compounds (salts, organic acids, proteins, free amino acids) with buffering capacity in the culture medium [20] and organic acids production by growing cells [17]. TN consumption during fermentation depends on the initial medium composition, mainly the type and concentration of the nitrogen source [18,19] and culture pH [16,19,21]. In fact, the consumption of TN [16] or amino acids [21] in *L. lactis* strains was maximal when the culture pH reached values between 5.8 and 6.5, and decreased abruptly for high and low pH values.

On the other hand, biomass production depends on different factors including the initial medium composition (concentration and type of nutrients (mainly carbon, nitrogen and phosphorous sources), initial and final pH values in the cultures, pH evolution and production of inhibitory compounds [17]. Nisin synthesis depends not only on the time course of the evolution of biomass concentration, but also on i) the amount of biomass produced, ii) initial concentration and type of nutrient (carbon, nitrogen and phosphorous sources), iii) initial and final pH value, pH evolution and pH drop generated in the cultures [5,8,12,17,19]. So that, the specific effect of these factors on the response variables (culture pH, TN consumption, biomass and nisin production) could be non-synchronous producing a different change in the time course of the latter variables and consequently, in their relationships.

For example, the buffering capacity (BC), which is a measure of the resistance of the culture medium to pH changes, affects biomass and nisin synthesis differently. On the one hand, the increase in BC favors biomass production since the cultures remain longer within the optimum pH range (between 5.8 and 6.5) for nutrient consumption for *L. lactis* CECT 539 [16,21]. On the other hand, these high pH values inhibit bacteriocin synthesis, which was higher at an optimum pH value of 4.90 in DW medium, due to the need of a low pH value to favor the maturation of the nisin molecule [5,16,22]. The value of this optimum final pH for nisin production depends on the producer strain and composition of culture medium [8,12,22–25].

In addition, it has been observed that higher pH drops (rpH) enhance nisin production [12,16,19,25] before the cultures reached an inappropriate pH for survival and cell growth of *L. lactis* [26]. For example, in the batch cultures conducted in DW medium adjusted to different initial pH values, the highest nutrient (sources of carbon, nitrogen

and phosphorous) consumption and biomass concentration were obtained at initial pH values of 6.0 and 6.5. However, the highest nisin concentration was obtained at an initial pH of 7.0, in which the highest final rpH was generated (see Figure 5). Thus, in the specific case of the series of fermentation in the DW media supplemented with glucose (Figure 1), it can be observed that the evolution of biomass production in each culture was different. The same trend was observed in the batch cultures in DW media supplemented with different levels of glucose and phosphorous (Figures 2 and 3), with nutrients of the MRS broth (Figure 4) or adjusted to different initial pH values (Figure 5). In these three series of cultures (DW-TS-TP, DW-MRS and DW-pH), the evolution of culture pH and nisin production was also different.

For these reasons, it is very difficult to develop a general four-dimensional LV-like equation to explain the variations in the time courses of the four variables (culture pH, TN, X and BT). In addition, with the use of a general four-dimensional equation, the effect of different initial culture conditions on the evolution of the four dependent variables could not be explained, leading to a misinterpretation of the kinetics of the cultures.

To solve this problem, we first fitted the four-dimensional LV-like equation (1)–(4) to each individual culture of each series of fermentation to accurately determine how the values of the different parameters change with changes in the initial culture conditions (concentrations of glucose, TP and TS, MRS broth nutrients and pH). Afterward, the four-dimensional LV-like equation (1)–(4) was modified (when this was possible) by including a term for the specific effect of the initial culture conditions (initial concentrations of glucose, TP and TS, MRS nutrients and initial pH) on the evolution of pH, TN, X and BT.

## 2.2. Modeling the Batch Nisin Production System in Individual Cultures Corresponding to Each Series of Fermentation

When the four-dimensional LV-like equation (1)–(4) was used to describe the relationships between the four response variables (pH, TN, X and BT) in each individual culture, both equations and values of the parameters were statistically significant ( $P < 0.050$ ), with  $R^2$  and  $F$ -values considerably higher, and  $B_f$  and  $A_f$  values  $\sim 1$  (Tables 4–8).

In addition, the predictions of the four-dimensional LV-like equation (1)–(4) for each response variable (solid lines Figures 1–5) were in perfect agreement with the corresponding experimental data. This indicates that the developed four-dimensional LV-like equation (1)–(4) is consistent and robust to accurately describe the trend observed in the experimental data of culture pH, TN, X and BT.

The results obtained for each series of fermentation are discussed below.

### 2.2.1. Series of fermentation DW-G

Table 4 shows the parameter values as well as the statistical analysis obtained when the four-dimensional LV-like equation (1)–(4) was fitted to the experimental data of culture pH, TN, X and BT in the DW-G cultures.

In this case, all values of the parameters in the equations (1)–(4) were significant ( $P < 0.05$ ) and considerably higher values for  $R_{pH^2}$  (between 0.9886 and 0.9916),  $R_{TN^2}$  (between 0.9938 and 0.9991),  $R_X^2$  (0.9999) and  $R_{BT^2}$  (between 0.9927 and 0.99829) were obtained. The  $B_f$  and  $A_f$  values calculated for equations (1)–(3) were  $\sim 1$  and the  $RPDM$  values were considerably lower than 10%. However, in the case of nisin production, the values of  $B_f$  (between 0.8207 and 0.9475) and  $A_f$  (between 1.0756 and 1.2185) obtained using equation (4) were the farthest from one and the  $RPDM$  values corresponding to the cultures in DW media supplemented with 15, 20, and 25 g glucose/L were slightly higher than 10% (Table 4).

**Table 4.** Statistically significant ( $P < 0.05$ ) parameter values (as estimates  $\pm$  confidence intervals) calculated with the four-dimensional LV-like equation (1)–(4) for each individual culture of the series of fermentation DW-G.

| Parameter        | Initial glucose concentrations (g/L) in the DW medium |                   |                   |                   |                   |                   |
|------------------|-------------------------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                  | 0                                                     | 5                 | 10                | 15                | 20                | 25                |
| $\alpha_{BC}$    | 0.157 $\pm$ 0.007                                     | 0.107 $\pm$ 0.006 | 0.086 $\pm$ 0.005 | 0.071 $\pm$ 0.004 | 0.055 $\pm$ 0.003 | 0.040 $\pm$ 0.003 |
|                  | ( $P < 0.0001$ )                                      | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $\alpha_{pH,X}$  | 0.949 $\pm$ 0.009                                     | 0.879 $\pm$ 0.007 | 0.760 $\pm$ 0.005 | 0.755 $\pm$ 0.004 | 0.721 $\pm$ 0.003 | 0.710 $\pm$ 0.002 |
|                  | ( $P < 0.0001$ )                                      | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $K_1$            | 4.970 $\pm$ 0.024                                     | 4.945 $\pm$ 0.026 | 4.996 $\pm$ 0.031 | 4.996 $\pm$ 0.031 | 4.992 $\pm$ 0.032 | 4.982 $\pm$ 0.031 |
|                  | ( $P < 0.0001$ )                                      | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $R_{pH^2}$       | 0.9886                                                | 0.9911            | 0.9916            | 0.9910            | 0.9894            | 0.9887            |
| $RPDM$           | 0.7314                                                | 0.5956            | 0.5031            | 0.5615            | 0.6400            | 0.6675            |
| $B_f$            | 1.0000                                                | 0.9993            | 0.9986            | 0.9993            | 0.9987            | 0.9975            |
| $A_f$            | 1.0073                                                | 1.0060            | 1.0051            | 1.0057            | 1.0064            | 1.0067            |
| $F\text{-ratio}$ | 353.78                                                | 206.96            | 145.19            | 120.56            | 90.80             | 62.61             |
| $P\text{-value}$ | $< 0.0001$                                            | $< 0.0001$        | $< 0.0001$        | $< 0.0001$        | $< 0.0001$        | $< 0.0001$        |
| $\alpha_{TN,X}$  | 0.283 $\pm$ 0.006                                     | 0.301 $\pm$ 0.005 | 0.287 $\pm$ 0.004 | 0.284 $\pm$ 0.022 | 0.288 $\pm$ 0.022 | 0.294 $\pm$ 0.021 |
|                  | ( $P < 0.0001$ )                                      | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $K_2$            | 0.244 $\pm$ 0.001                                     | 0.235 $\pm$ 0.001 | 0.230 $\pm$ 0.001 | 0.228 $\pm$ 0.001 | 0.227 $\pm$ 0.001 | 0.239 $\pm$ 0.001 |
|                  | ( $P < 0.0001$ )                                      | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $\alpha_{TN,BT}$ | 0.006 $\pm$ 0.000                                     | 0.007 $\pm$ 0.000 | 0.011 $\pm$ 0.000 | 0.011 $\pm$ 0.000 | 0.012 $\pm$ 0.000 | 0.014 $\pm$ 0.000 |
|                  | ( $P < 0.0001$ )                                      | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $K_3$            | 0.479 $\pm$ 0.006                                     | 0.498 $\pm$ 0.005 | 0.503 $\pm$ 0.003 | 0.505 $\pm$ 0.017 | 0.511 $\pm$ 0.033 | 0.558 $\pm$ 0.031 |
|                  | ( $P < 0.0001$ )                                      | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $R_{TN^2}$       | 0.9938                                                | 0.9948            | 0.9953            | 0.9945            | 0.9974            | 0.9991            |
| $RPDM$           | 0.8665                                                | 0.7341            | 0.6715            | 0.6011            | 0.4022            | 0.2316            |
| $B_f$            | 1.0018                                                | 1.0014            | 1.0019            | 1.0005            | 1.0011            | 0.9991            |
| $A_f$            | 1.0087                                                | 1.0074            | 1.0067            | 1.0060            | 1.0040            | 1.0023            |
| $F\text{-ratio}$ | 187.03                                                | 180.94            | 159.87            | 123.68            | 158.47            | 340.51            |

| P-value           | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              |
|-------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|
| $\alpha_{X,TN}$   | 3.299±0.220<br>( <i>P</i> < 0.0001)                   | 3.020±0.214<br>( <i>P</i> < 0.0001)                   | 2.861±0.199<br>( <i>P</i> < 0.0001)                   | 2.635±0.192<br>( <i>P</i> < 0.0001)                   | 2.368±0.180<br>( <i>P</i> < 0.0001)                   | 2.275±0.183<br>( <i>P</i> < 0.0001)                   |
| $\alpha^*_{X,BT}$ | 1.960×10 <sup>-5</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.960×10 <sup>-5</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.960×10 <sup>-5</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.960×10 <sup>-5</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.960×10 <sup>-5</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.960×10 <sup>-5</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $\alpha_{X,pH}$   | 0.106±0.012<br>( <i>P</i> < 0.0001)                   | 0.108±0.012<br>( <i>P</i> < 0.0001)                   | 0.108±0.011<br>( <i>P</i> < 0.0001)                   | 0.094±0.011<br>( <i>P</i> < 0.0001)                   | 0.085±0.010<br>( <i>P</i> < 0.0001)                   | 0.087±0.011<br>( <i>P</i> < 0.0001)                   |
| $K_4$             | 0.986±0.057<br>( <i>P</i> < 0.0001)                   | 0.985±0.058<br>( <i>P</i> < 0.0001)                   | 0.978±0.055<br>( <i>P</i> < 0.0001)                   | 0.883±0.052<br>( <i>P</i> < 0.0001)                   | 0.827±0.050<br>( <i>P</i> < 0.0001)                   | 0.837±0.050<br>( <i>P</i> < 0.0001)                   |
| $R_X^2$           | 0.9999                                                | 0.9999                                                | 0.9999                                                | 0.9999                                                | 0.9999                                                | 0.9999                                                |
| RPDM              | 1.3611                                                | 1.5037                                                | 1.4333                                                | 1.1965                                                | 1.0048                                                | 1.0636                                                |
| $B_f$             | 0.9872                                                | 0.9856                                                | 0.9867                                                | 0.9896                                                | 0.9924                                                | 0.9906                                                |
| $A_f$             | 1.0140                                                | 1.0155                                                | 1.0148                                                | 1.0123                                                | 1.0102                                                | 1.0108                                                |
| F-ratio           | 12730.46                                              | 9856.45                                               | 9580.19                                               | 10221.87                                              | 11087.76                                              | 10202.99                                              |
| P-value           | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              |
| $\alpha_{BT,X}$   | 1.679×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.679×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.679×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.679×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.679×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.679×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $\alpha_{BT,TN}$  | 2.080±0.012<br>( <i>P</i> < 0.0001)                   | 1.640±0.010<br>( <i>P</i> < 0.0001)                   | 1.519±0.010<br>( <i>P</i> < 0.0001)                   | 1.398±0.008<br>( <i>P</i> < 0.0001)                   | 1.161±0.008<br>( <i>P</i> < 0.0001)                   | 1.178±0.010<br>( <i>P</i> < 0.0001)                   |
| $\alpha^*_{BT,X}$ | 0.580±0.000<br>( <i>P</i> < 0.0001)                   | 0.580±0.000<br>( <i>P</i> < 0.0001)                   | 0.580±0.000<br>( <i>P</i> < 0.0001)                   | 0.580±0.000<br>( <i>P</i> < 0.0001)                   | 0.580±0.000<br>( <i>P</i> < 0.0001)                   | 0.580±0.000<br>( <i>P</i> < 0.0001)                   |
| $\alpha_{BT,pH}$  | 4.567×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.567×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.567×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.567×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.567×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.567×10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $K_5$             | 22.817±0.025<br>( <i>P</i> < 0.0001)                  | 21.029±0.025<br>( <i>P</i> < 0.0001)                  | 18.737±0.025<br>( <i>P</i> < 0.0001)                  | 15.999±0.024<br>( <i>P</i> < 0.0001)                  | 13.926±0.039<br>( <i>P</i> < 0.0001)                  | 11.146±0.027<br>( <i>P</i> < 0.0001)                  |
| $R_{BT}^2$        | 0.9982                                                | 0.9979                                                | 0.9979                                                | 0.9975                                                | 0.9927                                                | 0.9947                                                |
| RPDM              | 5.7645                                                | 7.7226                                                | 9.0138                                                | 10.4798                                               | 14.1249                                               | 11.5509                                               |
| $B_f$             | 0.9475                                                | 0.9023                                                | 0.8840                                                | 0.8657                                                | 0.8207                                                | 0.8513                                                |
| $A_f$             | 1.0756                                                | 1.1108                                                | 1.1312                                                | 1.1551                                                | 1.2185                                                | 1.1801                                                |

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|                 |          |          |          |          |          |          |
|-----------------|----------|----------|----------|----------|----------|----------|
| <i>F</i> -ratio | 18542.88 | 16247.73 | 15997.62 | 13717.05 | 4600.57  | 6330.41  |
| <i>P</i> -value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

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The parameter value is considered statistically significant if its corresponding *P*–value is lower than 0.05.



This was probably because nisin production is quantified by a photometric bioassay using an indicator strain and consequently, the experimental error in determining nisin titers could be greater than those of the analytical methods used in pH, total nitrogen and biomass measurements. So that, in the latter cultures, the differences between the experimental and predicted nisin values during the first 9 h of fermentation were low (Figure 1), but the experimental nisin data in this interval, used as the denominator in the equation (8) were also considerably low, increasing the *RPDM* value (Table 4).

Concerning the equation parameters, it can be noted that the values of  $\alpha_{BC}$  and  $\alpha_{pH,X}$  in equation (1) decreased with the increase in the initial concentration of the carbon source ( $G_0$ ) in the medium. This is mainly due to the inhibition that increasing glucose concentrations produced on the growth of *L. lactis* CECT 539 and consequently on lactic acid production [14], causing a gradual reduction in the pH drop in the culture media (Figure 1). Since the cultures reached almost the same final pH level (between 4.73 and 4.92), the values of  $K_1$  only varied between 4.945 and 4.996 (Table 4).

The values of  $\alpha_{TN,X}$  and  $K_2$  in equation (2) did not show a significant variation (mean values of  $0.296 \pm 0.010$  and  $0.238 \pm 0.009$ , respectively), indicating that the TN consumption for the growth was similar in the six glucose-supplemented cultures. In addition, slight increases in the values of  $\alpha_{TN,BT}$  (from 0.006 to 0.0014) and  $K_3$  (from 0.479 to 0.558) were observed, due to the different nisin titers (22.48, 20.64, 18.35, 15.60, 23.40, and 10.70 BU/mL) produced in each culture (Figure 1). So that, the high variability observed in the  $\alpha_{TN,BT}$ , and  $K_3$  values compared to those of  $\alpha_{TN,X}$  and  $K_2$  was due to the higher variations in the nisin concentrations synthesized compared to the levels of biomass produced (Figure 1).

Equation (3) also provides an appropriate description of biomass production in each culture. In this case, the values of  $\alpha_{X,TN}$  decreased due to the above-mentioned growth inhibition produced by the carbon source, that led to a reduction in the amounts of TN consumed. However, the efficiency of TN consumption for nisin production ( $\alpha_{X,BT}^*$ ) did not change, since the reduction in growth was proportional to that observed in nisin synthesis, because this bacteriocin was produced in this culture as a pH-dependent primary metabolite [14]. The value of  $\alpha_{X,pH}$  decreased for initial concentrations of glucose higher than 10 g/L, while the  $K_4$  value decreased with the increase in glucose supplementation, as a consequence of the decrease in biomass production and the increase in the final pH values in the cultures (Figure 1).

A constant value was obtained for the  $\alpha_{BT,X}$ ,  $\alpha_{BT,X}^*$  and  $\alpha_{BT,pH}$  in equation (4) for the different cultures. This corroborates the affirmation that bacteriocin was produced as a pH-dependent primary metabolite and indicates that the TN consumption for biomass production was proportional to the production of bacteriocin and biomass (Table 4). Decreasing values were obtained for  $\alpha_{BT,TN}$ , and  $K_5$  due to the reduction in nisin titers with an increase in the initial concentration of the carbon source in the medium.

From the detailed observation of the series of fermentation DW-G (Figure 1), it can be noted that the increase in the initial glucose levels ( $G_0$ ) produced an increase the final culture pH and TN concentration, and a decrease in the final biomass and nisin levels, but each variable evolved in the same way in the different cultures. In fact, the rates of culture pH ( $rpH = dpH/dt$ ) and TN ( $rTN = dTN/dt$ ) decrease and biomass ( $rX = dX/dt$ ) and nisin ( $rBT = dBT/dt$ ) production in the glucose-supplemented culture media exhibited an exponential decrease in comparison with the respective rates in the culture in the unsupplemented culture (Figure 6).

Then, when the modified four-dimensional equation (8)–(11) was used as a general equation to describe the batch nisin production in DW medium supplemented with different initial glucose concentrations, satisfactory results were obtained:

- For eq. (8):  $\alpha_{BC} = 0.172 \pm 0.004$  ( $P < 0.0001$ ),  $\alpha_{pH,X} = 0.365 \pm 0.056$  ( $P < 0.0001$ ),  $K_1 = 5.484 \pm 0.008$  ( $P < 0.0001$ ) and  $C = 0.021 \pm 0.004$  ( $P < 0.0001$ ).  $R^2 = 0.9848$ ,  $B_f = 0.9988$ ,  $A_f = 1.0087$ ,  $RPDM = 0.7285$ ,  $F\text{-ratio} = 56.35$ .

- For eq. (9):  $\alpha_{TN,X} = 0.548 \pm 0.052$  ( $P < 0.0001$ ),  $K_2 = 0.311 \pm 0.011$  ( $P < 0.0001$ ),  $\alpha_{TN,BT} = 9.249 \times 10^{-4} \pm 0.000$  ( $P < 0.0001$ ),  $K_3 = 0.472 \pm 0.052$  ( $P < 0.0001$ ) and  $C = 0.038 \pm 0.002$  ( $P < 0.0001$ ).  $R^2 = 0.9942$ ,  $B_f = 1.0021$ ,  $A_f = 1.0069$ ,  $RPDM = 0.6916$ ,  $F\text{-ratio} = 144.35$ .

- For eq. (10):  $\alpha_{X,TN} = 1.281 \pm 0.051$  ( $P < 0.0001$ ),  $\alpha_{X,BT}^* = 0.011 \pm 0.001$  ( $P < 0.0001$ ),  $\alpha_{X,pH} = 1.561 \times 10^{-4} \pm 0.000$  ( $P < 0.0001$ ),  $K_4 = 0.751 \pm 0.006$  ( $P < 0.0001$ ) and  $C = 0.035 \pm 0.003$  ( $P < 0.0001$ ).  $R^2 = 0.9945$ ,  $B_f = 1.0064$ ,  $A_f = 1.0480$ ,  $RPDM = 4.7563$ ,  $F\text{-ratio} = 260.33$ .

- For eq. (11):  $\alpha_{BT,X} = 0.005 \pm 0.000$  ( $P < 0.0001$ ),  $\alpha_{BT,TN} = 2.188 \pm 0.107$  ( $P < 0.0001$ ),  $\alpha_{BT,X}^* = 20.977 \pm 0.521$  ( $P < 0.0001$ ),  $\alpha_{BT,pH} = 0.013 \pm 0.001$  ( $P < 0.0001$ ),  $K_5 = 32.672 \pm 0.004$  ( $P < 0.0001$ ) and  $C = 0.053 \pm 0.003$  ( $P < 0.0001$ ).  $R^2 = 0.9977$ ,  $B_f = 0.7711$ ,  $A_f = 1.3249$ ,  $RPDM = 16.5046$ ,  $F\text{-ratio} = 403.79$ .

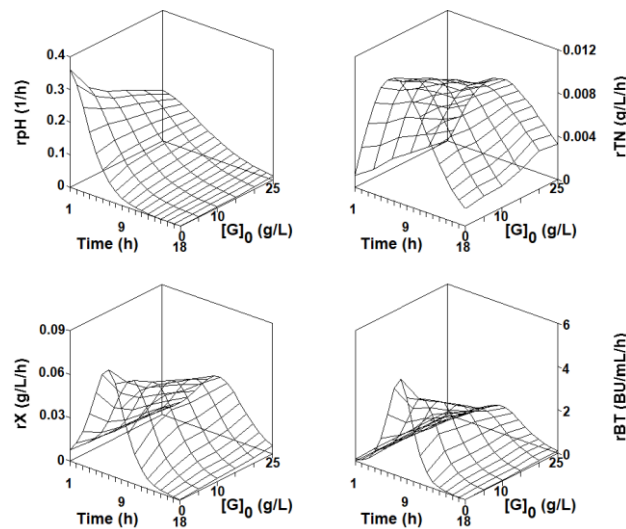


Figure 6. Response surfaces showing the time courses of the experimental rates of pH, TN, X and Nis as a function of the initial glucose concentration ( $[G]_0 = 0, 5, 10, 15, 20$ , and  $25$  g/L) in the DW medium. The different rates were obtained from the experimental data shown in Figure 1.

So that, the predictions of the modified four-dimensional equation (8)–(11) (solid lines in Figure 7) show its feasibility to be used as a general equation for describing the nisin production system in the series of fermentation DW-G.

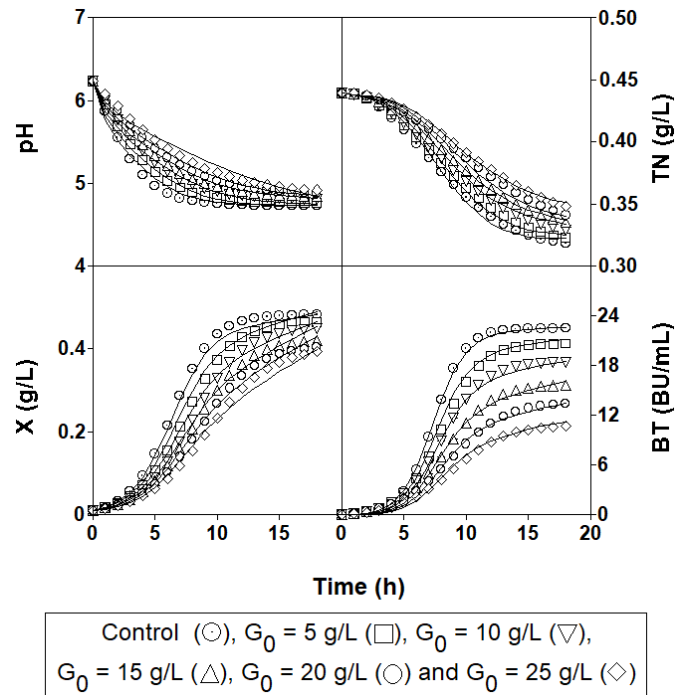


Figure 7. Modeling the nisin production system in batch cultures in DW media supplemented with different amounts of glucose to obtain 0, 5, 10, 15, 20, and 25 g glucose/L, according to the general four-dimensional LV-like equation (11)–(14). The experimental points are the culture pH, TN, X and BT data shown in Figure 1.

### 2.2.2. Series of fermentation DW-TS-TP

The results obtained when the four-dimensional equation (1)–(4) was fitted to each individual culture of the series of fermentation DW-TS-TP are shown in Tables 5 and 6. The predictions of equation (1)–(4) are shown as solid lines in Figures 2 and 3. As observed before for the series of fermentation DW-G, the DW-TS-TP cultures were satisfactorily described using this procedure (Tables 5 and 6).

The values of  $\alpha_{BC}$  in equation (1) were similar (between 0.010 and 0.014) and the highest  $\alpha_{pH,X}$  value was obtained under the optimum condition (OC), in which the highest pH drop (difference between the initial and final pH value) was observed (Figures 2 and 3). The calculated values for  $K_1$  varied between 4.525 and 5.363 (Tables 5 and 6), which are in perfect agreement with the range of final pH values (between 4.53 and 5.37) obtained in the cultures (Figures 2 and 3).

The highest values of  $\alpha_{TN,X}$  and  $\alpha_{TN,BT}$  in equation (2) were obtained in the culture performed under the optimum conditions (TS = 22.6 g/L, TP = 0.46 g/L), in which the highest amounts of TN were consumed (0.23 g/L), while a constant  $K_2$  and  $K_3$  varied slightly in accordance with the initial levels of TS and TP in the culture media (Figures 2 and 3). This suggests that the total nitrogen source consumption depended on the initial composition of the fermentation medium, as indicated before [2,5,14–16].

The values of  $\alpha_{X,TN}$  and  $K_4$  in equation (3) varied as a function of the initial media composition, but the values of  $\alpha_{X,BT}^*$  and  $\alpha_{X,pH}$  were constant in all cultures (Tables 5 and 6), indicating that the competition between the biomass and bacteriocin by the nitrogen source and the effect of pH on the growth were similar in the different fermentations.

As observed in the series of fermentation DW-G, the values of  $\alpha_{BT,X}$ ,  $\alpha_{BT,X}^*$ , and  $\alpha_{BT,pH}$  in eq. (4) were constant for the different cultures (Tables 5 and 6), meanwhile the values calculated for  $\alpha_{BT,TN}$ , and  $K_5$  depended on the initial TS and TP concentrations in the different culture media.

**Table 5.** Statistically significant ( $P < 0.05$ ) parameter values (as estimates  $\pm$  confidence intervals) calculated with the four-dimensional LV-like equation (1)–(4) for each individual culture corresponding to the four factorial points and the four axial points of the experimental matrix (Table 2) of the series of fermentation DW-TS-TP.

| Parameter        | Factorial points  |                   |                   |                   | Axial points      |                   |                   |                   |
|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                  | TS = 48.3 g/L     | TS = 48.3 g/L     | TS = 25.6 g/L     | TS = 25.6 g/L     | TS = 51.3 g/L     | TS = 22.6 g/L     | TS = 37.0 g/L     | TS = 37.0 g/L     |
|                  | TP = 0.59 g/L     | TP = 0.28 g/L     | TP = 0.59 g/L     | TP = 0.28 g/L     | TP = 0.43 g/L     | TP = 0.43 g/L     | TP = 0.63 g/L     | TP = 0.24 g/L     |
| $\alpha_{BC}$    | 0.014 $\pm$ 0.001 | 0.012 $\pm$ 0.001 | 0.014 $\pm$ 0.002 | 0.013 $\pm$ 0.001 | 0.013 $\pm$ 0.002 | 0.011 $\pm$ 0.002 | 0.013 $\pm$ 0.001 | 0.012 $\pm$ 0.001 |
|                  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $\alpha_{pH,X}$  | 0.852 $\pm$ 0.008 | 0.849 $\pm$ 0.008 | 0.855 $\pm$ 0.007 | 0.854 $\pm$ 0.007 | 0.851 $\pm$ 0.007 | 0.710 $\pm$ 0.005 | 0.703 $\pm$ 0.002 | 0.810 $\pm$ 0.004 |
|                  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $K_1$            | 5.186 $\pm$ 0.006 | 5.363 $\pm$ 0.006 | 4.626 $\pm$ 0.007 | 4.931 $\pm$ 0.006 | 5.351 $\pm$ 0.006 | 4.643 $\pm$ 0.011 | 4.860 $\pm$ 0.008 | 5.128 $\pm$ 0.010 |
|                  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $R_{pH^2}$       | 0.9931            | 0.9961            | 0.9987            | 0.9979            | 0.9948            | 0.9957            | 0.9912            | 0.9931            |
| $RPDM$           | 0.8326            | 0.5690            | 0.3595            | 0.5095            | 0.6495            | 0.8538            | 1.3258            | 1.0099            |
| $B_f$            | 1.0027            | 0.9997            | 1.0007            | 1.0017            | 1.0013            | 1.0035            | 1.0000            | 0.9951            |
| $A_f$            | 1.0083            | 1.0057            | 1.0036            | 1.0051            | 1.0065            | 1.0085            | 1.0134            | 1.0102            |
| $F\text{-ratio}$ | 1078.51           | 1189.17           | 1427.00           | 1377.82           | 1187.61           | 561.33            | 1214.71           | 615.38            |
| $P\text{-value}$ | < 0.0001          | < 0.0001          | < 0.0001          | < 0.0001          | < 0.0001          | < 0.0001          | < 0.0001          | < 0.0001          |
| $\alpha_{TN,X}$  | 0.264 $\pm$ 0.001 | 0.259 $\pm$ 0.003 | 0.377 $\pm$ 0.005 | 0.329 $\pm$ 0.011 | 0.265 $\pm$ 0.016 | 0.197 $\pm$ 0.003 | 0.310 $\pm$ 0.002 | 0.290 $\pm$ 0.018 |
|                  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $K_2$            | 0.294 $\pm$ 0.011 | 0.314 $\pm$ 0.007 | 0.261 $\pm$ 0.007 | 0.238 $\pm$ 0.007 | 0.295 $\pm$ 0.003 | 0.212 $\pm$ 0.009 | 0.328 $\pm$ 0.010 | 0.294 $\pm$ 0.006 |
|                  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $\alpha_{TN,BT}$ | 0.005 $\pm$ 0.000 | 0.005 $\pm$ 0.000 | 0.007 $\pm$ 0.000 | 0.007 $\pm$ 0.000 | 0.005 $\pm$ 0.000 | 0.005 $\pm$ 0.000 | 0.006 $\pm$ 0.000 | 0.006 $\pm$ 0.000 |
|                  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $K_3$            | 0.470 $\pm$ 0.049 | 0.470 $\pm$ 0.034 | 0.305 $\pm$ 0.039 | 0.470 $\pm$ 0.061 | 0.470 $\pm$ 0.025 | 0.442 $\pm$ 0.045 | 0.283 $\pm$ 0.033 | 0.395 $\pm$ 0.027 |
|                  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $R_{TN^2}$       | 0.9881            | 0.9946            | 0.9890            | 0.9901            | 0.9860            | 0.9892            | 0.9858            | 0.9956            |
| $RPDM$           | 1.3015            | 0.7133            | 1.9907            | 1.4406            | 1.5689            | 1.7611            | 2.2908            | 0.7884            |
| $B_f$            | 1.0050            | 1.0050            | 1.0072            | 1.0070            | 1.0088            | 1.0069            | 1.0073            | 1.0029            |
| $A_f$            | 1.0130            | 1.0071            | 1.0199            | 1.0143            | 1.0057            | 1.0176            | 1.0229            | 1.0079            |

|                                     |                      |                      |                      |                      |                      |                      |                      |                      |
|-------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| <i>F</i> -ratio                     | 89.73                | 111.61               | 109.92               | 129.73               | 747.64               | 46.27                | 40.04                | 205.86               |
| <i>P</i> -value                     | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             |
| $\alpha_{X,TN}$                     | 2.446±0.041          | 2.578±0.051          | 2.660±0.053          | 2.783±0.017          | 2.615±0.024          | 2.685±0.012          | 2.386±0.031          | 2.406±0.030          |
|                                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $\alpha^*_{X,BT}$                   | 0.006±0.000          | 0.006±0.000          | 0.006±0.000          | 0.006±0.000          | 0.006±0.000          | 0.006±0.000          | 0.006±0.000          | 0.006±0.000          |
|                                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $\alpha_{X,pH}$                     | 0.048±0.000          | 0.048±0.000          | 0.048±0.000          | 0.048±0.000          | 0.048±0.000          | 0.048±0.000          | 0.048±0.000          | 0.048±0.000          |
|                                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| <i>K</i> <sub>4</sub>               | 0.881±0.002          | 0.834±0.002          | 1.087±0.003          | 0.958±0.001          | 0.834±0.001          | 1.026±0.001          | 0.978±0.001          | 0.897±0.001          |
|                                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| <i>R</i> <sub>X</sub> <sup>2</sup>  | 0.9969               | 0.9955               | 0.9962               | 0.9998               | 0.9961               | 0.9999               | 0.9987               | 0.9986               |
| <i>RPDM</i>                         | 2.8967               | 3.3674               | 2.6718               | 0.5558               | 2.9667               | 0.4707               | 1.9843               | 2.0646               |
| <i>B<sub>f</sub></i>                | 0.9837               | 0.9812               | 0.9868               | 0.9997               | 0.9842               | 1.0021               | 0.9881               | 0.9871               |
| <i>A<sub>f</sub></i>                | 1.0301               | 1.0352               | 1.0277               | 1.0056               | 1.0308               | 1.0047               | 1.0204               | 1.0213               |
| <i>F</i> -ratio                     | 2823.29              | 2121.83              | 2174.82              | 19169.65             | 10058.71             | 35470.47             | 4642,57              | 4793.50              |
| <i>P</i> -value                     | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             |
| $\alpha_{BT,X}$                     | 0.002±0.000          | 0.002±0.000          | 0.002±0.000          | 0.002±0.000          | 0.002±0.000          | 0.002±0.000          | 0.002±0.000          | 0.002±0.000          |
|                                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $\alpha_{BT,TN}$                    | 1.529±0.001          | 1.511±0.009          | 2.061±0.023          | 2.539±0.023          | 1.547±0.005          | 2.607±0.023          | 1.998±0.015          | 1.885±0.014          |
|                                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $\alpha^*_{BT,X}$                   | 0.580±0.000          | 0.580±0.000          | 0.580±0.000          | 0.580±0.000          | 0.580±0.000          | 0.580±0.000          | 0.580±0.000          | 0.580±0.000          |
|                                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $\alpha_{BT,pH}$                    | 0.005±0.000          | 0.005±0.000          | 0.005±0.000          | 0.005±0.000          | 0.005±0.000          | 0.005±0.000          | 0.005±0.000          | 0.005±0.000          |
|                                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| <i>K</i> <sub>5</sub>               | 18.075±0.021         | 15.359±0.017         | 24.717±0.049         | 20.599±0.027         | 15.368±0.009         | 23.325±0.030         | 21.632±0.027         | 18.071±0.022         |
|                                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| <i>R</i> <sub>BT</sub> <sup>2</sup> | 0.9999               | 0.9999               | 0.9995               | 0.9997               | 0.9999               | 0.9997               | 0.9996               | 0.9997               |
| <i>RPDM</i>                         | 6.5207               | 6.5685               | 5.4800               | 4.5860               | 6.2595               | 4.8439               | 5.3122               | 5.4941               |
| <i>B<sub>f</sub></i>                | 0.9175               | 0.9163               | 0.9606               | 0.9847               | 0.9220               | 0.9921               | 0.9606               | 0.9531               |

|            |          |          |          |          |          |          |          |          |
|------------|----------|----------|----------|----------|----------|----------|----------|----------|
| $A_f$      | 1.0929   | 1.0939   | 1.0692   | 1.0529   | 1.0886   | 1.0550   | 1.0677   | 1.0719   |
| $F$ -ratio | 14078.78 | 15003.18 | 5399.44  | 9490.05  | 56340.20 | 10366.35 | 12532.03 | 12549.35 |
| $P$ -value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

**Table 6.** Statistically significant ( $P < 0.05$ ) parameter values (as estimates  $\pm$  confidence intervals) calculated with the four-dimensional LV-like equation (1)–(4) for each individual culture corresponding to the five center points (TS = 37.0 g/L, TP = 0.43 g/L) of the experimental matrix (Table 2) and to the optimum conditions (TS = 22.6 g/L, TP = 0.46 g/L) of the series of fermentation DW-TS-TP.

| Parameter        | Center points    |                  |                  |                  |                  | Optimum conditions |
|------------------|------------------|------------------|------------------|------------------|------------------|--------------------|
|                  | TS = 37.0 g/L    | TS = 37.0 g/L    | TS = 37.0 g/L    | TS = 37.0 g/L    | TS = 37.0 g/L    | TS = 22.6 g/L      |
|                  | TP = 0.43 g/L    | TP = 0.43 g/L    | TP = 0.43 g/L    | TP = 0.43 g/L    | TP = 0.43 g/L    | TP = 0.46 g/L      |
| $\alpha_{BC}$    | 0.012±0.001      | 0.012±0.001      | 0.012±0.001      | 0.012±0.001      | 0.012±0.001      | 0.010±0.001        |
|                  | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ )   |
| $\alpha_{pH,X}$  | 0.695±0.003      | 0.694±0.002      | 0.695±0.004      | 0.694±0.002      | 0.694±0.002      | 0.812±0.001        |
|                  | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ )   |
| $K_1$            | 4.894±0.007      | 4.896±0.007      | 4.866±0.007      | 4.891±0.008      | 4.927±0.001      | 4.525±0.006        |
|                  | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ )   |
| $R_{pH^2}$       | 0.9947           | 0.9938           | 0.9918           | 0.9958           | 0.9934           | 0.9992             |
| $RPDM$           | 0.9606           | 1.0312           | 1.1912           | 0.8968           | 1.1235           | 0.5352             |
| $B_f$            | 0.9999           | 0.9999           | 1.0011           | 0.9982           | 0.9972           | 0.9976             |
| $A_f$            | 1.0097           | 1.0104           | 1.0120           | 1.0090           | 1.0113           | 1.0054             |
| $F\text{-ratio}$ | 1351.81          | 1300.15          | 1338.04          | 1239.09          | 1234.48          | 2452.37            |
| $P\text{-value}$ | < 0.0001         | < 0.0001         | < 0.0001         | < 0.0001         | < 0.0001         | < 0.0001           |
| $\alpha_{TN,X}$  | 0.333±0.010      | 0.331±0.017      | 0.334±0.009      | 0.330±0.011      | 0.328±0.018      | 0.381±0.024        |
|                  | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ )   |
| $K_2$            | 0.307±0.006      | 0.298±0.007      | 0.307±0.006      | 0.298±0.005      | 0.312±0.007      | 0.318±0.007        |
|                  | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ )   |
| $\alpha_{TN,BT}$ | 0.007±0.000      | 0.007±0.000      | 0.007±0.000      | 0.007±0.000      | 0.007±0.000      | 0.008±0.000        |
|                  | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ )   |
| $K_3$            | 0.315±0.029      | 0.333±0.036      | 0.320±0.026      | 0.336±0.025      | 0.315±0.028      | 0.151±0.017        |
|                  | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ ) | ( $P < 0.0001$ )   |
| $R_{TN^2}$       | 0.9947           | 0.9928           | 0.9955           | 0.9964           | 0.9929           | 0.9848             |
| $RPDM$           | 1.0281           | 1.2965           | 0.8539           | 0.7589           | 1.3194           | 3.9910             |
| $B_f$            | 1.0037           | 1.0050           | 1.0027           | 1.0025           | 1.0038           | 1.0031             |
| $A_f$            | 1.0103           | 1.0129           | 1.0085           | 1.0076           | 1.0132           | 1.0403             |
| $F\text{-ratio}$ | 210.13           | 142.69           | 317.00           | 327.55           | 213.46           | 255.82             |



| P-value             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             |
|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| $\alpha_{X,TN}$     | 2.381±0.022          | 2.398±0.021          | 2.475±0.014          | 2.372±0.024          | 2.369±0.011          | 2.539±0.013          |
|                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $\alpha^{*}_{X,BT}$ | 0.006±0.000          | 0.006±0.000          | 0.006±0.000          | 0.006±0.000          | 0.006±0.000          | 0.006±0.000          |
|                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $\alpha_{X,pH}$     | 0.048±0.000          | 0.048±0.000          | 0.048±0.000          | 0.048±0.000          | 0.048±0.000          | 0.048±0.000          |
|                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $K_4$               | 0.959±0.001          | 0.962±0.001          | 0.968±0.001          | 0.956±0.001          | 0.961±0.001          | 1.081±0.001          |
|                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $R_X^2$             | 0.9994               | 0.9995               | 0.9998               | 0.9991               | 0.9999               | 0.9999               |
| RPDM                | 1.2540               | 1.0753               | 0.8266               | 1.4703               | 0.4259               | 1.3051               |
| $B_f$               | 0.9930               | 0.9944               | 1.0035               | 0.9930               | 0.9997               | 1.0066               |
| $A_f$               | 1.0128               | 1.0109               | 1.0083               | 1.0150               | 1.0043               | 1.0129               |
| F-ratio             | 8788.76              | 10035.77             | 23325.09             | 7397.12              | 32728.36             | 29518.63             |
| P-value             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             | < 0.0001             |
| $\alpha_{BT,X}$     | 0.002±0.000          | 0.002±0.000          | 0.002±0.000          | 0.002±0.000          | 0.002±0.000          | 0.002±0.000          |
|                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $\alpha_{BT,TN}$    | 1.983±0.018          | 1.971±0.017          | 2.102±0.0190         | 1.908±0.018          | 2.166±0.018          | 2.511±0.039          |
|                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $\alpha^{*}_{BT,X}$ | 0.580±0.000          | 0.580±0.000          | 0.580±0.000          | 0.580±0.000          | 0.580±0.000          | 0.580±0.000          |
|                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $\alpha_{BT,pH}$    | 0.005±0.000          | 0.005±0.000          | 0.005±0.000          | 0.005±0.000          | 0.005±0.000          | 0.005±0.000          |
|                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $K_5$               | 20.708±0.031         | 20.732±0.030         | 20.757±0.030         | 20.364±0.033         | 21.290±0.027         | 24.305±0.070         |
|                     | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) | ( <i>P</i> < 0.0001) |
| $R_{BT}^2$          | 0.9996               | 0.9997               | 0.9997               | 0.9997               | 0.9997               | 0.9990               |
| RPDM                | 5.5845               | 5.5226               | 5.1351               | 5.6615               | 4.9028               | 6.2342               |
| $B_f$               | 0.9637               | 0.9616               | 0.9671               | 0.9556               | 0.9693               | 0.9961               |
| $A_f$               | 1.0705               | 1.0702               | 1.0635               | 1.0728               | 1.0601               | 1.0693               |

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|                 |          |          |          |          |          |          |
|-----------------|----------|----------|----------|----------|----------|----------|
| <i>F</i> -ratio | 8816.37  | 9429.66  | 9190.09  | 8002.21  | 11225.47 | 3140.31  |
| <i>P</i> -value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

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In the series of cultures DW-TS-TP, the effects of initial TS and TP concentrations on both growth and bacteriocin production were described by empirical quadratic equations [15]. However, the inclusion of terms for explaining these effects in the four-dimensional LV-like equation (1)–(4) could contribute to obtaining a general equation for describing the evolution of the four response variables (pH, TN, X and BT). However, this approach has several drawbacks, since too large equations could be obtained, and information about the true relationship between the dependent variables ( $pH(t)$ ,  $TN(t)$ ,  $X(t)$ ,  $BT(t)$ ) and the own essence of the LV relationships would be lost.

In fact, the rates of culture pH drop (rpH), total nitrogen consumption (rTN), and biomass (rX) and nisin (rBT) production in the different experiments (1–14) did not show a clear dependence on changes in initial TS and TP concentrations (Figure 8).

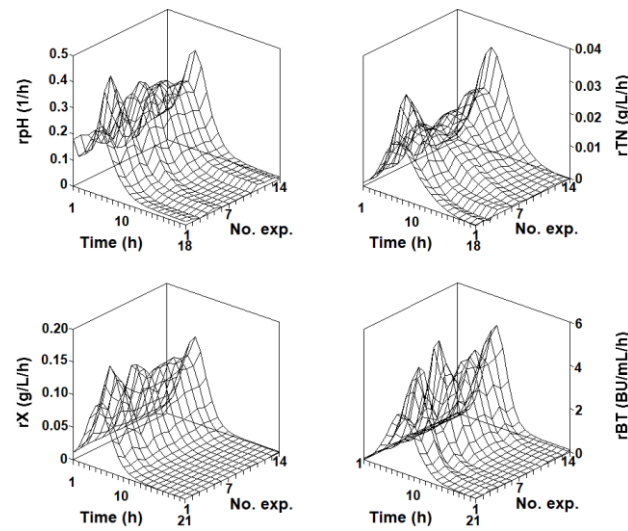


Figure 8. Response surfaces showing the time courses of the experimental rates of pH, TN, X and Nis in the 14 experiments (No. exp. = 1–14) performed at different initial TS and TP concentrations. The different rates were obtained from the experimental data shown in Figures 2 and 3.

### 2.2.3. Series of fermentation DW-MRS

In this series of fermentation (Figure 4), the values of  $\alpha_{BC}$  and  $\alpha_{pH,X}$  in equation (1) decreased with the increase in the initial MRS nutrients concentration in the medium (Table 7), due to the increase in the buffering capacity of the MRS nutrients-supplemented DW media [16]. The values of  $K_1$  varied slightly between 4.693 and 4.931 (Table 7) since the six individual cultures reached almost the same final pH value (between 4.64 and 4.73). The values of  $\alpha_{TN,X}$  and  $\alpha_{TN,BT}$  in equation (2) did not change, indicating that the TN source was proportionally used for growth and nisin synthesis in all cultures. Increased values of  $K_2$  and  $K_3$  were obtained because of the increasing final TN concentrations (0.311, 0.635, 1.315, 1.931, 2.700, and 3.430 g/L) in the MRS nutrients-supplemented media (Figure 4).

In equation (3),  $\alpha_{X,TN}$  decreased, suggesting that the nitrogen source was not consumed in parallel with the initial TN concentration in the medium. As observed in the series of fermentation DW-TS-TP, the values of  $\alpha_{X,BT}^*$  and  $\alpha_{X,pH}$  were constant in all cultures (Table 7).

**Table 7.** Statistically significant ( $P < 0.05$ ) parameter values (as estimates  $\pm$  confidence intervals) calculated with the four-dimensional LV-like equation (1)–(4) for each individual culture of the series of fermentation DW-MRS.

| Initial nutrient concentrations (% <i>, w/v</i> ) in the DW medium |                   |                   |                   |                   |                   |                   |
|--------------------------------------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Parameter                                                          | 0                 | 25                | 50                | 75                | 100               | 125               |
| $\alpha_{BC}$                                                      | 0.197 $\pm$ 0.021 | 0.165 $\pm$ 0.009 | 0.097 $\pm$ 0.006 | 0.038 $\pm$ 0.003 | 0.014 $\pm$ 0.001 | 0.006 $\pm$ 0.001 |
|                                                                    | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $\alpha_{pH,X}$                                                    | 1.518 $\pm$ 0.203 | 0.620 $\pm$ 0.059 | 0.431 $\pm$ 0.040 | 0.345 $\pm$ 0.027 | 0.338 $\pm$ 0.017 | 0.289 $\pm$ 0.009 |
|                                                                    | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $K_1$                                                              | 4.852 $\pm$ 0.027 | 4.931 $\pm$ 0.025 | 4.802 $\pm$ 0.024 | 4.751 $\pm$ 0.026 | 4.741 $\pm$ 0.021 | 4.693 $\pm$ 0.016 |
|                                                                    | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $R_{pH^2}$                                                         | 0.9910            | 0.9967            | 0.9965            | 0.9957            | 0.9972            | 0.9989            |
| $RPDM$                                                             | 0.8627            | 0.4439            | 0.5254            | 0.6755            | 0.5505            | 0.3856            |
| $B_f$                                                              | 0.9998            | 0.9995            | 0.9993            | 0.9992            | 0.9994            | 0.9997            |
| $A_f$                                                              | 1.0086            | 1.0045            | 1.0053            | 1.0068            | 1.0055            | 1.0039            |
| $F\text{-ratio}$                                                   | 278.56            | 312.67            | 182.47            | 79.77             | 98.92             | 227.65            |
| $P\text{-value}$                                                   | $< 0.0001$        | $< 0.0001$        | $< 0.0001$        | $< 0.0001$        | $< 0.0001$        | $< 0.0001$        |
| $\alpha_{TN,X}$                                                    | 0.077 $\pm$ 0.002 | 0.077 $\pm$ 0.001 | 0.077 $\pm$ 0.002 | 0.077 $\pm$ 0.003 | 0.077 $\pm$ 0.000 | 0.077 $\pm$ 0.000 |
|                                                                    | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $K_2$                                                              | 0.126 $\pm$ 0.002 | 0.398 $\pm$ 0.003 | 1.485 $\pm$ 0.006 | 1.781 $\pm$ 0.002 | 1.847 $\pm$ 0.001 | 1.974 $\pm$ 0.001 |
|                                                                    | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $\alpha_{TN,BT}$                                                   | 0.004 $\pm$ 0.000 | 0.004 $\pm$ 0.000 | 0.004 $\pm$ 0.000 | 0.004 $\pm$ 0.000 | 0.004 $\pm$ 0.001 | 0.004 $\pm$ 0.001 |
|                                                                    | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $K_3$                                                              | 0.669 $\pm$ 0.026 | 0.719 $\pm$ 0.069 | 1.266 $\pm$ 0.010 | 1.989 $\pm$ 0.017 | 2.998 $\pm$ 0.011 | 4.223 $\pm$ 0.072 |
|                                                                    | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  | ( $P < 0.0001$ )  |
| $R_{TN^2}$                                                         | 0.9952            | 0.9887            | 0.9965            | 0.9938            | 0.9809            | 0.9982            |
| $RPDM$                                                             | 1.1253            | 5.4775            | 1.1753            | 2.0317            | 2.0080            | 0.3172            |
| $B_f$                                                              | 1.0020            | 1.0544            | 0.9936            | 0.9885            | 1.0199            | 0.9992            |
| $A_f$                                                              | 1.0113            | 1.0544            | 1.0119            | 1.0208            | 1.0200            | 1.0032            |
| $F\text{-ratio}$                                                   | 1040.01           | 723.43            | 1055.35           | 1233.66           | 1209.41           | 1044.16           |

| P-value           | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              |
|-------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|
| $\alpha_{X,TN}$   | 1.065±0.010<br>( <i>P</i> < 0.0001)                   | 0.528±0.009<br>( <i>P</i> < 0.0001)                   | 0.240±0.003<br>( <i>P</i> < 0.0001)                   | 0.136±0.002<br>( <i>P</i> < 0.0001)                   | 0.095±0.001<br>( <i>P</i> < 0.0001)                   | 0.079±0.001<br>( <i>P</i> < 0.0001)                   |
| $\alpha^*_{X,BT}$ | 1.881x10 <sup>-6</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.881x10 <sup>-6</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.881x10 <sup>-6</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.881x10 <sup>-6</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.881x10 <sup>-6</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.881x10 <sup>-6</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $\alpha_{X,pH}$   | 4.824x10 <sup>-7</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.824x10 <sup>-7</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.824x10 <sup>-7</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.824x10 <sup>-7</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.824x10 <sup>-7</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.824x10 <sup>-7</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $K_4$             | 0.486±0.001<br>( <i>P</i> < 0.0001)                   | 0.586±0.002<br>( <i>P</i> < 0.0001)                   | 0.626±0.001<br>( <i>P</i> < 0.0001)                   | 0.780±0.003<br>( <i>P</i> < 0.0001)                   | 0.856±0.003<br>( <i>P</i> < 0.0001)                   | 0.857±0.003<br>( <i>P</i> < 0.0001)                   |
| $R_X^2$           | 0.9997                                                | 0.9985                                                | 0.9996                                                | 0.9997                                                | 0.9997                                                | 0.9998                                                |
| <i>RPDM</i>       | 1.3539                                                | 2.7984                                                | 1.5402                                                | 1.9298                                                | 6.1988                                                | 9.7202                                                |
| $B_f$             | 1.0090                                                | 1.0176                                                | 1.0105                                                | 1.0141                                                | 1.0171                                                | 1.0130                                                |
| $A_f$             | 1.0134                                                | 1.0275                                                | 1.0153                                                | 1.0192                                                | 1.0218                                                | 1.0171                                                |
| <i>F</i> -ratio   | 6655.68                                               | 1991.30                                               | 4543.13                                               | 2238.06                                               | 2418.03                                               | 3559.48                                               |
| P-value           | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              |
| $\alpha_{BT,X}$   | 1.617x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.617x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.617x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.617x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.617x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.617x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $\alpha_{BT,TN}$  | 1.321±0.031<br>( <i>P</i> < 0.0001)                   | 0.606±0.016<br>( <i>P</i> < 0.0001)                   | 0.248±0.005<br>( <i>P</i> < 0.0001)                   | 0.179±0.003<br>( <i>P</i> < 0.0001)                   | 0.123±0.002<br>( <i>P</i> < 0.0001)                   | 0.101±0.001<br>( <i>P</i> < 0.0001)                   |
| $\alpha^*_{BT,X}$ | 0.024±0.000<br>( <i>P</i> < 0.0001)                   | 0.024±0.000<br>( <i>P</i> < 0.0001)                   | 0.024±0.000<br>( <i>P</i> < 0.0001)                   | 0.024±0.000<br>( <i>P</i> < 0.0001)                   | 0.024±0.000<br>( <i>P</i> < 0.0001)                   | 0.024±0.000<br>( <i>P</i> < 0.0001)                   |
| $\alpha_{BT,pH}$  | 4.567x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.567x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.567x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.567x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.567x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.567x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $K_5$             | 22.658±0.104<br>( <i>P</i> < 0.0001)                  | 28.036±0.165<br>( <i>P</i> < 0.0001)                  | 36.898±0.213<br>( <i>P</i> < 0.0001)                  | 45.295±0.170<br>( <i>P</i> < 0.0001)                  | 56.945±0.266<br>( <i>P</i> < 0.0001)                  | 58.914±0.216<br>( <i>P</i> < 0.0001)                  |
| $R_{BT}^2$        | 0.9992                                                | 0.9990                                                | 0.9992                                                | 0.9995                                                | 0.9991                                                | 0.9993                                                |
| <i>RPDM</i>       | 8.8153                                                | 6.0134                                                | 10.0347                                               | 10.0256                                               | 15.6883                                               | 19.6949                                               |
| $B_f$             | 0.8966                                                | 0.9260                                                | 0.8697                                                | 0.8703                                                | 0.8497                                                | 0.8493                                                |
| $A_f$             | 1.1211                                                | 1.0853                                                | 1.1568                                                | 1.1562                                                | 1.1874                                                | 1.1875                                                |

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|                 |          |          |          |          |          |          |
|-----------------|----------|----------|----------|----------|----------|----------|
| <i>F</i> -ratio | 922,07   | 752.96   | 850.34   | 2046.28  | 1385.29  | 2313.89  |
| <i>P</i> -value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

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The values of  $K_4$  increased with the increase in MRS nutrients addition, in a perfect agreement with the increase in the final biomass concentrations (0.482, 0.582, 0.621, 0.759, 0.818, and 0.833 g/L) in the DW-MRS media (Figure 4).

As occurred in the two previous series of fermentation DW-G and DW-TS-TP, constant values for the parameters  $\alpha_{BT,X}$ ,  $\alpha_{BT,X}^*$  and  $\alpha_{BT,pH}$  were obtained in eq. (4) in the different cultures (Table 7). The values calculated for  $\alpha_{BT,TN}$  decreased, indicating that the TN consumption for nisin synthesis was not proportional to the initial TN concentration in the media. The increase in the  $K_5$  values (22.658, 28.036, 36.898, 45.295, 56.945, and 58.914) was in accordance with the final BT values (22.67, 28.07, 37.00, 45.45, 57.26, and 59.23 BU/mL) reached in the cultures (Figure 4).

In this series of cultures, the increase in the MRS nutrients addition to the DW medium affected both the evolution of the cultures and the final concentrations of biomass and nisin obtained (Figure 4), as well as the rates  $rpH$ ,  $rTN$ ,  $rX$  and  $rBT$  (Figure 9). The rates of culture pH decrease exhibited a transition from exponential decay-shaped curves (in the DW25, DW50, DW75 media) to bell-shaped curves (in the DW100 and DW125 media), meanwhile the  $rTN$ ,  $rX$  and  $rBT$  profiles showed bell-shaped curves. Although the  $rpH$  decreased exponentially with the increase in the initial MRS nutrients concentration ( $[Nut]_0$ ) in the medium (upper left part of Figure 9), the  $rTN$ ,  $rX$  and  $rBT$  profiles did not show an appreciable relationship (linear, quadratic, sigmoidal, etc.) with  $[Nut]_0$ .

Therefore, in this case, it is difficult to develop a general four-dimensional LV-like equation (e.g. equations (8)-(11)) describing the evolution of pH, TN, X and BT as a function of the initial concentrations of MRS nutrients.

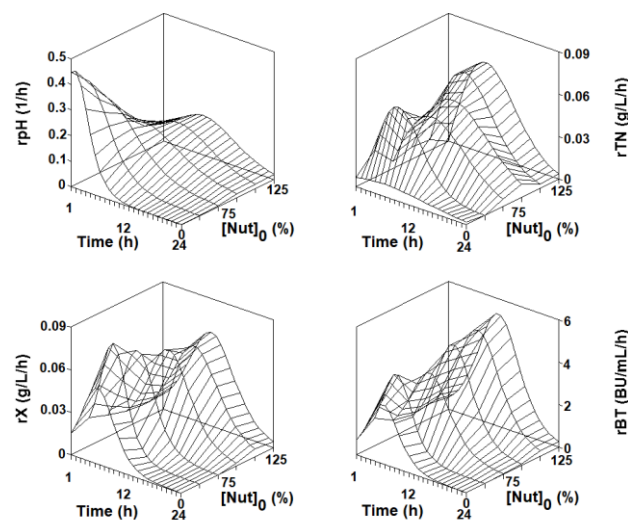


Figure 9. Response surfaces showing the time courses of the experimental rates of pH, TN, X and Nis as a function of the initial concentration of MRS both nutrients ( $[Nut]_0 = 0, 25, 50, 75, 100$ , and  $125\%$ ) in the DW medium. The different rates were obtained from the experimental data shown in Figure 4.

#### 2.2.4. Series of fermentation DW-pH

The results obtained in these series of cultures are shown in Table 8 and the predictions of equations (1)–(4) are shown as solid lines in Figure 5. In these fermentations, the lowest values of  $\alpha_{BC}$  were calculated for the cultures performed at pH values lower than 6.0, in which the lowest growth and pH decrease were observed (Figure 5). In contrast, the highest  $\alpha_{BC}$  values were obtained in the cultures conducted at pH values  $\geq 6.0$  (Table 8), in which the highest growths and pH decreases were observed (Figure 5).



**Table 8.** Statistically significant ( $P < 0.05$ ) parameter values (as estimates  $\pm$  confidence intervals) calculated with the four-dimensional LV-like equation (1)–(4) for each individual culture of the series of fermentation DW-pH.

| Parameter        | Initial pH values             |                               |                               |                               |                               |                               |                               |
|------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|                  | 4.5                           | 5.0                           | 5.5                           | 6.0                           | 6.5                           | 7.0                           | 7.5                           |
| $\alpha_{BC}$    | 2.341x10 <sup>-7</sup> ±0.000 | 1.431x10 <sup>-4</sup> ±0.000 | 2.895x10 <sup>-4</sup> ±0.000 | 0.003±0.000                   | 0.003±0.000                   | 0.003±0.000                   | 0.003±0.000                   |
|                  | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P = 0.0306$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              |
| $\alpha_{pH,X}$  | 0.141±0.004                   | 0.258±0.031                   | 0.512±0.006                   | 0.529±0.009                   | 0.535±0.016                   | 0.390±0.020                   | 0.381±0.013                   |
|                  | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              |
| $K_1$            | 4.457±0.000                   | 4.412±0.066                   | 4.375±0.001                   | 4.425±0.004                   | 4.546±0.010                   | 4.751±0.038                   | 5.343±0.021                   |
|                  | ( $P < 0.0001$ )              | ( $P = 0.0004$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              |
| $R_{pH^2}$       | 0.9982                        | 0.9958                        | 0.9841                        | 0.9993                        | 0.9944                        | 0.9948                        | 0.9958                        |
| $RPDM$           | 4.7481                        | 0.3412                        | 2.5536                        | 0.3283                        | 1.1378                        | 1.3306                        | 0.9188                        |
| $B_f$            | 1.0020                        | 1.0025                        | 1.0254                        | 0.9976                        | 1.0026                        | 0.9975                        | 1.0019                        |
| $A_f$            | 1.0020                        | 1.0034                        | 1.0254                        | 1.0033                        | 1.0114                        | 1.0134                        | 1.0092                        |
| $F\text{-ratio}$ | 1190.85                       | 1103.97                       | 752.90                        | 1133.28                       | 1175.91                       | 1132.60                       | 142.82                        |
| $P\text{-value}$ | < 0.0001                      | < 0.0001                      | < 0.0001                      | < 0.0001                      | < 0.0001                      | < 0.0001                      | < 0.0001                      |
| $\alpha_{TN,X}$  | 0.318±0.090                   | 0.324±0.011                   | 0.345±0.009                   | 0.373±0.035                   | 0.405±0.014                   | 0.337±0.011                   | 0.332±0.010                   |
|                  | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              |
| $K_2$            | 2.781±0.125                   | 2.692±0.026                   | 3.040±0.032                   | 3.036±0.063                   | 4.627±0.064                   | 2.577±0.140                   | 2.798±0.023                   |
|                  | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              |
| $\alpha_{TN,BT}$ | 1.226x10 <sup>-3</sup> ±0.001 | 1.235x10 <sup>-3</sup> ±0.000 | 1.259x10 <sup>-3</sup> ±0.000 | 1.303x10 <sup>-3</sup> ±0.000 | 1.467x10 <sup>-3</sup> ±0.000 | 1.508x10 <sup>-3</sup> ±0.000 | 5.121x10 <sup>-4</sup> ±0.000 |
|                  | ( $P = 0.0084$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              |
| $K_3$            | 1.795±0.038                   | 1.856±0.095                   | 1.861±0.089                   | 0.555±0.043                   | 0.587±0.227                   | 3.267±0.110                   | 3.603±0.064                   |
|                  | ( $P < 0.0001$ )              | ( $P = 0.0080$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              | ( $P < 0.0001$ )              |
| $R_{TN^2}$       | 0.9815                        | 0.9824                        | 0.9992                        | 0.9991                        | 0.9966                        | 0.9985                        | 0.9991                        |
| $RPDM$           | 5.0329                        | 0.6907                        | 0.1428                        | 0.4606                        | 1.0937                        | 0.3136                        | 0.1652                        |
| $B_f$            | 0.9974                        | 0.9996                        | 0.9995                        | 1.0016                        | 1.0050                        | 1.0000                        | 0.9991                        |
| $A_f$            | 1.0049                        | 1.0069                        | 1.0014                        | 1.0046                        | 1.0109                        | 1.0031                        | 1.0017                        |
| $F\text{-ratio}$ | 67.19                         | 78.53                         | 193.93                        | 134.41                        | 61.58                         | 39.43                         | 293.59                        |

|                   |                                                       |                                                       |                                                       |                                                       |                                                       |                                                       |                                                       |
|-------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------|
| <i>P</i> -value   | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              |
| $\alpha_{X,TN}$   | 0.014±0.007<br>( <i>P</i> = 0.0073)                   | 0.073±0.001<br>( <i>P</i> < 0.0001)                   | 0.113±0.001<br>( <i>P</i> < 0.0001)                   | 0.137±0.002<br>( <i>P</i> < 0.0001)                   | 0.138±0.002<br>( <i>P</i> < 0.0001)                   | 0.121±0.001<br>( <i>P</i> < 0.0001)                   | 0.120±0.001<br>( <i>P</i> < 0.0001)                   |
| $\alpha^*_{X,BT}$ | 8.625x10 <sup>-4</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.625x10 <sup>-4</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.625x10 <sup>-4</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.625x10 <sup>-4</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.625x10 <sup>-4</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.625x10 <sup>-4</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.625x10 <sup>-4</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $\alpha_{X,pH}$   | 4.579x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.579x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.579x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.579x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.579x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.579x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 4.579x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $K_4$             | 0.257±0.054<br>( <i>P</i> < 0.0001)                   | 0.288±0.001<br>( <i>P</i> < 0.0001)                   | 0.657±0.001<br>( <i>P</i> < 0.0001)                   | 0.878±0.003<br>( <i>P</i> < 0.0001)                   | 0.935±0.004<br>( <i>P</i> < 0.0001)                   | 0.821±0.004<br>( <i>P</i> < 0.0001)                   | 0788±0.001<br>( <i>P</i> < 0.0001)                    |
| $R_{X^2}$         | 0.9688                                                | 0.9999                                                | 0.9999                                                | 0.9997                                                | 0.9995                                                | 0.9953                                                | 0.9999                                                |
| $RPDM$            | 18.1531                                               | 9.5294                                                | 5.2973                                                | 1.8362                                                | 2.4203                                                | 11.7785                                               | 0.8993                                                |
| $B_f$             | 0.9712                                                | 1.0023                                                | 1.0053                                                | 1.0124                                                | 1.0179                                                | 1.1162                                                | 1.0068                                                |
| $A_f$             | 1.0471                                                | 1.0044                                                | 1.0075                                                | 1.0182                                                | 1.0239                                                | 1.1162                                                | 1.0090                                                |
| <i>F</i> -ratio   | 89.33                                                 | 4081.32                                               | 11349.44                                              | 3231.17                                               | 1841.10                                               | 1583.65                                               | 13438.81                                              |
| <i>P</i> -value   | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              | < 0.0001                                              |
| $\alpha_{BT,X}$   | 1.570x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.570x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.570x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.570x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.570x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.570x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 1.570x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $\alpha_{BT,TN}$  | 0.106±0.003<br>( <i>P</i> < 0.0001)                   | 0.114±0.001<br>( <i>P</i> < 0.0001)                   | 0.125±0.001<br>( <i>P</i> < 0.0001)                   | 0.130±0.002<br>( <i>P</i> < 0.0001)                   | 0.140±0.003<br>( <i>P</i> < 0.0001)                   | 0.147±0.001<br>( <i>P</i> < 0.0001)                   | 0.129±0.001<br>( <i>P</i> < 0.0001)                   |
| $\alpha^*_{BT,X}$ | 8.337x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.337x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.337x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.337x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.337x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.337x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) | 8.337x10 <sup>-3</sup> ±0.000<br>( <i>P</i> < 0.0001) |
| $\alpha_{BT,pH}$  | 0.060±0.003<br>( <i>P</i> < 0.0001)                   | 0.062±0.001<br>( <i>P</i> < 0.0001)                   | 0.058±0.002<br>( <i>P</i> < 0.0001)                   | 0.055±0.001<br>( <i>P</i> < 0.0001)                   | 0.043±0.002<br>( <i>P</i> < 0.0001)                   | 0.035±0.001<br>( <i>P</i> < 0.0001)                   | 0.048±0.002<br>( <i>P</i> < 0.0001)                   |
| $K_5$             | 1.301±0.019<br>( <i>P</i> < 0.0001)                   | 5.350±0.028<br>( <i>P</i> < 0.0001)                   | 10.489±0.017<br>( <i>P</i> < 0.0001)                  | 22.863±0.226<br>( <i>P</i> < 0.0001)                  | 44.980±0.389<br>( <i>P</i> < 0.0001)                  | 55.061±0.145<br>( <i>P</i> < 0.0001)                  | 38.428±0.159<br>( <i>P</i> < 0.0001)                  |
| $R_{BT^2}$        | 0.9993                                                | 0.9999                                                | 0.9960                                                | 0.9940                                                | 0.9978                                                | 0.9989                                                | 0.9968                                                |
| $RPDM$            | 33.6961                                               | 24.2919                                               | 15.1509                                               | 13.7076                                               | 11.6965                                               | 11.0016                                               | 13.3502                                               |
| $B_f$             | 0.8496                                                | 0.8685                                                | 0.8590                                                | 0.8555                                                | 0.8670                                                | 0.7973                                                | 0.7339                                                |
| $A_f$             | 1.3682                                                | 1.2580                                                | 1.1632                                                | 1.2138                                                | 1.1828                                                | 1.1691                                                | 1.2104                                                |

[illegible]

The highest  $\alpha_{pH,X}$  value was obtained at initial pH 6.5, which was the best initial pH for growth (Table 8, Figure 5). The  $K_1$  values in the cultures conducted at initial pH values of 4.5, 5.0, 5.5, 6.0, and 6.5 were between 4.37 and 4.55 (Table 8), which are in accordance with the final pH values (between 4.42 and 4.54) reached in these cultures (Figure 5). In the cultures performed at the highest pH levels (7.0 and 7.5), the calculated values of  $K_1$  (4.75 and 5.34, respectively) were also in accordance with the final values reached in these cultures (4.73 and 5.35, respectively).

The highest concentrations of biomass (0.855 g/L) and nisin (55.18 BU/mL) were, respectively, obtained in the cultures at initial pH 6.5 and 7.0 (Figure 5). Consequently, the highest  $\alpha_{TN,X}$  ( $0.405 \pm 0.014$ ) and  $\alpha_{TN,BT}$  ( $1.508 \times 10^{-3} \pm 0.000$ ) values were, respectively, obtained in these cultures (Table 8). Probably for this reason, the highest value of  $K_2$  ( $4.627 \pm 0.064$ ) was calculated at pH 6.5, meanwhile the values of  $K_3$  were higher ( $3.267 \pm 0.110$  and  $3.603 \pm 0.064$ ) at initial levels of pH 7.0 and 7.5, respectively (Table 8).

The values of  $\alpha_{X,TN}$  in equation (3) increased from pH 4.5 to 6.5 (the optimum pH for growth) and decreased at higher pH values. The constant values obtained for  $\alpha_{X,BT}^*$  and  $\alpha_{X,pH}$  were obtained indicating a constancy in the competition between biomass and nisin for the nitrogen source and a similar effect of the evolution of the culture pH on the time course of the growth in the different cultures (Table 8). The values of  $K_4$  increased from pH 4.5 to 6.5 and decreased for initial pH values of 7.0 and 7.5, in accordance with the same trend observed for the final biomass concentrations reached in the cultures (see Table 8 and Figure 5).

As the nisin showed pH-dependent primary metabolite kinetics in these cultures [16], the values calculated for  $\alpha_{BT,X}$  in equation (4) were similar (Table 8) and due to the above-mentioned constancy of the competition between biomass and nisin for the nitrogen source, a constant value for  $\alpha_{BT,X}^*$  was obtained.

The highest value for  $\alpha_{BT,TN}$ , and the lowest value for  $\alpha_{BT,pH}$  were obtained at pH 7.0 (the optimum pH for nisin synthesis) indicating that TN consumption for nisin synthesis was favored at initial pH 7.0 and, both pH and pH drop evolution were optimal for nisin production (Figure 5). The values obtained for  $K_5$  (1.301, 5.350, 10.489, 22.863, 44.980, 55.061, and 38.428) were in perfect agreement with the final nisin concentrations (0.83, 3.57, 10.27, 22.94, 45.14, 55.18, and 38.63 BU/mL) reached in the cultures performed at initial pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5, respectively (Table 8 and Figure 5), as observed in the previous series of batch fermentation (DW-G, DW-TS-TP, and DW-MRS).

Since there was no appreciable relation (*e.g.*, linear, quadratic, exponential) between the  $rpH$ ,  $rTN$ ,  $rX$  and  $rBT$  time courses with the initial pH values in the cultures (Figure 10), the use of modified four-dimensional LV-like equation (8)–(11) to explain the relationships between the evolution of the four variables (culture pH, TN, X and BT) was also unsatisfactory in the series of fermentation DW-pH.

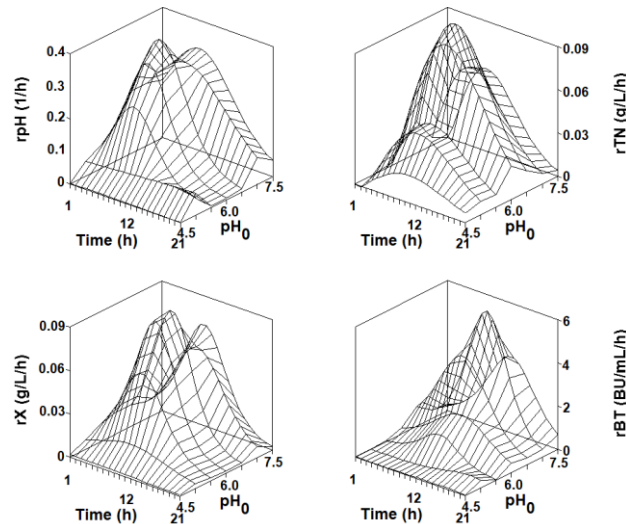


Figure 10. Response surfaces showing the time courses of the experimental rates of pH, TN, X and Nis as a function of the initial culture pH ( $pH_0 = 4.5, 5.0, 5.5, 6.0, 6.5, 7.0$ , and  $7.5$ ). The different rates were obtained from the experimental data shown in Figure 5.

### 3. Materials and Methods

#### 3.1. Microorganisms, Culture Media and Inoculum Preparation

In this work, *Lactococcus lactis* CECT 539 and *Carnobacterium piscicola* CECT 4020 were used as the nisin-producing strain and target bacterium (in the nisin activity bioassay), respectively. Both strains were obtained from the Spanish Type Culture Collection (CECT) and cultured at 30 °C in MRS (de Man Rogosa and Sharpe) agar slants or broth.

Diluted whey (DW) and concentrated mussel-processing waste (CMPW) were used to prepare the different culture media (Table 1). Sterilization (121 °C/15 min) of these substrates led to the precipitation of a protein fraction that interfered with biomass measurements. For this reason, the precipitated material was removed by acidification of the DW and CMPW substrates to pH 4.5 with 5 N HCl, heating (121 °C/15 min) and centrifugation ( $12,000 \times g$  for 15 min) [22,23].

Given that the nisin-producing strain is not an amylolytic bacterium, the glycogen contained in the CMPW was enzymatically hydrolyzed to produce a glucose-containing substrate as described in Costas et al. [27].

To prepare the different fermentation substrates, DW medium was supplemented with the following nutrients: i) different amounts of glucose to obtain 5, 10, 15, 20, and 25 g glucose/L of medium (series of fermentation DW-G) [14], ii) different volumes of CMPW medium (101.33 g glucose/L) and amounts of  $KH_2PO_4$  to obtain initial total sugars and phosphorous concentrations between 22.61 and 51.35 g/L, and 0.24 and 0.63 g/L, respectively (series of fermentation DW-TS-TP) [15], and iii) MRS broth nutrients at 25, 50, 75, 100, and 125% (w/v) of their standard concentrations in the complex substrate to produce the DW25, DW50, DW75, DW100, and DW125 media (series of fermentation DW-MRS) [16]. In the latter cultures, glucose and Tween 80 were not added to the DW medium because the addition of these compounds did not improve nisin production [16]. In these three series of fermentation, control cultures in unsupplemented DW medium were performed to obtain data for the comparisons [14–16].

The fourth series of batch cultures was conducted in DW100 medium adjusted to different initial pH values: 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 or 7.5 (series of fermentation DW-pH) [16]. Tables 1 and 2 show the mean compositions of the resulting culture media used in this work.

To prepare the preculture, cells of *L. lactis* CECT 539 were inoculate from MRS agar slants to sterile MRS broth (10 mL) and incubated at 30 °C for 12 h with shaking at 200

rpm. After that, 50 mL of inoculum medium (which was, in each case, similar to the corresponding fermentation substrate) were inoculated with 1 mL of the preculture and subsequently incubated for 12 h at 30 °C with shaking at 200 rpm. An appropriate volume of the latter culture was used to inoculate the corresponding fermentation medium used in the different batch cultures to give an initial viable cell count of  $1.5 \times 10^9$  colony-forming units/mL [14–16].

### 3.2. Batch Cultures

The different experimental data used in this research were collected from previous batch cultures of *L. lactis* CECT 539 [14–16]. The fermentations were conducted in 250-mL Erlenmeyer flasks that contained 50 mL of the corresponding DW-based medium. After inoculation, the flasks were incubated at 30 °C with shaking at 200 rpm for 18 h [14], 21 h [15] and 24 h [16].

### 3.3. Analytical Methods

The corresponding analytical methods used to measure culture pH and concentrations of total nitrogen, biomass and nisin were previously described in Costas et al. [14].

### 3.4. Modeling Procedures

The four-dimensional equation (Eqs. 1–4) based on the competitive Lotka–Volterra (LV)-like equations [13] was developed to describe the relationships between the dynamics of culture pH ( $pH(t)$ ), total nitrogen ( $TN(t)$ ) consumption, and biomass ( $X(t)$ ) and nisin ( $BT(t)$ ) production, with the following assumptions:

i) The culture pH decline could be described as the difference between the buffer capacity of the medium [28] and the logistic decrease of pH due to lactic acid production by the biomass because of its metabolic activity:

$$-\frac{dpH(t)}{dt} = \frac{\alpha_{BC}}{\delta pH(t)} - \alpha_{pH,X} \cdot pH(t) \cdot \left(1 - \frac{pH(t)}{K_1}\right) \cdot X(t) \quad (1)$$

ii) The total nitrogen, the limiting nutrient in these cultures [5] which is channeled into the cells and nisin molecules, could be considered as a prey that is consumed for both biomass and nisin production:

$$-\frac{dT_N(t)}{dt} = -\alpha_{TN,X} \cdot TN(t) \cdot \left(1 - \frac{TN(t)}{K_2}\right) \cdot X(t) - \alpha_{TN,BT} \cdot TN(t) \cdot \left(1 - \frac{TN(t)}{K_3}\right) \cdot BT(t) \quad (2)$$

iii) Biomass could be considered as one predator that grows logistically competing with nisin for the nitrogen source (the limiting nutrient) and depending on the culture pH:

$$\frac{dX(t)}{dt} = \alpha_{X,TN} \cdot X(t) \cdot \left(1 - \frac{X(t) + \alpha_{X,BT}^* \cdot BT(t) + \alpha_{X,pH} \cdot pH(t)}{K_4}\right) \cdot TN(t) \quad (3)$$

iv) Nisin could be considered as the second predator that is produced by the biomass but competes with it for the nitrogen source, and depends on the culture pH:

$$\frac{dBT(t)}{dt} = \alpha_{BT,X} \cdot X(t) \cdot BT(t) + \alpha_{BT,TN} \cdot BT(t) \cdot \left(1 - \frac{BT(t) + \alpha_{BT,X}^* \cdot X(t) + \alpha_{BT,pH} \cdot pH(t)}{K_5}\right) \cdot TN(t) \quad (4)$$

From these equations, it can be clearly noted that, in the absence of biomass (that means for  $X(t_0) = 0$ ), nisin could not be produced ( $BT(t_0) = 0$ ), and consequently,  $dpH(t_0)/dt = 0$  in eq. (1),  $dTN(t_0)/dt = 0$  in eq. (2),  $dX(t_0)/dt = 0$  in eq. (3) and  $dB T(t_0)/dt = 0$  in eq. (4).

The capability of the four-dimensional LV equation (1)–(4) to describe the batch nisin production system was first assessed by adjusting a unique global set of model parameters (general equation) to the entire set of experimental data in each series of fermentation (DW-G, DW-TS-TP, DW-MRS and DW-pH cultures).

Second, the values of the parameters of the four-dimensional LV equation were continuously adjusted to describe the time course of  $pH(t)$ ,  $TN(t)$ ,  $X(t)$  and  $BT(t)$  of each individual culture in different series of fermentation.

Before being used to fit the four-dimensional LV-like equation, the experimental data of the culture pH and remaining concentrations of total nitrogen, biomass and nisin [14–16] were smoothed using the following logistic equations (5–7):

For the culture pH ( $Q(t) = pH(t)$ ) and total nitrogen ( $Q(t) = TN(t)$ ) decrease, we modified the logistic decline equation presented by Goudar et al. [29] as follows:

$$Q(t) = \frac{A}{B - e^{(C_0 + C_1 \cdot t + C_2 \cdot t^2)}} \quad (5)$$

$$\text{Being } A = \frac{pH_0 \cdot pH_f}{pH_0 - pH_f} \text{ for culture pH or } A = \frac{TN_0 \cdot TN_f}{TN_0 - TN_f} \text{ for TN}$$

$$\text{and } B = \frac{pH_0}{pH_0 - pH_f} \text{ for culture pH or } B = \frac{TN_0}{TN_0 - TN_f} \text{ for TN}$$

For biomass ( $X(t)$ ) production, the logistic equation presented by Goudar et al. [29] was used by considering that the death cell rate was zero:

$$X(t) = \frac{D}{1 + E \cdot e^{-F \cdot t}} \quad (6)$$

$$\text{Being } D = X_{max} \text{ and } E = \frac{X_{max} - X_0}{X_0}$$

For nisin ( $BT(t)$ ) synthesis, we modified the logistic decline equation [29] as follows:

$$BT(t) = \frac{G}{1 + H \cdot e^{(-I \cdot t)}} - \frac{G}{1 + H} \quad (7)$$

$$\text{Being } G = BT_{max}$$

In an attempt to obtain a general equation for describing the batch nisin production system, the four-dimensional LV-like equation (1)–(4) was modified by including a term ( $\delta_i = \exp(-J \cdot F_0)$ ) to explain the effect of the initial culture conditions ( $F_0$ : concentrations of glucose, TS and TP, and MRS broth nutrients, or culture pH) on the evolution of the dependent variables  $pH(t)$ ,  $TN(t)$ ,  $X(t)$  and  $BT(t)$ . So that, the four-dimensional LV-like equation (1)–(4) was modified as follows:

$$-\frac{dpH(t)}{dt} = \left( \frac{\alpha_{BC}}{\delta pH(t)} - \alpha_{pH,X} \cdot pH(t) \cdot \left(1 - \frac{pH(t)}{K_1}\right) \cdot X(t) \right) \cdot \delta_i \quad (8)$$

$$-\frac{dT_N(t)}{dt} = \left( -\alpha_{TN,X} \cdot TN(t) \cdot \left(1 - \frac{TN(t)}{K_2}\right) \cdot X(t) - \alpha_{TN,BT} \cdot TN(t) \cdot \left(1 - \frac{TN(t)}{K_3}\right) \cdot BT(t) \right) \cdot \delta_i \quad (9)$$

$$\frac{dX(t)}{dt} = \alpha_{X,TN} \cdot \delta_i \cdot X(t) \cdot \left(1 - \frac{X(t) + \alpha_{X,BT}^* \cdot BT(t) + \alpha_{X,pH} \cdot pH(t)}{K_4}\right) \cdot TN(t) \quad (10)$$

$$\frac{dBT(t)}{dt} = \left( \alpha_{BT,X} \cdot X(t) \cdot BT(t) + \alpha_{BT,TN} \cdot BT(t) \cdot \left(1 - \frac{BT(t) + \alpha_{BT,X}^* \cdot X(t) + \alpha_{BT,pH} \cdot pH(t)}{K_5}\right) \cdot TN(t) \right) \cdot \delta_i \quad (11)$$

The statistics software SigmaPlot for Windows version 12.0 (Systat Software, Inc. 2012) was used to obtain the statistically significant ( $P < 0.05$ ) values of the constants in the different models, and the corresponding coefficient of multiple determination ( $R^2$ ), as well as the  $F$ -ratio and  $P$ -value from Fisher's  $F$ -test ( $\alpha = 0.05$ ). The goodness-of-fit of the different models was also checked using the mean relative percentage deviation modulus (RPDM) values [5] and the bias ( $B_f$ ) and accuracy ( $A_f$ ) factors [30]:

$$RPDM = \frac{100}{n} \sum \left| \frac{Y_{exp_i} - Y_{pred_i}}{Y_{exp_i}} \right| \quad (12)$$



$$B_f = 10^{\frac{\sum \log(\frac{Y_{pred_i}}{Y_{exp_i}})}{n}} \quad (13)$$

$$A_f = 10^{\frac{\sum |\log(\frac{Y_{pred_i}}{Y_{exp_i}})|}{n}} \quad (14)$$

Where  $n$  is the number of experimental data,  $Y_{exp_i}$  is the experimental value and  $Y_{pred_i}$  is the value predicted by the model. Values of  $R^2 \geq 0.95$ ,  $RPDM < 10\%$  [5], and  $B_f$  and  $A_f$  close to 1 [30] are indicative that the corresponding equation was accurately fitted to the experimental data.

### Nomenclature

|                     |                                                                                                                                                             |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| $t$                 | Time (h)                                                                                                                                                    |
| $pH(t)$             | Culture pH value over the time                                                                                                                              |
| $pH_0$              | Initial culture pH value                                                                                                                                    |
| $pH_f$              | Final culture pH                                                                                                                                            |
| $\alpha_{BC}$       | Specific coefficient related with the buffer capacity (BC) of the culture medium (dimensionless)                                                            |
| $\delta_{pH}(t)$    | pH gradient (dimensionless) = $pH_0 - pH(t)$                                                                                                                |
| $\alpha_{pH,X}$     | Intrinsic pH drop rate (L/g/h) caused by the biomass growth                                                                                                 |
| $K_1$               | Minimum final pH (dimensionless) that could be reached in the culture                                                                                       |
| $TN(t)$             | Total nitrogen concentration (g/L) over the time                                                                                                            |
| $TN_0$              | Initial total nitrogen concentration (g/L)                                                                                                                  |
| $TN_f$              | Final total nitrogen concentration (g/L)                                                                                                                    |
| $\alpha_{TN,X}$     | Intrinsic TN consumption rate (L/g/h) for biomass production                                                                                                |
| $K_2$               | Theoretical maximum TN concentration (g/L) that could be used for biomass production                                                                        |
| $\alpha_{TN,BT}$    | Intrinsic TN consumption rate (mL/BU/h) for nisin production                                                                                                |
| $K_3$               | Theoretical maximum TN concentration (g/L) that could be used for nisin production                                                                          |
| $X(t)$              | Biomass concentration (g/L) over the time                                                                                                                   |
| $X_0$               | Initial biomass concentration (g/L)                                                                                                                         |
| $X_{max}$           | Maximum biomass concentration (g/L)                                                                                                                         |
| $\alpha_{X,TN}$     | Intrinsic growth rate (L/g/h) based on nitrogen consumption                                                                                                 |
| $\alpha^{*}_{X,BT}$ | Efficiency of TN utilization (mg/BU) to be channelled into nisin rather than into biomass (competition coefficient)                                         |
| $\alpha_{X,pH}$     | Constant that represents the effect of pH time course on the growth ( $h^{-1}$ )                                                                            |
| $K_4$               | Theoretical maximum biomass concentration (g/L) affected by the competition between biomass and nisin production for the nitrogen source and pH time course |
| $BT(t)$             | Nisin concentration (BU/mL) over the time                                                                                                                   |

|                       |                                                                                                                                                                                               |
|-----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| $BT_{max}$            | Maximum nisin concentration (BU/mL)                                                                                                                                                           |
| $\alpha_{BT,X}$       | Intrinsic nisin production rate (L/g/h) based on the growth of the producer strain                                                                                                            |
| $\alpha_{BT,TN}$      | Intrinsic nisin synthesis rate (L/g/h) based on nitrogen consumption                                                                                                                          |
| $\alpha^*_{BT,X}$     | Efficiency of TN utilization (BU/mg) to be channelled into biomass rather than into nisin (competition coefficient)                                                                           |
| $\alpha_{BT,pH}$      | Constant that represents the effect of pH time course on nisin synthesis ( $h^{-1}$ )                                                                                                         |
| $K_5$                 | Theoretical maximum nisin concentration (g/L) affected by the competition between bacteriocin and biomass production for the nitrogen source and pH time course                               |
| $A, B, C_0, C_1, C_2$ | Constants in equation (5)                                                                                                                                                                     |
| $D, E, F$             | Constants in equation (6)                                                                                                                                                                     |
| $G, H, I$             | Constants in equation (7)                                                                                                                                                                     |
| $J$                   | Constant in equations (8)-(11)                                                                                                                                                                |
| $F_0$                 | Initial concentration of glucose (g/L), TS and TP (g/L), and MRS broth nutrients (g/L), or culture pH in equations (8)-(11)                                                                   |
| $\delta_i$            | Function to explain the effect of the initial culture conditions on the evolution of the culture pH, total nitrogen consumption, biomass production and nisin synthesis in equations (8)-(11) |
| $[G]_0$               | Initial glucose concentration (g/L)                                                                                                                                                           |
| $[Nut]_0$             | Initial concentration of MRS broth nutrients (g/L)                                                                                                                                            |
| $RPDM$                | Mean relative percentage deviation modulus                                                                                                                                                    |
| $B_f$                 | Bias factor                                                                                                                                                                                   |
| $A_f$                 | Accuracy factor                                                                                                                                                                               |
| $n$                   | Number of experimental data                                                                                                                                                                   |
| $Y_{exp_i}$           | Experimental values of culture pH and concentration of total nitrogen, biomass and nisin                                                                                                      |
| $Y_{pred_i}$          | Predicted values by the corresponding equation for culture pH and concentration of total nitrogen, biomass and nisin.                                                                         |
| $R^2$                 | Correlation coefficient                                                                                                                                                                       |

#### 4. Conclusions

The main contribution of this paper is the development, for the first time, of a four-dimensional LV-equation for an accurate description of the batch nisin production system by *L. lactis* CECT 539 in different series of fermentation (DW-G, DW-TS-TP, DW-MRS and DW-pH) in DW media supplemented with different concentrations of glucose (DW-G cultures), total sugars and phosphorous (DW-TS-TP cultures) or MRS broth nutrients (DW-MRS cultures) or adjusted to different initial pH values (DW-pH cultures).

The results obtained in this paper demonstrated that the microbiological bacteriocin production system could be explained by considering the biological approach used to describe the relationships between species that compete for the same nutrient sources. Thus,

further knowledge is provided about the relationship between the main culture variables (culture pH, total nitrogen consumption and the synthesis of biomass and bacteriocin) involved in nisin production, which is usually difficult to explain.

However, a general four-dimensional LV-equation (with a unique set of parameters) could only be appropriately developed to describe each series of fermentation when the change in the initial nutrient composition or pH in the media produced a clearly observable effect (e.g., linear, quadratic or exponential) on the evolution of the rates of culture pH decrease, TN consumption and production of biomass and nisin.

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