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Unstructured mathematical model for biomass, lactic acid and bacteriocin productions by lactic acid bacteria in batch fermentation.

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Abstract

Background: A simple macroscopical model was proposed to describe the fermentation kinetics of growth, bacteriocins and lactic acid production by *Lactococcus lactis* and *Pediococcus acidilactici* in a batch system. The equations used were: the logistic reparametrized for growth, Luedeking-Piret model for bacteriocin production, maintenance energy model for glucose consumption and homofermentative balance equation for lactic acid formation.

Results: In all the cultures, the mathematical models, consistents and robusts, adjusted, perfectly, the experimental kinetic profiles. Also, the corresponding kinetic parameters were significant, so much biological as statistically.

Conclusions: The group of integrated equations used, besides showing a high accuracy to predict the studied bioproductions, established an useful tool for the control of lactic acid bacteria kinetics in bioreactors in terms of its statistical consistency.

1. Introduction

From a metabolic point of view, lactic acid bacteria (LAB) produce a wide number of compounds with antimicrobial activity, of great interest in the alimentary industry, as they are: ethanol, hydrogen peroxide, diacetyl, butanediol, lactic and acetic acids and bacteriocins or lantibiotics.[1,2] Lactic acid has been usually employed as bacterial biopreservative in foods[3] and, also recently, as monomer for the plastic polymer synthesis, solvents and oxygenated chemicals.[4,5] Bacteriocins are antimicrobial peptides against Gram-positive bacteria, produced by different genres of LAB: *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Pediococcus* and *Leuconostoc* [2,6]; being the nisin produced by *Lactococcus lactis* one of the most studied.[2,7, 8]

On the other hand, an indispensable tool for the optimization, control, design and analysis of the combined production of lactic acid and bacteriocins to industrial scale, derive of the development of mathematical robust models, formulated with parameters of clear biological significance and statistically consistent, and which can be easily implemented in the bioreactors software. Among these models, we meet with the denominated "*structured*" or those that consider the group of intracellular metabolic pathways (with difficulties for the knowledge *in vivo* of the reaction rates of

the implied enzymes), and the simplest, but equally useful and tremendously descriptive in the experimental reality, denominated "*unstructured*" that describe the production of biomass mediating one variable global.

In this study, experimental data of biomass, lactic acid, bacteriocins and glucose from batch fermentations by *Lactococcus lactis* and *Pediococcus acidilactici* on MRS media were evaluated in order to establish an unstructured mathematical model, which can be used to describe the corresponding kinetics cultures and facilitating the estimation of the confidence intervals of the parameters with biological meaning.

2. Materials and methods

2.1. Microbiological methods

The micro-organisms used were *Lactococcus lactis* subsp. *lactis* (abbreviated key Lc HD1)[9] and *Pediococcus acidilactici* NRRL B-5627 (Pc 1.02)[10,11]. *Leuconostoc mesenteroides* ssp. *lysis* (kindly donated by Dr. Ray, University of Wyoming, Laramie, USA) and *Carnobacterium piscicola* CECT 4020 (Spanish Collection Type Culture) were employed as indicators for the nisin and pediocin bioassays, respectively. Stock cultures were stored at –50ºC in powdered skimmed milk suspension with 25% glycerol. Inocula (1% v/v) consisted of cellular suspensions from 12 (Lc HD1) and 24 (Pc 1.02) h aged in MRS medium, adjusted to an OD (700 nm) of 0.900.

The medium used for the cultures were commercial MRS (Pronadisa, Hispanlab S.A., Spain) with an initial pH adjusted to 7.0 with NaOH 5M and sterilised at 121ºC for 12 min. Micro-organisms were grown in 300 ml Erlenmeyer flasks with 200 ml of medium at 30ºC, with 200 rpm orbital shaking. The cultures were carried out in quadruplicate. At pre-established times, each culture was divided into two aliquots. The first was centrifuged at 5,000 rpm for 15 min, and the sediment washed twice and resuspended in distilled water to the adequate dilution for measuring the optical density (OD) at 700 nm. The dry weight can then be estimated from a previous calibration curve. The supernatant was used for the measure of glucose, lactic acid and acetic acid (data not shown). The second aliquot was used for the extraction and quantification of bacteriocin (nisin and pediocin). All assays were carried out in triplicate.

2.2. Analytical methods

Glucose, lactic and acetic acids were quantified by HPLC analysis (refractive-index detector), using an ION-300 column (Transgenomic, USA) with 6 mM sulphuric acid as a mobile phase (flow=0.4 mL/min), at 65ºC. Methods for the extraction and quantification of bacteriocin were described in detail by Cabo et al.[12] and Murado et al.[13], using *Leuconostoc mesenteroides* ssp. *lysis* (for nisin) and *Carnobacterium piscicola* CECT 4020 (for pediocin) as indicators.

2.3. Numerical methods

Fitting procedures and parametric estimations calculated from the results were carried out by minimisation of the sum of quadratic differences between observed and model-predicted values, using the non linear least-squares (quasi-Newton) method provided by the macro 'Solver' of the Microsoft Excel XP spreadsheet. Statistica 6.0 (StatSoft, Inc. 2001) and Simfit v.5.6.7 (kindly provided by Dr. W.G. Barsley of the Manchester University, UK and Dr. F.J. Burguillo of the Salamanca University, Spain) programs were used to evaluate the significance of the parameters estimated by the adjustment of the experimental values to the proposed mathematical models and the consistency of these equations.

3. Results and discussion

3.1 Unstructured kinetic models

The unstructured models which to describe microbial kinetics include the most fundamental observations relating microbial growth processes, this is[14]: a) the biomass concentration and the rate of cell mass production are proportional; b) the cells need substrate and can synthesize metabolic products even when the growth has finished; c) the evolution of the biomass throughout the culture time (growth rate) presents an asymptote as upper limit (saturation level) different for each substrate or level of substrate used. In the model that next is schematized the formation rate equations of biomass (X), bacteriocins (B), lactic acid (L) and glucose uptake (G) were used. The meaning and definition of the group of model parameters as well as its units are summarized in the symbolic notation table 1.

Microbial growth

The unstructured models more commonly used in the macroscopic description of growth processes are the Monod and the logistic equations.[15,16] The logistic model, independent of the substrate concentration, is easily managed and suitable for the adjustment of the typical sigmoid profiles of biomass production, also facilitating the calculation of parameters of biological and geometrical significance. This way, the logistic equation allows the biomass variation against time to be described by the following differential equation, typical for the mechanism of auto-catalytic reaction:

$$r_X = \frac{dX}{dt} = \mu_{mX} \cdot X \cdot \left(\frac{K - X}{K} \right) \tag{1}$$

which, integrated between $X_0 \rightarrow X$ and $0 \rightarrow t$, gives the explicit form of biomass as a function of the time:

$$X = \frac{K}{1 + \exp\left(c - \mu_{mX} \cdot t\right)} \ , \ \text{with} \ \ c = \ln\left(\frac{K}{X_0} - 1 \right) \tag{2}$$

Another parameter of great utility and robust in the sense that it is not very sensitive to experimental error [17], is the maximum growth rate (v_{mX}), or the slope of the straight tangent to the function at its inflection point (t_i). Making the second derivative equal to zero and isolating the abscissa of the inflection point ($t=t_i$), we obtain:

$$\frac{d^2X}{dt^2} = 0 \ \xrightarrow{t=t_i} \ \ t_i = \frac{c}{\mu_{mX}} \tag{3}$$

and therefore the value of the slope (v_{mX}) is:

$$v_{mX} = \left(\frac{dX}{dt} \right)_{t_i} = \frac{K \cdot \mu_{mX} \cdot e^{c - \mu_{mX} \cdot t_i}}{\left(1 + e^{c - \mu_{mX} \cdot t_i}\right)^2} = \frac{K \cdot \mu_{mX} \cdot e^{c - \mu_{mX} \cdot \frac{c}{\mu_{mX}}}}{\left(1 + e^{c - \mu_{mX} \cdot \frac{c}{\mu_{mX}}}\right)^2} = \frac{K \cdot \mu_{mX}}{4} \tag{4}$$

On the other hand, the lag period of the culture (λ_X) may be defined as the intersection of the tangent at the inflection point with the abscissa axis. Therefore the value for the biomass when $t=t_i$ is:

$$X(t_i) = X\Big|_{t=t_i} = \frac{K}{1+\exp\left(c-\mu_{mX}\cdot\dfrac{c}{\mu_{mX}}\right)} = \frac{K}{1+e^0} = \frac{K}{2}$$

and the equation of that tangent:

$$R = X(t_i) + f'(t_i)\cdot(t-t_i) = X(t_i) + \left(\frac{dX}{dt}\right)_{t_i}\cdot(t-t_i) = \frac{K}{2} + v_{mX}\cdot(t-t_i)$$

Therefore, the value for λ_X, or time (t) when R=0, is:

$$\frac{K}{2} + v_{mX}\cdot(\lambda_X - t_i) = 0 \;\Rightarrow\; \lambda_X = \frac{v_{mX}\cdot t_i - \dfrac{K}{2}}{v_{mX}} = \frac{\dfrac{K\cdot\mu_{mX}}{4}\cdot\dfrac{c}{\mu_{mX}} - \dfrac{K}{2}}{\dfrac{K\cdot\mu_{mX}}{4}} = \frac{c-2}{\mu_{mX}} \qquad (5)$$

Finally, in order to facilitate the calculation of the corresponding intervals of confidence, the parameters of interest should appear explicitly in the model. Thus, inserting the values of v_{mX} and λ_X given by equations (4) and (5) into (2), one obtains the definitive expression:

$$X = \frac{K}{1+\exp(c-\mu_{mX}\cdot t)} \xleftrightarrow[c=2+\frac{4\cdot v_{mX}\cdot\lambda}{K}]{\mu_{mX}=\frac{4\cdot v_{mX}}{K}} X = \frac{K}{1+\exp\left[2+\dfrac{4\cdot v_{mX}}{K}\cdot(\lambda_X - t)\right]} \qquad (6)$$

Bacteriocins formation

The kinetics of the bacteriocins production was based on the Luedeking and Piret model.[18] Originally used in the production of lactic acid by *Lactobacillus delbrucckii*, it has been of great utility, with modifications which allow the incorporation of other effects, in the description and

typify of the metabolites microbial formation.[9-11] Applied to the problem under study, said criteria permits the following assumptions:

1: The increase in biomass (X) over time can be described using the logistical equation (2 or 6).

2: The rate of production (r_B) of a metabolic product –in our case, bacteriocins (B)– obeys the Luedeking y Piret equation:

$$r_B = \frac{dB}{dt} = \alpha_B \cdot \frac{dX}{dt} + \beta_B \cdot X \tag{7}$$

developing this differential equation among $0 \to B$, $X_0 \to X$ and $0 \to t$, we obtain:

$$\int_0^B dB = \alpha_B \int_{X_0}^{X} dX + \beta_B \int_0^t X \cdot dt \quad \Rightarrow \quad B = \alpha_B \cdot (X - X_0) + \beta_B \int_0^t \frac{K}{1 + e^{c - \mu_{mX} \cdot t}} \cdot dt$$

where, after substituting X for (2) and to integrate the last term, the following analytic form is generated:

$$B = \frac{\alpha_B \cdot K}{1 + \left(\frac{K}{X_0} - 1 \right) \cdot e^{-\mu_{mX} \cdot t}} - \alpha_B \cdot X_0 + \frac{\beta_B \cdot K}{\mu_{mX}} \cdot \ln \left[\frac{X_0 \cdot \left(e^{\mu_{mX} \cdot t} - 1 \right) + K}{K} \right] \tag{8}$$

or, atending to the reparametrisation of (6), we have:

$$B = \frac{\alpha_B \cdot K}{1 + \left(\frac{K}{X_0} - 1 \right) \cdot e^{-\frac{4 \cdot v_{mX} \cdot t}{K}}} - \alpha_B \cdot X_0 + \frac{\beta_B \cdot K^2}{4 \cdot v_{mX}} \cdot \ln \left[\frac{X_0 \cdot \left(e^{\frac{4 \cdot v_{mX} \cdot t}{K}} - 1 \right) + K}{K} \right] \tag{9}$$

This function permits us to classify the metabolites as *primaries*, if the rate of formation depends solely on the growth rate of the biomass ($\alpha_B \neq 0$; $\beta_B = 0$); *secondaries*, if this depends solely on the biomass present ($\alpha_B = 0$; $\beta_B \neq 0$), and *mixed*, if this depends simultaneously on the rate of production of the biomass and on the biomass present ($\alpha_B \neq 0$; $\beta_B \neq 0$).

Also, making $\beta_B = -r_D$, we can obtain profiles in those that the bacteriocin production is inactivated at long time of culture, being obtained this way, a decreasing profile after reaching a maximum of formation.[19]

Glucose consumption

In general in all the heterotrophic cells, a carbon source, like in our case the glucose, is used to form cellular material (cellular growth as biomass formation), metabolic products (in this study lactic acid) and the maintenance of the rest of cellular functions. However, the nisin and pediocin production depend, basically, to the origin, type and concentration of the protein source used. [20]

This way, the consumption of glucose (*G*) throughout the time can be modelled using, another time, a Luedeking and Piret like equation, in which one keeps in mind the quantity of sugar that is metabolized to form cellular biomass and for the cellular maintenance (maintenance energy model [21]):

$$r_G = -\frac{dG}{dt} = \frac{1}{Y_{X/G}} \cdot \frac{dX}{dt} + m_X \cdot X \qquad (10)$$

that, developing the differential equation and integrating among $G_0 \rightarrow G$, $X_0 \rightarrow X$ and $0 \rightarrow t$, allows to establish, finally, the corresponding primitive function:

$$-\int_{G_0}^{G} dG = \frac{1}{Y_{X/G}} \int_{X_0}^{X} dX + \int_0^t m_G \cdot X \cdot dt \quad \Rightarrow \quad G = G_0 - \frac{1}{Y_{X/G}} \cdot \left(X - X_0\right) - m_G \int_0^t \frac{K}{1 + e^{c - \mu_{mX} \cdot t}} \cdot dt \quad \Rightarrow$$

$$G = G_0 + \frac{X_0}{Y_{X/G}} - \frac{1}{Y_{X/G}} \cdot \frac{K}{1 + \left(\frac{K}{X_0} - 1\right) \cdot e^{-\mu_{mX} \cdot t}} - \frac{m_G \cdot K}{\mu_{mX}} \cdot \ln\left[\frac{X_0 \cdot \left(e^{\mu_{mX} \cdot t} - 1\right) + K}{K}\right] \qquad (11)$$

which in reparametrized form it is expressed as:

$$G = G_0 + \frac{X_0}{Y_{X/G}} - \frac{1}{Y_{X/G}} \cdot \frac{K}{1 + \left(\frac{K}{X_0} - 1 \right) \cdot e^{-\frac{4 \cdot v_{mX}}{K} \cdot t}} - \frac{m_G \cdot K^2}{4 \cdot v_{mX}} \cdot \ln \left[\frac{X_0 \cdot \left(e^{\frac{4 \cdot v_{mX}}{K} \cdot t} - 1 \right) + K}{K} \right] \quad (12)$$

Lactic acid production

Finally, the lactic acid formation by *L. lactis* and *P. acidilactici* can be treated by means of a homofermentative LAB (in both cases, the acetic acid production according to the lactic formation was worthless and inferior at the 10%). This way, the equation which describes this production is given by:

$$r_L = \frac{dL}{dt} = -\frac{1}{Y_{G/L}} \cdot \frac{dG}{dt} \quad (13)$$

$$\int_0^L dL = -\frac{1}{Y_{G/L}} \int_{G_0}^{G} dG \;\Rightarrow\; L = -\frac{1}{Y_{G/L}} \cdot (G - G_0) = \frac{G_0 - G}{Y_{G/L}} \;\Rightarrow$$

$$L = \frac{-X_0}{Y_{X/G} \cdot Y_{G/L}} + \frac{1}{Y_{X/G} \cdot Y_{G/L}} \cdot \frac{K}{1 + \left(\frac{K}{X_0} - 1 \right) \cdot e^{-\mu_{mX} \cdot t}} + \frac{m_G \cdot K}{Y_{G/L} \cdot \mu_{mX}} \cdot \ln \left[\frac{X_0 \cdot \left(e^{\mu_{mX} \cdot t} - 1 \right) + K}{K} \right] \quad (14)$$

$$L = \frac{-X_0}{Y_{X/G} \cdot Y_{G/L}} + \frac{1}{Y_{X/G} \cdot Y_{G/L}} \cdot \frac{K}{1 + \left(\frac{K}{X_0} - 1 \right) \cdot e^{-\frac{4 v_{mX}}{K} \cdot t}} + \frac{m_G \cdot K^2}{4 \cdot Y_{G/L} \cdot v_{mX}} \cdot \ln \left[\frac{X_0 \cdot \left(e^{\frac{4 \cdot v_{mX}}{K} \cdot t} - 1 \right) + K}{K} \right] \quad (15)$$

3.2. Bioproductions and consumes by Lactococcus lactis and Pediococcus acidilactici

In order to demonstrate the usefulness of the mathematical model proposed the kinetic of production and derived consumptions of the *L. lactis* and *P. acidilactici* growths were studied under the conditions which are described in the materials and methods section. The experimental results with the corresponding fits to the models (2), (6), (9), (12) and (15) are represented in the figures 1 and 2. Quantitatively, these adjustments generated the parametric estimations summarized in the Table 2.

The acid lactic bacteria cultures showed a classical growth trend, this is, sigmoid profiles. After a lag phase (about 3.8 h and 4.4 h for Lc HD1 and Pc 1.02, respectively), the biomass entered the exponential growth phase, reaching the asymptotic level of maximum growth, 1.17 g/L (Lc HD1) and 1.27 g/L (Pc 1.02), around the 13 and 30 h. In the same way, the productions of bacteriocins and lactic acid took place simultaneously to the cellular growth following similar kinetic profile. The nisin and pediocin formation behaved as a mixed metabolite ($\alpha_B \neq 0$ and $\beta_B \neq 0$), with a little value of secondary component –β_B– with respect to the numeric value of the parameter α_B.

By adjusting the experimental data to equation (12), the parameter values of glucose consumption with the corresponding confidence intervals were as follows (table 2): 21.033±0.513, 0.139±0.018, 0.057±0.050 for S_0 (in g/L), $Y_{X/G}$ (in gX/gG) and m_G (in gG/gX.h), respectively, on the Lc HD1 cultures and 19.929±0.578, 0.149±0.021, 0.086±0.025 for Pc 1.02 fermentations. The fitting of results was satisfactory graphical and statistically. Regarding the lactic acid production, in both microorganisms, results of significant $Y_{G/L}$ were obtained, with yields compared non superiors to 12% of difference.

Globally, in all the kinetic processes, the mathematical equations were consistent (see Fisher's *F* and *p*-values in the table 2) and the parametric estimations passed Student's *t*-test (α=0.05). On the other hand, all the values foreseen in the non-linear adjustments produced high coefficients of linear correlation with the values really observed (r > 0.98).

3.3. Evaluation of the models

To test the microbial growth, products formation and substrate uptake models, a comparison of equations proposed above with another fermentative experimental data is shown in the figure 3. In this example, the numeric data generated in the work of Parente et al.[22] were used. This manuscript describes, kinetically, the biomass and enterocin productions as well as glucose uptake of the lactic acid bacterium *Enterococcus faecium* in batch culture. The non-lineal adjustment among experimental values and equations produced the significant following parametric results (see units in the table 1): K=1.363±0.058, v_m=0.315±0.052, λ=1.322±0.397, X_0=0.052±0.029, μ_m=0.924±0.171, α_B=1.718±0.208, r_D=0.069 ±0.020, G_0: 12.305±0.660, $Y_{X/G}$: 0.133±0.023 and

m_G: 0.097±0.094. Keeping in mind the value of $\beta_B \neq 0$ ($r_D \neq 0$), we can define the enterocin production like secondary. Equally to that exposed in the previous section, the temporary description of the culture was geometrically robust and satisfactory (figure 3), also statistically consistent (p-values=0.00000; r=[0.991-0.997]).

4. Conclusions

The quantitative and mechanistic study of fermentations is a complex process and, generally, it is very difficult or even impossible to obtain complete information of all the steps, balances, restrictions and characteristic that defines it. It is hence, that the models presented in this work were shown been worth to adjust the experimental data obtained in the cultures of LAB as well as those obtained from the bibliography. Statistically, the parameters, with biological and geometrical clear significance, were significant (*t-Student* test) and the mathematical models consistent (*F-Fisher* test). From the point of view of it possible application, the group of integrated equations establishes an useful resource and of easy implementation in a spreadsheet or in a simple software for the control of microbial kinetics in bioreactors.

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Figure captions

Figure 1: Production kinetics of biomass (■), nisin (●), lactic acid (□) and glucose uptake (○) by *Lactococcus lactis* on MRS medium. The experimental data was fitted to the models proposed in the point 3.1 of the results and discussion section.

Figure 2: Production kinetics of biomass (■), pediocin (●), lactic acid (□) and glucose uptake (○) by *Pediococcus acidilactici* on MRS medium. The experimental data was fitted to the models proposed in the point 3.1 of the results and discussion section.

Figure 3: Production kinetics of biomass (■), enterocin (●) and glucose uptake (○) by *Enterococcus faecium* from Parente et al.[22]. The experimental data was fitted to the models proposed in the point 3.1 of the results and discussion section.

Figure 1

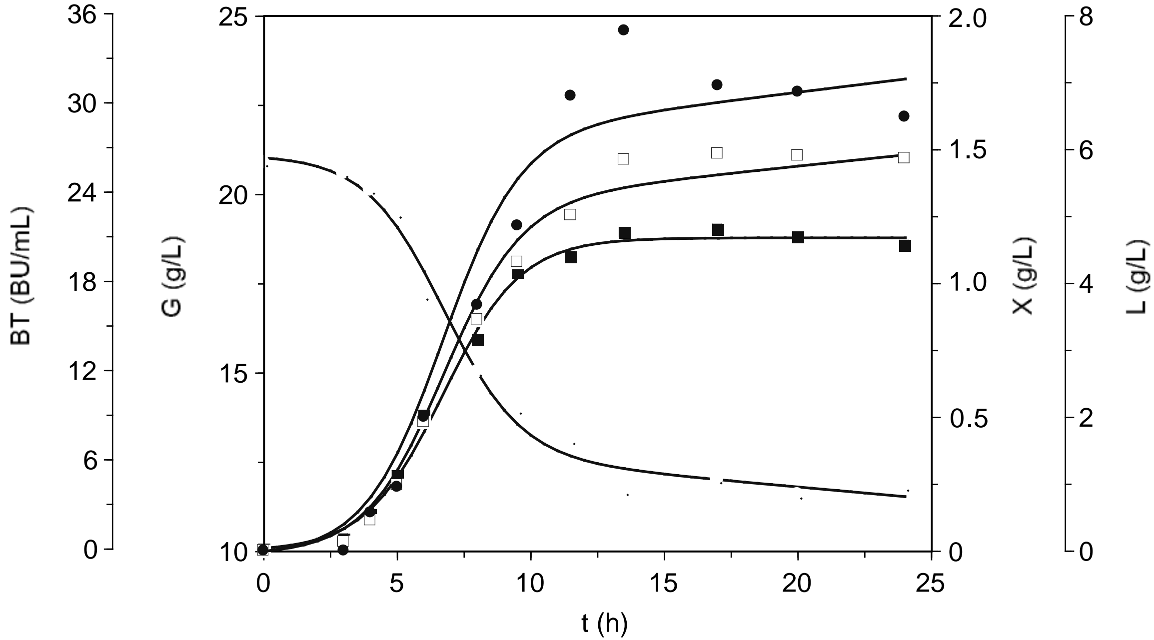


Figure 2

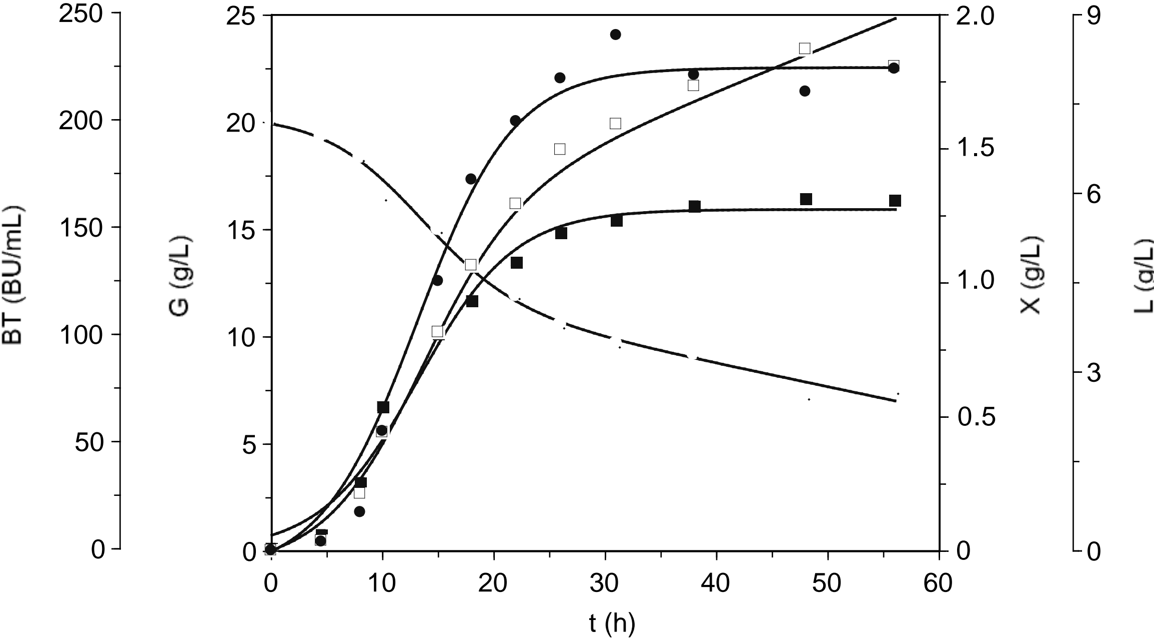
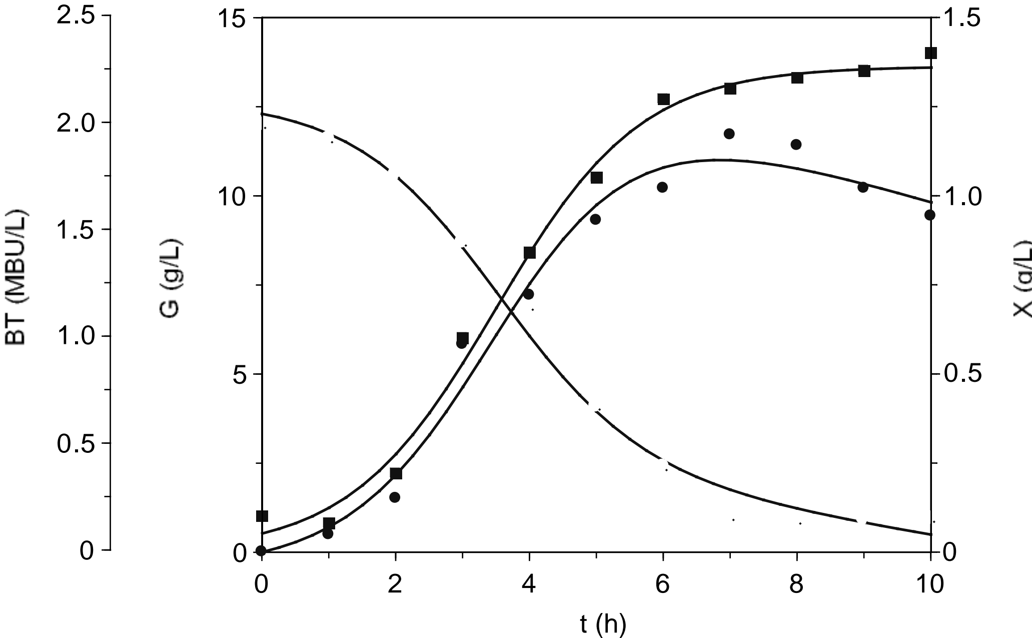


Figure 3



TABLES

Table 1: Symbolic notations used. BU: Bacteriocin arbitrary units.

r_X :	Growth rate. Dimensions: g/L.h
X :	Biomass. Dimensions: g/L
t :	Time. Dimensions: h
K :	Maximum biomass. Dimensions: g/L
μ_m :	Specific maximum growth rate (biomass production per unit of biomass and time). Dimensions: h^{-1}
X_0 :	Initial biomass. Dimensions: g/L
v_{mX} :	Maximum growth rate. Dimensions: g/L.h
λ_X :	Growth lag phase. Dimensions: h
r_B :	Bacteriocin (nisin or pediocin) rate production. Dimensions: BU/mL.h
B :	Bacteriocin (nisin or pediocin). Dimensions: BU/mL
α_B:	Luedeking and Piret parameter (growth-associated constant for bacteriocin production). Dimensions: g bacteriocin / g biomass or BU/mg
β_B :	Luedeking and Piret parameter (non growth-associated constant for bacteriocin production). Dimensions: g bacteriocin / g (biomass).h or BU/mg.h
r_D :	Bacteriocin inactivation rate. Dimensions: g bacteriocin / g (biomass).h or BU/mg.h
r_G :	Glucose rate consumption. Dimensions: g/L.h
G :	Glucose. Dimensions: g/L
G_0 :	Initial glucose. Dimensions: g/L
$Y_{X/G}$:	Yield factor for biomass formation on glucose. Dimensions: g biomass / g glucose
m_s :	Maintenance coefficient. Dimensions: g glucose / g (biomass).h
r_L :	Lactic acid rate production. Dimensions: g/L.h
L :	Lactic acid. Dimensions: g/L
$Y_{G/L}$:	Yield factor for glucose consumed per lactic acid production. Dimensions: g glucose / g lactic acid

Table 2: Parametric estimations (see Table 1) corresponding to the kinetic models (2, 6, 9, 12, 15), applied to the cultures of *L. lactis* and *P. acidilactici* on MRS medium. CI: confidence intervals (α=0.05). *F*: F-Fisher test (df: degrees freedom). r: correlation coefficient between observed (obs) and predicted (pred) data.

Lactococcus lactis (Lc HD1)		*Pediococcus acidilactici* (Pc 1.02)	
BIOMASS	values ± CI	BIOMASS	values ± CI
K	1.171 ± 0.039	K	1.274 ± 0.071
μ_{mX}	0.687 ± 0.109	μ_{mX}	0.230 ± 0.058
X_0	0.012 ± 0.008	X_0	0.060 ± 0.043
v_{mX}	0.201 ± 0.030	v_{mX}	0.073 ± 0.017
λ	3.778 ± 0.453	λ	4.409 ± 2.229
F (df=9; α=0.05)	2307.226	F (df=9; α=0.05)	896.648
p-value	0.00000	*p*-value	0.00000
r (obs-pred)	0.9979	r (obs-pred)	0.9938
NISIN	values ± CI	PEDIOCIN	values ± CI
α_B	24.082 ± 4.430	α_B	185.546 ± 8.584
β_B	0.189 ± 0.160	β_B	0.0010 ± 0.0008
F (df=10; α=0.05)	341.317	F (df=10; α=0.05)	2263.985
p-value	0.00000	*p*-value	0.00000
r (obs-pred)	0.9827	r (obs-pred)	0.9948
GLUCOSE	values ± CI	GLUCOSE	values ± CI
G_0	21.033 ± 0.513	G_0	19.929 ± 0.578
$Y_{x/G}$	0.139 ± 0.018	$Y_{x/G}$	0.149 ± 0.021
m_s	0.057 ± 0.050	m_s	0.086 ± 0.025
F (df=9; α=0.05)	5983.937	F (df=9; α=0.05)	4181.075
p-value	0.00000	*p*-value	0.00000
r (obs-pred)	0.9951	r (obs-pred)	0.9964
LACTIC ACID	values ± CI	LACTIC ACID	values ± CI
$Y_{G/L}$	1.601 ± 0.060	$Y_{G/L}$	1.432 ± 0.131
F (df=11; α=0.05)	3461.713	F (df=11; α=0.05)	578.321
p-value	0.000000	*p*-value	0.000000
r (obs-pred)	0.9961	r (obs-pred)	0.9950