Biochemical Engineering Journal 88 (2014) 115–130

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular Article

Modeling the batch bacteriocin production system by lactic acid bacteria by using modified three-dimensional Lotka–Volterra equations

Nelson Pérez Guerra*

Department of Analytical and Food Chemistry, Food Science and Technology Faculty, University of Vigo, Ourense Campus, As Lagoas s/n, 32004 Ourense, Spain

ARTICLE INFO

Article history:
Received 2 October 2013
Received in revised form 4 April 2014
Accepted 23 April 2014
Available online 4 May 2014

Keywords:
Fermentation
Growth kinetics
Lotka–Volterra models
Modeling
Bacteriocin production system
Whey

ABSTRACT

Different batch cultures of *Lactococcus lactis* CECT 539, a nisin-producing strain, were carried out in culture media prepared with whey and mussel processing wastes. From these cultures, a reasonable system of differential equations, similar to the three-dimensional Lotka–Volterra two predators-one prey model, was set up to describe, for the first time, the relationship between the absolute rates of growth, pH drop and nisin production.

Thus, the nisin production system was described as a three-species (pH, biomass and nisin) ecosystem. In this case, both nisin and biomass production were considered as two pH-dependent species that compete for the nitrogen source. Excellent agreement (R^2 values $\geq$0.9885) resulted between model predictions and the experimental data, and significant values for all the model parameters were obtained. The developed model was demonstrated (R^2 values $\geq$0.9874) for five batch cultivations of the strains *L. lactis* CECT 539 in MRS broth and *Lactobacillus sakei* LB 706 (sakacin A producer), *Pediococcus acidilactici* LB42-923 (pediocin AcH producer), *L. lactis* ATCC 11454 (nisin producer) and *Leuconostoc carnosum* Lm1 (leuconocin Lcm1 producer) in TGE broth. These results suggest that the batch bacteriocin production system in these culture media can be successfully described by using the Lotka–Volterra approach.

1. Introduction

Bacterial growth has been commonly modeled by using empirical algebraic expressions such as the *Gompertz* model, as well as rate equations, for instance, the Verhulst's logistic model, the Monod model or modifications of these [1–5]. Furthermore, the relationship between product formation and growth has been usually described by using the Luedeking and Piret (LP) model [6], which relates the absolute rate of product formation (dP/dt) to the absolute cell growth rate (dX/dt) and to the bacterial concentration (X) according to Eq. (A.1). By using this model, the product synthesized in a fermentation process can be classified as a primary ($a \neq 0, b = 0$), secondary ($a = 0; b \neq 0$), or mixed ($a \neq 0; b \neq 0$) metabolite [3,6–8]. In these cases, the rate of product synthesis depends on the growth rate alone (in case of a primary metabolite), on the bacterial concentration alone (in case of a secondary metabolite) or on both the growth rate and bacterial concentration (in case of a mixed metabolite) [6], respectively.

However, in the case of bacteriocin (*BT*) production, the use of the LP model did not always produce satisfactory results, mainly because bacteriocin synthesis depends not only on biomass production, but also on other factors, including the cultivation method, the type and concentration of the carbon, nitrogen and phosphorus sources, the type and composition of the culture media, the temperature, agitation and aeration, as well as the culture pH [9]. The latter variable plays a very important role in bacteriocin production since synthesis of this product depends on both the pH evolution and culture pH drop [7,10,11]. In addition, bacteriocin production is affected by several pH-dependent mechanisms including aggregation, adsorption of bacteriocin onto producer cells and proteolytic degradation by specific or non-specific proteases [12–18]. On the other hand, the post-translational processing of prebacteriocin to produce active bacteriocin depends on the final pH reached in the cultures [19].

For these reasons, the LP expression has been modified by introducing a term for the effect of pH on bacteriocin synthesis

* Correspondence to: Department of Analytical and Food Chemistry, Food Science and Technology Faculty, University of Vigo, As Lagoas, 32004 Ourense, Spain. Tel.: +34 988 387 053; fax: +34 988 387 001.
E-mail address: nelsonpg@uvigo.es

http://dx.doi.org/10.1016/j.bej.2014.04.010
1369-703X/

Nomenclature

P	product concentration
X	biomass concentration (g of cell dry matter (CDM)/L)
t	time (h)
a	growth associated constant in the Luedeking and Piret model (BU/mL/g)
b	non-growth associated constant in the Luedeking and Piret model (BU/mL/g/h)
H	rate constant (h^{-1}) for pH drop
X_{max}	maximum biomass concentration (g of CDM/L)
X_0	initial biomass concentration (g of CDM/L)
J	rate constant (h^{-1}) for biomass concentration increase
BT	nisin concentration (BU/mL)
K	maximum nisin concentration (BU/mL)
c, m, b_0	constants (dimensionless)
b_1	constant of proportionality (h^{-1})
α_{pH}	constant (h^{-1}) related with the stabilization of the final pH value (saturation level) in the culture medium.
β_{pH}	average rate of change (slope) of the culture pH curve before pH stabilization (dimensionless).
δ_{pH}	rate of change of culture pH (L/g/h) due to the production of organic acids
α_x	intrinsic growth rate (h^{-1})
β_x	efficiency of nitrogen source utilization (mL/BU/h) for growth rather than for bacteriocin production
δ_x	constant (h^{-1}) that represents the effect of the time course of pH on the growth
α_{BT}	efficiency of nitrogen source utilization (L/g/h) for bacteriocin production rather than for growth
β_{BT}	constant (h^{-1}) that represents the effect of the time course of pH on nisin production
δ_{BT}	coefficient adsorption of bacteriocin (h^{-1}) onto producer cells
R^2	correlation coefficient
OD	optical density
kAU	kiloactivity unit per milliliter = 10^3 activity units/mL

Generic name abbreviations

C.	*Carnobacterium*
L.	*Lactococcus*
Lb.	*Lactobacillus*
Ln.	*Leuconostoc*
Ped.	*Pediococcus*

[2–4,7,10,11]. With this procedure, for example, both pediocin and nisin have been classified as pH-dependent primary metabolites [2,3,10,11].

Taking into account that the three-dimensional Lotka–Volterra model has been used to describe three-species predator–prey interactions (two preys-one predator or one prey-two predators systems) [20], the development of a similar model able to describe the relationship between the variables X, BT and pH could give a new insight for a better interpretation and understanding of the bacteriocin production system. However, as far as we know, the bacteriocin production dynamic in relation to the growth of the producer strain and the culture pH has not been modeled by using three-dimensional rate equations.

Therefore, the aim of this study is to develop a model based on the three-dimensional predator–prey Lotka–Volterra systems [20] for describing the batch nisin production system by *Lactococcus lactis* CECT 539 in different culture media buffered with different concentrations of the buffering agent. To obtain the experimental data of culture pH, growth and bacteriocin production, the previously published batch cultures of *L. lactis* CECT 539 on diluted whey (DW) [21], concentrated whey (CW) [11] and mussel processing wastes (MPW) [22] media were repeated for 18 h, but with sampling periods of 2 h. The experimental data were smoothed by using the corresponding logistic models for each variable (pH, X and BT) to reduce the experimental error in the data and to accurately calculate the absolute rates of culture pH drop ($-dpH/dt$), growth (dX/dt) and nisin (dBT/dt) production. The calculated pH, X, BT, $-dpH/dt$, dX/dt and dBT/dt values were then used to develop the three-dimensional system of equations.

The model developed was also fitted to describe previously published experimental data from batch cultures of *L. lactis* CECT 539 in MRS broth [10], and *Lactobacillus sakei* LB 706 (sakacin A producer), *Pediococcus acidilactici* LB42-923 (pediocin AcH producer), *L. lactis* ATCC 11454 (nisin producer) and *Leuconostoc carnosum* Lm1 (leuconocin Lcm1 producer) in TGE broth [19].

2. Materials and methods

2.1. Bacterial cultures

L. lactis subsp. *lactis* CECT 539, the nisin-producing strain, and *Carnobacterium piscicola* CECT 4020, the nisin sensitivity indicator strain, were acquired from the Spanish Type Culture Collection (CECT, Valencia, Spain). Both bacteria were grown on MRS (De Man, Rogosa and Sharpe broth, Merck, Germany) and maintained as frozen stock held at −40 °C in Nutrient broth containing 15% (v/v) glycerol. Before their use in fermentation experiments, working cultures were grown (30 °C, 12 h) on MRS as agar slants. The latter cultures were maintained at 4 °C and propagated twice in liquid cultures in the same medium at 30 °C [10].

2.2. Culture medium preparation, inoculation and batch cultures

Different batch cultures of *L. lactis* CECT 539 in culture media prepared with whey and mussel processing wastes were carried out in the same conditions as those described in previous works [11,21,22]. However, in the present study, the samples were taken every 2 h to obtain sufficient experimental data for the development of mathematical models capable of describing the batch nisin production system.

The diluted (DW) and concentrated whey (CW) were obtained from a local dairy plant and the mussel processing wastes (MPW) were obtained from a local mussel processing plant. Before being used as culture media, both the DW and CW were deproteinized and the MPW was firstly deproteinized and then hydrolyzed by using procedures as previously described [21,22]. Subsequently, unbuffered DW, CW and MPW media (adjusted to initial pH 6.3) and the same media buffered to initial pH 6.3 with 0.03, 0.10 and 0.25 M potassium hydrogen phthalate–NaOH were sterilized at 121 °C for 15 min [11,21,22].

The different batch fermentations were performed in duplicate in 250 mL Erlenmeyer flasks containing 50 mL of medium, on a rotary shaker (Innova 4330, New Brunswick Scientific Co., Inc., New Jersey) with an agitation speed of 200 rpm and a temperature of 30 °C, for 18 h. The inoculum consisted in 2% (v/v) of a 12-h culture in the corresponding culture medium. Two flasks were withdrawn each 2 h and triplicate samples (runs) were taken from each flask to measure growth (X), pH and nisin activity (BT).