Pediocin production by *Pediococcus acidilactici* in solid state culture on a waste medium.

Process simulation and experimental results

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Keywords: pediocin production; solid-state-fermentation; waste medium.

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SUMMARY

The production of pediocin by *Pediococcus acidilactici* was comparatively studied in submerged and solid-state culture, using polyurethane foam particles soaked in commercial (MRS) and waste media with various supplements, where product concentrations were 15 times higher in MRS medium. For the solid state analysis, cultures were treated by successive compression and refilling of tubular mini-reactors equipped with a piston, without the need for re-inoculation. This method was found to be simple, reproducible and easily controllable, allowing culture productivity to be maintained for long periods of time without alterations in the basic properties of the system. In addition, yields were found to be superior compared to those from submerged culture. The system kinetics were modelled on the basis of widely accepted assumptions with a good fit to the experimental results and observed biomass fluctuations less evident than those predicted by the kinetic model.

Keywords: pediocin production; solid-state-fermentation; waste medium.

INTRODUCTION

Lactic acid bacteria produce a wide variety of anti-microbial factors that include as many metabolic end-products (lactic acid, diacetyl, acetic acid) as bacteriocin peptides (De Vuyst and Vandamme, 1992; Klaenhammer, 1988). The latter group plays an important role in food fermentation, preservation and intestinal ecology (Dunne et al., 1999; Gilliland, 1990; Fuller and Gibson, 1997). In recent years, there has been a notable rise in interest of the genus *Pediococcus*, several strains of which are commonly used in the manufacture of fermented sausages, due to

contributions to flavour and colour development (Stiles and Hastings, 1991). Bacteriocins from *Pediococcus* (pediocins) have been considered as potential food biopreservatives (Daeschel, 1990; Ray, 1992), and their applicability in effectively controlling food pathogenic microbiota has been investigated (Bhunia et al., 1988; Pucci et al., 1988; Liao et al., 1993; Cintas et al., 1995). Pediocin production has been mostly studied in submerged culture on commercial media, although some assays were based on residual substrates, such as milk whey or mussel processing wastes (Goulhen et al., 1999; Guerra and Pastrana, 2001; Vázquez, 2001).

According to the definition of Viniegra-González et al. (1994), solid state fermentation (SSF) is a microbial process occurring mostly on the surface of solid materials which can absorb or contain water, in the presence or absence of soluble nutrients. SSF comprises two very different modes (Murado et al., 1998). In the first, a divided and humidified solid (*e.g.* cereal grain, flour, bran, sawdust) behaves as both support and nutrient source, and the process essentially occurs in absence of free water (Kumar and Lonsane, 1987; Ramesh and Lonsane, 1990; Pastrana et al., 1995). In the second mode, a nutritionally inert solid (*e.g.* synthetic foam: Murado et al., 1997; Pintado et al., 1998) acting exclusively as a support, is soaked in a nutrient solution. Even if the presence of free water is minimized in the inter-particulate space, the microorganism has access to pore water that occupies the hollow fraction of the support. Such access is a complex function of the solute concentration, the degree of hydrophobicity of the foam, its density, the relationship between hollow fraction and pore size (affecting capillary retention), particle diameter (affecting packaging and intra- and inter-particulate restrictions to mass transfer), growth of the biomass and its adhesiveness to the support. It should be further remembered that if the system is subject to forced aeration, the evaporation rate of the liquid phase is an additional variable.

In spite of their complex interrelations, this grouping of factors is useful for directing the time-course of the basic variables of the culture, *i.e.* the water activity and the restrictions to mass

transfer. Moreover, assuming the non-additive character of the interactions between these factors, multivariable experimental plans have been used in diverse micro-fungal productions and have led to empirical equations capable of orientating the search of optimal conditions by gradient and surface response methods. This facilitates the treatment of these systems and provides useful indices for understanding their dynamics (Pastrana et al., 1995; Murado et al., 1997, 1998; Pintado et al., 1997, 1998).

Although the ideal microorganisms for SSF are microfungi, numerous lactic acid bacteria have been isolated from foods such as cheese, cured meat, ham and elaborated fish products, thus suggestive of a tolerance to low water activities. In addition, since water activity is not important in the second mode of SSF, it was decided to analyse this mode in cultures of *Pediococcus acidilactici*, studying biomass and pediocin productions in a commercial conventional medium (MRS). Furthermore, it was also assayed the application for the same purpose of a low-cost medium, useful in other bioproductions (González et al., 1992; Murado et al., 1993; Pastrana et al., 1995), and prepared from an industrial effluent (mussel processing waste: MPW) which is abundantly discharged to the coastal waters along the Galician Rías (NW Spain). The experimental results allowed to verify the mathematical model developed to simulate the culture kinetics starting from reasonable groups of work conditions.

MATERIALS AND METHODS

Microbiological methods

The pediocin-producing strain used was *Pediococcus acidilactici* NRRL B-5627, kindly provided by the Northern Regional Research Laboratory (Peoria, Illinois. USA). The basic medium

used (M) was prepared by saccharification of concentrated mussel processing wastes (MPW) by methods described in detail in previous papers (González et al., 1992; Murado et al., 1993; Pastrana et al., 1995), and further dilution to the following concentrations of essential nutrients (in g.l⁻¹): glucose: 10.8; proteins (Lowry): 1.5; total phosphorus: 0.3. Finally, M was supplemented with 6 g.l⁻¹ of peptone and 6 g.l⁻¹ of yeast extract, termed MP and MY, respectively. Commercial MRS medium was also used for comparative purposes. In all cases, initial pH was adjusted to 7.0.

The experimental units were mini-reactors (50 ml methacrylate veterinary syringes) with 0.45 g of a support consisting of ~120 cylindrical particles (6×6 mm) of polyurethane foam (density=20 g.dm⁻³). The nominal volume of this load approximately represented, including the inter-particulate space, a double effective volume (45 cm³) with a coefficient of occupation of ~90% of the syringe space. The support was soaked in 6 ml of culture medium or liquid phase, equivalent to 30% of the maximum load of the foam. Each loaded experimental unit was sterilized by steam flow for 60 min and inoculated with 0.3 ml (5% of liquid phase) of a cellular suspension from 20 hours-aged submerged culture on medium M, adjusted to an OD (700 nm) of 0.900. Cultures were grown in a roller incubator operating at 18 cycles per minute, at 30°C and 76% humidity (environment of a saturated NaCl solution).

At pre-established times and in sterile conditions, the load of each syringe was evacuated with the piston, thus obtaining approximately 95% of the incubated medium (or less if required). The system was subsequently recharged with fresh medium. It is prudent to carry out these steps twice, in order to achieve a good soaking and measure the new pH of the system (always lower than that of the fresh medium) before the final syringe refill.

For comparative purposes, all media were also assayed in conventional submerged culture using a 250 ml Erlenmeyer flasks with 160 ml (optimum load coefficient for pediocin production),

at 30°C, with orbital shaking (200 rpm) and inoculation with 1.6 ml of cell suspension as described for the solid state operation.

Analytical methods

Each sample (submerged or solid state) was divided into two aliquots. The first aliquot was centrifuged at 5,000 rpm for 15 min. The sediment was washed twice and resuspended in distilled water to the dilution required for OD measurement at 700 nm and dry weight estimation from a previous calibration curve. Supernatant was used for the determination of reducing sugars (Bernfeld, 1951), proteins (Lowry et al., 1951), and lactic acid by HPLC analysis (refractive-index detector), using an ION-300 (Teknokroma) column with 6mM sulphuric acid as a mobile phase (flow=0.4 ml.min⁻¹), at 65°C. The second aliquot was used for the extraction and quantification of bacteriocin, using *Carnobacterium piscicola* CECT 4020 (Spanish Type Culture Collection) as an indicator, according to methods described in detail previously (Cabo et al., 1999; Murado et al., 2002). All assays were carried out in triplicate.

Numerical methods

Fitting procedures and parametric estimations were carried out in all cases 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 97 spreadsheet.

RESULTS AND DISCUSSION

Preliminary experiments with submerged culture

The results from submerged cultures on M, MP, MY and MRS media are shown in Figures 1 and 2. Higher productions in MRS are evident. Supplementing medium M with peptone improves pediocin production, but not biomass, the contrary to yeast extract.

Simulation of solid-state fermentation

The simulation of fermentation in solid state proposed here assumes that between each successive compression the microorganism grows according to the logistical model starting from an inoculum X_0 determinable *a posteriori* from the extracted fraction. With this assumption, the operatory described in the methods is reducible to a succession of batch cultures with a global trajectory described by the specific growth rate μ and the maximum biomass K in the process conditions used. Furthermore, this trajectory can be controlled through the time interval between two successive extractions, Δt , and the dilution factor of the inoculum, h , equal to the relation between the volume that remains in the system after each compression and the initial volume (or subsequent to each refill).

On the other hand, in agreement with the model of Cabo et al. (2001) for nisin production by *Lactococcus lactis*, which adequately describes bacteriocin production by various lactic acid bacteria including the preset strain, a realistic system model should incorporate the effect of pH decrease per unit time (δpH) on growth rate. Consequently, pediocin production, considered a

primary metabolite in the sense of Luedeking and Piret (1959), will be influenced. The process can be described as follows (see notations in table 1):

1: Growth, or biomass production X , follows the logistical model:

$$X = \frac{K}{1 + e^{c - \mu t}}; \text{ where } c = \ln\left(\frac{K}{X_0} - 1\right) \quad [1]$$

2: The variable δpH affects the growth rate r_X so that:

$$R_X = r_X (1 + b \delta pH); \text{ with } r_X = \frac{dX}{dt} \text{ and } \delta pH = \frac{\Delta pH}{\Delta t} \quad [2]$$

Accordingly, and given that in differential terms the logistical equation takes the form:

$$\text{rate} = \frac{dX}{dt} = \mu X \left(\frac{K - X}{K} \right) \quad [3]$$

the model here proposed is described by:

$$\text{rate} = \frac{dX}{dt} = \mu X \left(\frac{K - X}{K} \right) (1 + b \delta pH) \quad [4]$$

If μ were constant, equation [4] permits an integral formulation of the type:

$$X = \frac{K}{1 + \text{EXP}[c - \mu(1 + b \delta pH)t]} \quad [5]$$

However, as [4] contains μ (a function of δpH and thus time), a dynamic situation develops admitting a numerical solution only.

3: The decrease in pH (being pH_0 and pH_f the initial and final values, respectively) can be described adequately with the von Bertalanffy equation:

$$pH = pH_f + ae^{-ct} \quad ; \quad \text{with } a = (pH_0 - pH_f) \quad [6]$$

4: The rate of bacteriocin production r_P can be described with the classical model of Luedeking and Piret (1959):

$$r_P = \alpha r_X + \beta X \quad [7]$$

commonly expressed by dividing both terms by biomass, so:

$$\frac{r_P}{X} = \alpha \frac{r_X}{X} + \beta \quad ; \quad \text{and:} \quad \frac{r_P}{X} = \alpha \mu + \beta \quad [8]$$

This formulation enables microbial metabolites to be classified as primary (production rate dependent on rate of biomass production: $\beta=0$), secondary (production rate dependent on biomass present: $\alpha=0$), and mixed (production rate simultaneously dependent on growth rate and biomass present: $\alpha \neq 0$ and $\beta \neq 0$).

5: In agreement with 2, the effect of δpH on growth leads to a situation that admits only a numerical solution. Accordingly, the numerical derivative of the logistical biomass provides an apparent rate r_X , from which the real rate, R_X , is obtained when the effect of δpH is considered. The numerical integration of R_X provides the real biomass X_R . From the substitution of R_X and X_R in the Luedeking and Piret equation, the real rate of bacteriocin production, R_P , can be obtained. Finally, numerical integration of the rates R_X and R_P gives the real values of biomass and bacteriocin (X_R and BT_R):

$$X_R = \sum_{t=0}^{t=t} R_X = \sum_{t=0}^{t=t} r_X (1 + b \delta pH) \quad [9]$$

$$BT_R = \sum_{t=0}^{t=t} R_P = \sum_{t=0}^{t=t} (\alpha R_X + \beta X_R) \quad [10]$$

6: The relationship h between the volumes immediately precedent and subsequent to compressions determines, together with the time interval Δt between two successive compressions, the magnitude X_0 of the inoculum for the following time interval. In this way (being $X_{t(n)}$ the biomass at the end of the cycle n , $X_{0(n+1)}$ the initial biomass of the cycle $n+1$, and L the logistic function), we can write:

$$h = \frac{X_{t(n)}}{X_{0(n+1)}} \quad ; \quad X_{t(n)} = L(\Delta t) \quad [11]$$

Moreover, $X_{t(n)}$ can be easily measured in an aliquot of liquid phase extracted by means of a small stroke of the piston. Therefore, h can be freely established in each cycle as a function of the initial biomass desired for the next cycle.

The preceding formulation facilitates the visualization of the process and permits the values of Δt and h which maximize the production extracted per time unit to be determined. Figure 3 shows, using an arbitrary set of parametric values, the simulations corresponding to three runs characterized by the relationship between Δt and the duplication time of the biomass ($t_D = \ln 2 / \mu$):

- a) $\Delta t < 2 t_D$: the system tends to a wash out.
- b) $\Delta t > 2 t_D$: the system is stabilised at a determined production level.
- c) Variable values of Δt , always superior to $2 t_D$: the system oscillates between stable values of production, and its behaviour is adjusted to those observed in experiments carried out with the same criterion (figs. 4, 5 and 6).

Experimental results

To experimentally verify the conclusions of the simulation, three series of cultures were carried out (in the media MRS, MY and MP) which were forced to follow the kinetic trajectories of the most complex type. This was done by maintaining h constant, and dividing the total incubation time in various periods characterised by the time interval Δt between two successive compressions.

The results, shown in figures 4, 5 and 6, confirm the described analysis. It should be noted that the irregularity of the profiles corresponding to the rates and productions for each time interval does not prevent the experimental accumulated productions (given the smoothing produced by the summation) to satisfactorily reproduce the model-predicted productions. In fact, the irregularity of the production per individual time interval is itself of interest. Such irregularity confirms the actual existence of the dampened oscillations which can be appreciated in the simulation after each change

in the Δt value (figure 3, down), despite the fact that no random variable was included to simulate the experimental error. On the other hand, the oscillations that precede to an asymptotic value are more sensitive to the experimental error than this value, which explains the different profiles of these transient phases when comparing the kinetic model with experimental data.

If the corresponding results from the different media are compared, the most significant point is the high consumption of carbohydrate promoted by the MRS medium, leading to greater production than the other two media by almost an order of magnitude. In addition, the peptone supplement again clearly promotes greater production than those obtained with yeast extract, as in submerged culture. Several authors (e.g. Jensen and Hammer, 1993; De Vuyst, 1995) have suggested that the production of bacteriocins requires complex protein sources containing peptides that act as precursors or inducers of the corresponding biosynthesis. Accordingly, the differences promoted by the supplements used, already detected in a previous work (Cabo et al., 2001), as well as the poor results on M medium, may be due to differences in the respective peptidic composition.

In spite of the efforts made to describe the SSF in a formally precise way, this type of culture still contains an important part of empiricism. When their results improve those from the submerged culture, it is common to justify them adducing that the SSF reproduces specially well the natural conditions in which the implied microorganisms grow (in introduction we have alluded to the aptitude of the lactic acid bacteria to grow in SSF). However, these different conditions also hinders a strict comparison between both modalities.

Keeping in mind these limitations, figures 1 and 2 represent only a reference illustrating the quantitative results of conventional submerged cultures in the same media used for the SSF. This reference could be useful for eventual decisions about the most efficient way of producing bacteriocins. In the case studied here, such results demonstrated the superiority of the solid state

operation for both MP and MRS media. If we compare values corresponding to equivalent volumes of medium (submerged culture) and liquid phase (SSF), the maximum pediocin production in MP is reached at 32 h, with 15.8 BU.ml^{-1} , whereas in solid state, operating at $\Delta t=12$ hours, the production in the same period is 41.3 BU.ml^{-1} (261%). For MRS, Δt values leading to maximum production were not used, although a conservative calculation (acceptable given the model reliability) produces an estimation of 532 BU.ml^{-1} in 48 hours. This is equal to 130% of the corresponding production with the same incubation period in submerged culture.

Furthermore, it should be noted that the solid state cultures were carried out with a conventional, sub-optimal value of h . It is thus foreseeable that the corresponding optimisation led to even more substantial differences in favour of this mode. Finally, the cycle of successive compression without the need for re-inoculation has been demonstrated, constituting a simple, reproducible, easily controllable operation without scaling problems. Culture can therefore be prolonged for long time periods without alterations to the basic properties of the system.

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REFERENCES

- Bernfeld P. 1951. Enzymes of starch degradation and synthesis. *Advances in Enzymology* 12:379-427.
- Bhunia AK, Johnson MC, Ray B. 1988. Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. *J Appl Bacteriol* 65:261-268.
- Cabo ML, Murado MA, González MP, Pastoriza L. 1999. A method for bacteriocin quantification. *J Appl Microbiol* 87:907-914.
- Cabo ML, Murado MA, González MP, Pastoriza L. 2001. Effects of aeration and pH gradient on nisin production. A mathematical model. *Enzyme Microb Technol* 29:264-273.
- Cabo ML, Murado MA, González MP, Vázquez JA, Pastoriza L. 2001. An empirical model for describing the effects of nitrogen sources on nisin production. *Lett Appl Microbiol* 33:425-429.
- Cintas LM, Rodríguez JM, Fernandez MF, Sletten K, Nes IF, Hernandez PE, Holo H. 1995. Isolation and characterization of Pediocin L50, a new bacteriocin from *Pediococcus acidilactici* with a broad inhibitory spectrum. *Appl Environ Microbiol* 61:2643-2648.
- Daeschel MA, 1990. Application of bacteriocins in food system. In: Bills, D.D., King, S.D. (eds) *Biotechnology and food safety*. Butterworth-Heinemann, Boston pp 19-103.
- De Vuyst L, Vandamme EJ. 1992. Nisin, a lantibiotic produced by *Lactococcus lactis* subsp. *lactis*: Properties, biosynthesis, fermentation, and applications. In: De Vuyst, L., Vandamme, E.J., editors. *Bacteriocins of lactic acid bacteria: Microbiology, genetics, and applications*. London: Blackie Academic & Profesional, p. 151-221.
- Dunne C, Murphy L, Flynn S, O'Mahony L, O'Halloran S, Feeney M, Morrissey D, Thornton G, Fitzgerald G, Daly C, Kiely B, Quigley EM, O'Sullivan GC, Shanahan F, Collins JK 1999. Probiotics: From myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials. *Antonie van Leeuwenhoek* 76:279-292.
- Fuller R, Gibson GR 1997. Modification of the intestinal microflora using probiotics and prebiotics. *Scand J Gastroenterol Suppl* 222:28-31.
- Gilliland SE. 1990. Health and nutritional benefits from lactic acid bacteria. *FEMS Microbiol Rev* 87:175-188.
- González MP, Siso MIG, Murado MA, Pastrana L, Montemayor MI, Mirón J 1992. Depuration and valuation of mussel-processing wastes. Characterization of amylolytic postincubates from different species grown on an effluent. *Bioresource Technol* 42:133-140.
- Goulhen F, Meghrou J, Lacroix C. 1999. Production of a nisin Z/pediocin mixture by pH-controlled mixed-strain batch cultures in supplemented whey permeate. *J Appl Microbiol* 86:399-406.
- Guerra N, Pastrana L. 2001. Enhanced nisin and pediocin production on whey supplemented with different nitrogen sources. *Biotech Lett* 23:609-612.
- Klaenhammer TR 1988. Bacteriocins of lactic acid bacteria. *Biochemie* 70:337-349.
- Kumar PKR, Lonsane BK 1987. Gibberellic acid by solid state fermentation: consistent and improved yields. *Biotechnol Bioeng* 30:267-271.
- Liao C-C, Yousef A-E, Richter ER, Chism GW. 1993. *Pediococcus acidilactici* PO2 bacteriocin production in whey permeate and inhibition of *Listeria monocytogenes* on foods. *J Food Sci* 58:430-434.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265-275.
- Luedeking R, Piret EL 1959. A kinetic study of the lactic acid fermentation batch process, and controlled pH. *J Biochem Microbiol Tech Eng* 16:52-55.

- Murado MA, González MP, Pastrana L. 1998. Production of microfungus metabolites on inert solid supports. In: Pandalai SG (edit). *Recent Res. Devel. In Biotech Bioeng*. India: Research Signpost 1:405-432.
- Murado MA, González MP, Torrado A, Pastrana L. 1997. Amylase production by solid state culture of *Aspergillus oryzae* on polyurethane foams. Some mechanistic approaches from an empirical model. *Process Biochem* 32(1):35-42.
- Murado MA, Siso MIG, González MP, Montemayor MI, Pastrana L, Pintado J. 1993. Characterization of microbial biomasses and amylolytic preparations obtained from mussel processing waste treatment. *Bioresource Technol* 43:117-125.
- Murado MA, González MP, Vázquez JA. 2002. Dose-response relationships: an overview, a generative model and its application to the verification of descriptive models. *Enzyme Microb Technol* 31:439-455.
- Pastrana LM, Gonzalez MP, Pintado J, Murado MA. 1995. Interactions affecting gibberellic acid production in solid-state culture: A factorial study. *Enzyme Microb Technol* 17:784-790.
- Pintado J, Gonzalez MP, Murado MA. 1997. Interactions between pretreatment and nutrient concentrations of mussel processing effluents for citric acid production. *Enzyme Microb Technol* 20(7):544-549.
- Pintado J, Torrado A, Gonzalez MP, Murado MA. 1998. Optimization of nutrient concentration for citric acid production by solid-state culture of *Aspergillus niger* on polyurethane foams. *Enzyme Microb Technol* 23(1/2):149-156.
- Pucci MJ, Vedamuthu ER, Kunka BS, Vandenberg PA. 1988. Inhibition of *Listeria monocytogenes* by using bacteriocin PA-1 produced by *Pediococcus acidilactici* PAC 1.0. *Appl Environ Microbiol* 54:1901-1906.
- Ramesh MV, Lonsane BK. 1990. Critical importance of moisture content of the medium in α -amylase production by *Bacillus licheniformis* M27 in a solid-state fermentation system. *Appl Microbiol Biotechnol* 33:501-505.
- Ray B. 1992. Bacteriocins of starter culture bacteria as food biopreservative. In: Ray B, Daeschel M, eds. *Food Biopreservatives of Microbial Origin*, Vol. 8. Florida: CRC Press, pp. 177-205.
- Stiles ME, Hastings JW. 1991. Bacteriocin production by lactic acid bacteria: potential for use in meat preservation. *Trends Food Sci Technol* 2:247-251.
- Vázquez JA. 2001. *Lactobacterias como probiontes e produtoras de bacteriocinas. Modelos de crecimiento e actividade. Aplicacións á acuicultura*. PhD thesis, University of Santiago de Compostela (Spain). ISBN 84-8121-996-7.
- Viniegra-González G, Larralde-Corona CP, López Isunza F. 1994. *Advances in bioprocess engineering*. In: Galindo E, Ramírez OT (eds). *Advances in bioprocess engineering*. Amsterdam: Kluwer Academic Publishers: 295.

TABLE 1: Symbolic notations used

X :	Biomass (X_0 : initial biomass). Dimensions ($g\ l^{-1}$).
K :	Maximum biomass. Dimensions ($g\ l^{-1}$).
μ :	Specific growth rate (biomass formed per unit of present biomass and per unit of time). Dimensions (h^{-1}).
b :	Constant ratio to be experimentally determined. Dimensions (h).
r_X :	Growth rate at constant pH. Dimensions ($g\ l^{-1}\ h^{-1}$).
R_X :	Growth rate induced by pH variation. Dimensions ($g\ l^{-1}\ h^{-1}$).
δpH :	Decrease in pH per unit of time. Dimensions (h^{-1}).
pH_0 :	Initial pH or upper level of the pH range. Dimensionless.
pH_f :	Final pH or lower (asymptotic) level of the pH range. Dimensionless.
c :	Rate of pH variation. Dimensions (h^{-1}).
r_p :	Production rates for P (product: bacteriocin). Dimensions ($BU\ ml^{-1}\ h^{-1}$).
α :	Parameter to be experimentally determined. Dimensions ($BU\ 10^{-3}\ g^{-1}$).
β :	Parameter to be experimentally determined. Dimensions ($BU\ 10^{-3}\ g^{-1}\ h^{-1}$).
X_R :	Production rate total biomass. Dimensions ($g\ l^{-1}\ h^{-1}$).
R_p :	Bacteriocin rate induced by pH variation. Dimensions ($BU\ ml^{-1}\ h^{-1}$).
BT_R :	Production rate total bacteriocin. Dimensions ($BU\ ml^{-1}\ h^{-1}$).

LEGENDS

FIGURE 1: Submerged culture of *P. acidilactici* in M (○), MY (◇) and MP (□) media. X: biomass; BT: bacteriocin; La: lactic acid; Rs: reducing sugars; Pr: protein.

FIGURE 2: Submerged culture of *P. acidilactici* in MRS medium. Notations as in figure 1.

FIGURE 3: Simulation of the main results of solid state cultures with intermittent compression, follow the criterion that specify in the text. A: biomass (○) before and after of each extrusion; B: biomass (○) and bacteriocin (●) extracted per unit of support weight; C: evolution of pH (▽) and acumulated sums of bacteriocin extracted per unit of support weight. Up: supposed of extrusions to intervals of time lower than the double time of duplicate ($t_D = \ln 2 / \mu$) of the microorganism. Center: similar, but with intervals higher. Down: variable intervals upper to $2t_D$, reproduced a similar experimental operatory that led to the results of figures 4, 5 and 6.

FIGURE 4: *P. acidilactici* cultures in polyurethane foams in MRS medium. X: biomass. BT: bacteriocin. ΣG : acumulated sums of glicose added (○) and consumed (●). ΣPr : acumulated sums of protein added (○) and consumed (●). ΣX and ΣBT : acumulated sums of biomass and bactericin take out, respectly. Sup: support.

FIGURE 5: *P. acidilactici* cultures in polyurethane foams in MY medium. Notations as figure 3.

FIGURE 6: *P. acidilactici* cultures in polyurethane foams in MP medium. Notations as figure 3.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6