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Modelling the fed-batch production of pediocin using mussel processing wastes

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Abstract

Cell growth and pediocin production by *Pediococcus acidilactici* NRRL B-5627 were compared using batch (on MRS broth and a culture medium from mussel processing wastes (MPW)) and two fed-batch fermentations on MPW with re-alkalization cycles. This last fermentation technique yielded the highest biomass and pediocin productions as compared to batch fermentations. Mathematical models were set up to describe fed-batch production of biomass and pediocin by *P. acidilactici*. While cell growth was dependent on pH change, nitrogen and phosphorous availability and product inhibition (lactic acid, ethanol and butane-2,3-diol), pediocin production was dependent on both growth and the final pH reached in each re-alkalization period. The models developed offer a better fit and a more realistic description of the experimental biomass and pediocin production data than the logistic and Luedeking and Piret model. Therefore, they could be used to design feeding strategies for enhancing and controlling fed-batch pediocin production.

Keywords: Pediocin; Fed-batch fermentation; Mussel processing wastes; Modelling; Homolactic fermentation; Mixed acid fermentation

1. Introduction

In recent years, there has been an increased interest in the use of bacteriocins as natural food preservatives and antimicrobial agents [1]. Bacteriocins are biologically active proteins that have an antibacterial activity against Gram-positive bacterial species related to the producer strain. Some of these have a very narrow spectrum of activity but others have a relatively broad spectrum of antibacterial activity [2–4].

Pediocins (that are produced by *Pediococcus* strains, organisms generally recognized as safe (GRAS)) have a wide inhibitory spectrum of activity which includes both spoilage and pathogenic organisms, such as *Listeria monocytogenes*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Clostridium perfringens* [5]. The use of these bacteriocins in combination with other stress inducing processes (such as heating, freezing, acid treatment, chelating agents, high hydrostatic pressure and electroporation) can also be effective against Gram-negative or resistant Gram-positive bacteria [6–9].

Since bacteriocin synthesis is considered to be growth associated, factors affecting biomass production (such as temperature, pH, media composition, availability of certain essential compounds, presence of inhibitory compounds) also affect bacteriocin production [10,11]. However, in some cases the pH can also produce a specific effect on bacteriocin synthesis without affecting biomass production [10,12–14]. In batch fermentations, when the optimal initial culture conditions for cell growth are fixed (pH, temperature and media composition), bacteriocin production was found to be strongly dependent on the initial pH [15–18], the pH-time course [13,19], the pH drop [12,19] and the final pH reached in the cultures [19–22]. However, this effect of pH on bacteriocin production depends on the culture medium [17,18] and the bacteriocin-producing strains or species [10,23].

Studies on factors affecting bacteriocin production by lactic acid bacteria (LAB) are usually carried out in rich, undefined media. However, such media are too expensive and have high peptone contents, which make difficult the subsequent purification of bacteriocins [10]. For these reasons, residual effluents from food industry have been used as inexpensive substrates for bacteriocin production [13,14]. In a previous work [14], we demonstrated the suitability of

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glycogen-rich mussel processing wastes [(MPW); average COD: 25 g O_2/l; glycogen as main component: 5–10 g/l] to be used as culture medium for batch production of nisin and pediocin. Since the production of some bacteriocins can be increased by employing fed-batch rather than batch fermentation methods [25–27], we used a fed-batch fermentation technique based on successive re-alkalizations of the culture medium [12] to improve nisin production on mussel processing wastes [24].

Some mathematical models have been proposed to describe bacteriocin production in batch cultivations [16,19]. These models were set up based on the Luedeking and Piret-like equation [28], with a term for growth associated bacteriocin production and a term for bacteriocin degradation or adsorption. However, models describing fed-batch bacteriocin production are lacking and those used in batch cultivations were not suitable for the simulation of fed-batch bacteriocin production [12,26].

In this paper, we report on the use of the fed-batch culture technique with re-alkalization cycles to increase pediocin production by *Pediococcus acidilactici* NRRL B-5627 on mussel-processing wastes. Furthermore, mathematical models were set up to describe the kinetics of *P. acidilactici* NRRL B-5627 cell growth and bacteriocin synthesis during fed-batch fermentation.

2. Materials and methods

2.1. Microorganisms

Pediococcus acidilactici NRRL B-5627, the pediocin-producing strain, and *Carnobacterium piscicola* CECT 4020, the target organism, were obtained from the Northern Regional Research Laboratory (NRRL, Peoria, IL, USA) and the Spanish Type Culture Collection (CECT), respectively. These bacteria were maintained at 4 °C on agar slants (MRS).

2.2. Culture medium, inoculum preparation and fermentation conditions

The composition of the mussel processing wastes used as fermentation medium is shown in Table 1. The preparation of this waste to be used as culture medium was described previously [14].

Table 1
Mean composition (g/l) of the culture media obtained from MPW

	MPW	CMPW
Total sugars	5.33	101.33
Reducing sugars	5.33	101.33
Proteins	1.82	3.47
Total nitrogen	0.65	0.54
Total phosphorous	0.14	0.06

Batch cultures of *P. acidilactici* on MRS and MPW medium were performed in 250 ml Erlenmeyer flasks containing 50 ml of the fermentation medium, on a rotary shaker (200 rpm) at 30 °C for 18 h. The inoculum consisted on 2% (v/v) of an exponentially growing culture on MRS broth or MPW medium. Samples were withdrawn (each 2 or 4 h) during incubation periods to perform the analytical determinations.

Fed-batch and re-alkalized cultures on MPW medium were carried out at a controlled temperature of 30 °C in a 6 l bench top fermentor (New Brunswick Scientific, New Jersey) with an agitation of 200 rpm and continuous-record of pH. The fermentor was filled with 4 l working volume of medium. The aeration level (0.5 l/h) was obtained by controlling the air supply by a flowmeter. Stepwise-pH profiles were obtained by re-alkalizing the cultures repeatedly up to initial pH (6.3) with 4N NaOH each 8 h. The feeding substrate was added at the same time as re-alkalizations. The inoculum consisted of 2% (v/v) of an exponentially growing culture on MPW medium. Samples (100 ml) were withdrawn at 8 h intervals to perform analytical determinations. The total sugar determination was used to calculate the fresh feeding substrate volumes needed to restore the initial total sugars concentration (5.33 g/l) in the fermentation medium. The culture volume was kept constant by feeding the fermentor with 100 ml fresh substrates containing the amounts of sugars consumed in each cycle and distilled water when this was necessary.

Two fresh substrates were used to feed the fermentor: a 240 g/l concentrated glucose (fermentation I), and a concentrated MPW (CMPW, Table 1) medium (fermentation II) which was prepared in the same conditions as the fermentation medium (MPW). The feeding media were added to the fermentor using a peristaltic pump (LKB, Pharmacia).

2.3. Analytical determinations

Methods for determination of cell growth, total phosphorus, nitrogen, protein and sugars were described or referred to in a previous paper [24].

The concentrations of lactic and acetic acids, butane-2,3-diol and ethanol were measured by HPLC using an ION-300 Organic Acids column (length 300 mm, internal diameter 7.8 mm) with a pre-column IONGUARD^{TM} (polymeric guard column), both obtained from Tecknokroma S. Coop. C. Ltda., Barcelona, Spain. The mobile phase consisted of 0.006N H_2SO_4 at a flow rate of 0.4 ml/min at (60–65) ° C and the refractive index of the peaks was measured by a refractometer with a refractive-index detector [24]. All analytical determinations were performed in triplicate.

2.4. Antibacterial activity assay

Aliquots from *P. acidilactici* NRRL B-5627 cultures were adjusted to pH 3.5 with 5N HCl to avoid the adsorption