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Mathematical modelling of cell suspension in high cell density conditions Application to L-lactic acid fermentation using *Lactobacillus casei* in membrane bioreactor

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Abstract

A modelling approach is proposed to represent the decreasing evolutions observed on cell concentration, biosynthetic activity and substrate consumption at late fermentation times during processing when using a cell recycling (membrane) apparatus and continuous feeding. Indeed, during such processes, which are over rather long time periods, the cell populations may have to withstand various stresses (physical, environmental and chemical) involving a decrease of the global activity of the cell populations. This study was carried out with L-lactic acid fermentation using *Lactobacillus casei* as a model system and the modelling approach was based on the definitions of a critical cell concentration and a stress zone. The results obtained corresponded to what was expected.
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1. Introduction

Cell cultivation using membrane bioreactors allows an increase in the productivity of the final substances obtained. However, during such processes, the living cells undergo stresses, which result in changes in physiology (decrease of global activity and of viability) and possibly in morphology (appearance of cell damage and lysis) [1–7]. Under these conditions, various stresses (physical, environmental and chemical, etc.) might be involved. A more thorough study of this could allow a better definition of process operation conditions in order to minimize stress effects and to optimize specific cultivation performances. This article concerns a modelling approach to the evolution of biomass, biosynthetic activity and substrate consumption during a fermentation process using a cell recycling (membrane) device and continuous feeding, and where, at the end, decreases due to cell injury are observed.

Under these conditions, three main types of stress phenomena can been distinguished. They are related to the influences of: (i) cultivation parameters; (ii) chemical stresses; and (iii) mechanical stresses. Among the cultivation parameters, the following changes or irregularities could be considered: temperature in the fermenter (cold or hot), of nutrient concentrations, partial pressures of oxygen and carbon dioxide, osmotic pressure and pH evolution. The chemical stresses include inhibition by high or low substrate concentrations, excess of products and cultivation in high cell concentration conditions. The mechanical stresses include those related to collisions between cells, shear stresses due to agitation and local turbulences and frictions in the reactor. They also involve the irregularities or the excessively severe conditions in the hydrodynamics due to the reactor design adopted in order to ensure good aeration (bubbling) and mixing for correct oxygen and nutrient transfers [8–16]. In these physical stresses, it is also very

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important to include those which are caused by the effects of the presence of additional or peripheral devices, such as pumps, membranes, modules, etc. Note that the latest review concerning this topic for microbial systems is rather old, dating from 1985 [17], and that more recent specific studies on this topic concern mainly animal cells [9,11–16,18], plant cells [2,5] and to a lesser extent filamentous microorganisms [19] or microbial floc microorganisms [20] but not unicellular bacteria (or yeasts).

For this study, we chose L-lactic acid fermentation as a model system. Lactic acid is one of the organic acids which has many applications in various types of industry: chemical; pharmaceutical; and food [21–23]. In 1987, the world production of lactic acid averaged approximately equal proportions being produced by chemical synthesis and fermentation processes [24]. Now, however, all lactic acid is produced using mainly biotechnological means, enzymic or fermentation, and in the latter case, with bacteria species, such as *Bacillus* or *Lactobacillus* [25–27]. Note also that one of the most promising fields of lactic acid application is the development of biodegradable plastics in industry [28,29].

This work presents an approach to modelling the decrease of biomass formation, the loss of biosynthetic activity and the decrease of substrate consumption during fermentation with cell recycling using a membrane apparatus and continuous feeding. It is applied to lactic acid fermentation in high cell density and mechanical stress conditions that are due to the use of a membrane bioreactor.

2. Materials and methods

The installation used in this study consisted of a bioreactor (volume 5 l) equipped with one blade stirrer. Agitation was at a rather low value −200 rpm—in order to avoid significant stress due to this agitation. Two peristaltic pumps (one for suspension feeding in the membrane cells and one for suspension drainage through the cleaning channel) providing viscous suspension pumping, were used at low flow rates in such a way as to minimize the damaging of biomass structure. The apparatus was implemented with a 7-channel microfiltration membrane tube module.

During the experiments, *Lactobacillus casei* subsp. *Rhamnosus* was used as the microorganism producer of L-lactic acid, and glucose was the carbon source substrate. The strain was stored on an MRS medium [30]. For the fermentations, an MRS medium with lactose was also used. The bioreactor operation conditions were the following: temperature 38 °C; pH 6.4; and agitation 200 rpm. The duration between two back-washings of the membrane module was 5 min.

The evolution of cellular activities (biomass, main substrate and metabolic evolution profiles) were evaluated under different stress conditions thanks to data obtained in fermentation experiments which allowed the definition of three different procedures, related to the different fermentation periods or steps which are progressive in stress impact. These procedures were the following:

1. For the first period of time (0–15 h), the bioreactor worked without a membrane module in batch regime, as an ordinary batch fermentor. The data for biomass, substrate and lactic acid concentration changes were monitored. Because of the absence of recycling, biomass concentration was rather low or moderate. This allowed the estimation of the kinetic curves of lactic acid fermentation without (or in conditions of low) stress. As stated above, agitation was 200 rpm.
2. For the second period of time (15–62 h), the fermentation using the bioreactor equipped with the outside membrane module operated with complete cell recycling and at a dilution rate of 0.6 h^{-1}. The corresponding cultivation principle is indicated in Fig. 1A. However the corresponding states were not stationary since there was no biomass going into the reactor. Due to cell

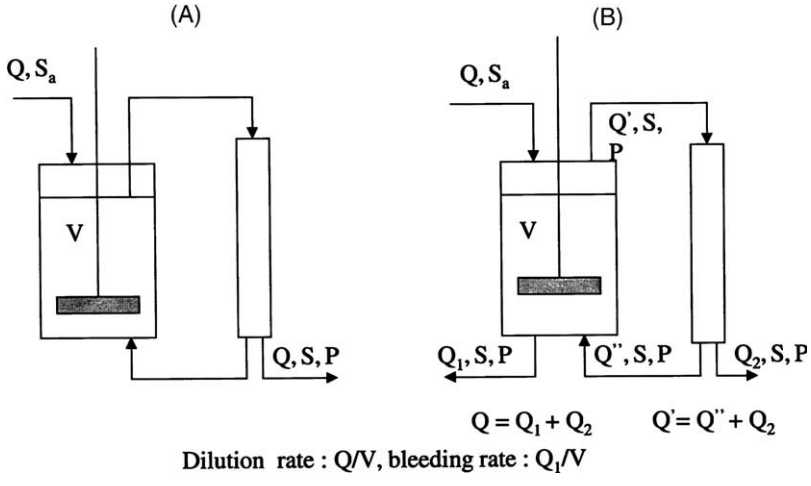

Fig. 1. Principles of cultivations during continuous fermentation using the bioreactor equipped with the membrane module operating under (A) total cell recycling or (B) partial cell recycling.

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