Production of Propionic Acid from Whey Permeate by Sequential Fermentation, Ultrafiltration, and Cell Recycling

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This article deals with the production by fermentation of a mycostatic and aromatic food additive based on propionic acid. Membrane bioreactors have been used from laboratory scale up to pilot and industrial production plants. Due to the high cell densities achieved by the sequential recycling mode of operation, a mixed acids solution was rapidly produced from whey permeate. The sterile fermented broth obtained was subsequently concentrated at different levels by evaporation and spray drying according to the projected use. Concentrated *Propionibacterium* cells (200 g · L^{-1} DW) were obtained from the process by periodic bleeds and could be used to good effect as cheese starters, silage preservatives, or probiotics. Propionic acid concentrations from 30 to 40 g · L^{-1} were easily achieved with no residual lactose. The highest volumetric productivity was 1.6 g · L^{-1} · h^{-1} for total acid and 1.2 g · L^{-1} · h^{-1} for propionic acid with a specific productivity of 0.035 h^{-1}. © 1993 John Wiley & Sons, Inc.
Key words: propionic acid • membrane bioreactor • semicontinuous • *Propionibacterium* • whey permeate

INTRODUCTION

Propionic acid and its salts are used in numerous processes such as in processes of esterification, in the production of cellulose propionate, in the manufacture of ester solvents, fruit flavors, perfume bases, and butyl rubber to improve processability and scorching resistance. In animal therapy, sodium propionate has been used in dermatoses, wound infections, and conjunctivitis. In the food industry, propionic acid and its salts are incorporated to suppress the growth of mold and rope in breads and cakes, on the surface of cheeses,[11] meats, fruits, vegetables, and tobacco, and to prevent the blowing of canned frankfurters. The Food and Drug Administration (FDA) lists the acid, the Na^+, Ca^{++}, and K^+ salts as preservatives in their summary of Generally Recognized As Safe (GRAS) additives.

The industrial production of propionic acid is almost entirely by petrochemical routes, although numerous fermentation processes have been patented since 1923,[21] mainly with strains of *Propionibacterium* genus. They have never passed the pilot plant level because: (i) propionic acid fermentation is a very fastidious task (1 to 2 weeks to be completed in batch); (ii) the production of organic acids by *Propionibacterium* is end-product-inhibited by acetic and propionic acid[17,18]; and (iii) separation and concentration of the acid is expensive (low concentration, small difference of volatility between acid and water, and presence of other acids (especially acetic acid).

The lower supplies and higher costs of oil, the opportunities to use byproducts of the food industry as cheap media, the increasing interest of consumers for organic natural products, and the emergence of more efficient fermentation processes[3] have led to a new opportunity for microbial production to be economically attractive.

Whey is a byproduct of the manufacture of cheese and casein, which is now largely treated by ultrafiltration for the recovery of proteins. But this process leads to a large proportion of effluents, named permeates, which are mainly constituted of water, lactose, and minerals. The disposal of these permeates is a critical problem in the dairy industry. However, they can be used as a base for biological transformations despite their low carbon and nitrogen contents.

The objectives of this study were to examine the technico-economic feasibilities of the production of an aromatic and mycostatic food additive by fermentation of a byproduct of dairy factories. We report the main results obtained on pilot and industrial plants by fermentation, in a sequential mode, of this whey permeate in a membrane bioreactor.

MATERIALS AND METHODS

Strains

The organisms used in this study were: *Propionibacterium acidici-propionici* (American Type Culture

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Collection, Rockville, MD, ATCC 4965; and Centre National Recherches Zootechniques, Jouy en Josas, France, CNRZ 287, 721, 733); *Propionibacterium thoenii* (ATCC 4871); *Propionibacterium jensenii* (ATCC 4870, 4867; and CNRZ 83, 731); *Propionibacterium freudenreichii* subsp. *freudenreichii* (National Collection of Industrial Bacteria, Aberdeen, Scotland, NCIB 5959 and CNRZ 89, 725, 726, 727, 728, 729); *Propionibacterium freudenreichii* subsp. *shermanii* (NICB 5964; CNRZ 433, 722, 724, and SO; 2908, 2910, 7916 from Standa Industrie, Caen, France; and PS1 from Boll, Arpajon, France); and *Lactococcus lactis* subsp. *lactis* (CNRZ 164).

The propionic strains were maintained in Yela medium[12] at −20°C. Three successive inoculations were carried out in the fermentation medium before inoculation of the bioreactor. The *L. lactis* strain was maintained in MRS medium[8] at −20°C.

Medium

Hydrochloric acid whey permeate was used as base medium. For the bioreactor up to 100 L and, for the Erlenmeyers, the dry matter of the medium was 6% (44 g · L^{-1} lactose). For the others, whey permease was either at 6% of dry matter (44 g · L^{-1} lactose) or concentrated: 10% of dry matter (73 g · L−1 lactose), or 16% (117 g · L^{-1} lactose).

Different types of additives were tested (see list Table II).

Analyses of Samples

Cell concentration was determined by optical measurement (650 nm, Shimadzu Spectrophotometer, Japan) correlated with dry weight. Lactose was determined by the Dubois method.[10]

Propionic acid and acetic acid concentrations were measured using an HPLC system (Spectra Physics, USA) equipped with an UV detector (215 nm). The ion exchange column (6 × 300 mm; Aminex A6, Biorad) was operated at room temperature with 0.01N H_2SO_4 (0.5 mL · min^{-1}) as eluent. The acids were measured after separation of the cells by centrifugation: 10 min at 1500*g*.

Experimental—Equipment and Procedure

Five kinds of bioreactors were used in this study:

- A 2-L bioreactor (SET 2M; Setric, France) with pH and temperature regulations. Sterilization was accomplished by autoclaving (120°C for 20 min).
- A 5-L pyrex vessel with a 3-L working volume. Sterilization was accomplished by autoclaving (120°C for 20 min).
- A 100-L bioreactor (Biolafitte, France) with a 50-L working volume and with direct steam sterilization (115°C for 30 min).
- A 750-L bioreactor with a 700-L working volume. The medium was sterilized by ultrafiltration (M9 from Tech-Sep, Rhône-Poulenc, France; surface area = 1.67 m^2; 2xS37, molecular cut-off = 300 kDa). A tangential velocity of 5 m · s^{-1} was achieved with a positive pump. The two units were steam sterilized.
- A 5.2-m^3 bioreactor (Guérin, France) coupled with an ultrafiltration unit (M9 from Tech-Sep; surface area = 34.2 m^2, 6xS252, molecular cut-off = 300 kDa). A tangential velocity of 5.2 m · s^{-1} was achieved with a positive pump (Brown Boveri, BBC, France). The two units were also steam sterilized. The medium was sterilized by ultra-high temperature: 140°C for 35 s (Vicarb, France; flow = 5 m^3 · h^{-1}) (Fig. 1).

Except for the 2-L one, each bioreactor was inoculated with a 10% (v/v) growth culture from the bioreactor

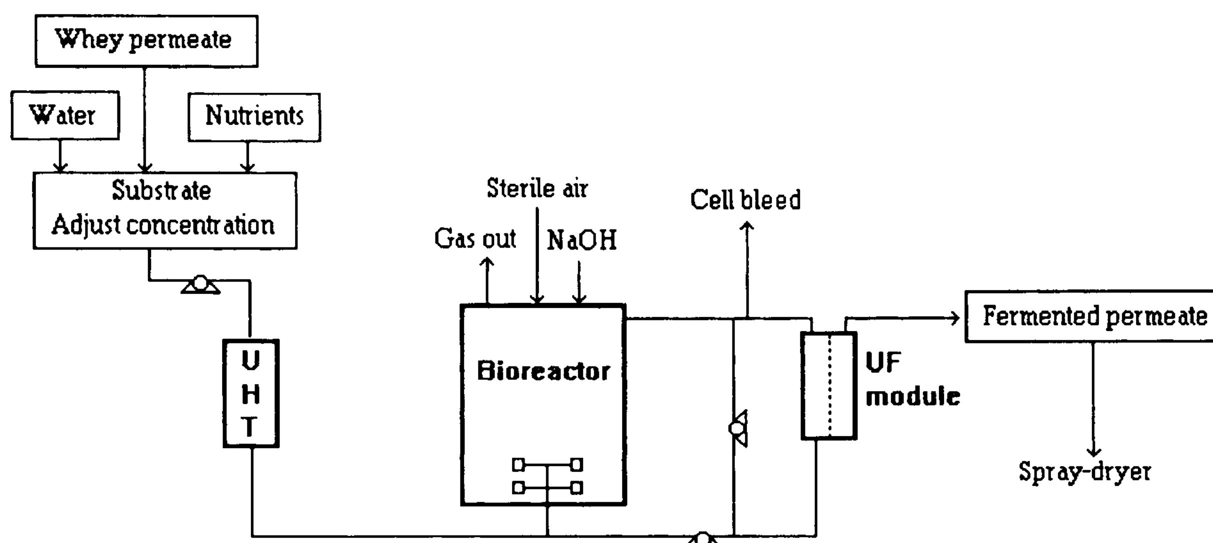

Figure 1. Schematic flow sheet diagram of the process (industrial pilot plant with UHT).

which was immediately smaller in size. The temperature of fermentation was maintained at 30°C and the pH was adjusted at 6.5 by automatic addition of 8N NaOH. The pressure in the fermentor was maintained at 0.2 bar with air.

Fermentation was first carried out batchwise until residual lactose concentration became less than 5% of the initial lactose concentration. Most of the residual lactose was assimilated by the bacteria during the time of ultrafiltration. Then the culture was passed through a sterile ultrafiltration module to produce a permeate fraction containing organic acids and residual lactose, and a retentate fraction including cells (because the pore size of the membrane was sufficiently small to prevent passage of cells). The permeate fraction was recovered and stored in a tank. It was subsequently concentrated and dried to 36% (w/w) propionic acid and 11% acetic acid (Laguilharre concentrator, France; Niro Atomizer, France).

The retentate fraction was recycled to the fermentation vessel. After partial withdrawal of fermented medium, fresh sterile medium was added (UF sterilization for the 750-L bioreactor or UHT sterilization for the 5-m^3 bioreactor) until the fermentor was full. This sterile medium passed through the UF unit (no filtration occurred) so that all the biomass remaining in the tube returned into the bioreactor. The biomass of retained cells served as mass inoculum for the succeeding charge or fermentable material, thus causing the fermentation to start quickly and proceed rapidly. Another ultrafiltration occurred when the lactose was exhausted.

As this process continued and repeated itself, there was an accumulation of biomass in the reactor. Finally, when a high cell concentration was achieved, it was necessary to bleed a part of the concentrated cells. Based on our previous results, and on the work of Crespo et al.,[7] a cell concentration of 50 $g \cdot L^{-1}$ was considered as the best compromise between the increased volumetric productivity and the increased clogging of the ultrafiltration membranes due to viscosity of the biomass. When the biomass achieved this concentration during the fermentation stage, the bleeding occurred every cycle or every second cycle.

For the first three cycles (or charges) the medium was 6.5% dry matter. For the further cycles it was between 10% and 16%. For the 750-L bioreactor, the total volume before recycling was 700 L. For the first two cycles, the permeate fraction was around 560 L and the retentate was 140 L. For the further cycles there was 450 L of permeate and 250 L of retentate.

RESULTS

Batch Experiments

The determination of fermentation parameters and optimization of the medium was achieved on a batch mode in the laboratory-scale bioreactors.

Screening

Selection of propionic bacteria strains causing the transformation of lactose into propionic acid was performed in an Erlenmeyer flask. One-hundred milliliters of whey permeate with 0.5% Corn Steep Powder (CSP; Amylum, Belgium, containing 43% proteins, 17% ash, 37% carbohydrates) was inoculated at 10%. The results are shown in Table I. On 24 strains tested, it was stated that the *Propionibacterium acidi-propionici* ATCC 5958 strain appeared to be the most productive. This bacteria was subsequently used throughout this study.

Temperature of Fermentation

Five temperatures were tested between 20° and 35°C. The highest productivity was obtained at 30°C (not shown).

pH

Five fermentations were achieved at 5.5, 6, 6.5, 7, and 7.5. The highest productivity was obtained at pH 6.5 (not shown).

Table I. Strains selection.[a]

Strain ref.	Species	Propionic acid (70 h) $g \cdot L^{-1}$	pH (40 h)
ATCC 4965	*acidi-propionici*	9.8	4.65
CNRZ 287		7.5	4.67
CNRZ 721		2.0	5.67
CNRZ 733		0.0	4.40
ATCC 4871	*thoenii*	8.0	4.62
ATCC 4870	*jensenii*	0.0	5.82
CNRZ 83		2.0	5.10
ATCC 4867		0.0	5.75
CNRZ 731		0.0	4.41
NCIB 5959	*freudenreichii*	0.0	6.02
CNRZ 89	subsp.	3.1	5.03
CNRZ 725	*freudenreichii*	2.9	5.29
CNRZ 726		3.0	5.36
CNRZ 727		3.2	5.10
CNRZ 728		3.2	5.24
CNRZ 729		3.2	5.31
NCIB 5964	*freudenreichii*	3.7	5.15
CNRZ 433	subsp.	2.3	5.18
CNRZ 722	*shermanii*	4.5	4.73
CNRZ 724		0.0	5.81
SO-STANDA		6.0	4.51
2908-STANDA		6.2	4.45
2910-STANDA		1.92	5.67
7916-STANDA		0.55	5.90
PSI-BOLL		0.82	5.20

[a] Propionic acid concentration after 70 h and pH reached in Erlenmeyers by the different strains after 40 h at 30°C.

Choice of the Neutralizing Agent

After testing NaOH, KOH, $CaCO_3$, and NH_3, the sodium hydroxide was selected. $CaCO_3$ was efficient, but it could not be used because it caused erosion of the UF membranes.

Nitrogen Additive

Previous experiments (not shown) have demonstrated the need to complement the medium (deproteinized whey) with nitrogen supplement to provide the cells with sufficient nutrients. Eighteen nitrogen additives were tested (Table II). They were added at 0.5% dry matter in whey permeate; each medium was at the same initial pH (7.0). Cultures were stopped after 70 h. Two were selected for their excellent productivity/price ratio: CSP from Amylum (Amylum, Belgium), and Yeast Extract LS65 (YE) from Lallemand. They can be used separately or together. In the runs presented, for the bioreactor, up to 750 L pilot plant, 0.5% (w/v) CSP was used, and 0.5% CSP + 0.25% YE were used in the 5-m^3 industrial plant. As the fermented medium will be ultrafilter, it is obvious that the "clogging capacity" of the nitrogen adjunction must be examined carefully (after sterilization in the whey permeate) to keep the filtration performance as high as possible. This consideration must be added to the well-known inhibitory effects on growth and loss of availability of nutrients observed after sterilization of media containing lactose and nitrogen.[1,2]

Inhibition of Lactic Contamination

As propionic fermentation is very favorable to many potential contaminants, like lactic acid bacteria, we tried to improve the safety of the process by the effect of the high density bacterial fence.

A medium containing whey permeate and 0.5% (w/v) CSP was inoculated with a very concentrated culture of *Propionibacterium* obtained by centrifugation. The initial biomass concentration varied between 5 and 50 $g \cdot L^{-1}$. The culture was also inoculated with a culture of *Lactococcus lactis* subsp. *lactis* at 0.1 $g \cdot L^{-1}$. The bottles were incubated at 30°C for 96 h. The results showed that, beyond an initial *Propionibacterium* concentration of 40 $g \cdot L^{-1}$, the growth rate and the cellular density reached by *Lactococcus lactis* subsp. *lactis* were lower than those observed in pure culture. Under 25 $g \cdot L^{-1}$, their development was not inhibited.

Continuous Experiments

In the first cycle, with the 750-L pilot plant, which is equivalent to a batch period, a volumetric productivity of 0.25 $g \cdot L^{-1} \cdot h^{-1}$ and a specific productivity of 0.035 $g \cdot g^{-1} h^{-1}$ were reached.

The biomass concentration increased in the bioreactor to reach 50 $g \cdot L^{-1}$ after 400 h. The biomass was subsequently maintained at this level by periodic bleeding in the retentate pipe. This concentration was chosen because previous

Table II. List of nutrients tested on *Propionibacterium*.[a]

Product	Society	Propionic acid ($g \cdot L^{-1}$)	Productivity/price
Yeast extract	Biokar (USA)	9.0	0.12
Yeast extract: Vitalarmor	Armor Protéines (France)	7.0	0.23
Yeast extract: Lacval 98F	Bioprox (France)	6.0	0.17
Yeast extract LS65	Lellemand (France)	9.5	0.26
Yeast extract SD20	Lallemand (France)	6.5	0.21
Inactivated yeast 2131	Lellemand (France)	6.6	0.33
Autolyzed yeast extract:	Armor Protéines (France)	5.5	0.14
Vitalyse H		6.0	1.00
Autolyzed yeast extract	Armor Protéines (France)	6.0	0.15
Urea	Prolabo (France)	4.6	1.84
Fish extract: DFB 55	ID Mer (France)	6.0	1.50
Sea fisch D.A.	Sea Fish (France)	3.9	1.60
Sea fish S.	Sea Fish (France)	3.7	1.42
Wheat hydrolysate	Doittau (France)	3.2	0.70
Corn steep powder	Roquette (France)	6.5	1.12
Corn gluten feed	Roquette (France)	6.0	3.00
Corn steep powder	Amylum (Belgium)	6.6	1.53
WPC	Triballat (France)	4.0	0.33

[a] Nitrogen additives tested for enhancement or propionic acid production by *P. acidi-propionici* ATCC 4965, at 30°C, in a whey permeate-based medium, with no pH regulation. Propionic acid concentration was measured after 70 h.

experiments have shown that potential contaminations were considerably lower with this, a biomass (see Results, Batch Experiments), and clogging of UF membrane was not too significant.

The propionic acid productivity increased drastically from the first step to the fifth and was subsequently maintained. The propionic acid concentration reached 20 $g \cdot L^{-1}$ in the fist two cycles and between 30 and 40 $g \cdot L^{-1}$ in the others. This was due to the higher lactose content of the medium. Higher lactose levels were tried, but problems of clogging arose in the UHT apparatus leading to a partial filling of the industrial bioreactor.

Lactose was exhausted at the end of each run. This point was one of the main economic cornerstones of the process. The final lactose content of the product had to be as low as possible. This point is particularly important when comparisons are made between our results and most of the current published results where high levels of expensive nutrient additives have been used and where a large proportion of the initial carbon content of the medium was lost in the product.[23] The high nutritive quality of these media enable them to achieve high dilution rates in continuous fermentation, leading to high volumetric productivity. But a rapid analysis of this type of process evidenced that it was economically nonviable.

The volumetric productivity increased to 1.2 $g \cdot L^{-1} \cdot h^{-1}$ with biomass until hour 400. Moreover, even if the biomass was maintained around 50 $g \cdot L^{-1}$ by cell bleeding, the volumetric productivity increased to hour 600 and was then stabilized at around 0.9 $g \cdot L^{-1} \cdot h^{-1}$. The total acid volumetric productivity reached 1.6 $g \cdot L^{-1} \cdot h^{-1}$, mainly by the contribution of acetic acid. The average volumetric productivity was 0.28 $g \cdot L^{-1} \cdot h^{-1}$ for the entire run.

Two cycles at 300 and 600 hours, deliberately longer than the others (100 h compared with 40 to 70 h) were conducted in order to observe the effects of lactose exhaustion on biomass activity. The subsequent cycles were not affected by this nutrient depletion as we observed the same lactose consumption and propionic acid accumulation. The specific productivity decreased from 0.035 to 0.017 h^{-1} at a biomass of 50 $g \cdot L^{-1}$.

Figure 2a is an example of a typical run on the 5-m^3 pilot plant. The biomass was maintained at around 40 $g \cdot L^{-1}$ when partial cell bleeding was performed. These concentrated cells (100 to 120 $g \cdot L^{-1}$) could be used as silage preservative and cow nutrients, or to inoculate a less sophisticated bioreactor without pH regulation and cell recovery device as a single tank. The 15 cycles presented in Figure 2b demonstrate clearly the regularity of the process at this biomass density. The lactose was exhausted after 20 to 50 h leading to a new ultrafiltration-feeding step.

The propionic and acetic concentrations reached, respectively, 43 and 13 $g \cdot L^{-1}$, but were normally around 35 and 9 $g \cdot L^{-1}$ (Fig. 2c). The ratio between the two was rather constant (around 3.7) and independent of the cell concentration or initial lactose content.

The propionic acid volumetric productivity reached 0.80 $g \cdot L^{-1} \cdot h^{-1}$ and stabilized at 0.65 $g \cdot L^{-1} \cdot h^{-1}$. The average volumetric productivity during the 950 h was 0.31 $g \cdot L^{-1} \cdot h^{-1}$. The total acid productivity reached 1.4 $g \cdot L^{-1} \cdot h^{-1}$. The yield was 0.54 g of propionic acid per gram of lactose (from 0.46 $g \cdot g^{-1}$ to 0.62 $g \cdot g^{-1}$).

Figure 2d shows the initial decrease in specific productivity from 0.025 to 0.010 h^{-1} in 200 h and a stabilization at 0.017 ± 0.003 h^{-1} throughout this run.

DISCUSSION

The screening evidenced the higher propionic acid production of the *acidi-propionici* strains. This fact was also noticed by other authors who chose them for organic acid biosynthesis.[3,25] The low pH sometimes obtained with low propionic acid content resulted from the effect of other organic acids. The temperature of 30°C and a pH of 6.5 are common points for most of the studies on propionic acid production.[21] The effectiveness of the different neutralizing agents to maintain the pH at 6.5 were rather similar. Nevertheless, with $CaCO_3$ we observed very high permeate fluxes in the ultrafiltration device. In fact, the zirconium membranes were eroded by the suspension of $CaCO_3$. Only the macroporous carbon support of the membrane remained, leading to a kind of rough filtration rather than UF.

Among the nitrogen additives tested, yeast extracts were the best. In our particular process, a clogging problem was sometimes observed. Previous investigations showed that the polarization layer was mainly built by the polysaccharides (mannanes) of the yeast cell wall. This technical problem was less pronounced with yeast extract LS65 which gave, moreover, the higher propionic acid content. Corn steep powder was also chosen as a good compromise between effectiveness, price, and ease of use.

Production of propionic acid from whey or other lactose effluents has been studied since 1923.[22,24] But, the conclusions of the studies were very often that the slowness of the process made its practical utility doubtful. A propionic acid-tolerant mutant has been developed,[25] but the reported yield is still too low to be economically attractive. Several new fermentation technologies have emerged and increased the volumetric productivity of the propionic acid fermentation. Successful immobilization of *Propionibacterium* cells were reported.[5,6,14] If the performances of immobilized cells are of interest on a laboratory scale, they present severe drawbacks, which impair their potential industrial utilization. The mechanical properties of the beads containing the cells are frequently too weak to allow packaging in a large plug flow reactor or are destroyed by long stirring in a continuous stirred tank reactor. Cell leakage is continuously encountered leading to a loss of catalytic activity and to the necessity of a cell separation step to obtain a cell-free

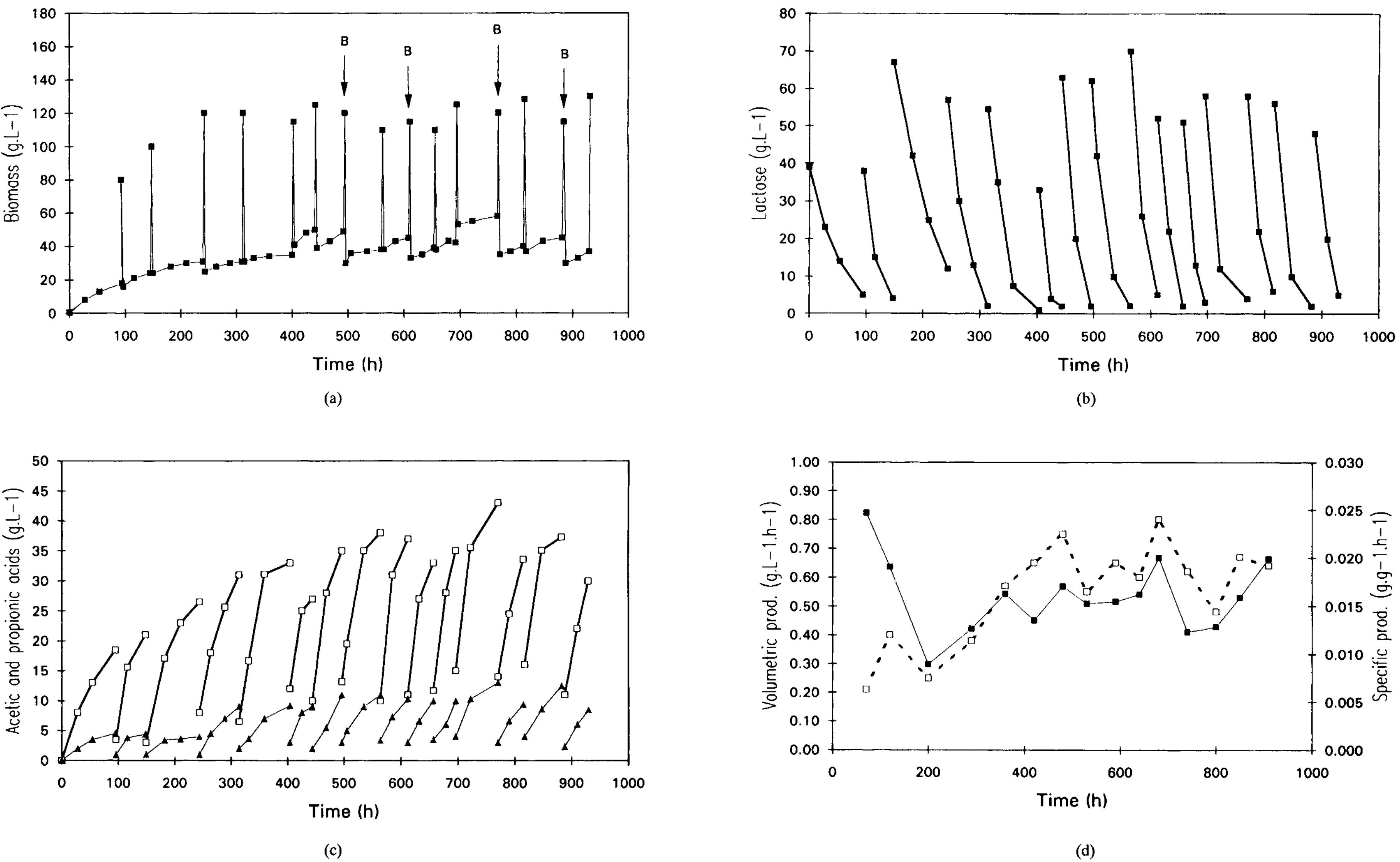

Figure 2. (a) Biomass evolution during a typical run on the 5-m^3 bioreactor. Each peak results of the concentration of the cells by ultrafiltration. At times, indicated by arrows, a cell bleed was done (B). (b) Lactose concentration during the same run. (c) Propionic (□) and acetic (▲) acid concentrations the same run. (d) Specific productivity (■) and volumetric productivity (□) during the cycles [same run as (a)].

product. However, the use of spiral-wound fibrous-bed bioreactors, with propionic acid bacteria attached to the fiber surfaces, seems promising.[16]

The best results in terms of productivity and product quality were obtained in continuous fermentation with cells recycled by ultrafiltration.[3,4] But continuous fermentation with such a sophisticated technology was not easy to scale-up and implant in a factory where only the batch process was well known.

The volumetric productivities obtained in this study were around 1 $g \cdot L^{-1} h^{-1}$. These results compete efficiently with the 2 $g \cdot L^{-1} \cdot h^{-1}$ of most of the laboratory-scale studies with continuous fermentation,[4,5] but are under the 10 $g \cdot L^{-1} \cdot h^{-1}$ sometimes reached by high performance trials.[3,7]

The sequential mode of operation offered several advantages on this basis. The technical operations were very similar to batch fermentation, therefore, the process was rapidly assimilated by the staff. This program allows utilization of only one membrane device for several bioreactors, which is very useful from an economic point of view. The time for filtration was shorter compared with the continuous use of a membrane bioreactor. The fouling of the membrane was less, so the permeate flux was higher and the cleaning procedures were less drastic. In addition, this membrane filter can be washed during fermentation, a step impossible to achieve in continuous runs, unless the bioreactor is coupled to two units: one being used for filtration as the other is being washed (but two pumps are used in this case). Finally, the filtration device can be used to sterilize the medium before fermentation between normal operating steps.

Nevertheless, only a few studies have been done in that type of operation. Semicontinuous processes have been used to improve the yield of propionic acid[25] in a conventional bioreactor. Repeated recycling culture has been already tested by Lee and Chang[15] to alleviate the acetic acid inhibition in *Escherichia coli* and by Denis and Boyaval[9] to produce an extracellular microbial enzyme in a membrane bioreactor. This mode of bioreactor operation will probably be more appreciated by professionals for industrial fermentation plants than the continuous fermentation, because it is easier to set it up in the factory.

The decrease in the specific productivity observed when the biomass concentration increased in the bioreactor has already been observed.[3] This decreased specific productivity at the high cell concentration was probably the result of physiological perturbations: inhibition, substrate limitation, or variations in the intracellular water content of the cell. This is one of the reasons for the cellular bleed.

The long-term stability of the process has been proved with the runs presented and with numerous others. At the present time, no phage has been found on *Propionibacterium* strains. We have observed no loss of catalytic activity of our strains. Moreover, it seems possible to increase the resistance of *P. acidi-propionici* to organic acid inhibition by long, periodic cultures with medium enriched in propionic and acetic acids.

At present, trials are performed with 30% dry matter (220 $g \cdot L^{-1}$ lactose) to try to achieve higher propionic acid contents and to increase the propionic acid/acetic acid ratio. These runs are conducted with a higher biomass content (>80 $g \cdot L^{-1}$). The aim of these experiments is to find a balance between an increased propionic acid concentration and the decreased specific productivity due to the higher biomass and acid contents of the medium, without neglecting a total lactose consumption.

Even if propionic and acetic acids are the two major end-products of lactose fermentation by propionic acid bacteria, other acids could be produced in the medium: lactic acid,[20] succinic and pyruvic acids,[13] malic and fumaric acids,[19] and *iso*-valeric and formic acids. These "byproducts" must be carefully examined, because variations in the process, and especially in the growth medium and agitation speed, lead to important variations in the concentration of these products. A decreased yield of propionic acid production and a modification of the final product of the fermentation are then observed.

Moreover, the recent work of Hsu and Yang,[13] shows that even if neutral pH is optimum for the growth of *Propionibacterium acidi-propionici,* the propionic acid yield is low. On the other hand, in the acidic pH range, the growth rate is low, but the yield is double. With our particular mode of operation, it will be easy to multiply the cells, in the first cycles, at a neutral pH, and to allow an acidification for the next ones to enhance the yield. Moreover, a safer process will be obtained at this lower pH.

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