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Feasibility of propionic acid production by extractive fermentation

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Abstract — Production of propionic acid by fermentation is hindered by low productivity and product inhibition. Cell immobilization to increase productivity and extractive fermentation to reduce product inhibition were investigated. Propionic acid concentration in the extractive fermentation was maintained at 13 g·L^{-1} by concurrent extraction with a liquid extractant consisting of 40 % (v/v) Alamine® 304-1 (trilaurylamine) in Witcohol® 85 NF (oleyl alcohol). A final concentration of 71 g·L^{-1} propionic acid was obtained in non extractive mode. Yields of propionic and acetic acids were doubled and higher overall productivities were obtained in the extractive fermentation. The extractant also exhibited selectivity for propionic acid over acetic acid, thus partially purifying the former. In both fermentation modes, productivity was enhanced by cell immobilization in calcium alginate beads. An economic analysis of a modified version of the fermentation based on several favorable assumptions showed that the extractive fermentation can, at best, approach economic feasibility at an annual production of 4.7×10^7 kg. For the assumed conditions, the production cost of the propionic acid was US$ 1.16·kg^{-1}; this cost was reduced to US$ 0.94·kg^{-1} when the value of the acetic acid byproduct was included. © Inra/Elsevier, Paris.

extractive fermentation / propionic acid / propionibacteria / economic

Résumé — Étude de faisabilité de la production d'acide propionique par fermentation extractive. La production d'acide propionique par fermentation rencontre deux obstacles : la faible productivité et l'inhibition par le produit. L'immobilisation de cellule pour augmenter la productivité et la fermentation extractive pour réduire l'inhibition par le produit ont été étudiées. La concentration en acide propionique en fermentation extractive était maintenue à 13 g·L^{-1} par extraction en co-courant avec un liquide d'extraction composé à 40 % (v/v) d'Alamine® 304–1 (trilaurylamine) dans du Witcohol® 85NF (oleyl alcool). Une concentration finale de 71 g·L^{-1} en acide propionique était obtenue en mode non-extractif. Les rendements en acides propionique et acétique étaient doublés et une productivité globale supérieure était obtenue en fermentation extractive. Le liquide d'extraction montrait de plus une sélectivité en faveur de l'acide propionique par rapport à l'acide acétique, conduisant à une purification partielle. Dans les deux modes de fermentation, la productivité était aug-

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mentée par l'immobilisation des cellules dans des billes d'alginate de calcium. Une analyse économique de la version modifiée de la fermentation basée sur ces hypothèses les plus favorables a montré que la fermentation extractive peut, au mieux, approcher la faisabilité économique pour une production annuelle de $4,7 \times 10^7$ kg. Dans les conditions envisagées, le coût de production de l'acide propionique était de 1,16 $\$\cdot kg^{-1}$; ce coût était réduit à 0,94 $\$\cdot kg^{-1}$ lorsque l'on incluait la valeur du coproduit acide acétique. © Inra/Elsevier, Paris.

fermentation extractive / acide propionique / bactérie propionique / économie

1. INTRODUCTION

Propionic acid is a commodity chemical with uses in animal feed, grain preservation, antifungal agents (calcium and sodium salts), plasticizers (cellulose acetate propionate) and herbicides. The synthesis of propionic acid has so far been dominated by petrochemical routes, including the oxidation of propane, propionaldehyde, and propanol. This makes the product very vulnerable to the sudden price fluctuations of propane and natural gas. On the other hand, synthesis by fermentation is able to utilize inexpensive and renewable biomass as substrate [3, 9, 10, 12, 14, 17, 25].

Commercialization of a propionic acid fermentation process must overcome three barriers. First, the fermentation is lengthy. A typical batch fermentation takes about 3 d to reach 20 $g\cdot L^{-1}$ propionic acid, with a yield (the mass of the acid formed per unit mass of the substrate consumed) usually less than 60 % [8]. Second, the fermentation is end-product-inhibited [26], which limits the final propionic acid concentration. Third, downstream separation and concentration of the acid are expensive because of its low concentration (usually less than 60 $g\cdot L^{-1}$ propionic acid) and the presence of acetic acid as a byproduct. The low volatility of propionic acid relative to water makes recovery by direct distillation problematic.

Integration of separation and fermentation as a means of overcoming some of these barriers has been considered for several carboxylic acids including propionic acid [1, 7, 22–24]. Among separation methods, liquid-liquid extraction is the most widely studied. Several extractive fermentation processes for propionic acid were developed using several complex solvent systems [13, 16, 21]. Solichien et al. [21] used a hollow-fiber module for membrane extraction of propionic acid with 200 $g\cdot L^{-1}$ TOPO (tri-*n*-octyl phosphine oxide, extractant) in kerosene (diluent). This solvent system was nontoxic to the propionibacteria. However, the integrity of the hollow-fiber module was hard to maintain because of solvent crystallization at room temperature. Propionic acid was also extracted with 100 $g\cdot L^{-1}$ TOPO in *n*-decane (as solvent) in a flat sheet, supported liquid membrane (SLM) apparatus by Ozadali et al. [16]. The stability of the SLM was limited by leaching of the *n*-decane. Lewis and Yang [13] used 40 % (w/w) Alamine® 336 in 2-octanol to extract propionic acid via two modes of extraction. With in situ extraction, direct contact of the microorganism and the solvent caused acid productivity to decrease; the fermentation was not inhibited by ex situ extraction.

The economic feasibility of the propionic acid fermentation process has been addressed [2, 13, 15]. Lewis and Yang [13] reported that 16 400 kg of calcium propionate could be produced daily from 450 000 kg of whey for about US$ $0.34\cdot kg^{-1}$ of product. However, they gave no details on downstream processing costs. Clausen and Gaddy [2] presented preliminary designs for a plant

with an annual production of 3.2×10^7 kg at costs of US$ 0.46·kg^{-1} and US$ 0.54·kg^{-1} for acetic and propionic acids, respectively (1981 values). Nishikawa et al. [15] studied co-production of other valuable products such as vitamin B_{12} with propionic acid to improve the economic viability of the fermentation process.

Previously we found that 40 % (v/v) Alamine® 304-1 (trilaurylamine) in Witcohol® 85 NF (oleyl alcohol) was nontoxic, provided good partitioning of propionic acid into the solvent, and was compatible with free acid recovery by distillation [6]. In the present study, this solvent system is used in a fed-batch extractive fermentation with immobilized cells to avoid product inhibition. Immobilized cells offer the advantages of high cell densities, elimination of the lag phase, and reduced exposure to solvent. Fed-batch mode also avoids catabolite repression.

2. MATERIALS AND METHODS

2.1. Materials

Propionibacterium thoenii P20 from the culture collection of the Department of Food Science and Human Nutrition at Iowa State University was used. Seed cultures were grown in sodium lactate broth (NLB) and sodium lactate agar (NLA) as described by Rickert et al. [20]. Fermentations were conducted in fermentation broth (FB) containing 75 g·L^{-1} glucose [20]. For extractive fermentation, 18 mmol·L^{-1} calcium chloride was added to FB to maintain alginate bead integrity. The solvent for extractive fermentation was 40 % (v/v) Alamine 304-1 (trilaurylamine, Henkel Corp., Tucson, AZ) in Witcohol 85 NF (> 90 % oleyl alcohol, Witco Corp., Dublin, OH).

2.2. Fermentation

Strain P20 was immobilized in calcium alginate beads (average diameter:l 2.5 mm in nonextractive and 2.7 mm in extractive processes) as described by Rickert et al. [20]. The bead load (% w/v of beads to fermentation medium) in all fermentations was 40 %. The fermenter and accessory controls were previously described [20]. A constant pH at 6 or 7 was maintained via the automatic addition of 6 mol·L^{-1} NaOH. The temperature was 32 °C and the culture was agitated at 150 rpm. Glucose was monitored with a YSI enzymatic glucose/lactate analyzer (Model 2700, Yellow Springs, Inc., Yellow Springs, OH) and was maintained between 35 and 75 g·L^{-1} by feeding appropriate amounts of a 500 g·L^{-1} glucose solution. An aliquot of 10 × glucose-free FB was also fed every 24 h to replenish other nutrients.

For extractive fermentation in 300 mL FB, the solvent reservoir contained 350 mL of 40 % (v/v) Alamine 304-1 in Witcohol 85 NF, and was replaced every other day. The hollow-fiber membrane extractor consisted of 224 hydrophobic, microporous polypropylene hollow fibers (Celgard® X20–400, Hoechst Celanese Corp., Charlotte, NC), each with an effective length of 25.4 cm, potted into a glass shell (Iowa State University Glass Blowing Shop, Ames, IA) with O.D., I.D., and length of 14, 12.7, and 305 mm, respectively. The total effective membrane surface area was 716 cm^2. Before the fermentation, the shell side of the membrane extractor was chemically sanitized with 500 mL of 3 % (v/v) H_2O_2 and then rinsed with 800 mL sterile deionized water.

Extractive fermentation was started in non extractive fed-batch mode for 22 h at pH 6. At this time, continuous circulation of the medium at 15 mL·min^{-1} on the shell side of the hollow-fiber membrane extractor was begun, while the organic solvent circulated countercurrently at 5 mL·min^{-1} on the tube side. In addition, the pH in the fermenter was allowed to fall to pH 5.5 and controlled there for better acid extraction. Solvent leakage from the membrane pores was prevented by applying back pressure (0.08–0.09 MPa) to the shell side of the extractor. The hydrophobic nature of the hollow fibers prevented the medium from penetrating into the solvent phase as long as the back pressure did not exceed approximately 0.13 MPa.

To enumerate viable immobilized cells, 8 beads were dissolved in 2 mL of sodium citrate (10 g·L^{-1}) solution at room temperature, diluted, and plated on NLA; colonies were counted after 4 d of anaerobic incubation at 32 °C. Samples were taken from the fermenter for HPLC analysis before and after glucose feedings. Before analysis, solvent-phase samples were back-extracted with NaOH. Detailed procedures were described previously [21].

Amounts of propionic and acetic acids were expressed as undissociated acids, and all calculations were performed using the molecular weights of the undissociated acids. It should be noted that above pH 6, most of these acids will exist in their anion form.

2.3. Economic evaluation

The economic evaluation was carried out, with some modifications on our part, using the process design software package BioPro Designer® (Intelligen Inc., Scotch Plaines, NJ). A batch process with an assumed annual capacity of 4.7×10^7 kg propionic acid was used as the basis for calculations. A 10-stage mixer-settler extractor replaced the hollow-fiber membrane extractor used experimentally. This substitution was viewed as technically feasible because of the low toxicity of the solvent for the cells [6]. Scaled-up cost of the hollow-fiber extractor was too great to be feasible for a commodity chemical such as propionic acid. The mixer-settler extractor was sized to maintain the fermenter propionic acid concentration at 3 $g \cdot L^{-1}$ and provide a contact time per stage that was sufficient for 90 % stage efficiency in similar extractions [11].

The fermentation batch time was assumed to be 210 h, the longest duration tested experimentally. During the fermentation, the medium would be recycled through the mixer-settler extractor, where the acids are removed by the 40 % (v/v) Alamine 304-1 in Witcohol 85 NF solvent system. The solvent extract from the extractor is continuously distilled under vacuum in an acid stripping column to recover the acids from the overhead. Any co-extracted water has been neglected. The acids are further purified by distillation to obtain the propionic and acetic acid product streams. The solvent lost in distillation (0.01 %) is replaced with fresh solvent before recycle to the extractor. The bead-immobilized cells for the production fermentation are supplied by a separate cell immobilization process, which includes fermenters for growing seed culture and a decanter-centrifuge for biomass recovery.

The equipment design parameters and chemical prices are detailed elsewhere [4]. All prices were values from 1996–1997, either available in the software or provided by the suppliers of the chemicals. For fermentation substrates, best case choices of NH_3 as the nitrogen source and low-cost 'fermentables' as the carbon source were used. The fermentables were assumed to be glucose equivalents with costs comparable to those of hydrolyzed cellulose or byproducts such as corn steep liquor. Fermentations utilizing corn steep liquor as the substrate achieved similar propionic acid production to that obtained from glucose [19]. The yield coefficients (mass of products produced per mass of fermentable substrate consumed) used were the values obtained from our earlier kinetic studies for a propionic acid concentration of approximately 3.0 $g \cdot L^{-1}$ [5].

The pH can be controlled in extractive fermentation by removal of acid instead of addition of base. Therefore, base consumption was assumed to be negligible in the current design. The costs of cell immobilization were evaluated based on the highest cell density achievable by free-cell fermentation and the initial immobilized-cell density required by the production fermentation at various bead usages (the number of consecutive batches in which the beads can be used in production fermentation without replacement). These costs were combined with the production fermentation costs to determine the overall process profitability.

3. RESULTS

3.1. Fermentation

Although growth of propionibacteria is optimum at pH 7, it has been previously observed that acid production by immobilized cells is comparable at pH 6 or pH 7 [20]. Thus, non extractive fermentations were performed at both pH values for comparison with the extractive fermentation, which was started at pH 6 and continued at pH 5.5 for better extraction performance. *Figure 1* shows acid production by nonextractive fermentation at pH 6. After 168 h fermentation, 71.0 $g \cdot L^{-1}$ propionic acid and 16.1 $g \cdot L^{-1}$ acetic acid were produced at pH 6; at pH 7, 66.2 $g \cdot L^{-1}$ propionic acid and 17.4 $g \cdot L^{-1}$ acetic acid were produced by 209 h.

Broth concentrations throughout a 202-h extractive fermentation are shown in *figure 2*. The solvent-side profiles show that propionic acid was extracted much faster than acetic acid (*figure 3* and *table I*); this resulted in partial purification of the propi-

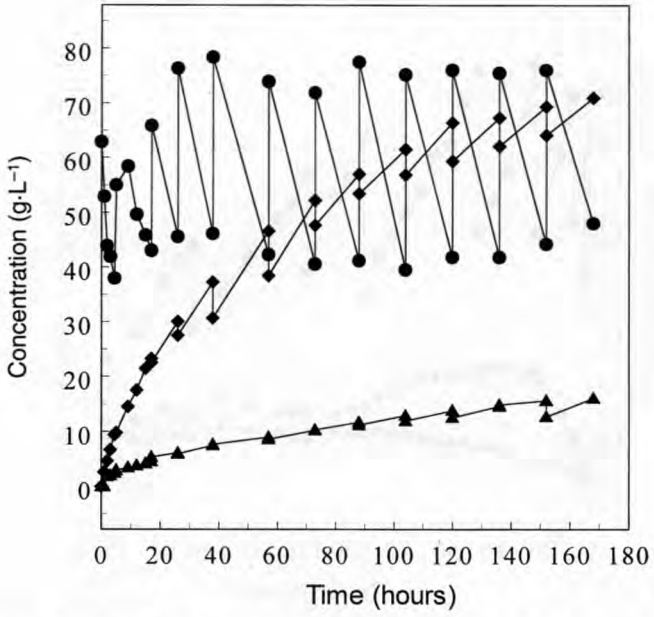

Figure 1. Substrate and products concentrations in the fermentation broth during the course of fed-batch fermentation at pH 6: (●), glucose; (◆), propionic acid; (▲), acetic acid. Decreases in products concentration coinciding with glucose addition are the result of dilution.

Figure 1. Concentration en substrat et produits dans le milieu de fermentation au cours d'une fermentation *fed-batch* à pH 6 : (●), glucose ; (◆), acide propionique ; (▲), acide acétique. Les diminutions en concentration des produits coïncident avec l'addition de glucose et sont le résultat de la dilution.

onic acid. During each of the four solvent replacement periods, the propionic acid extracted was always close to or more than 100 % of the amount produced in the fermenter during the same period. This shows that the membrane area/fermenter volume of 2.4 $cm^2 \cdot mL^{-1}$ used was adequate for propionic acid recovery. Less frequent solvent replacement or a need to maintain lower propionic acid concentration in the fermentation would increase the membrane area required. No solvent was detected in the final propionic acid solution.

As was the case for the nonextractive fermentation, no overall decrease of viable cells was observed for either immobilized or free cells; immobilized cell numbers remained above 10^{10} per mL and viable free cells increased from about 10^8 to 10^9 per mL during the course of the fermentation. This confirms the tolerance of strain P20 to the extraction solvent system. Free cells did not clog the hollow fiber extractor nor interfere with organic acid extraction.

The overall performance of the extractive fermentation compared to that of the nonextractive process is shown in *table II*. Acid yields and productivities were higher in the extractive fermentation. The influence of acid concentration on culture performance can be seen by following the variation in productivity and yields (*figure 4*) during the course of the fermentations. These data have been smoothed to eliminate some of the variability resulting from the need to calculate concentration differences over short time periods. Fluctuations that remain are not considered significant

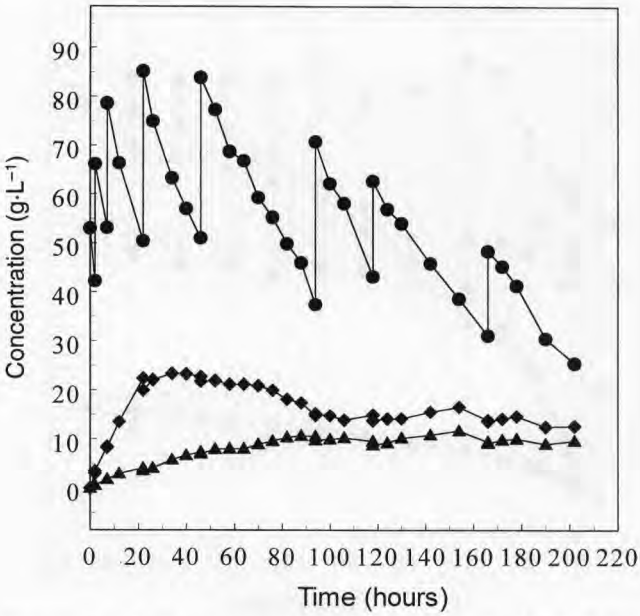

Figure 2. Substrate and products concentrations in the fermentation broth during the course of the extractive fermentation. Extractive mode began at 22 h. (●), glucose; (◆), propionic acid; (▲), acetic acid.

Figure 2. Concentrations en substrat et produits dans le milieu de fermentation au cours d'une fermentation extractive. Le mode extractif commençait à 22 h. (●), glucose ; (◆), acide propionique ; (▲) acide acétique.

but are likely due to unsteady state conditions in the fermenter combined with experimental error in analyses. For the extractive fermentation, differences were summed both from the broth and from the solvent reservoir. Productivity and yields were quite stable during the extractive fermentation, but decreased steadily during non extractive fermentation. As the acid con centration built up, performance declined. An added benefit of the extractive process was that the extraction of acids to the solvent phase reduced base consumption for fermentation pH control by 80 %.

3.2. Economic evaluation

Two major favorable extrapolations have been made for the model process. We have observed that 1.23 $g \cdot L^{-1}$ biomass can be produced in 48 h of free-cell fermentation (unpublished data). The initial cell density in an immobilized-cell fermentation with 40 % (w/v) bead load is about 24 $g \cdot L^{-1}$. Therefore, if beads are used for just a single fermentation, it would require four seed fermenters each producing five batches of free cells to produce enough biomass for an immobilized-cell fermenter of the same size. We have assumed reuse of immobilized cells for five consecutive batches of 210-h fermentation.

An acid productivity of 0.142 $g \cdot L^{-1} \cdot h^{-1}$ with 1.45 % (w/v) bead load was achieved at 2.77 $g \cdot L^{-1}$ propionic acid concentration in a previous study [5]. For this analysis we assumed that the same productivity per bead volume would be maintained at 40 % (w/v) bead load, giving a productivity of 3.9 $g \cdot L^{-1} \cdot h^{-1}$ based on fermenter volume.

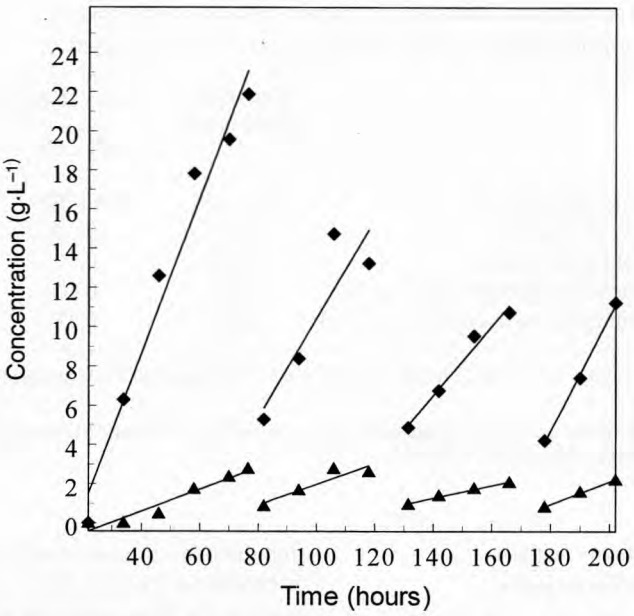

Figure 3. Products concentrations in the solvent reservoir during the course of the extractive fermentation. Breaks in the curves indicate points at which solvent was replaced: (◆), propionic acid; (▲), acetic acid.

Figure 3. Concentrations en produits dans le réservoir de solvant au cours de la fermentation extractive. Les cassures dans les courbes indiquent les points où le solvant était remplacé. (◆), acide propionique ; ▲), acide acétique.

Table I. Efficiency of acid extraction relative to acid production.

Tableau I. Efficacité de l'extraction d'acide relative à l'acide propionique.

Time Period[a] (h)	% Propionic Acid extracted[b]	% Acetic Acid extracted[b]
22–76.5	97.1	37.7
76.5–18	138.6	84.8
118–166	99.2	81.8
166–202	106.1	87.1

[a] Period of the fermentation in which a given batch of solvent was circulating in the extractor.
[b] In the given period, the percentage of propionic or acetic acid produced that was extracted.

[a] Période de la fermentation au cours de laquelle une quantité donnée de solvant circulait dans l'extracteur.
[b] Dans la période, % d'acides propionique et acétique extraits.

The resulting economic evaluation is shown in *table III*. Details of major equipment specifications and purchase costs as well as annual operating costs are presented elsewhere [4]. Of the total equipment cost, 61 % is for the fermenters and 16 % for the extractor. Based on the annual operating costs of cell immobilization and production, including annual depreciation of investment at 9.5 %, the profitability analysis indicates

Table II. Overall performance of extractive[a] and non-extractive fermentation.
Tableau II. Performance globale de fermentation extractive[a] et non extractive.

	Extractive fermentation	Non extractive fermentation	
		pH = 6.0	pH = 7.0
Immobilized cell density[b] (cfu·mL^{-1})	1.3×10^{11}	1.5×10^{11}	1.4×10^{11}
Acetic acid yield (g·g^{-1} glucose)	0.12	0.04	0.05
Propionic acid yield (g·g^{-1} glucose)	0.43	0.20	0.19
Acetic acid volumetric productivity (g·L^{-1}·h^{-1})	0.12	0.10	0.08
Propionic acid volumetric productivity (g·L^{-1}·h^{-1})	0.46	0.42	0.32

[a] Including the performance in the first 22 h non extractive mode. [b] Average viable cell counts of immobilized cells per medium volume.

[a] Comprenant la performance au cours de 22 premières heures en mode non extractif. [b] Moyenne des dénombrements de cellules immobilisées par volume de milieu.

Table III. Profitability analysis.
Tableau III. Analyse de profit.

Total investment (thousand US$·y^{-1})	146 671
Revenue streams flowrate	
Propionic acid (thousand kg·y^{-1})	47 203
Acetic acid (thousand kg·y^{-1})	16 561
Production unit cost[a]	
Propionic acid (US$·kg^{-1})	1.16
Selling price	
Propionic acid (US$·kg^{-1})	0.92
Acetic acid (US$·kg^{-1})	0.84
Revenue	
Propionic acid (thousand US$·y^{-1})	48 807
Acetic acid (thousand US$·y^{-1})	15 302
Annual operating cost* (thousand US$·y^{-1})	60 084
Gross profit (thousand US$·y^{-1})	718

[a] Including the annual costs for cell-immobilization process (assuming immobilized cells can be used for 5 consecutive batches of a 210 h fermentation) and depreciation of 9.5 % annually.

[a] Comprenant le coût annuel du procédé d'immobilisation des cellules (en considérant que les cellules immobilisées peuvent être utilisées pour 5 *batchs* consécutifs de 210 h de fermentation) et une dépréciation annuelle de 9,5 %.

that the overall process becomes profitable at production costs of US$ 1.16·kg^{-1} of propionic acid. When credit for acetic acid (US$ 0.84·kg^{-1}) is taken into account, the fermentation reaches marginal profitability at the current market price for propionic acid of US$ 0.94·kg^{-1}. The return on investment of the process before tax is 2.7 %, at that point.

4. DISCUSSION

4.1. Fermentation

The results from the pH 6 fermentation surpass those reported by Paik and Glatz [17] for a similar study of propionic acid fermentation with calcium alginate-immobilized cells. In their work, lower overall productivities of 0.26 and 0.05 g·L^{-1}·h^{-1} were achieved for propionic and acetic acids, respectively. A different bacterial strain and only a 10 % (w/v) bead load were used in their fed-batch fermentation. In addition, the glucose concentration in the medium was exhausted between feedings to 20 g·L^{-1}. The differences in bead load and prevailing glucose concentration were probably the major contributing factors to the differences in performance between the previous [17] and the current fermentation. However, acid

inhibition was still seen in the current fermentation. Propionic acid productivity and propionic and acetic acid yields decreased as propionic acid accumulated in the medium (*figure 4*). Some of the glucose was diverted to succinate, which accumulated to approximately 21 $g \cdot L^{-1}$ in the pH 6 fermentation. The propionic/acetic acid ratio decreased with increasing propionic acid concentration, which suggests that propionic acid production was more strongly affected by acid accumulation than was acetic acid production. The high concentration of acids in fed-batch fermentation was achieved at the cost of inefficient substrate use and lower productivity.

Improved performance was observed with extractive fermentation. Through continuous removal of propionic acid from the fermenter with liquid-liquid extraction, the propionic acid concentration was maintained at about 13 $g \cdot L^{-1}$. The overall acid yields were more than double those of the nonextractive process. Only 1.6 $g \cdot L^{-1}$ of succinate were produced during the extractive fermentation. According to the stoichiometry of glucose conversion to propionic acid through succinate [18], this decrease in succinate production would account for 15 % of the propionic acid yield improvement.

The lower acid concentration of the extractive fermentation also benefited productivity (except in the initial 22 h, *figure 4A*), even though the cell density was slightly lower. A similar effect has been observed during repeated batch fermentations with 40 % (w/v) bead-immobilized strain P20, where propionic aicd concentration never exceeded 15 $g \cdot L^{-1}$ [20]; up to 4.06 $g \cdot L^{-1} \cdot h^{-1}$ propionic acid productivity was achieved in that case. Response to lower acid concentrations also was observed in fermentation kinetic studies with the same bead-immobilized P20 cells [5].

4.2. Economic evaluation

The economic analysis demonstrates that even with favorable assumptions, the process only reaches the break-even point for current acid prices. Our favorable assumptions are justified. We observed that beads were undamaged and viable cell concentrations remained high at the end of the 202-h extractive fermentation process. Therefore, multiple usage of the beads is possible, although we have not done so under these conditions. Beads have been successfully reused in repeated batch fermentations [19]; up to 20 12-h batches were carried out without significant deterioration of the beads. However, there is no process that we are aware of for producing calcium alginate beads at the required scale.

The high acid productivity (3.9 $g \cdot L^{-1} \cdot h^{-1}$) assumed for 40 % (w/v) load of immobilized cells at 2.77 $g \cdot L^{-1}$ propionic acid concentration [5] significantly reduced the volume of fermenters required for industrial-scale production; hence, reasonable fermenter cost was achieved. Cost dictates use of a mixer-settler extractor rather than the membrane extractor used for our experiments.

Because the production cost of propionic acid is higher than its selling price (US$ 1.16·kg^{-1} vs US$ 0.94·kg^{-1}), co-production of acetic acid is very important to the process' economics. In the current study, the costs of substrates account for over 20 % of the overall operating costs; this would be higher if glucose at current market price was used. Therefore, the use of low-cost substrate without propionic acid productivity decline is very important for process feasibility. Research on substrates such as corn steep liquor, sulfite waste liquor and whey-based substrates has demonstrated the feasibility of such alternatives [3, 9, 10, 12, 14, 17, 25]. A common characteristic of these processes is that useful products such as organic acids can be produced from a waste material with a subsequent reduction in waste treatment cost. Sale of biomass for animal feed would further improve the economics.

In conclusion, extractive fermentation was successfully conducted using immobi-

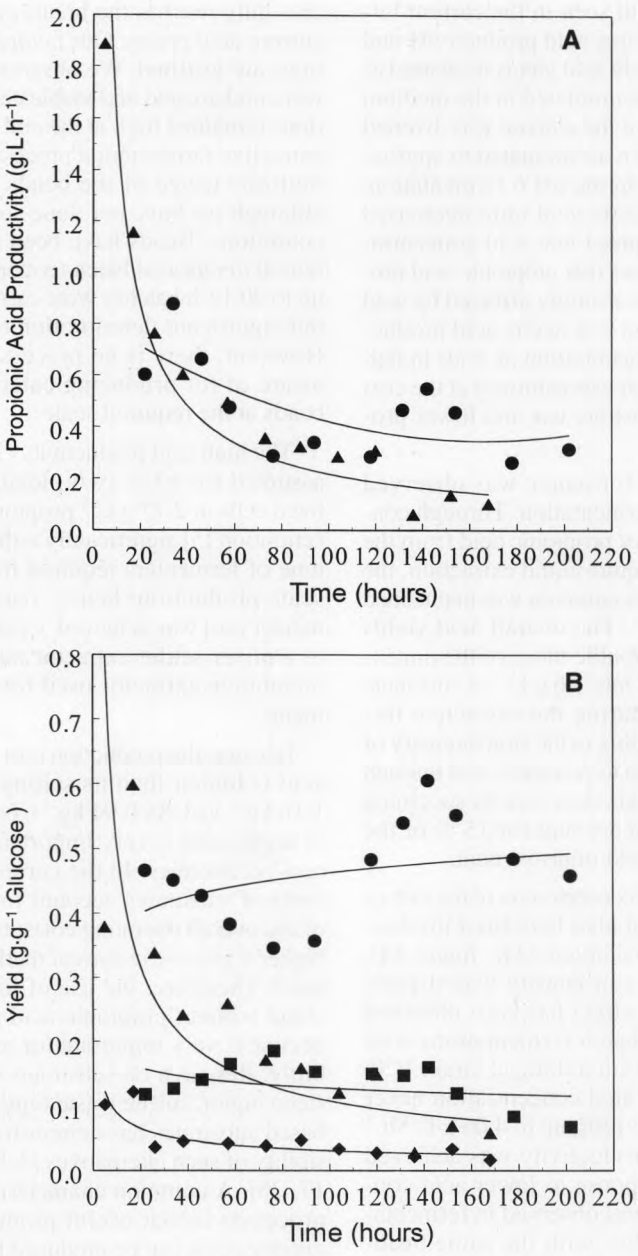

Figure 4. A) Propionic acid productivity during extractive (●) and non extractive (▲) fermentation at pH 6.0. **B**) Variation in product yield during extractive fermentation [(●), propionic acid; (■), acetic acid] and non extractive fermentation [(▲), propionic acid; (◆), acetic acid] at pH 6.

Figure 4. A) Productivité en acide propionique au cours de fermentation extractive (●) et non extractive (▲) à pH 6,0. **B**) Variation du rendement en produit au cours de la fermentation extractive [(●), acide propionique ; (■), acide acétique] et non extractive [(▲), acide propionique ; (◆), acide acétique] à pH 6.

lized cells and 40 % (v/v) Alamine 304-1 in Witcohol 85 NF as the solvent. Higher acid yields and productivities were achieved with the maintenance of low concentrations of propionic acid in the medium by extraction. Additional advantages included reduction of base consumption by 80 % and selective extraction of propionic acid over acetic acid. Economic analysis indicated that several favorable outcomes must be realized simultaneously for even marginal profitability. Whole-cell extraction in a mixer-settler extractor, multiple uses of the bead-immobilized cells, scale-up of productivity, use of an inexpensive substrate, and co-production of acetic acid were all necessary.

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