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THE APPLICATION OF CELL RECYCLE TO CONTINUOUS FERMENTATIVE LACTIC ACID PRODUCTION

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SUMMARY

A continuous stirred tank reactor (CSTR) with cell recycle was used to produce lactic acid from glucose using Lactobacillus delbreuckii NRRL-B445. A volumetric productivity of 76 gm/l-h was obtained with an effluent concentration of 35 gm/l lactic acid and a residual glucose concentration of less than 0.02 gm/l.

INTRODUCTION

The fermentation route to lactic acid presently competes with a synthetic route. Fermentation lactic acid is generally less expensive, but usually contains impurities such as carbohydrates, and consequently has less heat stability than synthetic lactic acid. The recovery processes required to produce fermented material comparable to synthetic lactic acid are expensive. A reduction in fermentation costs could enable these more expensive recovery techniques to be used to produce high quality lactic acid at comparable costs.

Reductions in fermentor size and cost could be accomplished by increasing the volumetric productivity of the fermentor. The volumetric productivity of a conventional batch or continuous fermentor is often limited by the low concentration of microbes in the system. Several methods to increase the concentration of viable microbes in a lactic acid fermentor have been examined. Stenroos et al. (1982) immobilized Lactobacillus delbreuckii in a calcium alginate bead column reactor. Vick Roy et al. (1982) immobilized L. delbreuckii on the outside of a hollow fiber bundle which was perfused with nutrient medium. Friedman and Gaden (1970) used a batch dialysis fermentor and Stieber and Gerhardt (1981) used a continuous dialysis fermentor to reduce the lactic acid concentration and increase the concentration of organisms.

In this work, L. delbrueckii NRRL-B445 was used to ferment glucose to lactic acid in a continuous stirred tank reactor (CSTR) with cell recycle. The cells were separated from the effluent and recycled back to the fermentor with a flat membrane, tangential flow, ultrafiltration unit. Similar systems have been used by Rogers et al. (1980) and Charley et al. (1983) to produce ethanol using Zymomonas mobilis. Sortland and Wilke (1969) used a rotating filter fermentor, which operates in an analogous manner to a CSTR with cell recycle, to produce lactic acid using Streptococcus faecalus. These studies showed increased volumetric production rates and high cell densities compared to a CSTR without cell recycle.

MATERIALS AND METHODS

Figure 1 schematically illustrates the fermentation system. The

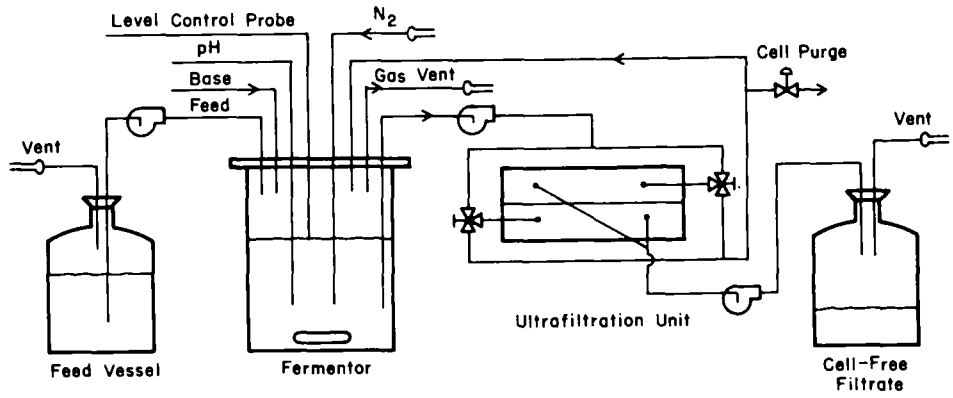

Figure 1. Cell recycle fermentation apparatus.

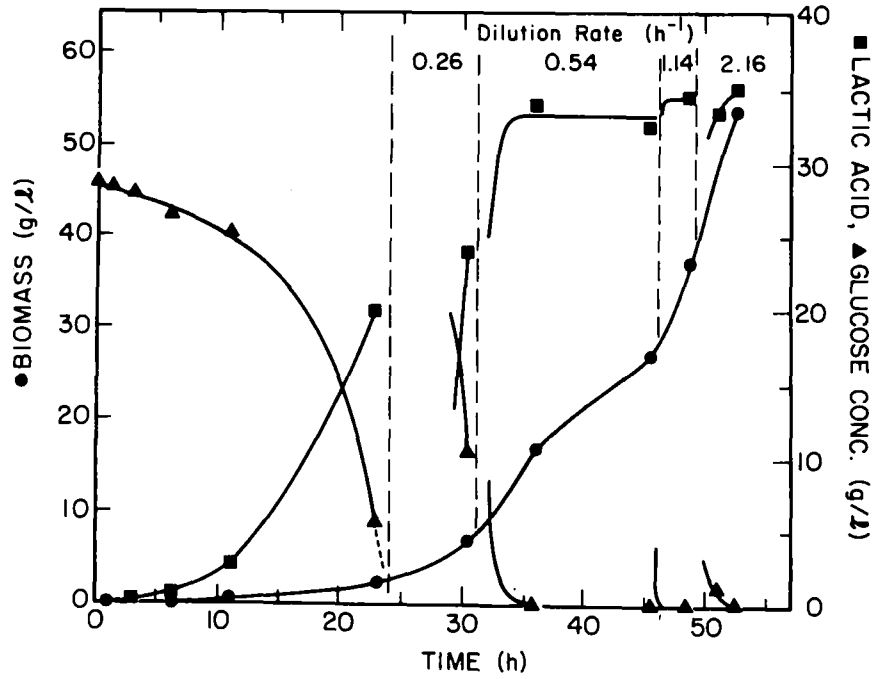

Figure 2. Fermentation profile of lactic acid production in a CSTR with cell recycle.

fermentor consisted of a 1.0 l glass vessel with internal baffles and a magnetic stirring bar. An AMF Cuno® H3791-121 diaphragm pump was used to recirculate the broth through the filter unit. Peristaltic pumps were used for the feed and the effluent. The effluent pump was controlled by a Dyna-Sense® model 7186 electronic liquid level controller. The total liquid volume in the fermentor and recycle loop was 700 ml, with 480 ml in the reactor and 220 ml in the recycle loop.

The filtration unit was an acrylic Pellicon® Cassette System (Millipore Corp.). The unit contained two 100,000 molecular weight cutoff, polysulfone membranes with a combined surface area of 0.046 m^2. The membranes were separated from the acrylic blocks by coarse retentate screens. Valves on the filtration unit allowed the flow over the membranes to be reversed. The fermentor and filtration unit were maintained at 42 C. All lines between the filter and fermentor were insulated.

The organism used was Lactobacillus delbreuckii NRRL-B445, a facultatively aerobic, gram positive, homofermentative, lactic acid producer. Mostly L(+) lactic acid is produced, with about 3% D(-) lactic acid. The medium consisted of the following: glucose (50 gm/l), yeast extract (30 gm/l), succinic acid (2.0 gm/l), NaOH (1.25 gm/l), K_2HPO_4 (0.2gm/l), KH_2PO_4 (0.2gm/l), $MgSO_4(7H_2O)$ (0.6 gm/l), $MnSO_4(H_2O)$ (0.03 gm/l), and $FeSO_4(7H_2O)$ (0.03 gm/l). The broth was controlled at pH 6.1 by the addition of 2N NH_4OH.

The filter unit was 'sterilized' with 3.7 wt % formaldehyde for 20 h and rinsed with 20 l of sterile water. A 70 wt % ethanol solution at pH 2 proved to be less satisfactory for this purpose in earlier experiments. The fermentor was charged with medium, the recycle pump was started, and then the system was inoculated with 7.0 ml of medium containing cells in exponential growth. The fermentation was initially carried out in a batch mode. Continuous operation was started after a sufficient cell mass had accumulated. The feed rate was increased when the residual glucose was less than 0.02 gm/l. The cell density continually increased because cells were not purged from the system. Samples were taken from the fermentor vessel and the filtrate line. Fermentor samples were centrifuged. Cell mass was determined by optical density measurements at a wavelength of 610 nm. Optical density was calibrated with dry weight.

The glucose concentrations were measured with an Instrumentation Laboratories model 919 glucose analyzer which uses a glucose oxidase-peroxidase procedure. Lactic acid concentration was measured using an enzymatic procedure described by the Sigma Chemical Company technical bulletin 726UV/826UV. The viability of the culture was measured at 6.0, 45.5, and 53.4 h by the slide culture technique of Postgate et al. (1961).

RESULTS AND DISCUSSION

The concentrations of lactic acid, biomass, and glucose versus time are shown in Figure 2. The responses of the lactic acid and glucose concentrations to changes in the feed rate were estimated from alkali addition rate data. The initial medium was diluted with the sterile water left in the filter unit and had a glucose concentration of 28.6 gm/l. The alkali addition diluted the effective feed concentration to 35.4 ± 0.8 gm/l during periods of complete glucose exhaustion. The final lactic acid concentration was 35 gm/l. The volumetric productivity of the system based on the total culture volume in the fermentor and filter was 76 gm/l-h at 52.5 h. The final cell mass concentration was 54 gm cells dry weight (DW)/l. The residual glucose was less than 0.02 gm/l. The final lactic acid and cell mass yields were 0.96 ± 0.06 gm lactic acid/gm glucose and 0.09 ± 0.06 gm cells DW/gm glucose, respectively.

The specific growth rate and specific acid production rate of the microbes at 52.5 h was 0.11 h^{-1} and 1.4 gm lactic acid/h-gm cells DW, respectively. Batch fermentation yields of lactic acid and cell mass obtained in our laboratory are 0.90 ± 0.05 gm lactic acid/gm glucose and 0.16 ± 0.04 gm cells DW/gm glucose. The maximum specific growth rate of the organism is approximately 0.65 h^{-1}. The maximum specific lactic acid productivity of the organism is 2.5 ± 0.5 gm lactic acid/h-gm cells DW. Compared to the batch data the cell growth appears to be suppressed relative to lactic acid production in the cell recycle reactor. However, the wide range of uncertainties in the data indicate that additional experimentation would be required to verify this statistically. Throughout the fermentation, greater than 95% of the microbes were viable.

At 52.5 h the liquid filtration rate was 1.5 l/h. The maximum flux of the membrane had been reached, and further increases in the feed flow rate could not be achieved without adding more filter area. The final transmembrane flux was 0.78 m^3/m^2-d with an inlet gauge pressure of 2.31 atmospheres. The recirculation rate over the membrane was approximately 10.1 m^3/m^2-d which corresponds to a tangential velocity of about 6 cm/s. The direction of fluid flow over the membranes was reversed every 2 to 6 hours. This reduced the inlet pressure. These same membranes had been used in four previous experiments. The membranes were cleaned between experiments with 0.2N NaOH.

After extended operation at any particular dilution rate, the system appeared to have an excess biocatalytic capacity. For example, when the dilution rate was raised from 0.54 h^{-1} to 1.14 h^{-1}, the productivity of the system doubled in less than one half hour. This productivity increase was not accompanied by an appreciable increase in the cell mass or its viability.

Earlier work with this recycle system produced a high cell mass of 50 gm/l but a low volumetric productivity of 16 gm lactic acid/l-h and a viability of only 20%. The increase in viability in subsequent experiments was believed to have been achieved by replacing the 5N NaOH (pH≈14.7) neutralizing solution with the 2N NH_4OH (pH≈11.8). This agrees with Reilly's (1964) observation that batch growth was faster using NH_4OH rather than NaOH for pH control. The high local hydroxide and/or sodium concentration at the point of introduction may have killed some cells. A peristaltic pump was used in earlier experiments for the recycle loop. It was replaced by a diaphragm pump because of tubing wear problems. The mechanical stresses on the microbes in the peristaltic pump may have also contributed to the loss of viability. Over many hours the dead cells could have accumulated to significant levels in the system.

The performance of the cell recycle CSTR is compared to other fermentor systems in Table 1. All but one of the listed studies used the same strain of *L. delbreuckii* and essentially the same medium. Residual glucose concentration, effluent lactic acid concentration, lactic acid productivity, and pH are key variables for the production of lactic acid. Low residual glucose and high lactic acid concentrations reduce the cost of the recovery steps. The volumetric lactic acid productivity is strongly influenced by the pH and the lactic acid concentration. The undissociated, electroneutral form of lactic acid rather than lactate appears to be the species that inhibits the fermentation (Viniegra-Gonzalez and Gomez, 1983).

The lactic acid productivity of the cell recycle system compares favorably to the other systems in Table 1. The batch and continuous stirred tank reactors have low cell mass concentrations and consequently low productivities. The hollow fiber reactor has a high cell mass concentration and a high productivity but a low conversion of glucose. Subsequent studies with this system indicated that one must sacrifice reactor productivity to increase the conversion. The calcium alginate gel immobilization used by Stenroos *et al.* (1982) has a similar behavior

to the hollow fiber reactor. As the fermentation broth passes through these reactors, lactic acid accumulates, pH decreases, and nutrients are depleted. The microbes at the end of the reactors are thus in a less favorable environment than those at the beginning of the reactor. The conversion achieved per pass and/or the productivity of these reactors is affected by the buffering capacity of the medium. Both a favorable growth environment and a high cell mass concentration are achieved in the CSTR with cell recycle. This permits both high productivities and complete conversion at the same time. The excess biocatalytic capacity present in the CSTR with cell recycle could help maintain a low residual sugar concentration in the effluent during minor feed rate, feed composition, and culture condition perturbations. A low residual sugar concentration is important in minimizing raw materials and recovery costs.

A preliminary economic analysis of this technology suggests that for a production rate of 5.0×10^6 kg/yr up to \$0.20/kg could be saved over conventional technology using batch fermentors. The current price of lactic acid is about \$2.1/kg. In this analysis, a batch process with five batch reactors and a rotary drum filter was compared to two cell recycle fermentors in parallel with a membrane filtration device for cell recycle and a rotary drum filter to remove the remaining water from a cell purge stream. A membrane life of one year was assumed, and costs were based on existing commercial membrane costs. A four day cycle time was assumed for the batch reactors, and a 25 gm lactic acid/l-h productivity was assumed for the cell recycle reactor. All equipment was assumed to be constructed out of 316 stainless steel because of the corrosive nature of lactic acid.

Further research is needed to verify some of the assumptions used in this preliminary economic analysis. Membrane life and performance under these conditions is unknown. Large scale, steam sterilizable units are not commercially available and their costs are not well known. The maximum productivity of the CSTR with cell recycle has not been established. As stated earlier, the productivity obtained in this work was limited by the surface area in the filter unit. Higher productivities could be reached with a greater surface area to culture volume ratio. Other means of achieving the cell recycle have not been explored for lactic acid production. Productivities and conversions with a typical industrial effluent concentration of 12-15 wt % lactic acid have not been experimentally evaluated. Projected lactic acid productivities with a 15 wt % lactic acid effluent concentration of pH 5.0, 6.0, and 7.0 are 0.25, 25, and 96 gm/l-h, respectively. These projections are based on unpublished batch kinetic studies of the effects of pH and lactic acid on the organisms. The application of this technology for commercial lactic acid production seems promising.

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Table 1.
Comparison of Lactic Acid Fermentation Systems

Organism	System	Percent conversion	Cell conc. (g/l)	Lactic acid conc. (g/l)	Volumetric productivity (g/l-h)	Reference
L. del-breuckii NRRL-B445	Cell Recycle CSTR	≈100	54	35	76	This work
"	Batch	≈100	≈7-8[c]	45	1-2[a]	Leudeking and Piret (1959a)
"	Batch with Dialysis	70[c]	≈11[c]	35	2-3[a]	Freidman and Gaden (1970)
"	CSTR	≈100	≈7-8[c]	38	7	Luedeking and Piret (1959b)
"	Hollow Fiber Reactor (300 fibers)	4[b]	350	2	100	Vick Roy et al. (1982)
L. del-breuckii "special strain"	Calcium alginate gel beads	≈100	≈67[c]	≈46[c]	≈3[c]	Stenroos et al. (1982)

a) adjusted for total cycle time
b) for a single pass with a residence time of 3 to 4 sec.
c) estimated from reported data