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LACTIC ACID PRODUCTION BY LACTOBACILLUS DELBREUCKII IN A HOLLOW FIBER FERMENTER

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SUMMARY

Lactic acid was produced by viable Lactobacillus delbreuckii NRRL-B445 in a hollow fiber fermenter. Final cell densities in the fluid surrounding the fibers in the fermenter were apparently as high as 480 gms DW/L, and volumetric productivities reached 100 gms/L-hr lactic acid. The observed cell yields were appreciably lower than batch cell yields.

INTRODUCTION

High density fermentations employing immobilized, viable cells have been accomplished in various ways. The different methods of immobilization, and the advantages and disadvantages of immobilized cells are discussed in a review by Abbott (1977). In a hollow fiber fermenter the microbes are gently immobilized around the outside of the fibers in the shell side of the device. The microporous walls of the hollow fibers allow the nutrients and products to diffuse freely, but the microbes are unable to penetrate them. Some advantages that may be realized with this type of immobilization are extremely high cell densities, high volumetric productivities, low susceptibility to process upsets, and a reduced requirement for feed sterilization.

Past experimental work with whole cells in hollow fiber reactors have been reported by Kan and Shuler (1978), Mattiasson and Ramstorp (1981), Inloes, Robertson and Michaels (1982), and Smith, Robertson and Michaels (1980). In this work the organisms were in a viable, densely packed cell mass occupying the entire volume around the fibers whereas the previously mentioned studies involved either cell suspensions used for a specific one step enzymatic reaction or did not make full use of the available shell volume.

Modeling of these reactors have been undertaken by Webster and Schuler (1978) (1979) (1981) for the case of no appreciable axial gradients and by Waterland, Michaels and Robertson (1974) and Kim and Cooney (1976) who treated axial gradients as well.

Experimental

A schematic of the apparatus used is shown in Figure 1. The hollow fiber fermenter was maintained at 45°C in a constant temperature incubator. The hollow fiber module consisted of an Amicon Vitafiber^{R} shell (9.5 cm x 0.65 cm dia.) and 60, 108 or 300 Celanese Celgard^{R} microporous polypropylene hollow fibers (type X-10 100 micron i.d., 150 micron o.d.).

The homofermentative lactic acid bacteria Lactobacillus delbreuckii NRRL-B445, was grown on a medium described by Friedman and Gaden (1970)

except 11.8 gm/L succinic acid, 1.0 gm/L K_2HPO_4 and 1.0 gm/L KH_2PO_4 were used in place of the acetate-phosphate buffer described.

The hollow fiber fermenters were sterilized by ethylene oxide and required wetting with a solution of 50% v/v ethanol prior to use. The fermenter was inoculated by injecting a growing cell suspension into the shell space through one of the inoculation ports. The pH was maintained at pH 6.0 with the addition of 5N NaOH. The pH of the exit stream was monitored. Medium flow rates ranged from 60 to 200 ml/hr.

Glucose was determined by glucose oxidase with Instrumentation Laboratories model 919 glucose analyser. Lactic acid was determined enzymatically according to a procedure described in Sigma Chem. Co. technical bulletin 726-UV/826-UV.

RESULTS AND DISCUSSION

The concentrations of glucose and lactic acid versus time are shown in Figures 2, 3 and 4 for the 60, 108 and 300 fiber reactors, respectively. The solid and open symbols represent the concentrations in the recycle vessel and in the stream exiting from the fermenter, respectively. It is apparent from the shape of the curves that the lactic acid production rate is limited by transport of glucose across the fiber wall and into the cell mass in all three cases. Because of the high cell densities and from measurements of the fibers mass transfer properties it appears that the major mass transfer resistance is in the cell mass itself. In the absence of mass transfer limitations one would expect an exponential increase in lactic acid concentration. Mechanical problems with the prefilters caused an early termination of the 60 and 108 fiber runs. In the 300 fiber run the fibers were contorted by the growing cell mass. The fibers were damaged so that the cells were able to penetrate the walls and contaminate the recycle vessel at about 100 hours. Problems with the pump during the 300 fiber experiment caused flowrate fluctuations which may be responsible for the erractic nature of the glucose and lactic acid curves. The pH change between the medium entering and exiting the fermenter did not exceed 0.4 pH units. Measurements inside the hollow fiber reactor were not attempted because of the difficulty in taking samples.

A comparison of the three experiments to each other and to controlled pH batch fermentation is shown in Table 1. The maximum volumetric acid production rate was greater than that for batch fermentation in all cases, and greater than the value of 16 gm/L-hr that could be expected for a CSTR. The rate did not increase proportionately with the increase in surface area as would be expected for a mass transfer limited situation. This may be because the fibers are not evenly spaced and thus the actual surface area available to the microbes may be different than the surface areas presentd in Table 1. The specific productivity of the organisms was only 10-15% of the specific productivity during the exponential period of batch growth. This appears to be further evidence for mass transfer limitations. The final cell densities achieved were extremely high compared to conventional batch fermentations, while the cell yields were much lower than normal. The value of 480 gms DW/L for the 300 fiber experiment is higher than 200 gms DW/L that one would expect for microbes that are roughtly 80% water. Thus, it appears that the H_2O content of the cells must be less than that of normal cells, or that cell debris may have accummulated in the fermenter. Although cell growth appears to be inhibited, it was not visually apparent that it was

TABLE 1

Comparison of Hollow Fiber Reactors to Batch Growth

# OF FIBERS	60	108	300	BATCH
Fiber Length (cm)	5.8	5.5	6.5	---
Surface Area (cm^2)	16.4	28.0	91.9	---
Shell Side Volume (cm^3)	3.4	3.2	2.6	---
Max Lactic Acid Prod. Rate g/L·h				
based on shell side vol.	29	50	138	---
based on fermenter vol. (3.6 cm^3)	28	44	100	5*
Specific Productivity				
$\frac{\text{g lactic acid}}{\text{g DW·h}}$	.25	---	.29	2.0*
Final Cell Density $\frac{gDW}{L}$				
based on shell side vol.	117	---	480	9
based on fermenter vol.	111	---	350	---
YIELDS $\frac{\text{g latic acid}}{\text{g glucose}}$	.9 ± .1	.95 ± .1	1.0 ± .1	.9 ± .05
$\frac{\text{gm DW cells}}{\text{gm glucose}}$	.055	---	.064	.19
flow rate ml/h	60	65	150-200	---

*In exponential phase, on identical medium. This compares well to the value quoted by Friedmann and Gaden (1970).

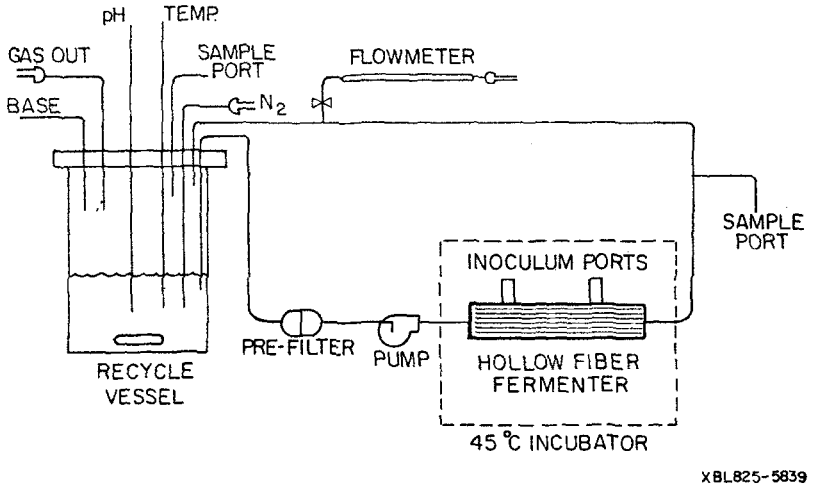

Fig. 1. Hollow Fiber Batch Recycle Apparatus.

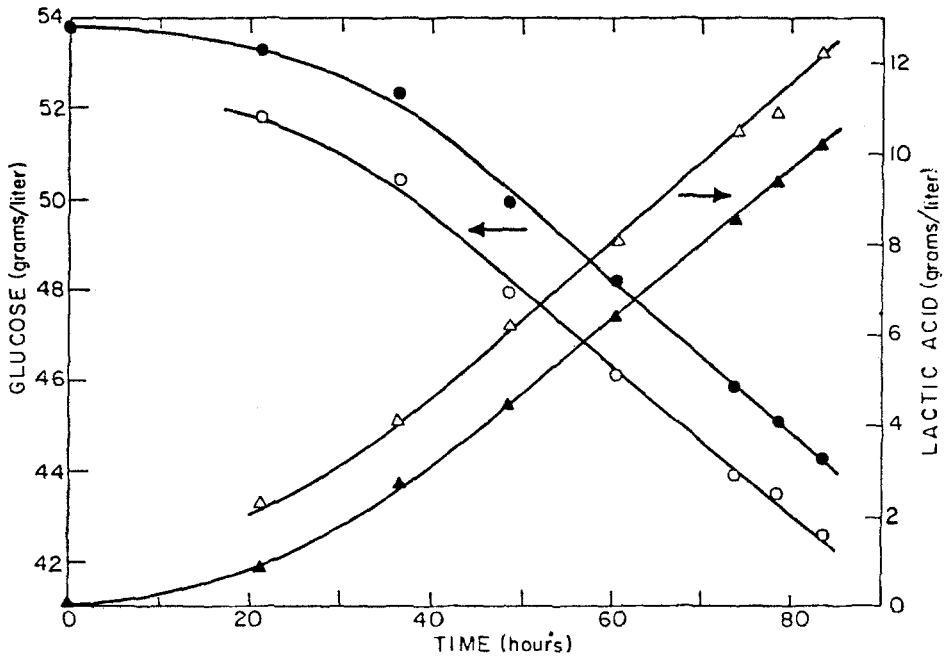

Fig. 2. 60 Fiber Fermenter. XBL 825-5840A

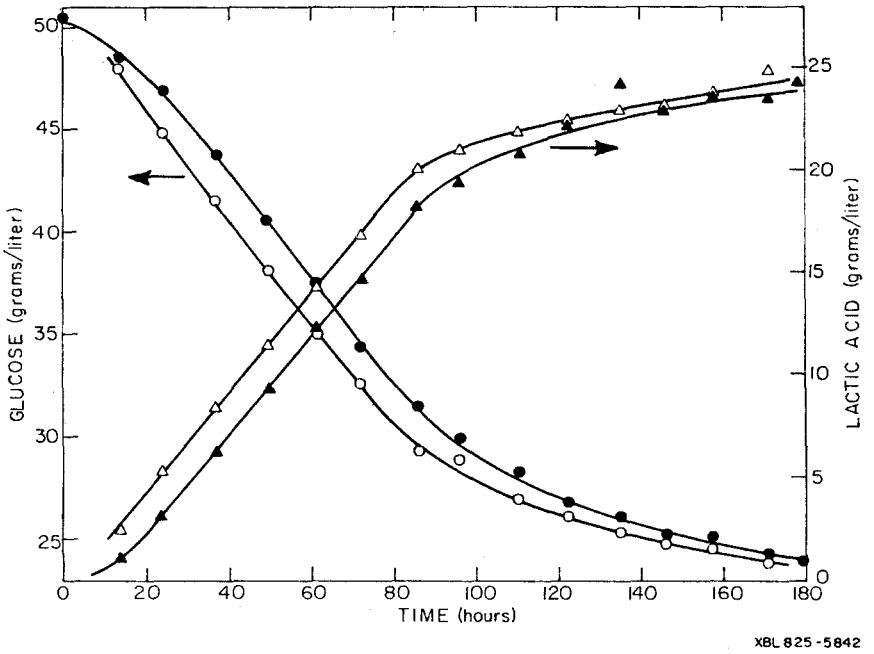

Fig. 3. 108 Fiber Fermenter.

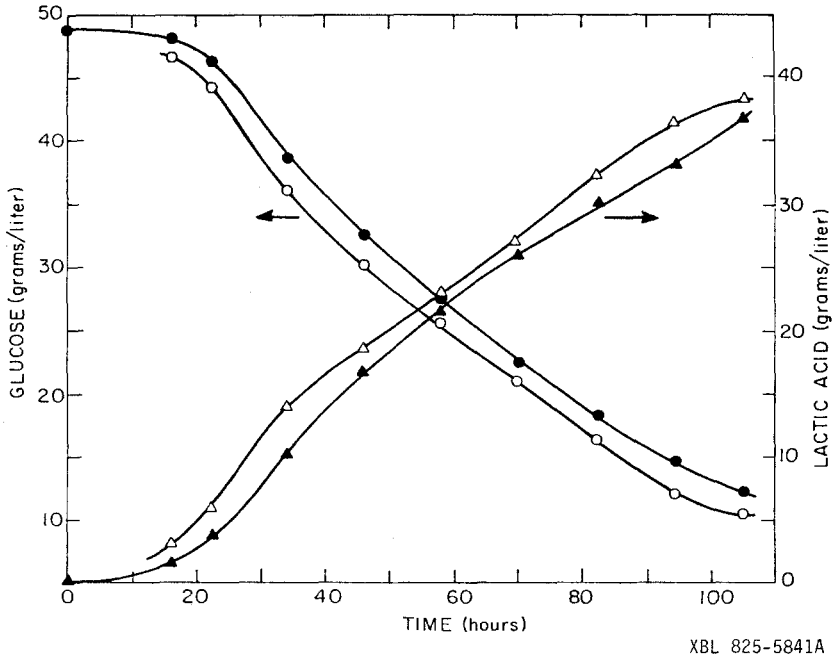

Fig. 4. 300 Fiber Fermenter.

completely halted during any of the experiments. After 100 hours in the 108 fiber experiment, some cavities appeared which were later filled with cells. It is possible that cells far from the fibers underwent lysis, and then other cells were pushed outward by the microbes multiplying near the fibers. It appears as though the lactic acid yield may have increased, but there is too much uncertainty in the data to be able to be sure. The uncertainty may be due to the glucose and lactic acid assays and in correcting for the base addition and sample withdrawal.

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