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Ethanol Fermentation in a Tower Fermenter Using Self-Aggregating *Saccharomyces uvarum*

Bioreactor Performance

CHEE-SHAN CHEN,[1] E. CHAN,[1]
S. L. WANG,[1] C. S. GONG,[2] AND L. F. CHEN*,[2]

[1]*Department of Food Engineering, Da-Yeh Institute of Technology, Chan-Hwa, Taiwan 51505, ROC: and* [2]*Department of Food Science, Purdue University, West Lafayette, IN 47906, USA*

ABSTRACT

A self-aggregating strain of *Saccharomyces uvarum* (U4) was used as a biocatalyst to carry out continuous ethanol fermentation in a tower fermentor equipped with a cell separator. Cell aggregates (2–3 mm) formed a stable packed bed in the fermentor, and the cell separator retained yeast cells effectively. Corn steep liquor was used as a nitrogen source for the fermentation of corn syrup and black strap molasses. An ethanol productivity of 54 g/L/h was reached using corn syrup at a dilution rate of 0.7/h, and sugar concentration in the feed was 15% (w/v). For molasses fermentation, an ethanol productivity of 22 g/L/h was obtained at a dilution rate of 0.7/h, and sugar concentration in the feed was 12.5% (w/v). Ethanol yields obtained from tower fermentation are higher than those obtained from flask fermentation (96% for corn syrup fermentation and 92% for molasses fermentation). No significant loss in fermentation activity was observed after 3 mo of operation.

Index Entries: Tower fermentor; *Saccharomyces uvarum*; ethanol fermentation; aggregates, molasses.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

One effective way to increase productivity in ethanol fermentation is to employ a continuous process using immobilized yeast cells. There are many reports on the utilization of immobilized cell systems for continuous ethanol fermentation. Living cells can be entrapped within porous solid support, such as κ-carrageenan, polyacrylamide, calcium alginate, and pectin (*1,6*). Surface adsorption on solid support is another way of cell immobilization that had been widely employed. In surface adsorption, yeast cells were absorbed onto the surface of wood chips, bricks, synthetic polymers, or other materials that have a large surface area (*7,9*). Immobilization of yeast cells in hollow fibers to increase cell density had also been studied (*10*). One of the simplest cell immobilization systems is to use self-immobilized heavy-yeast aggregates (agglomerates). Self-immobilization of yeast cells in a fermentor without a solid support can be achieved by using self-aggregating yeast mutants and similar systems (*11–19*).

Compared to conventional free-cell systems, immobilized cell systems have the advantage of being able to maintain a much higher cell density in the fermentor (about 40 g dry cell/L for cell immobilization systems to about 20 g dry cell/L for free-cell systems (*1–10*). Higher cell concentration can be obtained in a tower fermentor using self-aggregating cells (70–100 g dry cell/L for self-aggregating systems vs 30–50 g dry cell/L for traditional cell immobilization systems) (*11–13, 18,20*). In addition to higher cell concentration, self-aggregating yeast system are highly resistant to contaminations, active growing, and they have a long-term stability. In this article, the performances of a tower fermentor using self-aggregating *Saccharomyces uvarum* U4 to ferment corn syrup and molasses was studied.

MATERIALS AND METHODS

Organism

A strain of *Saccharomyces uvarum* (ATCC 26602) that formed large (2–3 mm) and stable aggregates was used for ethanol fermentation. The aggregated yeast was maintained on YMP agar slants containing yeast extract (0.3% w/v), malt extract (0.3% w/v), bacto-peptone (0.45% w/v), and glucose.

Materials

Black strap molasses was supplied by US Sugar Corporation and Savannah Foods and Industries. Corn dextrose syrup containing about 50% (w/v) glucose from corn starch hydrolysis and corn steep liquor were gifts from A. E. Staley Company.

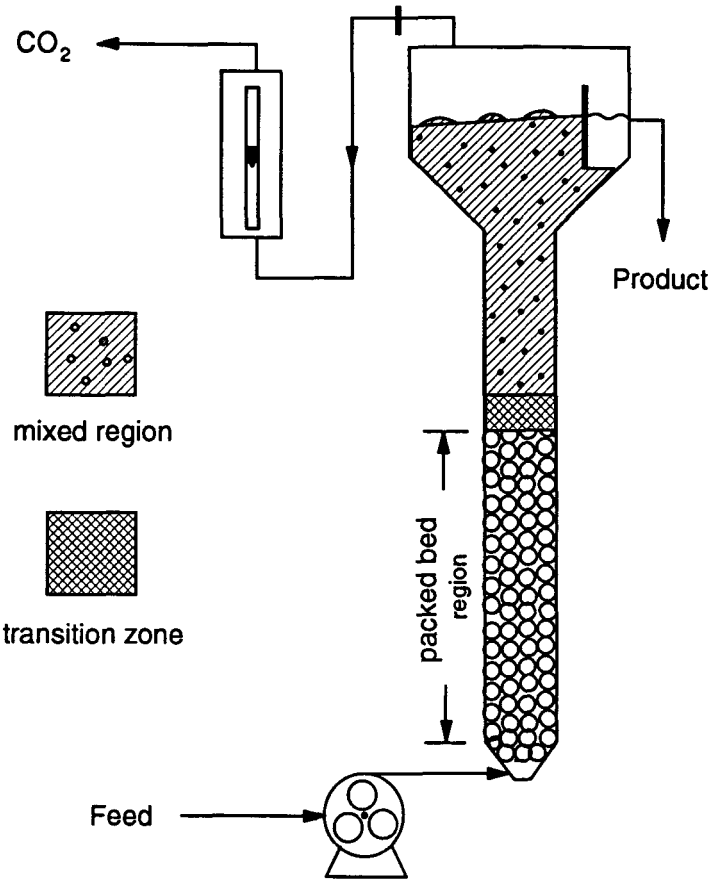

Fig. 1. Diagramatic configuration of tower fermentor using self-aggregating yeast.

Seed Culture

The aggregated yeast cells from an agar slant were inoculated into 200 mL of molasses growth medium containing 7% (w/v) of sugar and 2% (w/v) of corn steep liquor in a 500-mL flask, and incubated in a shaker at 30°C and 150 rpm. The inoculum grew to a cell mass of 3 g wet wt/flask (200 mL) after 3 d and was used as the seed culture for the tower fermentor.

Tower Fermentor

The fermentor was constructed by using clear polycarbonate tubing of 6.35 cm od, 5.715 cm id, and 115 cm in length as shown in Fig. 1. A cone-shaped cell separator that expands from 6.35 cm od to 20.32 cm od within 10 cm of height, using polycarbonate material, was attached to the top of the tower fermentor (Fig. 2). A baffle that separates rising carbon

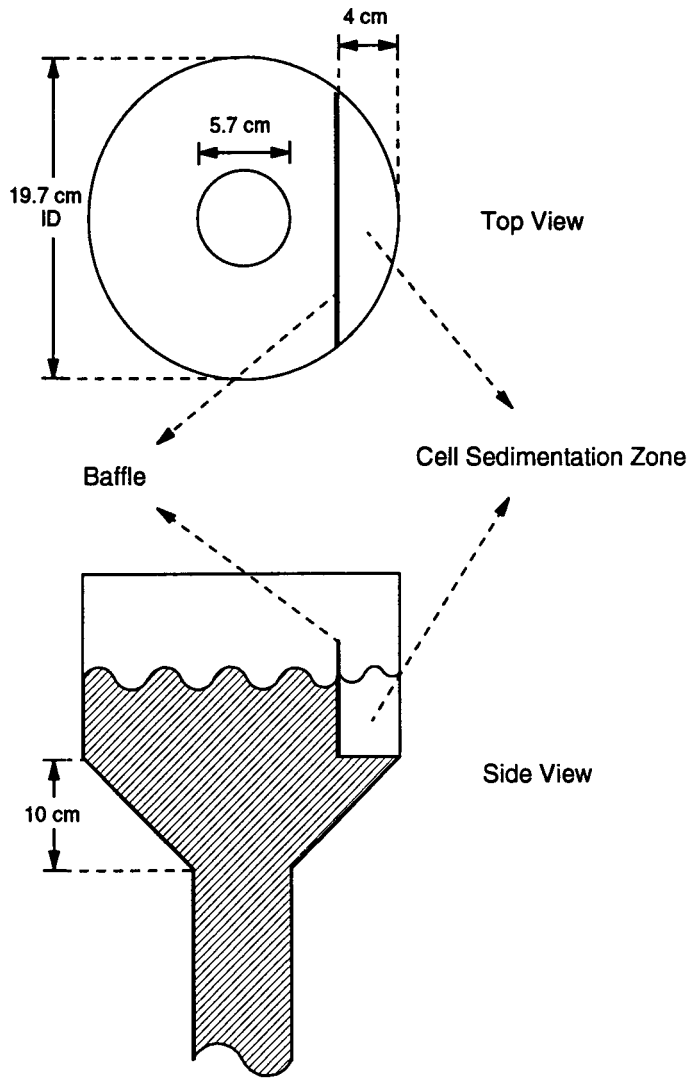

Fig. 2. Diagramatic configuration of the cell separator.

dioxide and exit stream was glued to the wall about 5.2 cm away from the center axis as shown in Fig. 2. The cell separator has a working volume of 2.7 L (3.3 L including the clear-cell sedimentation zone as shown in Fig. 2). Total working volume of the tower fermentor was 6.1 L (including the clear-cell sedimentation zone). Sampling ports were constructed from the base to the top of the tower fermentor at 5-cm intervals. Fermentation media were pumped into the lower fermentor from the bottom by a peristaltic pump and overflowed from the clear-cell sedimentation zone of the cell separator as shown in Fig. 1.

Before inoculation, the tower fermentor was sterilized with bleach solution (5% w/v) for 20 min, the fermentor was then rinsed with 4 L of sterilized water. Seed culture (containing about 3 g wet yeast cells) was pureed into the tower fermentor from the top, and fresh medium containing 7% (w/v) sugar and 2% (w/v) corn steep liquor was pumped into the tower fermentor. Medium flow rates were 100, 400, and 700 mL/h for the first, second, and third day, respectively. After 5 d, aggregated yeast cells (2–3 mm in diameter) accumulated and packed to form a stable packed-bed region as shown in Fig. 2. The height of the packed-bed region was about 50 cm.

The tower fermentor was operated at different flow rates, sugar concentration, and types of substrates. In order to assure steady-state operation, when flow rate was changed, 12 h of operation were allowed before taking data. When types of substrate were changed, 24 h of operation were allowed before taking data.

Analytical Methods

Samples taken from sampling ports were centrifuged immediately and frozen for later analysis. Sugars (glucose, fructose, and sucrose) were analyzed by HPLC (Waters Associates, with an Amino-RP column, and mobile phase was 75% (v/v) acetonitrile and 25% (v/v) water. Xylose was used as internal standard for HPLC analysis. Ethanol concentration was determined by gas chromatography (Varian aerograph series 1700). Acetone was used as internal standard.

RESULTS AND DISCUSSIONS

Cell Separator

Since the baffle in the cell separator was located about 3 cm away from the wall of the columnar body of the tower fermentor, yeast cells in the sedimentation zone were free from the disturbance of CO_2 bubbles. The cross-sectional area of the cell sedimentation zone was 45 cm^2. Linear velocity of the upward liquid in this region would only be 2.2 cm/min for a medium flow rate of 6 L/h (the highest medium flow rate expected by this series of experiment).

Large amounts of fresh and disintegrated cell aggregates were fluidized in the upper portion of the fermentor. The terminal velocity of these cell aggregates as measured in water using glass cylinder is about 70 cm/min, which is 30 times higher than liquid linear velocity in the sedimentation zone. Therefore, the cell separator attached to fermentor can retain the aggregate that is 0.1 mm or lager. Cell concentration in the effluent

was 10^3–10^4 cells/mL, and the fermentation broth in the cell sedimentation zone was clear (Figs. 1 and 2). A smaller cross-sectional area of the cell sedimentation zone had been tried, but the results were not satisfactory. The cell separator, which was designed based on the information of the terminal velocity of cell aggregates, successfully retained cell aggregates in the tower fermentor, and a packed bed of large (2–3 mm) cell aggregates was established in 5 d. The biomass in the packed-bed region was measured to be 95 g dry cell/L liquid, and that in the mixed region was about 65 g dry cell/L liquid.

Tower Fermentor

Figure 1 shows that the tower fermentor consists of two regions of different characteristics. Large-sized (2–3 mm) cell aggregates were able to settle down and formed a packed-bed region in the lower portion of the column. Above the packed-bed region was the mixed region, where small cell aggregates were fluidized by CO_2 and fluid flowing. In between was a short transition zone. The characteristics of these two regions are different, and the transition zone is short. A distinct boundary between these two regions was observed. The cell viability in the fermentor was measured to be 85% (±5%) viable.

Sugar and Ethanol Concentration Profiles

Sugar and ethanol concentration profiles for black strap molasses fermentation at different sugar and corn steep liquor concentrations and different medium flow rates are shown in Figs. 3, 4, and 5. Sugar and ethanol concentration profiles for corn syrup fermentation at different sugar concentrations and different medium flow rates are shown in Figs. 6–8. Dashed vertical lines in Figs. 3–8 indicates the observed positions of the boundary between the packed-bed region and the mixed region. The different characteristics of the two regions are shown in Figs. 4A–6D. In the packed-bed region, sugar and ethanol concentrations varied gradually, which is an important characteristic of a packed-bed region. Above the packed-bed region, sugar concentrations dropped drastically to a flat level of sugar concentration equal to the sugar concentration in the effluent, which is an important characteristic of a CSTR (mixed reactor). Such sharp variations of substrate and product concentrations happened in a short distance of 10 cm. This phenomenon explains the observed distinct boundary between the packed-bed region and mixed region.

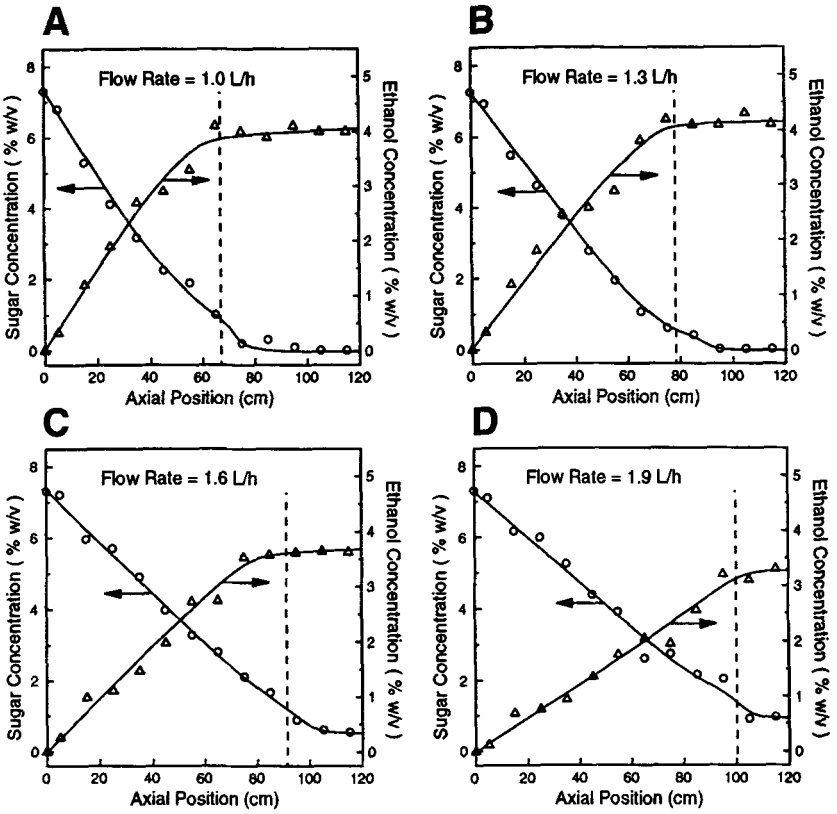

Fig. 3. Sugar and ethanol concentration profiles of black strap molasses fermentation at different flow rates; feed composition was 7.3% (w/v) sugar and 0.5% (w/v) corn steep liquor.

Ethanol Yield

Ethanol yield obtained from shaker-flask study fermentation was 96% of the theoretical value from corn syrup and 92% for black strap molasses. The ethanol yields for molasses fermentation in tower fermentor are higher under different fermentation conditions (*see* Figs. 3–5). Similar results were also obtained from corn syrup fermentation (Figs. 6–8). Table 1 lists the ethanol yield data at various operation conditions. The reason why ethanol yields were higher than theoretical value (0.51) was probably because of the CO_2 stripping effect and was partially contributed by sugars presented in corn steep liquor. Similar observation was also obtained by Peterson and Davidson (*6*).

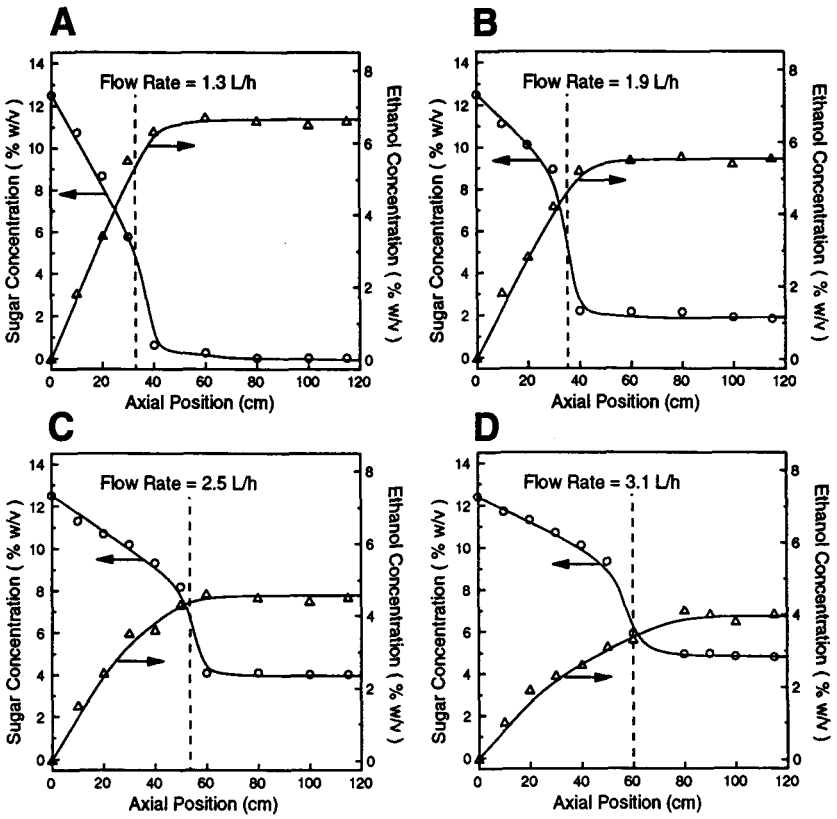

Fig. 4. Sugar and ethanol concentration profiles of black strap molasses fermentation at different flow rates; feed composition was 12.5% (w/v) sugar and 1.5% (w/v) corn steep liquor.

Ethanol Productivity

Ethanol productivity was calculated by multiplying ethanol concentration in the effluent by the flow rate and dividing by the working volume of the fermentor (5.5 L excluding the clear-cell sedimentation zone). The results are shown in Figs. 9A and B. An ethanol productivity of 22 g/L/h was obtained for molasses fermentation (feed composition was 12.5% w/v sugar and 1.5% w/v corn steep liquor) at a flow rate of 3.7 L/h (a dilution rate of 0.7/h). For corn syrup fermentation, an ethanol productivity of 54 g/L/h was obtained (feed composition was 15% w/v sugar and 1% corn steep liquor) at a flow rate of 3.7 L/h (a dilution rate of 0.7/h).

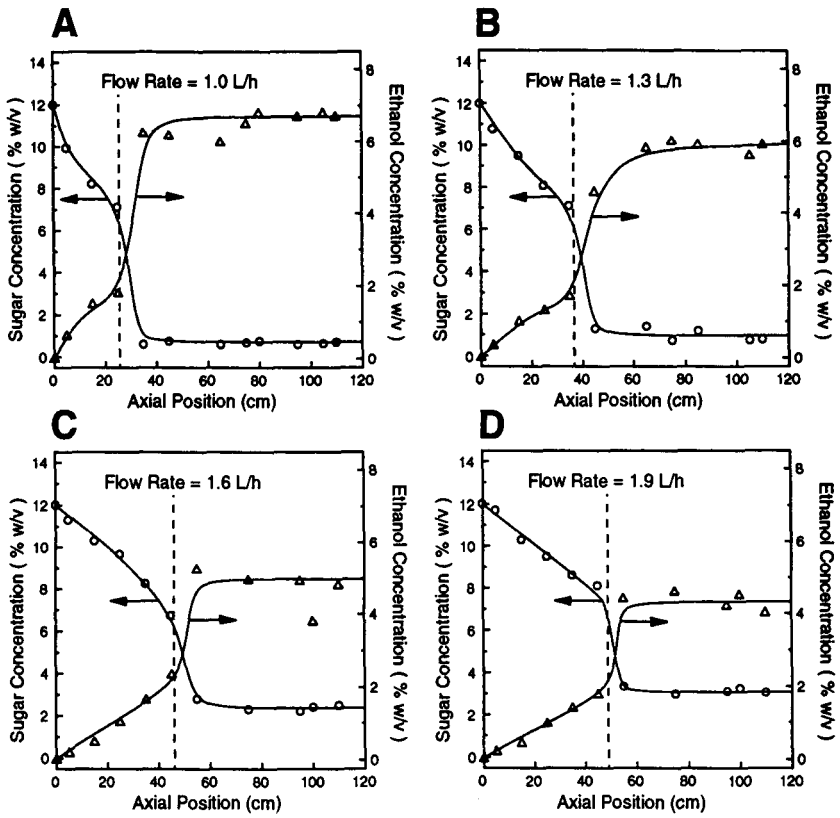

Fig. 5. Sugar and ethanol concentration profiles of black strap molasses fermentation at different flow rates; feed composition was 12% (w/v) sugar and 0.5% (w/v) corn steep liquor.

Effect of Corn Steep Liquor Concentration

Corn steep liquor has beneficial effect on molasses fermentation. The effects of two concentrations of corn steep liquor on molasses fermentation at different flow rates are shown in Figs. 4 and 5. Higher corn steep liquor concentration in the feed was found to increase ethanol productivity significantly, but it did not affect the height of the packed-bed region. The ethanol productivity was higher when 1.5% of corn steep liquor was present in the molasses as compared to the molasses with 0.5% of corn steep liquor (Fig. 9A).

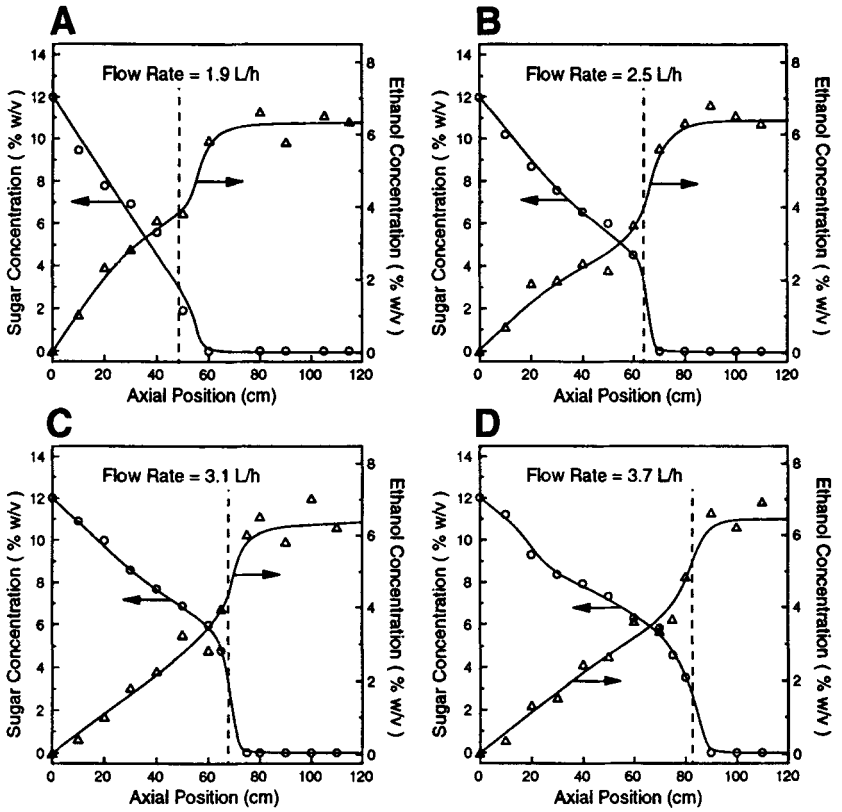

Fig. 6. Sugar and ethanol concentration profiles of corn syrup fermentation at different flow rates; feed composition was 12% (w/v) sugar and 0.5% (w/v) corn steep liquor.

Table 1
Ethanol Yield at Various Operation Conditions

Feed sugar conc., %w/v	Flow rate, L/h	Residual sugar conc., %w/v	Ethanol conc., %w/v	Yield[a], g/g EtOH/glucose
7.3	1.0	0.0	4.1	0.56
(Fig. 3)	1.3	0.0	4.2	0.58
	1.6	0.6	3.7	0.55
	1.9	0.9	3.3	0.52
12.5	1.3	0.0	6.7	0.54
(Fig. 4)	1.9	1.8	5.5	0.51
	2.5	4.0	4.6	0.54
	3.1	4.8	3.9	0.51
12.0	1.0	0.6	6.7	0.59
(Fig. 5)	1.3	1.0	5.9	0.54
	1.6	2.3	5.0	0.52
	1.9	3.0	4.3	0.48

(continued)

Table 1 (*continued*)

Feed sugar conc., %w/v	Flow rate, L/h	Residual sugar conc., %w/v	Ethanol conc., %w/v	Yield[a], g/g EtOH/glucose
12.0	1.9	0.0	6.3	0.52
(Fig. 6)	2.5	0.0	6.4	0.53
	3.1	0.0	6.3	0.52
	3.7	0.0	6.3	0.52
15.0	1.3	0.0	8.2	0.55
(Fig. 7)	2.5	0.0	8.3	0.55
	3.1	0.0	8.2	0.55
	3.7	1.1	8.0	0.53
18.0	1.3	0.0	12.1	0.67
(Fig. 8)	1.6	0.0	11.2	0.62
	1.9	1.5	9.1	0.55
	2.2	2.6	8.2	0.53

[a]Ethanol yield was higher than the theoretical value (0.51), probably because of the carbon dioxide stripping effect and was partially because of sugars presented in the corn steep liquor.

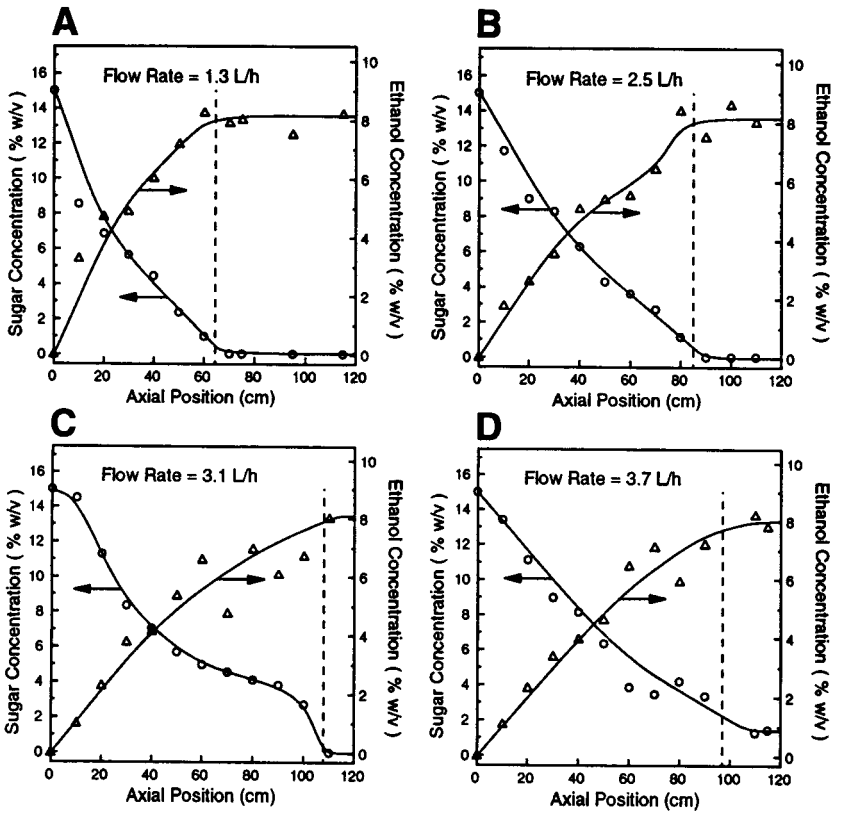

Fig. 7. Sugar and ethanol concentration profiles of corn syrup fermentation at different flow rates; feed composition was 15% (w/v) sugar and 1% (w/v) corn steep liquor.

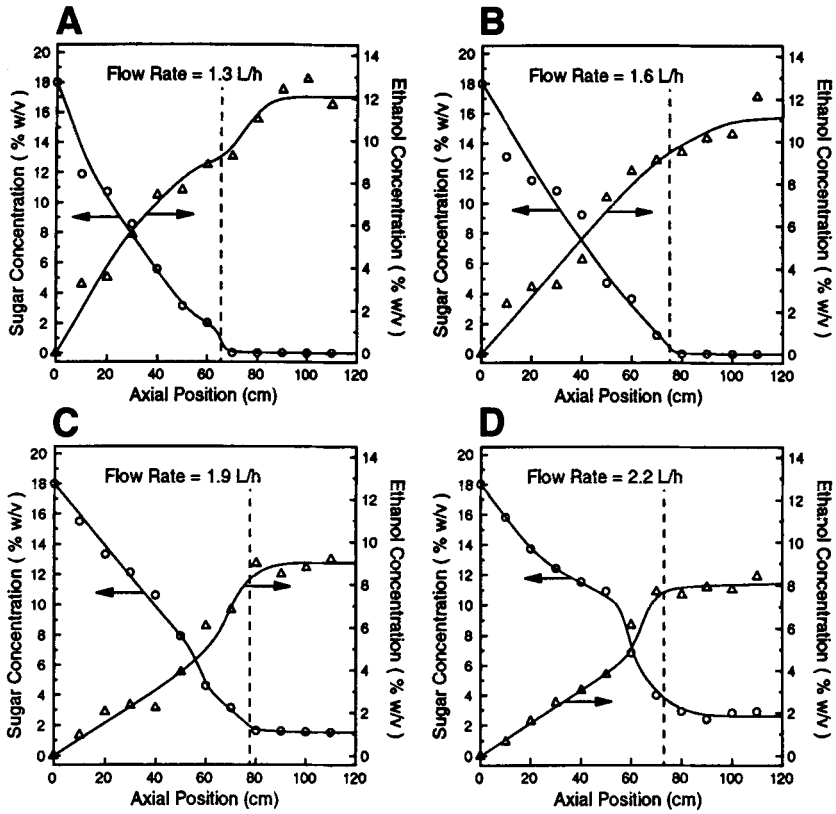

Fig. 8. Sugar and ethanol concentration profiles of corn syrup fermentation at different flow rates; feed composition was 18% (w/v) sugar and 1% (w/v) corn steep liquor.

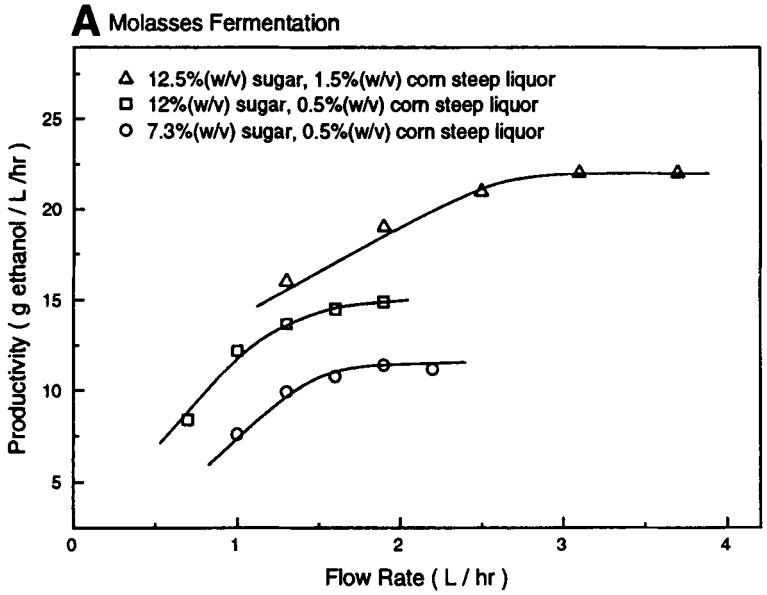

Fig. 9. Ethanol productivities at different flow rates.

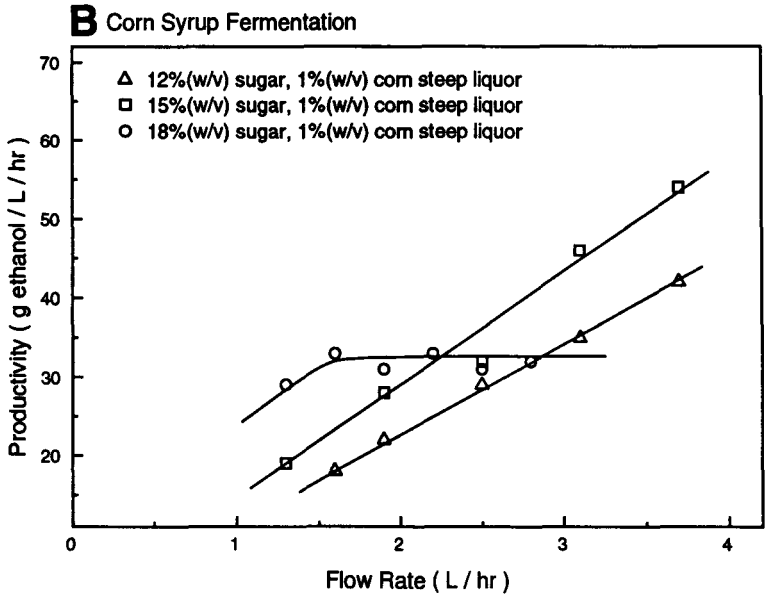

Fig. 9. (Continued)

Stability of the Bioreactor

The tower fermentor was operated continuously for a period of 3 mo. No significant loss of fermentation activity was observed.

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