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A Multichamber Tower Fermentor for Continuous Ethanol Fermentation with a Self-Aggregating Yeast Mutant

RODNEY CHRISTENSEN,*[,1] CHENG SHUNG GONG,[2] REN-TIAN TANG,[2] LI-FU CHEN,[2] AND NICHOLAS RIMEDIO[3]

[1]United States Sugar Corporation, PO Drawer 1207, Clewiston, FL 33440; [2]Department of Food Science, Purdue University, West Lafayette, IN 47907; and [3]Savannah Foods and Ind., PO Box 710, Savannah, GA 31402

ABSTRACT

A multichamber tower fermentor, with a combined working volume of 30 L, was used to ferment sugar cane molasses to ethanol using a self-aggregating yeast mutant, *Saccharomyces uvarum* U4, derived from *Saccharomyces uvarum* ATCC 26602. The column was constructed of plexiglass tubing divided into four chambers of different depths by conical shaped dividers. Gas generated during fermentation was allowed to escape from each chamber of the column through a small tube at the top of the chamber. The rate of escape was controlled by manually-operated diaphragm valves. This design significantly reduced the turbulence caused by CO_2 evolution and made it practical to maintain a total yeast bed depth double that previously sustained with this organism in long-term, single chamber tower fermentor tests. Using the multichamber tower fermentor, a 7–8% (w/v) ethanol-containing broth was continuously produced from molasses solution, with 160 g/L fermentable sugars, for more than 460 h of operation with a maximum dilution rate of 0.18 h^{-1}. Less than 10^3 cells/mL in the effluent was observed during most of the operation.

Index Entries: Multichamber tower fermentor; yeast aggregate; ethanol; *Saccharomyces uvarum* U4; continuous fermentation.

INTRODUCTION

Over the last decade, there has been continued interest in the use of biological systems for the production of ethanol from carbohydrate-con-

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

taining materials. Much of the recent research has focused on the use of continuous fermentation systems utilizing either immobilized cell systems (*1–4*), induced flocculation cell systems (*5,6*), or naturally flocculating cell systems (*7–10*) to increase cell densities and reduce residence time in the fermentor. A good review of additional work is given by Scott (*11*). In a typical immobilized or flocculating cell system, it is common to operate in a vertical fluidizied-bed tower fermentor similar to that first developed for the brewing industry (*12,13*). In this type of operation, carbon dioxide produced is allowed to escape upward through the cell bed and is released at the top of the column. Since CO_2 is released equimolarly with ethanol during the course of fermentation, the volume of carbon dioxide produced can be quite high. As an example, for each volume of an 18% sugar feed pumped to a tower fermentor, where the yield of ethanol is assumed to be 95%, approximately 46 vol of CO_2 (30°C, 1 atm.) is produced. The mechanical stress that accompanies the turbulence caused by carbon dioxide gas release is quite severe and can result in the fracture of immobilizing media or the break-up of cell aggregates. Channeling and backmixing of liquid medium, resulting from turbulent carbon dioxide flow through the cell system bed, also reduces the efficiency of fermentation by creating an uneven distribution of nutrients. These problems are magnified when an effort is made to scale-up most continuous fermentation systems. Therefore, it is important to design a bioreactor system that would allow produced gas to escape as rapidly as possible, while still allowing the retention of cells in the fermentor.

Several methods have been tested to overcome the problems caused by carbon dioxide generation during fermentation. One is to use multiple fermentors to accomplish a single fermentation. For example, Nagashima, et al. (*1*) used a system of three immobilized yeast columns in series to ferment cane juice. This helps diminish the mixing effect of evolving CO_2 gas, but has the disadvantage of greatly increasing the initial capital investment and complexity for a production facility. Another method tested is the horizontal packed-bed bioreactor used by Shiotani and Yamane (*3*). This system works well as a laboratory experiment, but may be difficult to scale up to a size that is useful in a production plant.

In this paper, we describe the use of a novel multichamber tower fermentor designed to overcome the problems caused by CO_2 evolution during the continuous production of ethanol using a self-aggregating mutant of *Saccharomyces uvarum*.

MATERIALS AND METHODS

Organism

The yeast strain employed is *Saccharomyces uvarum* U4, a mutant strain derived from *S. uvarum* ATCC 26602, using classical mutation-selection techniques. This mutant grows in a stable spherical aggregate form in liquid media. The characteristics of *S. uvarum* U4 have been described earlier (*7*).

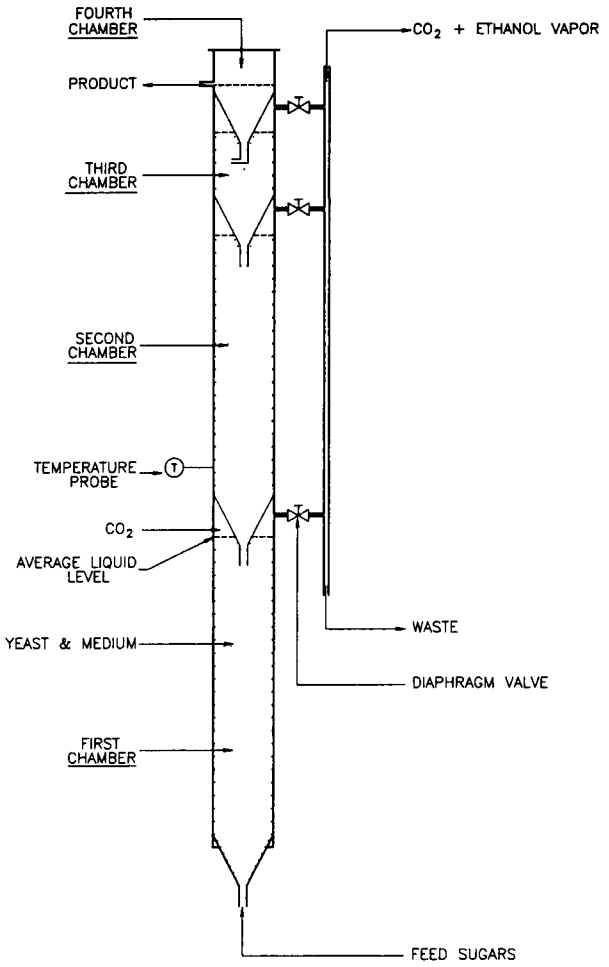


Fig. 1. Schematic configuration of the column fermentor employed in this study.

Medium

The feed medium was made from cane molasses (Everglades Sugar Reinery, Clewiston, FL) with the average composition: total solids 80%, invert sugars (glucose and fructose) 5.85% ds (dry solids), sucrose 77.11% ds, and ash 9.6% ds, pH 5.5–6.0. This was diluted to 18–20% solids with tap water and pasteurized at 100°C for 30 min in 17–20 L batches. To this was added: $ZnCl_2$, 0.04g/L; $MgCl_2 \cdot 6H_20$, 0.20 g/L; 85% H_3PO_4, 0.12g/L; Urea, 2 g/L; and penicillin-G (Sigma Chemical Co., St. Louis, MO), 0.002 g/L.

Fermentor

The fermentor was constructed of 15.2 cm diameter by 193 cm high plexiglass tubing separated into four chambers by polypropylene funnels (Fig. 1). The first two chambers, measuring 83.8 and 73.7 cm high, respectively, served as the main fermentation sites. The third chamber, measur-

ing 25.4 cm in height, was designed to provide an area for final fermentation and yeast settling. The funnels used had a conical section 12.8 cm deep and a downspout 5 cm long with a 1 cm opening at the bottom. The funnel separating the third and fourth chamber was also provided with a 5 cm perpendicular extension to the funnel downspout to act as a barrier to yeast movement into the top chamber, where product was removed from the column. The average liquid level in each of the first three chambers was maintained 5–15 cm below the top of the chamber by controlling CO_2 release through a 6.3 mm diameter tube located 2.5 cm below the top of the chamber with a manually-operated diaphragm valve. This gave an average column working volume of 30 L.

Startup

Sufficient inoculum for column operation was built up by first growing yeast for 24 h in six 250 mL shaker flasks containing a sterile mixture of 5 g/L YMP Broth (Difco, Detroit, MI), followed by 24 h growth in an air sparged (3 L/min), 14 L stirred tank fermentor containing a sterile medium consisting of 3 g/L yeast extract, 21 g/L fructose, and 29 g/L glucose. The yeast was decanted and transferred to a 75 L stirred tank fermentor for growth in a medium similar to that used in the 14 L fermentor. After 24 h, agitation was stopped, the yeast aggregates were allowed to settle, and supernatant was decanted off. The recovered yeast was then transferred to a 750 L stirred tank fermentor containing a medium consisting of cane molasses diluted to 10% solids and 3g/L yeast extract. This mixture was pasteurized at 100°C for 30 min by direct steam injection before yeast addition. Growth in this tank was facilitated by air sparging (35L/min) the medium. After 24 h of growth, yeast was recovered and stored at 8°C. No bacterial contamination was observed at any point during the growth process.

In preparation for inoculation, the entire tower fermentor was sterilized by soaking in hypochlorite (1%) solution for 24 h, followed by rinsing with sterile water. The column was inoculated by pouring 19 L of cold yeast mixed with a sufficient volume of feed medium into the top of the column. Column operation was then initiated by pumping feed to the column at the dilution rate of 0.11 h^{-1}. (For this paper all dilution rates reported are defined as flow rate/working volume.)

Operation

After starting at an initial dilution rate of 0.11 h^{-1}, the flow rate of feed to the column was slowly increased until a maximum dilution rate of 0.18 h^{-1} was reached at an elapsed time of 158 h and maintained for the remainder of the experiment. No provision was made to control either pH or temperature, and over the course of the experiment, the pH of the column effluent ranged between 5.7 and 6.4 and the column temperature averaged 30°C.

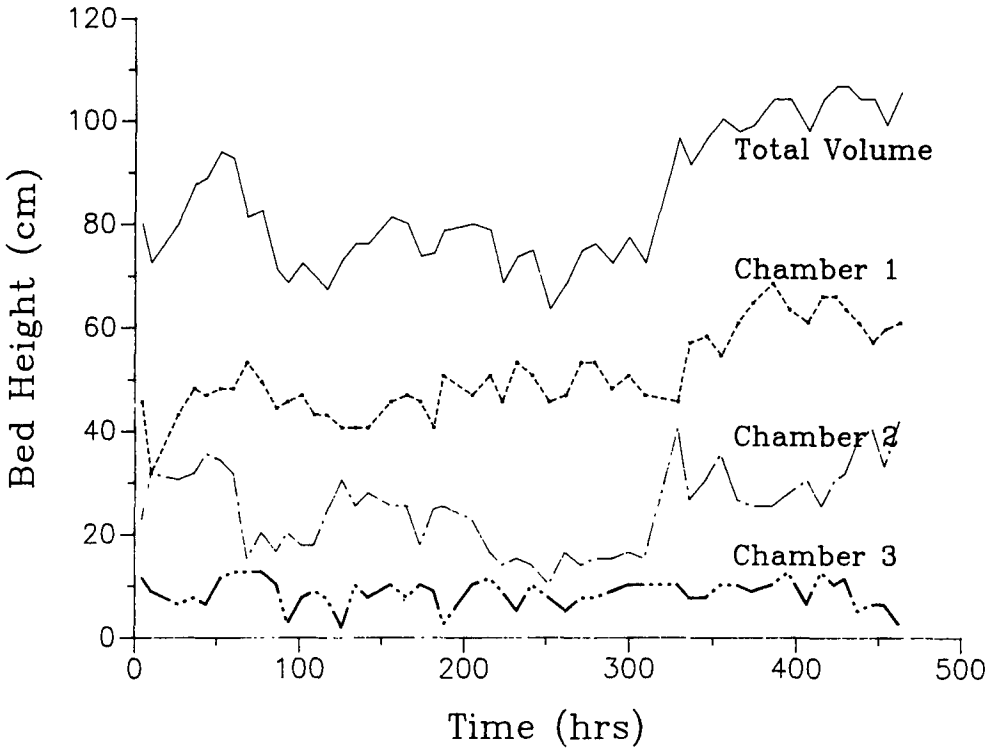


Fig. 2. Aggregate yeast packed-bed heights through 460 h of continuous operation.

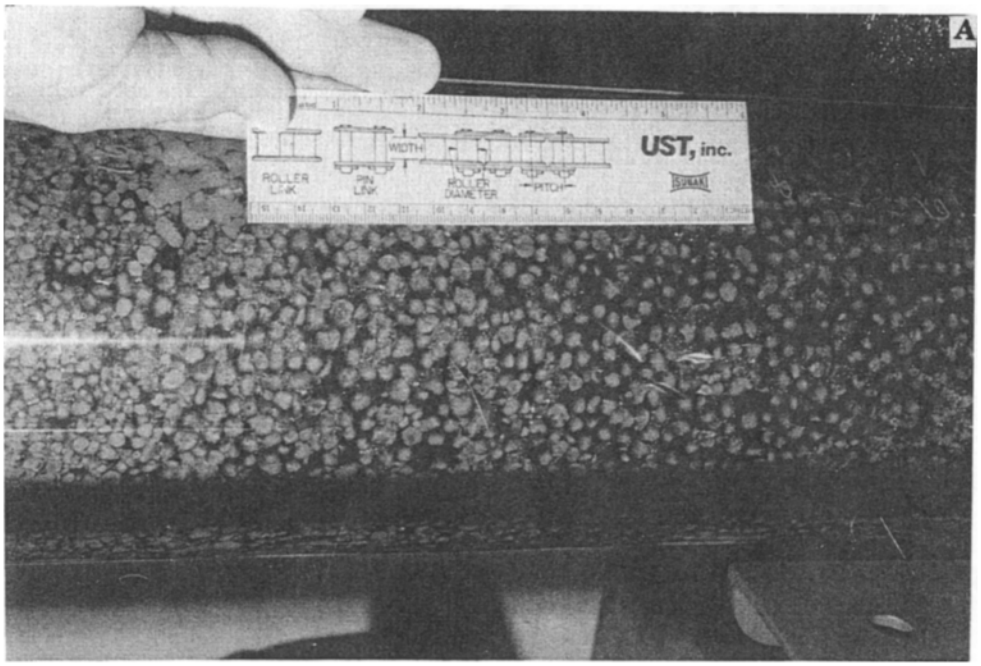
Analysis

Samples of the feed medium and column product were analyzed for sugars and ethanol by HPLC (Waters, Milgood, MA) using a Bio-Rad HPX87C column. Biomass levels (dry weight) were determined by centrifuging aliquots of fermentor contents, washing the cells with distilled water, centrifuging, and drying at 80°C for 48 h.

RESULTS AND DISCUSSION

Aggregate Yeast Bed

The choice of chamber size for the multichamber fermentor was made based on earlier single chamber fermentor work with *Saccharomyces uvarum* U4 that indicated that it was difficult to maintain a bed depth greater than 55 cm for extended periods of time. Following start-up of the fermentor, the yeast bed in each chamber had stabilized by 20 h elapsed time at an average cell density of 24 g/L dry wt. Figure 2 shows the yeast accumulation in each chamber through the course of the experiment. Between 20 h and 180 h, the yeast bed in the first chamber remained fairly steady at a level of between 40 and 50 cm; however, this was not true for the second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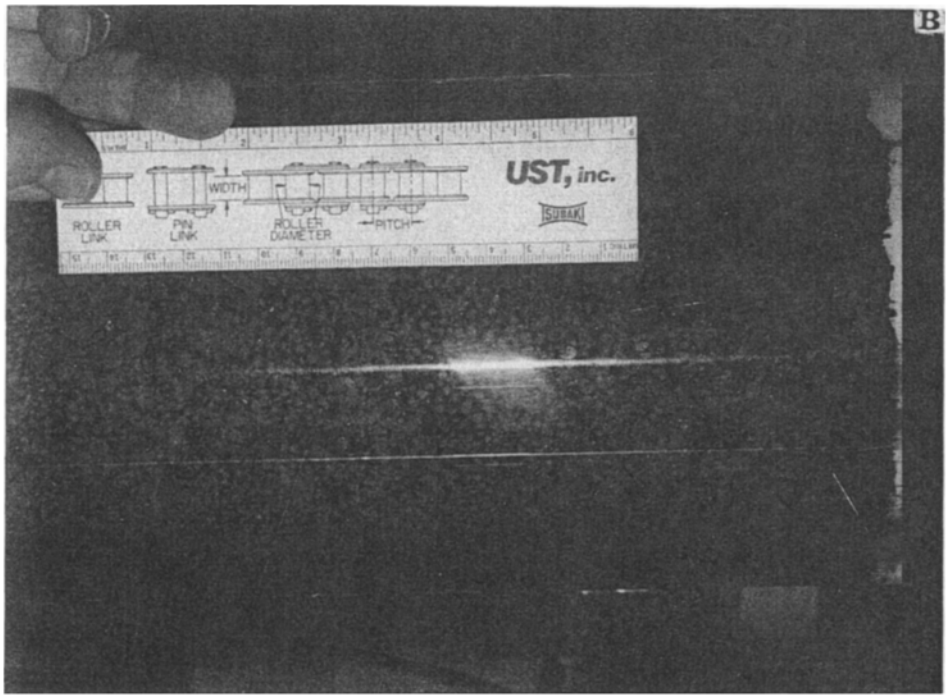
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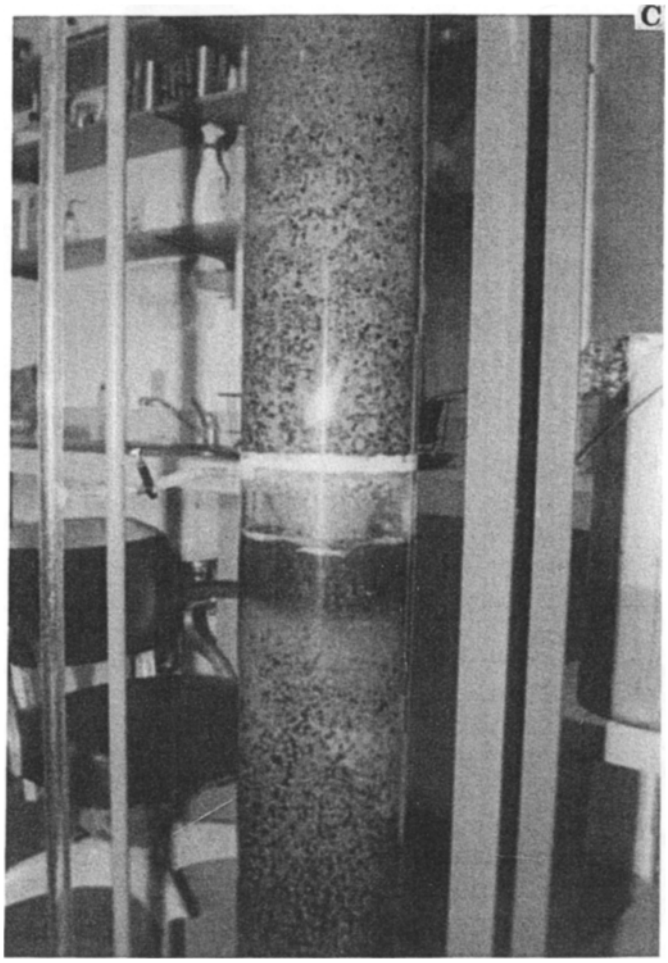

Fig. 3. (**A**) Aggregate size in first chamber; (**B**) aggregate size in second chamber; and (**C**) interface of first and second chamber showing the CO_2 bleed valve.

chamber, where the level varied dramatically during the same period over a range between 15 and 35 cm. In order to understand why this occurred, it is necessary to describe the physical characteristics of the yeast aggregates in each chamber. In the first chamber, the aggregates existed as well-defined spheres of between 1 and 7 mm in diameter, with an average diameter of 5 mm (Fig. 3A). In the second chamber, with an average diameter of 2 mm (Fig. 3B). In the nutritionally poor, high ethanol environment of the third chamber, the yeast did not exist as discrete aggregates, but as a large, loose, amorphous cake. This led to a steady accumulation of yeast in the third chamber, since the settling of yeast from the third chamber back to the second was inhibited by the "sticky" nature of the

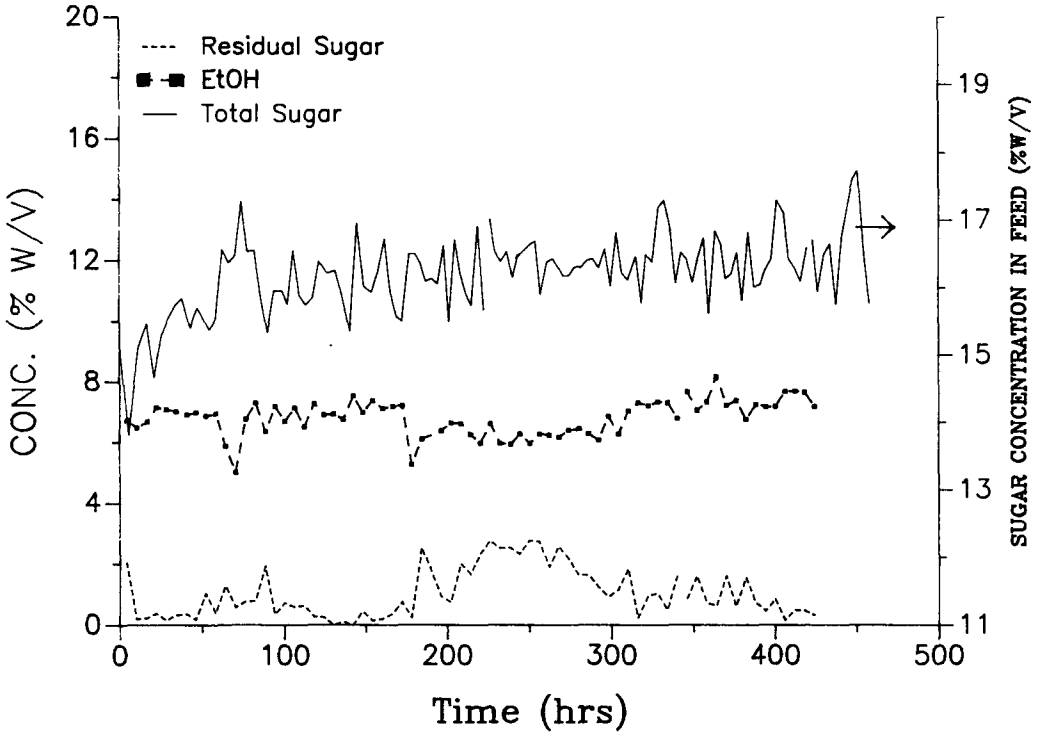

Fig. 4. Feed sugar and ethanol production through 460 h of continuous operation.

yeast mass in the third chamber. As the accumulation of yeast in the third chamber continued, the level would periodically reach a point where yeast was forced into the fourth (product outlet) chamber. When this occurred, a slug of yeast would wash out of the column. It should be possible to correct this problem by increasing the size of the downspout opening between the second and third chamber to the point where wall effects are minimized and gravity can overcome the binding force holding the yeast mass together in the third chamber.

Yeast growth in the fermentor was not great enough to replace the periodic loss of yeast, so at 314 h into the experiment, 5.5 L of yeast that had been recovered and stored at 8°C was added back to the column. Following this, the yeast levels in the first and second chambers steadied at between 54 and 68 cm, and 25 and 40 cm, respectively, for the final 125 h of the experiment.

Ethanol Production and Sugar Uptake

Figure 4 shows the feed sugar concentration and the residual sugar and ethanol concentrations of the column product during the course of this experiment. Previous experiments using a more ideal medium for fermentation, e.g., YMP-glucose, have shown that *S. uvarum* U4 is capable

of providing good ethanol productivity and high yields in a single chamber tower fermentor (*7*). In this experiment, cane molasses, a material with a high salt content and correspondingly high osmotic pressure, was used as a feedstock. This led to an increase in glycerol production (*14*) (0.6% in the product on average) and a drop in the rate of fermentation and attainable yield. Still, an average yield of 91% of theoretical on sugars fermented and 96.3% utilization of sugars was recorded for the final 125 h of the experiment at a dilution rate of 0.18 h^{-1} and an ethanol productivity of 14 g/L-h. This compares favorably to work done by Prince and Barford on sugar cane juice (*8*).

Fermentation proceeded most rapidly in the first chamber of the column, where nutrient levels were highest and ethanol concentration was still low. An average of 65–70% of sugar uptake occurred here. Twenty-five to thirty percent of sugar was utilized in the second chamber and less than 5% in the third.

CONCLUSIONS

The multichamber tower fermentor with continuous release of gas at top of each chamber offers a novel solution to the problems caused by CO_2 evolution in most other fluidized-bed continuous fermentation systems. The design is simple and should lend itself well to scale-up and use with either immobilized or other aggregate cell systems, thus making it an attractive alternative to batch fermentation. The only limitation to fermentor performance was the build-up of cell mass in the third chamber of the fermentor; however, this should be readily corrected with a minor change in the design of the cone distribution system for the yeast-settling chamber.

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REFERENCES

1. Nagashima, M., Azuma, M., and Noguchi, S. (1986), *Ann. NY Acad. Sci., Biochem. Eng.* 457–468.
2. Wade, M., Kato, J., and Chibata I. (1980), *Eur. J. Appl. Microbiol. Biotechnol.* **10,** 275–287.
3. Shiotani, T. and Yamane, T. (1981), *Appl. Microbiol. Biotechnol.* **13,** 96–101.
4. Davison, D. H. and Scott, C. D. (1988), *Appl. Microbiol. Biotechnol.* **18,** 19–34.
5. Chen, L. F. and Gong, C. (1986), *Appl. Microbiol. Biotechnol.* **25,** 208–212.

6. Prince, I. G. and Barford, J. P. (1982), *Biotechnol. Lett.* **4,** 621–626.
7. Gong, C. S. and Chen, L. F. (1984), *Biotechnol. Bioeng. Symp.* **14,** 257–268.
8. Prince, I. G. and Barford, J. P. (1982), *Biotechnol. Lett.* **4,** 469–474.
9. Comberbach, D. M. and Bu'lock, J. D. (1984), *Biotechnol. Lett.* **6,** 129–134.
10. Netto, C. B., Destruhaut, A., and Goma, G. (1985), *Biotechnol. Lett.* **7,** 355–360.
11. Scott, C. D. (1987), *Enzyme Microb. Technol.* **9,** 66–73.
12. Royston, M. G. (1966), *Process Biochem.* (July), 215.
13. Green Shields, R. N. and Smith, E. L. (1971), *Chem. Eng.* (London) **249,** 182.
14. Brumm, P. J. and Hebeda, R. E. (1988), *Biotechnol. Lett.* **10,** 677–682.