0273-2289/91/2829—0719$02.20

Prediction of Bed Height in a Self-Aggregating Yeast Ethanol Tower Fermenter

CHEESHAN S. CHEN,* ERRCHENG CHAN, CHENG-SHUNG GONG, AND LI-FU CHEN*

Laboratory of Advanced Bio-Processing Technology, Food Science Department, Purdue University, West Lafayette, IN 47907

ABSTRACT

A packed bed region and a mixed region were observed in an ethanol tower fermenter packed with flocs of self-aggregating yeast. Sizes of yeast flocs were 2–3 mm and 0.2–0.3 mm in diameter in the packed bed region and the mixed region, respectively. Three major factors were found to affect the height of the packed bed region significantly. They were dilution rate, nutrient limitation, and hydrodynamic limitation. An empirical method was proposed using these three factors to predict the height of the packed bed region in the fermenter.

Index Entries: Ethanol fermentation; yeast aggregates; *Saccharomyces uvarum;* tower fermenter; packed bed.

INTRODUCTION

In liquid substrate fermentation, self-aggregating yeast has advantages in many aspects over the yeast immobilized on a solid support. Self-aggregating yeast has a higher cell concentration (70–100 g dry cell/L) than the immobilized cell system (30–50 g dry cell/L) (*1,3,7,8,12*). Self-aggregating yeast systems provide no space for the contaminant cells to grow, unless the contaminant cells can adhere to the yeast cells. Unlike the immobilized cell system, the cell aggregates in the fermenter are self-maintained. No addition of cells is required.

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

Continuous ethanolic fermentation in a tower fermenter packed with flocculating yeast has been studied by many researchers in recent years (*1,3,8,9,12*). Admassu and Korus (*1*) studied reaction kinetics of ethanol formation and hydrodynamics in a tower fermenter, and a mathematical model was proposed to describe dispersion and kinetics in fermenting glucose.

Most of the tower fermenters consist of a mixed region and a near-plug flow region (*1*). In a plug flow reactor, only downstream yeast cells undergo product (ethanol) inhibition, whereas in a CSTR, all yeast cells are under the same product inhibition. Theoretically, a plug flow reactor is preferred to CSTR in terms of volumetric productivity (*10*); however, a large particle size of yeast aggregates is required to prevent fluidization. If the particle size is too large, the diffusion of the substrate may reduce the effectiveness of the fermentation ability. The effectiveness factor of the yeast aggregates with a diameter of 1 mm was reported as 0.87 (*5*). When the diffusion limitation is constant, it is desirable to maintain a packed bed region as high as possible in the tower fermenter. Unfortunately, there is a limit of bed height for the yeast cells to form a packed bed in the fermenter. One of the major limiting factors is the large amount of carbon dioxide generated from fermentation. This large amount of gas rises to the top at a high flow rate, which ruptures and/or fluidizes the cell aggregates. In order to maintain the reactor as a packed bed reactor, Christensen et al. (*6*) designed a vertical multichamber fermenter to release some of the accumulated carbon dioxide. For reactor design, whether it is a single-stage reactor or a multichamber reactor, the prediction of the bed height is essential.

One objective of this research was to study some parameters affecting the stability of cell aggregates under shear force. This stability affects the height of the packed bed region. Another objective was to propose a method for predicting the height of the packed bed region.

MATERIALS AND METHODS

A mutant of *Saccharomyces uvarum* (ATCC 26602) that formed good aggregates was used (*4*). Black strap molasses and high test molasses were gifts from the US Sugar Corp. and Savannah Sugar Foods and Industries, respectively. Corn steep liquor and corn dextrose syrup were gifts from the A. E. Staley Company. Yeast extract, malt extract, and bactopeptone were purchased from DIFCO.

Preparation of the Fermentation Medium

Corn steep liquor was first heated at 100°C for 2 h to remove excess sulfur dioxide. Then, it was mixed with water, corn syrup, or molasses to form a solution with the desired sugar concentration. The medium was

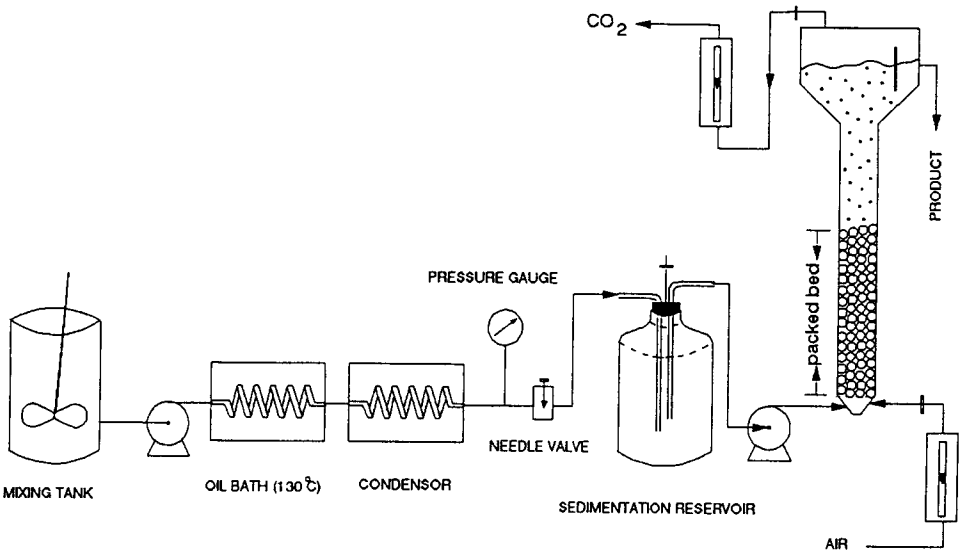

Fig. 1. Experimental setup of the tower fermenter constructed with polycarbonate tubing of 6.35-cm od, 5.715-cm id, and 115 cm in length.

heated at 125°C for 2 min, and then pumped into a fermenter shown in Fig. 1.

Apparatus for Continuous Fermentation

The tower fermenter was constructed with polycarbonate tubing of 6.35-cm od, 5.715-cm id, and 115 cm in length. A cell separator, which has a volume of 3.3 L, was attached to the top of the column. Total working volume of the column was 6.1 L. Sampling ports were 5 cm apart starting from the base to the top.

Inoculum

Yeast cells were grown in 200 mL of high test molasses growth medium containing 7% (w/v) of sugar and 2% (w/v) of corn steep liquor in a 500-mL flask. After 3 d of incubation, the flask contained about 3 g of wet yeast cells.

Column Start-Up

The tower fermenter was first sterilized with a bleach solution (5% w/v) for 20 min, and then rinsed with 4 L of sterilized water. The inoculum culture was poured into the column, and the fresh medium containing 7% (w/v) sugar and 2% (w/v) corn steep liquor was pumped into the bottom of the column. The flow rates were 100 mL/h, 400 mL/h, and 700 mL/h for the first, second, and third day, respectively. The height of the packed bed region reached a steady level (about 50 cm) in 5 d. Medium containing 12% (w/v) sugar was then pumped into the column. When substrates

were changed, 1 d was allowed before taking data to assure a steady-state operation.

Cell mass in the column was measured by draining a known volume of slurry through the sampling port. The slurry was then centrifuged and dried in an oven at 60°C.

Determination of Terminal Velocity of Yeast Flocs

Cell aggregates were obtained from the fermenter during operation. The diameter of aggregates was about 2–3 mm. The terminal velocity of these aggregates as a falling sphere was measured in a cylinder containing fermented broth according to Maxworthy (*11*).

Determination of Height of Packed Bed

Because of the formation of two distinct regions (packed bed and mixed region), the height of the packed bed region can be estimated visually as shown in Fig. 1.

Analytical Methods

Total sugar (glucose, fructose, and sucrose) and ethanol concentrations were analyzed by HPLC (Waters Associates, with an Amino-RP column) and gas chromatography (Varian aerograph series 1700), respectively. Gas flow rates were measured by Gilmont flow meter.

RESULTS AND DISCUSSION

In the start-up media, the cells grew into a fluffy mass in the first day. They were completely fluidized. After the cell concentration reached a critical point (40 g of dry cells/L), cells started to floc and formed spherical aggregates. The large aggregates settled to the bottom, and two distinct regions in the fermenter could be observed: a packed bed region and a CSTR region. At first, these cell aggregates were about 1–2 mm in diameter. Five days later, they grew to 3–5 mm. After the media were switched to fermentation substrates (containing more than 12% [w/v] of sugar), the aggregates in the packed bed region remained at 2–3 mm in diameter, and the cell density was 95 g/L. The yeast aggregates packed firmly inside the column and were not mobilized by the bypassing fluid. Admassu and Korus (*1*) described this region as the "near-plug flow" region. The aggregates in the upper level were fluidized. The cell aggregates were about 0.5 mm in diameter, and the cell density was 67 g dry cell/L.

Significant differences in floc size and in flow behavior made these two regions visually distinguishable. The height of the packed bed region can be easily measured. The distinct differences between the packed bed

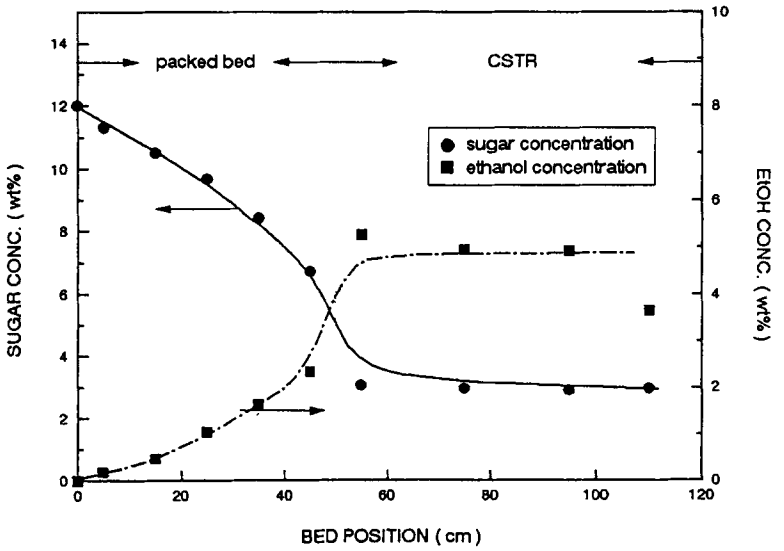

Fig. 2. Sugar and ethanol concentration profiles in the fermenter at a dilution rate of 0.46 h^{-1}. Feed: Black strap molasses, sugar concentration: 12% (w/v).

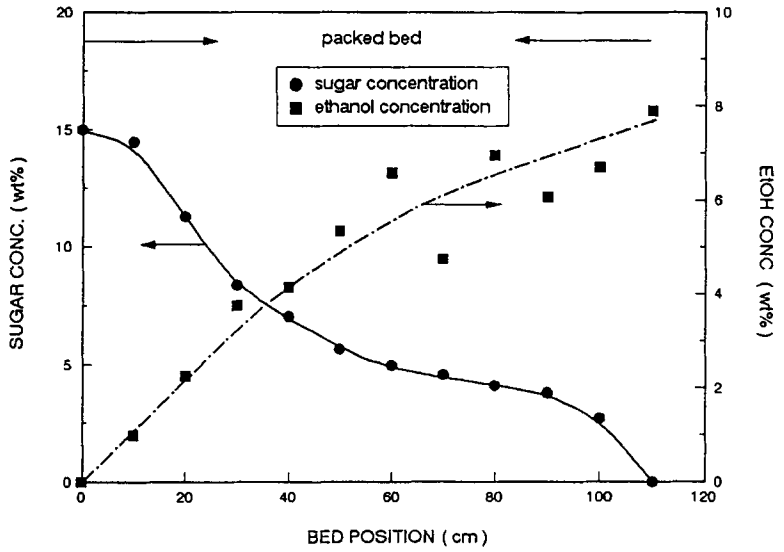

Fig. 3. Sugar and ethanol concentration profiles in the fermenter at a dilution rate of 0.46 h^{-1}. Feed: Corn dextrose syrup, sugar concentration: 15% (w/v).

region and the fluidized region also could be seen in the concentration profiles of the sugar and alcohol in the column. As shown in Figs. 2 and 3, in the packed bed region, the sugar concentration decreased gradually and the ethanol concentration also increased gradually, although in the fluidized region, both sugar and ethanol concentrations leveled off. The productivities of this fermenter to ferment corn syrup and black strap molasses at different dilution rates are shown in Fig. 4.

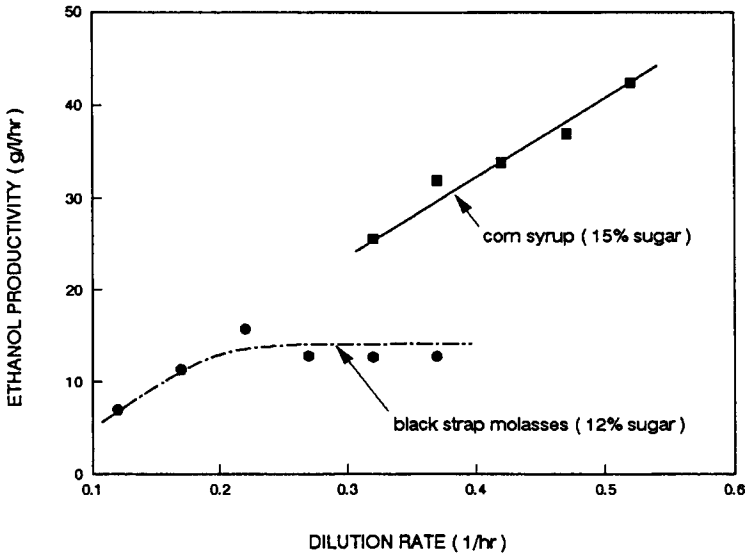

Fig. 4. Ethanol productivity as a function of dilution rate. Feed: corn syrup with a sugar concentration of 15% w/v; black strap molasses with a sugar concentration of 12% w/v.

Effect of Sugar Concentration on the Bed Height of Packed Bed Region

In the fermentation of sugar molasses, under a constant dilution rate (0.17 h^{-1}), the bed height of the packed bed region depended on the sugar concentration (Fig. 5). The yeast cells had the highest adhesiveness to maintain themselves as aggregates when the molasses substrate contained about 12% (w/v) of sugar. Further increase in the sugar concentration also increased the level of impurities, which interfered with the aggregation mechanism of yeast cells; therefore, the bed height decreased. Black strap molasses contained a large amount of colloidal material, which interfered with the aggregation ability of the yeast cells. These impurities can partly be removed after sterilization by settling overnight. When this decanted substrate was pumped into the fermenter, the bed height increased.

As shown in Fig. 6, when the substrates contained 12% (w/v) of sugar, the height of the packed bed region increased linearly with the increasing dilution rate for three different substrates. However, at the top of the packed bed region, the sugar concentrations were constant for each type of substrate despite the differences in dilution rates (Fig. 7). When air was pumped into the reactor bottom at a rate of 50 mL/min, the bed height decreased, and the sugar concentration on the top of the packed bed region increased. In another observation, big yeast flocs disintegrated into smaller ones of size 0.2–0.3 mm in diameter when they were suspended in the substrate collected from the top of the packed bed region. These

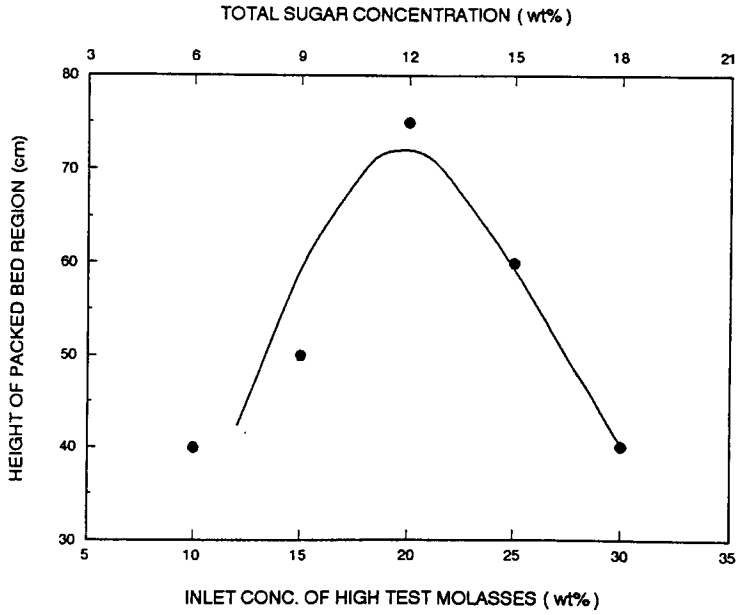

Fig. 5. Effect of molasses concentration on the bed height of packed bed region. Dilution rate was 0.17 h^{-1}.

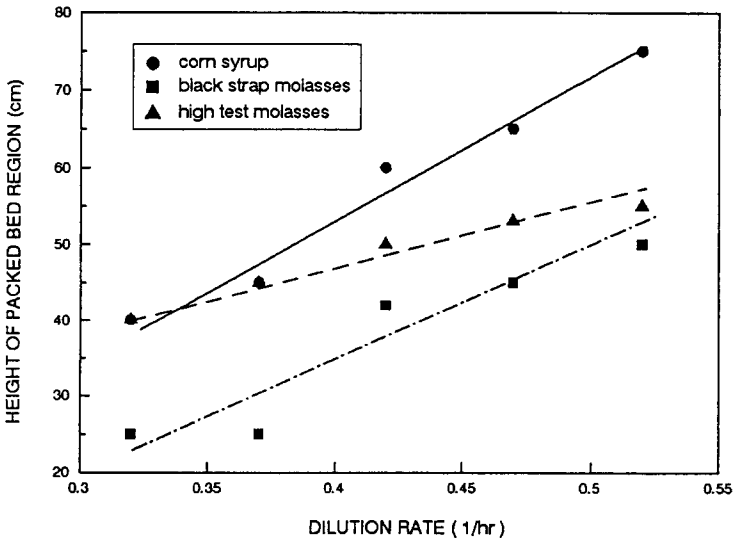

Fig. 6. Bed height of the packed bed region as a function of dilution rate. Feed: corn syrup, high test molasses, and black strap molasses. Sugar concentration was 12% (w/v).

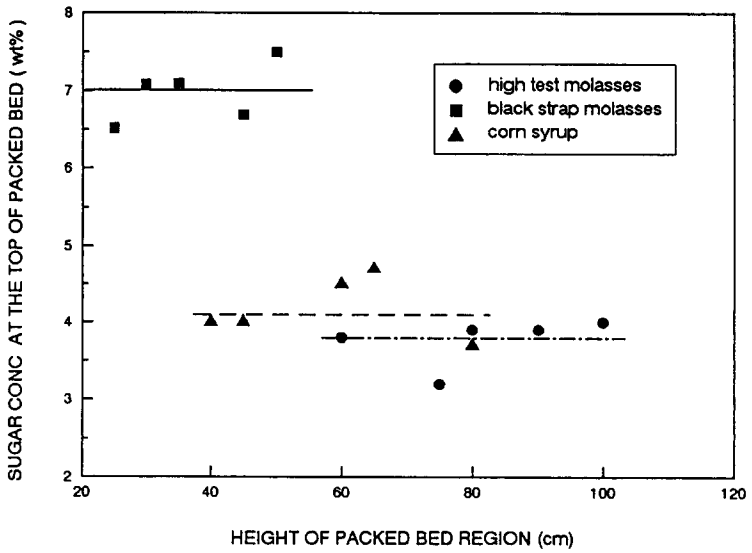

Fig. 7. Sugar concentration at the top of the packed bed region at various bed heights resulting from different dilution rates. Feed: corn syrup, high-test molasses, and black strap molasses. Sugar concentration was 12% (w/v).

data indicate that the stability of yeast aggregates is a function of the cell physiology corresponding to various sugar concentrations and the impurities in the substrate.

Bed Height Prediction for the Unstable Cell Aggregates Resulting from Inappropriate Level of Nutrition or Impurities

In a substrate containing low levels of nutrients and high levels of impurities, yeast aggregates are not stable. They can be disintegrated by little shear forces. The prediction of bed height of the packed bed region can only rely on the empirical data, which shows that in a fixed substrate the bed height is a function of dilution rate (Fig. 6). Because the sugar concentration at the bed top is independent of the dilution rate (Fig. 7), if a kinetic model in the fermenter is available, bed height can be predicted based on the sugar consumption.

Bed Height Prediction for the Yeast Aggregates in Clean Substrate

From the results of this study, it was found that, at a constant dilution rate, the height of the packed bed region was limited by two major factors: impurities in substrate and hydrodynamic limitation. When yeast cells were grown in corn syrup or pure sucrose medium containing yeast extract, malt extract, and bactopeptone or corn steep liquor, yeast cells formed aggregates of 2–5 mm in diameter. The aggregates thus formed

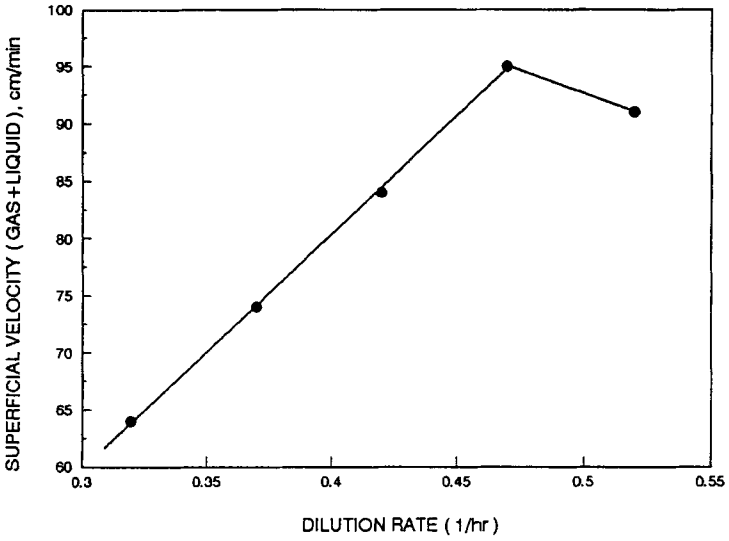

Fig. 8. Bed top superficial fluid velocity at various dilution rates. Feed: Corn dextrose syrup, sugar concentration: 15% (w/v).

could withstand a shear force at the terminal velocity. Therefore, the bed height limitation of the packed bed fermenter using corn syrup or pure sucrose as a substrate was controlled by hydrodynamic characteristics.

Hydrodynamic limitation comes from large amounts of carbon dioxide generated from fermentation. The maximal bed height in a packed bed occurs when the liquid velocity approaches the minimal fluidization velocity.

According to the calculation by Begovich and Watson's equation (2), yeast cell aggregates of 2.5 mm in diameter and a density of 1.26 g/cm^3 would begin to be fluidized at a total fluid (gas and liquid) superficial velocity of 97 cm/min. Figure 8 shows that, at a dilution rate of 0.48, the superficial total fluid (liquid and gas) velocity at the top of the packed bed is about 95 cm/min. At this point, an increase of gas flow by pumping 20 mL/min of air from the bottom decreased the height of the packed bed region.

In general, terminal velocity approximates minimal fluidization velocity, but is usually higher than the minimal fluidization velocity. Terminal velocity can be used to estimate the maximal height of a packed bed. The cell aggregates will be fluidized only when the intrinsic linear velocity approaches terminal velocity. For calculating the intrinsic linear velocity of the fluid, the cross-section area occupied by the solid fraction should be considered. Solid fraction near the bed top was estimated by collecting liquid (the volume of the liquid pumped into the column during the drainage was subtracted from the collected total liquid volume) and solid through a side opening with a diameter of 1.5 cm located at 15 cm below bed top. The volume of solid was measured after sedimentation of yeast cells. At a dilution rate of 0.48, solid volume fraction at the top 15 cm was 0.4.

In this system, the volumetric gas/liquid flow ratio was about 50 (48 mL/min for liquid and 2.4 L/min for gas), and gas existed in the fermenter as small bubbles. The liquid portion became a thin film surrounding the gas bubbles, and traveled along with the bubbles. This phenomena can be observed visually. Therefore, it can be assumed that the superficial liquid flow rate near the top of the packed bed is the summation of liquid and gas flow rates. The linear velocity of the total fluid at the bed top can be estimated by dividing the superficial fluid velcoity at the bed top by the average cross-sectional area of the fluid passage. This area equals the cross-sectional area of the fermenter times a factor E_f (E_f=1—solid volume fraction). Liquid and gas flow rates can be easily measured. It was calculated that the fluid linear velocity was 158 cm/min. This value is close to but lower than the measured terminal velocity (170 cm/min) of the yeast aggregates (2–3 mm in diameter) in the fermented molasses juice containing about 6% (w/v) of ethanol. When the dilution rate is lower than 0.48 h^{-1}, the fermenter maintains a packed bed (Fig. 8) until all sugar is consumed to a level below 0.3% (w/v).

With a proper mathematical model, it is possible to predict the sugar concentration profile as a function of the position of the packed bed and the dilution rate. By knowing the sugar concentration profile, sugar consumption rate can be estimated and the carbon dioxide generation rates at different heights can be calculated. Then the linear liquid velocity (equal to total linear fluid velocity) can be calculated from the flow rate of carbon dioxide at various positions of the packed bed. The height at which the liquid velocity approaches the terminal velocity is the maximal height of the packed bed region.

CONCLUSION

In designing a continuous ethanol fermenter using self-aggregating yeast, both the physiology of yeast cells and hydrodynamics in the fermenter should be considered. When the height of the packed bed region is dependent on nutrient and impurity levels, cell aggregates disintegrate before the liquid velocities reach terminal velocity or minimal fluidization velocity. The linear relationship between the bed height and dilution rate for different substrate shown in Fig. 4 will be useful information in the prediction of bed height. If the height of the packed bed region falls under control of hydrodynamic limitation, liquid velocity can be calculated assuming that the liquid velocity is equal to total fluid velocity.

ACKNOWLEDGMENT

This research has been supported by Savannah Food and Industries Inc. and US Sugar Corp.

REFERENCES

1. Admassu, W. and Korus, R. (1985), *The Chemical Engineering Journal* **31,** B1.
2. Begovich, J. M. and Watson, J. S. (1978), *Fluidization,* Davidson, J. F. and Keairns, D. L., eds., Cambridge Univ. Press, Cambridge, UK, pp. 190–195.
3. Bu'lock, J. D., Comberbach, D. M., and Ghommich, C. (1984), *The Chemical Engineering Journal* **29,** B9.
4. Chen. L. F. and Gong, C. S. (1985), *Biotechnol. Bioeng.* **14,** 257.
5. Chen, L. F. and Gong, C. S. (1986), *Appl. Microbiol. Biotechnol.* **25,** 208.
6. Christensen, R., Gon, C. S., Tang, R., Chen, L. F., and Rimedio, N. (1990), *Appl. Biochem. Biotechnol.* **24,** 25.
7. Hamamci, H. and Ryu, D. Y. (1988), *Appl. Microbiol. Biotechnol.* **28,** 515.
8. Jones, S. T., Korus, R. A., Admassu, W., and Heimsch, R. C. (1984), *Biotechnol. Bioeng.* **26,** 742.
9. Kida, K., Yamadaki, M., Asano, S., Nakata, T., and Sonoda, Y. J. (1989), *Fermentation and Bioengineering* **68,** 107.
10. Levenspiel, O. (1972), *Chemical Reaction Engineering,* 2nd Ed., John Wiley, NY, Ch. 6.
11. Maxworthy, T. (1965), *J. Fluid Mech.* **23,** 2, 369.
12. Prince, I. G. and Barford, J. P. (1982), *Biotechnol. Lett.* **4,** 7, 469.