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Design and Demonstration of an Immobilized-Cell Fluidized-Bed Reactor for the Efficient Production of Ethanol†

Scientific Note

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ABSTRACT

Initial studies have been carried out on the scale-up of a medium-scale (2–5 m tall with a 10.2-cm id), fluidized-bed reactor (FBR) designed for fuel ethanol fermentation using immobilized *Zymomonas mobilis*. These results suggest that further improvements in ethanol productivity along with good operability may be possible when compared with previous results at the bench scale (40–110 g ethanol/L/h) and present industrial reactors (2–10 g ethanol/L/h). On-line and off-line measurement and control systems are also described. *Z. mobilis* was immobilized in κ-carrageenan at cell loading of approx 60 g (dry wt)/L of biocatalyst. The system is designed for determining optimal operating conditions for achieving high conversion and productivity with variations in feedstocks, temperature, flow rate, and column sizes.

Index Entries: Ethanol fermentation; fluidized-bed reactor; immobilized cell; *Zymomonas mobilis*.

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INTRODUCTION

Domestic ethanol use and production are presently undergoing significant increases along with planning and construction of new production facilities. Increased demand is the result in part of ethanol's use as both a direct oxidant for petrochemical fuels and as a chemical feedstock for other oxidants. Significant efforts are ongoing to reduce ethanol production costs by investigating new inexpensive feedstocks (woody biomass) and by reducing capital, and energy costs through process improvements.

Ethanol is typically produced in batch and fed-batch (BFB) processes. BFB reactors are traditionally favored because high substrate conversion can be easily achieved along with control of bacterial contamination. Potential fermentation improvements include increases in reactor productivity to reduce capital costs and better choice of fermentation bacterium to improve rates, conversions, and final concentration.

Present research indicates that continuous fermentation systems have significant advantages over BFB fermentations, including increased productivity and control of microbial environments, while achieving required high substrate conversions. For example, a number of schemes have been developed to retain biomass for increasing productivity and substrate conversion. These strategies include cell recycle by filtration, sedimentation, entrapment by membranes, and immobilization in gel beads (*1,2*). These retention schemes can then be used with various reactor configurations, including continuous-stirred tank (CSTR), packed-bed (PBR), and fluidized-bed reactors (FBR). Typical BFBs have volumetric ethanol (EtOH) productivities between 2 and 5 g EtOH/L/h (*3,4*). On a total reactor volume basis, volumetric productivity for continuous systems are reported as approx 6–8 g EtOH/L/h for a free-cell CSTR, 10–16 g/L/h for an immobilized cell CSTR, 10–30 g/L/h for a hollow-fiber reactor, 16–40 g/L/h for a vertical PBR, and 50–120 g/L/h for an immobilized-cell FBR (*5*). Very high productivities approaching 190 g/L/h have been reported for FBRs using a flocculant *Z. mobilis* with cell recycle (*6*).

Z. mobilis has some advantages over *Saccharomyces cerevisiae*, which include:

1. A higher ethanol tolerance;
2. A dextrose conversion rate that is equal to or greater than that of *S. cerevisiae;* and
3. Behavior as an aerotolerant anaerobe, requiring neither strict anaerobe conditions nor a minimum oxygen level (*8,9*).

In this article, we report initial results of our efforts to scale up an immobilized-cell FBR for fuel ethanol production using immobilized *Z. mobilis*. The operability and control of the system are described and evaluated, along with preliminary productivity and yield results. Previous bench-scale studies demonstrated significant improvements in productivity, yield, and operability (*7*). These studies demonstrated:

1. Complete conversion of substrates;
2. More than a 10-fold productivity increase over a BFB;
3. Improved ethanol yield per mass dextrose; and
4. Improved operability and control.

Improved mass transfer and pH control, mitigation of gas-chanelling problems, and reduced product inhibition contributed to good operability and control at the bench scale. High volumetric flow rates and retained biomass concentrations, and, in some circumstances, pH and other factors, gave the desired bacteria a distinct ecological advantage and helped to control bacterial contamination (*7*). Conversions approaching 100% have been described previously (*7*). The present reactor (23,000 L/mo capacity) was scaled up from bench-scale FBRs having capacities of 400–2300 L/mo.

MATERIALS AND METHODS

Cultures of *Z. mobilis* NRRL-B-14023 were grown under nitrogen in media consisting of yeast extract (10 g/L) (Red Star Specialty Products, Milwaukee, WI), dextrose (20 g/L) (Staleydex 333, A. E. Staley Mfg. Co. Decatur, IL), and phosphate (2.0 g/L) at pH 6.0. The 300 L of broth for large-scale production of beads were grown in a 500-L fermentation system (New Brunswick Scientific, Edison, NJ) using a 1% inoculum.

The immobilization method is described in detail elsewhere (*10*). In summary, bacteria were removed from solution by centrifugation and then mixed with a solution of κ-carrageenan (4 wt%). Gel beads were formed by extrusion under an imposed pulsated flow into 0.3*M* KCl fixing solution. Iron oxide (3 wt%) was added to increase the specific gravity and improve bead retention in the column. Bead sizes were measured using a microscope. Volumetric bacterial concentrations were determined by dissolving the beads in distilled water and microscopically enumerating. Bead volume was measured by displacement.

Figure 1 depicts the major components of the FBR. Media components were stored as three separate solutions. Required quantities were supplied to the reactor using peristaltic pumps, whereas feed stream mixing was achieved using static mixers. The feed temperature was controlled using a 600-W heat tape and a model 76000 PID controller (Omega Engineering Inc., Stamford, CT). The feed constituents entered the reactor through a three-way valve at the bottom of the initial expansion section. The reactor main body consisted of 2-ft sections of 4-in.-id glass pipe connected by 1-in.-thick Lexan spacers. The total length of the reactor was 2.5 m with a 1.2-m disengagement section. The Lexan spacers were equipped with multiple ports for probes, sampling, and reagent addition. Internal heating and cooling of the FBR was accomplished by a U-shaped tube heat exchanger extending the length of the reactor constructed from 1/4-in. stainless-steel tubing. The heat-exchanger tube was connected to a model

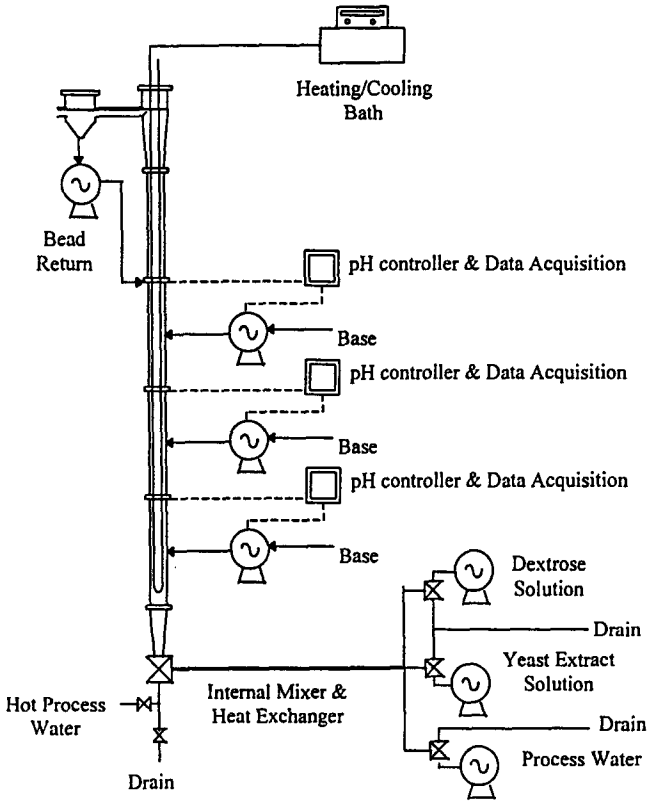

Fig. 1. Major components of the FBR. Desired feed concentrations are achieved by mixing individual substrates contained in separate reservoirs. Hot process water is available for rinsing feed lines. Systems are available for controlling pH and reactor temperature, along with returning small amounts of beads that flow over the weir at the top of the reactor.

1157 heating/cooling recirculating bath (VWR Scientific, Philadelphia, PA). Temperature and pH probes were installed at 0.6-m intervals along the length of the FBR in the Lexan spacers. Peristaltic pumps controlled by a model 3672 pH controller (Jenco Electronics, LTD., San Diego, CA) supplied NaOH to the reactor through addition ports in the Lexan spacer for controlling the pH between 4.9 and 5.1. The NaOH addition ports were located at axial positions of 0.45, 1.3, and 2.0 m. The disengagement section located at the top of the reactor was constructed using a 2-ft-long, 4- to 6-in. glass expansion. A weir installed at the entrance to the side arm retained beads in the reactor. A settling arm and peristaltic pump recovered stray beads that flowed over the weir. Liquid product exited the reactor by gravity drain. Gaseous coproduct exited the reactor through a port in the reactor cover. The volumetric flow rate of gas was measured using a flow sensor on the outlet. The reactor was also outfitted with catch pans and alarms to indicate leaks or overflow conditions. During the mechanical shake-down tests described here, only a partial bed was used (approx 0.8 m of settled bed).

The fluid dynamics within the reactor are a complex function of reaction rate, dextrose-feed concentration, solid loading, and gas–liquid–solid properties. The fluidization of the bed changes rapidly with axial position owing to significant changes in fluid flow rates and physical properties. For example, the entering liquid feed has a density of approx 1.07, whereas the effluent has a density of 0.98. Fluidization of the bed can be thought of as occurring in three zones that may be distinguished visually. The first zone, located at the bed entrance, acts as an expanded bed. The second zone, fluidized by gas product, starts a few centimeters above the entrance and encompasses most of the bed. The third zone, termed the disengagement section, is characterized by significant mixing produced by large quantities of gas and a relatively low biocatalyst population.

The reactor was not sterilized nor were the feed streams. Minimal procedures were used for mitigation of contaminant growth. These included:

1. Rinsing of feed lines twice daily with 60°C water (normal operation resumed with 10 min);
2. Refrigeration of yeast extract solution;
3. Replacement of feed containers with each charge of fresh feed; and
4. Medium stored as three separate solutions (Fig. 1).

Dextrose was stored as a 30–40% syrup at room temperature. Water was used without treatment. The yeast extract feed solution consisted of yeast extract (1.5%), KCl (0.3*M*), and Dow-Corning Antifoam B (0.01%), and was stored at 5°C. Final concentrations of the synthetic laboratory medium after mixing were 12–18 wt% dextrose, 0.5% yeast extract, and 0.1*M* KCl. The final concentration of dextrose could be adjusted by changing the ratios of the dextrose and process water feed streams.

A model was developed (*11*) describing the three-phase FBR for fermentation of dextrose to ethanol using immobilized *Z. mobilis*. This model is summarized to aid in understanding the reactor behavior. Diffusion was assumed to be the dominant transport mechanism inside the biocatalyst pellet. Axial reactor transport included convection and dispersion along with gaseous CO_2 effects. Dextrose was anaerobically converted to approximately equal molar quantities of ethanol and CO_2 (*12*). The fermentation kinetics of dextrose to ethanol by *Z. mobilis* have been extensively characterized (*13*) and are described using a modified Michealis-Menten-type mechanism with substrate and product inhibition. Biomass was also assumed not to be a function of radial position.

RESULTS AND DISCUSSION

Some of the conditions tested are detailed in Table 1. During the mechanical testing, the reactor was operated at less than half loading (approx 0.8 m of settled bed). Also, owing to the nature of the testing, the

Table 1
Experimental Conditions

Flow rate, L/h	Feed concentration, g/L	Temperature, °C	Internal heat exchanger set point, °C	pH
13.8	169	30–31°C	30	5.0–5.1
15.6	87	30–31°C	30	5.0–5.1
18.6	166	31–32°C	30.5	4.9–5.0
27.6	152	33–34°C	30.5	5.0–5.1

reactor operated at low flow rates for much of the trial (compared to scale-up calculations).

The beads were initially loaded with 15 g of *Z. mobilis* (dry wt)/L based on the biocatalyst volume. Cell concentrations in the beads at the conclusion of the experiments had increased to approx 80 ± 20 g (dry wt)/L beads. The bead diameter was approximately equal to previous bench experiments and was 1.6 ± 0.4 mm. The biocatalyst density was 1.04 g/mL in water. At the conclusion of the experiment, liquid phase biomass was high (1.25 g biomass [dry wt]/L relative to the bench experiments [< 0.3 g biomass/L for ref. 7]). There were no obvious signs of physical degradation of the biocatalyst.

The overall operability of the reactor system was good. For example, the reactor system operated successfully without operator intervention or attendance for hours. Reactor temperature and pH were consistently maintained between 30 and 34°C and between 4.9 and 5.2, respectively. Growth of *Z. mobilis* is observed at temperatures up to 36°C, whereas growth is rarely observed above 40°C (*12*). Most strains of *Z. mobilis* grow between the pH limits of 4 and 7.5 (*12*).

An improvement in hydrodynamics was observed with respect to previous experiments (*17*). During bench experiments, bubble fronts were observed that spanned the diameter of the reactor (*14*). Little or no such behavior was observed using the larger-diameter reactor. The character of gas and liquid flow through a small tube is a strong function of fluid physical properties and tube dimensions. Slug flow easily occurs in tube < 2 cm in id for water–air systems (*15*). The bench-scale experiments were performed in reactors with small internal diameters (maximum of 3.81-cm id) (*7*). Addition of a solid phase further reduces the effective tube diameter and may adversely affect hydrodynamics. In summary, the larger reactor diameter (10.2-cm id) may significantly improve hydrodynamics and reactor performance.

Dextrose concentrations as a function of axial position in the reactor bed are depicted in Fig. 2 for flow rates and feed concentrations indicated in Table 1. In all cases, very little conversion occurred after the first 0.75 m

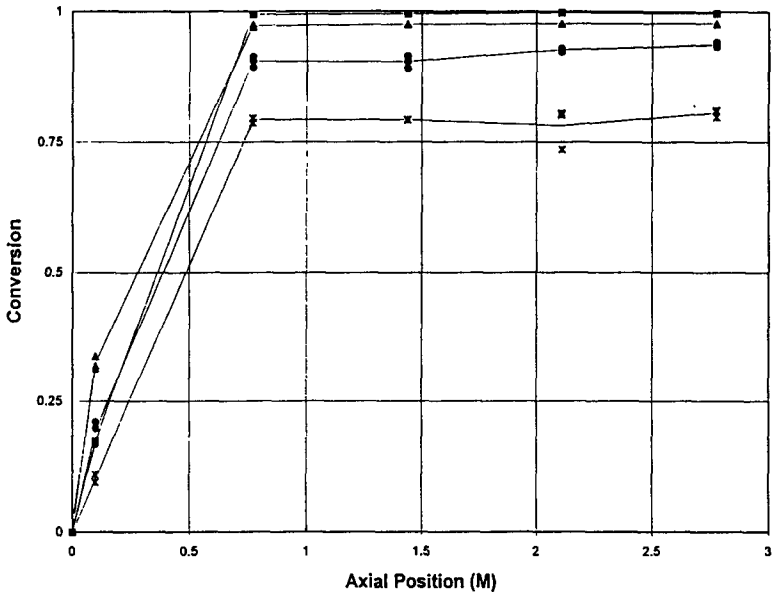

Fig. 2. Axial concentration of dextrose as a function of flow rate. Experimental flow rates were 13.8 L/h (▲), 15.6 L/h (■), 18.6 L/h (●), and 27.6 L/H (✕). Conditions are listed in Table 1. Lines are drawn through averages values.

of bed. The figure indicates that as flow increases, dextrose conversion is reduced because of lowered contact time between bacteria and substrate. Conversion ranged from 80 to 99.7%. As mentioned previously, the upper region of the bed had a noticeably low biocatalyst population and is further characterized by a very high degree of mixing relative to other parts of the bed. Thus, significant mixing in the upper bed along with a low biocatalyst population contributes to the low conversion occurring in the upper bed.

Productivity as a function of flow rate is depicted in Fig. 3 for the conditions in Table 1. The productivity calculations were based on the effective reactor volume (the first 1.2 m of bed). As mentioned previously, negligible conversion occurred above the second sample port in the remaining bed volume owing to nonoptimal bed loading. The productivity ranged from 59 to 170 g ethanol/L/h, which is equal to or greater than observed in previous experiments (40–110 g ethanol/L/h) (*7*). Two factors may contribute to this improvement, namely, slightly higher biomass concentration in the biocatalyst beads and the previously mentioned improved hydrodynamics.

Ethanol yields tended to increase with flow rate (Fig. 4), but were somewhat lower than previous bench results (*7*). Bench experiments demonstrated 97% of the theoretical 0.51 g ethanol/g dextrose. The lower yields may be the result of free-cell contaminant growth in the fluid phase as evidenced by significantly higher liquid-phase biomass levels. As

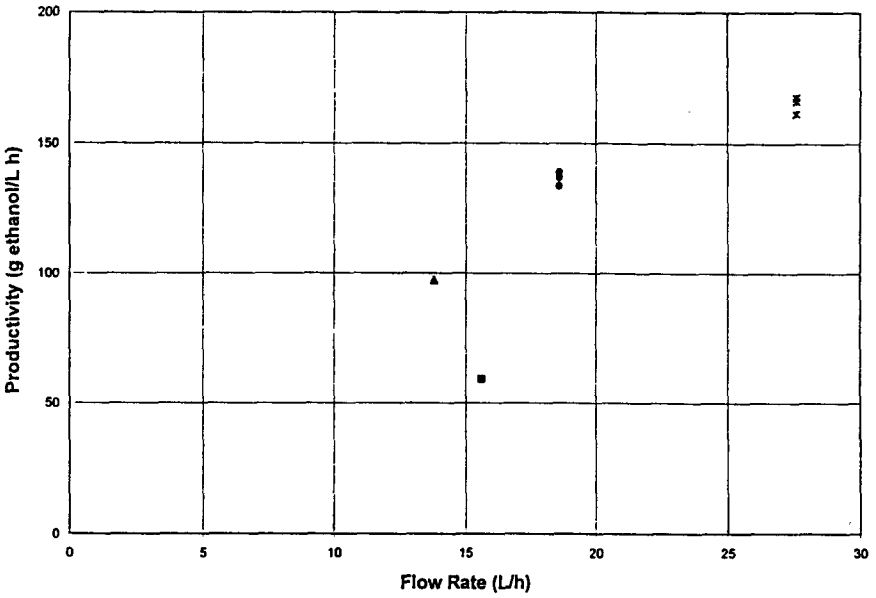

Fig. 3. Productivity as function of flow rate. Experimental flow rates were 13.8 L/h (▲), 15.6 L/h (■), 18.6 L/h (●), and 27.6 L/h (X). Conditions are listed in Table 1. The volumetric productivity increased with flow rate.

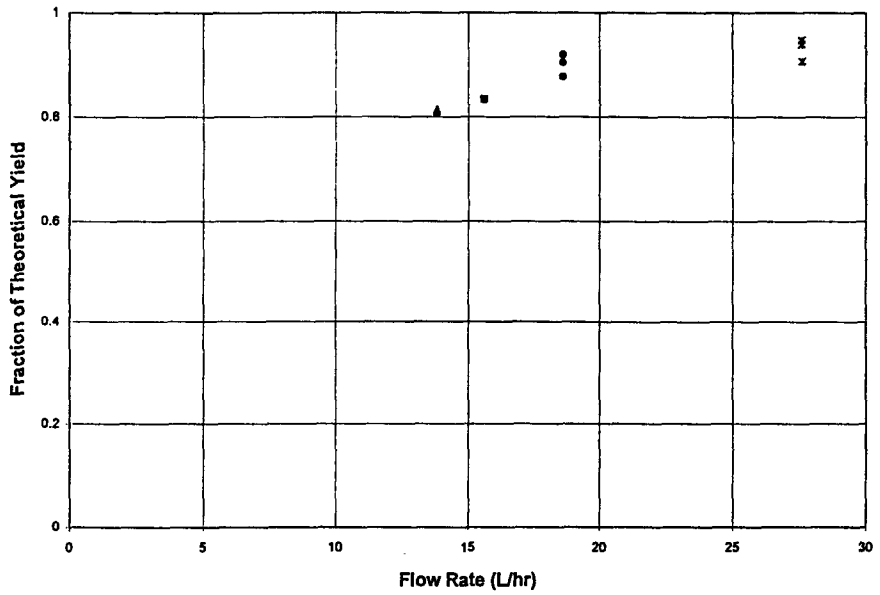

Fig. 4. Fraction of theoretical yield as a function of flow rate. Experimental flow rates were 13.8 L/h (▲), 15.6 L/h (■), 18.6 L/h (●), and 27.6 L/h (X). Conditions are listed in Table 1.

noted previously, the biomass in free solution was greater than four times that observed in bench-scale experiments. Possible factors that increased excessive cell growth in the liquid phase (Z. *mobilis* and bacterial contaminants) include:

1. Returned flocculated bacteria from the side arm via continual use of the bead return mechanism;
2. Unusually low flow rates during mechanical testing of the apparatus; and
3. Poor reactor inlet pH and temperature control.

The advantageous effects of lowered pH, low residence times, and large populations of immobilized Z. *mobilis* for mitigating contaminant growth have been previously noted (*7*). Also, the ability to wash out contaminants while maintaining the desired culture within the beads has been previously demonstrated (*7*).

CONCLUSIONS

Operation with a larger version of bench-scale reactors has been achieved (increase in capacity by an order of magnitude). Characteristics of the biocatalyst beads were similar to those of previous studies, whereas much higher quantities of free biomass were observed. Improved hydrodynamics were observed using the larger-id reactors. A quantitative evaluation of three-phase flow will clearly improve understanding and application during future activities with this reactor system.

This study demonstrated that the immobilized FBR continues to be a feasible alternative to conventional BFB fermentations. Advantages from fluidization and immobilized Z. *mobilis* include:

1. Good operability of the reactor system;
2. Control of bacterial contaminants;
3. Elimination of sterilization for feeds; and
4. Greatly improved productivities.

Future plans include a longer experiment for optimization and a more detailed investigation of operability, bed loading, kinetics, productivity, mass transfer, hydrodynamics, mitigation of free cell growth, and biocatalyst longevity. Column behavior will also be compared to predictive methods extended from Petersen and Davison (*11*). Strain improvements, including lowered substrate and product inhibition, along with higher ethanol tolerances will improve the attractiveness of this system.

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