1 of 1

DESIGN AND DEMONSTRATION OF AN IMMOBILIZED-CELL FLUIDIZED BED REACTOR FOR THE EFFICIENT PRODUCTION OF ETHANOL *

O. F. Webb**, Brian H. Davison, T. C. Scott, and C. D. Scott

Chemical Technology Division
Oak Ridge National Laboratory
PO Box 2008 MS-6226
Oak Ridge, TN 37831-6038

For presentation at the

16th Symposium on Biotechnology
for Fuels and Chemicals
Gatlinburg, TN
May 9-13, 1994

"The submitted manuscript has been authored by a contractor of the U.S. Government under contract No. DE-AC05-84OR21400. Accordingly, the U.S. Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or allow others to do so, for U.S. Government purposes."

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

Research supported by the Office of Transportation Technologies of the U. S. Department of Energy and administered by the National Renewable Energy Laboratory under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

** Author to whom all correspondence should be addressed.

MASTER

Design and Demonstration of an immobilized-cell fluidized-bed reactor for the efficient production of ethanol.

O. F. Webb, T. C. Scott, B. H. Davison, and C. D. Scott

ABSTRACT

Initial studies have been carried out using a 4 inch ID fluidized bed reactor (FBR). This medium scale FBR was designed for scale-up. Present performance was compared with results from experiments using smaller FBRs. On-line and off-line measurement systems are also described. *Zymomonas mobilis* was immobilized in κ -carrageenan at cell loadings of 15-50 g (dry weight) L^{-1} . The system is designed for determining optimal operation with high conversion and productivity for a variety of conditions including feedstocks, temperature, flow rate, and column sizes (from 2 to 5 meters tall). The demonstration used non-sterile feedstocks containing either industrial (light steep water) or synthetic nutrients and dextrose.

INTRODUCTION

Despite low fuel costs, ethanol continues to be an attractive alternative because of its oxygen content and because it can be produced by fermentation of renewable resources such as corn. Also, uncertainty over long term petrochemical costs and availability make ethanol attractive.

Batch and fed-batch processes (FBP) have been traditionally used for fermentation of ethanol, as well as other chemicals used for feed stocks, fuels, and other end products. Factors that traditionally favored batch and fed-batch fermentations include high substrate conversion, factors associated with separations, and mitigation of contamination problems.

Present research indicates that some continuous fermentation systems have some significant advantages over traditional batch operations while achieving required high conversions of substrates and controlling contamination problems. Potential advantages include significant productivity improvements and improved control of microbial environments. For example, a number of schemes have been developed for reducing washout to obtain and maintain high productivity. 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 reactor (CSTR), packed bed reactor (PBR), and fluidized bed reactors (FBR). For example, typical batch and FBPs have volumetric ethanol productivity's of between 2-5 g EtOH $L^{-1} h^{-1}$ (3,4). On a total reactor volume basis, volumetric productivity for continuous systems are reported as

approximately 6-8 g EtOH L⁻¹ h⁻¹ for a free cell CSTR, 10-16 g EtOH L⁻¹ h⁻¹ for an immobilized cell CSTR, 10-30 g EtOH L⁻¹ h⁻¹ for a hollow fiber reactor, 16-40 g EtOH L⁻¹ h⁻¹ for a vertical PBR, and 60-110 g EtOH L⁻¹ h⁻¹ for a immobilized cell FBR (5). Very high productivities approaching 190 g EtOH L⁻¹ h⁻¹ have been reported for FBRs using a flocculant *Zymomonas mobilis* with cell recycle (6). The high volumetric flow rates and retained biomass concentrations give cultured bacteria a distinct ecological advantage over unwanted microbes in these settings. Under some circumstances, pH and other factors can be easily controlled in continuous reactors for mitigating the growth of contaminants. Conversions approaching 100 percent have been described previously (7).

In this work, we report initial results of continuous fermentation of dextrose to ethanol using *Z. mobilis* immobilized in κ -carrageenan in a scaled-up FBR. The operability, control, and performance of the system are described and evaluated. The present reactor (23,000 liters per month capacity) was scaled-up from bench-scale FBRs having capacities of 400-2,300 liters per month. Previous bench-scale studies (7) demonstrated: (1) more than a 10 fold productivity increase to 50-120g (ethanol)h⁻¹ L⁻¹ over a FBP; (2) improved ethanol yield per mass dextrose; and (3) improved operability and control over PBR. The staged plug flow character of a FBR allows faster approach to reaction completion than well-mixed reactors. Potential advantages of a FBR over a PBR include improved mass transfer, pH control, and advantages related to inhibition by reactants and products. Also, formation of CO₂ coproduct makes the use of packed bed reactors less attractive due to channels formed by large amounts of gaseous coproduct. FBRs minimize mass transfer resistance and mitigate channeling and gas disengagement problems. *Z. mobilis* was used in this study because: (1) it has a high ethanol tolerance; (2) it's dextrose conversion rate is equal to or greater than *Saccharomyces cerevisiae* (8, 9); and (3) *Z. mobilis* is an aerotolerant anaerobe requiring neither strict anaerobe conditions nor a minimum oxygen level. Immobilization provides *Z. mobilis* with a distinct competitive advantage. Also, immobilization allows achievement and maintenance of very high productivities.

MATERIALS AND METHODS

Cultures of *Z. mobilis* NRRL-B-14023 were grown under nitrogen in media (YEG media) consisting of yeast extract (10g L⁻¹) (Red Star Specialty Products, Milwaukee, WI), dextrose (20g L⁻¹) (Staleydex 333, A. E. Staley Mfg. Co. Decatur, IL), and phosphate (2.0 g L⁻¹) at pH 6.0. The 300 L of broth for large scale production of beads was grown in a 500 L fermentation system (New Brunswick Scientific, Edison, NJ) using a 1 percent inocula. This strain of *Z. mobilis* was used for previous studies (7).

Z. mobilis was removed from the broth by centrifugation prior to immobilization in κ -carrageenan (4 % by weight). The immobilization method is described in detail elsewhere (10). In summary, bacteria are removed from solution by centrifugation and

then mixed with a solution of κ -carrageenan. Gel beads are formed by extrusion under an imposed vibration into 0.3M KCl fixing solution. Iron oxide (3% by weight) was added to increase the specific gravity. Bead sizes were measured using a dissecting microscope. Bacterial concentrations were determined by dissolving the beads in distilled water and microscopically measuring them in a counting chamber.

Figure 1 depicts the major components of the laboratory reactor. Media components were stored as three separate solutions. Required quantities are supplied to the reactor using peristaltic pumps, while feed stream mixing was achieved using internal mixers in the common feed line. The feed temperature was controlled using a 600 watt heat tape and a model 76000 PID controller (Omega Engineering Inc., Stamford, CT). The feed constituents then enter the reactor through a 3-way valve at the bottom of the initial expansion section. The reactor main body consists of 2 foot sections of 4 inch ID glass pipe connected by 1 inch thick Lexan spacers. The Lexan spacers are equipped with multiple ports for probes, sampling, and reagent addition. Internal heating and cooling of the reactor is accomplished by a u-shaped tube heat exchanger extending the length of the reactor constructed from 1/4" stainless steel tubing. The heat exchanger tube is connected to a model 1157 heating/cooling recirculating bath (VWR Scientific, Philadelphia, PA). Temperature and pH probes were installed along the length of the reactor 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 meters. The disengagement section located at the top of the reactor was constructed using a 2 foot long 4-6 inch, glass expansion pipe. 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 could be measured using a flow sensor on the outlet.

The synthetic laboratory media was stored as 3 separate solutions prior to use (Figure 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 (0.3M), and Dow-Corning Antifoam B (0.01%) and was stored at 5 °C in a walk in refrigerator. Final concentrations of the synthetic laboratory media after mixing was dextrose (between 12-18 percent by weight), yeast extract (0.5 %), and KCl (0.1M). The final concentration of dextrose could be adjusted by changing the ratios of the dextrose and process water feed streams. Feeds were used without sterilization. Feed lines and containers were periodically changed or rinsed with hot process water (60 °C).

Experiments were performed by varying flow rates and feed concentrations using a partially loaded reactor (approximately 1 meter of settled bed). The model describing a three-phase, FBR has been developed previously (11) for fermentation of dextrose to

ethanol using immobilized *Z. mobilis*. Dextrose is anaerobically converted to approximately equal molar quantities of ethanol and CO₂ (12). The fermentation kinetics of dextrose to ethanol by *Z. mobilis* are extensively characterized (13). In summary, a Michealis-Menton type mechanism with substrate and product inhibition is employed:

$$r_A = \frac{(q_p)_m}{Y_{\frac{p}{s}}} \frac{S}{K_s + S} \frac{K_i}{K_i + S} f_P x$$

where

$$f_P = \begin{cases} 0 & \text{for } P \geq P_m \\ 1 - \frac{P - P_i}{P_m - P_i} & \text{for } P_i \leq P \leq P_m \\ 1 & \text{for } P \leq P_i \end{cases}$$

In Peterson & Davison (11) diffusion was assumed the dominant transport mechanism inside the biocatalyst pellet. Biomass was also assumed not to be a function of radial position. Transport and reaction in the solid phase are representing by the boundary value problem:

$$\frac{d}{d\xi} \left(\xi^2 \frac{ds}{d\xi} \right) = - \left(\frac{R^2}{C_0 D_{glucose}} \right) \xi^2 r_A(s)$$

with boundary conditions

$$\begin{aligned} \frac{ds}{d\xi} &= 0 & \text{at} & \quad \xi = 0 \\ s &= s_{bulk} & \text{at} & \quad \xi = 1 \end{aligned}$$

Using a material balance on the liquid phase, Peterson & Davison (11) described the axial concentrations in the reactor using

$$\frac{d^2 s}{dz^2} = \frac{L}{\psi} \left(u_L - \frac{\alpha \beta e^{-\beta CO_2}}{L} \frac{dCO_2}{dz} \right) \frac{ds}{dz} + \frac{L^2}{\psi C_0} \eta \epsilon_s r_A$$

with boundary conditions

$$s - \frac{\psi}{L(u_L)} \frac{ds}{dz} - 1 = 0 \quad \text{at} \quad z = 0$$

$$\frac{ds}{dz} = 0 \quad \text{at} \quad z = 1$$

RESULTS AND DISCUSSION

These results will summarize findings made during an initial trial. The trial was performed with a partially loaded column (approximately 1 meter of settled bed). Volumetric flow rates were varied for the kinetic experiments which are detailed in Table 1. Temperature and pH were maintained at optimal conditions for *Z. mobilis*. Flow rates were varied between predicted bounds from scale-up calculations. Several important characteristics of the reactor system were evaluated during the initial trial including: (1) operability, (2) volumetric productivities, and (3) reaction kinetics.

The beads were made of 4% κ -carrageenan, 3% Fe_2O_3 , and loaded with 15 g of *Z. mobilis* (dry weight) L^{-1} . Bead volume was measured by displacement. Iron oxide was added to increase the bead density to improve retention of the beads in the column. Cell concentrations in the beads at the conclusion of the experiments had increased to approximately 80 ± 20 g (dry weight) L^{-1} beads. The bead diameter was on average 1.6 ± 0.4 mm. These results are similar to previous bench scale experiments (7). This set of experiments was performed with a partially loaded bed (approximately 1 meter of settled bed). Free floating biomass above the bed in the liquid phase was significantly higher (>1.25 g biomass (dry weight) L^{-1} with respect to the bench experiments ($<.3$ g biomass L^{-1} for reference 7).

The operability of the reactor system was good overall. The reactor system operated successfully without operator intervention or attendance for long periods of time. For example, reactor temperature was consistently maintained between 30 and 34 °C. Growth of *Z. mobilis* is observed at temperatures up to 36 °C while growth is rarely observed above 40 °C (12). Most strains of *Z. mobilis* grow between the pH limits of 4 and 7.5 (12). No problems were encountered with controlling the pH between 4.9 and 5.2. The reactor was also outfitted with catch pans and alarms to indicate leaks or overflow conditions.

Operational problems were insignificant using non-sterile feed for operation. Minimal procedures were used for mitigation of contaminant growth and included: (1) feed lines were rinsed twice a day using 140 °C water (normal operation resumed within 10 minutes), (2) refrigeration of yeast extract solution, and (3) replacement of feed containers with each charge of fresh feed.

An improvement in hydrodynamics was observed with respect to previous experiments (7). During the bench scale 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. Conversion of dextrose to ethanol is a complex function of biomass loading, contact between bacteria and substrate, and substrate concentration. The fluid dynamics and thus reaction rates are further influenced by the geometry of the reactor. The character of gas and liquid flow (dispersed or slug) through a tube may be significantly affected by the dimensions of the small conduit. The bench scale experiments were performed in reactors with small internal diameters (maximum of 3.81 cm ID) (7). Slug flow easily occurs in tubes approximately $<3/4$ inch in ID for water-air systems (15). Addition of a solid phase further reduces the effective tube diameter and may adversely effect hydrodynamics. In summary the larger reactor diameter (4 inch ID) may have a significant impact on the performance of the reactor (little or no bubbles spanning the diameter of the column).

Dextrose concentrations as a function of axial position in the reactor bed are depicted in Figure 2. In all cases the conversion was essentially complete by the first 0.75 meters of bed. The pathway used by the bacteria to ferment dextrose produces carbon dioxide in equimolar amounts to ethanol. Significant fluidization of the upper bed may occur from the gaseous coproduct with the effect of reducing the reaction rate in the upper section. Also, due to partial loading of the reactor, most of the biocatalyst beads are located in the lower bed. Little reaction occurred in the upper bed for all flow rates (Fig. 2).

Productivity as a function of flow rate is depicted in Figure 3. The productivities were calculated on the first 0.75 meters of bed. Negligible conversions were observed above the second sample port in the remaining bed volume. The productivity ranging from 150 to 320 g ethanol $L^{-1} hr^{-1}$ was equal to or several times greater when compared to previous experiments. The productivity for previous experiments ranged from 40 to 100 g ethanol $L^{-1} hr^{-1}$ (7). Two factors may contribute to this improvement, namely, slightly higher biomass concentration in the biocatalyst beads and the previously mentioned improved hydrodynamics. The effects of hydrodynamics, bed loading, mass transfer as a function of biomass loading and bead diameter, and fluidization are planned for future investigations and bed optimization.

Ethanol yields tended to increase with flow rate (Figure 4) but were somewhat lower than previous bench results (7). Triplicate measurements were made at each data point. Several explanations are possible for these observed tendencies: (1) the reactor was only partially full of biocatalyst, (2) excessive growth in the liquid phase reduced yield, and (3) the competitive advantage of immobilized *Z. mobilis* improved with flow rate due to contaminant wash out. As noted previously, the biomass in free solution was greater than four times that observed in bench scale experiments. Improvements that might be implemented to reduce excessive free biomass might include use of a fully loaded bed and control of the inlet feed pH to mitigate contaminant growth at the bed inlet. The advantageous effects of lowered pH for mitigating contaminant growth have been

previous noted (7). Immobilized *Z. mobilis* has an improved competitive advantage at high flow rates due to contaminant wash (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 previous studies while much higher quantities of free biomass were observed. The present studies were performed with a partially loaded bed.

The operability of the reactor system was good overall. For example, the integrated reactor system operated successfully for long periods of time without operator intervention or attendance. Also, individual and integrated systems maintained reactor conditions within desired limits. The reactor was equipped with safety systems for halting operation in the event of a leak or overflow situation.

The reactor operated well using non-sterile feed while only minimal procedures were used for mitigation of contaminant growth in feed containers and supply line. The advantages of the present operating conditions, lowered pH and temperature have previously been demonstrated. Thus the feeds need not be sterile but only clean.

Improved hydrodynamics were observed using the larger ID reactors. Hydrodynamic problems such as slug flow during gas liquid flow in small pipes and tubes is clearly demonstrated in the literature. A quantitative evaluation of 3 phase flow will clearly improve understanding and application during future activities with this reactor system.

Productivity was significantly higher in the present series of experiments than previously demonstrated. This improvement is probably due to a slightly higher biomass concentration in the biocatalyst beads and improved hydrodynamics. The effects of hydrodynamics, bed loading, mass transfer as a function of biomass loading and bead diameter, and fluidization are planned for future investigations and optimization.

Ethanol yields were somewhat lower in this work than in previous experiments. The lower yields may be the result of free cell growth as evidenced by the significantly higher biomasses in the fluid phase. Methods to further mitigate contaminant growth such as using a fully loaded bed, higher flow rates, and inlet pH control are scheduled for evaluation during the upcoming 1600 hr experiment. The ability to wash out contaminants while maintaining the desired culture within the beads has been previously demonstrated.

Future plans include a longer experiment for optimization and a more detailed investigation of operability, kinetics, productivity, mass transfer, hydrodynamics, and biocatalyst longevity. Column behavior will also be compared to predictive models

extended from Peterson & Davison (11). Strain improvements including lowered substrate and product inhibition along with higher ethanol tolerances will improve the attractiveness of this system.

This study demonstrated that the immobilized fluidized-bed reactor continues to be a feasible alternative to conventional batch and fed-batch fermentations. Strain improvements including lowered substrate and product inhibition along with higher ethanol tolerances will improve the attractiveness of this system. Advantages from fluidization and immobilized *Z. mobilis* are demonstrated.

REFERENCES

1. Crueger, W. and A. Crueger, Biotechnology: A Textbook of Industrial Microbiology, Science Tech, Inc. Madison, WI, 1982.
2. Inloes, D. S., Michaels, A. S., Robertson, C. R., and Matin, A. (1985), *Appl. Microbiol. Biotech.* **23**, 85-91.
3. Silman, R. W. (1984), *Biotech. Bioeng.* **26**, 247-51.
4. Bajpai, P. K. and Margaritis, A. (1985), *Enzyme Microb. Technol.*, **7**, 462-64.
5. Godia, F., Casa, C., and Sola, C. (1987), *Process Biochem.* **22**(2), 43-48.
6. Scott, C. D. (1983), *Biotech. Bioeng. Symp.* **13**, 287-298.
7. Davison, B. H. and Scott, C. D. (1988), *Appl. Biochem. Biotech.*, **18**, 19-34.
8. Rodger, P. L., Lee, K. J., and Tribe, D. E. (1980), *Process Biochem.* **15**(6), 7-11.
9. Wordem, R. M. (1982), "A Kinetic Study of Ethanol Production by *Zymomonas mobilis*," MS Thesis, Univ. of Tennessee, 1982. Also available as ORNL/TM-8722, Martin Marietta Energy Systems, Oak Ridge National Laboratory, Oak Ridge, TN.
10. Scott, C. D. (1987), *Ann. NY Acad. Sci.* **501**, 487-493.
11. Petersen, J. N. and Davison, B. H. (1991), *Appl. Biochem. and Biotech.* **28**, 685-698.
12. Swings, J. and De Ley, J. (1977), *Bacterial Reviews*, **41**, 1-46.
13. Lee, K. J. and Rogers, P. L. (1983), *Chem. Eng. J.* **27**, B31-B38.
14. Davison, B. H. and Donaldson, T. L. in Biotechnology Processes, Ho, C. S. and Oldshue, J. Y. eds., AIChE, New York, 1987.
15. Cliff, R., Grace, J. R., and Weber, M. E., Bubbles, Drops, and Particles, Academic Press, New York, 1978.

SYMBOLS

- C_0 _ Feed substrate concentration g L^{-1}
 CO_2 _ Carbon Dioxide flow rate L s^{-1}
 D_{dextrose} _ Effective diffusivity of dextrose $\text{dm}^2 \text{s}^{-1}$
 K_i _ Substrate inhibition constant for ethanol production g L^{-1}
 K_s _ Substrate limitation constant for ethanol production g L^{-1}
 L _ Total bed length dm
 P _ Ethanol concentration g L^{-1}
 P_i _ Threshold ethanol concentration for ethanol production g L^{-1}
 P_m _ Maximum ethanol concentration for ethanol production g L^{-1}
 $(q_p)_m$ _ Maximum specific ethanol production rate g L^{-1}
 R _ Radius of bead dm
 r_A _ Rate of dextrose conversion $\text{mol s}^{-1} \text{L}^{-1}$
 S _ Glucose concentration g L^{-1}
 u_L _ Bulk liquid velocity dm s^{-1}
 x _ Biomass concentration g L^{-1}
 $Y_{p/s}$ _ Ethanol yield g g^{-1}
 z _ Dimensionless axial position
 ψ _ Effective dispersion coefficient $\text{dm}^2 \text{s}^{-1}$
 α _ Dimensionless coefficient
 β _ Dimensionless coefficient
 ϵ_s _ Volume fraction solids
 η _ Effectiveness factor
 ξ _ Dimensionless bead position

Figure 1. Major components of the fluidized bed reactor. 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.

Figure 2. Axial concentration of dextrose for several reactor residence times. As the flow rate increases, less of the dextrose is converted. At high flow rates, the last portion of the reactor bed becomes well mixed by the gaseous coproduct and the aqueous streams.

Figure 3. Productivity as function of flow rate. All data points are shown with a feed concentration of approximately 150 g dextrose L⁻¹. The volumetric productivity increased with flow rate.

Figure 4. Theoretical yield as a function of axial reactor position. Triplicate measurements were made at each data point. The trend in theoretical yield improvement was possibly due to improved competitive advantage of the bacteria.

Table 1. Kinetic experiment conditions. Temperature and pH were maintained at optimal conditions for *Z. mobilis*.

Flow Rate (L hr ⁻¹)	Feed Concentration (g L ⁻¹)	Temperature (°C)	Internal Heat Exchanger Set Point (°C)	pH
13.8	169	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

-
-
-

-

-

-

-

-

-

-

-

-

-

DATE

FILMED

12 / 15 / 94

END