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Operability and Feasibility of Ethanol Production by Immobilized *Zymomonas mobilis* in a Fluidized-Bed Bioreactor

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ABSTRACT

Studies have been carried out using immobilized *Z. mobilis* in fluidized-bed bioreactors and have emphasized operation during high productivity and conversion. The bacteria are immobilized within small uniform beads (~1 to 1.5-mm diam) of κ-carrageenan at cell loadings of 15–50 g (dry wt)/L. Conversion and productivity were measured under a variety of conditions, including feedstocks, flow rates, temperature, pH, and column sizes (up to 2.5 m tall). Volumetric productivities of 50–120 g EtOH/h-L reactor volume have been achieved. Productivities of 60 g/h-L are demonstrated from a 15% feed with residual glucose concentrations of less than 0.1% and 7.4% EtOH in the tallest fermentor. Among feeds of 10, 15, and 20% dextrose, the 15% gave the highest productivity and avoided substrate inhibition. A temperature of 30°C and pH 5 were the optimum conditions. The ethanol yield was shown to be nearly constant at 0.49 g EtOH/g glucose, or 97% of the theoretical under a variety of conditions and transients. The biocatalyst beads have been shown to remain active for two months. Nonsterile feed has been used for weeks without detrimental contamination. The advantages of this advanced bioreactor system over conventional batch technology are discussed.

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

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INTRODUCTION

Ethanol fermentations are a proven way of creating liquid fuels, octane enhancers, and chemical feedstocks from renewable resources. Recently interest has temporarily waned as a result of the current oil miniglut. Despite this, some commercial interest and research continues because of the abundance of raw materials such as corn in the US, and the prediction that the energy economics will change again. Ethanol fermentations of grains have a long history and tradition of batch operations; however, most current research is directed toward novel continuous reactor designs and the improvement of the microbes to increase productivity, ethanol yields, and tolerance to inhibitors.

The utilization of a continuous system to ferment glucose to ethanol offers considerable advantages over the traditional batch technology. These advantages are largely related to improvements in volumetric productivity and in establishing a stable environment for the maximum microbial rates. Highly productive continuous culture typically requires some form of biomass retention to prevent washout and provide sufficient biomass loadings to allow complete conversion at high flow rates. For a recent survey, see Godia et al. (*1*). Many methods have been used for biomass retention in fermentation including: cell recycle by filtration (*2*), sedimentation (*3*), cell entrapment in membranes (*4*), or entrapment within gel beads as used here. These biocatalysts are then used in various reactor configurations ranging from chemostats, packed columns, and fluidized beds to more complex configurations. Each of these methods has resulted in some increase of the volumetric ethanol productivity above the value of 2–5 g EtOH/Lh typical for batch and fed-batch reactors (*5,6*). For comparison with other continuous systems, the volumetric productivity on a total reactor volume basis is approximately 6–8 g/Lh for a free cell CSTR, 10–16 g/Lh for an immobilized cell CSTR, 10–30 g/Lh for a hollow-fiber reactor, and 16–40 g/Lh for a vertical packed bed with immobilized cells (*1*). The highest productivity reported was 186 g/Lh with 95% conversion for a tapered column with a flocculant *Z. mobilis* and cell recycle (*7*).

A frequent practical difficulty of continuous reactors is lower substrate conversions and ethanol product concentrations. The productivities listed above from Godia et al. (*1*) are frequently reported for less than 99% conversion. For comparison, a substrate conversion of >99% is expected from a batch operation. High conversion is necessary from both an economic consideration of the raw material as the largest single element of the product cost (*8*), and from an operational consideration of the detrimental effect glucose has on distillation. Furthermore, a high ethanol concentration is recommended to compete with the existing industrial batch reactors so that the current distillation columns can be used. Also, a continuous system must retain operational long enough to compensate for the cost of the biomass retention and be able to deal with

the problem of contamination. In addition, the operational effects of flow rate and feed composition, as well as the environmental effects of temperature and pH, need to be examined to characterize and evaluate a new system. Most of the previous studies have examined some of these aspects but not all together. Some notable exceptions include Samejima et al. (*9*), who studied pilot-scale, packed-bed reactors with immobilized *Saccharomyces cerevisiae*, and the free cell culture work on *Z. mobilis* of Rogers and coworkers taken from many papers (includ ing *2,10–13*).

This process used *Z. mobilis* immobilized in κ-carrageenan in a fluidized-bed bioreactor (FBR). *Z. mobilis* has been shown (*2,14*) to be superior or equal to *S. cerevisiae* in its specific glucose conversion rate, its fermentative efficiency, and its ethanol tolerance. *Z. mobilis* is an aerotolerant anaerobe (*15*) that has a particular advantage over yeast, since there is no minimum oxygen requirement and also no need for stringent anaerobic conditions. *Z. mobilis* has been shown (*16*) to assimilate and convert unrefined industrial feedstocks as well as the pure laboratory medium. The entrapment within a natural hydrogel bead, such as carrageenan, appears to have little effect on the intrinsic biokinetics of *Z. mobilis* (*17*). Nevertheless, despite the stable innate cellular biokinetics after immobilization, mass transfer resistances can be significant at high cell loadings and should be avoided by the use of smaller beads (<2 mm diam) than those typically produced (3–5 mm diam).

A columnar reactor has strong advantages over a mixed reactor because of its plug-flow or multistage character. This configuration allows a faster approach to reaction completion since more of the reactor is at a higher reaction rate because of the overall higher substrate concentration and to the localization of product inhibition to the exit section. Packed-bed reactors have been successfully used for fermentation (*9,18*) but have had channeling problems caused by the large volumes of the gas coproduct, CO_2. Operation of a columnar reactor filled with biocatalyst beads as a fluidized-bed bioreactor will minimize mass transfer resistances and channeling, and allow improved disengagement of the CO_2.

In this work, the operability and feasibility of ethanol production using immobilized *Z. mobilis* in a FBR has been studied. The influence of operating conditions, such as flow rate and feed composition, on the system performance, has been examined. System performance was characterized by conversion, yield, and productivity. The yield of ethanol from glucose was compared to the theoretical stoichiometric limit of 2 mol ethanol/mol glucose. The volumetric productivity was calculated from the total rate of ethanol produced divided by the total column reactor volume, neglecting the disengagement section. Optimization with respect to temperature and pH was performed as well as longevity studies on the biocatalyst. Qualitative observations have been made on the operability, the effect of contamination from nonsterile feed, and design improvements made during the scaleup from a benchtop FBR to a large laboratory scale FBR with a 2.5-m tall bed.

MATERIALS AND METHODS

Inocula of *Z. mobilis* NRRL-B-14023 were used and grown in still Fernbach flasks containing 50 g/L glucose and 5 g/L yeast extract (DifCo). This strain has been used previously at ORNL and is a rapid fermentor of the desired media. The inocula were centrifuged to concentrate the cells before adding them to the gelling solution; the final gel concentration was 4% κ-carrageenan. Iron oxide (3%) was sometimes added to the gel to increase the specific density. The bead production technique has been described in detail elsewhere (*19*). In brief, the gel was extruded as a jet under an imposed vibration which dispersed the jet into small monodispersed droplets that were stabilized in a 0.3 *M* KCl solution. The bead diameters were measured visually and were made smaller than 2 mm.

The synthetic laboratory medium was, the desired concentration of refined glucose, 0.5% yeast extract, and 0.1 *M* KCl. The industrial feedstocks (provided by A. E. Staley Co., from their ethanol production plant at Loudon, TN) used were unrefined, saturated, corn–dextrose solution containing high lipid levels, and light steep water (LSW), an uncharacterized waste stream of corn milling that acts as a nutrient supplement. They were diluted to give the desired glucose value and 20% (v/v) LSW. All LSW typically has a dry wt of 5%. Sterile media was prepared in an autoclave at 121°C; unsterile media was stored at 4°C until used. Silicone Dow-Corning Antifoam B was added to control foaming.

The ethanol fermentation experiments were performed in a variety of laboratory-scale FBRs.

1. A medium columnar, reactor (91 cm in length with a uniform 2.54 cm diam) with a short tapered entrance section;
2. A medium, tapered reactor (93 cm in length with a uniform taper from 1.27–3.81 cm ID);
3. A short, tapered reactor (30 cm long, tapered from 1.27–2.54 cm ID); and
4. A large, columnar reactor (224 cm long with a 3.81cm ID after a short, tapered entrance section).

All FBRs had an expansion section to 7.62 or 10.16 cm ID above the bed to allow the biocatalyst beads to fall back into the column. The liquid was removed by overflow in a sidearm-settling chamber as indicated in the schematic of the medium, columnar reactor, FBR No. 1 (Fig. 1). Table 1 lists the lengths and effective volumes (volume to the disengagement expansion) of the four FBRs. FBR Nos. 1 and 2 have been described before in fermentations with a flocculant *Z. mobilis* (*7,20*).

The temperature was controlled at 30°C by a jacketed water bath and monitored near the top port with a thermistor. The pH was controlled (generally at pH 5) by the addition of KOH at multiple-axial ports along the FBR in response to a Chemtrix pH controller. The Orion pH probe

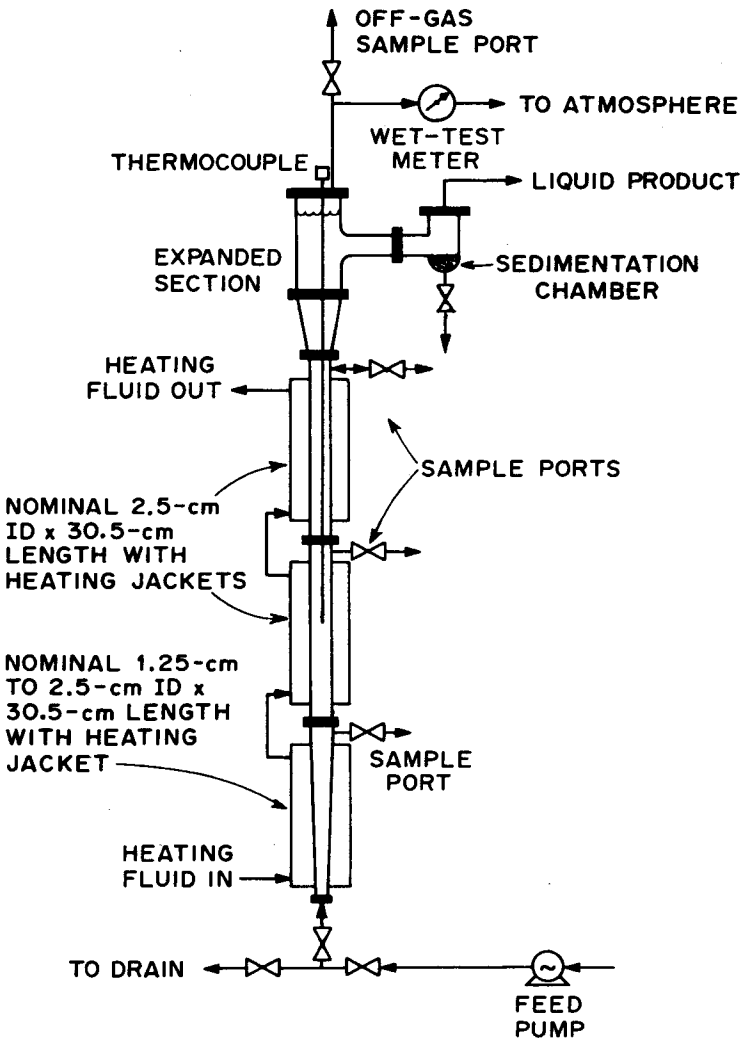

Fig. 1. Typical configuration for fluidized-bed bioreactor.

was positioned at the top port in FBR Nos. 1, 2, 3, and at the third port in FBR No. 4. The pH probe must be placed near the base addition to prevent pH overshoot caused by a lag in response because of the plug flow. The gas produced was vented out the top of the column and measured in a wet-test meter as the volumetric rate of CO_2 production.

Samples were taken from all ports of the reactors, quickly filtered, and frozen for later analysis. The pH of the samples was checked externally to ensure adequate pH control. The glucose was measured in a YSI Glucose Analyzer. The ethanol was measured by gas chromatography on a Chromosorb 101 column at 150°C with a FID.

Table 1
Laboratory-Scale FBRs

No. FBR	Length, cm	Volume mL	ID	No. ports
1. Medium	91	400	2.54	4
2. Tapered	93	675	1.27–3.81	4
3. Small	30.5	85	1.27–2.54	1
4. Large	224	2300	3.81	4

Cell concentrations were measured microscopically in a counting chamber for both beads and the broth. The conversion factor of $1.25 \times 10^{+12}$ cells/g (dry wt) was obtained from three different free-cell suspensions. Periodically some beads were removed, patted dry, and dissolved to a 100-fold dilution in distilled water and the cell number counted. Sterile water and 0.1 *M* KCl were used to wash and dissolve the beads for the streak cultures. Streak plates were made on YPG agar (10% glucose, 5% peptone, .5% yeast extract) and incubated overnight at 30°C.

RESULTS AND DISCUSSION

These results will summarize quantitative and qualitative observations drawn from 15 fermentation runs in various FBRs and of durations from 1 wk to 2 m. The amount of data allows only certain runs to be noted in detail. Design improvements in the experimental apparatus and protocols were also made during the scaleup to the large laboratory-scale reactor (No. 4) used in the last six experiments. Among these improvements was the modification of the sidearm settler from a horizontal to an inclined connector. This change greatly decreases the amount of beads lost into the sidearm by replacing a flat ledge where they can settle with an incline that drops them back into the main column.

Experiments in the large and medium FBRs were performed with or without iron oxide added to the biocatalyst beads to increase their density. The specific kinetics were very similar in all experiments but the rate of bead loss from the experiments with the plain beads was much greater than in the experiments where the beads contained 3% Fe_2O_3. This loss was surprising since 4% κ-carrageenan beads containing 5–40 g (dry wt)/L cells should be more dense than the feed. However, it was observed that some beads would float to the top of the FBR and be washed out. These same beads would immediately sink upon application of a vacuum, implying that this buoyancy is a result of entrapped carbon dioxide gas. When these identical beads were returned to a sugar feed solution, they would quickly rise again and would produce CO_2 bubbles. Microscopic examination of cross-sections of floating and nonfloating beads showed the presence of large void pockets in the floating beads. This bead loss effect caused by buoyancy was substantially reduced by high biomass levels and by the addition of more iron oxide.

A key experiment will be described in detail to illustrate the operability of this process. One 30-d test was made in the medium-tapered FBR in which both laboratory and industrial glucose concentrations of 10, 15, and 20% (w/v) were used at different flow rates, varying from almost packed-bed conditions to maximum productivity. The feed contained either .5% yeast extract or 20% LSW as a nutrient supplement. As mentioned above, it was previously shown that either feed was suitable for ethanol production in this system (*16*).

The beads were made of 4% κ-carrageenan and 3% Fe_2O_3, initially loaded with 18 g (dry wt)/L of *Zymomonas mobilis*. These beads (300mL) with an average diameter of 1.66 mm were placed in the FBR and the sterile feed flow started. Within 2 d the biocatalyst biomass had increased to and stabilized at the average value of 50 g/L. After 3 d, the feed was no longer sterilized but was kept at 4°C after preparation and warmed in a heat exchanger before entering the FBR. The FBR was then operated at each set of operating conditions for about a day. This procedure allowed flow stabilization because the residence times were less than 2 h.

The overall conversion and productivity data for this series of tests are presented in Fig. 2. As stated before, the productivity is calculated on a total reactor volume basis. These graphs show the expected trends, a steady decrease in conversion as flow rate is increased in a constant bed and an increase in productivity to a maximum in cases where the catalytic activity of the bed is fully utilized. Also, the percent conversion of the feed decreases with increasing feed concentration. Nearly complete conversion was achieved for both 10 and 15% glucose at the lowest flow rate; although, at this setting, the 15% glucose feed yielded a 50% increase in productivity. The lower productivity of the 20% feed can be explained by the increasing importance of substrate inhibition in the initial sections of the bed. Substrate inhibition in *Z. mobilis* has been shown to be negligible below 10% and significant above 17% glucose (*2,11,14*).

While the reactor was being operated with unsterilized feed, microscopic examination showed the presence of wild yeast in the feed and in the broth. Separate streak plate cultures were made on YPG agar of (*1*) the broth, (2) the surface layer of the biocatalyst, which was removed by washing in sterile .1 *M* KCl, and (3) the washed beads that were crushed in sterile water. The effluent broth contained <.3 g biomass (dry wt)/L with less than 5% of the wild yeast. The biocatalyst bead surface contained *Z. mobilis* with even less yeast, and the washed beads contained internally only *Z. mobilis* at >40 g biomass (dry wt)/L. These data confirm the operability of this process under unsterile conditions in the presence of a contaminant and the maintenance of the pure culture within an immobilized biocatalyst in a nonsterile environment since the contaminants were unable to colonize the biocatalyst.

Grote and Rogers (*11*) have found free cell *Z. mobilis* chemostat cultures to be resistant to bacterial contamination caused by high ethanol and low pH throughout. They did not test for wild yeast—a likely aci-

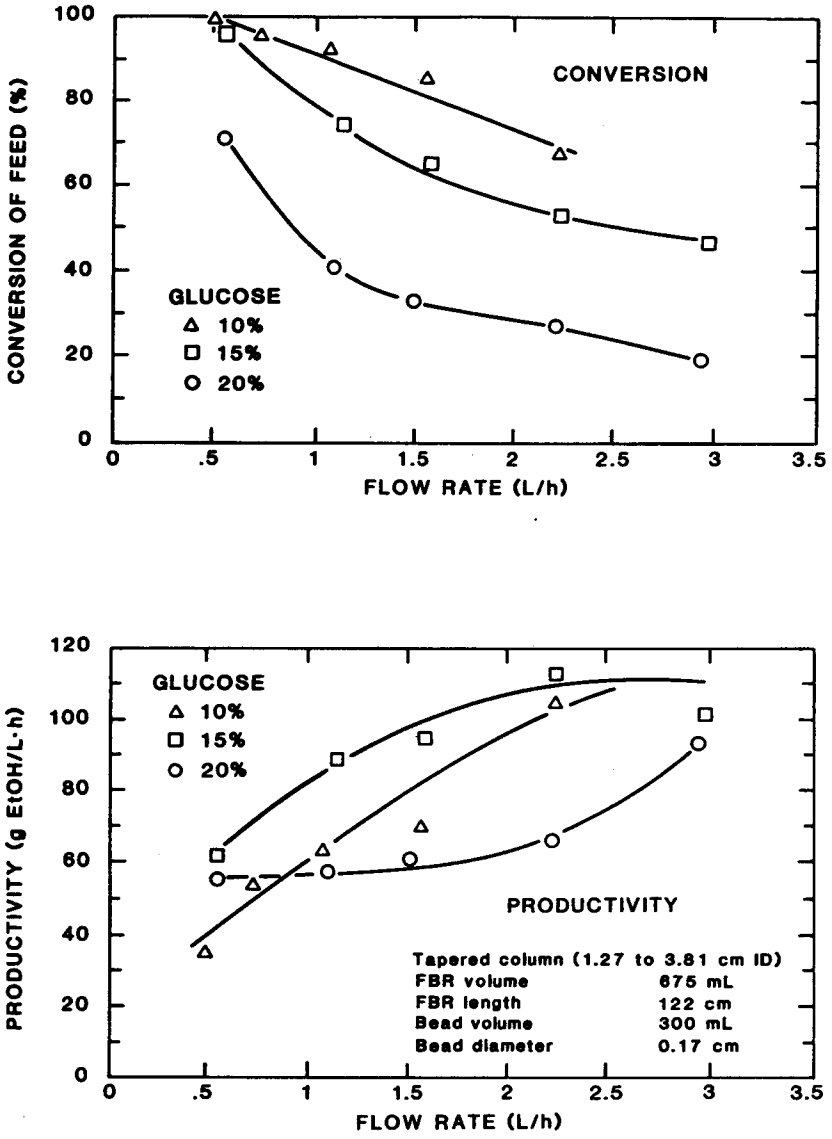

Fig. 2. Ethanol production in a medium laboratory FBR.

dophilic contaminant. Samejima et al. (*9*) found that an inlet pH of 4 allowed the nonsterile operation of a yeast packed-bed reactor without deleterious effects. Here, nonsterile operation was successful at pH 5 because of the higher flow rate and better mixing, which removed the contaminants.

The high ethanol yield has been cited as an additional advantage to the use of *Z. mobilis* over yeast in fermentation. The theoretical stoichiometric ratio is 2 mol of ethanol/mol dextrose consumed, or 0.51 g/g. At

best, yeast typically achieves 92–95% of this ratio (*21*). *Z. mobilis* yields have been reported as high as 99% of this ratio (*13,19*). In Fig. 3, all data points from each of the four ports of the previous one-month experiment are reported with the ethanol concentration plotted as a function of the glucose consumed. An average yield of .49 g/g or 97% of theoretical was achieved under a variety of flow rates, feeds, feedstocks, and transients. This advantage in ethanol production can be explained by the much lower level of glycerol production, as well as the observation that most of the carbon requirements can be satisfied by the yeast extract (*15*). Changes in yield are seen in yeast, under various operating conditions, because of the Crabtree effect (*21*); *Z. mobilis* avoids this variation by using the Entner-Doudoroff pathway (*15*). This advantage may be dependent on the presence of a rich nutrient supplement (*15*) and proper environmental conditions permitting *Z. mobilis* to use nearly all the glucose for energy and ethanol production. This is unclear, as other researchers (*22,23*) have seen no change in yields with a switch from complex to simple defined media, but have observed a small decrease in growth rate in free cell *Z. mobilis* culture. Under current economic condi-

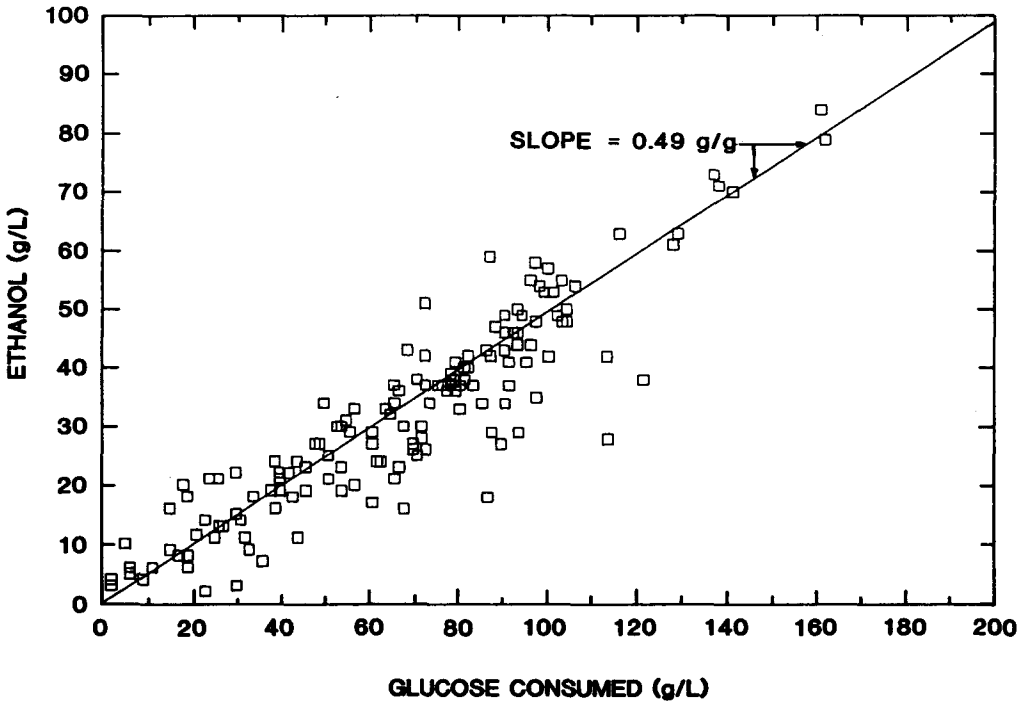

Fig. 3. Yield (g EtOH/g glucose) throughout FBR.

tions, the raw materials (i.e., dextrose from corn or other sources) are the largest single part of the costs; therefore, even a small, but consistent, increase in the yield can result in appreciable savings over the expected FBR operating lifetime of months. This small, but consistent, improvement in yield could mean a 5% increase (92–97%) in the gallons of ethanol produced per bushel of corn. Because raw materials are the major cost component in fermentative ethanol, this could be an appreciable saving.

After one month of operation reported above in the medium, tapered FBR, 30 mL of these active beads were transferred to a small FBR (No. 3) and fed with 10% glucose feed for another month. The beads remained actively fermenting and stable throughout the two-month period. The glucose conversion rate decreased by only 10% over the additional 800 h. The mean bead diameter increased from 1.7 to 2.3 mm during this period. Grote et al. (*13*), observed a 30% decline in biocatalyst activity over 800 h of operation in a similar packed-bed reactor. The reason for the more stable biocatalyst activity may be caused by better control of the local reactor conditions, namely the pH, in the FBR. Despite deactivation, a constant conversion effluent can be maintained by slowly decreasing the flow rate to compensate, as was done by Samejima (*9*) to maintain a 7% w/v ethanol effluent for 4000 h. Another possibility is the periodic replacement of part of the biocatalyst bed. Preliminary cost estimates indicate that with an active biocatalyst life of greater than 60 d, the biocatalyst cost becomes a negligible part of the total ethanol cost.

A larger reactor (No. 4) was constructed to test the ability to scaleup this process. All experiments in the large FBR were performed only with the unsterilized industrial feedstock. The feedstocks of dextrose, LSW, and water were metered into an in-line mixer. A small amount of silicone antifoam (1 g/L LSW) was added to the LSW to control foaming within the column. Experiments were performed with different bead formulations of 4% κ-carrageenan and cells with 0, 3, and 4.5% Fe_2O_3 added to the beads. As described above, the iron oxide was necessary to prevent bead loss caused by buoyancy from entrapped carbon dioxide.

The conversion and productivity for these runs are presented in Fig. 4. The scatter in this figure is greater than before because of the use of multiple runs instead of a single run. In both figures, it is clear, that of the operating conditions used, a feed with 10% dextrose consistently gives the highest conversions; however, a feed with 15% dextrose can also be used for equally high conversion and much higher productivities at slightly lower flow rates. As in the smaller FBR, the highest productivities and ethanol concentrations (up to 7.5% EtOH) were obtained with the 15% feed; substrate inhibition becomes important with the 20% feed. Selected results from these runs are presented in Table 2 and show that enhanced productivities and high conversions can be achieved in an immobilized cell FBR.

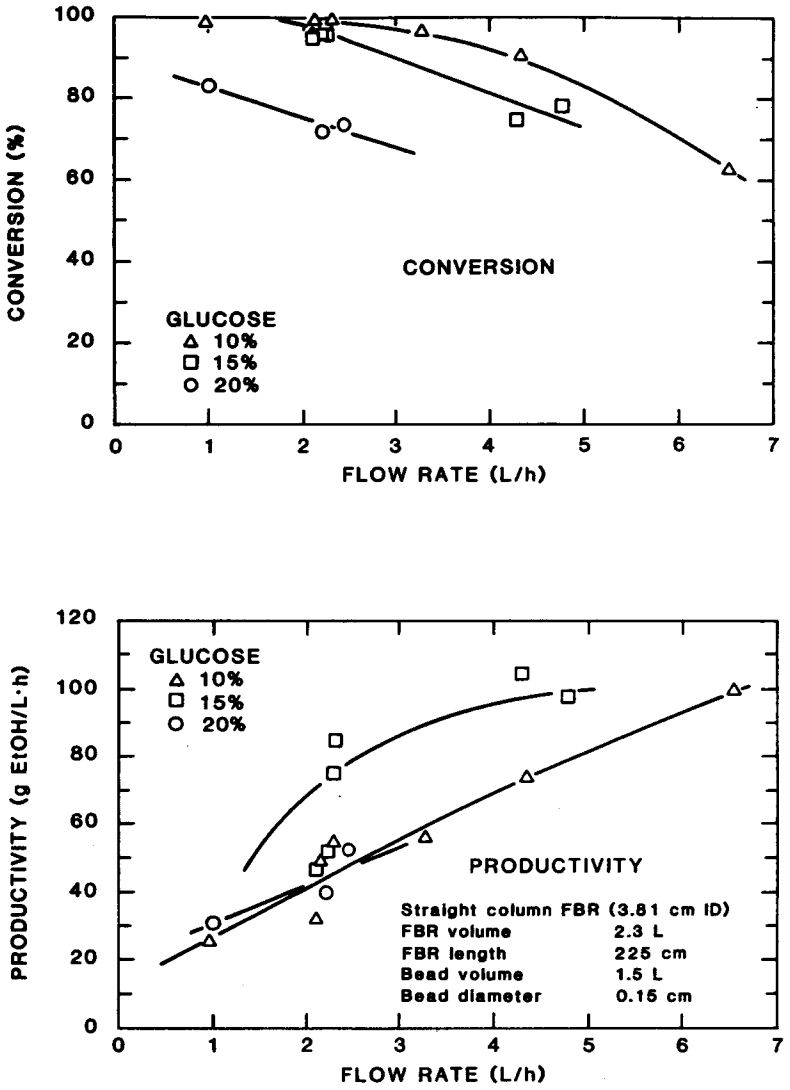

Fig. 4. Ethanol production in a large laboratory FBR.

Scaleup

Ultimate scaleup of a FBR system will require an increase in column diameter and the determination of an optimum column height. An advantage of FBRs is that traditionally they scale very well in diameter. Extensive studies have been performed in two FBRs with the indication that an increase in column height will increase conversion with only a slight decrease in overall columetric productivity. In the scaleup from FBR No. 2 to FBR No. 4, the volume was less than quadrupled and the biocatalyst

Table 2
Selected Results from the Large FBR with Industrial Feedstocks

Dextrose concentration		Feed		
Feed, g/L	Effluent, g/L	rate, L/h	Productivity, g EtOH/L·h	Conversion, %
101	.6	2.12	48.8	99.4
96[a]	.0	2.01	47.5	100.0
153	1.2	2.28	75.2	99.2
143[a]	.4	1.06	25.6	99.7

[a]Data points not on Fig. 5, since this was not the desired feed percent.

was quintupled. Judged on a four-fold increase of scale with respect to the flow rate, Figs. 2 and 4 show a quantitative similarity with regard to conversion and productivity. This result lends credence both to the reproducibility of the results and to claims for future scaleup and predictions of the needed column height.

Typical axial concentration profiles of glucose and ethanol from the large FBR are presented in Fig. 5. These data illustrate the multistage character of the FBR with substrate inhibition effects isolated in the entrance to the reactor and product inhibition near the exit; the highest re-

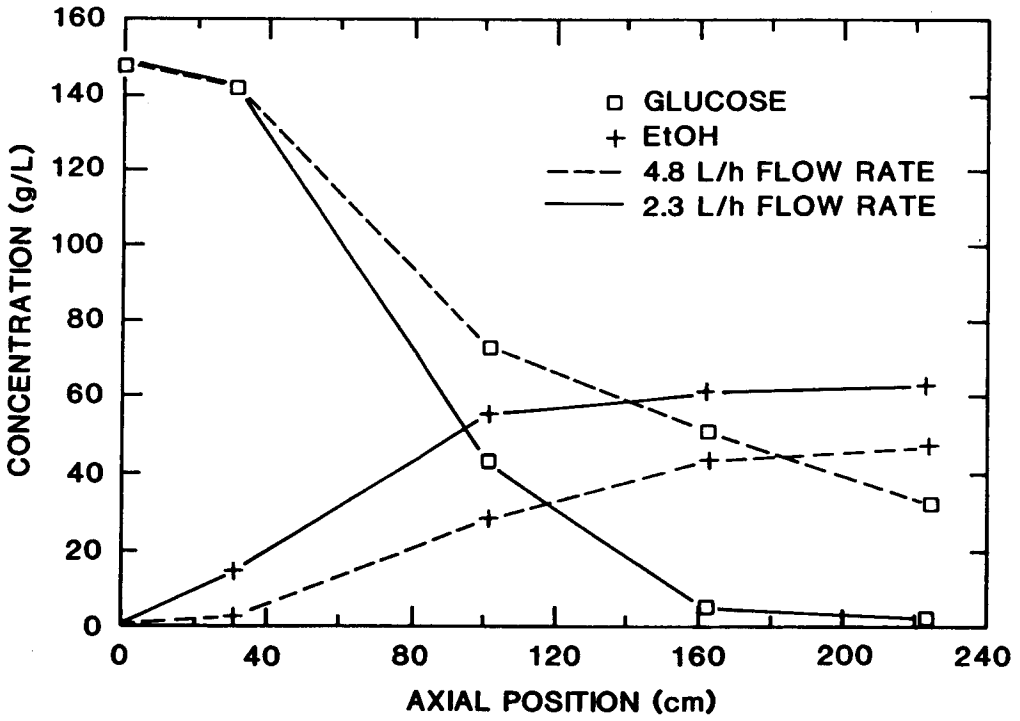

Fig. 5. Axial concentration profile in FBR.

action rate is in the middle of the reactor. The solid lines for a flow rate of 2.3 L/h show essentially complete conversion of glucose at a column height of only 160 cm. By increasing the flow rate to 4.8 L/h (dashed lines), the conversion capacity of this column was exceeded, even though the ethanol productivity increased from 75 to 95 g/(L·h). Incomplete conversion profiles can be used to estimate the column height needed for complete conversion.

Optimization of Environmental Conditions

In these experiments, the FBR was stabilized at the standard operating conditions of 30°C, pH 5, 15% glucose and 20% LSW/L feed. The variable of interest was changed and at least 3 d allowed to reach a new steady state.

Experiments were performed in both the medium (FBR No. 1) and large (FBR No. 4) where the temperature was changed from the standard temperature of 30°C. In the medium reactor a temperature increase to 35°C gave a 25% decrease in the rate that was largely reversed when the temperature was restored to 30°C. In the large FBR, the shift from 30 to 35°C resulted in a 50% decline in conversion rate that was sustained for 3 d. There was no significant change in the conversion rate between 28–32°C. Thus, the optimum temperature for operating this immobilized FBR appears to be near 30°C. This result contradicts the expectation from some free cell experiments (*14,24*) that found the optimum production temperature for *Z. mobilis* to be near 35°C; but it agrees with other data (*25,12*) that showed a flat optimum between 30–34°C and a decreased ethanol yield at high temperatures. It is unclear whether this difference is strain dependent or is inherent in the effects of immobilization.

An extensive set of pH shift experiments were performed in the large reactor. The pH control was changed and the pH of samples at all ports was checked at each reading. The pH was controlled throughout the column to within ± .3 pH units. The upshift from pH 5 to 6 caused no effect in operation or rates. However, the downshift back to pH 5 caused a sudden change in the buoyancy of part of the bed as discussed above. The change in buoyancy from this pH downshift may be due to a change in the solubility of CO_2 in water. Because of these floating beads, about 20% of the bed was lost with a resultant 20% decrease in the overall rate. A further downshift to pH 4.5 resulted in a 40% decrease in the conversion rate without the sudden bead loss. This deactivation was permanent. These observations agree with the effect of pH on free cells of *Z. mobilis* reported in the literature (*14*), where the rate is constant between pH 5 and 6 and the rate decreases sharply below pH 5 with cessation of activity near pH 4.

The optimum pH is near pH 5; at a lower pH, the rates decrease and the cells deactivate, at a higher pH, there is a greater chance of significant

contamination. The considerations of deactivation and bead loss because of buoyancy, emphasize that good pH control is important in fermentative FBRs. This idea is corroborated by the qualitative observations that failure of pH control usually would cause a FBR run to be aborted, while the FBR could recover from a failure of the flow or concentration variation of the feed.

The final environmental factor considered was the amount of LSW or nutrient supplement necessary for optimal operation and conversion of the dextrose. The composition of LSW in 15% dextrose feed was changed in the large FBR at constant flow rate. The productivity was constant and optimum for the conversion of 15% dextrose feed between 20–33% v/v LSW. Below 20% the productivity decreased because of some nutrient limitation, whereas above 33% the rate decreases, possibly because of high levels of other inhibitory constituents of the LSW. It appears that the unrefined corn dextrose syrup has some supplemental nutrient value itself, as shown by the nutrient limitation at low LSW.

CONCLUSIONS

Ethanol fermentations were successfully carried out in a number of FBRs using immobilized *Z. mobilis*. This bioreactor has been shown to be operationally robust with one run of more than 800 h and a biocatalyst life of more than 1600 h with a 10% decline in activity over the final 850 h.

The FBR can be operated under a wide variety of flow rates and feed composition. The conversion and productivity were determined as a function of flow rate and feed composition. The 15% dextrose feed is near optimum, allowing complete conversion and the highest productivities while avoiding extreme substrate and product inhibition. Using 15% dextrose feed, productivities more than 60 g EtOH/(L·h) were consistently achieved with essentially complete conversion, whereas a maximum of 120 g/(L·h) was observed with incomplete conversion (<50%). Operationally, the flow rate should be set at whatever is needed to achieve nearly complete conversion of the glucose. The immobilized *Z. mobilis* FBR was shown to have definite advantages over the typical yeast fed-batch reactor. Most important, there was a more than tenfold increase in the ethanol productivity with the same high (>99%) conversion of a 15% dextrose feed over the free cell yeast batch fermentation currently used commercially. This would allow a much smaller reactor with smaller capital costs to be used for the same alcohol output. When compared to most other continuous fermentors, as described in the introduction, this FBR has higher productivities at complete conversion. The exceptions are in the flocculant cell columnar reactors. These systems have reported higher productivities but have some still-to-be-studied difficulties with their slow approach to steady state on the floc particles. Ethanol concentrations of greater than 7% w/v can be concurrently

achieved. These conditions, with the negligible residual dextrose, should allow the use of current distillation technology and equipment. In addition, the current industrial feedstocks have been shown to be fully utilizable by this new process.

Contamination is a serious problem in the longterm operation of many continuous bioreactors. A second advantage of this system is the operation with little or no asepsis. The ability of an immobilized cell FBR to wash out contaminants while maintaining the desired pure culture within the beads has been conclusively demonstrated. This advantage counterbalances the absence of the low pH used in yeast fed-batch reactors to maintain sterility. As seen here, this advantage should be applicable to most immobilized cell FBRs, with definite advantages in operability and in operating costs since the feed need only be clean, not sterilized.

The third major advantage is the improved ethanol yield per g dextrose because of *Z. mobilis*. This increase to .49 g/g, or >97% of the stoichiometric limit (compared to the optimum 90–95% for yeast), is stable and consistent under a variety of conditions and transients.

Stable environmental conditions, especially pH, must be maintained continuously throughout the FBR to achieve these reactor lifetimes. The optimum environmental conditions were found to be a pH of 5, a temperature of 30°C, and a nutrient supplemental of 20% v/v LSW in a 15% dextrose feed. Precise environmental control does not appear necessary because a variation of pH between pH 4.7–5.5 has little effect, but conditions outside of this range will detrimentally affect the catalyst activity (*26,27*). Fortunately, a FBR with multiple ports and sensors should be relatively amenable to control.

FBRs 30–224 cm in length and 1.27–3.81 cm ID have been operated successfully. This indicates the amenability to scaleup of a fluidized bioreactor. A pilot scale reactor of 15.2 cm ID and 450–600 cm in length is suggested for the next level of scaleup, but is beyond the scope of this paper. Simulations and modeling of the profiles and hydrodynamics have been begun using plug-flow reactors and CSTRs-in-series as model reactors. Complex hydrodynamics have been observed under some conditions and are reported elsewhere (*28*).

This study demonstrates that the immobilized *Z. mobilis* fluidized-bed bioreactor is a feasible alternative to the conventional fed-batch yeast fermentation and a strong contender to other continuous fermentors. The advantages of both the use of fluidization and the use of *Z. mobilis* have been shown.

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