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Performance of Coimmobilized Yeast and Amyloglucosidase in a Fluidized Bed Reactor for Fuel Ethanol Production

MAY Y. SUN,[1,2] PAUL R. BIENKOWSKI,[1,2]
BRIAN H. DAVISON,[1,2] MERRY A. SPURRIER,[1]
AND OREN F. WEBB*,[1]

[1]*Oak Ridge National Laboratory, P.O. Box 2008, MS-6226, Chemical Technology Division, Oak Ridge, TN 37831-6226; and* [2]*University of Tennessee, Department of Chemical Engineering, Knoxville, TN 37916*

ABSTRACT

The performance of coimmobilized *Saccharomyces cerevisiae* and amyloglucosidase (AG) was evaluated in a fluidized-bed reactor. Soluble starch and yeast extracts were used as feed stocks. Conversion of soluble starch streams to ethanol has potential practical applications in corn dry and wet milling and in developmental lignocellulosic processes. The biocatalyst performed well, and demonstrated no significant loss of activity or physical integrity during 10 wk of continuous operation. The reactor was easily operated and required no pH control. No operational problems were encountered from bacterial contaminants even though the reactor was operated under nonsterile conditions over the entire course of experiments. Productivities ranged between 25 and 44 g ethanol/L/h/. The experiments demonstrated that ethanol inhibition and bed loading had significant effects on reactor performance.

Index Entries: Ethanol; glucose; starch; simultaneous saccharification and fermentation; fluidized-bed reactor.

INTRODUCTION

Domestic ethanol use and production are presently undergoing significant increases along with planning and construction of new production facilities. Raw material costs typically make up 55–75% of the final alcohol

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

selling price *(1)*. Significant efforts are ongoing to reduce ethanol production costs by investigating new inexpensive feedstocks (woody biomass) and by process improvements in the fermentation and separation steps. Increasing reactor productivity is a potent method for reducing capital costs associated with new construction and expansion of existing facilities. Selection of fermentative organism and the reactor configuration also affect operating cost factors, such as yield, energy demand, and control of bacterial infections.

A key element in the development of advanced bioreactor systems capable of very high conversion rates is the retention of high biocatalyst concentrations within the bioreactor and a reaction environment that ensures intimate contact between substrate and biocatalyst. Such strategies include cell recycle by filtration, sedimentation, entrapment by membranes, and immobilization in gel beads *(2,3)*. 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 batch reactors, commonly used in industry, have volumetric ethanol (EtOH) productivities between 2 and 5 g EtOH/L/h *(4,5)*. On a total reactor volume basis, volumetric productivity for continuous systems with high conversion is 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 *(6)*. One very effective method is to use an immobilized biocatalyst that can be placed into a reaction environment that provides effective mass transport, such as a fluidized bed. Previous studies have shown that such systems may be more than 10–50 times as productive as industrial benchmarks *(6,7)*. Economic impacts of the FBR for ethanol production may be significant *(8)*.

In this article, we describe FBR experiments for simultaneous saccharification and fermentation of starch, which employ entrapped yeast in a covalently crosslinked gelatin, chytosan, amyloglucosidase (AG) matrix. This study attempts to provide a comparison between yeast and previous FBR investigations. Previous studies with *Zymomonas mobilis* demonstrated significant performance advantages, such as very high productivities of 50–200 g ethanol/L/h and high yields around 97% of theoretical *(6,7)*. Yeasts have operational advantages, such as excellent pH tolerance. Such a simultaneous saccharification and fermentative approach has the advantage that AG and yeast are easily retained in the reactor for continuous use. Starch was used as raw material instead of glucose. Combining saccharification and fermentation in one vessel could reduce capital costs.

MATERIAL AND METHODS

The *Saccharomyces cerevisiae* cells were immobilized in covalently crosslinked gelatin (6 wt%) and chytosan (0.25 wt%) with AG. Crosslinking was accomplished by glutaraldehyde. Biocatalyst diameters

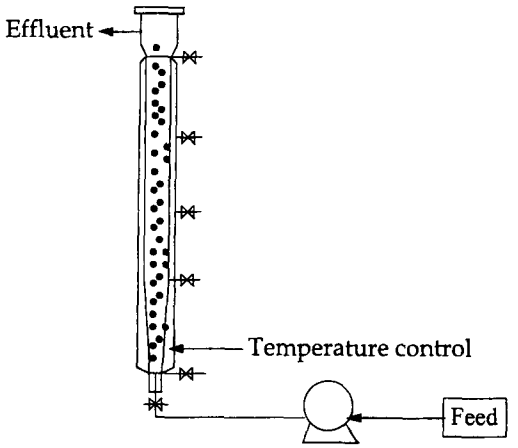

Fig. 1. FBR schematic. The reactor consisted of an expanded 30-cm inlet section (1.27–2.5 cm in id), three 30-cm sections of 2.5-cm ID jacketed glass pipe, and a 10-cm disengagement section of 9-cm ID with a screened sidearm for disengagement of beads from the reactor effluent.

ranged between 1.2 and 2.8 mm. The developmental biocatalyst was supplied by Genencor International.

Feed solutions consisted of various concentrations of StarDri 100 starch (A. E. Staley, Decatur, IL), 5 g/L Tastetone 900 AG yeast extract (Red Star, Juneau, WI), and 0.1 % w/v Antifoam B (Dow Corning, Midland, MI) in tap water.

The reactor depicted in Fig. 1 was constructed of a 30-cm inlet section, which expanded from 1.27–2.5 cm in ID, three 30-cm sections of 2.5-cm id jacketed glass pipe, and a 10 cm disengagement section of 9 cm id with a screened sidearm for disengagement of beads from the reactor effluent. Temperature was controlled at 34°C by a Haake A82 (Berlin, Germany) recirculating water bath. The feed was introduced at the reactor bottom using a model 7550-60 Masterflex peristaltic pump (Cole Parmer, Niles, IL). The pump was calibrated daily. The reactor was open to the atmosphere for gas-liquid biocatalyst disengagement at the outlet.

Starch, glucose, and ethanol concentrations were measured using a Shimadzu high-performance liquid chromatograph (HPLC) consisting of an RID6A refractive index detector, an SIL 10A autoinjector, an LC10AD pump, an SCL10A system controller, CTD10A column oven, and a CR501 integrator. An Aminex HPX-87H (Bio-Rad Laboratories, Hercules, CA) column with a 5-m*M* H_2SO_4 mobile phase separated analytes.

Minimal procedures were used for mitigation of contaminant growth. These included:

1. Changing feed lines with each new charge of feedstocks;
2. Replacement of feed containers with each charge of fresh feed; and

3. Medium was autoclaved prior to use, because significant amounts of contaminants existed in the yeast extract.

Previous investigations demonstrated that sterile operation is not necessary: however, the feed must be kept free of high levels of contamination (e.g., contamination $>10^8$ cells/mL). The reactor was not sterilized or cleaned after the 10-wk experiment began.

RESULTS AND DISCUSSION

The operability of the reactor was good throughout the experiment. The reactor system generally operated without operator intervention or attendance. The pH within the reactor was not controlled, and ranged from approx 6.5 at the inlet to about 3.5 at the column outlet. The temperature was maintained at 34°C throughout the experiment. The biocatalyst was used continuously for 10 wk in the FBR without recharging. There was no noticeable loss of biocatalyst from the bed. There were no obvious signs of physical degradation of the biocatalyst, except for very few beads that demonstrated significant diameter increases. The average AG activity was approx 8 μmol of glucose produced/min/mL of biocatalyst.

The fluid dynamics and reaction kinetics are coupled, and are complex functions of reaction rate, starch and glucose concentration, solids loading, and gas-liquid-solid properties *(9,10)*. The fluidization of the bed changes rapidly with axial position owing to significant changes in fluid flow rates and physical properties. 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, is fluidized by liquid. The second zone, fluidized by gas product, starts within the expansion section and encompasses most of the bed. The third zone, termed the disengagement section, is characterized by high gas holdup (e.g., 5–20%) and significant mixing. The axial reaction rate in the FBR is a strong function of reactor biocatalyst concentration. Gas and liquid holdup, and dispersion, which become larger in the upper parts of the reactor, reduce the biocatalyst concentration. Inside the biocatalyst matrix, starch is hydrolyzed by AG to form glucose and then converted to ethanol and carbon dioxide by yeast:

$$(C_6H_{10}O_5)_n + (n-1)H_2O \xrightarrow{AG} nC_6H_{12}O_6 \quad (1)$$

$$nC_6H_{12}O_6 \xrightarrow{Yeast} 2nCH_3CH_2OH + 2nCO_2 \quad (2)$$

The biocatalyst was loaded with sufficient AG to convert low concentrations of starch at significant rates. The AG reaction rate was thus expected to be rapid at higher starch concentrations.

Figure 2 depicts an example concentration profile for the FBR. The flow rate and mass flow rate were 10 mL/min and 60 g starch/h, respectively. Bed loading is moderate, since the range of starch mass loading for

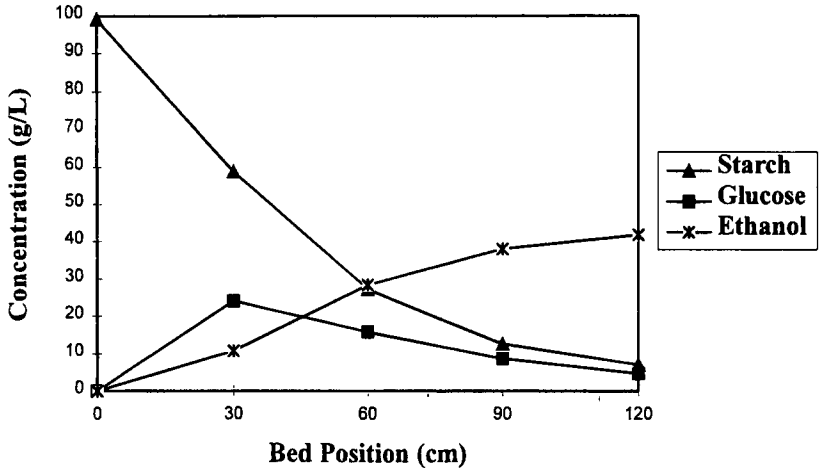

Fig. 2. Example of concentration profile. Starch concentration decreased with reactor position as AG conversion proceeded. Glucose is an intermediate between the AG and yeast reactions initially increased and then decreased. Ethanol concentration increased as the dextrose was converted.

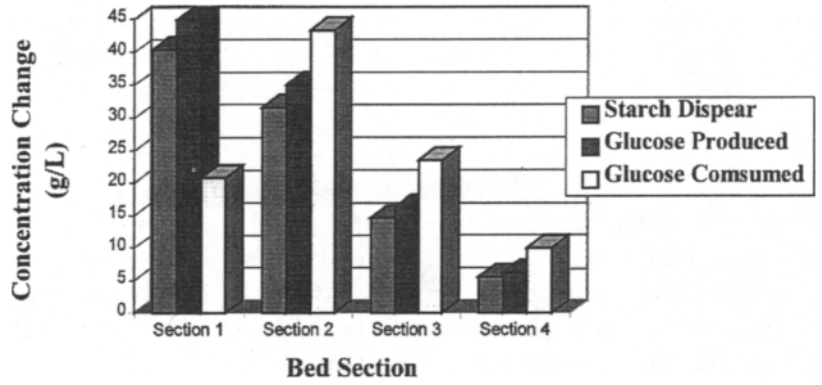

Fig. 3. The conversion and/or accumulation of starch, glucose, and ethanol as change within individual reactor sections.

the 10-wk experiment ranged from 20–120 g/h^{-1}. Starch concentration decreased with reactor position as AG conversion proceeded. Glucose is an intermediate between the AG and yeast reactions. Therefore, axial glucose concentration will be a function of both AG and yeast reaction rates. Glucose accumulates in the first reactor section because of high AG conversion rate (high starch concentration). The ethanol concentration increases as glucose is made available by the AG and then converted by the yeast. Figure 3 illustrates the conversion and/or accumulation of starch, glucose, and ethanol within individual reactor sections. Glucose accumulation in the first section indicates that the yeast reaction is slower than the

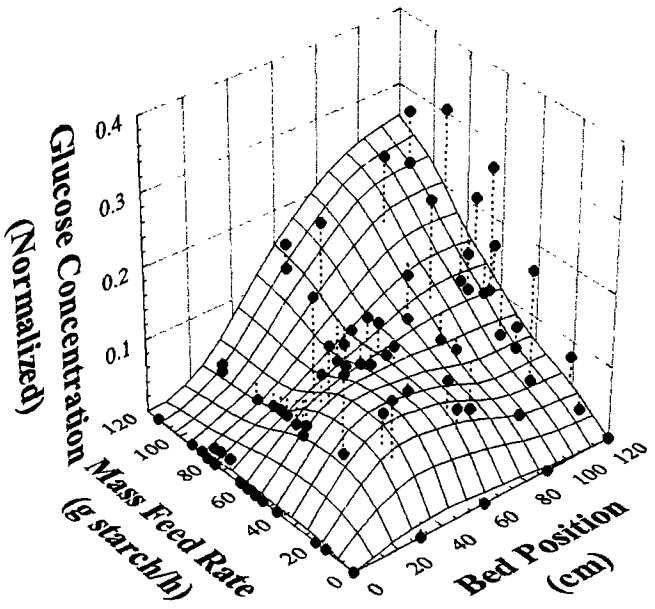

Fig. 4. Mass loading influence. As mass loading increased, glucose accumulation also increased. Bed position was also important as a variable to indicate the effect of contact time between substrates and the AG and yeast. Glucose is normalized to glucose equivalents in the feed.

AG reaction in this section. Yeast reaction rate is a function of glucose concentration, but is also affected by substrate and product inhibition. The conversion of starch to glucose drops in later sections as the available starch is reduced by prior reaction. Significant glucose conversion occurred in the second section owing to high glucose availability. Product may inhibit yeast in later sections of the reactor.

The surface in Fig. 4 allows visualization of the effect of mass loading on reactor performance. The data clearly demonstrate that as mass loading increased, glucose accumulation also increased. Bed position was also important as a variable to indicate the effect of contact time between substrates and the AG and yeast. Thus, as the contact time increased, the total conversion of starch and glucose also increased. The case depicted in Fig. 2 (previously discussed) is shown in Fig. 4 as the first data series closest to the Bed Position axis. In this case, glucose initially accumulated; however, as the starch supply rapidly decreased via AG conversion, almost complete conversion of substrates was achieved. The AG reaction is influenced greatly by starch concentration and to a minor extent by glucose concentration. The yeasts, on the other hand, are limited at the reactor inlet by a lack of glucose. As ethanol is formed, the yeasts become inhibited. The onset of ethanol inhibition begins at concentrations of approx 20 g/L^{-1} *(1)*. Figure 4 also indicates that the reaction was limited by yeast and not the

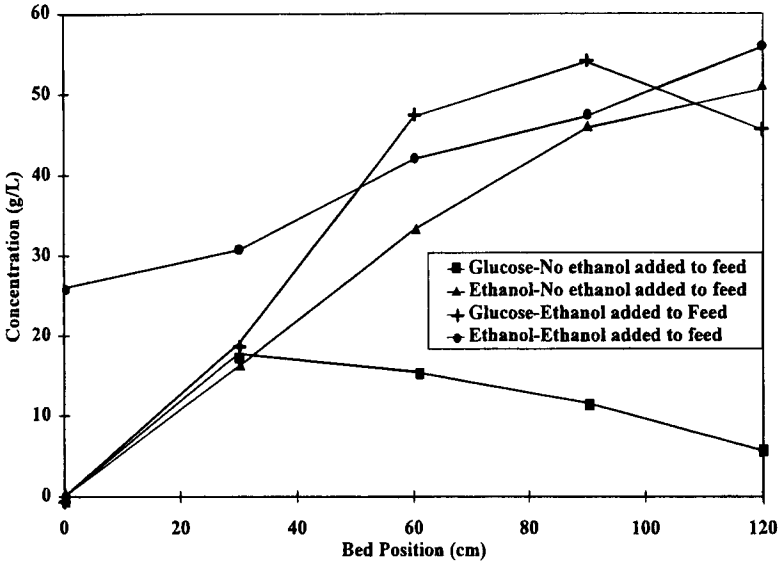

Fig. 5. Ethanol inhibition example. The concentration profile of glucose in the reactor was a function of ethanol inhibition demonstrated by addition of ethanol to the feed.

AG. The data clearly demonstrate that as mass loading increased, the accumulation of glucose increased. Under actual practice, the AG activity would ideally be balanced against the yeast activity to maintain a low glucose concentration throughout the reactor. Yeasts are primarily the greatest unknown in this system, because their performance not only depends on glucose and ethanol concentration, but also on nutrition, yeast, and biocatalyst age, and other factors. The surface was produced by fitting the data using the distance-weighted least-squares method in Statistica (StatSoft, Inc., Tulsa, OK) similar to the method of McLain *(11)*. The surface in Fig. 4 is qualitative because of the complex coupling of reactions (AG and yeast) and three-phase hydrodynamics. Figure 4 demonstrates that care must be taken to balance the activities of the yeast and the AG.

In a series of experiments, ethanol was added to the reactor feed solution for verifying model kinetics and for reactor scale-up. Ethanol inhibition at the top of the reactor is masked by three-phase hydrodynamic effects, gas holdup in particular. Figure 5 depicts an example of ethanol inhibition. Ethanol concentration increased slowly relative to the case without ethanol in the feed. As yeast ethanol inhibition increases, the difference in yeast rates and AG will increase. Ethanol is not expected to inhibit AG activity under fermentation conditions *(12,13,14)*. Thus, the ratio of glucose to starch concentrations at different points within the reactor was used as a general indicator of ethanol inhibition. Figure 6 allows visualization of the effects of ethanol inhibition on reactor performance. The data clearly show that as the ethanol in the feed increased, that rate differences between AG

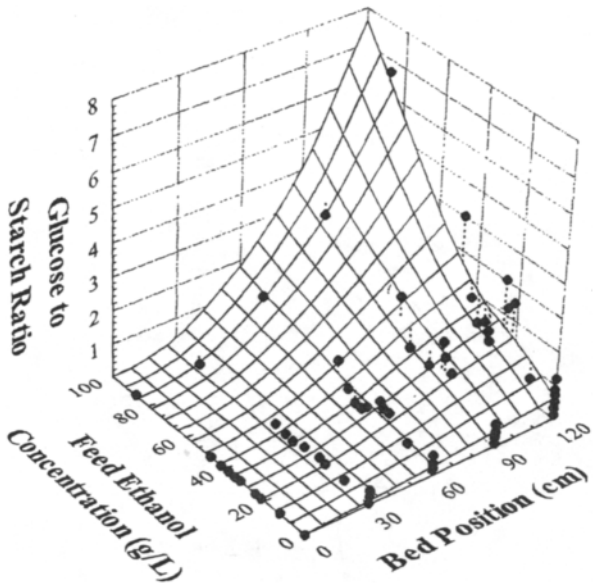

Fig. 6. Visualization of ethanol inhibition effects. As yeast ethanol inhibition increased, the difference in glucose and starch conversion rates increased, because AG was not inhibited. Under reactor conditions, the AG-catalyzed, starch-to-glucose equilibrium is greatly shifted to the dextrose product. The ratio of glucose to starch concentrations at different points within the reactor was used as a general indicator of ethanol inhibition.

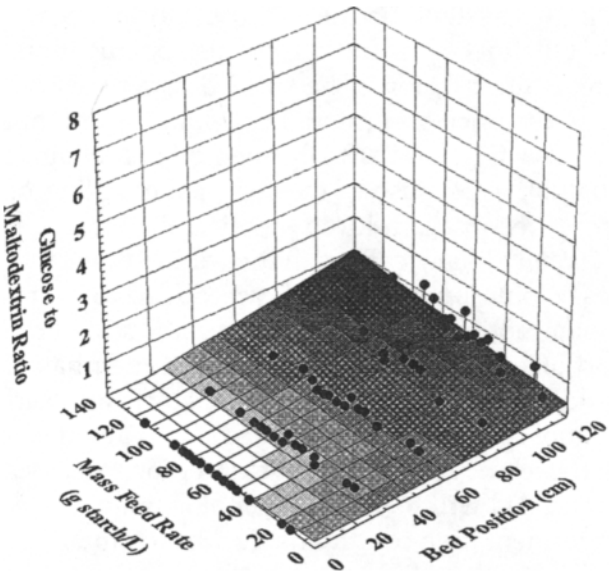

Fig. 7. Influence of mass loading on dextrose conversion and accumulation. The ratio of glucose to starch is a function of yeast and AG reaction rates. There is a small affect on the ratio of glucose to starch within the bed with bed position and mass feed rate.

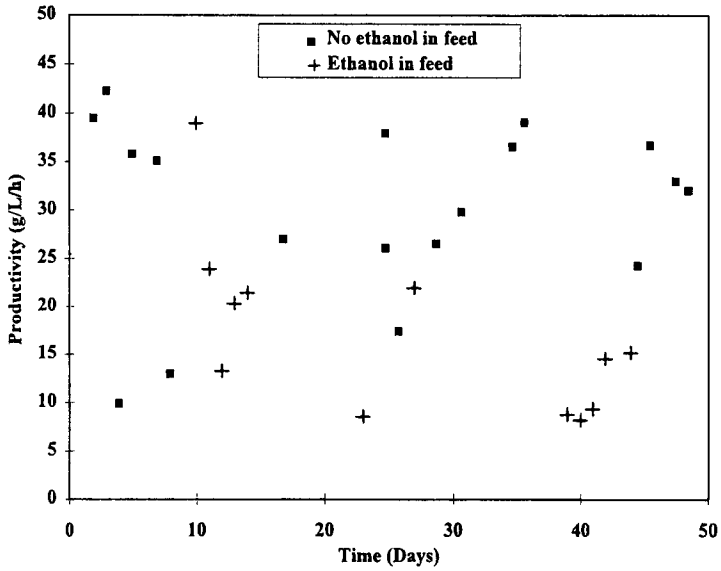

Fig. 8. Productivity profile. The data are grouped into two subsets: one subset containing feeds with ethanol and one subset containing feeds without ethanol. Most of the data with no ethanol in the feed were grouped in the range of 25–44 g ethanol/L/h. Low values were owing to very low loading of the reactor. When ethanol was included in the feed, the range of productivities generally ranged between 5 and 25 g ethanol/L/h. The differences in these productivities further demonstrate that ethanol inhibition had a significant effect on reactor performance. From both set of data, there were no obvious declines in productivity.

and yeast became more pronounced. The ratios also depend somewhat on feed concentrations and liquid feed rates. Figure 7 demonstrates that the mass loading effect was small compared to ethanol inhibition. No ethanol was added to the feed in Fig. 7. There was a small increase in the glucose-to-starch mass ratio with bed position and with mass loading when ethanol was not added to the feed. The data for Figs. 6 and 7 were generated using the same ranges of starch feed concentrations and flow rates. Statistica generated the surfaces for visualization in Figs 6. and 7.

Figure 8 depicts productivity over the course of the last 8 wk of the 10-wk experiment. Two weeks were allowed for stabilization of yeast activity. Mass loading varied throughout the experiment by varying feed concentrations and slow rates. The data are grouped into two subsets: one subset containing feeds with ethanol and one subset containing feeds without ethanol. Most of the data with no ethanol in the feed were grouped in the range of 25–44 g ethanol/L^{-1}/h^{-1}. Three points not falling in this group were owing to very low mass loading of the reactor. When ethanol was included in the feed, the range of productivities generally was between 5 and 25 g ethanol/L/h. The differences in these productivities further demonstrate that ethanol inhibition had a significant effect on reactor per-

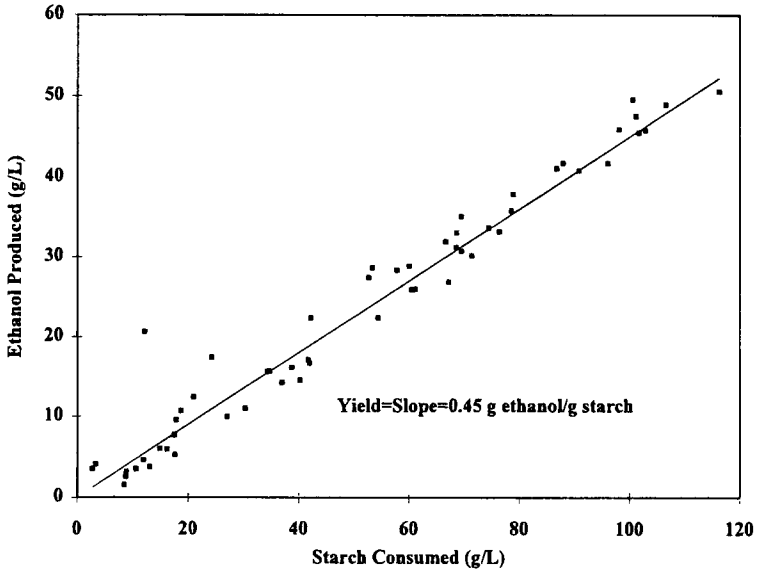

Fig. 9. Average yield. The yield for these experiments ranged around 80% of theoretical as measured by the slope of substrate converted to ethanol produced. Possibly, yeast nutrition limited yield.

formance. Also, there were no obvious declines in productivity in either set of data over time.

The average yield was calculated by the slope of substrate conversion to ethanol production (Fig. 9). The yield for these experiments ranged around 80% of theoretical. A yield of >90% of theoretical has been demonstrated with stirred tank reactors (STRs) and similar biocatalysts *(15)*. Possibly yeast nutrition played a significant role in this lower yield. In a batch reactor system, Cysewski *(16)* demonstrated that ethanol yield was severely restricted if the concentration of yeast extract was 4.0 g/L^{-1} or 6.0 g/L^{-1}. He suggested a yeast extract requirement of 8.5g L^{-1}. The physiology of immobilized yeast may differ from free-cell physiology. Yeast extract was used at 5 g/L^{-1} concentration levels for direct comparison with previous work with *Z. mobilis*. Under these conditions, *Z. mobilis* demonstrated yields of 97 and 96% of theoretical for experiments at the batch and pilot scales *(6,7)*. These data suggest that yeasts have higher nutritional requirement than *Z. mobilis* when immobilized. This may not be a significant issue in industrial processes where very rich nutrient streams, such as steep water, are routinely used in the fermentation.

SUMMARY

Coimmobilized yeast and AG were used to convert soluble starch to ethanol effectively in one reactor. This research demonstrates that yeast is very hardy and produces ethanol over a very long period of time. The bio-

catalyst performed well, and demonstrated no significant loss of activity or physical integrity during 10 wk of continuous operation. The reactor was easily operated and required no pH control. No operational problems were encountered from bacterial contaminants, even though the reactor was operated under nonsterile conditions over the entire course of experiments. Ethanol inhibition is an important factor. Productivities ranged significantly above industrial benchmarks. Coimmobilized yeast-AG biocatalyst continues to demonstrate the potential for FBR use.

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