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Ethanol Production from Corn Starch in a Fluidized-Bed Bioreactor†

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Abstract

The production of ethanol from industrial dry-milled corn starch was studied in a laboratory-scale fluidized-bed bioreactor using immobilized biocatalysts. Saccharification and fermentation were carried out either simultaneously or separately. Simultaneous saccharification and fermentation (SSF) experiments were performed using small, uniform κ-carrageenan beads (1.5–2.5 mm in diameter) of co-immobilized glucoamylase and *Zymomonas mobilis*. Dextrin feeds obtained by the hydrolysis of 15% dry-milled corn starch were pumped through the bioreactor at residence times of 1.5–4 h. Single-pass conversion of dextrins ranged from 54–89%, and ethanol concentrations of 23–36 g/L were obtained at volumetric productivities of 9–15 g/L-h. Very low levels of glucose were observed in the reactor, indicating that saccharification was the rate-limiting step. In separate hydrolysis and fermentation (SHF) experiments, dextrin feed solutions of 150–160 g/L were first pumped through an immobilized-glucoamylase packed column. At 55°C and a residence time of 1 h, greater than 95% conversion was obtained, giving product streams of 162–172 g glucose/L. These streams were then pumped through the fluidized-bed bioreactor containing immobilized *Z. mobilis*. At a residence time of 2 h, 94% conversion and ethanol concentration of 70 g/L were achieved, resulting in an overall process productivity of 23 g/L-h. At residence times of 1.5 and 1 h, conversions of 75 and 76%, ethanol concentrations of 49 and 47 g/L, and overall process productivities of 19 and 25 g/L-h, respectively, were achieved.

Index Entries: Ethanol; dry-milled corn starch; *Zymomonas mobilis*; glucoamylase; fluidized-bed reactor.

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Introduction

In recent years, interest has been revived in the use of ethanol as an octane booster and as an alternative transportation fuel. The economics of fuel ethanol production is significantly influenced by the cost of raw materials used in the production process, which accounts for 55–75% of the final ethanol selling price *(1)*. Corn starch has been the predominant feedstock for industrial ethanol production. Lignocellulosic materials such as agricultural residues and "energy" crops are also being considered as potential feedstocks *(2)*. Current bioconversion processes for fuel ethanol production from corn starch have a low volumetric productivity and require many hours of residence time in the fermentor. Development of high-productivity processes and reactors can potentially reduce capital costs associated with new construction and expansion of existing facilities. Proper selection of the reactor configuration and fermentative microorganism can be used to increase productivity, ethanol yields, and tolerance to inhibitors.

The use of continuous systems with high cell densities can improve ethanol volumetric productivities as compared with traditional batch systems *(1)*. Immobilization in gels is a method of biocatalyst retention in continuous systems *(3–7)*. Ethanol volumetric productivities of more than 60 g/L-h with essentially complete conversion of 15% dextrose feed have been achieved using immobilized *Zymomonas mobilis* in a fluidized-bed bioreactor (FBR) *(5)*. This is a significant improvement over typical productivities of 2–5 g/L-h obtained using batch and fed-batch processes. Apart from its plug flow characteristics, an FBR also provides effective mass transport by overcoming channeling and carbon-dioxide buildup. Economic impacts of the use of FBR for ethanol production from glucose have been estimated to be six cents/gallon *(8)*. Simultaneous saccharification and fermentation (SSF) of starch to ethanol has been investigated by several researchers and is also being practiced in commercial processes using "free" biocatalysts *(9–11)*. Applications of the FBR using co-immobilized glucoamylase-*Saccharomyces cerevisiae* and glucoamylase-*Z. mobilis* have shown that typical volumetric productivities in the range of 15–40 g/L-h could be achieved *(12,13)*. These studies were conducted using maltodextrin as the substrate and yeast extract or light steep water as the supplemental nutrient source. An SSF process facilitates the use of a single reactor for both hydrolysis and fermentation, and also minimizes the contamination risk because of the presence of ethanol in the fermentation broth. However, a drawback of this process is that the operating conditions cannot be optimized for both steps simultaneously. The maximum temperature at which *Z. mobilis* can ferment efficiently in a continuous process is 35°C *(5)*, which is well below the optimum temperature of glucoamylase at 55°C *(14)*; hence an SSF process will suffer from low enzyme activity. In fact, it has been observed that in such a process, the enzymatic hydrolysis was indeed the rate-limiting step *(9,10)*.

In this investigation, the production of ethanol from dry-milled corn starch was first studied in an SSF process using co-immobilized gluco-

amylase-*Z. mobilis* biocatalyst beads in an FBR. Ethanol production by separate hydrolysis and fermentation (SHF) was then studied using immobilized glucoamylase in a packed-bed reactor and immobilized *Z. mobilis* biocatalyst beads in an FBR. The results obtained with both process configurations are presented and compared in this report.

Materials and Methods

Microorganism

Z. mobilis NRRL-B-14023 was used in the fermentation studies. The stock culture was maintained in 25% glycerol at –70°C. For immobilization, cells were grown in a 75-L fermentor (New Brunswick Scientific Co., Edison, NJ) at 30°C and pH 5.0 (maintained using 2.5 *M* NaOH). A working volume of 50 L was used. The seed culture (4 L) was prepared in two fernbachs. The seed-culture media contained 50 g/L glucose, 5 g/L Tasteone 900AG yeast extract (Red Star, Juneau, WI), and 5 g/L KH_2PO_4. Concentrated phosphoric acid was used to adjust the pH of the medium to 5.0. The medium was sterilized at 121°C for 20 min and each fernbach was inoculated with 1.5 mL of stock culture. The seed culture was incubated in a shaker at 30°C and 50 rpm for 36 h. After inoculation, the cells were grown in the fermentor (medium was identical to the seed culture) until the glucose concentration dropped to between 5 and 10 g/L. The cells were then harvested with a Sharples centrifuge (Sharples Equipment Division, Philadelphia, PA). The cell pastes were stored at 4°C until ready for use in the immobilization step.

Enzymes

The α-amylase used for starch liquefaction and dextrinization was supplied by Morris Ag-Energy, (Morris, MI). Glucoamylase immobilized on porous diatomaceous earth as a support was supplied by Genencor International (Elkhart, IN). The immobilized enzyme particle diameter was between 1.0 and 1.5 mm. The specific activity of the immobilized enzyme at 55°C and pH 4.2 is 881 U/g on a dry weight basis *(15)*. One unit of activity represents the amount of enzyme that will produce one micromole of glucose in 1 min under the assay conditions.

Corn Starch Hydrolysis

Dry-milled corn starch was supplied by Morris Ag-Energy. The moisture content of the corn starch was determined to be 13%. Liquefaction and hydrolysis of the corn starch were carried out at 95°C and pH 6.3–6.5 using 0.2% (v/w) α-amylase for 1.5 h. Enzyme activity in the starch slurry was maintained by adding 150 ppm calcium ions in the form of $Ca(OH)_2$. The hydrolysis was carried out in 10-L batches in a 20-L steel container. Mixing was achieved by using a high-speed laboratory mixer equipped with a 3-in (7.6-cm) propeller (Cole Parmer Instrument Company, Vernon Hills, IL) at 700–1000 rpm. The starch hydrolysis was carried out until the slurry

gave a starch negative test (no violet coloration) with iodine solution. Experiments were conducted with 15% and 28% solids. For an industrial dry-milled corn starch-to-ethanol process, a solids loading of ~28% is commonly used (B. J. Jordan, personal communication). In preliminary experiments, it was observed that the solids in the 28% slurry could not move through the FBR. This prompted us to test the flow of a dilute slurry with 15% solids in the FBR. However, the solids caused plugging of the FBR after a few hours of continuous operation. Therefore, these solids were removed by centrifugation using a Sorvall centrifuge (DuPont Instruments, Newtown, CT) at 4000 rpm for 10 min. The wet solids cake was washed thoroughly with 1:1 (w/w) water and recentrifuged. The liquid recovered from this step was mixed with the liquid obtained from the first centrifugation step. This combined dextrins mixture was used as the substrate for the ethanol production experiments. It should be pointed out that a 1:1 (w/w) ratio of water to wet solids was used in the laboratory experiments to minimize feed dilution. Extraction experiments indicated that the dextrin recovery in this case was ~57% of the dextrins still trapped in the solid cake. In large-scale operation, a greater ratio of water to wet solids can be used to improve dextrin recovery. The dilute dextrin stream then obtained can be recycled to the starch-liquefaction tank. This process scheme will minimize the loss of fermentable substrate associated with the removed solids.

Biocatalyst Bead Preparation

Bead preparation was initiated by dissolving 40 g κ-carrageenan (FMC Corporation, Rockland, ME) in 600 mL of deionized water at about 75°C. The dissolved gel was then placed in a water bath at 35°C. To prepare the co-immobilized biocatalyst, 150 mL of immobilized glucoamylase was ground in a ceramic mortar that was placed in an ice bath to reduce the particle size to <0.1 mm. Earlier studies *(13)* showed no loss of enzyme activity from the support during the grinding process. The ground enzyme slurry was then added to the gel solution. To this was added 40 g (wet weight) of *Z. mobilis* cell paste. The final solution volume was brought up to 1 L with deionized water. In preparing the immobilized *Z. mobilis* beads, the immobilized glucoamylase was excluded from the gel and 30 g of Fe_2O_3 was added to increase the density of the beads. Bead formation was achieved using a previously developed technique in which the heated gel material was forced through a small nozzle using a peristaltic pump *(16)*. A vibration transducer was attached to the flexible delivery tube. By observing the nozzle exit stream under stroboscopic light, the vibrational frequency was tuned to produce monodispersed droplets. The gel droplets containing glucoamylase and *Z. mobilis* were collected in a stirred vessel containing 0.3 *M* KCl and were allowed to cure for 24 h at 4°C. Following this step, the beads were screened to remove those bigger than 2.8 mm in diameter. The remaining beads were stored in 0.3 *M* KCl at 4°C until use.

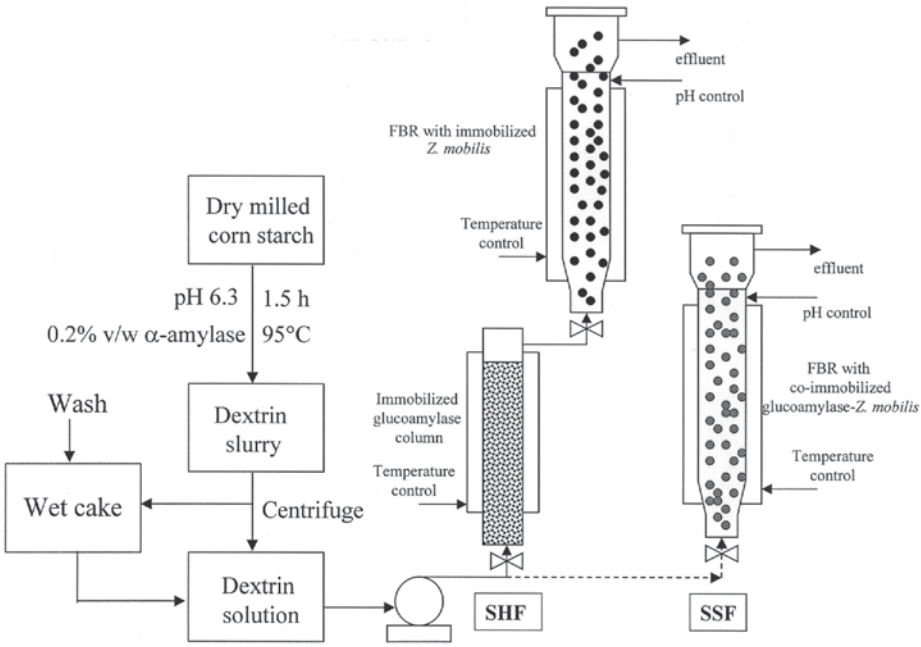

Fig. 1. Process schematic for conversion of dry-milled corn starch to ethanol by the SHF and SSF processes using immobilized biocatalysts. In the SHF process, an immobilized glucoamylase packed-bed reactor and FBR containing immobilized *Z. mobilis* beads are used. In the SSF process, an FBR containing co-immobilized glucoamylase-*Z. mobilis* biocatalyst beads is used.

Measurement of Protein Content in Biocatalyst Beads

The protein content of the fresh and spent beads was measured as follows. Ten mL of beads were mixed vigorously in deionized water. A spatula was used to crush the beads and release the cells. The suspension was centrifuged at 4000 rpm for 5 min and the supernatant was discarded. Ten mL of 1.0 *N* HCl was added to the pellet and thoroughly mixed. The suspension was centrifuged again and the supernatant was discarded. Ten mL of solution (1.0 *N* NaOH + 0.47 wt % NaCl) was added to the pellet and mixed thoroughly in a test tube, which was then placed in a boiling water bath for 15 min to dissolve the proteins. The solution was cooled in water. The suspension was centrifuged, and the supernatant was assayed for protein using the Bio-Rad (Hercules, CA) procedure, which determines protein by the Bradford method. Albumin was used as the standard.

Fluidized-Bed Bioreactor (FBR)

The FBR, as shown in Fig. 1, was a jacketed glass column with 5.1 cm inside diameter and 47 cm in length. The volume of the FBR was 0.9 L. The FBR was cleaned with 50% ethanol and hot water before the biocatalyst beads were loaded. The volume occupied by the beads was 600 mL. The pH

in the upper part of the FBR was controlled at 5.0 using 0.5 *M* NaOH. The base injection was placed very close to the pH probe in order to avoid pH overshoot. The temperature of the FBR was maintained at 35°C when the co-immobilized biocatalyst was used and at 30°C when only immobilized *Z. mobilis* was used.

Z. mobilis was allowed to grow within the beads for 4–5 d. When co-immobilized glucoamylase-*Z. mobilis* beads were used, this was accomplished by pumping feed solutions containing 150 g/L StarDri 100 starch (A. E. Staley Co., Decatur, IL), 0.05 *M* KCl, and 5 g/L yeast extract (Red Star) through the FBR at residence times of 2–3 h. The pH of the feed was adjusted to 5.8 using phosphate buffer. The feed was sterilized by autoclaving at 121°C for 1 h. When immobilized *Z. mobilis* beads were used, the soluble starch in the feed was replaced by 150 g/L dextrose (A. E. Staley Co.).

Following colonization of the ΩMocatalyst beads by the microorganism, feed solutions of maltodextrin with 5 g/L yeast extract or hydrolyzed corn starch (supplemented at times with 15% [v/v] light steep water) were pumped through the FBR to give residence times in the range of 1–4 h. All the feed solutions contained 0.05 *M* KCl for stabilization of the biocatalyst beads. The feed lines were changed when the empty feed reservoir was replaced. For each set of experimental conditions, at least six residence times were allowed for the FBR to reach steady state before samples were analyzed for dextrins, glucose, and ethanol.

Immobilized Glucoamylase Packed-Bed Reactor

The reactor was a jacketed glass column with a 2.54 cm inside diameter and 60 cm in length. The volume of the reactor was 0.3 L. Immobilized glucoamylase occupied 80% of the column volume. The column was operated in the upflow mode, and the temperature was maintained between 50° and 55°C. The pH of the feed was adjusted to 5.0 with phosphoric acid.

Analytical Methods

Dextrins, glucose, and ethanol were analyzed using a high-performance liquid chromatography (HPLC) system consisting of a Waters 410 RI detector, a Waters 717 Plus Autosampler, and an Alltech 425 HPLC pump. The column was an Aminex HPX-87H (Bio-Rad) column. The mobile phase was 5 m*M* H_2SO_4 pumped at a flow rate of 0.6 mL/min. Data acquisition and analysis were performed using the Waters Millennium software. The chromatograms of maltodextrin and hydrolyzed starch samples showed three distinct peaks. These peaks were quantified using pure maltotetraose, maltotriose, and maltose standards (Sigma Chemical Company, St. Louis, MO). Maltodextrin consisted of 91.9% (w/w) maltotetraose, 5.7% (w/w) maltotriose, and 2.4% (w/w) maltose. Maltotetraose was the predominant component of the hydrolyzed starch samples as well. The dextrin concentration was determined by adding the concentrations of all three components.

Results and Discussion

Activity of Co-Immobilized Biocatalyst Beads

A previous study with co-immobilized glucoamylase-*Z. mobilis* biocatalyst *(13)* showed that a maximum ethanol productivity of 37 g/L-h was achieved. However, the productivity declined with time even when the same experimental conditions were maintained. This prompted us to investigate the activity of the biocatalyst over an extended time period. A repeated batch experiment was conducted using maltodextrin as the substrate prior to experiments in the FBR. Each batch cycle lasted approximately 24 h. A total of 22 cycles were carried out. Samples were taken at periodic intervals to analyze for maltodextrin, glucose, and ethanol. The batch media consisted of 100 g/L maltodextrin, 25% (v/v) light steep water (nutrient source), and 0.05 *M* KCl. The experiment was conducted in a 250-mL Erlenmeyer flask at 35°C, 100 rpm, and pH 5.0. The diameter of the beads was between 1 and 2 mm. The flask contained 20 mL of biocatalyst beads in 100 mL of medium. At the end of each cycle, the beads were recovered and washed with deionized water before being placed in fresh medium for the next batch cycle. The pH of the fermentation medium dropped from 5 to 4.3 (on an average) during each batch run. Maltodextrin conversions and ethanol concentrations at the end of each cycle were in the range of 80–95% and 37–48 g/L, respectively. Average ethanol yield obtained was 0.46 g ethanol/g maltodextrin (this corresponds to 81% of the theoretical yield). Typical of batch processes, productivities of 3 g/L-h were obtained.

The cell loadings of the used beads (after 22 batch cycles) and fresh beads were determined by measuring their protein content. The protein contents of the fresh and used beads were 0.352 g/L and 0.444 g/L, respectively. This 26% increase in protein content indicated significant growth of *Z. mobilis* within the biocatalyst beads. For comparison of maltodextrin conversion, the cells in used and fresh beads were inactivated by treatment with 40% (w/v) ethanol. Under these conditions, the beads retained only the glucoamylase activity. Following inactivation of the immobilized *Z. mobilis*, the beads were tested for maltodextrin hydrolysis in batch experiments at 35°C and pH 5.0. The results of these experiments are shown in Fig. 2. Almost identical concentration profiles of maltodextrin and glucose were obtained with the fresh and used beads, indicating that the growth of *Z. mobilis* within the beads did not restrict the diffusion of the substrate into these beads. In addition, the data showed that the immobilized glucoamylase was stable and retained its activity over a period of 22 d at 35°C.

The biocatalyst beads were also tested in a batch experiment (one cycle) using the same medium as described previously, but at pH 4.0. The objective of this experiment was to determine whether it would be feasible to operate the FBR without pH control. The initial maltodextrin concentration was 91.4 g/L, and at the end of 23 h, an 80% maltodextrin conversion was obtained. However, only 0.8 g/L ethanol was produced and up to 76.7 g/L

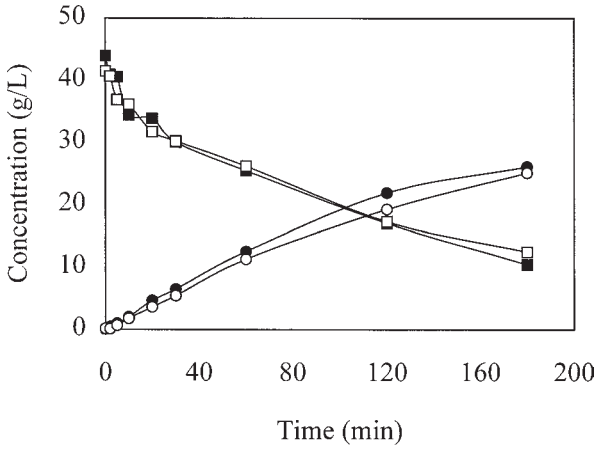

Fig. 2. Comparison of maltodextrin and glucose concentration profiles with fresh and used (through 22 batch cycles) co-immobilized biocatalyst beads. —■—, maltodextrin-fresh beads; —□—, maltodextrin-used beads; —●—, glucose-fresh beads; —○—, glucose-used beads.

glucose accumulated in the medium. Although the *Z. mobilis* cells showed very little activity, they were not irreversibly deactivated. This was verified qualitatively by the evolution of CO_2 when these beads were washed and resuspended in the medium at pH 5.0. These results indicate that a decline in ethanol production in the continuous FBR will occur when a pH of 5.0 is not maintained.

Simultaneous Saccharification and Fermentation (SSF)

In these experiments, both synthetic maltodextrin solutions and dextrin solutions obtained by hydrolysis of dry-milled corn starch were used as feeds for the FBR. In the latter case, a 15% dry-milled corn-starch feed was hydrolyzed to dextrins using α-amylase. After solids separation and washing of the wet cake, the solids-free feed was used in the FBR experiments. The results for synthetic maltodextrin and corn-starch hydrolyzate feeds are summarized in Table 1.

For synthetic maltodextrin, two feed concentrations of 108.7 and 188.1 g/L were used. These feeds are representative of the dextrins concentration obtained by the hydrolysis of 15 and 28% dry-milled corn starch, respectively. Yeast extract (5 g/L) was provided as a nutrient source. For the 108.7 g/L feed, a single-pass conversion of 66.6% was achieved at a residence time of 2.2 h. However, for a 188.1 g/L feed, the single-pass conversion was 31.3% at a similar residence time (1.9 h). By increasing the residence time to 3.7 h, the maltodextrin conversion improved to 53.1%. Volumetric ethanol productivities in the range of 12–15 g/L-h were obtained. Average ethanol yields of 0.47 g ethanol/g starch were obtained, corresponding to 84% of the theoretical yield (0.56 g ethanol/g starch).

Table 1
Simultaneous Saccharification and Fermentation (SSF) of Maltodextrin and Dextrins from Dry-Milled Corn Starch to Ethanol Using Co-Immobilized Glucoamylase-*Z. mobilis* in the FBR

Feed (g/L)	Dilution rate (l/h)	Conversion (%)	Ethanol (g/L)	Productivity (g/L-h)
Synthetic maltodextrin				
108.7	0.46	66.6	32.1	14.8
188.1	0.54	31.3	28.0	15.1
188.1	0.27	53.1	45.3	12.2
Dextrins in 15% starch hydrolyzate				
95.9	0.66	53.6	22.9	15.1
99.3	0.54	63.2	27.7	15.0
104.0	0.42	66.9	32.6	13.7
100.3	0.25	89.3[a]	36.44	9.1

[a]Glucose in the effluent during this run was 11.7 g/L owing to failure of the pH controller. The pH dropped to 4.60 before it was readjusted to 5.0.

For the starch hydrolyzate feed, no additional nutrients were supplied. The single-pass conversions ranged from 54–89% and volumetric ethanol productivities were in the range of 9.1–15.1 g/L-h. Average ethanol yields were identical to those obtained with the synthetic maltodextrin feed.

The above experiments with synthetic maltodextrin and starch hydrolyzate feeds were performed continuously over a period of 22 d. During this time period, no structural failure of the biocatalyst was observed. In all the experimental runs, steady-state glucose concentrations in the effluent remained at low levels (below 4 g/L). It was also observed that the glucose concentration in the effluent increased in the absence of pH control (i.e., when the pH dropped below 5.0). The steady-state data shown in Table 1 indicates that the hydrolysis of soluble starch to glucose was the rate-limiting step. The glucoamylase used has the highest activity at a temperature range of 55–65°C *(14)*. The experiments were performed at 35°C to avoid thermal inactivation of the immobilized *Z. mobilis*. At this temperature, the enzyme activity is only 16% of the maximum activity *(14)*. In order to achieve higher conversion of the hydrolyzed starch to ethanol, longer residence times are needed. This can be accomplished by either increasing the column length or decreasing the feed flow rates. However, at high residence times the chances of contamination during continuous operation of the FBR increase and channeling of the feed occurs owing to poor fluidization. A cascade arrangement of FBRs can also be used. Besides providing greater residence time, this configuration can also help in the disengagement of the CO_2. This can lower the gas hold-up in the FBR, thereby improving mass transfer between the substrate in the liquid phase and the biocatalyst beads.

Table 2
Fermentation of Glucose Obtained by Enzymatic Hydrolysis of Dextrins from Dry-Milled Corn Starch to Ethanol Using Immobilized *Z. mobilis* in the FBR

Glucose (g/L)	Dilution rate (1/h)	Conversion (%)	Ethanol (g/L)	Productivity (g/L-h)
162.4	0.50	94.2	70.3	35.2
162.4	0.64	59.3	45.3	29.0
153.6[a]	0.66	75.0	48.6	32.1
172.1	1.0	47.7	32.6	32.6
162.9[a]	1.1	75.6	47.2	51.9

[a]In these runs, 15% (v/v) light steep water was added to the feed to provide an additional nutrient source.

Separate Hydrolysis and Fermentation (SHF)

By carrying out the dextrins hydrolysis and fermentation steps sequentially, it is possible to perform both steps under more optimal conditions. Therefore, this process configuration was also investigated.

Solids-free solution of 150–160 g/L dextrins (obtained by hydrolysis of 28% dry-milled corn starch) at pH 5.0 was fed to the immobilized glucoamylase column at a rate of 0.3 L/h, thereby giving a residence time of 1 h. The temperature of the column was maintained at 55°C. The conversion of soluble starch to glucose was greater than 95% and gave a product stream of 162–172 g/L glucose. This glucose product stream from the immobilized enzyme column was used as the feed for the FBR containing immobilized *Z. mobilis* at 30°C. The pH of the feed to the FBR was adjusted to 5.8 using 50% (w/v) NaOH, and 0.05 *M* KCl was added for stabilization of the beads.

Table 2 summarizes the glucose feed concentrations (from the immobilized enzyme column) and the ethanol concentrations obtained at different dilution rates. The results are also plotted in Fig. 3. At a residence time of 2 h, a 94.2% single-pass conversion of the glucose feed and an effluent ethanol concentration of 70.3 g/L were achieved at steady state without adding any additional nutrients in the feed. At shorter residence times of 1.56 h and 1 h, the glucose conversion declined to 59.3 and 47.7%, respectively. Nutrient limitation was shown to be a possible explanation for this observation. Upon use of 15% (v/v) light steep water as an additional nutrient source, improvements in glucose-feed conversion to 75.0 and 75.6% were achieved at residence times of 1.5 and 0.9 h, respectively. These results showed that nutrient-limitation effects could limit feed conversions at short residence times in the continuous FBR. An average ethanol yield of 0.45 g ethanol/g glucose was obtained in the aforementioned experiments.

The performance of the immobilized *Z. mobilis* beads in the FBR was also investigated after switching to synthetic glucose feeds consisting of

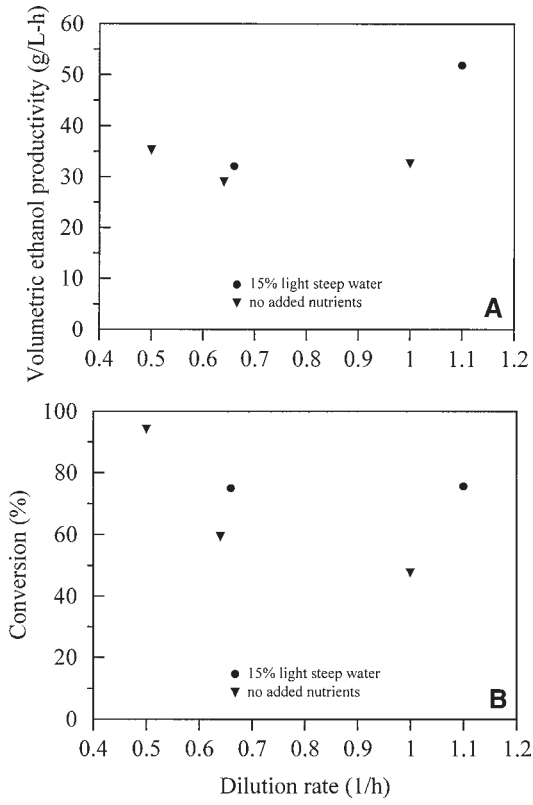

Fig. 3. **(A)** Volumetric ethanol productivities and **(B)** glucose feed conversions as a function of dilution rate for the SHF process.

Table 3
Fermentation of Synthetic Glucose Feed to Ethanol
Using Immobilized *Z. mobilis* in the FBR

Synthetic glucose (g/L)	Dilution rate (1/h)	Conversion (%)	Ethanol (g/L)	Productivity (g/L-h)
167.9	1.1	70.8	48.4	53.2
166.5	1.0	82.2	68.8	68.8
165.5	0.73	90.4	63.6	46.4
167.9	0.68	90.7	63.6	43.3
172.3	0.67	91.4	64.8	43.4
165.5	0.56	94.1	67.3	37.7
165.5	0.54	93.6	65.8	35.5
167.9	0.51	98.2	64.9	33.1

165–172 g/L glucose and 5 g/L yeast extract as a nutrient source. The process performance at different residence times in the FBR is summarized in Table 3. The results are also plotted in Fig. 4. Single-pass glucose conversions ranged from 98.2% at a residence time of 2 h to 70.8% at a residence

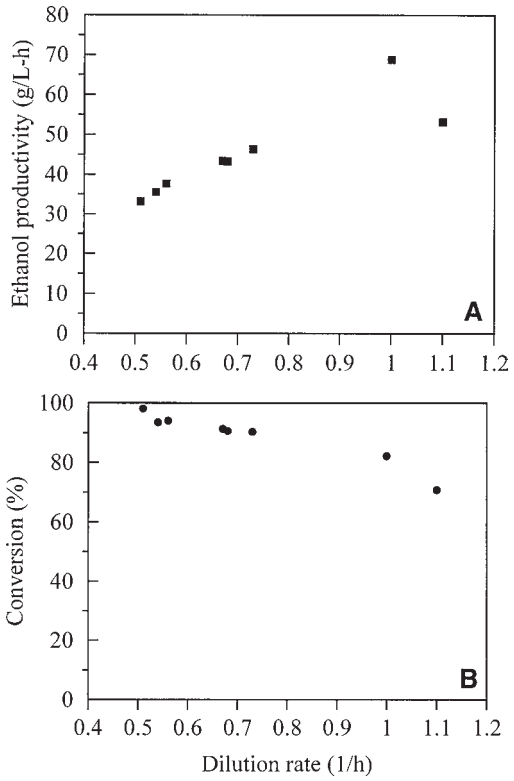

Fig. 4. **(A)** Volumetric ethanol productivities and **(B)** synthetic glucose feed conversions as a function of dilution rate for the SHF process.

time of 0.9 h. These results were comparable with those obtained with the hydrolyzed dextrins feed supplemented with 15% (v/v) light steep water. The highest volumetric ethanol productivity achieved was 68.8 g/L-h at a residence time of 1 h with 82.2% conversion of the synthetic glucose feed. An average ethanol yield of 0.43 g ethanol/g glucose was achieved in these experiments.

To control the pH in the FBR, a dilute NaOH solution (0.5 *M*) was used to avoid overshoot. The use of this dilute solution caused some dilution effect in the effluent owing to periodic injection of the base. Based on daily base consumption, it is estimated that the effluent was diluted between 5 and 10%. This might be the cause for the apparently low ethanol yield because lactic acid (<5 g/L) was the only other product detected in the effluent samples. It should also be noted that in some of these experimental runs, ethanol yields in the range of 0.47–0.49 g ethanol/g glucose (corresponding to 92–97% of the theoretical yield) have been obtained. This is in agreement with the yields reported by Davison and Scott *(5)*. The FBR with immobilized *Z. mobilis* beads was run continuously with synthetic glucose and hydrolyzed dextrins feeds over a period of 27 d without structural failure of the beads.

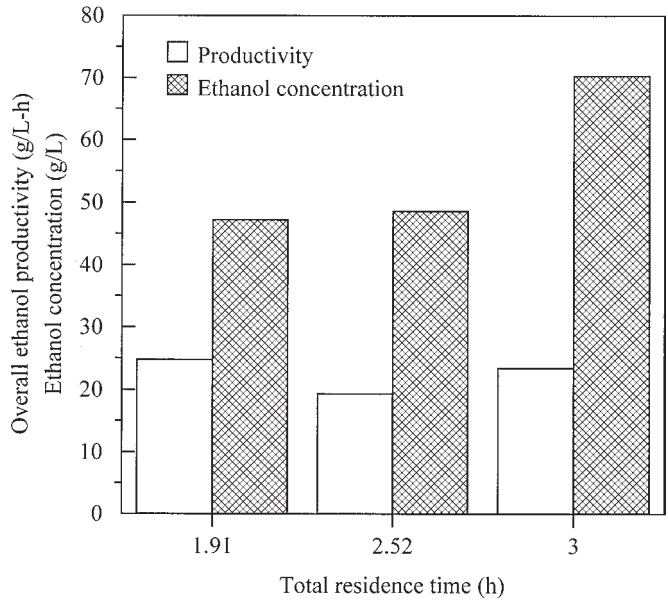

Fig. 5. Overall volumetric ethanol productivities and ethanol concentrations obtained from hydrolyzed corn-starch feed by the separate hydrolysis and fermentation (SHF) process using immobilized glucoamylase and *Z. mobilis*. Results are plotted as a function of the total residence time.

The overall volumetric ethanol productivity for the process was calculated by taking into account the residence time of the feed in the immobilized glucoamylase packed column and in the FBR. The overall productivities and ethanol concentration as a function of total residence time for the hydrolyzed starch feed is plotted in Fig. 5. At a total residence time of 3 h, a steady-state effluent ethanol concentration of 70.3 g/L was achieved at a feed conversion of 94.2%, resulting in an overall productivity of 23.4 g ethanol/L-h. This is a substantial improvement over the volumetric ethanol productivity obtained in typical industrial batch processes (2–3 g/L-h). These results also show that higher productivities and ethanol concentrations were achieved in the continuous SHF process in comparison with the continuous SSF process in the FBR.

Conclusions

Both SSF and SHF of dry-milled corn starch to ethanol were performed using immobilized glucoamylase and *Z. mobilis*. In the continuous SSF process using the co-immobilized biocatalyst, the hydrolysis of dextrins to glucose was the rate-limiting step, primarily owing to the low activity of glucoamylase at 35°C. In the SHF process, it was possible to perform both steps at their optimum temperatures of 55° and 30°C, respectively. Significant improvements in volumetric ethanol productivity were obtained as compared with traditional batch processes. An economic analysis of both

processes is being performed to determine the feasibility of scale-up and commercialization of the technology.

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