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M. S. Krishnan, N. P. Nghiem** and B. H. Davison

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Bioprocessing Research and Development Center
Oak Ridge National Laboratory
Bldg. 4505, MS 6226
Oak Ridge, TN 37831-6226
423-574-1184; ndp@ornl.gov

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**Author to whom all correspondence should be addressed.

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ETHANOL PRODUCTION FROM DRY-MILL CORN STARCH IN A FLUIDIZED-BED BIOREACTOR

M. S. Krishnan, N. P. Nghiem and B. H. Davison

Oak Ridge National Laboratory, Chemical Technology Division,
Oak Ridge, TN 37831-6226

ABSTRACT

The development of a high-rate process for the production of fuel ethanol from dry-mill corn starch using fluidized-bed bioreactor (FBR) technology is discussed. Experiments were conducted in a laboratory scale FBR using immobilized biocatalysts. Two ethanol production process designs were considered in this study. In the first design, simultaneous saccharification and fermentation was performed at 35°C using κ-carageenan beads (1.5 mm to 2.5 mm in diameter) of co-immobilized glucoamylase and *Zymomonas mobilis*. For dextrin feed concentration of 100 g/L, the single-pass conversion ranged from 54% to 89%. Ethanol concentrations of 23 to 36 g/L were obtained at volumetric productivities of 9 to 15 g/L-h. No accumulation of glucose was observed, indicating that saccharification was the rate-limiting step. In the second design, saccharification and fermentation were carried out sequentially. In the first stage, solutions of 150 to 160 g/L dextrins were pumped through an immobilized glucoamylase packed column maintained at 55°C. Greater than 95% conversion was obtained at a residence time of 1 h, giving a product of 165 to 170 g glucose/L. In the second stage, these glucose solutions were fed to the FBR containing *Z. mobilis* immobilized in κ-carageenan beads. At a residence time of 2 h, 94% conversion and ethanol concentration of 70 g/L was achieved, giving an overall productivity of 23 g/L-h.

Keywords: Ethanol, *Zymomonas mobilis*, glucaomylase, dry-mill corn starch, fluidized-bed reactor.

OVERVIEW

Corn starch is the predominant feedstock for industrial ethanol production. Batch and fed-batch processes for fuel ethanol production from corn starch have a low volumetric productivity of 2 to 5 g ethanol/L-h (Bajpai and Margaritis, 1985). The use of continuous systems having high biocatalyst loadings along with some form of biocatalyst retention mechanism can improve ethanol productivities compared to traditional batch systems. Some of these methods include cell recycle by filtration, sedimentation, entrapment in

membranes or entrapment in gels. These biocatalysts can then be used in different reactor configurations like continuous-stirred tank, packed-bed, and fluidized-bed reactors for improving volumetric productivity. Volumetric ethanol productivity (total reactor volume basis) for continuous systems with high conversion is in the range of 6-8 g/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 packed bed reactor with immobilized cells, and 50-120 g/L-h for an immobilized cell fluidized-bed reactor (FBR) (Davison and Scott, 1988). In this work, an FBR configuration with immobilized biocatalysts was used. Apart from its plug flow characteristics, an FBR also provides effective mass transport by overcoming channeling and carbon dioxide buildup; problems that are encountered in packed bed reactors. The economic impact of the FBR for ethanol production from glucose has been estimated to be 6 cents/gallon (Harshbarger et al., 1995).

In this paper, results of ethanol production from dry-mill corn starch by the simultaneous saccharification and fermentation (SSF) process and the separate hydrolysis and fermentation (SHF) process using immobilized biocatalysts are reported and compared. In the SSF process, a co-immobilized glucoamylase-*Z. mobilis* biocatalyst was used in an FBR. In the SHF process, immobilized glucoamylase was used in a packed bed reactor and immobilized *Z. mobilis* was used in an FBR.

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, New Jersey) at 30°C and pH 5. The seed culture media contained 50 g/L glucose, 5 g/L Tasteone 900AG yeast extract (Red Star, Juneau, Wisconsin), and 5 g/L KH_2PO_4. The seed culture (4 L) 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, Pennsylvania). The cell pastes were stored at 4°C until ready for use in the immobilization step.

Enzymes

Starch liquefaction and dextrinization was performed using a thermostable α-amylase supplied by Morris Ag-Energy, (Morris, Minnesota). Glucoamylase immobilized on porous diatomaceous earth as a support was supplied by Genencor International (Elkhart, Indiana). 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 units/g on a dry weight basis (Lantero, et al., 1995). One unit of activity represents the amount of enzyme that will produce one micromole of glucose in one minute under the assay conditions.

Corn Starch Hydrolysis

Dry-milled corn starch (13% moisture content) was supplied by Morris Ag-Energy (Morris, Minnesota). 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 with 150 ppm $Ca(OH)_2$ for 1.5 hours. 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. In both cases, 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, Connecticut) 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. 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.

Biocatalyst Bead Preparation

40 grams κ-carageenan (FMC Corporation, Rockland, Maine) was dissolved 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 (Sun et al., 1998) 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 (on which vibrational frequency is imposed) using a peristaltic pump (Scott et al., 1988). The gel beads 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.

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. 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. 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.

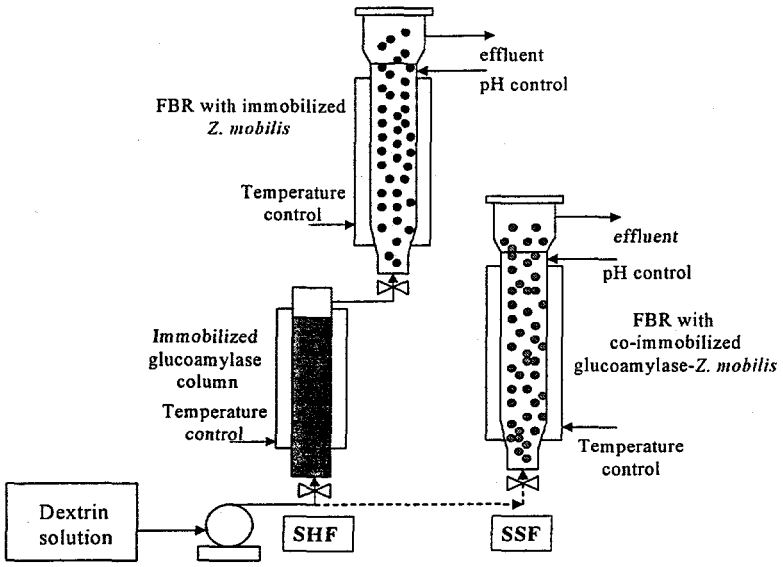

Figure 1. Process Schematic of SHF And SSF Processes for Ethanol Production from Dry-Milled Corn Starch.

Immobilized Glucoamylase Packed-Bed Reactor

The reactor was a jacketed glass column with a 2.54 cm inside diameter and 60 cm 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 dextrins feed was adjusted to 5 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 (BioRad Laboratories, Hercules, California) column. The mobile phase was 5 mM H_2SO_4 pumped at a flow rate of 0.6 mL/min. Data acquisition and analysis were performed using the Waters Millenium software.

RESULTS AND DISCUSSION

Simultaneous Saccharification and Fermentation (SSF)

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

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

Feed (g/L)	Dilution rate (h^{-1})	Conversion (%)	Ethanol (g/L)	Productivity (g/L-h)
Dextrins				
95.9	0.66	53.6	22.9	15.1
99.3	0.54	63.2	27.7	15.0
104	0.42	66.9	32.6	13.7
100.3	0.25	89.3[a]	36.44	9.1
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

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

For the 15% starch hydrolyzate feed , no additional nutrients were supplied. The single-pass conversions ranged from 54 to 89% and volumetric ethanol productivities were in the range of 9.1 to 15.1 g/L-h. 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). For synthetic maltodextrin, two feed concentrations of 108.7 g/L 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 to 15 g/L-h were obtained. Average ethanol yields were identical to those obtained with the starch hydrolyzate feed. The above experiments with starch hydrolyzate and synthetic maltodextrin feeds were performed continuously over a

period of 22 days. 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 below 4 g/L. 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 (Product data sheets, Genencor International, Elkhart, Indiana). Since the maximum temperature at which *Z. mobilis* can ferment glucose to ethanol efficiently is 35°C, the continuous SSF was also carried out at 35°C. Consequently, the SSF process suffers from low enzyme activity. 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. 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.

Separate Hydrolysis and Fermentation (SHF)

This process configuration allows the dextrin hydrolysis and fermentation steps to be carried out sequentially, thus making it possible to perform both steps under more optimal conditions. Solids-free solution of 150-160 g/L dextrins (obtained by hydrolysis of 28% dry-milled corn starch) at pH 5 was fed continuously to the immobilized glucoamylase column (maintained at 55°C) with a residence time of 1 h. 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.

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	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.

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. 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 h and 0.9 h, respectively. An average ethanol yield of 0.45 g ethanol/g glucose was obtained in the above experiments.

The performance of the immobilized *Z. mobilis* beads in the FBR was also investigated after switching to synthetic glucose feeds supplemented with 5 g/L yeast extract as a nutrient source. The process performance at different residence times in the FBR is summarized in Table 3.

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	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

Single-pass glucose conversions ranged from 98.2% at a residence time of 2 h to 70.8% at a residence 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 1h 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.

In order to avoid pH overshoot, the pH in the FBR was controlled by using a dilute NaOH solution (0.5 M). This caused some dilution effect in the effluent due to periodic injection of the base. Based on daily base consumption, it is estimated that the effluent was diluted between 5-10%. This might be the cause for the apparently low ethanol yield since lactic acid (<5 g/L) was the only other product detected in the effluent samples. The FBR with immobilized *Z. mobilis* beads was run continuously with synthetic glucose and hydrolyzed dextrins feeds over a period of 27 days without structural failure of the beads.

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. At a total residence time of 3 h, a steady-state effluent ethanol concentration of 70.3 g/L was achieved with a feed conversion of 94.2%, resulting in an overall productivity of 23.4 g ethanol/L-hr. This is a significant improvement over the volumetric ethanol productivity achieved 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. Current efforts aim at improvements in process performance, evaluation of process economics and scale-up in pilot-scale bioreactors.

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