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Enzyme Recovery and Recycling Following Hydrolysis of Ammonia Fiber Explosion–Treated Corn Stover

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Abstract

Both cellulase and cellobiase can be effectively recovered from hydrolyzed biomass using an ultrafiltration recovery method. Recovery of cellulase ranged from 60 to 66.6% and for cellobiase from 76.4 to 88%. Economic analysis shows that cost savings gained by enzyme recycling are sensitive to enzyme pricing and loading. At the demonstrated recovery of 60% and current loading of 15 Filter paper units of cellulase/g of glucan, enzyme recycling is expected to generate a cost savings of approx 15%. If recovery efficiency can be improved to 70%, the savings will increase to >25%, and at 90% recovery the savings will be 50%.

Index Entries: Enzyme recycle; biomass; ammonia fiber explosion; ethanol; corn stover.

Introduction

Enzyme cost is one of the primary expenses in a biomass-to-ethanol process. Leading enzyme development companies are focusing efforts on reduction of enzyme-manufacturing costs; however, even at estimated cost reduction levels (10- to 12-fold reduction of current cost of $5/gal of ethanol), enzymes still represent approx $0.40–$0.50/gal of ethanol produced *(1,2)*. The ammonia fiber explosion (AFEX) process treats biomass with liquid ammonia at elevated pressure in a batch or continuous-flow reactor and then flashes the ammonia and biomass out explosively *(3)*. In this process, the combination of the chemical effect of ammonia pretreatment and the physical effect of rapid pressure release causes the ammonia to "boil" violently, thereby disrupting and expanding the accessible surface area of the biomass. This disruption and the increase in the accessible surface area have been shown to enhance the susceptibility of biomass to enzymatic hydrolysis *(4)*.

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Several investigators have studied the technical feasibility of enzyme recovery and recycling *(5–8)* during biomass hydrolysis. The objective of the present study was to evaluate methods to recover and recycle enzymes for hydrolysis of AFEX-treated biomass and determine the economic contribution of recovery and recycle to overall process economics in a biomass-to-ethanol process. Two recovery methods were examined: (1) a method using ultrafiltration, and (2) recovery by soaking with fresh biomass. Binding of cellulase to the biomass could lead to loss of enzyme during filtration; therefore, we examined the effect of adding low concentrations of a nonioinic surfactant to the hydrolysis, a process that was shown to enhance recovery of enzyme and increase yield by other researchers *(9,10)*. This is believed to be owing to the surfactant reducing the nonspecific binding of the enzyme.

Materials and Methods

AFEX Process and Equipment

The AFEX Reactor is a 1-gal high-pressure reactor manufactured by Parr (Moline, IL). It is rated for use up to 1900 psig at 350°C. The head, cylinder, internal wetted parts, external fittings, and magnetic drive are all T316 stainless steel. The reactor has a variable-speed, 0.5-hp motor with a 5:1 gear drive. It is fitted with an aluminum block heater with cooling capabilities. A controller maintains the temperature and agitation set points. Temperature, pressure, and agitation are recorded on a data logger.

The ammonia batch injection system provided by American Lea (Holliston, MA) consists of a heat exchanger, a diaphragm-metering pump, and a Coriolis-type flow sensor. The heat exchanger is designed to cool the ammonia to keep it in liquid form. The diaphragm-metering pump has a 0.5-hp, 1730-rpm motor. The ammonia flash tank is a 500-L pressure vessel designed to flash the ammonia. The tank is T-316L stainless steel and is rated for 30 psig at 250°F.

The raw material was corn stover composed of 36% cellulose, 21% xylan, and 18% lignin. Corn stover was premixed with water to 60% moisture, preheated to 70°C in the 1-gal reactor. Anhydrous ammonia was added to provide a 1:1 ammonia/dry biomass mass ratio. The mixture was further heated to 90°C and allowed to react for 30 min. The pressure was then quickly released to 15 psig. The wet biomass was dried under atmospheric condition for over 12 h and stored at 4°C.

Biomass Pretreatment and Hydrolysis

Three similarly treated (AFEX) lots (60% moisture; treatment time of 30 min at 90°C; ammonia loading of 1.5 g/g of biomass) of corn stover were mixed and samples taken (average moisture content of 9.05%). The pretreated corn stover was stored at 4°C until use. Enzymatic hydrolysis was performed in 200-mL shake flasks at 50°C for 24 h using 15 Filter

paper units (FPU) of cellulase (Spezyme CP; Genencor, Cedar Rapids, IA)/g of glucan and 40 cellobiase units (CBU) of cellobiase (Novo 188; Novo, Wilton, CT)/g of biomass in 50 m*M* citrate buffer, pH 5.0, with 5% solid loading (5 g of dry stover/100 mL of solution). One-milliliter samples of the hydrolysate were collected following hydrolysis and analyzed.

Enzyme Assays

Cellobiase (β-glucosidase) was assayed by the method of Zaldivar et al. *(11)* with some modifications. The substrate consisted of 5.0 mg of cellobiose (Sigma, St. Louis, Mo) in 1 mL of 50 m*M* citrate buffer, pH 5.0. Enzyme (0.1 mL) was added to 0.5 mL of substrate and incubated for 10 min at 50°C.

Cellulase (β-1,4-endoglucanase) was assayed by the method of Konig et al. *(12)* with some modifications. The substrate consisted of 10 mg of carboxymethylcellulose (Sigma) in 1 mL of 50 m*M* citrate buffer, pH 5.0. Enzyme (0.1 mL) was added to 0.5 mL of substrate and incubated for 10 min at 50°C.

The reaction in both assays was stopped by placing the assay mixture in a boiling water bath for 5 min. Samples were stored on ice for glucose analysis. Enzyme controls (enzyme + buffer) and substrate controls (substrate with no enzyme) were run with each sample. All analytical work was performed in triplicate. Glucose was analyzed using a YSI Biochemistry Analyzer (YSI, Yellow Springs, OHi). The glucose concentrations of the controls were subtracted from the treatment sample glucose concentrations to yield the actual glucose released for each sample. Units of enzyme activity are defined as micromoles of glucose released per minute per milliliter of enzyme.

Enzyme Recovery by Ultrafiltration

The protocol for enzyme recovery is shown in the process flow diagram of Fig. 1. The filtrate from the initial filtration (Whatman GF/D glass fiber filter) is further processed by ultrafiltration (polyethersulfone 76-mm, 10,000-Dalton membrane; Millipore, Bellerica, MA). The ultrafiltration retentate was combined with the wash stream from the glass fiber filtration and recycled for fresh biomass hydrolysis. Fresh enzyme was added to the recycled enzyme to achieve standard enzyme-loading conditions. This recycle was repeated three times. The ultrafiltration filtrate contained the hydrolyzed sugars. Two treatments were examined. Treatment A consisted of enzymatic hydrolysis in the presence of Tween-20 (ICI Americas, Bridgewater, N J) added at 0.1% loading by weight to the hydrolysis mix. Treatment B was a standard hydrolysis mix with no surfactant added.

Recovery by Soaking Method

Recovery by soaking fresh biomass with the enzyme stream was performed as described by Moniruzzaman et al. *(8)* with minor modifications. A mix (50 mL) of cellulase and cellobiase was added to AFEX-treated

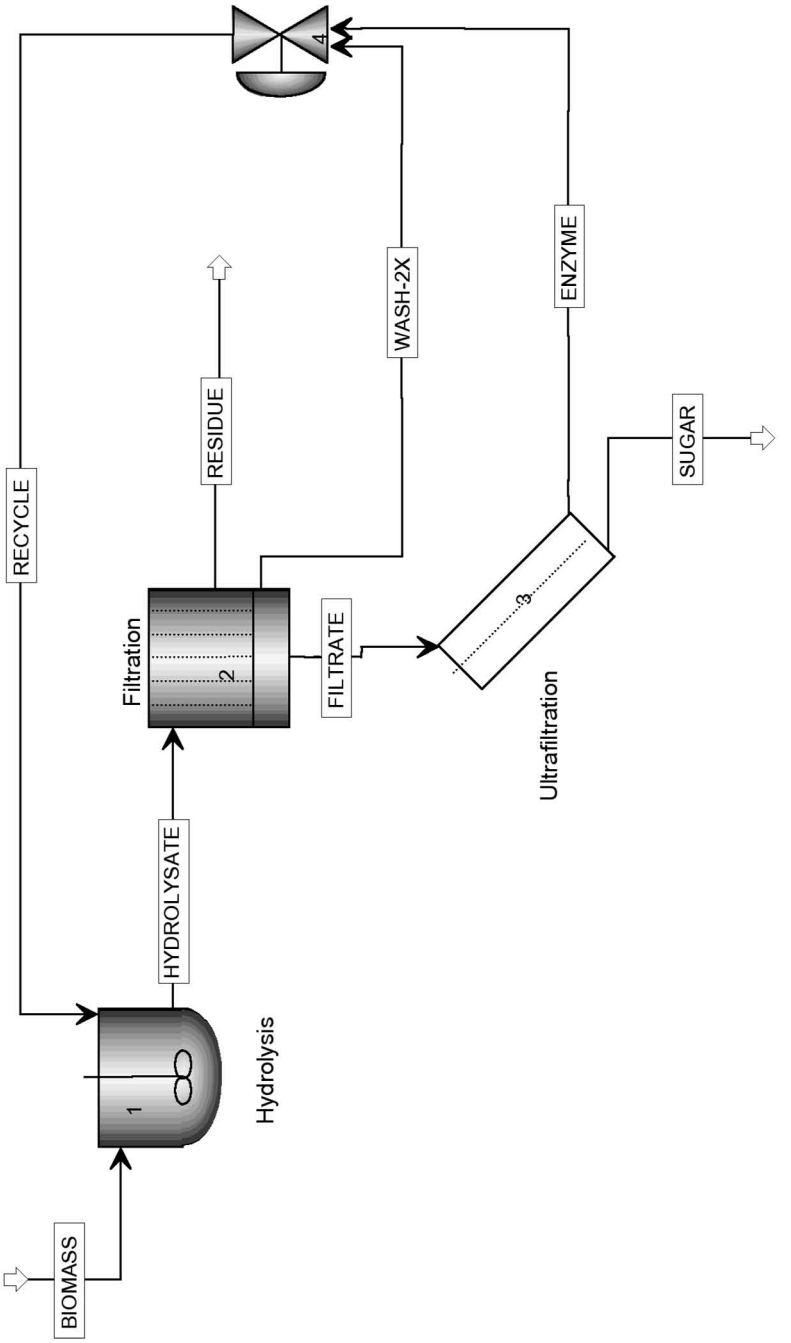

Fig. 1. Process flow diagram of enzyme recovery process using ultrafiltration.

corn stover at concentrations equivalent to the standard loading for hydrolysis. This mixture was incubated for 15 min at room temperature with mixing. Following incubation the enzyme plus biomass was filtered using a glass fiber filter under vacuum. This experiment was conducted with both (1) fresh enzymes and (2) recycled enzymes with fresh enzyme added to achieve standard loading concentrations. All fluid volumes were recorded and the percentage of fluid recovery was calculated.

Data Analysis

For both methods of enzyme recovery, the percentage of recovery of enzyme was calculated based on enzymatic activity as determined on the individual samples. The amount of activity of each sample was expressed as a ratio of sample activity/initial activity × 100 = percentage of activity recovered. All experiments were performed in duplicate and all analyses in triplicate. The reported values are the mean of sample replicates ±SD.

Economic Modeling

An ASPEN Plus–based (Aspen, Cambridge, MA) process model (MBI dry mill ethanol process model) was previously developed to evaluate technical and economic performance of ethanol production from AFEX-treated biomass. Basic engineering and economic parameters have been established for a 50-million-gallon-per-year ethanol process. Table 1 summarizes the design basis and model assumptions. Table 2 provides all the raw materials used for the process. Simulation runs provided the mass and energy balances for the process. Ethanol production rate is 52.8 million gallons /yr and the annual revenue is $65,434,496. The cost contribution of the enzymes (no recycle) toward ethanol production costs is dependent on the unit cost of enzymes assumed in the model. A price assumption of $0.05/lb of cellulase results in a cost of $0.12/gal of ethanol.

The stover is directly fed to the AFEX Reactor. The model AFEX Reactor is an extruder that mixes the stover and recycled liquid ammonia. Recycled ammonia is added to adjust the ammonia:dry biomass weight ratio to 1:1. The stover and liquid ammonia mixture is depressurized into an AFEX knockout drum. The liquids flash and the stover is exploded. The overhead vapor is compressed to 150 psig, condensed to a liquid, and then stored. A multistage centrifugal compressor is used. The treated solids were hydrolyzed using cellulase and cellobiase enzymes for the required period.

For this study the base case model was modified for enzyme recovery and recycle. Table 3 provides the design basis for the enzyme recycle model. Enzymes are recovered from the hydrolysate following a 24-h hydrolysis. As shown in Fig. 1. the first step in the process is prefiltration of solid residue from the sugar and enzyme fractions. The sugars are separated from the enzymes using ultrafiltration. The recovered enzymes are mixed with wash water and freshly made-up enzymes and recycled to the hydrolysis tank. Sugar solution is concentrated using a multiple-effect

Table 1
Design Basis and Assumptions for an AFEX-Treated Corn Stover–to–Ethanol Process

Technical assumption	Value
Cellulose conversion (%)	80
Xylan conversion (%)	50
Ethanol yield (w/w dry biomass)	0.21
Ethanol yield (gal/dry t)	62.8
Maximum yield (gal/dry t)	91.0
Ammonia loading (w/w dry biomass)	1
Ammonia loss (% of feed ammonia)	1.5
Moisture loading (w/w dry biomass)	1.5
AFEX temperature (°C)	90
AFEX pressure (psi)	300
AFEX reaction time (min)	30
Cellulase loading (FPU/g glucan)	15
Cellobiase loading (CBU/g biomass)	40

Table 2
Raw Material Consumption in AFEX-Treated Corn Stover–to–Ethanol Process

Material	Amount (kg/h)
Corn stover	100,000
Clarifier polymer	28
Ammonia	1275
Liquid feed syrup	42,500
Cellulase	6630
Cellobiase	11,900
Ammonium phosphate	1700
Potassium phosphate	1700
NaOH	850
Boiler chemicals	1
CT chemicals	2
Wastewater chemicals	3
Wastewater polymers	0.2

evaporator with vapor recompression and converted into ethanol in a fermentor using yeast or bacteria.

Based on the (PFD) process flow diagram just described, a simple process and equipment design analysis was performed to determine the sizing and pricing for each piece of equipment required. Installation factors for equipment were estimated based on MBI's dry mill ethanol process model. Utilities used in the process are electricity, steam, process water, and deionized water. Energy utilization was estimated using standard energy balance calculations.

Table 3
Design Basis and Assumptions for Enzyme Recycle Model with Ultrafiltration

50 mmgal/yr ethanol plant
Recycle process replenished every 3 mo
Overall cellulase recycle = 60%
Overall cellobiase recycle = 80%
Ethanol plant–based capital estimate
Glucose yield = 80%
Xylose yield = 50%
Ethanol plant–based utilities
$5/MCF of gas and $0.05/kWH
Cellulase cost range = $0.05 to $2/lb of enzyme
Cellobiase cost range = $0.10 to $4/lb of enzyme
Cellulase loading = 15 or 3 FPU/g of glucan
Cellobiase loading = 40 or 0 CBU/g of biomass
Ultrafiltration membrane life = 2 yr
Ultrafiltration scaling factor = 0.66
Deionized water = $1.50/mgal
Process water = $0.50/mgal

mgal, thousand gallons; MCF, 1000 cubic feet.

The cost of the ultrafiltration unit was determined using vendor quotes and standard scaling factors. Annual membrane replacement costs were determined using a membrane life of 2 yr. Key process variables such as the raw material costs, utilities costs, fixed-operating costs, byproducts revenue, and annual depreciation were estimated using standard methods. A straight line annual depreciation for 10 yr of project life was assumed. No salvage value was considered at the end of the project life. The overall cost of enzyme recycle was compared with the nonrecycle enzyme costs. The actual savings were estimated as a percentage of the nonrecycled costs.

Results and Discussion

The results indicate that both cellulase and cellobiase can be effectively recovered from hydrolyzed biomass using an ultrafiltration recovery method. The percentage recovery of cellulase ranged from 60 to 66.6%, and of cellobiase from 76.4 to 88% and recovery was consistent over three recycle events (Fig. 2). Glucose yield from enzyme hydrolysis averaged 70% of theoretical over the three recycle events. The addition of Tween-20 to the hydrolysis mixture did not improve the recovery of cellulase. The effect of the addition of Tween-20 to cellobiase recovery was inconclusive.

The results from the soaking method of recovery show that approx 35% of the enzyme remained with the biomass following filtration (Fig. 3). It is not clear whether the enzyme was actually bound to the biomass, because approx 30% of the liquid was also retained with the biomass, and

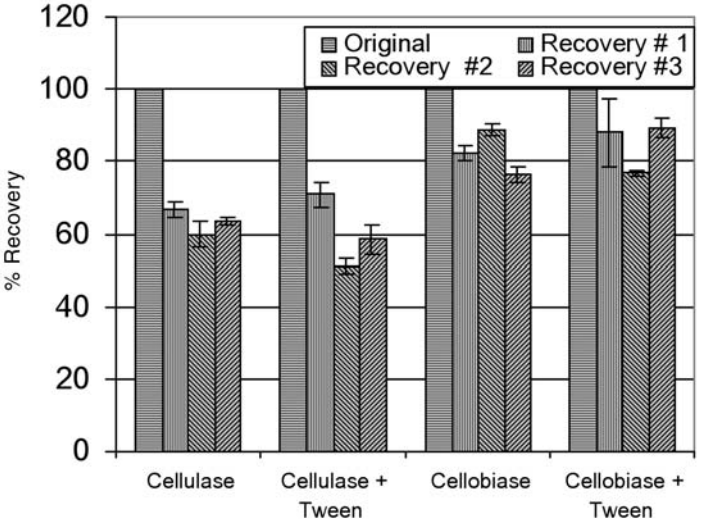

Fig. 2. Percentage of recovery of cellulase and cellobiase activity following hydrolysis of AFEX-treated corn stover with and without addition of Tween-20 (0.1%) to hydrolysis.

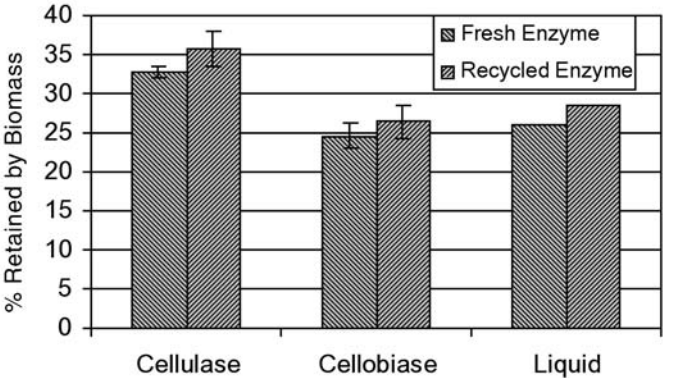

Fig. 3. Biomass retention of cellulase, cellobiase, and liquid following soaking method of enzyme recovery and recycle.

not recovered following filtration. At 35% recovery, this method is less efficient than the ultrafiltration method. There was no observed difference in recovery with this method when using fresh enzyme vs recycled enzyme.

The base case Aspen model for AFEX conversion of corn stover into ethanol was used for this study to determine the economic effect of enzyme recovery and recycle. The effect of enzyme loading and enzyme cost on ethanol production costs was determined using the basis and assumptions specified in Table 3. The results are presented as percentage savings per gallon of ethanol produced using enzyme recovery and recycle over the base case model (no enzyme recovery/recycle). First, the effect of enzyme cost was determined for three enzyme loadings (case 1 = 15 FPU of cellulase, 40

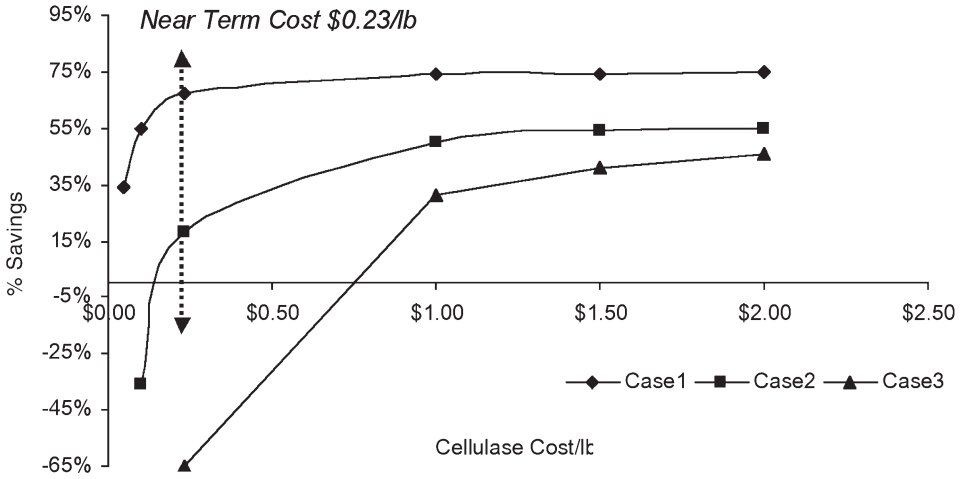

Fig. 4. Effect of enzyme loading and cost on economy of enzyme recycle in conversion of AFEX-treated corn stover into ethanol. Case 1 = 15 FPU of cellulase/g of glucan, 40 CBU of cellobiase/g of biomass, 60% cellulase, and 80% cellobiase recycle; case 2 = 15 FPU of cellulase/g of glucan, 0 CBU of cellobiase, 60% cellulase recycle; case 3 = 3 FPU of cellulase/g of glucan, 0 CBU of cellobiase, 60% cellulase recycle.

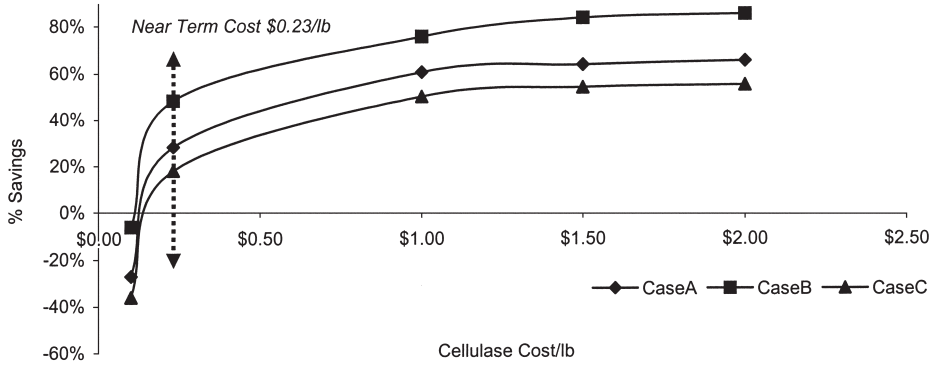

Fig. 5. Effect of enzyme loading, cost, and recovery efficiency on economy of enzyme recycle in conversion of AFEX-treated corn stover into ethanol. Case A = 15 FPU of cellulase/g of glucan, no cellobiase, and 70% cellulase recycle; case B = 15 FPU of cellulase/g of glucan, no cellobiase, and 90% cellulase recycle; case C = 15 FPU of cellulase/g of glucan, no cellobiase, and 60% cellulase recycle.

CBU of cellobiase; case 2 = 15 FPU of cellulase; case 3 = 3 FPU of cellulase) and is shown in Fig. 4. At the current recycle efficiency (60% for cellulase and 80% for cellobiase), case 1 resulted in savings between 35–75% for all enzyme prices considered. For cellulase costs >$0.10/lb, case 2 resulted in positive savings up to a maximum of 55%. Case 3 provided positive savings up to a maximum of 35% for all prices >$0.75/lb of cellulase. At a projected cost of $0.23/lb of cellulase, case 3 will not be economical.

As a near-term projection, cellobiase was eliminated from the process. This is based on the current Department of Energy goal of engineering future cellulase products to provide sufficient cellobiase activity for efficient hydrolysis—a goal not yet achieved. Figure 5 shows that at a fixed loading of 15 FPU of cellulase/g of glucan and zero cellobiase, all considered cases (case A = 70% cellulose recycle; case B = 90% cellulose recycle; case C = 60% cellulose recycle) resulted in positive savings for cellulase prices over >$0.15– $0.20/lb. Case A provided maximum savings of 80%, case B provided maximum savings of 60%, and case C provided maximum savings of 50%. At the projected cost of $0.23/lb of cellulase, all cases will be economical. Future studies will address optimizing recovery efficiency and the effect of enzyme recycle on the economics of converting other types of biomass into ethanol.

Acknowledgment

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