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Research paper

Countercurrent saccharification of lime-pretreated corn stover – Part 1

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ABSTRACT

Using the sugar platform, enzymes are a major cost contributor in biofuel production. Conventionally, enzymatic saccharification is performed in batch. To more efficiently use enzymes, a new continuous countercurrent method is explored. Pseudo-continuous countercurrent saccharification was performed on lime-pretreated corn stover at enzyme loadings of 1 mg CTec3/g dry biomass and (1 mg CTec3 + 1 mg HTec3)/g dry biomass and the results were compared with batch. To achieve the same glucan conversion as compared to batch, countercurrent saccharification reduced enzyme loading by 1.6 and 1.4 times at 1 mg protein/g biomass and 2 mg protein/g biomass, respectively. In batch saccharification, the effect of hemicellulase addition was investigated. In countercurrent saccharification, as compared to only 1 mg CTec3/g biomass loading, adding 1 mg HTec3/g biomass increased glucose and xylose yields by 6% and 12%, respectively. The effect of product sugars on enzyme inhibition was studied in batch saccharification.

1. Introduction

Growing energy demands, limited fossil reserves, and concerns over global warming are incentives to use renewable sources of energy. Biofuels can replace conventional liquid fuels obtained from petroleum sources. For biofuels to compete economically with petroleum-derived fuels, it is important to have inexpensive feedstocks. Lignocellulosic biomass is the least expensive and most abundant renewable material available on earth. Comprised of cellulose, hemicellulose, and lignin, it can be converted to biofuels through three different platforms: thermochemical, sugar, and carboxylate [1–3]. In the sugar platform, pretreated biomass is enzymatically hydrolyzed to sugars. Enzymes are a major cost in a biomass-to-ethanol process. The contribution of enzyme cost to biofuels depends on various factors, such as type of feedstock, pretreatment effectiveness, enzyme loading, and biofuel yield. The production cost of cellulase is approximately $5–10/kg [4–6]. The challenge of enzyme cost can be addressed by using them more efficiently.

Conventionally, enzymatic saccharification is performed in batch with typical reaction times of 3–7 days. At the end of batch saccharification, enzyme activity remains, but leftover enzymes are usually discarded. To reduce the enzyme costs by reusing the leftover enzymes, recycle strategies have been studied in the past [7–9]. This paper investigates the reduction in enzyme requirements for lime-pretreated corn stover by using countercurrent saccharification rather than batch.

For chemical processes, countercurrent systems are generally more efficient than batch and offer advantages such as more efficient utilization of substrates, continuous processing that avoids loading and unloading idle times, and less product inhibition. In a countercurrent saccharification system, the biomass and liquid flow in opposite directions. The fresh biomass encounters product liquid at one end and digested biomass encounters fresh liquid at other end. The enzyme addition point can be selected strategically to maximize enzyme utilization. The enzymes present in the product liquid are used by fresh active biomass at one end, thus reducing the impact of product inhibition. At the other end, digested biomass is washed with fresh liquid to recover spent enzymes and product sugars, thus improving process efficiency. The liquid product can potentially reach high sugar concentrations because it last contacted fresh highly reactive biomass.

The benefits of countercurrent saccharification have been investigated in the past [10–12]. As compared to batch saccharification, Fox et al. [10] and Jeffries and Scharman [11] observed yield improvement by a factor of 1.27 and 1.39 in countercurrent

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saccharification, respectively. These studies used only three stages in the simulated countercurrent system and enzymes were added in the terminal stage. Zentay et al. [12] compared countercurrent and batch saccharification of pure cellulose substrates and reported significantly higher yields in countercurrent system than batch. An eight-stage countercurrent train with 5 mg protein/g biomass (Train 1) achieved 87.8% glucose conversion, whereas a train with 2 mg protein/g biomass (Train 2) achieved 56.1% glucose conversion [12]. Compared to 5-day batch saccharification, to achieve the same glucose conversion, enzyme requirements were reduced by factors of 16.8 and 8 for Trains 1 and 2, respectively. Also, the eight-bottle trains employed in this study had substantial remaining enzyme activity at both ends. Based on these lessons, a new set of experiments was designed to investigate countercurrent saccharification of real substrate (pretreated corn stover). To ensure complete utilization of enzyme, a sixteen-stage train was used in the study reported in this paper.

This paper describes pseudo-continuous countercurrent saccharification of lime-pretreated corn stover. Instead of adding enzymes to a terminal stage, the enzymes were added to a fixed intermediate stage. This study uses Novozymes' newest commercial enzymes (CTec3 and HTec3) available in the market. Sugar yields were calculated at steady state, which was validated using the Slope Method [12]. The countercurrent saccharification sugar yield was compared with batch results. The sugar concentration distribution across the countercurrent train was analyzed to determine where the enzyme is active in the system. Part 2 describes an economic analysis and the use of a continuous column reactor as a commercial version of countercurrent saccharification [13].

2. Materials and methods

2.1. Submerged-lime-pretreatment (SLP)

Corn stover was pretreated using long-term submerged lime pretreatment [14]. The water and lime loadings were 10 kg water/kg dry biomass and 0.15 kg $Ca(OH)_2$/kg dry biomass, respectively. CO_2-free air was used for the pretreatment. The pretreatment time was 30 days and the temperature was maintained at 50 °C. The pretreated corn stover was washed with water, air dried at room temperature, and stored in Ziploc bags.

2.2. Shock pretreatment

Submerged-lime-pretreated corn stover was shock treated in a 20-L vessel with a conical section and run-up tube. The shock vessel was loaded with 1.4 kg dry corn stover and 14 L water (including water in biomass). Stoichiometric H_2 and O_2 were added to the head space of the apparatus and ignited using a glow plug. Detonation causes a rapid pressure increase to 12 MPa within 19 μs. The resulting shock wave transferred through the aqueous slurry and mechanically disrupted the structure of corn stover. Finally, the lime + shock treated biomass slurry was air dried at room temperature and stored in Ziploc bags.

2.3. Compositional analysis of biomass

The composition of raw and pretreated biomass was determined using standard NREL procedure [15]. The biomass used for Sections 1 and 2 of the countercurrent saccharification contains 42.59% glucan and 19.79% xylan.

2.4. Saccharification

2.4.1. Substrate

Raw, lime-pretreated, and lime + shock treated corn stover was saccharified.

2.4.2. Citrate buffer

Optimal performance of cellulase CTec3, cellulase CTec2, and hemicellulase HTec3 occur at pH 4.75–5.25, pH 5.0–5.5, and pH 4.8–5.2, respectively [16–18]. Citrate buffer at 0.1-M concentration and pH of 4.8 was used to maintain relatively high enzyme activity. To prepare the buffer, citric acid monohydrate and trisodium citrate dihydrate were added to deionized (DI) water.

2.4.3. Antibiotics

To prevent growth of contaminating microorganisms that could consume produced sugars, an antibiotic cocktail was added to each bottle. The cocktail was composed of tetracycline and cycloheximide solutions. Tetracycline solution (10 g/L) was prepared in an aqueous solution of 70% ethanol. Cycloheximide solution (10 g/L) was prepared in deionized water. To each batch saccharification vial, 40 μL of tetracycline and 30 μL of cycloheximide solution were added per 10 mL of solution.

2.4.4. Enzyme solutions

Three different Novozymes enzymes were used in this study: Cellic® CTec2, CTec3, and HTec3. CTec2 is a blend of aggressive cellulases with high levels of β-glucosidases and hemicellulases that degrade lignocellulose into sugars [16]. CTec3 is Novozymes' newest commercial enzyme product for effective hydrolysis of cellulose. It contains proficient cellulase components boosted by proprietary enzyme activities and a new array of hemicellulase activities [17]. HTec3 is the newest commercial enzyme product from Novozymes for effective hydrolysis of insoluble and soluble hemicelluloses [18].

2.4.5. Incubator

Optimal performance of CTec2, CTec3, and HTec3 occur at temperatures of 45–50 °C, 50–55 °C, and 40–45 °C, respectively. In this study, a standing incubator cabinet was used. The incubator is a roller apparatus with a rotational speed of 2 rpm maintained at constant temperature (50 °C).

2.5. Countercurrent saccharification

Countercurrent saccharification of lime-pretreated corn stover was performed using 16 1-L centrifuge bottles (Thermo Fisher Scientific, catalog# 05-562-25). All 16 bottles were started as batch saccharification with the same initial solid concentration (100 g/L) and total volume of 250 mL. Tables 1 and 2 show the enzyme loadings and experimental details, respectively. Fig. 1 shows the schematic of the experiment. In this paper, the 16-bottle countercurrent system is often described as a countercurrent “train” and the monitoring procedure is referred as a “transfer.”

2.5.1. Monitoring of the countercurrent saccharification

The countercurrent train was monitored every other day (48 h) to take samples and transfer solids and liquids. During every transfer, each bottle was centrifuged to achieve phase separation of liquid and solid wet cake (70–80% moisture content). For each bottle, the volume and mass of separated liquid and weight of wet cake were recorded. The pH of the liquid was measured to ensure it was compatible with the enzymes. Liquid samples (1 mL) were taken from every bottle and analyzed by HPLC to determine sugar concentrations. When the sugar concentrations from each bottle