Glucose to Fuel: Investigating Renewable Resources for Glucose Production

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Abstract: Cellulose is a key component in plant cell walls, and is the most abundant biopolymer in the world. Enzymes can be used to break cellulose down into glucose, which can then be fermented to ethanol fuel. Avicel (a purified form of cellulose), corn stover, acid treated corn stover, and paper pulp were tested using commercially available enzymes to determine the effectiveness of the enzyme and which substrate was the most efficiently converted to glucose. Results indicate that paper pulp produces the highest glucose concentration, yielding 654.4mg total sugar per ml of reaction in the form of glucose and trace amounts of xylose. Differences could be seen between the untreated and the pretreated stover, with pretreatment leading to more sugar production in the form of xylose. These commercial enzymes were then compared to a set of enzymes being isolated from Clostridium Thermocellum (CelFRS) with and without a chaperonin-like ring structure attached (SC151). A new enzyme (CelA) was added into the existing mixture and a pilot experiment was done to determine ratios that would maximize glucose production. While the CelA addition showed promise, no considerable increase in glucose production could be obtained. Comparatively, the enzymes being isolated still fell short of commercially available enzymes in glucose production. These results demonstrate the current state of the art in commercial conversion of cellulose to fermentable sugars, and set the benchmark for the continuing investigation toward maximizing glucose yields.

Introduction

Cellulose can be broken down into glucose using various methods, including enzymatic digestion. The cell wall is made up of 15-40% Cellulose, 30-40% hemicellulose and pectin, and 20% lignin (1). Each of these components can be broken down into individual sugars which can be fermented into ethanol. Finding a way to efficiently break down cellulose would lead to a vast set of new resources for fuel. In this study, the effect of pretreatment was investigated, and a saccharification technique using commercially available enzymes was used to determine efficiency on parts of corn crop, recycled paper stock, and a commercially available cellulose product for comparison.

Methods

Enzymatic Digests:
- 434g/L Novozyme Celluclast Mixture
- 75ug Substrate, delivered in slurry suspension
- Enzyme concentration tested from 1.5ug to 20.5ug enzyme.
- Digested at 50 degrees C for 15 hrs.

HPLC:
- Standards from 2mg/mL to 12mg/mL prepared for comparison
- HPX-87P column, 20mm data collection 15mm post run at 6ml/min
- Filtered deionized water mobile phase

Results

Results averaged, finding maximum conversion with 10.416ug enzyme (56.6% conversion in avicel)

Next step: Clostridium thermocellum enzymes, with and without attachment to a "chaperonin-like" molecule: SC151

Discussion

- Effect of pretreatment: increased xylose production
- Avicel plateau seen at less than 60% (product inhibited?)
- CelA incorporation shows promise
- Clostridium thermocellum enzymes fall below commercial levels

Conclusion

This experiment demonstrated that pretreatment is very effective in increasing sugar yields. The potentially product inhibited reaction indicates that procedures must be perfected in order to maximize glucose production. CelA shows promise, but it is clear that more glucosidases/vylenases/hemicellulases must be isolated to reach commercial levels of production.