Purification and Biochemical Characterization of a Xylanolytic Glycoside Hydrolase from *Caldicellulosiruptor saccharolyticus*

Caroline Wise and Donald A Comfort, Ph.D.

Chemical and Materials Engineering

**Background**

The current global energy crisis is a result of increasing energy demands coupled with depleting fossil fuel reserves. One solution to this crisis is the use of lignocellulosic biomass for conversion into biofuels. Biomass is comprised of cellulose and hemicellulose, which are polysaccharides that can be broken down via enzymatic hydrolysis into simple sugars and then converted to biofuels. Glycoside hydrolases are enzymes that have already proven to be effective in metabolizing carbohydrates.

**Introduction**

The objective of this research is to determine if a glycoside hydrolase from the thermophilic bacterium *Caldicellulosiruptor saccharolyticus* is effective at enzymatic hydrolysis of cellulose and hemicellulose. An enzyme from *C. saccharolyticus*, Csac_2410, was cloned, expressed as a protein, purified, and biochemically characterized for pH optima, temperature optima, and substrate specificity. DNS assays for reducing sugars were performed for the biochemical characterization.

**Results**

- **Incubation Temperature (°C)**
  - Specific Activity (μmol xylose/mg enz/min)
  - pH

**Conclusions**

- *C. saccharolyticus* is a thermophilic bacterium containing over 60 glycoside hydrolases
- The Csac_2410 gene effectively hydrolyzes xylan at 80°C and pH 6.25
- Csac_2410 could potentially be a part of a suite of enzymes that work together to hydrolyze the cellulose and hemicellulose in lignocellulosic biomass for the upstream processing of bioethanol and other biofuels

**Acknowledgments**

UD University Honors Program
UD Chemical and Materials Engineering