

**Southeastern Sun Grant Center Quarterly Progress Report
(Template)**

Project Title: Enzymatic and Multiphase Solution Processing of Lignocellulosic Biomass

Recipient Organization: Florida Agricultural and Mechanical University

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Project Location: Florida A&M – Florida State University College of Engineering, Tallahassee, FL 32310

Lafayette College

[Location of project activities; if multiple locations, please list all in use.]

Reporting Period: March 15, 2008 June 14, 2008

Date of Report: July 30, 2008]

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IMPORTANT NOTE: If any part of your quarterly report contains **proprietary/confidential information**, or details that should not be released to the general public, the specific sections of the report should be marked as such, by clearly marking the beginning and end of the confidential information. The marked sections will not be released to the general public or any unauthorized parties.

The report is in the following pages.

1. **Planned Activities:** [This section should include the planned activities that were stated in the previous quarterly report for the task being discussed, including subtasks, milestones, deliverables, and go/no go decision points. *If this is the first quarterly report, outline planned activities for the last quarter.*]

Planned activity in previous report

4.1 Developing and testing a protocol for enzyme activity (from Genencor) in the NMMO solvent with different loadings of cellulose: This will be a key focus in the next quarter.

2. **Actual Accomplishments:** [The discussion should include all significant work completed in the past quarter to support the project and accomplish goals.]

2.1 Development of Dinitrosalicylic (DNS) acid assay for measurement of simple sugars: An assay was developed for measuring the total sugars released during enzymatic reactions with the help of a UV-vis spectrophotometer. The steps in the assay are in the attached word file (Reducing Sugar Measurement by DNS Method). Calibration of the absorbance was done with the help of D-glucose.

Figure 1 is a calibration plot of absorbance as a function of glucose concentration as measured by the DNS assay. The goal is to use DNS assay coupled with the calibration plot to measure the total sugars released during the enzymatic reactions. This is demonstrated in the sections below.

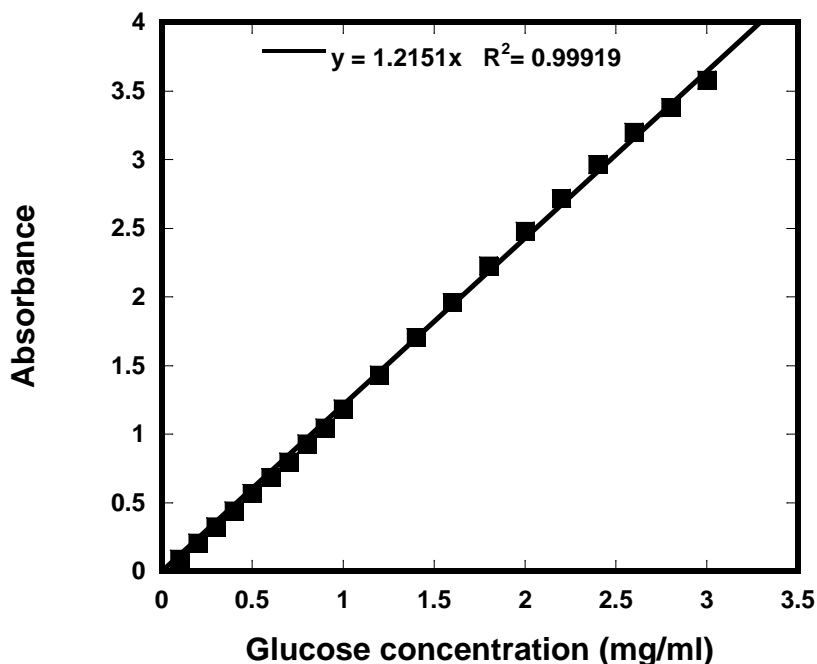


Figure 1: Calibration plot of Absorbance as a function of Glucose concentration for the DNS assay.

2.2 Development of Filter Paper (FP) Assay for Enzyme Activity: Filter paper tests were also developed to test the enzyme activity in water and in the N-methyl morpholine oxide (NMMO). The steps of this assay are given in the attached word file (FilterPaperMethod).

The following was achieved in laboratory flask test as the conditions for extrusion reaction conditions are being determined.

- 1. The reaction rate of commercial mixed cellulase enzymes in NMMO is an order of magnitude higher than it is on dispersed pretreated and regenerated cellulose from NMMO and filter paper.** At 50°C with one percent cellulose in 40% NMMO/ 60% deionized water (pH 7.46) , 70% conversion was achieved in 1.5 hours, 80% conversion was achieved in 2.5 hours and 85% in 5 hours. When 10% acetic acid in deionized water was used instead of deionized water, (pH 5.75) 90 % conversion was achieved in 1.5 hour, 90 % in 1.5 hours and 95 % in 5 hours. To achieve an 80% conversion on pretreated and regenerated cellulose from NMMO and filter paper, the pH was decreased to under 5 by using just deionized water and 24 hours were required. However the pretreated and regenerated cellulose from NMMO is about twice as reactive as dispersed cellulosic fibers from filter paper.
- 2. The reactivity of commercial mixed cellulase enzymes in NMMO is strongly pH sensitive.** In a series of runs with mixtures of NMMO and deionized water ranging from 30 % water to 100% water at 50°C, indicated that with 60 % deionized water (pH 7.22) and dispersed filter paper fibers only 10% of the cellulose is converted to glucose after 24 hours. With 100% deionized water, no NMMO (pH 4.74) and dispersed filter paper fibers only 70 % conversion was achieved after 24 hours.
- 3. The reactivity of commercial mixed cellulase enzymes in NMMO is temperature dependent and better below At 70°C.** As noted above at 50°C after 1.5 hours the conversion for 60% deionized water was 70% and with 10% acetic acid was 90%. Whereas at 70°C only 35% conversion occurred after 1.5 hours and in 10% acetic acid 50% conversion occurred. At 70°C these conversions were reached after 0.5 hours with very little additional reactivity at longer times.

Future work will include:

1. Determination of reactivity in 60% deionized water and 10% acetic acid at 60°C and 40°C.
2. Once reasonable concentrations, pH and temperatures for near optimum reactivity are achieved in the lab, then the twin screw reactor will be used.
3. Scouting experiments with the ionic liquid 1-Butyl-3-methylimidazolium acetate ([Bmim]Ac) that is reported to be a solvent for cellulose. [Bmim]Ac has a pH of 6.1 compared to the monohydrate form of NMMO required for dissolving cellulose of 10. It may be possible to add the commercial enzymes that are already in an acetate buffer (pH 4.9) directly to the [Bmim]Ac without causing the cellulose to phase separate, or at least not as quickly. Furthermore use of acetic acid to buffer the [Bmim]Ac to a lower pH may also not have a detrimental effect on solubility.