STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Cellulase Activity Measurement

AUTHOR: Troy Tian
DATE: September 10th, 2013
APPROVALS:
DATE: January 1st, 2014

A. Scope

This SOP describes the procedure to measure cellulase activity using Cellulase T tablets supplied by Megazyme.

B. Safety and Training Requirements

Refer to UF lab safety policies regarding equipment listed in section D below before starting any process work.

Review the location of fire extinguishers, fire blankets, safety showers, spill cleanup equipment and protective gear before beginning any process work.

During operations in the Lab, the following safety gear will be utilized at all times:

- Lab Coat
- Safety Glasses
- Protective Gloves

C. Related Documents and SOPs

1. Megazyme Cellazyme T Booklet (T-CTZ200-0503)
2. MSDS Binder

D. Equipment/Materials

1. Spectrophotometer
2. Glacial acetic acid
3. 5 M sodium hydroxide solution
4. 1 M hydrochloric acid solution
5. Sodium azide
6. 2% trisodium phosphate solution (w/v, pH 11.0)
7. Cellulase enzyme sample
8. DI water
9. Whatman No. 1 filter paper
10. 1 L volumetric flask
11. 16 x 120 mm glass tube
12. Water bath
13. pH meter

TITLE: Cellulase Activity Measurement

E. Detailed Procedure

E.1. Preparation of buffer stock solution (sodium acetate buffer, pH 4.5, 2M)

1. Add 120 g of glacial acetic acid to 1600 ml of DI water (final concentration equals to 1.05 g/ml).
2. Adjust the pH to 4.5 by adding 5 M sodium hydroxide solution.
3. Adjust the volume to 1 L.
4. Date and store the buffer stock at room temperature (stable for 2 years).

E.2. Preparation of assay buffer (sodium acetate buffer containing sodium azide, pH 4.5, 100 mM)

1. Add 50 ml of the buffer stock solution in 850 ml of DI water.
2. In the chemical hood, adjust the pH to 4.5 by dropwise addition of 1 M hydrochloric acid solution.

   CAUTION: Do NOT add sodium azide until the pH is adjusted. Acidification of sodium azide releases hydroazoic acid, which is highly TOXIC and VOLATILE.

3. Add 0.2 g of sodium azide and adjust the volume to 1 L.
4. Date and store the buffer stock at room temperature (stable for 1 month).

E.3. Assay

1. Add 1 ml of cellulase sample into 49 ml of the assay buffer and mix it thoroughly (the solution is referred as **Original Extract** in **F.2. Calculation of Activity**).
2. Dilute this solution 10-fold by addition to 9 ml of the assay buffer and mix it thoroughly.
3. Repeat the process of dilution until a concentration of enzyme suitable for the assay is obtained (10 to 50 milliUnit on 10 mg/mL β–glucan. Refer to **F.1. Standardization**).
4. Add 0.5 mL of suitably diluted enzyme solution in the assay buffer (100 mM, pH 4.5) to a glass test-tube and pre-equilibrate to 40°C in a water bath for 5 min.
5. Initiate the assay by the addition of a Cellazyme T tablet. The tablet hydrates rapidly. **Do not stir the tube**.
6. Incubate the reaction exactly 10 min at 40°C
7. Terminate the reaction by adding 10.0 mL of trisodium phosphate solution with vigorous stirring on a vortex mixer.
8. Prepare a blank by adding trisodium phosphate to the diluted enzyme solution without the addition of the Cellazyme T tablet.
9. Allow the tubes to stand for approximately 4-5 min at room temperature and then stir the contents again.

10. Filter the slurry through a Whatman No. 1 (9 cm) filter circle.
11. Measure the absorbance of the filtrate at 590 nm against the blank.

F. Data Archival and Analysis

F.1. Standardization

A standard curve relating the activity of purified Trichoderma sp. cellulase (EG II) on barley β-glucan and Cellazyme T tablets is shown in Figure 1. Activity on barley β-glucan was determined at a substrate concentration of 10 mg/mL in 100 mM sodium acetate buffer (pH 4.5) at 40°C using the Nelson/Somogyi reducing sugar procedure.

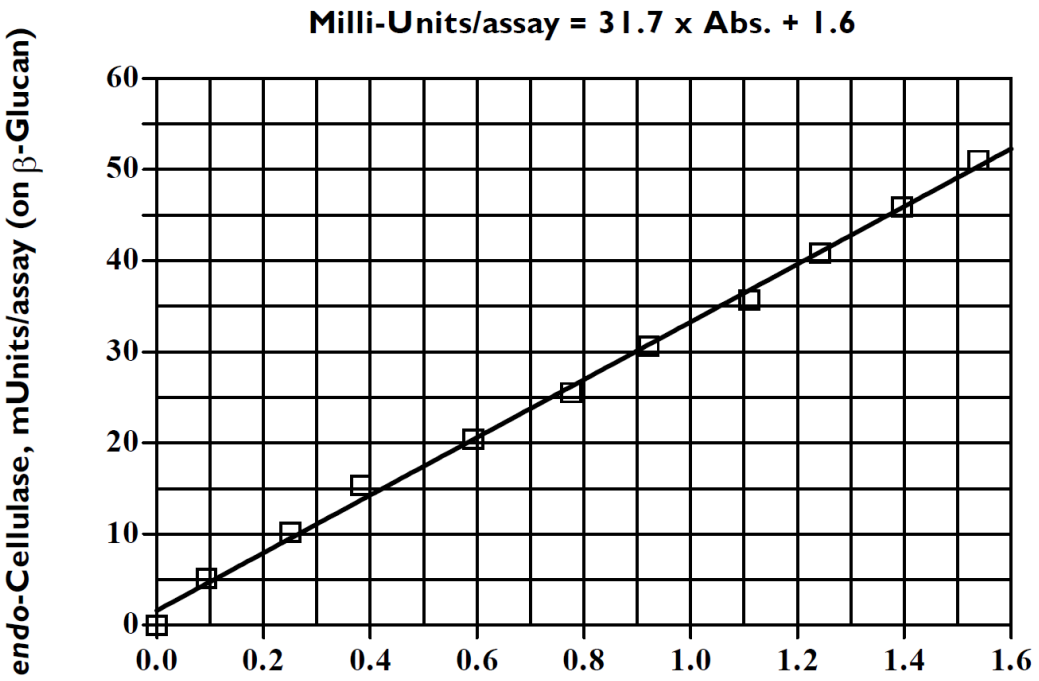

Fig. 1 Trichoderma sp. endo-Cellulase (EG II) standard curve on Cellazyme T (Lot 00601) in 100 mM sodium acetate buffer Enzyme standardised on barley β-glucan (at pH 4.5). One Unit of activity is defined as the amount of enzyme required to release one micromole of glucose reducing-sugar-equivalents per minute from either barley β-glucan (10 mg/mL) at pH 4.5 and 40°C.

F.2. Calculation of activity

endo-Cellulase activity is determined by reference to the standard curve to convert absorbance to milliUnits of activity per assay on barley β-glucan, and then calculated as follows:

TITLE: Cellulase Activity Measurement

Units/mL or gram of Original Preparation:

= milliUnits (per assay i.e. per 0.5 mL) x 2 x 50 x $\frac{1}{1000}$ x Dilution

where:

2 = conversion from 0.5 mL to 1.0 mL.

50 = the volume of buffer used to extract the original enzyme (1.0 mL of enzyme added to 49 mL of buffer assay).

$\frac{1}{1000}$= conversion from milliUnits to Units.

Dilution = further dilution of the **Original Extract**.

Record all measurements in the laboratory notebook including the date, time, vessel, and batch number of the sample.