STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Cellulase System

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APPROVALS: Process Change Committee

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A. Scope

Enzymes are used to breakdown the carbohydrate portion of biomass into simple sugars. Enzymes are pumped at a specified rate proportional to the biomass in the Liquefaction step. This SOP describes the preparation of the cellulase addition system.

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 plant, the following safety gear will be utilized at all times:

- Hard Hat
- Safety Goggles
- Protective Gloves

CAUTION: Enzyme solutions can cause allergic reactions.

Proper PPE should be used for handling enzyme solutions including:

- face shield
- protective gloves
- chemical resistant apron or rain suit

C. Related Documents and SOPs

1. Liquefaction SOP-2325
2. Cellulase Product Data Sheet
3. Beta-Glucanase Product Data Sheet

D. Preparation/Materials/Equipment

1. Sampling Containers
2. 5 gallon pale

3. Stop watch or watch.
4. 15 mL Sample Tube
5. Peristaltic Pump

E. Detailed Procedure

1. Filling the Cellulase Tote
    a. Determine the amount of enzyme in the cellulase tote by visual inspection.
    b. Verify that there is sufficient enzyme to complete a process batch according to the Experimental Plan.
    c. If the amount of enzyme is sufficient, proceed to Section E.2 Start Up.
    d. If there is not enough enzyme to complete a batch, refill the cellulase tote with sufficient enzyme solution by:
        i. Close valve 8303-V-01 at the cellulase tote.
        ii. Obtain the stock enzyme solution from the Cold Room.
        iii. Set up a peristaltic pump and transfer line from the stock solution to the cellulase tote by:
            1) Connect the transfer line to the stock solution container hose connect and the drain valve.
            2) Thread the transfer line through the peristaltic pump.
            3) Open the port on top of the cellulase tote and insert the transfer line.
            4) Turn on peristaltic pump and fill the cellulase tote.
            5) Make sure transfer line stays in cellulase tote.
            6) Turn off peristaltic pump when desired amount has been transferred.
            7) Replace cap on cellulase tote top port.
            8) Disconnect transfer line from stock enzyme container.
            9) Return stock enzyme container to Cold Room.
    e. Open valve 8303-V-01
2. Start Up
    a. Charge the cellulase system:
        i. Assure valve 8303-V-09 is closed.
        ii. Open valve 8303-V-01 at the cellulase tote.
        iii. Verify that the tote is vented by loosen top port cap.
        iv. Open valve 8303-V-03 and V-04 at the Calibration Cylinder.
        v. Allow enzyme solution to fill the cylinder.
        vi. Close valve 8303-V-04 to stop filling the cylinder.
        vii. Open valve 8303-V-06 downstream of the Cellulase Metering Pump (PT-8304).
        viii. Open the valve on the feed line 8303-V-08.
3. Operation
    a. Assure the Liquefaction Tank is running normally according to SOP-2325

b. Assure that the power switch on the back of the Cellulase Metering Pump PT-8304 is ON.
c. At the HMI, set the controller SIC-8304-01 to CASCADE and turn on the pump PT-8304.

4. Calibration of the Cellulase Metering Pump PT-8304
    a. At the HMI, turn off the Cellulase Metering Pump PT-8304.
    b. Open valves 8303-V-03 and V-04 to fill the calibration column.
    c. Once the calibration column is full, close valve 8303-V-01.
    d. At the HMI, set the controller SIC-8304-01 to MANUAL and the OUTPUT to 25%.
    e. At the HMI, turn on the Cellulase Metering Pump PT-8304.
    f. Record the amount of time for the calibration column to empty.
    g. At the HMI, turn off the Cellulase Metering Pump PT-8304.
    h. Calculate the flow rate of enzyme for the specific pump speed using the volume of the calibration column and the time recorded.
    i. Repeat steps E.4.a. – E.4.h. using 50%, 75%, and 100% in the OUTPUT of the controller SIC-8304-01 (step E.4.d.).
    j. Create a linear regression using the pump flow rate vs. the pump speed.
5. Sampling
    a. Use Sampling SOP-0511 to obtain a sample of the enzyme solution at valve 8303-V-07.
    b. Obtain a 5 – 10 mL sample of enzyme solution into a 15 mL sample tube.
    c. Immediately take sample to lab for analysis of enzyme activity.