STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Sugars, organic acids, and inhibitors by HPLC

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APPROVALS: Process Change Committee **DATE: March 22nd, 2012**

A. Scope

This procedure describes the sample preparation and subsequent measurement of sugars, organic acids, and inhibitors from fermentors, propagators, liquefaction and hydrolysate tanks using High Pressure Liquid Chromatography (HPLC).

B. Safety and Training Requirements

Refer to UF Environmental Health and Safety (EH&S) policies when handling chemicals and disposing of waste.

Review the Material Safety Data Sheets (MSDS) for each material 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 laboratory, the following safety gear will be utilized at all times:

- Safety Goggles or Face Shield
- Protective Gloves (nitrile, neoprene)
- Lab coat

C. Related Documents and SOPs

1. Agilent Technologies HPLC System operating manual (1260 Infinity)
2. Sampling SOP-0511
3. Eppendorf microcentrifuge manual (5418/5418R)
4. HPLC Calibration SOP-0516

D. Preparation/Materials/Equipment

1. Pipet tips (101-1000 µl range)

2. 1 mL pipetter
3. Eppendorf microcentrifuge (5418)
4. 1.5 mL microcentrifuge tubes
5. National Scientific HPLC sample vials
6. National Scientific HPLC sample vial caps
7. 3 mL syringes
8. 0.45 µm syringe filters
9. 4 mM sulfuric acid solution
10. Nanopure water
11. Computer with Chemstation software

E. Detailed Procedure

1. Make sure the HPLC has been calibrated according to HPLC System Operation Manual and HPLC Calibration SOP-0516.
2. The HPLC pump should be started at least two hours before starting the measurements to make sure you have a stable baseline.
    a. Verify that the pump's flow rate is 0.6 mL/min for the sugars and inhibitors column (BioRad Aminex HPX-87P).
    b. Verify that the pump's flow rate is 0.4 mL/min for the organic acids and inhibitors column (BioRad Aminex HPX-87H).
    c. Record the column pressure in the HPLC log book once a stable baseline is achieved.
3. If measuring sugars and inhibitors, make sure the nanopure water bottles are full (~1 L).
4. If measuring organic acids, make sure the 4 mM sulfuric acid bottles are full (~1 L).
5. Verify that the liquid waste container for the HPLC is not full. If it is full, neutralize the solution and dispose of it according to appropriate UF EH&S policies.
6. Prepare the samples to be analyzed by HPLC by filtering the sample into an HPLC vial.
    a. If frozen, take the sample out of the freezer and thaw at room temperature, otherwise wait for the sample to be at room temperature.
    b. Mix well the sample (by inverting at least 6 times), place 1 mL of the sample in a 1.5 mL microcentrifuge tube, and centrifuge at 14,000 rpm (~17,000 x g) for 2 min.

c. Using a pipetter and appropriate pipette tips, transfer the supernatant to a 3 mL syringe equipped with a 0.45 µm syringe filter and filter the sample into the HPLC sample vial.

d. The sample needs to be at least 400 µL. If not, repeat step E.4.a., E.4.b., and E.4.c.

7. Seal the vial with a crimper using the vial caps.
8. Run the samples in the HPLC by following the instructions given in the Agilent Technologies HPLC System operating manual (1260 Infinity).
    a. Label samples according to Batch Number, time, and date.
9. Once the samples have run, obtain the results from the Chemstation software.
    a. Verify the key peaks are integrated properly.

F. Data Archival and Analysis

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