STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Water Insoluble Solids Determination

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APPROVALS: Process Change Committee **DATE: October 3rd, 2012**

A. Scope

This procedure describes how to measure Water Insoluble Solids (WIS) as a percentage of initial biomass.

B. Safety and Training Requirements

Refer to UF lab safety policies and review the Material Safety Data Sheets (MSDS) for each material listed in section D below before starting any process work.

Refer to UF Biosafety guidelines and the NIH Guidelines whenever handling biological cultures/genetically modified organisms.

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 Goggles
- Protective Gloves (nitrile, neoprene)

C. Related Documents and SOPs

1. UF Biosafety Manual (November 2008)
2. UF Lab Safety Manual (November 2003)
3. Dry weight measurement by moisture balance SOP-0503
4. Oven manual
5. Kern Moisture Balance manual

D. Preparation/Materials/Equipment

1. Moisture balance (Kern MLB_N model)

2. 3 magnetic filter units
3. 3 2-L side arm flasks
4. Whatman filter paper #4
5. 3 1-L plastic beaker
6. 60 °C oven (Model)
7. Desiccator
8. Bleach
9. Balance (up to 0.01 g)
10. HEPA filters (Whatman HEPA vent filter – Fisher 0974479)

E. Detailed Procedure

1. Measure the percentage dry weight of the sample using the moisture balances (see SOP- 0503).
2. Calculate the amount of sample needed to obtain 5 g dry weight;

$$Sample\ WW = \frac{5\ g\ DW}{\%\ DW}$$

3. Measure the calculated amount in each of the three 1-L plastic beakers not varying the weight more than 0.01 g.
4. Set-up the filter units with the filter paper and the side arm flasks.
    a. Place the filter at the bottom part of the filter unit. Wet the filter with a few drops of DI water to make sure it stays in place.
    b. If the sample contains GMO's, place 20 mL of bleach into the vacuum flask to inactivate the GMO.
    c. Place the top part of the filter holding on top of the filter.
    d. Place the filter unit on top of a side arm flask using a rubber stopper. Make sure to seal the top of the flask using the stopper.
    e. Connect the side arm flask to the vacuum valve using rubber tubing with the HEPA filter in line.
    f. Open the vacuum valve.
5. Add ~200 mL of DI water to the plastic beaker containing the 5 g DW of sample.
6. Swirl the beaker to assure all of the solids are suspended, and pour the water and sample slurry into the filter unit. The water should flow through with the solubles and the solids should remain in the filter.
7. Add another 200 mL of DI water to the plastic beaker, swirl and pour again into the filter unit.

8. Keep repeating step 7 until ~4 L of DI water have run through the sample. The filtrate can be discarded in the sink.
9. Measure the pH of the filtrate. The pH should be 7.0 ± 0.5 If not, keep adding water to the filter unit until the pH of the filtrate is neutral.
10. Place the filter unit with the remaining solids in 60 °C oven for 48 h.
11. Take the filter unit out of the oven and place it inside a desiccator to cool down.
12. Measure the weight of the filter unit and dried solids (WFUS).
13. Remove the solids from the filter and the filter unit and measure the weight of the filter unit and filter paper (WFUF).
14. Calculate the % WIS of your sample;

$$\%WIS = \frac{WFUS - WFUF}{WW\ Sample * \%\ DW}$$

F. Data Archival and Analysis

Record all measurements in their corresponding batch records and store records in a folder labeled with the run number.