

**STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT**

TITLE: Sampling SOP

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APPROVALS: Process Change Committee

DATE: March 4th, 2012**A. Scope**

This SOP describes the procedure to obtain samples from different stages in the process in order to assess overall performance. Sterile sampling should be used whenever there is a possibility of contaminating the process with undesired microorganisms and/or when it is needed to assess the microbial population at a certain stage in the process (i.e., fermentation, propagation, liquefaction). In all other cases, non-sterile sampling is appropriate.

B. Safety and Training Requirements

Refer to UF lab safety policies regarding equipment 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 plant, the following safety gear will be utilized at all times:

- Safety Goggles or Face Shield
- Protective Gloves
- Hard Hat

C. Related Documents and SOPs

1. Phosphate determination SOP-0501
2. Moisture by moisture balance SOP-0503
3. Autoclave operation SOP-0504
4. Sugars, organic acids, and inhibitors concentration SOP-0505
5. Viable plate count SOP-0507
6. Primary seed flask culture SOP-0509

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7. Biomass composition SOP-0512
8. Optical density measurement SOP-0513
9. pH measurement SOP-0514
10. Conductivity SOP-0515
11. Biomass storage and handling SOP-1215
12. Biomass pretreatment SOP-2110
13. Sample cyclone SOP-2115
14. C5 Hydrolysate storage SOP-2135
15. Biomass liquefaction SOP-2325
16. Primary propagator #1 SOP-3210
17. Primary propagator #2 SOP-3215
18. Secondary propagator #1 SOP-3220
19. Secondary propagator #2 SOP-3225
20. Fermentation tank A SOP-3230
21. Fermentation tank B SOP-3235
22. Fermentation tank C SOP-3240
23. Chemical inactivation inside vessel SOP-3501
24. Beer well SOP-4000
25. Thermal inactivation inside vessel SOP-5002
26. Phosphoric acid system SOP-8110
27. CIP system SOP-8205
28. Wastewater system SOP-9530
29. Steam supply SOP-9305

D. Preparation/Materials/Equipment

1. 1 gal Ziplock bag
2. Sample flask
3. Sample tube with screw-cap
4. Sterile sample tube with screw-cap
5. Sample bottle with screw-cap
6. Sterile sample bottle with screw-cap
7. Sharpie
8. Refrigerator (40 °F)
9. Freezer (-4 °F)

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E. Detailed Procedure

E.1 Sterile Sampling

1. Make sure the system you are going to sample from is operating appropriately according to their respective SOP.

CAUTION: Do not take a sample if the tank/line is pressurized.

2. Make sure the Steam Supply is ready according to SOP-9305.

CAUTION: Whenever dealing with steam, make sure you wear high temperature protective gloves and face shield.

3. If possible, apply steam to the sample port according to the respective SOP in order to sterilize the sample valve.
 - a. Make sure the sample port is closed.
 - b. Open the valve that controls the flow of low pressure steam to the sample port.
 - c. Allow steam to flow through the sampling port for at least 10 min in order to sterilize it.
 - d. Close the valve that controls the flow of low pressure steam to the sample port.
4. Open the sample valve and discard the first few seconds of material released into appropriate waste container. For samples of fermentations, the waste container must be inactivated prior to disposal.
5. Collect the sample in a sterile screw-cap tube for samples up to ~45 mL or in screw-cap sterile bottles for samples up to ~250 mL.
6. Label the tube/bottle with a sharpie indicating the date, time, vessel, and batch number.
7. If doing plate counts, mix the contents by inversion 6 times and aseptically take a sample from the tube/bottle and plate according to Viable Plate Count SOP-0507. This should be done as quickly as possible after taking the sample.
8. If measuring optical density of the culture, mix the contents by inversion 6 times and aseptically take a sample from the tube/bottle and measure the cell mass according to Optical Density Measurement SOP-0513. This should be done as quickly as possible after taking the sample.

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9. Make the appropriate measurements (ethanol, sugars, inhibitors, organic acids, ethanol, dry weight, water insoluble solids, phosphate content, pH, conductivity) according to their respective SOPs and the experiment being carried out.
 - a. In between measurements, place the sample in the refrigerator at 40 °F (4 °C) to prevent further degradation of the sample.
 - b. For HPLC measurements, make sure to mix the sample by inversion at least 6 times.
 - c. Before measurements for pH, conductivity, or dry weight, wait for the sample to reach room temperature.
10. After analysis is complete, place the sample in the freezer at -4 °F (-20 °C) for long-term storage.

E.2 Non-sterile Sampling***E.2.a Liquid/Slurry Samples***

1. Make sure the system you are going to sample from is operating appropriately according to their respective SOP.

CAUTION: Do not take a sample if the tank/line is pressurized.

2. Open the sample valve and discard the first few seconds of material released into an appropriate waste container.
3. Collect the sample in a screw-cap tube for samples up to ~45 mL or in screw-cap bottles for samples up to ~250 mL.
4. Label the tube/bottle with a sharpie indicating the date, time, vessel, and batch number.
5. Make the appropriate measurements (sugars, inhibitors, organic acids, ethanol, dry weight, water insoluble solids, phosphate content, pH, conductivity) according to their respective SOPs and the experiment being carried out.
 - a. In between measurements, place the sample in the refrigerator at 40 °F (4 °C) to prevent further degradation of the sample.
 - b. For HPLC measurements, make sure to mix the sample by inversion at least 6 times.
 - c. Before measurements for pH, conductivity, or dry weight, wait for the sample to reach room temperature.

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6. After analysis is complete, place the sample in the freezer at -4 °F (-20 °C) for long-term storage.

E.2.b Solid Samples

1. Make sure the system you are going to sample from is operating appropriately according to their respective SOP.
2. If using the sample cyclone during pretreatment, please refer to the Sample Cyclone SOP-2115.
3. Collect the sample in a plastic 1 gal Ziplock bag.
4. Label the bag with a sharpie indicating the date, time, vessel, and batch number.
5. Make the appropriate measurements dry weight, water insoluble solids, phosphate content, pH, conductivity) according to their respective SOPs and the experiment being carried out.
 - a. In between measurements, place the sample in the refrigerator at 40 °F (4 °C).
 - b. Wait for the sample to reach room temperature before making any measurements.
6. Place the sample in the freezer at -4 °F (-20 °C) for long-term storage.

F. Data Archival and Analysis

Record the sample date and time in the respective vessel batch record.