

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

TITLE: Validation of Thermal Inactivation by Distillation

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APPROVALS: Process Change Committee

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A. Scope

This SOP describes the procedure for the verification and validation of the inactivation of the ethanol producing strain in beer feed using the Distillation and Dehydration System. Once the inactivation has been verified at least three times, the validation should be performed at least once a month under normal operation.

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 glasses
- Protective gloves
- Hard hat

C. Related Documents and SOPs

1. Air System Operation SOP-9405
2. Potable Water System SOP-9705
3. Ethanol Distillation SOP-4905
4. Differential Plating SOP-0522
5. Beer Well SOP-4600
6. Sampling SOP-0511

D. Preparation/Materials/Equipment

E. Detailed Procedure

1. Ensure that the Air System is operational according to Air System SOP-9405.
2. Ensure that the Potable Water System is operational according to Potable Water System SOP-9705.

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3. Ensure the beer is actively feeding to the Distillation and Dehydration system according to the Ethanol Distillation SOP-4900.
 4. Take a sterile sample before distillation process using drain valve 4601-V-40 according to Beer Well SOP-4600 and Sampling SOP-0511.
 5. Take a sterile sample from the stillage stream by:
 - a. Open the valve located at the bottom of the stripping column, and allow to flow into 5 gal bucket for 10 seconds.
 - b. Collect a sample according the Sampling SOP-0511.
 - c. Ensure the slurry from the distillation and dehydration system is contained in the Decanter Feed Tank and **do not operate the decanter** while the sample is in process. If the Decanter Feed Tank is full, stop the distillation and dehydration system according to the Ethanol Distillation SOP-4900 to stop the feed of stillage to the Decanter Feed Tank.
 6. Take each sample immediately to the lab and process for differential plating count according to the Differential Plating SOP-0522.
 7. Inactivate the broth in the bucket from Step 5.a by adding bleach to 1% final concentration.
 8. The plated samples should give negative results.
 - a. If the test is negative, the operation of the decanter can take place and the distillation process can be resumed according to the Ethanol Distillation SOP-4900.
 - b. If the test is positive, inactivate the microorganism in the Decanter Feed Tank by addition of bleach solution.