

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

TITLE: Validation of Thermal Inactivation by Decanter Pick Heater

AUTHOR: Troy Tian
APPROVALS: Process Change Committee

DATE: March 20th, 2013
DATE: December 16th, 2014

A. Scope

This SOP describes the procedure for the verification and validation of the inactivation of the ethanol producing strain in Beer Well streams with the use of the pick heater. Once the inactivation has been verified at least three times, the validation should be performed at least once a month under normal operation or when a significant change in the media takes place.

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. Decanter SOP-4900
2. Air System Operation SOP-9405
3. Differential Plating SOP-0522
4. Potable Water System SOP-9705
5. Beer Well SOP-4600
6. Biomass Liquefaction SOP-2325
7. Distillation SOP-4905
8. Process Water System Operation SOP-9505
9. Steam Supply SOP-9305
10. Sampling SOP-0511

D. Preparation/Materials/Equipment

- 1.

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E. Detailed Procedure

1. Ensure the Beer Well system is operating normally and actively pumping beer through the direct steam injection heater and into the Decanter Feed Tank (TS-4901) according to the Beer Well SOP-4600.
2. Take a sterile sample before the pick heater using sampling valve SSP-4601-01 according to Beer Well SOP-4600 and Sampling SOP-0511 and measure the presence of the production strain using the Differential Plating SOP-0522.
3. Take a sterile sample after the pick heater using drain valve 4901-V-03 according to Sampling SOP-0511 and measure the presence of the production strain using the Differential Plating SOP-0522.
4. Ensure the decanter feed pump and the decanter are turned off before the plating results (24 hours) are verified.
5. The final count of the production strain after the direct steam injection heater should be ZERO.
 - a. If the cell count is greater than expected, contact the supervisor and inactivate the microorganism in the Decanter Feed Tank by addition of bleach solution.
 - i. The bleach solution can be added through the top of the decanter by removing the cap of the extra opening at the top of the tank.
 - ii. Depending on the level of the tank, add enough bleach to reach a concentration of at least 0.6%.
 - iii. Maintain agitation in the Decanter Feed Tank for at least 2 h to ensure inactivation by the bleach solution.
6. Empty Decanter Feed Tank according to Decanter SOP-4900.