STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Validation of thermal inactivation using the waste water pick heater

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APPROVALS: Process Change Committee **DATE: June 30th, 2014**

A. Scope

This process describes the procedure pertaining to the thermal inactivation of the ethanol producing strain using a direct steam injection heater while pumping out of the Waste Water Tank TS-9502. This procedure should be performed at the system startup and at least monthly after that during normal operation.

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 plant, the following safety gear will be utilized at all times:

- Hard Hat
- Safety Glasses
- Protective Gloves (nitrile, neoprene)

C. Related Documents and SOPs

1. UF Laboratory Safety Manual
2. UF Biosafety Manual
3. Potable Water System SOP-9705
4. Air System Operation SOP-9405
5. Sampling SOP-0511
6. Differential Plating SOP-0522
7. Steam Supply SOP-9305
8. Autoclave SOP-0504
9. Waste Water System SOP-9530

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D. Preparation/Materials/Equipment

1. Differential media plates
2. Sodium Chloride
3. Sterile 250 mL-500 mL polypropylene bottle with cap
4. Sterile snap-cap micro centrifuge tubes
5. Sterile Air Laminar Flow Bench
6. Sterile pipette tips (1 mL)
7. Pipette (1 mL)
8. Vortex mixer
9. Nitrile gloves
10. Absorbent wipes
11. 5 gallon bucket

E. Detailed Procedure

1. Obtain a sample before the pick heater by:
    a. Open valve 9502-V-17 and allow to flow for 1 minute prior to sample collection.
    b. Collect a sterile sample according the Sampling SOP-0511.
2. Perform a viable plate count on the samples before the direct steam injection heater according to Differential Plating SOP-0522.
3. The final count for the ethanologenic strain should be ZERO.
    a. If the cell count is greater than zero, do not proceed and contact the supervisor.
4. Operate the waste water system normally and actively pump waste water through the direct steam injection heater according to the Waste Water System SOP-9530.
5. Obtain a sample after the pick heater by:
    a. Open the valve in the waste water line located after the direct steam injection heater, and allow to flow into 5 gal bucket for 10 seconds. The valve is located in the Buckeye area parallel to the retention area.
    b. Collect a sterile sample according the Sampling SOP-0511.
6. Perform a viable plate count on the sample after the direct steam injection heater according to Differential Plating SOP-0522.
7. The final count after the direct steam injection heater should be ZERO.
8. If the cell count is greater than expected, make sure the waste water pump is turned off and contact the supervisor. This would be considered a loss of containment and the University of Florida Biosafety Office has to be contacted within 24 hours.

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F. Data Archival and Analysis

Record date, time and the name of the person taking the sample as well as the flow rate and the temperature of the waste water at time of sampling