STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Fermentation Tank A VS-3204A

AUTHOR: Troy Tian **DATE: September 24th, 2012**

APPROVALS: Process Change Committee **DATE:**

A. Scope

This SOP describes the procedure to clean, sterilize, and operate Fermentation Tank A during normal operation in order to ferment the biomass slurries and produce ethanol.

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 for Research Involving Recombinant DNA Molecules whenever biological cultures/genetically modified organisms are handled or present in the equipment.

Review the location of fire extinguishers, fire blankets, safety showers, spill cleanup equipment and protective gear before beginning any process work.

When performing any work above 6 feet from the ground, make sure to properly use a harness to prevent injury in case of a fall.

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

- Safety Goggles
- Protective Gloves
- Hard Hat

C. Related Documents and SOPs

1. Experimental Plan
2. Ethanol Concentration SOP-0500
3. Moisture by Moisture Balance SOP-0503
4. Sugars, Organic Acids and Inhibitors Concentration SOP-0505
5. Viable Plate Count SOP-0507
6. Sampling SOP-0511
7. pH Measurement SOP-0514
8. Conductivity Measurement SOP-0515
9. Plant pH Probe Calibration SOP-0519
10. Media Preparation SOP-2155

11. Biomass Liquefaction SOP-2325
12. Secondary Propagator 3A SOP-3220
13. Secondary Propagator 3B SOP-3225
14. Trace Element Storage SOP-3250
15. Beer Well SOP-4000
16. Phosphoric Acid System Operation SOP-8110
17. Clean In Place (CIP) SOP-8205
18. Antifoam System Operation SOP-8310
19. Lime Slurry System Operation SOP-8405
20. Base B System Operation SOP-8565
21. Refrigeration System Operation SOP-9210
22. Steam Supply System Operation SOP-9305
23. Air System Operation SOP-9405
24. UV Water System Operation SOP-9555
25. Hot Water System Operation SOP-9605
26. Potable Water System SOP-9705

D. Preparation/Materials/Equipment (Saved for future use)

E. Detailed Procedure

E.1 Vessel Preparation

1. At the HMI and at local devices, ensure that the Fermentation Tank A vessel is empty, at atmospheric pressure, clean and ready for operation.
2. Ensure that the Potable Water System is operational according to Potable Water System SOP-9705.
3. Ensure that the Steam Supply system is operational according to Steam System SOP-9305.
4. Ensure the Refrigeration System is functioning properly according to SOP-9210.
5. Ensure that the Air System is operational according to Air System SOP-9405.
6. Ensure the Hot Water System is functioning properly according to SOP-9605.
7. Instrument Calibration;
    a. Pressure Measurement
        i. Verify that the local indicator is reading ambient conditions (PIT-3204A-33).

ii. On the HMI, verify the reading of PI-3204A-33 is the same as local indicator.

b. Temperature Measurement
   i. On the HMI, verify that the following temperature probes are reading current ambient conditions: TIC-3204A-10.
   ii. Verify that the reading of the local indicator (TI-3204A-18) is the same as displayed on the HMI.

c. Vessel Level Measurement
   i. On the HMI, verify that level indicator LT-3204A-03 is reading zero level.
   ii. Configure the following valves to their appropriate position to ensure there is no liquid in the vessel:
      i) Close valves: 3205-V-29, -32, -34.
      ii) Open valves: 3205-V-19, -30, -31, -35, -36, -58.
      iii) If liquid/slurry starts to exit the drain, close valve 3205-V-30 and contact the supervisor.

d. pH Measurement
   i. Calibrate pH probes AE-3204A-01A and AE-3204A-01B according to Plant pH Probe Calibration SOP-0519.
   ii. Verify that the pH readings after calibration are the same as displayed on the HMI tag AIC-3204A-01.

8. Verify that all side ports on the vessel are filled and secure.
9. At the HMI, switch the temperature control TIC-3204A-10B to MANUAL, the OUTPUT to -5, and mode to SIP.
10. Visually verify that the jacket does not have hot/cooling water flowing through it by ensuring that XV-3204A-29, XV-3204A-30, XV-3204A-31, and XV-3204A-32 are closed.
11. Verify that the jacket is at ambient temperature and pressure using PI-3204A-16 and TI-3204A-17.
12. Verify the initial valve settings according to the table below:

Table 1. Initial valve positions settings for sterilization

Fermentor A system

TITLE: Fermentation Tank A VS-3204A

Line	Application	Valve	Position	Check
CIP Supply	Spare valve	3205-V-01	Closed	
	Slurry line cleanse	3205-V-02	Closed	
		3205-V-03	Closed	
	Vent line cleanse	3205-V-57	Closed	
	To tank bottom inlet	3205-V-05	Closed	
		3205-V-07	Closed	
		3205-V-10	Closed	
		3205-V-45	Closed	
		3205-V-55	Closed	
	Spray ball valves	3205-V-15	Closed	
		3205-V-16	Closed	
	Tank cleanse	3205-V-20	Closed	
CIP Return	To CIP return	3205-V-41	Closed	
	Spare valve	3205-V-44	Closed	
UV Sterilizer	To propagator transfer line	3205-V-09	Closed	
Sterile Air	Pressure indicator	3205-V-12	Open	
	To tank bottom inlet	3205-V-21	Closed	
	To tank top inlet (vent)	3205-V-17	Closed	
	Vacuum relief valve	3205-V-18	Open	
Vent	Vent outlet	3205-V-19	Closed	
	To beer well	3205-V-58	Closed	
	To vacuum pump	3205-V-56	Closed	

TITLE: Fermentation Tank A VS-3204A

Fermentor A system				
Line	**Application**	**Valve**	**Position**	**Check**
Vessel jacket	Jacket inlets/outlets	3205-V-59	Open	
		3205-V-60	Open	
		3205-V-61	Open	
		3205-V-62	Open	
		3205-V-63	Open	
		3205-V-64	Open	
	High point vent	3205-V-53	Open	
	Recirculation	3205-V-26	Closed	
		3205-V-52	Closed	
	Jacket recirculation loop	3214-V-37	Closed	
		3214-V-38	Open	
		3214-V-40	Open	
		3214-V-41	Closed	
		3214-V-42	Open	
	Pressure indicator	3205-V-25	Open	
	Steam trap	3205-V-27	Open	
	Drain	3205-V-28	Open	
Steam	To vessel jacket	3205-V-51	Closed	
	Pressure indicator	3205-V-49	Open	
	To tank bottom inlet	3205-V-48	Closed	
	Steam inlet	3205-V-33	Closed	
	To sample port	3205-V-22	Closed	
	Drain	3205-V-46	Closed	
Cooling water/hot water	Cooling water/hot water return	3205-V-50	Closed	
Base B	To Base/lime slurry inlet	3205-V-13	Open	
Hose connection	To floor hose connection	3205-V-54	Closed	

TITLE: Fermentation Tank A VS-3204A

Fermentor A system				
Line	**Application**	**Valve**	**Position**	**Check**
Transfer line	Bottom drain	3205-V-30	Closed	
	To Fermentor A pump	3205-V-31	Open	
		3205-V-35	Closed	
	Drain	3205-V-36	Closed	
	Steam trap	3205-V-32	Closed	
	To beer well	3205-V-38	Open	
		3205-V-42	Open	
		4601-V-12	Closed	
	To hose connection	3205-V-40	Closed	
	Drain	3205-V-39	Closed	
	Drain	3205-V-43	Closed	
	To CIP return	3205-V-41	Open	
	Drain	3205-V-44	Closed	
	Spare line (Propagator 1A)	3205-V-34	Open	
	From Propagator 3A	3205-V-06	Open	
	Spare valve	3205-V-08	Closed	
	From Propagator 3B	3205-V-11	Closed	
	To tank bottom inlet	3205-V-29	Closed	
	From Hydrolysate pH Adjustment Tank	3205-V-04	Closed	
	Separate valve	3205-V-23	Closed	

E.2 Sterilization (SIP)

1. Ensure the Fermentor A jacket has been fully drained by visual verification at the end of the line after valve 3205-V-28.
2. Add Low Pressure Steam to the tank jacket by:
    a. Close High Point Vent valve 3205-V-53.
    b. Open the control-line-valve of PRV-3204A-13.
    c. Verify steam pressure in PI-3204A-15 is below 30 psi.
        i. If not, contact supervisor.
    d. Slowly open steam supply valve 3205-V-51.
    e. When steam exits drain at 3205-V-28, open the steam trap T1-3205-03 and close the drain valve 3205-V-28.
    f. Verify steam pressure in PI-3204A-15 is 15 psi ± 2 psi.
        i. If not, contact supervisor.

3. Add Low Pressure Steam to the tank by:
    a. Open valve 3205-V-48 and verify steam pressure in PI-3204A-20 is 30 psi.
    b. Open the steam trap T1-3205-06.
    c. Open steam supply valve 3205-V-33 on the bottom of the tank.
    d. Open tank drain to trap valve 3205-V-32.
    e. Open tank drain valve 3205-V-30.
    f. On the HMI, set sterilization temperature on TIC-3204A-10B to 250 °F (121 °C) and set the MODE to AUTO.
4. When the tank reaches 5 psi of pressure, remove residual air by:
    a. On the HMI, stop steam to the tank by setting MODE to MANUAL on TIC-3204A-10B and OUTPUT to -5.
    b. Close tank drain valve 3205-V-30.
    c. Open vent line to vacuum pump valve 3205-V-19 and V-56.
    d. At the HMI, open solenoid valve XV-3201-01 and turn on vacuum pump PV-3201.
    e. At the HMI, monitor the pressure on PI-3204A-33 until it reaches -10 psi.
    f. At the HMI, turn off the vacuum pump PV-3201 and close the solenoid valve XV-3201-01.
    g. Close the vacuum line valves 3205-V-19 and V-56.
    h. On the HMI, set MODE to AUTO on TIC-3204A-10B.
    i. Once the pressure on PI-3204A-33 is above 5 psi, open the tank drain valve 3205-V-30.
5. When the tank reaches 250 °F, record the time.
6. Maintain sterilization temperature for 60 minutes.
7. After sterilization time is completed, cool the tank by:
    a. On the HMI stop steam to the tank by setting MODE to MANUAL on TIC-3204A-10B and OUTPUT to -5
    b. Stop steam to the jacket by closing valve 3205-V-51.
    c. Start adding cooling water to the jacket by:
        i. Relieve pressure in the jacket by slowly opening jacket drain valve 3205-V-28.
        ii. After steam has stopped exiting drain, open jacket high point vent valve 3205-V-53.
        iii. After the jacket has been drained, close valves 3205-V-27, V-28, V-53.
        iv. Open valve 3205-V-50.
        v. At the HMI, set Temperature control at TIC-3204A-10B to NORMAL, and on TIC-3204A-10A enter the set point to 98.6 °F (37 °C), and the MODE to AUTO.
    d. On the HMI, monitor the tank pressure at PI-3204A-33.

e. On the HMI, monitor the tank temperature at TIC-3204A-10A.

8. Once the tank temperature reaches 98.6 °F (37 °C), open valves 3205-V-26, -52 to Fermentor A Jacket Pump.
9. At HMI, start Fermentor A Jacket Pump PT-3214.
10. Begin normal operation.

E.3 Operation of Fermentation Tank A

E.3.1 Startup

1. Ensure the Base B System is functioning properly according to SOP-8565.
2. Ensure the Phosphoric Acid System is functioning properly according to SOP-8110.
3. Ensure the Antifoam System is functioning properly according to SOP-8310.
4. Ensure media solution is ready according to Media Preparation SOP-2155.
5. Ensure Trace Metal Storage System is ready according to the Trace Metal Storage SOP-3250.
6. If needed, ensure the lime system is operational according to the Lime System SOP-8405
7. Close valve 3205-V-30 to prevent flow through main drain.
8. Open valves 3205-V-19 and V-58 to the Beer Well vent line.
9. On the HMI, open valves XV-3204A-35 and XV-3204A-36.
10. Add the desired amount of Trace Metals, Magnesium Sulfate and Sodium Metabisulfite solutions according to the Experimental Plan by:
    a. Ensure Trace Metals, Magnesium Sulfate and Sodium Metabisulfate solutions are ready to transfer according to the Trace Metal Storage SOP-3250.
    b. At HMI, set the speed of the pump using SIC-3202-02 (according to the experimental plan), switch the pump speed controller SIC-3202-02 to AUTO, and turn on Trace Metals Pump 1 (PT-3202).
    c. At HMI, monitor pressure in PI-3202-04.
    d. At HMI, turn off Trace Metals Pump 1 when transfer is completed. This may take up to several hours.
11. Add desired amount of biomass slurry according to the Experimental Plan by:
    a. Ensure biomass slurry is ready to transfer according to the Biomass Liquefaction SOP-2325.
    b. Open valve 3205-V-04 to open the transfer line.
    c. On the HMI, open valve XV-3204A-37.

d. Start adding biomass slurry to the tank according to the Biomass Liquefaction SOP-2325.
e. At the HMI, monitor the flow rate of the pH Adjustment Pump (PT-2302) using SIC-2302-14. Determine the amount of slurry added using the level sensor LI-3204A-03.
f. On the HMI, verify the temperature is set to 98.6 °F (37 °C) in TIC-3204A-10A.
g. Once the level of the vessel is above ~15% turn on agitation at the HMI using XS-3204A-05, and on SIC-3204A-05 set the speed according to the Experimental Plan.
h. Turn on the air sparger by:
    i. Open valve 3205-V-21.
    ii. On the HMI, open valves XV-3204A-39.
    iii. Set the desired sterile air flow rate according to the experimental plan in the rotameter FI-3204A-26.
    iv. Monitor the air pressure in PI-3203A-08.
i. Verify the temperature in Fermentor A is around 98.6 °F (37 °C) in TI-3204A-18.
j. Configure valves to inoculate Fermentor A from either Secondary Propagator 3A or 3B:
    i. If inoculating from Propagator 3A:
        i). Ensure Propagator 3A is operational and ready to deliver the seed according to the Secondary Propagator 3A SOP-3220.
        ii). Close valve 3205-V-09 and open valves 3205-V-06, -29, -30, -31.
        iii). Transfer the desired amount of seed solution from Propagator 3A to Fermentor A according to the Experimental Plan and Secondary Propagator 3A SOP-3220.
        iv). Close valves 3205-V-06, -29, -30, -31 when inoculation is complete
    ii. If inoculating from Propagator 3B:
        i). Ensure Propagator 3B is operational and ready to deliver the seed according to the Secondary Propagator 3B SOP-3225.
        ii). Close valve 3205-V-09 and open valves 3205-V-11, -29, -30, -31.
        iii). Transfer the desired amount of seed solution from Propagator 3B to Fermentor A according to the Experimental Plan and Secondary Propagator 3B SOP-3225

iv). Close valves 3205-V-11, -29, -30, -31 when inoculation is complete.

iii. Record time and date of inoculation in fermentation log book.

k. When the tank level reaches 20 % to cover pH probe AE-3204A-01A , turn on the pH control by:
   i. On the HMI, set the pH to 6.3 in AIC-3204A-01 using AE-3204A-01A.
   ii. On the HMI, switch the controller AIC-3204A-01 to AUTO.
   iii. Take a sterile sample using sample valve SSP-3205-01 and the steam valve 3205-V-22 according to Sampling SOP-0511.
   iv. Measure the pH of the sample according to pH Measurement SOP-0514.
   v. If the pH is not 6.3 $\pm$ 0.20, contact supervisor.

l. Start antifoam control by:
   i. Ensure the antifoam is ready to transfer according to the Antifoam System Operation SOP-8310.
   ii. On the HMI, turn on Antifoam Pump 2 (PT-3215).
   iii. On the HMI, turn on antifoam control by switching the controller to AUTO.

m. Once the tank has reached the appropriate level according to the experimental plan, switch to the next tank according to the Liquefaction SOP-2325 and close valve 3205-V-04.

12. On the HMI, close valve XV-3204A-37.
13. Take a sterile sample using sample valve SSP-3205-01 and the steam valve 3205-V-22 according to Sampling SOP-0511.
    a. Measure sugars, organic acids, and inhibitors concentration according to SOP-0505.
    b. Measure ethanol according to Ethanol Measurement SOP-0500
    c. Perform viable plate counts according to SOP-0507.

E.3.2 Operation

1. On the HMI, monitor the temperature and pH regularly in TIC-3204A-10A and AIC 3204A-01, respectively.
    a. Should temperature or pH be different from the set-point (±10%) contact supervisor.

2. Every 24 hours, take a sterile sample using sample valve SSP-3205-01 and the steam valve 3205-V-22 according to Sampling SOP-0511 and the Experimental Plan.
    a. Measure sugars, organic acids, and inhibitors concentration according to SOP-0505.
    b. Measure ethanol concentration according to Ethanol Concentration SOP-0500.
    c. Perform viable plate counts according to SOP-0507.
3. Allow for fermentation to complete according to the experimental plan before proceeding to **E.3.3** ***Transfer to Beer Well***.

E.3.3 Transfer to Beer Well

1. At the HMI, turn off the temperature control (TIC-3204A-10A), antifoam (PT-3215), and pH control (AIC-3204A-01) by switching to MANUAL and setting the OUTPUT to -5 for the temperature and the pH controllers, and by switching to OFF the antifoam control.
2. Open valves 3205-V-30, -31, -35, and -42.
3. Ensure Beer Well is ready to receive the beer according to the Beer Well SOP-4000.
4. On the HMI, start the Fermentor A Pump (PC-3204A).
5. When the transfer is complete, turn off the Fermentor A Pump (PC-3204A).
6. Close valve 3205-V-30.

E.4. Cleaning (CIP)

1. Ensure that the CIP system is operational and ready according to CIP system SOP-8205
2. Ensure valves are set according to the table below.

NOTE: Before configuring these valves, ensure the CIP system is not supplying pressure to the CIP header but only to the CIP loops.

Table 2**.** Initial valve positions settings for CIP

Fermentor A system				
Line	**Application**	**Valve**	**Position**	**Check**
CIP Supply	Spare valve	3205-V-01	Closed	
	Slurry line cleanse	3205-V-02	Closed	

		3205-V-03	Open
	Vent line cleanse	3205-V-57	Open
	To tank bottom inlet	3205-V-05	Closed
		3205-V-07	Open
		3205-V-10	Closed
		3205-V-45	Closed
		3205-V-55	Closed
	Spray ball valves	3205-V-15	Closed
		3205-V-16	Closed
	Air sparger	3205-V-20	Closed
CIP Return	To CIP return	3205-V-41	Open
	Spare valve	3205-V-44	Closed
UV Sterilizer	To propagator transfer line	3205-V-09	Closed
Sterile Air	Pressure indicator	3205-V-12	Open
	To spargers	3205-V-21	Open
	To tank top inlet (vent)	3205-V-17	Closed
	Vacuum relief valve	3205-V-18	Open
Vent	Vent outlet	3205-V-19	Open
	To beer well	3205-V-58	Closed
	To vacuum pump	3205-V-56	Closed
Steam	To vessel jacket	3205-V-51	Closed
	Pressure indicator	3205-V-49	Open
	Pressure indicator	3205-V-47	Open
	To tank bottom inlet	3205-V-48	Closed
	Steam inlet	3205-V-33	Closed
	To sample port	3205-V-22	Closed
	Drain	3205-V-46	Closed
Cooling water/hot water	Cooling water/hot water return	3205-V-50	Closed
Base B	To Base/lime slurry inlet	3205-V-13	Closed

Fermentor A system				
Line	**Application**	**Valve**	**Position**	**Check**
Overflow	To floor hose connection	3205-V-54	Closed	

Transfer line	Bottom drain	3205-V-30	Open
	To Fermentor A pump	3205-V-31	Open
		3205-V-35	Open
	Drain	3205-V-36	Closed
	Steam trap	3205-V-32	Closed
	To beer well	3205-V-38	Open
		3205-V-42	Closed
	To hose connection	3205-V-40	Closed
	Drain	3205-V-39	Closed
	Drain	3205-V-43	Closed
	To CIP return	3205-V-41	Open
	Drain	3205-V-44	Closed
	Spare line (Propagator 1A)	3205-V-34	Closed
	From Propagator 3A	3205-V-06	Closed
	Spare valve	3205-V-08	Closed
	From Propagator 3B	3205-V-11	Closed
		3204-V-42	Closed
		3204-V-45	Closed
		3204-V-46	Closed
	To tank bottom inlet	3205-V-29	Open
	From Hydrolysate pH Adjustment Tank	3205-V-04	Closed
	Separate valve	3205-V-23	Closed

3. On the HMI, open valves XV-3204A-38 and XV-3204A-39 to open the air sparger lines to Fermentor A.

   Note: Air should be flowing constantly through the spargers during the entire CIP process in order to prevent clogging.

4. On the HMI, open valve XV-3204A-37 to open the slurry line to Fermentor A.
5. Set CIP system to deliver Rinse Water by:
   a. Slowly opening valve 8201-V-20 and then closing valve 8201-V-21.
   b. On the HMI, turn on Fermentor A Pump PC-3204A.
   c. Locally monitor the pressure in PI-3204A-22 to be around 20 PSI.

d. To rinse out the vent line and the slurry line, let the CIP system run with the current valve configuration for 5 min.
e. After 5 min, open CIP supply spray ball valves 3205-V-15 and -16.
f. Close valves 3205-V-03, -07, -29 and -57.
g. Open valve 3205-V-58.
h. Run the rinse cycle for 15 min.
i. Open valve 8201-V-21 and then close valve 8201-V-20.
j. On the HMI, turn off Fermentor A Pump PC-3204A.

6. Set CIP system to deliver Caustic water by:
   a. Close CIP supply spray ball valves 3205-V-15 and 16.
   b. Close vent valve 3205-V-58.
   c. Open CIP supply valve 3205-V-03, -07, -29 and -57 to allow CIP solution into the vent line and slurry line.
   d. Close 8201-V-31 and open 8201-V-04 to return CIP solution to the Dilute Caustic Tank.
   e. Slowly open valve 8201-V-13 and then close 8201-V-14.
   f. On the HMI, turn on Fermentor A Pump PC-3204A.
   g. Locally monitor the pressure in PI-3204A-22 to be around 20 PSI.
   h. To clean the vent line and slurry line, let the CIP system run with the current valve configuration for 5 min.
   i. After 5 min, open CIP supply spray ball valves 3205-V-15 and -16.
   j. Close valves 3205-V-03, -07, -29 and -57.
   k. Open valves 3205-V-58.
   l. Run the rinse cycle for 15 minutes.
   m. Open valve 8201-V-14 and then close valve 8201-V-13.
   n. On the HMI, turn off Fermentor A Pump PC-3204A.
7. Set CIP system to deliver UV water by:
   a. Close CIP supply spray ball valves 3205-V-15 and 16.
   b. Close vent valve 3205-V-58.
   c. Open CIP supply valve 3205-V-03, -07, -29 and -57 to allow UV water into the vent line and slurry line.
   d. Ensure the UV Water System is running according to SOP-9555.
   e. Close valve 8201-V-04 and open valve 8201-V-30 to return UV water to the Rinse Tank.
   f. Slowly open valve 8201-V-12.
   g. On the HMI, turn on Fermentor A Pump PC-3204A.

h. Locally monitor the pressure in PI-3204-22 to be around 20 PSI.
i. To rinse the vent line and the slurry line, let the UV Water run with the current valve configuration for 5 min.
j. After 5 min, open CIP supply spray ball valves 3205-V-15 and V-16.
k. Close valves 3205-V-03, -07, -29 and -57.
l. Open valves 3205-V-58.
m. Run the UV water cycle for 15 minutes.
n. Close valve 8201-V-12
o. Close the CIP supply spray ball valves 3205-V-15 and V-16.
p. Once the tank is empty, on the HMI, turn off Fermentor A Pump PC-3204A.
q. Close valve 8201-V-30 and open valve 8201-V-31.

8. On the HMI, close valves XV-3204A-37, XV-3204A-38 and XV-3204A-39.
9. Close main drain valve 3205-V-30.
10. Fermentation Tank A is now clean and ready for sterilization.

F. Data Archival and Analysis

Date of inoculation:
Time of inoculation:
Experimental Plan Number:

Date	Time of Day	Time Elapsed	Temp (°F) (TI-3204-18)	pH (AE-3204A-01A)	Agitation (%)	Air flow rate (L/min)	Comments

Take notes of all calculations and measurements. Store the data obtained in the appropriate Log Book.