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STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Primary Propagator #2 VS-3202B

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APPROVALS: Process Change Committee **DATE:**

A. Scope

This SOP describes the procedure to clean, sterilize, and operate Primary Propagator 2B (VS-3202B) during normal operation in order to propagate the seed .

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.

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. Transfer Vessel SOP-0510
7. Sampling SOP-0511
8. Optical Density SOP-0513

9. pH Measurement SOP-0514
10. Conductivity Measurement SOP-0515
11. Plant pH Probe Calibration SOP -0519
12. C5 Hydrolysate Storage SOP-2135
13. Media Preparation SOP-2155
14. Secondary Propagator 3A SOP-3220
15. Secondary Propagator 3B SOP-3225
16. Beer Well SOP-4000
17. Phosphoric Acid System Operation SOP-8110
18. Clean In Place (CIP) SOP-8205
19. Antifoam System Operation SOP-8310
20. Base B System Operation SOP-8565
21. Refrigeration System Operation SOP-9210
22. Air System Operation SOP-9405
23. UV Water System Operation SOP-9555
24. Hot Water System Operation SOP-9605
25. Steam Supply System Operation SOP-9305
26. Potable Water SOP-9705

D. Preparation/Materials/Equipment

E. Detailed Procedure

E.1 Preparation

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 SOP-9705.
3. Instrument Calibration;
    a. Pressure Measurement
        i. On the HMI, verify that pressure indicator (PIT-3202B-33) is reading ambient conditions.

ii. Verify that the reading of the local indicator (PI-3202B-33) is the same as displayed on the HMI.

b. Temperature Measurement
   i. On the HMI, verify that temperature probe (TIC-3202B-10B) is reading current ambient conditions:
   ii. Verify that the reading of the local probe (TI-3202B-02) is the same as displayed on the HMI.

c. Vessel Level Measurement
   i. On the HMI, verify that level indicator LT-3202B-03 is reading zero level.
   ii. Configure the following valves to their appropriate position to ensure there is no liquid in the vessel:
      1. Close valves: 3210-V-31, 3210-V-33, 3210-V-37, 3203-V-08, 3203-V-34, 3203-V-33, and steam trap valve T1-3203-04.
      2. Open valves: 3210-V-04, 3210-V-44, 3210-V-29, 3210-V-32, 3210-V-36, 3203-V-09, 3203-V-26, 3203-V-30, and 3203-V-29.
   iii. If any liquid remains let it fully drain from the tank then set valves back to their original position.

d. pH Measurement
   i. Calibrate pH probe AE-3202B-01A according to Plant pH Probe Calibration SOP-0519
   ii. Verify that the pH reading after calibration is the same as displayed on the HMI tag AIC-3202B-01.

4. Verify that all side ports on the vessel are filled and secure.
5. Visually verify that the jacket does not have hot/cooling water flowing through it by assuring XV-3202B-29, XV-3202B-30, XV-3202B-31, and XV-3202B-32 are closed.
6. Verify the jacket is at ambient temperature and pressure using PI-3202B-16 and TI-3202B-17.
7. **Verify the following automatic valves are in the closed position: XV-3202B-23, XV-3202B-V-24, and XV-3202B-V-27.**
8. Verify that steam trap T1-3210-06 is open.
9. Verify the initial valve settings according to the table below:

STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Primary Propagator #2 VS-3202B

Primary Propagator 2B
VS-3202B

Line	Application	Valve	Position	Check
CIP Header	Spray Ball Valve	3210-V-05	Closed	
	Spray Ball Valve	3210-V-06	Closed	
	Vent Line Cleanse	3210-V-45	Closed	
	C5 and Prep Tank Line Cleanse	3210-V-10	Closed	
	To Main Transfer Line	3210-V-12	Closed	
	To Main Transfer Line	3210-V-13	Closed	
	To Main Transfer Line	3210-V-14	Closed	
	Air Sparger Line Cleanse	3210-V-19	Closed	
UV Water	Intermediate	3210-V-15	Closed	
	To Main Transfer	3210-V-31	Closed	
Sterile Air	Outlet to atmosphere	3210-V-16	Closed	
	Pressure Indicator	3210-V-17	Open	
	Intermediate to spager	3210-V-18	Closed	
	To vent	3210-V-01	Closed	
	To vent	3210-V-03	Open	
	Vacuum Relief Valve	3210-V-07	Closed	
Phosphoric Acid	Inlet to tank	3210-V-21	Open	
Prep Tank	Inlet to tank	3210-V-11	Closed	
C5 Pump	Inlet to tank	3210-V-09	Closed	
Vessel Jacket	High Point Vent	3210-V-46	Open	
	Recirculation Pump	3210-V-42	Closed	
	Drain	3210-V-47	Closed	
	Steam Inlet	3210-V-40	Closed	
	Steam Pressure Gauge	3210-V-39	Open	
	Recirculation pump	3210-V-26	Closed	
	Cool/Hot water return	3210-V-41	Open	
	Pressure Indicator	3210-V-25	Open	

	Drain to steam trap	3210-V-27	Closed
	Drain to floor	3210-V-28	Open
	Recirculation pump supply	3214-V-17	Open
	Recirculation pump return	3214-V-20	Open
	Recirculation pump pressure indicator	3214-V-18	Open
Steam Inlet to Tank	Inlet to tank	3210-V-34	Closed
	Pressure Indicator	3210-V-38	Open
	Sample Port	3210-V-48	Open
	Sample Port	3210-V-20	Closed
Vent	Main	3210-V-04	**Open**
	To Beerwell	3210-V-44	Closed
	To Vacuum Pump	3210-V-43	Closed
Transfer Line	Bottom Drain Valve	3210-V-29	Closed
	Steam Trap	3210-V-35	Closed
	Intermediate	3210-V-32	Open
	Secondary Propagator 3A	3210-V-36	Closed
	Secondary Propagator 3B	3210-V-37	Closed

E.2 Sterilization

1. Ensure the Steam System is operational according to Steam System SOP-9305.
2. Ensure the Hot Water System is functioning properly according to SOP-9605.
3. Ensure the Refrigeration System Operation is function properly according to SOP-9210.Ensure the Primary Propagator 2B Jacket has been fully drained by visual verification at the floor drain exit, located down the line from valve 3210-V-28.
4. Add Low Pressure Steam to the tank jacket by:
    a. At the HMI, switch the temperature control TIC-3202B-10B to SIP mode.
    b. Close Jacket High Point Vent valve 3210-V-46.
    c. Verify steam pressure in PI-3202B-15 is below 30 psi.
        i. If not, contact supervisor.
    d. Slowly open steam supply valve 3210-V-40.

e. When steam exits drain downline from 3210-V-28, open the drain to the steam trap (valve 3210-V-27) and close the drain valve 3210-V-28.
f. Verify the pressure in PI-3202B-15 is 15 ± 2 psi.
    i. If not, contact supervisor.

5. Add Low Pressure Steam to the tank by:
    a. Open steam supply valve 3210-V-34 on the bottom of the tank.
    b. Open tank drain to trap valve 3210-V-33.
    c. Open tank drain valve 3210-V-29.
    d. On the HMI, set sterilization temperature on TIC-3202B-10B to 250 °F (121 °C).
    e. On the HMI, set MODE to AUTO on TIC-3202B-10B.
6. When the tank reaches 5 psi of pressure, remove residual air by:
    a. On the HMI, set MODE to MANUAL on TIC-3202B-10B and OUTPUT to -5.
    b. Close tank drain valve 3210-V-29.
    c. Open the vent line to vacuum pump valves 3210-V-04 and 3210-V-43.
    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-3202B-33 until it reaches -10 psi.
    f. At the HMI, turn off vacuum pump PV-3201, close the solenoid valve XV-3201-01, and then immediately move to the next step.
    g. Close the vent line to vacuum pump valves 3210-V-04 and 3210-V-43.
    h. On the HMI, set MODE to AUTO on TIC-3202B-10B.
    i. Once the pressure reads 0 psi on PI-3202B-33, open the tank drain valve 3210-V-29.
7. When the tank reaches 250 °F, record the time.
8. **Ensure the tank pressure is above the vacuum relief set point, then open vacuum relief valve 3210-V-07 to sterilize the portion of pipe between the relief valve and the tank. This valve will remain open throughtout fermentation.**
9. Maintain sterilization temperature (250 °F or higher) for 60 minutes.
10. 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-3202B-10B and OUTPUT to -5.
b. Close valve 3210-V-34.
c. Stop steam to the jacket by closing valve 3210-V-40.
d. Introduce sterile-air to the tank by slowly opening valve 3210-V-01. This will prevent a vacuum from forming while the tank cools rapidly. (This step may not be needed depending on the vacuum relief vent efficiency)

NOTE: It is imperative that the pressure inside the tank does not drop below atmospheric from here on.

e. Start adding cooling water to the jacket by:
    i. Relieve pressure in the jacket by slowly opening jacket drain valve 3210-V-28.
    ii. After steam has stopped exiting drain, open jacket high point vent valve 3210-V-46.
    iii. After the jacket has been drained, close valves 3210-V-27, 3210-V-28, 3210-V-46.
    iv. At the HMI, set MODE to NORMAL on TIC-3202B-10B.
    v. At the HMI, set Temperature control to 98.6 °F (37 °C) and AUTO on TIC-3202B-10A.
f. Monitor the tank pressure on the HMI at PI-3202B-33.
g. Monitor the tank temperature on the HMI at TIC-3202B-10A.
h. Once the tank temperature reaches 98.6 °F (37 °C):
    i. Close valve 3210-V-01, which will then put the responsibility of maintaing a positive pressure differential onto the vacuum relief vent only.
    ii. Open valve 3210-V-26 and 3210-V-42 to the Primary Propagator 2B Jacket pump.
    iii. At HMI, start Primary Propagator 2B Jacket Pump PT-3212.

E.3 **Operation of Primary Propagator 2B**

TITLE: Primary Propagator #2 VS-3202B

a. Start Up

1. Ensure the UV Water System is running according to SOP-9555.
2. Ensure the Base B System is functioning properly according to SOP-8565.
3. Ensure the Phosphoric Acid System is functioning properly according to SOP-8110.
4. Assure the Antifoam System is functioning properly according to SOP-8310.
5. Close valve 3210-V-29 to prevent flow through the bottom of tank transfer line.
6. Open valves 3210-V-04 and V-44 to the Beer Well vent line.
7. Open valve 3210-V-21 to allow for addition of Phosphoric Acid.
8. Add desired amount of Hydrolysate according to the Experimental Plan by:
    a. Ensure C5 Hydrolysate Storage is ready according to SOP-2135.
    b. Open valve 3210-V-09 to start adding C5 hydrolysate to the tank.
    c. At the HMI, corroborate the amount of slurry added using the level sensor LIC-3202B-03.
    d. Once the desired level has been achieved, stop the transfer of hydrolysate according to SOP-2135.
    e. Close valve 3210-V-09.
9. Add the amount of UV water necessary for operation according to the Experimental Plan by:
    a. Close Valve 3210-V-32.
    b. Open Valves 3210-V-15 and V-31.
    c. Open Valve 3210-V-29 to add water through the bottom of the tank.
    d. Once the volume reaches 20%, turn on agitation (AG-3202A) using XS-3202A-05, and set speed to 100% in MANUAL on the HMI using SIC-3202A-05.
    e. Monitor the liquid level on the HMI LIC-3202B-03 until it reaches the desired level.
    f. Turn off UV Water addition by closing valves 3210-V-29 and 3210-V-31.
10. Verify that the temperature is set to 98.6 °F (37 °C) on the HMI (TIC-3202B-10A).
11. When the temperature reaches the set point, turn on the pH control by:
    a. Set the pH to 9.0 at the HMI for AIC-3202A-01A and switch to AUTO.
    b. Once the pH is at setpoint, turn off the pH control at the HMI by switching to MANUAL and setting the OUTPUT to -5 (AIC-3202B-01).

c. Take a sample using sample valve SSP-3210-01 and the steam valve 3210-V-20 according to Sampling SOP-0511.
d. Meaure the pH of the sample according to pH Measurement SOP-0514.
    i. If the pH is not 9.0 ± 0.20, contact supervisor.

12. Allow the Hydrolysate/UV Water solution to sit for 20 – 24 h.
13. Add the desired amounts Trace Metals, Magnesium Sulfate and Sodium Metabisulfite solution according to the Experimental Plan by:
    a. Open valve 3210-V-11.
    b. Add the desired amounts of Trace Metal, Magnesium Sulfate and Sodium Metabisulfite solution according to the Media Preparation SOP-2155.
    c. Once the desired amounts have been added, close valve 3210-V-11.
14. Turn on the air sparger by:
    a. Open valve 3210-V-18.
    b. Set the desired sterile air flowrate in the rotameter FI-3202B-26.
15. Turn on Antifoam Control by:
    a. Open valves 3213-V-20.
    b. At the HMI, switch the antifoam to AUTO.
16. Set the pH to 6.3 at the HMI using AIC-3202B-01.

CAUTION: This procedure requires the use of genetically modified bacteria, vessel sterility and microbial containment is imperative.

17. Inoculate the propagator using sterile transfer vessel according to the experimental plan and Transfer Vessel SOP-0510.
    a. Record time and date of inoculation in fermentation log book.
18. Take a sterile sample using sample valve SSP-3210-01 and the steam valve 3210-V-20 according to Sampling SOP-0511.
    a. Measure ethanol according to Ethanol Measurement SOP-0500.
    b. Measure sugars, organic acids, and inhibitors concentration according to SOP-0505.
    c. Measure cell density according to SOP-0513.

d. Viable plate counts according to SOP-0507.

b. Operation

1. Monitor the temperature and pH regularly on HMI using TIC-3202B-10A and AIC-3202B-01.
2. Should temperature or pH be different from the set-point (±10%) contact supervisor.
3. Take sterile samples according to the Experimental Plan using the sample valve SSP-3210-01 and the steam valve 3210-V-20 according to Sampling SOP-0511.
    a. Measure ethanol according to Ethanol Measurement SOP-0500.
    b. Measure sugars, organic acids, and inhibitors concentration according to SOP-0505.
    c. Measure cell density according to SOP-0513.
    d. Viable plate counts according to SOP-0507.
4. After 24 h, take a sterile sample according to the Experimental Plan using the sample valve SSP-3210-01 and the steam valve 3210-V-20 according to Sampling SOP-0511.
    a. Measure ethanol according to Ethanol Measurement SOP-0500.
    b. Measure sugars, organic acids, and inhibitors concentration according to SOP-0505.
    c. Measure cell density according to SOP-0513.
    d. Viable plate counts according to SOP-0507.
5. If the analysis of the samples taken in step 4 fall within the expected parameters (according to Experimental Plan), proceed to section E.3.d: *Transfer of seed to Secondary Propagator 3A/3B.*

c. Broth Contamination or Disposal

1. In the case that the Propagation is not carried out forward to the secondary propagation, transfer the contents to the Beer Well by:
    a. Make sure the Beer Well is ready to receive the broth according to the Beer Well SOP-4000.

b. At the HMI, turn off temperature (TIC-3202B-10A) and pH control (AIC-3202B-01) by switching to MANUAL and setting the OUTPUT to -5.
c. At the HMI, turn off the antifoam by switching to OFF.
d. Configure valves for transfer from Primary Propagator 2B to Beer Well by:
    i. Ensure valves 3210-V-29, 3210-V-31 and 3210-V-37 are closed.
    ii. Close valve 3210-V-33.
    iii. Open valves 3210-V-32 and 3210-V-36.
    iv. Close valves 3203-V-06, 3203-V-07, 3203-V-08, 3203-V-30, 3203-V-34, 3203-V-36, 3203-V-38 and 3203-V-41.
    v. Close valves 3205-V-05, 3205-V-07, 3205-V-08, 3205-V-09, 3205-V-11, 3205-V-31, 3205-V-32, 3205-V-36, 3205-V-39, 3205-V-40, 3205-V-41, 3205-V-43.
    vi. Open valves 3203-V-09, 3203-V-26, 3203-V-33, 3203-V-40, and 3203-V-42
    vii. Open valves 3205-V-06, 3205-V-29, 3205-V-35, 3205-V-38, 3205-V-42.
    viii. Open valve 3210-V-29 to allow broth to fill the lines up to Fermentor A pump.
e. At the HMI, turn on the Fermentor A Pump (PC-3204A) on XS-3204A-21.
f. Monitor the pressure in PI-3204A-22.
g. Once the pressure drops significantly the propagator is empty and the pump (PC-3204A) can be turned off on the HMI (XS-3204A-21).
h. Transfer the newly added contents from the Beer Well to the Decanter Feed Tank according to Beer Well SOP-4000.

d. Transfer of seed to Secondary Propagator 3A/3B

1. At the HMI, turn off temperature (TIC-3202B-10A) and pH control (AIC-3202B-01) by switching to MANUAL and setting the OUTPUT to -5.
2. At the HMI, turn off the antifoam by switching to OFF.
3. Configure valves for transfer from Primary Propagator 2B to either Secondary Propagator 3A or 3B:
    a. If transferring to Secondary Propagator 3A (VS-3203A):

i. Close valve 3203-V-08 and 3203-V-09.
ii. Open valve 3210-V-36.

b. If transferring to Secondary Propagator 3B (VS-3203B):
i. Close valve 3204-V-08 and 3204-V-09.
ii. Open valve 3210-V-37.

4. Ensure valves 3210-V-09, 3210-V-10, 3210-V-11, and 3210-V-31 are closed.
5. Close valve 3210-V-21.
6. Ensure XV-3202B-23 is in the closed position on the HMI.
7. Close valve 3210-V-44.
8. Open valve 3210-V-32.
9. Close valve 3210-V-33.
10. Open valve 3210-V-29.
11. Pressurize Primary Propagator 2B to 30 PSI by:
    a. Slowly open valve 3210-V-03 while having someone at the HMI watch and relay the pressure reading on PI-3202B-33.

CAUTION: Make sure you do not over-pressurize the vessel. When the vessel is pressurized to 50 PSI, the rupture disc breaks (PSE-3202B-28) and the entire contents of the tank are transferred to the Beer Well in order to release the pressure.

    b. Once the pressure of the vessel has reached 30 PSI, close valve 3210-V-03.
    c. Complete the SOP for operation of either Secondary Propagator 3A or 3B (SOP-3220 or SOP-3225).

c. Shutdown

1. Ensure the necessary amount of inoculum has been transferred into either Secondary Propagator 3A or 3B according to the Experimental Plan.
2. Ensure the main drain valves for either Secondary Propagator (3A: 3203-V-34, 3B: 3204-V-30) are closed.
3. Open vent valve 3210-V-44 to release any remaining pressure in the vessel.

d. Cleaning

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.

Rinse Tank TS-8203				
Line	**Application**	**Valve**	**Position**	**Check**
CIP Return	Rinse Tank Return	8201-V-30	Closed	
	Caustic Tank Return	8201-V-04	Closed	
	To Drain	8201-V-31	Open	
Primary Propagator 2B VS-3202B				
Line	**Application**	**Valve**	**Position**	**Check**
CIP Header	Spray Ball Valve	3210-V-05	Closed	
	Spray Ball Valve	3210-V-06	Closed	
	Vent Line Cleanse	3210-V-45	Open	
	C5 and Prep Tank Line Cleanse	3210-V-10	Open	
	To Main Transfer	3210-V-12	Closed	
	To Main Transfer	3210-V-13	Closed	
	To Main Transfer	3210-V-14	Closed	
	Air Sparger Line Cleanse	3210-V-19	Open	
Vent	Main	3210-V-04	Open	
	To Beerwell	3210-V-44	Closed	
	To Vacuum Pump	3210-V-43	Closed	
UV Water	Intermediate	3210-V-15	Closed	
	To Main Transfer	3210-V-31	Closed	
Sterile Air	Outlet to Atmosphere	3210-V-16	Closed	
	Intermediate to	3210-V-18	Closed	

	sparger			
	Intermediate to top of tank	3210-V-03	Closed	
	Vacuum Relief Valve	3210-V-07	Closed	
Phosphoric Acid	Inlet to tank	3210-V-21	Closed	
Prep Tank Cooler	Inlet to tank	3210-V-11	Closed	
C5 Pump	Inlet to tank	3210-V-09	Closed	
Steam Inlet to Tank	Inlet to tank	3210-V-34	Closed	
	Sample Port	3210-V-20	Closed	
Transfer Line	Bottom Drain Valve	3210-V-29	Open	
	Steam Trap	3210-V-33	Closed	
	Intermediate	3210-V-32	Open	
	Secondary Propagator 3A	3210-V-36	Closed	
	Secondary Propagator 3B	3210-V-37	Closed	
Fermentor A VS-3204A				
Line	**Application**	**Valve**	**Position**	**Check**
CIP Header	Secondary Propagator 3A/3B Transfer Line Cleanse	3205-V-05	Closed	
	Secondary Propagator 3A/3B Transfer Line Cleanse	3205-V-07	Closed	
	Secondary Propagator 3A/3B Transfer Line Cleanse	3205-V-10	Closed	
Main Transfer	UV Water Addition	3205-V-09	Closed	
	Outlet to atmosphere	3205-V-08	Closed	
	Secondary Propagator 3A/3B Transfer Line	3205-V-29	Open	
	Main Drain	3205-V-31	Closed	
	Drain to Trap	3205-V-32	Closed	
	Outlet to atmosphere	3205-V-36	Closed	
	Outlet to atmosphere	3205-V-39	Closed	

Outlet to atmosphere	3205-V-40	Closed
Outlet to atmosphere	3205-V-44	Closed
Beer Well Transfer Line	3205-V-42	Closed
Main Return Line	3205-V-35	Open
CIP Return Line	3205-V-38	Open
CIP Return Line	3205-V-41	Open

3. Clean Primary Propagator 2B (VS-3202B) by:
   a. If inoculum was transferred to Secondary Propagator 3A (VS-3203A):
      i. Verify valves 3203-V-06, 3203-V-07, 3203-V-08, 3203-V-30 and 3203-V-34 are closed.
      ii. Verify valves 3203-V-09 and 3203-V -26 are open.
      iii. Close valves 3203-V-36, 3203-V-38, and 3203-V-41.
      iv. Open valves 3203-V-33, 3203-V-40, and 3203-V-42.
      v. Close valve 3205-V-11.
      vi. Open valve 3205-V-06.
      vii. Allow for transfer of CIP waste to Fermentor A pump PC-3204A by opening valve 3210-V-36.
   b. If inoculum was transferred to Secondary Propagator 3B (VS-3203B):
      i. Verify valves 3204-V-06, 3204-V-07, 3204-V-08, 3204-V-30, and 3204-V-33 are closed.
      ii. Verify valves 3204-V-09 and 3204-V-32 are open
      iii. Close valves 3204-V-40, 3204-V-42, and 3204-V-45.
      iv. Open valves 3204-V-35, 3204-V-44, and 3204-V-46.
      v. Close valve 3205-V-06.
      vi. Open valve 3205-V-11.
      vii. Allow for transfer of CIP waste to Fermentor A pump PC-3204A by opening valve 3210-V-37.
4. Set CIP system to deliver Rinse Water to drain by:
   a. Slowly opening valve 8201-V-20 and then closing valve 8201-V-21.

b. At the HMI, turn on pump PC-3204A.
c. Locally monitor the pressure in PI-3204-22 to be around 20 psi.
    i. If pressure acts eratically below 15 psi then notify supervisor in the control room.
d. To rinse out the vent line, air sparger line, and C5/Prep tank line let the CIP system run with the current valve configuration for 1 minute.
e. After 1 minute, open CIP supply spray ball valves 3210-V-05 and 3210-V-06.
f. Close CIP supply valves 3210-V-45, 3210-V-10, and 3210-V-19.
g. Open vent line valve 3210-V-44 and allow the rinse cycle to run for 15 mintues.
h. After 15 minutes, open valve 8201-V-21 and then close valve 8201-V-20 and quickly turn off pump PC-3204A at the HMI.

5. Set CIP system to deliver Caustic water by:
    a. Close CIP supply spray ball valves 3210-V-05 and 3210-V-06.
    b. Close vent valve 3210-V-44.
    c. Open CIP supply valves 3210-V-45, 3210-V-10, and 3210-V-19 to allow CIP solution into the vent line, air sparger line, and C5/Prep tank 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. At the HMI, turn on pump PC-3204A.
    g. Locally monitor the pressure in PI-3204-22 to be around 20 psi.
        i. If pressure acts eratically below 15 psi then notify supervisor in the control room.
    h. To clean out the vent line, air sparger line, and the C5/Prep tank line let the CIP system run with the current valve configuration for 1 minute.
    i. After 1 minute, open CIP supply spray ball valves 3210-V-05 and 3210-V-06.
    j. Close CIP supply valves 3210-V-45, 3210-V-10, and 3210-V-19.
    k. Open vent line valve 3210-V-44 and allow the clean cycle to run for 15 mintues.
    l. After 15 minutes, open valve 8201-V-14 and then close valve 8201-V-13 and quickly turn off pump PC-3204A at the HMI.
6. Set CIP system to deliver UV water by:

a. Ensure the UV Water System is running according to SOP-9555.
b. Close CIP supply spray ball valves 3210-V-05 and 3210-V-06.
c. Close vent valve 3210-V-44.
d. Open CIP supply valves 3210-V-45, 3210-V-10, and 3210-V-19 to allow UV Water into the vent line, air sparger line, and C5/Prep tank line.
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.
a. At the HMI, turn on pump PC-3204A.
b. To rinse out the vent line, air sparger line, and the C5/Prep tank line let the CIP system run with the current valve configuration for 1 minute.
c. After 1 minute, open CIP supply spray ball valves 3210-V-05 and 3210-V-06.
g. Close CIP supply valves 3210-V-45, 3210-V-10, and 3210-V-19.
h. Open vent line valve 3210-V-44 and allow the UV rinse cycle to run for 15 mintues.
i. After 15 minutes, close valve 8201-V-12 and quickly turn off pump PC-3204A at the HMI.

7. Close main drain valve 3210-V-29.
8. Primary Propagator 2B is now clean and ready for use.

STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Primary Propagator #2 VS-3202B

Fermentation Data Log:

Experimental plan number:_____ Vessel:_____ Biomass type:_____

Date	Time of Day	Elapsed Time (h)	Temp (deg F)	pH (Bottom Probe| Top Probe)	Airflow Rate (L/min)	Pressure (psi)	Comment
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