

**STANDARD OPERATING PROCEDURE  
STAN MAYFIELD BIOREFINERY PILOT PLANT****TITLE: Biomass Liquefaction**

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**A. Scope**

This SOP describes the procedure to clean and operate the Liquefaction Tank (VS-2301), Liquefaction Cooler (HP-2301), and pH Adjustment Tank (VS-2302) during normal operation in order to liquefy pretreated biomass with enzymes to produce slurries rich in C6 sugars for fermentation.

**B. Safety and Training Requirements**

Refer to UF lab safety policies regarding equipment listed in section D below before starting any process work.

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 Goggles
- Protective Gloves
- Hard Hat

**C. Related Documents and SOPs**

1. Experimental Plan
2. Dry Weight Measurement by Loss on Drying SOP-0502
3. Dry Weight Measurement by Moisture Balance SOP-0503
4. Sugars, Organic Acids, and Inhibitors Concentration SOP-0505
5. Sampling SOP-0511
6. Plant pH Probe Calibration SOP-0517
7. Biomass Pretreatment SOP-2110
8. Secondary Propagator 3A SOP-3220
9. Secondary Propagator 3B SOP-3225
10. Fermentation Tank A SOP-3230
11. Fermentation Tank B SOP-3234

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12. Fermentation Tank C SOP-3240
13. Beer Well SOP-4000
14. CIP System SOP-8205
15. Cellulase System SOP-8355
16. Aqueous Ammonia System SOP-8510
17. Refrigeration System SOP-9210
18. Steam System SOP-9305
19. Air System SOP-9405
20. UV Water System SOP-9555
21. Hot water system SOP-9605
22. Potable Water System SOP-9705
- 23.

**D. Preparation/Materials/Equipment**

1. Pretreated Biomass
2. UV water
3. Aqueous Ammonia
4. Cellulase enzyme cocktail

**E. Detailed Procedure**

**NOTE:** At the HMI and at local devices, ensure that the Liquefaction vessel is empty, at atmospheric temperature and pressure, clean, and ready for operation.

**CAUTION:** This procedure requires the use of enzyme solutions that may cause allergic reactions.

***E.1 Start-up***

1. Ensure that the air system is operational according to Air System SOP-9405.
2. Ensure that the potable water system is ready according to the Potable Water System SOP-9705.
3. Instrument calibration and verification;
  - a. Pressure Measurement

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- i. Verify the readings on the HMI (PI-2301-08, PI-2302-10) are at ambient conditions and are the same as displayed in the local transmitter screen.
  - b. Temperature Measurement
    - i. On the HMI, verify that the following temperature probes are reading current ambient conditions: TIC-2301-04A, TIC-2302-04A
    - ii. Verify that the readings of the local probes (TI-2301-18, TI-2302-18) are the same as displayed on the HMI.
  - c. pH Measurement
    - i. Calibrate pH probes 2302-16A/B according to Plant pH Probe Calibration SOP-0517
    - ii. Verify that the pH readings on the local pH control unit are the same as displayed on the HMI (AIC-2103-16).
- 4. Verify that all side ports on the vessel are filled and secure.
- 5. Verify the initial valve settings for sterilization according to the table below:

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Liquefaction Tank VS-2301				
Line	Line Number	Valve	Position	Check
Cellulase Supply		2301-V-13	Closed	
CIP Header		2301-V-09	Closed	
		2301-V-12	Closed	
Sterile Air		2301-V-11	Closed	
Aqueous Ammonia		2301-V-02	Closed	
UV Water	Return header supply	2301-V-03	Closed	
	Biomass cooling #1	2301-V-04	Open	
	Biomass cooling #2	2301-V-05	Open	
	Biomass cooling #3	2301-V-06	Open	
	Biomass cooling #4	2301-V-07	Open	
	Direct addition to tank	2301-V-08	Closed	
Vessel Jacket Top	High Point Vent	2301-V-20	Open	
	outlet	2301-V-21	Open	
	recirculation pump	2301-V-19	Closed	
	water return	2301-V-18	Closed	
	Steam	2301-V-22	Closed	
	Steam pressure gauge	2301-V-16	Open	
Vessel Jacket Bottom	Main valve	2301-V-26	Open	
	recirculation pump	2301-V-25	Closed	
	Drain to steam trap	2301-V-30	Open	
	Drain	2301-V-31	Open	
Tank Bottom Inlet	Steam pressure gauge	2301-V-32	Open	
	tank Inlet	2301-V-27	Open	

***Initial Valve Settings Table – cont.***

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Liquefaction Tank VS-2301				
Line	Line Number	Valve	Position	Check
Hydrolyzate pH Adjustment Return		2301-V-14	Closed	
Vent	to Beer Well	2301-V-44	Closed	
		2301-V-XX	Closed	
	to Vacuum Pump	2301-V-43	Open	
Transfer Line	Bottom Drain Valve	2301-V-28	Closed	
	drain to trap	2301-V-33	Closed	
	Future connection	2301-V-34	Closed	
	line to pump	2301-V-35	Open	
	Drain	2301-V-36	Closed	
	Sample	2301-V-45	Closed	
	Distribution	2301-V-37	Open	
	to pH adjustment tank	2301-V-38	Open	
	to CIP return	2301-V-39	Closed	
Jacket Recirc Pump	to Decanter Feed tank	2301-V-40	Closed	
	From Liquefaction Tank	3214-V-47	Open	
	Pressure Gauge	3214-V-48	Open	
Sample Valve	To Liquefaction Tank	3214-V-50	Open	
	Low Pressure Steam Header	2301-V-15	Open	
	Steam to valve	2301-V-42	Closed	
	Sample Valve	SSP-2301-01	Closed	

#### E.2 Sterilization of the Liquefaction Tank

1. Ensure that the steam system is operational according to Steam System SOP-9305.
2. Ensure that the refrigeration system is operational according to Refrigeration System SOP-9201.
3. Ensure that the hot water system is operational according to the Hot Water System SOP-9605.
4. Ensure that the Screw Press Transfer Conveyor (CV-2106) is set to REVERSE direction to the C6 Dump Bin according to the Biomass Pretreatment SOP-2110 and verify that HV-2106-04 is CLOSED.
5. On the HMI, set MODE to SIP on TIC-2301-04B.
6. Once the jacket is drained, close the high point vent valve 2301-V-20.
7. Verify steam pressure in PI-2301-03 is below 30 psi
  - a) If not, contact supervisor.

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8. Slowly open valve 2301-V-22 to allow steam to enter the jacket.
9. Once steam exits the drain, close valve 2301-V-31 and open drain to trap valve 2301-V-30.
10. Verify the pressure in PI-2301-03 is 15 PSI  $\pm$  2 PSI.
  - a) If not, contact supervisor.
11. On the HMI, set sterilization temperature on TIC-2301-04B to 250 °F.
12. On the HMI, set mode to AUTO on TIC-2301-04B.
13. When the tank reaches 5 PSI of pressure, remove residual air by:
  - a) Stop steam to the tank by closing valve 2301-V-27.
  - b) Close tank drain valve 2301-V-28.
  - c) Open vent line using 2301-V-XX
  - d) Close trap valves at bottom drains TI-3201-02 and -03
  - e) On the HMI, open cooling water supply valve XV-3201-01.
  - f) On the HMI, turn on vacuum pump PV-3201
  - g) Monitor the pressure in the Liquefaction tank PI-2301-08 until it reaches -10 PSI
  - h) Close the vent line using valve 2301-V-XX.
  - i) Open the steam valve 2301-V-27.
  - j) On the HMI, turn off vacuum pump PV-2301
  - k) Once pressure reaches 5 PSI, open trap valves TI-3201-02 and -03.
14. When the tank reaches 250 °F, record the time.
15. Maintain sterilization temperature for 60 minutes.
16. After sterilization time is over, stop steam to the tank by closing valve 2301-V-27.
17. Stop steam to the jacket by closing valve 2301-V-22.
18. Monitor the tank pressure on the HMI at PI-2301-08.
19. Monitor the tank temperature on the HMI at TIC-2301-04B.

**CAUTION: The jacket has been pressurized with ~15 PSI steam. Make sure to cordon off the area and wear heat resistant gloves when performing the next step.**

20. Relieve pressure in the jacket by slowly opening jacket drain valve 2301-V-31
21. After steam has stopped exiting drain, open jacket high point vent valve 2301-V-20
22. When jacket is empty, close drain valve 2301-V-31, steam trap 2301-V-30, and the vent valve 2301-V-20.
23. Open Water return valve 2301-V-18.
24. Open Cooling Water supply to jacket valve 2301-V-26.
25. On the HMI, set Temperature control at TIC-2301-04A to 122 °F (50 °C).

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26. On the HMI, set MODE to NORMAL and AUTO on TIC-2301-04A.
27. When the temperature drops below 212 °F, open the sterile air valve 2301-V-11 to add air to the tank via the vacuum breaker.
28. Once the tank temperature reaches 122 °F (50 °C):
  - a) Open valve 2301-V-19 and V-25 to Liquefaction jacket recirculation pump
  - b) At HMI, start Liquefaction Recirculation Pump PT-3204
29. Begin normal operation.

***E. 3 Sterilization of the pH Adjustment Tank***

1. Ensure that the Steam system is operational according to Steam System SOP-9305
2. Ensure that the Refrigeration System is operational according to Refrigeration System SOP-9201
3. Ensure valves are positioned according to the Initial Valve Setting Table below.

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<b>pH Adjustment Tank VS-2302</b>				
<b>Line</b>	<b>Line Number</b>	<b>Valve</b>	<b>Position</b>	<b>Check</b>
CIP Header	CIP upstream to Liquefaction	2303-V-02	Closed	
	CIP Liquefaction feed line	2304-V-03	Closed	
	to spray ball	2303-V-09	Closed	
	to spray ball	2303-V-11	Closed	
Sterile Air	tank inlet	2303-V-07	Closed	
Liquefaction Pump	feed line	2303-V-05	Open	
	tank inlet	2303-V-08	Closed	
Vessel Jacket Top	Jacket outlet	2303-V-16	Open	
	High Point Vent	2303-V-17	Open	
	Recirculation pump	2303-V-15	Closed	
	Water return	2303-V-13	Closed	
	Pressure gauge	2303-V-12	Open	
Vessel Jacket bottom	Jacket Drain	2303-V-23	Open	
	Jacket Inlet	2303-V-25	Open	
	Jacket Drain to trap	2303-V-29	Closed	
	Hot Water	2303-V-27	Closed	
	Cool water Pressure gauge	2303-V-28	Open	
	Recirculation pump	2303-V-24	Closed	
Tank Bottom Inlet	tank drain to trap	2303-V-22	Closed	
		2303-V-20	Open	
Low Pressure Steam Header	Pressure gauge	2303-V-18	Open	
	Drain to trap	2303-V-19	Open	
	to Jacket	2303-V-14	Closed	
	To tank	2303-V-22	Closed	
	Pressure gauge	2303-V-20	Open	

***Initial Valve setting table – cont.***



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pH Adjustment Tank VS-2302				
Line	Line Number	Valve	Position	Check
Transfer Line	Tank Drain	2303-V-26	Closed	
	Tank drain to trap	2303-V-30	Closed	
	transfer line to pump	2303-V-31	Open	
	Drain	2303-V-32	Closed	
	Drain	2303-V-33	Closed	
	transfer to header	2303-V-34	Open	
	to liquefaction tank	2303-V-35	Open	
	to fermentation distribution	2303-V-36	Closed	
	to CIP return	2303-V-37	Closed	
	spare	2303-V-38	Closed	
	to Fermentor A	2303-V-39	Closed	
	to Fermentor B	2303-V-40	Closed	
	spare	2303-V-41	Closed	
	to Propagator 3A	2303-V-42	Closed	
	to Fermentor C	2303-V-43	Closed	
	To Propagator 3B	2303-V-44	Closed	
Aqueous Ammonia	Feed to liquefaction feed line	2303-V-04	Closed	
	feed to tank	2303-V-46	Closed	
Tank Vent	Tank vent	2303-V-10	Open	
	to vacuum pump	2303-V-50	Closed	
	to beer well	2303-V-49	Open	
Sample Valve	Steam supply	2303-V-45	Closed	
	Sample Valve	SSP-2303-01	Closed	

4. On the HMI, set MODE to SIP on TIC-2303-04B.
5. Add Low Pressure Steam to the tank jacket by:
  - a. Close high point vent valve 2303-V-17.
  - b. Verify steam pressure in PI-2302-07 is less than 30 PSI.
  - c. Slowly open steam supply valve 2303-V-14.
  - d. When steam exits the drain, close drain valve 2303-V-23 and open drain to trap valve 2303-V-29
6. Add Low Pressure Steam to the tank by:
  - a. Open steam supply valve 2303-V-22 on the bottom of the tank.
  - b. Close Liquefaction transfer line valve 2303-V-31

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- c. Open tank drain to trap valve 2303-V-30.
  - d. Open main tank drain valve 2303-V-26.
7. Close tank vent to Beer Well valve 2303-V-49.
8. On the HMI, set sterilization temperature on TIC-2303-04B to 250 °F.
9. On the HMI, set MODE to AUTO on TIC-2303-04B.
10. When the tank reaches 5 PSI of pressure, remove residual air by:
  - a. Stop steam to the tank by closing valve 2303-V-22
  - b. Open vent line to vacuum pump valve 2303-V-50
  - c. Close valves to the steam traps T1-2303-02 and T1-2303-04
  - d. On the HMI, open solenoid valve XV-3201-01.
  - e. On the HMI, turn on vacuum pump PV-3201.
  - f. Monitor the pressure in the pH Adjustment Tank PI-2302-10 until it reaches -10 PSI
  - g. Close the vacuum line using valve 2303-V-50
  - h. Open the steam valve 2303-V-22
  - i. When the pressure reaches 5 PSI, open valves to the steam traps T1-2303-02 and T1-2303-04
  - j. On the HMI, turn off vacuum pump PV-3201.
  - k. On the HMI, close solenoid valve XV-3201-01
11. When the tank reaches 250 °F, record the time.
12. Maintain sterilization temperature for 60 minutes.
13. Cool the tank jacket by:
  - a. Stop steam to the tank by closing valve 2303-V-22
  - b. Stop steam to the jacket by closing valve 2303-V-14
  - c. Start cooling water by:
    - i. Relieve pressure in the jacket by slowly opening jacket drain valve 2303-V-23
    - ii. After steam has stopped exiting drain, open jacket high point vent valve 2303- V-17
    - iii. When jacket is empty, close drain 2303-V-23 and vent 2303-V-17 valves.
    - iv. Open cooling water return valve 2303-V-13 and hot water supply valve 2303-V-27.
    - v. Open Cooling Water supply to jacket valve 2303-V-25
    - vi. On the HMI, set Temperature control on HMI at TIC-2302-04 to 122 °F (50 °C)
    - vii. On the HMI, set MODE to NORMAL and AUTO on TIC-2302-04A.
14. Cool the tank by:
  - a. Monitor the tank pressure on the HMI at PI-2302-10.

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- b. Monitor the tank temperature on the HMI at TIC-2302-04.
  - c. When the pressure drops below 10 PSI, open the sterile air valve 2303-V-07 to add air to the tank via the vacuum breaker.
  - d. Once the tank temperature reaches 122 °F (50 °C):
    - i. Open valve 2303-V-15 and V-24 to pH Adjustment Tank recirculation pump.
    - ii. At HMI, start pH Adjustment Tank Recirculation Pump PT-3205.
15. Begin normal operation.

***E.4 Operation of Liquefaction Tank***

1. Ensure UV Water System is operational according to UV Water System SOP-9555.
2. Add the minimum volume of UV water for operation (670 gal ) to the Liquefaction Tank (VS-2301) by;
  - a. Open Valve 2302-V-04 to supply UV water to the Liquefaction Cooler.
  - b. Close vacuum line using valve 2301-V-43.
  - c. Open the vent line to the Beer Well valve 2301-V-44 and valve 2301-V-XX.
  - d. Close line to the vacuum breaker 2301-V-11.
  - e. Ensure valve 2302-V-02 and V-09 are open to supply water from the cooler to the Liquefaction Tank.
  - f. Open valve 2301-V-03 on the Liquefaction Tank to add water directly to the tank.
  - g. On the HMI, set UV Water flow controller (FIC-2301-01) to 100% in MANUAL mode.
  - h. Once the volume reaches 30%, on the HMI, turn on the agitation (AG-2301), switch the speed controller (SIC-2301-06) to MANUAL, and set the Output of SIC-2301-06 to 100%.
  - i. Monitor the liquid level on the HMI LIC-2301-09 until it reaches 36%.
  - j. Turn off UV Water addition by setting the flow controller FIC-2301-01 to -5 and MANUAL mode.
3. Verify that the temperature set point is 122 °F (50 °C) in TIC-2301-04A on the HMI according to the Experimental Plan.
4. Set the Liquefaction Tank level control according to Experimental Plan on HMI LIC-2301-09
5. Turn on the Liquefaction Tank Pump PT-2301 and set SIC-2301-14 to CASCADE on HMI

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6. When the Liquefaction Tank VS-2301 temperature reaches set point, start adding biomass by:
  - a. On the HMI, open valve HIC-2106-04
  - b. Set the Screw Press Transfer Conveyor (CV-2106) to the “Forward” and immediately perform next step E.4.2.c
  - c. On the HMI, Restart the Transfer Conveyor in FORWARD WITHIN 5 SECONDS, using XS-2106-01.
  - d. Start Liquefaction Cooler (HP-2301) by:
    - i. Open valves 2301-V-03 to add UV water to the Liquefaction tank.
    - ii. On the HMI, set Liquefaction Cooler temperature controller (TIC-2301-19) to      °F
    - iii. Set the flow rate of UV Water according to the Experimental Plan on FIC-2301-01.
  - e. Turn on Base Addition
    - i. Prepare Aqueous Ammonia System according to SOP-8510 to deliver ammonia to Liquefaction tank.
    - ii. Open valve 2301-V-10 to deliver Aqueous Ammonia to the Liquefaction Tank.
    - iii. Set pH set point to 5.0 on HMI using AIC-2301-16 with lower pH probe AE-2301-16A, and set the controller to AUTO.
    - iv. Set the speed control SIC-8501-01 to CASCADE.
  - f. Turn on Cellulase Addition
    - i. Ensure that the pH is at setpoint.
    - ii. Prepare enzyme system according to Cellulase System SOP-8355
    - iii. Open valve 2301-V-13 to start cellulase flow to the tank.
    - iv. At the HMI, set flow rate of enzyme solution according to Experimental Plan using FIC-8304-03 and switch the controller to AUTO.
7. Monitor temperature (TIC-2301-04A) and liquid volume (LIC-2301-09) levels using the HMI.
8. Set the output of the Liquefaction Tank to feed the Decanter Feed Tank by opening valve 2301-V-40.

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9. When the liquid level reaches set point (approximately 6 h), open valve 2301-V-28. The pump will start the transfer of material out of the Liquefaction tank to the Decanter Feed Tank.
10. On the HMI, increase the UV water supply set point according to Experimental Plan using FIC-2301-01
11. Take a sterile sample of the Liquefaction material using the Sampling SOP-0511 and valve SSP-2301-01 by:
  - a. Verify valve SSP-2301-01 is closed.
  - b. Open steam supply valve 2301-V-42

**CAUTION: Steam vents into the room. Use protective gloves and rope off area.**

- c. Allow steam to flow through the valve for 3 minutes.
- d. Close steam supply valve 2301-V-42
- e. Wait 10 minutes for the valve to cool
- f. Place sample container below drain valve 2301-V-41
- g. Open sample valve SSP-2301-01 to fill container.
- h. Close sample valve SSP-2301-01
- i. Repeat steaming the valve by Steps E.4.11.a – d.
- j. Take sample to the laboratory and:
  - i. Determine Moisture using the Moisture by Moisture Balance SOP-0503
    1. Target range is set by the Experimental Plan
  - ii. Determine pH using pH Measurement SOP-0514
    1. Target range is set by the Experimental Plan
  - iii. Determine Sugars using Sugar Concentration SOP-0505
    1. Target range is set by the Experimental Plan
12. Monitor the Liquefaction process by taking samples every 30 min and process according to Step 11 above.

**CAUTION: The Decanter Feed Tank capacity is 6 hours of flow. Do NOT overfill.**

13. If the values do not fall with the acceptable range within 5 hours, contact Supervisor.

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14. When values are within the range according to the Experimental Plan, begin transfer feed to the pH Adjustment Tank VS-2302 by:
  - a. Open valve to pH Adjustment Tank 2301-V-38
  - b. Open valve 2303-V-08 on the Liquefaction Pump line on the pH Adjustment Tank VS-2302 to add slurry to the tank.
  - c. Close valve to the Decanter Feed Tank 2301-V-40

#### E.4 Operation of the pH Adjustment Tank

1. According to the Experimental Plan, determine the desired Tank to receive the biomass slurry after pH Adjustment.
2. Make sure the receiving tank is clean and ready to receive biomass according to their respective SOP.
3. Set the valves to the desired receiving tank according to the Table below.

Target Tank	Valve Numbers 2303									
	V-35	V-36	V-37	V-38	V-39	V-40	V-41	V-42	V-43	V-44
Return to Liquefaction	O	C	C	C	C	C	C	C	C	C
Propagator 3B	C	O	C	C	C	C	C	C	C	O
Propagator 3A	C	O	C	C	C	C	C	O	C	C
Fermentor A	C	O	C	C	O	C	C	C	C	C
Fermentor B	C	O	C	C	C	O	C	C	C	C
Fermentor C	C	O	C	C	C	C	C	C	O	C

4. Once the biomass slurry is flowing to the pH Adjustment Tank in Step E.4.14 above, monitor the liquid level until it reaches 30% using the HMI LIC-2302-09.
5. Once the target level is achieved in Step 4;
  - a. Turn on agitation to 100% using HMI (XS-2302-06).
  - b. At the HMI, set the level in the tank according to the Experimental Plan by entering the desired level in LIC-2302-09 and setting the controller to AUTO.
  - c. Turn on pH Adjustment Tank Pump PT-2302 on the HMI.

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- d. On the HMI, turn on Temperature Control by setting the temperature to 98.6 °F (37 °C) on TIC-2302-04 and switching the controller to AUTO.
  - e. Turn on pH Control by:
    - i. Ensure Aqueous Ammonia System is ready according to SOP-8510
    - ii. On the HMI, set pH Set point to 6.3 using AIC-2302-16 and set to AUTO.
    - iii. Set the speed control SIC-8503-01 to CASCADE.
6. Once the tank level reaches set point, the pump will start pumping to the target tank.
7. Monitor liquid level in the tank using the HMI (LIC-2302-09).
8. Take sterile samples according to the Experimental Plan and to the Sampling SOP-0511 using SSP-2303-01, the procedures in Step E.4.11 and:
  - a. Determine Moisture using the Moisture by Moisture Balance SOP-0503
    - i. Target range is set by the Experimental Plan
  - b. Determine pH using pH Measurement SOP-0514
    - i. Target range is set by the Experimental Plan
  - c. Determine Sugars using Sugar Concentration SOP-0505
    - i. Target range is set by the Experimental Plan
9. If any of the values are out of range, contact Supervisor.

***E.5 Shutdown***

1. When the fermentation tank reaches 78% full (6,784 gal), stop Liquefaction by:
  - a. Set the Screw Press Transfer Conveyor (CV-2106) to the REVERSE direction on the HMI (HIC-2106-04) to deliver pretreated biomass to the C6 Dump Bin according to SOP-2110.
  - b. Turn off UV Water addition to Liquefaction by closing supply valve 2301-V-03
  - c. Turn off pH Control at HMI AIC-2301-16 to Manual with OUTPUT at -5
  - d. Turn off enzyme addition to liquefaction tank according to Cellulase System SOP-8355.
2. Run the contents out of the Liquefaction to pH Adjustment Tank by:
  - a. Continue operation until the Liquefaction volume decreases to 15%
  - b. Agitation will automatically turn off.

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- c. Monitor the pressure at the Liquefaction Tank pump PT-2301 discharge at PI-2302-15. When the pressure drops to zero:
      - i. Visually confirm that there is no Liquefaction material in the clear tubing at pump PT-2301.
      - ii. Turn off pump PT-2301 at the HMI
3. Run contents out of pH Adjustment tank into fermentation by:
  - a. Continue operation until the pH Adjustment Tank volume decreases to 15%
  - b. Agitation will automatically turn off.
  - c. Turn off pH Control at HMI AIC-2302-16 by switching to Manual with OUTPUT at -5.
  - d. Monitor the pressure at the pH Adjustment Tank pump PT-2302 discharge at PI-2302-15.
  - e. When the pressure drops to zero:
    - i. Visually confirm that there is no Liquefaction material in the clear tubing at pump PT-2302.
    - ii. At the HMI, turn off pump PT-2302
  - f. Close the valve to fermentation (per Table in Section E.4.3.)
  - g. Close valve 2301-V-38 to close the line from Liquefaction to pH Adjustment.

***E.5 Cleaning (CIP) Liquefaction Tank***

1. Ensure that the CIP system is operational and ready according to CIP System SOP-8205.
2. Ensure the UV Water System is running according to UV Water System SOP-9555.
3. Open CIP System drain valve 8201-V-31.
4. Open CIP spray ball valves 2301-V-09, -12 on top of the Liquefaction Tank.
5. Open tank drain valves 2301-V-28, -35.
6. Open CIP return valves 2301-V-37, -39.
7. Ensure distribution valves 2301-V-38, -40 are closed.
8. Set CIP system to deliver Rinse Water by:
  - a. slowly open valve 8201-V-20 and close 8201-V-21



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- b. At HMI, turn on Liquefaction Tank Pump PT-2301 and set it to MANUAL with speed at 100%.
  - c. Run the rinse cycle for 15 minutes.
  - d. Open valve 8201-V-21 and then close valve 8201-V-20.
  - e. Continue running pump PT-2301 to empty the tank
  - f. At the HIMI, turn off the pump (PT-2301) when the tank is empty.
9. Set CIP system to deliver caustic solution by:
  - a. Close valve 8201-V-31 and open valve 8201-V-04 to return caustic solution to the Dilute Caustic Tank.
  - b. Slowly open valve 8201-V-13 and close valve 8201-V-14
  - c. At HMI, turn on Liquefaction Tank Pump (PT-2301) and set it to MANUAL with speed at 100%.
  - d. Run the CIP system for 15 minutes.
  - e. Open valve 8201-V-14 and then close valve 8201 V-13.
  - f. Continue running the pump (PT-2301) to empty the tank.
  - g. At the HIMI, turn off the pump (PT-2301) when the tank is empty.
10. Set CIP system to deliver UV water by:
  - a. Close valve 8201-V-04 and then open valve 8201-V-30 to return UV water the rinse tank.
  - b. Slowly open valve 8201-V-12.
  - c. At HMI, turn on Liquefaction Tank Pump (PT-2301) and set it to MANUAL with speed at 100%.
  - d. Run the CIP system for 25 minutes.
  - e. Close valve 8201-V-12
  - f. Continue running pump PT-2301 to empty the tank
  - g. At the HIMI, turn off the pump (PT-2301) when the tank is empty.
11. Close spray ball valves 2301-V-09, -12.
12. Close valves 8201-V-30 and 2301-V-39.
13. Liquefaction Tank is now clean and ready for sterilization.

***E.6.Cleaning (CIP) pH Adjustment Tank***

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1. Ensure that the CIP system is operational and ready according to CIP System SOP-8205.
2. Ensure the UV Water System is running according to UV Water System SOP-9555.
3. Open CIP spray ball valves 2303-V-09 on top of the pH Adjustment Tank.
4. Open tank drain valves 2303-V-26, -31.
5. Open CIP return valves 2303-V-34, -37.
6. Close distribution lines 2303-V-35 and V-36.
7. Set CIP system to deliver Rinse Water according to CIP System SOP-8205.
8. At HMI, turn on pH Adjustment Tank Pump (PT-2302) and set it to MANUAL with speed at 60%.
  - a. Run the rinse cycle for 15 minutes.
  - b. Open another CIP spray ball valve 2303-V-11 and close valve 2303-V-09.
  - c. Run the rinse cycle for 15 minutes.
  - d. Open valve 8201-V-21 and then close valve 8201-V-20.
  - e. Continue running pump PT-2302 to empty the tank.
  - f. At the HMI, turn off the pump (PT-2302) when the tank is empty.
9. Set CIP system to deliver caustic solution according to CIP SOP-8205.
  - a. At HMI, turn on pH Adjustment Tank Pump (PT-2302) and set it to MANUAL with speed at 60%.
  - b. Run the CIP system caustic solution for 15 minutes.
  - c. Open the other CIP spray ball valve 2303-V-09 and close valve 2303-V-11.
  - d. Run CIP Caustic cycle for another 15 minutes.
  - e. Stop the caustic cycle by opening CIP System valve 8201-V-14 and then close valve 8201-V-13.
  - f. Continue running pH Adjustment pump PT-2302 to empty the tank.
  - g. At the HMI, turn off the pump (PT-2302) when the tank is empty.
10. Set CIP system to deliver UV water according to CIP SOP-8205.
  - a. At HMI, turn on pH Adjustment Tank Pump (PT-2302) and set it to MANUAL with speed at 60%.
  - b. Run the CIP system for 15 minutes.
  - c. Open the other CIP spray ball valve 2303-V-11 and close valve 2303-V-09.

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- d. Run UV Water cycle for another 15 minutes.
- e. Close valve 8201-V-12.
- f. Continue running pump PT-2302 to empty the tank.
- g. At the HIMI, turn off the pump (PT-2301) when the tank is empty.
- 11. Close the spray ball valve 2303-V-11.
- 12. Close valves 2303-V-37.
- 13. pH Adjustment Tank is now clean and ready for sterilization.

**E.6 Cleaning (CIP) Transfer Lines**

- 1. Set the CIP system to supply Rinse water according to CIP System SOP-8205
- 2. Clean the transfer line from Liquefaction tank to pH Adjustment tank by:
  - a. Close Liquefaction transfer line valve 2301-V-37
  - b. Close Liquefaction transfer line at the pH Adjustment tank valve 2303-V-05
  - c. Open CIP Header valve at pH Adjustment tank 2303-V-02
  - d. Run the rinse cycle for 5 minutes.
  - e. Set the CIP System to Caustic according to the CIP System SOP-8205.
  - f. Run the Caustic cycle for 10 minutes.
  - g. Set the CIP System to UV Water according to CIP System SOP-8205
  - h. Run the UV Water for 15 minutes.
  - i. Stop the CIP of the Liquefaction transfer line by:
    - i. Close CIP Header valve at pH Adjustment tank 2303-V-02
    - ii. Open Liquefaction transfer line at the pH Adjustment tank valve 2303-V-05
    - iii. Open Liquefaction transfer line valve 2301-V-37
- 3. Clean the transfer line from the pH Adjustment Tank to the receiving tank by:
  - a. Close the pH Adjustment tank transfer line valve 2303-V-34.
  - b. Close pH Adjustment transfer line at the receiving tank downstream from the CIP Header connection (as done in step E.6.2.b above).
  - c. Open CIP Header valve at the receiving tank CIP Header connection to the transfer line (as done in step E.6.2.c above).
  - d. Run the rinse cycle for 5 minutes.
  - e. Set the CIP System to Caustic according to the CIP System SOP-8205.

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- f. Run the Caustic cycle for 10 minutes.
- g. Set the CIP System to UV Water according to CIP System SOP-8205.
- h. Run the UV Water for 15 minutes.
- i. Stop the CIP of the pH Adjustment transfer line by:
  - i. Close CIP Header valve at the receiving tank.
  - ii. Open pH Adjustment transfer line at the receiving tank.
  - iii. Open pH Adjustment transfer line valve 2303-V-34
- 4. The transfer lines are clean and ready to use.