STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PLANT

TITLE: Applikon 140 L Fermentor Heat Kill Operating Procedure

---

AUTHOR: **Claudia C. Geddes** **DATE: April 1, 2011**
APPROVALS: **Process Change Committee** **DATE:**
UF EH&S **DATE:**

CONTENTS

A. Scope

This procedure describes how to kill microorganisms present in the fermentation broth after completion of ferementation run.

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.

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:

- Lab Coat
- Safety Goggles or Face Shield
- Protective Gloves (nitrile, neoprene)
- Autoclave Gloves

Avoid inhalation of vapors and wear nitrile or neoprene rubber gloves. Contain spills by using spill kits next to fermentors.

C. Related Documents and SOPs

1. Sampling procedure SOP
2. Enzymatic saccharification of pretreated sugarcane bagasse SOP
3. Transfer of enzyme saccharified slurry to 140 L fermentor SOP
4. UF Biosafety manual
5. MSDS sheets for chemicals listed in section D

D. Preparation/Materials/Equipment

The equipment used in this SOP is listed below:

1. Applikon In-situ sterilizable 140 L fermentor

The chemicals/materials used in this SOP are listed below:

1. Fermented enzyme saccharified cake slurry

E. Detailed Procedure

The working volume for this fermentor is 20 to 140 liters. It is composed of a Bio controller (ADI010), fermentation vessel, jacket, tubing, frame, and Pilot System (ADI1075).

<u>STERILIZATION</u>:

1. Cover spouts on the left top side of the fermentor with aluminum foil.

2. Make sure all steam valves to fermentors are closed.

3. Start up small boiler. It should take 20-25 min. to heat up and get up to pressure.

4. Make sure water lines and air valves are OPEN. They are located on the wall behind the fermentors.

5. Turn power switch ON by turning clockwise all the way; there should be two clicks (like turning a car on). An alarm will go off for ~10 seconds (this is normal). If power on does not work then release/turn OFF the emergency stop button by turning clockwise.

6. Make sure stirrer is OFF. The stirrer will automatically be off if it is set on Remote and the loop is OFF.

7. Make sure the fermentor light is OFF.

8. Make sure every clamp and valve that needs to be closed is closed tight before starting the sterilization procedure.

9. Close the exhaust valve V11.02.

10. Make sure valves V10.07 and V10.08 are closed.

11. Make sure the three spouts in the front of the fermentor are closed. They are closed when they are pushed out. Push them out by pressing the pin on the side of the spout and pull the spout out. Pushing in opens the spouts.

12. Remove water return tubing (red tube). Close water valve to condenser (this is the red tubing) and move the tube from the water supply and hook up to the drain.

13. Close valves **V14.01**, V14.02, V11.02 (exhaust line), V14.03. During fermentation when you are sampling, open V14.02.

14. Close valve V10.08 (sparger) and V10.07 (overlay). These two valves provide steam to the fermentation chamber.

15. Slowly Open steam valve (in back of the fermentor) all the way.

16. Activate Temp. loop.

17. Menu → Sterilization Settings Value (use knob) → choose values by pressing menu after entering the desired temperature (e.g. 121°C) and time (e.g. 20 min.). The steam to filter is preset at 90°C. Menu → Sterilization → Start. Menu → Sterilize Routine → Sterilization Activation.

18. When you see steam coming out of the lines going into the drain behind the fermentor, OPEN the cooling water valve (by the wall in back of the fermentor) to help with the steam sterilization.

19. During sterilization, pressure should be between 17 and 20 psi. Use exhaust valve V11.02 to maintain pressure below 20 psi. Open and close it accordingly.

    - Valve V10.19 shuts OFF during sterilization which then shuts OFF air (blue tube) also.

20. Turn valve V11.03 ON. Turn it ON during growth also when steam is ON to superheat HEO2 to prevent filter F03 from getting wet. If the filter gets wet then F03 will not be able to close properly.

21. At 100°C-102°C crack valve V10.07 (overlay) open to sterilize the line. Pay attention to the pressure. It must be kept below 25 psi by opening exhaust valve V11.02. The exhaust valve may need to slightly

cracked open during steps 20-21. Open valves V11.01 (exhaust filter condensate) and V14.04. Close valve V10.07 (overlay) at 112°C-115°C.

22. Open valve V10.08 (sparger). Close the valve at 121°C.

23. Close the exhaust valve V11.02 (exhaust valve) if it was open.

24. Once the sterilization cycle is done, close valves V14.04, V11.01, V14.03, and V11.03.

25. Activate Temp. loop.

26. Hook the red tubing back the way it was. Put the tube into "water return" and into "water supply." Open the water supply valve.

27. Close the main steam valve.

28. Open overlay valve V10.07 to make sure you have 15 psi air to prevent a vacuum from building up during cool down. It will take about 45 minutes for the fermentor to cool to 37°C.