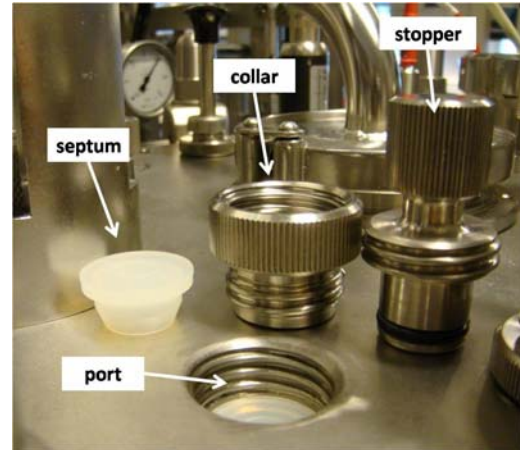
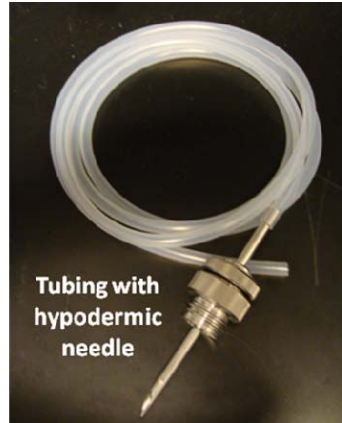


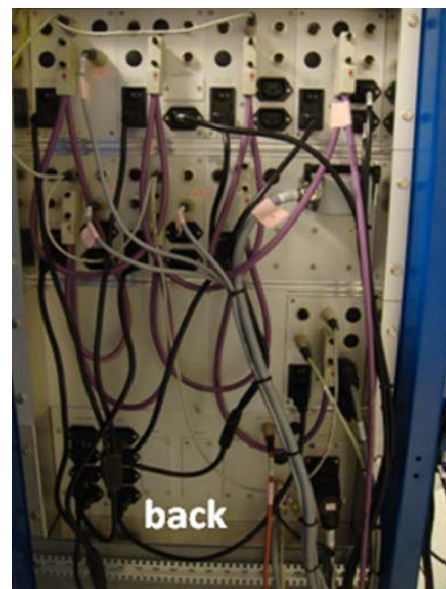
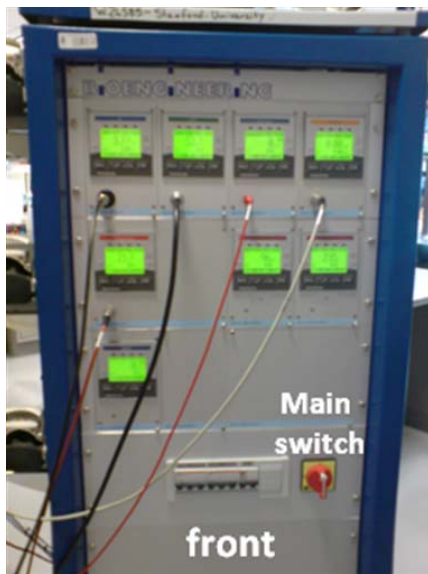
## Lab Pilot Fermenter Type LP351, 75 Liters OPERATING INSTRUCTIONS

### Before you start...

1. Autoclave antifoam and base solution (in glass bottles) along with three feeder tubing lines with hypodermic needles attached on one end. There is no need to sterilize the acid solution.
2. Secure a disposable septum, metal collar and stopper onto four free ports on the lid of the vessel.



3. Make sure there is enough lubricant (80% glycerol in water) in the canister at the bottom of the vessel.
4. Connect the cables from the fermenter to the front and back panels of the Intelligent Front Module (IFM) control box. Turn on the main switch on the IFM.



## 5. Calibrate the pH probe through the IFM

- On the pH panel, press "Menu" and use the < > buttons to select CALI. Press OK.
- Select CALIB. MEAS, press OK.
- Press OK on password.
- Enter 1ST REF = 7.00 pH (Always do pH 7 first), and dip the probe into the appropriate pH solution with the white dot facing the current. Wait until the current stabilizes, then select CALIBRATE. Rinse the probe with deionized water.
- Enter 2ND REF = 4.00 pH, and repeat the previous step with the appropriate pH solution. Select CALIBRATE when the reading stabilizes.
- Select CALIB DONE and press OK.
- Mount the pH probe to the side port of the fermenter vessel - white dot on the probe facing the flow current.

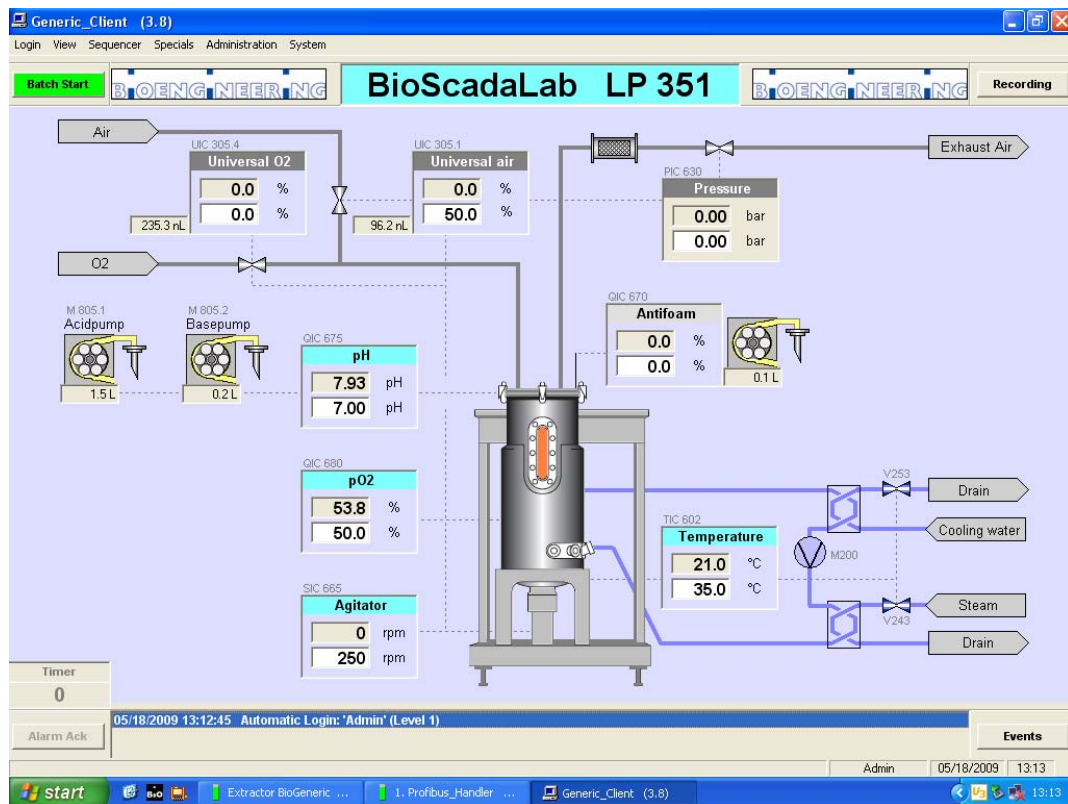
### Turning on the energies:

#### 1. In the Autoclave room:

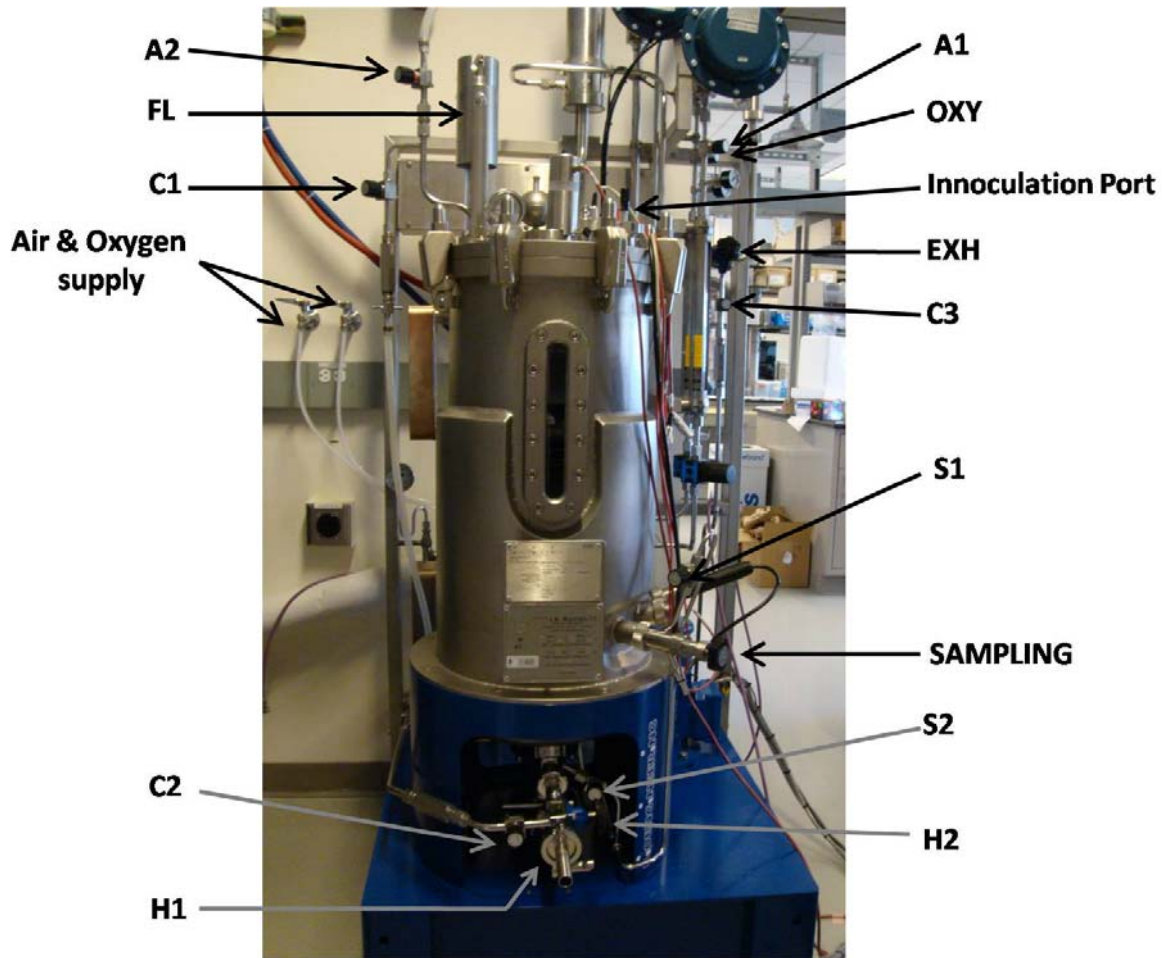
- Turn the Autoclave switch to POWER and wait for the steam pressure to build up to ~ 4 bars (Open the door underneath the autoclave chamber to check the steam pressure gauge).
- Turn the yellow steam valve at the right side of the Autoclave to align in parallel with the pipeline. CAUTION: The steam pipe is hot!
- Check that all industrial cold water valves on the back wall are open (parallel to the pipelines)



- Turn on the computer (Username: user; Password: 123456). The BioScadaLab software should open automatically. Make sure that the IFM is on before turning on the computer.

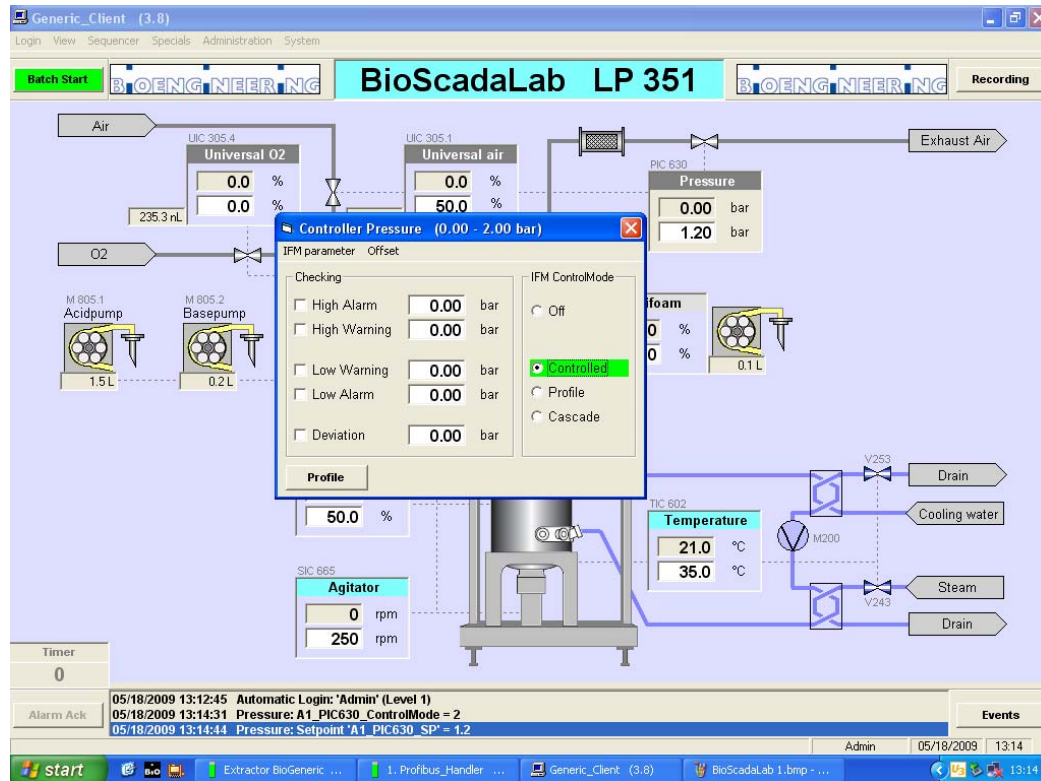


- Click on "Batch Start" and enter the fermentation information.
- Turn on the air and oxygen supply lines on the wall.



### Pressure Leak Test

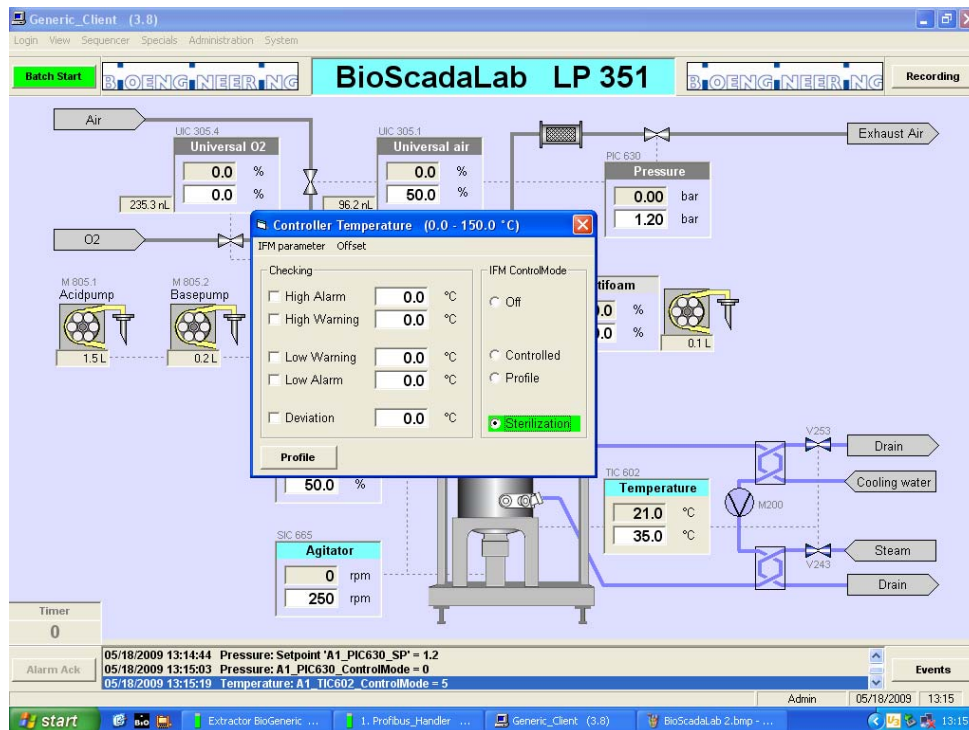
1. Drain residual liquid:
  - Open the harvest valves (H1 and H2) to drain residual liquid from the vessel.
  - With the SAMPLING valve closed, open S1 until all residual condensate has drained.  
CAUTION: Hot steam will be released.
2. Open A1, A2, EXH
3. Close all other valves: OXY, C1, C2, C3, S1, S2, H1, H2, CW, SAMPLING
4. The air filter (FL) should be in a raised position (handle points to the back)
5. In BioScadaLab, click on the Pressure panel and select "Controlled". Repeat for "Universal Air" and "Universal O2"



6. Enter the following set-points in the corresponding white fields:
  - Pressure: 1.2 bar
  - Universal Air: 100%
  - Universal O2: 0%
7. Once the actual pressure (indicated in the gray field) has reached 1.2 bar, close A1, C2, and EXH.
8. Wait for 5-10 minutes, and check that there is no significant reduction in the actual pressure.

### Vessel Sterilization

1. Open C1, C3, EXH (fully)
2. Close all other valves: A1, OXY, C2, S1, S2, H1, H2, CW, SAMPLING
3. Lower FL (handle points to the front)
4. Add culture media into the vessel via the Inoculation Port.
5. In BioScadaLab, click on the Agitator panel and select "Controlled". Enter 250 rpm in the white field. The agitator should now be activated.
6. In BioScadaLab, click on the Temperature panel and select "Sterilization". The sterilization cycle will now start. (The sterilization parameters have been pre-set to return to 37°C at the end of the cycle.)



7. Wait for the system to heat up. Once 95°C has been reached, the IFM display will read "CLOSE THE VALVE, WHEN DONE PRESS OK".
  - Turn EXH to 90% closed, then press OK
8. The controller will heat up to 121°C, at which point sterilization will continue for 20 minutes.
9. After the sterilization time is finished, the system will start cooling to 100°C, and the IFM display will read "OPEN THE VALVE, WHEN DONE PRESS OK".
  - Open A1, EXH, CW
  - Close C1, C3, and all other valves
  - Lift FL (handle points to the back), then press OK
10. Temperature will now decrease and be maintained at 37°C.

### **Installation of Antifoam, Acid and Base Feeder Solutions**

1. Install the tubing leading from the antifoam, acid and base solutions onto the corresponding rotatory pumps at the side of the IFM.
2. On the lid of the vessel, unscrew a stopper and pour ethanol on top of the septum. Light a small flame to sterilize. Hold the hypodermic needle from the tubing above the flame and remove the needle case. When the flame is almost extinguished, pierce the needle through the septum and screw it down in place. Repeat for each tubing.

### **Fermentation Parameters Settings**

- On BioScadaLab (PC), click on each control panel and select "Controlled". Enter the desired parameters on the corresponding white fields. The recommended Agitator speed is ~250 rpm.

### **Innoculation**

1. Open A1, A2, OXY, EXH, C1, and C3
2. Remove the stopper on the Innoculation Port and flame the mouth briefly. Introduce the inoculum directly into the vessel. Maintain sterility as much as possible.

### **Sampling**

1. Open S1 for 5-10 seconds to sterilize the sampling pipe. CAUTION: Steam and condensate will be released.
2. Open the SAMPLING valve to collect sample.

### **Harvesting**

1. Open C2 and then S2 for 5 seconds to sterilize the condensate line.
2. Open H1 and then S2 to sterilize the harvest line.
3. Close S2 and open both H1 and H2 to harvest the fermentation batch.

### **Wrapping Up**

1. On BioScadaLab, click on each control panel and select "Off"
2. Stop the batch run on BioScadaLab. Username: Admin, Password: admin
3. Close all valves, and turn off all energies (air, oxygen, steam).
4. Rinse the vessel by filling it with water. On BioScadaLab, right click on Agitator and select "Controlled". Enter 250 rpm as the set point. Drain the water by repeating the steps described in the Harvesting section. Turn the Agitator off when done.
5. Turn off the PC and the main switch on the IFM.

Contact person:

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