

Title: New Brunswick BioFlo 3000 Bioreactor SOP

Approvals:

Preparer: Kari Britt Date 15May09
Reviewer: Bob O'Brien Date 15May09

1. **Purpose:** Operation of the New Brunswick BioFlo 3000 Bioreactor.
2. **Scope:** Applies to growing yeast or bacteria in the New Brunswick BioFlo 3000 Bioreactor.
3. **Responsibilities:**
 - 3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
 - 3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
 - 4.1. New Brunswick BioFlo 3000 Bioreactor instrumentation manual
 - 4.2. BioCommand Lite Software Manual
 - 4.3. autoclave SOP
5. **Definitions:** N/A
6. **Precautions:** N/A
7. **Materials:**
 - 7.1. pH 4.0 buffer standard
 - 7.2. pH 7.0 buffer standard
 - 7.3. laboratory tissues
 - 7.4. silicone tubing
 - 7.5. PharMed tubing
 - 7.6. clamps
 - 7.7. aluminum foil
 - 7.8. air filters
 - 7.9. glass wool
 - 7.10. probe stand with clamps
 - 7.11. glycerol
 - 7.12. laboratory glassware detergent
 - 7.13. deionized water
 - 7.14. DO electrolyte
 - 7.15. 3M potassium chloride (KCl) solution for pH probe storage
 - 7.16. bleach
 - 7.17. Optional: antifoaming agent (such as Antifoam A)
 - 7.18. personal computer to run BioCommand Lite Software and Microsoft Excel
 - 7.19. **Bioreactor**
 - 7.19.1. pH probe
 - 7.19.2. DO probe
 - 7.19.3. RTD temperature probe
 - 7.19.4. harvest tube
 - 7.19.5. sparger tube
 - 7.19.6. thermowell tube

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- 7.19.7. glass vessel
- 7.19.8. condenser
- 7.19.9. feed solution flask assembly
- 7.19.10. sampler assembly
- 7.19.11. heat exchanger base
- 7.19.12. baffle
- 7.19.13. thumb screws

8. Procedure:

Note: Refer to Section 9 (Attachments) for diagrams of the bioreactor parts as needed throughout this procedure.

8.1. Clean All Bioreactor Parts

- 8.1.1. Gather all bioreactor vessel parts, including the DO probe, pH probe and calibrating buffers.
- 8.1.2. Clean all bioreactor vessel parts (except pH and DO probes) with a soft cloth, warm water, and glassware detergent. Rinse thoroughly with warm tap water, and then with several changes of deionized water.
Note: A soft brush may be used on difficult to clean parts of the bioreactor vessel.
- 8.1.3. Gently clean the pH and DO probes using a soft cloth and deionized water. Be careful not to harm the sensory tips of these probes.
- 8.1.4. Rinse the inside of the sparger tube to remove clogs and prevent damage to the bioreactor.

8.2. Assemble the Vessel

Note: All fittings are hand-tightened except the DO probe adapter.

- 8.2.1. Attach the DO, pH, and temperature cables to their appropriate outlets on the front side of the cabinet of the BioFlo 3000.
- 8.2.2. If necessary, insert the plastic plugs into the holes in the glass vessel.
 - 8.2.2.1. Make sure that the top steel ring is lifted above the holes before inserting the plugs or the headplate will not be able to be attached.
 - 8.2.2.2. Insert the plastic bolt through the hole from the inside of the glass vessel. Place the rubber plug over the end of the bolt on the outside of the vessel (with the tapered side facing into the vessel), secure the plug with a plastic nut, and tighten the nut. Repeat this process for each of the four plugs as needed.
- 8.2.3. If the plugs are already attached to the vessel, make sure that they are tightly secured or the vessel will leak.
- 8.2.4. Place the glass vessel onto the heat exchanger base, making sure that the vessel is centered within the bottom steel ring to prevent leaking.
- 8.2.5. Secure the glass vessel to the heat exchanger base by connecting the bottom steel ring to the heat exchanger base using the small thumb screws. Tighten the screws in a crosswise manner.
- 8.2.6. Check for leaks by pouring approximately 3L of water into the vessel and allowing it to sit for at least 5 minutes while observing the vessel for a leak.
 - 8.2.6.1. If a leak is observed, detach the vessel and the heat exchanger base, and repeat steps 8.2.3. through 8.2.6., as needed, until no leaks are observed.

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- 8.2.7. If no leaks are observed, remove the water from the vessel by lifting the vessel (still attached to the heat exchanger base) and pouring the water out into a sink or container.
- 8.2.8. Place the baffle into the glass vessel.
- 8.3. **Assemble the Headplate - Underside (Figure 3)**
 - 8.3.1. If necessary, attach the impellers to the agitator shaft. The lower impeller should be flush with the base of the agitator shaft, and the upper impeller should be approximately 6cm above the lower impeller.
 - 8.3.2. Inspect the integrity of the O-rings on the thermowell, harvest tube and sparger tube. Replace if worn or cracked.
 - 8.3.3. Insert the thermowell into the thermowell port from the underside of the headplate.
 - 8.3.4. Adjust the height of the thermowell so that the base is approximately 2 cm above the lower impeller.
 - 8.3.5. Secure the thermowell in place with a nut by lowering the nut over the top of the thermowell and hand-tightening it to the threads on the topside of the headplate.
 - 8.3.6. Insert the harvest tube into the harvest tube port from the bottom of the headplate.
 - 8.3.7. Adjust the height of the harvest tube so that the base is directly below the lower impeller. Rotate the lower impeller by hand to make sure that there is enough clearance for rotation without collision with the harvest tube.
 - 8.3.8. Secure the harvest tube in place with a nut by lowering the nut over the top of the harvest tube and hand-tightening it to the threads on the topside of the headplate.
 - 8.3.9. Insert the sparger tube into the sparger tube port from the bottom of the headplate.
 - 8.3.10. Adjust the height of the sparger tube so that the base is directly below the lower impeller. Rotate the lower impeller by hand to make sure that there is enough clearance for rotation without collision with the sparger tube.
 - 8.3.11. Secure the sparger tube in place with a nut by lowering the nut over the top of the sparger tube and hand-tightening it to the threads on the topside of the headplate.
- 8.4. Pour the cell culture media that will be used for the run into the vessel (refer to the process SOP for media composition).
- 8.5. **Attach the Headplate to the Vessel**
 - 8.5.1. Place the headplate onto the vessel.
 - 8.5.2. Secure the headplate to the top steel ring using the large thumb screws. Tighten the screws in a crosswise manner.
- 8.6. **Assemble the Headplate – Top Side (Figure 4)**
 - 8.6.1. Insert the tube of the sampler assembly into the sampler assembly port. Lower the tube until the base is approximately half way between the upper and lower impellers. Hand tighten the nut at the top of the tube.
 - 8.6.2. Inspect the integrity of the O-ring at the bottom of the condenser. Replace if worn or cracked.
 - 8.6.3. Insert the condenser into the condenser port and secure in place by hand-tightening the nut to the threads on the topside of the headplate. Make sure that the barbed connectors are pointing to the left. Refer to Figure 1 for proper orientation of the condenser.

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- 8.6.4. Lower the foam probe into the foam probe port as far as it will go and then hand-tighten the nut to the threads on the topside of the headplate.

8.7. Connect Bioreactor to Cabinet

- 8.7.1. Place the vessel and heat exchanger base on the platform in front of the cabinet so that the heat exchanger base fits on the three steel plugs. The heat exchanger connectors should face the front left corner of the platform. See Figure 1 for proper orientation of the heat exchanger.
- 8.7.2. Make sure that the two-way valve of the sampling assembly is closed. The valve is closed when the position of the two-way valve handle is perpendicular to the sample tube, as shown in Figure 2.
- 8.7.3. Connect the “Jacket Water Out” water line to the top heat exchanger connector using the quick connects adaptors.
- 8.7.4. Connect the “Jacket Water In” water line to the bottom heat exchanger connector using the quick connects adaptors.
- 8.7.5. Connect the “Condenser Water Out” water line to the top condenser connector using the quick connects adaptors.
- 8.7.6. Connect the “Condenser Water In” water line to the bottom condenser connector using the quick connects adaptors.
- 8.7.7. Turn on the water. The water valve is located under the lab bench where the BioFlo 3000 is located. The pressure gauge should read between 15 and 20psi when the water is turned on.

8.8. Calibrate the pH Probe

- 8.8.1. Turn on the BioFlo 3000.
- 8.8.2. Select fermentation (2) and press the ENTER key.
- 8.8.3. Check the pH probe for any damage. Replace the probe if damaged.
- 8.8.4. Remove the shorting cap from the top of the pH probe and connect the pH cable to the pH probe.
- 8.8.5. Press the SCREEN key to select the <Calibration> screen and then press the ENTER key.
- 8.8.6. Remove the protective cap from the bottom of the pH probe. Carefully pour the 3M potassium chloride storage solution into a test tube with a screw cap. Label the tube as: Bioflo 3000 pH Probe Storage Solution, 3M potassium chloride. Store in a test tube rack at room temperature until needed.
- 8.8.7. Carefully immerse the pH and RTD temperature probes into a pH 7 standard buffer solution. Allow a few minutes for the electrode to equilibrate.
- 8.8.8. Use the arrow keys to select the box in the “pH” row of the “Function” column. This box should be displaying “Read” initially.
- 8.8.9. Change “Read” to “Zero” by pressing the ALTER key until “Zero” appears. Then press the ENTER key.
- 8.8.10. Use the arrow keys to select the box located in the “pH” row of the “Zero” column. Enter “7.0” using the number key pad and then press the ENTER key. Do this step even if “7.0” was already displayed when selecting the box.
- 8.8.11. Rinse the pH and RTD temperature probes thoroughly with deionized water. Pat them dry with a laboratory tissue.

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- 8.8.12. Immerse the pH and RTD temperature probes into a pH 4 standard buffer solution. Allow a few minutes for the electrode to equilibrate.
- 8.8.13. Use the arrow keys to choose the box located in the “pH” row of the “Span” column. Enter “4.0” using the number keypad and then press the ENTER key. Do this step even if “4.0” was already displayed when selecting the box.
- 8.8.14. Rinse the pH and RTD temperature probes with deionized water and pat dry with a laboratory tissue.
- 8.8.15. Disconnect the pH cable from the pH probe, and replace the shorting cap.
- 8.8.16. Inspect the integrity of the internal O-ring of pH probe port. Replace if worn or cracked.
- 8.8.17. Apply a small amount of deionized water to the pH probe and then insert it into the pH probe port.
- 8.8.18. Ensure that the pH probe electrode is not touching the baffle.
- 8.9. **Install Dissolved Oxygen Probe**
 - 8.9.1. Remove the protective cap from the bottom of the DO probe and inspect the screen. Replace the screen if damaged.
 - 8.9.2. Remove the bottom housing that encases the DO probe tip.
 - 8.9.3. Inspect the integrity of the O-ring and replace if worn or cracked.
 - 8.9.4. Replenish the DO electrolyte in the bottom housing.
 - 8.9.5. Replace the bottom housing.
 - 8.9.6. Carefully insert the DO probe into the DO port of the headplate. Tighten the adaptor with a wrench.
 - 8.9.7. Ensure that the DO probe is not touching the baffle.
- 8.10. **Attach Tubing**
 - 8.10.1. Attach silicone tubing to the harvest port, bend the tube in half and secure with a clamp. Wrap glass wool and aluminum foil around the exposed end.
 - 8.10.2. Place a piece of PharmMed tubing (approximately 1.5 meters in length) onto each addition port, bend the tubes in half and secure with clamps. Wrap glass wool and aluminum foil around the exposed ends.
 - 8.10.3. Place silicone tubing on the top outlet of the condenser and connect an air filter. Wrap glass wool and aluminum foil around the exposed end of the filter.
 - 8.10.4. Place silicone tubing on the top of the sparger tube and connect an air filter. Bend the tube in half and secure with a clamp. Wrap glass wool and aluminum foil around the exposed end of the tubing.
 - 8.10.5. Remove the rubber bulb from the sampler assembly. Insert glass wool into the bulb and reattach it to the sampler assembly.
- 8.11. **Autoclave the vessel**
 - 8.11.1. Turn off the BioFlo 3000.
 - 8.11.2. Turn off the water valve (under the lab bench).
 - 8.11.3. Disconnect the water lines to the heat exchanger.
 - 8.11.4. Disconnect the water lines to the condenser.
 - 8.11.5. Double check all tubes and ports to ensure that the vessel is completely sealed.
 - 8.11.6. Autoclave the entire vessel and heat exchanger assembly for at least 30 minutes per autoclave SOP.

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8.12. Prepare for Operation

- 8.12.1. Remove the clamp from the tubing attached to the top outlet of the condenser.
- 8.12.2. Place the vessel and heat exchanger base on the platform in front of the cabinet so that it fits on the three steel plugs. The heat exchanger connectors should face the front left corner of the platform. See Figure 1 for proper orientation of the heat exchanger base.
- 8.12.3. It is advantageous to begin polarization of the DO probe at this point in the procedure. Follow directions in step 8.14.1 to do this, and then return to step 8.12.4. to connect the water lines.
- 8.12.4. Connect the “Jacket Water Out” water line to the top heat exchanger connector using the quick connects adaptors.
- 8.12.5. Connect the “Jacket Water In” water line to the bottom heat exchanger connector using the quick connects adaptors.
- 8.12.6. Connect the “Condenser Water Out” water line to the top condenser connector using the quick connects adaptors.
- 8.12.7. Connect the “Condenser Water In” water line to the bottom condenser connector using the quick connects adaptors.
- 8.12.8. Turn on the water. The water valve is located under the lab bench. The pressure gauge should read between 15 and 20psi.
- 8.12.9. Add approximately 1mL of glycerol to the thermowell and insert the RTD temperature probe into the thermowell.
- 8.12.10. Remove the shorting cap from the top of the pH probe and connect the pH cable to the pH probe.
- 8.12.11. Connect the air line from the sparger tube to the sparger gas port located on the front of the cabinet, near the top.
- 8.12.12. Turn on the air supply.
- 8.12.13. The air pressure gauge should not exceed 10psi (5psi is the ideal pressure level).
- 8.12.14. Remove the agitator cover from the top of the headplate and place the agitator motor securely in the agitator shaft.
- 8.12.15. Turn on the BioFlo 3000.
- 8.12.16. Select Fermentation mode (2) and press the ENTER key.
- 8.12.17. If the Master screen does not automatically appear use the SCREEN key to select the <Master> screen and then press the ENTER key.
- 8.12.18. Set the temperature control mode to “Prime” by using the arrow keys to select the box in the “Control” row of the “Temp 1” column. Press the ALTER key until “Prime” is displayed and then press the ENTER key.
- 8.12.19. Wait 5 minutes before setting the working temperature. Refer to the process SOP for the desired working temperature.
 - 8.12.19.1. Set the working temperature by using the arrow keys to select the box in the “Set” row of the “Temp 1” column. Use the number keypad to enter the desired temperature and then press the ENTER key. Do this step even if the desired temperature was already displayed when selecting the box.

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- 8.12.20. Set the control mode to P-I-D by selecting the box in the “Control” row of the “Temp 1” column. Press ALTER until “P-I-D” appears and then press the ENTER key.
- 8.12.21. Set the agitation loop to P-I-D control by selecting the box in the “Control” row of the “Agit 1” column. Press the ALTER key until “P-I-D” appears and then press the ENTER key.
- 8.12.22. Set the agitation setpoint to 100rpm by selecting the box in the “Set” row of the “Agit 1” column. Use the number keypad to enter 100 and then press the ENTER key.
- 8.12.23. Set up the feed solution flasks on the appropriate feed pumps, as indicated by the process SOP. Acidic or basic solutions should be set up on Feed 1. Refer to the process SOP for indication of an acidic or basic solution.
 - 8.12.23.1. Unclamp the tube attached to an addition port and remove the glass wool and aluminum foil.
 - 8.12.23.2. Thread the free end of the tube through the bottom of the feed pump, counter clockwise around the pump, and out the top.
 - 8.12.23.3. Place the free end of the tube on the top of the glass pipet of the feed solution flask.

Note: The pump moves in a clockwise direction. Therefore the solution will flow from right to left in the tube.
- 8.12.24. Repeat step 8.12.23 for additional feed solution flasks, if needed.
- 8.12.25. Set the pH setpoint to the desired value. Refer to the process SOP for the desired pH.
 - 8.12.25.1. Set the pH setpoint by selecting the box in the “Set” row of the “pH 1” column. Use the number key pad to enter the desired pH and then press the ENTER key. Do this step even if the desired pH was already displayed when selecting the box.
- 8.12.26. Set the pH loop to P-I-D control by selecting the box in the “Control” row of the “pH 1” column. Press the ALTER key until “P-I-D” appears and then press the ENTER key.
- 8.12.27. Designate Feed 1 as acid or base. Refer to the process SOP to determine if the Feed 1 solution is acidic or basic.
 - 8.12.27.1. Designate Feed 1 by using the arrow keys to select the box in the “Loop Name” row of the “pH 1” column. This box should be displaying the column heading “pH 1”. Press the ALTER key until the “Feed 1” column heading appears then press the ENTER key.
 - 8.12.27.2. Use the arrow keys to select the box in the “Control” row of the “Feed 1” column. Use the ALTER key to select either “Base” or “Acid” and then press the ENTER key.
 - 8.12.27.3. Set the setpoint to 100 by choosing the box in the “Set” row of the “Feed 1” column. Use the number keypad to enter “100” and then press the ENTER key.
- 8.12.28. Return to the pH loop display by selecting the “Feed 1” column heading. Press the ALTER key until “pH 1” appears and then press the ENTER key.

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8.12.29. Wait for the pH value to reach its setpoint before proceeding.

8.13. Activate additional feed loops at the appropriate time as indicated by the process SOP.

8.13.1. Activate a feed loop by selecting the box in the “Loop Name” row of the “pH 1” column. This box should be displaying the column heading “pH 1”. Press the ALTER key until the “Feed 2” column heading appears (or the appropriate feed column heading if not “Feed 2”).

8.13.2. Enter the appropriate setpoint for the feed solution, as indicated by the process SOP, by choosing the box in the “Set” row of the “Feed 2” column. Use the number keypad to enter the appropriate setpoint value and then press the ENTER key.

8.13.3. In the “Control” row of the “Feed 2” column press the ALTER key until “on” is displayed, then press ENTER.

8.14. Calibrate the Dissolved Oxygen Probe

Note: The DO probe should not be calibrated until the desired working temperature has been reached.

8.14.1. Polarize the DO probe for 30 minutes prior to performing calibration.

8.14.1.1. If necessary, connect the DO cable to the DO electrical outlet on the front of the cabinet.

8.14.1.2. Remove the protective cap from the top of the DO probe and connect the DO Cable to the DO probe.

8.14.1.3. If necessary, turn on the BioFlo 3000.

8.14.1.4. Allow the probe to sit for at least 30 minutes.

8.14.1.5. Verify that the DO level is above 0. If the DO display reads 0 or below there may be a problem with the DO probe. Contact the lab manager if this occurs.

8.14.2. Use the SCREEN key to choose the <Calibration> screen and then press the ENTER key.

8.14.3. Use the arrow keys to choose the box in the “DO” row of the “Function” column. This box should be displaying “Read”.

8.14.4. Change “Read” to “Zero” by pressing the ALTER key until “Zero” appears and then pressing the ENTER key.

8.14.5. Use the arrow keys to choose the box in the “DO” row of the “Zero” column. Enter “0.0” using the number keypad and then press the ENTER key.

8.14.6. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.

8.14.7. Use the arrow keys to choose the box in the “Set” row of the “Agit 1” column.

8.14.8. Enter “1000” (for 1000rpm) using the number keypad and then press the ENTER key. This will turn on the agitation motor.

8.14.9. Press the SCREEN key and then use the arrow keys to select the <Gases> screen and then press the ENTER key.

8.14.10. Set the gases mode to “Manual” by selecting the box in the “Mode” row (it is the only box available in the “Mode” row). Press the ALTER key until “Manual” is displayed, then press the ENTER key.

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- 8.14.11. Allow ten to thirty minutes for the vessel to equilibrate.
- 8.14.12. Press the SCREEN key and then use the arrow keys to select the <Calibration> screen and then press the ENTER key.
- 8.14.13. Use the arrow keys to choose the box in the “DO” row of the “Function” column. This box should be displaying “Read”.
- 8.14.14. Change “Read” to span by pressing the ALTER key until “Span” is displayed and then press the ENTER key.
- 8.14.15. Choose the box in the “DO” row of the “Span” column. Enter “100.0” using the number keypad and then press the ENTER key.
- 8.15. **There are three possible control modes for dissolved oxygen. Refer to the process SOP for the desired mode.**
 - 8.15.1. **Controlling by Agitation Only**
 - 8.15.1.1. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.
 - 8.15.1.2. Set the DO loop to P-I-D by selecting the box in the “Control” row of the “DO2 1” column. Press the ALTER key until “P-I-D” appears and then press the ENTER key.
 - 8.15.1.3. Temporarily set the agitation loop to P-I-D by selecting the box in the “Control” row of the “Agit 1” column. Press the ALTER key until “P-I-D” appears and then press the ENTER key.
 - 8.15.1.4. To set the minimum rpm select the box in the “Set” row of the “Agit 1” column. Use the number keypad to enter the desired minimum rpm, as indicated by the process SOP and then press the ENTER key.
 - 8.15.1.5. Set the agitation loop to DO control mode by selecting the box in the “Control” row of the “Agit 1” column. Press the ALTER key until “DO” appears and then press the ENTER key.
 - 8.15.1.6. To set the maximum rpm select the box in the “Set” row of the “Agit 1” column. Use the number keypad to enter the desired maximum rpm, as indicated by the process SOP and then press the ENTER key.
Note: The controller will automatically adjust the rpm, therefore after the maximum value is entered the displayed set value may change immediately afterward.
 - 8.15.1.7. To input the DO setpoint select the box in the “Set” row of the “DO2 1” column. Use the number keypad to enter the desired setpoint, as indicated by the process SOP and then press the ENTER key.
 - 8.15.2. **Controlling by % Oxygen Sparged**
 - 8.15.2.1. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.
 - 8.15.2.2. Set the DO loop to P-I-D by selecting the box in the “Control” row of the “DO2 1” column. Press the ALTER key until “P-I-D” appears and then press the ENTER key.
 - 8.15.2.3. Press the SCREEN key and then use the arrow keys to choose the <Gases> screen and then press the ENTER key.

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- 8.15.2.4. Set the gases mode to DO control by selecting the box in the “Mode” row (it is the only box available in the “Mode” row). Press the ALTER key until “DO” is displayed and then press the ENTER key.
- 8.15.2.5. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.
- 8.15.2.6. To input the DO setpoint select the box in the “Set” row of the “DO2 1” column. Use the number keypad to enter the desired setpoint, as indicated by the process SOP and then press the ENTER key.

8.15.3. Controlling by Agitation and % Oxygen

Note: In this mode agitation will be increased first until maximum rpm is reached and more oxygen is required, then control by % oxygen will begin.

- 8.15.3.1. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.
- 8.15.3.2. Set the DO loop to P-I-D by selecting the box in the “Control” row of the “DO2 1” column. Press the ALTER key until “P-I-D” appears and then press the ENTER key.
- 8.15.3.3. Temporarily set the agitation loop to P-I-D by selecting the box in the “Control” row of the “Agit 1” column. Press the ALTER key until “P-I-D” appears and then press the ENTER key.
- 8.15.3.4. To set the minimum rpm select the box in the “Set” row of the “Agit 1” column. Use the number keypad to enter the desired minimum rpm, as indicated by the process SOP and then press the ENTER key.
- 8.15.3.5. Set the agitation loop to DO control mode by selecting the box in the “Control” row of the “Agit 1” column. Press the ALTER key until “DO” appears and then press the ENTER key.
- 8.15.3.6. To set the maximum rpm select the box in the “Set” row of the “Agit 1” column. Use the number keypad to enter the desired maximum rpm, as indicated by the process SOP and then press the ENTER key.
Note: The controller will automatically adjust the rpm, therefore after the maximum value is entered the displayed set value may change immediately afterward
- 8.15.3.7. Press the SCREEN key and then use the arrow keys to choose the <Gases> screen and then press the ENTER key.
- 8.15.3.8. Set the gases mode to agitation and % oxygen control by selecting the box in the “Mode” row (it is the only box available in the “Mode” row). Press the ALTER key until “Ag02DO” is displayed and then press the ENTER key.
- 8.15.3.9. Use the SCREEN key to choose the <Master> screen and then press the ENTER key.
- 8.15.3.10. Navigate to the air monitor loop by selecting the column heading “Agit 1” in the “Loop Name” row. Press the ALTER key until “Air 1” appears.
- 8.15.3.11. Input the desired air pressure by selecting the box in the “Set” row of the “Air 1” column. Use the number keypad to enter the desired air flow rate (usually between 1.5 and 2 L/min.) and then press the ENTER key.

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8.15.3.12. Return to the agitation display by selecting the “Air 1” column heading. Press the ALTER key until “Agit 1” appears and then press the ENTER key.

8.15.3.13. To input the DO setpoint select the box in the “Set” row of the “DO2 1” column. Use the number keypad to enter the desired setpoint, as indicated by the process SOP and then press the ENTER key.

8.16. Use of Foam Sensor

8.16.1. Set up an autoclaved feed solution flask containing an antifoam agent according to the process SOP.

8.16.2. Set up the feed solution flask containing and antifoam agent on Feed 2 following directions in step 8.12.23.

8.16.3. Connect the foam sensor cable (black) to the Level 1 connector on the front of the cabinet.

8.16.4. Connect the foam sensor cable to the foam sensor.

8.16.5. Connect the red cable to the ground stud located on top of the headplate.

8.16.6. Use the SCREEN key to select the <Master> screen and then press the ENTER key.

8.16.7. Use the arrow keys to select the “Agit 1” column heading. Press the ALTER key until the “Feed 2” column heading appears and then press the ENTER key.

8.16.8. Use the arrow keys to select the box in the “Control” row of the “Feed 2” column.

8.16.9. Set the control mode to Level 1 by pressing the ALTER key until “Lvl 1” appears and then press the ENTER key.

8.16.10. Determine the foam percentage setpoint at which the antifoam agent should be added by referring to the process SOP.

8.16.11. Input the desired foam percentage setpoint by selecting the box in the “Set” row of the “Feed 2” column. Use the number keypad to enter the desired setpoint and then press the ENTER key.

8.16.12. Select the “Feed 2” column heading. Press the ALTER key until “Lvl 1” appears and then press the ENTER key.

8.16.13. Set the Level 1 control mode to add by selecting the box in the “Control” row of the “Lvl 1” column. Press the ALTER key until “On” appears and then press the ENTER key.

8.16.13.1. Return to the agitation display by selecting the “Lvl 1” column heading. Press the ALTER key until “Agit 1” appears and then press the ENTER key.

8.17. Fermentation Procedure

8.17.1. At this point we are ready to do the final preparation before inoculation. Our fermentation run is going to be monitored and controlled by a computer using the BioCommand Lite Software.

8.17.2. Check that the personal computer is connected to the BioFlo 3000 cabinet. If they are not connected, request that the lab manager or course instructor connect the computer to the cabinet before proceeding.

8.17.3. Turn on the computer.

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- 8.17.4. From the desktop open BioCommand Lite by double clicking on the BCLite icon.
- 8.17.5. You can choose to use an existing recipe or create a new recipe. To use an existing recipe follow the procedure in step 8.17.6. To create a new recipe follow the procedure in step 8.17.7.
- 8.17.6. To use an **existing recipe**, choose “Open an Existing Recipe” and then click “Continue”.
 - 8.17.6.1. Select the appropriate recipe (i.e., Pichia Master 1.rcp).
 - 8.17.6.2. Verify that data is collected at 60min intervals in the “Time Data” tab under “Log Interval (Mins)”. If the Log Interval is not 60, change it to 60 using the following procedure.
 - 8.17.6.2.1. Click on “Change Interval”
 - 8.17.6.2.2. Select “60” from the pull down menu.
 - 8.17.6.2.3. Click “Save”.
 - 8.17.6.3. Click on the “Views” tab, and double click “Trend.3” in the column on the left under “Current Views”.
 - 8.17.6.4. Verify that the Trend.3 ranges are appropriate per the process SOP.
 - 8.17.6.5. On the main screen, click “Start Batch”.
 - 8.17.6.6. Save batch file as the product lot number (i.e. 21Feb08HSA).
 - 8.17.6.7. Skip step 8.17.7. and continue with the SOP at step 8.17.8.
- 8.17.7. To create a **new recipe**, from the “BioCommand Lite” window select “Create a new recipe with Batch Wizard” and click “Continue”.
 - 8.17.7.1. In the “BioCommand Batch Wizard-Introduction” window click “Next”.
 - 8.17.7.2. In the “BioCommand Batch Wizard –Step 1” window type in “Recipe1” under “Enter Recipe Display Name:” and click “Next”.
 - 8.17.7.3. In the “BioCommand Batch Wizard-Step 2” window click “Select Loops”.
 - 8.17.7.4. Click on “Browse Local Server”.
 - 8.17.7.5. Under “Available Loops:” highlight “BIOLAB01” then click “>>”. This adds “BIOLAB01” to the “Loops in Batch” box.
 - 8.17.7.6. Under “Available Loops:” highlight “Controller 1” and click “Browse Local Server.”
 - 8.17.7.7. After the + sign appears next to “Controller 1” click “>>”.
 - 8.17.7.8. Click “Close”.
 - 8.17.7.9. When the “Biocommand Batch Wizard –Step 2” window reappears, click “Next”.
 - 8.17.7.10. The “Biocommand Batch Wizard –Step 3” window should appear with the recipe file name. File name format should be:
“C:\BIOCOM~1\BIORcp[#].rcp”. Click “Next”.
 - 8.17.7.11. In the “Biocommand Batch Wizard –Step 4” window click “Next”.
 - 8.17.7.12. In the “Biocommand Batch Wizard – Finished!” window, click “Finish”.
 - 8.17.7.13. In the “Recipe[#].B1:Setup.[#]” window, select “Start Batch”.
 - 8.17.7.14. If needed, type in “Recipe1” in the “Batch Display Name” window.
 (“Recipe1” may automatically appear.). Click “OK”.
 - 8.17.7.15. In the “Biocommand Save Files” window save batch file as:

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- BT220 [semester] [year]. Record file name in the batch record, then click “OK”.
- 8.17.7.16. From the “Recipe[#].B1:Setup.[#]” window, click on the “Views” tab.
- 8.17.7.17. In the “New Views:” box, double click on “Trend”.
- 8.17.7.18. The “Recipe[#].B1:Trend.[#]” window should appear.
- 8.17.7.19. Display trend data using the following procedure:
- 8.17.7.19.1. From the “Recipe[#].B1:Trend.[#]” window, select the “Loops” tab.
 - 8.17.7.19.2. Click on “Setup Loop 1” (or the appropriate Loop number).
 - 8.17.7.19.3. From the “Select Loop” window, highlight one of the parameters to be monitored (i.e.: Agit 1). Then click “OK”. The parameter name chosen is automatically transferred to the “Recipe[#].B1:Trend.[#]” window.
 - 8.17.7.19.4. Click “>>”.
 - 8.17.7.19.5. Click “Setup Loop2”.
 - 8.17.7.19.6. From the “Select Loop” window, highlight another parameter.
 - 8.17.7.19.7. Click “OK”.
 - 8.17.7.19.8. Click “>>”.
- 8.17.7.20. Repeat step 8.17.26. until all parameters needed are added to the “Recipe[#].B1:Trend.[#]” window. (AGIT1, DO2 1, TEMP 1 and pH 1 should always be added.)
- 8.17.7.21. Set the graph range for each parameter using the following procedure.
- 8.17.7.21.1. In the “Recipe[#].B1:Trend.[#]” window select the “Graphs” tab. Use “<<” to navigate all the way back to the first parameter (Loop 1). Enter the values listed below into the appropriate box for each parameter then select “>>” to apply the change to the graph.

Parameter	Low:	High:
AGIT1	100	1100
DO21	0.0	100
TEMP1	0.0	40.0
pH1	1.0	7.0
Feed1	0	10
Feed 2	0	10

- 8.17.8. **Do NOT close any windows during a run.**
- 8.18. **Inoculation Procedure**
- 8.18.1. Allow the bioreactor time to reach all of its setpoints.
 - 8.18.2. Attach the bulb to sample port (be sure there is glass wool in tube before attaching).
 - 8.18.3. Verify that all of the setpoint parameters are within range and then aseptically add the inoculum through the inoculation port per the process SOP.
 - 8.18.4. Take immediate sample per process SOP for baseline determination.
- 8.19. **Monitor Parameters per process SOP.**
- 8.20. **Harvest Culture per process SOP.**
- 8.21. **Shut Down Procedure**

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- 8.21.1. Select "End Batch."
- 8.21.2. Exit BCLite program.
- 8.21.3. On the desktop, select DBViewer32.
- 8.21.4. Select browse and choose the file used during the run.
- 8.21.5. Export the data to Microsoft Excel.
- 8.21.6. Turn all the control modes (Temp, pH, Agit, DO, Feed 1-5) on the Bioflo 3000 controller to off.
- 8.21.7. Turn off the BioFlo 3000.
- 8.21.8. Turn off the water flow.
- 8.22. Clean the BioFlo 3000.**
 - 8.22.1. Make up approximately 4 liters of 10% bleach solution. Label each vessel containing the bleach solution as: 10% Bleach, [date], [initials], Store: Room Temperature, Dispose: Drain.
 - 8.22.2. Disconnect all cords and tubes on the outside of the vessel assembly.
 - 8.22.3. Pour 3L of 10% bleach solution into the vessel through the inoculation port.
 - 8.22.4. Soak the vessel in the 10% bleach solution for approximately 15 minutes.
 - 8.22.5. Remove the agitator motor and wipe down with a sponge using 10% bleach solution.
 - 8.22.6. Remove the headplate from the vessel.
 - 8.22.7. Remove the baffle from the vessel. Clean the baffle with water and glassware detergent. Scrub clean with a soft brush if needed. Rinse thoroughly with tap water and then with deionized water.
 - 8.22.8. Remove the vessel from the heat exchanger base and pour the bleach solution down the drain. Rinse the vessel thoroughly.
 - 8.22.9. Remove the plastic plugs from the vessel.
 - 8.22.10. Clean the vessel carefully with warm water, glassware detergent and a sponge. Rinse thoroughly with tap water and then deionized water.
Note: Handle the vessel gently, the glass is very brittle.
 - 8.22.11. Clean the plastic plugs and thumb screws (large and small) thoroughly with warm water and glassware detergent. Scrub clean with a soft brush if needed. Rinse thoroughly with tap water and then with deionized water.
 - 8.22.12. Remove all probes and tubes from the topside and underside of the headplate.
 - 8.22.13. Place DO and pH probes securely in the probe stand and place the shorting caps on the top of each probe.
 - 8.22.14. Clean all bioreactor tubes with a soft cloth, warm water, and glassware detergent. Rinse thoroughly with warm tap water, and then with several changes of deionized water.
Note: A soft brush may be used on difficult to clean parts of the bioreactor vessel.
 - 8.22.15. Clean the inside of the sparger tube to remove clogs and prevent damage to the bioreactor on a future run.
 - 8.22.16. Remove the pH and DO probes one at a time from the probe stand and gently clean the probes using a soft cloth and deionized water. Be careful not to harm

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the sensory tips of these probes. Return each probe to the probe stand after cleaning.

- 8.22.17. Add pH probe storage solution (3M potassium chloride) to the protective cover of the pH probe. The cover should be approximately half full of solution.
- 8.22.18. Carefully cover the pH probe with the protective cap. The probe should be immersed in pH probe storage solution.
- 8.22.19. Place the DO probe protective cap on the bottom of the DO probe.
- 8.22.20. DO and pH probes should be stored in an upright position in the probe stand.
- 8.22.21. Clean the headplate and impellers with water and glassware detergent. A soft brush or sponge can be used. Rinse thoroughly with tap water and then deionized water.
- 8.22.22. Remove the O-ring from the headplate and heat exchanger base and clean thoroughly.
- 8.22.23. Clean the heat-exchanger base and rinse thoroughly.
- 8.22.24. Ensure that all vessel parts have been cleaned and rinsed thoroughly. Allow them to air dry.
- 8.22.25. Wipe down the BioFlo cabinet with 10% bleach solution.

9. Attachments:

- 9.1. Figure 1: BioFlo 3000 Vessel and Cabinet
- 9.2. Figure 2: Vessel Parts
- 9.3. Figure 3: Headplate, Underside
- 9.4. Figure 4: Assembled Headplate, Top Side
- 9.5. Figure 5: BioFlo 3000 Side View
- 9.6. Figure 6: BioFlo 3000 Controller

10. History:

Name	Date	Amendment
Sonia Wallman	1997	Initial release
Sue Penney	2003	Updated.
Karl J. Cresswell	12/19/05	Removed all grease steps and added inspection of DO probe.
Deb Audino	20July07	Added Feed 1 and 2 low and high values. Added inputing DO setpoint.
Deb Audino	04Apr08	College name change. Added steps for using an existing recipe.
Kari Britt	15May09	Changed directions for calibration of the pH meter and dissolved oxygen probes. Changed directions in "Prepare for Operation" section. Changed directions for assembling the headplate. Changed directions for the three types of control modes. Clarified directions for using BCLite. Removed figures and added additional figures in the attachments section.

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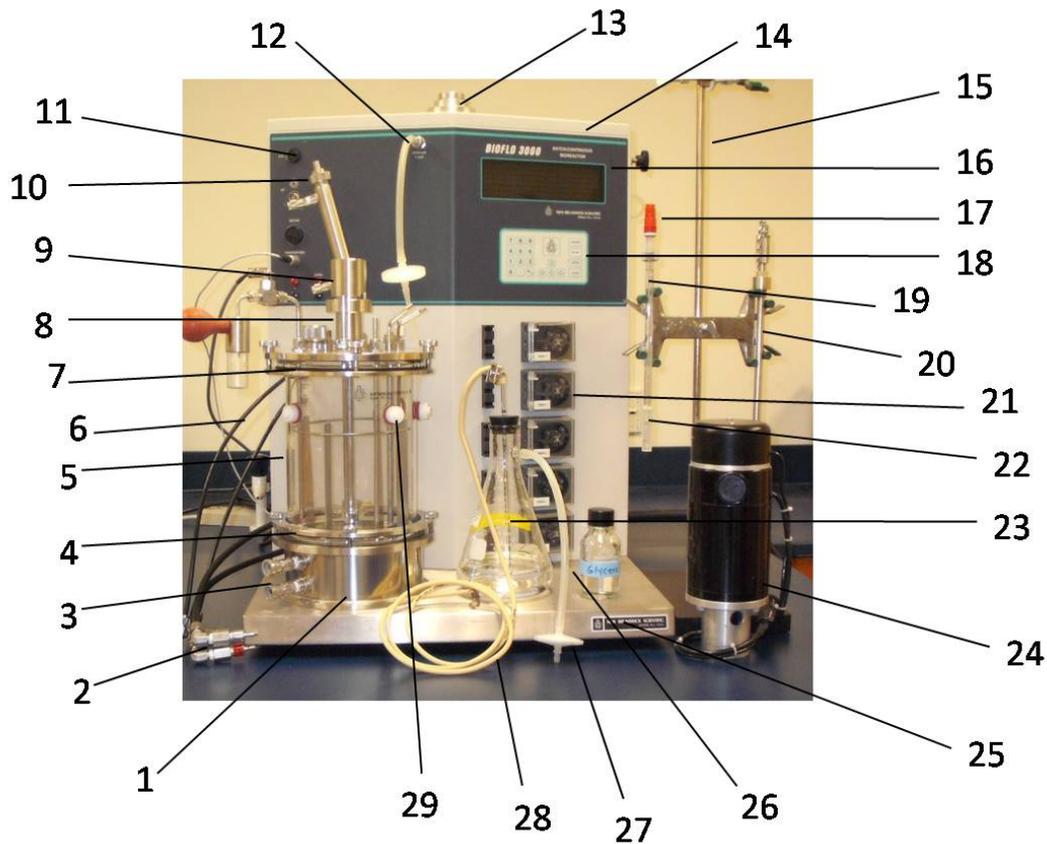


Figure 1: BioFlo 3000 Vessel and Cabinet

- | | |
|-------------------------------|-----------------------------|
| 1. Heat Exchanger Base | 15. Probe Stand |
| 2. Jacket Water Line Adaptors | 16. Controller Display |
| 3. Heat Exchanger Connectors | 17. pH Probe Shorting Cap |
| 4. Bottom Steel Ring | 18. Controller Keypad |
| 5. Glass Vessel | 19. pH Probe |
| 6. Condenser Water Lines | 20. DO Probe |
| 7. Top Steel Ring | 21. Feed Pump |
| 8. Agitator Shaft | 22. pH Probe Protective Cap |
| 9. Agitator Cover | 23. Feed Bottle Assembly |
| 10. Condenser Top Outlet | 24. Agitator Motor |
| 11. Electrical Outlet | 25. Platform |
| 12. Sparger Gas Port | 26. Silicone Tubing |
| 13. Agitator Motor Stand | 27. Air Filter |
| 14. Cabinet | 28. PharMed Tubing |
| | 29. Plastic Plug |

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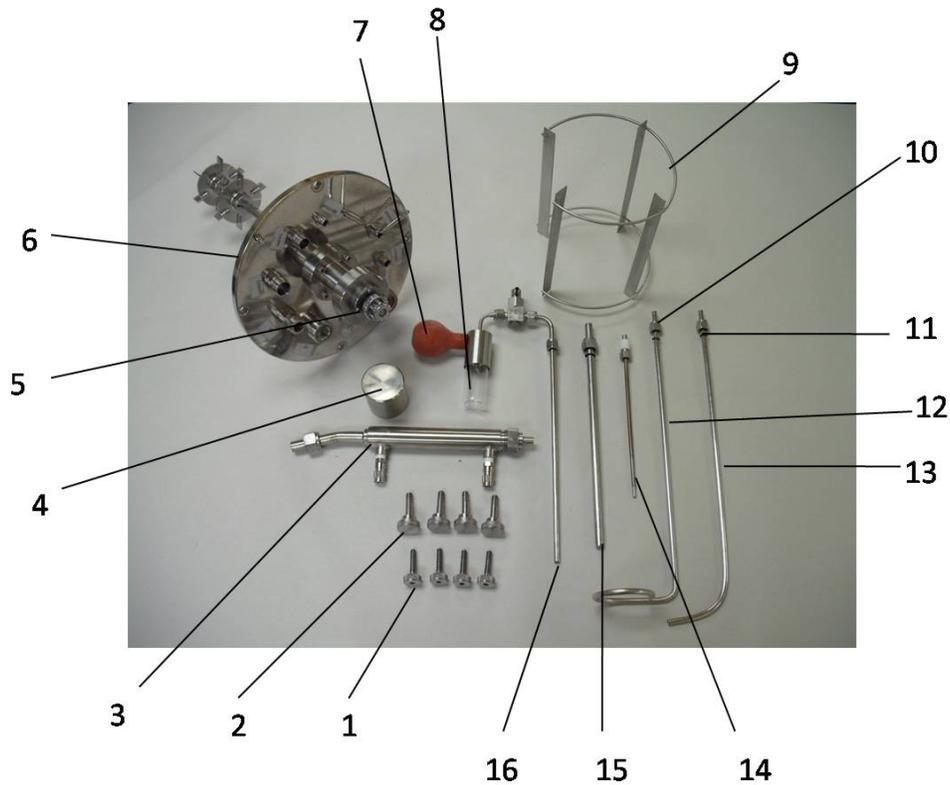


Figure 2: Vessel Parts

- | | |
|--------------------------|----------------------|
| 1. Small Thumb Screw | 9. Baffle |
| 2. Large Thumb Screw | 10. Nut |
| 3. Condenser | 11. O-ring |
| 4. Agitator Cover | 12. Harvest Tube |
| 5. Agitator | 13. Sparger Tube |
| 6. Headplate | 14. Foam Sensor |
| 7. Sampler Assembly Bulb | 15. Thermowell |
| 8. Sample Bottler | 16. Sampler Assembly |

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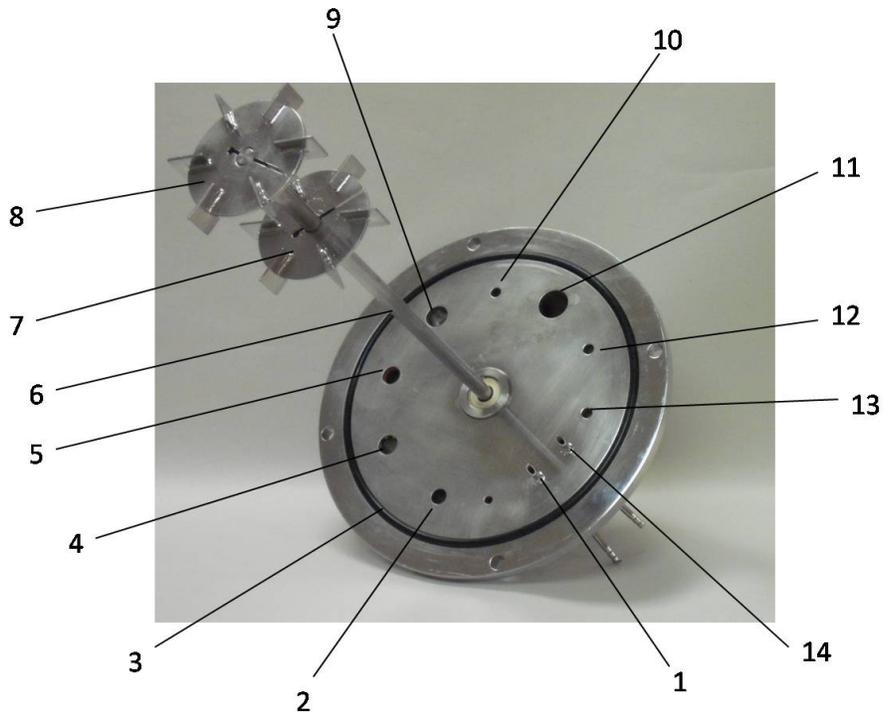


Figure 3: Headplate, Underside

- | | |
|---------------------|---------------------------|
| 1. Addition Port | 8. Lower Impeller |
| 2. Thermowell Port | 9. DO Probe Port |
| 3. O-ring | 10. Sampler Assembly Port |
| 4. Foam Sensor Port | 11. Inoculation Port |
| 5. pH Probe Port | 12. Harvest Tube Port |
| 6. Agitator Shaft | 13. Sparger Tube Port |
| 7. Upper Impeller | 14. Addition Port |

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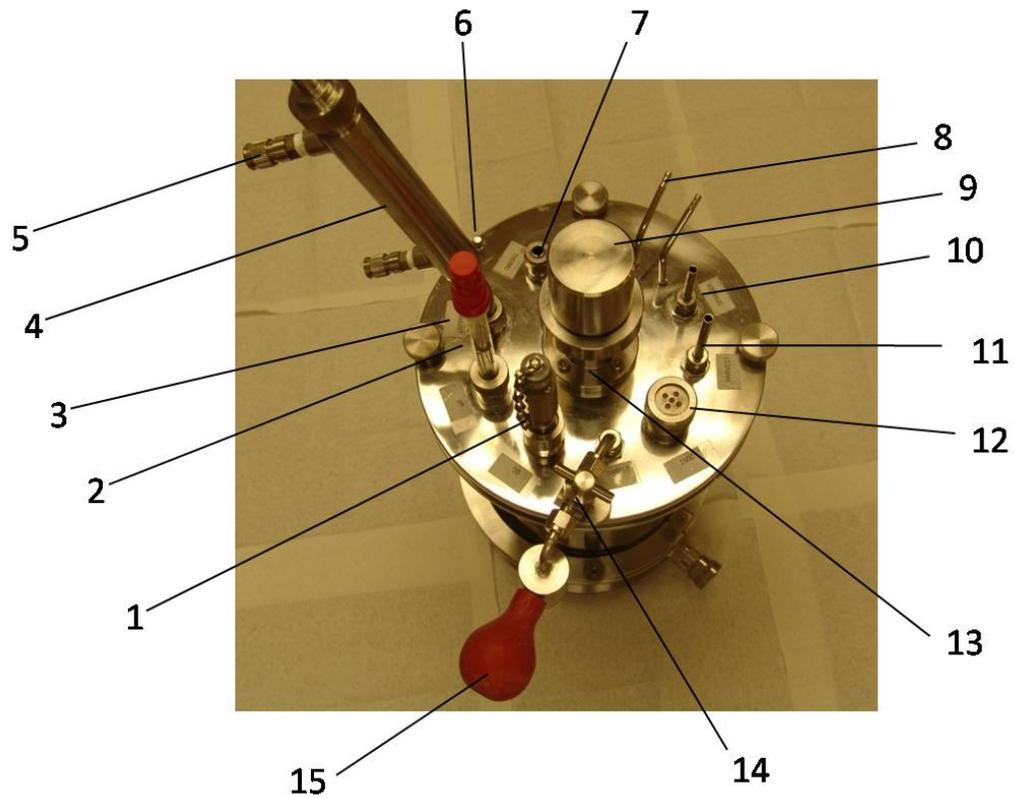


Figure 4: Assembled Headplate, Top Side

- | | |
|----------------------------------|---|
| 1. DO Probe | 9. Agitator Cover |
| 2. pH Probe | 10. Sparger Tube |
| 3. Foam Sensor (behind pH Probe) | 11. Harvest Tube |
| 4. Condenser | 12. Inoculation Port |
| 5. Condenser Barbed Connectors | 13. Agitator Shaft |
| 6. Ground Stud | 14. Sampler Assembly Valve (in closed position) |
| 7. Thermowell | 15. Sampler Assembly Bulb. |
| 8. Addition Port | |

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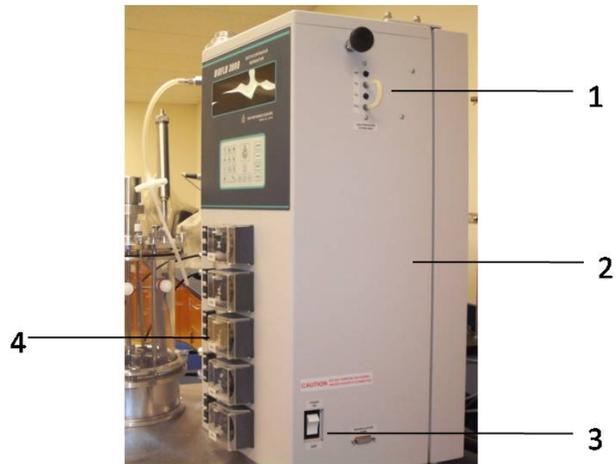


Figure 5: BioFlo 3000 Side View

1. Gas and Air Console
2. Cabinet
3. Power Switch
4. Feed Pump

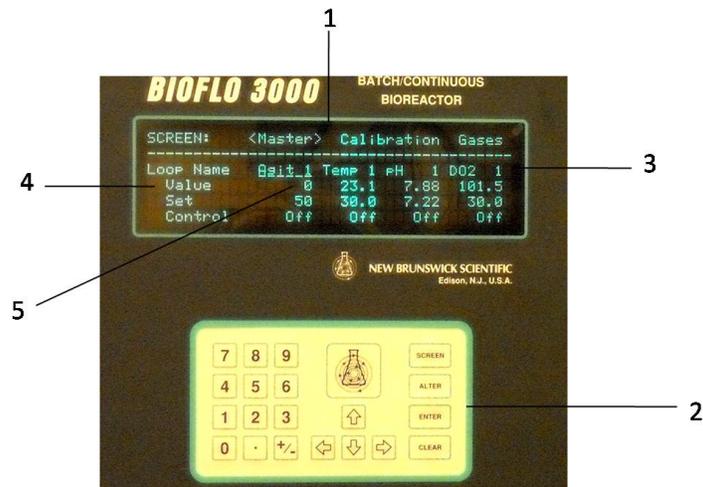


Figure 6: BioFlo 3000 Controller

1. Display Screen
2. Keypad
3. Column Heading
4. Row Name
5. Box (Where the Column Heading and Row Name coordinates meet.)