

Title: Applikon Bioreactor Operation SOP

Approvals:

Preparer: Bob O'Brien Date 03Apr08
Reviewer: Deb Audino Date 03Apr08

1. Purpose:

1.1. Operation of the Applikon 3L Benchtop Bioreactor.

2. Scope:

2.1. Applies to growing mammalian or insect cells in the Applikon 3L Benchtop 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. Applikon Benchtop Bioreactor manufacturer instrumentation manuals
- 4.2. autoclave SOP
- 4.3. biological safety cabinet SOP

5. Definitions: N/A

6. Precautions:

- 6.1. The sodium bicarbonate solution is very basic/caustic. Prevent contact with skin.
- 6.2. The O₂ electrolyte used in the DO probe is a strong Alkaline (pH13) solution. Avoid contact with skin, mucous membrane, or eyes. If contact does occur flush effected area with water.
- 6.3. The heat blanket is very warm when in use. Use caution.

7. Materials :

- 7.1. pH 4.0 buffer standard
- 7.2. pH 7.0 buffer standard
- 7.3. lab towels
- 7.4. 1X PBS
- 7.5. 1M sodium bicarbonate
- 7.6. silicone tubing
- 7.7. PharMed tubing
- 7.8. clamps
- 7.9. aluminum foil
- 7.10. air filters (4)
- 7.11. glass wool
- 7.12. probe stand with clamps
- 7.13. 15mL conical tube
- 7.14. O₂ electrolyte (for DO probe)
- 7.15. 3M potassium chloride (KCL) solution for pH probe storage

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- 7.16. Bioreactor
 - 7.16.1. glass pH probe
 - 7.16.2. stainless steel DO probe
 - 7.16.3. Pt-100 temperature probe
 - 7.16.4. harvest tube
 - 7.16.5. sparger
 - 7.16.6. thermowell tube
 - 7.16.7. glass vessel
 - 7.16.8. condenser
 - 7.16.9. feed bottle
 - 7.16.10. sample bottle assembly
 - 7.16.11. heater blanket
- 7.17. autoclave
- 7.18. biological safety cabinet
- 7.19. glycerol
- 7.20. transfer pipets
- 7.21. source of CO₂
- 7.22. source of air
- 7.23. 1L sterile bottles
- 7.24. 500mL sterile bottles

8. Procedure:

8.1. Assemble Vessel Stand (Figure 1)

- 8.1.1. Inspect the integrity of the large O-rings on the vessel stand and headplate.
- 8.1.2. Replace if worn or cracked.
- 8.1.3. Lower glass vessel into the vessel stand, making contact with the O-ring.
- 8.1.4. Verify that the bolts are threaded completely into the vessel stand. Thread if necessary.
- 8.1.5. If preparing for a run, add approximately 25mL 1X PBS to the vessel and approximately 250mL of 1M Sodium bicarbonate to the feed bottle.

8.2. Assemble Headplate –Underside (Figure 2)

- 8.2.1. Inspect the integrity of the O-rings on the harvest tube, sparger tube, and thermowell tube. Replace if worn or cracked.
- 8.2.2. Put two inch long piece of tubing on the non-barbed end of the harvest tube.
- 8.2.3. Insert the harvest tube from the underside of the headplate between the inoculation port and 3 addition port.
- 8.2.4. Place a washer and nut onto the harvest tube on the top of the headplate. Tighten the nut until the retainer of the harvest tube is flush with the underside of the headplate.
- 8.2.5. Insert the sparger tube from the underside of the headplate, next to blind stopper.
- 8.2.6. Rotate sparger tube so it is aligned beneath the stirrer impeller.

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- 8.2.7. Place a washer and nut onto the sparger tube on the top of the headplate. Tighten the nut until the retainer of the sparger tube is flush with the underside of the headplate.
- 8.2.8. Insert the thermowell tube from the underside of the headplate, in between the pH and DO probe ports.
- 8.2.9. Place a washer and nut onto the thermowell tube on the top of the headplate. Tighten the nut until the retainer of the thermowell tube is flush with the underside of the headplate.
- 8.3. **Attach Headplate to Vessel Stand (Figure 3)**
 - 8.3.1. Place the headplate onto the vessel stand, positioning the holes on the outer edge of the headplate with the bolts on the vessel stand.
 - 8.3.2. Place the sample bottle assembly onto the bolt located by the 3 addition port and attach with a mill fastener.
 - 8.3.3. Secure the headplate with the remaining 5 mill fasteners by hand in a crosswise pattern.
- 8.4. **Assemble Headplate – Top Side (Figure 3)**
 - 8.4.1. Inspect the integrity of the O-ring in the condenser port of the headplate. Replace if worn or cracked.
 - 8.4.2. Inspect the black seal at the bottom of the condenser underneath the retainer nut. Replace if worn or cracked.
 - 8.4.3. Place the condenser into the condenser port making sure that the barbed connectors are facing out.
 - 8.4.4. Tighten the retainer nut.
 - 8.4.5. **DO Probe**
 - 8.4.5.1. Remove the protective cap from the bottom of the stainless steel DO probe.
 - 8.4.5.2. Inspect the screen at the bottom of the probe tip. Replace if damaged.
 - 8.4.5.3. Holding the probe in a vertical position, unscrew the membrane module from the bottom of the probe.
 - 8.4.5.4. Inspect the integrity of the O-ring underneath the module and replace if worn or cracked.
 - 8.4.5.5. Replenish DO electrolyte. There should be approximately 1mL of O₂ electrolyte solution in the membrane module.
 - 8.4.5.6. Replace membrane module.
 - 8.4.5.7. Inspect the integrity of the O-ring at the top of the stainless steel DO probe. Replace if worn or cracked.
 - 8.4.5.8. Inspect the black seal at the top of the DO probe under the retainer nut. Replace if worn or cracked.
 - 8.4.5.9. Place the DO probe into the DO probe port and tighten retainer nut.
- 8.5. **pH Probe Calibration**
 - 8.5.1. Remove the protective caps from the top and bottom of the glass pH probe.
 - 8.5.2. Pour the 3M potassium chloride (KCL) storage solution from the bottom protective cap into the 15mL conical tube.
 - 8.5.3. Connect the pH Controller cable to the top of the pH probe.

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- 8.5.4. Turn on the ADI1025 unit by pressing the large green button on the front of the unit.
- 8.5.5. Wait for instrument to initialize (approximately one minute).
- 8.5.6. The set point main screen will appear on the display screen.
- 8.5.7. On the ADI1010, press the "pH" key then the "Calib." key.
- 8.5.8. Use the dial on the ADI1010 to highlight "reset to default calibration values."
- 8.5.9. Press the "Calib." key to input the factory defaults into the ADI1010 controller.
- 8.5.10. Press the "pH" key then the "Calib." key.
- 8.5.11. Use the dial on the ADI1010 to highlight the calibration option.
- 8.5.12. Press the "Calib." key to select the calibration option. This will display the pH calibration screen.
- 8.5.13. Rinse the pH and Pt-100 temperature probes with DI water and blot dry.
- 8.5.14. Place the pH and Pt-100 temperature probes into the pH 7 buffer standard.
- 8.5.15. Allow the pH and temperature readings to stabilize.
- 8.5.16. Press "Start/Stop" key to input the temperature value.
- 8.5.17. Press the "Calib." key.
- 8.5.18. Use the dial on the ADI1010 to input the pH value of the standard (7.00).
- 8.5.19. Press the "Calib." key.
- 8.5.20. Rinse the pH and Pt-100 probe with DI water and blot dry.
- 8.5.21. Place the pH and Pt-100 probe in to the pH 4 buffer standard.
- 8.5.22. Allow the pH reading to stabilize.
- 8.5.23. Use the dial on the ADI1010 to input the pH value of the standard (4.00).
- 8.5.24. Press the "Calib." Key. This will display the pH calibration slope and offset values.
 - 8.5.24.1. Expected values:
Slope: 0.95-1.05
Offset: $< \pm 0.3$
 - 8.5.24.2. See supervisor if not within this range.
- 8.5.25. Press the "Setp." key twice to return to the main screen.
- 8.5.26. Turn off power by pressing the large green button on the ADI1025 unit.
- 8.5.27. Remove both probes from pH 4 buffer standard.
- 8.5.28. Rinse both probes with DI water and blot dry.
- 8.5.29. Disconnect the pH probe from the controller cable and replace the top protective cap.
- 8.5.30. Inspect the integrity of the O-ring at the top of the glass pH probe. Replace if worn or cracked.
- 8.5.31. Inspect the black seal at the top of the pH probe under the retainer nut. Replace if worn or cracked.
- 8.5.32. Place the pH probe into the pH probe port and tighten the retainer nut.

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8.6. Attach Tubing/ Autoclave Preparation

- 8.6.1. Connect labeled end of silicone tubing with air filters on the following :
 - sparger tube
 - condenser top outlet
 - CO₂ overlay port
- 8.6.2. Use a small piece of silicone tubing to connect together two of the ports on the 3 addition port.
- 8.6.3. Connect the PharMed tubing from the feed bottle to the 3 addition port.
- 8.6.4. Connect the sample bottle tubing to the harvest tube.
- 8.6.5. Clamp off all tubing (near the headplate) except the condenser top outlet. The condenser top outlet must remain unclamped to release pressure during autoclaving.
- 8.6.6. Close all open ends with glass wool and cover with aluminum foil.

8.7. Autoclave

- 8.7.1. Prepare autoclave per autoclave SOP.
- 8.7.2. Transfer bioreactor vessel to autoclave.
- 8.7.3. Autoclave at 121°C for 20 minutes, using slow exhaust.
- 8.7.4. Remove from autoclave when cycle is complete and return to bench.

8.8. Polarize DO Probe

- 8.8.1. Remove protective caps from the pH and DO probes.
- 8.8.2. Connect the pH and DO probes to the appropriate power cable.
- 8.8.3. Wrap the thermal blanket around the vessel and plug into the ADI1025.
- 8.8.4. Turn on power by pressing the large green button on the ADI1025 unit.
- 8.8.5. Allow DO probe to polarize for at least 6 hours before performing DO calibration.

8.9. Media Addition

- 8.9.1. Aseptically add media (per the process SOP) to the bioreactor vessel.

8.10. Run Preparation

- 8.10.1. Place stirrer motor on stirrer motor mount.
- 8.10.2. Remove the clamps on the following tubing:
 - feed bottle
 - CO₂ overlay port
 - sparger
- 8.10.3. Place tubing from the feed bottle through the feed pump on the ADI1025.
- 8.10.4. Connect the tubing from the CO₂ overlay port to the overlay outlet on the ADI1025.
- 8.10.5. Connect the tubing from the sparger to the sparger outlet on the ADI1025.
- 8.10.6. Using a transfer pipet, add a small amount (1.5-2mL) of glycerol to the thermowell.
- 8.10.7. Place the Pt-100 temperature probe into the thermowell.

8.11. Establish Set Points

- 8.11.1. On the ADI1010, press a parameter key (pH, Temp, dO₂, or Stirrer) then the "Setp." key.

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- 8.11.2. Use the dial to set the desired set point per the process SOP.
- 8.11.3. Press the “Setp.” key to return to the Main Menu.
- 8.11.4. Repeat until each set point in the process SOP is established per the process SOP.
- 8.12. **Establish Limits**
 - 8.12.1. On the ADI1010, press a parameter key.
 - 8.12.2. Press the “limits” key to go to the upper limit.
 - 8.12.3. Use the dial to set the upper limit per the process SOP.
 - 8.12.4. Press the “limits” key again to go to the lower limit.
 - 8.12.5. Use the dial to set the lower limit per the process SOP.
 - 8.12.6. Press the “Setp.” key to return to the Main Menu
 - 8.12.7. Repeat until an upper and lower limit is set for each parameter per the process SOP.
- 8.13. **Activate Control Loops**
 - 8.13.1. Press the pH key then the “Start/Stop” key to activate the pH control loop.
 - 8.13.2. Press the “Temp” key then the “Start/Stop” key to activate the temperature control loop.
 - 8.13.3. Press the “Stirrer” key then the “Start/Stop” key to activate the stirrer control loop.
 - 8.13.4. Lights on the pH, temperature, and stirrer keys should turn green.
 - 8.13.5. NOTE: Do NOT activate the dO₂ control loop at this time.
- 8.14. **DO Probe Calibration (after six hours of polarization)**
 - 8.14.1. On the ADI1010, press the “dO₂” key then the “Calib.” key.
 - 8.14.2. Four options are displayed: (View Calibration Values, Temperature Compensation, Calibration, and Reset to Default Calibration Values.)
 - 8.14.3. Use the dial on the ADI1010 to highlight the calibration option.
 - 8.14.4. Press the “Calib.” key to select the calibration option.
 - 8.14.5. Use the dial on the ADI1010 to set the value to 100.
 - 8.14.6. Press the “Calib.” key.
 - 8.14.7. The DO probe calibration slope should be on the ADI1010 display.
 - 8.14.7.1. Expected values are:
Slope: 8-15 at 37°C or 10-20 at 25°C
 - 8.14.7.2. See supervisor if not within this range.
 - 8.14.8. Press the “Setp.” key to return to the main screen on the ADI1010 display.
 - 8.14.9. Press the “dO₂” key then the “Start/Stop” key to activate the dO₂ control loop.
 - 8.14.10. Lights on the “dO₂” key should turn green.
 - 8.14.11. Disconnect the DO probe BRIEFLY (<1min) from the DO cable.
 - 8.14.11.1. The DO reading should quickly decrease to 0.
 - 8.14.11.2. If the DO value does not drop below 0.5% the probe requires maintenance (refer to the user manual for the DO probe).
 - 8.14.11.3. Reconnect the DO cable to the DO probe.
 - 8.14.12. Turn on Air and CO₂ supplies to the bioreactor.

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8.15. Add cell culture through the Inoculation port per the process SOP.

8.16. BioXpert Lite

- 8.16.1. Turn on power to computer.
- 8.16.2. Open BioXpert Lite software.
- 8.16.3. Choose: Username: NHCTC, Mode: Cultivation, and Fermentor no.:1.
- 8.16.4. Select “OK”.
- 8.16.5. To ensure the computer is communicating with the controller, on the menu bar, click on Run, Test. Next to each variable, a number should be displayed in the values column representing the value of that parameter.
 - 8.16.5.1. If no numbers are displayed, notify your instructor.
- 8.16.6. Select “OK”.
- 8.16.7. On the menu bar, click on Run, New.
- 8.16.8. In the Organism box, type in the name of the cells being grown. Type any comments in the Comments box.
- 8.16.9. Select “OK”.
- 8.16.10. Choose the access interval between readings.
 - 8.16.10.1. This can be changed during a run without changing the start moment.
- 8.16.11. Under Protocol in the Data box type “F” (for fall) or “S” (for spring) and the year (i.e. F2006).
 - 8.16.11.1. Note: The Data box allows a maximum of 8 characters.
- 8.16.12. Select “OK”.
 - 8.16.12.1. Note: Selecting “OK” begins the data collecting process.
- 8.16.13. **Important:** Do NOT close (i.e. “X-out of) the On-line Session window or select “END” at **any** time during the run. Doing this will cause the **loss** of **all** data for the run.
- 8.16.14. To view the Chart window during a run, simply click on the blue bar at the top of the Chart window. This will bring the chart to the front. Do the same to view the On-line Session window if it is behind the Chart window.

8.17. Setting the scale of the graph

- 8.17.1. View the Chart window in the BioXpert light application by clicking on the blue bar at the top of the chart window.
- 8.17.2. View the “Y” axis variables by clicking on a parameter in the Variables window. All of the variables can be viewed at once.
- 8.17.3. Use the mouse to hold the cursor over any of the Y axis variable lines (pH, Temp, dO₂, or Stirrer) in the chart. Left click to open the Scale Settings window.
- 8.17.4. Check the box next to “Manually Scale”.
- 8.17.5. Choose the Y axis you would like to change in the “List of Variables” pull-down menu.
- 8.17.6. Change the scale by entering the desired values in the Scale range boxes.
- 8.17.7. Select “OK”.

8.18. Ending a Run

- 8.18.1. On the menu bar, click on File, Save as. Delete the (*) and enter a file name.

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- 8.18.2. Select “OK”.
- 8.18.3. Export the data to EXCEL by choosing File, Export, Data on the menu bar.
 - 8.18.3.1. Select the following options for export:
 - Format: SYLK (for EXCEL)
 - Variables: On-lines (Off-lines should not be checked.)
 - Time Interval: On-line
 - Time Range: All
 - 8.18.3.2. Note: In the Time Interval option, On-line will export all data collected at the interval period chosen in section 8.16.10 while Off-line will export data points that were collected every 15 minutes. “Other” is used to export a custom interval.
 - 8.18.3.3. Select “OK”.
 - 8.18.3.4. Under File Name delete the (*) and type a file name. Make note of the directory where the file is saved before selecting “OK”.
 - 8.18.3.5. Select “OK”.
- 8.18.4. Turn off the control loops on the ADI1010by selecting a parameter key (“pH”, “Temp”, “dO₂”, or “Stirrer”), and pressing the “Start /Stop” key. Repeat for each control parameter.
- 8.18.5. Turn off the Air and CO₂ supplies to the ADI1025 controller.
- 8.18.6. Aseptically remove cultivation through the harvest port, utilizing the sample bottle.
- 8.18.7. Turn off power switch on the ADI1025.
- 8.18.8. Remove stirrer motor.
- 8.18.9. Unplug the heater blanket and remove from the vessel. Store flat.
- 8.18.10. Disconnect the power cords for the pH and DO probes.
- 8.18.11. Remove the pH and DO probes and place in storage clamps.
- 8.18.12. Place protective caps on the pH and DO probes.
- 8.18.13. Remove the Pt-100 probe, rinse with DI water and blot dry.
- 8.18.14. Remove all tubing.
- 8.19. **Cleaning the Bioreactor**
 - 8.19.1. Clean pH and DO probes.
 - 8.19.1.1. Remove pH and DO probes from the headplate.
 - 8.19.1.2. Wipe down DO and pH probes with a lab towel damp with 10% bleach solution.
 - 8.19.1.3. Rinse DO and pH probes with DI water and blot dry.
 - 8.19.2. Remove the headplate from the vessel (with harvest tube, thermowell tube and sparger tube still attached).
 - 8.19.3. Add 1L of 10% bleach solution to the vessel.
 - 8.19.4. Replace the headplate and soak the sparger tube, harvest tube and thermowell tube for approximately 15 minutes.
 - 8.19.5. Remove headplate from vessel.

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- 8.19.6. Disassemble the entire headplate assembly and wash all of the components with 10% bleach solution. Rinse with DI water, and spray with 70% IPA. Set out on lab towels to air dry.
- 8.19.7. Clean all tubing with 10% bleach solution, rinse with DI water, and spray with 70% IPA. Set out on lab towels to air dry.
- 8.19.8. Wipe down controller with a lab towel damp with a 10% bleach solution, then with a towel damp with DI water.
- 8.19.9. Place headplate and components out on lab towels to dry.
- 8.19.10. After the components have dried, reassemble the bioreactor for storage (leaving out the pH and DO probes).

8.20. pH probe Storage

- 8.20.1. After cleaning the pH probe, verify that no broth or media residue remains on the membrane surface or diaphragm.
- 8.20.2. Pour the 3M potassium chloride (KCl) solution from the 15mL conical tube into the protective cap. Add more 3M potassium chloride (KCl) solution if necessary to fill the cap ½ full with the solution.
- 8.20.3. Insert the electrode end of the pH probe into the protective cap. The electrode should be completely immersed in the 3M potassium chloride (KCl).
- 8.20.4. Place the probe in a storage clamp and store in vertical position.

8.21. DO probe Storage

- 8.21.1. After cleaning the DO probe, verify that no broth or media residue remains on the membrane surface or diaphragm.
- 8.21.2. Holding the probe in a vertical position, unscrew the membrane module from the bottom of the probe and verify that approximately 1mL of O₂ electrolyte solution is present in the membrane module.
- 8.21.3. Replace the protective cap. Place probe in storage clamp, and store in vertical position.

9. Attachments:

- 9.1. Figure 1: Vessel and Headplate
- 9.2. Figure 2: Underside of the Headplate
- 9.3. Figure 3: Assembled Headplate
- 9.4. Figure 4: ADI1010 Controller

10. History:

Name	Date	Amendment
Bob O'Brien Deb Audino	072705	Initial Release
Deb Audino Bob O'Brien	100605	Deb Audino and Bob O'Brien, 100605, Removed all grease steps, reduced amount of PBS to add to vessel, removed PBS removal step, modified adding media in BSC, modified cleaning steps.
Bob O'Brien	042706	Add O ₂ in precaution, additional materials for probe storage, modify section 8.5 for calibration. Add section 8.20 pH probe storage; add section 8.21 DO probe storage.
Kari Britt	09Oct06	New directions for BioXpert Lite section to correspond with

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		upgrade to new version to computers. Moved Export the Data section to the Ending a run section in order to consolidate BioXpert Lite directions.
Bob O'Brien	04Apr08	College name change

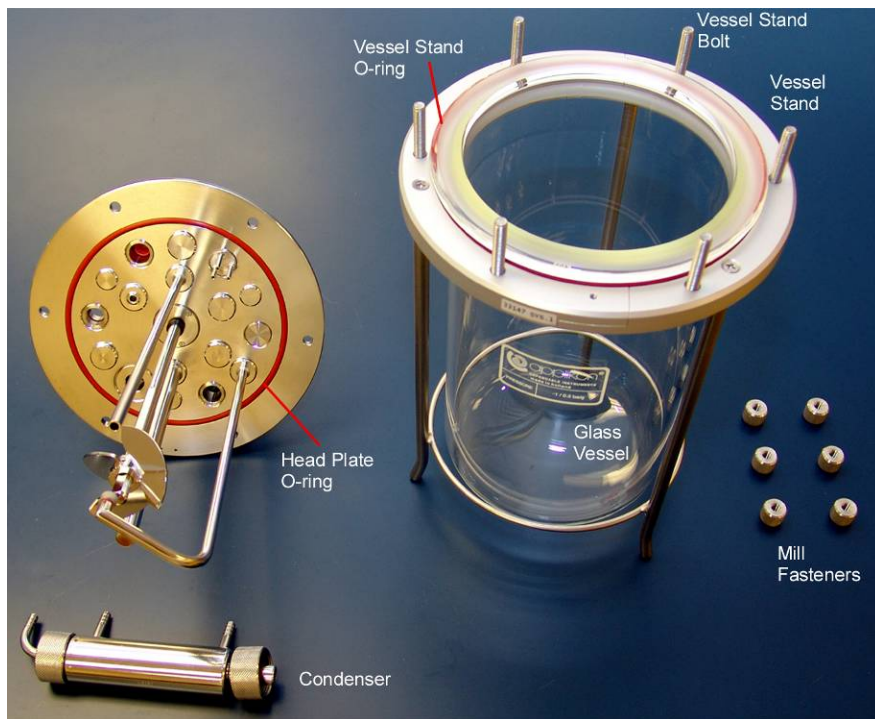


Figure 1: Vessel and Headplate

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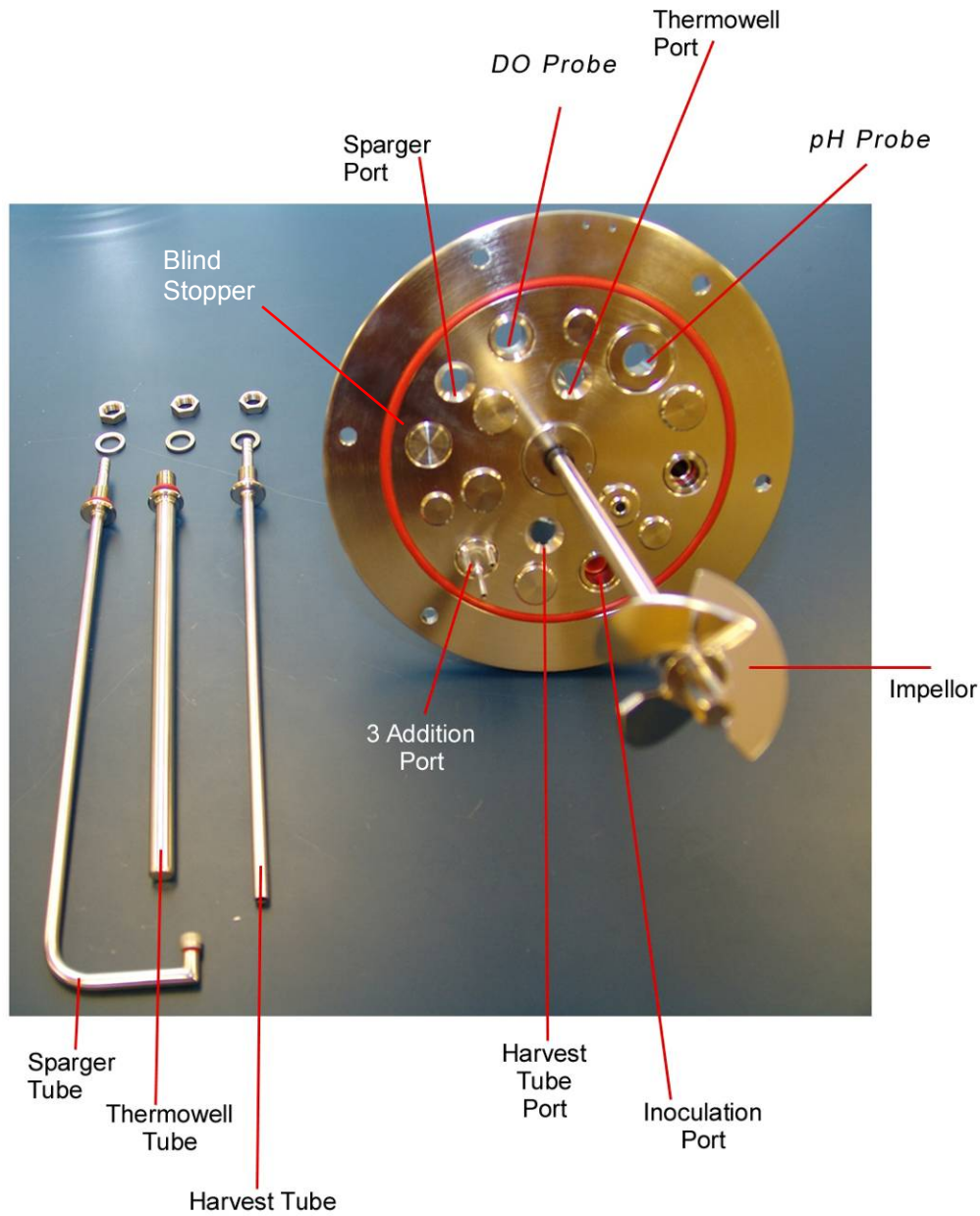


Figure 2: Underside of Headplate

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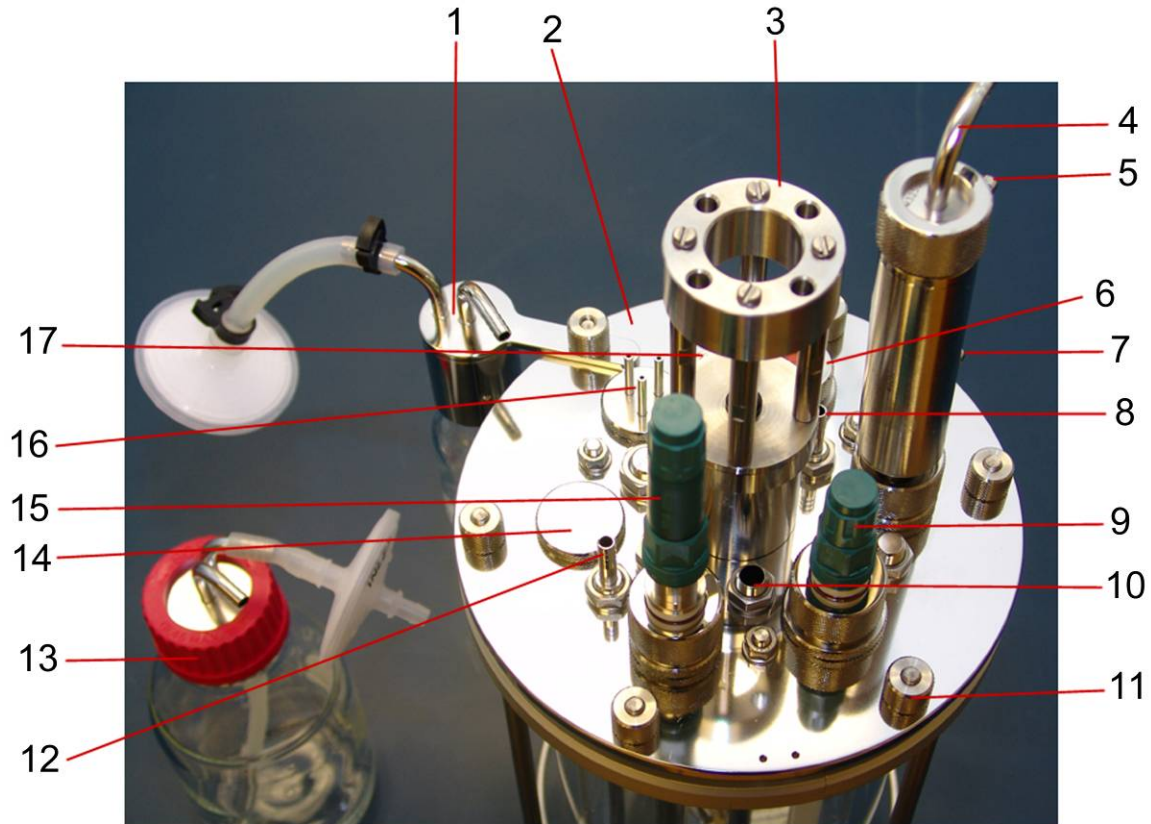


Figure 3: Assembled Headplate

- | | |
|----------------------------------|---------------------|
| 1. Sample bottle assembly | 10. Thermowell port |
| 2. Head plate assembly | 11. Mill fastener |
| 3. Stirrer motor mount | 12. Sparger |
| 4. Condenser air outlet | 13. Feed bottle |
| 5. Condenser water outlet (from) | 14. Blind stopper |
| 6. Inoculation port | 15. DO probe |
| 7. Condenser water inlet (to) | 16. 3 Addition port |
| 8. CO ₂ overlay port | 17. Harvest tube |
| 9. pH probe | |

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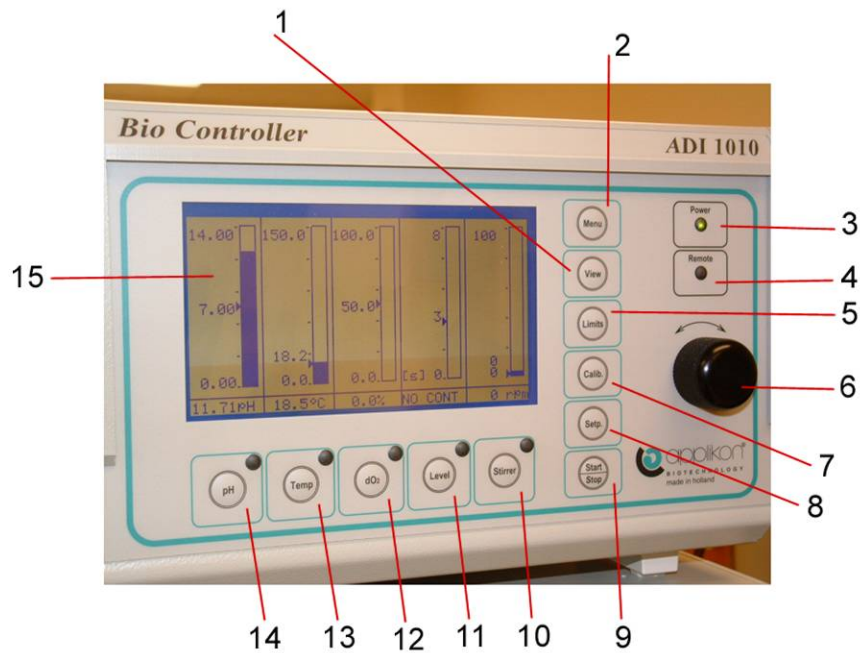


Figure 4: ADI1010 Controller

1. View function key.
2. Menu function key
3. Power indicator LED
4. Remote indicator LED
5. Limits function key
6. Dial: digital potentiometer
7. Calib. Key
8. Setup key
9. Start/Stop key
10. Stirrer parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, and alternating Green/Red out of range
11. Level parameter key; has a dual color LED, off, Green on no alarm, Red out of range, and alternating Green/Red out of range
12. DO parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, alternating Green/Red out of range
13. Temp parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, alternating Green/Red out of range
14. pH parameter key; has a dual-color LED, off, Green on no alarm, Red out of range, alternating Green/Red out of range
15. Display screen