# **Guide to Operations**

## BioFlo 110 Modular Benchtop Fermentor

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## WARNING! High voltage.

Always make sure this equipment is properly grounded.



## WARNING!

This product is not designed to contain gases within the range of their lower explosion limit (LEL) and their upper explosion limit (UEL). If your process requires or produces gases, be sure to verify their LEL and UEL concentration range (available online).

## 

This equipment *must* be operated as described in this manual. If operational guidelines are not followed, equipment damage and personal injury *can* occur. Please read the entire User's Guide before attempting to use this unit.

Do not use this equipment in a hazardous atmosphere or with hazardous materials for which the equipment was not designed.

New Brunswick Scientific Co., Inc. (NBS) is not responsible for any damage to this equipment that may result from the use of an accessory not manufactured by NBS.

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## **Manual Conventions**

NOTE:	Notes contain essential information that deserves special attention.
	<i>Caution</i> messages appear before procedures which, if caution is not observed, could result in damage to the equipment.
WARNING!	<i>Warning</i> messages alert you to specific procedures or practices which, if not followed correctly, could result in serious personal injury.
Bold	Text in boldface type emphasizes key words or phrases.
	This particular <i>Warning</i> message, whether found in the manual or on the unit, means HOT SURFACE–and therefore represents a potential danger to touch.
CRUSH WARNING!	<i>Crush Warning</i> messages alert you to specific procedures or practices <u>regarding heavy objects</u> which, if not followed correctly, could result in serious personal injury.

V

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Every Instrument manufactured by the New Brunswick Scientific Co., Inc. is warranted to be free from defects in material and workmanship. This apparatus with the exception of glassware, lamps and electrodes (where supplied), is warranted for 1 year against faulty components and assembly and our obligation under this warranty is limited to repairing or replacing the instrument or part thereof, which shall with-in 1 year after date of shipment, prove to be defective after our examination. This warranty does not extend to any NBS products which have been subjected to misuse, neglect, accident or improper installation or application; nor shall it extend to products which have been repaired or altered outside the NBS factory without prior authorization from the New Brunswick Scientific Co., Inc.





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# 1 OVERVIEW

## 1.1 Design Concept

The BioFlo 110 is a modular fermentation system for microbial and cell culture applications. It features a modern, easy-to-use control interface, plus an available family of vessels, controller modules, and other accessories that address the diverse needs of the bioprocessing community.

A complete range of water-jacketed and non-jacketed fermentation and cell culture vessels is available to cover 0.4 through 14.0 liters. For temperature control, non-jacketed vessels use an external heating blanket and an internal cooling coil. Jacketed vessels use an external jacket water temperature controller.

Modularity means you can position components to suit your workspace and working style. The controller modules can be vertically stacked to conserve bench space. Modularity also makes it easy and economical to adapt your system for different tasks at any time.

As an autoclavable fermentor, the BioFlo 110 requires an external sterilizing apparatus that is large enough to accept the complete vessel assembly.

### 1.2 Process Control

The heart of the BioFlo 110 is the Primary Control Unit (PCU). The PCU serves as the operator interface for one to four vessels. Its bright graphic display and clearly marked keypad serve as the control center for all attached vessels and their associated loop controllers. Four-channel output to a strip-chart recorder is available from the PCU.

## 🖎 NOTE:

#### No more than 16 modules (any combination) can be supported by one PCU.

The BioFlo 110 Power Controller is required for temperature and agitation control. It also provides power for liquid pumps and other control modules. The rotameter and cooling water valve are mounted on the outside of the Power Controller.

Additional control modules and sensors enable expanding a basic temperature/agitation/ airflow fermentor to include control of pH, dissolved oxygen (dO2), antifoam addition, removal and addition of nutrients and other liquids, and blending inlet air with up to three other gases.

## 1.3 Computer-Ready

The BioFlo 110 is compatible with both ModBus and New Brunswick Scientific's *BioCommand* communications protocols. Optional *BioCommand* software adds automatic data logging, centralized monitoring and control, and process programming capabilities to one or more fermentors.

## 1.4 Enhancing Your Fermentor

Other accessories are available and new ones will be added to extend the capabilities of the BioFlo 110. Whenever your bioprocessing needs or applications change, contact your New Brunswick Scientific sales representative for a current description of BioFlo 110 products.

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# 2 INSPECTION & UNPACKING

### 2.1 Inspection of Boxes

After you have received your order from New Brunswick Scientific, inspect the boxes carefully for any damage that may have occurred during shipping. Report any damage to the carrier and to your local NBS Sales Order Department immediately.

#### 2.2 Packing List Verification

Unpack your order, saving the packing materials for possible future use. Also be sure to save the User's Guide, for instruction and reference.

Verify against your NBS packing list that you have received the correct materials, and that nothing is missing. The part numbers on the packing list correspond to part numbers and/or manufacturing numbers. You will find the number affixed to each item, either on a paper sticker on the packaging or on the serial number identification sticker on the equipment itself.

If any part of your order was damaged during shipping or is missing, or fails to operate, please fill out Customer Satisfaction Form 6300 (packed in the envelope with your warranty card) and return it by fax or mail. You can also call the New Brunswick Scientific Service Department.

# **3** PREPARING THE LOCATION

## 3.1 Physical Location

The surface where you place the BioFlo 110 should be smooth, level and sturdy. Ensure that there is enough space around the back and the front of the combined system for proper operation and access.

#### 3.2 Environment

The BioFlo 110 operates best under the following conditions:

- Ambient temperature range of  $10^{\circ}$ C to  $35^{\circ}$ C
- Relative humidity up to 80% non-condensing

#### 3.3 Utilities

Utility		Requirements (minimum-maximum)	
Water (50 µm filtration)		5-10 PSIG (0.34-0.68 kg/cm <sup>2</sup> )	
Air		3-10 PSIG (0.20-0.68 kg/cm <sup>2</sup> )	
Gases (optional)	02	3-10 PSIG (0.20-0.68 kg/cm <sup>2</sup> )	
	CO2	3-10 PSIG (0.20-0.68 kg/cm <sup>2</sup> )	
N2Electricity100V		3-10 PSIG (0.20-0.68 kg/cm <sup>2</sup> )	
		90-100 VAC 50/60 Hz 10 Amp	
	120V	108-132 VAC 50/60 Hz 10 Amp	
	220V	198-242 VAC 50/60 Hz 6 Amp	
	240V	216-264 VAC 50/60 Hz 6 Amp	

## NOTE:

All gases supplied should be medical grade.

# 4 INSTALLATION, ASSEMBLY & SET-UP

## 4.1 Introduction to Control Module Rear Panels

## 4.1.1 Primary Control Unit (PCU)





Refer to Figure 1 above to visually locate the following features on the rear panel:

•	FLOPPY DRIVE	An internal A: drive for field upgrades to the interface software.
•	POWER SWITCH	On/Off rocker switch.
•	AC CONNECTION	Inlet for power cord (supplied), either for connection to Power Controller outlet or for electrical service outlet.

**FUSE HOLDER** Easy access to replace 250V 2-amp Slo-Blo<sup>®</sup> fuse.

- AFS/MODBUS CONNECTOR 25-pin D connector for any supervisory software you may add.
  - CONTROLLER BUS<br/>CONNECTORSTwo RS-485 9-pin D connectors, one above the other.<br/>Use one to connect the Power Controller to the PCU with<br/>the cable supplied.
- **RECORDER CONNECTOR** One 9-pin D connector, reserved for use with any analog recorder you may add. 2.5V output supplies data records.

#### 4.1.2 Power Controller



## Figure 2: Power Controller Rear Panel

Refer to Figure 2 above to visually locate the following features:

WATER SOLENOID VALVE	Located on the side panel (not visible in drawing). Provides water from outside source for cool temperature control within the vessel and to the optional exhaust condenser.
WATER SOLENOID CONNECTOR	Located on the rear panel. Control connection for the water solenoid valve.
VOLTAGE SELECTOR	Located in the fuse drawer in the rear panel, next to the power cord connection.
AC CONNECTION	Located on the rear panel. Input connection, using the power cord supplied, to the electrical service.
PCU CONNECTION	Located on the rear panel. Output connection, using the power cord supplied, to power the PCU.
CONTROLLER BUS CONNECTORS	Located on the rear panel. Two RS-485 9-pin D connectors, one above the other. Use one to connect the Power Controller to the PCU with the cable supplied.
UNIT ADDRESS SELECTOR	Located on the rear panel. Four two-position (on/off) switches used to identify the vessel address, uniquely associating this module to one particular vessel.

## 4.1.3 dO2/pH Controller



## Figure 3: dO2/pH Controller – Rear Panel

### 4.1.4 Level Controller



## Figure 4: Level Controller – Rear Panel

4.1.5 Gas Mix Controller





## 4.1.6 Pump Module

A four-pump module is available to stack with the other BioFlo 110 modules, or to stand freely, adjacent to the module stack and vessel.

## ROTE:

Unlike the other modules, the pump module is wired for either 110V or 220V electric service only. Be sure that your module is correct for your electrical supply.

Refer to Figure 6 below to locate the following feature:

• **POWER INLET PLUGS** Located on the rear panel. Each pump is individually connected to the Power Controller by cables provided.





## 4.1.7 Other Optional Modules

Currently, an optional Thermal Mass Flow Controller is available. See Section 14 for all pertinent details (installation, operation, maintenance, troubleshooting and specifications).

### 4.2 Stacking Modules

The Power Controller, the largest and heaviest of the BioFlo 110 modules, is intended to be the base unit of any stack. You may stack any of the other modules on top of it, in any order. Every module has indentations on top to accommodate the feet of any module stacked on it.

Each Power Controller and any of the control modules (dO2/pH, level, gas mix and/or 4-pump) associated with it should be stacked beside the vessel they support. The modules should be within easy reach of the sensor cables and other leads that will plug into the stack.

If you are running more than one vessel from the PCU, you may wish to place the PCU at the top of the stack most convenient to the operator.

## 🖄 NOTE:

Figure 7 below is an illustration of a sample installation of two vessels with their control modules. For easy access to the touchpad and display screen, and for your working comfort, we suggest that you place the PCU at the top of the stack closest to the operator.

The illustration below is more schematic than realistic.

## NOTE:

No more than 16 modules (any combination) can be supported by one PCU.





## (For clarity, cables and tubing are not shown in this drawing.)

## 4.3 Interconnecting the Modules

All BioFlo 110 control modules are interconnected, to share power and communications.

## WARNING!

Make sure the Power Controller and Primary Control Unit (PCU) are switched off and unplugged from the power source before connecting or disconnecting any equipment.

## NOTE:

The PCU comes with two Module Bus terminator plugs that cap its 9-pin D-connectors on the rear panel. As you plug RS-485 (ModBus) cables into these connectors, move the terminator plugs to the open end of the cables, to close them off.

To connect the modules to each other, with the modules stacked in the preferred order:

- 1. Use a short RS-485 communication cable (provided) to connect the Power Controller from its top controller bus 9-pin connector to the bottom controller bus connector of the next module above. When you remove the terminator plug from the connector, set it aside for reuse.
- 2. In similar fashion, continue to daisy chain each module to the next, using the additional RS-485 cables provided.
- 3. Cap off the final module's unused controller bus connector with the terminator plug.

You can interconnect as many as three additional module stacks, each with its own Power Controller. Through this interconnection, all stacks will communicate with the PCU, giving it control over all four associated vessels. *See Figure 8 on the following page for a sample installation.* 

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Figure 8: Interconnecting the Modules

To connect the first stack to another:

- 1. Use a long RS-485 communication cable. Connect it to either the topmost controller bus on the first stack (i.e., the upper connector on the PCU) or to the bottommost controller bus (i.e., the lower connector on the Power Controller). Save the terminator plug from the controller bus for reuse.
- 2. Connect the other end to either the topmost or the bottommost controller bus on the next stack.
- 3. Using short RS-485 cables, continue to daisy chain each module to the next in the second stack, as you did before. Be sure that a terminator plug is on each unused controller bus connector.

Repeat steps 1-3 above for stacks three and four, if appropriate.

## 

A terminator plug must be fastened on both unused Controller Bus connectors in the system for the communication links to work properly.

## 4.4 \*\*Important Warnings\*\*

Before you begin to assemble or operate your vessel, whether it is non-jacketed or waterjacketed, be sure to read this section, for it contains essential information, cautions and warnings to protect your safety and the safety of your equipment.

# WARNING!

## **NEVER PRESSURIZE A GLASS VESSEL!**

- Always use eye protection, and exercise caution in the vicinity of glass. If the vessel exhaust becomes blocked, pressure can build up, possible shattering the vessel and endangering personnel.
- As soon as you open the airflow valve(s), verify by feel that air is flowing freely from the exhaust. If not, <u>immediately</u> close the valve(s) or turn off the air/gas supplies.
- <u>Never</u> intentionally block the exhaust to raise vessel pressure.
- Use the minimum air/gas pressure that will provide adequate airflow for the application. <u>Never</u> exceed the maximum air pressure of 10 psi. This maximum pressure is necessary only to obtain the highest gas flow rates. It is capable of breaking the vessel if the vessel is not properly vented.

## 

To protect the integrity of your glass vessel and to avoid damage, familiarize yourself with these cautions:

- Never allow hot glass to touch cold water or a cold surface.
- Never rest the vessel on an uneven surface.
- Never drag or roll the vessel across any surface.
- Avoid metal-to-glass contact. With the exception of occasional contact with baffles inside a vessel used for fermentation, avoid touching the glass with any metal object.
- Use non-abrasive cleaners only, and clean with soft brushes (no sharp ends or bristles).
- Any surface that comes into contact with any portion of the vessel must be clean and non-abrasive.
- Only finger-tighten the knurled headplate bolts and port adapters. Overtightening puts undesirable pressure on the glass.
- Keep the glass free from contact with any diamond material (diamong jewelry, industrial diamonds or diamond dust from grinding wheels.

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Whenever you assemble or disassemble the vessel components, if you need to lay the drive assembly aside while it is still attached to the headplate and the agitation impeller shaft, note that there is a correct and an incorrect way to position the assembly on a flat surface.

The wrong way, which is resting the headplate and impeller shaft on a surface (*see Figure 9a below*) puts the impeller shaft at risk for damage:

HEADPLATE DRIVE ASSEMBLY IMPELLER SHAFT

Figure 9a: WRONG Handling of Drive Assembly

The correct way, which is resting the drive assembly and headplate on the surface (*see Figure 9b below*), protects the impeller shaft from bearing weight. Naturally, you will have to take care not to hit the shaft as you work around it





### 4.5 Vessel Assembly: Non-Jacketed

One of the most versatile features of the BioFlo 110 is the wide variety of glass vessels available. There are two types of vessels, non-jacketed—which are provided with the basic models—and water-jacketed. Each type of vessel is available in four sizes: 1.3 liters, 3.0 liters, 7.5 liters and 14.0 liters (total volume; *for more detail, see Specifications in Appendix A*). If you have a water-jacketed vessel, skip to Section 0.

Every single-walled, non-jacketed vessel comes with a stainless steel stand from which the vessel is suspended. The stand has four rubber feet to provide stability. An electric heat blanket provides temperature control for the contents of the vessel. The blanket has two large viewing windows so the culture remains visible for inspection. (*See Figure 10*)

Figure 10 below shows a typical installation of the non-jacketed vessel, in its vessel stand, with the most commonly used accessory equipment. To provide a full view of how the internal components are arranged, the heat blanket is not shown.





Familiarize yourself with the arrangement of the headplate ports, as shown in the following diagrams, before proceeding with the vessel assembly. You may find it more practical to change the arrangement; the variety of ports and adapters will easily accommodate your needs.

For easy reference, see Appendix E for a mix and match summary of port sizes and the types of adapters and inserts available.

## 4.5.1 Headplate











Figure 13: 7.5L & 14.0L Headplate

### 4.5.2

#### **Install Heat Blanket**

- 1. Wrap the heat blanket *as snugly as possible* around the vessel, taking care to leave one of the viewing windows facing forward. You will probably want to orient the blanket so the power cord connection is out of the way.
- 2. Secure the blanket by overlapping the Velcro strips, and pressing them together.

## 🥙 WARNING!

NEVER cut any portion of the heat blanket. NEVER fold the heat blanket or place any weight upon it. For storage, always lay the heat blanket flat.

### Install Vessel in Vessel Stand

4.5.3

- 1. Place the clamping ring on the vessel stand: align the clamping ring holes with the vessel stand pillars, then slide it into place. It will come solidly to rest on the shoulder of each pillar.
- 2. Place sections of U-shaped rubber bumper *equidistantly* around the inside of the clamping ring: there are three pieces for 1.3L & 3.0L vessels, and two larger pieces for 7.5L and 14.0L vessels. Press each section securely against the inner edge of the ring.

## Figure 14: Upper Vessel Bumper Installation



- **3**. Gently lower the glass vessel through the center of the clamping ring, until the vessel flange rests snugly against the rubber bumpers.
- 4. Orient the vessel so the gradations on the glass are clearly visible at the front, facing the user, and situated between two vessel stand pillars.

## 4.5.4 Install Baffle (14.0L Fermentation Vessels ONLY)

For installation of the 1.3L, 3.0L and 7.5L vessel baffle, see Section 4.5.21.

If you are using a 14.0L vessel, install the baffle assembly inside the glass vessel:

- 1. Gently compress the baffle ring at its ends (to avoid scratching the vessel walls). You may find it convenient to squeeze the tab with your thumb.
- 2. Slide the assembly inside, with the tab facing up, until it comes to rest at the bottom of the vessel.

3. Orient the baffle so the opening is opposite the gradations on the vessel, and the tab is aligned with the back vessel stand pillar.

## 4.5.5 Install Impeller(s)

Install the impeller(s) as follows:

A. **For Cell Culture:** Slide the impeller onto the agitation drive shaft (from the bearing housing). Position the impeller at least 10 mm above the sparger. Clamp it down in place.

## NOTE:

# It is normal for the agitation impeller shaft to be very resistant to turning by hand. The shaft seal resistance ensures sterile operation.

B. For Fermentation: Slide one impeller onto the agitation drive shaft (from the bearing housing). Position this lower impeller according to the table below. Clamp it down in place. Then install the second (upper) impeller in the same manner.

Distance from Bottom of Headplate to Top of Impeller Blade				
	1.3L	3.0L	7.5L	14.0L
Lower Impeller	4 1/8 in.	6 11/16 in.	8 7/8 in.	12 in.
	105 mm	170 mm	225 mm	305 mm
Upper Impeller	2 5/8 in.	4 in.	6½ in.	9¼ in.
	67 mm	102 mm	165 mm	235 mm

## NOTE:

The distances indicated above provide a recommenced starting point. As working volumes and agitation rates change, you may wish to adjust the impeller location(s).

## NOTE:

It is good practice to lightly lubricate all O-rings, port threads and adapter threads with silicone grease before you install equipment in the headplate. Also inspect the headplate O-ring to be sure it is securely seated in its groove.

## 4.5.6 Install Cooling Coil

#### 1.3L Vessel Cooling Coil/Sparger Assembly

The cooling coil and sparger connections are welded into one special 12mm triport assembly.

- 1. From beneath the headplate, insert the assembly into the appropriate ports (*see Figure 11*).
- 2. From above the headplate, lock the assembly in place with a knurled 12mm to 12mm adapter. Finger tighten.
- 3. There are three set screws in the adapter. If you need to raise or lower the adapter/tri-port assembly, use the Allen key provided to adjust the set screw that is easiest to access. You only need to adjust one.

### 3.0L, 7.5L & 14.0L Vessel Cooling Coil

- 1. From beneath the headplate, insert both ends of the coil into the Cooling Coil (In) port and the Cooling Coil (Out) port.
- 2. From above the headplate, finger tighten the knurled adapter on each side of the cooling coil.

## 4.5.7 Install Sparger (3.0L, 7.5L & 14.0L Vessels)

- 1. From *beneath the headplate*, insert the sparger tube into the sparger port (*see Figures 12 & 13 for reference*).
- 2. Finger tighten the knurled adapter on the sparger, then use the Allen key provided to tighten the set screw. Do not overtighten.

## CAUTION!

Finger tighten only any adapter that has a white Teflon ferrule (tapered, cone-shaped insert under the Teflon washer). The ferrule can deform under too much pressure.

### 4.5.8 Install Harvest Tube

- 1. Working from beneath the headplate, install the harvest tube in the harvest port (*see Figures 11, 12 & 13 for reference*). If you are using the 1.3L vessel, the harvest tube and sampler tube are welded into the same tri-port to save space. When the headplate is in place on the vessel, the bottom of the harvest tube should rest at the bottom of the vessel.
- 2. Finger tighten the knurled adapter on the harvest tube, then use the Allen key provided to tighten the set screw. Do not overtighten.

### 4.5.9 Install Sampler Tube

- 1. Working from beneath the headplate, install the optional sampler tube in the sample port (*see Figures 11, 12 & 13 for reference*). If you are using the 1.3L vessel, the sampler tube and harvest tube are welded into the same tri-port to save space.
- 2. Finger tighten the knurled adapter on the sampler tube, then use the Allen key provided to tighten the set screw.

### 4.5.10 Install Thermowell

1. Working from *above the headplate*, insert the thermowell tube into the RTD port (*see Figures 11, 12 & 13 for reference*).

## CAUTION!

Make sure that the thermowell does not touch the cooling coil.

2. Finger tighten the knurled adapter on the thermowell.

### 4.5.11 Install Foam Probe

If you are using a foam sensor with a foam trap kit:

- 1. Working from above the headplate, insert the foam sensor into the appropriate port (*see Figures 11, 12 & 13 for reference*).
- 2. Finger tighten the knurled adapter.
#### 4.5.12 Install Foam Exhaust Tube

If you are using a foam trap, install the foam exhaust tube:

- 1. Working from beneath the headplate, insert the foam exhaust tube into the appropriate port, close to a headplate clamping nut (*see Figures 11, 12 & 13 for reference*) where you will later mount the foam trap.
- 2. Finger tighten the knurled adapter. If you need to raise or lower the tube at any time, use the Allen key provided to adjust the adapter's set screw.

#### 4.5.13 Install Level Probe(s)

If you are using a level probe as part of the antifoam system and/or a level probe to detect media level, one at a time:

- 1. Working from above the headplate, insert the level probe into the appropriate port (*see Figures 11, 12 & 13 for reference*).
- 2. Finger tighten the knurled adapter.

#### 4.5.14 Install Addition Tube(s)

Insert addition tubes and/or tri-ports in the appropriate ports for any or all of the following additions: media, nutrients, acid, base, antifoam. For each insertion:

- 1. Finger tighten the knurled addition or tri-port adapter.
- 2. Working from above the headplate, insert the addition tube or tri-port into the appropriate port (*see Figures 11, 12 & 13 for reference*).

#### 4.5.15 Install pH Probe

#### NOTE:

Prior to installation, any pH probe you are using should be inspected for damage, and replaced if necessary.

#### NOTE:

To avoid damage to the probes during operation, be sure that there is no interference between the probes and the baffle assembly, impeller blades, or cooling coil.

2. Lightly coat the pH probe with glycerol.

# 

Do not install the pH port adaptor in the headplate before inserting the probe. Follow the steps below to fit the pH port adapter onto the probe first, then insert the probe and adapter into the headplate.





#### With reference to Figure 15 above:

- 3. Gently slide the top portion of the knurled port adapter (part of the probe kit) onto the probe.
- 4. Slide the two white ferrules onto the probe, the narrower one on top of the deeper, cup-shaped one.
- 5. Gently slide the bottom portion of the port adapter onto the probe, taking care to orient the longer threaded section toward the top of the probe.
- 6. Remove the two O-rings installed in the pH port; first slide the white Teflon O-ring onto the probe, then follow with the black 12mm port adapter O-ring.
- 7. Do not yet close up all the elements of the port adapter.
- 8. Gently insert the probe into the appropriate port (*see Figures 11, 12 & 13 for reference*), allowing the O-rings to seat fully into the port.

# NOTE:

The fit may be snug. Gently rotate the probe as you press it into the port to avoid breakage.

- 9. Finger tighten the bottom portion of the port adapter into the port.
- 10. Adjust the probe to the desired height; then, nesting the ferrules, close the top portion of the adapter onto the bottom portion.
- 11. Finger tighten the knurled adapter assembly.

#### 4.5.16 Install dO2 Probe

### NOTE:

Prior to installation, any dissolved oxygen probe you are using should be inspected for damage and replaced if necessary.

## 🖎 NOTE:

To avoid damage to the probes during operation, be sure that there is no interference between the probes and the baffle assembly, impeller blades or cooling coil.

- 1. Wear protective gloves to protect yourself in case of accidental breakage.
- 2. Lightly coat the dO2 probe with glycerol.



Do not install the dO2 port adaptor in the headplate before inserting the probe. Follow the steps below to fit the dO2 port adapter onto the probe first, then insert the probe and adapter into the headplate.





- 3. Gently slide the top portion of the knurled port adapter (part of the probe kit) onto the probe.
- 4. Slide the two white ferrules onto the probe, the narrower one on top of the deeper, cup-shaped one.
- 5. Gently slide the bottom portion of the port adapter onto the probe, taking care to orient the longer threaded section toward the top of the probe.
- 6. Remove the two O-rings installed in the dO2 port; first slide the white Teflon O-ring onto the probe, then follow with the black 12mm port adapter O-ring.
- 7. Do not yet close up all the elements of the port adapter.
- 8. Gently insert the probe into the appropriate port (*see Figures 11, 12 & 13 for reference*), allowing the O-rings to seat fully into the port.

# NOTE:

# The fit may be snug. Gently rotate the probe as you press it into the port to avoid breakage.

- 9. Finger tighten the bottom portion of the port adapter into the port.
- 10. Adjust the probe to the desired height; then, nesting the ferrules, close the top portion of the adapter onto the bottom portion.
- 11. Finger tighten the knurled adapter assembly.

### 4.5.17 Install Exhaust Condenser

# WARNING!

<u>Never</u> intentionally block the exhaust to raise vessel pressure.

If you are using the optional exhaust condenser:

- 1. Unscrew the spare/exhaust port plug from the headplate, saving it for reuse.
- 2. Place the 12mm exhaust condenser adapter into the port.
- 3. Place the exhaust condenser inlet (*see Figures 18a & 18b below*) into the port, and finger tighten the knurled adapter.
- 4. Tighten it with the Allen key provided, until it is secure.
- 5. Attach the exhaust filter (respecting the direction of flow if stamped on the filter) to the condenser outlet. Secure the filter with a plastic tie.
- 6. Connect silicone tubing to the inlet port of the exhaust condenser. Secure with a plastic tie.
- 7. Connect the other end of the tubing to the water inlet (see **NOTE** below and Figure 17 on the following page), split from the water solenoid valve on the side of the Power Controller. Secure with a plastic tie.

# NOTE:

The water solenoid valve's brass body has two hose barbs, identified as "1" and "2" by numbers stamped on the body, just above the barb. Hose barb 1 is the water inlet. Hose barb 2 is the water outlet. The valve body swivels to facilitate access.

8. Connect silicone tubing to the top outlet port of the exhaust condenser. Allow enough tubing to run to the water drain. Secure the tubing with a plastic tie.



Figure 17: Water Solenoid Valve

# 🖄 NOTE:

If the weight of the exhaust filter kinks the tubing, fasten a short length of stiffening material to the tubing, using rubber bands or tie wraps, to support the filter.





#### 4.5.18 Install Sampler

The optional BioFlo 110 sampler system is designed to aseptically remove batch samples from the vessel. The entire installation is easily autoclaved in place on the vessel. If you are using the sampler, install the kit as follows, using Figures 19a & 19b below for reference:

- 1. Remove a headplate clamping nut adjacent to the location of the sampler tube.
- 2. Mount the metal sampler bottle holder arm on the clamping screw, and secure it in place with the clamping nut.



Figure 19a: Sampler/Harvest System (1.3L Vessel)

Figure 19b: Sampler System (3.0L, 7.5L & 14.0L Vessels)



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- 3. Connect a length of silicone tubing to the sampler tube on the headplate. Secure it in place with a plastic tie.
- 4. Slip a thumb clamp onto the tubing.
- 5. Connect the other end of the tubing to the tall sampler inlet pipe. Secure it in place with a plastic tie.
- 6. Connect a short length of silicone tubing to the short sampler outlet pipe. Secure it in place with a plastic tie.
- 7. Connect the sterile syringe filter to the other end of the tubing, taking care to respect the direction of flow if stamped on the filter. Secure the tubing in place with a plastic tie.
- 8. Insert the tip of the sampler syringe as far as it will go into the open end of the filter. Although the syringe will lodge there and hang freely in place, you can add a plastic tie for security.
- 9. Close the plunger.
- 10. Remove the cap from one of the sample bottles and screw the bottle into the metal holder.
- 11. Position the entire assembly to your satisfaction, then finger tighten the clamping nut.

#### 4.5.19 Install Foam Trap

If you are using a foam trap kit (see Figure 20 below):

- 1. Unscrew the headplate clamping nut (or base clamping nut, if you prefer to mount the trap at the base of the vessel) closest to the foam exhaust tube.
- 2. Mount the foam trap bottle holder on the clamping screw, using the hole at the end of the holder's mounting arm.
- 3. Secure the holder in place with the clamping nut. Leave the nut loose enough to swivel the holder.
- 4. Firmly place the foam trap bottle (250 ml or 500 ml) in the holder.

- 5. With the bottle cap in place, as eptically install a sterile  $(0.2 \ \mu)$  filter on the shorter tube that penetrates the cap. Be sure to respect the proper flow direction if stamped on the filter.
- 6. Connect a length of silicone tubing to the longer tube in the other bottle cap penetration. Secure the tubing with a plastic tie, and clamp it off on the top.
- 7. Connect the tubing, securing it with a plastic tie, to the foam exhaust tube in the headplate.
- 8. After autoclaving, you will position the bottle holder where you want it, then finger tighten the clamping nut.





#### 4.5.20 Plug Unused Ports

Close off unused ports:

- 1. Install a blind plug (without a hole) in any headplate port that will not be used.
- 2. Install silicone tubing, secured with a plastic tie and clamped shut, on any access tube (i.e., harvest tube) that will not immediately be used.

# 🖎 NOTE:

It is good practice to lightly lubricate the underside of the headplate with silicone before installing it on the vessel.

#### 4.5.21 Install 1.3L, 3.0L or 7.5L Fermentation Vessel Baffle

- 1. Gently place the baffle, tab facing up, around all of the other instruments protruding from the headplate, including the cooling coil.
- 2. Position the tab between the two uprights of the cooling coil.

Hold the baffle in place with two fingers when you lift the headplate assembly.

#### 4.5.22 Install Headplate

- 1. Orient the cooling coil uprights toward the back, opposite the gradations marked on the vessel glass. If this is a 1.3L, 3.0L or 7.5L fermentation vessel, squeeze and hold the baffle in place (opening toward the back) with thumb and forefinger. You may find it convenient to squeeze the tab with your thumb.
- 2. Carefully lower the headplate, easing all of its attachments into the vessel without hitting the glass (or the baffle inside, if this is a 14.0L fermentation vessel).

## NOTE:

If you are using a baffle, after installing the headplate, insert any convenient length of wood, plastic or stainless steel (do not use any other kind of metal) through an unused port to push the baffle down as far as it will go.

The baffle is stainless steel; repeated installations may cause it to retain a compressed position. Expand it before you squeeze it for installation, so it will spring back against the vessel walls.

- 3. Align the headplate holes with the vessel stand pillars, then slide it down until it rests securely against the vessel flange.
- 4. Finger tighten each clamping nut a little at a time to secure the headplate on the vessel stand, working diagonally from one to another (rather than working around the circle) to apply equal pressure.

See important **NOTE** on following page.

# **CAUTION!** To avoid vessel stress cracks, especially during autoclaving, never overtighten vessel clamping nuts.

#### 4.6 Vessel Assembly: Water-Jacketed

One of the most versatile features of the BioFlo 110 is the wide variety of glass vessels available. There are two types of vessels, non-jacketed—which are provided with the basic models—and water-jacketed. Each type of vessel is available in four sizes: 1.3 liters, 3.0 liters, 7.5 liters and 14.0 liters (maximum working volume; *for more detail, see Specifications in Appendix A*). If you have a non-jacketed vessel, and have already completed Section 4.5 above, skip down to Section 4.7.

Water-jacketed vessels need no stand; the water jacket, which is part of the vessel, is flared and flat at the bottom to provide secure, stable support. At the bottom is a metal base plate, to provide additional security against breakage. In operation, the jacked vessel sits on the Jacket Water Heater. The jacket water heater is designed so that the vessel water inlet and outlet fit in a notch at the rear, and the vessel feet fit into the four holes at the perimeter of the heater plate.

Figure 21 on the following page shows a typical installation of the double-walled, waterjacketed vessel, with the most commonly used accessory equipment.



Figure 21: Water-Jacketed Vessel Assembly

Familiarize yourself with the arrangement of the headplate ports, as shown in the following diagrams, before proceeding with the vessel assembly. You may find it more practical to change the arrangement; the variety of ports and adapters will easily accommodate your needs.

For easy reference, see Appendix E for a mix and match summary of port sizes and the types of adapters and inserts available.

4.6.1 Headplate



#### Figure 22: 1.3L Headplate





Figure 24: 7.5L & 14.0L Headplate

#### 4.6.2 Install Headplate Clamping Ring

The clamping ring that secures the headplate to the vessel is split in half to facilitate installation under the vessel flange. They are joined with two rectangular mounting plates.

1. As shown in Figure 25 below, install one mounting plate with two Phillips head screws (provided) on the end of one ring half so that the plate extends beyond the ring.

#### Figure 25: Installing Headplate Clamping Ring



- 2. In the same manner, install the second mounting plate on the other end of the ring half.
- 3. Bring the two halves of the headplate clamping ring together under the vessel flange, with the mounting plates on the bottom for easy access from below.
- 4. Align the mounting plates with their corresponding holes on the other ring half, and drop in the remaining Phillips head screws. Tighten the screws to fasten the ring in place.

#### 4.6.3 Install Vessel on Base Plate

- 1. Place the base plate on a level surface.
- 2. Lightly lubricate the base plate O-ring, and seat it securely in its groove.
- 3. Fit the one-piece water jacket guard (rubber gasket) around the outside of the bottom vessel flange, against the water jacket (*see Figure 26 on the following page*).
- 4. With the clamping screws in place on the ring, fit the bottom clamping ring onto the base plate.



Figure 26: Water Jacket Guard Installation (top view)

- 5. With the gradations marked on the glass facing front (toward the user), slide the vessel into the bottom clamping ring, until it rests securely against the base plate. Make sure the water inlet tube stands free (not kinked) inside the water jacket.
- 6. Finger tighten the six knurled thumb screws, to securely attach the clamping ring to the base plate. This seals the water jacket.

#### 4.6.4 Install Baffle (14.0L Fermentation Vessels ONLY)

For installation of the 1.3L, 3.0L & 7.5L vessel baffle, see Section 4.6.21.

If you are using a 14.0L vessel, install the baffle assembly inside the glass vessel:

- 1. Gently compress the baffle ring at its ends (to avoid scratching the vessel walls). You may find it convenient to squeeze the tab with your thumb.
- 2. Slide the assembly inside, with the tab facing up, until it comes to rest at the bottom of the vessel.
- 3. Orient the baffle so the opening is opposite the gradations on the vessel.

#### 4.6.5 Install Impeller(s)

Install the impeller(s) as follows:

A. **For Cell Culture:** Slide the impeller onto the agitation drive shaft (from the bearing housing). Position the impeller at least 10 mm above the sparger. Clamp it down in place.

### NOTE:

# It is normal for the agitation impeller shaft to be very resistant to turning by hand. This resistance ensures sterile operation.

B. For Fermentation: Slide one impeller onto the agitation drive shaft (from the bearing housing). Position this lower impeller *according to the table below*. Clamp it down in place. Then install the second (upper) impeller in the same manner.

Distance from Bottom of Headplate to Top of Impeller Blade						
	1.3L	3.0L	7.5L	14.0L		
Lower Impeller	4 1/8 in.	6 11/16 in.	8 7/8 in.	12 in.		
	105 mm	170 mm	225 mm	305 mm		
Upper Impeller	2 5/8 in.	4 in.	6½ in.	9¼ in.		
	67 mm	102 mm	165 mm	235 mm		

# NOTE:

The distances indicated above provide a recommenced starting point. As working volumes and agitation rates change, you may wish to adjust the impeller location(s).

# NOTE:

It is good practice to lightly lubricate all O-rings, port threads and adapter threads with silicone grease before you install equipment in the headplate. Also inspect the headplate O-ring to be sure it is securely seated in its groove.

#### 4.6.6 Install Sparger

- 1. From *beneath the headplate*, insert the sparger tube into the sparger port (*see Figures 22, 23 & 24 for reference*).
- 2. Finger tighten the knurled adapter on the sparger, then use the Allen key provided to tighten the set screw. Do not overtighten.

# CAUTION!

Finger tighten only any adapter that has a white Teflon ferrule (tapered, cone-shaped insert under the Teflon washer). The ferrule can deform under too much pressure.

#### 4.6.7 Install Harvest Tube

- 1. Working from beneath the headplate, install the harvest tube in the harvest port (*see Figures 22, 23 & 24 for reference*). If you are using the 1.3L vessel, the harvest tube and sampler tube are welded into the same tri-port to save space. When the headplate is in place on the vessel, the bottom of the harvest tube should rest at the bottom of the vessel.
- 2. Finger tighten the knurled adapter on the harvest tube, then use the Allen key provided to tighten the set screw. Do not overtighten.

#### 4.6.8 Install Sampler Tube

- 1. Working from beneath the headplate, install the optional sampler tube in the sample port (*see Figures 22, 23 & 24 for reference*). If you are using the 1.3L vessel, the sampler tube and harvest tube are welded into the same tri-port to save space.
- 2. Finger tighten the knurled adapter on the sampler tube, then use the Allen key provided to tighten the set screw.

#### 4.6.9 Install Thermowell

1. Working from *above the headplate*, insert the thermowell tube into the RTD port (*see Figures 22, 23 & 24 for reference*).

# *CAUTION!* Make sure that the thermowell does not touch the cooling coil.

2. Finger tighten the knurled adapter on the thermowell.

#### 4.6.10 Install Foam Probe

If you are using a foam sensor with a foam trap kit:

- 1. Working from above the headplate, insert the foam sensor into the appropriate port (*see Figures 22, 23 & 24 for reference*).
- 2. Finger tighten the knurled adapter.

#### 4.6.11 Install Foam Exhaust Tube

If you are using a foam trap, install the foam exhaust tube:

1. Working from beneath the headplate, insert the foam exhaust tube into the appropriate port, close to a headplate clamping nut (*see Figures 22, 23 & 24 for reference*) where you will later mount the foam trap.

Finger tighten the knurled adapter. If you need to raise or lower the tube at any time, use the Allen key provided to adjust the adapter's set screw.

#### 4.6.12 Install Level Probe(s).

If you are using a level probe as part of the antifoam system and/or a level probe to detect media level, one at a time:

- 1. Working from above the headplate, insert the level probe into the appropriate port (*see Figures 22, 23 & 24 for reference*).
- 2. Finger tighten the knurled adapter.

#### 4.6.13 Install Addition Tube(s)

Insert addition tubes and/or tri-ports in the appropriate ports for any or all of the following additions: media, nutrients, acid, base, antifoam. For each insertion:

- 1. Working from above the headplate, insert the addition tube or tri-port into the appropriate port (*see Figures 20, 21 & 22 for reference*).
- 2. Finger tighten the knurled addition or tri-port adapter.

#### 4.6.14 Install pH Probe

#### NOTE:

Prior to installation, any pH probe you are using should be inspected for damage and replaced if necessary.

#### NOTE:

To avoid damage to the probes during operation, be sure that there is no interference between the probes and the baffle assembly or impeller blades.

- 1. Wear protective gloves to protect yourself in case of accidental breakage.
- 2. Lightly coat the pH probe with glycerol.

# 

Do not install the pH port adaptor in the headplate before inserting the probe. Follow the steps below to fit the pH port adapter onto the probe first, then insert the probe and adapter into the headplate.





#### With reference to Figure 27 above:

- 3. Gently slide the top portion of the knurled port adapter (part of the probe kit) onto the probe
- 4. Slide the two white ferrules onto the probe, the narrower one on top of the deeper, cup-shaped one.
- 5. Gently slide the bottom portion of the port adapter onto the probe, taking care to orient the longer threaded section toward the top of the probe.
- 6. Remove the two O-rings installed in the pH port; first slide the white Teflon O-ring onto the probe, then follow with the black 12mm port adapter O-ring.
- 7. Do not yet close up all the elements of the port adapter.
- 8. Gently insert the probe into the appropriate port (*see Figures 22, 23 & 24 for reference*), allowing the O-rings to seat fully into the port.

## 🖎 NOTE:

# The fit may be snug. Gently rotate the probe as you press it into the port to avoid breakage.

9. Finger tighten the bottom portion of the port adapter into the port.

- 10. Adjust the probe to the desired height; then, nesting the ferrules, close the top portion of the adapter onto the bottom portion
- 11. Finger tighten the knurled adapter assembly.

#### 4.6.15 Install Dissolved Oxygen Probe

#### NOTE:

Prior to installation, any dissolved oxygen (dO2) probe you are using should be inspected for damage and replaced if necessary.

#### 🖎 NOTE:

To avoid damage to the probes during operation, be sure that there is no interference between the probes and the baffle assembly or impeller blades.

- 1. Wear protective gloves to protect yourself in case of accidental breakage.
- 2. Lightly coat the dO2 probe with glycerol.



Do not install the dO2 port adaptor in the headplate before inserting the probe. Follow the steps below to fit the dO2 port adapter onto the probe first, then insert the probe and adapter into the headplate.





#### With reference to Figure 28 above:

- 3. Gently slide the top portion of the knurled port adapter (part of the probe kit) onto the probe.
- 4. Slide the two white ferrules onto the probe, the narrower one on top of the deeper, cup-shaped one.
- 5. Gently slide the bottom portion of the port adapter onto the probe, taking care to orient the longer threaded section toward the top of the probe.
- 6. Remove the two O-rings installed in the dO2 port; first slide the white Teflon O-ring onto the probe, then follow with the black 12mm port adapter O-ring.
- 7. Do not yet close up all the elements of the port adapter.
- 8. Gently insert the probe into the appropriate port (*see Figures Figures 22, 23 & 24 for reference*), allowing the O-rings to seat fully into the port. If you are using an Ingold probe in a 1.3L, 3.0L or 7.5L vessel, be sure to use the seal washer provided.

### NOTE:

# The fit may be snug. Gently rotate the probe as you press it into the port to avoid breakage.

- 9. Finger tighten the bottom portion of the port adapter into the port.
- 10. Adjust the probe to the desired height; then, nesting the ferrules, close the top portion of the adapter onto the bottom portion.
- 11. Finger tighten the knurled adapter assembly.

#### 4.6.16 Install Exhaust Condenser

#### NARNING!

<u>Never</u> intentionally block the exhaust to raise vessel pressure.

If you are using the optional exhaust condenser:

- 1. Unscrew the spare/exhaust port plug from the headplate, saving it for reuse.
- 2. Place the 12mm exhaust condenser adapter into the port.
- 3. Place the exhaust condenser inlet (*see Figures 30a & 30b below*) into the port, and finger tighten the knurled adapter.
- 4. Tighten it with the Allen key provided, until it is secure.

- 5. Attach the exhaust filter (respecting the direction of flow if stamped on the filter) to the condenser outlet. Secure the filter with a plastic tie.
- 6. *With reference to Figures 30a and 30b*, connect a length of silicone tubing between the lower (cooling water inlet) port of the exhaust condenser and the T-connector (upstream of the water solenoid valve). Secure at both ends with a plastic tie.
- 7. Connect the T-connector inlet (*see Figure 30b*) to hose barb 2 (see **NOTE** and Figure 29 below) on the water solenoid valve. The valve is on the side of the Power Controller. Secure with a plastic tie.

## NOTE:

The water solenoid valve's brass body has two hose barbs, identified as "1" and "2" by numbers stamped on the body, just above the barb. Hose barb 1 is the water inlet. Hose barb 2 is the water outlet. The valve body swivels to facilitate access.

8. Connect silicone tubing to the top (cooling water outlet) port of the exhaust condenser. Allow enough tubing to run to the water drain. Secure the tubing with a plastic tie.



#### Figure 29: Water Solenoid Valve



Figure 30a: Exhaust Condenser (1.3L, 3.0L & 7.5L Vessels)

# NOTE:

If the weight of the exhaust filter kinks the tubing, fasten a short length of stiffening material to the tubing, using rubber bands or tie wraps, to support the filter.

Figure 30b: Exhaust Condenser (14.0L Vessel Only)



#### 4.6.17 Install Sampler

The optional BioFlo 110 sampler system is designed to aseptically remove batch samples from the vessel. The entire installation is easily autoclaved in place on the vessel. If you are using the sampler, install the kit as follows, using Figures 31a and 31b for reference:

- 1. Remove a headplate clamping nut adjacent to the location of the sampler tube.
- 2. Mount the metal sampler bottle holder on the clamping screw, and secure it in place with the clamping nut.



Figure 31a: Sampler/Harvest System (1.3L Vessel)



Figure 31b: Sampler System (3.0L, 7.5L & 14.0L Vessels)

- 3. Connect a length of silicone tubing to the sampler tube outlet on the headplate. Secure it in place with a plastic tie.
- 4. Slip a thumb clamp onto the tubing.
- 5. Connect the other end of the tubing to the tall sampler inlet pipe. Secure it in place with a plastic tie.
- 6. Connect a short length of silicone tubing to the short sampler outlet pipe. Secure it in place with a plastic tie.
- 7. Connect the sterile syringe filter to the other end of the tubing, taking care to respect the direction of flow if stamped on the filter. Secure the tubing in place with a plastic tie.
- 8. Insert the tip of the sampler syringe as far as it will go into the open end of the filter. Although the syringe will lodge there and hang freely in place, you can add a plastic tie for security. Close the plunger.
- 9. Remove the cap from one of the sample bottles and screw the bottle into the metal holder.
- 10. Position the entire assembly to your satisfaction, then finger tighten the clamping nut.

#### 4.6.18 Install Foam Trap

If you are using a foam trap kit (see Figure 32 below):

- 1. Unscrew the headplate clamping nut (or base clamping nut, if you prefer to mount the trap at the base of the vessel) closest to the foam exhaust tube.
- 2. Place a washer on the clamping screw, then mount the foam trap bottle holder on the screw, using the hole at the end of the holder's mounting arm.
- 3. Secure the holder in place with the clamping nut. Leave the nut loose enough to swivel the holder.
- 4. Firmly place the foam trap bottle (250 ml or 500 ml) in the holder.
- 5. With the bottle cap in place, as eptically install a sterile  $(0.2 \ \mu)$  filter on the shorter tube that penetrates the cap. Be sure to respect the proper flow direction if stamped on the filter.



Figure 32: Foam Trap

6. Connect a length of silicone tubing to the longer tube in the other bottle cap penetration. Secure the tubing with a plastic tie, and clamp it off on the top.

- 7. Connect the tubing, securing it with a plastic tie, to the foam exhaust tube in the headplate.
- 8. After autoclaving, you will position the bottle holder where you want it, then finger tighten the clamping nut.

#### 4.6.19 Install Antifoam System

If you are using a level probe to activate the addition of chemical defoamer, install the antifoam addition bottle following the procedure for installing a foam trap, with the following differences:

- Install the bottle holder close to the antifoam addition tube in the headplate.
- Connect the longer tube that penetrates the bottle cap to the antifoam addition tube in the headplate.

#### 4.6.20 Plug Unused Ports

Close off all unused ports:

- 1. Install a blind plug (without a hole) in any headplate port that will not be used.
- 2. Install silicone tubing, secured with a plastic tie and clamped shut, on any access tube (i.e., harvest tube) that will not immediately be used.

## NOTE:

It is good practice to lightly lubricate the headplate O-ring with silicone before installing it on the vessel.

#### 4.6.21 Install 1.3L, 3.0L or 7.5L Fermentation Vessel Baffle

- 1. Gently place the baffle, tab facing up, around all of the other instruments protruding from the headplate.
- 2. Position the tab and baffle opening toward the back of the headplate.

# CAUTION!

Hold the baffle in place with two fingers when you lift the headplate assembly.

#### 4.6.22 Install Headplate

- 1. If this is a 1.3L, 3.0L or 7.5L fermentation vessel, be sure to squeeze and hold the baffle in place (opening toward the back) with thumb and forefinger. You may find it convenient to squeeze the tab with your thumb.
- 2. Carefully lower the headplate, easing all of its attachments into the vessel without hitting the glass walls (or the baffle inside if this is a 14.0L fermentation vessel).

# NOTE:

If you are using a baffle, after installing the headplate, as indicated below, insert any convenient length of wood, plastic or stainless steel (do not use any other kind of metal) through open ports to push the baffle down as far as it will go.

The baffle is stainless steel; repeated installations may cause it to retain a compressed position. Expand it before you squeeze it for installation, so it will spring back against the vessel walls.

- 3. Align the headplate holes with clamping ring holes, then slide it down until it rests securely against the vessel flange.
- 4. Finger tighten each clamping screw a little at a time to secure the headplate, working diagonally from one to another (rather than working around the circle) to apply equal pressure.

# CAUTION!

To avoid vessel stress cracks, especially during autoclaving, never overtighten vessel clamping screws.

#### 4.7

#### Install the Agitation Motor

- 1. Make sure the motor coupling O-ring is lubricated.
- 2. Position the motor sleeve on the bearing housing (*see Figure 33*), taking particular care to align the side slots with the shoulder studs as you ease the sleeve down until it rests securely on the bearing housing. You may have to walk it down by rotating it slightly left and right.



# WARNING!

<u>Never</u> remove or install the motor while it is running.

# NOTE:

Periodically check the condition of the plastic cushioning (#P0740-3111) as you remove or reinstall the motor. Replace it when it begins to wear.

## 4.8 Install the Rotameter

# CAUTION!

Verify that the source of air is regulated at 10 PSI MAXIMUM.

- 1. Hang the rotameter on the left or right side of the Power Controller or PCU, whichever is most convenient. Using a Phillips head (+) screwdriver, mount the meter bracket using the screws provided.
- 2. Use silicone tubing to connect the air source to the bottom air inlet hose barb on the rotameter (*see Figure 34*).



- 3. Attach another piece of silicone tubing to the top air outlet hose barb (*see Figure 34 above*). Insert the other end of this tubing into the inlet sterile filter on the top of the vessel headplate.
- 4. Verify that the exhaust port is connected to a filter. If you are using the optional exhaust condenser, the filter should be attached to its outlet.
- 5. Read the airflow at the top flat edge of the float.

To increase airflow, turn the adjusting knob counterclockwise. To decrease airflow, turn it clockwise.

Rotameter Maximum Flow Rate					
Application	Vessel Size				
	1L	3.0L	7.5L	14.0L	
Fermentation	2.5 Lpm	5 Lpm	11.4 Lpm	20 Lpm	
Cell Culture	0.5 Lpm	1.2 Lpm	2.5 Lpm	5 Lpm	

# NOTE:

Adjust the airlow according to your specific application. A commonly suggested airflow for a typical culture is 1 VVM (vessel volume per minute) or less for fermentation, and 0.05 VVM or less for cell culture; unnecessarily high airflow can induce foaming.

#### 4.9 Install Liquid Addition Systems

Addition ports are used to feed base, acid, nutrients and media into the vessel. To install an addition system:

- 1. Install a sterile  $(0.2 \ \mu)$  filter on one of the two penetrations on the addition bottle cap. Be sure to respect the proper flow direction if stamped on the filter.
- 2. Connect a length of silicone tubing, securing it with a plastic tie, to the addition tube in the other penetration of the bottle cap. Clamp it off on the top.
- 3. After autoclaving, thread the tubing through the desired peristaltic feed pump.
- 4. Aseptically connect the tubing, securing it with a plastic tie, to the appropriate addition port on the headplate.
- 5. Remove the clamp.

#### 4.10 Making Connections

#### 4.10.1 Power

Confirm that the voltage selection of your BioFlo 110 matches the voltage rating of your electrical service by reading the voltage selection in the window of the fuse drawer (*see Figure 45*). Also confirm that the power requirements of all control modules (marked on each module's serial number sticker) are met by your electrical service.

Plug in the Power Controller: connect power cord P0720-2053 to the Power Controller's power inlet. Do not plug into your electrical service until you are ready to run the unit.

Plug in the Primary Control Unit (PCU):

- 1. **To support a single vessel assembly**, connect power cable P0720-2054 to the Power Controller's rear panel power outlet, labeled "PCU", and plug the other end into the PCU's rear panel power inlet.
- 2. To support multiple vessel assemblies (with multiple Power Controllers), either:
  - (a) follow step 1 above, choosing the most convenient Power Controller, or
  - (b) use the supplied power cord to plug the PCU directly into your electrical supply, thus making it independent of the Power Controllers.

#### 4.10.2 Water

The BioFlo 110 water solenoid valve is located on the side of the Power Controller. Water pressure should be 10 PSIG maximum, with 50  $\mu$ m filtration.

Be sure to secure all tubing connections for both the water and the drain lines with the fiberglass-reinforced hose clamps provided. Attach the <sup>1</sup>/<sub>4</sub> inch ID braided PVC tubing provided (see **NOTE** below) to the water solenoid valve outlet (look for a **2** stamped in the brass valve body, just above the hose barb—*see Figure 17 or 29*), and to the vessel cooling coil inlet (for non-jacketed vessels) or the vessel water jacket inlet.

## 🖄 NOTE:

There is a distinct, visible difference between this PVC tubing and the silicone tubing used elsewhere; be careful not to confuse them. The PVC tubing is clear, with white cross-hatched reinforcement braiding. Silicone tubing is milky.

# PVC tubing is NOT autoclavable and is not for use with hot liquids; it will melt.

- 1. Attach sufficient PVC tubing to the cooling coil outlet (for non-jacketed vessels) or the vessel water jacket outlet to reach the drain.
- 2. Connect to the water supply:
  - If you are using the optional exhaust condenser, connect tubing from the valve splitter outlet (marked 2 on the brass valve body) to the exhaust condenser water inlet. Connect pressure-regulated clean water to the water splitter inlet (marked 1 on the brass valve body).
  - If you are not using the exhaust condenser, connect pressure-regulated clean water directly to the inlet of the water solenoid valve (marked 1 on the brass valve body).
- 3. If you are using the optional Water Filter/Regulator kit (*see Section 10.3*), install it according to the kit instructions.

#### 4.10.3 Probes and Sensors

#### **Power Controller**

Familiarize yourself with the connections and controls on the front panel of the Power Controller, as illustrated in Figure 35 below:

- **POWER SWITCH** On/Off rocker switch.
- **OPERATING LIGHT** Lights when the Power Controller is on.
- AGITATION Connection for agitation motor.

- **TEMPERATURE** Connection for RTD temperature probe, to sense temperature inside the vessel.
- **HEATER CONNECTOR** Connection for the heat blanket (non-jacketed vessels) or for the jacket water heater (water-jacketed vessels).



Figure 35: Power Controller Front Panel

Note the following features and connections on the side of the Power Controller:

•	ROTAMETER	Can be mounted on either side. Manually adjusted rotameter regulates the flow of air/gases into the vessel.
•	WATER INLET & OUTLET	Via the water solenoid valve, located on the side panel, which provides the connection between the water supply and the vessel water inlet (either cooling coil or water jacket). Inlet is marked <b>1</b> and outlet <b>2</b> .

• SPARGE AIR INLET Via the rotameter mounted on the side panel or the Thermal Mass Flow Controller, either of which provides the connection between the air/gas supply and the sparger.

Attach all cables to their appropriate probes and sensors, then attach the cables to the appropriate module (not necessarily in this order):

Into the front panel of the Power Controller:

- Plug the RTD (temperature probe) cable where it is marked "Temperature".
- Plug the agitation motor cable where it is marked "Agitation".

#### WARNING! Agitation and Heater connection sites are high voltage. Never insert anything but the appropriate cable plug.

You will also plug into the front panel, when you are ready to begin operation:

- For non-jacketed vessels: the heat blanket cable where it is marked "Heater".
- For water-jacketed vessels: the jacket water heater cable where it is marked "Heater", **but only after** the jacket is filled with water, the vessel is filled with media, and the vessel is properly installed on the heater.

#### dO2/pH Controller

If you are using the BioFlo 110 dO2/pH Controller, familiarize yourself with the connections and controls on the front panel, as illustrated in Figure 36 below:



#### Figure 36: dO2/pH Controller – Front Panel
Plug the following into the front panel of the dO2/pH Controller:

- The dissolved oxygen probe cable ("dO2").
- The pH probe cable (right connector marked "pH"), and the pH probe ground (left connector marked "pH"). Attach the ground clip to the top of a headplate screw.

#### Level Controller

If you are using the BioFlo 110 Level Controller, familiarize yourself with the connections and controls on the front panel, as illustrated in Figure 37 below:

Figure 37: Level Controller – Front Panel



Plug the following into the front panel of the Level Controller:

- Foam probe (left connector marked "Foam"). When this probe gets wet, its associated pump turns *on*.
- Wet On level sensor (middle connector marked "Wet On"). When this probe gets wet, its associated pump turns *on*.
- Wet Off level sensor (right connector marked "Wet Off"). When this probe gets wet, its associated pump turns *off*.

#### 4.10.4 Pumps

If you are using the BioFlo 110 4-Pump Module, familiarize yourself with these features (*see Figure 38 below*):

- **ON/OFF SWITCH** An individual power switch for each pump is located on the front panel.
- **PUMP DOOR** Behind each hinged door is access to a peristaltic pump.



Figure 38: 4-Pump Module

Place the pump module adjacent to the vessel and its Power Controller. Install the tubing according to the instructions in the pump manual. You may want to consult the Pump Flow Rates listed in Section 11.2.

The Power Controller also provides power to the peristaltic pumps. Plug the pumps into the back panel of the Power Controller (*see Figure 2*):

- 1. The pumps used for pH control have dedicated sockets labeled "Acid" and "Base".
- 2. Other pumps can be plugged into the sockets labeled "Pump A", "Pump B" and "Pump C".

# CAUTION!

Plug the pump cables ONLY into power controller sockets marked "Pump". DO NOT use the socket marked "PCU".

For further details, see Section 7.12, Setting the Pumps.

#### 4.10.5 Air and Optional Gases

All gases should be medical grade. Gas pressure should not exceed 10 PSIG. Be sure to secure all tubing connections with plastic ties.

If you are using the optional Air Filter/Regulator kit (*see Section 10.3*), install it according to the kit instructions.

- 1. Connect pressure-regulated clean air to the inlet port of the rotameter using 5/16-inch (7.9 mm) OD silicone tubing, NBS #P0740-2505.
- 2. Connect the outlet of the rotameter to a sterile filter, taking care to observe the flow direction requirements if stamped on the filter.
- 3. Connect the filter outlet to the sparger inlet.

#### With the Gas Mix Controller:

Familiarize yourself with the front panel of the controller and its features:

 GAS INLETS Four push-in inlets for delivery of Oxygen, Carbon Dioxide, Air and Nitrogen from your source.
GAS STREAM OUTLET One push-in outlet for sequential output of various individual gases, as controlled by the Gas Mix Controller, and for delivery to the rotameter or Thermal Mass Flow Controller (TMFC) inlet. The rotameter or TMFC outlet delivers

air/gases to the sparger via the vessel headplate.



- 1. Attach pressure-regulated clean air and other gases to the push-in inlets of the 4-Gas controller using the blue polyurethane tubing supplied (NBS #P0470-3111, 0.125 inch ID/0.250 inch OD).
- 2. Connect the gas stream outlet port of the 4-Gas controller to the inlet of the rotameter.
- 3. Connect the outlet of the rotameter to a sterile filter, taking care to observe the flow direction requirements if stamped on the filter.
- 4. Connect the filter outlet to the sparger inlet.

#### 4.11 Install Cable Guides

Bolt-on cable guide clamps are provided to dress probe and sensor cables from the front of their control modules toward the back of the stack, where they naturally hang from the top of the vessel. Their use is optional. You may prefer to tuck the cables under their module, directing them toward the back of the stack, or to simply allow them to hang.

The cable guides can be mounted on either side of each module, wherever they will best suit your needs. They can also be mounted with the top jaw facing up or down. The two jaws of the guide snap together, so the tip of the top jaw zips along the tracks on the bottom jaw to expand or contract the cable enclosure. The guide functions rather like a plastic tie, with the difference that it can be opened and closed repeatedly. As shown in the drawing below, to open a clamp:

- 1. Place your thumb and forefinger on either side of the top jaw, at the boss.
- 2. Grip tightly and pull up.

#### NOTE:

Because the guide clamps down tightly, you may have to encourage unclamping with the tip of a screwdriver under the leading edge. Do not use undue force, however, to avoid damage to the plastic clamp or, if already present, to the cable inside.



#### 4.12 Voltage Selection and Fusing

## 🖎 NOTE:

If you move your BioFlo 110 to a new location that requires a change in voltage and/or fusing, consult your NBS representative.

It is possible to change the voltage on your BioFlo 110. It is also possible to change the fusing arrangement from a single fuse to a double fuse. These procedures, however, should only be performed by a qualified technician, referring to Appendix B in this manual.

#### 4.13 Calibrating the pH Probe

If you are using a pH probe, be sure to calibrate it before autoclaving the vessel assembly:

- 1. Check that the Power Controller and PCU are plugged in and powered on.
- 2. Make sure that the pH electrode is plugged into the pH connector on the dO2/pH Controller front panel, using the appropriate cable. Also check that the pH ground wire is plugged into the adjacent ground connector, and the ground clip is grounded.
- 3. When the PCU is powered on, the display says: New Brunswick Scientific BioFlo 110 Scanning Hardware .....
- 4. When the system detects the hardware, the display shows the following screen:



#### SELECTOR BUTTONS

The default language setting is *English*. If you prefer to use another screen language, press the selector button next to your preferred language.

Section 5, *Before First Use*, and Section 7, *Operation*, explain the Application (*Use*) settings and the choices offered by the *More*... selection. They also explain how to use all other screens and available settings.

Loop	Name	V V	alue	Loop Setpoint	Loop Control Mode
B	Name	Value	Setpoint	Control	
0	Temp	23.4	30.0 °C	Auto	
F	Agit	134	350 rpm	Auto	
0	Pump A	0.0	0.0 %	Manual	
1	Pump B	0.0	0.0 %	Manual	
1	Pump C	0.0	0.0 %	Manual	
0	рН	6.68	7.00 pH	Auto	
	dO2	77.1	0.0 %	Auto	

5. Press the  $\Box \Rightarrow$  button to open the Main Screen (see sample screen below):

The above screen is only one sample. The loop names will reflect the specific modules in your system.

Press the selector button in the pH row. The pH Loop Details screen opens:



6. In the pH Loop Details screen, press the *Calibrate* selector button. The Calibrate pH screen opens:



- 7. Rinse the pH electrode with distilled water, then immerse it in a pH 7.00 buffer solution. Allow a few minutes for the system to equilibrate, as indicated by a nearly stable Raw Input reading.
- 8. Press the Set Zero selector button. A highlighted edit box opens:

B i	Calibrate pH 6.52	
0 F	Zero Std: 7.00	Set Zero
 0		Set Span
1	Raw Input: 5500	Reset
1 0	Select "Reset" at any time in the Calibration process to return to the factory-set default Zero Standard and Span settings.	

- 9. Using the touchpad, key in **7.0**, the pH of the buffer.
- 10. Reconfirm that the Raw Input reading is stable, then press the *Enter* key to save the value (or press  $\times$  to cancel).
- 11. Press the Set Span selector button. As above, a highlighted edit box will open in the Set Span row.
- 12. Rinse the electrode with distilled water, then immerse it in a second pH buffer solution which is several pH units below pH 7.00 (e.g., 4.00) and allow a few minutes for the system to equilibrate, as indicated by a nearly stable Raw Input.

- 13. Using the touchpad, enter the new Span value, which is the buffer pH.
- 14. Reconfirm that the Raw Input is stable, then press the *Enter* key to save the value (or press ★ to cancel).
- 15. To ensure accuracy, repeat the above steps, using the same two buffer solutions.
- 16. After the values have been set, press the  $\Box \Rightarrow$  button to return to the Main Screen.

# CAUTION!

Do not use hydrochloric acid (HCI) with the BioFlo 110 for pH control or any other purpose, because HCI corrodes stainless steel. Over time, HCI will damage the stainless steel components.

Phosphoric and sulfuric (10% maximum concentration) acids are acceptable and are commonly used for pH control.

# 5 BEFORE FIRST USE

This section provides critical pre-operation instructions.

The BioFlo PCU display screens are available in English, French, German and Spanish. Before you operate your BioFlo 110 for the first time, be sure to select the preferred language for your PCU display screens.

Be sure also to configure the PCU and all other control modules so they can communicate with each other and, if desired, with an outside computer.

#### 5.1 Select PCU Display Language



When the PCU is powered on, the display shows the following screen:

SELECTOR BUTTONS

The default language setting is *English*. If you prefer to use another screen language, this is your opportunity to change the setting, if you have not already done so:

1. Press the selector button next to your preferred language.

#### 5.2 Assign Vessel Address

Assign one address to all control modules associated with a single vessel. As the PCU can control as many as four vessels, it is essential that each vessel's control module stack have one unique address.

The address is set using the unit address selector switches on the back panel of each module. The module is shipped with all four switches On (facing down). As you can see in the reference table below, which is also posted on the back panel of each module next to the unit address switches), this is the address for Unit 1 (the first vessel and its associated control modules).



Make sure that each module is set to the same address for Unit 1. A unit address switch can be flipped up or down with a pencilpoint, or the tip of a screwdriver.

If you have a second stack with a second vessel, set those modules for the Unit 2 address. Do the same for Units 3 and 4, if present.

#### 5.3 For PC Supervision

#### 5.3.1 Connect to Supervisory System

Connect the AFS/ModBus 25-pin connector on the PCU, using the appropriate cable, to the PC's serial port. Be sure to consult the user's manual that accompanies the supervisory software program and any interface box or converter you may need to complete the communications link.

The AFS/ModBus connector pin-outs are assigned as follows for RS-232 or RS-422 input and output:

Pin #		Signal
2	RS-232	Transmit Data
3	RS-232	Receive Data
7	RS-232/ RS-422	Ground
21	RS-422	Enable (Jumper to Pin #7 to enable RS-422)
13	RS-422	Transmit Data +
25	RS-422	Transmit Data -
24	RS-422	Receive Data +
12	RS-422	Receive Data -

#### 5.3.2 Select Communications Protocol

If a computer will be used for automatic data logging or process supervision, select an appropriate digital communications protocol, either ModBus or, if you are using NBS *BioCommand* supervisory software, AFS.

1. Press the *More*... selector button from the Application/Language screen, or press the □ ℝ key (once from the Main Screen, or twice from the opening Language Selection Screen) to open the System Details screen:



2. Press the selector button next to Comm. The Communication Settings screen will open:

S e t	Comm. Settings		
u	Comm. Protocol:	AFS	Mod Bus
р	Baud Rate:	9600	AFS
	Data Bits:	8	
	Parity:	Even	
	Stop Bits:	2	
	Flow Control:	None	

3. Press the selector button for your choice: AFS or ModBus.

There is no need to press the Enter key. Use the  $\times$  key to return to the Systems Detail screen, or the  $\Box \Rightarrow$  key to return to the Main Screen.

#### 5.3.3 Set Base Addresses

You must set base addresses, or ID numbers, for AFS or your other supervisory software, so the software recognizes each fermentor group as a distinct unit.

- 1. Press  $\Box \mathbf{k}$  to open the Systems Details screen (see sample screen above).
- 2. Press the selector button next to BaseAdd. The Base Address screen will open:

S	System Details	0
e t	Unit Base Address: 0	4
u	Unit 1 ID: 0	8
р	Unit 2 ID: 1	12
	Unit 3 ID: 2	16
	Unit 4 ID: 3	20
	Comm. Protocol: AFS	24
	Recorder Status: Off	28

3. Press the selector button for the number you wish to assign to the first unit. The system will automatically assign the next consecutive numbers to the other units you may be running from this PCU. In this way, if you choose to give Unit 1 the address of 8, for example, Unit 2 will be automatically assigned #9, Unit 3 will be #10 and Unit 4 will be #11.

## NOTE:

# Regardless of the actual number of fermentors being used, all four addresses are occupied by the PCU. Do not assign any other fermentor in your system a multidrop address within the range used by the PCU.

4. Press the × key to return to the Systems Detail screen, or press the □ ⇒ key to return to the Main Screen.

#### 5.3.4 Set Chart Recorder for Data Logging

You may choose to use an optional analog data recorder, which you connect via user-supplied cable to the PCU's 9-pin male "D" recorder connector. Four outputs (per PCU) are available to send data to the supervisory system for logging. Following are the pin designations for those four outputs:

From PCU	To Recorder		
Pin 2	+	Channel 1	
Pin 1	-		
Pin 3	+	Channel 2	
Pin 5	-		
Pin 4	+	Channel 3	
Pin 7	-		
Pin 6	+	Channel 4	
Pin 8	-		
Pin 9	Shield		

#### **Data Logging Pin Outputs**

To choose assign the outputs to the loops of choice:

- 1. Press  $\Box \mathbf{k}$  to open the Systems Details screen.
- 2. Press the selector button next to Recorder. The Recorder screen will open:

S e t u	Recorder Status: Off	Status	
р	Output 1: None	Out 1	
	Output 2: None	Out 2	
	Output 3: None	Out 3	
	Output 4: None	Out 4	

3. Press the selector button for Output 1. A screen with eight available loops opens:

S	Recorder		None
e t	Recorder Status:	Off	Temp-U1
u			Agit-U1
р	Output 1:	None	PumpA-U1
	Output 2:	None	PumpB-U1
	Output 3:	None	PumpC-U1
	Output 4:	None	pH-U1
			More

Initially, all of the Output are assigned to "None".

4. Press the selector button next to the loop you wish to assign to Output 1. If you do not see the loop you want, press the selector button next to More... to see the next group of available loops. In this example, we have selected Temp-U1, which is the Temperature loop from Unit 1. The previous screen returns, with the selected loop now assigned to Output 1:



- 5. Press the selector button next to On to turn Output 1 on.
- 6. Repeat steps 4 and 5 above to assign Output 2, Output 3 and Output 4.
- 7. Press the  $\Box \Rightarrow$  key to return to the Main Screen.

In future, should you wish to change an output assignment or to return it to None, follow steps 3-5 above to make new selections.

#### **STERILIZATION & REINSTALLATION** 6

# NOTE:

Before proceeding, consult the dimensions outlined in Sections 11.4.1 and 11.4.2 to be sure your autoclave is large enough to accommodate the vessel with its various components.



During autoclaving, the vessel exhaust filter and, if present, the water jacket drain must be vented to avoid explosion.



# 🦄 WARNING!

Use protective gloves when handling hot components.



**During sterilization:** 

- The bearing housing cap must be installed on the motor bearing housing, to keep steam from damaging the internal bearings.
- On water-jacketed vessels, the jacket must be half-filled with water.

# 

#### Never autoclave PVC tubing (clear with white braiding).

There are four objectives to preparing a vessel for sterilization:

- A. To minimize pressure differences throughout the sterilization process by ensuring that the air can transfer freely between the inside and the outside of the vessel;
- B. To ensure that minor pressure differences do not expel liquid from the vessel by clamping off all penetrations that go below liquid level;
- C. To protect hydrophobic filters from blockage, which would occur if condensation were allowed to wet and block the filter surface;
- D. To protect susceptible vessel assembly components from steam damage.

The first objective is met by leaving at least one vessel port open, the second by clamping shut flexible tubing attached to immersed penetrations, and the third by wrapping filters with a protective cap of aluminum foil. Use protective caps on probes and bearings to meet the fourth objective.

#### 6.1 Initial Preparation for Autoclaving

If this is a **water-jacketed vessel, the jacket must be half-filled with water** prior to autoclaving. Follow the steps in Section 6.1.1 below.

If this is a non-jacketed vessel, proceed to Section 6.2.

#### 6.1.1 Filling the Water Jacket

To fill the water jacket:

- 1. After the tubing and water supply are connected, make sure the solenoid valve cable and the RTD cable are plugged into the Power Controller.
- 2. Set the temperature control mode to Off (for instructions, see Section 7.4.1).
- 3. Check that the temperature reading is higher than 5°C.
- 4. Allow water to enter the piping system; it will stop at the solenoid valve.
- 5. Set the temperature loop control mode to *Auto*.
- 6. Enter a temperature setpoint (**SP**) that is at least 12°C below the current value (**CV**). The controller will respond to the call for cooling by opening the solenoid valve, filling the jacket with water.
- 7. When the jacket is halfway filled, set the temperature control mode to Off.

#### 6.2 Additional Preparation for Autoclaving

To continue preparing the vessel for sterilization:

- 1. Remove the motor from the top of the vessel and carefully put it aside.
- 2. Lubricate the vinyl bearing housing cap with silicone grease to facilitate sliding the cap securely onto the housing.
- 3. Place the bearing housing cap on the top of the bearing housing.
- 4. Disconnect the air and/or gas lines from the inlet filter on the sparger.
- 5. Disconnect the water lines. Remove all PVC tubing.
- 6. Clamp off the harvest tube, the sample tube and all other penetrations that are immersed in the media.
- 7. Remove the RTD from the thermowell.
- 8. Disconnect all probes and sensors, and remove their cables.
- 9. If you are using pH and dO2 probes, install each probe's shorting cap (provided in the probe kit).

- 10. Before placing the vessel into the autoclave, loosen the glass sample bottle by  $\frac{1}{2}$  turn.
- 11. Wrap all filters with aluminum foil to protect them from steam.
- 12. Attach a piece of tubing, wrapped with some non-absorbent material (such as glass wool or non-absorbent cotton) to one of the addition ports. Wrap foil around the end of the tubing, shaped like a funnel, to allow the vessel to vent more easily during autoclaving. Place a clamp on the tubing.

# NOTE:

#### Be sure to leave one clamp open during autoclaving to equalize pressure. If this is a water-jacketed vessel, also leave the jacket water inlet clamp open.

If you have addition, foam trap or harvest bottles mounted at the base of the vessel, you can autoclave them with the vessel. Without detaching their tubing from the headplate:

- 13. Remove the bottle holder(s) and reinstall each on one of the headplate clamping screws.
- 14. Reinsert the bottle and turn the holder until the bottle and holder are positioned over the headplate, rather than extended over the edge.
- 15. Finger tighten the knurled nut.
- 16. Clamp off the tubing, and, where appropriate, remove it from the pump.

#### Probe tips must be moist during sterilization:

- If you will be doing batch fermentation, be sure the vessel is filled with media so the media will also be sterilized.
- If you will be doing cell culture, use at least 100 ml of a balanced salt solution (such as phosphate-balanced saline solution). Sterilize the media separately, after autoclaving the vessel.

#### 6.3 Autoclaving the Vessel

- 1. If you have a 7.5L or 14.0L vessel assembly that is too tall for your autoclave, carefully lay the vessel, still mounted in its stand if present, in the optional angled autoclave rack (*part number M1273-9266—see Figure 41 below*). Secure it in place with the strap.
- 2. Insert the entire vessel assembly (glass jar, vessel stand if present, headplate and all headplate components) into an autoclave and sterilize. **Do not sterilize the jacket water heater**.
- 3. When you remove the vessel from the autoclave, immediately crimp the foil funnel on the addition port and close off the vent tubing to maintain sterility.



Figure 41: Angled Autoclave Rack Option for 7.5L & 14.0L Vessels

#### 6.3.1 Sterilization Time and Temperature

Sterilization time varies with autoclave characteristics, temperature settings, vessel size and contents (i.e., media properties). As a starting point, **autoclave for 25** minutes after the autoclave reaches 121° C.

# 

During autoclaving, the vessel and the water jacket (if present) must be vented at all times.

Release autoclave pressure only when the temperature has dropped below 90° C. Use slow exhaust (30-60 minutes).

Autoclave should be on liquid cycle pressure release, if it has such a feature.

# NOTE:

Filter manufacturers generally advise limiting filter sterilization to 30 minutes, but the longer time required for slow exhaust is essential to protecting the vessel integrity. NBS' long experience has shown no adverse effects at all on filters exposed to longer autoclaving times.

Adjust the time and temperature as needed. If you have a water-jacketed vessel and the jacket is not half-filled, the vessel may not reach sterile temperature.

If, after autoclaving, most of the liquid has left the vessel, the autoclave is exhausting too quickly. Adjust the autoclave to exhaust more slowly.

#### 6.4 Reinstalling the System

- 1. Position the vessel back on the benchtop. **If this is a water-jacketed vessel**, place the vessel on the jacket water heater: carefully align the water jacket inlet and outlet with the heater plate's rear notch, then place the vessel on the heater so the vessel's rubber feet fit into the holes provided for them.
- 2. Connect the heater to the heater connector on the front of the Power Controller.
- 3. Reconnect the water lines to the cooling coil (non-jacketed vessel) or the water jacket inlet and to the exhaust condenser, if present.

# 🖄 WARNING!

Cold water and hot glass is a potentially dangerous mix! Be sure to let the vessel cool for a few minutes before reconnecting the water line.

- 4. If present, remove the pH probe shorting cap and reconnect the pH cable to the probe and the dO2/pH Controller.
- 5. If present, remove the dO2 probe shorting cap and reconnect the dO2 cable to the probe and the dO2/pH Controller.
- 6. Remove the bearing housing cap (inspect for wear and replace for next use, if necessary), and reinstall the motor.
- 7. Reconnect the temperature probe and any other probe/sensor cables.
- 8. Remove addition, foam trap and/or harvest bottle assemblies from the headplate, and reinstall them on the base. Be sure to finger tighten all nuts.
- 9. Reinstall the addition, foam trap and/or harvest tubing in the appropriate pump(s) and release the clamp(s).
- 10. Reconnect the air and/or gas lines.
- 11. Add 1-2 ml of glycerin to the thermowell and reinstall the RTD temperature probe. Connect the cable to the Power Controller.

# WARNING!

If the temperature probe is plugged into its controller but not inserted into the thermowell, the vessel will overheat, resulting in risk to the operator and to the culture.

#### 6.5 Confirming pH Calibration

Autoclaving can alter the zero characteristics of pH probes, typically by 0.1 to 0.3 pH. To check, and to compensate for any discrepancy, you will need an accurate external pH meter. If one is not available, skip this section.

- 1. Following sterilization, with the media at room temperature, note the pH value on the BioFlo 110 Main screen.
- 2. Take a sample of media, and measure the pH on the external meter.
- 3. If the two values disagree, return to the BioFlo 110 Calibrate pH screen and set Zero to the value reported by the external meter. Do not change the Span or you will invalidate the entire calibration.

The pH value will now agree with the external meter's reading.

#### 6.6 Polarizing & Calibrating the dO2 Probe

# NOTE:

# If you are using a polargraphic dO2 probe, it must be polarized after autoclaving and before calibration.

Polargraphic dissolved oxygen probes must be polarized for several hours after autoclaving and before calibration, according to the manufacturer's instructions. Polarization can be accomplished by plugging the dO2 probe cable into the dO2/pH Controller and leaving it on for several hours. Alternatively, battery-powered polarizers are available for this purpose.

From the Main Screen of the appropriate fermentor, press the selector button in the dO2 row. The dO2 Loop Details screen opens:



The procedures to set the Control Mode, the Setpoint, and the Graphic Display High and Low parameters are the same as for the Temperature and Agitation Loops.

- 1. After autoclaving, wait until the media reaches operating temperature. You can speed the cooling process by entering the Temperature setpoint, agitating and sparging.
- 2. In the dO2 Loop Details screen, press the *Calibrate* selector button. The dO2 Loop Calibration screen opens:



3. Press the Set Zero selector button. A highlighted edit box opens:

B i	Calibrate dO2 0	
o F	Zero Std: 0.00	Set Zero
і 0		Set Span
1 1 0	Raw Input: 1022	Reset

- 4. Using the touchpad, key **0** into the highlighted edit box. **Do not press** *Enter* yet.
- 5. Establish a reference "zero" signal from the dissolved oxygen probe, using one of the following choices:
  - (a) More accurate: Sparge with nitrogen until the dO2 Raw Input stops decreasing.
  - (b) Widely used alternate: Momentarily disconnect the dO2 cable from the probe.

- 6. When the Raw Input stops decreasing, press the *Enter* key to save the value (or press ★ to cancel).
- 7. Press the Set Span selector button. A highlighted edit box opens:



- 8. Using the touchpad, key **100** for the new Span value into the highlighted edit box. **Do not press** *Enter* yet.
- 9. Establish a reference 100% level of dissolved oxygen in the media: sparge with air at the appropriate flow rate for your application.
- 10. When the Raw Input stops increasing, press the *Enter* key to save the 100% dO2 reference value.
- 12. After the values have been set, press the  $\Box \Rightarrow$  button to return to the Main Screen.

#### 6.7 Powering the Jacket Water Heater

**If you have a non-jacketed vessel, skip this section.** After sterilization, you should have installed the vessel on the jacket water heater and connected the heater to the Power Controller (see Section 6.4). *Never set the temperature control loop to the desired working temperature* (refer to Section 7.4.1) *until all water connections have been made, the jacket is filled with water, the vessel is filled with media, and the RTD has been installed in the thermowell and connected to the Power Controller.* Never plug the jacket water heater into the controller before the vessel is filled. When the heater is connected properly and the PCU is turned on, the jacket water stirrer should spin

freely.

# WARNING!

When the jacket water heater is plugged in, do not touch the top surface. It is very hot.

# 🖎 NOTE:

#### Always unplug the jacket water heater when not in use.

Whenever you remove the vessel, be sure to turn the temperature control *Off* (refer to Section 7.4.1) and unplug the jacket water heater. If you do not, the jacket water heater could reach excessive temperatures, posing a burn danger to personnel and causing the heater's internal safety cutoff switch to interrupt power to the heater.

If the safety cutoff is activated, power will not be restored until you do the following:

- 1. Wait for all surfaces to cool.
- 2. Turn the jacket water heater over and press the Thermostat Reset button, located on the bottom (*see Figure 42 below*).
- 3. Reinstall the vessel (jacket filled with water and vessel with media; temperature probe in the thermowell and plugged into the Power Controller).



#### Figure 42: Jacket Water Heater—Bottom View

# 7 OPERATION

# NOTE:

Before operating a water-jacketed vessel, make sure it is properly installed on the vessel jacket water heater, and that the heater is properly connected (see Section 6.4) before you set the desired working temperature.

#### 7.1 Primary Control Unit Display & Controls

The Primary Control Unit (PCU) serves as the operator interface. A single PCU can control up to four sets of control modules (but no more than sixteen total) for operating four vessels simultaneously. If you plan to run more than four vessels, another PCU must be added for each group of four.

The PCU has universal AC power input that can be set to accommodate electrical services between 100 and 240 Volts. It can be plugged into the outlet on the back of the Power Controller (*see Figure 2*), or plugged directly into your electrical service. The PCU is equipped with battery back-up to safeguard memory in case of power loss.



Figure 43: PCU Front Panel

The user interface touchpad has 12 numeric keys. As you can see in the drawing below, these keys are the numbers 0 through 9, a decimal point, and a  $\pm$  sign.

There are also 8 selector keys, which allow the user to choose specific loop detail screens from the main screen, and to select specific interactive screens for operational input.



There are also four function keys on the touchpad:



This key allows you to **toggle** the display from Main screen to Main screen, if you have more than one fermentor on the system. It also allows you to return to the Main screen from any other screen.



This key allows you to **retrieve** the Language screen or the Systems Detail screen.



This key allows you to **go back** to the previous screen. It also allows you to **cancel** any numerical input (e.g., setpoint, etc) that has not yet been entered.



This key allows you to **enter** any touchpad input that you wish to save.

#### 7.2 Selecting the Application

The default Application selection is *Fermentation*. The *Use*... selection on the initial setup screen will allow you to verify and/or change the application selected for each vessel (identified as Unit 1, Unit 2, etc.):

1. Press the *Use*... selector button.



2. The Application screen appears. To change the application for any vessel, press the *Use* button next to that unit. For this example, we will change Unit 2 from *Fermentation* to *Cell Culture*.



3. Press Unit 2's *Use* selector button. The display changes to Application selection screen (see sample screen below). Note that the alignment of choices (*Fermentation* or *Cell Culture*) on this screen does not relate to the units that appear on the left. You have already selected Unit 2, therefore your choice in this new screen will only affect Unit 2.

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4. Press the *Cell Culture* selector button. The display automatically returns to the previous screen, showing Unit 2's application as Cell Culture.



Follow the same steps any time you wish to verify or change application for a vessel ("Unit").

#### 7.3 Main Screen

1. Press the  $\Box \Rightarrow$  button to open the Main Screen (see sample screen below).

Loop	Name		Current Value	Loop Setpoint	Loop Control Mode
B	Name	Value	Setpoint	Control	
0	Temp	23.4	30.0 °C	Auto	
F	Agit	134	350 rpm	Auto	
0	Pump A	0.0	0.0 %	Off	
1	Pump B	0.0	0.0 %	Manual	
1	Pump C	0.0	0.0 %	Off	
0	рН	6.68	7.00 pH	Auto	
	dO2	77.1	0.0 %	Auto	

The above screen is only one sample. The loop names will reflect the modules in your system.

- 2. If you have more than one fermentor running off the PCU, press the □ ⇒ button again to open the Main Screen for Unit 2 (see sample screen below).
- 3. Press again for Unit 3, and again for Unit 4.

U	Name	Value	Setpoint	Control
n i	Temp	25.7	30.0 °C	Auto
t	Agit	253	350 rpm	Auto
2	Pump A	0.0	0.0 %	Manual
	Pump B	0.0	0.0 %	Manual
	Pump C	0.0	0.0 %	Manual
	рН	6.59	7.00 pH	Auto
	dO2	81.2	0.0 %	Auto

4. Press once more to return to the Unit 1 (labeled "BioFlo 110") Main Screen.

#### 7.4 Set the Temperature Loop

All loops have three parameters that you can specify through the PCU. They are the Control Mode, Setpoint and Graphic Display. As you go through the following steps to set the Temperature loop, you will learn how to set the others as well.

#### 7.4.1 Control Mode

To change the control mode of the temperature loop, starting from the Main Screen (see above) of the appropriate fermentation unit:

				Press here —	
B	Name	Value	Setpoint	Control	
0	Temp	23.4	30.0 °C	Off	
F	Agit	134	350 rpm	Auto	
0	Pump A	0.0	0.0 %	Manual	
1	Pump B	0.0	0.0 %	Manual	
1	Pump C	0.0	0.0 %	Manual	
0	рН	6.68	7.00 pH	Auto	
	dO2	77.1	0.0 %	Auto	

1. Press the selector button in the *Temp* row (see sample screen below).

The Temperature Loop Details screen appears (see sample below):



If the *Control* mode is *Off*, the PCU is not controlling temperature. To turn the control mode on:

• Press the selector button next to *Control*.



The Temperature Loop Control Mode screen opens:



• Press the selector button next to *Auto*.

The control mode is automatically updated as the Temperature Loop Details screen returns (see sample screen below):



#### 7.4.2 Setpoint

To set the temperature setpoint:

1. Press the selector button next to Setpoint. A highlighted edit box appears:



2. Using the numeric touchpad, enter the new setpoint:



3. Press the *Enter* key to save the new setpoint.

The Temperature Loop Details screen returns, showing the new setpoint on the graphic display's X axis:



#### 7.4.3 Graphic Display

To change the range of the Y axis on the graphic display:

- 1. Press the Disp Hi selector button. A highlighted edit box appears.
- 2. Using the touchpad, enter the desired High value.
- 3. Press the *Enter* key to save the new value.
- 4. Press the Disp Lo selector button. A highlighted edit box appears.
- 5. Using the touchpad, enter the desired Low value.
- 6. Press the *Enter* key to save the new value.

Press the  $\Box \Rightarrow$  button to return to the Main Screen.

#### 7.5 Setting the Agitation Loop

From the Main Screen of the appropriate fermentor, press the selector button in the *Agit* row. The Agitation Loop Details screen appears:



Set the Control Mode, the Setpoint, and the Display High and Low range in the same way as you did for the Temperature loop.

# A CAUTION! Never exceed 200 rpm unless at least one impeller is immersed in liquid.

#### 7.6 Setting the pH Loop

From the Main Screen of the appropriate fermentor, press the selector button in the pH row.

The pH Loop Details screen opens:



The procedures to set the Control Mode, the Setpoint, and the Graphic Display High and Low parameters are the same as for the Temperature and Agitation Loops.

# NOTE:

Be sure to calibrate the pH probe, following the procedure outlined in Section 4.13, before autoclaving it with the vessel.

#### 7.6.1 Setting a Deadband for pH control

A deadband is a tight plus and minus tolerance around the setpoint. This is a useful feature, for example, to trigger an alarm via supervisory software.

1. In the pH Loop Details screen, press the *Setup* selector button. The pH Setup screen opens:



2. Press the *Deadband* selector button. A highlighted text box opens:



3. Using the touchpad, enter the desired deadband value:

B i	рН 6.53 рН	Defaults
o F	Deadband: 0.00	0.20 Deadband
 0	Output Multiplier: 100.00	Multiplier
1 1		
<u>0</u>	Advanced Control P-Gain: 140.00	P Gain
	I-Gain: 14.00	I Gain

4. Press the *Enter* key (or press  $\times$  to cancel). The new value appears onscreen.

## Setting the Output Multiplier for pH Control

Later, when you begin your batch, you may notice that pH remains steady at the setpoint, yet the acid and base pumps are continually alternating in making additions. This is an indication that the controller is overcompensating for minor fluctuations in pH. *Output Multiplier* is a feature that attenuates controller output to the acid and base pumps and the  $CO_2$  gas line, providing more nuanced control of additions to maintain pH.

We recommend that you begin by implementing a multiplier of 25%. This means that if the controller's output to the base pump, for example, is 100%, then the 25% multiplier will reduce pump output to 25%. If the controller's output to the pump is 50%, the 25% multiplier factor will reduce pump output to 12.5%.

7.6.2

If, after applying an Output Multiplier of 25%, you find the results are attenuated but the controller seems unable to maintain the setpoint, increase the Multiplier by small increments until the controller is able to maintain setpoint.

To set the Multiplier:

1. In the pH Loop Details screen, press the *Setup* selector button. The pH Setup screen opens:



2. Press the *Multiplier* selector button. A highlighted text box opens:



3. Using the touchpad, enter the desired Multiplier value (note that you can set this percentage to the hundredths):


4. Press the Enter key to save the new value (or press  $\times$  to cancel). The new value appears onscreen.

#### 7.6.3 Setting P & I Gains for pH Control

#### NOTE:

## We recommend that you do not change the factory P & I settings unless you are an advanced user of P & I algorithms.

P and I values are numbers that determine how the fermentor responds to changing growth conditions and new setpoints. Because growth characteristics of bacterial and cell cultures are very different, they require some different P and I values for proper control. Incorrect P and I values can cause poor control, which may actually mimic component failure. For this reason, we recommend that you use the default factory-set P and I gains.

If you are very experienced with P and I algorithms and wish to change the default values, use this procedure:

- 1. In the pH Loop Setup screen, press the *P Gain* selector button.
- 2. Using the touchpad, enter the new *P Gain* value in the highlighted edit box.
- 3. Press the Enter key to save the new value (or press  $\times$  to cancel). The new value appears onscreen.
- 4. Press the *I Gain* selector button. A highlighted edit box will open.
- 5. Using the touchpad, enter the new *I Gain* value.
- 6. Press the Enter key to save the new value (or press  $\times$  to cancel). The new value appears onscreen.

Anytime you wish to return to the default P & I Gain settings, open the pH Loop Setup screen, and press the *Defaults* selector button.

See Section 12.5.1 for the pH P & I control equation.

#### 7.7 Setting the dO2 Loop

When your system includes the dO2/pH Controller, the PCU can supervise dissolved oxygen (dO2) in the media. If you are using a dO2 probe, you will need to set the dO2 loop. You will calibrate the probe after autoclaving.

#### NOTE:

After autoclaving, be sure to calibrate the dO2 probe *before* setting the dO2 loop. If you are using a polarographic dO2 probe, it must be polarized before calibration, but after autoclaving. To polarize and calibrate the dO2 probe, see Section 6.6.

#### 7.8 Setting dO2 Control Cascades

Cascading brings several systems together to work jointly to achieve your goal. There are four possibilities for Fermentation, and four other choices for Cell Culture, if you are using the Gas Mix Controller. Without the Gas Mix Controller, there are two cascades available to either Fermentation or Cell Culture. In all cases, when you first set up the system, the default choice is set to *None* (no cascade).

#### 7.8.1 Cascades for Fermentation without Gas Mix Controller

For Fermentation, you have two choices when you are not using the Gas Mix Controller: *None* or *Agitation*. (If you have the optional TMFC module, *see Section 14 for details*.)

Cascading to Agitation is designed to control dissolved oxygen through automatically controlled agitation speed and oxygen output (via P & I Gains—*see Section 7.9 below*). This is how it functions: when the actual dO2 value rises above the dO2 setpoint, the agitation speed will automatically decrease until the dO2 setpoint is reached. Conversely, when the actual dO2 value drops below the setpoint, the cascade system acts to bring it back up.

The default setting is *Cascade To: None*, which means that dissolved oxygen will be controlled only by the addition of oxygen when the actual dO2 level drops below setpoint.

To cascade dissolved oxygen to Agitation:

1. In the dO2 Details screen, press the *Setup* selector button. The Setup screen opens:

B i F I 0 1	dO2 73.1 % Cascade To: None Agit Hi Limit: 1200 Agit Lo Limit: 250	Defaults Cascade Agit Hi Agit Lo
1 0	<u>Advanced Control</u> P-Gain: 0.20 I-Gain: 1.00	P Gain I Gain

#### ROTE:

Selecting *Defaults* at any time (during setup or after settings have been changed) will return all settings EXCEPT *Cascade To:* to the default setting. Cascades remain in force until you select otherwise.

2. Press the *Cascade* selector button. The Cascade screen opens:



3. Press the *Agit* selector button. The Setup screen returns, showing the new cascade parameter:

B i o F I o 1	dO2 73.1 % Cascade To: Agit Agit Hi Limit: 1200 Agit Lo Limit: 250	Defaults Cascade Agit Hi Agit Lo
1 0	<u>Advanced Control</u> P-Gain: 0.20 I-Gain: 1.00	P Gain I Gain

4. Press ★ to return to the dO2 Details screen or □ ⇒ to return to the Main Screen.

#### 7.8.2 Cascades for Fermentation with Gas Mix Controller

When you are using the Gas Mix Controller, you have four choices to cascade to for Fermentation: *Agitation, Oxygen, Agitation/Oxygen* or *None*. (If you have the optional TMFC module, these screens are different. *See Section 14 for details.*)

Cascading to:

- *Agitation* controls dissolved oxygen through automatically (via P & I Gains *see Section 7.9 below*) controlled agitation speed and oxygen output. This is how it functions: when the actual dO2 value rises above the dO2 setpoint, the agitation speed will automatically decrease until the dO2 setpoint is reached. Conversely, when the actual dO2 value drops below the setpoint, the cascade system acts to bring it back up.
- *Oxygen* controls dissolved oxygen by automatically adjusting the mix of air and oxygen. (This is not available without the Gas Mix Controller.)
- *Agitation/Oxygen* controls dissolved oxygen by automatically running Agitation to the maximum speed, then, if dissolved oxygen still has not reached the setpoint, increasing the oxygen flow through the sparger. This cascade is the most frequently used in fermentation. (This is not available without the Gas Mix Controller.)
- The default setting is *Cascade To: None*, which means that dissolved oxygen will be controlled only by the addition of oxygen when the actual dO2 level drops below setpoint.

#### To cascade dissolved oxygen to Agitation:

opens:

В dO2 73.1 % i **Defaults** 0 F Cascade Cascade To: None Agit Hi Agit Hi Limit: 1200 0 Agit Lo Limit: 250 Agit Lo 1 1 **Advanced Control** 0 P-Gain: 0.20 P Gain I-Gain: 1.00 I Gain

1. In the dO2 Details screen, press the Setup selector button. The Setup screen

- 2. Press the *Cascade* selector button. The Cascade screen opens:



3. Press the *Agit* selector button. The Setup screen returns, showing the new cascade parameter:

B i	dO2 73.1 %	
0		Defaults
F	Cascade To: Agit	Cascade
	Agit Hi Limit: 1200	Agit Hi
	Agit Lo Limit: 250	Agit Lo
1		
0	Advanced Control	
	P-Gain: 0.20	P Gain
	I-Gain: 1.00	l Gain

- 4. If you wish to change the high and low limits for Agitation speed, use the *Agit Hi* and *Agit Lo* selector buttons to open an edit box for each, use the touchpad to input a value, and press the *Enter* button to save the new value to memory. The Hi Limit may not exceed 1200 and the Lo Limit may not go below 250.
- 5. Press ★ to return to the dO2 Details screen or □ ⇒ to return to the Main Screen.

To cascade to Oxygen: follow steps 1-4 above, but select O2 instead of Agitation.

**To cascade to** *Agitation/O2***:** follow steps 1-4 above, but select *Agit/O2* instead of *Agitation*.

#### 7.8.3 Cascades for Cell Culture without Gas Mix Controller

For Cell Culture, without the Gas Mix Controller, you have the same two choices explained for Fermentation without the Gas Mix Controller: *None* or *Agitation*. *See Section 7.8.1 above*. (If you have the optional TMFC module, *see Section 14 for details*.)

#### 7.8.4 Cascades for Cell Culture with Gas Mix Controller

When you are using the Gas Mix Controller, you have four choices to cascade to for Cell Culture: *Agitation, 4 Gas, Demand* or *None*. (If you have the optional TMFC module, *see Section 14 for details.*)

Cascading to:

• *Agitation* controls dissolved oxygen through automatically (via P & I Gains *see Section 7.9 below*) controlled agitation speed and oxygen output. This is how it functions: when the actual dO2 value rises above the dO2 setpoint, the agitation speed will automatically decrease until the dO2 setpoint is reached. Conversely, when the actual dO2 value drops below the setpoint, the cascade system acts to bring it back up.

- *4 Gas* controls dissolved oxygen by automatically adjusting the feed of all gases introduced to the culture. (This is not available without the Gas Mix Controller.)
- *Demand* controls dissolved oxygen by automatically adjusting the feed of carbon dioxide, nitrogen and oxygen. (This is not available without the Gas Mix Controller.)
- The default setting is *Cascade To: None*, which means that dissolved oxygen will be controlled only by the addition of oxygen when the actual dO2 level drops below setpoint.

#### To cascade dissolved oxygen to Agitation:

6. In the dO2 Details screen, press the *Setup* selector button. The Setup screen opens:

В	dO2 73.1 %	
- О Б		Defaults
	Cascade To: None Agit Hi Limit: 300	Agit Hi
1	Agit Lo Limit: 25	Agit Lo
1 0	Advanced Control	
	P-Gain: 0.20 I-Gain: 1.00	P Gain
		l Gain

7. Press the *Cascade* selector button. The Cascade screen opens:

B i	dO2 73.1 %	
0 F I 0	Cascade To: None Agit Hi Limit: 300	Agit 4 Gas
1	Agit Lo Limit: 25	Demand None
<u>0</u>	Advanced Control P-Gain: 0.20 I-Gain: 1.00	

8. Press the *Agit* selector button. The Setup screen returns, showing the new cascade parameter:

B i 0 F I 0 1	dO2 73.1 % Cascade To: Agit Agit Hi Limit: 300 Agit Lo Limit: 25	Defaults Cascade Agit Hi Agit Lo
1 0	<u>Advanced Control</u> P-Gain: 0.20 I-Gain: 1.00	P Gain I Gain

- 9. If you wish to change the high and low limits for Agitation speed, use the *Agit Hi* and *Agit Lo* selector buttons to open an edit box for each, use the touchpad to input a value, and press the *Enter* button to save the new value to memory. The Hi Limit may not exceed 300 and the Lo Limit may not go below 25.
- 10. Press ★ to return to the dO2 Details screen or □ ⇒ to return to the Main Screen.

**To cascade to 4** *Gas***:** follow steps 1-4 above, but select 4 *Gas* instead of *Agitation*.

**To cascade to** *Demand***:** follow steps 1-4 above, but select *Demand* instead of *Agitation*.

#### NOTE:

After a Cascade To: Gas Mix or to Cascade To: Demand has been selected, if the Gas Mix function is turned off through the O2 Loop Gas Mix screen, the cascade will be overridden.

#### 7.9 Setting P & I Gains for dO2 Control

#### NOTE:

## We recommend that you do not change the factory P & I settings unless you are an advanced user of P & I algorithms.

P and I values are numbers that determine how the fermentor responds to changing growth conditions and new setpoints. Because growth characteristics of bacterial and cell cultures are very different, they require some different P and I values for proper control. *For example, for cell culture, the P value should be 2.00 and the I value 0.67, while the factory-set values for fermentation are P* =0.20 *and I* = 1.00.

Incorrect P and I values can cause poor control, which may actually mimic component failure. For this reason, we recommend that you use the default factory-set P and I gains.

If you are very experienced with P and I algorithms and wish to change the default values, use this procedure:

- 1. In the dO2 Loop Setup screen, press the *P Gain* selector button.
- 2. Using the touchpad, enter the new *P Gain* value in the highlighted edit box.
- 3. Press the Enter key to save the new value (or press  $\times$  to cancel). The new value appears onscreen.
- 4. Press the *I Gain* selector button. A highlighted edit box will open.
- 5. Using the touchpad, enter the new *I Gain* value.
- 6. Press the Enter key to save the new value (or press  $\times$  to cancel). The new value appears onscreen.
- 7. After the values have been set, press the  $\Box \Rightarrow$  button to return to the Main Screen.

Anytime you wish to return to the default P & I Gain settings, open the dO2 Loop setup screen, and press the *Defaults* selector button.

See Section 12.5.2 for the dO2 P & I control equation.

#### 7.10 Setting the Gas Mix for Fermentation

**If you are using Gas Mix Controller**, your system's Main Screen will include an oxygen (O2) loop. From the Main Screen of the appropriate fermentor, press the selector button in the *O2* row to open the O2 loop screen:



- 1. Press the *Control* selector button.
- 2. In the next screen, press the *Manual* selector button.
- 3. To cascade O2 to your dO2 loop, see Section 7.8.2.
- 4. **To set a manual O2 percentage without cascading**, press the Setpoint selector button in the O2 loop screen (see sample screen above) and enter the percentage of oxygen needed.

The controller will automatically feed oxygen as needed to maintain the setpoint you have entered.

#### 7.11 Setting the Gas Mix for Cell Culture

**If you are using Gas Mix Controller**, your system's Main Screen will include a Gas loop. From the Main Screen of the appropriate fermentor, press the selector button in the *Gas* row to open the Gas Mix Screen. "Gas Mix" for this controller is not actually a mixing process; it is an automatically sequenced feeding of various gases.

Four control modes are possible: *4-Gas, Demand, 100% Feed* and *Off.* Selecting *Off* will turn the Gas Mix Controller off.

#### 7.11.1 4-Gas Mode

Because your system has the Gas Mix Controller, when you select 4-Gas as the control mode the PCU will control the levels of all gases (carbon dioxide, nitrogen, oxygen and air) being introduced to the culture, to maintain dO2 and pH at their respective setpoints.

B :	Gas Mix		4 Gas
	Control: 4 Gas		Demand
F			Off
   0	Gas	%	
	CO2	20.0	100% CO2
1 1	N2	49.7	100% N2
0	02	0.0	100% O2
	Air	30.3	100% Air

Select this control mode by pressing the *4-Gas* selector button. There are no further choices to program; in this mode, the 4-Gas controller will automatically control the sequential introduction of gases according to need.

#### 7.11.2 Demand Mode

Selecting *Demand* as the control mode will provide automatic "on demand" sparging of carbon dioxide, nitrogen and/or oxygen to maintain dO2 and pH at their respective setpoints.

Press the Demand selector button. There are no further choices to program.

#### 7.11.3 100% Feed Mode

If you need to feed 100% of any one of the four gases for a period of time, you can manually override the 4-Gas controller using this feature.

To override the Gas Mix Controller's control of gas sequencing in order to feed 100% of one gas: in the Gas Mix screen, press the selector button next to the gas of choice. The screen reflects your choice (in this case, CO2):

B :	Gas Mix		4 Gas
і О	Control: CO2		Demand
F			Off
і 0	Gas	%	
	CO2	100.0	100% CO2
1 1	N2	0.0	100% N2
0	O2	0.0	100% O2
	Air	0.0	100% Air

#### NOTE:

The gas will continue to feed at 100% until you manually select another gas, return to automatic *4-Gas* Mode, to *Demand* mode or choose *Off*.

#### 🖎 NOTE:

If, at any time after establishing a dO2 cascade, you turn the Gas Mix controller *Off* using the Gas Mix screen, the cascade will be overridden.

#### 7.12 Setting the Pumps

#### 7.12.1 Pump Operation

The BioFlo 110 supports five peristaltic pumps. On the rear panel of the Power Controller, the pump outlets labeled Acid and Base are integrated with the pH control loop. This is why they do not appear as individual pumps on the Main Screen. Assignable pumps A, B and C, do appear individually on the display. Each of these can be operated manually, by assigning a setpoint, or be set to respond automatically to signals from conduction probes.

#### 7.12.2 Pump Setpoint and Multiplier

Each of the three assignable pumps (A, B & C) accepts a setpoint. The setpoint determines the percentage of On time during each ten-second period while the pump is set to On. For example, if Pump A has a setpoint of 40%, every ten seconds it will turn on for four seconds.

Instead of a setpoint, the Acid and Base Pumps use a Multiplier value which can be assigned in the pH Control screen. A multiplier of 50%, for example, has the base pump run for 5 seconds every 10 seconds if the pH controller is calling for base addition.

#### 7.12.3 Control Mode

Pumps A, B and C accept a user-assigned Control Mode. The available control modes are Manual, Wet On, Wet Off and Antifoam. *Wet On, Wet Off* and *Antifoam* are the designations given, for convenience, to the probes that are plugged into the socket of the same name on the Level Module. Control modes affect pump operation in the following ways:

- Manual: pump operates at the setpoint
- Wet On: when the probe is immersed in media or foam, the pump turns on at its assigned setpoint. This is useful to remove media when additions accumulate to an undesired level, for example.
- Wet Off: when the probe is not immersed in media or foam, the pump turns on at its assigned setpoint. This is useful for media addition.
- Antifoam: when the probe is immersed in media or foam, the pump turns on at its assigned setpoint. This is useful to add chemical defoamer.

#### 7.12.4 Setting Pumps

To set the Multiplier for pH pumps (Acid and Base), see Section 7.6.2. Be sure also to set the Control Mode, Setpoint and Graphic Display (Hi and Lo limits) for those pumps.

Following the instructions below:

- 1. Set the Control Mode,
- 2. Enter a Setpoint,
- 3. Select the Hi and Lo limits of the Graphic Display presented in the Loop Details screen

for each assignable Pump A, B and C.

To set up the first peristaltic pump (Pump A) under the PCU's supervision:

1. In the Main Screen, press the selector button in the Pump A row to open this screen:



The procedures are generally the same as for the Temperature and Agitation Loops, to set the Graphic Display Hi and Lo parameters, the Setpoint, and the Control Mode (if you wish to choose Manual—which allows the operator to manually set pump output—or Off).

For the three automatic Control Modes unique to the pump loops (Antifoam, Wet On and Wet Off), *see Section 7.13 below for details*.

2. Repeat the setup procedure for Pump B and Pump C.

#### 7.13 Setting the Pump Control Mode: Antifoam, Wet On & Wet Off

If your system includes the Level Controller, the PCU can supervise the three level loops identified as Antifoam, Wet On and Wet Off, each of which is actually a control mode for the assigned pump, as mentioned in the section above.

1. To set up the defoamer pump, from the pump's Details screen, press the *Control* selector button for its designated pump. The Control screen opens:



2. Press the Antifoam selector button. The pump Details screen screen returns; after a short delay, the control mode will reflect your choice. Note that a new parameter, Probe Sensitivity, also appears:



is activated, CV is current percent output (either 0 .0 or the setpoint).

- 3. Probe sensitivity reflects the density of foam necessary to activate the antifoam pump. To set a sensitivity value (we recommend starting with 75.0), press the *Sensitivity* selector button, input the desired value using the touchpad, and press the *Enter* key.
- 4. Press the  $\Box \Rightarrow$  button to return to the Main Screen.
- 5. Follow the same procedure to set the Wet On pump and the probe sensitivity value, and the Wet Off pump and probe sensitivity.

#### 7.14 Inoculation

When you are ready to inoculate the media to start a batch, observe aseptic techniques to do the following:

1. Using a syringe, inject the inoculum through the self-sealing rubber seal of the septum port.

#### 🖄 NOTE:

Always inspect the condition of the septum seal and the bearing housing cap prior to autoclaving. Replace as necessary.

#### 7.15 Sterile Sampling

To remove a sample from an active batch:

- 1. Make sure the sampler syringe plunger is completely closed.
- 2. Confirm that the sample bottle is in place and finger-tight against its holder.
- 3. Open the thumb clamp.
- 4. Pull back on the syringe plunger to fill the bottle.
- 5. Close the thumb clamp. Close the plunger.
- 6. Observe aseptic techniques to remove the filled bottle and cap it.
- 7. Immediately install a new sample bottle in the holder.

#### 7.16 Automatic Foam Control

If you have installed a foam probe and set its control mode to *Antifoam*, the addition of antifoam is automatic. When the probe detects the presence of foam, its signal prompts the Level Controller to signal the Power Controller. The Power Controller activates the Antifoam pump to add chemical defoamer from the addition bottle through an addition port. When the probe no longer detects foam, the pump will be turned off.



At the end of a batch, to harvest the entire culture:

- 1. Aseptically prepare an autoclaved harvest bottle large enough to hold the entire contents of the vessel. The bottle cap should be equipped with a sterile filter on one penetration. There should also be sufficient silicone tubing on the other penetration to reach from the bottle, placed on the floor below the benchtop, to the harvest tube outlet. Secure both the tubing and the filter with plastic ties.
- 2. Aseptically install the harvest tubing on the harvest tube outlet, with a thumb clamp on the tubing. Close the clamp. Secure the tubing on the harvest tube with a plastic tie.
- 3. Set the harvest bottle on the floor below the vessel.
- 4. There are two ways to prime the harvest process: (a) with a pump, or (b) without a pump.
  - a. Thread the tubing through one of the peristaltic pumps, unclamp the tubing and manually turn the pump on. Harvesting will begin.
  - b. Install a rubber sampler bulb on the other end of the harvest bottle filter. Unclamp the harvest tubing, then squeeze and release the bulb. Harvesting will begin.
- 5. Once harvesting has been primed, there are two ways to complete the process:
  - a. Allow the pump to operate until the vessel is exhausted.
  - b. Allow gravity to do the work.

Note that a small amount of residue will probably remain in the vessel no matter how you harvest.

## 8 MAINTENANCE

Preventive maintenance keeps your equipment in proper working condition. When performed routinely, maintenance results in longer life for your equipment. It also reduces time lost due to equipment failure.

#### WARNING!

Always disconnect the power cord from the Power Controller and the PCU before performing maintenance.

Never immerse any BioFlo 110 module in liquid of any kind.

#### 8.1 Cleaning the Modules

Use a sponge dampened with mild detergent and water to wipe the exterior of each BioFlo 110 module. Rinse the sponge in clean water, then wipe the exterior again.

#### 8.2 pH Probe Maintenance and Storage

The probe should be stored standing upright, with the electrode tip immersed in a solution of 3 molar KCl or a buffer solution between pH 4.00 and pH 7.00.

#### CAUTION!

Never let a pH probe rest on its tip. Never leave a pH probe in DI water.

#### 8.3 dO2 Probe Maintenance and Storage

Use soft facial tissue to clean the dO2 probe.

Check the probe's Teflon membrane to be sure there are no punctures, puckers or wrinkles. If there are, the probe should be replaced.

When it is not in use in the vessel, the dO2 probe should be stored standing upright with the shorting cap in place and the membrane isolated from the air environment. At no time should the probe be allowed to rest on its membrane.

CAUTION! Never let a dissolved oxygen probe rest on its tip.

#### 8.4 Cleaning the Vessels

After each use, the vessel and the vessel components should be thoroughly cleaned. If necessary, decontaminate the vessel by autoclaving. If you are autoclaving your vessel, fill it with water to the maximum working volume. This will allow for easier clean-up later.



Never allow the drive assembly to rest on the impeller shaft (see Section 4.4 ).

#### 8.4.1 14.0L Vessel Disassembly

Special care must be taken when you remove the headplate assembly from the 14.0L vessel because the vessel narrows at the neck:

- 1. After you remove the headplate nuts and washers, lift the headplate assembly a few inches, and hold it up with one hand.
- 2. With the other hand, reach into the vessel, squeeze the baffle (between thumb and forefinger) tightly enough to clear the vessel neck.
- 3. Lift the assembly straight up until the entire assembly is clear.

#### 8.4.2 Vessel Cleaning Methods

There are three ways to clean your vessel:

Method 1	1. Drain all the liquid from the vessel.
	2. Disassemble and wash the headplate, glass vessel, impellers, baffles
	and other components in hot water and an appropriate laboratory
	detergent.
	3. If necessary, use a very small test tube brush to clean the ports and
	orifices. A soft brush may also be used to remove dried-on material
	from the glass.
	4. Rinse all components in tap water.
	5. Rinse twice in purified (deionized, RO, distilled water). Allow
	everything to dry thoroughly before reassembling the vessel.
Method 2	1. Drain the liquid from the vessel.
	2. Fill the vessel to the maximum working volume with water.
	3. Reassemble the vessel and components, and reconnect the agitation
	shaft to the motor.
	4. Press the Agitation key On.
	5. After approximately ten minutes, press the Agitation key Off.
	6. Remove the vessel and disassemble its components.
	7. Wash all elements in detergent and not water.
Method 3	1. Drain the liquid from the vessel.
	2. Fill the vessel to just above the maximum working volume with hot
	water and the recommended amount of a suitable detergent.
	3. Allow the vessel with its component parts to soak (for a short time or overnight).
	4. Drain the vessel.
	5. Disassemble and wash the headplate, glass vessel, impellers, baffles
	and other components in hot water and an appropriate laboratory
	detergent.
	6. If necessary, use a very small test tube brush to clean the ports and
	orifices. A soft brush may also be used to remove dried-on material
	trom the glass.
	/. Rinse all components in tap water.
	8. Kinse twice in purified (de-ionized, RO, distilled water). Allow
	everything to dry thoroughly before reassembling the vessel.

#### NOTE:

For easy cleaning, the ring sparger has a removable plug at the tip.

#### 8.5 Port & Adapter O-Ring Replacement

Each time you clean the vessel assembly, check all port and adapter O-rings for wear, distortion, rips or any other damage to their integrity. Replace them as needed.

8.6

When it needs to be replaced, pull the old O-ring out with a knife or a flat-bladed screwdriver. Lubricate the new O-ring with a light coat of silicone grease, then insert it, making sure it is well-seated in the groove.

#### 8.7 Bearing Housing Cap Replacement

Headplate O-Ring Replacement

Check the bearing housing cap after each use for distortion, tears or any other damage to its integrity. Replace the cap as needed.

#### 8.8 Replacing PCU Fuses

To replace a fuse in the PCU:

## **CAUTION!** Always disconnect the unit from the power source first.

- 1. Remove the cover/fuse block located on the rear of the unit with a small screwdriver.
- 2. Remove the old fuse.
- 3. Insert a new fuse of the same type.
- 4. Replace the cover/fuse block into the Fuse Holder/Power Inlet (see Figure 45 below).



#### Figure 45: Fuse Holder/Power Inlet

## 9 SERVICE

If any problems occur with your BioFlo 110 system or its individual components, do not attempt to perform any service on it. Unauthorized servicing may void the warranty. Please contact your local NBS Service Department or your local NBS distributor.

In any correspondence with NBS, please refer to the Model Number (BioFlo 110), Manufacturing Part Number(s) and Serial Number (s) of the problem module. This information is located on the bottom of each unit.

#### 9.1 Troubleshooting

If no information appears on the display, check the following before you call for service:

- 1. Verify that the power cord is correctly connected to the Power Controller and to an outlet of appropriate output.
- 2. If the PCU is powered via the Power Controller, make sure the power cord is correctly connected between both modules. If not, verify that the power cord is correctly connected to the PCU and to an outlet of appropriate output.
- 3. Verify that the Power Controller's power switch is turned On.
- 4. Confirm that the PCU power switch is turned On.
- 5. Check that all control modules are correctly connected to each other.
- 6. Make sure that all cables are attached properly.
- 7. Verify that each module's unit address selector switches are correctly assigned (per Section 5.2).

# 10 REPLACEMENT PARTS & ACCESSORIES

When you order replacement parts, accessory parts, or when you request service information, please provide the Model Number (BioFlo 110), Manufacturing Part Number(s) and Serial Number(s) of your unit. This information is located on the bottom of each module.

#### 10.1 Replacement Parts

Vessel Assembly	NBS Part Number		
FERMENTATION VESSEL ASSEMBLY: glass vessel, headplate, clamping nuts, baffle,			
thermowell, RTD probe, O-rings, port plugs & adapters, exhaust tube, sparge tube, harvest			
tube, direct drive motor & agitation shaft, bearing housing, set of bearing housing caps,			
rotameter, 2 six-bladed Rushton-type impeller	s. Non-jacketed v	essel assemblies also	
include cooling coil, heater blanket, vessel sta	and water valve	e. Jacketed vessel	
assemblies also include jacket water heater.			
Non-Jacketed	1.3L	M1273-1001	
	3.0L	M1273-1002	
	7.5L	M1273-1005	
	14.0L	M1273-1010	
Water-Jacketed	1.3L	M1273-1011	
	3.0L	M1273-1012	
	7.5L	M1273-1015	
	14.0L	M1273-1020	
CELL CULTURE VESSEL ASSEMBLY: glass vessel, headplate, clamping nuts, baffle,			
thermowell, RTD probe, O-rings, port plugs &	adapters, exhaust	tube, sparge tube, harvest	
tube, motor & agitation shaft, bearing housing	, set of bearing ho	using caps, rotameter, 1	
pitched blade impeller. Non-jacketed vessel	assemblies also in	clude cooling coil, heater	
blanket, vessel stand and water valve. Jacketed vessel assemblies also include jacket water			
heater.			
Non-Jacketed, with direct-drive motor	1.3L	M1273-1041	
	3.0L	M1273-1042	
	7.5L	M1273-1045	
	14.0L	M1273-1050	
Non-Jacketed, with magnetic drive motor	1.3L	M1273-1051	
	3.0L	M1273-1052	
	7.5L	M1273-1055	
	14.0L	M1273-1060	
Water-Jacketed, with direct drive motor	1.3L	M1273-1021	
	3.0L	M1273-1022	
	7.5L	M1273-1025	
	14.0L	M1273-1030	
Water-Jacketed, with magnetic drive motor	1.3L	M1273-1031	
	3.0L	M1273-1032	
	7.5L	M1273-1035	
	14.0L	M1273-1040	

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Angled Autoclave Rack	NBS Part Number
For 7.5L & 14.0L vessels	M1273-9266

Batteries & Fuses	NBS Part Number
Power Controller Fuse, 250V, 1.25 Amps	P0380-3750
Power Controller Fuse, 250V, 3.15 Amps	P0380-3751
PCU Fuse, Slo-Blo <sup>®</sup> , 250V, 2 Amps	P0380-3533
Battery, Alkaline, 1.5 VDC	P0480-9030

Exhaust Condenser	NBS Part Number
Exhaust Condenser, 1.3L, 3.0L & 7.5L	M1273-9945
Exhaust Condenser, 14.0L	M1273-9957

Foam Trap Kits	NBS Part Number
Foam Trap Kit, 500 mL bottle (all vessels)	M1273-9943
Foam Trap Kit, 250 mL bottle (all vessels)	M1273-9942

Headplate Port Fittings	NBS Part Number
6.35mm port to 4.76mm tube Adapter (compression)	M1273-5042
12mm port to 4.76mm tube Adapter (compression)	M1273-5055
6.35mm port to 6.35mm tube Adapter (set screw)	M1273-5054
12mm port to 6.35mm tube Adapter (set screw)	M1273-5056
12mm Probe Adapter (compression)	M1273-5040
12mm to 12mm Adapter (set screw)	M1273-5058
6.35mm Plug	M1273-9405
12mm Plug	M1273-9406
19mm Plug	M1273-9407

Heaters & Heat Blankets	NBS Part Number
Jacket Water Heater, 1.3L & 3.0L vessels	M1273-3107
Jacket Water Heater, 7.5L & 14L vessels	M1273-3108
Heat Blanket, 1.3L vessel	M1273-9931
Heat Blanket, 3.0L vessel	M1273-9932
Heat Blanket, 7.5L vessel	M1273-9930
Heat Blanket, 14.0L vessel	M1230-9933

Impellers	NBS Part Number
6-bladed Rushton-type, 52 mm, 1.3L & 3.0L	M1273-9291
6-bladed Rushton-type, 59 mm, 7.5L	M1273-9292
6-bladed Rushton-type, 74 mm, 14.0L	M1273-9293
Pitched Blade, 1.3L & 3.0L	M1273-9290
Pitched Blade, 7.5L & 14.0L	M1230-9212
Spin Filters: see following page	

Motors	NBS Part Number
Direct Drive Agitation Motor, Cell Culture, all vessels	M1273-3135
Direct Drive Agitation Motor, Fermentation, 1.3L & 3.0L	M1273-3120
Direct Drive Agitation Motor, Fermentation, 7.5L & 14.0L	M1273-3125
Magnetic Drive Agitation Motor, all vessels	M1273-3130

Spin Filters		NBS Part Number
Spin Filter, Suspension Cells	1.3L	M1273-3201
	3.0L	M1273-3202
	7.5L	M1273-3205
	14.0L	M1273-3210
Spin Filter, Microcarriers	1.3L	M1273-3211
	3.0L	M1273-3212
	7.5L	M1273-3215
	14.0L	M1273-3220

Tubing	NBS Part Number
Blue Polyurethane Tubing, <sup>1</sup> / <sub>8</sub> -inch (3.2 mm) ID,	P0740-3111
1/4-inch (6.35 mm) OD, 50 feet	
White Silicone Tubing, 3/16-inch (4.8 mm) ID, 5/16-inch	P0740-2505
(7.9 mm) OD, 25 feet	
White Silicone Tubing, 1/16-inch ID (1.6 mm), 3/16-inch	P0740-2396
(4.8 mm) OD, 50 feet	
White, Braided PVC Tubing, 1/4-inch (6.35 mm) ID, 0.438-	P0740-1631
inch (11 mm) OD, 15 feet	

Probes & Sensors	NBS Part Number
dO2 Probe Kit, Ingold polarographic, 1.3L	M1273-9974
dO2 Probe Kit, Ingold polarographic, 3.0L	M1273-9879
dO2 Probe Kit, Ingold polarographic, 7.5L	M1273-9886
dO2 Probe Kit, Ingold polarographic, 14.0L	M1273-9888
dO2 Probe, Ingold polarographic, 1.3L	P0720-6281
dO2 Probe, Broadley James polarographic, 1.3L	P0720-6370
dO2 Probe, Ingold polarographic, 3.0L	P0720-6282
dO2 Probe, Broadley James polarographic, 3.0L	P0720-6371
dO2 Probe, Ingold polarographic, 7.5L	P0720-6283
dO2 Probe, Broadley James polarographic, 7.5L & 14.0L	P0720-6372
dO2 Probe, Ingold polarographic, 14.0L	P0720-6284
Foam Level Probe Kit, 1.3L	M1273-9947
Foam Level Probe Kit, 3.0L	M1273-9951
Foam Level Probe Kit, 7.5L & 14.0L	M1273-9960
pH Probe Kit, Ingold gel-filled, 1.3L	M1273-9970
pH Probe Kit, Ingold gel-filled, 3.0L	M1273-9977
pH Probe Kit, Ingold gel-filled, 7.5L	M1273-9982
pH Probe Kit, Ingold gel-filled, 14.0L	M1273-9985
pH Probe, Ingold gel-filled, 1.3L	P0720-5582
pH Probe, Broadley James gel-filled, 1.3L	P0720-5741
pH Probe, Ingold gel-filled, 3.0L	P0720-5584
pH Probe, Broadley James gel-filled, 3.0L	P0720-5747
pH Probe, Ingold gel-filled, 7.5L	P0720-5580
pH Probe, Broadley James gel-filled, 7.5L	P0720-5742
pH Probe, Ingold gel-filled, 14.0L	P0720-5583
pH Probe, Broadley James gel-filled, 14.0L	P0720-5743
RTD Assembly, all vessels	M1273-8019

Sampler System	NBS Part Number
Sampler Kit, 1.3L	M1273-9946
Sampler Kit, 3.0L	M1273-9949
Sampler Kit, 7.5L	M1273-9953
Sampler Kit, 14.0L	M1273-9956
Syringe, 60 mL	P0440-0054
Disposable Filter	P0200-0970

Spargers	NBS Part Number
Sparger Ring, 1.3L	M1273-9259
Sparger Ring, 3.0L	M1273-9256
Sparger Ring, 7.5L	M1273-9246
Sparger Ring, 14.0L	M1273-9251
Porous Sparger, 1.3L, non-jacketed vessel	M1273-5007
Porous Sparger, 1.3L, water-jacketed vessel	M1273-5003
Porous Sparger, 3.0L, jacketed or non-jacketed vessel	M1273-5004
Porous Sparger, 7.5L, jacketed or non-jacketed vessel	M1273-5005
Porous Sparger, 14.0L, jacketed or non-jacketed vessel	M1273-5006

#### 10.2 Replacement Vessels

Part Description	NBS Part Number
1.3L Glass Vessel, non-jacketed	M1273-9907
1.3L Glass Vessel, water-jacketed	M1273-9908
3.0L Glass Vessel, non-jacketed	M1273-9909
3.0L Glass Vessel, water-jacketed	M1273-9915
7.5L Glass Vessel, non-jacketed	M1273-9916
7.5L Glass Vessel, water-jacketed	M1273-9917
14.0L Glass Vessel, non-jacketed	M1273-9918
14.0L Glass Vessel, water-jacketed	M1273-9919

#### 10.3 Miscellaneous Parts & Optional Accessories

Description	NBS Part Number
Addition/Harvest Bottle Kit, 250 mL	M1273-9989
Addition/Harvest Bottle Kit, 500 mL	M1273-9990
Air Filter /Regulator Kit (single manifold for one vessel)	M1230-3030
Air Filter /Regulator Kit (four manifolds for up to four vessels)	M1230-5002
Bearing Housing Cap, disposable (for autoclaving), pack of 10	M1273-9936
O-Ring Kit (washers & O-rings, including headplate), ALL vessels	M1273-9900
Septum Kit for 12mm port	M1273-3031
Spare Parts Kit, 1.3L – 3.0L non- jacketed vessel	M1273-9991
Spare Parts Kit, 7.5L – 14.0L non- jacketed vessel	M1273-9992
Spare Parts Kit, 1.3L – 3.0L water-jacketed vessel	M1273-9998
Spare Parts Kit, 7.5L – 14.0L water-jacketed vessel	M1273-9999
Water Regulator/Filter Kit (single manifold for one vessel)	M1117-2040
Water Regulator/Filter Kit (four manifolds for up to four vessels)	M1273-5001

Power Controller	NBS Part Number
Kit contains the power controller, 4 cable guides, power cord.	
100-120V	M1273-3100
200-240V	M1273-3110

Primary Control Unit (PCU)	NBS Part Number
Kit contains PCU, display & keypad, floppy disk drive,	M1273-3101
battery back-up, 2 cable guides, power line.	

dO2/pH Controller	NBS Part Number
Kit contains the dO2/pH controller, 1 Ingold gel pH probe & 1	ngold polarographic dO2 probe,
2 headplate probe adapters, 2 probe cables, 2 probe shorting	caps, module entry cable.
For 1.3L Vessel	M1273-9850
For 3.0L Vessel	M1273-9851
For 7.5L Vessel	M1273-9852
For 14.0L Vessel	M1273-9853
dO2 /pH Controller only	M1273-3102
Level Controller	NBS Part Number
Kit contains the level controller, 1 level sensor, 1 probe cable,	1 headplate probe adapter, 2
cable guides, module entry cable.	
For 1.3L Vessel	M1273-9841
For 3.0L Vessel	M1273-9842
For 7.5L or 14.0L Vessel	M1273-9843
Level Controller only	M1273-3103

Gas Mix Controller	NBS Part Number
Kit contains gas mix controller, module entry cable,	
polyurethane tubing, 2 cable guides.	M1273-3104

4-Pump Module	NBS Part Number
Kit contains the pump housing box, 4 peristaltic pumps, power	r line, 2 cable guides.
100-120V	M1273-3106
200-240V	M1273-3116

Thermal Mass Flow Controller	NBS Part Number	
Kit contains the thermal mass flow controller, module entry cal transformer, polyurethane tubing, 2 cable guides.	ble, 24VDC power line with	
Fermentation application, 20 SLPM	M1273-3109	
Cell Culture application, 5 SLPM	M1273-3112	

## 11 APPENDIX A: SPECIFICATIONS

#### 11.1 Modules

BioFlo 110 PCU				
Weight, Overall	5 lb. (2.3 kg)			
Dimensions	Height 6 in. (1			15.3 cm)
	Depth*		13¾ ir	n. (35 cm)
	Width		12 in.	(30.5 cm)
	* allow 3 inches	(7.6 cm) mo	re, front and rear,	for cable access
Display	3.4" x 4.5" (86.4	mm x 114.3	mm) electrolumir	escent monochrome, bright
	white (on blue so	creen), ¼ V0	GA resolution	-
Control	Loop Name		Display Range	Control Modes
	Agitation		0-1200 rpm	Off, Automatic
<ul> <li>Per vessel,</li> </ul>			(fermentation)	
up to four			0-300 rpm (cell	
vessels total			culture)	
	Temperature		0-70.0° C	Off, Automatic
• Up to 32	pH (with dO2/pH	Module)	2-12.0 pH units	Off, Automatic:
loops total				PID, adjustable deadband,
				liquid additions, 4-gas
				blending
	dO2 (with dO2/pH Module)		0-100%	Off, Automatic: PID,
				agitation cascade, sparge-on-
				demand, 4-gas blending
	Pump 1		0-100.00%	Off, Manual, Automatic
				(with conduction probes)
	Pump 2		0-100.00%	Off, Manual, Automatic
				(with conduction probes)
	Pump 3		0-100.00%	Off, Manual, Automatic
	Duran 4		0.400.000/	(with conduction probes)
	Pump 4		0-100.00%	
			0.4000/	(with conduction probes)
	OZ (WITH Gas IVII.	x	0-100%	Off, Manual, Automatic
Touchpad	Controller)	Lloor intorf	l 200: 24 touch cor	l sitivo huttons
		Supporte flace. 24 louch-sensitive bullons		
Computer connectivity		Supports II	asire-promupyia	d ModBus protocols
Computer connectivity		Senar port, supports AFS and MOOBUS protocols		
Recorder connectivity FOUF U-2.5			/ ^	
Power Switch	toh		$C \frac{30}{60} = \frac{100}{12}$	/A
Power Switch Located of Apple Angle		alino hattorios		
Eucing	inory back-up I wo AA all			
Dowor Indicator Li	2 Amp, 250V SIO-BIO"			
Other	Calor Light Located on front.			
Other				6
Cabling/tubing organizers				

BioFlo 110 Power Controller				
Weight	16 lb. (7.2 kg)			
Dimensions	Height	8 9/16 in. (21.8 cm)		
	Depth*	12 in. (30.5 cm)		
	Width	12 in. (30.5 cm)		
	*allow 3 inches (7	6 cm) more, front and rear, for cable access		
Agitation	Drive	Lift-off electric motor.		
		<ul> <li>Self-aligning motor and vessel shafts.</li> </ul>		
		Direct drive with surface seal for fermentation and		
		cell culture.		
		Magnetic drive for cell culture.		
	Speed Range	Fermentation: 50-1200 rpm		
		Cell culture: 30-300 rpm		
	Impellers	Standard Rushton; optional pitched blade		
Temperature	Range	5°C above ambient temperature to 70°C		
	System	$20-40^{\circ}C \pm 0.1^{\circ}C$ (± 0.5°C at other temperatures)		
	Accuracy			
	Control Stability	0.1°C		
	Sensor	One 100 platinum RTD, input located on front of console		
	Control	P-I control algorithm		
	SSR PWM	For heat blanket and for cooling solenoid valve		
	Outputs			
Electrical		115/230 VAC, 50-60 Hz, 1500 VA		
Output Connector	S	Five controlled (PWM) IEC outlets for pumps		
		Vessel Heater		
		Vessel Cooling Solenoid Valve		
		One switched mains outlet for PCU		
		Agitation motor		
Inputs		<ul> <li>Tachometer (joint with agitation output)</li> </ul>		
		RTD (temperature probe)		
Power Switch		Located on front.		
Pilot Light		Located on front. Illuminates when power is on.		
Other		Hangers for 2 Rotameters.		
		Hanger for cooling water valve.		
		Cable/tubing organizers		

	BioFlo	o 110 dO2/pH Controller	
Weight		5 lb. (2.3 kg)	
Dimensions	Height	2 5/8 in. (6.7 cm)	
	Depth*	12 in. (30.5 cm)	
	Width	12 in. (30.5 cm)	
	*allow 3 inches (7.	6 cm) more, front and rear, for cable access	

	allow 5 mones (7.6 cm) more, noncand real, for cable access		
Inputs	pH Probe	NBS-supplied cable	
	Dissolved	Polarographic electrode with NBS-supplied cable.	
	Oxygen Probe		
Control Options	See PCU Specification		
Power	12 VDC (supplied by PCU)		

BioFlo 110 Antifoam/Level Controller			
Weight		4 lb. (1.8 kg)	
Dimensions	Height	4 in. (10.2 cm)	
	Depth*	12 in. (30.5 cm)	
	Width	12 in. (30.5 cm)	
	*allow 3 inches (7.6 cm) more, front and rear, for cable access		
Inputs	Three 3-pin DIN	Operating Modes	
	connectors for	Antifoam additions	
	conduction	Wet ON: pump turns on when probe is wetted	
	probes.	Wet OFF: pump turns off when probe is wetted	
Power	12 VDC (supplied	by PCU)	

BioFlo 110 Four-Pump Module					
Weight	14 lb. (6.4 kg)				
Dimensions	Height	12 in. (30.5 cm)			
	Depth* 12 in. (30.5 cm)				
	Width	12 in. (30.5 cm)			
	*allow 3 inches (7.6 cm) more, front and rear, for cable access				
Pumps	Four peristaltic type pumps, independently powered				
Power	Line voltage (as I	abeled), supplied through Power Controller			

BioFlo 110 Gas Mix Controller					
Weight		5 lb. (2.3 kg)			
Dimensions	Height	4 in. (10.2 cm)			
	Depth*	10 in. (25.4 cm)			
	Width	10 in. (25.4 cm.)			
	*allow 3 inches (7.6 cm) more, front and rear, for cable access				
Range	0-100%				
Valves	4 solenoid valves				
Inputs	4 gas inputs (oxygen, air, carbon dioxide and nitrogen)				
Output	1 gas stream output (to sparger)				
Power	12 VDC (supplied by PCU)				
Maximum Gas	10 PSI				
Pressure					

#### 11.2 Pump Flow Rates

The following table lists pump flow rates according to tubing size, pump frequency and rotation speed.

Tubing ID (silicone only)	50 Hz / 12 RPM	60 Hz / 14.4 RPM
1/50 inch / 0.5 mm	0.25 ml/min	0.30 ml/min
1/32 inch / 0.8 mm	0.59 ml/min	0.71 ml/min
1/16 inch / 1.6 mm	2.62 ml/min	3.14 ml/min
1/8 inch / 3.2 mm	9.60 ml/min	11.5 ml/min
3/16 inch / 4.8 mm	19.6 ml/min	23.5 ml/min

#### 11.3 Tubing

Description	ID	OD	Length	NBS Part Number
Blue polyurethane	1/8 inch	<sup>1</sup> / <sub>4</sub> inch	50 feet	P0740-3111
	3.2 11111 2/10 in ab	0.33 IIIII		
vvnite sliicone	4.8 mm	5/16 Inch 7.9 mm	25 teet	P0740-2505
White silicone	1/16 inch	3/16 inch 4 8 mm	50 feet	P0740-2396
White braided PVC	1 <sup>/</sup> 4 inch	0.438 inch	15 feet	P0740-1631
	6.35 mm	11.13 mm		

The following tubing sizes are provided with your BioFlo 110 system:

#### 11.4 Vessels

BioFlo 110 Non-Jacketed Vessels						
Vessel Volume	Total Volume		1.3 L	3.0 L	7.5 L	14.0 L
	Working	Max.	1.0 L	2.2 L	5.6 L	10.5 L
	Volume	Min.	0.4 L	0.8 L	1.5 L	4.0 L
Vessel Weight	with Headpl	ate,	15 lb.	20.5 lb.	39.5 lb.	43 lb.
	Stand, Drive	Э,	(6.8 kg)	(9.3 kg)	(18 kg)	(19.5 kg)
	Inserts & Pl	ugs				
Stand	Non-Jacket	ed	Stainless steel, four legs for stability, high friction feet to			riction feet to
	Vessels		eliminate "walking".			
Aeration	Max. Flow F	Rate	Fermentation	: 2 VVM		
			Cell Culture:	0.5 VVM		
	Sparger		Stainless ste	el ring sparger	with removable	EPDM plug
	Maximum S	Sparge	10 PSIG			
	Pressure					
	Filters		0.2µ absolute filters, resterilizable			
Exhaust	Condenser		Optional, mounted in headplate			
	Filter		0.2µ absolute filter, resterilizable			

#### 11.4.1 Non-Jacketed Vessel Dimensions for Autoclaving (Vertical)

Non-Jacketed Vessel Assembly with supplied vertical stand & as prepared for autoclaving					
Total Vessel Volume         1.3 L         3.0 L         7.5 L         14.0 L					
Height without Exhaust Condenser	16.5 in.	16.5 in.	19.5 in.	24 in.	
(to top of bearing housing)	(42 cm)	(42 cm)	(49.5 cm)	(61 cm)	
Diameter without Exhaust Condenser	8.5 in.	8.5 in.	11.5 in.	11.5 in.	
	(22 cm)	(22 cm)	(29 cm)	(29 cm)	

...continued...

Non-Jacketed Vessel Assembly with supplied vertical stand & as prepared for autoclaving						
Total Vessel Volume	Total Vessel Volume         1.3 L         3.0 L         7.5 L         14.0 L					
Height with Exhaust Condenser	22 in.	22 in.	25.5 in.	29 in.		
(to top of bent-over exhaust filter)	(56 cm)	(56 cm)	(65 cm)	(74 cm)		
Width with Exhaust Condenser	8.5 in.	8.5 in.	11.5 in.	11.5 in.		
	(22 cm)	(22 cm)	(29 cm)	(29 cm)		
Length with Exhaust Condenser	9.5 in.	9.5 in.	14.5 in.	11.5 in.		
	(24.1 cm)	(24.1 cm)	(37 cm)	(29 cm)		

The above dimensions do not include optional addition bottles or optional sampler, which may be mounted to the vessel assembly for autoclaving; they can also be autoclaved unattached.

- If addition bottles are attached, add the following approximate dimensions to vessels *without* exhaust condenser:
  - $\blacktriangleright$  +4 cm to diameter
  - $\blacktriangleright$  +8 cm to height of 1L and 3L vessels
  - $\rightarrow$  +10 cm to height of 7.5L and 14L vessels.
- Addition bottles add no height to vessels with condenser.
- Sampler adds approximately +4 cm to diameter.

#### 11.4.2 Non-Jacketed Vessel Dimensions for Autoclaving (Inclined)

#### NOTE:

## For use with an Angled Autoclave Rack, the attachment of addition bottles is not recommended.

Non-Jacketed Vessel Assembly with optional Angled Autoclave Rack (M1273-9266) & as prepared for autoclaving					
Total Vessel Volume   7.5 L   14 L					
Height with Exhaust Condenser	18 in. (46 cm)	20 in. (51 cm)			
Length with Exhaust Condenser	28 in. (71 cm)	31.5 in. (80 cm)			
Width with Exhaust Condenser	11.5 in. (29 cm)	11.5 in. (29 cm)			

#### 11.4.3 Water-Jacketed Vessel Dimensions for Autoclaving (Vertical)

Water-Jacketed Vessel Assembly as prepared for autoclaving						
Total Vessel Volume         1.3 L         3.0 L         7.5 L         14.0 L						
Height without Exhaust Condenser	16 in.	18 in.	20.5 in.	27 in.		
(to top of bearing housing)	(41 cm)	(46 cm)	(52 cm)	(69 cm)		
Diameter without Exhaust Condenser	9.25 in.	9.25 in.	11.5 in.	11.5 in.		
	(23.5 cm)	(23.5 cm)	(29 cm)	(29 cm)		
Height with Exhaust Condenser	20.5 in.	22.5 in.	24.5 in.	31 in.		
(to top of bent-over exhaust filter)	(52 cm)	(57 cm)	(62 cm)	(79 cm)		
Width with Exhaust Condenser	9.5 in.	9.5 in.	11.5 in.	11.5 in.		
	(24.1 cm)	(24.1 cm)	(29 cm)	(29 cm)		
Length with Exhaust Condenser	11.5 in.	11.5 in.	11.5 in.	11.5 in.		
	(29 cm)	(29 cm)	(29 cm)	(29 cm)		

## CAUTION! Never autoclave the jacket water heater. Unplug and remove it.

The above dimensions do not include optional addition bottles or optional sampler, which may be mounted to the vessel assembly for autoclaving; they can also be autoclaved unattached.

- If addition bottles are attached, add the following approximate dimensions to vessels *without* exhaust condenser:
  - $\blacktriangleright$  +4 cm to diameter
  - $\rightarrow$  +8 cm to height of 1L and 3L vessels
  - $\rightarrow$  +10 cm to height of 7.5L and 14L vessels.
- Addition bottles add no height to vessels with condenser.
- Sampler adds approximately +4 cm to diameter.

#### 11.4.4 Non-Jacketed Vessel Dimensions for Autoclaving (Inclined)

#### NOTE:

For use with an Angled Autoclave Rack, the attachment of addition bottles is not recommended.

Water-Jacketed Vessel Assembly					
with optional Angled Autoclave Rack (M1273-9266) & as prepared for autoclaving					
Total Vessel Volume   7.5 L   14 L					
Height with Exhaust Condenser	18.5 in. (47 cm)	20.5 in. (52 cm)			
Length with Exhaust Condenser	26 in. (66 cm)	28.5 in. (72 cm)			
Width	11.5 in. (29 cm)	11.5 in. (29 cm)			

## 

Never autoclave the jacket water heater. Unplug and remove it.

## 12 APPENDIX B: TECHNICAL INTERVENTIONS

#### NOTE:

Only a qualified technician should perform the procedures contained in this chapter.



Always disconnect the power cord from the Power Controller and the PCU before undertaking any of the following procedures.

#### 12.1 Replacing Power Controller Fuses

There are eight replaceable fuses inside the power controller:

Fuse Number	Item Controlled	Fuse Number	Item Controlled
F1	Pump A	F5	Acid Pump
F2	Pump B	F6	Water Solenoid Valve
F3	Pump C	F7	Heater
F4	Base Pump	F8	Heater

To replace one of these fuses:

- 1. Unplug all power cords from the system.
- 2. Disconnect the Power Controller from any other modules.
- 3. Unplug the water solenoid valve from the rear panel.
- 4. Unplug the motor, RTD and heater cables from the front panel.
- 5. Loosen the two screws that secure the water solenoid valve assembly in place, then remove the assembly by sliding the mounting plate up until the slotted holes allow you to lift the assembly away from the screws.
- 6. Unscrew the ten side mounting screws that hold the Power Controller cover in place. Retain them for reuse.
- 7. Carefully pull the cover straight up until it separates from the base. There will be some resistance until it comes unclipped. Be careful not to pull on the ground wire that is attached to the front of the left side (from the rear).
- 8. Rest the cover on its front panel, adjacent to the base.

- 9. Grasp the fuse and pull it straight up until it separates from its base.
- 10. Insert a new fuse, taking care to align its two pins with the pinholes in the base. (If you are replacing one of the heater fuses, use a continuity meter to determine which fuse is blown.)
- 11. Ease the cover back in place, lowering it straight down, until it fits tightly. Take care to tuck the ground wire inside, clear of both the cover and the lip of the base.
- 12. Reinstall the side mounting screws. Tighten securely.
- 13. Reinstall the water solenoid valve assembly, tightening the screws against the mounting plate.
- 14. Reconnect the motor drive, RTD and heater to the front panel.
- 15. Reconnect the water solenoid valve to the rear panel.
- 16. Reconnect the Power Controller to the other module(s).
- 17. Reconnect the power cord.

#### WARNING!

Always disconnect the power cord from the Power Controller and PCU before undertaking the following procedures.

#### 12.2 Replacing PCU Batteries

- 1. Unplug all power cords from the system.
- 2. Disconnect the PCU from any other modules.
- 3. Unscrew the eight side mounting screws that hold the cover in place. Retain them for reuse.
- 4. Carefully pull the cover straight up until it separates from the base. Be careful not to pull on the ground wire that is attached to the front of the left side (from the rear), or on the ribbon cables that are attached to the front of the cover.
- 5. Rest the cover on its front panel, adjacent to the base.
- 6. The two AA alkaline batteries are housed in the battery holder at the front of the base. Cut the plastic tie wrap. Remove, then replace the batteries, taking care to align them properly (the polarity is marked in the bottom of the battery holder).
- 7. Replace the tie wrap (this is optional).
- 8. Ease the cover back in place, lowering it straight down, until it fits tightly. Take care to tuck the ground wire and the ribbon cables inside, clear of both the cover and the lip of the base.
- 9. Reinstall the side mounting screws. Tighten securely.

### WARNING!

Always disconnect the power cord from the Power Controller and PCU before undertaking any of the following procedures.
## NOTE:

# If you move your BioFlo 110 to a new location that requires a change in voltage and/or fusing, be sure to consult your NBS representative.

Locate the fuse drawer on the back panel of the Power Controller, next to the power plug. The voltage selection is displayed in a small window in the middle of the fuse drawer. The Power Controller is preset at 115 Volts.

If the voltage needs to be changed to 230V:

1. Using a narrow-tipped, flat-bladed screwdriver, introduce the blade into the small slot to the left of the fuse drawer.



Figure 46: Voltage Selection

- 2. Use the blade to pry the lip of the plastic lock from left to right, then gently lift up toward you. The drawer will pop out.
- 3. Pull the entire drawer assembly out and set it aside.
- 4. Use the screwdriver blade to pull the selector cube out (from the left side, insert the blade into the hollow of the cube).
- 5. Turn it 180° to display **115**, then snap it back into place. Be sure to press it firmly until it snaps.
- 6. Reinsert the fuse drawer, and snap it into place.

## NOTE:

If you are using pumps (with or without the Pump Module), you will need to replace them with pumps configured for the new voltage.

## ROTE:

Although the voltage selector is located in a housing termed "fuse drawer", it contains no fuses. The fuse drawer contains a spring and a blank. Be sure to keep these intact and in place when you reinstall the fuse drawer.

## 12.4 Changing PCU Fusing

If you are changing the voltage on your Power Controller, it may also be necessary to modify the fusing arrangement on the PCU.

## NOTE:

Be sure to discuss this with your NBS representative before a qualified technician makes any modifications to the unit.

## 12.4.1 Converting from Single Fuse to Double Fuse

Your BioFlo 110 PCU was initially set up with a single fuse arrangement. To change to a double fuse:

- 1. Open the cover of the power module using a small blade screwdriver and remove the cover/fuse block assembly (*see Figure 47 below*).
- 2. Loosen the Phillips screw behind the fuse block 2 full turns(*see Figure 48 below*).
- 3. Remove the fuse block by sliding it up and away from the screw shaft and lifting it off the pedestal (*see Figure 48 below*).
- 4. Invert the fuse block and slide it back onto the Phillips screw and pedestal. Tighten the Phillips screw.









- 5. Verify the correct fusing arrangement (see Figures 49a and 49b below).
- 6. Insert fuses.
- 7. Replace the cover unit into the fuse holder.



Figure 49b: Single Fusing Arrangement



12.5 P&I Control Equations

## NOTE:

The following information is provided for the use of advanced P & I algorithm users only.

## 12.5.1 pH

As mentioned in Section 7.6.3, following is the P & I control equation for pH:

**Out%** = **P** \* 
$$\frac{100\%(SP - CV)}{14pH}$$
 + **I** \*  $\frac{100\%}{14pH}$  **J** (SP-CV) dt

Where P is dimensionless and I is in min<sup>-1</sup>.

## 12.5.2 dO2

As mentioned in Section 7.9, following is the P & I control equation for dO2:

**Out%** = **P** \* 
$$\frac{100\%}{100dO2}$$
 (**SP-CV**) + **I** \*  $\frac{100\%}{100dO2}$   $\int$  (**SP-CV**) dt

Where P is dimensionless and I is in min<sup>-1</sup>.

# 13 APPENDIX C: COMMUNICATIONS PROTOCOLS

## L CAUTION!

This section is provided FOR ADVANCED PROGRAMMERS ONLY. Do not attempt to apply or change any of this information unless you are professionally trained in these matters.

Interventions by unskilled operators may severely compromise the operation of your BioFlo 110 system and may also void the warranty.

## 13.1 Communications Settings

The onboard computer has two RS-232 serial ports, labeled Comm1 and Comm2. Both ports are connected to the keyboard interface board (KIB), which converts them to RS-485.

**Comm1** is set up for connection to an NBS AFS (BioCommand) supervisory software interface box. (*See also Section 13.2.*)

**Comm1** can also be used for connection to a computer equipped with an RS-485 (ModBus) port. This allows the operator to control the BioFlo 110 with the remote computer. (*See also Section 13.3.*)

Comm1 Settings	For AFS Use	For ModBus Use
Baud Rate	9600	19200
Data Bits	8	8
Stop Bits	2	1
Parity	Even	None

**Comm2** is set up to communicate with the other BioFlo 110 control modules, which are daisy-chained together. Terminators **must** be installed on any and all unused connectors.

Comm2 Settings				
Baud Rate	38400			
Data Bits	8			
Stop Bits	1			
Parity	None			

## 13.1.1 AFS/ModBus Pin Connections

Pin	Signal	Observations
Number		
13	RS422 Tx+	
25	RS422 Tx-	
24	RS422 Rx+	
12	RS422 Rx -	
7	Ground	
2	RS232 Tx	
3	RS 232 Rx	
21	Mode	Open for RS232.
		Connect to 7 for RS422

The AFS/ModBus 25-pin "D" connector on the rear panel is pinned out as follows:

## 13.2 AFS (BioCommand) Communications Protocol

This RS422 communications protocol specification is for "Slave" equipment. "Slave" refers to any instrument that is interfaced to the NBS BioCommand (also called AFS) system, and "Master" refers to the BioCommand supervisory interface module.

### 13.2.1 General Specifications

- All line drivers and receivers must conform to IEEE RS-422 or RS-485 standards.
- Communications settings are listed in Section 13.1 above. There is no CTS/RTS or XON/XOFF handshaking.
- The Master initiates all transactions.
- A Slave transmit driver must be held in a high impedance state (tri-stated) at all times, except when it is responding to a transaction initiated by the Master.
- All transactions are carried out sequentially (one at a time).
- All transactions are prefixed with a single byte that indicates the multidrop ID of the Slave station involved.
- Valid Slave unit ID numbers are 0 through 99.
- All transmissions from the Master are terminated with a carriage return (0DH) character.
- Al responses to data requests are terminated with a carriage return (0DH) character.
- The Slave shall respond to a Change command from the Master with an ACK (06H) character.
- If the Slave detects an error in the transmission from the Master, it shall make no response.
- If the Slave successfully receives a transmission from the Master, it must begin the appropriate response in less than 200 mSec.

## 13.2.2 Protocol

- All Slaves must respond to the Requests for data shown in Section 13.2.3 below.
- The Response Header for each Request must be as shown in Section 13.2.3 below.
- All Headers must contain 9 characters; where necessary, spaces are used to complete the 9 characters. In Section 13.2.3, a space is represented by "(Sp)".
- The format of the Response must be as shown in Section 13.2.5 below.
- All Messages consist of a Header plus 10 Fields.
- All Fields must contain 6 characters; where necessary, spaces are used to complete the 6 characters.
- If there is no data for a particular Field, the Slave must fill that Field with space (20H) characters.
- All Slaves must respond to Change commands as shown in Section 13.2.4 below.
- The Headers for each Change command are shown in Section 13.2.4 below.
- The Slave response to a Change command shall be an ACK (06H) character.
- Even if a Change command is not applicable or not implemented by the Slave, the Slave must still acknowledge the command.
- When a Field in a Change command is blank, the Slave shall not change the settings for the loop or parameter associated with that Field.
- Numeric values in Fields must be ASCII decimal format.

## 13.2.3 Requests & Response Headers

Request Type		Request Format	Response Header†
Get Loop Names	*	(MD#)RA(CR)	Loop(Sp)(Sp)(Sp)(Sp)(Sp)
Get Current Values	*	(MD#)RC(CR)	Cur:Value
Get Setpoints		(MD#)RD(CR)	OP Points
Get Controller Outputs		(MD#)RE(CR)	Output(Sp)%(Sp)
Get Control Modes	*	(MD#)RJ(CR)	Control(Sp)(Sp)
Get Units	*	(MD#)RL(CR)	Main(Sp)Unit

\* All controllers must respond to these four requests or they will not be recognized by BioCommand.

† All Headers must contain a total of 9 characters

(MD#) = Multidrop ID Number	(CR) = Carriage Return	(Sp) = Space
-----------------------------	------------------------	--------------

### 13.2.4 Change Commands

Change Command	Command Header†
Change Setpoints	MS(Sp)(Sp)(Sp)(Sp)(Sp)(Sp)(Sp)
Change Controller Output	MO(Sp)(Sp)(Sp)(Sp)(Sp)(Sp)(Sp)
1 11 11 1 1	

 $\dagger$  All Headers must contain a total of 9 characters (Sp) = Space

### 13.2.5 Message Formats

#### **Response and Change command message format:**

MD#	Header 9 chars	S p	Field 1 6 chars	S p	Field 2 6 chars	S p	Field 3 6 chars	S p	•	•••	Field 9 6 chars	S p	Field 10 6 chars	S p	CR
		а		а		а		а				а		а	
		С		С		С		С				С		С	
		е		е		е		е				е		е	

All Headers must contain a total of 9 characters.

All Fields must contain a total of 6 characters.

(MD#) = Multidrop ID Number Chars = Characters (CR) = Carriage Return

### **Request format:**

MD#	Request	CR
	2 chars	

(MD#) = Multidrop ID Number Chars = Characters (CR) = Carriage Return

### Change command acknowledge format:

MD# ACK

(MD#) = Multidrop ID Number ACK = Acknowledgement character

### 13.3 ModBus Communications Protocol

- All BioFlo110 parameters are represented by Register assignments (see Register Map in Section 13.3.1 below).
- The BioFlo 110 implements Modbus functions 03, 06 and 16 decimal.
- All registers are transmitted high byte first then low byte (standard Modbus format).
- All floating point values are stored in two-Register pairs, with the higher half in the first (even) Register and the lower half in the second (odd) Register. Therefore, floating point values are transmitted as four bytes, starting with the highest and ending with the lowest.

- Character strings are stored in registers, with one character per register. The high byte of the returned Register value will contain 0 and the low byte will contain the character code.
- Integer values are stored in a single Register.

CONTROL TYPE VALUES				
Control Type	Value			
OFF	= 0X00			
Auto	= 0X01			
Manual	= 0X02			
ON/OFF	= 0X03			
P-DB*	= 0X04			
Total	= 0X0B			

\* P-DB means Proportional with Deadband

## 13.3.1 Register Map

## NOTE:

Programmers take note that all of the following are decimal addresses.

Loop Number:	0	1	2		31	Formula				
Parameters	Decimal Address									
1-Curr_val:	0	2	4		62	0 + (N Loop * 2)				
2-Setpoint:	64	66	68		126	64 + (N Loop * 2)				
3-Control_out:	128	130	132		190	128 + (N Loop * 2)				
4-Integral:	192	194	196		254	192 + (N Loop * 2)				
5-Derivative:	256	258	260		318	256 + (N Loop * 2)				
6-Raw_float:	320	322	324		382	320 + (N Loop * 2)				
7-Cal_gain:	384	386	388		446	384 + (N Loop * 2)				
8-Zero_std_off:	448	450	452		510	448 + (N Loop * 2)				
9-Eng_units:	512	516	520		636	512 + (N Loop * 4)				
10-Raw_input:	642	643	644		673	642 + (N Loop)				
11-Cal_offset:	676	677	678		707	676 + (N Loop)				
12-Loop_name:	740	756	772		1236	740 + (N Loop * 16)				
13-Control_mode:	1268	1276	1284		1516	1268 + (N Loop * 8)				

## NOTE:

Parameters 1 through 8 use two registers each, Parameter 9 uses four registers, Parameters 10 & 11 use one register each, Parameter 12 uses sixteen registers and Parameter 13 uses eight registers.

# 14 APPENDIX D: THERMAL MASS FLOW CONTROLLER

The Thermal Mass Flow Controller (TMFC) replaces the rotameter. The TMFC will give you the ability to display gas flow rate on the PCU. This digital gas flow rate can also be logged to data acquisition software, such as *BioCommand Lite* or *BioCommand Plus*.

When you use it with the dO2/pH Controller, you can automatically adjust the gas flow into the system, using either direct operator interaction, setpoint manipulation, or cascade control from the DO loop.

## NOTE:

In Section 1 of this manual, you read that the PCU serves as the operator interface for one to four vessels. Because the PCU cannot support more than a total of 16 modules, adding the Thermal Mass Flow Controller limits the PCU to a maximum of three vessels.

**Section 2** (*Inspection & Unpacking*), **Section 3** (*Preparing the Location*) in this manual provide essential information; be sure to read them prior to installing this optional module.

## WARNING!

Be sure you are familiar with all warnings and precautions in the body of this manual, which are provided for your safety and for the protection of your BioFlo 110 system.

## 14.1 Installation

The Thermal Mass Flow Controller (TMFC) has the same footprint as the Level Controller. You can place it on your module stack, or beside other modules.

With reference to Figure 8 (*Inconnecting the Modules*), repeated on the following page for reference, and Figure 50 (*TMFC Module Rear Panel*), daisy chain the TMFC module to the others in your stack. Remember that it is powered by the Power Controller and controlled by the PCU, so it must be daisy chained.



Figure 8: Interconnecting the Modules





## NOTE:

Be sure to check the setting of the Unit Address Selector on the rear panel, to be sure it is the same as the other control modules for this system. For details, see Section 5.2.

With reference to Figure 51 below:

- 1. **If you have the Gas Mix module**: connect the Gas Mix module output to the TMFC Gas Input; **if you do not have the Gas Mix module**: connect your air supply to the TMFC Gas Input.
- 2. Connect the TMFC Gas Output connector to the sparger inlet with the tubing provided.



Figure 51: TMFC Front View

## 14.2 Loading Software Update

If you purchased the optional TMFC module with the rest of your BioFlo 110 system, the supervisory software is fully configured for its presence; **skip to Section 14.3**, *Operation*.

If you are adding the TMFC module to a pre-established BioFlo 110 system:

- 1. In the PCU's floppy drive (see Figure 1), load the update disk shipped with the TMFC.
- 2. After the system boots, then follow any instructions that appear on the display screen.
- 3. When the initial setup screen appears (*see sample on the following page*), you are ready for operation.



## 14.3 Operation

Before proceeding through the following instructions, be sure to familiarize yourself with the PCU display, selector buttons, and the various interface screens described in Section 7 (*Operation*) of this manual.

With a dO2/pH Controller is present in the system, there are four possible controller combinations that determine the operation of the eighth loop on the fermentor, as recapped in the following table:

Module(s)	Module(s) Present 8th Loop		Operation
Gas Mix	TMFC		
NO	NO	N/A	Only option for dO2 control is a cascade to the Agitation loop.
YES	NO	"O2"	As explained in the Operation section of this manual.
NO	YES	"Gas"	Pressing the selector key for the 8th loop opens the <i>Gas</i> <i>Loop Details</i> screen, where you can set the Control mode to <b>Auto</b> or <b>Off</b> ; enter <b>Setpoint</b> (0-20 SLPM in Fermentation mode or 0-5 SLPM in Cell Culture mode) & set <b>Hi</b> and <b>Lo</b> display limits.
			The <i>dO2 Setup</i> screen shows Gas Flow cascade <b>Hi</b> and <b>Lo</b> limits in addition to the above information. Press the appropriate selector keys to change these limits. Pressing the <b>Cascade</b> key in the <i>dO2 Setup</i> screen reveals dO2 cascade selections of <b>Agitation</b> ("Agit"), <b>Gas Flow</b> ("Gas"), <b>Agitation/Gas Flow</b> ("Agit/Gas"), or <b>None</b> .

...continued...

Module(s) Present		8th Loop	Operation
Gas Mix	TMFC		
YES	YES	"Gas"	In Fermentation mode, the system is configured for gas flow control with oxygen supplementation. Press the <i>Gas</i> loop selector key to open the <i>Gas Control</i> screen, where you can use the selector keys to set these parameters for gas flow: Control mode ( <b>Auto</b> or <b>Off</b> ), Setpoint (0-20 SLPM), oxygen enrichment ( <b>O2 Mix</b> ) Control mode and output percentage ( <b>O2%</b> ).
			The <i>dO2 Setup</i> screen shows Gas Flow cascade <b>Hi</b> and <b>Lo</b> limits in addition to the above information. Press the appropriate selector keys to change these limits. Pressing the <b>Cascade</b> key in the <i>dO2 Setup</i> screen reveals dO2 cascade selections of <b>Agitation</b> ("Agit"), <b>Gas Flow</b> ("Gas"), <b>Gas Flow/Oxygen Enrichment</b> ("Gas/O2"), <b>Agitation/Gas Flow</b> ("Agit/Gas"), <b>Agitation/Gas Flow</b> ("Agit/Gas"), <b>or None</b> .
YES	YES	"Gas"	In Cell Culture mode, the system is configured for dO2/pH control using the NBS gas mixing algorithm (see Section 7.9) and separate gas flow control. Press the Gas loop selector key to open the Gas Mix screen. This screen works as explained in Section 7.11.1, but there is also one additional element: <b>Gas Flow control</b> . Pressing the Gas Flow selector key allows you to select the gas flow Control mode and Setpoint (0-5 SLPM).
			The <i>dO2 Setup</i> screen shows Gas Flow cascade <b>Hi</b> and <b>Lo</b> limits in addition to the above information. Press the appropriate selector keys to change these limits. Pressing the <b>Cascade</b> key in the <i>dO2 Setup</i> screen reveals the same dO2 cascade possibilities as with the Gas Mix module alone: <b>4-Gas</b> ("4 Gas"), <b>On Demand</b> ("Demand"), <b>Agitation</b> ("Agit") or <b>None</b> ( <i>see Section 7.8.4</i> ).

## 14.3.1 Fermentation Mode Setup

1. In the main screen (*see sample screen on the following page*), select the *Gas* loop, which represents oxygen and air:

B	Name	Value	Setpoint	Control	
і О	Temp	23.4	30.0 °C	Off	
F	Agit	134	350 rpm	Auto	
і О	Pump A	0.0	0.0 %	Manual	
1	Pump B	0.0	0.0 %	Manual	
1	Pump C	0.0	0.0 %	Manual	
0	PH	6.68	7.00 pH	Auto	
	dO2	77.1	0.0 %	Auto	
	Gas	0.3	20.0 SLPM	Auto	
				Press here -	

2. In the *Gas* loop screen that opens, set the *O2 Mix* in *Auto* Mode, as reflected in the resulting screen:

B i o F I o	Gas 0.0 Control: Off Setpoint: 0.0	SLPM	Control Setpoint
1 1 0	O2 Mix: Auto Output: 0.0		O2 Mix Set O2%

- 3. The default gas flow setpoint is 20. If you wish to change it, select *Setpoint*, then type in the desired value (from 0 to 20 SLPM—the system will automatically limit the gas setpoint to 20).
- 4. Select *Set O2%* to set the percentage of oxygen.

## NOTE:

After you set the O2%, the PCU will provide the complementary percentage of air. For example, if you set O2% at 25, the PCU will provide 75% air.



5. Finally, press the *Control* selector button, then set the *Gas* loop control mode to *Auto*. You will hear the solenoid click, and gas will come through the sparger.

## NOTE:

It is important to set the O2 Mix and O2% *before* turning the Gas loop control on, to avoid a sudden burst of large bubbles into the vessel.

## 14.3.2 Fermentation Mode Cascades

Before you set cascades, first review the table at the top of Section 14.3 to identify the cascades available, according to the combination of modules in your system.

**If you have the TMFC without the Gas Mix Module**, the *dO2 Cascade* screen will look like this:

B i	dO2 73.1 %		
0 F I 0 1 1 0	Cascade To: Agit Hi Limit: Agit Lo Limit: Gas Hi Limit : Gas Lo Limit: P-Gain: I-Gain:	None 1000 250 20.0 5.0 0.05 0.25	Agit Gas Agit/Gas None

B	dO2 73.1 %	
0		Agit
F	Cascade To: None	Gas
 0	Agit Hi Limit: 1000	Gas/O2
	Agit Lo Limit: 250	Agit/Gas
1	Gas Hi Limit : 20.0 Gas Lo Limit: 5.0	Aq/Ga/O2
0	P-Gain: 0.05	None
	I-Gain: 0.25	None

If you have the TMFC with the Gas Mix Module, the *dO2 Cascade* screen will look like this:

Make your choices as explained in Section 7.8.2.

## 14.3.3 Cell Culture Mode Setup

1. In the main screen, select the Gas loop. The Gas loop screen opens:

B	Gas 0.0		SLPM	Gas Flow
0	Control:	Off		4 Gas
F	Gas Mix:	Off		Demand
і О	Gas	%		Off
4	CO2	0.0		100% CO2
י 1	N2	0.0		100% N2
0	O2	0.0		100% O2
	Air	0.0		100% Air

- 2. While the *Control* mode and *Gas Mix* are *Off*, select *%Air* (or any other gas) to change the gas mix.
- 3. Select *Gas Flow*, then use the following screen to select a new gas flow *Setpoint*, if so desired:



- 4. The default setpoint is 5. If you wish to change it, select *Setpoint*, then type in the desired value (from 0 to 5 SLPM—the system will automatically limit the gas setpoint to 5).
- 5. If you wish to adjust the display's Y axis, use the *Disp Hi* and *Disp Lo* selections (*as explained in Section 7.4.3*).
- 6. Finally, set *Control* to *Auto* to turn on the gas flow. You will hear the solenoid valve click, and gas will come through the sparger.

## NOTE:

## It is important to set the O2 Mix and O2% before turning the Gas loop control on, to avoid a sudden burst of large bubbles into the vessel.

7. Press the  $\times$  key to return to the *Gas* loop screen. The *Demand* and *4 Gas* options work as explained in Sections 7.11.1 and 7.11.2. The presence of the TMFC does not affect these controls.

## 14.3.4 Cell Culture Mode Cascades

Before you set cascades, first review the table at the top of Section 14.3 to identify the cascades available, according to the combination of modules in your system.

**If you have the TMFC without the Gas Mix Module**, the *dO2 Cascade* screen will look like this (*see sample screen on following page*):

<sup>B</sup> i dO2 73.1 %	
<ul> <li>Cascade To: None</li> <li>Agit Hi Limit: 150</li> <li>Agit Lo Limit: 25</li> <li>Gas Hi Limit: 5.0</li> <li>Gas Lo Limit: 0.0</li> <li>P-Gain: 0.05</li> <li>I-Gain: 0.25</li> </ul>	Agit Gas Agit/Gas None

If you have the TMFC with the Gas Mix Module, the *dO2 Cascade* screen will look like this:



Make your choices as explained in Section 7.8.4.

## 14.4 Cleaning

See Section 8.1 for instructions on cleaning the module.

## 14.5 Specifications

BioFlo 110 Thermal Mass Flow Controller				
Weight	4 lb. (1.8 kg)			
Dimensions	Height	4 in. (10.2 cm)		
	Depth*	12 in. (30.5 cm)		
	Width	12 in. (30.5 cm.)		
	*allow 3 inches (7.	allow 3 inches (7.6 cm) more, front and rear, for cable access		
Range	0-100%			
Input	1 gas input			
Output	1 gas output			
Power	24 VDC power transformer (supplied)			
Maximum Gas 10 PSI				
Pressure				

### 14.6 Troubleshooting

If no information from the Thermal Mass Flow Controller appears on the display, check the following *before you call for service*:

- 1. Verify that the 24VDC power transformer cable is properly connected to the TMFC and to a grounded electrical outlet.
- 2. Confirm that the TMFC is properly connected (daisy-chained with RS-485 cables) to the Power Controller, dO2/pH Controller and the PCU. There must be a dO2/pH Controller or the TMFC will not be recognized by the PCU.
- 3. Check that the Power Controller's power switch is turned On.
- 4. Verify that the PCU power switch is turned On.
- 5. Verify that the TMFC's unit address selector switches are correctly assigned (per Section 5.2).

## NOTE:

If gas *Output*, which is now controlled by the TMFC, does not stabilize, check the levels in your supply tanks.

## 15 APPENDIX E: DRAWINGS

## 15.1 *Module Schematics*

Figure 52: PCU Schematics









Figure 54: dO2/pH Controller Schematics



Figure 55: Level Controller Schematics



Figure 56: Gas Mix Controller Schematics



Figure 57: 4-Pump Module Schematics (110V)



Figure 58: 4-Pump Module Schematics (220V)



Figure 59: Thermal Mass Flow Controller Schematics

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# 16 APPENDIX F: PORTS & ADAPTERS

One of the flexibility features of the BioFlo 110 is the variety of adapters that allow you to set up the headplate to suit your needs. The summary below will help you determine any adjustments you may wish to make to the recommended headplate arrangement.

PORT SIZE	APPLICATION	PART NUMBER			
PLUGS					
6.35mm	Unused port	M1273-9405			
12mm	Unused port	M1273-9406			
19mm	Unused port	M1273-9407			
INSERTS					
12mm	Septum	M1273-3031			
12mm	Thermowell/RTD	M1273-9200 (1.3L)			
		M1273-9201 (3.0L)			
		M1273-9202 (7.5L)			
		M1273-9203 (14.0L)			
12mm	Tri-port	M1273-9961			
SET SCREW ADAPTERS:					
CAUTION! Never use a set screw on a probe.					
12mm port to 12mm tube	For Exhaust Tube or Condenser	M1273-5058			
6.35mm port to 6.35mm tube	For Sparger, Dip Tube, Harvest, Cooling Coil	M1273-5054			
12mm port to 6.35mm tube	To make 12mm port universal for 12mm & 6.35mm diameters	M1273-5056			
COMPRESSION ADAPTERS	<b>6</b> :				
Compression adapters allow you to easily raise or lower the probe or tube, while					
maintaining a safe seal.					
6.35mm port to 4.76mm	For Sample Tube, Foam Probe, Level	M1273-5042			
tube	Probe				
12mm port to 4.76mm tube	To make 12mm port universal for	M1273-5055			
	12mm, 6.35mm & 4.76mm diameters				
12mm port to 12mm tube	For dO2 & pH probes	M1273-5040			

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