Guide to Operations

BioFlo®/CelliGen® 115
Benchtop Fermentor & Bioreactor

MANUAL No: M1369-0050
Revision B
June 2, 2009
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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).

CAUTION!
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 (NBS) is not responsible for any damage to this equipment that may result from the use of an accessory not manufactured by NBS.

WARNING!
High voltage. Always make sure this equipment is properly grounded.
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New Brunswick Scientific reserves the right to change information in this document without notice. Updates to information in this document reflect our commitment to continuing product development and improvement.

Manual Conventions

**NOTE:**

Notes contain essential information that deserves special attention.

**CAUTION!**

*Caution* messages appear before procedures which, if caution is not observed, could result in damage to the equipment.

**WARNING!**

*Warning* 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.

**CRUSH WARNING!**

This particular *Warning* message, whether found in the manual or on the unit, means HOT SURFACE—and therefore represents a potential danger to touch.

*Crush Warning* messages alert you to specific procedures or practices regarding heavy objects which, if not followed correctly, could result in serious personal injury.
Every instrument manufactured by New Brunswick Scientific 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, within 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 New Brunswick Scientific.
FERMENTOR/BIOREACTOR

INFORMATION SHEET

On this page, record the information for your fermentor/bioreactor and retain this for future reference.

MODEL NUMBER: ________________________________
VOLTAGE: ________________________________
SERIAL NUMBER: ________________________________

The above information can be found on the electrical specification plate.

Purchased with the following installed options:

__________________________________________________
__________________________________________________
__________________________________________________
__________________________________________________
__________________________________________________
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# 1 Warnings & Cautions

The following section is a recap of all WARNING and CAUTION messages contained in this manual. This information is essential to the safe operation of your BioFlo/CelliGen 115. Please take a moment to acquaint yourself with the content of each message.

Page numbers are provided so you can review the message and its application within its overall context.

## 1.1 WARNINGS

<table>
<thead>
<tr>
<th>WARNING</th>
<th>Page</th>
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</thead>
<tbody>
<tr>
<td>High voltage. Always make sure this equipment is properly grounded.</td>
<td>iv, 15</td>
</tr>
<tr>
<td>This product is not designed to contain gases within the range between their LEL &amp; their UEL.</td>
<td>iv</td>
</tr>
<tr>
<td>Never block the exhaust to pressurize the vessel.</td>
<td>7, 37, 44</td>
</tr>
<tr>
<td>Do not use this equipment in a hazardous atmosphere or with hazardous materials for which the equipment was not designed.</td>
<td>14, 16</td>
</tr>
</tbody>
</table>

**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, immediately close the valve(s) or turn off the air/gas supplies.
- **Never** intentionally block the exhaust to raise vessel pressure.
- Use the minimum air/gas pressure that will provide adequate airflow for the application. **Never** exceed the maximum air pressure of 10 psi. This maximum pressure is necessary only to obtain the highest gas flow rates.

**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.

During autoclaving, the vessel exhaust filter must be vented to avoid explosion.

Use protective gloves when handling hot components.

Be sure to let the vessel cool...before reconnecting the water line.

Be careful not to pinch your fingers in the pump head levers.

Always turn your BioFlo/CelliGen 115 off and disconnect the power cord before performing maintenance.

**NO ONE BUT A PROFESSIONAL SERVICE PERSON** should touch electric or electronic parts or assemblies in the electrical cabinet.

<table>
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<tr>
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<td>7, 37, 44</td>
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<td>111, 114</td>
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<td>112</td>
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</tbody>
</table>
1.2 **CAUTIONS**

<table>
<thead>
<tr>
<th>CAUTION</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>This equipment <em>must</em> be operated as described in this manual. If operational guidelines are not followed, equipment damage and personal injury can occur. … <em>Do not use this equipment in a hazardous atmosphere or with hazardous materials for which the equipment was not designed.</em></td>
<td>iv</td>
</tr>
<tr>
<td>Before making electrical connections, verify that the supply voltage matches the voltage and the power requirements marked on the electrical specification plate … and the control schematics supplied with the unit.</td>
<td>12, 15</td>
</tr>
<tr>
<td>Make sure all utility connections have been securely made before connecting to WATER-IN and before turning on the main water supply.</td>
<td>15</td>
</tr>
<tr>
<td>Before connecting or disconnecting the water hoses to/from the vessel and/or cabinet at any time, make sure the main water supply is closed.</td>
<td>16, 72, 102</td>
</tr>
<tr>
<td>To protect the integrity of your glass vessel and to avoid damage, familiarize yourself with these cautions… … do not fill the water jacket or operate the vessel until you have cut the cable ties and released the stir bar.</td>
<td>18</td>
</tr>
<tr>
<td>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.</td>
<td>31</td>
</tr>
<tr>
<td>Make sure that the thermowell does not touch the cooling coil.</td>
<td>32</td>
</tr>
<tr>
<td>Do not install the pH port adaptor in the headplate before inserting the probe…</td>
<td>34, 64</td>
</tr>
<tr>
<td>Do not install the dO2 port adaptor in the headplate before inserting the probe…</td>
<td>35, 67</td>
</tr>
<tr>
<td>To avoid vessel stress cracks, especially during autoclaving, make vessel clamping screws finger tight.</td>
<td>43</td>
</tr>
<tr>
<td>Before turning on the main power switch, make sure that: (1) the input water hose is connected, the drain line is connected and the water supply is turned on; (2) the vessel is in place and the quick-connect water lines are connected to the vessel’s heat exchanger; (3) the power cord is properly connected to the control cabinet and plugged into a suitable power outlet.</td>
<td>45</td>
</tr>
<tr>
<td>Be sure to wear protective gloves when installing a glass electrode.</td>
<td>63</td>
</tr>
<tr>
<td>We recommend that you avoid the use of hydrochloric acid (HCl) with the BioFlo/CelliGen 115 for pH control or any other purpose, because HCl corrodes stainless steel. Over time, it will severely damage the headplate, a costly component to replace, and other stainless steel components. Phosphoric and sulfuric (10% maximum concentration) acids are acceptable and are commonly used for pH control.</td>
<td>65</td>
</tr>
<tr>
<td>Never let a pH probe rest on its tip, and never leave a pH probe in DI water.</td>
<td>65, 111</td>
</tr>
<tr>
<td>During sterilization: the bearing housing cap must be installed on the fermentor bearing housing, to keep steam from damaging the internal bearings.</td>
<td>72</td>
</tr>
<tr>
<td>Never autoclave PVC tubing (clear with white braiding).</td>
<td>72</td>
</tr>
</tbody>
</table>

...continued...
<table>
<thead>
<tr>
<th>CAUTION</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>During autoclaving, the vessel must be vented at all times. Release the</td>
<td>75</td>
</tr>
<tr>
<td>autoclave pressure only when the temperature has dropped below 90º C.</td>
<td></td>
</tr>
<tr>
<td>Use slow exhaust.</td>
<td></td>
</tr>
<tr>
<td>Proper pH control is critically dependent on tubing size, which should</td>
<td>79</td>
</tr>
<tr>
<td>be as small as possible.</td>
<td></td>
</tr>
<tr>
<td>Never clean the vessel or its components or the control cabinet with</td>
<td>110</td>
</tr>
<tr>
<td>abrasive chemicals or materials.</td>
<td></td>
</tr>
<tr>
<td>Never let a DO probe rest on its tip.</td>
<td>111</td>
</tr>
</tbody>
</table>
2  INSPECTION & UNPACKING OF EQUIPMENT

2.1 Inspection of Box(es)

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

2.2 Packing List Verification

Verify against your NBS packing list that you have received the correct materials. Report any missing parts to your local NBS Sales Order Department.

2.3 Basic Components

You should have at least the following components, which will be described in greater detail later in this manual:

- Control Cabinet with Touchscreen*
- Vessel
- Thermowell & RTD
- Baffles (for fermentation only)
- Impellers
- Probe Kits (i.e., pH, DO, Foam, Level)
- Motor
- Bearing Housing
- Filters & connectors
- Inoculation/Addition System
- Sampling System
- Harvesting System
- Sparging System

*While you may have multiple units, each with its own components, you will have ordered only one touchscreen.

**NOTE:**

The assembled Control Cabinet/Touchscreen assembly is called a Control Station. For purposes of clarity in this manual, however, the control cabinet (which houses the controller) and the touchscreen will be referred to separately by their component names.
3 Introduction & Overview

3.1 System

BioFlo/CelliGen 115 is a versatile fermentor/bioreactor that provides a fully equipped system in one compact package. It can be employed for batch, fed batch or continuous culture with process control for pH, dissolved oxygen (DO), agitation, temperature, pump feed, antifoam and foam/level.

Systems can be configured as either control stations or utility stations. Each individual stand-alone system is a control station. One control station can run up to two additional utility stations, which are dependent on the control station.

3.2 Vessels

One of the most versatile features of the BioFlo/CelliGen 115 is the wide variety of glass vessels available. There are two types of vessels, non-jacketed (heat-blanketed) 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. Ports in the headplate are provided for, but not limited to, the following purposes: inoculation; base and acid addition; a thermowell for a resistance temperature detector (RTD); a foam probe; a sparger; a harvest tube; a sampling tube; an exhaust condenser; and dissolved oxygen (DO) and pH electrodes. The drive bearing housing is also located on the headplate (see Figures 3 & 8).

3.3 Agitation System

A removable agitation motor located on top of the bearing housing on the headplate is connected to the agitation shaft with a direct drive coupling or a magnetic coupling.

It can be easily disconnected before autoclaving the vessel and easily replaced after sterilization. The motor will provide a speed range from 50 to 1200 RPM for fermentation with direct drive, from 25 to 400 RPM for cell culture with direct drive, or from 25 to 200 RPM for cell culture with magnetic drive. The process control software ensures agitation speed control throughout the speed range.

It is possible to cascade Dissolved Oxygen (DO) to Agitation (AGIT) so the agitation speed will vary between the user-specified minimum and maximum setpoints in order to maintain the set percentage of DO. (See Section 10 for further information on setting up cascades.)

Default P & I (proportional & integral) values are preset at the factory. We strongly recommend that you maintain the factory-set parameters.
3.4 Temperature Control

The culture temperature setpoint may be selected within the range from 20°C above coolant temperature to 70°C for 1.3- to 7.5-liter vessels, and from 20°C above coolant temperature to 65°C for 14.0-liter vessels. It is controlled by the process control software which then sends information to either a heater blanket and cooling coil or to a water jacket. The media temperature is sensed by a Resistance Temperature Detector (RTD) submerged in the thermowell.

Default P & I (proportional & integral) values are preset at the factory. We strongly recommend that you maintain the factory-set parameters.

3.5 Aeration

Up to four gases, including air, nitrogen, carbon dioxide and oxygen, can be introduced into the media through the ring sparger or optional microsparger. The flow rate is controlled manually by one, two, three or four rotameter(s) or automatically by thermal mass flow controller (TMFC), according to the definition of your system. The TMFC is regulated automatically according to values set via the control station touchscreen.

The gas mix can either be controlled manually by adjusting the flow of gases through their rotameters or automatically if 4-gas mixing was purchased as an option. (For further information on cascading, see Section 10.) 4-gas mixing allows the system to automatically calculate the gas mix in response to culture needs.

Default P & I (proportional & integral) values are preset at the factory. We strongly recommend that you maintain the factory-set parameters.

3.6 pH Control

pH is controlled in the range of 2.00-14.00. The pH is sensed by a gel-filled pH probe (see Figures 11 & 28). Control is maintained by a P & I (proportional & integral) controller which operates peristaltic pumps, assigned to perform acid or base addition, or which controls the use of gas(es) for this purpose. The user can also select a deadband value to control pH within the user-assigned range: no acid or base will be added when the pH value falls within the deadband tolerance above or below the setpoint.

Default P & I (proportional & integral) values are preset at the factory. We strongly recommend that you maintain the factory-set parameters.
3.7 **DO Control**

Dissolved oxygen (DO) is controlled in the range of 0-200%. It is sensed by the DO electrode and control is maintained by the P & I controller by changing the speed of agitation, the thermal mass flow controller-regulated flow rate (if your system is so equipped), and/or the percentage of oxygen in aeration.

Default P & I (proportional & integral) values are preset at the factory. **We strongly recommend that you maintain the factory-set parameters.**

The DO probe is a polarographic probe. Be sure to inspect the DO probe before every run, changing the electrolyte solution and membrane as needed.

3.8 **Foam/Level Control**

Foam can be monitored during batch fermentation by a foam/level probe located in the headplate. The controller operates the antifoam-assigned pump that adds chemical defoamer into the vessel as needed. The internal level can also be controlled by using this feature. Pumps can be triggered to turn on or off in response to the presence or absence of liquid.

3.9 **Exhaust System**

The exhaust gases pass into the exhaust condenser (see Figures 13 and 14) where moisture is removed, then returned to the vessel. The remaining gases then pass through a 0.2 μm exhaust filter. Be sure to inspect filters before every run, replacing them as needed.

**WARNING!**

NEVER block the exhaust to pressurize the vessel!

3.10 **Recommended Accessories & Supplies**

Before you begin to assemble your BioFlo/CelliGen 115, it would be prudent to verify that you have all of the following accessories and supplies readily at hand:
• An autoclave
• Rubber gloves
• Silicone tubing
• A tie gun
• Plastic ties (multiple colors can be helpful)
• Plastic tubing connectors
• Addition bottles
• A liquid trap
• Polysulfone quick-connects

• An inoculation syringe
• Media
• Antifoam agent
• Aluminum foil
• Rubber bands
• pH 4 buffer
• pH 7 buffer
• Silicone O-ring lubricant (for fermentation only)

User’s kits and start-up kits are available from NBS with many of the commonly required items (including a selection of tubing, clamps, filters, connectors and addition vessels). Speak to your NBS sales representative for more information.

3.11 Supervisory Software

In addition to the built-in software that you interface with through the touchscreen, your BioFlo/CelliGen 115 system can be remotely controlled from a PC via NBS BioCommand optional supervisory software (see Section 4.10). Consult your NBS representative for details; be sure to ask for ModBus protocol.
4 INSTALLATION

4.1 Physical Location

The surface on which you place the BioFlo/CelliGen 115 should be smooth, level and sturdy. Ensure that the surface can bear the weight of the system (see Section 5, Specifications, for weights) plus vessel contents and any applicable ancillary equipment.

Also ensure that there is enough space around the back and the front of the BioFlo/CelliGen 115 for proper operation and access. Allow at least 4 inches of clearance behind the unit for heat dissipation.

**Figure 1a: Dimensions**

4.2 Environment

The BioFlo/CelliGen 115 fermentor operates properly under the following conditions:

- Ambient temperature range 10°C to 35°C
- Relative humidity up to 80% non-condensing
4.3 Installing the Control Cabinet

Position the BioFlo/CelliGen 115 control station cabinet on a firm, level surface in an area where utilities are readily available.

Connect the power cord to the rear of the control cabinet. At a later time, once the unit is completely assembled and all connections have been made, you will plug the power cord into a suitable electrical outlet.

Figure 1b: Front View

NOTE:
Figures 1a, 1b, 1c & 1d represent one possible control station cabinet configuration. Your control cabinet may look different, depending on the particular model and options you have purchased.
Figure 1c: Rear View

- Gas Connections (see Section 4.5.3)
- Cabinet Input port (see Section 4.4)
- Cabinet Output port (see Section 4.4)
- SCADA port (see Section 4.10)
- Plug for Power Cord

Cooling Vent

**NOTE:** Utility stations do not have a fan.

Service Connections (see Figure 1d)

Label with electrical specifications & unit serial number
**CAUTION!**

Before making electrical connections, verify that the supply voltage matches the voltage and the power requirements marked on the electrical specification plate (located on the rear panel of the cabinet) and the control schematics supplied with the unit.

### 4.4 Connecting Utility Cabinets

If you have a control station and one or two utility stations, use the bus cable provided in the following way:
1. Verify that the utility station is not yet connected to the control station, and that both are powered off.

2. Connect the RS-495 cable provided to the control station’s output COM port and to the utility station’s input COM port, as shown in Figure 1e. Verify that the cable is securely connected to both cabinets.

**Figure 1e: Connecting Cabinets**

To add a second utility station, repeat Steps 1 & 2.

Section 12.3 explains how to set up the control station and the utility station(s) to work together.
4.5 Utilities

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

All control and utility stations must be properly connected to gases, water supply, vessel water, electrical power and an open drain. The gas connections are located on the rear panel of the cabinet (see Figure 1g in Section 4.5.3). All other service connections are on the lefthand side of the cabinet (see Figure 1f in Section 4.5.2).

Using standard plant practices and respecting all applicable codes, connect services to the appropriate connections, as recapped in Table 1 and explained in greater detail in Sections 4.5.1 - 4.5.3.

Table 1: Service Connections

<table>
<thead>
<tr>
<th>Service/Utility</th>
<th>Requirement</th>
<th>Connection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical</td>
<td>120 VAC, 50/60 Hz., Single Phase, 10 Amp (fluctuations not to exceed ±10%)</td>
<td>120 VAC 1ph field wired to 15 Amp disconnect in panel</td>
</tr>
<tr>
<td></td>
<td>230 VAC, 50/60 Hz., Single Phase, 6 Amp (fluctuations not to exceed ±10%)</td>
<td>230 VAC 1ph field wired to 15 Amp disconnect in panel</td>
</tr>
<tr>
<td>Facility Water</td>
<td>5 - 10 PSIG</td>
<td>Quick Connect</td>
</tr>
<tr>
<td>Process Air</td>
<td>3 -10 PSIG</td>
<td>Push-in tube</td>
</tr>
<tr>
<td>Oxygen</td>
<td>3 - 10 PSIG</td>
<td>Push-in tube</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>3 - 10 PSIG</td>
<td>Push-in tube</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>3 - 10 PSIG</td>
<td>Push-in tube</td>
</tr>
<tr>
<td>Exhaust</td>
<td>1/2 PSIG maximum backpressure</td>
<td></td>
</tr>
</tbody>
</table>

4.5.1 Electrical Requirements

<table>
<thead>
<tr>
<th>Volts</th>
<th>Hertz</th>
<th>Amps</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>50/60</td>
<td>10</td>
</tr>
<tr>
<td>230</td>
<td>50/60</td>
<td>6</td>
</tr>
</tbody>
</table>

NOTE:
The electrical requirements vary depending on the part number that has been ordered. Model, Part Number and Electrical Power Requirements for each fermentor appear on a metal label affixed to the rear of the unit just above the connection for the power cord.
CAUTION!
Before making electrical connections, verify that the supply voltage matches the voltage and the power requirements marked on the electrical specification plate (located on the rear panel of the cabinet) and the control schematics supplied with the unit.

WARNING!
High voltage. Always make sure this equipment is properly grounded.

4.5.2 Water and Drain Connections

CAUTION!
Make sure all utility connections have been securely made before connecting to WATER-IN and before turning on the main water supply. Failure to observe these precautions will result in water leaking out of the unconnected hoses and the cabinet.

The water inlet and drain connections are located on the left side of the control cabinet (see Figure 1f, a detail from Figure 1d). Water pressure should be from 5 to 10 PSIG, with 50 μm filtration. 7.5-foot lengths of tubing are supplied with an open end for water in and the drain and with quick-connect fittings to attach to the cabinet. The tubing (NBS part number P0740-1631) has an inner diameter of ¼ inch and an outer diameter of 7/16 inch.

Figure 1f: Water Connections

For the EXHAUST CONDENSER IN & RETURN connections, 3-foot lengths of 3/16-inch ID silicone tubing (NBS part number P0740-2505) are pre-assembled with a quick-connect on one end, to be connected to the cabinet, and open at the other end to connect to the exhaust condenser’s inlet and outlet. The connection points should be secured with cable ties.

For the COOLING LOOP IN & RETURN connections, 3-foot lengths of 3/16-inch ID silicone tubing (NBS part number P0740-2505) are pre-assembled with a quick-connect on one end, to be connected to the cabinet. The other end is to be connected to (1) the cooling coil’s inlet and outlet on the headplate of heater blanket vessels or (2) to the water inlet and outlet lines coming from water jacketed vessels. The connection points on the open ends should be secured with cable ties.

BE SURE TO READ THE CAUTION ON THE NEXT PAGE.
4.5.3 Gas Connections

Gas inlets are located on the rear panel of the control cabinet (see Figure 1g on the following page). The sparge outlet is located on the left side of the cabinet (see Figure 1h on the following page).

There are push-in tube connectors for air, nitrogen, oxygen and carbon dioxide. These connectors accept flexible \( \frac{1}{8} \)-inch ID tubing; a 25-foot length of blue polyurethane tubing (NBS part number P0740-3113C3) is supplied with the cabinet; it can be cut to the appropriate sizes to attach to the utilities. Other soft, flexible-walled, chemically inert tubing (such as Marprene, Pharmed, etc.) may be used as well.

Gas inlets plugged with black plastic are unavailable to your configuration and must remain plugged.

All gases should be regulated using a two-stage regulator. The scale of the regulator gauge for gases going into the fermentor should be such that one can regulate pressure from 3 to 10 PSIG maximum.

Connect the barbed sparge connector (NBS part number P0242-0600) to the SPARGE outlet at top left side of the cabinet (see Figure 1h on the following page); connect the silicone tube attached to the sparge connector to the inlet filter on the vessel headplate. The sparge connector/tubing assembly is found in the tubing kit provided with your unit.
**Important Warnings**

Before you begin to assemble or operate your vessel, 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, possibly 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, immediately close the valve(s) or turn off the air/gas supplies.
- Never intentionally block the exhaust to raise vessel pressure.
- Use the minimum air/gas pressure that will provide adequate airflow for the application. Never exceed the maximum air pressure of 10 psi. This maximum pressure is necessary only to obtain the highest gas flow rates.

---

**CAUTION!**
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. Over-tightening puts undesirable pressure on the glass.
- Keep the glass free from contact with any diamond material (diamond jewelry, industrial diamonds or diamond dust from grinding wheels).

---

>Note:
Clean the vessel thoroughly after each run with detergent, otherwise debris could build up thus providing a place for bacteria to grow and produce toxins. This can result in low cell viability.
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 2a) puts the impeller shaft at risk for damage:

**Figure 2a: WRONG Handling of Drive Assembly**

![Diagram of incorrect handling](image)

The **correct** way, which is resting the drive assembly and headplate on the surface (see Figure 2b 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.

**Figure 2b: CORRECT Handling of Drive Assembly**

![Diagram of correct handling](image)

### 4.7 Vessel Assembly: Non-Jacketed

The vessels are available in four sizes: 1.3 liters, 3.0 liters, 7.5 liters and 14.0 liters (total volume; for more detail, see Specifications).
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 (shown in the smaller vessel views in Figure 3 on the following page) has two large viewing windows so the culture remains visible for inspection.

**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.

Figure 3 on the following page shows a typical installation of the 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 in the larger vessel view.
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.
4.7.1 Headplate

Figure 4: 1.3L Headplate

![Diagram of 1.3L Headplate]

**NOTE:**
The RTD thermowell port should only be used for its intended purpose.
Figure 5: 3.0L Headplate

NOTE:
The RTD thermowell port should only be used for its intended purpose.

Each bolt is a possible mounting position for a bottle holder.
NOTE:
The RTD thermowell port should only be used for its intended purpose.

4.7.2 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.
4.7.3 Install Vessel in Vessel Stand

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 7: 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.
4.7.4 **Install Baffle (14.0L Fermentation Vessels ONLY)**

For installation of the 1.3L, 3.0L and 7.5L vessel baffle, see Section 4.8.21.

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.

4.8 **Vessel Assembly: Water-Jacketed**

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 8 on the following page shows a typical installation of the double-walled, water-jacketed vessel, with the most commonly used accessory equipment.

![CAUTION!]

The Jacket Water Heater base (see Figure 8) includes a magnetic stir bar and plate. For stability during shipping, the stir bar is tied to the inner cage by cable: do not fill the water jacket or operate the vessel until you have cut the cable ties and released the stir bar.

Familiarize yourself with the arrangement of the headplate ports, as shown in Figures 4, 5 & 6, 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.
Figure 8: Water-Jacketed Vessel Assembly

NOTE:
This vessel is shown installed on the Jacket Water Heater.

AGITATION MOTOR

BEARING HOUSING

LIFTING HANDLE

HEADPLATE

TOP CLAMPING RING

BAFFLE

WATER JACKET

THERMOWELL

BASE PLATE

COOLING WATER INLET (COOL LOOP IN)

COOLING WATER OUTLET TUBE

SPARGER

BOTTOM CLAMPING RING

COOLING WATER OUTLET (COOL LOOP RETURN—tubing connected inside the jacket)
4.8.1 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 9 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 9: Installing Headplate Clamping Ring](image)

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.

4.8.2 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 10 on the following page).

4. With the clamping screws in place on the ring, fit the bottom clamping ring onto the base plate.
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.

### 4.8.3 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**.
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.

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

*For installation of the 1.3L, 3.0L & 7.5L vessel baffle, see Section 4.8.21.*

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.

4.8.5 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.

<table>
<thead>
<tr>
<th>Table 2: Impeller Positions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Distance from Bottom of Headplate to Top of Impeller Blade</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Lower Impeller</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Upper Impeller</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**NOTE:**

The distances indicated above provide a recommended 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* (NBS part number P0860-1050) before you install equipment in the headplate. Also inspect the headplate O-ring to be sure it is securely seated in its groove.

*For cell culture, you may want to use IPA or glycerol instead of silicone.
4.8.6 Install Cooling Coil

1.3L Vessel Cooling Coil/Sparger Assembly

The cooling coil and sparger connections are welded into one special 12mm tri-port assembly.

1. From beneath the headplate, insert the assembly into the appropriate ports.

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.

4.8.7 Install Sparger (3.0L, 7.5L & 14.0L Vessels)

1. From beneath the headplate, insert the sparger tube into the sparger port.

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.
4.8.8 Install Harvest Tube

1. Working from beneath the headplate, install the harvest tube in the harvest port. 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.

4.8.9 Install Sampler Tube

1. Working from beneath the headplate, install the optional sampler tube in the sample port. 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.

4.8.10 Install Thermowell

1. Working from above the headplate, insert the thermowell tube into the RTD port. Be sure to use the port designated for the RTD to avoid damaging the glass.

   CAUTION!
   Make sure that the thermowell does not touch the cooling coil or come into contact with the glass vessel.

2. Finger tighten the knurled adapter on the thermowell.

4.8.11 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.

2. Finger tighten the knurled adapter.
4.8.12 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 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.

4.8.13 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.

2. Finger tighten the knurled adapter.

4.8.14 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.

4.8.15 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.
1. Wear protective gloves to protect yourself in case of accidental breakage.
2. Lightly coat the pH probe with glycerol.

---

**CAUTION!**

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.

---

**Figure 11: pH Probe with Port Adapter (exploded)**

With reference to Figure 11 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, 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.

---

**4.8.16 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.

---

**CAUTION!**

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, 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.
4.8.17 Install Exhaust Condenser

**WARNING!**

Never 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 13a & 13b 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.

**Figure 13a: Exhaust Condenser (1.3L, 3.0L & 7.5L Vessels)**

![Diagram of Exhaust Condenser](image)

**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.

Ensure that gas flow through the exhaust condenser is unobstructed during runs and during autoclaving.
4.8.18 Install Sampler

The optional BioFlo/CelliGen 115 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 14a & 14b 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 14a: Sampler/Harvest System (1.3L Vessel)**

- **THUMB CLAMP**
- **SYRINGE FILTER**
- **SYRINGE**
- **SAMPLER BOTTLE HOLDER**
- **SAMPLER BOTTLE**
- **HEADPLATE CLAMPING NUT**
- **HEADPLATE CLAMPING SCREW**
- **SAMPLE TUBE**
- **1/8” SILICONE TUBING**
- **REDUCING ELBOW** (to 3/16” tubing on sampler holder)
- **SAMPLER/HARVEST PORT**
- **HEADPLATE**
- **SPARE**
- **HARVEST TUBE**

**Figure 14b: Sampler System (3.0L, 7.5L & 14.0L Vessels)**

- **THUMB CLAMP**
- **SYRINGE**
- **SYRINGE FILTER**
- **SAMPLER BOTTLE HOLDER**
- **SAMPLER BOTTLE**
- **HEADPLATE CLAMPING NUT**
- **HEADPLATE CLAMPING SCREW**
- **SAMPLE TUBE**
- **3/16” SILICONE TUBING**
- **SAMPLER PORT**
- **HEADPLATE**
- **SAMPLER TUBE**
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.

### 4.8.19 Install Foam Trap

If you are using a foam trap kit (see Figure 15 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, aseptically install a sterile (0.2 μ) 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.

Figure 15: Foam Trap

4.8.20 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.

4.8.21 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.*

4.8.22 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.
4.8.23 Install Vessel

Position the vessel next to the control cabinet, in the rounded cut-out designed for vessel placement between pumps and connectors. Be sure to keep the water line quick-connects to the left.

Figure 16: Vessel Location

Place vessel here

4.8.24 Install Motor Assembly

1. Position the motor assembly on top of the bearing housing, using the locating pin (or locating slot, if applicable) to orient it properly.
2. Connect the motor cable to the receptacle on the face of the control cabinet.

4.8.25 Make All Connections

1. Connect cables from all probes to their respective sockets on the face of the control cabinet (see Figure 1d).
2. Connect the exhaust condenser to the exhaust condenser port.
3. Using flexible tubing, connect the exhaust filter to the top of the condenser. Secure it with tubing ties.
4. Insert the RTD into the thermowell.
5. If you have not already done so, connect the sparge line (silicone tubing) to the inlet filter.

**WARNING!**
Never block the exhaust to pressurize the vessel (see Section 4.6).

### 4.9 Main Power Switch

The main power switch is located on the righthand side of the control cabinet, as you face the touchscreen (see Figure 17). Be sure to read the CAUTION on the following page before you turn the power on.

![Figure 17: Main Power Switch](image-url)
CAUTION!
Before turning on the main power switch, make sure that:
(1) The input water hose is connected, the drain line is connected and
the water supply is turned on;
(2) The vessel is in place and the quick-connect water lines are
connected to the vessel’s heat exchanger;
(3) The power cord is properly connected to the control cabinet and
plugged into a suitable power outlet.

4.10 Optional BioCommand Software

If you are using NBS supervisory software, be sure to consult your BioCommand
user’s manual for installation and start-up instructions in addition to the general
instructions provided below.

A 25-pin RS232/422 “D” connector com port, labeled SCADA, is provided on the rear
panel of the control cabinet (see Figure 17a) to connect the BioFlo/CelliGen 115 to a
supervisory host computer.

Figure 17a: RS232/422 Interface
Communication to *BioCommand* software is assured via an optional RS-232 interface cable:

1. Connect the 25-pin end of the RS-232 cable to the **SCADA** port, and make sure that the connection is secure.
2. Hand tighten the thumbscrews.
3. Refer to the *BioCommand* user’s guide for instructions on connecting the RS-232 interface cable to the supervisory host computer.

An NBS *BioCommand* advanced supervisory software program is available which will enable the operator to interface with a computer that has a Windows® 2000 (or higher) operating system. With this software, you will be able to establish or change the setpoints for temperature, pH, DO, agitation speed and pump flow rate. You will also be able to read and log the current values of any parameters (temp, pH, DO, air flow, pump flow rate, levels and agitation) that are monitored. The data can also be stored, plotted and, afterwards, transferred to other commonly available programs, to be manipulated and analyzed in various ways.

Table 3 identifies the pin designations for this 25-pin RS232/422 connector:

<table>
<thead>
<tr>
<th>Pin Number</th>
<th>Signal</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 4-6, 8-11, 14-20, 22-23</td>
<td>NC</td>
<td>not assigned</td>
</tr>
<tr>
<td>2</td>
<td>TXD</td>
<td>RS232 Data Output from fermentor</td>
</tr>
<tr>
<td>3</td>
<td>RXD</td>
<td>RS232 Data Input to fermentor</td>
</tr>
<tr>
<td>7</td>
<td>GND</td>
<td>Ground reference for all signals</td>
</tr>
<tr>
<td>12</td>
<td>IRXD+</td>
<td>RS422 paired data input to fermentor</td>
</tr>
<tr>
<td>24</td>
<td>IRXD-</td>
<td>RS422 paired data output from fermentor</td>
</tr>
<tr>
<td>13</td>
<td>ITXD+</td>
<td>RS422 paired data output from fermentor</td>
</tr>
<tr>
<td>25</td>
<td>ITXD-</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>IOS</td>
<td>Open selects RS232 Grounded selects RS422</td>
</tr>
</tbody>
</table>

Unless otherwise requested, the baud rate is factory-selected at 19200 and the connector is configured as an RS232 port: i.e., no jumper between pin #7 and pin #21. The factory-set address for the machine is 8.
# 5 Specifications

## BioFlo/CelliGen 115 System

<table>
<thead>
<tr>
<th>Vessels</th>
<th>Total Volume</th>
<th>1.3 L</th>
<th>3.0 L</th>
<th>7.5 L</th>
<th>14.0 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Volume</td>
<td></td>
<td>0.4-1.0L</td>
<td>0.8-2.2L</td>
<td>2.0-5.6L</td>
<td>4.0-10.5L</td>
</tr>
<tr>
<td>Design</td>
<td>Heat-blanketed and Water-jacketed All vessels are borosilicate glass, autoclavable, with dished-bottom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Station/Utility Station</td>
<td>Design</td>
<td>Advanced compact controller with integrated utility station capable of supporting 2 additional utility stations and vessels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Display</td>
<td>8.4” industrial color touchscreen display is standard with control station but not included with for 2nd or 3rd utility station</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Function</td>
<td>Fermentation and cell culture monitoring and control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>Indication</td>
<td>Digital display in 0.1°C increments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>20°C above coolant temperature to 70°C max temperature (65°C max temperature for 14.0L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>PID for heating and cooling Heat-blanketed Vessels: External heating blanket and immersed stainless steel coiling coil Water-jacketed Vessels: Water jacket heater and circulation loop</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensor</td>
<td>Platinum RTD probe (Pt 100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agitation</td>
<td>Drive</td>
<td>Magnetic Drive or Direct Drive.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indication</td>
<td>Digital display in 1 RPM increments.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>Direct Drive: 50-1200 RPM for fermentation, 25-400 for cell culture Magnetic Drive : 25-200 RPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>PID control; manual, automatic, or cascade settings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impellers</td>
<td>Rushton-style standard with fermentation system Pitched blade standard with cell culture Optional: Spin filter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baffles</td>
<td>Removable 316L stainless steel; fermentation only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aeration</td>
<td>Gas Flow options</td>
<td>0-4 Rotameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 0-150 mLpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 250-2500 mLpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 1-5 Lpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 1-20 Lpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 Thermal Mass flow Controller (TMFC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 0-20 SLPM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas Mixing options</td>
<td>Automatic 4-Gas Mixing (via 4 solenoids) Manual Gas mixing (via 4 gas manifold)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sparger</td>
<td>Standard: Ring sparger Optional: Microsparger</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inlet Filter</td>
<td>0.2μm interchangeable cartridge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₂ Gas</td>
<td>For calibration of DO probe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>Indication</td>
<td>Digital display in 0.01 pH increments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2-14 pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>PID, link to pumps or gases, adjustable deadband</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensor</td>
<td>pH probe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

...continued...
### BioFlo/CelliGen 115 System

<table>
<thead>
<tr>
<th>DO</th>
<th>Indication</th>
<th>Digital display in 0.1% increments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>0-200%</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>PID, Cascade to Agitation, Gases, GasFlo if equipped with TMFC</td>
</tr>
<tr>
<td></td>
<td>Sensor</td>
<td>Polargraphic DO probe</td>
</tr>
<tr>
<td>Exhaust</td>
<td>Filter</td>
<td>0.2μm interchangeable cartridge</td>
</tr>
<tr>
<td></td>
<td>Condenser</td>
<td>Stainless steel, water-cooled in headplate</td>
</tr>
<tr>
<td>3 Pumps</td>
<td>Control</td>
<td>12 RPM</td>
</tr>
<tr>
<td>Utilities</td>
<td>Water</td>
<td>10 PSIG maximum, 50 μm filtration</td>
</tr>
<tr>
<td></td>
<td>Gases</td>
<td>10 PSIG maximum</td>
</tr>
<tr>
<td>Electrical requirements</td>
<td>120VAC</td>
<td>50/60 Hertz</td>
</tr>
<tr>
<td></td>
<td>230VAC</td>
<td>50/60 Hertz</td>
</tr>
<tr>
<td>Control station/ Utility station dimensions</td>
<td>1.3L Vessel</td>
<td>9.5 24 8.5 22 22 56</td>
</tr>
<tr>
<td></td>
<td>3.0L Vessel</td>
<td>9.5 24 8.5 22 22 56</td>
</tr>
<tr>
<td></td>
<td>7.5L Vessel</td>
<td>14.5 37 11.5 29 23 65</td>
</tr>
<tr>
<td></td>
<td>14.0L Vessel</td>
<td>11.5 29 11.5 29 29 74</td>
</tr>
<tr>
<td>Heat-Blanketed</td>
<td>1.3L Vessel</td>
<td>11.5 29 11.5 29 20.5 52</td>
</tr>
<tr>
<td></td>
<td>3.0L Vessel</td>
<td>11.5 29 11.5 29 22.5 56</td>
</tr>
<tr>
<td></td>
<td>7.5L Vessel</td>
<td>11.5 29 11.5 29 26.8 68</td>
</tr>
<tr>
<td></td>
<td>14.0L Vessel</td>
<td>11.5 29 11.5 29 31.5 80</td>
</tr>
<tr>
<td>Dimensions</td>
<td>Water-Jacketed</td>
<td>With Exhaust Condenser</td>
</tr>
<tr>
<td></td>
<td>1.3L Vessel</td>
<td>11.5 29 11.5 29 20.5 52</td>
</tr>
<tr>
<td></td>
<td>3.0L Vessel</td>
<td>11.5 29 11.5 29 22.5 56</td>
</tr>
<tr>
<td></td>
<td>7.5L Vessel</td>
<td>11.5 29 11.5 29 26.8 68</td>
</tr>
<tr>
<td></td>
<td>14.0L Vessel</td>
<td>11.5 29 11.5 29 31.5 80</td>
</tr>
<tr>
<td>Net Weight</td>
<td>Control Station</td>
<td>USB for easy firmware upgrades (Control station only)</td>
</tr>
<tr>
<td></td>
<td>Communications:</td>
<td>BioCommand Port for communication with optional BioCommand/SCADA software</td>
</tr>
<tr>
<td></td>
<td>Regulatory Compliance</td>
<td>Certified to:</td>
</tr>
<tr>
<td></td>
<td>Conforms to:</td>
<td>UL Standard UL 61010-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAN/CSA C22.2 No. 61010-1</td>
</tr>
<tr>
<td>Ambient Operating Conditions</td>
<td>10-35°C, up to 80% relative humidity, non-condensing</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** Specifications are subject to change without notice.

### 5.1 Certifications

The BioFlo/CelliGen 115 has been tested and meets the requirements of U.S. and Canadian electrical and safety standards. And, as attested in the CE Declaration of Conformity reproduced on the following page, they also conform to the appropriate electrical and safety requirements set out in European Directives.
DECLARATION OF CONFORMITY

New Brunswick Scientific, hereby declares that the product(s) listed below conform to the European Union directive and standards identified in this declaration.

Product(s)
BioFlo 115 Fermentor System
CeliGen 115 Bioreactor System

EU Directive(s)
Low Voltage (73/23/EEC/93/68/EEC)

Standard(s)
EN61010-1: 2001 (2nd Edition) EN61000-4-2
EN61326-1:2006 Emissions EN61000-4-4
EN61326-1:2006 Immunity EN61000-4-5
EN61000-3-2: 2001 EN61000-4-11
EN61000-3-3: 1995

The conformity assessment procedures were performed at the following notified body:


The technical documentation relevant to the above equipment will be held at:
New Brunswick Scientific Company
PO Box 4005
44 Talmadge Road
Edison, New Jersey 08818-4005 U.S.A
Tel. (732) 287-1200
Fax. (732) 287-4222

Joe Violin
Director of QA RA

9/30/2009 Date
6 OPERATING CONTROLS

6.1 Touchscreen

Your primary interface with the BioFlo/CelliGen 115 is the touchscreen on the control cabinet.

Figure 18: Touchscreen

6.2 Display Screens

6.2.1 Touchscreen Calibration

The first time you power up, you may be prompted to calibrate the screen to your touch. Follow the onscreen instructions to touch and hold the target each time it appears. Usually you will be prompted to touch the four corners of the screen, twice in succession.

NOTE:

For optimal results, be sure to stand or sit in the position from which you are most likely to work. Height and angle of reach will affect calibration.
6.2.2 Start-Up Screen

The Start-Up screen, which tells you which operating software version is installed in your BioFlo/CelliGen 115, is first screen you see each time you turn on the power, if you have already calibrated the touchscreen (see Section 6.2.1). This screen remains in view for a few seconds, then it is replaced by the SUMMARY Screen.

6.2.3 Summary Screen

The SUMMARY screen (see Figure 19a) is command central; it puts all the available loops at your fingertips.

Your BioFlo/CelliGen 115 controller can run as many as three stations; the dark blue Unit Tab identifies which vessel’s operating parameters are being displayed (in the sample screen, the unit being displayed is labeled “Unit 2”); if you have more than one unit, pressing another Unit tab will move you to the SUMMARY screen for Unit 1 or, if present, Unit 3.

Figure 19a: Sample SUMMARY Screen (Fermentation with Auto Gas Mix)

Your BioFlo/CelliGen 115 comes with pre-assigned loop names. The available loops will change depending on your system’s configuration. This one is equipped with automatic Gas Mix.

NOTE:
The dark blue button usually represents the screen being displayed.
Your BioFlo/CelliGen 115 comes with pre-assigned loop names. The available loops will change depending on your system's configuration. This one is equipped with manual Gas Mix.

NOTE:
The dark blue button usually represents the screen being displayed.

Figure 19b: Sample SUMMARY Screen (Fermentation with Manual Gas Mix)

Figure 19c: Sample SUMMARY Screen (Cell Culture without TMFC)

NOTE:
The dark blue button usually represents the screen being displayed.
Figure 19d: Sample SUMMARY Screen (Cell Culture with TMFC)

Table 4 identifies the other interactive features of the SUMMARY screen:

<table>
<thead>
<tr>
<th>Parameter Column</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LoopName</td>
<td>Each unit comes with standard factory-assigned loops (e.g., Agitation, Temperature, pH, DO, etc.). As indicated in Figures 19a-19c, loops are factory-assigned according to the configuration of your system.</td>
</tr>
<tr>
<td>PV</td>
<td>Process Variable: here the display reflects the current value for each loop, in comparison to its setpoint (displayed in the next column).</td>
</tr>
<tr>
<td>Setpoint</td>
<td>The current setpoint (default or user-set) for each loop.</td>
</tr>
<tr>
<td>Out%</td>
<td>The current percent output for each loop. This is an automatic control function to maintain current readings within the setpoint tolerance range.</td>
</tr>
<tr>
<td>Control Mode</td>
<td>Depending on the loop, the control mode may be Off, Auto, Manual, On, or O2 Enrich.</td>
</tr>
<tr>
<td>Unit (of measure)</td>
<td>This is the unit of measure used for the PV and Setpoint.</td>
</tr>
<tr>
<td>Cascade</td>
<td>If any cascades have been programmed, they will be displayed here.</td>
</tr>
</tbody>
</table>

NOTE:
The dark blue button usually represents the screen being displayed.

…continued…
<table>
<thead>
<tr>
<th>Navigation Buttons (for screen access)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summary</td>
<td>This screen is command central; it shows all your loops, their current readings, setpoints and what has been programmed for them.</td>
</tr>
<tr>
<td>Calibration</td>
<td>This screen allows you to calibrate the pH, DO &amp; Level probes and the gas flow.</td>
</tr>
<tr>
<td>Cascade</td>
<td>A cascade is a control function that uses the output of one loop to influence the action and output of one or more other loop(s). This screen allows you to set up cascades, to view current settings, and to make changes to those settings.</td>
</tr>
<tr>
<td>Pumps</td>
<td>This screen gives you access to the Pump Gauges screen, where the three pump gauges are displayed, providing both current readings and the opportunity to change pump settings.</td>
</tr>
<tr>
<td>Setup</td>
<td>This screen allows you to make changes to your system settings, hardware setup &amp; controller setup</td>
</tr>
</tbody>
</table>

6.2.4 Keypads

When an alphanumerical or a numerical keypad is needed for you to put information into edit boxes, clicking in the edit box will open the required keypad (see Figures 19e & 19f).

**Figure 19e: Alphanumeric Keypad**

This keypad is used to designate a unit name.

What you type on the keypad appears here.

Pressing the Clear key clears the entry without closing the keypad.

When you have finished typing the entry, pressing the OK key saves the entry and closes the keypad.

Pressing the Cancel key clears the entry and closes the keypad.
6.2.5 Gauge Screens

Every loop has its own gauge screen. To access it, in the SUMMARY screen, touch the screen in the appropriate blue box in the LoopName column. Your touch will open that loop's GAUGE screen (see Figure 20).

Figure 19f: Numeric Keypad

This keypad is used to designate a setpoint speed for Agitation.

What you type on the keypad appears here.

When you have finished typing the entry, pressing the OK key saves the entry and closes the keypad.

Pressing the Clear key clears the entry without closing the keypad.

Figure 20: Sample GAUGE Screen (Agit)

Units:
The action of this loop, Agitation, is measured in RPMs.

LoopName:

Limits:
Here you adjust the high & low settings for this specific loop. When adjusted, the scaling for the gauge will also be adjusted to reflect the high & low limits selected.

Decimal Places:
Press the appropriate button to display values with 0, 1, 2 or 3 decimal places.

Gains:
Proportional & Integral values are what the software uses to calculate output based on differences between setpoint and PV. Changing these may seriously affect your system’s performance. If you think you may have accidentally changed the P&I values, press the Factory Default button to return to their original settings. (See Section 19.4 for more information on P&I Gains.)
6.2.6 Selecting Loop Control Modes

A control mode is the logic by which a controller generates the desired control signal. The operator has a choice of control modes, the most common of which are **ON**, **OFF**, **AUTO** and **MANUAL**. Other available control modes, in certain cases, are **O2ENRICH**, **2-GAS**, **3-GAS** or **4-GAS**.

In cascaded control, one sensor influences an actuator that is normally associated with a different sensor. The onscreen control mode choice will be the name of the loop chosen to have influence on the actuator. *(See Section 10 for details.)*

Control modes vary according to the loop and process mode. *(There are also operating modes for all of the pumps; see Section 11.3 for details.)*

To change operating modes for any of the displayed loops:

1. Press either the **LoopName** or the **Control Mode** box in the row for the appropriate loop, to open the loop’s **GAUGE** screen.

   ![Figure 21: Sample GAUGE Screen (pH)](image)

   **Deadband** is a user-definable **pH** value within which, + or – the setpoint, no response will be triggered.

   - 2. Press the button that corresponds to the desired operating mode.
   - 3. To save the new operating mode and return to the **SUMMARY** screen, press the **SUMMARY** button.

6.2.7 Entering Loop Setpoints

The setpoint is the value you want each loop to attain. When the loop control mode is **AUTO**, the fermentor will automatically make appropriate adjustments to maintain the value at the setpoint.

To enter a setpoint for any loop, follow these steps:

1. Touch either the **LoopName** box or the **Setpoint** box for the desired loop on the **SUMMARY** screen. In this example, we have selected **AGIT**.
2. The loop GAUGE screen opens (see Figure 20, repeated below for easy reference):

![Figure 20: Sample GAUGE Screen (Agit)](#)

3. Press inside the SETPOINT box to open the touchpad.

   If you select MANUAL, you will control the loop by adjusting the Output%, which offers a range of 0-100%, corresponding to the loop’s range. For example, selecting 100% for Agitation will cause the motor to run at 200 RPM (the High Limit set in the Limits pane of this screen).

4. Use the touchpad number keys to enter the desired setpoint.

   Use the white CLEAR button at any time before Step 5 to empty the setpoint edit box.

5. Press OK to save the setpoint and return to the GAUGE screen, or CANCEL to return without saving the setpoint.

### 6.2.8 Modifying Setpoints

This process is the same as entering setpoints. See Section 6.2.7 above.

**NOTE:**

Sections 6.2.9 - 6.2.12 will acquaint you with the primary screens accessed from the blue buttons at the bottom of each screen.
6.2.9 Calibration Screen

This screen is used to calibrate the pH, DO and level probes. For details on probe calibration, see Sections 7.2 (pH probe), 7.3 (DO probe) and 7.4 (Level probes).

![Calibration Screen](image)

These last two “loops” are input from the Level probes to the Level1 and Level2 loops.

6.2.10 Cascade Screen

A cascade is a control function that uses the output of one loop to influence the action and output of one or more other loop(s). This screen (see Figure 24) allows the user to set up cascades, to view current cascade settings and to change those settings. For details on setting cascades, see Section 10.1.

![Cascade Screen](image)
6.2.11 Pump Screen

This screen (see Figure 25) allows the user to access the pump gauges screens, where the three standard pumps are displayed, providing both current readings and the opportunity to change pump settings. For details on using the PUMP screen, see Section 11.1.

Figure 25: Pump Screen

6.2.12 Setup Screen

This master screen is actually comprised of three screens (see Figures 26, 26a & 27), accessed by tabs, which are used to set up the controller, system settings and hardware for the BioFlo/CelliGen 115 system. This section will introduce you to those screens and their features. For details on using the SETUP screen, see Section 12.

When you press the SETUP button, the screen that opens is actually the first tab, the CONTROLLER SETUP (see Figure 26) screen:
The UNIT NAME is user-selected. Press this box, then use the pop-up keypad to type in the name.

The VESSEL SIZE is user-selected by pressing the ▼ to access the dropdown menu, then pressing on the desired vessel size. *Choosing the vessel size here assures the application of accurate PID values.*

The TMFC Range and the number of TMFCs (0 means manual gas flow, usually by rotameter) are factory-set.

The default Operating Mode is Fermentation. To select Cell Culture, click on the ▼, then click on Cell Culture.

English is the default language. When other choices (Français, Deutsch, Español) become available, the user will select the language here.

Use this pane to change Date and Time *(see Section 12.2.1).*

Use this pane to view the Software/Firmware version installed, and to update Software via the USB port *(see Section 12.2.2).*

Use this pane to calibrate the touchscreen (see Section 12.2).
Figure 27: Hardware Setup Screen

Use this screen to view and add hardware for as many as 3 units installed in the system, and to set Unit IDs for software (see Section 12.3).

Use this pane to choose software connections (as explained in Section 12.3).
7 PROBE PREPARATION & CALIBRATION

7.1 pH Probe Inspection

Inspect probe for possible shipping damage. If damage is observed, notify the New Brunswick Scientific Service Department immediately.

Check the electrode tip for trapped air bubbles. To remove any air bubbles, hold the electrode upright and shake gently. NEVER REST THE PROBE ON ITS TIP.

7.2 pH Probe Calibration

NOTE:
Calibrate the pH probe before autoclaving it with the vessel.

1. If you have not already done so, connect the pH probe to the pH connector on the control cabinet (see Figure 1d), using the appropriate cable.
2. Turn the main power switch ON.
3. Press the CALIBRATION button to display the CALIBRATION screen.

NOTE:
The pH probe is calibrated using two external buffer solutions of known pH, usually 7.00 and 4.00.

4. Rinse the pH electrode with distilled water, then immerse it into pH 7.00 buffer solution and allow a few minutes for the system to equilibrate.
5. Open the CALIBRATION screen (see Figure 23, repeated on the following page for easy reference). Steps 6-8 are indicated as callouts around this screen:
9. Rinse the pH electrode with distilled water.
10. Immerse pH electrode into a second pH buffer solution which is several pH units above or below pH 7.00 (e.g., pH 4.00) and allow a few minutes for the system to equilibrate.
11. Similar to step 7 above, touch the SET SPAN edit box. Use the touchpad that opens to enter the value of the second buffer solution (e.g., 4.00), then press the OK button.
12. When the CURRENT VALUE reading stabilizes, press the SET SPAN button.
13. To ensure accuracy, repeat Steps 4-11 a few times, using the same two buffer solutions.

**NOTE:**
The pH calibration should be checked after autoclaving, immediately prior to inoculation. Take a sample from the vessel and compare the pH value displayed on the control cabinet screen to the pH recorded by an external pH meter. Any discrepancy should be adjusted with the SET ZERO procedure.

### 7.2.1 pH Probe Installation

**CAUTION!**
Be sure to wear protective gloves when installing a glass electrode.
**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.

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

---

**CAUTION!**
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.

---

**Figure 28: pH Probe with Port Adapter (exploded)**

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, 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.

---

**CAUTION!**

We recommend that you avoid the use of hydrochloric acid (HCl) with the BioFlo/CelliGen 115 for pH control or any other purpose, because HCl corrodes stainless steel. Over time, it will severely damage the headplate, a costly component to replace, and other stainless steel components.

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

---

7.2.2 pH Probe Maintenance & Storage

Check for any trapped air bubbles in the electrode’s tip to remove bubbles, hold electrode upright and shake electrode gently.

The probe should be stored standing upright, and the electrode tip should be immersed in the solution of 3 molar KCl or a buffer solution between pH 4.00 and pH 7.00. If the probe is so equipped, the two rubber T stoppers should be inserted.

---

**CAUTION!**

Never let a pH probe rest on its tip, and never leave a pH probe in DI water.
7.3 Dissolved Oxygen (DO) Probe Preparation

7.3.1 Inspecting the DO Probe

Inspect the probe for possible shipping damage. Immediately report any damage you may observe to the New Brunswick Scientific Service Department.

Remove the protective cap from the electrode end. The membrane is delicate and care must be exercised to prevent accidental damage. **NEVER REST THE PROBE ON ITS MEMBRANE.**

7.3.2 DO Probe Preparation

To ensure stable output, the probe should be sent through two or three sterilization (autoclaving) cycles prior to use. The probe will be operable after the second cycle, but it will be more stable with additional sterilizations. **The shorting plug should be installed on the probe during autoclaving or sterilization.**

Default P & I (proportional & integral) gains are preset at the factory. They are different for each operating mode, fermentation and cell culture. **It is strongly recommended that you maintain the factory-set parameters.**

Nevertheless, P & I gains for the DO loop can be modified by the operator, using the touchpad on the front of the control cabinet.

As noted above, fermentation mode and cell culture mode require different P & I values to ensure proper DO control. Whether you choose to use (as recommended) the factory-set values or to alter them, it is highly unlikely that you will ever need to re-set or change them. Even if the power fails during a run, the P & I values (pre-set if you do not change them, or your settings when you do) are stored in memory and should still be in effect when the power is restored. **For details regarding P & I settings, see Section 19, Appendix B.**

Nevertheless, it is always prudent to check these values at the beginning of a run, especially if the fermentor has not been used for a while or if other people have access to the unit.

**NOTE:**

It is recommended that you use the factory-set P & I values. Do not attempt to change the settings unless you are experienced with P & I control.
7.3.3 DO Probe Installation

**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.

**CAUTION!**
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 29 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, 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.

### 7.3.4 DO Probe Polarization

**NOTE:**
If the probe has been disconnected from a voltage source (either the unit’s O2 amplifier or a separate polarizing module) for longer than 5 minutes, it will need to be re-polarized.

To re-polarize:
Connect the probe to the operating O2 amplifier (or polarizing module).
Allow SIX HOURS FOR POLARIZATION prior to calibrating the probe.

### 7.3.5 DO Probe Calibration: Setting Zero

**NOTE:**
The DO probe is calibrated AFTER sterilization.

There are two methods to obtain zero for calibrating the DO probe. Review both methods and use the one you prefer:

**Method 1:**

1. Remove the DO cable from the DO electrode.
2. Go to the **CALIBRATION** screen (see Figure 23) and select DO.
3. Enter 0 in the **SET ZERO** edit box (see Figure 30 on the following page), then press **SET ZERO**.
4. Reconnect the DO cable to the DO electrode.

**NOTE:**

If you use Method 1, make sure the probe is not disconnected for more than one minute.

Method 2:

**NOTE:**

Nitrogen is needed for Method 2. There is an N₂ gas inlet on the control cabinet for this purpose; make sure that your nitrogen source is connected to this inlet.

1. Connect the DO cable to the DO electrode and the control cabinet.
2. Go to the CALIBRATION screen (see Figure 23) and select DO.
3. Press the N₂ (3) ON button. Depending on your system’s configuration, however, this button may not be present. In this case, manually turn the N₂ loop on from the SUMMARY screen, or manually turn on the rotameter, and set it to 1-20 SLPM (depending on vessel size and flow controller).
4. In approximately 10-30 minutes, the current value reading will stabilize.
5. Press the SET ZERO edit box (see Figure 30 on the following page), use the touchpad to enter 0, press the OK button, then press the SET ZERO button.
6. Press N₂ (3) OFF (or, if in Step 3 you manually turned the N₂ loop on, now manually shut off the nitrogen flow to the vessel).

**Figure 30: Calibrating DO**

RAW VALUE corresponds to the signal directly received from the probe, before it is converted to a DO value by the controller.

As explained in Step 4 above, these buttons may not be present for your system’s configuration.
7.3.6 DO Probe Calibration: Setting Span

1. In the AGIT GAUGE screen, set the AGIT speed to 50 RPM.
2. Set the AGIT mode to AUTO.
3. Vigorously sparge air into the vessel via the filter on the headplate until the display is stable for approximately 10 minutes (this may take up to 30 minutes total).
4. In the CALIBRATION screen, select DO.
5. Enter 100 in the SET SPAN edit box (see Figure 30), then press the SET SPAN button.

7.4 Level Probe Calibration

Each level sensor is connected to a conductivity probe that is sensitive to wet contact. According to the use you assign to the level probe, it will turn its assigned pump on or off.

For example, if you assign the probe to be Dry, you will position it in the space above the top of the media and calibrate it to be very sensitive to wetness (see Figure 30a). If the wetness is expected to be the result of foam, associate this level probe with the pump you assign to add antifoam (see Section 11.1 for details about pump assignment). When the foam is gone, the probe, sensing that it is no longer wet, will shut off the antifoam addition pump.

If, on the other hand, you assign the probe to be Wet, you will position it within the media and calibrate it to be sensitive to dryness (see Figure 30a). Associate this level probe with the pump you assign to add media so that if the probe becomes dry, it will turn the pump on until the probe is wet again.

Figure 30a: Calibrating Level Probes

To calibrate the level sensor as Dry, expose the dry probe to foam or media, depending on the element you wish to control, until the Raw Value changes to Wet.

To calibrate the level sensor as Wet, immerse it in media to show Wet as the Raw Value, then remove it from the media until the Raw Value changes to Dry.

Sensitivity is the level at which the probe will turn its associated pump on or off.
7.5 About Pump Calibration

To assure the most accurate flow rate, calibrate the pump each time you change tubing. See Section 11.6.4 for details.
8 VESSEL STERILIZATION

NOTE:
Before proceeding, consult the dimensions of your vessel assemblies to be sure your autoclave is large enough to accommodate the vessel with its various components.

WARNING!
During autoclaving, the vessel exhaust filter must be vented to avoid explosion.

WARNING!
Use protective gloves when handling hot components.

CAUTION!
Before connecting or disconnecting the water hoses to/from the vessel and/or cabinet at any time, make sure the main water supply is closed.

CAUTION!
During sterilization, the bearing housing cap must be installed on the fermentation vessel bearing housing, to keep steam from damaging the internal bearings.

CAUTION!
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.

8.1 Initial Preparation for Autoclaving

To prepare 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 DO 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 ½ 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 each 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 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 using heat-labile media, use at least 100 ml of a balanced salt solution (such as phosphate-balanced saline solution). Sterilize the media separately, after autoclaving the vessel.

8.2 Autoclaving the Vessel

1. If you have a 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 XMF-8624/M1227-9231—see Figure 31 below). Secure it in place with the strap.

2. **If the vessel is not water-jacketed, skip to Step 3.** If the vessel is water-jacketed, the jacket should be half full for autoclaving (see Section 4.8.3 for instructions on filling the jacket). Make sure that the Water In line connected to the bottom of the jacketed vessel is pinched closed, to avoid water leaking from the jacket during autoclaving.

3. Insert the entire vessel assembly (glass jar, vessel stand if present, headplate and all headplate components) into an autoclave and sterilize.

4. 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.
8.2.1 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.

**CAUTION!**
During autoclaving, the vessel must be vented at all times. Release the autoclave pressure only when the temperature has dropped below 90°C. Use slow exhaust (30-60 minutes). If available, the autoclave should be on liquid cycle pressure release.

**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, after autoclaving, most of the liquid has left the vessel, the autoclave is exhausting too quickly. Adjust the autoclave to exhaust more slowly.
9 \hspace{1cm} REINSTALLING THE VESSEL ASSEMBLY

9.1 \hspace{1cm} Reinstall the Vessel Assembly

**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.

1. Position the vessel next to the BioFlo/CelliGen 115 control cabinet. Connect the water lines to the heat exchanger and the exhaust condenser (see Vessel Assembly section).
2. Connect the drain line.
3. Connect the Cooling Loop In and Cooling Loop Return between the cabinet and the vessel.
4. Connect Exhaust In and Return between the cabinet and the exhaust condenser (if present).
5. Secure all connections.
6. Connect the Water In to your water supply.
7. Turn your water utility on to 10 PSIG.
8. Carefully place the motor on the bearing housing, on top of the vessel assembly.
9. Remove the pH shorting cap and connect the pH cable to the pH connector on the control cabinet.
10. Remove the DO shorting cap and connect the DO cable to the DO connector on the control cabinet.
11. Connect the foam probe cable to the foam connector on the control cabinet.

9.2 \hspace{1cm} Load Pump Tubing

The three standard pumps are located on the front of the control cabinet (see Figure 32):
Before you insert tubing into the **PUMP CHANNEL**, verify that the **PUMP** is in the **OFF** control mode. *With reference to Figure 33 below, follow these steps to properly load tubing into the **PUMP HEAD**.*

1. Open the **PUMP** cover to gain access to the interior of the pump.
2. Select the desired tubing size (*see Table 6 in Section 11.4 for reference*) and cut a length sufficient to reach from the inlet source, through the pump, and to the outlet recipient, allowing a few extra inches.
3. Form a loop large enough to go around the pump head.
4. Fit one side of the tubing loop into one of the spring-loaded clamps, pulling the clamp open with your finger.

**WARNING!**

Be careful not to pinch your fingers in the pump head levers.

5. Then, as you rotate the pump’s rotor by hand in a clockwise direction to clear the channel, lay the tubing in the channel around the pump head.
6. Fit the other end of the tubing loop into the second spring-loaded clamp, making sure the tubing fits tightly around the pumphead.
7. Press and hold the pump mode **Prime** button or change the pump mode to **ON** at 100% setpoint and ensure that the pump operates smoothly.

See Section 11.1 for details on pump assignment and Section 11 for details on pump set-up and operation.

### 9.3 Confirm pH Calibration

Autoclaving can alter the zero characteristics of pH probes, typically by 0.1-0.3 pH. To check, and to compensate for any discrepancy, you will need an accurate external pH meter.

1. Following sterilization, with the media at room temperature, note the pH value on the BioFlo/CelliGen 115 **SUMMARY** screen.
2. Take a sample of media and measure the pH using the external meter.
3. If the two values disagree, return to the pH calibration screen (see Section 7.2) 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.

### 9.4 Install Liquid Addition Systems

Figure 34 is a simple depiction of a typical addition system. Depending on the liquids (base, acid, nutrients, media) to be added, your system may be slightly different.

1. Aseptically install (if applicable) a sterile (0.2µm) filter in one of the two penetrations on the addition bottle cap.
2. Aseptically connect the tubing, securing it with a plastic tie, to the harvest tube in the addition bottle. Clamp it off at the top.
3. If you have not already done so, thread the tubing through the selected feed pump.
4. Connect the tubing, securing it with a plastic tie, to the appropriate addition port on the headplate.
5. Remove the clamp.

**CAUTION!**
Proper pH control is critically dependent on tubing size, which should be as small as possible. Consult Table 6, the flow rate/tubing size chart, for guidance.

### 9.4.1 Addition Tubing Size

pH can be controlled by automatic additions of liquid acid and base. Additions are triggered by the BioFlo/CelliGen 115 controller, which is constantly comparing current pH value with the pH setpoint and making adjustments as necessary.

**Figure 34: Typical Liquid Addition System**
The concentrations of acid and base, and the inner diameter of the acid and base addition tubing (where they pass through the peristaltic pumps), are critical parameters in the proper operation of a P&I pH control system. If the tubing is too large, excessive doses will be added. The result is that the system will “overcontrol,” alternating in close succession between adding one liquid, then the other, providing little or no change in pH reading. A user-selected deadband value is an aid to control pH within the user-assigned range: no acid or base will be added when the pH value falls within the deadband tolerance above or below the setpoint.

5-normal solutions make a good trade-off between moderate addition volume and good control characteristics. The correct tubing diameter varies a little with process, but inside diameters as small as 0.2 mm sometimes eliminate overcontrol while supplying sufficient liquid during high-demand culture phases.

**NOTE:**

Whatever the tubing ID, the tubing wall thickness must be 1/16-inch (1.6 mm).

NBS suggests that you begin with the supplied tubing, which is correct for most applications. If the system oscillates, reduce the tubing ID where it passes through the pump. Use commonly available step-up/step-down adapters and narrower bore tubing to make the tubing modifications, if required. Consult Table 6, the flow rate/tubing size chart, for further information.

### 9.5 Reconnect Gases

Ensure that all gas lines (air, oxygen, etc.) are routed to the appropriate ports and secured at both ends with plastic ties.

### 9.6 Install Temperature (RTD) Probe

1. Turn the power switch **ON**.
2. Add 1-2 ml of glycerin to the thermowell and insert the RTD temperature probe.
3. Attach the RTD cable to the RTD connector on the control cabinet.
4. Set agitation (**AGIT**) to the desired speed and then set its control mode to **AUTO**.
5. Set **TEMP** to the desired working temperature, and set its control mode to **AUTO**.
10 Cascade Control

A cascade is a control scheme in which the output of one control loop influences the setpoint of one or more other loops. In other words, it uses one or more parameter(s) to influence others. For example, if the DO control loop is cascaded to Agitation, whenever the DO process variable drops below its setpoint causing an increase in DO control loop output, the agitation setpoint will increase. This is effective, because agitation strongly influences DO. With this type of cascade, errors in DO are corrected by changes in agitation RPM.

The BioFlo/CelliGen 115 controller allows cascading from the DO loop to as many as three other loops, usually agitation, gas flow and oxygen (each complete loops with their own probes and actuators).

When more than one loop is configured as the recipient of a cascaded loop, they respond sequentially: as one maxes out, the next begins to ramp up.

Depending on the options installed in your system you will have the ability to select one of the following cascades. Those unavailable will be greyed out and not selectable.

- **None**, which means that dissolved oxygen will not be controlled by means of a cascade

- **Agitation** controls dissolved oxygen through automatically controlled agitation speed. When the actual DO2 value drops below the setpoint, the system will increases the agitation speed up to as much as the high limit to meet the culture demands. Once the DO setpoint is reached or exceeded, the agitation will fall back down to the low limit.

- **Oxygen** controls dissolved oxygen by automatically adjusting the mix of air and oxygen. (This is not available without the Automatic Gas Mix Option.) When the actual DO2 value drops below the setpoint, the system will increases the percentage of O2 to as much as the high limit to meet the culture demands.

- **Agitation/Oxygen** controls dissolved oxygen by first increasing Agitation to the high limit, then, if DO still has not reached the setpoint, increasing the oxygen percentage being entered through the sparger to as much as the high limit. This cascade is most frequently used in fermentation. (This is not available without the Automatic Gas Mix Option.)

- **Agitation/GasFlo** controls dissolved oxygen by first increasing Agitation to the high limit, then, if DO still has not reached the setpoint, increasing the GasFlo entering through the sparger to as much as the high limit. This cascade is most frequently used in fermentation. (This is not available without the Automatic GasFlo Option.)
• **GasFlo/O2** controls dissolved oxygen by first increasing GasFlo to the high limit, then, if DO still has not reached the setpoint, increasing oxygen percentage entering the system through the sparger to as much as the high limit. (This is not available without the Automatic GasFlo Option and Automatic Gas Mix Option.)

• **Agitation/GasFlo/O2** controls dissolved oxygen by first increasing Agitation to the high limit, then, if DO still has not reached the setpoint, increasing the GasFlo entering through the sparger to as much as the high limit. If the DO setpoint is still not achieved, the cascade will begin to increase the O2 percentage of the gas mix. (This is not available without the Automatic GasFlo Option and Automatic Gas Mix Option.)

### 10.1 Creating a Cascade

To create a DO cascade:

1. Press the **CASCADE** button to open the **CASCADE** screen (see Figure 24, repeated here for easy reference):

![Figure 24: Cascade Screen](image)

Before a selection is made, the default selection is **None**, indicated both by a dot in its option button (●) and by the loop name in **blue**. Any unavailable cascade will be greyed out, not selectable.
2. Select the Cascade To loop (or series of loops) here. Your selection will now have a dot in the option button (●) and will have changed from black to blue.

3. Enter the desired Low and High limits for the Cascade To loop(s) in the appropriate edit boxes.

In this sample cascade, as the system demands an increase of DO, agitation will increase from 25 to 200. If there is still a need for more DO, the GasFlo loop will kick in until the need is satisfied.
11 ABOUT PUMPS

After assigning the pumps (see Section 11.1), you will need to select a setpoint and a control mode for each, calibrate their flow rates, and select their pulse periods. This section will walk you through those operations.

There are three standard pumps on the front right of your control cabinet (see Figure 32, repeated here for easy reference).

**Figure 32: Standard Pump Array**

---

11.1 Pump Assignment

The user has the ability to assign each pump present in the system.

To assign a pump:

1. From any screen, press the PUMPS button at the bottom to open the PUMP screen (see Figure 25, repeated on the following page for easy reference):
5. Press SUMMARY to save the pump assignment(s) and to return to the SUMMARY screen.

⚠️ NOTE:

For details on the choice of Level Wet and Level Dry, see Section 11.6.1.

### 11.2 Pump Setpoint

To enter a setpoint for a pump:

1. Open the PUMP screen. Gauges for Pumps 1-3 are displayed in this screen (see Figure 36 on the following page).
This sample PUMP screen shows Pump 1 assigned to Acid (see Section 11.1 for details on assigning a pump). Instead of a Setpoint edit box, there is an “Out Mult” (Output Multiplier) edit box. The output for this pump is calculated through PID.

It is common, when a batch is running, to see that pH remains steady at the setpoint, yet the acid and/or 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 CO₂ gas line, providing more nuanced control of additions to maintain pH.

Contrary to the number shown in the Figure 36 Pump 1 Out Mult edit box, 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%.

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.
11.3 **Pump Control Mode**

There are three available control modes for each pump, as explained in Table 5:

<table>
<thead>
<tr>
<th>Control Mode</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off</td>
<td>The pump will receive no input and will not operate.</td>
</tr>
<tr>
<td>On</td>
<td>The pump will operate according to the parameters you have set.</td>
</tr>
<tr>
<td>Prime</td>
<td>This button toggles the pump on or off manually: as long as you press the button, the pump will run continuously. When you release the button, the pump will stop running.</td>
</tr>
</tbody>
</table>

⚠️ **NOTE:**

If pumps are linked to a cascade, this may affect the ability to manually change setpoints and control modes.

To select a Control Mode for any pump, press the appropriate button in the Control Mode pane of the **PUMPS** gauge screen (see Figure 36).

11.4 **Pump Flow Rate & Calibration Methods**

The pump will always run at the same speed, but its flow rate depends on the diameter of tubing you use. Table 6 provides the pump flow rates according to various tubing diameters:

<table>
<thead>
<tr>
<th>Tubing Wall Thickness</th>
<th>1/16 inch (1.6mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside Diameter: inch (mm)</td>
<td>1/50 (0.5)</td>
</tr>
<tr>
<td>12 RPM* Flow ml/minute (50 Hz)</td>
<td>0.25</td>
</tr>
<tr>
<td>12 RPM* Flow ml/minute (60 Hz)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*Pump speed will vary slightly depending on frequency

To calibrate any pump with the tubing you have selected:

1. Load approximately three feet of the tubing into the pump head.
2. Set up a reservoir with water at the input end of the tubing and an empty graduated cylinder, capable of measuring small quantities, at the output end of the tubing.
3. **Read this step completely before you do it:** with the input end of the tubing in the water reservoir, prime the tubing line by pressing the pump’s **Prime** button, but allow it to run only until liquid **starts** to flow into the tubing: **DO NOT** allow the liquid to run into the graduated cylinder yet.
4. *If you are not using a scale, skip to Step 5.* If you are using a scale, place the graduated cylinder (with the tubing) on the scale and press Zero on the scale.

5. In the Flow Rate pane of the PUMP screen (see Figure 36) for that pump, press the **Calibrate** button to open the Calibration pane (see Figure 37):

![Figure 37: Calibrating the Pump Flow Rate](image)

6. Press your choice of Run Time (15, 30 or 60 seconds); that button will turn green.

7. Press Start. The button will turn green and the pump will start running.

8. When the Run Time has elapsed, record the amount of liquid accumulated in the cylinder; enter that number (or the number registered on the scale) in the Amount Pumped edit box.

9. Press the Set button to save this data to the PUMP screen.

**NOTE:**

Calibration must be performed at operating setpoint.

The pump is now calibrated. As the pump runs, you will see that the total will increase by this calibration standard.

**NOTE:**

Each pump and each tubing size will need its own calibration.

### 11.5 Pump Period

At the bottom of each pump gauge is the Period(sec) pane (see Figure 38):

![Figure 38: Pump Period(sec)](image)

Use this edit box, and its associated touchpad, to enter a pump cycle time in seconds. For example, if the pump setpoint is 30%, setting a period of 5 seconds (as illustrated) will cause the pump to run 1.5 seconds, stop for 3.5 seconds, then cycle back on again. (Be sure to read the important **NOTE** on the following page.)
NOTE:
Running at a very low percentage renders the totalizer’s results inaccurate. We recommend the use of smaller tubing to avoid choosing a very low percentage for the pump setpoint.

11.6 Using Level Probes to Program Feed Pumps

11.6.1 Setting a Feed Pump to Add Liquid

A feed pump can be set to add liquid whenever the associated level probe, installed in the vessel, informs the pump that an addition is needed to maintain level.

Prior to autoclaving the vessel, make sure that the level probe that you wish to use is fully inserted into the vessel. When the vessel is set up at the control cabinet, raise the probe to the level at which you want addition to begin. Never lower a probe after autoclaving!

1. Open the PUMP screen.
2. Select the feed pump you wish to pump liquid into the vessel, and press that pump’s ASSIGNMENT button to open the PUMP ASSIGNMENT screen (see Figure 35, repeated below for easy reference).

Figure 35: Pump Assignment Screen

3. Press the Lvl2 Dry button, which corresponds to the probe’s connection on the control cabinet.
4. Press SUMMARY to save the pump assignment and to return to the SUMMARY screen.

In DRY control mode:

- when the liquid is not in contact with the probe, the feed pump is turned on so that more liquid will be added.
- when the liquid is in contact with the probe, the pump is turned off.
11.6.2 Setting a Feed Pump to Harvest

A level probe can also be used to set up a feed pump to harvest.

Prior to autoclaving the vessel make sure that the level probe that you wish to use is fully inserted into the vessel.

When the vessel is set up at the control cabinet, raise the probe to the level at which you want harvesting to begin (i.e., above the current liquid level). Never lower a probe after autoclaving!

1. Open the PUMP screen.
2. Select the feed pump you wish to pump liquid out of the vessel, and press that pump’s ASSIGNMENT button to open the PUMP ASSIGNMENT screen (see Figure 35, repeated above).
3. Select the Lvl2 Wet button, which corresponds to the probe’s connection on the control cabinet.

In WET mode:

- when the liquid is not in contact with the probe the pump is turned off.
- when the liquid is in contact with the probe the pump is turned on.

11.6.3 Level Control Off

When OFF is selected from any level (Foam, HiFoam, Lvl2 Wet, Lvl 2 Dry, Acid or Base) control mode menu, the pump is off.

11.6.4 Pump Calibration

Pump flow rates are provided in Table 6 (Section 11.4). However, more accurate flow rates through the various lines may be established by pre-calibrating the pumps, using the PUMP screen. This screen controls all pump parameters for the three standard fixed speed pumps supplied with each control cabinet and for any additional pumps added through the available analog input and output connections.

Using the PUMP screen, you can view total pump flow rate in ml/second and set the pump’s cycle time, and assign each pump to one of eight functions (None, Acid, Base, Foam/Lvl1, Lvl2Wet, Lvl2Dry—bearing in mind that the “level dry” function turns the pump on when the probe is not in contact with liquid; see Section 11.1 for details).
NOTE:
To assure the most accurate flow rate, calibrate the pump (see Section 11.4) each time you change tubing.
12 USING THE SETUP SCREEN

The SETUP screen (see Figure 39) is used to change Controller Setup (see Section 12.1), to adjust System Settings (select onscreen language when available, change date & time, update software and calibrate the touchscreen; see Section 12.2), and to check or change the Hardware Setup (see Section 12.3).

In addition, if you need to contact NBS Customer Service about your BioFlo/CelliGen 115, you may wish to access this screen to check, in the Hardware Setup pane, the status of installed modules and the firmware version (which you also see briefly in the START-UP screen).

12.1 Controller Setup

When you open the SETUP screen, normally the Controller Setup screen will display first (see Figure 39). If you find any other Setup screen in the display, press the Controller Setup tab to open this screen.

1. Press here to open a touchpad. The name you write in this box will appear on a dark blue button tab on the top menu line.

2. New Unit Name button tab appears here.

3. Currently, the Operating Control Mode is factory-set to Fermentation. See Figure 40 to change this.

4. See Figure 40b to select another Vessel Size.

5. Press the Save Changes button to save new selections (see details in text below).

This pane indicates the options installed on your system. See Section 12.3.1 for details.

If you have more than one unit, the dark blue Unit Name button tab (see callout 2 in Figure 39) is the unit actively represented in the screen. To move to another unit’s setup parameters, press the light blue button. When that button changes to dark blue, its parameters will be actively represented in the screen and you can make changes.
Controller Operating Mode settings (in the Figure 39 sample screen, the controller is set to “O2 Enrich – Direct or Cascade Driven”) depend on the number of thermal mass flow controllers (TMFCs) in your system. See Section 12.1.1 for details on gas control through the Controller Setup screen and the gas process loop gauge screens.

The Save Changes button saves your new selections and reconfigures all control loops accordingly. Although you can save each change one at a time in this screen by pressing it, you can also wait until all changes have been selected. If you leave this screen, however, and wish to save your changes, be sure to press the Save Changes button before you move to another screen.

**Figure 40: Changing Operating Control Mode**

To change the Operating Control Mode, press the ▼ button, then press the desired mode.

The mode will also change in the upper righthand corner of the SETUP screen when you press the Save Changes button (see Figure 40a).

**Figure 40a: Operating Control Mode Changed**

The new Operating Control Mode also appears here.
If you run the unit with various vessel sizes or the size indicated is incorrect for the Unit indicated, use the **Vessel Size** dropdown menus to change to the new vessel size (*see Figure 40b*), then press the **Save Changes** button to allow the system to reset to new parameters.

**Figure 40b: Changing Vessel Size**

To change the Vessel Size, press the ▼ button. In the dropdown menu, press the appropriate size, which will appear in the edit box. Press the Save Changes button to save this selection to memory.

### 12.1.1 Gas Control

Depending on your system’s configuration, you may have the following possibilities for gas control: 1-4 rotameters with manual gas mixing, 1 rotameter with automatic gas mixing, 1 TMFC with manual gas mixing or 1 TMFC with automatic gas mixing.

If your system is equipped with no TMFC or one TMFC, the system will be preconfigured to one *Operating Mode* in the **Controller Setup** screen: O2 Enrich-Direct or Cascade-Driven for Fermentation (*see Figure 39*) or 4 Gas Mix for Cell Culture (*see Figures 40 & 40a*).

Your system has 4 gas solenoid valves.

No TMFC means that all gas flow is manually controlled using one or more rotameter(s).
When you have Fermentation as the Control Mode and O2 Enrich as the Operating Mode, the gas process loops you will find in the SUMMARY screen are labeled Air (1)—as shown in Figure 41—and O2 (2).

**Figure 41: Air (1) Gauge Screen with O2 Enrich**

This gas loop is currently set to O2Enrh (O2 Enrich).

When you have Cell Culture as the Control Mode and 3-Gas mix as the Operating Mode, the process loops are labeled Air (1), O2 (2) and N2 (3) as selected in Figure 41a, or Air (1), O2 (2) and CO2 (4). The loops’ numbers, 1, 2, 3 & 4, correspond to the gas connections on the cabinet (see Figure 1g).

**Figure 41a: Air (1) Gauge Screen with 3-Gas**

Available Cell Culture Gas Mix selections. Choosing O2, N2 gives you a 2-Gas button; choosing Air, O2, CO2 or Air, O2, N2 (as shown) gives you a 3-Gas button. Choosing 4 Gas gives you a 4-Gas button, as shown in Figure 41b.

Selecting Manual in any other gas gauge screen allows you to adjust the percentage of that gas; air always makes up the remainder (if any) of 100%.
When you have Cell Culture as the Control Mode and 4-Gas mix as the Operating Mode, the process loops are labeled Air (1)—as shown in Figure 41b—O2 (2), N2 (3) and CO2 (4). The loops’ numbers, 1, 2, 3 & 4, correspond to the gas connections on the cabinet (see Figure Ig).

**Figure 41b: Air (1) Gauge Screen with 4-Gas**

There is also a GasFlo loop when one TMFC is present; settings in this loop’s gauge screen turn the TMFC on and off and control the gas flow rate. The GasFlo gauge screen (see Figure 42) allows you to set parameters for the TMFC that controls this gas. The gauge screen for any of the gases allows you to set parameters for the TMFC that controls the gas.

**Figure 42: GasFlo Gauge Screen**
12.2 System Settings

Press the third tab in the SETUP screen to open the System Settings screen (see Figure 43). Use this feature to select the onscreen language you prefer, to reset the date and/or time, to update the software, and to calibrate the BioFlo/CelliGen 115 touchscreen.

Figure 43: System Settings Screen

12.2.1 Resetting Date/Time

To reset the onscreen date and/or time (displayed in the lower righthand corner of every screen):

1. In the System Settings screen press the edit box for the numeric parameter you wish to change.
2. Use the pop-up touchpad to input the new number and press the OK button.
3. To change the month, press the down arrow (▼) and press the month you wish to select from its associated drop-down menu.
4. Press the Set button to save the new information. You can do this after each change, or after all changes have been made.

12.2.2 Updating Software

To update the system software, obtain a new version of the software in a USB drive and plug the drive into the USB port on the control cabinet.
1. In the **System Settings** screen, press the **Refresh** button to update the current software status and to search for a new USB drive.
2. The name of the new drive folder appears in the **Update File** box.
3. Press the **Update** button to install the file. The file will reboot twice; this may take a little time.
4. The **Software pane** will reflect the changes.

**Updating software will not affect any previous user settings.**

### 12.3 Hardware Setup

The BioFlo/CelliGen 115 system you purchase is preset in the factory as “Unit1” with all the accompanying hardware. In the Unit1 hardware list shown in the sample **Hardware Setup** screen (*Figure 44*), the system has the Base Power module, the Main pH/DO module, and one TMFC. This system is also set to NBS Modbus communication mode (*see the SCADA pane in Figure 44*), and has the Unit ID number of 2. This is the unit’s multidrop identification number. Remember, when you add units, that no two nodes on the network can have the same multidrop identification number.

**Figure 44: Hardware Setup Screen**

To add new hardware (such as a new utility station), after you connect the module(s) to the system:

1. Press the **Scan Hardware** button in this screen. All new hardware scanned will appear in the **New Hardware** box (*see Figure 45a*).
2. Press the >> button for the Unit name you wish to assign (Unit2, for example), and the new hardware list will move into that unit’s Module box (*see Figure 45b*).
3. To reassign a Unit name, press the << button next to the original unit’s Module box, then press the >>> button for the Unit name you wish to assign. This name will appear at the top of the screen.

4. Each unit needs a unique ID number: in the SCADA pane, assign the correct Communication Mode and Unit ID number, then press the Set button.

Figure 45a: Adding New Hardware

When you press the Scan Hardware button, new hardware appears here.

Figure 45b: New Hardware Added

When you press the >>> button for the Unit involved, the new hardware moves into this pane.
12.3.1 Identifying Utility Station(s) Added

Now that you have added one or more utility station(s) using the Hardware Setup screen, return to the Controller Setup screen to name the new unit(s) as desired, and to identify the vessel size, the operating mode and the options installed on the unit.

Figure 45c: Controller Settings for New Hardware Added

12.3.2 Removing a Utility Station

If at any time you wish to remove a utility station (one which has already been assigned a unit number) from the system, following these instructions:

1. Verify that the utility station is still connected to the control station and both are powered on.
2. Press the SETUP button to open the SETUP screen, then press the Hardware Setup tab (see Figure 44).
3. Press the Scan Hardware button and wait until all items are listed in the Unit panes on the right side of the screen.
4. Press the <<< button corresponding to the Unit you wish to remove. Wait until all the hardware assigned to that unit appear in the New Hardware box.
5. Power off the unwanted utility station and disconnect the RS-485 cable from its COM port and from the control station’s COM port.
13 PERFORMING A RUN

13.1 Set Up Foam Control

Before you fill the vessel with medium, confirm that the foam probe is working properly:

1. Fill the vessel with tap water or saline solution. **DO NOT USE DISTILLED WATER:** an ionic solution is necessary for conductivity.
2. Fill an addition bottle with the antifoam you will use. Attach small bore tubing to the bottle. Plug the end with cotton, and wrap the cotton with aluminum foil. Autoclave the bottle and tubing.
3. Thread the tubing through the pump, then aseptically connect the tubing to the headplate antifoam addition port.
4. Turn the pump on to prime the line.
5. Install the foam probe in its headplate port.
6. Connect the foam probe cable to Lvl 1 on the control cabinet, then attach the cable to the foam probe.
7. Open the PUMP screen.
8. Select the feed pump you are using by assigning Foam to that pump.
9. Enter the pump setpoint and press the ON button.
10. Remove the water/saline solution from the vessel.
11. Add medium to the vessel.
12. Ensure that all appropriate sensors and feed/harvest tubes, including the foam probe and antifoam addition system, are properly inserted and secure.
13. Make sure the DO probe and the pH probe are capped.
14. Ensure that the temperature probe is not in the thermowell; it cannot be autoclaved.
15. Close off all connectors with cotton and aluminum foil, clamp off all tubing, and autoclave the entire assembly.
16. After the vessel has cooled, connect all probes to the control cabinet and all addition tubes to the appropriate pumps. Make sure that all harvest and sample tubes are at the right level.
17. Make sure the impeller shaft is correctly and completely seated into the bearing housing.
18. Make sure that any unused ports are plugged with the supplied penetration plugs.

13.2 Preparing for a Fermentation Run

1. Connect water to the unit and turn it on.
2. Make sure the drain line is properly connected to the unit.
3. Connect the quick-connect plastic water lines to the exhaust condenser.
4. Add glycerin to the thermowell and insert the temperature probe.
5. Make sure the motor is not connected. Turn the power ON.
6. Set the TEMP setpoint to the desired working temperature.
7. Check that agitation (Agit) is in OFF mode. Connect the motor, then set agitation to the desired speed, and select Auto as its control mode.
8. Remove the shorting cap from the pH probe. Connect the pH cable to the pH probe.
9. Remove the protective cap from the DO probe and connect the DO cable to the DO probe.
10. If you have a water-jacketed vessel, be sure to refill the water jacket if required.

**NOTE:**
The DO polarographic probe will need to be connected for a minimum of six hours, to be properly polarized, before it can be correctly calibrated.

11. Calibrate the DO probe (see Section 7.3).
12. Set pH and DO to the desired setpoints
13. Set the pH control mode to Auto.
14. Set the DO control mode to Auto.
15. Open the PUMP screen and assign a pump to Acid and another pump to Base. Turn the pumps ON.
16. If you are using oxygen, set the O2 control loop to the desired setpoint for oxygen enrichment. If, however, you are using Air only, set the O2 setpoint to 0 (zero).
17. Set the O2 (or Air) control loop control mode to O2 Enrich.
18. Enable the pumps.
19. Go to the CASCADE screen and select the DO loop.
20. Set up cascades as desired.

**NOTE:**
Aeration is required whenever the agitation setpoint is greater than 750 RPM. NBS suggests a minimum airflow rate of 0.25 VVM when running at speeds ≥750 RPM.

### 13.3 Inoculation

Using the septum port:

1. Aseptically remove the inoculum from its flask with the inoculation syringe.
2. Inject the inoculum through the septum in the inoculation port.
If you prefer to inoculate via an addition port, be sure to flame the connectors and use an inoculum flask as your “addition vessel” (see Figure 34 for reference).

13.4 Start BioCommand (if present)

1. Start the BioCommand supervisory software on your computer, reset the EFT (Elapsed Fermentation Time) to zero, make appropriate program selections to begin logging data.
2. Make sure all gas pressures are 10 PSI and the water pressure is 10 PSI.
3. If your BioFlo/CelliGen 115 has rotameter air flow control, adjust the airflow to the desired rate. Check to see that flow is stable and that all gases are properly connected.

13.5 Sampling Procedure

Referring to Figure 14a or 14b, whichever represents your sampling system:

1. Check to be sure that the sample bottle is slightly loose, not tight against the gasket.
2. Close the valve on the sampler tube, if it is open.
3. Squeeze the bulb and, holding it compressed, tighten the sample bottle against the gasket.
4. Open the valve and gradually let go of the rubber bulb to obtain the desired sample volume.
5. When you have obtained the desire volume, close the valve.
6. Unscrew the sample bottle from the sampler. Take the cap from a new bottle, and place it on the sample-filled bottle.
7. Install the new bottle in the sampler and make sure that the sample bottle is firmly sealed against the sampler gasket. Always use aseptic techniques.
8. Repeat the above steps until you have the desired number of samples.

13.6 Fermentation Phases

In a typical fermentation run, you can expect to see four characteristic phases: (1) the Lag phase, (2) the Exponential Growth phase, (3) the Steady State phase, and (4) the Decline phase.

13.6.1 Lag Phase

This initial phase is aptly named because it is the slow beginning of your fermentation run, while the microbes become accustomed to their medium.
13.6.2 Exponential Growth Phase

After the initial lag, a sudden spurt in growth will indicate that the environment is fully hospitable to the microbes. Compared to the nearly inanimate lag phase, this activity will appear to be nearly uncontrolled.

13.6.3 Steady State Phase

Most of your run will be the desired steady state of growth. As long as the temperature, pH, DO and other essential parameters are stable and you feed your batch appropriately, this phase can last, for a standard e.coli fermentation, for example, approximately 2-3 hours. Eventually, however, you must expect your batch to decline.

13.6.4 Decline Phase

This final phase is marked by a slow dying off, which is, of course, inevitable.

13.7 Batch Operation

A batch operation is a closed growth environment in the sense that it contains a finite amount of media. The inoculum grows through the various phases of fermentation until it begins to decline and you harvest the desired product. It is easy to run and yields results quickly.

13.8 Fed Batch Operation

A fed batch operation (see Figure 46 on the following page) includes the addition of media to feed the batch fresh nutrient and to dilute any build-up of toxic by-products in the broth, thereby extending the life and growth of the desired product.
13.9 Continuous Operation

A continuous operation could be likened to an assembly line process: fresh medium is added as batch broth is harvested. The fermentation vessel contains, at all times, the optimum amount of media with an established, thriving culture.

13.10 Anaerobic and Microaerophilic Culture

When growing anaerobic organisms, oxygen must be excluded from the media, and when growing microaerophilic organisms, oxygen must be limited to a very low level in the media.
For anaerobes, several strategies can be used to eliminate oxygen:

- Reducing agents can be added to the media.

- Vigorous agitation (normally used to increase dissolved oxygen in the media) is not required. A low agitation rate, however, is required to keep the cells in suspension and to provide mixing of the liquid to maintain good temperature control. An inert gas such as nitrogen can be sparged into the media to provide the necessary anaerobic conditions.

- Additionally, a gas overlay can be installed to introduce the inert gas into the headspace. The gas introduced via the gas overlay can come from splitting of the sparge gas (by using a T or Y fitting).

For the growth of microaerophiles, a premixed gas is introduced into the sparge line and overlay. The gas mixture is dependent on the particular organism that you are culturing.

13.11 Harvesting Procedure

When the vessel is set up on the control cabinet, adjust the level probe’s tip to the level at which you want harvesting to stop (i.e., below the current liquid level):

1. Assign a feed pump as Lvl2 Wet, to pump liquid out of the vessel.
2. Aseptically connect the feed pump’s tubing to the harvest port.
3. Turn the pump ON. Since the liquid is in contact with the probe, the circuit will close, and the pump will begin pumping liquid out of the vessel.
4. When the liquid drops below the probe tip, the pump will stop.

See also Section 11.1, Pump Assignment. If you assign the pump to None instead of Lvl2 Wet, it will harvest as much as possible.

13.12 Shutdown Procedure

At the end of a run, to shut down the system, follow these steps:

1. Set GasFlo to OFF.
2. Set Agit and Temp to OFF.
3. Set all other control loops to OFF.
4. Turn off the power.
5. If the system is not to be used for several days, disconnect the power plug.
6. Remove, drain and clean the vessel *as outlined in Section 15*.

See also Section 21.7.5 for shutdown and cleaning tips.

**NOTE:**

Never wash the filters or get them wet.
14  **ESSENTIAL OPERATING TIPS**

14.1  *Precautions for Glass Vessel Assembly*

There are certain precautions you should take to avoid cracking or breaking the glass vessel during assembly and autoclaving:

- Glass can crack or break during assembly if the clamping screws are overtightened. As a precaution, tighten the screws only finger tight prior to autoclaving. You should be able to insert a business card between the glass and the metal.

- If the vessel is not sufficiently vented during autoclaving, it can crack or break. As a precaution, make certain that the exhaust filter(s) is (are) not wet or clogged. Also loosen the inoculation diaphragm cap for additional venting.

- After autoclaving, tighten the inoculation cap. When the vessel is installed on the control cabinet and air is freely flowing through it, you may retighten all nuts and screws, again taking care not to overtighten.

*NOTE:*

To maintain the best possible seal, O-rings should be replaced every six months or more frequently if needed.

14.2  *Exhaust Condenser & Exhaust Filters*

The inner assembly of the exhaust condenser can be removed for cleaning:

1. Pass warm water and detergent through the top of the condenser, but not through the quick-connects. Do this twice.
2. Run clear water through once.
3. Blow out with air.
4. Autoclave.

Clean the exhaust condenser after each run. This is most critical when operating as a chemostat for protracted fermentation times.
14.3 **Install a Double Filter System**

Double exhaust and double inlet filters are recommended. To install them:

1. Attach a Y fitting to the top of the condenser with a piece of tubing. Be sure to secure the tubing with a tie at each end.
2. Attach an exhaust filter to each branch of the Y. This allows you the flexibility to exchange sterilized filters during a run should one filter become clogged: all you have to do is pinch off the unused line with a clamp.
15 CLEANING

**CAUTION!**
Never clean the vessel or its components or the control cabinet with abrasive chemicals or materials.

15.1 Cleaning the Vessel

尽可能：
If applicable, be sure to follow the bio-safety regulations regarding the release of microorganisms into the environment.

1. Fill the vessel with a mild detergent and water solution.
2. Let it stand for one hour, then brush it thoroughly with a soft brush. Use the brush both on inside and on outside surfaces.
3. Drain the vessel and rinse several times with tap water.
4. Repeat rinsing with distilled water and let it dry.

15.1.1 List of Wetted Parts

For further reference in your choice of cleaning detergents, Table 7 provides a list of wetted parts in the vessel assembly and the materials they are made of:

<table>
<thead>
<tr>
<th>Wetted Parts</th>
<th>Material</th>
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</thead>
<tbody>
<tr>
<td>Headplate O-ring</td>
<td>EPDM</td>
</tr>
<tr>
<td>O-ring lubricant</td>
<td>Silicone</td>
</tr>
<tr>
<td>Headplate penetration O-rings</td>
<td>EPDM</td>
</tr>
<tr>
<td>Metal surfaces</td>
<td>316L or 316 stainless steel</td>
</tr>
<tr>
<td>Vessel glass</td>
<td>Borosilicate glass</td>
</tr>
<tr>
<td>Inoculation septum</td>
<td>Pure gum rubber, color tan</td>
</tr>
</tbody>
</table>

15.2 Cleaning the Cabinet

At least once a month, clean all the metal and plastic parts of your unit. Use a soft, damp cloth moistened with water or mild detergent. If a detergent is used, remove all residue by rinsing them with clean water.
16 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 turn your BioFlo/CelliGen 115 off and disconnect the power cord before performing maintenance.

16.1 pH Probe Maintenance and Storage

The pH 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.

16.2 DO Probe Maintenance and Storage

Use soft facial tissue to clean the DO 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 DO 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 DO probe rest on its tip.
16.3 Vessel & Tubing

After each and every run, clean the vessel and the headplate with its associated parts. All tubing and filters should be replaced.

16.4 Periodic Inspection

At three-month intervals, perform the following checks and inspections.

⚠️ NOTE:
Before you begin, make sure that the power switch is in the OFF position and that the power supply has been disconnected.

1. Check all controls and accessible items (power switch, connectors, screws, nuts and bolts) to make sure they are properly tightened. Tighten any loose item(s).
2. Check that all controls and connectors are free of dust.
3. Check that all O-rings in the headplate and impellers are intact and in good condition. Replace those that are not.

16.5 Agitator Bearing Housing

Every 3-6 months, the ball bearings and the shaft seals in the bearing housing should be checked and cleaned. Replace any worn-out bearings and/or shaft seals.

16.5.1 Motor Assembly Replacement

⚠️ WARNING!
NO ONE BUT A PROFESSIONAL SERVICE PERSON should touch electric or electronic parts or assemblies in the control cabinet.

If the motor assembly should require replacement, call for an authorized NBS service technician.
16.6 Replacement Parts

The following lists of replacement parts are provided for your convenience. Using the NBS part number will facilitate processing of your order by your local NBS distributor. For a complete list of spare parts, please visit the NBS website (www.nbsc.com) or contact your local sales representative.

<table>
<thead>
<tr>
<th>Heaters &amp; Heater Blankets</th>
<th>1.3L, 3.0L</th>
<th>M1369-3107</th>
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<td>Water Jacket Heaters</td>
<td>7.5L, 14.0L</td>
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<td></td>
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<td>M1369-8022</td>
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<td>14.0L</td>
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<table>
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<tr>
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<td>Direct Drive Fermentation</td>
<td>1.3L, 3.0L</td>
<td>M1369-3120</td>
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<td></td>
<td>7.5L, 14.0L</td>
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<table>
<thead>
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<th>Impellers</th>
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<tr>
<td>6-Blade Rushton type 74mm</td>
<td>1.3L, 3.0L</td>
<td>M1273-9206</td>
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<tr>
<td>Pitched Blade</td>
<td>7.5L, 14.0L</td>
<td>M1273-9207</td>
</tr>
</tbody>
</table>
If any problems occur with your BioFlo/CelliGen 115 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/CelliGen 115), and the Manufacturing Part Number and Serial Number of the unit.

### 17.1 Troubleshooting

**WARNING!**

Always turn your BioFlo/CelliGen 115 off and disconnect the power cord before performing maintenance.

As with any equipment, difficulties sometimes arise. If you experience a problem with the operation of your BioFlo/CelliGen 115, consult the following list of symptoms. You may be able to resolve the situation easily and quickly yourself.

If the problem is not listed below, or if the suggested solutions do not work, please call your NBS representative to request a service technician. **Other than the solutions proposed below, do not attempt to fix the equipment yourself.**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Solution</th>
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<tbody>
<tr>
<td><strong>TEMPERATURE:</strong></td>
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| Readout is a negative value (typically \(-225^\circ\) C). | - Inspect the temperature probe for obvious damage; replace it if necessary.  
- Make sure the temperature probe is connected to the cabinet jack. |
| The unit will not heat up.                   | - Make sure the unit was primed at start-up.  
- Make sure the temperature probe is plugged into the vessel thermowell.  
- Water pressure may be too low; raise pressure within recommended range.  
- Verify correct connection (click to lock) of the water inlet and outlet lines on the vessel heat exchanger.  
- Hit reset button on hot plate (if appropriate). |
| The unit is leaking water.                   | - Inlet water pressure may be too high; lower pressure within the recommended range.  
- Check for any loose connection of inlet hoses; tighten if necessary. |

...continued...
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Solution</th>
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<tr>
<td>AGITATION:</td>
<td></td>
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| Agitator does not turn, or turns only slowly. | • The motor drive coupling may not be installed properly; read the motor adaptation instructions in this manual, then check the coupling.  
• Remove/replace the O-ring.  
• Make sure the motor is plugged into the cabinet receptacle; TURN OFF MAIN POWER BEFORE CONNECTING THE PLUG. |
| DO and pH PROBES: | |
| DO probe readings are erratic. | • Recalibrate the probe, carefully following instructions in this manual.  
• Recharge the probe, carefully following instructions in this manual.  
• Probe may need a new membrane and a refill of electrolyte.  
• Check for a secure connection.  
• Replace probe cable or DO probe. |
| pH probe readings are erratic. | • Recalibrate the probe, carefully following instructions in this manual.  
• Check for a secure connection.  
• Gel-filled probe may need replacement.  
• Liquid-filled probe may need a refill of electrolyte.  
• Probe cable may need replacement. |
| Probe does not hold calibration. | • Probe may be defective; replace it.  
• pH/DO board may be defective; call for service. |
| GASFLOW: | |
| There is insufficient gas flow. | • Inlet or exhaust sterile air filter may be wet or clogged; replace it.  
• Check that the air pressure is within the specified range.  
• Make sure the control mode for DO and for pH is set to AUTO (not OFF).  
• Make sure that the GasFlo loop is ON.  
• Make sure that the Air loop is in O2 Enrichment mode.  
• Make sure that the DO cascades are Enabled. |
| GENERAL: | |
| Touchscreen is not responding. | • Calibrate touchscreen. |
## 18 Drawings

### 18.1 List of Drawings

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19  APPENDIX A: SOME GENERAL CONCEPTS

NOTE:
In this section, all discussions of P-I-D control are to explain the theory on which it is based. This product uses only P (proportional) & I (integral) control, not D (derivative).

19.1 What is a Controller?

The local process controller is a multi-loop controller, which means it can control several process parameters simultaneously. It compares current values with setpoints and creates independent control signals for each controlled parameter. The control signals are used to drive appropriate actuators that maintain the various parameters at their setpoints.

Using temperature as an example, the controller compares the output of a temperature sensor to the user-entered temperature setpoint, and generates a signal to activate either a heater or a cooler to maintain vessel temperature at the temperature setpoint. The controller provides the logic that generates appropriate drive signals to various actuators so that process parameters remain at their setpoints.

19.2 What is a Control Loop?

A control loop is the basic element of automatic process control. Three components comprise one control loop: a sensor, a controller, and an actuator. Based on information from a sensor, the controller generates an actuator control signal that maintains a parameter at its setpoint. Control will fail if any element in the control loop fails.
19.3 **What is Probe Calibration?**

In bioprocess control, *calibration* generally refers to establishing a correspondence between a probe’s output and the actual value of whatever that probe senses. For example, pH probes are often calibrated with pH 7.0 and pH 4.0 buffers to establish a “zero” (pH 7.0) and a “span” (pH 4.0). Other buffers can be used, but the principle is always the same. For any probe calibration, two values—a zero and a span—are required for the controller to correctly translate inputs from that probe. DO and pH probes are routinely calibrated before each use. Most other probes need be calibrated only infrequently.

19.4 **What are P-I-D Constants?**

The mathematics of P-I-D control is familiar to most control and process engineers.

In P-I-D mode, the controller creates a control signal that is based upon the deviation between the setpoint and input from a sensor. The magnitude of the control signal is determined by a mathematical formula that can include proportional (“P”), integral (“I”) and derivative (“D”) terms. The P, I and D constants are three numbers that determine the relative sizes of the proportional, integral and derivative terms, respectively. To use a temporal analogy, the P or proportional part of the control signal reflects present deviations between setpoint and current value. The I or integral component reflects past deviations, and the D or derivative term anticipates future values of the error.

Generally, with noisy or slow-responding sensors, such as dissolved oxygen and pH probes, the D constant should be set to zero. If the constants for a loop are too large, that loop will oscillate, displaying extreme swings in actuator output. If, for example, agitation changes suddenly and frequently between minimum and maximum RPM, one should suspect incorrect P, I and D values for the agitation control loop. This condition can easily be mistaken for a defective component when it actually results from incorrect settings.

If the constants are too small, control response will be slow, and setpoints may never be reached. Again, this can be mistaken for defective components. P-I-D constants are usually established by methodical trial and error.
19.5 What is P-I-D Tuning?

Tuning consists of establishing controller settings (the proportional, integral, and derivative constants) such that the controller provides proper control. If the P-I-D constants are incorrect, the control signal may be too weak for the parameter to ever reach setpoint or, at the other extreme, the controller may respond excessively to small errors, causing the actuator to oscillate between high and low values. Usable P-I-D constants must be determined for each P-I-D loop. The process is largely one of calculated trial and error.

All loops that are configured with the P-I-D control mode must be tuned. When delivered as part of an NBS system, P-I-D loops will have been tuned at the factory to work correctly with the NBS-controlled instruments. For other applications, the user is responsible for P-I-D tuning.

Tuning can be a complex task for those unfamiliar with the process, which is why a trained engineer or technician normally performs this task. A number of textbooks¹ that explain the theory and describe the process could be useful for the mathematically-inclined novice. The Ziegler-Nichols method, described in the footnoted reference, is used at our production facilities.

The following suggestions are intended for novices. Be sure to refer to a textbook, and consider utilizing the services of a technician.

- Allow sufficient time for the task. Tuning is an iterative process. It consists of configuring a loop with trial P, I and D values, evaluating loop response, then readjusting the constants. The process is repeated until the loop responds fully and without oscillation.

- One usually begins with a trial P, setting I and D to zero. After P is established, a similar iterative process establishes I.

- Most fermentor probes respond too slowly or are too noisy to utilize the D term to advantage. In most cases, D should remain at zero. Agitation is sometimes an exception.

- The magnitude of the control signal depends on the P, I and D constants. It also depends inversely on a Normalizing Constant.

19.6 What Do the Constants Mean?

The control signal, $S_N$, for a loop that is $N$ seconds into a run is expressed mathematically as:

$$S_N = P\left(\frac{e_N}{k}\right) + \Sigma \left(\frac{I}{60}\right)\left(\frac{e_n}{k}\right) + \left[\frac{(e_N-e_{N-1})}{k}\right]$$

Where:

- **P**, **I**, and **D** are, respectively, the proportional, integral and derivative constants
- **e** is the loop setpoint minus the current value, or error
- **k** is a normalizing constant for the loop

The controller reevaluates $S_N$ every second. **I** is divided by 60, so any value entered by the user should be in reciprocal minutes.

The normalizing constant $k$ can be set to any non-zero value, but is usually set to the full-scale reading of the loop. For example, if the range of expected temperatures is 0 to 125, setting $k$ to 125 results in a **P** term value of $P$ when the error is at a maximum, i.e.:

$$P\left(\frac{e_N}{k}\right) = P\left(\frac{125}{125}\right) = P$$

Similarly, with a full-scale error, the **I** term (after 1 minute) and the **D** term will be $I$ and $D$ respectively.
20 APPENDIX B: OTR

20.1 Determining an Oxygen Transfer Rate

The oxygen transfer rate (OTR) of all NBS fermentors is determined by a standard sulfite oxidation test.

The standard operating conditions for determining OTR are:

- Temperature: 30°C
- Agitation: 1000 RPM
- Aeration: 1 VVM

20.1.1 OTR Calculations

OTR can be estimated by titrating a fixed amount of sodium sulfite, Na$_2$SO$_3$, with air, Cu$^+$2:

$$2\text{SO}_3 + \text{O}_2 \rightarrow 2\text{SO}_4$$

**The Procedure**

Calibrate the DO electrode:

- Set zero on DO.

Fully oxygenate the fermentor with agitation and airflow.

- Set span to 100%.

Introduce a known amount of Na$_2$SO$_3$ into the fermentor when fully oxygenated.

- OTR = \(\frac{30,000 \ n}{V \Delta T}\) mM O$_2$/L/hr

  - \(n\) = number of moles of sodium sulfite
  - \(V\) = vessel volume in liters
  - \(\Delta T\) = time taken from DO curve at two points of 50% DO min.
20.2 Some Factors that Affect OTR And Horsepower

Many factors influence OTR, not the least of which are type, size and placement of impellers in the reactor. (Factors which effect OTR are vessel dimensions, impeller diameter, type of impeller, i.e. turbine, marine, pitched blade, etc.). New Brunswick Scientific selects and recommends the placement of impellers in the vessel to attain a minimum of 350 mM O$_2$/L/hr of OTR.

The BioFlo/CelliGen 115 fermentor is supplied with two properly sized Rushton Impellers. Placement of the impellers should be as indicated in Figure 3.

In some processes, users may wish to use a third impeller. Should this be the case, however, a smaller impeller diameter is required, since the systems are specifically designed such that the vessel diameter, motor, impellers, to produce a specific OTR. When any of the factors is changed, other features may also change.

For example, the standard impeller used on the 10-liter BioFlo/CelliGen 115 has a 3.24-inch ($\pm$ 0.015) diameter. If three impellers are to be used, 3.06” diameter impellers are required. This size impeller is normally used in a 7.5L BioFlo/CelliGen 115 vessel. These impellers should be placed such that the bottom impeller is placed one impeller diameter from the bottom of the vessel. The second impeller should be placed one impeller diameter above the bottom impeller, and the third impeller should be placed one impeller diameter above the second.

To determine the horsepower utilized by a given number of impellers, the following formula can be used. The impeller diameter varies to the 5th power with respect to horsepower. A very slight change in the diameter of an impeller can make a great deal of difference in the HP required to drive that impeller.

The approximate horsepower utilized to drive a given set of impellers is determined as follows:

$$ HP = D^5 \times RPM^3 \times (4.5 \times 10^{-13}) \times I $$

Where:

- HP = Horsepower
- D = Impeller diameter in inches
- RPM = Agitator speed in RPM
- $4.5 \times 10^{-13}$ = Constant (factor based on unaerated water at 20°C with a six-bladed Rushton impeller)
- I = factor based on the number of impellers used in the vessel:
  - Use 1 for one impeller
  - Use 1.8 for two impellers
• Use 2.4 for three impellers

⚠️ **NOTE:**

The HP requirements are substantially affected by aeration. An airflow rate of one vessel volume per minute (VVM) may produce as much as 40% reduction in the horsepower used. It is required that some air/gas flow be utilized when running at speeds above 750 RPM. The relationship in the reduction of horsepower when gas is added into the system is not linear. A small amount of air can produce a 20% reduction in horsepower.
The following section outlines step-by-step procedures for carrying out a benchtop fermentation. Provided in a question and answer format, this discussion covers such topics as which media formulation, tubing size, and concentration of various additives should be used. It also addresses the preparation, autoclaving and clean-up procedures for the vessel and accessories. While this example refers specifically to an E. coli fermentation in a BioFlo/CelliGen 115, the information is generally applicable for any fermentation.

## 21.1 Media Formulation

**Question:** What kind of media should be used, and does it differ from media used in shake flasks?

**Answer:** The media used in shake flasks does differ from the standard media used in a fermentation vessel. Shake flask media is generally of a much simpler composition. LB Broth and Tryptic Soy Broth are standard shake flask media.

Here is an example of a more complex media used in a recombinant *E. coli* fermentation:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>3.5</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>5.0</td>
</tr>
<tr>
<td>(NH₄)₂HPO₄</td>
<td>3.5</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.0 (for fed batch)</td>
</tr>
<tr>
<td></td>
<td>30.0 (for batch)</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>5.0</td>
</tr>
<tr>
<td>Trace Metals</td>
<td>1.0 ml/L</td>
</tr>
<tr>
<td>Antifoam</td>
<td>0.5 ml/L</td>
</tr>
</tbody>
</table>

**Trace metals formulation:**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl₃</td>
<td>1.6</td>
</tr>
<tr>
<td>CoCl₂.6H₂O</td>
<td>0.2</td>
</tr>
<tr>
<td>CuCl₂</td>
<td>0.1</td>
</tr>
<tr>
<td>ZnCl₂.4H₂O</td>
<td>0.2</td>
</tr>
<tr>
<td>NaMoO₄</td>
<td>0.2</td>
</tr>
<tr>
<td>H₃BO₄</td>
<td>0.05</td>
</tr>
<tr>
<td>HCl</td>
<td>10 ml</td>
</tr>
<tr>
<td>H₂O</td>
<td>to 1000 ml</td>
</tr>
</tbody>
</table>
For fermentation, the glucose solution is usually sterilized in a separate flask. It is then added aseptically to the other (heat labile) components that cannot be subjected to autoclaving, such as Ampicillin and the trace metal solution. These are prepared in advance by sterile filtration so that they are available as stock solutions. The magnesium sulfate is sometimes sterilized separately.

Most materials are available from a variety of vendors. Note that Sigma and Difco are often the best sources for the more unusual biological and chemical materials. The exact formulations of the trace metals solution and the fermentation media for the fermentors will depend on the precise fermentation you wish to conduct. Various formulations can be found in the handbooks and literature.

### 21.2 Antifoam Formulation

**Question:** What kind of antifoam should be used, and in what concentration?

**Answer:** Please visit our website at www.nbsc.com (click on the FAQs tab, then click on Fermentation and Cell Culture) for recommendations on types of antifoam agents to use. The initial concentration of antifoam is usually 0.1-0.5 ml/L. When the foam probe is used, the pumping of antifoam is controlled by the unit.

The pump should be set to add the minimum amount of antifoaming agent required to prevent foaming in your particular process. That amount varies depending on the amount of protein in the media, the amount of protein secreted by the microorganism, agitation speed, and other factors. Therefore, you will have to experiment to get the proper pump setting.

### 21.3 Tubing Size

**Question:** What is the correct tubing size for acid, base, antifoam and nutrient feed for a fed-batch run?

**Answer:** For vessels up to 5 liters, NBS part number TU202. This is Marprene tubing with an inside dimension (ID) of 1.6 mm. It has an OD of 4.8mm (3/16"NOM) and a wall thickness of 1.6mm. Larger tubing will be required for vessels over 5 liters. It may also be necessary to use a connecting fitting to allow two different tubing sizes to be used (in cases when the tubing size required for the pump and the size required for the direct connection to the vessel differ).

NBS recommends silicon tubing for use with the pump heads provided as standard on BioFlo fermentors. However, Marprene tubing may be used as well, as long as the tubing size does not exceed 3/16” bore x 1/16” wall. Marprene tubing of this size or smaller can be used with Watson-Marlow 101 pump heads under low pressure and with clockwise rotation.
Take note that silicon tubing should not be used with hydrochloric acid (HCL), sulfuric acid (H2SO4) or sodium hydroxide solutions since this material deteriorates rapidly when in contact with such solutions. Another reason for avoiding HCL is that HCL (and to a lesser extent H2SO4) causes corrosion of stainless steel. NaOH solutions equal to or less than 20% can be used in silicon tubing at temperatures less than 120 °F without destroying the tubing. Solutions of sulfuric acid less than 10% can cause moderate damage to silicon tubing.

21.4 Acid & Base

Question: What concentration and type of acid and base should be used?

Answer: The acid solution is 2 - 3N H2SO4. The base solution is either 5N NaOH or NH4OH ~ 29% (which is the standard commercially available concentration.) Note that these are fairly concentrated. The acid can affect the stainless steel parts of the fermentor vessel. To avoid damage to the entry ports, it is a good idea to use a sterile, disposable needle at the end of the addition tubing and to add the acid (or base) through the disposable needle. The needle will corrode, but it saves the fermentor vessel. Insert the needle though a septum port so that the drip point is away from stainless steel components and fairly close to the liquid level. You may also use a more diluted solution of the acid or base. However, take note that this may cause the complication of adding a larger volume of liquid to the vessel. Also, it is not a good idea to add acid and base through a single double or triple port adapter. The combined effects of both causes rapid corrosion of the adapter.

The pump setting is usually 20.0 - 25.0 under acid or base mode. For these concentrations of acid and base, Marprene tubing should be used. To avoid damage to the stainless steel headplate, use a septum port for introduction of these strong solutions into the vessel. If you are using silicon tubing, reduce the concentration of H2SO4 to less than 8% (about 5%) and use a 20% solution of NaOH. When selecting an acid for use in fermentation, select the lowest possible concentration that allows for pH control.

21.5 Glucose Feed

Question: What is the proper concentration of glucose feed?

Answer: The glucose is 50% concentration. The feed rate is not usually a constant value as this will differ not only from run to run, but it will vary greatly over the course of a run, depending upon the organism's growth. This operation can be controlled automatically by BioCommand, NBS' proprietary Windows®-based software.
Glucose feeding can be set to respond to other sensor cues (such as DO level, the pH reading, the turbidity measurement, the glucose measurement, etc.). The pumping profile to be used must generally be determined through experimental experience.

21.6  

**Recommended Process Control Settings**

**Question:** What are the recommended process control settings (i.e., temperature, pH, agitation speed, DO & gas sparge rate)?

**Answer:** For *E. coli*, temperature is usually set to 32° - 35°C and pH is set at 7.0 - 7.2. For yeast the values are 30°C and a pH value of 5.0. Agitation speed is usually set to a minimum of 200 - 300 rpm with a maximum value of 1000 rpm. Dissolved oxygen (or DO) level is usually 30%. The gas sparge rate is generally 0.5 to 1.0 VVM.

21.7  

**Typical Fermentation Run**

**Question:** Can you review the steps involved in set-up through shutdown of a fermentation run?

**Answer:** To answer properly, let's break the process down, as follows:

21.7.1  

**Vessel Preparation Before Autoclaving**

It is advisable to rinse the previously cleaned vessel prior to use. When doing this, remember that all clamps must be open and the valve for the sampling tube must be in the open position. If the glass wool is going to be replaced for the run, then remove it (and the rubber sampler bulb) prior to rinsing. The protective bearing housing cap must also be in place. It will be necessary to hold the protective cap in place if you plan to invert the headplate while rinsing it. In this case it is usually advisable to also remove the clamps that hold the headplate onto the rest of the vessel, as failure to do so will result in their falling out during inversion. The pH and DO probes should not be in the headplate while you rinse it. All gas filters must be removed prior to rinsing. The sparger must, in particular, be checked to ensure that it isn't clogged. The headplate must be oriented in combination with the vessel and the internal baffle so as to allow for the exhaust condenser lines to be connected. Also, the baffle must be positioned so that it does not interfere with the insertion of the pH and DO probes into their ports. Do not place the sample port to the rear of the vessel, and position it so that ample room is available to take a sample. It is advantageous to have the addition ports for acid, base, etc., on the same side as (or at least not opposite) the pumps. The old grease on the top of the glass cylinder should be wiped clean. Reapply grease (Dow Corning silicone grease) prior to installing the headplate: smear a very thin layer around the top
of the cylinder with your fingertip. (Take care to ensure that no residual grease remains on your hands when you touch other parts of the vessel.) When the headplate is in place, be sure to properly tighten the headplate clamps.

All tubing connected to the headplate should be secured at the headplate connection point, as well as to any addition bottles or other connectors. A tie-gun is useful for this purpose. Note that both the air sparger and the exhaust line will have a terminal filter. (For the BioFlo/CelliGen 115 vessel, the NBS part numbers are P0200-0491 for the sparge line’s small filter, and P0200-0490 for the exhaust line’s large filter.) All tubing connected to ports that have their terminus within the vessel below the liquid level (i.e., the harvest and sparge ports) must be clamped prior to autoclaving. The sampler valve must be in the closed position. Other hoses, such as those attached to base or addition ports, should be clamped to facilitate sterile hook-ups. NBS primarily uses the following clamps: a Hosecock Clamp (Fisher catalog number 05-847) and a Hoffman Side Tubing Clamp (Fisher catalog number 05-875B).

Do not rely on polymer clamps to survive autoclaving; they often pop open in the autoclave. If you wish to use the newer polymer clamps during the running of your fermentation, then place them onto the tubing but leave them open. Use easily removable metal clamps to actually close the line during autoclaving. These may be removed after the vessel has been autoclaved. Be sure to use the polymer clamp to close off the tubing BEFORE you remove the metal clamp.

Clamps can be placed at any point on tubing, but be sure they don’t clamp down onto a port or connector, because that would interfere with proper sealing. The open end of the tubing should be covered with cotton, then with aluminum foil. The clamp on the tubing be below the foil & cotton. The sparger filter should also be covered, but not quite as tightly. The exhaust filter is usually not covered. All tubing should be inspected both prior to and after autoclaving to insure integrity.

The above description also applies to any side harvest ports in use. Note that this type of port is often below the media fill line. It is also possible to use a hose that has been tied off and crimped at one end to provide a cap for the base port & addition port, as well as other ports. These caps must fit very securely over the port, in order to avoid loss of sterility due to displacement while autoclaving.

All O-rings should be checked for damage prior to autoclaving. All fittings must be checked for tightness. A loose fitting is often an indication that the small O-ring in the fitting assembly requires replacement.
Verify that the bottom of the glass cylinder is properly secured to its base. The agitation shaft must have its protective cap on prior to autoclaving. It is advisable to check that the connectors from the unit to the vessel (exhaust gas condenser) are compatible. This is a good time to check that the air and water lines to the unit are open and that (if required) an oxygen source is available and correctly connected.

The pH probe must be inspected prior to insertion: enough electrolyte must be present and in good condition, and the rubber stoppers must be securely in place. The pH probe must be properly calibrated prior to insertion in the headplate. (Be sure to carefully follow the manufacturer's instructions for probe calibration, or the instructions in this manual.) It is often necessary to coat the probe with a very thin layer of glycerin or deionized water in order to avoid jamming or breaking it during insertion. The pH probe must be inserted carefully, using two hands, with one hand holding the base of the probe near the port opening.

Never force the probe, and never insert the pH or the DO probe until the headplate is properly secured. It is absolutely critical that both the pH and DO probes have their protective caps on prior to autoclaving; in fact, the caps should always be on except when the probe is being hooked up to the unit. NEVER autoclave a pH probe or a DO probe without its protective cap.

Check the DO probe to be sure the required amount of electrolyte is present prior to insertion; NBS usually replaces electrolyte for each new run. The DO probe's membrane must also be inspected prior to use.

The glass wool for the sampler is prepared by rolling a small quantity up and inserting it into the small tube that attaches to the bulb. It may be necessary to trim any glass wool fibers that stick out. Note that it is undesirable to pack glass wool too tightly; use the bulb and a sampling tube to see if a vacuum can be held and released properly, as when a sample is normally taken. Attach a sample tube prior to autoclaving. This tube should be ¼ to ½ turn loose to avoid explosion or implosion. The glass wool should be covered with a piece of foil.

**21.7.2 Vessel Sterilization**

When autoclaving, the unit exhausts through the exhaust filter, so it is essential that the line be prevented from crimping and that the filter be good (unplugged). To ensure that crimping does not occur, use a short piece of fairly rigid tubing. If rigid tubing is not available, use a small splint to support the tubing. The vessel is normally sterilized for 45 minutes. Note, however, that certain media formulations cannot be sterilized for this length of time, as degradation will occur (check the media manufacturer's instructions). The probes must never be autoclaved dry.
If it becomes necessary to sterilize the vessel without media, use a balanced salt (phosphate-buffered saline) solution to cover the ends of the probes. Aseptically remove the PBS prior to filling the vessel with the desired media. NEVER PLACE PROBES IN DISTILLED OR DEIONIZED WATER: THIS WILL CAUSE YOUR PROBE TO LOSE ELECTROLYTE. The maximum fill is ~70% of the vessel's maximum volume. Autoclaving should be done (when liquid is present in the vessel) on a slow exhaust setting (see autoclave manufacturer’s instructions for autoclaving liquids). Sterilization is at 121°C. When sterilization is complete, check the exhaust line to verify that it didn't crimp, and check the vessel's integrity.

21.7.3 Post-Sterilization Vessel Set-Up

The vessel must be handled gently when removed from the autoclave, to prevent the media from boiling up. Confirm that any unprotected vented lines are clamped off upon removing the vessel from the autoclave. Check the vessel's integrity again, then transport it to the bench unit.

Place the vessel next to the control cabinet. The orientation must allow for proper hook-up to the the exhaust gas condenser lines. Connect the water lines, connecting the outgoing (return) lines before the incoming (delivery) lines, and ensuring that the delivery and return lines are not inverted. Insert the temperature probe into the thermowell. Check that the water lines to the unit are open. Set the temperature value below ambient temperature and set the control to Auto. After ~2-5 minutes, the unit can be switched to the desired temperature setting. This can be checked by making sure that water is truly leaving the unit: observe the water drained through the Drain or Water Out port.

Remove the protective caps from the pH and DO probes and connect the probes to the unit. Be careful with the pH probe: do not twist the probe into its connection to the unit, as this can compromise sterility. The connection must be screwed onto the probe. The pH probe should also be checked to ensure that its rubber stoppers have not been displaced. Note the time that the DO probe is connected, since the probe requires a minimum of 6 hours for polarization.

Remove the bearing housing cap and attach the motor. Open the SUMMARY screen and set the air from OFF to O2 Enrichment. Return to the SUMMARY screen and make sure that GasFlo is in ON mode. Connect the air line from the unit to the sparger’s terminal filter as aseptically as possible (although the filter will prevent external contamination, good technique is always a good idea).
Open the clamp on the sparger line and visually observe the vessel to ensure that air is flowing properly. Then set the agitation to the minimum desired value.

After set-up, the unit should be carefully observed to ensure that there are no problems, (especially no water line leaks).

21.7.4 Vessel Operation

The vessel must have any and all necessary addition bottles connected prior to use. If another bottle, such as the glucose feed, is not initially required, it can be hooked up later. The pH will probably need to be adjusted. This is done by setting the pH control to Auto. Note that due to the unit’s tendency to overshoot the target pH during this initial adjustment, it is desirable to set the initial pH setpoint a little conservatively. (For example: post-sterilization pH reading is 6.8, and desired setpoint is 7.2. Set the unit to setpoint 7.0 when conducting the initial adjustment.) Note that the pH reading must be taken from a vessel that has already cooled down.

Additional media components that are not autoclaved can be added once the vessel has cooled sufficiently. The protocol for this is the same as for inoculation, as described below.

Inoculation can be performed by aseptically pouring liquids into the vessel through the inoculation port, although NBS normally uses the harvest port to inoculate. A peristaltic pump or gravity is used to introduce the inoculum. The shake flask is connected to the port terminus using aseptic techniques, and then the clamps are opened to allow for addition. Once the material is all in (except for any residual inoculum which must be retained for testing), secure the clamps and disconnect the shake flask. At this point, the harvest port terminus must be covered up again, using aseptic techniques, with sterile cotton and foil.

To harvest from the vessel, attach a line to the harvest port and use a peristaltic pump to pump the culture broth out.

21.7.5 Vessel Shutdown & Cleaning

When the fermentation run is complete, it is necessary to carefully shut the process down. First, all operating parameters (agitation, temperature, DO level, pH, and gas feed) must be set from their current control modes (such as Auto, Manual, or ON) to the OFF mode.
Additionally, if a supplemental oxygen feed was used, it will be necessary to close the gas tank valve and its lines to the unit. If a recirculating chiller is in use, it should be shut off when the temperature control is shut off. Clamp off the feed lines (from any addition bottles used) prior to detaching them from the vessel.

The next step is to disconnect the vessel from the unit. Remove the temperature probe from the thermowell. Remove the motor and place the protective cap over the agitation shaft/bearing housing. When you disconnect the water lines, always disconnect the incoming lines prior to the outgoing lines. Disconnect the air line from the sparger.

Disconnect the pH and DO probes from the unit, and put on their protective caps. The DO probe presents an easy removal as you simply unscrew the thread and gently pull it out. Immediately rinse it off, then gently wipe it dry, always remembering to never touch the membrane at the tip. Some runs will result in an accumulation of biomaterial on the probe, so and it may be necessary to wipe the probe down more vigorously; nevertheless, in no case should the tip be touched. After cleaning the DO probe, visually inspect the tip for damage. (If it is damaged, replace the probe.) Store the probe in a clean area in such a way as to protect the sensitive tip.

Removing the pH probe is usually not so difficult inserting it because the shaft is still very real, however, so extreme care must be taken while removing it. Be sure to use two hands, with one hand at the top of the port acting as a guide to ensure proper removal. A gentle pace is required; if at any point in the process the probe should jam, absolutely avoid forcing. It may be necessary to reinsert the probe partway, and to apply a lubricant such as glycerin to the shaft and port in order to effect the removal. In extreme cases, it may be necessary to remove the headplate with the probe still inside so that you can approach the problem from both ends. In such a case, it is critical to remove the headplate very carefully. (We recommend that you have a spare probe available at all times, in case of breakage.)

Once the pH probe has been removed, it should be immediately washed off with warm water. If biomaterial has accumulated on the probe, use a sponge (or an equivalent that will not scratch glass) with gentle pressure to clean the surface. The very tip of the probe should be handled with extreme care and a Kimwipe should be used to gently dry it off after washing. The probe should be stored with the tip immersed in either electrolyte or pH 7 buffer. This electrolyte/buffer can be reused, but it should always be inspected prior to each use for precipitation or contamination.
Now that the vessel is detached from the unit, it can be cleaned. Remove any remaining cotton and foil covering the ports. The rubber sampler bulb should be removed and rinsed separately. The glass wool can be removed at this point, too. Detach the sampling tube and wash it separately. Open the valve on the sampling port and all clamps on all tubing connected to ports for proper washing (be sure to remove the media prior to unclamping any tubing below liquid level, such as a side harvest line). The headplate should be detached by loosening and then removing the clamps that hold it to the rest of the vessel. Those clamps may require rinsing. The remaining culture broth should be sterilized, or emptied into a bucket and disinfected by using bleach or other accepted disinfectant prior to disposal. Note that some media may be incompatible with this procedure, in which case the media can be placed into another container for sterilization prior to disposal.

The headplate should be washed thoroughly with warm water and then with deionized (DI) water. It may be necessary to scrub off any accumulations of biomaterial. A pad that won't scratch the steel is required for this. The agitation shaft, thermowell, harvest and sparger tubes, and the short beveled tips of the interior portion of the base-type addition ports will often require special attention. All tubes and shafts must be cleaned. Note that there may be some residual base or acid left in those lines, so extreme caution and the use of chemically-resistant gloves is highly recommended for this procedure. It is often necessary to hand wipe surfaces with a paper towel in order to fully remove residual traces of small particulate debris.

The washing of the bottom portion of the vessel requires the same procedures as the headplate. Note that the sides of the vessel, particularly near the baffle, may require special attention.

The vessel can now be cleaned by washing with detergent, or by using a cleaning solution. If the vessel is to be sterilized, all standard precautions must be taken. Note that for this purpose, the vessel does not need to be sealed except for those previously cited valves and tubing which run under the liquid level. It will be necessary to use water in the vessel. We recommend the use of DI (deionized) water, and the fill should be at least as high as your standard level for a run.

Unless you have already specifically wiped the residual grease off the top of the glass cylinder, there should be enough so that the headplate can be clamped to the glass vessel. DO NOT tighten the headplate clamps with the same force used to install the headplate prior to a run, as this could lead to vessel damage. Instead, the lightest possible pressure should be used.
The advantage to sterilization is that not only are residual viable organisms killed, but also residual debris will loosen and become removable by washing after the vessel has cooled. If a cleaning solution is required, we recommend a 10% dilution of Micro cleaning solution (International Products Corporation, catalog number 6732). Alternatively, if you are using the vessel for consecutive runs with the same media, rinsing it with warm tap water and with DI water may suffice. Note that if water will run over a vessel surface that is greased, the grease should be removed: wipe it off with a wet paper towel.

In cases where the vessel must be decontaminated prior to cleaning, add water so that the liquid level reaches the maximum working volume of the vessel. This will help prevent biological materials from adhering.
22 APPENDIX D: CORROSION RESISTANCE

Websites such as www.outokumpu.com provide up-to-date information about the 316 type stainless steel used in your BioFlo/CelliGen 115 vessels.
23 APPENDIX E: GENERAL CHARACTERISTICS OF EPR

23.1 Identifying EPR

<table>
<thead>
<tr>
<th>Common Names</th>
<th>EPR, EPT, EPDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trade Names</td>
<td>Resist-O (NordleR) - Compound No. AX-60660</td>
</tr>
<tr>
<td>ASTM D-2000 Classification</td>
<td>CA</td>
</tr>
<tr>
<td>Military (MIL STD 417)</td>
<td>RS</td>
</tr>
<tr>
<td>Chemical Definition</td>
<td>Ethylene Propylene</td>
</tr>
</tbody>
</table>

23.2 General Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Durometer Range (Shore A)</td>
<td>30-90 (NBS uses 80 for most O-rings)</td>
</tr>
<tr>
<td>Tensile Range (P.S.I.)</td>
<td>500-2500</td>
</tr>
<tr>
<td>Elongation (Max. %)</td>
<td>600</td>
</tr>
<tr>
<td>Compression Set</td>
<td>Good</td>
</tr>
<tr>
<td>Resilience - Rebound</td>
<td>Good</td>
</tr>
<tr>
<td>Abrasion Resistance</td>
<td>Good</td>
</tr>
<tr>
<td>Tear Resistance</td>
<td>Fair</td>
</tr>
<tr>
<td>Solvent resistance</td>
<td>Poor</td>
</tr>
<tr>
<td>Oil resistance</td>
<td>Poor</td>
</tr>
<tr>
<td>Low Temperature Usage</td>
<td>-20 to -60°F (-29 to -51°C)</td>
</tr>
<tr>
<td>High Temperature Usage</td>
<td>to 350°F (177°C)</td>
</tr>
<tr>
<td>Aging Weather - Sunlight</td>
<td>Excellent</td>
</tr>
<tr>
<td>Adhesion to Metals</td>
<td>Fair to Good</td>
</tr>
</tbody>
</table>

Ethylene Propylene is a polymer with outstanding properties. It has exceptionally good weather aging and ozone resistance; excellent water and chemical resistance; excellent resistance to gas permeability, and excellent temperature usage range up to 350°F (177°C). Ethylene Propylene is a polymer where oil and solvent resistance is poor, however, it is fairly good in ketones and alcohols. It is not recommended for exposure to aromatic hydrocarbons.
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