Bioprocess Equipment

New Brunswick
an eppendorf company

New Brunswick™ BioFlo® 310
Benchtop Fermentor/Bioreactor

Operating Manual
M1287-0054
Revision H

eppendorf

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January, 2014
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***CAUTION!** Explosion hazard!*

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 flammable gases, be sure to verify the LEL and UEL range of the gases used with this product.

Eppendorf does not warrant the accuracy and flow control of any gases in this product other than Air, N_2, O_2 and CO_2.

Eppendorf is not responsible for any and all hazards created by the use of any gases in this product other than Air, N_2, O_2 and CO_2. The use of flammable or toxic materials in the product without the appropriate monitoring or safety devices could restrict Eppendorf from providing warranty repair, technical advice or application support on this product.

ALERT!

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 Operating Manual before attempting to use this equipment.

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

Eppendorf is not responsible for any damage to this equipment that may result from the use of an accessory not manufactured by Eppendorf.

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:

_____

_____

_____

_____

_____

TABLE OF CONTENTS

1 USER INSTRUCTIONS

1.1 Hazard icons

	General hazard		Risk of burns
	Electrical shock hazard		Risk of material damage
	Explosion hazard		

1.2 Danger levels

The following danger levels are used in safety messages throughout this manual.
Acquaint yourself with each term and the potential risk if you disregard the safety message.

DANGER	Will lead to severe injuries or death.
WARNING	May lead to severe injuries or death.
CAUTION	May lead to light or moderate injuries.
ALERT	May lead to material damage.

1.3 Manual conventions

Depiction	**Meaning**
►	This prompts you to complete an action.
1. 2.	Perform these actions in the sequence described.
▪	List
	**NOTICE:** References useful information.

1.4 Abbreviations

DO	Dissolved Oxygen
LEL	Lower Explosion Limit
RTD	Resistance Temperature Detector
UEL	Upper Explosion Limit
VAC	Voltage in Alternating Current

2 INSPECTION & UNPACKING OF EQUIPMENT

2.1 Inspection and unpacking

- Unpack your order, saving the packing materials for possible future use. Save the operating manual for instruction and reference.
- Verify against your packing list that you have received the correct materials, and that nothing is missing.
- If any part of your order was damaged during shipping, is missing, or fails to operate, contact Eppendorf.

2.2 Basic components

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

- Control Cabinet
- Touchscreen*
- Vessel
- Thermowell & RTD
- Baffles
- Impellers
- Sensor 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.

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 310 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, foam/level, and additional analog/digital inputs and outputs.

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

3.2 Standard vessels

The fermentation vessels are designed for total volumes of 1, 3, 5 and 10 liters. Each vessel consists of a stainless steel headplate, a flanged glass tube (thick-walled) vessel body which is detachable from the stainless steel bottom-dished head. The dished head is jacketed for the circulation of temperature-controlled water. 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 sensor; 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.

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 multi-jaw coupling for fermentation.

It can be easily disconnected for autoclaving the vessel and easily replaced after sterilization. The motor will provide a speed range from 50 to 1200 rpm. 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 11 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 5°C above coolant temperature to 80°C (± 0.1°C). It is controlled by the process control software. 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

You will regulate the airflow rate by inputting values through the touchscreen on the control cabinet.

Up to four gases, including air, nitrogen, carbon dioxide and oxygen, can be introduced into the media through the ring sparger. The flow rate is controlled automatically by one, two, three or four thermal mass flow controller(s), according to the definition of your system. The thermal mass flow controller is regulated automatically according to values set via the control cabinet touchscreen. If you wish to use a Rotameter, there is an option for no thermal mass flow controller.

The percentage of oxygen blended with the sparge air can be controlled manually by the user or automatically through the controller by applying the O_2 enrichment function. *(For further information on cascading, see Section 11.)*

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 - 12.00 (± 0.01). The pH is sensed by a gel-filled pH sensor. Control is maintained by a P & I (proportional & integral) controller which operates two peristaltic pumps, assigned to acid and base addition ports, or 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

DO is controlled in the range of 0 - 200% (± 1%). 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 sensor is a polarographic sensor.

3.8 High foam control

Foam can be controlled during batch fermentation by a foam/level sensor, located in the headplate. The controller operates the antifoam-assigned pump that adds chemical defoamer into the vessel as needed.

3.9 Exhaust system

The exhaust gases pass into the exhaust condenser where moisture is removed, then returned to the vessel. The remaining air passes through the 0.2 μm exhaust filter.

CAUTION! Risk of explosion!
NEVER block the exhaust to pressurize the vessel!
See Section 4.9 for further details.

3.10 Sampling system

SYSTEM I

This system consists of a sampler attached to a sampling tube that extends to the lower portion of the vessel. The sampler has a rubber suction bulb to facilitate collection of representative samples without contamination. A 25 mL or 40 mL screw cap container serves as a reservoir for the sample collected.

SYSTEM II

This system consists of a sample line and a peristaltic pump. The **ON/OFF** function of the pump serves to operate the sampling system.

3.11 Recommended accessories & supplies

Before you begin to assemble your BioFlo 310, 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
- Plastic tubing connectors
- Addition bottles
- A liquid trap
- An inoculation syringe
- Media
- Antifoam agent
- Aluminum foil
- Rubber bands
- pH 4 buffer
- pH 7 buffer
- Silicone O-ring lubricant

A user's kit is available from Eppendorf with many of the commonly required items (including a selection of tubing, clamps, filters, connectors and addition vessels). Speak to your sales representative for more information.

3.12 Supervisory software

In addition to the built-in software that you interface with through the touchscreen, your BioFlo 310 system can be remotely controlled from a PC via Eppendorf *BioCommand*® optional supervisory software (*see Section 4.11*). Consult your sales representative for details; be sure to ask for your choice of AFS or ModBus protocol.

4 INSTALLATION

4.1 Physical location

The surface on which you place the BioFlo 310 fermentor should be smooth, level and sturdy. Ensure that the surface can bear the weight of the fermentor plus vessel contents and any applicable ancilliary equipment.

Also ensure that there is enough space around the back and the front of the BioFlo 310 for proper operation and access. Allow at least 4 inches of clearance behind the unit for heat dissipation. See Section 5, *Specifications*, for weights and dimensions.

4.2 Environment

The BioFlo 310 fermentor operates properly under the following conditions:

- Ambient temperature range 10°C to 30°C
- Relative humidity up to 80% non-condensing

4.3 Installing the control cabinet

Position the BioFlo 310 control cabinet on a firm, level surface in an area where utilities are readily available.

Level the horizontal surface of the base with four leveling glides if necessary.

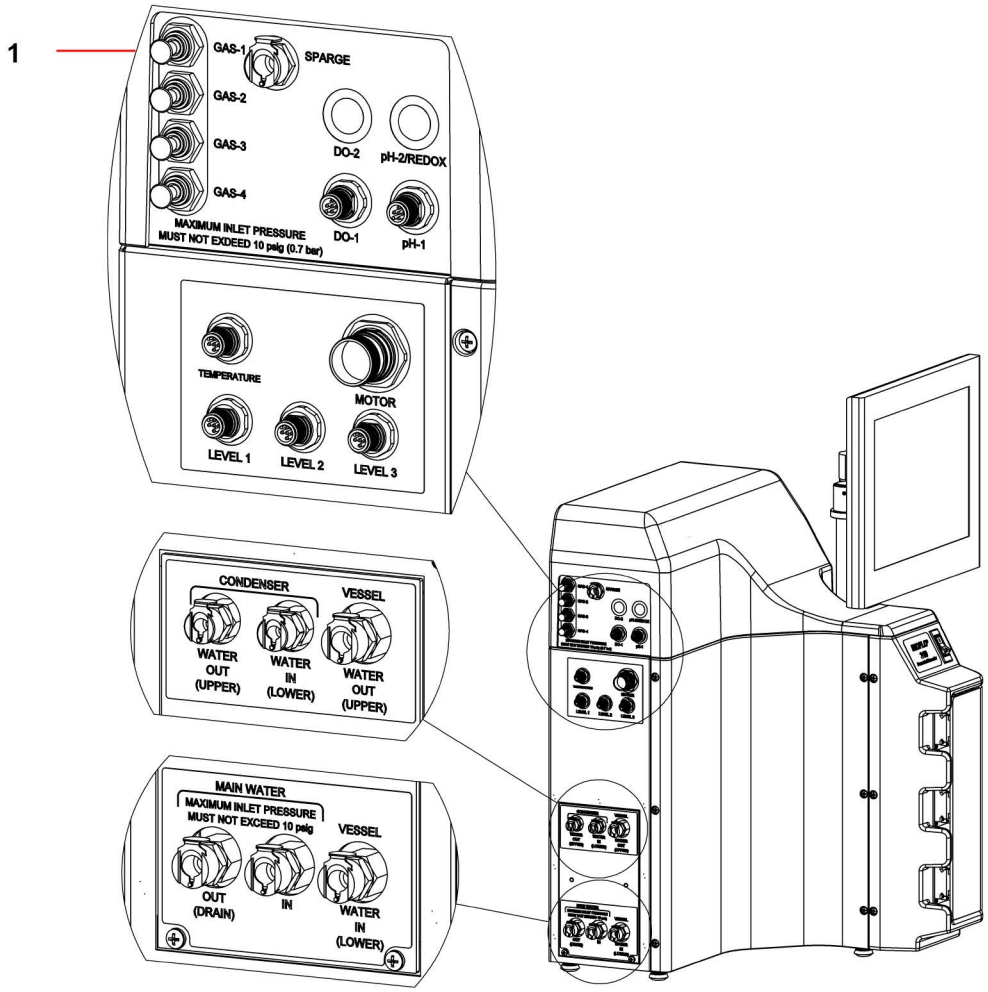
Connect the mains/power cord to the rear of the control cabinet. At a later time, once the unit is completely assembled and all connections have been made, you will plug the mains/power cord into a suitable electrical outlet.

4.4 Installing the touchscreen

With reference to the drawings on the following two pages, align the monitor with the mounting rack on the cabinet, and use the four screws provided with the monitor to securely fasten it to the rack. The mounting rack swivels for easy access.

Connect the cabinet's mains/power cord plug, com port connector and VGA monitor connector to the bottom of the touchscreen monitor.

Figure 1: Control Cabinet Service Connections

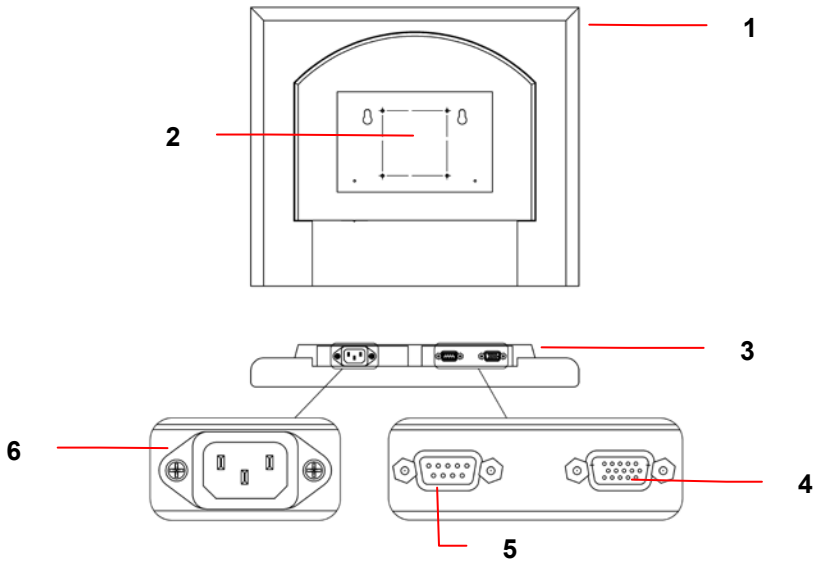


1	The equipment is shipped with the gas inlets plugged. Be sure to remove each metal plug before you insert tubing. IF ANY GAS INLET WILL NOT BE USED, KEEP IT CLOSED OFF with the metal plug provided. To remove the plug, press the ring that surrounds the plug toward the cabinet while simultaneously pulling the plug away from the cabinet.

ALERT!

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

Figure 2: Touchscreen-to-Control Cabinet Connections



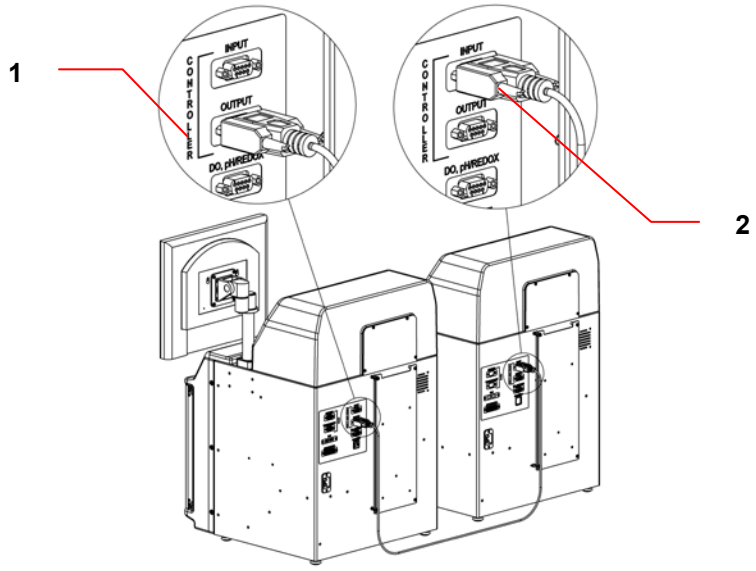
1	Touchscreen (rear view)	3	Touchscreen (bottom view)	4	VGA monitor connection
2	Attach the monitor to the Control Cabinet mounting rack with the four screws provided, using these holes.			5	Com port connector
				6	Mains/power cord plug

4.5 Connecting control cabinets

If you have more than one control cabinet, use the bus cable provided to connect the first's Controller Output port, located on the rear panel of the cabinet (see the drawing on the following page), to the second's Controller Input port.

Do the same to connect each additional cabinet (second to third, then third to fourth).

Figure 3: Connecting Control Cabinets



1	Connect OUTPUT of 1st cabinet…	2	…to INPUT of 2nd cabinet.

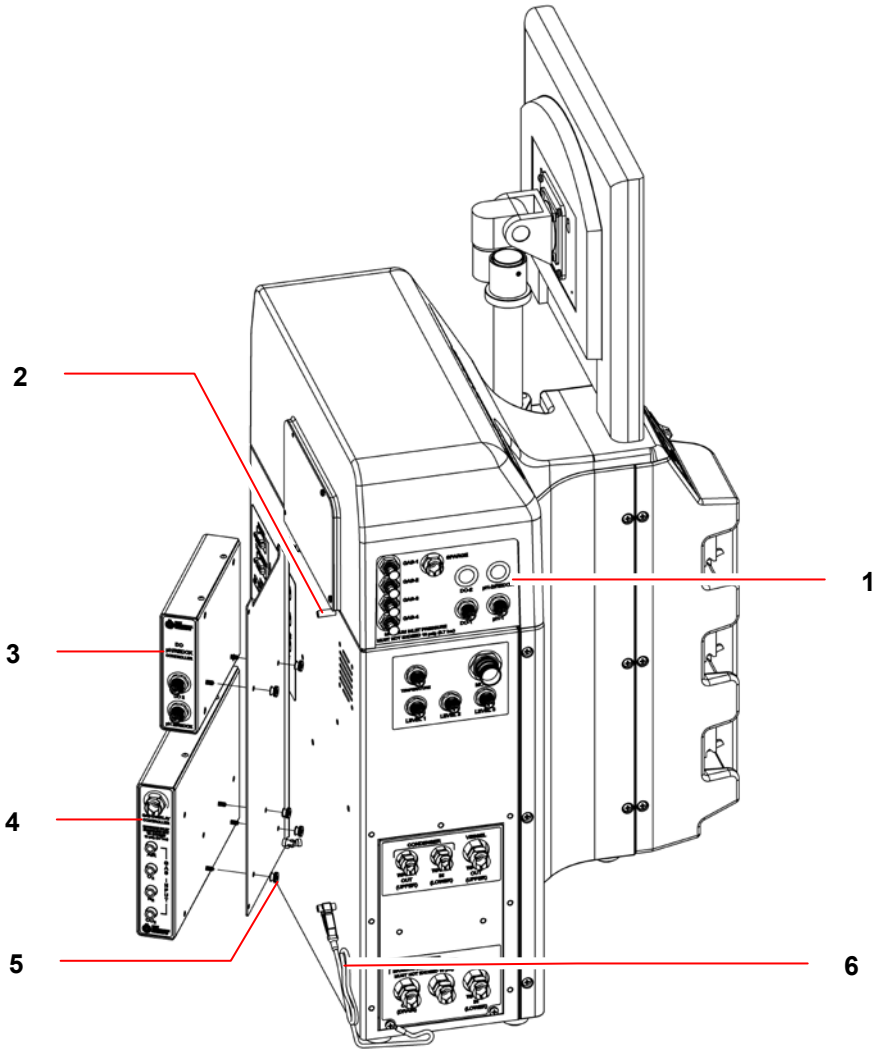
4.6 Adding optional controllers

If you have purchased an optional controller (DO & pH/REDOX and/or Gas Overlay), follow these instructions *with reference to the drawing on the following page* to install them:

1. Remove the 8-32 x ¼-inch Phillips pan head screws from the standoffs, and swing the rear plate open as shown. Reserve the screws for reuse.
2. Mount the controller to the rear plate through the corresponding mounting holes, securing it in place with the thumbscrews provided (2 for the DO & pH/REDOX controller and 3 for the Gas Overlay controller).
3. Swing the backplate and controller(s) back to the standoffs and reinstall the 8-32 x 1/4-inch screws.

If you have purchased the Microbial to Cell Culture/Gas Overlay Conversion Kit (part number M1287-3501), see Appendix A for installation instructions.

Figure 4: Mounting Optional Controllers



1	Connections are here for the optional 2nd DO & pH/Redox controller when it is factory-installed	4	Optional gas overlay controller (retrofitted in field)
2	Standoff	5	Thumbscrew
3	Optional 2nd DO & pH/Redox controller (retrofitted in field)	6	Earth/grounding strap

4.7 Earth/grounding strap

There is an earth/grounding strap, shown in the drawing above as anchored to the lower left mounting screw on the control cabinet's side panel.

When you install the vessel, the clip end of this earth/grounding strap must be clipped to the vessel headplate to earth/ground the motor to the cabinet. This is a requirement to meet safety regulations.

4.8 Utilities

CAUTION! Risk of explosion!

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

The control cabinet assembly must be properly connected to gases, water supply, vessel water, electrical mains/power and an open drain. All service connections are located on the lefthand side of the cabinet.

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 4.8.1 - 4.8.3.

Table 1: Service Connections

Service/Utility	*Requirement*	*Connection*
Electrical	100 - 120 VAC, 50/60 Hz., Single Phase, 15 Amp (fluctuations not to exceed ±10%)	100 - 120 VAC 1ph field wired to 15 Amp disconnect in panel
	208 - 230 VAC, 50/60 Hz., Single Phase, 15 Amp (fluctuations not to exceed ±10%)	208 - 230 VAC 1ph field wired to 15 Amp disconnect in panel
Water Return	Maximum backpressure 5 PSIG	Quick Connect
Facility Water	3 GPM must be regulated to 10 PSIG	Quick Connect
Process Air	10 PSIG	Push on
Oxygen	10 PSIG	Push on
Nitrogen	10 PSIG	Push on
Carbon Dioxide	10 PSIG	Push on
Exhaust	1/2 PSIG maximum backpressure	Quick Connect

4.8.1 Electrical requirements

ALERT!

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

100 - 120 Volts	50/60 Hertz	15 Amp
208 - 230 Volts	50/60 Hertz	15 Amp

CAUTION!* *High voltage!
Always make sure this equipment is properly earthed/grounded.

The electrical requirements vary depending on the part number that has been ordered. Model, Part Number and Electrical Mains/Power Requirements for each fermentor appear on a metal label affixed to the rear of the unit just above the connection for the mains/power cord.

4.8.2 Water and drain connections

The water inlet and drain connections are located on the left side of the control cabinet. **Water pressure should be 10 PSIG, with 50 µm filtration.** Connectors are quick-connect fittings that accept a six-foot long utility hose, which is supplied with the fermentor.

ALERT!
Before connecting or disconnecting the water hoses to/from the vessel at any time, be sure to follow these instructions in the order indicated:
To connect: **(1) Connect vessel Water Out line,**
(2) Connect vessel Water In line,
(3) Connect cabinet Main Water In line,
(4) Turn ON mains/power switch on cabinet.
To disconnect: **(1) Turn OFF mains/power switch on cabinet,**
(2) Disconnect Main Water In line from cabinet,
(3) Disconnect vessel Water In line,
(4) Disconnect vessel Water Out line.
Failure to heed these instructions may lead to hose leakage and/or pressure build-up inside the vessel jacket.

4.8.3 Gas connections

Gas inlets are located on the left side of the control cabinet.

There are push-in connectors for air, nitrogen, oxygen and carbon dioxide. These connectors accept flexible tubing (Part No.P0740-3113C3 polyurethane tubing); tubing is supplied with the fermentor. Other soft, flexible-walled, chemically inert tubing (such as Marprene®, PharMed®, etc.) may be used as well. **Be sure to remove the metal inlet plug first.**

***CAUTION!* Risk of explosion!**

- **Do not use this equipment in a hazardous atmosphere or with hazardous materials for which the equipment was not designed.**
- **All gases supplied should be medical grade.**
- **No gas pressure should rise above 10 PSIG (*see also page iv*).**
- **Never leave a gas inlet open; if no tubing will be connected, keep the inlet 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 between 0 - 10 PSIG maximum.

4.9 Vessel assembly

***CAUTION!* Risk of explosion!**

NEVER OVER-PRESSURIZE A GLASS CULTURE VESSEL!

- **Always use eye protection, and exercise caution in the vicinity of glass vessels. If the vessel exhaust becomes blocked, pressure can build up, possibly shattering the vessel and endangering personnel.**
- **Before opening the airflow valve(s), visually confirm that the vessel exhaust is not blocked by kinked tubing, clamps or a wet filter.**
- **After opening the airflow valve(s), verify by feel that air is flowing freely from the exhaust. If not, <u>immediately</u> close the valve(s) or turn off the air/gas supplies.**
- **<u>Never</u> intentionally block the exhaust to raise vessel pressure.**
- **Use the minimum air/gas pressure that will provide adequate airflow for the application. <u>Never</u> exceed the maximum pressure specified in this manual.**

Clean the vessel thoroughly after each run with detergent, otherwise debris will build up thus providing a place for bacteria to grow and produce toxins. This can result in low cell viability.

ALERT!

To avoid leaks and/or pressure build-up inside the vessel jacket, see *CAUTION* regarding connecting & disconnecting hoses in Section 4.8.2.

ALERT!

Always turn the cabinet mains/power OFF when the vessel and/or the water lines are not connected to the supply or the vessel. Failure to do this will result in premature failure of the pump.

ALERT!* *Risk of damage to vessel!

Prime the water system before the first use of the vessel and every time the vessel has been detached then reattached.

To prime the water system:

- **Make sure all water connections are intact.**
- **Set the Temp setpoint (*see Section 10.3.1*) 10°C below process value.**
- **Turn Temp to AUTO for 3 minutes (*see Section 10.1*) to run 100% cooling.**

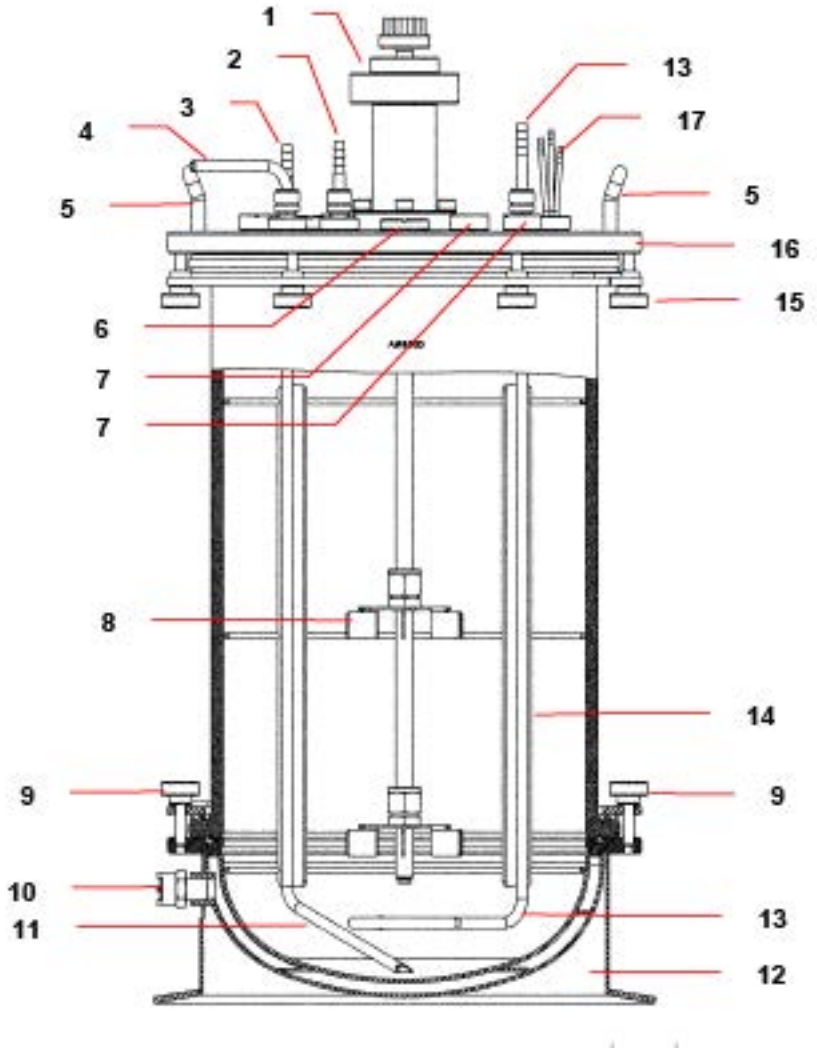
The cooling water will drive out any air that was left in the water system lines and the vessel heat exchanger.

1. Clean the jacketed glass vessel with a cell culture compatible detergent. After cleaning, rinse several times with distilled water.
2. If microcarriers are being used, the glass vessel should be siliconized. A silicone solution such as Sigmacote® may be used according to the manufacturer's instructions.

As you follow the instructions for vessel assembly, use the drawing on the following page as a guide. **Bear in mind that this illustration is for information only; vessel size and your choice of optional components may affect arrangements.**

You may also have a personal preference for the location of some components, which is why, as the size of the headplate allows, you have several same-size ports to choose from.

Figure 5: Fermentation Vessel Assembly



1	Bearing housing	7	DO/pH port	13	Sparger
2	Addition port	8	Rushton impeller, 6-bladed	14	Baffle
3	Harvest port	9	Base heater bolt	15	Headplate bolts
4	Sample port	10	Water in/out quick-connect	16	Headplate
5	Headplate handle	11	Harvest tube	17	Tri-port/addition
6	19mm port	12	Heat exchanger		

If you need to insert the baffle (*see Section 4.9.1*) and/or impellers (*see Section 4.9.2*), **remove the headplate** by unscrewing the headbolts, each a little at a time, working diagonally rather than around in a circle. Set the headbolts aside for reuse. Carefully lift the headplate (using the handles if they are present) and set it aside.

4.9.1 Insert baffle

For fermentation only: If you are using a baffle, and it is not already installed, install the baffle assembly inside the glass vessel by gently compressing the baffle ring at its ends and then sliding the assembly into the vessel until it rests on the bottom. Make sure the baffle's vertical opening faces the location where you plan to install the pH and DO sensors.

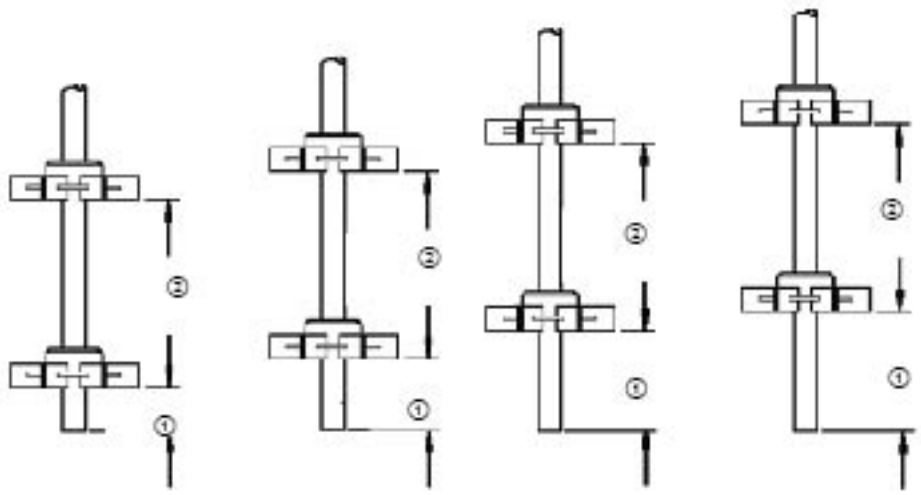
4.9.2 Insert impellers

Drive-shaft-mounted impellers are used for fermentation. To install your drive-shaft-mounted marine or pitched blade impellers:

1. Slide the impellers onto the agitation drive shaft (from the bearing housing).
2. The lower impeller should be positioned about 1/4 inch above the bottom of the baffle.
3. The upper impeller should be one to one-and-one-half impeller diameters above the lower impeller *(see the drawing below and the key on the next page)*.
4. Clamp the impellers down in place.

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

Figure 6: Impeller Location on Agitation Drive Shaft



See key to ① & ② on the next page.

	① To Bottom Impeller	② Between Bottom Impeller & Top Impeller	① To Bottom Impeller	② Between Bottom Impeller & Top Impeller
	mm			
1 L	12.7	76.2	0.5	3
3 L	25.4	101.6	1	4
5 L	0	114.3	0	4.5
10 L	12.7	127	0.5	5

ALERT!
Never exceed 200 rpm unless at least one impeller is immersed in liquid.

4.9.3 Install retention rings

1. Lubricate the headplate O-ring and the lower retention ring O-ring with a light coat of silicone grease.
2. Make sure each O-ring is well seated in its groove.
3. Position the glass vessel in its lower retention ring/heat exchanger.
4. Tighten each clamping screw a little at a time, working diagonally from one screw to another rather than working around the circle, to avoid misalignment.
5. Secure the clamping screws by making them evenly **finger tight**.

ALERT!
To avoid vessel stress cracks, especially during autoclaving, make vessel clamping screws finger tight; there should be just enough flex in the O-ring for you to be able to introduce a business card between the steel headplate and the glass vessel flange.

4.9.4 Install sparger

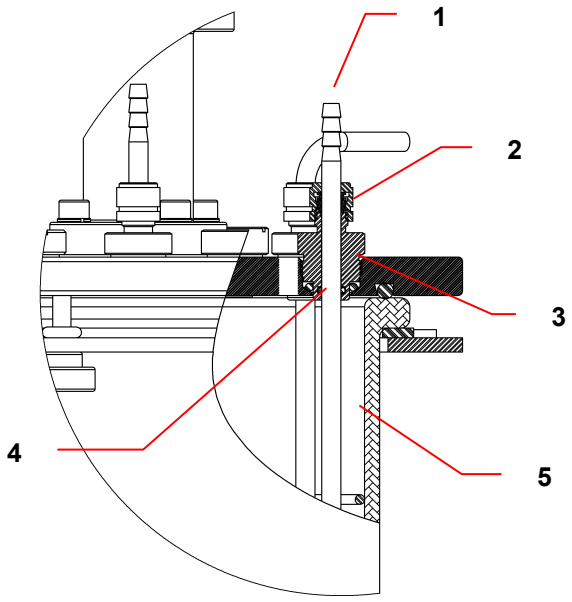
If you are using a sparger for gas delivery and if it is not already installed:

1. Working from inside the headplate, insert the sparger tube into the sparger port adapter (*see the drawings on the following pages for reference*).
2. Finger tighten the lock nut on the sparger.

ALERT!

Only finger tighten the lock nut on the sparger, harvest tube or thermowell. These lock nuts have ferrules that can extrude under too much pressure.

Figure 7: Sparger Installation

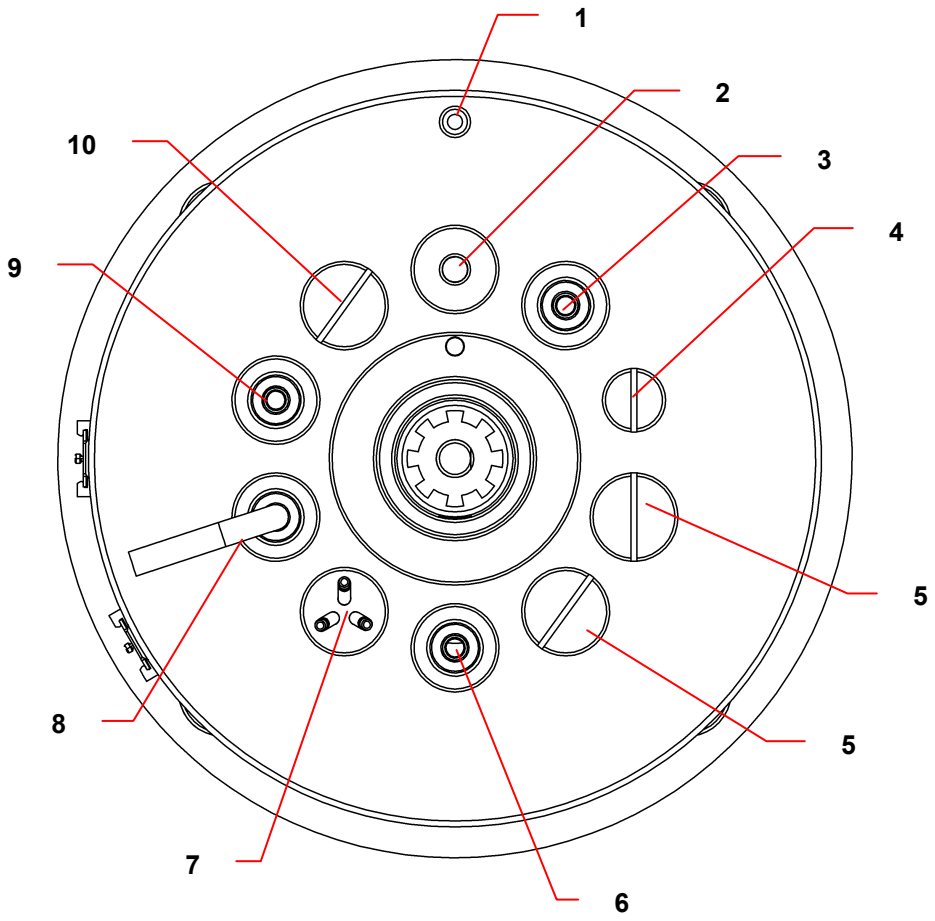


1	Add filter here	3	Port adapter	5	Sparger
2	Ferrule	4	O-ring		

4.9.5 Headplate penetrations

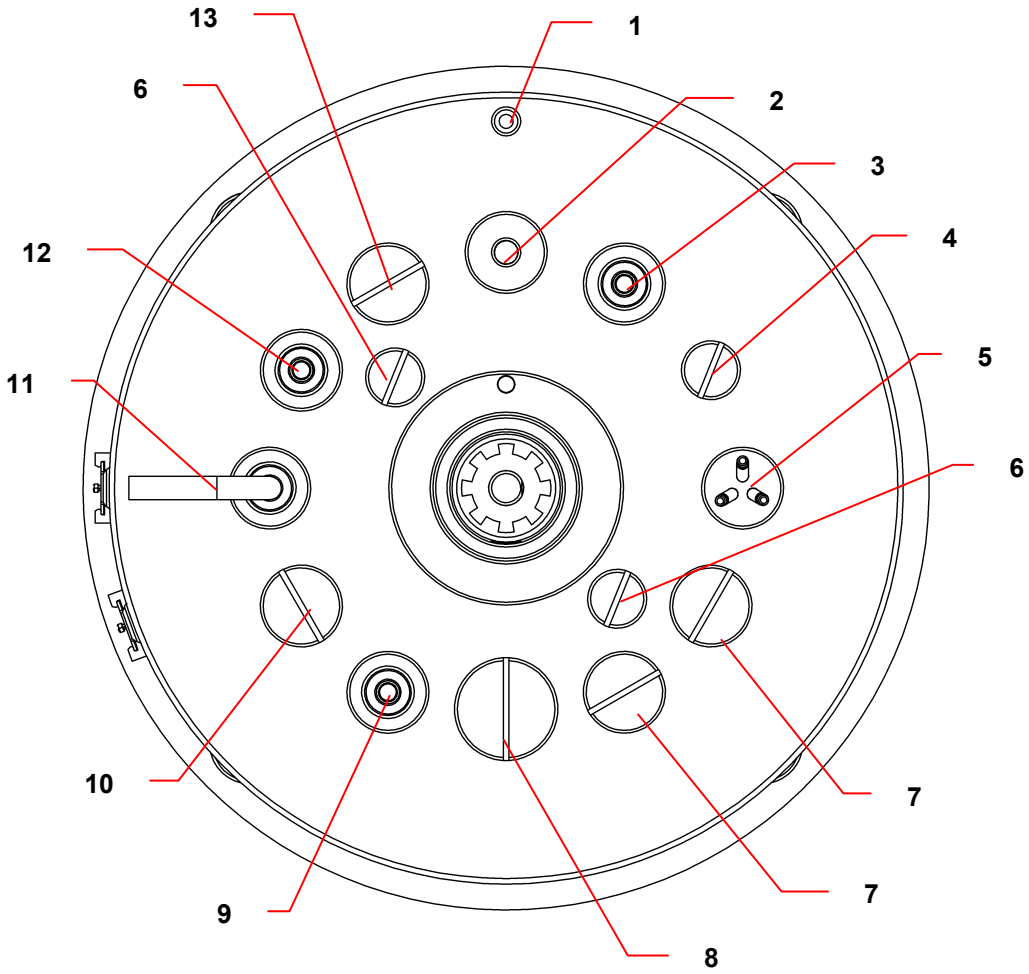
While the drawings on the following pages are not definitive maps, they will serve to guide your use of the various headplate ports.

Figure 8: 1 L Headplate Arrangement



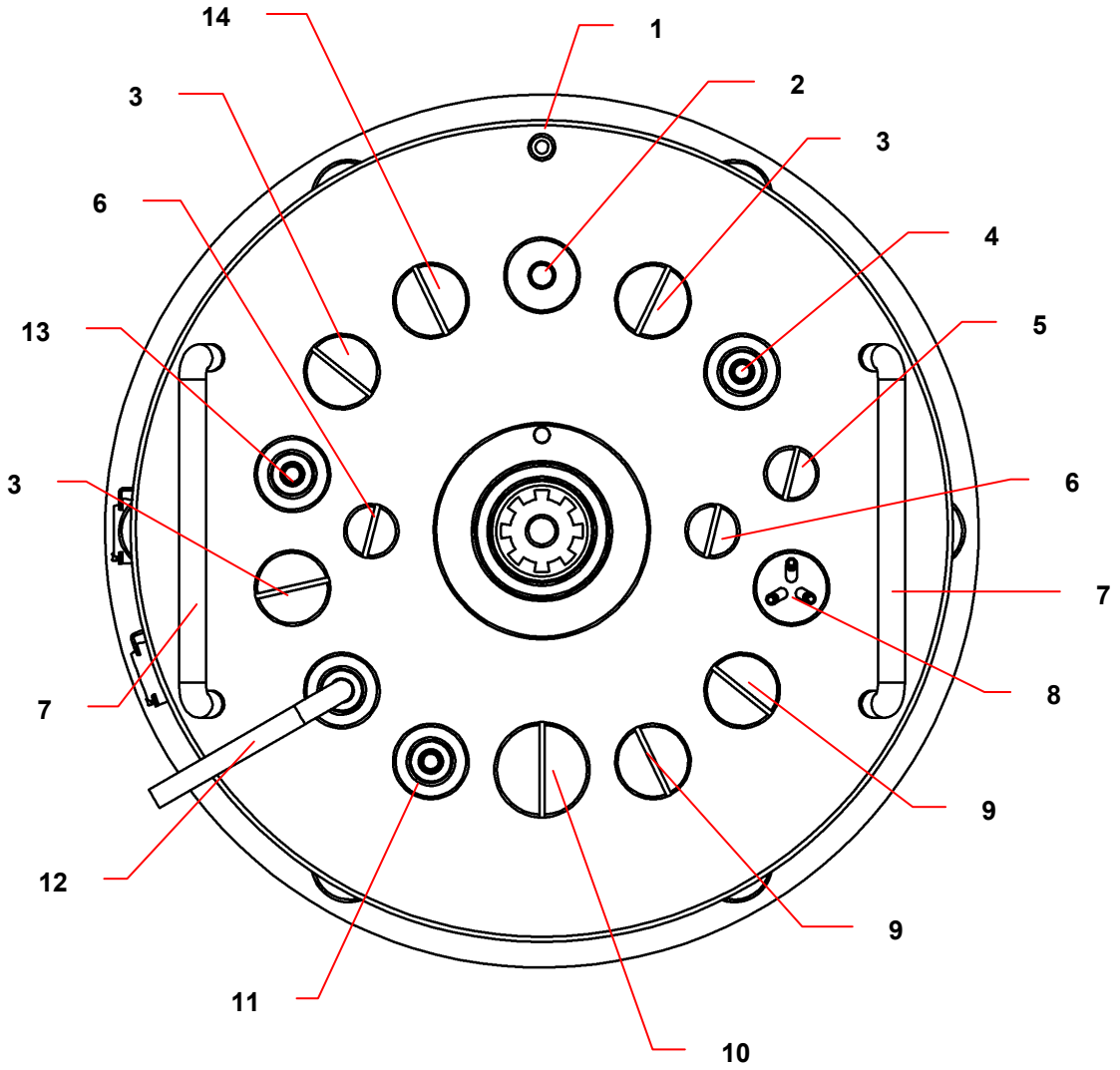
1	Earth/grounding lug	5	DO/pH	9	Harvest
2	Thermowell/RTD	6	Addition (single)	10	Exhaust
3	Sparger	7	Tri-port/addition		
4	Level/foam	8	Sampler		

Figure 9: 3 L Headplate Arrangement



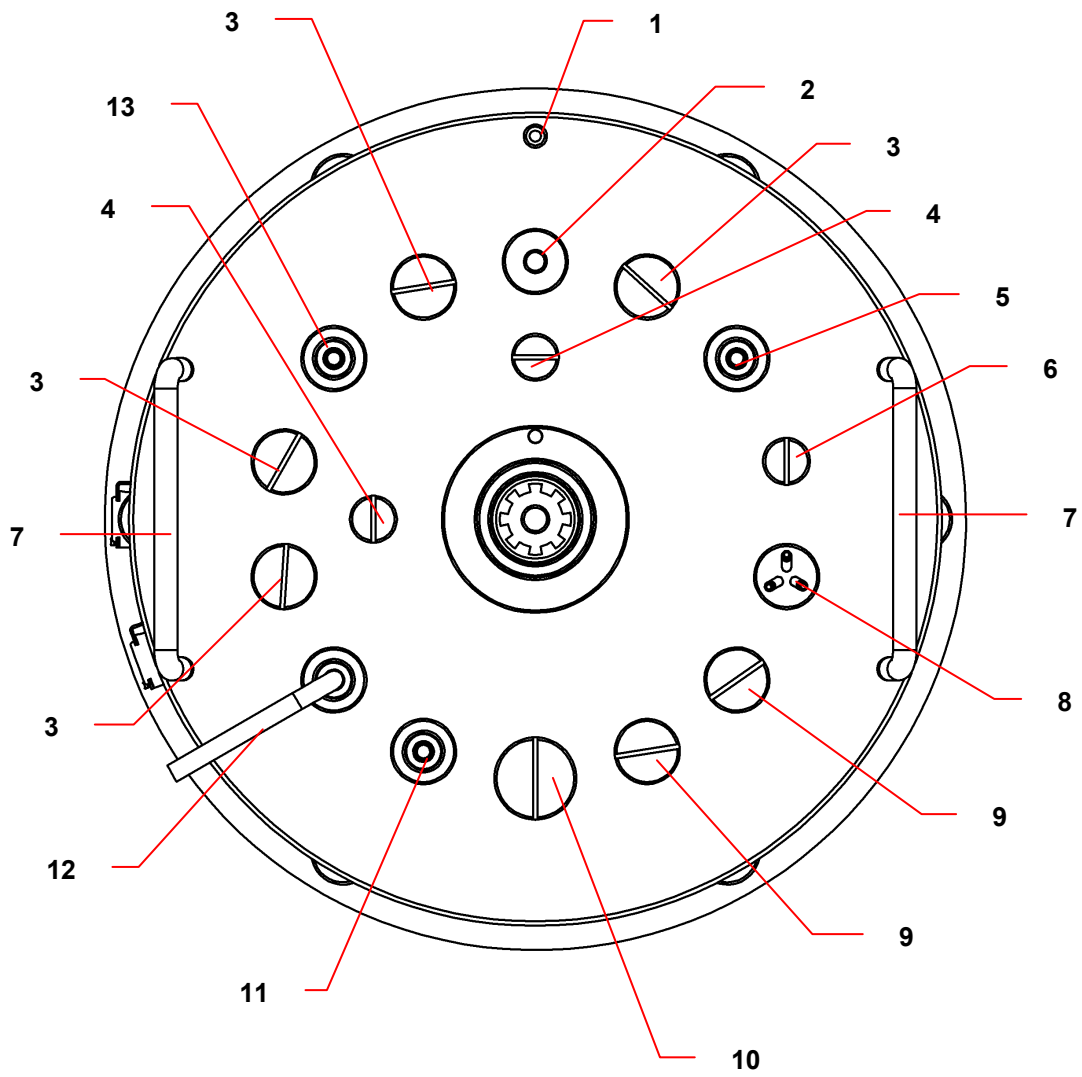
1	Earth/grounding lug	6	Spare 6.35 mm	11	Sampler
2	Thermowell/RTD	7	DO/pH	12	Harvest
3	Sparger	8	19 mm port	13	Exhaust
4	Level/foam	9	Addition (single)		
5	Tri-port/addition	10	Spare PG13.5 port		

Figure 10: 5 L Headplate Arrangement



1	Earth/grounding lug	6	Spare 6.35 mm	11	Addition (single)
2	Thermowell/RTD	7	Headplate handle	12	Sampler
3	Spare PG13.5 port	8	Tri-port/addition	13	Harvest
4	Sparger	9	DO/pH	14	Exhaust
5	Level/foam	10	19 mm port		

Figure 11: 10 L Headplate Arrangement

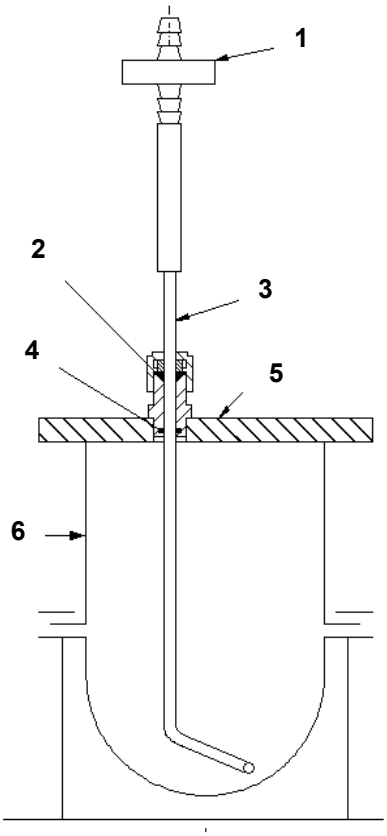


1	Earth/grounding lug	6	Spare 6.35 mm	11	Addition (single)
2	Thermowell/RTD	7	Headplate handle	12	Sampler
3	Spare PG13.5 port	8	Tri-port/addition	13	Harvest
4	Sparger	9	DO/pH		
5	Level/foam	10	Exhaust		

4.9.6 Install harvest tube

If it is not already installed, insert the harvest tube in the harvest port:

Figure 12: Harvest Tube Installation



1	Filter	3	Harvest tube	5	Headplate
2	Ferrule	4	O-ring	6	Vessel

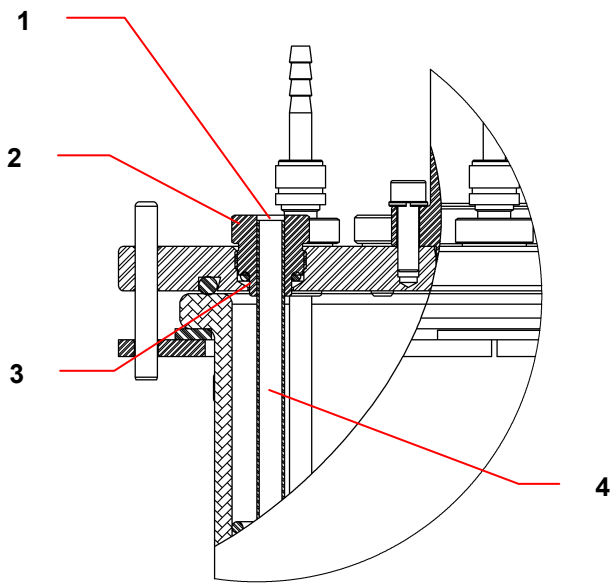
ALERT!

Only finger tighten the lock nut on the sparger, harvest tube or thermowell. These lock nuts have ferrules that can extrude under too much pressure.

4.9.7 Insert thermowell

Working from *outside* the headplate, insert the thermowell tube into the thermowell port:

Figure 13: Thermowell Installation



1	Insert RTD here	3	O-ring
2	Port adapter	4	Thermowell

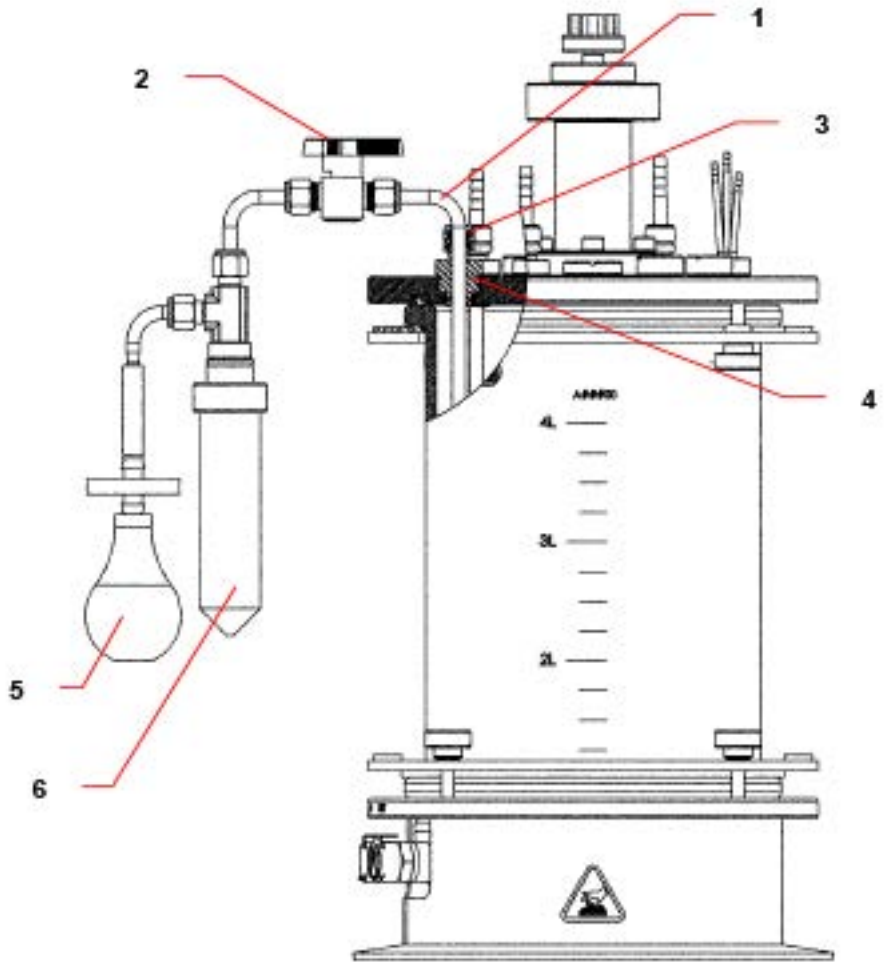
ALERT!

Only finger tighten the lock nut on the sparger, harvest tube or thermowell. These lock nuts have ferrules that can extrude under too much pressure.

4.9.8 Install sampler

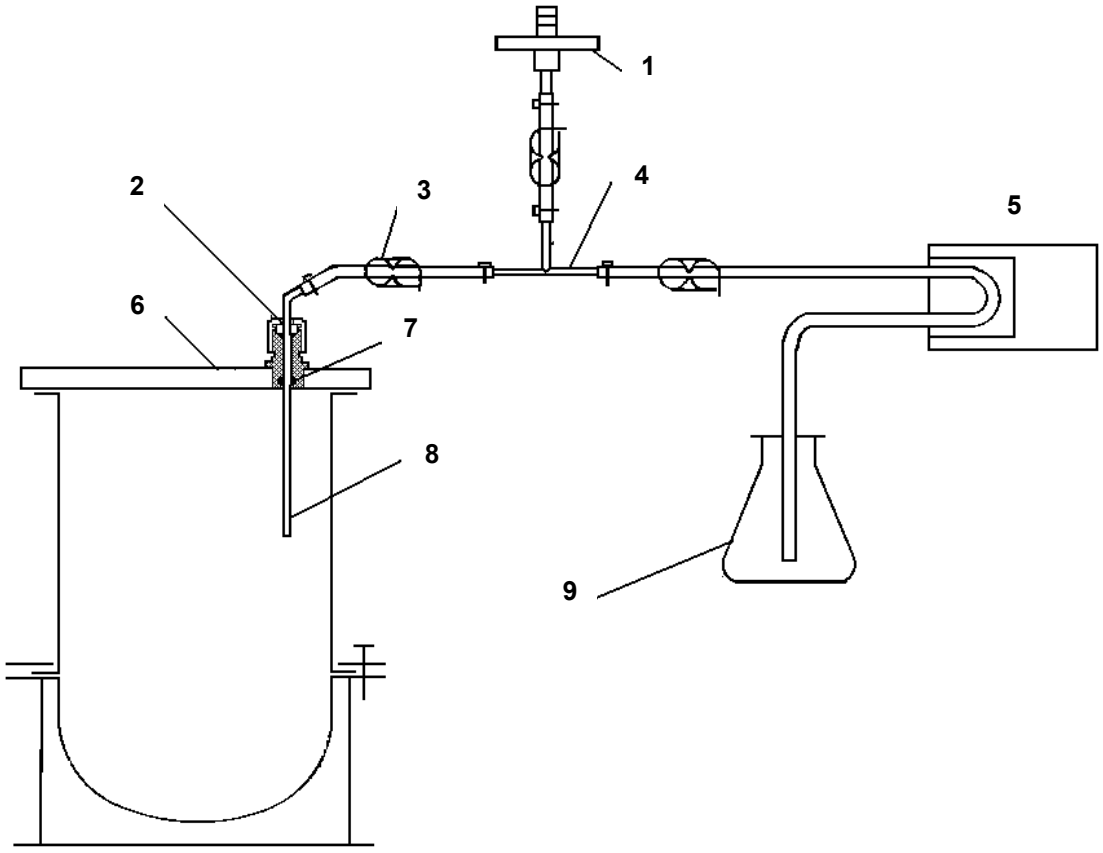
The sample tube should already be installed. Insert the sampler assembly into the sample port, as shown in the following drawings:

Figure 14: Sampling System I



1	Sample tube	4	Port adapter
2	Valve	5	Rubber bulb
3	Ferrule and O-ring	6	Bottle or vial

Figure 15: Sampling System II

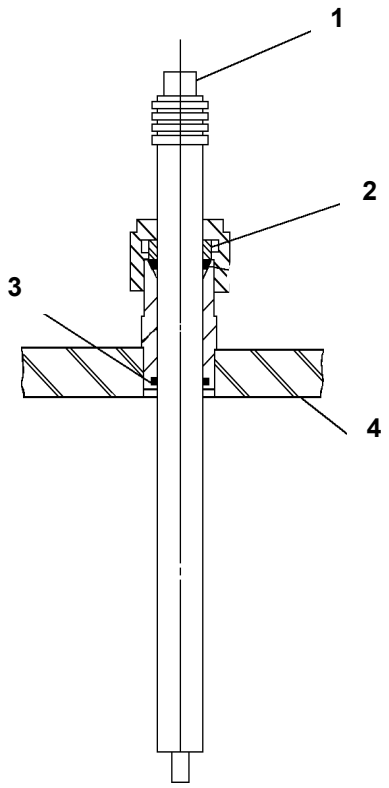


1	Filter	4	T Connector	7	O-ring
2	Ferrule	5	Peristaltic pump	8	Sample tube
3	Clamp	6	Headplate	9	Bottle

4.9.9 Install foam/level sensor

Install the foam/level sensor through the headplate:

Figure 16: Foam/Level Sensor Installation



1	Foam sensor	3	O-ring
2	Ferrule	4	Headplate

4.9.10 Install headplate on vessel

Anytime you need to install the headplate, follow these steps:

1. Position the headplate on the vessel flange and secure it to the upper retention ring. Use the headplate handles if they are present.
2. Tighten the clamping screws, taking care to tighten each screw a little at a time and to work diagonally across rather than around the circle.

DO NOT OVERTIGHTEN.
(See Operating Tips in Section 17 for further reference.)

Prior to installation, the pH and DO sensors should be properly prepared. *(See Section 4.9.11 for the pH sensor and Section 4.9.12 for the DO sensor).*

To avoid damage to the sensors during operation, be sure that there is no interference between the sensors and the baffle assembly, or between the sensors and the impeller blades. We recommend installation of the sensors at the vertical opening of the baffle.

4.9.11 Install pH sensor

1. Wear protective gloves to protect yourself in case of accidental breakage.
2. Lightly coat the pH sensor with glycerol or DI water to reduce friction.
3. With reference to the appropriate headplate drawing, gently insert the sensor into the appropriate port.

The fit may be snug; gently turn the sensor as you press it into the port to avoid breakage.

4.9.12 Install DO sensor

1. Wear protective gloves to protect yourself in case of accidental breakage.
2. Lightly coat the DO sensor with glycerol or DI water to reduce friction.
3. Gently insert the sensor into its adapter.
4. With reference to the appropriate headplate drawing, gently insert the sensor & adaptor into the appropriate port.

The fit may be snug; gently turn the sensor as you press it into the port to avoid breakage.

4.9.13 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.**

4.9.14 Install motor assembly

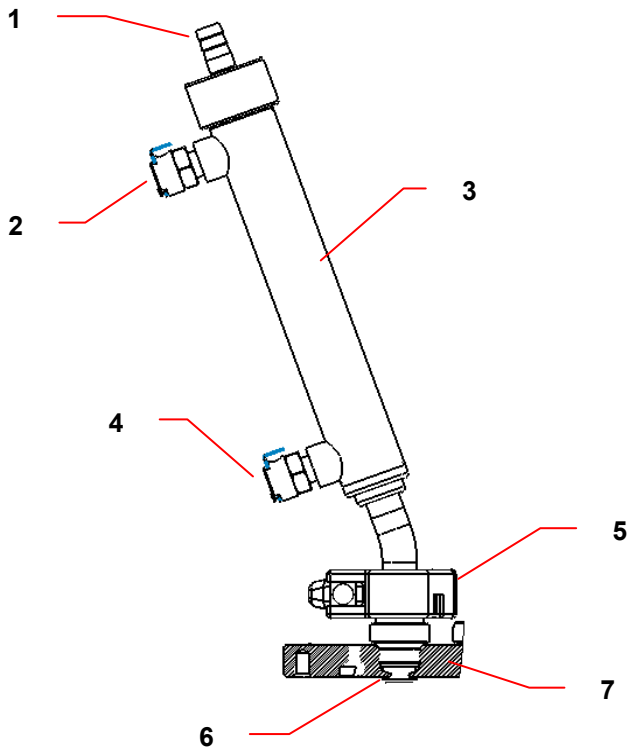
1. Position the motor assembly on top of the bearing housing, using the locating pin (or locating slot, if applicable) to orient it properly.
2. Connect the motor cable to the receptacle on the face of the control cabinet.

4.9.15 Make all connections

1. Connect cables from all sensors to their respective sockets on the face of the control cabinet.

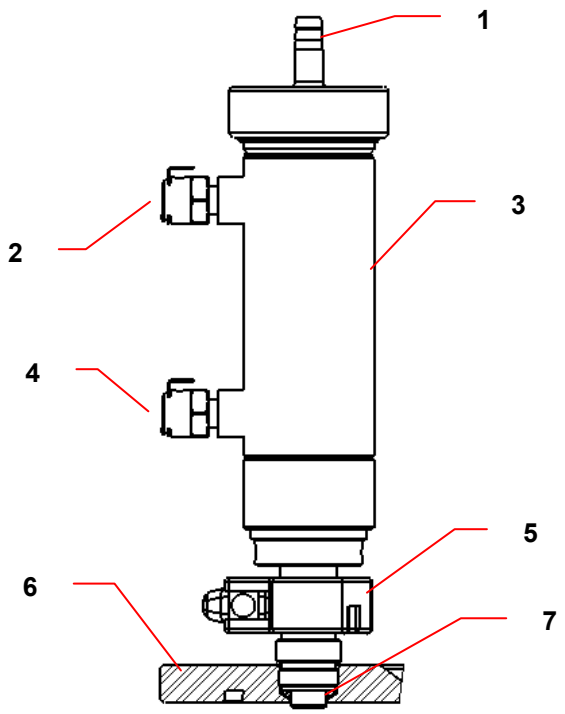
2. Connect the earth/ground lead from the antifoam socket on the face of the control cabinet to the pin in the headplate.
3. Connect the exhaust condenser to the exhaust condenser port.
4. Using flexible tubing, connect the exhaust filter to the top of the condenser. Secure it with tubing ties.
5. Connect the earth/ground wire from the cabinet to the vessel headplate.

Figure 17: Exhaust Condenser (1 L, 3 L & 5 L Vessels)



1	Install filter here	4	Condenser inlet	7	Headplate
2	Condenser outlet	5	Tri-Clamp®		
3	Exhaust condenser	6	O-Ring		

Figure 18: Exhaust Condenser (10 L Vessels)



1	Install filter here	4	Condenser inlet	7	O-Ring
2	Condenser outlet	5	Tri-Clamp®		
3	Exhaust condenser	6	Headplate		

WARNING! ***Risk of explosion!***
Never block the exhaust to pressurize the vessel (*see page 24*).

6. Slide a 2-inch long piece of 0.25-inch ID silicone tubing on the top of the sparger tube, then connect the air filter to it.
7. With flexible tubing, connect the other side of the filter to the sparger hose barb on the face of the control cabinet.
8. Secure both sides with tubing ties.

Always connect first the WATER OUT line to the upper quick-connect on the vessel heat exchanger and the WATER OUT line to the upper quick-connect on the exhaust condenser; then connect the WATER IN lines to the lower quick-connects in both locations.

…continued…

Never disconnect water lines when the mains/power is on, especially if the temperature control mode is AUTO. Be sure to change the temperature mode to OFF before removing the water lines. It is also prudent to turn the mains/power off.

When you do remove the water lines, disconnect the WATER IN line first, to protect yourself from water spraying from the heat exchanger or exhaust condenser.

Prime water lines before first use and every time the vessel has been disconnected (*see Section 4.9*).

4.10 Mains/power switch

The mains/power switch is located on the righthand side of the control cabinet, below the touchscreen and above Pumps 1 - 3.

ALERT!

Before turning on the mains/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 mains/power cord is properly connected to the control cabinet and plugged into a suitable mains/power outlet.
Failure to observe these cautions may lead to premature pump failure.

4.11 Optional BioCommand® *software*

If you are using Eppendorf 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 RS-232/-422 Modbus com port is provided on the rear panel of the control cabinet (*see Figure 33 in Section 6.3*) to connect the BioFlo 310 to a supervisory host computer. Communications to *BioCommand* software are via an optional RS-232 interface cable:

1. Connect the 25-pin end of the RS-232 cable to the *AFS/Modbus* port, and ensure that the connection is secure.
2. Hand tighten the thumbscrews.
3. Refer to the *BioCommand* operating manual for instructions on connecting the RS-232 interface cable to the supervisory host computer.

4.12 Inputs/outputs for ancillary devices

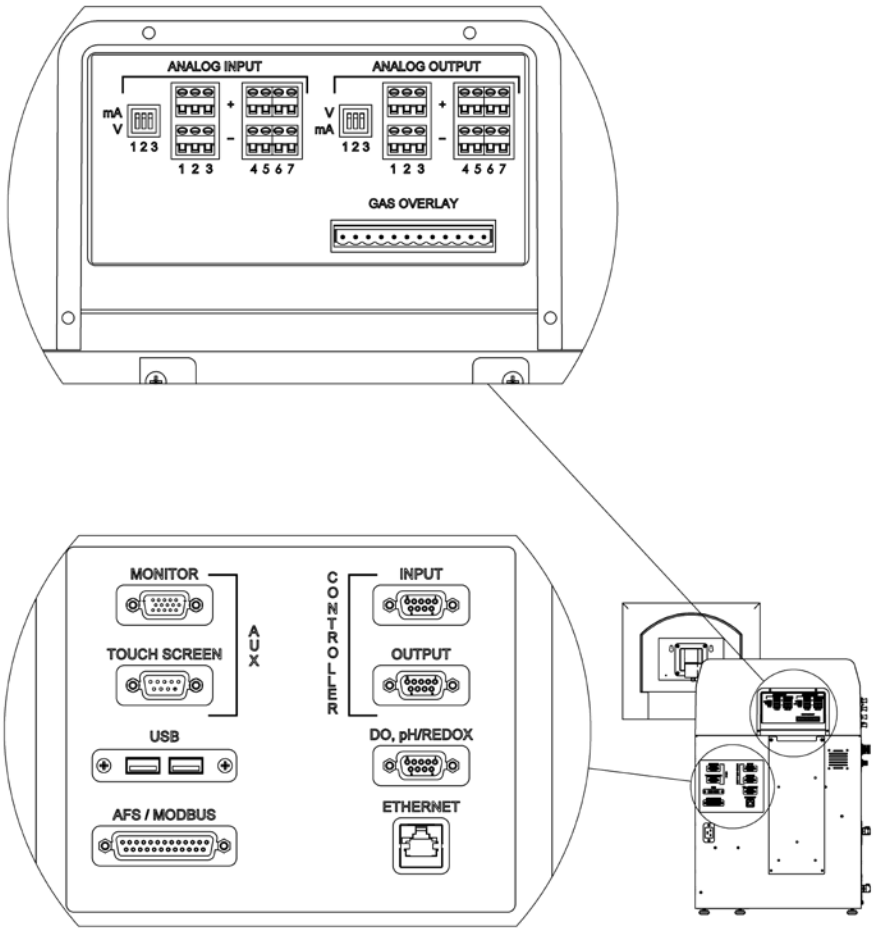
Each thermal mass flow controller (TMFC) uses one 0 - 5 V analog input and one 0 - 5 V analog output port. There is also an analog input/output port reserved (and labeled) for the Gas Overlay option available for cell culture.

Additional analog input and output ports are available on the control cabinet rear panel (*see the drawing below*) for the connection of analog ancillary devices such as additional pumps, turbidity sensors, gas analyzers and glucose analyzers. After the inputs are connected to the control cabinet, the collected information will be viewed and controlled via the touchscreen display.

Three of these additional analog input and output ports have dip switches to allow selection of either 4 - 20 mA or 0 - 5 V. The other four are 0 - 5 V dedicated.

As illustrated below, two USB serial ports are available on the control cabinet rear panel for the connection of serial ancillary devices such as scales for vessel and addition bottles. You can connect a box with eight serial (RS-232) inputs and outputs to one USB port to allow you to connect and control up to eight scales or other ancillary equipment.

Figure 19: Inputs & Outputs for Ancillary Equipment



5 SPECIFICATIONS

BioFlo 310 System					
Vessel	**Name**	1 L	3 L	5 L	10 L
	Working Volume	0.6 - 1.4 L	1.2 - 3.5 L	1.5 - 5.0 L	3.5 - 10.0 L
	Total Volume	2.5 L	5.0 L	7.5 L	14.0 L
Controller	**Master Control Station**	Controls 1 - 4 vessels, 32 control loops per vessel.; stores 10 recipes & 8 process variables per vessel for trend graphing. Includes an industrial touchscreen monitor/user interface, 3 built-in pumps & connectors for all utilities & communications signals used by fermentor/bioreactor 1.			
	Utility Station	One each required for optional 2nd, 3rd or 4th slave fermentors or bioreactors. Each includes 3 built-in pumps & connectors for all utilities & communications signals for its individual fermentor/bioreactor.			
	Touchscreen Interface/Display	15-inch industrial monitor capable of supporting up to 4 fermentors/bioreactors. One is standard with the Master Control Station. Optional 2nd touchscreen available for use with slave fermentors/bioreactors, to replicate the image shown on the Master display.			
Temperature	**Indication**	Digital display in 0.1°C increments			
	Range	From 5°C above coolant temperature to 80°C (setting range: 4 - 80°C).			
	Control	PI control employing PWM of heater and cooling water			
	Sensor	Platinum RTD sensor			
Agitation	**Drive**	Permanent magnet motor with high torque input.			
	Indication	Digital display in 1 rpm increments.			
	Range	50 - 1200 rpm			
	Control	PI-controlled			
	Sensor	Optical photoplastic disc 500 lines/rev with quadrature output.			
	Impellers	2 six-bladed Rushton turbine impellers provided			
Exhaust	**Filter**	0.2 µm disposable filter			
	Condenser	Stainless steel, water-cooled in headplate			
Aeration	**4-Gas System**	Up to 4 gases, including air, N_2, CO_2 & O_2, delivered to ring sparger			
	Sparger	Ring sparger			
	Inlet Filter	0.2 µm absolute disposable filter			
	N_2 Gas	For calibration of DO sensor			

...continued...

BioFlo 310 System					
pH	**Indication**	Digital display in 0.01 pH increments			
	Range	2 - 12 pH			
	Control	P&I			
	Sensor	pH gel-filled sensor			
DO	**Indication**	Digital display in 0.1% increments			
	Range	0 - 200%			
	Control	P&I, Agitation, O_2 Enrichment. Also GasFlow Rate if equipped with mass flow controller			
	Sensor	Polargraphic sensor			
Other Sensors	**Foam/Level**	One foam/level sensor is standard			
	Options	Redox or second pH and second DO sensors available			
Pumps[1]	**Pumps 1 & 2**	Assignable peristaltic pumps Fixed speed (12 rpm) or variable duty cycle Available control modes: Off, Prime, Base, Acid, Foam, Levl2 Wet, Lvl2 Dry, Lvl 3 Wet or Lvl3 Dry.			
	Pump 3	Assignable peristaltic pump Fixed speed (100 rpm) or variable duty cycle Available control modes: Off, Prime, Base, Acid, Foam, Levl2 Wet, Lvl2 Dry, Lvl 3 Wet or Lvl3 Dry.			
Utilities	**Water**	10 PSIG maximum, 50 µm filtration			
	Gas	10 PSIG maximum			
Electrical Requirements	**100 – 120 VAC**	50/60 Hertz	Single phase	15 Amps	
	208 - 230 VAC	50/60 Hertz	Single phase	15 Amps	
Net Weight	**Control Station**	40 kg (88 lb) with touchscreen			
	Touchscreen	6.8 kg (15 lb)			
	Vessel empty, without motor[2]	**1 L**	**3 L**	**5 L**	**10 L**
		10 kg (22 lb)	11 kg (24 lb)	15.5 kg (34 lb)	23 kg (51 lb)
Overall Dimensions with Touchscreen		63 cm wide X 61 cm deep X 86 cm high (25 in wide X 24 in deep X 34 in high)			
Overall Dimensions without Touchscreen		46 cm wide X 61 cm deep X 71 cm high (18 in wide X 24 in deep X 28 in high)			
External Computer Connections		Port supplied for remote connection of interface computer			
***BioCommand* Connections**		Port supplied for connection of *BioCommand* supervisory host computer.			
Fuses		One 5 A glass tube, fast-acting fuse			
Regulatory Compliance		*See Section 5.1*			
Ambient Operating Conditions		10 - 30°C, up to 80% relative humidity, non-condensing			

[1] See Table 6 (*Section 13.3*) for pump flow rates according to tubing size

[2] Vessel weight does not include sensors, exhaust condenser or other options.

5.1 Certifications

The BioFlo 310 has been tested to ETL standards, to comply with all appropriate safety standards.

As attested in the CE Declaration of Conformity reproduced on the following page, they also conform to the appropriate CE standards.

eppendorf

Declaration of Conformity

The product named below fulfills the requirements of directives and standards listed. In the case of unauthorized modifications to the product or an unintended use this declaration becomes invalid.

Product name:

New Brunswick™ BioFlo® / CelliGen® 310

including accessories

Product type:

Bench top bioreactor

Relevant directives / standards:

2006/95/EC: EN 61010-1, EN 61010-2-010, UL 61010-1, CAN/CSA-C22.2 No. 61010-1

2004/108/EC: EN 61326-1, EN61000-3-2, EN61000-3-3

2011/65/EU

2012/19/EU

Management Board | **Portfolio Management**

Date: December 16, 2013

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eppendorf@eppendorf.com

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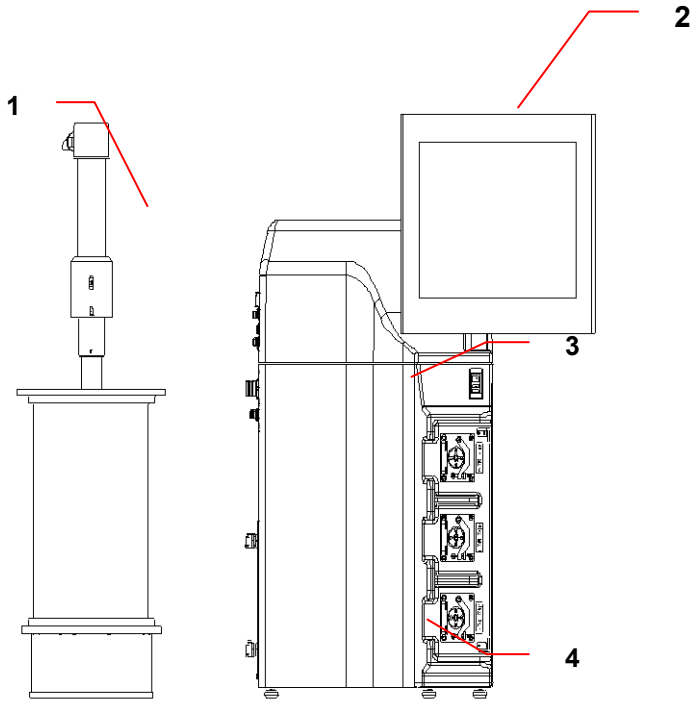
6 OPERATING CONTROLS

6.1 Touchscreen

Your primary interface with the BioFlo 310 is the touchscreen on the control cabinet.

Figure 20: Touchscreen



1	Control Cabinet	3	ON/OFF Mains/power Switch
2	Touchscreen Display	4	Pumps

6.2 Display screens

6.2.1 Touchscreen calibration

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

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 310, is first screen you see each time you turn on the mains/power, if you have already calibrated the touchscreen (*see Section 6.2.1*):

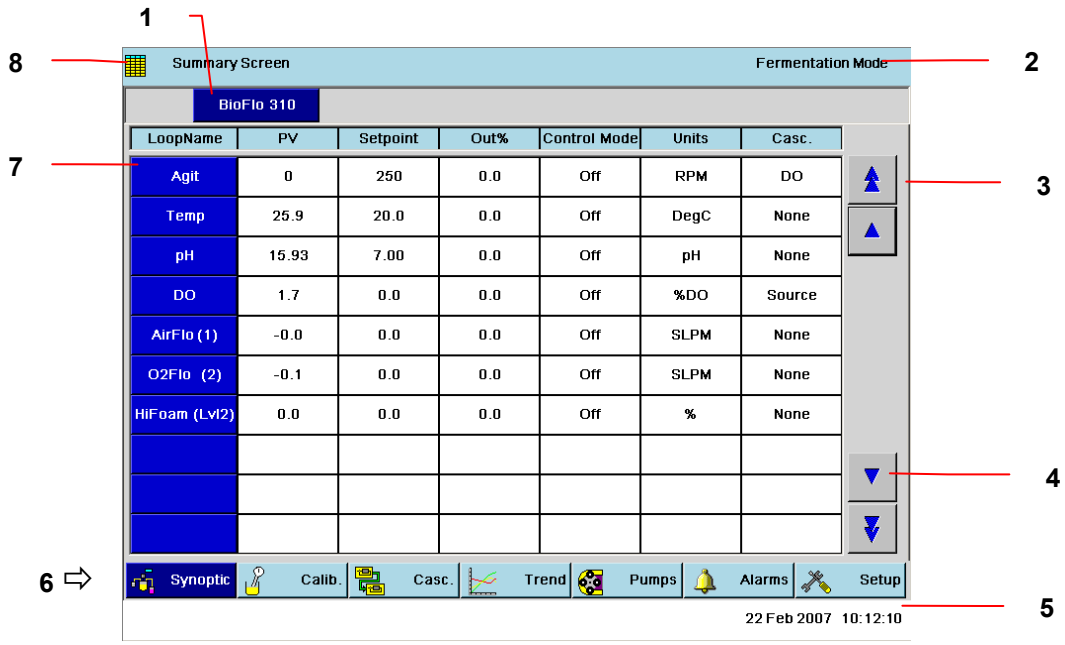
This screen remains in view for a few seconds, then it is replaced by the **SUMMARY** Screen.

6.2.3 Summary screen

The **SUMMARY** screen (*see sample screen below*) is command central; it puts as many as 32 loops at your fingertips.

Your BioFlo 310 controller can run as many as four stations; the Unit Tab identifies which vessel's operating parameters are being displayed (in the sample screen, Unit 1 has been labeled "BioFlo 310"); if you have more than one unit, pressing another Unit tab will move you sequentially to the **SUMMARY** screen for Unit 2, Unit 3 or Unit 4.

Figure 21: Sample SUMMARY Screen



The dark blue button usually represents the screen being displayed. Here it shows a new screen, SYNOPTIC, accessible from this screen (*see Section 6.2.4 for details*).

1	Unit Tab	4	Scroll Down Buttons	7	Assigned Loop Name
2	Operating Mode	5	Current Date & Time	8	Screen Name and Icon
3	Scroll Up Buttons	6	Access Screen Buttons		

Table 2 identifies the other interactive features of the **SUMMARY** screen:

Table 2: SUMMARY Screen Features

Parameter Column	Description
LoopName	Each unit comes with standard factory-assigned loops (e.g., Agitation, Temperature, pH, DO, etc.). There are also unassigned loops available, to be named and set up by the user when adding external equipment, for a maximum total of 32 loops.
PV	Process Variable: here the display reflects the current value for each loop, in comparison to its setpoint (displayed in the next column).
Setpoint	The current setpoint (default or user-set) for each loop.
Out%	The current percent output for each loop. This is an automatic control function to maintain current readings within the setpoint tolerance range.
Control Mode	Depending on the loop, the control mode may be Off, Auto, Manual, On, or O2 Enrich.
Unit (of measure)	This is the unit of measure used for the PV and Setpoint.
Cascade	If any cascades have been programmed, they will be displayed here.
Summary*	This screen is command central; it shows all your loops, their current readings, setpoints and what has been programmed for them.
Synoptic*	This screen is a graphical alternative to the SUMMARY screen. It shows your loops, their current readings and their setpoints. It also displays the current state of the fixed speed pumps, level sensors and process valves.
Calibration	This screen allows you to calibrate the pH, DO & Level sensors and the gas flow.
Cascade	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.
Trend	This screen allows you to set the parameters for plotting trend graphs and to view the graphs that track the activity of the selected loops during an entire fermentation run.
Pumps	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.
Alarms	In this screen you can turn alarms on and off, read the alarm history and acknowledge any alarm.
Setup	This screen allows you to load & save recipes and to make changes to your system settings, hardware setup & controller setup
Other Buttons	**Description**
Scroll Up	Press this button to scroll upwards, one loop at a time.
Scroll Down	Press this button to scroll downwards, one loop at a time.

*** see the following page**

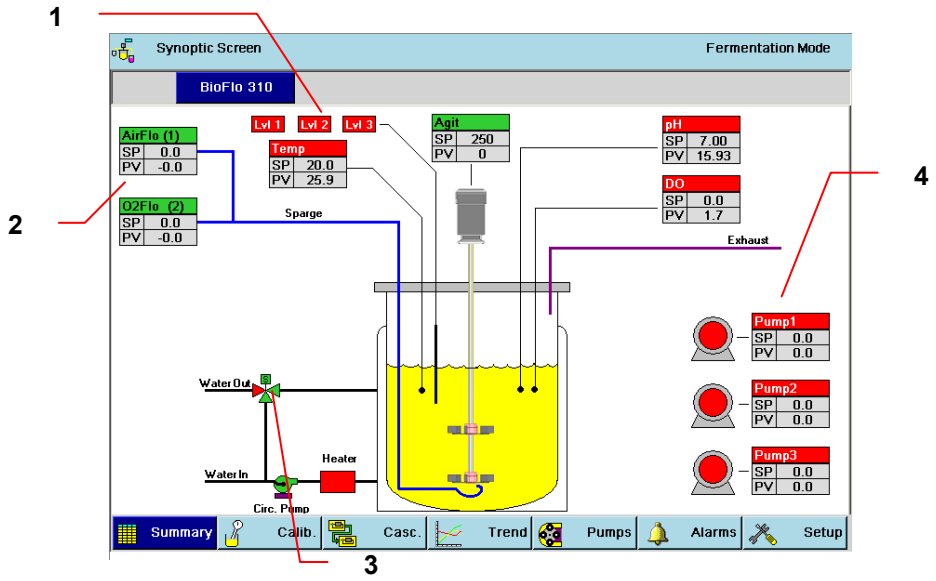
* The far left navigation button at the bottom of all main screens is a toggle between the **SUMMARY** and the **SYNOPTIC** screens. When viewing one, the button will be labeled for the other. Upon leaving either for one of the other screens, the default selection shown on the button will be the most recently visited of the two. That is, if you leave the **SUMMARY** screen to view the **TREND** screen, for example, the far left button will be labeled **SUMMARY**.

6.2.4 Synoptic screen

From any main screen, press the far left **SYNOPTIC** button to open the **SYNOPTIC** screen. If the far left button says **SUMMARY**, press it to open the **SUMMARY** screen. The button will now be labeled **SYNOPTIC**; press it again.

This screen provides a visual representation of all the loops, their settings and current process values. This screen provides all the functionality of the **SUMMARY** screen with the exception of the ability to add loops.

Figure 22: Sample Synoptic Screen

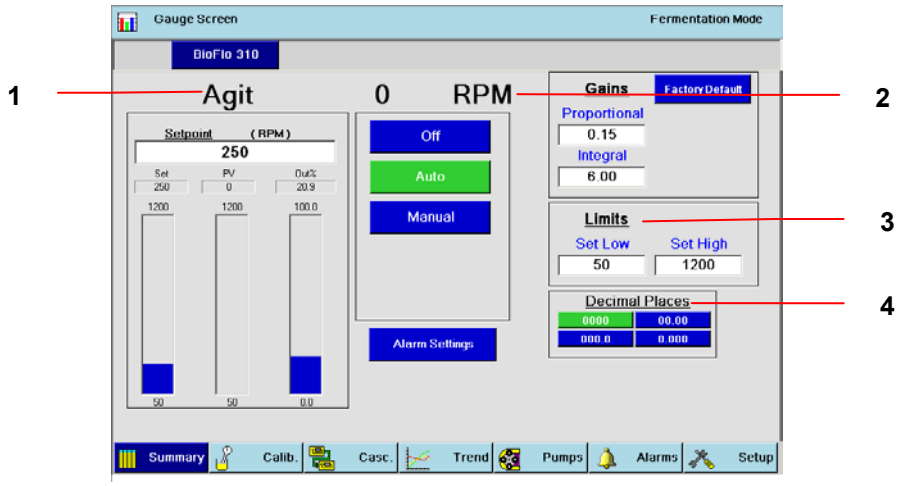


1	LEVEL SENSORS: color indicates sensor status (red = Dry, green = Wet)
2	LOOPS: each gauge indicates setpoint (SP) and process variable (PV). Title color indicates loop status (red = OFF, green = ON, blue = Manual). Touch a loop gauge to go to the full gauge screen.
3	VALVE or HEATER: color indicates valve status (red = Closed, green = Open). Touch a valve icon to go to the STERILIZATION screen.
4	PUMPS: each gauge indicates setpoint (SP) and process variable (PV). The pump icon and the gauge title color indicates that pump's status (red = OFF, green = ON).

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:

Figure 23: Sample GAUGE Screen



1	Loop Name
2	Unit of measurement: the action of this loop, Agitation, is measured in rpms.
3	**Limits**: adjust the high and low settings for this specific loop. When adjusted, the scaling for the gauge will also be adjusted to reflect the high and low limits selected.
4	**Decimal Places**: Press the appropriate button to display values with 0, 1, 2 or 3 decimal places.

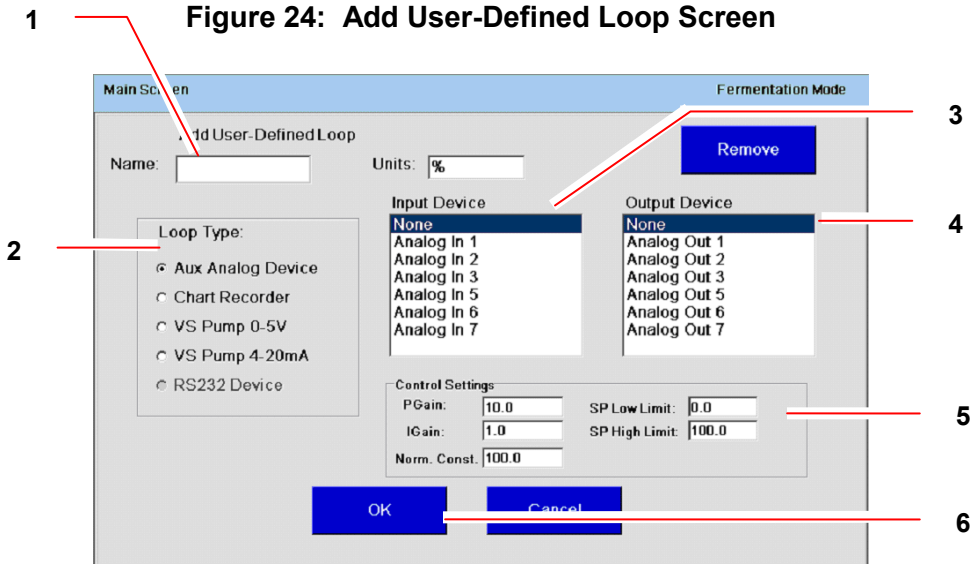
6.2.6 Adding loops

The BioFlo 310 comes to you with standard factory-assigned loops and the possibility to add more loops, which are related to external auxiliary equipment, added via standard analog and optional serial (RS-232) input/outputs located on the rear panel.

To add a new loop:

1. Scroll down in the **SUMMARY** screen beyond the last pre-assigned loop, and press on a blank **LoopName** box.
2. The **Add User-Defined Loop** screen will open:

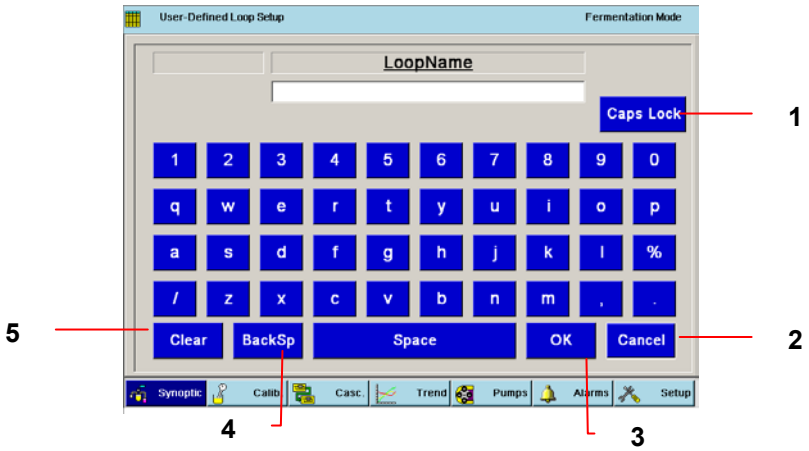
Figure 24: Add User-Defined Loop Screen



1	Step 3: Press here and use the touchpad (see sample screen below) to name the loop.
2	Step 4: Press the appropriate option button. The corresponding measurement units will automatically appear (% in this sample screen).
3	Step 5: Press the appropriate input device designation.
4	Step 6: Press the appropriate output device designation.
5	Step 7: Input the desired control settings.
6	Step 8: After making all of your selections, press the **OK** button to save them.

Each TMFC in the system will utilize one of the 0 - 5 V Input/Output devices on the board. You can see in the sample screen above, for example, that I/O 4 is missing from the Input Device & Output Device lists. That is because this system was configured with 1 TMFC, assigned as I/O Device 4.

Figure 25: LoopName Touchpad



1	Press **Caps Lock** to shift to CAPITAL letters; press it again to shift back to lower case.
2	Press **Cancel** to return to the GAUGE screen **without** saving work done in the Loop Name touchpad screen.

…continued on the following page…

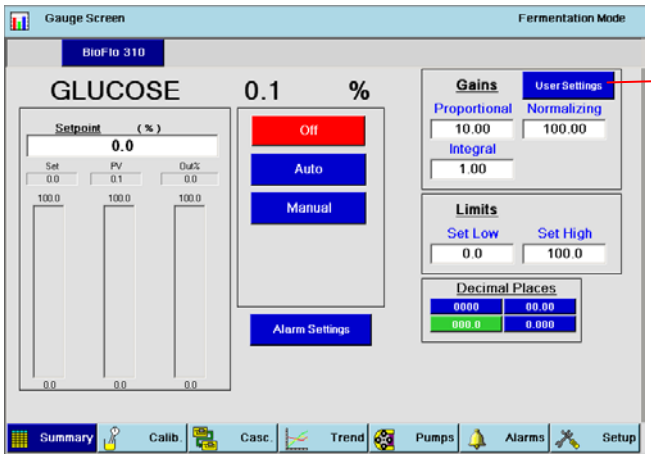
3	Press **OK** to save the work done in the Loop Name touchpad screen and to return to the GAUGE screen
4	Press **BackSp** to backspace, cancelling one character at a time.
5	Press **Clear** to clear the LoopName edit box in this screen, allowing you to begin again.

6.2.7 Deleting loops

Only user-added loops can be deleted. If you wish to delete a loop:

1. In the **SUMMARY** screen press the **LoopName** box for the loop you wish to delete.
2. In the loop's **GAUGE** screen, and **if this is not a pump control loop**, press the **UserSettings** button:

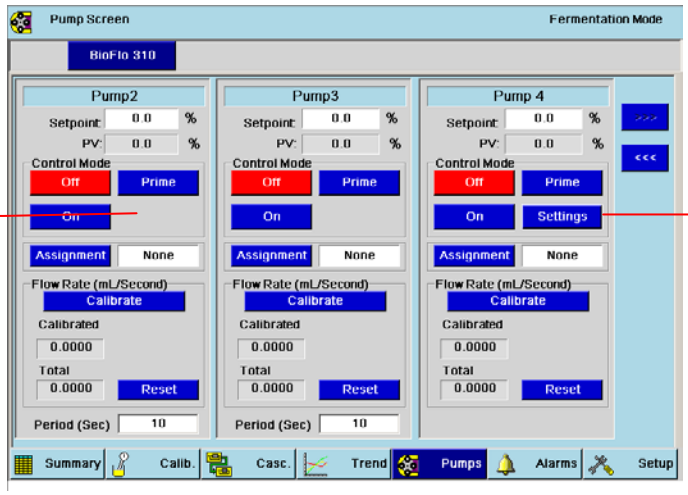
Figure 26: Deleting a Control Loop



1	Press the **UserSettings** button here to open the Add User-Defined Loop screen.

3. In the **Add User-Defined Loop** screen, press the **Remove** button.
4. **If the loop is a pump:** only optional pumps have a **Settings** button that provides access to their **Remove** button (*see the sample screen on the following page*).

Figure 27: Deleting a Pump Control Loop



1	Pumps 1, 2 & 3 cannot be removed; these are factory-installed on each Control Station. Only optional pumps (such as Pump 4 shown here) have a **Settings** button.
2	Press the **Settings** button here to open the screen where you can delete the optional pump control loop by pressing on the **Remove** button.

5. When you return to the **SUMMARY** screen, the loop will be deleted.

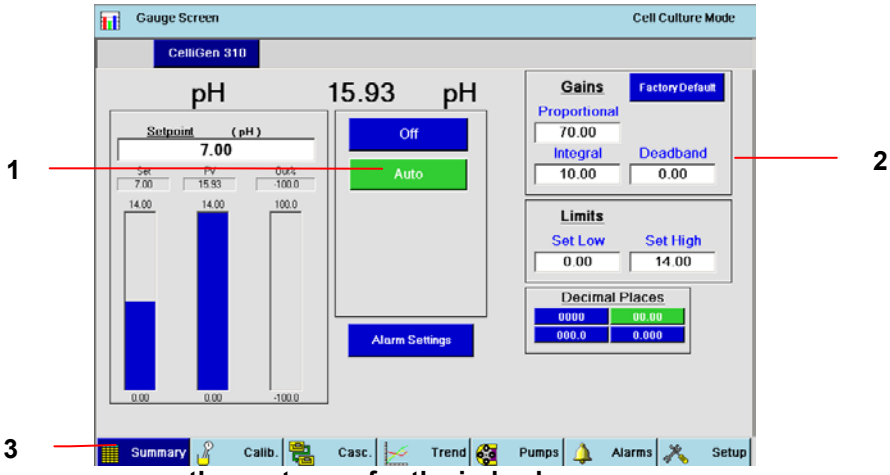
6.2.8 Selecting loop control modes

Control modes vary according to the loop and process mode. (There are also operating modes for all of the pumps; *see Section 13.2* 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 28: Sample GAUGE Screen (pH)



…see the next page for the index key…

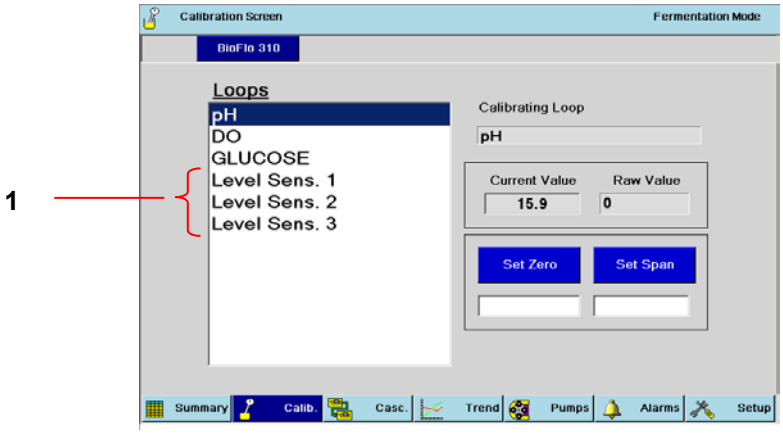
1	Step 2: Press the button that corresponds to the desired operating mode.
2	**Deadband** is a user-definable pH value within which, above or below the setpoint, no response will be triggered.
3	Step 3: To save the new operating mode and return to the SUMMARY screen, press the **Summary** button.

Sections 6.2.9 - 6.2.14 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 and the DO sensors. For details on sensor calibration, see Section 7.2 (pH sensor) and Section 7.3 (DO sensor).

Figure 29: Calibration Screen

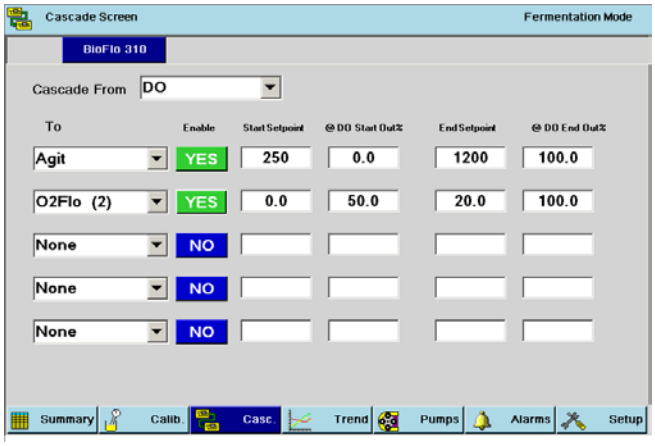


1	These last three “loops” are input from the Level sensors to the Level and HiFoam loops.

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 the following page*) allows the user to set up cascades, to view current cascade settings and to change those settings. For details on setting cascades, *see Section 11*.

Figure 30: Cascade Screen

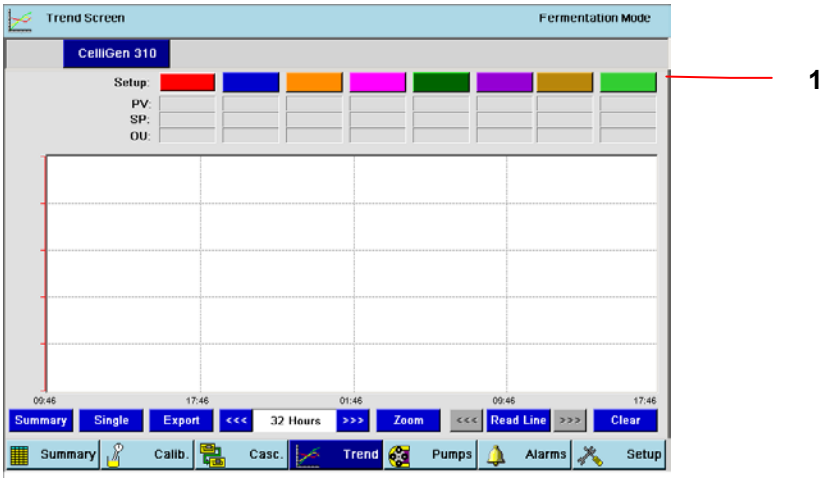


6.2.11 Trend screen

This screen allows the user to set the parameters for plotting trend graphs and to view the graphs that track the activity of up to 8 selected loops during an entire process run. The data can be exported through the USB port in Excel format to a PC.

For details on using the **TREND** screen, see Section 12.

Figure 31: Trend Screen

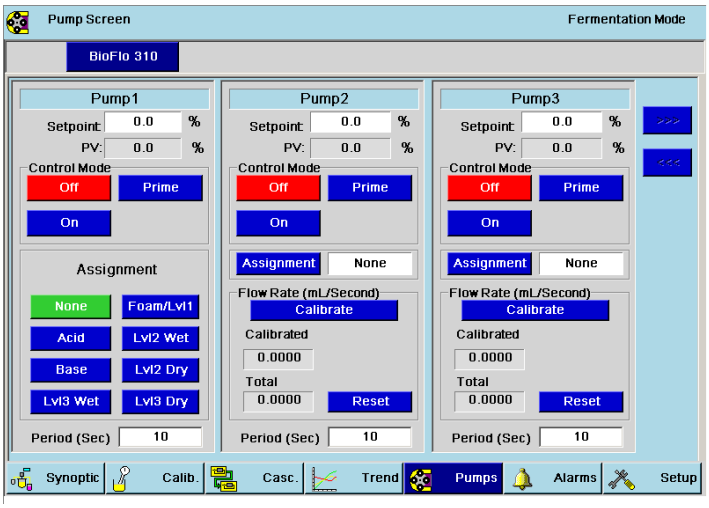


1	The user will assign a tracking color to each loop.

6.2.12 Pumps screen

This screen allows the user to access the pump gauges screens, where the three standard pumps (plus any optional pumps) are displayed, providing both current readings and the opportunity to change pump settings. For details on using the **PUMPS** screen, see Section 10.5.

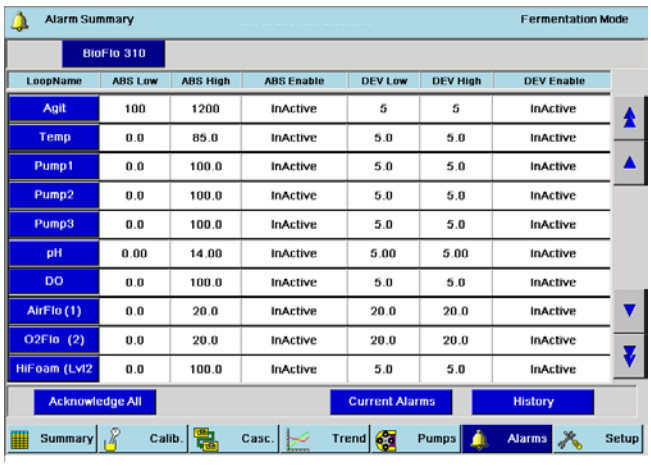
Figure 32: Pumps Screen



6.2.13 Alarms screen

This screen allows the user to turn alarms on and off, to read the alarm history and to acknowledge any alarm while it is active. For details on using the **ALARMS** screen, *see Section 14*.

Figure 33: Alarms Screen

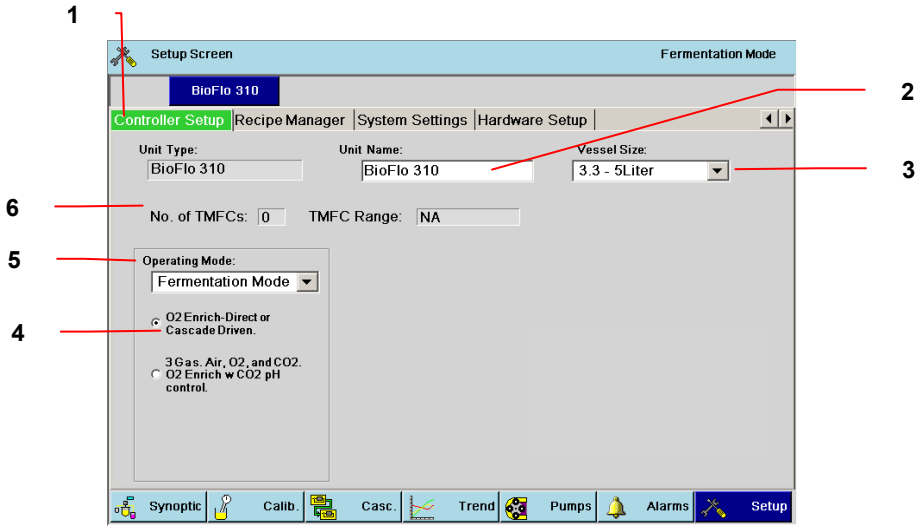


6.2.14 Setup screen

This master screen is actually comprised of four screens, accessed by tabs. These screens are used to set up the controller, recipe management, system settings and hardware for the BioFlo 310 system. This section will introduce you to those screens and their features. For details on using the **SETUP** screen, see Section 15.

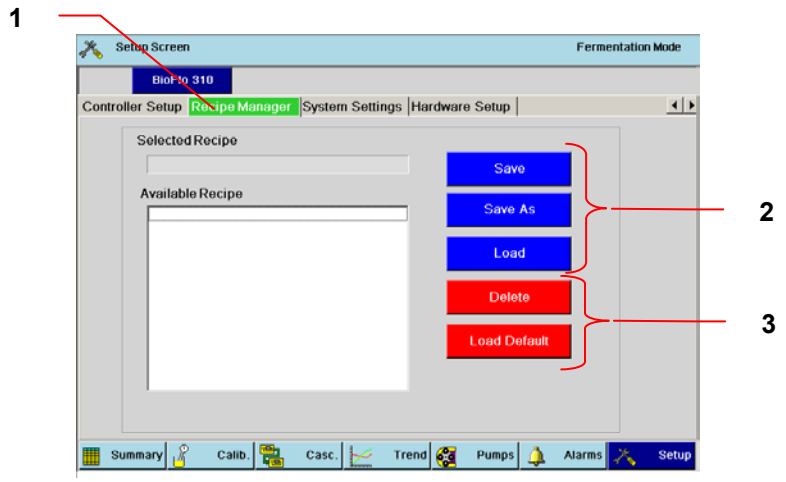
When you press the **SETUP** button, the screen that opens is actually the first tab, the **CONTROLLER SETUP** screen:

Figure 34: Controller Setup Screen



1	Tab
2	The Unit Name is user-selected. Press this box, then use the pop-up touchpad to type in the name.
3	The Vessel Size is user-selected by pressing the down arrow to access the dropdown menu, then pressing on the desired vessel size. *Choosing the vessel size here assures the application of accurate PI values.*
4	The user selects gas mixing options here. Options will vary for cell culture. The gas overlay option (for cell culture only) is available only when the optional overlay box is attached through the I/O port (see Section 0).
5	The default Operating Mode is Fermentation. The select Cell Culture, click on the down arrow, then click on Cell Culture.
6	The TMFC (thermal mass flow controller) Range and the number of TMFCs (0 means manual gas flow, usually by Rotameter) are factory-set.

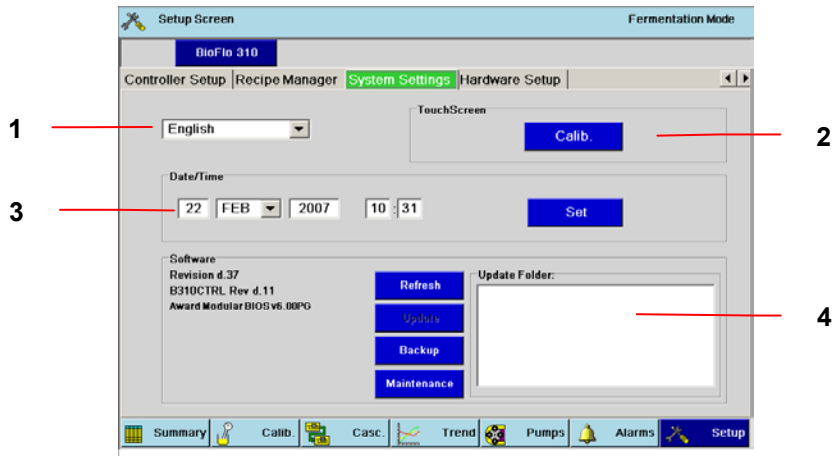
Figure 35: Recipe Manager Screen



1	Use the RECIPE MANAGER screen to save and load up to 10 recipes.
2	Recipes can be saved and loaded using these buttons.
3	The **Delete** button removes the recipe currently selected. The **Load Default** button restores factory settings.

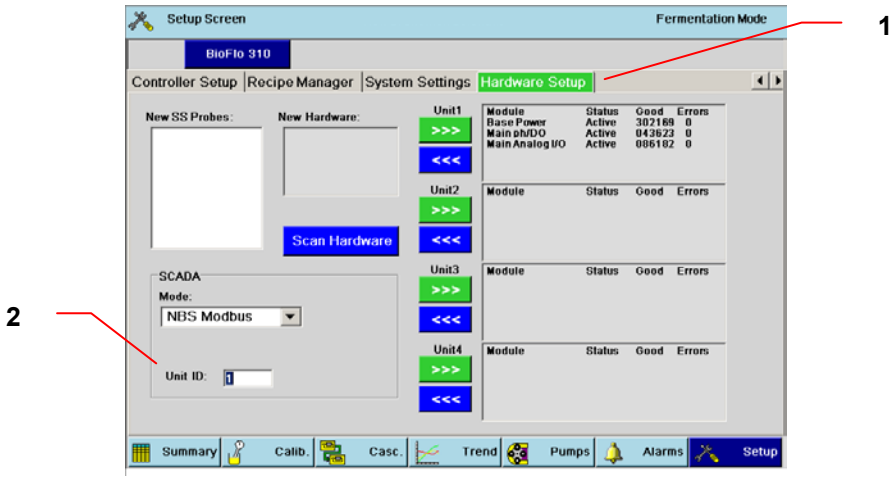
A **recipe** consists of all setpoints, controller settings, control modes, calibration data and cascades set on a system.

Figure 36: System Settings Screen



1	English is the default language. When other choices (Français, Deutsch, Español) become available, the user will select the language here.
2	Use this pane to calibrate the touchscreen (see Section 6.2.1).
3	Use this pane to change Date and Time (see Section 15.3.1).
4	Use this pane to view the Software/Firmware version installed, and to update Software via the USB port (see Section 15.3.2).

Figure 37: Hardware Setup Screen

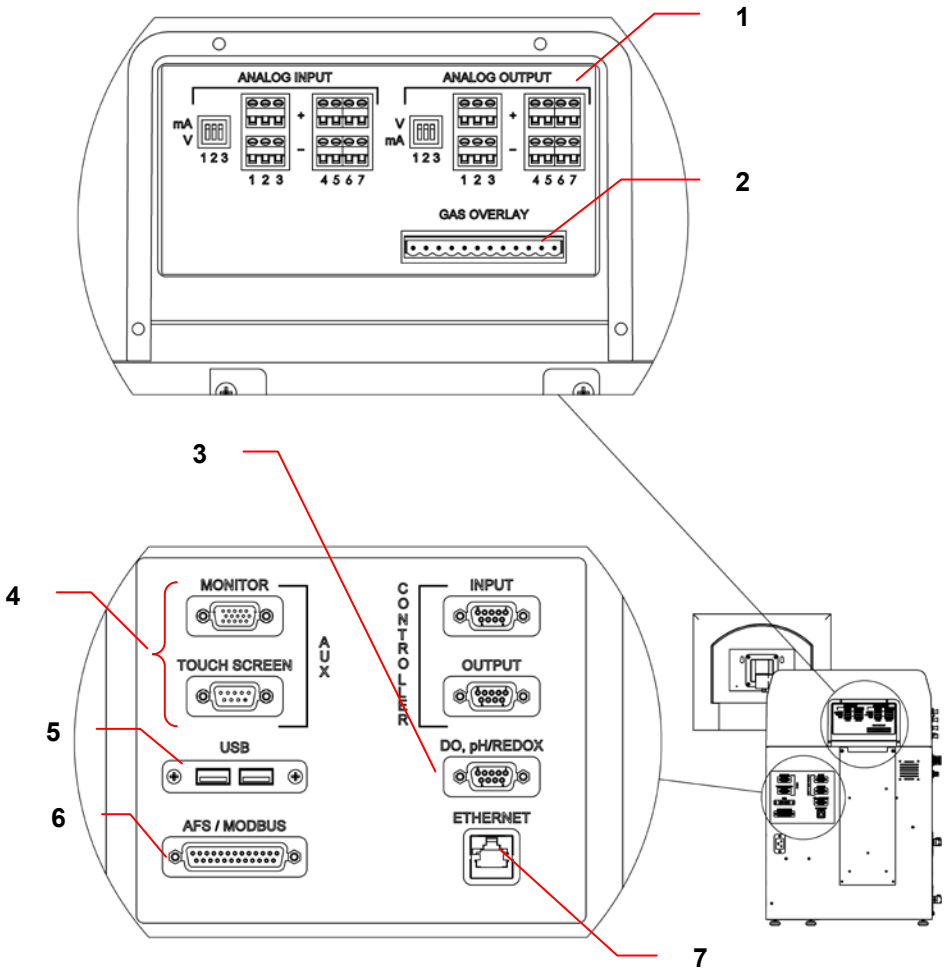


1	Use this screen to view and add hardware for as many as 4 fermentors installed in the system.
2	Use this pane to choose software connections and to set Unit IDs for software.

6.3 RS-232/-422 computer interface

An RS-232/-422 com port has been provided; there is a 25-pin “D” connector located on the lower rear panel of the control cabinet (see the drawing on the following page). The connector is labeled **AFS/Modbus**.

Figure 38: Control Cabinet Rear Panel



1	Analog inputs & outputs are easily accessible here.
2	Gas overlay connection is optional for cell culture users only.
3	For the user's addition of non-factory-installed REDOX or a 2nd pH/DO controller.
4	For the connection of a 2nd display.
5	2 USB ports
6	For use with New Brunswick *BioCommand*® supervisory software.
7	For future expansion.

A New Brunswick *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 RS-232/-422 connector:

Table 3: AFS/Modbus Com Port Pin Designation

Pin Number	Signal	Comments
1, 4 - 6, 8 - 11, 14 - 20, 22 - 23	NC	not assigned
2	TXD	RS-232 Data Output from fermentor
3	RXD	RS-232 Data Input to fermentor
7	GND	Earth/ground reference for all signals
12	IRXD+	RS-422 paired data input to fermentor
24	IRXD-	
13	ITXD+	RS-422 paired data output from fermentor
25	ITXD-	
21	IOS	Open selects RS-232 Earthed/grounded selects RS-422

Unless otherwise requested, the baud rate is factory-selected at 9600 (AFS) or 19200 (Modbus) and the connector is configured as an RS-232 port: i.e., no jumper between pin #7 and pin #21.

7 SENSOR PREPARATION & CALIBRATION

7.1 pH sensor inspection

Inspect sensor for possible shipping damage. If damage is observed, notify the Eppendorf Service Department immediately.

If you have a liquid-filled sensor, check the level of the reference electrolyte. It should be about 1cm below the filling orifice. To add reference electrolyte, take the filling pipette (P0740-4820) and fill it with electrolyte solution.

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

Both chambers of the electrode are filled with the same reference electrolyte, for a total volume of approximately 30 mL. For normal operation, remove the rubber T stoppers if the sensor is so equipped, saving them for use during sterilization.

7.2 pH sensor calibration

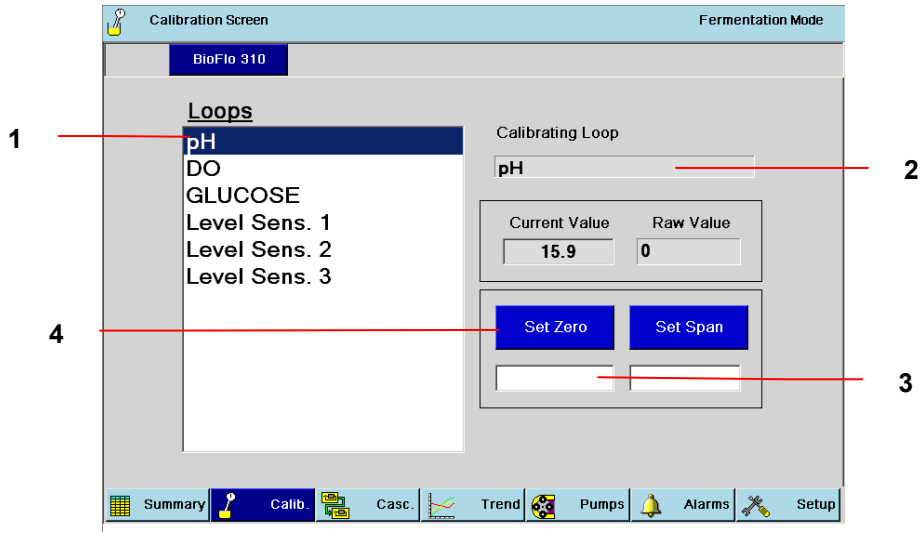
Calibrate the pH sensor before autoclaving it with the vessel.

1. If you have not already done so, connect the pH sensor to the pH connector on the control cabinet, using the appropriate cable.
2. Turn the mains/power switch **ON**.
3. Press the **CALIBRATION** button to display the **CALIBRATION** screen.

The pH sensor 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:

Figure 39: Calibration Screen



1	Step 6a: Press pH here…
2	Step 6b: …and pH appears here.
3	Step 7: Touch inside the Set Zero edit box. Use the touchpad that opens to enter 7.0, then press the **OK** button.
4	Step 8: When the Current Value reading stabilizes, press the **Set Zero** button.

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.

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 sensor installation

ALERT!

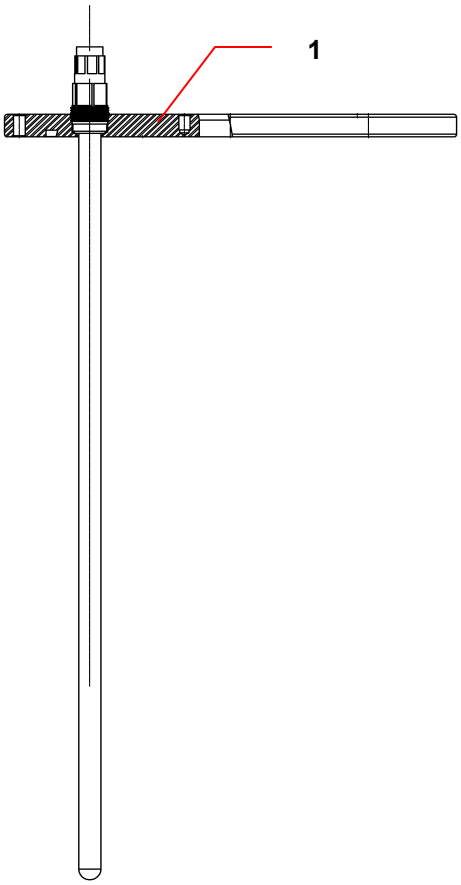
Be sure to wear protective gloves when installing a glass electrode.

To install the pH sensor in the headplate:

1. Apply a small amount of silicone grease or glycerol to the electrode body.

2. Install the pH electrode as shown below. Gently turn the sensor as you press it into the port, to ease it into the vessel without forcing. Also make sure that there is no interference inside the vessel.

Figure 40: pH Sensor



1	Headplate

ALERT!

We recommend that you avoid the use of hydrochloric acid (HCl) with the BioFlo 310 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 sensor maintenance & storage

If you have a liquid-filled sensor, check the level of the filling solution. It should be about 1cm below the filling orifice.

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

The sensor should be stored standing upright. 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 sensor is so equipped, the two rubber T stoppers should be inserted.

ALERT!

Never let a pH sensor rest on its tip, and never leave a pH sensor in DI water.

7.3 Dissolved oxygen (DO) sensor preparation

7.3.1 Inspecting the DO sensor

Inspect the sensor for possible shipping damage. Immediately report any damage you may observe to the Eppendorf 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 SENSOR ON ITS MEMBRANE.***

7.3.2 DO sensor preparation

To ensure stable output, the sensor should be sent through two or three sterilization (autoclaving) cycles prior to use. The sensor will be operable after the second cycle, but it will be more stable with additional sterilizations. ***The shorting plug should be installed on the sensor 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 touch pad 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 reset or change them. Even if the mains/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 mains/power is restored. *For details regarding P & I settings, see Section 22, 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.

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.

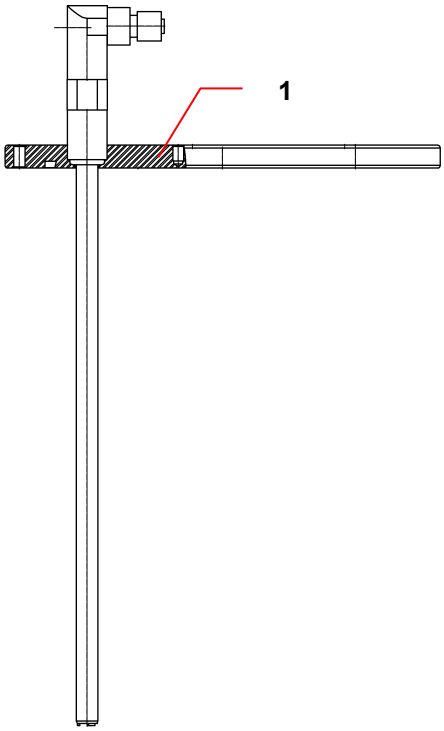
7.3.3 DO sensor installation

Install the DO sensor into the vessel headplate assembly, ensuring that there is no interference inside the vessel and taking care to never strike or bump the tip of the sensor.

To install the sensor without an adapter, with reference to the drawing on the following page:

1. Unscrew the lock nut from the port.
2. Gently slide the lock nut onto the DO sensor, from bottom to top.
3. Apply a light coat of glycerol to the sides of the sensor.
4. Carefully insert the sensor into the port, gently rotating it as you glide it into place.
5. When it is fully seated, finger tighten the lock nut.

Figure 41: DO Sensor



1	Headplate

7.3.4 DO sensor polarization

If the sensor has been disconnected from a voltage source (either the unit's O_2 amplifier or a separate polarizing module) for longer than 5 minutes, it will need to be re-polarized.

To re-polarize:
Connect the sensor to the operating O_2 amplifier (or polarizing module). Allow SIX HOURS FOR POLARIZATION prior to calibrating the sensor.

7.3.5 DO sensor calibration: setting zero

The DO sensor is calibrated AFTER sterilization.

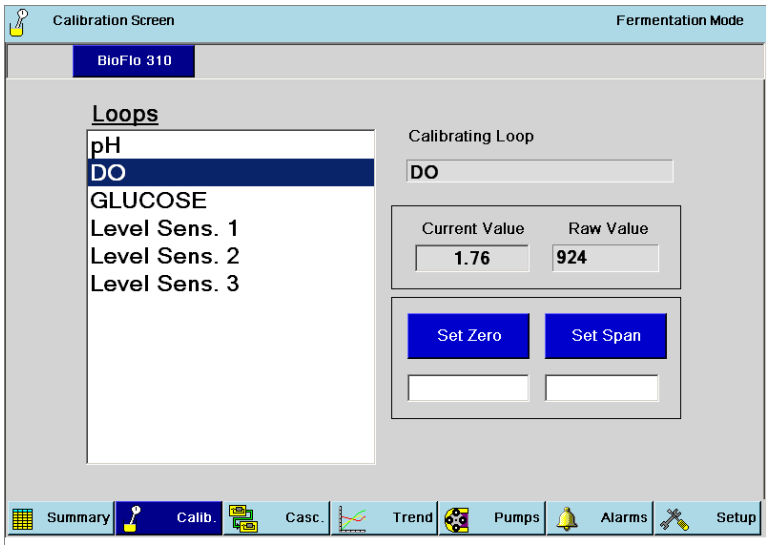
There are two methods to obtain zero for calibrating the DO sensor. Review both methods and use the one you prefer:

Method 1:

1. Remove the DO cable from the DO electrode.
2. Go to the **CALIBRATION** screen and select **DO**.
3. Enter **0** in the **SET ZERO** edit box, then press **SET ZERO**.
4. Reconnect the DO cable to the DO electrode.

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

Figure 42: Calibrating DO



Method 2:

Nitrogen is needed for Method 2. There is an N_2 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 and select **DO**.
3. Press the **N2 (3) ON** button. If your system has 3 or 4 TMFCs, however, this button will not be present. In this case, manually turn the N_2 loop on from the **SUMMARY** screen 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, 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 N_2 loop on, now manually shut off the nitrogen flow to the vessel).

7.3.6 DO sensor 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, then press the **SET SPAN** button.

7.3.7 About pump calibration

To assure the most accurate flow rate, calibrate the pump each time you change tubing. *See Section 10.6.4 for details.*

8 VESSEL STERILIZATION

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! Risk of explosion!

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

CAUTION! Risk of personal injury!

Use protective gloves when handling hot components.

ALERT!

Before connecting or disconnecting the water hoses to/from the vessel at any time, be sure to follow these instructions in the order indicated:

To connect: **(1) Connect vessel Water Out line,**
(2) Connect vessel Water In line,
(3) Connect cabinet Main Water In line,
(4) Turn ON mains/power switch on cabinet.

To disconnect: **(1) Turn OFF mains/power switch on cabinet,**
(2) Disconnect Main Water In line from cabinet,
(3) Disconnect vessel Water In line,
(4) Disconnect vessel Water Out line.

Failure to heed these instructions may lead to hose leakage and/or pressure build-up inside the vessel jacket.

ALERT!

During sterilization:

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

ALERT!

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 sensors and bearings to meet the fourth objective.

8.1 Initial preparation for autoclaving

If this is a non-jacketed vessel, proceed to Section 8.2.

If this is a **water-jacketed vessel for Cell Culture, the jacket must be half-filled with water** prior to autoclaving. Follow the steps in Section 8.1.1 below.

8.1.1 Filling the water jacket

To fill the water jacket on a Cell Culture vessel:

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 5ºC.
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 12ºC below the process variable (**PV**). The controller will respond to the call for cooling by opening the solenoid valve, filling the jacket with water.
7. When the jacket is halfway filled, set the temperature control mode to **OFF**.

8.2 Additional preparation for autoclaving

To continue preparing the vessel for sterilization:

1. **Turn off mains/power to the controller.**
2. Remove the motor from the top of the vessel and carefully put it aside.
3. Lubricate the vinyl bearing housing cap with silicone grease to facilitate sliding the cap securely onto the housing.
4. Place the bearing housing cap on the top of the bearing housing.
5. Disconnect the air and/or gas lines from the inlet filter on the sparger.
6. Disconnect the water lines. Remove all PVC tubing.
7. Clamp off the harvest tube, the sample tube and all other penetrations that are immersed in the media.
8. Remove the RTD from the thermowell.
9. Disconnect all sensors and sensors, and remove their cables.
10. If you are using pH and DO sensors, install each sensor's shorting cap (provided in the sensor kit).
11. Before placing the vessel into the autoclave, loosen the glass sample bottle by ½ turn.
12. Wrap all filters with aluminum foil to protect them from steam.
13. 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.

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

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

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

Sensor tips must be moist during sterilization:

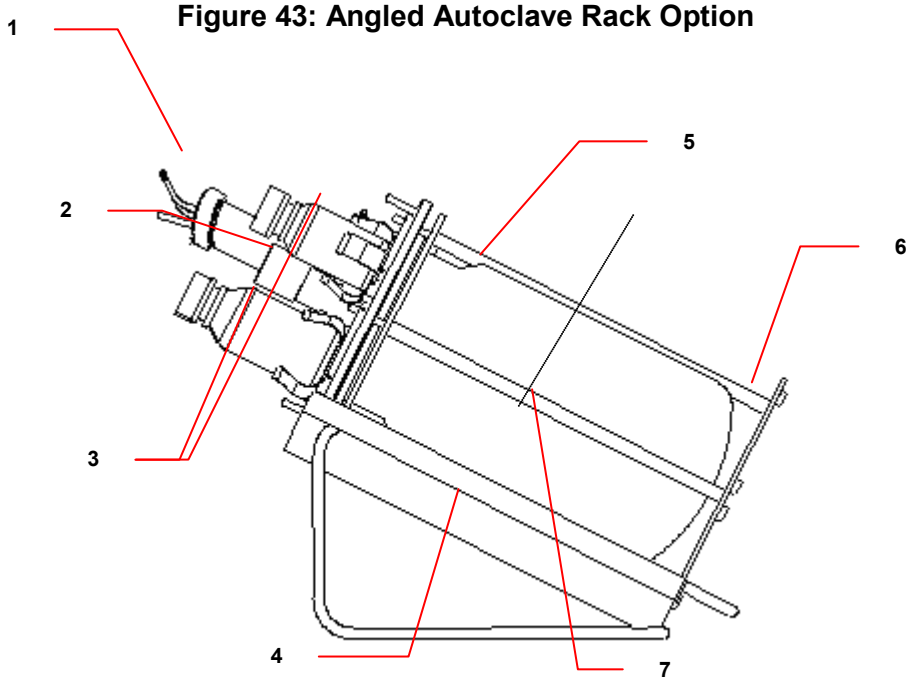
- If you will be doing batch fermentation, be sure the vessel is filled with media so the media will also be sterilized.
- If you will be using heat-liable media, use at least 100 ml of a balanced salt solution (such as phosphate-balanced saline solution). Sterilize the media separately, after autoclaving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

8.3 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 the drawing below)*. Secure it in place with the strap.
2. Insert the entire vessel assembly (glass jar, vessel stand if present, headplate and all headplate components) into an autoclave and sterilize.

3. When you remove the vessel from the autoclave, immediately crimp the foil funnel on the addition port and close off the vent tubing to maintain sterility.

Figure 43: Angled Autoclave Rack Option



1	Exhaust condenser: must point UPWARD	5	Non-jacketed vessel assembly
2	Bearing housing cap	6	Vessel stand
3	Foam trap and/or addition bottles	7	Retention strap
4	Autoclave rack		

8.3.1 Sterilization time and temperature

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

ALERT!

During autoclaving, the vessel and the water jacket (if present) 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.

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. No adverse effects have been shown filters exposed to longer autoclaving times.

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

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

9 REINSTALLING THE VESSEL ASSEMBLY

9.1 Reinstall the vessel assembly

WARNING! Risk of explosion!

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 310 control cabinet. Connect the water lines to the heat exchanger and the exhaust condenser *(see Vessel Assembly, Section 4.9)*. **Remember to connect the WATER OUT lines first**.

ALERT!

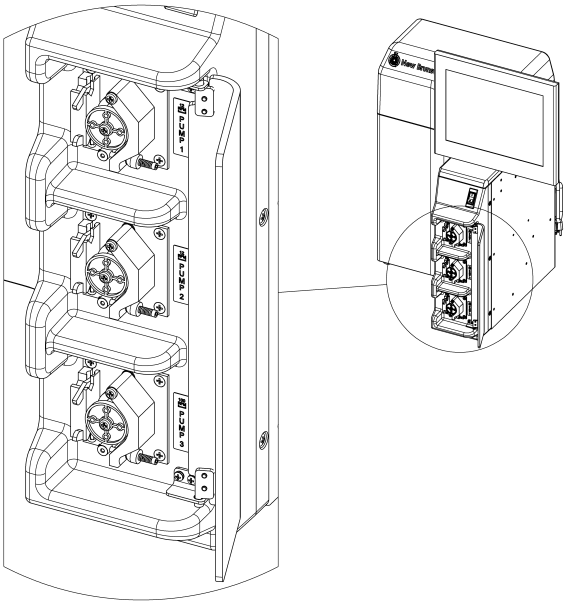
To avoid leaks and/or pressure build-up inside the vessel jacket, see *CAUTION* regarding connecting & disconnecting hoses in Section 4.8.2.

2. Carefully place the motor on the bearing housing, on top of the vessel assembly.
3. Remove the pH shorting cap and connect the pH cable to the pH connector on the control cabinet.
4. Remove the DO shorting cap and connect the DO cable to the DO connector on the control cabinet.
5. Connect the foam sensor cable to the foam connector on the control cabinet.
6. Be sure to attach the earth/grounding strap clip to the vessel headplate.

9.2 Load pump tubing

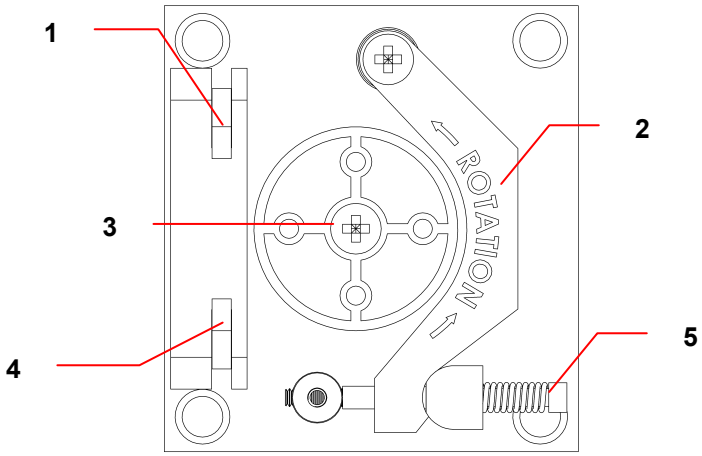
The three standard pumps are located on the front of the control cabinet*:*

Figure 44: Standard Pump Array



Before you insert tubing into the **PUMP CHANNEL**, verify that the **PUMP** is in the **OFF** control mode. *With reference to the drawing below*, follow these steps to properly load tubing into the **PUMP HEAD**:

Figure 45: Loading Pump Tubing



1	Upper spring-loaded clip	4	Lower spring-loaded clip
2	Tubing guide	5	Spring-loaded lever
3	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 13.3 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. Pull the right side of the spring-loaded lever down to release the tubing guide.
5. Hold the upper spring-loaded clip open and load the upper clip channel; allow the upper clip to close over the tubing.
6. Lift the tubing guide up to the right, and thread the tubing around the pump head, anchoring the tubing with one finger.
7. Pulling the loop taut, open the lower spring-loaded clip, load the lower clip channel.

> ***CAUTION!*** ***Risk of personal injury!***
> **Be careful not to pinch your fingers in the pump head levers.**

8. Let the tubing guide drop down to hold the tubing in place, and close the spring-loaded lever. Make sure it snaps shut.
9. 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 10.5 for details on pump assignment and Section 13 for details on pump set-up and operation.

9.3 Confirm pH calibration

Autoclaving can alter the zero characteristics of pH sensors, 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 310 **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.

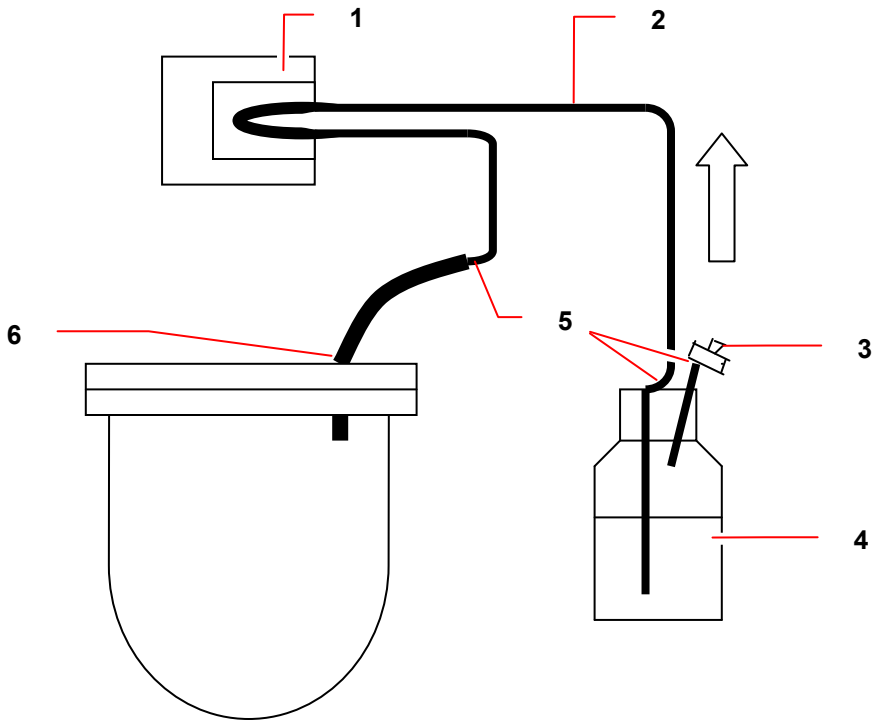
9.4 Install liquid addition systems

The drawing on the following page 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 (0.2 µm) 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.

Figure 46: Typical Liquid Addition System



1	Peristaltic pump	4	Addition bottle
2	Tubing	5	Tubing connectors
3	Breathing port with sterile filter (0.2μm)	6	Access to addition port

ALERT!

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 310 controller, which is constantly comparing current pH value with the pH setpoint and making adjustments as necessary.

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 over- control while supplying sufficient liquid during high-demand culture phases.

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

Eppendorf 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.

If any gas inlet will remain unused, make sure it is plugged. Use the metal plug inserted at the factory for shipping.

9.6 Install temperature (RTD) sensor

ALERT!

Proper installation of the RTD sensor is essential to temperature control.

1. Turn the mains/power switch **ON**.
2. Add 1 - 2 ml of glycerin to the thermowell and insert the RTD temperature sensor.
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 GETTING STARTED

10.1 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**.

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.

10.2 Setting P & I values

P & I values are numbers that determine how the fermentor responds to changing growth conditions and new setpoints. These are listed in each loop's **GAUGE** screen. You may need to modify P&I values to suit your particular process. To do so, press inside the Proportional & Integral edit boxes, each time entering the desired value using the popup keypad.

If you change P&I values, you can return to the original settings at any time by pressing the Factory Default button in the loop's GAUGE screen.

10.3 Loop setpoints

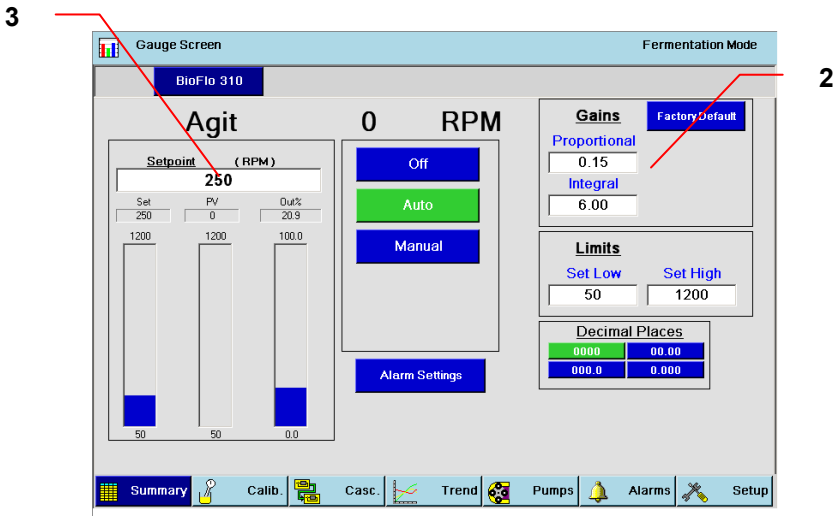
The setpoint is the value you want each loop to attain. When the loop control mode is **AUTO**, the fermentor will automatically make appropriate adjustments to maintain the value at the setpoint.

10.3.1 Entering setpoints

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

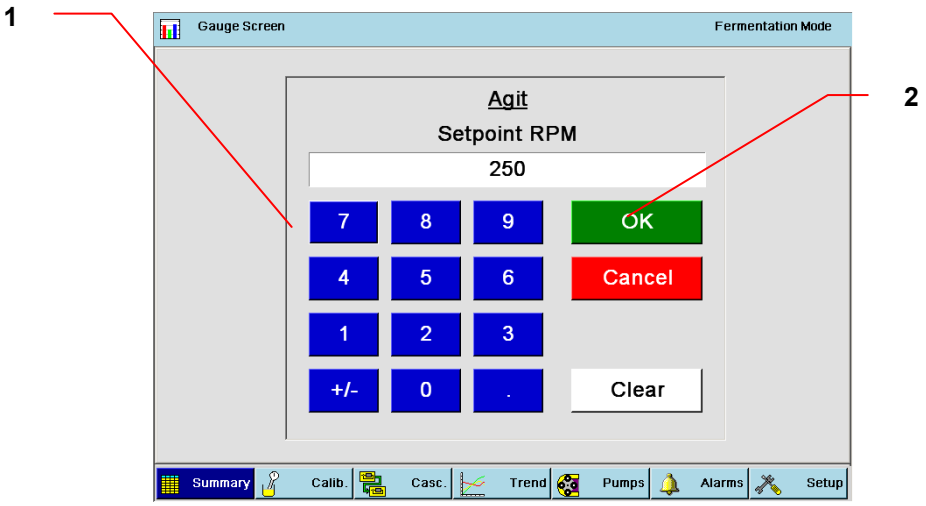
1. Touch either the **LoopName** box or the **Setpoint** box for the desired loop on the **SUMMARY** screen. In this example, we have selected **AGIT**.
2. The loop **GAUGE** screen opens:

Figure 47: Sample GAUGE Screen



1	Step 3: Press inside the Setpoint box to open the setpoint touchpad.
2	PI Values: adjusting these values will determine how your system responds to changes in your culture. For details, see Section 23.5.

Figure 48: Setpoint Touchpad



1	Step 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.
2	Step 5: Press **OK** to save the setpoint and return to the GAUGE screen, or press **Cancel** to return *without* saving the setpoint.

10.3.2 Modifying setpoints

This process is the same as entering setpoints. *See Section 10.3.1 above.*

10.4 DO cascade system

Cascading brings several systems together to work jointly to achieve your goal. This cascade is designed to control DO through P&I-controlled agitation speed and oxygen output. This is how it functions: when the actual DO value rises above the DO setpoint, the agitation speed will automatically decrease until the DO setpoint is reached. Conversely, when the actual DO value drops below the setpoint, the cascade system acts to bring it back up. Agitation will not fall below its setpoint, even if DO rises above the DO setpoint. *See Section 11 for details about setting cascades.*

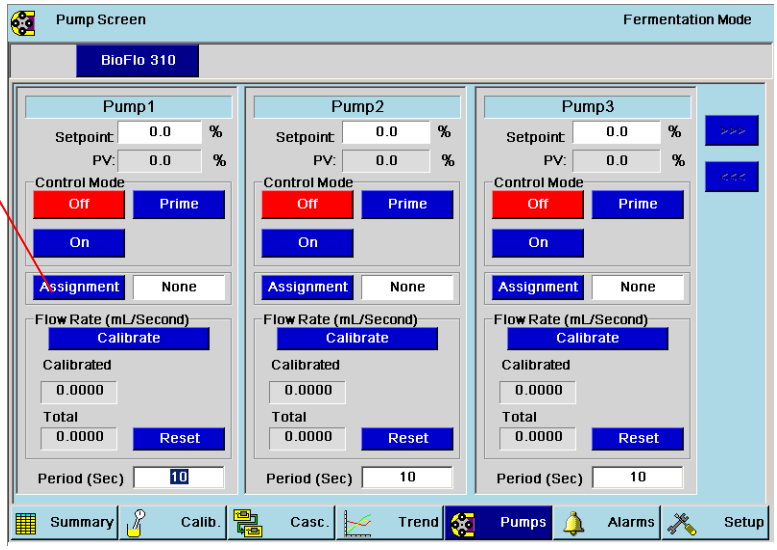
10.5 Pump assignment

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 **PUMPS** screen:

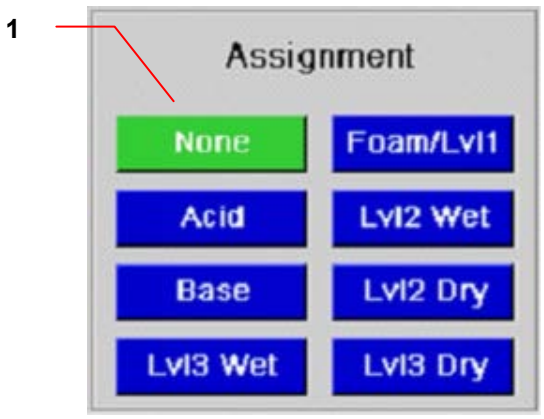
Figure 49: Pumps Screen



1

1	Step 2: Press the Pump 1 **Assignment** button to open the Pump Assignment screen (see the following page).

Figure 50: Pump Assignment Screen



1	Step 3: Press the button that corresponds to your choice of assignment for Pump 1.

4. Repeat Steps 2 – 3 for the other pumps to be assigned.
5. Press **SUMMARY** to save the pump assignment(s) and to return to the **SUMMARY** screen.

For details on the choice of Level Wet and Level Dry, see Section 10.6.1.

10.6 Using level sensors to program feed pumps

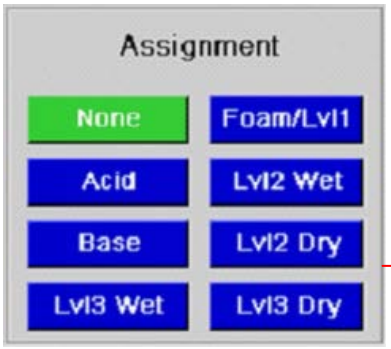
10.6.1 Setting a feed pump to add liquid

A feed pump can be set to add liquid whenever the associated level sensor, 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 sensor that you wish to use is fully inserted into the vessel. When the vessel is set up at the control cabinet, raise the sensor to the level at which you want addition to begin. **Never lower a sensor after autoclaving!**

1. Open the **PUMPS** 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:

Figure 51: Pump Assignment Screen



1	Step 3: Press the **Levl2Dry** or **Lvl3Dry** button, whichever corresponds to the sensor'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 sensor, the feed pump is turned **on** so that more liquid will be added.
- when the liquid is **in contact** with the sensor, the pump is turned **off**.

10.6.2 Setting a feed pump to harvest

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

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

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

1. Open the **PUMPS** 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.
3. Select the **Lvl2 Wet** or **Lvl3 Wet** button, whichever corresponds to the sensor's connection on the control cabinet.

In **WET** mode:

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

10.6.3 Level control off

When **OFF** is selected from any level (Foam, HiFoam, Lvl2 Wet, Lvl3 Wet, Lvl 2 Dry, Lvl3 Dry, Acid or Base) control mode menu, the pump is off.

10.6.4 Pump calibration

Pump flow rates are provided in Table 6 (*Section 13.3*). However, more accurate flow rates through the various lines may be established by pre-calibrating the pumps, using the **PUMPS** 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 **PUMPS** 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, Lvl3Wet or Lvl3Dry—any "level dry" function turns the pump on when the sensor is not in contact with liquid; *see Section 10.5 for details*).

To assure the most accurate flow rate, calibrate the pump (*see Section 13.3*) each time you change tubing.

11 CASCADE CONTROL

Cascades are control schemes in which the Output % of one process control loop influences the setpoint of one or more other loops. In other words, it uses feedback from one parameter to influence others. In BioFlo 310 bioreactors, the output % value is mathematically determined by evaluating the error between measured present values and desired setpoints, and integrating these values into a PID-based control algorithm.

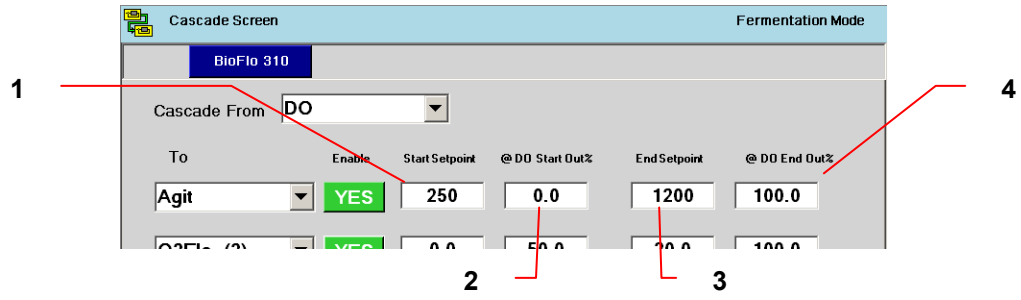
The BioFlo 310's RPC controller allows cascading from any loop to as many as five other loops. DO and pH are the most commonly *cascaded-from* loops; oxygen and nitrogen commonly receive the cascade from DO, and CO_2 and Base pump usually receive the cascade from pH, altering their respective setpoints to correct errors in DO. Systems with 2 or more TMFCs require the writing of cascades for pH and DO control, where systems with 1 TMFC can be set to automatically adjust the mixtures of the sparge gas depending on the need to make adjustments.

When more than one loop is configured as the recipient of a cascaded loop, they may respond in parallel, at the same time, or in series, one after the other, depending on how the cascade has been set up. Cascades set up to run in series generally give more predictable control responses. Sometimes a small region of overlap, where two loop setpoints vary simultaneously, is used to smooth the transition from one loop to another.

11.1 Creating a cascade

The screen detail below shows the headers from the **CASCADE** screen (set to "Cascade From DO"), with an explanation of the settings those headers represent:

Figure 52: Cascade Screen (detail)



1	**Start Setpoint** is the loop value the user defines for the system to be at when initial DO Start Out% is reached. Typically this value will be close to the normal operating setpoint.

…continued on the next page…

See important NOTICE on the next page.

2	**@DO Start Out%** represents the DO output % value where the user wants the cascade to begin. When this output % is reached, the setpoint of the Cascade To loop will change to the value entered as Start Setpoint. The current DO output % can be found on the **SUMMARY** screen at the intersection of the Output% column and the DO loop row. This value is calculated by using the integrated PI values. It is essentially a mathematical calculation of setpoint "error" from PV (current process value), "error" meaning any readings that are above or below the programmed setpoint. As the "error" discrepancy increases, or as the duration of such a discrepancy remaining static increases, the Output% also increases.
3	**End Setpoint** is the loop value the user defines as the maximum allowable value when the DO End Out% is reached. Typically this value will also be the same as the system's maximum allowable setpoint for the loop.
4	**@DO End Out%** represents the DO output % where the user wants the cascade to stop. This value can be set to any integer from 0 to 100% as long as it is greater than the Start Out%. The greater it is than the Start Out%, the smoother the increase in setpoints.

It is important to remember that cascades are based on the loop (whether DO or pH) Out% value posted in the SUMMARY screen. These numbers are the basis for all cascades involving that loop. See the examples below for more explanation.

It can be a very beneficial exercise to watch how the values on the **SUMMARY** screen change to reflect differences between the present value (PV), the setpoint, and the DO output percentage (Out%).

When the PV is greater than the setpoint, the system will be generating a negative Out% because the controller senses a need to decrease DO:

LoopName	PV	Setpoint	Out%	Control Mode	Units	Casc.
DO	74.8	35.0	-38.6	Auto	%DO	Source

When the PV is less than the setpoint, the system will be generating a positive Out%, because the controller senses a need to increase DO:

LoopName	PV	Setpoint	Out%	Control Mode	Units	Casc.
DO	25.2	35.0	51.6	Auto	%DO	Source

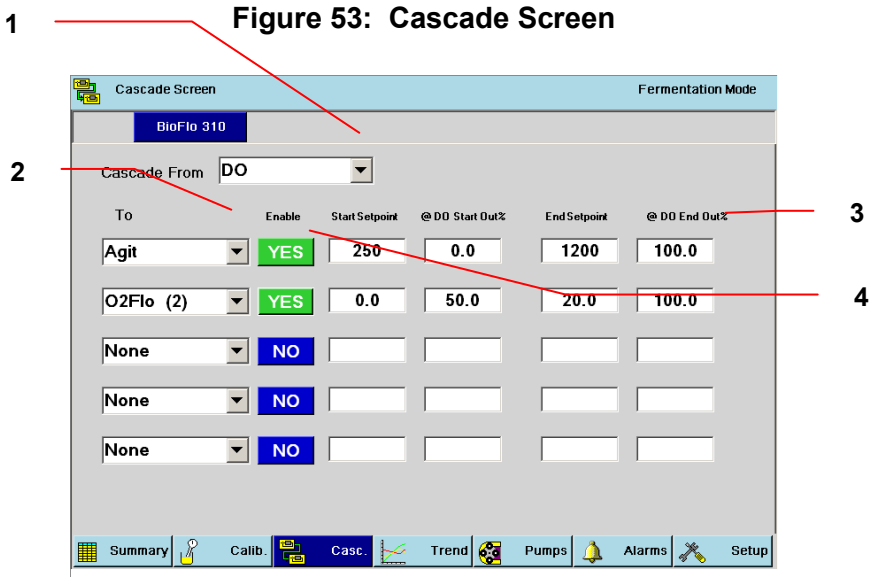
When the PV equals the setpoint, the Out% should be approximately 0, as the controller senses no need to make adjustments to DO:

LoopName	PV	Setpoint	Out%	Control Mode	Units	Casc.
DO	35.2	35.0	0.0	Auto	%DO	Source

To create a cascade:

1. Press the **CASCADE** button to open the **CASCADE** screen:

Figure 53: Cascade Screen

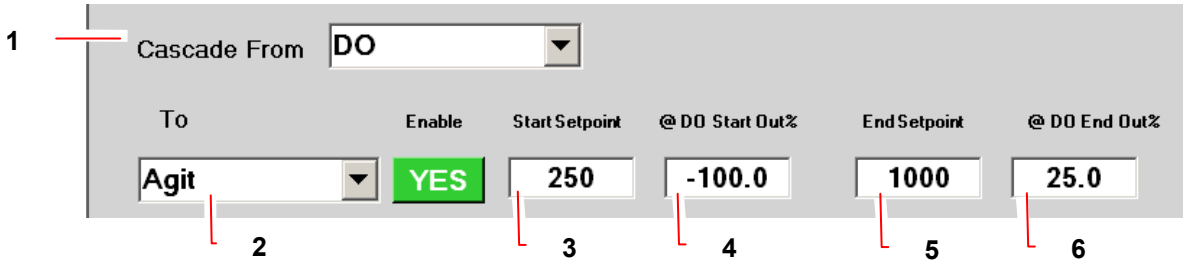


1	Step 2: Use this ▼ dropdown menu to select the "Cascade-From" loop.
2	Step 3: Use the first ▼ dropdown menu to select the first "Cascade-To" loop.
3	Step 4: Set the Start Setpoint, @ DO Start Output%, End Setpoint and @DO End Output% values one by one by pressing the edit box, entering the desired value on the touchpad and pressing the **OK** button.
4	Step 5: Press the corresponding Enable button to select **YES**.

11.2 Controlling DO by cascade

Example: Cascading DO to Agitation, GasFlo and O2 (2).

In the example below, errors in **DO** are corrected by increasing agitation, gas flow, and oxygen concentration:

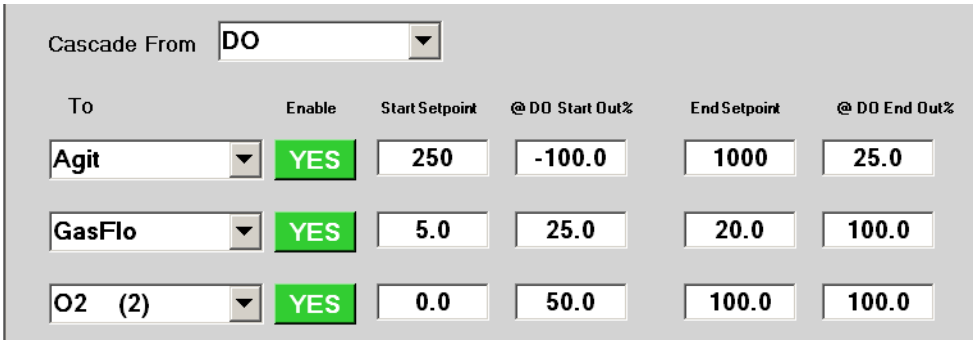


…see the next page for the index key…

1	**DO** is the source of the cascade.
2	**Agit** is the loop being influenced by the cascade.
3	**Start Setpoint** is the value to which the system will set the loop setpoint when the DO Start Out% is reached.
4	**@DO Start Out%** is the DO output % value at which the user wants the cascade to begin. When this output value is reached, the loop's setpoint will be the value of the start setpoint. In this example, when the DO output is -100.0, the setpoint will be 250.
5	**End Setpoint** is the maximum loop setpoint value the user defines, once the @DO End Out% is reached. Typically this value is the maximum allowable value the process can handle. In this example, when the DO End Out% reaches 25, the agitation will have increased to 1000 rpm.
6	**@DO End Out%** represents the DO output % where the user wants the cascade to stop. This value can be set to any integer from 0 to 100% as long as it is greater than the Start Out%. The greater it is than the Start Out%, the smoother the increase in setpoints.

The following cascade is an example of the setup for multiple loops being used to control one source loop:

Figure 54: Sample DO Cascade



The cascade scheme shown above can be read as follows: as DO output% increases, prompted by a need to increase DO, agitation will increase from 250 rpm to 1000 rpm over the DO output range of -100% (*see the following paragraphs for guidelines on setting* **DO Start Out%**) to 25%. If this response is not enough to correct the deviation between setpoint and present value of DO, the system will then begin to respond by increasing GasFlo. The setpoint will increase from 5 SLPM to 20 SLPM over the DO output % range of 25% to 100%. If these two events are still not enough to correct the error the system, will then respond by increasing the O2 percent of the gas mix. The O2 percent will increase from 0% to 100% over a DO output% range of 50% to 100%

When to use -100 as DO Start Out% in a Cascade:

Commonly after calibrating DO sensors, the system will have a DO present value higher than the setpoint. This happens because of calibration methods and because the culture has not had enough time or has not grown to a significant enough density to start to consume the available DO. This difference, as described earlier, will cause the DO output % to plummet to -100% because the system thinks it needs to decrease the DO. When you set -100 as the **DO Start Out%**, the loop being cascaded to begins increasing as soon as the PV dips below the setpoint. Once the PV dips below the setpoint, the Output % will begin to increase.

When to use 0 as DO Start Out% in a Cascade:

Zero should be used as the **DO Start Out%** if the media is equilibrated to the desired setpoints before inoculation or before the cascade is enabled. In other words, if the DO setpoint and DO PV are close to equal at the start of a run, it will work best if the cascade **DO Start Out%** is 0. When configured this way, any drop in DO below the setpoint will be compensated by the cascade loop.

- **Regardless of DO (*cascaded-from*) output, the setpoint of any *cascade-to* loop will not go below its own Minimum Setpoint value.**
- **Minimum Output% corresponds to the minimum value that will produce the minimum setpoint; lower outputs will not affect setpoint.**
- **Regardless of DO output, the setpoint of any cascade-to loop will not rise above its own maximum setpoint.**

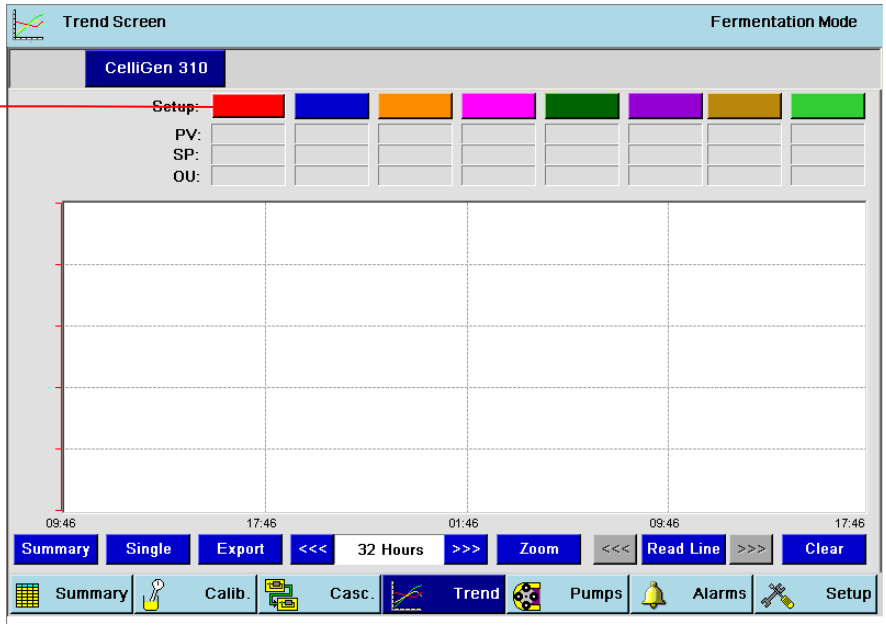
12 PLOTTING TRENDS

Opening the **TREND** screen allows you to plot and display a graph of ongoing fermentation data, viewing from 30 minutes to 144 hours of input. Up to 8 loops can be plotted on the graph, each in its own distinctive user-selected color. The graph and data are only available while the fermentor is running. Data cannot be stored in the controller, but can be archived remotely on an auxiliary PC via the RS-232/-422 Modbus interface (*see Section 4.11 for details*) or saved to a USB storage device.

12.1 Creating a trend graph

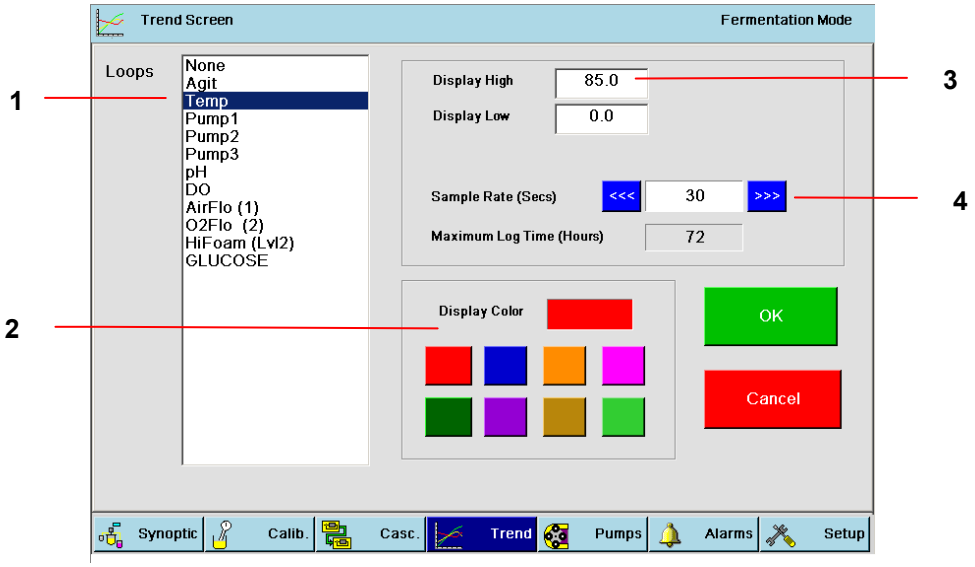
1. From any screen, press the **TREND** button to open the **TREND** screen:

Figure 55: Trend Screen



1	Step 2: To select the first loop you wish to display, press the red Setup button.

Figure 56: Trend Setup Screen



1	Step 3: Select the first loop. The program will automatically place it in the red box.
2	Step 4: If you wish to change the color of this loop, press the new color choice here.
3	Step 5: Press the Display High box to enter (using the touchpad) the high limit for the Y axis, then use the Display Low edit box to set the low limit.
4	Step 6: Press the ramp up **>>>** or ramp down **<<<** button to select the desired data sampling interval: 5, 15, 30 or 60 seconds.

7. Press **OK** to save your choice and return to the **TREND** screen, or Cancel to return to the **TREND** screen without saving any changes.
8. Repeat Steps 2 - 7, selecting a different color for each loop, up to a maximum total of 8 loops.
9. With reference to the screen on the following page (a sample Trend Graph in progress) and Table 4, acquaint yourself with the Trend Graph buttons at the bottom of the graph:

Figure 57: Trend Graph

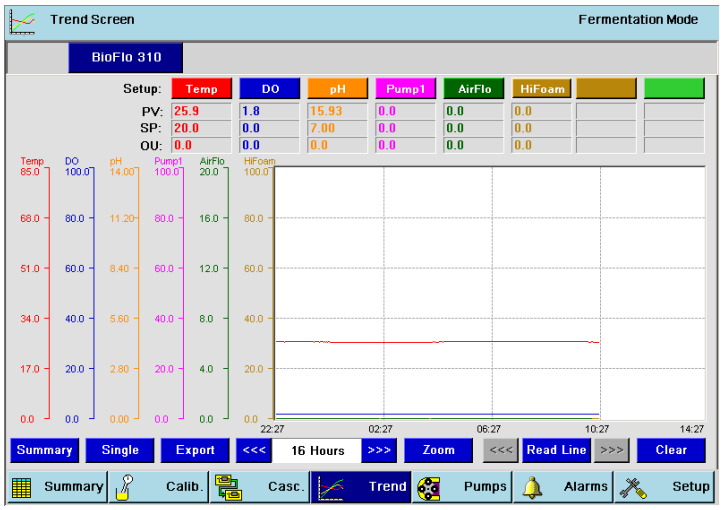


Table 4: Trend Graph Buttons

Button/Feature	Description
Summary	Press this button to cycle through three summary display modes: all eight loops at once, loops 1 - 4 (red-fuschia), and loops 5 - 8 (dark green-light green).
Single	Press this button to display the graph for one loop at a time, in the order (left to right) they are displayed in the colored buttons at the top of the screen.
Export	Press this button to export a text file containing all of the Trend data. See Section 12.1.1 for detailed instructions.
<<< Ramp Down	Press this button to select a lower Timespan or to move the Read Line toward the left of the screen.
[Timespan Indicator]	Using the Ramp Down or Ramp up button on either side of this edit box, select the timespan to display onscreen. Preset increments range from 30 Minutes to 144 Hours.
>>> Ramp Up	Press this button to select a higher Timespan or to move the Read Line toward the right of the screen.
Zoom	Press this button to open an interactive mode where you can zoom in on a section of interest on one plot. The button turns red when you touch it, indicating you are in Zoom mode. See Section 12.1.2 below for detailed instructions.
Read Line	Press this button to open an interactive mode where you can move a vertical (cross-sectional) line across the graph to aid in determining a particular reading. See Section 12.1.3 below for detailed instructions.

12.1.1 Using the export button

To export Trend data as a text file to a USB external memory device for use with a PC program (e.g., Microsoft Excel®):

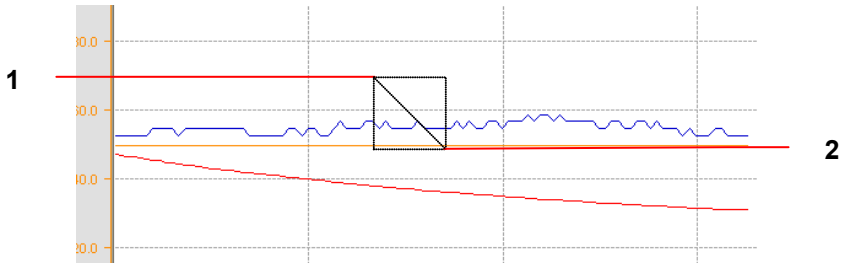
1. Install the USB external memory device into one of the USB connections on the back of the Control Cabinet.
2. Open the **TREND** screen and push the **Export** button.
3. In the screen that opens, select the USB external memory device from the list of available drives.
4. Touch the empty **FileName** box. Using the touchpad that appears, enter the desired file name, then press the **OK** button.
5. Press the **Save** button to save the file to the USB external memory device.
6. Remove the USB external memory device and use it to download the data to your PC.

12.1.2 Using the zoom button

To zoom in on a particular section of one loop plot:

1. Press the Zoom button at the bottom of the **TREND** screen. It will turn red to indicate that the zoom mode is active.
2. Press, in succession, two diagonal locations that would frame, left to right, the section of interest. For example, press the upper left corner, then the lower right corner as shown below. **NOTE:** The rectangle does not appear onscreen; it is indicated here for reference only.

Figure 58: Selecting Zoom Coordinates



1	Press first here, in the upper left corner of an imagined box.
2	Press second here, in the lower right corner of an imagined box. The rectangle and the diagonal line shown here do not appear onscreen; this image is for reference only.

3. The Trend view will display the data between the two points selected, and will adjust the time axis to match the elapsed time represented by this close-up.
4. Press the Zoom button again to return to the regular trend graph.

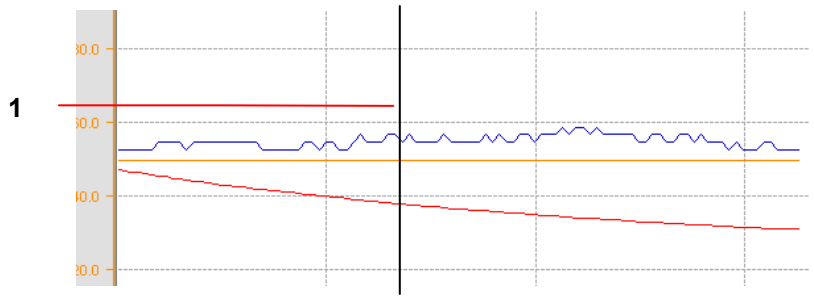
Minimum axis time in zoom mode is 120 seconds. If you wish to use the zoom mode and the read line (*see Section 12.1.3*) at the same time, you must enter Zoom mode first.

12.1.3 Using the read line

The Read Line mode allows you to read PV values from the graph (displayed at the top of the screen) at a position you select. To use the Read Line:

1. Press the Read Line button at the bottom of the **TREND** screen. It will turn red to indicate that the read line mode is active, and black vertical line will appear at the current time position on the graph.
2. To move the line to a time of your choosing, press the graph at the desired point. You can also press the Read Line <<< or >>> button (both are now red and active) to move the line one click at a time for more precision:

Figure 59: Selecting a Read Line Location



1	Press anywhere along the desired vertical axis to locate the Read Line.

3. Press the **Read Line** button again to return to the regular trend graph.

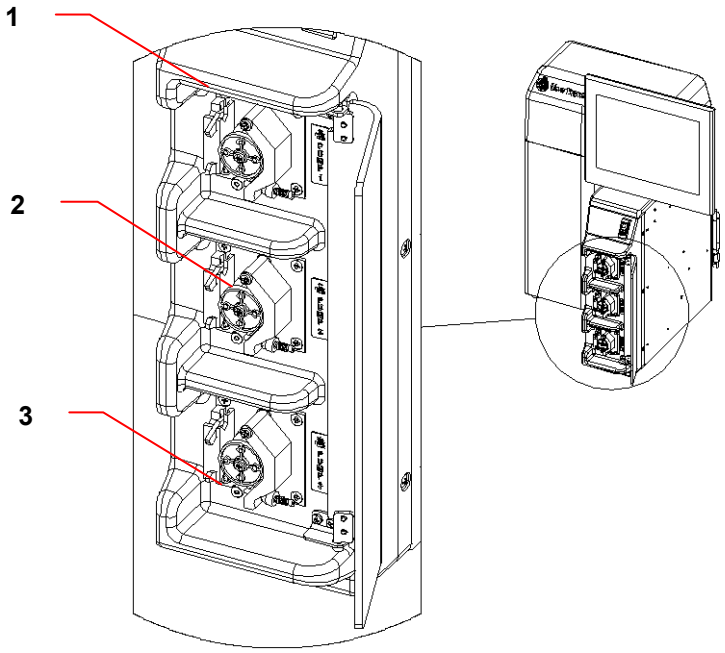
If you wish to use the zoom mode (*see Section 12.1.1*) and the read line at the same time, you must enter Zoom mode first.

13 ABOUT PUMPS

After assigning the pumps (*see Section 10.5*), 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. Remember to set up any optional pumps you may have added to your system (*see Section 13.5 to install an external variable speed pump*).

Figure 60: Standard Pump Array



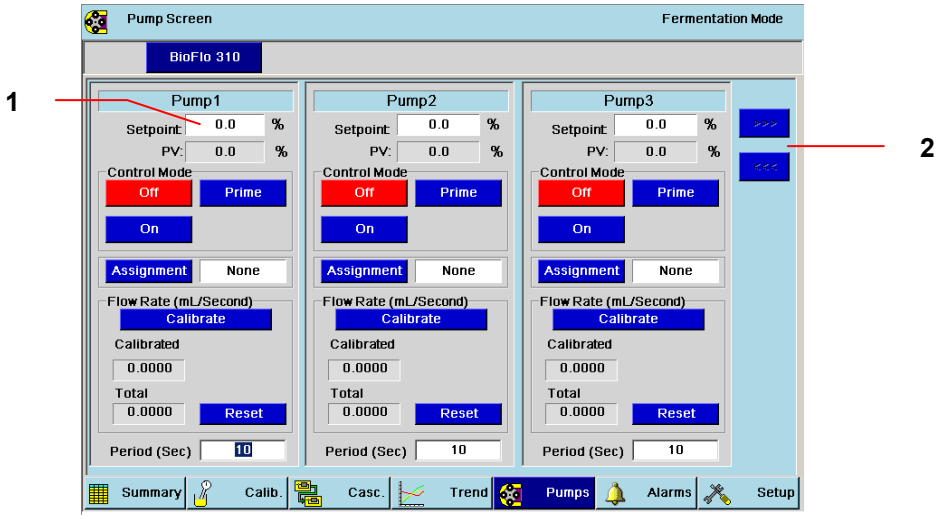
1	Pump 1 (12 rpm)	2	Pump 2 (12 rpm)	3	Pump 3 (100 rpm)

13.1 Pump setpoint

To enter a setpoint for any pump:

1. Open the **PUMPS** screen. Gauges for Pumps 1 - 3 are displayed in this screen. If you have one or more additional pumps, press the >>> button to continue past Pump 3.

Figure 61: Setting Pump Setpoint



1	Step 2: Press the Setpoint edit box for Pump 1. Step 3: Use the touchpad that opens to enter the desired setpoint, then press **OK** to save it and return to this screen (or press **Cancel** to return to this screen without saving a setpoint).
2	If you have optional pumps installed, these buttons will be marked **>>>** and **<<<**, and they will be active, allowing you to scroll to the next page or back.

4. Repeat Steps 2 - 3 for each pump.

13.2 Pump control mode

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

Table 5: Pump Control Modes

Control Mode	Description
Off	The pump will receive no input and will not operate.
On	The pump will operate according to the parameters you have set.
Prime	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.

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.

13.3 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:

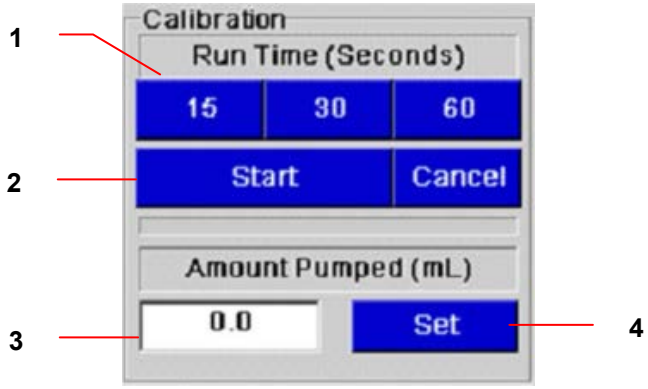
Table 6: Flow Rate per Tubing Size

Tubing Wall Thickness	**1.6mm (1/16 in)**			
Inside Diameter: inch (mm)	**0.8 (1/32)**	**1.6 (1/16)**	**2.4 (3/32)**	**3.2 (1/8)**
Flow ml/revolution	0.03	0.11	0.24	0.41
12 rpm Flow ml/minute	0.360	1.32	2.88	4.92
100 rpm Flow ml/minute	3.00	11.0	24.0	41.0

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 **PUMPS** screen for that pump, press the **Calibrate** button to open the Calibration pane:

Figure 62: Calibrating the Pump Flow Rate



1	Step 6: Press your choice of Run Time (**15**, **30** or **60**); that button will turn green.
2	Step 7: Press **Start**. The button will turn green and the pump will start running.
3	Step 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.
4	Step 9: Press the **Set** button to save this data to the PUMPS screen.

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.

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

13.4 Pump period

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

Figure 63: Pump Period(sec)

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.

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.

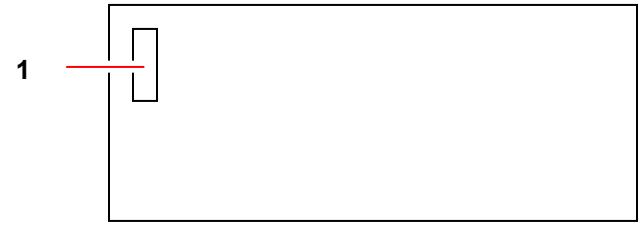
13.5 Installing an external variable speed pump

ALERT!

When selecting an external pump to operate with your system, please consult with your local sales representative to be sure the model you choose is compatible with your BioFlo 310.

1. Connect the D connector of the pump's 3-wire cable at **Interface** on the rear panel of the pump. Your pump will be marked 115 V or 230 V depending on the electric supply you specified when you purchased the pump.

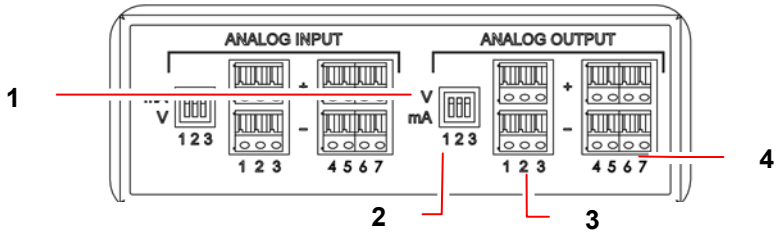
Figure 64: Variable Speed Pump



1	Use **Interface** to connect the cable provided.

2. Locate the Analog Output Connections on the rear of the BioFlo 310 cabinet:

Figure 65: Rear Panel of BioFlo 310 Cabinet



1	Dip switches: provided to switch connectors 1 - 3 between mA (down) and V (up).
2	Analog Output dip switch 1 is reserved for pressure control
3	1 - 3 for mA or V
4	4 - 7 for V only

3. The preferred connection for pumps is 4 - 20 mA. *If you are using a 0 - 5 V connection, skip to step 5.* To use 4 - 20 mA, connect the end of the green cable wire to one of the three (1, 2 or 3) negative (-) **outputs** at the bottom.

4. Connect the end of the white cable wire to the positive (+) output at the top: *be sure to use the same number* (1, 2 or 3) as you used for the green wire.

5. If, *and only if*, you are using a 0 - 5 V connection instead of 4 - 20 mA, connect the end of the green wire (return) to one of the four (4, 5, 6 or 7) negative (-) outputs at the bottom and connect the end of the black wire (0 - 5 V input) to the matching positive (+) outputs at the top.

6. Set up the pump control loop using a loop for external equipment.

After a pump is added, it will appear on the **PUMPS** screen.

ALERT!

Be sure to set the dip switches correctly when using either 4 - 20 mA or 0 - 5 V inputs/outputs.

14 ABOUT ALARMS

14.1 ABS and DEV alarms

There are two types of alarm modes you can set, Absolute (ABS) and Deviation (DEV):

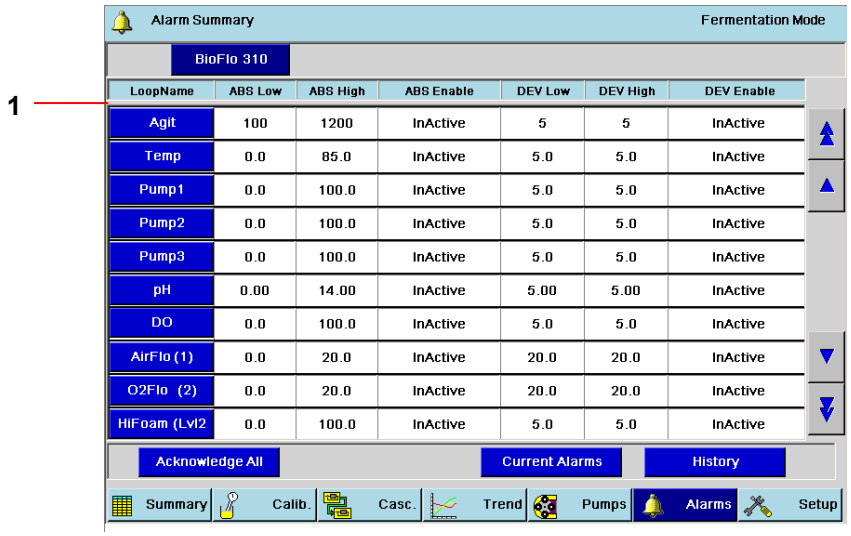
- An Absolute alarm is triggered when the control loop's Process Variable falls below the absolute Low limit or rises above the absolute High limit that you set.
- A Deviation alarm is triggered when the control loop's Process Variable falls below or rises above the control band that you specify around the loop's setpoint (e.g., a tolerance of 10 rpm above or 5 rpm below the Agitation setpoint).

14.2 Setting alarms

To set alarms:

1. Press the **ALARMS** button to open the **ALARMS** screen (*see the sample screen below and Table 7*, which explains the features of this screen).

Figure 66: Alarms Screen

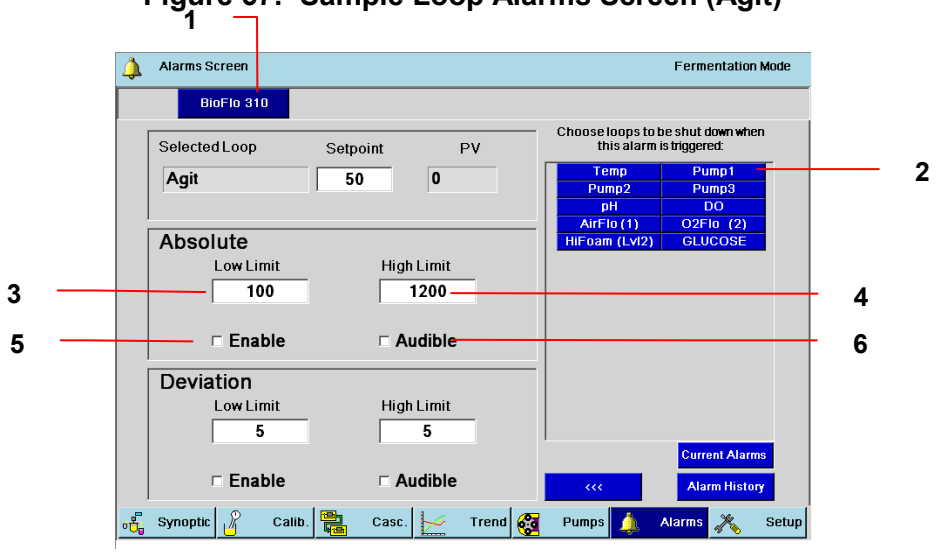


1	Step 2: Press the first loop for which you want to enable an alarm. That loop's individual Alarms screen will open. For this example, we use the Agitation loop (**Agit**).

Table 7: Alarms Screen Features

Feature Name	Description
LoopName	Like the BioFlo 310 name box, this box is blue under normal operating conditions, and red when there is an alarm condition. Press a LoopName box to open that control loop's alarm screen.
ABSLow	This column indicates the Absolute low limit you program for control loops. An alarm is triggered if the loop PV falls below this point.
ABSHigh	This column indicates the Absolute high limit you program for control loops. An alarm is triggered if the loop PV rises above this point.
ABSEnable/ABSAudible	This column indicates whether the Absolute alarm limits have been enabled ("Active") or not ("InActive") for visible (ABSEnable) and/or audible (ABSAudible) alarms.
DEVLow	This column indicates any tolerance you have set below the control loops' setpoints.
DEVHigh	This column indicates any tolerance you have set above the control loops' setpoints.
DEVEnable/DEVAudible	This column indicates whether the Deviation alarm limits have been enabled ("Active") or not ("InActive") for visible (DEVEnable) and/or audible (DEVAudible) alarms.
Acknowledge All button	Press this button to acknowledge (and stop) all alarms.
Current Alarms button	Press this button to open a screen that addresses any current alarm condition.
History Button	Press this button to open the historical record of alarms for the current run.
Scroll Up or Scroll Down	Use this button to scroll upwards or downwards in the table onscreen.
Scroll Back	Use this button to return to a previous screen.

Figure 67: Sample Loop Alarms Screen (Agit)



…see the next page for the index key…

1	In an alarm condition, this name box turns red,
2	Pressing any of these loop buttons will cause the selected loop(s) to shut down when an alarm is triggered for (in this sample) the Agit loop.
3	Step 3: If you wish to set an Absolute alarm, enter an Absolute Low limit here…
4	…and enter an Absolute High Limit here.
5	Step 4: Press here to enable the Visual Absolute alarm.
6	Step 5: Press here to enable the Audible Absolute alarm.

6. If you wish to set a **Deviation** alarm, use the **Deviation** pane and follow the same procedure as outlined in Steps 3 - 5 above.
7. Use the **Scroll Back** button (<<<) to return to the main Alarms screen and follow these steps for any other alarms you wish to set, or press the **SUMMARY** button to return to the **SUMMARY** screen.

14.3 Acknowledging an alarm

When an alarm condition develops, the **LoopName** box on the **SUMMARY** screen for the control loop involved will turn from blue to red, as will the BioFlo 310 name box. This is the Visual alarm. A footnote, written in red, will also appear in order to identify the nature of the alarm (e.g., Unit 1—Deviation Low Error).

The Visible alarm will remain onscreen until the alarm condition is rectified. If the Audible alarm is also enabled, beeping will occur until the alarm is acknowledged.

There are three ways to acknowledge alarms: (1) one alarm at a time, (2) all alarms for one control loop at a time, and (3) all alarms for all control loops at a time, for the rare occasion such a condition should arise.

To acknowledge **one alarm at a time**:

1. Press the **ALARMS** screen button to open the **ALARMS** screen.
2. Press the red **LoopName** box to open that control loop's **ALARMS** screen.
3. Press the **Current Alarms** button to open the **Current Alarms Summary** screen.
4. Press the Index box for the alarm you wish to acknowledge. It will turn green.
5. Press the **Acknowledge** button. The alarm will be deleted from the screen.
6. Repeat Steps 4 & 5 for any other alarms recorded for this loop.
7. Press the **Scroll Back** (**<<<**) button to return to the **ALARMS** screen.
8. Repeat Steps 2 - 7 for any other control loop alarms.

To acknowledge **all alarms simultaneously for one control loop**:

1. Press the **ALARMS** screen button to open the **ALARMS** screen.
2. Press the red **LoopName** box to open that control loop's **ALARMS** screen.
3. Press the **Current Alarms** button to open the **Current Alarms Summary** screen.
4. Press the **Acknowledge All** button. All alarms will be deleted from this screen.
5. Press the **Scroll Back** (**<<<**) button to return to the **ALARMS** screen.

To acknowledge **all alarms for all control loops** at the same time:

1. Press the **ALARMS** screen button to open the **ALARMS** screen.
2. Press the **Acknowledge All** button. All alarms will be deleted from this screen.
3. Press the **Scroll Back** (**<<<**) button to return to the **ALARMS** screen.

ALERT!

Acknowledging alarms is NOT a replacement for correcting the condition that triggered the alarm. Diagnose the cause of the alarm condition and rectify the situation to ensure proper operation of your BioFlo 310.

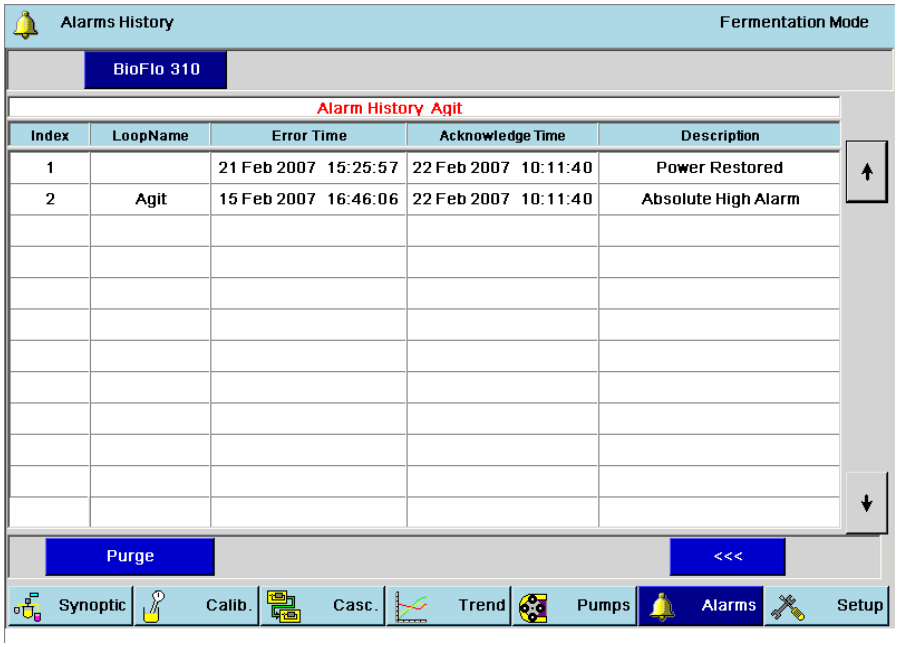
14.4 Alarms history

Each time an alarm is triggered, whether Visible and/or Audible, the controller records the event. The controller also records each alarm acknowledgement. You can access the Alarms History screen (1) to consult the data, (2) to save the data to an optional auxiliary PC, and/or (3) to purge the records once the condition has been rectified.

To access the Alarms History screen **to consult data**:

1. Press the desired control loop's **LoopName** box in the main **ALARMS** screen.

Figure 68: Sample Alarms History Screen



2. In the control loop's **Alarms Screen** that opens, press the **Alarm History** button.
3. Press the **Scroll Down** (↓) or **Scroll Up** (↑) button to read through the data.
4. Press the **Scroll Back** (**<<<**) button to return to the **ALARMS** screen.

To access the Alarms History screen **to purge the history**:

1. Press the desired control loop's **LoopName** box in the main **ALARMS** screen.
2. In the control loop's **Alarms Screen** that opens, press the **Alarm History** button.
3. Press the **Purge** button to erase all records.

You cannot delete one record at a time; you can only purge all records simultaneously.

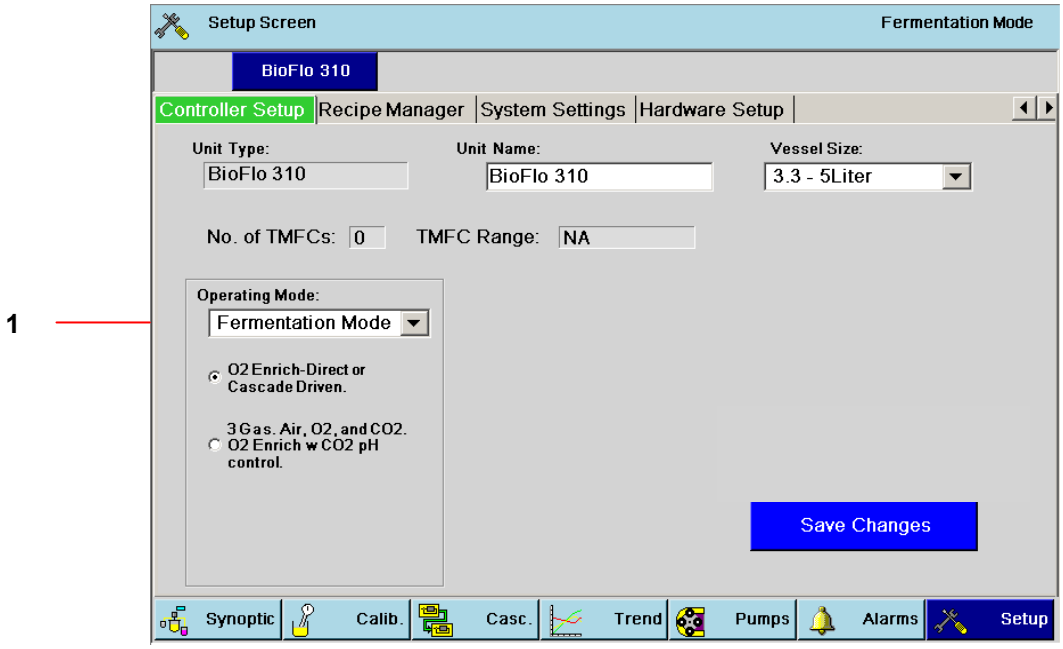
4. Press the **Scroll Back** (**<<<**) button to return to the **ALARMS** screen.

15 USING THE SETUP SCREEN

The **SETUP** screen has one feature that you will use with frequency, the **Recipe Manager** (*see Section 15.2*). You can also use this screen to change **Controller Setup** (*see Section 15.1*), to adjust **System Settings** (select onscreen language when available, change date & time, update software and calibrate the touchscreen; *see Section 15.3*), and to check or change the **Hardware Setup** (*see Section 15.4*).

In addition, if you need to contact Eppendorf Customer Service about your BioFlo 310, 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).

Figure 69: Controller Setup Screen

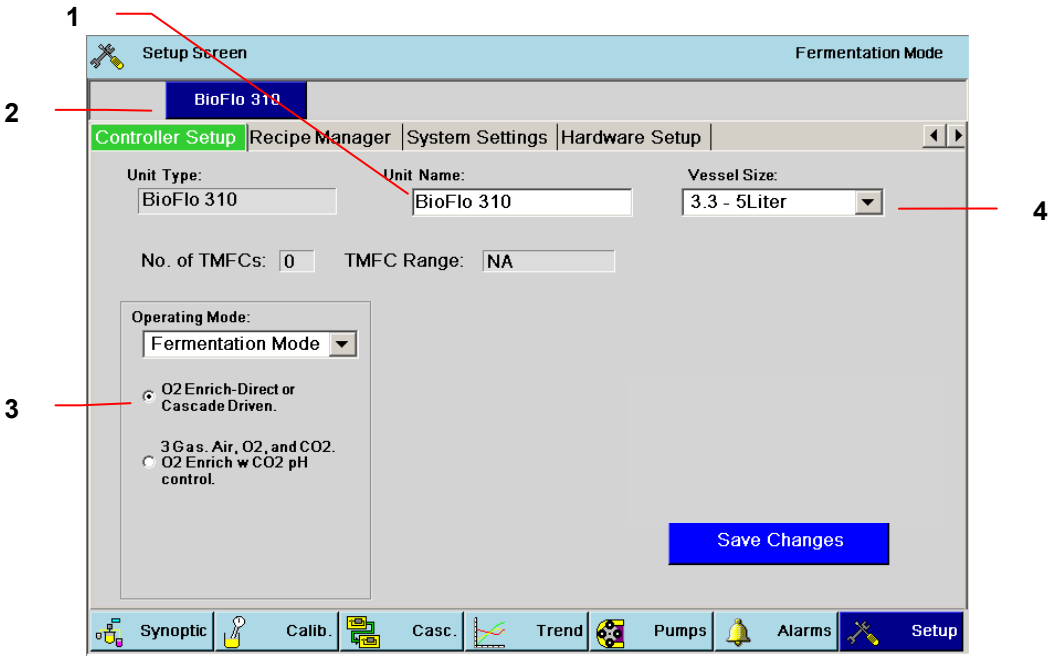


1	The equipment is factory-set in Fermentation mode.

15.1 Controller setup

When you open the **SETUP** screen, normally the **Controller Setup** screen will display first. If you find any other Setup screen in the display, press the **Controller Setup** tab to open this screen.

Figure 70: Controller Setup Screen



1	Step 1: Press here, in the Unit Name box, to open a touchpad. The name you write in this box using the touchpad will appear on a colored button tab on the top menu line.
2	Step 2: This is where the new Unit Name button tab appears.
3	Step 3: Press the appropriate option button in the Operating Mode pane. Options change depending on the number of TMFCs present. See Sections 15.1.1–15.1.4 for details.
4	Step 4: Press ▼ to select the vessel size.

Name each unit in your system using the Unit Name edit box touchpad; a corresponding colored button tab. In this sample screen, the Unit Name, *BioFlo 310*, is also written on the menu line button tab. You may wish to simply name the units by their designation in the **Hardware Setup** screen (*see Section 15.4*): Unit1, Unit2, etc.

Controller *Operating Mode* settings (in the sample screen above 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 Sections 15.1.1 - 15.1.4 for details on gas control, through the **Controller Setup** screen and the gas process loop gauge screens.

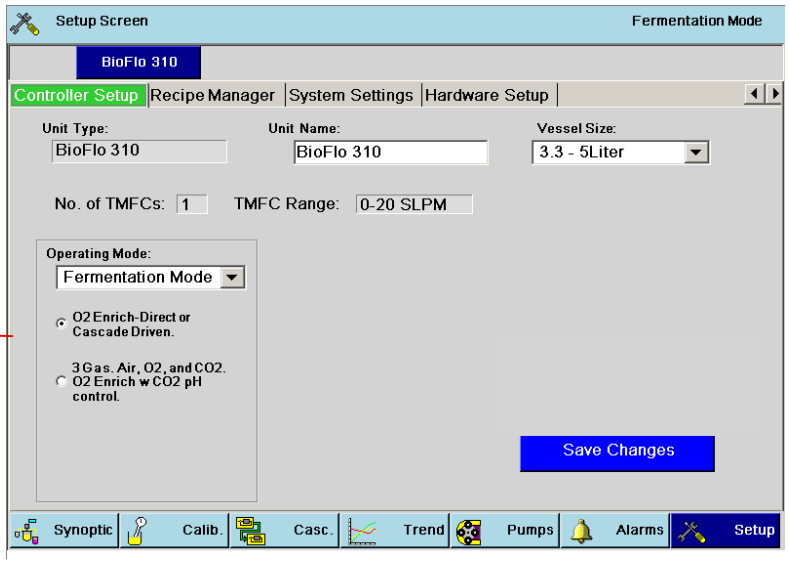
If you run the unit with various vessel sizes, use the **Vessel Size** dropdown menus to change to the new vessel size, then press the **Save Changes** button to allow the system to reset to new parameters.

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.

15.1.1 Gas control with 1 or no TMFC

If your system is equipped with no TMFC or one TMFC, you have the choice of two *Operating Modes* in the **Controller Setup** screen: O2 Enrich and 3-Gas in Fermentation mode. Your system has 4 gas solenoid valves. No TMFC means that all gas flow is manually controlled using a Rotameter.

Figure 71: Controller Setup Screen (0 - 1 TMFC)



1	Select **O2 Enrich** for Air and O2. Select **3 Gas** for Air, O_2 & CO_2.

If you select O2 Enrich as the *Operating Mode*, the gas process loops you will find in the **SUMMARY** screen are labeled Air (1) and O2 (2). If you select 3Gas, the process loops are labeled Air (1), O2 (2) and CO2 (4). The loops' numbers, 1, 2 & 4, correspond to the gas connections on the cabinet.

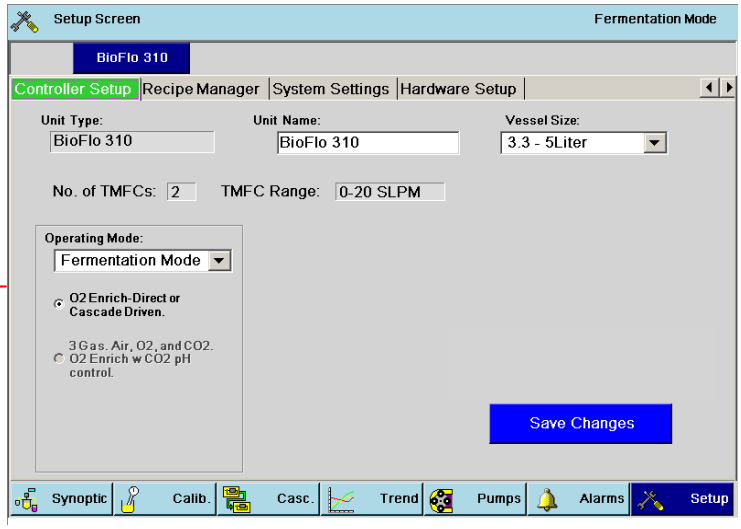
There is also a GasFlo loop when one TMFC is present; settings in this loop's gauge screen turn the TMFC on and off and control the gas flow rate.

15.1.2 Gas control with 2 TMFCS

If your system is equipped with two TMFCs, the only *Operating Mode* available in the **Controller Setup** screen is O2 Enrich, because only two gases can be brought into the unit (*see the following page*).

The gas process loops you will find in the **SUMMARY** screen are labeled AirFlo (1) and O2Flo (2). The loops' numbers, 1 - 2, correspond to the gas connections on the cabinet.

Figure 72: Controller Setup Screen (2 TMFCs)

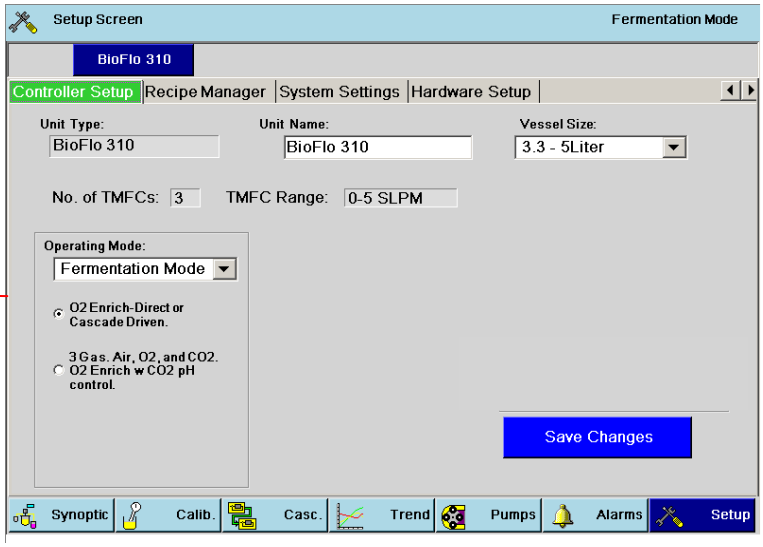


1	With 2 TMFCs, you can only select **O2 Enrich** as the Operating Mode. 3Gas mode is greyed out (inaccessible).

15.1.3 Gas control with 3 TMFCS

If your system is equipped with three TMFCs, you can select either O2 Enrich or 3Gas as the *Operating Mode*:

Figure 73: Controller Setup Screen (3 TMFCs)



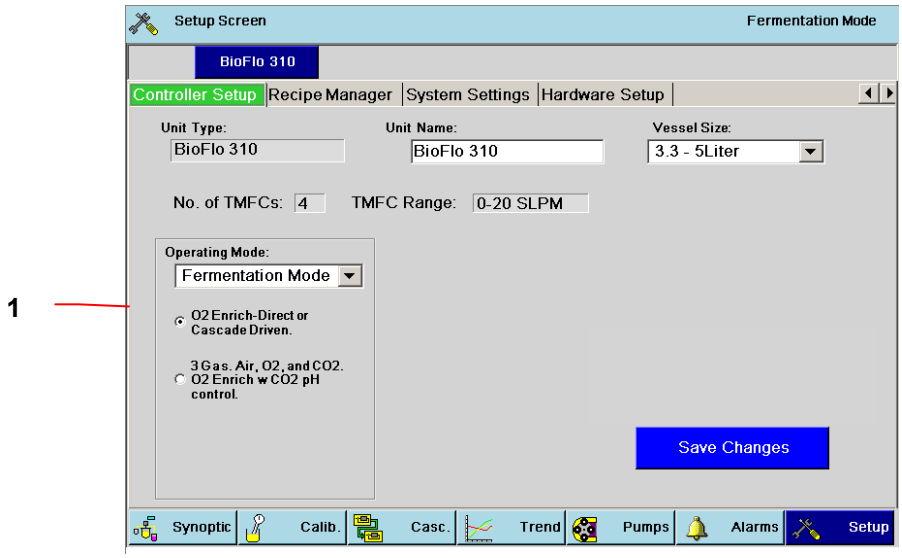
1	With 3 TMFCs, you can select either **O2 Enrich** or **3Gas** as the Operating Mode.

The gas process loops you will find in the **SUMMARY** screen are labeled AirFlo (1), O2Flo (2), and CO2Flo (3). The loops' numbers, 1 - 3, correspond to the gas connections on the cabinet.

15.1.4 Gas control with 4 TMFCS

If your system is equipped with four TMFCs, you can select either O2 Enrich or 3Gas as the *Operating Mode* for Fermentation:

Figure 74: Controller Setup Screen (4 TMFCs)



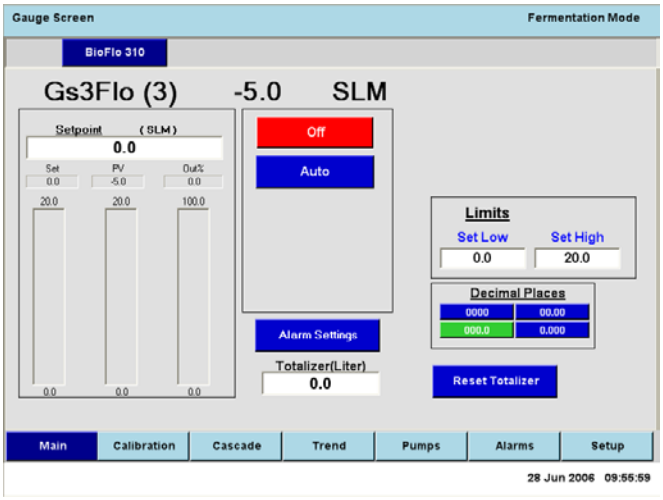
1	With 4 TMFCs, you can select either **O2 Enrich** or **3Gas** as the Operating Mode.

The gas process loops you will find in the **SUMMARY** screen are labeled AirFlo (1), O2Flo (2), and CO2Flo (4). There is also a third loop called Gs3Flo (3), for the addition of a gas of your choosing. The loops' numbers, 1 - 4, correspond to the gas connections on the cabinet.

Any loop name ending in Flo represents a certain gas (e.g., AirFlo, O2Flo, etc.) with a dedicated TMFC. If the gas loop name does not end with "Flo", it represents the presence of a gas solenoid valve rather than a TMFC.

The Gs3Flo (3) gauge screen (*see the following page*) allows you to set parameters for the TMFC that controls this gas.

Figure 75: Gs3Flo (3) Gauge Screen



15.2 Recipe manager

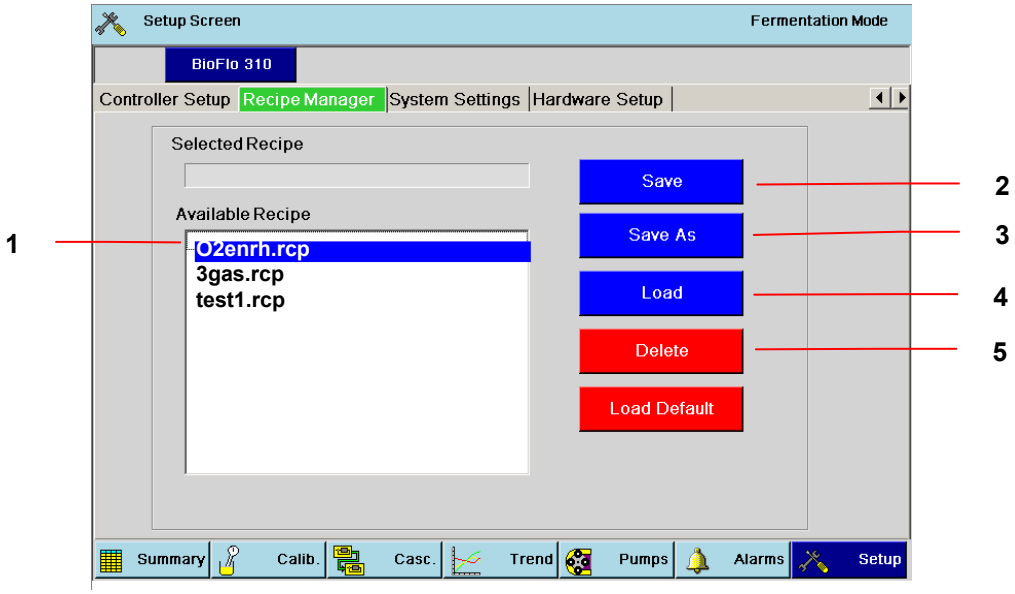
Press the second tab in the **SETUP** screen to open the **Recipe Manager** screen (*see the following page*). Use this feature to access, rename, save, load and delete recipe files for your fermentation runs.

Recipes consist of all user-definable variables available on the **CONTROL** screens. When a recipe is saved, all the current settings on the controller (including but not limited to setpoints, control modeas, alarms, P&I values, and cascades) are saved to the controller's memory.

You can save this data with a unique name using the **Save As** button, or overwrite an existing recipe using the **Save** button.

Each controller is capable of storing up to 10 recipes. You can retrieve these recipes by opening the **Recipe Manager** screen, where all saved recipes are listed in the Available Recipes pane. Select the desired recipe by following Step 1, as explained on the following page, then load it as shown in Step 3.

Figure 76: Recipe Manager Screen

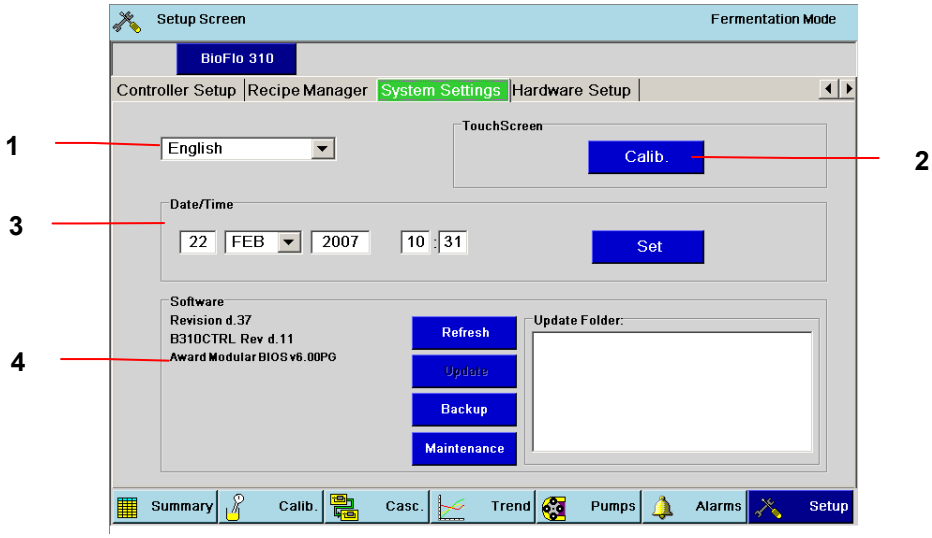


1	Step 1: Press the recipe file of choice from the list in this box. Its name will appear in the Selected Recipe box, as shown.
2	Step 2: Press the **Save** button to save the recipe as is, or…
3	…if you wish to rename the file, press the **Save As** button and use the pop-up touchpad to designate a new name.
4	Step 3: Press the **Load** button to load the Selected Recipe file.
5	To delete a recipe from the system, select is (see Step 1), then press the **Delete** button.

15.3 System settings

Press the third tab in the **SETUP** screen to open the **System Settings** screen (*see the following page*). Use this feature select the onscreen language you prefer, to reset the date and/or time, to update the software, and to calibrate the BioFlo 310 touchscreen.

Figure 77: System Settings Screen



1	Other languages are not available at this time.
2	To recalibrate the system's touchscreen, press the **Calib.** button, then touch the onscreen target each time it appears. You will be guided through the process.
3	To change the Date and/or Time, see Section 15.3.1.
4	Listed here are the current User Interface and Control Program versions. To update the software, see Section 15.3.2.

15.3.1 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.

15.3.2 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:

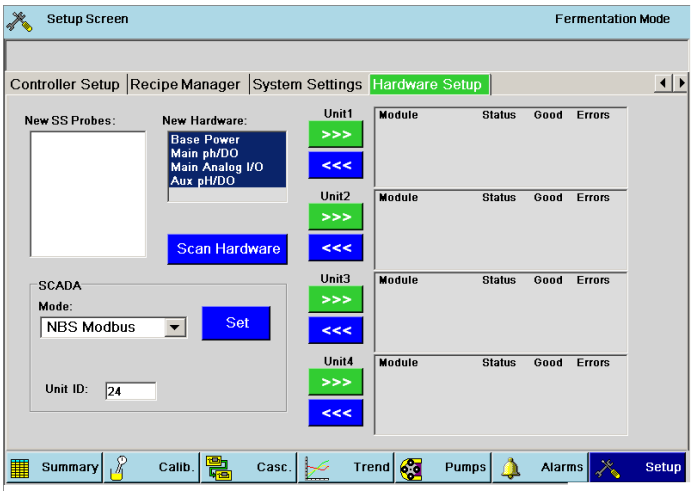
1. In the **System Settings** screen, press the **Refresh** button to update the current software status and to search for a new USB drive.
2. The name of the new drive folder appears in the **Update File** box.
3. Press the **Update** button to install the file. The file will reboot twice; this may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

Updating software will not affect any previous user settings.

15.4 Hardware setup

The BioFlo 310 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, the system has the Base Power module, the Main Analog module and the Auxiliary pH/DO module. This system is also set to AFS communication mode (*see the SCADA pane in Figure 58*), 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 78: Hardware Setup Screen

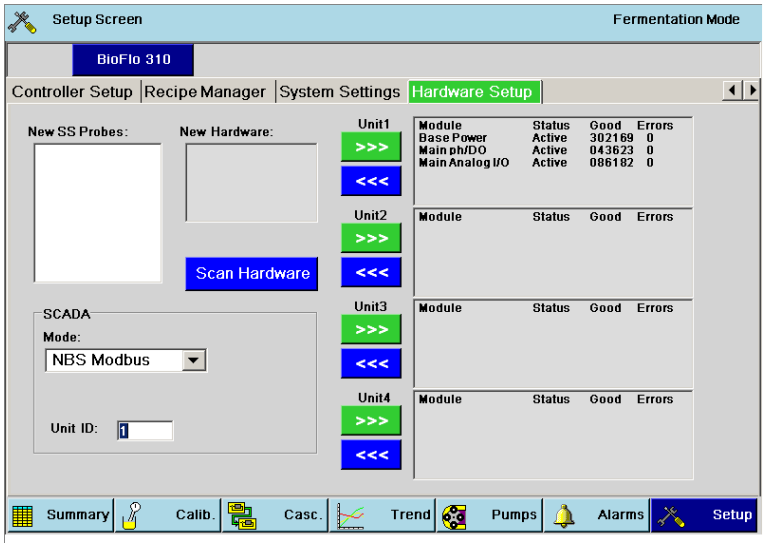


To add new hardware (including a new utility station), after connecting the module(s) to the system:

1. Press the **Scan Hardware** button in this screen. All new hardware scanned will appear in the **New Hardware** box (*see the following page*).

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.
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 (*see "BioFlo 310" in the sample screen below*).
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 79: Adding New Hardware



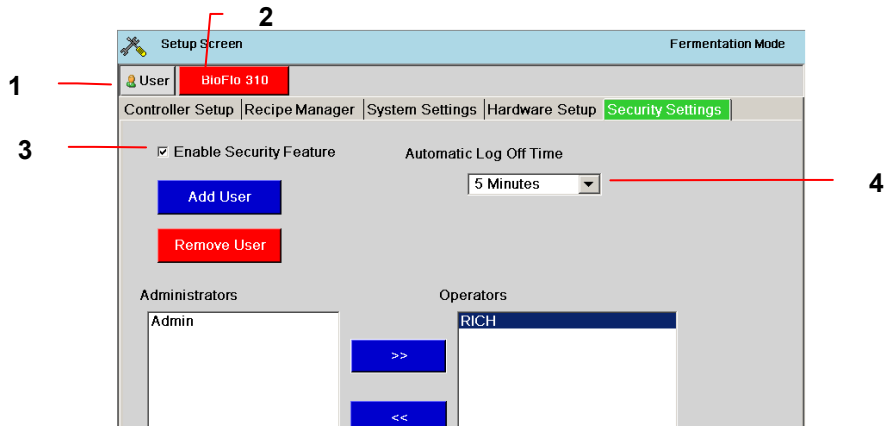
15.5 Security settings

The security feature on the 310 provides two user access levels:

- **Operators** have access to routine operations but they cannot change security settings.
- **Administrators** have access to all operations including defining new users (operators and administrators) and setting security parameters.

Press the fifth tab in the **SETUP** screen to open the **Security Settings** screen:

Figure 80: Security Settings Screen

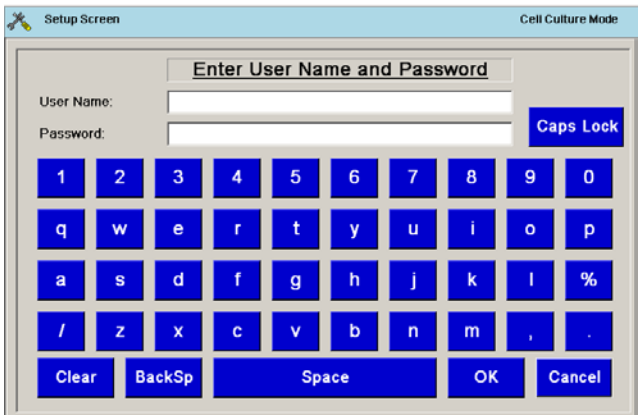


1	When security is enabled, the **User** button appears in this corner of all main screens.
2	This block shows the name of your bioreactor: BioFlo 310.
3	Selecting "Enable Security Feature " (☑) turns security on; deselecting it (☐) turns security off. Only a user in the Administrator group has access to this feature.
4	Use the dropdown menu to define the time before the system automatically logs off, leaving only the SUMMARY, SYNOPTIC and TREND screens available.

In this screen, a user with Administrator status can move users from the Operators group to the Administrators group (or vice versa) by highlight the user name in the Administrators or Operators pane, then pressing the **>>** or **<<** button to move that user from one pane to the other.

An Administrator can also add users to or remove users from the system using the **Add User** and **Remove User** buttons. To remove a user, press the user name to select it in the pane where it appears, then press the **Remove User** button. To add a user, press the **Add User** button, use the keypad screen that opens to type in the user name (and assign a Password if desired), then press the **OK** button.

Figure 81: Security Keypad

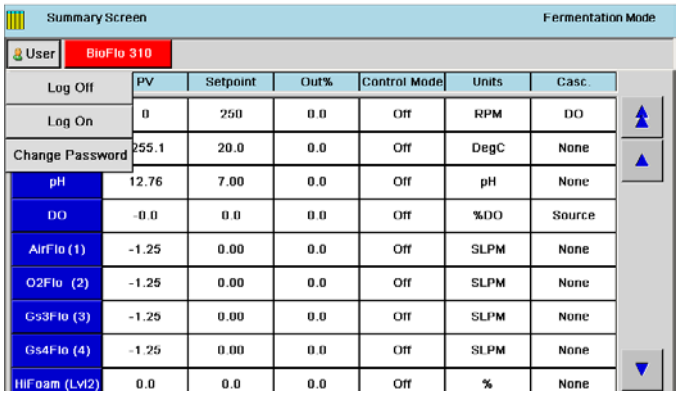


As shown in the Security Settings Screen on the previous page and in the Summary Screen below, when security is enabled, the **User** button appears in the top left corner of all major screens.

When a user presses the **User** button, that user can use the popup buttons (as shown here) to **Log Off** or to **Log On** by pressing the appropriate button.

If the user has Administrator status, the **Change Password** button will be available. Pressing it will open the security keypad to change his/her password. ADMIN is the default password.

Figure 82: User Button



When Security is enabled, only the Synoptic/Summary **and** Trend **navigation buttons remain active at the bottom of the screen; the other navigation buttons will be greyed out and inaccessible.**

16 PERFORMING A RUN

16.1 Set up foam control

Before you fill the vessel with medium, confirm that the foam sensor 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 sensor in its headplate port.
6. Connect the foam sensor cable to Lvl 1 on the control cabinet, then attach the cable to the foam sensor. Attach the earth/ground.
7. Open the **PUMPS** 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 sensor and antifoam addition system, are properly inserted and secure.
13. Make sure the DO sensor and the pH sensor are capped.
14. Ensure that the temperature sensor 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 sensors 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.

16.2 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 vessel jacket and exhaust condenser.

ALERT!

Before connecting or disconnecting the water hoses to/from the vessel at any time, be sure to follow these instructions in the order indicated:

To connect: **(1) Connect vessel Water Out line,**
(2) Connect vessel Water In line,
(3) Connect cabinet Main Water In line,
(4) Turn ON mains/power switch on cabinet.

To disconnect: **(1) Turn OFF mains/power switch on cabinet,**
(2) Disconnect Main Water In line from cabinet,
(3) Disconnect vessel Water In line,
(4) Disconnect vessel Water Out line.

Failure to heed these instructions may lead to hose leakage and/or pressure build-up inside the vessel jacket.

4. Add glycerin to the thermowell and insert the temperature sensor.
5. Make sure the motor is not connected. Turn the mains/power **ON**.
6. Set the **TEMP** loop sepoint below the ambient temperature and select **Auto** as the **TEMP** control mode. This will prime the water jacket.
7. Set the **TEMP** setpoint to the desired working temperature.
8. 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.
9. Remove the shorting cap from the pH sensor. Connect the pH cable to the pH sensor.
10. Remove the protective cap from the DO sensor and connect the DO cable to the DO sensor.

The DO polarographic sensor 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 sensor (*see Section* ***Error! Reference source not found.***).
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 **PUMPS** 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. Open the **CASCADE** screen and select the **pH** loop.
19. Enable the pumps.
20. Return to the **CASCADE** screen and select the **DO** loop.
21. Set up cascades for **Agit**, **GasFlow** (**if** your unit has automatic thermal mass flow control) and **O2**. You can also cascade a pump for glucose addition.
22. Enable the cascades.
23. Set the **GasFlow** loop's control mode to **ON**.

Aeration is required whenever the agitation setpoint is greater than 750 rpm. Eppendorf suggests a minimum airflow rate of 0.25 VVM when running at speeds ≥750 rpm.

16.3 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”.

16.4 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 310 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.

16.5 Sampling procedure

Referring to Sampling System I or Sampling System II, 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.

16.6 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.

16.6.1 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.

16.6.2 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.

16.6.3 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.

16.6.4 Decline phase

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

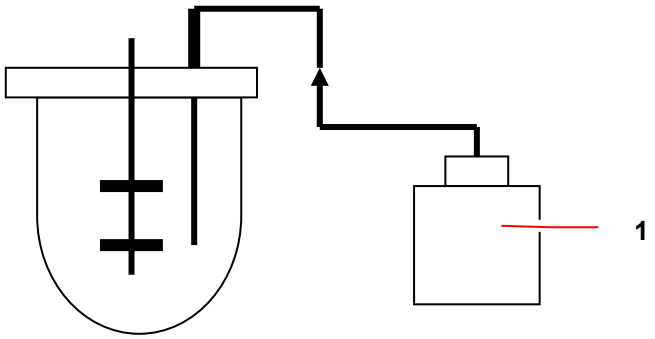
16.7 Batch operation

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.

16.8 Fed batch operation

A fed batch operation (*see 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.

Figure 83: Fed Batch Operation

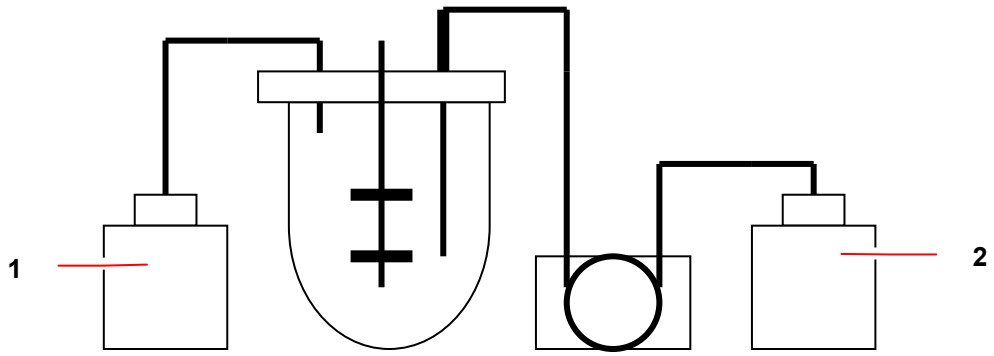


1	Fresh media

16.9 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.

Figure 84: Continuous Operation



1	Fresh media	2	Harvested broth

16.10 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.

16.11 Harvesting procedure

When the vessel is set up on the control cabinet, adjust the level sensor'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** or **Lvl3 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 sensor, the circuit will close, and the pump will begin pumping liquid out of the vessel.
4. When the liquid drops below the sensor tip, the pump will stop.

See also Section 10.5, *Pump Assignment*. If you assign the pump to **None** instead of **Lvl2 Wet** or **Lvl3 Wet**, it will harvest as much as possible.

16.12 Shutdown procedure

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

1. Set **GasFlow** to **OFF**.
2. Set **Agit** and **Temp** to **OFF**.
3. Set all other control loops to **OFF**.
4. Turn off the mains/power.
5. If the system is not to be used for several days, disconnect the mains/power plug.
6. Remove, drain and clean the vessel *as outlined in Section 18*.

See also Section 25.7.5 for shutdown and cleaning tips.

Never wash the filters or get them wet.

17 ESSENTIAL OPERATING TIPS

17.1 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.

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

17.2 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.

17.3 Install a double filter system

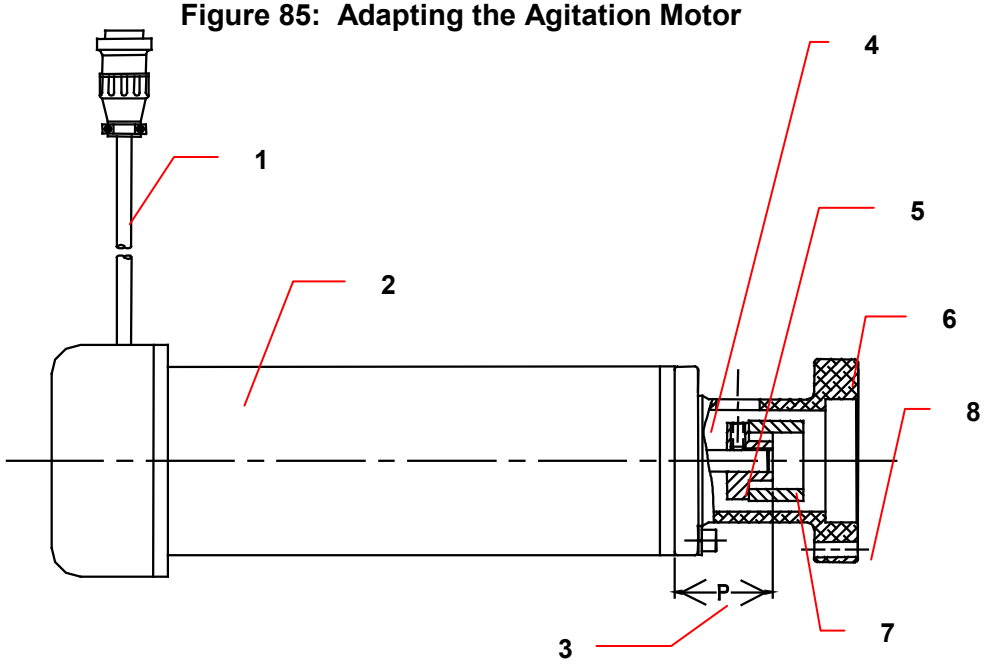
Double exhaust and double inlet filters are recommended. To install them:

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.

17.4 Adapting the motor to BioFlo® 3000 vessels

Figure 85: Adapting the Agitation Motor



1	Motor Cable	4	Four Screws	7	Sleeve
2	Motor	5	Half Coupling	8	NOTE: Mount spacer with this hole *away from* motor cable.
3	Dimension P	6	Spacer Housing		

To use BioFlo 3000 vessels with your BioFlo 310, you will need the following spacer housing(s):

- 1L and/or 3L Vessel: M1226-9402 spacer housing
- 5L Vesse Use the BioFlo 310 spacer housing
- 10 L Vessel M1226-9458 spacer housing

All BioFlo 310 vessels use the same spacer housing.

18 CLEANING

ALERT!

To avoid leaks and/or pressure build-up inside the vessel jacket, see *ALERT* regarding connecting & disconnecting hoses in Section 4.8.2.

ALERT!

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

18.1 Cleaning the vessel

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.

18.1.1 List of wetted parts

For further reference in your choice of cleaning detergents, Table 8 provides a list of wetted parts in the vessel assembly and the materials they are made of:

Table 8: Wetted Parts

Wetted Parts	Material
Purple headplate O-ring	EPDM
O-ring lubricant	Silicone
Headplate penetration O-rings	EPDM
Metal surfaces	316L or 316 stainless steel
Vessel glass	Borosilicate glass
Inoculation septum	Pure gum rubber, color tan

18.2 Cleaning the cabinet

At least once a month, clean all the metal 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.

19 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.

CAUTION! High voltage!

Always turn your BioFlo 310 off and disconnect the mains/power cord before performing maintenance.

19.1 pH sensor maintenance and storage

The pH sensor 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.

ALERT!

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

19.2 DO sensor maintenance and storage

Use soft facial tissue to clean the DO sensor.

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

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

ALERT!

Never let a DO sensor rest on its tip.

19.3 Vessel & tubing

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

19.4 Periodic inspection

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

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

1. Check all controls and accessible items (mains/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.

19.5 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.

19.5.1 Motor assembly replacement

CAUTION!* *High voltage!
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 Eppendorf service technician.

19.6 Fuse replacement

There is one replaceable 5-Amp fast-acting glass tube fuse.

19.7 Replacement parts

The following list of replacement parts is provided for your convenience. Using the part number will facilitate processing of your order by your local Eppendorf distributor.

This list also includes some parts for cell culture use.

Part Description	Part Number
Fermentation Glass Vessel (1 L)	M1287-9930
Fermentation Glass Vessel (3 L)	M1287-9931
Fermentation Glass Vessel (5 L)	M1287-9932
Fermentation Glass Vessel (10 L)	M1287-9933
Cell Culture Glass Vessel (1 L)	M1287-9920
Cell Culture Glass Vessel (3 L)	M1287-9921
Cell Culture Glass Vessel (5 L)	M1287-9922
Cell Culture Glass Vessel (10 L)	M1287-9923
Inlet Filter (1 L, 3 L, 5 L Vessels) (10 L CelliGen Only)	P0200-0491
Exhaust Filter (1 L, 3 L, 5 L Vessels) (10 L CelliGen Only)	P0200-0495
Inlet Filter (10 L) Fermentation only	P0200-0495
Exhaust Filter (10 L) Fermentation only	P0200-4130
RTD Assembly (All Sizes)	M1294-8013
pH Cable (All Sizes)	P0720-2273
pH Autoclave Cap	P0720-5317
pH Sensor (1 L) * Not used with Basket Impeller	P0720-5582
pH Sensor (3 L) *Not used with Basket Impeller	P0720-5584
pH Sensor (5 L) *Not used with Basket Impeller	P0720-5584
pH Sensor (10 L) *Not used with Basket impeller	P0720-5580
DO Sensor (1 L) * Not used with Basket Impeller	P0720-6282
DO Sensor (3 L) *Not used with Basket Impeller	P0720-6282
DO Sensor (5 L) *Not used with Basket Impeller	P0720-6283
DO Sensor (10 L) *Not used with Basket impeller	P0720-6283
pH Sensor (1 L, 3 L, 5 L) **Used with Basket Impeller	P0720-5582
pH Sensor (10 L) **Used with Basket impeller	P0720-5584
DO Sensor (1 L, 3 L) **Used with Basket Impeller	P0720-6280
DO Sensor (5 L, 10 L) **Used with Basket Impeller	P0720-6282
DO Sensor Compression fitting	M1287-5030
DO Cable (All Sizes)	P0720-2333

…continued…

Part Description	Part Number
Replacement DO Membrane Kit (4 Membranes)	P0720-6268
Replacement DO Membrane Kit (1 Membrane)	P0720-6339
Teflon Washer for DO Sensor	P0100-9780
O-Ring for DO Sensor	P0280-6702
Foam/Level Sensor (All Sizes)	F5-137C
O-Ring for 6.35 mm Port	P0280-5882
O-Ring for PG 13.5 Port	P0280-5912
O-Ring for 19 mm Port	P0280-5952
6.35 mm Port Plug	M1294-9534
PG 13.5 Port Plug	M1294-9540
19 mm Port Plug	M1294-9536
Replacement Septum Kit (Includes Headplate Fitting)	M1287-5031
Replacement Rubber Septum	P0280-2690
Tri-Port adapter	M1287-9212
Single Addition Adapter/Tube	M1287-5043
Compression Fitting for ¼-in Tube	M1287-5033
1 L (Ferm) 1/4" (0.635 cm) Sample Tube 9.9" (25.1 cm)	M1287-9486
1 L (Ferm) 1/4" (0.635 cm) Harvest Tube 11.6" (29.6 cm)	M1287-9482
3 L (Ferm) 1/4" (0.635 cm) Sample Tube 12.4" (31.4 cm)	M1287-9487
3 L (Ferm) 1/4" (0.635 cm) Harvest Tube 14.1" (35.9 cm)	M1287-9483
5 L (Ferm) 1/4" (0.635 cm) Sample Tube 13.1" (33.3 cm)	M2187-9488
5 L (Ferm) 1/4" (0.635 cm) Harvest Tube 15.3" (38.8 cm)	M1287-9484
10 L (Ferm) 1/4" (0.635 cm) Sample Tube 16.5" (41.9 cm)	M1287-9489
10 L (Ferm) 1/4" (0.635 cm) Harvest Tube 19.0" (48.3 cm)	M1287-9485
Water-Out Vessel Quick Connect (Cell Culture Vessels)	P0240-0943C3
Water-In Vessel Quick Connect (Cell Culture Vessels)	P0240-0940C3
Water-Out Vessel Quick Connect (Fermentation Vessels)	P0240-0940C3
Water-In Vessel Quick Connect (Fermentation Vessels)	P0240-0943C3
Heater, 750 W, (100 - 240 V)	P0620-1320
Fuse, 5 Amp (Motor Drive)	P0380-3451
Bearing Housing Autoclave Cap (10 pack)	M1273-9936
Fermentation Motor Assembly (1 L, 3 L, 5 L, 10 L)	M1287-0800
Cell Culture Motor Assembly (1 L, 3 L, 5 L, 10 L)	M1287-0750
Ball Bearing (1 L, 3 L, 5 L, 10 L)	P0180-0451C3
Shaft Seal (1 L, 3 L, 5 L)	P0280-0072
Shaft Seal (10 L)	P0280-0420
1/4" (0.635 cm) Polysulfone Quick Connect (Female)	P0240-2680
1/4" (0.635 cm) Polysulfone Quick Connect (Male)	P0240-2670

…continued…

Part Description	Part Number
250 mL Addition Bottle	M1273-9989
500 mL Addition Bottle	M1273-9990
3/16" ID Silicone Tubing (25' Length)	M0740-2505
1/8" ID Silicone Tubing (25' Length)	M0740-2445
Replacement Headplate Thumb Screws	M1287-9466
Silicone Grease (FDA Approved)	P0860-1050

20 SERVICE

If any problems occur with your BioFlo 310 system or its individual components, do not attempt to perform any service on it. Unauthorized servicing may void the warranty. Please contact your local Eppendorf Service Department or your local distributor.

In any correspondence with Eppendorf, please refer to the Model Number (BioFlo 310), and the Manufacturing Part Number and Serial Number of the unit.

20.1 Troubleshooting

CAUTION! High voltage!

Always turn your BioFlo 310 off and disconnect the mains/power cord before performing maintenance.

As with any equipment, difficulties sometimes arise. If you experience a problem with the operation of your BioFlo 310, 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 Eppendorf representative to request a service technician. **Other than the solutions proposed below, do not attempt to fix the equipment yourself.**

Problem	Possible Solution
TEMPERATURE:	
Readout is a negative value (typically –225° C).	• Inspect the temperature sensor for obvious damage; replace it if necessary. • Make sure the temperature sensor 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 sensor 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.
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...

Problem	Possible Solution
AGITATION:	
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. • Make sure the motor is plugged into the cabinet receptacle; TURN OFF MAINS/POWER BEFORE CONNECTING THE PLUG.
DO and pH SENSORS:	
DO sensor readings are erratic.	• Recalibrate the sensor, carefully following instructions in this manual. • Recharge the sensor, carefully following instructions in this manual. • Sensor may need a new membrane and a refill of electrolyte. • Check for a secure connection. • Replace sensor cable or DO sensor.
pH sensor readings are erratic.	• Recalibrate the sensor, carefully following instructions in this manual. • Check for a secure connection. • Gel-filled sensor may need replacement. • Liquid-filled sensor may need a refill of electrolyte. • sensor cable may need replacement.
Sensor does not hold calibration.	• Sensor may be defective; replace it. • pH/DO amplifier on superboard may be defective; call for service.
GASFLOW:	
There is insufficient gas flow.	• Inlet 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 GasFlow 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.

21 DRAWINGS

Figure 86: Control Schematics, BioFlo 310 Controller

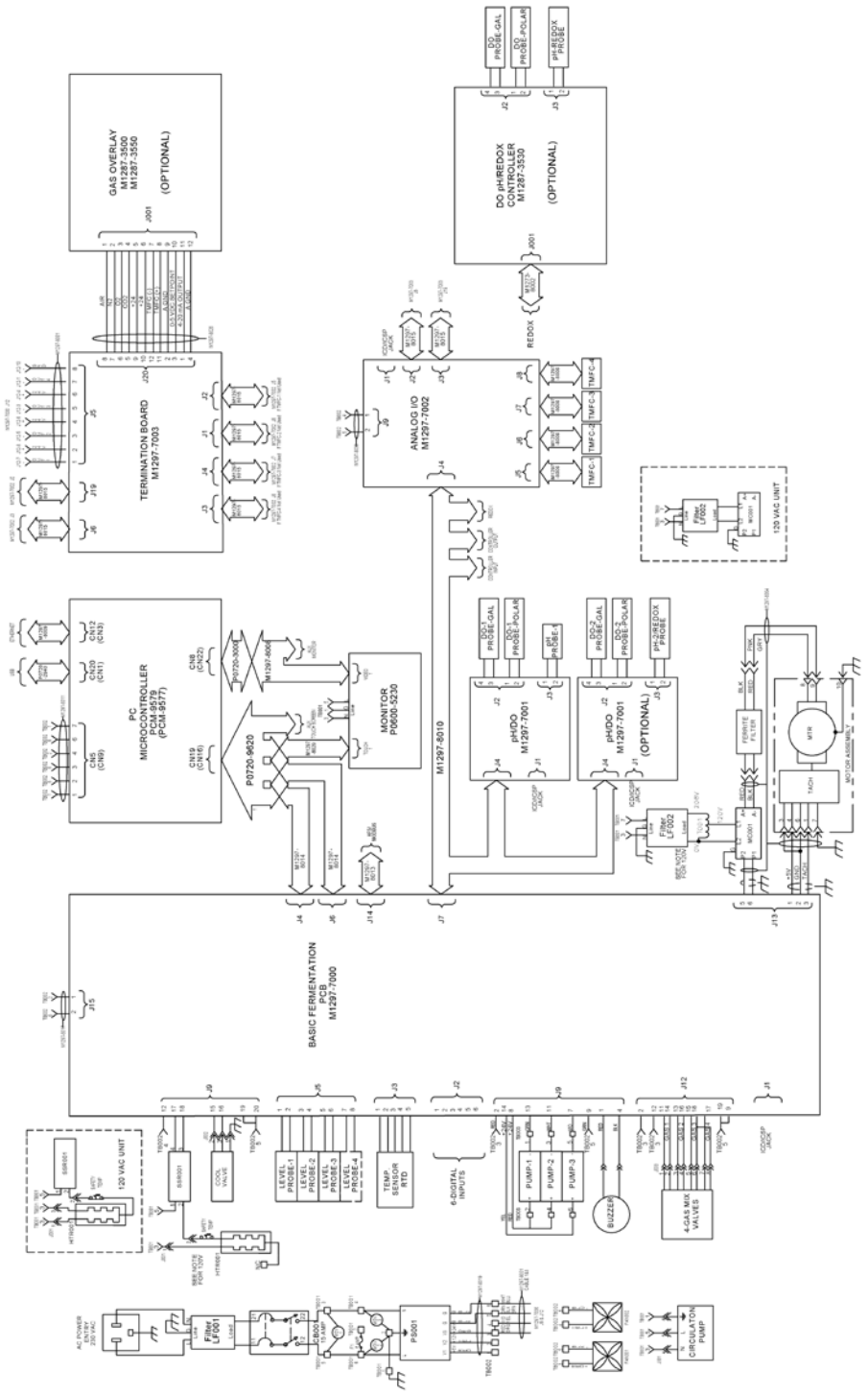


Figure 87: Control Schematics, BioFlo 310 Controller with Single-Use Vessel

The use of an optional New Brunswick CelliGen BLU single-use vessel with the BioFlo 310 controller requires removal of the water connectors and their replacement a heat blanket connector. This schematic shows that change:

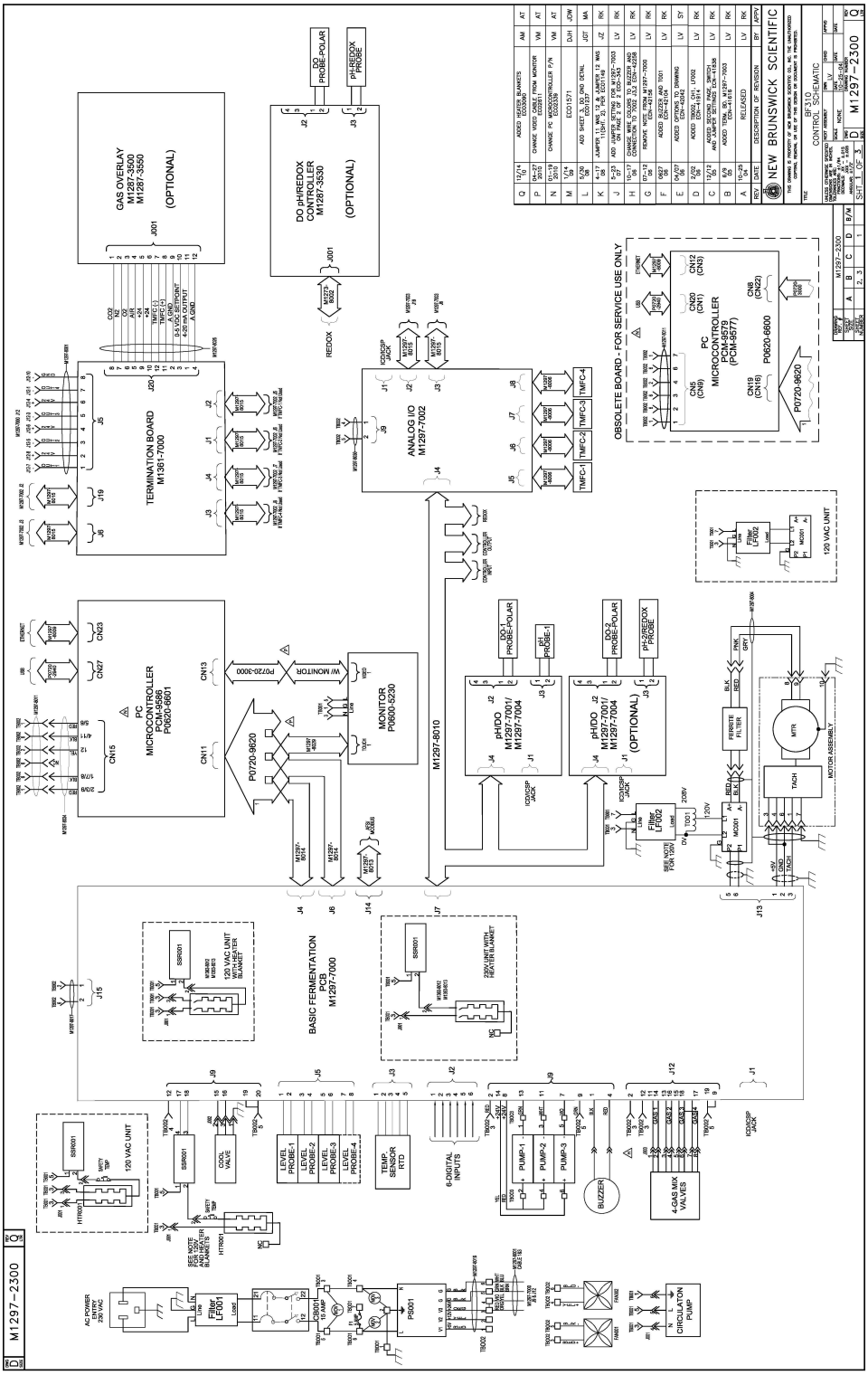
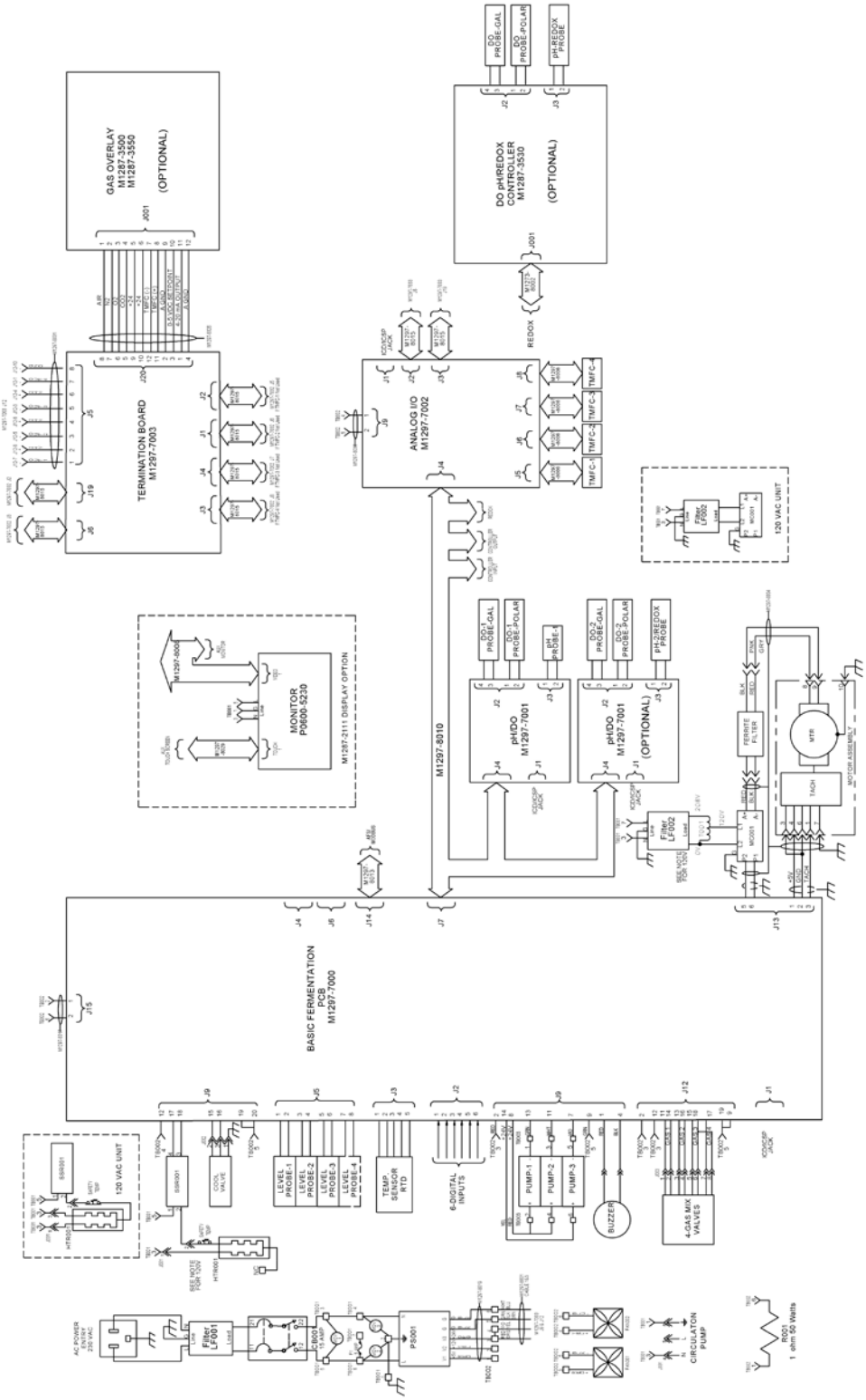


Figure 88: Control Schematics, BioFlo 310 Utility Station



21.1 List of drawings

21.2 List of tables

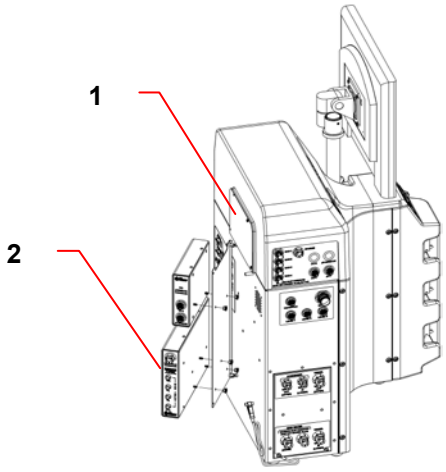
22 APPENDIX A: MICROBIAL TO CELL CULTURE CONVERSION KIT

The Microbial to Cell Culture/Gas Overlay Conversion Kit (part number M1287-3501) makes it possible for the BioFlo 310 fermentation control cabinet to run cell culture. Because this kit was developed from the cell culture gas overlay kit, it can still be used as a fully functioning gas overlay kit for cell culture.

To install the conversion kit:

1. Mount the gas overlay conversion controller box to the rear panel of the BioFlo 310, using the thumbnuts and the standoffs (if needed) that are provided:

Figure 89: Mounting Microbial to Cell Culture Conversion Controller Box

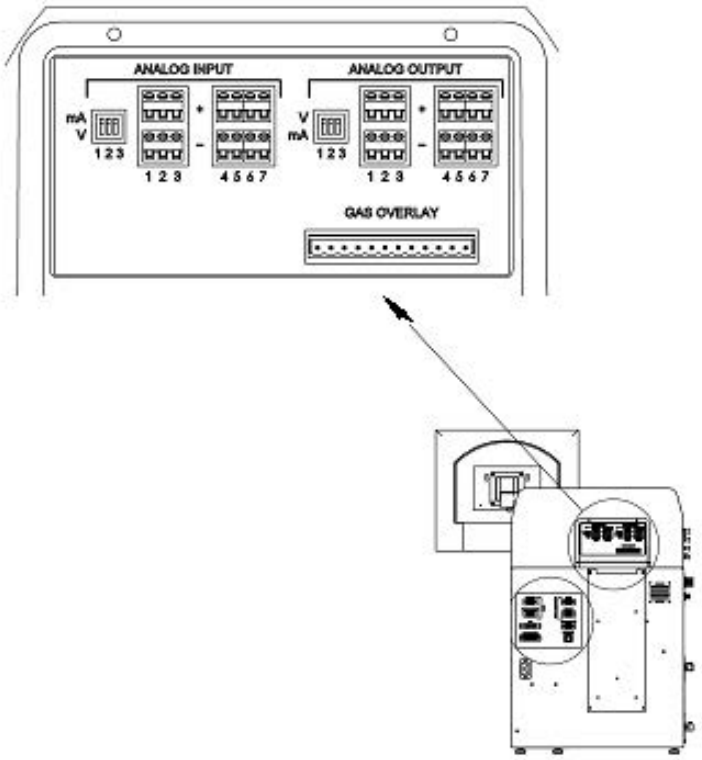


1	Stainless Steel Cover Plate
2	Mount conversion controller box here

.

2. Remove the stainless steel plate (*see the drawing above*) that covers the connectors, and connect the supplied cable from the conversion controller box to the **Gas Overlay** connector (*see the following page*):

Figure 90: Gas Overlay Connector



3. Connect the gases to the gas overlay conversion box's gas inputs.

22.1 For gas overlay only

1. Keep the tubing connected between **4GAS MIX** and **TMFC** on the conversion box.
2. Use the sparge silicone tubing (provided) to connect from **Overlay/Sparge** to the vessel's overlay gas port.

22.2 For conversion from fermentation to cell culture

To use the conversion controller box to run cell culture on your BioFlo 310 fermentor:

1. Remove the tubing that connects **4GASMIX** and **TMFC** on the conversion box.
2. Connect tubing from the control cabinet's **Sparge** connector to **TMFC** on the conversion box.
3. Connect **Sparge/Overlay** to the vessel's sparger tube. This will lower your sparge gas flow range to 0.1 to 5 slpm from the original built-in TMFC's gas flow range of 0.4 to 20 slpm.

4. Using the ¼-inch tubing adaptor provided, connect 4GASMIX to the bottom of your external Rotameter. Connect the top of the Rotameter to the vessel for overlay gas. The gas overlay is now manually controlled via the Rotameter and the conversion box's 4-gas manifold.
5. On the touchscreen display, choose the Setup Screen; make sure the control mode is set to **Cell Culture** and make sure the gas overlay is selected with the automatic gas flow control. In the Setup Screen, press the **Save Changes** button to generate the **OvlFlo** and **OvlMix** loops.
6. From the Gauge Screen, set the **GasFlo** loop setpoint to 20 slpm and set the control mode to **AUTO**.
7. From the Gauge Screen, set the **OvlFlo** loop setpoint to your preferred sparger gas flow rate, as it is now the sparger flow rate controller.
8. **OvlMix** still controls the mixing of the overlay gas. The overlay gas flow rate is manually controlled via the Rotameter; adjust the Rotameter to get the preferred overlay gas flow rate.

23 APPENDIX B: SOME GENERAL CONCEPTS

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).

23.1 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.

23.2 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.

23.3 What is sensor calibration?

In bioprocess control, *calibration* generally refers to establishing a correspondence between a sensor's output and the actual value of whatever that sensor senses. For example, pH sensors 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 sensor calibration, two values—a zero and a span—are required for the controller to correctly translate inputs from that sensor. DO and pH sensors are routinely calibrated before each use. Most other sensors need be calibrated only infrequently.

23.4 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 sensors, 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.

23.5 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 a New Brunswick system, **P-I-D** loops will have been tuned at the factory to work correctly with the New Brunswick-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 textbooks[1] 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.

[1] For example, Chinks, F.G., Process Control Systems: Application, Design, and Tuning, McGraw-Hill (1988), New York, Auckland, Bogota, London, Toronto, Sydney, Tokyo, Montreal.

- 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 sensors 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.*

23.6 What do the constants mean?

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

$$\mathbf{S_N = P(e_N/k) + \Sigma(I/60)(e_n/k) + D[(e_N-e_{N-1})/k]}$$

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.:

$$\mathbf{P(e_N/k) = P(125/125) = P}$$

Similarly, with a full-scale error, the **I** term (after 1 minute) and the **D** term will be **I** and **D** respectively.

24 APPENDIX C: OTR

24.1 Determining an oxygen transfer rate

The oxygen transfer rate (OTR) of all New Brunswick 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

24.1.1 Otr calculations

OTR can be estimated by titrating a fixed amount of sodium sulfite, Na_2SO_3, with air, CU+2:

$$2SO_3 + 0_2 \rightarrow 2SO_4$$

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_2SO_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
  ΔT = time taken from DO curve at two points of 50% DO min.

24.2 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.). Eppendorf 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 310 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 310 has a 3.24-inch (± 0.015) diameter. If three impellers are to be used, 3.06" diameter impellers are required. This size impeller is normally used in a 5 L BioFlo 310 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 5^{th} 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:

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

Where:

HP	=	Horsepower
D	=	Impeller diameter in inches
rpm	=	Agitator speed in rpm
4.5×10^{-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

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.

25 APPENDIX D: FERMENTATION TECHNIQUES

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 310, the information is generally applicable for any fermentation.

25.1 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:

Chemical	**g/L**
KH_2PO_4	3.5
K_2HPO_4	5.0
$(NH_4)_2HPO_4$	3.5
$MgSO_4 \cdot 7H_2O$	0.5
Glucose	5.0 (for fed batch) 30.0 (for batch)
Yeast Extract	5.0
Trace Metals	1.0 ml/L
Antifoam	0.5 ml/L

Trace metals formulation:	
$FeCl_3$	1.6
$CoCl_2 \cdot 6H_2O$	0.2
$CuCl_2$	0.1
$ZnCl_2 \cdot 4H_2O$	0.2
$NaMoO_4$	0.2
H_3BO_4	0.05
Hcl	10 ml
H_2O	to 1000 ml

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.

25.2 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 sensor 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.

25.3 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, 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).

Eppendorf 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 (H_2SO_4)) 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 H_2SO_4) 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.

25.4 Acid & base

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

Answer: The acid solution is 2 - 3N H_2SO_4. The base solution is either 5N NaOH or NH_4OH ~ 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 H_2SO_4 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.

25.5 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*, New Brunswick's 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.

25.6 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.

25.7 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:

25.7.1 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 sensors 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 and the cooling jacket water lines to be connected. Also, the baffle must be positioned so that it does not interfere with the insertion of the pH and DO sensors 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 310 vessel, the 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. Eppendorf 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 (cooling jacket water line and 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 sensor 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 sensor must be properly calibrated prior to insertion in the headplate. (Be sure to carefully follow the manufacturer's instructions for sensor calibration, or the instructions in this manual.) It is often necessary to coat the sensor with a very thin layer of glycerin or deionized water in order to avoid jamming or breaking it during insertion. The pH sensor must be inserted carefully, using two hands, with one hand holding the base of the sensor near the port opening.

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

Check the DO sensor to be sure the required amount of electrolyte is present prior to insertion; Eppendorf usually replaces electrolyte for each new run. The DO sensor'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.

25.7.2 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 sensors 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 sensors. Aseptically remove the PBS prior to filling the vessel with the desired media. NEVER PLACE SENSORS IN DISTILLED OR DEIONIZED WATER: THIS WILL CAUSE YOUR SENSOR 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.

25.7.3 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 cooling jacket water lines and 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 sensor 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**. If using an external recirculating chiller, it also must be turned on at this time, and the water level in the chiller should also be checked prior to use; this will prime the jacket. 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 sensors and connect the sensors to the unit. Be careful with the pH sensor: do not twist the sensor into its connection to the unit, as this can compromise sterility. The connection must be screwed onto the sensor. The pH sensor should also be checked to ensure that its rubber stoppers have not been displaced. Note the time that the DO sensor is connected, since the sensor 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 **GasFlow** 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).

25.7.4 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 Eppendorf 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 asceptic 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.

25.7.5 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 sensor 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 sensors from the unit, and put on their protective caps. The DO sensor 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 sensor, so and it may be necessary to wipe the sensor down more vigorously; nevertheless, in no case should the tip be touched. After cleaning the DO sensor, visually inspect the tip for damage. (If it is damaged, replace the sensor.) Store the sensor in a clean area in such a way as to protect the sensitive tip.

Removing the pH sensor is usually not so difficult inserting it because the shaft is wet and should be relatively easy to remove. The danger of sensor breakage 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 sensor should jam, absolutely avoid forcing. It may be necessary to reinsert the sensor 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 sensor 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 sensor available at all times, in case of breakage.)

Once the pH sensor has been removed, it should be immediately washed off with warm water. If biomaterial has accumulated on the sensor, use a sponge (or an equivalent that will

not scratch glass) with gentle pressure to clean the surface. The very tip of the sensor should be handled with extreme care and a Kimwipe should be used to gently dry it off after washing. The sensor 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.

26 APPENDIX E: CORROSION RESISTANCE

Websites such as www.outokumpu.com provide up-to-date information about the 316 type stainless steel used in your BioFlo 310 vessels.

27 APPENDIX F: GENERAL CHARACTERISTICS OF EPR

27.1 Identifying EPR

Common Names	EPR, EPT, EPDM
Trade Names	Resist-O (NordleR) - Compound No. AX-60660
ASTM D-2000Classification	CA
Military (MIL STD 417)	RS
Chemical Definition	Ethylene Propylene

27.2 General characteristics

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

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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eppendorf

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