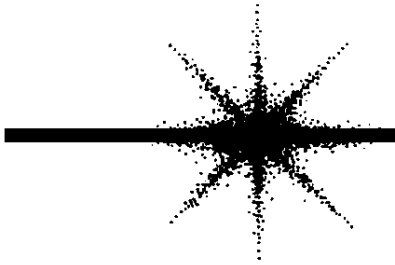

Guide to Operations

BioFlo 6000 Mobile Pilot Plant Fermentor with Automatic Sterilization

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WARNING

The BioFlo 6000 *must* be operated as described in this manual. If guidelines are not followed, equipment damage and personal injury can occur. Please read the entire User's Guide before attempting to use the BioFlo 6000.

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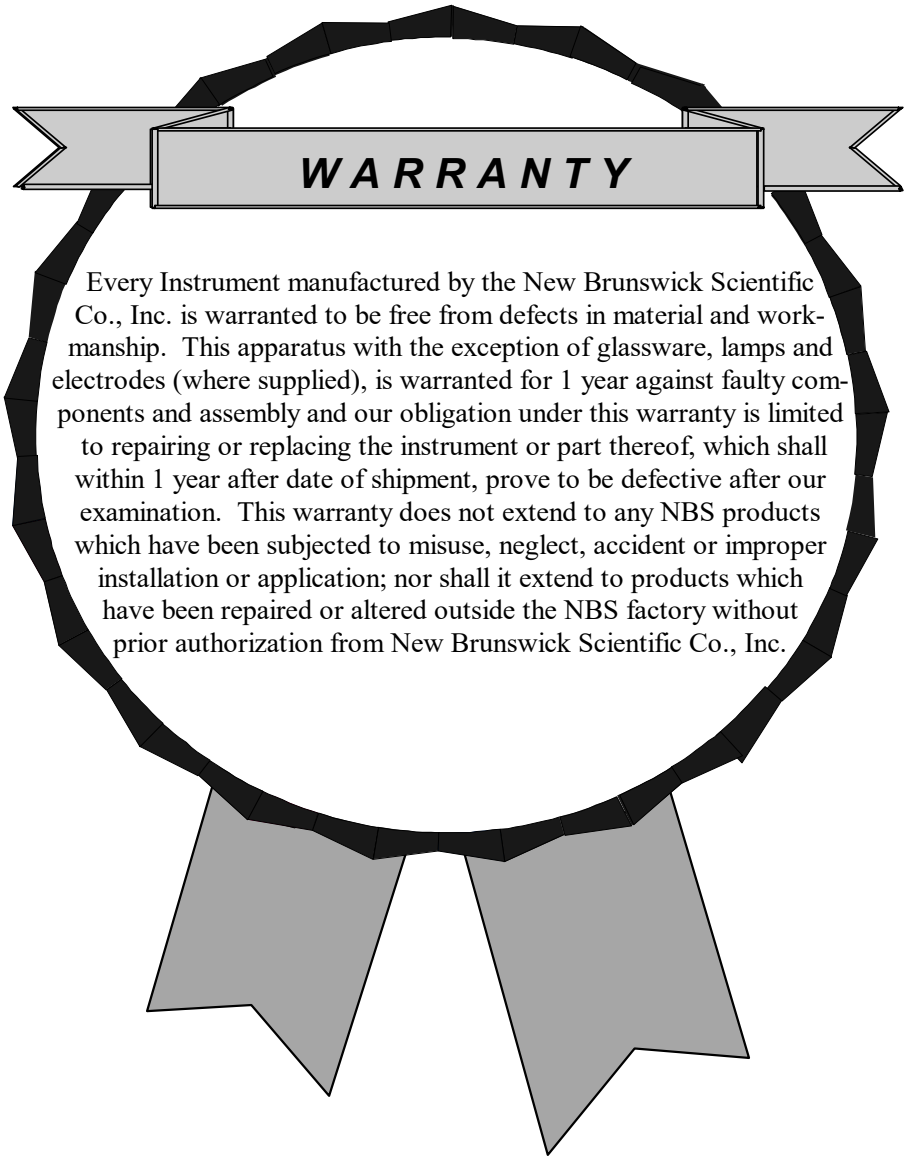
Manual Conventions

NOTE: Notes contain important and useful information separate from text.

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

Warning messages alert you to specific procedures or practices which, if not followed correctly, could result in serious personal injury.

Bold Text in **Bold** face type emphasizes key words or phrases.



WARRANTY

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

Accessories Supplied With This Unit As Checked-Off Below:

- Gas Overlay ☐
- pH Probe System ☐
- Polaraographic D.O. Probe System ☐
- Two Gas Control System ☐
- Four Gas Control System ☐
- Level Control System ☐
- High Level Foam Safety System ☐
- Peristaltic Pump System
  - Pumps (1) ☐
  - Pumps (2) ☐
  - Pumps (3) ☐
  - Pumps (4) ☐
- Addition Vessel System ☐
- Aseptic Transfer System ☐
- Exhaust Gas Analysis (O_2 And Co_2) ☐
- Redox Control (Analysis) System ☐
- Pall Filters (In Place Of Standard Domnick Hunter) ☐
- Absolute Exhaust Filter ☐
- Weight Measurement System ☐
- Marine Propellers ☐
- Pitched Blade Impeller ☐
- Extra Resterilizable Sample Port, 19 mm ☐
- Extra Resterilizable Port, 25 mm ☐
- Exhaust Incinerator ☐
- Transformer Package ☐
- Double Mechanical Seals ☐
- Pressure Alarm On Mechanical Seal ☐
- Turbidity Monitor ☐
- Dissolved Co_2 Monitor ☐
- Nutrient Addition With Pump ☐
- Chilled Water Connections ☐
- Air Prefilter ☐

Accessories Supplied With This Unit As Checked-Off Below:

Ingold DO Kit	☐
Broadley-James DO Kit	☐
pH Probe, Ingold Liquid Kit	☐
pH Probe, Ingold Gel Kit	☐
pH Probe, Broadley James Gel Kit	☐
Septum Kit, 1.5" Tri-clamp	☐
Sterile Quick Connect, 19 mm	☐
Sterile Quick Connect, 25 mm	☐
Water Prefilter	☐
Steam Prefilter	☐

CONTENTS

1. OVERVIEW 13

1.1 Specifications 17

2. BIOFLO 6000 FEATURES 19

2.1 Vessel 19

2.2 Vessel and Jacket Penetrations 20

2.3 Process Piping 22

2.4 Agitation / Drive System 23

2.5 Temperature Control System 23

3. GETTING STARTED 24

3.1 Positioning the Fermentor 24

3.2 Service/Utility Connections 24

3.3 ML-6100 Connections 27

4. INITIAL PREPARATION OF FERMENTOR 28

4.1 Initial Preparation 28

4.2 Drain Valve 29

4.3 Water Priming 30

5. AGITATION CONTROL SYSTEM 32

5.1 Operation 33

6. AIR SYSTEM 36

6.1 Thermal Mass Flowmeter 37

6.2 Rotometer (Optional) 38

6.3 Thermal Mass Flowcontroller (Optional) 40

6.4 Vessel Pressure 40

6.5 Exhaust Gas Treatment 43

6.6 Operation of Sparge/Overlay Air (Gas) System 44

7. FOUR GAS CONTROL MODULE (OPTIONAL) 46

7.1 Valving 47

7.2 Factory Settings For Four Gas Mix 49

7.3 Viewing Gas Mix Status 51

8. PNEUMATIC HEADLIFT (OPTIONAL) 54

8.1 Operation of Headlift 54

9. PUMPS (OPTIONAL) 58

9.1 Pump Capacity 59

9.2 Tubing Selection 59

9.3 Loading Tubing into Pump Head 61

10. FOAM CONTROL SYSTEM (OPTIONAL) 62

10.1 Chemical Foam Control System (with Peristaltic Pump) 62

10.2 Setup of Foam Probe 63

10.3 High Foam Safety System (Optional) 64

11. pH CONTROL SYSTEM (OPTIONAL) 66

11.1 System with Pumps (Optional) 66

11.2 Calibration of pH Probe 67

11.3 Recalibration After Sterilization 70

11.4 Setting Up A Cascade From pH To Pumps 71

12. DO CONTROL (OPTIONAL) 74

12.1 Calibration of DO probe 75

13. PREPARING FOR FERMENTATION 78

13.1 Systems Check 78

13.2 Adding Media to the Vessel 79

13.3 Sterilization of Vessel Using Heat Labile Media 80

14. AUTOMATIC STERILIZATION 82

14.1 Sterilization (With Liquid In Vessel) 82

14.2 Valve Sequencing 86

14.3 Shutdown During Sterilization 88

14.4 Enabling sterilization 89

14.5 Aborting Sterilization 90

14.6 Sterilization (Vessel Empty) 91

15. INOCULATION AND ADDITION VALVES (OPTIONAL) 92

15.1 Sterilization 92

15.2 Addition and Inoculation 95

16. SAMPLING VALVES (OPTIONAL) 96

16.1 Sterilization 97

16.2 Sampling 97

17. SHUTDOWN 98

17.1 Shutdown Procedure 98

18. PREVENTIVE MAINTENANCE 100

18.1 Three Month Intervals 100

18.2 One Year Intervals 101

18.3 Cleaning 102

18.4 Water Filter (Water Prefilter and Regulator Kit) 102

18.5 Steam Filter (Steam Prefilter and Regulator Kit) 103

18.6 Sterile Air Filter, Inlet and Exhaust 104

18.7 Motor and Gear Reducer 105

19. TROUBLESHOOTING 108

20. REPLACEMENT PARTS AND ACCESSORY INFORMATION 110

20.1 Replacement Parts Descriptions and Part Numbers 110

20.2 Accessory Descriptions and Part Numbers 110

21. APPENDIX A 112

21.1 Motor Control Schematic 112

21.2 ML-6100 Schematic with Auto Sterilization 113

21.3 ML-6100 Schematic with Auto Sterilization & 4-Gas Option 114

21.4 ML-6100 Schematic with Auto Sterilization & 2-Gas Option 115

21.5 ML-6100 Optional Recorder Kit Schematic 116

21.6 Piping & Instrumentation Diagrams Auto Sterilization 117

22. SYSTEM GAINS 120

23. APPENDIX B 121

23.1 Corrosion Resistance Tables 121

24. APPENDIX C 129

24.1 General Characteristics of EPR 129

25. APPENDIX D 131

25.1 Factory Sterilization Valve Sequencing Settings 131

26. INDEX 133

1. OVERVIEW

The BioFlo 6000 Mobile Pilot Plant is a versatile fermentor offering the choice of either 75, 100 or 130-liter vessels (total volumes). The vessel, piping and the fabricated sheet metal components are made entirely of stainless steel to reduce contamination.

In order to accommodate processes of varying complexity, NBS offers several options to adapt the BioFlo 6000 for specific needs. The available options are listed below:

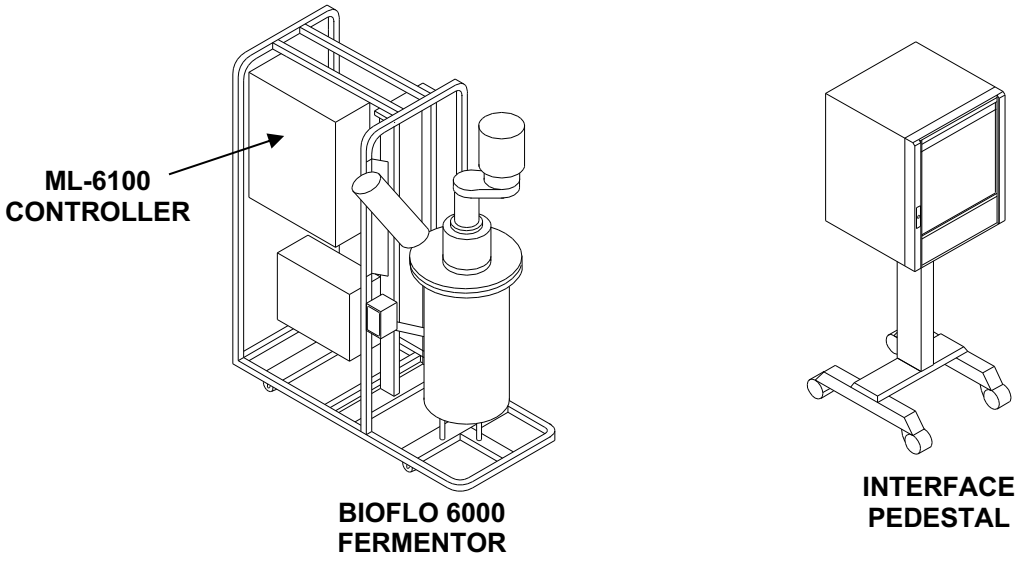
Part No.	*Description*
M1238-4004	Vessel, 75 L
M1238-4002	Vessel, 100 L
M1238-4003	Vessel, 130 L
P0200-2434	Marine Blade Impeller, LH, 75 L
P0200-2234	Marine Blade Impeller, RH, 75 L
P0200-2444	Marine Blade Impeller, LH, 100 L
P0200-2244	Marine Blade Impeller, RH, 100 L
P0200-2444	Marine Blade Impeller, LH, 130 L
P0200-2244	Marine Blade Impeller, RH, 130 L
M1180-9349	Pitched Blade Impeller, 75 L
M1238-5019	Pitched Blade Impeller, 100 L
M1238-5024	Pitched Blade Impeller, 130 L
M1238-3107	Seal, single mechanical, 75 L
M1238-3106	Seal, double mechanical, 75 L
M1238-3101	Seal, single mechanical, 100 L
M1238-3100	Seal, double mechanical, 100 L
M1238-3104	Seal, single mechanical, 130 L
M1238-3105	Seal, double mechanical, 130 L
M1238-2590	Agitator Seal Alarm
M1238-2530	Sterilization, manual
M1238-2531	Sterilization, automatic
M1238-3006	Headlift, pneumatic, 75 L
M1238-3003	Headlift, pneumatic, 100 L
M1238-3004	Headlift, pneumatic, 130 L
M1238-2710	Rotameter, manual sterilization, 75L
M1238-2711	Rotameter, automatic sterilization, 75L
M1238-2510	Rotameter, manual sterilization, 100L
M1238-2511	Rotameter, auto sterilization, 100L
M1238-2610	Rotameter, manual sterilization, 130L
M1238-2611	Rotameter, auto sterilization, 130L
M1238-2714	Mass flow meter, manual sterilization, 75 L
M1238-2715	Mass flow meter, automatic sterilization, 75 L
M1238-2514	Mass flow meter, manual sterilization, 100 L
M1238-2515	Mass flow meter, auto sterilization, 100 L
M1238-2614	Mass flow meter, manual sterilization, 130 L
M1238-2615	Mass flow meter, auto sterilization, 130 L
M1238-2712	Mass flow controller, manual sterilization, 75 L
M1238-2713	Mass flow controller, automatic sterilization, 75 L

M1238-2512	Mass flow controller, manual sterilization, 100 L

Part No.	*Description*
M1238-2513	Mass flow controller, auto sterilization, 100 L
M1238-2612	Mass flow control, manual sterilization, 130 L
M1238-2613	Mass flow control, auto sterilization, 130 L
M1238-2722	Automatic Back Pressure Control with manual sterilization, 75 L
M1238-2723	Automatic Back Pressure Control with automatic sterilization, 75 L
M1238-2522	Automatic Back Pressure Control with manual sterilization, 100 L
M1238-2523	Automatic Back Pressure Control with automatic sterilization, 100 L
M1238-2622	Automatic Back Pressure Control with manual sterilization, 130 L
M1238-2623	Automatic Back Pressure Control with automatic sterilization, 130 L
M1238-2720	Manual Back Pressure Control with manual sterilization, 75 L
M1238-2471	Manual Back Pressure Control with automatic sterilization, 75 L
M1238-2520	Manual Back Pressure Control with manual sterilization, 100 L
M1238-2521	Manual Back Pressure Control with automatic sterilization, 100 L
M1238-2620	Manual Back Pressure Control with manual sterilization, 130 L
M1238-2621	Manual Back Pressure Control with automatic sterilization, 130 L
M1238-0302	Air/ Pressure Control Hardware
M1238-0300	Condenser Port Plugged
M1238-2581	Exhaust Condenser
P0200-0751	Exhaust Filter, Depth, 100 L
P0200-0754	Exhaust Filter, Absolute, 100 L
M1238-3010	Touch Screen Controller mounted in a mobile cabinet
M1238-3020	Touch Screen Controller mounted on a swing arm
M1238-2340	I/O Card Only (4-20mA)
M1238-2580	Gas Overlay
M1238-0500	High Foam
M1238-0502	Level Kit*
M1238-0501	Foam Control*
M1238-0600	Ingold DO Kit
M1238-0602	Broadley-James DO Kit
M1238-0610	pH probe, Ingold liquid kit**
M1238-0611	pH probe, Ingold gel kit**
M1238-0612	pH probe, Broadley James gel kit **
M1238-6013	Transmitter for DO and pH
M1238-5010	Pumps- 1
M1238-5014	Pumps- 2
M1238-5015	Pumps- 3
M1238-5016	Pumps- 4
M1238-2585	Sample Valve, NBS, 25 mm
M1238-2586	Sample Valve, NBS, 19 mm
M1238-2587	Addition Valve, NBS, 25 mm (a maximum of four may be selected)
M1238-2584	Addition Valve, NBS, 19 mm (a maximum of four may be selected)
M1154-3017	Aerosol Containment System for 25 mm Sample Valve
M1154-3016	Aerosol Containment System for 19 mm Sample Valve
M1238-0614	Septum Kit, 1.5" Triclamp
M1153-9633	Sterile Quick Connect, 19 mm
M1153-9639	Sterile Quick Connect, 25 mm
M1154-2030	Air Prefilter
M1238-2582	Water Prefilter
M1238-2583	Steam Prefilter

The BioFlo 6000 Fermentor is either manually (standard) or automatically (optional) sterilized and consists of an open-frame, skid-mounted piping console and a stainless steel vessel that is mounted to the left of the piping. This manual only covers the operation of the BioFlo 6000 with the automatic sterilization option.

A ML-6100 Controller is mounted on the rear of the piping skid and connects via a cable to a computer that is housed on a swing arm on the front of the skid or in a mobile Interface Pedestal so that it may be moved separately from the piping skid.



The piping skid is mounted on lockable casters so as to be completely mobile.
The skid includes all of the plumbing and valves for in place sterilization, cooling and operation of the fermentor. The system connects to all required services (air, steam, water and drain).

The fermentor vessel is a jacketed ASME coded pressure vessel with ports and wells sufficient to insert all the required sensors, and provide aeration and agitation. The system permits the heating and cooling necessary for in-place sterilization and fermentation. All process piping, the vessel and the vessel jacket are constructed of 316L stainless steel.

An ASEPCO diaphragm drain valve is provided as standard. Addition valves (NBS type plunger or other) and sample valve (NBS spool valve or other) are optional.

The standard version of the BioFlo 6000 consists of a piping skid and vessel having airflow regulated via a thermal mass flowmeter with manual valving, manual sterilization and manual pressure control. The fermentor comes with a ML-6100 Controller, and a computer HMI which may be either mounted on the front of the unit or in an Interface Pedestal which houses a computer and touchscreen monitor.

Several other controller options are available upon request. The standard fermentor is equipped with sensors for temperature and agitation only. Sensors for level, pH, DO antifoam and nutrient addition are optional. All ports are plugged as standard. All headplate port connections are Tri-Clamp. All vessel side wall ports have Ingold connections. Sufficient ports are provided on the side wall for the insertion of an optional pH probe, an optional DO probe and other optional probes or sensors. The type and size of addition valves and sample valves must be selected. Pumps, headlift, exhaust condenser as well as a number of other features are optional.

The fermentor is supplied with separate operating manuals for the fermentor and ML-6100 controller, a set of drawings (piping schematics, electrical schematics, etc.) and a full listing of disposable and replacement parts.

Typically the fermentor is filled through the powder or liquid charging/light port (located in the headplate), raised to sterilization temperature, held at sterilization temperature for an operator determined period of time, and cooled to operating temperature. It is then pressurized and aerated for an operator determined period; harvested and ultimately shutdown. Addition of aseptic inoculum, as well as aseptic sampling may be accomplished by the use of optional steam-sterilizable inoculation and sampling ports.

1.1 SPECIFICATIONS

BioFlo 6000 Fermentor			
Weight	75L 1300 lbs. (590 kg)	100L 1350 lbs. (612 kg)	130L 1390 lbs. (630 kg)
Overall Dimensions	81" to 102" High x 68" Long x 28.5" Wide 2060 mm to 2590 mm High x 1730 mm Long x 720 mm Wide		
Mobile Piping Skid	Fermentation system assembled on a stainless steel skid mounted on casters for easy mobility.		
Fermentor Vessel **External Finish** **Internal Finish**	Heavy gauge, polished 316L stainless steel, jacketed vessel. 32 RA 20 RA		
Total Capacity: **Maximum Working Volume (L)** **Minimum Working Volume (L)** **Height-to-diameter ratio**	75L 55 15 2.21:1	100L 75 25 2.25:1	130L 100 30 1.91:1
Vessel Dimensions: **Height, inches** **ID** **OD, measured at flange** **Impeller Dia.**	75L 33.48 in. (850.4 mm) 15.12 in. (384.0 mm) 20 in. (508 mm) 5.12 in. (130.0 mm)	100L 35.19 in. (893.8 mm) 15.62 in. (396.7 mm) 22 in. (558.8 mm) 6.44 in. (138.2 mm)	130L 33.69 in. (855.7 mm) 17.62 in. (447.5 mm) 23 in. (584.2 mm) 7.23 in.(183.6 mm)
Impellers	3 six-blade Rushton-type turbine impellers of 316L stainless steel, adjustable on drive shaft.		
Pressure Rating	ASME-coded—rated for 40 psig (2.8 kg/cm^2) in the vessel, and 35 psig (2.5 kg/cm^2) in the jacket.		
Vessel Viewing Window	Large rectangular window, sidewall mounted. Area of visibility -11.5" High x 2.5" wide (292 mm x 64 mm). Internal visibility, above and below the liquid level		
Baffles	Four stainless steel baffles		
Rupture Disk	Vessel pressure safety device with Tri-Clamp connector conveying any spillage safely away. Disk set to rupture at 40 psi		
Diaphragm Drain Valve	ASEPCO valve installed in bottom of vessel as standard. Size 1.0" ID (25.4 mm), in 75 &100 liter vessels, and 1.5" ID (38.1 mm) 130 liter vessels		
Sparger Inlet	Mounted in upper side wall of vessel. Connects to standard ring sparger		
Ports	All ports are plugged as standard for optional insertion of addition lines, sensors and penetrations. All headplate ports are Tri-Clamp all vessel wall ports are Ingold-type ports (25 mm and 19 mm OD), supplied as standard		
Acid & Base	Ports for connecting optional addition line kits to acid and base reservoirs standardly supplied as plugged.		
Inoculation/Addition/ Sample Valves	Optional resterilizable valves, 19 mm and 25 mm OD size.		

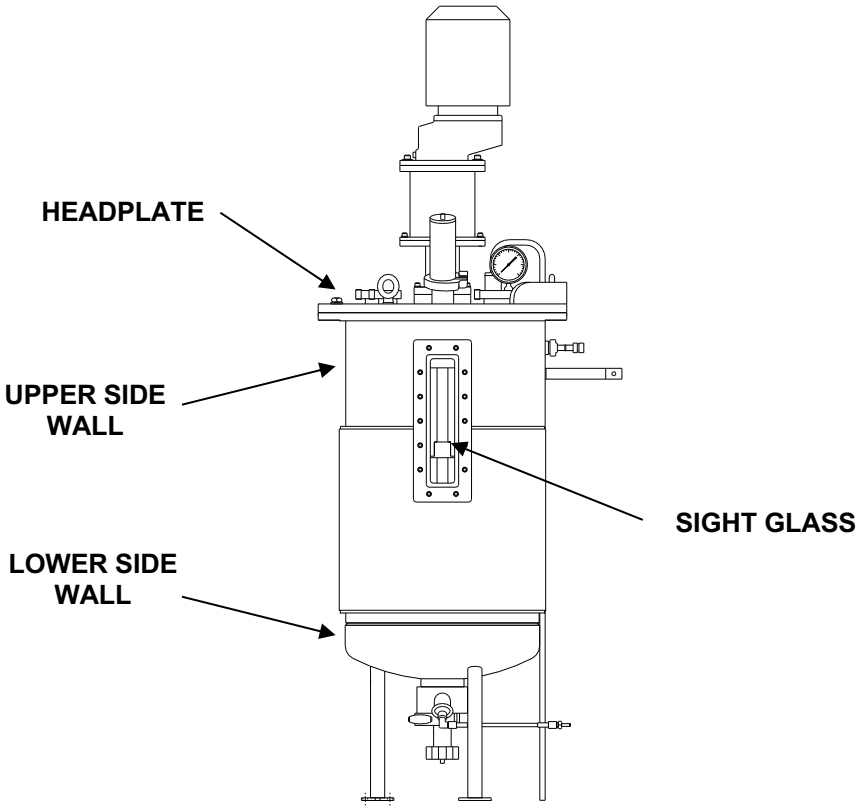
BioFlo 6000 Fermentor			
Agitation **Speed Range** **Control** **Motor**	75L 80-800 ±2 rpm 2 hp Industrial AC motor, top-entry. Optical sensor maintains precise speed over entire range	100L 50-550 ±2 rpm 2 hp	130L 50-550 ±2 rpm 2 hp
Fiber Optic Speed Sensor	PID regulation of speed in microprocessor feedback circuit		
Bearing Housing	Single bearing housing seal, standard. Double mechanical seal with sterile steam bleed, optional.		
Back-Pressure Regulator	Manual as standard, Automatic Back Pressure control is optional		
Air Flow	A thermal mass flowmeter is standard for monitoring airflow, a manual valve is provided for control.		
Air Flow Range	75L 5-100 SLPM	100L 10-200 SLPM	100L 10-200 SLPM
Air Inlet Filter	0.2μ absolute, PTFE material		
Sterilization **Manual** **Automatic**	Manual control of steam, air, and cold water is standard. Automatic control of sterilization and flow of cold water, optional. Sterilization is programmable from 5 to 180 minutes with automatic cool down to preset operating temperature.		
Sterilization Temperature	105°C-130°C		
Steam **Clean Condensate Drain** **Contaminated Condensate Drain**	1/2" female NPT 3/4" female NPT		
Electrical Service	200 -240 V, 50/60Hz, single phase, 8 KVA. Meets UL, CSA, VDE & British standards		
Chart Recorder Package	Optional 4-20mA connection for six-channel programmable strip-chart recorder.		
Temperature Control	Thermowell and RTD sensor are standard. Temperature controllable from 5°C above ambient to 60°C.		
Probe Calibration	Digital calibration of optional pH and DO probes via touchscreen monitor.		
Addition Lines	For feeding of primary and supplementary nutrients are optional.		
Pre-Filter and Regulator Kits	Water and steam kits for removal of particulate and impurities and control of pressure are optional.		
Foam/Level Probe	Ports plugged. Optional foam/level probe includes sensor and cable for control of antifoam addition or liquid level.		
High Foam Probe	Optional probe for detecting foam in exhaust line. Includes sensor with cable.		
Aerosol Containment	Optional aerosol containment device to prevent escape of aerosols when sampling. (Available only when ordered with optional sampling valve.)		
Air Exhaust Filter	Depth filter (polypropylene material). Optional 0.2μ absolute exhaust filter also available.		
Validation	Multiple options available to meet specific customer validation requirements		

2. BIOFLO 6000 FEATURES

2.1 VESSEL

Each vessel conforms to the latest ASME Section VIII Pressure Vessel Code and is supplied with copies of the Manufacturer's Data Report (U-3). The vessel includes ASME-Code Stamp.

Vessel



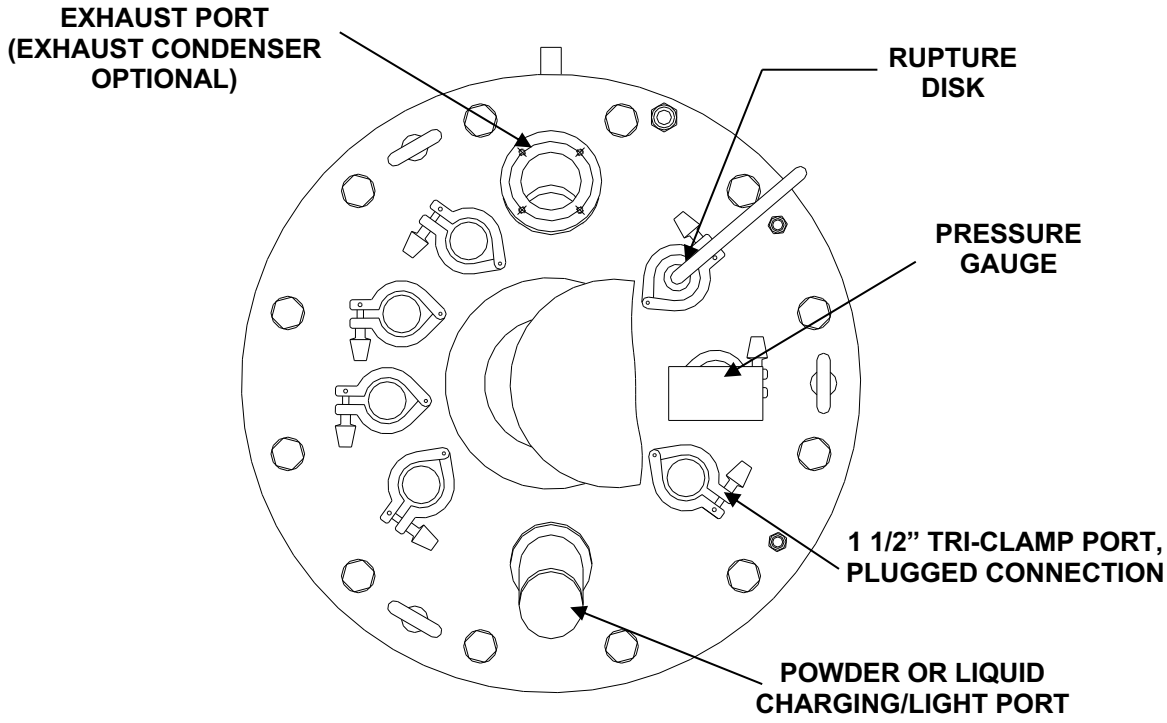
The vessel has an internal design pressure of 40 psig (2.72 Atm) and full vacuum and a jacket design pressure of 35 psig (2.38 Atm), each at 300°F (149°C) design temperature. Working volume of the vessels is approximately 75% of their stated capacity, i.e., 55, 75 and 100 liters maximum working volume for each of the above vessels. All ports are normally plugged. The headplate is removable from the body. An optional, pneumatic headlift is available as well.

2.2 VESSEL AND JACKET PENETRATIONS

Headplate:

- 2-1/2" ID addition port for powder addition, with Tri-Clamp connection and sight glass built into the port cap (non resterilizable). A removable light source is supplied for illumination through this port.
- Seven 1-1/2” Tri-Clamp connections for foam probe, level probe, pressure gauge, rupture disk, etc.
- Bearing Housing
- Pressure Gauge: 1-1/2” Tri-clamp connection
- Rupture Disk: 1-1/2” Tri-clamp connection
- Exhaust: Bolted with O-ring seal for either plain exhaust or optional exhaust condenser

Headplate Penetrations

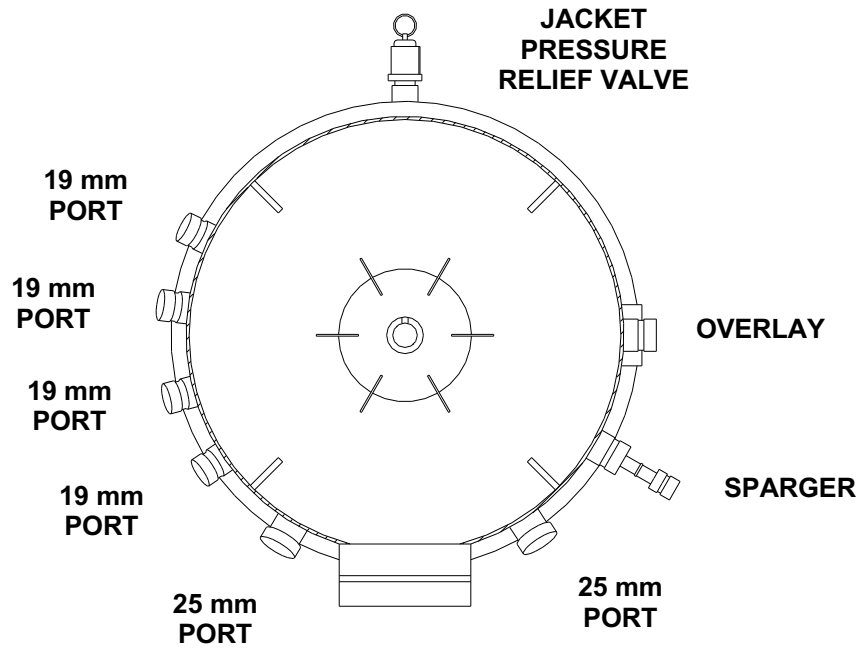


All headplate ports with the exception of those for the exhaust condenser and bearing housing have Tri-Clamp connections.

Upper Side Wall:

- Sparger Inlet
- Two 25 mm ports (plugged) for optional steam resterilizable inoculation/ addition valves.
- Air/Gas Overlay (plugged, optional connection)
- Four 19 mm ports (plugged), septum ports or resterilizable addition valves are optional.

Side Wall Penetrations (All Vessels)



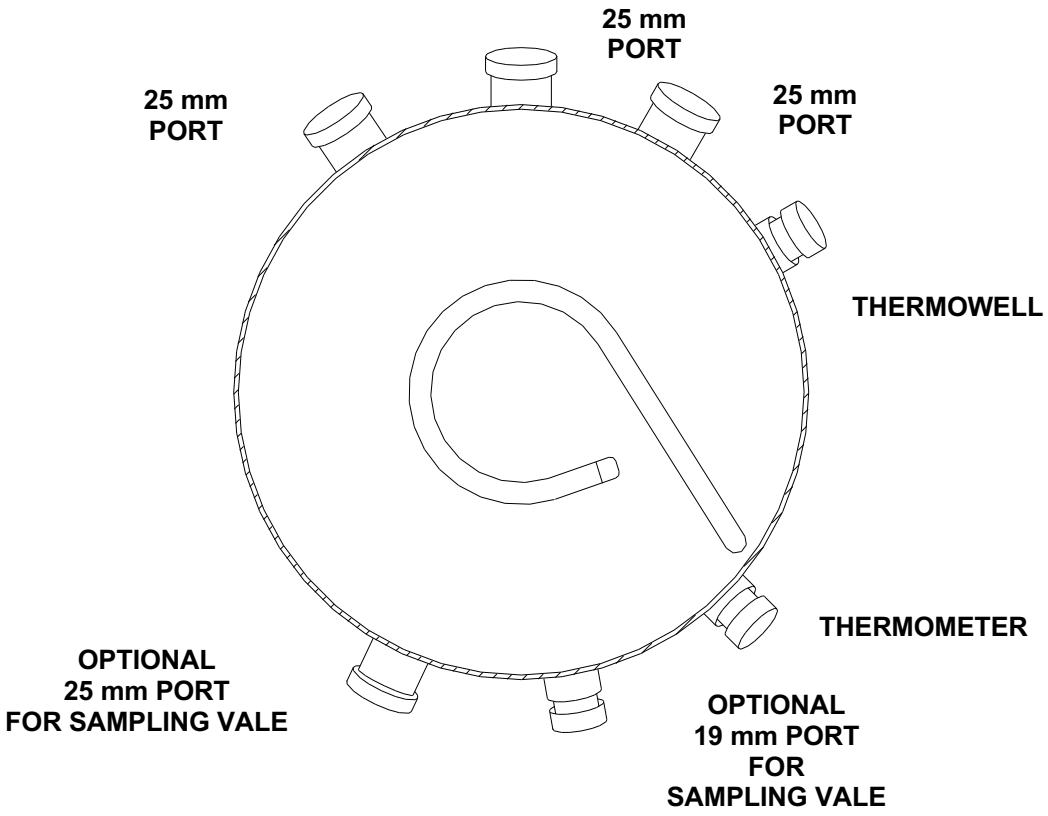
Sight Glass:

Large rectangular, sight glass with vertical orientation, viewing area extends above the surface of the liquid at working volume and extends below the liquid (approx. 11-1/2" L x 2-1/2" W viewing surface).

Lower Side Wall:

- Two 19 mm ports (thermowell and thermometer)
- 19 mm (or 25 mm also available) port for optional steam resterilizable sample valve (plugged).
- Three 25 mm Ingold ports (for optional probes, DO, pH, etc.), plugged

Lower Side Wall Penetrations (All Vessels)



All connections are Ingold type (modified for cleanability).

Jacket:

Jacket pressure relief valve port. Jacket water/steam inlet and outlet.

2.3 PROCESS PIPING

All process piping, valves and associated components are of type 316 stainless steel. All other piping is of type 316L or type 304 stainless steel. The inlet air and exhaust line both have steam-sterilizable, replaceable, cartridge filters. The exhaust filter housing is oversized to accommodate filters from a variety of filter manufacturers. Normally an absolute air inlet filter and depth exhaust filter are provided.

2.4 AGITATION / DRIVE SYSTEM

Agitation speed is capable of being set to any value between the specified minimums and maximums of 50 to 550 rpm for the 100 and 130 liter BioFlo 6000 and 80 to 800 rpm for the 75 liter fermentor. A PID control loop holds the setting to within ±2 rpm of the setpoint.

The system is top drive and comes complete with an AC variable speed motor with optical speed pickup. Three Rushton impellers are standard and are sized to give a minimum OTR of 350 mM O_2 /L/ hr without O_2 supplementation.

A bearing housing with a single seal is provided as standard and features a double permanently lubricated bearing mounted in a housing with a precision machined and straightened shaft. The bearings are protected with dust seals. Included within the bearing housing assembly is a single mechanical seal consisting of a tungsten carbide seat and a carbon rotating face that is spring loaded to insure maximum sealing ability.

A double mechanical seal is optional. The optional double mechanical seal bearing housing is as described above but the double seal arrangement is lubricated and cooled by a sterile condensate system. This system consists of a condenser for providing the sterile condensate along with control valves for both the steam and water to the condenser. Also included is a backpressure regulator with a gauge for controlling the pressure of the condensate between the two seals. This pressure can be adjusted to allow for higher than atmospheric pressure conditions.

A power switch for agitation is provided on the front of the fermentor. To operate, the switch must be in the “on” position. Setpoints may then be entered on the touchscreen. Control modes are selected via the ML-6100 Controller.

2.5 TEMPERATURE CONTROL SYSTEM

Vessel temperature control during the growth cycle is controlled from 5°C above the coolant supply temperature to a maximum of 85°C. An RTD temperature sensor and thermowell are provided as standard. The RTD probe provides the signal for a PID loop capable of holding the vessel temperature to within ±0.1°C. Temperature control is via the ML-6100 Controller. Setpoint entry is at the ML-6100 touchscreen.

Piping to connect to an optional closed loop chiller or cooling tower is provided as standard.

3. GETTING STARTED

3.1 POSITIONING THE FERMENTOR

Carefully transport the fermentor to the exact position in which it will be operated. Position the skid and lock the casters by depressing the locking foot pad.

Position the Interface Pedestal in a convenient location, close to the fermentor, to allow for easy access during operation.

3.2 SERVICE/UTILITY CONNECTIONS

Before making connections to the BioFlo 6000, become familiar with each service connection and its location. The following table describes each service connection. The BioFlo 6000 must be connected to regulated utilities as described in the order that they appear.

Service/Utility	*Requirement*	*Connection*
Process Air	75L 5-100 SLPM @ 60-80 psig (4.1 - 5.4 Atm Ga.) 100L 10-200 SLPM 100L 10-200 SLPM	1/2” FNPT
Instrument Air	75 psig (60 psig Min.,100 psig Max.).	1/4” FNPT
Water Return	Maximum backpressure 10 psig	1/2” FNPT
Water	6 GPM (23 LPM), must be regulated to 30 psig	1/2” FNPT
Electrical	200/240 V, 50/60 Hz., Single Phase, 8KVA	240V AC Plug
Clean Condensate Return	Gravity Type - Open	1/2” FNPT
Contaminated Condensate Return	Gravity Type - Open	3/4” FNPT
Process Steam (Pure Steam)	25 lbs/hr(11.4 Kg/hr) @ 60-80 psig (4.1 - 5.4 ATM Ga.). Regulated to 25-30 psig.	1/2” FNPT
Utility Steam	125 lbs/hr(57 Kg/hr)@ 60-80 psig (4.1 - 5.4 Atm Ga.), must be regulated to 30 psig	1/2” FNPT
Exhaust	1/2 psig maximum backpressure	1/2” FNPT

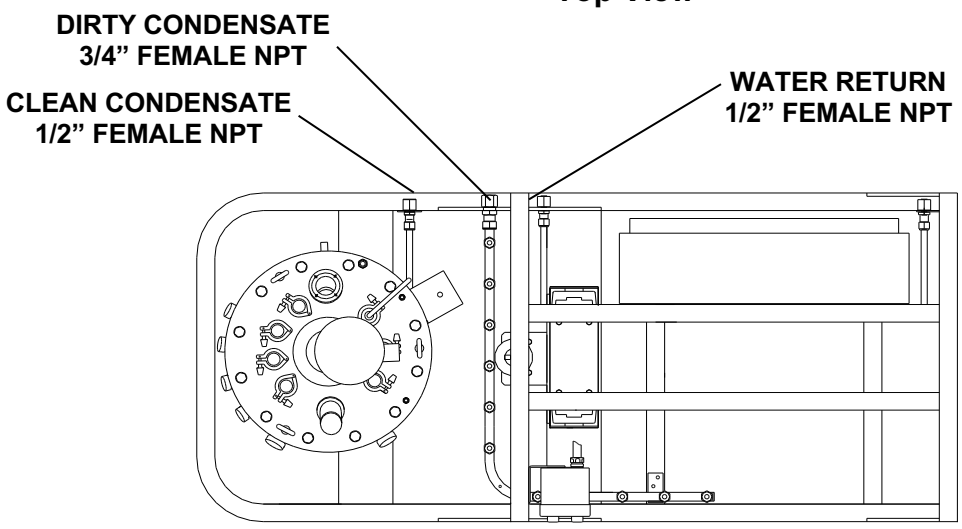
Prior to making connections to the piping module, check that the following inlet service valves are turned off.

- **MAIN AIR (HS-1)**
- **INSTRUMENT AIR (HS-8)**
- **WATER (HS-4)**
- **MAIN STEAM (HS-6A)**

Using standard plant practices and all applicable codes, connect services to the piping skid

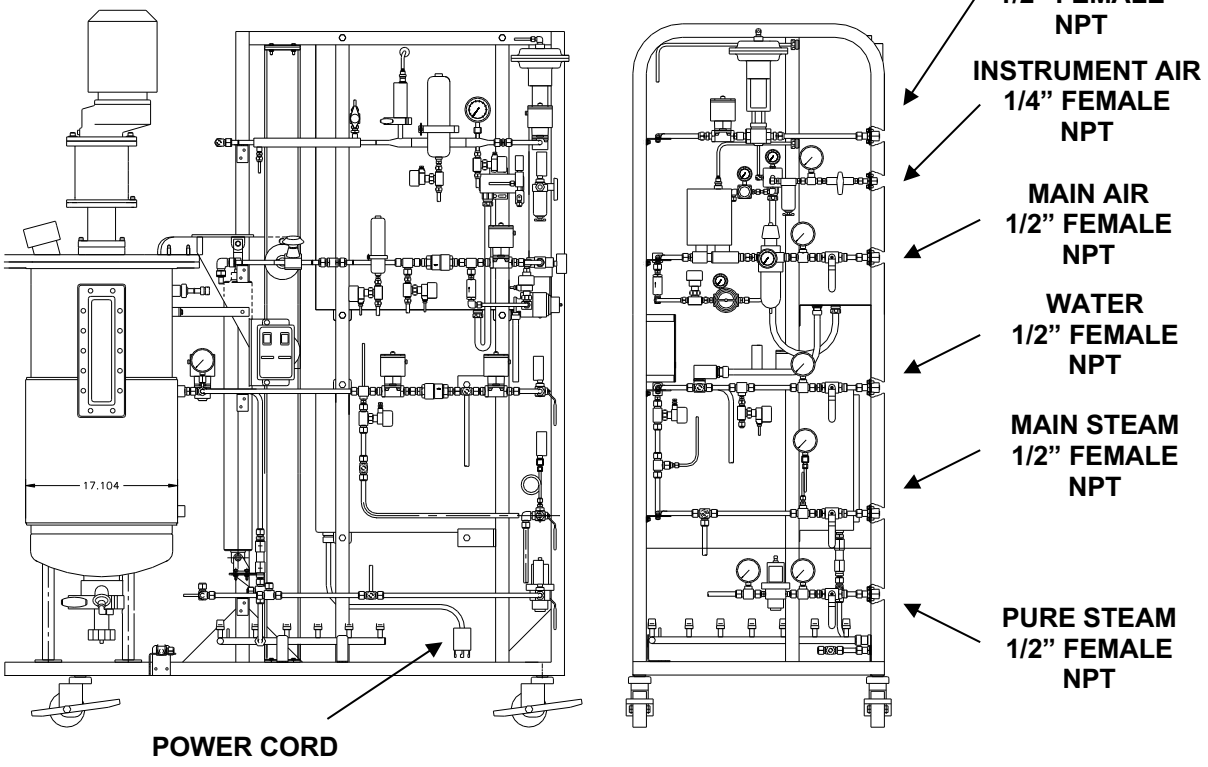
Service Connections

Top View



FRONT VIEW

SIDE VIEW



NOTE:

Water to the fermentor should be free of particulate. Passage through a 20µ filter is acceptable. If the water is heavily laden with particulate, a prefilter kit is recommended. Refer to the *Replacement Parts and Accessory Information* section of this manual or contact your local NBS parts distributor or sales office.

Steam Supply:

The fermentor is designed to be operated from a clean steam supply. If it is known that the steam supply is dirty or contains large particles, it is recommended that a steam prefilter be installed in the steam line leading to the unit. Such a prefilter should consist of a bronze or stainless steel filter body rated for maximum available inlet pressure and temperature.

The filter element may be any of the following: glass or nylon fiber wound on a stainless steel support screen, sintered metal, or graphite. Flow capacity must equal or exceed the maximum steam flow requirement of the unit.

When a prefilter is used, install the filter in the vertical position with valves on the inlet and the outlet side. Steam traps should be connected on the filter drain and down stream of the discharge valve.

If the steam supply line to the unit is of considerable length, it is recommended that a drip leg with a steam trap be installed in the steam line as close as possible to the steam service connection. This will prevent steam condensate from collecting in the line.

A steam pressure regulator and filter kit is available as an option.

Exhaust:

The Exhaust connection may be run to an open floor drain if the gases exiting from the Fermentor are not harmful and have no significant odor. If local codes allow, the line may be run directly from the building. If it is necessary to clean the gases, before exhausting them to the atmosphere, the line can be run to a filter or scrubber. If the gases are piped away a drip leg (larger in size so that a slight reservoir is formed) should be put in the line because the gases leaving a fermentor are moisture laden. A drain valve should be put in the drip leg and it should be opened periodically to allow the condensate to drain.

NOTE:

If the water return line is to be connected so that water is returned to a chiller or cooling tower, then the maximum allowable back pressure in this line is 10 psig with unit in rapid cooling.

Electrical:

The BioFlo 6000 is equipped with a 240 VAC plug, attached to the line cord or, the fermentor may be hardwired. Refer to the Control Schematics supplied with the unit and connect a suitable power line as indicated on the electrical specification plate, located on the side panel opposite of the vessel on the ML-6100 controller cabinet.

CAUTION !

Before making electrical connections, verify that the supply voltage matches the voltage and power requirements on the electrical specification plate and control schematic

3.3 ML-6100 CONNECTIONS

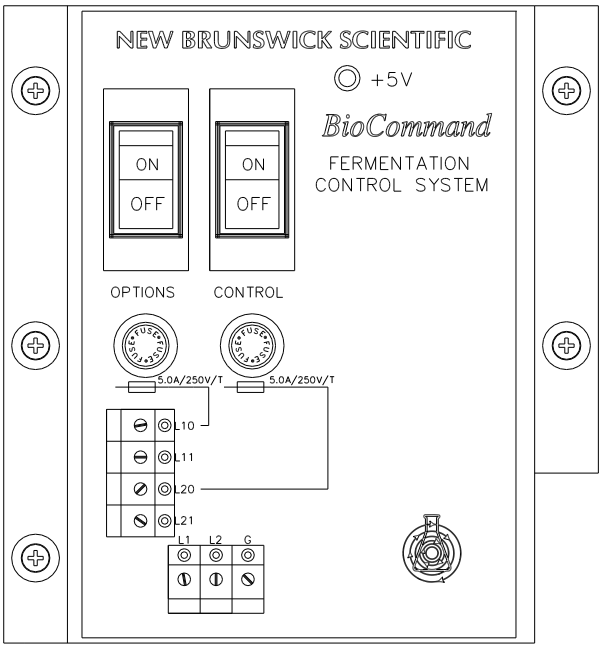
Refer to the *ML-6100 Multi-Loop BioProcess Controller, User's Guide* for detailed instructions on connecting the Interface computer to the ML-6100 Controller mounted on the BioFlo 6000 piping skid.

4. INITIAL PREPARATION OF FERMENTOR

4.1 INITIAL PREPARATION

4.1.1 Turn the power on by setting both rocker switches located in the ML-6100 Controller cabinet to the **ON** position.

Main Control Switch Detail



4.1.2 Open the following hand valves:

- **MAIN AIR (HS-1)**
- **WATER (HS-4)**
- **MAIN STEAM (HS-6A)**
- **PURE STEAM (HS-6B)**

4.1.3 Adjust both plant and pure steam pressure at source to 25-30 psig.

4.1.4 Adjust water pressure regulator at source to 30 psig.

4.1.5 Adjust **AIR PRESSURE REGULATOR (PCV-1)** to read 25 psig on gauge downstream of regulator. It is factory set and will probably not need to be adjusted.

4.1.6 Adjust **VESSEL PRESSURIZATION REGULATOR (PCV-7A)** to read 18-20 psig on nearby gauge. It is factory set and will probably not need to be adjusted.

4.1.7 Adjust **INSTRUMENT AIR** at source to 75 psig. Open **INSTRUMENT AIR SERVICE VALVE (HS-8)**.

4.1.8 Adjust **INSTRUMENT AIR REGULATORS** on the fermentor to 20 psig.

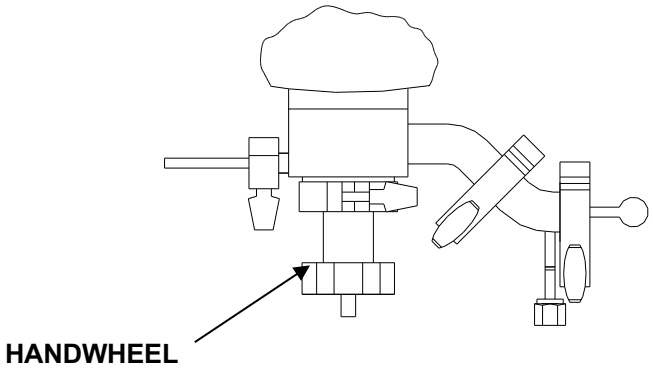
4.1.9 If the double mechanical seal is supplied, open seal steam condensate and water valves before operation of agitator. Refer to the *Agitation Control System* section of this manual.

4.1.10 The system is now ready for operation.

4.2 DRAIN VALVE

The BioFlo 6000 is provided with a manually operated resterilizable, ASEPCO drain valve. This is a flush mounted diaphragm valve which has both position and leak indicators. The valve is designed without deadlegs and so that process fluids are absolutely isolated. A steam port in the valve allows cleaning or resterilization of the valve while the tank valve is closed. A sanitary clamp holds the replaceable diaphragm in place. The diaphragm is EDPM. The connection on the outlet end is a sanitary flange. The valve is opened and closed via a handwheel. To open the valve, turn the handwheel counterclockwise when viewed from the top. To close the valve turn the handwheel clockwise.

Drain Valve Detail



4.3 WATER PRIMING

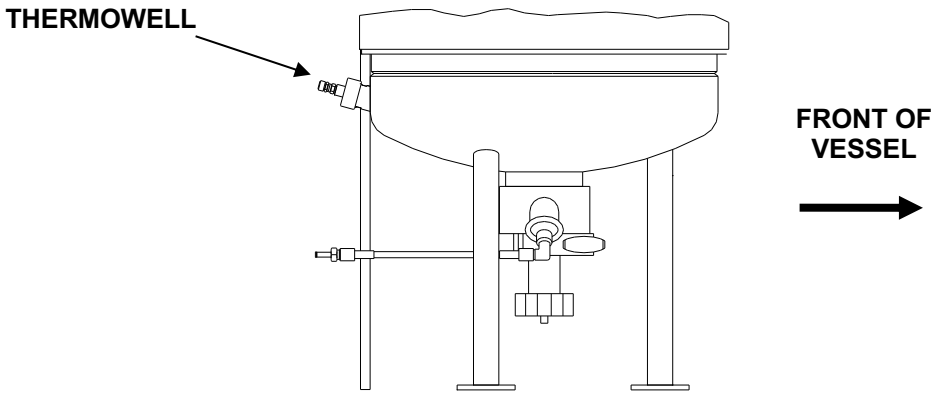
The water system must be primed before proceeding with any temperature control adjustments. Once the system has been primed, it is not necessary to repeat these procedures unless the system is drained.

To prime the system, perform the following:

4.3.1 Measure out 2 to 3 cc of mineral oil or glycerin, and insert it into the **THERMOWELL** using an eyedropper. The mineral oil or glycerin should only cover the bottom 1-1/2" of the well.

4.3.2 Verify that the **RTD TEMPERATURE PROBE** is connected to the **ML-6100 CONTROLLER** and insert the probe into the **THERMOWELL** and secure.

Thermowell Location



4.3.3 At the Interface Pedestal, turn on both the computer and the touchscreen controller.

4.3.4 Verify that the temperature control loop in the display is set to **ON-OFF** or **PID** control mode.

4.3.5 Set the operating temperature setpoint 10°C or more below the indicated temperature.

4.3.6 Observe the water return line. The jacket is primed when a steady stream of water runs out of the line. If the line is not exposed, wait approximately two minutes.

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5. AGITATION CONTROL SYSTEM

Agitation is accomplished by means of a motor rotating the shaft with impellers. Shaft revolution (speed**)** is indicated on the display gauge of the agitation control loop. Power for the motor operation is controlled by the agitation switch located on the switch panel, located on the side opposite of the ML-6100 Controller. Agitation setpoints and operating mode are user defined at the interface touchscreen monitor.

CAUTION !

The Agitator should be used whenever the fermentor is in the sterilize or temperature control mode.

A speed of 150-200 rpm is recommended during operation of the sterilization or temperature control mode. Agitation improves heat transfer between the liquid in the vessel and water or steam in the vessel jacket.

NOTE:

Agitation is not required during sterilization if the vessel is sterilized empty.

CAUTION !

On initial set up prior to operation, check that all impellers are tightly fastened to the drive shaft.

NOTE:

The following should be noted when a **Double Mechanical Seal** is present:

- The **SEAL STEAM VALVE (TCV-24B**) which supplies steam condensate to the bearing housing should be opened 1/2 to 1-1/2 turns and the **SEAL STEAM CONDENSATE WATER VALVE (TCV-24A**) which supplies water to the condenser to form the steam condensate should also be open such that the condensate line is warm to the touch while the Agitator is operating. It is not necessary to close these valves unless the Fermentor is not in use for a long period of time.

- **SEAL STEAM PRESSURE REGULATOR (PCV-24**) should be adjusted to10 psig to balance the mechanical seals in the bearing housing. If vessel pressure is higher than 15 psig, then this regulator must be adjusted to above, but not greater than 5 psig of the operating pressure.

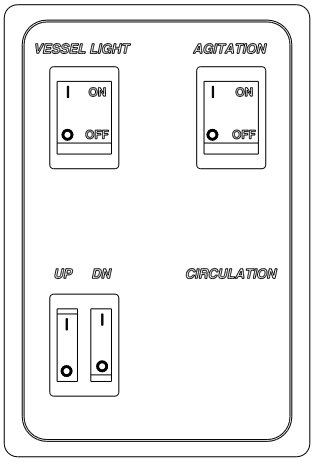
5.1 OPERATION

To operate the agitation control system, perform the following steps:

5.1.1 Ensure that the two main power switches are **ON** and that there is power to the fermentor.

5.1.2 Set the **AGITATION CONTROL SWITCH** to the **ON** position.

Agitation Control Switch (Shown with Headlift Option)



5.1.3 Set the agitation control loop on the touchscreen to **PID** control.

NOTE:

If the agitation control loop is cascaded from the DO control loop, the DO control loop may provide the setpoint for the Agitation control loop. Refer to the *ML-6100 Multi-Loop BioProcess Controller User's Guide* for detailed information on Cascade Control.

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6. AIR SYSTEM

Air (gas) is supplied from the customer source through a prefilter and then a sterile filter. The air (gas) is introduced into the culture vessel via a multi-orifice ring sparger located below the bottom most impeller or through an overlay connection above the tank liquid level. The sparger is a standard feature of the BioFlo 6000. The gas overlay is an option.

Sparge Air Pressure: is regulated by a **PRESSURE REGULATOR (PCV-I)** and indicated on the attached gauge.

Inlet Gas Flow Rate: is controlled by one of three ways depending on the option selected at the time of purchase. Control may be manual via a rotometer or thermal mass flowmeter, or automatic via a thermal mass flowcontroller.

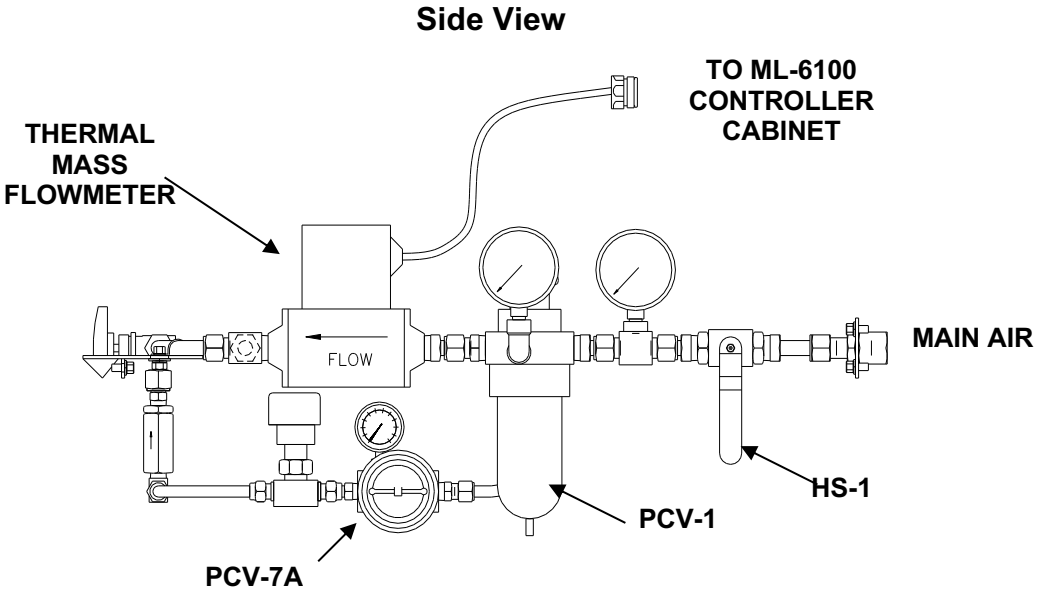
The minimum and maximum allowable air flow rates are dependent on the vessel size. Airflow can be adjusted between the minimum and maximum allowable setpoints for each vessel size. Refer to the following table:

Air Flow Rate Range

Vessel Size	*Min. Air Flow Rate SLPM*	*Max. Air Flow Rate SLPM*
75 L	5	100
100 L	10	200
130 L	10	200

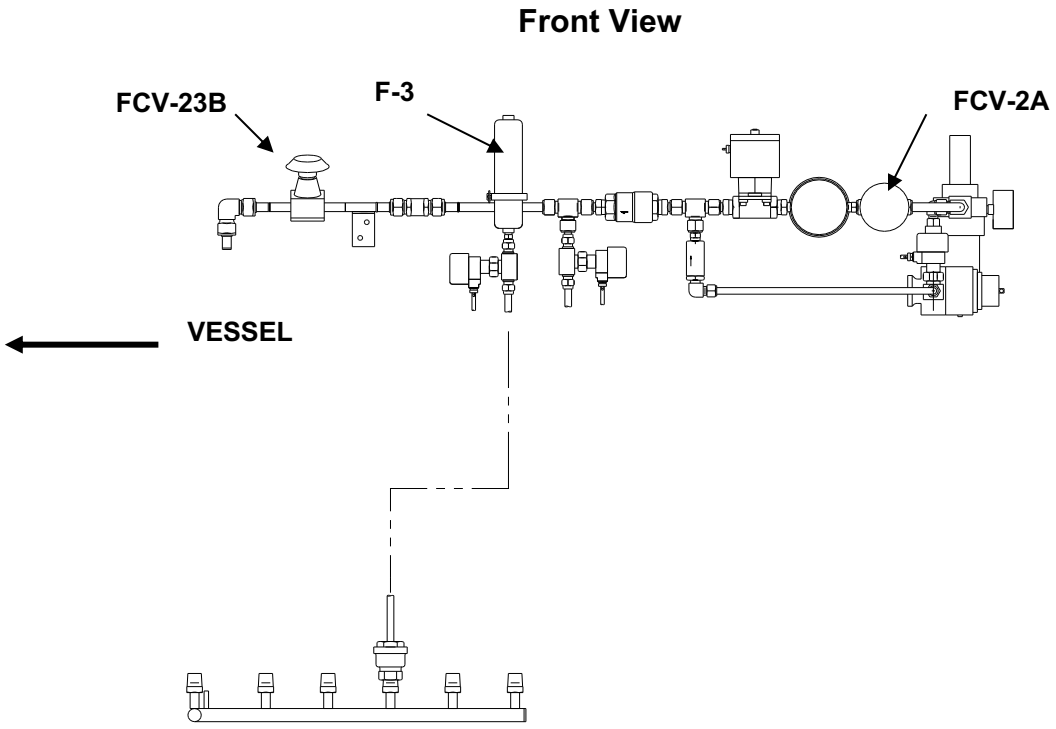
6.1 THERMAL MASS FLOWMETER

The thermal mass flowmeter is the standard control system of the BioFlo 6000. Perform the following to control air flow using the thermal mass flowmeter:



6.1.1 Ensure that the **MAIN AIR VALVE (HS-1)** is open.

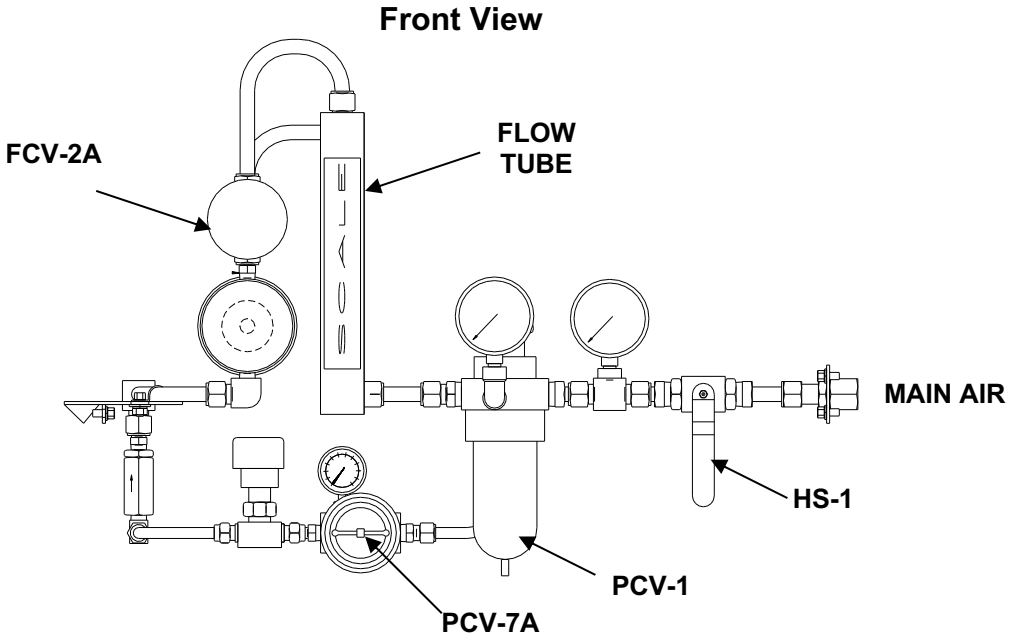
6.1.2 Airflow is adjusted through the tee handle **(FCV-2A)**. Turning the handle counterclockwise increases the air flow. Turning the handle clockwise decreases the airflow.



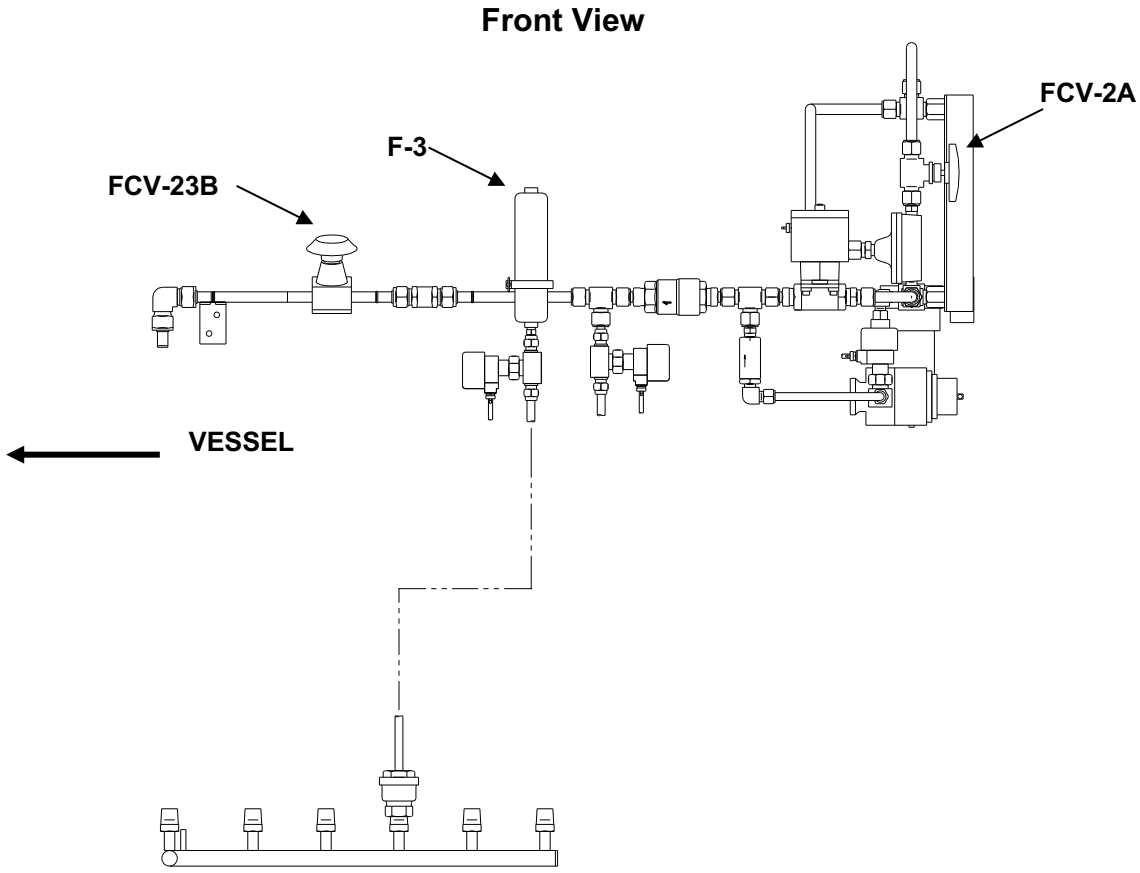
The air flow rate will appear on the touchscreen in the airflow control loop display gauge. The controller is configured at the factory to display this loop.

6.2 ROTOMETER (OPTIONAL)

The rotometer is located at the far right of the piping skid. To control airflow using the rotometer, perform the following:



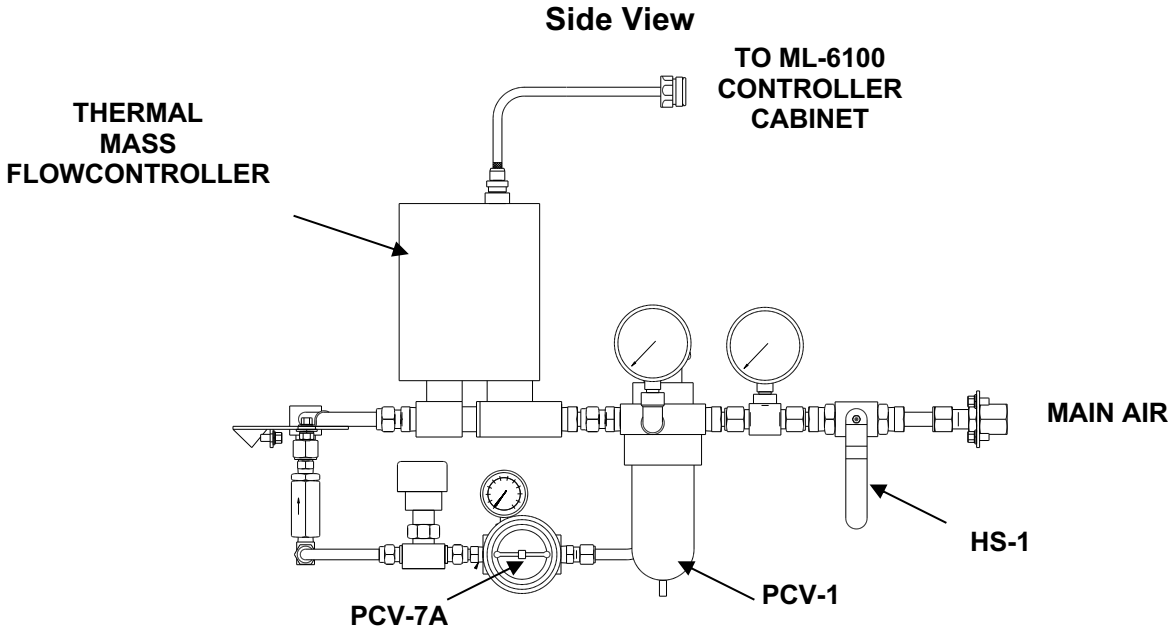
6.2.1 Assure that the **MAIN AIR VALVE (HS-1)** is open.



6.2.2 Air flow rate is adjusted by the tee handle **(FCV-2A)**, and indicated on the flow tube. Turning the handle of **(FCV-2A)** counterclockwise increases the air flow. Turning the handle clockwise decreases the airflow.

6.3 THERMAL MASS FLOWCONTROLLER (OPTIONAL)

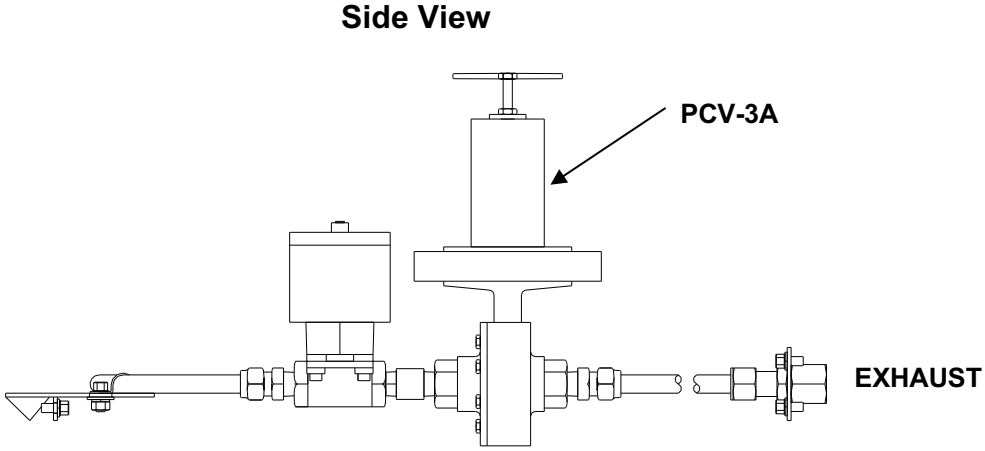
Ensure sure that the **MAIN AIR VALVE (HS-1)** is open. Airflow rate is set on the touchscreen. The controller has been configured at the factory to display this loop.



6.4 VESSEL PRESSURE

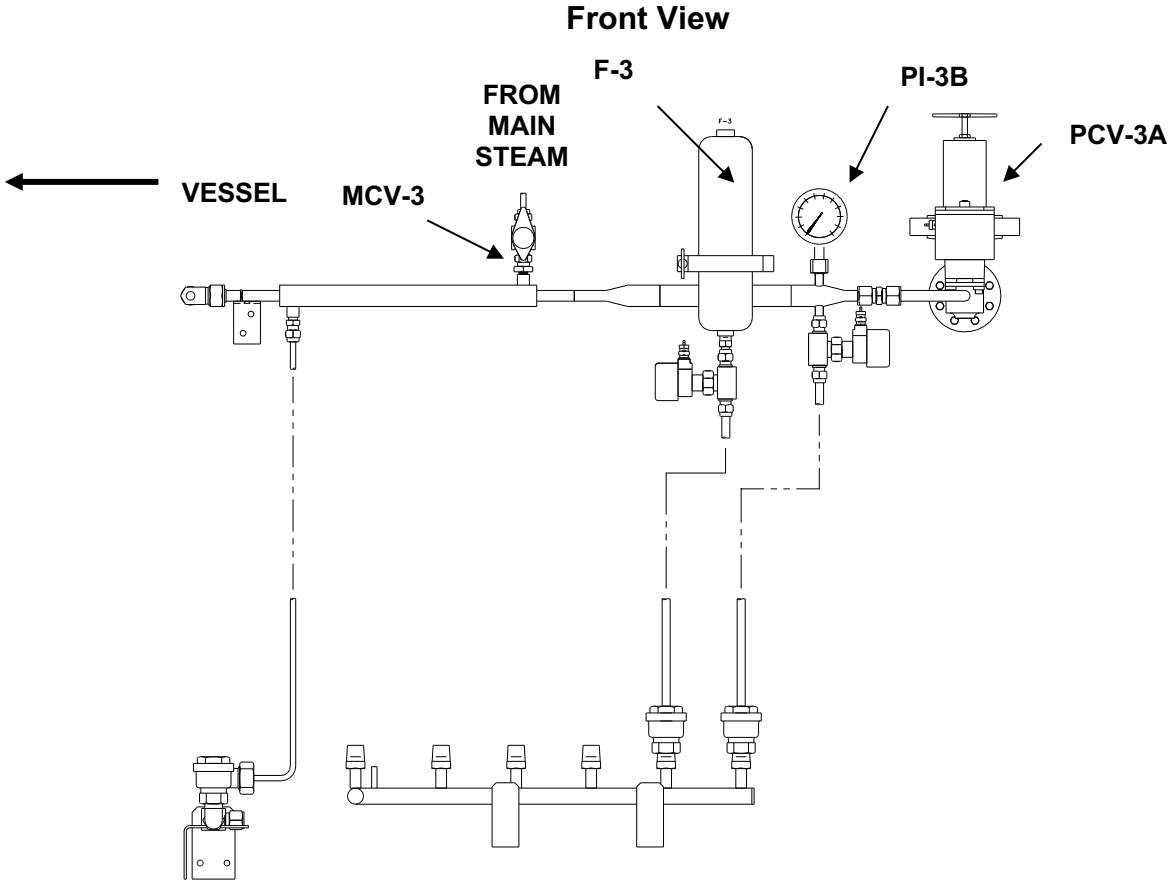
Systems with Manual Backpressure Control:

Vessel pressure is manually adjusted using the **BACKPRESSURE REGULATOR VALVE (PCV-3A)**. The vessel pressure is monitored on the pressure gauge which is mounted on the vessel headplate.



NOTE:

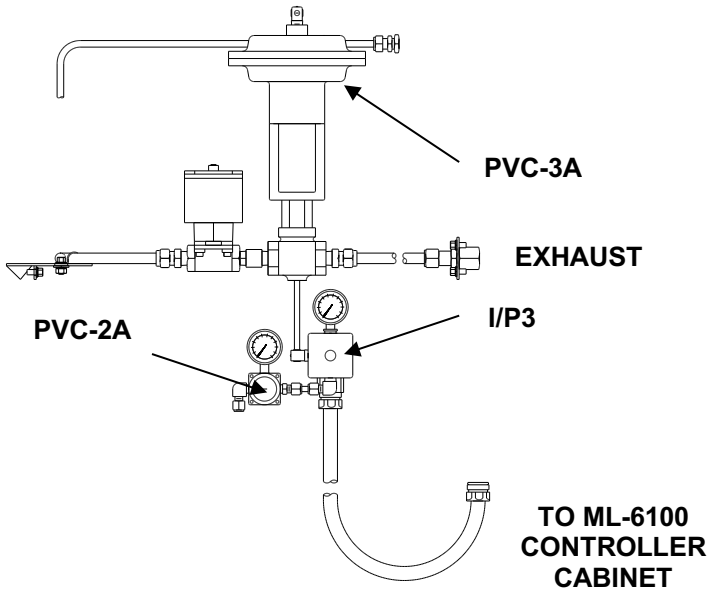
Do not use the gauge mounted **(PI-3B)** just downstream of the **EXHAUST FILTER (F-3)** to monitor backpressure since there is a pressure differential as a result of the filter placement.



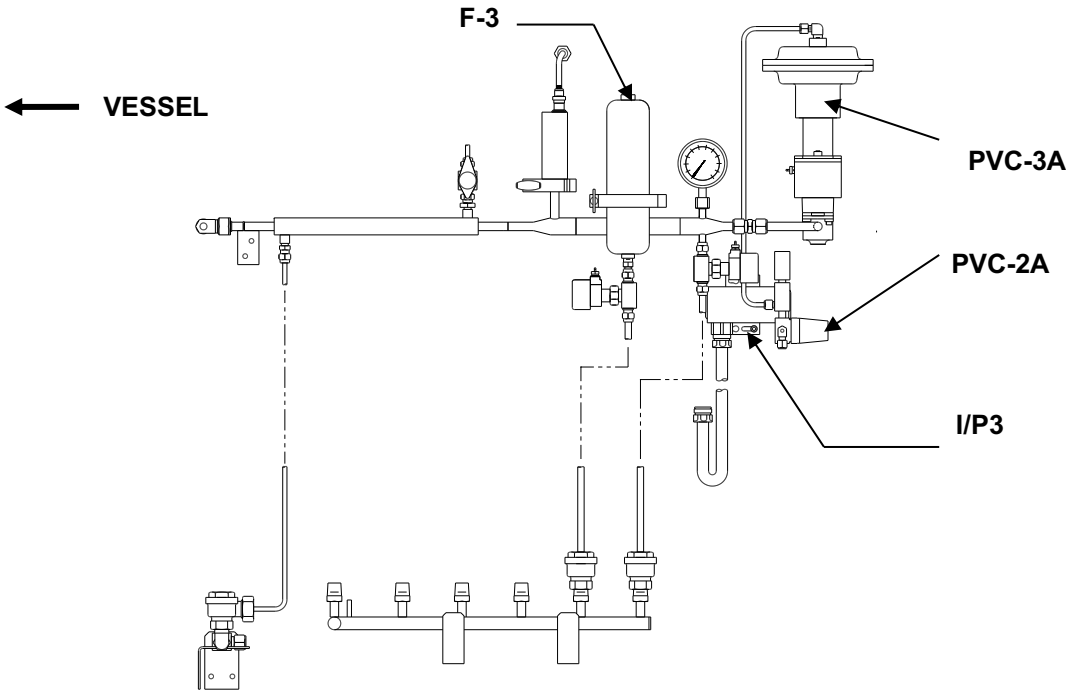
Systems with Optional Automatic Backpressure Control:

Vessel pressure is regulated by pressure control loop of the ML-6100. This controller receives a signal from the pressure transducer and controls **I/P3** which in turn controls valve **(PCV-3A)**. The control valve **(PCV-3A)** is "air to close" and, therefore, fails open.

Side View



Front View



Sterile Inlet and Exhaust Air (Gas) Filters:

The incoming and (exiting) air (gas) is filtered by a cartridge type filter (supplied with the system). The elements are located each in its own stainless steel housing in the front of the unit. The elements are sterilized during the last phase of vessel sterilization by passing steam through the filter.

The exhaust filter is heated to raise the exhaust gas above its dew point. The heater upstream of the exhaust filter has a valve associated with it. This valve MCV-3 must be fully open during operation of the fermentor, and is only closed when the filter housing needs to be cleaned due to a foam over.

NOTE:

The filter element used must be changed periodically to maintain proper filter operation. Since many operating and environmental variations exist between fermentations, a periodic check of filter condition is required to determine when replacement is necessary. Initially, the filter element should be examined on a monthly basis and changed when:

- The filter is found to have deteriorated from repeated sterilizations.
- Oil or media is observed on the element.

6.5 EXHAUST GAS TREATMENT

The gases and moisture leaving the fermentor via a pipeline located in the top of the vessel are introduced into an exhaust condenser when present and filter, and then exit from the unit.

When present the exhaust condenser is mounted on the headplate of the fermentor. It minimizes liquid loss and evaporation of the fermentor culture medium by working as a heat exchanger. The exhaust air of the fermentor is passed over the cooling coil thereby condensing vapor and aerosols and allowing condensate to return to the vessel.

The **EXHAUST CONDENSER WATER VALVE (MCV-4)** should be fully open at initiation of the growth cycle. It must be closed when opening the headplate.

6.6 OPERATION OF SPARGE/OVERLAY AIR (GAS) SYSTEM

When the optional gas overlay is present inlet gas it is split into two streams. One stream goes to the sparger and the inlet gas is introduced into the medium below the bottom impeller. The second stream is directed to the gas overlay. Cut off valves are supplied so that inlet gas can be directed to either the sparger or the gas overlay or both.

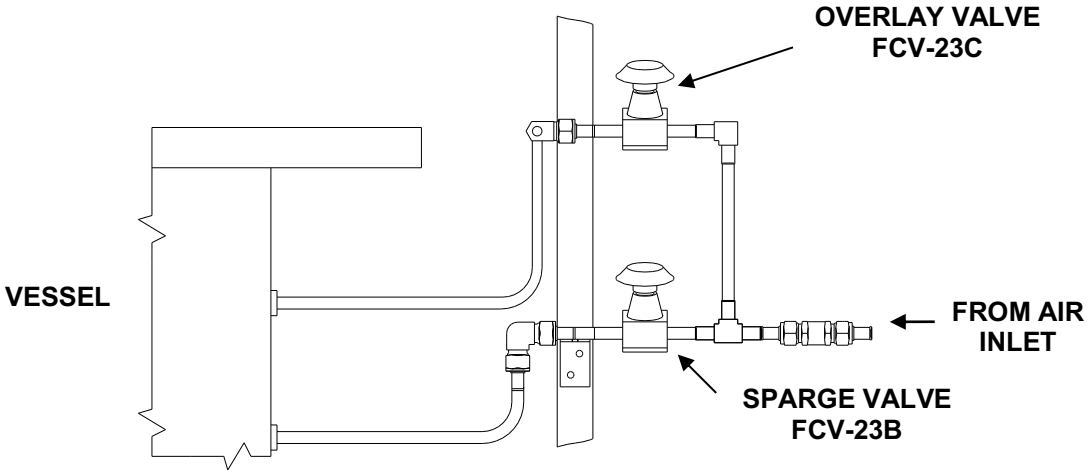
In systems with a gas overlay, both the gas overlay piping and the sparge line must be sterilized subsequent to a fermentation. Ensure that the valve **(FCV-23C)** to the gas overlay and the sparge line valve are open during sterilization.

To operate the Sparger/Overlay Air (Gas) System, perform the following:

6.6.1 Ensure that connections of air (gas) pipelines into and out of the fermentor are tight and the **INLET FILTER CARTRIDGE** checked as above.

6.6.2 Open **SPARGE VALVE (FCV-23B)** and **OVERLAY VALVE (FCV-23C)** prior to sterilization.

Sparge/Overlay Air (Gas) System Detail



6.6.3 Adjust the air flow rate and the vessel pressure either manually or automatically (when the automatic option has been ordered) at the touchscreen in the air/(gas) flow and pressure control loops. Make sure that another loop is not attempting to control vessel pressure or air (gas) flow.

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7. FOUR GAS CONTROL MODULE (OPTIONAL)

The four gas module is activated when the **GAS MIX CONTROLLER OUTPUT TYPE** is selected on a specific loop. With the **GAS MIX CONTROLLER OUTPUT TYPE** selected, the controller mixes combinations of air, oxygen, nitrogen and carbon dioxide as required to control DO and pH. A constant a constant gas flow rate is maintained during this process. The composition of the gas mixture is determined by the duty cycle of the solenoid valves which control the flow of the respective gases. The time required to cycle once through all four valves is called the Pulse Width Modulation Period or PWM Period. It is typically set to 10 seconds, but the time period can be made shorter for smoother control, or longer for extended valve life.

The gas mix control mode is determined by the controller output action and the presence of a cascade input to the loop.

Control Mode	*Cascade Input*	*Output Action*	*Gases Mixed*	*Application*
2 Gas	No	Direct	Air and O_2	Oxygen enrichment
2 Gas	Yes	2 Ch Comp	Air and O_2	Cascade driven oxygen enrichment
3 Gas	No	Center Off	Air, N_2 and O_2	Bi-directional DO Control
3 Gas	Yes	Direct	Air, O_2, and CO_2	Oxygen enrichment w/ CO_2 addition for pH control
4 Gas	Yes	Center Off	Air, N_2, O_2, and CO_2	Bi-directional DO Control w/ CO_2 addition for pH control

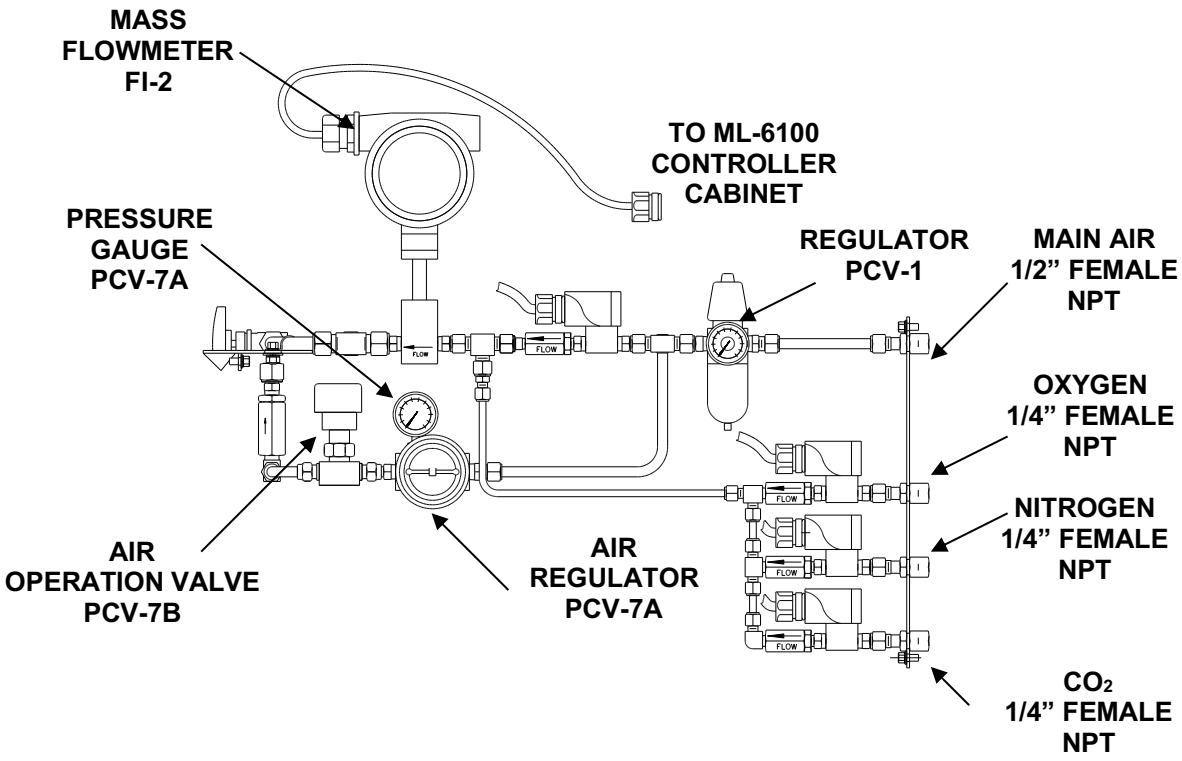
The configuration of a control loop for gas mixing is completed by selecting the output device type as SSR4 and the output device connector ID. SSR4 is a set of four dedicated solid state relays (SSRs) which must be chosen for this operation. For the gas mix to work appropriately, the relay pairs must be connected to the gas solenoid valves. The controller causes the valves to pulse open for different fractions of a user-specified time to obtain different mixing ratios. A constant and continuous flow is maintained for all mixtures.

SSR	*Gas*
1	CO_2
2	Nitrogen
3	Oxygen
4	Air

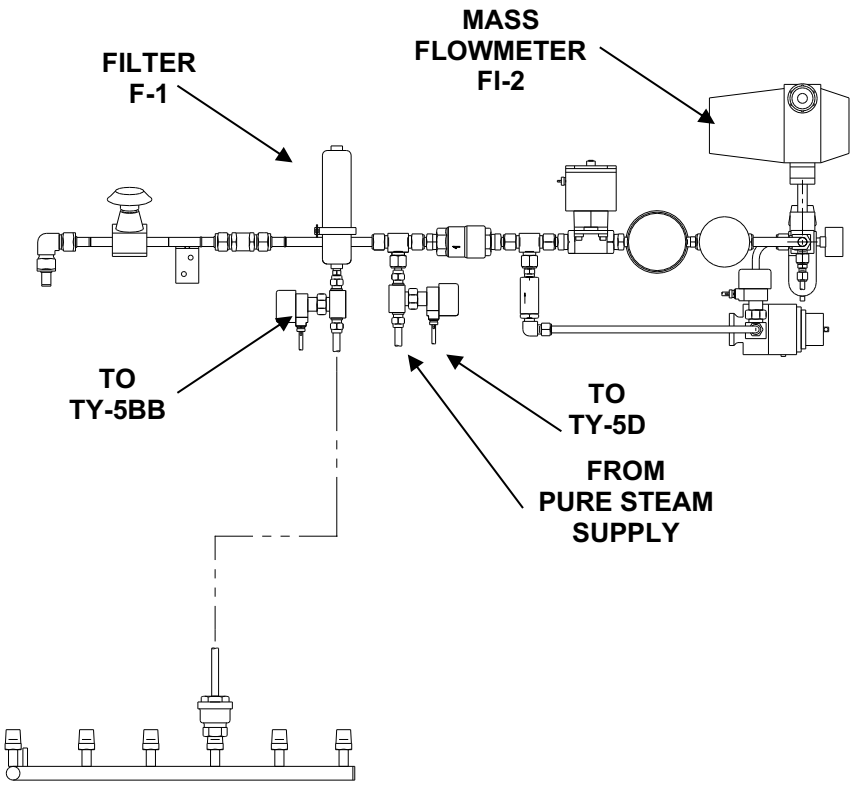
7.1 VALVING

Each gas is fed to the BioFlo 6000 through its own solenoid valve. The valves are located on the side of the piping skid below the main air connection.

Side View



Front View


MASS
FLOWMETER
FI-2
FILTER
F-1
TO
TY-5BB
TO
TY-5D
FROM
PURE STEAM
SUPPLY

7.2 FACTORY SETTINGS FOR FOUR GAS MIX

For units with the Four Gas Module installed, the ML-6100 is factory configured to run a four gas mix. The settings include the pH, DO and Pump1(base addition) control loops. The configurations for the pH and DO control loops are as follows:

pH Control Loop:

The pH controller is set to the following:

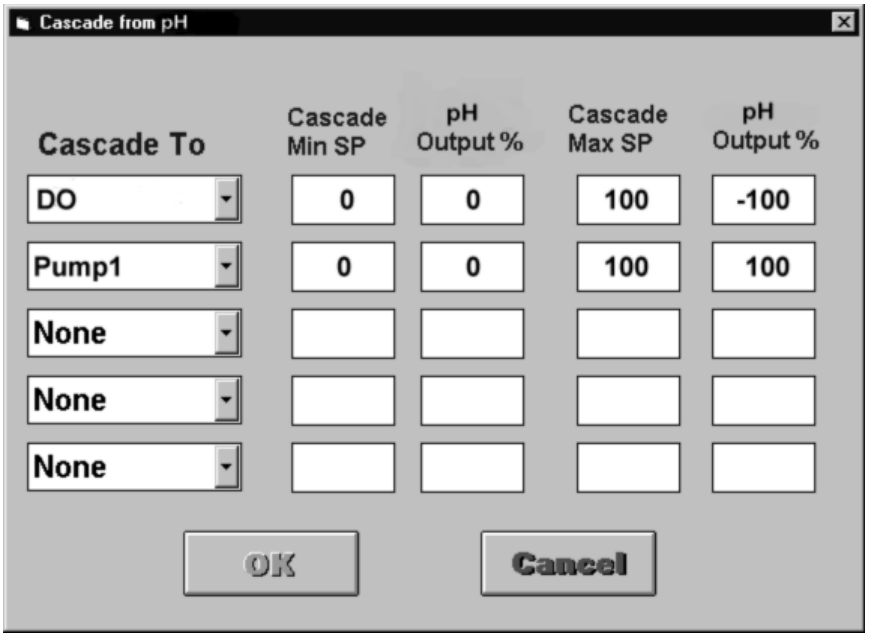
Control Mode	*Output Type*	*Output Action*
PID	-	-

with PID gains:

Proportional (P)	*Integral (I)*	*Derivative(D)*
10	2	0

A cascade from the pH control loop to the DO and Pump1 (base addition) control loops is also present. The cascade scheme is setup as follows:

Cascade To	*Cascade Min SP*	*pH Output %*	*Cascade Max SP*	*pH Output %*
DO	0	0	100	-100
Pump1	0	0	100	100



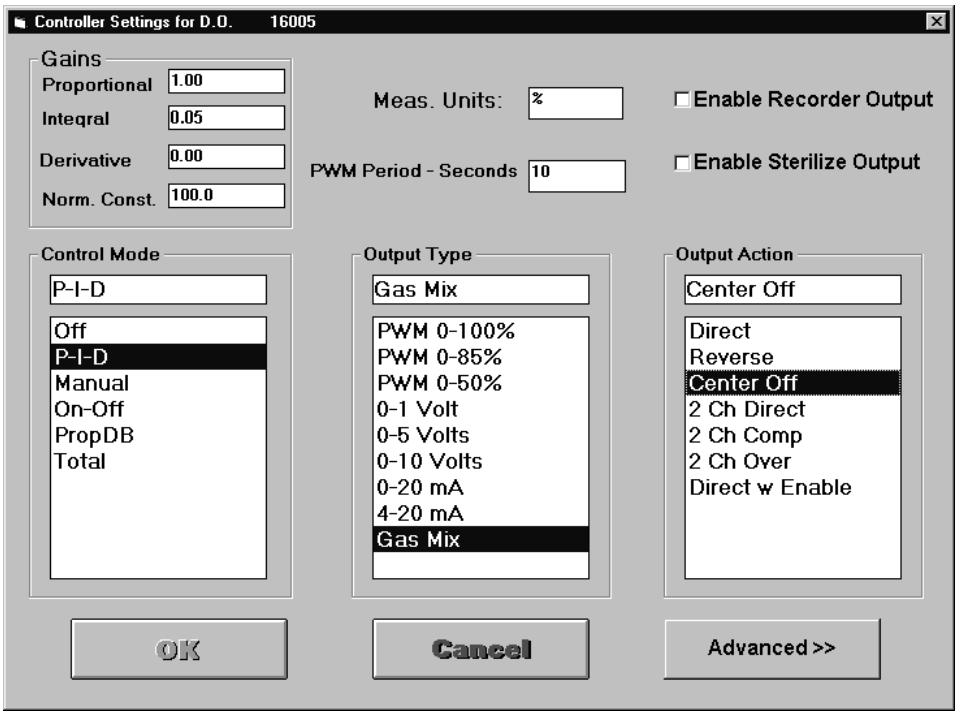
DO Control Loop:

The DO controller is set to the following:

Control Mode	*Output Type*	*Output Action*
PID	Gas Mix	Center Off

with PID gains:

Proportional (P)	*Integral (I)*	*Derivative(D)*
1	0.5	0

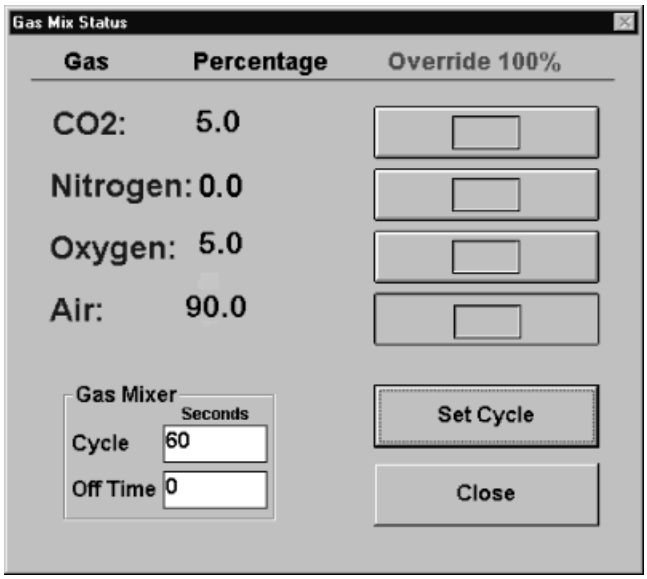


7.3 VIEWING GAS MIX STATUS

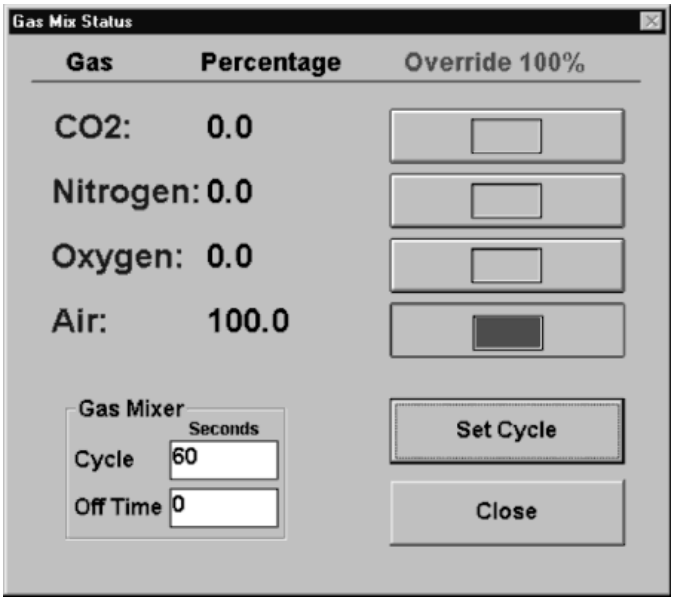
To view the current status of a gas mix, perform the following:

7.3.1 In the **GUAGE SCREEN**, press the control mode area of the gas mix loop display gauge.

7.3.2 The **GAS MIX STATUS WINDOW** will appear.



7.3.3 The current composition of the gas mixture is displayed in the percentage column. To override the gas mix controller, press the appropriate override button to force the selected gas to 100%.



7.3.4 To set the gas mix cycle, press the area inside the cycle text box. Enter the desired time that you wish to mix gases in seconds. To set the time that the gas mix will be off, press the area inside the off time text box. Once the cycle time and off time have been entered, press the **SET CYCLE BUTTON** to start the gas mix cycle.

NOTE:

The gas mix cycle time is used to ensure proper operation of the gas mix impeller. The exact settings are process dependent and must be empirically established for each process.

7.3.5 To exit this window, press the **CLOSE BUTTON**.

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8. PNEUMATIC HEADLIFT (OPTIONAL)

8.1 OPERATION OF HEADLIFT

In order to clean the inside of the vessel, the headplate must be raised. This can be done by means of the operation of the headlift system.

CAUTION !

If a double mechanical seal is installed, close Headplate Steam Valve (TCV-24A), Headplate Steam Water (TCV-24B), and Exhaust Condenser Water Valve (MCV-4) before opening headplate.

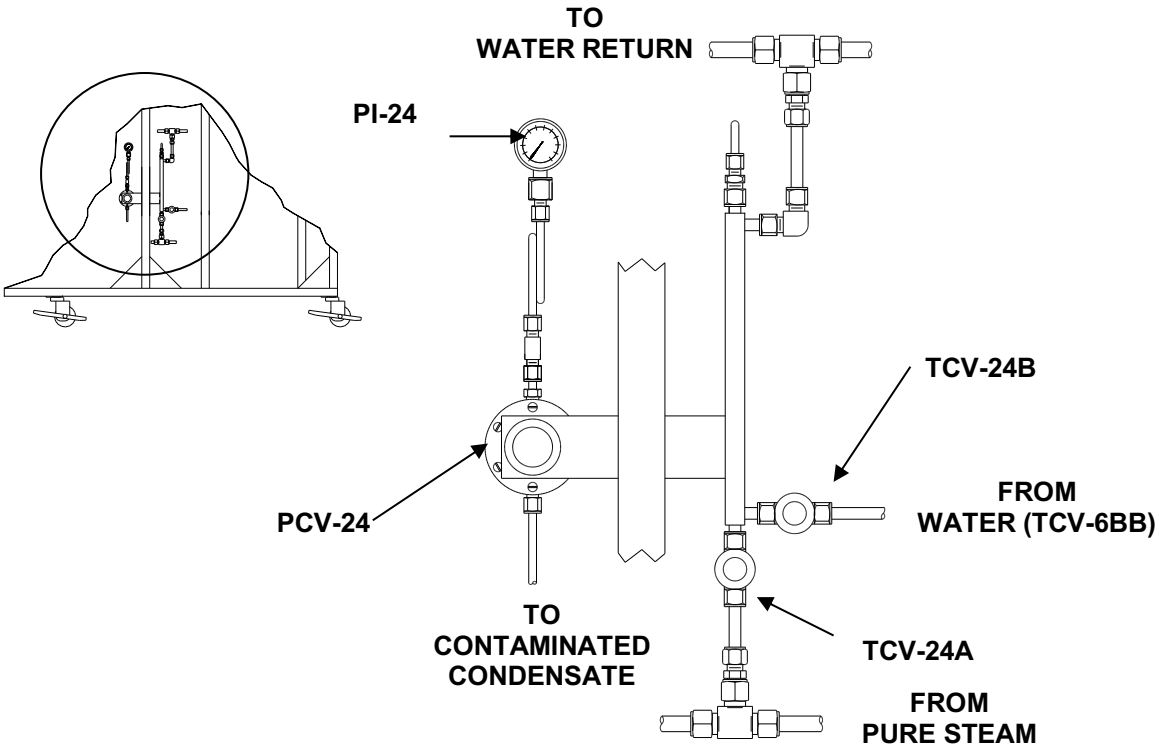
To raise the headplate:

8.1.1 Set all controller switches and systems to "OFF".

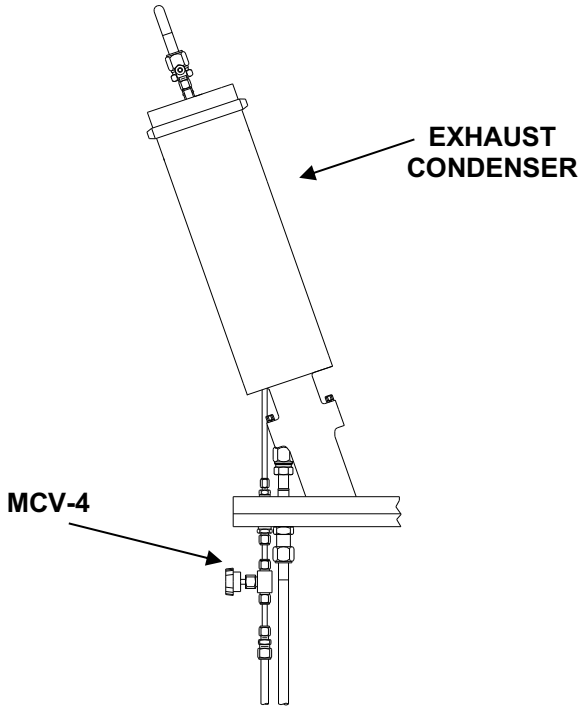
8.1.2 Close the following valves:

- **EXHAUST CONDENSER WATER (MCV-4)**
- **HEADPLATE STEAM (TCV-24B)**
- **HEADPLATE STEAM WATER (TCV-24A)**

TCV-24A AND TCV-24B DETAIL



MCV-24 DETAIL



8.1.3 Verify that the main power switches inside the ML-6100 controller cabinet are on.

8.1.4 Turn pressure control loop **OFF** and verify that vessel pressure is 0 psig.

8.1.5 Vessel and headplate cooled sufficiently for handling.

8.1.6 Remove the headplate bolts.

8.1.7 Remove safety pin from upper track.

8.1.8 Verify that the headplate is clear.

8.1.9 Set the headlift switch to the **UP** position.

NOTE:

As headplate raises, check for any mechanical interference. When the headplate reaches full height, an internal switch will limit further travel.

8.1.10 Insert the safety pin in upper track.

8.1.11 Turn the main power switches off.

8.1.12 To lower headplate reverse foregoing procedures. Make sure the headlift shuts off automatically or else the agitator will not operate, since it is interlocked to prevent operator injury.

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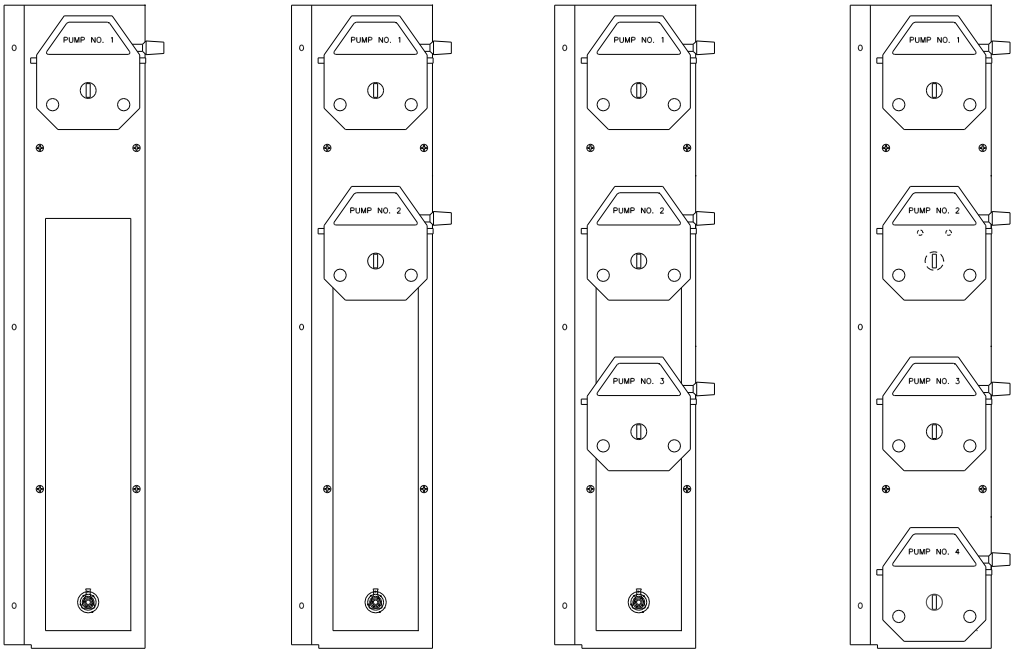
9. PUMPS (OPTIONAL)

The BioFlo 6000 is designed to accommodate up to four peristaltic pumps for nutrient addition, pH control, foam control, or other processes. When installed as an option, these pumps are mounted on the piping skid between the ML-6100 controller cabinet and the vessel.

Optional Pump Configurations



Each pump is labeled from one to four, depending on the number of pumps installed. In addition, the pumps may be assigned any one of a variety of functions through the ML-6100 controller. Refer to the *ML-6100 Multi-Loop BioProcess Controller, User's Guide* for detailed instructions. The standard pump configuration above uses 6 RPM motors which are PWM controlled.

9.1 PUMP CAPACITY

The following chart lists pumping capacities for the pumps that are available:

Masterflex L/S™ Tubing	*Flow Rate ml/rev*	*Discharge Pressure* Contin. psig(bar)*	*Discharge Pressure* Interm. psig(bar)*
L/S 13	0.06	25 (1.7)	40 (2.7)
L/S 14	0.21	25 (1.7)	40 (2.7)
L/S 16	0.80	25 (1.7)	40 (2.7)
L/S 25	1.70	20 (1.4)	35 (2.4)
L/S 17	2.80	15 (1.0)	20 (1.4)
L/S 18	3.80	15 (1.0)	15 (1.4)

* As tested Noprene, Pharmed, and Tygon Tubing. Values will be less for silicone and C-Flex Tubing.

9.2 TUBING SELECTION

Tubing Cross Sections	*L/S 13*	*L/S 14*	*L/S 16*
Inside Diameter in. (mm)	0.03 (0.8)	0.06 (1.6)	0.12 (3.1)
Hose Barb Size in. (mm)	1/16 (1.6)	1/16 (1.6)	1/8 (3.2)
Flow Range ml/min with 6 to 600 rpm drive	0.36-36	1.3-130	4.8-480
Max. Continuous Pressure	20 psig (1.4 bar)	20 psig (1.4 bar)	20 psig (1.4 bar)
Max. Intermittent Pressure	35 psig (2.4 bar)	35 psig (2.4 bar)	35 psig (2.4 bar)
Max. Vacuum	26 in. Hg (660 mm Hg)	26 in. Hg (660 mm Hg)	26 in. Hg (660 mm Hg)
Suction Lift	29 ft. H_2O (8.8 m H_2O)	29 ft. H_2O (8.8 m H_2O)	29 ft. H_2O (8.8 m H_2O)

Tubing Cross Sections	*L/S 25*	*L/S 17*	*L/S 18*
Inside Diameter in. (mm)	0.19 (4.8)	0.25 (6.4)	0.31 (7.9)
Hose Barb Size in. (mm)	3/16 (4.8)	1/4 (6.4)	3/8 (9.5)
Flow Range ml/min with 6 to 600 rpm drive	10-1000	17-1700	23-2300
Max. Continuous Pressure	20 psig (1.4 bar)	20 psig (1.4 bar)	20 psig (1.4 bar)
Max. Intermittent Pressure	35 psig (2.4 bar)	35 psig (2.4 bar)	35 psig (2.4 bar)
Max. Vacuum	26 in. Hg (660 mm Hg)	20 in. Hg (510 mm Hg)	20 in. Hg (510 mm Hg)
Suction Lift	29 ft. H_2O (8.8 m H_2O)	22 ft. H_2O (6.7 m H_2O)	22 ft. H_2O (6.7 m H_2O)

Tubing Information	*L/S 13*	*L/S 14*	*L/S 16*	*L/S 25*	*L/S 17*	*L/S 18*
*NBS Silicone**	P0740-2331	P0740-2325	P0740-2405	P0740-2505	P0740-2540	P0740-2646
Silicone (Peroxide)	96400-13	96400-14	96400-16	96400-25	96400-17	96400-18
Silicone (Platinum)	96410-13	96410-14	96410-16	96410-25	96410-17	96410-18
Tygon Lab (R-3603)	6409-13	6409-14	6409-16	6409-25	6409-17	6409-18
Tygon LFL	6429-13	6429-14	6429-16	6429-25	6429-17	6429-18
Tygon Fuel (F-4040-A)	6401-13	6401-14	6401-16	6401-25	6401-17	6401-18
Tygon Food (B-44-4X)	6419-13	6419-14	6419-16	6419-25	6419-17	6419-18

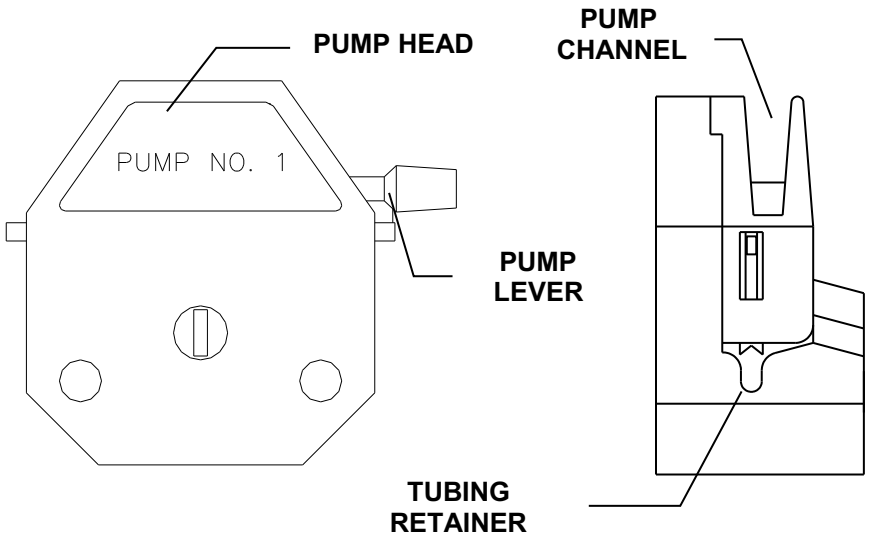
* Can only be ordered through NBS, the remaining are Masterflex part numbers

9.3 LOADING TUBING INTO PUMP HEAD

Before you insert the tubing into the **PUMP CHANNEL**, verify that the **PUMP LEVER** and the **TUBING RETAINERS** are in the open position. Perform the following steps to properly load tubing into the **PUMP HEAD**:

9.3.1 To open the **PUMP HEAD**, flip the black **PUMP LEVER** to the left.

Loading Tubing



9.3.2 On the right (outlet) side of the **PUMP HEAD,** press the black **TUBING RETAINER** inward and up until **TUBING RETAINER** is in the up position (retracted).

9.3.3 On the left (inlet) side of the **PUMP HEAD**, press the black **TUBING RETAINER** inward and up until **TUBING RETAINER** is in the up position (retracted).

9.3.4 Guide tubing into, and through the **PUMP CHANNEL**.

9.3.5 Flip the black **PUMP LEVER** on the **PUMP HEAD** to the right side and verify that the lever locks into place.

9.3.6 On the right (outlet) side of the **PUMP HEAD**, press the black **TUBING RETAINER** inward and down until **RETAINER** pinches the tubing.

9.3.7 On the left (inlet) side of the **PUMP HEAD**, press the black **TUBING RETAINER** inward and down until **RETAINER** pinches tubing.

10. FOAM CONTROL SYSTEM (OPTIONAL)

Aeration and agitation may cause foaming before inoculation and during fermentation. This unit may be supplied with the following system for control of the foam:

10.1 CHEMICAL FOAM CONTROL SYSTEM (WITH PERISTALTIC PUMP)

In order to control foam, a foam/level control system and an optional pump is required. A conductivity-type foam sensing probe is installed through a Tri-clamp fitting in the headplate of the fermentor. Contact with foam activates a peristaltic pump, which is connected aseptically to an appropriate port on the vessel by tubing, and introduces a chemical defoaming agent into the fermentor.

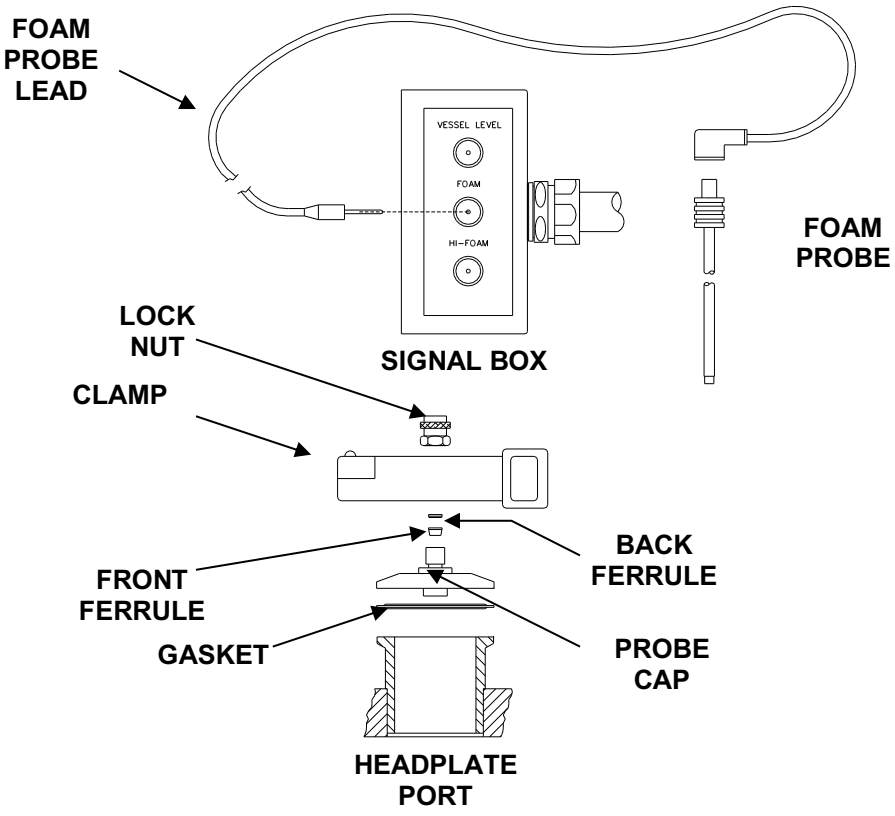
The control circuit includes a sensitivity adjustment to prevent minor splashing from causing spurious operation. Control in PWM mode allows for addition and mixing time, as desired. (The customer provides sterile antifoam in a suitable container for this system.) A steam-sterilizable port or a septum port may be provided in the upper wall of the vessel for antifoam addition.

When the foam control option is ordered, the ML-6100 is configured to include an antifoam control loop.

10.2 SETUP OF FOAM PROBE

Attach the **FOAM PROBE LEAD** to **FOAM PROBE** and plug other end into **FOAM PROBE JACK** on the **SIGNAL BOX** mounted on the bearing housing. Adjust the foam probe to the desired level and tighten the lock nut sufficiently to firmly hold the probe. Do not overtighten the lock nut. (This lock nut has Teflon ferrules and will extrude and the nut will then cut through the Teflon sleeve on the probe thus activating the system.

Foam Probe Setup



NOTE:

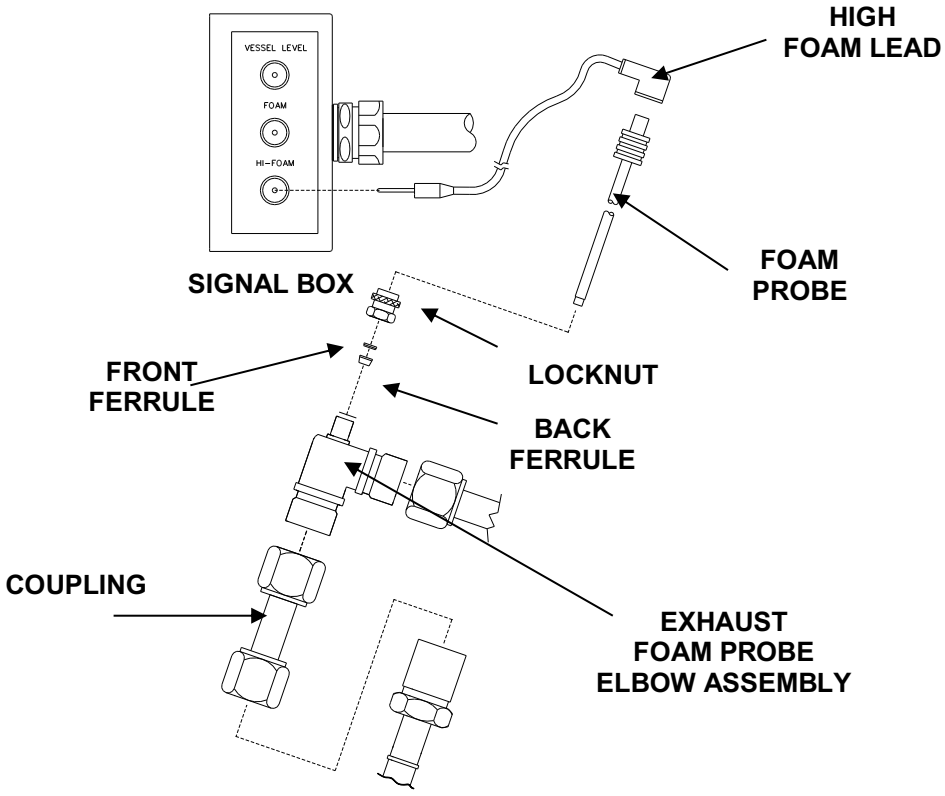
It is recommended to set up and adjust the foam probe before the sterilization cycle. The height of the probe may be adjusted after sterilization by loosening the ferrule and holding a swab of cotton that has been soaked with a suitable anti-bacterial solution around the probe.

Applications of antifoaming agents are at the discretion of the user. This agent should be miscible with water and thermally stable at sterilization temperatures (121°C-130°C) in order to sterilize it.

10.3 HIGH FOAM SAFETY SYSTEM (OPTIONAL)

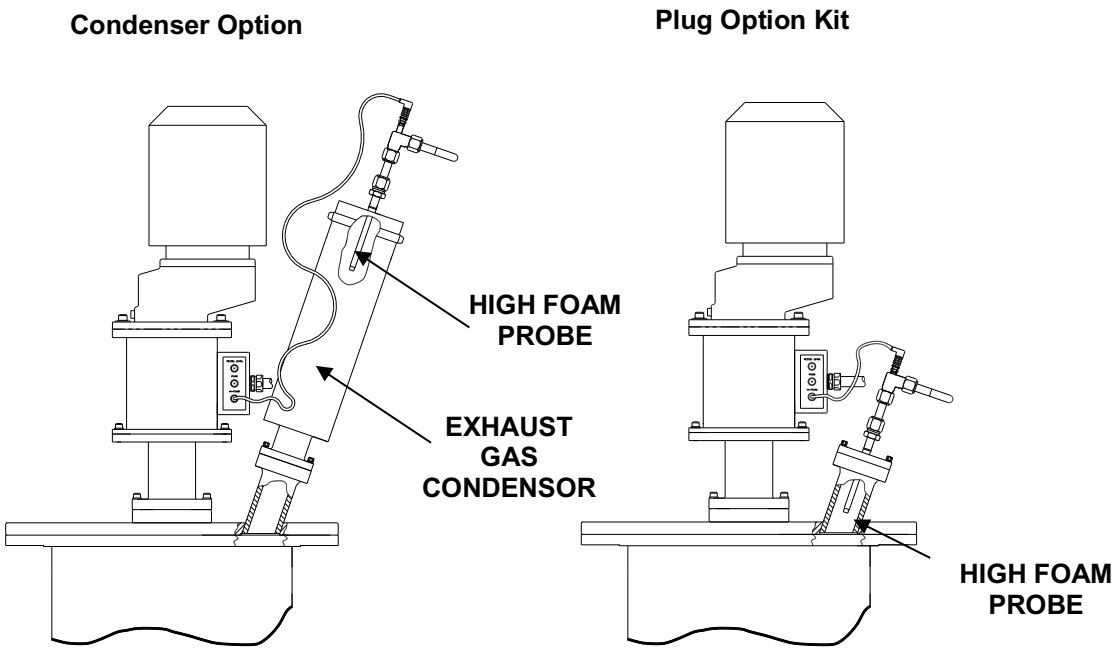
A separate foam probe may be supplied as an option to provide a check on the normal antifoam control system(s). This option involves a separate foam probe which should be adjusted at a higher level than the normal foam control probe. This device allows for the stopping of airflow and agitation (or any other loop) should a high foam condition occur.

High Foam Probe Detail



When the high foam control option is ordered, the ML-6100 is configured to include an high foam control loop.

High Foam Probe Mounting Options



Refer to the *ML-6100 Multi-Loop Bioprocessing Controller, User's Guide* for detailed instructions on setting up control loop alarm conditions.

The same precautions for adjusting and care of the regular foam probe applies in this case.

11. pH CONTROL SYSTEM (OPTIONAL)

During the growth cycle, control of the pH may be required. When a BioFlo 6000 is purchased with the option for pH control, the ML-6100 is preconfigured at the factory to include a pH control loop. Refer to the *ML-6100 Multi-Loop BioProcess Controller*, *User's Guide* for detailed instructions on changing setpoints and control of pH.

11.1 SYSTEM WITH PUMPS (OPTIONAL)

A pH electrode is furnished for side-mounting in a mating penetration in the lower side-wall of the fermentor vessel. The electrode assembly is steam-sterilizable-in-place at the same time the medium and vessel are sterilized. A signal from the pH electrode is conditioned and amplified near the probe for input to the ML-6100 which monitors pH in the fermentor vessel. When the pH exceeds the desired value (as preset) an addition of acid is introduced automatically into the vessel; when pH is too low an addition of base is introduced automatically into the vessel. In the PWM mode of control a timed addition can be used to permit sufficient time for the acid or base to be properly mixed with the fermentation both prior to a subsequent addition.

The pH value is also continuously displayed on the touchscreen. Two peristaltic addition pumps are required; one for acid addition and one for base addition. The customer provides sterile acid and base reagent and their containers. Optional steam-sterilizable capped ports with appropriate valving and traps installed in the upper side-wall of the vessel may be used for introduction of the acid and base to the fermentor vessel. Alternatively, optional hose barb ports or septum ports located in the headplate or upper side wall may be used for introduction of acid and base.

Operation, maintenance and troubleshooting of pH Probe and pH Controller are described in relevant manuals which accompany this manual.

11.2 CALIBRATION OF pH PROBE

Inspect the probe for possible shipping damage. If damage is observed or parts are missing immediately, notify the Service Department of New Brunswick Scientific Co., Inc.

Liquid Filled Probes:

Check the level of the reference electrolyte as described in the manufacturer's instructions. It should be about 1 cm below the filling orifices that are closed with rubber "T" stoppers. To add reference electrolyte take the filling pipette (P0740-4820) and fill them to 1cm below the orifices with Viscolyte B (P0860-0130) electrolyte.

NOTE:

The two chambers are filled with the same reference electrolyte. During normal operations the two rubber stoppers are removed. Liquid filled probes should then be assembled into the housing and pressurized. Refer to the *pH probe manual* supplied with the probe.

Gel Filled Probes:

Refer to manufacturer's instructions when using gel filled probes. These probes do not require pressurizable housings.

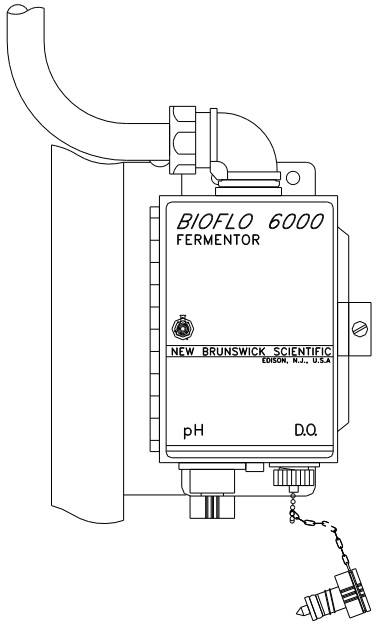
Calibration:

A two point calibration of the pH probe is performed with buffer solutions of known pH. Optimum results will be achieved if the buffer solutions are at the expected growth temperature.

Calibrate the pH probe before sterilizing vessel. Ensure that the calibration buffers are at the correct temperature. Calibrate the pH probe as follows:

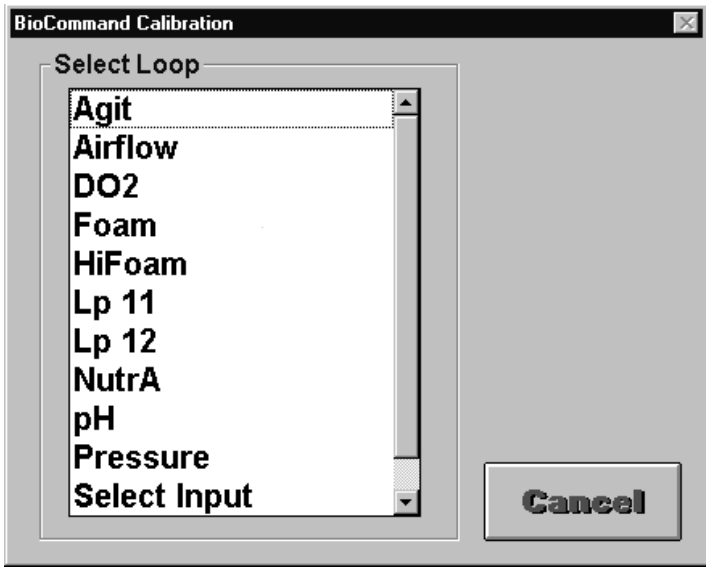
11.2.1 Connect pH probe to the coaxial connection on the DO/pH amplifier with pH cable. The DO/pH amplifier is located on the upright frame near the vessel. The coaxial connector is inserted into the amplifier connection that is located underneath the amplifier.

DO/pH Amplifier



11.2.2 In the **GUAGE SCREEN** of the touchscreen, Press the **CAL. BUTTON**.

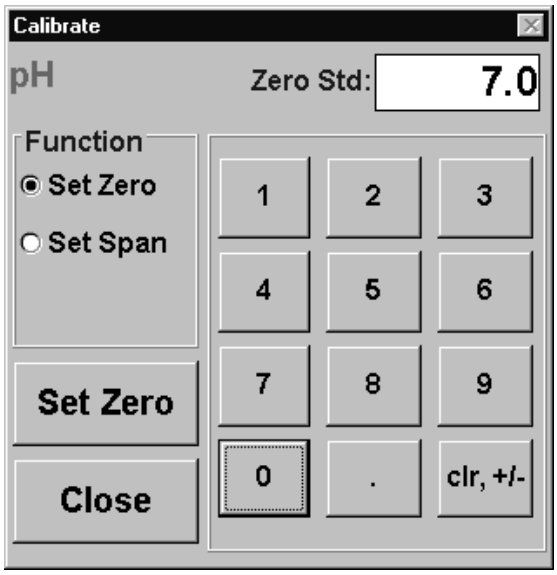
11.2.3 The **CALIBRATION SELECTION WINDOW** will appear.



11.2.4 Select the pH control loop from the list by pressing on pH.

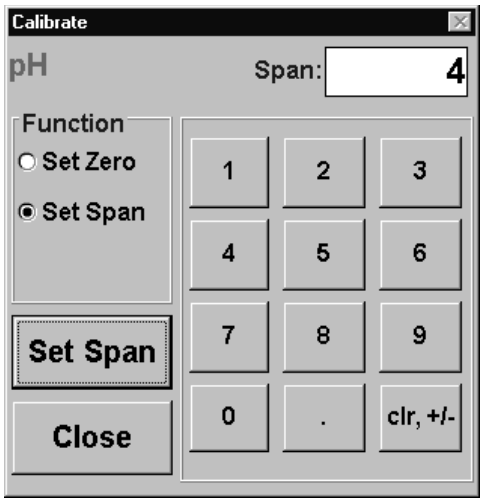
11.2.5 To set a “zero”, ensure that the set zero function is selected, and use the keypad to enter the appropriate value into the **ZERO STD** text box. Use the **Clr, +/- BUTTON** to clear any unwanted information from the text box.

11.2.6 Immerse the pH probe into an external buffer solution with a nominal pH of 7.00. Enter 7.00. Once the reading has stabilized, press the set **ZERO BUTTON**.



11.2.7 Immerse pH probe into a second buffer solution with a nominal pH of 4.00 or 10.00.

11.2.8 To set span, ensure that the **SET SPAN FUNCTION** is selected, and enter the appropriate value into the span text box. Use the **Clr, +/- BUTTON** to clear any unwanted information from the text box. Once the reading has stabilized. Press the **SET SPAN BUTTON**. The value entered will then become the span value.



11.2.9 After sterilization recheck the pH probe calibration by sampling the vessel contents and measuring its pH. Any small change may be compensated by adjusting the "zero" setting to the measured value.

11.2.10 Press **CLOSE** to exit this window.

11.3 RECALIBRATION AFTER STERILIZATION

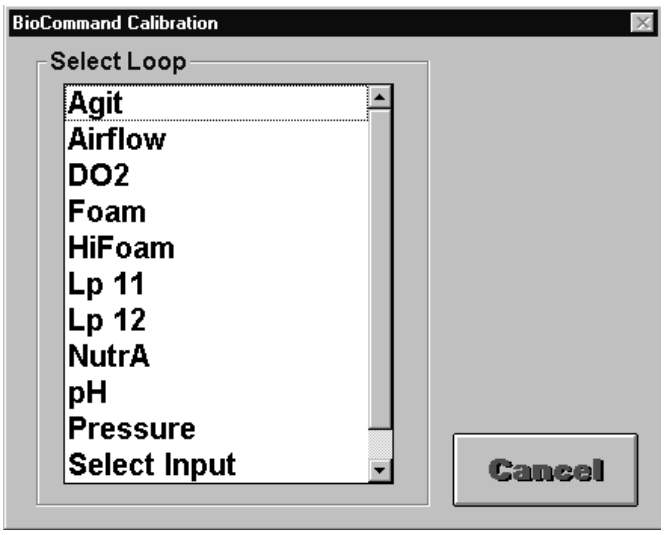
To recalibrate a pH probe after sterilization, perform the following:

11.3.1 Aseptically sample the culture medium. Refer to the *Sampling Valves* section of this manual for detailed instructions.

11.3.2 Using an external pH meter, check the pH of the sample.

11.3.3 In the **GUAGE SCREEN** of the touchscreen, Press the **CAL. BUTTON**.

11.3.4 The **CALIBRATION SELECTION WINDOW** will appear.



11.3.5 Select the pH control loop from the list by pressing on pH.

11.3.6 At the **CALIBRATE WINDOW**, ensure that the set zero function is selected, and use the keyboard to enter the measured pH value into the **ZERO STD** text box. Use the **Clr, +/- BUTTON** to clear any unwanted information from the text box. Press the set **ZERO BUTTON**.

11.3.7 Press **CLOSE** to exit this window.

11.4 SETTING UP A CASCADE FROM PH TO PUMPS

On the BioFlo 6000, installed pumps are numbered 1 through 4, with any number of pumps from 0-4 being installed .

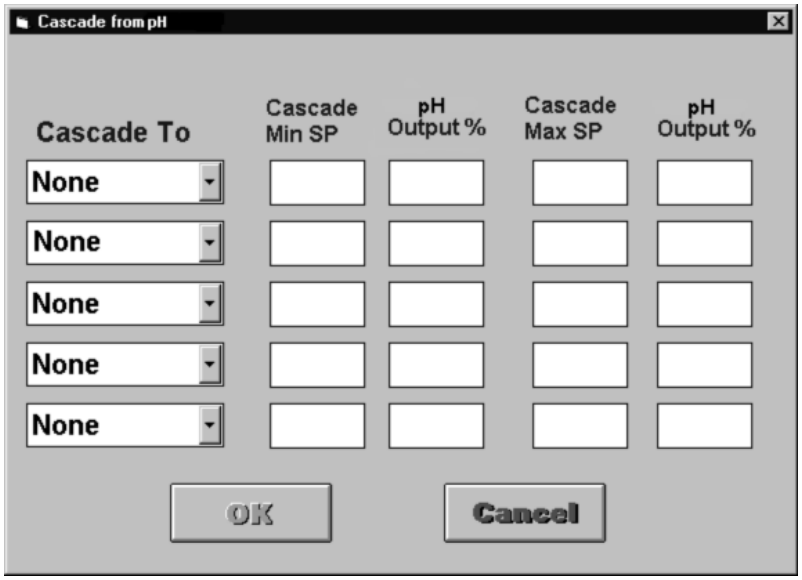
When setting up a pump for a particular function, pumps may be set up individually and controlled individually or cascaded from an appropriate control function such as the control of pH. For example, for the control of pH using an acid feed and a base feed, the pumps which control acid and base feed can be cascaded from pH.

In this example, **PUMP 1** will function as a base feed and **PUMP 2** will function as the acid feed pump from pH.

To cascade from the pH control loop to **PUMP 1** and **PUMP 2** perform the following:

11.4.1 At the touchscreen, go to the **GUAGE SCREEN**. Press the **CASC BUTTON**. The **CASCADE CONTROL SCREEN** will appear.

11.4.2 Select the control loop which you want to cascade from, in this case pH. The **CASCADE SCREEN** will appear.



11.4.3 The control loop selected, i.e. pH, will appear in the upper left as **CASCADE FROM pH**. The loop name, pH, will also appear above the columns labeled **OUTPUT%**.

11.4.4 In the column labeled **CASCADE TO**, select **PUMP 1** in the first data box. If **PUMP 1** is not a selection, refer the instructions for adding a loop in the *ML-6100 Multi-Loop Bioprocessing Controller, User's Guide*.

11.4.5 In the column labeled **CASCADE SP**, enter the minimum setpoint for pH. Regardless of output of the cascade-from loop, the setpoint of this cascade to loop will not go below the value you enter here.

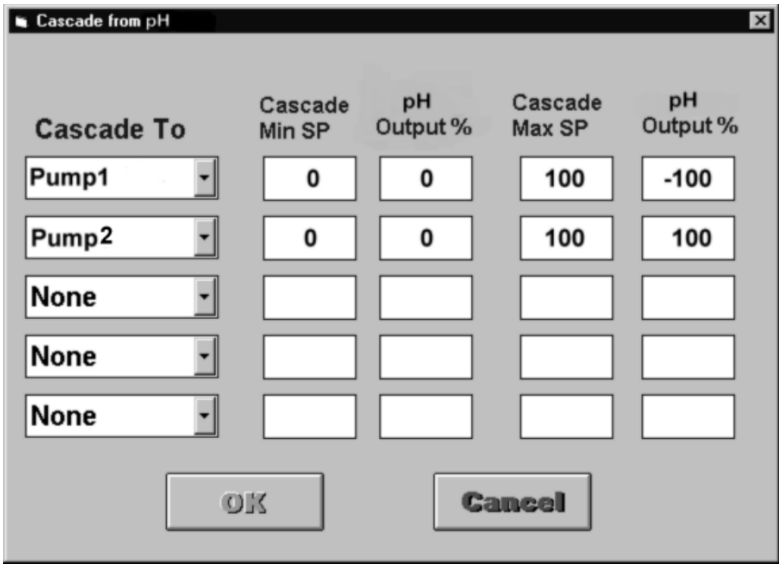
11.4.6 In the first data entry box of the **OUTPUT %** column (immediately to the right of the **CASCADE MIN SP** column), enter the minimum *cascade-from* loop output that will produce the minimum setpoint entered in the previous column. Lower outputs will not change the setpoint.

11.4.7 In the first data entry box of the of the **CASCADE MAX SP** column, enter the maximum setpoint for this loop. Regardless of output of the cascade-from loop, the setpoint of this *cascade-to* loop will not go above the value you enter here.

11.4.8 In the first data entry box of the last **OUTPUT %** column, enter the maximum c*ascade-from* loop output that will produce the maximum setpoint entered in the previous column. Higher outputs will not change the setpoint.

11.4.9 This completes the setup for **PUMP 1** cascaded-to the pH control loop.

11.4.10 **PUMP 2** is cascaded from pH in the same manner as described above by entering **PUMP 2** in the data box.



11.4.11 When data entry is complete, press **OK**.

12. DO CONTROL (OPTIONAL)

During the growth cycle, control of the dissolved oxygen (DO) level may be required. The BioFlo 6000 may be supplied with an optional system for control of DO. When a BioFlo 6000 is purchased with the option for DO control, the ML-6100 is preconfigured at the factory to include a DO control loop.

An optional side entering polarographic dissolved oxygen electrode manufactured by either Broadley James or Ingold may be provided. The DO probe with replaceable membrane is inserted into a side wall port subsequent to filling the vessel. Alternately, a DO probe which can be inserted through a headplate port may be used.

Once inserted into the vessel, the DO probe is connected via a probe cable to the DO/pH amplifier that is located on the upright frame at rear of the piping skid near the vessel. The connector is inserted into the amplifier connection that is located underneath the amplifier.

The DO probe generates a current signal that is amplified and conditioned near the probe to become the input to the ML-6100 System that monitors the dissolved oxygen. Dissolved oxygen is read on a scale of 0-l00% that represents the percentage of saturation at a particular pressure and temperature of the medium. See the ML-6100 Manual to easily calibrate the instrument at the operating pressure and temperature.

When the dissolved oxygen level deviates from the desired value, the controller changes the agitation rate (the aeration rate or the nutrient flow rate) as set into a control strategy by operator. In some systems, that is, those with automatic backpressure control, the controller can change the vessel pressure when the dissolved oxygen level deviates from the desired value. Refer to the *ML-6100 Multi-Loop Bioprocessing Controller, User's Guide*, for instructions on setting up DO cascades.

The probe is steam sterilized in place at the same time the medium and vessel are sterilized.

Operation, maintenance and troubleshooting of DO Probe and ML-6100 controller are described in relevant manuals that accompany this manual.

12.1 CALIBRATION OF DO PROBE

Inspect the probe for possible shipping damage. If damage is observed or parts are missing, immediately notify the Service Department of New Brunswick Scientific Co. Inc.

The optional DO probe provided may be an Ingold polarographic probe or a Broadley James polarographic probe and should be set up in accordance with its operating manual.

Polaraographic probes require a polarization period of at least 6 hours before calibration can be attempted. Polarization is accomplished by connecting the probe to the DO/pH amplifier and turning the power to the fermentor on. The probe is polarizing as long as the power is turned on and the probe is connected. Alternately, a polarizing device is available from NBS or the probe manufacturer, that keeps the probe polarized when disconnected from the fermentor. This module may be connected directly to the probe to maintain the probe in a polarized state.

A two point calibration of the DO probe should be performed after each sterilization. Since the DO reading varies with temperature, calibration should be carried out at the growth temperature where DO control is required.

The DO zero point may be calibrated in one of two ways:

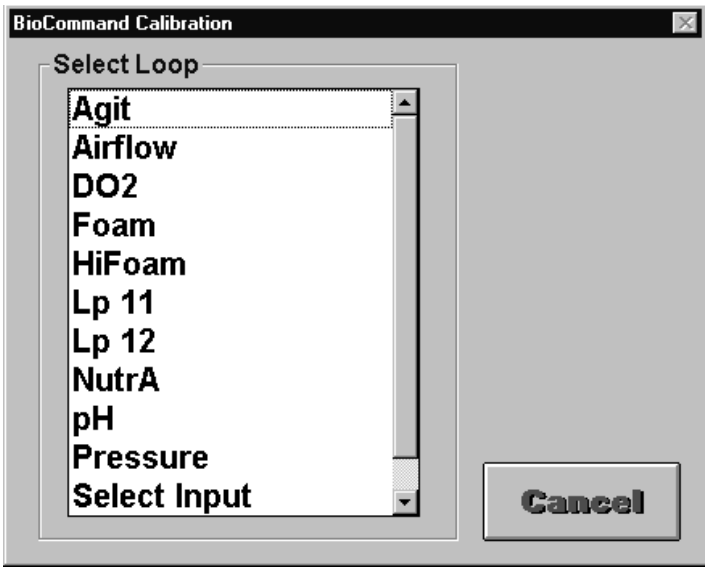
- An electronic method
- Sparging nitrogen.

Electronic Method:

12.1.1 Polarize the probe for a minimum of six hours.

12.1.2 At the touchscreen, press the **CAL. BUTTON** in the **GAUGE SCREEN** of the touchscreen.

12.1.3 The **CALIBRATION SELECTION WINDOW** will appear.



12.1.4 Select the DO control loop from the list by pressing on it.

12.1.5 Unplug the DO probe from the amplifier.

12.1.6 With the probe unplugged, set the "zero". Ensure that the set zero function is selected, and use the keypad to enter the appropriate value into the **ZERO STD** text box. Use the **Clr, +/- BUTTON** to clear any unwanted information from the text box. Once the desired number is entered, press the set zero number.

NOTE:

Do not leave the DO disconnected from the amplifier for more than one minute. Prolonged disconnection will result in depolarization of the probe.

Sparging with Nitrogen:

A more accurate zero setting is obtained by assuring the probe has stabilized at the desired growth temperature and then by setting agitation speed to 500 rpm and sparging nitrogen into the vessel. After the DO reading stabilizes for at least 5 minutes, enter the "zero" on the **PROBE CALIBRATION SCREEN**.

To calibrate the span:

To calibrate the span value, the fermentor should be on and operating at the desired growth temperature. Then proceed as follows:

12.1.7 Set the agitation speed to 500 rpm and **vigorously sparge air** into the vessel.

12.1.8 Carefully monitor the current value reading of DO control loop display gauge. Once the reading has stabilized, press the **CAL BUTTON**.

12.1.9 The **CALIBRATION SELECTION WINDOW** will appear.

12.1.10 Select the DO control loop from the list by pressing on it.

12.1.11 The **LOOP CAILBRATION SCREEN** will appear.

12.1.12 To set the span, ensure that the **SET SPAN FUNCTION** is selected, and enter 100 into the span text box using the keyboard. Once the number is entered, press the set span button. The value entered will then become the span value.

12.1.13 Press **CLOSE** to exit this window.

NOTE:

The probe is calibrated in percentage saturation at a given temperature, a given pressure (calibration conditions). If pressure is raised, then readings may be obtained which are higher than 100%.

13. PREPARING FOR FERMENTATION

After becoming familiar with the components and operation of each of the systems, subsystems, options and controller that are included on your BioFlo 6000, you are ready to set up the fermentor for a run.

NOTE:

When preparing the fermentor for a run always make sure that all hardware is setup to control the process prior to entering any setpoints or activating the system at the controller.

13.1 SYSTEMS CHECK

Prior to running a fermentation make sure that all standard and any optional systems provided with the BioFlo 6000 are operational:

- Exhaust condenser
- Gas Overlay
- Foam Control .
- High Foam Control
- Level Control
- Pneumatic Headlift
- pH control
- DO control
- Automatic Back Pressure Control
- Pumps
- Addition Valves
- Sample Valves

In addition, perform the following:

13.1.1 Ensure that the fermentor is clean and ready for operation, and confirm that all O-rings, gaskets, seals, filters and septa (when present) are in place and in good condition.

13.1.2 Check to make sure that the drain valve and any sample and addition valves present are closed.

13.1.3 Make sure that the **RTD TEMPERATURE PROBE** is securely in place in the vessel.

13.1.4 If sensors such as pH, DO, foam, high foam and level probes are to be used, make sure that they have been properly inserted and are secure.

13.1.5 Connect the pH and DO probe cables to the probes and connect the connector to the pH and DO amplifier.

13.1.6 Connect the foam, high foam and level probe cables to the appropriate probes and connect the coaxial connector end to the appropriate connection point in the signal box that is mounted on the bearing housing.

13.1.7 Any other sensors, such as cell mass sensors, etc., or sample devices, should also be inserted at this time and properly connected to the appropriate set of terminals inside the ML-6100 controller cabinet.

13.1.8 Ensure that fermentor is ready to run by confirming that all the services have been properly connected and regulated.

13.1.9 At the touchscreen make sure the main screen has been configured to allow for all the process parameters to be monitored and controlled during the run.

13.1.10 Enter all set points and confirm that you have selected the desired mode of operation for each process loop.

13.1.11 The vessel is now ready for fermentation.

13.2 ADDING MEDIA TO THE VESSEL

Media may be added to the vessel in several ways. Usually the headplate is left in place and media is added through the powder or liquid charging/light port. To do so, remove the light element from the powder or liquid charging/light port by opening the Tri-clamp connector.

Dry components may be added through this port. Liquid may then be added and the solids dissolved in the vessel. (The unit and agitator may be turned on to facilitate dissolution of the solids.) Liquid should be added to a level not greater than the maximum working volume allowable for the vessel, with allowances for the inoculum which will be added after sterilization.

Liquid media may also be added through the powder/ light port or through any of the other normally plugged ports in the headplate.

Once the media has been added, the port that was used, must be closed. Verify that all valves are closed.

NOTE:

Media, liquid and solid can be also added to the vessel by removing the headplate and adding the required components. If media is added in this manner, make sure that the headplate is securely in place prior to turning on the system.

13.3 STERILIZATION OF VESSEL USING HEAT LABILE MEDIA

Cell culture media is often composed of a variety of heat labile components. If filter sterilized media is to be added to the vessel, the vessel may be sterilized empty or with phosphate buffered saline (PBS) in the vessel. After sterilization the PBS can be aseptically removed and presterilized liquid media (or media components) can be added aseptically though a resterilizable addition port.

If the vessel is to be sterilized empty, the instructions for sterilization with liquid in the vessel should be followed with the exception that the drain valve should be open instead of closed during sterilization

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14. AUTOMATIC STERILIZATION

Automatic Sterilization is activated at the touchscreen interface. The ML-6100 controller is preconfigured for this feature.

14.1 STERILIZATION (WITH LIQUID IN VESSEL)

This procedure utilizes the temperature control elements of the System. Before proceeding, the Water Priming Procedure must be complete. Then proceed as follows:

14.1.1 Insert pH, DO, Foam, and level probes (if used) into Vessel and connect probes into proper jacks the piping skid.

14.1.2 Verify that mineral oil or glycerin has been added to the thermowell and that the RTD Temperature probe is properly inserted and connected

14.1.3 Close the **DRAIN VALVE (FCV-19A)**, the **SAMPLE VALVE (FCV-18A)** and all lower vessel valves. Check that all lower vessel ports are sealed. Make sure that ring nuts on ports are tight.

14.1.4 Open the **POWDER OR LIQUID CHARGING/LIGHT PORT CAP** or other suitable port and add water and/or nutrient that is not heat labile. Make allowances for inoculum to be added later. Replace fill port cap used and tighten securely (hand tight is sufficient).

14.1.5 Verify that both control switches inside the ML-6100 controller cabinet are turned on and that the touchscreen and computer are also on and operational.

14.1.6 At the touchscreen, set temperature control loop for the desired operating temperature after sterilization. Turn the temperature control loop **ON** by setting the temperature control loop to **PID** control mode.

14.1.7 Set the sparge air flow rate and the vessel pressure desired for operation after sterilization. For units equipped with a thermal mass flowcontroller or automatic back pressure control, these two control loops should set to **PID** control mode.

14.1.8 Ensure all headplate ports and upper side-entry ports are closed, such as:

- **INOCULATION (FCV-21A)**
- **ACID ADDITION (FCV-22A)**
- **BASE ADDITION (FCV-25A)**
- **ANTIFOAM ADDITION (FCV-20A)**
- **NUTRIENT ADDITION (FCV-26A)**
- **ADDITION VESSEL SUPPLY VALVES (FCV34,44,54,etc.)**

All ports should have a piece of equipment inserted or be capped or plugged. All clamps on ports should be secure.

14.1.9 Turn on the **AGITATION SWITCH** (on switch panel) and set the agitation rate at the touchscreen. During sterilization, agitation control loop should be set to a minimum rate of 150 rpm. Turn on the agitation control loop by putting loop in **PID** control.

14.1.10 If there is a double mechanical seal on the fermentor, do not operate agitation unless there is some liquid in the vessel and **SEAL STEAM CONDENSATE (TCV-24B)** and **SEAL STEAM CONDENSER WATER (TCV-24A) VALVES** are open.

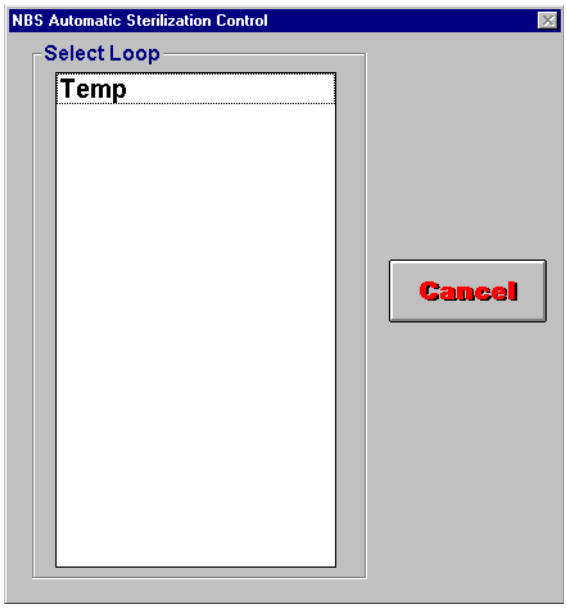
NOTE:

The **SEAL STEAM CONDENSER WATER VALVE (TCV-6BB)** is shut off automatically during sterilization and will be turned back on immediately afterwards.

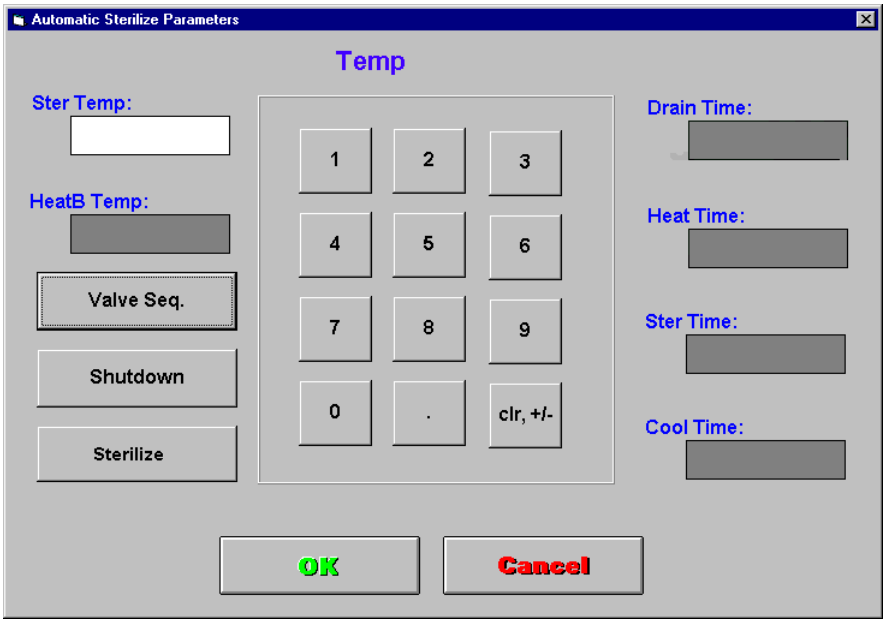
14.1.11 Open the following valves:

- **SPARGE VALVE (FCV-23B)**
- **OVERLAY VALVE (FCV-23C)**
- **DRAIN VALVE STEAM (TCV-19A)**
- **SAMPLE STEAM (TCV-18A)**
- **STEAM VALVES TO ALL INSTALLED RESTERILIZABLE ADDITION VALVES**

14.1.12 Go to the touchscreen. From the **GAUGE SCREEN**, press the **STERIL. BUTTON**. The **NBS AUTOMATIC STERILIZATION CONTROL DIALOG** will appear.



14.1.13 Select the temperature control loop by pressing on the its name. The **AUTOMATIC STERILIZE PARAMETERS WINDOW** will appear. The selected control loop name will appear at the top of the window.



Enter the parameters by pressing on each of the text boxes and entering the values on the keyboard. Press **OK** to accept the parameters. The parameters are as follows:

- **STER TEMP** Sterilization Temperature, range: 0°C-140°C.
- **HEAT B TEMP** Temperature at which steam is allowed to directly enter the vessel, in °C. The must be less than or equal to (≤) the **STER TEMP**.
- **DRAIN TIME** Drain Time, range: 0-500 minutes.
- **HEAT TIME** Heat Time, range: 0-500 minutes. For the fastest heat time possible, set to 0.
- **STER TIME** Sterilization Time, 0-500 minutes.
- **COOL TIME** Cool Time, 0-500 minutes. For the fastest cool time possible, set to 0.

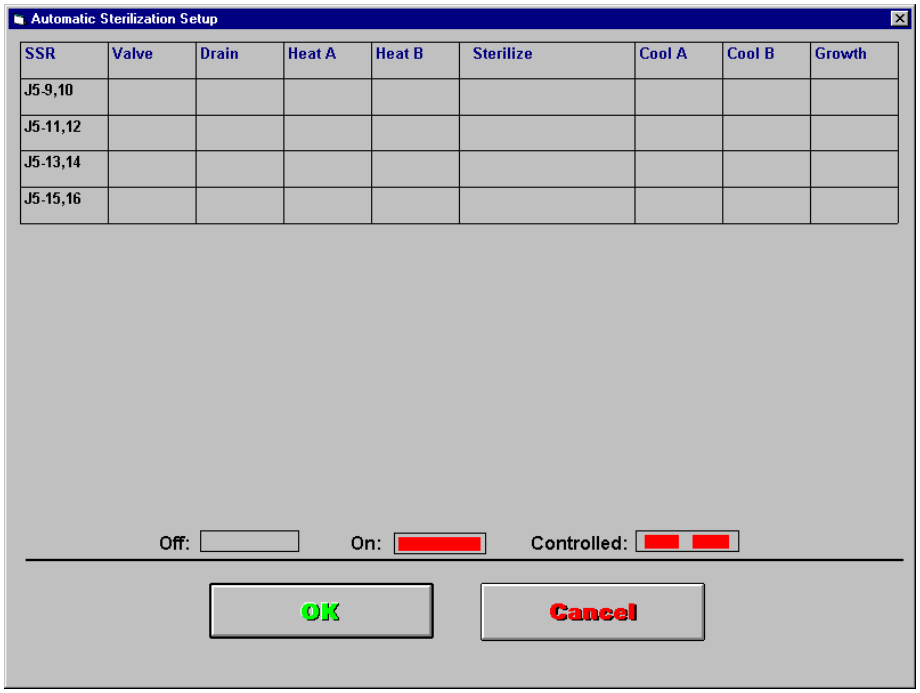
Press **OK** to accept the parameters. The **SYSTEM CONFIGURATION PASSWORD PROMPT** will appear if the controller configuration is passworded. Enter the **SYSTEM CONFIGURATION PASSWORD** to accept the changes to the **AUTOMATIC STERILIZE PARAMETERS WINDOW**.

14.2 VALVE SEQUENCING

The ML-6100 Controller has been preconfigured to sequence a set of valves on the BioFlo 6000 during the automatic sterilization cycle. Refer to *Appendix D* for *Factory Set Valve Sequencing Settings*. The operator may make changes to the valve sequencing by following these instructions.

To sequence a set of valves for automatic sterilization, press the **VALVE SEQ. BUTTON** in the **AUTOMATIC STERILIZE PARAMETERS WINDOW** to access the **AUTOMATIC STERILIZATION SETUP WINDOW**. Valve sequencing is done by labeling each valve with its appropriate connection and placing the specified operation of the valve in each column or step in the sterilization process. This is accomplished by touching the screen at the appropriate valve box. The screen will change to indicate whether the value is off or controlled.

After the valve operation data has been entered, press **OK**. The **SYSTEM CONFIGURATION PASSWORD** prompt will appear. Enter the password on the keyboard and press **OK**. If the **SYSTEM CONFIGURATION PASSWORD** is set to blanks, the prompt will not appear. The software will then return to the **AUTOMATIC STERILIZE PARAMETERS WINDOW**.

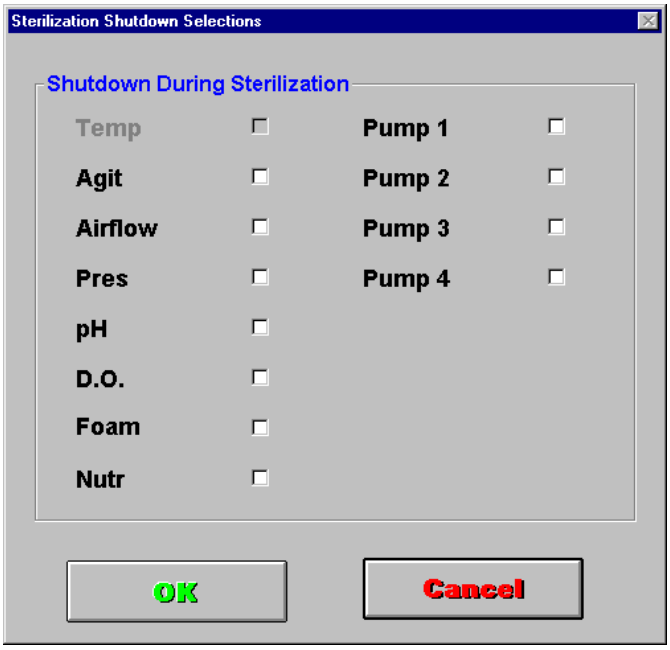


The column headings or operations for valve sequencing are as follows:

- **VALVE** Valve name or ID.
- **DRAIN** Jacket drain operation.
- **HEAT A** Heating at temperatures below the **HEAT B** temperature setting in the **AUTOMATIC STERILIZE PARAMETERS WINDOW** and which steam does not directly enter the vessel.
- **HEAT B** Heating at temperatures above the **HEAT B** temperature setting in the **AUTOMATIC STERILIZE PARAMETERS WINDOW** and which steam directly enters the vessel.
- **STER** Heating operation at sterilization temperature
- **COOL A** Cooling with temperature more than 10°C above normal operation temperature setpoint.
- **COOL B** Cooling with temperature within than 10°C above normal operation temperature setpoint.
- **GROWTH** Cooling with temperature within than 1°C above normal operation temperature setpoint.

14.3 SHUTDOWN DURING STERILIZATION

The **AUTOMATIC STERILIZATION PARAMETERS WINDOW** also includes a shutdown feature. This allows the user to shutdown or disable specific loops during the sterilization process. Once the sterilization process is enabled, selected loops are in operative until the sterilization process is completed and the sterilization control loop has returned to its normal operating temperature setpoint.

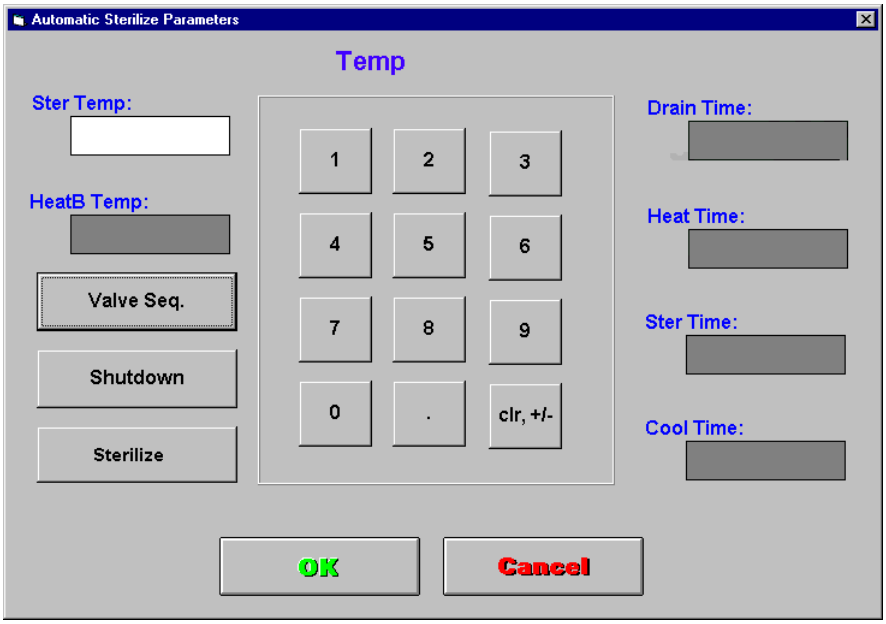


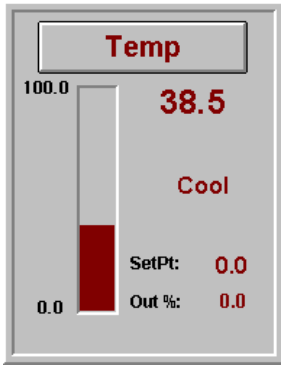
To shutdown or disable loops during sterilization, press the **SHUTDOWN BUTTON**. The **STERILIZATION SHUTDOWN SELECTIONS WINDOW** will appear. Select the loops you wish to disable by pressing on the check box next to each loop. The sterilization loop will be grayed out in the loop list to prevent it from being selected. Make your selections and press **OK** to accept the selections.

It is recommended that the airflow and pressure control loops always be shutdown during sterilization. Shutdown of all other control loops is optional depending on whether or not they are connected to the vessel or not. If liquid is in the vessel, the agitation loop should not be shutdown.

14.4 ENABLING STERILIZATION

To start the sterilization process, press the **STERILIZE BUTTON**. The software will then return to the **GAUGE SCREEN**. Each phase of the sterilization process will be displayed in the control mode of display gauge of the selected control loop.

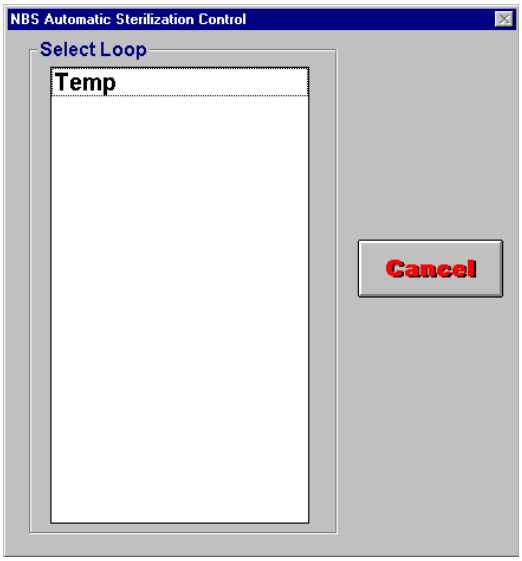




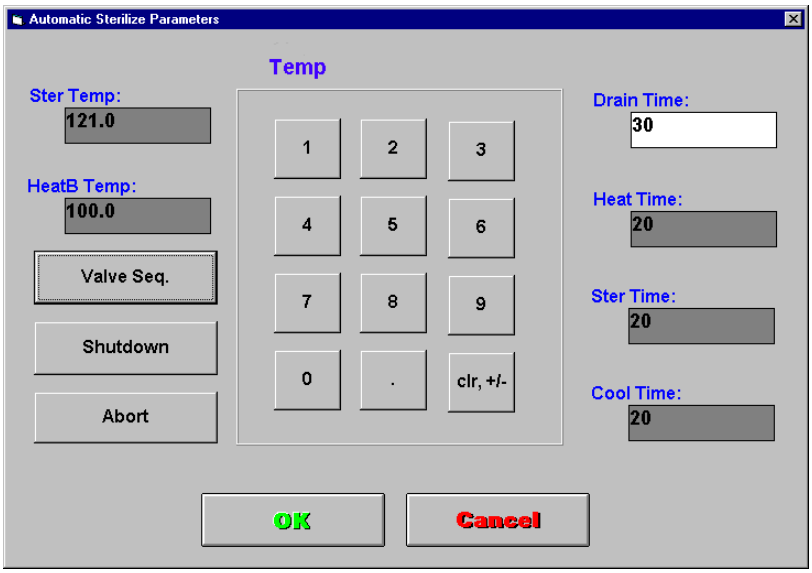
14.5 ABORTING STERILIZATION

The sterilization process may be stopped or aborted at any given time after the process has been started. To abort the sterilization process perform the following:

14.5.1 From the **GAUGE SCREEN**, press the **STERIL. BUTTON**. The **NBS AUTOMATIC STERILIZATION CONTROL DIALOG** will appear.



14.5.2 Select the control loop by pressing on the loop name. The **AUTOMATIC STERILIZE PARAMETERS WINDOW** will appear, but will be slightly modified. Once the sterilization process is enabled, the **STERILIZE BUTTON** is replaced by the **ABORT BUTTON**.



14.5.3 To cease the sterilization process, press the **ABORT BUTTON**. The software will return to the **GAUGE SCREEN**.

14.5.4 The word **COOL** will be displayed in the control mode of the control loop display gauge until the current temperature of the loop is within its setpoint limits.

14.6 STERILIZATION (VESSEL EMPTY)

If the vessel is to be sterilized empty, the instructions for sterilization with liquid in the vessel should be followed with the exception that the drain valve should be open instead of closed during sterilization

15. INOCULATION AND ADDITION VALVES (OPTIONAL)

Resterilizable valves are available for 19 mm and 25 mm ports. These two position (poppet) addition valves are designed so that attachment to the vessel is via retaining ring which holds the valve on to a welded fitting. A steam inlet line delivers pressurized steam to the interior and exterior of the valve before and after each use. Liquid is transferred via a quick-connect fitting. A stainless steel cap maintains sterility of the fitting. Outlet steam is fed to a condensate trap. The valve is designed to eliminate blind pockets and crevices. The two position valve is spring loaded so that the entrance port opens a steam path though a hollow tube. When the valve in the forward position, the tube is extended into the vessel so that sterile liquid may be introduced into the vessel.

The prerequisites for sterile inoculum and addition transfer are:

- Sterile culture medium in the culture vessel cooled down to the operating temperature.
- Adjustment of suitable airflow rate and setup of sterile inlet and exhaust conditions.
- Adjusted agitation speed.
- Adjusted pH of the culture medium.
- Suitable seed culture conditions.

15.1 STERILIZATION

To make an addition aseptically, the valve must be sterilized prior to use. It is a good habit to resterilize the valve immediately after each use so that is ready for any subsequent addition.

To sterilize the valve, perform the following:

15.1.1 Ensure sure that the stainless steel cap is in place.

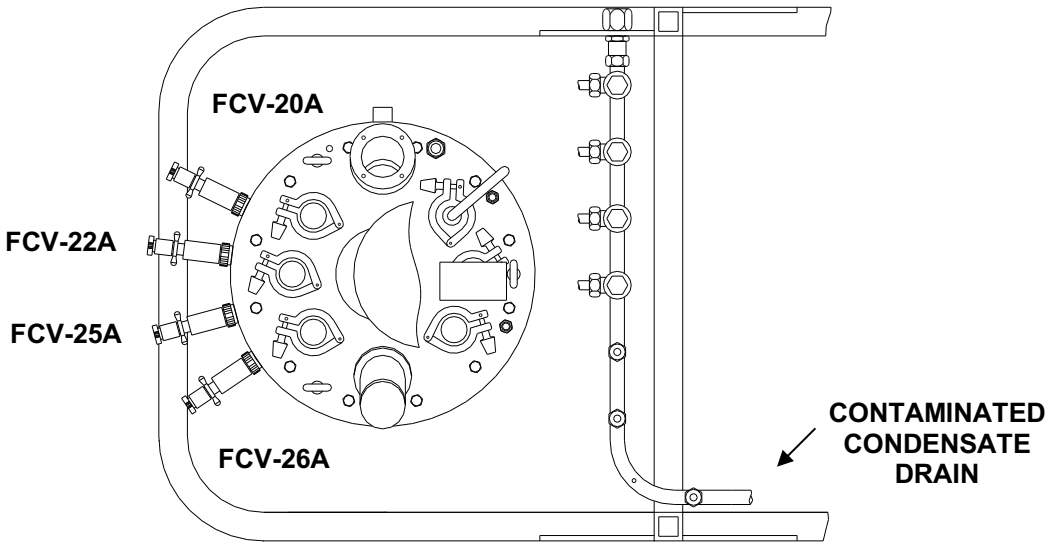
15.1.2 Open the **STEAM INLET VALVE** (for 19 mm ports: **TCV-20A**, **TCV-22A**, **TCV-25A**, or **TCV-26A** and **TCV-22A** for the 25 mm port).

15.1.3 Allow steam to pass through the valve for about 15 minutes.

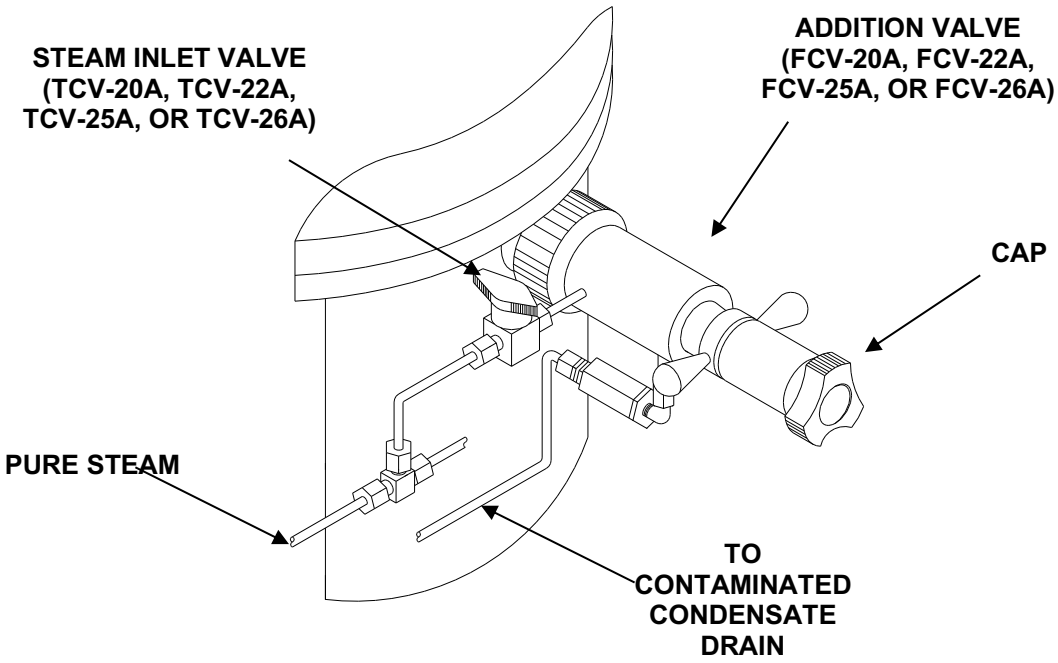
15.1.4 Close the **STEAM INLET VALVE**.

15.1.5 Allow the valve to cool thoroughly before using.

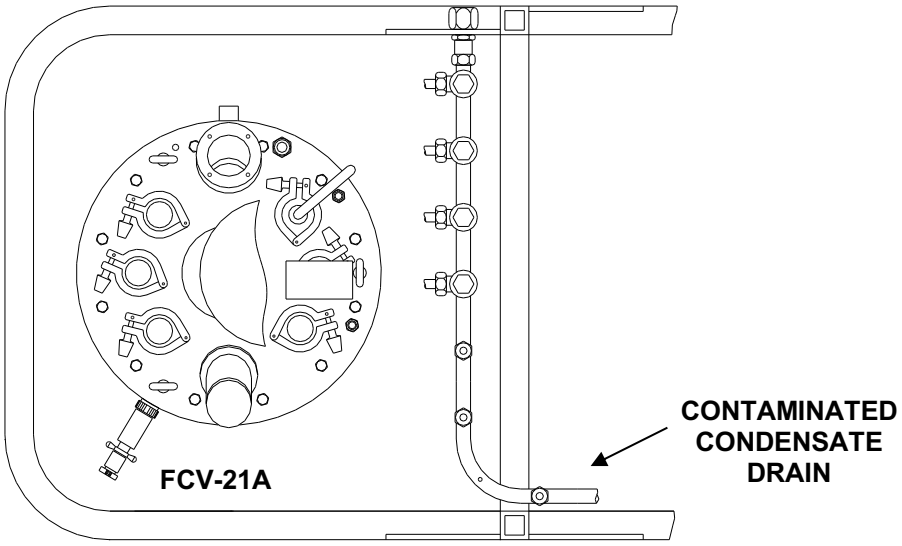
19 mm Addition Valve Locations



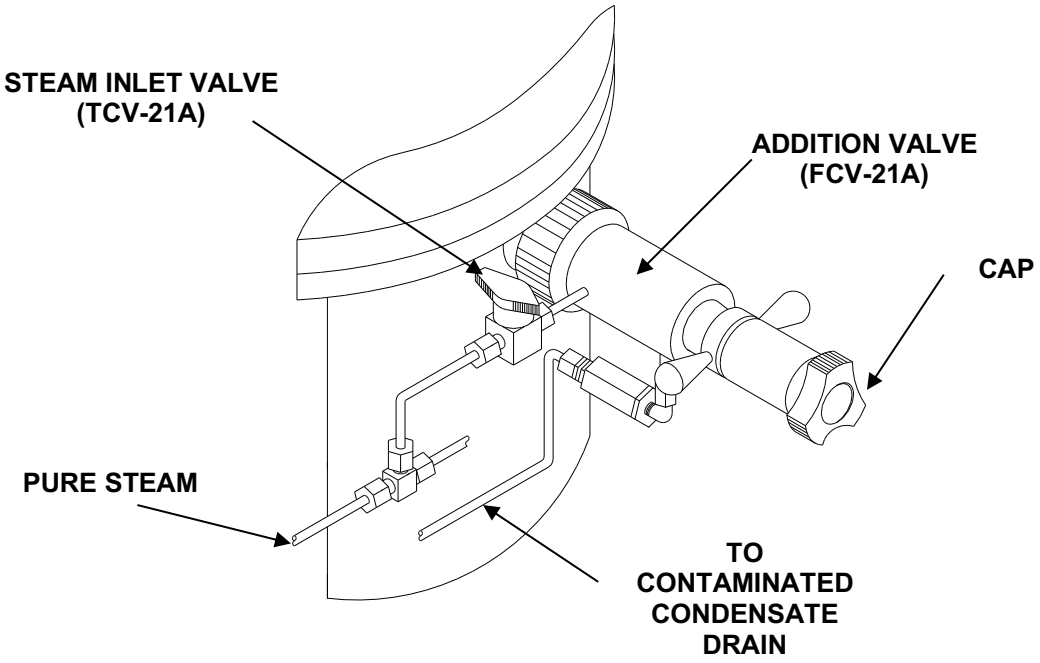
19 mm Addition Valve Detail



25 mm Addition Valve Location



25 mm Addition Valve Detail



15.2 ADDITION AND INOCULATION

15.2.1 Prepare a presterilized additive in an appropriate addition vessel fitted with tubing (and a fitting which allows easy connection to the quick-connect)

15.2.2 Reduce the vessel pressure to 0 psig manually or at the touchscreen

15.2.3 Assuming a previously sterilized port, carefully remove the inoculation port steam cap and aseptically attach an inoculation container using silicone or tygon type tubing.

15.2.4 To open the inoculation valve, press the black, polymer handles in (toward the vessel) and turn clockwise. This brings the valve's entrance port forward into the vessel.

15.2.5 Addition of the liquid may be accomplished by gravity feed or by a peristaltic pump.

15.2.6 After inoculation, close the inoculation valve by pressing the black polymer handles in turn counterclockwise and release.

15.2.7 Break the addition vessel connection by removing the connecting tubing from the quick connect fitting.

15.2.8 Replace the stainless steel cap. Resterilize the port.

Follow this procedure for any other additives.

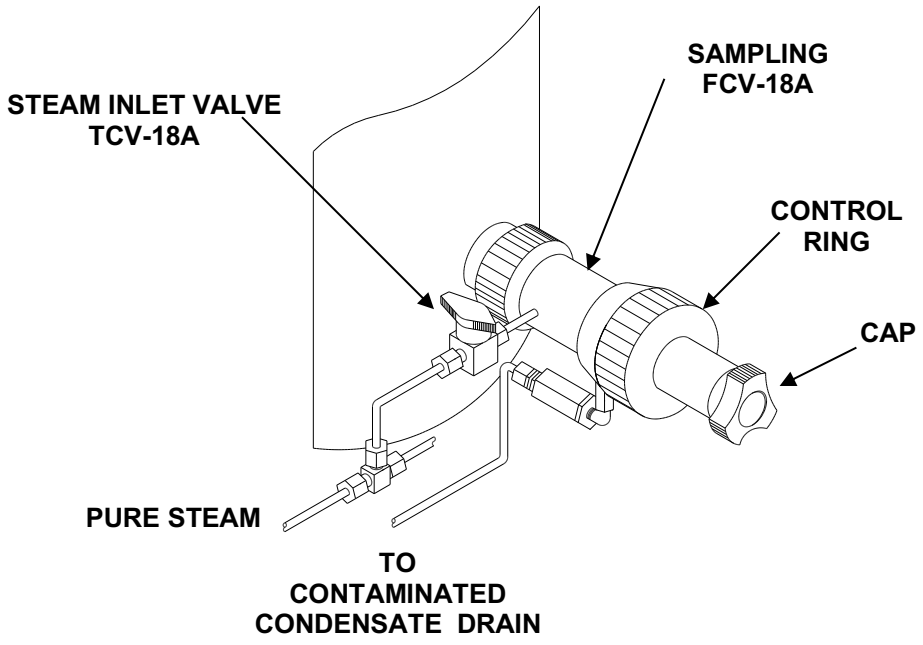
16. SAMPLING VALVES (OPTIONAL)

Resterilizable sample valves are available for 19 mm and 25 mm ports. The variable flow sample valves are designed so that attachment to the vessel is via retaining ring which holds the valve on to a welded fitting. A steam inlet line delivers pressurized steam to the interior and exterior of the valve before and after each use. Liquid is transferred via a quick-connect fitting. A stainless steel cap maintains sterility of the fitting. Outlet steam is fed to a condensate trap. The valve is designed to eliminate blind pockets and crevices. The multiposition valve is spring loaded so that the entrance port opens a steam path though a hollow tube. When the control ring is turned clockwise, the tube is extended into the vessel so that sterile liquid may be extracted.

To take a sample aseptically the valve must be sterilized prior to use. It is a good habit to resterilize the valve immediately after each use so that is sterile and cooled for any future sampling.

In situations where aerosol containment is necessary, an aerosol containment system is available for both the 19 and 25 mm sampling ports. Refer to the *Replacement Parts and Accessory Information* section of this manual or contact your local NBS sales representative or parts distributor for details.

Sampling Valve Detail



16.1 STERILIZATION

16.1.1 Make sure that the stainless steel cap is in place.

16.1.2 Open the **STEAM INLET VALVE (TCV-18A)**.

16.1.3 Allow steam to pass through the valve for about 15 minutes.

16.1.4 Close the **STEAM INLET VALVE**.

16.1.5 Allow the valve to cool thoroughly before using.

16.2 SAMPLING

16.2.1 Prepare an appropriate sample vessel ready to receive the sample (or vessel fitted with tubing and a fitting which allows easy connection to the quick-connect connected to the quick-connect).

16.2.2 Remove the protective stainless steel cap.

16.2.3 Position the sample vial (tube, etc., sampling of liquid is usually accomplished by gravity), and aseptically connect the sample line onto the quick-connect fitting

16.2.4 To open the channel into the vessel, turn the clear polymer **CONTROL RING** clockwise. This brings forward the valve's entrance port into the vessel. The adjustment is variable so that the flow can be controlled so that a sample of a few milliliters can be taken or the flow can be increased so that a larger sample may be taken.

16.2.5 To close the valve, turn the clear polymer **CONTROL RING** counterclockwise.

16.2.6 Remove the connecting tubing from the sample vessel from the quick connect fitting.

16.2.7 Replace the stainless steel cap, and sterilize the valve.

17. SHUTDOWN

17.1 SHUTDOWN PROCEDURE

After the fermentation, instrument operation and services supplied must be terminated. It is recommended to proceed with the following steps:

17.1.1 Set the pH control loop to off.

17.1.2 Set the DO control loop to off.

17.1.3 Set the foam control loop to off.

17.1.4 Set the pressure control loop to off.

17.1.5 Set the airflow control loop to off.

17.1.6 Stop agitation.

17.1.7 Stop steam supply to vessel.

17.1.8 To drain the contents of the vessel, remove the vessel drain port cap. Using the Tri-clamp fitting on the port connect an appropriate hose for transferring.

17.1.9 If the fermentor is to remain off for an extensive period of time, all service supply valves should be closed.

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18. PREVENTIVE MAINTENANCE

Preventive maintenance is performed to keep equipment in proper working order. When periodically performed, preventive maintenance will result in longer life for the equipment and will reduce time lost due to equipment failure.

18.1 THREE MONTH INTERVALS

Perform the following checks and inspections:

18.1.1 Turn off all power to the fermentor and close all service supply valves.

18.1.2 Inspect O-rings on the headplate and their mating surfaces for resilience, tears, cuts or other conditions that may interfere with sealing.

18.1.3 Check all fuses on the equipment to make sure the fuses and holders have not corroded.

18.1.4 Check all controls and accessible items (switches, knobs, connections, pilot lights and fuse holders) to make sure that they are properly tightened. Tighten any component that is loose.

18.1.5 With steam **OFF**, open and examine steam traps. In order to clean them, unscrew the top. Examine the interior. Remove any foreign matter from the trap.

18.1.6 Open and clean all inline check valves which may see media from the vessel, especially **(FCV-23A)**. This valve may need to be cleaned more often depending on media.

18.1.7 Check all hand valves, both on the vessel and piping skid for tightness of the valve packing (just beneath the handle), which should be tightened to produce a slight drag on the valve handle when operated.

18.1.8 Open and inspect, clean, or replace all prefilters on services (i.e., steam, water and air).

18.1.9 Remove Vessel bottom drain valve and inspect the gaskets and packing for excessive wear.

18.1.10 Replace all O-rings on the Inoculation, **DRAIN** and **SAMPLING PORTS**. Coat O-rings with **LIGHT** film of silicone grease.

18.1.11 Check air flow for any variation from maximum with vessel empty and with water in vessel.

18.1.12 Set back pressure regulator or pressure control loop for 20 psig vessel pressure and turn off the entire unit once this pressure is reached. Note any vessel pressure decrease. If there is more than a 2 psi drop in one hour, check for leakage.

18.2 ONE YEAR INTERVALS

Perform all the preceding checks and inspections plus the following:

18.2.1 Check and clean all check valves.

18.2.2 Install repair kits in air operated valves.

18.2.3 Check all O-rings used in penetrations in the vessel.

18.3 CLEANING

18.3.1 After each fermentation, remove any dirt from the ML-6100 controller and motor controller cabinets, wiring cables and connectors.

18.3.2 Use a clean, dry, lint-free cloth or a dry brush for cleaning of the outer part of the fermentor. If necessary, wipe any parts on the headplate with a cloth saturated with water. The vessel may be scoured and flushed clean. Do not use steel wool to clean the vessel.

18.3.3 To maintain better life from O-rings and gaskets, be sure all surfaces are clean and that gaskets and O-rings are wiped clean before reassembling filter covers, fermentor tops, vessel and all gasket openings. A **LIGHT** coat of silicone grease should be applied to all O-rings before reassembling.

18.4 WATER FILTER (WATER PREFILTER AND REGULATOR KIT)

When filter is clogged and flow is restricted replace filter element as follows:

18.4.1 Close the **WATER VALVE (HS-4)**.

18.4.2 Place a container for water under the pet-cock drain valve on the water filter.

18.4.3 At the bottom of the filter, open the pet-cock valve to release residual pressure, then close it.

18.4.4 Located on top of the filter, unscrew the hex head bolt until the body of the filter is free.

18.4.5 Remove the cotton filter element(s) and replace with a new one(s).

18.4.6 Reassemble the filter, making sure the O-ring is seated properly between the filter body and head.

18.4.7 Replace the hex head bolt, check that the small O-ring is in place, then tighten the bolt securely.

18.4.8 Check that the pet-cock valve is closed, then open the **WATER VALVE (HS-4)**.

18.5 STEAM FILTER (STEAM PREFILTER AND REGULATOR KIT)

The steam filter is normally removed and cleaned when a noticeable pressure drop in steam pressure is indicated on the steam gauge.

18.5.1 At the rear of the unit, close the **MAIN STEAM VALVE (HS-6A)**.

18.5.2 Make sure the valve is tightly closed. Check the steam gauge. Wait until the filter cools down before proceeding.

18.5.3 Open any steam valve to release pressure within the steam filter, then close the valve.

18.5.4 On top of the filter, unscrew the hex head bolt until the filter body is separate from the top.

18.5.5 Raise the filter body up to clear the head.

18.5.6 Unscrew the large wing-nut on top of the filter element.

18.5.7 Clean off the outside of the filter element with water.

18.5.8 Insert a water hose into the large opening of the element and plug-up the smaller hole. Flush the element with water.

18.5.9 At this time, check the following components for wear, cuts and fit:

- Large O-ring on head of filter
- Small O-ring of shaft
- Sealing Washer
- Head Gasket

If the large O-ring, sealing washer or head gasket is damaged, replace as required. If the small O-ring requires replacement, perform the following:

NOTE:

Applicator is supplied with the unit.

18.5.10 Be sure that the old O-ring has been removed, and the center rod is clear and free of any foreign particles, dirt, etc.

18.5.11 Lubricate the new O-ring with a good commercial O-ring lubricant or silicone grease.

18.5.12 Place the applicator over the threads at the lower end of the center rod.

18.5.13 Place the O-ring over the tapered end of the applicator, then roll the O-ring over the applicator until the O-ring rolls off the end of the applicator and into the O-ring groove.

18.5.14 After all components have been checked or replaced, reassemble the filter in the reverse manner in which it was disassembled.

18.5.15 Open the **MAIN STEAM VALVE (HS-6A)**.

18.6 STERILE AIR FILTER, INLET AND EXHAUST

To replace an element, unscrew the clamp at center of filter and release top cover from filter base.

When replacing an element, be careful not to damage filter element or the O-ring at base of element.

Remove the cartridges from the sterile air inlet and exhaust filters, and check them for damage or signs of blockage. Also check the O-ring on the cartridge for tears, resilience and other conditions that may interfere with sealing. Apply a small amount of silicone grease to this O-ring before reassemble. If this O-ring is found to be damaged, ensure its replacement is made of Ethylene Propylene.

These cartridges have a minimum use of 20 sterilizations and it is difficult to tell their condition from visual inspection; bubble point, DOP testing or other tests of this type are the only way to guarantee integrity.

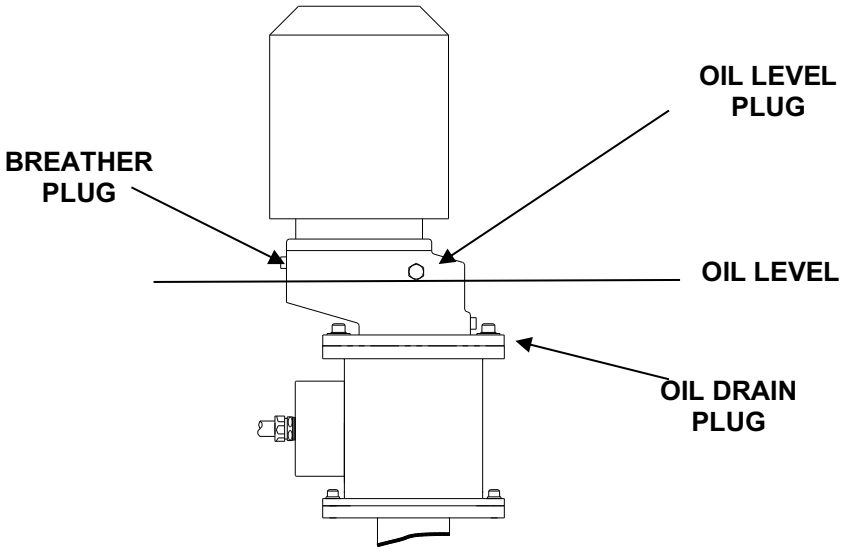
One should also check the color of these cartridges, the exhaust cartridge will normally show signs of media color, if the inlet cartridge or inner filter body show signs of media penetration, check valve **(FCV-23A)** in the air line should be cleaned or replaced.

This check valve can be a major problem area especially if medias with solids are being used.

18.7 MOTOR AND GEAR REDUCER

Oil levels and quality should be checked at regular intervals, depending on usage. Oil changes are required at intervals of 10,000 operating hours, or every two years, which ever comes first. If a synthetic oil lubricant is used then this period can be extended to 20,000 operating hours or every four years. In applications where hostile operating conditions exist, such as high humidity, corrosive environment, or large temperature changes, the lubricant should be changed at more frequent intervals.

Motor and Gear Reducer Detail



Oils for Motor and Gear Reducer

Ambient Air Temperature °F	*kin viscosity at 40 °C (cSt)*	*Gulf Oil Co.*	*Chevron Oil Co.*	*American Oil Co.*	*Mobil Oil Co.*	*Shell Oil Co.*	*Texaco Oil Co.*
+104 to +32	220	Gulf EP. Lubricant S100	Chevron Non-Leaded Gear Compound 220	Permagear EP220	Mobilgear 630	Shell Omala Oil 220	Meropa 220
+77 to +5	155	Gulf EP. Lubricant S60	Chevron Non-Leaded Gear Compound 220	Permagear EP150	Mobilgear 629	Shell Omala Oil 100	Meropa 150

Grease for Ball and Roller Bearings

Gulf Oil Co.	*Chevron Oil Co.*	*American Oil Co.*	*Mobil Oil Co.*	*Shell Oil Co.*	*Texaco Oil Co.*
Gulfcrown Grease EP. No. 2	Chevron Dura-Lith EP2	Amoilth Grease No. 2 EP	Mobilux EP2	Alvania Grease R3	Multifak EP2

Grease packed bearings should be cleaned and regreased every 10,000 hours or 20,000 hours for synthetic grease. Input (high speed) bearings should not be overgreased. They should be filled with grease not to exceed 1/3 of the bearing's fee volume. For output bearings and bearings with replaceable grease shields, fill 2/3 of their free volume.

NOTE:

When the recommended lubricant is not available, it is permissible to use a lubricant having equivalent characteristics but it is not recommended to mix different brands. Under no circumstances should synthetic lubricants be mixed with one another, or with one having a mineral base.

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19. TROUBLESHOOTING

If any problems occur with your fermentor, do not attempt to perform any service on the equipment. Unauthorized servicing may void the warranty. Please contact your local NBS Representative. In any correspondence with NBS, please refer to the Model Number, and Serial Number of your **BIOFLO 6000.** This information is on the **ELECTRICAL SPECIFICATION PLATE** on the side panel of the **ML-6100 CONTROLLER**, opposite the vessel.

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20. REPLACEMENT PARTS AND ACCESSORY INFORMATION

When ordering replacement or accessory parts, or requesting service information, please provide the Model Number, and Serial Number of your **BioFlo 6000.** This information is on the **ELECTRICAL SPECIFICATION PLATE** on the side panel of the ML-6100 controller, opposite the vessel.

20.1 REPLACEMENT PARTS DESCRIPTIONS AND PART NUMBERS

Part Description	*NBS Part Number*
Plug Kit, Exhaust Condenser	M138-3000
Agitator Seal Cond. Alarm Kit	M1238-0506
Spare Parts Kit	M1238-9920
Wearables Parts Kit	M1238-9923

20.2 ACCESSORY DESCRIPTIONS AND PART NUMBERS

Accessory Description	*NBS Part Number*
DO Kit, Ingold	M1238-0600
DO Kit, Broadley James	M1238-0602
pH Kit, Ingold Liquid	M1238-0610
pH Kit, Ingold Gel	M1238-0611
DO Kit, Ingold	M1238-0601
pH Kit, Broadley-James Gel	M1238-0612
pH/DO Amplifier Kit	M1238-0613
Ingold Polarization Module	P0720-5610
DO Simulator	P0720-5620
Valley Instruments Polarization Module	P0720-5750
Valley Instruments DO/pH Simulator	P0720-5631
Kit, Septum 1-1/2” Tri-Clamp	M1238-0614
Steam Prefilter and Regulator Kit	M1238-2283
Exhaust Condenser Kit	M1238-2581
Water Prefilter and Regulator Kit	M1238-2582
High Foam Kit	M1238-0500
Foam Control Kit	M1238-0501
Level Control Kit	M1238-0502
Nutrient Addition Kit	M1238-0505
Aerosol Containment, 19 mm	M1154-3016

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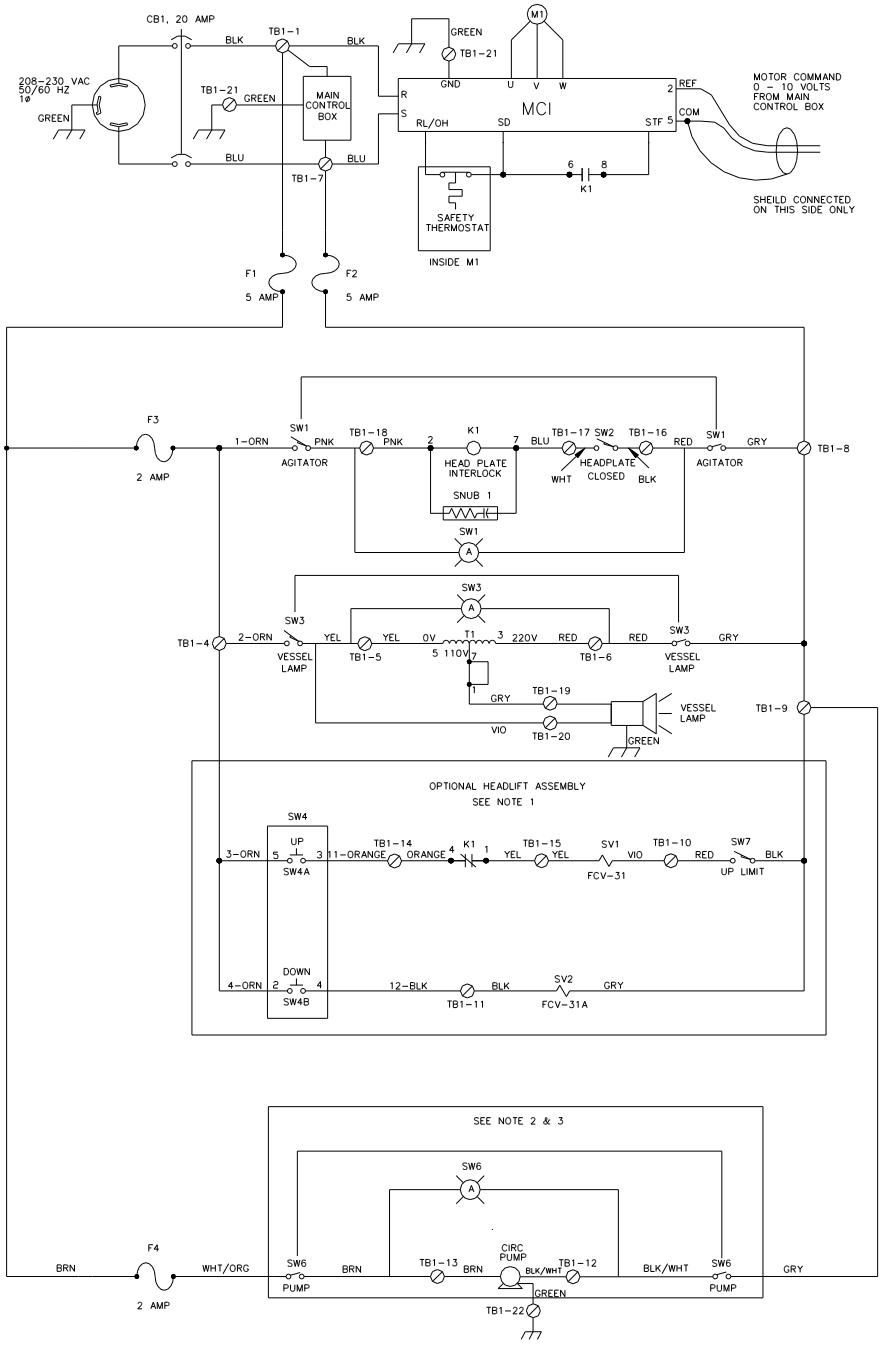
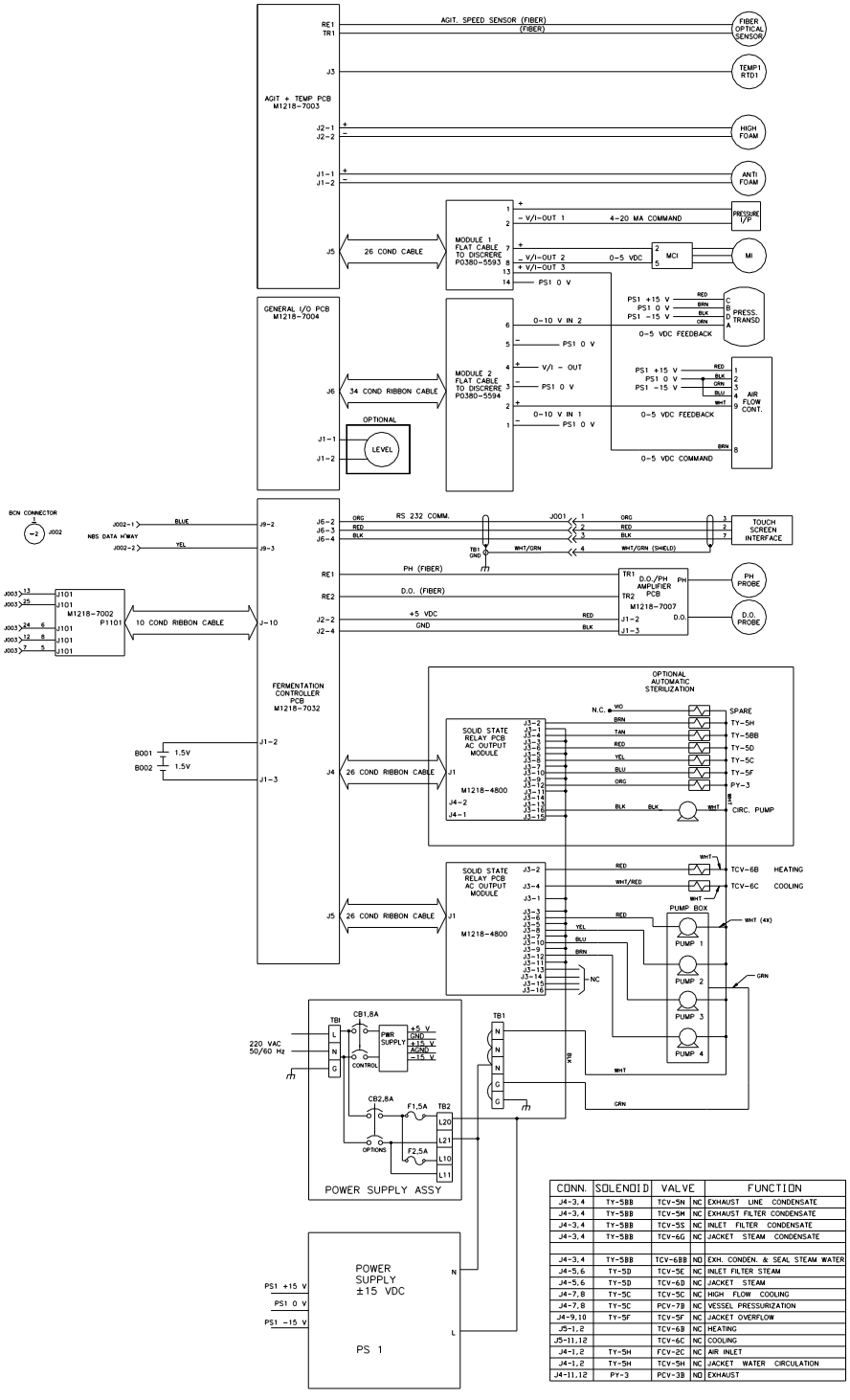
21. APPENDIX A

21.1 MOTOR CONTROL SCHEMATIC



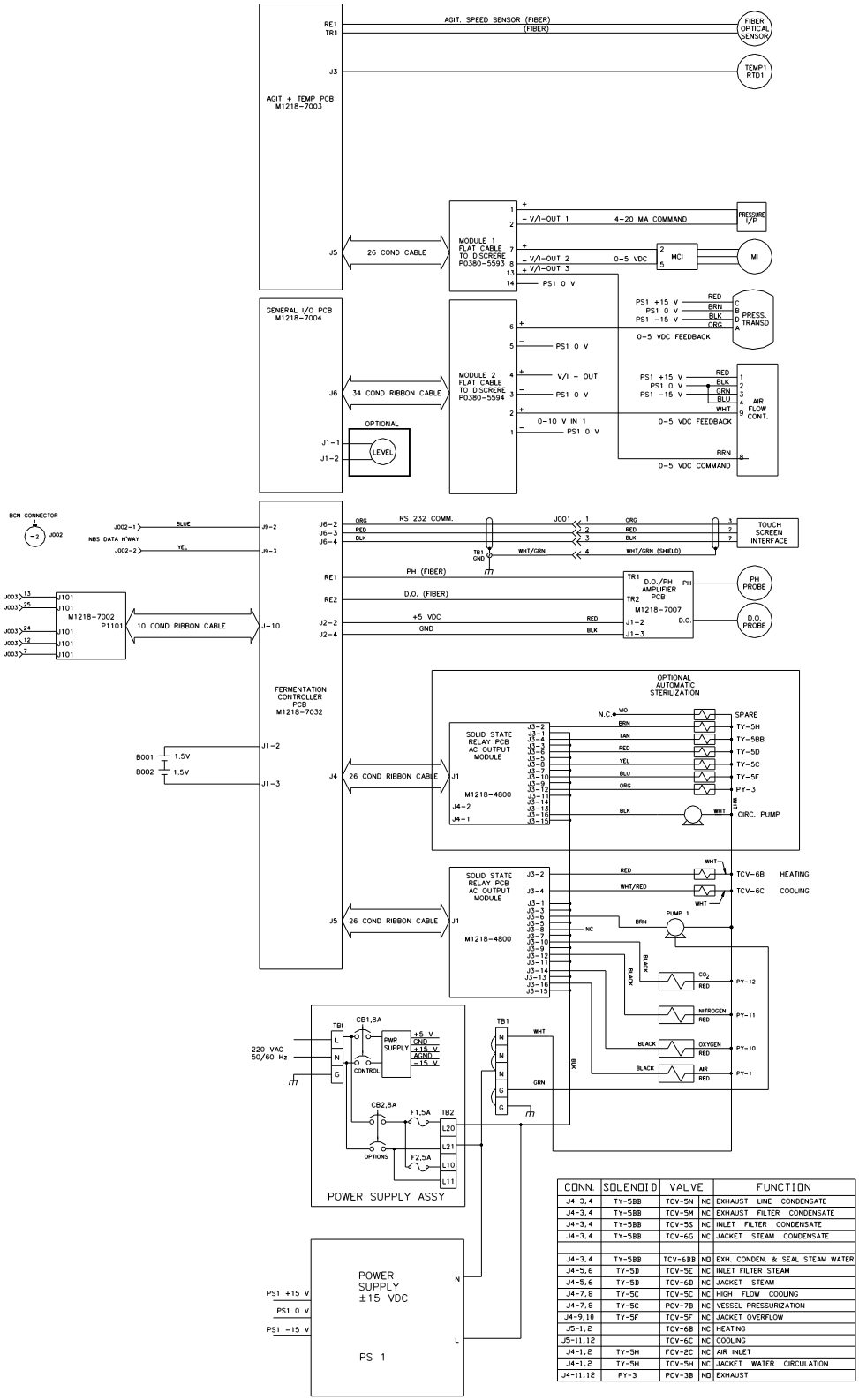
NOTES:
1. THESE COMPONENTS ARE ONLY USED WITH THE OPTIONAL HEAD LIFT ASSEMBLY.
2. SW6 IS ONLY USED WHEN THE AUTOMATIC STERILIZATION OPTION IS NOT INSTALLED.
3. SEE CONTROL BOX CONTROL SCHEMATIC FOR PUMP WIRING, WHEN THE OPTIONAL AUTOMATIC STERILIZATION OPTION IS INSTALLED.

21.2 ML-6100 SCHEMATIC WITH AUTO STERILIZATION



CONN.	SOLENOID	VALVE		FUNCTION
J4-3,4	TY-5BB	TCV-5N	NC	EXHAUST LINE CONDENSATE
J4-3,4	TY-5BB	TCV-5M	NC	EXHAUST FILTER CONDENSATE
J4-3,4	TY-5BB	TCV-5S	NC	INLET FILTER CONDENSATE
J4-3,4	TY-5BB	TCV-6G	NC	JACKET STEAM CONDENSATE
J4-3,4	TY-5BB	TCV-6BB	NO	EXH. CONDEN. & SEAL STEAM WATER
J4-5,6	TY-5D	TCV-5E	NC	INLET FILTER STEAM
J4-5,6	TY-5D	TCV-6D	NC	JACKET STEAM
J4-7,8	TY-5C	TCV-5C	NC	HIGH FLOW COOLING
J4-7,8	TY-5C	PCV-7B	NC	VESSEL PRESSURIZATION
J4-9,10	TY-5F	TCV-5F	NC	JACKET OVERFLOW
J5-1,2		TCV-6B	NC	HEATING
J5-11,12		TCV-6C	NC	COOLING
J4-1,2	TY-5H	FCV-2C	NC	AIR INLET
J4-1,2	TY-5H	TCV-5H	NC	JACKET WATER CIRCULATION
J4-11,12	PY-3	PCV-3B	NO	EXHAUST

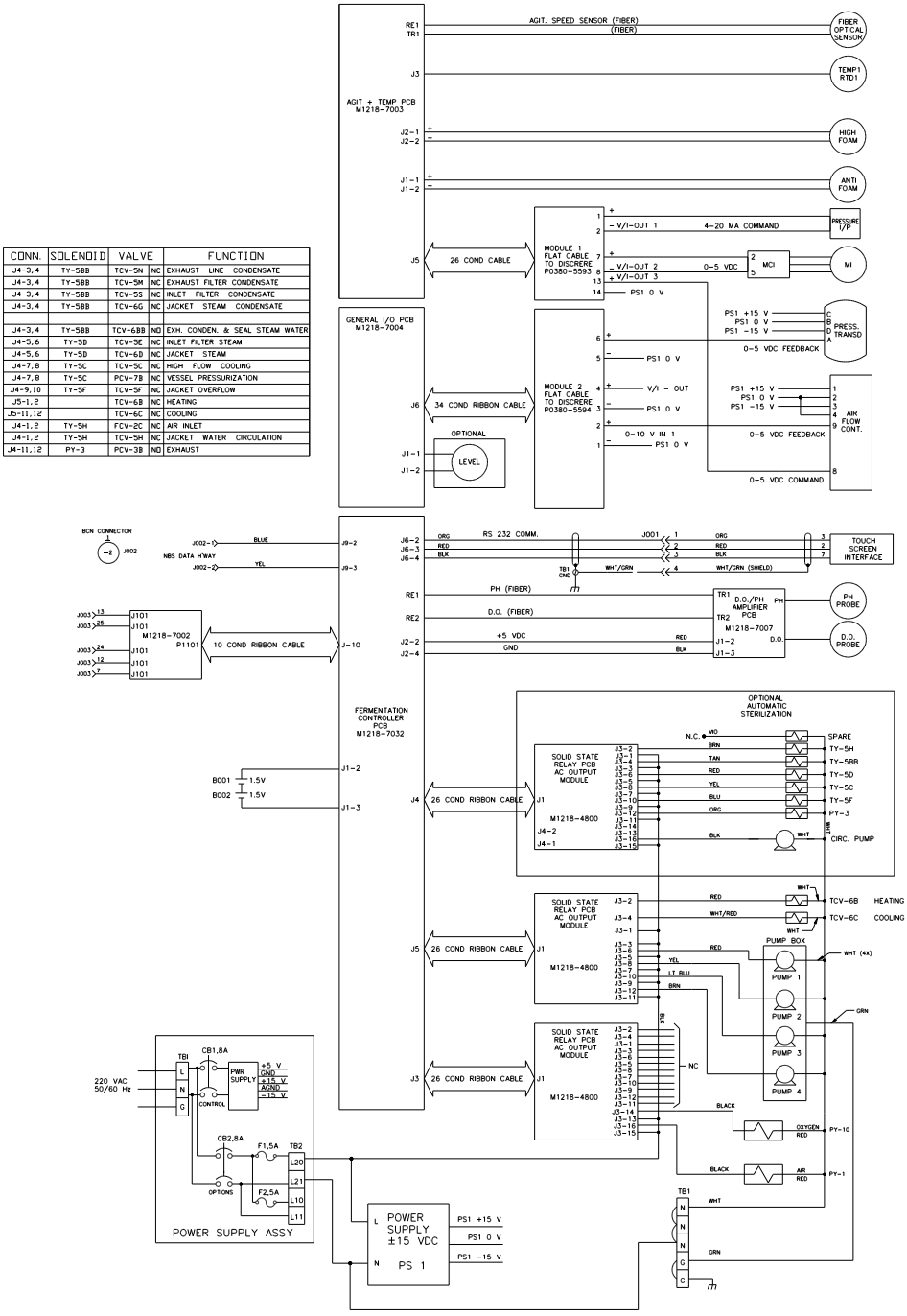
21.3 ML-6100 SCHEMATIC WITH AUTO STERILIZATION & 4-GAS OPTION



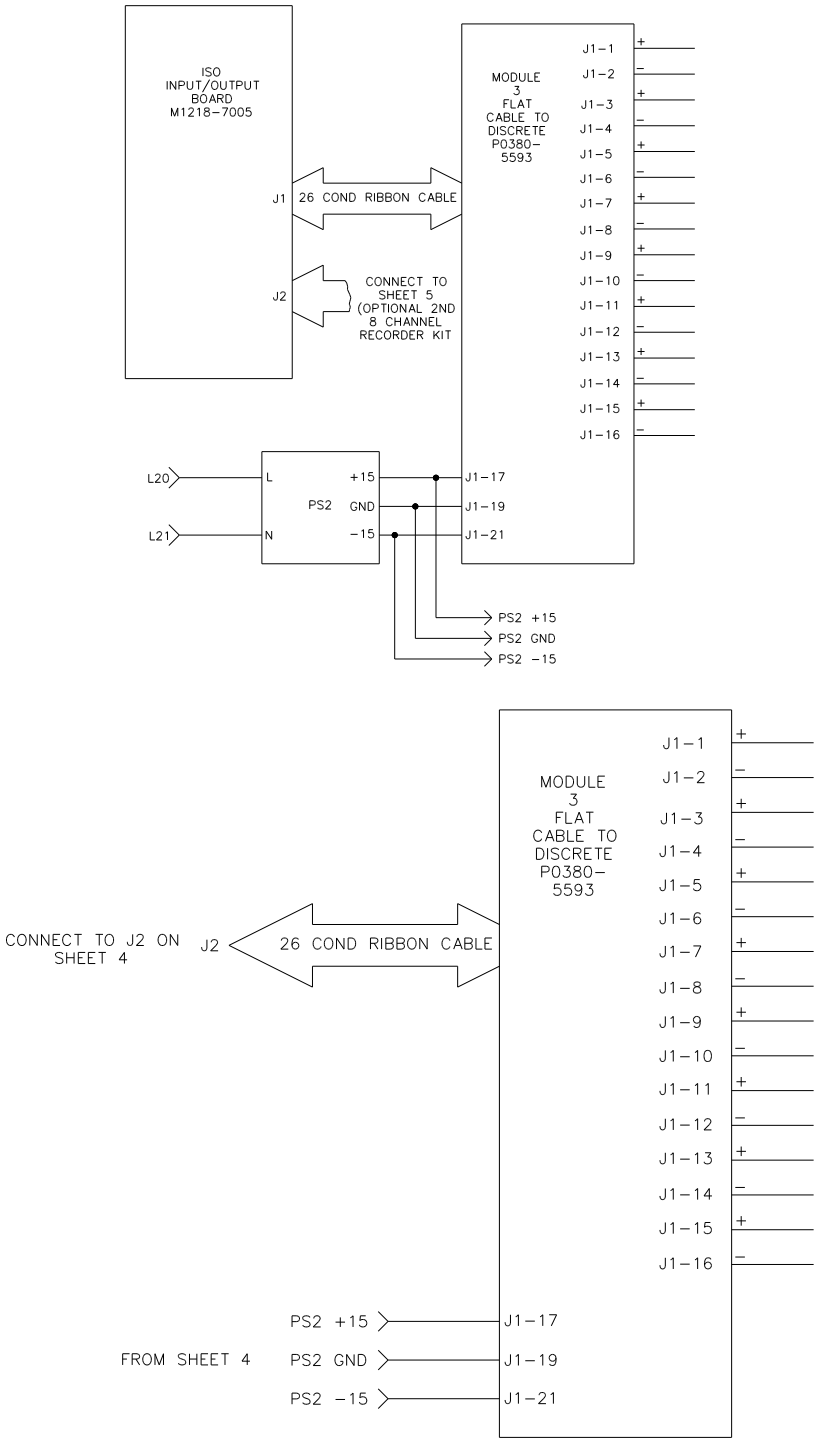
CONN.	SOLENOID	VALVE		FUNCTION
J4-3,4	TY-5BB	TCV-5N	NC	EXHAUST LINE CONDENSATE
J4-3,4	TY-5BB	TCV-5M	NC	EXHAUST FILTER CONDENSATE
J4-3,4	TY-5BB	TCV-5S	NC	INLET FILTER CONDENSATE
J4-3,4	TY-5BB	TCV-6G	NC	JACKET STEAM CONDENSATE
J4-3,4	TY-5BB	TCV-6BB	NO	EXH. CONDEN. & SEAL STEAM WATER
J4-5,6	TY-5D	TCV-5E	NC	INLET FILTER STEAM
J4-5,6	TY-5D	TCV-6D	NC	JACKET STEAM
J4-7,8	TY-5C	TCV-5C	NC	HIGH FLOW COOLING
J4-7,8	TY-5C	PCV-7B	NC	VESSEL PRESSURIZATION
J4-9,10	TY-5F	TCV-5F	NC	JACKET OVERFLOW
J5-1,2		TCV-6B	NC	HEATING
J5-11,12		TCV-6C	NC	COOLING
J4-1,2	TY-5H	FCV-2C	NC	AIR INLET
J4-1,2	TY-5H	TCV-5H	NC	JACKET WATER CIRCULATION
J4-11,12	PY-3	PCV-3B	NO	EXHAUST

21.4 ML-6100 SCHEMATIC WITH AUTO STERILIZATION & 2-GAS OPTION

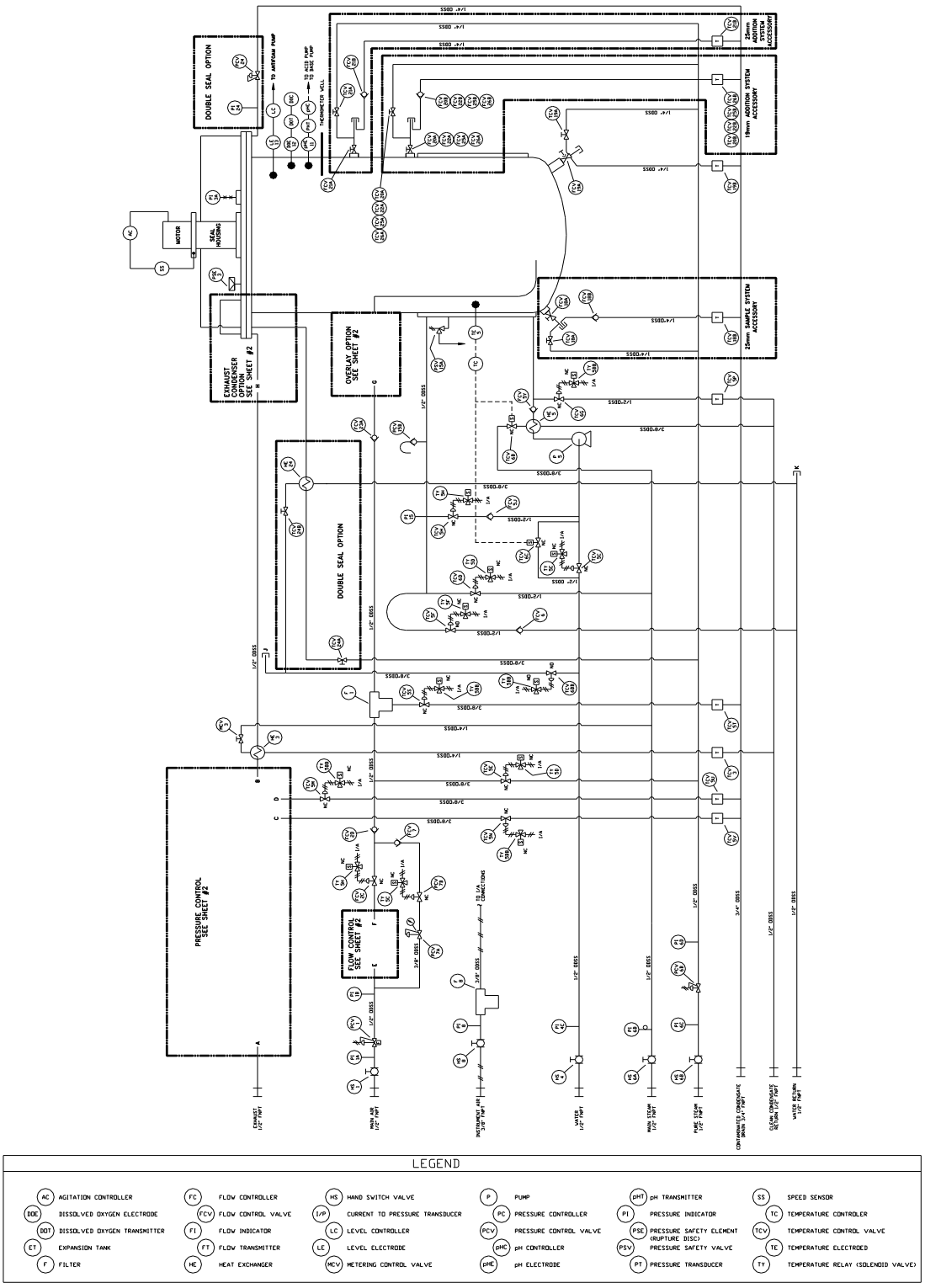
CONN.	SOLENOID	VALVE		FUNCTION
J4-3,4	TY-5BB	TCV-5N	NC	EXHAUST LINE CONDENSATE
J4-3,4	TY-5BB	TCV-5M	NC	EXHAUST FILTER CONDENSATE
J4-3,4	TY-5BB	TCV-5S	NC	INLET FILTER CONDENSATE
J4-3,4	TY-5BB	TCV-6G	NC	JACKET STEAM CONDENSATE
J4-3,4	TY-5BB	TCV-6BB	NO	EXH. CONDEN. & SEAL STEAM WATER
J4-5,6	TY-5D	TCV-5E	NC	INLET FILTER STEAM
J4-5,6	TY-5D	TCV-6D	NC	JACKET STEAM
J4-7,8	TY-5C	TCV-5C	NC	HIGH FLOW COOLING
J4-7,8	TY-5C	PCV-7B	NC	VESSEL PRESSURIZATION
J4-9,10	TY-5F	TCV-5F	NC	JACKET OVERFLOW
J5-1,2		TCV-6B	NC	HEATING
J5-11,12		TCV-6C	NC	COOLING
J4-1,2	TY-5H	FCV-2C	NC	AIR INLET
J4-1,2	TY-5H	TCV-5H	NC	JACKET WATER CIRCULATION
J4-11,12	PY-3	PCV-3B	NO	EXHAUST



21.5 ML-6100 OPTIONAL RECORDER KIT SCHEMATIC



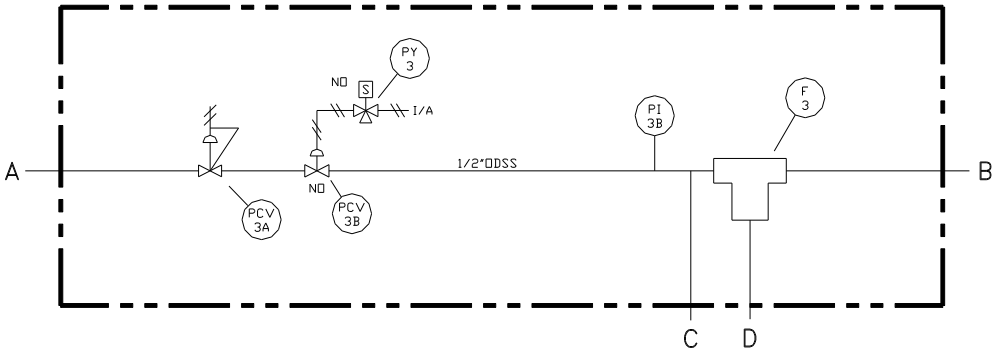
21.6 PIPING & INSTRUMENTATION DIAGRAMS AUTO STERILIZATION



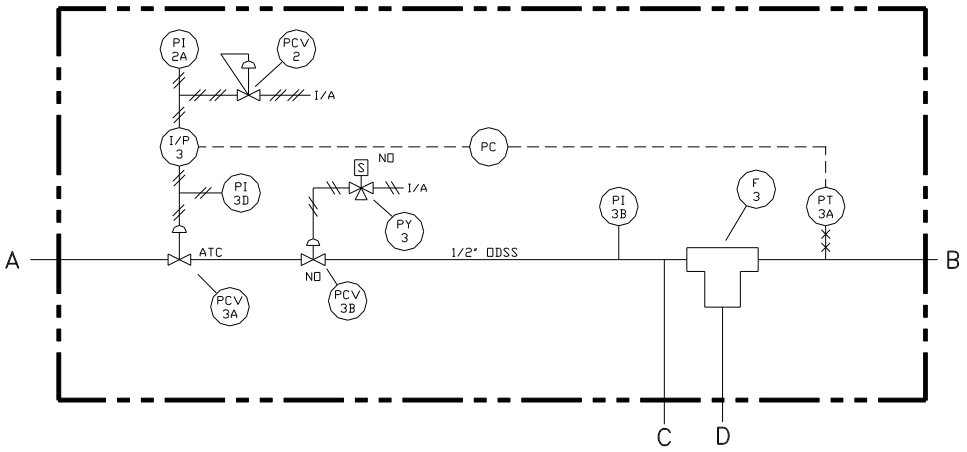
LEGEND

AC AGITATION CONTROLLER	FC FLOW CONTROLLER	HS HAND SWITCH VALVE	P PUMP	pHT pH TRANSMITTER	SS SPEED SENSOR
DOE DISSOLVED OXYGEN ELECTRODE	FCV FLOW CONTROL VALVE	I/P CURRENT TO PRESSURE TRANSDUCER	PC PRESSURE CONTROLLER	PI PRESSURE INDICATOR	TC TEMPERATURE CONTROLER
DOT DISSOLVED OXYGEN TRANSMITTER	FI FLOW INDICATOR	LC LEVEL CONTROLLER	PCV PRESSURE CONTROL VALVE	PSE PRESSURE SAFETY ELEMENT (RUPTURE DISC)	TCV TEMPERATURE CONTROL VALVE
ET EXPANSION TANK	FT FLOW TRANSMITTER	LE LEVEL ELECTRODE	pHC pH CONTROLLER	PSV PRESSURE SAFETY VALVE	TE TEMPERATURE ELECTRODED
F FILTER	HE HEAT EXCHANGER	MCV METERING CONTROL VALVE	pHE pH ELECTRODE	PT PRESSURE TRANSDUCER	TY TEMPERATURE RELAY (SOLENOID VALVE)

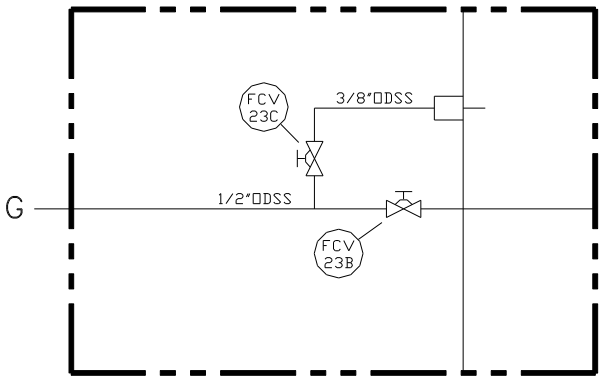
MANUAL PRESSURE CONTROL



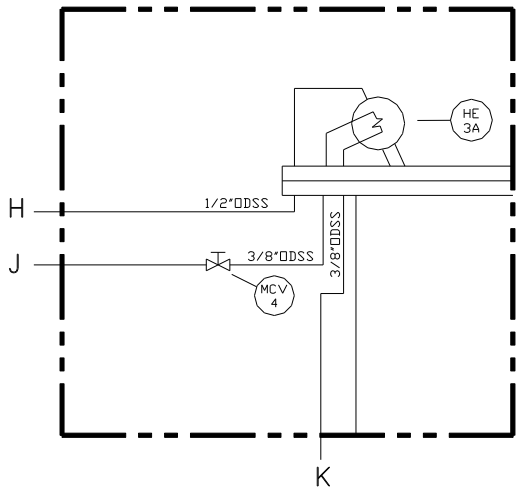
AUTOMATIC PRESSURE CONTROL



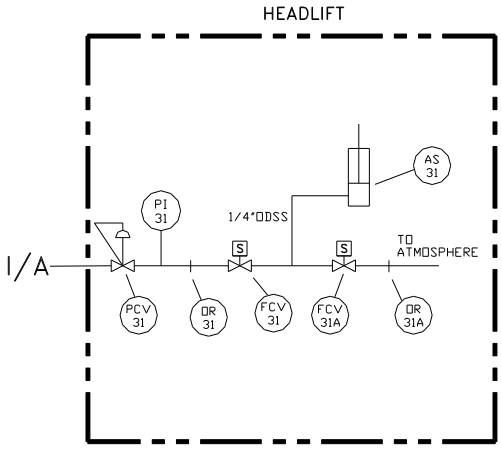
OVERLAY



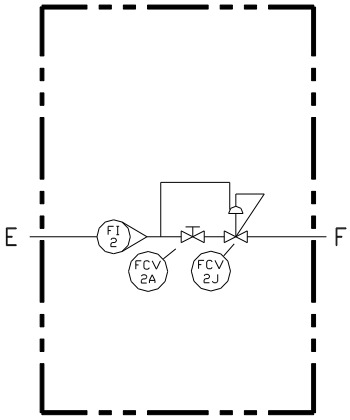
EXHAUST CONDENSER



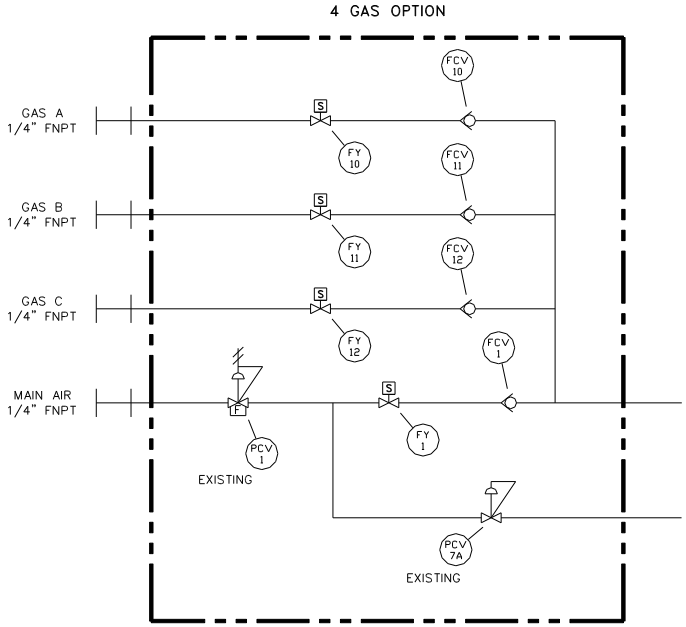
HEADLIFT



MANUAL FLOW CONTROL



4 GAS OPTION



22. SYSTEM GAINS

Loop Name	P Gain	I Gain	D Gain	Normaliz. Const
Temperature	30.00	0.20	0.00	130
Agitation	0.40	4.00	0.00	800
pH	70.00	5.00	0.00	14
Dissolved Oxy.	2.50	1.25	0.00	100
Airflow	0.75	3.00	0.00	100

23. APPENDIX B

23.1 CORROSION RESISTANCE TABLES

● Corrosion Rate less than 0.002" per year
❍ Corrosion Rate less than 0.020" per year
☐ Corrosion Rate from 0.020" to 0.050" per year
× Corrosion Rate greater than 0.050" per year

This table shows the resistance of Type 316 stainless steel to the more common chemicals. Many factors influence the resistance of materials to various solutions Factors which must be given consideration for service in corrosive environments are: temperature, concentration, aeration, influence of inhibiting or accelerating contaminents, influence of recirculation, solids in suspension, velocity; frequency of use, and equipment design. The corrosion data is reprinted from Corrosion Data Survey, 1967 and 1974 Editions, published by the National Association of Corrosion Engineers

C	F	20	40	60	80	100
350	662					
300	- - -					
250	482					
212	- - -					
175	347					
150	- - -					
125	257					
100	212					
75	167					
50	- - -					
25	77					
C	F	20	40	60	80	100
		Percent Concentration in Water				

The influence of contaminants is probably the most important from a commercial standpoint. Corrosive solutions are seldom found that will be free to all contaminates. However, the majority of these contaminants have no influence on corrosion, but the ones that do generally affect the conditions greatly.

When reviewing these corrosion tables, it is good to keep the following in mind:
Stainless Type 316 is resistant to chemicals.

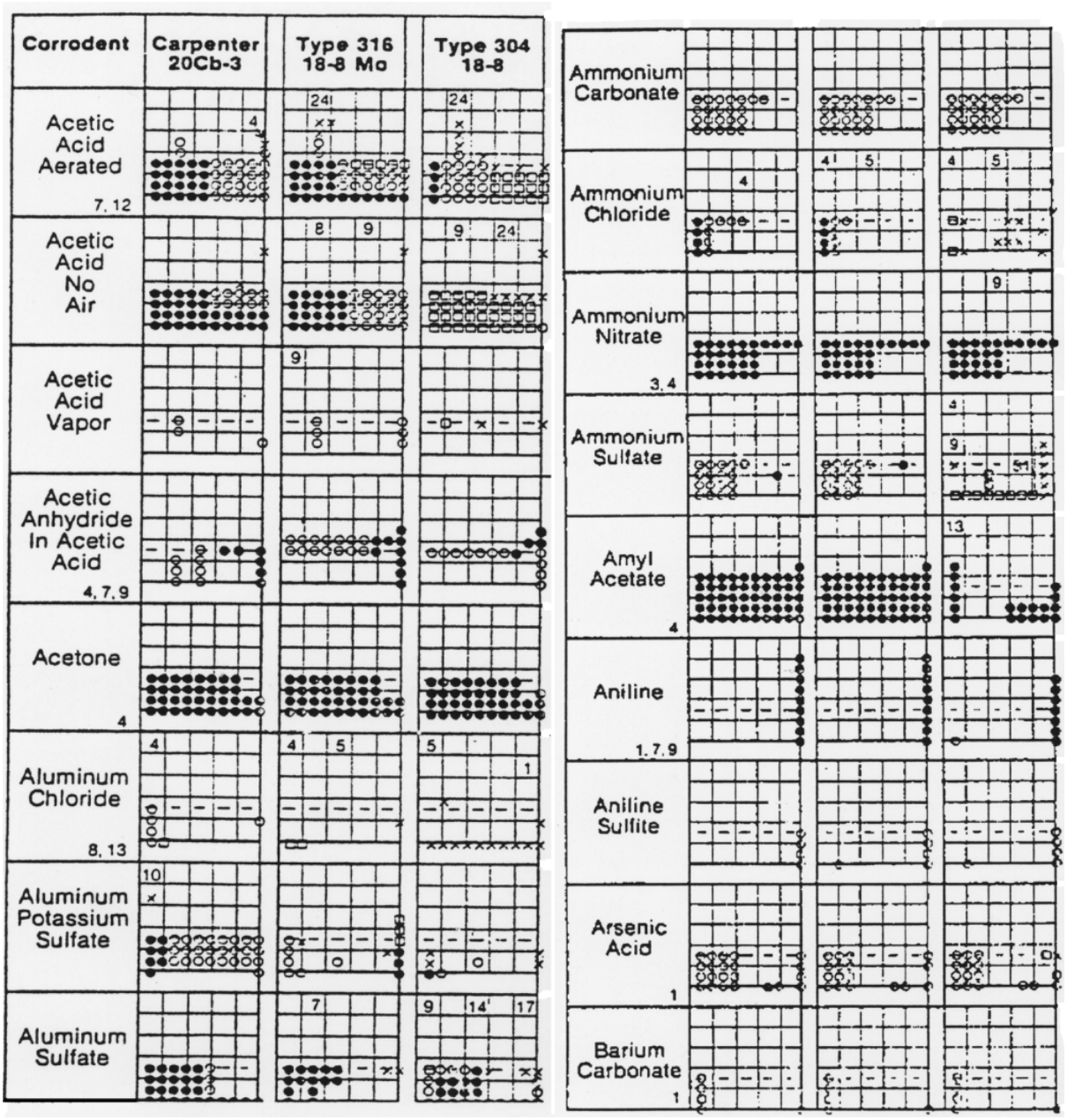
FOOTNOTES

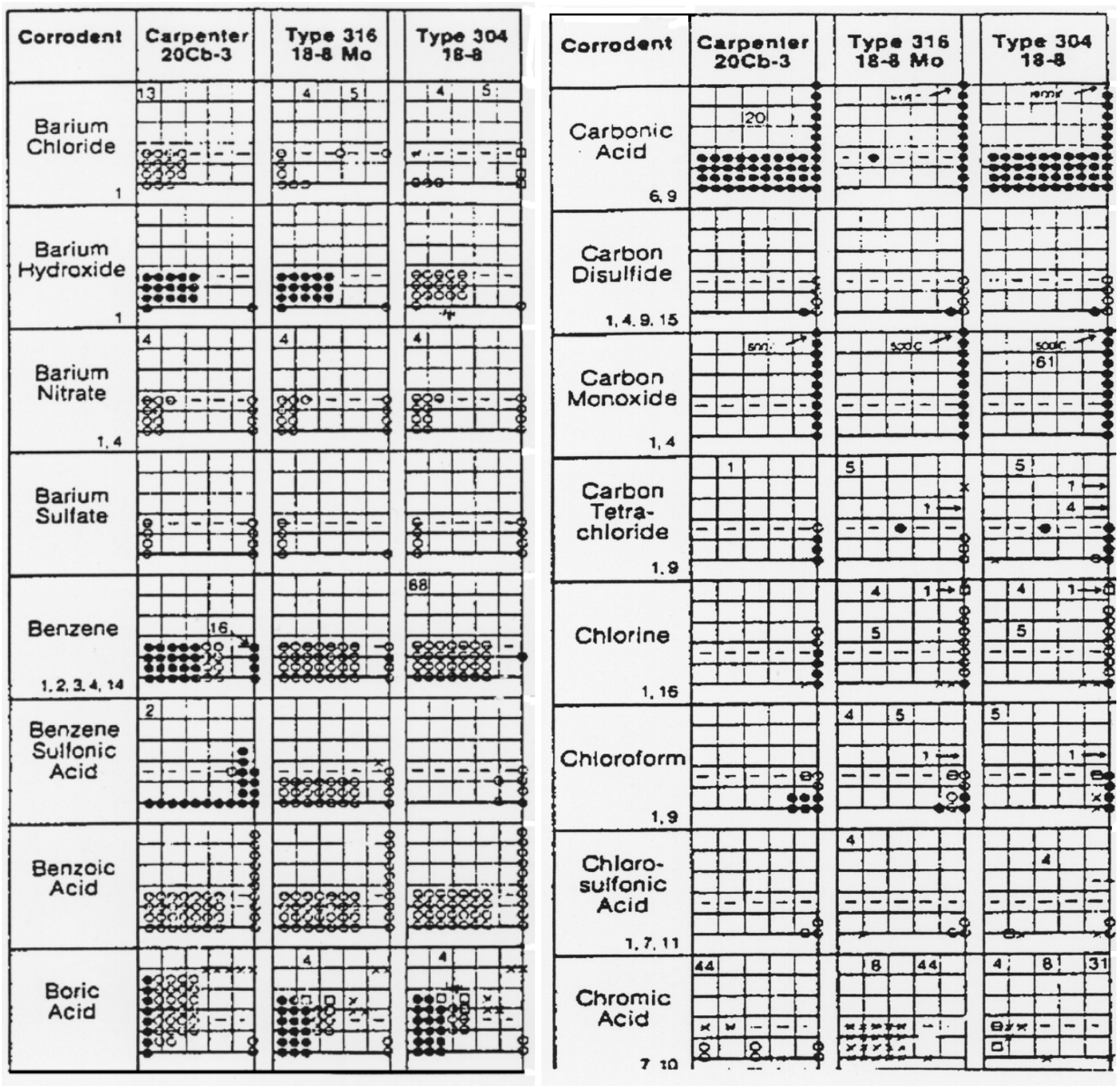
Footnotes for Corrosives:

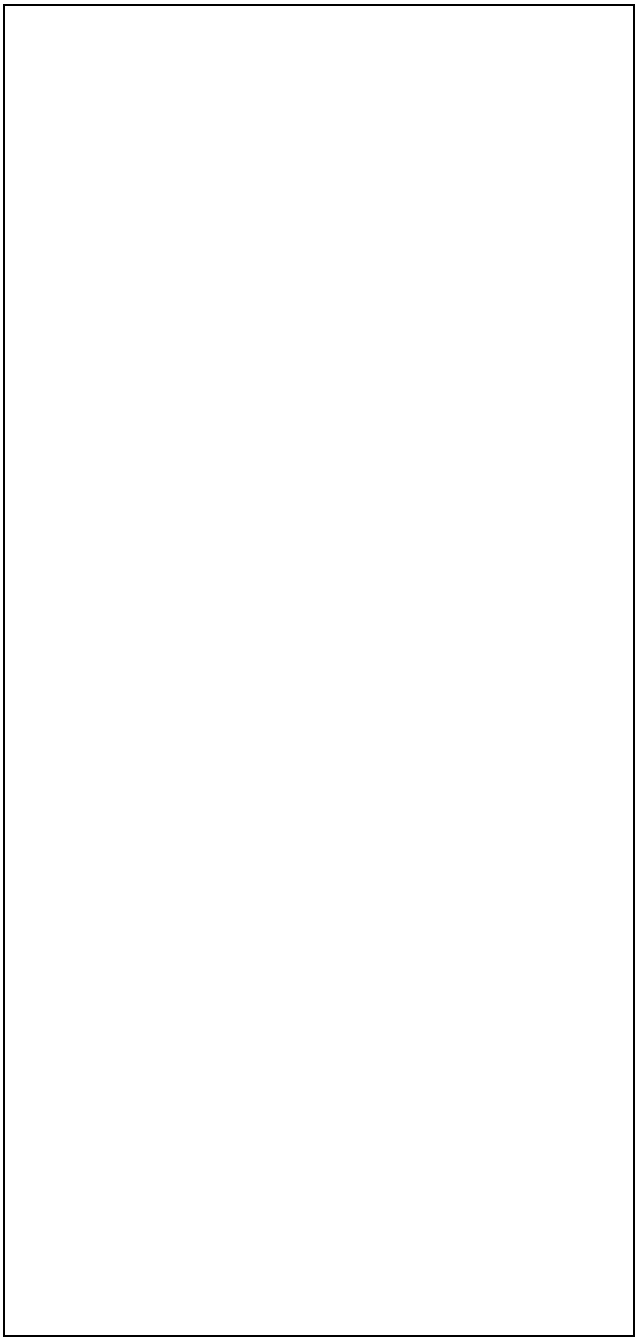
1 Poison
2 Toxic
3 Explosive
4 Flammable
5 Ingestion poison
6 Inhalant poison
7 Attacks skin
8 Irritant
9 Vapor harmful
10 Ignites Organics
11 Fuming liquid
12 Hygroscopic
13 Liberates HCL in water
14 Narcotic
15 Volatile
16 Hazardous under pressure
17 Ignites combustibles
24 Exothermic with water

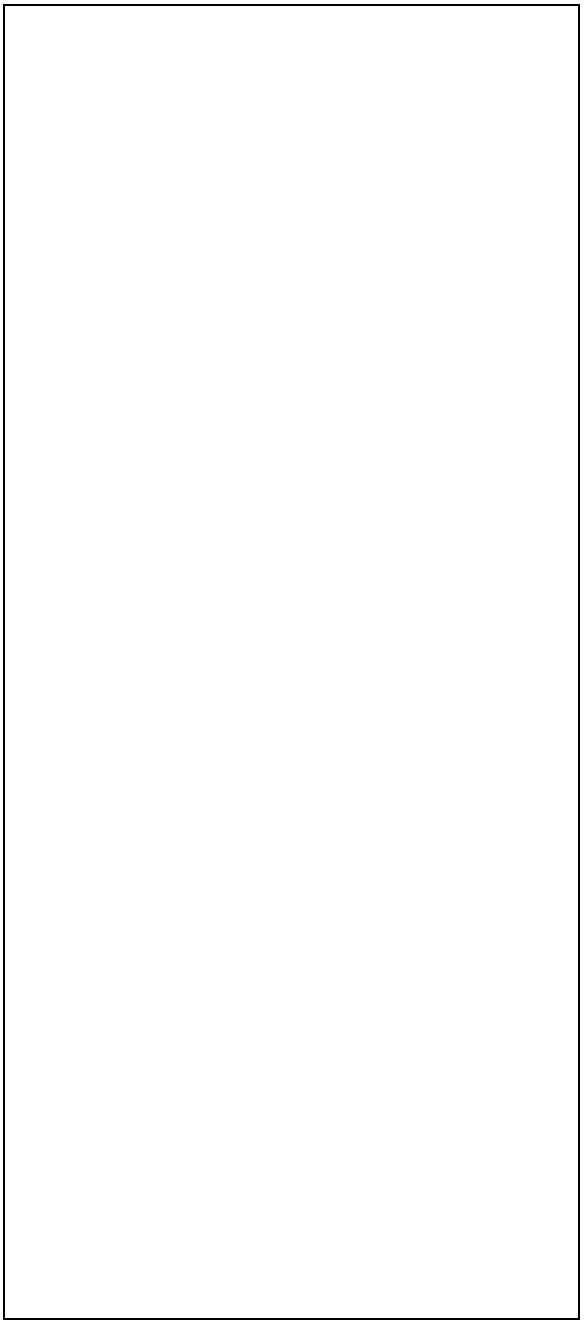
Footnotes for Data Squares:

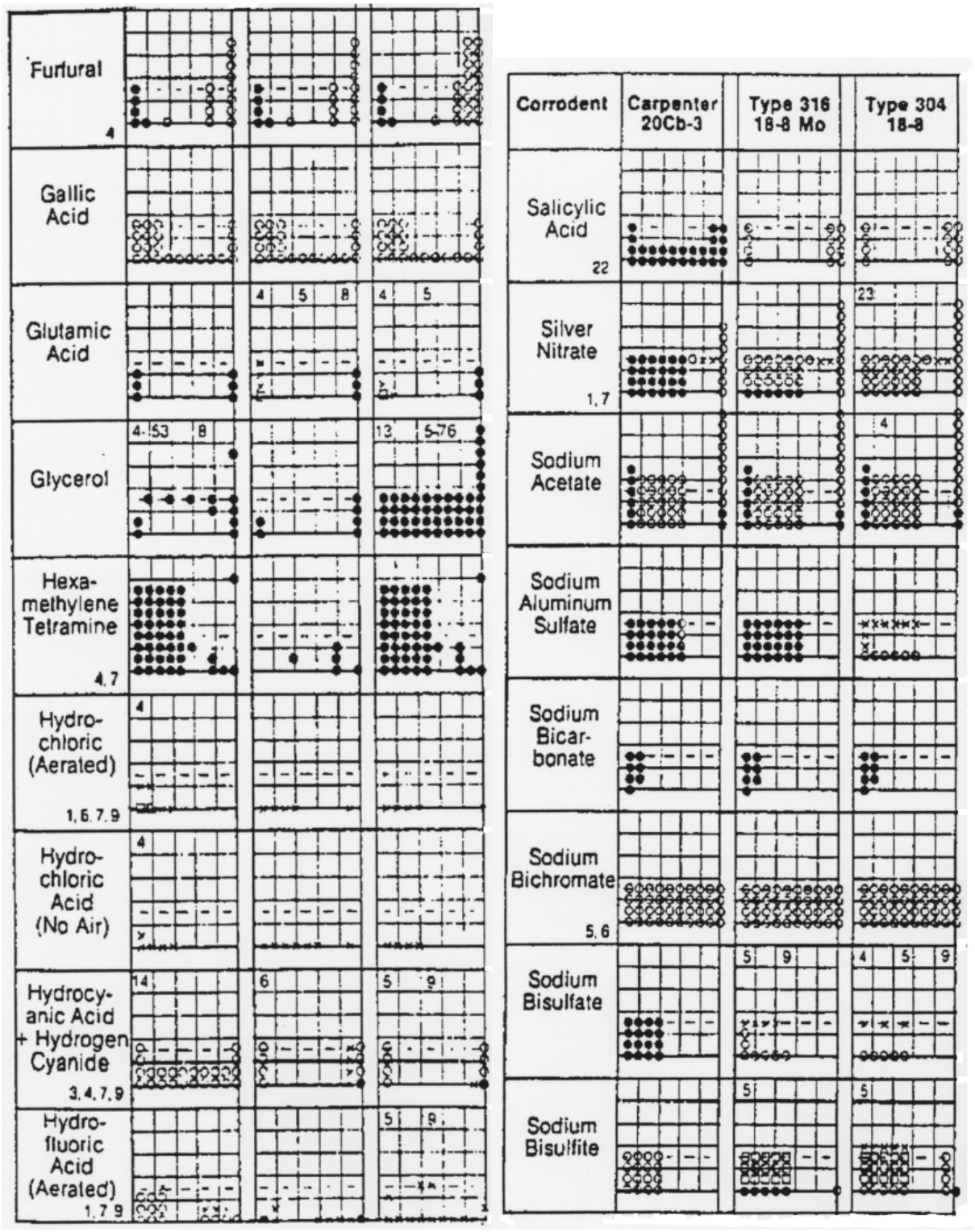
20 Agitated
21 7 pH
22 < 7 pH
23 > 7 pH
24 No HCI, H_2SO_4, HaC1
34 No stress
36 300 psi
37 Stress relieved
45 < 60 psi
51 > 2.25% Mo
72 over 400ºC
79 300 psi
80 No SO_3

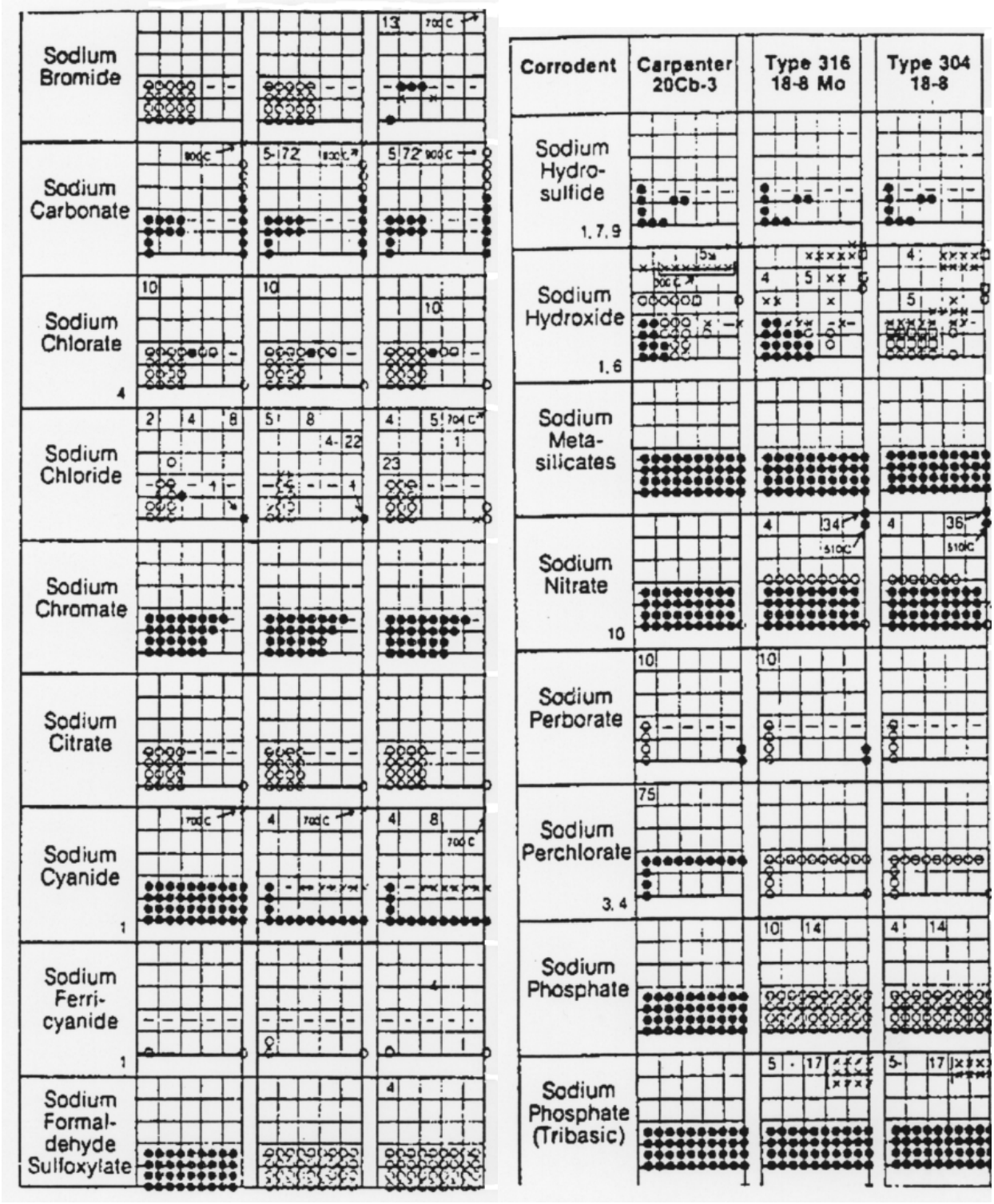

Corrodent
Carpenter 20Cb-3
Type 316 18-8 Mo
Type 304 18-8
Acetic Acid Aerated 7, 12
Acetic Acid No Air
Acetic Acid Vapor
Acetic Anhydride In Acetic Acid 4, 7, 9
Acetone 4
Aluminum Chloride 8, 13
Aluminum Potassium Sulfate
Aluminum Sulfate
Ammonium Carbonate
Ammonium Chloride
Ammonium Nitrate 3, 4
Ammonium Sulfate
Amyl Acetate 4
Aniline 1, 7, 9
Aniline Sulfite
Arsenic Acid 1
Barium Carbonate 1

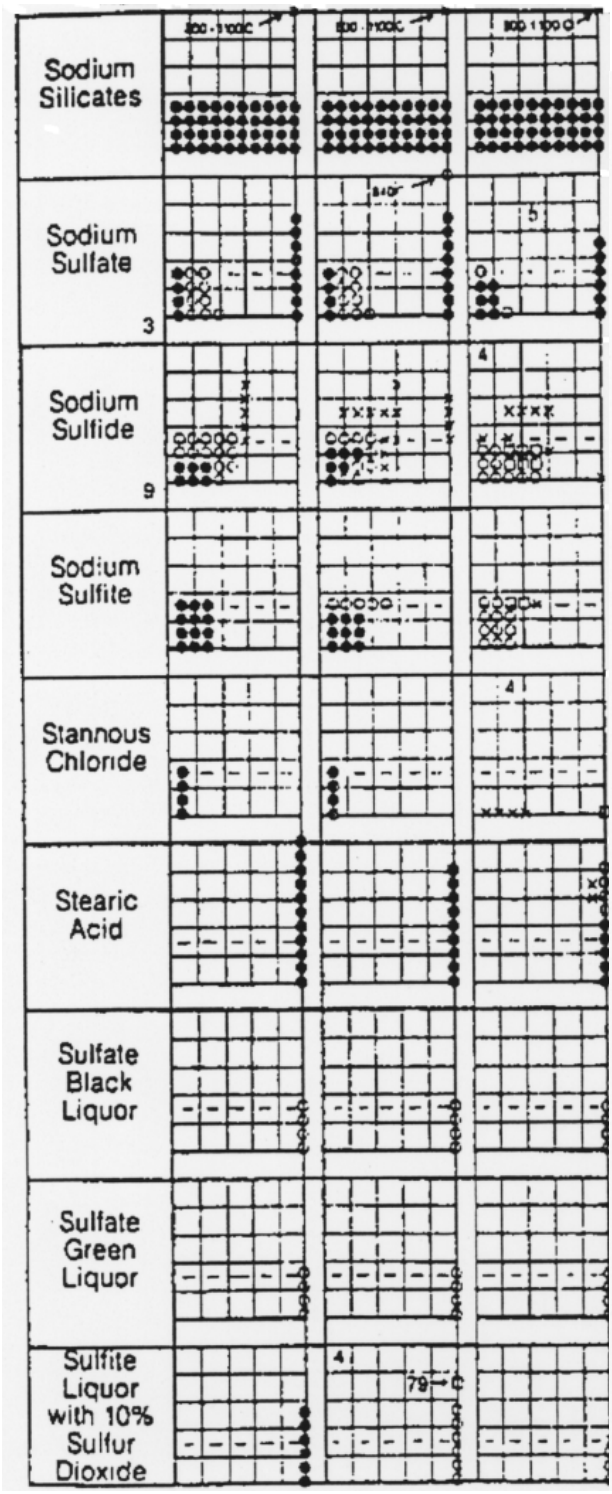

Corrodent
Carpenter 20Cb-3
Type 316 18-8 Mo
Type 304 18-8
Barium Chloride
1
Barium Hydroxide
1
Barium Nitrate
1, 4
Barium Sulfate
Benzene
1, 2, 3, 4, 14
Benzene Sulfonic Acid
Benzoic Acid
Boric Acid
Carbonic Acid
6, 9
Carbon Disulfide
1, 4, 9, 15
Carbon Monoxide
1, 4
Carbon Tetra-chloride
1, 9
Chlorine
1, 16
Chloroform
1, 9
Chloro-sulfonic Acid
1, 7, 11
Chromic Acid
7, 10

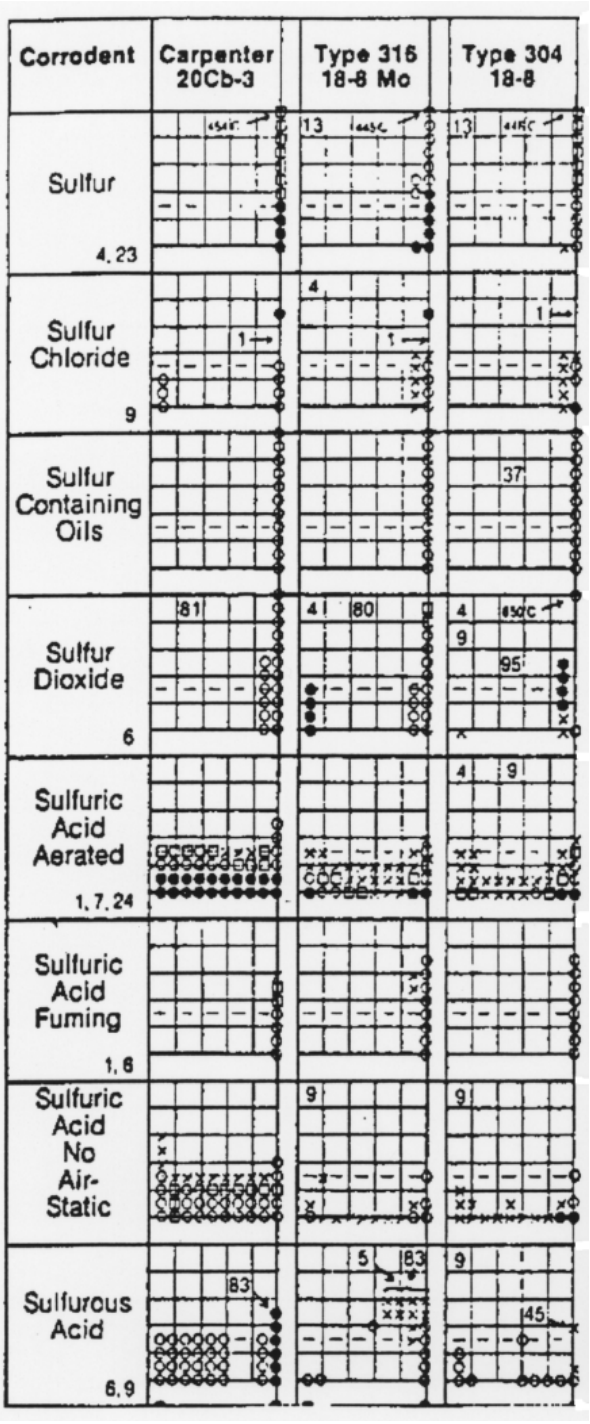


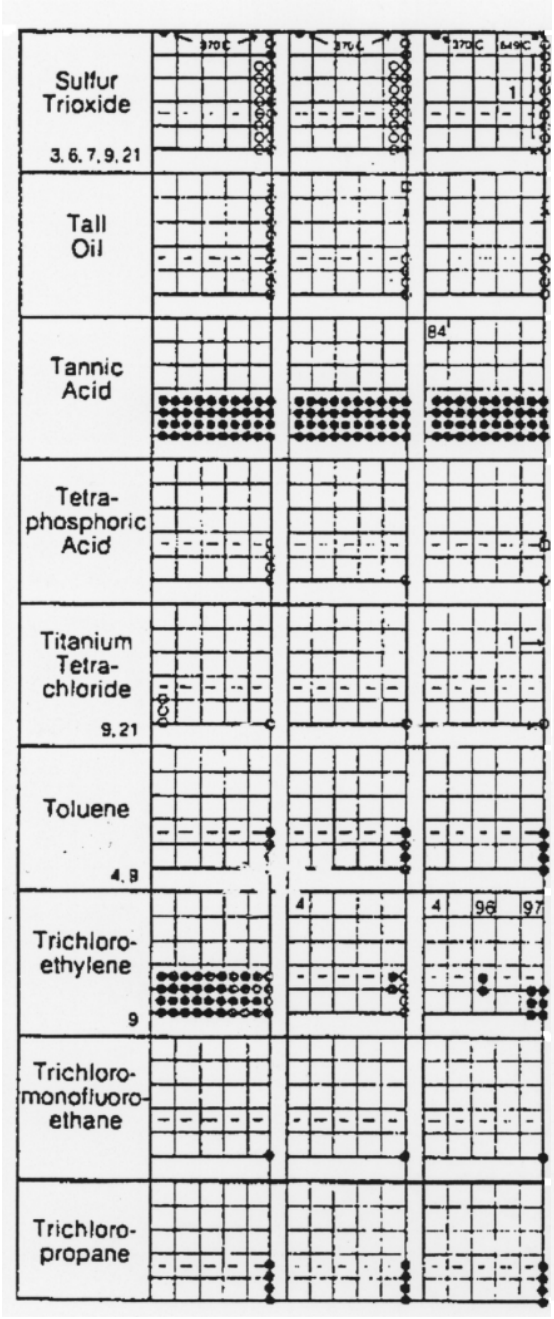


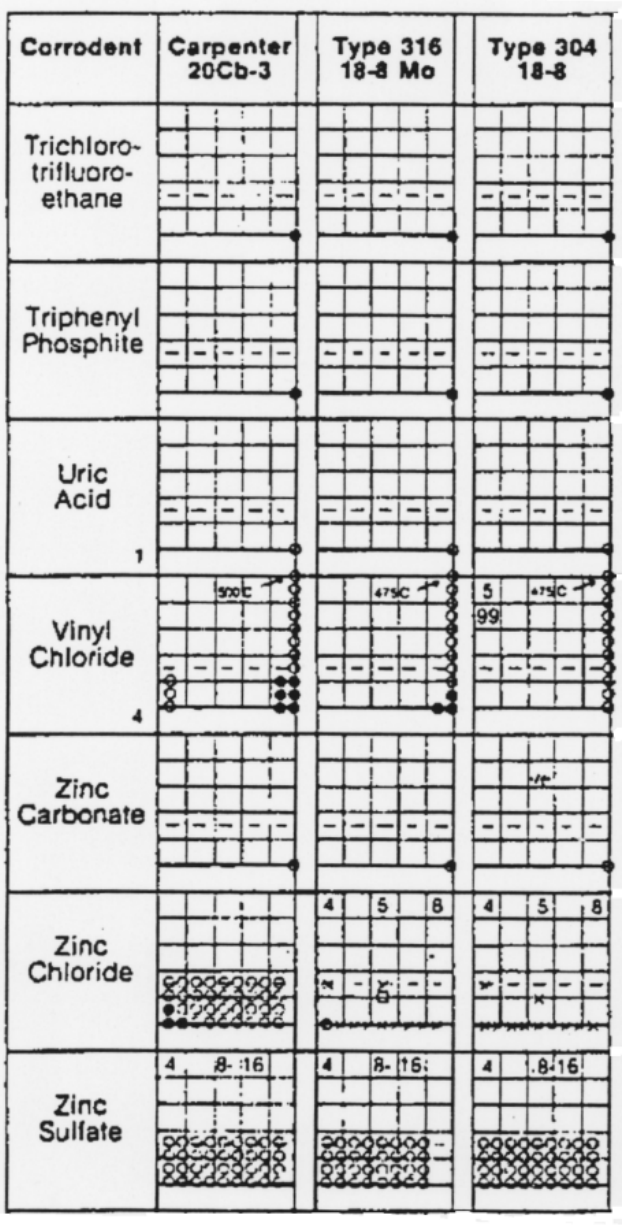

Furfural
4
Gallic Acid
Glutamic Acid
4 5 8
4 5
Glycerol
4 5 3 8
13 5 7 6
Hexa-methylene Tetramine
4, 7
Hydro-chloric (Aerated)
4
1, 6, 7, 9
Hydro-chloric Acid (No Air)
4
Hydrocy-anic Acid + Hydrogen Cyanide
14
6
5 9
3, 4, 7, 9
Hydro-fluoric Acid (Aerated)
5 9
1, 7, 9
Corrodent
Carpenter 20Cb-3
Type 316 18-8 Mo
Type 304 18-8
Salicylic Acid
22
Silver Nitrate
23
1, 7
Sodium Acetate
4
Sodium Aluminum Sulfate
Sodium Bicar-bonate
Sodium Bichromate
5, 6
Sodium Bisulfate
5 9
4 5 9
Sodium Bisulfite
5
5


Corrodent
Carpenter 20Cb-3
Type 316 18-8 Mo
Type 304 18-8
Sodium Bromide
Sodium Carbonate
Sodium Chlorate
4
Sodium Chloride
Sodium Chromate
Sodium Citrate
Sodium Cyanide
1
Sodium Ferri-cyanide
1
Sodium Formal-dehyde Sulfoxylate
Sodium Hydro-sulfide
1, 7, 9
Sodium Hydroxide
1, 6
Sodium Meta-silicates
Sodium Nitrate
10
Sodium Perborate
Sodium Perchlorate
3, 4
Sodium Phosphate
Sodium Phosphate (Tribasic)


Sodium Silicates
Sodium Sulfate
3
Sodium Sulfide
9
Sodium Sulfite
Stannous Chloride
Stearic Acid
Sulfate Black Liquor
Sulfate Green Liquor
Sulfite Liquor with 10% Sulfur Dioxide


Corrodent
Carpenter 20Cb-3
Type 316 18-8 Mo
Type 304 18-8
Sulfur
4, 23
Sulfur Chloride
9
Sulfur Containing Oils
Sulfur Dioxide
6
Sulfuric Acid Aerated
1, 7, 24
Sulfuric Acid Fuming
1, 6
Sulfuric Acid No Air-Static
Sulfurous Acid
6, 9


Sulfur Trioxide
3, 6, 7, 9, 21
Tall Oil
Tannic Acid
Tetra-phosphoric Acid
Titanium Tetra-chloride
9, 21
Toluene
4, 8
Trichloro-ethylene
9
Trichloro-monofluoro-ethane
Trichloro-propane


Corrodent
Carpenter 20Cb-3
Type 316 18-8 Mo
Type 304 18-8
Trichloro-trifluoro-ethane
Triphenyl Phosphite
Uric Acid
1
Vinyl Chloride
4
Zinc Carbonate
Zinc Chloride
Zinc Sulfate

24. APPENDIX C

24.1 GENERAL CHARACTERISTICS OF EPR

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

GENERAL CHARACTERISTICS

Durometer Range (Shore A)	30-90 (NBS 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 (F°)	-20 to -60°
High Temperature Usage (F°)	to 350°
Aging Weather - Sunlight	Excellent
Adhesion to Metals	Fair to Good

COMMENT

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 up to 350°F. 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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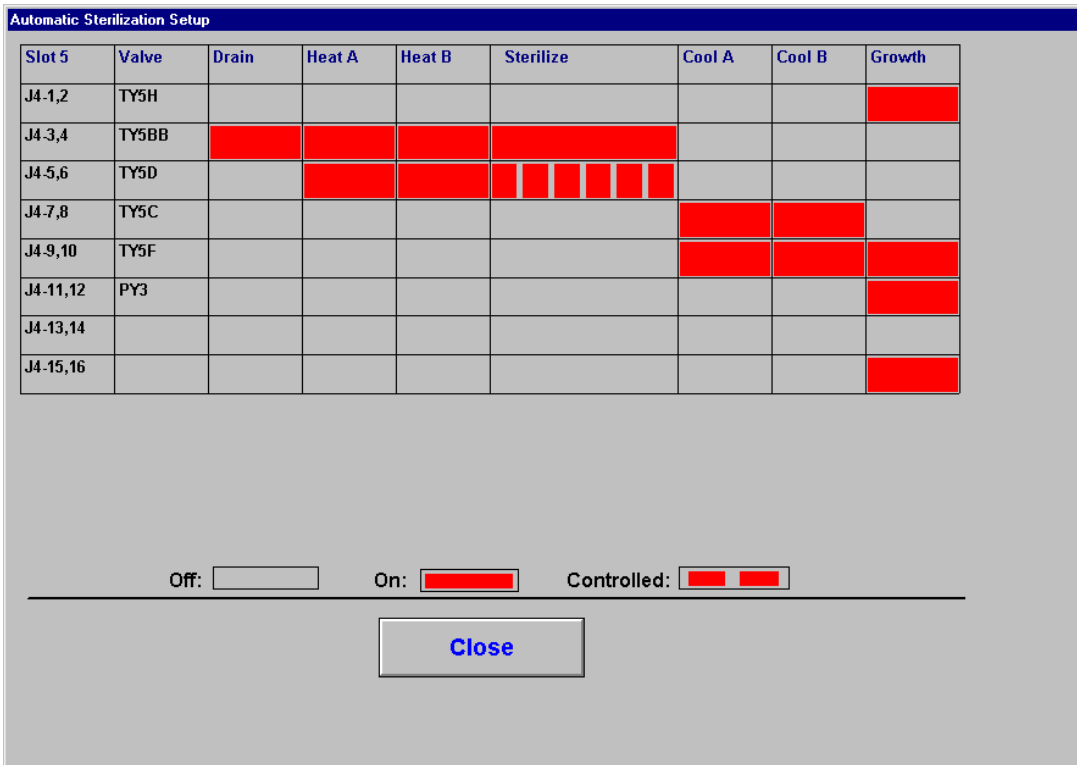
25. APPENDIX D

25.1 FACTORY STERILIZATION VALVE SEQUENCING SETTINGS



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26. INDEX

A

Abort Button · 90
Aborting Automatic Sterilization · 90
Accessory
  Descriptions · 110
  Part Numbers · 110
Adding Media to the Vessel · 79
Addition Valve · 92
  Operation · 95
  Sterilization · 92
Agitation Control System · 23, 32
  Operation · 33
Air System · 36
  Overview · 36
  Rotometer · 38, 39
  Thermal Mass Flowcontroller · 40
  Thermal Mass Flowmeter · 37, 38
Automatic Backpressure Control · 42
Automatic Sterilization · 82
Automatic Sterilization Control Dialog · 83

B

BioFlo 6000 Features
  Agitation/Drive System · 23
  Headplate Penetrations · 20
  Jacket · 22
  Lower Side Wall Penetrations · 21
  Process Piping · 22
  Sight Glass · 21
  Temperature Control System · 23
  Upper Side Wall Penetrations · 21
  Vessel · 19
  Vessel and Jacket Penetrations · 20

C

Calibration
  DO Probe · 75
  pH Probe · 69
Chemical Foam Control System with Pump · 62
Cleaning · 102
Cool A Operation · 87
Cool B Operation · 87
Cool Time · 85

D

DO Control System · 74
  Overview · 74
  Probe Calibration · 75
  Probe Polarization · 75
DO Probe
  Calibration · 75
  Calibration by Sparging with Nitrogen · 76
  Electronic Calibration · 75
  Polarization · 75
  Span Calibration · 77
  Zero Calibration · 76
Double Mechanical Seal · 33
Drain Operation · 87
Drain Temp · 85
Drain Valve · 29

E

Electrical Connections · 27
Exhaust · 26
Exhaust Gas Treatment · 43

F

Foam Control System · 62
  Chemical Foam Control · 62
  Foam Probe Setup · 63
  High Foam Probe Mounting Options · 65
  High Foam Probe Setup · 64
  High Foam Safety System · 64
Foam Probe · 63
Four Gas Control Module · 47

G

Gas Mix Status Window · 51
Gas Mixing
  Controller Connections · 47, 48
  Four Gas Control Module · 47
  Valving · 47, 48
Gas Overlay · 44
Gauge Screen · 83, 89, 90, 91
General Characteristics Of Epr · 129
Growth Operation · 87

H

Heat A Operation · 87
Heat B Operation · 87
Heat B Temp · 85
Heat Labile Media · 80
Heat Time · 85
High Foam Probe · 64, 65

I

Initial Preparation · 28
Inoculation and Addition · 92, 95
Inoculation Valve · 92

M

Manual Backpressure Control · 40
ML-6100 Connections · 27

O

Overview · 13, 14, 15, 16
  Options List · 13, 14
  Specifications · 17

P

pH Control System
  Cascade Setup with Pumps · 71, 72
  Overview · 66
  pH Probe · 67
  pH Probe Calibration · 67
  System with Pumps · 66, 71, 72
pH Probe · 67
  Calibration · 67
  Gel Filled · 67
  Gel Filled Calibration · 67
  Liquid Filled · 67
  Liquid Filled Calibration · 67
  Recalibration After Sterilization · 70
  Span Calibration · 69
  Zero Calibration · 69
Pneumatic Headlift · 54, 55, 56
  Operation · 54, 55, 56
Positioning the Piping Skid · 24
Preparing for Fermentation · 78
Preventive Maintenance · 100
  Cleaning · 102
  Motor and Gear Reducer · 105, 106
  One Year Intervals · 101
  Steam Prefilter and Regulator Kit · 103, 104
  Sterile Inlet and Exhaust Filters · 104
  Three Month Intervals · 100
  Water Prefilter and Regulator Kit · 102
Pump Capacity · 59
Pumps · 58
  Loading Tubing · 61
  Optional Configurations · 58
  Pump Capacity · 59
  Tubing Selection Chart · 59, 60

R

Replacement Parts
  Descriptions · 110
  Part Numbers · 110
Rotometer · 38, 39

S

Sampling Valve · 96
  Operation · 97
  Sterilization · 97
Schematic
  ML-6100 Optional Recorder Kit · 116
  ML-6100 with Auto Sterilization · 113
  ML-6100 with Auto Sterilization and 2 Gas Option · 115
  ML-6100 with Auto Sterilization and 4 Gas Option · 114
  Motor Control · 112
  Piping and Instrumentation with Auto Sterilization · 117, 118, 119
Select Calibration Window · 68, 70, 75, 77
Service/Utility
  Connections · 24, 25
  Electrical · 27
  Exhuast · 26
  ML-6100 · 27
  Requirements · 24
  Steam Supply · 26
Set Cycle Button · 52
Setting Gas Mix Cycle · 52
Shutdown During Sterilization · 88
Shutdown Procedure · 98
Span Calibration
  DO Probe · 77
  pH Probe · 69
Sparge/Overlay Air (Gas) System · 44
Steam Supply · 26
Ster Operation · 87
Ster Temp · 85
Ster Time · 85
Sterile Inlet and Exhaust Air (Gas) Filters · 43
Sterilization
  Aborting · 90
  Automatic · 82
  Automatic Sterilization Control Dialog · 83
  Cool A Operation · 87
  Cool B Operation · 87
  Drain Operation · 87
  Enabling · 89
  Growth Operation · 87
  Heat A Operation · 87
  Heat B Operation · 87
  Heat Labile Media · 80
  Liquid in Vessel · 82, 83
  Overiew · 82
  Presterilization Systems Check · 78
  Shutdown · 88
  Ster Operation Operation · 87
  Sterilize Button · 89
  Valve ID · 87
  Valve Sequencing · 86
  Vessel Empty · 91
Sterilization Process, Enabling · 89
Sterilization Shutdown Selections Window · 88
Sterilize Button · 89
Systems Check Prior to Fermentation · 78

T

Thermal Mass Flowcontroller · 40
Thermal Mass Flowmeter · 37, 38
Troubleshooting · 108

V

Valve ID · 87
Valve Sequencing · 86
Vessel Pressure · 40
    Automatic Backpressure Control · 42
    Exhaust Gas Treatment · 43
    Manual Backpressure Control · 40
    Sterile Inlet and Exhaust Air (Gas) Filters · 43
Viewing Gas Mix Status · 51

W

Water Priming · 30

Z

Zero Calibration
    DO Probe · 76
    pH Probe · 69