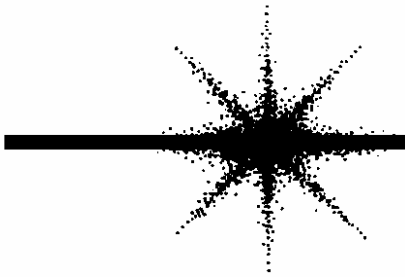

Guide to Operations

BIOFLO III
THE MICROPROCESSOR CONTROLLED LABORATORY-SCALE FERMENTOR

MANUAL NO.: M1226-0050/N

NEW BRUNSWICK SCIENTIFIC CO., INC.
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TELEPHONE: 800-631-5417 . FAX: 732-287-4222 . TELEX: 4753012 NBSCO
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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 one year against faulty components and assembly and our obligation under this warranty is limited to repairing or replacing the instrument part thereof, which shall within one 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 of 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.. In addition to the above, all biological shakers shipped to the U.S.A. and Canada carry an additional one-year warranty.

IMPORTANT NOTICE:

RETURN MATERIAL AUTHORIZATION POLICY

IF SERVICE IS REQUIRED:

PLEASE CALL OUR SERVICE DEPARTMENT AT
1-800-237-2298

PLEASE DO NOT RETURN ANY EQUIPMENT FOR SERVICE WITHOUT A RETURN AUTHORIZATION AND NUMBER WHICH CAN BE OBTAINED FROM OUR SERVICE DEPARTMENT.

THE RETURN AUTHORIZATION NUMBER MUST APPEAR ON THE OUTSIDE OF ALL CARTONS.

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CHAPTER 1
INTRODUCTION

1.1 SCOPE OF MANUAL

This manual contains a description of the vessel, installation, operating and maintenance instruction for the BioFlo III System 1.25, 2.5 and 5L Models manufactured by the New Brunswick Scientific Co., Inc., 44 Talmadge Road, P.O. Box 4005, Edison, New Jersey 08818-4005, USA.

1.2 DESCRIPTION OF EQUIPMENT

BioFlo III is a versatile bioreactor that provides a fully equipped state of the art fermentation system in one compact package. BioFlo III can be employed for batch or continuous culture with microprocessor control of pH, DO, agitation, temperature, nutrient feed, and electronic foam control.

1.3 DESCRIPTION OF VESSEL

The vessel parts consist of a stainless steel head plate, a flanged glass tube (thick walled) vessel body which is detachable from the bottom dished head. The dished head is jacketed for circulation of temperature controlled water. Four sterilizable polypropylene compression ports are provided in the glass wall for the addition of antifoam and nutrients, as well as for vessel overflow in continuous culture studies. Ports are provided in the headplate for: Inoculation, base and acid addition, a thermowell for Resistance Temperature Detector, a Sparger, a Harvest Tube, a Sampler, an Exhaust Condenser, Dissolved Oxygen and pH Electrodes. The drive bearing housing is also located on the headplate.

1.4 AGITATION SYSTEM

A removable agitation motor located on top of the bearing housing is connected to the agitation shaft with a multi-jaw coupling. It can be easily disconnected while autoclaving the vessel and replaced after sterilization. The motor will provide agitation speed range of 50 to 1000 ± (2 RPM + 0.5% setting).

1.5 TEMPERATURE CONTROL

The culture temperature may be selected in the range from 4°C to 80°C (± 0.1°C) and is controlled by a microprocessor based PI (Proportional and Integral) controller. The media temperature is sensed by an RTD (Resistance Temperature Detector) submerged in the thermowell.

1.6 AERATION

Sterile air is introduced into the medium through the ring sparger, and is controlled by the needle valve of the flowmeter. It is able to provide 1.5 (working volume) of sterile air thru 0.2 μm replaceable cartridge filter (Fig. 1). The filter is sterilizable with the vessel. With the system, Oxygen Transfer Rates of 350 mMO_2/L/Hr may be obtained.

1.7 pH CONTROL

pH is controlled in the range of 2.00-12.00 (± 0.01). The pH is sensed by a glass electrode. Control is maintained by PID controller which operates two peristaltic pumps, connected to acid and base addition ports (Fig. 1).

1.8 DO CONTROL

DO is controlled in the range of 5-95% (± 1%). It is sensed by the DO electrode and control is maintained by the PID controller which changes the speed of agitation.

1.9 FOAM CONTROL

Foam is controlled during batch fermentation by the antifoam probe which is located in the headplate. The controller operates the antifoam addition pump that adds chemical defoamer through the port in the wall of the vessel (Fig. 1). Sensitivity and time adjustments are made on potentiometers located on a small printed circuit board mounted on the underside of the electronic shelf, easily accessible when the rear door is open. The potentiometer on the right (P1) is for sensitivity adjustment and the potentiometer on the left (P2) is for percent pump on time adjustment.

1.10 MEDIUM FLOW CONTROL

A nutrient feed peristaltic pump is provided for use during continuous culture fermentation. The maximum flow rate can be found in section 3.8.

1.11 EXHAUST SYSTEM

The exhaust gases pass into the exhaust condenser where moisture is removed and returned to the vessel. The air remaining passes through the 0.2 μm exhaust filter and goes to the atmosphere (Fig. 1).

1.12 SAMPLING SYSTEM

The sampler is attached to a sampling tube that extends to the liquid of the vessel. The sampler has a rubber suction bulb to facilitate collection of representative samples

without contamination. A 25mL screw-cap container serves as a reservoir (Fig. 2).

1.13 FLOWMETER

The flowmeter is a simple, precise means of indicating flow rates in fluid systems. Their design is based on a variable area principle. BioFlo III is equipped with a 0.2 to 4.0 SLPM flowmeter.

NOTE: *5L Unit uses an interchangeable tube to allow increased flow to 7.5 SLPM. Tube is replaced by removing plug on top of flowmeter (Fig. 3).*

CHAPTER 2
INSTALLATION

2.1 INSPECTION

Unpack the BioFlo III and carefully inspect for any apparent damage which may have occurred during transit. Report any obvious damage to the carrier and to New Brunswick Scientific Co., Inc..

Verify that the cabinet, its accessory kit (M1151-0010) and manual are correct. Inspect the vessel assembly, probe kit and vessel accessory kit (M1226-0014).

For initial set up refer to Chapter 3.

2.2 DESCRIPTION OF THE BIOFLO III CONSOLE

1. Front Panel (Fig. 4)

   Right hand side part
   - Agitation Switch - to start or stop the agitation motor.
   - Increase/Descrease Switch - to raise or lower the set point of the parameter selected. Also used during calibration of pH and DO.
   - Selector Switch - to select pH, DO, agitation, temperature or nutrient readings to be displayed.
   - Mode Switch - to select control, setpoint, span or zero mode.
   - DO Control Active Switch - push in this switch to "ON" position. DO will cascade with agitation.

   Left hand side part
   - DO electrode receptacle.
   - pH electrode receptacle.
   - RTD (Resistance Temperature Detector) receptacle.
   - Antifoam (on level) and ground lead sockets.
   - Agitation motor connector.
   - Flowmeter, Flowrate adjustable valve and barbed connector for air.

2. Rear of Cabinet (Fig. 5)

   - Input water line barbed connector.
   - Drain line barbed connector.
   - Air inlet line barbed connector.
   - Line cord.
   - Fuses

3. Left Hand Side of the Console

- 9 pin D-connector for recorder output.
- 25 pin D-connector RS232/422.

4. Right Hand Side of the Console

   - Switch panel (Fig. 6).

   The switch panel located on the right-hand side of the console includes: the Main Power Switch which controls the power to the system; the Prime Switch which fills the recirculation system with water before normal operation, and after each autoclaving. Antifoam, nutrient, base and acid switches operate the addition pumps. The top position "ON", is for continuous pumping to prime the tubing. The bottom switch position "AUTO" is for automatic pump operation. The center position is "OFF".

   - External nutrient pump control (Fig. 7).

The 6 lug terminal strip allows for external control of the nutrient pump mounted on the front panel.

Requirements: A controlled DC voltage source to supply a voltage of +5 VDC to 15 VDC and have a minimum current rating of 20mA.

Connections:
a. Remove the three jumpers from lugs 1 and 2, 3 and 4, 5 and 6 of the terminal strip.
b. Connect the positive control source of lug 3 of terminal strip.
c. Connect the ground lead from control source to Lug 1 of terminal strip.

NOTE: *The nutrient pump switch adjacent to the nutrient pump will not function with these jumpers removed.*

2.3 INSTALLATION OF CONSOLE

1. Position the BioFlo III console on a firm and level surface in an area where services are readily available.

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

3. Set the power and agitation switches to their off position.

4. Check the specification plate on the rear of the unit and plug the line cord into a suitable electrical outlet.

2.4 SERVICE CONNECTIONS

Water: 20 max. PSIG, 50 μm filtration, trim valve adj. 1 1/2 turns open.
Air: 10 PSIG max.

2.5 RECORDER CONNECTIONS

Recorder Output	Option:	Recorder Cable
0.0 - 1.0 Volt DC		M1132-3022

PINS

1, 2	TEMP
3, 4	pH
5, 6	D.O.
7, 8	AGITATION

Grounds are pins 2, 4, 6 and 8 pins.

CHAPTER 3
PREPARATION AND OPERATION

3.1 CLEANING OF VESSEL

1. Fill the vessel with a mild detergent and water solution. Let stand for one hour then scour thoroughly. Use a brush on both inside and outside surfaces.

2. Drain the vessel and rinse several times with tap water. Repeat rinsing with distilled water and let dry.

3.2 VESSEL ASSEMBLY

1. Slide impellers on the shaft of the bearing housing and clamp them down. Lower impeller should be positioned about 1/4 inch above the bottom of the baffle. Upper impeller one to one and half impeller diameters above lower impeller (Fig. 8).

Working From the Underside of the Headplate (Fig. 9)

2. Insert the sparger tube in the sparger port (Fig. 10).

3. Insert the harvest tube in the harvest port (Fig. 11).

Working From the Top of the Headplate

4. Insert the thermowell tube into the temperature port* (Fig. 12).

5. Insert the sampler assembly into the sample port (Fig. 2).

6. Install foam probe in the headplate (Fig. 13).

7. Install the baffle assembly inside of the glass jar by pressing the two edged baffle together (Fig. 14).

8. Install four polypropylene ports or plugs in the wall of the glass jar. Be sure to use a blind plug when a port is not in use (Fig. 15).

9. Lubricate the headplate O-ring with silicone grease.

10. Place the headplate on the flange of the vessel and lock it to the clamping ring with knurled screws.**

11. Install pH and DO probes (Fig. 16, 17, 18).*** Coat probe with glycerin

before inserting into the port in the headplate.****

12. Insert exhaust condenser into the exhaust condenser port. Connect the exhaust filter on the top of the condenser with flexible tubing (Fig. 20).

13. Slide 2" long, 1/4" inside diameter silicone tubing on the top of the sparger tube, then connect air filter to it (Fig. 10).

NOTES:

* *Add a few drops of glycerin into the thermowell tube before inserting RTD.*

** *Do not overtighten the clamping ring to the headplate. Same to the clamping screws for the glass tube reactor and the vessel base (vessel heater).*

*** *When the inoculation port is used for installation of Phoenix DO probe, the DO port is converted to the inoculation port (Fig. 19).*

**** *Membrane of the probe must be free of glycerin.*

3.3 PREPARATION BEFORE STERILIZATION

Before sterilization, perform the following procedure:

1. Fill vessel with water to its working volume.

2. Place the vessel assembly on the three pins located in the base of the cabinet.

3. Position the motor assembly on the top of the bearing housing. Connect the motor cable to the receptacle on the face of the cabinet.

4. Connect cables from all probes (pH, DO and antifoam) to their respective sockets on the front face of the cabinet.
Ground lead from the antifoam socket is to be connected to the ground pin on the headplate.

5. Insert RTD in the thermowell on the headplate and connect it to the socket on the cabinet.

6. Connect the inlet filter on the top of the sparger tube to the hose barb in the face of the cabinet with flexible tubing.

7. Connect water line to rear of the cabinet. Adjust water pressure to 20 PSIG max.

8. Connect drain line to the rear of the cabinet.

9. Connect air line to the rear of the cabinet. Adjust air pressure to 10 PSIG max.

10. Connect the quick connects of plastic water lines to the vessel base and the exhaust condenser.

11. As soon as the power switch is turned ON, press the prime switch ON and hold for approximately 10 seconds or until water runs out the drain line at the rear of the unit.

IMPORTANT NOTE:

When turning the power switch ON, particularly at initial installation. It is important that the unit is properly primed by filling the water circulation loop to prevent the circulating pump from running dry for any period of time.

12. Check that the machine functions properly.

13. Pull out the pH probe from the pH port and calibrate it (see Section 3.6).

3.4 STERILIZATION PROCEDURE

1. Remove the motor from the top of the vessel and place it on the motor mount at the top of the cabinet.

2. The housing cover is to be positioned on the top of the bearing housing before sterilization (Fig. 26).

3. Disconnect air line of the inlet filter side.

4. Disconnect all probes and remove probe cables.

5. Disconnect vessel heater and exhaust condenser water lines.*

6. Remove the sampler rubber bulb then insert glass wool into sampler port and close sampler valve.

7. Empty and clean the vessel.

8. Fill vessel with medium or sterilized water.**
9. Sterilize the complete assembly consisting of jar, headplate and components of headplate by inserting in an autoclave.

10. Autoclave at a temperature of 121°C at 15 PSIG for 25 minutes.

NOTES:

* *When disconnecting the water lines, always disconnect the Water-In line first.*

CAUTION:

NEVER DISCONNECT THE QUICK CONNECT WATER LINES WITH VESSEL BASE AND EXHAUST CONDENSER WHEN THE POWER IS "ON" AND THE CURRENT READING OF THE VESSEL TEMPERATURE IS HIGHER THAN THE SET POINT.

** *For continuous culture, vessel should not be sterilized empty. Use at least 100mL of sterilized water. Probe tips must be moist during sterilization. After autoclaving medium will be brought from the medium reservoir, sterilized separately. For batch fermentation the medium is sterilized with the vessel.*

CAUTION:

WHEN AUTOCLAVING THE GLASS VESSEL, IT MUST BE VENTED AT ALL TIMES. DURING STERILIZATION OF THE GLASS REACTOR, VESSEL, CONTENTS, AND ELECTRODES IT IS IMPORTANT THAT THE PRESSURE BUILT UP IN THE AUTOCLAVE IS RELEASED ONLY WHEN TEMPERATURE HAS DROPPED BELOW 90°C. SLOW EXHAUST IS REQUIRED.

If after autoclaving, most of the liquid has exhausted from the vessel, the autoclave is exhausting too quickly. Adjust the autoclave to exhaust more slowly. Attach a piece of tubing with some non-absorbent material such as glass wool or non-absorbent cotton and some foil wrapped on the ends to one of the addition ports. This helps the vessel to vent more easily during autoclaving. Immediately crimp the foil and close off the vent tubing to maintain sterility.

3.5 pH PROBE PREPARATION

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

Check the level of the reference electrolyte. It should be about 1cm below the filling orifice, which is closed with a rubber T stopper. To add reference electrolyte, take the filling pipette (P0740-4820) and fill it with Viscolyte B (P0860-0130) Electrolyte.

NOTE: *The two chambers are filled with same Reference Electrolyte. The total volume of Reference Electrolyte held be the electrode is approximately 30mL. During normal operation the two rubber stoppers are to be removed.*

3.6 pH ELECTRODE PROBE CALIBRATION

pH probe is to be calibrated prior to autoclaving vessel.

Connect electrode to probe cable.

Turn the agitation switch OFF.

Turn ON power switch.

NOTE: *The pH measuring system is calibrated using two buffer solutions of a known pH.*

1. Set the selector switch to pH.

   Set the mode switch to "ZERO".

   Immerse the pH probe into an external pH 7.00 buffer solution.

   Set the display to read the pH value of the buffer with INC/DEC switch.

2. Immerse the pH electrode in a second external buffer solution which is several pH units above or below the pH selected in the previous step.

   Set the mode switch to "SPAN".

   Set the display to read the value of the second buffer solution with the INC/ DEC switch.

NOTE: *After autoclaving it is recommended that the calibration is checked by taking a sample from the vessel and measuring the pH with an external pH meter.*

3.7 DISSOLVED OXYGEN PROBE CALIBRATION

Probe is to be calibrated after autoclaving vessel.

1. There are two methods of obtaining zero on DO.

   METHOD 1 (Less Accurate)
   Remove the DO probe cable from the DO probe. Set the selector switch to "DO".

   Set the mode switch to "ZERO". With the INC/DEC switch adjust the display reading on the DO to read zero. Connect the DO probe cable to the probe.

   METHOD 2
   Connect the DO probe cable to the DO probe. Set the selector to DO.

   Set the agitation speed to 500 RPM.

   Sparge Nitrogen into the vessel until the display is stable for approximately 5 minutes.

   Set the mode switch to the "ZERO" position.

   With the INC/DEC switch adjust the display for zero reading.

2. Set the "SPAN".

   Set the selector to DO.

   Set the mode switch to "SPAN". Vigorously sparge air into the vessel. Set the agitation speed to 500 RPM.

   After about 30 minutes observe the reading on the display. After the DO reading has stabilized. With the INC/DEC switch adjust the DO display reading to 100.

3.8 SELECTION OF MEDIUM FLOW RATE

Medium flow rate depends on the bore size of the tubing and percentage of the time that nutrient pump will be running (at constant speed).

Nutrient Pump (Seconds):

Set Point	Pump ON (Sec.)	Pump OFF (Sec.)
100.0	10	3
90.0	9	4
80.0	8	5
70.0	7	6
60.0	6	7
50.0	5	8
40.0	4	9
30.0	3	10
20.0	2	11
10.0	1	12
00.0	0	00

Pump flow rates at 100% set point.

Flow rates (mL/min) Silicone tubing only

		Tubing internal diameter				
Freq.	RPM	0.5mm 1/50"	0.8mm 1/32"	1.6mm 1/16"	3.2mm 1/8"	4.8mm 3/16"
50 Hz	5	.083	.19	.84	3.05	6.10
60 Hz	6	0.10	.23	1.00	3.65	7.32

3.9 PREPARATION FOR OPERATION

1. Position the vessel on BioFlo III console. Connect the vessel base and the exhaust condenser with water lines.*

2. Carefully place the DC motor on the vessel assembly.

3. Add glycerin to the thermowell and insert (RTD) temperature probe.

4. Turn power to "ON" position and "PRIME" the system.

5. Connect all probe cables to their respective receptacles.

6. Calibrate DO probe (see sec. 3.7).

7. Set agitation to desired speed and then depress agitation selector to the "ON" position.

8. With "INC/DEC" switch adjust the displayed value of temperature, pH, DO and Nutrient to the desired set point.

9. Set the "DO ACTIVE" switch to "DO ON" or "DO OFF" position.
10. Adjust the airflow rate on the flow meter to the desired flow rate.

NOTE: *Aeration is required whenever the agitation setpoint is greater than 750 rpm. NBS suggests a minimum airflow rate of 0.25 vvm when running at speeds of 750 rpm or more.*

****NOTE:*** *Always connect the water out line first.*

3.10 D.O. ACTIVE SYSTEM

The system is designed to control D.O. within a range of 5-95%. D.O. regulation is accomplished by PID control of agitation speed between 50 and 1000 rpm. When the D.O. actual value is above the D.O. set point, the agitation rpm will decrease until the D.O. set point is reached.

As the D.O. level drops below the set point value, agitation will continue to increase until the D.O. set point is reached. Any further increases in agitation, if needed, must be introduced manually.

To set up and use D.O. Mode proceed as follows:

1. Set selector to Agitation.
2. Set mode to set point.
3. Adjust rpm with Inc/Dec to the minimum rpm value you wish for D.O. control.
4. Press D.O. control active and observe LED is now on for both D.O. control active and D.O. control max rpm.
5. Adjust rpm with Inc/Dec to maximum value you wish for D.O. control.
6. Set mode switch to control.
7. D.O. mode is now controlled by regulation of the Agitation (rpm) between the limits that have been set (minimum/maximum set points).

3.11 SAMPLING PROCEDURE (Fig. 2)

To sample the culture, proceed as follows:

1. Check that the value on the sampler is closed.

2. Loosen the sample bottle so that it is not sealed against the sampler gasket.

3. Squeeze the rubber bulb and then tighten the bottle to seal against the sampler port.

4. Open sampler value and slowly release the bulb.

5. When desired volume of sample is obtained, close the valve.

6. Remove bottle with sample from the sampler. Place the cap from a new bottle onto the bottle containing sample and install the new bottle in the sampler and make sure that the sample bottle is firmly sealed against the sampler gasket. Use aseptic techniques.

7. Repeat step 1 through 6 until desired number of samples are taken.

3.12 SHUT-DOWN PROCEDURES

To shut-down the system proceed as follows:

1. Rotate flowmeter dial to zero.

2. Set the Agitation and Power switches to OFF.

3. If the system is not to be used for several days, disconnect power plug. Remove and clean vessel, and associated components.

NOTE: *DO NOT WASH THE FILTERS OR GET THEM WET.*

CHAPTER 4
MAINTENANCE

4.1 GENERAL

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

4.2 CONSOLE CLEANING

At least once a month, clean all metal parts of unit. Use a damp cloth moistened with water or mild detergent. If a detergent is used, remove all excess by clean water washing.

4.3 PERIODIC INSPECTION

At three month intervals perform the following checks and inspections with all switches "OFF" and incoming power disconnected.

1. Check the fuse(s) for clean contact.

2. Check all controls and accessible items (switches, knobs, fuse holders, screws, nuts and bolts) to make sure they are properly tightened. Tighten any item which is loose.

3. Check that all controls are free of dust and operate easily.

4.4 VESSEL AND TUBING CLEANING

After each run clean the vessel, headplate and associated parts.

4.5 PROBE MAINTENANCE AND STORAGE

Carefully read the instruction manual of probes before use.

4.6 THE AGITATOR BEARING HOUSING (Fig. 25)

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

4.7 REPLACEMENT PART LIST

Vessel (1.25L) Assy.	M1226-2000
Vessel (2.5L) Assy.	M1226-2003
Vessel (5L) Assy.	M1226-2005
Inlet Filter	P0200-0491 (37mm disc)
Exhaust Filter	P0200-0490 (50mm disc)
RTD Assy.	M1169-8002
pH Probe (1.25L, 2.5L) Liquid	P0720-5325
pH Probe (1.25L, 2.5L) Gel, Ingold	P0720-5582
pH Probe (1.25L) Gel, Broadley James	P0720-5731
pH Probe (2.5L) Gel, Broadley James	P0720-5744
pH Probe (5L) Liquid	P0720-5021
pH Probe (5L) Gel, Ingold	P0720-5580
pH Probe (5L) Gel, Broadley James	P0720-5732
pH Cable, Liquid, Gel, Ingold	P0720-2095
pH Cable, Gel, Broadley James	P0720-2270
pH Cap, Liquid, Gel, Ingold	P0720-5317
pH Cap, Gel, Broadley James	P0720-5737
pH Probe, Electrolyte, Liquid	P0860-0130
DO Probe (1.25L) (Phoenix)	P0720-5440
DO Probe (2.5L) (Phoenix)	P0720-5450
DO Probe (5L) (Phoenix)	P0720-5460
DO Probe Cable	P0720-2202
* DO Probe (1.25L) (Ingold)	P0720-5565
* DO Probe (2.5L) (Ingold)	P0720-5561
* DO Probe (5L) (Ingold)	P0720-5562
DO Probe Cap (Ingold)	P0720-5567
DO Service Kit (Ingold)	P0720-5569
DO Adapter (Ingold) (1.25L, 2.5L)	M1016-0900
DO Adapter (Ingold) (5L)	M1226-9446
Seal Washer for Ingold DO Probe	M1016-0890
Teflon Washer for Ingold DO Probe	P0100-9780
O-Ring for Ingold DO Probe	P0280-9163
Antifoam Probe	F5-137
Hose Connect Plug	M1151-9418
Hose Connect Open	M1151-9430
Seal, Vessel Side Port	M1151-9419
Nut, Vessel Side Port	M1151-9420
Fuse, 12 Amp (100V, 120V)	P0380-3122
Fuse, 2.5 Amp (100V, 120V)	EF-141
Glass Vessel 1 1/4L	M1151-9904
Glass Vessel 2 1/2L	M1152-9903
Glass Vessel 5L	M1155-9904
Fuse, 6 1/4 Amp, 220V	EF-118
Fuse, 2 Amp, 220V	P0380-3260

Bearing Housing Cover	M1151-9444
Bearing Housing Cover O-Ring	P0280-6093
Motor Assembly (1.25L, 2.5L)	M1226-0700
Motor Assembly (5L)	M1226-0800

Units shipped before April 1, 1996 (Mfg. No. M1226-3004, -3005, -3006)

Heater, 400W, 100/120V Units	P0620-1330
S.S.R. (240D10), 10 Amp, 100-240 VAC Unit	P0400-3011
Heater, 400W, 220/240V Units	P0620-1331

Units shipped after April 1, 1996

Heater, 750W, 100/120 VAC Units	P0620-1320
S.S.R. (240D45), 45 Amp, 100-240 VAC Units	P0400-3151
S.S.R. (240D10), 10 Amp, 100-240 VAC Units	P0400-3011
Heater, 750W, 220/240V Units	P0620-1321

***NOTE: Ingold DO Probes must be used with DO Adapters.**

O-RING

Description	1 1/4L	2 1/2L	5L
Head Plate	P0280-8002	P0280-8062	P0280-8132
Bumper, Flat Gasket	M1151-9905	M1152-9904	M1155-9902
Knob	P0280-5322	Same	Same
DO Port	P0280-5932	Same	Same
DO Port (Ingold) Washer	M1016-0890	Same	Same
Teflon Washer (Ingold DO)	P0100-9780	Same	Same
O-Ring (Ingold DO Probe)	P0280-9163	Same	Same
pH	P0280-5912	Same	Same
3/16" Port	P0280-5302	Same	Same
1/4" Port	P0280-5322	Same	Same
3/8" Port	P0280-5342	Same	Same
1/2" Port	P0280-5912	Same	Same

4.8 WARNING:

Do not attempt to change boards or electronic components unless you are a qualified service technician. Integrated circuits are extremely susceptible to damage from electrostatic discharge. Read and follow the precautions in Section 4.9 before you begin.

4.9 ESD PRECAUTIONS

1. Do not remove components from their antistatic packaging until you are ready to insert them into their sockets or install the board.
2. Before handling components or boards, touch an unpainted portion of the system unit chassis for a few seconds.
3. Wear a wrist grounding strap, available from most electronic component stores.

APPENDIX I

BIOFLO III OPERATION TIPS

GLASS VESSEL ASSEMBLY

Recommendations for prevention of cracking glass during assembly and autoclaving:

- Cracking of glass due to overtightening of assembly screws will occur during *tightening*, not during autoclaving. Therefore,

    1. Prior to autoclaving, tighten screws "finger tight". If a wrench is applied at this point, turn each nut 1/2 revolution not to exceed 1 revolution.
    2. Place unit in autoclaving making certain that the exhaust filter(s) is not wet or clogged. Also, loosen the inoculation diaphragm cap to achieve further ventilation.
    3. After autoclaving, tighten inoculation port cap. Further tightening of nuts can be done at this time with air flowing through vessel.

EXHAUST CONDENSER

1. DESCRIPTION
    - There are two quick connects on the condenser body, the lower one has valve, the upper one is without valve.
    - To connect the water lines, connect the upper quick connect first, then the lower one.
      To disconnect the water lines, disconnect the lower one first, then the upper one.
    - A restriction (M1151-0140) has been installed in the water line as a water saving device (Fig. 21). If maximum water flow to the condenser is desired, the restriction may be removed.

2. CLEANING
    - The inner assembly of the condenser can be removed for cleaning (Fig. 22).
    - To clean, pass warm water and detergent through the top of the condenser twice (not through quick connects).
    - Run clean water through once.
    - Blow out with air.
    - Clean exhaust condenser after each fermentation. This is most critical when operating as a chemostat for protracted fermentation time.

INSTALL A DOUBLE FILTER SYSTEM

Double exhaust and double inlet filters are recommended.

- Attach a "Y" fitting to the top of the condenser with a piece of tubing. Attach an exhaust filter on each branch. This allows you the flexibility to exchange sterilized filters during a run should one filter become clogged, (pinch off unused line with a clamp).

NOTE: *Fifty generations of recombinant E. coli have been propagated by BioFlo III in continuous culture. Prolonged operation as a chemostat requires double filters.*

OPERATION AS A CHEMOSTAT, SUGGESTIONS

Switch the relative positions of the antifoam and acid pumps on the console so that the antifoam pump is now on the bottom and the acid pump is on the top.

- "Switching" pumps is achieved by switching electrical connections in the back of the unit.
- Simply exchange the 4 plugs which go to one pump board with the 4 plugs from the other pump board.
- It is convenient to use the newly positioned antifoam pump as the harvest pump, (gravity is now in your favor; a continuously filled harvest tube will prevent "grow back").
- Place L-shaped harvest tube through a side port, 3/4 of the way down to the bottom of the vessel.

  Use the antifoam probe as a level probe to actuate the harvest pump.

OTR CALCULATIONS

OTR can be estimated by titrating fixed amount of sodium sulfite, Na_2SO_3, with air,

- $2SO_3 + O_2 \xrightarrow{CU+2} 2SO_4$

Calibrate D.O. electrode.

- Set zero on DO

Fully oxygenate fermentor with agitation and airflow.

- Set span to 100%

Introduce a known amount of Na_2SO_3 into fermentor when fully oxygenated.

- $OTR = \frac{30{,}000\ n}{V\ \Delta T}$ mmoles/L/hr

  n = number of moles of sodium sulfite
  V = vessel volume, liter
  ΔT = time taken from DO curve at two points of 50% DO min.

  Actual OTR Data:
  n = .7 moles (88.27 gms)
  V = 5 liters (filled to center line of addition ports)
  ΔT = 10 minutes
  OTR = 420 mmoles/1/hr

  Impeller Scheme: Rushtons, 1 diameter off bottom, 1 1/2 diameter separation
  Viscosity: 1 cp
  RPM: 998
  air flow: 7.5 SLPM, 1 1/2 vvp (volume air per volume liquid)
  catalyst: .014 moles $CuSO_4 \bullet 5H_2O$ (3.5 gms)

FACTORS AFFECTING CONTROL

The BioFlo III, BioFlo IV, BioFlo 3000, CelliGen Plus, MP40 and 80 controls most functions using a PI (Proportional & Integral), control algorithm. The proportional term of this algorithm is straight forward and provides a larger output for a greater error (set point minus current value). The integral term however, is not as straight-forward. This term integrates the error as a function of time to provide a larger output if an error exists for a time period. Each of these terms have constants which are used in each term of the equation. These constants have been picked based on New Brunswick Scientific's experience with this product and several cell lines. The integral constant is very much dependent on the response time of the system. This response time is affected by many variables. These include medium viscosity, sensor response time, control element response time and cell response time. These factors affect each control loop but are especially important in the control of pH. In general, this flow rate and acid/base concentrations for particular application must be determined empirically by the investigator.

The first factor to consider is the pH probe. pH probes as they age and are affected by many factors, e.g., protein accumulation, diaphragm clogging, and electrolyte contamination. All of these things lengthen the response time of the probe. This builds lag time into the control loop and causes the controller not to work properly. The probe should be cleaned and reactivated on a regular basis.

The next factor is strength or concentration of the acid or base (caustic) being used. Obviously, the higher the concentration the faster the system will respond to a given

addition. If the system is overshooting the set point in either direction the system could be made to control better by diluting the appropriate reagent (acid if undershooting and base if overshooting).

The last factor that can be controlled is the amount added over a given period of time. Since in this system the pump is a fixed speed pump and the "ON" time is controlled over a fixed pulse width if the flow rate is too small or too large, the controller cannot adjust properly. Therefore, the flow rate should be adjusted by changing the bore size of the tubing used for the peristaltic pump addition. Usually the tubing is too large and should be adjusted downward to effect better control if unit is not controlling properly.

Under proper conditions, pH may be controlled to within ± .02 units. However, if the flow rate or acid/base concentration are too high, the control shown in Figure 23A may be observed. Here, control at pH 6.00 (from 7.18) after 24-48 hours of Saccharomyces cerevisiae fermentation was attempted using 2N HCL and 2N NaOH with a flow rate of approximately 7.3 mL/min (9.5 mL/min for maximum flow rate). After an initial period of accurate control, it became apparent that the ratio of flow rate (or concentration) to bioreactor volume was too high. In this case, control to ± 0.12 unit was obtained. If the flow rate is increased still further to 13.5 mL/min by increasing the internal diameter (I.D.) of the tubing, then control to ± 0.19 units results. This is shown in Figure 23B. On the other hand, if the flow rate is decreased to 4.1 mL/min by a reduction in tubing I.D., then accuracy is increased to ± 0.02 units, after a slightly longer initial lag time (Fig. 23C).

The effects of agitation rate (or inversely, viscosity) may also be seen in Figure 23D, where the pH control of a 1L fermentation of the same organism is attempted. The difference in control accuracy at different agitation rates is evident (flow rate = 1.9 mL/min). Although a less optional deviation from the set point value, as shown in the examples above, may be more than acceptable for a particular application, it is generally desirable to eliminate such deviations in order to minimize the excessive use of acid and base. Furthermore, the addition of excess Na+ and CL- ions (as NaOH and HCL), along with increase in medium volume, are factors which one might also wish to consider.

The dissolved oxygen control loop may have similar control difficulties if the probe or electrode is not maintained on a regular basis.

The temperature control loop might not control well in very vicious applications because the heat transfer rate was decreased so much the controller loses its ability to adjust the heating and cooling times properly. Most times this can be cured or improved by increasing the agitation and/or airflow rates and thus improving the heat transfer. Improper temperature control might also happen after a long period of use because there is a buildup of mineral deposits inside the cooling system. This can be cured by flushing the system with a de-liming solution.

APPENDIX II
COMMUNICATION PROTOCOLS FOR NBS RS232/422

COMMUNICATIONS

The fermentor system has serial communications signals compatible with both the RS232C and RS422 standards which allow a host computer to be interfaced directly to the fermentor system. These signals are accessed via the 25 pin "D" connector (serial port). Table 4 provides detailed information on pin assignments for the serial port connector and associated signal definitions.

COMMUNICATION SET-UP - (See Table 1 and 2)
All the switch settings are read only once at fermentor power-up time. So any change of the switch settings will not take effect until next power-up.

MODE
Non-Multi-Drop Mode - The fermentor responds to inquiry and modify set points commands.

Multi-Drop Mode - The fermentor responds to inquiry and modify set points commands which are prefixed with a unit number. When using the RS422 multi-drop format, the mating connector for each fermentor unit on the RS422 link should be fitted with a 1000 ohm, 1/2w terminating resistor. It should be connected from IRXD+
(pin 12) to IRXD- (pin 24). The ITXD lines should be terminated similarly at the host.
Consult the host computer manual for details and appropriate pin numbers.

HANDSHAKE - None

BAUD RATE
Baud rates are selectable and include standard rates 1200, 2400, 4800, 9600.

WORD LENGTH
In the non-multi-drop mode, 7-bit or 8-bit word length may be used.

In the multi-drop mode, 8-bit word length is required.

PARITY
Parity may be selected as none or even.

STOP BITS
2 stop bits.

UNIT NUMBER

The multi-drop mode requires a unique unit number assignment for each fermentor in the multi-drop chain. All inquiries and directives and their responses in the multi-drop mode are prefixed with the unit number. The unit number is formatted as a single hexadecimal byte where the low-order nibble (bits 3-0) is set to a number between 0 and 15 and the high-order nibble (bits 7-4) is set to 8. Table 5 shows the valid hexadecimal values for fermentor unit numbers. The format of a multi-drop operating setpoint inquiry from unit 1 in hexadecimal notation is

81 52 44 0D

where 81 is the unit number
52 is the code for R
44 is the code for D
and 0D is the code for carriage return.

The response of unit 1 to this inquiry is shown in Figure 1.0d. A directive to change the setpoints of unit 1 is shown in Figure 1.0h.

EOL CHARACTERS

The EOL (end of line character) is CR (0DH).

SERIAL PORT PROTOCOL

Two types of commands are accepted by the fermentor: Inquiry or Directive.

INQUIRIES

An inquiry command causes a requested report to be transmitted by the fermentor. All inquiry request strings are of the form:

52H	xxH	0DH
R	X	Carriage Return

Where R and X are standard ASCII and X is as shown in Table 3.

Table 3 - Inquiry Responses

X	xx	Reponse	Response Header
A	41H	Loop Names	“Loop ”
B	42H	Blank Line	
C	43H	Current Values	“Cur:Value”
D	44H	Operating Points	“OP Points”
E	45H	Output Percentage	“Output % ”
J	4AH	Control Modes	“Control ”
K	4BH	Blank Line	
L	4CH	Current Value Units	Main Unit

Inquiries are acknowledged by the requested report in the format described below. If parity is selected and a parity failure occurs, no response is made.

REPORT FORMAT

Each report begins with an identifying header (9 characters) followed by a space. The identifier for each report is as shown in Table 3. The requested data for each installed loop is then printed in a six character field followed by a space.

When no loop is installed, the data field for that loop is filled with spaces so that all responses total 80 characters. Finally the response is terminated with a CR.

Figure 1.0e shows a typical response to a request for current values. The response is shown in ASCII as well as the actual hexadecimal character string transmitted for clarity. Similarly Figure 1.0c shows a typical response to a request for operating points.

ONE DIRECTIVE IS SUPPORTED BY THE FERMENTOR

Modify Setpoints - Figure 1.0g shows the required format for a "Modify Set points" directive. Note that the numeric data fields are positioned exactly the same as in a report generated by the fermentor. The data field for each loop must contain a valid ASCII numeric string (i.e., be composed of characters 0 thru 9 and may include a "+" or "-" sign, a decimal point, and leading or trailing spaces) or may be filled with spaces. A space-filled data field (20H 20H 20H 20H 20H 20H) for an installed loop does not modify the set point of this loop. If parity has been selected and a parity failure is detected in the directive, it will be ignored. The directive must be terminated with a carriage return (0DH) after the 80th character. If the directive is received properly the fermentor executes the command and acknowledges by sending a 06H.

TABLE 1
SETTINGS OF SWITCH 1 (S1) ON THE CONTROL BOARD

1. S1-2 and S1-1 are used for setting baud rate:

Baud Rate	S1-2	S1-1
1200	ON	ON
2400	ON	OFF
4800	OFF	ON
9600	OFF	OFF

2. S1-3 is used for setting parity check:

Parity	S1-3
even	ON
no	OFF

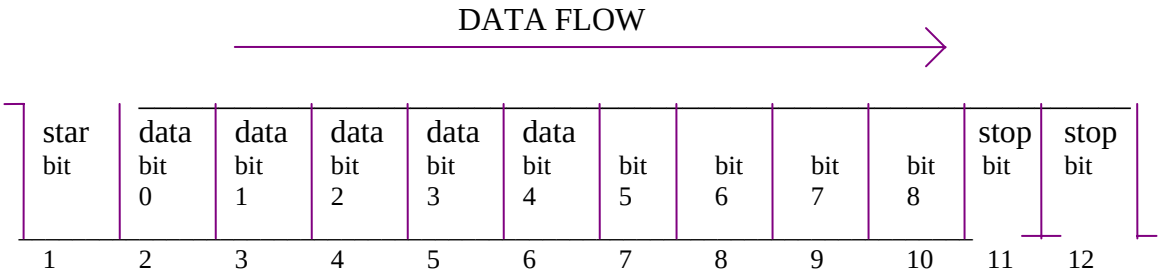
3. S1-4 is used for setting mode:

Mode	S1-4
multidrop	ON
non-multidrop	OFF

4. S1-8, S1-7, S1-6, S1-5 are used for setting unit number:

Unit #	S1-8	S1-7	S1-6	S1-5
0	ON	ON	ON	ON
1	ON	ON	ON	OFF
2	ON	ON	OFF	ON
3	ON	ON	OFF	OFF
4	ON	OFF	ON	ON
5	ON	OFF	ON	OFF
6	ON	OFF	OFF	ON
7	ON	OFF	OFF	OFF
8	OFF	ON	ON	ON
9	OFF	ON	ON	OFF
10	OFF	ON	OFF	ON
11	OFF	ON	OFF	OFF
12	OFF	OFF	ON	ON
13	OFF	OFF	ON	OFF
14	OFF	OFF	OFF	ON
15	OFF	OFF	OFF	OFF

TABLE 2
SERIAL DATA FORMAT



Mode and Parity	bit 7	bit 8
multidrop, parity	adata *	parity bit
multidrop, no parity	adata *	1 **
non-multidrop, parity	parity bit	1 **
non-multidrop, no parity	0 **	1 **

* adata - 1 indicates that the current date is a unit number.
- 0 indicates that the current date is a 7-bit data.

** This is the value the transmitter of Superboard sends out. The receiver of Superboard does not care this bit.

TABLE 3

NBS Serial Communication Message Format

- unit number character; S - blank space character (20H); C - CR character (0DH)

MesHeader	S	Loop#1	S	Loop#2	S	Loop#3	S	Loop#4	S	Loop#5	S	Loop#6	S	Loop#7	S	Loop#8	S	Loop#9	S	Loop10	S	C
01		09	10	11		16	17	18		23	24	25		30	31	32		37	38	39		44	45	46		51	52	53		58	59	60		65	66	67		72	73	74		79	80	81

Figure 1.0a Message Format - Non-multidrop

#	MesHeader	S	Loop#1	S	Loop#2	S	Loop#3	S	Loop#4	S	Loop#5	S	Loop#6	S	Loop#7	S	Loop#8	S	Loop#9	S	Loop10	S	C
01	02		10	11	12		17	18	19		24	25	26		31	32	33		38	39	40		45	46	47		52	53	54		59	60	61		66	67	68		73	74	75		80	81	82

Figure 1.0b Message Format - Multidrop

```
O P  P o i n t s        9 0 0      1 0 2 . 5       1 5 . 0       1 0 . 0       6 . 8 0       0 . 0 0       3 7 . 0      - 1 0 0 0      1 2 1 . 5          0 . 0 .
4F 50 20 50 6F 69 6E 74 73 20 20 20 20 39 30 30 20 20 31 30 32 2E 35 20 20 20 31 35 2E 30 20 20 20 20 31 30 2E 30 20 20  20 36 2E 38 30 20 20 20 20 30 2E 30 30 20 20 20 33 37 2E 30 20 20 2D 31 30 30 30 20 20 31 32 31 2E 35 20 20 20 20 30 2E 30 20 0D
```

Figure 1.0c Operating Points Report Format - Non-multidrop

```
# O P  P o i n t s        9 0 0      1 0 2 . 5       1 5 . 0       1 0 . 0       6 . 8 0       0 . 0 0       3 7 . 0      - 1 0 0 0      1 2 1 . 5          0 . 0
23 4F 50 20 50 6F 69 6E 74 73 20 20 20 20 39 30 30 20 20 31 30 32 2E 35 20 20 20 31 35 2E 30 20 20 20 20 31 30 2E 30 20  20 20 36 2E 38 30 20 20 20 20 30 2E 30 30 20 20 20 33 37 2E 30 20 20 2D 31 30 30 30 20 20 31 32 31 2E 35 20 20 20 20 30 2E 30 20 0D
```

Figure 1.0d Operating Points Report Format - Multidrop

```
C u r : V a l u e        8 9 9      1 0 2 . 2       1 5 . 0       1 0 . 1       6 . 7 8       0 . 1 0       3 6 . 9      - 1 0 0 5      1 2 1 . 6          0 . 0
43 75 72 3A 56 61 6C 75 65 20 20 20 20 38 39 39 20 20 31 30 32 2E 32 20 20 20 31 35 2E 30 20 20 20 20 31 30 2E 31 20 20  20 36 2E 37 38 20 20 20 30 2E 31 30 20 20 20 33 36 2E 39 20 20 2D 31 30 30 35 20 20 31 32 31 2E 36 20 20 20 20 30 2E 30 20 0D
```

Figure 1.0e Current value Report Format - Non-multidrop

```
# C u r : V a l u e        8 9 9      1 0 2 . 2       1 5 . 0       1 0 . 1       6 . 7 8       0 . 1 0       3 6 . 9      - 1 0 0 5      1 2 1 . 6          0 . 0
23 43 75 72 3A 56 61 6C 75 65 20 20 20 20 38 39 39 20 20 31 30 32 2E 32 20 20 20 31 35 2E 30 20 20 20 20 31 30 2E 31 20  20 20 36 2E 37 38 20 20 20 30 2E 31 30 20 20 20 33 36 2E 39 20 20 2D 31 30 30 35 20 20 31 32 31 2E 36 20 20 20 20 30 2E 30 20 0D
```

Figure 1.0f Current value Report Format - Multidrop

```
M S                      8 5 0      1 0 0 . 0       1 2 . 5       1 0 . 0       6 . 8 0       0 . 0 0       3 5 . 0      - 1 0 0 0      1 2 1 . 0          0 . 0
4D 53 20 20 20 20 20 20 20 20 20 20 38 35 30 20 20 31 30 30 2E 30 20 20 20 31 32 2E 35 20 20 20 31 30 2E 30 20 20  20 36 2E 38 30 20 20 20 20 30 2E 30 30 20 20 20 33 35 2E 30 20 20 2D 31 30 30 30 20 20 31 32 31 2E 30 20 20 20 20 30 2E 30 20 0D
```

Figure 1.0g Modify Setpoints Directive Format - Non-multidrop

```
# M S                      8 5 0      1 0 0 . 0       1 2 . 5       1 0 . 0       6 . 8 0       0 . 0 0       3 5 . 0      - 1 0 0 0      1 2 1 . 0          0 . 0
23 4D 53 20 20 20 20 20 20 20 20 20 20 38 35 30 20 20 31 30 30 2E 30 20 20 20 31 32 2E 35 20 20 20 31 30 2E 30 20 20  20 36 2E 38 30 20 20 20 20 30 2E 30 30 20 20 20 33 35 2E 30 20 20 2D 31 30 30 30 20 20 31 32 31 2E 30 20 20 20 20 30 2E 30 20 0D
```

Figure 1.0h Modify Setpoints Directive Format - Multidrop

TABLE 4
SERIAL INTERFACE CONNECTOR

PIN #	SIGNAL	COMMENTS
1	NC	
2	TXD	RS232 Data Output from Fermentor
3	RXD	RS232 Data Input to Fermentor
4	NC	
5	NC	
6	NC	
7	GND	Ground Reference for all Signal
8	NC	
9	NC	
10	NC	
22	NC	
11	NC	
23	NC	
12	IRXD+	RS422 Paired Data Input
24	IRXD-	to Fermentor
13	ITXD+	RS422 Paired Data Output
25	ITXD-	from Fermentor
14-19	NC	
20	NC	
21	IOS	Open Selects RS232; Grounded Selects RS422

TABLE 5
HEXADECIMAL CODES
FOR
MULTIDROP UNIT NUMBERS

Unit Number	Hexadecimal Code
0	80
1	81
2	82
3	83
4	84
5	85
6	86
7	87
8	88
9	89
10	8A
11	8B
12	8C
13	8D
14	8E
15	8F

```
100  '-------------------------------------------------------------;
110  '                                                             ;
120  '       PC To BIOFLOW III Communications Test/Sample Program  ;
130  '                        (Dec. 07, 1989)                      ;
140  ' This program works on COM1. Switch 1 to 4 of DIP SW1 in BIOFLOW ;
150  ' should be OF,OF,ON,OF, which means non-multidrop and even parity;
160  ' check. If communication is OK, the loop name line, current value;
170  ' line, and the setpoint line are displayed. Then, they should be ;
180  ' updated every 5 seconds. Agitation setpoint ramps up 1 rpm every;
190  ' time, starting from 80 rmp. To stop running, use CTRL-BREAK.    ;
200  '-------------------------------------------------------------;
210  'Setup Communication Port COM1
220  CLOSE #1
230  OPEN "COM1:9600,E,7,2,RS,CS,DS" AS #1
240  'Definitions
250  '-----------
260  DIM SETPT(10),SETPT$(10),LOOP$(10),VALUE(10),VALUE$(10)
270  DIM OPRPT$(10),OPRPT(10)
280  'Initializations
290  '--------------
300  AGIT%=1:TEMP%=2:DO%=3:PH%=4:NUTR%=5
310  MS$="MS         "     'MS$ IS THE MODIFY SETPOINT DIRECTIVE
320  RA$="RA"+CHR$(13)     'RA$ IS THE LOOP NAME INQUIRY COMMAND
330  RC$="RC"+CHR$(13)     'RC$ IS THE CURRENT VALUE INQUIRY COMMAND
340  RD$="RD"+CHR$(13)     'RD$ IS THE OPERATING POINT INQUIRY COMMAND
350  RB$="RB"+CHR$(13)     'RB$ IS THE BLANK LINE INQUIRY COMMAND
360  SETPT(AGIT%)=80
370  SETPT(TEMP%)=37.5
380  SETPT(NUTR%)=4.3
390  SETPT(PH%)=6.54
400  SETPT(DO%)=38
410  'START COMMUNICATIONS LOOP
420  T0=TIMER:RATE=5      'RATE IS THE APPROXIMATE DATA SAMPLING RATE
430  IF (TIMER-T0)<RATE THEN 430
440  GOSUB 550
450  'Define User Program Below
460  PRINT "    "
470  PRINT LOOP$(0);
480  PRINT VALUE$(0);
490  PRINT OPRPT$(0);
500  FOR I=1 TO 5         'CONVERT VALUE$ TO VECTOR OF CUR.VALUES
510  GOSUB 690
520  NEXT I
530  SETPT(AGIT%)=SETPT(AGIT%)+1   'RAMP AGITATION SPEED
540  GOTO 420
550  'COMMUNICATIONS SUBROUTINE
560  PRINT #1,RA$;          'READ LOOP LIST FROM BIOFLOW
570  LINE INPUT #1,X$:LOOP$(0)=X$
580  PRINT #1,RC$;          'READ CURRENT VALUE STRING FROM BIOFLOW
590  LINE INPUT #1,X$:VALUE$(0)=X$
600  PRINT #1,RD$;          'READ OPERATING POINT STRING FROM BIOFLOW
610  LINE INPUT #1,X$:OPRPT$(0)=X$
620  SP$=""                 'BUILD SET POINT COMMAND LINE
630  FOR N=1 TO 10
640  SP$=SP$+LEFT$(STR$(SETPT(N))+"        ",7)
650  NEXT N
660  SP$=MS$+SP$+CHR$(13)
670  PRINT #1,SP$;:Z$=INPUT$(1,#1)
680  RETURN
690  VALUE(I)=VAL(MID$(VALUE$(0),(11+7*(I-1)),7)):RETURN
```

PARTS LIST - 1 1/4L, 2 1/2L AND 5L
BIOFLO III (Refer to Fig. 27A, 27B, 27C & 27D)

ITEM	PART NUMBER	DESCRIPTION	QTY.
1a	P0160-6450	Half Coupling	1
1b	P0160-6450	Half Coupling	1
1c	P0160-6451	Half Coupling	1
2a	M1151-4100	Bearing Housing Assy.	1
2b	M1152-4100	Bearing Housing Assy.	1
2c	M1155-4100	Bearing Housing Assy.	1
3a	P0280-5322	O-Ring	6
3b	P0280-5322	O-Ring	7
3c	P0280-5322	O-Ring	7
4a	M1151-9412	Knob	3
4b	M1151-9412	Knob	4
4c	M1151-9412	Knob	4
5a	P0280-8002	O-Ring	2
5b	P0280-8062	O-Ring	2
5c	P0280-8132	O-Ring	2
6a	M1226-9204	Headplate	1
6b	M1226-9206	Headplate	1
6c	M1226-9208	Headplate	1
7a	M1151-9905	Bumper, Flat Gasket	2
7b	M1152-9904	Bumper, Flat Gasket	2
7c	M1155-9902	Bumper, Flat Gasket	2
8a	M1151-9437	Clamping Ring	1
8b	M1226-9426	Clamping Ring	1
8c	M1226-9432	Clamping Ring	1
*9	M1151-9436	Connector, Clamping Ring	4
10a	M1151-9904	Glass Vessel	1
10b	M1152-9903	Glass Vessel	1
10c	M1155-9904	Glass Vessel	1
*11	M1169-0610	Knob Screw	4
12a	M1151-9435	Clamping Ring, Bottom	1
12b	M1152-9412	Clamping Ring, Bottom	1
12c	M1155-9434	Clamping Ring, Bottom	1
13a	M1151-9201	Vessel Heater Assy.	1
13b	M1152-9201	Vessel Heater Assy.	1
13c	M1155-9206	Vessel Heater Assy.	1
*14	P0240-0854	Quick Connect Body	1
*15	P0240-0856	Quick Connect Body	1

| ITEM | PART NUMBER | DESCRIPTION | QTY. |

16a	M1151-9226	Baffle	1
16b	M1152-9207	Baffle	1
16c	M1155-9205	Baffle	1
17a	M1151-9224	Impeller	2
17b	M1152-9208	Impeller	2
17c	M1155-9205	Impeller	2
* 18	M1151-9518	Plug, Glass Vessel	4
* 19	M1151-9520	Nut, Hose Connector	4
* 20	M1151-9419	Seal, Hose Connector	4
21a	M1151-9227	Sparger Tube	1
21b	M1152-9407	Sparger Tube	1
21c	M1155-9203	Sparger Tube	1
22a	M1226-9434	Collar Nut	1
22b	M1151-9512	Collar Nut	1
22c	M1151-9512	Collar Nut	1
* 23	M1151-9511	Plug, Inoc. Port	1
* 24	M1077-1040	Rubber, Syringe	1
* 25	M1226-9414	Plug 1/4”	3
* 26	M1132-946T	Ferrule 1/4”	3
* 27	P0280-5302	O-Ring	4
* 28	M1132-9433	Plug, pH	1
* 29	M1132-9431	Cap	1
* 30	P0280-5912	O-Ring	2
* 31	M1226-9418	Plug 1/2”	1
* 32	H-727	Plug, DO	1
* 33	P0280-5932	O-Ring	1
* 34	M1176-9241	Plug, 3/8”	1
* 35	P0280-5342	O-Ring	1
* 36	M1226-9416	Plug 3/16”	1
* 37	M1226-9430	Ferrule	1
* 38	P0280-5292	O-Ring	1
* 39	S2217-2100	Cap Screw 8-32 x 5/8”	4
* 40	W2212-3088	Washer #8 Lock	4
* 41	S2114-3041	Screw 8-32 x 1/4”	8
* 42	W2217-3120	Washer 1/4” Lock	4
45a	M1132-9144	Sampler Tube	1
45b	M1132-9144	Sampler Tube	1
45c	M1132-9141	Sampler Tube	1
* 46	M1151-9519	Hose Connector	4
47a	M1151-9150	Nutrient Tube	2
47b	M1152-9217	Nutrient Tube	2
47c	M1155-9525	Nutrient Tube	2

ITEM	**PART NUMBER**	**DESCRIPTION**	**QTY.**

48a	M1151-940P	Harvest Tube	1
48b	M1152-9405	Harvest Tube	1
48c	M1155-9416	Harvest Tube	1
49a	M1132-9252	Well Thermister	1
49b	M1132-9254	Well Thermister	1
49c	M1132-9253	Well Thermister	1
*50	P0280-5362	O-Ring	1
*56	P0240-8270	Nut	1
*57	P0240-7970	Ferrule	1
*58	P0240-8260	Nut	1
*59	P0240-7960	Ferrule	1
*60	P0240-8240	Nut	3
*61	P0240-7940	Ferrule	3
*62	P0240-8230	Nut	1
*63	P0240-7930	Ferrule	1

NOTES:

*** indicates common to all three sizes**
a indicates parts for 1 1/4L vessel
b indicates parts for 2 1/2L vessel
c indicates parts for 5L vessel

VESSEL SPARE PARTS
1.25L (M1226-0100)

PART NO.	DESCRIPTION	WHERE USED	QTY
P0280-5322	O-Ring 2-010 EPRP	For knob assy., 3 per headplate, 3 for ¼" ports	18
M1016-0890	Washer, D.O. Probe	Used with probe adapter for 12mm probe	2
P0280-5912	O-Ring 2-111 EPRP	On pH port (on top and bottom)	6
P0280-5292	O-Ring 2-007 EPRP	When foam probe used	3
P0280-5302	O-Ring 2-008 EPRP	On foam probe; harvest port; sampling port; antifoam port	12
P0280-5342	O-Ring 2-012 EPRP	On thermowell port	3
P0280-5932	O-Ring 2-113 EPRP	On D.O. port for 14mm probe	3
M1077-1040	Diaphragm	On inoculation port	2
M1132-946T	Ferrule	On ¼" port assy. with tubing	6
M1226-9430	Ferrule 3/16"	On 3/16" port assy. with foam probe	2
P0240-7960	Ferrule Frt SS 3/8 tube	On water in/out jacket to console (item #2) flexible hose assy	2
P0160-6389	Coupling Sleeve	On motor assy., connects to bearing housing	2
P0540-0150	Chipboard	Packaging for all these parts	1
M1151-9419	Seal - Silicone	On hose connector plugs on glass side, goes inside sampler assy.	6
P0280-5362	O-Ring 2-014 EPRP	On condenser port	3
P0180-0170	Ball Bearing	On bearing housing	3
P0280-5422	O-Ring 2-020 EPRP	On cap of bearing housing	3
P0280-5472	O-Ring 2-025 EPRP	On bearing housing	3
P0280-8002	O-Ring 2-353 EPRP	On head plate and base plate	4
M1151-9905	Bumper	Protects glass vessel	2

VESSEL SPARE PARTS
2.5L (M1226-0101)

PART NO.	DESCRIPTION	WHERE USED	QTY
P0280-5322	O-Ring 2-010 EPRP	For knob assy., 4 per headplate, 3 for ¼" ports	18
M1016-0890	Washer, D.O. Probe	Used with probe adapter for 12mm probe	2
P0280-5912	O-Ring 2-111 EPRP	On pH port (on top and bottom)	6
P0280-5292	O-Ring 2-007 EPRP	When foam probe used	3
P0280-5302	O-Ring 2-008 EPRP	On foam probe, harvest port, sampling port, antifoam port	12
P0280-5342	O-Ring 2-012 EPRP	On thermowell port	3
P0280-5932	O-Ring 2-113 EPRP	On D.O. port for 14mm probe	3
M1077-1040	Diaphragm	On inoculation port	2
M1132-946T	Ferrule	On ¼" port assy. with tubing	6
M1226-9430	Ferrule 3/16"	On 3/16" port assy. with foam probe	2
P0240-7960	Ferrule Frt SS 3/8 tube	On water in/out jacket to console (item #2) flexible hose assy.	2
P0160-6389	Coupling Sleeve	On motor assy., connects to bearing housing	2
M1151-9419	Seal-Silicone	On hose connector plugs on glass side, goes inside sampler assy.	6
P0280-5362	O-Ring 2-014 EPRP	On condenser port	3
P0180-0170	Ball Bearing	On bearing housing	3
P0280-5422	O-Ring 2-020 EPRP	On cap of bearing housing	3
P0280-5472	O-Ring 2-025 EPRP	On bearing housing	3
P0280-8062	O-Ring 2-359 EPRP	On head plate and base plate	4
M1152-9904	Bumper	Protects glass vessel	2
P0540-0150	Chipboard	Packaging for all these parts	1

VESSEL SPARE PARTS
5L (M1226-0102)

PART NO.	DESCRIPTION	WHERE USED	QTY
P0280-5322	O-Ring 2-010 EPRP	For knob assy., 4 per headplate, 3 for ¼" ports	18
M1016-0890	Washer, D.O. Probe	Used with probe adapter for 12mm probe	2
P0280-5912	O-Ring 2-111 EPRP	On pH port (on top and bottom)	6
P0280-5292	O-Ring 2-007 EPRP	When foam probe used	3
P0280-5302	O-Ring 2-008 EPRP	On foam probe, harvest port, sampling port, antifoam port	12
P0280-5342	O-Ring 2-012 EPRP	On thermowell port	3
P0280-5932	O-Ring 2-113 EPRP	On D.O. port	3
M1077-1040	Diaphragm	On inoculation port	2
M1132-946T	Ferrule	On ¼" port assy with tubing	6
M1226-9430	Ferrule 3/16"	On 3/16" port assy. with foam probe	2
P0240-7960	Ferrule Frt SS 3/8 tube	On water in/out jacket to console (item #2) flexible hose assy.	2
P0160-6389	Coupling Sleeve	On motor assy., connects to bearing housing	2
M1151-9419	Seal - Silicone	On hose connector plugs on glass side, goes inside sampler assy.	6
P0280-5362	O-Ring 2-014 EPRP	On condenser port	3
P0180-0170	Ball Bearing	Bearing housing	3
P0280-5422	O-Ring 2-020 EPRP	On cap of bearing housing	3
P0280-5472	O-Ring 2-025 EPRP	On bearing housing	3
P0280-8132	O-Ring 2-366 EPRP	On head plate and base plate	4
M1155-9902	Bumper	Protects glass vessel	2
P0540-0150	Chipboard	Packaging for all these parts	1

DRAWING LIST

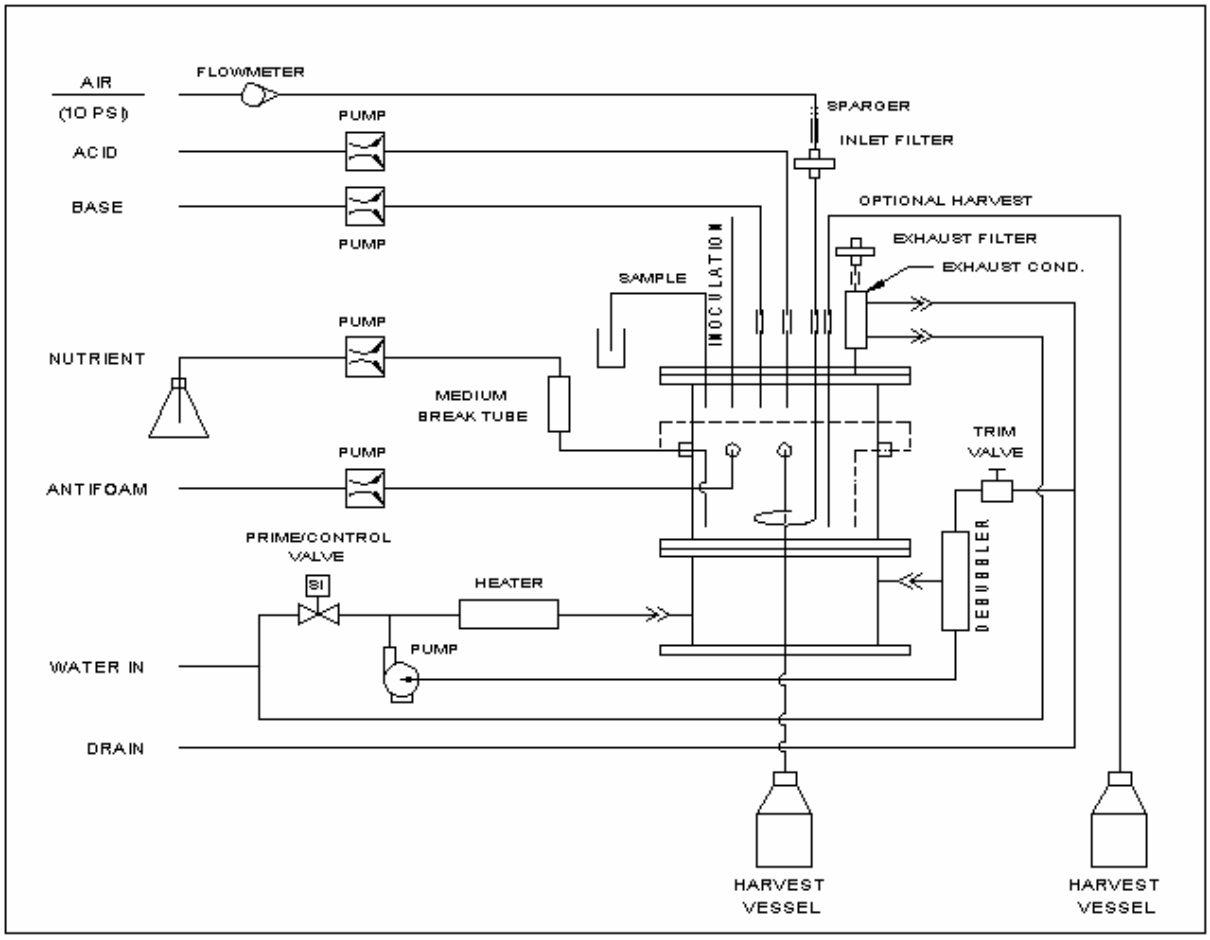


FIGURE 1 - VESSEL SCHEMATIC

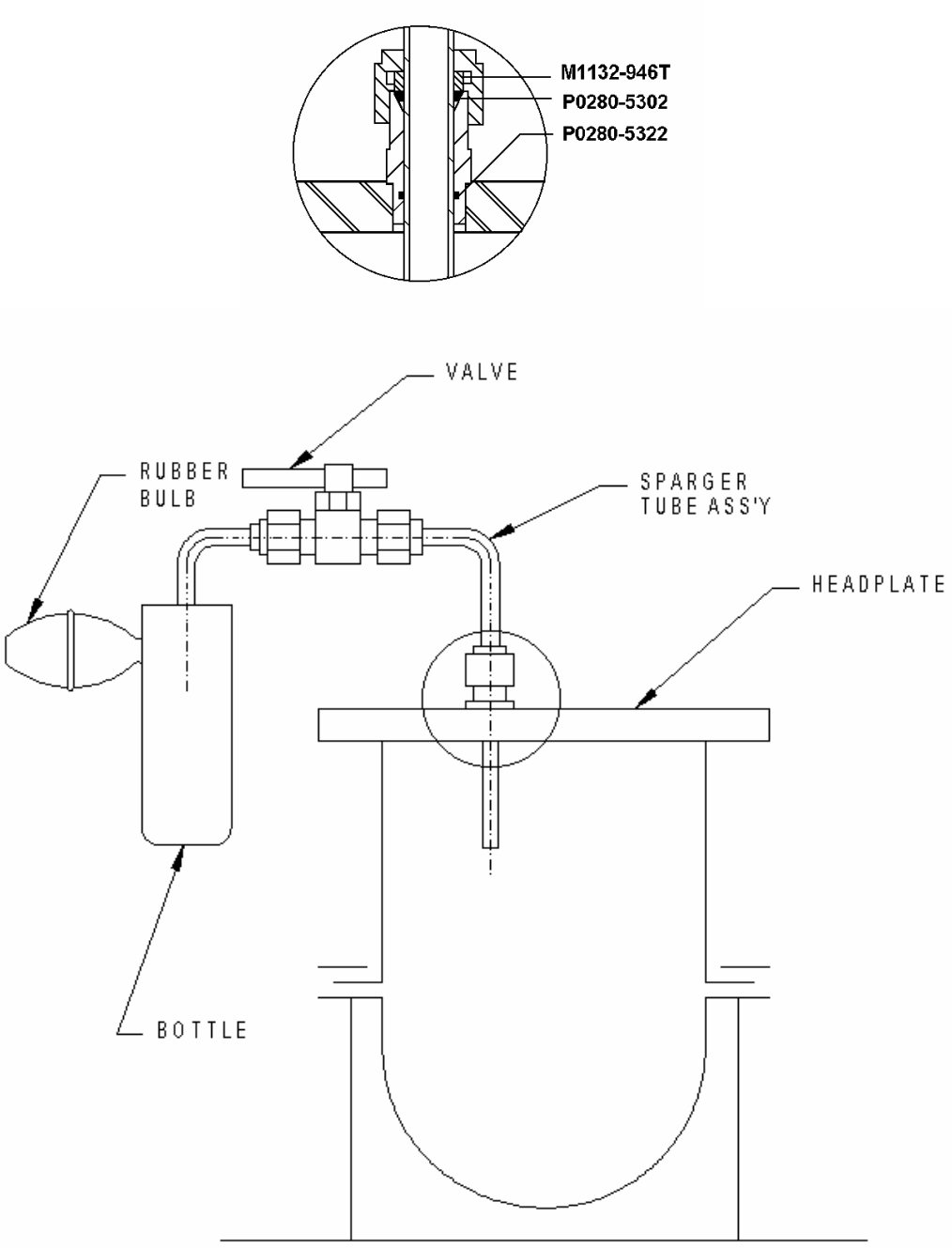


FIGURE 2 - SAMPLING SYSTEM

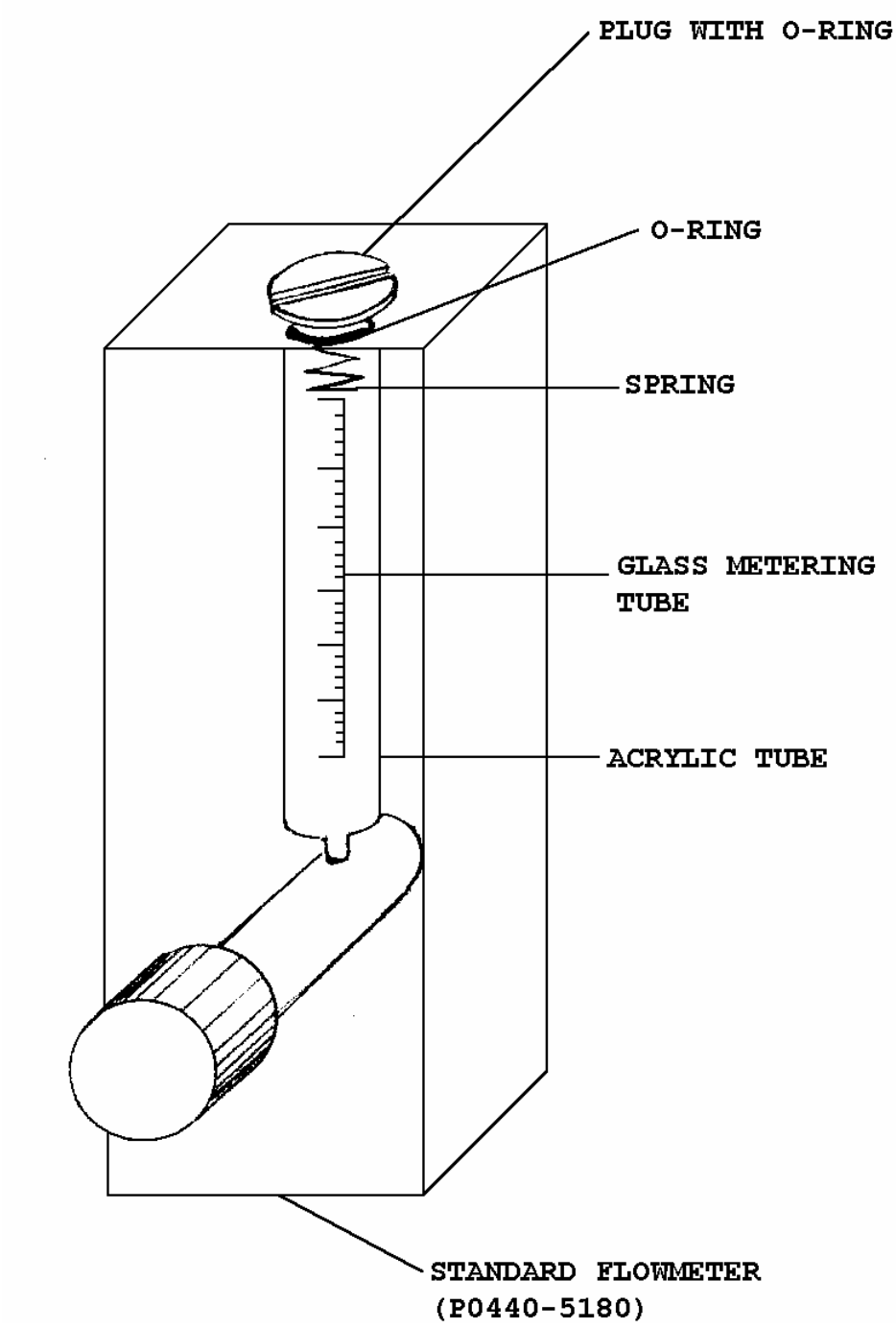


FIGURE 3 - FLOWMETER CONVERSION

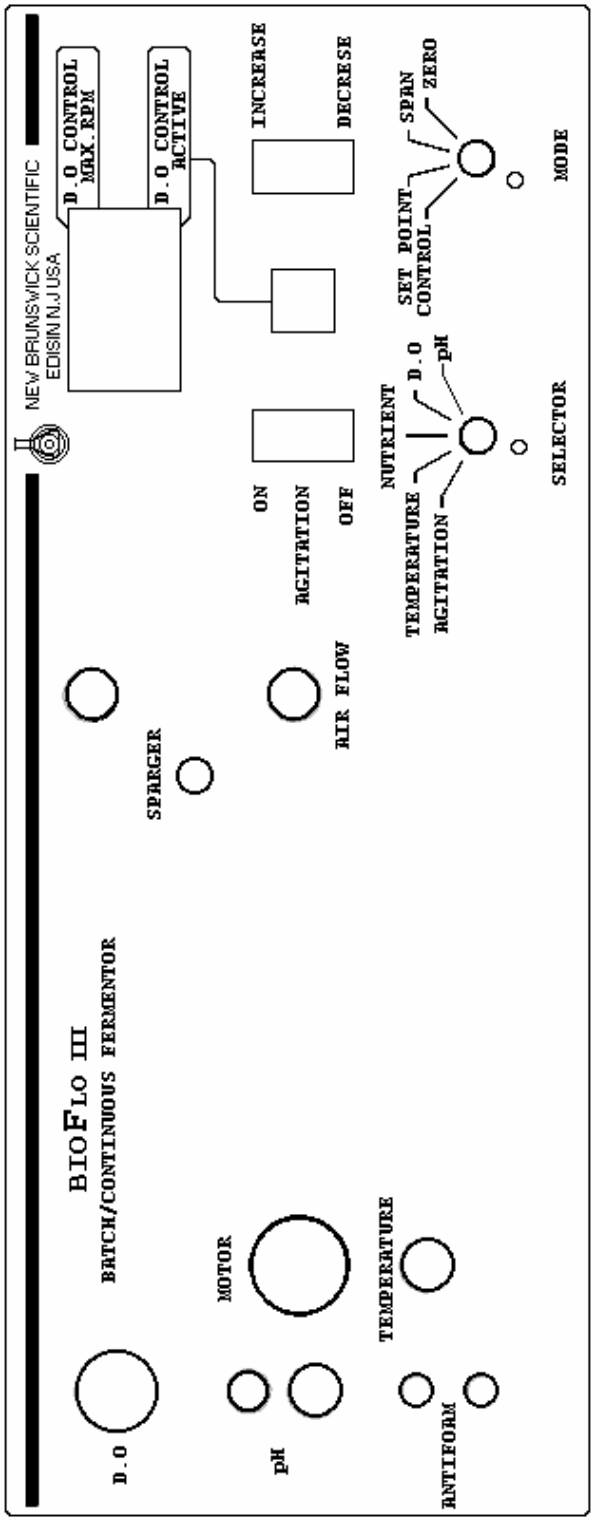


FIGURE 4 - FRONT PANEL

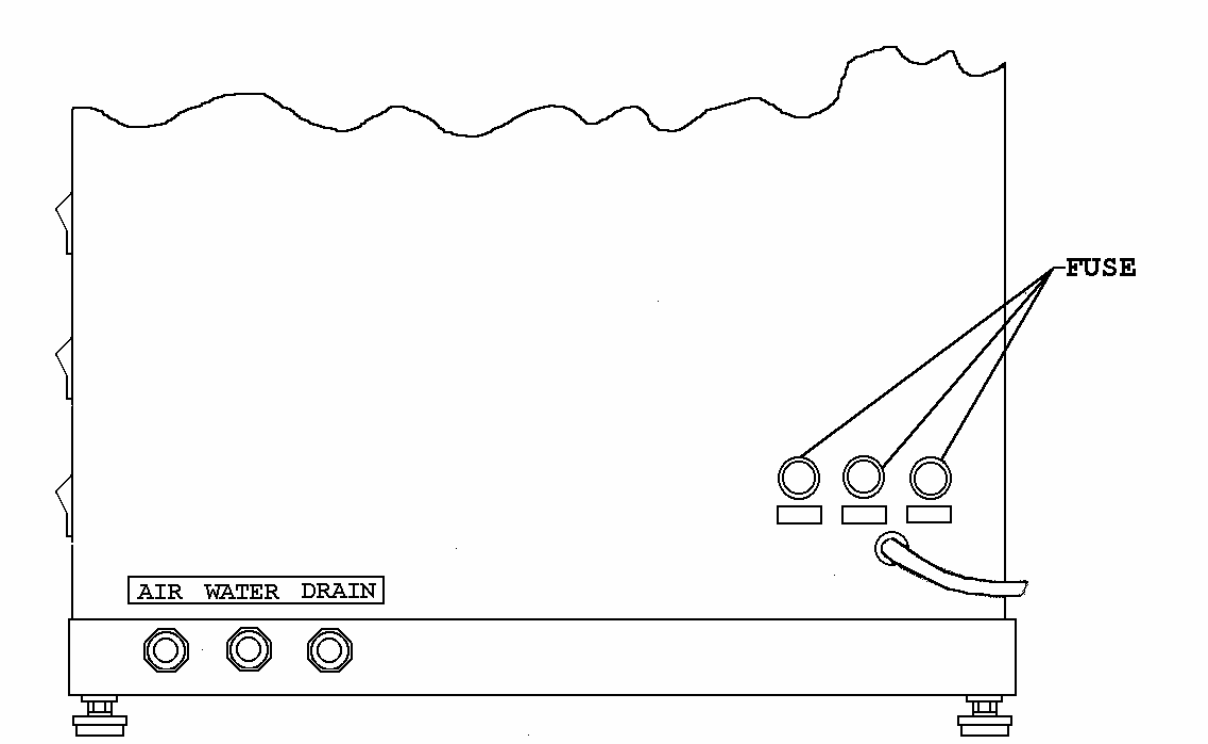


FIGURE 5 - REAR OF CABINET

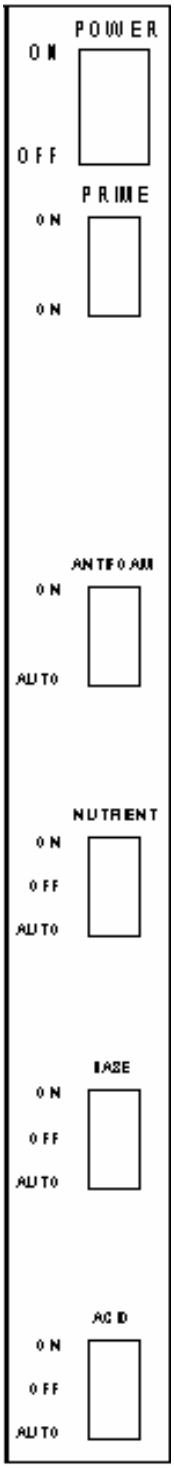


FIGURE 6 - SWITCH PANEL

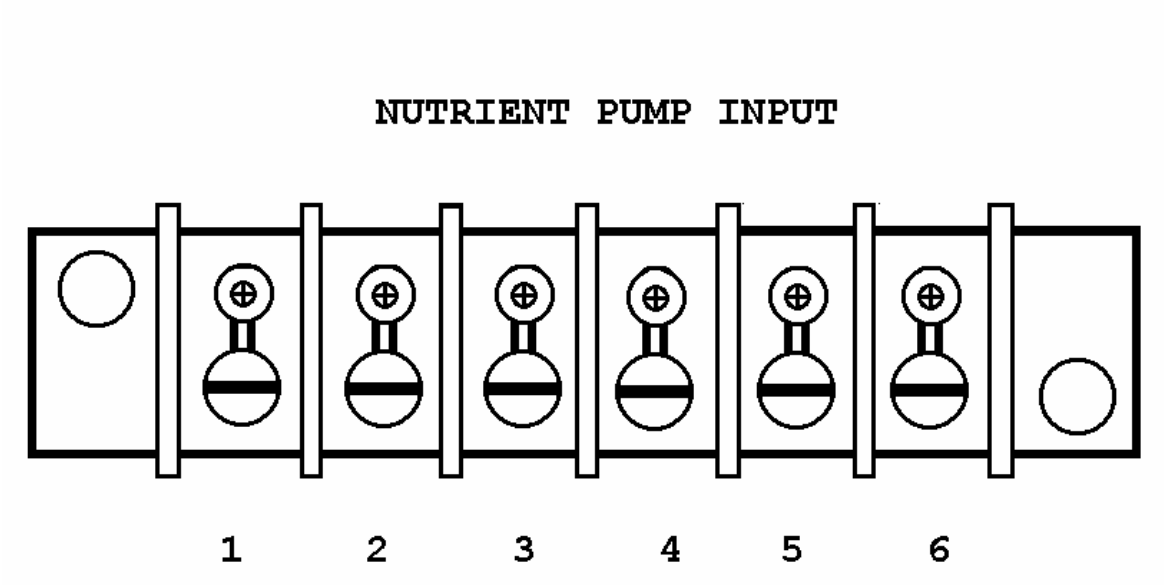


FIGURE 7 - EXTERNAL NUTRIENT PUMP TERMINAL

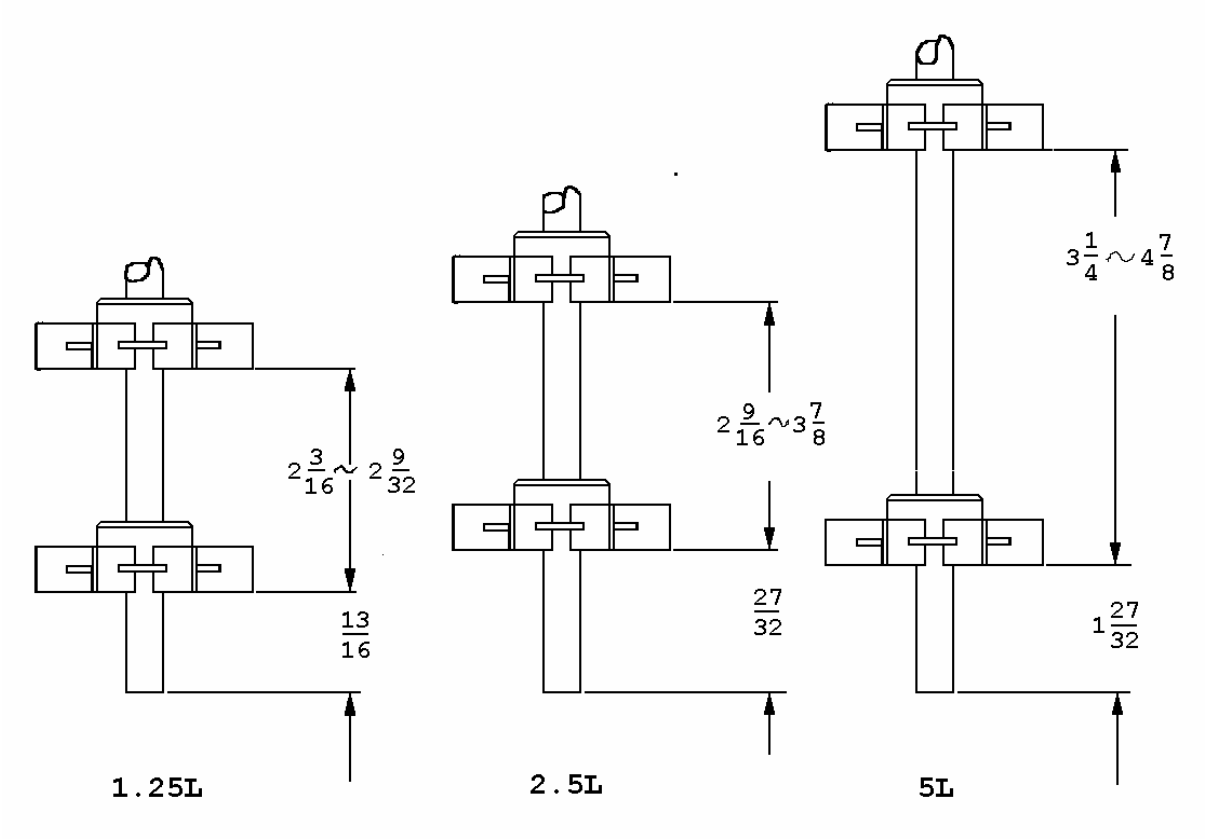


FIGURE 8 - SUGGESTED IMPELLER LOCATION

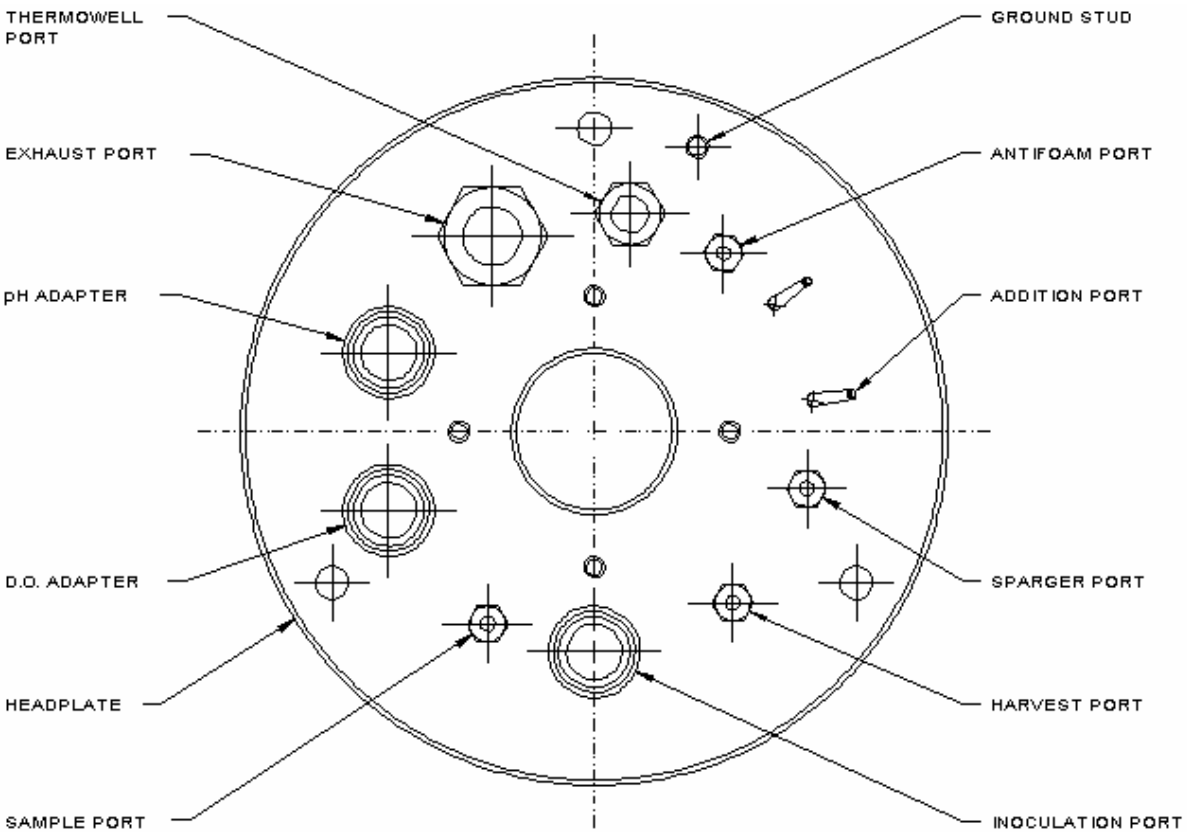


FIGURE 9 - HEADPLATE

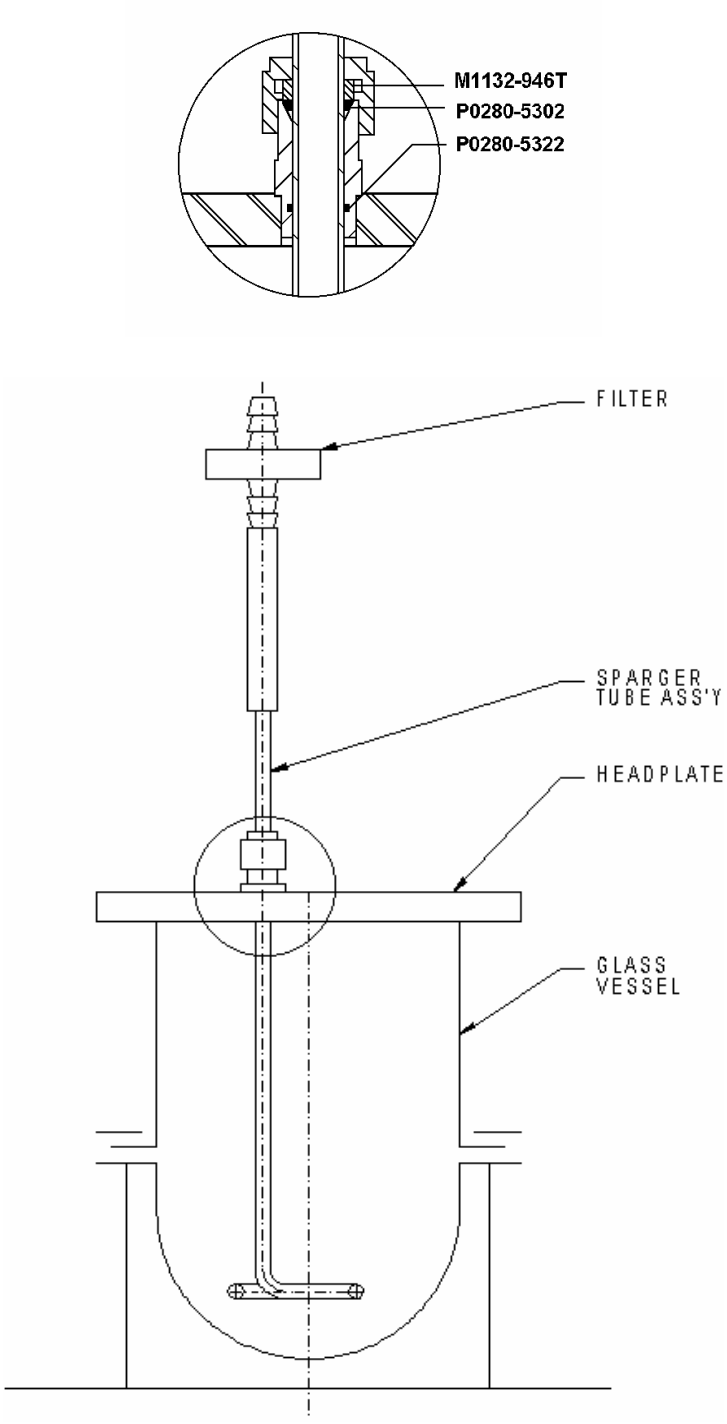


FIGURE 10 - SPARGER TUBE

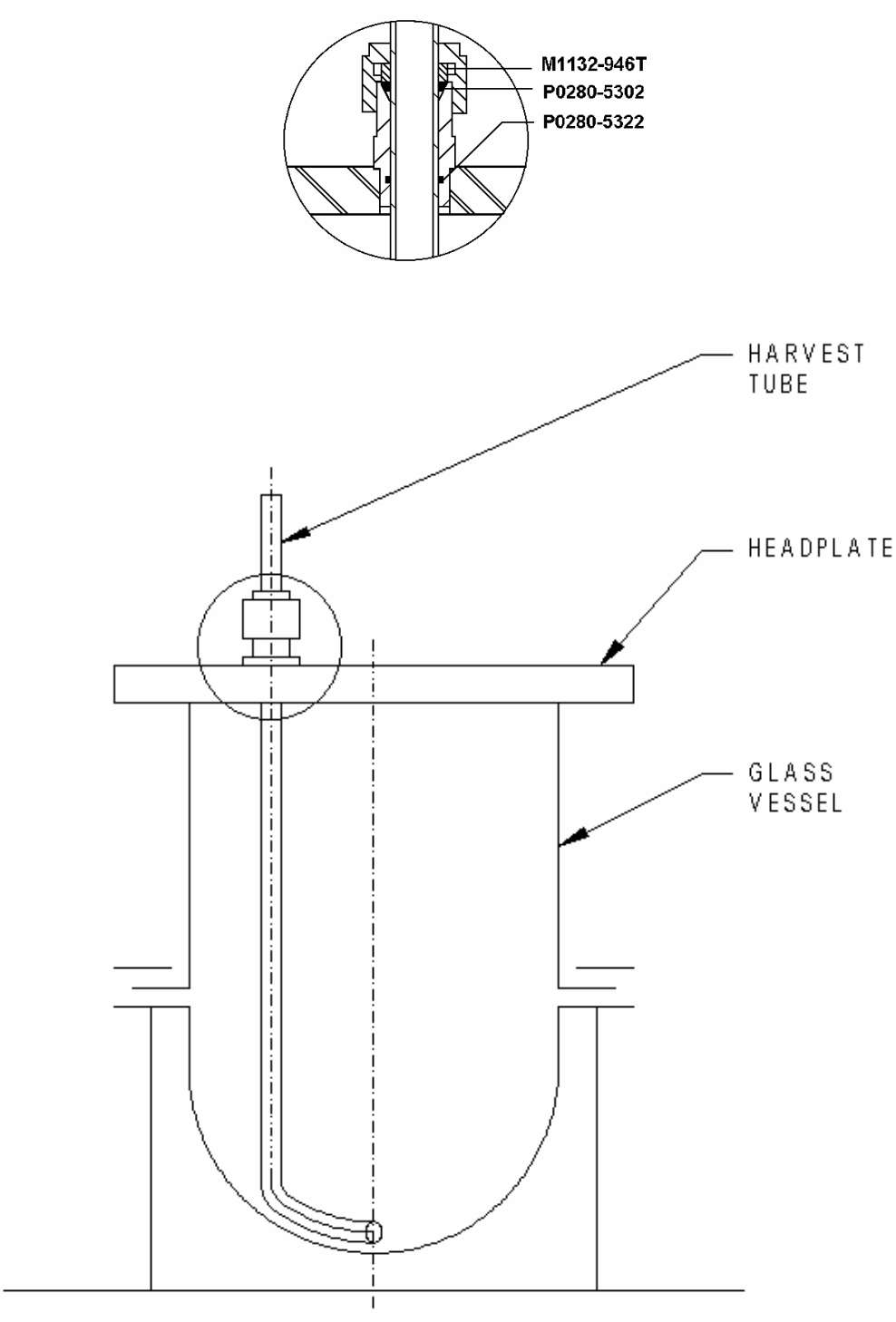


FIGURE 11 - HARVEST TUBE

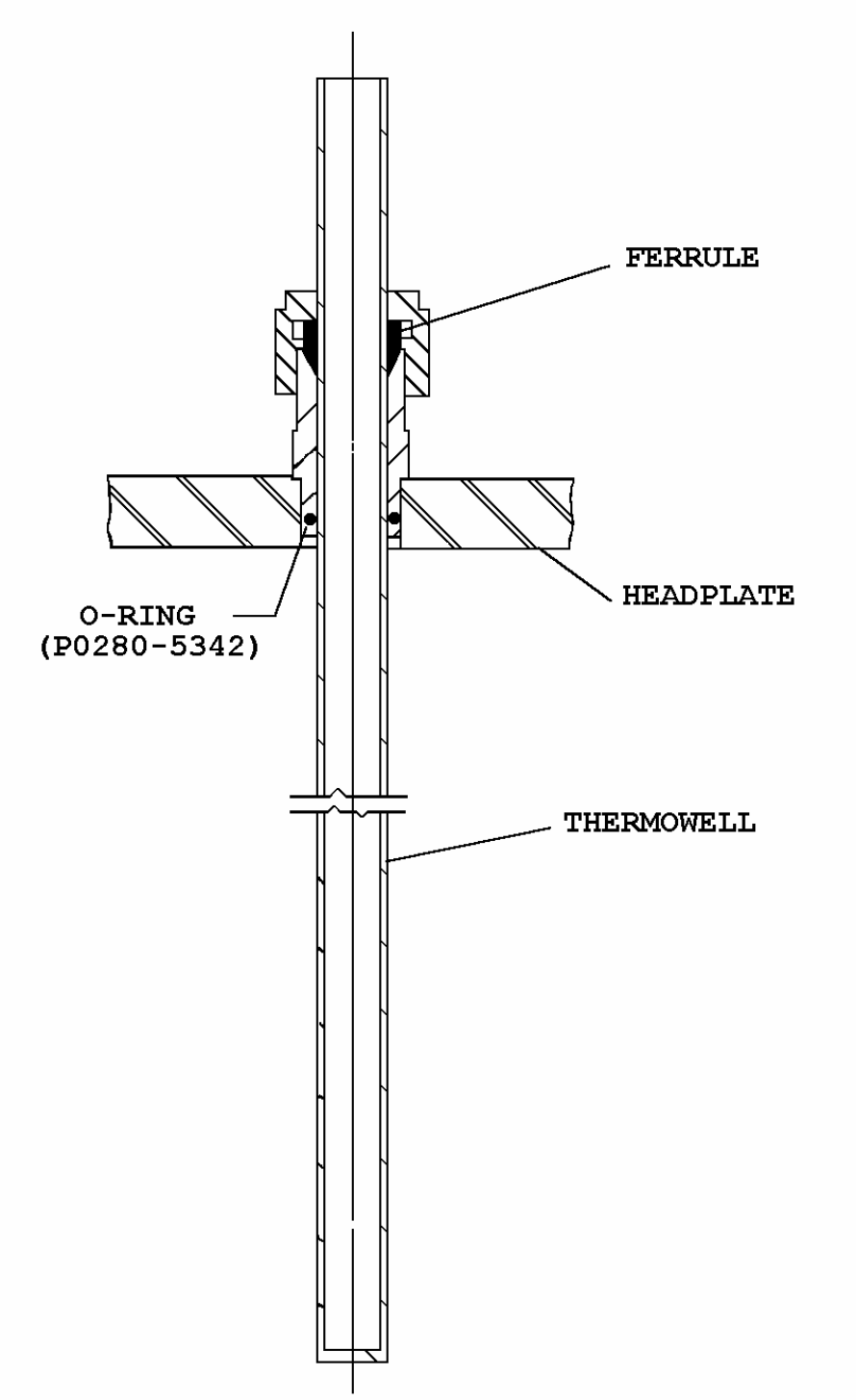


FIGURE 12 - THERMOWELL

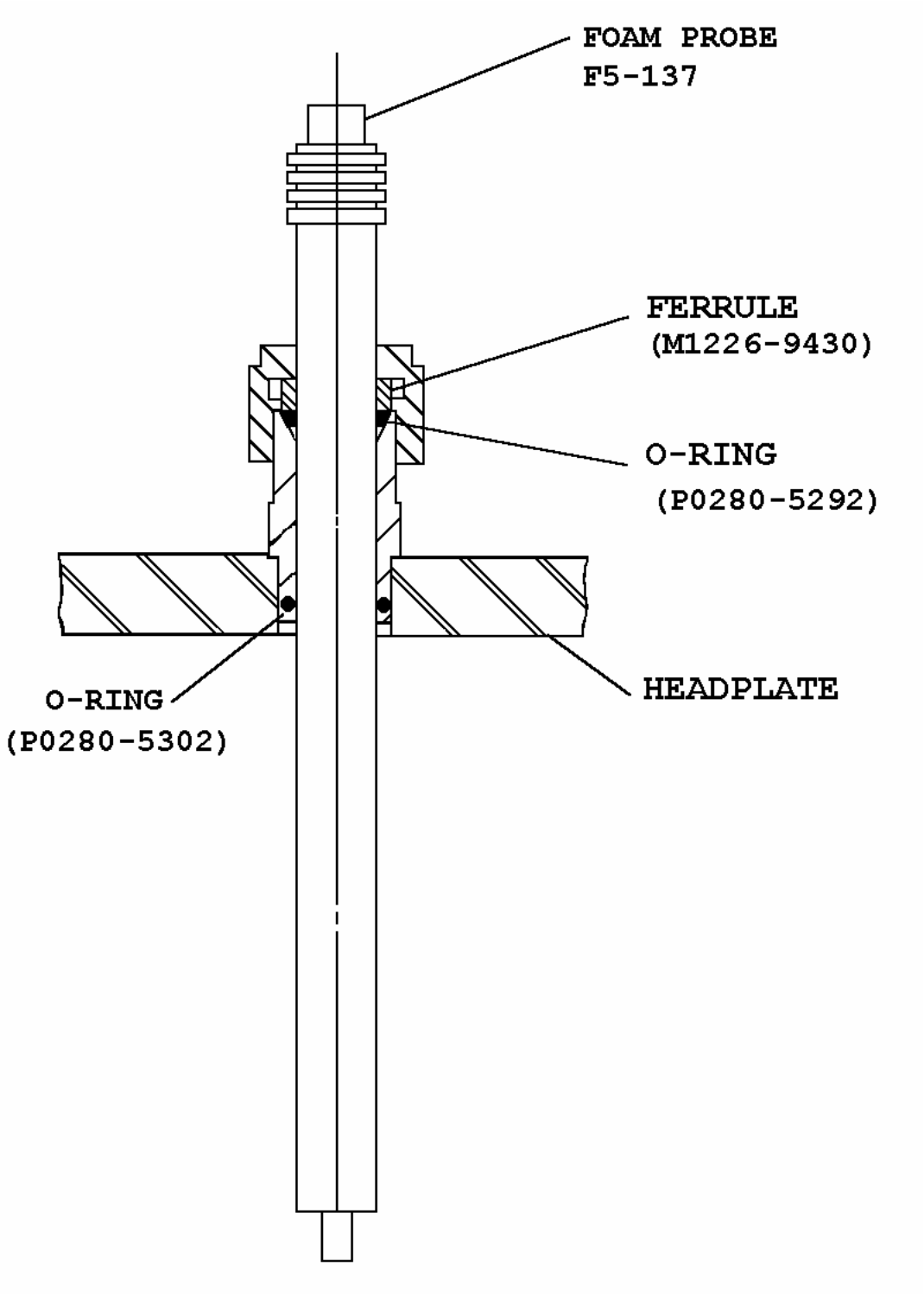


FIGURE 13 - FOAM PROBE

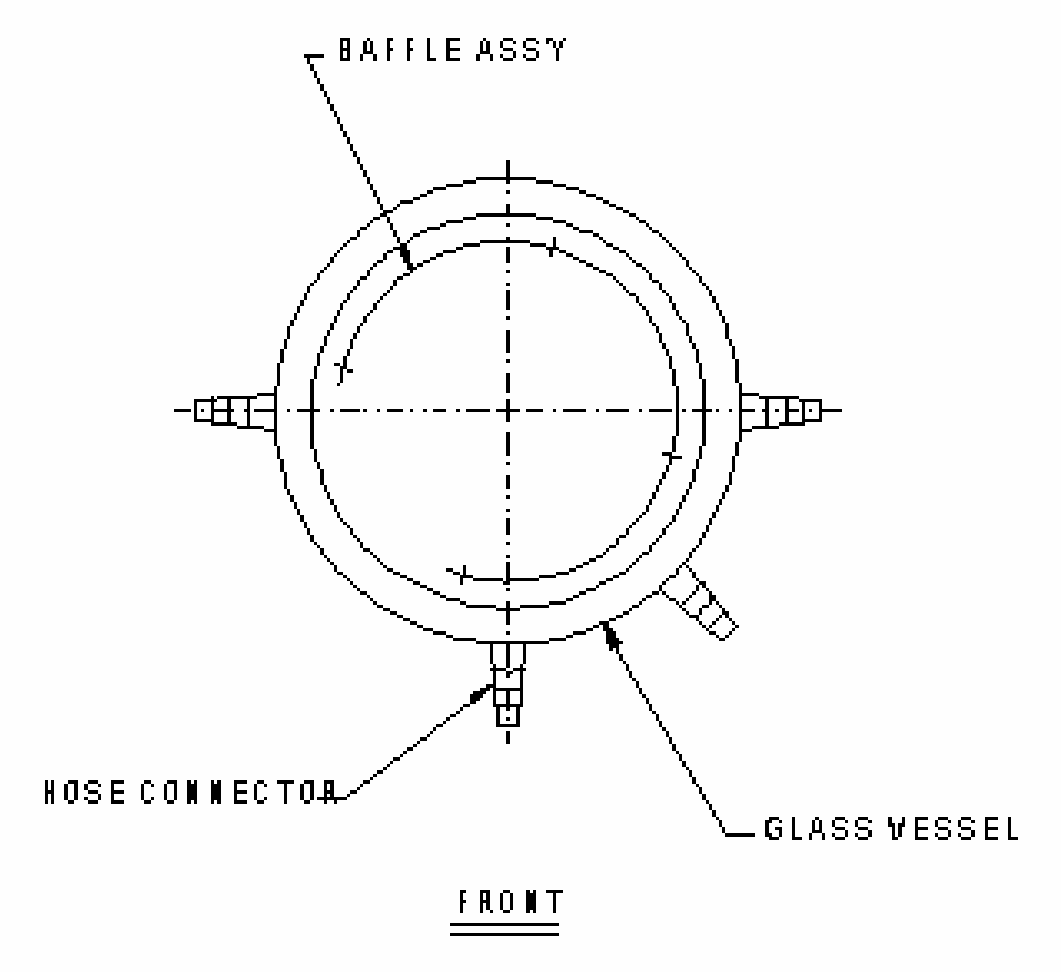


FIGURE 14 - BAFFLE INSTALLATION

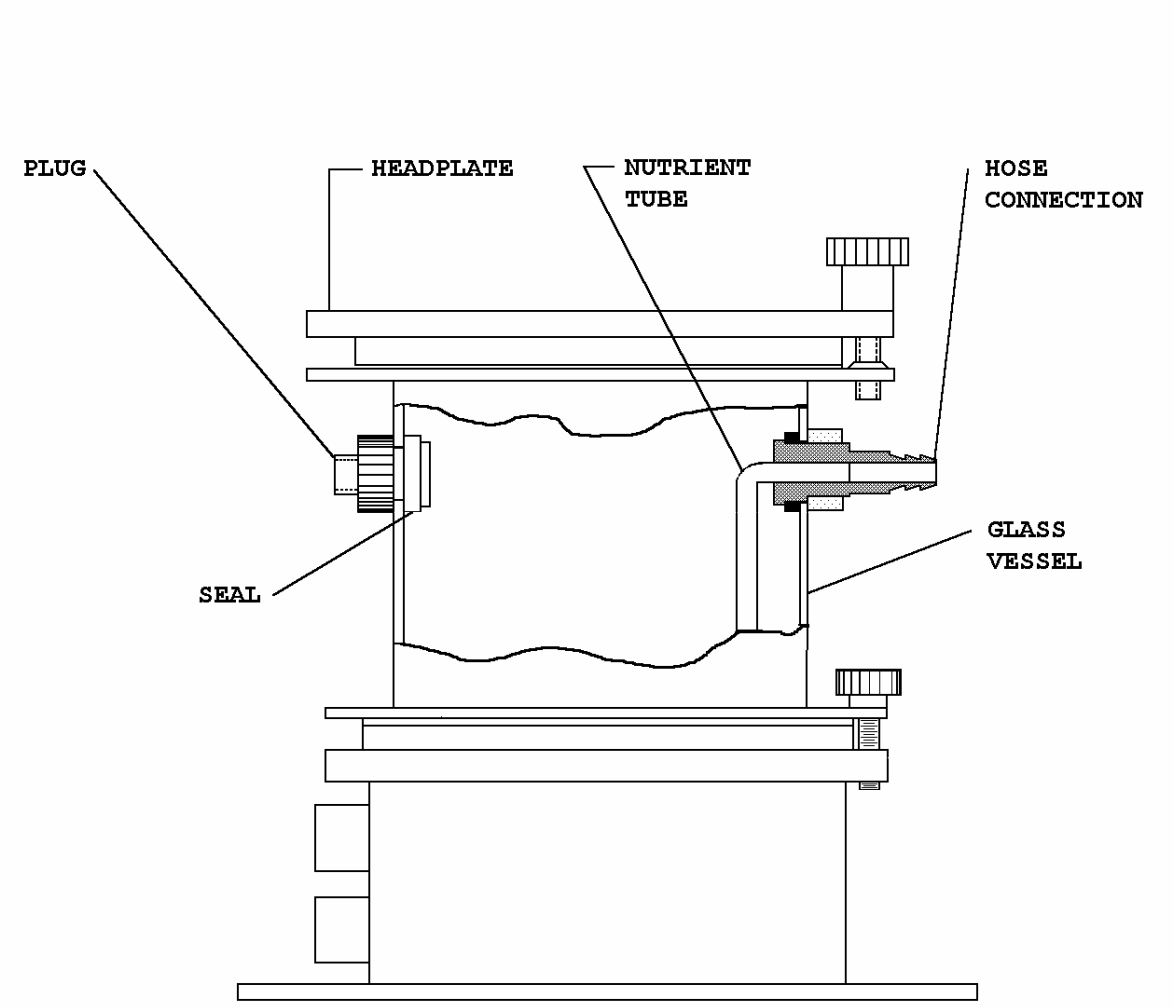


FIGURE 15 - SIDE PORTS

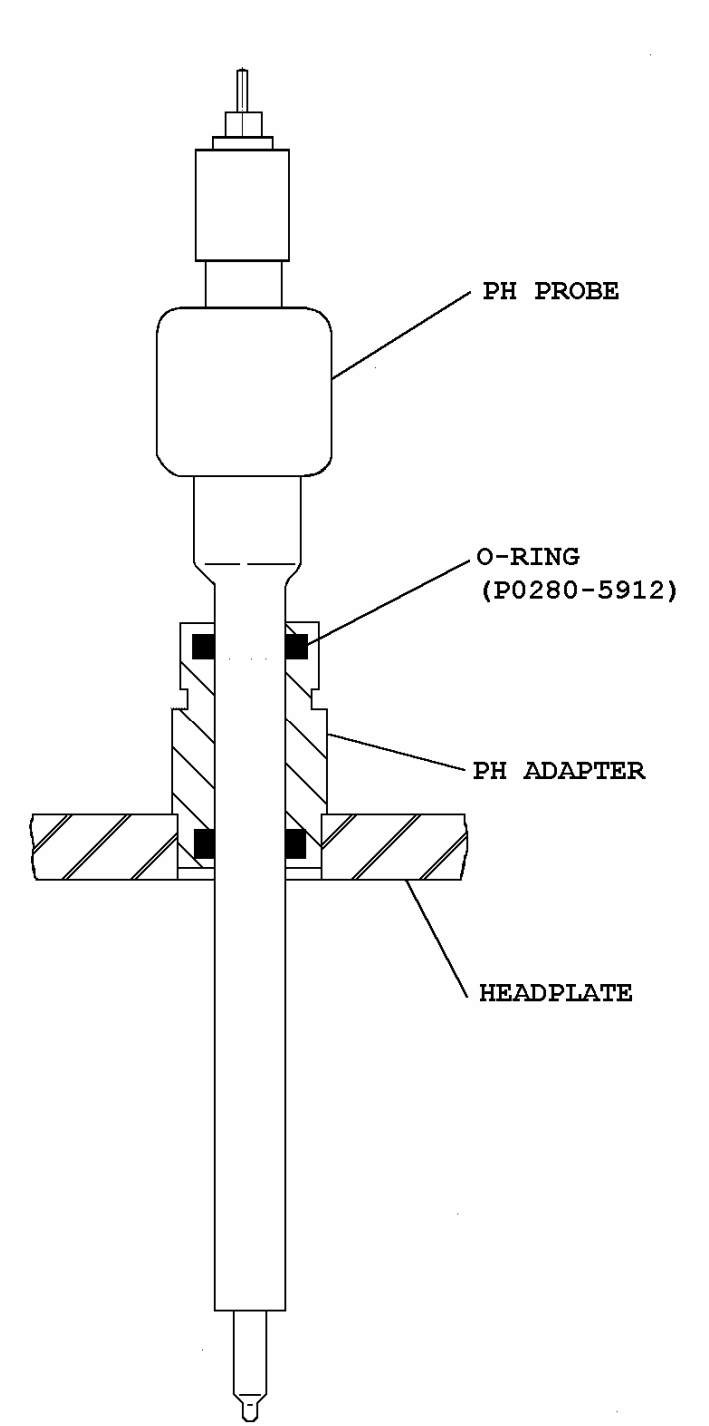


FIGURE 16 - pH PROBE INSTALLATION

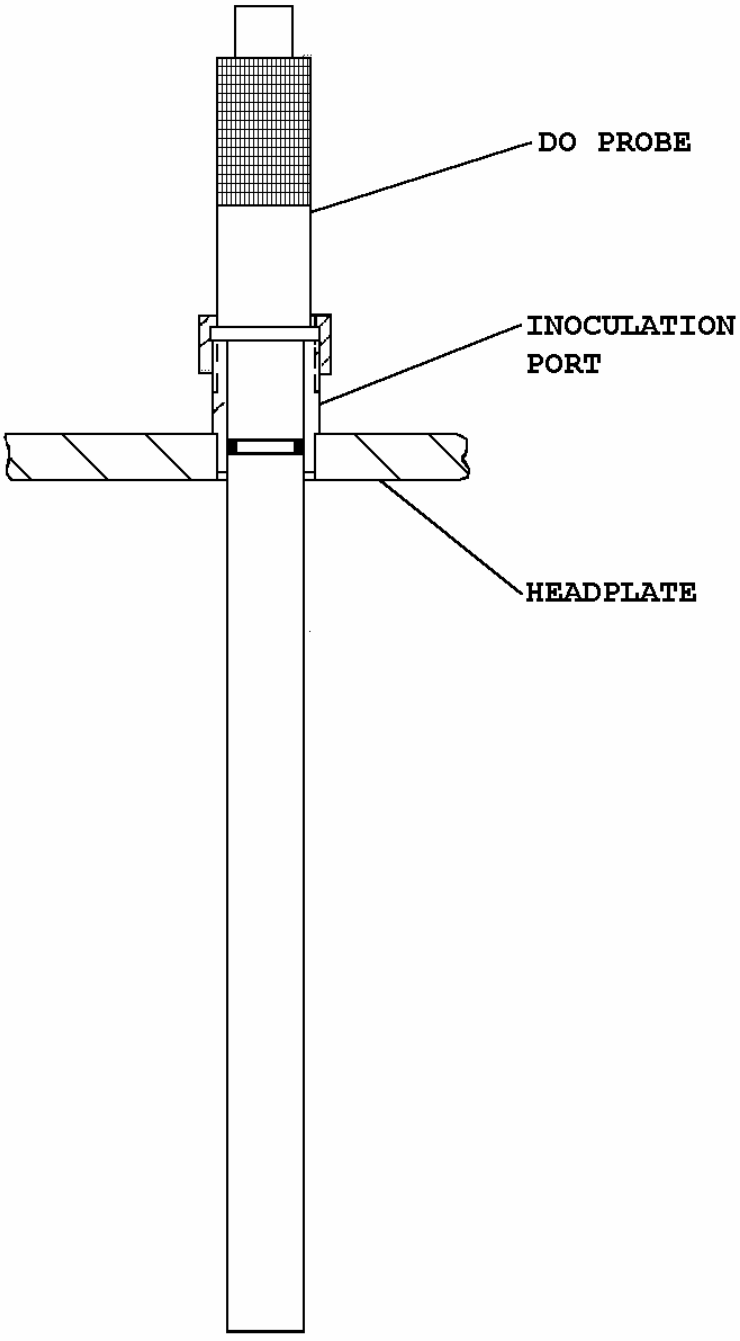


FIGURE 17 - D.O. PROBE (PHOENIX) INSTALLATION

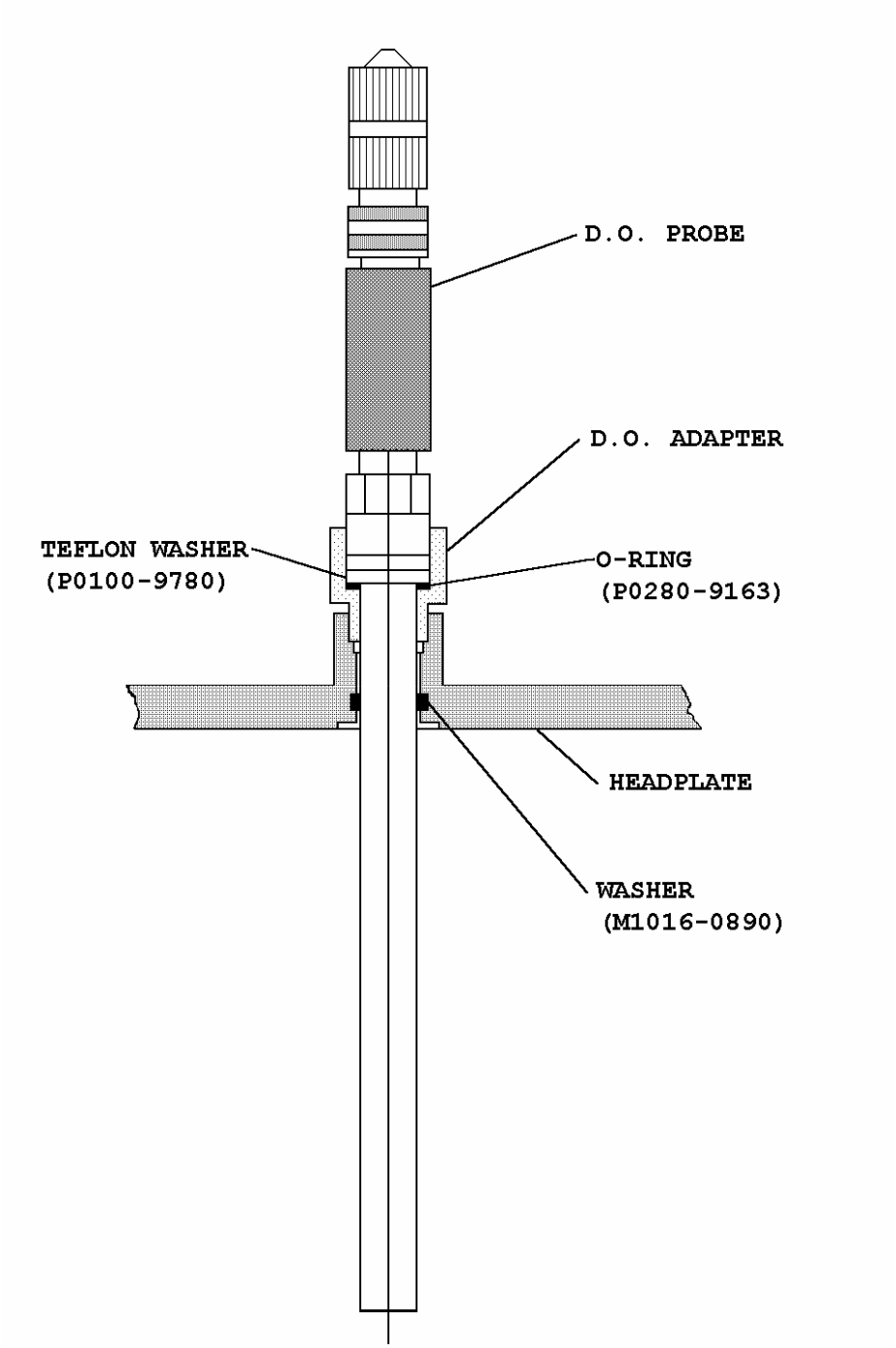


FIGURE 18 - D.O. PROBE (INGOLD) INSTALLATION

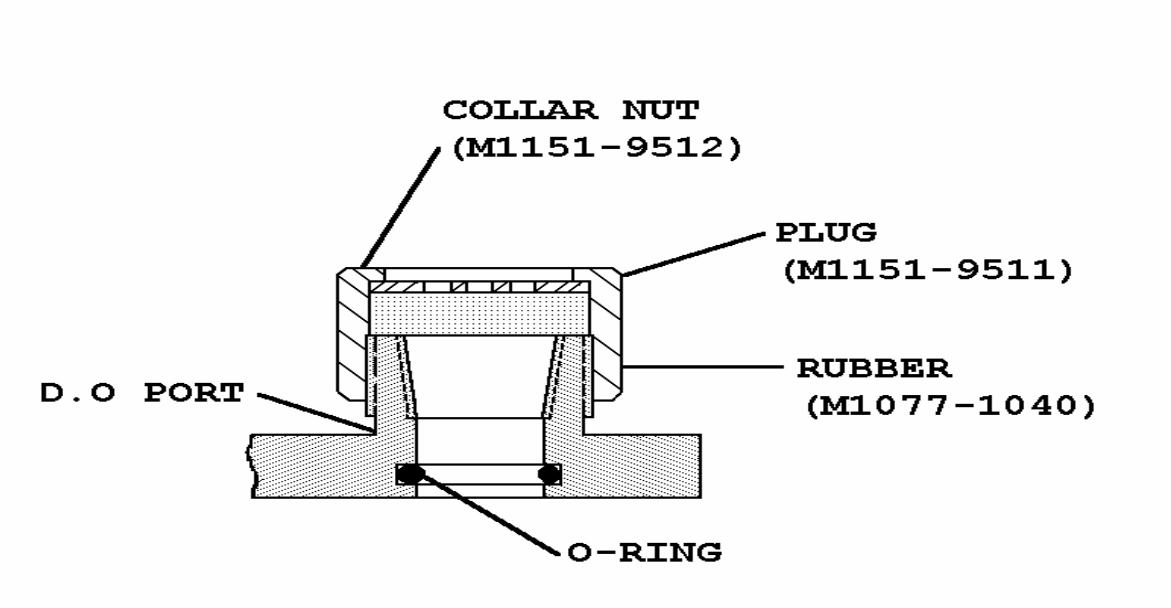


FIGURE 19 - CONVERSION OF D.O. PORT TO INOCULATION PORT

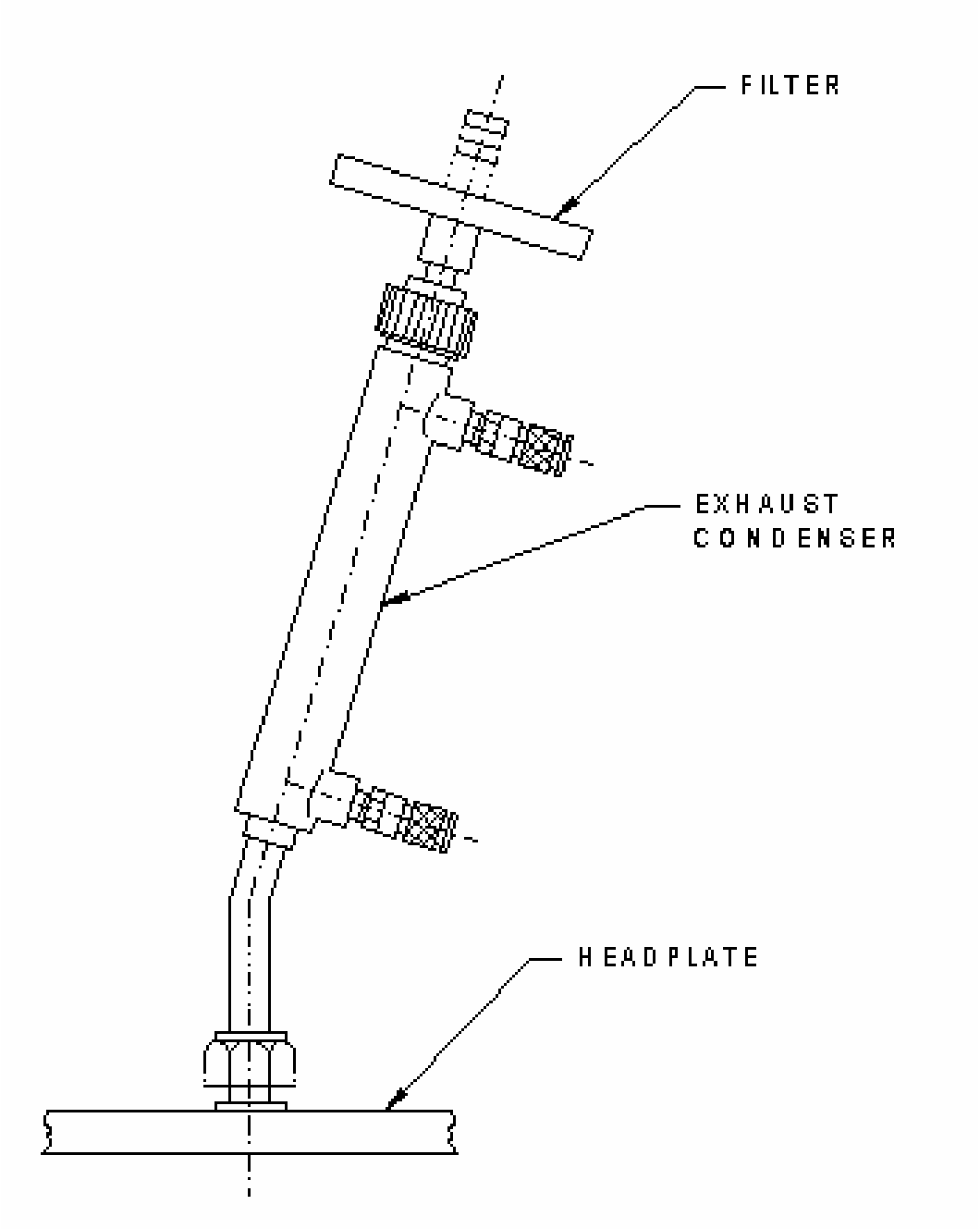


FIGURE 20 - EXHAUST CONDENSER INSTALLATION

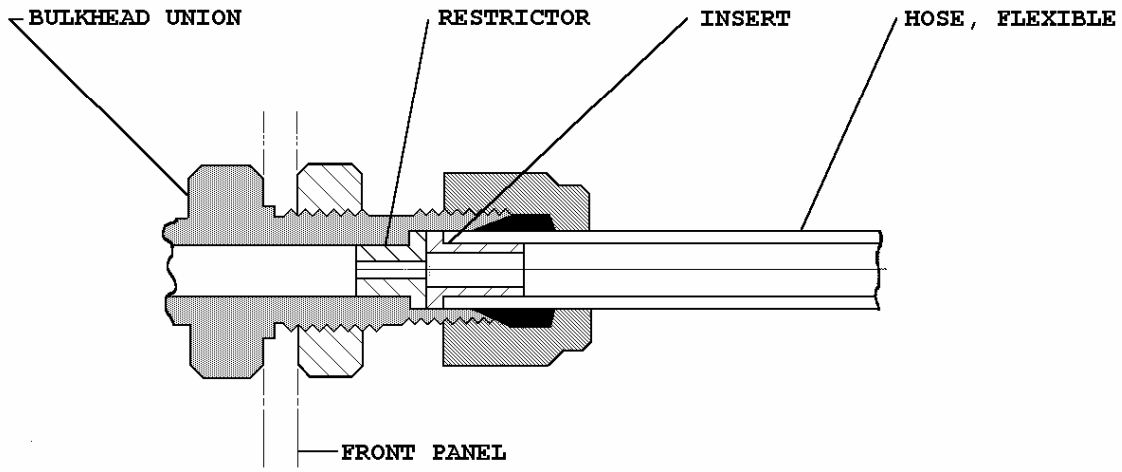


FIGURE 21 - RESTRICTOR IN CONDENSER “WATER IN” LINE

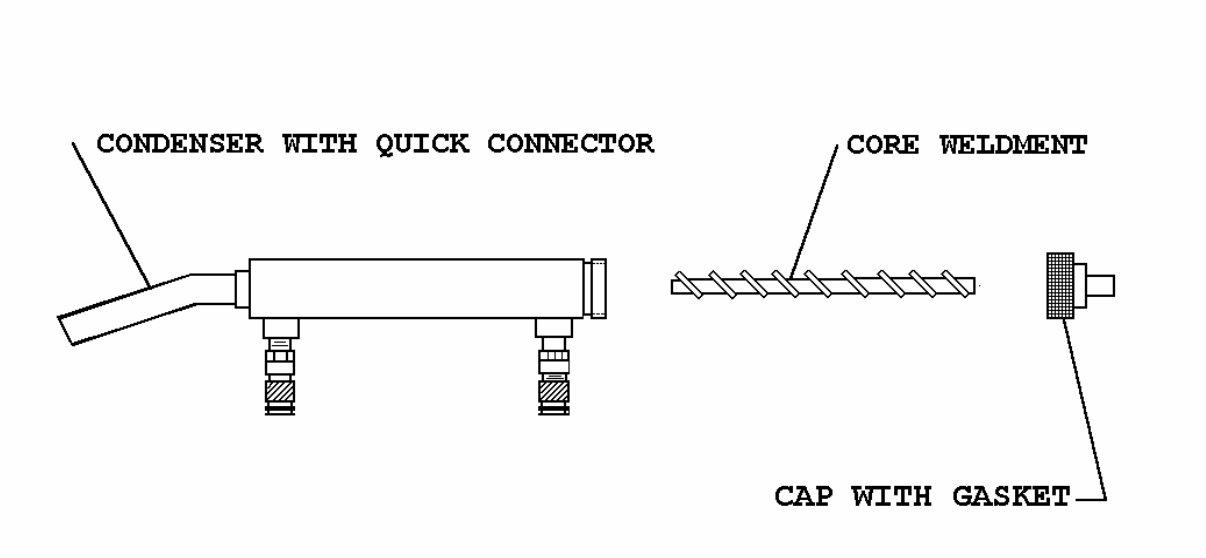


FIGURE 22 - DISASSEMBLY OF CONDENSER

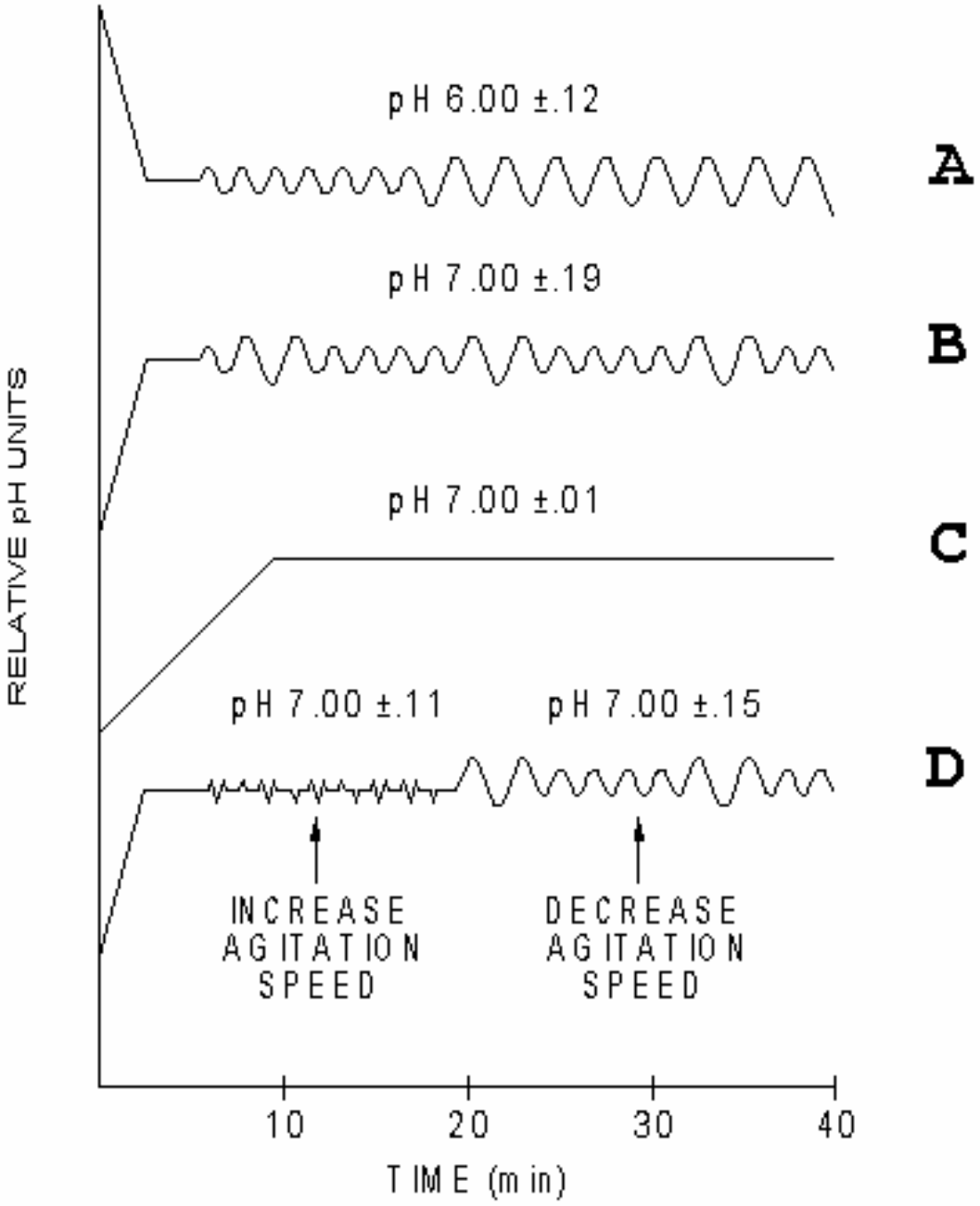


FIGURE 23 - FACTOR AFFECTING pH CONTROL

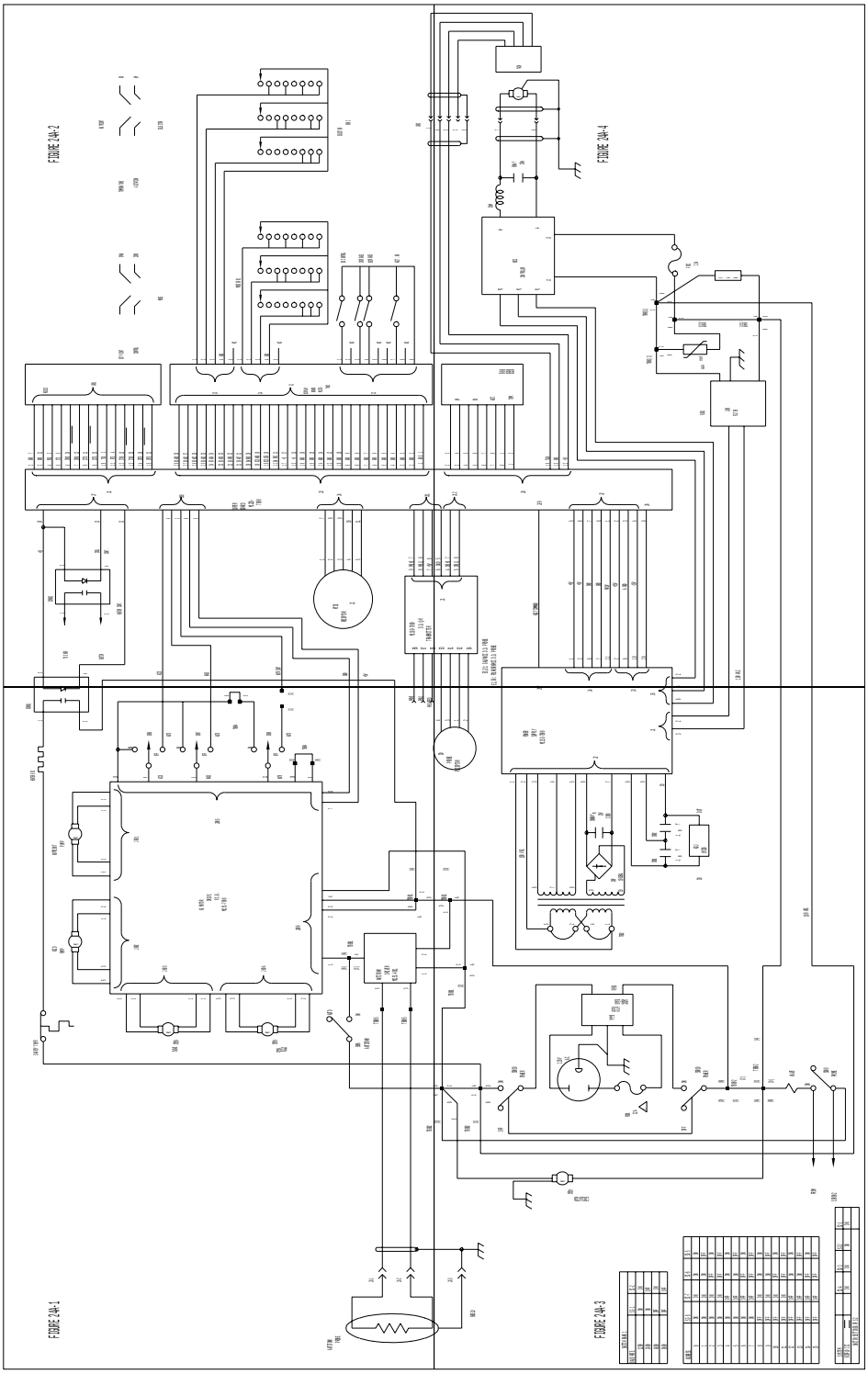


FIGURE 24A - CONTROL SCHEMATIC (100V)

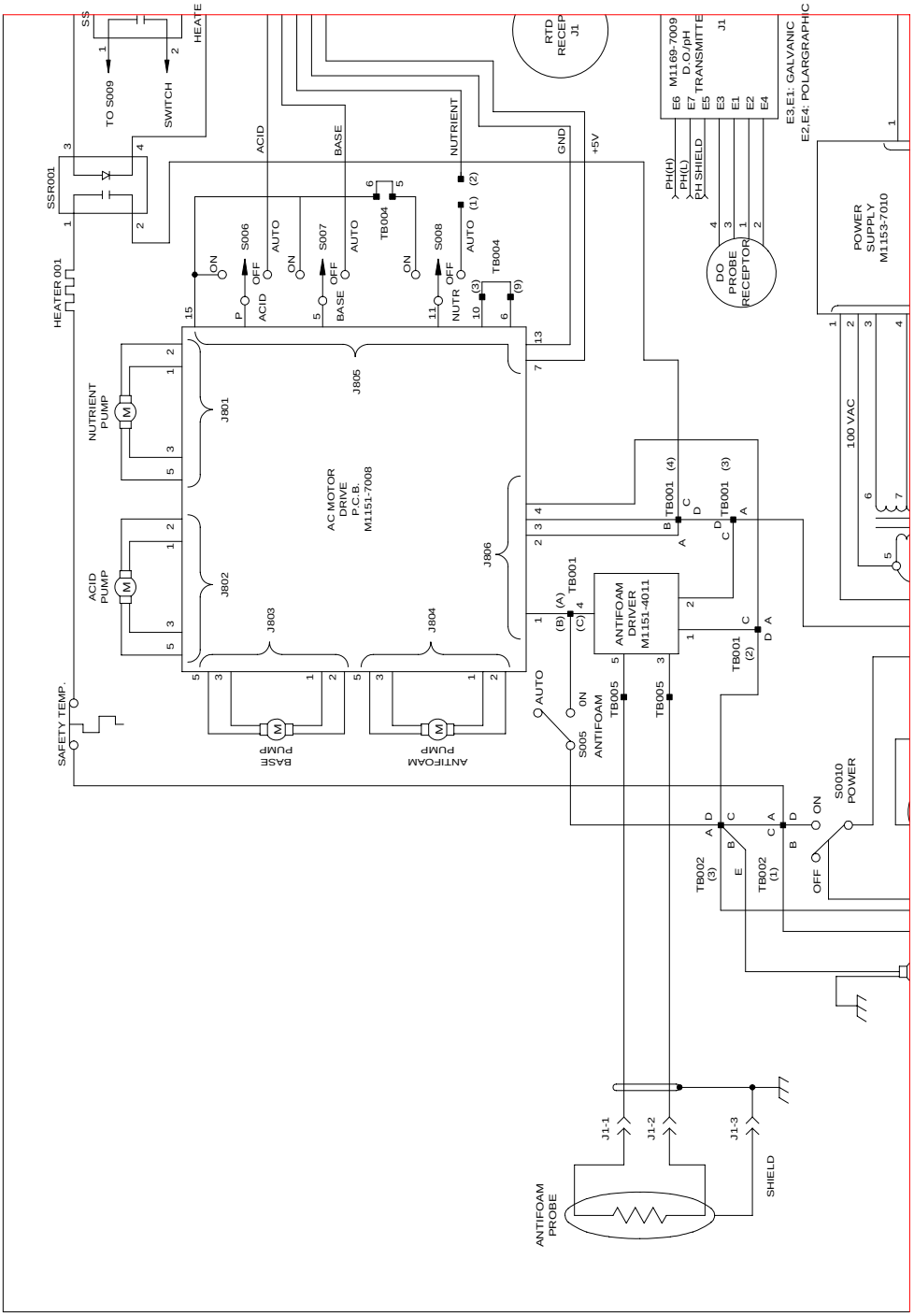

SS
TO S009
SWITCH
HEATE
SSR001
HEATER 001
SAFETY TEMP.
ACID
BASE
NUTRIENT
GND
+5V
RTD
RECEP
J1
M1169-7009
D.O./pH
TRANSMITTE
PH(H)
PH(L)
PH SHIELD
E3,E1: GALVANIC
E2,E4: POLARGRAPHIC
DO
PROBE
RECEPTOR
POWER
SUPPLY
M1153-7010
100 VAC
S006
S007
S008
TB004
NUTR
NUTRIENT
PUMP
ACID
PUMP
J801
J802
J803
J804
J805
J806
A.C.MOTOR
DRIVE
P.C.B.
M1151-7008
BASE
PUMP
ANTIFOAM
PUMP
TB001
ANTIFOAM
DRIVER
M1151-4011
TB005
S005
ANTIFOAM
S0010
POWER
TB002
J1-1
J1-2
J1-3
SHIELD
ANTIFOAM
PROBE

FIGURE 24A-1

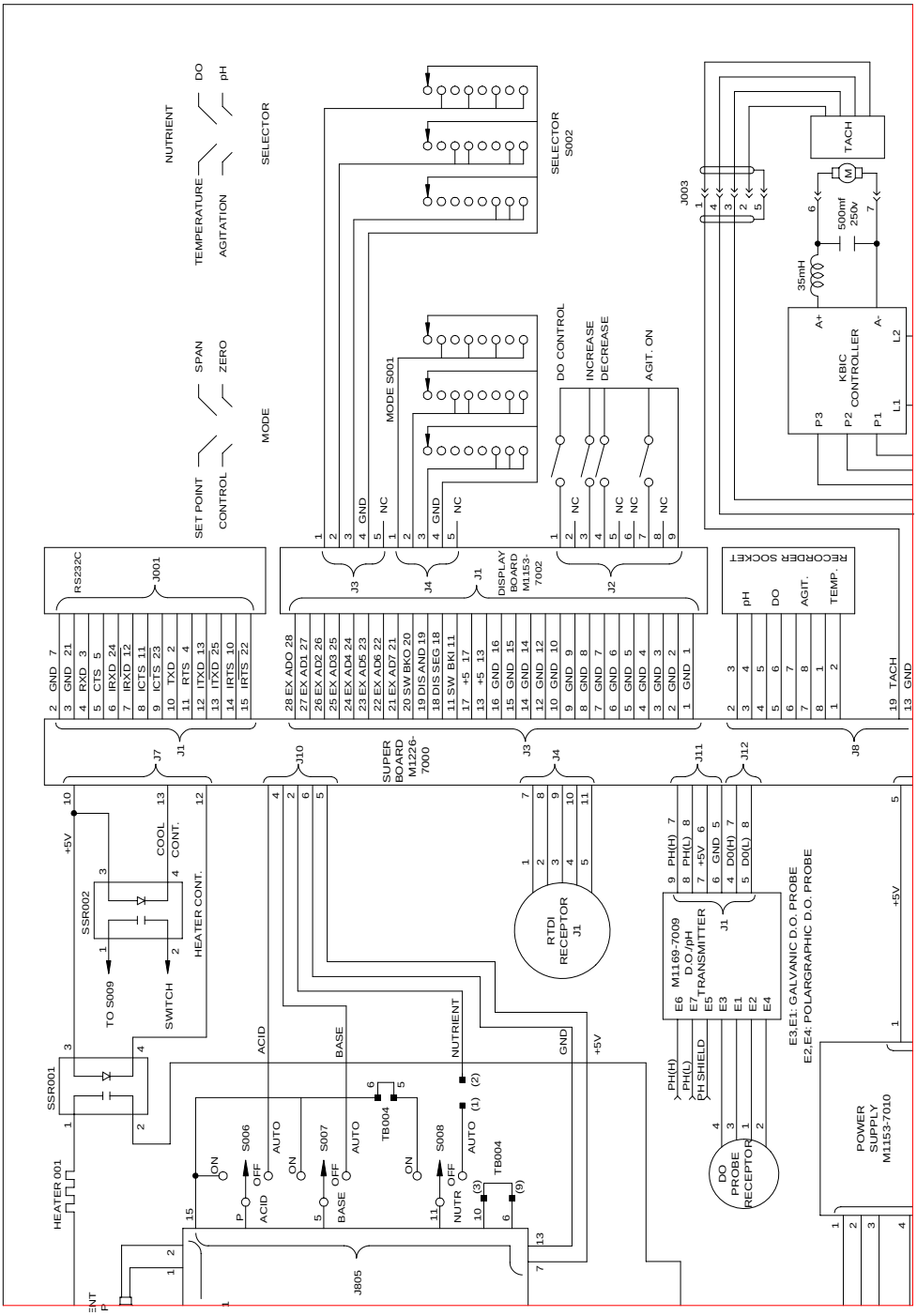

SUPER BOARD M1226-7000
DISPLAY BOARD M1153-7002
RECORDER SOCKET
RS232C
J001
J1
J2
J3
J4
J7
J8
J10
J11
J12
J805
MODE
SET POINT
CONTROL
SPAN
ZERO
MODE S001
SELECTOR
SELECTOR S002
NUTRIENT
TEMPERATURE
AGITATION
DO
pH
DO CONTROL
INCREASE
DECREASE
AGIT. ON
TACH
J003
35mH
500mf
250v
KBIC CONTROLLER
A+
A-
L1
L2
P1
P2
P3
HEATER 001
SSR001
SSR002
TO S009
SWITCH
HEATER CONT.
COOL CONT.
+5V
ACID
BASE
NUTRIENT
GND
S006
S007
S008
TB004
RTDI RECEPTOR J1
M1169-7009 D.O./pH TRANSMITTER
PH(H)
PH(L)
PH SHIELD
DO PROBE RECEPTOR
E3,E1: GALVANIC D.O. PROBE
E2,E4: POLARGRAPHIC D.O. PROBE
POWER SUPPLY M1153-7010

FIGURE 24A-2

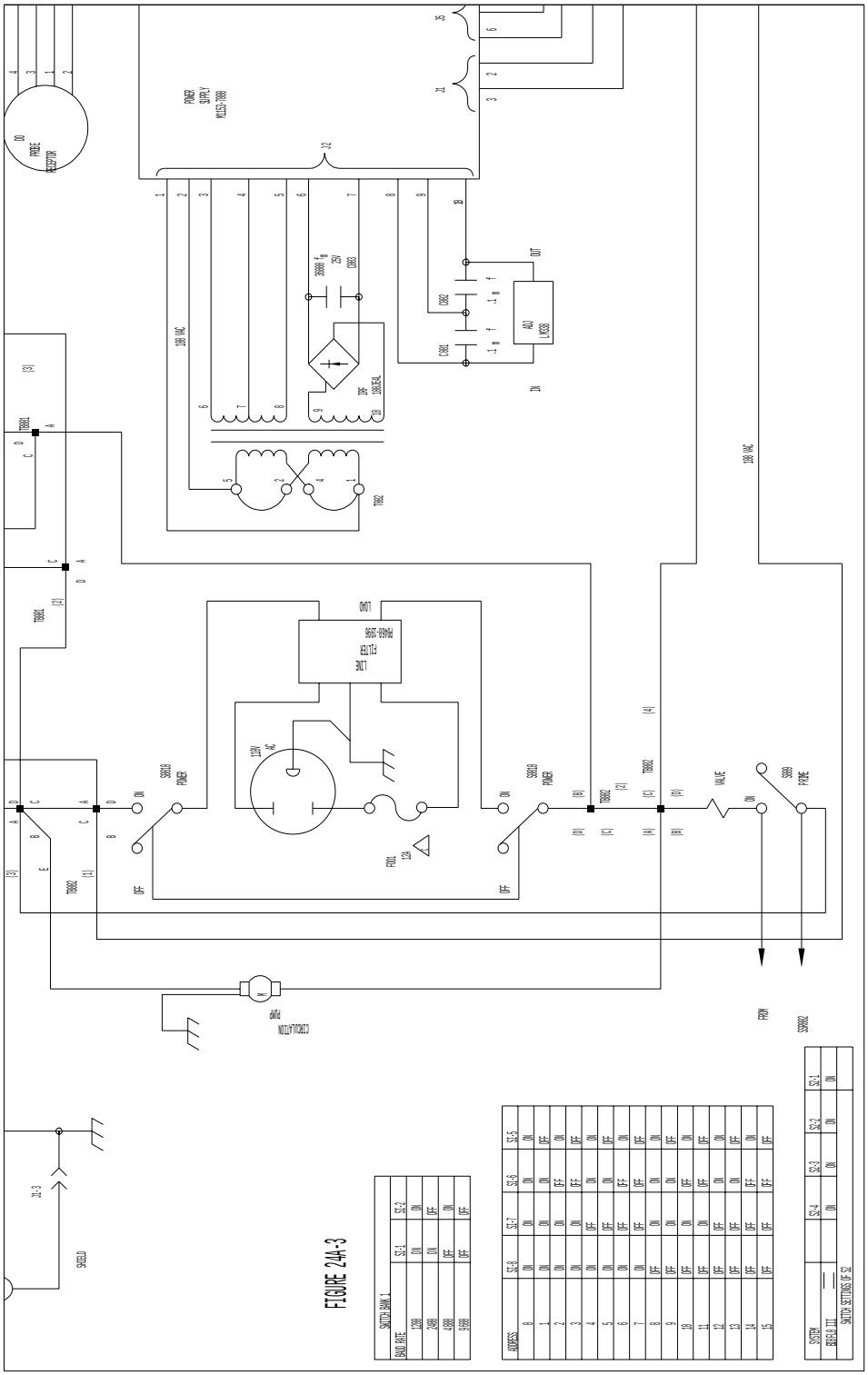

POWER SUPPLY
LINE FILTER
LOAD
CIRCULATION PUMP
SHIELD
SWITCH BANK 1
SWITCH SETTINGS
ADDRESS
SYSTEM

FIGURE 24A-3

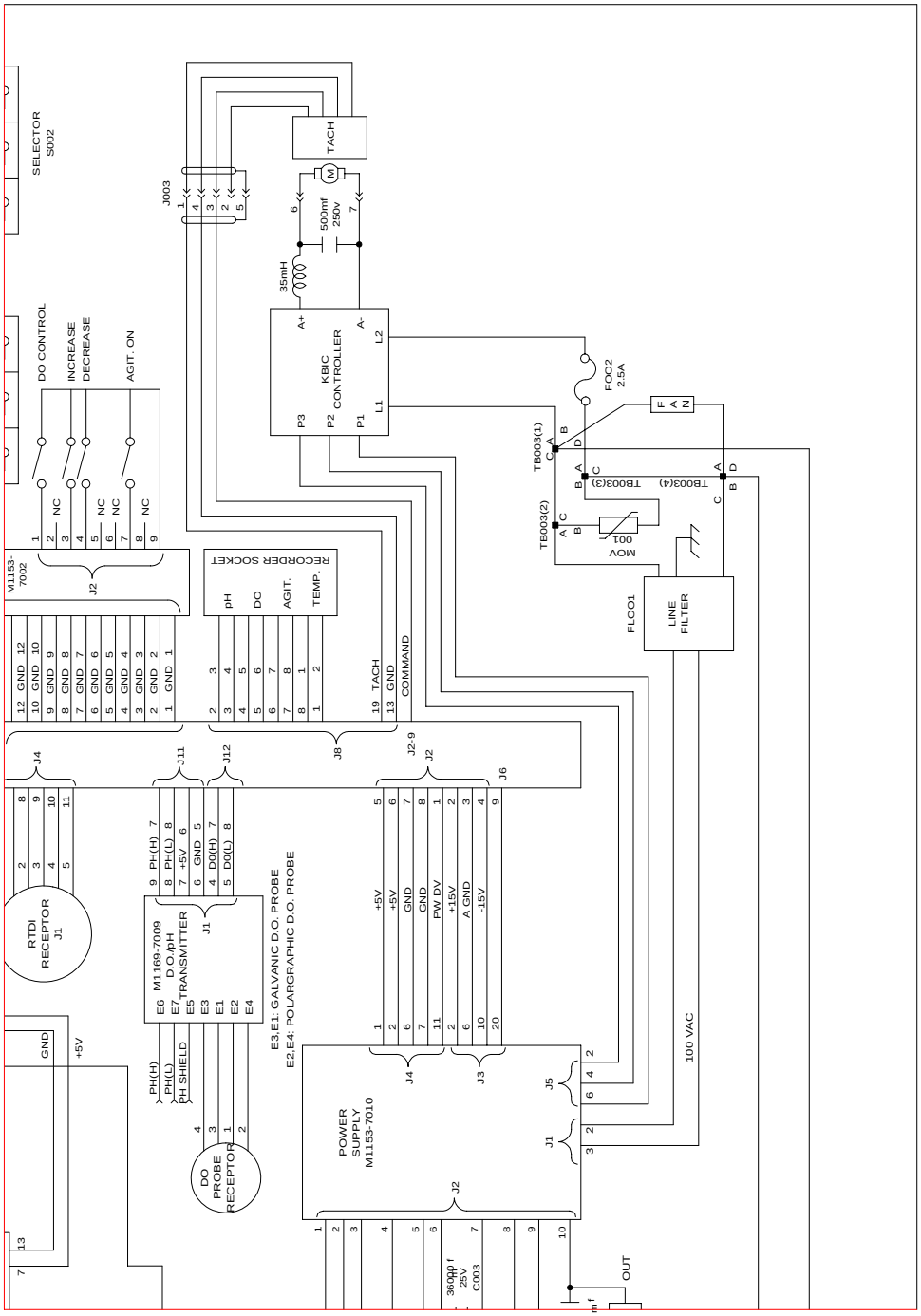

SELECTOR S002
TACH
J003
500mf 250v
35mH
KBIC CONTROLLER
A+
A-
L2
L1
P3
P2
P1
DO CONTROL
INCREASE DECREASE
AGIT. ON
NC
FOO2 2.5A
F A N
TB003(1)
TB003(3)
TB003(4)
TB003(2)
MOV 001
FLOO1
LINE FILTER
M1153-7002
J2
RECORDER SOCKET
pH
DO
AGIT.
TEMP.
GND
TACH
COMMAND
J8
J4
J11
J12
J2-9
J6
RTDI RECEPTOR J1
M1169-7009 D.O./pH TRANSMITTER
PH(H)
PH(L)
+5V
GND
D0(H)
D0(L)
PH SHIELD
E1
E2
E3
E4
E5
E6
E7
E3,E1: GALVANIC D.O. PROBE
E2,E4: POLARGRAPHIC D.O. PROBE
DO PROBE RECEPTOR
POWER SUPPLY M1153-7010
PW DV
A GND
-15V
J1
J2
J3
J4
J5
100 VAC
OUT
C003

FIGURE 24A-4

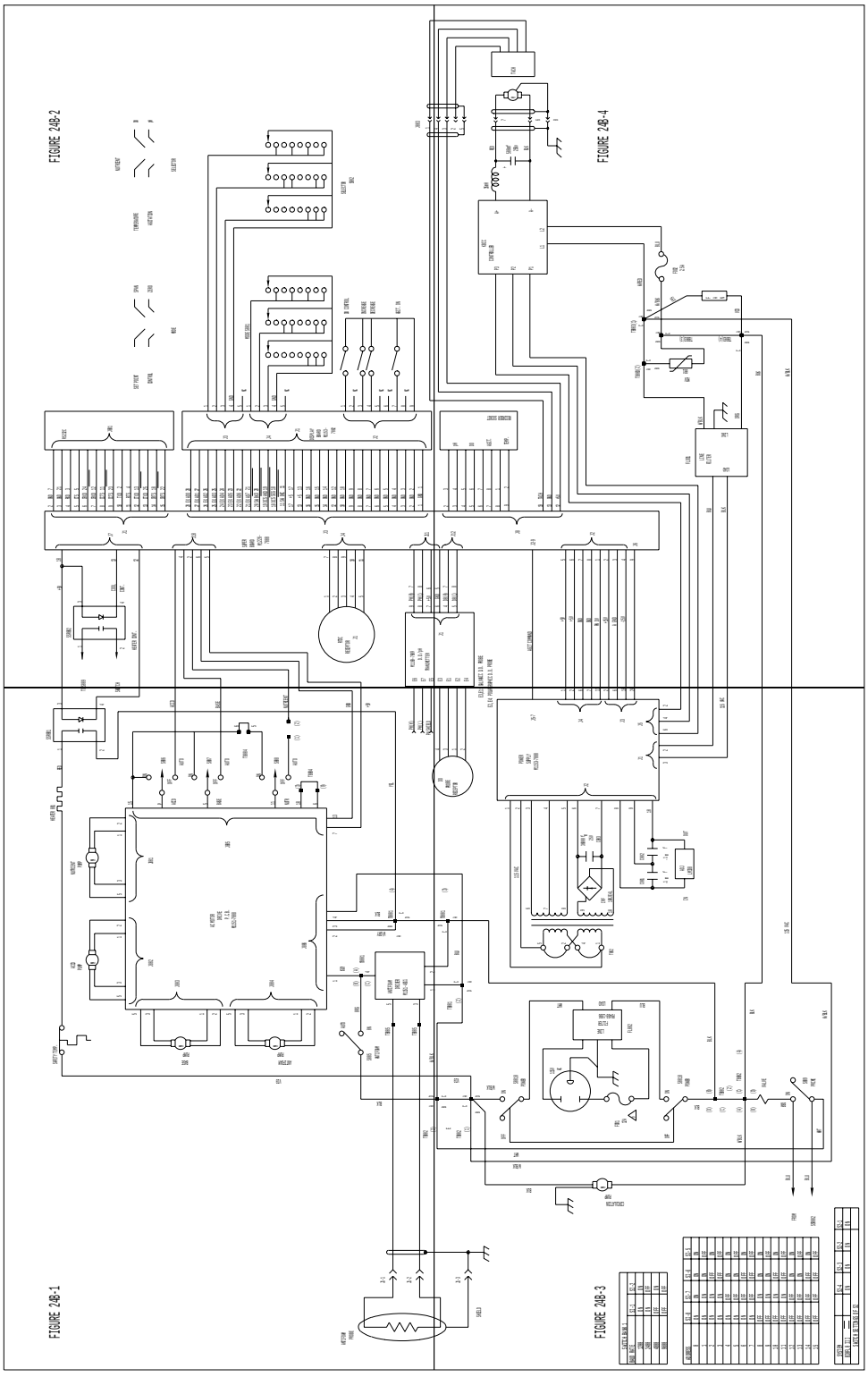


FIGURE 24B - CONTROL SCHEMATIC (115V)

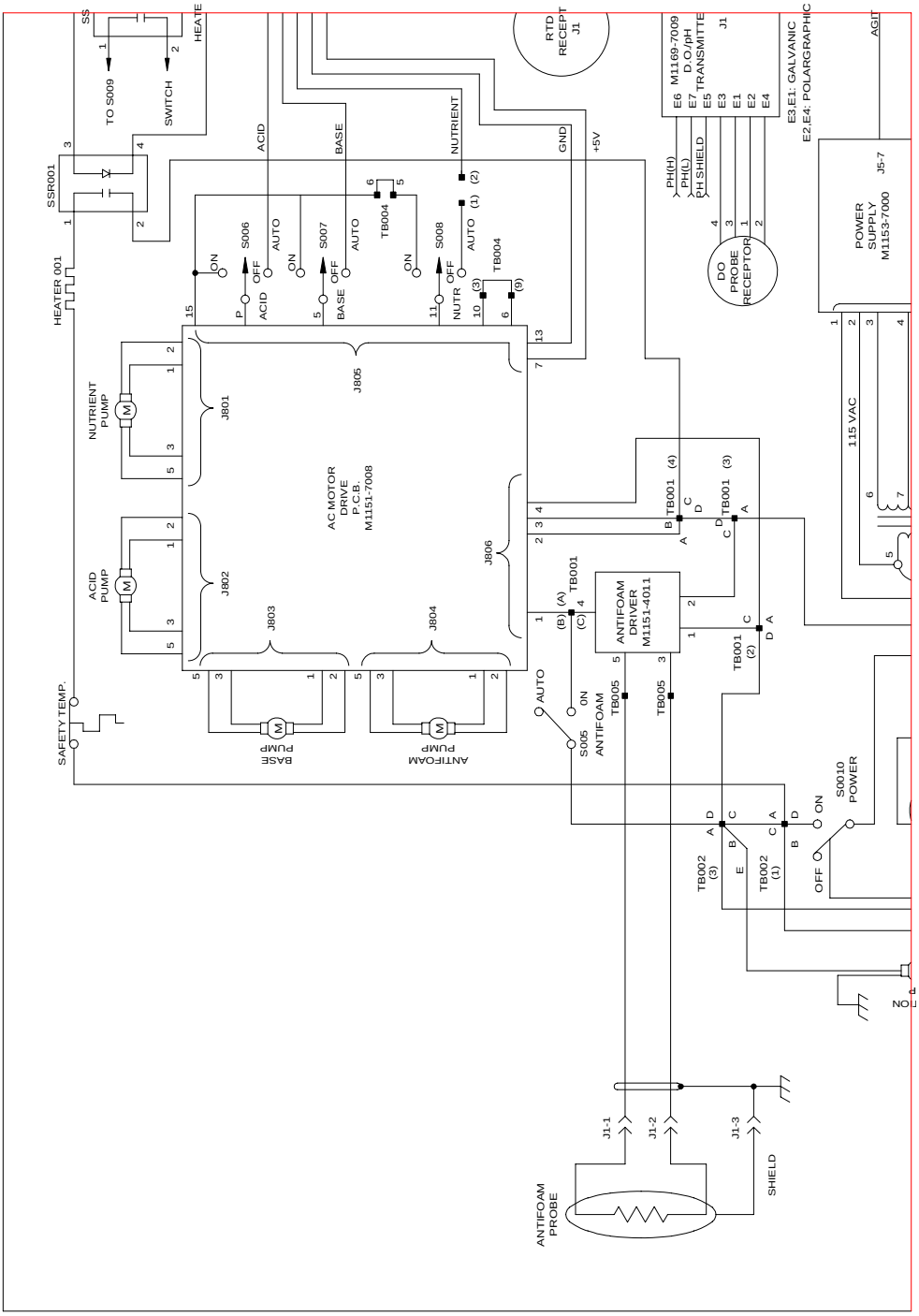

SSR001
HEATER 001
SAFETY TEMP.
NUTRIENT PUMP
ACID PUMP
BASE PUMP
ANTIFOAM PUMP
AC MOTOR DRIVE P.C.B. M1151-7008
J801
J802
J803
J804
J805
J806
ACID
BASE
NUTRIENT
GND
+5V
RTD RECEPT J1
M1169-7009 D.O./pH TRANSMITTER
PH(H)
PH(L)
PH SHIELD
DO PROBE RECEPTOR
E3,E1: GALVANIC
E2,E4: POLARGRAPHIC
POWER SUPPLY M1153-7000
115 VAC
ANTIFOAM DRIVER M1151-4011
TB001
TB002
TB004
TB005
S005 ANTIFOAM
S006
S007
S008
S0010 POWER
ANTIFOAM PROBE
SHIELD

FIGURE 24B-1

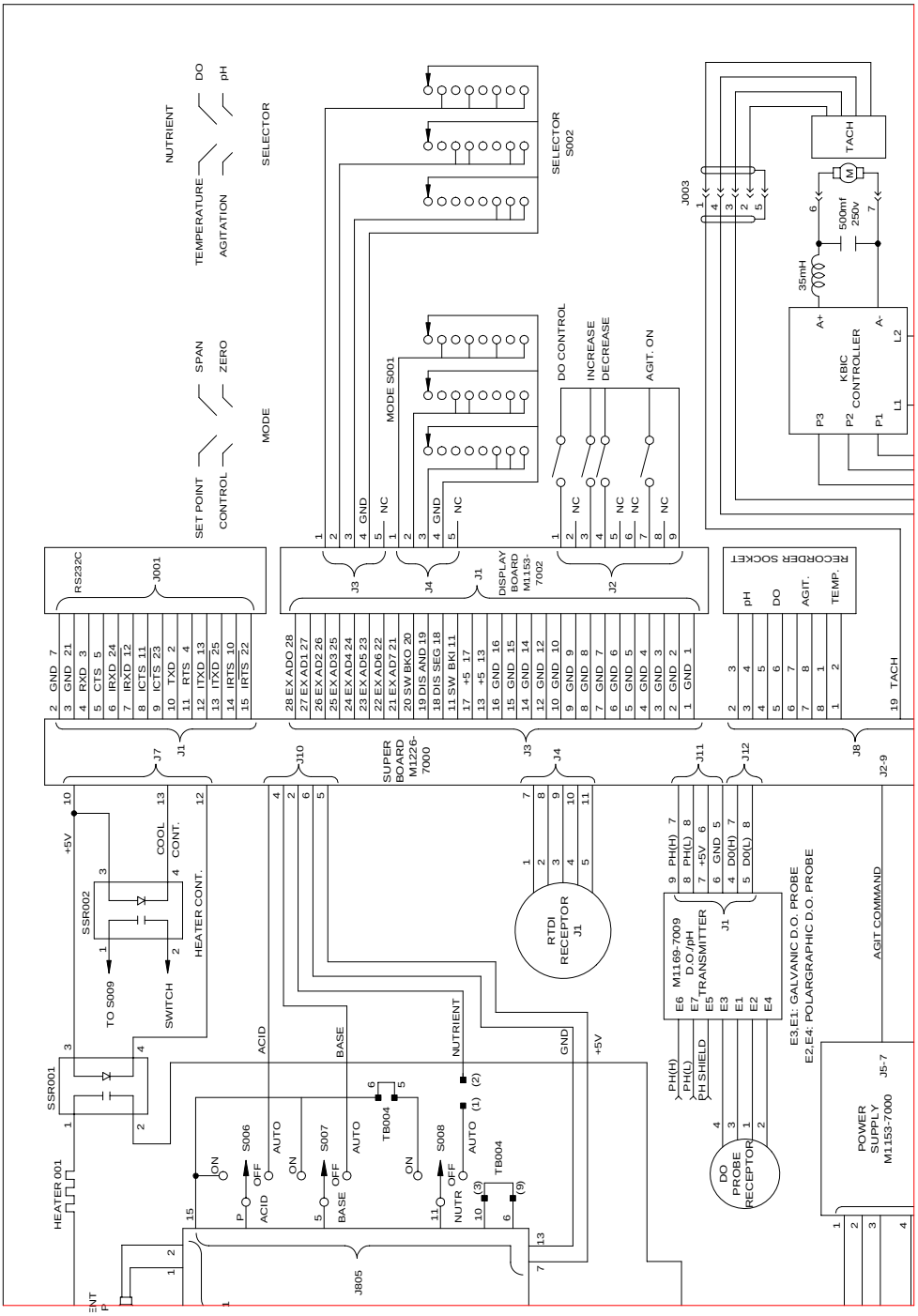

SUPER BOARD M1226-7000
DISPLAY BOARD M1153-7002
RECORDER SOCKET
pH
DO
AGIT.
TEMP.
RS232C
J001
J1
J2
J3
J4
J7
J8
J10
J11
J12
J2-9
J5-7
J805
SELECTOR S002
MODE S001
NUTRIENT
TEMPERATURE
AGITATION
SELECTOR
SET POINT
CONTROL
SPAN
ZERO
MODE
DO CONTROL
INCREASE
DECREASE
AGIT. ON
TACH
J003
35mH
500mf
250v
KBIC CONTROLLER
A+
A-
L1
L2
P1
P2
P3
HEATER 001
SSR001
SSR002
TO S009
SWITCH
HEATER CONT.
COOL CONT.
+5V
ACID
BASE
NUTRIENT
GND
S006
S007
S008
TB004
AUTO
ON
OFF
RTDI RECEPTOR J1
M1169-7009 D.O./pH TRANSMITTER
PH(H)
PH(L)
PH SHIELD
DO PROBE RECEPTOR
E3,E1: GALVANIC D.O. PROBE
E2,E4: POLARGRAPHIC D.O. PROBE
POWER SUPPLY M1153-7000
AGIT COMMAND

FIGURE 24B-2

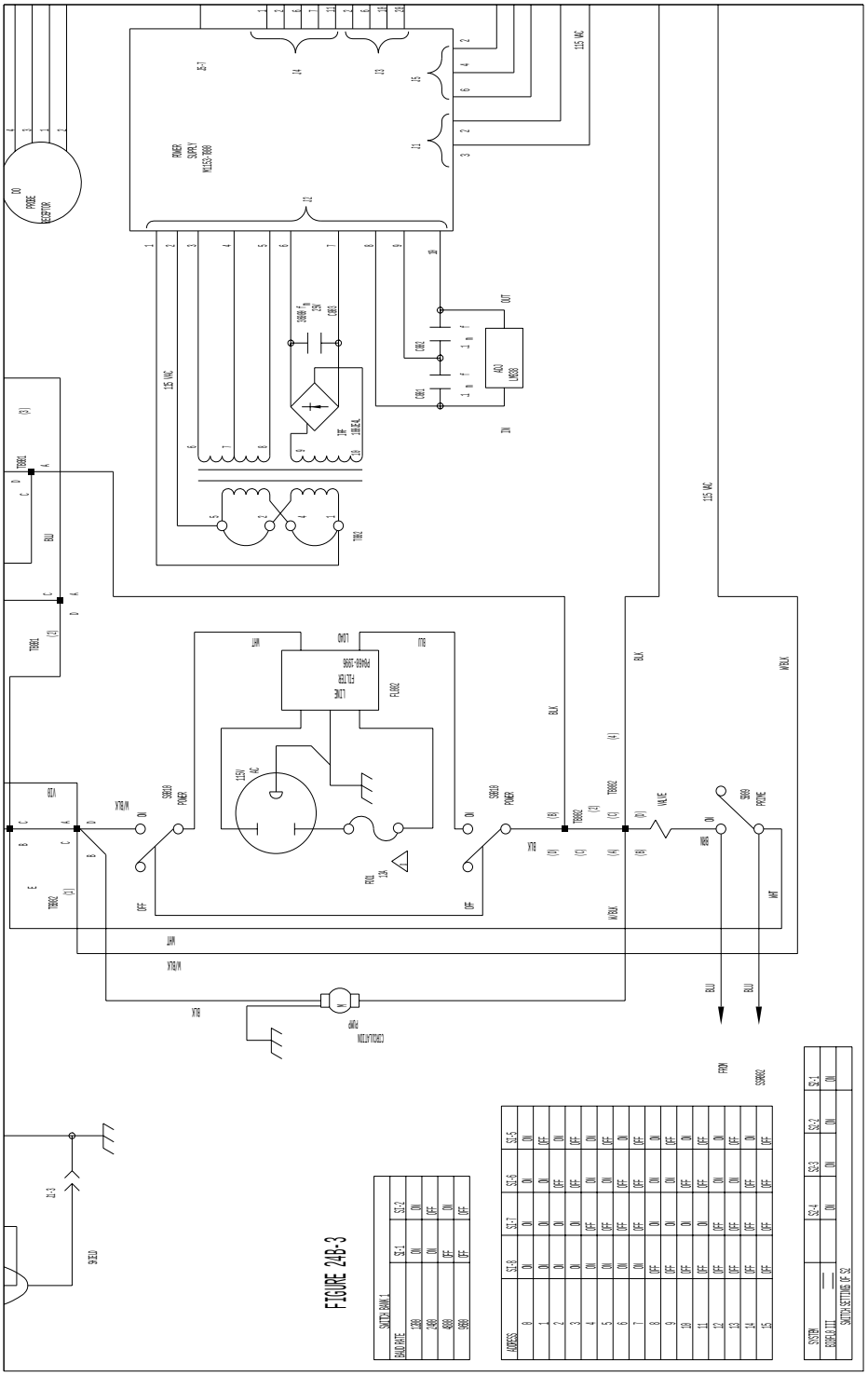


FIGURE 24B-3

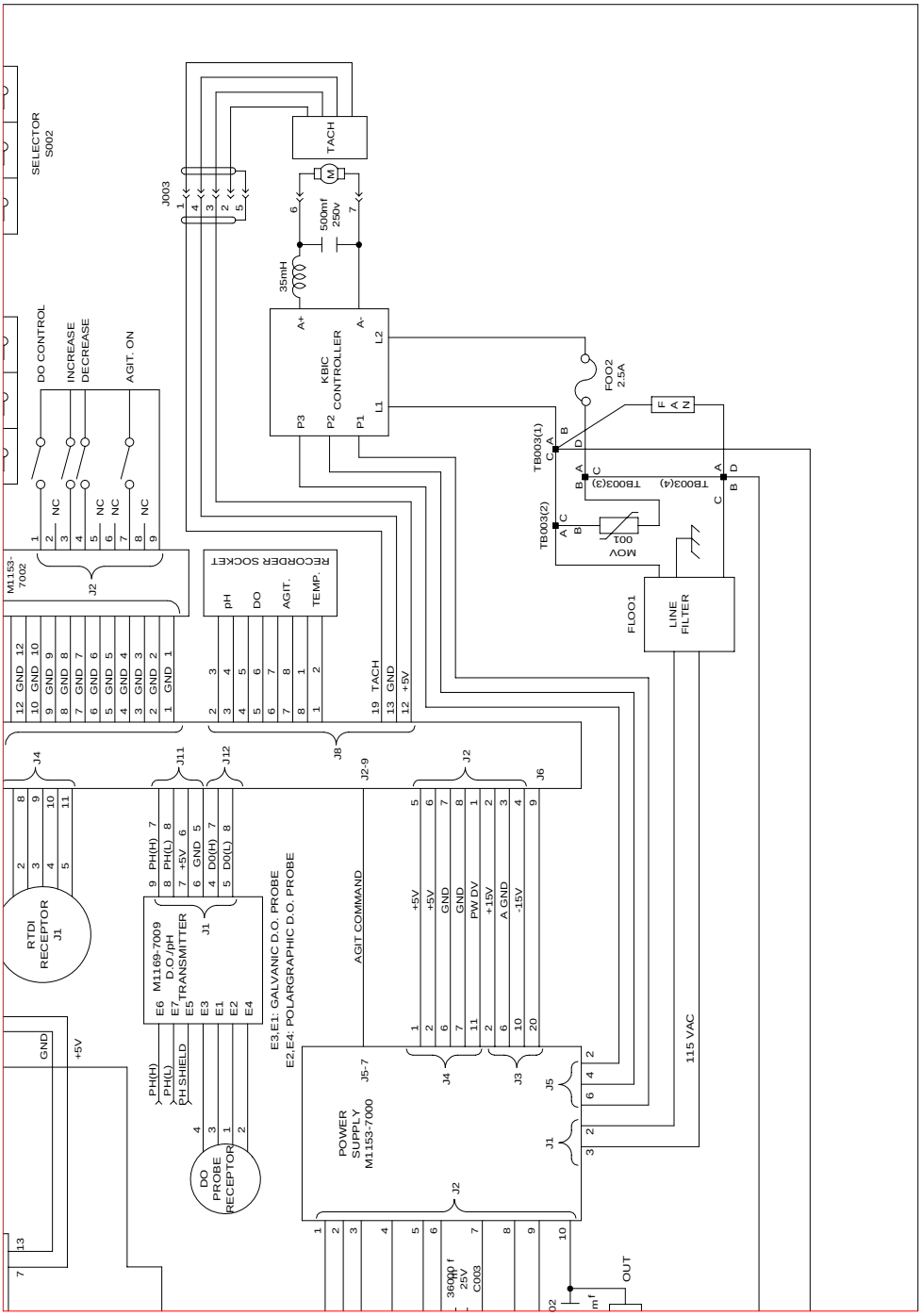

SELECTOR S002
TACH
J003
500mf 250v
35mH
KBIC CONTROLLER
A+
A-
L2
L1
P3
P2
P1
FOO2 2.5A
F A N
TB003(1)
TB003(3)
TB003(4)
TB003(2)
MOV 001
FLOO1
LINE FILTER
DO CONTROL
INCREASE DECREASE
AGIT. ON
NC
M1153-7002
J2
RECORDER SOCKET
pH
DO
AGIT.
TEMP.
GND
TACH
+5V
J4
J11
J12
J8
J2-9
J6
RTDI RECEPTOR J1
M1169-7009 D.O./pH TRANSMITTER
PH(H)
PH(L)
+5V
GND
DO(H)
DO(L)
PH SHIELD
E6
E7
E5
E3
E1
E2
E4
E3,E1: GALVANIC D.O. PROBE
E2,E4: POLARGRAPHIC D.O. PROBE
DO PROBE RECEPTOR
AGIT COMMAND
POWER SUPPLY M1153-7000
J5-7
J3
J5
J1
115 VAC
OUT
PW DV
A GND
-15V

FIGURE 24B-4

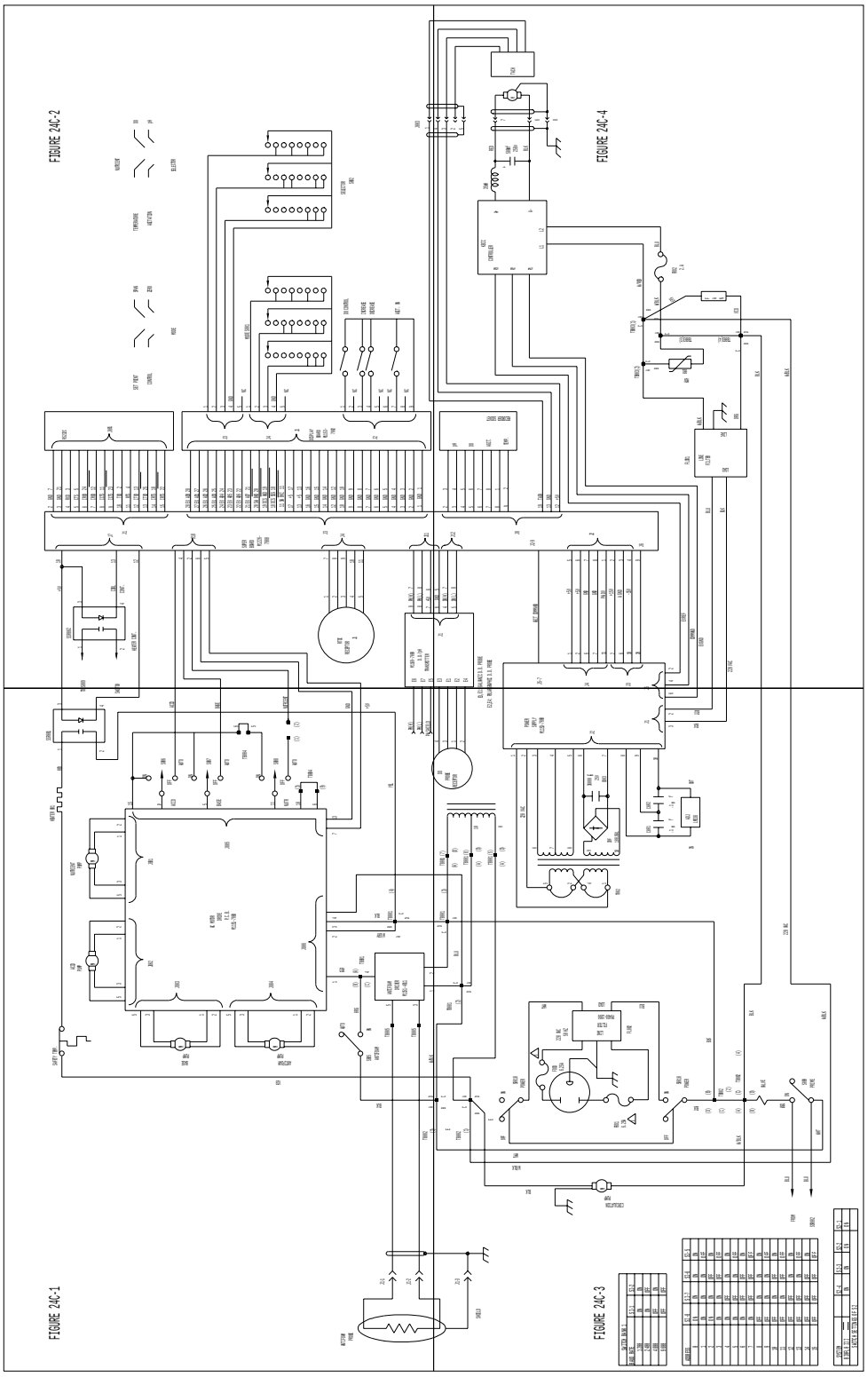


FIGURE 24C - CONTROL SCHEMATIC (220V)

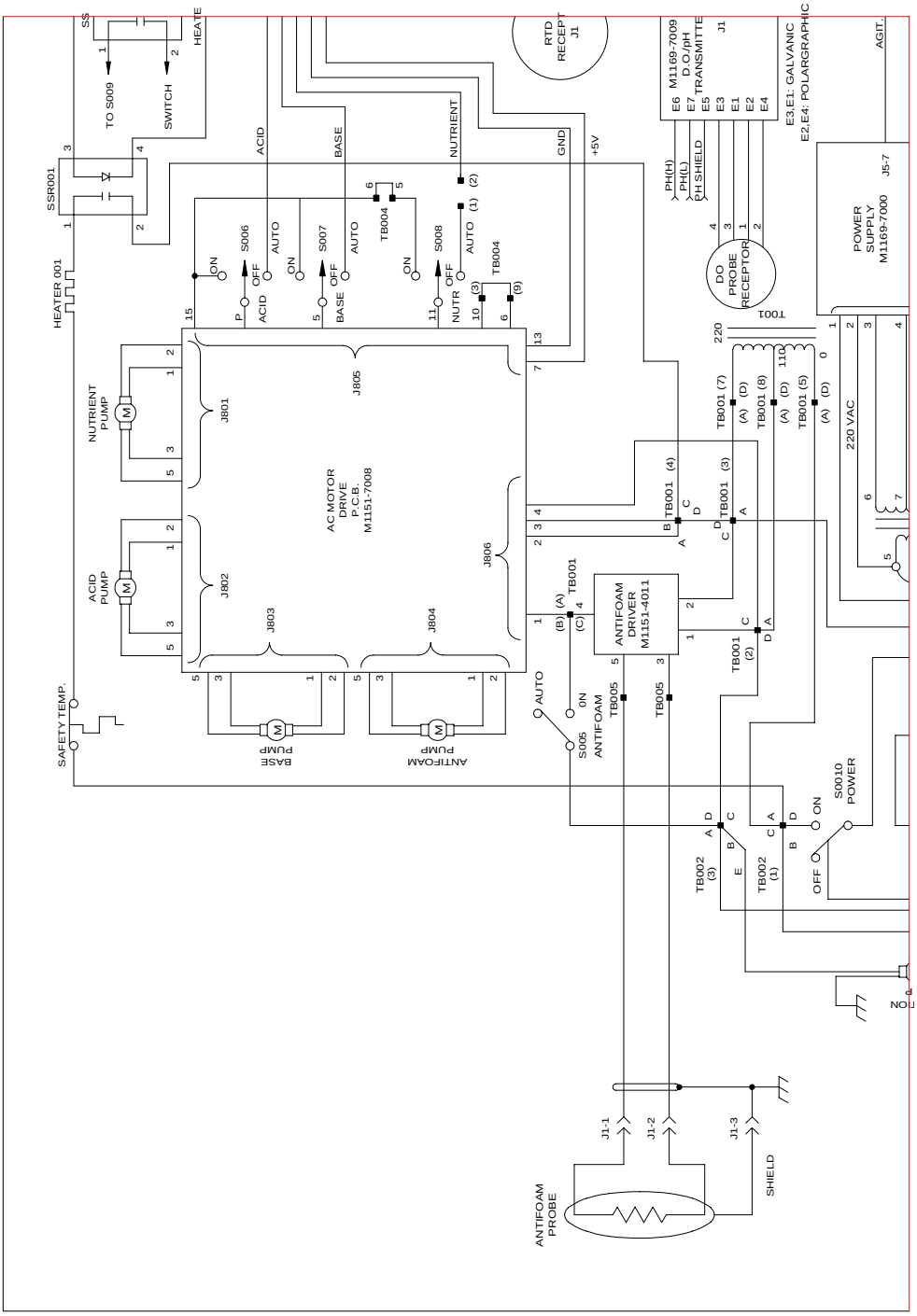

SSR001
HEATER 001
SAFETY TEMP.
NUTRIENT PUMP
ACID PUMP
BASE PUMP
ANTIFOAM PUMP
AC MOTOR DRIVE P.C.B. M1151-7008
ANTIFOAM DRIVER M1151-4011
ANTIFOAM PROBE
SHIELD
SWITCH
TO S009
ACID
BASE
NUTRIENT
GND
+5V
RTD RECEPT J1
M1169-7009 D.O./pH TRANSMITTE
PH(H)
PH(L)
PH SHIELD
E3,E1: GALVANIC
E2,E4: POLARGRAPHIC
DO PROBE RECEPTOR
POWER SUPPLY M1169-7000
AGIT.
220 VAC
S0010 POWER
S005 ANTIFOAM
S006
S007
S008
TB004
TB005
TB001
TB002
T001
J801
J802
J803
J804
J805
J806

FIGURE 24C-1

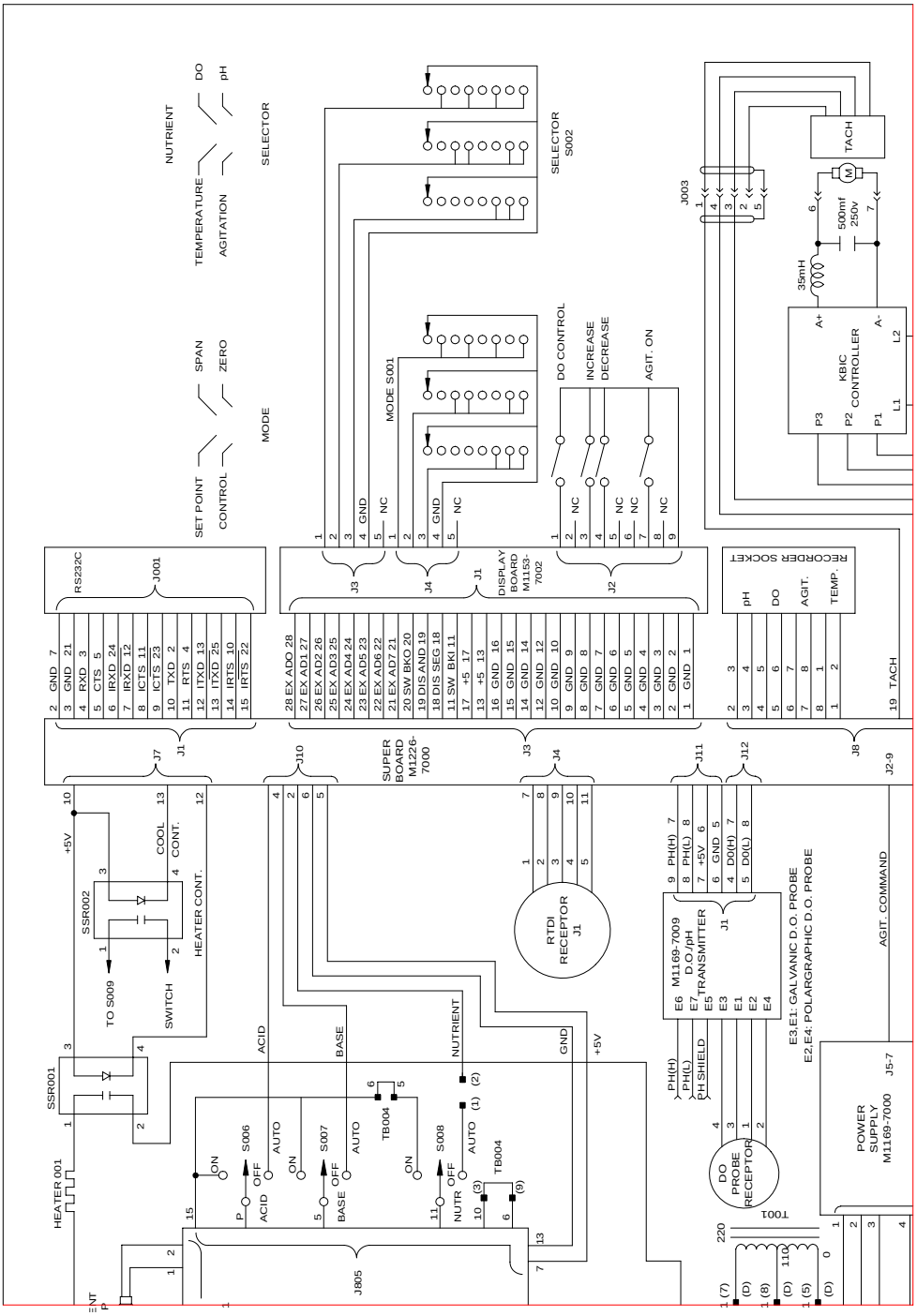

SUPER BOARD M1226-7000
DISPLAY BOARD M1153-7002
KBIC CONTROLLER
RECORDER SOCKET
SELECTOR S002
MODE S001
DO CONTROL
INCREASE
DECREASE
AGIT. ON
SET POINT
CONTROL
SPAN
ZERO
MODE
NUTRIENT
TEMPERATURE
AGITATION
SELECTOR
DO
pH
TACH
35mH
500mf
250v
SSR001
SSR002
HEATER 001
TO S009
SWITCH
HEATER CONT.
COOL.
CONT.
+5V
ACID
BASE
NUTRIENT
GND
RTDI RECEPTOR J1
M1169-7009 D.O./pH TRANSMITTER
PH SHIELD
DO PROBE RECEPTOR
T001
E3,E1: GALVANIC D.O. PROBE
E2,E4: POLARGRAPHIC D.O. PROBE
AGIT. COMMAND
POWER SUPPLY M1169-7000
J805

FIGURE 24C-2

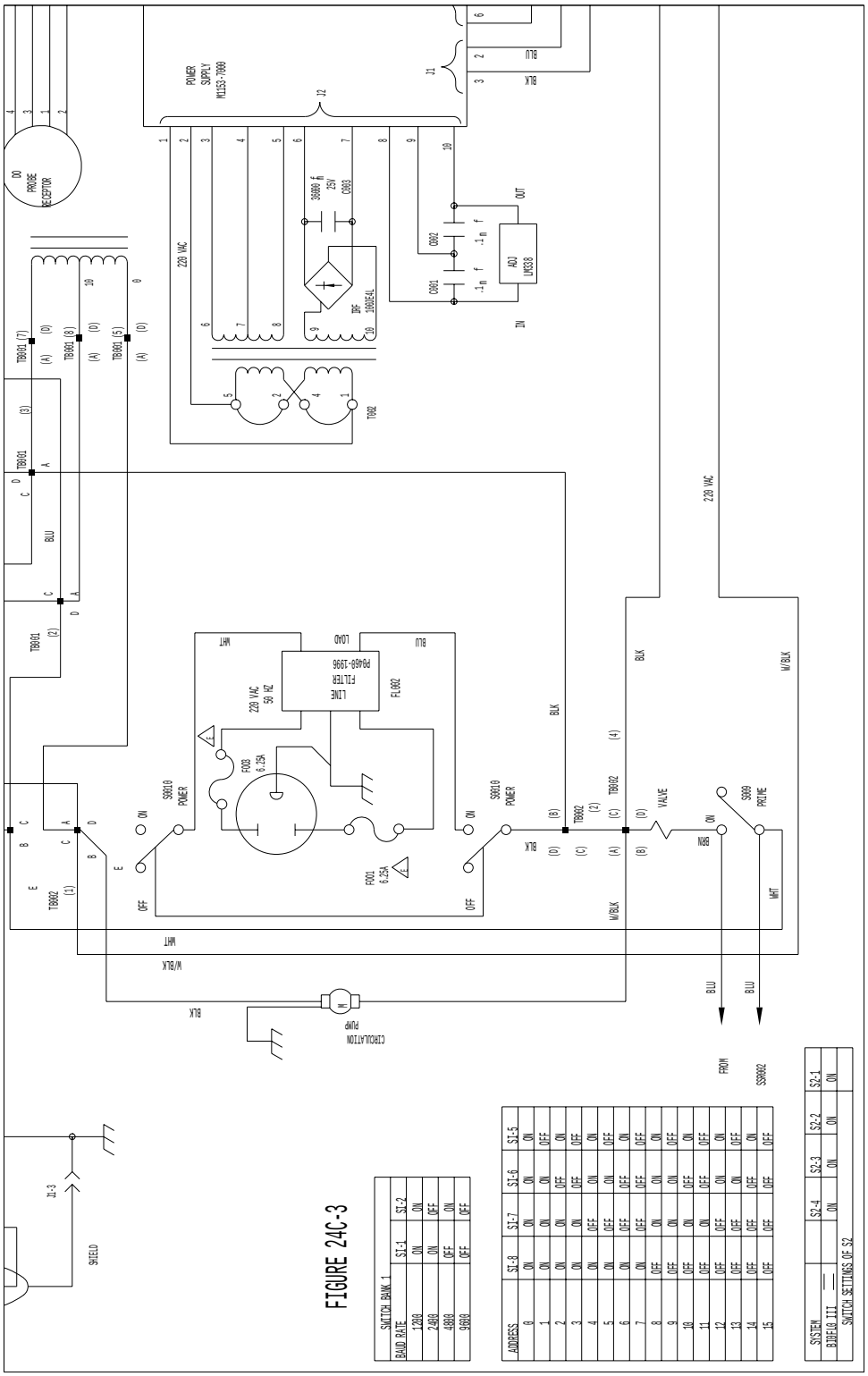


FIGURE 24C-3

FIGURE 24C-3

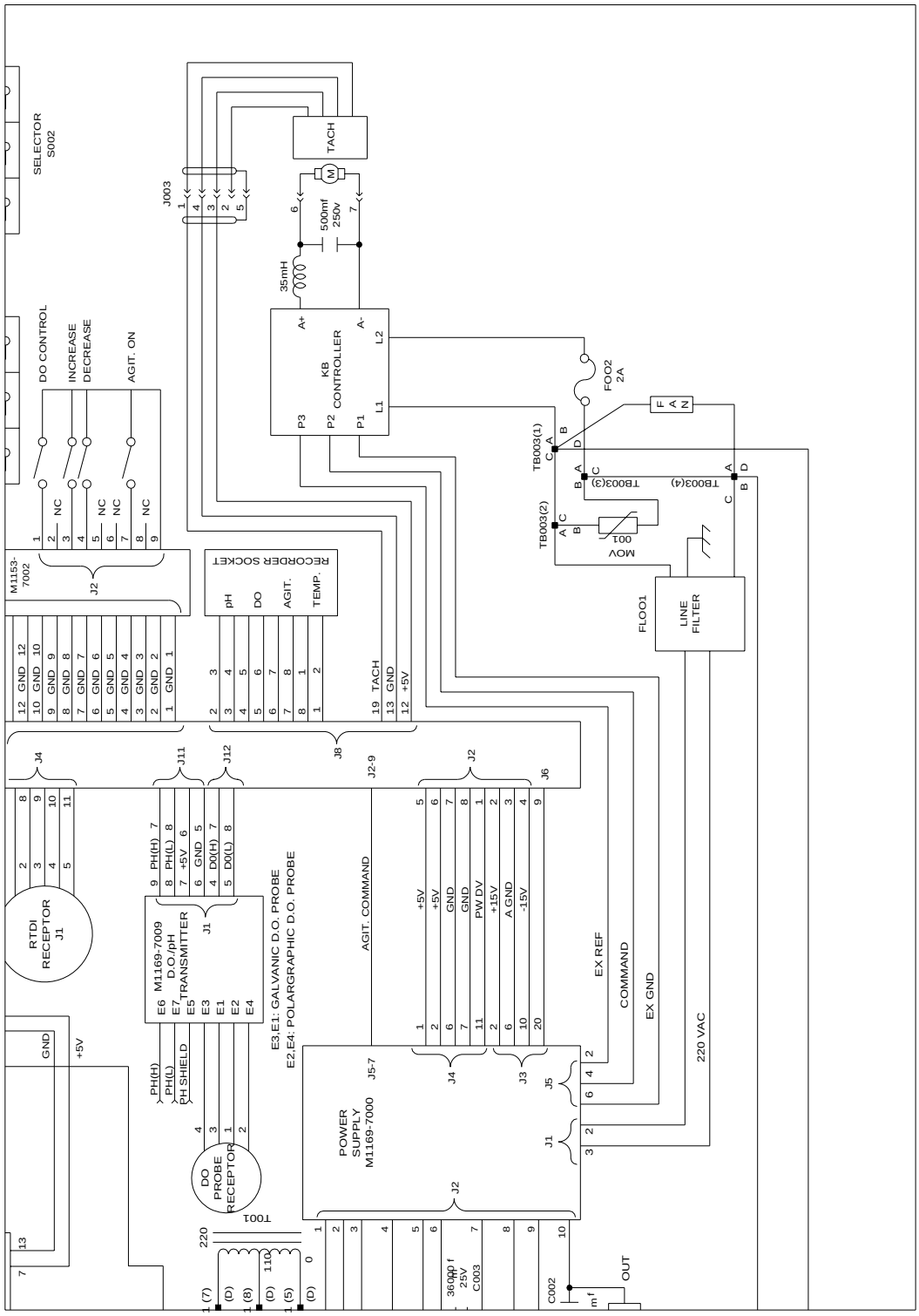

SELECTOR S002
DO CONTROL
INCREASE
DECREASE
AGIT. ON
NC
M1153-7002
J2
RECORDER SOCKET
pH
DO
AGIT.
TEMP.
TACH
J003
500mf 250v
35mH
KB CONTROLLER
A+
A-
L2
L1
P3
P2
P1
FOO2 2A
F
A
N
TB003(1)
TB003(2)
TB003(3)
TB003(4)
MOV
001
FLOO1
LINE FILTER
J8
J4
J11
J12
J2-9
J6
RTDI RECEPTOR J1
PH(H)
PH(L)
+5V
GND
DO(H)
DO(L)
M1169-7009 D.O./pH TRANSMITTER
PH SHIELD
E6
E7
E5
E3
E1
E2
E4
E3,E1: GALVANIC D.O. PROBE
E2,E4: POLARGRAPHIC D.O. PROBE
DO PROBE RECEPTOR
T001
220
110
0
AGIT. COMMAND
PW DV
A GND
-15V
EX REF
COMMAND
EX GND
220 VAC
POWER SUPPLY M1169-7000
J5-7
J3
J5
J1
J2
C002
C003
OUT

FIGURE 24C-4

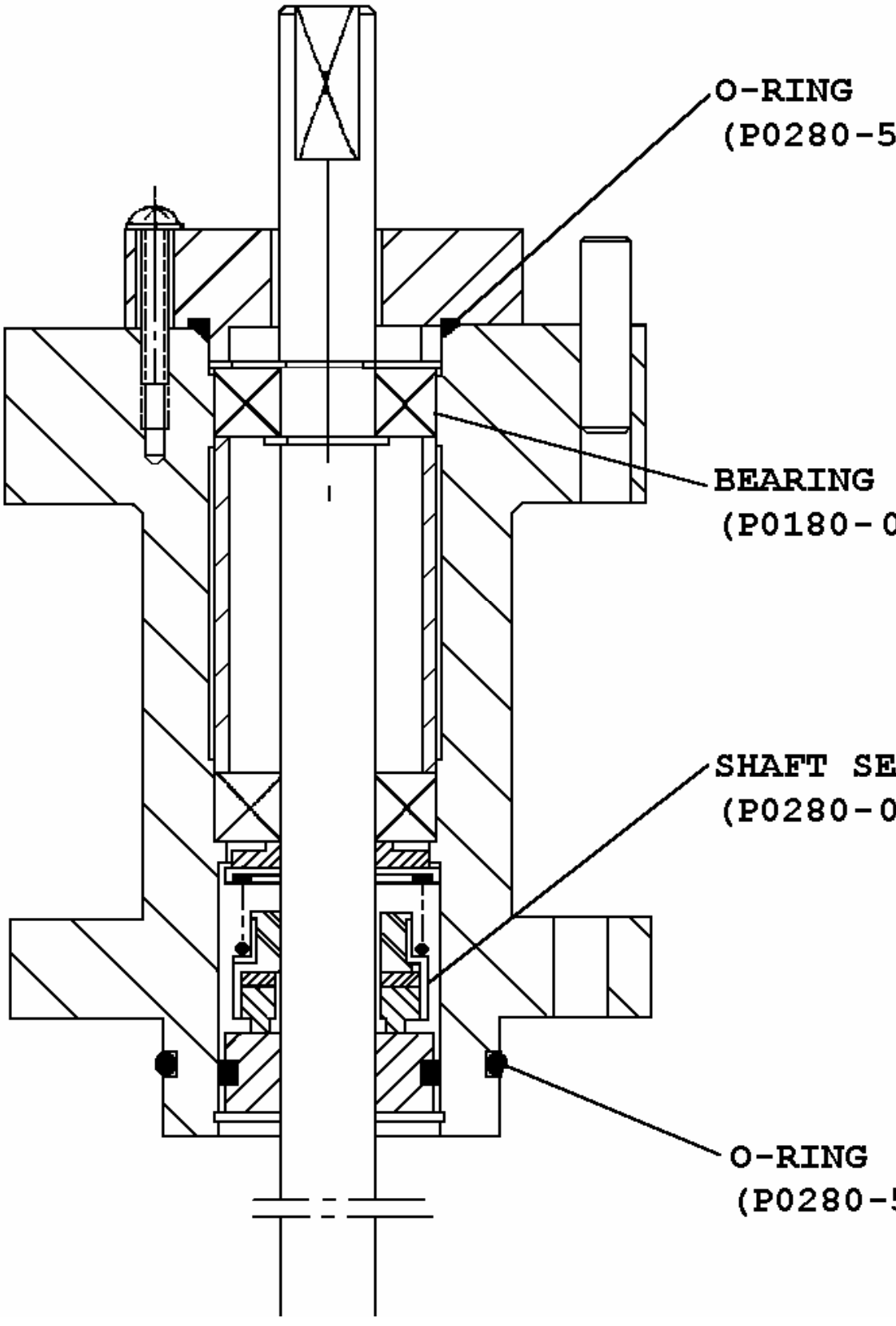

O-RING
(P0280-5
BEARING
(P0180-0
SHAFT SE
(P0280-0
O-RING
(P0280-5

FIGURE 25 - BEARING HOUSING ASSEMBLY

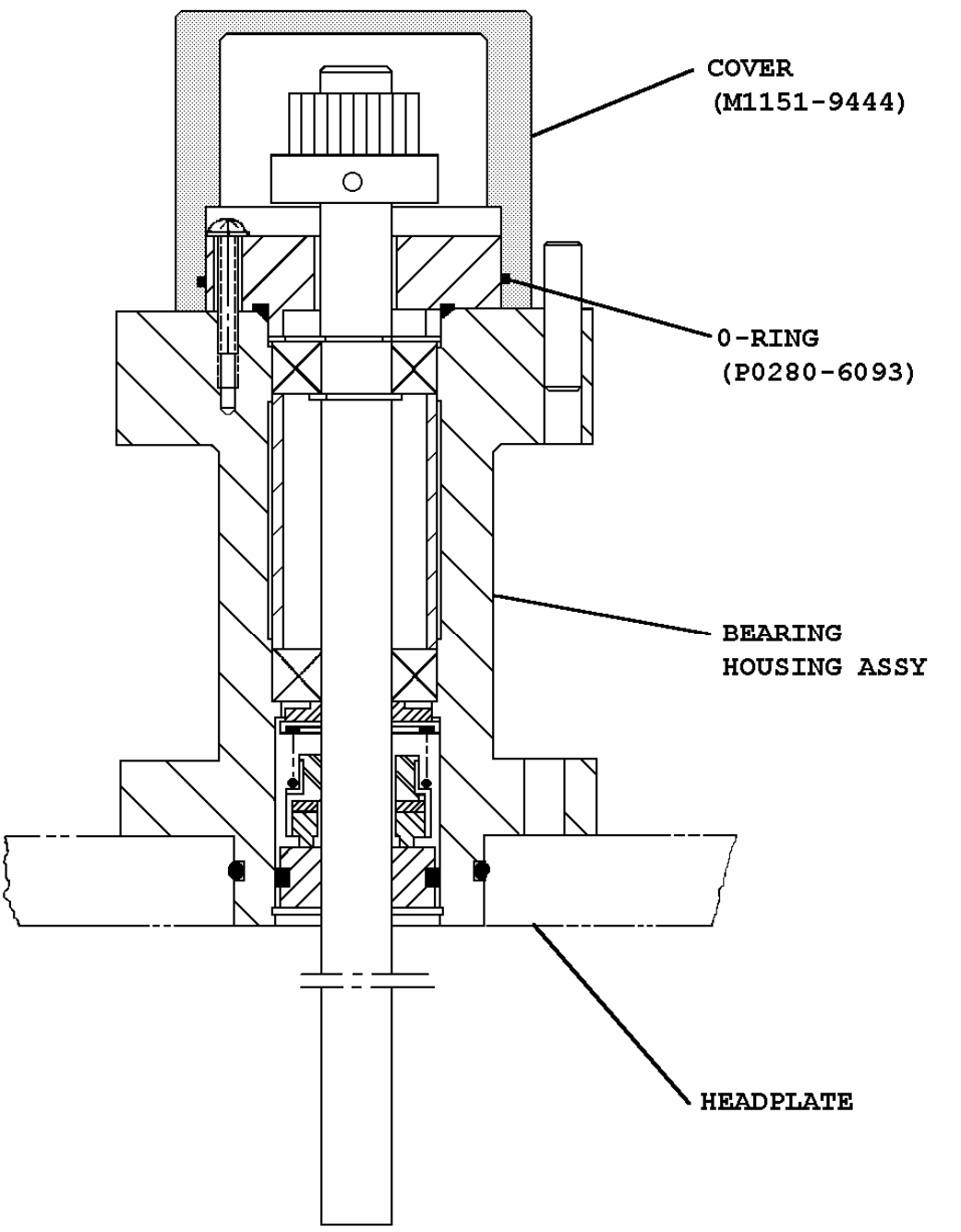


FIGURE 26 - BEARING HOUSING IN STERILIZATION

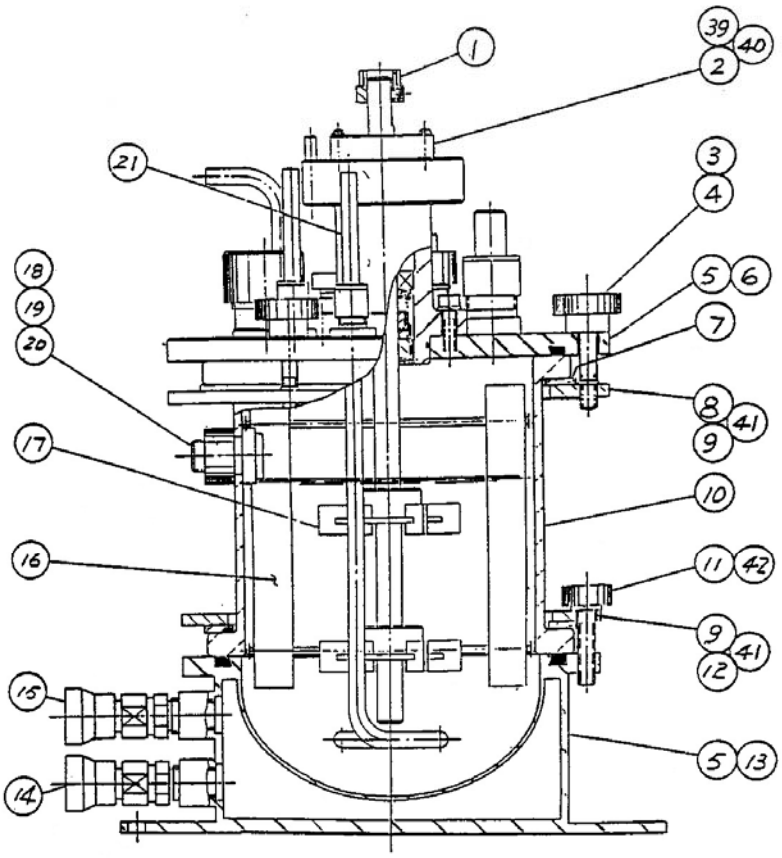


FIGURE 27A - VESSEL ASSEMBLY

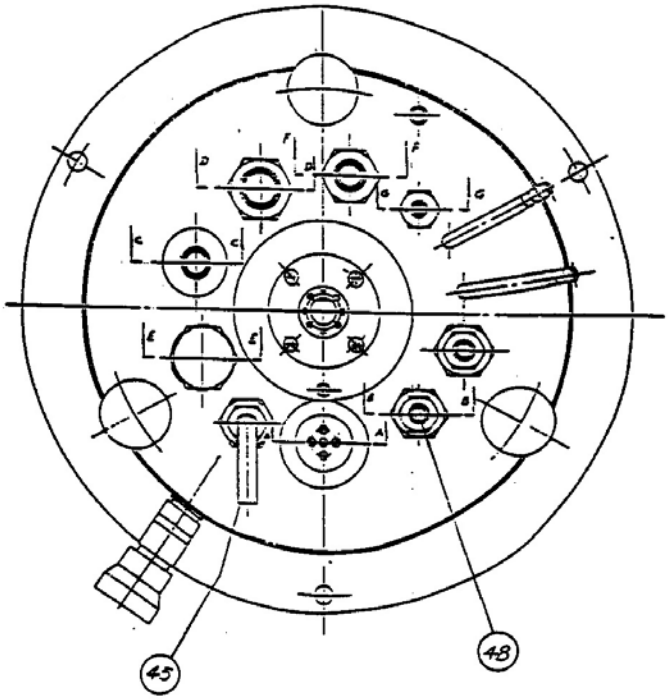


FIGURE 27B - HEADPLATE ASSY. (1 1/4L, 2 1/2L, 5L)

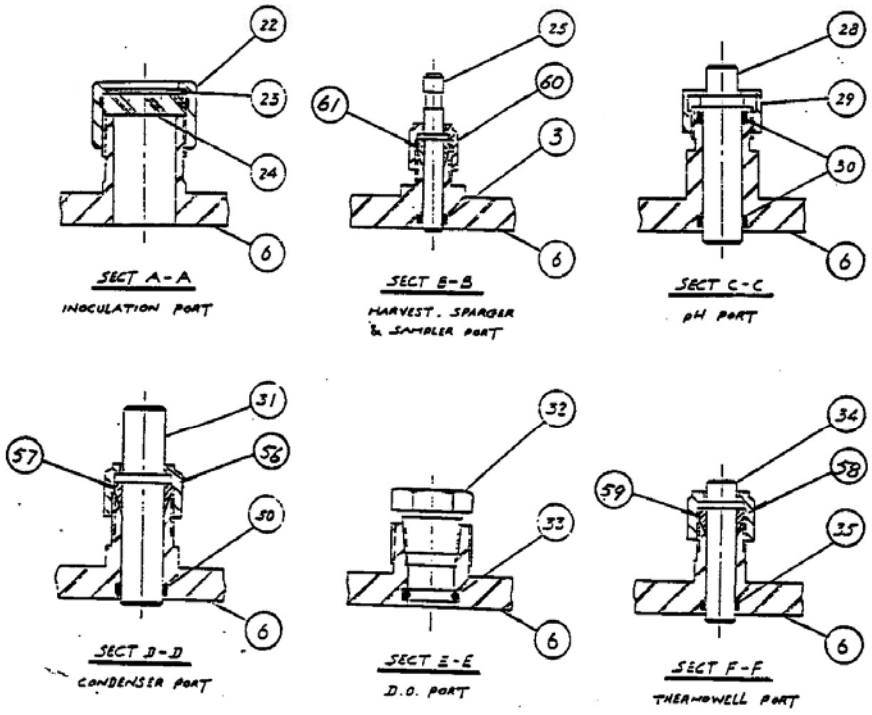


FIGURE 27C - PORT ASSEMBLY II

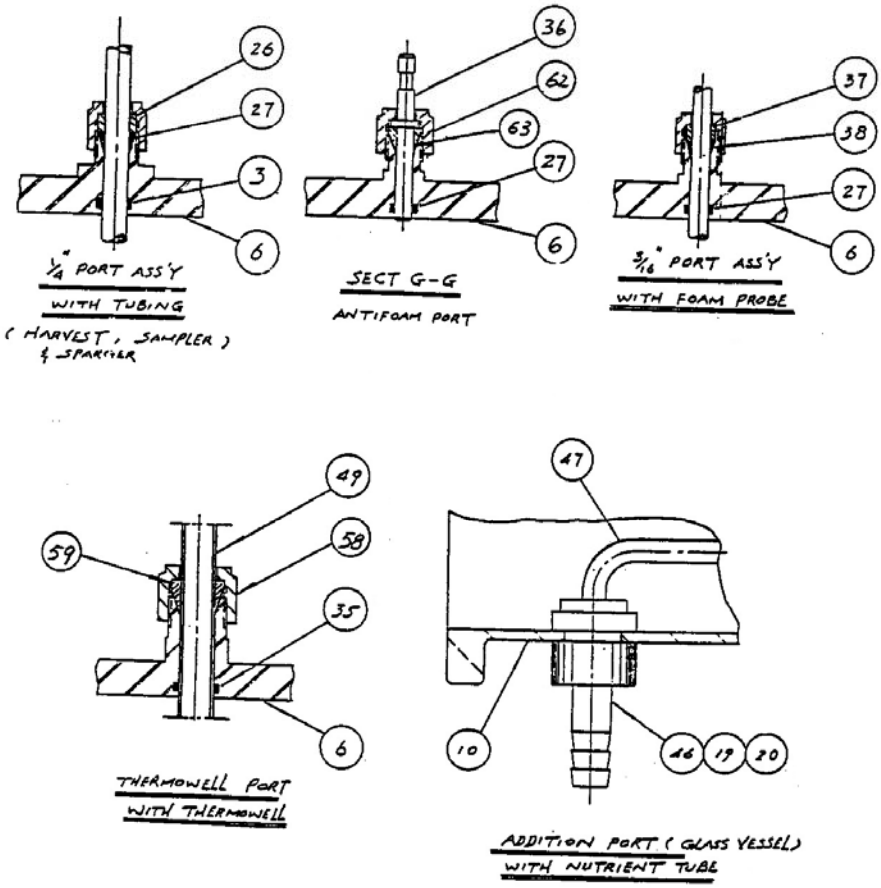


FIGURE 27D - PORT ASSEMBLY I