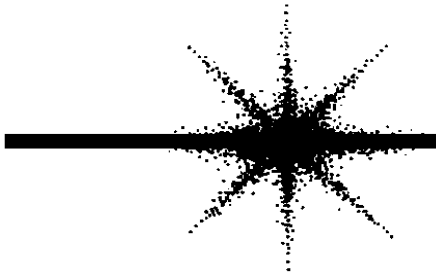
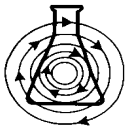


Guide to Operations

BIOFLO 3000
BENCH-TOP
FERMENTOR

MANUAL NO.: M1217-0050/K

NEW BRUNSWICK SCIENTIFIC CO., INC.
BOX 4005 . 44 TALMADGE ROAD . EDISON, NJ 08818-4005 . 732-287-1200
TELEPHONE: 800-631-5417 . FAX: 732-287-4222 . TELEX: 4753012 NBSCO
INTERNET: http://www.nbsc.com/ . E-MAIL: bioinfo@nbsc.com



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BIOFLO 3000 MANUAL

CHAPTER 1 - INTRODUCTION

1.1 SCOPE OF MANUAL

This manual contains a description of the equipment, the vessel, installation, operating and maintenance instruction for the BioFlo 3000 System manufactured by the New Brunswick Scientific Co., Inc., 44 Talmadge Road, P.O. Box 4005, Edison, New Jersey 08818-4005, U.S.A..

1.2 DESCRIPTION OF EQUIPMENT

BioFlo 3000 is a versatile bioreactor that provides a fully equipped fermentation and cell culture system in one compact package.* It can be employed for batch or continuous culture with microprocessor control of pH, DO2, agitation, temperature, pump feed, antifoam and vessel level.

* <u>NOTE:</u> *This manual only contains a description of the vessel, installation, operating and maintenance instructions for the fermentation system. For cell culture system please read CelliGen Plus instruction manual (M1176-0050).*

1.3 DESCRIPTION OF VESSEL

The vessels are designed for working volumes of 1.25, 2.5 and 5.0 liters. It consists of a stainless steel head plate, a flanged glass tube (thick walled) vessel body which is detachable from the stainless steel 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 a resistance temperature detector; a foam probe; a sparger; a harvest tube; a sampling tube; an exhaust condenser; dissolved oxygen and pH electrodes. The drive bearing housing is also located on the headplate (Fig. 1, 2).

1.4 AGITATION SYSTEM

A removable agitation servo motor located on the 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 20 to 1200 RPM *(see important note in section 2.6)*. A PID control loop holds the setting to within 1 RPM.

1.4.1 When Dissolved Oxygen (D.O.) is in the PID mode and the agitation is in the D.O. mode the agitation is cascade-connected to the D.O. so that the agitation speed is varied between the minimum (set by user) and the maximum setpoint of agitation (set by user) to maintain the set percentage of the D.O..

1.5 TEMPERATURE CONTROL

The culture temperature may be selected in the range from 5C above coolant temperature to 80C (.1C) 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 (Fig. 3).

1.6 AERATION

Air and oxygen can be introduced into the medium through the ring sparger and the flow rate is controlled by a needle valve located on the right hand side of the control cabinet (Fig. 4).

Percentage of oxygen introduced into the medium can be determined manually by the user. For high density culture, 100% of oxygen can be applied.

Oxygen can be introduced into the medium automatically through D.O. cascade system. When DO2 loop is in PID mode and "2 GAS" is in "DO" mode, oxygen will be introduced automatically into the inlet gas flow to maintain the set percentage of the D.O..

When DO2 loop is in PID mode, agitation loop is in DO mode and 2-Gas is in AgO2DO mode, oxygen can also be introduced automatically into the medium after agitation speed reaches its maximum setting value, yet the dissolved oxygen is still lower than the set point.

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. 5). Available as an option is the ability to provide control with a deadband of 0.1 about a setpoint (see switch 2 settings at the end of section 2.6). This will provide for only allowing the pumps to be activated when you are either 0.1 pH units above or below the setpoint chosen.

1.8 D.O. CONTROL

D.O. is controlled in the range of 5-95% (1%). It is sensed by the D.O. electrode and control is maintained by the PID controller which can change the speed of agitation and the percentage of oxygen in aeration.

The D.O. probe used on BioFlo 3000 can be Phoenix polarographic or Ingold polarographic (Fig. 6, 7).

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 into the vessel (Fig. 8).

1.10 EXHAUST SYSTEM

The exhaust gases pass into the exhaust condenser where moisture is removed and returned to the vessel. The air remaining passes into the 0.2 m exhaust filter (Fig. 9).

1.11 SAMPLING SYSTEM

1.11.1 System I

This system has a sampler which is attached to a sampling tube extending to the lower portion 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. 10).

1.11.2 System II

This system consists of a sample line and a peristaltic pump. Use "ON/OFF" mode of the

pump to operate the pump (Fig. 11).

1.12 SPECIFICATIONS

Vessel	a. Total Volume	1.6 Liters/3.3 Liters/6.6 Liters
	b. Working Volume	1.25 Liters/2.5 Liters/5 Liters
Temperature	a. Indication	Digital display in 0.1C increments.
	b. Range	From 5C above coolant temperature to 80C (setting range: 4C-80C. Temperature under that of coolant can be obtained with optional refrigeration unit.)
	c. Control	PID control employing PWM of heater & cooling water.
	d. Sensor	Platinum RTD.
Agitation	a. Drive	Permanent magnet servo motor with high torque output.
	b. Range	20-1200 RPM *(see important note in section 2.6)*
	c. Sensor	Optical photoplastic disc 1000 lines/rev with Quadrature output.
	d. Control	Microprocessor based with PID.
	e. Impellers	Six-blade turbine impeller.
	f. Indication	Digital display in 1 RPM increments.
Exhaust	a. Filter	0.2 m interchangeable cartridge.
	b. Condenser	Stainless steel counterflow water cooled in headplate.
Aeration	a. 2-Gas System	Air, O2, delivered to ring sparger.
	b. Flowmeter	0-10 SLPM mass flowmeter.
	c. Sparger	Ring sparger
	d. Inlet Filter	0.2 m interchangeable cartridge.
pH	a. Indication	Digital display 0.01 pH increments.
	b. Range	2-12 pH
	c. Control	PID
	d. Probe	pH Ingold
Dissolved Oxygen	a. Indication	Digital display 0.1% increments.
	b. Range	0-200%
	c. Probe	Polarographic (Ingold or Phoenix)
	d. Control	PID via superboard/Agit, 2-Gas
Pumps	See attached matrix.	
Voltage	Universal 100V 120V 220V 240V	

There are five assignable peristaltic pumps on BioFlo 3000, namely, Feed 1, Feed 2, Feed 3, Feed 4 and Feed 5.

Pumps	Modes	Operation
Feed 1-5	Off, Manual, Base,	Fixed Speed,

	Acid, Lvl 1, Lvl 2, Lvl 3, On	Duty Cycle Variable

Flowrates of Pumps

Flowrates (mL/min)* Silicone tubing only

		Tubing internal diameter				
		0.5mm	0.8mm	1.6mm	3.2mm	4.8mm
Frequency	RPM	1/50"	1/32"	1/16"	1/8"	3/16"
50 Hz	12	.252	.59	2.62	9.60	19.6
60 Hz	14.4	.300	.71	3.14	11.5	23.5

* *NOTE: The numbers shown in the table are the flowrates when the control mode of the feed pump is "ON". When the feed pump is set in other modes (manual, base, acid, lvl 1, lvl 2 and lvl 3) the flowrates will be 20% less than the numbers shown in the table.*

1.13 OPERATING CONTROLS AND DISPLAYS

The main operating controls of the BioFlo 3000 is the membrane keypad. There are twenty keys on the keypad. The function of "Screen" key is to select the screen. There are three screens which can be selected on the display. The first one is the "Master" screen, the other two are: a "Calibration" and a "Gases" screen (Fig. 12, 14, 15, 16).

Besides above three screens, there is a mode selection screen which displays only after the power switch is turned on. The "Mode Selection" (Fig. 13) will display on the screen for 20 seconds. Operator can select cell culture or fermentation by pressing "1" or "2" within this period of time. Then the screen will automatically turn to the "Master Screen".

MODE SELECTION

1. CELL CULTURE
2. FERMENTATION

Your Selection: 2

Figure 13

On the top of each selective screen, three screen names are listed, the one in brackets is the screen currently displaying, the other two screens are to be selected. Press "Screen" key, the middle one will blink, this means that this screen is ready to be selected, press "Enter" key, the selection of a new screen is done. If another screen is to be selected, don't press "Enter" key but arrow () key first, then press "Enter" key.

The function of the "Alter" key is to select the loop and the mode of each loop. When

making these selections, move the cursor to the loop or the mode to be changed, then press the "Alter" key to get the desired selection and then press "Enter" key. If you have a loop set already, you cannot change into that loop.

SCREEN	<MASTER>	CALIBRATION	GASES

Loop Name	Agit 1	Temp 1	pH 1	DO2 1
Value	100	37.0	7.00	100
Set	100	37.0	7.00	100
Control	PID	PID	PID	PID

NOTE: *The control mode of each loop are shown in the following table.*

						FEED 1 TO	LVL 1 TO
LOOP	AGIT 1	TEMP 1	pH 1	DO2 1	AIR	FEED 5	LVL 3
	OFF	OFF	OFF	OFF	MON	OFF	OFF
	PID	PRIME	PID	PID		MAN	ADD
	DO					BASE	
		PID				ACID	HRVST
CONT						LVL1	
MODE						LVL2	
						LVL3	
						ON	

Figure 14

SCREEN	<2-GAS>	MASTER	CALIBRATION

Oxygen Enrichment		Air		O2
Output %		75.5		24.5
Mode	DO			
Control Gains	P	0.25	I	2.50

Figure 15

NOTE:

1. *Mode can be off, Do, AgO2DO or manual. DO mode can be selected when DO2 loop is in PID mode. AgO2DO mode can be selected when DO2 loop is in PID mode and agitation is in DO mode.*
2. *P (Proportional) I (Integral) value can be selected by the operator. Move cursor to O2, press code "9XXXX", then P I value can be changed.**

* *NOTE: P I values have been set in the factory before shipping. Under normal conditions, it is not necessary to reset these values.*

SCREEN	<CALIBRATION>	MASTER	GASES

Calibration

	Value	Zero	Span	Function
pH	7.00	7.00	4.00	Read
D.O.	100	0	100	Read

NOTE:

1. *Change function to zero, calibrate zero.*
2. *Change function to span, calibrate span.*

Figure 16

There is a recorder output connector on the left hand side of the control cabinet. An external recorder with 0-1 volt high impedance input may be connected to the output.

An RS232/422 computer interface has been provided. A 25 pin "D" connector is located on the left hand of the control cabinet. An advanced fermentation software program is available which will enable the operator to interface with an IBM or suitably compatible MS-DOS computer. It will enable one to establish or change the set points for temp, pH, D.O., agitation speed and pump flow rate. The operator may also read and log the current values of those parameters (temp, pH, DO, air flow, pump flow rate, levels and agitation) which are monitored. By having the data available in this form it can be stored, plotted and, afterwards, transferred to other commonly available programs, manipulated and analyzed in various ways.

CHAPTER 2 - INSTALLATION

2.1 INSPECTION

Unpack the BioFlo 3000 and carefully inspect for any apparent damage which may have occurred during transit. A component list is enclosed. Check the component list against the parts shipped. Report any missing parts to New Brunswick Scientific Co. Report any obvious damage to the carrier and to New Brunswick Scientific Co., Inc..

2.2 UTILITY REQUIREMENTS

Water: 20 PSIG max., 50m filtration
Gas: 10 PSIG max.

Electrical Requirements: 100/120 Volt - 50/60 Hz - 10 Amp
220/240 Volt - 50/60 Hz - 6 Amp

NOTE: See Fig. 24 for fuse replacing.

2.3 INSTALLATION OF CONSOLE

A. Position the BioFlo 3000 console on a firm level surface in an area where services are readily available.

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

C. Check the specifications plate on the rear of the unit. Connect the line cord to the console and plug the line cord into a suitable electrical outlet (Fig. 17).

2.4 POWER SWITCH

The main power switch which controls the power to the system is located on the left hand rear of the console.

Prior to turning on the main switch make sure that:

A. The input water hose is connected at the rear of the unit and the water supply is turned "ON". The drain line must also be connected.
B. The vessel base is in place and the quick connect plastic lines are connected to the vessel in front.

2.5 Recorder Output: 0.0 - 1.0 Volt DC

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

Grounds are pins 2, 4, 6 and 8.

PIN #	PIN FUNCTION	FOR 0-1 VDC OUTPUT
1	TEMPERATURE +	0-100C
2	TEMPERATURE -	
3	pH +	2-12 pH
4	pH -	
5	D.O. +	0-200%
6	D.O. -	
7	AGITATION +	0-1500 RPM
8	AGITATION -	

2.6 RS232/422 COMPUTER INTERFACE

The RS232/422 Computer Interface is a 25 pin "D" type connector mounted on the left hand side of the electrical cabinet. It is by means of this connector that an external computer may communicate with and control the BioFlo 3000 Fermentor. Pin designations are as follows:

PIN #	PIN FUNCTION	PIN #	PIN FUNCTION
7	JUMPER	23	ICTS
	(ONLY REQ.FOR		
21	RS422 CONFIG.)	2	TXD
3	RXD	4	RTS
5	CTS	13	ITXD
12	IRXD	25	ITXD
24	IRXD	10	IRTS
11	ICTS	22	IRTS

Unless requested otherwise the baud rate is factory selected at 9600 and the connector is configured as an RS232 port: i.e. no jumper between pin #7 and pin #21. Furthermore, the machine has been assigned an address location of 0.

In addition to the two available connector configurations, there are four baud rates and 16 addresses which may be selected by changing dip switch settings. The switches to effect both these types of changes are internally mounted in the same array on the superboard.

If a change is requested, first turn the power off, then open the back door, the superboard is located on the top of the shelf. The single array for both functions is an assembly of eight dip switches labeled SW1.

To select one of the four available baud rates the first two switches in the array should be set as shown in the following table:

BAUD	S1-1	S1-2

1200	ON	ON
2400	OFF	ON
4800	ON	OFF
* 9600	OFF	OFF

Switch positions 3 and 4 are used as follows:

	SW1-3	SW1-4
* Multidrop (8 data bits) and even parity check	ON	ON
Multidrop (8 data bits) and no parity check	OFF	ON
No Multidrop (7 data bits) and even parity check	ON	OFF
No Multidrop (7 data bits) and no parity check OFF		OFF

To select one of the sixteen addresses for the machine it is necessary to set the last four switches in accordance with the following table:

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

* SW1 of the machine has been set as "OFF-OFF-ON-ON-ON-ON-ON-ON".

The switch settings of SW2 are:

	ON	OFF
SW2-1	pH Control without dead band	pH Control with dead band
SW2-2	Agitation 20-1000 RPM	Agitation 20-1200 RPM

SW2-3 and SW2-4 can be set either "ON" or "OFF".

IMPORTANT NOTE:

SW2-1 and SW2-2 have been set at "ON" position in the factory before shipping. If 1200 RPM agitation speed is required, please consult with New Brunswick Scientific Co. before taking action.

CHAPTER 3 - PREPARATION & OPERATION

(For preparation and operation of Cell Culture, please read CelliGen Plus Manual M1176-0050 - included with conversion kit - see p. 23)

3.1 CLEANING OF VESSEL

A. 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.

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

C. Sterilization *(see 3.8)*.

3.2 VESSEL ASSEMBLY

A. 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. 18)*.

* Lubricate vessel O-ring with silicone grease, position glass tube reactor and secure clamping screws, perform the same for head plate. ***DO NOT OVERTIGHTEN.***

Working From the Inside of the Coverplate.

B. Insert the sparger tube in the sparger port, (Fig. 19).

C. Insert the harvest tube in the harvest port, (Fig. 20).

Working From the Outside of the Coverplate.

D. Insert the thermowell tube in the temperature control port, (Fig. 3).

E. Insert the sampler assembly into the sample port (Fig. 10).

F. Install foam probe in the head plate, (Fig. 8).

G. Install the baffle assembly inside of the glass jar by pressing the two edged baffles together.

H. Install four polypropylene ports or plugs in the wall of the glass jar. Be sure to use a blind plug (without hole) when a port is not in use, (Fig. 21).

Place head plate on the flange of the vessel and lock it to the clamping ring with knurled screws *(see operation tips)*. Install pH and DO probes (coat probe with glycerin before insertion into head plate). Prior to installation, probes should be well prepared *(see sec. 3.3, 3.4, 3.5 and 3.7)*.

NOTE: To avoid damage to the probes make sure that no interference exists between the probes and the baffle assembly.

Place the vessel on the three pins located on the base of the cabinet.
Position the motor assembly on top of the bearing housing locating pin. Connect the motor cable to the receptacle on the face of the cabinet.

Connect cables from all probes to their respective sockets on the face of the cabinet. Ground lead from the antifoam socket on the face of the cabinet is to be connected to the pin in the headplate.

Connect exhaust condenser to the exhaust condenser port. Connect the exhaust filter on the top of the condenser with flexible tubing.

Slide 2" long, 0.25"ID silicone tubing on the top of the sparger tube, then connect air filter to it. One side of the filter is to be connected to the hose barb in the face of the cabinet with flexible tubing.

3.3 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.

Check the electrode tip for trapped air bubbles. To remove any air bubbles hold the electrode upright and shake gently.

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

3.4 INSTALLATION OF THE pH ELECTRODE

CAUTION:
WEAR PROTECTIVE GLOVES WHEN INSTALLING ANY GLASS ELECTRODES.

1. Apply a small amount of silicone grease or glycerol to the electrode body.
2. Install the pH electrode as shown in Figure 5.

3.5 PROBE MAINTENANCE AND STORAGE

pH

1. Check the level of the filling solution. It should be about 1cm below the filling orifice. To add solution, *see Section 3.3.*
2. Check for any trapped air bubbles in the electrode's tip to remove bubbles, hold electrode upright and shake electrode gently.
3. The probe should be stored standing upright. The electrode tip should be immersed in the solution of 3 molar KC1 or a buffer solution between pH 4 and pH 7. The two rubber "T" stoppers should be inserted. At no time should the electrode be allowed to rest on the tip.

3.6 pH ELECTRODE CALIBRATION

NOTE: pH electrode is calibrated before autoclaving vessel.

1. Connect electrode to the pH connector using the appropriate cable.
2. Turn ON the main power switch.
3. Display calibration screen.

NOTE: The pH measuring system is calibrated using two external buffer solution of known pH.

4. Immerse pH electrode into pH 7.00 buffer solution and allow a few minutes for the system to equilibrate.
5. Set the pH function zero.
6. Set the display to read 7.00.
7. Rinse the pH electrode with distilled water.
8. Immerse pH electrode into a second pH buffer solution which is several pH units above or below pH 7.00 (eg. 4.00) and allow a few minutes for the system to equilibrate.
9. Set the pH function "Span".
10. Set the display to read the value of the second buffer solution.
11. Repeat steps 5-10 using the same buffer solutions.

NOTE: The pH calibration should be checked after autoclaving immediately prior to inoculation. This is performed by taking a sample from the vessel and comparing the value of pH displayed on the screen with that of an external pH meter. Any discrepancy should be adjusted with the function set to "Zero".

3.7 OPERATION OF DISSOLVED OXYGEN (DO) PROBE

1. Remove protective cap from electrode end. The membrane is delicate and care must be exercised to prevent accidental damage. Never rest probe on membrane.

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

3. Install probe into vessel head plate assembly (Fig. 6,7). If an Ingold probe is to be used, wrap the adapter threads with teflon tape, screw into headplate and tighten with a wrench. Carefully insert the probe into the adapter. Finger tighten enough to compress the o-ring to insure a tight seal.

3.8 STERILIZATION PROCEDURE

Remove the motor drive from the top of the vessel and place it on the motor mount at the top of the cabinet. Place the housing cover on the top of the bearing housing before sterilization *(Fig. 22)*.

Disconnect air line of the inlet filter side.

Disconnect all probes and remove probe cables.

Rubber T-stoppers should be in place in the pH probe during autoclaving. (Rubber bands can be placed around them to prevent blow out during sterilization.)

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

sampler valve.

Sterilize the complete assembly consisting of jar, headplate and components of headplate by inserting in an autoclave.

NOTE: *For continuous culture, vessel should not be sterilized empty. Use at least 100 mL 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.*

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

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 90C. SLOW EXHAUST IS REQUIRED.

CAUTION:

AT NO TIME SHOULD YOU SUBJECT THE PROBE TO CONDITIONS WHICH WOULD CAUSE THE ELECTROLYTE TO BOIL. PARTICULAR CARE SHOULD BE EXERCISED DURING THE COOLING CYCLE FOLLOWING AUTOCLAVING WHEN LOCALIZED BOILING TENDS TO OCCUR. EITHER MAINTAIN A SLOW COOLING RATE OR PRESSURIZE THE AUTOCLAVE DURING THIS PERIOD. CONSULT YOUR AUTOCLAVE MANUFACTURER FOR INFORMATION REGARDING A PRESSURE BALANCING FEATURE AND PROPER AUTOCLAVING TECHNIQUE TO ELIMINATE LOCALIZED BOILING DURING THE COOLING CYCLE.

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.

3.9 DISSOLVED OXYGEN ELECTRODE (D.O.) CALIBRATION

NOTE: D.O. electrode is calibrated after autoclaving vessel.

IMPORTANT NOTE:

When the system is operated for the first time, or when the electrode has been disconnected from the voltage source (amplifier or polarization module) for longer than 5 to 10 minutes, the electrode must be connected to the operating O2 amplifier for polarization purpose prior to calibration.

*The electrode is polarized and ready for operation after **six** hours of polarization time.*

1. There are two methods of obtaining zero on the BioFlo 3000. Use either Method 1 or Method 2.

   Method 1
   a. Remove the D.O. electrode cable from the D.O. electrode.
   b. Display calibration screen.
   c. Set the D.O. function to "Zero".
   d. Set the display to read zero by setting "Zero" to 0.
   e. Re-connect the D.O. electrode cable to the D.O. electrode.

   Method 2
   a. Connect the D.O. electrode cable to the D.O. electrode.
   b. Set agitation speed to 500 RPM.
   c. Sparge nitrogen into the vessel via the filter on the head plate until the D.O. display is stable for approximately 10 minutes (this may take up to 30 minutes).
   d. Display calibration screen.
   e. Set the function to "Zero".
   f. Set the display to read zero by setting "Zero" to 0.

2. Setting the "Span".
   a. Set the agitation rate of 500 RPM and agitation mode PID.
   b. Vigorously sparge air or oxygen into the vessel via the filter on the head plate until the display is stable for approximately 10 minutes (this may take up to 30 minutes).
   c. Set the function to "Span".
   d. Set the display to read 100 by setting "Span" to 100.

3.10 PREPARATION FOR OPERATION

A. Position and secure the vessel on BioFlo 3000 console. Connect the heat exchanger and exhaust condenser.

B. Carefully place the DC servo motor on the vessel assembly.

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

D. Turn on the water valve, adjust water pressure to 20 PSI.

E. Connect air and O2 to the unit, adjust gas pressure to 10 PSI.

CAUTION:

NEVER ATTACH TUBINGS MADE OF HARD MATERIAL, SUCH AS TEFLON, DIRECTLY TO THE PLASTIC HOSE BARBS ON THE GAS ENTRY.

IN CASE HARD MATERIAL TUBING IS GOING TO BE USED, ATTACH A SHORT SOFT TUBING (NBS P/N P0740-2430) BETWEEN THE HOSE BARB AND THE HARD TUBING.

F. Turn on the power switch.

G. Select fermentation mode by pressing key "2".

H. On master screen, set temp loop control mode to "Prime" for 1 minute.

I. Set agitation to desired speed and then set mode to PID.

J. Set temp to the desired working temperature set mode to PID.

K. Remove plugs and tape (if any) and shorting cap from the pH probe and connect the pH cable to the pH probe.

L. Remove the protecting cap from the D.O. probe and connect the D.O. cable to the D.O. probe.

M. When the vessel reaches desired working temperature, calibrate the D.O. probe.

N. Set pH and D.O. to the desired set points and set pH and D.O. mode to PID.

O. Set agitation to D.O. if applying D.O. cascade system *(see 3.11)*.

P. Set 2-Gas mode to D.O. or AgO2DO, if applying oxygen enrichment/D.O. cascade system *(see 3.11.2)*. Otherwise, set 2-Gas mode to manual.

Q. Adjust air flow rate to the desired value.

3.11 D.O. CASCADE SYSTEM

The system is designed to control D.O. by PID control of agitation speed and oxygen output. When D.O. actual value is above the D.O. setpoint, the agitation speed will automatically decrease until the D.O. setpoint is reached.

As the D.O. level drops below the setpoint, there are three ways to pick it up.

3.11.1 Agitation/D.O. Cascade System

To set up, proceed as follows:

a. Set agitation and D.O. loop to PID mode.
b. Set agitation to the minimum RPM you wish for D.O. control.
c. Change agitation mode to D.O..
d. Set agitation speed to maximum RPM you wish for D.O. control.
e. D.O. is now controlled by regulation of the agitation speed between the limits that have been set (minimum/maximum setpoints).

3.11.2 Oxygen Enrichment/D.O. Cascade System

To setup, proceed as follows:

a. Set D.O. loop to PID mode.
b. Set 2-Gas to D.O. mode.
c. Select proper PI value*
d. D.O. is now controlled by regulation of the percentage of oxygen output between 0 and 100.

* <u>NOTE:</u> *P.I. values have been set in the factory before shipping. Under normal conditions, it is not necessary to reset these values.*

3.11.3 Agitation/Oxygen/D.O. Cascade System

To setup, proceed as follows:

a. Set agitation and D.O. loop to PID mode.
b. Set agitation to the minimum RPM you wish for D.O. control.
c. Change agitation mode to D.O.
d. Set agitation speed to maximum RPM you wish for D.O. control.
e. Set 2-Gas to "AgO2DO" mode.
f. Select proper PI value *(see note of 3.11.2.C)*.
g. D.O. is now controlled by the regulation of agitation speed first. After the maximum set speed is reached, then D.O. is controlled by the regulation of the percentage of oxygen output.

3.12 SAMPLING PROCEDURE

To sample the culture proceed as follows: (Fig. 10)

1. Check to be sure that the sample bottle is loose not sealed against sampler gasket.

2. Close the valve on the sampler tube, if open.

3. Squeeze the bulb and then see that sample bottle is sealed against sampler gasket. Open valve and obtain desired volume of sample. Close valve.

4. Remove bottle from sampler. Place the cap from a new bottle on 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.

5. Repeat step 1 through 4 until desired number of samples are taken.

3.13 SHUT-DOWN PROCEDURES

To shut-down the system proceed as follows:

1. Shut-off gas flow.

2. Set the agitation, temperature to "OFF".

3. Turn off the power.

4. If the system is not to be used for several days, disconnect power plug. Remove and clean vessel, and associated components as outlined in previous steps.

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

3.14 OPERATION TIPS

A. 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.

NOTE: If you use a wrench in step 1, DO NOT TIGHTEN AGAIN in step 3. (One revolution maximum after finger tight.)

B. Exhaust Condenser/Exhaust Filters

The inner assembly of condenser can be removed for cleaning.
Pass warm water and detergent through the *top* of the condenser, (NOT through quick disconnects), twice.
Run clear water through once.
Blow out with air. Autoclave.
Clean exhaust condenser after each fermentation. This is most critical when operating as a chemostat for protracted fermentation times.

C. 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).

D. OTR (Oxygen Transfer Rate) Calculation

$$OTR = \frac{30{,}000\ m}{V\ T} \quad \text{m.moles/L/hr}$$

Where m = number of moles of Sodium Sulfite Na_2SO_3

Weight of 1 mole of Na_2SO_3 = 126.04 gm
V = vessel working volume L
T = time taken from DO curve at two points of 50% DO min.

Procedures:

Operate machine at 37C, 1000 RPM, air flow = (1/2 - 1 V) LPM.
Calibrate DO to 100 and allow it to stabilize.
Add Na2SO3 (with a small amount about 5gm of Cu SO4).
The DO reading drops very quickly. At DO = 50, start counting time.
When DO returns to 50, stop counting time.

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. Check that all O-rings in the headplate and impeller are intact. Replace those that are not.

4.4 VESSEL AND TUBING CLEANING

After each run clean the vessel, headplate and associated parts. The tubings and filters should be replaced.

4.5 PROBE MAINTENANCE AND STORAGE

D.O. PROBE

To clean D.O. probe, use soft facial tissue.

Check the D.O. probes teflon membrane. (Make sure there is no wrinkling or punctures.)

Check the vent tubes, make sure they are clean.

The probe should be stored standing upright with the shorting plug in place and the membrane isolated from the air environment. At no time should the probe be allowed to rest on the electrode's membrane.

As soon as the probe is filled with electrolyte, it begins aging and its output can be expected to decline gradually. Depending on the operating conditions, a filled probe should last through 15 to 30 sterilization cycles, and/or 6 months.

The probe should not be stored at a temperature greater than 60C.

pH PROBE

Check the level of the filling solution. It should be about 1cm below the filling orifice. To add solution, *see section 3.3*.

The probe should be stored standing upright. The electrode tip should be immersed in a solution of 3-Molar KCL or a Buffer Solution between pH 4 and pH 7. The two rubber "T" stoppers should be inserted. At no time should the electrode be allowed to rest on the tip.

NOTE: The rubber T-stoppers should be left in place during autoclaving, but removed during regular usage.

Certain types of Mycelia can grow on the liquid junction. These growths can be prevented from forming by the addition of a few drops of Formaldehyde (up to 1%) to the 3-Molar KCL solution.

4.6 THE AGITATOR BEARING HOUSING (Fig. 23)

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) Assembly	M1169-2000
Vessel (2.5L) Assembly	M1169-2001
Vessel (5L) Assembly	M1169-2003
Inlet Filter	P0200-0491 (37mm Disc)
Exhaust Filter	P0200-0490 (50mm Disc)
RTD Assembly	M1169-8002
pH Probe (1.25L, 2.5L)	P0720-5325
pH Probe Cable (1.25L, 2.5L)	P0720-2095
pH Probe (5L)	P0720-5021
pH Probe Cable	P0720-2095
pH Electrode K9 Cap	P0720-5317
pH Probe Electrolyte	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 (Phoenix)	P0720-2202
DO Probe (1.25L) (Ingold)	P0720-5560
DO Probe (2.5L) (Ingold)	P0720-5561
DO Probe (5L) (Ingold)	P0720-5561
DO Cable (Ingold)	P0720-2202
DO Service Kit, Ingold	P0720-5569
DO Electrode Cap, Ingold	P0720-5567
Teflon Washer for Ingold DO Probe	P0100-9780
O-Ring for Ingold DO Probe	P0280-9163
Seal Washer for Ingold DO Probe	M1016-0890
Antifoam Probe	F5-137
Hose Connect Plug	M1151-9518
Hose Connect Open	M1151-9519
Seal, Vessel Side Port	M1151-9419
Nut, Vessel Side Port	M1151-9420
Fuse, 10 Amp, 115V	EF-143
Fuse, 6 Amp, 220V	P0380-3140
Glass Vessel 1 1/4L	M1151-9904
Glass Vessel 2 1/2L	M1152-9903

Glass Vessel 5L	M1155-9904
Fuse, 10 Amp, Motor Drive	P0380-3160
Bearing Housing Cover	M1151-9444
Bearing Housing Cover O-Ring	P0280-6093
Motor Assy. (1 1/4, 2 1/2 & 5L)	M1169-0600
Flat Gasket, Vessel (1.25L)	M1151-9905
Flat Gasket, Vessel (2.5L)	M1152-9904
Flat Gasket, Vessel (5L)	M1155-9902
Ball Bearing	P0180-0170
Shaft Seal	P0280-0072
Silicone Grease (FDA Approved)	P0860-1050

O-RINGS

Used On	Qty/Unit	1 1/4L	1/2L	5L
Headplate	1	P0280-8002	P0280-8062	P0280-8132
Knob	3	P0280-5322	P0280-5322	P0280-5322
D.O. Port	1	R-135	R-135	R-135
pH Port	2	P0280-5914	P0280-5914	P0280-5914
Antifoam Port	1	P0280-5292	P0280-5292	P0280-5292
1/4" Plug	3	R-131	R-131	R-131
3/8" Plug	1	P0280-5333	P0280-5333	P0280-5333
1/2" Plug	1	R-274	R-274	R-274
1/4" Plug #2	1	P0280-5280	P0280-5280	P0280-5280
Quick Conn. Heater	2	P0280-5332	P0280-5332	P0280-5332
Quick Conn. Condenser	2	P0280-5302	P0280-5302	P0280-5302
Bearing Hsg.	1	P0280-5422	P0280-5422	P0280-5422
Bearing Hsg., Lower	1	P0280-5472	P0280-5472	P0280-5472

APPENDIX
CELL CULTURE ON BIOFLO 3000

When BioFlo 3000 is used for cell culture, the following items should be prepared.

A. Vessel Assembly (Basic)

NBS Part No.	Total Volume
M1176-0006	2.2L
M1176-0008	5L
M1176-0003	7.5L

B. One of the following items should be selected.

1. Fixed Bed Kit (Packed Bed Reactor)

NBS Part No.	Total Volume
M1176-1012	2.2L
M1176-1013	5L
M1176-1016	7.5L

2. Cell Lift Impeller Kit

NBS Part No.	Total Volume
M1176-1000	2.2L
M1176-1001	5L
M1176-1002	7.5L

3. Marine Blade Impeller Kit

NBS Part No.	Total Volume
M1176-1003	2.2L
M1176-1004	5L
M1176-1005	7.5L

4. Pitched Blade Impeller Kit

NBS Part No.	Total Volume
M1176-1006	2.2L
M1176-1007	5L
M1176-1008	7.5L

C. One of the following probe kits should be selected.

1. Probe kit for Cell Lift, Marine Blade and Pitched Blade Impeller Applications.

NBS Part No.	Total Volume
M1176-0115	2.2L
M1176-0135	5L
M1176-0150	7.5L

2. Probe Kit for Fixed Bed (Packed Bed Reactor)

NBS Part No.	Total Volume
M1176-0115	2.2L
M1176-0213	5L
M1176-0215	7.5L

D. One of the following Perfusion Kits could be selected.

1. Perfusion Kit for Cell Lift Application.

NBS Part No.	Voltage
M1176-0120 - Plus optional pump P0620-0485	110/120V
M1176-0120 - Plus optional pump P0620-0486	220/240V

2. Perfusion Kit for Fixed Bed Application.

NBS Part No.	Total Volume
M1176-1011	All sizes

E. Conversion Kit M1217-0076. This includes a CelliGen Plus manual, a motor, a motor stand, two jacket water lines and two condenser cooling water lines.

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