YSI *incorporated*

YSI 5300A

Biological Oxygen Monitor System

Operations Manual

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1. Introduction

The YSI 5300A Biological Oxygen Monitor is designed to measure oxygen uptake and evolution in biological systems. It utilizes Clark type polarographic oxygen probes immersed in magnetically stirred sample chambers, and produces oxygen uptake or evolution curves in 2 to 15 minutes. This two-channel instrument offers simultaneous display, recorder outputs, and RS-232 serial outputs for monitoring the results of each evaluation of samples.

Two different sensor systems are available. The "Standard System", for the measurement of samples of 3 to 8 ml, consists of a 5300A Monitor, a 5301B Standard Bath, two 5331A Oxygen Probes, and other supplies. The 5304 Micro Adapter Kit will modify the 5301B for use with 1 ml samples. The "Micro System," for the measurement of a fixed volume sample of 600 µL or for a continuous flowing sample, includes a 5300A Monitor, one or two 5356 Micro Oxygen Chambers, one or two 5357 Micro Oxygen Probes, and other supplies. The Micro Oxygen Chamber and the Micro Probe are manufactured by Instech Laboratories, Inc..

The YSI 5331A and YSI 5357 Oxygen Probes are a complete polarographic system consisting of a platinum cathode, silver anode and KCl solution held captive around electrodes by a Teflon membrane fastened with an o-ring. When a polarizing voltage is applied across the probe, all oxygen in the probe is consumed (reduced) at the cathode and current flows in direct stoichiometric relation to the rate of oxygen consumption (reduction). Oxygen then diffuses through the membrane at a rate proportional to the oxygen pressure outside the probe, since oxygen pressure in the probe is near zero. When steady state conditions are reached in about 30 seconds, current flows through the probe at a rate in proportion to the external oxygen pressure.

The YSI 5301B Bath Assembly has three sample chambers with built-in magnetic stirrers surrounded by a water bath. The YSI 5301B is able to measure sample size from 3 to 8 mL with the YSI 5331A probes. The YSI 5304 Micro Adapter Kit modifies the YSI 5301B for use with 1-mL samples and requires the YSI 5331 probes as well. The YSI 5301B Bath Assembly is connected with a constant temperature circulator (YSI 5310) and together they provide a temperature controlled environment where the oxygen uptake and evolution can be accurately monitored in a sample. The Lucite plunger included with the YSI5301 Bath Assembly provides an access slot for the introduction of samples, inhibitors and activators into the sample chamber.

2. Installing and Initial Setup

Except when operating at ambient temperatures, a constant temperature circulator is necessary to control the temperature of the sample chambers (YSI recommends a constant temperature circulator with a +/- 0.02 °C control for maximum accuracy). A 2 channel chart recorder, serial printer, or computer (equipped with an RS232 port or data acquisition system) is necessary for a hard copy of experimental results. Your YSI dealer can recommend and offer suitable circulators (YSI 5310) and chart recorders (YSI 5320).

2.1 Unpacking the System

When you unpack your new YSI 5300A Biological Oxygen Monitor for the first time, check the packing list to make sure you have received everything you should have. If there is anything missing or damaged, call the dealer from whom you purchased the system. If you do not know which of our authorized dealers sold the system to you, call YSI Customer Service at 800-659-8895 or 937-767-7241, or email us at lifesciences@ysi.com and we'll be happy to help you.

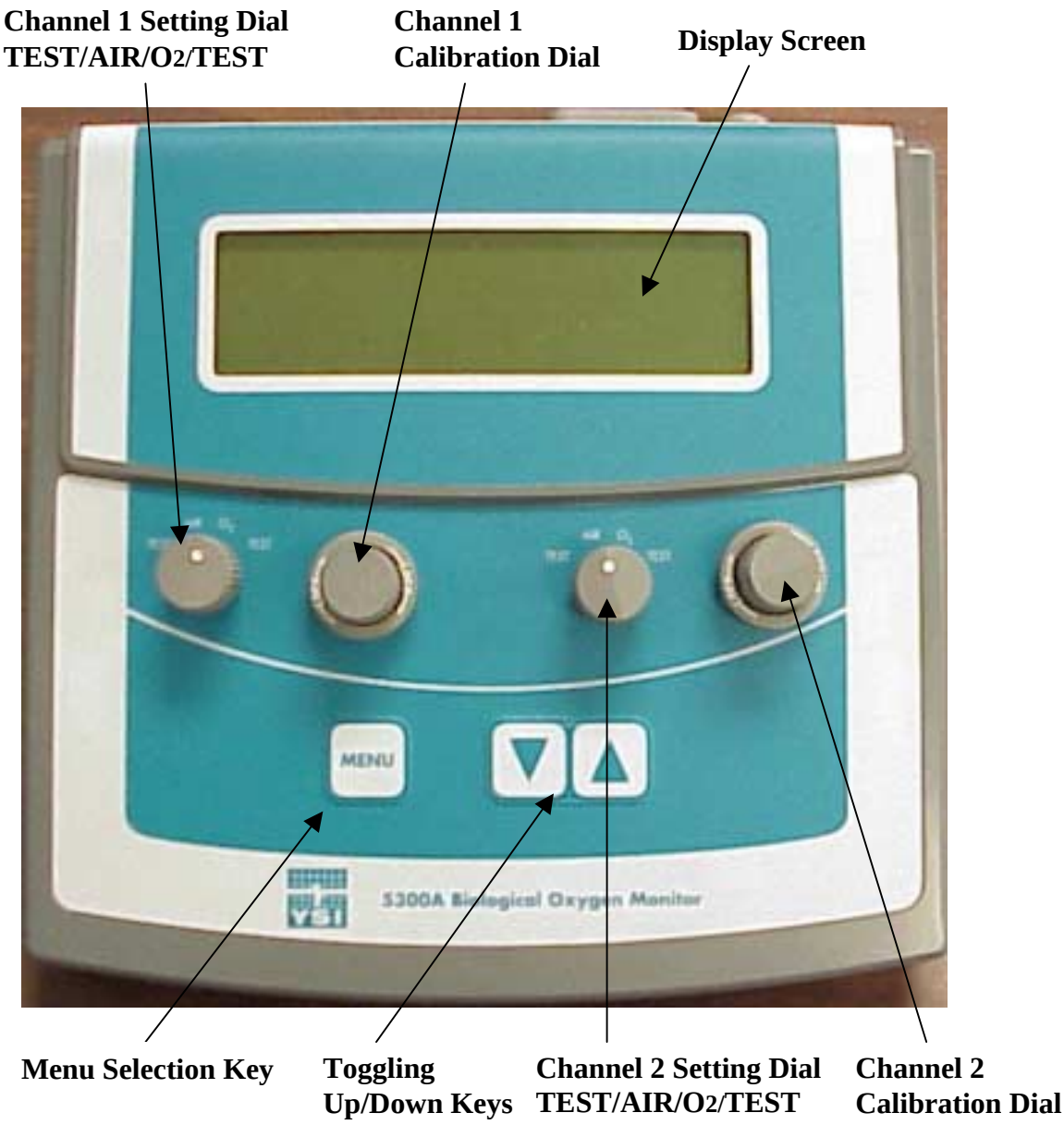
2.2 Completing the Warranty Card

Please complete the Warranty Card and return it to YSI. This will record your purchase of this instrument in our computer system. Once your purchase is recorded, you will receive prompt, efficient service in the event any part of your YSI 5300A should ever need repair.

2.3 Viewing the Front and Back Panels

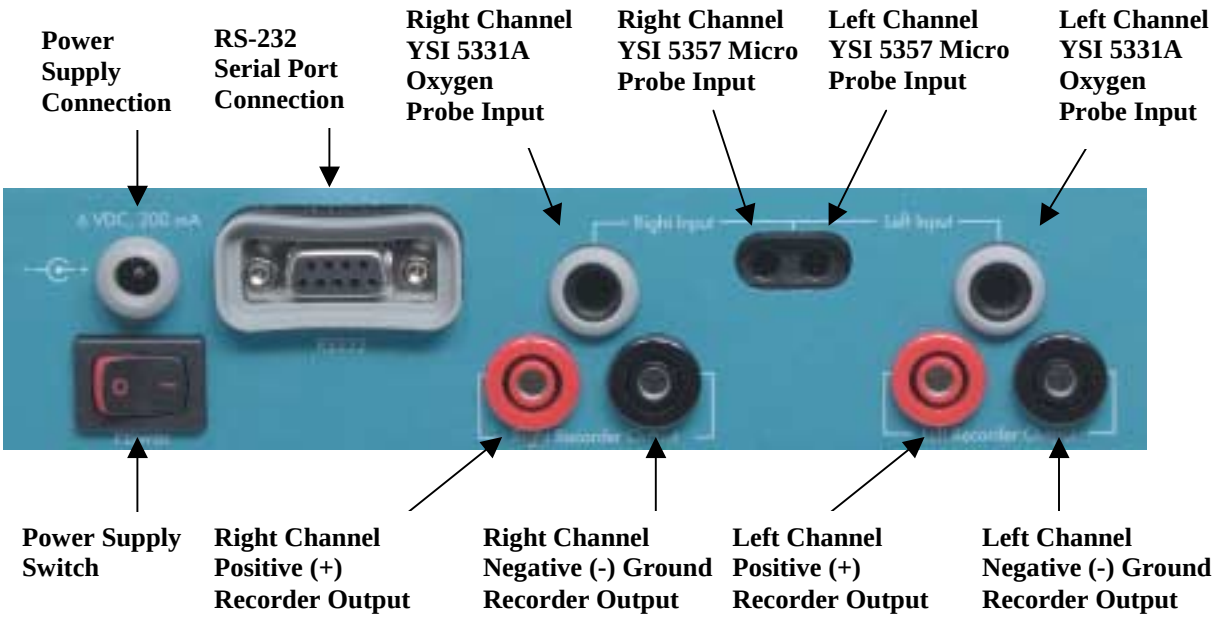
2.3.1 Front Panel

The front panel of the YSI 5300A contains the display screen, TEST/AIR/ O_2/ TEST setting dials, calibration adjustment dial, Menu, and toggling keys as shown below.



2.3.2 Rear Panel

The rear panel contains the connections for the power supply, power supply switch, YSI 5331A probes, YSI 5357 Micro Oxygen Probes, RS232 serial port, and recorder outputs.



Power Supply Connection

The power supply connection requires a 6 VDC power supply with a least 200mA current.

Oxygen Probe Connections

The YSI 5331A has a 0.25” Stereo Phone Plug. The YSI 5357/ 5358 Micro Probe System has a 0.173" miniature telephone plug.

Note: The YSI 5331 Oxygen Probes are not compatible with the YSI 5300A Monitoring System. Only the YSI 5331A oxygen probe and the YSI 5357/ 5358 micro oxygen probe system are compatible with the YSI 5300A Monitor.

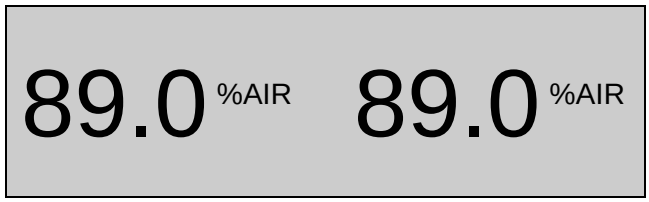
RS232 Connection

The RS232 connection is a standard DB9 connector. Use a straight serial cable (not a null modem) to connect the YSI 5300A to a computer serial port or a serial printer. See Accessories and Replacement Parts, Sec. 11 for the YSI item number.

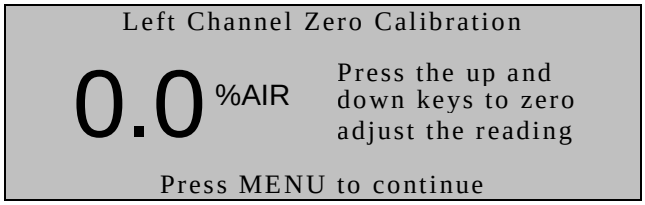
2.4 Setting Up the Software

The YSI 5300 has two primary modes: monitoring/calibration mode and the zero calibration/ RS-232 setup mode.

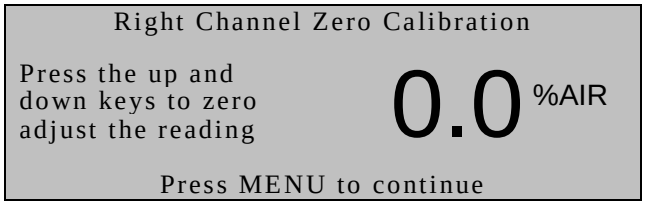
1. The monitoring/ calibration mode is the default mode and is shown below. In this mode the analyzer is constantly measuring oxygen. The analyzer can also be calibrated in this mode by unlocking the calibration dial and setting the analyzer to the appropriate calibration value. (See Sec. 6 for calibration instructions.)



2. To adjust the contrast on the display screen, press the [▲] up soft-key or the [▼] down soft-key in the sampling/ calibration mode.
3. To enter the zero calibration/ RS-232 setup mode press the [MENU] soft-key.
4. The Left Channel Zero Calibration screen will first be displayed. A zero percent sample will be used to adjust at the zero point calibration at this menu. Adjust the zero point to reflect **0.0%** with this zero percent oxygen sample. The left channel zero calibration will display actual oxygen readings. (See Sec 6.1 for zero calibration instructions.)

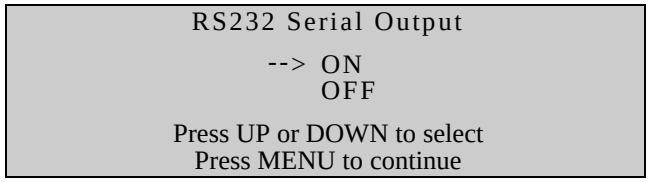


5. Press the [MENU] soft-key again and following screen will be displayed.

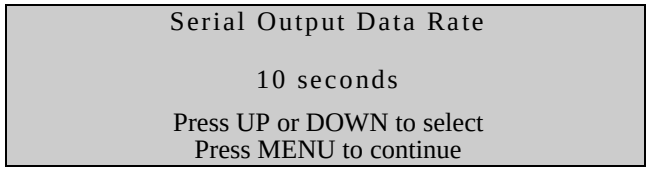


A zero percent sample will be used to adjust at the zero point calibration at this menu. Adjust the zero point to reflect **0.0 %** with this zero percent oxygen sample. The left channel zero calibration will display actual oxygen readings. (See Sec 6.1 for zero calibration instructions.)

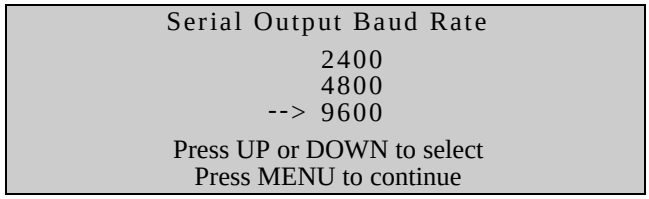
6. Press the [MENU] soft-key again and following screen will be displayed



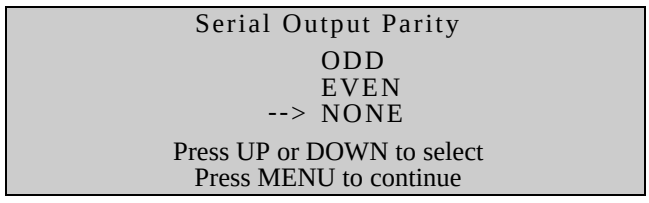
7. Select On or Off to activate or disable the RS232 Serial Output option for serial port data acquisition and collection.
8. Press the [MENU] soft-key again and following screen will be displayed.



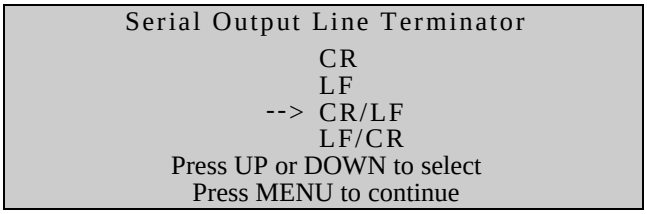
9. Select the serial output data rate via the RS-232 serial output for your data collection needs. (Output data rate ranges from 1 to 60 seconds). Compare your computer or serial printer's requirements with YSI 5300A setting.
10. Press the [MENU] soft-key again and following screen will be displayed.



11. Select the appropriate serial output baud rate for your data acquisition requirements. Compare your computer or serial printer's requirements with YSI 5300A setting.
12. Press the [MENU] soft-key again and following screen will be displayed.

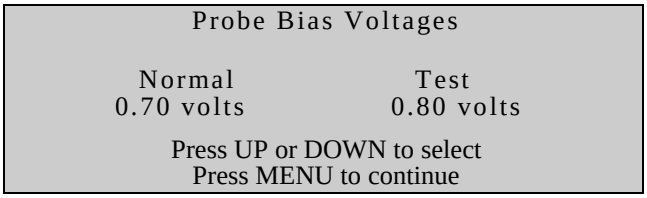


13. Select the appropriate serial output parity per your data acquisition requirements. Compare your computer or serial printer's requirements with YSI 5300A setting.
14. Press the [MENU] soft-key again and following screen will be displayed.



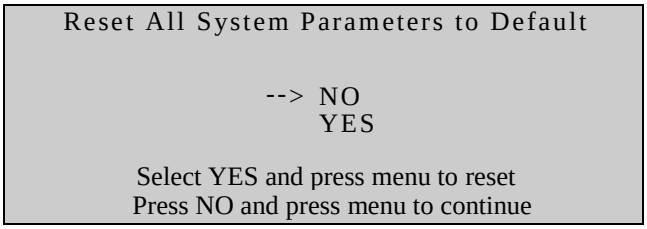
15. Select the appropriate serial output line terminator per your data acquisition requirements. Compare your computer or serial printer's requirements with YSI 5300A setting.

16. Press the [MENU] soft-key again and following screen will be displayed.



17. The probe bias voltages screen shows the difference in the voltages between the normal mode and the test mode for both probes.

18. Press the [MENU] soft-key again and following screen will be displayed.



The Reset All System Parameters to Default screen allows the user to reset the analyzer to the factory default settings.

NOTE: Inactivity in the zero calibration/ RS-232 setup mode will cause the analyzer to default to the calibration/ sampling mode after ~15 seconds.

2.5 Setting Up the Standard System

Description

The YSI 5300A Biological Oxygen Monitor standard system consists of the following separate YSI products:

- 1 YSI 5300A Biological Oxygen Monitor
- 1 YSI 5301B Standard Bath Assembly (includes 1 5350 Membrane Mounting Kit, 1 5225 Sample Chamber kit, tubing, etc.)

- 2 YSI 5331 Oxygen Probes, each with a 5775 Standard Membrane/KCl Kit

The assembled 5301B Standard Bath Assembly has three sample chambers with magnetic stirrers, surrounded by a water chamber. A constant temperature circulator is connected to the bath. Two 5331 Oxygen Probes are prepared for operation using the 5350 Membrane Mounting Kit to install the parts found in the 5775 Standard Membrane/KCl Kit. The probes are held in plungers that fit closely into the sample chambers. The probes plug into the back of the instrument.

Assembling the Standard System:

1. Insert three glass sample chambers from the box marked 5215 (included with the 5301B) into the holes in the bath cover.
2. Wet three of the large rubber o-rings with distilled or de-ionized water.
3. Place one rubber o-ring over each of the sample chambers near its top (the o-rings should seat against the top cover).
4. Slide each sample chamber into the bath until it rests on its stop.
5. Place a white nylon hold down ring over each chamber (above the o-ring), push it down and twist clockwise to secure it under the stainless steel shoulder screws (this will seat the o-rings against the top cover for a water-tight seal).

Caution: Over-tightening the hold down ring may break the sample chamber.

6. Make sure that the power switches on the 5301B Bath Assembly and 5300A Monitor are in the off (0) position, then plug the power cords into a properly grounded power receptacle.
7. If the constant temperature circulator is used for your application, connect the 5301B Bath Assembly to the circulator using the YSI 5315 Plumbing Kit. (Wetting the tubing ends with water or alcohol will ease the tubing installation). Though it is not critical which 5301B tubing fitting is used as the inlet, YSI recommends using the bottom fitting.
8. Fill the circulator with distilled water and set the controls to the desired temperature. Operate the circulator according to its instruction manual. Note: YSI recommends using only distilled water in the bath. If distilled water is not used, minor rust stains may appear in the 5301B Bath chamber. They may be removed using a mildly abrasive pad.
9. If the chart recorder is used for your application, connect a recorder to the recorder output port on the back of the 5300A Monitor. The 5300A Monitor has a self-zeroing feature with no probes plugged in; the recorder zero reference can be set with this zero output.

NOTE: The red port on the 5300A Monitor back panel is positive and the black port is negative.

10. If the RS-232 serial cable is used for your application, connect the RS-232 serial cable to both the YSI 5300A RS-232 port and the acquisition serial data port.
11. Prepare the 5331 Oxygen Probes for operation as described in section 4.1.

12. Plug the 5331 Oxygen Probes into the jack marked Input on the rear of the 5300A Monitor.

NOTE: When a 5331 Oxygen Probe and a Micro Oxygen Probe are both plugged into the same channel's input jacks, the display and output values reflect the YSI 5357 Micro Oxygen Probe response.

13. Numbered marking tape is supplied with the 5301B Bath Assembly to identify each probe if desired.

2.6 Setting Up the Micro System

Description

The YSI 5300 Biological Oxygen Monitor Micro System consists of the following components:

- 1 YSI 5300A Biological Oxygen Monitor
- 1 or 2 YSI 5356 Micro Oxygen Chambers
- 1 or 2 YSI 5357 Micro Oxygen Probes
- 1 or 2 YSI 5358 Cable Assemblies
- 1 YSI 5775 Standard Membrane Kit

You can use the 5356 Micro Oxygen Chamber for fixed-volume single samples or for continuous, flow-through monitoring. You should use a constant temperature circulator to control the block temperature. A remote magnetic stirrer controller keeps the sample chamber stirred when you measure fixed-volume samples.

Assembling the Micro System as follows:

1. Assemble the YSI 5356 Micro Oxygen Chamber as described in the Instech Laboratories Model 600A Instruction Manual provided.
2. Connect the Oxygen Chamber to a constant temperature circulator (tubing not supplied).
3. Fill the circulator with distilled or de-ionized water and set it to the desired temperature. Operate the circulator according to its instruction manual.
4. If the chart recorder is used for your application, connect a recorder to the recorder ports on the rear of the monitor. **NOTE: The red port on the 5300A Monitor back panel is positive and the black port is negative.**
5. If the RS-232 serial cable is used for your application, connect the RS-232 serial cable to both the YSI 5300A RS-232 port and the acquisition serial data port.
6. Prepare the Micro Probe for operation as described in the Instech Laboratories Model 125/05 manual.
7. Thread the probe into the 5358 Cable Assembly.
8. With the monitor off, plug the Cable Assembly into the appropriate jack on the back of the YSI 5300A.

NOTE: When a Standard Oxygen Probe and a Micro Oxygen Probe are both plugged into the same channel's input jacks, the display and recorder values reflect the Micro Probe's response.

9. Immerse the probe in distilled water up to 1" above the rubber o-ring until you are ready to insert it into the sample chamber.

3. Preparing your Bath Assembly

3.1 Choosing your Bath Assembly

Three bath assemblies are available with for use with the YSI 5300A Biological Oxygen Monitor. Each is unique and should be considered for the user's particular need.

3.1.1 YSI 5301B Bath Assembly

The YSI 5301B Bath Assembly (See Figure 3.1) provides relatively airtight sample chambers that are stirred magnetically and controlled in temperature when connected to a constant temperature circulator. The YSI 5301B Bath Assembly is designed to test sample sizes ranging from 3 to 8 milliliters in volume.

Figure 3.1 YSI 5301B Sample Chamber

The three sample chambers are held in place with locking nylon hold down rings and sealed in the bath with two rubber o-rings (See Figure 3.2).

The nylon hold down ring also serves as a tensioning device to hold the Lucite plunger in position while allowing a very fine adjustment regardless of sample size.

The white Lucite plunger has a slanting face and an access slot along the side for removal of gas bubbles from the sample. The removal of bubbles is of the greatest importance because of the 20 to 1 increase in the amount of oxygen per unit volume in the gas phase over that in saturated solution.

The solution level in the access slot should be between the overflow groove and the lower end of the Lucite plunger. The small amount of unstirred solution in the access slot and around the Lucite plunger then serves as a barrier to oxygen diffusion into or out of the stirred solution in the sample chamber. The error caused by diffusion thorough this path is no more than ½ % per 15 minutes for sample chamber oxygen pressures between 50 and 100% of the outside oxygen pressure. Errors due to oxygen leakage can be minimized by operating with the small possible oxygen atmosphere, and by limiting the running time of the sample.

If saturating gases other than air are being used, leakage errors may be minimized by flushing the overflow groove with the saturating gas.

The access slot can also be used for the introduction of samples, inhibitors and activators into the sample chamber with the aid of a hypodermic syringe.

Stirring is necessary to prevent oxygen depletion by the probe of the solution directly adjacent to the cathode. The stirring constantly renews the solution in front of the cathode and results in a steady reading.

In situations where more or less sample agitation is desired other stirrers can be used, the probe operation should be checked with stirrers interchanged to see that readings are not changed.

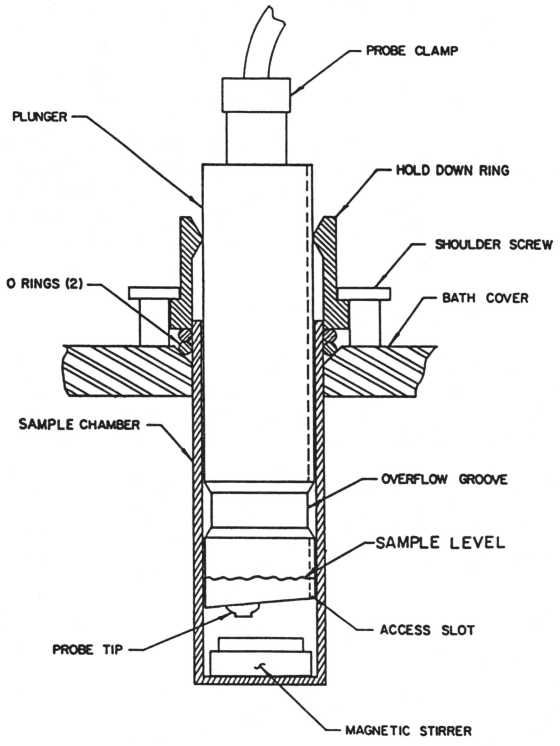


Figure 3.2 YSI 5301B Sample Chamber Assembly

3.1.2 YSI 5304 Micro Adapter Kit

This kit gives the YSI 5301B Bath Assembly greater sensitivity for measuring small sample volumes (1 milliliter) in experiments where limited sample is available, or where the uptake rate is low in relation to the total oxygen in the system. Two specially modified Lucite plungers and magnetic stirrers are included to use with the YSI 5331A Oxygen Probes.

3.1.3 YSI 5356 Micro Oxygen Chamber

The YSI 5356 Micro Oxygen Chamber is designed for a 600-microliter sample and can be converted to measure a continuous flowing sample. Please refer to your Instech manual for additional details.

3.2 Setting up your Bath Assembly

The following discussion is based on use of the YSI 5301B Bath Assembly (Figure 3.1 and 3.2), but it applies generally to any of the bath assemblies offered by YSI.

3.2.1 Warm-up and Equilibrium

When first turned on, most circulators are slow to reach the desired temperature. Allow sufficient time for the bath assembly to reach temperature before starting measurements.

To minimize the equilibration time, the substrate may be brought to temperature before introducing the sample. The probe and plunger can be kept at temperature in one of the spare sample chambers. In this way, meaningful data can be gathered within a minute after sample introduction.

3.2.2 Saturation of Solution

As an air-saturated solution is heated to the operating temperature of the system, the solution is automatically kept at saturation by the constant evolution of gas. In such a situation, it is only necessary to wait 2 or 3 minutes for temperature equilibration.

The appearance of gas bubbles in the sample chamber after the insertion of the Lucite plunger is an indication that the solution has not completely equilibrated..

Saturation of the solution with oxygen mixtures other air in the YSI 5301B can be achieved (See Appendix B: Tables). First, the Lucite plunger is withdrawn part way up the sample tube. Then the saturating gas can be bubbled through the solution and into the space between the Lucite plunger and the solution (a 0.05” diameter plastic tube will fit down the Lucite plunger through the access slot). With the stirrer turned on, the solution will saturate in a few minutes. After the solution has been saturated, the plastic tubing can be withdrawn back into the overflow groove. Flushing the overflow groove with the saturating gas will prevent the introduction of air into the sample chamber during the insertion of the Lucite plunger into the solution.

To prevent excessive evaporation, the saturating gas can be bubbled through water before being introduced into the chamber.

3.2.3 Position of the Lucite Plunger

The Lucite plunger should be inserted into the sample chamber so as to expel all gas through the access slot. Bubbles that tend to stick to the Lucite plunger surface can be removed by raising the plunger to let enough air (or saturating gas) into the sample chamber to gather all the bubbles, and then reinserting the plunger into the solution to expel the gas.

Solution level in the access slot should be between the lower end of the Lucite plunger and the overflow groove, See Figure 3.2. **It is important that no solution be in the overflow groove during a measurement.** Solution in the overflow groove reduces the amount material being observed in the sample chamber and increases the liquid surface in contact with the air. A larger liquid surface increases air diffusion into the solution.

With materials that foam when stirred, twisting the Lucite plunger slightly helps gather small bubbles at the access slot.

3.2.4 Small Samples, Inhibitors, and Activators

Additions of a few tenths of a milliliter can be made to the sample chamber without removing the Lucite plunger by using a hypodermic needle inserted down the access slot.

The needle is inserted well into the sample chamber and the injection is made. After the introduction is completed, the solution level in the sample chamber is lowered so that it is once again between the overflow groove and the lower end of the Lucite plunger.

When the injection is made, the sample solution in both the needle and the syringe should be free of air bubbles.

A short length of 0.05” diameter plastic tubing can serve as a flexible extension to the needle. Teflon tubing is recommended because it is chemically inert and remains stiff at 37°C.

4. Preparing your Oxygen Probe

When preparing either the 5331 Standard Oxygen Probe or the 5357 Micro Oxygen Probe, you should take certain precautions to ensure repeatable and reliable performance. ***Do not touch the membrane except at its edges where it will not come in contact with the probe's electrodes.*** The oils on your skin can cause contamination. With reasonably careful handling, a membrane can be expected to last a week or more. Because the membrane is water permeable, the probe tip should be kept immersed in distilled water after it has been prepared and until it is ready to be used. This will eliminate the need for frequent recharging of the probe with electrolyte.

When storing the probe for extended periods, rinse and dry it well, then install a new membrane (no KCl) to protect the electrodes.

The routine daily probe test is described in section 6.2.

4.1 Standard Oxygen Probe

Each YSI 5331A Oxygen Probe comes with a YSI 5775 Standard Membrane/KCl Kit. The YSI 5331A Oxygen Probe is designed to be used with the 5301B Bath Assembly.

The YSI 5350 Membrane Mounting Kit (Figure 4.1) is used to install membranes on the 5331A Oxygen Probe.

Figure 4.1

The 5775 Standard Membrane/KCl Kit contains two packs of membranes. Each pack contains 15 of 0.001″ FEP Teflon membranes and a squeeze bottle containing dry KCl for preparing the electrolyte.

The 5776 High Sensitivity Membrane/KCl kit contains two packs of membranes. Each pack contains 15 of 0.0005" FEP Teflon membranes. These membranes allow twice the sensitivity of the 5775 Standard Membrane Kit. This kit also includes the squeeze bottle containing dry KCl for preparing the electrolyte.

Preparing the probe/ Membrane Installation :

The following instructions describe the probe's preparation using the YSI 5350 Membrane Mounting Kit (Figure 4.1) supplied with the bath.

1. Fill the squeeze bottle (marked O_2 Probe Solution) with distilled water and shake until the KCl crystals are completely dissolved.
2. Remove the rubber o-ring and membrane from the probe.
3. Place a new membrane over the unflared end of the brass membrane holder.
4. Slip the large rubber o-ring from the Membrane Mounting Kit down over the membrane and membrane holder to form a "drum" (the membrane should be taut with no wrinkles).
5. Place the o-ring from the probe over the small end of the o-ring applicator and slide it to the large end.
6. Seat the probe (cable end down) firmly into the slot in the front face of the probe holder. The probe should rest on the bottom of the slot.
7. Wet the whole tip of the probe, including the o-ring grove, with electrolyte solution (there should be no bubbles in the electrolyte on the probe).
8. Position the membrane holder (membrane up) directly over the probe. Pass the membrane holder down over the probe until it rests on the probe holder. The membrane should now be stretched over the end of the probe and be wrinkle free.
9. Keeping the membrane tight, place the large end of the Teflon o-ring applicator down against the end of the probe and push the rubber o-ring down off the applicator and onto the probe until it seats in the o-ring grove.
10. Remove the large rubber o-ring from the membrane holder and slip the holder off the probe by raising it straight up. Be careful not to disturb the membrane.
11. Inspect the probe carefully. The membrane should be free of wrinkles and holes. No air bubbles should be present under the membrane (a loupe or microscope will aid this inspection).
12. Cut off the excess membrane material close to the rubber o-ring with scissors.
13. Rinse the excess KCl solution from the outside of the probe with distilled water.
14. Wet the rubber o-ring on the probe with distilled water and insert the probe into the plunger supplied with the bath assembly.
15. By hand, screw the nylon probe clamp into the plunger until it is snug (Do Not Overtighten). The rubber o-ring can be observed through the Lucite plunger. A black ring

will appear and widen as the clamp is tightened. Inspect this black ring for continuity (a true seal is required). If the membrane has not been properly trimmed, it could interfere with the seal.

4.2 Micro Oxygen Probe

Please refer to the Instech Laboratories Model 125/05 manual for instructions for preparing the YSI 5357 Micro Probe.

5. Operating your Oxygen Probe

5.1 Principles of Operation

A Clark type oxygen probe is used in this complete polarographic system. A thin membrane stretched over the end of the probe isolates the electrodes from the environment. The membrane is permeable to gases and allows them to come in contact with the probe face. When a suitable voltage is applied across the electrodes, oxygen will react at the cathode causing a current to flow. The current is proportional to the amount of oxygen that permeates the membrane. The probe actually measures oxygen pressure. Since oxygen is rapidly consumed at the cathode, it can be assumed that the oxygen pressure inside the membrane is zero. Thus, the force causing the oxygen to diffuse through of oxygen outside the membrane. If the oxygen pressure increases, more oxygen diffuses through the membrane and more current flows through the electrodes. A lower pressure results in less current. Diffusion thorough the membrane is directly proportional to pressure. The oxygen pressure/probe current relationship is stoichiometric. That is, the relationship between external oxygen pressure and probe current is linear.

The YSI 5331A Standard Oxygen Probe contains a .025" diameter platinum cathode and a silver anode encased in an epoxy block. The 5357 Micro Oxygen Probe contains a epoxy body covered with a silver anode sleeve. These configurations facilitate cleaning, minimize the volume of filling solution required, and insure membrane tension. A Teflon membrane is secured with a rubber o-ring.

5.2 Electrical Characteristics

The current output of the probe is dependent upon the cathode area and the permeability of the membrane directly above the cathode. Individual probe variations can be attributed to differences in membrane characteristics; both film irregularities and variations of installation methods contribute to such differences. Variations of +/- 10% may be experienced with membranes from the same package. Wider variations should be suspect. Look for gross membrane imperfections, membrane rupture or damage during installation.

5.3 Voltage Plateau

When the system is operating correctly, the current output of the probe is nearly flat between 0.6 to 0.8 volts input. A long, flat "plateau" region permits current to be relatively independent of applied voltage, and results in linearity of output signal for a wide range of oxygen pressures.

The probes are operated with a polarizing voltage of 0.8 VDC. The plateau specification is that the output signal shall change less than 3% when polarizing voltage is lowered to 0.7 VDC. In terms of system performance, this translates to a departure from linearity of less than 0.2%, at worst case conditions.

The TEST function of the YSI 5300A provides a means for checking the plateau. (See Sec. 6.2 Daily Probe Test for additional instructions.)

The plateau test should show a change of less than 3% for new and freshly cleaned probes. Determine this by comparing the displayed values at AIR (0.8 volts) and TEST (0.7 volts). Probes showing slopes of 5 to 8% (older probes or those needing cleaning) may prove entirely serviceable. Check the probe recovery time when subjected to a step change in the polarizing

voltage (make the observation when changing from the AIR to the TEST position). The signal should be within 3% of the AIR value 2 ½ minutes after switching.

5.4 Noise

Noise can be attributed to many parts of the system, to poor grounding, to pick-up from high voltage machines, etc., but two kinds of noise can originate from the probe.

a) The occasional burst or spike occurring frequently but randomly. Check a second probe; if both probes behave in the same way, the trouble is probably elsewhere.

b) Continual noise of several percent of full scale which may increase in magnitude with time. The membrane may be in perfect condition. The silver anode may not be not be making a good reference contact with the solution. Cleaning with ammonia is recommended. (See Probe Care and Maintenance Sec. 5.9)

If the noise originates in the probe, a damaged membrane may be the cause (folds or creases in the membrane are always suspect). Examination of the probe under a low power microscope or a jeweler loupe can be useful. Check for holes, creases, KCl growths, or drying out under the membrane.

5.5 Temperature

The current from the probe is highly dependent on the temperature, and in particular, the temperature of the membrane adjacent to the cathode as well as the sample chamber temperature. The permeability of the membrane is temperature sensitive. The FEP Teflon membrane has a temperature coefficient of permeability of 2%/°C. Accurate temperature control is therefore required, and temperature equilibrium time must be considered when making changes in the setup. An additional 2%/°C change occurs in a sealed sample chamber because of the change in oxygen solubility. So, it can be seen that a 1°C change in temperature can cause a change of as much as 4% from the 100.0% calibration value.

When the nature of the experimental samples necessitates measurement of temperatures from 5°C to 15°C, it is recommended that the Model 5301B Bath Assembly be used. At these lower temperatures it will be necessary to use a more sensitive membrane due to the lower oxygen content in your sample. It is recommended that the YSI 5776 KCl and High Sensitivity Membrane Kit be used. The membranes that come in this kit are 0.0005” thick and allows twice the sensitivity of the 5775 Standard Membrane Kit.

5.6 Oxygen Consumption by the Probe

The polarographic probe consumes oxygen.

$$O_2 + 2H_2O + 4e = 4\ OH^-$$

The rate of oxygen consumption by the probe is in direct proportion to the current produced by the electrodes. The most practical way to minimize the errors due to oxygen consumption by the probe is to use a small area cathode.

A probe current of 1 microamp is equivalent to 8.3 X 10-11 grams of oxygen per second consumed by the probe.

The YSI 5331 Standard Oxygen Probe has a probe current of about 0.33 microamps in air at 37°C. For a biological system containing 19 micrograms of oxygen (3 milliliters at 37°C), about 0.13% error would accrue per 15 minutes of operation. The YSI 5357 Micro Oxygen Probe has a probe current of about 0.018 microamps in air at 37°C. For biological systems containing 3.8 micrograms of oxygen (0.6 milliliters at 37°C) about 0.04% error would accrue per 15 minutes of operation.

For most work, the errors described above can be neglected, but when using very small samples or conducting experiments for extended periods, significant offsets may accumulate.

5.7 Electrolyte Saturation and Bubbles in Electrolyte

The oxygen probe must be considered a part of the total system under study. For example, the ½ saturated KCl electrolyte which fills or covers the probe has a definite volume and contains dissolved gases including oxygen (only the electrolyte covering the platinum cathode is free of oxygen). The amount of oxygen "stored" in the electrolyte depends on the volume of the electrolyte and its immediate history.

When the oxygen level of the sample is decreased during a run, oxygen is induced to leave the electrolyte, pass through the membrane and enter the sample, thus introducing possibility significant errors.

The YSI 5331 Standard Oxygen Probe has an electrolyte volume of 2 to 3 microliters while the YSI 5357 Micro Oxygen Probe has less than 1 microliter. Errors caused by oxygen in the electrolyte in either probe, therefore, may be disregarded except for very small samples.

More serious is the presence of gas bubbles under the membrane. Volume for volume, an air bubble contains 20 times more oxygen than air-saturated water. Thus, if a large gas bubble is present, significant error can occur with normal size samples. Furthermore, since equilibrium must be attained by diffusion thorough the membrane, the system may be sluggish and exhibit slow drifts.

5.8 Drift of Calibration

A probe in good condition operating in a well-controlled environment will typically exhibit drifts of less than 5% per hour. A drift rate of only 1 % per hour is not uncommon, but external factors influence the test and some of them are difficult to identify or control.

Factors that may influence the drift rate of the system: changes in air composition in the laboratory (barometric pressure changes, sudden changes in humidity), mold or bacteria breeding in a solution that has been left in the chamber, or organic material picked up by the probe (as from hands, or from contract with the bench).

5.9 Probe Care and Maintenance

When properly used, the YSI 5331A Oxygen Probe requires very little maintenance. It may occasionally be necessary to clean the silver anode to remove contamination. To clean the anode, immerse the probe in a 1:1 solution of ammonia and distilled water for 10 to 60 seconds, then wipe it dry with a cotton tipped swab. Only clean the probe when necessary.

The platinum cathode requires servicing after long periods of use. The plateau within which the cathode is mounted must be kept flat and smooth. To re-establish this surface, the probe end should be rubbed in a circular motion on a wet frosted microscope slide. Wet the slide with distilled water, use special care to keep the probe body perpendicular to the slide and rotate the probe in only one direction, either clockwise or counterclockwise.

Store the probe for short periods submersed in distilled water. For extended periods, clean well and cover with a membrane (no KCl).

6. Operating your Oxygen System

For practical purposes, calibration of 100% saturation in air saturated water or in an experimental medium such as Ringers solution at known temperature and pressure and of known composition, will provide reliable, accurate and repeatable results. The oxygen depletion assay described in section 6.3 is based on a calibration in air-saturated water at the same pressure and temperature as the sample. Where comparative results are sought, the procedures described above will provide reliable, accurate and repeatable results. However, if absolute values under specific conditions are needed, numerous factors affecting measurement must be carefully controlled.

The ability of a liquid to hold oxygen (or any gas) varies according to three important parameters: its constitution, its temperature and the ambient pressure. Oxygen solubility decreases as the salt content or temperature of a solution increases. The tables in Appendix D will provide data to help calculate oxygen solubility.

The probe membrane itself has a temperature coefficient of 2%/°C, while temperature changes in a sample chamber sealed by an inserted probe can cause another 2%/°C change in oxygen solubility. Barometric variation between the normal daily extremes (25 mm in a 760 mm scale) can cause 3% of change. (A 343 meter elevation above sea level will cause as much as a 4% decrease in oxygen solubility. See Appendix B.)

It is important for the experimenter to be aware that "100% oxygen saturation" can mean very different things not only for different solutions, but for the same solution under different conditions.

6.1 Calibrating

BEFORE YOU CALIBRATE you must setup the monitoring system, as discussed in the *Installation and Initial Setup* section of this manual, and prepare the oxygen probe as discussed in the probe operations section.

Dissolved oxygen calibration must be done in an environment with a known oxygen content. Two such environments will be discussed here: calibration in air saturated water (AIR dial setting) and calibration in oxygen saturated water (O_2 dial setting). Choose one which best fits your application and set each channel dial to the appropriate setting.

This section is intended for use as a general guide for use of the YSI 5300A Biological Oxygen Monitor. System operation is as follows:

1. Turn on the circulator and allow sufficient time for it to come to the desired temperature per your circulator manual instructions.
2. Turn on the 5300A Monitor and the 5301B Bath Assembly stir motor.
3. Into one of the sample chambers of the bath, place 3 mL of air saturated (or oxygen saturated) distilled water and the magnetic stirrer supplied with the bath.
4. Allow 3 minutes for temperature equilibration then turn off the bath stir motor.
5. Insert the prepared Channel 1 5331 Probe into the sample chamber. Remove all of the air from the sample chamber through the access slot in the Lucite plunger (a slight twisting of

the plunger helps gather the bubbles at the access slot). The solution level in the access slot should be between the lower end of the Lucite plunger and its overflow groove.

6. Rotate the function switch on the 5300A Monitor to AIR, then set the display to read 100.0% for your air saturated distilled water solution (O_2 dial setting, 21.0% oxygen saturated distilled water solution) using the Channel 1 CAL control knob. Turn the CAL control locking knob clockwise to lock the knob.

7. Set the recorder (if applicable) to full scale. Note that the 5300A Monitor's recorder output for 100% is equal to 1.000 volt full scale.

8. Observe system stability as indicated by the recorder trace. The trace should be noise free and no more drift than ½% in 15 minutes.

9. Repeat above steps for channel 2 calibration.

NOTE: Any result over 199.99% will display OL (over limit) on the display screen.

Zero Oxygen Calibration

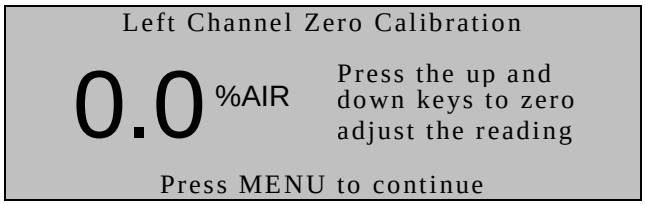
In some small volume applications, the accuracy of the calibration can be improved by performing a zero calibration as all oxygen probes have a small background current, even in the absence of oxygen (See Sec. 5.6 for additional probe information). For highest accuracy measurements, a zero calibration should be performed to compensate for the specific background current of the probe in use.

To calibrate to a true zero, place the probe in a zero oxygen environment and adjust the calibration value to zero.

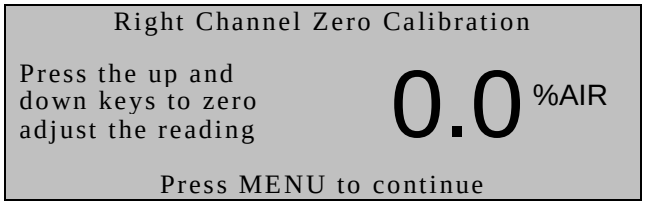
A standard method for creating such an zero oxygen environment is to dissolve excess sodium sulfite (Na_2SO_3) and a trace of cobalt chloride ($CoCl_2$) in water. Preferably, the water should come from the sample to be measured. (See Standard Methods for the Examination of Water & Wastewater, method 4500-O G, 19th edition). The addition of a very small amount of sodium dithionite or sodium borohydride will also remove all oxygen from the sample. Alternatively, you may place the probe in a sample that has been bubbled with 100% nitrogen gas. After using these agent the chamber should be cleaned immediately and thoroughly. It may be necessary to change the electrode membrane before some studies are conducted.

1. Place this zero oxygen sample in the temperature controlled YSI 5301B Bath Assembly sample chamber and allow the probe to equilibrate.

2. Press the [MENU] soft-key and the following screen will be displayed.



1. After the readings are stable, press the [▲] up soft-key or the [▼] down soft-key to enter the calibration value of 0.0%.
2. Press the [MENU] soft-key and following screen will be displayed.



1. If applicable, after the right channel readings are stable, press the [▲] up soft-key or the [▼] down soft-key to enter the calibration value of 0.0%.

NOTE: When you change the zero point calibration, you offset the other calibration value, so after zeroing the probe for zero oxygen , you must recalibrate in an oxygen environment.

After ~15 seconds of inactivity the YSI 5300A will reset to the main display, but the zero point calibrations will remain active.

6.2 Daily Probe Test

(Does not have to be repeated from each measurement).

a. After calibration, set the 5300A Monitor function switch to AIR and wait until the recorder traces a steady value.

b. Unlock the CAL control knob, set the reading to 90.0% and relock the knob.

c. Turn the function switch counterclockwise to the TEST position and wait for a steady trace.

d. The test trace should be no lower than 87.0% of full scale after 2.5 minutes. (See Sec. 5.3, Voltage Plateau for additional information) If the probe does not meet this specification, the membrane should be replaced (see section 4.1). If performance still does not improve, the probe should be cleaned (see *Probe Care and Maintenance, Sec. 5.9*).

After performing the probe test and determining that the probe is functioning correctly, turn the function switch to the AIR position, unlock the Cal control knob and reset the display to 100.0%. Lock the CAL control knob. Repeat above steps for channel 2 daily probe test.

6.3 Sample Monitoring

After calibration and the daily probe test your system is ready to monitor oxygen.

1. Turn off the 5301B Bath stir motor and place a 3 mL sample and a magnetic stirrer into the sample chamber.
2. Turn on the 5301B Bath stir motor. Allow 3 minutes for temperature equilibration, then turn the 5301B Bath stir motor off.
3. Insert a prepared probe into this sample chamber as in step 5.

4. Allow from 2 to 15 minutes for the recorder to produce a trace defining the oxygen uptake curve.

5. Determine the actual oxygen consumption rate as illustrated in the following example:

An air saturated sample of Ringers solution at 1 atmosphere and 37°C contains 5.02 µL O_2/mL (see Table 1). Therefore, a 3 mL sample contains 5.02 µL O_2/mL x 3 mL = 15.06 µL O_2. If the oxygen concentration were to change from 82% to 64% in 5 minutes, the air saturation would have been reduced by 18%.

A change of 18% = 2.71 µL O_2. On an hourly basis, 2.71 µL O_2 x 12/hr = 32.52 µL/hr.

6. To remove a magnetic stirrer from a sample chamber, use the magnet retriever (YSI 5225) supplied with the 5301B Bath Assembly.

NOTE: Any result over 199.99% will display OL (over limit) on the display screen.

7. Troubleshooting

Problem/ Symptoms	Possible Cause	Correction
YSI 5300A Biological Monitor		
Blank Display	Power supply not connected	Connect power supply
Data not logging on chart recorder	Chart recorder unplugged	Connect power supply
	Chart recorder not connected with YSI 5300A Monitor	Connect recorder with monitor
		Refer to chart recorder manual for further direction
Data not logging via RS232 serial port connection	RS232 Serial Output not turned on	Correct monitor setup
	Serial cable not connected	Connect serial cable
	Serial cable is faulty	Replace cable
	Monitor and data acquisition system not setup correctly	Check and compare monitor and data acquisition software setup
Test setting greater than 3% of Air/ O2 value 2 ½ minutes after switching	Membrane integrity failure	Install new YSI Teflon membrane and fresh KCl solution, sec.
	KCl solution has bubbles	Install new YSI Teflon membrane and fresh KCl solution, sec.
	Probe face fouled	Clean probe face, see Sec. 5.9
	Air bubbles on membrane	Slightly twist plunger to remove bubbles at access slot
	Temperature fluctuations	Use appropriate temperature controlled circulator with water bath, see Sec. 5.5
	Improper stirring	Check stirring
Slow probe response	Membrane integrity failure	Install new YSI Teflon membrane and fresh KCl solution, sec.
	KCl solution has bubbles	Install new YSI Teflon membrane and fresh KCl

		solution, sec.
	Probe face fouled	Clean probe face, see Sec. 5.9
Display values not changing when exposed to oxygen	Oxygen probes not plugged in	Connect oxygen probes
Calibration drift	Membrane and KCl solution failing	Install new YSI Teflon membrane and fresh KCl solution, sec.
	Probe face fouled	Clean probe face, see Sec. 5.9
		See Sec. 5.8 for further direction
Readings inconsistent	Membrane and KCl solution failing	Install new YSI Teflon membrane and fresh KCl solution, sec.
	Probe face fouled	Clean probe face, see Sec. 5.9
	Air bubbles on membrane	Slightly twist plunger to remove bubbles at access slot
	Temperature fluctuations	Use appropriate temperature controlled circulator with water bath, see Sec. 5.5
	Improper stirring	Check stirring
YSI 5301B Bath Assembly		
Magnetic stirrers not moving	YSI 5301B power supply not connected	Connect power supply
	Debris or residue accumulation	Clean sample chamber and magnetic stirrer with isopropanol
		Replace magnetic stirrer

8. Warranty and Repair

YSI 5300A Biological Oxygen Monitors, YSI 5301B Bath Assembly and YSI 5331A probes are warranted for one year from date of purchase by the end user against defects in materials and workmanship. Within the warranty period, YSI will repair or replace, at its sole discretion, free of charge, any product that YSI determines to be covered by this warranty.

To exercise this warranty, write or call your local YSI representative, or contact YSI Customer Service in Yellow Springs, Ohio. Send the product and proof of purchase, transportation prepaid, to the Authorized Service Center selected by YSI. Repair or replacement will be made and the product returned, transportation prepaid. Repaired or replaced products are warranted for the balance of the original warranty period, or at least 90 days from date of repair or replacement.

Limitation of Warranty

This Warranty does not apply to any YSI product damage or failure caused by (i) failure to install, operate or use the product in accordance with YSI's written instructions, (ii) abuse or misuse of the product, (iii) failure to maintain the product in accordance with YSI's written instructions or standard industry procedure, (iv) any improper repairs to the product, (v) use by you of defective or improper components or parts in servicing or repairing the product, or (vi) modification of the product in any way not expressly authorized by YSI.

THIS WARRANTY IS IN LIEU OF ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. YSI's LIABILITY UNDER THIS WARRANTY IS LIMITED TO REPAIR OR REPLACEMENT OF THE PRODUCT, AND THIS SHALL BE YOUR SOLE AND EXCLUSIVE REMEDY FOR ANY DEFECTIVE PRODUCT COVERED BY THIS WARRANTY. IN NO EVENT SHALL YSI BE LIABLE FOR ANY SPECIAL, INDIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES RESULTING FROM ANY DEFECTIVE PRODUCT COVERED BY THIS WARRANTY.

YSI Factory Service Centers

United States

YSI Incorporated • Repair Center • 1725 Brannum Lane • Yellow Springs, OH • 45387 • Phone: 937 767-7241 • Fax: 937 767-9353

Endeco/YSI Inc. • 13 Atlantis Drive • Marion, MA • 02738 • Phone: 508 748-0366 • Fax: 508 748-2543

Europe

YSI LTD • Lynchford House • Lynchford Lane • Farnborough, Hampshire • GU14 GLT • Phone: 441 252 514711 • Fax: 441 252 511855

YSI Authorized Service Centers

California

EviroServices & Repair • 1110 Burnett Avenue, Suite D • Concord, CA • 94520 • Phone: 510 609-1088 • Fax: 510 674-8655

Fisher Scientific ISD • 2822 Walnut Avenue, Suite E • Tustin, CA • 92681 • Phone: 800 395-5442

Florida

Aquatic Eco Systems, Inc. • 1767 Benbow Court • Apopka, FL • 32703 • Phone: 407 886-3939 • Fax: 407 886-6787

Georgia

Fisher Scientific ISD • 2775 Horizon Ridge Court • Suwanee, GA • 30174 • Phone: 800 395-5442

Illinois

Fisher Scientific ISD • 1600 West Gleenlake Avenue • Itasca, Ill • 60143 • Phone: 800 395-5442

Maine

Q. C. Services • P.O. Box 68 • Harrison, ME • 04040 • Phone: 207 583-2980 • Fax: 207 583-6936

Mississippi

Aquacenter • 166 Seven Oaks Road • Leland, MS • 38756 • Phone: 601 378-2861 • Fax: 601 378-2862

CC Lynch and Associates • 212 E. 2nd Street • Suite 203 • Pass Christian, MS • 39571 • Phone: 601 452-4612 • Fax: 601 452-2563

New Jersey

Fisher Scientific ISD • 52 Fadem Road • Springfield, NJ • 07081 • Phone: 800 395-5442

Oregon

Q. C. Services • P.O. Box 14831 • Portland, OR • 97293 • Phone: 503 236-2712 • Fax: 503 235-2535

Pennsylvania

Fisher Scientific ISD • 585 Alpa Drive • Blawnox, PA • 15238 • Phone: 800 395-5442

8.1 Cleaning Instructions

NOTE: Before they can be serviced, equipment exposed to biological, radioactive, or toxic materials must be cleaned and disinfected. Biological contamination is presumed for any instrument, probe, or other device that has been used with body fluids or tissues, or with waste water. Radioactive contamination is presumed for any instrument, probe or other device that has been used near any radioactive source.

If an instrument, probe, or other part is returned or presented for service without a Cleaning Certificate, and if in our opinion it represents a potential biological or radioactive hazard, our service personnel reserve the right to withhold service until appropriate cleaning, decontamination, and certification has been completed. We will contact the sender for instructions as to the disposition of the equipment. Disposition costs will be the responsibility of the sender.

When service is required, either at the user's facility or at YSI, the following steps must be taken to insure the safety of our service personnel.

1. In a manner appropriate to each device, decontaminate all exposed surfaces, including any containers. 70% isopropyl alcohol or a solution of 1/4 cup bleach to 1 gallon tap water are suitable for most disinfecting. Instruments used with waste water may be disinfected with .5% Lysol if this is more convenient to the user.
2. The user shall take normal precautions to prevent radioactive contamination and must use appropriate decontamination procedures should exposure occur.
3. If exposure has occurred, the customer must certify that decontamination has been accomplished and that no radioactivity is detectable by survey equipment.
4. Any product being returned to the YSI Repair Center, should be packed securely to prevent damage.
5. Cleaning must be completed and certified on any product before returning it to YSI.

8.2 Packing Instructions

1. Clean and decontaminate items to insure the safety of the handler.
2. Complete and include the Cleaning Certificate.
3. Place the product in a plastic bag to keep out dirt and packing material.
4. Use a large carton, preferably the original, and surround the product completely with packing material.
5. Insure for the replacement value of the product.

Cleaning Certificate

Organization _____

Department _____

Address _____

City _____ State _____ Zip _____

Country _____ Phone _____

Model No. of Device _____ Lot Number _____

Contaminant (if known) _____

Cleaning Agent(s) used _____

Radioactive Decontamination Certified?

(Answer only if there has been radioactive exposure)

___ Yes ___ No

Cleaning Certified By _____

Name Date

9. Required Notice

The Federal Communications Commission defines this product as a computing device and requires the following notice:

This equipment generates and uses radio frequency energy and if not installed and used properly, may cause interference to radio and television reception. There is no guarantee that interference will not occur in a particular installation. If this equipment does cause interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- re-orient the receiving antenna
- relocate the computer with respect to the receiver
- move the computer away from the receiver
- plug the computer into a different outlet so that the computer and receiver are on different branch circuits.

If necessary, the user should consult the dealer or an experienced radio/television technician for additional suggestions. The user may find the following booklet, prepared by the Federal Communications Commission, helpful: "How to Identify and Resolve Radio-TV Interference Problems." This booklet is available from the U.S. Government Printing Office, Washington, DC 20402, Stock No. 0004-000-00345-4.

10. Accessories and Replacement Parts

The following parts and accessories are available from YSI or any Franchise Dealer authorized by YSI.

{PRIVATE }YSI Order Number	Description
5092	Nylon Hold Down Ring
5093	Lucite Plunger (standard 5301B)
5215	Chamber Pack (6 chambers, 12 O-rings)
5222	Magnetic Stir Bar (standard 5301B)
5225	Magnetic Retriever
5301B	Standard Bath Assembly
5304	Micro Adapter Kit
5307	Lucite Plunger (5301B w/5304 Micro Adapter Kit)
5309	Magnetic Stir Bar (5301B w/5304 Micro Adapter Kit)
5310	Constant Temperature Circulating Bath (includes YSI 5315)
5313	Magnetic Retriever
5315	Plumbing Kit (used to connect YSI 5310 with the YSI 5301B)
5320	2 Channel Chart Recorder (includes YSI 5320)
5325	Wire Set (used to connect YSI 5320 with YSI 5300A)
5331A	Oxygen Probe
5338	O-ring Applicator
5350	Membrane Mounting Kit
5356	Micro Oxygen Chamber
5357	Micro Oxygen Probe
5358	Cable Assembly
5775	Membrane/KCl Kit, Standard (30 membranes)
5776	Membrane/KCl Kit, High Sensitivity (30 membranes for higher permeability below 15ºC
5793	Standard Membranes (150 membranes)
5794	High-Sensitivity Membranes (150 membranes)
5945	O-ring Kit (12 O-rings)
052021	RS-232 Cable (used to connect YSI 5300A with data acquisition serial port)

11. Appendix A - Specifications

YSI 5300A System (YSI 5300A, YSI 5331A, and YSI 5301B)

System Stability: Maximum 5% of full scale per hour at 50% saturation or greater. (Accounts for probe, temperature, electronic and O_2 leak effects.)

System Linearity: ±1% of full scale (full scale is 200%)

Environmental Requirements:

- Laboratory setting
- Ambient temperature 15 to 35° C
- Relative Humidity up to 65% (non-condensing), low signal readings at 90% humidity affects the readings.

UL3101-1 compliance (5300A and 5301B): Pollution degree 2, Installation category 2

YSI 5300A Monitor

Channels: Each of the two channels are independently operated and each channel has a dedicated 0 to 2.000 Volt recorder output. Each channel has two input jacks, one for the standard probe and one for the micro probe.

Display: LCD, oxygen results displayed in % oxygen saturation, four digits maximum with a resolution of 0.1%

Instrument Size: 15.8 H x 28.5 W. x 22 D cm, 2.4 kg

6.2 H x 11.2 W x 8.7 D in, 5.3 lb

Power: 115 vac ±10%, 60 hz, 0.16 amp

230 vac ±10%, 50 hz, 0.08 amp

Approval: CE

Size: 9 X 9.5 X 4.4 inches 22.9 X 24.1 X 11.2 cm

Weight: 2.6 pounds 1.1 kg

Recorder Output: 0 to 2.000 volts corresponds to full scale. 20 K ohms minimum load impedance required. The display is tracked within ±0.2% of full scale; the differential output capability (Channel 1 minus Channel 2) is accurate to ±0.4% of full scale.

YSI Oxygen Probes

Oxygen Consumption Rate Range: 3 to 250 µL O_2/hr in air-saturated solutions;:15 to 250 µL O_2/hr in oxygen saturated solutions.

Probe Linearity: ±1.0% of full scale. (full scale is 200%)

Oxygen Consumption of the 5331A Standard Oxygen Probe: less than $6x10^{-7}$ grams O_2/hr (<0.1 µL 0_2/hr) in air.

Oxygen Consumption of 5357 Micro Oxygen Probe: less than $6X10^{-9}$ grams O_2/hr (<0.005 µL O_2/hr) in air.

Stabilization Time: 60 seconds maximum with probe and solution at operating temperature.

Response Time: 90% of final reading in approximately 10 seconds (± 2 seconds). Assumes probe and solution are at operating temperature.

YSI 5301B Bath Assembly

Sample Chamber Size: 600 µL with the 5357 Micro Oxygen Probe, 1 mL with YSI 5304 Micro Adapter Kit and 3 to 8 mL with 5331 Standard Oxygen Probe.

Temperature Stability: With suitable circulator, ±0.02°C of circulator temperature in the sample chamber.

Stirring Speed: 480 RPM with 5301B Standard Bath and variable to 1600 RPM with 5356 Micro Oxygen Chamber

Sample Temperature Range: 5 to 40°C

Approvals: 115V 5301B: UL

230V 5301B: CE

12. Appendix B – Tables

Volume of Oxygen Dissolved in Aqueous Medium

(microliters of oxygen per milliliter at 1 atmosphere)

Temp ºC	*Equilibrated with 100% O_2*		*Equilibrated with 21% O_2*	
	H_2O[1]	*Ringers Solution*[2]	*H_2O*[2]	*Ringers Solution*[2]
15	34.2	34.0	7.18	7.14
20	31.0	31.0	6.51	6.51
25	28.5	28.2	5.98	5.92
28	26.9	26.5	5.65	5.56
30	26.1	26.0	5.48	5.46
35	24.5	24.5	5.14	5.14
37	23.9	23.9	5.02	5.02
40	23.1	23.0	4.85	4.83

[1] From "Handbook of Chemistry and Physics" 40th Ed., Chemical Rubber Pub. Co., Cleveland, 1958-1959.

[2] Recalculated from Umbriet et al (1964). "Manometric Methods", 4th Ed. Burgess Pub. Co.

Solubility of O_2 in Buffered Mitochondrial Medium Equilibrated with Air (20.9 % O_2)

Temperature ºC	*µg atoms O_2/ mL* [3]	*µMoles/ mL (mM)*
15	0.575	0.288
20	0.510	0.255
25	0.474	0.237
30	0.445	0.223
35	0.410	0.205
37	0.398	0.199
40	0.380	0.190

[3] Solubility of O_2 experimentally determined by Chappell (1964) "Biochem." J. 90, 225., in a buffered mitochondrial medium containing $NADH_2$ inorganic phosphate, and isolated mitochondria.

Bunsen Coefficients for Solubility of Oxygen in Plasma and Blood [4]

Temperature ºC	*Plasma*	*Blood Hb g/ 100mL*			
		5g	*10g*	*15g*	*20g*
15	0.0302	0.0310	0.0312	0.0316	0.0323
20	0.0277	0.0282	0.0284	0.0287	0.0293
25	0.0257	0.0261	0.0263	0.0265	0.0271
28	0.0246	0.0249	0.0251	0.0253	0.0259
30	0.0238	0.0241	0.0243	0.0245	0.0251
35	0.0220	0.0226	0.0227	0.0229	0.0234
37	0.0214	0.0220	0.0221	0.0223	0.0228
40	0.0208	0.0211	0.0212	0.0214	0.0219

[4] From Christofordes et al "J. Appl. Physiol." 26:56, 1969 and "J. Appl. Physiol." 27:592, 1969.

Solubility of Oxygen in Water and Various Solvents

Relative Solubility Solvent Solubility at 20°C		Effect of Certain Electrolytes on Solubility = Solubility Concentration Per Unit M Concentration	
1 M NaCl	0.724	NaCL	0.0073
1 M KCl	0.737	KCl	0.0069
1M HCl	0.932	KF	0.0078
½ M H_2SO_4	0.894	$NaNO_2$	0.0053
1 M KOH	0.670	1/3 x $K_3Fe(CN)_6$	0.0060
1 M NaOH	0.662	½ x $K_2C_2O_4$	0.0071
1 M Sucrose	0.554	NaH_2CO_3	0.0081
Methyl Alcohol	7.670	½ Na_2CO_3	0.0085
Ethyl Alcohol	7.700	NaOH	0.0090
Acetone	6.910	Lactic Acid	0.0003

Sources:

Sendroy, Dillion & Van Slyke, “J. Biol. Chem.” 105:597, 1939.

Van Slyke et al, “J. Biol. Chem.” 78:765, 1928.

Handbook of Respiration, Wright Air Development Center Report, 58-352, Page 6.

Atmospheric Pressure Vs. Altitude Correction

Atmospheric Pressure (mm Hg)	Altitude		Correction Factor
	Feet	Meters	
768	-276	-84	101
760	0	0	100
752	278	85	99
745	558	170	98
737	841	256	97
730	1126	343	96
722	1413	431	95
714	1703	519	94
707	1995	608	93
699	2290	696	92
692	2587	789	91
684	2887	880	90
676	3190	972	89
669	3496	1066	88
661	3804	1160	87
654	4115	1254	86
646	4430	1350	85
638	4747	1447	84
631	5067	1544	83
623	5391	1643	82
616	5717	1743	81
608	6047	1843	80
600	6381	1945	79
593	6717	2047	78
585	7058	2151	77
578	7401	2256	76
570	7749	2362	75
562	8100	2469	74
555	8455	2577	73
574	8815	2687	72
540	9187	2797	71

Derived from the 16th Edition of “Standard Methods for the Examination of Water and Wastewater”.

13. Appendix C - Required Notice

The Federal Communications Commission defines this product as a computing device and requires the following notice:

This equipment generates and uses radio frequency energy and if not installed and used properly, may cause interference to radio and television reception. There is no guarantee that interference will not occur in a particular installation. If this equipment does cause interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- re-orient the receiving antenna
- relocate the computer with respect to the receiver
- move the computer away from the receiver
- plug the computer into a different outlet so that the computer and receiver are on different branch circuits.

If necessary, the user should consult the dealer or an experienced radio/television technician for additional suggestions. The user may find the following booklet, prepared by the Federal Communications Commission, helpful: "How to Identify and Resolve Radio-TV Interference Problems." This booklet is available from the U.S. Government Printing Office, Washington, DC 20402, Stock No. 0004-000-00345-4.

14. Appendix D: Application Notes

Application notes for the YSI 5300A are currently under development. For a current listing, please contact YSI Life Sciences Customer Service (800-659-8895 or 937-767-7241 extension 2) or visit the YSI Website at www.YSI.com.

15. Appendix E: Gas Solubility Relationships

Gas Solubility Relationships

µg/ mL= 0.031 µMole/mL

µg/ mL= 0.700 µL/ mL at 0ºC

µM/ mL= 32.000 µg/mL

µM/mL= 22.400 µL/mL

1 µL/mL= 1.430 µg/ml at 0ºC

1 µL/mL= 0.045 µMole/mL at 0ºC

To correct for temperatures other than 0ºC when using values in microliters:

µL at 0ºC X [(273 + TºC) / 273] = µL at TºC

Sources:

Sendroy, Dillon & Van Slyke, op cit.

Van Slyke et al, op cit.

Handbook of Respiration, op cit.

16. Appendix F: Interfering Gases

H_2S, SO_2, halogens, nitrous oxide and CO are interfering gasses. If you suspect erroneous readings, it may be necessary to determine if these are the cause. The following gases have been tested for response.

100% Carbon Monoxide	Less than 1%
100% Carbon Dioxide	Less than 1%
100% Hydrogen	Less than 1%
100% Chlorine	2/3 O_2 response
100% Helium	None
100% Nitrous Oxide	1/3 O_2 response
100% Ethylene	None
100% Nitric Oxide	1/3 O_2 response

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