GENERAL INFORMATION

This family of dissolved oxygen electrodes is of the steam sterilizable polarographic type, designed to be interchangeable with NBS oxygen amplifiers and other manufacturer's amplifiers designed to the same specifications. The probe is designed to withstand severe conditions, like high temperature, high pressure, and moisture during sterilization.

SPECIFICATIONS

Electrode body : Polished 316L stainless steel.
Membrane : PTFE/Silicone (stainless steel reinforced).
Cathode : Platinum, diameter 0.10mm.
Anode : Tubular Ag.
Output in air-saturated DI water : 50 nA.
Residual Signal : less than 1 nA.
Response time : less than 60 seconds to 98% of full response.

THEORY OF OPERATION

The polarographic dissolved oxygen electrode was first described by Clark in 1956. It consists of polarized platinum and silver electrodes, with the electrolyte separated from the sample by a PTFE/silicone gas permeable membrane. Oxygen diffuses across the electrode membrane and is reduced to hydroxyl ions at the platinum cathode according to the reaction:

\[ \text{(1) } O_2 + 2H_2O + 4e^- = 4OH^- \]

The electrons necessary for this process are produced by a reaction at the silver anode. Because the electrolyte contains chloride ions, this reaction occurs as:

\[ \text{(2) } 4Ag + 4Cl^- = 4AgCl + 4e^- \]

At any given temperature, the current flow between cathode and anode is directly proportional to the level of oxygen outside the membrane.

ZERO POINT OF AMPLIFIER

The electrical zero point of the amplifier must be first set. (Follow the manufacturer’s operating instructions).
TEMPERATURE COMPENSATION

Temperature compensation is not required when calibration is made at the same temperature as the controlled fermentation temperature. The fermentation must be temperature controlled, with no temperature variation.

POLARIZATION

When the amplifier is switched on, polarization voltage is applied to the anode and the cathode. Initial current is very strong, but falls off exponentially and settles down to a steady state after a few hours.

Since the equilibrium period of the polarization current is relatively long, it is advisable to keep the electrode connected to the amplifier when not in use. The small current flowing through the electrode will not shorten the life of the electrode. If, for any reason, the electrode must be disconnected, or the amplifier switched off, the electrode must be repolarized before it can be used. During the polarization period, the electrode current will decrease even in oxygen free solutions. An excessive zero current may indicate incomplete polarization.

ELECTRODE CONSTRUCTION

The external portion of the electrode is constructed of a polished stainless steel cylinder. The functional portion of the electrode is constructed of a ryton chamber enclosing an ultra-pure tubular Ag anode and a 0.10 mm diameter cathode. A thin PTFE/silicone membrane, reinforced by a stainless steel mesh at the tip, provides for efficient sealing of the system and is in contact with the platinum cathode and electrolyte. The membrane is permeable to oxygen but impermeable to water and electrolyte.

PREPARATION

The electrode is shipped filled with electrolyte and with PTFE/silicone membrane in place. The protective vinyl cap on the sensing tip must be removed before and during sterilization. Upon receiving the electrode, inspect for damage. If any damage is noticed, notify pHoenix Electrode Company Service Department immediately.

ELECTRODE CHECK

Remove protective vinyl cap.

Connect the detachable electrode cable to the probe and amplifier.

Follow polarization procedures (see POLARIZATION SECTION)

Leave electrode in air saturated DI water. Set the range selector on the amplifier to zero. Adjust the zero current to read zero on the indicator. Then calibrate the meter to 100% saturation degree. Pass nitrogen gas over the electrode membrane. The electrode will react immediately increasing the reading, which will decrease after 45 seconds. The reading should be under 10%. (Refer to the amplifier manufacturer’s procedure on checking the electrode).
ZERO POINT OF THE ELECTRODE

The zero current of the dissolved oxygen electrode (electrode current in pure \( \text{N}_2 \)) is usually negligibly small and almost identical with the amplifier zero point. Nonetheless, the electrode zero point should be periodically checked as some electrode faults result in excessive zero current. Moreover, checking the zero point calibration is necessary before measurement of low oxygen concentration. The zero current (residual current) of the electrode may be compensated by adjusting the zero button on the meter. After zeroing the electrode, recalibrate it in air-saturated water.

ELECTRODE SLOPE

Zero point adjustment must precede slope calibration. Unlike zero point calibration, the aqueous phase is preferred for slope adjustment. The following problems arise in calibration in air:

1) Membrane permeability slightly differs in air and water.

2) Relative air humidity rarely attains 100%.

3) The temperature is poorly defined.

When calibrating in a fermentor, slope calibration should be performed only after sterilization or the electrode slope may be altered. This alteration may be comparatively large, particularly when a new membrane is used for the first time.

After cooling, allow the vessel to reach operating temperature. This is very important when a temperature compensator is not used. Saturate the aqueous phase with air in the vessel by purging air at the maximum flow rate for approximately 20 minutes, allowing the output signal to stabilize. Adjust the display module to 100%.

Calibration is usually most effective at an air saturation of 100%. Since calibration depends on pressure, the 100% air saturation should be done under operating pressure. (Refer to amplifier manufacturer’s operating procedures).

STERILIZATION

Electrodes should be installed in the headplate of the fermentor or in the side port, depending on the type of fermentor. During sterilization, particular care should be exercised during autoclaving when localized boiling tends to occur. Either maintain a slow cooling rate or pressurize the autoclave during sterilization. Consult your autoclave manufacturer for information regarding a pressure balance feature and proper autoclaving procedures to eliminate localized boiling during the cooling cycle. After sterilization and cool down, electrolyte may be replenished by following the filling procedure. DO NOT remove the electrode from the fermentor.
CALIBRATION

Connect the electrode cable to the DO probe and to the DO amplifier. Follow the polarization procedure as described, along with the amplifier manufacturer’s operating manual.

Dissolved oxygen electrodes should be recalibrated prior to every measurement. If work is performed under sterile conditions, the system must be calibrated after sterilization, but prior to inoculation.

MEMBRANE REPLACEMENT

The membrane should be examined routinely after each fermentation cycle and replaced if any deterioration is evident.

To remove the membrane, unscrew the bottom stainless steel cap with the membrane in place. (Figure 1).

Inspect the glass part and platinum cathode at the very tip of the glass with tissue paper.

Remove a new membrane from it’s package. Use a screwdriver to replace membrane as shown in Figure 1.

Fill the membrane cap with electrolyte and screw the cap onto the tip of the electrode.

After the membrane cap is securely in place, the PTFE/silicone membrane should be stretched across the glass cathode. The electrode is to be filled with electrolyte, following the above procedures (see PREPARATION SECTION).

After re-assembling the membrane and filling the electrode with electrolyte, it must be checked. The polarization and sterilization procedures should be followed.

TROUBLESHOOTING

Problem: The probe when in air-saturated water generates no potential (zero output).

Solution: Check that all signal cables connections are correct. Check that the probe is filled with electrolyte.

Problem: The output signal is reversed.

Solution: Reverse the signal wires connected to the display module or recorder.

Problem: The calibration potentiometer is on maximum but the signal does not reach 100%.

Solution: Change the membrane. Change the electrolyte.
Problem: The output signal is unstable and shows random drift.

Solution: Check the grounding of the fermentor and of the instrumentation.

NOTE: The output signal may not reach 100% if the ambient air temperature is less than 15%.

TROUBLESHOOTING DURING FERMENTATION

During the sterilization process, 0.5 - 1.0 ml of electrolyte will be lost due to expansion.

Check that the signal is not being affected by external switching gear, power relays, etc.

Check that the slow random drift is not due to poor mixing of culture medium.

If the fault persists, it may be due to a badly fitted or incorrectly tightened membrane.

Problem: The output signal is stable, but shows a regular sinusoidal drift.

Solution: Check the proportional band setting of the DO control.

Check the DO control on the fermentor.

Problem: During a long fermentation, the signal drifts to a value that appears incorrect.

Solution: Check to see if the membrane is split or has been fouled by the culture medium.

MAINTENANCE

After the electrode has been used for a period of time, (at least five (5) cycles), the residual current may rise. To minimize the residual current, the following cleaning method is recommended:

Gently unscrew the membrane cover from the body, rinse the inside of the membrane with DI water. Flush the electrolyte chamber with DI water. Soak the silver anode and the glass tip in silver cleaning solution (commercially available as jewelry cleaning solution) for about 10 minutes. Thoroughly rinse the soaked portion with DI water, blot dry with tissue paper. Fill the electrode with electrolyte as described above.

PACKING LIST

pHoenix Electrode Company polarographic DO probe

Electrolyte 1
Spare membrane 1
Membrane Mounting tool 1
O-rings 2
Plastic syringe 1
Polarographic Oxygen Electrode Membrane Replacement Procedure

A. Detachable Lead
B. O-Ring Seal
C. 316 Stainless Steel Body
D. O-Ring Seal
E. Platinum Cathode
F. Ag/AgCl anode
G. Replaceable PTFE/silicone & stainless steel mesh membrane
H. Membrane Cover
I. 16mm OD with M18 x 1.5 thread
J. Membrane replacement tool