STERILIZABLE DISSOLVED OXYGEN SENSORS

USER MANUAL

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1. INTRODUCTION:

The ADI dO_2 sensors are used for measuring the partial pressure of dissolved oxygen in media for biotechnological processes.
The sensors are available in two versions: a standard version with a stainless steel membrane tip and a "low-drift" version with a titanium membrane tip.
Both types of dO_2 sensors are available in different sizes for application in the head plate of a 2 - 20 liter autoclavable bioreactor and in the bottom DN25 insert of the 20 - 200 liter Bio Bench / Pilot In Situ reactors.
Control of dissolved oxygen tension can be obtained in combination with the ADI 1030 (autoclavable systems) or the ADI 1060 (in situ systems) Bio Controller.

The polarographic ADI dO_2 sensor is based on the principle of a Clark-cell. It consists of a platinum working electrode (cathode) and a silver counter (reference) electrode (anode); the gas permeable membrane separates the electrode from the medium.
The polarographic amplifier supplies a constant polarization voltage of - 675 mV.
The oxygen molecules that diffuse through the semi-permeable membrane (and the thin layer of electrolyte) are reduced at the cathode while the silver anode is oxidized simultaneously.
Polarization causes a current to flow which is measured by the amplifier; this current is proportional to the partial pressure of oxygen in the medium.
The slope of the O_2 sensor is temperature, pressure and medium dependent. Therefore calibration must be performed under operating conditions (same temperature, pressure and medium!).
To enable automatic temperature correction the standard version sensor with the AppliSens logo on the connector is fitted with an integrated NTC resistor (k = -4%, R = 22 kOhm @ 25 °C, B25/50 = 3560)

2. OPERATION:

The sensor comes with a protective cap to avoid mechanical damage of the sensor tip and prevent evaporation of the electrolyte; this cap must be removed before using the sensor.
AppliSens sterilizable dO_2 sensors are supplied factory-tested and certified with membrane module in place.
When the system is operated for the first time or when the sensor has been disconnected from the controller for longer than 5 minutes, the sensor must be polarized (connected to the dO_2 amplifier) for at least 6 hours prior to calibration. If the sensor is only disconnected for a couple of minutes, less than 6 hours will be sufficient.
Insufficient polarization will cause an increased residual signal and therefore lead to measurement errors (see section 4: Electrode Verification).

Note:
In case of autoclavable systems, the electrode cable must be removed before sterilization; cover the electrode connector with a cap or cotton with sterilizable (aluminum) foil!

3. CALIBRATION:

If the measurement is performed under sterile conditions, the system should be calibrated after sterilization since sterilization may alter the sensor slope.
After cooling, the fermentor must be aerated long enough to reach a constant oxygen pressure; the signal is allowed to stabilize and the reading of the amplifier adjusted to the desired value (e.g. 100 % air saturation, 20.9% oxygen saturation or e.g. 9.17 mg O_2/l pure water at 20°C and 760 mmHg).
Note:
- In case of autoclavable systems, the sensor must be connected to the amplifier immediately after sterilization in order to polarize it; it is recommended to polarize the electrode for at least several (6) hours before calibration. Preferably calibration should take place just before inoculation (the longer the polarization period, the higher the measuring accuracy!).
- Homogenize the medium by stirring during calibration in order to prevent a diffusion gradient.

A "one point" calibration is normally enough to enable accurate measurements over the full range between 0 and 100% dO_2 (at 0% dO_2 the polarized sensor system does not generate any current). However, it is good practice to verify the measured signal at 0% dO_2. Strip all oxygen from the medium by pure N_2 gas (99.98%) and follow the reading of the sensor until it is stabilized. In case the final reading is > 0.5% of air saturation, the sensor needs maintenance (refer to section 10).

The sensor should be pre-sterilized once before calibrating in order to prevent measurement errors.
After the first sterilization, new membrane modules may show a slope-alteration of some percent of the measuring signal; after a second sterilization only a fraction of the first alteration will be observed.

4. ELECTRODE VERIFICATION:

It is good practice to verify the sensor before it is put to use. An elevated residual signal (signal at 0% oxygen saturation) indicates malfunctioning of the O_2 sensor. If the reading at 0% dO_2 is too high (> 0.5% of air saturation) the sensor should be inspected; see section 10, Trouble Shooting.
Verify the sensor by placing it in an oxygen-free environment, either in pure N_2 gas or in a solution from which all oxygen was stripped with N_2 (99.98%).
Note:
- Do not use CO_2 gas for stripping oxygen from the calibration/verification solutions since this will introduce an error.

After 5 to 10 minutes the reading should be stable. In case the bioreactor is sparged with N_2, take enough time to strip all oxygen from the solution.

5. MOUNTING:

When mounting the sensor in the reactor (head plate or bottom insert), be careful not to damage it; both the membrane and inner body are fragile.

Note:
The “low drift” type has a PG 13.5 thread that can be mounted into the DN25 port sensor holder for pH and DO electrodes for In Situ applications.

6. MAINTENANCE:

Visually inspect the membrane for damage prior to each calibration. If the membrane is contaminated, clean it with a moist, soft cloth.
Depending on the nature of the medium, the electrolyte may have to be changed periodically; it must be refreshed at least every six months.
Note:
If the medium contains components that diffuse through the membrane (like acetic acid, aldehyde or neutral mercaptanes), the electrolyte must be refreshed more frequently!
The membrane module must be replaced when it starts to show:
- elevated residual signal,
- sluggish response,
- drifting or noisy readings,
- mechanical damage, etc. (see section 10, Trouble Shooting).

7. STORAGE:

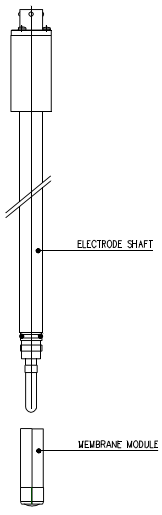
Filled with electrolyte for the ADI dO_2 sensor, the electrode can be stored for several months provided that the protective cap is covering the membrane module (to prevent evaporation of the electrolyte). During this period of time, the electrode can be kept polarized so it is always ready for use. For storage over longer periods, the sensor should be stored dry e.g. in the membrane module without electrolyte.

8. INSTRUCTIONS FOR EXCHANGE OF ELECTROLYTE AND MEMBRANE:

WARNING: The O_2 electrolyte is strongly alkaline (pH = 13). Contact of electrolyte with skin, especially mucous membrane or eyes, must be avoided (use safety glasses and gloves). If such contact occurs, the affected area must be thoroughly rinsed with water. Get medical attention if adverse symptoms appear.

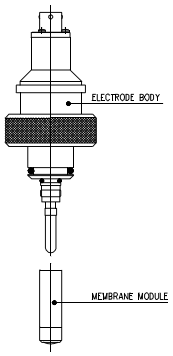
When the membrane starts to shows signs of failure (refer to section 6, Maintenance), it must be replaced.

When replacing the membrane module and electrolyte, strictly follow the instructions below:
- Hold the sensor in a vertical position (membrane downwards) and unscrew the old membrane module,
- Rinse the interior electrode body and the annular space between this and the lower part of the steel shaft with d.i. water and dry it carefully with a lint free tissue,
- Visually inspect the O-ring for mechanical defects and replace if necessary,
- Hold the membrane module with a tissue and fill it with **1 ml (40 drops)** of electrolyte.



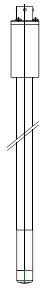
Hold the electrode **vertically** and screw the membrane module **very slowly** onto the shaft.

- After refreshing electrolyte for the ADI dO_2 sensor, the sensor has to be repolarized (see paragraph 2),
- After replacing the membrane module, the sensor has to be sterilized, calibrated (see paragraph 3) and verified (see paragraph 4).



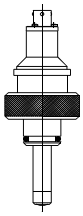
Note:
When this instruction is not followed carefully, the membrane in the module may be damaged!

9. ORDERING INFORMATION:



Normal autoclavable dO_2-sensor in different lengths, detachable cable (head plate application):

Z010023510:	Sensor Dissolved Oxygen L=235MM 1-5L ⌀ = 12mm
Z010038510:	Sensor Dissolved Oxygen L=385MM 7-15L ⌀ = 12mm
Z010059010:	Sensor Dissolved Oxygen L=590MM 20L ⌀ = 12mm
Z110200010:	Cable D. Oxygen sensor L=2.0m BNC
Z110200020:	Cable DO sensor + NTC L=2.0m leads



Normal In Situ sterilizable dO_2-sensor with detachable cable (DN25 port application):

Z010007010	Sensor Dissolved Oxygen L=70mm DN-25
Z110380010	Cable D. Oxygen sensor L=3.8m BNC
Z110500010	Cable D. Oxygen sensor L=5.0m BNC
Z110500020	Cable DO sensor + NTC L=5.0m leads

"Low drift" autoclavable dO_2-sensor in different lengths, detachable cable and titanium module (head plate application):

Z010006521	Sensor Dissolved Oxygen L= 65 ⌀ = 12mm 2P (fixed PG13.5 for 250L BioSep)
Z010023520	Sensor Dissolved Oxygen L=235 1-5L ⌀ = 12mm 2P
Z010038520	Sensor Dissolved Oxygen L=385 7-15L ⌀ = 12mm 2P
Z010059020	Sensor Dissolved Oxygen L=590 20L ⌀ = 12mm 2P
Z100200010	Cable pH/DO-2p sensor L=2.0m BNC

"Low drift" In Situ sterilizable dO_2-sensor with detachable cable and titanium module (DN25 port application):

Z010011020	Sensor Dissolved Oxygen L=110 ⌀ = 12mm 2P for
Z101002510	DN25 pH/DO Sensor Holder or for
Z100002520	Sensor holder DN25 A Tracfix
Z010015420	Sensor Dissolved Oxygen L=154 ⌀ = 12mm 2P for
Z100002530	Sensor holder DN25 B Tracfix
Z100380010	Cable pH/DO-2P sensor L=3.8m BNC
Z100500010	Cable pH/DO-2P sensor L=5.0m BNC

Accessories and spare parts:

Z110000410	Membrane kit Dissolved Oxygen sensor (4 modules, electrolyte 50ml, 4 O-rings)
Z110000420	Membrane kit Dissolved Oxygen sensor titanium (4 modules, electrolyte 50ml,4 O-rings)
Z110005010	Electrolyte Dissolved Oxygen sensor 50ml

10. TROUBLE SHOOTING:

PHENOMENON		REASON	VERIFICATION	WHAT TO DO
1. Slope out of range (Slope >0.6 at 37°C)	a	Short circuit in cable	Check reading with connected cable but without sensor	Replace cable if reading more than 0.5 % of air saturation
	b	Short circuit in sensor	Check reading with detached module and carefully dried inner body	If reading more than 0.5% of air saturation, the sensor is defective
	c	Ground loop	Earthing potential difference between fermentor head plate and measuring device / controller	Earth fermentor and measuring device at the same earth point
	d	Damaged membrane	Visually inspect the membrane	Replace membrane module
2. No sensitivity	a	No polarization voltage	Amplifier	Verify polarisation voltage
	b	Cable rupture	Verify resistance of cable	Replace cable
	c	Not enough electrolyte in membrane module	Visual inspection. Electrolyte must contact anode	Refresh electrolyte solution
	d	Membrane completely fouled	Impermeable layer on membrane	Clean membrane module or replace it
	e	Malfunction in sensor	Sensor	Replace sensor
3. Sensitivity too low (Slope <0.3 at 37°C)	a	Depleted electrolyte	After long term measurements of more than 6 months	Replace electrolyte solution
	b	Contaminated anode	Black silver ring (brown is o.k.) (e.g. caused by S^{2-})	Clean with tooth paste Rinse thoroughly
	c	Contaminated cathode	Not visible (can only be identified by recording a current-voltage curve)	Clean with tooth paste Rinse thoroughly
	d	Membrane fouled	Layer on membrane	Clean with soft tissue or replace module
4. Elevated residual signal (Verify with O_2-free medium)	a	Short circuit in cable	Check reading with connected cable but without sensor	Replace cable if reading more than 0.5 % of air saturation
	b	Short circuit in sensor	Check reading with detached module and carefully dried inner body	If reading more than 0.5% of air saturation, the sensor is defective
	c	Ground loop	Earthing potential difference between fermentor head plate and measuring device / controller	Earth fermentor and measuring device at the same point
	d	Damaged membrane	Visually inspect the membrane	Replace membrane module
	e	Test with CO_2 gas instead of N_2	The dO_2 sensor shows a CO_2 interference in absence of O_2	Use N_2 or Bisulphite solution (catalysed by Co(II)salt)
	f	Contaminated cathode	Not visible (can only be identified by recording a current-voltage curve)	Clean with tooth paste Rinse thoroughly
	g	Sensor not polarized long enough	Polarization < 6 hours	Polarize more than 6 hours

PHENOMENON		REASON	VERIFICATION	WHAT TO DO
5. Unexpected high reading during measurement	a	O_2-free and CO_2 saturated medium	CO_2 interference of dO_2 sensor	
	b	Interference of air/O_2 bubbles	May be observed with air lift reactors or in cultures of high cell density	Place sensor in a region with less air/O_2 bubbles
	c	Elevated pressure in the bioreactor	E.g. caused by an increased flow resistance in sterile filter	Avoid increased pressure
6. Sluggish response	a	Membrane fouled	Layer on membrane	Clean with soft tissue or replace module
	b	Damaged membrane	Visually inspect the membrane	Replace membrane module
	c	Contaminated cathode	Not visible (can only be identified by recording a current-voltage curve)	Clean with tooth paste Rinse thoroughly
	d	Very viscous medium		Sluggish response is normal in case of a viscous medium
7. Unstable signal	a	Air/O_2 bubbles sticking on membrane	May be observed with air lift reactors or in cultures of high cell density	Change stirring and/or aeration conditions or change probe location
	b	Damaged membrane	Visually inspect the membrane	Replace membrane module
	c	Crack in glass body at the cathode	Visual inspection. Unstable signal especially at low pO_2	If crack in glass body, sensor is defective
	d	Electrostatic interference		Apply equipment according to certified conditions
8. Drift too large	a	After sterilization (In-Situ systems only)	Time between cooling down and calibration too short	Increase cooling interval
	b	Membrane fouled	Layer on membrane	Clean with soft tissue or replace membrane module
	c	Damaged membrane	Visually inspect the membrane	Replace membrane module
	d	Contaminated cathode	Not visible (can only be identified by recording a current-voltage curve)	Clean with tooth paste Rinse thoroughly
	e	Contaminated anode	Black silver ring (brown is o.k.) (e.g. caused by S^{2-})	Clean with tooth paste Rinse thoroughly
	f	Ground loop	Earthing potential difference between fermentor head plate and measuring device / controller	Earth fermentor and measuring devices at the same point
	g	Change of pressure in bioreactor	E.g. caused by an increase of the flow resistance in sterile filter	Avoid changes in pressure
	h	Change of medium temperature	Verify medium temperature	Avoid changes in temperature
9. Membrane torn		Too much electrolyte in the module		Add only 1 ml (40 drops of electrolyte and screw the module very slowly onto the shaft.