UNISENSE

NO_x^- BIOSENSOR USER MANUAL

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Version October 2012

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WARRANTY AND LIABILITY

Notice to Purchaser

This product is for research use only. Not for use in human diagnostic or therapeutic procedures.

Warning

Microsensors have very pointed tips and must be handled with care to avoid personal injury and only by trained personnel.
Unisense A/S recommends users to attend instruction courses to ensure proper use of the products.

Warranty and Liability

Microsensors are a consumable. Unisense will only replace dysfunctional sensors if they have been tested according with the instructions in the manual within 14 days of receipt of the sensor(s).
The warranty does not include repair or replacement necessitated by accident, neglect, misuse, unauthorized repair, or modification of the product. In no event will Unisense A/S be liable for any direct, indirect, consequential or incidental damages, including lost profits, or for any claim by any third party, arising out of the use, the results of use, or the inability to use this product.

Unisense mechanical and electronic laboratory instruments must only be used under normal laboratory conditions in a dry and clean environment. Unisense assumes no liability for damages on laboratory instruments due to unintended field use or exposure to dust, humidity or corrosive environments.

Repair or Adjustment

Sensors and electrodes cannot be repaired. Equipment that is not covered by the warranty will, if possible, be repaired by Unisense A/S with appropriate charges paid by the customer. In case of return of equipment please contact us for return authorization.
For further information please see the document General Terms of Sale and Delivery of Unisense A/S as well as the manuals for the respective products.

CONGRATULATIONS WITH YOUR NEW PRODUCT!

Support, ordering, and contact information

The NOx- sensor is a brilliant tool for on-line measurement in experiments where the resolution of time and concentrations are important.

If you wish to order additional products or if you encounter any problems and need scientific/technical assistance, please do not hesitate to contact our sales and support team. We will respond to your inquiry within one working day.

E-mail: sales@unisense.com

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Tueager 1
DK-8200 Aarhus N, Denmark
Tel: +45 8944 9500
Fax: +45 8944 9549

Further documentation and support is available at our website www.unisense.com.

REPLACEMENT OF SENSORS

Unisense will replace sensors that have been damaged during shipment provided that:

- *The sensors were tested immediately upon receipt in accordance with the delivery note and the manual*
- *The seal is still intact.*
- *The sensors are returned to Unisense for inspection within two weeks.*
- *The sensors are correctly packed for return to Unisense, in accordance with the note included in the sensor box.*

RECOMMENDED AMPLIFIERS

One-channel amplifier: Microsensor Monometer
Multi-channel amplifiers: Microsensor Multimeter

OVERVIEW

This manual covers the use of the biosensor for nitrate/nitrite (NO_x^-) and for nitrite (NO_2^-). For simplicity, this will be refered to as NO_x^- in this manual.
The NO_x^- biosensor consists of an electrochemical component (the nitrous oxide transducer) and a biological component (the biochamber). In order to optimize the lifetime of the sensor the biochamber is replaceable.

IMPORTANT
Read the technical description of the NO_x^- biosensor before starting

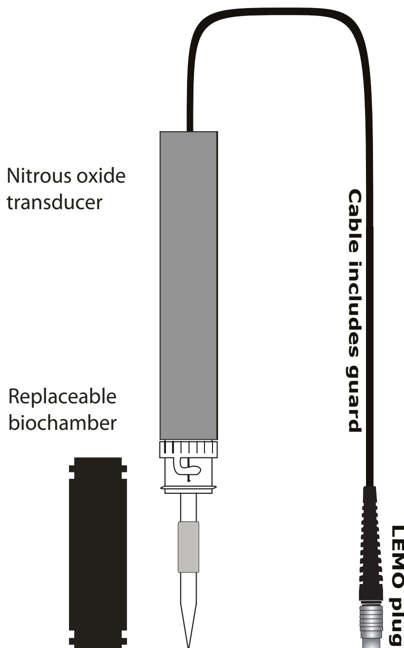

Fig. 1. Nitrate biosensor with replaceable biochamber

Nitrate or nitrite diffuses into the NO_x^- biosensor from the external environment through a selective membrane (Fig. 2). Bacteria situated in a reaction chamber behind this membrane reduce the nitrate or nitrite to nitrous oxide (N_2O), which is detected by an electrochemical nitrous oxide transducer. The amount

of N_20 reduced on the cathode surface is proportional to the concentration of NO_x^- in the external environment. The respiration of the bacteria oxidizes carbon (the energy source) to form CO_2 and water and there is a simultaneous reduction of nitrate and nitrite to nitrous oxide. This process is quite similar to humans' respiration of carbon with oxygen. In microbiology, the use of nitrate and nitrite in place of oxygen for respiration is called denitrification.

The NO_x^- biosensor is equipped with a reservoir containing the carbon source. This carbon compound diffuses into the reaction chamber (Fig. 2), where the bacteria are positioned in opposing gradients of the two substrates, carbon and NO_x^-, required for their growth. Excess cells formed by the growth of the bacteria are pushed upwards into the reservoir, where there is no NO_x^- present. As these cells are unable to respire, growth will cease, and the cells degenerate, releasing cell components, which are used as a carbon source for other living cells.

The bacteria are facultative aerobic microorganisms, meaning that they are able to use both oxygen and NO_x^- for respiration. Respiration with oxygen results in the highest energy yield, and oxygen is therefore used preferentially over NO_x^-. However, oxygen respiration by the bacteria does not affect the sensor's response to NO_x^-.

The N_2O transducer is a Clark-type sensor, in which N_2O is reduced to nitrogen gas (N_2) on the surface of a polarized cathode. As two electrons are used to reduce a N_2O molecule, two electrons are taken from a silver/silver iodide reference electrode (the anode). An picoammeter measures the transport of electrons between the anode and cathode, and the measured current is proportional to the amount of N_2O reduced on the cathode surface. The latter is again proportional to the concentration of NO_x^- diffusing into the sensor from the external environment.

A silicone membrane in the tip of the N_2O transducer electrically separates bacterial production and electrochemical detection of N_2O. The silicone membrane allows the passage of gasses and

small, uncharged molecules, while ions are unable to pass. The electrolyte of the N_2O transducer is therefore efficiently shielded from the environment containing the bacteria. Electrical noise from the surrounding environment thus has a limited effect on the sensor's signal.

The bacteria performing the process inside the sensor is a pure culture and must not be contaminated with bacteria from an external source. Therefore the bacteria are separated from the external environment by an ion-permeable membrane, which only allows the passage of small ions, including NO_x^-. Larger molecules and other bacterial cells cannot enter the sensor through this membrane.

The NO_x^- biosensor measures NO_x^- in almost all aqueous solutions: mud, soil, and sediment; fresh, brackish, and saline water; serum, urine, etc. However, this of course excludes samples that contain substances toxic to the bacteria. The sensor functions at temperatures between 10-38ºC, but the response time, calibration and detection limits are significantly affected by temperature. At temperatures below 10ºC, the bacterial conversion of NO_x^- to N_2O becomes too slow, and at temperatures higher than 38ºC the cells die.

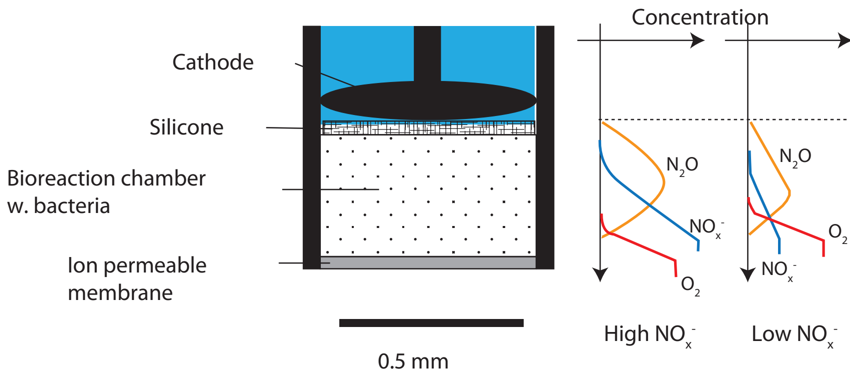

Fig. 2. Principle of the nitrate biosensor.

GETTING STARTED

BEFORE USING THE BIOSENSOR

The wrapping around the sensor will ensure that the membrane in the tip of the sensor does not dry out. Additionally, the sensor should be stored upright with the biochamber pointing downwards (See Fig. 3).

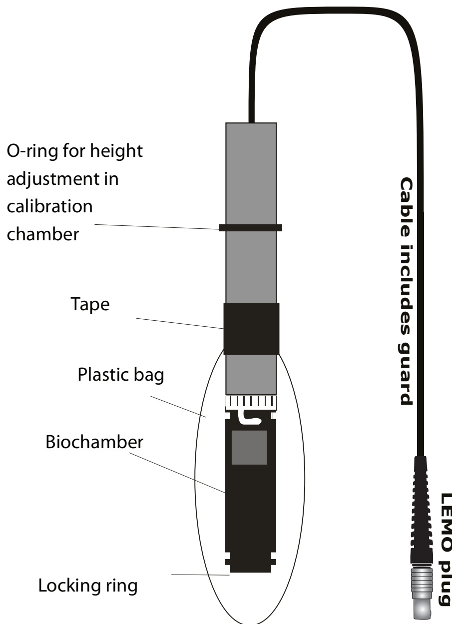

Fig. 3: NO_x^- sensor with protective wrapping (plastic bag).

WARNING

- *The sensor must only be used in aqueous solutions*
- *Do not expose the sensor to toxic compounds of any sort. These include detergents, sulfide, alcohol, and solutions with a pH lower than 3 or higher than 10*
- *Do not use the sensor in solutions with more than 3.8% salinity*
- *The temperature during storage and use must never exceed 38°C*
- *Avoid contact with pointed or sharp objects that may puncture the membrane*
- *Ensure the membrane is kept moist*
- *Follow the storage instructions carefully.*

Polarization of the N_2O transducer

The signal from the sensor is generated in picoampere. Therefore it must be connected to a Unisense picoampere amplifier during measurements. Biosensors (or the N2O transducer part of the biosensors) should be polarized with -0.80 V. On the Microsensor Multimeter and Microsensor Monometer, lemo biosensors are recognized as a "sensor for manual settings". For details on how to set the polarization, consult the user manual of the amplifier that you are using.
In the following, we assume that the sensor is connected to a Unisense picoampere amplifier like the Unisense Microsensor Multimeter.

Remove the gas bubbles from the electrolyte

Holding the biochamber downwards, shake the sensor to get bubbles inside the sensor to rise to the top (as you would shake an old-style Mercury thermometer to reset it). Mount the sensor on a stand. Do not remove the wrapping around the biochamber.

Pre-polarization of the N_2O transducer

The N_2O transducer part of the biosensor must be pre-polarized before measurements to ensure correct functioning of the sensor.

1. Connect the sensor and adjust the pre-polarization voltage to -1,3 V and adjust the pre-amp range (mV/pA) to 0,01. Wait for approximately 5 min.
2. Adjust the polarization voltage to –0.8 V and the pre-amp range (mV/pA) to 0,1. Immediately after the sensor is connected, the signal will be very high and fluctuate (possibly out of the picoammeter's measuring range for some minutes). The signal should decrease to less than 200 mV within 2 hours of connecting the sensor to the picoammeter. If this is not the case, consult Troubleshooting.

Calibration

Please consult the relevant software manual for instruction on how to calibrate in the software.

IMPORTANT
Always calibrate at the same temperature, salinity, and stirring velocity as the measurements.

The sensor must be calibrated in standard NO_x^- solutions before measurements can be performed. The sensor responds linearly to low concentrations of NO_x^- (0-1000 µM) at temperatures above 20ºC and thus it is normally sufficient to use a two-point calibration, e.g. at 0 and 200 µM. However, it is a good idea to make calibration curves with more data points in order to develop a routine and become acquainted with the use of the sensor. Always make sure that the concentrations used for calibration cover the expected range of NO_x^- concentrations in your sample.

Consider the stirring conditions under which the sample analysis will be conducted, and use the same conditions for calibration. The best results are obtained in stirred liquid. Stirring is best obtained using a magnetic stirrer, but be aware of possible heating of the sample by the stirrer. It may be necessary to use a heat-insulating plate on top of the stirrer. If the sample volume is very small, stirring during measurements may not be possible and in this case the sensor should also be calibrated in still liquid. For measuring in such small samples, it is recommended to use the flow cell for the biosensor (please see Advanced use of the biosensor).

Remove the plastic bag from the biochamber and place the sensor in the Unisense calibration chamber containing a calibration solution (200 µM NO_x^-). The O-ring on the shaft of the sensor should be adjusted so that the tip of the sensor is well submersed in the liquid. Wait for a stable signal (usually between 3-8 nA at room temperature). This may take up to an hour.

Now the sensor is ready to be calibrated.

The sensor's response to NO_x^- is affected by changes in the ionic composition of the samples being analyzed (this is caused by the so-called "Donnan-effect") and the signal can differ by up to 30% from seawater to fresh water. This sensor must be calibrated in a

solution with approximately the same ionic composition as the sample. Here are two ways to prepare the calibration solution: using a prepared calibration solution and using water from the sampling site. If you are working with samples with unknown ionic composition, we recommend to use water from the sampling site and prepare solutions by the "known additions method". For samples from salt or brackish waters, it is possible to prepare a calibration solution by adding NaCl to demineralized water to obtain the right salinity. This is because NaCl will account for the majority of ions in the sample.

1. Using a prepared calibration solution
   a. Prepare a NO_x^--free solution with approximately the same ionic composition as the sample by adding NaCl. Place the sensor in the solution and wait for a stable signal (approximately 2 min). Beware of gas bubbles that may be caught under the tip of the sensor and affect calibration.
   b. Add NO_x^- to some of the above solution resulting in a known concentration and observe the increase in signal from the sensor. Remember that both temperature and stirring during calibration must be the same as those during measurements. Wait until the signal is stable. This procedure can be repeated with several concentrations of NO_x^-.
   c. When the calibration is completed, a calibration curve can be made, and the sensor is ready for use.

The calibration should be repeated regularly (e.g. every two hours) when starting using the sensor. A quick calibration check can be

IMPORTANT

The sensor signal is affected by the ionic composition of the sample due to the so-called "Donnan effect" across the membrane. Exact measurements can therefore only be obtained if calibration is performed in a liquid with approximately the same ionic composition as the sample. Demineralized water should thus only be used for the zero calibration value,.

performed from time to time between samples with a control measurement in a solution of known NO_x^- concentration.

2. Using water from the sampling site
(”Known additions calibration method)
By using the same liquid as the samples, the ionic composition will be identical in the sample and the calibration solution.

a. If measurements are performed in fresh water, the signal for zero NO_x^- can be determined by placing the sensor in demineralized water. If measurements are performed in brackish or saline water, the zero NO_x^- solution can be prepared using demineralized water with added NaCl corresponding to the same salinity as the sample to be analyzed. Wait for a stable signal (approximately 2 min). This is the zero-value.

b. In order to find the other point for the two-point calibration, the sensor is now placed in a known volume of water from the sampling site. Wait for a stable signal. Again, beware of small gas bubbles getting caught on the tip of the sensor.

c. Add NO_x^- to the calibration solution to obtain a known concentration (e.g. 100 µM). Now, the concentration of the solution will be the background concentration of the sample plus 100 µM. Watch the signal increase and stabilize. The increase corresponds to the added concentration of NO_x^-, and the conversion factor between

the response in pA and the NO_x^- concentration in µM can be calculated. Remember that the stirring velocity and temperature of the calibration solution must be identical to the samples to be analyzed.
The calibration procedure can be repeated with several concentrations of NO_x^-. in order to obtain a better estimate of the conversion factor.

d. The background NO_x^- concentration in the calibration solution can be determined from the difference in signal obtained for zero NO_x^- in the solution, multiplied by the conversion factor calculated from the calibration procedure (se Fig. 5).

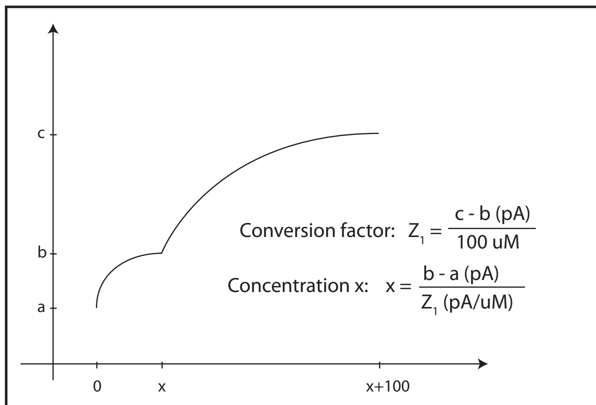

Figure 5. Example of calibration procedure calculations

The water from the sampling site can now be used as a standard - with or without addition of NO_x^- from a stock solution. If the NO_x^- concentration in the water from the sampling site is more than 25 µM, we recommend calibrating the sensor with a prepared solution as described above.

When the calibration is completed, a linear calibration curve can be plotted and the sensor is ready for use. Repeat the calibration procedure regularly in the beginning, for example every second hour. A quick calibration check can also be made with regular control measurements in a standard solution with a known NO_x^- concentration.

If the water from the sampling site has high biological activity, it is necessary to filter it through a 0.2 µm filter before using it as a calibration solution. This will prevent a change in the NO_x^- concentration over time, caused by the activity of microorganisms in the sample.

Calibration chamber CAL300

GENERAL USE OF THE BIOSENSOR

Measurements

The sensor measures NO_x^- online with a 90% response time of 1-2 minutes. It can therefore be used to measure a change in the NO_x^- concentration of a particular solution over time or to measure the NO_x^- concentration in different samples.
The membrane of the sensor should be wiped daily with a piece of cloth or tissue. This will prevent the growth of bacteria on the outside of the membrane, which can otherwise affect the functioning of the sensor.

If possible, measurements should therefore be planned so that the measured NO_x^- concentration does not vary greatly between successive individual measurements. When moving the sensor from very high (e.g. 200 μM) to very low (a few μM) concentrations in one step, the accuracy of the measurements is decreased because it takes longer for the signal to stabilize at the low concentration after exposure to very high levels of NO_x^-.

IMPORTANT
Always wipe the membrane with a wet piece of cloth or paper before use. Wiping cannot damage the membrane, and the paper or cloth should be rubbed against the membrane to ensure removal of any polymers on the outside of the membrane. Biofilm/polymers on the outside of the membrane can reduce the diffusion of NO_x^- into the reaction chamber and thereby decreases the sensitivity of the sensor.

Changing salinity of the sample solution

The sensor needs a period of acclimatization when shifting between sample solutions with different salinity or ionic composition, for example when shifting from fresh to saline water or vice versa. The length of the acclimatization period depends on the properties of the solutions that the sensor was exposed to before and after the shift.

Very high signals can occur during the acclimatization period as the activity of the bacteria in the biochamber is temporarily inhibited, which may cause oxygen to penetrate through the biomass and interfere with the N_2O transducer. This interference from oxygen disappears again after the bacteria have acclimatized.

Procedure:

1. Place the sensor in a solution with the new salinity and containing nitrate. Remember to stir the solution.
2. Await a stable signal and perform the proper calibration procedures.

If stability is not reached within 10 hours, we recommend changing the biochamber (See ”Replacement of biochamber”).

Interference

Exposure to high concentrations of sulfide should be avoided as it can severely affect the sensitivity of the biosensor.
Do not expose the sensor to toxic compounds of any sort. These include detergents, sulfide, alcohol, and solutions with a pH lower than 3 or higher than 10. On suspicion of sensor damage, repeat calibration and consult 'Trouble-shooting'.

IMPORTANT
Small gas bubbles are easily caught on the tip of the sensor but should always be removed. They may cause erroneous measurements by impeding the diffusive transport of NO_x^- into the sensor.

IMPORTANT
Always rinse the sensor with water of the same ionic composition as the samples. Do not use demineralised water.

ADVANCED USE OF THE BIOSENSOR

Measurements with a flow cell

It is possible to measure NO_x^- or NO_2^- concentrations in very small samples (down to 5 µL), by using a flowcell mounted on the biosensor (fig. 7).

The flowcell outlet tube (the longest) must be connected to a high quality peristaltic pump able to produce a stable and low flow rate. Using the tubes that came with the flowcell (0.1 CC/M) the optimal flow range is 5-100 µL/minute but flow rates down to 1 µL/minute can be used.

TIP

If you use the sensor regularly during a longer period, leave it connected to the amplifier and do not turn this off. This will spare you for the time consuming prepolarization period.

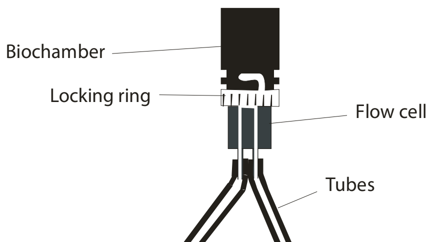

Fig. 7: Biosensor equipped with a flow cell.

Mounting the flow cell

1. Mount the flow cell on the "dummy" biochamber that came with the flow cell. The flow cell must be mounted according to Fig. 7. There are 4 stubs on the flow cell that fit to 4 depressions on the biochamber. The flow cell is fastened with the white locking ring. Ensure that the depressions on the biochamber of the sensor, are free of membrane material.

2. Fill the in- and outlet tubes with water (to get rid of air) and place a droplet of water on the surface of the flow cell to ensure that the membrane of the biosensor does not

dry out. Use zero standard water (NO_x^--free).

3. Mount the flow cell on the biochamber. If the sensor has been in an upturned position during handling, hold the biochamber downwards and shake the sensor to get bubbles in the electrolyte of the nitrous oxide transducer to rise to the top (as you would shake an old-style Mercury thermometer to reset it).
4. Mount the outlet tubes on the pump and place the end of the inlet tube in zero standard (samples must be sucked through the flow cell, not pumped).
5. Adjust the peristaltic pump to a flow rate of 5-100 µL/minute.
6. Start the pump and confirm that the sample is sucked though the flow cell by observing whether droplets are formed at the end of the outlet tube.

Calibration and measurement

To obtain accurate calibration and measurements it is important to ensure a stable temperature. Optimal temperature control is obtained by immersing the samples, flow cell, and a part of the inlet tube in a temperature controlled water bath, and using a low flow rate (5-20 µL/minute). The optimal temperature (highest sensitivity, fastest response) is obtained at 25-30°C.

TIP

Bubbles passing through the flow cell create erratic signals. In order to prevent entrapment of gas bubbles in the flow cell it is recommended to switch off the pump when changing standards or samples.

Calibration

Await a stable signal and calibrate the sensor by placing the inlet tube in different standards. To avoid intrusion of gas bubbles it is recommended to switch off the pump when changing standards. Measure the time delay from switching standards to a stable signal is obtained at the chosen flow rate.

Measurement

The biosensor equipped with a flowcell can either be used as a remote online sensor or to measure NO_x^- concentrations in discrete samples.

Online measurement:
Flow rates from 5-100 µL/minute should be used and the flow rate should be kept at a fixed level. The inlet tube can be extended if necessary. Substances from the biochamber will diffuse into the sample that passes through the flow cell. Therefore the sample, which has passed the flow cell, does not have the same chemical composition as the inlet sample.

Measurement in discrete samples:
Mixing of samples in the tubes does not happen and with known retention time in the tubes, samples can be changed with intervals of 2.5 to 3 minutes (response time of the sensor plus 1 minute). Bubbles can accumulate in the flow cell and prevent correct measurements. If there are indications of this, (erratic signals) the bubbles can be removed by briefly speeding up the flow rate. To ensure correct functioning and accurate measurements it is advisable to recalibrate during measurements by using standards.

Maintenance and storage

To prevent biofilm formation in the flow cell it must be dismounted once a day in order to clean the membrane of the biosensor.
During long-term storage, remove the flow cell and follow the instructions in "Storage and Maintenance".

STORAGE AND MAINTENANCE

WARNING

The sensor must NOT be placed in dry air, as this will cause the membrane to dry out. The sensor can be placed in the calibration solution between measurements.

Storage of the biosensor

Short-term storage (2-3 days)

During short-term storage, the sensor should remain polarized so that it can be used again immediately. The sensor should be continuously immersed in aerobic water of the same salinity as the samples to be analyzed. A simple option is to use the Unisense calibration chamber and aerate the solution. It is also advisable that the storage solution contains approx. 100 µM NO_x^- .

Long-term storage

If the sensor will not be used for some days, it should be disconnected from the amplifier. During long-term storage the sensor should be continuously immersed in aerobic water of the same salinity as the samples to be analyzed but without NO_x^-. If the sensor is used for freshwater analysis demineralized water can be used. A simple option is to use the Unisense calibration chamber and aerate the solution. When restarting the NO_x^- sensor, repeat the start-up procedures.

Storage of the biochamber

Biochambers should be refrigerated immediately after receipt to ensure the longest possible lifetime. The biochambers can be stored for at least one month under these conditions.

Replacement of the biochamber

The biochamber should be replaced if any of the following is observed, and cannot be solved by consulting Trouble-shooting:

- Non-linear calibration curve
- Very long 90% response time (> 2-3 min)
- Small or no response to NO_x^-
- Stability is not regained after changing the sample solution

Removal of the biochamber

1. Remove the tape on the biochamber.
2. Remove the biochamber from the N_2O transducer by unscrewing the white locking ring clockwise until the biochamber is unlocked, and pull the biochamber off the N_2O transducer. The biochamber should fit tightly to the transducer.
3. Rinse the N_2O transducer with demineralized water and put it down on a flat surface. Take care not to damage the exposed tip of the transducer. If the N_2O transducer is connected to the picoammeter, the signal will be very high because the transducer is sensitive to oxygen.

Test mounting of the biochamber

Optional: for users that are unsure of how to mount the chamber. Enclosed with the shipment from Unisense is a ”dummy” biochamber with no membrane, media, or bacteria. This can be used to practice mounting of the biochamber.

1. The black plastic of the N_2O transducer has a raised seam, which fits into a positioning slit on the inside of the biochamber (Fig. 9). The N_2O transducer is inserted into the biochamber by fitting this seam into the positioning slit, and guiding the tip of the transducer into of the biochamber.
2. The biochamber and the transducer are then fixed together by turning the white locking ring counter-clockwise until the flexible hooks lock onto the knobs on the biochamber. If the transducer and

biochamber are assembled correctly, the metal cathode can be observed through the small hole in the tip of the sensor when held up to a light.

Sterilizing the N_2O transducer.

1. Wipe off the exposed N_2O transducer with a piece of soft cloth or tissue.
2. Heat some demineralized water to approximately 70°C in an Erlenmeyer flask and immerse the sensor into this water for 10 seconds, ensuring that the sensor is immersed all the way to the white locking ring.
3. Dip the sensor into 70% alcohol for 10 seconds and place it on a clean surface that has been wiped with alcohol. Be careful not to damage the tip of the N_2O transducer.

Mounting of the biochamber on the N_2O transducer

1. Take the biochamber from the refrigerator. Ensure that the membrane is kept moist. The two white rings hold a membrane protective part and a lid, respectively (Fig. 9).
2. Remove one of the two pieces of tape, so that a small hole is exposed to enable pressure equilibration during handling of the biochamber.
3. Unscrew the white ring in the top of the biochamber, remove the lid and mount the chamber on the N_2O transducer as described above (Test mounting of the biochamber). Excess medium can

subsequently be pressed out of the hole for pressure equilibration. Wipe the area around the exposed hole with alcohol and cover it carefully with the enclosed black tape.

4. Place the sensor in a Unisense calibration chamber containing a calibration solution with 200 µM NO_x^-. Wait for a stable signal, typically 3-8 nA at 20°C, which can take some hours. When a stable signal is obtained, the sensor is ready for calibration and use.

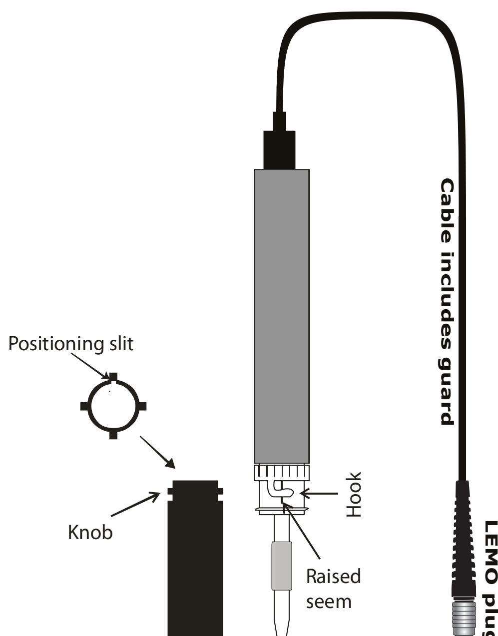

Fig.8: Biochamber and transducer separated

Switching specificity

The sensor can switch specificity from nitrate/nitrite (NO_x^-) to nitrite (NO_2^-) and vice versa by replacing the biochamber of the

sensor. However, each chamber can only be used once. After a biochamber has been used, the pressure has changed inside and it can not be re-used. Follow the procedures in the section “Replacement of biochamber”.

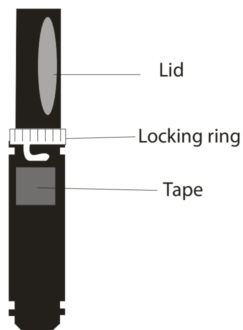

Fig. 9: Biochamber

SPECIFICATIONS

Effective measuring range

The effective measuring range is defined as the range of NO_x^- concentrations over which the sensor has a linear response to NO_x^-. Higher temperatures result in a larger measuring range, because the activity of the bacteria is positively correlated to temperature. Toxic compounds in the external environment have the opposite effect, as they reduce the bacterial activity.

Measuring range at 20°C

0-1000 µM NO_x^- at maximum 3.8% salinity

Measuring range at 10°C

0-200 µM NO_x^- at maximum 3.8% salinity

Output

1,25-4 nA per 100 µM NO_x^- (net signal) at 20°C.
Signal as a function of NO_x^- concentration is positively correlated with temperature. The temperature coefficient is 2-4% per °C. The calibration of the sensor is dependent on the composition of ions in the external environment. The net signal can vary up to 30% depending upon the ionic composition of the sample.

Temperature range at maximum 3,8 % salinity

10-38°C
Sensors that are able to measure down to 5°C can be supplied.

Response time

The response time is highly dependent on whether the sample is stirred. The fastest response is obtained in a stirred sample.
In as stirred sample, the 90% response time is less than 90 seconds at 20°C. At higher temperatures, the response time is faster while at lower temperature it is slower.

Sensitivity to stirring of the sample

The sensitivity towards stirring depends on the temperature and salinity of the sample and can be up to 20% of the signal.

Interferences

Nitrous oxide: N_2O in the external environment diffuses through the reaction chamber and is detected by the N_2O sensor. The sensitivity to N_2O is approximately 2.5 times the sensitivity for NO_x^-.
Sulfide: Sulfide gas can destroy the NO_x^- sensor. Do not use the sensor in solutions containing sulfide.

Signal drift

When the sensor is handled correctly, the signal drift is normally less than 20% per week.

Lifetime of the N_2O transducer

The N_2O transducer has a guaranteed lifetime of 800 hours and an expected lifetime of 1000-1500 hours, at constant polarization. The lifetime is prolonged if the sensor is used for short-term measurements and disconnected between periods of measurements. For long-term storage, the sensor should therefore be disconnected from the amperemeter (see section on Storage and Maintenance).

Life time of the biochamber

The lifetime of the biochamber mounted on a nitrous oxide transducer depends on the environment the sensor is exposed to. When handled correctly, it is normally several weeks.
The lifetime for an un-mounted biochamber is more than one month if is stored at 4°C.

Detection limits

At 10°C: < 0.3 µM
At 20°C: < 0.2 µM
At 30°C: < 0.2 µM

TROUBLE SHOOTING

Problem The signal is very high (>2 nA) and not stable in a NO_x^- free solution.

Possible cause Oxygen interference.

Solution Test for sensitivity to oxygen: Place the sensor in a calibration solution free of oxygen and NO_x^-. Oxygen can be removed by aerating with an inert gas (such as N_2). If the signal decreases below 2 nA, the sensor has become oxygen sensitive.
Place the sensor in a stirred, NO_x^- free calibration solution aerated with atmospheric air. Wait until the signal stabilizes at a low value (< 2 nA) and repeat calibration (see Section on Calibration). If the sensor is still sensitive to oxygen after 24 hours, the biochamber itself has been damaged and should be replaced

Problem No or negative signal from the sensor

Possible cause Bubbles in the electrolyte have disconnected the electrical circuit of the N_2O transducer.

Solution Shake the sensor while holding it upright so that bubbles within the electrolyte rise (as you would shake an old style mercury thermometer). Prevent the membrane from drying out by holding a piece of moist tissue in front of it. The sensor will need some hours to stabilise following this procedure and calibration should be repeated.

Problem High and unstable zero current (more than 2 nA) in solutions free of oxygen and NO_x^-.

Possible cause The N_2O transducer is defective.

Solution Replace the N_2O transducer and send the defective transducer back to Unisense. The N_2O transducer is guaranteed for one month after receipt and defective sensors will be replaced within this period.

Problem The sensor is not sensitive to NO_x^-.

Possible cause 1 Bubbles caught on the tip of the sensor block the diffusion of NO_x^- into the biochamber.

Solution Remove the bubbles. It is not necessary to recalibrate the sensor.

Possible cause 2 Biofilm or precipitations on the membrane block the diffusion of NO_x^- into the biochamber.

Solution Wipe the membrane thoroughly with a moist cloth or tissue. When the sensor has a stable signal, calibration should be repeated. If the signal does not stabilise, the biochamber should be replaced.

Problem Non-linear calibration (higher response per µM NO_x^- - at higher concentration).

Possible cause Contamination of the biochamber or precipitation in the biochamber medium.

Solution Replace the biochamber.

If you encounter other problems and need scientific/technical assistance, please contact sales@unisense.com for online support (we will answer you within one workday)

APPENDIX: EQUILIBRIUM N_2O CONCENTRATIONS

Equilibrium nitrous oxide concentrations (mmol/liter) at ambient partial pressure of 1 atm. in water as a function of temperature and salinity.

Sources:
Weiss, R.F; Price, B. A.: Marine, Chemistry, 1980, 8, 347-359.

Table 1

(‰) / °C	0	5	10	16	20	25	30	36	40
0	59.35	48.46	40.16	32.66	27.05	24.09	21.61	18.61	16.98
10	55.85	45.73	37.99	30.96	25.69	22.91	20.57	17.73	16.18
20	52.58	43.15	35.93	29.35	24.40	21.78	19.58	16.89	15.42
30	49.50	40.73	33.98	27.82	23.18	20.71	18.63	16.09	14.70
35	48.03	39.56	33.05	27.09	22.59	20.19	18.18	15.70	14.35
38	47.17	38.88	32.50	26.66	22.24	19.89	17.91	15.48	14.15
40	46.60	38.43	32.14	26.37	22.01	19.69	17.73	15.33	14.01

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