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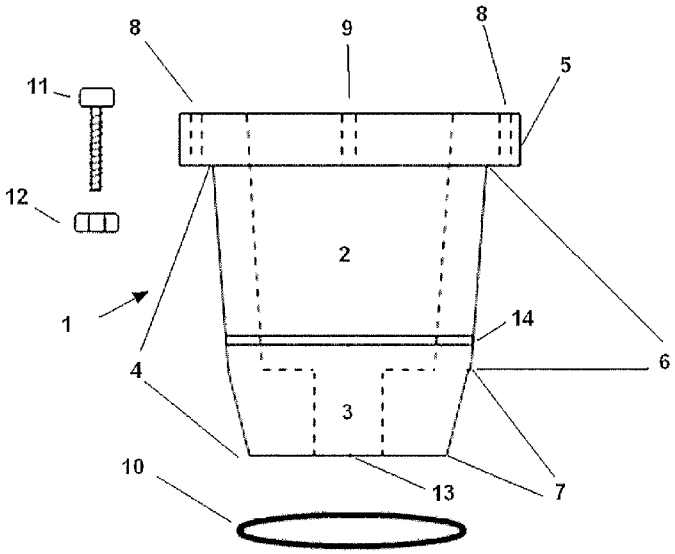


Fig. 1

(57) Abstract: There is provided a stopper device for applying a sensor, wherein said stopper comprises: a first section providing a first tapered section for fitting to a correspondingly tapered section of a sample container, said first section also comprises a second tapered section adjacent said first tapered section. The first section is provided with a sealing means such that a gastight seal is provided between the stoppers first tapered section and the sample container. The first section is provided with a void wherein at least part of the sensor can be fitted in an gas tight manner. A second section is provided with a channel for a sensor cable. There is also provided a sample container and method for measurement.

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STOPPER SAMPLE CONTAINER AND METHOD OF MEASUREMENT USING SAID STOPPER

DESCRIPTION

The following invention is directed towards a stopper according to claim 1, a sample container according to claim 8, and a method according to claim 9.

BACKGROUND

Sensors are rapidly developed for continuous measurements of different dissolved quantities in aqueous samples. Placing and removing a sensor in a gas tight container may pose problems. The sensor has to be attached to the container in a gas tight manner and sensors cables should be possible to connect from the outside. A gastight seal between the container, the sensor, and the ambient environment has to be established when the sensor is placed in the container, and broken again when the sensor is removed. In case of liquid samples, air bubbles should not be trapped inside the container as they may interfere with the measurement. The sensor should be easy to remove after measurement and not get stuck in the container. In sample containers which are filled with sample fluids and emptied with regular intervals the problem manifests itself regularly.

SUMMARY

It is an object of the present invention at least to alleviate the problems outlined above.

According to an aspect of the invention the object is achieved by a stopper device for applying a sensor, wherein said stopper comprises:

a first section providing a first tapered section for fitting to a correspondingly tapered section of a sample container, said first section also comprises a second tapered section adjacent said first tapered section, said second tapered section has a more acute angle than the first tapered section for avoiding trapping of air bubbles during measurement, when said stopper is fitted to a sample container,

the first section is provided with a sealing means, preferably in the form of at least one o-ring, such that a gastight seal is provided between the stoppers first tapered section and the sample container,

the first section is provided with a void wherein at least part of the sensor can be fitted in an gas tight manner, said void runs through the first section,

a second section is provided with a channel for a sensor cable, said second section is also provided with removal means, arranged to raise the stopper from the sample container, thus facilitating removal of said stopper.

The stopper device may be used to close a container, specifically a sample container such as a sample bottle. Alternatively, the stopper device may be referred to as a stopper. Such a container may be used for measuring water samples using a sensor. Water samples may be take e.g. from lakes and the sea, from process water used in various processing industries, from sewage, and water purification plants.

According to embodiments said void may be adapted for an optode sensor, preferably being capable of making online registration of respiration values from a sample of water from a coastal region.

According to embodiments said respiration values may be measured in the form of oxygen content.

According to embodiments said sensor may be able to record any of the following parameters, pH, temperature, salinity, conductivity, or other parameters.

According to embodiments the removal means may be constituted by at least one screw, being arranged to raise said stopper by contacting the sample container when screwed, preferably the top of the sample container.

According to embodiments said sample container may be a sample bottle.

According to embodiments said stopper device may be arranged to fit a sample bottle of the size of at least 100 ml preferably at least 500 ml, more preferably of 1000 ml.

According to a further aspect of the invention there is provided a sample container comprising a stopper device according to any one of aspect or embodiments described herein.

According to a further aspect of the invention there is provided a method for measurement, comprising the following steps:

- providing a sample of water,
- providing a sensor for the measurement,
- applying said sensor to a sample bottle by means of an stopper device according to any one of aspects or embodiments disclosed herein,
- attaching said sensor to a control unit,
- registration of measurement values from said sensor by said control unit.

The method may be used for measurement of pelagic respiration in aquatic samples, such as samples of coastal waters. The registration of measurement values by said control unit may be a registration of respiration values.

According to embodiments the sample may be provided in the sample bottle.

According to embodiments said sample may have a size which is larger than 100 ml, preferably larger than 500 ml, more preferably a size of 1000 ml.

According to embodiments said control unit may be portable, preferably in the form of a portable computer such as a laptop, such that online measurement can be performed outside a normal laboratory environment.

According to embodiments measurement of the following parameters may be provided, pH, temperature, salinity, conductivity, or others.

Further features of, and advantages with, the present invention will become apparent when studying the appended claims and the following detailed description. Those skilled in the art will realize that different features of the present invention may be combined to create embodiments other than those described in the following, without departing from the scope of the present invention, as defined by the appended claims.

LIST OF DRAWINGS

Fig. 1 Shows a stopper, release means and a seal, for an embodiment of the invention

Fig. 2 Shows a stopper according to an embodiment of the invention.

Fig. 3 Shows an experimental set-up for measurement with stopper, sample bottle and computer.

Fig. 4 Shows agreement of respiration measurement with established current method.

Fig. 5 Shows accuracy of respiration measurement.

Fig. 6 Shows detection limit and precision of respiration measurement.

DETAILED DESCRIPTION

The present invention will now be described more fully with reference to the accompanying drawings, in which example embodiments are shown. However, this invention should not be construed as limited to the embodiments set forth herein. Disclosed features of example embodiments may be combined as readily understood by one of ordinary skill in the art to

which this invention belongs. Like numbers refer to like elements throughout. Well-known functions or constructions will not necessarily be described in detail for brevity and/or clarity.

A stopper 1 for providing a sensor in a gastight manner to a sample container is described. The stopper 1 may also be referred to as a stopper device 1. Said stopper has a first section 4 provided with two tapered sections 6 and 7. The first tapered section 6 is arranged to be able to fit to a sample container's neck. The second tapered section 7 is provided for avoiding trapping of gas bubbles during sampling. The angle of the second tapered section 7 is more acute than that of the first tapered section 6. A second section 5 is provided with non tapered sides. The second section has through holes 8 for receiving release means 11. Said release means can optionally be provided with an adjustment means 12. The release means 11 is arranged to be able to contact the upper surface of a sample container. The release means 11 is preferably constituted by at least one screw. The adjustment means 12 is preferably constituted by at least one nut.

The stopper has a first void divided into two void sections 2 and 3. On of the void sections 3 is arranged to be able to receive the sensor discussed above. Preferably a section of the sensor is arranged to be fitted into said void section 3. Said void section 3 preferably has the form of a cylinder and is a through hole with a diameter that allows a sensor to be fitted thereto in a gas tight manner. The shape of the void section 3 can also be tapered. Adjacent and linked to said void section 3 is a larger void section 2 positioned. Said void section 2 is optimized for housing wiring and contacts to the said sensor. This void section 2 can be tapered or have cylinder shape.

The stopper can be provided with a notch 14 for receiving a sealing means 10, preferably in the form of an O-ring. Said notch can be suspended with if another type of seal is provided.

The main application of said stopper is for an Optode sensor. Said sensor is preferably used in an online measurement of respiration in coastal waters. However adjustments of the stopper would allow it to be used with other sensors as well.

In a method of measurement of respiration of coastal waters said stopper 1 may be utilized, the method comprises the following steps:

- providing a sample of water,
- providing a sensor for the measurement,
- applying said sensor to a sample bottle by means of an stopper device according to claim 1 or 2,

- attaching said sensor to a control unit,
- registration of said respiration values by said control unit

The sample size can be larger than 100 ml, preferably larger than 500 ml. However should a smaller sensor be used a smaller sample size could be allowed.

Said control unit is preferably provided with a computer program product able to store the sensors measurement values, and evaluate the said values, such that an online measurement is possible.

It should be understood that the above described stopper and method also are applicable for fresh water, sewage water, and process water application. It has been shown to be possible to record oxygen content in fresh water as well.

The invention is further described in the following:

Respiration is one of the most important processes in the biosphere, from the level of metabolism in individual organisms to regulation of CO_2 and O_2 levels in the atmosphere. Respiration is also fundamental to understand occurrence of hypoxic waters and the balance between auto- and heterotrophic processes in the sea and its influence of the downward transport of biomass through sedimentation (i.e. the marine biological pump). Measurement of biological oxygen demand (BOD) is also a standard method in controlling the quality of waste waters. Thus, estimates of respiration are important to understand mechanisms behind current societal concerns like the increasing levels of CO_2 in the atmosphere (i.e., the Greenhouse effect) and oxygen deficiency in coastal water bodies (hypoxia). Understanding the level and control of respiration is, therefore, an important issue for natural science. One reason for the insufficient knowledge about this process is that respiration studies currently are scarce, compared with CO_2 fixation measurements, partly due to methodological reasons.

Consequently, accurate and robust measurements of respiration are important for the advancement of this research field. Especially for marine pelagic ecosystems that require high levels of precision for respiration rate measurements because the ambient oxygen pool may change of as little as 0,01 % day^{-1}. In absolute units, this demands that rates between 0.02 to 75 mmol O_2 m^{-3} day^{-1} have to be measured. For coastal waters, the observed rates are higher, ranging from 1.7 to 84 mmol O_2 m^{-3} day^{-1}.

One of the commonly applied techniques to measure respiration in water samples is to analyze changes in dissolved oxygen concentrations by end point titration with the high-precision Winkler titration technique. The precision and detection limit of this technique are also adequate for pelagic measurements in the ocean. A theoretical detection limit of 0.07 mmol O_2 m^{-3} day^{-1} has been reported for automated Winkler titration, while practical precision reported from the field ranges between 0.1 and 2 mmol O_2 m^{-3} day^{-1}. Analysis of low respiration rates with this technique requires a relatively high level of sample replication per rate estimate, involving several titrations. The time consuming wet chemistry and meticulous handling that are required hamper spatial and temporal coverage of respiration measurements and make this technique difficult for a layperson to use. Additionally, a linear decline in oxygen is typically assumed, and incubation times are relatively long. Furthermore, sample volumes are typically 60-120 ml, excluding sufficient numbers of larger zooplankton to determine their contribution to the measured respiration rate. Still, the in vitro dark bottle incubation measuring change in oxygen concentration with the Winkler technique is the most commonly used method, comprising more than 90 % of the values reported in marine pelagic environments.

A sensor technique to measure oxygen is based on the Clark electrode. It has a reported precision of 0.1 µM when measuring oxygen concentration. However, the electrode has an inherent oxygen consumption corresponding to 0.3 mmol $m^{-3}d^{-1}$, which is higher than the detection limit of the high-precision Winkler technique. Lower inherent oxygen consumption is reported at the Unisense® web page, however, no published reference or calculation is provided. In the original reference to the method a precision of 2.4 mmol $m^{-3}d^{-1}$ or higher is reported for respiration measurement. This is a more than 10 times higher uncertainty than the results of the high precision Winkler technique.

Dynamic luminescence quenching (DLQ) has recently been introduced in marine sciences as an optical method to accurately determine oxygen concentrations with long-term stability and high precision (<1µM). Oxygen measurements can be made with high frequency (i.e., seconds – minutes) and precision by a computer in an inert manner devoid of inherent oxygen consumption (c.f. Clark electrode above), suggesting that changes in oxygen over time can be measured with confidence. In addition, temporal changes in oxygen levels can be monitored without assuming linearity over longer time periods.

To the knowledge of the inventor, the use of DLQ-based systems to directly measure plankton respiration without preconcentration of organisms has not been reported. Herein a novel application of the DLQ-technique and a new measurements system to more readily

and precisely estimate respiration in aquatic environments than currently possible is presented. However, in other applications, DLQ has recently been used to measure respiration in cultures, concentrated samples and sediment-water interfaces.

Herein it is established whether the accuracy, detection limit and precision of one type of commercially available Optode are sufficient to provide absolute respiration rates in unmanipulated marine pelagic samples. Samples were taken from coastal and offshore environments over a wide salinity range. An analytical unit was developed that allows the Optode to be mounted to a sample bottle that can be maintained under stable temperature conditions. Efforts were made to maximize precision and minimize handling while allow measurements to be made on a routine basis. There is also discussed the potential advantages and limitations of the present technique when performed in the field aboard a ship.

An example embodiment may be set up as follows:

Oxygen concentration in the respiration experiments were measured with four commercially available Optodes with an integrated temperature sensor (Optode 3835, Aanderaa Data Instruments AS,www.aadi.no). This type of sensor has been frequently used. As with most other oxygen sensors Optodes measure the partial pressure of oxygen dissolved in water. By tuning the sensor to the salinity of the water sample, data were automatically converted to the absolute oxygen concentration ($[O_2]$, µmol dm^{-3}) using reported formulas. Sensor readings were plotted and logged in real-time using the software package Oxyview by Aanderaa Data Instruments AS (www.aadi.no).

One portable computer was used to log data from up to 4 Optodes simultaneously (Fig. 3). An 8-port serial hub with an external power supply (VScom, USB-8COM, www.vscom.de) was used to collect RS232 signals, and an 8-port hub (D-link, DUB-H7 7-PORT USB 2.0 HUB, www.dlink.com) provided electrical power (5V) from the computer to the four Optodes. If desired, the same system could run four additional Optodes, allowing further sample replication. Fig. 3 shows a schematic diagram of the respiration measurement unit: 21, Immersion cooler; 22, Water bath with thermostat; 23, Sample bottles with cables connected to the sensor plug in the stopper; 24, Magnetic stirrers; 25, Serial Hub; 26, USB Hub for 5 V power to sensors; 27, Portable computer.

According to example embodiments a sample container, such as an analysis bottle, and mounting of the Optode may be utilized as follows:

A 1 dm^3 clear glass bottle (Cat. No. HAVA23231607, VWR) with a NS60 grounded neck, (inner ∅ 60 mm) was selected as the incubation vessel. This provides a sample volume about 10 times larger than is typically used in Winkler titrations. An important advantage of the present technique is that, at least in coastal areas (5-10 individuals dm^{-3}), large zooplankton may also be sampled in a statistically acceptable number to be included in the respiration estimate.

A stopper device 1 may be constructed from acrylic plastic (poly(methylmethacrylate)) to mount the Optode in, forming an air-tight seal by an o-ring, e.g.Nitrile 50.52 x 1.78 mm, while still allowing easy removal of the stopper after incubations. The latter is further aided by release means 11, such as three screws placed in the upper part of the stopper device 1. The air-tight seal ensures that minimum gas exchange between the sample and the surrounding air. The stopper device 1 has a conical shape, similar to the design of typical Winkler bottles (Biochemical Oxygen Demand bottles), to allow air bubbles to readily escape. The stopper device 1 allows simple mounting and dismounting of the Optode onto the sample bottle, without the trapping of air bubbles. The Optode adaptor foot is inserted into the stopper tube and sealed by 2 o-rings towards the tube wall. The cable attachment screw will lock the Optode in the stopper device 1 from the outside.

A magnetic stirrer (Ikamag RET, www.ika.com) and 2 cm magnetic rods were used for each analysis bottle. The magnetic stirrer was set at 80 rpm to mix the water in the analysis bottle. A stand may be provided to keep bottles in position during rough seas and maintain proper stirring and temperature control of the samples.

In example embodiemtns, the thermostat heated water bath may have inner dimensions of 32.5 cm (length), 30 cm (width) and 14.5 cm (depth) (Julabo 12 B, www.vwr.com), allowing the sample bottle to be covered by liquid up to the lower part of the neck. The bath was used in conjunction with an immersion cooler (Julabo FT 200, www.vwr.com) to control the incubator bath e.g. in a range of 0-30 °C with a precision of ±0.05 °C. To prevent freezing at temperatures approaching zero °C, 30 % polypropylene glycol may be used as the bath liquid. The bottles and immersion cooler probe may be placed in a specially designed plastic holder to keep them in a fixed position and promote stable temperatures.

Samples may be protected from light with a cardboard box and black plastic bag. The level of light irradiation inside the cardboard box may be below the detection limit (< 0.04 µmol m^{-2} s^{-1}) at an ambient room light of 17.6 µmol m^{-2} s^{-1}. Light inside the incubators may be measured with a Li-Cor data logger (LI-1000) and a spherical quantum sensor for

photosynthetic active radiation (Biosperical Instruments, QSPL 2101, www.biospherical.com) immersed in the water bath.

According to example embodiemnts drift-corrected Optode calibrated with Winkler measurements may be performed as follows:

The Optode measurements that were corrected for drift showed good agreement with respiration rates determined by the Winkler titration method, Fig. 4. The Optode-based rates were 105 % and 101 % that of the Winkler-based values at the in situ and + 3° temperatures, respectively. This supported the conclusion that Optode measurements required drift correction to be comparable to measurements made by the Winkler method. It also shows that the estimated size of the mean short-term system drift was reasonable. Fig. 4 shows comparison between Winkler titration (n=2×8) and the Optode sensor. The Optode values were corrected for system drift. The comparison was done at two temperatures to obtain different rates in parallel incubations. Error lines show the 95 % confidence intervals. For the Optode, the sum of regression and drift confidence intervals were used for calculation of the 95 % C.I. (N=2).

According to example embodiments expected increase with temperature may be as follows:

As a positive control, measurements of respiration rates with the Optode method were performed at different temperatures. This provided a controlled way to test the response of the method since an increase in the respiration rate is expected with elevated temperatures. Using samples from the same Niskin bottle, there was found the expected exponential increase in oxygen consumption with increasing temperature, Fig. 5. The temperature dependence of oxygen consumption corresponded to a Q_{10} –value of 3.0, comparable to the levels found in earlier studies. This result suggests that the Optode method accurately measures respiration rates with good precision when a system drift correction is applied. Fig. 5 shows the temperature dependence of oxygen consumption in sea water is shown. A system drift correction was applied. The sample was from the Bothnian Sea (Stn B3) taken at 1 m depth and incubated in parallel at 4 different temperatures in a temperature controlled room. The in situ temperature was 19 °C. Error bars for respiration rates show the 95 % confidence interval, including the system drift error. Error bars for the temperature were hidden by the vertical bars.

The precision of respiration estimates (i.e., slope coefficient) was, on average, 0.3 mmol m^{-3} d^{-1} (95 % C. I.) and varied little between different measurements. The method, therefore,

seemed to have good reproducibility, despite the variability observed in the time series. At this level of precision, one-third of the offshore measurements were at or below the detection limit. Pre-conditioning of the stopper and Optode in low oxygen water could, however, improve the detection limit to 0.15 mmol m^3 d^{-1}, close to the high precision Winkler titrations. The reason for the reproducibility of the precision was that the error of the applied system drift correction accounted for the majority of the uncertainty. The uncertainty of the oxygen change in each time series, excluding contribution from drift correction, was one third of the error (median 95 % C. I. ±0.15 mmol m^{-3} d^{-1}).

The Optode with dynamic luminescence quenching (DLQ) technique showed a detection limit and precision similar to those obtained with the high-precision Winkler titration method (Fig. 6). Typical rates of respiration found in coastal waters and oceanic surface water during the productive seasons should, therefore, be above the detection limit. This method does however not currently have sufficient precision to routinely monitor respiration rates below the euphotic zone or in oligotrophic oceanic sites that often fall below 0.3 mmol m^{-3} d^{-1}. The same limitation applies to surface and coastal waters during the winter when productivity is lower. The detection limit of the Optode in the field was about 3 times higher than the best reported high-precision Winkler titrations from two field studies, while being comparable to other studies using Winkler titrations. The Optode sensor showed the best precision for sensor-based techniques that have been reported in the literature. Pre-treatment of the stopper and Optode in low oxygen also show a potential to approach the best precision reported (i.e. 0.15 mmol m^{-3} d^{-1}).

The primary advantage of the Optode method is that it provides a simple and operator-independent protocol to measure respiration in natural waters without inherent oxygen consumption. The Optod can run for several years with annual calibration and the sensor foil alone can be renewed to extend the life-time of the Optode, promoting good economy. This may promote the incorporation of respiration measurements in field studies and make the use of respiration data in environmental monitoring programs feasible. Due to the ability to easily perform many measurements, the Optode technique could lead to new insights about factors that affect respiration rates. Additionally, with a higher rate of data collection in time and space, a better basis for establishing regional and global carbon budgets may be gained. Including respiration measurements in marine monitoring programs would also provide more reliable assessments of ecosystem productivity and, thereby, management of eutrophication.

The field estimates for a Clark electrode reported in Langdon et al. 1995 was relatively high (6-8 µmol $dm^{-3} d^{-1}$) and lacked precision estimates or presentation of time series. In the original reference to the method by Langdon (1984) a precision of 2.4 µmol $dm^{-3}d^{-1}$ or higher is reported. This is about 10 times higher than results presented for the Optode, and even higher compared to high-precision Winkler titration.

According to the power analysis for the Optode time series a theoretical detection limit of 0.04 mmol $m^{-3} d^{-1}$ at a 250 µM oxygen concentration in 24 hour incubation is possible. This calculation was based on the median coefficient of variation around the regression line of autoclaved samples (CV=0.066 %). Robinson and Williams calculated a theoretical detection limit of 0.07 mmol $m^{-3} d^{-1}$ for Winkler titrations. The dynamic luminescence quenching technique therefore has the potential to provide continuous measurements of respiration of highest known precision today. The possibility to investigate short-term variation is therefore an advantage of this method. However, according to the power analysis shorter incubation times elevate the detection limit, and rate differences as high as 1 mmol $m^{-3} d^{-1}$ must e.g. occur to be detected in 3-h incubations.

The developed analytical unit and protocol was able to be used aboard research vessels, even during rough seas. The time from sampling to the start of 4 incubations was typically less than 20 minutes. The working time was limited to emptying, rinsing and filling sample bottles, followed by setting up the measurements in the Optode software. The avoidance of wet chemistry minimized operator influence on the results, resources, waste, and improved safety for technical staff. The Optode method, however, required stringent temperature control and evaluation of time series. The stable temperature (±0.05 °C) that was routinely achieved minimized the temperature influence on oxygen measurements.

One further advantage of the presented Optode method is the 3-20 times larger sample volume (1 dm^{-3}) obtained compared to Winkler titrations (0.05-0.3 dm^{-3}). A larger sample includes more organisms. This is advantageous if a general estimate of plankton oxygen consumption. A larger sample also may reduce other containment effects and allows extended incubation time. The current setup of present technique would, therefore, give an accurate estimate of microbial plankton respiration comprising, on average, 99 % of aquatic respiration.

The online continuous monitoring of the oxygen concentration by computer software precluded assumptions of linearity of oxygen change. This also enabled a correct

mathematical model to be applied for rate determination. However, approximately linear development with time was typically observed at most sites investigated.

According to some embodiments, the present Optode method is applicable to other aquatic environments where respiration rates are above 0.3 mmol m^{-3} d^{-1}, Fig. 6. The same is true for culture studies. For application in deep and cold water environments, however, the detection limit must be lowered. Pre-incubating the stopper and Optode in low oxygen water 24 hours prior to measurements, to remove oxygen bound to the plastic material may be performed. This improved the detection limit to 0.19 mmol m^{-3} d^{-1}. According to some embodiments the stopper device 1 and Optode may be without plastic material. Fig. 6 shows performance of the dynamic luminescence technique as measured with the Optode sensor. Accuracy shows the relative deviation from Winkler titrations when the drift correction is applied. Precision shows the combined 95 % confidence intervals for field incubations and the drift subtraction. The detection limit is defined as one 95 % confidence interval. The system drift is the mean of 37 measurements in autoclaved sea water samples.

Example embodiments described above may be combined as understood by a person skilled in the art. Although the invention has been described with reference to example embodiments, many different alterations, modifications and the like will become apparent for those skilled in the art. Therefore, it is to be understood that the foregoing is illustrative of various example embodiments and that the invention is defined only the appended claims.

CLAIMS

1. Stopper device for applying a sensor, wherein said stopper comprises:
a first section providing a first tapered section for fitting to a correspondingly tapered section of a sample container, said first section also comprises a second tapered section adjacent said first tapered section, said second tapered section has a more acute angle than the first tapered section for avoiding trapping of air bubbles during measurement, when said stopper is fitted to a sample container,

the first section is provided with a sealing means, preferably in the form of at least one o-ring, such that a gastight seal is provided between the stoppers first tapered section and the sample container,

the first section is provided with a void wherein at least part of the sensor can be fitted in an gas tight manner, said void runs through the first section,

a second section is provided with a channel for a sensor cable, said second section is also provided with removal means, arranged to raise the stopper from the sample container, thus facilitating removal of said stopper.

2. Stopper device according to claim 1, wherein said void is adapted for an optode sensor, preferably being capable of making online registration of respiration values from a sample of water from a coastal region.

3. Stopper device according to claim 2 wherein, said respiration values are measured in the form of oxygen content.

4. Stopper device according to claim 1, wherein said sensor is able to record any of the following parameters, pH, temperature, salinity, conductivity, or other parameters.

5. Stopper device according to any of the claims above wherein, the removal means is constituted by at least one screw, being arranged to raise said stopper by contacting the sample container when screwed, preferably the top of the sample container.

6. Stopper device according to any of the claims above wherein, said sample container is a sample bottle.

7. Stopper device according to claim 6, wherein said stopper device is arranged to fit a sample bottle of the size of at least 100 ml preferably at least 500 ml, more preferably of 1000 ml.

8. A sample container comprising a stopper device according to any one of claims 1 – 7.

9. Method for measurement, comprising the following steps:
- providing a sample of water,
- providing a sensor for the measurement,
- applying said sensor to a sample bottle by means of an stopper device according to claim 1 or 2,
- attaching said sensor to a control unit,
- registration of measurement values from said sensor by said control unit.

10. Method according to claim 9 wherein, said sample has a size which is larger than 100 ml, preferably larger than 500 ml, more preferably a size of 1000 ml.

11. Method according to claim 9 or 10, wherein said control unit is portable, preferably in the form of a portable computer such as a laptop, such that online measurement can be performed outside a normal laboratory environment.

12. Method according to any of the claims 9 – 11 wherein, measurement of the following parameters is provided, pH, temperature, salinity, conductivity, or others.

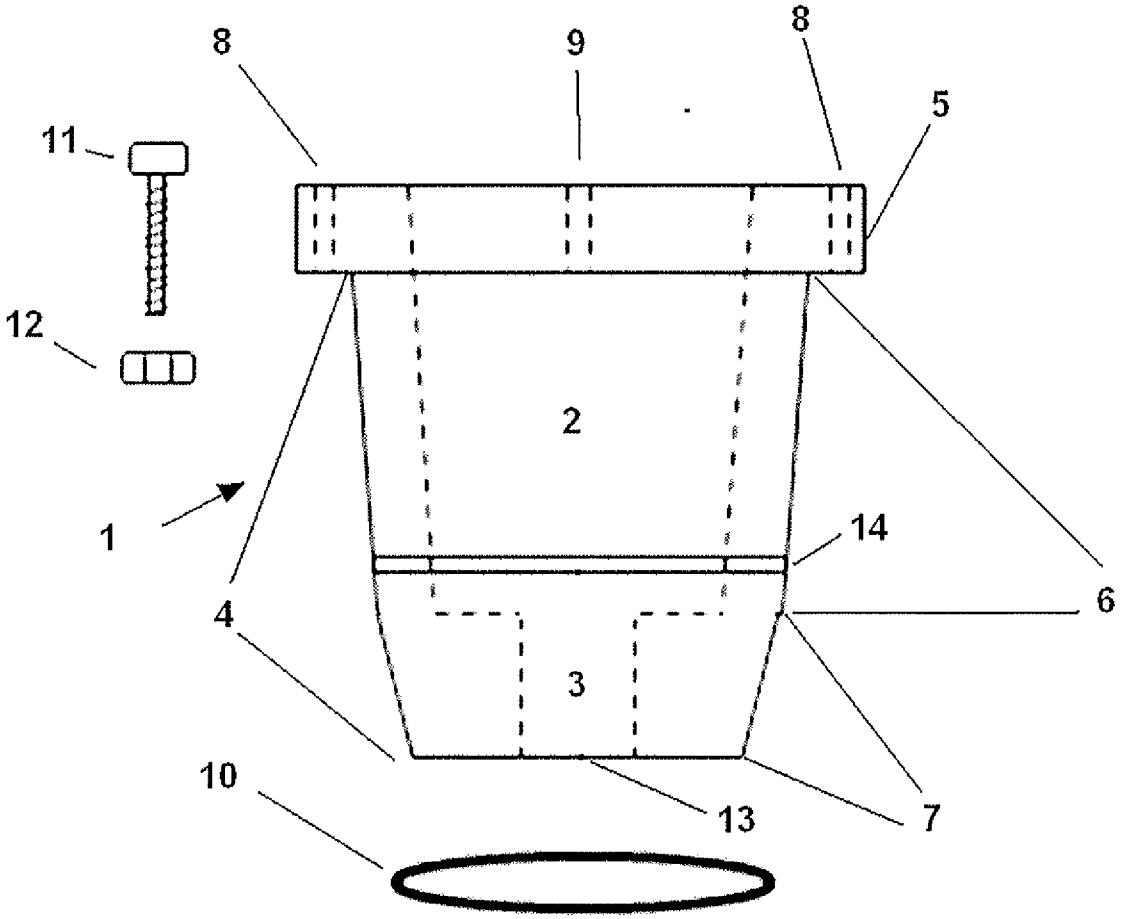


Fig. 1

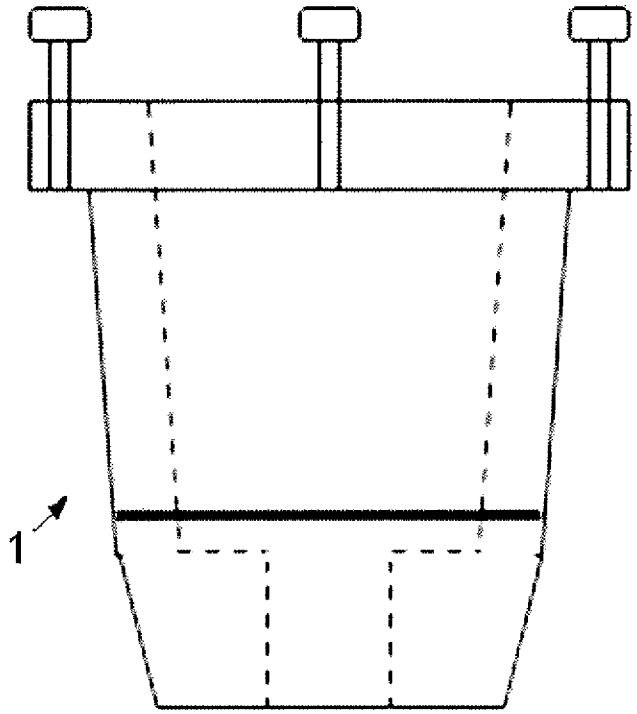


Fig. 2

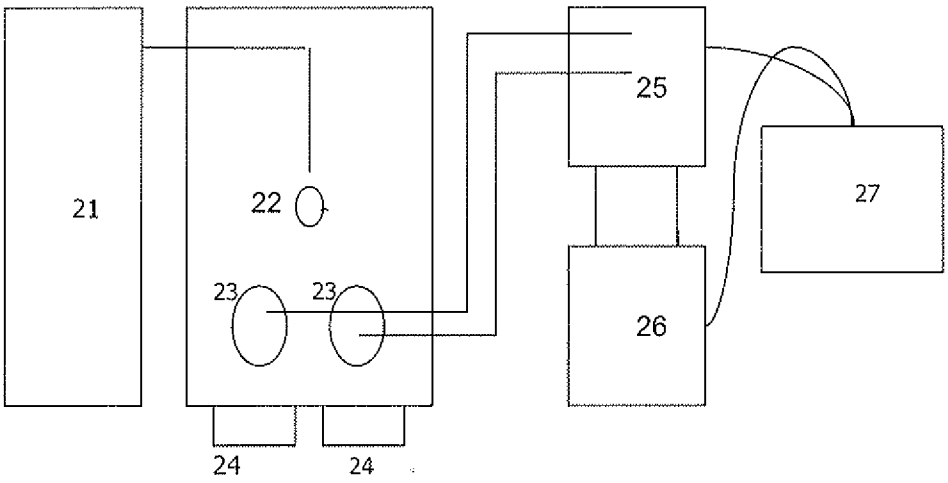


Fig. 3

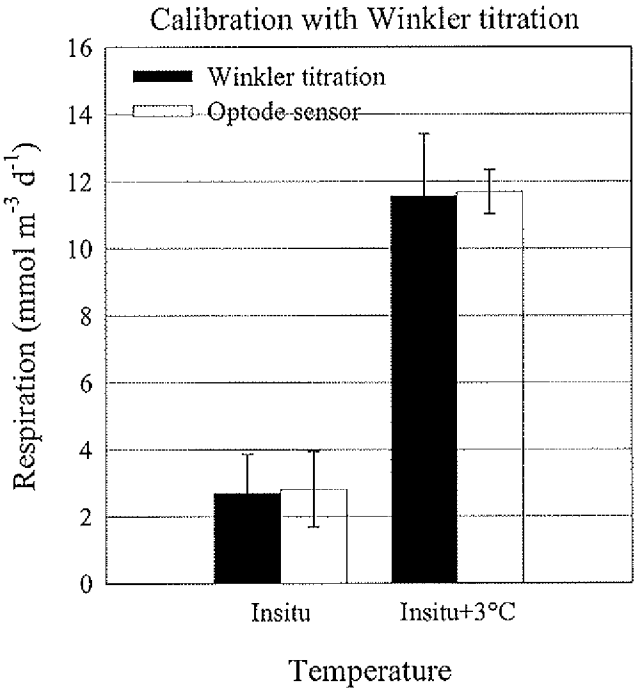

Calibration with Winkler titration
Winkler titration
Optode sensor
Respiration (mmol m-3 d-1)
16
14
12
10
8
6
4
2
0
Insitu
Insitu+3°C
Temperature

Fig. 4

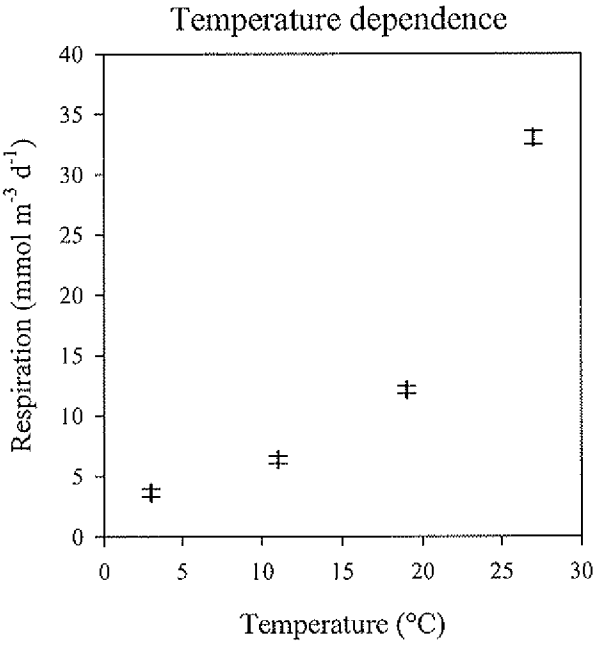

Temperature dependence
Respiration (mmol m-3 d-1)
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
Temperature (°C)

Fig. 5

Accuracy	Precision	Detection limit	System drift
(%)	(mmol m^{-3} d^{-1})	(mmol m^{-3} d^{-1})	(mmol m^{-3} d^{-1})
103	±0.3	0.3	2.1 (±0.3)[a]

[a] 95 %Confidence interval.

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2012/050846

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: C12M, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, PAJ, BIOSIS, COMPENDEX, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5447374 A (FRASER DOUGLAS S ET AL), 5 September 1995 (1995-09-05); abstract; figures 1-8 --	1-12
A	US 5085759 A (HARKER ALAN R), 4 February 1992 (1992-02-04); abstract; figures 1,3A --	1-12
A	EP 0208949 A2 (PLISCHKE & BUHR KG), 21 January 1987 (1987-01-21); abstract; figure 1 --	1-12
A	US 5770153 A (WAGNER INGEBORG), 23 June 1998 (1998-06-23); abstract; figures 1,2 --	1-12

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27-11-2012	28-11-2012

Name and mailing address of the ISA/SE	Authorized officer
Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Lars Jakobsson Telephone No. + 46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2012/050846

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 3958572 A (LAWHEAD CREIGHTON M), 25 May 1976 (1976-05-25); abstract; figures 1,3 --	1-12
A	US 3974930 A (GIZARD PIERRE ET AL), 17 August 1976 (1976-08-17); abstract; figure 1 --	1-12
A	US 3136440 A (KRUG ALBERT E ET AL), 9 June 1964 (1964-06-09); figures 1,2 -- --------	1-12

International application No.
PCT/SE2012/050846

Continuation of: second sheet

International Patent Classification (IPC)

G01N 33/18 (2006.01)
C12M 1/24 (2006.01)
C12M 1/34 (2006.01)

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US	5085759	A	04/02/1992	NONE			
EP	0208949	A2	21/01/1987	DE	3524345	A1	08/01/1987
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US	3974930	A	17/08/1976	NONE			
US	3136440	A	09/06/1964	CH	410298	A	31/03/1966
				DE	1498600	C2	26/03/1970
				FR	1393651	A	26/03/1965
				GB	984149	A	24/02/1965