

Fully automated spectrophotometric approach to determine oxygen concentrations in seawater via continuous-flow analysis

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Abstract

Oxygen consumption measurements are the most common approach to estimate the remineralization of organic carbon to CO₂. A refined protocol of the spectrophotometric Winkler approach is presented, where a continuous-flow analyzer is coupled with a custom-made autosampler holding up to 30 oxygen bottles. The time required for analysis is 2 min per sample, and the precision is 0.05% at ~200 mmol O₂ m⁻³. Thus, analysis speed and quality are significantly improved compared to the classic Winkler titration approach to determine O₂ concentrations. The accuracy of the method is 99.7% ± 0.2% as determined by comparing the measured versus the theoretical oxygen concentration of saturated seawater at 20°C. The measured absorbance of the iodine at 460 nm wavelength was linear up to an equivalent of 320 mmol O₂ m⁻³, which is within the range of open-ocean oxygen concentrations. The instrument was tested on a cruise in the subtropical North Atlantic where community respiration (CR) and bacterial respiration (BR) were determined. Both CR and BR decreased by ~85% from the Mauritanian upwelling region and the oligotrophic gyre. Along this transect, the contribution of BR to CR increased from 36% to 76%. The instrument proved highly suitable for work at sea and should allow more rapid and precise oxygen concentration measurements under open-ocean conditions.

Introduction

Measurements of oxygen concentrations are used to characterize water masses in physical and chemical oceanography and to estimate the respiratory activity of single specimens or entire subsystems. The Winkler method (Winkler 1888) is considered the most accurate and cost-effective method to measure dissolved oxygen in water. The Winkler approach is also the recognized standard to calibrate the Clark-type oxygen sensors on conductivity-temperature-depth rosettes in seagoing research and limnology.

Recently, renewed interest in the respiratory activity of plankton emerged, as it might be one of the missing key parameters in ocean carbon-cycling models (Del Giorgio and

Williams 2005). Because the remineralization of organic matter to CO₂ is of particular interest, the most direct way of measuring respiration is to follow the evolution of dissolved inorganic carbon (DIC) in incubations. Similarly, differences in oxygen concentrations can be measured—requiring, however, the use of a respiratory quotient to convert oxygen consumption to CO₂ production. The small changes in concentration of either CO₂ or O₂ over time during these incubations, against a usually high background concentration, demand high precision of the method.

Unfortunately, for respiration measurements the coulometric CO₂ analysis (Johnson et al. 1993; Robinson and Williams 1999) is feasible only in oceanic systems with fairly high plankton activity. This is mainly due to the high DIC background in seawater (~2000 mmol DIC m⁻³), which allows only differences of ~1 mmol DIC m⁻³ to be measured. The lower average oxygen concentration in seawater of ~200 mmol O₂ m⁻³ results in a resolution of 0.06 to 0.2 mmol O₂ m⁻³ for the Winkler method (Williams and Jenkinson 1982). This is sufficient for most oligotrophic systems with generally low plankton activity, and the small oxygen changes are detectable within ecologically meaningful incubation times.

In the Winkler method, manganese chloride is added to a known amount of seawater, followed by the addition of an alkaline sodium hydroxide-potassium iodide solution. The resulting

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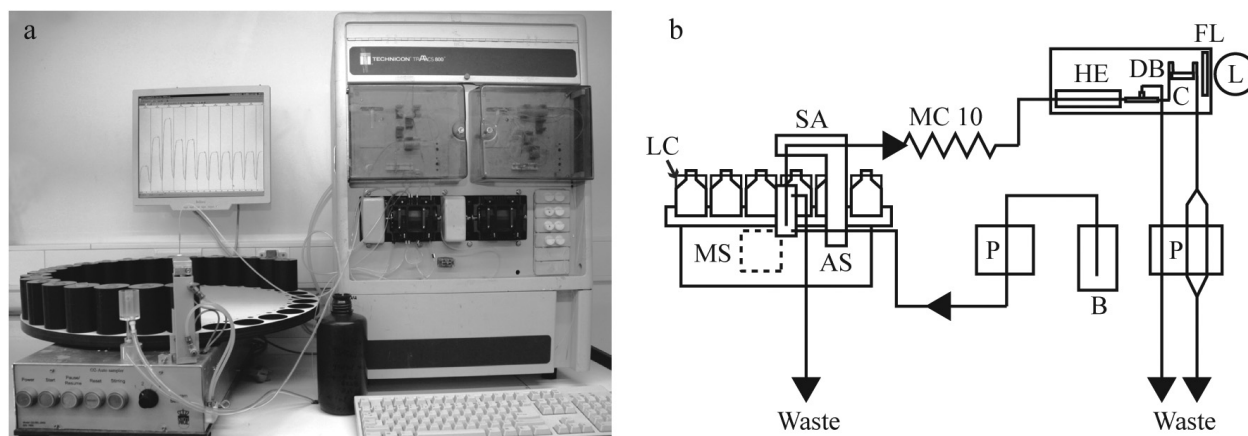


Fig. 1. Setup of the autosampler and the TRAACS 800 continuous-flow analyzer in the temperature controlled container (a) and schematic diagram of the sample flow path through the analysis system (b). Autosampler (AS), sampling arm (SA), light cover for the bottles (LC), magnetic stirrer (MS), mixing coil (MC; 10 turns), heat exchange element (HE), debubbler (DB), flowthrough cuvette (C), filter (FL; cutoff at 460 nm), light source (L), peristaltic pump (P), wash solution container (B). Arrows indicate the flow of sample, wash, and waste water through the system.

manganous hydroxide precipitate reacts with the dissolved oxygen in the water and forms a hydrated tetravalent oxide of manganese. Upon acidification, the manganese hydroxides dissolve, and the trivalent manganese acts as an oxidizing agent and liberates iodine from the iodide ions.



The iodine is equivalent to the dissolved oxygen in seawater and present as free iodine (I_2) and tri-iodide (I_3^-) (Burger and Liebafsky 1973).

In the classic Winkler method the iodine is determined titrimetrically with standard thiosulfate; however, despite improved automation of the end-point detection (Bryan et al. 1976; Williams and Jenkinson 1982; Furuya and Harada 1995), it commonly takes ~4 to 6 min to titrate a sample. A faster spectrophotometric approach to determine O_2 was introduced by Broenkow and Cline (1969) and is based on measuring the absorbance of the colored I_2 and I_3^- . The concentration of oxygen is then calculated by comparing the absorbance in a sample against standards of known oxygen content made from potassium iodate (KIO_3) solutions. After several improvements in analysis speed and standardization (Pai et al. 1993; Roland et al. 1999) and the choice of wavelength (Labasque et al. 2004), this technique might have the potential to replace the Winkler titration for most applications in biological oceanography and aquatic ecology, particularly if the particle load of the water is low such as in coastal oligotrophic regions and open-ocean systems.

To further improve the spectrophotometric approach for measuring oxygen concentrations, we developed a fully automated analysis system using a custom-made autosampler in conjunction with a continuous-flow analyzer. With this inexpensive system, the time required to perform high-quality oxygen concentration measurements is significantly reduced

and, ultimately, the analysis is coupled to an analytical control commonly not possible in conventional Winkler titration.

Materials and procedures

Adaptation of the TRAACS system—For the oxygen analysis, we used a custom-made autosampler in combination with a standard Technicon TRAACS 800 autoanalyzer (Bran + Luebbe, Germany). The autosampler consists of an electric motor, a pneumatic sampling arm driven by compressed air at ~4 bar, and a magnetic stirrer (Figure 1). The timing of the motor and the sampling arm with the needle can be programmed via an RS232 connection with a simple DOS program. The parameters were adjusted to 30-s flushing with wash solution, followed by 3 picks of a sample and 90-s aspiration of the sample. The platform holds up to 30 bottles, and a pneumatic pin fixes the platform firmly in place when the sample is in picking position. A sensor at the sampling arm stops the autosampler at empty tray positions. A separate button starts the autosampler, and a reset button interrupts the sampling sequence and turns the platform to the default start position. Thus, the autosampler is completely independent from the TRAACS analyzer and its software.

The TRAACS analyzer was equipped with a standard tungsten filament lamp and a fixed bandpass filter of 460 ± 10 nm (TRAACS part no. 165B04446) (Figure 1). The flow cell (TRAACS part no. 165B03001) had a volume of 7.85 mm^3 , and the flow rate was set to $\sim 1 \text{ cm}^3 \text{ min}^{-1}$ via the internal peristaltic pump. To maintain a stable temperature in the flow cell, a heat exchange element was installed in front of the cuvette. The analyzer was controlled via the commercial TRAACS analysis software (AAACE version 5.40 for Windows).

Chemicals—We used the common Winkler reagents to determine oxygen concentrations: manganese chloride ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 600 g dm^{-3} ; 3 mol L^{-1}) (A), alkaline iodide reagent (NaOH ;

250 g dm⁻³; 6 mol L⁻¹ + KI; 350 g dm⁻³; 2 mol L⁻¹) (B), and sulfuric acid (H₂SO₄; 10 mol L⁻¹) (C). After preparation, the reagent-grade chemicals were filtered through Whatman GF/F filters and subsequently stored in polycarbonate bottles at ~20°C in the dark. The standard stock solution was prepared with potassium iodate (KIO₃) (Malinckrodt Baker; primary standard). KIO₃ was dried at 180°C for 6 h, and 2.5 g KIO₃ was dissolved in 250 cm³ ultrapure Milli-Q water. Thus, 1 cm³ KIO₃ stock solution is equivalent to 70.1 mmol O₂ m⁻³. The prepared stock solution was divided into small 50-mL polycarbonate bottles and stored in a chamber with 100% humidity to prevent evaporation of water and therefore an increase in the concentration of the stock solution over long storage periods.

Glass bottles—Custom-made oxygen bottles made from borosilicate glass with a nominal volume in the range of 116 to 122 cm³ were calibrated to the mm³ level according to the recommendations of the World Ocean Circulation Experiment (WOCE) (see Culbertson 1991). Each borosilicate glass bottle and the corresponding ground-glass stopper were engraved with a unique number for later identification of the exact volume. A set of these bottles was used to prepare the calibration standards.

Respiration measurements involve the incubation of many bottles at the same time (up to 400 in our case), and serially numbered bottles frequently lead to confusion in the different experiments. With a system of a few numbers and color-coded oxygen bottles, we find it easier to keep track of the samples, and administrative tasks are minimized. We found that a difference in volume of ≤ 0.5 cm³ between bottles, analyzed in a single run on the flowthrough analyzer, results in an uncertainty in oxygen concentration of ≤ 0.02%. This uncertainty is below the detection limit of the Winkler method, and oxygen bottles not used for the calibration procedure (see below) were sorted in classes of ≤ 0.5 cm³ and coded with rubber tape in different colors. In the analysis software of the instrument, we apply a single volume-correction factor calculated from the mean of the volume class (see Eq. 3), resulting in the automatic output of final oxygen concentrations. Thus, when measuring bottles of one volume class on the flowthrough analyzer, there is no need to note the different bottle numbers and find the corresponding volumes.

Oxygen measurements of t_0 - and t_1 -bottles were usually performed in triplicate. For simple identification of the different respiration experiments in the incubation baths, each batch of 6 oxygen bottles was labeled with a unique number, and finally, t_0 -bottles were marked with black rubber tape.

Sampling procedure and handling—The general sampling procedure and handling of samples follow the recommendations of Carritt and Carpenter (1966). For community respiration measurements, the seawater was tapped directly from 10-L NOEX bottles mounted on the conductivity-temperature-depth (CTD) rosette. The seawater for bacterial respiration measurements was filtered over 0.8- μ m polycarbonate filters and equilibrated with oxygen by shaking. Generally, triplicate samples were taken for t_0 and t_1 measurements. Seawater was

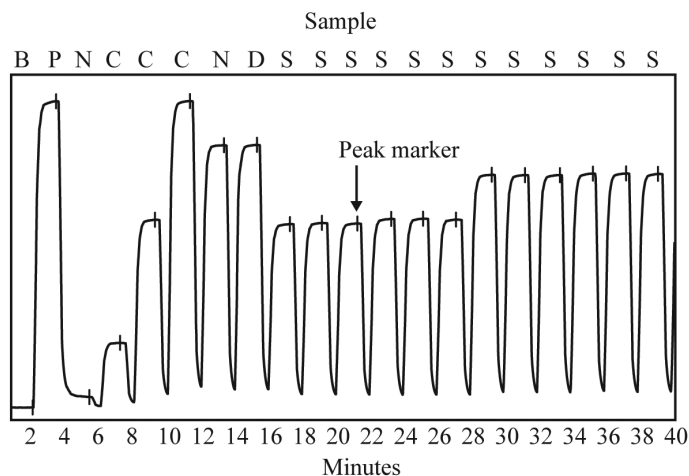


Fig. 2. Example of a peak chart from respiration measurements on the continuous-flow analysis system conducted during the BADE-2 cruise. Baseline readings of the wash solution (B), primer as indicator for maximum expected peak height (P), instrument calibration standards (C), sensitivity drift standards (D), and samples (S) are shown. N denotes peaks used to minimize the carryover effect and are not used in calculations. The peak marker is set in a predefined peak window. min, analysis time.

siphoned into 6 oxygen bottles (from a single volume class) with Tygon tubing overflowing each bottle by at least 3 times its volume. The oxygen content in a bottle was fixed with 1 cm³ reagent (A), followed by 2 cm³ reagent (B), both added near the bottom of the bottle with high-precision dispensers (Fortuna Optifix basic; precision ± 0.1%). The precise addition of chemicals (A) and (B) is important because they dilute the sample. Therefore, a dummy sample was spiked with the reagents before the actual samples to ensure that no air was in the dispensers. After adding the reagents, the bottles were stoppered and shaken vigorously to mix the chemicals. For samples directly tapped from the CTD, the addition of reagents and stoppering of the bottles were done as quickly as possible to prevent contamination of undersaturated samples by atmospheric oxygen. For incubation and storage, the bottles were immersed in water baths (kept at in situ temperature) to avoid drying of the stopper seal. After ~20 min, the fixed bottles were shaken again to ensure complete reaction of the chemicals. Before starting the measurements on the TRAACS system, 1 cm³ reagent (C) was added to the fixed samples. Subsequently, a small magnetic stirring bar was introduced carefully, and the bottle openings were covered with parafilm to avoid loss of volatile compounds.

As an improvement on standard procedures, the bottles were immediately covered with dark plastic cylinders shielding ambient light. The samples were gently stirred for a few seconds with an external magnetic stirrer (Metrohm) until the precipitate in the bottles was dissolved. Finally, the bottles were placed on the autosampler. Before aspiration of the sample into the flowthrough analyzer, the sample was agitated again with the built-in magnetic stirrer of the autosampler to

ensure complete mixing of the solution, thereby preventing chemical stratification.

Calibration procedure—Instrument calibration involves the measurement of the baseline or wash solution, a primer, instrument calibration standards, and sensitivity drift standards (Figure 2). For the wash solution and standards used during work at sea, particle-poor seawater from below the euphotic zone was collected into an 80-L polycarbonate carboy and acclimatized at 20°C.

For the instrument calibration standards and the primer, seawater was poured into oxygen bottles with known volume. Subsequently, reagents A (1 cm³), B (2 cm³), and C (1 cm³) were added in reverse order with the high-precision dispensers. After the addition of each reagent, the bottles were stoppered and vigorously shaken. Finally, the KIO₃ standard solution was added with highly accurate adjustable volume electronic pipettes (Biohit) of 100, 250, and 1000 mm³ (precision < 0.05% for the 100 and 250 mm³ pipettes and < 0.15% for the 1000 mm³ pipette). A magnetic stirring bar was inserted into the bottle and the bottle immediately covered with parafilm and the dark plastic cylinders. The primer is equal to the highest standard and is used to adjust the baseline and gain setting of the photomultiplier to prevent the sample peaks from going off scale. Generally, calibration was done in the range of expected oxygen concentrations.

For flowthrough systems, it is necessary to provide a low-concentration marker or baseline to separate consecutive peaks. To minimize carryover effects between the baseline and the samples, the wash solution was adjusted to an oxygen concentration slightly lower than the expected lowest value in the samples. The baseline is measured at the start and the end of an analytical run to correct for baseline drift if necessary. To correct for changes in the sensitivity of the photomultiplier (e.g., due to slight temperature variations), sensitivity drift standards were prepared with an O₂ concentration between the highest and lowest sample in the batch. The drift standards were placed (a) after the instrument calibration standards, (b) after 30 samples, and (c) at the end of each run. Both wash solution and sensitivity drift standards were prepared similarly to the calibration standards. A conventional blank is not required for calibration because standards and references include all the chemicals also used for regular samples. All preparations and measurements were done in a temperature-controlled container set at 20°C.

Software and calculation—From the linear regression of the calibration standards and the drift corrections the samples are quantified via the software with the following simplified equation:

$$O_2 \text{ (mmol m}^{-3}\text{)} = [(\text{slope} \times Abs_{corr} + \text{intercept}) \times Bot_f] - O_{2r} \quad (2)$$

where Abs_{corr} is the absorbance at 460 nm corrected for baseline drift, sensitivity drift, and the carryover effect (exact formulas for correction terms in Bran + Luebbe 2002); O_{2r} (1.05 mmol m⁻³) is the amount of dissolved O₂ introduced via the two reagents, measured via photometrical titration accord-

ing to Murray et al. (1968). Bot_f is the volume correction factor for bottles in a range of ≤ 0.5 cm³ and was calculated by

$$Bot_f = \frac{Bot_v + R_C}{Bot_v - (R_A + R_B)} \quad [3]$$

where Bot_v is the average bottle volume of the respective volume class in cm³ and $R_{A,B,C}$ is the volume of the added specific reagents in cm³. The absorbance measurements of standards and samples are rendered as peaks in the chart window of the TRAACS analysis software (Figure 2). Peaks are automatically detected as the highest reading within a preset peak window, and an adjustable smoothing parameter averages successive data points for improved peak identification in case of rough sea state. Before completed calibration of the analyzer, uncorrected oxygen concentrations are displayed. At the end of a run, the data are exported as an ASCII file and contain the final O₂ concentrations as well as the raw data for manual calculation.

Assessment

Sample collection and analysis—Initial tests of the method were performed in the laboratory. The performance of the setup at sea was tested during the BADE-2 cruise (September to October 2004), where we followed the dynamics of microbial respiration and prokaryotic production from the Mauritanian coastal upwelling into the subtropical North Atlantic gyre at ~20°N (Figure 3).

Choice of wavelength—Because one of the most important aspects in oxygen determinations is the total iodine, Labasque et al. (2004) suggested measuring the absorbance of I₂ and I₃⁻ at the intersection point at 466 nm. Due to limitations in the availability of fixed-wavelength filters for the TRAACS system, we measured at 460 ± 10 nm. While at 466 nm, oxygen concentrations of up to 900 mmol O₂ m⁻³ can be measured

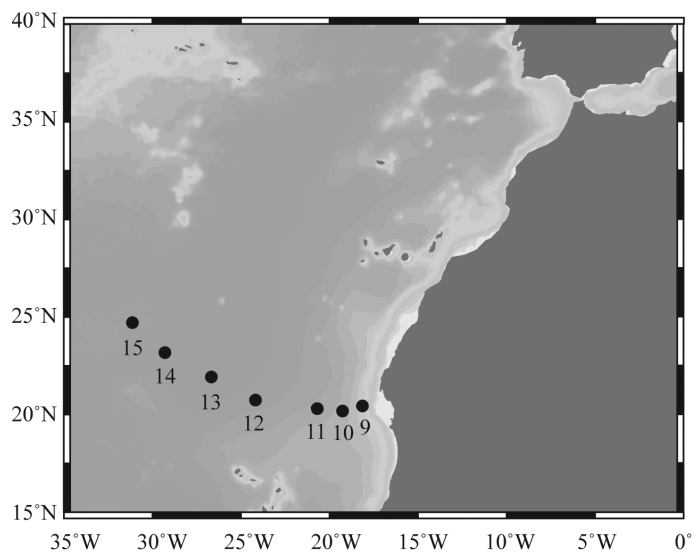


Fig. 3. Map of the cruise track during BADE-2 from the Mauritanian upwelling (Station 9) into the subtropical North Atlantic gyre (Stations 13–15).

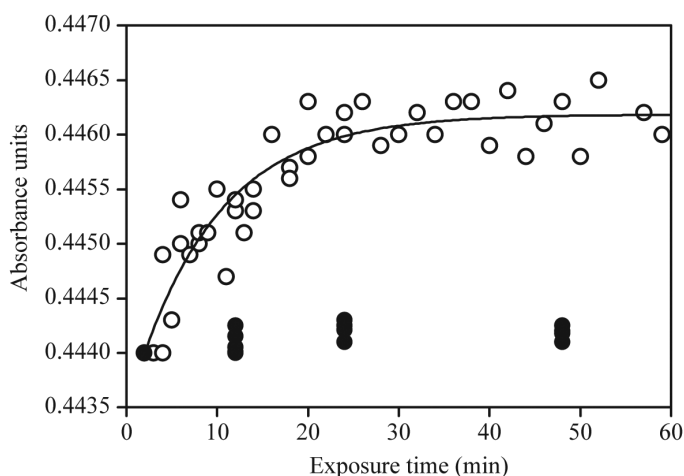


Fig. 4. Photochemical effect of ambient light on absorbance over time (O; 3 experiments). The model is for illustration purposes and recalculated to oxygen concentrations; it indicates an O_2 increase of $\sim 0.9 \text{ mmol m}^{-3}$ within 30 min. ●, measurements in bottles covered with black plastic cylinders; 6 samples were measured at 0, 12, 24, and 48 min.

(Labasque et al. 2004); the limit of linearity of the calibration at 460 nm is probably lower. However, most open-ocean profiles show oxygen concentrations $< 320 \text{ mmol } O_2 \text{ m}^{-3}$ (eWOCE Data, 2002). Up to $320 \text{ mmol } O_2 \text{ m}^{-3}$, our calibration lines measured at 460 nm were always linear (data not shown). Additionally, on a moving ship the absorbance reading is more stable when a fixed-wavelength filter is used rather than a movable mirror, as in many general-purpose spectrophotometers.

Volatilization of iodine—Volatilization of iodine is recognized as a potential problem in the Winkler method, especially when exposing the sample to air (Carritt and Carpenter 1966). In closed systems, with the lower end of the sampling tube placed close to the bottom of the bottle, vaporization of iodine is not detectable over short time periods (Pai et al. 1993). However, the samples spend up to 60 min on the autosampler. Therefore, the bottles were covered immediately with parafilm after addition of sulfuric acid. Parafilm itself is not completely gas tight, but we found no systematic decrease in absorbance over a period of 60 min.

Photochemistry in fixed samples—After acidification with the sulfuric acid, the color of the samples darkens considerably due to ambient laboratory light. The influence of light on the analysis has not been emphasized before. In experiments ($n = 3$), the influence of light exposure of samples on the absorbance was measured at 2-min intervals for 60 min (Figure 4). The maximum increase in absorbance over this 60-min period corresponded to an average O_2 increase of $\sim 0.9 \text{ mmol m}^{-3}$, which would add a significant and variable error to the analysis. No increase in absorbance was detected, however, in bottles held in the dark (Figure 4). Although the reasons for this photochemical effect are not clear, traces of copper in the $MnCl_2 \cdot 4H_2O$ solution could be responsible for oxidation processes during light exposure (Van Bennekom, unpublished observa-

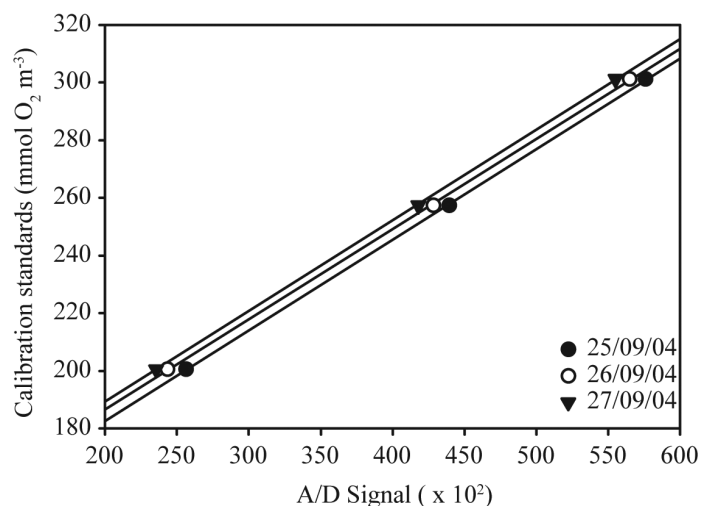


Fig. 5. Example of calibration lines obtained on 3 consecutive days using stored standards. The slopes of the 3 calibration lines are not significantly different. The calculations to test for differences among the slopes were done according to Zar (1999). The x -axis shows the raw analog-to-digital output of the spectrophotometer (A/D values $\times 10^2$).

tions). To prevent a light-induced increase in absorbance, we immediately covered the bottles with black plastic cylinders after acidification (see Figure 1).

Calibration—The continuous-flow system has to be calibrated with known concentrations of oxygen for each run. The preparation of calibration standards requires particular care and is time consuming. If the calibration of oxygen sensors is the ultimate goal, a new set of calibration standards should be used for each run to ensure the highest possible accuracy. If differences in oxygen concentrations between samples are the main purpose of the measurement such as for the determination of respiration rates, the slope of the calibration line is important. We tested whether the standards of a calibration line can be stored and used for several days. The slopes of the calibration lines determined with standards stored for up to 3 days were not significantly different from each other ($F_{3,4} = 0.41$, $P = 0.757$; Figure 5).

Accuracy and precision—Certified reference material for oxygen measurements is not available, which would be important to intercalibrate the various oxygen methods applied in different laboratories. A simple, though not ideal, way to check whether our protocol shows acceptable agreement with the true oxygen value is to compare measured samples of O_2 -saturated seawater to the theoretical oxygen concentration derived from gas saturation tables. Seawater (20 L) was UV sterilized, kept in the dark at 20°C (temperature was monitored with a high-precision sensor), and saturated with oxygen via an aerator and stirring bar for 2 days. The aerator was turned off 1 h before sampling to prevent collection of oversaturated water. Samples were taken for salinity (measured on a Guildline Autosol 8400B), and air pressure readings were retrieved from the Royal Netherlands Meteorological Institute. Finally, 8 oxygen bottles

Table 1. Precision of triplicate measurements during the BADE-2 cruise in the subtropical Atlantic over a range of oxygen concentrations.

<i>n</i>	O_2 , mmol m^{-3}		PSD, mmol m^{-3}		RSD, %
	Average	SD	Average	SD	
SAT	78	213.62	8.21	0.07*	0.04
CTD	66	200.13	24.44	0.09†	0.06

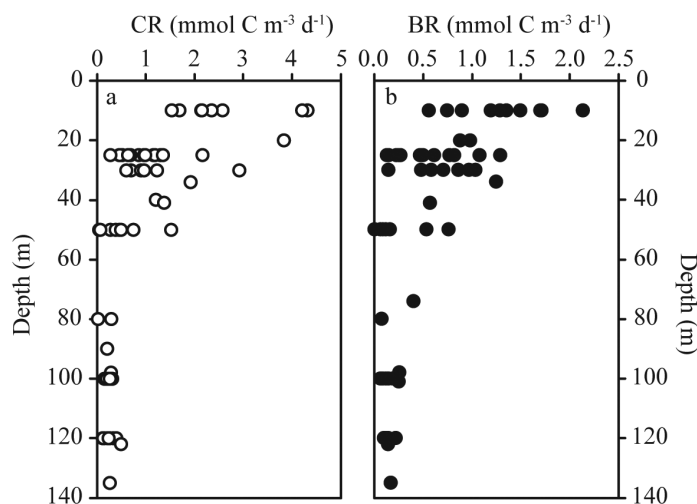
For the saturated samples (SAT) and samples directly tapped from the CTD, the pooled standard deviation (PSD) and relative standard deviation (RSD) were calculated according to McNaught and Wilkinson (1997). * $df=99$; † $df=80$.

with known volumes were filled and measured according to the procedure described above. Assuming 100% saturation, the theoretical oxygen concentration in seawater was calculated taking the water temperature, salinity, and air pressure into account (UNESCO 1973). Following this approach, our measurements reached $99.7\% \pm 0.2\%$ ($n=40$; 5 experiments) of the calculated oxygen concentration, which is similar to the estimated accuracy reported for the Winkler titration approaches.

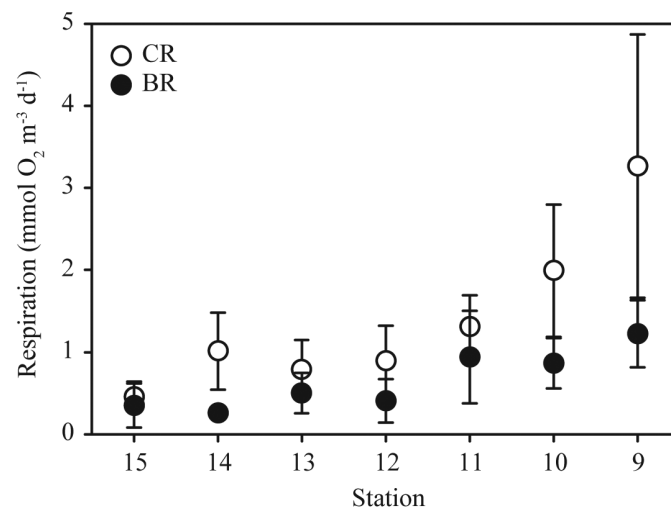
The precision of our method and the instrument was tested at sea, which is the main application site of our system. For calibration of oxygen sensors, WOCE demands a Winkler method precision of $< 0.2\%$ (Culbertson 1991). For respiration measurements, however, the precision should be better. The precision of triplicate seawater samples used as t_0 for bacterial respiration measurements was on average 0.04% (Table 1) including errors due to preparation and handling of the samples. The precision of triplicate samples tapped directly from the CTD was 0.06% (Table 1) and is thus slightly lower than that for the measurements on saturated samples.

Field study of microbial production and respiration—We measured community respiration (CR) in unfiltered seawater and bacterial respiration (BR) in 0.8- μ m filtered seawater (assuming that respiration in the 0.8- μ m filtered fraction is dominated by bacteria) collected in the euphotic layer along a transect from the Mauritanian upwelling into the subtropical North Atlantic gyre. Samples for nutrients, DOC, and primary and bacterial production were taken as well but will be presented elsewhere. For the respiration measurements, we incubated triplicate oxygen bottles in a water bath in the dark at in situ temperature ($\pm 1^\circ\text{C}$). t_0 bottles were fixed immediately with the Winkler reagents, and t_1 bottles were fixed after an incubation time of 12 to 24 h depending on the expected microbial activity. CR and BR decreased significantly with depth down to ~ 120 m ($r^2 = 0.72$, $P < 0.0001$, $n = 61$ for CR and $r^2 = 0.52$, $P < 0.0001$, $n = 62$ for BR; Figure 6). Both CR and BR were within the range of reported respiration rates for upwelling and oligotrophic systems (Del Giorgio and Cole 2000; Gonzalez et al. 2003; Robinson and Williams 2005).

Most of the variability in respiration between the upwelling stations and the oligotrophic gyre waters was found in the top 40 m. Thus, only the near-surface layer was analyzed to assess lateral trends. CR and BR decreased by $\sim 86\% \pm 56\%$ ($3.26\text{--}0.46$ mmol O_2 m^{-3} d^{-1}) and $\sim 84\% \pm 41\%$ ($1.22\text{--}0.20$ mmol O_2 m^{-3} d^{-1}),

**Fig. 6.** Depth profiles of total community respiration (a) (CR; mmol O_2 m^{-3} d^{-1}) measured in unfiltered seawater and bacterial respiration (b) (BR; mmol O_2 m^{-3} d^{-1}) measured in 0.8- μ m filtered seawater.

respectively, from the upwelling area to the oligotrophic gyre (Figure 7). BR explained $\sim 61\%$ of the variability in CR (Figure 8). This suggests that BR comprises a major fraction of CR over a broad trophic spectrum. The contribution of BR to CR increased from the upwelling region ($36\% \pm 12\%$) toward the oligotrophic gyre, reaching $76\% \pm 24\%$ (Figure 9). Over the entire transect, BR contributed $55\% \pm 19\%$ to CR, which is in agreement with the few studies available for open-ocean systems (Robinson and Williams 2005). However, as indicated by

**Fig. 7.** Lateral trend in total community respiration (CR; mmol O_2 m^{-3} d^{-1}) and bacterial respiration (BR; mmol O_2 m^{-3} d^{-1}) from the Mauritanian upwelling (Station 9) toward the subtropical North Atlantic gyre (Stations 13–15; see Figure 3). Data points are averages of the respiration measurements over a depth range of 10–40 m ($n=5$ per station); error bars show standard deviations.

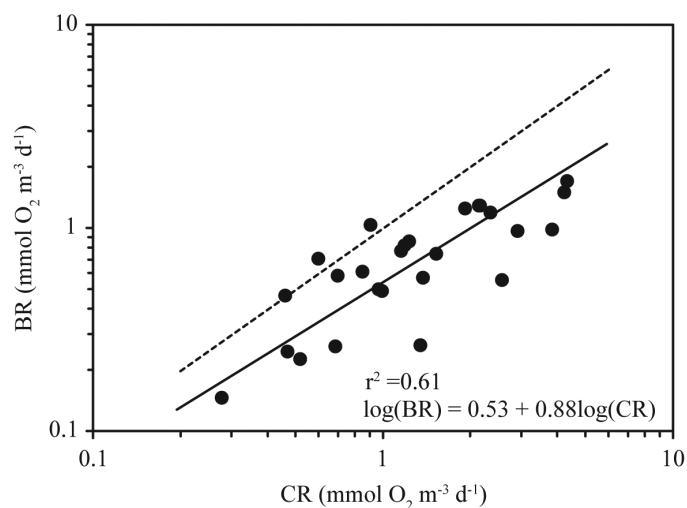


Fig. 8. Relationship between total community respiration (CR; $\text{mmol O}_2 \text{ m}^{-3} \text{ d}^{-1}$) and bacterial respiration (BR; $\text{mmol O}_2 \text{ m}^{-3} \text{ d}^{-1}$). The solid line represents a reduced major axis (RMA) regression; dotted line indicates 1:1 relation.

our data, bacterial respiration might not always be the dominant fraction of oceanic community respiration.

Discussion

Recently, respiration was proposed to be a better estimator for system productivity than primary production (Del Giorgio and Williams 2005). Thus, in the open ocean, mapping of respiration and primary production are of similar importance. For large parts of the ocean, data on respiration do not exist, and the lack of spatial resolution makes it impossible to draw firm conclusions on systems' remineralization rates on a global scale (Robinson and Williams 2005). One reason there are orders of magnitude more estimates of organic matter production (i.e., primary production) than remineralization is probably that measurement of oxygen consumption by Winkler titration is considered tedious and labor intensive. Our main motivation was therefore to develop a continuous-flow analysis setup to increase the number of high-precision oxygen determinations.

An alternative method allowing high-precision oxygen determinations is membrane inlet mass spectrometry (MIMS). With transportable MIMS instruments, it is possible to measure a variety of oceanic trace gases with high sample throughput. MIMS measures oxygen more directly than methods based on chemistry. This is important in situations where the addition of chemicals to a sample is problematic, e.g., in pressurized samples from the deep sea. Further advantages include the small volume of sample needed per analysis and the possibility of conducting underway measurements of gases from the surface ocean (Tortell 2005). The determination of oxygen gas in an aspirated sample with MIMS depends on the stability of the flow rate across the silicone membrane. To eliminate the flow rate-dependent fluctuations, oxygen concentrations

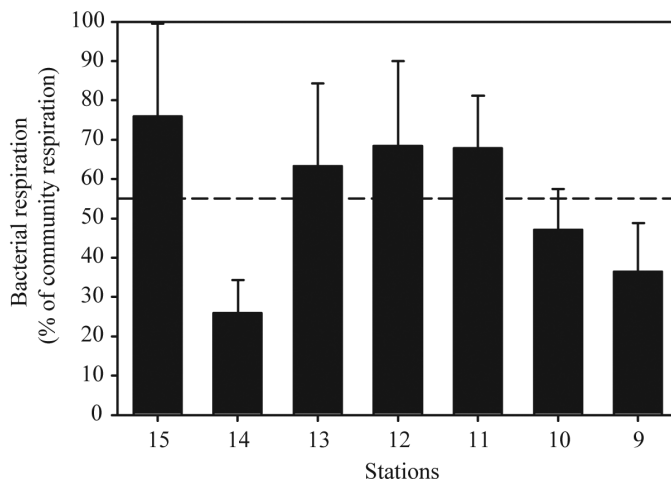


Fig. 9. Bacterial respiration (BR) expressed as percentage of total community respiration (CR) at the different stations from the Mauritanian upwelling to the subtropical North Atlantic gyre. Error bars show standard errors ($n = 5$); the broken line indicates the grand average over all the stations.

are normalized to parallel measurements of the biologically inert argon (Kana et al. 1994; Tortell 2005). Consequently, absolute oxygen concentrations frequently do not compare well with the Winkler approach. Whereas MIMS seems a promising tool for future open ocean work measuring oxygen and other gases, there are only a few direct comparisons with standard methods employed in oceanography available at the moment, and ship-board experience with these instruments is rather limited thus far.

Recently, a method for measuring respiration with oxygen microprobes was presented (Briand et al. 2004). With a reported precision of 0.05% measured at $239.9 \text{ mmol O}_2 \text{ m}^{-3}$, the method apparently performs quite well in the laboratory; however, shipboard performance has not yet been tested. Uncertainty in the linearity of oxygen consumption over time represents a potential problem in endpoint measurements and can be detected by continuous recording of the oxygen concentration with microprobes (Briand et al. 2004).

Although the above-mentioned approaches open new ways to measure oxygen, the spectrophotometric method is based on the accepted principles of Winkler chemistry. Unlike titration with either photometric or potentiometric endpoint detection, the spectrophotometric method directly measures the concentration of total iodine in a sample. The quantification of oxygen concentrations does not rely on the concentration of standardized thiosulfate but is directly calibrated with potassium iodate. Spectrophotometric methods are relatively easy to adapt to full automation, and the instruments are usually rugged and simple to repair on board. Depending on the spectrophotometer, a reading is recorded when the absorbance is stable as judged by the analyst, or alternatively, at a preset time, the instrument averages over a certain time. Nevertheless, several readings per sample should be taken. The continuous-

flow analysis allows for more control on the actually recorded concentration. The data are saved automatically and can be exported in various ways, either as raw data for manual calculations or as drift- and baseline-corrected concentrations calculated from the calibration line in either ASCII or Excel format. Furthermore, optical control of the peak chart helps to quickly identify outliers. The measurement is fast (~2 min per sample), and only a minimum of supervision is necessary.

So far, the precision may have distracted scientists from using the spectrophotometric approach. Labasque et al. (2004) and Pai et al. (1993) report a shipboard precision of ~0.12%, whereas the overall precision of the data reported here is 0.05%. We suggest that this improved precision is the result of the automation of the measurement and the correction possibilities for the sensitivity and baseline drift of the instrument.

Comments and recommendations

Calibration of the analysis system has been tested at O₂ concentrations ranging from 50 to 350 mmol m⁻³. Thus, when encountering lower oxygen concentrations, as in oxygen-depleted zones, the calibration needs to be extended to lower concentrations. A possibility is to prepare the wash solution without addition of KIO₃ and include the resulting low baseline in the instrument calibration line.

During experiments in the subtropical Atlantic, we experienced only moderate sea state. A problem with spectrophotometers on board ships is the unstable filament in the tungsten lamps. When measuring during rough sea state, the readings can be considerably biased. To avoid this problem, we recently installed a blue light-emitting diode (LED) that eliminates this source of erroneous measurements.

The tubing and the cuvette diameter in our apparatus are small and potentially can get clogged by particles in the sample. Therefore, it is necessary to flush the system with a cleaning solution (2% Hellmanex, Hellma, Germany) and Milli-Q water after each run. The manganese hydroxide that forms on the dispenser tip of the bottle containing the NaOH/KI reagent contaminates the blanks. For cleaning, we recommend using a dilute solution of sulfuric acid with a few milliliters of NaOH/KI.

Although we used a TRAACS system, in principle any spectrophotometer adapted for flowthrough analysis and appropriate software for peak detection can be used. Oxygen measurements as proposed here, with a continuous-flow analyzing system in conjunction with an autosampler, should be a useful tool for open-ocean expeditions where high sample throughput and high precision are essential.

References

Bran + Luebbe. 2002. AACE Software 5.40 Operation Manual.
 Briand, E., O. Pringault, S. Jacquet, and J.-P. Torreton. 2004. The use of oxygen microprobes to measure bacterial respiration for determining bacterioplankton growth efficiency. *Limnol. Oceanogr. Methods* 2:406-416.
 Broenkow, W. W., and J. D. Cline. 1969. Colorimetric deter-

minations of dissolved oxygen at low concentrations. *Limnol. Oceanogr.* 14:450-454.
 Bryan, J. R., J. P. Riley, and P. J. I. B. Williams. 1976. A Winkler procedure for making precise measurements of oxygen concentration for productivity and related studies. *J. Exp. Mar. Biol. Ecol.* 21:191-197.
 Burger, J. D., and H. A. Liebhafsky. 1973. Thermodynamic data for aqueous Iodine solutions at various temperatures. *Anal. Chem.* 45:600-602.
 Carritt, D. E., and J. H. Carpenter. 1966. Comparison and evaluation of currently employed modifications of Winkler method for determining dissolved oxygen in seawater: a NASCO Report. *J. Mar. Res.* 24:287-318.
 Committee, W. d. p. 2002. WOCE global data. WOCE international project office.
 Culberson, C. H. 1991. Dissolved oxygen, p. 15. *In* WOCE Hydrographic Operations and Methods. T. M. Joyce [ed.] Woods Hole, Massachusetts, USA.
 Del Giorgio, P. A., and J. J. Cole. 2000. Bacterial energetics and growth efficiency, p. 289-325. *In* *Microbial Ecology of the Oceans*. D. L. Kirchman [ed.] Wiley-Liss.
 ——— and P. J. I. B. Williams. 2005. The global significance of respiration in aquatic ecosystems: from single cells to the biosphere, p. 267-303. *In* *Respiration in Aquatic Ecosystems*. P. A. Del Giorgio and P. J. I. B. Williams [eds.] Oxford University Press.
 Furuya, K., and K. Harada. 1995. An automated precise Winkler titration for determining dissolved oxygen on board ship. *J. Oceanogr.* 51:375-383.
 Gonzalez, N., R. Anadon, and L. Viesca. 2003. Carbon flux through the microbial community in a temperate sea during summer: role of bacterial metabolism. *Aquat. Microb. Ecol.* 33:117-126.
 Johnson, K. M., K. D. Wills, D. B. Butler, W. K. Johnson, and C. S. Wong. 1993. Coulometric total carbon dioxide analysis for marine studies: maximizing the performance of an automated gas extraction system and coulometric detector. *Mar. Chem.* 44:167-187.
 Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennett, and J. C. Cornwell. 1994. Membrane inlet mass spectrometer for rapid high precision determination of N₂, O₂, and Ar in environmental water samples. *Anal. Chem.* 66:4166-4170.
 Labasque, T., C. Chaumery, A. Aminot, and G. Kergoat. 2004. Spectrophotometric Winkler determination of dissolved oxygen: re-examination of critical factors and reliability. *Mar. Chem.* 88:53-60.
 McNaught, A. D., and A. Wilkinson. 1997. *Compendium of Chemical Terminology: IUPAC, 2nd ed.* Blackwell Science.
 Murray, C. N., J. P. Riley, and T. R. S. Wilson. 1968. Solubility of oxygen in Winkler reagents used for determination of dissolved oxygen. *Deep-Sea Res. Part I* 15:237-238.
 Pai, S.-C., G.-C. Gong, and K.-K. Liu. 1993. Determination of

- dissolved oxygen in seawater by direct spectrophotometry of total iodine. *Mar. Chem.* 41:343-351.
- Robinson, C., and P. J. I. B. Williams. 1999. Plankton net community production and dark respiration in the Arabian Sea during September 1994. *Deep-Sea Res. Part II* 46:745-765.
- and P. J. I. B. Williams. 2005. Respiration and its measurement in surface marine waters, p. 147-180. // *Respiration in Aquatic Ecosystems*. P. A. Del Giorgio and P. J. I. B. Williams [eds.] Oxford University Press.
- Roland, F., N. F. Caraco, J. J. Cole, and P. A. Del Giorgio. 1999. Rapid and precise determination of dissolved oxygen by spectrophotometry: evaluation of interference from color and turbidity. *Limnol. Oceanogr.* 44:1148-1154.
- Tortell, P. D. 2005. Dissolved gas measurements in oceanic waters made by membrane inlet mass spectrometry. *Limnol. Oceanogr. Methods* 3:24-37.
- UNESCO. 1973. International oceanographic tables, p. 195. NIO-UNESCO.
- Williams, P. J. I. B., and N. W. Jenkinson. 1982. A transportable microprocessor-controlled precise Winkler titration suitable for field and shipboard use. *Limnol. Oceanogr.* 27:576-584.
- Winkler, L. W. 1888. Die Bestimmung des im Wasser gelösten Sauerstoffes. *Chem. Berichte* 27:2843-2855.
- Zar, J. H. 1999. *Biostatistical Analysis*, 4th ed. Prentice-Hall.

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