Gas exchange system for extended in situ benthic chamber flux measurements under controlled oxygen conditions: First application—Sea bed methane emission measurements at Captain Arutyunov mud volcano

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Abstract

Mass transfer rates of many gases, nutrients, and trace metals across the sediment water interface are dependent on environmental oxygen conditions. In this article, a novel gas exchange system for extended in situ flux and respiration measurements in benthic chambers under defined oxygen conditions is described. Integrated within a GEOMAR modular lander, the gas exchange system was used to perform in situ measurements of the total oxygen uptake and sea bed methane emission rates under constant oxygen conditions at Captain Arutyunov mud volcano (Gulf of Cadiz) in a water depth of 1320 m. During two separate lander deployments, the oxygen concentration within the benthic chambers was kept constant for 37 and 47 h, respectively. Under these conditions total oxygen uptake rate remained constant at 4.4 and 13.2 mmol m⁻² d⁻¹. Seabed methane emission was low, in the range 0 to 0.2 mmol m⁻² d⁻¹. The system is suited for prolonged (days) in situ flux determinations under natural background oxygen conditions and offers a wide range of experimental applications.

In aquatic sciences, benthic chambers designed as boxes, bell jars, and cylindrical micro-/mesocosms have become a generally accepted approach for in situ measurements of fluxes of nonconservative elements that are involved in the biological and geochemical turnover of organic carbon, such as dissolved gases, nutrients, natural, and anthropogenic trace metals, and alternative electron acceptors (cf. Viollier et al. 2003). Since the end of the 1970s, autonomous chamber deployments were conducted successfully in deep-sea environments for in situ respiration measurements to estimate benthic carbon turnover (Smith 1978, Smith and Baldwin 1984, Smith and Kaufmann 1999, Wenzhöfer and Glud 2002, Witte and

Acknowledgments

Pfannkuche 2000) or for food pulse experiments to study bentho-pelagic coupling (Levin et al. 1999, Moodley et al. 2002, Witte et al. 2003a, 2003b). In the deep sea, rates of organic carbon degradation are usually assumed to depend on the availability and composition of organic carbon rather than on the concentration of oxygen. The contribution of oxygen consumption caused by secondary redox reactions to the total oxygen uptake (TOU) can be neglected in such organically poor habitats. The organic carbon mineralization can be deduced from the constant TOU defined by the linear decrease of the oxygen concentration inside the benthic chamber.

However, more and more, this approach is also applied for organically enriched and biogeochemically active environments at continental margins such as upwelling areas (Glud et al. 1999), mud volcanoes (Linke et al. 2005), or methane seep sites (Sommer et al. 2006). In these habitats, the basic assumption made for deep-sea TOU measurements—that the rate of oxygen uptake is independent of the oxygen concentration in the bottom water—is untenable (Hall et al. 1989). At cold seep sites, oxygen consumption can be extremely fast. From cold seep sediments at Hydrate Ridge, covered with sulfide oxidizing microbial mats, Sommer et al. (2006) report that the oxygen content inside benthic chambers became completely depleted within hours or even less after placement of the chamber on the sea floor. In these environments, the oxidation of

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Figure 1. Schematic drawing of the gas exchange system. For explanation, see text.

reduced inorganic solutes, such as sulfide, can contribute greatly to the TOU.

For such environments, oxygen availability becomes a limiting factor to conduct prolonged flux measurements under natural conditions. Severe changes of the oxygen concentration inside the chamber will not only affect community respiration and benthic carbon turnover, but also the flux of redox sensitive elements across the sediment water interface. Flux measurements conducted under such conditions are questionable and cannot be considered as reflecting natural conditions. Fluxes and turnover of gases, nutrients, and major ions take place on different temporal scales. For their determination, as well as for ecological studies such as food pulse experiments, prolonged chamber measurements under controlled oxygen conditions become necessary.

First attempts to control biogeochemical conditions inside benthic chambers include manual addition of solutes and gases from external sources (Anderson et al. 1986, Hall et al. 1989) and artificial "gill" systems providing oxygen from a diving tank (Chadwick et al. 1994) or the ambient sea water (Morse et al. 1999) into the benthic chamber. In shallow water studies, the lid of the chamber has been opened from time to time to replenish oxygen using the ambient sea water. A similar approach is conducted in a deep-sea respirometer described by Okuda et al. (2005) and Sommer et al. (unpubl. results) where the water body inside the chamber is exchanged with the ambient sea water via a pump. However, none of these systems allow measurement of respiration and fluxes under experimentally defined and stable oxygen conditions in depths down to 6000 m. In these systems except the flux chamber described by Chadwick et al. (1994), loss of solutes will occur when the chambers are opened periodically or when gas exchange takes place in the ambient sea water (as in Morse et al. 1999).

In this study, we describe a novel gas exchange system that allows the control of oxygen conditions inside autonomous benthic chambers for several days. The system is completely separated from the ambient sea water, thus no loss of gases will occur, enabling the calculation of their respective mass transfer rates. Results from first deployments of the gas exchange system at methane seep sites atop of Captain Arutyunov mud volcano in the central Gulf of Cadiz are presented.

Materials and procedures

Gas exchange system—In principal, the gas exchange system transfers dissolved oxygen from a cylindrical reservoir (PVC, volume: 29.53 L), containing air-saturated seawater into the benthic chamber (Figure 1). The design of this system is based on an earlier prototype as described by Sommer et al. (2006). The earlier system had shortcomings with respect to handling, accurate regulation of oxygen content, bacterial contamination, and leakages of gases and tracers. In this earlier system, the gas exchange site was represented by a stack of 5 silicone membranes (total exchange area: 393 cm²; cf. Sommer et al. 2006, Figure 4). Many tubes and distributors were involved to generate the water transport from the chamber and the reservoir to the gas exchange sites. Thus, this system was difficult to clean, and there was the risk of developing biofilms and particles being deposited in the tubes and on the membranes. Microorganisms of these biofilms or attached to the particles consume oxygen, which would contribute to the TOU. Due to its mechanical design, it was time consuming to fill the system with sea water without enclosing air bubbles being trapped in the tubes or fittings. Two deep-sea pumps were involved to transport the water from the reservoir and the water from the chamber to the gas exchange sites. Hence, the oxygen concentration inside the chamber was difficult to regulate. Lastly, the gas exchange system was attached to the outside of the reservoir and exposed to the ambient sea water, thus there was a risk that gases are lost to the environment.

In the new system, diffusive gas exchange between the chamber- and the reservoir water takes place across the wall of a 20-m long standard laboratory silicone tube (i.d. 5 mm; wall thickness 1 mm) (Figure 1). Between the chamber water and the reservoir water, there is no direct contact. The silicone

Oxygen controlled benthic flux measurements

tube is coiled around a framework made of polyoxymethylene (POM) in a way that the coils do not touch each other and that the contact area between tubing and carrier is minimized to provide sufficient area (approximately 3142 cm²) for gas exchange. The maximum exchange rate of oxygen that can be achieved with the system depends on the length of the silicone tube and the concentration gradient of gases between chamber and reservoir water. The tube can be easily replaced or cleaned after each deployment to reduce the effects of biofilm formation. To minimize leakage of the gas exchange system, the silicone tubing arrangement is housed within the reservoir. Silicone is permeable to gases but not to ions. Hence, exchange of other gases such as methane, helium, or argon will also take place between the chamber and the reservoir. Mass transfer rates of these gases can be calculated when sequential water samples from the reservoir and the chamber are taken. For the connection between the chamber and the silicone tube arrangement in the reservoir, gas tight tubes (ISO VERSINIC i.d. 12 mm, wall thickness: 2 mm) were used.

Prior to the deployment of the system, the reservoir was filled with filtered (0.4 μ m) aerated sea water from the site of investigation to achieve the same salinity and, in effect, solubility of gases in the chamber and reservoir water. The reservoir sea water was oxygenated by bubbling it with air under in situ temperature (~9°C). To reduce bacterial contamination, the reservoir was lined out with a plastic sack into which the sea water was poured. The plastic sack was replaced for each deployment. The silicone tubing for gas exchange and the tubes connecting the chamber and the reservoir were also filled with filtered sea water. Care was taken to avoid enclosure of air bubbles. Transfer of chamber water to the gas exchange site in the reservoir is facilitated by means of a SEABIRD pump (SBE Mod. 5T).

During operation of the gas exchange system, the oxygen concentration inside the benthic chamber, the reservoir and in the ambient seawater is continuously monitored by optical sensors (Aanderaa Instruments, Norway; Model 3830). The functional principle and calibration procedure of the optode is described by Tengberg et al. (2006) and the operating manual provided by Aanderaa Instruments AS (2003). Briefly, the sensing foils of the optodes are delivered with calibration data to be used for the internal processor describing their behavior with respect to oxygen concentration and temperature. The ambient temperature is measured by the optode, and its effect on the oxygen concentration measurement is automatically compensated for by the internal processor of the optode. The sensitivity of the sensors is better at lower concentrations (± 0.5 µmol L⁻¹) than at higher concentrations of 300–500 µmol L^{-1} (± 1 µmol L^{-1}). The effect of salinity on the measured oxygen concentration was corrected internally by the optode using a setting of 0 psu for the laboratory measurements and 35 psu for the in situ applications. Pressure exerts an influence on oxygen detection of ~ 4% lower response per 100 bar pressure. As shown by Tengberg et al. (2006), this response is reversible and predictable. The effect of pressure on oxygen levels was compensated by applying the following equation:

$$O_{2c} = O_2 \left(1 + \frac{0.04 * d}{1000} \right)$$

where d is depth (m) and O_2 the measured oxygen concentration (Aanderaa AS 2003). The performance of the optodes during the in situ applications was checked using parallel water samples for automated Winkler titration (Grasshoff et al. 1983). Mean deviation between the concentration determined by the optodes and Winkler titration was 7.6 ± 2.6 µmol L⁻¹ (n = 10). Data logging and regulation of the oxygen concentration inside the chamber is conducted by a control unit developed in the IFM-GEOMAR electronic workshop.

The gas exchange system can be operated in two different modes. The oxygen concentration in the chamber can be adjusted to the outside level. In the alternative mode of operation, the gas exchange system can be set to maintain a defined oxygen concentration inside the chamber. In this mode, three time periods with different oxygen concentration settings can be defined for the entire duration of the flux measurements. This option allows the investigation of the response kinetics of the enclosed sediment community and associated mass transfer rates to different oxygen levels within the same chamber. Oxygen consumption of the habitat enclosed by the chamber will reduce the oxygen concentration inside the chamber until the defined oxygen concentration is reached or until it falls below the external level, then the controlling unit activates the pump to transfer oxygen poor chamber water to the gas exchange site in the reservoir. When the oxygen level inside the chamber rises above the defined oxygen concentration, the pump is switched off. A second pump (SBE Mod. 5T) permanently recirculates the reservoir water counter to the flow direction of the chamber water through the silicone tube to enhance gas exchange.

Laboratory tests-Laboratory tests using silicone tubes with a wall thickness of 0.25 mm (Riesbeck, Germany) and 1 mm (Rehau, Germany) were conducted to evaluate the performance of the gas exchange system. In order to measure the rate of oxygen transfer between the reservoir and the chamber the starting oxygen concentrations were ~ 275 μ mol L⁻¹ in the reservoir and close to zero in the chamber. During the laboratory tests, the benthic chamber was replaced by a glass bottle (vol. 11.5 L). Instead of seawater, distilled water was used, and the tests were conducted at a temperature of about 19°C. Oxygen poor water was transferred from the glass bottle to the gas exchange site in the reservoir using a SEABIRD pump (SBE Mod.5T). Except for the different wall thickness, the set up of the silicone tubing was as described above. Oxygen concentrations inside the reservoir and the glass bottle were monitored using optical sensors (Aanderaa Instruments AS) and logged via a computer at a sampling rate of 1 min. The water bodies within the reservoir and the glass bottle were well mixed using pumps. Three replicate measurements for each wall thickness of the tube were made.



Figure 2. Left panel, BIGO prior to deployment. Mounted on its top is the TV-guided launching system that allows the smooth placement of the lander at selected sites at the sea floor. Right panel, close up showing the arrangement of the reservoir and the benthic chamber.

In situ application—To determine sea bed methane emission under controlled oxygen conditions, the gas exchange system was integrated within the GEOMAR modular lander BIGO (Biogeochemical Observatory, Figure 2). The functional principle of the lander has been described by Pfannkuche and Linke (2003) and Sommer et al. (2006). During a research cruise of the RV *MS Merian* 1/3 in April/May 2006, BIGO was deployed for a maximum duration of 61.7 h atop of Captain Arutyunov mud volcano at sites with active mud flow (Table 1). Smooth placement of BIGO on selected sites on the sea floor was conducted by means of a TV-guided launching system. One to two hours after BIGO was placed on the sea floor, the benthic flux chamber (i.d. 28.8 cm, area: 651.4 cm²) was slowly driven into the sediment (~ 30 cm h⁻¹).

To follow sea bed methane emission, eight sequential water samples (vol. ~ 47 mL) were taken from the chamber as well as from the reservoir by means of glass syringe water samplers (Figure 2). The syringes were connected to the chamber using 1-m long Vygon tubes (vol 5.2 mL). Prior to the deployment, these tubes were filled with distilled water, and great care was taken to avoid enclosure of air bubbles. To monitor the ambient bottom water an extra syringe water sampler (8 samples) was employed. The position of the sampling inlet for the ambient bottom water was about 30 cm above the sediment surface. Chamber incubation time periods were defined as the time interval between the first and the last water sample (Table 1). During these deployments the gas exchange system was operated in the mode to maintain defined oxygen concentrations inside the benthic chamber (BIGO 2: 160 µmol L⁻¹, BIGO 3: 170 µmol L⁻¹). These lower than the ambient oxygen concentrations (~ 222 - 231 µmol L-1) were chosen to assess the difference between the TOU measured in the chamber when the gas exchange system is switched off in comparison to the TOU measured when the gas exchange system is operating. Furthermore, during the initial phase when the gas exchange system is switched off, the time course of the oxygen concentration can be used to assess whether the chamber is tightly sealed or not.

Table 1. Station data, total oxygen uptake rates (TOU), and sea bed methane emission rates measured during BIGO deployments during RV *Merian* cruise 1/3*

Gear &			Depth		TOU	CH ₄ emission
station nr	Date	Position	(m)	T _{inc} (h)	(mmol m⁻² d⁻¹)	(mmol m ⁻² d ⁻¹)
BIGO 2, 223	5 May 2006	35°39.700´N 07°20.010´E	1320	56.2	13.2	0.2
BIGO 3, 246	7 May 2006	35°39.706´N 07°20.001´E	1321	61.7	4.4	0
						CH₄ input
						(mmol m ⁻² d ⁻¹)
MUC 10, 296	1 May 2006	35°39.700´N 07°20.010´E	1318	_	_	3.4

 T_{inc} denotes the incubation time (h) of the benthic chambers. The oxygen concentration (µmol L⁻¹) inside the chamber was set as follows: BIGO 2: 160; BIGO 3: 170.

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Component/Control unit	Energy demand (mA)	Energy source	Max. time of operation (h)
Chamber CU	140	12 V/28 Ah	100
Syringe water sampler CU	140	12 V/28 Ah	100
Gas exchange CU (incl. 4 optodes)	200 (340)	12 V/28 Ah	70 (41)
Pumps (reservoir + chamber)	90 + 120	12 V/28 Ah	67†
Motor (chamber)	Idle running: 300 – 600	6 V/28 Ah	_
	Max. load: 3000		
Motor (syringe water sampler)	Idle running: 300 – 600	6 V/28 Ah	_
	Max. load: 3000		

Table	Energy	scheme	of the Bl	GO with	its different	components	and cont	trol units	(CU)*
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*As energy sources, NiCd rechargeable batteries were used, which under in situ conditions provide ~ 14 Ah instead of 28 Ah.

[†]The maximum time of operation was calculated presuming that the pump (chamber), which transports the chamber water to the gas exchange site, is operating permanently.

The energy consumption is a limiting factor for autonomous lander deployments. To cover the energy demand of the different control units, optodes, motors, and pumps of the BIGO, NiCd rechargeable batteries (12 V/28 Ah and 6 V/28 Ah) were used from which about 14 Ah are available under in situ conditions (Table 2). The motors, which move the chamber into the sediment or trigger the water sampling, are not permanently operating. Thus no maximum time of operation was calculated.

After retrieval of the landers, the enclosed sediment cores in the chambers appeared undisturbed with a clear overlying water body and intact sediment topography. The volume of the enclosed cylindrical water column was determined geometrically, using an average height that was carefully measured from the surface of the sediment to the lower edge of the chamber lid at the front, rear, left and right side of the chamber (BIGO 2: 15.6 L; BIGO 3: 12.8 L). The sediment surfaces were not very rugged or inclined. The determination of the height might involve an error of ± 1 cm, which results in a precision of the flux determination of $\pm 4\%$. Indications for sediment compaction due to the insertion of the chamber into the sea floor were not detected.

Methane analysis-Methane concentrations were determined by "head space" analysis modified after Linke et al. (2005). Immediately after retrieval of the BIGO, 10 mL syringe water samples were carefully transferred into a septum stoppered glass vial (vol. 21.6 mL), which contained 3 g sodium chloride. The volume of the resulting headspace was 10.2 mL. Methane concentrations in sediment cores were determined in 1 cm intervals down to a depth of 14 cm followed by 2 cm intervals. From each depth horizon, a subsample (vol. 1.57 mL) was transferred into a septum-stoppered glass vial that contained 6 mL saturated sodium chloride solution and 1.5 g sodium chloride in excess. Volume of headspace was 13.33 mL. Within 24 h, methane concentration in the headspace was determined using a Shimadzu GC 14A gas chromatograph fitted with a flame ionization detector and a 4 m 1/8' Poraplot Q (mesh 50/80) packed column. Prior to the measurements, the samples were equilibrated for 2 h in a shaking table. Analytical accuracy to reproduce a methane standard of 9.98 ppm was \pm 0.15% (corresponding to \pm 6.7 nmol L⁻¹ for water samples and \pm 8.8 nmol L⁻¹ for sediment samples). Porewater methane concentrations of the sediment samples were corrected for the respective water content (range: 54% – 68% by volume). Methane concentrations were corrected for the methane content in the air (1.8 ppm) present in the head-space, and for the dilution of the water samples by the distilled water (vol: 5.2 mL) enclosed in the Vygon tube during in situ water sampling. Methane concentrations determined during this study are well below saturation (1.2 mmol L⁻¹ at 1 bar, 35 psu, 20°C; calc. after Tishchenko et al. 2005) hence loss of methane due to pressure release during recovery of the lander can be excluded.

Determination of methane and oxygen fluxes across the sediment water interface—The TOU and sea bed methane emission, $CH_{4/lux}$ were calculated from linear regressions of the total O₂ and CH_4 inventories enclosed by the chamber and the reservoir versus time

TOU,
$$CH_{4flux} = (dI_{(res + chamber)}/dt)/A_{ch}$$

where dI_(res+chamber)/dt (mmol d⁻¹ for O₂, µmol d⁻¹ for CH₄) is the change of the total inventory of the respective gas over the time period where the oxygen concentration inside the ben-thic chamber was kept constant. A_{ch} is the area of the chamber (m²). The oxygen or methane inventories enclosed by the chamber or the reservoir denote the concentration of O₂ or CH₄ multiplied with the volume of the overlying water in the chamber or the water volume in the reservoir at a given time.

Assessment and discussion

Laboratory tests—An equilibrium oxygen concentration between the glass bottle (Ch) used as chamber and the reservoir (Res) was established after about 300 min for both silicone tubes (wall thickness 0.25 and 1.0 mm) tested (Figure 3A). For both tubes, oxygen transfer was fastest at the start of the measurement when the concentration gradient (delta C) between the reservoir and the chamber was highest. Concurrently with a decreasing concentration gradient, the oxy-



Figure 3. (A) Mean oxygen concentration in the reservoir (Res) and the glass bottle (Ch) used as chamber during laboratory tests using 20 m long silicone tubes with 0.25 and 1 mm wall thickness. Delta C denotes the mean concentration difference between the reservoir and the glass bottle for each tube. For each tube, three replicate measurements were conducted. (B) Mean difference of oxygen concentration between the chamber supplied via the thin-walled tube and that via the thick-walled tube plotted versus time.

gen exchange strongly decreased. The transfer of oxygen across the thin-walled silicone tube is slightly faster than that across the thick-walled tube. However, 100 min after the start of the measurements the maximum difference between the oxygen concentration inside the chamber using a 0.25 mm and a 1.0 mm tube reaches only a maximum of 14 µmol L⁻¹ and decreases rapidly as the oxygen concentrations of the reservoir and the chamber approach equilibrium (Figure 3B). For field applications, both silicone tubes are equally appropriate. The oxygen transfer rate between the reservoir and the chamber water can be further adjusted by using different lengths of the silicone tube. At the starting conditions with a maximum concentration gradient between the reservoir and the chamber, the gas exchange system (tube wall: 1 mm) provides 256 µmol L-1 h-1 oxygen. This would be sufficient to cover a high oxygen demand of 14.4 μ mol L⁻¹ h⁻¹ (corresponding to 56.8 mmol m⁻² d⁻¹) measured at a highly active seep site in the northern Gulf of Mexico (Sommer et al. unpubl. data).

In situ application at Captain Arutyunov mud volcano—Sites with active mud flow at Captain Arutyunov mud volcano were indicated by the occurrence of numerous unsorted clasts of mostly mudstones, high porewater concentrations of methane of up to 10.6 mmol L⁻¹ (Figure 4), and at some locations high sulfide levels of up to 5.7 mmol L⁻¹ in the uppermost 30 cm of the sediment (Sommer et al. unpubl. data). Atop of Captain Arutyunov, we detected gas hydrates buried only a few centimeters below the sediment surface. Maximum methane concentrations in the sediments (max. sampling depth 14.5 cm) retrieved by the chambers of BIGO 2 and 3 were 1.0 and 1.4 µmol L⁻¹ respectively (Figure 4). All of the sampled mud flow sediments were colonized by siboglinid tube worms and the bivalve mollusc *Thyasira sarsi* populated deeper sulfidic sediment strata sporadically. *Oxygen*—During the deployment of BIGO 2 and BIGO 3, the gas exchange system worked very well, showing similar time courses of the oxygen concentration inside the benthic chambers (Figure 5). In BIGO 2, the defined oxygen concentration of 160 μmol L⁻¹ was reached within 14 h. Subsequently, the oxygen concentration inside the chamber remained constant over 46 h until retrieval of the lander, whereas the oxygen content in the reservoir decreased. During deployment of BIGO 3, the defined oxygen concentration of 170 μmol L⁻¹ was reached after 28 h, followed by a constant oxygen concentration inside the chamber for about 37 h. The oxygen



Figure 4. Methane concentrations in surface sediments retrieved during the deployments of BIGO 2 and 3 in comparison to a deeper reaching sediment core (MUC 10) from the mud volcano Captain Arutyunov. Analytical accuracy: \pm 9 nmol CH₄ L⁻¹.



Figure 5. Left panels, oxygen concentration over time in the benthic chamber (EX), the reservoir (RES) and the ambient sea water (AMB) during the deployments of BIGO 2 and 3. Right panels, time course of the combined oxygen inventory of the benthic chamber and the reservoir in BIGO 2 and BIGO 3. The regression lines indicate the total oxygen uptake (TOU) of the enclosed community during the defined and constant oxygen condition of 160 and 170 μ mol L⁻¹ respectively. Accuracy of the optode: $\pm 1 \mu$ mol O₂ L⁻¹.

concentration never fell below the defined oxygen concentration, indicating that gas exchange proceeded rapidly without a noticeable lag phase. Overshooting of the oxygen concentration inside the chamber after initiating the pump was not more than 5 μ mol L⁻¹ above the defined oxygen level. The oxygen concentration of the ambient bottom water, which was measured about 80 cm above the sediment surface ranged between 222 and 231 μ mol L⁻¹.

During the phase where the oxygen concentration inside the chamber was kept constant the total oxygen inventory decreased at a constant rate (Figure 5, left panels). The effect of decreasing oxygen concentration on TOU as often observed during benthic respiration measurements was removed by means of the gas exchange system, and specific TOU rates of 13.2 ± 0.5 and 4.4 ± 0.2 mmol m⁻² d⁻¹ were calculated for the defined oxygen conditions established during the deployment of BIGO 2 and BIGO 3 respectively.

Oxygen consumption in BIGO 2 was relatively high resulting in a nonlinear decrease of the oxygen concentration during the initial phase until the defined oxygen concentration of 160 µmol L^{-1} was reached and the gas exchange system was switched on. At this site the oxygen uptake rate was dependent on oxygen availability inside the chamber. TOU calculated for a 1 h time period prior to switching on the gas exchange system equates to 13.9 mmol m⁻² d⁻¹ and only slightly exceeds the TOU, which was determined under constant oxygen conditions. Oxygen uptake in BIGO 3 was low, and the decrease of the oxygen concentration during the initial phase (gas exchange system not operating) was linear. During this period, TOU was 4.0 mmol $m^{-2} d^{-1}$ and similar to the TOU determined under constant oxygen conditions.

The maximum duration of the maintenance of constant oxygen conditions inside the chamber depends on the biological and geochemical activity of the sediment and on the oxygen inventory provided by the reservoir. With the initial oxygen inventory of 8.7 mmol in the reservoir, an oxygen concentration of 160 μ mol L⁻¹ inside the chamber of BIGO 2 and BIGO 3 could have been maintained for 13.0 d and 37.5 d, respectively.

Resolution of oxygen fluxes/maximum duration to maintain oxic conditions inside the chamber—Bottom water oxygen concentration and the water volume enclosed by the chamber and the reservoir determines the ability of the system to resolve oxygen fluxes as well as the maximum time period of the system to maintain oxic conditions inside the chamber. As our system encloses a large water volume (chamber + reservoir), an important aspect is whether the change of oxygen concentration, which in organically poor environments can be very small, can be reasonably well detected. For the following considerations, we assume that the gas exchange between the reservoir and the chamber takes place all the time, which would be the case when the ambient oxygen concentration shall be maintained during the flux measurement. In Figure 6A, Sommer et al.



Figure 6. (A) Change of the oxygen concentration (μ mol L⁻¹ d⁻¹) in dependence on different volumes of water enclosed by the chamber and the reservoir for five different total oxygen uptake rates (TOU) representing an environmental range from the deep-sea to methane seeps. (B) Time period until the oxygen inventory of the reservoir and the chamber (open symbols) is completely consumed at the three different TOU in dependence on the bottom water oxygen concentration. Filled symbols indicate time of oxygen depletion if only a chamber enclosing a water volume of 10 L is considered.

the effect of different volumes of the water body enclosed by our system on the change of oxygen concentration (μ mol L⁻¹ d⁻¹) for five different TOU is investigated. The total oxygen uptake rates selected cover a range from the deep-sea (0.5 – 1.0 mmol m⁻² d⁻¹) to highly active habitats such as methane seep sites (40 mmol m⁻² d⁻¹). The volumes considered in Figure 6A were selected according to mechanical design of our system where the volume of the reservoir is 29.5 L and that of water body enclosed in the chamber can vary between 10 to 20 L.

At the first sight, the effect of increasing volume on the change of the oxygen concentration at low oxygen consumption rates (0.5 - 1 mmol m⁻² d⁻¹) appears to be of minor importance in comparison to higher TOU. However, the change of oxygen concentration at a TOU of 0.5 and 1 mmol m⁻² d⁻¹ is 3.3 and 6.5 μ mol L⁻¹ d⁻¹ for a volume of 10 L, which in our system would represent the volume of the chamber not including the reservoir. If the volume increases to 50 L, the change of oxygen concentration is only 1.3 and 0.7 µmol L⁻¹ d⁻¹. These changes of the oxygen concentration are close to or beyond the detection limit of the optode and the Winkler titration. To resolve very small total oxygen uptake rates, a small volume is required to detect changes of the oxygen concentration within a reasonable time period. Since the reservoir increases the overall volume, our system is not suited for oxygen flux measurements in such low activity environments. However, the system is versatile and for low oxygen fluxes the system can be set for below ambient oxygen concentrations. With this set up, the system may never turn on resulting in a regular benthic chamber experiment. As the environments become more active in terms of oxygen consumption (e.g., chemosynthetic habitats, continental margin sediments high in organic matter) increasing volume lowers the change of oxygen concentration but it is still clearly detectable. In these environments the gas exchange system despite its increased volume offers the possibility to conduct measurements under natural oxic conditions for longer time periods without losing precision (Figure 6B).

For a given constant TOU, the oxygen concentration in the bottom water determines the time period until the oxygen inventory enclosed by the chamber is completely depleted (Figure 6B). When considering a chamber enclosing a water volume of 10 L (Figure 6B, filled symbols), then in active environments or in habitats with low oxygen bottom water levels (e.g., in oxygen minimum zones) the oxygen inventory of the chamber can be depleted within hours. Under such conditions the accurate determination of the oxygen consumption and of oxygen sensitive fluxes of e.g., methane, dinitrogen, ammonium, or phosphate is not possible. When the oxygen inventory provided by the reservoir (9000 μ mol, Figure 6B, open circles) is included, the time until complete oxygen depletion is prolonged for 13.8 d at a TOU of 10 mmol m⁻² d⁻¹ and 3.5 d at a TOU of 40 mmol m⁻² d⁻¹.

Sea bed methane emission—During constant oxygen conditions inside the chamber of BIGO 2, the sea bed methane emission was 0.2 ± 0.008 mmol m⁻² d⁻¹ (Figure 7). The initially enhanced increase of the methane concentration inside the chamber within the first 14 h is likely due to the disturbance of the sediment. Although the chamber is slowly driven into the sediment, the sediment matrix is slightly disturbed, particularly when clasts or shell fragments are displaced causing small fractures along which methane can easily escape. The enhanced methane release during the initial phase can be also observed in the ambient bottom water. During deployment of BIGO 3, no methane release has been observed. In the reservoir (150 ± 19 nmol L⁻¹) and the chamber (166 ± 19 nmol L⁻¹)



Figure 7. Left panels, methane concentration over time in the benthic chamber (EX), the reservoir (RES), and the ambient sea water (AMB) during the deployments of BIGO 2 and 3. Right panels, time course of the combined methane inventory of the benthic chamber and the reservoir in BIGO 2 and BIGO 3. The regression lines indicate sea bed methane emission during the defined and constant oxygen conditions of 160 μ mol L⁻¹ during BIGO 2. Analytical accuracy: \pm 7 nmol CH₄ L⁻¹.

the methane concentration remained relatively constant. Methane emission from Arutyunov mud volcano sediments is further indicated by elevated methane concentrations in the bottom water, which during deployment of BIGO 2 and 3 were 177.4 ± 9.7 and 167.9 ± 55.7 nmol L⁻¹, respectively. Temporal variability of the methane bottom water concentration was high during BIGO 3. Background methane concentration measured 5 m above the sediment surface at a nearby reference station (CTD 28) in a water depth of 1185 m was 1.8 nmol L⁻¹ (Schneider v. Deimling pers comm.).

Except methane emission rates from Hydrate Ridge, Cascadia convergent margin (Sommer et al. 2006), these are to the best of our knowledge the first in situ sea bed emission rates measured under controlled oxygen conditions. From the sediment core MUC 10 (Figure 4) taken at the Captain Arutyunov mud volcano during the same cruise using a multiple corer, Sommer et al. (unpubl. data) calculated a methane input at a sediment depth of 14 cm of 3.4 mmol m⁻² d⁻¹. Using the maximum methane emission rates measured in this study, only 6% of the methane input at deeper sediment horizons is emitted into the water column. The remaining fraction of 94% is presumed to be consumed in the sediment due to anaerobic methane oxidation. From the Captain Arutyunov mud volcano, Niemann et al. (2006) report an anaerobic turnover rate of 1 mmol m⁻² d⁻¹. Based on the numerical modeling of methane porewater concentration profiles obtained from gravity coring, Hensen et al. (2007) calculated much higher methane emission rates for Captain Arutyunov mud volcano in the range of 10.7 to 18.0 mmol m⁻² d⁻¹ than those measured during this study. In addition to other reasons such as strong spatial and temporal variability, differences in fluid flow velocities, these rates might be overestimated due to the coring technique. For gas-charged sediments covered with microbial mats at Hydrate Ridge and a mud volcano of Costa Rica continental margin, methane emissions in the range of 1.9 to up to 100 mmol m⁻² d⁻¹ were measured in situ using benthic chambers (Linke et al. 2005, Sommer et al. 2006, Torres et al. 2002). For Hydrate Ridge sediments covered with clam beds where the methane and sulfide front is depressed several centimeters deep into the sediment these authors report emission rates $< 1 \text{ mmol m}^{-2} \text{ d}^{-1}$, which is similar to the emission rates found in this study. The lower methane emission rates measured at the clam beds (Hydrate Ridge) and Captain Arutyunov mud volcano correlate well with the respective slow porewater fluid flow in the range of 0.02–0.1 m y⁻¹ at Hydrate Ridge (Torres et al. 2002, Tyron et al. 2002) and 0.1 m y^{-1} at the Captain Arutyunov mud volcano (Hensen et al. 2007).

Comments and recommendations

The gas exchange system in connection to a benthic chamber has a wide range of application in process- and experimentally orientated biogeochemical and ecological studies in aquatic environments. Particularly in organically rich environments or sediments underlying oxygen minimum zones the oxygen inventory inside the chamber is quickly depleted. Below a threshold level of the ambient oxygen concentration TOU becomes nonlinear and the kinetics of redox sensitive reactions is strongly affected and release rates of e.g., methane, sulfide, or ammonium are changed, which under the natural ambient oxygen conditions would not be the case. The gas exchange system is capable to maintain these natural conditions for time periods of days (cf. Figure 6B). Climate change projections predict a 4% to 7% decline of the dissolved oxygen in the ocean until the end of this century (Joos et al. 2003). This is partly related to the warming of the ocean and associated lower solubility of oxygen, but particularly to enhanced stratification and a decrease in ventilation of the ocean interior (Bopp et al. 2002, Keeling and Garcia 2002). Alterations of the oxygenation state of the ocean and the sea floor might strongly affect release rates of methane but also nutrients and trace metals. To test such environmental scenarios, the described gas exchange system provides a suitable tool for measurements of fluxes across the sediment water interface under controlled oxygen conditions.

References

- Aanderaa Instruments AS. 2003. TD 218 Operating manual oxygen optode 3830 and 3930.
- Anderson, L. G., P. O. J. Hall, A. Iverfeldt, M. M. R. van der Loeff, B. Sundby, and S. F. G. Westerlund. 1986. Benthic respiration measured by total carbonate production. Limnol. Oceanogr. 31:319-329.
- Bopp, L., C. LeQuéré, M. Heinemann, A. C. Manning, and P. Monfray. 2002. Climate-induced oceanic oxygen fluxes: Implications for the contemporary carbon budget. Glob. Biogeochem. Cycl. 16:10.10292001GB001445.
- Chadwick, D. B., S. D. Stanley, and S. H. Lieberman. 1994. Autonomous benthic lander for polluted bays, harbors. Sea Tech. 10:10-15.
- Glud, R. N., J. K. Gundersen, and O. Holby. 1999. Benthic in situ respiration in the upwelling area off central Chile. Mar. Ecol. Prog. Ser. 186:9-18.
- Grasshoff, K., M. Ehrhardt, and K. Kremmling. 1983. Methods of seawater analysis. Weinheim, Germany: Verlag Chemie GmbH.
- Hall P. O. J., L. G. Anderson, M. M. Rutgers van der Loeff, B. Sundby, and S. F. G. Westerlund. 1989. Oxygen uptake kinetics in the benthic boundary layer. Limnol. Oceanog. 34:734-746.
- Hensen, C., M. Nuzzo, E. Hornibrook, L. M. Pinheiro, B. Bock, V. H. Magalhães, and W. Brückmann. 2007. Sources of mud volcano fluids in the Gulf of Cadiz—indications for hydrothermally imprint. Geochim. Cosmochim. Acta. 71: 1232-1248.
- Joos, F., G. K. Plattner, T. F. Stocker, A. Körtzinger, and D. W. R. Wallace. 2003. Trends in marine dissolved oxygen:

Implications for ocean circulation change and the carbon budget. EOS Trans. AGU 84:197-204.

- Keeling, R. F., and H. Garcia. 2002. The change in oceanic O₂ inventory associated with recent global warming. Proc. Natl. Acad. Sci. U.S.A. 99:7848-7853.
- Levin, L. A., N. E. Blair, C. M. Martin, D. J. Master, G. Plaia, and C. J. Thomas. 1999. Macrofaunal processing of phytodetritus at two sites on the Carolina margin: in situ experiments using ¹³C-labeled diatoms. Mar. Ecol. Prog. Ser. 182:37-54.
- Linke, P., K. Wallmann, E. Suess, C. Hensen, and G. Rehder. 2005. In situ benthic fluxes from an intermittently active mud volcano at the Costa Rica convergent margin. Earth Planet. Sci. Lett. 235:79-95.
- Moodley, L., J. J. Middelburg, H. T. S Boschker, G. C. A. Duineveld, R. Pel, P. M. J. Hermann, and C. H. R. Heip. 2002. Bacteria and foraminifera: key players in a short-term deep sea benthic response to phytodetritus. Mar. Ecol. Prog. Ser. 236: 23-29.
- Morse, J. W., G. Boland, and G. T. Rowe. 1999. A "gilled" benthic chamber for extended measurement of sediment water fluxes. Mar. Chem. 66:225-230.
- Niemann, H., and others. 2006. Microbial methane turnover at mud volcanoes of the Gulf of Cadiz. Geochim. Cosmochim. Acta. 70:5336-5355.
- Okuda, C., K. Buck, C. Levesque, J. Barry, and M. Risi. 2005. Benthic in situ oxygen consumption measured with an optode-based respirometer [conference abstract]. ASLO 2005, Aquatic Sciences Meeting, Salt Lake City, USA
- Pfannkuche, O., and P. Linke. 2003. GEOMAR landers as long-term deep-sea observatories. Sea Tech. 44:50-55.
- Smith, K. L. 1978. Benthic community respiration in NW Atlantic Ocean – in situ measurements from 40 to 5200 m. Mar. Biol. 47:337-347.
 - and R. J. Baldwin. 1984. Seasonal fluctuations in deep-sea sediment community oxygen consumption: central and eastern North Pacific. Nature. 307:624-626.
- and R. S. Kaufmann. 1999. Long-term discrepancy between food supply and demand in the deep eastern North Pacific. Nature 284:1174-1177.
- Sommer, S., and others. 2006. Efficiency of the benthic filter: Biological control of the emission of dissolved methane from sediments containing shallow gas hydrates at Hydrate Ridge. Global. Biogeochem. Cycl. 20:GB2019, doi:10.1029/ 2004 GB002389.
- Tishchenko, P., C. Hensen, K. Wallmann, and C. S. Wong. 2005. Calculation of the stability and solubility of methane hydrate in seawater. Chem. Geol. 219:37-52.
- Tengberg, A., and others. 2006. Evaluation of a lifetime-based optode to measure oxygen in aquatic systems. Limnol. Oceanogr: Methods. 4:7-17.
- Torres, M. E., and others. 2002. Fluid and chemical fluxes in and out of sediments hosting methane hydrate deposits on Hydrate ridge, OR, I: Hydrological provinces. Earth Planet. Sci. Lett. 201:525-540.

- Tyron, M. D., K. M. Brown, and M. E. Torres. 2002. Fluid and chemical flux in and out of sediments hosting methane hydrate deposits on Hydrate Ridge, OR. II. Hydrological processes. Earth Planet. Sci. Lett. 201:541-557.
- Viollier, E., and others. 2003. Benthic biogeochemistry: state of the art technologies and guidelines for the future of in situ survey. J. Exp. Mar. Biol. Ecol. 285-286: 5-31.
- Wenzhöfer, F., and R. N. Glud. 2002. Benthic mineralization in the Atlantic: a synthesis based on in situ data from the last decade. Deep-Sea Res. I 49:1255-1279.

Witte, U., and O. Pfannkuche. 2000. High rates of benthic

carbon remineralisation in the abyssal Arabian Sea. Deep-Sea Res. II 47:2785-2804.

- and others. 2003a. In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. Nature 424:763-766.
- —, N. Aberle, M. Sand, and F. Wenzhöfer. 2003b. Rapid response of a deep-sea benthic community to POM enrichment: An in situ experimental study. Mar. Ecol. Prog. Ser. 251:27-36.

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