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Use of voltammetric solid-state (micro)electrodes for studying biogeochemical processes: Laboratory measurements to real time measurements with an *in situ* electrochemical analyzer (ISEA)

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Abstract

Solid-state voltammetric (micro)electrodes have been used in a variety of environments to study biogeochemical processes. Here we show the wealth of information that has been obtained in the study of sediments, microbial mats, cultures and the water column including hydrothermal vents. Voltammetric analyzers have been developed to function with operator guidance and in unattended mode for temporal studies with an *in situ* electrochemical analyzer (ISEA). The electrodes can detect the presence (or absence) of a host of redox species and trace metals simultaneously. The multi-species capacity of the voltammetric electrode can be used to examine complex heterogeneous environments such as the root zone of salt marsh sediments. The data obtained with these systems clearly show that O_2 and Mn^{2+} profiles in marine sedimentary porewaters and in microbial biofilms on metal surfaces rarely overlap indicating that O_2 is not a direct oxidant for Mn^{2+}. This lack of overlap was suggested originally by Joris Gieskes' group. In waters emanating from hydrothermal vents, Fe^{2+}, H_2S and soluble molecular FeS clusters (FeS_{aq}) are detected indicating that the reactants for the pyrite formation reaction are H_2S and soluble molecular FeS clusters. Using the ISEA with electrodes at fixed positions, data collected continuously over three days near a *Riftia pachyptila* tubeworm field generally show that O_2 and H_2S anti-correlate and that H_2S and temperature generally correlate. Unlike sedimentary environments, the data clearly show that *Riftia* live in areas where both O_2 and H_2S co-exist so that its endosymbiont bacteria can perform chemosynthesis. However, physical mixing of diffuse flow vent waters with oceanic bottom waters above or to the side of the tubeworm field can dampen these correlations or even reverse them. Voltammetry is a powerful technique because it provides chemical speciation data (e.g.;

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oxidation state and different elemental compounds/ions) as well as quantitative data. Because (micro)organisms occupy environmental niches due to the system's chemistry, it is necessary to know chemical speciation. Voltammetric methods allow us to study how chemistry drives biology and how biology can affect chemistry for its own benefit.

Keywords: Solid state electrodes; Voltammetry; *In situ* analyses; Water column; Sediments; Hydrothermal vents

1. Introduction

Electrochemical techniques have often been used to study environmental processes. However, in the last couple of decades, there has been much interest in making real time measurements with these and other techniques. In this paper, we review and update our group's use of solid-state voltammetric solid-state (micro)electrodes. These electrodes are able to measure multiple analytes simultaneously at fine scale resolution. They were originally designed to detect a suite of important redox chemical species in sediments but have now been used for water column work in anoxic basins and near hydrothermal vents.

We also show representative scans for a variety of chemical species to introduce the reader to all that is possible with the technique; discuss standardization and calibration of the electrodes; document that these electrodes can be used for longer term measurements in the environment; and provide representative examples of data from different environments. Before we review some principles of electrochemical methods for measurements, we note the breadth of work performed by Joris Gieskes during his career. Joris performed work on samples from the water column, sediments, brines, hydrothermal vents among other environments. In this paper, we show how our electrode technology can be applied to some of these environments.

The major methods using electrodes (including microelectrodes) in environmental chemical analysis are of three general types (1) conductimetric, (2) potentiometric and (3) amperometric/voltammetric (Taillefert et al., 2000a). Conductimetric electrodes are used to measure salinity and are incorporated into a variety of designs commercially and will not be further discussed. Examples of the potentiometric type are pH (Cai and Reimers, 1993), sulfide as the Ag_2S electrode (e.g., Revsbech et al., 1983) and pCO_2 (Cai and Reimers, 1993), and of the amperometric type are O_2 and N_2O (Revsbech et al., 1980, 1988). In the potentiometric type, potential is measured at near zero current passing through an electrochemical cell consisting of the working electrode and a reference electrode, and the potential is proportional to the concentration of the analyte. In amperometric types (based on voltammetry), current is measured at a fixed potential between the working and reference electrodes, and the current is proportional to the concentration of the analyte. Initial environmental research has centered on the use of microelectrodes for a single species in microbial ecology (e.g., Revsbech and Jorgensen, 1986) and in deep-sea sediments (e.g., Reimers et al., 1986) although two to three separate microelectrodes housed together in one unit have been reported (Revsbech and Jorgensen, 1986; Visscher et al., 1991).

In voltammetric work, current is measured while scanning the entire voltage range of the solid-state electrode (I vs E curves), which allows the measurement of more than one species at a given time in the same region of space (Brendel and Luther, 1995). Voltammetry is analogous to absorbance versus wavelength curves in spectroscopy. Measured electrode currents for peaks during voltage scans are proportional to the concentration(s) of the analyte(s). An advantage of voltammetry with full voltage scan over amperometry at one fixed potential is that voltammetry provides a better measure of background or zero current. Voltammetry is thus a non-selective method that can measure many chemical species. In Voltammetry, a reference electrode (e.g., Ag/AgCl) is used to apply voltage against a working electrode (e.g.; Au/Hg) while a counter electrode (Pt) is used to measure current so that no current passes through the reference electrode.

The need for voltammetric sensors arose in the late 1980's because of intense interest in studying anaerobic oxidation processes of sulfide with Fe and Mn oxide minerals (e.g., Aller and Rude, 1988; Burdige and Nealson, 1986), bacterial reduction of Fe and Mn oxides during organic matter decomposition (Lovley, 1991; Nealson and Myers, 1992) and bacterial oxidation of Fe^{2+} and Mn^{2+}(e.g.; Tebo, 1991). It was clear that another sensor was needed to measure these metals in conjunction with other chemical species in real time. Until our development of the solid-state Au/Hg (micro)electrode (Brendel and Luther, 1995), it was not possible to measure metals and redox species such as O_2 and H_2S simultaneously and on the same electrode. Using a single (micro) electrode we could determine multiple species including the metals, Fe and Mn, which are important in sedimentary processes on the same (sub)millimeter scale as O_2 and at larger scales (meter) in the water column of lakes, estuaries (e.g., Chesapeake Bay); major basins (e.g., Black Sea) and hydrothermal vents. Our single Au/Hg electrode has the capability of determining all the key soluble redox species except for nitrate and ammonia. Table 1 shows the chemical species that have been analyzed to date in the environment. The solid-state Au/Hg voltammetric microelectrode can measure rapidly, at the same time, and with (sub)millimeter spatial resolution, five of the principal redox species involved in early diagenesis (O_2, Mn^{2+}, Fe^{2+}, HS^-, and I^-) as well as FeS and Fe(III) species. I^- is a proxy for nitrate reduction because bacterial iodate and nitrate oxidation of organic mater have similar free energies (Luther et al., 1998). This tool can be used to measure the distribution of these redox components in natural waters and in the porewaters of marine and freshwater sediments as well as temporal changes at any given location or depth. In addition, the technique can measure several trace metals and the direct measurement of Zn(II) (Nolan and Gaillard, 2002) and Pb (II) (Sundby et al., 2005) in sediments has now been accomplished. Lastly a flow cell device with either the gold plated mercury electrode (Luther et al., 2002) or a iridium plated mercury electrode encased in a gel membrane has been used to measure these and other electroactive metals in natural waters (Tercier-Waeber et al., 2002).

Table 1
Electrode reactions at the Au/Hg electrode vs the saturated calomel electrode (SCE)

		E_p ($E_{1/2}$) (V)	MDL (μM)
$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	(1a)	−0.33	3
$H_2O_2 + 2H^+ + 2e^- \rightarrow H_2O$	(1b)	−1.23	3
$HS^- + Hg \rightarrow HgS + H^+ + 2e^-$	(2a)	adsorption onto Hg < −0.60	
$HgS + H^+ + 2e^- \leftrightarrow HS^- + Hg$	(2b)	~ −0.60	<0.1
$S(0) + Hg \rightarrow HgS$	(3a)	adsorption onto Hg < −0.60	
$HgS + H^+ + 2e^- \leftrightarrow HS^- + Hg$	(3b)	~ −0.60	<0.1
$Hg + S_x^{2-} \rightarrow HgS_x + 2e^-$	(4a)	adsorption onto Hg < −0.60	
$HgS_x + 2e^- \leftrightarrow Hg + S_x^{2-}$	(4b)	~ −0.60	<0.1
$S_x^{2-} + xH^+ + (2x-2)e^- \rightarrow xHS^-$	(4c)	~ −0.60	<0.1
$2RSH \leftrightarrow Hg(SR)_2 + 2H^+ + 2e^-$	(5)	typically more positive than H_2S/HS^-	
$2S_2O_3^{2-} + Hg \leftrightarrow Hg(S_2O_3)_2^{2-} + 2e$	(6)	−0.15	10
$2I^- + Hg \leftrightarrow HgI_2 + 2e$	(7)	−0.28	<0.1
$FeS + 2e^- + H^+ \rightarrow Fe(Hg) + HS$	(8)	−1.15	molecular species
$Fe^{2+} + Hg + 2e^- \leftrightarrow Fe(Hg)$	(9)	−1.43	10
$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$	(10)	−0.2 to −0.9 V	molecular species
$Mn^{2+} + Hg + 2e^- \leftrightarrow Mn(Hg)$	(11)	−1.55	5
$Cu^{2+} + Hg + 2e^- \leftrightarrow Cu(Hg)$	(12)	−0.18	<0.1
$Pb^{2+} + Hg + 2e^- \leftrightarrow Pb(Hg)$	(13)	−0.41	<0.1
$Cd^{2+} + Hg + 2e^- \leftrightarrow Cd(Hg)$	(14)	−0.58	<0.1
$Zn^{2+} + Hg + 2e^- \leftrightarrow Zn(Hg)$	(15)	−1.02	<0.1
$S_4O_6^{2-} + 2e^- \rightarrow 2S_2O_3^{2-}$	(16)	−0.45	<50
$2HSO_3^- + Hg \leftrightarrow Hg(SO_3)_2^{2-} + 2H^+ + 2e^-$ requires low pH (~4.7) as sulfite (SO_3^{2-}) is not electroactive	(17)	−0.50	<50

All data were obtained with a 100-μm diameter electrode ($A = 7.85 \times 10^{-3}$ mm^2). O_2 data were collected by Linear Sweep Voltammetry at 200 mV s^{-1}; all others were collected by Square Wave Voltammetry at 200 mV s^{-1}. Potentials can vary with scan rate and concentration; e.g., on increasing concentration, the sulfide signal becomes more negative. Detection limit can be enhanced with faster scan rates (Bond, 1980).

When applying potential from a positive to negative scan direction, sulfide and S(0) react in a two step process (1 — adsorption onto the Hg surface; 2 — reduction of the HgS film) and polysulfides react in a three step process (1 — adsorption onto the Hg surface; 2 — reduction of the HgS_x film; 3— reduction of the S(0) in the polysulfide). Increasing the scan rate separates electrode reactions 4b and 4c into two peaks because Eq. (4c) is an irreversible process (increasing scan rate shifts this signal; Bond, 1980). MDL = Minimum detection limit.

2. Experimental section

2.1. Solid state working (micro)electrodes construction and analytical methods

Gold amalgam glass electrodes are constructed as described in Brendel and Luther (1995) with modifications to insure a waterproof seal. Briefly, the end of a 15 cm section of 5 or 6 mm-diameter glass tubing is heated in a small flame and the tip pulled to a diameter of less than 0.4 mm for a length of about 3–5 cm. The 100 µm-diameter Au wire is soldered to the conductor wire of a BNC cable (RG-174U/type 50 Ω coax cable; 0.095 in diameter) and inserted through the larger inside diameter part of the glass so that the Au/Cu/solder junction is at the bottom of the large diameter part of the glass. Then, the solder junction is fixed within the tubing with West System 105 epoxy resin and 206 hardener to form a high-purity, optical-grade, nonconductive fill. The epoxy is injected into the larger diameter of the glass tubing with a syringe and Teflon cannula (20 gauge). The epoxy drains slowly through the open smaller diameter of the glass tip. On setting, the epoxy seals the tip and the top end can be refilled with epoxy if necessary. After final setting of the epoxy, the tip is sanded and polished. Then the top end is coated with Scotchkote (3M) electrical coating and Scotchfil (3M) electrical insulation putty. These glass tipped electrodes are durable but can break when dropped or when they hit clam shells located in sediments. The wire is then mated to an appropriate connector for attachment to the analyzer.

Gold amalgam PEEK™ (polyethyletherketone) electrodes are made by fixing 100 µm-diameter Au wire soldered to the conductor wire of BNC cable within a body of 0.125″-diameter PEEK™ tubing, which is commercially available as standard HPLC high-pressure tubing. The metal is fixed within the tubing with the West System epoxy as above. The epoxy is injected into the tip of the PEEK™, which contained the gold wire that was previously soldered to the conductor wire of the BNC cable. The epoxy comes out the end where the BNC cable is and the cable is pushed flush to make contact with the PEEK™ tubing. The PEEK™ tubing is slightly smaller than the diameter of the BNC cable so does not drain although the epoxy has a moderate setting time (~1 h). On setting, the epoxy seals the tip and the top end can be refilled with epoxy. Then the BNC cable end is coated with Scotchkote (3M) electrical coating and Scotchfil (3M) electrical insulation putty. After final setting of the epoxy, the tip is sanded and polished. We have tested this design in the lab at a pressure of 200 atm without failures. PEEK™ and high-purity epoxy fill permits the determination of metal concentrations without risk of contamination, and at temperatures as high as 150 °C.

Once constructed each working electrode surface was sanded with 400 grit paper, and then polished with four diamond polishes of successively smaller size (15; 6; 1; and 0.25 µm). Finally each electrode is plated with Hg by reducing Hg(II) from a 0.1 N Hg/0.05 N HNO_3 solution, for 4 min at a potential of −0.1 V, while purging with N_2. The mercury/gold amalgam interface was conditioned using a 90 s −9 V polarization procedure in a 1 N NaOH solution (Au/Hg on the negative terminal and Pt wire on the + terminal). The electrode was then run in linear sweep mode from −0.05 to −1.8 V versus a Saturated Calomel Electrode (SCE) or a solid-state Ag/AgCl electrode several times in oxygenated seawater to obtain a reproducible O_2 signal. The SCE and solid-state Ag/AgCl reference electrodes are similar in potential.

Fig. 1A shows the two working electrode designs — one in glass for sediment work and another in the durable polymer PEEK™ for water column and hydrothermal vent work. Fig. 1B shows a glass electrode after fabrication but before sanding and polishing, and Fig. 1C shows the same electrode after sanding and final polishing. In all our work, the electrodes provide reproducible data because of the application of *in situ* conditioning potentials to clean the electrode electrochemically after each voltage scan.

Solid state Pt (counter) and Ag/AgCl (reference) electrodes were fabricated in a similar way but 500 µm-diameter or larger Pt and Ag wire are used. Also, the metal is permitted to protrude from the tip by at least 20 mm. To make a AgCl coating on the Ag wire, the Ag wire is placed into a chloride solution and polarized for 20 s. using a 9 V battery (Pt wire on the negative terminal and Ag wire on the + terminal).

To maintain reproducibility during analysis, a conditioning step was used between each potential scan. When O_2 is present, conditioning is not necessary and a value more negative than −0.1 V will result in partial consumption of O_2. We have found that a potential of −0.05 V for a period of <5 s restores the electrode surface while measuring Fe and/or Mn in anoxic environments when H_2S is not present. If H_2S or a soluble Fe(III) species are present, a potential of −0.9 V is used to remove any Fe, Mn and sulfide deposited on the electrode, since none of these species is electroactive at this potential. Standardization of the electrodes is described in Brendel and Luther (1995) and Luther et al. (2002).

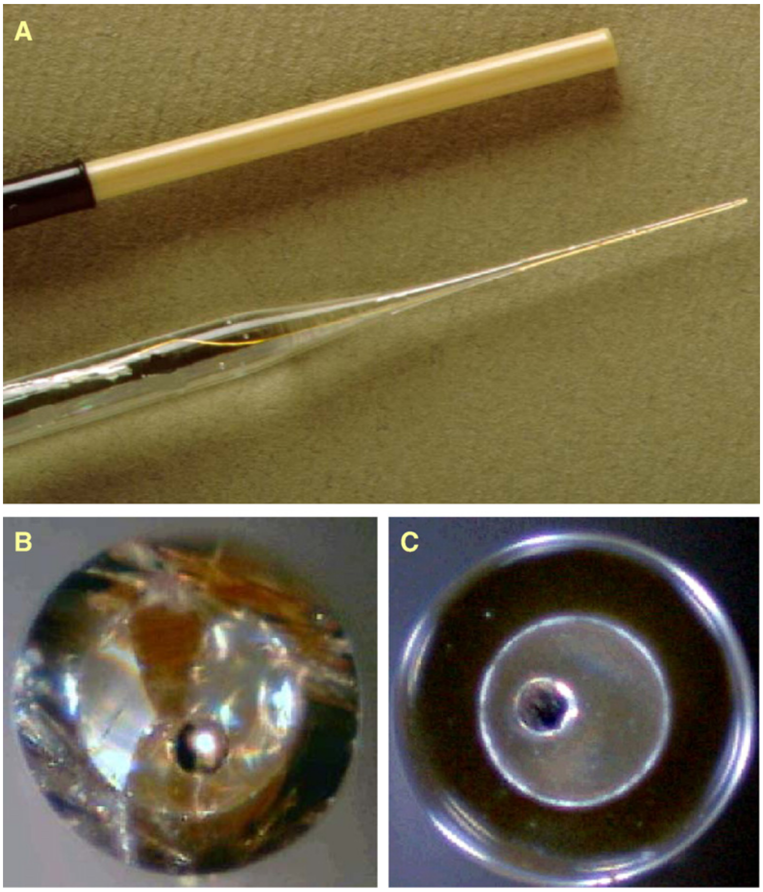

Fig. 1. (A) A glass encased and PEEK electrode — the Au wire is soldered to the conductor wire and fixed with a non-conductive epoxy. (B) The tip of a 100 µm diameter Au electrode in glass after initial construction but before sanding and polishing. (C) After final polishing with 0.25 µm diamond polish and prior to plating with Hg solution.

In our early sediment work (Brendel and Luther, 1995; Luther et al., 1997, 1998), cores were brought back to the lab or to the shipboard laboratory. However, due to the strong interest in making real time measurements to better document biogeochemical processes, we have now deployed the solid-state electrodes in a variety of environments with the aid of industrial cooperation (Analytical Instrument Systems, Inc.). *In situ* studies range from sediment studies in lakes, bays and estuaries to water column studies in the Black Sea (Konovalov et al., 2003) to hydrothermal vent studies (Luther et al., 2001a,b) that document the chemistry that constrains life in extreme environments. Recent local work documents the chemistry in inland bays that leads to fish kills (Luther et al., 2004).

2.2. Temperature studies

A thermostatted voltammetric cell was attached to a water bath. For O_2 temperature dependence experiments, a solution of air saturated seawater was added to the cell. The current was determined and the solution was then analyzed by traditional Winkler titration after addition of reagents. New solutions were used for each temperature/current measurement.

For HS^-, Mn(II) and Fe(II), 25 mL of seawater was added to the cell, which was then degassed with Argon and adjusted to the appropriate temperature. The seawater pH was adjusted to 8.5, 8.1 and 5.7 for HS^-, Mn(II) and Fe(II), respectively. These pH adjustments insured for sulfide that only one species (HS^-) was present and that oxidation of Fe(II) was minimized. Hydroxylamine was also added to Fe(II) solutions to prevent any oxidation from traces of O_2. For each analyte, degassed standards were then added to the deaerated seawater, and scans showed that O_2 was not detectable (detection limit ~3 µM) and the current of the analyte was then obtained by performing 5–10 CV scans. Fresh solutions were made for each species at each temperature measurement.

2.3. In situ voltammetric analyses

For shallow work, voltammetric analyses were carried out in real-time using an Analytical Instrument Systems, Inc. (AIS) DLK-100A laboratory electrochemical analyzer, controlled by a microcomputer aboard ship and using software provided by the manufacturer. The analyzer and computer were connected to a 12 V D.C. marine deep cycle battery. For profiles <5 m water depth, the working electrode can be lowered while keeping the counter and reference electrode in surface water. For water depths to 30 m, a long Cable Receiver, AIS Model DLK-LCR-1, was connected directly to the analyzer as an interface with a 30-meter waterproof cable. The cable contained three shielded wires [center conductor with shield] for the working (Au/Hg), reference (Ag/AgCl) and counter (Pt) electrodes as well as DC power (+15 V, −15 V) and ground. The other end of the cable was connected to a transmitter, AIS Model DLK-LCT-1, enclosed in a pressure housing and mounted on a stainless steel frame connected to a winch with durable rope. The transmitter was connected via 1-meter lengths of shielded cable directly to the working, reference and counter electrodes mounted on a stainless steel frame. The working electrode acted as the virtual ground for the system. This setup allows signals to be transmitted through 30 meters of cable without signal degeneration. We typically used linear sweep voltammetry (≥1000 mV/s) waveforms for these *in situ* analyses. Any other electrochemical waveform can also be applied to this electrode system. The potential range scanned was typically −0.05 to −1.8 V.

For deep-water work, DLK-SUB electrochemical analyzers from AIS were used to collect data. There are two designs. The first, which is used for water column work or survey work, is powered via a cable from remotely operated vehicles (ROV) or deep-sea submersibles (Nuzzio et al., 2002) or by internal 12 V DC batteries (Konovalov et al., 2003). Briefly, this instrument contains a potentiostat, a multiplexer to monitor up to four electrodes one at a time, and a computer, which communicates with another computer aboard ship. All waterproof connections were made with connectors from Impulse, Inc. and the aluminum housing (1 m length; 20 cm outside diameter) of the instrument is rated for operation at full ocean depth (~6000 m; 600 atm). The instrument is a stand-alone package capable of being deployed from ships, DSV *Alvin*, or remotely operated vehicles (ROV) with tethers containing RS-232 cable up to 1500 m without the need for signal amplification. The voltammetry hardware is linked to an IBM compatible computer inside the housing. The internal computer communicates with another computer via the RS 232 cable and is controlled by an operator, who can reprogram waveforms to respond to the radically different environments found at vent sites. A separate 1-meter cable is used to make connections with the working, counter, and reference electrodes and the pressure housing. This cable has four inputs for working electrodes, one input for the counter electrode and another for the reference electrode. Another input for grounding the reference electrode insured signal integrity. For DSV *Alvin* the working electrode wand or holder is constructed of Delrin encasing a stainless steel handle so that the manipulator (arm) can hold and deploy the electrodes without breaking them. The counter and reference electrodes are place in the submersible's basket and are not moved by any manipulator.

The second design is similar in design but is modified to be a totally unattended *in situ* electrochemical analyzer (ISEA), which can be powered by a separate battery or by solar panels. The internal computer is a 1 GB memory card that can be programmed to run a sequence of experiments and store the data until the analyzer is recovered. This lowers power consumption relative to the system described above. The ISEA can be deployed for longer periods of time in an unattended mode of operation.

3. Results and discussion

3.1. Chemical species measurable

Table 1 gives pertinent information on electrode reactions of analytes of interest in marine chemistry. Representative cyclic voltammogram (CV) scans are given in Fig. 2 for dissolved O_2 (Fig. 2A), Mn^{2+} (Fig. 2B), H_2S/HS^- (Fig. 2C), Fe^{2+} (Fig. 2D), a solution with both Fe^{2+} and H_2S/HS^- to produce FeS molecular clusters (FeS_{aq}, Fig. 2E), a solution where Fe^{2+} was allowed to form Fe(III) (Fig. 2F), I^- (Fig. 2G), and $S_2O_3^{2-}$ (Fig. 2H). For most analytes, a distinct "s" shaped curve is observed. However, for H_2S/HS^- (Fig. 2C), a peak shaped curve results because the scan begins at positive potential where H_2S/HS^- electrochemically oxidizes the Hg to form a HgS film. On scanning to negative potentials, the sulfide is stripped (cathodically). On the return scan in the positive direction, a small "s" shaped curve is noted as the HgS film is reformed. When Fe^{2+} is added to H_2S/HS^- (Fig. 2E) the peak decreases and signals for both FeS_{aq} and Fe^{2+} are observed. The H_2S/HS^- peak also shifts positively due to complexation (Luther et al., 1996a).

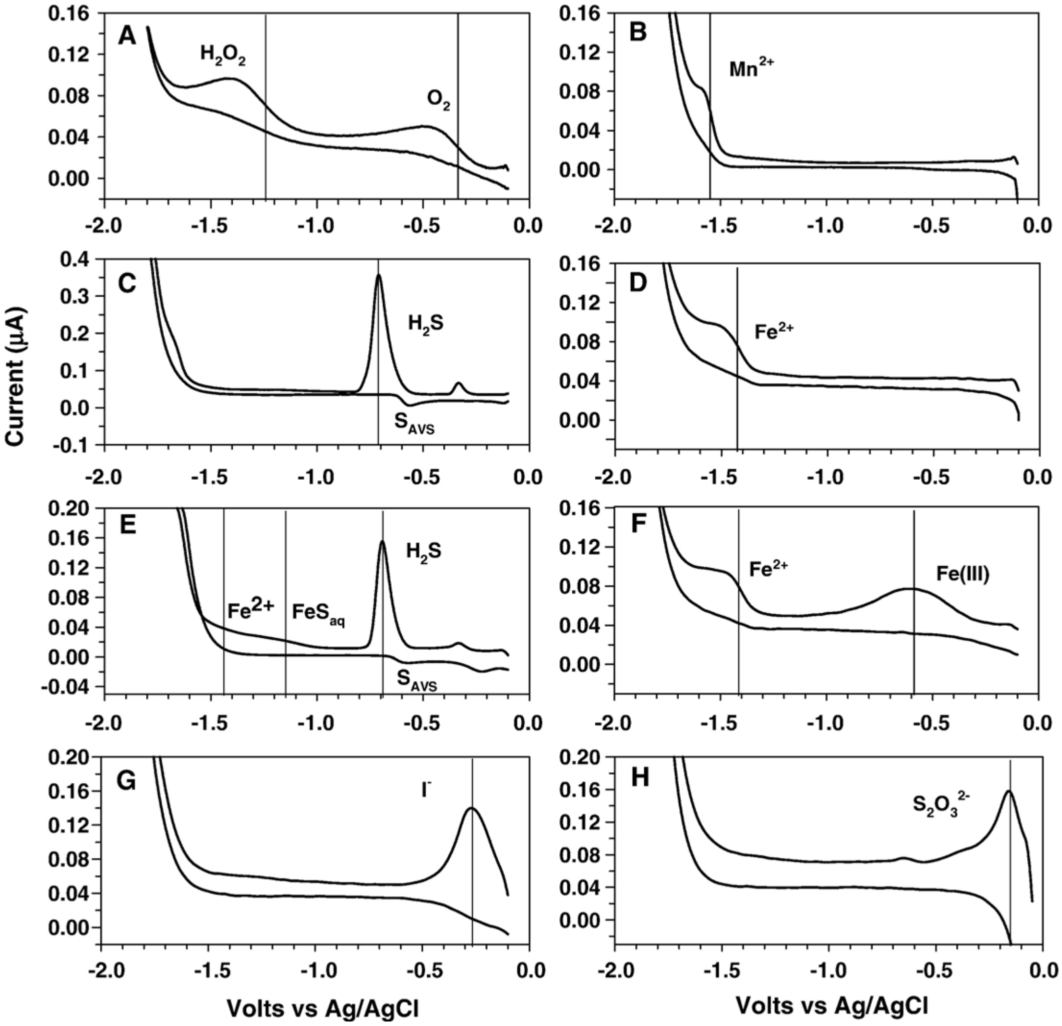

Fig. 2. Representative cyclic voltammograms scans at a scan rate of 1000 mV s^{-1} for the following chemical species in Sargasso Sea seawater (pH 8.00 unless other wise noted). Direction of voltage scan is from positive V to negative V back to positive V. (A) O_2 saturated in seawater, (B) 98 µM Mn^{2+}, (C) 50 µM H_2S, (D) 200 µM Fe^{2+}(pH 5.09), (E) 25 µM $Fe^{2+}$and 50 µM H_2S (pH 7.00), (F) 200 µM Fe^{2+}(pH 8.00 — note Fe^{2+}oxidation), (G) 100 µM I^-, (H) 100 µM $S_2O_3^{2-}$. The lines indicate the half-wave potential ($E_{1/2}$) or the peak potential (Ep) for the analyte.

3.2. Standardization of working electrodes

Several factors need to be considered to standardize the working electrode. The current of an electrode reaction is dependent on (i) temperature as the diffusion coefficient and gas solubility (in the case of dissolved O_2,) vary with temperature, (ii) salinity as diffusion of the analyte to the electrode increases with low salinity, (iii) pH as the chemical speciation (e.g., of a metal) may change with pH, (iv) the (flow rate)$^{1/2}$ and (v) the (scan rate)$^{1/2}$. Items (ii) and (iii) were discussed in Brendel and Luther (1995) whereas items (iv), (v) and the independence of current with pressure were discussed in Luther et al. (2002). We have found that the 100 µm-diameter working electrodes give constant current once the flow rate reaches 0.01 cm s^{-1} as the rate of mass transport is constant above that flow. In addition, scan rates above 1000 mV s^{-1} are necessary to obtain full scans without noise from stochastic mixing. Fig. 3 shows that the noise becomes significantly less at high scan rates. We also note the peroxide wave increases with scan rate relative to the O_2 wave as scan rate increases. At very high scan rates, it can double and this signal increase can be used to obtain better O_2 detection limits.

As an example of standardization based on (i), Fig. 4 shows the effect of dissolved O_2 in seawater with temperature. As temperature increases, the concentration of dissolved O_2 decreases, whereas the diffusion of

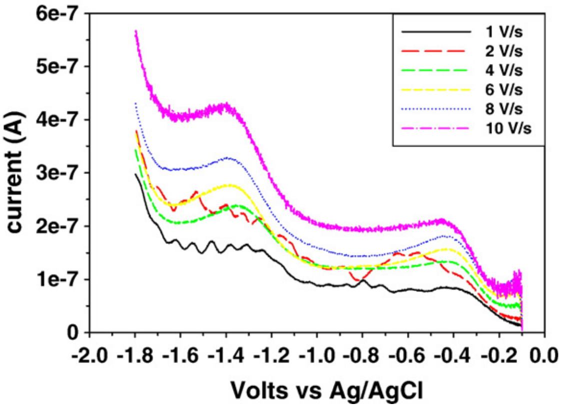

Fig. 3. *In situ* cyclic voltammograms (O_2 scans) at different scan rates in Delaware Bay surface waters (near 14 foot Lighthouse).

dissolved O_2 to the working electrode increases. These two parameters compensate each other in such a way that the current for 100% saturation of dissolved O_2 remains the same over the temperature range from 2 to 50 °C. Thus, standardization and calibration of the Au/Hg electrode for dissolved O_2 over temperature requires only one temperature measurement.

Fig. 5A shows the current from cyclic voltammetry scans as a function of the temperature for HS^-, Mn(II) and Fe(II). For all analytes, the current increases with temperature. The curves obtained are not linear as the Randles-Sevcik Eq. (1) indicates that current is proportional to the square root of the diffusion coefficient, D. [The other parameters were kept constant and are defined as n = the number of electrons for the electrode reaction; A = area of the working electrode, C = concentration of the analyte; and v = scan rate]. Fig. 5B shows that for all analytes the current is proportional to $D^{1/2}$ where D values are calculated for each temperature from Eq. (2) (Boudreau, 1997) using parameters in Li and Gregory (1974) and Boudreau (1997). Standard curves are performed at room temperature and for field temperatures the appropriate slopes are used based on the data in Fig. 5A and B.

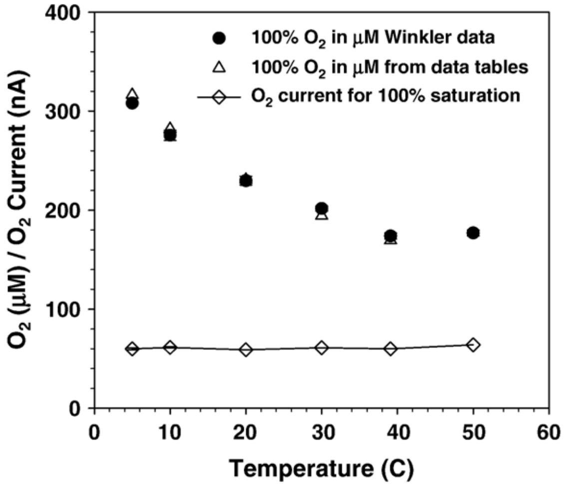

Fig. 4. Current data (◊) for O_2 in Sargasso Sea seawater as temperature is varied. The Winkler titration data (●) and expected O_2 saturation (Δ) are also provided.

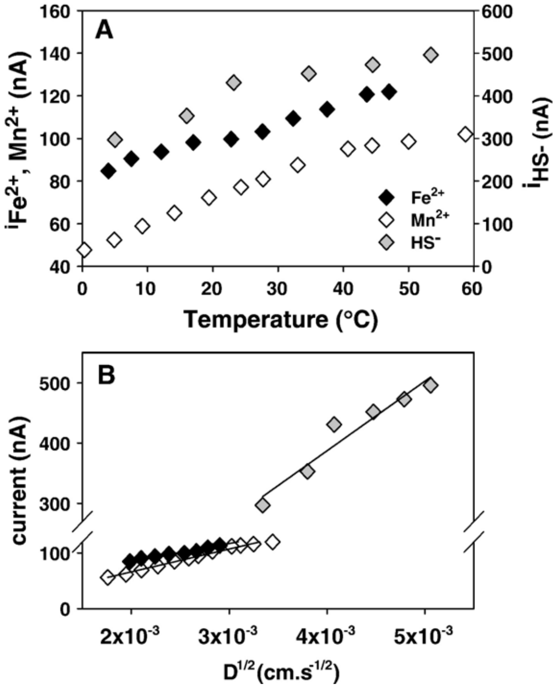

Fig. 5. (A) Variation of current with temperature for 140 µM HS^-, 480 µM Mn(II) and 900 µM Fe(II) in Sargasso Sea seawater. (B) Current as a function of the square root of the diffusion coefficient, D, for HS^-, Mn(II) and Fe(II) in Sargasso Sea seawater. Linear regressions have respective r^2 of 0.94, 0.99, 0.98.

$$i = 2.69 \times 10^5 n^{3/2} A C D^{1/2} v^{1/2} \tag{1}$$

$$D^0(T) = (m_0 + m_1 T)10^{-6} \tag{2}$$

3.3. Electrode stability

Fig. 6 shows the stability of the Au/Hg electrode for O_2 determination in filtered seawater which was replaced everyday in an electrochemical cell in the laboratory over a 2 month period. The total number of scans for this experiment is 7909; the average current is 37.94 nA with a relative standard deviation (RSD) of 0.723. A thermostatted electrochemical cell was not used for this work so the spread in the RSD

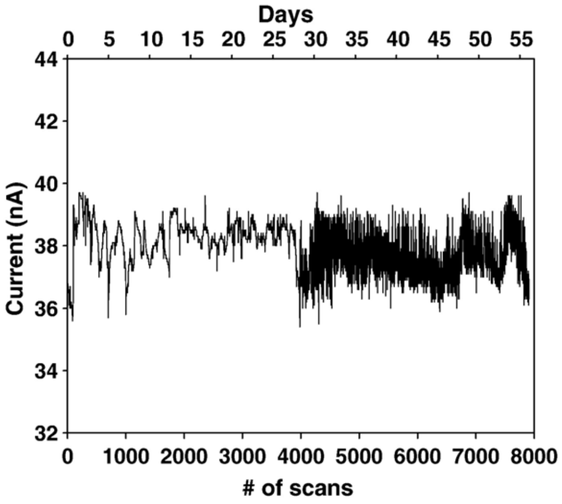

Fig. 6. Au/Hg electrode data in filtered Sargasso Sea seawater saturated with O_2 over a two month period. The linear sweep scan rate was 200 mV s^{-1}.

is mainly related to temperature fluctuations in the laboratory.

In another *in situ* field experiment, a Au/Hg working electrode was placed in a stainless steel cage and lowered with a boom off of the University of Delaware dock for about five days. The electrode was held in a horizontal position to the sediment about 30 mm from the sediment/water interface and 2 m from the dock's wall. In this experiment, a 12 V DC battery was coupled to a solar panel so that the battery could be recharged during the day. The electrode was run every 15 min for four scans and then was held at the −0.9 V conditioning step until the next set of measurements. Fig. 7 shows the concentration decreases over the first three days but shows a sinusoidal pattern that follows the water height variation with the tides. On the fourth day, a rainstorm with significant mixing occurred, and the concentration increased followed by a decrease. Because of the low light levels during the storm the battery did not recharge and the experiment was stopped. The filled black circles are the current data for the electrode, which was brought onto the dock twice daily and tested with a 100% saturated dissolved O_2 seawater standard in a standard electrochemical cell. The reproducibility and stability over this period are excellent and demonstrate that the working electrode should be able to be used for much longer periods. There are two reasons for this stability (i) the conditioning step used between each set of scans cleans the electrode and (ii) the scanning of potential from −0.1 to −1.8 V prevents biofouling of the tip. Interestingly, the first scan of each set showed that a trace of sulfide was measurable (data not shown). These observations indicate that wave and tidal motion affect the sediment-water interface and permit H_2S from the porewaters to be released to the water column.

3.4. Sediments

Our early salt marsh (Brendel and Luther, 1995; Luther, 1995; Luther et al., 1996b) work showed the importance of the Au/Hg electrode in measuring dissolved Mn^{2+}, Fe^{2+} and Fe^{3+} in addition to O_2 and H_2S, but work in the St. Lawrence estuary and continental margin sites (Luther et al., 1997, 1998; Anschutz et al., 2000) clearly documented the importance of Mn and Fe dynamics in the sedimentary environment. In this work, we showed that the O_2 profile rarely overlaps with the Mn^{2+} profile in marine sedimentary environments (Fig. 8). Their overlap had generally been assumed (e.g. Froelich et al., 1979; Cai and Sayles, 1996) [although not by Gieskes' group (see Shaw et al., 1990)] because direct measurement of all redox species without sampling artifacts was not an easy task by traditional methods. Thus, another oxidant is required to oxidize Mn^{2+} and our results showed that nitrate and iodate are excellent candidates linking the Mn and N cycles to the formation of N_2 (see also Anschutz et al., 2000; Boudreau et al., 1998). Our sediment profiles show remarkable similarity to water column profiles of the Black Sea (Murray et al., 1995) indicating that redox processes are similar in anoxic basins and sediments. Finally, the ability of the electrode

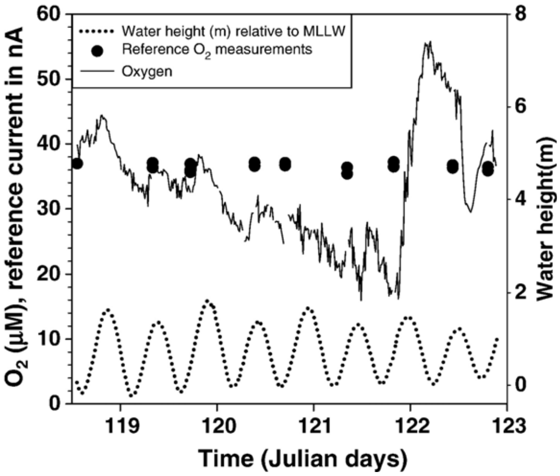

Fig. 7. *In situ* Au/Hg electrode data (solid line) above the sediment-water interface at the University dock over a five day period. The solid circles are the filtered seawater saturated with O_2 standard (scan rate of 200 mV s^{-1}) and match the data in Fig. 6. The dashed line is the relative water depth due to tidal influence.

to resolve the 3D variability of pore water solutes in the vicinity of biological structures are shown in Fig. 8D. Note the subsurface maximum in O_2 as the electrode penetrated a worm burrow (Luther et al., 1998).

The multispecies capacity and the small size of the electrodes have been exploited to investigate redox processes in salt marsh sediments where the presence of roots creates a complex heterogeneous mosaic of redox environments (Sundby et al., 2003, 2005). Roots of aquatic plants are conduits of oxygen to the interior of otherwise anoxic sediments, and the sediment that surrounds individual roots constitutes a microenvironment characterized by high reaction rates and intense fluxes. Ideally one would like to probe pore water properties in the immediate vicinity of a root and at known distances from the root surface, but roots are hidden, and it is not possible to know *a priori* where a root is located. However, the voltammetric solid state electrode can be used to probe the sediment for these microenviroments and characterize their properties. The multispecies capacity of voltammetric probe can determine whether the pore water in the microenvironment is oxidizing (oxygen present) or reducing [Fe(II) present].

Sediment work carried out in flumes (Huettel et al., 1998), in mesocosms (Luther et al., 1998) and in real time (Luther et al., 1999) show that soluble Fe^{3+} (Luther et al., 1996b; Taillefert et al., 2000b, 2002) and FeS_{aq} clusters (Theberge and Luther, 1997; Rickard et al., 1999; Rozan et al., 2002) are formed in sediments. The signal for Fe^{3+} is typically broad (Fig. 2F) but comparison of this signal with several laboratory prepared Fe(III) organic complexes indicates that the porewater signal represents Fe(III) organic complexes (Taillefert et al., 2000b, 2002). On aging, the Fe(III) signal shifts from about −0.4 V to more negative potentials. The Fe(III) signal is not generally observed when sulfide is present in porewaters as known Fe(III) complexes react with sulfide in a matter of minutes. Because S(0) and H_2S give a signal at the same potential (Table 1), S(0) and Fe(III) signals can be present. Performing several scans shows whether these signals decrease and are indicative of the reaction of Fe(III) with H_2S.

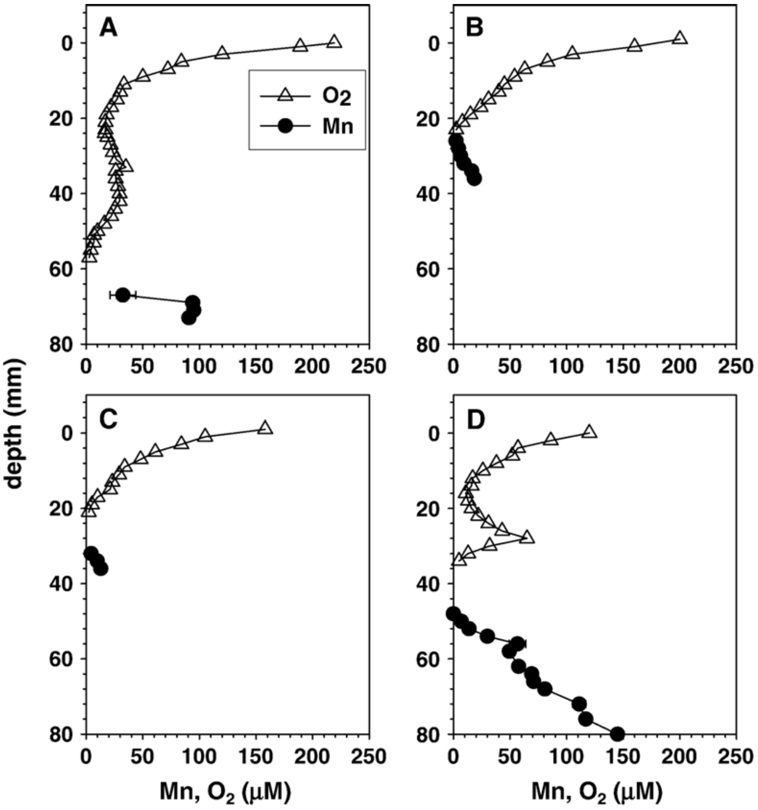

Fig. 8. Four plots of O_2 (Δ) and Mn^{2+} (●) from continental margin sites. Plots A, C and D show no overlap but plot B does (this is a rarity in marine sediments). Plot D shows an increase in O_2 at depth and indicates that a worm burrow was intersected. Several profiles coupled to make three-dimensional plots reveal the extent of the burrow (Luther et al., 1998). If a chemical species is not detected, a value is not plotted.

The solid-state microelectrode has also been mounted on a ROV equipped with microprofiling instrumentation and been used *in situ* to measure pore water profiles of dissolved O_2, Mn, Fe, iodide and sulfide in nearshore sediments (Luther et al., 1999). The electrode was connected to the shipboard voltammetric analyzer with a 30 m cable, which had receiver–transmitter transducers on each end to preserve the signal quality along the cable. The sensor was thus controlled and monitored in real-time from the research vessel anchored at the study site. Single analyte O_2, pH and resistivity microsensors provided by Dr. Clare Reimers were inserted into the sediment next to the voltammetric sensor. Excellent agreement was found between the Clark amperometric O_2 electrode and the Au/Hg voltammetric electrode (Luther et al., 1999). Again these profiles showed no detectable overlap of O_2 and Mn^{2+} in the sediments similar to observations on cores from the continental margin sites of Canada. A subsurface Mn^{2+} peak was observed at about 2 cm and coincided with a subsurface pH maximum as expected based on Eq. (3). The Au/Hg electrode and pH data can be explained by

$$236MnO_2 + C_{106}H_{263}O_{110}N_{16}P + 364H^+ \rightarrow 8N_2 + 106HCO_3^- + HPO_4^{2-} + 236Mn^{2+} + 636H_2O \quad (3)$$

organic matter decomposition with alternative electron acceptors and by secondary reactions involving the products from organic matter decomposition. Similar data are observed in the upper few sediments of lakes (Cai et al., 2002), but in this case pH and Mn^{2+} do not decrease after the Mn/pH maximum.

Another interesting in-situ application of these electrodes derives from their ability to measure low concentrations of iodide. The use of iodide as a tracer of water flow into sediment was exploited by Reimers et al. (2004). They added iodide to the bottom water upstream of a microelectrode inserted into the sediment and monitored the arrival of the tracer and the penetration of the iodide labeled water into the permeable sediment.

3.5. Microbial cultures, mats and biofilms

Direct insertion of the Au/Hg electrode into cultures (Farrenkopf et al., 1997; Dollhopf et al., 2000) documented the conversion of iodate to iodide, Mn(IV) to Mn(II) and Fe(III) to Fe(II) during the microbially mediated oxidation of organic matter. Increasing the crystalline nature of the electron acceptor's phase slows down the rate of reduction. Also, we have documented the change of sulfur chemistry in salt marsh microbial mats (Glazer et al., 2002; Luther et al., 2001a) and showed that soluble elemental sulfur, $S_2O_3^{2-}$ and polysulfides (S_x^{2-}) form in discrete layers of (sub) millimeter thickness. The detection of S_x^{2-} is readily observed as two peaks [one more positive for S(−2) and the other more negative for the $(x-1)$ S(0) atoms; Table 1; Rozan et al., 2000]. The electrochemical data in microbial mats correlate well with the data obtained from molecular biology and microscopic methods (Glazer et al., 2002).

In studies of biofilm corrosion in seawater, a 25 μm solid-state electrode was used to study the chemistry in biofilms (Xu et al., 1998; Dexter et al., 2003). The biofilm had a thickness of 125 μm and the computer manipulator had a resolution of better than 5 μm. The distributions of solutes within the biofilm were similar to those in sediments, including the non-overlap of O_2 and Mn^{2+} distributions. Thus, the chemistry of the biofilm is similar to that found in sediments and anoxic basins.

3.6. Water column data including hydrothermal vents

We have applied the technology to the study of the water column by mounting the electrodes on a CTD (Luther et al., 2002), by pumping water through a flow cell (Luther et al., 2002) and by deployment from the Deep Sea submersible *Alvin* (Luther et al., 2001b, 2002b; Nuzzio et al., 2002). Fig. 9A shows the deployment of the electrodes from *Alvin*. The electrodes are housed in a white wand containing a stainless steel handle for the submersible's manipulators to position. Fig. 9B, C show voltammetric scans and the analyzed data obtained while deploying the electrodes 0.5 m above a black smoker at the East Pacific Rise (9°50′). The reproducibility is excellent. The one scan in green (Fig. 9B) was affected by electrical noise from the submersible. We are aware of no other method that can provide data in such a turbid environment (analogous to sediments) because 30 cm of the tip of the wand cannot be seen in the black smoke. The data obtained indicate that the reactants for the pyrite formation reaction at vents (Wächterhäuser, 1988) are H_2S and FeS_{aq} clusters (Eq. (4)) as suggested by Rickard (1997) and Rickard and Luther (1997).

$$H_2S + FeS_{aq} \rightarrow FeS_2 + H_2 \quad (4)$$

Other data collected with the Au/Hg electrode showed that the tubeworm, *Riftia pachyptila*, resides in waters that are rich in H_2S whereas the polychaete, *Alvinella pompejana*, lives in waters rich in FeS clusters with little or no free H_2S. Because *Riftia* has an endosymbiont that performs chemosynthesis that requires H_2S and *Alvinella* does not, our data indicate that chemical speciation drives hydrothermal vent ecology.

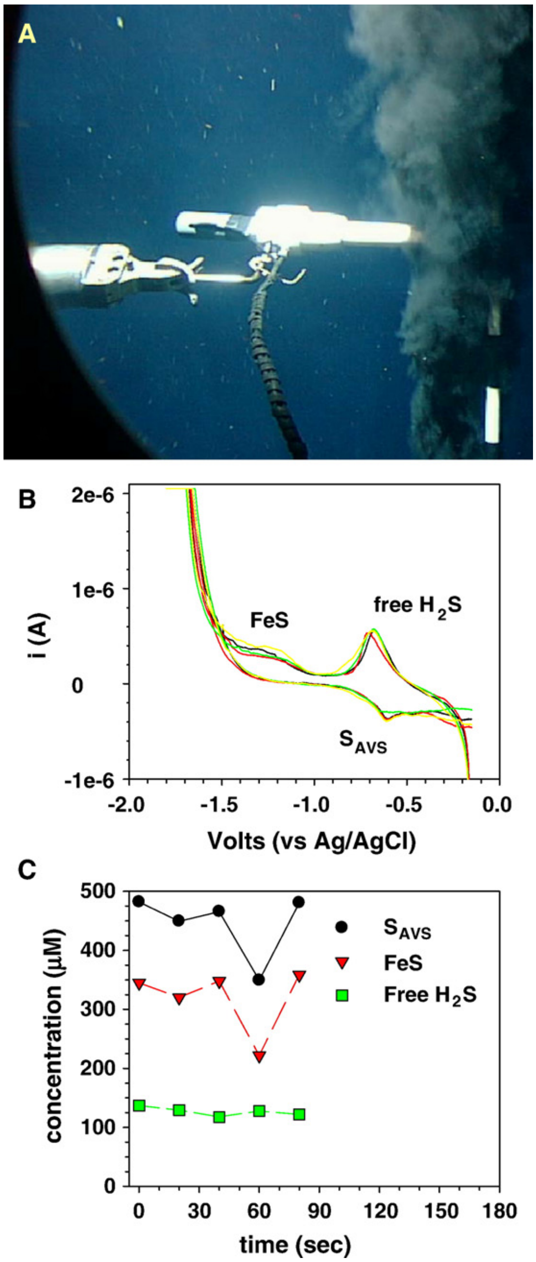

Fig. 9. (A) Positioning of the electrodes 0.5 m above (25 °C) the orifice of a black smoker chimney (360 °C). (B) Cyclic voltammograms (1 V/s scan rate; scan direction is from positive to negative and back to positive) show the measurement of free H_2S, FeS clusters and total sulfide (sum of free H_2S and FeS). (C) Time course of the measurements. A conditioning step of 15 sec at −1.0 V was used to clean the surface of the Au/Hg electrode between each scan.

Fig. 10A shows the unattended ISEA device deployed near a *Riftia* field (the TICA site which has been destroyed due to a recent eruption) and the data obtained over three days, respectively. In this experiment, each working electrode was programmed sequentially to perform four scans (analyses) every 3.5 min. The data in Fig. 10B indicate that H_2S can change over two orders of magnitude (from non-detectable to ~30 µM) in a day whereas O_2 varies by a factor of three. The data also indicate that H_2S increases generally correlate with O_2 decreases as well as temperature increases (Fig. 10C) as first shown by Johnson et al. (1986) over short time periods (<2 h). After day two, there was an average increase for H_2S and a decrease for O_2. Close inspection does show that H_2S and O_2 sometimes positively correlate whereas H_2S and temperature do not closely correlate. These observations are likely due to physical mixing of diffuse flow vent waters with oceanic bottom waters of unknown current magnitude and direction. A main conclusion from these data is that *Riftia* live in an area where they can uptake both H_2S and O_2 simultaneously so that their endosymbiont bacteria can perform chemosynthesis. [The co-existence of H_2S and O_2 is not observed in sedimentary porewaters and in the Black Sea water column.] These are among the first results showing the rapid changes of the chemical environment at a diffuse flow vent site with a fixed sensor over an extended period of time and without sampling the waters prior to measurement. Previous work usually had the *Alvin* manipulator move the orifice of a tube to pump water into an autoanalyzer for subsequent measurements.

4. Conclusions

The voltammetric method with solid-state electrodes is suitable for the study of chemistry of a wide variety of environmental systems in real time, but requires that the analyst be versed in the principles of electrochemistry including voltammetry. Voltammetry is a powerful technique because it provides chemical speciation data (e.g.; oxidation state and different elemental compounds/ions) as well as quantitative data. Because (micro) organisms occupy environmental niches due to the system’s chemistry, it is necessary to know the speciation. In addition, it is a multi-species method that provides information on the relationship and reactivity between different chemical species. Voltammetric methods allow us to relate how chemistry and biology interact; that is, how chemistry can sometimes drive biology and ecology, and how living organisms can affect the chemistry of their environment for their own

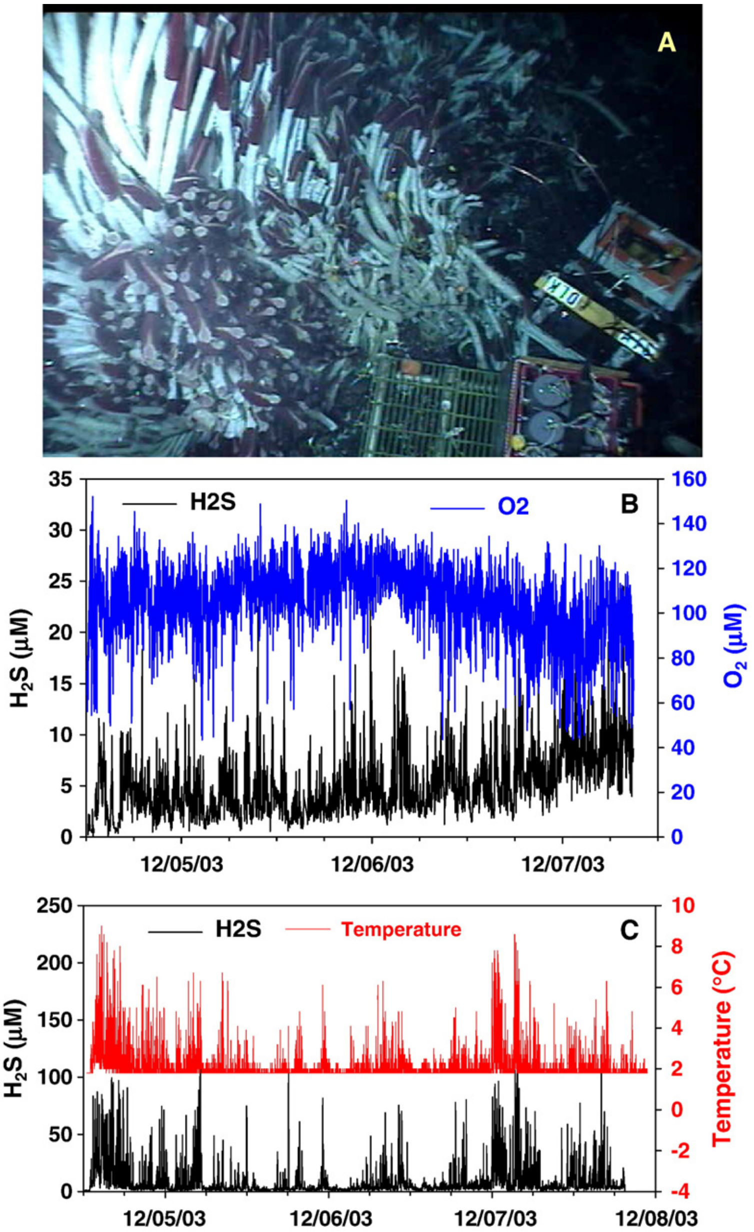

Fig. 10. (A) Deployment of electrodes with the ISEA near a field of *Riftia pachyptila* at the TICA site, December 2003. (B) O_2 and H_2S data from the same electrode during the deployment. (C) H_2S data from another electrode and temperature data during the deployment.

benefit. We have applied the electrodes to study a variety of ecosystems including salt marshes, microbial mats, nearshore to pelagic sediments, polluted aqueous environments, hydrothermal vents, stratified lakes, estuaries, and bays. These environments span the range of oxic to seasonally anoxic to permanently aoxic. As a result of our initial electrode longevity tests, we are pursuing the use of these electrodes off of moored systems.

Acknowledgements

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encouragement over the years, and we all express our best wishes to him on the occasion of his retirement. The breadth of Dr. Gieskes' work throughout his career has been an example for us to follow and has encouraged us to strive for breadth in our own research as we use our electrode technology.

References

Aller, R.C., Rude, P.D., 1988. Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. Geochim. Cosmochim. Acta 52, 751–765.

Anschutz, P., Sundby, B., Lefrançois, L., Luther III, G.W., Mucci, A., 2000. Interactions between metal oxides and species of nitrogen and iodine in bioturbated marine sediments. Geochim. Cosmochim. Acta 64, 2751–2763.

Bond, A.M., 1980. Modern Polarographic Methods in Analytical Chemistry. Marcel Dekker, Inc., p. 518.

Boudreau, B.P., 1997. Diagenetic Models and their Implementation. Springer-Verlag, Berlin, p. 414.

Boudreau, B., Mucci, A., Sundby, B., Luther III, G.W., Silverberg, N., 1998. Comparative diagenesis at three sites on the Canadian Continental Margin. J. Mar. Res. 56, 1259–1284.

Brendel, P.J., Luther III, G.W., 1995. Development of a gold amalgam voltammetric microelectrode for the determination of dissolved Fe, Mn, O_2, and S(-II) in porewaters of marine and freshwater sediments. Environ. Sci. Technol. 29, 751–761.

Burdige, D.J., Nealson, K.H., 1986. Chemical and microbiological studies of sulfide-mediated manganese reduction. Geomicrobio. J. 4, 361–387.

Cai, W.-J., Reimers, C.E., 1993. The development of pH and pCO_2 microelectrodes for studying the carbonate chemistry of porewaters near the sediment-water interface. Limnol. Oceanogr. 38, 1762–1773.

Cai, W.-J., Sayles, F.J., 1996. Oxygen penetration depths and fluxes in marine sediments. Mar. Chem. 52, 123–131.

Cai, W.J., Zhao, P., Wang, Y., Theberge, S.M., Witter, A., Luther III, G.W., 2002. Porewater redox species, pH and pCO_2 in aquatic sediments. In: Taillefert, M., Rozan, T. (Eds.), Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry. American Chemical Society Symposium Series, vol. 811. American Chemical Society, Washington, D.C., pp. 188–209. Ch. 10.

Dexter, S.C., Xu, K., Luther III, G.W., 2003. Mn cycling in marine biofilms: effect on the rate of localized corrosion. Biofouling 19, 139–149.

Dollhopf, M.E., Nealson, K.H.D., Simon, D., Luther III, G.W., 2000. *In situ* solid state Au/Hg voltammetric microelectrodes to analyze microbial reduction. Mar. Chem. 70, 171–180.

Farrenkopf, A.M., Dollhopf, M.E., Chadhain, S.N., Luther III, G.W., Nealson, K.H., 1997. Reduction of iodate in seawater by bacterium *Shewanella putrefaciens* strain MR-4. Mar. Chem. 57, 347–354.

Froelich, P.N., Klinkhammer, G.P., Bender, M.L., Luedtke, N.A., Heath, G.R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B., Maynard, V., 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochim. Cosmochim. Acta 43, 1075–1090.

Glazer, B.T., Cary, S.C., Hohmann, L., Luther III, G.W., 2002. *In situ* sulfur speciation using Au/Hg microelectrode as an aid to microbial characterization of an intertidal salt marsh microbial mat. In: Taillefert, M., Rozan, T. (Eds.), Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry. American Chemical Society Symposium Series, vol. 811. American Chemical Society, Washington, D.C., pp. 283–305. Ch. 14.

Huettel, M., Ziebis, W.S., Forster, S., Luther III, G.W., 1998. Advective transport affecting metal and nutrient distributions and interfacial fluxes in permeable sediments. Geochim. Cosmochim. Acta 62, 613–631.

Johnson, K.S., Beehler, C.L., Sakamoto-Arnold, C.M., Childress, J.J., 1986. *In situ* measurements of chemical distributions in a deep-sea hydrothermal vent field. Science 231, 1139–1141.

Konovalov, S.K., Luther III, G.W., Friederich, G.E., Nuzzio, D.B., Tebo, B.M., Murray, J.W., Oguz, T., Glazer, B., Trouwborst, R.E., Clement, B., Murray, K.J., Romanov, A.S., 2003. Lateral injection of oxygen with the Bosporus plume: fingers of oxidizing potential in the Black Sea. Limnol. Oceanogr. 48, 2369–2376.

Li, Y.-H., Gregory, S., 1974. Diffusion of ions in sea water and in deep-sea sediments. Geochim. Cosmochim. Acta 38, 703–714.

Lovley, D.R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. Microbiol. Rev. 55, 259–287.

Luther III, G.W., 1995. Trace metal chemistry in porewaters. In: Allen, H.E. (Ed.), Metal Contaminated Aquatic Sediments. Ann Arbor Press, Chelsea, MI, pp. 65–80. Ch. 3.

Luther III, G.W., Rickard, D., Theberge, S.M., Olroyd, A., 1996a. Determination of metal (bi)sulfide stability constants of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, $Cu^{2+}$and Zn^{2+} by voltammetric methods. Environ. Sci. Technol. 30, 671–679.

Luther III, G.W., Shellenbarger, P.A., Brendel, P.J., 1996b. Dissolved organic Fe(III) and Fe(II) complexes in salt marsh porewaters. Geochim. Cosmochim. Acta 60, 951–960.

Luther III, G.W., Sundby, B., Lewis, B.L., Brendel, P.J., Silverberg, N., 1997. Interactions of manganese with the nitrogen cycle: alternative pathways to dinitrogen. Geochim. Cosmochim. Acta 61, 4043–4052.

Luther III, G.W., Brendel, P.J., Lewis, B.L., Sundby, B., Lefrançois, L., Silverberg, N., Nuzzio, D.B., 1998. Simultaneous measurement of O_2, Mn, Fe, I^- and S(-II) in marine porewaters with a solid-state voltammetric micro-electrode. Limnol. Oceanogr. 43, 325–333.

Luther III, G.W., Reimers, C.E., Nuzzio, D.B., Lovalvo, D., 1999. *In situ* deployment of voltammetric, potentiometric and amperometric microelectrodes from a ROV to determine O_2, Mn, Fe, S(-2) and pH in porewaters. Environ. Sci. Technol. 33, 4352–4356.

Luther III, G.W., Glazer, B.T., Hohman, L., Popp, J.I., Taillefert, M., Rozan, T.F., Brendel, P.J., Theberge, S.M., Nuzzio, D.B., 2001a. Sulfur speciation monitored *in situ* with solid state gold amalgam voltammetric microelectrodes: polysulfides as a special case in sediments, microbial mats and hydrothermal vent waters. J. Environ. Monit. 3, 61–66.

Luther III, G.W., Rozan, T.F., Taillefert, M., Nuzzio, D.B., Di Meo, C., Shank, T.M., Lutz, R.A., Cary, S.C., 2001b. Chemical speciation drives hydrothermal vent ecology. Nature 410, 813–816.

Luther III, G.W., Bono, A., Taillefert, M., Cary, S.C., 2002. A continuous flow electrochemical cell for analysis of chemical species and ions at high pressure: laboratory, shipboard and hydrothermal vent results. In: Taillefert, M., Rozan, T. (Eds.), Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry. American Chemical Society Symposium Series, vol. 811. American Chemical Society, Washington, D.C., pp. 54–73. Ch. 4.

Luther III, G.W., Ma, S., Trouwborst, R., Glazer, B., Blickley, M., Scarborough, R.W., Mensinger, M.G., 2004. The roles of anoxia, H_2S, and storm events in fish kills of dead-end canals of Delaware Inland Bays. Estuaries 27, 551–560.

Murray, J.W., Codispoti, L.A., Friederich, G.E., 1995. The suboxic zone in the Black Sea. In: Huang, C.P., O'Melia, R., Morgan, J.J. (Eds.), Aquatic Chemistry: Interfacial and Interspecies Processes. Adv. Chem. Ser., vol. 244. American Chemical Society, Washington DC, pp. 157–176.

Nealson, K.H., Myers, C.R., 1992. Microbial reduction of anganese and iron: new approaches to carbon cycling. Appl. Environ. Microbio. 58, 439–443.

Nolan, M.A., Gaillard, J.-F., 2002. Probing zinc speciation in contaminated sediments by square wave voltammetry at a Hg/Ir microelectrode. In: Taillefert, M., Rozan, T. (Eds.), Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry. American Chemical Society Symposium Series, vol. 811. American Chemical Society, Washington, D.C, pp. 210–226. Ch. 11.

Nuzzio, D.B., Taillefert, M., Cary, S.C., Reysenbach, A.L., Luther III, G.W., 2002. *In situ* voltammetry at hydrothermal vents. In: Taillefert, M., Rozan, T. (Eds.), Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry. American Chemical Society Symposium Series, vol. 811. American Chemical Society, Washington, D.C, pp. 40–53. Ch. 3.

Reimers, C.E., Fischer, K.M., Merewether, R., Smith, K.L., Jahnke, R.A., 1986. Oxygen microprofiles measured *in situ* in deep ocean sediments. Nature 320, 741–744.

Reimers, C.E., Stecher, H.A., Taghon, G.L., Fuller, C.M., Huettel, M., Rusch, A., Ryckelynck, N., Wild, C., 2004. *In situ* measurements of advective solute transport in permeable shelf sands. Cont. Shelf Res. 24, 183–201.

Revsbech, N.P., Jorgensen, B.B., 1986. Microelectrodes: their use in microbial ecology. Adv. Microb. Ecol. 9, 293–352.

Revsbech, N.P., Sorensen, J., Blackburn, T.H., Lomholt, J.P., 1980. Distribution of oxygen in marine sediments measured with microelectrodes. Limnol. Oceanogr. 25, 403–411.

Revsbech, N.P., Jorgensen, B.B., Blackburn, T.H., Cohen, Y., 1983. Microelectrode studies of the photosynthesis and O_2, H_2S, and pH profiles of a microbial mat. Limnol. Oceanogr. 28, 1062–1074.

Revsbech, N.P., Nielsen, L.B., Christensen, P.B., Sørensen, J., 1988. A combined oxygen and nitrous oxide microsensor for denitrification studies. Appl. Environ. Microbiol. 54, 2245–2249.

Rickard, D.T., 1997. Kinetics of pyrite formation by the H_2S oxidation of iron(II) monosulfide in aqueous solutions between 25 °C and 125 °C: the rate equation. Geochim. Cosmochim. Acta 61, 115–134.

Rickard, D.T., Luther III, G.W., 1997. Kinetics of pyrite formation by the H_2S oxidation of iron(II) monosulfide in aqueous solutions between 25 °C and 125 °C: the mechanism. Geochim. Cosmochim. Acta 61, 135–147.

Rickard, D., Oldroyd, A., Cramp, A., 1999. Voltammetric evidence for soluble FeS cluster complexes in anoxic estuarine muds. Estuaries 22, 693–701.

Rozan, T.F., Theberge, S.M., Luther III, G.W., 2000. Quantifying elemental sulfur (S^0), bisulfide (HS^-) and polysulfides (S_x^{2-}) using a voltammetric method. Anal. Chim. Acta 415, 175–184.

Rozan, T.F., Taillefert, M., Trouwborst, R.E., Glazer, B.T., Ma, S., Herszage, J., Valdes, L.M., Price, K.S., Luther III, G.W., 2002. Iron, sulfur and phosphorus cycling in the sediments of a shallow coastal bay: Implications for sediment nutrient release and benthic macroalgal blooms. Limnol. Oceanogr. 47, 1346–1354.

Shaw, T.J., Gieskes, J.M., Jahnke, R.A., 1990. Early diagenesis in differing depositional environments: the response of transition metals in pore water. Geochim. Cosmochim. Acta 54, 1233–1246.

Sundby, B., Vale, C., Caetano, M., Luther III, G.W., 2003. Redox chemistry in the root zone of a salt marsh sediment in the Tagus Estuary, Portugal. Aquat. Geochem. 9, 257–271.

Sundby, B., Caetano, M., Vale, C., Gobeil, C., Luther III, G.W., Nuzzio, D.B., 2005. Root induced cycling of lead in salt marsh sediments. Environ. Sci. Technol. 39, 2080–2086.

Taillefert, M., Luther III, G.W., Nuzzio, D.B., 2000a. The application of electrochemical tools for *in situ* measurements in aquatic systems: a review. Electroanalysis 12, 401–412.

Taillefert, M., Bono, A.B., Luther III, G.W., 2000b. Reactivity of freshly formed Fe(III) in synthetic solutions and marine (pore) waters: voltammetric evidence of an aging process. Environ. Sci. Technol. 34, 2169–2177.

Taillefert, M., Rozan, T.F., Glazer, B.T., Herszage, J., Trouwborst, R.E., Luther III, G.W., 2002. Seasonal variations of soluble organic-Fe(III) in sediment porewaters as revealed by voltammetric microelectrodes. In: Taillefert, M., Rozan, T. (Eds.), Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry. American Chemical Society Symposium Series, vol. 811. American Chemical Society, Washington, D.C., pp. 247–264. Ch. 13.

Tebo, B.M., 1991. Manganese(II) oxidation in the suboxic zone of the Black Sea. Deep-Sea Res. 38 (Suppl. 2), S883–S905.

Tercier-Waeber, M.-L., Buffle, J., Koudelka-Hep, M., Graziottin, F., 2002. Submersible voltammetric probes for real-time continuous monitoring of trace elements in natural aquatic systems. In: Taillefert, M., Rozan, T. (Eds.), Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry. American Chemical Society Symposium Series, vol. 811. American Chemical Society, Washington, D.C., pp. 16–39. Ch. 2.

Theberge, S.M., Luther III, G.W., 1997. Determination of the electrochemical properties of a soluble aqueous FeS cluster present in sulfidic systems. Aquat. Geochem. 3, 191–211.

Visscher, P.T., Beukema, J., van Gemerden, H., 1991. *In situ* characterization of sediments: measurements of oxygen and sulfide profiles with a novel combined needle electrode. Limnol. Oceanogr. 36, 1476–1480.

Wächterhäuser, G., 1988. Pyrite formation, the first energy source for life: a hypothesis. Syst. Appl. Microbiol. 10, 207–210.

Xu, K., Dexter, S.C., Luther III, G.W., 1998. Voltammetric microelectrodes for biocorrosion studies. Corrosion 54, 814–823.