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Submersible Online Oxygen Removal System Coupled to an in Situ Voltammetric Probe for Trace Element Monitoring in Freshwater

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A new online oxygen removal system specifically built to allow in situ deoxygenation of freshwater before in situ voltammetric detection of trace elements using a submersible probe is described. It is based on the permeation of oxygen through a silicone tubing surrounded by an enzymatic cross-linked O_2 scavenging gel. The main advantages of the enzyme cross-linked gel compared to the chemical O_2 reducing solutions proposed in the literature for laboratory online deoxygenation coupled to other devices are as follows: (i) ease of preparation and storage under normal room condition, (ii) good long-term stability (maximum time tested 1 month), (iii) no formation of insoluble oxidized chemical compounds adsorbed on the external silicone tubing wall that may interfere with the O_2 diffusion, and (iv) easy in situ applications thanks to the convenience of chemical gel for transport and pressure compensation. Detailed description of the construction of the system as well as the systematic laboratory tests performed to optimize its performance are reported. Examples of environmental applications are also given. In particular, in situ monitoring and profiling of Cu(II), Pb(II), and Zn(II) in oxygenated lake water have been performed using this new online oxygen removal device coupled to a submersible voltammetric probe. The results indicate that the online oxygen removal system is robust and efficient for in situ, online deoxygenation and allows reliable subsequent voltammetric measurements of trace metals present at sub-nM level in oxygen saturated freshwater.

Introduction

The impacts of human activities on aquatic systems, and in particular chemical pollution, are becoming increasingly important issues. To define efficient remedial action, the role and the fate of chemical pollutants need to be better understood. For this purpose, the development of new analytical instrumentation capable of performing in situ, real time monitoring of specific forms of compounds in continuous and reproducible manner, on a wide spatial network, is required (*1*). Recently we reported the development of a sophisticated, compact, reliable, submersible voltammetric probe, for in situ trace element measurements in natural aquatic systems. This system called Voltammetric In situ Profiling System (VIP System) is based on advanced microprocessor, telemetry, and microsensor technologies (*2*). It has been successfully applied for in situ monitoring and profiling of Cu(II), Pb(II), Cd(II), and Zn(II) at sub-nM level and Mn(II) at the sub-μM level in both oxygen saturated seawater and in anoxic lake and groundwaters (*2*−*4*). However, its application to in situ trace element measurements in oxygenated freshwater was limited until now due to the interference of the oxygen signal.

Indeed, O_2 is an electroactive compound in oxic water, and it gives rise to two reduction waves at Hg electrodes, corresponding to the reduction of O_2 to H_2O_2 ($E \sim -0.1$ V vs Ag/AgCl/saturated KCl) and of H_2O_2 to H_2O ($E \sim -0.9$ V vs Ag/AgCl/saturated KCl) successively. Thus, for in situ trace metal voltammetric measurements where purging of the sample with inert gases to remove O_2 is too unwieldy, the reduction of O_2 may produce two types of interference: (a) production of a signal 4−6 orders of magnitude larger than that produced by the trace metals of interest and (b) pH increase at the electrode surface due to the consumption of H^+ during the reduction of O_2. It has been demonstrated that the interference (a) can be minimized by using subtractive voltammetry under appropriate conditions or square wave anodic stripping voltammetry (SWASV) with relatively high frequency (*5*) and eliminated by the combination of both techniques (*2*) in well pH buffered aquatic media, such as seawater, where interference (b) is not encountered. However, in low pH buffer capacity (i.e., typical natural HCO_3^- concentration $\leq 5 \times 10^{-3}$M) oxygenated freshwaters, the reduction of O_2, in particular during the preconcentration step of SWASV, may lead to an increase in pH of up to 11 (oxygen saturated sample) at the electrode surface and thus in turn to the formation of hydroxide, carbonate, or sulfide precipitates during the stripping step (*6*). Voltammetric signal may be drastically deformed or even suppressed by these reactions. To overcome this problem, either (i) a pH buffer must be added to the sample, which is not recommended for ultratrace analysis because of possible contaminations, or (ii) O_2 must be eliminated from the sample stream before the voltammetric cell. Several systems have been proposed for laboratory online oxygen removal, based on chemical (*7*−*9*), electrolytic (*10*), photochemical (*11*), or physical methods (*12*−*18*), in particular in combination with luminescence spectroscopic detection or liquid chromatography coupled to UV or amperometric detectors. Advantages and limitations of these systems for in situ applications have been discussed elsewhere (*1*). Briefly, chemical and photochemical methods are not suitable for trace metal analysis since they require the addition of reducing agents or organic acids to the sample which may introduce contaminations as well as induce changes in metal speciation. Online electrolytic reduction of oxygen is also not suitable since the test metal ions will also be removed at negative potentials. In contrast, physical methods based on diffusion of O_2 through a gas-permeable tubing, often silicone, surrounded by chemical reducing agents, inert gases at reduced pressure or vacuum are more suitable (*11*−*18*). They have been successfully applied in the laboratory and in field flow analysis of trace metals present at nM levels in tap and seawaters (*15, 18*). However, the use of inert gases or vacuum to eliminate the O_2 after its diffusion through silicone tubing is not applicable for in situ deoxygenation. Another problem of these systems for online deoxygenation of freshwater is that CO_2 will also be eliminated (i.e., CO_2 permeability is much higher than the O_2 permeability in silicone tubing), and thus the pH of the sample may increase drastically during the deoxygenation process. The use of chemical O_2 scavenging solution outside the tubing minimizes both problems, but the chemical

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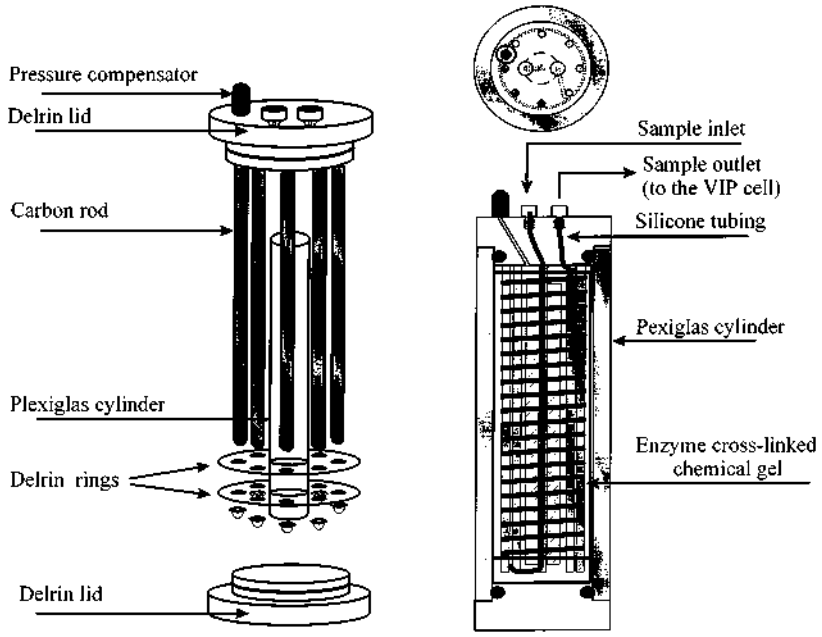


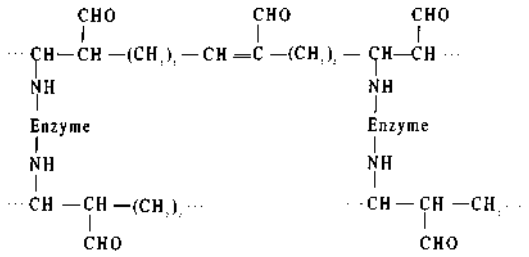
FIGURE 1. Schematic diagram of the GOD-module for in situ online O_2 removal.

solutions utilized until now present some other drawbacks. In particular they cannot be easily prepared and stored and/or they form insoluble oxidized chemical compounds which adsorb on the external silicone tubing wall surface and interfere with the O_2 diffusion (*18*).

A new gas permeation device specifically built to overcome the problems mentioned above and allow in situ online deoxygenation of freshwater is described in this paper. The novelty of this system is that an enzyme cross-linked chemical gel, obtained by mixing glucose oxidase (GOD), bovine serum albumin, and glutaraldehyde has been used for oxygen removal. The free aldehyde functional groups in the structure of this gel are responsible for O_2 consumption. Systematic laboratory tests were done to optimize online deoxygenation with the glucose oxidase module (GOD-module) prior to voltammetric measurements of Cu(II), Pb(II), Cd(II), and Zn(II) in weakly buffered media using the VIP probe. The GOD-module coupled to the submersible voltammetric probe of the VIP System has been applied for in situ trace metal monitoring and profiling in oxygenated lake water to demonstrate its efficiency for online, in situ removal of oxygen.

Experimental Section

The Online Oxygen Removal Module. A schematic diagram of the online oxygen removal module (GOD-module) is shown in Figure 1. The housing consists of a Plexiglas cylinder (4.5 cm/6 cm internal/external diameters, 32 cm length) closed at the ends with acetalic copolymer (Delrin) lids having O-ring. Eight carbon rods are fixed with equal distance on the top Delrin lid. A second Plexiglas cylinder (0.45 cm/2.5 cm internal/external diameters, 25 cm length) is positioned concentrically to the eight carbon rods using a Delrin ring. Rigidity of the whole system is ensured by screwing the carbon rods on a second Delrin ring. The silicone tubing (see below) is introduced in the system via the hole of the internal Plexiglas cylinder and then wound around the carbon rods (Figure 1). This design allows for optimizing the surface of the silicone tubing in contact with the enzyme cross-linked chemical oxygen scavenging gel and minimizing the internal volume of the module. The fittings for the silicone tubing on the upper lid were standard thread fittings made of inert, metal free Teflon material. The whole part is then immersed in the Plexiglas reservoir (i.e., formed by the external Plexiglas cylinder and the bottom Delrin lid) containing 400 mL of enzymatic solution obtained by mixing 50 mL of 50 mg/mL glucose oxidase (glucose oxidase from *Aspergillus niger* (EC 1.1.3.4): ≈ 25 U/mg, catalase ≤ 50%; Fluka n*o*. 49178), 250 mL of 80 mg/mL bovine serum albumin (Fluka no. 05480), and 100 mL of 2.5% (vol/vol) glutaraldehyde (Fluka n*o*. 49626) freshly prepared solutions. This must be done by gently lowering it with swinging motions to remove any bubbles entrapped around the silicone tubing. The excess of solution in the reservoir (≈1 mL) is expelled via the rubber pressure compensator while closing the system. The system was left overnight to ensure complete cross-linking of the gel and then used for online removal of oxygen. The structure of the gel is as follows:



The free aldehyde functional groups are oxidized by oxygen to give peroxy acids as intermediaries which in turn disproportionate to give carboxylic acid (*19*).

Silicone Tubings. Four different silicone tubing with 2.5 mm/3.5 mm, 1.5 mm/3 mm, 1 mm/2 mm, and 0.5 mm/1.5 mm internal/external diameters respectively and an oxygen permeability coefficient of $\approx 6000 \times 10^{-11}$ $cm^3 \cdot cm/cm^2 \cdot s \cdot cmHg$ were tested (Maagtechnic-Switzerland). The length of each tubing chosen was such that the internal volume of the tubing was three times the volume of the flow-through voltammetric cell of the submersible VIP probe (i.e., 3 × 1.5 mL). This ensures that for each renewal of the sample in the online GOD-module tubing, the voltammetric cell is flushed with three times its volume, i.e., the minimum volume required for complete renewal of the sample in the voltammetric cell to avoid carry-over effects (*2*).

Instrumentation and Experimental Conditions. Voltammetric measurements were performed using the VIP System

described in detail elsewhere (*2*). The inlet of the GOD-module was connected to the submersible peristaltic pump and the outlet to the inlet of the VIP flow-through voltammetric cell. This latter is based on a three electrode system (*2*): a homemade manufactured Ag/AgCl/KCl saturated agarose gel/1 M $NaNO_3$ agarose gel reference electrode, a built-in platinium ring auxiliary electrode, and an Agarose membrane-covered Hg-plated Ir-based microelectrode arrays (μ-AMMIA) working electrode described in detail in ref *20*. Mercury semidrops were plated through the gel layer onto Ir substrates at −400 mV (vs the above reference electrode) in a deoxygenated 5 mM $Hg(CH_3COO)_2$ in 10^{-2} M $HClO_4$ solution. Reoxidation of the mercury was carried out also through the agarose gel by scanning the potential linearly from −300 mV to +300 mV, at 5 mV/s, in a degassed 1 M KSCN solution. In both cases, the currents were recorded and the radius of the mercury semidrops were determined, from the measured electric charge, by assuming they are parts of spheres (*20*). The same agarose protective membrane was used over a period of about 1 month. Voltammetric measurements with gel integrated microsensors consist of two successive steps: equilibration of the agarose gel with the test solution (typically 5 min for a gel thickness of 300 μm) and voltammetric analysis inside the gel. Features of gel-integrated microsensors for in situ measurements in natural aquatic systems are discussed in detail elsewhere (*3, 20, 21*). The dissolved O_2 in the samples before and after online deoxygenation using the GOD-module was measured using Linear Sweep Voltammetry (LSV). The LSV conditions used were as follows: precleaning $E = -100$ mV; precleaning $t = 60$ s; initial $E = -100$ mV; final $E = -1000$ mV; scan rate = 20 mV/s; sampling time = 50 ms. A plateau, with a current proportional to the oxygen concentration, is observed in the potential range of −500 to −900 mV (*15*). SWASV trace metal were performed under the following conditions: precleaning $E = -100$ and + 50 mV; precleaning $t = 60$ s; deposition $E = -1000$ to −1200 mV; deposition time = 5 to 15 min; final $E = -100$ and + 50 mV; pulse amplitude = 25 mV; step amplitude = 8 mV; frequency = 200 Hz. $NaNO_3$ of suprapur grade and reagent grade $Hg(CH_3COO)_2$, $HClO_4$, and KSCN were used. Lake Bret and Greifensee (Switzerland) freshwater samples, filtered through 0.2 μm pore size membrane, having an alkalinity of 2.25 and 2.94 mM and a pH of 8.2 and 8.7, respectively, were used for laboratory tests. The temperature effect study was carried out at two different temperatures, i.e., 22° and 4 °C, by immersing the GOD-module in a water tank connected to a Cryostat with automatic control of the water temperature (Cryostat model F34-E, Julabo-Germany). Long-term efficiency of online deoxygenation as well as the reproducibility of trace metal measurements as a function of time in lake sample were studied at both temperatures as follows: LSV O_2 measurements were performed two to three times per day and replicate SWASV trace metal measurements were performed every 1−1.5 h between LSV measurements using the automatic mode of the VIP System (for detail see ref *2*). The spiked lake water sample was renewed each morning with a freshly prepared one. A μ-AMMIA with the same mercury layer was used during the whole measurement period.

Field tests were performed in Lake Leman (Switzerland-June 1999) near the city of Versoix, located at about 25 km north of Geneva. A multiparameter probe, operated by the voltammetric VIP probe, was incorporated into the submersible system to control exact immersion at depth of the system and to allow simultaneous measurements of *T*, pH, O_2, conductivity, and redox potential (for details see ref *2*). Samplings were performed using a peristaltic pump, and laboratory total metal concentration measurements were performed using inductively coupled plasma mass spectrometry (ICP-MS; Hewlett-Packard model 4500). The sampling polyethylene tubing was washed by pumping 10^{-2} M HNO_3 suprapur solution for 2 h and rinsed with demineralized water overnight before the first field test. Then after and before each field test, it was rinsed with demineralized water for about 20 h. The 50 mL polyethylene tubes used for sampling were systematically washed three times successively in 10^{-2} M HNO_3 suprapur solution and in milli-Q water overnight and day, respectively. For ICP-MS measurements, the maximum metal contamination level in blank solutions (milli-Q water + HNO_3 suprapur) was found to be 0.6, 0.2, and 6 nM for Cu(II), Pb(II), and Zn(II), respectively, and a standard deviation in the range 2−10% was obtained for five replicate measurements performed in each natural samples.

Laboratory Optimization of the GOD-Module

Silicone Tubing Geometry and Flow-Rate of the Sample. The influence of the silicone tubing geometry, the flow-rate of the sample, and the residence time on the efficiency of the GOD-module for online O_2 removal were first studied. For this purpose, O_2 content in a 0.1 M $NaNO_3$ solution was measured by LSV before and after online deoxygenation under the various conditions. Efficiency of the O_2 removal by the GOD-module, expressed as %, was calculated as follows

$$\text{efficiency [\%]} = \left(1 - \frac{i_{\text{lim}}^{O_2\text{res.}}}{i_{\text{lim}}^{O_2\text{sat.}}}\right) \times 100 \qquad (1)$$

where $i_{\text{lim}}^{O_2\text{res.}}$ = limiting current of residual O_2 at $E = -800$ mV after online deoxygenation with the GOD-module and $i_{\text{lim}}^{O_2\text{sat.}}$ = limiting current of O_2 at $E = -800$ mV in oxygen saturated solution.

Effect of the residence time of the solution in two different silicone tubings on the efficiency of the GOD-module is shown in Figure 2a. The minimum residence time corresponds to the time required for a complete renewal of the solution in the whole fluidic system (i.e., sampling tubing, GOD-module, and flow-through voltammetric cell) for a given flow-rate. Longer residence times are obtained by stopping the pump and leaving the renewed solution in the GOD-module for various time intervals before pumping it in the voltammetric cell. The results showed that increasing the residence time under quiescent conditions only slightly improved the efficiency of the GOD-module. In contrast, the efficiency of the GOD-module was found to depend strongly on the flow-rate and tubing geometry as shown in Figure 2b. An efficiency ≥98% was reached for silicone tubings with a wall thickness of 0.5 mm and an internal diameter ≤1 mm using a flow-rate ≤5 mL/min. Note that for the silicone tubing with an internal diameter of 0.5 mm, the maximum flow-rate that can be used was found to be 2.5 mL/min. In all the subsequent experiments, a silicone tubing with 1 mm/2 mm internal/external diameter and a flow rate of 4−5 mL/min were used since under these conditions a maximum efficiency of the GOD-module with a faster renewal of the sample in the fluidic system is achieved.

SWASV Trace Metal Measurements After Online Deoxygenation. The reliability of SWASV trace metal measurements in low pH buffer capacity media after online deoxygenation with the GOD-module was checked by performing calibration by successive standard additions as well as measurements of trace metal peak currents as a function of the deposition time (t_{dep}) in 0.1 M $NaNO_3$ solution, 5×10^{-3}M $NaNO_3$ solution, and lake Bret water samples filtered through 0.2 μm pore size membrane. The results obtained after online deoxygenation were compared with results obtained for similar measurements performed in N_2 degassed $NaNO_3$ solutions and 0.2 μm filtered lake samples degassed with a mixture of N_2/CO_2 to maintain the pH at its natural value (i.e., 8.2). For the measurements made in lake water samples,

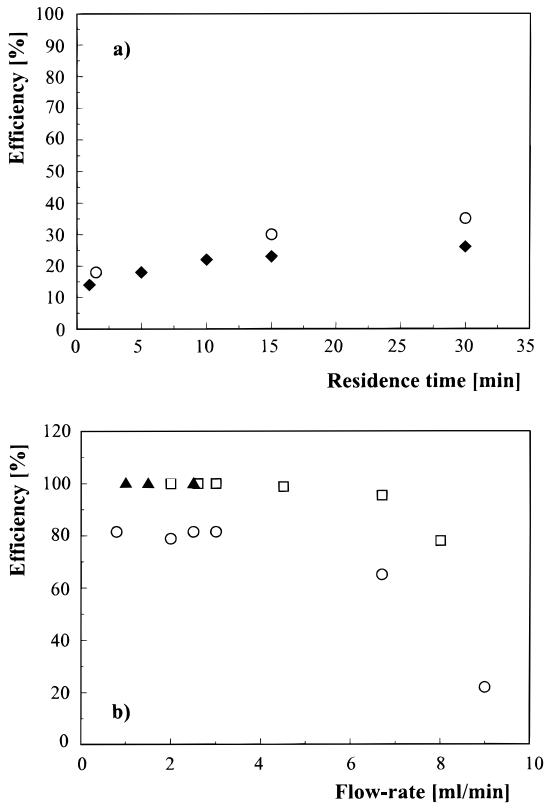


FIGURE 2. (a) Effect of the residence time on the online deoxygenation efficiency, determined from eq 1, of the GOD-module. ○: silicone tubing with 2.5 mm/3.5 mm internal/external diameter and 1 m length, flow-rate = 9 mL/min; ◆: silicone tubing with 1.5 mm/3 mm internal/external diameter and 3 m length, flow-rate = 8.2 mL/min. (b) Effect of the flow-rate and internal diameter of the silicone tubing on the online deoxygenation efficiency, determined from eq 1, of the GOD-module. ○: silicone tubing with 2.5 mm/3.5 mm internal/external diameter and 1 m length; □: silicone tubing with 1 mm/2 mm internal/external diameter and 6 m length; ▲: silicone tubing with 0.5 mm/1.5 mm internal/external diameter and 20 m length.

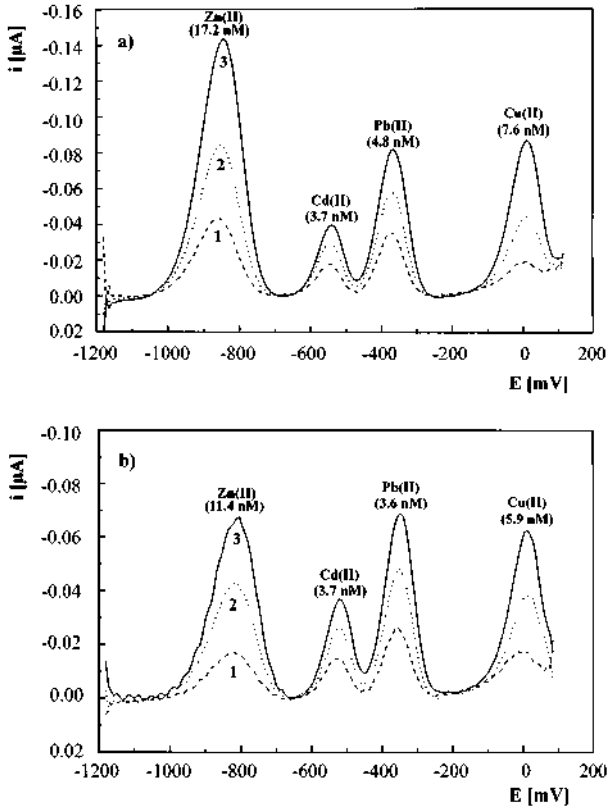


FIGURE 3. Typical SWASV trace metal voltammograms measured using 5 min (1), 10 min (2), and 15 min (3) deposition time in 5 mM $NaNO_3$ solution: (a) degassed with N_2 gas and (b) after online deoxygenation with the GOD-module (silicone tubing with 1 mm/2 mm internal/external diameter and 6 m length; flow rate = 4.5 mL/min).

pH of the samples were measured before and after online deoxygenation (i.e., before and after SWASV measurements) to check an eventual concomitant elimination of CO_2.

Typical SWASV voltammograms of the four metals measured, at various deposition times, in 5×10^{-3} M $NaNO_3$ solution degassed with either N_2 gas or the GOD-module are shown in Figure 3 (parts a and b, respectively). Plots of peak currents vs t_{dep} in the range 5−15 min for each of the tested metals, and the corresponding calibrations curves of these metals in the various media were found to be linear irrespective of the deoxygenation mode. For comparison purpose, the slopes obtained from the graphs i_p vs t_{dep} and the calibration curves were normalized by dividing them by the metal concentrations and the deposition times, respectively. The calculated normalized slopes, expressed in nA/nM min, are reported in Table 1. These data show that the normalized slopes of each of the metals are similar for both types of experiments and deoxygenation mode and thus confirm the effectiveness of the online deoxygenation process of the GOD-module. Finally, a slight decrease in pH, i.e., ≤0.1 pH unit, was observed for the Lake Bret water samples after online deoxygenation. These results demonstrate that there is no concomitant elimination of CO_2 during online oxygen removal with the GOD-module (i.e., elimination of CO_2 will lead to an increase of the pH samples).

Effect of Time and Temperature on the Efficiency of the GOD-Module. Long-term efficiency of the GOD-module for online O_2 removal is an important factor to consider as the VIP System allows both daily in situ monitoring/profiling or autonomous, continuous monitoring over extended period of time (*2*, *22*). Another important factor that can affect the online deoxygenation efficiency of the GOD-module in situ is the temperature. Indeed, the temperature of natural waters may vary over a wide range, typically 4−25 °C, depending on the season and the depth. The influence of these two factors were studied by performing LSV and SWASV measurements in 0.2 μm filtered Lake Greifensee water samples spiked with various concentrations of Cu(II), Pb(II), and Cd(II) using the GOD-module maintained at a temperature of 22° and 4 °C (see Experimental Section for conditions).

For the tests at 22 °C, efficiency of the GOD-module as well as Pb(II) and Cd(II) peak current intensities obtained from LSV and SWASV measurements respectively over 2 weeks measurements are shown in Figure 4a. It can be seen that the deoxygenation efficiency of the GOD-module is close to 98% over the whole measurement period. The average peak currents for both metals over the studied period were found to be the following: (i) Pb(II), 42.09 ± 3.81 μA and 53.67 ± 5.7 μA for an added concentration of 6 nM (days 1−5 and 12−14) and 8.4 nM (days 6−11), respectively, and (ii) Cd(II), 18.09 ± 1.75 μA and 29.93 ± 3.65 μA for an added concentration of 4.9 nM (days 1−5) and 8.6 nM (days 6−14), respectively. The low standard deviation, ca. 10%, obtained for the average peak currents of both metals over the 14 days of continuous SWASV measurements confirm the efficiency of the GOD-module for long term online deoxygenation at room temperature and demonstrates also the excellent reproducibility and reliability of the μ-AMMIA for continuous, automatic measurements over extended period (i.e., no renewal of the Hg layer over the whole measurement period).

TABLE 1. Normalized Slopes of Trace Metals Calculated from i_p vs t_{dep} Graphs and Calibration Curves Obtained by SWASV Measurements in Different Media Degassed Using Either N_2 Gas or the GOD-Module

media	deoxygenation	meas. type	slopes [nA/nM min] Cu(II)	Pb(II)	Cd(II)	Zn(II)
0.1 M $NaNO_3$	nitrogen	calibration	0.82	0.98	0.70	0.40
0.1 M $NaNO_3$	GOD-module	calibration		1.08	0.76	0.35
5×10^{-3} M $NaNO_3$	nitrogen	calibration	0.80	1.05	0.63	0.38
5×10^{-3} M $NaNO_3$	GOD-module	calibration	0.85	1.08	0.68	0.37
0.2 μm filtered lake Bret sample	GOD-module	calibration	0.80	1.10	0.65	
5×10^{-3} M $NaNO_3$	nitrogen	$i = f(t_{dep})$	0.89	1.11	0.70	0.50
5×10^{-3} M $NaNO_3$	GOD-module	$i = f(t_{dep})$	0.75	1.14	0.63	0.44

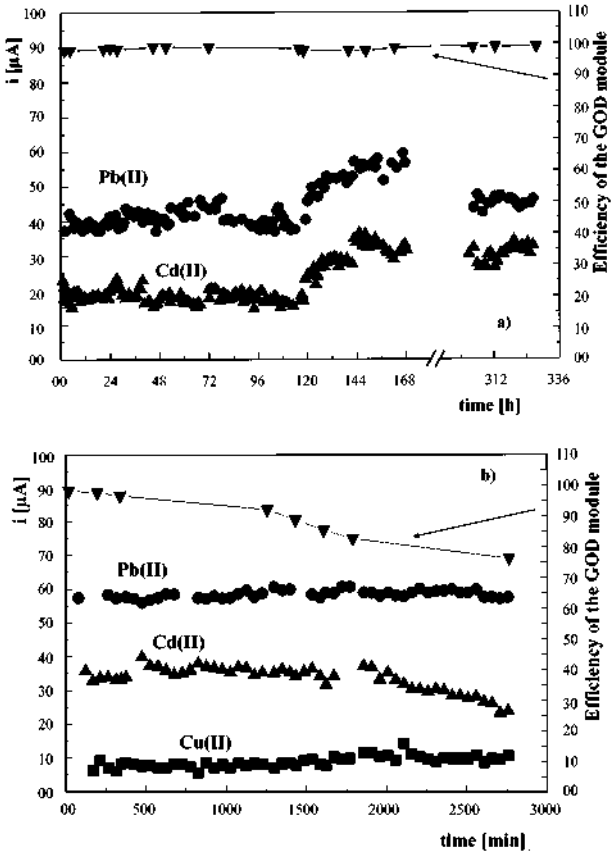


FIGURE 4. Efficiency of the GOD-module for online deoxygenation (▼), determined from eq 1, and SWASV trace metal peak currents (● Pb(II); ▲ Cd(II); ■ Cu(II)) as a function of the measurement time. GOD-module conditions used as in Figure 3; SWASV $t_{dep} = 5$ min. (a) GOD-module maintained at a temperature of 22 °C. Lake Greifensee water sample filtered through 0.2 μm pore size membrane spiked with 6 nM (days 1−5 and 12−14) and 8.4 nM (days 6−11) of Pb(II); 4.9 nM (days 1−5) and 8.6 nM (days 6−14) Cd(II). (b) GOD-module with insulating housing maintained at a temperature of 4 °C; first measurement cycle. Lake Greifensee water sample filtered on 0.2 μm pore size membrane spiked with 8.4 nM Pb(II), 8.6 nM Cd(II), and 4.5 nM Cu(II).

On the other hand at 4 °C, LSV O_2 measurements showed that the efficiency of the GOD-module declined to 80% within 3 h of immersion in a 4 °C water bath. When the temperature of the GOD-module was left to rise to room temperature, an increase in the efficiency was observed. These results show that, as expected, the rate of O_2 consumption by the enzyme cross-linked chemical gel decreases with decrease in temperature. To minimize the cooling of the reductive gel, the GOD-module was placed in a Plexiglas housing padded with an insulating polymer foam. This simple insulating housing system lengthens the efficiency of the GOD-module, and 80% efficiency was observed only after ~35 h (Figure 4b).

TABLE 2. SWASV Average Trace Metal Peak Currents as a Function of Various Efficiency of the GOD-Module Calculated from Three Replicate Continuous Measurements[a]

test no.	efficiency [%]	average peak current [μA] Cu(II)	Pb(II)	Cd(II)
1	99−80	9.8 ± 0.3	58.9 ± 1.4	35.1 ± 0.8
1	80−75	10.2 ± 0.3	59.0 ± 1.8	27.2 ± 4.5
2	99−80	18.6 ± 0.1	65.0 ± 0.8	37.4 ± 3.2
2	80−70	18.9 ± 0.5	63.5 ± 0.2	27.0 ± 3.5
2	70−65	18.9 ± 0.4	57.6 ± 0.2	20.2 ± 0.3
3	99−80	26.8 ± 0.6	66.1 ± 0.5	44.9 ± 0.4
3	80−70	25.4 ± 0.3	65.8 ± 0.4	33.8 ± 0.8
3	70−65	26.7 ± 0.6	56.2 ± 0.5	23.3 ± 0.9

[a] GOD-module with insulating housing maintained at 4 °C. Lake Greifensee water sample filtered through 0.2 μm pore size membrane spiked with 8.4 nM Pb(II) and 8.6 nM Cd(II) for tests 1−3 and 2.5, 4.5, and 6.5 nM Cu(II) for tests 1−3, respectively.

After this first test, the GOD-module was left to warm to room temperature at least for 10 h before starting a new cycle of LSV and SWASV replicate measurements at 4 °C and this was repeated twice. The decrease in efficiency of the GOD-module were found to be similar in both cases, in particular: slower than without the insulating housing but faster than in the first test, i.e., efficiency of 80% was observed after an immersion time of ~15 h. SWASV average peak currents of Cu(II), Pb(II), and Cd(II) for various efficiency ranges of the GOD-module and for the three replicate tests at 4 °C are reported in the Table 2. The results show that (i) variations in peak currents are within the μ-AMMIA experimental errors, i.e., maximum ± 5% (*20*), for the three elements tested for deoxygenation efficiency of the GOD-module ≥80%, (ii) decrease in Cd(II) and Pb(II) peak currents are observed when the deoxygenation efficiency of the GOD-module was <80% and <70%, respectively, and (iii) Cu(II) peak currents remain constant within experimental error over the deoxygenation efficiency range tested, i.e., efficiency ≥65%. A possible explanation for the sensitivity of the trace metals to O_2 in the order Cu(II) < Pb(II) < Cd(II) is that in this case the decreases in peak current intensities are due to a chemical oxidation of the reduced metal in the amalgam by O_2 (*23*) rather than to an increase in pH, due to the reduction of O_2, at the electrode surface. It must be noted however that the effect of O_2 will depend on sample composition, pH, and buffering. One salient feature of the results observed is that for an efficiency of the GOD-module ≤80%, residual currents of O_2 was detected on the SWASV stripping and background voltammograms measured with the VIP voltammetric probe (for details see ref *2*). Thus this characteristic can be used to diagnose the efficiency of the online deoxygenation during in situ SWASV measurements of trace metals. Finally, the results showed that the simple insulating housing can be used for in situ daily monitoring at 4 °C (i.e., efficiency of the GOD-module > 80% for a measurement period of about 15 h), but for long-term

Station	Metal	Conc. [nM] VIP	Conc. [nM] ICP-MS
1	Cu	5.40	97.0
	Pb	0.48	5.5
	Zn	--	197.0
2	Cu	2.45	19.6
	Pb	0.17	1.1
	Zn	0.81	--
3	Cu	3.08	64.7
	Pb	0.27	9.8
	Zn	1.97	229.3
4	Cu	2.85	36.1
	Pb	--	3.2
	Zn	0.65	49.7
5	Cu	2.38	16.9
	Pb	0.26	0.9
	Zn	0.50	15.8
6	Cu	1.68	22.0
	Pb	--	1.4
	Zn	0.84	61.0
7	Cu	0.30	20.6
	Pb	0.16	0.8
	Zn	0.12	41.4

FIGURE 5. Concentrations of the mobile fraction of trace metals, measured in situ using the VIP voltammetric probe connected to the GOD-module, and total trace metal concentrations, measured in pH 2 acidified lake water samples using ICP-MS, in various stations near the city of Versoix. Lake Leman, Switzerland: June 9, 11, and 16, 1999. GOD-module conditions used as in Figure 3; SWASV t_{dep} = 15 min.

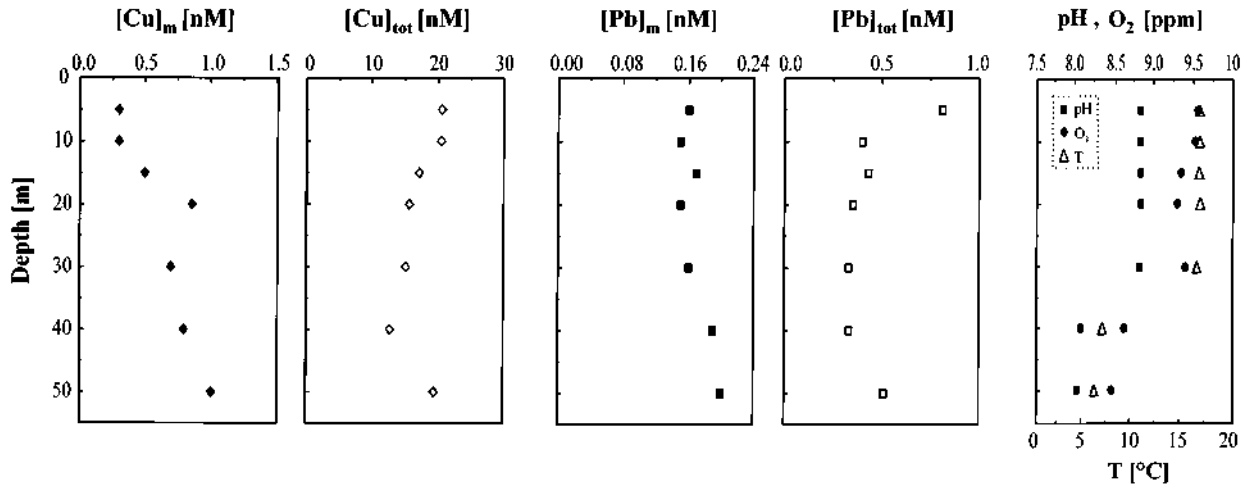


FIGURE 6. Results of profiling performed at station 7 (see Figure 5). $[M]_m$ = concentration of the mobile fraction of trace metal measured in situ using the VIP voltammetric probe connected to the GOD-module; $[M]_{tot}$ = total metal concentrations measured in pH 2 acidified lake water samples using ICP-MS; *T*, O_2 and pH were measured, simultaneously to in situ trace metal measurements, with the multiparameter probe. Lake Leman, Switzerland: June 16, 1999. GOD-module conditions used as in Figure 3; SWASV conditions t_{dep} = 30 min.

autonomous in situ monitoring a more sophisticated insulating housing must be developed. This work is under way.

Environmental Application

To check the efficiency of online deoxygenation in real conditions, the GOD-module connected to the voltammetric probe of the VIP System was tested in lake Leman (Switzerland) near the city of Versoix. In situ monitoring of the mobile fraction of trace metal (i.e., the fraction selectively measured with the VIP voltammetric probe in complex media and defined as free metal ions and small labile complexes of few nanometers size (*3*)) was performed in surface water (depth 1 and 5 m; $T = 14-17$ °C) on June 9, 11, and 16, 1999 at various stations. During the three field trials, efficiency of the GOD-module, determined from LSV O_2 measurements just after the first deployment and at the end of each monitoring day, was found to be in the range 98−95%, and a good resolution of the SWASV voltammograms was observed. These results demonstrate the reliability of the submersible GOD-module for in situ online deoxygenation.

No significant trace metal concentration variations were observed between measurements performed at 1 and 5 m at this period of the year. Average concentration of the mobile fraction of trace metals, determined by SWASV in situ monitoring at both depths after temperature effect correction (for details see ref *4*), together with total metal concentrations, determined in the laboratory in unfiltered samples acidified to pH 2 (HNO_3 suprapur) using ICP-MS, are reported in Figure 5. It can be seen that relatively high concentrations are observed in the main and in the external harbor of a resort (i.e., station 1 and 3, respectively), and relatively rapid dilution are observed over the first 2 km for Cu(II), Pb(II), and Zn(II). Cd(II) was found to be below the detection limit of both techniques. The total Cu(II) and Zn(II) concentrations found by ICP-MS were in the same range as those reported previously in lake Leman (i.e., Cu(II): 18−377 nM; Zn(II): 40−560 nM (*24*)).

Profiling was attempted at station 7 (maximum depth 60 m), and the results obtained are shown in Figure 6. Even if this was not the aim of this study, interesting observations can be made from these preliminary results. In particular the ratio of mobile to total concentrations of Pb(II) ($[Pb]_m/[Pb]_{tot}$) was similar over the entire water column, while the mobile fraction as well as the ratio of mobile to total concentration of Cu(II), and Zn(II) (not shown), were found to be much smaller in the upper water layer (typically the first 15 m) than in the bottom layer. The salient feature is that available annual data on the biophysicochemical conditions of Lake Leman show that high primary productivity (i.e., production rates: 22−166 mg C/m^3 day and chlorophyll *a* concentrations: 1.5−7.5 mg/m^3) is also observed over the first 15 m of the water column at this period of the year (*25*). This suggests that an important fraction of the mobile Cu(II) and Zn(II) species is either assimilated by the phytoplankton or complexed by their exudates (*26*) as nonlabile or nonmobile species, or even both, in the upper layer of the water column. The role of biota is supported by the fact that Pb(II), which is known to be not easily assimilated, does not show the same trends as Cu(II) and Zn(II). Of course more systematic studies are required for rigorous interpretations of these data. In particular extended studies of the variation of the mobile fraction of the different elements as a function of seasonal primary productivity should enable one to get useful information on bioavailability of trace metals provided that reliable analytical measurements can be performed. For this purpose the application of the VIP submersible voltammetric probe for in situ measurements in oxic freshwater, thanks to the submersible GOD-module developed, is particularly useful. Indeed, it was previously shown that the VIP probe allows measurements of mobile concentrations as low as 50 pM for Pb(II) and Cd(II), 150 pM for Cu(II), and 300 pM for Zn(II) using a deposition time of 15 min (*2*, *4*). In addition, in situ measurements minimize or eliminate most of the important analytical artifacts encountered during sampling and sample handling in separation techniques such as cascade ultrafiltration or ultracentrifugation which must be used before laboratory measurements of the trace metal mobile fraction concentrations using classical techniques.

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