Aquatic Sciences 53/1, 1991

1015–1621/91/010055-19 $ 1.50 + 0.20/0

Evaluation and application of dialysis porewater samplers for microbiological studies at sediment-water interfaces

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Key words: Equilibrium diffusion technique; porewater gradients; sediment microbiology.

ABSTRACT

The equilibrium diffusion technique has become a valuable tool for ecological and biogeochemical studies in aquatic environments. In sediment ecosystems, changes in concentration of microbial metabolites with increasing depth can be determined dependably and reproducibly with this technique. Since the permeation characteristics of the membranes employed are crucial, selecting the proper membrane requires knowledge about its behavior under conditions which prevail in the natural environment. Thirteen polymer sheets were evaluated comparing permeation terms for biogeochemically relevant solutes, biodegradability, and mechanical strength. Cellulose-based dialysis membranes are most satisfactory when employed in low temperature anoxic environments. For this membrane, correction terms were calculated to account for diffusion losses during retrieval and sampling. Optimal incubation times can now be predicted from experimentally determined permeation coefficients for several porewater solutes. Dialysis porewater samplers (DPS) have been successfully applied during more than 100 independent experiments for the collection of interstitial water from surface sediments. DPS were used for water depths as deep as 290 meters.

1. Introduction

Sediments of lakes, oceans, rivers, and certain groundwater aquifers harbor active microbial populations which participate in the cycling of nutrients, geochemically reactive elements and many pollutants. In sediments, microorganisms catalyze key reactions of early diagenetic events during compaction thereby regulating the composition of the water in the interstitial spaces (Berner 1980). Qualitative and quantitative information about microbial metabolism in surface sediments and groundwater aquifers can best be gained from in situ studies of indicative compounds dissolved in the interstitial water (Sholkovitz 1973, Emerson 1976). Their changes represent sensitive indicators for microbially mediated processes. It is essential to use

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sampling procedures that collect chemically unaltered porewater uncontaminated by solid components of the sediment matrix. Presently five different types of porewater samples are most often used for analyses: (i) Supernatants after centrifugation of sliced sediment cores (Sholkovitz 1973, Emerson 1976); (ii) pressure filtrates of sliced sediment cores (sqeeze-water) (Staub 1981, Presley et al. 1967); (iii) porewater expelled through sampling ports from pressurized intact sediment cores (Bender et al. 1987, Jahnke 1988); (iv) samples obtained by suction of porewater into a sampling device (Sayles et al. 1973); and (v) in situ sampling of porewater with the equilibrium diffusion technique (Hesslein 1976, Brandl and Hanselmann 1981, Adams et al. 1982, Carignan 1984, Brandl et al. 1990). We have employed all five methods for appropriate applications in limnic and marine sediments. For microbiological and biogeochemical studies the equilibrium diffusion technique has worked most satisfactorily. The sampling device usually consists of small chambers which are filled with salt- and gas-free water, covered with a semipermeable membrane and incubated in different sediment horizons until equilibrium is reached. For reliable application and the determination of optimal incubation times, it is essential to know the permeability characteristics of each type of membrane, its adsorptive and desorptive properties for chemical compounds and microorganisms, and its resistance to microbial degradation. Reliablility and reproducibility of the method depend on the knowledge of these characteristics. In addition, quantitative information is needed on artifacts which are introduced during the time span between retrieval of the sampler and final porewater conservation (exposure to lake water, to air etc.) such that losses or additions can be corrected for.

Here we summarize experiences with the equilibrium diffusion sampling procedure which we use for investigations of sedimentary microbial habitats. The objectives of the present study are: (i) to evaluate the permeation properties of different types of membranes for porewater-specific substances and the resistance to biodegradation; (ii) to determine diffusive artifacts introduced during the retrieval procedure and present appropriate corrections; and (iii) to apply the technique to microbial studies at the sediment-water interface. The technique is applicable to ecosystems whose steady state does not change in the course of the experiment.

2. Materials and methods

Porewater samplers

We employ dialysis porewater samplers (DPS) which are modified versions of the one originally described by Hesslein (Hesslein 1976) (see appendix for details). The sampler we employed most often consists of an $80 \times 12 \times 1.5$ cm Plexiglas sheet containing 41 rows of cylindrical dialysis chambers, each 1.5 cm in diameter and 1.5 cm deep. This construction allows for individual preservation treatment in each compartment if necessary. The chambers are covered on both sides with membranes held in place by 3 mm thick Plexiglas overlays. The membranes seal the single chambers and prevent exchanges between them. Up to five individual samples of 2.6 ml can be collected from each row.

For in situ studies we implant DPS perpendicular to the sediment surface. We leave 8 to 10 chamber rows exposed to the water above the sediment-water interface. Incubation times are determined according to Eq. 5 given in the appendix. For experiments in great depths the samplers are positioned with the aid of a small research submarine equipped with a hydroelectric manipulator.

Calculation of membrane permeability

In laboratory studies to determine membrane permeation characteristics the samplers were incubated at 5 °C in a 50 l chromatography tank filled with distilled water. The outside volume was approximately 100 times larger than the sum of the compartment volumes. Specific compounds of interest were added to the tank in amounts which closely simulated in situ conditions. Methane was continuously bubbled through the aqueous phase in order to maintain saturation. Stirring assured homogeneous distribution of solutes in the tank. The dialysis chambers of the DPS were originally filled with distilled water. Samples were periodically withdrawn by syringe and analyzed for specific compounds.

Permeation properties of different membranes were also evaluated in a laboratory assay with two adjacent dialysis chambers made out of Plexiglas (Northrop and Anson 1929). The two compartments (20 ml each) were separated by the membrane which was to be evaluated. The exchange surface was 9.1 cm^2. The content of each compartment was stirred to assure homogeneous conditions. Substances of interest were added to one compartment and samples were periodically withdrawn from the other or from both. Concentration changes could thus be determined as a function of time and the membrane-specific permeation properties could be calculated by the procedure outlined below. The permeation coefficients obtained have to be considered as maximal values.

Permeation coefficients of membranes (k^M) are calculated from the permeation kinetics of the equilibrium experiments according to

$$k^M = V_i/A \cdot 1/t \cdot \ln (C_0/(C_0 - C_i(t)))$$

where V_i and A are the volume and the membrane covered exchange area of the diffusion chamber, respectively. C_0 is the approximately constant concentration in compartment 1 and $C_i(t)$ the concentration in compartment 2 at sampling time t (Crank et al. 1981). The actual value for k^M was determined from the best fit of the measured data pairs ($C_i(t)$ vs. t) to the saturation function

$$C_i/C_0 = 1 - \exp (-k^M \cdot A/V_i \cdot t)$$

The same type of equation was applied for the calculation of diffusion-dependent alterations during retrieval of the samplers.

Degradation of membranes

For degradation experiments, membrane disks (47 mm in diameter) were dried for 24 hours at 90 °C and weighed. For degradation tests under anoxic conditions, a

small tank was filled with lake sediment (approx. 2 l) and membrane probes were positioned in duplicate on the sediment surface. A layer of sediment (2 cm thick) was then added to cover the membranes completely. The sediment tank was incubated at 28 °C. After an incubation period of 15 days the membranes were retrieved from the sediment, thoroughly rinsed with distilled water and wiped in order to remove any material that adhered to the membrane, dried and weighed. The weight loss was taken as a rough indication for degradation. Analogous experiments were performed under oxic conditions and at 5 °C under either oxic or anoxic conditions. Control samples were incubated in sterile tap water at 28 °C under both conditions.

On a second set of membranes permeation characteristics (k^M) were determined before and after incubation. Dialysis membranes were incubated in sediment slurry at 5 °C and 25 °C under either oxic or anoxic conditions. After 15 days the membranes were removed from the slurry, rinsed thoroughly and again subjected to permeation tests at 5 °C.

Analytical methods

Particle free samples for the determination of sulfate and nitrate were preserved in formalin (2% v/v). The tubes for methane analyses contained 100 µl of nitric acid (10% v/v) since earth alkali, alkali and transition metals were also analyzed in the same samples. Free hydrogen sulfide was precipitated as ZnS with zinc acetate (4% w/v in 2% v/v acetic acid) and free organic acids were transformed into their sodium salts with 2 M NaOH.

Nitrate and sulfate were determined by ion chromatography (Hertz and Baltensperger 1984); iron, manganese, calcium, and magnesium in some samples by flame atomic absorption spectroscopy (AAS). Potential contamination from the membrane matrix was measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after extraction of membrane pieces in 5% (v/v) nitric acid. ICP-AES was also used to determine a series of porewater constituents such as calcium, magnesium, sodium, potassium, iron, manganese, and phosphorus in the acidified samples and silica in alkaline (10% w/v NaOH) samples. Sulfide was quantified colorimetrically with the methylene blue method (Gilboa-Garber 1971). Gas chromatography with flame ionization detection was used for the determination of methane (Rudd et al. 1974) and volatile fatty acids (VFA) (Du Preez and Lategan 1978). It should be remembered that hydrogen sulfide must be removed from samples before phosphate and ammonium can be determined colorimetrically.

3. Results and discussion

Membrane permeability

Selecting an appropriate membrane is the most crucial step in the application of the equilibrium diffusion technique. Stability of the membrane matrix and minimal changes in permeability are important for membranes used in long-term experiments

Table 1. Some specifications of membranes evaluated

#	Type	Material	Thickness [μm]	Pore size [μm]	Transparency	Manufactured by, purchased from
1	Dialysis membrane	Cellulose	80	none	yes	Union Carbide
2	Saran wrap	Polyethylene	10	none	yes	DOW Chemical
3	Saranex 11	Polyethylene	38	none	yes	DOW Chemical
4	Saranex 14	Polyethylene	50	none	yes	DOW Chemical
5	Saranex 19	Polyethylene	100	none	yes	DOW Chemical
6	Saranex 23	Polyethylene	50	none	yes	DOW Chemical
7	Polycarbonate foil	Polycarbonate	10	none	yes	Nuclepore
8	Polyamide tubing	Polyamide	35	none	yes	Kleiner, Switzerland
9	Polyamide microfilter	Polyamide	200	8	no	Sartorius
10	Versapor 200	Acrylic polymer	229	0.2	no	Gelman
11	Cuprophan	Cellulose	40	none	yes	Kleiner, Switzerland
12	HT-200 Tuffryn	Polysulfone	165	0.2	no	Gelman
13	Cellophane wrap	Cellulose	20	none	yes	Migros, Switzerland

under adverse and differing environmental conditions. For the purpose of this discussion we define membrane permeability k_j^M [cm s^{-1}] as the solute (j) specific molecular "filtration" ability of a particular polymer sheet of thickness l_M under a given set of boundary conditions (temperature, solvent, ionic strength, pH etc.). Knowledge about the variability of k^M is a prerequisite for plausible interpretation of data collected with this technique.

We have evaluated a set of commercially available polymer sheets (Table 1), at least 12 cm wide and obtainable at reasonable costs. Characterization was carried out with solutes which reflect microbial activities in aquatic ecosystems: (i) CO_2, HCO_3^-, CO_3^{2-}, H^+, and OH^- to follow changes in the carbonate buffering capacity and the acid neutralizing ability; (ii) NH_4^+, NH_3, NO_3^-, NO_2^-, HPO_4^{2-}, $H_2PO_4^-$, HS^-, S^{2-}, H_2S, SO_4^{2-}, O_2, CH_4, and organic acids and other gaseous, dissolved, neutral and charged small organic molecules which are microbial substrates, nutrients, electron accpetors or products; (iii) Ca^{2+}, Mg^{2+}, Na^+, K^+, and Cl^- as the major inorganic ions which assure charge balance in the lacustrine environment; and (iv) Fe^{2+} and Mn^{2+} as redox indicator compounds.

Of the membranes listed in Table 1 the very thin polyethylene (#2) and polycarbonate sheets (#7) showed insufficient mechanical stability for our applications. Collection of particle free water could not be guaranteed with porous membranes (membrane type #9, #10, #12). Membranes of the Saran and Saranex series (#2 to #6) were impermeable to protons (Fig. 1 a for #2), the polycarbonate foil (#7) and the polyamide tubing (#8) were only slightly permeable for H^+. Since protons have a high diffusion coefficient in water (approx. $70 \cdot 10^{-6}$ $cm^2 \cdot s^{-1}$ at 283 K) their permeation through membranes can serve as a primary evaluation criterion. The polyamide tubing material (#8) selectively excluded fatty acid anions (Fig. 1 c) and showed only poor permeability for methane (Fig. 1 b). Therefore, only membrane types #1 and #10 to #13 were further evaluated. When appropriate, other materials were included again for comparative reasons.

Certain membranes contain acid leachable compounds such as boron, calcium, magnesium, potassium, silicon, sodium, or sulfur; all probably remnants of the fabrication process. When released, these compounds could alter the near-field metabolism or feign a false composition of the interstitial water. Membranes with a high content of contaminants (type #10, #12, #13; data not shown) were later excluded from use. For the cellulose dialysis membrane routinely used in most of the experiments we adopted a conditioning procedure in which the membrane was first soaked in HCl (10^{-4} M) for 20 min and then rinsed thoroughly with salt free water. Pretreated membranes were also free of phosphorus containing contaminants.

Incubation times of dialysis porewater samplers

From an experimental standpoint one prefers incubation times which are as short as possible yet long enough to assure equilibrium even for the compound with the lowest permeation coefficient. Incubation times depend not only on k_j^M, but are also determined by the sampler design factor f (for determination see appendix). The lower f, the sooner equilibrium is reached between the volume of the sampler

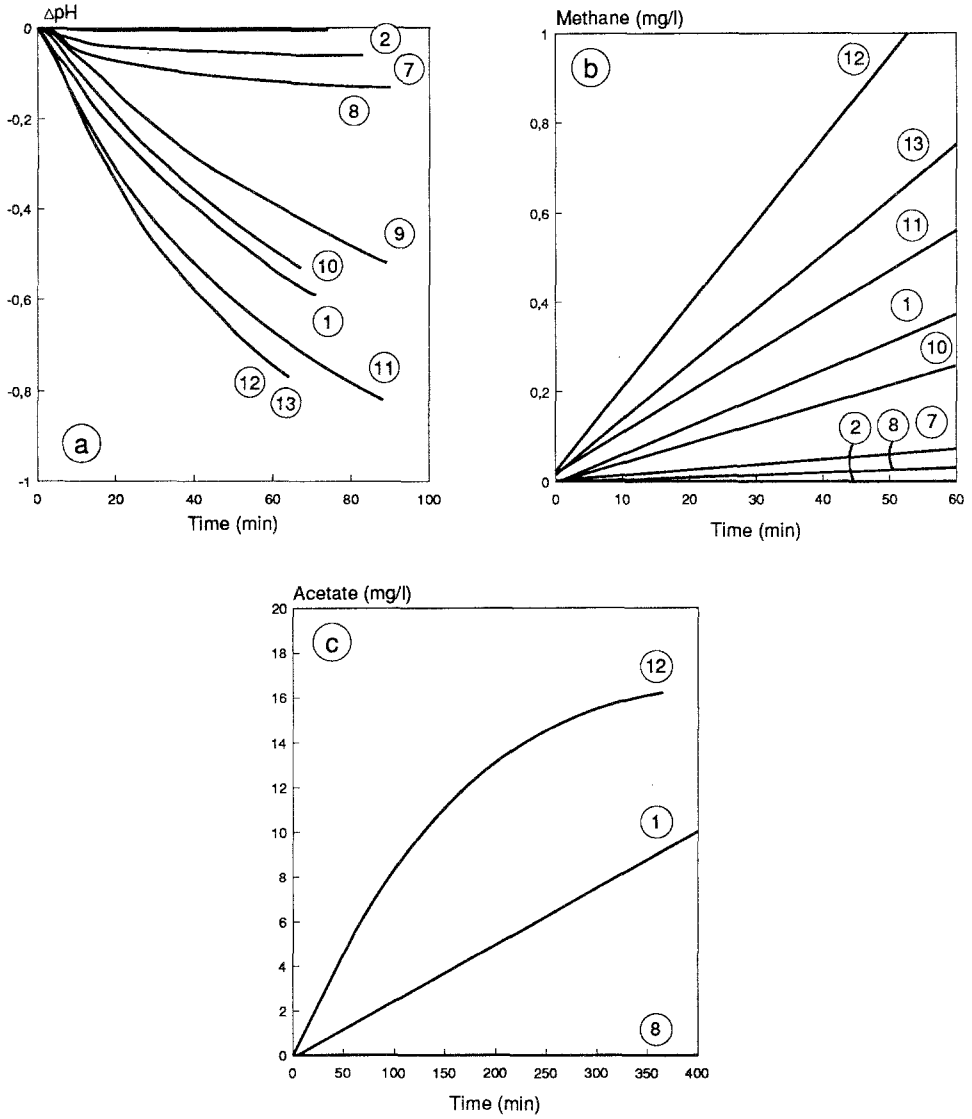


Figure 1. Diffusion properties of different types of membranes at room temperature (22 °C). a) Permeability for protons. The initial pH in both dialysis compartments was between 7.5 and 8.0. To start the experiment 1 ml 10% HCl was added to one of the dialysis chambers. b) Diffusion of methane. Initially, the distilled water of one dialysis compartment was saturated with methane. c) Diffusion of acetate. Initially, the acetate concentration was 1 mM in one of the dialysis compartments. The numbers correspond to the membrane types described in Table 1

Table 2. Molecular diffusion coefficients (D_j) and membrane-retardation factors (α_j) for cellulose membrane (#1)

Species j	Diffusion coefficients in water (D_j^W) $[cm^2 \cdot s^{-1}] \cdot 10^{-6}$	sediment[b] (D_j^S) $[cm^2 \cdot s^{-1}] \cdot 10^{-6}$	Membrane retardation factor[a] α_M^W
SO_4^{2-}	5.88	5.31	193.4
Na^+	7.39	6.67	12.9
Ca^{2+}	4.40	3.97	15.4
Mg^{2+}	4.11	3.71	14.8
Fe^{2+}	3.97	3.58	31.5
CH_4	12.8	11.55	20.7

[a] Retardation of the sediment $\alpha_S^W = 1/\phi^2 = 1.108$; retardation of the membrane $\alpha_M^W = D_j^W/D_j^M$. D_j^W is the diffusion coefficient for membrane of thickness l_M. $D_j^M = k_j^M \cdot l_M$. For k_j^M see Table 4; $l_M = 80\ \mu m$

[b] Calculated for a homogeneous porosity (ϕ) of 0.95. $D_j^S = D_j^W \cdot \phi^2$

chamber (V_i) and the environment. The design factor also determines the spatial resolution and the sample volume available for analyses. Permeation coefficients for a few selected inorganic ions and a neutral compound of environmental interest are listed in Table 4. The coefficients shown are valid for cellulose membrane (#1) incubated at 5 °C.

Practicable incubation times (t) for the porewater samplers have been calculated by considering the design of the sampler and the diffusion characteristics of specific chemical compounds (see Appendix, Eq. 5). For sulfate one obtains 50% of the environmental concentration inside the compartment of a type-3 sampler (see Appendix) incubated at 5 °C after 1.6 days, 90% after 5.3 days, and 99% after 10.6 days. Almost 130 days would be required to reach 99% of the equilibrium concentration of SO_4^{2-} using our high resolution sampler (design factor $f = 9.14$). This type of sampler is applied more successfully for the determination of methane profiles and to obtain more detailed gradients at the sediment-water interface.

Permeation values for different compounds have to be derived experimentally for each membrane since no generally applicable relationship exists between membrane permeabilities and a molecular parameter of the compounds of interest. Table 2 contains the retardation factors (α) which describe the changes in diffusion coefficients between water (W) and membrane matrix (M) as $\alpha_M^W = D_j^W/D_j^M$. Their differences indicate the permeation selectivity of the membrane for the chemical species listed. It may be seen from the listing that diffusion into the chambers is regulated by membrane characteristics rather then by retardation through sediment components.

It is misleading to derive optimal incubation times from the permeation coefficients of single ions. Since the charge balance inside the compartment has to be maintained at all times, the ion with the lowest permeability coefficient determines the duration of the incubation. Thus, if Ca^{2+} and SO_4^{2-} form an ion pair in the environment under investigation, sulfate determines the correct incubation time, even if one is interested only in the Ca^{2+}-equilibrium. It is essential, therefore, to

consider all major ions in the environment being sampled with the equilibrium diffusion technique.

Since there are neither biogeochemical sources nor major sinks of Na^+ in the lake sediments we were studying, this ion offers an internal standard for equilibrium evaluations. The nearly constant concentration of Na^+ in the overlaying water and at all sediment depths (porosity decreases from 0.93 at the surface to 0.69 at a depth of 40 cm) indicates that equilibrium conditions have been reached for this ion (Fig. 2g). If charged species dissolved in the interstitial water were of interest, we adopted an incubation time of 12 to 20 days for type-2 and type-3 samplers (see Appendix) in the lake sediment ecosystems studied.

Sampling artifacts

As soon as a membrane covered sampler is exposed to different conditions the diffusion process will proceed toward a new equilibrium. Thus, porewater samples are altered during the retrieval time between removal of the sampler from the sediment and the final preservation of the samples. Dissolved gases (CH_4, H_2S, O_2, CO_2, N_2) require special attention since their solubility decreases rapidly as the sampler warms up and since membranes normally have high k^M-values for gases, a consideration of particular importance for investigations in great depths. In order to make the proper corrections, the changes were evaluated quantitatively for losses of CH_4 and H_2S and for contaminations with SO_4^{2-}, NO_3^-, and O_2 with the cellulose membrane (#1).

At 5 °C, 17% of the methane originally present can escape from the porewater sampler within 30 min in water. After 2 h at 5 °C there are only 48%, and at 22 °C only 33% of the original methane left. The original concentration at $t = 0$ ($C_i(t_0)$) for samples collected at time t after removal of the sampler from the sediment is calculated according to:

$$C_i(t_0) = C_i(t) \cdot \exp(q \cdot t)$$

where $C_i(t)$ is the concentration still present at the time of sample preservation; q is a correction term which depends on the chemical of interest, the type of sampler, the membrane, the temperature, the gradient between the sample in the chambers, and the medium of the environment; t is the time which elapses between retrieval and final sample fixation. The correction term must be determined experimentally for each membrane type and it is then applied to the sampling procedure which is routinely followed. For a type-3 sampler (see Appendix) covered with a cellulose membrane (#1) q is 0.2874 h^{-1} for CH_4, if the sampler is kept in air at 22 °C. If the sampler is kept in a protective casing for the time of exposure to water the losses are 20% to 25% less than without the casing; q is 0.234 h^{-1}. Similar corrections are necessary for H_2S if the pH of the porewater is below 8 ($q = 0.5556\ h^{-1}$ for 22 °C against air). Sulfate and nitrate are normally present in the interstitial water at very low concentrations or they are depleted in porewater from deeper sediment horizons. Exposure of a porewater sampler to sulfate and nitrate containing lake water leads to higher values which can be corrected to the conditions at the time of sampler removal according to

$$C_i(t_0) = C_i(t) - C_0 \cdot (1 - \exp(-q \cdot t))$$

where $C_i(t_0)$ is the true concentration, $C_i(t)$ the concentration measured, C_0 the concentration of sulfate or nitrate in the lake water to which the samples are exposed for the time span t after retrieval from the incubation site, and q ($=0.0181\ h^{-1}$ for sulfate) is an experimentally determined correction factor. No corrections were necessary for the low nitrate concentrations in the lake water. Due to the low k^M of the cellulose membrane for SO_4^{2-}, contaminations with this compound are 4.5 μmolar (C_i) after 30 min at 5 °C and 18 μmolar within two hours for $C_0 = 500$ μmolar.

Contamination with oxygen is a particular problem for samplers retrieved from anoxic sediments. The content of extremely redox labile compounds (Fe^{2+}, Mn^{2+}, HS^-) can be altered at low oxygen concentrations. Originally anoxic samples exposed for 30 min to water containing 12 mg O_2/l at 5 °C and then for another 30 min to air at 25 °C acquired a final O_2-concentration of approximately 3.2 mg/l in an in vitro experiment. In samplers retrieved from hypertrophic sediments and exposed to air for as little as 30 min we have observed turbid white sulfur and brown metal oxide and hydroxide precipitates in the compartment and on membrane surfaces.

Artifacts can be minimized if one follows standardized procedures during positioning and retrieval of the samplers. In order to keep these unavoidable changes under control, we followed a reproducible sampling sequence which was particularly helpful for field sampling: (i) DPS were transported under water in protective casings and were immediately wrapped with Saran foil when retrieved at the surface; (ii) dissolved gases and other volatile compounds were sampled with syringes and injected through rubber membranes into gas tight vials; (iii) conductivity, pH and temperature were measured directly in those sampler compartments from which the samples for the alkalinity titration were collected; (iv) redox labile metal ions were optimally preserved by injecting concentrated mineral acid ($pH \leqq 1$) directly into the sampler compartment. Hydrogen sulfide was precipitated as ZnS. (v) Microbial metabolites (NO_3^-, SO_4^{2-}, HPO_4^{2-}, NH_4^+) were conserved with formalin or in acid; (vi) more inert ions (Ca^{2+}, Mg^{2+}, Na^+, K^+) were preserved in concentrated acid. Steps (ii) to (iv) could be carried out within 30 min, the complete sampling procedure for the 5 types of samples from a type-3 sampler can thus be done by two people within 60 min. Alkalinity titrations and the colorimetric determinations of phosphate, ammonium, and hydrogensulfide ought to be carried out within a few hours after sampling.

Degradation of membranes

Membranes for in situ studies were also evaluated for their mechanical and chemical stability and for their resistance to microbial degradation. Thin membranes and those with a brittle glass fiber matrix were mechanically unsatisfactory. All of the membranes considered were chemically and mechanically stable at $4 < pH < 10$, in reducing and oxidizing environments between $-500\ mV < E_h < +800\ mV$, at temperatures $0\,°C < T < 30\,°C$ and when exposed to carboxylic acids (acetic, propionic, isobutyric) and alcohols (ethanol, methanol) in concentrations up to 50 mM. Polysulfone membranes showed a slight darkening when incubated in H_2S-containing environments.

Table 3. Biodegradation of different membrane types under various conditions [a]

# [b]	Membrane material [c]	Weight loss [% dry wt] incubated at 5 °C in lake water (oxic)	sediment (anoxic)	incubated at 28 °C in lake water (oxic)	sediment (anoxic)
1	Cellulose	0	0	2.7	4.8
2	Polyethylene	0	0	0	0
8	Polyamide	1.2	0.5	1.5	0.3
10	Acrylic polymer	1.0	0.6	1.9	0.9
11	Cellulose	2.5	1.6	8.0	11.3
12	Polysulfone	0	0.2	1.5	0
13	Cellulose	1.1	0	3.8	34.6

[a] Weight loss [% dry wt] after incubation under the conditions listed for 15 days. Each value represents the average of duplicate experiments. Controls for each membrane were incubated under sterile conditions in distilled water

[b] Numbers correspond to Table 1

[c] See Table 1 for specification

Resistance to microbial attack was of major importance since membrane digesting microbes can weaken the mechanical stability and change the permeability of the membrane or can block membrane pores. A false porewater composition would be achieved, if microorganisms were metabolically stimulated by an easily digestible membrane polymer. We have followed weight losses during incubation under a variety of conditions (Table 3) and changes in permeability as measures for membrane integrity (Table 4). Artificial polymers were most resistant to microbial attack, but cellulose based membranes (#1 and #13) showed significant weight losses only when incubated at elevated temperatures (Table 3). The extent of degradation varied for cellulose membranes from different manufacturers. Of the cellulose membranes, the dialysis membrane (#1) was most resistant against microbial attack at least at low temperatures. Hydrophobic membranes tend to offer preferred surfaces for non-specific microbial adsorption (SEM pictures not shown).

We could not observe significant changes in permeability after cellulose membrane #1 had been incubated in sediment slurries at 5 °C under either oxic or anoxic conditions (Table 4). Anoxic incubations at elevated temperatures however increased permeability for all 6 chemical species tested.

4. Applications

Geochemical gradients as expressions of microbial activities

Examples which illustrate applications of the equilibrium diffusion method in lake sediment ecosystems are summarized in Fig. 2. Mass gradients are consequences of spatially separated source and sink processes. The shape is maintained as long as the sustaining transport and transformation mechanisms continue. Certain gradients depict differences in chemical potentials which represent energy stores. These may be accessible to microbes able to live by converting metabolically the energy

Table 4. Changes in permeation coefficients $k_{j(5\,°C)}^{M}$ of the cellulose dialysis membrane #1 through incubation in sediment

	Permeation coefficient $k_{j(5\,°C)}^{M}$ [cm · h^{-1}]					
	SO_4^{2-}	Na^+	Ca^{2+}	Mg^{2+}	Fe^{2+}	CH_4
Before incubation[a]	0.0136	0.259	0.128	0.125	0.0570	0.278
± Standard deviation	0.0007	0.013	0.006	0.006	0.0028	0.019
99% conf. intervall ($\pm 3\sigma$)	0.0021	0.039	0.018	0.018	0.0084	0.057
After incubation						
oxic (5 °C)[b]	0.0146	0.243	0.134	0.129	0.0605	0.248
anoxic (5 °C)[c]	0.0136	0.236	0.133	0.119	0.0591	0.260
anoxic (28 °C)[d]	0.0561 (+313)[e]	0.346 (+34)	0.156 (+22)	0.148 (+18)	0.0737 (+29)	0.338 (+22)

[a] Permeation coefficients are calculated from 15 measurements
[b] Incubation of membranes for 15 days in aerated sediment slurry from cold adapted lake sediment at 5 °C
[c] Incubation of membranes for 15 days under anoxic conditions at 5 °C
[d] Incubation of membranes for 15 days under anoxic conditions at 28 °C
[e] Values in parentheses represent permeability changes (in %) when compared with the k_j^M of the membranes before incubation. All permeation tests with membranes before and after incubation were carried out at 5 °C

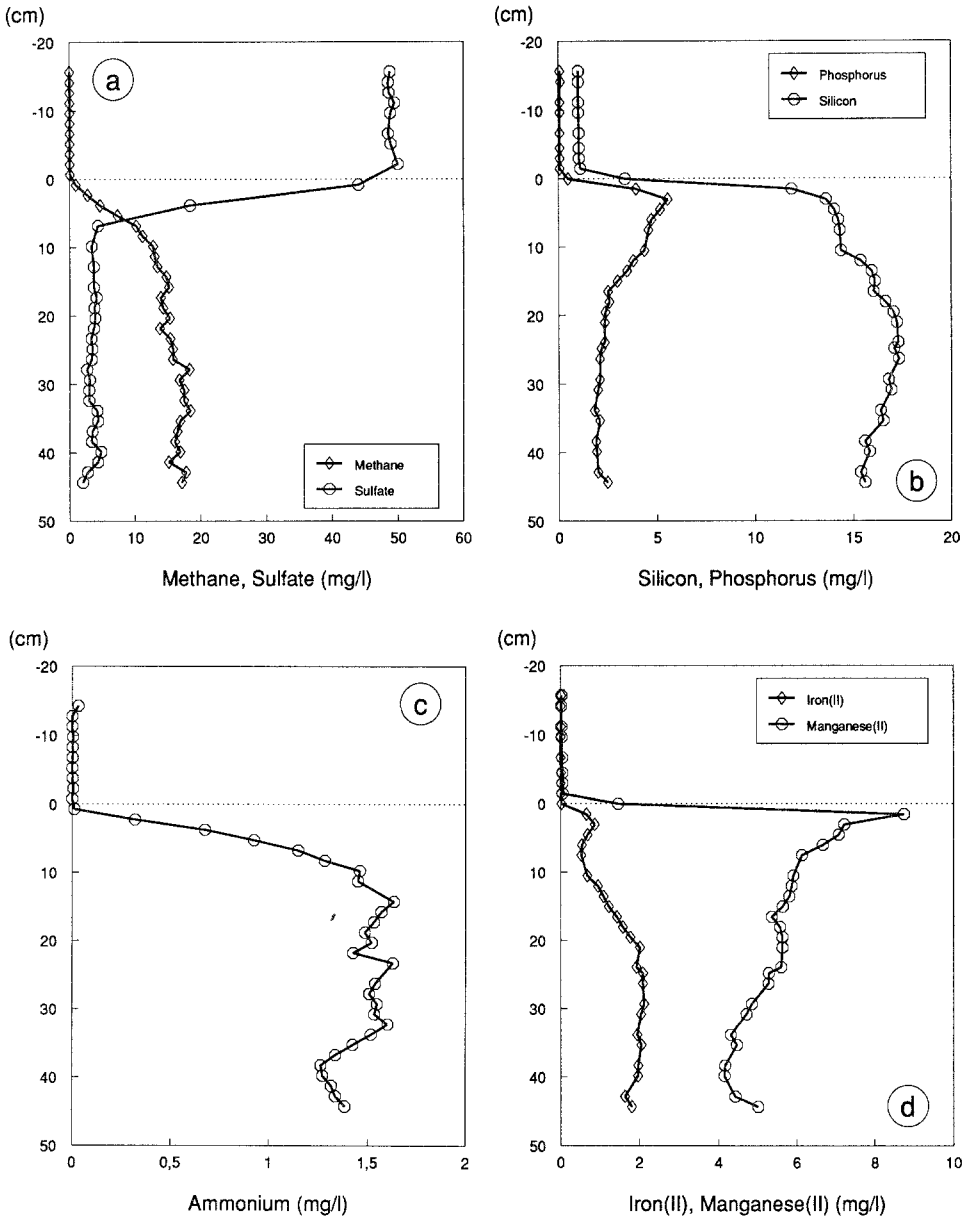
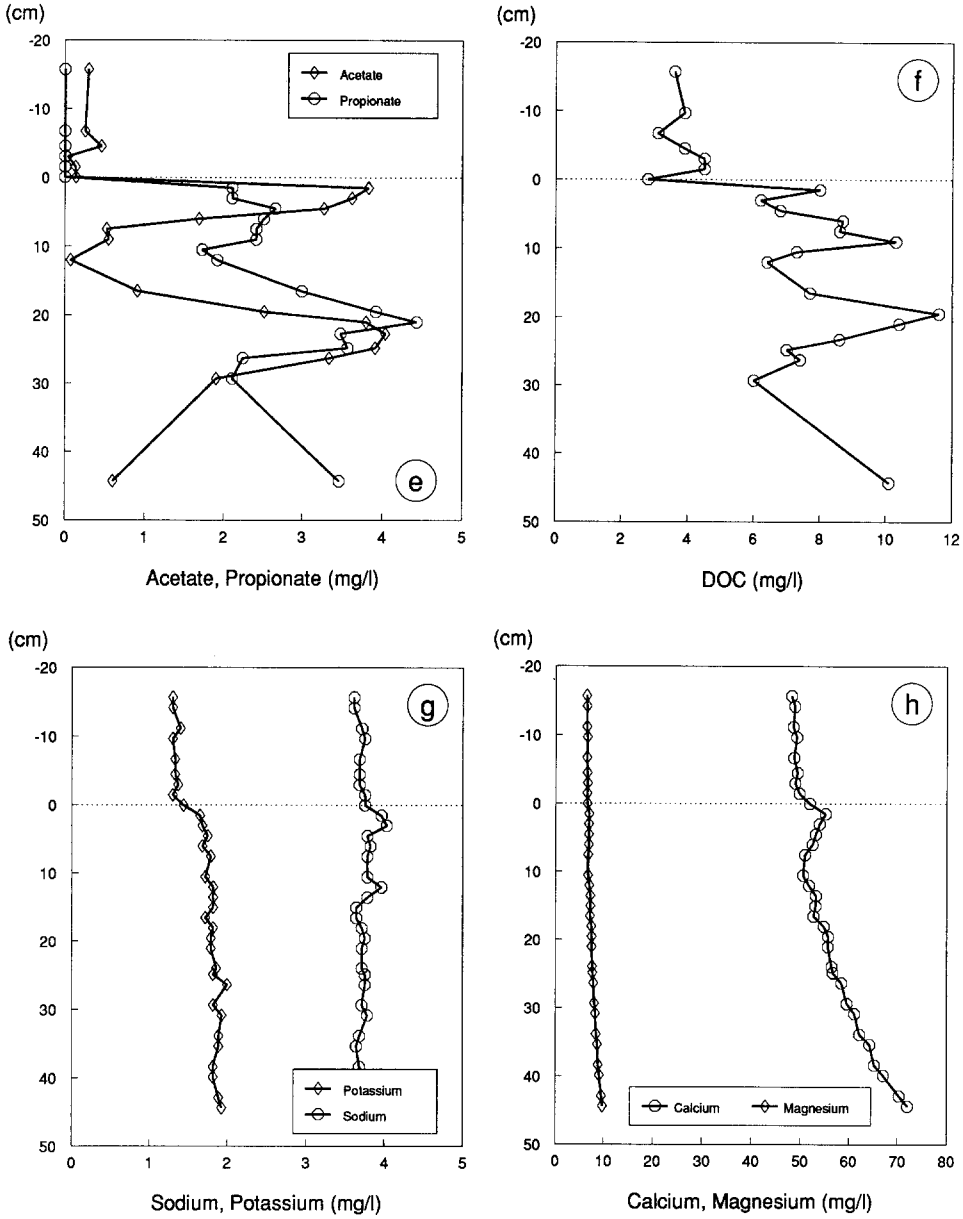


Figure 2. Depth profiles of various microbially and geochemically relevant compounds in the surface sediments of Lake Geneva at a depth of 251 m (17. 12. 85). Incubation time of the type-3 sampler was 14 days



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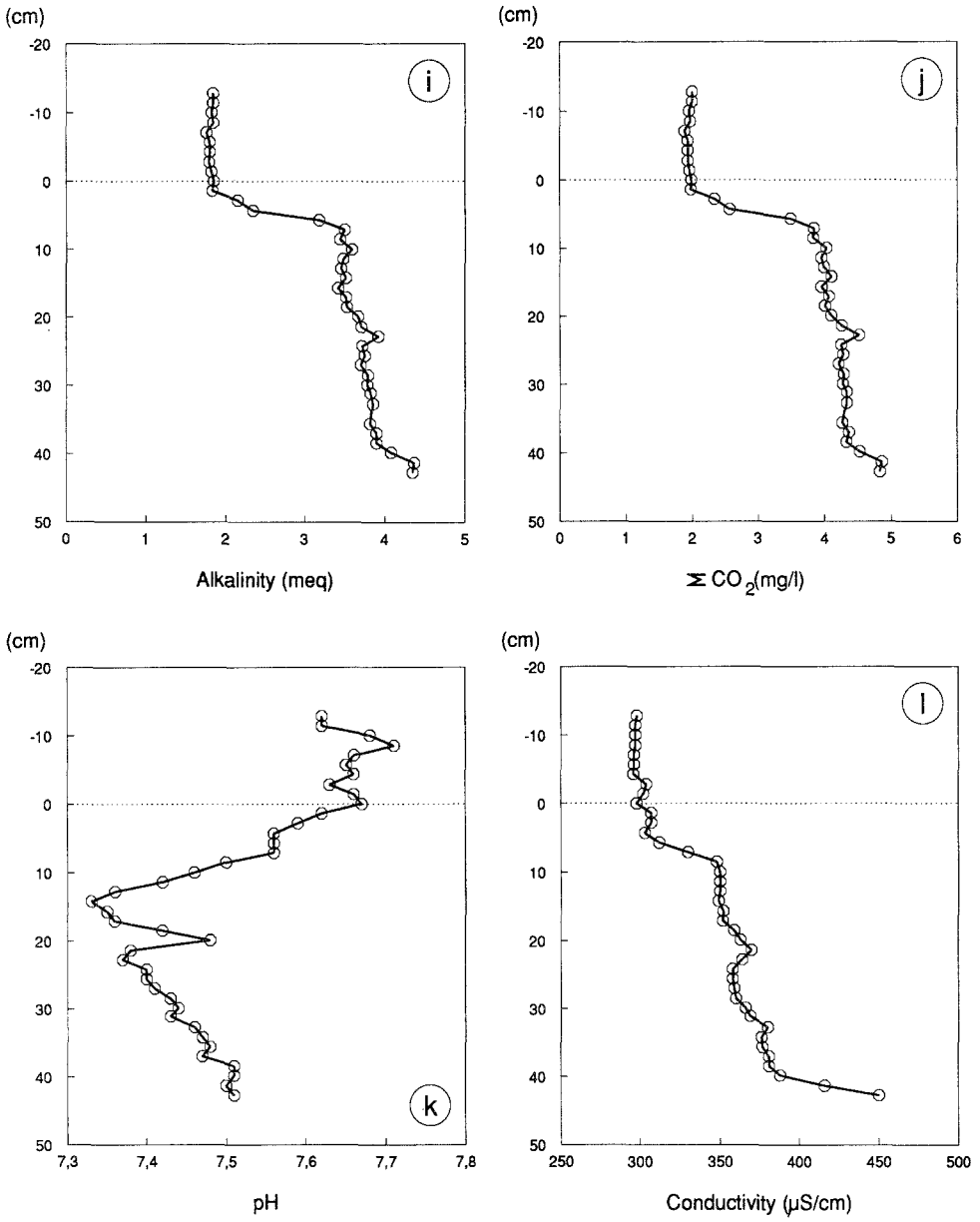

Fig. 2. continued

supplied. Therefore, the shape of a mass gradient can reflect the microbial involvement in its creation and maintenance. The following information may be deduced from the concentration-depth-profiles of Fig. 2.: (i) changes in intensity of certain ecosystem state parameters (Fig. 2a, 2k, 2l); (ii) mass fluxes between sediment compartments and across the sediment water interface (obtained by applying Fick's first law); (iii) reaction rates for consumption or production (calculated according to the modified mass conservation equation (Brandl et al. 1990)); (iv) concentrations of biologically more conservative ions (e.g. Na^+ in Fig. 2g) which serve as indicators for equilibrium concentrations in the sampling chambers; (v) horizons of sinks and sources of dissolved materials as well as sites where specific biogeochemical reactions such as sorption, accumulation, depletion, scavenging, precipitation, reduction, or consumption take place (Fig. 2a to 2e and 2h to 2k); and (vi) changes in ecosystem capacity parameters for proton and electron buffering (Fig. 2i to 2k), ligand exchange and characterization of the complexes formed (Fig. 2b, 2d, and 2g to 2l), and mineral dissolution and formation (Fig. 2b, 2d, 2h, 2j, 2k).

Lake Geneva, even at its greatest depths, is considered to be oxic. At the experimental site the gradients show no evidence for bioturbation by sediment dwelling animals. The gradients are maintained by bottom currents as well as spacially separated chemical and biologically mediated reactions. Sulfate reducing activities are mostly confined to the top 9 cm while methanogenesis is most active at greater depths (Fig. 2a). Indications of free hydrogen sulfide could not be observed in these sediments. Phosphorus, iron, and manganese release is most pronounced in the sediment horizon below the surface (Fig. 2b, 2d). Manganese and phosphorus also diffuse into deeper strata where they might be involved in mineral formation. Although the concentration of free ferrous iron decreases slightly in the sulfidogenic zone (Fig. 2d), the shape of the gradient still points toward a large reservoir of ferric iron compounds in older sediment strata. Most likely fermentative and methanogenic processes are linked to the reduction of ferric iron in greater depths. A net release of NH_4^+ occurs below 10 cm (Fig. 2c); and in the biologically most active layer above, all is oxidized or assimilated. Ammonium and probably also potassium (Fig. 2g) which are bound to clay minerals might comprise reservoirs in deeper zones. Of the organic carbon released into the interstitial water, up to 90% (w/w) is made up of acetate and propionate which are, indubitably, fermentation products (Fig. 2e, 2f). The concentrations of Ca^{2+} and Mg^{2+} (Fig. 2h) are related to the non-biogenic contributions of alkalinity and the total content of various carbonate species (Fig. 2i, 2j). All interpretations are valid for gradients obtained under steady state conditions. The diffusion equilibrium technique may not be applied to ecosystems where steady state cannot be expected throughout the incubation period of the sampler.

5. Conclusions

We consider membrane #1 the best choice for a broad spectrum of applications in lake sediments. At low temperatures its biostability is high, its chemical and

mechanical integrity is maintained, and its permeability is not altered. Under conditions which prevail in sediments of carbonate buffered hard water lakes this membrane is permeable to many microbial metabolites and to the most pertinent geochemical indicator compounds. It is non-porous and impermeable to colloids and bacteria and has a molecular exclusion limit of 12000 Daltons. It is suited therefore for the collection of particle-free water samples which can be processed without further filtration. Its transparency permits a visual control of the compartment contents for eventual precipitate formation during sampling. Before use, small amounts of contaminants can be leached from the membrane with weak acid without hydrolyzing the matrix polymer. Though permeation coefficients for ionic, neutral and gaseous compounds allow for relatively short incubation times, backdiffusion is slow enough for the method to be applied in great depths from which retrieval times are longer.

ACKNOWLEDGEMENTS

We thank D. Bollier, R. Bögli, and H. P. Schmidhauser for the construction of the diffusion samplers, Prof. J. Piccard and his crew for the safe operation of the submersible, Mrs. M. Guecheva for the analyses with the ICP-AES, and Prof. R. Bachofen for his interest in our work. For parts of this work we included data collected by H. B. and Beat Jegerlehner in the course of their Ph. D. and diploma theses, respectively. The work was supported by the Swiss National Science Foundation (grant 3.520-0.83) and by the canton of Zürich.

Appendix: Determination of incubation times for equilibrium dialysis samplers

We routinely employ four types of dialysis porewater samplers (DPS). Important design features are summarized below. The type-1 sampler consists of a PVC post ($680 \times 25 \times 14$ mm) with sampling intervals (depth resolution) of 30 mm. The cylindrical dialysis chambers have volumes of 2.47 cm^3 (V_i) and membrane covered total exchange areas ($2A$) of 3.53 cm^2. The type-2 sampler consists of a polypropylene sheet ($1360 \times 160 \times 15$ mm) with sampling intervals of 19 mm. The slot-shaped dialysis chambers have volumes of 18.44 cm^3 (V_i) and a total exchange area of 24.58 cm^2. The type-3 sampler consists of a Plexiglas sheet ($800 \times 120 \times 15$ mm) with sampling intervals of 15 mm and cylindrical dialysis chambers with volumes of 2.65 cm^3 and a total exchange area of 3.53 cm^2. The type-4 sampler consists of a Plexiglas sheet ($800 \times 120 \times 15$ mm) with sampling intervals of 3.4 mm. The cylindrical dialysis chambers have volumes of 2.65 cm^3 and a total exchange area of 0.29 cm^2. The chamber volume is the same as in a type-3 sampler, but the chambers are arranged in a pattern which gives the fine resolution. The chambers of all samplers are covered on both sides with dialysis membranes.

Mass balance for chemical species of interest which diffuse freely through the matrix of the dialysis membrane:

$$dQ_i/dt = 2\mathrm{A} \cdot \mathrm{J} \quad \text{or} \quad V_i \cdot dC_i/dt = 2A \cdot J \tag{1}$$

with

Q_i = mass of species of interest inside the compartment [mole] = $V_i \cdot C_i$

V_i = compartment volume [cm^3]

A = membrane covered exchange area [cm^2] on one side of compartment

h = height of compartment [cm]

C_i = concentration of chemical species of interest inside the compartment [$mole \cdot cm^{-3}$]

t = incubation time [s]

J = Mass flux of chemical species of interest across the membrane surface [$mole \cdot cm^{-2} \cdot s^{-1}$]

Mass flux according to Fick's first law

$$J = -D_M \cdot \Delta C/\Delta l \tag{2}$$

D_M = Diffusion coefficient for species considered in membrane matrix M under a given set of environmental conditions [$cm^2 \cdot s^{-1}$].

Δl = thickness of dialysis membrane [cm]

ΔC = concentration difference across the membrane [$mole \cdot cm^{-3}$] = $C_i - C_0$, with C_0 = concentration in the interstitial spaces of the surrounding sediment. C_0 is assumed to remain constant during the sampling period. Equilibrium is attained when $C_i = C_0$.

For thin membranes we may use the relation

$$D_M \simeq k^M \cdot \Delta l$$

k^M = permeation speed for a particular chemical species across membrane matrix M [$cm \cdot s^{-1}$]

From Eq. (1) and (2) follows for steady state conditions

$$dC_i/dt + 2(A/V_i) \cdot k^M \cdot (C_i - C_0) = 0$$

which can be rearranged to the differential equation

$$dC_i/dt + 2(A/V_i) \cdot k^M \cdot C_i - 2(A/V_i) \cdot k_M \cdot C_0 = 0$$

whose solution becomes

$$C_i/C_0 = 1 - \exp(-2(A/V_i) \cdot k^M \cdot t) \tag{3}$$

for the boundary conditions $C_i = 0$ at $t = 0$ and $C_i \to C_0$ for $t \to \infty$.

For V_i/A = design factor f[cm], Eq. (3) is reduced to:

$$C_i/C_0 = 1 - \exp(-2/f \cdot k^M \cdot t) \tag{4}$$

For the incubation time follows from Eq. (4):

$$t = f/2 \cdot 1/k^M \cdot \ln(C_0/(C_0 - C_i)). \tag{5}$$

REFERENCES

Adams, D. D., G. Matisoff and W. J. Snodgrass, 1982. Flux of reduced constituents (Fe^{2+}, Mn^{2+}, NH_4^+ and CH_4) and sediment oxygen demand in Lake Erie. Hydrobiologia 91/92:405–411.

Bender, M., W. Martin, J. Jess, F. Sayles, L. Ball and C. Lambert, 1987. A whole-core squeezer for interfacial pore-water sampling. Limnol. Oceanogr. 32:1214–1225.

Berner, R. A., 1980. Early diagenesis: a theoretical approach, Princeton University Press, Princeton.

Brandl, H. and K. W. Hanselmann, 1981. An equilibrium diffusion technique for measuring concentration gradients of small soluble molecules applied to in situ determination of anaerobic mineralization processes in lakes. Experientia 37:1225–1226.

Brandl, H., K. W. Hanselmann and R. Bachofen, 1990. In situ stimulation of bacterial sulfate reduction in sulfate limited freshwater lake sediments. FEMS Microbiol. Ecol. 74:21–32.

Carignan, R., 1984. Interstitial water sampling by dialysis: Methodological notes. Limnol. Oceanogr. 29:667–670.

Crank, J., N. R. McFarlane, J. C. Newby, G. D. Paterson and J. B. Pedley, 1981. Diffusion processes in environmental systems, Macmillan Press, New York.

Du Preez, J. C. and P. M. Lategan, 1978. Gas chromatographic analysis of C_2–C_6 fatty acids in aqueous media using Carbopack B – Carbowax 20M – phosphoric acid. J. Chromatogr. 150:259–262.

Emerson, S., 1976. Early diagenesis in anaerobic lake sediments: Chemical equilibria in interstitial waters. Geochim. Cosmochim. Acta 40:925–934.

Gilboa-Garber, N., 1971. Direct spectrophotometric determination of inorganic sulfide in biological materials and other complex mixtures. Anal. Biochem. 43:129–133.

Hertz, J. and U. Baltensperger, 1984. Determination of nitrate and other inorganic anions in salad and vegetables by ion chromatography. Fresenius' Z. Anal. Chem. 318:121–123.

Hesslein, R. H., 1976. An in situ sampler for close interval pore water studies. Limnol. Oceanogr. 21:912–914.

Jahnke, R. A., 1988. A simple, reliable, and inexpensive pore-water sampler. Limnol. Oceanogr. 33:483–487.

Northrop, J. H. and M. L. Anson, 1929. A method for the determination of diffusion constants and the calculation of the radius and weight of the hemoglobin molecule. J. Gen. Physiol. 12:543–554.

Presley, B. J., R. R. Brooks and H. M. Kappel, 1967. A simple squeezer for removal of interstitial water from ocean sediments. J. Mar. Res. 25:355–357.

Rudd, J. W. M., R. D. Hamilton and N. E. R. Campbell, 1974. Measurement of microbial oxidation of methane in lake water. Limnol. Oceanogr. 19:519–524.

Sayles, F. L., T. R. S. Wilson, P. N. Hume and P. C. Mangelsdorf Jr., 1973. In situ sampler for marine sedimentary pore waters: Evidence for potassium depletion and calcium enrichment. Science 181:154–156.

Sholkovitz, E., 1973. Interstitial water chemistry of the Santa Barbara Basin sediments. Geochim. Cosmochim. Acta 37:2034–2073.

Staub, E. A., 1981. Diagenese im rezenten Sediment des Vierwaldstättersees und ihre Veränderung durch die Eutrophierung: Tiefenprofile biologisch-chemischer Parameter im Sediment und Porenwasser. Dissertation 6841, Federal Technical Institute, Switzerland.

Received 30 August 1990;
revised manuscript accepted 28 December 1990.