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SULFUR AND CARBON CYCLING IN A STRATIFYING FRESHWATER LAKE

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SULFUR AND CARBON CYCLING IN A STRATIFYING FRESHWATER LAKE

Proefschrift

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in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
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in het openbaar te verdedigen
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Subject headings: acetate kinetics ; freshwater sediments

/ sulfur cycling ; freshwater sediments.

Front page: A composition of alchemistic symbols.

STELLINGEN

1. Om het verband tussen sulfaatreductie en de oxidatie van lagere vetzuren onbetwistbaar in sedimenten aan te tonen, zou men electronen moeten kunnen labelen.

2. Berekeningen van opnamesnelheden van nutriënten door bacteriën in het sediment met behulp van diagenetische modellen blijken in het algemeen lagere resultaten te geven dan die verkregen uit kinetische experimenten in batches.

   Literatuur: Jørgensen, B. B., (1978), J. Geomicrobiol. 1: 29-47.

3. Accumulatie van lagere vetzuren na toevoeging van molybdaat aan sediment is geen bewijs dat sulfaatreductie een rol speelt bij de consumptie van vetzuren in zoetwater sedimenten.

   Literatuur: Parkes R.J., Gibson G.R., Mueller-Harvey I., Buckingham W.J., and Herbert R.A. (1989) J. of Gen. Microbiol., 135: 175-189.

4. Theoretische modellen die lagere concentraties van electrondonoren in de toplaag van het sediment voorspellen op grond van de grotere energetische efficiëntie van respiratieve processen zijn ongeldig, indien de locale productie van electronendonoren de consumptie overtreft.

   Literatuur: Lovley D.R., and Klug MJ. (1986) Geochim. Cosmochim. Acta 50: 11-18.

5. Bij het bepalen van adsorptie van lagere vetzuren aan sediment door middel van verdelingscoëfficiënten van toegevoegde labels dient men rekening te houden met een oververzadiging van de actieve plaatsen door vetzuren die vrijkomen tijdens de sterilisatie van het sediment.

   Literatuur: Sansone et al. (1987) Geochimica et Cosmochimica Acta 51: 1889-1896

6. In het algemeen zijn polaire verbindingen gemakkelijker te bepalen als men ze derivatiseert; de spore-analyse van carbonzuren is echter een "dramatische" uitzondering op deze regel.

   Literatuur: Grob, R. L. (1977) Modern practice of gas chromatography, page 386, John Wiley & Sons, New York.

7. De allereerste publicatie over gas-vloeistofchromatografie ging over de analyse van vetzuren en de laatste zal waarschijnlijk ook hierover gaan.

Literatuur: James, A.T., and Martin, A.J.P. (1952) Biochem. J. 50: 679-690.

8. Vanwege de alom aanwezige acetaat-verontreiniging in reagentia zou men deze stof als de "quinta essentia" van de organische chemie kunnen beschouwen.

9. Bij de analyse van waterige oplossingen met gaschromatografie is de injector vaak letterlijk de 'bottleneck' van de bepaling.

10. Gezien de hoogte van sommige verkeersdrempels zouden sportfietsen van schokdempers moeten worden voorzien.

11. Men hoeft niet terug te gaan tot de tijd van de *Australopithecinae* om aan te tonen dat de mens waarschijnlijk reeds rechtop kon lopen, voordat hij zich menselijk ging gedragen. Ook tegenwoordig kan men nog levende bewijzen tegenkomen.

12. Het papierverbruik zou wel eens kunnen verminderen, indien men de bediening van fotocopieëer-apparaten zou vereenvoudigen.

13. Een tweesprong van een pad op een paddestoel kan een padvinder in een patstelling doen verzanden.

Stellingen behorende bij het proefschrift *Sulfur and carbon cycling in a stratifying lake*. C.A. Hordijk, Wageningen, 13 oktober 1993.

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 THE LAKE VECHTEN PROJECT

Lake Vechten lies in the central part of the Netherlands in the municipality of Bunnik at 52°04'N and 5°05'E. The lake was formed in 1941 by excavation of sand necessary for building a nearby highway. It has a surface area of 4.7 ha and a maximum depth of 11.9 m. (Fig. 1)

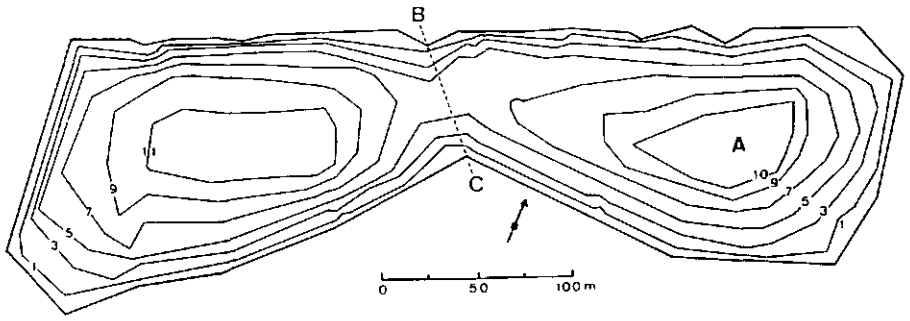

Figure 1: Bathymetric map of Lake Vechten (contours in meters). Point A is the mean sampling area. Broken line BC represents the boundary between eastern and western basin (Steenbergen and Verdouw 1982).

Lake Vechten has been used constantly as a research site of the Limnological Institute since about 1964 (Steenbergen and Verdouw 1982). Excavated and isolated lakes have become common part of the Dutch landscape and some of them are used for fish breeding or for dumping waste. Lake Vechten has a simple hydrology. The lake has no surface inlet or outlet and can be classified as a seepage lake with a water residence time of five years. The deepest basin of the lake has a reduced black sedimentary layer of about 30 cm

thick, which overlies a non-permeable clay layer. The sediment can be classified as Gyttja sediment (Wetzel 1982). Gyttja is a coprogenous sediment containing the remains of all particulate organic matter, inorganic precipitations, and minerogenic matter. In a fresh state, gyttja is very soft and hydrous, usually with a black color. In contrast to dy and peaty sediments, gyttja sediment is never brown. Gyttja sediments are usually found in lakes in which phytoplanktonic productivity dominates above littoral productivity by macrophytes. Data on the chemical constitution of the sediment are given in Chapter 3.3 Table 4; Chapter 3.4 Table 1, and in the Progress report of the Limnological Institute of 1987.

Lake Vechten is a meso-eutrophic, warm, monomictic lake. Thermal stratification starts at the end of April and lasts till the end of November. The stratification period of Lake Vechten is characterized by a gradual and successive depletion of oxygen, nitrate, and sulfate with depth and time in the hypolimnion (Steenbergen and Verdouw 1984). When oxygen and nitrate are depleted by the end of April, Fe(II) and reduced sulfur are released from the sediment into the hypolimnion (Chapter 3.3). Sulfate depletion is completed at the end of August (Chapter 3.2). At the end of November temperatures decrease in the epilimnion and stratification ends. In winter, when the lake water column is completely mixed ("circulation period"), oxygen, nitrate and sulfate diffuse into the surface sediment where they are reduced by respiratory processes (Chapter 3.1). The succession in which these electron acceptors are reduced is related to the net energy yield gained by the bacterial oxidation of organic matter (McCarty 1978). This implies that the sediment can be divided into one horizon in which reduction of oxygen, iron, and nitrate dominates, a sulfate reducing horizon, and a methanogenic horizon. The successive depletion of oxygen, nitrate and sulfate with increasing depth and with season provides an opportunity to study the respiratory activity of the benthic microcosms under conditions when different electron acceptors are limiting.

Anaerobic mineralization

Heterotrophic bacteria may, by various types of fermentation processes, release the potential energy stored in organic material by using organic compounds as electron acceptor while oxidizing other substances. These species produce, in addition to CO_2 and H_2O, reduced compounds such as lactate, alcohols, H_2S, NH_4, etc (Fenchel 1969). Other anaerobic bacteria can use inorganic compounds as electron acceptors for the oxidation of organic material. Thus, SO_4^{2-} may be reduced to H_2S, NO_3^- to N_2, CO_2 to CH_4, and H_2O to H_2. The sequence in which such processes occur depends on the availability of the various hydrogen acceptors and the thermodynamic efficiency of the processes (McCarty 1978).

Mineralization in the near surface sediment of in Lake Vechten sediment is hindered by a lack of electron acceptors able to oxidize organic matter (Chapter 3.1). The supply of oxygen, nitrate, and sulfate is hindered by the slow molecular diffusion from the superficial water layer into the sediment. Therefore these compounds are called "external electron acceptors" (Lovley and Klug 1986). Deeper in the sediment generation of CH_4 from H_2 with CO_2 as electron acceptor can be expected (Fig. 2).

Electron acceptors formed in the sediment, e.g. CO_2 from fermentative processes and sulfate from the hydrolysis of estersulfates (King and Klug 1980), are called "internal electron acceptors (Lovley and Klug 1986). Due to the high concentration of HCO_3^- (about 4 mM: Verdouw and Dekkers 1980) in the sediment makes it unlikely that CO_2 is limiting electron acceptor in Lake Vechten. Within the mineralization processes occurring in the anoxic sediment, sulfate reduction and methanogenesis play a major role.

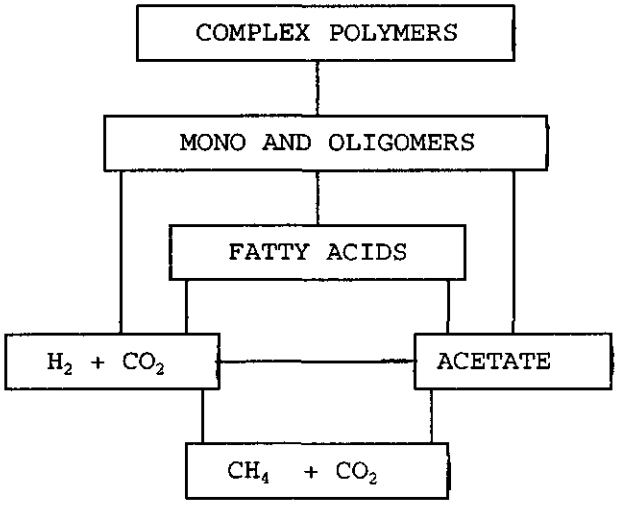

Figure 2. Scheme for the flow of carbon in the complete anaerobic conversion of organic matter of methane (Zinder 1984).

1.2 THE SULFUR CYCLE

Sulfur is a biogenically active element. About 0.1% of the earth's crust and about 1.0% of the biomass consist of sulfur. So sulfur undergoes a tenfold enrichment when being incorporated into biogenic matter. The biogenic sulfur pool originates from two processes; *assimilatory sulfate reduction* and *dissimilatory sulfate reduction*. Assimilatory sulfate reduction can be important in freshwater ecosystems. A shortage of sulfate can limit the bloom of algae and macrophytes and so indirectly affects fish growth (Lake Victoria, Hesse 1958). As a result of continuous deposition of atmospheric sulfur originating from industrial emission, the occurrence of sulfur deficient freshwater biotopes in North-west Europe is unlikely.

Many different sulfur bearing compounds are produced during assimilation by benthic organism (Freney 1961). Most of them, however, are susceptible to decomposition, so then do not accumulate in uncombined form and are not readily detected in a free state. However a few "free" organic sulfur compounds originating from decay products of algae and macrophytes can be found in nature. Examples are osmolytes originating form seaweeds (Visscher 1992), estersulfates (King and Klug 1980), amino acids, thioethers, and mercaptans formed during putrefaction in waste waters (Dunette et al. 1985).

The second source of biogenically reduced sulfur in nature is dissimilatory sulfate reduction by heterotrophic anaerobic bacteria of the genera *Desulfovibrio* and *Desulfotomaculum* (Postgate, 1979). In anoxic condition *Desulfovibrio* reduces sulfate to sulfide with electrons released during the oxidation of molecules like acetate and lactate or hydrogen (Pfennig and Widdel 1982).

$$2\ CH_3CHOHCOO^- + SO_4^{2-} \text{ ---> } 2\ CH_3COO^- + 2\ HCO_3^- + H_2S$$
$$4\ H_2 + SO_4^{2-} + 2\ H^+ \text{ ---> } 4\ H_2O + H_2S$$

The released energy by this process can be used for assimilation. Although sulfate reduction is intense in Lake Vechten (Chapter 3.3, Table 5), concentrations of free sulfide in the sediment were low (about 5 µM; Chapter 2.4). An explanation for the low sulfide concentrations is that the sulfide generated in the sediment quickly reacts to form a variety of organic and inorganic sulfur compounds (Davison and Heanley 1978, Moers et al. 1988; Fig. 3).

In lake Vechten, the ΣH_2S ($H_2S + HS^- + S^{2-}$) produced by dissimilatory sulfate reduction appear to react primarily with iron to form iron sulfides (Chapter 3.2. Fig. 5). The high concentrations of reactive Fe in the sediment (Chapter 3.3: Verdouw and Dekkers 1980) is expected to maintain concentrations of dissolved ΣH_2S concentrations low. About 60% of the sedimentary sulfur pool is acid-volatile sulfide (Chapter 3), and the deep black color is an indication of FeS precipitates. Sulfide can however also react with the

sediment matrix to form organic sulfur compounds (Moers et al. 1988; Fig. 3).

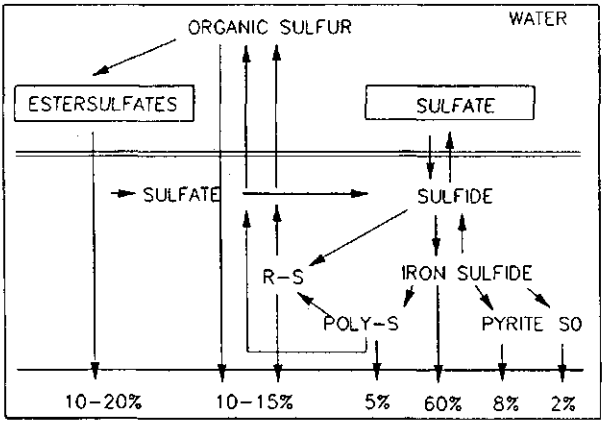

Figure 3. The major pathways with their related S-constituents in the sulfur cycle in the sediment of Lake Vechten (Hordijk unpublished).

In conclusion, sulfur species in the sediment originate either direct or indirect from dissimilatory sulfate reduction or from deposited organic material. In either case there is a direct interaction between the carbon and sulfur cycle.

The interaction between the carbon and sulfur cycle is not limited to dissimilatory sulfate reduction only. Energy stored in reduced sulfur compounds can be released by sulfur oxidizing species of the genera like *Beggiatoa* and *Thiobacilli* that are blooming

at the sediment-water interface shortly after fall overturn in lake Vechten (Sweerts et al. 1990). Blooms of sulfur oxidizing phototrophes (*Chloronema, Chlorobium, and Chromatium*) were also found in the metalimnion during summer stratification (Steenbergen et al. 1987).

There is little insight in the total amount of sulfur species that actually cycles in hypolimnia of freshwaters (Stuiver 1967; Chapter 3.3). The study in Lake Vechten indicated that about 85% of the initially buried sulfur is released again in the superficial water layer (Chapter 3.3). Especially during summer stratification, cycling between sediment and hypolimnion appeared to be intense. Sulfur cycling can affect the sulfur composition of the sediment. Thus care should be taken to identify trends in sedimentary sulfur as a reflection of historical changes in lake water chemistry. The cycling of substantial amounts of sulfur species in the hypolimnion, indicates that these species can play an important role in the energy flow between sediment and the lake water column. Whole-lake modeling indicated that in Lake Vechten about 0.6 to 2.6 Kmol carbon per year is oxidized by sulfate reducers (Steenbergen et al. 1987).

1.3 THE CARBON CYCLE

Fatty acid generation

Detritus is a major input of organic matter to the sediment in productive lakes like Vechten (Chapter 3.3). On annual base about 570 mmol m^{-2} sestonic carbon is deposited in the pelagial horizon of the lake (Chapter 3.3; Table 5). During breakdown, detritus is initially decomposed to macromolecules and sequentially to smaller fragments by various fermentative and respiratory processes (Fig. 2). Finally, short-chain organic acids (SCOA) are formed which can serve as substrates for dissimilatory sulfate reduction and methanogenesis (Sansone and Martens 1982). SCOA can be divided into two types of compounds, namely, 1) volatile fatty acids (VFA; e.g. acetate, propionate, butyrate), and 2) non-volatile fatty acids (NVFA; e.g. lactate, succinate and oxalate; Chapter 2).

Dissimilatory sulfate reduction is frequently the dominant mineralization process in marine sediments (Jφrgensen 1978). This is illustrated by the fact that inhibiting sulfate reduction directly caused accumulation of VFA in such sediments (Parkes 1989). In lake Vechten, inhibition of both sulfate reduction and methanogenesis only resulted in an accumulation of acetate and not of other VFA (data not shown).

Fatty acid consumption

SCOA's are important intermediates in the anoxic breakdown of organic matter with acetate playing a key role (Shaw and McIntosh 1990, Lovley and Klug 1986). The two main processes in the sediment involving consumption of acetate are sulfate reduction and methanogenesis (Lovley and Klug 1986). Acetate is a direct precursor for acetoclastic methanogenesis, and lactate has been noted as a potential important electron donor for sulfate reducers (Cappenberg 1974; Widdel and Pfennig 1977). There is a lack of insight in the actual turnover of lactate or other short chain hydroxy-acids in sediments, mainly due to the analytical difficulties to access *in situ* concentrations. The concentration profiles of lactate and formate with depth in the sediment of Lake Vechten may be the only one published for freshwater systems (Chapter 2.4). More is known about the kinetics in which the SCOA's are consumed (Chapter 3.4). Radioactive tracer experiments revealed that acetate forms a branch point in the anaerobic breakdown of organic material (Shaw and McIntosh 1990). Thus, information about the kinetics of acetate may help to provide quantitative insights in the carbon flow. This study is focused mainly on the kinetics of acetate.

An estimation of the amount of carbon mineralized with acetate as intermediate, requires accurate measurements of acetate concentrations and good insights in the actual kinetics. An evaluation of the importance of acetate as 'key metabolite' in the carbon cycle, requires a comparison with mineralization processes at different seasonal periods. In this study we have developed techniques to study the steep concentration gradients of

acetate and sulfate in the near-surface sediments (Chapter 2). The observed concentration gradients of these 'key metabolites' apparently reflected a balance between diffusion, production, and consumption by dissimilatory processes. Accurate measurement of these profiles has opened a new way for estimating consumption rates by mathematical modeling (Chapter 3). The simultaneously measured profiles of oxygen, nitrate, and sulfate presented here (Chapter 3.1) are among the first ever published for freshwater sediments. These profiles support the hypothesis that the net energy yield of each respiratory process determines the sequence in which external electron acceptors are depleted in the sediment (Chapter 3.1). It is likely that the most energetically favourable substrates are firstly oxidized in an environment limited by electron-acceptors. Thus, substrate competition may occur in the respiring horizon, explaining the accumulation of acetate in this layer (Chapter 3.4).

1.4 METHODS TO STUDY THE SULFUR AND CARBON CYCLE

In Lake Vechten different dominant metabolic processes can be observed in the top two cm of the sediment. To study the breakdown kinetics within each metabolically definable horizon requires sampling and analytical techniques with a high depth resolution. Techniques like chromatography and microelectrodes that have become available in the past decade allow measurement of trace concentrations in small volumes. A major part of this study deals with the adaption of new chromatographical techniques to sedimentary research. Of the shelf methods for a reliable assessment of trace concentrations of ions involved in the anaerobic respiratory processes were not available at the beginning of this study.

Fatty acid analysis

Special attention has been paid to the analysis of short-chain organic acids (SCOA), compounds that are important organic electron donors in the breakdown of organic

material, and to compounds related to the sulfur cycle. SCOA's are usually divided into two groups based on thermal stability in respect to gaschromatographic analysis (Chapter 2.4). SCOA's like acetate, propionate, butyrate, valerate and caproate and their iso-acids can be volatilized, after acidification, without decomposition and are usually called volatile fatty acids (VFA). Substituted SCOA's e.g. lactate, oxalate, succinate, and pyruvate, cannot be volatilized without thermal breakdown in the gaschromatograph. These acids are classified as non-volatile fatty acids (NVFA). Prior to gas chromatographic analysis, NVFA had to be derivatized to improve volatilization (Chapter 2.4). Trace analysis of NVFA derivatives are also complicated by their sensitivity to hydrolysation and the presence of contaminants in reagents and solvents (Chapter 2.4).

Kinetic studies, using radioactive labels, demonstrated the fast turnover rates of NVFA's in marine and freshwaters (Jones and Simon 1984; Shaw and McIntosh 1990). We have developed a method to detect *in situ* lactate and formate pools in freshwater sediments (Chapter 2.4). The kinetics of lactate in Lake Vechten has also been studied previously using radioactive labels (Cappenberg and Jongejan 1978). However, more information is needed to understand the role of NVFA's in sediments than the sited studies can give.

The introduction of water-resistant capillary columns simplified assessment of the VFA's by gaschromatography (Chapter 2.5) and mass-selective detection. Capillary gas chromatography with mass-selective detection is superior to liquid chromatography in respect to specificity and separation power (Chapter 2.4). The gas chromatographic method developed in this study is a fast and relative easy to use technique for a direct assessment of VFA in pore waters.(Chapter 2.5). This has enabled us to do many experiments to unravel VFA kinetics under different physiological conditions. In this study we are dealing mainly with acetate kinetics, a 'key metabolite' in the anaerobic mineralization (Chapter 3.4).

Electron acceptor analysis

The carbon flux across the sediment water interface can be estimated by following the consumption rates of the electron donor acetate (Chapter 3.4), uptake of electron acceptors (oxygen, nitrate, sulfate: Chapter 3.1) and by the production of the end products CO_2 and CH_4 (Chapter 2.4 and Chapter 4). Much attention has been paid in this study to the development of techniques to estimate electron acceptor consumption by batch incubations or by modeling of natural concentration profiles (Chapter 2). Net sulfate reduction rates were also estimated from the total sulfur pool accumulated in the sediment.

1.5 OUTLINE OF THIS THESIS

This thesis is dealing with the final steps of anaerobic breakdown of organic matter in a stratifying lake. Chapter 2 deals with the development of new sampling and detection techniques to study mineralization in the near-surface sediment. Special attention is being paid to the kinetics of sulfate and acetate, which are key metabolites in the anaerobic breakdown of organic matter. Chapter 3 deals with nitrate reduction, sulfate reduction and acetate uptake in the sediment. Chapter 4 includes a discussion on the impact of seasonal effects, sulfur sedimentation, sulfur reduction, sulfur oxidation, sulfur release, and sulfur accumulation rates. Chapter 4 also includes a discussion on acetate uptake rates under different experimental conditions. I have compared sulfate reduction and acetate metabolism by studying seasonal changes in concentrations in the sediment and by batch experiments. To examine the role of sulfate in the oxidation of acetate, specific inhibitors were used. The results of these experiments are included in the final discussion at the end of this thesis (Chapter 4).

CHAPTER 2

METHODS TO DETERMINE RESPIRATORY PROCESSES IN SEDIMENTS

CHAPTER 2.1

Analysis of sulfate at the mud-water interface of a freshwater lake sediments using indirect photometric chromatography

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Analysis of sulfate at the mud-water interface of freshwater lake sediments using indirect photometric chromatography

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Summary

A high pressure liquid chromatographic technique to determine sulfate in fresh-water sediment interfaces is described. Indirect photometric detection was applied, which afforded a detection limit of 300 pmol, using 500 µl sediment samples. The choice of the detector and eluent effects are discussed. The agreement between the results obtained by the method described and those obtained using suppressed conductometric detection was good. Steep sulfate profiles (20–200 µM) were observed in the sulfate reducing layer. The method developed will be applied to quantify sulfate reduction.

Key words: *Indirect photometric chromatography – Mud-water interfaces – Sulfate profiles – System peaks*

Introduction

Studies on the anaerobic mineralization of organic matter in both freshwater and marine environments have stressed the importance of the respiratory sulfate reduction [1–3]. To quantify this reduction using either radioactive sulfate ($^{35}SO_4^{2-}$), batch experiments or mathematical models, accurate measurement of sulfate in situ at the mud-water interface is needed; this layer is most active. Sulfate in lake samples has traditionally been determined using turbidimetric techniques in combination with barium [4]. As these methods require large volume samples, the microlayers within the interface are disturbed so that accurate measurements in this active area are not possible. Recently, several high pressure liquid chromatographic (HPLC) methods to determine anions have been developed and reviewed [5–7]. As sulfate has no UV absorbance, direct UV monitoring is excluded. In the

present study sulfate was detected indirectly by using the decrease in the background absorbance of a strongly UV-absorbing aromatic eluent buffer. This recently developed method for monitoring transparent anions is called indirect photometric chromatography (IPC) [5]. Separation is achieved by competition between a strongly UV-absorbing buffer anion and the solute anions for the ion exchange sites on the column. It follows that elution of a solute anion will be accompanied by a localized deficiency of the UV-absorbing anions in the eluent. The appearance of an anion-component of the solute is then shown by a drop in the base line [5, 6, 8]. The increase of transmission, expressed in the depth of the negative peak, is proportional to the amount of solute injected. This new method gave well defined sulfate profiles in the μM range in the sediments and overlying water of Lake Vechten (The Netherlands).

Materials and Methods

Apparatus

The modular liquid chromatograph consisted of a model 1330 Bio-Rad dual piston pump with a model 1305 Bio-Rad UV detector (band width, 10 nm; zero suppression range, 5.12 absorbance units full scale (AUFS); output filter, 1 s). Samples were introduced using a Rheodyne 7125 valve. The column (15 × 0.46 cm, Chrompack, Middelburg, The Netherlands) was packed with 5 μm Nucleosil SB anion exchange particles. A 7.5 × 0.21 cm guard column (type B for anion exchange chromatography, Chrompack) and a 3 × 0.46 cm pre-saturation column packed with 10 μm Partisil SAX particles (cartridge; Brownlee labs, Santa Clara, USA) was used. The columns were covered with cotton wool for temperature stability.

Chemicals

Trimesic acid (1,3,5-benzene-tricarboxylic acid; Merck AG, Darmstadt, FRG; zur synthese); potassium-hydrogen-phthalic acid (Merck, zur analyse); 2-sulfobenzoic acid, ammonium salt (Merck, zur synthese) and other common chemicals were obtained from Merck and used without purification.

Buffer preparation

Sulfobenzoic acid, ammonium salt (219 mg) was diluted in 500 ml double-distilled water. The pH was adjusted to 5 ± 0.3 after 15 min of stirring. The column was equilibrated for at least 1 h, using this buffer. After analysis the column was stored in a buffer of the same composition at pH 4. For preparing the mixed buffer, 50 ml 1 mM trimesic acid was added to 200 ml 2 mM sulfobenzoic acid solution, the pH of this mixture being adjusted to 5.5.

Sample collection and pretreatment

Undisturbed mud cores were sampled, using a modified Jenkin surface-mud sampler, from the deepest part in the eastern depression of Lake Vechten [9]. Subsamples (minimum 0.5 g) were drawn by piercing the syringe through the 2

mm holes (covered with Scotch tape no 471) in the acrylic glass sampling tube. The samples were twice centrifuged in tubes with short conical bottoms at 1000 × *g*. The supernatant was stored at −20°C until analysis. Before injection, the samples were filtered through a 2 μm frit mounted in a 1 ml syringe.

Quantification

Concentrations were evaluated by external standardization. For calibration, a series of gravimetric standard solutions in the range of 0–250 μM (0–25 ppm) sulfate were made in double distilled water. Calibration curves were made by plotting the peak heights obtained from the chromatograms of the standards against the original standard solutions. The peak heights obtained from the sample chromatograms were compared mathematically with those of the standards for analysis. The molar response factors used were obtained from the calibration curves.

Results and Discussion

In IPC several aromatic buffers with a high extinction coefficient have been described [5]. For the separation of sulfate we preferred sulfobenzoic to phthalic and trimesic for its higher elution strength and solubility at pH 4.5. Also pH did not affect the sulfobenzoic UV spectrum but it did remarkably influence the phthalic UV spectrum (Fig. 1). At low background absorbance (0.4) the sensitivity decreased and at high background levels (1.5) noise occurred. Optimal signal to

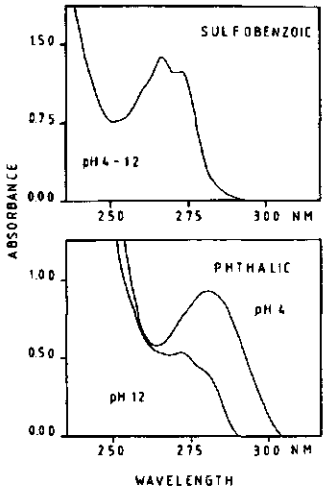

Fig. 1. Absorption spectra of 2 mM sulfobenzoic acid and 1 mM phthalic acid measured on a Pye Unicam SP8–500 spectrophotometer with a 1 nm slit and a 1 cm cuvet.

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noise ratios were obtained when the transmission ranged from 10 to 20% which corresponded with 2.5 mM sulfobenzoic acid on the Bio-Rad detector. To nullify this high background absorbance a large zero suppression range is needed. Electronic zero suppression was preferred to optical dual beam suppression for stability of the baseline. The background can also be reduced by setting the detector to a lower absorbing wavelength. The broad spectra of the applied eluents, however, afforded the use of large band width detectors (10 nm) to obtain sufficient transmission.

The lower background level of these detectors has the advantage that high concentrations of UV absorbing-buffers can be used in order to reduce retention time. The background absorbance can also be reduced by measuring on a slope of an absorption peak, which might be optically less accurate than measuring on the top of the peak.

Eluent effects

With 2 mM sulfobenzoic acid the separation of sulfate and other anions was good (Fig. 2A) but the elution of sulfate was strongly retarded. For sulfate analysis, retention time was reduced by applying a buffer mixture of 0.2 mM trimesic and 1.6 mM sulfobenzoic acid (Fig. 2B). The pH of the buffer mixture was adjusted

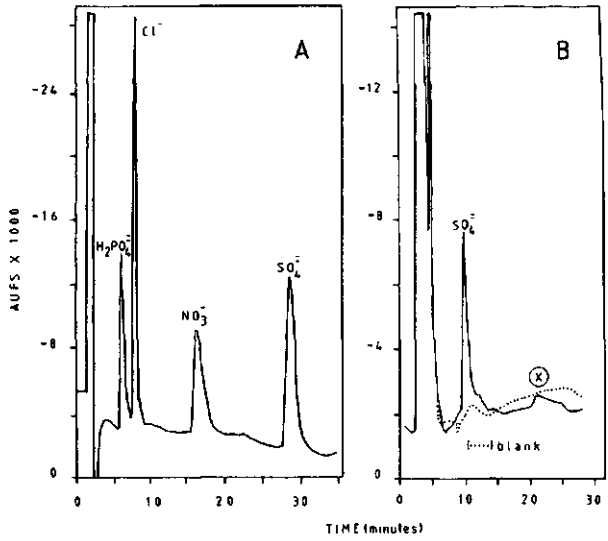

Fig. 2. (A) Liquid chromatogram of the separation of phosphate, chloride, nitrate and sulfate (1 μg of each acid injected) Conditions: buffer composition, 2 mM sulfobenzoic acid (pH 4.7); wavelength, 274 nm; flow, 1.5 ml/min; range, 0.04 absorbance units full scale. (B) Liquid chromatogram of a sulfate standard (100 μM) in distilled water (1 μg in 100 μL) injected; X, system peak. Conditions: buffer composition, 1.6 mM sulfobenzoic acid and 0.2 mM trimesic acid (pH 5.5); wavelength 258 nm; flow, 1.0 ml/min; range, 0.02 absorbance units full scale.

to 5.5 to ionize the carbonic groups of the trimesic acid for a higher elution strength. Retention time can also be reduced by applying shorter columns. However, very short columns (3 cm) easily gave non-reproducible, non-linear results due to pollution of the column head. At pH 4.5 (Fig. 2A) a slow eluting unidentified peak occurs behind the sulfate peak (data not shown) in the moderate saline sediment samples. This peak is called system peak, while its response depends on the chromatographic system used. The peak is strongly reduced at pH 5.2 (Fig. 2B). At pH 5–6 a pre-saturation column is needed to protect the analytical column from slow dissolution of the silica backbone. The column was stored at pH 4.0 to prolong its lifetime.

The chromatograms of pore water samples, which were made organic-free by heating at 450°C, still showed a system peak. By correct timing of the injection, interference of the sample peak by the system peak of a previous injection was avoided.

Linear range and detection limits

The linear range of the chromatograph under conditions of Fig. 2B was limited to 1–100 nmol per injection. Below 1 nmol measurements were irreproducible and at higher concentrations calibration curves were non-linear. An injection of 10 nmol (100 μM in 100 μl) gave a signal change of 3.35 ± 0.20 mV (n = 8). The electronic noise was 0.05 mV which indicated a detection limit of 300 pmol (2 S/N). A small band width detector with a slit of 2.5 nm (Perkin Elmer type LC 75) afforded a detection limit of 600 pmol under similar conditions. For sulfate, the same detection limits as with the Bio-Rad detector were found with a non-suppressed conductometric detector (Perkin Elmer type LC 21). Sulfobenzoic acid is not a suitable buffer for conductometric detection, since it has a high conductivity. It was replaced by phthalic acid in combination with a low capacity column. This low capacity column (Vydac 302 IC, 0.1 meq) is needed to obtain an acceptable retention time with the low concentrations of phthalic acid (2 mM) applied, to reduce conductivity [10]. The column was covered with cotton wool to reduce temperature changes, which affected the conductometric detection more than the photometric one. Conductometric detection in which the conductance of the eluent is reduced by a suppressor column [11] afforded a lower detection limit (30 pmol) but required special equipment. The detection limits obtained by indirect refractive index detection [12] are the same as for IPC (300 pmol) but IPC is less sensitive to the formation of system peaks.

Recovery

For determining the recovery of the sulfate fraction from the sediments, two gravimetric standard series of sulfate were made, one in distilled water and one by standard addition to the sediment (Fig. 3). The sediment samples were pre-treated as under Materials and Methods. After pre-treatment, calibration curves of each series were made and the slopes were compared. The two curves were parallel and well correlated, indicating a recovery of 92%. The curves also indicate that no sulfate is formed from FeS oxidation during sample pre-treatment. Standards

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stored for 3 months at room temperature did not deteriorate. The variation in the slope of the calibration curves made with these standards in about 2 weeks was within 14% (n = 4).

Application

Sulfate concentration profiles were measured in the upper cm of the sediment of Lake Vechten by the method described above as well as by suppressed conductometric ion chromatography (Fig. 4). The similarity of the profiles confirmed the reliability of both methods for lake sediment samples. The steep gradient profile in the upper 2 cm of the sediment indicates that the active sulfate-reducing zone occurs in the same layer of the maximum abundance of sulfate-reducing bacteria, as found earlier [9]. The developed IPC method for sediment samples will be used for quantifying bacterial sulfate reduction by measurements with radio-tracer techniques and calculations from mathematical models. The seasonal role of sulfate reduction as a terminal process in anaerobic carbon mineralization in freshwater sediments will be compared with the turnover of various important electron donors in this respect, measured by a recently described high pressure liquid chromatographic fluorescence method [13].

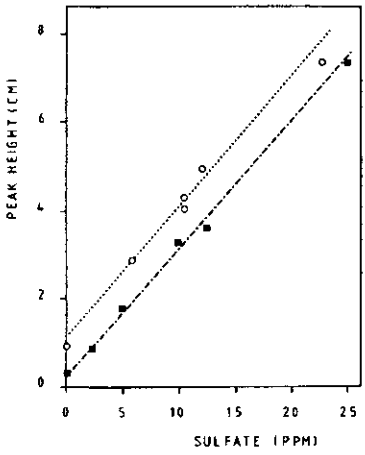

Fig. 3. Sulfate recovery during pre-treatment. (---) Untreated standard series (0–25 ppm) in distilled water (r, 0.9979; b, 0.288). (.....) Standard series (0–25 ppm) made in sediment mixture of the first 5 cm (r, 0.9918; b, 0.266) Conditions: buffer composition, 2 mM sulfobenzoic acid (pH 5.0); wavelength, 258 nm; flow 1.0 ml/min; loop, 100 µl; range, 0.01 absorbance units full scale.

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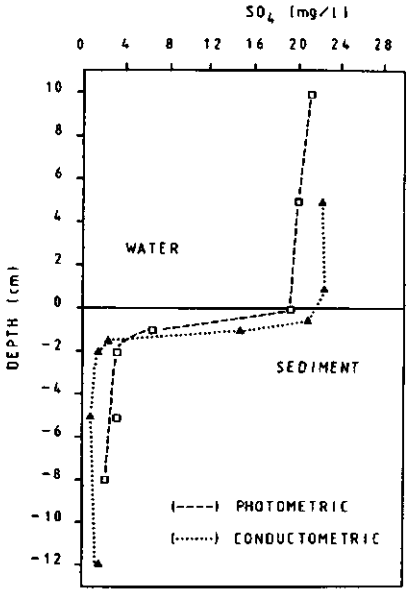

Fig. 4. Sulfate gradient profiles at the sediment-water interface of Lake Vechten, measured by indirect photometric- and suppressed conductometric detection. Samples taken on 9 February 1983.

Acknowledgements

We wish to thank J. Slanina and M.J. van Os (Netherlands Energy Research Foundation (ECN), Petten, The Netherlands) for the conductometric measurements in Fig. 4 and P.R. Haddad (University of New South Wales, Australia) for his interest in our work.

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CHAPTER 2.2

Sulfate analysis in pore water by radio-ion chromatography employing 5-sulfoisophthalic acid as a novel eluent.

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Sulfate analysis in pore water by radio-ion chromatography employing 5-sulfoisophthalic acid as a novel eluent

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Summary

The chromatographic properties of a new eluent, 5-sulfoisophthalic acid, in indirect photometric chromatography is described. Detection limits below 50 pmol were obtained for sulfate pore-water analysis of freshwater sediments. The small injection volumes (20 μl) reduced column head contamination and the pH 4 of the buffer improves column stability without system-peak formation. Bacterial sulfate reducing activities in freshwater sediments could well be followed by high-pressure liquid chromatography with indirect photometric detection in conjunction with off-line scintillation counting.

Key words: *Indirect photometric chromatography - Sulfate reduction - System peaks*

Introduction

Unlike marine systems [1], sulfate respiration in freshwater sediments is limited by diffusion of sulfate into the sediment rather than by electron donor limitation [2]. For this reason bacterial sulfate conversion processes in freshwater sediments are focussed in a narrow horizon just below the sediment-water interface. Steep sulfate concentration gradients develop in this layer as the result of an equilibrium between sulfate diffusion and respiration processes [3]. To study the sulfate conversion processes along this steep gradient, the analytical method employed needs to be accurate and sensitive. High sensitivity is needed to reduce the sample size to a minimum. Only small samples (0.5 g wet weight sediment) can prevent distortion of the steep sulfate profile during sampling. Ion chromatography is a sensitive technique which enables handling of small samples under anaerobic conditions.

Until recently, ion chromatography was generally performed with conductometric detection [4–7], while photometric detection was restricted to those anions which ab-

sorbed sufficiently in the low UV region [8–11]. For example a very low detection limit (8 pmol) was obtained for nitrate with detection at 190 nm [11], but even at this wavelength the UV absorption of sulfate is inconvenient for trace analysis (detection limit 30 nmol). Sensitive photometric measurements of the transparent sulfate anion can be achieved indirectly by interaction with a component added to the eluent.

One way to visualize sulfate indirectly is by the addition of a UV-absorbing counter ion. This technique is called reverse phase ion-pair chromatography [12–14] and has detection limits up to 100 pmol [14]. The occurrence of slow eluting vacancy peaks induced by the injection complicate sulfate analysis in this technique. These vacancy peaks are called system peaks [12] as their appearance depends on the system used and their origin remains unknown. These slow eluting system peaks easily interfered with sequential sulfate injections and also have been reported with non-suppressed conductometric detection [8] and refractive index detection [15].

A second, indirect photometric method for visualizing transparent anions is called Indirect Photometric Chromatography (IPC) [16–21]. Separation is achieved by the competition between the strongly UV-absorbing anion and the solute anions for the ion exchange sites on the column. It follows that UV elution of sulfate will be accompanied by a localized deficiency of the UV-absorbing anions in the eluent. The presence of the anion in the solute is shown by a drop in the baseline, which is proportional to the amount injected.

Phthalic acid was the first eluent used in IPC [14, 15]. It is mostly used with low capacity columns (<0.1 meq g^{-1}) to reduce retention time of the strongly retarded sulfate peak [17, 18]. Despite the low capacities of these columns, relative large concentrations of phthalic acid (up to 5 mM) must be used to elute sulfate within 10 min and the eluent strength of phthalic acid is strongly pH dependent [5]. High buffer concentration leads to a highly noisy background absorption (1.5 absorbance units full scale; AUFS). The wavelength must be increased to a less absorbing region to stay within the zero suppression range of the detector [17]. System peak formation, the remarkable pH influence on the phthalic acid spectrum [3], and eluent strength [5] due to the dissociation of the second carboxylic group (pK, 4.6–5.5) complicate the application of this buffer for sulfate analysis.

Sulfonic acid anions are permanently dissociated in the pH range at which the silica backbone of the column is stable (up to 6), resulting in better dissolution properties and a less pH dependable absorption spectrum and eluent strength. Replacing phthalic acid by sulfobenzoic acid [3] decreased the retention time of SO_4. Trimesic acid was added to the eluent as accelerator. However, the relative high pH (5.5) of the mixed buffer tends to shorten column life, especially with large ($>100\ \mu l$) soft alkaline pore-water injections. This high pH is essential to activate the accelerator. The substitution of sulfobenzoic acid by the stronger 5-sulfoisophthalic acid (SIPA) buffer makes accelerating additives superfluous. Column life is improved because the pH of the buffer can be reduced to pH 4 without system peak induction. A second advantage with SIPA as an eluent is that the detection limit is reduced from 300 to below 50 pmol. This permits smaller sample sizes (0.1 ml sediment) and thus less pollution of the column head. The buffer also proved to be very suitable in $^{35}SO_4^{2-}$ analysis.

Material and Methods

Apparatus

The liquid chromatograph consisted of a model 1330 Bio-Rad pump with a model 1305 Bio-Rad UV detector. Samples were introduced by a Rheodyne 7125 valve with a 20 μl loop, which was replaced by a 200 μl loop for the radioactivity measurements. The column (75×4.6 mm: HPLC Technology, Cheshire, England) was packed with 5 μm Nucleosil SB anion exchange particles (ion exchange on porous silica basis; 1 meq g^{-1}). A 75×2.1 mm guard column (type B for anion exchange chromatography, Chrompack, Middelburg, The Netherlands) was used. The columns were insulated with cotton wool for temperature stability.

Chemicals

Trimestic acid (1,3,5-benzene-tricarboxylic acid), potassium-hydrogen-phthalic acid, 2-sulfobenzoic acid-ammonium salt were obtained from Merck AG (Darmstadt, West Germany). Ortho-benzene disulfonic dipotassium salt hydrate and 5-sulfoisophthalic acid monosodium salt were obtained from Aldrich (Beerse, Belgium). Buffers were diluted in double distilled water or de-ionized water filtered through a Milli-Q water purification system (Millipore Corp.) and pH adjusted after 15 min of stirring.

Calibration

Sulfate concentrations were evaluated by external standardization [2]. For calibration, a series of mixtures containing gravimetric $^{32}SO_4^{2-}$ standard solutions (0–250 μM; 0–25 ppm) and calculated $^{35}SO_4^{2-}$ amounts (5000–25000 dpm ml^{-1}) were used. $^{32}SO_4^{2-}$ calibration curves were made by plotting the peak heights obtained from the chromatograms of the UV detector against the sulfate concentration of the original standard solutions. $^{35}SO_4^{2-}$ calibration curves were made by collecting 6 ml fractions, which included the peak volume. After eluting from the chromatograph in scintillation vials, 11 ml Instagel II (Packard) was added. The radioactivity in the vials was counted for 20 min at a window breadth of 4–167 Kev in a Packard Tri Carb 4530 liquid scintillation spectrometer. Quench curves were made according to the prescription of the manufacturer of the instrument. Chloroform (0–0.25 ml) was added as quencher to a series of scintillation vials containing 5 ml SIPA buffer (0.5 mM; pH 4); 1 ml $^{35}SO_4^{2-}$ standard (8.5×10^4 dpm ml^{-1}), and 9 ml Instagel II.

Sample collection

Sample collection was done with the exclusion of oxygen until the reduced sulfur fraction was separated from the pore-water by centrifugation. This prevented any error in sulfate analysis by oxidation of sulfur to sulfate. Undisturbed sediment cores were collected from the deepest part of the eastern depression of Lake Vechten [2] with a modified Jenkin surface mud sampler. During the non-stratified period the upper water layer was removed to prevent mixing of the sulfate rich and oxygen-containing water-layer with the sulfate-poor interstitial water of the sediment. Subsamples (0.2–1.5 g wet weight, depending on the amount of pore water released) were horizontally drawn by piercing the syringe through the 2 mm holes in the acrylic glass sampling tube covered

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with tape (Scotch no 471). The sediment samples were immediately centrifuged at 1000×g at room temperature for 5–10 min in capped 0.4 ml or 1.5 ml polypropylene centrifuge tubes with short conical bottoms (Tamson, Zoetermeer, The Netherlands).

The supernatant was separated from the sediment immediately after centrifugation and stored at −20°C until analysis. The SO_4^{2-} concentration in the frozen samples remained unchanged for at least one month. A second centrifugation of the samples before analysis removed excess of organic material by coprecipitation with the formed Fe(III) colloids. Twenty μl of this supernatant was injected into the liquid chromatograph for sulfate analysis. Details on the recovery of the sulfate fraction from the sediment are given elsewhere [2].

Results and Discussion

The chromatographic properties of three sulfonic acid buffers are given in Table I. The shortened retention time showed that SIPA is a much stronger eluent than ortho-benzenedisulfonic- and sulfobenzoic acid. The principle of stoichiometric exchange applies and, therefore, when sulfate desorbs from the column a SIPA anion absorbs to replace it. Hence the sensitivity of the detector system is directly proportional to the extinction coefficient of the absorbing compounds present in the mobile phase [16, 21]. Thus, the peak height of the sulfate peak is determined by the molar response factor of the buffer while sulfate itself has no UV absorption. The higher eluent strength of the SIPA buffer allows lower buffer concentration. This permits us to work at a lower, more sensitive wavelength yet remain below the 1.5 AUFS level, the point at which excessive detector noise occurs. At buffer concentrations below 0.2 mM baseline instability occurs with 20 μl pore-water injections. Sample anions become attached to active sites for longer periods without competition from the SIPA anions. Above 1.0 mM SIPA no separation was achieved between sulfate, nitrate and the injection peaks. Good separation and optimum sensitivity for sulfate was found with 0.4 mM SIPA at pH 4.5 for 20 μl injections (Figs. 1 and 2).

The detection limit varies between 20 and 50 pmol (twice the signal-to-noise ratio)

TABLE I

CHROMATOGRAPHIC PROPERTIES OF SOME ACID ELUENTS

Peak heights recalculated to a 50 μl 25 ppm injection. Transmission, 10 ± 1%; t_r, relative retention time at a flow of 1.2 ml min^{-1}. Abbreviations: SBA, sulfobenzoic acid; OBDSA, ortho-benzene disulfonic acid; SIPA, 5-sulfo-isophthalic acid.

Eluent	Concentration (mM)	pH	Peak height (AUFS × 100)	t_r (min)	Wavelength (nm)
SBA	2.25	5.5	0.61	16	269
OBDSA	1.5	5.5	0.36	15	271
	1.7	5.3	0.39	10	271
SIPA	0.5	4.5	3.10	3	240
	1.0	5.3	4.15	2	248

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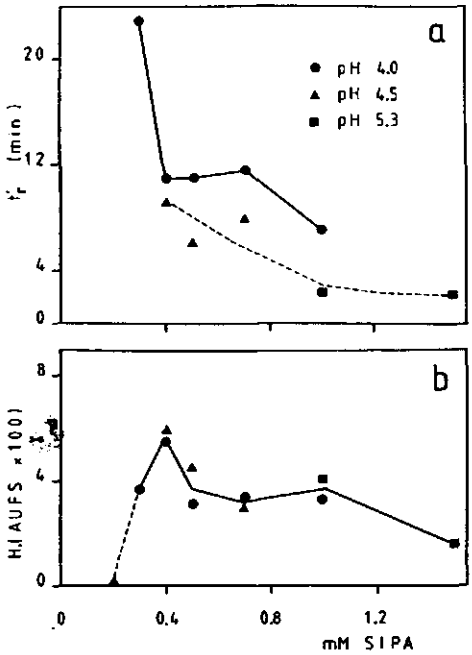

Fig. 1. Optimalization of the IPC detection of sulfate with SIPA buffer as eluent. (a) Retention times (t_r') are measured from the start of the first injection peak (Fig. 2). (b) Peak heights (H) were recalculated to 50 µl (25 ppm) injection at a range of 0.02 AUFS and an eluent flow of 0.8 ml min^{-1}. Wavelength was adjusted for each eluent concentration from 237 nm (0.3 mM SIPA) to 245 nm (1.5 mM SIPA), to obtain a 10% transmission with maximum gain.

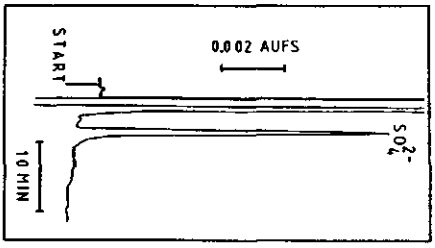

Fig. 2. Sulfate chromatogram of a sedimental pore water sample (14.4 ppm). Conditions: range, 0.02 AUFS; loop, 20 µl; eluent, 0.4 mM SIPA (pH 4.5); flow rate, 0.8 ml min^{-1}; wavelength, 240 nm.

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for a 20–200 μl loop; 120 pmol for a 300 μl loop; and 200 pmol for a 500 μl loop. With the 20 μl loop a molar response factor of 0.966 AUFS/μmol for nitrate (t_r 3.25 min) and 1.48 AUFS/μmol for thiosulfate (t_r 15.2 min) was found. For sulfate analysis of the sediment samples below 0.2 g wet weight, the 20 μl loop was used and calibration curves were linear (r, 0.998) from 0.3 to 25 ppm.

Oxidation of the reduced sulfur during sample pretreatment was negligible. Free dissolved sulfide pools (10^{-9} mol/l) [2] are small in relation to the sulfate pools (10^{-6} mol/l) [3] in the freshwater sediments of Lake Vechten. The low sulfide concentration is a result of the large excess of free $FeCO_3$ ($\alpha Fe = 3.6$ mmol/l) [22] in Lake Vechten which immediately precipitates any free sulfide complexes [23]. These complexes were quantitatively precipitated during the centrifugation step. Artifact formation by chemical oxidation of reduced sulfur to sulfate was for this reason easily excluded by storing the pore-water fraction separately. In sediments with a significant free sulfide pool precautions (i.e., the addition of metal salts) are advisable. The stability of metal sulfide complexes (i.e., FeS, FeS_2, ZnS) is controlled by their low solubility ($pK > 18$) rather than by the presence or absence of oxygen [24]. Cheng and Morris [25] investigated the initial velocities of sulfide oxidation in freshwater and found an induction period of 0.2–6 h and a half-life of 50 h. Under strongly forced aerobic conditions only 2–5 ppm SO_4 per h was released from Lake Vechten sediment.

A peak which coelutes with thiosulfate resulting from this oxidation indicates the formation of stable intermediates from reduced sulfur species. Thus, as already noted by other authors [26], the oxidation of sulfide to sulfate is probably a complex process. The excess of free dissolved Fe(II) CO_3 in the sediment had a second important advantage. By freezing the separated pore-water, humic acids and salts are concentrated. When the sample is melted Fe(III) colloids are formed which precipitate most of the organic compounds [27].

These colloids, which included the humic acids, could easily be separated from the pore-water by centrifugation. The humic acids had to be removed since they tend to pollute the column head by irreversible absorption which results in a reduced column life. Sample filtration through a 2 μm frit [3] is superfluous. This allows the sample volume to be reduced to just the amount needed to flush the sample injection loop.

IPC forms a good combination with on-line and off-line scintillation detection of radioactive solutes. On-line scintillation detection was done by a Ramona IM 20220 (Isomeas, West Germany) radioactivity detector with a flow cell tightly packed with solid scintillator particles [28–31]. The radiation-induced fluorescence pulses were detected by two photomultiplier tubes and were translated to dpm by an Apple 2^c data processor. With a flow rate of 0.8 ml/min (flowcell 400 μl) and a transit time of 0.5 min, a detection limit below 500 dpm per injection (200 μl) (peak height equal to double the signal-to-noise ratio) was obtained. All the radioactivity injected was recovered in the sulfate peak. Alternatively, this peak could easily be collected by hand in a scintillation vial for off-line scintillation counting. The off-line scintillation counting was more accurate (standard error within 1%) because the counting time was not limited. A ten-fold improvement of the detection limit over the on-line detector was achieved with 20 min counting time. The accuracy of the off-line detection enables one to measure small decreases of the added radioactive sulfate during incubation. Quench curves of the elu-

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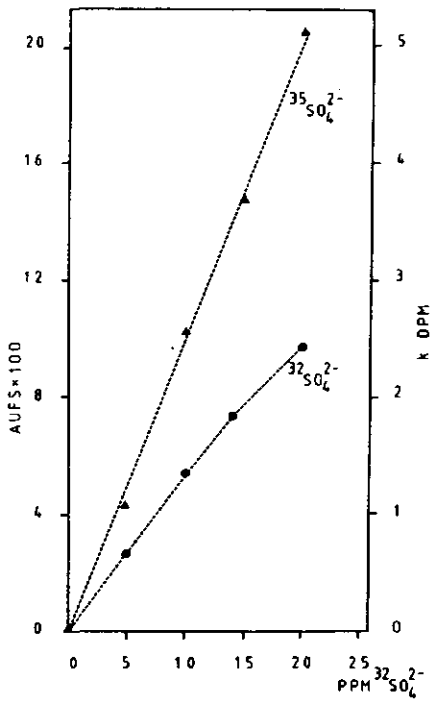

Fig. 3. Calibration curves of mixed standards of $^{32}SO_4^{2-}$ (5000–25000 dpm ml^{-1}; r, 0.999) and $^{32}SO_4^{2-}$ (5–20 ppm; r, 0.998) Conditions: range, 0.16 AUFS; loop, 200 μl; wavelength, 239 nm; flow rate, 0.8 ml min^{-1}; eluent, 0.5 mM SIPA.

ent (0.4 mM SIPA, pH 4.5) showed a counting efficiency of >95% in contrast to 60% or less for the unpurified pore-water. The recovery of $^{35}SO_4^{2-}$ from the chromatograph was 100% and the calibration curves were linear (Fig. 3). Optical detection of $^{32}SO_4^{2-}$ became non-linear above 15 ppm.

The combination of $^{32}SO_4^{2-}$ measurements by IPC and off-line $^{35}SO_4^{2-}$ scintillation detection facilitates the study of sulfate conversion processes. The depletion of $^{35}SO_4^{2-}$ (Fig. 4A) followed the production of $^{35}S^{2-}$ in small sediment batches taken from the Jenkin core. The $^{35}S^{2-}$ in Fig. 4 represents the acid volatile sulfide pool measured by a modified version of the distillation method of Jørgensen [32] and is described in detail elsewhere [2]. The total radioactivity in the batches, calculated as the sum of the $^{35}SO_4^{2-}$ and $^{35}S^{2-}$ pools, remained constant in this experiment. The calculated amount of $^{35}SO_4^{2-}$ added at t_0 and the measured $^{35}SO_4^{2-}$ pool at t_0 were the same. These results indicate that 100% of the added $^{35}SO_4^{2-}$ pool was recovered from the sediment. The results were confirmed with molybdate inhibited batches. No significant $^{35}SO_4^{2-}$ absorption occurs in

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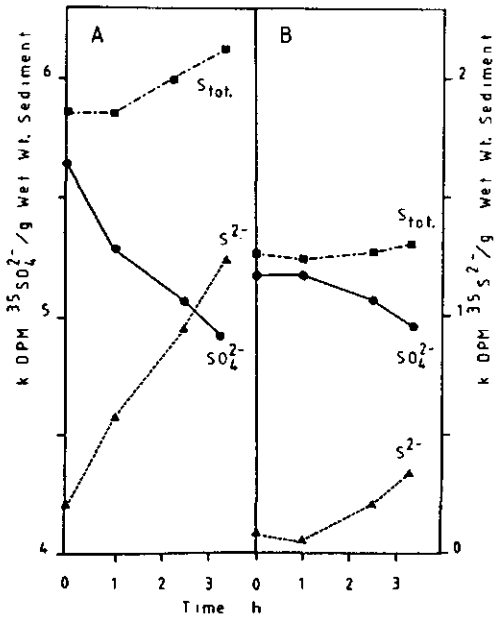

Fig. 4. Bacterial $^{35}SO_4^{2-}$ reduction (●——●) and $^{35}S^{2-}$ production (▲- - -▲) in anaerobic incubated batches taken at a sediment depth of -2 cm (A) and -5 cm (B), sampled in Lake Vechten (The Netherlands). Note that the ^{35}S label balance (■ - · - ■) recovered in the inactive -5 cm sample remains constant.

the clay and organically rich freshwater sediments like Lake Vechten. This lack of absorption greatly simplifies sulfate reduction estimations based on modelling or measuring actual turnover rates. A significant difference in sulfate-reducing activity between -2 cm (Fig. 4A) and -5 cm (Fig. 4B) was observed. The higher sulfate-reducing activity occurred in the same horizon as the maximum abundance of sulfate-reducing bacteria and was directly correlated to the turnover rates of various important electron donors [33, 34].

Estimations of sedimentary bacterial sulfate reduction rates based on direct measurement of sulfate depletion by HPLC has advantages over the distillation method [2, 32], where $^{35}S^{2-}$ produced as a product of sulfate reduction is released by acidification of the sediment. The H_2S gas is distilled into a ZnAc trap and counted. An important disadvantage of this distillation method is that not all the reduced sulfur is recovered. A part of the reduced sulfur could be incorporated by a sequential reaction into non-acid-volatile products like FeS_2 [35, 36]. Not measuring these products could easily lead to a two-fold underestimation of the sulfate-reducing activity. Long-term incubations of Lake Vechten sediment indicate the formation of these products [2].

Measurement of the non-acid-volatile pool is laborious and anaerobiosis should be

maintained during the whole procedure [35]. The method of quantifying sulfate reduction detailed in this paper is direct, simple and sufficiently sensitive for application to low sulfate environments.

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CHAPTER 2.3

Determination of total sulfur in freshwater sediments by ion chromatography.

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DETERMINATION OF TOTAL SULFUR IN FRESHWATER SEDIMENTS BY ION CHROMATOGRAPHY

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Abstract—A method to determine total sulfur in freshwater sediments has been developed. This method involves oxidation of the sediment by a half fusion reaction with a Na_2CO_3/KNO_3 mixture, purification with cation exchange chromatography and detection of the released sulfate by indirect photometric chromatography. Total sulfur estimates from the sediment of Lake Vechten were similar to those obtained by induced coupled plasma spectrometry ($r = 0.998$, $n = 35$) and roentgen-fluorescence spectrometry. The method gives reliable results with difficult-to-oxidize R–S–R bonds such as those present in methionine. The detection of sulfate by the sensitive technique of ion-chromatography (detection limit 5 pmol) enables sulfur analysis in μg quantities. The method can be combined with trace determination of radioactive ^{35}S. Total sulfur in the 47-year old sediment of Lake Vechten varied between 1100 and 1600 mg l^{-1} wet sediment, representing approx. 0.4% of the dry weight. The total sulfur pool is comprised of 3% elemental sulfur, 8% pyrite, 60% acid volatile sulfide and 12% ester sulfates, leaving 17% non-identified. From a comparison of total sulfur estimates in the 28 cm thick sedimented layer (125 g S m^{-2}), sulfate reduction rates (11.4 g S m^{-2} $year^{-1}$) and sedimentation rates (3.21 g S m^{-2} day^{-1}), it appears that most of the sulfur reduced during the year is re-oxidized.

Key words—total sulfur, ion chromatography, freshwater sediments, sulfur accumulation

INTRODUCTION

The dynamic role of sediments in controlling the sulfur cycle of freshwater ecosystems has been stressed in recent publications (Hordijk *et al.*, 1985; Mitchell *et al.*, 1984; Nriagu and Soon, 1985; Rudd *et al.*, 1986). When oxygen and nitrate are depleted in the sediments sulfate becomes an important inorganic electron acceptor in the degradation of organic material (Hordijk *et al.*, 1987). The formed hydrogen sulfide interacts with photosynthesis, algal respiration, iron cycle, and is a regulating factor of electron and proton activities in the hypolimnion (Dunette *et al.*, 1985; Stauffer, 1987). Reduced sulfur, originating from sulfate reduction, can accumulate in a large variety of forms in freshwater sediments. In the sediment of Lake Vechten, sulfide formed during dissimilatory sulfate reduction primarily reacts with free Fe^{2+} to form FeS which is acid volatile (Hordijk *et al.*, 1985). In previous studies (Hordijk and Cappenberg, 1985) we observed that during incubation of the sediment with $^{35}SO_4^{2-}$ a part of the freshly formed radioactive acid volatile sulfide (AVS) was converted to a less reactive compound. Besides FeS, other end-products of the sulfur cycle observed in freshwater lakes are pyrite (Psenner, 1983), elemental sulfur (Heim *et al.*, 1984) and organic sulfur (Mitchell *et al.*, 1984; Mopper and Taylor, 1986; Nriagu and Soon, 1985). Organic sulfur in limnetic sediments can originate from a reaction of sulfide with the organic-rich matrix (Mopper and Taylor, 1986), but also by the input of detritus (King and Klug, 1980; Nriagu and Soon, 1985). For instance, macrophytes contain a high amount of ester-sulfates (King and Klug, 1980). A reliable total sulfur (S_t) method is essential to unravel the complex sulfur pool present in freshwater systems, as well as to estimate the net sulfur deposition rate. Significant amounts of ester-sulfates, FeS, FeS_2 and S° were found in Lake Vechten sediment. These compounds were examined by the total sulfur procedure to test its applicability to a complex freshwater system.

Low detection limits are obtained with anion chromatography and photometric detection (Hordijk *et al.*, 1987). This facilitates the study of nitrate and sulfate kinetics, in sulfate-poor freshwater sediments (Hordijk *et al.*, 1985). Ion chromatography is suitable for use in $^{35}SO_4^{2-}$ tracer experiments (Hordijk *et al.*, 1985) as well as for analyzing relevant intermediates such as $S_2O_3^{2-}$ or $S_4O_6^{2-}$ (Hordijk *et al.*, 1985; Moses *et al.*, 1984). Busman *et al.* (1983) used ion chromatography to estimate total sulfur. This method, together with the Johnson Nishita distillation (Tabatabai and Bremner, 1970) are often applied to the study of organic rich sediments with low sulfur contents (<1%). We combined dry combustion (Grant and Yeung, 1971) with indirect photometric

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chromatography (I.P.C.), to produce an effective procedure which requires no special glassware. Sulfate detection by I.P.C. can be automated and conveniently replaces traditional turbidimetric procedures, which were never entirely satisfactory. It can be applied with success to sediments with low sulfur (<1%) and high carbon contents (>8%). Values for total sulfur agree with those measured by the independent techniques of Induced Coupled Spectrometry and roentgen fluorescence spectrometry (Oenema, 1988).

METHODS

Apparatus

Sulfate and thiosulfate concentrations were measured by I.P.C. (Hordijk *et al.*, 1985). The high performance liquid chromatograph (HPLC) consisted of a model 1330 Bio-Rad pump with a model 1305 Bio-Rad u.v. detector. Samples were introduced by a Rheodyne 7125 valve with a 10 μl loop. The column was a Chromsep system (Chrompack, Middelburg, The Netherlands) which consisted of a metal holder, a 10 × 3 mm pre-column and a glass cartridge (100 × 3 mm). The cartridge was filled with 5 μM IonoSpher A (Chrompack; anion exchange particles on porous silica basis, 1 mequiv g^{-1}). The eluent was made by dissolving 0.4 mM 5-sulfoisophthalic acid (Aldrich, Beerse, Belgium) in de-ionized water filtered through a Milli-Q purification system (Millipore Corp.). This buffer was carefully adjusted to pH 4.7 with NaOH and stored in the dark to prevent algal growth. Initial column priming requires about 1 day of eluent flushing. Sulfate concentrations were evaluated by external standardization. The calibration curves were constructed by plotting the area of the sulfate peaks obtained, using an IBM computer in combination with a CI-10 integrator (LDC; Milton Roy, Fla), against the concentrations of the original standard solutions.

Sample manipulations

Sampling was done in the eastern basin of Lake Vechten, an isolated meso-eutrophic, monomictic lake (4.7 ha). The eastern basin has a maximum depth of 10.5 ± 0.35 m and a strongly reduced (< −200 mV) sediment which overlies a non-permeable clay layer. The sediment pore water has a pH of 7.7; and a concentration of 4.3 mequiv l^{-1} HCO_3^-. Relevant aspects of the limnology of Lake Vechten are described in Verdouw *et al.* (1985).

Surficial sediment was collected in the eastern basin by a modified Jenkin sampler (Cappenberg, 1974). Cores which included the sediment–clay interface were sampled by scuba diving. Subsamples (1–5 g) were withdrawn by syringe or by slicing the sediment core at depth-intervals ranging from 1 to 2 cm. The sediment samples were freeze-dried or dried at 70°C overnight. A pre-oxidation involving the addition of 0.5 ml H_2O_2 (30% v/v, p.a. quality, Merck Darmstadt, F.R.G.) is recommended for strongly reduced and organic rich sediments. Pre-oxidation prevents liberation of gaseous sulfur compounds during the drying process, and reduces the amount of nitrate needed for the final oxidation. After drying, dry weight was determined and samples were ground and sieved through 85 μm. For total sulfur analysis, 100 mg of ground dry sediment was transferred into 20 ml glass scintillation vials (Packard, Brussels, Belgium) and covered with 1.0 g of a Na_2CO_3/KNO_3 mixture (10:1). For a reagent blank, the mixture alone was used. It is essential to sieve both the sediment and the reagents to obtain a good recovery with the half-fusion. The Na_2CO_3/KNO_3 mixture layer must be dry and completely cover the sediment. It was essential to use high quality Na_2CO_3 to obtain low blanks [blank of Suprapur quality (Merck) was 5.6 mg S/kg Na_2CO_3; blank of p.a. quality (Merck) was 35 mg S/kg Na_2CO_3].

The samples were oxidized in a muffle furnace for 4 h at 620°C. After oxidation, 10 ml of Milli-Q water was added to the pellet and the freshly formed sulfate extracted by sonification (30 min). The vials were centrifuged (10 min, 100 *g*). An aliquot of 1.0 ml from the supernatant was eluted through a cation-exchange column which was made of a disposable syringe (B-D, Dublin, Ireland) with a glass frit.

The column was filled with 2 ml Dowex 50-X8 cation exchange resin (100–200 mesh; Fluka AG, Buchs, Switzerland). Before packing the columns, this resin was regenerated by washing 3 times with 1 M HCl, and with Milli-Q water 4 times. To flush the dead volume, two volumes of 5 ml Milli-Q water were pushed through the syringe. An aliquot of 10 μl of the eluate was injected into the ion chromatograph for sulfate analysis.

For comparison, total sulfur was estimated by I.C.P. (Oenema, 1988). Sediment was taken from the Jenkin core, treated with peroxide and freeze-dried. This sediment sample (250 mg) was then oxidized in a mixture of perchloric acid (40%) and nitric acid (25%) at 170°C in scintillation vials. The mixture was re-dissolved in 50 ml of HCl (10%) before injecting it into the plasma spectrometer.

To investigate the release of sulfate by wet digestion and half fusion, sediment samples and small amounts of some relevant sulfur compounds (Table 1) were used. The half fusion procedure was also tested for pyrite. The molar S/Fe ratio of the crystalline pyrite standard was 2.007 (purity >99%; Oenema, 1988). Pyrite and FeS (Merck; purity 75%) were obtained in crystalline form. These chemicals were ground and sieved through 85 μm before use. Elemental S (Baker, N.J.; purity 99.8%), cysteine (BDH, Poole, England; purity 99.9%); methionine (BDH; 98.5%) were not sieved. The reagents were incubated overnight at 130–150°C in 35 ml Teflon vials with air-tight screw caps. The oxidants were H_2O_2 (Chriswell *et al.*, 1986). HNO_3–$HClO_4$ mixture (Oenema, 1988), and NaOBr (Tabatabai and Bremner, 1970). The released sulfate was measured by turbidimetry in a 5 cm cell on a Zeiss PHQ2 single beam spectrometer at 380 nm using a slit of 0.03 mm. For each sample a separate calibration curve had to be made. This was done by standard addition of a known amount of sulfate to the oxidized samples. Standard addition of sulfate was necessary as the remaining surplus of oxidation reagent varies with each sample and affects the formation of the barium crystals, and thereby the results of the turbidimetric method. This makes the turbidimetric method laborious.

Table 1. Recovery as sulfate of some sulfur chemicals (1 mg) after oxidation by half fusion with Na_2CO_3/KNO_3, peroxide, hypobromite and perchloric/nitric acid

	Oxidation procedure			
Compound	Na_2CO_3/KNO_3	H_2O_2	NaOBr	$NClO_4/HNO_3$
FeS	95	28	20	30
S^o	97	6	21	20
Methionine	100	1	1	2
Cysteine	102	2	3	5

Total sulfur in freshwater sediments

HCl hydrolyzable sulfur fraction

The HCl hydrolyzable sulfur fraction was estimated by the method of King and Klug (1980) for freshwater sediments. Sediment was taken by syringe, weighed (1 g) and transferred into 20 ml scintillation vials. Five ml 5 N HCl was added to the vials. After all the FeS had been volatilized, the vials were carefully heated (4 h, 110°C). The volume was re-adjusted and the formed sulfate in the supernatant was measured by turbidimetry.

Acid volatile sulfur fraction

Acid volatile sulfur was determined by a mini-distillation set up (Hordijk *et al.*, 1985) consisting of 7 ml scintillation vials with rubber stoppers (Packard, Brussels, Belgium). Fresh sediment (0.5 ml) which was anaerobically collected by syringe from Jenkin Cores, sampled from the lake in the non-stratified period, was transported in nitrogen-flushed scintillation vials. The samples were stirred with 7 mm long magnetic bars driven by tape recorder motors which were mounted under an acrylic glass plate. The acid volatile sulfide was released by the addition of 1 ml of anoxic HCl (1 M) and sequentially distilled in a stream of oxygen-free nitrogen into a second scintillation vial containing 4 ml of 2% zinc acetate. The trapped sulfide was determined spectrophotometrically using the *p*-phenylenediamine color reaction (Golterman *et al.*, 1978). Standard addition experiments in which a series of $Zn^{35}S$ (6.4–25.4 Bq) was added to the sediment showed a linear recovery of $89 \pm 7\%$ ($r = 0.9981$) of the liberated H_2S in the first trap of the distillation apparatus (Hordijk *et al.*, 1985). To determine the recovery of crystalline FeS (Merck), a series of FeS standards (31–184 μg S) were distilled. The recovery of the FeS standards was $86 \pm 6\%$, measured by the color reaction.

Pyrite sulfur fraction

Pyrite in the sediment was determined using the selective dissolution method (Begheijn *et al.*, 1978). A subsample of 100 mg was first treated with 1 ml H_2SO_4 (96%) and 3 ml HF (48%) for 1 min and then with hot 4 M HCl for 10–30 min to dissolve all non-pyritic iron. After centrifugation and washing 3 times with 1 M HCl the pyritic residue was dissolved in HNO_3 (68%). The released Fe was determined by atomic absorption spectrometry.

Elemental sulfur fraction

Estimation of elemental sulfur in the sediment was based on the reaction of elemental sulfur (S°) with sulfite in a phosphate buffer (Gooyer *et al.*, 1984). The thiosulfate which was formed was measured by ion chromatography. Sediment samples were anaerobically taken from the Jenkin core and freeze-dried in 60 ml serum vials with screw-caps and rubber inlets. The sediment was successively extracted 4 times by shaking with 15 ml de-aerated hexane (Heim *et al.*, 1984). The 4 hexane fractions were combined and carefully dried by rotary-evaporation under slightly reduced pressure. The dried residue was transferred into 20 ml vials and dissolved in 10 ml of buffer consisting of 2.5 mM sulfite and 4 mM sodium phosphate. The vials were vigorously shaken for 7 min in a water-bath at 85°C and transferred directly into an ice bath to stop the oxidation of sulfite to sulfate. The freshly formed thiosulfate was analyzed by I.P.C. Chromatographic conditions were similar to those used for the sulfate determinations.

RESULTS

Total sulfur

Complete separation of the sulfate peak from the nitrate peak was achieved within 10 min (Fig. 1). Recoveries of sulfate standards (0.5 ml, 1000 μM; $n = 6$) eluted from a 2 cm high cation-exchange column were $95 \pm 4\%$. Recoveries of $95 \pm 7\%$ (Table 1) of the reduced sulfur as sulfate were found for FeS, S°, methionine, and cysteine for the whole procedure. The recovery for pyrite was $96 \pm 5\%$. In order to investigate if the amount of KNO_3 (0.1 g) added before half fusion was enough to oxidize all sedimental sulfur to sulfate, a series of methionine standards (0.22–1.5 mg S) was added to 100 mg dried sediment (containing 0.12 mg S). A linear and quantitative recovery ($97 \pm 3\%$; $r = 0.994$) of added methionine-sulfur (up to 0.86 mg S) as sulfate was found, indicating that sufficient KNO_3 was added. The nitrate peak in the chromatogram (Fig. 1) is a second indication that there is sufficient KNO_3 to oxidize the sedimental sulfur.

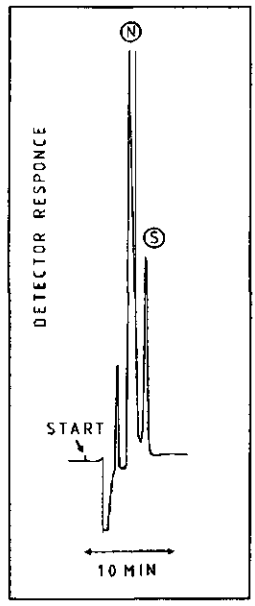

Fig. 1. Chromatogram of a total sulfur sample. S, sulfate; N, nitrate. Original concentration, 1300 mg S kg^{-1} wet sediment. Chromatographic conditions: 0.04 absorbance units full scale; loop 10 μl, eluent 400 μM sulfoisophthalic acid pH 4.7; flow rate 0.3 ml min^{-1}; wavelength 243 nm.

Total sulfur analyzed by I.C.P. and by ion chromatography in sediment samples taken from -1 to -27 cm were well correlated ($r = 0.994$; $n = 35$). In addition, the 1305 mg of total S/kg of wet Lake Vechten sediment found using roentgen fluorescence spectrometry is comparable to values found by I.C.P. (1398 mg S/kg wet sediment) and HPLC (1397 mg S/kg wet sediment in a -8 cm sample).

Total sulfur can theoretically be underestimated by the applied procedure, if significant concentrations of barium are liberated during sediment digestion and precipitate a part of the formed sulfate. The maximum effect should be 5% in the deeper sediment layers and about 1.3% in the top layers according to

the sediment barium concentrations (approx. 12 mg Ba kg^{-1} wet sediment; measured by I.C.P.). The good agreement with the roentgen fluorescence measurements, and the standard addition experiments with methionine provides support for absence of any matrix effect in the recovery of sulfate.

Elemental sulfur

The extraction of S° into hexane was quantitative ($97 \pm 3\%$) when measured by u.v. detection, but after rotary evaporation and reaction with sulfite only 70–75% was recovered as thiosulfate. The thiosulfate peak (retention time 8 min) was well separated from the sulfite and sulfate peaks (retention time 6 min). The sensitivity of the system for thiosulfate was 3.16×10^{-4} absorbance units per mol. The detection limit of the procedure was 2 mg S° kg^{-1} of wet sediment using a 20 μl injection. Elemental sulfur concentrations (2–36 mg S° kg^{-1} wet sediment: Table 2) in the sediment were close to the detection limit. Estimated values (20–70 mg S° kg^{-1}) obtained by measuring S° in hexane by u.v. absorption at 276 nm (absorption coefficient 0.92×10^{-3} cm l mol^{-1}; detection limit 4 mg l^{-1}) were higher, but this latter method can easily be influenced by co-extracted pigments.

Other sulfur compounds

Sulfate (3–220 μM) was only observed in the top 3 cm of the sediment (Hordijk *et al.*, 1985) and only during the non-stratified period. Thiosulfate concentrations in the sediment were below the detection limit (4–20 mg $S_2O_3^{2-}$ kg^{-1} wet sediment). Sediment estimates for pyrite, HCl hydrolyzable sulfur and acid volatile sulfide are given in Table 2. Free sulfide concentrations were very low ($< 10^{-6}$ M) and showed a peak in the most active sulfate reducing zone of the sediment (−1 to −3 cm). This layer corresponds to the maximum abundance of sulfate-reducing bacteria (Cappenberg, 1974).

DISCUSSION

Chemical oxidation of sulfide to sulfate involves complex reaction mechanisms (Almgren and Hagstrom, 1974; Cline and Richards, 1969). To estimate total sulfur from the release of sulfate from the sediment all reduced sulfur compounds must be oxidized. We found that even with powerful digestion reagents, oxidation of small amounts (1 mg) of some ground (15 μm) sulfur chemicals to sulfate was incomplete (Table 1). Shaw (1959) noted that changes in sample size, reaction containers, and heating devices greatly influence the recovery of S by wet digestion procedures. There was no significant difference between the oxidation of FeS with $HClO_4$, HNO_3, or H_2O_2 (Table 1). Addition of acid leads to a quick quantitative dissociation of the crystalline FeS, and release of H_2S as observed by the anoxic distillation. Apparently the crystalline structure of the reagent FeS had no effect on its chemical oxidation in acid.

The low recoveries (1–30%; Table 1) of sulfur as sulfate of FeS and other chemicals after wet digestion are not in agreement with recoveries of sulfur from Lake Vechten sediment after wet digestion (up to 70% S released with H_2O_2 digestion; up to 83% S released from HNO_3 digestion; compared with roentgen fluorescence measurements). The sedimental sulfur forms are obvious easier to oxidize then their related chemicals.

Chemical sulfur oxidation is a complex process. Stable inorganic intermediates that can be formed are polysulfides, S°, thiosulfate, thionates and sulfite (Almgren and Hagstrom, 1974; Cline and Richards, 1969; Moses *et al.*, 1984). Stable organic intermediates to be expected are mainly sulfonic acids from thiols, and sulfones from thioethers (Mopper and Taylor, 1986; Roberts, 1971). These partly oxidized sulfur compounds are detectable by I.C.P. but not by the ion chromatographic procedure. We investigated an oxidation procedure for I.C.P. samples involving $HNO_3/HClO_4$ digestion at 140°C (Oenema, 1988). After oxidation of the sediment samples sulfate represented only 55% ($n = 10$) of the total dissolved sulfur measured by I.C.P. Apparently more dissolved sulfur compounds had been formed. Specific analysis for thiosulfate, polythionates, sulfite, and polysulfides (Van Gemerden, 1987) on an I.C.P. solute, originating from Lake Vechten sediment, showed besides SO_4^{2-} (55%), mainly polysulfides (40%). From this we concluded that the I.C.P. procedure cannot be used to analyze total sulfur as sulfate in freshwater sediments.

In limnetic sediments R–S bonds can be expected (Mitchell *et al.*, 1984; Mopper and Taylor, 1986; Nriagu and Soon, 1985). We have tested oxidation procedures with representatives of R–S–CH_3 bonds (methionine) and R–S–H bonds (cysteine) which are notoriously difficult to measure. Oxidation of the thioether, methionine, is particularly difficult (Callan and Toennies, 1941; Shaw, 1959). Oxidation with H_2O_2 (Chriswell *et al.*, 1986) was incomplete for 1 mg methionine and S° (3.1 and 2.2%, respectively at pH 1; 11.5 and 6.4% at pH 7; 5.8 and 2.1% at pH 13). Alkaline oxidation with NaOBr to *sulfate* in destruction flasks at 280°C (Tabatabai and Bremner, 1970;

Table 2. Concentration of some sulfur compounds in the sediment of Lake Vechten measured in the early spring. Concentrations are given in mg S l^{-1} wet sediment. ES, HCl hydrolyzable sulfur fraction (mainly estersulfates); AVS, acid volatile sulfide (mainly FeS)

Depth (cm)	S°	Pyrite	ES	AVS
2	36	75	90	743
3	24	73	86	874
4	25	93	126	947
5	24	127	172	989
6	15	124	141	725
7	—	119	178	810
8	—	128	232	910
9	—	106	302	755

data not shown) or in closed Teflon vials at 140°C was also incomplete (Table 1). To circumvent this problem Tabatabai and Bremner (1970) estimated total sulfur as H_2S after sequential reduction of the digest. An additional problem is that residual aggressive reagents interfere with detection by ion chromatography. Even after 100-fold dilution excessive distortion of the baseline occurs and the silica-based chromatographic column tends to decompose. We conclude that a convenient oxidation procedure and efficient removal of the excess reagent, essential for a successful HPLC application, could not be achieved using wet digestion.

Fusion with Na_2CO_3 is an old and established dry oxidation technique for estimating total sulfur in soils (Begheijn *et al.*, 1978; Grant and Yeung, 1971). Dry oxidation has the advantage over wet oxidation that elevated temperatures can be used. With Na_2CO_3, temperatures can be raised up to 600–800°C, sufficient to destroy R–S bonds, followed by a complete oxidation of the reduced sulfur. Complete fusion is not essential if a small amount of KNO_3 is added to the sediment–carbonate mixture (Grant and Yeung, 1971). With half fusion at 620°C, glass scintillation vials could be used instead of the platinum crucibles and excessive decomposition of sedimentary silica was avoided. Added nitrate is reduced during the oxidation and released as gas from the mixture. Any surplus nitrate is separated from the sulfate peak on the high capacity column (Fig. 1), and Na_2CO_3 is removed by the cation exchange step. The Na^+ is exchanged against H^+ and, due to the pH shift, CO_3^{2-} is released as CO_2.

In contrast to suppressed conductometric chromatography (Busman *et al.*, 1983; Moses *et al.*, 1984), I.P.C. uses a high capacity column (1 mequiv g^{-1}). This reduces overloading effects, enabling short run times (10 min) for saline aliquots. The absence of an extra suppressor column as well as temperature effects associated with optical detection allows trace sulfate analysis to be performed on a simple multipurpose chromatographic system. The use of disposable glassware, the absence of a suppressor column, as well as the waste being liquid is ideal for $^{35}SO_4^{2-}$ tracer experiments. There are no vacancy peaks in the chromatogram (Fig. 1) which can interfere with sulfate analysis. The pH of the sulfoisophthalic acid buffer (pH 4.7) is optimal for the lifetime of inexpensive silica backbone columns relative to resin backbone. The high sensitivity (4 pmol equals 3 times signal to noise ratio) allows small injection volumes (5–10 μl). This improves column life (approx. 1000 injections) as less contaminants are introduced with each injection of interstitial water. The concentration of the eluent (400 μM) is so low that continued flushing of the system to prevent crystallization in the pump system is not necessary. A presaturation column, as required by buffers running at higher pH (6.0) is not needed. The reliability of the proposed method is demonstrated by the high correlation with the I.C.P. measurements ($r = 0.9981$) and good agreement with roentgen fluorescence spectrometry.

Sediment measurements

Estimates of total sulfur (Fig. 2) in the sediment of Lake Vechten are similar to values found in a large variety of freshwater lakes (Baudo and Muntau, 1986; Davison *et al.*, 1985; Mitchell *et al.*, 1984; Nriagu and Soon, 1985). Estimates show accumulation of reduced sulfur in the eastern basin of Lake Vechten (C/S_t ratio of 20). Acid volatile sulfide forms the major part (60%) of the sulfur pool of Lake Vechten, with S° (3%) and FeS_2 (8%) representing only a minor part. Apparently conditions in the sediment (intensive respiration, pH 7.6; αFe 3.6 mM; Eh < -200 mV) did not favor conversion of FeS (Davison *et al.*, 1985) to FeS_2 (Psenner, 1983) or the formation of S° as observed in some other ecosystems (Heim *et al.*, 1984). Our findings confirm those of Davison (C/FeS_2 ratio of 250) who concluded that freshwater pyrite formation is stimulated in reducing sediments with either a low turnover of organic carbon or periodic incursions of oxygen. These conditions are absent in Lake Vechten. The residual fraction ($S_t - FeS_2 - AVS - S°$) is a rough indication of the carbon-bound fraction in freshwater systems (Nriagu and Soon, 1985). Although the residual fraction forms a considerable part of the total sulfur pool (10–30%) it did not dominate the sediments as in non-polluted lake sediments in Ontario (Nriagu

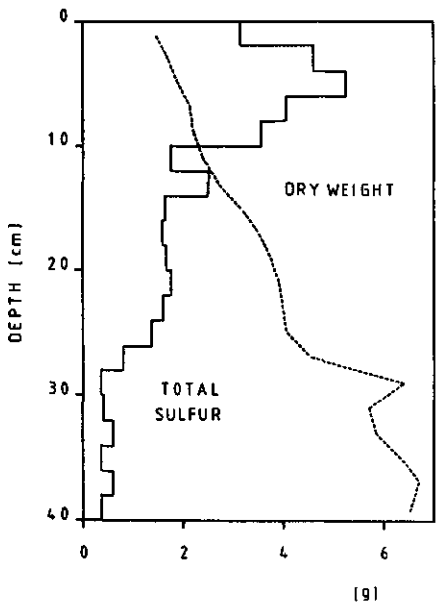

Fig. 2. Depth profiles of dry weight [percentage/10 (-----)] and of total sulfur [g S kg^{-1} dry wt (——)] in the sediment of Lake Vechten measured by HPLC as well as by I.C.P. in the stratification period (July 1986).

and Soon, 1985). The HCl-hydrolyzable sulfur pool (12%) suggests that ester sulfates ($R–O–SO_3H$) may be present (King and Klug, 1980). Ester sulfates can contribute to an internal sulfur cycle which is independent of molecular diffusion. To provide more detailed interpretation of the residual pool, specific techniques to determine functional organic sulfur groups (Wieder *et al.*, 1985) may be helpful.

During the excavation of Lake Vechten in 1941, the clay layer originating from the soil surface was dumped at the bottom. Estimation of the thickness (±28 cm) of the accumulated sediment in the eastern depression (−10.5 m) of the lake is therefore straightforward. The interface between the accumulated sediment and the original soil-layer was characterized by increases in the dry weight, organic C, and organic N (Verdouw *et al.*, 1985) and decreases in the concentration of some trace metals such as Pb, Sr, Zn and Mn (data not shown). From the dry weight (gradually increasing from 14.8% of the wet weight at 0–2 cm to 45.7% at −28 cm), specific gravity (1.1–1.2) and sulfur content (Fig. 2) we estimated that 125 g S has been deposited beneath 1 m^2 sediment since 1941. In 1985, 11.4 g S m^{-2} was reduced as estimated from the average sulfate reduction rates through the whole year. The input of particulate organic sulfur was 3.5 g S m^{-2} $year^{-1}$ (Steenbergen *et al.*, 1987). Addition of both processes yielded a potential input of 15 g S m^{-2} $year^{-1}$, which is relatively high compared with the sulfur accumulated (125 g) over 45 years. The rates of sulfate reduction and the accumulation of organic sulfur will probably have changed since the lake was formed and so the apparently much higher recent rates of supply to the sediment may be due to these changes. However, this argument cannot be applied to near-surface sediment. The top 3 cm of the sediment was formed in 4.5 years, as estimated from average sedimentation rates (0.65 cm $year^{-1}$), dry weight (15%), and specific gravity (1.1–1.2). In this time interval, reduction and input together yielded a potential fixation of about 67.5 g S m^{-2} but only 24 g S m^{-2} was present in the top 3 cm. From these values we conclude that only one-seventh of the dissimilatory reduced sulfur is immobilized in the sediment. The main part is re-released into the water of the lake.

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CHAPTER 2.4

Quantitative high-pressure liquid chromatography fluorescence determination of some important lower fatty acids in lake sediments.

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Quantitative High-Pressure Liquid Chromatography-Fluorescence Determination of Some Important Lower Fatty Acids in Lake Sediments

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For the quantitative determination of traces of fatty acids in pore water, several gas and liquid chromatographic methods were tested and discussed. Direct determination by gas-liquid chromatography with the use of formic acid-saturated carrier gas was found to be the least laborious method, but it is only recommended for the determination of volatile acids such as acetate and higher homologs. For the determination of lactate and formate, a derivatization procedure is necessary. The determination of these acids as phenacyl or benzyl esters was complicated by contaminants in the reagents. For this reason, a high-pressure liquid chromatography procedure with 4-bromomethyl-7-methoxycoumarin as a fluorescent labeling reagent is preferred. With this method, lactic, acetic, and formic acids could be demonstrated simultaneously at the nanogram level in 5-ml samples. Profiles of these acids in the sediment of Lake Vechten were measured, and they showed correlations with sulfate-reducing and methanogenic bacterial activities.

In freshwater lake sediments, the anaerobic degradation of complex organic inputs involves an initial fermentation which produces volatile fatty acids (VFA), hydrogen, and carbon dioxide. These products are subsequently utilized by methanogenic bacteria and sulfate-reducing bacteria. It has been shown that in Lake Vechten (The Netherlands), acetate and formate are the major precursors of methane as in other aquatic ecosystems, and lactate is the major precursor of sulfate reduction (5–7, 17, 22, 23). To study this process, accurate measurements of these acids are important. This paper describes the gas-liquid chromatography (GLC) and high-pressure liquid chromatography (HPLC) methods necessary for accurate measurement of formate and acetate as well as lactate.

VFA can be determined without pretreatment by GLC methods (8) as modified for mud samples (6). The main advantages of these methods (method I) are the short analysis time and the absence of interference by derivatization reagents. For acetate and homologs to C_8, this is the preferred method. Formate gives no flame ionization detector response, and lactate is not sufficiently volatile. Consequently, these acids must be chemically modified before chromatographic analysis. Higher fatty acids have been analyzed as their methyl esters in marine sediments (18, 21). The nonquantitative formation and the low boiling points of the lower fatty acid-methyl derivatives make this method unsuitable for nanogram-level analyses in organic pore sediments. Several less volatile derivatives have been synthesized and separated by GLC or HPLC as described in this paper (method III). However, during the derivatization period, side products are formed which introduce interfering peaks in the chromatogram (10). The use of solid-liquid-phase transfer catalysts may reduce the reaction time, but the catalyst itself could be a source of impurities and interfering peaks in the GLC analyses (13).

HPLC methods for VFA require initial derivatization for UV detection. Formation of phenacyl esters (2) introduces artifacts, making interpretation difficult. Recently, several fluorescent derivatives of fatty acids were synthesized (10, 11, 14, 16, 20). In particular, 4-bromomethyl-7-methoxycoumarin (BMC) is a very suitable alkylating reagent since it shows no fluorescence itself and the reaction product formed is a highly fluorescent derivative which can be determined by HPLC at the picomole level. With BMC as the alkylating reagent, a pretreatment procedure was developed (method II), resulting in well-defined concentration gradients of acetic, lactic, and formic acids in the sediment of Lake Vechten.

MATERIALS AND METHODS

Apparatus. The modular liquid chromatograph consisted of a model 100 A Altex dual piston pump combined with a Pye model LCXP low-pressure gradi-

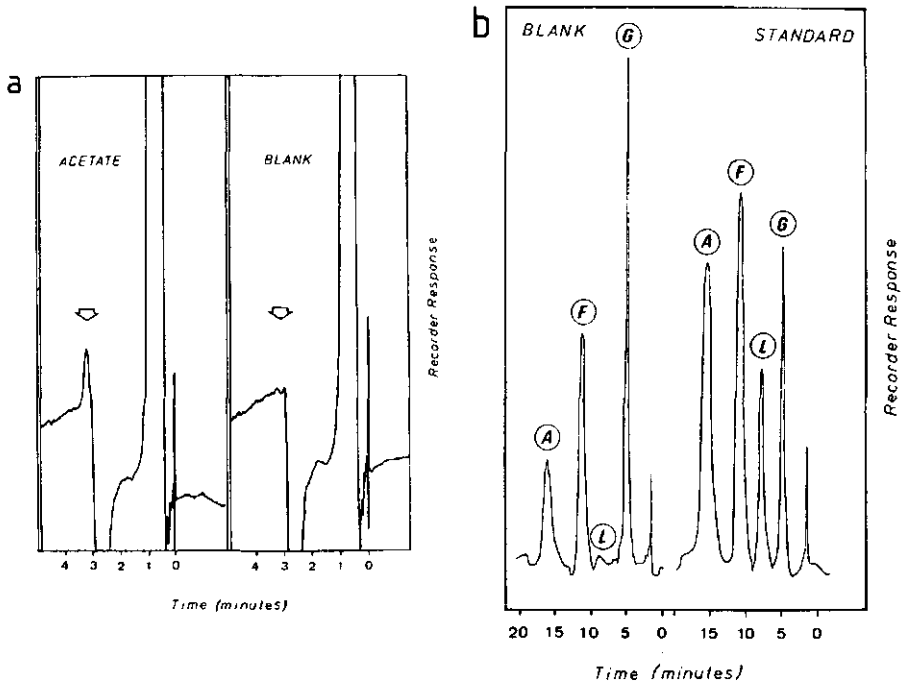

FIG. 1. (a) Gas chromatogram of an aqueous acetate standard of 20 μM (200 pmol injected). Column, Glass (2 m by 2 mm) packed with 20% neopentyl glycol adipate on Chromosorb WAW DMCS, 80 to 100 mesh; injector temperature, 200°C; detector temperature, 200°C; carrier gas flow rate, 30 ml/min (He-HCOOH), 115°C. (b) Liquid chromatogram of a mixture of fatty acids as their BMC esters. Symbols: A, acetate; F, formate; L, lactate; G, side product. A sample of 10 μl containing 300 pmol of each ester was injected. Attenuation, 40×. Chromatographic conditions: mobile phase, 26% MeCN (Merck zür Rückstandsanalyse) in He-purged distilled water (pH 7); flow rate, 1.2 ml/min.

ent programmer. Samples were introduced by means of a Rheodyne 7125 valve with a 10-μl loop. The detector was an Aminco-Bowman spectrophotofluorometer equipped with an ellipsoidal condensing system and a built-in 9-μl quartz flow cell. The column (250 by 4.6 mm) was packed with 5-μm Hypersil ODS particles and was thermostated (40 ± 1°C) with a Memmert waterbath. The GLC analyses were performed with a Packard-Becker 417 or 428 gas chromatograph equipped with a flame ionization detector and glass columns. Quantitation was carried out by peak height measurements of standard curves or by integration with a Spectra Physics System I integrator.

Reagents. The alkylating reagents BMC and *p*-bromophenacyl bromide were obtained from Chrompack (Middelburg, The Netherlands). The outer alkylating reagents and the crown ethers were purchased from Aldrich Europe (Beerse, Belgium). The Cryptand Kryptofix 221 and KF were obtained from E. Merck AG, Darmstadt, West Germany. The dry reagents were used without further purification. Reference acids were lithium lactate (BDH, Poole, England; analytical grade), natrium acetate (Merck Suprapur), and natrium formate (J. T. Baker Chemical Co., Phillipsburg, N.J.; analytical grade). Acetonitrile (Merck, zür Rückstand Analyse) and methanol (Merck, Lichrosolv) were used as the HPLC eluents. Acetonitrile (Merck Usavol) was used as a solvent in the derivatization reactions. The other organic solvents and common chemicals tested were obtained from Merck or Baker. The cation exchanger (Dowex 50W-X8, 100 to 200 mesh) was obtained from Fluka AG, Buchs, Switzerland (analytical grade quality).

Analytical procedures. Method I: GLC determination of acetate and other volatile acid homologs with formic acid vapor. Sample collection and pretreatment. Undisturbed mud cores were sampled by means of a modified Jenkin surface-mud sampler from the deepest part of the eastern depression of Lake Vechten (7). Subsamples of 5 to 10 g of wet mud were drawn by piercing with a syringe through the 2-mm holes (covered with Scotch tape no. 471) in the Perspex sampling tube. The samples were centrifuged for 5 min at 8,000 rpm. The supernatant was adjusted to pH 10 with KOH, and 3 ml was freeze-dried overnight. The next day, the freeze-dried salts were dissolved in 0.5 ml of double-distilled water, and 5 μl of the solution was injected on a column for GLC (Fig. 1a). Quantifica-

TABLE 1. Temperature programming for the lactate ester

Reagent ester	Temp (°C)		Isothermal preperiod		Programming rate (°C/min)	Isothermal final period		He flow (ml/min)
	Injection	Detection	Temp (°C)	Time (min)		Temp (°C)	Time (min)	
p-Bromophenacyl	205	235	140	6	5	195	7	23
p-Nitrobenzyl	250	250	140	2	5	190	6	25
Naphtacyl	260	260	150	3	4	210	3	22

tion was done by an external standard method with gravimetric standard mixtures (0 to 25 μM) of acetate and higher homologs made in double-distilled water. The standards were freeze-dried under exactly the same conditions as the samples. External standardization was used to save analysis time. Calibration curves and molar response factors were obtained by plotting the peak heights of the standard chromatograms against the original concentrations of the standard solutions. After correction of the double-distilled water blank and the apparatus blank, the peak heights of the sample chromatograms were compared mathematically, by using the response factors, with those of the standards for analysis.

GLC column preparation and conditioning. Liquid-phase neopentyl glycol adipate (NPGA; Chrompack); 10% (wt/vol), was dissolved in chloroform and coated on deaerated Chromosorb WAW (80 to 100 mesh). After slow mixing with reflux in a rotary evaporator, the solvent was removed in vacuo. A further period at 80°C in a vacuum oven removed the last traces of chloroform. This material was packed in a glass column (2 mm × 1.35 m) and conditioned by slowly increasing the oven temperature at 1°C min to 200°C with the carrier gas on. The column was left overnight at 200°C with a carrier gas flow of 15 ml of helium containing no formic acid vapor. The next day, the column was connected to the detector, and a small stainless steel vessel 9 cm long with an internal diameter of 1.2 cm was placed on line before the injection port. The vessel was filled with 2 ml of formic acid (Merck Suprapur). The column was bought to 115°C with no carrier gas flow, preventing condensation of the formic acid vapor on the cold column. The column was stabilized overnight with formic acid vapor in the carrier gas phase at 130°C.

Method II: HPLC-BMC determination of lactate, formate, and acetate. Samples were taken from mud cores as described above and centrifuged for 5 min at 8,000 rpm. The supernatant was filtered over a Gelman A glass fiber filter and eluted over the cation-exchange resin Dowex 50 × 8, in potassium form. The ion-exchange procedure is necessary when a crown ether or cryptand is used for derivatization (2). Resin clean-up was conducted by successive stirring with 1 N HCl (for 10 min), distilled water (three times for 10 min each), and KCl (pH 7; twice for 15 min each). A glass column 10 cm long with an inside diameter of 1.5 cm was filled with 2 ml of the prepared resin, which was adjusted just before use to pH 10. The column was twice washed with distilled water and dried with compressed air. One bed volume of pore water was used to wash the column. A second bed volume was eluted and collected in a glass serum vessel. The eluate was adjusted to pH 10 with KOH, and 2 ml was transferred into brown glass ampoules, which served as the reaction flasks for esterfication. The ampoules were freeze-dried overnight at a vacuum of 30 μBar.

Derivatization. The next day, the freeze-dried residue was dissolved in 1 ml of actonitrile containing 1,000 nmol of BMC as the alkylating reagent and 500 nmol of Kryptofix 221 as catalyst. To prevent hydration of the salts, the ampoules had to be immediately flame sealed and incubated for 1 h at 85°C with vigorous stirring. Sealed ampoules were utilized to prevent loss of solvent at 85°C. They are readily cleaned by heating at 450°C, which removes contaminating acetic acid. The reaction mixture was allowed to cool before opening the ampoules, and 5 μl of the acetonitrile solution was injected into the HPLC column. The samples were chromatographed as described in the legend to Fig. 1b.

Quantification. Concentrations were evaluated by external standardization. For calibration, series of gravimetric standard solutions of a mixture of lactate, acetate, and formate in the range of 5 to 25 μM were made in double-distilled water which was adjusted to pH 10 with KOH. The standards were freeze-dried and derivatized under the same conditions used for the samples. A reagent blank was made by incubating the used reagents at 85°C together with the samples. Calibration curves were made by plotting the peak heights obtained from the chromatograms of the derivatized standards against the original concentrations of the standard solutions. After correction for the reagent blank, the peak heights obtained from the sample chromatograms were compared mathematically with those of the standards for analysis. The molar response factors used were obtained from the calibration curves.

Method III. GLC determination of *p*-nitrobenzyl, *p*-bromophenacyl, and naphtacyl esters. The sample pretreatment was the same as for the HPLC-BMC (method II). The derivatization method used was that of Kimura et al. (13) as modified. A series of fatty acid standards (C_1–C_7) and a lactic acid standard were made in double-distilled water, adjusted with KOH to pH 10, and freeze-dried. For derivatization, the freeze-dried acid standards were dissolved in 1 ml of acetonitrile solution containing 2.5×10^{-3} M alkylation reagent and 2.5×10^{-4} M 18-crown-6 ether. The ampoules were flame sealed and incubated for 1 h at 85°C. The reaction mixture was allowed to cool before injection into the GLC system. Quantification was done much as in method II. Chromatographic conditions; Column, glass (2 m by 4 mm) packed with 3% OV 1 on Gaschrom Q, 80 to 100 mesh; carrier gas, helium. For temperature programming, see Table 1.

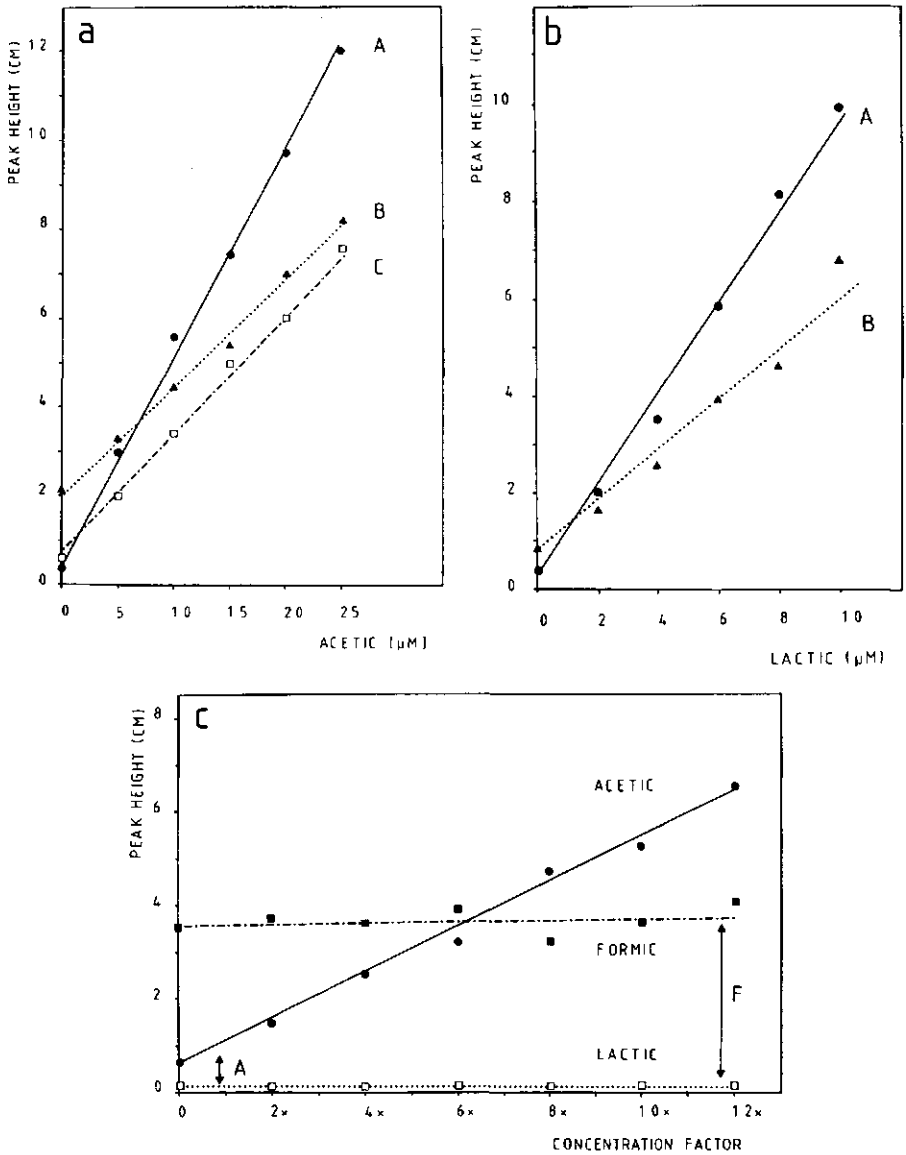

FIG. 2. Recovery and organic acid blanks of acetic, formic, and lactic acids in the pretreatment procedure of method II. (a) Acetate recovery of the different steps of the pretreatment procedure of method II, measured after each step by method I. Symbols: ●, untreated standard series (0 to 25 µM) (r, 0.9976; b, 0.474); ▲, standard series (0 to 25 µM), filtered, eluted over the ion exchanger, and freeze-dried (r, 0.9974; b, 0.274); □, standard series (0 to 25 µM), freeze-dried (r, 0.9984; b, 0.260). (b) Recovery of lactate in the pretreatment procedure of method II. Symbols: ●, calibration curve (0 to 100 µM) untreated (r, 0.9973; b, 0.0961); ▲, calibration curve (0 to 10 µM), 10 times concentrated by freeze-drying, after the filtration and ion-exchange step of the pretreatment procedure (r, 0.9838; b, 0.0552). (c) Determination of organic acid blanks. The concentrations increase of lactic, formic, and acetic acids are plotted against the increased volume reduction by freeze-drying of double-distilled water to a final volume of 1.5 ml. Reagent blanks: A, Acetic acid; F, formic acid.

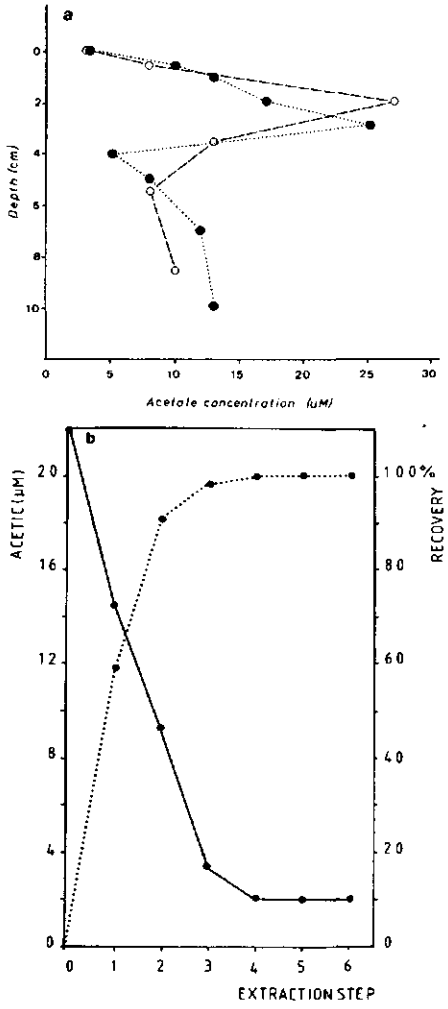

FIG. 3. Concentration profile of acetate in the sediment of Lake Vechten. (a) The sample was taken on 13 January 1981 and was analyzed directly by GLC without derivatization (method I) (●). Duplicate core taken on the same date was analyzed after 1 week by the HPLC-BMC method (method II) (○). Previous observations showed no variation of depth profiles after storing the cores for at least 1 week in the dark at in situ temperature (7). (b) Recovery of acetate from the mud. Symbols: Solid line, concentration decrease in the supernatant; dotted line, recovery of the water exchangeable pool.

RESULTS AND DISCUSSION

Method I. Direct GLC determination of VFA. The use of formic acid saturated with carrier gas in the analysis of VFA is well known (1, 6, 8). Adsorption (which generally gives rise to the tailing of the peaks), irregularly shaped peaks, and ghosting are prevented by the formic acid vapor in such a way that internal standardization is not needed. The highest-purity formic acid (Merck Suprapur) is essential in maintaining a stable base line. The neopentyl glycol adipate column is extremely stable to water injections and can be used for more than 2,000 injections without showing any deterioration. For external standardization, calibration curves were used (Fig. 2a). The linear range of the method is from 3 to 200 μM. Above 200 μM, adsorption is not sufficiently suppressed by the formic acid vapor. The GLC afforded a minimal practical detectable quantity of 15 pmol of acetate per injection, which makes this method suitable for in situ analysis of freshwater sediments (Fig. 3).

Method II. HPLC-fluorescence determination of lactate, formate, and acetate BMC esters. Chromatographic properties. The use of acetonitrile allowed the separation of the ester derivatives shown in Fig. 1b. A solvent of methanol (14) did not give this resolution. One side product with the retention time of glycolate (G in Fig. 1b) was produced. The peaks with the same retention time as the formate (F) and acetate (A) esters originate mainly from fatty acid impurities (see below). The excitation and emission spectra of the BMC esters were recorded in situ by stopping the flow when the ester peak arrived in the detector cell. The optimal excitation wavelength was 329 nm, and the emission wavelength was 403 nm. These results agree with the spectra made by Lloyd (15) with methanol. No change in the spectra was observed when changing the acetonitrile concentration in the chromatographically useful range. The relative fluorescence intensity of the three BMC esters was the same (0.115 mV/pmol). The electornic noise of the detector was below 0.05 mV, indicating a detection limit below 100 fmol. The practical useful range of the described procedure was limited to a concentration of 3 to 100 μM (30 to 1,000 pmol injected). The linear range could be increased to 500 μM without detecting effects of quenching. However, high BMC concentrations in the ampoule favor the formation of decay products of the reagent, making analysis below 10 μM inaccurate. In general, to obtain reproducible reaction results, the absolute BMC amount must be three times higher than the total acid, with a minimum of 1,000 nmol of BMC per ampoule.

Recovery experiments. When acetate was added to four mud samples to a final concentration of 500 μM, only 60 ± 5% was recovered by centrifugation in the pore water fraction. In a second experiment, two mud samples with no added acetate were used. The samples were

TABLE 2. Retention times of some lactic esters and the catalyst 18–crown 6 ether as determined by GLC method III

Reagent ester	Lactic ester		Crown ether	
	Retention time (min)	Acid with same retention time (±1 min)	Retention time (min)	Acid with same retention time (±1 min)
p-Bromophenacyl	13.8	Valeric	15.2	Lactic
p-Nitrobenzyl	17.5	Propionic	24.0	Isocaproic
Naphtacyl	24.9	Isobutyric	17.7	Acetic

centrifuged to separate the pore water fraction from the mud. The mud pellet was suspended in double-distilled water and centrifuged again. After centrifugation, the increase of the acetate concentration in the supernatant as a result of the extraction of the mud pellet was measured by GLC (method I). The centrifugation and extraction were successively repeated until no acetate was released from the pellet (Fig. 3b). The results of both experiments indicate that only 60% of the water-exchangeable acetate pool was recovered in the pore water fraction after a single centrifugation.

The HPLC-BMC method (II) was used to determine the recovery of lactate, formate, and acetate. Standards (0 to 10 μM) were concentrated 10 times by freeze-drying after elution over the ion-exchange column. A calibration curve (Fig. 2b, curve B) was made with these standards. A second calibration curve (Fig. 2b, curve A) was made with untreated standards in the range of 0 to 100 μM. The ratio of the slopes of the curves indicate a recovery of 57% (B/A = 0.0552/0.0961) for lactate, 68% for acetate, and 59% for formate. To investigate the recovery of each step of the HPLC-BMC procedure, gravimetric acetate standards (0 to 25 μM) were made in double-distilled water and divided into three parts.

The first part was analyzed directly by GLC (method I), and a calibration curve was made (Fig. 2a, curve A). A second part was freeze-dried and dissolved in an equal amount of double-distilled water (Fig. 2a, curve B). A third part was filtered, eluted over the ion-exchange column, and freeze-dried (Fig. 2A, curve C, total procedure). From the differences in the slopes of the three calibration curves, a recovery of 58% was found for the freeze-drying step, which is nearly equal to the total procedure (56%). Thus, the freeze-drying step seems to be the only step in which appreciable losses occur. The recovery of acetate was also found to be pH dependent. At pH 8, the recovery of the freeze-drying step was reduced to 20%. At pH 2, no acetate remained. A higher pH such as pH 11 did not improve the recovery and might have affected the derivatization reaction.

Derivatization studies. The ability of crown ethers and cryptands to catalyze derivatization reactions of carboxylic acids in organic aprotic solvents is well known (2, 10). These species, called phase transfer catalysts, have the ability to strongly solvate alkali metal cations. The net effect of this solvation is to enhance the solubility of alkali metal salts in the solvent and to increase the nucleophility of the anion in the salt. The use of crown ethers to form BMC esters is described in detail by Lam and Grushka (14). According to them, the reaction proceeds to completion in 10 min. We found that at the nanomole level the incubation time was insufficient as only 30% of the acids had reacted after 15 min with K221 as catalyst. After 1 h of incubation, the derivatization yield was increased to 85%. A 1-h incubation time was utilized as a compromise between the completeness of the derivatization and the increased formation of side products. Other ion pair-forming phase transfer catalysts such as tetra-*n*-butyl-ammonium bromide (12) and CeF (19) gave bad results because of water introduction since the freeze-dried salts are very hygroscopic.

As only small amounts of pore water are available in the sediment analysis, calibration curves were made in distilled water. It is known that Ca^{2+} Na^{+}, and Mg^{2+} in the pore water decrease the derivatization yields (2). These cations were removed by exchange with potassium in the ion-exchange step. To determine the derivatization yield of the pore water samples, two gravimetric standard series of lactate (0 to 50 μM) were made, one in distilled water and one in pore water. Both series were eluted over the ion-exchange resin before derivatization (method II). Calibration curves of each series were made, and the slopes were compared. The two calibration curves were parallel and well correlated (r, 0.9997; $n = 2 \times 5$), indicating that the derivatization yield was the same in the pore water series and in the distilled water series (data not shown). In conclusion, calibration curves made in distilled water can be used for quantification of pore water samples.

Blank reduction. To obtain a good resolution

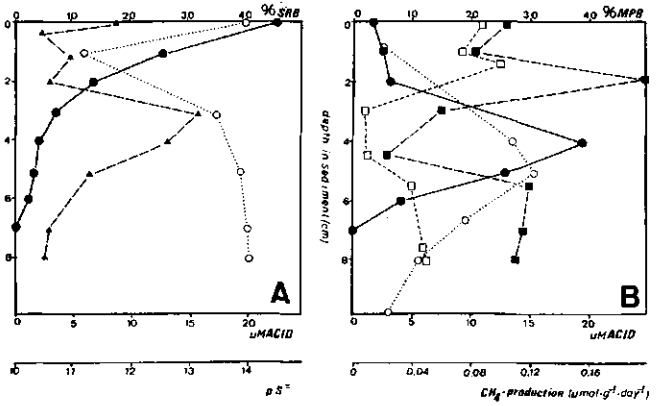

FIG. 4. Fatty acid gradient profiles in pore water of the upper 8 cm of the sediment of Lake Vechten, measured as BMC esters (method II). The results are compared with previously observations of bacterial activity (3). Symbols: (A) ●, Sulfate-reducing bacteria; ○, dissolved sulfide; ▲, lactate. (B) ●, Methane-producing bacteria; ○, methane production; ■, acetate; □, formate.

of the concentration gradient in the sediment, it was necessary to take small samples. Consequently, a decrease of the detection limit had to be obtained by blank reduction. During the development of the procedure, three main sources were found to be responsible for high acetate and formate blanks. First, the glassware is a regular source of acid contamination and must be heated overnight at 450°C before use. Use of small disposable brown ampoules for derivatization protects photolabile BMC, allows decontamination by heating at 450°C, prevents leakage of acetonitrile, and does not require thorough cleaning of reflux apparatus to prevent cross-contamination. The use of previously heated, dry glassware eliminated traces of acid contamination.

A second source of impurities is the solvents and reagents used for the derivatization (reagent blank). The reaction requires polar aprotic solvents as the reaction is much faster than in protic solvents. Polar solvents are difficult to purify of all traces of water and acid. The presence of moisture can introduce hydrolysis of the BMC alkylation reagent (9, 10) and the reaction solvent acetonitrile. In contrast to acetone, acetonitrile can be easily dried over 0.3-nm mol sieve. Acetonitrile (Merck Uvasol) was satisfactory without further purification when stored over the mol sieve. Replacement of acetonitrile by propionitrile did not reduce the acetate blank. This indicates that no acetate was formed from hydrolysis during the incubation. The concentration of acid impurities found in dimethylformamide and acetone was unacceptably high (up to 50 µM). Dimethyl sulfoxide was too difficult to dry. The crown ether was dried in a vacuum oven at 150°C, and the alkylation reagents were dried over P_2O_5 plus carrier (Sicapent, Merck).

The third source of impurities is the distilled water blank. Calibration standards were made up fresh in quartz double-distilled water (pH 10). This introduced a double-distilled water blank in the calibration curves which was superimposed on the reagent blank. This distilled water blank is not present in the samples. To determine the acid impurities in the distilled water blank and the reagent blank, a series of different volumes of distilled water was freeze-dried. The residues were dissolved in a constant volume of acetonitrile solution and analyzed. The peak heights were plotted against the increased volume reduction of the double-distilled water (Fig. 2c). Some formic acid (15 µM; Fig. 2c, F) and acetic acid (5 µM; Fig. 2c, A), but no lactic acid, were found in the reaction solvent. The increase of acetic acid concentration indicated a distilled water blank of 3 µM. The blank was not reduced significantly by further distillation with several kinds of fractionating columns. Distillation from acidic permanganate solution or UV radiation did not reduce the blank either. For accurate measurements of the acetic acid concentrations in the sediment, a double-distilled water blank as well as a reagent blank must be determined at each run.

Method III: GLC determination of fatty acid esters. The derivatization of VFA to their phenacyl-related derivatives is well known (2, 12).

Unfortunately, the moderate volatility of some of these compounds precludes the use of polyester GLC columns, and the more stable silicone columns lack sufficient resolution for accurate analysis of lactate in the presence of other VFA (Table 2). Reagent peaks easily overlap with the formic and acetic esters with various temperature programs (Table 1). The use of the more volatile benzyl esters on the neopentyl glycol adipate column produced a double ester peak. GLC calibration plots of the *p*-bromophenacyl ester of lactate on a OV 1 column were found to be linear from 40 to 400 μM or 120 to 1,200 pmol (r, 0.9998; n = 6). This detection limit is insufficient for in situ analysis of fresh water samples (0 to 20 μM) without concentration before analysis.

Application. The VFA analyses described in this paper were developed to define the interactions between sulfate-reducing and methanogenic bacteria. Acetate concentration profiles in the sediment were measured by methods I and II (Fig. 3). The similarity of the profiles confirmed the reliability of both methods with environmental samples. For the measurement of lactate and formate concentration profiles, the HPLC-BMC method II was used (Fig. 4). A point source of lactate lies at approximately 3 cm in the mud layer, and a sink of lactate exists in both the deeper and the sediment-interface layers (Fig. 4A). The relatively higher concentration of acetate and the lower concentration of lactate found in the upper layers in the sediment of Lake Vechten are in agreement with our earlier observation of a maximum abudance of sulfate-reducing bacteria (3). These sulfate-reducing bacteria oxidize lactate to acetate and produce H_2S (6, 7). The highest H_2S concentration corresponded to the minimal lactate concentration (Fig. 4A). In addition, in this layer the turnover rate constant of lactate is relatively high (k = 2.37/h) in comparison with that of acetate (k = 0.07/h) (4, 6). In the deeper sediment layers, the highest numbers of acetate-utilizing methanogenic bacteria are found at a depth of 4 to 6 cm (3), concomitantly with the highest production of methane and turnover rate constant of acetate (k = 0.24/h). The horizon of maximum methane-forming bacteria and methanogenic activity corresponded to the minima of acetic and formic acid recoveries (Fig. 4B).

Conclusion. Both GLC method I and HPLC method II are suitable for freshwater analysis of fatty acids in the range of 3 to 20 μM. Method I is preferable for all volatiles except formate and lactate because of its speed and simplicity. For the determination of lactate and formate, method II must be used. As the practical detection limit of method III is at least five times higher than for methods I and II, preconcentration steps must be applied for freshwater analysis. Methods I and II will be used in our studies of seasonal measurements of carbon fluxes and with turnover rates of carbon at the sediment-water interface.

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CHAPTER 2.5

Trace determination of lower volatile fatty acids in sediments by gaschromatography with chemical bonded FFAP columns.

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Trace determination of lower volatile fatty acids in sediments by gas chromatography with chemically bonded FFAP columns

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ABSTRACT

A capillary gas–liquid chromatography method was developed for the quantification of free lower volatile fatty acids (LVFA) in freshwater sediments. The method is based on the application of water-resistant FFAP (free fatty acid phase) columns and splitless injection. An important feature is the ability to determine LVFA directly at picomole levels in 1–3 μl of water without sample extraction, clean-up or derivatization. Continuous saturation of the carrier gas with formic acid is superfluous, making this method compatible with mass-selective detection. Detection limits of 0.2 pmol can be obtained for propionate with mass-selective detection and 1 pmol for acetate with flame ionization detection. The ability to study LVFA metabolism using stable isotope tracers is discussed. The method allows the measurement of well defined concentration profiles (4–70 μM) in sediment pore waters and is a good alternative to existing techniques for determining trace amounts of LVFA in very small volumes of organic-rich matrices.

INTRODUCTION

Lower volatile fatty acids (LVFA) such as acetate and propionate, generated during diagenesis of organic matter, play a key function in aquatic ecosystems as precursors of methanogenesis and respiration[1–3]. Respiration in meso-eutrophic lakes is limited mainly to the top few centimeters of the sediment–water interface[4] while methanogenesis occurs in deeper strata, making spatial differences in LVFA metabolism with sediment depth expected. A study of natural concentration profiles of LVFA and their actual turnover rates will lead to a better understanding of complex interactions between fermentative and respiratory processes. A knowledge about the actual conversion of LVFA to carbon dioxide and methane in freshwaters is still limited, however, owing to the lack of analytical methods[2] for measuring low LVFA concentrations (4–70 μM) routinely in very small sample volumes of porewater.

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Gas–liquid chromatography (GLC) is routinely applied to the determination of LVFA of food[5], clinical[6], atmospheric[7] and waste-water samples[8]. Ion-exclusion chromatography (IEC) is a alternative for determining LVFA in relatively clean matrices such as Antarctic ice water[9], but trace measurements in sediments can be complicated by interfering peaks[10]. To analyse sediments by IEC requires sample clean-up methods such as vacuum distillation[11] or ultrafiltration[12]. In an earlier study we determined LVFA, including formate and lactate, after derivatization to a fluorophore[2]. Detection limits below 100 fmol can be achieved but complex sample pretreatment is required[2]. For determining volatile acids only, GLC without derivatization or sample purification is preferable for its speed and simplicity[13].

Recently, FFAP-CB (free fatty acid phase chemical bond; Varian) capillary columns have been introduced[15]. These columns tolerate aqueous injections, making them very suitable for the determination of hydrophilic LVFA. Capillary columns facilitate the application of mass-selective detectors and consequently the use of stable isotopes to trace metabolic processes as discussed in this paper.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 5890 gas chromatograph with a split–splitless injection port, a 90 × 4 mm I.D. glass liner and a flame ionization detector was used. The capillary columns were a 10 m × 0.53 mm I.D. column coated with 1-μm FFAP-CB wax (Hewlett-Packard) and a 25 m × 0.32 mm I.D. column coated with 0.33-μm FFAP-CB wax (Chrompack, Middelburg, The Netherlands). Chromatographic conditions are given in Table I. The column was primed daily by ten injections of 1.0% formic acid (Suprapur; Merck, Darmstadt, F.R.G.) in Milli-Q water.

TABLE I

CHROMATOGRAPHIC CONDITIONS FOR THE DETERMINATION OF LVFA USING 25 m × 0.32 mm I.D. AND 10 m × 0.53 mm I.D. CAPILLARY FFAP-CB COLUMNS

Programme	*Column I.D. (mm)*		
	0.32 (FID)	*0.32 (MS)*	*0.53 (FID)*
Injector temperature	225°C	150°C	150°C
Detector temperature	260°C	250°C	200°C
Isothermal period	80°C (1.10 min)	80°C (1.10 min)	70°C (1.10 min)
First ramp rate	15°C min^{-1}	15°C min^{-1}	10°C min^{-1}
Isothermal period	105°C (1.00 min)	105°C (1.00 min)	105°C (0.25 min)
Second ramp rate	10°C min^{-1}	15°C min^{-1}	n.d.
Isothermal period	140°C (1.00 min)	140°C (1.00 min)	n.d.
Purge on time	1.1 min	1.1 min	1.1 min
Purge off time	2.5 min	2.5 min	4.8 min
Gases (ml min^{-1})			
Carrier gas (He)	4	4.5	20
Make up gas (He)	35	27	20
Septum vent	1	1	1
Purge vent	30	30	30

For peak identification, a C_1–C_{10} fatty acid mixture (Supelco, Bellefonte, PA, U.S.A.) and gravimetric standards (Suprapur; Merck) were analysed with an HP 5970 mass-selective detector, to which a 25 m × 0.32 mm FFAP (Chrompack) column was linked. Samples (0.5 μl) were introduced by splitless injection. Chromatographic conditions are included in Table I; the vacuum in the mass-selective detector was below $8 \cdot 10^{-5}$ Torr and the ionization energy was 70 eV. During elution, continuous scans were made from *m/z* 44 to 550. The mass spectral data were processed with an HP-300 computer to trace molecular ions (M^+) or specific fragments originating from LVFA. Sediment and stable isotope analysis were performed by selective ion monitoring (SIM).

Sediment analysis

Undisturbed sediment cores were taken with a Jenkin sampler from Vechten, a 10-m deep lake with a clay-rich anoxic sediment[1]. Samples of 0.35 g were drawn by syringe through 2.8-mm holes (covered with Scotch tape No. 471) in the acrylic glass sampling core. The samples were centrifuged at 1000 *g* for 5 min in 0.4-ml polypropylene sampling tubes (Emergo, The Netherlands). The supernatant was separated and frozen for storage. Before injection, 100 μl of supernatant were adjusted with 1 μl of 50% formic acid (Suprapur; Merck). A volume of 3 μl was injected in duplicate or triplicate into the gas chromatograph. Calibration was done by external standardization with gravimetric standards (0–50 μ*M*) of acetate (Suprapur; Merck), [$^2H_3$]acetate (99.5% pure; Aldrich, Beerse, Belgium) and propionate (analytical-reagent grade; J. T. Baker, Philipsburgh, NY, U.S.A.) in Milli-Q water, acidified with 0.5% (v/v) formic acid. The glass insert in the injector needs regular cleaning; pushing a wetted ball of crumpled paper though the insert is usually sufficient to pick up deposits.

RESULTS

With FFAP-CB, good separation of acetate and propionate from other LVFA was achieved (Figs. 1 and 2). Only intense molecular ion peaks of acetate (*m/z* 60 and 61), propionate (*m/z* 73–77) and butyrate (*m/z* 88) were recovered in scans of the fatty acid mixture. Other LVFA were identified by their retention times and interpretation of related spectra. Formate (*m/z* 44–46) was not recovered in the ion chromatograms. [$^2H_3$]Acetate signals (*m/z* 63) were of same intensity as those of unlabelled acetate.

Sediment samples (natural pH 7.5–8; 5–10 mequiv l^{-1} HCO_3^-) require acidification (pH < 4.5) to improve volatilization and suppress adsorption in the instrument. At pH 4.5–5.5 the peak heights were already 70% lower. New columns require 0.5% formic acid acidification, but contaminated columns need a higher concentration. Only the acetate blank was affected by formic acid addition; an increase in formic acid concentration by 0.5% leads to a *ca.* 2 μ*M* rise in the acetate blank. With 0.5% formic acid, the acetate blanks for Milli-Q water (1.5–4 μ*M*) were similar to those previously found (2–5 μ*M*[2]). The reproducibility improved with increasing concentration (Table II), column diameter and with injection volume [0.53 mm I.D. column and 25 μ*M*: 0.5 μl, 10.5%; 3 μl, 1.2%; 5 μl, 0.6% (n = 5)]. With the 0.32 mm I.D. column the relationship between injection volume and peak height was linear up to 8 μl (r = 0.993) and with the 0.53 mm I.D. column until at least 5 μl (r = 0.997) for acetate.

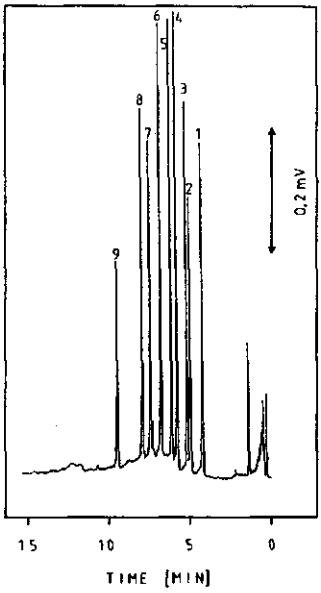

Fig. 1. Chromatogram of a mixture of volatile fatty acids (Supelco; 3 μl 100 μM or 300 pmol each) by GLC with a 25 m × 0.32 mm I.D. FFAP column and FID. Peaks: 1 = acetic acid; 2 = propionic acid; 3 = isobutyric acid; 4 = butyric acid; 5 = valeric acid; 6 = isovaleric acid; 7 = capronic acid; 8 = isocapronic acid; 9 = heptanoic acid.

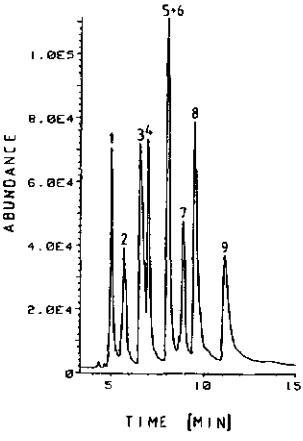

Fig. 2. Composed ion chromatogram of a mixture of volatile fatty acids (0.6 μl, 100 μM or 60 pmol each) by GLC on a 25 m × 0.32 mm I.D. FFAP column and MS processed in the SIM mode. The ion chromatogram was composed by summation of intensities of ions of m/z 45 and 60. Peaks as in Fig. 1.

TABLE II

RELATIONSHIP BETWEEN RELATIVE STANDARD DEVIATION, CONCENTRATION (n = 10) AND COLUMN DIAMETER FOR ACETATE ANALYSIS WITH SPLITLESS INJECTION (3 μl) AND FID

Standards were made in Milli-Q water (blank 3 μM) which was acidified with 0.5% formic acid.

Concentration (μM)	*Relative standard deviation (%)*	
	0.32 mm I.D. column	*0.53 mm I.D. column*
0	19	7
5	14	9
10	8	4
25–100	8	3

The detection limits, defined as a signal change equal to three times the signal-to-noise ratio, were 1–3 pmol for flame ionization detection (FID), and 0.2–0.7 pmol for mass-selective detection (MS) (Table III). Acetate calibration graphs [signal obtained by summation of ABU (abundance units or relative intensity) for m/z 45 and 60) were linear from 20 to 400 pmol (r = 0.947). Below 20 pmol the curves flatten. The [$^2H_3$]acetate (m/z 63; r = 0.998) and propionate calibration graphs (m/z 45 and 75; r = 0.957) were linear from 0.2 to 400 pmol.

DISCUSSION

Capillary FFAP-CB columns can be applied to the trace determination of LVFA (<200 pmol) in aqueous samples. Gross sediment sample sizes of 300 μl can be processed with ease as only 3 μl of centrifugate are needed for GLC. Continuous

TABLE III

COMPARISON OF DETECTION LIMIT, LINEAR RANGE AND SENSITIVITY OF THE DETERMINATION OF ACETATE AND PROPIONATE BY GLC

The signal of acetate was composed of m/z 45 and 60 and that of propionate m/z 45 and 74.

Detection	*Column I.D. (mm)*	*Detection limit (pmol)*	*Linear range*		*Sensitivity (attenuation 32)*[a]
			pmol	*r*	
Acetate					
FID	0.32	1	0–150	0.990	5.7 μV pmol^{-1}
FID	0.53	1	0–125	1.000	6.7 μV pmol^{-1}
MS	0.32	0.2	30–400	0.947	261 ABU pmol^{-1}
Propionate					
FID	0.32	3	n.d.	n.d.	4.7 μV pmol^{-1}
FID	0.53	2.5	0–125	0.999	5.8 μV pmol^{-1}
MS	0.32	0.7	0–400	0.957	86 ABU pmol^{-1}

[a] ABU, abundance units.

saturation of the carrier gas with formic acid vapour to suppress ghosting[2,13,14] was unnecessary. Sample pretreatment requires no purification or concentration step, thereby avoiding losses due to ineffective extraction or freeze-drying[2]. Derivatization of LVFA in pore water requires excess of reagent, as part of the reagent will be neutralized by dissolved organic carbon[2]. Addition of an excess of reagent implies that reproducibility of the reagent blanks rather than the detection limit of the derivative determines the accuracy at trace levels. Lactate, for example, does not appear as an impurity in reagents and can be well determined as a derivative. Acetate, however, is a common contaminant in reagents and solvents[2]. Using the FFAP-CB column, reagent interference is circumvented and only the instrument blank limits the linear dynamic range in which acetate can be accurate determined (Table II). The absence of a reagent label has for MS a second advantage that stable isotope signals originate only from isotope in the LVFA molecules themselves. Note that ^{13}C in aromatic reagent labels can contribute considerably to "blank" signals.

The practical advantages of using stable isotope labelling instead of radioactive isotopes[3] are obvious. Stable isotope tracers can be used for fieldwork or in ordinary laboratories without special licenses. We previously applied on-line gas proportional counting of radioactively labelled LVFA[3]. In the proportional counter most of the chromatographic resolution was lost, which is not the case with MS (Fig. 2). Similar MS chromatograms of free LVFA (Fig. 2) have, to our knowledge, not yet been published. Of the LVFA, only formate (*m/z* 44–46[16]) could not be detected by MS or FID and derivatization or other techniques[2,10,17] may be required for this compound.

Sediment analysis

Profiles of free acetate concentrations measured in 1988 and 1989 in sediment porewater from Lake Vechten (Fig. 3) regularly showed similarly shaped concentration profiles to those observed in 1978[4] and 1983[2], but the peaks in the profiles

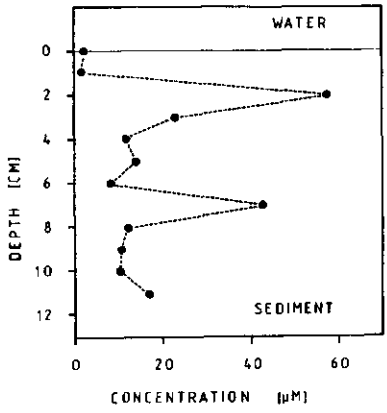

Fig. 3. Profile of free acetate concentration with sediment depth in pore water collected in Lake Vechten on April 24th, 1989.

measured with the FFAP-CB column were much sharper. Although changes in LVFA metabolism in the lake cannot be excluded, the lower concentrations found in 1978 and 1983 can be ascribed to dilution effects due to the larger sample sizes (5–10 ml instead of 0.35 ml) used in previously applied techniques. The sharp sediment acetate peaks obviously reflect intense acetogenesis under well defined metabolic conditions. The acetate peak just below the sediment–water interface can be explained by respiratory and/or diffusion processes[2,3]; the peak at −6 cm, however, hints at as yet unexplained fermentative processes. To unravel these processes, [$^2H_3$]acetate can be used as a tracer.

In conclusion, GLC on capillary FFAP columns is a good alternative to existing techniques for the determination of trace amounts of acetate and higher homologues in small sample volumes of organic-rich matrices such as sedimental porewater.

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CHAPTER 3

THE SULFUR AND CARBON CYCLE IN LAKE VECHTEN

CHAPTER 3.1

Estimation of bacterial nitrate reduction rates at in situ concentrations in freshwater sediments.

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Estimation of Bacterial Nitrate Reduction Rates at In Situ Concentrations in Freshwater Sediments

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A method was developed to follow bacterial nitrate reduction in freshwater sediments by using common high-performance liquid chromatographic equipment. The low detection limit (14 pmol) of the method enabled us to study concentration profiles and reaction kinetics under natural conditions. Significant nitrate concentrations (1 to 27 μM) were observed in the sediment of Lake Vechten during the nonstratified period; the concentration profiles showed a successive depletion of oxygen, nitrate, and sulfate with depth. The profiles were restricted to the upper 3 cm of the sediment which is rich in organics and loosely structured. Nitrate reduction in the sediment-water interface followed first-order reaction kinetics at in situ concentrations. Remarkably high potential nitrate-reducing activity was observed in the part of the sediment in which nitrate did not diffuse. This activity was also observed throughout the whole year. Estimates of K_m varied between 17 and 100 μM and V_{max} varied between 7.2 and 36 μmol cm^{-3} day^{-1} for samples taken at different depths. The diffusion coefficient of nitrate ($[10 \pm 0.4] \times 10^{-6}$ cm^2 s^{-1}) across the sediment-water interface was estimated by a constant-source technique and applied to a mathematical model to estimate the net nitrate reduction during the nonstratified period. In this period, observed nitrate reduction rates by the model, 0.2 to 0.4 mmol m^{-2} day^{-1}, were lower than those found for oxygen (27 mmol m^{-2} day^{-1}) and sulfate (0.4 mmol m^{-2} day^{-1}). During the summer stratification, nitrate was absent in the sediment and reduction could not be estimated by the model.

Anaerobic respiration in limnic sediment plays an important role in nutrient remineralization (3). Good insight into the mechanism involving these electron transfer processes is essential for a reliable quantification of the role of sediment in the carbon cycle. Recent studies demonstrated important differences between terminal metabolic pathways in marine and freshwater ecosystems (8, 12). The main difference is the continuous electron acceptor limitation in most freshwater sediments. In most submerged marine sediments electron donor limitation is more likely to occur due to the permanent high sulfate concentration in the water layer above the sediment. Anions that can serve as terminal electron acceptors in freshwater sediments are, in order of net energy yield, oxygen, nitrate, and sulfate. The concentrations of these compounds in the water layer of Lake Vechten showed the typical seasonal fluctuation of a mesotrophic monomictic lake (21). In the summer season stratification occurs, and concentration profiles of oxygen, nitrate, and sulfate (8, 21, 28) in the water phase showed that these electron acceptors were already depleted in the hypolimnion before they could diffuse to the sediment in the deeper parts of the lake. In this season dead organic material, originating from primary production and detritus, deposits in the deeper part of the lake, where it accumulates on the sediment surface. In the nonstratified period complete circulation of the lake occurs, and the electron acceptors diffuse to the deeper parts of the lake. In this period steep concentration profiles of the electron acceptors develop in the sediment, reflecting an equilibrium between transport by diffusion from the overlying water layer and their successive consumption by intense respiration.

Natural nitrate concentrations in marine as well as in most freshwater sediments are low, and analytical tools to measure these low nitrate concentrations are lacking (10, 18). This is one of the reasons why nitrate reduction is generally quantified from the accumulation of ^{15}N-labeled reaction products rather than by directly following nitrate depletion (14, 20). The relevance of the reported rates may be questioned in many cases, since nitrate is generally added at concentrations much higher then those found in nature (13, 18). Sediment enrichment with nitrate might artificially induce zero-order kinetics, normally not occurring in situ. Applying the sensitive and simple ion-chromatographic technique developed during this study enabled us, first, to measure nitrate reduction kinetics in small (5- to 10-ml) sediment batches at in situ concentrations and, second, to estimate nitrate reduction by mathematical modeling. The small sample volumes needed by this technique allow the study of steep, nondisturbed concentration profiles of nitrate essential for quantification by modeling.

MATERIALS AND METHODS

Apparatus. The liquid chromatograph consisted of a model 1330 pump and a model 1305 UV detector (Bio-Rad Laboratories, Richmond, Calif.). Samples were introduced by a Rheodyne 7125 valve with a 50-μl loop. The column (75 by 4.6 mm; HPLC Technology, Cheshire, England) was packed with 5 μm of Nucleosil 5-SB anion-exchange particles. A guard column (75 by 2.1 mm) (type B for anion-exchange chromatography; Chrompack, Middelburg, The Netherlands) was used. The columns were insulated with cotton wool for temperature stability. The eluent was made by diluting sulfuric acid to 1 mM in deionized water filtered through a Milli-Q purification system (Millipore Corp.). The nitrate blank of the Milli-Q water was <0.08 μM (5 ppb). The pH of the eluent was carefully adjusted with NaOH (pH 4.5). The eluent was stored in the dark. Nitrate and nitrite concentrations were evaluated by external standardization.

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For calibration, a series of mixtures containing gravimetric standard solutions of nitrate (1.6 to 24 μM [0.1 to 1.5 ppm]) were used. The calibration curves were made by plotting the peak heights obtained from the chromatograms against the concentration of the original standard solutions. In routine analysis, chromatographic peaks were integrated with a combination of an IBM computer and a CI-10 integrator (LDC Milton Roy, Riviera Beach, Fla.).

Sample collection. Undisturbed sediment cores were taken by a modified Jenkin mud sampler from the deepest part of the eastern depression of Lake Vechten (2). The lake is described in detail elsewhere (21). The upper water layer was carefully removed to prevent mixing of the nitrate-rich and oxygen-containing water layer with the nitrate-poor interstitial water of the sediment. Sediment subsamples (1.3 g, wet weight) were drawn by piercing a syringe through the 2-mm holes (covered with Scotch tape [no. 471]) in the acrylic glass sampling tube. The sediment samples were immediately anaerobically transferred into screw-capped vials (Pierce Chemical Co., Rockford, Ill.) and centrifuged at 1,000 × *g* at room temperature for 2 min. The supernatant was separated from the sediment and immediately stored at −20°C upon analysis in small (0.4-ml) polypropylene vials (Tamson, Zoetemeer, The Netherlands). Before analysis, the supernatant was thawed and centrifuged to remove excess organic material by coprecipitation with the formed Fe(III) colloids. A 50-μl portion of the supernatant was injected into the liquid chromatograph for nitrate analysis. For nitrite analysis, the pore water was directly injected under anaerobic conditions. The eluent of the liquid chromatograph was continuously flushed with helium to suppress any nitrite oxidation artifacts and formation of bubbles in the pump head during elution. Sulfate analyses were performed simultaneously by indirect photometric chromatography (6).

Recovery of nitrate. To determine the recovery of nitrate after sampling and centrifugation, sediment was withdrawn from the Jenkin core at −1-cm depth and homogenized. Nitrate reduction was suppressed by gamma radiation (2.5 Mrad) or anaerobic autoclaving (30 min at 125°C) or by adding Halamid (final concentration, 4%). The inactivated slurry was divided into five batches of 10 ml each, which were spiked with nitrate to obtain a standard series with final concentrations in the pore water of 0 to 64 μM nitrate. The batches were incubated anaerobically in the dark at 8°C. Subsamples were withdrawn after 15 h of incubation. Nitrate concentration series in the pore water were curve compared with gravimetric standard series made in distilled water. The recovery was calculated from the ratio of the slopes of both calibration curves made from the plots of peak height against concentration. Nitrate recovery in aerated sediment was determined in the same way. The slurry was, however, not deactivated but flushed overnight with air. The sediment batches were spiked with nitrate (16 to 64 μM) and shaken under air for 3 h. In between, subsamples were taken from each batch for nitrate analyses. The slope of the obtained calibration curve made in the sediment was compared with that made in distilled water to estimate the recovery.

Sediment characterization. Sediment samples were collected at different depths of the Jenkin core as mentioned and centrifuged in calibrated (1.3-ml) reaction vials (Pierce) at 1,000 × *g*. The pore water was withdrawn for nitrate and sulfate analyses. The remaining sediment was dried at 110°C to determine the dry weight per milliliter of wet sediment. Nitrogen and carbon per milliliter of wet sediment were measured with a CHN-analyzer (model 240; The Perkin-Elmer Corp., Norwalk, Conn.) (28). Redox potential and oxygen measurements were done by dipping microelectrodes into the Jenkin core at 1-mm intervals (16).

Model of NO_3^- dynamics. The model applied to estimate nitrate reduction is derived from the one used by Berner (1) to estimate sulfate reduction in marine sediments. The basic assumption of the model is that the concentration change of nitrate with time at a given depth x is a reflection, mainly, of three processes: (i) diffusion according to Fick's second law; (ii) deposition; (iii) nitrate reduction.

$$\left.\frac{\partial C}{\partial t}\right|_x = D_s \frac{\partial^2 C}{\partial x^2} - \omega \frac{\partial C}{\partial x} - f(x) \qquad [1]$$

In this equation C is the nitrate concentration measured in situ, D_s ($=D_{NO_3}$) is the diffusion coefficient, ω is the rate of sedimentation in Lake Vechten (1.8×10^{-3} cm day^{-1} [22]), and $f(x)$ is a function describing the nitrate reduction at depth x. The definition of this function $f(x)$ depends on the reaction kinetics of nitrate reduction in the sediment-water interface. Assuming that nitrate reduction is limited by diffusion means that first-order kinetics is to expected and the rate of nitrate reduction at depth x will be correlated with the concentration at this depth: $d[NO_3]_x/dt = -K \cdot [NO_3]_0$ or after intergration. $\ln [NO_3]_t = -K \cdot t \cdot \ln[NO_3]_0$. From this equation we can derive that nitrate reduction will decrease exponentially with depth or

$$f(x) = a \cdot e^{-b(x)} \qquad [2]$$

in which x represents the depth in the sediment; a and b are unknown constants. Solving these constants will reveal a function from which at each depth nitrate reduction can be calculated. Under steady-state conditions D_{NO_3} and $f(x)$ are independent of time and $dC/dt = 0$ at depth x. According to Berner (1) and with the border conditions of $C = C_0$ at $x = 0$ and $C = C_\infty$ at $x = \infty$, two solutions can be obtained for differential equation 1

$$C_{(x)} = \frac{a}{D_{NO_3} \cdot b^2 + \omega \cdot b} \cdot e^{-b(x)} + C_\infty \qquad [3]$$

$$C_{(x)} = (C_0 - C_\infty) \cdot e^{-bx} + C_\infty \qquad [4]$$

where C_0 is the nitrate concentration of the overlying water layer and C_∞ is the minimum nitrate concentration. The a term of function $f(x)$ can be found by substitution of solution 3 in equation 4. The b term of function $f(x)$ can be estimated by fitting computer-generated plots of solution 4 on natural measured nitrate concentration profiles. Nitrate reduction can now be estimated by substitution of a and b terms in function $f(x)$ and successive integration, yielding the practical formula

$$\int_{x_1}^{x_2} f(x)dx = -(C_0 - C_\infty)(\omega + D_s b)e^{-b(x_2 - x_1)} \qquad [5]$$

Determination of diffusion coefficient. The model requires an independent estimate of D_{NO_3}, which was determined by the constant-source technique of Duursma and Hoede (4). We previously applied this technique to estimate the diffusion constant of sulfate and proved its realibility by comparison with an instantaneous-source technique (4) and intact whole-core incubations (8). To estimate D_{NO_3}, subcores were filled with 4 cm of inactivated sediment collected from the upper 2 cm of the Jenkin core with a porosity of 0.9. A water layer of 15 cm containing 160 μM NO_3^- was carefully placed above the sediment, and the subcore was incubated at

8°C. Incubation times were carefully optimized until a pure diffusion profile was formed within ±2 cm of sediment. The overlying water layer was occasionally mixed with care to maintain a constant concentration above the sediment. After incubation, the sediment pore water was sampled at 0.5-cm intervals and analyzed for nitrate. The diffusion constant D_{NO_3} was determined by plotting the nitrate concentration against the depth on an error function graph (4).

Sediment slurry incubations. For better insight into the kinetics at the sediment-water interface, subsamples (8 ml) were anaerobically collected at −0.75 cm, using the Jenkin core, and transferred into 20-ml serum vessels. The serum vessels were capped with butyl rubber stoppers and the headspace was flushed with oxygen-free nitrogen. The sediment batches were spiked with nitrate until two or three times the natural concentration and anaerobically incubated at the in situ temperature (8°C) of the sediment. No pH change was observed during incubation. Six subsamples were withdrawn by piercing the syringe through the butyl rubber stopper at 5-min intervals, immediately centrifuged (30 s at 1,000 × *g*), and analyzed or frozen and stored. Instead of serum vessels, 10-ml plastic syringes were also applied for short-term incubation.

RESULTS

Nitrate analysis. We compared the sensitivity of several detection modes in combination with a relatively inexpensive, high-capacity Nucleosil 5-SB anion-exchange column (Table 1). The application of a strong high-capacity column excluded conductometric detection (6, 7). Nonsuppressed conductometric detection with low-capacity columns and indirect photometric detection proved too insensitive (detection limit, <200 pmol [6]), with injection volumes below 100 μl. Direct UV detection with methane sulfonic acid as eluent (9) yielded a detection limit of 80 pmol. Detection limits significantly improved (15 pmol) by replacing methane sulfonic acid with pure sulfate as eluent (pH 4.5) in a concentration range of 0.5 to 2.5 mM. Stable base lines were obtained within 2 h of flushing the column (Fig. 1).

Nitrate and nitrite were well separated at an eluent concentration of 1 mM H_2SO_4 (nitrate retention time, 10 min; nitrite retention time, 7 min), without interference of sulfate, thiosulfate, thionate, sulfide, sulfite, or a dissolved organic carbon pool (DOC). Calibration curves were linear between 1.6 and 65 μM (0.1 to 1.4 ppm) with a 50-μl loop (r, 0.999) and peak-height measurements. The linearity improved about twofold with electronic integration. The sensitivity of the nitrate analysis is proportional to the volume injected. With 300-μl injections, linear calibration curves for nitrate or nitrite in the range of 0.16 to 1.6 μM (10 to 100 ppb; r, 0.999) can be obtained with ease. However, nonpurified pore water injections of this size quickly poison the analytical column head. The analytical column could be used for at least 1,000 analyses within 0.5 year with 50-μl injections. Sequential 50-μl injections of an 8 μM nitrate solution yielded a standard error of 8% with peak-height measurements. An absolute detection limit of 20 pmol was calculated from three times the noise of the detector (1.5×10^{-4} absorbance units full scale) divided by the sensitivity (13.4 absorbance units full scale μmol^{-1}). Standard addition of nitrate to the sample matrix indicated a recovery of 98 ± 2% for both the aerobic and the anaerobic parts of the sediment. Nitrate concentrations did not increase when sediment slurries were mixed under air for 1 h.

TABLE 1. Comparison of different ion chromatographic techniques for nitrate determination, using optical detection

Eluent[a]	Retention time (min)	Molar response (AUFS μmol^{-1})[b]	Detection limit (pmol)
0.4 mM SIPA	4	1.0	200
10 mM MSA	9	5.0	80
0.5 mM H_2SO_4	14	8.1	25
2.5 mM H_2SO_4	8	15.6	15

[a] SIPA, sulfoisophthalic acid; MSA, methane sulfonic acid.
[b] AUFS, Absorbance units full scale.

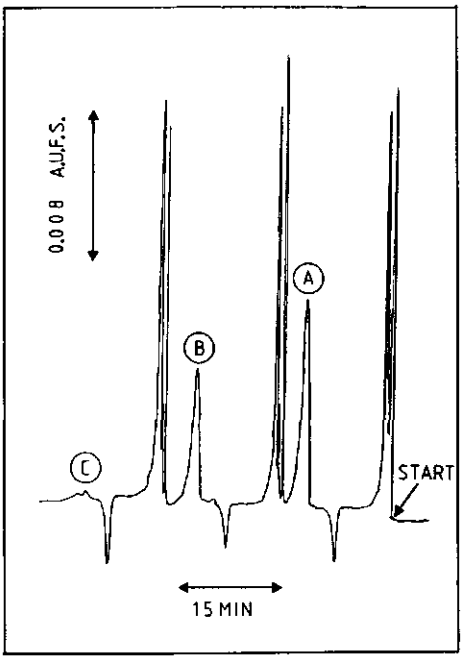

FIG. 1. Nitrate chromatograms of sedimental pore water. Samples were taken in the nonstratified period at 0 (A), −0.5 (B), and −1.0 (C) cm. Concentrations: A, 27.5 μM; B, 17 μM; C, 1.9 μM. Conditions: Range, 0.04 absorbance units full scale (AUFS); loop, 50 μl; eluent, 0.75 mM H_2SO_4 (pH 4.5); flow rate, 0.8 ml min^{-1}; wavelength, 204 nm.

Field observations. The concentrations of oxygen, nitrate, and sulfate in the deeper parts of Lake Vechten showed the typical seasonal fluctuations of a mesotrophic monomictic lake (21) (Table 2). The highest concentrations of nitrate (25 μM) and sulfate (220 μM) in the water layer just above the sediment were observed in winter and early spring (Table 2), and the lowest concentrations (nitrate, <1 μM; sulfate, <20 μM) were seen at the end of the summer stratification. In the summer no nitrate or nitrite could be detected in the sediment. In the upper 2 cm of sediment there was clearly a successive depletion with depth of oxygen, nitrate, and sulfate in the time that the lake underwent complete circulation (Fig. 2a). Other anions which could serve as potential electron acceptors such as nitrite, sulfite, thiosulfate, or tetrathionate were not found in the sediment. Dry weight (Fig. 2d) frequently increased rather steeply from 50 g dm^{-3}

TABLE 2. Seasonal fluctuation of oxygen, nitrate, and sulfate concentrations in the water layer 1 m above the sediment surface in the lowest part of the eastern depression of Lake Vechten

Fluctuation of:	Concn (μM) in:											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Oxygen	360	340	340	350	250	0	0	0	0	0	238	375
Nitrate	21	21	20	20	18	0	0	0	0	0	7	11
Sulfate	210	200	200	200	140	80	20	10	10	200	200	200

at the surface of the sediment to 200 g dm^{-3} (at −3 cm). Redox potential (Fig. 2c) dropped sharply, i.e., 170 mV in the upper 4 mm of the sediment and 280 mV at −2.5-cm depth. Note that absolute redox potentials are difficult to estimate as the dissolved iron in the pore water interferes with calibration with the normal electrode. The percentages of N and C in the total dry weight were a little higher in the top layer (Fig. 2b) compared with those in the deeper layers, but the absolute amount of carbon increased from 0.3 M at −0.5 cm to 1.1 M at −3 cm and total N increased from 33 mM at −0.5 cm to 74 mM at −3-cm depth.

Reaction kinetics. Nitrate addition to the sediment collected from the sediment-water interface at −1 cm resulted in a complete nitrate depletion within 0.5 h (Fig. 3). Plots of incubation time versus nitrate concentration on a linear (Fig. 3a) or logarithmic (Fig. 3b) scale showed a shift from zero- to first-order kinetics when the surplus of added nitrate was consumed up until natural concentrations were reached. Intensive nitrate reduction was observed in the sediment during the whole year, including the summer stratification when nitrate is absent in the deeper layers of the lake. Nitrate reduction was also observed in the deeper layers of the sediment. V_{max} decreased with depth from 20.0 (−1 cm) to 18.7 (−3 cm) to 8.4 (−5 cm) to 6.0 (−8 cm) μM day^{-1}. Michaelis-Menten plots were made to estimate when nitrate-reducing rates became concentration dependent. K_m esti-

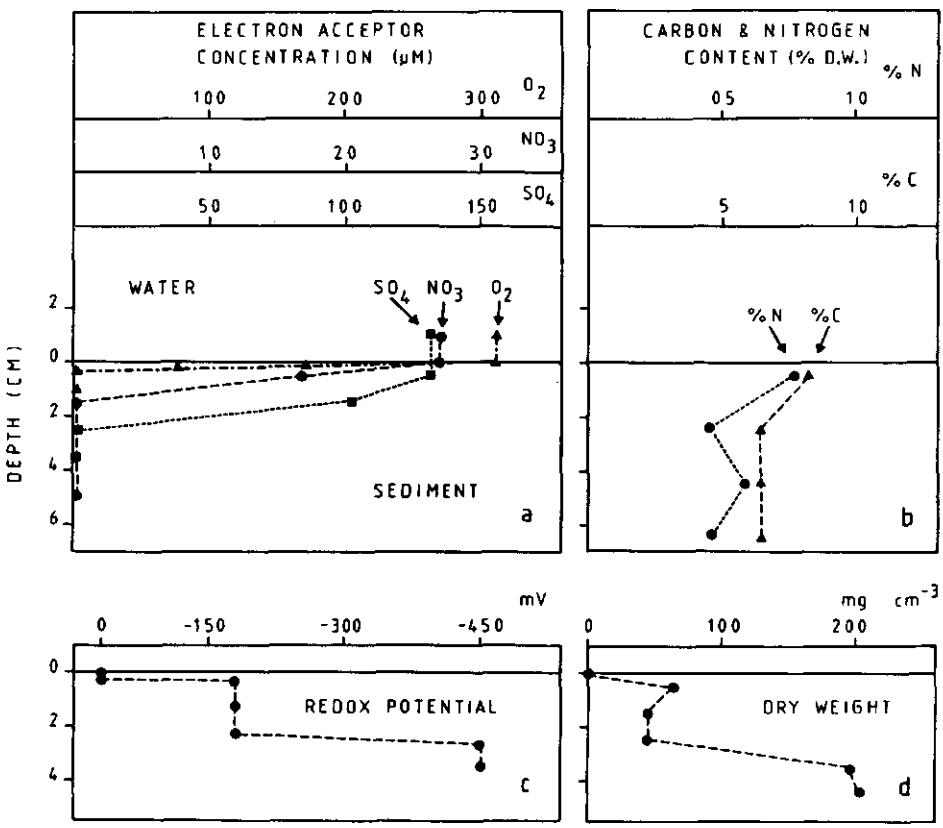

FIG. 2. Concentration profiles at the sediment-water interface of Lake Vechten during the circulation period. (a) Electron acceptors: oxygen (▲); nitrate (●); sulfate (■). (b) Carbon and nitrogen content as percent dry weight. (c) Redox potential. (d) Dry weight. The oxygen and redox potential were measured at 1-mm interval.

mates were 17 (−1 cm), 26 (−1 cm), and 110 (−2 cm) μM in the nonstratified period and 26 μM (−1 cm) during stratification. Figure 4 shows a Michaelis-Menten plot made with the values from Fig. 3a.

Model calculations. The estimated diffusion coefficient of nitrate (D_{NO_3}) in the sediment-water interface was 10.1 ± 0.4 × 10^{-6} cm^2 s^{-1}. The D_{NO_3} values were simular for anaerobic autoclaved, radiated, or Halamid-inactivated sediment. The latter suppressed nitrate reduction only temporarily. In the diffusion experiments, nitrate concentrations in sediment incubated simultaneously remained constant with Halamid as the suppressor. The estimated D_{NO_3} values were comparable to literature values measured in distilled water (15) if corrected for temperature (8°C) and porosity (±0.9). No literature values of D_{NO_3} at the sediment-water interface are known to us. Application of the model to concentration profiles in the circulation period, using the estimated D_{NO_3}, yielded a maximum net reduction rate of 0.3 ± 0.1 mmol m^{-2} day^{-1} (n = 5). The model could not be applied during summer stratification while nitrate is absent in the sediment and in the water layer above the sediment.

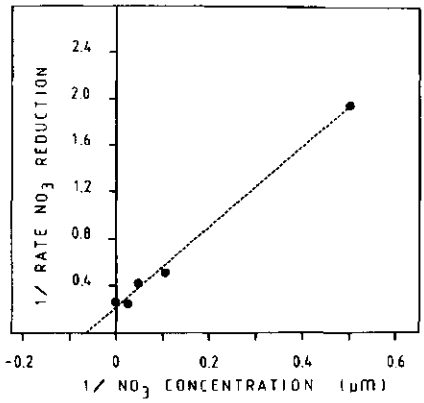

FIG. 4. Lineweaver-Burk plot of nitrate reduction rate as a function of nitrate concentration in the sediment of Lake Vechten. Units of 1/V are given in minutes $micromole^{-1}$ $liter^{-1}$.

DISCUSSION

Nitrate analysis. As in many other Dutch freshwater lakes, the concentration of DOC such as humic acids and other organic compounds in Lake Vechten is high. DOC values vary between 4 and 6 mg of C $liter^{-1}$ in open water and between 12.5 and 15.5 mg of C $liter^{-1}$ in pore water (after filtration through a 0.45-μm filter [3]). For a direct measurement at low-UV wavelengths, the nitrate peak had to be separated well from other UV-absorbing interfering substances present in the DOC pool. A high-capacity column like the Nucleosil 5-SB is capable of separating nitrate from high amounts of organics without overloading. The DOC pool did not interfere with nitrate analysis due to the low affinity of these compounds at low pH for the silica-based anion-exchange column. The DOC pool cannot compete well with the sulfate buffer on the ion-exchange sites of the column, and most of this pool is flushed directly through the column with the void volume. The nitrate anion interacts intensively with the column material and a well-shaped peak evolves. Injection of nonpurified samples into the liquid chromatograph, however, induces a quick decline (1 month) in the column performance by an irreversible absorption of a small part of the DOC pool on the column head with each injection. The column deteriorates; its resolution decreases, dissolution of the column head occurs, and back pressure increases. When large injection volumes (1 to 2 ml) were applied to compensate for insensitive detection, natural profiles were disturbed.

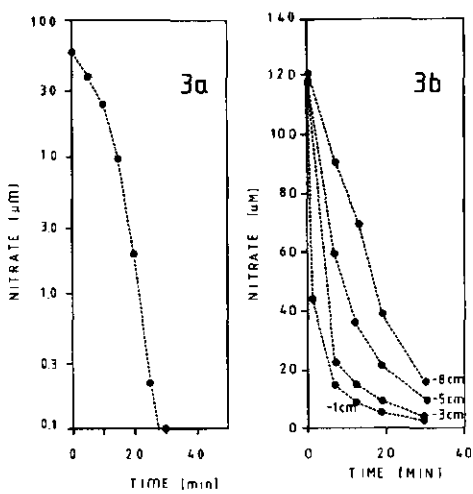

FIG. 3. Semilog plot of nitrate depletion in an 8-ml sediment batch taken at −1-cm depth of the Jenkin Core in the nonstratified period (a) and linear plots of nitrate depletion during the summer stratification at various depths in the sediment (b).

The procedure in this paper circumvents most of these difficulties, first, by improving the detection limit, allowing smaller injection volumes (<50 μl) which reduced the amount of DOC brought onto the column; and second, by applying a sample purification procedure, Sample clean-up of organic-rich pore water can be done by eluting the pore water through an absorption column (25). This technique is inconvenient for sample sizes of <200 μl as, although dead volumes can be minimized, the pore water dilutes during elution though the absorption column and must be reconcentrated after collection. A much simpler way of purifying pore water is by slowly freezing out. The DOC pool concentrates and reacts easier in this way with the dissolved Fe(III) present in the pore water. Excess Fe(III) in the pore water is formed by the oxidation of dissolved $Fe(II)CO_3$ (free dissolved Fe pool = 3.6 mM [27]). After thawing, the humic acids and dissolved Fe(III) complexes can be coprecipitated by centrifugation.

Reaction kinetics. Nitrate addition to the sediment immediately stimulates an intensive nitrate-reducing activity. In aerated sediments, however, complete nitrate reduction did not occur until all oxygen was consumed. These experiments together with the observation of steep nitrate concentration profiles indicate that, in the organic-rich sediment of Lake Vechten, nitrate limitation occurs, which restricts respiratory nitrate reduction to a small zone immediately below the oxidized surface. It was remarkable that nitrate reduction was also stimulated immediately by addition of nitrate to deeper parts of the sediment (Fig. 3b) where, in situ, no nitrate was detected. The origin of this potential nitrate-

reducing activity is unknown to us, but simular observations were noted for other sediments (17, 23). Nitrate-reducing activity also occurred during the summer stratification immediately after nitrate addition to the sediment. At this time nitrate was already absent for several months in the eastern depression of the lake (Table 2), implying that an active nitrate-reducing flora is permanently present in the sediment. A nutrical need for assimilatory nitrate reduction is superfluous regarding the amount of NH_4^+ present in the sediment (0.025% of the dry weight [20, 26]), and nitrate reduction in the sediment may therefore be entirely dissimilatory, including the deeper parts of the sediments. We previously also observed a potential sulfate-reducing activity in deeper parts of the sediment (8) in which sulfate no longer diffused.

The reaction kinetics of the nitrate reduction was studied directly by following its depletion in the sediments as no chemical absorption of ntirate was observed in the controls. The lack of significant nitrification was remarkable and agrees with the results of van Kessel (24) that at low temperatures (±4°C) no or little growth of nitrifying bacteria occurs. Potential nitrate-reducing activity decreased with depth, so batch sizes had to be kept to a minimum to prevent mixing up the top layer with less active sediment. Semilogarithmic (Fig. 3a) and linear (Fig. 3b) plots indicated that in enriched sediments nitrate reduction initially follows zero-order kinetics up to natural concentrations, with a sequential shift to first-order kinetics until nearly complete depletion is reached. The K_m values obtained from Michaelis-Menten plots (Fig. 4) varied between 17 and 100 μM and are comparable to those found by Oremland et al. (17) in a marine estuary. Estimation of K_m values or turnover rates requires about 6 ml of sediment as a time course had to be made, and one subsample is needed for each time interval. Taking the steep concentration profiles into account, it cannot be excluded that some mixing of the sediment, even with this sample size, might occasionally occur and influence a precise estimate of K_m or V_{max} values. More important, however, is that mixing the sediment with nitrate excludes the in situ existing limitation by diffusion, which complicates interpretation of the measured reduction rates. Estimation of the actual nitrate reduction by modeling may, therefore, be more reliable than estimations obtained from incubation of slurried sediment batches.

Modeling. The increase of dry weight at ±3-cm depth observed in many Jenkin cores indicates that the sediment-water interface is less consolidated than the layer below. The lack of redox potential fluctuations at millimeter intervals in the sediment-water interface indicates the absence of microniches as reported in marine sediments (11). The similar C/N ratios found in the sediment and the sediment-water interface also indicate a homogeneous structure, ideal for mathematical modeling. In Lake Vechten sediment, respiration processes were found to be restricted to the loosely bound sediment-water interface as methanogenesis mainly occurs in the more compact layer (Fig. 2d) marked by the sharp decrease in redox potential (Fig. 2c). The steep concentration profiles of the investigated electron acceptors (Fig. 2a) are obviously a reflection of a steady state between an intensive reduction and diffusion. Their successive depletion with depth might be explained by the net energy yield of the involved electron transfer processes (19). For example, the energy yield gained by the oxidation of acetate, an important potential electron donor (5), is much higher with the reduction of oxygen (27 kcal [113 kJ] electron mol^{-1}) or nitrate (17 kcal [71 kJ] electron mol^{-1}) than with that of sulfate (3 kcal [13 kJ] electron mol^{-1}). Nitrate reduction apparently outcompetes sulfate reduction in the anaerobic zone.

Applying the model with the estimated diffusion coefficient ($D_{NO_3} = 10.10^{-6}$ cm^2 s^{-1}) yielded a nitrate reduction of 0.3 ± 0.1 mmol m^{-2} day^{-1}, a rate much lower than the potential nitrate reduction rate expected for batch experiments (±74 mmol m^{-2} day^{-1}). The latter was estimated by the formula $v = V_{max} \cdot C/(K_m + C)$ given by Oren and Blackburn (18), in which v is the nitrate reduction rate, C is the mean nitrate concentration at −1 cm, and K_m is the Michaelis-Menten constant from Fig. 4. The estimated nitrate reduction by the model is in the same range as the simultaneously estimated sulfate reduction (0.4 mmol m^{-2} day^{-1}) but much lower than the estimated oxygen consumption (±27 mmol m^{-2} day^{-1}). More information is needed to translate these numbers in terms of carbon mineralization throughout the whole year because internal remineralization processes (especially for sulfate) in the sediment are not taken into account by the model.

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CHAPTER 3.2

Kinetic studies of bacterial sulfate reduction in freshwater sediments by high-pressure liquid chromatography and microdistillation.

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Kinetic Studies of Bacterial Sulfate Reduction in Freshwater Sediments by High-Pressure Liquid Chromatography and Microdistillation

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Indirect photometric chromatography and microdistillation enabled a simultaneous measurement of sulfate depletion and sulfide production in the top 3 cm of freshwater sediments to be made. The simultaneous measurement of sulfate depletion and sulfide production rates provided added insight into microbial sulfur metabolism. The lower sulfate reduction rates, as derived from the production of acid-volatile $^{35}S^{2-}$ only, were explained by a conversion of this pool to an undistillable fraction under acidic conditions during incubation. A mathematical model was applied to calculate sulfate reduction from sulfate gradients at the sediment-water interface. To avoid disturbance of these gradients, the sample volume was reduced to 0.2 g (wet weight) of sediment. Sulfate diffusion coefficients in the model were determined ($D_s = 0.3 \times 10^{-5}$ cm^2 s^{-1} at 6°C). The results of the model were compared with those of radioactive sulfate turnover experiments by assessing the actual turnover rate constants (2 to 5 day^{-1}) and pool sizes of sulfate at different sediment depths.

An understanding of the coupling between sediments and the overlying water requires a knowledge of the mechanisms controlling both nutrient remineralization rates and chemical exchange across the sediment-water interface. The respiratory reduction of sulfate to sulfide is one of the dominating processes in the metabolism and diagenesis of anoxic sediments, although the quantitative importance of detrital breakdown by sulfate-reducing organisms is just beginning to be appreciated. Studies on brackish sediment in Denmark (12, 21) have shown the importance of bacterial sulfate reduction in the annual mineralization of organic matter in marine sediments. Sulfate reduction in freshwater sediments may also be very significant (4, 10, 18–20) if, despite low concentrations of free sulfate, large pools of alkyl-sulfate esters are produced photosynthetically in the water layer and an internal sediment-surface recycling process is operative. Recent data obtained in our laboratory (2a) suggest that sulfate reduction as a terminal process in anaerobic carbon mineralization plays a more important role in freshwater sediments than was previously understood. In the mesotrophic stratifying Lake Vechten, The Netherlands, 8 to 20% of the amount of sedimented particulate organic carbon is converted to CO_2-carbon by sulfate reduction. The lake is described in detail elsewhere (22).

As a result of analytical difficulties in determining the pool sizes of sulfate in interstitial water in freshwater sediments, data on the quantification of sulfate reduction by existing ^{35}S radiotracer methods (13) or mathematical modeling (1) are essentially lacking for low-sulfate environments. Recently, we have developed a method to determine sulfate in freshwater-sediment interfaces by indirect photometric chromatography (IPC), which gives well-defined sulfate profiles in the micromolar range for the sediments in Lake Vechten (7). In this method, separation is achieved by competition between the strongly UV-absorbing anion and the solute anions for the ion-exchange sites on the column. It follows that elution of sulfate will be accompanied by a localized deficiency of the UV-absorbing anions in the eluent. The presence of the anion in the solute is then shown by a drop in the base-line value, which is proportional to the amount injected.

In this paper we describe the dynamics of sulfate reduction with the use of a mathematical model involving vertical sulfate gradients as a function of diffusion, bacterial reduction, and sedimentation. The rates of sulfate reduction and sulfide production were also determined directly in sediment cores by a $^{35}SO_4^{2-}$ radiotracer technique that involves the use of microdistillation, which is assumed not to disturb the most active superficial sediment layer during sampling by minimizing the total amount of mud needed for analyses. Special attention was paid to the calculation of the turnover rates by assessing the actual turnover rate constants (k values) and actual pool sizes of sulfate at different sediment depths; this was done by determining the in situ concentration profiles and the depletion of $^{35}SO_4^{2-}$ by IPC. Finally, the rates were compared with those calculated from the model as a means of understanding the kinetics of bacterial sulfate reduction and internal sediment sulfate recycling.

MATERIALS AND METHODS

Sulfate analysis. The liquid chromatograph consisted of a model 1330 Bio-Rad pump with a model 1305 Bio-Rad UV detector (Bio-Rad Laboratories, Richmond, Calif.). Samples were introduced by using a Rheodyne 7125 valve with a 20-μl loop, which was replaced by a 200-μl loop for the radioactivity measurements. The column (75 by 4.6 mm; HPLC Technology, Cheshire, England) was packed with 5-μm Nucleosil SB anion exchange particles. A guard column (75 by 2.1 mm, type B for anion exchange chromatography; Chrompack, Middelburg, The Netherlands) was used. The columns were insulated with cotton batting for temperature stability. An eluent of 5-sulfoisophthalic acid buffer (0.4 mM, monosodium salt diluted in distilled water [pH 4.5]; Aldrich, Beerse, Belgium) was used. Detection was at 239 nm with a flow rate of 0.8 ml/min; the attenuation was 0.16 absorbance units at full scale with the 200-μl loop. Concentrations were evaluated by external standardization. For calibration, a series of gravimetric standard solutions in the range of 0 to

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250 μM (0 to 25 μg/ml) sulfate were made in double-distilled water. Calibration curves were made by plotting the peak heights obtained from the chromatograms of the standards against the original standard solutions. The peak heights obtained from the sample chromatograms were compared mathematically with those of the standards for analysis. The molar response factors used were obtained from the calibration curves. More details on eluent effects, reduction of retention time, and recovery of the sulfate fractions from the sediments are given in a description of an earlier version of the method (7).

Sample collection. Samples were collected with the exclusion of oxygen to prevent possible oxidation of reduced sulfur to sulfate. A modified Jenkin surface mud sampler was used to collect undisturbed mud cores from the deepest part of the eastern depression of Lake Vechten (2). For the measurement of sulfate concentration profiles, subsamples of 0.2 to 1.5 g, depending on the water content of the sediments, were horizontally drawn by piercing the syringe through the 2-mm holes (covered with Scotch tape no. 471) in the acrylic glass sampling tube. The upper water layer was removed to prevent mixing of the sulfate-rich water layer with the sulfate-poor sediment water. The subsamples were transferred into mini-vials (Pierce Chemical Co., Rockford, Ill.) with different volumes (maximum volume, 1.5 ml) and short, conical bottoms. The vials were completely filled with sediment and immediately closed with screw-capped Teflon-faced disks and centrifuged for 5 to 10 min at 1,000 × *g* at room temperature. After centrifugation, the supernatant was immediately separated from the sediment and stored at −20°C on analysis. After thawing, organic Fe(III) complexes were formed. The Fe(III) cations originated from the excess of free $Fe^{II}CO_3$ (23) dissolved in the pore water of Lake Vechten. The formed iron coprecipitates were removed by a second centrifugation, and 20 μl of the supernatant was injected into the high-pressure liquid chromatograph for sulfate analysis. Injection of unfreezed supernatant showed that the freezing procedure had no measurable influence on the sulfate concentration in the supernatant, as all the reduced sulfur was precipitated in the first centrifugation step.

Model of SO_4^{2-} dynamics. Berner (1) described a theoretical model to estimate the rate of sulfate reduction from measured sulfate profiles by the equation

$$\left.\frac{\partial C}{\partial t}\right|_x = D_s \frac{\partial^2 C}{\partial x^2} - \omega \frac{\partial C}{\partial x} - f(x)$$

In this formula C is the measured sulfate concentration at time t, D_s is the diffusion coefficient, ω is the rate of sedimentation, and $f(x) = a \cdot e^{-bx}$, a function describing first-order sulfate reduction. When the system is in steady state, a practical solution of $f(x)$ in this diffential equation is given by:

$$\int_{x_1}^{x_2} f(x)dx = -(C_o - C_\infty)(\omega + D_s b)e^{\frac{\omega}{D_s}(x_2 - x_1)}$$

where C_o is the sulfate concentration of the overlying water layer, C_∞ is the minimum sulfate concentration, and b could be computed by fitting the function $C_x = (C_o - C_\infty) \cdot e^{-bx} + C_\infty$ on the measured sulfate profile.

Diffusion measurements. The application of the model requires an independent determination of the diffusion constant D_s. One method of estimating D_s is the instantaneous source technique (5). Here the diffusion profile developed from a momentary release from a thin layer placed above the sediment is used. Measurements for the instantaneous source technique were done in a subcore (12 by 3 cm) at the environmental temperature of 6°C. To suppress sulfate reduction, the sediment was mixed with Na_2MoO_4 at a final concentration of 20 mM, which is 100 times higher than the concentration necessary for complete inhibition of sulfate-reducing activity in freshwater sediments (20). The subcore was incubated overnight at 6°C; on the following day a 0.5-cm layer of water containing 10 mM Na_2SO_4 was carefully placed above the sediment, and the core was incubated for 3.75 h before the sulfate concentration profiles were measured. The diffusion coefficient is found from the equation

$$\log_{10} C_{(x,t)} = a(t) - \frac{x^2}{4\,D_s t} \cdot \log_{10} e$$

where $C_{(x,t)}$, the concentration at depth x, is obtained from the sulfate concentration profile at time t and $a(t)$ is a function of time. For a constant t, $a(t)$ is constant and D_s can be calculated from the slope of the regression line of a plot of x^2 against $C_{(x)}$.

A second method of estimating D_s is the constant source technique (5). Here the diffusion profile developed from a constant sulfate input is used. The measurements were done in an intact Jenkin core with a water layer containing 20 mM Na_2MoO_4 35 cm above the sediment. After 24 h, the permeation of MoO_4^{2-} suppressed sulfate reduction in the sediment. At this time sulfate was added to the upper water layer to a final concentration of 10 mM. The column was incubated overnight. On the following day the sulfate concentration profile in the upper 4 cm of the sediment was measured. The diffusion constant D_s is then determined from the equation

$$C_{(x,t)} = C_o \cdot \text{erfc}\,[x/2(D_s t)^{1/2}]$$

where at time t the concentration C_x at depth x is obtained from the sulfate concentration profile. C_o is constant in time, and erfc denotes the complementary error function. D_s was estimated from the error function graph described by Duursma and Hoede (5).

$^{32}SO_4^{2-}$ batch experiments. For estimating sulfate reduction rates, sediment samples (20 ml) were taken anaerobically at different depths from a Jenkin core by syringe and transferred into 30-ml serum vessels. The serum vessels were capped with butyl rubber stoppers, and the headspace was flushed with oxygen-free nitrogen. The sediment batches were mixed with Na_2SO_4 (final concentration, 2 mM) and placed into an Perspex jar (GasPak; BBL Microbiology Systems, Cockeysville, Md.) for anaerobic incubation for 14 days at 4°C with gentle mixing. During incubation, subsamples of the sediment were taken and analyzed for sulfate.

$^{35}SO_4^{2-}$ tracer experiments. Undiluted sediment samples (6 ml) from various depths were taken horizontally from the Jenkin core, as described above, for incubation with $Na_2{}^{35}SO_4$. The samples were anaerobically transferred into 7-ml pico-vials (Packard, Brussels, Belgium). Each pico-vial was capped with a butyl rubber stopper, and the headspace (1 ml) was flushed for 5 min with oxygen-free nitrogen which had passed through a column of BASF catalyser R3-11 at 150°C. After the headspace had been flushed, an anaerobic solution containing 1.78×10^5 dpm of $Na_2{}^{35}SO_4$ in 0.1 ml of distilled water (Amersham Corp., Little Chalfont, England) was added by piercing the rubber stopper with a syringe. The sediment samples in the pico-vials were stirred vigorously on a Vortex mixer for a uniform distribution of the

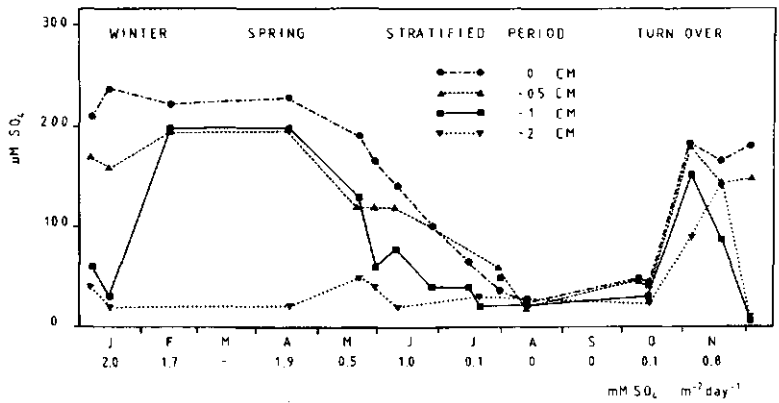

FIG. 1. Sulfate concentrations in the pore water of the upper 2 cm of sediment in Lake Vechten in 1983. The sulfate reduction rates obtained from the profiles and calculated from the model are given for each month. The horizontal axis has two scales; the upper scale shows time in months and the lower scale shows sulfate reduction rates in millimoles of SO_4^{2-} per square meter per day.

$^{35}SO_4^{2-}$ label. The samples were incubated in the dark at 6°C (in situ temperature). During the incubation, 1-ml samples were taken with a syringe at hourly intervals. No change in the natural pH (7.2) was observed. The samples were transferred into pico-vials which were previously flushed with nitrogen.

The pico-vials were immediately weighted and centrifuged at 1,000 × *g* for 1 min at room temperature. After centrifugation, 300 μl of pore water was withdrawn by syringe, transferred into centrifuge tubes, and stored at −20°C for sulfate analysis. The centrifuged pico-vials with the precipitated sediment were frozen by immersion in an acetone-dry ice mixture for 15 min and stored under nitrogen for subsequent distillation and radiochemical analysis. For simultaneous $^{35}SO_4^{2-}$ and $^{32}SO_4^{2-}$ measurements, the frozen pore water was thawed and centrifuged before injection into the high-pressure liquid chromatograph. The $^{32}SO_4^{2-}$ concentration was measured by the UV detector as described above. For $^{35}SO_4^{2-}$ measurements, a 6-ml fraction which included the sulfate peak volume (3 ml) was collected in a scintillation vial to which 11 ml of Instagel II (Packard) was added. The radioactivity in the vials was counted in a Packard Tri Carb 4530 liquid scintillation spectrometer for 20 min at a window breadth of 4-167 KeV. Quench curves of the eluent (0.4 mM 5-sulfoisophthalic acid) showed an excellent count efficiency (95% or more), in contrast to that of the untreated pore water (below 60%). This efficiency is explained by the complete separation of the sulfate peak from potential quenching components (organics and cations) and the lack of absorbance of the eluent above 300 nm, in which spectrum region the scintillator emits. Calibration curves with a calculated amount of radioactivity (1,000 to 1,500 dpm per 200-μl injection) that were used for external standardization and for correction of the decay time (87.9 days) were linear (r = 1.00000). Standard addition of $^{35}SO_4^{2-}$ to the sediment showed a recovery of 98.5% from the pore water.

The $^{35}S^{2-}$ produced from the same sediment samples were measured by a modification of the method of Jørgensen (13) on the day of incubation. The modification was the substitution of a microdistillation apparatus which consisted of the pico-vial containing the frozen sediment sample after centrifugation and two pico-vial traps in series connected by a short segment of thick-wall Teflon tubing. Seven units were placed parallel on a Perspex glass plate for simultaneous distillation. The traps contained 2 ml of zinc acetate solution, 0.5 ml of NaOH, and 0.1 ml of isoamyl alcohol. It proved essential to add the isoamyl alcohol as antifoam in the microdistillation apparatus. The samples were stirred with microbars and tape-recorder motors which were mounted under a Perspex glass plate. Before distillation, the system was flushed with oxygen-free nitrogen. At this stage the samples were still frozen. The nitrogen flow (± 5 ml min^{-1}) was adjusted for each sample unit to prevent cross-contamination by siphoning. Distillation of the acid-volatile sulfide was initiated by the addition of 1 ml of 4 N HCl, which reduced the pH of the sediment to 0. The liberated $H_2{}^{35}S$ was carried with the nitrogen into the pico-vial traps within 40 min. A solution of 5 ml of Instagel II (Packard) and 0.1 ml of NaOH was added to the traps for measurement with the scintillation counter. Standard addition experiments in which a series of $Zn^{35}S$ (382 to 1,520 dpm) was added to the sediment showed a linear recovery of 89 ± 7% (r, 0.9981) of the liberated $H_2{}^{35}S$ in the first trap. Quench curves of $Zn^{35}S$ made in the trap mixture showed an efficiency of 95%. We used ZnS to see whether the added amount of HCl was sufficient to release the volatile metal-bound sulfide pool and whether the released $H_2{}^{35}S$ was quantitatively trapped in the distillation train. The results agree with those found by Ingvorsen et al. (10).

Sulfate reduction rates were calculated from the formula

$$\text{rate} = k \cdot [^{32}SO_4^{2-}] = \frac{\ln(N_0/N)}{t} \cdot [^{32}SO_4^{2-}]$$

where N_0 is the added $^{35}SO_4^{2-}$ (disintegrations per minute per cubic centimeter), N is the $^{35}SO_4^{2-}$ rest activity at time t (days), and $[^{32}SO_4^{2-}]$ is the sulfate concentration obtained from the sulfate profile in the sediment (nanomoles per cubic centimeter).

Sulfide production rates calculated from the formula (13)

$$\text{rate} = \frac{[^{32}SO_4^{2-}] \cdot (^{35}S^{2-})}{(^{35}SO_4^{2-}) \cdot t}$$

where $[^{32}SO_4^{2-}]$ is the sulfate concentration (nanomoles per

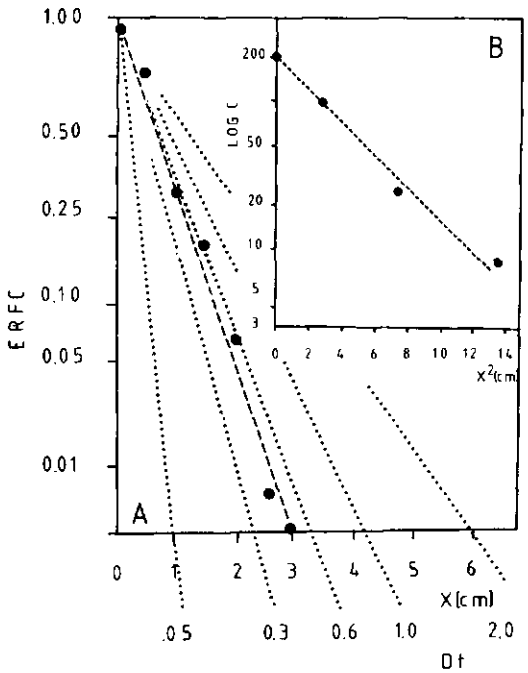

FIG. 2. (A) Plot of the inward diffusion of sulfate (stripes) from the water column into the sediment of a Jenkin core (constant source model). The scale on the vertical (y) axes of the graph was calculated from the complementary error function (ERFC) to arrive at a linear relation between the depth x (cm) and the fraction C_x/C_o (y coordinate). The diffusion coefficient D_s can be estimated from $D_s \times t$ by interpolation between lines (dotted) drawn for different Dt values (5). (B) Plot of the inward diffusion of sulfate from a momentary release from a thin (0.5-cm) water layer into a sediment subcore by using the instant source model. C (concentration of SO_4^{2-}) is plotted on a vertical logarithmic scale to arrive at a linear relation with the square of the depth (centimeters), from which slope D_s can be calculated.

cubic centimeter) of the sediment, ($^{35}SO_4^{2-}$) and ($^{35}S^{2-}$) are the sulfate and sulfide radioactivities (disintegrations per minute), and t is the incubation time (days).

RESULTS

Sulfate concentrations in the top 2 cm of the sediment decrease at the beginning of the summer stratification (Fig. 1). In the summer and early autumn the sulfate concentrations are low, and early in October the sulfate concentrations increase again. The deeper layers (1 and 2 cm deep) showed a second fall in sulfate concentration in the autumn and early winter. This is not observed in the more superficial layers of the water column just over the sediment. With the vertical sulfate profiles at the sediment-water interface, sulfate-reducing activities (Fig. 1) were estimated by the diffusion model. In the model, the diffusion constant calculated from the constant source ($[0.3 \pm 0.05] \times 10^{-5}$ cm^2 s^{-1}) (Fig. 2A) and from the instant source ($[0.3 \pm 0.03] \times 10^{-5}$ cm^2 s^{-1}) (Fig. 2B) were used. These values are similar to those for marine sediments if corrected for temperature (16, 17). The sedimentation rate (1.8×10^{-3} cm day^{-1}) was estimated from the thickness of the organic layer in the lake (20 to 30 cm) and the time in which this layer was deposited, namely about 40 years (22). The sulfate reduction rates obtained from the model (Fig. 1) at the 1-cm-deep layer roughly parallel the sulfate concentration. The highest sulfate reduction rates (2 mM m^{-2} day^{-1}) were observed in winter, whereas during summer stratification in August and September no sulfate reduction in the sediment was found.

A second estimation of sulfate-reducing activity was derived from the decrease in sulfate in a stagnant water column over a sediment core (Fig. 3). During the first 13 days of the incubation at 4°C, sulfate gradients were developed in the water phase, and from these profiles, a sulfate reduction of 0.6 mM m^{-2} day^{-1} was estimated. When the water column was mixed by gentle air bubbling, release of sulfate (5 mM m^{-2} day^{-1}) from the sediment occurred.

A third estimate of the sulfate-reducing activity was derived by following sulfate depletion in $^{32}SO_4^{2-}$-enriched sediment samples (10 times the natural concentration). During the first 7 days, sulfate reduction rates were linear, the highest being 107 to 110 nmol cm^{-3} day^{-1} in the batches from the upper 2 cm of the sediment, followed by 22 to 30 nmol cm^{-3} day^{-1} in batches from 2 to 6 cm and 6 nmol cm^{-3} day^{-1} in batches from 6 to 10 cm. After 7 days, the sulfate reduction rates in the batches increased, resulting in complete depletion of the sulfate pool in the batches from the upper few centimeters within 14 days. The total sulfate reduction from the sediment surface (1.7 mmol m^{-2} day^{-1}) was calculated by summation of the areal sulfate reduction rates.

The fourth estimate of sulfate-reducing activity is derived from the conversion of $^{35}SO_4^{2-}$ to $^{35}S^{2-}$. A logarithmic decrease of $^{35}SO_4^{2-}$ with time at various depths was observed for at least 3 h (Fig. 4A). Using the turnover rate constants derived from Fig. 4A (Table 1) and the actual in situ pool sizes which remained constant during incubation,

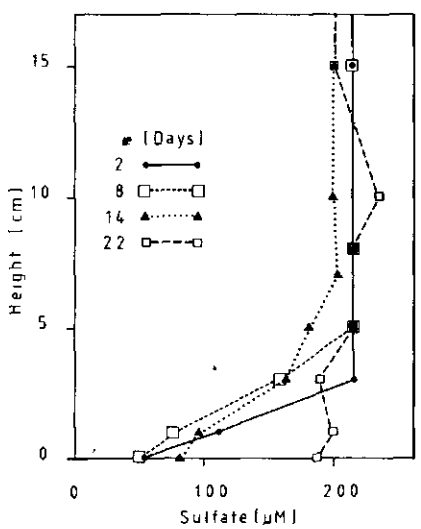

FIG. 3. Sulfate profiles in a stagnant water column above the sediment of a Jenkin core measured at different time intervals during the 22-day incubation at 4°C.

we calculated sulfate reduction rates for different depths at the sediment-water interface (Table 1). The summed sulfate-reducing activity for the different depths was 3.6 mM m^{-2} day^{-1}. A logarithmic increase of acid-volatile $^{35}S^{2-}$ was simultaneously observed in the same samples (Fig. 4B). the $^{35}S^{2-}$ generated in this experiment represents a significant proportion (33% or more) of the $^{35}SO_4^{2-}$ added to the sediment. The efficiency of conversion appears to increase with depth (Table 1). Summation of the turnover rates of $^{35}SO_4^{2-}$ obtained from the production rate constants of $^{35}S^{2-}$ (Table 1) gives a total sulfate reduction rate of 1.6 mM m^{-2} day^{-1}.

To determine the variation in sampling, sediment batches were taken from five different Jenkin cores at a depth of 2 cm. The batches were incubated at in situ temperature, and the conversion of $^{35}SO_4^{2-}$ into $^{35}S^{2-}$ was followed until the added $^{35}SO_4^{2-}$ (±21,000 dpm cm^{-3}) was nearly depleted (Fig. 5). A maximum of 80% of the added $^{35}SO_4^{2-}$ was recovered in the acid-volatile $^{35}S^{2-}$ pool after 1 h. The sulfate reduction rate in these samples was much faster than those shown in Fig. 4. The $^{35}S^{2-}$ in the sediment generated from the reduction of $^{35}SO_4^{2-}$ showed a loss with time after nearly all the $^{35}SO_4^{2-}$ had been depleted. The same phenomenon was observed in the inhibition experiments with 20 mM Na_2MoO_4 (Fig. 5). The addition of 20 mM Na_2MoO_4 completely inhibited the depletion of $^{35}SO_4^{2-}$ in the sediment batches after 0.5 h, after which the acid-volatile $^{35}S^{2-}$ pool decreased logarithmically and the $^{35}SO_4^{2-}$ remained constant.

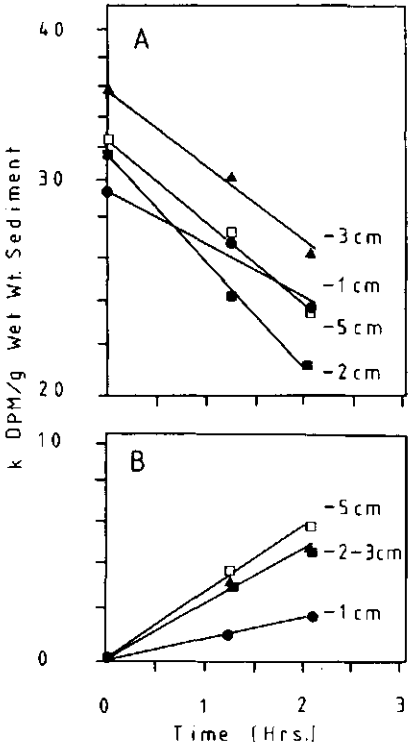

FIG. 4. Depletion of $^{35}SO_4^{2-}$ (A) and the production of $^{35}S^{2-}$ (B) on semi-log scale in sediment batches from several depths in the upper 5 cm of a Jenkin core.

TABLE 1. Sulfate depletion and sulfide production in sediment batches from Lake Vechten, calculated from the data in Fig. 4

Sample depth (cm)	Pool size of sulfate (μM)	Turnover rate constant (day^{-1})	Sulfate reduction (nmol cm^{-3} day^{-1})	Sulfide production (nmol cm^{-3} day^{-1})	Recovery (%)[a]	Recovery (%)[b]
1 ± 0.2	86 ± 10	2.1 ± 0.5	185 ± 65	61 ± 7	33	98
2 ± 0.2	32 ± 5	3.2 ± 0.4	107 ± 23	70 ± 22	65	108
3 ± 0.2	12 ± 5	3.2 ± 0.4	41 ± 20	24 ± 10	59	104
5 ± 0.2	3 ± 1	5.1 ± 0.4	24 ± 5	9 ± 3	38	101

[a] Percentage of sulfate recovered in the sulfide fraction.
[b] Recovery of $^{35}SO_4^{2-}$ at time t_0 after 1 min of mixing.

DISCUSSION

The dynamics of sulfate reduction in sediments were generally investigated by following the sulfide production (8, 10, 14, 15, 18–20), since a sensitive (microliter) method for determining sulfate was not available. With the recent introduction of liquid chromatography in sedimental sulfate analysis (7), it is now possible to measure sulfate depletion directly rather than analyze one of its conversion products (S^{2-}). Also, for accurate measurement of the easily disturbed steep gradients of sulfate (Fig. 1), only small samples (0.2 g [wet weight]) as used by IPC prevented mixing of the sediment layers. IPC is preferred for studying the conversion processes of sulfate in freshwater, since the technique is

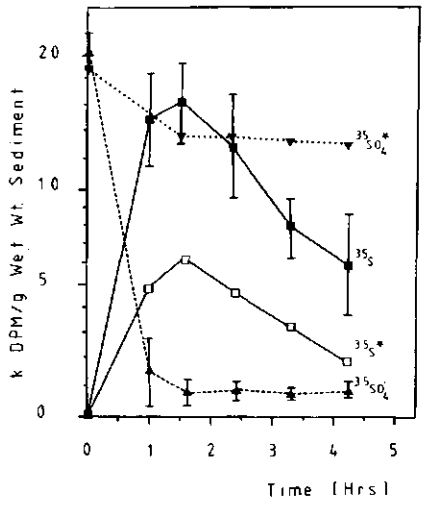

FIG. 5. Average depletion of acid-volatile ^{35}S in anaerobically incubated sediment batches of samples (from a depth of 2 cm) in five different Jenkin cores. The acid-volatile ^{35}S was generated by the conversion of $^{35}SO_4^{2-}$. (*) Inhibition effect of Na_2MoO_4 addition (after 1 h of incubation) on the conversion of $^{35}SO_4^{2-}$ into acid-volatile ^{35}S. Note the depletion of ^{35}S while the $^{35}SO_4^{2-}$ concentration remains constant.

TABLE 2. Comparison of sulfate reduction rates obtained from the model and from batch experiments

Method	Sulfate reduction rate (mM m^{-3} day^{-1})
Diffusion model	1.8
Incubation of stagnant cores	0.6
Batch incubation with $^{35}SO_4^{2-}$	1.7
Batch incubation with $^{35}SO_4^{2-}$	
Depletion of $^{35}SO_4^{2-}$	3.6
Production of $^{35}S^{2-}$	1.6

neither laborious nor difficult to set up. Since the sediments are not exposed to air, the risk of oxidation artifacts is considerably reduced. The reduction rates obtained from the model are mainly determined by the steepness of the measured concave-down profile, which were normally not found in marine sediments (14), since mixing within the core will smooth the natural gradient, resulting in an underestimation of the sulfate-reducing activity.

In Lake Vechten, sulfate-reducing activities were correlated with the seasonal fluctuation of the sulfate concentration in the lower layers of the lake (23). During summer stratification the sulfate in the hypolimnion is supplied mainly through diffusion from the metalimnion. In this period, sulfate reduction is low in the sediment and occurs in the hypolimnion. This is also evident from the sulfate depletion in the hypolimnion and the presence of sulfate-reducing bacteria in this layer (2). The relation between carbon input and sulfate consumption in the deepest water stratum and in the sediments of Lake Vechten was studied with the help of diffusion flux calculations and sedimentation measurements. At the start of the summer stratification, about 19% of the input carbon was mineralized by sulfate reduction, but at the end of the summer stratification this was about 8% (22a).

During the autumnal overturn, both increased turbulence and oxygen supply to the lower water layers result in a net production of sulfate that reappeared in the overlying water of the sediment. In winter, when the water layers in the lake are remixed, an equilibrium between sulfate conversion processes is established so that the concentration of sulfate in the lake does not change. The turbulence effect was imitated by the aerobic incubation of intact Jenkin cores (Fig. 3), which showed that the amount of sulfate withdrawn from the overlying water is controlled by the turbulence of the water layer. Oxygen and redox potential measurements by microelectrode techniques in the sediments of intact Jenkin cores showed a rapid decrease of 10 μg/ml to 0.5 μg of O_2 per ml and a redox potential fall of 170 mV in the upper 4 mm of the sediment, excluding chemical reoxidation of the reduced sulfur below this layer. These observations confirms the results of the model which showed the highest sulfate-reducing activity in the same small layer (0.5 to 2.5 cm deep) of the maximum abundance of sulfate-reducing bacteria (2).

Anaerobic incubation of sulfate-enriched sediment batches from this layer with sulfate concentrations 10 times the natural ones showed that the sediment can reduce this excess of sulfate completely without the limitation of the organic pool. This was indicated previously by assessing the pool sizes and turnover rates of important lower fatty acids (3, 6). The absence of the organic limitation permits first-order kinetics in the model, and no sulfate adsorption to the sediment was found (7). Sulfate reduction in the anaerobic sediment of Lake Vechten is therefore limited by the available sulfate concentration supplied by diffusion.

Comparison of the results of the model (Table 2) with those from the batch experiments in the same sulfate-reducing zone (0 to 5 cm deep) showed that reducing rates observed by incubation with $^{35}SO_4^{2-}$ (Fig. 4 and 5) were higher. This may be explained by the direct availability of sulfate from recycling processes to the sulfate-reducing bacteria in the upper 5 mm of the sediment. These recycling processes are not measured by the diffusion model. The applied model gives an estimate of the net sulfate reduction rates and may be useful in routine studies for comparing sulfate-reducing activities in different freshwater sediments.

The most reliable sulfate reduction rates in sediments are obtained by measuring the depletion rate of sulfate simultaneously with their pool size; this gives the actual turnover rates. From the acid-volatile sulfide depletion shown in Fig. 5, it may be concluded that sulfate reduction rates in freshwater sediments are easily underestimated if only sulfide production is followed. This is because the addition of molybdate (Fig. 5) shows that when sulfate reduction is completely inhibited, the formed sulfide slowly depletes. The lower sulfate reduction rates derived from the production of acid-volatile $^{35}S^{2-}$ can be explained by a slow conversion of this pool to an undistillable fraction. The acid distillation does not include $^{35}S^{2-}$ label incorporated into compounds as pyrite and elemental sulfur, which must be determined by other means (9). It was recently reported (8, 9, 11, 15) that only 30% of the converted $^{35}SO_4^{2-}$ ends up in the acid-volatile pool which is recovered by distillation. In Lake Vechten sediment, 50% of the acid-volatile pool is converted into the non-acid-volatile pool within 3 h (Fig. 5). Howarth and Merkel (9) reported the rapid formation of pyrite in salt marsh sediments. From Fig. 5 we can conclude that after 1.5 h more than 85% of the reduced sulfur was present as $Fe^{35}S$. For this reason, we believed as a result of sequential reactions the reduced ^{35}S is more likely to end up in inorganic (FeS_2,S_0) instead of organic compounds. The incomplete recovery of all the reduced sulfur compounds by the acid distillation complicates an accurate estimation of sulfate-reducing activity in the sediment. For this reason, IPC is an additional, nearly indispensable, tool for studying the conversion processes of sulfate in freshwater.

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CHAPTER 3.3

Factors affecting the sulfur cycle in a stratifying freshwater lake : Indications for substantial cycling of unknown sulfur species.

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Factors affecting the sulfur cycle in a stratifying freshwater lake: Indications for substantial cycling of unknown sulfur species.

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Running title: Sulfur cycling in a freshwater lake

Key words: sulfur cycle, lakes, sediments, sulfur sedimentation.

ABSTRACT

Non-sulfate sulfur species represented 50-90% of the lake water sulfur pool (80-200 µM) of Vechten, a meso-eutrophic monomictic freshwater lake. Only 5-10% of the lake water sulfur pool was ΣH_2S or FeS and as little as 2% was associated with sestonic matter. Thus, the role of sulfur as electron carrier in freshwaters may not be limited only to ΣH_2S and sulfate cycling; the unidentified sulfur pool may be an important electron donor for phototrophs in the metalimnion. Pronounced seasonal trends in the concentrations of the sulfur pools were observed only in the lake water column. The seasonal trends in sulfur sedimentation rates (up to 1.1 mmol m^{-2} d^{-1}) resemble the trends in the oxygen-, sulfate- and iron concentrations. About 40% of the sulfur sedimentation rate can be assigned to precipitation of FeS.

INTRODUCTION

Sulfur species play an important role in the energy flow during synthesis and breakdown of organic matter in lakes (Hordijk and Cappenberg 1985). Sulfate acts as an electron acceptor in dissimilatory processes in the sediment, and sulfide as an electron donor for phototrophs in the metalimnion (Steenbergen and Verdouw 1984). Steep sulfate concentration gradients in near-surface sediment of sulfate- limited freshwaters are caused by dissimilatory sulfate reduction (Hordijk et al. 1987; Bak and Pfennig 1991). Sulfide generated by this process may react with the sediment to form a variety of organic and inorganic sulfur compounds (Nriagu and Soon 1985; Moers et al. 1988; Feijtel et al. 1989). In sediments with labile Fe concentrations exceeding 350 mmol (kg [dry weight])$^{-1}$, sulfide reacts with Fe^{2+} to form FeS, which accumulates in the sediment (Baker et al. 1989; Hordijk et al. 1989). In lake Vechten, over 60% of the profundal sediment-sulfur pool consists of amorphous FeS precipitates, giving the sediment a deep black color. Not all the reduced sulfur is permanently immobilized in the sediment. The sediment sulfur pool is affected by input due to sulfate reduction and seston-sedimentation, but also by

releases of sulfide and sulfate into the hypolimnion. These processes may also temporary alter the sulfur content of the lake water column. Insight in sulfur cycling is essential to discriminate between seasonal variations in sulfur pools and anthropogenic enrichments due to sulfur pollution.

Many studies on sulfur cycling are focused on sulfur accumulation in sediments and/or SO_4^{2-} and ΣH_2S transformations (Gorham 1969; Davison and Heaney 1978; Nriagu and Soon 1985; Baudo and Muntau 1986; Stauffer 1987). Studies dealing with seasonal cycling of total sulfur between lake water column and sediment are scarce (Stuiver 1967; Sholkovitz and Copland, 1982; David and Mitchell 1985). Even few deal with sulfur sedimentation-rate data (Baker et al. 1989; King and Klug 1982; David and Mitchell 1985).

Compared to marine systems, sulfate concentrations in freshwaters are low, but other sulfur species may be relatively more important. Methods like induced coupled plasma spectrometry and ion chromatography have simplified the analysis of total sulfur and sulfate. This enables a simple assessment of the non-sulfate pool in the lake water column. Such information is, however, only functional if it could be related with lake's hydrology and biochemistry. The study presented here relates trends in lake water sulfur with sulfur sedimentation rates, Fe cycling and sedimentary sulfur pools. It gives a synopsis of those processes that directly affect sulfur cycling between the sediment and lake water. The relationships between the S cycle and other limnological features of lake Vechten are discussed.

METHODS

Study area

This study was carried out in the eastern basin of Lake Vechten, an isolated meso-eutrophic lake (52°04'N and 5°05'E; 4.7 ha) with limnetic properties that have been constant for many years (see in Gulati and Parma 1982; Verdouw et al. 1987). The lake

was formed in 1941 by excavation of sand for construction of a nearby highway. The lake has no surface inlet or outlet and may be classified as a seepage lake; water residence time is approximately 5 years. It is warm monomictic; thermal stratification begins at the end of April and lasts until late October. The eastern basin has a maximum depth of 10.5 m, with a strongly reduced black sediment layer of about 30 cm, overlying an impermeable clay layer. Chemical characteristics of the profundal sediment are given in Table 1.

Table 1. Visual inspections and dry mass profiles of sediment cores indicated that the sediment in the sampling area was homogeneous. The sediment pore water has a pH of 6.5 to 6.8, and 4.3 mM HCO_3^-. The lake surface water has a pH of 7.7 to 9.0 and 2.6 mM HCO_3^-. (Verdouw and Dekkers, 1980).

depth	Dm	C_i	C_o	Fe_t	Fe_d	FeS_2
cm	%	%	%		mmol kg dm^{-1}	
1.5	13	1.7	6.7	114	0.7	23
4.5	19	1.5	5.7	159	1.0	30
7.5	24	1.4	4.9	193	1.1	25
10.5	28	5.9	4.3	225	1.2	24

Table 1. Depth distribution of some compounds in the sediment of Lake Vechten. Abbreviations: Dm, dry mass; C_i, inorganic carbon (% of the dry mass); C_o, organic carbon (% of the drymass); Fe_t, total iron; Fe_d, dissolved iron; FeS_2, pyrite; FeS, acid volatile sulfide. Values are give in mmol (kg [dry mass])$^{-1}$

Sulfate analysis

Sulfate concentrations were analyzed by ion chromatography with indirect-photometric detection (Hordijk et al. 1985). The chromatograph consisted of a model 3000

LDC-MILTON ROY pump with a model 3100 LDC-MILTON ROY UV detector. Samples were introduced by a Rheodyne 7125 valve with a 10 µl loop. The column consisted of a metal holder, a 10 * 3 mm pre-column and a 100 * 3 mm glass cartridge filled with 5 µM Ionospher-A (Chrompack, Middelburg, the Netherlands). The eluent was 0.4 mM 5-sulfoisophthalic acid (Aldrich, Beerse, Belgium) in Milli-Q water (pH 4.7). Sulfate concentrations were evaluated by external standardization (detection limit < 20 pmol; C.V. = 6%, for 3-250 µM).

Sediment sulfur analysis

Profundal sediment was collected using a Jenkin sampler (Cappenberg 1974). With this device, acrylic-glass sampling cores (50 cm long and 7 cm I.D.) were sunk gently into the sediment. The cores included about 15 cm of sediment and 35 cm overlying water. Cores were collected at monthly intervals from August 1987 to September 1988 (except January). Sediment samples were taken from the top 12 cm of the Jenkin core at least eight different depths. The samples (1-3 g wet weight) were drawn by a 5-ml syringe through 2.8 mm holes (covered with Scotch tape No. 471) in the core at 1.0 cm depth-intervals. The sediment was dried at 70 °C, ground, and stored in screw-cap glass bottles after the dry mass was determined. At 70 °C losses of sulfur are minimal (Baudo and Muntau 1986).

Total sulfur was measured as sulfate after oxidation of the sediment with KNO_3 at 610°C (Hordijk et al. 1989). Before the assay, 0.5 ml H_2O_2 (30% v/v, P.A. quality, Merck Darmstadt, West Germany) was added to the sediment for oxidation (1 h). The oxidized sediment was dried (70°C), ground (<85 µM) and 100 mg was transferred into 20-ml glass scintillation vials (Packard, Brussels, Belgium). The sediment was mixed with 0.5 g ground Na_2CO_3 (Merck, Suprapur Quality) and 0.05 g KNO_3. The vials were ignited at 610 $\pm$ 10°C for 4.5 hours. The residue was extracted with 10 ml Milli-Q water by sonification for 0.5 h. The extract was centrifuged for 10 min at 100 g. A 1-ml aliquot of

the supernatant was eluted through a cation exchanger (Dowex 50-x8 100-200 mesh: Fluka AG, Buchs, Switzerland). The eluate was diluted 10 times and 5 µl was injected into the ion chromatograph for SO_4^{2-} analysis. Total sulfur in the sediment was estimated from the SO_4^{2-} concentrations in the solute and the dry mass of the sediment.

Sulfur sedimentation rate estimates

Sulfur sedimentation rates were estimated from material periodically collected by sediment traps (Verdouw et al. 1987). The sediment traps consisted of three acrylic-glass cylinders (35 cm long by 7-cm diam), mounted on a disk. Four discs were installed in the centre of the lake at different depths in March 1982 (Steenbergen and Verdouw 1984). These depths were in the epilimnion (4 m), metalimnion (5 m), and hypolimnion (7.2 and 9.0 m). Trapped material was collected every 1-2 weeks from April 1982 to January 1983. Seston collected from traps submerged at the same depth was pooled, dried, and stored in screw-capped vials. The samples were ground, and 100 mg of each sample was withdrawn for analysis of total sulfur. Sulfur-sedimentation rates were estimated by multiplying previously published seston sedimentation rates (Verdouw et al. 1987) with the total sulfur contents of the seston.

Sulfate reduction rate estimates

Sulfate reduction rates in the lake water column were estimated by following the depletion of added $^{35}SO_4^{2-}$ in anoxic water batches. In summer of 1984 water samples were collected from the hypolimnion at 7.5 m, 8.1 m and 8.7 m in duplicate using a Friedinger sampler (Golterman 1970). A Jenkin sampler was used to collect water just above the sediment surface. Five sub-samples were withdrawn from the Jenkin core by syringe and stored in 100 ml serum flasks faced with rubber stoppers (no head space). To two of these samples 0.5 ml of a 0.6 M MoO_4^{2-} stock solution was added to inhibit sulfate reduction (Oremland and Capone 1988). After transport to the laboratory, about 15 ml of

water was withdrawn by syringe and transferred into 17 ml test-tubes faced with 1.5 cm thick butyl-rubber stoppers. The test-tubes were pre-flushed with nitrogen that had passed through a column of BASF catalyst R3-11 at 150 °C to remove oxygen. The test-tubes were incubated at 7 °C in the dark (in situ conditions) for 2 hours. Then, 1 ml of anoxic water containing 6667 Bq of $^{35}SO_4^{2-}$ (sodium salt: Amersham Corp., Little Chalfont, England) was added by syringe. The spiked solutions were incubated for 3 days at 7 °C and sampled periodically (0.5 ml) to follow the depletion of sulfate.

Sulfate was separated from other sulfur species by ion chromatography (injection volume, 200 µl: Hordijk and Cappenberg 1985). The effluent of the ion chromatograph was continuously flushed with He to prevent oxidation of sulfur species to SO_4^{2-} during elution. Effluent (5 ml), including only the separated SO_4^{2-} peak, was collected at the outlet of the UV detector in a 20-ml scintillation vial and mixed with 11 ml scintillator fluid (Instagel II; Packard, Brussels, Belgium). Radioactivity of the mixture was counted for 10 min using a Packard Tri Carb 4530 ß liquid-scintillation counter (window size 4-167 KeV; accuracy better than 1% for 16.7 Bq and 16 ml gel).

Sulfate reduction was also assessed by following the generation of radioactive acid volatile sulfide (AVS) in $^{35}SO_4^{2-}$ spiked lake water batches. Lake water was collected at 30-cm depth intervals from 6.0 to 10.2 m depth In September 1985. The water was collected in a serum vial of 100 ml using a Vålas sampler (Steenbergen et al. 1987) After transport to the laboratory, the serum vials were spiked with $^{35}SO_4^{2-}$ (final concentration, 333 Bq ml^{-1}) and incubated at 7 °C in the dark for 155 min. Thereafter, a 4.5-ml sample was withdrawn by syringe and transferred into a micro-distillation apparatus to separate AVS released after acidification of the sediment with HCL (Hordijk and Cappenberg 1985). The radioactivity of the trapped AVS was counted for 15 min.

Lake water sulfur analysis

Concentrations of total sulfur were determined in unfiltered lake water. Lake water (2 l) was collected from 1.5 m to 10.5 m at 1.5 m intervals in the middle of the lake by a Friedinger sampler. Water just above the sediment was collected with the Jenkin sampler. Nine profiles were obtained between March 1989 and March 1990. The lake water column was sampled 3 times in winter, 4 times in summer and 2 times in autumn of 1989. A subsample (0.5 ml) was withdrawn for SO_4^{2-} analysis for each depth.

For analysis of total sulfur 2 l water of each depth was evaporated at 70 °C. The dry mass of the residue (about 250 mg l^{-1}) was determined and 100 mg of this was mixed with 0.5 g Na_2CO_3/KNO_3 mixture and ignited at 610 °C for 4.5 h. The formed sinter was extracted with 10 ml Milli-Q water by sonification for 0.5 h. One ml suspension was transferred into a 20-ml teflon vial and a mixture of 6-ml HCl (37%) and 2-ml HNO_3 (65%) was added. The vials were screw-capped and heated for one hour at 100 °C on a sand bath. The acid mixture was diluted to 1 l with Milli-Q water before analysis by inductively coupled plasma emission spectrometry (ICP; Atom Scan 25) for sulfur and iron. The dry masses of the lake water samples were used to estimate the actual total iron and sulfur concentrations in the lake. The residual sulfur pool (S_{res}) was defined as the total sulfur pool minus the sulfate pool.

Equilibrium calculations

Solubility calculation offers a method to evaluate possible precipitation of metastable iron sulfides in hypolimnetic lake waters. Saturation indices (SI) with respect to amorphous FeS, mackinawite ($FeS_{0.94}$) and greigite (Fe_3S_4) were calculated using ion activity products (IAP) of the measured Fe^{2+}, ΣH_2S concentrations and pH in the hypolimnion, assuming equilibrium with orthorhombic sulfur (Schoonen and Barnes 1991). The equations used for equilibrium calculations are summarized in Table 2.

$$Fe_xS_xS_y(s) + xH^+(aq) \rightleftharpoons xFe^{2+}(aq) + xHS^-(aq) + yS(s)$$

$$\log(IAP) = \log\left(\frac{[Fe^{2+}]^x \cdot [HS^-]^x}{\{H^+\}^x}\right) \cdot \gamma_{Fe^{2+}} \cdot \gamma_{HS^-}$$

$$\log\gamma_i = -0.5 \cdot z_i \cdot \sqrt{I}/(1 + \sqrt{I})$$

$$I = 0.5\ \Sigma c_i z_i^2$$

(i= HCO_3^-, Cl^-, SO_4^{2-}, PO_4-P, Ca^{2+}, $Mg2^+$, Fe^{2+}, Na^+, NH^{4+}, Mn^{2+}, K^+; pH=7.0: data from Gulati and Parma 1982)

$$SI = \log(IAP) - \log(K_{Fe_xS_xS_y})$$

$$\ln K_T = \ln K_{298} + \Delta H_r/R\ (1/298 - 1/T)$$

{ } = Species activity (mol l^{-1})

[ ] = Species concentration (mol l^{-1})

$K_{FexSxSy}$ = Solubility product for an iron sulfide phase

I = Ionic strength of the solution (mol l^{-1})

z_i = charge of the ion species i

c_i = concentration of the ion species i (mol l^{-1})

γ_i = Activity constant of the ionic species i

ΔH_r = Enthalpy of reaction (J mol^{-1})

Table 2. Summary of equations used for equilibria calculations

A saturation index, SI>0 indicate supersaturation with respect to $Fe_xS_xS_y$ and SI<0 indicates undersaturation. Concentrations of HS^- were calculated from ΣH_2S concentrations using the first and second dissociation constants of H_2S. pK1 was corrected for temperature (Millero 1986). The solubility products of iron sulfides are given in Table 3.

	pK
Amorphous FeS	2.99
Mackinawite ($FeS_{0.94}$)	3.60
Greigite (Fe_3S_4)	12.85

Table 3. Solubility products of iron sulfides at 25°C.
Free energy data from Berner (1967).

The reaction enthalpies could not be calculated for mackinawite and greigite, so solubility products were not corrected for temperature. We applied the temperature correction for amorphous FeS by using ΔH_r of troilite, a crystalline FeS phase (ΔH_r = -5193 J mol^{-1}, Robie et al. 1978).

Sulfate release estimates in slurries

The generation of SO_4^{2-} was followed under several conditions; total sulfur was not examined. One to 10 g surface sediment were sampled using a 10-ml syringe during the anoxic period. The samples were diluted 5, 10 or 20 times and the slurries were continuously flushed with air for 6 days. The release of SO_4^{2-} in the slurries was followed by periodic sampling.

For discriminating between chemical and microbial oxidation, two slurries were sterilized by gamma-irradiation (4.5 MRad). The release rates of SO_4^{2-} during aeration of both sterilized slurries were compared with those in untreated slurries.

To examine the effect of inhibiting sulfate reduction, MoO_4^{2-} (final concentration 0.5 mM) was added to two slurries and the release of SO_4^{2-} during aeration was compared with those in untreated slurries.

Estimates of total sulfur and sulfate release rates from cores

To study the SO_4^{2-} release rates from the sediment into the lake during the oxic period, three sediment cores, including about 35 cm superficial water and 15 cm sediment, were collected just before the fall overturn in October 1991. The overlying water was flushed gently with a stream of finely dispersed air bubbles (1-2 ml min^{-1}). Subsamples were taken every 2-3 days to follow the sulfate increase in the superficial water for 15-30 days. The release rate of total sulfur was also measured in the superficial water layer of the sediment column. Water samples (10 ml) for total sulfur analysis were withdrawn after 4, 6, 12, and 30 days. The samples were analyzed by ICP without further sample preparation.

The effect of MoO_4^{2-} on the release of total sulfur and sulfate was assessed in parallel experiments. Two sediment cores were collected and MoO_4^{2-} (final concentration of 0.5 mM) was added to the overlying water before the release of total sulfur and SO_4^{2-} was followed under oxic conditions.

RESULTS

Sediment sulfur

Sediment samples collected in 1987 and 1988 had an average total sulfur concentration of 93.8 mmol (kg [dry mass])$^{-1}$ in the top 10 cm (coefficient of variation [C.V.] = 20%; n = 12; Table 4).

Depth	Dwt	TS	S_o	FeS_2	ES	AVS	S_{res}
cm	g l^{-1}	mmol S l^{-1}					
0-1	26	45.6	0.4	3.8	4.6	31.5	5.3
1-2	138	46.8	1.1	4.6	2.8	23.2	14.9
2-3	148	53.6	0.8	4.6	2.7	27.3	18.3
3-4	152	65.8	0.8	4.8	3.9	29.6	25.7
4-5	162	67.2	0.8	8.0	5.4	30.9	22.2
5-6	174	55.3	0.5	7.8	4.4	22.7	20.0
6-7	181	61.7	NA	7.4	5.6	25.3	23.4
7-8	193	55.8	NA	8.0	7.3	28.4	12.1
8-9	203	47.2	NA	6.6	9.4	23.6	7.5
9-10	208	54.1	NA	4.4	9.7	32.4	7.5

Table 4: Major sulfur fractions in a sediment core of Lake Vechten collected in spring 1986. For methods see in Hordijk et al. 1989. Abbreviations Dwt, dry weight; TS, total sulfur; S°, elemental sulfur; FeS_2, pyrite; ES, estersulfates; AVS, acid volatile sulfide; S_{res}, residual sulfur; and NA, not analyzed.

The average sediment sulfur values in July 1987 and in November 1988 are in the same range as those measured in July 1986 by plasma spectrometry and in November 1986 by

roentgen-fluorescence spectrometry (Hordijk et al. 1989). The sediment sulfur pool did not have a clear seasonal trend.

Lake water

Lake water profiles of total sulfur, SO_4^{2-}, S_{res} and total Fe did not vary with depth during winter 1989 (Fig. 1A).

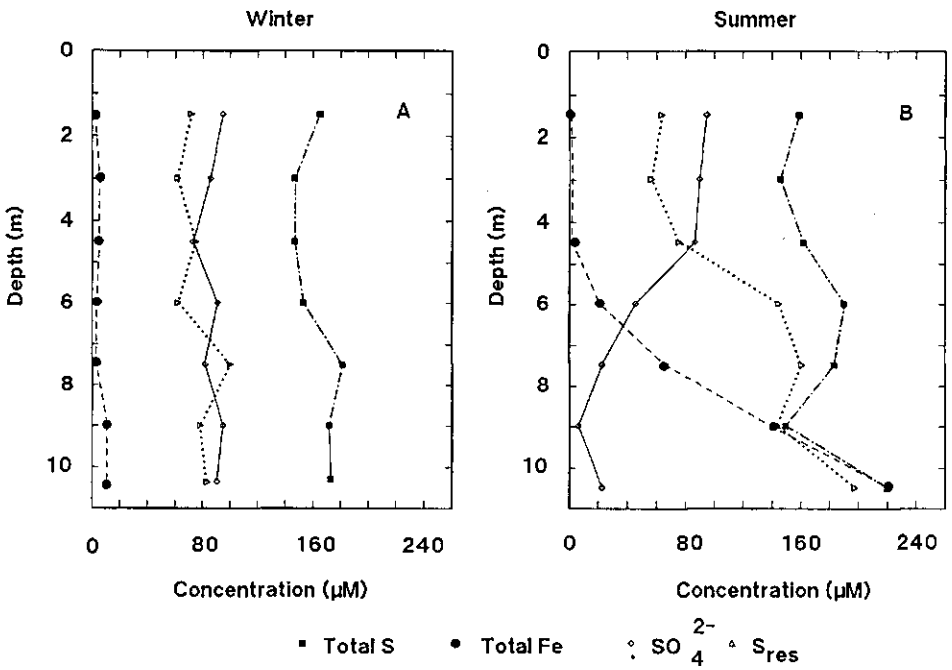

Fig. 1. Concentrations of total S, sulfate-S, non-sulfate S and total-Fe in the lake water column sampled during: A) the circulation period in April 1988 and, B) the stratification period in August 1988. As much as 50% of total sulfur in the

lake water consisted of SO_4^{2-} in the oxic period. During stratification, SO_4^{2-} decreased while S_{res} and total Fe increased in the hypolimnion (Fig. 1B). Similar trends in SO_4^{2-} and total Fe concentrations were seen in 1963-1965, 1973-1975, and 1982 in the hypolimnion. The seasonal trends in Fe concentrations in the hypolimnion in 1973-1975 closely resembled those in 1989 (Fig. 2A).

Hypolimnetic Fe concentrations reached their maximum (230 µM Fe) near the sediment (10.5 m) at the advent of fall overturn (November 1989). Highest ΣH_2S concentrations ($\pm$ 10 µM) were found in September-November and represented about 5-10% of the hypolimnetic total sulfur. No ΣH_2S was detectable in the lake water column in the oxic period. In the sediment, ΣH_2S concentrations remained below 1 µM. Thiosulfate, tetrathionate, or elemental sulfur were below the detection limits (< 1 mg l-1: Hordijk et al. 1989). Trends in O2 and SO_4^{2-} in 1982 (Fig. 3) and 1989 corresponded well to those found previously in Lake Vechten (Van Gemerden 1967; Verdouw and Dekkers 1980; Steenbergen and Verdouw 1984).

Apparently, the major limnetic aspects of the lake have shown minor variations over the last 20 years and the data collected at the same period of different years are, therefore, comparable. Total dry mass recovered after evaporation of the lake water samples collected from 0-10 m depth at 70 °C varied between 200 and 360 mg l^{-1} in 1989 (average 250 mg; n = 60) and showed no clear trends with season or depth.

Sulfur sedimentation rate estimates

Sulfur sedimentation rates based on material collected in traps installed at 4.0 m (not shown), 5.0 m and 7.2 m depth (Fig. 4A-B) remained low. The rates at a depth of 9.6 m, however, were much higher and had a seasonal variation (Fig. 4C), increasing from 0.19 mmol S (kg [dry weight])$^{-1}$ d^{-1} in the oxic period to about 1.1 mmol S (kg [dry weight])$^{-1}$ d^{-1} in the anoxic period (Fig. 4C). During fall overturn, S-sedimentation decreased again down to winter values (0.2 mmol S (kg [dry mass])$^{-1}$ d^{-1}). The second increase in S-

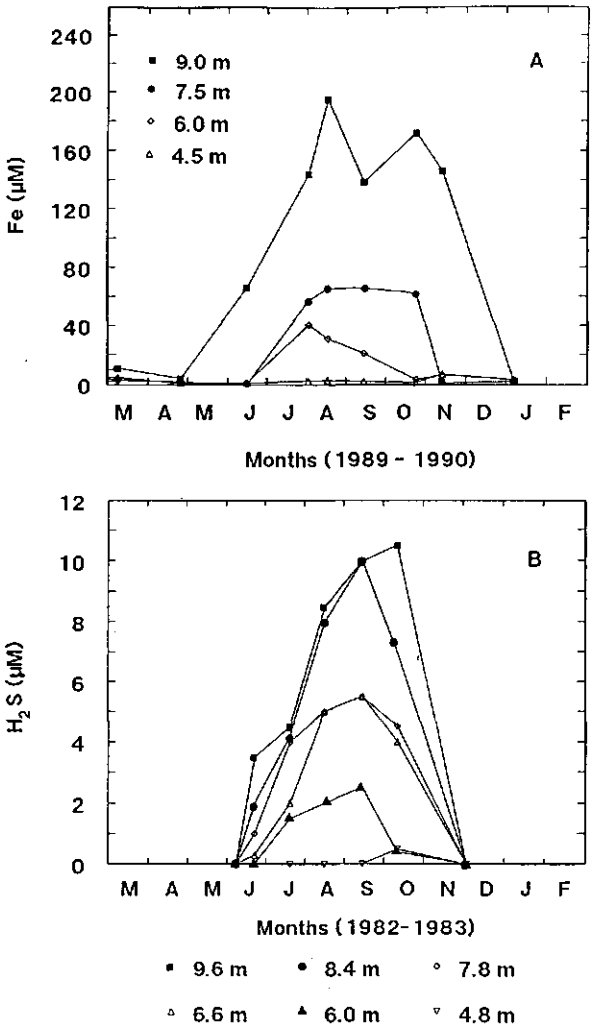

Fig. 2. A) Concentrations of total Fe in 1989-1990 and, B) ΣH_2S concentrations in 1982-1983 in the bathylimnion of Lake Vechten.

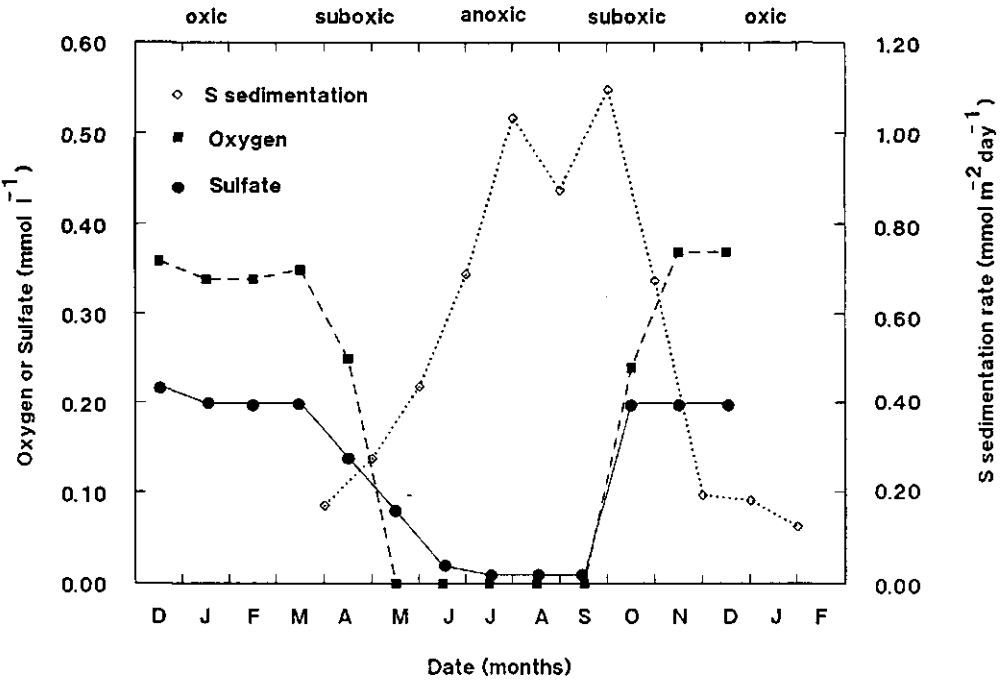

Fig. 3. Comparison of the seasonal trends in oxygen and sulfate concentrations in the lake water column (measured in 1982) with the sulfur-sedimentation rates at 9.6 m depth in Lake Vechten (measured in 1989).

sedimentation was apparently related with an increase in seston sedimentation at the end of stratification (Fig. 5). Notable is also the close resemblance of Fig. 2A and Fig. 4C. Sedimentation rates for total sulfur and organic carbon sedimentation were unrelated. The annual seston-sedimentation rate at 9.6 m was 570 g dry mass m^{-2} yr^{-1} and the seston-sulfur sedimentation rate was 178 mmol S m^{-2} yr^{-1} (Table 5). Sedimentation rates estimates based on trapped material may be altered by mineralization in the traps. This problem was minimized by collecting the trapped material every 1-2 weeks (Callieri et al. 1991). In Lake Vechten, less than 10% of the trapped material was lost by mineralization during a 2-weeks incubation period

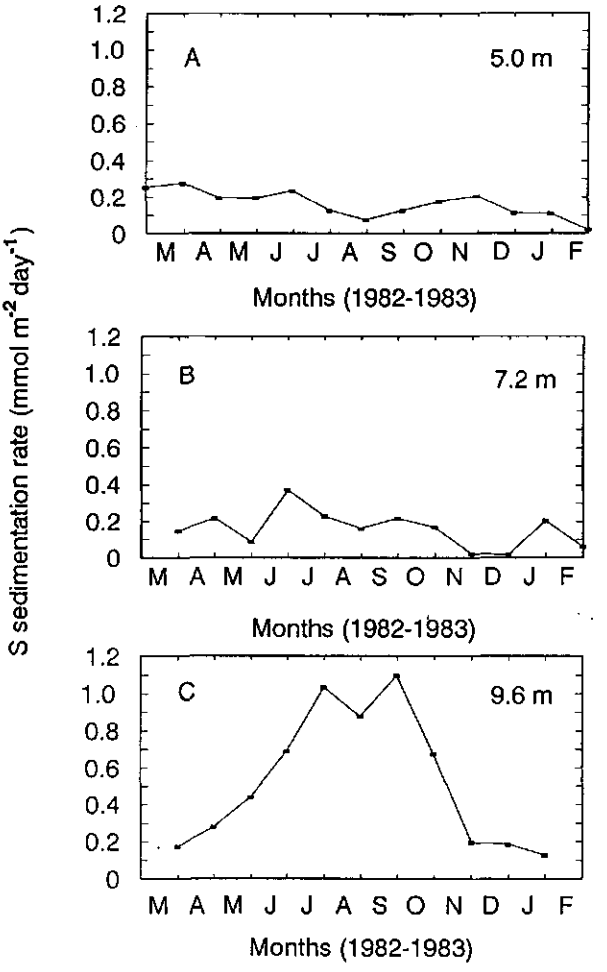

Fig. 4. Seasonal variations in S-sedimentation rates in the lake water column of Vechten in 1982-1983. The sediment traps were incubated for 14 days at 5.2 m (metalimnion); 7.2 m; and 9.6 m (hypolimnion).

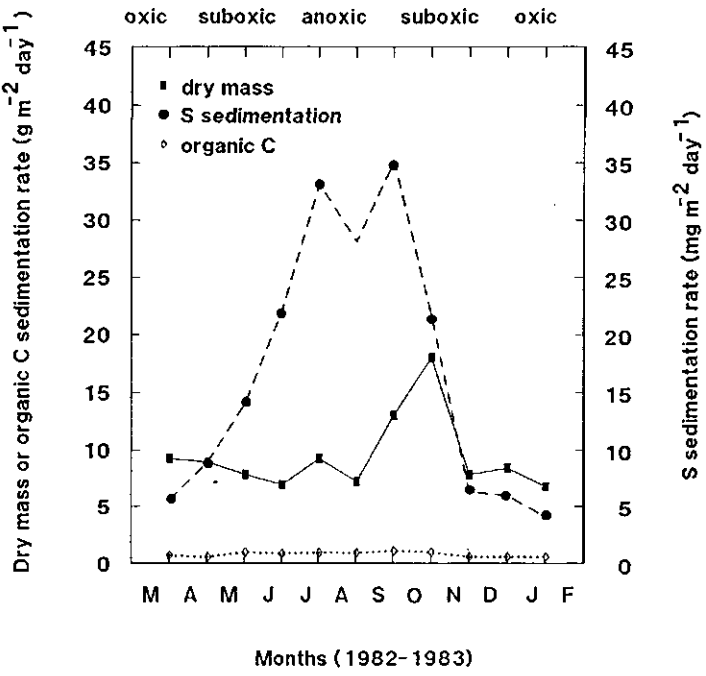

Fig. 5. Dry mass, total organic carbon, and total sulfur sedimentation rates derived from sediment traps installed at 9.6 m depth in the lake in 1982-1983.

(Steenbergen et al. 1987). The amount of sulfate that can be reduced in 14 days is insignificant in relation to the total amount of sulfur that is trapped in the same period (Hordijk et al. 1985). Thus the traps provided reliable estimate of sulfur sedimentation rates in the pelagic region of the lake.

	Wintergreen	South Lake	Vechten
Sedimentation rates in the hypolimnion			
Seston (g m^{-2} yr^{-1})	1030	70	570
TS (mmol m^{-2} yr^{-1})	266	21	178
Sediment			
SR (mmol m^{-2} yr^{-1})	2490	2	1068
TS (µmol g^{-1})	204	167	940
AVS (% of TS)	4	13	60
Limnetic aspects			
pH	6.5-7.0*	4.9-5.4	7.6-9.0
Max. depth	6.5	18	11
Anoxia	Yes	No	Yes

Table 5. Comparison of seston sedimentation rates and relevant field data of 3 types of lakes. Abbreviations: SR, sulfate reducing activity; AVS, acid volatile sulfide. (* = sediment pH).

Saturation of iron precipitates

Vechten lake water was supersaturated (SI>0) with respect to amorphous FeS at 7.5 m and 9.0 m depths during the anoxic period from June until November (Fig. 6).

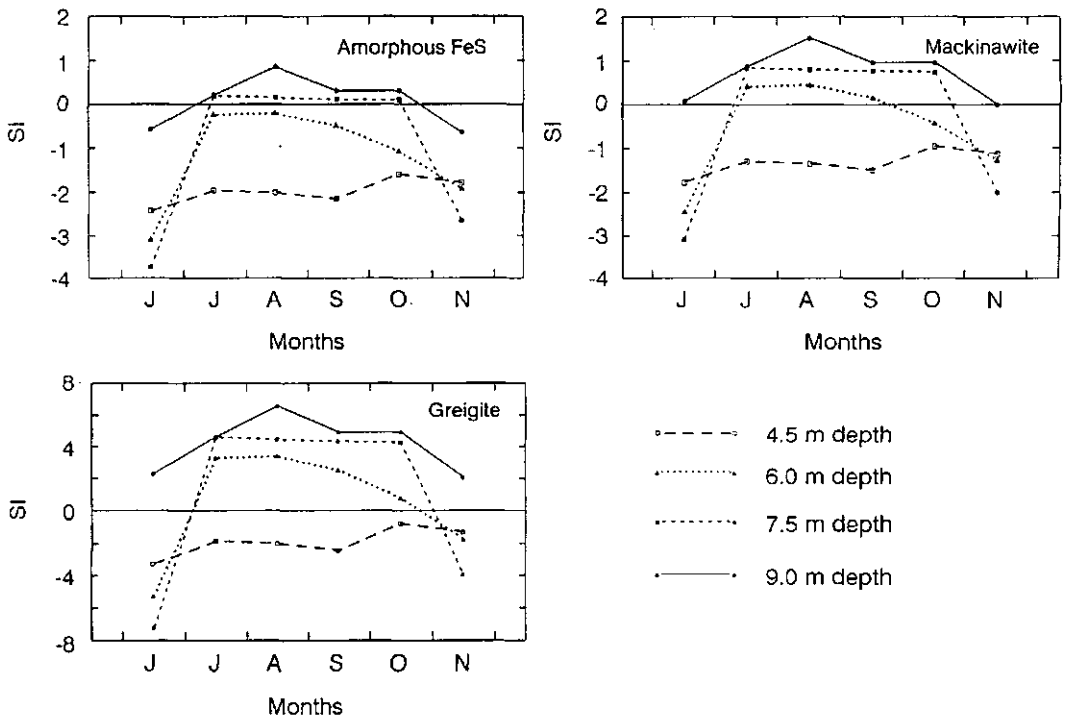

Fig. 6. Seasonal variations in saturation indexes in the hypolimnion of Lake Vechten estimated from field data collected in the summers of 1982 and 1989.

Supersaturation also occurred for the crystalline iron sulfides, mackinawite and greigite, at 6.0, 7,5 and 9.0 m during the anoxic period (Fig. 6). The SI values are upper limits because 1) solubility products were not corrected for temperature (or for amorphous FeS only partly corrected) and 2) complexation of Fe with dissolved organic matter was not considered. The presence of FeS precipitates was however evident from the blackening of 0.45 µ filters, and the disappearance of this color after the addition of HCl.

Sulfate reduction estimates in the hypolimnion

In the first experiment in which anoxic water from the hypolimnion was spiked with $^{35}SO_4^{2-}$, no decrease of SO_4^{2-} and $^{35}SO_4^{2-}$ concentrations occurred (not shown). During the first three hours, concentrations either remained constant or fluctuated anomalously. Thereafter, SO_4^{2-} and $^{35}SO_4^{2-}$ remained constant for two days. In the lake-water samples to which MoO_4^{2-} was added as an inhibitor for sulfate reduction, SO_4^{2-} and $^{35}SO_4^{2-}$ also remained constant during the two days incubation. In the second experiment, in which sulfate reduction was estimated from the generation of ΣH_2S, no radioactive ΣH_2S was found in any of the 14 water samples spiked with $^{35}SO_4^{2-}$ during 2.5 h incubation.
In conclusion, SO_4^{2-} reduction was not detectable in the hypolimnion.

Sulfate release rates in sediment slurries

Sulfate release in aerated sediment slurries was linear and increased with dilution (undiluted, < 15 mmol S (kg [dry weight])$^{-1}$ d^{-1}; 5 times diluted, 68 (SE = 4) mmol S (kg [dry weight])$^{-1}$ d^{-1}; 10 times diluted 103 (SE = 5) mmol S (kg [dry weight])$^{-1}$ d^{-1}). The sulfate release rates did not differ for the sediment collected at 1, 2, 3, and 5 cm. Sulfate release represented 36 $\pm$ 7% of the total sulfur release rate in the pore water. Traces of thiosulfate were also released when undiluted sediment was intensively aerated. The release rate of thiosulfate was about 1.7 mmol (kg [dry weight])$^{-1}$ d^{-1} for slurries collected from the top 5 cm. Thiosulfate, an intermediate in sulfur oxidation in freshwater sediments (Jørgensen 1990), was not found in untreated lake samples (detection limit < 5 µM).

To estimate how much sulfur could actually be converted to sulfate, surface sediment was 10-times diluted and aerated for 6 days. Aliquots were withdrawn periodically to follow the increase of the sulfate concentration in the slurry. Sulfate concentrations increased linearly (33.9 mmol S (kg [dry weight])$^{-1}$ d^{-1}; r, 0.986; SE = 4) and reached

constant values after 2.2 days until the end of the experiment. The total sulfur release into the pore water was 68 (SE = 4) mmol S (kg [dry weight])$^{-1}$ during the first day. The total amount of sulfate released after six days was 103 $\pm$ 5 mmol S (kg [dry weight])$^{-1}$. The total sulfur concentration of the sediment was 99.5 mmol S (kg [dry weight])$^{-1}$. Apparently, complete oxidation of the sedimentary sulfur pool to sulfate can be accomplished by intensive aeration of slurries for several days. The complete recovery of the sedimentary sulfur pool as SO_4^{2-} indicated that losses due to volatilization of sulfur were negligible. During the incubations, pH of the slurries dropped from 6 to 5. The effect of pH decrease on sulfur release rates was not investigated.

Addition of MoO_4^{2-} to sediment slurries reduced the release rate of SO_4^{2-} by about 20%. For example, during a 40-hour experiment, the SO_4^{2-} release rate was 38.4 (SE = 5.0) mmol SO_4^{2-} (kg [dry weight])$^{-1}$ d^{-1} in non-treated slurry and 57.1 (SE = 3.0) mmol SO_4^{2-} (kg [dry weight])$^{-1}$ d^{-1} in a slurry with 0.5 mM MoO_4^{2-}. Gamma-irradiation completely stopped the release of SO_4^{2-} in two slurries of sediment collected at 1 cm depth in the cores. Even after 25 hours of vigorous stirring and aerating, SO_4^{2-} release was undetectable.

Oxygen is depleted within the first 2-3 mm of the sediment under *in situ* conditions (Hordijk et al. 1987). Oxidation of reduced sulfur species is, therefore, not to be expected below this horizon. With an oxygen penetration depth of 2-3 mm and a SO_4^{2-} release rate of 15 mmol (kg [dry weight])$^{-1}$ for undiluted sediment, potential sulfate release rates from the surface sediment into the lake water column will be between 4 and 41 mmol m^{-2} d^{-1} during the oxic period.

Sulfate and sulfur release rates from sediment cores

Release rates of sulfate into the overlying water were measured in three untreated cores with aerated water. The cores were collected in October 1991. The sulfate concentrations

in the overlying water of the sediment cores increased linearly ($r > 0.96$) for 15 days incubation (average release rate 3.3 (SE = 0.3) mmol S m^{-2} d^{-1}). The release rates or sulfate in two reference cores treated with MoO_4^{2-} were 1.15 (SE = 0.12) and 1.22 (SE = 0.03) mmol SO_4^{2-} m^{-2} d^{-1}. The release rates of total sulfur in two untreated aerated cores, collected in October 1991, were 4.8 (SE = 0.1) and 7.2 (SE = 1.0) mmol m^{-2} d^{-1} and 3.1 (SE = 0.07) mmol m^{-2} d^{-1} in the reference core treated with MoO_4^{2-}. Thus, similar to the slurry experiments, release rates of sulfate and total sulfur in the MoO_4^{2-} treated cores were lower as those in the untreated ones. The pH of the superficial water in the cores gradually increased from 7.1 to 8.0 during the 15-days experiment,

The release rates of sulfate and total sulfur found in the batches and cores together with the sulfate-reduction rate estimates, measured earlier in the lake (Hordijk et al. 1985) are summarized in Table 6.

DISCUSSION

Seasonal variations in the lake

The sulfur cycle in lake Vechten is strongly affected by seasonal variations in O_2 and SO_4^{2-} concentrations in the hypolimnion. In winter (November-March), the lake water column was mixed and concentrations of O_2 and SO_4^{2-} were constant with depth (Fig. 3). Shortly after spring overturn (April-May), O_2, NO_3^- and SO_4^{2-} concentrations decrease in the hypolimnion (Hordijk et al. 1987). When the hypolimnion becomes anoxic, Mn^{2+}, Fe^{2+} and ΣH_2S increased in the hypolimnion (Fig. 2; Verdouw and Dekkers 1980). Sulfate continues to be present in the sediment until September (Fig. 3). The seasonal trend in the sulfur sedimentation rates at 9.6 m resembles those of O_2, NO_3^-, SO_4^{2-}, ΣH_2S and Fe concentrations at this depth (Fig. 1-4). Total sulfur sedimentation rates could, however, not be linked to organic-carbon (Fig. 5) or organic-nitrogen sedimentation rates (not shown).

	Release rate		Reduction rate
	Sulfate	Total S	Sulfate
		mmol m^{-2} d^{-1}	
Untreated slurry	9.0	NA	3.6
10 times diluted slurry	36.0	NA	NA
Untreated core	3.3	4.8-7.2	0.6
Molybdate treated core	1.2	3.1	NA

Table 6. Comparison of release rate estimates of SO_4^{2-} and TS with sulfate reduction rate estimates in sediment collected at 10 depth in the eastern depression of Lake Vechten. The release rates were estimated in samples collected in the advent of fall turnover (1991; anoxic period). The sulfate reduction estimates were derived from previous estimates done in the same sampling area in early spring (oxic period; Hordijk et al. 1985). NA; not analyzed.

Seasonal variations in the sediment sulfur pool

Sulfate reduction and fixation (119 mmol S m^{-2} yr^{-1}; Hordijk et al. 1985) and sulfur sedimentation (178 mmol S m^{-2} yr^{-1}: this study) are the main pathways for sulfur to the profundal sediment. Releases of sulfur species (H_2S in summer and sulfate in winter) are the main pathways for sulfur from the sediment to the water column. The resultant of these processes, together with the net sediment accumulation rate, will determine the sulfur concentration of the sediment. Considering the average total sulfur concentration of the sediment (93.8 mmol (kg [dry mass])$^{-1}$) and an average sediment accumulation rate

of 0.65 cm yr^{-1}, seasonal variations in S-input or S-output should be discernible as fluctuations in the S-concentration in the near-surface sediment.

Pronounced seasonal changes in the relation between the sulfur content of the 0-1 cm stratum and the 1-10 cm stratum were not found. Apparently, the sulfur flux across the sediment-water interface was nearly in balance during all seasons. The absence of a temporary total-sulfur accumulation in the sediment during summer also implies that substantial amounts of sulfur had been released into the hypolimnion to maintain this balance. Hordijk et al. (1989) reported that in Lake Vechten about 15% of the sulfur input is permanently buried in the sediment and 85% recycles into the lake water.

Seasonal variations in sulfur sedimentation

Sholkovitz and Copland (1982) noted that the release of sedimentary Fe during stratification will stimulate the formation of FeS precipitates. The formation of these mineral phases may ultimately lead to increased sulfur sedimentation rates. Our data support this conclusion. The grey color of the hypolimnetic water suggests FeS precipitates. Supersaturation conditions for amorphous FeS and crystalline iron sulfides occurred from June until November below the 7.5 m depth (Fig. 6). The highest sulfur-sedimentation rates correlated with this depth and period (Fig. 4 and 6). Formation of more oxidized precipitates as greigite (Fe_3S_4) may occur in the sub-oxic metalimnion (about 5 m depth). If these species control sulfur sedimentation, one may expect that sedimentation rates at 7.2 m (anoxic) would be higher than at 5 m (suboxic). There was, however, no increase in S-sedimentation rates between these depths (Fig. 4). This indicates that FeS, formed between 7.2 m and 9.6 m is the main iron-sulfur precipitate.

The average Fe sedimentation rate at the 9.6 depth is 0.35 mmol $m^{-2} d^{-1}$ between July and October (Verdouw and Dekkers 1980). The average sulfur sedimentation rate is about 0.9 mmol $m^{-2} d^{-1}$ at 9.6 m in the same period. Based on a FeS stoichiometry of 1:1, only 40% of the sulfur trapped at 9.6 m can originate from FeS precipitates. Thus, about 60% of the trapped sulfur appeared to consist of sulfur species other than FeS.

There was no difference between sulfur sedimentation rates at 4.0 m, 5.0 m and 7.2 m depth between July and October (Fig. 4). This indicates that the sedimenting sulfur pool measured at these depths (about 0.2 mmol m^{-2} d^{-1}) originates from the epilimnion and cannot be attributed to FeS precipitation. Assuming that this pool will also pass the 9.6 m plane, we can expect that about 20% of the sedimenting sulfur pool originates from the epilimnion.

Sulfur release rates

Penetration of oxidizers (O_2, NO_3^- and Fe^{3+}) into the sediment is limited to a few mm in Lake Vechten (Hordijk et al. 1987). Slurry incubations are useful to investigate how easily sediment sulfur can be oxidized. Incubation of intact sediment cores is, however, a more realistic approach to estimate actual sulfur release rates in the lake. The addition of MoO_4^{2-} to sediment cores or slurries to inhibit sulfate reduction (Oremland and Capone 1988) did not promote SO_4^{2-} release rates. On the contrary, MoO_4^{2-} appears to reduce sulfur and sulfate releases in all experiments. Possibly, ΣH_2S reacts with MoO_4^{2-}, hampering oxidation (Sϕrgensen et al. 1981; Oremland and Capone 1988).

There was, however, no release of SO_4^{2-} in gamma-irradiated slurries, in the absence of MoO_4^{2-}. Apparently sulfur is oxidized mainly by microbes and MoO_4^{2-} inhibits this process. It has been suggested that Mo species may inhibit microbial sulfur oxidation (Oremland and Capone 1988). Our results support this hypothesis. Thus the use of MoO_4^{2-} to discriminate between biological and chemical sulfur oxidation is not a reliable method. That microbes are involved in sulfur oxidation in Vechten is elucidated by the annual bloom of *Beggiatoa* species at the sediment surface in early winter (Sweerts et al. 1990). Populations of *Beggiatoa* can accelerate sulfur oxidation by four orders of magnitude (Larkin 1983).

The sediment core incubations indicated that under oxic conditions over 60% of the sulfur is released as non-sulfate species (Fig. 7).

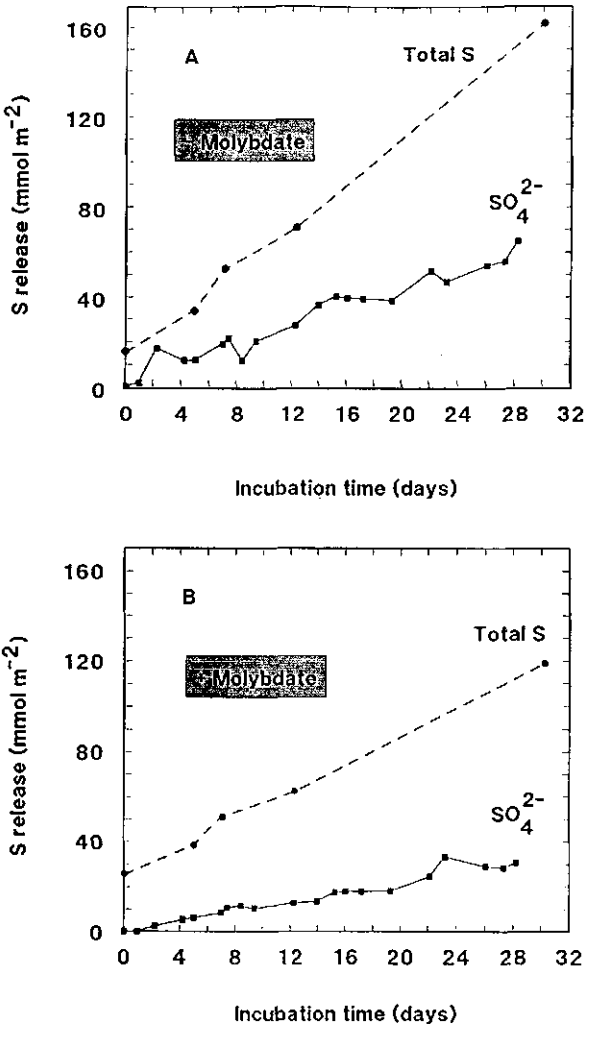

Fig. 7. Release rates of total sulfur and sulfate in sediment cores when the superficial water was aerated. The cores collected just before fall overturn when the superficial water layer was still anoxic. A) without inhibitor; B) with MoO_4^{2-} as sulfate reduction inhibitor.

Releases of non-sulfate species were also seen in sediment cores collected in a shallow oligotrophic acid moorland pool (pH 4.5) in which radioactive H_2S was added to the sediment (Marnette et al. 1992). Over 90% of the radioactivity released from the spiked sediment could not be attributed to sulfate or ΣH_2S. This stresses the need to include total sulfur estimates for lake water when studying sulfur cycling in both acid and neutral lakes.

Lake water sulfur

Sulfate reduction and sulfate reducing bacteria were not detectable in the hypolimnion (Cappenberg and Verdouw 1982), which make it likely that the residual sulfur pool (S_{res} = Total Sulfur - sulfate) originated from sedimentary sulfur release. This is supported by the observations of Stuiver (1967), who found that primary immobilized radio-active-labeled sulfur species were released from the sediment into the hypolimnion during the next summer stratification.

The nature of the lake-water sulfur pool should be elucidated to estimate the contribution of each sulfur species in the electron transfer between sediment and metalimnion. The S_{res} pool did not oxidize fast to SO_4^{2-}. Aerating hypolimnetic water for three hours did not produce SO_4^{2-}. This resistance to chemical oxidation may explain why phototrophes compete successfully for reduced sulfur. In Lake Vechten, there is a dense population of sulfur-oxidizing phototrophs at the thermocline (Steenbergen et al. 1987) where a decrease of S_{res} occurs (Fig. 1). It has been previously estimated that 1.2 to 1.9 mmol S m^{-2} d^{-1} migrates towards this horizon (Steenbergen et al. 1987).

ΣH_2S in the lake water (Fig. 2B) makes up at most 5 to 10% of the S_{res} pool in the hypolimnion. The S_{res} in the lake water column during in winter is neither ΣH_2S nor particulate sulfur. Concentrations of total sulfur for lake water, filtered over 7-µm, during the summer algae bloom, were quite similar to unfiltered lake water. Considering that S accounted for 0.15-1.96% of the dry mass of sestonic matter (Baker et al. 1989), and a

maximal seston mass of 3.1 mg dry weight l^{-1} (in the epilimnion in August: Verdouw et al. 1987), sestonic-S was at most 0.7 µM S or 1.3% of the S_{res} pool.

Mass spectra of dichloromethane extracts of Lake Vechten water indicated only traces of polysulfides (S_1 to S_{10}) in the hypolimnion. Literature data on polysulfide concentrations in comparable lakes , however, are lacking and theoretical conditions do not favour the presence of substantial concentrations of polysulfides (Cheng and Gupta 1973).

Organic sulfur species occurring in freshwaters are estersulfates, thiols, thioethers, or sulfurized geopolymers (R-O-SO_3 or R-S bonds; Bechard and Rayburn 1979; Jenkins et al. 1979; King 1980; Moers et al. 1988). Low-molecular weight thiols or thioethers (i.e. dimethyl sulfide) were not found in the mass spectra of lake water samples. Moers et al. (1988) demonstrated that mixing cellulose and H_2S resulted in a slow formation of wide variety of sulfur-containing organic compounds. Such reactions may lead to a difficult-to-specify mixture of sulfur compounds associated to geopolymers. In conclusion, although we can quantify the size of the S_{res} pool, its nature remains enigmatic.

Comparison of Lake Vechten with other lakes

Field data estimations of sulfur-sedimentation rates in freshwater lakes are scarce. Three sites for which sulfur-sedimentation rates are known to us are Wintergreen Lake, a hyper-eutrophic lake (King and Klug 1982), South Lake, an oligotrophic lake (David and Mitchell 1985) and Little Rock Lake, an acidified seepage lake (Baker et al. 1989). The differences in sediment-sulfur pools, sulfur-sedimentation rates and sulfate-reduction rates between the first two lakes and Lake Vechten are obviously related to differences in hydrology, pH and, degree of trophy (Table 5). The most noticeable difference in sedimental-sulfur composition between the three lakes is the relative high acid-volatile sulfide concentration in Vechten (Table 5).

David and Mitchell (1985) suggested that the high concentrations of organic-sulfur in the sediment of South Lake are due to the absence of mineralization of seston-sulfur. In

Lake Vechten, with summer anoxia and a total primary production in summer of over 92 mmol C m^{-2} d-1 (Steenbergen 1982), seston-sulfur in the sediment can be expected. However, in contrast to Wintergreen Lake and South Lake, sedimentary sulfur was about 60% FeS (Table 5). High FeS concentrations are typical for sediments with high Fe contents (>350 mmol (kg [dry weight])$^{-1}$: Baker et al. 1989). Lake Vechten with a total Fe content in the profundal surface sediment of 775 $\pm$ 100 mmol (kg [dry weight])$^{-1}$ (Verdouw and Dekkers 1980) is not exceptional in this aspect.

CONCLUSIONS

Pronounced seasonal fluctuations in the sulfur cycle were seen only in the hypolimnion and appeared to be closely related with the iron cycle in Lake Vechten. Interactions between iron and sulfur determine the nature of the sediment sulfur pool. The role of non-sulfate and non-ΣH_2S species in the cycling of sulfur between sediment and metalimnion should be elucidated in freshwaters. The resistance of the S_{res} pool to chemical oxidation leads us to hypotheze that sulfur-oxidizing microbes can use this pool as electron donor in suboxic environments. This hypothesis is circumstantially supported by the occurrence of the dense populations of sulfur-oxidizing phototrophs in the metalimnion during summer, and Beggiatoa at the sediment surface in early winter.

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CHAPTER 3.4

Kinetic studies of acetate in freshwater sediments: use of stable isotopic tracers.

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Kinetic studies of acetate in freshwater sediments: use of stable isotopic tracers

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running head: acetate kinetics in sediments

ABSTRACT

The kinetics of acetate uptake in the sediment of Lake Vechten, a meso-eutrophic, monomictic freshwater lake, was investigated by monitoring the disappearance of deuterated acetate tracers. Steep acetate concentration gradients were observed in the top cm when the sediment was analyzed at mm scale. The gradients revealed not only the dynamic nature of the acetate pools but also indicated substantial mass transport between sediment layers. Acetate uptake could conveniently be modeled using first order reaction kinetics. Uptake rates ranged from 30 μM day^{-1} (winter) to 546 μM day^{-1} (early spring) in the sulfidogenic horizon (2 cm) and from 30 μM day^{-1} (winter) to 1176 μM day^{-1} (late summer) in the methanogenic horizon (5 cm). The integrated acetate uptake rate in the top 7 cm was 39.8 $\pm$ 20 (2 * SE) mmol acetate m^{-2} day^{-1} during early spring. More than 40% of the total acetate pool was reversibly adsorbed in oxic sediment slurries. The presence of hydrous Fe(III) oxides appeared to determine the occurrence of acetate adsorption.

INTRODUCTION

The C_2-C_6 volatile fatty acids (VFA) are key intermediates in the diagenesis of organic matter in aquatic sediments (Lovley and Klug, 1982; Sansone, 1986). They serve as precursors for methanogenesis and are used as respiratory substrates by sulfate-reducing bacteria (Cappenberg, 1974). A study of natural concentrations of VFA with their actual turnover rates provides an insight to the complex interactions between fermentative and respiratory processes. Since acetate is the main fermentation intermediate in freshwater sediments (Lovley and Klug, 1982), a reliable estimate of acetate turnover can help to gauge total mineralization.

The role of acetate in the carbon flow within the sediment is complex. Not only is acetate generated from the decomposition of organic matter, but also from acetogenesis using H_2 and CO_2 as substrates (Dolfing, 1988). Aceticlastic methanogenesis and dissimilatory sulfate reduction with generation of CO_2 are the main acetate-consuming processes in anoxic marine sediments (Shaw et al., 1984). The availability of sulfate may determine which of these processes dominate in freshwaters (Lovley and Klug, 1986). In respiring freshwater sediments a successive depletion of oxygen, nitrate,

sulfate, and ferric iron may occur within a few cm (Hordijk et al., 1987). This implies that acetate profiles near the sediment surface will reflect a complex balance between acetogenesis and acetate utilizing processes.

Acetate uptake in freshwaters may include mineralization (Lovley and Klug, 1986), incorporation (Fredrickson et al., 1988) and adsorption (Balba and Nedwell, 1981). Acetate consumption rates can be estimated from the appearance of $^{14}CO_2$ and $^{14}CH_4$ from ^{14}C-acetate unless there is a considerable CO_2 fixation (Dolfing, 1988). Acetate can also condense to other VFA with H_2 uptake (Dolfing, 1988; Thauer et al., 1977). When the relative contribution of these acetate-utilizing processes is unknown, it may be advisable to estimate acetate turnover directly by following its depletion, rather than tracing and summing its endproducts. The obvious means to do this is to measure the depletion of labeled VFA together with their natural pool sizes. This requires a technique that can separate and detect VFA and their isotopic labels simultaneously. The detection method has to be sensitive enough to measure *in situ* concentrations (<100 µM in freshwaters: Lovley and Klug, 1982; Hordijk et al., 1983; Jones and Simon, 1984; Phelps and Zeikus, 1985).

Chromatography has frequently been used to separate and detect radioactively labeled VFA in sediments (Cappenberg and Jongejan, 1978; Balba and Nedwell, 1982; Jones and Simon, 1984). Recently, we published a method to analyze VFA by capillary chromatography (Hordijk et al., 1990). Capillary columns can separate closely related fatty acids and require a sample size of a few µl, which is a major advantage for studying flocculent sediments (Hordijk et al., 1987). Capillary gas chromatography combined with mass-selective detection (GC-MS) can detect several metabolites with their isotopes simultaneously, and thus provides useful information to unravel kinetics in complex biochemical mixtures (Schulman et al., 1972; Barrie et al., 1984). The ability of GC-MS to detect stable isotopes can also be practical in view of the regulatory limitations concerning the use of radioactive isotopes. Stable isotope labeled VFA have been used as tracers in medical science (Tserng et al., 1984; Walter et al., 1989), but rarely in sedimentary studies (Schulman et al., 1972; Krzycki et al., 1987).

This paper describes the use of deuterated acetate to study acetate kinetics and acetate adsorption in freshwater sediments, using batch volumes of 5-10 ml. Pool sizes

and uptake rates thus obtained are related to overall biochemistry of the sediment and the lake-water column. The study is focused on anoxic sediment in which sulfate reduction and methanogenesis are assumed to be the main acetate-consuming processes. The applied techniques may, however, also be useful for studying the suboxic horizon of the sediment.

MATERIAL AND METHODS

Site description

The sediment examined was collected in 1988-1991 from the eastern depression (depth 10.5 m) of Lake Vechten. Vechten is a meso-eutrophic and monomictic lake (52°04'N and 5°05'E; area 4.7 ha; mean depth 6.5 m). It was formed in 1941 by excavation of sand needed for constructing a nearby highway and has been a site of limnological research for more than 30 years (Gulati and Parma, 1982). The lake has no surface drainage and may be classified as a seepage lake. Above the seven-meter depth horizon, where the sediment is sandy and permeable, there is a slow lateral influx of ground water penetrating the lake from the east side and leaving the lake at the west side. The water residence time is approximately five years. Below 9 m depth, the bottom of the lake is covered with approximately 30 cm black silty sediment, overlying a non-permeable clay layer.

Sediment description

The profundal sediment at 10 m depth has a fine and homogeneous silty structure without an apparent spatial variability. Sedimented detritus is the main carbon source of the profundal sediment. Average sedimentation rate is 0.5 cm $year^{-1}$ (Verdouw et al., 1987). Total organic carbon input in the bathylimnion was 58 $\pm$ 17 mmol C m^{-2} day^{-1} and showed no seasonal variations (Steenbergen and Verdouw, 1984). Carbon (4-7%) and iron (ca. 5%) both form a substantial part of the dry mass of the sediment (Table 1).

Depth	Dry Mass	Fe_t	Fe_x	FeS	Fe_d	C_i	C_o
cm	%	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}	%	%
1.5	13	114	7	23	0.7	1.7	6.7
4.5	19	159	9	30	1.0	1.5	5.7
7.5	24	193	11	25	1.1	1.4	4.9
10.5	28	225	11	24	1.2	5.9	4.3

Table 1. Depth distribution of some relevant compounds in the sediment of Lake Vechten in the non-stratified period: dry mass (% of wet sediment); Fe_t, total iron, Fe_x, KCL exchangeable iron; Fe_d, dissolved iron (mmol kg^{-1} wet sediment; Verdouw and Dekkers, 1980); C_i, carbonate carbon; C_o, Organic carbon (% of the wet sediment mass: Dekkers, unpublished).

Other characteristic components are aluminum (6.2%), calcium (5.8%), silicon (24.6%), nitrogen (0.6%) and sulfur (0.3%). About 5-7 % of the iron pool is in a water- extractable form (presumably mainly as $FeCO_3$: Verdouw and Dekkers, 1980). More than 0.2 % of the dry mass may be present as FeS, giving the sediment a deep black color (Table 1).

Thermal cycle of the lake

Lake Vechten is a monomictic lake. The lake water column is completely mixed in the period December-April. The sediment surface is brown colored while the rest of the sediment is black. Oxygen, nitrate and sulfate diffuse into the top cm of the sediment (Fig. 1A).

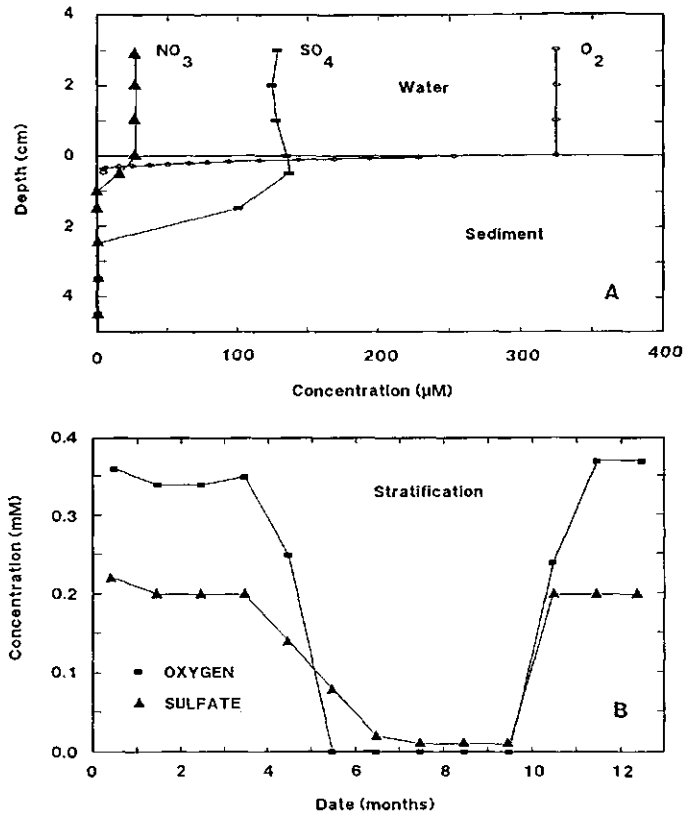

Fig. 1. A) Depth profiles of some external electron acceptors at the sediment-water interface during winter (Hordijk et al., 1987); B) Seasonal fluctuation of oxygen and sulfate in the eastern depression of Lake Vechten in 1982 (9m; Hordijk, unpublished).

Their concentration gradients in the sediment are a result of molecular diffusion, consumption, recycling, and tortuosity effects (Hordijk et al 1985). The sediment can be divided into an oxic horizon (2-3 mm), a sulfidogenic horizon (1-3 cm) and a methanogenic horizon (3-7 cm, Fig. 2) during the non-stratified period.

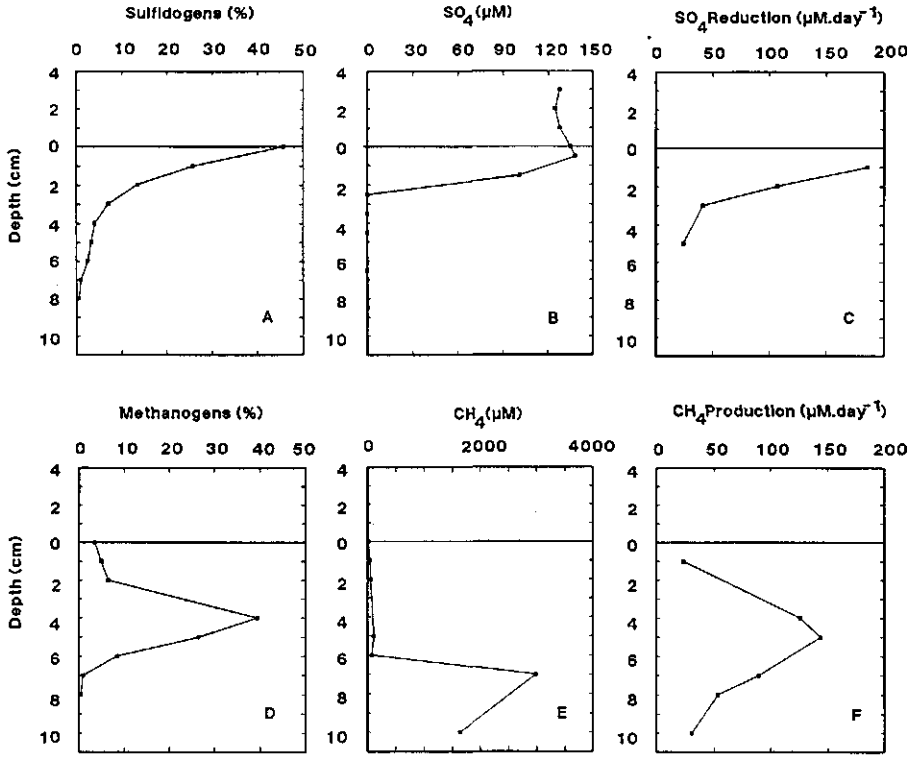

Fig. 2. Distribution of sulfidogenic and methanogenic activities in the sediment of Lake Vechten during winter. A. Proportional distribution of the sulfidogenic population with depth (Hordijk and Cappenberg, 1983). B. Sulfate profile (Hordijk et al. 1987). C. Sulfidogenic activity (Hordijk et al., 1985). D. Proportional distribution of the total methanogenic population with depth (Hordijk and Cappenberg, 1983). E. Dissolved methane (November 1981, unpublished). F. Methanogenic activity (Hordijk and Cappenberg, 1983).

A stable stratification period begins after spring overturn (April) and lasts until November. During the stratification period, oxygen and nitrate are rapidly depleted in the hypolimnion (below 7 m; cf. Fig. 1B), while sulfate remains present in the sediment until September. During October-November (fall) stratification becomes unstable (pre-overturn period) and ends in December. After this transition period the

lake water column is completely mixed. Average temperatures at the lake surface varied between 5 °C (mid winter) and 20 °C (mid summer). Temperatures near the bottom of the lake (9-10 m depth; sampling area) varied between 5 °C (mid winter) and 11 °C (mid summer: Steenbergen and Verdouw, 1982).

Apparatus

Acetate analysis was performed using a Hewlett-Packard (HP) Model 5890 gas chromatograph. A flame ionization detector (FID) or an HP 5970 mass-selective detector (MSD) was used (Hordijk et al., 1990). The capillary column was 10 m * 0.53 mm I.D. and coated with 1 µm FFAP-CB wax (HP). Samples of 3 µl were introduced manually by splitless injection on a 4 * 90 mm glass liner. For CD_3COOH analysis, an 11.5 m * 0.32 mm I.D. column coated with 0.33 µm FFAP-CB wax (HP) was used and sample volume was limited to 1 µl. The molecular ion of CD_3COOH was detected as m/z 63. Mass spectral data were processed using a HP-300 computer, and ion chromatograms (m/z 63) were calibrated using standards (99.5% pure, Aldrich, Beerse, Belgium). Chromatographic conditions for GC-MS detection of CD_3COOH were: carrier gas (He), 4.5 ml min^{-1}; injector-purge flow, 5.7 ml min^{-1}; septum-purge flow, 0.5 ml min^{-1}; temperature, 70 °C (1.10 min); rate of temperature increase, 10 °C min^{-1} up to 90 °C (4.10 min); injection port, 150 °C; solvent delay, 2.50 min; purge off, 1.10 min; MSD transfer-line, 150 °C; vacuum, 1 * 10^{-4} Torr; ionization energy, 70 eV. Manual tuning was done on m/z 29, 69 and 100 with perfluorotributylamine used as the calibration gas.

External standardization was carried out with gravimetric standards (0-50 µM) of acetate and propionate (Suprapur, Merck) in acidified Milli-Q water (Millipore Corp). Molar response factors (FID, 5.7 µV $pmol^{-1}$: MSD, 261 abundance units $pmol^{-1}$) were obtained by plotting peak heights (FID) or area (MSD) of standards against their original concentrations. The CH_3COOH calibration curves (signal obtained by integration) for m/z 45 and 60 were linear from 20 to 400 µM for 1 µl injections (r, 0.947) for mass selective detection. Below 20 µM CH_3COOH curves flatten with mass selective detection. The CD_3COOH (m/z 63; r, 0.998) and CH_3CH_2COOH calibration graphs (m/z 45 and m/z 75) were, however, linear from 0.2 to 400 µM for 1 µl

injections (r, 0.957) with mass selective detection. Total acetate concentrations below 20 µM were measured by FID. The relative standard deviation of a 5 µM acetate solution was 14 % (n = 10) for a 0.32 mm I.D. column (MSD) and 9 % (n = 10) for a 0.53 mm I.D. column (FID). In a range of 25-100 µM, the relative standard deviation was 8% (n = 10) for a 0.32 mm I.D. (FID) and 3% (n = 10) for a 0.53 mm I.D. column (FID). The pore water samples were injected in triplicate and detector responses were averaged before comparing with the calibration curves. The detection limit, defined as a signal change equal to three times the signal to noise ratio, was 0.5-1.5 µM for 2 µl injections (FID) and 0.2-0.7 µM for 1 µl injections (MSD) for acetate and propionate. There was a linear relation between the injected volume (0.5 - 5 µl) and the signal of the FID (5.7 µV per pmol acetate) using a 0.53 mm column. Injections using a 0.32 mm column need to be limited to 1 µl. Detection characteristics of other VFA are in the same order of magnitude as for propionate (Hordijk et al., 1990).

Sulfate was assessed by liquid chromatography using indirect photometric detection at 254 nm and 5-sulfoisophthalic acid as the eluent (Hordijk and Cappenberg, 1985). The detection limit was 2 µM for 10 µl injections; relative standard deviation 6%; r, 0.998 for 3-250 µM.

Sample collection

Sediment cores (acrylic glass tubes, 50-cm long and 7-cm I.D.) were taken by a Jenkin surface-mud sampler from the deepest part (ca. 10 m) of the eastern depression of Lake Vechten (Cappenberg, 1974; Golterman, 1970). Two parallel series of holes (I.D. 2 mm) were drilled in the acrylic glass of the core in a vertical line at 1 cm intervals. One series was shifted 5 mm from the other, allowing sampling at 5 $\pm$ 1.5 mm intervals. The holes in the core were covered with Scotch tape 471 prior to sampling. The sampled cores contained about 15 cm of flocculent sediment and 35 cm of superficial water. The superficial water was carefully removed just before sediment subsamples were taken from the core. About 0.35 g (wet weight) sediment was sub-sampled with a 1-ml syringe. A 1-mm I.D. needle, placed on the syringe, was pierced through the tapered holes in the core. The subsamples were transferred into 0.4 ml

capped polypropylene tubes (Emergo, Netherlands) and centrifuged at 1000 g for five min at 7 °C. One hundred µl of the supernatant was acidified with one µl of 30% formic acid (Suprapur quality; Merck, Darmstadt, Germany) and 1-3 µl of the aliquot was injected in duplicate or triplicate into the gas chromatograph for analysis. The VFA concentrations were assessed in 23 sediment cores collected in 1989-1991.

Lake water was collected at 1.20-m depth intervals during summer and winter in 1982 using a Friedinger water sampler with a 60 cm x 10 cm I.D. sample tube (Golterman, 1970). The VFA were analyzed by liquid chromatography with fluorescence detection (Detection limit, 0.1 pmol; relative standard deviation, 8%: Hordijk and Cappenberg, 1983).

Adsorption kinetics

Sediment samples (10-15 g) were withdrawn by a 10-ml syringe from a Jenkin core. The sediment samples were diluted with anoxic pore water to get a homogeneous slurry. The dilution of the sediment was done in an anaerobic glove box. The pore water used for dilution was collected from centrifuged sediment of another core. The diluted samples were autoclaved in closed vials with nitrogen for 30 min at 120 °C. For comparison, sediment was also sterilized with gamma irradiation (4 MRad: Sansone et al., 1987). During sterilization VFA were formed. The surplus of VFA was removed by washing the slurry with 0.2 µm filtered pore water until *in situ* concentration level was reached. An aliquot of the slurry was withdrawn by syringe to determine the dry mass per volume. The dry mass was determined by ignition at 110 °C. The slurries were spiked with CD_3COOH and gently shaken for one hour. After incubation, adsorption was estimated by comparing the CD_3COOH concentrations in the pore water with those of the standards used for the spiking. Similar experiments were done with sediment slurries which had been flushed with air for a few hours. During flushing the color changed from deep black to brown.

To demonstrate that acetate may interact with hydrous Fe(III)-oxides at *in situ* concentrations (0-50 µM acetate), chemically pure colloid-suspensions were synthesized by diluting $FeCl_3$ in sterile Milli-Q water. The suspension of Fe(III)-colloids was

centrifuged three times, replacing each time the supernatant with Milli-Q water, to extract the residual chloride. The extracted suspension was adjusted to pH 7.5. Five suspensions were made, ranging between 0.9 mM Fe and 9.0 mM Fe (Table 2).

	Fe (mmol l^{-1})			
	0.90	2.24	4.48	8.96
Acetate (µM)	Recovery (%)			
10	72	74	51	49
20	70	75	50	43
30	81	69	49	42
40	80	65	56	41
50	84	69	61	42

Table 2. Recovery of dissolved acetate from suspensions of iron coagulates at pH 7.5 (relative standard deviation = 6%).

This concentration range included the dissolved Fe fraction and the Fe fraction that can be extracted with 2 M aqueous KCL solution (Verdouw and Dekkers, 1980; Table 1). A series of gravimetric CD_3COOH solutions was made in the range from 0 to 50 µM for each Fe(III)-colloid suspension. The suspensions were incubated for 1.5 h at room temperature with gentle shaking. After incubation, adsorption in the colloid suspensions was estimated by comparing the CD_3COOH concentrations in the solutions with those of the standards in distilled water.

Bacterial kinetics

First-order uptake-rate constants were estimated in sediment batches using CD_3COOH as tracer. The sediment for the batches was collected using a Jenkin sampler during early spring (March-April), summer (May-September), just before fall overturn (September-mid November), shortly after fall overturn (end of November) and

during winter (December-February) over 1989-1991. The sediment batches (10-15 g wet weight) were sampled at 2-cm and 5-cm depths from the Jenkin core using a 10-ml syringe. The sediment was transferred into 30-ml serum vials under a stream of nitrogen which had passed through a column of BASF catalyst R3-11 at 130 °C to remove traces of oxygen. The vials were capped with butyl-rubber stoppers and the headspace was flushed with nitrogen for 5 min. The pH of the sediment was not affected (pH 7-7.7). After flushing, the vials were transferred into an environmental cabinet (Fisons, BR 185). The batches were gently shaken at 7 °C for 45 min to acclimate to lake temperature. The batches were spiked with CD_3COOH and homogenized on a Vortex mixer for a few min to achieve an uniform distribution of the label. Samples were withdrawn before and after spiking to assess the acetate concentration. The batches were incubated at 7.0 °C for about 4 h and the depletion of CD_3COOH was followed by periodic sub-sampling. The sub-samples were immediately centrifuged at 1000 x g for 5 min at 7 °C in capped centrifuge tubes. The supernatant was withdrawn by syringe and acidified with formic acid to a final concentration of 0.3% before injecting it into the gas chromatograph. Formic acid was added to dissolve Fe(III)-coagulates. Acetate consumption (mmol m^{-2} day^{-1}) was estimated for the early spring period (March-April) by integration of acetate uptake over a 7-cm depth interval. Averaged acetate concentrations of the sediment surface (0-1.5 cm), the sulfidogenic (1.5-2.5 cm; cf. Fig. 2C) and methanogenic horizon (2.5-7 cm; cf. Fig. 2F) were used for these calculations. The results were compared with data on methanogenesis and sulfate reduction rates collected earlier (Hordijk and Cappenberg, 1983; Hordijk et al., 1985; Hordijk et al., 1987).

RESULTS AND DISCUSSION

Lake water profiles

Acetate concentrations increased with depth in the lake water column during the non-stratified period (November-mid May 1982; Fig. 3A-C). Dilution by lateral influx of ground water above the 7 m depth contour may explain the relatively lower acetate concentrations in the surface water in this period. Other fatty acids were below the detection limit (< 1 µM) in the lake water column.

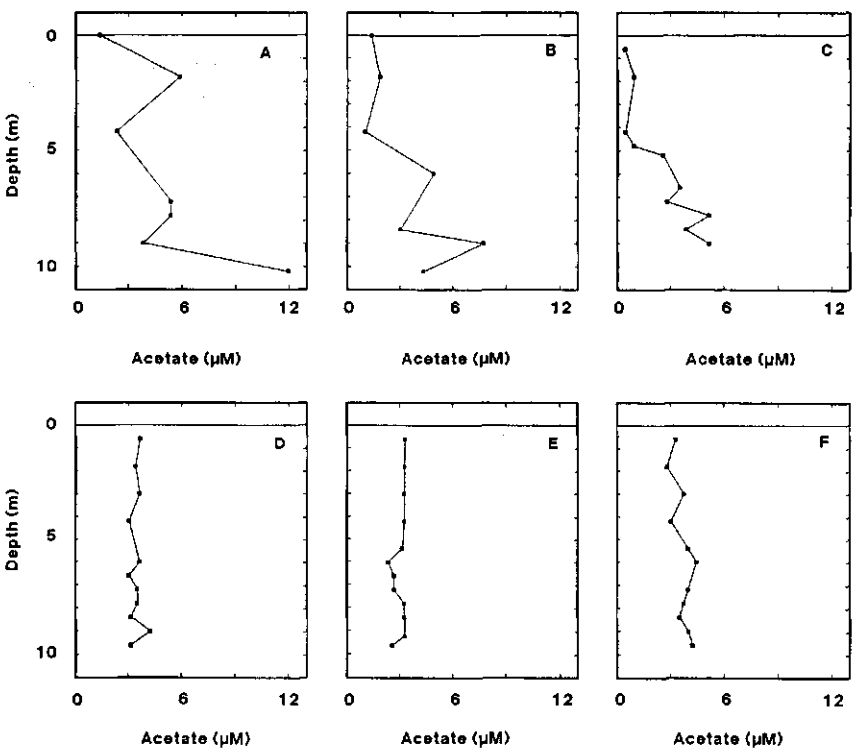

Fig. 3. Seasonal variations of acetate concentrations with depth in the lake-water column of Lake Vechten. Samples were collected using a Friedinger water sampler in the year 1982. (A) 21 November; (B) 10 February; (C) 25 May; (D) 22 June; (E) 20 July; (F) 8 August.

Acetate concentrations were about constant with depth during the stratification period (June-August; Fig. 3D-F). In this period also traces of formate (1-6 µM) and lactate (0.2-3.5 µM) were found in the lake (data not shown), but other VFA remained undetectable.

In November, just before the fall turnover, acetate concentrations increased in the samples collected with the Friedinger sampler near the lake bottom (Fig. 3A). An increase in acetate concentrations was also seen in the water layer of sediment cores collected in September-November 1990 (Table 4; Fig. 4C).

In this overturn period, oxygen, nitrate, and sulfate were depleted at 9.6 m depth

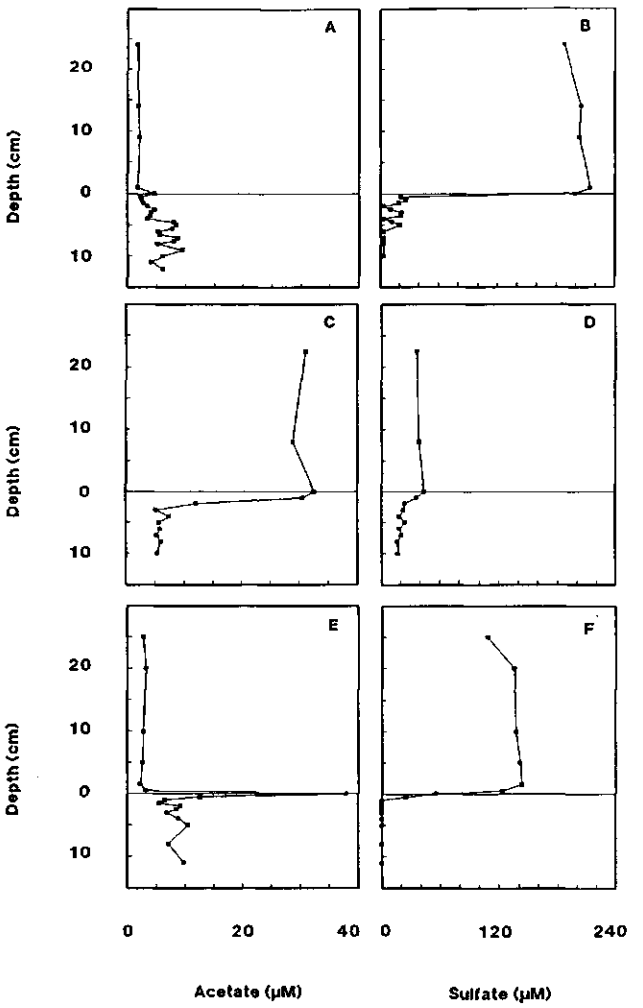

Fig. 4. Seasonal variations of porewater acetate and sulfate concentrations in the sediment of Lake Vechten in 1990. A and B, in the non-stratified period (26 March); C and D, before fall turnover (25 September); E and F, after fall turnover (10 December).

(Fig. 1B). Changes in acetate concentrations in the water column were not associated with total organic carbon sedimentation rates as these values remain constant through the year (58 ± 17 mmol total organic C m^{-2} d^{-1} at the 9.6 m plane; Verdouw et al., 1987).

Pore water profiles

The variations in acetate concentrations near the bottom of the lake were related with depth and season (Table 4). In winter (December-February) no increases in acetate concentrations were found in the near surface sediment, where steep concentration profiles of oxygen, nitrate and sulfate indicated intense respiration (Fig. 1A). The acetate generated in this layer may be consumed by respiratory processes.

In early spring (March-April) primary production starts to increase (Steenbergen and Verdouw, 1982). In this period acetate concentration peaks at 1-cm depth were found in four of eight cores collected. Steep acetate peaks (42 µM) were also found at 7-cm depth in a core collected on 24 April 1989, and in a core collected on 1 May 1989 (48 µM). A third and extreme high peak was seen at 7 cm (140 µM) in a core collected on 25 September 1989. We excluded these three observations from further evaluation since such peak concentrations at 7 cm were not observed in 1990 and 1991.

During the summer stratification (May-September), five out of six profiles showed an acetate increase in the top cm. Acetate concentrations in the superficial water increased from 6.3 µM in May up to 23 µM in the end of October (Fig. 4C). During the same period overlying water sulfate concentrations gradual decreased at 9.6 m depth (Fig. 1B).

In November, stratification became unstable, acetate concentrations decreased to 1.5-4 µM (Table 4) and oxygen, nitrate, and sulfate reappeared in the overlying water (Fig. 1). Steep acetate gradients occurred in the near surface sediment during these early stages of mixing (Fig. 4C and 4E). Such gradients can be attributed only to intense acetogenesis concentrated in a narrow horizon, considering the pore water diffusion coefficient of acetate of 7 ± 1 * 10^{-3} cm^2 h^{-1} (Hordijk, unpublished) and the high porosity of the sediment (Table 1).

The steep gradients also indicate that substantial amounts of acetate are likely to diffuse from the top cm into the methanogenic horizon. The ability to measure such

gradients opens ways to assess acetogenesis by mathematical modeling. However, to support model approaches, actual kinetics in the oxic sediment first need to be investigated. Sampling at small depth intervals also reveals the dynamic nature of the acetate pore water pool in the near surface sediment. This dynamic nature explains most of the variations in acetate uptake rate estimates seen in this study.

Acetate concentrations were assessed previously in Vechten using liquid chromatography with fluorescence labeling (Hordijk and Cappenberg, 1983). This method allows a simultaneously assessment of acetate, formate, and lactate in sediments but it requires larger sample sizes (5-10 ml). This complicates sampling of cores with high depth resolution. The trends in acetate concentrations with depth in 1989-1991 are, however, comparable with those seen in 1982. Except for traces of propionate (1 µM) other VFA (butyrate, valerate, caproate and their iso-acids) were not found in sediment collected in 1982 and 1989-1991 (detection limit < 1 µM).

Distribution of acetate consuming processes

The anoxic sediment of Lake Vechten can be divided into a sulfidogenic and a methanogenic horizon (Fig. 2). Lovley and Klug (1986) expected, based on physiological characteristics of involved microbes, the lowest acetate concentrations in the sulfidogenic horizon. Their model anticipates a dual limitation of acetate and sulfate for the sulfate reducers, and that sulfidogens will out-compete methanogens for acetate. Lovley and Klug (1986) noted a lack of field data to verify their model for freshwater sediments.

The appearance of acetate peaks suggests a surplus rather than a limitation of acetate in the sulfidogenic horizon of lake Vechten sediment. A second indication of a surplus of electron donors for sulfidogens is the occurrence of first order kinetics for sulfate reduction (Hordijk et al., 1985). Also addition of acetate to the sediment had no stimulating effect on sulfate reduction (data not shown). Furthermore, methanogenesis in the sulfidogenic horizon also indicated a surplus of acetate (Fig. 2). Capone and Kiene (1988) noted that in marine systems methanogenesis can occur in the presence of sulfate at the expense of substrates not utilized by sulfate reducers. In freshwaters, competition for electron donors could also be uncoupled if rate-limiting electron donors

were generated in excess due to seasonal effects (Winfrey and Zeikus, 1979). Such effects may also occur in Lake Vechten.

Seasonal effects may lead to rapid variations in metabolic activities in stratifying lakes during late summer (Jones and Simon, 1985; Phelps and Zeikus; 1985). The rapidly varying acetate pools in Lake Vechten sediment also indicate changes in metabolic activities during late summer.

During fall (November), acetogenesis in the near-surface sediment apparently provides more acetate than locally is consumed. In an electron-acceptor limited environment respiring microbes will prefer those substrates providing the most energy for assimilation (Lovley and Klug, 1986). If the respiring microbes are already saturated with energetically more favourable substrates, acetate can accumulate or be used by methanogens (Capone and Kiene, 1988). In Lake Vechten, the surplus of acetate apparently diffuses deeper into the sediment or into the superficial water layer (cf. Fig. 4C). Thus acetate produced in the top cm may finally be consumed deeper in the sediment, where conditions apparently are more preferable for methanogenesis (cf. Fig. 2 D-F). This explains why high acetate uptake rates can be found in the methanogenic horizons, which is not related with the total organic carbon pool at that depth.

Michelson et al. (1989) predicted that differences in the depth distribution of acetogenesis and acetate consumption in near surface sediments may result in diffusion of acetate along sharp concentration gradients in marine sediments. They also noted a lack of techniques having sufficient depth resolution (2.5-5.0 mm) to study *in situ* acetate profiles. With a depth resolution of 5 $\pm$ 1.5 mm, *in situ* profiles could be measured accurately in Lake Vechten (Fig. 4C and 4E). The steepness of the profiles confirms the idea of Michelson et al. (1989) that rapid transport of acetate between layers differing in respiratory activity could be important in sedimentary systems. This stresses the need of measuring at narrow depth intervals in strongly respiring freshwater sediments.

Acetate adsorption

Insights into the chemical interaction of VFA with the sediment are essential to

evaluate estimates of *in situ* uptake rates. We know that up to 40 % of the total acetate pool can be attached reversibly to the sediment matrix (Hordijk and Cappenberg, 1983). Also in marine sediments acetate adsorption has been reported (Christensen and Blackburn, 1982; Shaw et al., 1984; Parkes et al., 1988; Sansone et al., 1987). However, Jones and Simon (1984) found no acetate adsorption in Blelham Tarn, a freshwater pond. The redox state and organic composition of the sediment may influence VFA adsorption (Sansone et al., 1987; Liang and Morgan, 1990). The Fe^{3+} cation may have a higher affinity to complex with organics than Fe^{2+} (Bader et al., 1960). Thus the redox couple Fe^{3+}/Fe^{2+} may not only control the oxidation state of Lake Vechten sediments (Verdouw and Dekkers, 1980), but also its adsorption capacity for VFA.

The change in color of the surface sediment from black to brown in winter is obviously due to oxidation of FeS. The sulfidogenic horizon (Fig. 2A-C) remains deep black, because iron oxidation is limited to the top mm of the sediment only. Determination of whether adsorption occurs in the near-surface sediment is not simple because ferric iron may also serve as an electron acceptor for VFA oxidation (Champine and Goodwin, 1991). Therefore we synthesized sterile Fe(III)-colloid particles in a range relevant for Lake Vechten (0.9-9 mM Fe: Table 1). Adsorption of acetate increased with the concentration of colloids (Table 2), thus VFA adsorption on iron may occur under *in situ* conditions. Adsorption on Fe(III)-coagulates may also have analytical consequences. Dissolved Fe(II) may oxidize to form Fe(III) coagulates during sample handling and ,subsequently, adsorb acetate (Hordijk et al., 1990). To exclude such artifacts, contact of sediments with air was avoided.

To discriminate between acetate consumption by the benthic community and chemical adsorption, the sediment had to be sterilized. Sterilization may however also affect the sediment matrix and alter its adsorption characteristics. Thus adsorption data obtained using sterilized sediments must be regarded as tentative. Two sterilization methods, gamma-irradiation and autoclaving, were examined. Sansone et al. (1987) noted that 2.3 MRad was needed for effective sterilization and therefore, they used exposures of $\geq$ 2.5 MRad for their experiments. When Lake Vechten sediment was gamma-irradiated using 4 MRad, more acetate (2-7 µmol g^{-1} dry mass) was formed than if the sediment were autoclaved (0.4-0.6 µmol g^{-1} dry mass). Other VFA were

formed by gamma-irradiation in smaller quantities: propionate, 0.2 µmol g^{-1}; butyrate, 0.03 µmol g^{-1}. Considering the amount of acetate formed by radiolysis, it appears that gamma-irradiation affects the sediment matrix more than autoclaving does. During gamma-irradiation radiolysis of VFA to CO_2 also occurs (Negron-Mendosa and Navarro-Gonzalez, 1990; Sansone et al., 1987). Thus not all VFA formed from the sediment matrix may be recovered at the end of the experiment. This implies that gamma-irradiation may damage the sediment matrix more than could be concluded by measuring the release of VFA only. The fluctuating acetate recoveries and the substantial radiolysis indicate that gamma-irradiation at dosages of 4 MRad may not be a reasonable method to use with studies of adsorption kinetics in organic rich sediments. Using lower dosages than 4 MRad may be an alternative (Sansone et al. 1987).

Recoveries of acetate, propionate, and butyrate were nearly quantitative in autoclaved and in gamma-irradiated anoxic sediments (Table 3), and those of the last two were also quantitative in oxic sediments. Acetate recoveries in oxic sediments were, however, not quantitative (Table 3).

Gamma irradiation

	Anoxic (%)	Oxic (%)	Concentration (µM)
Acetate	91 ± 9 (5)	52 ± 17 (5)	10-60
Propionate	103 ± 10 (5)	107 ± 5 (5)	10-60
Butyrate	104 ± 12 (5)	108 ± 6 (5)	10-60

Heat Sterilization

	Anoxic (%)	Oxic (%)	Concentration (µM)
Acetate	100 ± 5 (9)	68 ± 10 (9)	103

Table 3. Recovery of acetate from sterilized oxic and anoxic sediment. The number of replicates is given in parenthesis.

About 0.24 µmol acetate g^{-1} dry mass was adsorbed. Apparently, only adsorption of acetate is affected by the redox potential of the sediment, which agrees well with the results derived from the sterile Fe(III)-coagulate experiments. The nearly 100% recovery of acetate in anoxic sediment batches (Table 3) suggested that corrections for adsorption may be omitted when using anoxic sediments of Lake Vechten.

Acetate turnover kinetics

Acetate oxidation by sulfidogens is important in marine systems with a surplus of sulfate (Parkes et al., 1988). In freshwaters, however, the supply of sulfate is frequently

limited to the near-surface sediment (Fig. 2A) and methanogenesis is expected to be the main acetate-consuming process (Lovley and Klug, 1982). Thus the sediment of Lake Vechten can be divided into a mainly sulfidogenic (1-3 cm; Fig. 2A-C) and a mainly methanogenic (3-7 cm; Fig. 2D-F) horizon when the lake is not stratified. Acetate uptake rates in the two horizons were estimated by multiplying average first-order uptake-rate constants with average acetate concentrations for five distinct time periods (Table 4).

First-order uptake-rate constants were obtained by monitoring the depletion of CD_3COOH tracers in spiked sediments sampled at 2 and 5 cm depth (Fig. 5). Depletion curves obtained from the batch experiments ($n > 15$) could conveniently be modeled by first-order kinetics. Only one depletion curve measured in sediment at 2 cm depth tended to be clearly parabolic (Fig. 5)

In Lake Vechten, estimated uptake rates in December-February (2-5 cm layer, 0.03 mM day^{-1}: Table 5) are similar to those obtained earlier using ^{14}C as tracer in the same period (1 cm, 0.02 mM day^{-1}; 5 cm, 0.04 mM day^{-1}: Cappenberg and Jongejan, 1978). The acetate uptake rates in Lake Vechten were low (<1.2 mmol l^{-1} day^{-1}: Table 5) if compared with literature values for other locations (3.8 mM day^{-1}, Lovley and Klug, 1982; 2.0 mM l^{-1} day^{-1}, Phelps and Zeikus, 1985).

Integration over the top 7 cm for early spring, using values of Table 4-6 and depth intervals 0-1.5 cm, 1.5-2.5 cm, and 2.5-7 cm, yielded an average uptake rate of 39.8 $\pm$ 20 (2 * SE) mmol acetate m^{-2} day^{-1} for the early spring period. Based on carbon equivalents, this uptake rate is about 0.6 to 1.5 times the organic carbon sedimentation rate (58 $\pm$ 17 mmol C m^{-2} day^{-1}: Steenbergen and Verdouw, 1984). This comparison supported the idea that acetate is a key intermediate in carbon mineralization, as previously demonstrated by Lovley and Klug (1982) for freshwaters.

Period		Acetate Concentration (µM)				
Season	Month	SW	SE	1 cm	SE	N
Early spring	Mar-Apr	3.5	[0.7]	17.9	[5.7]	8
Summer	May-Sep	6.3	[1.8]	13.1	[3.0]	6
Pre-overturn	Sep-Nov	23.0	[4.6]	27.0	[2.1]	3
Post-overturn	Nov	3.0	[0.8]	20.0	[7.2]	5
Winter	Dec-Feb	1.5	[0.4]	0.75	[0.5]	3

Period		Acetate concentration (µM)				
Season	Month	2 cm	SE	5 cm	SE	N
Early spring	Mar-Apr	6.5	[1.0]	6.4	[0.7]	8
Summer	May-Sep	8.4	[3.1]	6.8	[1.8]	6
Pre-overturn	Sep-Nov	8.3	[1.8]	7.0	[0.6]	3
Post-overturn	Nov	3.1	[1.1]	4.1	[1.3]	5
Winter	Dec-Feb	0.5	[0.2]	0.8	[0.4]	3

Table 4. Seasonal changes in acetate concentrations in the superficial waterlayer and in the sediment at 1, 2, and 5 cm depth. Abbreviations: SW, superficial waterlayer; SE, standard error of the mean.

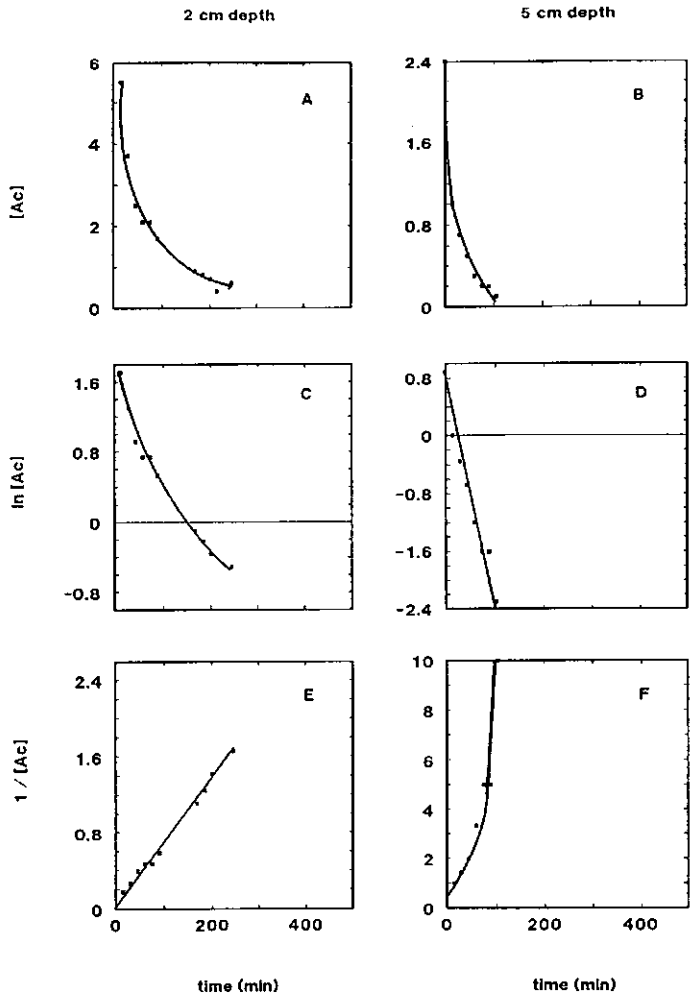

Fig. 5. Linear, semi-log, and reciprocal plots from which acetate uptake-rate constants were obtained, using the slopes of the lines. The left panels show data from a batch experiment using sediment collected from 2 cm (sulfate reducing horizon); the right panels show data from a batch experiment using sediment collected from 5 cm (methanogenic horizon). Samples were collected in the non-stratified period (3 April 1990).

2 cm depth

Period		K_1	Ac	Uptake	n
Season	Months	day^{-1}	µM	µM day^{-1}	
Early spring	Mar-Apr	84 (59-109)	6.5	546	2
Summer	May-Sep	36 (31-41)	8.4	302	2
Pre-overturn	Sep-Nov	49	8.3	408	1
Post overturn	Nov	65	3.1	201	1
Winter	Dec-Feb	60 (58-61)	0.5	30	2

5 cm depth

Period		K_1	Ac	Uptake	n
Season	Months	day^{-1}	µM	µM day^{-1}	
Early spring	Mar-Apr	82 (72-92)	6.4	522	2
Summer	May-Sep	163	6.8	1108	1
Pre-overturn	Sep-Nov	168	7.0	1176	1
Post overturn	Nov	55	4.1	226	1
Winter	Dec-Feb	37 (35-39)	0.8	30	2

Table 5. Uptake-rate constants and acetate uptake-rate estimates at two and five-cm depth during different seasonal periods. Uptake rates are calculated using the average K_1 for the period and the average acetate concentrations in the same period.

Acetate Consuming Processes

A comparison of acetate uptake rates with sulfate reduction and methane production measured in early spring is given in Table 6.

	Turnover rates		
Depth	Acetate	Sulfate	Methane
cm	μM day^{-1}	μM day^{-1}	μM day^{-1}
1	715	180	20
2	546	146	50
5	522	30	150

Table 6. Comparison of average turnover rates (μM day^{-1}) of acetate, sulfate and methane at 1, 2, and 5 cm depth in the profundal sediment of Lake Vechten during early spring.

The acetate uptake rate integrated over the top seven cm (39.8 $\pm$ 20 mmol m^{-2} day^{-1}) appeared to be high in relation to the sum of sulfate-reduction (0.6-3.6 mmol S m^{-2} day^{-1}: Hordijk et al., 1985) and methane-production (7.2 $\pm$ 1.5 mmol CH_4 m^{-2} day^{-1}: Fig. 2F) based on carbon equivalents. Discrepancies between acetate consumption and sulfate reduction have been reported in marine sediments (Christensen and Blackburn, 1982; Shaw and McIntosh, 1990). Christensen and Blackburn (1982) proposed that acetate forms complexes with solutes that have sufficient stability to render the acetate unavailable to immediate microbial attack. Therefore pore water analysis will overestimate the biologically available acetate pool size. We cannot exclude that such a complex forming may also occur in freshwaters and affect our results. A possible interaction of acetate with Fe(III)-coagulates in *oxic* environments has been revealed in this study. On the other hand we found a fast and complete depletion of labeled

acetate in non-sterile *anoxic* sediments and a complete recovery of labeled acetate in sterile *anoxic* sediments. This indicates a rapid exchange between complexed and free acetate pools in anoxic environments. Thus a chemical interaction of acetate with other compounds may not severely limit the bio-availability of acetate in anoxic sediments.

Overestimates of acetate uptake rates have also been ascribed to a catalytic exchange of the carboxylic group of acetate with porewater CO_2 (Schauder et al., 1986; Shaw and McIntosh, 1990). This reaction cannot affect our results as we used deuterium labels. Uptake rates estimated from [2-^{13}C]acetate and CD_3COOH tracers did not differ significantly (data not shown). Thus loss of deuterium label due to exchange reactions with the solute can also be excluded. Thus we were unable to explain our results by chemical artifacts.

Two acetate-consuming microbial processes that may occur in anoxic sediments, besides sulfate reduction and methanogenesis, are: 1) assimilatory uptake; and 2) 'condensation' reactions. The first was demonstrated in Lake Vechten by fast incorporation of VFA labels into cell lipids (Fredrickson et al., 1988). Also some methanogens require acetate as a carbon source (Jetten et al., 1990). Harvey et al. (1989) reported an assimilatory uptake of 2 µmol acetate l^{-1} day^{-1} in marine sediments with a methanogenic activity of 0.5 µmol CH_4 g^{-1} day^{-1}. Considering the methanogenic activity in Lake Vechten (about 120 µmol CH_4 l^{-1} day^{-1}: Fig. 2F), we can assume that acetate incorporation by methanogens is quantitatively insignificant.

The second possible acetate-consuming process, 'condensation' of acetate to higher VFA with H_2 uptake (Thauer et al., 1977; Dolfing, 1988), does not seem to be important in lake Vechten. The absence of butyrate (C_4) and caproate (C_6), and the relatively low uptake-rate constant of butyrate (k_1, 3.12 day^{-1}: Hordijk unpublished), indicate that 'condensation' reactions to higher VFA could not explain the high acetate uptake rates in Lake Vechten.

Conclusions

This study showed the utility of using stable isotopic tracers to study microbial processes. The small sample volumes (1-3 µl) needed for detection of these tracers by gas-chromatography mass-spectrometry is a main advantage when studying processes in near- surface sediments.

In Lake Vechten sediments, the average acetate uptake rate (39.8 $\pm$ 20 mmol acetate m^{-2} day^{-1}) appear to be high, if compared with previous estimates of methanogenesis (7.2 $\pm$ 1.5 mmol CH_4 m^{-2} day^{-1}) and sulfate reduction (0.6-3.6 mmol SO_4^{2-} m^{-2} day^{-1}) done in early spring. It indicates that, in early spring, most of the total organic carbon sedimentation (58 $\pm$ 17 mmol C m^{-2} day^{-1}) may be metabolized with acetate as an intermediate. The depth profiles revealed the dynamic nature of the acetate pore water pool.

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CHAPTER 4

DISCUSSION, CONCLUSIONS, AND PERSPECTIVES

4.1 EVALUATION OF THE LAKE WATER COLUMN DATA

Sulfur species concentration profiles

The concentration profiles of iron and sulfur species in the hypolimnion of Lake Vechten illustrated the importance of processes in bottom sediments on lake water chemistry in stratifying lakes. The presence of a substantial pool unknown sulfur-bearing species indicated that sulfur studies in the lake water column should not be limited to FeS, ΣH_2S, and SO_4^{2-} only. Other, yet unknown sulfur-bearing species, may also be important intermediates in the energy transfer between sediment and metalimnion (Steenbergen, et al. 1987; Chapter 3.3). Reduced sulfur species in the hypolimnion may also act as a temporary sink of energy. This energy may be released when oxygen becomes available shortly after fall overturn.

During the anoxic period sulfate reduction continues while oxygen is depleted in the hypolimnion. Thus sulfide formed by sulfate reduction cannot be re-oxidized at the sediment water interface and may accumulate as FeS in the sediment during this period. Because, moreover there is a continuous supply of sulfate into the lake by a horizontal ground water influx (Chapter 3.3), considerable amounts of sulfur may have been stored in the sediment. The percentage of the total sulfur in Lake Vechten sediment is ,however, similar to those found in most other European lake sediments (about 0.35%: Baudo and Muntau 1986).

Significant seasonal trends in total sulfur concentrations in the sediment were not found (Chapter 3.3). From data on sulfate reduction rates and sedimentation rates, we concluded that more than 85% of the sulfur input into the sediment is released again into the

hypolimnion (Chapter 2.3). Thus there may be a continuous cycling of sulfur species between the sediment and hypolimnion during the whole year.

Only few sulfur studies have related lake water chemistry and sedimentation data with total sulfur pools in the sediment (South Lake, David and Mitchell 1985; Wintergreen Lake, King and Klug 1982). Comparison of the field data of Lake Vechten and the other two lakes indicated considerable differences in the sulfur cycle (Chapter 3.3; Table 5). For example, seston sedimentation rates (1030 g m^{-2} yr^{-1}) and sulfate reduction rates (2490 mmol S m^{-2} yr^{-1}) suggest that Wintergreen lake is more productive than Lake Vechten (seston sedimentation rate, 570 g m^{-2} yr^{-1}; sulfate reduction rate 1068 mmol S m^{-2} yr^{-1}). If Fe was not a limiting factor for sulfur precipitation, one may speculate that more sulfur will accumulate in Wintergreen Lake sediment than in Lake Vechten sediment. Total sulfur concentrations, however, were 4.5 times higher in Lake Vechten than in Wintergreen (Chapter 3.3 Table 5). This illustrates that higher rates of sulfate reduction do not always result in higher concentrations of sedimentary sulfur.

The lake water sulfur species

The field data of Lake Vechten indicated a close relation between the sulfur and iron cycle in the hypolimnion (Chapter 3.3). Davison et al. (1992) discussed the formation of FeS in the hypolimnion. Based on a FeS stoichiometry of 1:1 at most 40% of the sulfur trapped at 9.6 m depth can be explained by the presence of FeS precipitates (Chapter 3.3). Thus, more sulfur bearing species as FeS may be present in the lake water column. By subtracting the SO_4^{2-}, ΣH_2S and FeS concentrations from the total sulfur concentration, the existence of a pool unknown sulfur-bearing species was revealed in the lake water

column (Chapter 3.3).

Revealing the nature of unknown sulfur-bearing species other than FeS, ΣH_2S, or SO_4^{2-} in the hypolimnion may give better insights in the electron transport between sediment and metalimnion. Sulfur bearing species may be associated with or incorporated into the dissolved organic carbon (DOC) or particulate material (Freney 1961; Moers et al. 1988). Trends in sedimentation rates of carbon and total sulfur were, however, not correlated in Lake Vechten (Chapter 3.3). Also the DOC pool did not follow the seasonal trends seen in the lake water sulfur pools (Chapter 3.3). Lake water DOC concentrations (about 5 mg l^{-1}: J. Olie, unpublished) and a C:S relation of about 100:1 for organic material (Chapter 3.3) makes it unlikely that the DOC pool contributes significantly to the missing sulfur fraction. For similar reasons the biomass (algae and bacteria) could explain at most a few percent of the total sulfur pool in the lake water (Chapter 3.3). It is however still possible that the unknown sulfur pool is associated with the DOC and/or particulate pool. For example, sulfur enrichment of organics can occur due to the chemical reaction with mineral sulfur (Moers 1988). Information on sulfur enriched species in hypolimnia is however absent in literature.

Ways to specify lake-water sulfur species

There are two basic approaches to specify sulfur bearing species in the lake-water column: functional group analysis (e.g. C-S and C-O-S bonds) and molecular size fractionation. Reductants as HI (Kowalenko 1985) and Raney Nickel (Freney 1970) have been frequently used for functional group analysis. Unfortunate, none of these methods are really specific (Freney 1970). Also the precision of the assessments of inorganic

compounds like FeS_2 (Lord 1982), S^o (Devai 1984) and polysulfides (Chen and Gupta 1973) are still a point of discussion. Majchrowics et al. (1991) published a method for sulfur analysis by temperature programmed reduction. It would be interesting to use this method on Vechten sediment and to compare the results with other methods. Besides that methods to differentiate between sulfur species in sediments are far from perfect, they also may need modifications before their application to lake water samples.

Fractionation of sulfur-bearing species on molecular size in freshwaters has never been done, to our knowledge. Using dialysis membranes may also be a way to study the interaction between sulfur species with particulate matter in the lake water column.

It is important to know if reduced sulfur compounds can be formed in the lake water column by local microbial activity as it may help to unravel the origin of these compounds. Therefore we have measured sulfate reduction and sulfate production in the lake water column (Chapter 3.3), but did not obtain conclusive results. It is possible that the applied methods were not sensitive enough to detect microbial activities in the lake water column. It is more likely that the sulfur species in the hypolimnion are originating from benthic microbial processes. This hypothesis is supported by the gradual increase of sulfur bearing species and iron with depth during summer stratification (Chapter 3.3: Fig. 1-2) and field studies done by other workers at comparative sites (Stuiver 1967).

A goal for future research may be to investigate if unknown sulfur bearing species could serve as an electron donor for the dense population of sulfur oxidizing phototrophes blooming at the thermocline of Lake Vechten. Lake balance studies indicated a discrepancy between ΣH_2S supply by diffusion and the primary production of the sulfur oxidizing phototrophes in the metalimnion (Steenbergen and Korthals 1982). To study if there are more sulfur species than ΣH_2S and FeS that could serve as electron donor,

ΣH_2S free lake water should be added to cultures of sulfur oxidizing phototrophes.

4.2 EVALUATION OF THE SEDIMENT DATA

In contrast to the lake water column, microbial activities like the uptake of VFA and sulfate reduction rates can be well detected in the near surface sediment (Chapter 3). This study is focused on acetate kinetics, an important substrate for sulfidogens in marine sediments (Widdel and Pfennig 1977; Skyring 1988). The addition of MoO_4^{2-} to marine sediments induced the accumulation of acetate and other VFA (Parkes et al. 1989).
A problem was however that sulfate reduction rates were too low to explain the high acetate uptake rates in marine sediments (Shaw et al. 1984). A second problem is that sulfate-reduction rate estimates obtained from batch experiments are usually higher than those obtained by mathematical modeling, both in marine and freshwater sediments (Chapter 3.2; Jørgensen 1978; Marnette et al. 1992). Discrepancies between in vitro methane production rate estimates and in situ measurements have also been reported for freshwaters (Kelly and Chynoweth 1979).

We have tried to relate acetate uptake rates and sulfate reduction rates by comparing field data and batch experiments. Lovley and Klug (1986) have proposed a theoretical model that sulfidogens and methanogens compete for acetate in near-surface sediments. Thus, this model suggests that acetate is a limited substrate for sulfate reduction. On stoichiometric base of 1:1 rates of sulfate reduction in the near surface sediment (4 ± 3 mmol m^{-2} day^{-1}; Chapter 3.4) were much lower than those of acetate consumption (40 ± 20 mmol m^{-2} day^{-1}; Chapter 3.4) measured at the same time and depths. This indicates that acetate does not limit sulfate reduction. Further evidence that the availability of acetate

does not limit sulfate reduction is 1) the occurrence of relative high acetate concentrations at the sediment-water interface, and 2) the lack of stimulation of sulfate reduction by the addition of acetate (data not shown). It seems that diffusion of sulfate into the sediment, rather than availability of acetate, limits sulfate reduction. That sulfate reduction is limited in Lake Vechten sediment is indicated appearance of first order reaction kinetics (Chapter 3.2).

Low acetate concentrations and first order kinetics for acetate uptake (Chapter 3) indicate that acetate is a limited substrate in the methanogenic horizon. Thus, the availability of H_2 and acetate appears to control methanogenesis deeper in the sediment. Like CO_2, acetate and H_2 are released endogenously during fermentation of biopolymers (Winfrey and Zeikus 1979). It is also possible that these compounds diffuse from the sediment-water interface into the methanogenic horizon (Michelson et al., 1989). Hydrogen concentrations can have a strong impact on acetate kinetics in the methanogenic horizon (Dolfing 1988). An objective of future research may be to study the effect of H_2 on VFA kinetics under *in situ* conditions (Lovley and Goodwin 1988).

The hypothesis that electron acceptors limit respiration at the sediment surface and electron donors limit methanogenesis deeper in the sediment, agrees well with our field observations (Chapter 3.4). In situ concentration profiles of acetate contradict with those predicted by Lovley and Klug (1986), but resemble well with those predicted by Michelson et al. (1989). Lovley and Klug expected the highest concentrations of acetate in the methanogenic horizon and the lowest in the sulfate reducing area. Michelson et al. (1989) expected the highest acetate concentrations at the sediment surface. Both workers noted the inability to verify their models with field measurements. This illustrates the

need for analytical methods as introduced in this thesis.

Acetate uptake in the sulfidogenic horizon

We have used specific inhibitors to get insights in the interaction between acetate kinetics and sulfate reduction. Molybdate (Parkes et al. 1989; Michelson et al. 1989) was used to stop sulfate reduction and BES (2-bromoethanesulfonic acid; Oremland and Capone 1988) to stop methanogenesis. The inhibitors were added to sediment batches collected from the sulfidogenic (2 cm) and from the methanogenic (5 cm) horizon. The sediment was collected during early spring when the lake was completely mixed. Turnover rates of acetate were compared with those previously estimated in the same batch without inhibitor added. Thus, experiments were sequentially done in the same batch and differences in microbial activities due to spacial effects were excluded in this way. Addition of 5 mM MoO_4^{2-} completely blocked sulfate reduction, and acetate uptake was inhibited for 60%. So sulfate reduction could be an important acetate consuming processes. However, inhibition of sulfate reduction may also stimulate other processes to use acetate normally used by sulfidogens. This will mask the effect that MoO_4^{2-} has on the oxidation of acetate by sulfidogens. Thus, assuming that molybdate inhibits only sulfate reduction, acetate utilization by sulfidogens may be more as 60%.

The conclusions obtained from the inhibition experiments become equivocal, if *in situ* sulfate reduction rates are compared with acetate uptake rates in the top cm of the sediment. On a 1:1 stoichiometric base, sulfate reduction rates represented at most about 25% of the acetate uptake rates (Chapter 3.4, Table 6). So at most 25%, not 60%, inhibition of acetate uptake rates had to be expected with MoO_4^{2-} as inhibitor and sulfate

as the main acetate consuming process. Thus MoO_4^{2-} not only affected sulfate reduction, but also affected other acetate consuming processes. So this type of inhibition experiments may lead to erroneous conclusions for freshwater sediments.

Acetate uptake rates in the sulfidogenic horizon are much higher as the sulfate-reducing rates (Chapter 3.4). This indicates that acetate is mainly consumed by non-sulfidogenic processes.This does however not exclude that, like in marine systems, acetate is one of the main electron donors for sulfate reduction in Lake Vechten.

There are however indications that acetate is not even very important in sulfate reduction. These indications are 1) incorporation of ^{14}C-acetate in cell lipids of benthic microbes was inhibited by only 32% by MoO_4^{2-} at 2 cm (lactate > 95% inhibition: Fredrickson et al., 1988), and 2) supply of exogenous sulfate (up to 350 µM) to sediment collected at 1 or 2-cm depth did not stimulate acetate uptake (data not shown). So non-sulfidogenic processes may be primarily responsible for acetate uptake in the respiring horizon.

The role of acetate-oxidizing sulfidogens in natural freshwater systems is also dubious. Spores of *Desulfotomaculum acetoxidans* (Widdel and Pfennig 1977) and *Desulfobacter postgatei* (Widdel and Pfennig 1981) have been isolated from both marine and freshwater sediments using saline media. Several other authors were however not able to isolate active microbes from freshwater sediments (Laanbroek and Geerligs 1983; Bak and Pfennig 1991). The optimum growth temperature for *D. acetoxidans* was 37 °C and it was isolated from animal manure and dung contaminated habitats suggesting that it was of intestinal origin (Gibson 1990). *D. postgatei* has a K_m of 200 µM sulfate (Ingvorsen et al. 1984). Such growth conditions indicate that Lake Vechten is not an optimal habitat for these species. The average temperature in the hypolimnion of Lake Vechten is 10 °C and

acetate may have to compete with energetic preferable substrates as lactate and H_2 in the sulfidogenic horizon (Chapter 2.4).

Acetate uptake in the methanogenic horizon

The role of acetoclastic methanogenesis is undisputed in freshwater sediments (Cappenberg 1974; Winfrey and Zeikus 1979). Experiments in which the formation of ^{14}C-CO_2 and ^{14}C-CH_4 were followed from 2-^{14}C-CH_3COOH indicated that in the methanogenic horizon about 85% of the methyl group of acetate was converted to ^{14}C-CH_4 at 5 cm depth (Cappenberg and Jongejan 1978).

We also used specific inhibitors to study acetate kinetics in the methanogenic horizon (3-10 cm). Molybdate (Parkes et al. 1989; Michelson et al., 1989) was used to inhibit sulfate reduction and BES (2-bromoethanesulfonic acid; Oremland and Capone 1988) to inhibit methanogenesis. We used cocktails of BES (1-10 mM) and MoO_4^{2-} (5 mM) in batches from the methanogenic horizon. The cocktails did not completely block acetate consumption at 5 cm depth (data not shown). To stop methanogenesis, 50 to 100 mM BES and 20 hours incubation was needed, which agrees with results in King (1984). Although acetate uptake was inhibited by more than 90% using 50-100 mM BES, results cannot be conclusive. BES concentrations were so high that also non-methanogenic processes may have been affected. Thus, experiments with BES as inhibitor did not exclude the possibility that other processes than methanogenesis were important for acetate uptake.

Discrepancy between acetate uptake, methanogenesis and sulfate reduction.

Comparison of acetate uptake rates with methanogenesis (Chapter 3.4) indicates that deeper in the sediment other processes than acetoclastic methanogenesis may play a role in acetate uptake. Pilot studies indicated that the uptake of CD_3COOH was higher that the formation of $^{13}C\text{-}CH_4$ from $^{13}C\text{-}CH_3COOH$ (data not shown). Note that uptake rates of $^{13}C\text{-}CH_3COOH$ and CD_3COOH were comparable (Chapter 3.4). Corresponding results were obtained with $^{14}C\text{-}CH_3COOH$ (Cappenberg and Jongejan 1978). These supported the conclusion made from the field measurements that there is a discrepancy between methanogenesis and acetate utilization deeper in the sediment.

In several marine studies acetate-uptake rates exceeded sulfate-reduction rates and carbon-input rates (Christensen and Blackburn 1982; Shaw and McIntosh 1990) while sulfate was the dominant electron acceptor. In Lake Vechten, acetate uptake rates were also high in relation to other benthic processes, but uptake rates did not significantly exceeded carbon input rates (Chapter 3.4). Comparative freshwater studies are lacking to our knowledge. Differences in the proportional distribution of label between the bioavailable and non-bioavailable acetate is noted as a potential artefact causing an overestimation of acetate uptake-rates in marine sediments (Christensen and Blackburn 1982). The fast and complete turnover and the lack of adsorption do not indicate that such artifacts may have affected our results.

Another explanation for the high acetate turnover rates may be the artificial induction of latent enzymes during the batch incubations. Jørgensen (1978) demonstrated that experimental effects of mixing, diluting, and otherwise handling the sediment may strongly influence the natural metabolic processes. Especially small changes in H_2 concentrations due to sample processing may affect acetate metabolism (Dolfing 1988). A detailed study on what effect mixing and dilution might have on acetate metabolism

may be a goal for new research.

Acetate uptake rates showed considerable variations (Chapter 3.4). The reason for this is that turnover rates are estimated by multiplying turnover rate constants with natural pool sizes of acetate. These pool sizes result from the balance of two fast processes: acetogenesis and acetate uptake. Thus small variations in acetogenesis or acetate uptake may be the reason for the variations in acetate pool sizes and turnover-rate estimates.

Estimated acetate turnover rates are in the same range as the total organic carbon sedimentation rates in Lake Vechten (Chapter 3.4). This is in contrast to marine studies where acetate turnover rate estimates exceeded carbon input (Christensen and Blackburn 1982; Shaw and McIntosh 1990). In conclusion, acetate uptake rates in Lake Vechten are not significantly higher than the carbon input rates, although they are high in relation to methanogenesis and sulfate reduction.

4.3 SOME ANALYTICAL CONSIDERATIONS

Recently King (1991) has developed an enzymatic method to determine acetate in marine and freshwaters that coupled the synthesis of acetyl coenzyme A to AMP production. The resulting AMP was assayed by liquid chromatography. He claimed that his technique measures only acetate that reacts enzymatically and not acetate fractions that are kinetically slow to equilibrate or that require some harsh chemical conditions to desorb bound pools are not measured. It would be interesting to apply this enzymatic technique in Lake Vechten and to compare its results with the methods used in this study. The application of enzymatic techniques in sediments has however a few limitations: 1)

the inability to detect isotopic labels, 2) the inability to measure more fatty acids simultaneously and, 3) enzyme activity maybe affected by sulfide in the sediment matrix. Careful examination of side effects will be needed to test the reliability of an enzymatic method in Gyttja sediments.

In Chapter 3.4 we have demonstrated that acetate can complex with Fe^{3+}. Thus, if there is an interaction between acetate and Fe^{3+}, it will depend on the redox condition of the sediment. Gel chromatography has been used to separate complexed and non-complexed acetate pools in marine matrices (Christensen and Blackburn 1982; Michelson et al. 1989). This was done to evidence the existence of a complexed non-bioavailable pool. There are however no indications that these experiments were done under strict anoxic conditions, thus preventing pore water Fe^{2+}, if present, to oxidize. This implies that Fe^{3+} can be formed from Fe^{2+} during elution on the gel column. Sequentially, the formed Fe^{3+} may have reacted with acetate to form complexes giving rise to multiple peaks in the chromatogram. For this reason it would useful to eluate the Fe^{2+} containing pore water of Lake Vechten under strict anoxic conditions and compare the results with those achieved with oxygenated eluent.

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SAMENVATTING

Dit proefschrift is een samenvatting van ca. 10 jaar onderzoek aan de anaerobe mineralisatie van organisch materiaal in het stratificerend meertje Vechten. Het onderzoek is toegespitst op de zwavelkringloop en op de relatie tussen de zwavelkringloop en de koolstofkringloop. In hoofdstuk 1 wordt de limnologie van het meertje Vechten in relatie tot deze twee cycli besproken.

In zoetwater systemen speelt het sediment een belangrijke rol in de afbraak van organisch materiaal. Tot voor enige jaren terug was er geen ruimtelijk inzicht in de gelaagheid van de verschillende mineralisatie processen in het sediment. Ideeën over deze gelaagheid waren gebaseerd op fysiologische eigenschappen van potentiëel dominante bacterie populaties.

Nieuwe analytische ontwikkelingen hebben het mogelijk gemaakt om de microbiële activiteit in dunne laagjes sediment te meten. In hoofdstuk 2 wordt op deze technieken ingegaan. Hoofdstuk 3 is meer toegespitst op de evaluatie van de veldwaarnemingen

Uit veldmetingen bleek dat de penetratiediepte van zuurstof, nitraat en sulfaat in het sediment van Vechten slechts enkele mm tot enkele cm bedraagt. Door het meten van de scherpe concentratie profielen was het mogelijk om de opnamesnelheden van electron acceptoren modelmatig te bepalen in de toplaag van het sediment. Ter ondersteuning van de model berekeningen werden diffusie coefficienten, adsorptie effecten en reactie kinitiek van relevante verbindingen onafhankelijk bepaald. De omzetting van nitraat en sulfaat in de toplaag van het sediment verliep via een eerste orde kinetiek, wat duidt op een electron-acceptor limitatie. Met deze aanname werden, met behulp van de gemeten concentratie profielen, sulfaat en nitraat opnamesnelheden door het sediment geschat

volgens het model van Berner (1964). De geschatte opnamesnelheden bleken voor sulfaatreductie ongeveer een factor twee lager dan de waarden verkregen uit batch experimenten.

Een opmerkelijk resultaat van de batch experimenten is de snelle omzetting van nitraat en sulfaat in sediment batches uit de methanogene zone (3-7 cm). Onder *in situ* omstandigheden zijn sulfaat en nitraat namelijk niet detecteerbaar in deze laag. Toch doet de afwezigheid van een "lag-time" vermoeden dat er een vitale nitraat en/of sulfaat reducerende populatie aanwezig is in een sediment laag die reeds 6-14 jaar oud is.

In een tweede fase van het onderzoek in Vechten werd de accumulatie van zwavel in het sediment vergeleken met de gemeten sulfaatreductie snelheden. Slechts 15% van de totale hoeveelheid gereduceerde zwavel blijkt permanent te worden opgeslagen in het sediment en de rest lijkt te circuleren tussen sediment en hypolimnion.

In de laatste fase van het onderzoek is getracht een verband te leggen tussen de zwavelkringloop en koolstofkringloop door de kinetiek van de 'sleutel-metabolieten' acetaat en sulfaat met elkaar te vergelijken. Uit deze vergelijking blijkt dat de rol van sulfaat als electronen acceptor in de oxidatie van acetaat in zoetwater systemen als Vechten dubieus is. Accumulatie van acetaat na toevoeging van molybdaat is in het verleden vaak aangevoerd als bewijs dat acetaat beschikbaarheid is als electronen donor voor sulfaatreductie in marine sedimenten. Vergelijkbare experimenten in Vechten gaven aan dat molybdaat niet alleen sulfaatreductie remt maar mogelijk ook andere acetate consumerende processen beïnvloedt.

Acetaat opname in het sediment verloopt snel in vergelijking met sulfaatreductie en methaanproductie. De snelheid van acetaat opname overtreft echter niet de koolstofsedimentatie, zoals wel is aangetoond in marine systemen. De hoge opname snelheid van acetaat wijst op de mogelijke aanwezigheid van meer acetaat consumerende

processen dan alleen sulfaatreductie en methaan productie. Meer onderzoek in deze richting lijkt ons wenselijk.

SUMMARY

This thesis is a synopsis of a ten-years research on the anaerobic breakdown of organic matter in the stratifying Lake Vechten. Special attention has been paid to the sulfur cycle and how this cycle interacts with carbon mineralization. The sediment plays an important role in the breakdown of organic matter in freshwater ecosystems. Until a few years ago, there was no insight in how mineralization processes were spatial organized in the sediment. New analytical developments have enabled to measure the steep concentration profiles in flocculent sediment layers of a few cm. These techniques are discussed in the second chapter of this thesis. Chapter 3 is more focused on field data collection and evaluation and Chapter 4 is discussing perspectives for future research.

The study in Lake Vechten demonstrated that the penetration depth of O_2, NO_3^-, and SO_4^{2-} into the sediment can be limited to a few mm till a few cm in freshwaters. This is a crucial difference with marine systems where SO_4^{2-} usually penetrates much deeper. This difference makes it also expectable that microbial kinetics in freshwaters differ from those in marine sediments.

The ability to measure concentration profiles across narrow depth intervals has open new ways to estimate electron acceptor consumptions by mathematical modeling. To support modeling, diffusion coefficients, adsorption effects, and kinetics were determined independently. The uptake of sulfate and nitrate in sediment in batches collected form the respiring horizon followed a first order kinetics. This indicates that electron limitation occurs in the top layer of the sediment. Assuming first order kinetics and using the measured concentration profiles, sulfate and nitrate consumption rates were estimated by the model of Berner. The estimated rates for sulfate reduction were twice lower that the

values obtained from the batch experiments.

A notable result of the batch experiments was the fast uptake rate of nitrate and sulfate in the batches collected from the methanogenic horizon (3-7 cm). Under in situ conditions, nitrate and sulfate will not penetrate till this horizon. The absence of a 'lag time' indicated the presence of a vital sulfate and nitrate reducing community in sediment of approximately 6-14 years old.

In the second stage in this study we examined if the accumulated total sulfur in the sediment could give an indication on sulfate reduction rates integrated over a longer period. By comparison sulfate reduction rates and total sulfur sedimentation rates with the actual amount of sulfur present in the sediment, it appears that only about 15% of the annual sulfur input is permanently buried in the sediment. Thus, more than 80% of the sulfur is released again into the lake water column. This idea was supported by the concentration profiles of sulfur species seen in the lake-water column. The lake-water measurements also revealed that substantial more sulfur bearing species were present in the lake water column as could be explained by the sulfate, FeS, and ΣH_2S pool only.

In the last stage of the study we have related the sulfur and carbon cycle by comparing the kinetics of the 'key metabolites' acetate and sulfate. This comparison indicated that the role of sulfate as electron acceptor in the oxidation of acetate in freshwaters is dubious. Accumulation of acetate after addition of molybdate has several times been used as evidence that sulfidogens uses mainly acetate as electron donor in marine systems. Comparable experiments in Lake Vechten indicated however that molybdate also affected other acetate consuming processes besides sulfate reduction, making conclusions from this type of inhibition experiments tentative. In conclusion, sulfate reduction does not play

an important role in acetate consumption in freshwater like it does in marine systems.

Acetate uptake rates are relative high if compared with sulfate reduction rates and methane production rates. This indicated the existence of other quantitative important acetate consuming processes in the anaerobic part of the sediment. The acetate uptake rates did however not exceed total carbon sedimentation rates as in marine systems.

This study was carried out at the Netherlands Institute of Ecology,

Centre for Limnology, Rijksstraatweg 6, 3631 AC Nieuwersluis.

NAWOORD

Toen ik in 1978 solliciteerde bij het Limnologisch Instituut wist ik niet wat limnologie betekende en wat ik mij van het instituut voor moest stellen. Het bleek al gauw dat ik binnen het instituut een eigen weg moest zoeken. Ik ben Thomas Cappenberg dan ook erkentelijk voor vrijheid en vertrouwen die mij toen is geschonken. Het heeft de basis gelegd voor mijn verdere ontwikkeling. Via het "zure regen project" ben ik in 1986 in contact gekomen met Ton Feytel en Nico van Breemen van de "Vakgroep bodemkunde en Geologie" (LUW). Hierna volgde een intensieve samenwerking en vruchtbare gedachten uitwisseling tussen de vakgroep bodemkunde en de MOS werkgroep. Tijdens deze periode werd het via een nieuwe EEG wetgegeving mogelijk gemaakt om ook als HBO'er te kunnen promoveren. Met name door de aanmoedigingen van Ton, Yigal en Emille heb ik aan Nico van Breemen gevraagd wat hij hiervan vond. Met zijn hulp werden de juiste voorwaarden voor een promotie geschapen. Zijn interesse en kritische houding hebben sterk bijgedragen aan de kwaliteit van dit proefschrift. Het uitvoeren van de experimenten was echter niet mogelijk geweest zonder de hulp van de werkplaats. Daarom wil ik ook vooral Tinus Roling bedanken voor het realizeren van de ingewikkelste gaskronkels, Piet, Marien, van Asdonk, Hafkamp, Henny, en Petra voor geleverde kunstwerken en 'meedenk-inspiraties'; Hep en Steven voor de monsternames. Natuurlijk vergeet ik hierbij ook niet mijn collega's van de werkgroep MOS, de administratie en bibliotheek. Binnen het instituut heb ik vooral respect gekregen voor Ramesh Gulati en Onno van Tongeren voor hun ruime interesse. Tenslotte wilde ik vermelden dat ik met veel plezier heb samengewerkt met alle stagiares en studenten die ik in de afgelopen jaren heb mogen begeleiden. Ik wil dan ook Gert, Johan, Charles, Johan, Han, Marchel, Frank, Ilse, Henk, Susan, Dian, Gerton en Michiel bedanken voor hun bijdrage.

CURRICULUM VITAE

Kees Hordijk werd op 26 december 1953 in Zwijndecht geboren. In juni 1978 studeerde hij af aan de vierjarige opleiding Hoger Natuur Wetenschappelijk Onderwijs (HNWO) te Breda (niveau vergelijkbaar met HTS chemie). In 1978 trad hij op contract basis in dienst van het Limnologisch Instituut te Nieuwersluis (thans onderdeel van het Nederlands Instituut voor Oecologisch Onderzoek) als research-analist in de werkgroep Mineralisatie van Organische Stof. In 1980 volgde een vaste aanstelling aan dit instituut. Hij werd in 1986 bevorderd tot eerste laboratorium assistent mede op grond van zelfstandig onderzoek dat deels in ecologische deels in analytische tijdschriften onder eigen naam werd gepubliceerd. Op 1 februari 1990 werd hem vrijstelling van het doctoraalexamen van de LUW verleend om te kunnen promoveren.