Dynamics of $\Sigma$CO$_2$ in a surficial sandy marine sediment: the role of chemoautotrophy

Uffe Thomsen, Erik Kristensen*
Institute of Biology, Odense University, Campusvej 55, DK-5230 Odense M, Denmark

ABSTRACT: Net consumption and production of CO$_2$ in the surface layers of a sandy marine sediment were examined with a depth resolution of 1 mm. A transient state diagenetic model fitted to measured porewater profiles of total inorganic carbon ($\Sigma$CO$_2$) in open incubated sediment plugs revealed 3 distinct zones. The first was an upper oxic/suboxic zone of 5 to 8 mm depth with high net $\Sigma$CO$_2$ production rates (4910 to 5570 nmol cm$^{-3}$ d$^{-1}$). The second zone (8 to 9 mm) below the suboxic layer showed a net CO$_2$ uptake (161 to 191 nmol d$^{-1}$) which coincided with the zone of maximum $^{14}$C-labeled bicarbonate fixation ($R_{\Sigma}$CO$_2$). This implies that anoxic CO$_2$ fixation is associated with anoxic processes probably involving sulfur species, since both NO$_3^-$ and metal oxides are absent. The CO$_2$ fixation processes were, however, dependent on supply of oxidation equivalent from above, since they were completely inhibited under anoxic conditions in the overlying water. A third zone, situated below 16 mm in the deepest reduced sediment, had low net production rates of $\Sigma$CO$_2$ (55 to 97 nmol cm$^{-3}$ d$^{-1}$). The role of $\Sigma$S$_2$O$_3^{2-}$ in CO$_2$ fixation was examined in completely anoxic and closed sediment incubations (jars). The presence of 0.5 mM $\Sigma$S$_2$O$_3^{2-}$ did not induce higher CO$_2$ fixation rates than $\Sigma$S$_2$O$_3^{2-}$-free controls. When thiosulfate was increased to 2 mM, a stimulation of CO$_2$ fixation occurred, indicating chemoautotrophy by e.g. disproportionation. The fact that significant CO$_2$ fixation also occurred initially in thiosulfate-free, anoxic control sediment indicated that hetero-/chemolithotrophic CO$_2$ fixation may be higher in marine sediment than previously thought.

KEY WORDS: Mineralization · Carbon fixation · Diagenetic modelling · Sulfate reduction · Thiosulfate · Marine sediment

INTRODUCTION

Coastal marine sediments are supplied by organic carbon allochthonously via sedimentation or autochthonously via benthic micro- and macrophytes. Chemoautotrophic fixation of CO$_2$ in sediments can also be considered an autochthonous source of highly labile organic matter (Fenchel & Blackburn 1979). Howarth (1984) estimated that chemoautotrophic production consumed 3 to 6% and 10 to 18% of the heterotrophic CO$_2$ production in 2 coastal sediments of low and high metabolic activity, although this may be an overestimation (Jørgensen 1988). Chemolithotrophic bacteria obtain energy by oxidizing inorganic substrates derived from anaerobic decomposition of photosynthetically produced organic material (Jørgensen 1989). Therefore, these bacteria are found in transitional environments where electron acceptors and reduced inorganic compounds are present simultaneously. One classic example is the extremely narrow $O_2$-$H_2S$ interface in chemoautotrophic Beggiatoa mats that oxidize sulfide at very high rates (Jørgensen & Revsbech 1983, Nelson et al. 1986).

In coastal marine sediments, deficiency of total inorganic carbon ($\Sigma$CO$_2$) in discrete layers below the oxic surface has been ascribed to chemoautotrophic assimilation (Aller & Yingst 1985, Kristensen & Hansen 1995). The most likely electron acceptors for chemoautotrophy in the suboxic zone of these sediments are Mn$^{4+}$ and Fe$^{3+}$ (Howarth 1984, Aller & Rude 1988). S-species like $\Sigma$S$_2$O$_3^{2-}$ and SO$_4^{2-}$ can be produced as intermediates in the chemoautotrophic catalyzed oxidation of HS$^-$ or FeS to SO$_4^{2-}$ by MnO$_2$ (Aller & Rude 1988,
Spontaneous oxidation of HS\(^-\) by Fe-oxides can also produce S\(_2\)O\(_3\)\(^2^-\), SO\(_3\)\(^2^-\), and SO\(_4\)\(^2^-\) (Dos Santos Afonso & Stumm 1992). The complex of partially oxidized S-compounds may also by themselves be important for carbon dynamics in anaerobic sediments. The role of these compounds is supported by reports of autotrophic disproportionating and chemolithotrophic bacteria (Tuttle & Jannasch 1973, 1977, Jørgensen et al. 1979, Kepkay & Novitsky 1980, Bak & Cypionka 1987, Bak & Pfennig 1987, Thamdrup et al. 1993). The quantitative role of chemolithotrophic bacteria in marine ecosystems, however, is still not fully understood.

The aim of this study was to locate and quantify net rates of \(\Sigma CO_2\) production and consumption with a depth resolution of 1 mm in subsurface layers of open incubated organic-poor marine sediment. Estimates of net \(\Sigma CO_2\) consumption from profile modelling were compared with rates of gross uptake of \(^{14}\)C-labeled bicarbonate. Closed sediment incubations were used to evaluate the potential role of S\(_2\)O\(_3\)\(^2^-\) for \(^{14}\)CO\(_2\) incorporation under reduced conditions.

**MATERIALS AND METHODS**

**Sediment sampling.** Subtidal sediment was collected in June (plugexp 1), August (plugexp 3), and December (plugexp 2) 1994 from the shallow (0.2 to 0.6 m) marine lagoon Fællesstrand, on the northeast coast of Fyn, Denmark. The sediment was homogeneous and consisted of well-sorted sand with a low content of silt and clay (<0.5%). The organic content was low (loss-on-ignition ~0.5%), originating primarily from benthic diatoms. The macrophyte vegetation was poor in the area with the seagrass *Ruppia maritima* as the most abundant species. Benthic macrofauna were numerous with the polychaete *Nereis diversicolor*, the gastropod *Hydrobia neglecta*, and the crustacean *Corophium volutator* as the most common species. A detailed description of the area is given by Kristensen (1993).

On each sampling date surface sediment (0 to 5 cm) was sieved through a 1 mm mesh in the field to remove benthic macrofauna. In the laboratory the sediment was further sieved through a 500 μm mesh and stored at 5°C in a polyvinylchloride (PVC) container with 1 cm of overlying water until further use within 3 d.

**Plug experiments (plugexp).** Of the 3 plug experiments conducted, plugexp 1 and 2 were aerobic while plugexp 3 consisted of both aerobic and anaerobic incubations. The sediment was homogenized by hand and placed in cylindrical plug holders, 5.5 cm high and 8.0 cm in diameter (Fig. 1). The sediment plugs were adjusted by a movable bottom to obtain a depth of 3 cm (L), with the sediment surface flush with the upper edge of the plug holders. During each incubation temperature was 15°C and salinities were 18, 27, and 24% in plugexp 1, 2, and 3, respectively.

**Plug exp 1 and 2:** In each of plugexp 1 and 2, 6 to 10 plugs were gently submerged into a darkened and aerated 300 l reservoir. A submerged centrifugal pump provided continuous water circulation. The incubation time was 20 to 28 d with renewal of 1/6 of the seawater every fourth day. Before a set of 3 preincubated plugs were sectioned, flux measurements on each were performed by incubation in circular plexiglass chambers (11 cm diameter) with an effective volume of 1.25 l (i.e. corrected for plug volume). The chambers were closed with O-ring sealed lids that had inlet and outlet equipped with 3-way sampling valves and an adjustable stirring bar (Teflon-coated) receiving momentum from an external rotating magnet at ~100 rpm. Stirring was kept below the resuspension level and a dye test confirmed the stirring efficiency. Three to four sampling routines were done over a total flux incubation time of 9 to 24 h. Samples for CO\(_2\) were taken by 10 ml glass syringes (Fortuna) and preserved by adding 0.2% saturated HgCl\(_2\). Analysis was done within 6 h by flow injection/diffusion cell analysis (Hall & Aller 1992) on a Kontron HPLC (high-performance liquid chromatography) system with 30 mM HCl as carrier and 10 mM NaOH as receiver (precision <2%). Parallel samples were taken and analyzed for O\(_2\) by the standard Winkler technique (precision <0.3%). Fluxes (directly measured: \(J_{\text{O2}}\)) were determined from the accumulation rate of \(\Sigma CO_2\) (\(C_t\)) over time \(t\). \(C_t\) was corrected for dilution of sample by compensation water. The correction was <0.5%.
Subsequently, the plugs were cut into 1, 2, and 3 mm sections in the intervals 0–12, 12–20, and 20–26 mm, respectively, for porewater extraction. During sectioning, a cylindrical collar with the same diameter as the plug holders and a height of 1 mm was placed on top of the holders. Plugs were then elevated within the holder by a screw-based piston with a 1 mm pitch. The sediment was pushed until the surface was flush with the upper edge of the collar. A steel plate with a thickness of 0.5 mm was used to cut off the sediment inside the collar. The plugs were cut successively and each slice was transferred into a 20 ml polystyrene (PS) vial. The screwcaps of the vials were penetrated in the circumference by a needle leaving a 0.5 mm hole. Before the vials were capped, a 0.45 µm Millipore filter supported by a Whatman GF/C filter was placed inside each cap. All vials were then transferred bottom-up into centrifuge tubes and centrifuged for 10 min at 2200 rpm (800 x g). The extracted porewater was analyzed immediately for pH (plugexp 2) and ΣCO$_2$ (plugexp 1 and 2). Remaining porewater was stored at -18°C for later analysis of either NO$_3$ (plugexp 1) or SO$_4^{2-}$ (plugexp 2).

Porewater pH was measured with a pH-electrode (Orion, 81-03) and ΣCO$_2$ was determined by flow injection/diffusion cell analysis after interferring sulfide had been precipitated with 100 µl of 50% saturated HgCl$_2$ per 900 µl sample and removed by centrifugation at 14000 rpm (13000 x g) for 5 min. Samples of NO$_3$ were analyzed using the standard autoanalyzer method of Armstrong et al. (1967). SO$_4^{2-}$ was analyzed by Dionex ion chromatography using an AG4A precolumn and an IonPac AS4A-SC anion column with a self-regenerating suppressor (ASRS-I). The eluent was carbonate (1.8 mM)-bicarbonate (1.7 mM). Precision was better than 3%.

Solid phase Fe (plugexp 1) was determined by HCl extractions according to Lovley & Phillips (1987) with a slightly modified hydroxylamine hydrochloride reduction technique (Thamdrup et al. 1994). Three plugs were sectioned and handled in a glovebag with a N$_2$ atmosphere. Each section was homogenized and subsamples (~1 g) were extracted in 5 ml of 0.5 M HCl for 30 min. After centrifugation the supernatant was analyzed spectrophotometrically for HCl extractable Fe(II) using the Ferrozine color reaction (Stookey 1970). Total extractable Fe [Fe(II) + Fe(III)] was analyzed similarly after hydroxylamine hydrochloride reduction of supernatant subsamples. The concentration of Fe(III) was calculated as the difference between total Fe and Fe(II) concentrations.

**Plugexp 3:** The oxic and anoxic preincubations in plugexp 3 were made simultaneously for 21 d (Fig. 1). Four aerobic plugs were incubated as mentioned above for plugexp 1 and 2. Four other plugs were preincubated under anoxic conditions in 4 plexiglass chambers (11.5 cm high and 9.1 cm in diameter) that had a continuously recycled flow of anoxic water supplied from a 10 l N$_2$ purged reservoir (renewed every fourth day). Bottom and top of the chambers were closed with O-ring sealed plexiglass lids. The flow of water through the chambers was maintained by a peristaltic pump (Ismatec, mp-ges) at a rate of 4.3 to 5.6 l d$^{-1}$. All tubing and connections were made of glass, PVC, or tygon. Frequent tests revealed that O$_2$ was absent in the reservoir water. Water above the sediment (390 ml) was stirred as described previously. Valves before the inlet and after the outlet in the sediment chambers were used for sampling of water for CO$_2$ analysis.

After the preincubation period a short-term [¹⁴C]HCO$_3^-$ ([¹⁴C]CO$_2$) incubation was conducted. Oxic plugs were placed in chambers similar to those used for the anoxic sediment. The overlying water was removed, leaving a 1 mm water film above the sediment surface before 8 vertical injections of 25 µl [¹⁴C]HCO$_3^-$ (9 µCi ml$^{-1}$) with a 250 µl SGE syringe were applied to each plug (both oxic and anoxic). Care was taken to assure a homogeneous vertical distribution of tracer. Oxic plugs were incubated with air as headspace, while anoxic plug chambers were purged with N$_2$ for 5 min before lid closure. After 2 h of acclimatization, anoxic and oxic plugs were sectioned and porewater extracted at approximately 16 h intervals over a 2 d period. Porewater and overlying water were preserved by adding 0.5 M NaOH to pH >12 and stored in 20 ml PS vials at 5°C for later analysis of [¹⁴C]CO$_2$. Fresh sediment subsamples (~0.5 g) were acidified by adding 0.7 ml of 0.5 M HCl and dried at 105°C for 24 h to remove dissolved and precipitated [¹⁴C]-labeled inorganic carbon. The dried samples were then ground and analyzed for total organic C (TOC) and N (TON) and [¹⁴C]-labeled TOC ([¹⁴C]TOC).

Porewater and overlying water samples of 0.5 to 1 ml were diluted with distilled water to a final volume of 7 ml. [¹⁴C]CO$_2$ was then separated by acidification with 0.5 ml of 2 M HCl and trapped in 25% (vol/vol) ethanolamine in 2-ethoxyethanol after purging with air as a carrier gas (Andersen & Kristensen 1992). TOC and TON subsamples were analyzed on a Hewlett-Packard 185B CHN-analyzer and [¹⁴C]CO$_2$ in the exhausted gas was trapped as mentioned above to obtain incorporated [¹⁴C]TOC (Kristensen & Andersen 1987). [¹⁴C]CO$_2$ trap samples (7 ml) were mixed with 10 ml Luma Safe Plus (Packard) scintillation liquid and counted on a Packard 2200CA Tri-Carb scintillation analyzer (liquid scintillation counting, LSC). The total carbon incorporation (TOC) in each sample was calculated from incorporated [¹⁴C]TOC and specific [¹⁴C] activity of porewater CO$_2$. 
Independently incubated plugs were used for determination of porosity profiles, determined from wet density (weight of known volume) and water content (water loss after drying at 130°C for 6 h).

**Jar experiment (jarexp).** A volume of 2250 ml reduced anoxic sediment (the same batch as used in plugexp 2) was homogenized under N₂. A subsample was taken for determination of porosity, as in the plug-exp, before the sediment was split into 3 portions of 750 ml. To each portion was added 900 μl of a [14C]HCO₃⁻ solution (25 μCi ml⁻¹) under continuous mixing. Subsequently, 3.0 ml of either 0, 50, or 200 mM SO₄²⁻ solution was added to the 3 portions to obtain final concentrations of -0 (C), 0.5 mM (T1/2) and 2 mM (T2). After thoroughly mixing again the sediment from each of the 3 portions was transferred to either 10 (C) or 20 (T1/2 and T2) 20 ml acid-washed glass vials (borosilicate, Packard). The vials were filled completely allowing no headspace, closed with foil-lined screw caps, and sealed with tape. In order to assure completely anoxic conditions the vials were incubated half-submerged bottom up in anoxic sediment. Incubation temperature was 15°C.

Frequent sampling by sacrificing 1 (C) or 2 (T1/2 and T2) vials were done during the first 2 d and once a week during the remaining 29 d experimental period. Fresh sediment subsamples (~0.5 g) were taken for determination of TOC, as in plugexp 3. The vials were then centrifuged for porewater extraction through a GF/C filter (Whatman, 25 mm) as described earlier. The porewater was subsequently filtered through a 0.2 μm polycarbonate membrane filter (Whatman, 25 mm). After determining the porewater pH, 1000 μl subsamples were transferred to 1.5 ml Eppendorf tubes, capped and stored at 5°C for less than 24 h before analysis for ΣCO₂ (as in plugexp 1 and 2). Other 1000 μl subsamples were preserved with 0.5 M NaOH to pH >12 and stored in 20 ml PS vials at 5°C for later analysis of 14C-labeled dissolved inorganic ([14C]CO₂) and organic ([14C]DOC) carbon. Isolation of [14C]CO₂ from porewater was performed as described in plugexp 3. In addition, the acidified and flushed porewater samples were radioassayed for [14C]DOC. Dissolved volatile organic compounds may have been lost by the flushing treatment, but they usually constitute only a minor part of the total DOC pool (Sugimura & Suzuki 1988). The radioactivity was determined by addition of 10 ml Ultima Gold XR (Packard) scintillation liquid and counted by LSC as mentioned earlier. The total carbon incorporation in dissolved form (DOC) was calculated from incorporated [14C]DOC and specific 14C activity of CO₂ in each sample. Remaining porewater (1.5 to 2 ml) was stored at -18°C for later analysis of SO₄²⁻ and S₂O₃²⁻. SO₄²⁻ was analyzed as described in plugexp 2 and S₂O₃²⁻ was quantified colorimetrically after cyanolysis (Nor & Tabatabai 1975).

The temporal variation in porewater solutes was described by least squares linear regressions and rates or ratios are presented as the slope with standard error of the coefficient (SE). Statistical comparisons were performed by either Student’s t-test or analysis of covariance (ANCOVA) and multiple comparisons (Zar 1984).

**RESULTS**

**Sediment description**

There was a distinct colour zonation in oxic sediment plugs. The surface was covered by a patchy fluff layer, indicating meiofauna activity (Aller & Aller 1992). The upper 2 mm orange-brown zone was followed by a 2 mm grey zone. Below 1 cm depth, the sediment was greyish-black with a distinct sulphide odour. The anoxic plugs were, like the closed incubated sediment (jar-exp), homogeneously greyish-black.

Porosity in the plugs ranged from 0.28 to 0.35, with highest values in the uppermost and bottom layers. Depth averaged TOC and TON were 104 ± 0.5 (kSD, N = 60) μmol g⁻¹ dry wt, respectively, providing a C:N ratio of 11.

**Plugexp**

**Sediment-water fluxes.** Flux incubations showed a linear ΣCO₂ concentration change with time (r² = 0.69 to 0.85), indicating a constant production rate. Short-term flux incubations (9 h) in plugexp 1 provided a ΣCO₂ flux of 25.0 ± 3.3 mmol m⁻² d⁻¹ (± SE, N = 3), but with poor precision <50% due to insufficient analytical sensitivity. Precision in the long-term (24 h) plugexp 2 was <21% and the ΣCO₂ flux was 35.3 ± 4.0 mmol m⁻² d⁻¹ (± SE, N = 3). The simultaneously measured O₂ concentration changes were highly linear (r² > 0.98), providing fluxes of 17.5 ± 1.5 and 19.4 ± 0.7 mmol m⁻² d⁻¹ (± SE, N = 3) in plugexp 1 and 2, respectively.

**Porewater solutes.** The general depth pattern of ΣCO₂ was similar in plugexp 1 and 2, although the actual shape of the profiles was different. Concentrations increased from ~2.0 at the surface to 3.6 mM at 23 to 26 mm depth with a subsurface minimum at 5 to 14 (plugexp 1) and 8 to 16 mm (plugexp 2) depth (Figs. 2A & 3A), indicating a net production of ΣCO₂ in the upper layers, net consumption in the midzone, and net production in the deepest part. A test showed that there was no significant difference between ΣCO₂ profiles incubated for 22 and 29 d (p >
randomized block ANOVA: multiple comparison), indicating that the systems were close to steady state. Calculations based on the concentration-independent reaction model according to Aller & Mackin (1989) showed that steady state actually should be attained after 29 d with the \( \Sigma CO_2 \) rates presented here (see ‘Discussion’).

Porewater \( SO_4^{2-} \) in plugexp 2 increased from 24 mM in the overlying water to 27 mM at 4 mm depth. Below this depth the concentration decreased and reached 25 mM in the deepest layers. \( NO_3^- \) decreased rapidly with depth from 200 \( \mu \)M at the interface to <10 \( \mu \)M at 5–6 mm in plugexp 1, suggesting an intense consumption (Fig. 2C). The profile showed no real sign of net \( NO_3^- \) production, although nitrification must have occurred close to the sediment-water interface. The generally high \( NO_3^- \) concentration in the upper layers may have masked any production in this zone. The decrease in pH with depth in the sediment, although with bumps around 5 and 13 mm, showed that acid producing processes dominated throughout the plugs in plugexp 2.

**Particulate iron.** Profiles of extractable Fe are shown in Fig. 2B (plugexp 1). Total Fe [Fe(II)\(_{HCl}\) + Fe(III)\(_{HCl}\)] was highest in the surface sediment (2.6 \( \mu \)mol g\(^{-1}\) dry wt) where Fe(III)\(_{HCl}\) constituted 84%. There was a simultaneous decline in Fe(III)\(_{HCl}\) and increase in Fe(II)\(_{HCl}\) in the 1 to 6 mm depth zone. From 12 to 13 mm depth the Fe(II)\(_{HCl}\) level increased abruptly from 1.3 to 1.7 \( \mu \)mol g\(^{-1}\) dry wt, which coincided with the greyish to black colour transition. The lower boundary of the Fe(III)\(_{HCl}\) and \( NO_3^- \) zones, which were almost identical (5–6 mm), defines the suboxic-anoxic transition (Froelich et al. 1979).

\(^{14}\)CO\(_2\) incorporation. Incorporation of \(^{14}\)CO\(_2\) into TOC\(_i\) [R\(_{TOC}\)] was highly dependent on the presence of O\(_2\) in the overlying water (Fig. 4). Significant \(^{14}\)C incorporation was distributed over a wide zone (5 to 18 mm) in oxic plugs, whereas no significant (p > 0.05) incorporation was evident at any depth in anoxic plugs. The bell-shaped incorporation profile in the oxic incubation showed a maximum rate of 240 nmol cm\(^{-3}\) d\(^{-1}\) at 13 mm depth.

**Jarexp**

Porewater solutes. Accumulation of \( \Sigma CO_2 \) showed a general 2-phase linear pattern in all treatments (p \( \leq \) 0.05), although not significant in C (p = 0.12). The transition between phase 1 and 2 occurred about after about 55 h (Fig. 5). The addition of thiosulfate in T1/2 and T2 did not significantly (p > 0.05) stimulate net \( \Sigma CO_2 \) production in the initial phase (phase 1), although the C and T1/2 net production rates of 530 and 514 nmol cm\(^{-3}\) d\(^{-1}\), respectively, were low compared with T2, 800 nmol cm\(^{-3}\) d\(^{-1}\). Net rates of \( \Sigma CO_2 \) production in phase 2 decreased 3 to 4 times compared with phase 1 and reached 173 nmol cm\(^{-3}\) d\(^{-1}\) in T1/2, and 203 nmol cm\(^{-3}\) d\(^{-1}\) in T2.

Consumption of \( SO_4^{2-} \) in C and T1/2 was rapid in phase 1 with rates of 1340 and 1170 nmol cm\(^{-3}\) d\(^{-1}\), respectively, but decreased dramatically in phase 2 to 142 and 115 nmol cm\(^{-3}\) d\(^{-1}\), respectively (Fig. 5). In T2 the \( SO_4^{2-} \) pattern was clearly affected by thiosulfate, leading to a slightly increasing \( SO_4^{2-} \) concentration.
Fig. 4. Plugexp 3. Rates of CO₂ incorporation into TOC, \( R_{\text{TOC}} \), as a function of depth in anoxic and oxic incubated cores. Data presented as the slope ± SE of coefficient (N = 4) from linear regression of time series incubations.

Fig. 5. Jar experiment (jarexp). Porewater \( \Sigma CO₂ \) and \( SO₄^{2-} \) of control (C), 0.5 mM \( S₂O₃^{2-} \) (T1/2), and 2 mM \( S₂O₃^{2-} \) (T2) jars as a function of time. C is represented by single determination while T1/2 and T2 are mean ± range (N = 2). Lines represent linear regressions of phase 1 (<55 h) and phase 2 (>55 h), respectively.

Table 1. Jar experiment (jarexp). C:S stoichiometry of net \( \Sigma CO₂ \) production and net \( SO₄^{2-} \) reduction in phase 1 and 2 (see text). Values are given as slopes ± SE (N) and correlation coefficients are presented as \( r^2 \).

<table>
<thead>
<tr>
<th></th>
<th>Phase 1</th>
<th></th>
<th>Phase 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C:S</td>
<td>( r^2 )</td>
<td>C:S</td>
<td>( r^2 )</td>
</tr>
<tr>
<td>C</td>
<td>0.26 ± 0.09 (4)</td>
<td>0.83</td>
<td>1.17 ± 0.17 (6)</td>
<td>0.92</td>
</tr>
<tr>
<td>T1/2</td>
<td>0.37 ± 0.06 (10)</td>
<td>0.84</td>
<td>1.40 ± 0.17 (12)</td>
<td>0.86</td>
</tr>
<tr>
<td>T2</td>
<td>-</td>
<td>-</td>
<td>1.40 ± 0.10 (12)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

during the initial phase. \( S₂O₃^{2-} \) was consumed linearly (\( r^2 = 0.87 \)) at a rate of 250 nmol cm\(^{-2}\) d\(^{-1}\) during this phase (data not shown). Subsequently, the \( SO₄^{2-} \) reduction (141 nmol cm\(^{-2}\) d\(^{-1}\)) in phase 2 approached the level found in C and T1/2. At the end of phase 1 the \( S₂O₃^{2-} \) concentration in T2 jars was close to the level found in C jars (<30 µM). Unfortunately, \( S₂O₃^{2-} \) samples from T1/2 were lost.

Net \( SO₄^{2-} \) reduction and net \( \Sigma CO₂ \) production showed a good correlation in both phase 1 and 2 of C and T1/2 jars (C:S; Table 1). The C:S stoichiometry in phase 1 was significantly lower (p < 0.01) than in phase 2 for C and T1/2. The added thiosulfate did not significantly (p > 0.05) alter the C:S ratio in T1/2 compared with C in phase 1. There was no significant (p > 0.05) C:S correlation in phase 1 of T2 when based on \( SO₄^{2-} \), but net \( S₂O₃^{2-} \) consumption and net \( \Sigma CO₂ \) production showed a good correlation (\( r^2 = 0.85 \)), leading to a \( \Sigma CO₂:S₂O₃^{2-} \) ratio of 2.85 ± 0.42 (N = 10). All jars had similar C:S ratios (p > 0.05) in phase 2.

\( ^{14}CO₂ \) incorporation. Incorporation of inorganic carbon in the jar experiment was evident from both TOC\(_i\) and DOC\(_i\) pools (Fig. 6). \( ^{14}CO₂ \) incorporation into TOC\(_i\) was only determined until 60 h (phase 1), whereas incorporation into DOC\(_i\) was followed throughout the entire 744 h incubation period. All jars showed a net TOC\(_i\) increase until 35 to 45 h. Between 45 and 60 h, however, the TOC\(_i\) inventory declined. Estimates of \( R_{\text{TOC}} \) during the initial 35 to 45 h were similar in C and T1/2 (see Table 2), whereas T2 had a 5 to 6 times larger input than C and T1/2.

Table 2. Jarexp. \( CO₂ \) incorporation rates calculated from linear regressions of time-dependent changes in TOC\(_i\), \( R_{\text{TOC}} \), and \( R_{\text{POC}} \) (<35 to 45 h). Values are given as slopes ± SE (N). Correlation coefficients are assigned as \( r^2 \). \( \Delta \) indicates the average fraction \( (R_{\text{TOC}i} - R_{\text{POC}i})/R_{\text{TOC}i} \) in %.

<table>
<thead>
<tr>
<th></th>
<th>( R_{\text{TOC}i} )</th>
<th>( r^2 )</th>
<th>( R_{\text{POC}i} )</th>
<th>( r^2 )</th>
<th>( \Delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>99 ± 25 (4)</td>
<td>0.88</td>
<td>70 ± 21 (4)</td>
<td>0.71</td>
<td>29</td>
</tr>
<tr>
<td>T1/2</td>
<td>87 ± 14 (8)</td>
<td>0.86</td>
<td>77 ± 13 (8)</td>
<td>0.85</td>
<td>11</td>
</tr>
<tr>
<td>T2</td>
<td>490 ± 270 (8)</td>
<td>0.35</td>
<td>490 ± 270 (8)</td>
<td>0.35</td>
<td>0</td>
</tr>
</tbody>
</table>
Thomsen & Kristensen: Role of chemoautotrophy in sediment $\Sigma$CO$_2$ dynamics

Figure 6. Jars show the temporal pattern of CO$_2$ incorporation into DOC and TOC in C, T1/2, and T2 jars. C is represented by a single determination while T1/2 and T2 are mean ± range (N = 2). Note the axis break on the abscissa and different scales of the ordinates.

higher rate. DOC$_{1}$ peaked simultaneously or even earlier than TOC$_{1}$, reaching 43 nmol C cm$^{-3}$ at 35 h in C, 30 nmol C cm$^{-3}$ at 45 h in T1/2, whereas a maximum of 23 nmol C cm$^{-3}$ was attained as early as 14 h in T2. The produced DOC$_{1}$ accounted for about 10% of TOC$_{1}$ in C and T1/2 treatments, while DOC$_{2}$ constituted only 1 to 5% in T2. The concentration of DOC$_{1}$ reached a minimum of 4 to 19 nmol C cm$^{-3}$ at the transition between phase 1 and 2 in all jars. Subsequently, the DOC$_{1}$ concentration was constant until 200 h followed by an increase in all jars to a level of about 30 nmol C cm$^{-3}$.

**DISCUSSION**

**Subsurface carbon cycling**

Mineralization of organic matter produces $\Sigma$CO$_2$ at the expense of terminal electron acceptors and therefore $\Sigma$CO$_2$ accumulates with depth in sediments. When autotrophic microbial and other CO$_2$ consuming processes are present, profiles may locally be deflected (Aller & Yingst 1985, Kristensen & Hansen 1995). The boundaries between production and/or consumption rates can be defined from convex-concave transitions or marked differences in curvature of profiles. The $\Sigma$CO$_2$ and SO$_4^{2-}$ profiles measured in the present plug experiments indicate that reactions were distinctively different in at least 3 depth zones: an upper CO$_2$ and SO$_4^{2-}$ production zone, a middle CO$_2$ and SO$_4^{2-}$ consumption zone, and a lower CO$_2$ production and SO$_4^{2-}$ consumption zone (Figs. 2A & 3A, B).

Reaction rates of $\Sigma$CO$_2$ and SO$_4^{2-}$ in non-bioturbated sediments can be estimated from measured porewater profiles. Assuming that profiles are the result of reaction and diffusion processes, i.e. neglecting sedimentation, compaction, and externally impressed flow, the distribution of any solute, $C_{x,t}$, can be described by the 1-dimensional diagenetic equation (Berner 1980):

$$\frac{\partial C}{\partial t} = \frac{D_s}{1 + K} \frac{\partial^2 C}{\partial x^2} + \frac{R}{1 + K}$$  \hspace{1cm} (1)

where $t$ = time, $x$ = depth into the sediment, $K$ = adsorption coefficient, $D_s$ = molecular diffusion coefficient in sediment, and $R$ = net reaction rate of solute.

In the present plug experiment, a 3-layer case is assigned to the diffusion-reaction model by separating the sediment column into an upper zone 1 ($0 < x < z_1$), middle zone 2 ($z_1 < x < z_2$), and lower zone 3 ($z_2 < x < L$). The reaction rates and diffusion coefficients are assumed constant within each zone. The adsorption coefficient $K$ for $\Sigma$CO$_2$ (almost entirely HCO$_3^-$ at the pH 7.5 ± 0.3 (± SD)) and SO$_4^{2-}$ is assumed to be 0. The sediment diffusion coefficient $D_s$ is derived from the free solution diffusion coefficient, $D_{so}$, by correction for tortuosity by the empirical relation for sandy sediments $D_s = \Phi D_{so}$ (Li & Gregory 1974, Ullman & Aller 1982), where $\Phi$ is the average porosity in the zone of interest.

By applying the appropriate conditions (time $T$, depth $n$ and time increment $\delta T$; initial porewater concentration $C_{x,t}$; overlying water concentration $C_{x,t+\delta T}$; depth of the zones $z_1$, $z_2$, $L$; and molecular diffusion coefficients in each zone $D_{1s}$, $D_{2s}$, and $D_{3s}$), the 3 reaction rates $R_1$, $R_2$, and $R_3$ can be deduced by solving Eq. (1) with the following initial and boundary conditions:

1. $t = 0$, $C = C_{x,t}^{ntr}$, $0 < x < L$
2. $t > 0$, $C = C_{x,t}$, $x = 0$
3. $\delta C/\delta x = 0$, $x = L$

Reaction rates were estimated by solving Eq. (1) using the implicit Crank-Nicolson numerical method. Every time step ($\delta T$) until time $T$ involves the solution of a tridiagonal matrix with a vertical depth increment entries by Gaussian elimination (Crank 1975, Vemuri & Karplus 1981). The transient state model is widely applicable because exact information about initial conditions and reaction time does not necessitate steady-state assumptions. By assigning $z_1$ and $z_2$ as the depths of transition between the 3 zones mentioned above,
Table 3. Plug experiments (plugexp) 1 and 2. Parameters used in the 3-layer diagenetic model (Eq. 1) for best fit $\Sigma CO_2$ and $SO_4^{2-}$ profiles (Figs. 2A & 3A, B). $x_1$, $x_2$: depths of zone borders; $D_{x1}$, $D_{x2}$, $D_{x3}$: estimated sediment diffusion coefficients in the 3 zones; $C_{x1}$: initial porewater concentration; $C_T$: overlying water concentration; $R_1$, $R_2$, $R_3$: estimated volume specific rates. Positive rates indicate production.

<table>
<thead>
<tr>
<th></th>
<th>$x_1$ (cm)</th>
<th>$x_2$ (cm)</th>
<th>$D_{x1}$ (cm$^2$ d$^{-1}$)</th>
<th>$D_{x2}$ (cm$^2$ d$^{-1}$)</th>
<th>$D_{x3}$ (cm$^2$ d$^{-1}$)</th>
<th>$C_{x1}$ (mM)</th>
<th>$C_T$ (mM)</th>
<th>$R_1$ (nmol cm$^{-3}$ d$^{-1}$)</th>
<th>$R_2$ (nmol cm$^{-3}$ d$^{-1}$)</th>
<th>$R_3$ (nmol cm$^{-3}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Sigma CO_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plugexp 1</td>
<td>0.5</td>
<td>1.4</td>
<td>0.311</td>
<td>0.298</td>
<td>0.322</td>
<td>3.2</td>
<td>1.75</td>
<td>543</td>
<td>-191</td>
<td>37</td>
</tr>
<tr>
<td>Plugexp 2</td>
<td>0.8</td>
<td>1.6</td>
<td>0.315</td>
<td>0.296</td>
<td>0.325</td>
<td>3.5</td>
<td>2.00</td>
<td>543</td>
<td>-161</td>
<td>55</td>
</tr>
<tr>
<td>$SO_4^{2-}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plugexp 1</td>
<td>0.8</td>
<td>1.6</td>
<td>0.196</td>
<td>0.191</td>
<td>0.222</td>
<td>19.5</td>
<td>23.7</td>
<td>885</td>
<td>-96</td>
<td>-17</td>
</tr>
</tbody>
</table>

reaction rates in each zone ($R$) were approximated from the visually best fit to each of the measured porewater profiles. Parameters used in the 3-layer diagenetic model are shown in Table 3.

The model-estimated profiles were in good agreement with the measured data, except for a slight overestimation at 1 to 5 mm depth in plugexp 2 (Fig. 3A, B). The concave bend in the upper 4 mm of $\Sigma CO_2$ and $SO_4^{2-}$ profiles in plugexp 2 (Fig. 3A, B) suggested that enhanced transport of solutes occurred in this part of the sediment. Accordingly, the depth-integrated $CO_2$ production based on porewater modelling ($J_{x2}$) was 10 times lower than the directly measured $CO_2$ flux ($J_{DM}$; Table 4). Further, the diffusive flux can be estimated from Fick's first law by linear regression of the steepest concentration gradient in the surface sediment by (Berner 1980):

$$J_x = -\phi D_x \frac{\partial C}{\partial x}$$

where $J_x$ and $J_{x2}$ were 9 to 13 times lower than $J_{DM}$ (Table 4). $J_{DM}$ was, despite the limited thickness (3 cm) of the sediment plugs, within the range previously reported for Fællesstrand sediment (Kristensen et al. 1992, Kristensen & Hansen 1995). The rates may, however, be slightly overestimated due to bacterial contribution in the water phase and along chamber walls.

The apparent deficit between $J_{DM}$ and the profile estimates can be explained by (1): $D_x$ is underestimated in the upper few millimeters due to meiofaunal activity (Aller & Aller 1992); (2) stirring during flux incubation significantly decreased the diffusive boundary layer. Further, stirring introduced pressure gradients that induced advective flushing of porewater solutes (Huettel & Gust 1992, Glud et al. 1995, 1996). By the diffusion analogy, convective transport processes can simply be incorporated into the diffusion-reaction model (Eq. 1) as an effective transport coefficient ($\bar{D_x}$) according to:

$$\bar{D_x} = D_x \times J_{DM}/J_{x2}$$

Assuming that advection is restricted to zone 1 and here discretely distributed with depth and horizontally uniform, $\bar{D_x}$ equals 3.23 and 2.85 cm$^2$ d$^{-1}$ (plugexp 1 and 2). Hence, reaction rates estimated by Eq. (1) may be significantly underestimated in proportion to the enhancement of the transport coefficient. Using $\bar{D_x}$ in Eq. (1), $\Sigma CO_2$ reaction rates of 5570 and 4910 nmol cm$^{-3}$ d$^{-1}$ are obtained in the upper zone 1 of plugexp 1 and 2, respectively, which are 60 to 90 times higher than zone 3. Calculating $J_{x2}$ gives 27.7 and 38.8 mmol m$^{-2}$ d$^{-1}$ for plugexp 1 and 2, respectively, which for obvious reasons are in good agreement with $J_{DM}$. Similarly, a net $SO_4^{2-}$ production rate of 8010 nmol cm$^{-3}$ d$^{-1}$ is obtained in zone 1 of plugexp 2. However, porewater flow induced by pressure gradients decreases strongly with depth and also varies horizontally; accordingly, a higher degree of variation in $\bar{D_x}$ is expected within the upper few millimeters of the sediment.

Table 4. Plugexp 1 and 2. Measured and estimated net fluxes of $\Sigma CO_2$, $J_{DM}$; directly measured flux. Values represent average ± SE (N); $J_x$: diffusion-estimated $CO_2$ flux (Eq. 2) based on $\Sigma CO_2$ profiles; $J_{NO_3}^-$: diffusion-estimated $CO_2$ flux (Eq. 2) based on $NO_3$ profiles using a 5/4 conversion factor; $J_{x2}$: depth-integrated production rate estimated from diagenetic modelling (Eq. 1).

<table>
<thead>
<tr>
<th></th>
<th>$J_{DM}$ (mmol m$^{-2}$ d$^{-1}$)</th>
<th>$J_x$ (mmol m$^{-2}$ d$^{-1}$)</th>
<th>$J_{NO_3}^-$ (mmol m$^{-2}$ d$^{-1}$)</th>
<th>$J_{x2}$ (mmol m$^{-2}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plugexp 1</td>
<td>25.0 ± 3.3 (3)</td>
<td>2.33e$^{+0.2}$</td>
<td>2.41e$^{+0.2}$</td>
<td>1.07e$^{+1.4}$</td>
</tr>
<tr>
<td>Plugexp 2</td>
<td>35.3 ± 4.0 (3)</td>
<td>2.67e$^{+0.5}$</td>
<td>3.90e$^{+1.3}$</td>
<td>3.53</td>
</tr>
</tbody>
</table>

*Depth of concentration gradient (mm)
1993, Brandes & Devol 1995). Denitrification estimated from the steepest NO$_3^-$ concentration gradient (Eq. 2) and converted to CO$_2$ production (e.g. Seitzinger et al. 1980) is shown in Table 4. Correcting $J_{NO_3^-}$ for enhanced solute transport using $D_r$ reveals a CO$_2$ production of 10.86 mmol m$^{-2}$ d$^{-1}$. Thus, denitrification appeared to account for $\sim$40% of the heterotrophic CO$_2$ production, but part of the NO$_3^-$ consumption in the deeper parts of the suboxic zone may be coupled to chemical or chemolithotrophic oxidation of reduced sulfur compounds (e.g. Sørensen et al. 1979, Jørgensen & Sørensen 1985, Sørensen & Jørgensen 1987, Canfield et al. 1993b). As a result, SO$_4^{2-}$ is produced in the upper zone 1 (Fig. 3B, Table 3), although SO$_4^{2-}$ reduction and concomitant $\Sigma$CO$_2$ production may proceed simultaneously.

Plugexp 2 and 3 suggested that the middle zone 2, in addition to the consumption of CO$_2$, was characterized by high rates of sulfate reduction. Hence, sulfitolithoautotrophic bacteria seems available in this sediment despite the small height (L). Both the net CO$_2$ uptake estimated from the diffusion-reaction model in zone 2 and gross $^{14}$C uptake showed extensive and almost similar rates (Fig. 4, Table 3), which indicated that transport of solutes here is strictly diffusional. The $^{14}$CO$_2$ incorporation rates are comparable to these of Enoksson & Samuelsson (1987) in sediments of the Gullmar fjord (Sweden). The maximum CO$_2$ fixation rate observed in the oxic environment below the suboxic zone only in sediment underlying oxic water substantiated that incorporation of CO$_2$ is strongly dependent on the presence of oxygen (Kepkay & Novitsky 1980). The high CO$_2$ fixation rate below the suboxic zone can therefore not be explained by heterotrophic assimilation, but rather by chemolitho- and mixotrophic incorporation. These 2 biologically driven CO$_2$ fixation processes cannot be distinguished from each other based on $^{14}$CO$_2$ uptake alone and are here simply designated as chemoolithotrophic activity. Since our $^{14}$CO$_2$ incorporation data in the oxic/suboxic zone (Fig. 4) were rather low compared with the deeper layers, CO$_2$ fixation by chemoolithotrophic oxidation of e.g. NH$_4^+$ and HS$^-$ were of limited importance (Kepkay & Novitsky 1980). Furthermore, anaerobic chemolithotrophic processes utilizing NO$_3^-$ as electron acceptor are not likely to involve significant autotrophic CO$_2$ fixation (Aller 1994, Mulder et al. 1995). Chemoolithoautotrophic CO$_2$ fixation (estimated from $^{14}$CO$_2$ fixation and $J_{DM}$) was equivalent to 8–11% of total CO$_2$ efflux from plugs. This is comparable to the estimates of Howarth (1984) based on sulfate reduction and total respiration rates of coastal sandy sediments. Based on a net CO$_2$ uptake of 161 nmol cm$^{-2}$ d$^{-1}$ (plugexp 2; Table 3) and a gross CO$_2$ uptake of 180 nmol cm$^{-2}$ d$^{-1}$ (plugexp 3) in the middle zone, the CO$_2$ produced by sulfate reducing bacteria (SRB) was only 19 nmol cm$^{-3}$ d$^{-1}$. The high net SO$_4^{2-}$ reduction rate, but low gross CO$_2$ production rate in zone 2, suggested that H$_2$ could be an important substrate for chemolithoautotrophic SRB. Accordingly, Novelli et al. (1988) generally found maximum rates of H$_2$ production in the upper 3 cm of various coastal sediments. However, the SO$_4^{2-}$ profile reflected a complex sulfur dynamics (Fig. 3B) and a higher degree of variation with depth than described by the 3-layer model.

### Role of H$_2$ and SO$_4^{2-}$

The 2 phase temporal pattern of $\Sigma$CO$_2$ and SO$_4^{2-}$ in jars indicated a change in substrate availability for SRB. A similar rapid initial SO$_4^{2-}$ decrease in sediment enclosures was reported by Goldhaber et al. (1977). Aller & Yingst (1980) suggested that variations in sulfate reduction following sediment mixing are due to excessive short-term substrate supply to microbial populations depleted for a specific substrate in the unmixed case. Depending on the type of substrate made available, the C:S ratio may vary. The examples given below illustrate the variability in C:S ratios of important metabolic processes involving SO$_4^{2-}$ commonly found in marine sediments (e.g. Laanbroek & Pfennig 1981, Sørensen et al. 1981, Widdel 1988):

**Acetate:**

$$\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} \rightarrow \text{C}_2\text{H}_4\text{O}_3^- + \text{H}_2\text{S}_2\text{O}_6^{-}$$

**Propionate:**

$$\text{CH}_3\text{CH}_2\text{COO}^- + 3\text{SO}_4^{2-} \rightarrow \text{C}_4\text{H}_7\text{COO}^- + 4\text{CH}_3\text{COO}^- + 3\text{H}_2\text{S}_2\text{O}_6^{-}$$

**Hydrogen:**

$$\text{H}_2 + \text{SO}_4^{2-} + \text{H}^+ \rightarrow \text{C}_2\text{H}_4\text{O}_3^- + \text{H}_2\text{O} + \text{H}_2\text{S}_2\text{O}_6^{-}$$

The rapid SO$_4^{2-}$ consumption and relatively slower $\Sigma$CO$_2$ evolution in phase 1 with a C:S 0.26 to 0.37 (C and T1/2) indicate that H$_2$ may be an important substrate used by SRB (Eq. 6). As H$_2$ does not usually accumulate in sediments, an initial supply of H$_2$ in phase 1 could be related to mixing-induced enhancement of H$_2$ production by fermentation processes. Several species of SRB have been reported to grow by autotrophic or mixotrophic metabolism with H$_2$ as the key electron donor (e.g. Badziong et al. 1978, 1979, Klemps et al. 1985, Widdel 1988). The $^{14}$CO$_2$ incorporation rate in unamended sediment (C) was relatively high compared with the $R_{\text{DM}}$, 0 obtained in the deep layers of anaerobic plug incubation, plugexp 3, which also supports the contention that H$_2$ utilization by SRB in phase 1 is an important electron donor for chemoolithotrophy. But the CO$_2$ fixation did not severely affect the $\Sigma$CO$_2$ production deficit, i.e. low C:S ratio, as carbon incorporation was only 12 to 15% of net CO$_2$ production.
Fig. 7. Jarexp. Average gross $\Sigma$CO$_2$ production calculated as the sum of $R_{\text{OC}}$ and net $\Sigma$CO$_2$ production ($R_{\text{SOC}}$).

The similarity in C:S ratios in phase 2 among the 3 jar treatments indicated that the influence of S$_2$O$_3^{2-}$ had diminished and that substrate availability had normalized. All C:S ratios in this phase were, however, still significantly lower ($p < 0.001$) than the theoretical C:S ratio of 2 for SRB using e.g. acetate as obtained in long-term jar incubations for 59 to 230 d (Burdige 1991, Kristensen & Hansen 1995). Low C:S ratios may indicate that SRB oxidize organic carbon incompletely by e.g. the reaction shown in Eq. (5) or continues to fix CO$_2$ driven by the reaction shown in Eq. (6).

The dependence of CO$_2$ fixation in reduced sediment on the presence of O$_2$ in the overlying water can be related to chemoautotrophic disproportionation of thiosulfate that has been produced by oxidation of sulfide in the suboxic layers (Fossing & Jørgensen 1990). The addition of 0.5 mM S$_2$O$_3^{2-}$, which is well above control (C) level <30 $\mu$M, did not induce any excessive CO$_2$ fixation. But in T2 jars the 2 mM S$_2$O$_3^{2-}$ pool caused a 5 times increase in $^{14}$CO$_2$ incorporation while gross CO$_2$ production doubled (Fig. 7), which indicated that both chemoautotrophic and hetero-/chemolithotrophic processes can be stimulated by thiosulfate (Tuttle & Jannasch 1977). The stimulation was probably due to S$_2$O$_3^{2-}$ disproportionation and facultatively autotrophic growth by SRB (Bak & Cypionka 1987) or replacement of SO$_4^{2-}$ in the H$_2$-consuming reaction shown in Eq. (6) by S$_2$O$_3^{2-}$ with concomitant assimilation of $^{14}$CO$_2$ (Badziong & Thauer 1978). Net SO$_4^{2-}$ consumption during phase 1 in T2 treatments was competitively inhibited by S$_2$O$_3^{2-}$ due to preferential consumption of thiosulfate by SRB (Postgate 1984, Widdel 1988) and production of SO$_4^{2-}$ from S$_2$O$_3^{2-}$ may occur by disproportionation and oxidation (Jørgensen 1990, Elsgaard & Jørgensen 1992).

The quantitative role of DOC$_i$ in jars was generally low and decreased in the sequence: C > T1/2 > T2 (Table 2), implying that the presence of S$_2$O$_3^{2-}$ caused a reduction in DOC$_i$ production or increase in DOC consumption. The positive correlation between S$_2$O$_3^{2-}$ additions and net production of POC$_i$, on the other hand, substantiated that S$_2$O$_3^{2-}$ can be responsible for $^{14}$CO$_2$ incorporation into bacterial biomass. The wider CO$_2$ fixation zone observed in plugexp 3 ($^{14}$CO$_2$ assay) than in plugexp 1 and 2 (porewater model) can be explained by the presence of mobile DOC$_i$ that have dispersed the spatial distribution of estimated $R_{\text{OC}}$, in plugexp 3 due to vertical diffusion and subsequent adsorption or biological uptake of DOC$_i$ in layers without CO$_2$ fixation. The gradual increase of DOC$_i$ during phase 2 (2 nmol cm$^{-3}$ d$^{-1}$) suggested a continued CO$_2$ fixation or formation of refractory DOC from the old POC pool.

More work is required, however, to fully elucidate the relative role of autotrophic S$_2$O$_3^{2-}$ oxidation/disproportionation and H$_2$ oxidation with respect to carbon dynamics in sub-suboxic sediment layers.

**CONCLUSIONS**

The present study provided evidence for a significant autochthonous primary production in a subsurface sandy sediment. The strong dependence of inorganic carbon uptake on the presence of oxygen in the overlying water, despite the fact that incorporation was located in totally anoxic sediment layers, suggested a coupling between oxic/suboxic and reduced sediment. A distinct 2 phase $\Sigma$CO$_2$ and SO$_4^{2-}$ pattern with a low C:S ratio in the initial phase (2 d) of the jarexp indicated that H$_2$ could be an important electron donor for chemoautotrophic SRB. Hence, H$_2$ consumption by SRB might be important in the zone of CO$_2$ fixation, as C:S attained a similar value here. The presence of high concentrations of S$_2$O$_3^{2-}$ (2 mM, jarexp) stimulated both hetero-/chemolithotrophic activity and autotrophic CO$_2$ fixation. Accordingly, very intense production rates of thiosulfate should prevail in the suboxic layers to support significant autotrophic CO$_2$ fixation in anoxic layers below by e.g. thiosulfate disproportionation.

**Acknowledgements.** We thank Hanne Brandt and Ove Larsen for technical assistance in the laboratory and the Ecology Group at the Institute of Biology for valuable discussions. Furthermore, we thank T. H. Blackburn and R. N. Glib for helpful suggestions and constructive criticism of the manuscript.

**LITERATURE CITED**


Badziewg W, Ditter B, Thauer RK (1979) Acetate and carbon dioxide assimilation by Desulfovibrio vulgaris (Marburg), growing on hydrogen and sulfate as sole energy source. Arch Microbiol 123:301–305


Responsible Subject Editor: T. H. Blackburn, Aarhus, Denmark


Manuscript first received: May 29, 1996
Revised version accepted: January 3, 1997