Microbiological and Biogeochemical Studies of Microbial Mn(IV) and Fe(III) Reduction in Arctic Sediments (Svalbard)

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Picture of polar bear: Agata Zaborska during CABANERA cruise 2004

Cover picture: Bo Barker Jørgensen during Svalbard cruise 2003 (grey mud Station J, red mud Station F)

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SUMMARY

This study extends the small database of studies that emphasize the importance of microbial Mn(IV) and Fe(III) reduction for the degradation of organic material in Arctic sediments of Svalbard. Furthermore it improves the understanding of the regulation of microbial Mn and Fe reduction in marine sediments. The investigations of pure cultures of Mn- and Fe-reducing bacteria provide an important approach to assess their potential *in situ* activities and to study their adaptation to environmental settings.

Microbial Mn(IV) and Fe(III) reduction were studied at four sites between 79° and 81° north. These include a fjord of the west coast of Svalbard (Smeerenburgfjorden) with 212 m water depth and three sites of the northeast coast with 286 to 503 m water depth. All sites were characterized by low bottom water temperatures between 2 and 3°C. Total oxygen uptake and sulfate reduction rates were determined by whole core incubations. Anaerobic carbon oxidation and the contribution of sulfate reduction to carbon oxidation were studied in sediment bag incubations of different depth intervals. Anaerobic mineralization rates were compared to the vertical distribution of pore water and solid phase constituents, to determine the importance and zonation of the different respiratory processes in the sediments.

Total oxygen uptake (4.2 mmol m^{-2} d^{-1}) and anaerobic carbon oxidation rates of the bag incubations (10.0 mmol m^{-2} d^{-1}, 0-5 cm interval) of Smeerenburgfjorden sediment were as high as in comparable permanently cold and temperate sediments indicating that the microbial community is adapted to the permanently low temperature. In contrast oxygen uptake (1.5-3.5 mmol m^{-2} d^{-1}) and anaerobic carbon oxidation rates (1.5-4.3 mmol m^{-2} d^{-1}, 0-10 cm intervals) of the three sediments at the northeast coast of Svalbard were lower than at Smeerenburgfjorden. At the northeast coast of Svalbard the carbon supply to the sediments is very limited due to long periods of ice coverage, restricting primary production. In contrast the west coast is influenced by relatively warm Atlantic water and a higher deposition of organic material to the sea floor. Thus, the benthic microbial community of the northeast coast of Svalbard is probably limited by the organic carbon contents of the sediments.

In bag incubations of Smeerenburgfjorden sulfate reduction accounted for 57% of the anaerobic carbon oxidation at 0-5 cm depth and the remaining 43% were attributed to microbial Fe reduction. The zone of microbial Fe reduction overlapped in the surface sediment of the uppermost 2 cm with the zone of sulfate reduction. High rates of organic carbon mineralization lead to a rapid depletion of Fe(III). Thus, the bacterial Fe-reducing

community seemed to be mainly limited by Fe(III). Below 2 cm depth sulfate was sole electron acceptor. Due to low concentrations of solid Mn, Mn was suggested to be mainly reduced chemically by Fe(II) and sulfide. Calculations for the total carbon oxidation of the top 10 cm revealed that Fe reduction contributed to 13%, while sulfate reduction accounted for 34% and aerobic respiration was the most important respiratory process accounting for 53%.

High concentrations of particulate Fe(III) (≥108 μmol cm^{-3}) and unusually high concentrations of solid Mn (≥60 μmol cm^{-3}) were measured at the three stations at the northeast coast of Svalbard. Consequently, microbial Mn and Fe reduction were the most important anaerobic respiratory processes in the sediments contributing to 69 to at least 90% of anaerobic carbon oxidation. In two of three sediments, sulfate reduction rates were below the detection limit (<10-13% of carbon oxidation) and Mn and Fe(III) were present over 0-10 cm depth. Mn and Fe reduction were probably the only important electron accepting processes in these sediments. At the third station a typical depth distribution of anaerobic respiratory pathways occurred: dissimilatory Mn reduction in the surface 3 cm of the sediment, followed by dissimilatory Fe reduction concurrent with sulfate reduction at 3-5 cm, and finally below 5 cm solely sulfate reduction.

The relative contribution of Mn and Fe reduction at all four sites to carbon oxidation seemed to be regulated by the concentrations of particulate Mn and Fe(III), the carbon oxidation rate, and bioturbation. Thus, low organic carbon contents and high concentrations of particulate Mn and Fe at the northeast coast of Svalbard compared to Smeerenburgfjorden seemed to favor microbial Mn and Fe reduction. *In situ*, the contribution of Mn and Fe reduction to carbon mineralization in the surface sediments will vary due to the competition with oxygen- and nitrate-reducing bacteria.

Anoxic slurry incubations of Smeerenburgfjorden sediment were prepared to study the role of volatile fatty acids (VFA) as substrates for Fe- and sulfate-reducing bacteria. Combined turnover rates of ^{14}C-acetate and ^{14}C-lactate accounted only for 15% of the anaerobic dissolved inorganic carbon (DIC) production in the surface layer (0-2 cm), where Fe and sulfate reduction occurred simultaneously, and for 42% in the sulfate reduction zone (5-9 cm). The inhibition of sulfate reduction with selenate resulted in lower DIC production rates compared to the uninhibited slurries. The accumulation of VFA (acetate, propionate, and isobutyrate) in the inhibited slurries indicated that sulfate-reducing bacteria were the main consumers. This was supported by similar acetate accumulation rates in the inhibited slurries compared to initial turnover rates measured by radiotracer in the uninhibited slurries.

However, VFA turnover accounted only for a maximum of 43% of the sulfate reduction and 6% of the Fe reduction in the sediment. Thus, other electron donors have to be utilized by the two bacterial populations.

Psychrophilic and psychrotolerant Fe-reducing bacteria were enriched and isolated at 4, 10, and 17°C from two fjord stations at the west coast of Svalbard. The strains were related to species of the genera *Shewanella*, *Desulfuromusa*, *Desulfuromonas*, and *Desulfovibrio* within the γ- and δ-*Proteobacteria*. The isolates reduced ferric citrate and poorly crystalline Fe oxide, however, strains related to *Desulfovibrio* gained no energy for growth from this process. All isolates were able to grow at –2°C, the freezing point of sea water, showing that they are adapted to the permanently low temperatures of Arctic sediments. As electron donors all strains utilized important fermentation products found in marine sediments. In addition to Fe the bacteria reduced other electron acceptors such as oxygen, manganese, elemental sulfur and sulfate. A possible contribution of sulfate-reducing bacteria to benthic Fe reduction was indicated by the isolation of Fe(III)-reducing *Desulfovibrio*-related strains.

The strains related to species of *Desulfuromusa*, *Desulfuromonas* and *Desulfovibrio* represent new species of their respective genera. The description of these as new species, *Desulfuromusa ferrireducens* sp. nov., *Desulfuromonas svalbardensis* sp. nov., *Desulfovibrio ferrireducens* sp. nov. and *Desulfovibrio frigidus* sp. nov., extends the group of Fe-reducing for psychrophilic and psychrotolerant species in pure culture.

Additionally, a moderately thermophilic, spore-forming, sulfate-reducing bacterium was enriched and isolated at 28°C from a fjord sediment of the west coast of Svalbard. The isolated strain is related to species of the Gram-positive genus *Desulfotomaculum* and is proposed as new species, *Desulfotomaculum arcticum* sp. nov. Due to its inability to grow below 26°C the bacterium must have been present inside the sediment as an inactive spore.

ZUSAMMENFASSUNG

Die vorliegende Doktorarbeit unterstreicht die Bedeutung von mikrobieller Mangan (Mn)- und Eisen (Fe)-Reduktion für den Abbau von organischem Material in arktischen Sedimenten von Svalbard. Weiterhin verbessert sie das Verständnis über die Regulation von mikrobieller Mn- und Fe-Reduktion in marinen Sedimenten. Die Untersuchung von Mn- und Fe-reduzierenden Bakterien stellen einen wichtigen Ansatz dar, um die potentielle *in situ* Aktivität und die Anpassung an bestimmt Umweltbedingungen zu bestimmen.

Die vier untersuchten Standorte, ein Fjord (Smeerenburgfjorden) der Westküste Svalbards mit einer Wassertiefe von 212 m und drei Standorte an Svalbards Nordostküste mit Wassertiefen zwischen 286 und 503 m, zeichneten sich durch niedrige Bodenwassertemperaturen von 2-3°C aus. Sauerstoffzehrungs- und Sulfatreduktionsraten der Sedimente wurden in Kerninkubationen gemessen. Die anaerobe Kohlenstoffmineralisierung und der Anteil von Sulfatreduktion wurden in Tüteninkubationen von Sedimenten aus unterschiedlichen Tiefenstufen untersucht. Die anaeroben Kohlenstoffoxidationsraten wurden mit der vertikalen Verteilung von Porenwasser- und Festphasenbestandteilen der Sedimente verglichen, um die Bedeutung und Zonierung der unterschiedlichen Respirationswege zu bestimmen.

Die Raten der Sauerstoffzehrung (4.2 mmol m^{-2} d^{-1}) und anaeroben Kohlenstoffoxidation der Tüteninkubationen (10.0 mmol m^{-2} d^{-1}, 0-5 cm Intervall) von Smeerenburgfjorden waren ähnlich zu Raten, die für vergleichbare permanent kalte und temperierte Sedimente gemessen wurden. Dies deutet darauf hin, dass die mikrobielle Gemeinschaft an die permanent niedrigen Temperaturen arktischer Sedimente angepasst ist. Im Gegensatz dazu waren die gemessenen Raten von Sauerstoffzehrung und anaerober Kohlenstoffoxidation (1.5-3.5 mmol m^{-2} d^{-1} bzw. 1.5-4.3 mmol m^{-2} d^{-1} für ein Intervall von 0-10 cm) in den Sedimenten der Nordostküste Svalbards niedriger als die von Smeerenburgfjorden. Vermutlich hängt dies mit den unterschiedlichen dominierenden Wassermassen an der Ost- und Westküste zusammen. Der Ostspitzbergenstrom transportiert arktisches Meerwasser entlang der Ostküste und bestimmt durch die langen Perioden mit Eisbedeckung die kurzen Perioden der Primärproduktion, in denen relativ wenig organische Biomasse produziert wird. Hingegen wird die Westküste durch warmes, nährstoffreiches atlantisches Wasser des Westspitzbergenstroms beeinflusst, und eine größere Menge des in der Wassersäule produziertem organischem Material sinkt zum Meeresboden. Die benthische mikrobielle Gemeinschaft der Nordostküste ist wahrscheinlich durch den Gehalt an organischen Kohlenstoff in den Sedimenten limitiert.

In Tüteninkubationen mit Sediment aus Smeerenburgfjorden wurde der Anteil der Sulfatreduktion an der anaeroben Kohlenstoffoxidation in den oberen 5 cm des Sediments mit 57% bestimmt, während die restlichen 43% der mikrobiellen Fe-Reduktion zugerechnet wurden. Die Zone von Fe-Reduktion überlappte in den obersten 2 cm des Sediments mit der Sulfatreduktionszone. Hohe Mineralisierungsraten von organischem Material führten zu einer raschen Abnahme von Fe(III), das darauf schließen lässt, dass die Fe-reduzierenden Bakterien hauptsächlich Fe(III) limitiert waren. Unterhalb von 2 cm war Sulfat der einzige Elektronenakzeptor. Aufgrund der geringen Mn-Konzentration wurde Mn wahrscheinlich hauptsächlich chemisch von Fe(II) und Sulfid reduziert. Die Abschätzung der gesamten Kohlenstoffoxidation in den oberen 10 cm des Sediments ergab, dass 13% des Kohlenstoffs über Fe remineralisiert wurden. Aerobe Respiration war der wichtigste mikrobielle Prozess (53% der Kohlenstoffoxidation), und Sulfatreduktion war mit 34% wichtigster anaerober Prozess.

Hohe Konzentrationen von Fe(III) (≥108 µmol cm^{-3}) und ungewöhnlich hohe Konzentration von Mn in der Festphase (≥60 µmol cm^{-3}) wurden in den drei Sedimenten von der Nordostküste Svalbards gemessen. Dies stimmte mit der Bedeutung von Mn- und Fe-Reduktion als wichtigste mikrobielle Respirationsprozesse mit einem Anteil von 69 bis wenigstens 90% an der anaeroben Kohlenstoffoxidation überein. In zwei der drei untersuchten Sedimente waren die Sulfatreduktionsraten unterhalb des Detektionslimits (<10–13% der Kohlenstoffoxidation), und Mn und Fe(III) wurden über die gesamten untersuchten 10 cm Sedimenttiefe detektiert. Somit waren Mn und Fe wahrscheinlich die wichtigsten Elektronenakzeptoren für die anaerobe Kohlenstoffoxidation. An der dritten Station wurde eine typische Abfolgen von Respirationsprozessen mit der Sedimenttiefe beobachtet: 0-3 cm Mn-Reduktion, gefolgt von gleichzeitiger Fe- und Sulfatreduktion zwischen 3 und 5 cm und in 5-10 cm ausschließlich Sulfatreduktion.

Die relative Bedeutung von mikrobieller Mn- und Fe-Reduktion in den Sedimenten der Nordostküste von Svalbard bzw. nur Fe-Reduktion im Sediment von Smeerenburgfjorden schienen durch die Konzentrationen von Mn und Fe(III) in der Festphase, die Kohlenstoffmineralisationsrate und die Bioturbation reguliert zu werden. Somit schienen die Bedingungen in Sedimenten der Nordostküste gegenüber denen von Smeerenburgfjorden, mikrobielle Mn- und Fe-Reduktion zu begünstigen. *In situ* wird die Bedeutung von Mn- und Fe-Reduktion in den Oberflächensedimente durch die Konkurrenz mit aeroben und Nitrat-reduzierenden Bakterien beeinflusst.

Die Rolle kurzkettiger Fettsäuren als Substrate für Fe- und sulfatreduzierende Bakterien wurde an anoxischen, mit Meerwasser verdünnten Sedimentinkubationen

untersucht. 15% der anaeroben Produktion von anorganischem Kohlenstoff (DIC) konnten auf den Umsatz von ^{14}C-Acetat und ^{14}C-Laktat in der Oberfläche (0-2 cm), wo Fe- und Sulfatreduktion gleichzeitig auftraten, zurückgeführt werden. In der Sulfatreduktionszone (5-9 cm) betrug der Anteil 42%. Die Hemmung der Sulfatreduktion durch Selenat führte zu einer verminderten DIC Produktion verglichen zu den ungehemmten Inkubationen. Die Akkumulation kurz-kettiger Fettsäuren (Acetat, Propionat und Isobutyrat) in den gehemmten Inkubationen wies darauf hin, dass sulfatreduzierende Bakterien für den Umsatz verantwortlich sind. Dies wurde gestützt durch die Beobachtung, dass die Akkumulations-raten von Acetat in den gehemmten Ansätzen ähnlich den anfänglichen Umsatzraten in den ungehemmten Ansätzen waren. Der Umsatz von Acetat und Laktat war für maximal 43% der gemessenen Sulfatreduktion und 6% der Fe-Reduktion im Sediment verantwortlich. Folglich müssen andere Elektronendonatoren von den beiden Bakterienpopulationen genutzt werden.

Psychrophile und psychrotolerante Fe-reduzierende Bakterien wurden bei 4, 10 und 17°C von zwei Fjordsedimenten der Westküste Spitzbergens angereichert und isoliert. Die Stämme waren verwandt zu Arten der Gattungen *Shewanella, Desulfuromusa, Desulfuromonas* und *Desulfovibrio* der γ- und δ-Proteobakterien. Alle Isolate reduzierten Fe(III)-Citrat und schlecht kristallines Fe-Oxid, jedoch wurde bei den mit *Desulfovibrio*-verwandten Stämmen kein Wachstum beobachtet. Die Isolate wuchsen bei –2°C, dem Gefrierpunkt von Meerwasser, was zeigt, dass sie an die permanent kalten Temperaturen von arktischen Sedimenten angepasst sind. Als Elektronendonatoren wurde von allen isolierten Stämmen wichtige Fermentationsprodukte mariner Sedimenten genutzt. Zusätzlich zu Fe reduzierten die Bakterien Elektronenakzeptoren wie Sauerstoff, Mangan, elementarer Schwefel und Sulfat. Da alle *Desulfovibrio*-verwandten Isolate Fe(III) reduzieren konnten, ist eine Beteiligung von sulfatreduzierenden Bakterien an der benthischen Fe-Reduktion möglich.

Die Stämme der Gattungen *Desulfuromusa*, *Desulfuromonas* und *Desulfovibrio* repräsentieren neue Arten. Durch die Beschreibung als neue Arten, *Desulfuromusa ferrireducens* sp. nov., *Desulfuromonas svalbardensis* sp. nov., *Desulfovibrio ferrireducens* sp. nov. und *Desulfovibrio frigidus* sp. nov., wird die Gruppe der Fe-reduzierenden Bakterien in Reinkultur um neue psychrophile und psychrotolerante Arten erweitert.

Zusätzlich wurde ein moderat thermophiles, Sporen-bildendes, sulfatreduzierendes Bakterium bei 28°C aus einem Fjordsediment der Westküste angereichert und isoliert. Das Isolat gehört zur Gattung der Gram-positiven *Desulfotomaculum* und wird als neue Art, *Desulfotomaculum arcticum* sp. nov., vorgeschlagen. Da dieses Bakterium nicht unterhalb von 26°C wächst, war es im Sediment wahrscheinlich als inaktive Spore vorhanden.

1 INTRODUCTION

1.1 The Arctic Ocean around Svalbard

The Arctic Ocean has the widest continental shelf and is the smallest of the world's five oceans. The sun, not seen at all during the Arctic winter, shines for 24 hours a day in the summer months. The polar climate is characterized by persistent cold, and the sea floor, in particular, experiences permanently low temperatures. This is the rule rather than the exception, since 90% of the ocean's floor is permanently colder than 5°C (Levitus & Boyer, 1994).

Svalbard is an archipelago located in the Arctic Ocean with the main islands Spitsbergen and Nordaustlandet (Fig. 1). The islands are located in a transition zone between different water masses of Atlantic and Arctic origin (Gerdes & Schauer, 1997) (Fig. 1). Relatively warm, nutrient-rich Atlantic water flows northwards with the West Spitsbergen Current along the west coast, supporting the melting of sea ice in spring (Hebbeln & Wefer, 1991, Falk-Petersen *et al.*, 2000). Waters of the Arctic Ocean determine the conditions at the north and east coast of Svalbard (East Spitsbergen Current), where the ice coverage retreats only for a short annual period (Falk-Petersen *et al.*, 2000). Accordingly, the coast around Svalbard can be geographically grouped into an area south and west which is influenced by warmer waters and shorter periods of ice coverage compared to the area north and east.

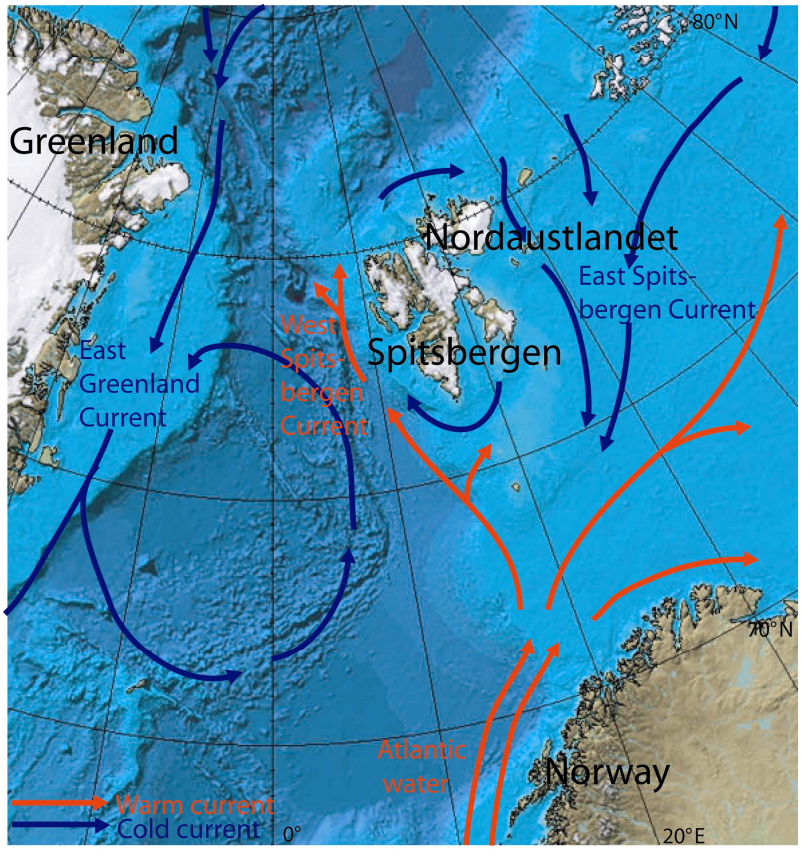

Fig. 1. Svalbard with the main islands Spitsbergen and Nordaustlandet and surrounding currents. (Modified after Hisdal (1998))

1.2 Organic carbon mineralization

The bulk organic material in the sediment is derived from the photic zone of the water column (Jørgensen, 1983). The spring phytoplankton bloom in waters around Svalbard typically develops with the melting of sea ice (Wassmann & Slagstad, 1993; Strass & Nöthig, 1996), and accordingly sedimentation of organic carbon to the sediment is highest at the ice margin (Hebbeln & Wefer, 1991). In the deep sea most of the organic material is degraded in the water column, whereas in shelf regions 10-50% of the primary productivity settles to the sediment (Jørgensen, 1983).

1.2.1 Pathways of benthic carbon mineralization

Most of the deposited organic material in the sediments is degraded completely to CO_2 through activity of the benthic microorganisms and animals. The decomposition of organic material by bacteria starts with the hydrolysis of the macromolecular compounds outside the bacterial cells. This depolymerization is mediated by exoenzymes which are either excreted by bacteria or associated with the bacterial cell wall. The smaller molecules can be taken up and degraded by the bacteria for energy generation. In general the complete oxidation of carbon to CO_2 in the sediment is mediated by a consecutive sequence of respiratory processes with depth, where different inorganic electron acceptors (O_2 → NO_3^- → Mn(IV) → Fe(III) → SO_4^{2-} → CO_2) are reduced (Froelich *et al.*, 1979). This redox cascade corresponds to the decrease in free energy ($\Delta G°$) available for respiration with the different electron acceptors (Table 1). Electron acceptors with higher energy yields are commonly preferentially used (Lovley & Phillips, 1987; Chapelle & Lovley, 1992; Achtnich *et al.*, 1995). As a result of this pattern and the limited availability of most electron acceptors a vertical zonation of the pathways in the sediment is observed (Jørgensen, 2000). The different electron accepting pathways can spatially overlap with each other, depending on substrate availability or reactivity (Canfield *et al.*, 1993a; Achtnich *et al.*, 1995; Jakobsen & Postma, 1999; Kostka *et al.*, 1999b; Thamdrup, 2000; Jensen *et al.*, 2003).

Aerobic respiration produces the largest $\Delta G°$ (Table 1), and occurs in the top sediment layer. In shelf sediments the oxic zone is generally only mm to cm thick (e.g. Revsbech *et al.*, 1980; Jørgensen *et al.*, 2005). In sediments with high organic input the oxygen uptake is not exclusively due to carbon oxidation coupled to aerobic respiration but also to reoxidation of inorganic species such as Fe(II), Mn(II), and sulfide, which have been reduced in lower layers. The supply of oxygen and nitrate from the bottom water to the sediment is mainly

diffusive. Denitrification starts directly in the oxygen-depleted zone. While bacteria using oxygen or nitrate as electron acceptors are in general able to oxidize carbon molecules completely to CO_2, most metal- (Mn(IV) and Fe(III)) and sulfate-reducing bacteria are dependent on the activity of fermenting bacteria, which degrade high molecular weight compounds primarily to volatile fatty acids, H_2, and CO_2. The products of fermentation (e.g., formate, acetate, lactate, propionate, butyrate, isobutyrate, and H_2) serve as electron donors for the bacterial reduction of Mn(IV), Fe(III), or sulfate. The reduced products of respiration, Fe(II), Mn(II), and H_2S, are transported upwards by diffusion, advection, or bioturbation to the surface layer of the sediments where they are reoxidized chemically or bacterially. Methanogens use H_2 and CO_2, C_1-compounds or acetate to form methane, gaining only a small amount of energy (Table 1).

Table 1. Free energy yield, ΔG°, per mol organic carbon for carbon oxidation reactions in marine sediments. (Data from Jørgensen (2000))

Process	**Reaction**	**ΔG° (kJ mol^{-1})**
Aerobic respiration	$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-479
Denitrification	$5CH_2O + 4NO_3^- \rightarrow CO_2 + 2N_2 + 4HCO_3^- + 3H_2O$	-453
Mn(IV) reduction	$CH_2O + 3CO_2 + H_2O + 2MnO_2 \rightarrow 2Mn^{2+} + 4HCO_3^-$	-349
Fe(III) reduction	$CH_2O + 7CO_2 + 4Fe(OH)_3 \rightarrow 4Fe^{2+} + 8HCO_3^- + 3H_2O$	-114
Sulfate reduction	$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$	-77
Methanogenesis	$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$	-28

The quantitative importance of each pathway for carbon oxidation has been studied intensively. In general aerobic respiration and sulfate reduction are the most important metabolic pathways in shelf sediments. Sulfate reduction typically accounts for 25-50% of the total carbon oxidation in coastal sediments (Jørgensen, 1982). With decreasing carbon input and increasing water depth the significance of sulfate reduction decreases. In most marine sediments the significance of nitrate as oxidant for organic matter is of minor importance (≤4% of total carbon oxidation) (Jørgensen, 1983; Canfield *et al.*, 1993b; Rysgaard *et al.*, 1998; Kostka *et al.*, 1999b; Glud *et al.*, 2000). The Mn oxide content in marine sediments in general is small and microbial Mn(IV) reduction is suggested to be globally insignificant (Thamdrup, 2000). In contrast the Fe oxide content is usually higher, and Fe(III) reduction contributes in average 17% to total carbon mineralization in a wide selection of continental marine sediments (Thamdrup, 2000).

1.2.2 Regulation of carbon oxidation in sediments around Svalbard

a) Organic carbon deposition. Large interseasonal variations of primary production around Svalbard are due to limitation in light availability by ice coverage as well as complete darkness in winter. Primary productivity is also effected by the different water masses around the coast off Svalbard. At the south and west coast the warm and nutrient-rich Atlantic water of the West Spitsbergen Current leads to early melting of the ice in spring and stimulates primary production and subsequently sedimentation (Hebbeln & Wefer, 1991). Thus, primary production in fjords of the west coast is comparable to that of more southern latitudes where there is no significant ice cover (Eilertsen *et al.*, 1989). In contrast, colder water masses of Arctic origin influence the east coast of Svalbard where the ice melting proceeds northwards throughout the summer. Along the north coast the retreat of the ice proceeds eastwards so that the ice coverage generally persists longest at the northeast coast of Svalbard (Falk-Peterson *et al.*, 2000). Correspondingly, primary production and the flux of organic material to the sediment were found to correlate with the duration of ice coverage and were significantly lower in areas influenced by polar waters than in areas influenced by Atlantic water (Wassmann & Slagstad, 1993; Hulth *et al.*, 1996; Sakshaug, 1997). In addition to light availability and ice coverage, the amount of organic material reaching the sea floor is dependent on hydrographic regimes, water column temperature, and the pelagic food web structure (Grebmeier & Barry, 1991). Benthic mineralization determined as oxygen uptake rates correlated with the ice cover duration and water depth: i.e., higher rates in sediments south and southwest as well as in shallow sites of Svalbard, compared to rates of deeper sites north and northeast (Hulth *et al.*, 1994).

b) Temperature. Generally, decreasing temperature slows down chemical processes as well as bacterial metabolism. However, biogeochemical processes do not appear to be slower or less efficient in polar regions than in temperate habitats. Rates of carbon turnover in fjord sediments of Svalbard were found to be as high as in comparable temperate sediments (Table 2) (Arnosti *et al.*, 1998; Sagemann *et al.*, 1998; Thamdrup & Fleischer, 1998; Kostka *et al.*, 1999b; Arnosti *et al.*, 2005). Benthic microbial communities in Arctic and Antarctic sediments do not seem to be limited by temperature but rather by organic carbon availability (Nedwell *et al.*, 1993; Glud *et al.*, 1998; Rysgaard *et al.*, 1998). However, temperature adaptation of benthic mineralization processes is difficult to determine due to differences in substrate availability between habitats (Thamdrup & Fleischer, 1998). Bacteria isolated from permanently cold habitats such as sea ice or sediments of the Arctic and Antarctic revealed adaptation to low temperatures by growing below 0°C (Bowman *et al.*, 1997a; Knoblauch *et*

al., 1999; Bowman *et al.*, 2003; Knittel *et al.*, 2005). Psychrophilic sulfate-reducing bacteria showed almost constant growth yields between –1.8°C and their respective optimum growth temperature (T_{opt}), whereas mesophilic bacteria show decreasing growth yields below their T_{opt}, indicating a special adaptation to low temperature of psychrophiles (Knoblauch & Jørgensen, 1999).

In cold marine sediments it was shown that, in addition to the expected bacterial population able to grow at *in situ* temperatures, thermophilic populations of oxygen- and sulfate-reducing bacteria are present (Isaksen *et al.*, 1994; Thamdrup *et al.*, 1998). In these sediments optimum temperatures for thermophilic aerobic respiration and sulfate reduction were 55 and 60°C, respectively. Recent studies also determined a (second) temperature optimum for sulfate reduction in the thermophilic temperature range for permanently cold sediments of Svalbard (Maren Nickel, unpublished data). The isolation and characterization of spore-forming aerobic and sulfate-reducing bacteria from cold environments led to the suggestion that these bacteria are not active at the low *in situ* temperatures of their habitats and are present only as spores (Bartholomew & Paik, 1966; Isaksen *et al.*, 1994; Barnes *et al.*, 1998).

1.3 Mn and Fe reduction in marine sediments

Mn and Fe in marine sediments exist in the form of various metal oxides. Reduction of Mn(IV) and Fe(III) can be mediated chemically or bacterially. Although studying the two processes is rather complex and laborious, the database of studies where dissimilatory Mn and Fe reduction was quantified has grown in the last 10 to 20 years. In marine sediments the importance of dissimilatory Fe reduction for the oxidation of organic carbon was found to range from below detection to >50% (Thamdrup, 2000). Whereas, microbial Mn reduction is of minor importance in most marine sediments (Thamdrup, 2000). The contribution of dissimilatory Mn and Fe reduction to carbon mineralization in sediments is dependent on various factors (Thamdrup, 2000).

1.3.1 Mn and Fe in marine sediments

Due to their low solubility, Mn(III), Mn(IV), and Fe(III) are present in the solid phase in the sediments. In most coastal sediments Mn and Fe oxides and oxyhydroxides (hereafter called Mn and Fe oxides) constitute a continuum of various forms of amorphous to crystalline structures (Burdige, 1993). These differ among sediments considerably in their availability and reactivity (Canfield, 1989). Both metal oxides can be reduced either biotically or

abiotically by sulfide, and in the case of Mn, also by Fe(II) or pyrite (Postma, 1985; Lovley & Phillips, 1988; Myers & Nealson, 1988; Schippers & Jørgensen, 2001).

The reduced species of Mn and Fe are more soluble than the oxidized species and occur in the marine pore water as dissolved Mn^{2+} and Fe^{2+}. But both species usually strongly adsorb to surfaces of metal oxide or other particles or precipitate with carbonates, phosphates, or sulfides (Murray *et al.*, 1984; Canfield *et al.*, 1993a; Kostka & Luther III, 1994; Roden & Zachara, 1996). The extent of precipitation and adsorption strongly depends on the sediment and pore water constituents. Mn(II) is reoxidized by oxygen and nitrate at the sediment-water interface where it is transported by bioturbation. If rates of oxidation are slow, it can be transported in the dissolved form by diffusion. Thus, typical Mn oxide enrichments are produced in the surface sediments. When oxygen and nitrate are abundant only in a small zone in the sediment, Mn^{2+} can diffuse through this zone and is then oxidized in the water column where it can be transported elsewhere (Canfield *et al.*, 1993b; Aller, 1994). As Fe(II) is more reactive than Mn(II) it generally does not escape the sediment, but is oxidized in the surface sediment with Mn(IV), nitrate, or oxygen.

1.3.2 Methods to quantify Mn and Fe reduction rates

In the past decades methods for measuring rates of oxygen consumption, denitrification, sulfate reduction and methanogenesis have been improved and are currently standard techniques. Compared to these techniques, Mn and Fe reduction rate measurements are laborious and complicated due to the chemical complexity of the Mn- and Fe-cycles.

For dissolved species such as oxygen, nitrate, sulfate, and methane, simple flux calculations often produce realistic estimates for their turnover, because their transport is controlled by diffusion. For the solid Mn and Fe oxides, transport by diffusion is impeded. The size of the Mn and Fe oxide pool is usually relatively large compared to the amounts of Mn and Fe, which are reduced during an incubation. Thus, changes in the pool of oxidized compounds are too small to be detected. The reduced forms are more soluble but usually adsorb strongly or precipitate on metal oxides and other sediment particles. Therefore, measurements of changes in Mn^{2+} and Fe^{2+} concentrations over time would easily underestimate the actual rates (e.g., Canfield *et al.*, 1993b; Thamdrup & Canfield, 1996; Glud *et al.*, 2000).

Additionally, it is not possible to distinguish between microbially and chemically produced Mn(II) and Fe(II). The addition of specific inhibitors for sulfate reduction such as molybdate or selenate (Oremland & Capone, 1988) provides an alternative approach to

overcome the chemical reduction of Mn(IV) and Fe(III) by sulfide. But the inhibition of such an important mineralization pathway of marine sediments as sulfate reduction might cause an imbalance in the close coupling of different bacterial processes in the sediment, and therefore turnover rates might not reflect *in situ* conditions.

The addition of radiolabeled tracers is a standard technique to measure precise rates of sulfate reduction (Jørgensen, 1978) and methonogenesis (Alperin & Reeburgh, 1985). Rates of denitrification are similarly determined by the addition of nitrate labeled with the stable isotope ^{15}N (Nielsen, 1992). To apply this approach to Mn and Fe reduction measurements is complicated by the need for labeled Mn and Fe oxide minerals, representative of the complexity of the different, naturally occurring minerals (Canfield, 1989; Van Der Zee & Van Raaphorst, 2004). In addition, the quantification of Fe reduction rates with radiolabeled ^{55}Fe has failed because isotopic exchange between Fe(II) and radiolabeled ferrihydrite resulted in radiolabeled Fe(II) without net Fe reduction (Roden & Lovley, 1993).

Today the method most commonly used to quantify rates of dissimilatory Mn and Fe reduction is to determine total carbon oxidation rates and subtract the contribution of sulfate reduction (Canfield *et al.*, 1993a; Thamdrup, 2000). The excess of carbon oxidation is attributed to Mn and Fe reduction. In this technique sediment is sliced in discrete depth intervals and incubated anoxically in gastight bags (Hansen *et al.*, 2000). Total carbon oxidation rates are determined by the accumulation of dissolved inorganic carbon (DIC) and/or ammonia and sulfate reduction rates are measured simultaneously with the $^{35}SO_4^{2-}$-tracer technique (Jørgensen, 1978). The carbon oxidation not attributed to sulfate reduction can be assigned to other possible electron acceptors. Due to anoxic incubation the supply of oxygen and nitrate from the water column is cut off, and in general *in situ* concentrations of these molecules are low and should be depleted fast. Thus, the excess of carbon oxidation can be attributed to dissimilatory Mn or Fe reduction depending on the availability of Mn and Fe oxides. However, stimulation of carbon oxidation rates in such incubations has been repeatedly observed (Kostka *et al.*, 1999b; Arnosti *et al.*, 2005). This has been explained by the mixing of the sediment resulting in a more homogeneous distribution of substrates available to a greater number of bacteria. The detection limit of this method is estimated with ~10% of total anaerobic carbon oxidation (Thamdrup, 2000).

1.3.3 Regulation of dissimilatory Mn and Fe reduction

Several factors have been identified to control microbial Mn and Fe reduction in marine sediments including sediment reworking, availability of organic material, and concentrations

and reactivity of Mn(IV) and Fe(III) oxides. These parameters influence the competition between different microbial populations and, if the competition is not effective, might result in the coexistence of different electron accepting pathways such as Fe and sulfate reduction (Thamdrup, 2000).

a) Concentrations of Mn(IV) and Fe(III) oxides. Rates of dissimilatory Mn and Fe reduction were shown to strongly depend on the availability of Mn(IV) and Fe(III) (Thamdrup, 2000). The distribution and relative contribution of microbial Mn and Fe reduction to carbon oxidation is in general closely correlated with the concentrations of Mn(IV) oxides and poorly crystalline Fe(III) oxide. With abundant microbially reducible Mn(IV)and Fe(III) oxides, Mn- and Fe-reducing bacteria should outcompete sulfate reducers for common substrates (Lovley & Phillips, 1987). However, sulfate reduction is never completely inhibited but takes place simultaneously with Fe reduction and the shift from Fe to sulfate reduction occurs generally gradually with depth (Canfield *et al.*, 1993a; Van Cappellen & Wang, 1996; Thamdrup, 2000; Jensen *et al.*, 2003). One possible explanation is that the Fe-reducing bacteria are limited by the available area of Fe oxide surfaces. Alternatively, Fe and sulfate reduction might be spatially separated in the sediment as Fe oxides are heterogeneously distributed and Fe-reducers have to be close to the Fe oxides in contrast to sulfate reducers, which have the ability to cluster around organic rich areas in the sediment (Thamdrup, 2000; Jensen *et al.*, 2003).

b) Reactivity of Mn and Fe oxides. "Reactive Fe" ("chemical reactivity") has traditionally been defined as the fraction of Fe in marine sediments which reacts with sulfide to form FeS and pyrite (Berner, 1970). Alternatively, reactive Fe was determined by reductive dissolution with dithionite, as this extraction has been shown to extract a large portion of Fe contained in crystalline and amorphous Fe oxides, reactive Fe silicates, and a small portion of FeS (Canfield, 1989; Kostka & Luther III, 1994; Kostka & Luther III, 1995). The rates of reductive dissolution decreases for different Fe oxides in the order ferrihydrite > lepidocrocite > goethite > hematite (Larsen & Postma, 2001; Bonneville *et al.*, 2004). Although in the last years the interest in the development and description of extraction techniques has increased, today there is no simple and straightforward method to measure the pool and reactivity of Mn and Fe oxides.

The rates of microbial Mn and Fe oxide reductions are among other things influenced by the type of Mn or Fe oxides, i.e., their reactivity (Jakobsen & Postma, 1999; Van Der Zee & Van Raaphorst, 2004). Beside amorphous ferrihydrite commonly used for cultivation of Fe-reducing bacteria, some bacteria have been shown to be able to reduce more crystalline phases

such as goethite and magnetite (Lovley & Phillips, 1986; Kostka & Nealson, 1995; Roden & Zachara, 1996) or iron in clay minerals (Kostka *et al.*, 1999a; Kostka *et al.*, 2002a). Recently Bonneville *et al.* (2004) showed that both chemical dissolution rates and microbial reduction rates correlate to the solubility of Fe oxides. In contrast, Roden (2003; 2004) determined significant differences in rates of enzymatic reduction, and abiotic reductive dissolution, and thus concluded that abiotic reductive dissolution cannot be used as an indicator for enzymatic reduction. Instead the authors suggested that the surface area of oxides is more important for the significance of microbial Fe reduction than the crystal structure (Roden & Zachara, 1996; Roden, 2003; Roden, 2004).

Various Mn oxides differ in their contents of Mn(III) and Mn(IV) (Burdige, 1993). North Sea sediments show wider ranges of Mn oxide reactivities in the oxic layer of the sediment compared to deeper sediment zones (Van Der Zee & Van Raaphorst, 2004). Microbial Mn reduction was suggested to be affected by the reactivity of Mn oxides similar to Fe reduction (Van Der Zee & Van Raaphorst, 2004).

c) Availability of organic carbon. In sediments with low carbon deposition, the mineralization of organic material is dominated by aerobic respiration as in deep sea sediments (Glud *et al.*, 1994). Increased concentrations of carbon divert more organic carbon into the anaerobic pathways. Modeling can show that, at high Fe reduction rates, most Mn(IV) is reduced by Fe(II), and at high rates of sulfate reduction, Fe is consumed by hydrogen sulfide (Van Cappellen & Wang, 1996; Wang & Van Cappellen, 1996; Wijsman *et al.*, 2002). Consequently, less Mn or Fe are available for organic matter mineralization. Correspondingly, Mn and Fe reduction contribute most to organic carbon oxidation at intermediate carbon loading, whereas at higher mineralization rates sulfate reduction is more important (Thamdrup & Canfield, 1996; Kostka *et al.*, 1999b; Wijsman *et al.*, 2002).

d) Mixing processes. The reduction rates of Mn and Fe oxides are controlled to a large part by sediment mixing processes, which enable the recycling of the metal oxides (Thamdrup, 2000). In most sediments, bioturbation by abundant fauna causes this mixing. This process transports organic matter and Mn and Fe oxides downwards as well as reduced Mn(II) and Fe(II) upwards. This results in reoxidation of Mn(II) and Fe(II) to Mn and Fe oxides by oxygen and nitrate, and of Fe(II) also by Mn(IV) in the surface sediment. The overall effect of bioturbation is a faster mixing and turnover of Mn and Fe. Therefore, high rates of Mn and Fe reduction corresponded to high rates of bioturbation in the respective sediments (Aller, 1990; Canfield *et al.*, 1993a; Aller, 1994; Van Cappellen & Wang, 1996; Kostka *et al.*, 1999b; Kostka *et al.*, 2002b; Jensen *et al.*, 2003).

1.3.4 Rates of dissimilatory Mn and Fe reduction

Fe reduction is an important electron acceptor in many marine habitats. Bag incubation studies of sediments from Svalbard, Greenland, Denmark, Norway, and Chile, have demonstrated the relative importance of Fe reduction (0-75%) to anaerobic organic carbon oxidation (Table 2). With an average contribution of 17% to the total carbon mineralization in a wide selection of coastal marine sediments, Fe reduction is of similar importance as oxic respiration (18%) (Thamdrup, 2000). The relative contribution of Fe reduction to carbon oxidation of 0-26% in permanently cold sediments of Svalbard and Greenland was similar to temperate environments (Table 2). Anaerobic carbon oxidation and sulfate reduction rates of Arctic sediments were also similar to rates of comparable temperate habitats (Table 2), indicating that the benthic activities and processes can be similar in permanently cold and temperate marine sediments.

Table 2. Carbon oxidation rates and contribution of Fe reduction in marine sediments determined by anoxic bag incubations

Location	Water depth (m)	Temperature (°C)	Anaerobic C oxidation (mmol m^{-2} d^{-1})	Sulfate reduction rate (mmol m^{-2} d^{-1})	% Fe reduction to carbon oxidation	Reference[a]
Svalbard	155, 115, 175	2.6, 0.2, -1.7	24, 12, 11	12, 2.6, 4.4	0, 26, 10	(1)
Norway	329	7	13	5.0	16	(1)
Greenland	36, 85	-1.3	12, 6.1	2.9, 2.2	26, 21	(2), (3)
Chile[#]	1015, 2000	3.9, 6	12, 9.2	4.8, 2.7	12, 29	(4)
Denmark*	190, 380	9	14, 8.4	4.1, 1.5	32, 51	(5)
Denmark	25, 56	-	11, 40	5.4, 5.0	5[§], 75[§]	(6)

[a]References (1) Kostka *et al.* (1999b), (2) Rysgaard *et al.* (1998), (3) Glud *et al.* (2000), (4) Thamdrup & Canfield (1996), (5) Canfield *et al.* (1993b), (6) Jensen *et al.* (2003)
[#]only slope stations shown, at the four shelf stations Fe reduction was insignificant for carbon mineralization
*Mn-rich station S9 not included
[§]% Fe reduction to anaerobic carbon oxidation

Fe(III) is also a significant electron acceptor for microbial carbon oxidation in freshwater sediments (Jones *et al.*, 1984; Kappler *et al.*, 2004), salt marshes (Kostka & Luther III, 1994; Kostka *et al.*, 2002b), tropical mangrove forest sediments (Kristensen *et al.*, 2000), rice fields (Achtnich *et al.*, 1995), and aquifers (Chapelle & Lovley, 1992; Jakobsen & Postma, 1999).

Recently in deep subsurface sediments of the Pacific Ocean, maximum concentrations of dissolved Mn and Fe as deep as 300 mbsf marked successive zones of Mn and Fe reduction (D'Hondt *et al.*, 2004). The deep introduction of oxygen and nitrate through underlying

basaltic aquifers may here enable the oxidation of Mn and Fe and sustain continued metal cycling.

The relative importance of Fe reduction is dependent on Fe oxide contents, carbon oxidation rates, and mixing processes. In sediments with high carbon oxidation rates such as the Hornsund fjord of Svalbard and shelf stations off Chile Fe reduction was found to be unimportant for carbon mineralization (Thamdrup & Canfield, 1996; Kostka *et al.*, 1999b). Modeling of mineralization pathways in sediments characterized by low bioturbation rates and/or Fe oxide contents estimated a contribution of <4% of microbial Fe reduction to total mineralization in most North Sea sediments (Slomp *et al.*, 1997) and <5% of anaerobic carbon oxidation in Aarhus bay sediments (Thamdrup *et al.*, 1994).

Usually concentrations of Mn oxide are relatively low compared to other electron acceptors such as Fe oxide and sulfate, therefore, the contribution of Mn reduction to carbon oxidation in most marine sediments is small and estimated to be globally insignificant (Thamdrup, 2000). However, the redox cycle of Mn is often intense and may contribute significantly to the reoxidation of reduced Fe and sulfur species (Canfield *et al.*, 1993a; Aller, 1994; Thamdrup *et al.*, 1994). Therefore, increasing Mn^{2+} concentrations do not necessarily imply the bacterial mediated process coupled to carbon oxidation (Postma, 1985; Lovley & Phillips, 1988; Myers & Nealson, 1988; King, 1990) and in many studies Mn reduction is assumed to occur purely chemically (Canfield *et al.*, 1993a; Thamdrup *et al.*, 1994; Kostka *et al.*, 1999b). Only a few areas of the oceans have been identified with very high Mn oxide concentrations (≥100 μmol cm^{-3}), such as the Panama basin, where Mn has a hydrothermal origin, and the deep parts of the Skagerrak, where Mn, released from more reducing sediments, is trapped at the surface. In these sediments dissimilatory Mn reduction was determined to be the most important anaerobic terminal electron accepting process contributing >90% to carbon mineralization in 0-10 cm depth (Aller, 1990; Canfield *et al.*, 1993a). In Black Sea sediments with high Mn contents (~10-50 μmol cm^{-3}) in the surface microbial Mn reduction was determined to be the most important electron acceptor in the upper ~1 cm (Thamdrup *et al.*, 2000). In Mn oxide rich sediment zones Fe reduction is not detectable due to the rapid oxidation of Fe(II) with Mn(IV) (Myers & Nealson, 1988). Therefore, it is not possible to distinguish between the two processes when Mn(IV) is abundant.

1.4 Mn- and Fe-reducing prokaryotes

A wide phylogenetic diversity of *Bacteria* and *Archaea* are known to reduce Fe(III) and Mn(IV) in a dissimilatory manner (Fig. 2). As Fe reduction is typically the more important process compared to Mn reduction in marine habitats (Thamdrup, 2000), more bacteria have been isolated by Fe than by Mn reduction. The process of Fe reduction especially has been studied with species of the genera *Geobacter* and *Shewanella* belonging to the δ- and γ-*Proteobacteria*, respectively.

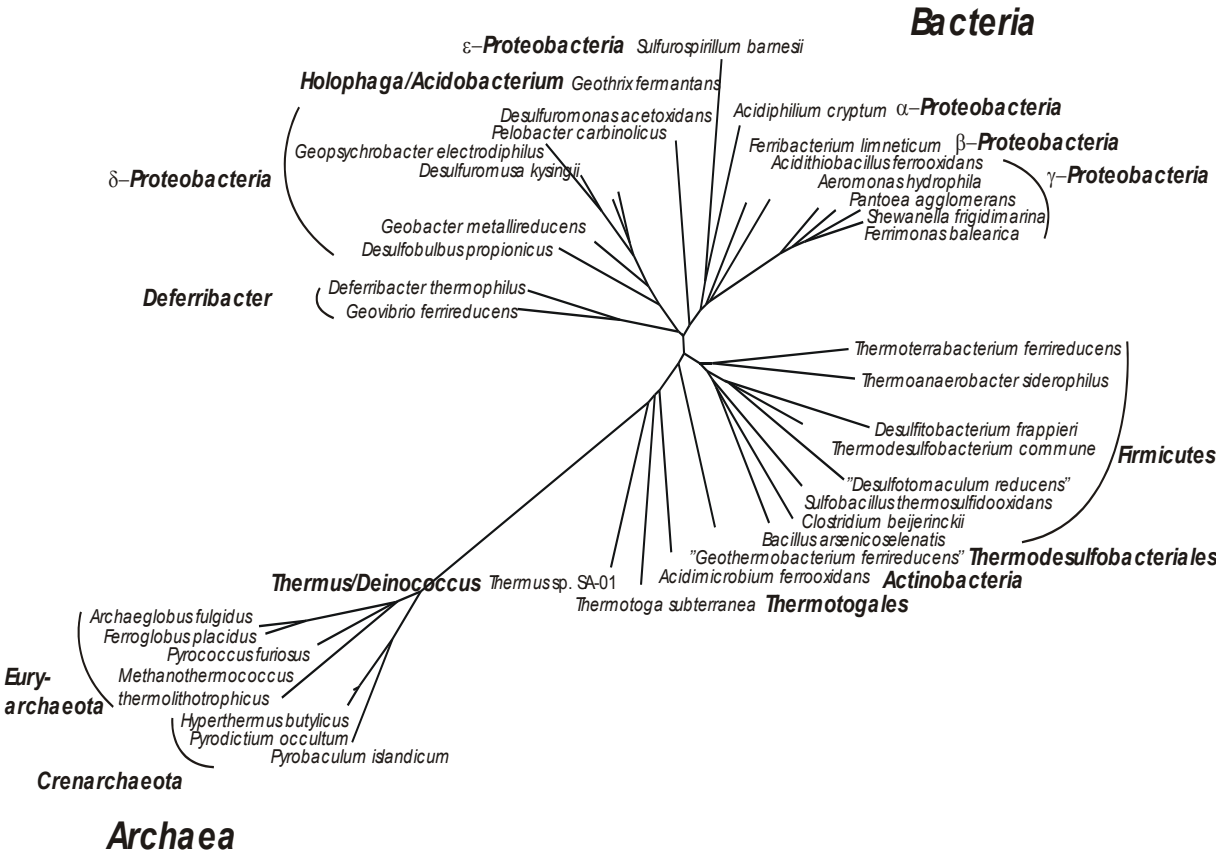

Fig. 2. Phylogenetic tree, based on 16S rDNA sequences, of prokaryotes gaining energy by Fe(III) reduction.

1.4.1 Pure cultures

Fe-reducing bacteria belong to many different phylogenetic groups (Fig. 2). The largest taxonomic groups of Fe-reducing bacteria are the *Geobacteraceae* and the genus *Shewanella.* The *Geobacteraceae* within the δ-*Proteobacteria* includes the genera *Geobacter, Desulfuromonas, Malonomonas, Pelobacter,* and *Desulfuromusa* (Lonergan *et al.*, 1996; Holmes *et al.*, 2004b). For most species of the *Geobacteraceae* the ability to reduce Fe(III) and S° was shown including species of the genera *Pelobacter* (Lovley *et al.*, 1995) and *Malonomonas* (Holmes *et al.*, 2004b), which for a long time have been believed to grow only

by fermentation (Stackebrandt *et al.*, 1989; Kolb *et al.*, 1998). Facultatively anaerobic species of the genus *Shewanella* within the γ-*Proteobacteria* have been isolated mainly by aerobic respiration from a variety of habitats including coastal, open and deep sea environments, and invertebrate intestines (Ivanova *et al.*, 2004; and references therein).

Beside Fe-reducing prokaryotes where the reduction supports growth, microorganisms classified to other physiological groups like sulfate reducers (Coleman *et al.*, 1993; Lovley *et al.*, 1993a), methanogens (Bond & Lovley, 2002; Van Bodegom *et al.*, 2004), and fermenting bacteria (Lovley, 1991) were proven to reduce Fe. While fermenting bacteria channel only a fraction of the reducing equivalents to Fe, sulfate reducers and methanogens are able to use Fe as sole electron acceptor. But in most cases it has not been definitively shown that these bacteria conserve energy for growth from this process. The potential reduction of Fe by sulfate-reducing bacteria has been extensively studied in the last few years. A number of sulfate-reducing bacteria are able to reduce Fe (*Desulfovibrio desulfuricans, Desulfovibrio vulgaris, Desulfovibrio sulfodismutans, Desulfovibrio baarsii, Desulfovibrio* spp. strain G-11, *Desulfomicrobium baculatum,* and *Desulfobacterium autotrophicum*) (Coleman *et al.*, 1993; Lovley *et al.*, 1993a; Li *et al.*, 2004). So far growth by Fe reduction was only shown for the two sulfate-reducing species *Desulfobulbus propionicus* (Holmes *et al.*, 2004a) and *"Desulfotomaculum reducens"* (Tebo & Obraztsova, 1998).

1.4.2 Metabolic versatility

Most Fe-reducing bacteria in pure cultures are able to use Mn as alternative electron acceptor (Thamdrup, 2000). Usually also electron acceptors such as oxygen, nitrate, or elemental sulfur can be reduced by these bacteria. Furthermore, some of these bacteria can directly reduce humic substances or analogous, which shuttle electrons to Fe(III) (Lovley *et al.*, 1996; Lovley *et al.*, 1998). Fe-reducing bacteria may enhance the bioremediation of contaminated sites e.g. by transferring electrons to chlorinated compounds (Krumholz *et al.*, 1996; Krumholz, 1997; Sung *et al.*, 2003), or metals such as uranium, cobalt, technetium, and chromium (Lloyd *et al.*, 2000; Heidelberg *et al.*, 2002; Holmes *et al.*, 2002; Anderson *et al.*, 2003).

Species of the genus *Desulfovibrio* can reduce Fe(III) and U(VI), but do not gain energy for growth (Lovley *et al.*, 1993a; Lovley *et al.*, 1993b). Growth is observed during reduction of sulfate, sulfite, thiosulfate, or elemental sulfur. But some species are additionally able to reduce oxygen and nitrate (e.g., Marschall *et al.*, 1993; Krekeler & Cypionka, 1995; Sass *et al.*, 1998), or grow by disproportionation of sulfur compounds (Bak & Pfennig, 1987).

Fe-reducing bacteria in culture utilize important fermentation products such as volatile fatty acids, H_2, and small alcohols (Lovley, 2000). Additionally a few Fe-reducing bacteria can oxidize sugars (Coates *et al.*, 1998; Küsel *et al.*, 1999; Kashefi *et al.*, 2003) or amino acids (Caccavo *et al.*, 1996; Kashefi *et al.*, 2003), and others have been shown to metabolize aromatic hydrocarbons (Anderson *et al.*, 1998; Coates *et al.*, 1999). Some Fe-reducing bacteria can also grow by fermentation or synthrophically (Schink & Pfennig, 1982; Schink & Stieb, 1983; Schink, 1984; Lovley *et al.*, 1997; Cord-Ruwisch *et al.*, 1998).

In situ volatile fatty acids and hydrogen have been determined as important electron donors for sulfate-reducing bacteria in marine sediments (e.g., Sørensen *et al.*, 1981; Finke, 2003). Turnover rates in Arctic fjord sediments were highest for acetate followed by lactate and propionate (Finke, 2003). To date comparable studies on the substrates for Mn- and Fe-reducing bacteria are missing.

1.4.3 In situ *abundance*

High *in situ* abundance of members of the *Geobacteraceae* was repeatedly shown by 16S rDNA sequences in clone libraries and by DGGE bands of diverse habitats such as freshwater sediments (Stein *et al.*, 2001), aquifers (Röling *et al.*, 2001), temperate marine sediments (Llobet-Brossa *et al.*, 2002; Mußmann *et al.*, 2005), permanently cold sediments of the Antarctic and Arctic (Ravenschlag *et al.*, 1999; Bowman & McCuaig, 2003; Purdy *et al.*, 2003), and on rice roots (Ikenaga *et al.*, 2003; Scheid *et al.*, 2004). A 16S rDNA clone library of a fjord sediment of Svalbard included a significant portion of sequences related to *Desulfuromonas palmitatis* indicating that bacteria belonging to the *Geobacteraceae* may be abundant in these sediments (Ravenschlag *et al.*, 1999). Members of the *Geobacteraceae* might be involved in the reduction/oxidation of contaminants as high abundance was reported in contaminated environments e.g. with petroleum, arsenic, or uranium (Anderson *et al.*, 1998; Röling *et al.*, 2001; Holmes *et al.*, 2002; Anderson *et al.*, 2003; Petrie *et al.*, 2003; Islam *et al.*, 2004; North *et al.*, 2004; Suzuki *et al.*, 2005). Bacteria belonging to *Geobacteraceae* can easily be enriched and isolated by amendment of acetate (Myers & Nealson, 1988; Coates *et al.*, 1996; Straub *et al.*, 1998; Snoeyenbos-West *et al.*, 2000; Thamdrup *et al.*, 2000).

While psychrophilic strains of *Geobacteraceae* are not available in culture, psychrophilic strains related to *Shewanella* could be isolated from permanently cold habitats such as Arctic, Antarctic, and deep sea sediments (Jensen *et al.*, 1980; Bowman *et al.*, 1997b; Bozal *et al.*, 2002; Bowman *et al.*, 2003). 16S rDNA sequences related to *Shewanella* were

found in clone libraries of Antarctic sediments (Bowman & McCuaig, 2003) and Arctic sea ice (Brinkmeyer *et al.*, 2003). However, the quantitative importance of *Shewanella* in biogeochemical cycles is not well understood, since only a few studies have investigated their *in situ* abundance (DiChristina & DeLong, 1993; Brinkmeyer *et al.*, 2003), and most clone libraries lack sequences related to *Shewanella* including clone libraries of fjord sediments from Svalbard (Ravenschlag *et al.*, 1999).

The *in situ* Fe reduction by species related to *Desulfovibrio* was suggested by lipid biomarker analysis from siderite ($FeCO_3$) concretions in a salt marsh (Coleman *et al.*, 1993). Biomarkers specific for the genera *Desulfovibrio* and *Desulfobacter* indicated their abundance, while those specific for typical Fe reducers of the genera *Shewanella* and *Geobacter* were absent.

1.4.4 Temperature tolerance

Bacteria are assigned to different groups according to their temperature tolerance (Morita, 1975), defined by T_{min}, the lowest temperature for growth, T_{opt}, the temperature for fastest growth, and T_{max}, the highest temperature for growth.

psychrophiles	T_{min} <0°C	T_{opt} <15°C	T_{max} <20°C
psychrotolerants	T_{min} ≤5°C	T_{opt} >15°C	T_{max} >20°C
mesophiles		T_{opt} 25-40°C	T_{max} 40-45°C
thermophiles		T_{opt} 55-70°C	T_{max} ~75°C
hyperthermophiles		T_{opt} 80-110°C	

Mn- and Fe-reducing bacteria actively reduce metal oxides in a temperature range from below 0°C in polar oceans to ≥100°C in hydrothermal waters. Thermophilic and hyperthermophilic prokaryotes with the ability of Fe reduction include several *Archaea* (*Ferroglobus placidus, Pyrobaculum islandicum*) and deeply branching *Bacteria* (*Thermotoga maritima, Thermodesulfobacterium commune*) (Fig. 2) (Lovley *et al.*, 2004). This led to the suggestion that Fe reduction may have been among the earliest forms of metabolism on earth (Vargas *et al.*, 1998).

Most Mn- and Fe-reducing bacteria in pure culture are mesophilic (Lovley *et al.*, 2004), psychrophilic and psychrotolerant isolates are rare. Psychrophilic species have been isolated within the genus *Shewanella* from Antarctic water and sediment (*Shewanella gelidimarina, Shewanella frigidimarina,* and *Shewanella livingstonensis*) (Bowman *et al.*, 1997b; Bozal *et al.*, 2002) and *Geobacter psychrophilus* within the *Geobacteraceae* of δ-

Proteobacteria from groundwater (Nevin *et al.*, 2005). The psychrotolerant Fe-reducing bacteria able to grow at 4°C, are *Rhodoferax ferrireducens* within the β-*Proteobacteria* isolated from a coastal aquifer sediment at 20°C (Finneran *et al.*, 2003) and *Geopsychrobacter eletrodiphilus* within the *Geobacteraceae* isolated from a sediment fuel cell incubated in the laboratory at 15°C (Holmes *et al.*, 2004c).

1.5 Aim of the present study

Only a few psychrophilic and psychrotolerant Fe-reducing bacteria are available in cultures. However, dissimilatory Fe reduction has been shown to be an important process for the degradation of organic matter in permanently cold Arctic sediments. Hence, in these sediments a community of Fe-reducing bacteria adapted to low temperatures must be abundant. Additionally sulfate-reducing bacteria have been shown to reduce Fe(III) and might therefore be directly involved in Fe reduction. The primary aim of this study was to investigate Fe reduction in permanently cold sediments of Svalbard, by isolating Fe- and sulfate-reducing bacteria. The electron donors and acceptors, as well as the temperature adaptation for each isolate were determined. This not only provides information on potential *in situ* activities of the isolates, but also improves our knowledge on adaptations of benthic communities to environmental conditions. Secondly the contribution of Fe reduction to total carbon oxidation was quantified in the sediment of Smeerenburgfjorden at the northwest coast of Svalbard to extend our knowledge on Fe reduction in permanently cold sediments. Volatile fatty acids and hydrogen have been found to be important substrates for sulfate-reducing bacteria in marine sediments. Similar studies are not available for Fe-reducing bacteria. Therefore, we conducted inhibition experiments to determine the role of volatile fatty acids as substrates for Fe- and sulfate-reducing bacteria in Smeerenburgfjorden sediment.

Compared to the benthic mineralization in fjord sediments of the west coast of Svalbard, which have relatively high fluxes of organic carbon, the processes in sediments of the north and east coast have rarely been studied. Since primary production in this area is limited by long periods of ice coverage the benthic community is probably affected by low carbon availability. As part of the Norwegian CABANERA project (Carbon flux and ecosystem feed back in the northern Barents Sea in an era of climate change) the most important electron accepting processes (oxygen, Mn, Fe, and sulfate reduction) involved in the benthic carbon mineralization were identified and turnover rates determined in these sediments.

1.6 Sampling sites

Samples were collected from three fjord sediments (Tempelfjorden, Stations CC & CD; Nordfjorden, Station BC; Smeerenburgfjorden, Station J) (Fig. 3) on the west coast of Svalbard during a cruise with MS "FARM" in 2001 to isolate Fe-reducing and sulfate-reducing bacteria from permanently cold sediments. During a second cruise to Svalbard in 2003, we measured Fe reduction rates in a fjord sediment (Smeerenburgfjorden, Station J) (Fig. 3). In 2004 Maren Nickel and I participated in a cruise with RV "JAN MAYEN" of the Norwegian CABANERA project, where we sampled the sediments of the northeast coast of Svalbard to investigate the benthic carbon mineralization (Stations VIII, X, and XII) (Fig. 3). Following this cruise we collected sediments with MS "FARM" at Smeerenburgfjorden, for the inhibition experiment in cooperation with Niko Finke.

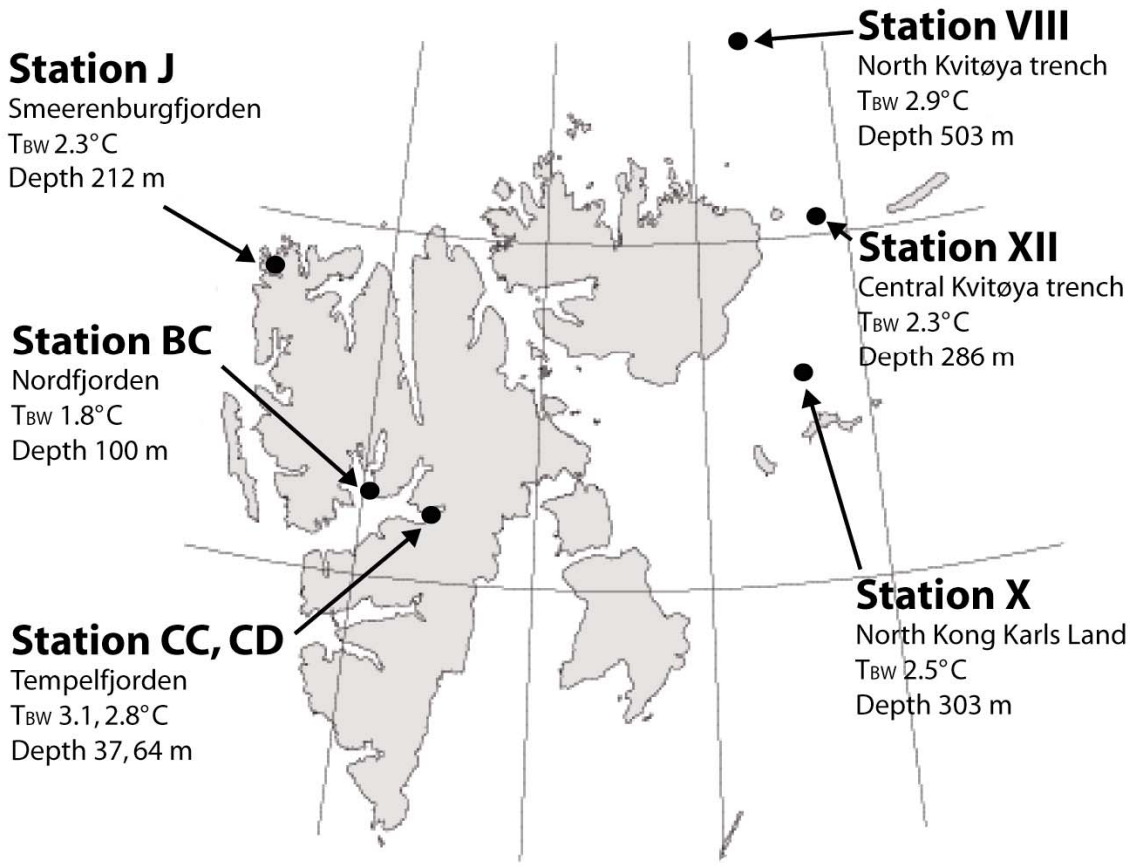

Fig. 3. Sampling sites off Svalbard.

T_{BW}, bottom water temperature; Depth, water depth.

1.7 References

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2 MANUSCRIPTS

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2.6 *Desulfotomaculum arcticum* sp. nov., a new spore-forming, moderately thermophilic sulfate-reducing bacterium isolated from a permanently cold fjord sediment of Svalbard

Verona Vandieken, Christian Knoblauch & Bo Barker Jørgensen

2.1

Pathways of carbon oxidation in an Arctic fjord sediment (Svalbard) and isolation of psychrophilic and psychrotolerant Fe(III)-reducing bacteria

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The main mineralization pathways were determined in a permanently cold fjord sediment of the west coast of Svalbard. In whole core incubations the total oxygen uptake rate was 4.2 ± 0.4 mmol m^{-2} d^{-1} and the sulfate reduction rate 2.6 ± 0.6 mmol m^{-2} d^{-1}. Sulfate reduction was determined as most important anaerobic carbon oxidation pathways with 57% in anoxic bag incubations of the top 5 cm of the sediment, the remaining 43% were attributed to microbial Fe(III) reduction. Both processes occurred simultaneously in the uppermost 2 cm, and the Fe-reducing community seemed to be limited mainly by the availability of Fe(III). Below 2 cm sulfate reduction was the sole electron accepting process. Calculations for the uppermost 10 cm of the sediment yielded a contribution of the different respiratory pathways as follows: aerobic respiration 53%, Fe(III) reduction 13%, and sulfate reduction 34%. *In situ* the importance of Fe(III) reduction might vary due to competition for substrate with oxygen- and nitrate-reducing bacteria in the surface sediment. From enrichment cultures of two fjord sediments of Svalbard Fe(III)-reducing bacteria were isolated and belong to the genera *Desulfuromonas*, *Desulfuromusa*, *Shewanella*, and *Desulfovibrio.* Strains related to *Desulfovibrio* reduced Fe(III) without energy generation for growth. All isolates were psychrophilic or psychrotolerant and grew at –2°C, the freezing point of seawater, indicating adaptation to permanently cold temperatures.

Introduction

The exploration of permanently low temperature environments is motivated by their quantitative importance, as 90% of the ocean's sea floor permanently experience temperatures of <5°C (Levitus & Boyer, 1994). Benthic microbial processes are controlled among others by temperature and substrate availability. The degradation of organic matter arriving at the sea floor involves a complex bacterial food web. Metabolic end products of some bacteria can serve as substrates for others (e.g. fermentation products are substrates for Mn-, Fe- and sulfate-reducing bacteria). Responsible for the complete degradation to CO_2 is a cascade of respiratory processes with different inorganic electron acceptors (O_2, NO_3^-, Mn(IV), Fe(III) and SO_4^{2-}) involved. Studies of metabolic rates of the different processes in the sediment led to the conclusion that the rates of organic carbon turnover in Arctic coastal sediments were as high as in comparable temperate sediments (Arnosti *et al.*, 1998; Sagemann *et al.*, 1998; Thamdrup & Fleischer, 1998; Kostka *et al.*, 1999; Arnosti *et al.*, 2005). The benthic microbial

communities in these habitats do not seem to be limited by temperature but rather by organic carbon availability (Glud *et al.*, 1998; Rysgaard *et al.*, 1998; Nedwell *et al.*, 1993).

In three permanently cold fjord sediments of Svalbard sulfate reduction was the dominant terminal electron accepting process (58-92% of total mineralization) (Kostka *et al.*, 1999). In two of the three sediments studied microbial Fe(III) reduction was the second most important anaerobic respiration pathway (10 and 26%), while it was below detection at the third station. A similar contribution of Fe reduction was determined in permanently cold sediments of the east coast of Greenland with 21-26 % of total carbon oxidation (Rysgaard *et al.*, 1998; Glud *et al.*, 2000). These studies together with studies in temperate coastal sediments determined the average contribution of 17% of Fe(III) reduction to total carbon mineralization reviewed by Thamdrup (2000).

Bacteria able to respire with Fe(III) are phylogenetically diverse (Lovley *et al.*, 2004). Most species of the family *Geobacteraceae* within the δ-*Proteobacteria* (including the genera *Desulfuromonas, Geobacter, Pelobacter, Malonomonas,* and *Desulfuromusa*) are able to reduce both Fe(III) and elemental sulfur (Lonergan *et al.*, 1996; Holmes *et al.*, 2004b). A large fraction of the 16S rRNA gene sequences in a Svalbard sediment clone library was closely related to *Desulfuromonas palmitatis* (Ravenschlag *et al.*, 1999), indicating that this group might contribute to the Fe- and/or sulfur-cycle in the permanently cold fjord sediment. Psychrophilic, Fe-reducing bacteria of the genus *Shewanella* within the γ-*Proteobacteria* have been isolated from Antarctic and Arctic sea ice as well as Antarctic sediments (Bowman *et al.*, 1997b; Bozal *et al.*, 2002; Bowman *et al.*, 2003; Brinkmeyer *et al.*, 2003). A contribution of sulfate-reducing bacteria to benthic Fe-reduction was suggested from results of pure culture studies and biomarker analysis of a salt marsh sediment (Coleman *et al.*, 1993; Lovley *et al.*, 1993; Tebo & Obraztsova, 1998; Holmes *et al.*, 2004a; Li *et al.*, 2004).

Fe-reducing species belonging to the genera *Shewanella* and *Desulfovibrio* as well as the family *Geobacteraceae* can alternatively use other environmentally important electron acceptors like oxygen, nitrate, Mn(IV) oxide, elemental sulfur, or sulfate or they may grow by fermentation. Therefore it is mostly difficult to link environmentally relevant subpopulation of bacteria to biogeochemical processes *in situ*. The study of pure cultures provides a good approach to indicate their potential activities and study the adaptation of bacteria to environmental settings. Bacteria isolated from permanently cold habitats such as sea ice or sediments have revealed adaptation to low temperatures (Bowman *et al.*, 1997a; Knoblauch & Jørgensen, 1999; Bowman *et al.*, 2003). From fjord sediments of Svalbard sulfate-reducing and sulfur-oxidizing bacteria with temperature optima between 7 and 18°C and the ability to

grow at –2°C, the freezing point of sea water, have been isolated (Knoblauch *et al.*, 1999b; Knittel *et al.*, 2005).

The aim of the present study was to identify bacteria potentially contributing to the Fe- and sulfur-cycles in fjord sediments of Svalbard's west coast and to investigate the importance of Fe(III) reduction for the degradation of organic carbon in one of the fjord sediments.

Material and Methods

Site and sampling. Marine sediments were sampled during three cruises with MS "Farm" in fjords along the west coast of Svalbard. Details for the stations are given in Table 1. The enrichment and isolation work was started with marine sediment from a cruise in September 2001 to stations CC, CD and J. Anoxic bag incubation experiments of Station J sediment were performed on the second cruise in August 2003. Pore water and solid phase constituents were measured 2003 and 2004, total oxygen uptake rates, dissolved inorganic carbon (DIC) production rates and sulfate reduction rates were measured in sediment cores from Station J in August 2004. The concentrations of DIC, Fe^{2+}, sulfate, and sulfide in the pore water and the content of solid phase Fe and Mn were measured in sediment cores from the cruises to Station J in 2003 and 2004, while ammonium and elemental sulfur were analyzed only in 2004. In general, the concentrations and depth distributions were similar in 2003 and 2004. Therefore, we present only the complete data set of 2004.

Table 1. Sample site information and Fe content

Stations	**CC**	**CD**	**J**
Position	Tempelfjorden	Tempelfjorden	Smeerenburgfjorden
Latitude	78°26'039" N	78°25'267" N	79°42'006" N
Longitude	17°19'722" E	17°08'277" E	11°05'199" E
Water depth (m)	37	64	212
Bottom water temperature (°C)	3.1	2.8	2.3
Sampled in	2001	2001	2001, 2003 & 2004
Average total Fe content (μmol cm^{-3})	nd	91.9[a]	79.2

nd not determined
[a]data measured 2001

Sediment cores of 14 cm diameter and up to 40 cm long, were retrieved by a HAPS corer and subcores were stored on the ship at *in situ* temperature. In the laboratories in Ny

Ålesund and Longyearbyen the sediments were stored at 0°C and handled outdoors at air temperatures between 0 and 5°C to prevent warming of the sediment.

The sediment of Station J was characterized by an extensive bioturbation apparent from many polychaete tubes and polychaetes in the upper 20 cm of the sediment. On the surface of the sediment brittle stars were observed.

Incubations in anoxic bags. (2003) For anoxic bag incubations sediment of Station J from 10 cores was sliced in 1 cm depth intervals, and parallel sediment sections were pooled and transferred into gastight plastic bags (Hansen *et al.*, 2000) under a constant stream of N_2. The bags were incubated at 0°C in the dark inside a larger N_2-filled bag to ensure anoxia. Subsamples for pore water and solid phase analyses were withdrawn 10 times over 7 days from each bag.

Sampling. Pore water was squeezed by a pore water press under N_2 through GF/F filters. Porewater was filtered directly into Ferrozine-solution to measure Fe^{2+} (see below). 1.8 ml aliquots for DIC analysis were collected in glass vials without headspace and measured within 2 days, or samples were fixed with $HgCl_2$ and stored at 4°C until analysis. 1-2 ml of pore water was frozen for NH_4^+-, NO_3^--, and NO_2^--analysis. For Mn^{2+} and Ca^{2+} determination 0.5-2 ml were acidified with 6 M HCl and stored at 4°C. Pore water for sulfate and sulfide analyses was preserved with Zn acetate or $ZnCl_2$. Sediment for analysis of reactive Fe was extracted in HCl from a separate core. For the extraction of Fe and Mn with dithionite subsamples were stored frozen at –21°C. For the analysis of elemental sulfur a subsample of 0.5-2 g sediment was mixed in 2 ml 20% Zn acetate and stored frozen at –21°C. Bottom water of Station J was frozen for determination of NO_3^- and NO_2^-.

Pore water analyses. DIC was analyzed by flow injection with conductivity detection (Hall & Aller, 1992). Fe(II) was measured according to Stookey (1970) with Ferrozine (1 g l^{-1} in 50 mM HEPES buffer, pH 7) spectrophotometrically at 562 nm (Shimadzu UV 1202). NO_3^- and NO_2^- were measured using a NO_x-Analyzer (Thermo Environmental Instruments, Franklin, USA) (Braman & Hendrix, 1989). NH_4^+ samples were analyzed spectrophotometrically at 630 nm (Shimadzu UV 1202) (Grasshoff *et al.*, 1999). Ca^{2+} and Mn^{2+} were measured by inductively coupled plasma atomic emission spectrometry (Perkin Elmer Optima 3300 RL). Sulfate was analyzed by non-suppressed ion chromatography (Waters, column IC-Pak™, 50 x 4.6 mm) (Ferdelman *et al.*, 1997). Sulfide was determined by

the methylene blue spectrophotometric method at 670 nm (Shimadzu, UV 1202) (Cline, 1969).

Solid phase analyses. Particulate Fe and Mn was quantified after extraction with dithionite-citrate-acetic acid (Canfield, 1989). The extract was analyzed by flame atomic absorption spectrometry for Mn concentrations (Perkin Elmer, Atomic Absorption Spectrometer 3110). Quantification of Fe from the dithionite-citrate-acetic acid extraction resulted in a lower Fe content compared to the HCl extraction (0.5 M HCl for 1 h), therefore only the results of the HCl extraction are presented. The HCl-extracts were analyzed for Fe(II) with Ferrozine and for total Fe (Fe(III) + Fe(II)) with Ferrozine + 1% (w/v) hydroxylamine hydrochloride. Elemental sulfur was analyzed according to Zopfi *et al.* (2004). A subsample of the sediment frozen in Zn acetate for elemental sulfur analysis was extracted with 5 ml methanol. The sulfur was separated by HPLC with a Zorbax ODS column (125 x 4 mm, 5 µm; Knauer, Germany) with methanol as eluent at a flow of 1 ml min^{-1}. The sulfur was determined from the absorption at 265 nm.

Sulfate reduction rates. (2004) Sulfate reduction rates of whole cores incubations were measured in three parallel cores of 3 cm diameter using the $^{35}SO_4^{2-}$ core injection technique (Jørgensen, 1978). Sulfate reduction in the anoxic bags was determined at every sampling point in subsamples incubated with $^{35}SO_4^{2-}$ radiotracer in 5 ml glass tubes. The incubations were stopped with 10% or 20% Zn acetate. Total reduced inorganic sulfur was analyzed by a cold chromium distillation (Kallmeyer *et al.*, 2004).

Oxygen consumption rates. (2004) Oxygen consumption rates were measured in three sediment cores with an inner diameter of 54 mm, which were closed without gas phase with rubber stoppers. The cores were incubated in the dark for 42-46 h at 0°C with continuous stirring of the water column by a magnetic stirring bar placed at the top of the water column. Oxygen consumption of the sediment was measured during the whole incubation with a micro-optode (Holst *et al.*, 1997; Klimant *et al.*, 1997). Samples for DIC were taken from the water column of the core before and at the end of the oxygen measurement and fixed with $HgCl_2$. The DIC samples were analyzed on a coulometer (CM5012, CO_2 coulometer; CM5130 Acidification module, UIC Inc. Coulometrics). The volume of the water column was determined by addition of a NaBr solution. Concentrations of NaBr were analyzed by anion

chromatography (Dionex DX500, eluent: 9 mM $NaCO_3$, precolumn: AG9 HC, column: AS9 HC).

Calculation of Fe^{2+} flux. From the Fe^{2+} pore water profile the flux (J) of Fe^{2+} at 0°C was calculated by Fick's first low of diffusion: $J = \phi\ D_S\ dC/dz$, where ϕ is the porosity, D_S is the diffusion coefficient in sediment, and C is the solute concentration at a given depth (z). D_S was calculated according to Iversen & Jørgensen (1993): $D_S = D_0/(1+3(1-\phi))$, where D_0, the diffusion coefficient in seawater, was taken from Schulz & Zabel (2000, p. 92): D_0 (Fe^{2+}) = 3.15 10^{-6} cm^2 s^{-1} (at 0°C).

Enrichment and isolation of pure cultures. (2001) Enrichment of bacteria was started with sediments from stations CC, CD and J. Isolates of Fe-reducing bacteria were obtained in artificial seawater medium (Widdel & Bak, 1992) with low sulfate concentration (0.4 mM instead of 28 mM to prevent growth of sulfate-reducing bacteria). As electron donors acetate (20 mM) or lactate (20 mM) were used, and as electron acceptor for the enrichment poorly crystalline Fe oxide (~30 mM), which was later replaced by Fe citrate (~30 mM) for the isolation in agar dilution series (Isaksen & Teske, 1996). Sulfate-reducing bacteria were enriched and isolated similar to Fe-reducing bacteria but with sulfate (28 mM) instead of Fe(III) as electron acceptor and with lactate (20 mM) or formate (10 mM) as electron donor. Cultures were enriched, isolated, and incubated at temperatures of 4, 10, and 17°C. Growth of bacteria was indicated by the production of Fe^{2+} for Fe-reducing bacteria (Stookey, 1970) or the production of sulfide for sulfate-reducing bacteria (Cord-Ruwisch, 1985).

Some of the isolates have been deposited in culture collections: strain 102 (DSM 16956, JCM 12926), strain 112 (DSM 16958, JCM 12927), strain 61 (DSM 16995, JCM 12925), strain 18 (DSM 17176, JCM 12924).

Growth parameters and physiology. Temperature regulation of respiration of the strains was determined in a temperature gradient block at temperatures between –2 and 32°C (Sagemann *et al.*, 1998). Growth was monitored in duplicates by measuring production of Fe^{2+} or sulfide. The ability of the strains to use alternative electron acceptors and donors was determined in duplicate tubes after growing cultures had been transferred to new medium for verification. The following electron donors were tested (in combination with Fe citrate or sulfate) (mM): acetate (20), lactate (20), formate (10), propionate (10), butyrate (10), H_2/CO_2 (80/20, v/v), ethanol (10), propanol (10), butanol (10), fumarate (10), and succinate (10). As

electron acceptors the following were tested (in combination with lactate or acetate) (mM): poorly crystalline Fe oxide (~30), Fe citrate (~30), Mn oxide (~30), elemental sulfur, sulfate (30), thiosulfate (10), sulfite (2), oxygen (air), and fumarate (20).

Fe(III) reduction by *Desulfovibrio*-related strains. Cells of strains 18, 61 and 77 were transferred from sulfate-reducing cultures to sulfate-free medium. As substrates lactate and Fe(III) citrate or poorly crystalline Fe oxide were added. If Fe(III) was reduced, 10% of the culture was transferred to new medium.

Fe reduction by strain 61 was investigated in more detail. Cells of strain 61 were grown by sulfate reduction (400 ml medium with 28 mM sulfate, 20 mM lactate). In the log phase of growth the culture was centrifuged for 10 min and washed twice with 100 ml sulfate-free medium for which the reducing agent sulfide was replaced by $Fe(II)Cl_2 \cdot 4H_2O$ (2-3 mM end concentration). The cells were resuspended in sulfate-free medium in an anoxic glove box and the suspension, 50 ml each, was filled into serum vials. Lactate (20 mM) and Fe citrate (~30 mM) or Fe oxide (~30 mM) were added to the vials. The incubation temperature was 0 or 20°C (in triplicates).

Phylogenetic analysis. Universal bacterial primers 8F and 1492R (Buchholz-Cleven *et al.*, 1997) were used to amplify 16S rDNA of the isolated strains. The PCR products were amplified with primers 8F, 341F, 518F, 534R, 1099F, and 1492R (Buchholz-Cleven *et al.*, 1997) for sequence analysis (Applied Biosystems model 3100 Genetic Analyzer DNA sequencer). The sequences were analyzed with the ARB program package (Ludwig *et al.*, 2004). Phylogenetic trees were calculated with the ARB program applying neighbor-joining, maximum-parsimony, and maximum-likelihood methods with different sets of filters to a subset of data that included only complete sequences of representative members of *Proteobacteria*.

Results

Solid phase and pore water chemistry of Station J. The nitrate and nitrite concentrations in the bottom water were 12 and 0.5 µM. The pore water dissolved inorganic carbon (DIC) and ammonium concentrations increased with depth (Figs. 1A & 1B). A peak in Fe^{2+} concentration occurred at 3-4 cm (59 µM) (Fig. 1C). Based on this Fe^{2+} pore water profile we calculated the Fe^{2+} fluxes from the interval of 1-4 cm depth. The fluxes were 49.9 µmol m^{-2} d^{-1} and 27.9 µmol m^{-2} d^{-1} through the top and bottom layer of the interval yielding a net Fe^{2+}

production of 2.6 µM d^{-1} for the 1-4 cm interval. The sulfate concentration in the pore water was constant in the uppermost 21 cm of the sediment, and free sulfide did not exceed the detection limit of 1 µM. Extractable S° increased with depth to a concentration of 7.4 µmol cm^{-3} between 11-17 cm (Fig. 1D). Mn oxide was enriched near the surface (1.5 µmol cm^{-3}) and dropped to a stable background level of ≤0.8 µmol cm^{-3} below 2 cm (Fig. 1E). The HCl-extractable total Fe concentration was in average 79.2 µmol cm^{-3} (Fig. 1F).

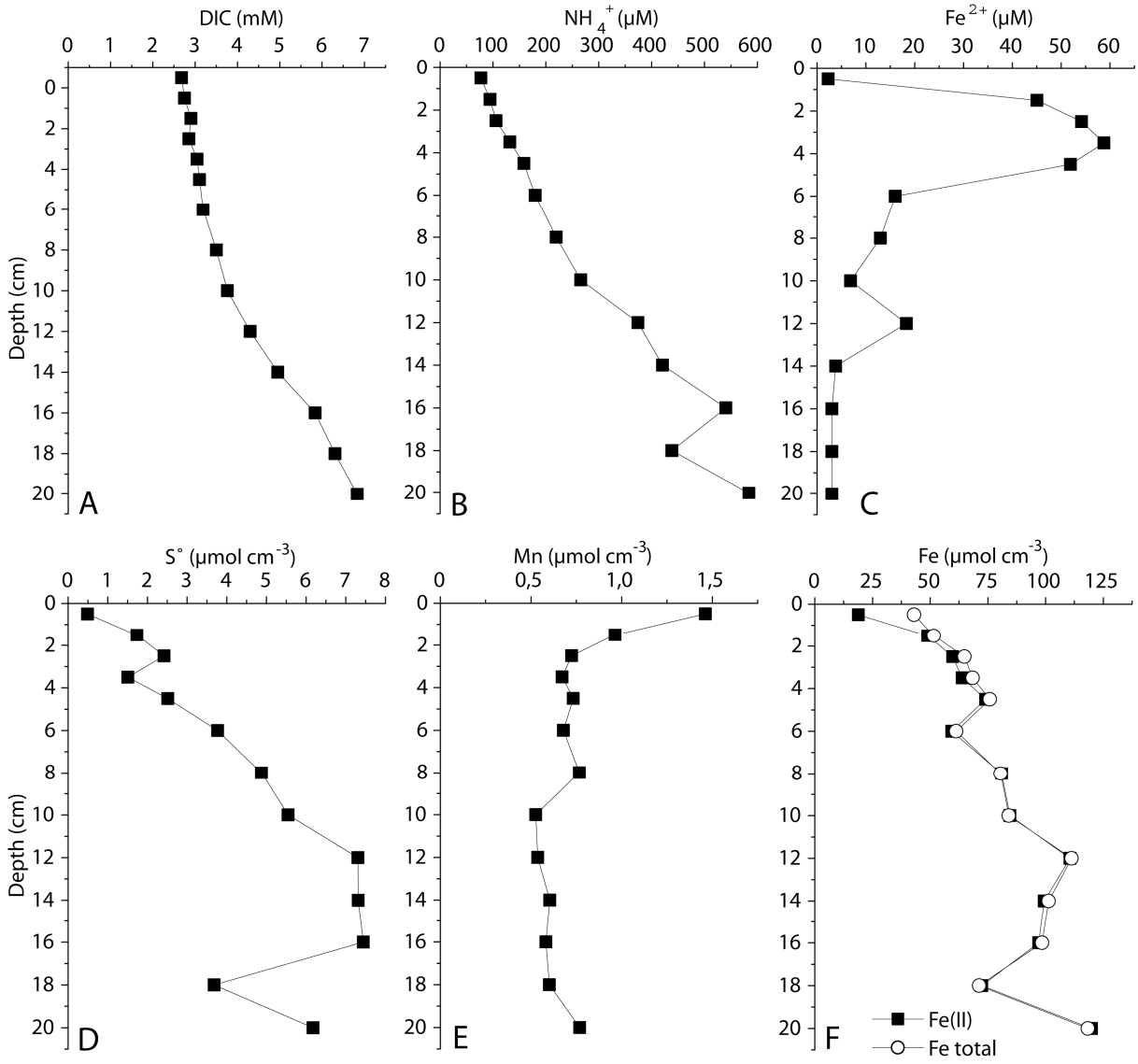

Fig. 1. Pore water (DIC, NH_4^+, Fe^{2+}) and solid phase (S°, Mn, Fe) constituents of the sediment.

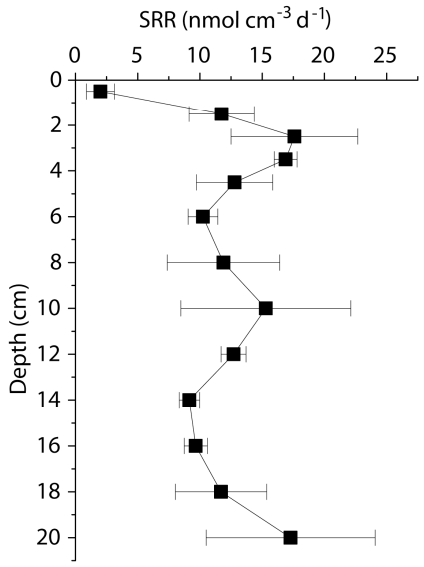

Fig. 2. Sulfate reduction rates with standard errors of triplicates.

Oxygen consumption and sulfate reduction rates of whole core incubations. The mean total oxygen uptake rate was 4.2 ± 0.4 mmol m^{-2} d^{-1} and the DIC release rate 7.1 ± 1.6 mmol m^{-2} d^{-1}. Highest sulfate reduction rates were measured between 2-4 cm (Fig. 2), and the mean depth-integrated rate for 0-21 cm was 2.6 ± 0.6 mmol m^{-2} d^{-1}.

Anaerobic carbon mineralization in bag incubations. Anoxic bags of pooled sediment sections each of 1 cm depth interval from the uppermost 5 cm were incubated at 0°C. The Fe^{2+} concentration in the pore water increased in all bags over the whole incubation time. The rate of Fe^{2+} accumulation was highest in the surface sediment at 0-1 cm, 14.9 µM d^{-1}, and decreased with depth to 0.8 µM d^{-1} at 4-5 cm (Fig. 3A). The Mn^{2+} concentrations increased only in the bags from the upper 2 cm with rates of 3.0 and 0.8 µM d^{-1} (Fig. 3A), which scaled with the distribution of reactive Mn (Fig. 1E). In all 5 bags the pore water concentrations of DIC increased linearly throughout the 7 days of incubation, indicating that the reaction rates remained constant and that no precipitation of carbonate occurred. This was supported by constant Ca^{2+} concentrations in the pore water during the incubations (data not shown). The DIC production rate was highest in the surface layer of the sediment and decreased with depth (Fig. 3B), whereas sulfate reduction rates showed no change with depth (Fig. 3B) and were constant with incubation time. The depth-integrated sulfate reduction rates of the bag incubations (2.8 mmol m^{-2} d^{-1}) was 4.7-fold higher than the rates of whole core incubations for the same sediment interval, indicating a stimulation that is probably due to sediment mixing. We do not expect that the stimulation was different for the anaerobic pathways of respiration. A similar stimulation was reported recently for fjord sediments of Svalbard (Kostka *et al.*, 1999; Arnosti *et al.*, 2005). DIC accumulation rates and sulfate reduction rates for the bag incubations were in agreement for the sediment depth between 2 and 5 cm (Fig. 3B), assuming an overall stoichiometry of 2:1 for DIC production to sulfate reduction as terminal electron acceptor (Thamdrup & Canfield, 1996). In contrast, both rates diverged significantly in the top 0-2 cm of the sediment and indicated that respiration pathways other than sulfate reduction were in operation.

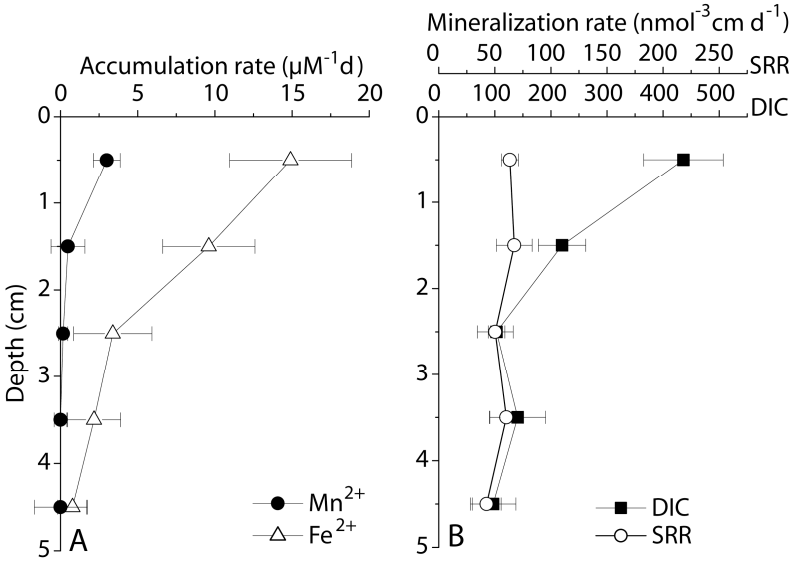

Fig. 3. Rates measured during anoxic bag incubations. (A) Depth distribution of dissolved Fe^{2+} and Mn^{2+} accumulation rates during incubations. Error bars indicate standard errors from linear regression. (B) Depth profiles of anaerobic mineralization of organic carbon. Error bars indicate standard errors from linear regression of DIC production and standard deviation of sulfate reduction rates (SRR). Scales are plotted at a ratio of 2:1 for DIC production and SRR.

Enrichment and isolation of Fe- and sulfate-reducing bacteria. For the enrichment of bacteria from three stations of two fjord sediments of Svalbard we chose as electron donors acetate, lactate, and formate as they are important fermentation products in marine sediments. With acetate and Fe(III) we isolated two strains (strains 112 and 102) related to species of the *Geobacteraceae* within the δ-*Proteobacteria* (Fig. 4). Within the γ-*Proteobacteria* and closely related to species of the genus *Shewanella* strains 62, 86, 104, and 109 were isolated by lactate oxidation coupled to Fe reduction (Fig. 4).

Strain 61, related to species of the genus *Desulfovibrio* within the δ-*Proteobacteria* (Fig. 4), was isolated under Fe(III) reducing conditions with lactate as electron donor. So far nearly all species of this genus have been isolated by sulfate reduction, but the ability to reduce Fe(III) was demonstrated for some species (Coleman *et al.*, 1993; Lovley *et al.*, 1993; Li *et al.*, 2004). Lovley *et al.* (1993) suggested, however, that in these experiments *Desulfovibrio desulfuricans* obtained energy for growth from the 300 μM sulfate present in the medium and not from reduction of Fe(III). Correspondingly, it could not be unequivocally determined whether strain 61 grew during the enrichment and isolation by Fe(III) reduction or by reduction using the low concentration of sulfate in the medium (400 μM). Two strains closely related to strain 61 (strains 18 and 77) (Fig. 4) were isolated by sulfate reduction (28 mM sulfate) with formate and lactate.

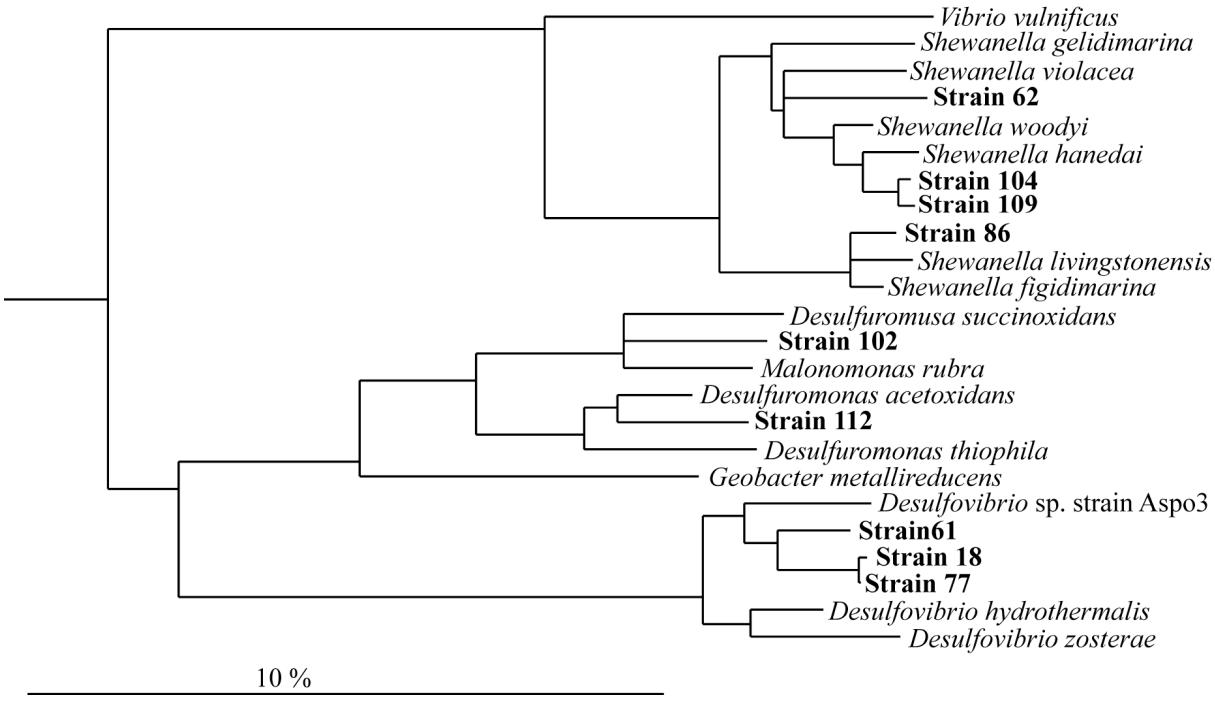

Fig. 4. Phylogenetic tree based on 16S rDNA sequences. The bar indicates 10% sequence divergence. The consensus tree was evaluated according to the results of maximum likelihood, neighbor joining, and maximum parsimony analyses. Multifurcation indicate topologies that could not be unambiguously resolved.

Characterization of the isolated strains. All isolates of the present study were able to grow at –2°C (Table 2). For the strains related to *Shewanella*, *Desulfuromonas*, and *Desulfuromusa* highest Fe reduction rates were measured at ~15°C (Table 2), and the bacteria are therefore true psychrophiles. The three strains related to *Desulfovibrio* grew best (by sulfate reduction) between 20 and 23°C, although they were isolated at different temperatures (4, 10, and 17°C) (Table 2), and can be characterized as psychrotolerants.

The substrate characteristics of the isolated strains are listed in Table 2. In addition to Fe(III) the strains reduced electron acceptors such as oxygen, Mn(IV) oxide, elemental sulfur, or sulfate. Beside acetate and lactate the strains oxidized other important fermentation products such as formate, hydrogen, or propionate.

Table 2. Characterization of the isolated strains

Strains	**102**	**112**	**62, 86, 104 & 109**	**18, 61 & 77**
Related to species of	*Desulfuromusa*	*Desulfuromonas*	*Shewanella*	*Desulfovibrio*
Isolated from station	CD	J	CC, CD & J	CC, CD& J
Optimum temperature (°C)	14-17	14	~15	20-23
Growth range (°C)	-2-23	-2-20	-2-20	-2-30
Electron acceptors				
Fe oxide / Fe citrate	+	+	+	+*
Mn oxide	+	+	+	-
elemental sulfur	+	+	-	(+)
sulfate	-	-	-	+
thiosulfate	-	-	-	(+)
sulfite	-	-	-	+
oxygen	-	-	+	-
fumarate	+	+	(+)	-
Electron donors				
acetate	+	+	(+)	-
lactate	+	-	+	+
formate	+	-	+	+
propionate	-	+	-	-
butyrate	-	-	-	-
hydrogen	+	-	+	+
ethanol	+	+	-	+
propanol	+	+	-	(+)
butanol	+	+	-	-
fumarate	+	-	-	+
succinate	+	-	-	(+)

+, substrate was used for growth; -, substrate was not used for growth; (+), substrate used by some strains; +*, substrate was reduced but no growth was observed

For all three *Desulfovibrio* related strains 18, 61, and 77 the reduction of ferric citrate and poorly crystalline Fe oxide was tested at their respective isolation temperature, and two to four transfers with significant Fe reduction in sulfate-free medium were possible (data not shown). Nevertheless the cultures reduced the Fe slower with every transfer, and we suggested that it was due to dilution and no growth occurred. The ability of strain 61 to reduce Fe was studied in more detail. Cells grown under sulfate reducing conditions were washed with sulfate-free medium and incubated with ferric citrate or poorly crystalline Fe oxide as sole electron acceptor at 0 or 20°C. The medium was, besides being sulfate free, reduced with

Fe(II) instead of sulfide to exclude an internal S-cycle with Fe(III) as oxidant for sulfide. The reduction of ferric citrate without sulfate was fast at temperatures of 0 and 20°C (Fig. 5). With poorly crystalline Fe oxide reduction of Fe(III) was at 20°C as fast as the reduction of ferric citrate at 0°C (Fig. 5). Fe oxide was not reduced at 0°C. We suppose that the low temperature prevented the establishment of certain threshold conditions in the medium (e.g. redox potential) or of microniches necessary for strain 61 to start the reduction of Fe oxide. However, in all Fe-reducing cultures strain 61 did not seem to gain energy for growth, as indicated by bacterial counts (data not shown).

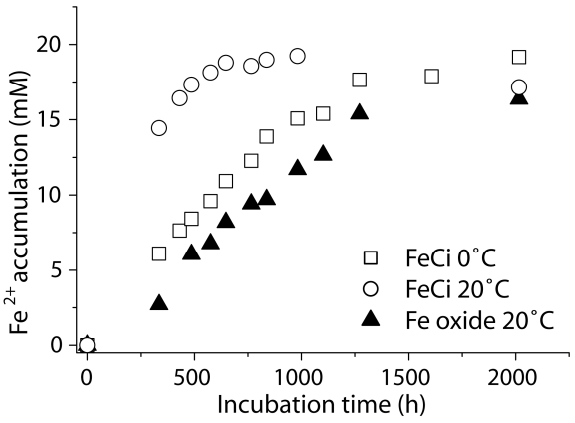

Fig. 5. Fe reduction of ferric citrate (FeCi) or Fe oxide by strain 61 in the absence of sulfate at 0 and 20°C (average of triplicates). The initial Fe(II) concentration due to $FeCl_2$ utilized as reducing agent of the culture medium was subtracted.

Discussion

Benthic carbon mineralization. The areal oxygen uptake rate for Smeerenburgfjorden sediment was 4.2 ± 0.4 mmol m^{-2} d^{-1}, which is similar to rates measured in other fjord sediments of Svalbard (3.6-8.1 mmol m^{-2} d^{-1}) (Glud *et al.*, 1998). In another Arctic area along the northeast coast of Greenland oxygen uptakes rates were within the same range at conditions comparable to our study site with 3.6 mmol m^{-2} d^{-1} at 60-163 m water depth and 5 mmol m^{-2} d^{-1} a few weeks after the settling of an algal bloom, while rates were higher at shallower water depths and directly after the phytoplankton bloom (Rysgaard *et al.*, 1998; Glud *et al.*, 2000). The DIC release rate of Station J was 7.1 ± 1.6 mmol m^{-2} d^{-1}, higher than the oxygen consumption rate. A similar trend was found in coastal sediments of Denmark (Jørgensen, 1996).

The depth-integrated sulfate reduction rate of Station J of 2.6 ± 0.6 mmol m^{-2} d^{-1} was within the range measured in other fjord sediments from the west coast of Svalbard (0.9-4.2 mmol m^{-2} d^{-1}) (Sagemann *et al.*, 1998; Knoblauch *et al.*, 1999a; Finke, 2003).

Also the anaerobic carbon oxidation rate of sediment incubations was similar to rates for permanently cold sediments as well as comparable temperate sediments: The depth-integrated anaerobic DIC production rate was 10.0 mmol m^{-2} d^{-1} for 0-5 cm, while previous studies measured for intervals of 9 or 10 cm rates of 11-24 mmol m^{-2} d^{-1} in fjord sediments of

Svalbard (Kostka *et al.*, 1999), 6 and 12 mmol m^{-2} d^{-1} in coastal sediments of Greenland (Rysgaard *et al.*, 1998; Glud *et al.*, 2000), 8-40 mmol m^{-2} d^{-1} in sediments of Denmark and Norway (Canfield *et al.*, 1993b; Kostka *et al.*, 1999; Jensen *et al.*, 2003), and 9.2 and 12 mmol m^{-2} d^{-1} in sediments of the Chilean slope (Thamdrup & Canfield, 1996). We conclude that rates of carbon mineralization of Smeerenburgfjorden are similar to comparable permanently cold and temperate sediments and the benthic microbial community is adapted to the permanently cold temperature.

Fe and Mn reduction. In contrast to oxygen uptake and sulfate reduction rates a method for a direct quantification of Fe reduction rates in sediment cores is currently not available. Today the most commonly used approach is to perform anoxic sediment incubations where the excess of anaerobic carbon oxidation, that is not coupled to sulfate reduction, is attributed to other available electron acceptors such as Mn or Fe oxides (Thamdrup, 2000).

Sulfate reduction rates matched DIC production rates (assuming a stoichiometry of 2:1 moles of carbon oxidized to sulfate reduced) in the anoxic bag incubations at 3-5 cm sediment depth (Fig. 3B). At 0-2 cm the carbon oxidation rates clearly exceeded the carbon oxidation coupled to sulfate reduction (Fig. 3B). The excess DIC production must be attributed to electron acceptors other than sulfate. We excluded oxygen and nitrate reduction because oxygen consumption was fast in the sediment cores and the low concentrations of nitrate and nitrite (12 and 0.5 µM in the bottom water) probably also had been reduced within a few hours in our bags. *In situ* these processes are dependent on the continuous supply from the water column, which was cut off during bag incubations. Because of the low content of reactive Mn oxide in comparison to Fe, Mn reduction was probably mainly mediated by abiotic oxidation of Fe(II) and microbial Mn reduction could be only of minor importance (Canfield *et al.*, 1993a; Thamdrup *et al.*, 1994). Therefore, we attribute the sulfate reduction independent carbon oxidation in the uppermost 2 cm to microbial Fe(III) reduction (Fig. 3B). This is supported by the Fe^{2+} accumulation rates in the pore water, which decreased with depth (Fig. 3A). To the depth-integrated DIC production rate in the bags of 10.0 mmol m^{-2} d^{-1} sulfate reduction contributed with 5.7 mmol m^{-2} d^{-1} to the DIC accumulation, while the remaining 4.3 mmol m^{-2} d^{-1} or 43% of the total anaerobic DIC production could be attributed to microbial Fe(III) reduction.

Zones of Mn and Fe reduction are in general indicated by the accumulation of Mn^{2+} and Fe^{2+} in the pore water. Metal liberation rates are, however, often one or two orders of magnitude lower than the gross production rates due to adsorption and/or precipitation

(Sørensen, 1982; Canfield *et al.*, 1993a; Thamdrup & Canfield, 1996; Glud *et al.*, 2000; Thamdrup *et al.*, 2000; Jensen *et al.*, 2003). In our incubation experiment, Fe^{2+} accumulation rates were similar to rates measured in previous studies with a comparable relative contribution of Fe reduction to carbon oxidation (Canfield *et al.*, 1993a; Glud *et al.*, 2000). Additionally the calculated net production rate of Fe^{2+} from the pore water profile of 2.6 µM d^{-1} at 1-4 cm sediment depth was in good agreement with the Fe^{2+} production rates in the bags of the same depth interval (9.6, 3.4, and 2.2 µM d^{-1}) considering a stimulation by 5-fold for the bags (see above). Nevertheless, the Fe^{2+} accumulation rates in bags from 0-1 and 1-2 cm accounted for less than 2% of the calculated Fe reduction rates based on the sulfate reduction independent DIC production rates. Roden (2004) showed with a pure Fe(III)-reducing culture growing on Fe containing sands and soils that 28-78% of the total Fe(II) production was accounted for by solid-phase Fe(II) accumulation. A potential sink for Fe(II) in sediments is the precipitation with sulfide, which is produced during sulfate reduction, to form FeS or pyrite. An indication for this was the black color of the sediment and that H_2S was not detected in the pore water throughout the incubation despite high rates of sulfate reduction. Additionally Fe^{2+} might adsorb to Fe or Mn oxides, be reoxidized by Mn(IV) oxide, or react through other unknown pathways (Van Cappellen & Wang, 1996). On the other hand, we cannot exclude that Fe(III) oxide was abiotically reduced by sulfide and a quantification was not possible.

In the bag incubation, dissimilatory sulfate and Fe reduction were measured simultaneously in the uppermost 2 cm, whereas below 2 cm sulfate reduction was the sole respiration process (Fig. 3B). The overlap of sulfate reduction and metal reduction was reported for many habitats, suggesting that the competition for substrates was not effective for the one or the other (Canfield *et al.*, 1993a; Achtnich *et al.*, 1995; Jakobsen & Postma, 1999; Kostka *et al.*, 1999; Jensen *et al.*, 2003). For Fe-reducing bacteria the availability of reactive Fe oxide is often important in limiting turnover rates (Lovley & Phillips, 1987; Thamdrup, 2000), and competitive inhibition of sulfate reduction by Fe-reducing bacteria is not complete. The overlap of Fe and sulfate reduction was in accordance to the rapid depletion of Fe(III) in the uppermost 2 cm (Fig. 1F). Thus, the bacterial Fe-reducing community in Smeerenburgfjorden sediments seemed to be mainly limited by Fe(III) rather than carbon.

Additionally the relative contribution of microbial Fe reduction to carbon oxidation depends on the faunal activity and sediment accumulation rate, with a high importance of Fe reduction if the faunal activity is high and the organic carbon deposition intermediate to guarantee a deeper mixing zone (Wang & Van Cappellen, 1996; Kostka *et al.*, 1999). The

presence of fauna indicated a high bioturbation and a fast turnover of Fe, which probably have a favorable effect for Fe-reducing bacteria in Smeerenburgfjorden sediments.

Pathways of carbon mineralization. To estimate the relative contribution of the different mineralization pathways for the depth interval of 0-10 cm (Table 3) we based the anoxic degradation on whole core sulfate reduction rates (Rysgaard *et al.*, 1998), since the processes in the bag incubations were stimulated by the mixing of the sediment. Sulfate reduction was assumed to be the sole terminal respiration pathway below 5 cm. Bacterial oxygen consumption was determined from the difference between anaerobic DIC production and DIC release of the whole core incubations. As the denitrification rate was not measured we do not include this pathway in our calculations but assume that it contributed 2-3% to carbon oxidation as described for other fjord sediments of Svalbard (Kostka *et al.*, 1999). These calculations determined aerobic respiration to be the most important mineralization pathway in 0-10 cm sediment depth of Station J (53%), and sulfate reduction as second most important (34%). The contribution of Fe reduction to total carbon oxidation was 13%. *In situ* the contribution of Fe reduction might vary due to a competition with oxygen- and nitrate-respiring bacteria for substrate, depending on the supply of oxygen and nitrate from the water column to the sediment by diffusion and bioturbation.

Table 3. Rates and contribution of the different mineralization pathways to carbon oxidation calculated for 0-10 cm

Pathway	**rate**[a]	**%**[b]
O_2 respiration	3.8	53
Denitrification	nd	nd
Mn(IV) reduction	0	0
Fe(III) reduction	0.9	13
SO_4^{2-} reduction	2.4	34

[a](mmol C m^{-2} d^{-1})
[b]% total organic carbon oxidation by each pathway
nd not determined

A similar contribution of Fe reduction to carbon oxidation (10 and 26%) was shown for sediments of Van Mijenfjorden and Storfjorden at the southwest and southeast coast of Svalbard (Kostka *et al.*, 1999). In another fjord (Hornsund), however, no contribution of dissimilatory Fe reduction to carbon mineralization was detected, which was explained by a high sedimentation rate and a shallow mixing zone. In permanently cold sediments of Greenland Fe reduction accounted for 21-26% of total mineralization and at temperate sites

like the coasts of Denmark, Norway and Chile for 0-50% (Canfield *et al.*, 1993b; Rysgaard *et al.*, 1998; Thamdrup & Canfield, 1996; Glud *et al.*, 2000).

Fe-reducing bacteria. To investigate the bacteria responsible for Fe reduction in permanently cold sediments of Svalbard in more detail we isolated Fe-reducing strains from two fjord sediments with similar Fe contents (Table 1). The isolates belong to the δ- and γ-subclasses of *Proteobacteria* and were characterized concerning temperature tolerance and substrate usage (Table 2). As all isolates grew well at the freezing point of sea water at –2°C, the strains are adapted to low *in situ* temperatures of their habitat. The isolation of bacteria from different physiological groups from sea ice and sediments of Antarctic and Arctic found a high abundance of psychrophilic strains indicating the adaptation to permanently low temperature (Knoblauch *et al.*, 1999a; Bowman *et al.*, 2003; Brinkmeyer *et al.*, 2003; Knittel *et al.*, 2005).

Two isolates (strains 102 and 112) belong to the genera *Desulfuromusa/Malonomonas* and *Desulfuromonas* (Fig. 4). These genera, together with *Geobacter* and *Pelobacter*, form an important group of Fe-reducing bacteria: the family *Geobacteraceae* within the δ-*Proteobacteria* (Lonergan *et al.*, 1996; Holmes *et al.*, 2004b). Recently the first psychrophilic and psychrotolerant species within *Geobacteraceae* were isolated (Holmes et al. 2004c, Nevin et al. 2005). The presence of members of the *Geobacteraceae* was shown for various habitats including freshwater and marine sediments in temperate and permanently cold habitats by cultivation-independent as well as -dependent methods (Ravenschlag *et al.*, 1999; Röling *et al.*, 2001; Stein *et al.*, 2001; Llobet-Brossa *et al.*, 2002; Bowman & McCuaig, 2003; Purdy *et al.*, 2003; Lovley *et al.*, 2004, and references therein; Mußmann *et al.*, 2005). In sediments of Smeerenburgfjorden (Station J) FISH analysis showed the highest abundance of *Desulfuromonas-Pelobacter* (up to 2.2% of DAPI cell counts) between 0.5 and 3 cm depth (Ravenschlag *et al.*, 2000), which was within the Fe reduction zone determined in this study. For the major group of sulfate-reducing bacteria, *Desulfosarcina-Desulfococcus*, highest cell numbers were found below the Fe reduction zone at 2.25 cm depth (Ravenschlag *et al.*, 2000). However, recent studies indicate that the FISH probe for the *Desulfuromonas-Pelobacter* group might be unspecific. Nevertheless, a dominant group of clone sequences in a clone library of another Svalbard fjord sediment was closely related to *Desulfuromonas palmitatis* (Ravenschlag *et al.*, 1999). Together with the isolation of the psychrophilic strains 102 and 112 we suggest that the group *Geobacteraceae* is present in fjord sediments at the west coast of Svalbard. An important characteristic of the isolated strains 112 and 102 and of other species of this family is the ability to reduce Fe(III) and elemental sulfur (Table 2). Hydrogen

sulfide is the product of sulfate reduction, the most important anaerobic pathway in most marine sediments (e.g., Jørgensen, 1982; Canfield *et al.*, 1993a; Thamdrup & Canfield, 1996). Elemental sulfur is a major product of abiotic oxidation of hydrogen sulfide by Fe(III) (Yao & Millero, 1996). The concentration of S° increased from 0.5 µmol cm^{-3} at the surface to 7.4 µmol cm^{-3} at 16 cm in the sediment of Station J (Fig. 1D) and provides an alternative electron acceptor for the strains related to *Desulfuromonas* and *Desulfuromusa*. Most species of the *Geobacteraceae* are able to couple the reduction of Fe(III) and sulfur to the oxidation of acetate (Table 2), which is an important fermentation product in marine sediments including sediments of Svalbard (e.g., Sørensen *et al.*, 1981; Finke, 2003). Thus, these bacteria might play an important role in the sulfur- and Fe-cycles of marine sediments.

Shewanella is a genus with known psychrophilic Fe-reducing species. All strains isolated with lactate (strains 62, 86, 104, and 109) in the present study were closely related to the psychrophilic species *Shewanella gelidimarina, Shewanella frigidimarina, Shewanella hanedai, Shewanella violacea,* and *Shewanella livingstonensis* isolated from Antarctic, Arctic or deep sea sediments (Jensen *et al.*, 1980; Bowman *et al.*, 1997b; Nogi *et al.*, 1998; Bozal *et al.*, 2002) (Fig. 4). Further psychrophilic strains and clone-sequences related to *Shewanella* were identified in Antarctic and Arctic sea ice and Antarctic shelf sediments (Bowman *et al.*, 1997b; Junge *et al.*, 2002; Bowman & McCuaig, 2003; Bowman *et al.*, 2003; Brinkmeyer *et al.*, 2003). Besides permanently cold habitats strains of this genus were isolated from a variety of habitats including coastal, open and deep sea environments and in invertebrates (Ivanova *et al.*, 2004; and references therein) and a wide distribution of this genus is likely. However, their quantitative importance in biogeochemical cycles is not understood, since sequences related to *Shewanella* are generally not found in clone libraries of marine sediments including the clone library of fjord sediment of Svalbard (Ravenschlag *et al.*, 1999). Facultatively anaerobic strains of *Shewanella* are well adapted to conditions in surface sediments. Here, oxygen might be an important alternative electron acceptor. Oxygen in coastal sediments of Svalbard was found to penetrate 3-11 mm into the sediment (Glud *et al.*, 1998; Kostka *et al.*, 1999) and oxygenated water can be introduced into deeper sediment layers by bioirrigating fauna (Jørgensen *et al.*, 2005). Reactive Mn oxide is typically enriched in surface sediments (Fig. 1E), which can be reduced by the isolated strains related to *Shewanella, Desulfuromonas,* or *Desulfuromusa*.

We isolated strains belonging to the genus *Desulfovibrio* under Fe(III) reducing as well as sulfate reducing conditions from sediments of all three stations, and demonstrated their ability to reduce poorly crystalline and soluble Fe(III) forms in sulfate-free medium. Fe

reduction without growth was shown also for several other species of *Desulfovibrio, Desulfobacterium* and *Desulfomicrobium* (Coleman *et al.*, 1993; Lovley *et al.*, 1993; Li *et al.*, 2004). Growth of sulfate-reducing bacteria with Fe as electron acceptor was, however, so far reported only for *Desulfobulbus propionicus* and "*Desulfotomaculum reducens"* (Tebo & Obraztsova, 1998; Holmes *et al.*, 2004a). *In situ* reduction of Fe(III) by populations of *Desulfovibrio* was suggested based on the presence of biomarkers characteristic for *Desulfovibrio* in a salt marsh sediment (Coleman *et al.*, 1993). From our results we conclude that our strains did not grow during Fe reduction. The Fe reduction rate of strain 61 was 12 µM h^{-1} at *in situ* temperatures (Fig. 5) (0°C, Fe citrate) compared to e.g. 69 µM h^{-1} for the *Desulfuromonas*-related strain 112 under the same conditions. Therefore, *Desulfovibrio*-related strains are able to reduce Fe at modestly lower rates compared to other Fe-reducing bacteria and might be involved in Fe reduction in their habitat. In previous investigations neither *Desulfovibrio* related strains were isolated nor were related sequences found in 16S rDNA libraries of fjord sediments of Svalbard (Knoblauch *et al.*, 1999a; Ravenschlag *et al.*, 1999). However, we isolated psychrotolerant strains of *Desulfovibrio* from sediments of three stations of Svalbard with the ability to reduce quantitative important electron acceptors sulfate and Fe.

Conclusions

Fe reduction is an important process for the mineralization of organic material in Smeerenburgfjorden (Station J) sediments. The isolated strains 112, 109, and 77 of Smeerenburgfjorden, representing organisms of all three isolated phylogenetic groups (*Geobacteraceae*, *Shewanella*, and *Desulfovibrio*). The relatively high Fe content provides supportive conditions for microbial Fe reduction in the uppermost 2 cm. The importance of Fe reduction at Stations CC and CD in Tempelfjorden, from where the other strains were isolated, was not determined, but the Fe content was similar to Smeerenburgfjorden. However, not only a high Fe content is important for microbial Fe reduction but additionally a high bioturbation and an intermediate sedimentation rate are supportive. The isolation of psychrophilic dissimilatory Fe-reducing bacteria from all three stations shows that this physiological group is present and adapted to low temperatures of permanently cold sediments on the west coast of Svalbard. In marine sediments Fe additionally might be reduced by sulfate-reducing bacteria.

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2.2

Psychrophilic Fe-reducing bacteria isolated from Arctic sediments, Svalbard: *Desulfuromonas svalbardensis* sp. nov. and *Desulfuromusa ferrireducens* sp. nov.

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From permanently cold Arctic marine sediments we isolated psychrophilic, Gram-negative, rod-shaped, motile bacteria (strains 112^T and 102^T) that conserved energy from dissimilatory Fe(III) reduction concomitant to acetate oxidation. Both strains grew at temperatures down to –2°C with temperature optima of 14°C and 14-17°C for strains 112^T and 102^T, respectively. The isolated strains reduced Fe(III) using common fermentation products such as acetate, lactate, propionate, formate, or hydrogen as electron donors, and they also fermented fumarate. Alternatively to Fe(III) they reduced fumarate, S°, and Mn(IV). Based on 16S rDNA sequence similarity, strain 112^T was most closely related to *Desulfuromonas acetoxidans* (97.0%) and *Desulfuromonas thiophila* (95.5%), and strain 102^T to *Malonomonas rubra* (96.3%) and *Desulfuromusa succinoxidans* (95.9%) within the δ-*Proteobacteria*. Strains 112^T and 102^T therefore represent new species, for which the names *Desulfuromonas svalbardensis* sp. nov. with the type strain 112^T (= DSM 16958^T = JCM 12927^T), and *Desulfuromusa ferrireducens* sp. nov. with the type strain 102^T (= DSM 16956^T = JCM 12926^T) are proposed.

The genus *Desulfuromonas* was first described by Pfennig & Biebl (1976), who isolated the marine species, *Desulfuromonas acetoxidans*, that reduces elemental sulfur with acetate. The genus contains four further species, which had been isolated with reduction of Fe, sulfur, or tetrachloroethylene from marine and freshwater sediments: *Desulfuromonas palmitatis* (Coates *et al.*, 1995), *Desulfuromonas acetexigens* (Finster *et al.*, 1994), *Desulfuromonas thiophila* (Finster *et al.*, 1997), and *Desulfuromonas chloroethenica (*Krumholz, 1997; Krumholz *et al.*, 1996). The genus *Desulfuromusa* is represented by three species: *Desulfuromusa bakii, Desulfuromusa kysingii,* and *Desulfuromusa succinoxidans*, isolated by elemental sulfur reduction (Liesack & Finster, 1994). Together with the genera *Pelobacter, Malonomonas*, and *Geobacter*, *Desulfuromusa* and *Desulfuromonas* form the family *Geobacteraceae,* a monophyletic group within the δ-*Proteobacteria* (Holmes *et al.*, 2004a; Lonergan *et al.*, 1996). An important characteristic of species within this group is the ability to reduce Fe(III) and/or elemental sulfur. Additionally some species grow by fermentation or syntrophically (Cord-Ruwisch *et al.*, 1998; Schink, 1984; Schink & Pfennig, 1982; Schink & Strieb, 1983). Due to the variety of metabolic pathways performed by isolated species of the *Geobacteraceae*, the *in situ* activity of this group remains unclear since several electron

donors and acceptors available in freshwater and marine sediments usually can be utilized by these bacteria.

The isolated strains were obtained from enrichment cultures inoculated with surface sediments of two fjords along the west coast of Svalbard with bottom water temperatures of 2-3°C. Strains 49, 60, 102^T, and 103 originated from Tempelfjorden, Station CD (78°25.267 N, 17°08.277 E; water depth 64 m) and strain 112^T from Smeerenburgfjorden, Station J (79°42.006 N, 11°05.199 E; water depth 212 m). The enrichment, and isolation was performed in artificial sea water medium (Widdel & Bak, 1992) with a reduced $MgSO_4 \cdot 7H_2O$ concentration of 0.4 mM to avoid growth of sulfate-reducing bacteria. Acetate (20 mM) and synthetically produced poorly crystalline Fe oxide (~30 mM) (Lovley, 2000) was added for enrichments at 10°C. For the isolation in deep agar dilution technique (Isaksen & Teske, 1996) Fe oxide was displaced by soluble Fe(III) citrate (~30 mM). For the determination of alternative substrates, salt, pH, and vitamin requirements for growth medium with a lower salt concentration was used (saltwater medium) (Widdel & Bak, 1992). All physiological tests were performed in duplicates at 10°C. Cultures growing with alternative substrates were transferred into fresh test medium for verification. Temperature tolerance of the strains was determined in an aluminum temperature gradient block at 13 different temperatures between –2 and 30°C (Sagemann *et al.*, 1998). The salt requirement was determined in media with 12 different NaCl concentrations between 0.05 and 5% (w/v) and 10 different $MgCl_2 \cdot 6H_2O$ concentrations between 0.02 and 3.6% (w/v). The pH optima of the strains were determined in media with 12 different pH values (in triplicates) that covered a range from pH 5.5 to 8.3. For all tests growth was monitored spectrophotometrically (Shimadzu, UV 1202) by measuring OD at 580 nm for cells grown on fumarate/acetate and by measuring Fe^{2+} accumulation (Stookey, 1970) for cells grown on Fe(III) citrate/acetate. Reduction of Fe(III) citrate was also tested in media with $FeCl_2 \cdot 4H_2O$ (2-3 mM end concentration) or cysteine (1 mM end concentration) as reducing agents instead of sulfide.

Malonomonas rubra (DSM 5091^T) obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) was grown in saltwater medium (Widdel & Bak, 1992) with malonate as substrate. To test the ability of cells to grow by S°, Fe(III) or Mn(IV) reduction, malonate was replaced with ferric citrate, poorly crystalline Fe oxide, Mn oxide, or S° as electron acceptor and acetate as electron donor.

Fatty acids were analyzed by GC and GC-MS (Elvert *et al.*, 2003). Lipoquinones (Tindall, 1990) and the G + C content of genomic DNA (Mesbah *et al.*, 1989) were determined at the DSMZ.

PCR amplification of 16S rDNA was performed with the primers 8F and 1492R, and amplified for sequence analysis with primers 8F, 341F, 518F, 534R, 1099F, and 1492R (Buchholz-Cleven *et al.*, 1997). For the phylogenetic analysis the ARB program (Ludwig *et al.*, 2004) was used. The topology of the tree was evaluated by using maximum parsimony, neighbor joining, and maximum likelihood algorithms without and with filters, which excluded positions with less than 50% conservation within the δ-*Proteobacteria.* The 16S rDNA sequences of *Desulfuromonas svalbardensis* strain 112^T, strain 49, strain 60, strain 103, and *Desulfuromusa ferrireducens* strain 102^T have been deposited in the GenBank database under accession numbers AY835388-AY835392.

Purity of cultures of the strains 49, 60, 103, 112^T, and 102^T was checked microscopically and by inoculating the cultures into media with yeast extract, casein, glucose or fructose. Strains 49, 60, 103, and 112^T were all phylogenetically closely related (99.4-99.7% 16S rDNA sequence similarity). The strains were tested for growth with a selection of environmentally important electron acceptors and donors and showed a similar substrate spectrum (data not shown). Furthermore, the strains all revealed similar optimum growth temperatures around 15°C and growth at 0°C (data not shown). Due to the similarities of strains 49, 60, 103, and 112^T, strain 112^T was selected for further detailed characterization. Strain 102^T was also characterized in detail.

Cells of both strains grew as thin rods (Fig. 1). Strain 112^T was 0.7 µm x 2-3.5 µm and of strain 102^T 0.7-1 µm x 3-5 µm in size. The latter formed clumps in liquid culture. Both strains stained Gram-negative, were non-sporeforming and motile. Electron microscopy (Zeiss EM 10 A, at the UFT, University of Bremen) revealed for strain 112^T a peritrichous flagellation and for strain 102^T a monopolar lophotrichous flagellation (Fig. 1C).

Strain 112^T grew fastest at 14°C and did not grow above 20°C. The temperature optimum of strain 102^T was between 14 and 17°C, and the maximum temperature 23°C. The ability of strain 112^T and 102^T to grow at –2°C showed that the isolates were adapted to the permanently low *in situ* temperature of the Arctic Ocean. According to their temperature range of growth both strains were defined as psychrophiles. Strain 112^T had an optimum for NaCl at 2.6%, growing between 0.7 and 4.5%. The optimum for $MgCl_2 \cdot 6H_2O$ was between

0.02 and 0.8%, and growth was inhibited at a concentration >1.9%. For strain 102^T the optimum for NaCl was 2.6-4%, with growth ranging from 1.5-4.5%. The strain grew equally well over the range of 0.4 to 3.6% $MgCl_2 \cdot 6H_2O$. Thus, both strains grew at seawater concentrations of NaCl and $MgCl_2 \cdot 6H_2O$ which are 2.5% for NaCl and 1.1% for $MgCl_2 \cdot 6H_2O$. Strain 112^T grew at pH 6.5-7.5, with an optimum at pH 7.3. Strain 102^T showed a similar growth range of pH 6.5-7.9 and an optimum at pH 7.0-7.3.

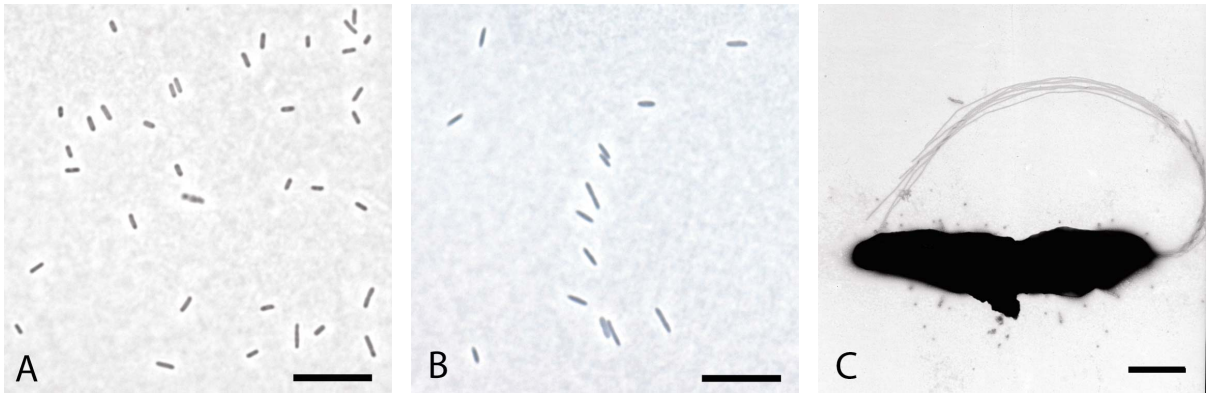

Fig. 1. (A, B) Phase contrast micrographs of (A) *Desulfuromonas svalbardensis* 112^T and (B) *Desulfuromusa ferrireducens* 102^T. (C) Electron micrograph of *Desulfuromusa ferrireducens* 102^T, showing the rod shape and the monopolar lophotrichous flagellation. The scale bar indicates 10 µm for (A) and (B), and 1 µm for (C).

Strain 112^T grew in the presence of ferric citrate with acetate, propionate, pyruvate, ethanol, propanol, butanol, proline, and cholin chloride as electron donors, and strain 102^T with acetate, lactate, formate, H_2/CO_2, succinate, pyruvate, fumarate, ethanol, propanol, butanol, and proline. Electron donors not used by both strains were butyrate, hexanoate, malate, succinate, fructose, glucose, glycerol, glycin, glutarate, alanine, serine, proline, betaine, sorbite, nicotinate, yeast extract, and casein; substrates not used by strain 112^T were lactate, formate, fumarate, succinate, and H_2/CO_2; and strain 102^T did not use propionate and cholin chloride. Both strains grew by reduction of Fe(III) compounds (ferric citrate and Fe oxide tested), and fumarate in the presence of acetate. Additionally, the strains slowly reduced elemental sulfur and Mn oxide. Both strains did not reduce sulfate, thiosulfate, sulfite, nitrate, nitrite, oxygen, and malate. Ferric citrate was also reduced in media with $FeCl_2$ or cysteine as reducing agents instead of sulfide. No reduction of Fe(III) in the presence of oxygen was observed for either strain. Disproportionation of sulfur or thiosulfate was not observed. Both strains fermented fumarate, but not lactate, malate, malonate,

pyruvate, glucose, and fructose. Strain 102^T did not require vitamins for growth. Strain 112^T required biotin as vitamin.

Table 1. Fatty acid abundancies of strains 112^T and 102^T.

Fatty acids	**112^T**	**102^T**
$C_{13:0}$	0.01	0.01
i-$C_{14:0}$	0	trace
$C_{14:0}$	0.07	0.07
i-$C_{15:0}$	0.02	0
ai-$C_{15:0}$	0	0.01
$C_{15:0}$	0.01	0.01
$C_{16:1}\omega9c$	0.01	0.01
$C_{16:1}\omega7c$	**0.35**	**0.39**
$C_{16:1}\omega7t$	0	0.02
$C_{16:1}\omega5c$	0.04	0.01
$C_{16:0}$	**0.43**	**0.036**
10Me-$C_{16:0}$	0	trace
i-$C_{17:0}$	trace	trace
ai-$C_{17:0}$	trace	trace
$C_{17:0}$	0	0.01
$C_{18:2}$	0	0.02
$C_{18:1}\omega9$	0.01	0.02
$C_{18:1}\omega7$	0.01	0.01
$C_{18:1}\omega5$	trace	0
$C_{18:0}$	0.02	0.06

trace: <0.01

The phospholipid derived ester-linked fatty acid composition of strains 112^T and 102^T is listed in Table 1. $C_{16:1}\omega7c$ and $C_{16:0}$ were dominant as fatty acids in both strains similar to the fatty acid composition of *Geobacter metallireducens* (Lovley *et al.*, 1993). Cells of strain 112^T contained as major menaquinone MK8 and traces of MK9 (2%), cells of strain 102^T contained only MK8. The G + C content of the DNA of strain 112^T was 50.1 mol% and for strain 102^T 52.3 mol%.

Comparative analysis of the 16S rDNA showed that both strains belong to the δ-*Proteobacteria* (Fig. 2). Strain 112^T was related to *Desulfuromonas acetoxidans* (level of 16S rDNA similarity, 97.0%), *Desulfuromonas thiophila* (95.5%), *Pelobacter venetianus* (93.7%), and *Desulfuromonas chloroethenica* (93.1%). The two strains differed mainly in their temperature tolerance, with *Desulfuromonas acetoxidans* being mesophilic growing between 25 and 35°C and strain 112^T being psychrophilic growing between –2 and 20°C. Physiological differences between the two strains were the ability of strain 112^T to oxidize propionate, to ferment fumarate, and the inability to reduce malate (Table 2).

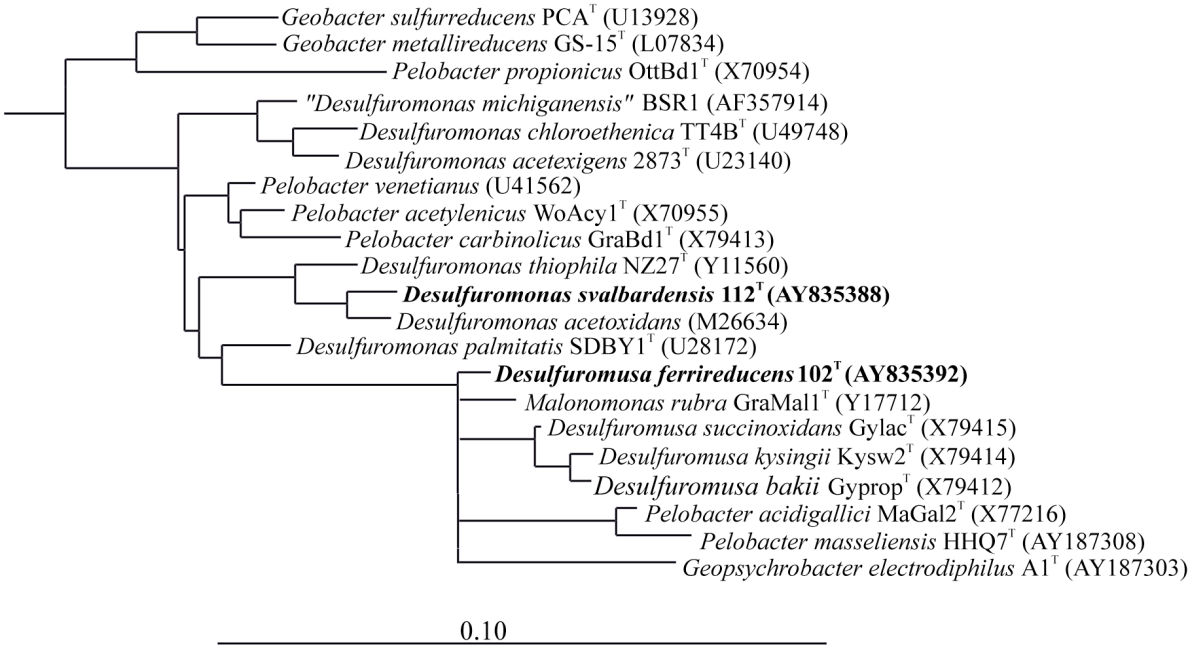

Fig. 2. Phylogenetic tree of 16S rDNA sequences. Multifurcations indicate tree topologies that could not be resolved unambiguously. Multifurcations are not shown for all ambiguous topologies, then the tree is based on the tree calculated by maximum likelihood analysis. The scale bar indicates 10% estimated sequence divergence.

The closest relatives of strain 102^T were *Malonomonas rubra* (level of 16S rDNA similarity, 96.3%), *Desulfuromusa succinoxidans* (95.9%), *Desulfuromusa kysingii* (95.5%), and *Desulfuromusa bakii* (95.4%). *M. rubra* was so far the only described species of this genus, and the genus was established due to the ability of this species to grow by fermentation of malonate (Dehning & Schink, 1989), but cells did not grow by anaerobic respiration (Kolb *et al.*, 1998). The ability of *M. rubra* to reduce Fe compounds was recently described, and the authors suggested that *M. rubra* should be renamed a member of the genus *Desulfuromusa* (Holmes *et al.*, 2004a). This is supported by our results that *M. rubra* (DSM 5091^T) reduced ferric citrate, Fe oxide, elemental sulfur and Mn oxide with acetate as electron donor, similar to species of *Desulfuromusa*. Therefore we propose strain 102^T to be placed as new species of the genus *Desulfuromusa*.

Table 2. Major characteristics of species of the genus *Desulfuromonas* and strain 112^T. Data taken from Pfennig & Biebl (1976), Finster *et al.* (1994), Coates *et al.* (1995), Finster *et al.* (1997), Krumholz (1997), Sung *et al.* (2003).

	112^T	***D. acet-oxidans***	***D. acet-exigens***	***D. thiophila***	***D. chloro-ethenica***	***D. palmitatis***	***"D. michi-ganensis"***
Temperature optimum (°C)	14	30	30	26-30	21-31	40	25
Temperature range (°C)	-2-20	25-35	nd	10-40	21-31	nd	5-45
Electron donors							
acetate	+	+	+	+	+	+	+
propionate	+	-	-	-	nd	-	-
Electron acceptors							
Fe(III) compounds	+	+	nd	+*	+	+	+
Mn(IV) oxide	+	+	nd	n d	nd	+	nd
sulfur	+	+	+	+	+	+	+
fumarate	+	+	+	-	+	+	+
malate	-	+	+	-	nd	-	+
Fermentation							
fumarate	+	-	nd	-	nd	nd	+

nd: not determined; + = substrate was used for growth, - = substrate was not used for growth; +* = substrate was reduced but no growth was observed; in this table not all electron donors and acceptors used by the species are listed.

The new isolated strain 102^T was psychrophilic growing between –2 and 23°C, whereas *Desulfuromusa* species did not grow below 4°C and optimum temperatures were ≥25°C (Finster & Bak, 1993; Liesack & Finster, 1994). Recently, the first psychrotolerant species, *Geopsychrobacter electrodiphilus,* within the *Geobacteraceae* was isolated, which was closely related to species of *Desulfuromusa* and *Malonomonas rubra* but represented a unique phylogenetic cluster (Holmes *et al*., 2004b). Most species of *Desulfuromusa*, *M. rubra*, strain 102^T, and *G. electrodiphilus* shared the ability to reduce Fe(III), elemental sulfur, Mn(IV), and fumarate and oxidize acetate, succinate, and pyruvate, but differed in the usage of other substrates (Table 3).

Table 3. Major characteristics of species of the genus *Desulfuromusa, Malonomonas rubra, Geopsychrobacter electrodiphilus* and strain 102^T. Data taken from Liesack & Finster (1994), Dehning & Schink (1989), Holmes *et al.* (2004b).

	102^T	***D. succin-oxidans***	***D. kysingii***	***D. bakii***	***M. rubra***	***G. electro-diphilus***
Temperature optimum (°C)	14-17	30-35	30-35	25-30	28-30	22
Temperature range (°C)	-2-23	4-35	4-35	8-32	22-45	4-30
Electron donors						
acetate	+	+	+	+	+	+
formate	+	-	-	-	nd	-
propionate	-	+	+	+	nd	-
alcohols[#]	+	-	-	-	nd	only ethanol
lactate	+	+	+	+	nd	-
Electron acceptors						
Fe(III) compounds	+	-	+	-	+	+
Mn(IV) oxide	+	nd	nd	nd	+	+
sulfur	+	+	+	+	+	+
fumarate	+	+	+	+	nd	-
malate	-	-	+	-	nd	-
nitrate	-	-	+	-	nd	nd
fermentation						
fumarate	+	+	+	+	+	nd
malate	-	+	+	+	+	nd
malonate	-	-	-	-	+	nd

nd: not determined; + = substrate was used for growth, - = substrate was not used for growth; + no growth = substrate was reduced but no growth was observed; in this table not all electron donors and acceptors used by the species are listed.
[#]alcohols: ethanol, propanol, butanol

The *in situ* abundance of members of the *Geobacteraceae* had been demonstrated for temperate as well as permanently cold marine sediments of Arctica and Antarctica as in 16S rRNA clone libraries of these sediments several sequences closely related to strains of *Geobacteraceae* previously had been found (Ravenschlag *et al.*, 1999; Bowman & McCuaig, 2003; Purdy *et al.*, 2003; Mußmann *et al.*, 2005). The isolation of the new strains 102^T and 112^T from marine sediments of Svalbard suggests that this group of bacteria is present in diverse freshwater and marine environments. Yet, the significance and *in situ* activity of the sulfur-/Fe-reducing *Geobacteraceae* remains unclear for most habitats. As reviewed by

Thamdrup (2000), Fe reduction is the second most important anaerobic respiration pathway in a wide range of habitats. In Arctic marine sediments of Svalbard, Fe reduction accounted for 0-26% of the total carbon respiration (Kostka *et al.*, 1999). Marine surface sediments that have a zone of reactive Fe and Mn as well as accumulation of elemental sulfur provide optimal conditions for bacteria able to reduce these compounds, such as the isolated strains. Such a sediment setting was, for example, described from the Danish coast where the concentration of sulfur was highest in the zone of Fe/Mn reduction (Sørensen & Jørgensen, 1987), due to the rapid reaction of H_2S with Mn(IV) or Fe(III) to form elemental sulfur.

Possible substrates for Fe-reducing bacteria are the common fermentation products such as volatile chain fatty acids and hydrogen. Strains 112^T and 102^T oxidized important fermentation products acetate, lactate, formate, and/or hydrogen concomitant with the reduction of elemental sulfur or Fe. Acetate is an important substrate for sulfate-reducing bacteria in temperate as well as Arctic marine sediments (e.g. Sørensen *et al.*, 1981; Finke, 2003). Turnover rates in Arctic fjord sediments were highest for acetate followed by lactate and propionate (Finke, 2003).

Psychrophilic sulfate-reducing bacteria isolated from Svalbard sediments showed constant growth yields between –2°C and their optimum growth temperature (Knoblauch & Jørgensen, 1999). Among the Fe-reducing bacteria, psychrophiles of the genus *Shewanella* were isolated from the Antarctic, the Alboran Sea, or deep sea sediments of the Pacific Ocean, e.g. *Shewanella frigidimarina, Shewanella gelidimarina, Shewanella woodyi,* or *Shewanella violaceae* (Bowman *et al.*, 1997; Makemson *et al.*, 1997; Nogi *et al.*, 1998). The strains isolated by Fe reduction in the present study grew at *in situ* temperatures just above the freezing point of seawater and were accordingly well adapted to the permanently low temperatures of the Arctic Ocean. Recently the first psychrophilic and psychrotolerant species within the family *Geobacteraceae* have been isolated, *Geopsychrobacter electrodiphilus* and *Geobacter psychrophilus* (Holmes *et al.*, 2004b; Nevin *et al.*, 2005). Our isolates extend the group of psychrophiles within the *Geobacteraceae*.

In summary, the isolated strains, *Desulfuromonas svalbardensis* and *Desulfuromusa ferrireducens*, were well suited for life in anoxic permanently cold sediments of Svalbard. The abundance and diversity of Fe- and sulfur-reducing bacteria in this environment have, however, not been investigated. More studies on the microbial communities and their *in situ* activities are needed to fully understand the importance of sulfur and Fe reduction in marine sediments.

Description of *Desulfuromonas svalbardensis* sp. nov. sval.bard.en'sis, N.L. fem. adj. *svalbardensis*, from Svalbard, group of islands in the northern Barents Sea from where the type strain was isolated.

Cells are rod-shaped, 0.7 µm x 2.5-3 µm, motile by peritrichous flagella; Gram-negative; strict anaerobic; chemoorganotrophic. Biotin as a vitamin is required. *Desulfuromonas svalbardensis* grows by oxidation of acetate, propionate, ethanol, propanol, butanol, cholin chloride, or pyruvate with concomitant reduction of Fe(III). Fe(III) compounds, Mn oxide, elemental sulfur, and fumarate serve as electron acceptors. Fermentative metabolism with fumarate. The pH range for growth is between pH 6.5 and 7.5; optimum pH is 7.3. *Desulfuromonas svalbardensis* is psychrophilic with an optimum growth temperature of 14°C and a temperature growth range of –2 to 20°C. 50.1 mol% of G + C of the DNA.

The habitat is a permanently cold fjord sediments of the west coast of Svalbard. Type strain is 112^T (DSM 16958^T = JCM 12927^T). The 16S rDNA sequence has been deposited in GenBank (AY835388).

Description of *Desulfuromusa ferrireducens* sp. nov. fer.ri.re.du'cens. L. n. *ferrum,* iron; L. part. adj. *reducens,* leading back, bringing back, and in chemistry converting to a reduced oxidation state; N.L. part. adj. *ferrireducens* reducing Fe(III) to Fe(II).

Cells are rod-shaped, 0.7-1 µm x 3-5 µm, motile by monopolar lophotrichous flagella; Gram-negative; strict anaerobic; chemoorganotrophic. No vitamins are required for growth. *Desulfuromusa ferrireducens* oxidizes acetate, lactate, succinate, fumarate, pyruvate, proline, ethanol, propanol, butanol, formate, or H_2/CO_2 with the reduction of Fe(III). Fe(III) compounds, elemental sulfur, Mn oxide, and fumarate serve as electron acceptors. Fermentative metabolism with fumarate. The pH range of growth is between pH 6.5 and 7.9; optimum pH is 7.0-7.3. *Desulfuromusa ferrireducens* is psychrophilic with an optimum growth temperature of 14-17°C and a temperature growth range of -2-23°C. 52.3 mol% of G + C of the DNA.

The habitat is a permanently cold fjord sediments of the west coast of Svalbard. Type strain is 102^T (DSM 16956^T = JCM 12926^T). The 16S rDNA sequence has been deposited in GenBank (AY835392).

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2.3

Desulfovibrio frigidus sp. nov. and *Desulfovibrio ferrireducens* sp. nov., two psychrotolerant bacteria isolated from Arctic fjord sediments (Svalbard) with the ability to reduce Fe(III)

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Strains 18^T, 61^T and 77 were isolated from two permanently cold fjord sediments at the west coast of Svalbard. The three psychrotolerant strains with temperature optima at 20-23°C were able to grow at the freezing point of sea water, –2°C. The strains oxidized important fermentation products such as hydrogen, formate, and lactate with sulfate as electron acceptor. Sulfate could be substituted by sulfite, thiosulfate, or elemental sulfur. Poorly crystalline and soluble Fe(III) compounds were reduced in sulfate-free medium, but no growth occurred under these conditions. In the absence of electron acceptors fermentative growth was possible. The pH optima for the strains were around 7.1. The G + C contents of DNA were 43.3 and 42.0 mol% for strains 18^T and 61^T, respectively. Strains 18^T, 61^T, and 77 were most closely related to *Desulfovibrio hydrothermalis* (95.0-95.7% 16S rDNA sequence similarity). Strains 18^T and 77, exhibiting 99.9% sequence similarity, are proposed as new species, *Desulfovibrio frigidus* sp. nov. (type strain 18^T, DSM 17176^T, JCM 12924^T). Strain 61^T was closely related to strains 18^T and 77 (97.6 and 97.5% 16S rDNA sequence similarity), but on the basis of DNA-DNA hybridization it is proposed to represent a new species, *Desulfovibrio ferrireducens* (type strain 61^T, DSM 16995^T, JCM 12925^T).

Dissimilatory sulfate reduction is the most important anaerobic mineralization pathway in many temperate and permanently cold marine sediments (e.g., Jørgensen, 1982; Canfield *et al.*, 1993; Thamdrup & Canfield, 1996; Rysgaard *et al.*, 1998; Kostka *et al*,. 1999; Glud *et al.*, 2000). Most sulfate-reducing bacteria are phylogenetically placed within the δ-*Proteobacteria*, among them the genus *Desulfovibrio*, which comprises 44 described species. A special characteristic of some *Desulfovibrio* strains is the ability to reduce Fe(III) compounds but without gaining energy for growth (Coleman *et al.*, 1993; Lovley *et al.*, 1993; Li *et al.*, 2004). Here we report the isolation of three novel psychrotolerant *Desulfovibrio*-related strains with the ability to reduce Fe(III).

Strain 61^T was isolated as described recently for the isolation of psychrophilic Fe(III)-reducing bacteria (Vandieken *et al.*, submitted). An enrichment culture of artificial sea water medium (Widdel & Bak, 1992) with ~30 mM poorly crystalline iron oxide, 0.4 mM sulfate, and 20 mM lactate was inoculated with surface sediment of Tempelfjorden, Station CD (78°25'267" N, 17°08'277" E, bottom water temperature 2.8°C) and incubated at 10°C. The Fe oxide was replaced by ferric citrate for isolation in deep agar dilution series. Cells of strain

61^T were motile vibrios, and the 16S rDNA sequence was 95.7% similar to the sequence of *Desulfovibrio hydrothermalis*. The ability of *Desulfovibrio desulfuricans* to reduce Fe(III) for several consecutive transfers has been shown previously (Lovley *et al.*, 1993), however, the authors suggested that the strains grew during the experiments with the 0.3 mM sulfate in the medium and not by Fe reduction. Correspondingly, we could not unequivocally determine whether strain 61^T grew during the isolation by Fe(III) reduction or with the 0.4 mM sulfate in the medium. Strains 18^T and 77 were enriched and isolated under sulfate reducing conditions with 28 mM sulfate and 20 mM lactate and 10 mM formate at 4 and 17°C from sediment of Station CC (Tempelfjorden, 78°26'039" N, 17°19'722" E, bottom water temperature 3.1°C) and Station J (Smeerenburgfjorden, 79°42'006" N, 11°05'199" E, bottom water temperature 2.3°C), respectively. 16S rDNA sequencing showed that strains 18^T and 77 were closely related to *D. hydrothermalis* and to strain 61^T.

Strains 61^T and 77 showed a vibrioid or sigmoid morphology, 2.5-5.5 x 0.5-0.7 µm in size, whereas cells of strain 18^T were straight rods, 3.5-4.5 x 0.5-0.7 µm in size. Cells of all strains were motile by means of a single polar flagellum as indicated by electron microscopy (Fig. 1). The Gram stain was negative for all strains.

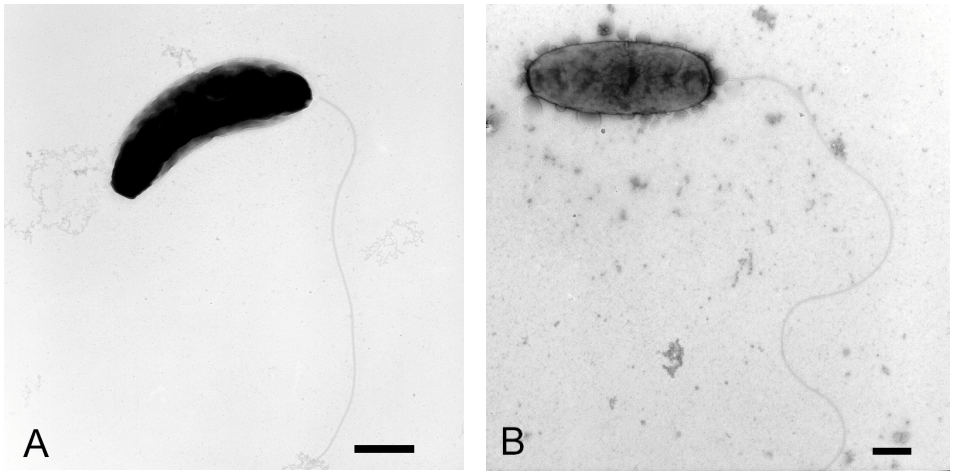

Fig. 1. Electron micrographs (negative stain with uranyl acetate) of *D. ferrireducens* 61^T (A) and *D. frigidus* 18^T (B), showing the sigmoid shape of strain 61^T and the straight rod shape of strain 18^T. Both strains are motile by a single monopolar flagellum. The scale bars indicate 0.5 µm.

The general physiological characteristics of strains 18^T, 61^T, and 77 were evaluated under sulfate reducing conditions with lactate as electron donor at their respective isolation temperature. The tests used have been described previously (Vandieken *et al.*, submitted). Neither strain needed vitamins for growth. The strains grew at NaCl and $MgCl_2$

concentrations of sea water. NaCl optima were 2-3% for strains 18^T and 77 and 1-2.5% for strain 61^T, and strains 18^T, 61^T, and 77 grew with NaCl concentrations of 2-3.5%, 0.7-4% and 1.5-4%, respectively. $MgCl_2$ optima were 0.04-1.9%, 0.02-2.5%, and 0.4% and $MgCl_2$ growth ranges were 0.02% to 2.5%, to 3.5%, and to 1.9% for strains 18^T, 61^T, and 77. The pH optima were 6.9-7.2, 7.1-7.5, and 7.1 and growth was observed at pH 6.9-7.5, 6.3-7.5, and 6.7-7.5 for strains 18^T, 61^T, and 77 respectively. Common end products of fermentation such as lactate, formate, and hydrogen served as electron donors (Table 1). The strains reduced sulfate and other sulfur compounds like sulfite, thiosulfate, or elemental sulfur (Table 1). Reduction of ferric citrate or poorly crystalline Fe oxide in sulfate-free medium was observed in 2-4 consecutive transfers for all three strains. Reduction of Fe(III) became slower with every transfer, however, and we suggest that the strains did not conserve energy for growth. The ability of Fe(III) reduction was previously described for several species of *Desulfovibrio* (*Desulfovibrio desulfuricans, Desulfovibrio vulgaris, Desulfovibrio sulfodismutans, Desulfovibrio baarsii, Desulfovibrio* spp. strain G-11) as well as for *Desulfomicrobium baculatum* and *Desulfobacterium autotrophicum* (Coleman *et al.*, 1993; Lovley *et al.*, 1993; Li *et al.*, 2004). Growth by Fe(III) reduction was so far only shown for the two sulfate-reducing bacteria *Desulfobulbus propionicus* and *"Desulfotomaculum reducens"* (Tebo & Obraztsova, 1998; Holmes *et al.*, 2004).

Although isolated at different temperatures (4, 10 and 17°C) all three strains showed similar temperature optima for growth of 20-23°C (Table 1). All strains were able to grow at the freezing point of sea water, –2°C. According to their temperature range the strains can be considered as psychrotolerant.

The G + C contents were determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, and were 42.0 mol% for strain 61^T and 43.3 mol% for strain 18^T (Table 1).

Table 1. Comparison of the characteristics of *D. ferrireducens* strain 61^T, *D. frigidus* strains 18^T and 77 and closely related species *D. hydrothermalis, D. zosterae,* and *D. salexigens.* Data taken from Postgate & Campbell (1966), Postgate (1984), Zellner *et al.* (1989), Nielsen *et al.* (1999), and Alazard *et al.* (2003).

strains	**61^T**	**18^T**	**77**	***D. hydro-thermalis***	***D. zosterae***	***D. sal-exigens***
Temperature range (°C)	-2-30	-2-25	-2-26	20-40	up to 34.5	up to 42-45
Temperature optimum (°C)	23	20-23	21-22	35	32.5-34.5	34-37
Electron acceptors						
sulfate	+	+	+	+	+	+
thiosulfate	+	-	-	+	+	nd
sulfite	+	+	+	+	+	nd
elemental sulfur	-	+	-	-	+	nd
Fe(III) citrate or oxide	+*	+*	+*	nd	nd	nd
Electron donors						
lactate	+	+	+	+	+	+
formate	+	+	+	+	-	+
formate + acetate	+	+	+	+	(+)	+
hydrogen	+	+	+	+	-	+
hydrogen + acetate	+	+	+	+	+	+
ethanol	+	+	+	+	+	+
propanol	+	+	-	-	-	+
fumarate	+	+	+	+	+	+
succinate	+	-	-	-	-	+
alanine	-	+	+	-	+	nd
pyruvate	-	-	-	+	+	+
malate	-	-	-	+	+	+
choline	-	-	-	+	+	+
glycerol	-	-	-	+	nd	+
fructose	-	-	-	-	+	nd
Fermentation						
malate	+	+	-	nd	-	-
pyruvate	+	-	+	+	+	-
fumarate	+	+	-	(+)	+	nd
glucose	-	-	+	nd	-	nd
fructose	-	-	-	-	+	nd
G + C content (mol%)	42.0	43.3	nd	47	42.7	45.5

+ = substrate was used for growth, - = substrate was not used for growth, +* = substrate was reduced but no growth, (+) = substrate was poorly utilized, nd = not determined; electron acceptors tested but not reduced by strains 18^T, 61^T, and 77: nitrate, nitrite, oxygen, malate, fumarate, manganese oxide; electron donors tested but not oxidized: acetate, butyrate, propionate, hexanoate, malate, butanol, pyruvate, fructose, glucose, glycerol, glycin, glutarate, serine, proline, betaine, sorbite, nicotinate, yeast extract, casein, cholin chloride; substrates tested for fermentation but not used: lactate, fructose.

16S rDNA sequences were amplified and analyzed as previously described (Vandieken *et al.*, submitted). The phylogenetic positions of the three strains were evaluated by using the ARB program (Ludwig *et al.*, 2004) with the neighbor joining, maximum likelihood and maximum parsimony algorithms in combination with different sets of filters (Fig. 2). Strains 18^T and 77 were closely related to each other (99.9% 16S rDNA sequence similarity), therefore we suggest that these two strains belong to the same species. The 16S rDNA sequence similarities between strains 61^T and 18^T were 97.6% and between strains 61^T and 77 97.5%. DNA-DNA hybridization was done by the DSMZ, and DNA-DNA similarities were 14.5% between strains 61^T and 18^T and 18.3% between strains 61^T and 77. Therefore, we propose the description of two new species: *Desulfovibrio ferrireducens* (type strain 61^T) and *Desulfovibrio frigidus* (type strain 18^T). Both strains 61^T and 18^T are closely related to the undescribed *Desulfovibrio* species strain Aspo3 (97.4 and 95.4% 16S rDNA sequence similarity, respectively) isolated from subterranean groundwater (Pedersen *et al.*, 1996), as well as to *Desulfovibrio hydrothermalis* (95.7 and 95.0%) isolated from a deep-sea hydrothermal chimney (Alazard *et al.*, 2003), *Desulfovibrio zosterae* (94.8 and 94.3%) isolated from marine seagrass roots (Nielsen *et al.*, 1999), and *Desulfovibrio salexigens* (94.6 and 95%) (Fig. 2).

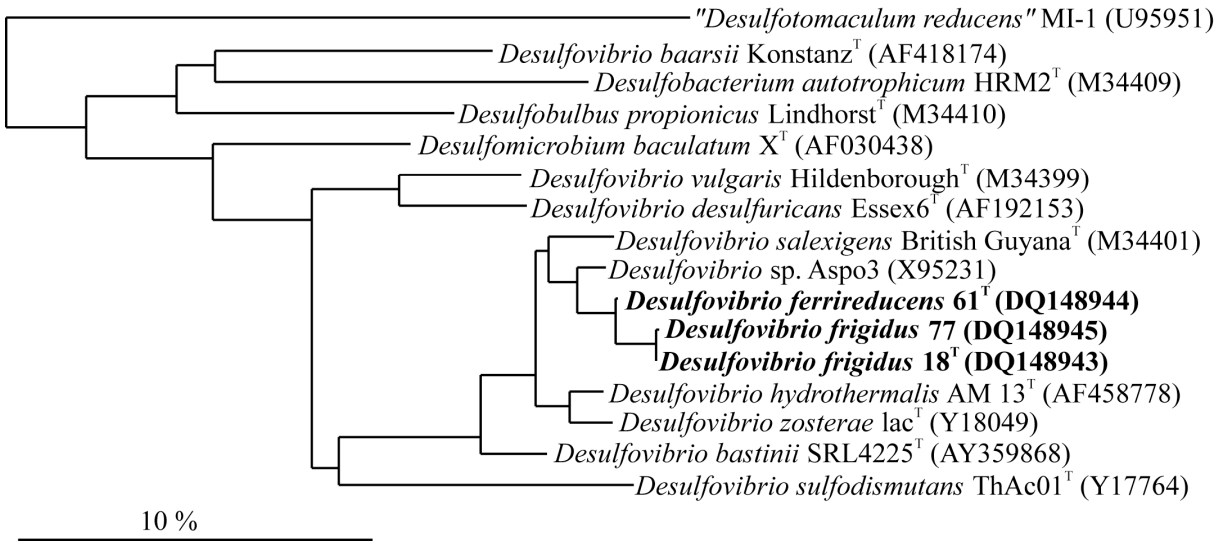

Fig. 2. The phylogenetic tree shows the position of the isolated strains 18^T, 61^T and 77 within the genus *Desulfovibrio* and to other sulfate-reducing bacteria with the ability to reduce Fe(III). The tree is based on 16S rDNA sequences and calculated by Maximum Likelihood algorithm with a 50%-filter for δ-*Proteobacteria*. The scale bar indicates 10% estimated sequence divergence.

The isolated strains and their closest relatives, *D. hydrothermalis*, *D. zosterae*, and *D. salexigens*, can be distinguished by their substrate usage and additionally by their temperature ranges of growth (Table 1). *D. hydrothermalis*, *D. zosterae*, and *D. salexigens* are mesophiles with temperature optima at 33-37°C (Postgate, 1984; Nielsen *et al.*, 1999; Alazard *et al.*, 2003), whereas strains 18^T, 61^T, and 77 are psychrotolerant (temperature optima at 20-23°C) and able to grow at –2°C, the freezing point of sea water. As the three strains were isolated from fjord sediments with temperatures of 2-3°C at the time of sampling, they are able to grow at the permanently low *in situ* temperature of Svalbard sediments.

Description of *Desulfovibrio ferrireducens* sp. nov.

Desulfovibrio ferrireducens (fer.ri.re.du'cens. L.n. *ferrum*, iron; L. part. adj. *reducens* leading back, bringing back, and in chemistry converting to a different oxidation state; N.L. part. adj. *ferrireducens,* reducing Fe(III) to Fe(II)). Cells are vibrioid or sigmoid, 2.5-5.5 x 0.7 µm, motile by a single polar flagellum. Gram-negative. No vitamins required for growth. *D. ferrireducens* oxidizes lactate, formate, hydrogen, ethanol, propanol, fumarate, and succinate with sulfate reduction. Sulfate, thiosulfate, and sulfite serve as electron acceptors. Iron compounds (Fe(III) oxide and Fe(III) citrate) are reduced without growth. Fermentative growth with malate, pyruvate, and fumarate. Optimum NaCl concentration is 1-2.5%, and growth occurs between 0.7-4% NaCl; for $MgCl_2$ the optimum concentration is between 0.02-2.5% and growth occurs up to a concentration of 3.5%. pH optimum is 7.1-7.5 and pH range is 6.3-7.5. Temperature optimum is 23°C and growth range is between –2 and 30°C. 42.0 mol% of G + C content of the DNA.

The type strain is 61^T (DSM 16995^T, JCM 12925^T), isolated from a permanently cold sediment of the west coast of Svalbard. The 16S rDNA sequence has been deposited in GenBank (DQ148944).

Description of *Desulfovibrio frigidus* sp. nov.

Desulfovibrio frigidus (fri'gi.dus. L. masc. adj. *frigidus*, cold, bacterium living in the permanently cold sediment of Svalbard). Cells are rod-shaped or vibrioid, 2-5 x 0.7 µm, motile by a single polar flagellum. Gram-negative. No vitamins required for growth. *D. frigidus* oxidizes lactate, formate, hydrogen, ethanol, fumarate, and alanine with sulfate reduction, one strain oxidizes propanol. Sulfate and sulfite serve as electron acceptors, one strain reduces elemental sulfur. Iron compounds (Fe(III) oxide and Fe(III) citrate) are reduced without growth. Fermentative growth with malate, pyruvate, fumarate, and glucose is possible

for one or the other strain. The growth range for NaCl and $MgCl_2$ is different for both strains, but the optimum NaCl concentration is 2-3%, and growth occurs at 2-3.5% NaCl; for $MgCl_2$ the optimum concentration is around 0.4% and growth occurs up to a concentration of 1.9%. pH optimum is 7.1 and pH range is 6.9-7.5. Temperature optimum is at 20-23°C and growth range is –2 to 25°C. 43.3 mol% of G + C content of the DNA.

Strains 18^T and 77 represent strains of the same species, *Desulfovibrio frigidus*, with strain 18^T (DSM 17176^T, JCM 12924^T) as type strain. Both were isolated from a permanently cold sediment at the west coast of Svalbard. The 16S rDNA sequences have been deposited in GenBank (strain 18^T, DQ148943; strain 77, DQ 148945).

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2.4

Low contribution of acetate and lactate to iron and sulfate reduction in Arctic sediments, Svalbard

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Sediments from an Arctic fjord, Svalbard, were incubated to determine iron and sulfate reduction rates and the contribution of acetate and lactate as electron donors for the terminal oxidation of organic matter. Sediment slurries from two depth intervals (Top 0-2 cm, Deep 5-9 cm) were incubated for 28 days and dissolved inorganic carbon (DIC) production, sulfate reduction, Fe^{2+} accumulation and volatile fatty acid (VFA) concentrations were monitored. In the Top layer 2/3 of the mineralization could be attributed to sulfate reduction and the remaining 1/3 to iron reduction. In the Deep layer sulfate reduction was the sole important process. Acetate and lactate turnover rate measurements with radiotracer incubations, revealed a combined contribution of only 15 and 42% to the anaerobic mineralization in the Top and Deep layer, respectively. Up to 6% of the iron reduction and a maximum of 22 and 43% of the sulfate reduction in the Top and Deep layer, respectively, could be attributed to combined acetate and lactate oxidation. Similar acetate oxidation rates as measured with the radiotracer incubations were obtained from the acetate accumulation after inhibition of sulfate reduction with selenate. Additionally, the accumulation of VFA's in the selenate inhibited slurries did not enhance the iron reduction. Thus, a minimum 94% of the iron reduction and 78 and 57% of the sulfate reduction in the Top and Deep layer, respectively, must be coupled to the oxidation of alternative electron donors.

Introduction

Anaerobic degradation of complex organic material in aquatic systems is a multi step process with several, physiologically specialized organisms involved (e.g. Blackburn, 1987; Capone & Kiene, 1988). The products of the fermentative bacteria serve as electron donors for the terminal oxidizing bacteria, that use inorganic electron acceptors for the complete oxidation of the organic matter. In marine sediments generally Fe(III) reduction and sulfate reduction are the most important respiratory processes in the anoxic zone (Thamdrup, 2000).

Microorganisms that reduce sulfate and iron may use a broad range of electron donors. They range from simple compounds such as hydrogen, short alcohols and volatile fatty acids (VFA), longer alcohols and VFA (Widdel, 1980), sugars (Coates *et al.*, 1998; Sass *et al.*, 2002; Kashefi *et al.*, 2003), amino acids (Stams *et al.*, 1985; Kashefi *et al.*, 2003) to aromatic (Widdel, 1980; Anderson *et al.*, 1998), and aliphatic hydrocarbons (Aeckersberg *et al.*, 1991).

These potential substrates give only little information on the substrates used *in situ* by the iron and sulfate reducers in their habitat. Investigations on the substrates for sulfate reducing bacteria in temperate marine sediments have been carried out using two methods. The substrate turnover can be measured by following the degradation of radiolabeled substrates (Christensen & Blackburn, 1982; Sansone, 1986; Wellsbury & Parkes, 1995; Wu *et al.*, 1997) or by measuring the accumulation of potential substrates after inhibition of the sulfate reduction by molybdate (Sørensen *et al.*, 1981; Fukui *et al.*, 1997). These investigations showed that VFA (acetate in particular) were the major substrates for sulfate reduction. Similar investigations for sediments dominated by iron reduction or with simultaneous iron and sulfate reduction are missing. Additionally, little is known about the substrates for sulfate reducers in permanently cold environments, which account for >90% of the ocean (Levitus & Boyer, 1994). Selenate can be used as specific inhibitor for sulfate reduction (Oremland & Capone, 1988). Bacterial populations of marine sediments are known to often carry the potential to reduce oxidized trace elements such as Se (e.g. Stolz & Oremland, 1999). Thus, the addition of selenate to marine sediments does not only serve to inhibit sulfate reduction (Oremland & Capone, 1988) but also induces selenate reduction (Steinberg & Oremland, 1990).

We investigated sediment samples from a permanently cold, Arctic sediment to reveal the relative contribution of iron reduction and sulfate reduction to terminal oxidation. Dissolved inorganic carbon (DIC) production, sulfate reduction, and VFA concentrations were monitored for 28 days of incubation. Combined VFA turnover measurements using radiotracer incubations and sulfate reduction inhibition studies using selenate were performed to determine the importance of acetate and lactate as electron donors for iron and sulfate reduction. To our knowledge, this is the first study investigating the contribution of VFA's as substrates for iron reducing bacteria in marine sediments.

Material and Methods

Sediment. Sediment samples were taken with a HAPS corer at Station J (79°42'006N 11°05'199E) in Smeerenburgfjorden on the northwest coast of Svalbard, Norway, in August 2004. The *in situ* temperature was 2.3°C, the water depth 212 m. The average HCl extractable Fe (Fe(II) + Fe(III)) content was 79.2 µmol cm^{-3} and the average dithionite extractable Mn content 0.7 µmol cm^{-3}. The nitrate concentrations in the bottom water were 12 µM (Vandieken *et al.*, in prep.).

Incubations. Sediment of the two depth intervals, 0-2 cm and 5-9 cm, was mixed with an equal amount of anoxic seawater, homogenized under N_2, and filled in two glass bottles each. A 1.4 M sodium selenate solution was added to one parallel for both depths to a final concentration of 5 mM. In previous experiments this concentration had proven to be sufficient to inhibit sulfate reduction in Svalbard sediments (data not shown). The slurries were incubated at 0°C for 28 days. After 1 hour, 1, 2, 3, 4, 7, 8, 10, 12, 14, 21, and 28 days subsamples were taken for sulfate reduction rate (SRR), pore water and solid phase analysis.

Pore water analyses. Pore water for the determination of concentrations of Fe^{2+}, Mn^{2+}, sulfide, sulfate, selenate, selenite, and $\sum CO_2$ was obtained by centrifuging triplicate sediment samples in glass centrifuge tubes without headspace for 10 minutes at 2500 *g* at 4°C. Pore water for volatile fatty acid (VFA) analysis was obtained by centrifugation in Spinex© (Phenomenex) filter units at 2500 *g* at 4°C for 10 minutes.

DIC was analyzed by flow injection with conductivity detection (Hall & Aller, 1992). Fe^{2+} was measured according to Stookey (1970) with Ferrozine (1 g l^{-1} in 50 mM HEPES buffer, pH 7) spectrophotometrically (Shimadzu UV 1202) at 562 nm. Mn^{2+} was measured by inductively coupled plasma atomic emission spectrometry (Perkin Elmer Optima 3300 RL). Sulfate was measured by non-suppressed ion chromatography (Waters, column IC-Pak™, 50 x 4.6 mm) (Ferdelman *et al.*, 1997). Sulfide was determined by the methylene blue spectrophotometric method (Cline, 1969) (Shimadzu, UV 1202) at 670 nm (detection limit 1 µM). To determine the selenate reduction rate as a result of selenate addition, selenate and selenite concentrations were analyzed by anion chromatography (Dionex DX500, eluent: 9 mM $NaCO_3$, precolumn: AG9 HC, column: AS9 HC). The detection limit is 2 µM for selenite and selenate.

Volatile fatty acids were measured after derivatization with n-phenylhydrazin by absorbance at 400 nm on an HPLC (Albert & Martens, 1997). The other VFA shown in Figure 5 are calculated based on their molar C-bases standardized to acetate. The concentration is multiplied with the number of C-atoms and divided by C- atoms in acetate.

Sulfate reduction rates (SRR). At each time point duplicate subsamples of the slurries were incubated with 50 kBq ^{35}S-sulfate. After 6 h the incubations were stopped with 20% ZnAc. The reduced sulfur fraction was extracted by the cold chromium distillation (Kallmeyer *et al.*, 2004). SRR was calculated as described by Jørgensen (1978).

Volatile fatty acid (VFA) turnover rates. After 1, 4, 8, and 14 days of incubation subsamples of the slurries were taken to measure VFA turnover rates. Approximately 10 ml slurry was filled into N_2 flushed syringes. 300 kBq $^{14}C_2$-acetate or $^{14}C_u$-lactate were injected into triplicate syringes for each slurry. After 10, 20, 30, 40, 50, and 60 minutes approximately 1.5 ml of the sample was withdrawn into 5 ml 2% NaOH. This served to stop the reaction and fix the ^{14}C-DIC produced. Blank samples were prepared by addition of the tracer to the NaOH previous to addition of the sediment. ^{14}C-TIC and ^{14}C-acetate were separated by the shaker method (Joye *et al.*, 2004). In brief, 6 M HCl was added to the sample to drive out the DIC as CO_2, which was trapped in phenylethylamine/NaOH. The trapped ^{14}C-DIC and remaining ^{14}C in the sample were measured by scintillation counting. The turnover rate constant was determined as the slope of the fraction of tracer turned over per time. Multiplication with the concentration yielded the turnover rate.

Acetate oxidation rates were calculated for the uninhibited slurries from the acetate accumulation. The accumulation rates of the uninhibited slurries were subtracted from the rates of the inhibited slurries giving the maximum acetate oxidized by sulfate reduction in the uninhibited slurries.

Results

Pore water chemistry. Dissolved inorganic carbon (DIC) concentrations showed a steady increase with time (Fig. 1). From this increase mineralization rates of organic carbon were calculated (Fig. 2). The DIC production for both depths was approximately twice as high for the uninhibited compared to the selenate inhibited slurry, and twice as high for the Top (0-2 cm) compared to the Deep (5-9 cm) slurry. Fe^{2+} accumulation in the pore water of the slurries was linear over the duration of the incubation. The accumulation rates were 7 nmol cm^{-3} d^{-1} for the Top and 0.5 nmol cm^{-3} d^{-1} for the Deep slurries with no significant differences between the inhibited and uninhibited slurries. The rates presented in Table 1 are based on a stoichiometry of 4 Fe(III) reduced for every DIC produced. Mn^{2+} and sulfate concentrations stayed constant over time in all slurries. Sulfide concentrations stayed below detection limit throughout the incubation.

Sulfate reduction rates (SRR) in the Top slurry varied between 48 and 70 nmol cm^{-3} d^{-1} with rather constant rates after an initial increase (Fig. 3). In the Deep slurry the SRR was 18 nmol cm^{-3} d^{-1} right after preparation of the slurry, increased to 62–70 nmol cm^{-3} d^{-1} on day 1 through 4, and then decreased to values between 18 and 31 nmol cm^{-3} d^{-1}. Addition of ~5 mM

selenate led to a decrease of SRR below the detection limit of 2 nmol cm^{-3} d^{-1} in both depth intervals.

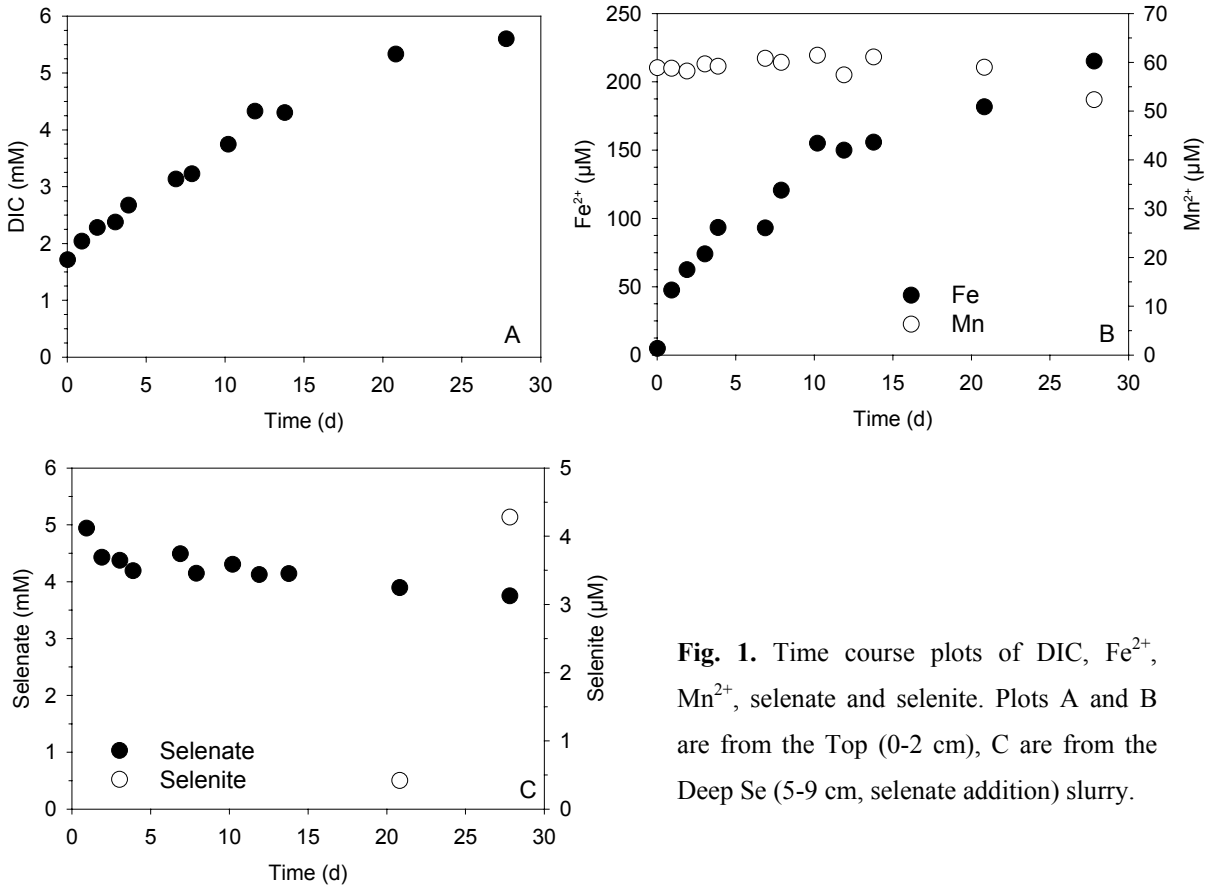

Fig. 1. Time course plots of DIC, Fe^{2+}, Mn^{2+}, selenate and selenite. Plots A and B are from the Top (0-2 cm), C are from the Deep Se (5-9 cm, selenate addition) slurry.

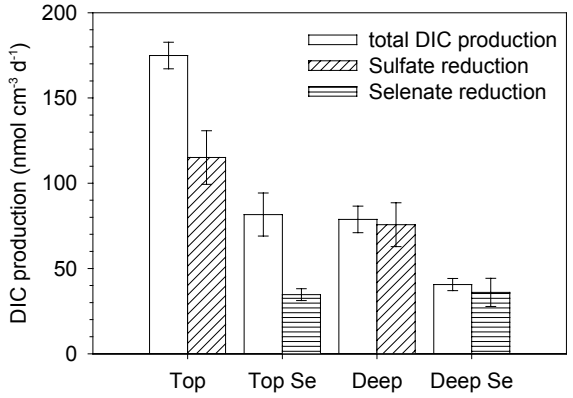

Fig. 2. Dissolved inorganic carbon (DIC) production in the Top (0-2 cm), Top Se (0-2 cm, selenate addition), Deep (5-9 cm), and Deep Se (5-9 cm, selenate addition) slurries. DIC production is shown as anaerobic DIC production as calculated from DIC accumulation and calculated based on sulfate reduction and selenate reduction.

Volatile fatty acid (VFA) concentrations showed an increase in all slurries at the beginning of the experiment (Fig. 4). In all samples concentrations of acetate were highest followed by propionate and isobutyrate. Lactate concentrations remained low (around 1-2 µM) throughout the incubation. In the uninhibited slurries the acetate concentrations increased from 45 µM to 78 µM after 2 days for the Top and to 93 µM after 3 days for the Deep slurry followed by a

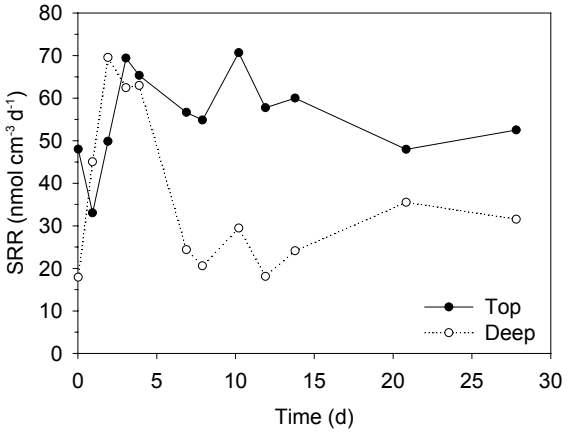

Fig. 3. Sulfate reduction rates (SRR) measured in the uninhibited Top (0-2 cm) and Deep (5-9 cm) slurries. In the selenate amended slurries the rates were below the detection limit of 2 nmol cm^{-3} d^{-1}.

decrease to 3-8 µM in both. In the Top slurry the concentrations showed a second transient increase with maximum concentrations after 12 days. In the inhibited Top (Top Se) slurry the VFA concentrations increased throughout the entire incubation reaching 680, 60, and 22 µM for acetate, propionate, and isobutyrate, respectively. In the inhibited Deep (Deep Se) slurry VFA concentrations increased for the first 12 days, reaching 335, 18, 7.5 µM for acetate, propionate, and isobutyrate, respectively, and decreased towards the end of the incubation.

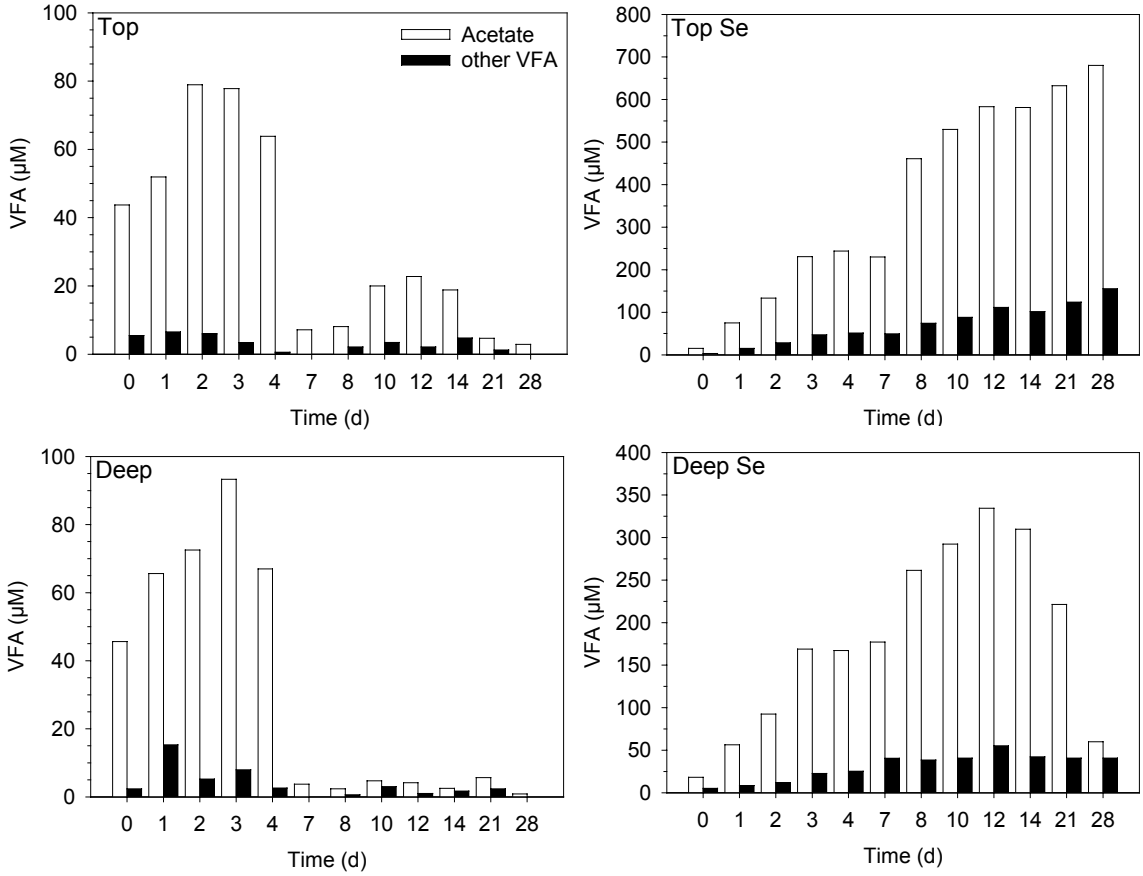

Fig. 4. Volatile fatty acids (VFA) concentrations measured in the pore water of the Top (0-2 cm), Top Se (0-2 cm, selenate addition), Deep (5-9 cm), and Deep Se (5-9 cm, selenate addition) slurries. Other VFA concentrations were calculated as described in the method section. Note the different scales of the y-axis.

^{14}C-acetate and -lactate turnover rates were measured after 1, 4, 8, and 14 days of incubation (Fig. 5). Turnover rate constants for lactate were generally 3- to 15-fold higher than for acetate, but due to the higher concentrations of acetate the respective turnover rates were higher than for lactate. In the uninhibited slurries the turnover rate of acetate was 1.5- to 9-fold higher than the lactate turnover rate, in the inhibited slurries 3- to 120-fold higher. The turnover rates calculated as mol DIC produced during VFA oxidation are shown in Figure 5. After 1 and 4 days the combined acetate and lactate turnover rate was about 6-fold higher in the uninhibited compared to the inhibited slurries. Together with the steady increase in VFA concentrations the acetate turnover rate in the Top Se slurry increased during the whole incubation. For the uninhibited slurries the rate decreased after 4 days again parallel to decreasing VFA concentrations. In the Deep Se slurry the rates only changed slightly for the last three measurements. The lactate turnover rates were generally 2- to 10-fold higher in the uninhibited compared to the inhibited slurries. The rates did not show a clear trend with time, which is in agreement with stable lactate concentrations in the slurry. Acetate oxidized by sulfate reduction was determined from the accumulation in the inhibited versus uninhibited slurry. The oxidation rates are 14 and 38 nmol C cm^{-3} d^{-1} in the Top and Deep slurry, respectively, similar to the rates determined by radiotracer incubations (Fig. 5)

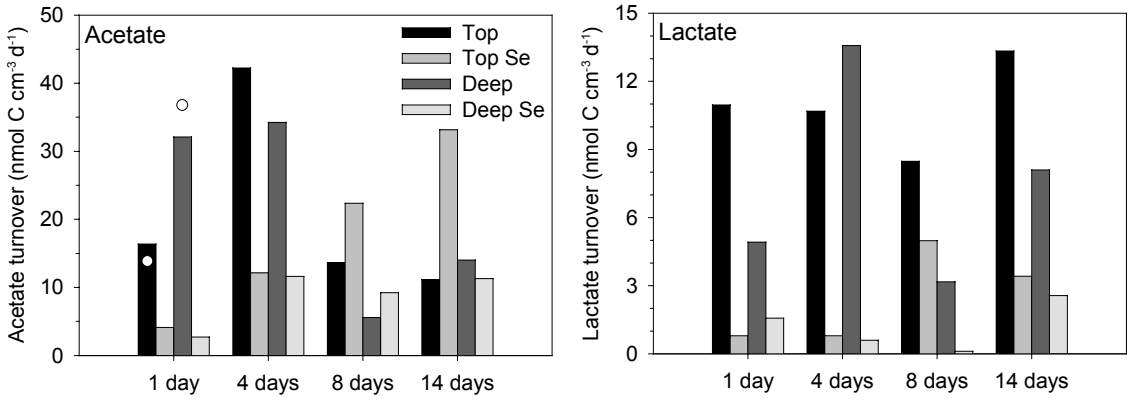

Fig. 5. ^{14}C-acetate and ^{14}C-lactate turnover rates measured in the Top (0-2 cm), Top Se (0-2 cm, selenate addition), Deep (5-9 cm), and Deep Se (5-9 cm, selenate addition) slurries. The rates are given in mol C produced during acid oxidation. The open symbols in the acetate turnover graph represent the rates calculated from the difference in the acetate accumulation in the inhibited compared to the uninhibited slurries.

Selenate addition inhibited sulfate reduction and allowed VFA analysis. Molybdate, the commonly used inhibitor of sulfate reduction, complexes VFA's (Rosenheim, 1893; Finke, 1999), thus preventing their determination with the n-phenylhydrazin derivatization technique

(Mueller Harvey & Parkes, 1987; Albert & Martens, 1997). Selenate can be microbially reduced to selenite, Se°, or selenide (Stolz & Oremland, 1999; Lloyd *et al.*, 2001). In culture and in sediment studies selenate is usually reduced to selenite or Se° (Majers *et al.*, 1988; Steinberg & Oremland, 1990; Oremland *et al.*, 1994; Stolz & Oremland, 1999; Herbel *et al.*, 2000; Lucas & Hollibaugh, 2001; Knight *et al.*, 2002). The addition of selenate resulted in selenate reduction, which was reflected in decreasing selenate concentrations. The measured potential selenate reduction rates were 23 and 25 nmol cm^{-3} d^{-1} for the Top and Deep slurry, respectively (Fig. 2). In our study selenite was only detected in the Deep slurry after 20 days of incubation. The concentrations increased towards the end of the incubation to 4 µM. Due to the low or missing selenite production and based on the literature available, a reduction of the selenate to elemental selenium was assumed. The contribution of dissimilatory selenate reduction to DIC production was based on a 2:3 stoichiometry of selenate:DIC.

Comparing the DIC production in the uninhibited and inhibited slurries the only two major effects of the selenate addition seem to be the inhibition of sulfate reduction and initiation of selenate reduction. A general toxic effect of the high selenate concentrations to the microbial community was not seen, as the time course of the DIC, Fe^{2+}, and Mn^{2+} were the same in the inhibited and uninhibited slurry.

Discussion

Mineralization of organic matter (measured as dissolved inorganic carbon (DIC) production) in the uninhibited slurries was about twice as high in the Top (0-2 cm) as in the Deep (5-9 cm) interval. This is probably due to the higher reactivity of the freshly deposited organic material at the surface compared to the sediment in the deep interval. The sulfate reduction rate was approximately 50% higher in the Top compared to the Deep slurry. The initial increase in sulfate reduction in the Top slurry was probably due to the observed increase in volatile fatty acids (VFA) concentrations (Fig. 4) and indicates an electron donor limitation for sulfate reduction. Iron reduction rates were calculated from the measured DIC production and sulfate reduction rates according to Canfield *et al.* (1993a) (Table 1), and are referred to as calculated iron reduction rates. For this the DIC production from sulfate reduction (assuming a stoichiometry of 2 moles carbon oxidized per mole of sulfate reduced (Thamdrup & Canfield, 1996)) is subtracted from the measured DIC production. The remaining DIC production is attributed to Fe reduction (Table 1) (Canfield *et al.*, 1993b) as alternative electron acceptor concentrations such as solid Mn and nitrate concentrations of Smeerenburgfjorden sediment were low (Vandieken *et al.*, in prep.). The sulfate reduction

rates were similar to previous investigations at the same site, but DIC production rates are about 30% lower resulting in a lower calculated contribution of iron reduction to the total mineralization (Vandieken *et al.*, in prep.). In the previous investigation bag incubations with homogenized sediments were used instead of slurry incubations in this study. One possible explanation is that the dilution of the sediment resulted in an increasing distance of the iron reducers to the solid iron oxides, reducing their activity.

Table 1. DIC production in the Top (0-2 cm), Top Se (0-2 cm, selenate addition), Deep (5-9 cm), and Deep Se (5-9 cm, selenate addition) slurries. Contribution of the different electron accepting processes to the production of dissolved inorganic matter (DIC) calculated on a C-molar basis (nmol C cm^{-3} d^{-1}).

	Total DIC production	**Sulfate reduction**	**Selenate reduction**	**Calculated iron reduction**		**Iron accumulation**
Top	175 ± 7.8	115 ± 16	-	60 ± 23.8	34% [a]	1.7 ± 0.20
Top Se	82 ± 13	< 4	35 ± 3.4	47 ± 16.4	57% [a]	1.9 ± 0.10
Deep	79 ± 7.8	76 ± 13	-	3 ± 20.8	-	0.16 ± 0.03
Deep Se	41 ± 3.5	< 4	38 ± 8.3	3 ± 11.8	-	0.11 ± 0.09

[a] Relative contribution of iron reduction to DIC production

The addition of ~5 mM selenate was sufficient to inhibit sulfate reduction in the two sediments. Concurrently the mineralization of organic matter, measured as DIC production, decreased (Fig. 2, Table 1). The decrease in DIC production was, however, smaller than the DIC production from sulfate reduction in the uninhibited slurry (Table 1). Taking DIC produced from selenate reduction into account, the calculated iron reduction rates in the inhibited slurries were not statistically different from the uninhibited slurries. In the Deep slurry the calculated Fe reduction rates of 3 nmol cm^{-3} d^{-1} are less than the error of the method (Table 1). The increase in dissolved Fe^{2+} was similar in the inhibited and uninhibited slurries, but about 30-fold lower compared to the calculated production rates (Table 1). Similar results were found in earlier studies on iron reduction in marine sediments (Canfield *et al.*, 1993a). Inhibition of sulfide production from sulfate reduction did not lead to a faster increase in dissolved Fe^{2+} indicating that adsorption rather than precipitation as FeS is the main sink for iron from the pore water. Alternatively, the lack of sulfide production could have resulted in a decrease in chemical iron reduction and stopped the precipitation of FeS. As the Fe^{2+} accumulation is the same in the inhibited and uninhibited slurries, the effect on the chemical iron reduction and the FeS precipitation must have been very similar. Several previous studies using molybdate to inhibit sulfate reduction also did not result an increased dissolved Fe^{2+}

accumulation in marine sediments (Sørensen, 1982; Canfield *et al.*, 1993a) or in enrichment cultures (Tugel *et al.*, 1986).

Volatile fatty acids (VFA) are fermentation products and serve as important substrates for terminal oxidation (Sørensen *et al.*, 1981; Christensen & Blackburn, 1982; Wellsbury & Parkes, 1995). The increase of the VFA after the mixing of the sediment (Fig. 4) showed that terminal oxidation could no longer balance the fermentation rate and has been reported before (Finke, 1999; Finke, 2002; Arnosti *et al.*, 2005). This is probably caused by an enhancement of the fermentation due to the mixing and not by a decrease in terminal oxidation as seen in the steady increase in DIC independent of increasing or decreasing VFA concentrations. In the uninhibited slurries the concentrations of VFA decreased again after approximately 3 days of incubation.

In the selenate slurries, sulfate reduction was inhibited leading to a longer period of increasing VFA concentrations. In the Deep slurry the selenate reduction, initiated by the selenate addition, was sufficient to lower the VFA concentrations again towards the end of the incubation (Fig. 4). In the Top slurry the initial microbial activity was twice as high (determined as DIC production), whereas the potential selenate reduction was similar to the Deep slurry. Thus, the selenate reduction was not sufficient to compensate for the lack of sulfate reduction resulting in an increase in VFA concentrations throughout the experiment.
The increased VFA concentrations as a result of the selenate inhibition did not result in a stimulation of the iron reduction. Thus, the iron reducing bacteria were apparently not electron donor limited or they used other electron donors than VFA.

VFA turnover rates in the uninhibited Top and Deep slurries after 1 day of incubation accounted for only about 15 and 42% of the DIC production, respectively. The difference between the initial acetate accumulation rates in the selenate inhibited and the uninhibited slurries were similar to the turnover rates measured after one day in the uninhibited slurries (Fig. 5), indicating that most of the acetate is utilized by sulfate reducers. Additionally, the measurement of the same rate of acetate oxidation with the tracer turnover measurements and the selenate inhibition strengthen the validity of the measured rates. The maximum contribution of VFA to sulfate reduction were 22 and 43% for the Top and Deep slurry, respectively. Alternative electron donors must have been responsible for the remaining 78 and 57% of sulfate reduction in these sediments. Previous investigations of temperate sediments showed that VFA's were the most important electron donors for sulfate reduction (Christensen & Blackburn, 1982; Skyring, 1988; Parkes *et al.*, 1989). Earlier studies in

permanently cold sediments from Svalbard, however, indicated a lower contribution of VFA to the terminal oxidation (Finke, 2002). Detailed investigations of potential alternative electron donors such as amino acids, sugars, or alcohols are necessary to solve this problem.

Initial turnover rates of VFA in the selenate inhibited samples were 5- and 15-fold lower than in the uninhibited Top and Deep slurries and accounted for 3 and 2% of the DIC production, respectively. This was due to the inhibition of the sulfate reduction, the major sink for the VFA. A maximum contribution of the VFA turnover to iron reduction can be calculated assuming iron reduction to be the only sink for VFA's in the inhibited slurries. For the Top slurry a maximum contribution of 6% of the iron reduction can be coupled to the oxidation of acetate and lactate. In the Top slurry the turnover rate of acetate increased with increasing VFA concentrations indicating an electron donor limitation, whereas in the Deep slurry the rate increased from day 1 to day 4 and stayed constant thereafter. This might be due to a lag phase in the selenate reduction of several hours although that was not obvious from the measurements of the selenate concentrations. The stronger reduction in the lactate turnover in the inhibited slurries compared to the acetate turnover indicates that lactate is mainly oxidized by sulfate-reducing bacteria.

Potential selenate reduction was previously detected in sediments ranging from polluted sites with measurable concentrations in the porewater (up to 144 nM selenite) to pristine sites (Steinberg & Oremland, 1990; Lucas & Hollibaugh, 2001). Dissimilatory selenate-reducing bacteria are widespread throughout the bacterial domain (Stolz & Oremland, 1999; Lucas & Hollibaugh, 2001). Selenate might be reduced as alternative substrate by nitrate reductases, but the specific activity is much higher for dedicated selenate reductases (Watts *et al.*, 2005). Some sulfate-reducing bacteria can reduce selenate without growth in the presence sulfate (Lloyd *et al.*, 2001).

The selenate reduction in the Smeerenburgfjorden sediments did not show a lag phase (of more than several hours). Due to the missing lag phase and the low temperatures we exclude growth of selenate reducing bacteria over the time course of our experiments. Previous investigation on sulfate reducing bacteria showed no growth after substrate addition in permanently cold sediments from Svalbard within two weeks of incubation (M. Mußmann, unpublished data). Previous investigations in pristine temperate sediments showed a lag phase of 2 to 6 days (Lucas & Hollibaugh, 2001). The formation of selenite in the deep slurry, however, did show a lag phase of approximately 20 days (Fig. 1). In culture studies selenite production showed no or only a short (several hours) lag phase (Majers *et al.*, 1988;

Oremland *et al.*, 1994; Knight *et al.*, 2002). A possible explanation for the long lag phase for the selenite production is the presence of two different populations of selenate reducers in the Deep slurry. Bacteria, capable of reducing selenate to elemental selenium or selenide were present and active in the sediment, living on an alternative metabolism for example nitrate reduction. Whereas bacteria reducing selenate to selenite were present in the sediment, but were either initially inactive, or the change of the metabolism required substantial time. The measured potential selenate reduction rates of 23 and 25 nmol cm^{-3} d^{-1} lie in the middle of the reported range of potential selenate reduction rates in pristine and contaminated sediments of 1.7 to 530 nmol cm^{-3} d^{-1} (Steinberg & Oremland, 1990; Lucas & Hollibaugh, 2001).

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2.5

Carbon mineralization in Arctic sediments northeast of Svalbard: Mn(IV) and Fe(III) reduction as most important anaerobic respiratory pathways

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Carbon oxidation rates and pathways were determined in three sediments at 79°-81° north in the Barents Sea where the ice cover restricts primary production to a few months in the year. Oxygen uptake rates (1.5-3.5 mmol m^{-2} d^{-1}) and sulfate reduction rates (<0.2-0.46 mmol m^{-2} d^{-1}) were measured by whole core incubations. Pathways of anaerobic carbon oxidation were determined by combining results of anaerobic sediment incubations with pore water and solid phase analyses of the sediments. In accordance with the high contents of solid Mn (≥60 µmol cm^{-3}) and Fe(III) (≥108 µmol cm^{-3}), dissimilatory Mn(IV) and Fe(III) reduction contributed between 69 and at least 90% to the anaerobic carbon mineralization. At two of the three stations, sulfate reduction rates were below our detection limit of 1 nmol cm^{-3} d^{-1}. Solid Mn and Fe(III) were abundant from the surface to 10 cm sediment depth and probably were the only important anaerobic electron acceptors. At the third station a typical vertical zonation of anaerobic mineralization was observed, with Mn(IV) reduction at 0-3 cm followed by concurrent Fe(III) and sulfate reduction at 3-5 cm and sulfate reduction at 5-10 cm. *In situ* Mn(IV) and Fe(III) reduction have to compete with oxic respiration as calculations and the distribution of pore water constituents indicated oxygen penetration depths of 1-4 cm into the sediments. Rates of microbial carbon oxidation were found to be lower compared to studies from fjords of the west and south coast. This is in accordance with limited organic carbon supply by primary and secondary productivity caused by long periods of ice coverage, meaning that the benthic microbial community is primarily limited by carbon availability.

Introduction

Benthic microbial communities in sediments around Svalbard in the Arctic Ocean experience permanently low temperature whereas the flux of organic matter to the sea floor varies seasonally. Phytoplankton productivity depends here on the short ice-free periods in the summer when light is available for photosynthesis (Hebbeln & Wefer, 1991; Hebbeln & Berner, 1993; Wassmann & Slagstad, 1993). The west and south coast of Svalbard are influenced by the relatively warm Atlantic water of the West Spitsbergen Current that flows northwards and keeps the coast ice-free most of the year. The north and east coast are characterized by cold polar water from the Arctic Ocean resulting in a later seasonal thawing of the sea ice. Correspondingly, primary production was found to be significantly lower in

areas influenced by polar waters and long periods of ice coverage than in areas influenced by Atlantic water (Wassmann & Slagstad, 1993; Sakshaug, 1997). The differences in primary production are reflected in higher C_{org} contents and oxygen uptake rates of sediments southwest of Svalbard compared to sediments off the northeast coast (Hulth *et al.*; 1994; Hulth *et al.*, 1996).

The fjord sediments along the south and west coast of Svalbard are characterized by relatively high organic carbon deposition (Eilertsen *et al.*, 1989) and mineralization rates are as high as in comparable temperate environments (Arnosti *et al.*, 1998; Sagemann *et al.*, 1998; Thamdrup & Fleischer, 1998; Kostka *et al.*, 1999; Arnosti *et al.*, 2005). In these sediments, as well as in sediments at the east coast of Greenland, dissimilatory Fe(III) and sulfate reduction were found to be important anaerobic pathways of carbon oxidation (Rysgaard *et al.*, 1998; Kostka *et al.*, 1999; Glud *et al.*, 2000). Studies from a wide range of coastal marine sediments have shown that Fe reduction is an important carbon oxidation pathway whereas microbial Mn reduction is in general insignificant due to low Mn oxide concentrations and shallow depth distribution of Mn(IV) (Thamdrup, 2000). As an exception, dissimilatory Mn reduction was found to be quantitatively important for benthic carbon mineralization in sediments of Skagerrak, the Panama Basin, and the Black Sea due to high Mn oxide contents and high rates of bioturbation (Aller, 1990; Canfield *et al.*, 1993a; Canfield *et al.*, 1993b; Thamdrup *et al.*, 2000).

We studied for the first time pathways of microbial respiration in Arctic sediments northeast of Svalbard**.** The sediments had high concentrations of particulate Mn and Fe(III) and are exposed to low organic carbon deposition. Rates of Mn, Fe, and sulfate reduction were determined in relation to the distribution Mn and Fe in the solid phase. The present study shows that besides aerobic respiration, Mn(IV) and Fe(III) reduction were most important for carbon mineralization.

Material and Methods

Sampling sites. This study was part of the Norwegian CABANERA project (Carbon flux and ecosystem feed back in the northern Barents Sea in an era of climate change). Sediments were sampled during the second CABANERA cruise from 20 July to 3 August 2004 on board RV "Jan Mayen" off the northeast coast of Nordaustlandet. An overview of the location and characteristics of the benthic stations are given in Figure 1 and Table 1. Stations VIII and XII were located in trenches and were ice-free at the time of sampling. Station X was positioned in an area with melting ice floes and was coupled to a pelagic sampling station in

the CABANERA project. The sediments of stations VIII and X were sampled with a multiple corer that retrieved up to 4 cores with 10 cm inner diameter. At station XII sediment was subsampled from a 50 cm x 50 cm box core into the same cores.

Fig. 1. Chart of sampling stations northeast of Svalbard.

Anoxic bag incubations. Sediment from the upper 10 cm of the cores was sliced into the following depth intervals: Station VIII 0-3 and 6-12 cm, stations X and XII 0-1, 1-2, 2-3, 3-4, 4-5, 5-7, and 7-10 cm. Sediment from the same depth interval of several cores was filled under a constant stream of N_2 into gastight plastic bags (Hansen *et al.*, 2000) outdoors at an air temperature around 0°C. The incubation bags were closed without gas phase and incubated near *in situ* temperature at 0°C inside larger N_2 filled plastic bags to ensure anoxia. Ten times over 55-60 days of incubation subsamples were withdrawn in a cold room at 4°C.

Pore water and solid phase sampling. Pore water of whole cores and pore water from the bags were retrieved by a pore water press under N_2 through GF/F filters. Pore water was filtered directly into Ferrozine-solution to measure Fe^{2+} (see below). 1.8 ml aliquots for dissolved inorganic carbon (DIC) and alkalinity analyses were collected in glass vials without headspace capped with Viton septa, fixed with $HgCl_2$ and stored at 4°C until analysis in Bremen. 1.5 ml of pore water was frozen for NH_4^+, NO_3^-, and NO_2^- analysis. For Mn^{2+} and Ca^{2+} determination 0.5 ml was acidified with 6 M HCl and stored at 4°C. Pore water for sulfate and sulfide analyses was preserved with Zn acetate or $ZnCl_2$. Bottom water of the stations was stored frozen for determination of NH_4^+, NO_3^-, and NO_2^- and for Mn^{2+} analysis acidified with HCl.

For the extraction of solid phase Fe and Mn with dithionite and for the determination of total organic C and N subsamples were stored frozen at –21°C. For the analysis of elemental sulfur a subsample of 0.5-2 g sediment was mixed with 2 ml 20% Zn acetate and stored frozen at –21°C.

Pore water analyses. DIC was analyzed by flow injection with conductivity detection (Hall & Aller, 1992). Fe(II) was measured spectrophotometrically according to Stookey (1970) with Ferrozine (1 g l^{-1} in 50 mM HEPES buffer, pH 7) at 562 nm (Shimadzu UV 1202). NH_4^+ was determined spectrophotometrically at 630 nm (Shimadzu UV 1202) (Grasshoff *et al.*, 1999). NO_3^- and NO_2^- were measured using a NO_x-Analyzer (Thermo Environmental Instruments, Franklin, USA) (Braman & Hendrix, 1989). Ca^{2+} and Mn^{2+} in pore water were measured by inductively coupled plasma atomic emission spectrometry (Perkin Elmer Optima 3300 RL). Sulfate was measured by non-suppressed ion chromatography (Waters, column IC-Pak™, 50 x 4.6 mm) (Ferdelman *et al.*, 1997). Sulfide was determined by the methylene blue spectrophotometric method at 670 nm (Shimadzu, UV 1202) (Cline, 1969). Alkalinity was determined by Gran titration using 0.02 M HCl.

Solid phase analyses. Fe was extracted by HCl (0.5 M HCl for 1 h) and the extract analyzed for Fe(II) with Ferrozine and for total Fe with Ferrozine plus 1% (w/v) hydroxylamine hydrochloride. Fe(III) concentrations were calculated by subtracting concentrations of Fe(II) from total Fe concentrations. Solid Mn in the sediment was quantified after freeze drying and extraction with dithionite-citrate-acetic acid (Canfield, 1989) by flame atomic absorption spectrometry (Perkin Elmer, Atomic Absorption Spectrometer 3110). Freeze dried samples for determination of total organic C and N contents were pretreated with HCl, dried again and analyzed using a CNS analyzer (Fisons™ Na1500 elemental analyzer). Elemental sulfur was analyzed according to Zopfi *et al.* (2004). A subsample of the sediment frozen in Zn acetate for elemental sulfur analysis was extracted with 5 ml methanol. The sulfur was separated by HPLC with a Zorbax ODS column (125 x 4 mm, 5 µm; Knauer, Germany) with methanol as eluent at a flow of 1 ml min^{-1}. The sulfur was detected from the absorption at 265 nm with a detection limit of 1 µM.

Sulfate reduction rates. Sulfate reduction rates were measured in three parallel cores of 3 cm diameter using the $^{35}SO_4^{2-}$ whole core injection technique (Jørgensen, 1978). Sulfate reduction of the anoxic bags was determined at every sampling time point in subsamples incubated with 100 kBq $^{35}SO_4^{2-}$ radiotracer in 5 ml glass tubes. The incubations were stopped after 6 h with 20% Zn acetate. Total reduced inorganic sulfur was analyzed by a cold chromium distillation (Kallmeyer *et al.*, 2004).

Oxygen consumption rates. Sediment cores with an inner diameter of 54 mm were closed without gas phase with rubber stoppers. The cores were incubated in the dark at 0°C with continuous stirring of the water column by a magnetic stirring bar placed at the top of the water column. Oxygen consumption of the sediment was measured during the whole incubation with a micro-optode (Holst *et al.*, 1997; Klimant *et al.*, 1997). The volume of the water column was determined by addition of a NaBr solution. Concentrations of NaBr were analyzed by anion chromatography (Dionex DX500, eluent: 9 mM $NaCO_3$, precolumn: AG9 HC, column: AS9 HC). Total oxygen consumption rates were calculated from duplicate cores for station VIII, and triplicates for stations X and XII.

Calculations. The precipitation of $CaCO_3$ during bag incubation was calculated according to Thamdrup *et al.* (2000) from decreasing Ca^{2+} concentrations: $\Delta CaCO_3 = \Delta[Ca^{2+}]_{sol} (1+K_{Ca})$, where K_{Ca} is the adsorption constant for Ca^{2+} (K_{Ca} = 1.6) (Li & Gregory, 1974). The production of DIC was calculated as follows: DIC production = DIC accumulation + $CaCO_3$ precipitation.

The saturation of pore waters by rhodocrocite was calculated with the program PHREEQC using the thermodynamic constants of the database (Parkhurst, 1995). Measured alkalinity and concentrations of DIC, Ca^{2+}, and Mn^{2+} were included in the calculations.

The penetration depth of oxygen, h, was estimated according to Revsbech *et al.* (1980): $h = 2D_S C_0 \phi / J$, where D_S is the diffusion coefficient in the sediment, C_0 is the oxygen concentration at the sediment surface, ϕ is the porosity, and J is the oxygen uptake rate. D_S was calculated according to Iversen & Jørgensen (1993): $D_S = D_0/(1+3(1-\phi))$, where D_0, the diffusion coefficient in seawater, was taken from Schulz & Zabel (2000): $D_0(O_2)$ = 1.25 10^{-9} m^2 s^{-1} (at 0°C).

Results

Pore water and solid phase chemistry. The total organic nitrogen and carbon contents of the sediments were similar for the three stations with 0.17-0.18% organic nitrogen and 1.41-1.46% organic carbon (Table 1). At all stations the sulfide concentration in the pore water was below the detection limit of 1 µM and the sulfate concentration stayed constant over 0-20 cm depth. Dissolved inorganic carbon (DIC) and NH_4^+ concentrations increased with depth from the bottom water into the sediment at all three stations (Figs. 2A, 2D, & 2G). In the following, we will present data for NO_3^-, NO_2^-, Mn^{2+}, Fe^{2+}, and solid Mn and Fe from each station individually and highlight differences between the stations.

Table 1. Sample site information and sediment characteristics

Station	VIII	X	XII
Position	northern Kvitøya trench	north of Kong Karls Land	central Kvitøya trench
Latitude	81°16.65' N	79°26.50' N	80°09' N
Longitude	26°51.18' E	28°48.43' E	29°36' E
Water depth (m)	503	303	286
Bottom water temperature (°C)	2.9	2.5	2.3
Total organic carbon (%)	1.46	1.41	1.45
Total organic nitrogen (%)	0.17	0.17	0.18
Oxygen consumption rates (mmol m^{-2} d^{-1})	2.1 ± 0.7	1.5 ± 0.4	3.5 ± 1.6
Sulfate reduction rates (0-19 cm) (mmol m^{-2} d^{-1})	<0.2	<0.2	0.46 ± 0.13

Station VIII. The bottom water concentrations of nitrate and nitrite were 17 μM and 0.7 μM, respectively, whereas highest concentrations of nitrate were measured in the pore water at 0-1 cm depth (Fig. 2B). Below 6 cm where nitrate concentrations decreased to a background level of <2 μM, Mn^{2+} concentrations increased to a maximum at 12-19 cm (Fig. 2B). Fe^{2+} concentrations in the pore water increased with depth, similar to Mn^{2+}, but stayed low (<20 μM) (Fig. 2B). The extractable Mn and Fe(III) contents of the sediment were low in the top 3 cm but increased with depth (Fig. 2C). The Fe(III) content was highest at 6 cm and was depleted at 12 cm depth. Mn was present from the surface to 21 cm depth with a maximum concentration of 65 μmol cm^{-3} at 8 cm.

Station X. The nitrate and nitrite concentrations of the bottom water were 17 μM and 0.4 μM, respectively. Nitrate was depleted at 3 cm sediment depth below which Mn^{2+} increased to a maximum at 4-19 cm (Fig. 2E). Concentrations of soluble Fe^{2+} were mostly below detection (Fig. 2E). Solid Mn and Fe(III) had high concentrations in the surface sediment (up to 108 μmol cm^{-3}) and were depleted at 8 cm depth (Fig. 2F).

Station XII. The nitrate concentration in the bottom water was 5 μM and the nitrite concentration 0.4 μM. The maximum nitrate concentration was 12 μM at 0-2 cm depth (Fig. 2H). While nitrate was depleted below 3 cm, Mn^{2+} concentrations in the pore water increased below 1 cm to a maximum at 4-5 cm depth (Fig. 2H). Similar to Mn^{2+}, Fe^{2+} concentrations peaked at 3-6 cm (Fig. 2H). Extractable Mn and Fe(III) were enriched in the surface sediment, similar to station X. The maximum Mn concentration of 61 μmol cm^{-3} was measured at 1-2 cm depth and Mn was depleted below 3 cm (Fig. 2I). Fe(III) penetrated to 5 cm depth with a maximum concentration of 128 μmol cm^{-3} at 3-4 cm (Fig. 2I). The elemental sulfur content at

station XII increased from zero at the surface to 0.26 µmol cm^{-3} at 12 cm (Fig. 3A). At stations VIII and X sulfur concentrations were below detection limit.

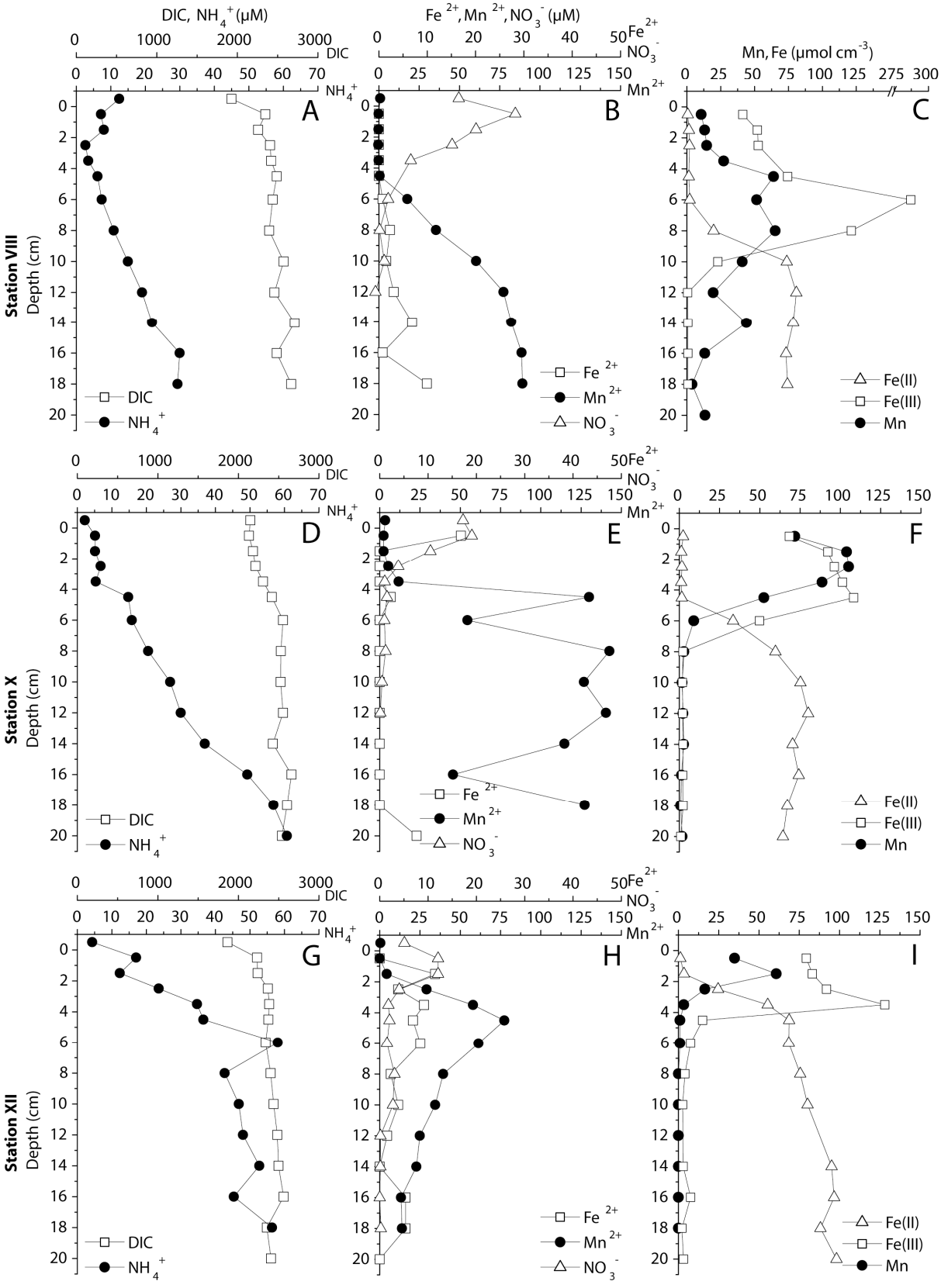

Fig. 2. Concentrations of the pore waters (DIC, NH_4^+, NO_3^-, Fe^{2+}, and Mn^{2+}) and solid phase (Mn, Fe(III), and Fe(II)). Note different scale for plot C.

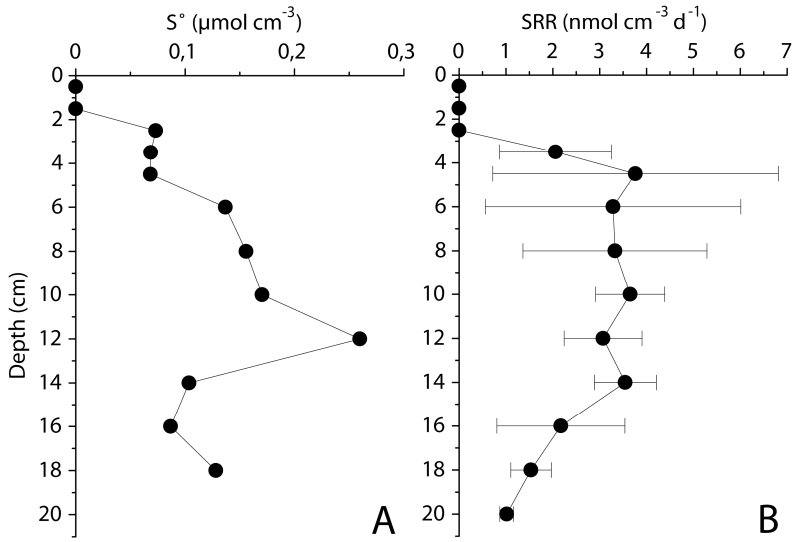

Fig. 3. (A) Solid phase S° of station XII. (B) Sulfate reduction rates (SRR) at station XII. Error bars indicate standard deviations of triplicate measurements. At the other stations S° concentrations and sulfate reduction rates were below detection.

Oxygen consumption and sulfate reduction rates of whole cores. Oxygen uptake rates of the sediments were lowest at station X with 1.5 mmol m^{-2} d^{-1}, 2.1 mmol m^{-2} d^{-1} at station VIII, and highest at station XII with 3.5 mmol m^{-2} d^{-1} (Table 1). Based on these rates the calculated oxygen penetration depths were 1.4, 1.9, and 0.8 cm for stations VIII, X, and XII, respectively. Sulfate reduction rates were below our detection limit of 1 nmol cm^{-3} d^{-1} at 0-21 cm depth in the cores of stations VIII and X. At station XII sulfate reduction was below detection in the top 3 cm and increased to rates of 3-4 nmol cm^{-3} d^{-1} at 4-15 cm depth (Fig. 3B).

Carbonate precipitation during anoxic bag incubations. DIC concentrations in bag incubations are expected to increase linearly during constant degradation of organic carbon. In this study linear increases of DIC concurrent with constant Ca^{2+} concentrations were measured only in the pore water of the two bags of station X from 0-1 and 1-2 cm depth, for station VIII in the bag from 6-12 cm, and for station XII in the four bags from 3 to 10 cm (data not shown). Decreasing or constant DIC concentrations, either from the beginning or after an initial increase, were measured in all other bags (station VIII 0-3 cm, station X between 2 and10 cm, station XII between 0 and 3 cm) (Fig. 4A, only data for station XII shown). This was related to decreasing Ca^{2+} concentrations in the respective bags (Fig. 4B) indicating a precipitation of $CaCO_3$. DIC accumulation rates were corrected for $CaCO_3$ precipitation according to Thamdrup *et al.* (2000). $CaCO_3$ precipitation, however, did not seem to account for the complete carbonate precipitation. Additional precipitation of carbonate with Mn^{2+} was indicated by supersaturation of pore waters with rhodocrocite in

most bags (ion activity products generally exceeded the solubility constant >2-fold) and decreasing Mn^{2+} accumulation rates towards the end of the incubations in the bags of station XII from 0 to 3 cm (Fig. 4C). Correction for $MnCO_3$ precipitation was not done, as precipitation is dependent on the Mn^{2+} concentrations, which increased due to Mn reduction. Therefore, the corrected DIC production rates represent minimum rates (Thamdrup *et al.*, 2000).

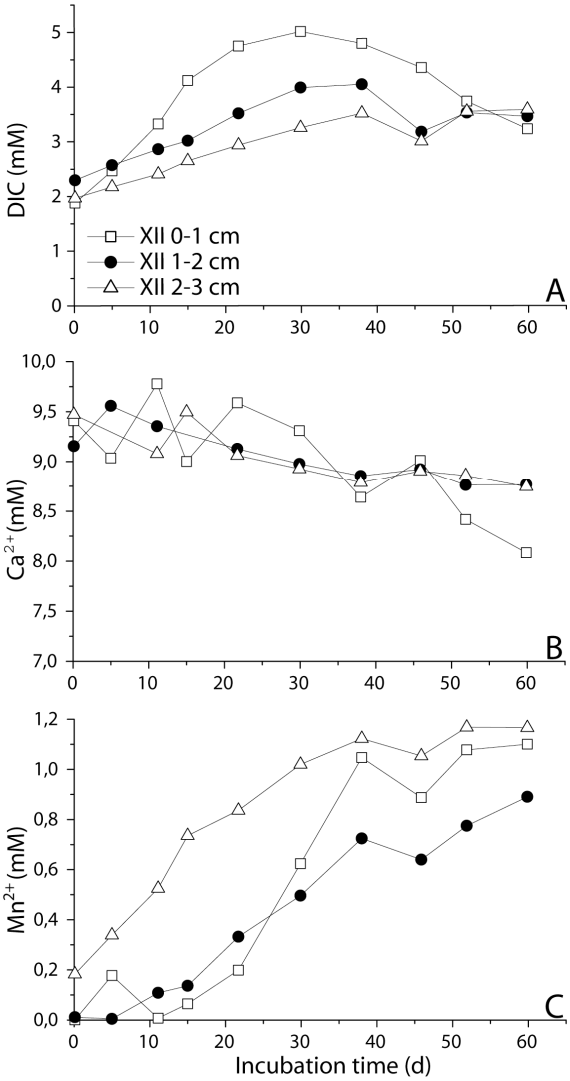

Fig. 4. Changes in pore water concentrations in bags from 0-1, 1-2, and 2-3 cm depth of station XII during two months of anoxic incubation: DIC (A), Ca^{2+} (B), and Mn^{2+} (C).

Anaerobic carbon oxidation. The results of the anoxic bag incubations are in the following presented for each station individually and depth-integrated DIC and NH_4^+ production rates are given in Table 2.

Station VIII. Rates of pore water DIC, NH_4^+, and Mn^{2+} production were higher in the bag of 0-3 cm sediment depth compared to the bag from 6-12 cm (Fig. 5A). Fe^{2+} concentrations were low (below the background level of ≤16 μM) throughout the incubation in both bags. Sulfate reduction rates were below the detection limit similar to the whole core incubations indicating that other respiration processes dominated the carbon oxidation in this zone.

Station X. DIC and NH_4^+ production rates showed the same depth trends with maximum rates between 1 and 5 cm (Fig. 5B). Mn^{2+} concentrations at 0-1 and 1-2 cm sediment depth did not increase significantly throughout the incubations. At 2-3 cm Mn^{2+} started to accumulate after 15 days (data not shown), and in the bags from 3 to 10 cm the

concentrations increased linearly throughout the incubation, resulting in highest Mn^{2+} accumulation rates at 2-4 cm (Fig. 5B). Similar to station VIII, sulfate reduction rates were below the detection limit and Fe^{2+} concentrations stayed low throughout the incubation.

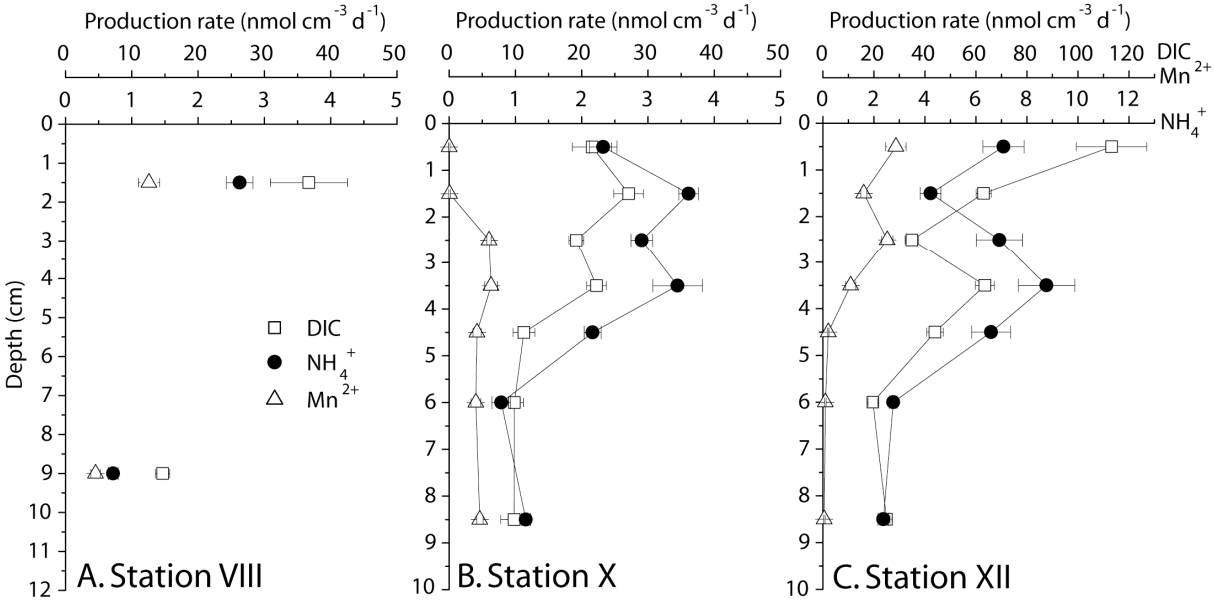

Fig. 5. Anaerobic mineralization rates (DIC and NH_4^+) and soluble Mn^{2+} accumulation rates for stations VIII (A), X (B), and XII (C). Error bars indicate standard errors from linear regression of concentration over time. Note different scales.

Station XII. DIC production rates were highest in the surface sediment and decreased with depth (Fig. 5C). Mid-depth maxima were detected for DIC and NH_4^+ production rates at 3-4 cm (Fig. 5C). Mn^{2+} accumulation rates were highest in the surface sediment and decreased with depth (Fig. 5C). When Mn^{2+} stopped to accumulate in the bag from 3-4 cm sediment depth after half of the incubation time, Fe^{2+} started to accumulate in the pore water (Fig. 6). Fe^{2+} concentrations increased also in the two bags from 4-5 and 5-7 cm towards the very end of the incubation time (Fig. 6), but stayed low in the remaining bags.

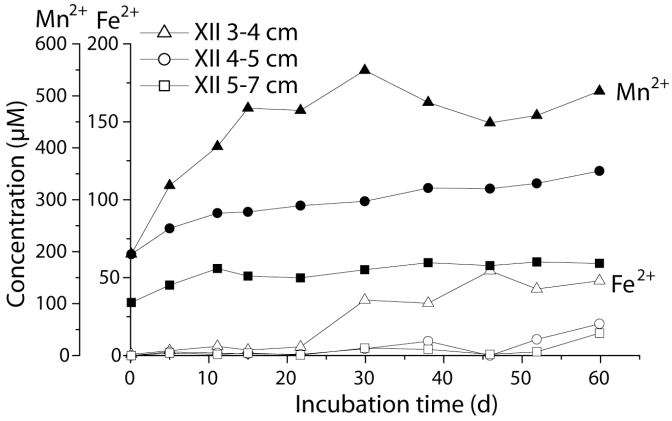

Fig. 6. Accumulation of Fe^{2+} (open) and Mn^{2+} (filled) in pore water during anoxic incubation of three bags from 3-4, 4-5 and 5-7 cm of station XII.

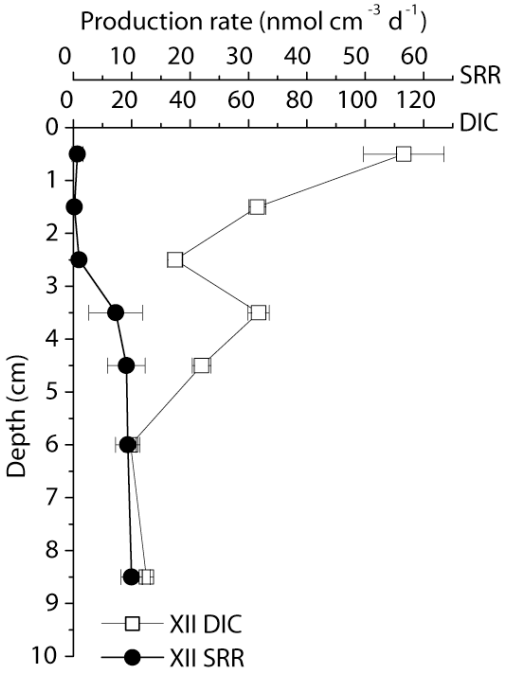

Fig. 7. Vertical profiles of anaerobic carbon mineralization at station XII: sulfate reduction rates (SRR) and DIC production rates. Error bars indicate standard errors from linear regression of DIC production and standard deviation of SRR. Scales are plotted at a ratio of 2:1 for DIC production to SRR.

Sulfate reduction was depressed in the surface sediment from 0-3 cm while rates below 3 cm increased with depth (Fig. 7). Sulfate reduction rates were almost constant in all bags over the incubation (data not shown) so that average rates were taken. The depth-integrated sulfate reduction rate for bag incubations (Table 2) was 3-fold higher than in whole core incubations for the same depth interval, probably due to a stimulation of carbon oxidation by mixing of the sediment (Kostka *et al.*, 1999; Arnosti *et al.*, 2005). Assuming a stoichiometry of 2:1 moles of carbon oxidized (= DIC produced) to sulfate reduced (Thamdrup & Canfield, 1996), there was a good agreement between DIC production based on sulfate reduction rates and the measured DIC production rates at 5-10 cm (Fig.7). At 0-5 cm measured DIC production rates exceeded the sulfate reduction-based DIC production. Thus, other electron acceptors than sulfate were utilized for microbial carbon oxidation.

Discussion

Anaerobic carbon oxidation and sulfate reduction. Highest rates of anaerobic DIC production were measured at station XII with a depth integrated rate of 4.3 mmol m^{-2} d^{-1} for 0-10 cm (Table 2). Only at this station could sulfate reduction be detected in the sediment. While sulfate reduction was depressed at 0-3 cm the measured DIC production could be partially attributed to sulfate reduction at 3-5 cm and completely at 5-10 cm (assuming a stoichiometry of 2:1 DIC produced to sulfate reduced) (Fig. 7). Accordingly, over the complete depth interval of 0-10 cm 31% of the anaerobic carbon oxidation was coupled to sulfate reduction. Although at stations VIII and X sulfate reduction rates were below the detection limit, we assume that sulfate reduction might occur at these stations. As anaerobic carbon oxidation rates were low (Figs. 5A & 5B, Table 2) either the incubation time with the $^{35}SO_4^{2-}$ tracer might have been too short or not enough tracer has been injected to detect

sulfate reduction. However, based on the detection limit we calculated the maximum contribution of sulfate reduction to anaerobic carbon oxidation integrated over 0-10 cm sediment depth to be <0.1 mmol m^{-2} d^{-1} or <10% for station VIII and <13% for station X (Table 2).

A higher contribution of sulfate reduction might have been masked by biologically catalyzed oxidation of HS^- to SO_4^{2-} concomitant to Mn reduction (Aller & Rude, 1988; King, 1990). Thamdrup *et al.* (1993) suggested that in those experiments the chemical oxidation of HS^- to S° by Fe(III) or Mn(IV) was followed by microbial disproportionation of S° to SO_4^{2-} and HS^-. However, in general with abundant easily reducible Mn and Fe oxides Mn- and Fe-reducing bacteria should outcompete sulfate reducers for common substrates (Lovley & Phillips, 1987). Nevertheless, Fe and sulfate reduction usually occur simultaneously and the gradual shift from Fe to sulfate reduction with depth is related to decreasing concentrations of poorly crystalline Fe(III) (Canfield *et al.*, 1993a; Kostka *et al.*, 1999; Thamdrup, 2000; Jensen *et al.*, 2003). We conclude that electron acceptors other than sulfate, most likely Mn(IV) and Fe(III), were the most important for anaerobic carbon oxidation at 0-12 cm sediment depth at stations VIII, at 0-10 cm at station X, and at 0-5 cm at station XII (Table 2).

Table 2. Depth-integrated rates from anoxic bag incubations (mmol m^{-2} d^{-1}) at 0-10 cm (station VIII 0-3 and 6-12 cm)

Station	VIII	X	XII
DIC production	2.0	1.5	4.3
NH_4^+ production	0.12	0.20	0.46
Sulfate reduction	<0.1	<0.1	0.67
Mn or Fe reduction[a], % of anaerobic carbon oxidation	>90	>87	69

[a]calculated from sulfate-independent DIC production rates

Mn and Fe reduction. The importance of Mn and Fe as microbial electron acceptors was supported by high contents of solid Mn and Fe(III) present in the surface sediments of all stations over 5-10 cm depth (Figs. 2C, 2F, & 2I). High concentrations of Mn^{2+} in the pore water indicated zones of Mn reduction and corresponded to increasing Mn^{2+} concentrations during incubation in most bags (Figs. 2B, 2E, 2H, & 5). Mn^{2+} and Fe^{2+} liberation rates might indicate the dominating respiration pathway, but as other reactions like precipitation, adsorption, and chemical oxidation occur simultaneously, these usually underestimate microbial reduction rates. The rates of dissimilatory Mn and Fe reduction can be calculated by subtraction of sulfate reduction based DIC production from the total DIC production where

the excess of carbon oxidation can be attributed to Mn and/or Fe reduction (Thamdrup, 2000). In the following, we will look at the results of bag incubations from each station individually.

Station XII. At 0-5 cm depth the excess of anaerobic carbon oxidation, which was not coupled to sulfate reduction, was attributed to microbial Mn and Fe reduction while below 5 cm sulfate reduction was the only significant electron acceptor (Fig. 7). We suggest that in the surface sediment at 0-3 cm Mn reduction was an important anaerobic respiration pathway, based on high Mn^{2+} liberation rates and solid Mn concentrations of 16-61 μmol cm^{-3} (Figs. 5C & 2I). Additionally, Fe(III) was present in 2-fold higher concentration than Mn in this zone (Fig. 2I). Heterotrophic Fe(III) reduction in the presence of Mn oxide might be masked by the reoxidation of produced Fe^{2+} by Mn(IV) (Postma, 1985; Lovley & Phillips, 1988; Myers & Nealson, 1988). Correspondingly, we could not exclude simultaneous microbial Fe and Mn reduction in the presence of high concentrations of solid Mn for all stations. At station XII at 3-4 cm, low Mn concentrations of 3 μmol cm^{-3} probably limited microbial Mn reduction. In combination with high Fe(III) concentrations in this zone, microbial Fe reduction was assumed to be the dominant process. We suggest that produced Fe^{2+} first was abiotically oxidized by Mn(IV), which resulted in Mn^{2+} accumulation during the first half of the incubation (Fig. 6). After complete Mn(IV) reduction by Fe(II) after 30 days, Fe^{2+} could accumulate, similar to observations with pure cultures by Myers & Nealson (1988). In the two bags of 4-5 and 5-7 cm the accumulation of Fe^{2+} was also detected toward the very end of the incubations (Fig. 6), but, with decreasing Fe(III) concentration, sulfate reduction became the dominating respiration pathway (Fig. 5C). Altogether, Mn was probably the sole anaerobic terminal electron acceptor in the surface sediment while below 3 cm a switch from microbial Mn to Fe reduction occurred. Fe and sulfate reduction occurred simultaneously at 3-5 cm and at 5-10 cm sulfate was the sole important electron acceptor (Table 2).

Station X. A contribution of sulfate reduction to anaerobic carbon oxidation was not detected, thus the complete DIC production at 0-10 cm was attributed to Mn and Fe reduction. However, a correlation between solid Mn and Fe contents and accumulation rates of soluble Mn^{2+} was only determined for the interval of 2-6 cm depth (Figs. 2F & 5C). At 6-10 cm depth Mn^{2+} accumulated in the pore water, even though the Mn content was low. In contrast to the profile in Figure 2F, the Mn content measured in the bags from 6-10 cm depth was ≥20 μmol cm^{-3} (data not shown). This difference is probably due to local heterogeneity and the fact that sediment from 10 cores was pooled for the bag incubations. In Black Sea sediments Mn concentrations above ~10 μmol cm^{-3} led to the inhibition of sulfate reduction by microbial Mn

reduction (Thamdrup *et al.*, 2000). Thus Mn concentrations of ≥20 μmol cm^{-3} between 0-10 cm depth at station X should allow dissimilatory Mn reduction.

Despite high concentrations of solid Mn and Fe(III), Mn^{2+} and Fe^{2+} did not accumulate in the pore water of the top 2 cm. High rates of DIC and NH_4^+ production indicated active microbial carbon oxidation (Fig. 5B), yet it was not clear which alternative electron acceptors other than Fe and Mn could have been used. Oxygen and nitrate should have been completely depleted within the first 13 days of incubation (calculations based on the DIC production rates and bottom water concentrations of oxygen (334 μM), nitrate (17 μM) and nitrite (0.4 μM)). The lack of another obvious electron acceptor responsible for the continuous DIC and NH_4^+ production leads us to conclude that Mn reduction was also active at 0-2 cm but that the produced Mn^{2+} was totally adsorbed.

Similar results were found in Skagerrak sediments of Denmark, where the lack of Mn^{2+} liberation in bag incubations of surface sediments was observed for two stations, whereas at a third station Mn^{2+} accumulated in the top sediment with high rates (Canfield *et al.*, 1993a). Adsorption experiments showed that dissolved Mn^{2+} could be completely adsorbed onto sediments containing high concentrations of Mn oxide with oxidation levels of 3.6-3.8 (Murray *et al.*, 1984; Canfield *et al.*, 1993a). It was assumed that Mn^{2+} adsorbed on surface sites of Mn oxides with high affinity for Mn^{2+} and could only accumulate after these sites had been saturated. Based on such experiments, Canfield *et al.* (1993a) suggested that the surface sediment where Mn^{2+} did not accumulate, differed in adsorption behavior for Mn^{2+} from the sediment of the third station where Mn^{2+} accumulated and where oxidation level of Mn oxides was probably less than 3.6-3.8. Correspondingly, we propose that the surface sediment from 0-2 cm depth of station X contained oxidized Mn oxides onto which Mn^{2+} produced during the incubation completely adsorbed. The initial complete adsorption was also indicated for the bag from sediment below (2-3 cm), where Mn^{2+} started to accumulate after 15 days of incubation (data not shown). Due to highest Mn^{2+} accumulation rates in the surface sediment at station XII, we propose that Mn phases were there in a more reduced state than at station X allowing Mn^{2+} to accumulate from the beginning of the incubations. We conclude that at station X Mn was the most important electron acceptor at 0-10 cm depth (Table 2).

Station VIII. The complete anaerobic carbon oxidation could be attributed to Mn and Fe reduction as extractable Mn and Fe(III) were present over the entire sediment depth of 0-12 cm and sulfate reduction was not detected. Additionally, the Mn^{2+} accumulation rate was

higher at 0-3 cm than at 6-12 cm matching with higher surface rates of DIC and NH_4^+ production (Fig. 5A).

***In situ* significance of Mn and Fe reduction.** It is likely that *in situ* Mn and Fe reduction in the surface sediment are suppressed due to the presence of oxygen and nitrate. Hulth *et al.* (1994) measured oxygen penetration depths of 1.7 to ≥5.9 cm in sediments off the north and northeast coast of Svalbard. Based on oxygen uptake rates we calculated oxygen penetration depth of 0.8-1.9 cm for the three sediments. The presence of oxygen in the surface sediments was also indicated by the distribution of pore water constituents. Maximum concentrations of nitrate below the surface indicated a zone of nitrification which requires the presence of oxygen (Figs. 2B, 2E, & 2H). The depletion of oxygen in the sediments was indicated by decreasing concentrations of nitrate, probably due to denitrification, and increasing concentrations of Mn^{2+} (Figs. 2B, 2E, & 2H). The calculated oxygen penetration depth of 0.8 cm for station XII was in accordance with the depletion of nitrate and the accumulation of Mn^{2+} below 1 cm depth. For station VIII the distribution of nitrate and Mn^{2+} in the pore water indicated the deepest penetration of oxygen of ~4 cm, whereas the calculated oxygen penetration depth was 1.4 cm compared to 1.9 cm for station X. A deeper penetration of oxygen might result from active bioirrigation by polychaetes, which introduce oxygen into deeper sediment layers (Jørgensen *et al.*, 2005) and might cause spatial heterogeneity of porewater constituents. We conclude that oxygen and nitrate reduction were probably active in surface sediment intervals of 1 to 4 cm of the three sediments. Because nitrate reduction is in general of minor importance for the degradation of organic matter and accounted for only 2-3% of total carbon oxidation in permanently cold sediments of Svalbard (Kostka *et al.*, 1999), oxic respiration was the most important process in surface sediments of the three stations.

Although oxygen was probably abundant over several cm in the surface sediments the anoxic bag incubations revealed a high potential for anaerobic carbon oxidation also in this zone for all stations indicating that the *in situ* microbial population was able to mineralize organic carbon also by anaerobic respiration. The Mn- and Fe-reducing bacteria may be either facultatively anaerobic, such as strains of the genus *Shewanella* (Ivanova *et al.*, 2004) and *Rhodoferax* (Finneran *et al.*, 2003), or micro-aerotolerant/-aerophilic such as strains of the family *Geobacteraceae* (Dehning & Schink, 1989; Methé *et al.*, 2003; Lin *et al.*, 2004).

Oxygen and nitrate reoxidize the products of Mn and Fe reduction, Mn(II) and Fe(II), and their abundance in surface sediments is therefore important for the Mn- and Fe-cycle.

Calculations for Danish coastal sediments determined that Mn and Fe atoms were recycled 100-300 times before their ultimate burial (Canfield *et al.*, 1993a; Thamdrup *et al.*, 1994). Especially for deep sediment zones the maintenance of Mn and Fe reduction is dependent on mixing processes (Aller, 1990; Canfield *et al.*, 1993a; Thamdrup *et al.*, 1994). Bioturbation enables the downward transport of organic matter and Mn and Fe oxides as well as upward transport of Fe(II) and Mn(II) for the reoxidation by oxygen, nitrate and, in the case of Fe(II), by Mn(IV) and the formation of new oxides. We observed highest abundance of polychaetes and polychaete tubes at station XII indicating bioturbation, which correlates with highest rates of carbon oxidation and fastest turnover of Mn and Fe at 0-5 cm.

Dissimilatory Mn reduction is insignificant for the oxidation of carbon in most marine shelf sediments due to low Mn oxide contents and a shallow penetration depths (Thamdrup, 2000). In fjord sediments of the west coast of Svalbard measured Mn concentrations were low (<4 μmol cm^{-3}) or intermediate (~18 μmol cm^{-3} in sediments of Van Mijenfjorden at the west coast and in Storfjorden at the southeast coast). Microbial Mn reduction was therefore assumed to be insignificant for carbon oxidation (Kostka *et al.*, 1999). Sediments with very high Mn oxide concentrations (≥100 μmol cm^{-3}) are restricted to small areas of the oceans such as the Panama Basin, where the Mn has a hydrothermal origin, or the deep parts of the Skagerrak, where Mn, released from more reducing sediments, is trapped. At these sites microbial Mn reduction was shown to account for more than 90% of the mineralization and could be detected as deep as 10 cm in these sediments (Aller, 1990; Canfield *et al.*, 1993a). The relatively high Mn contents of ≥60 μmol cm^{-3} and Fe(III) contents of ≥108 μmol cm^{-3} in sediments of the three stations off the northeast coast of Svalbard were related to the significance of dissimilatory Mn and Fe reduction for carbon oxidation. Our study supports the importance of metal reduction for carbon mineralization in permanently cold sediments, as previous studies determined that Fe reduction accounted for 0-26% of the total carbon oxidation in fjord sediments of the west coast of Svalbard (Kostka *et al.*, 1999) and for 21-26% in sediments of east Greenland (Rysgaard *et al.*, 1998; Glud *et al.*, 2000). This is in agreement with contributions of 0 to >50% of Fe reduction to total carbon mineralization found in a wide selection of coastal sediments (Thamdrup, 2000).

Rates of carbon oxidation in permanently cold sediments. Total oxygen uptake rates were 1.5-3.5 mmol m^{-2} d^{-1} at the three stations northeast of Svalbard (Table 1). Similar rates of 1.9-4.1 mmol m^{-2} d^{-1} have been measured north and northeast of Svalbard whereas significantly higher rates (3.6-11.2 mmol m^{-2} d^{-1}) were determined in sediments of the south and west coast

(Hulth *et al.*, 1994; Glud *et al.*, 1998). The difference was suggested to result from different water masses of Atlantic and Arctic origin determining the duration of the annual ice coverage and, thus, the phytoplankton production (Hulth *et al.*, 1994). Benthic oxygen consumption can also vary interannually as a response to the settling of fresh organic material from the water column (Grebmeier & McRoy, 1989; Rysgaard *et al.*, 1998; Glud *et al.*, 2003).

As aerobic and anaerobic rates of carbon oxidation were highest for the middle station XII and lowest at the southernmost station X (Tables 1 & 2), we propose that the sediment of station XII is supplied annually with more organic carbon. A possible explanation is a semi-permanent polynya observed west and southwest of Kvitøya (Vinje & Kvambekk, 1991; Falk-Petersen *et al.*, 2000). This region is ice-free earlier than the surrounding area and an earlier spring bloom and enhanced biomass production over the whole year was suggested (Strass & Nöthig, 1996; Falk-Petersen *et al.*, 2000).

The retreat of the sea ice during late spring in general proceeds from two sides towards the northeast of Svalbard: along the east coast northwards and along the north coast eastwards (Falk-Petersen *et al.*, 2000). Although station VIII was the deepest and northernmost station (Fig. 1, Table 1), higher aerobic and anaerobic carbon oxidation rates were measured compared to the southernmost station X. Station VIII was situated in a trench, which extends along the east coast of Nordaustlandet. Sediment might be derived from the slopes and be transported northwards in the trench, as sedimentation rates were twice as high at station VIII (1.3 mm y^{-1}) compared to station X (0.6 mm y^{-1}) (Agata Zaborska, unpublished data). Observations and satellite images over several years showed that in some years the area around Kong Karls Land (station X) was ice covered longer than the area further north which may restrict primary production. Thus, station X may be supplied with less organic material than the other two stations. In conclusion, the primary production is probably be similar or higher at station VIII than at station X, and additional organic material may be transported to station VIII to become available to benthic oxidation and supporting higher mineralization rates in the north. However, the difference in carbon oxidation rate between the two stations was not that distinct and, due to the complexity of the ice coverage, currents and bottom topography, not easy to explain.

Most studies of anaerobic benthic mineralization around Svalbard have been carried out in fjord sediments at the south and west coast (Glud *et al.*, 1998; Rysgaard *et al.*, 1998; Sagemann *et al.*, 1998; Thamdrup & Fleischer, 1998; Kostka *et al.*, 1999; Arnosti & Jørgensen, 2003; Arnosti *et al.*, 2005). Anaerobic carbon oxidation rates of sediment incubations (11-24 mmol m^{-2} d^{-1}) and sulfate reduction rates (0.9-4.2 mmol m^{-2} d^{-1}) in those

fjord sediments were considerably higher than rates measured in this study (Tables 1 & 2) (Sagemann *et al.*, 1998; Knoblauch *et al.*, 1999; Kostka *et al.*, 1999; Finke, 2003). We suggest that temperature did not account for these differences since the *in situ* temperature in sediments around Svalbard varies only from –1 to +3°C. Water depths of ≥300 m and the annually low primary production at the northeast coast (Wassmann & Slagstag, 1993; Sakshaug, 1997) result in less organic carbon settling to the sea floor and might be responsible for the low rates of carbon oxidation in these sediments. In contrast, fjord sediments on the west coast receive a relatively large flux of organic carbon by primary productivity in the water column (Eilertsen *et al.*, 1989; Hop *et al.*, 2002). Therefore we suggest that the supply and availability of carbon matter limits benthic activity at the northeast coast off Svalbard rather than low temperatures.

High carbon deposition favors sulfate reduction and the produced hydrogen sulfide consumes Fe and Mn leaving less Fe and Mn for microbial reduction. Intermediate organic carbon deposition increases the relative importance of suboxic respiration pathways (nitrate, Mn, and Fe reduction) (Van Cappellen & Wang, 1996; Wang & Van Cappellen, 1996; Wijsman *et al*., 2002). In conclusion intermediate carbon supply and high concentrations of particulate Mn and Fe(III) are favorable for the Mn- and Fe-reducing community in the sediments of the northeast coast of Svalbard.

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2.6

Desulfotomaculum arcticum sp. nov., a new spore-forming, moderately thermophilic sulfate-reducing bacterium isolated from a permanently cold fjord sediment of Svalbard

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Strain 15^T is a new spore-forming, sulfate-reducing bacterium isolated from a permanently cold fjord sediment of Svalbard. Sulfate could be substituted by sulfite or thiosulfate. Hydrogen, formate, lactate, propionate, butyrate, hexanoate, methanol, ethanol, propanol, butanol, pyruvate, malate, succinate, fumarate, proline, alanine, and glycin were used as electron donors in the presence of sulfate. Fermentative growth with pyruvate was observed. Optimal growth was observed at pH 7.1-7.5 and concentrations of 1-1.5% NaCl and 0.4% $MgCl_2$. Strain 15^T grew between 26-46.5°C and the optimal temperature for growth was 44°C. Therefore strain 15^T was not able to grow at *in situ* temperatures of its original habitat in Arctic sediments and we propose that it was present in the sediment in the form of spores. The G + C content of DNA was 48.9 mol%. Strain 15^T was most closely related to *Desulfotomaculum thermosapovorans* (93.5% 16S rDNA sequence similarity). We propose that strain 15^T is a new species of the genus *Desulfotomaculum*, *Desulfotomaculum arcticum* (type strain 15^T, DSM 17038^T, JCM 12923^T).

The genus *Desulfotomaculum* includes meso- and thermophilic species mainly isolated from thermal sites. Under unfavorable environmental conditions that do not permit the organisms to grow or metabolize substrates, they may survive in the form of spores. Spores are resistant to drying, oxygenation, starvation, and extreme temperatures. Thus, *Desulfotomaculum*-related strains have been isolated from environments that do not support growth of the organisms such as extreme temperature environments. *Desulfotomaculum halophilum* grows between 30 and 40°C and was isolated from 85°C hot fluids (Tardy-Jacquenod *et al.*, 1998). Strains of the spore-forming genera *Desulfotomaculum* and *Desulfosporosinus* were isolated from cold sediments of the coast of Denmark (Isaksen *et al.*, 1994), from permanently cold deep sea sediments (Barnes *et al*., 1998), and from permafrost soil (Vainshtein *et al.*, 1995), although these bacteria are not able to grow at *in situ* temperatures of those localities.

Strain 15^T was isolated at 28°C from sediment of Nordfjorden, Station BC (water depth 100 m, bottom water temperature 1.8°C) on the west coast of Svalbard. The enrichment culture was started in artificial sea water medium (Widdel & Bak, 1992) with 28 mM sulfate and a suspension of lyophilized cyanobacteria (*Spirulina*) as carbon and energy source. For isolation in deep agar dilution series the *Spirulina* suspension was replaced by a fatty acid mix of acetate, lactate, butyrate, and propionate.

Cells of strain 15^T were rods, 2-3 x 1 µm in size, and when endospores were formed cells appeared lemon-shaped (Fig. 1). Endospores were spherical and located in the center of the cells. Motility was, under the culture conditions used, not observed. Gram staining was negative. A negative Gram stain has been repeatedly described for species of *Desulfotomaculum*, yet the ultrastructure of the cell wall of these species, examined by electron microscopy, is usually typical for gram-positive bacteria (Stackebrandt *et al*., 1997), but was not tested for strain 15^T.

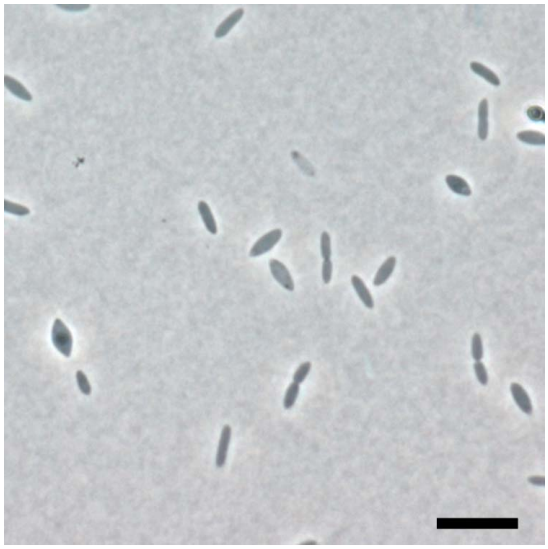

Fig. 1. Phase contrast micrograph of *Desulfotomaculum arcticum* strain 15^T. The scale bar indicates 10 µm.

The tests for physiological characterization have been described recently (Vandieken *et al*., submitted). Vitamins were not required for growth. Strain 15^T used (mM): sulfate (30), sulfite (2), and thiosulfate (10) in the presence of lactate. As electron acceptors strain 15^T did not reduce (mM): ferric citrate (30), poorly crystalline iron oxide (30), manganese oxide (30), malate (20), fumarate (20), nitrate (20), nitrite (10), oxygen (air), or elemental sulfur. With sulfate as electron acceptor the strain oxidized the following substrates (mM): H_2/CO_2, formate (10), lactate (20), propionate (10), butyrate (10), hexanoate (3), methanol (10), ethanol (10), propanol (10), butanol (10), pyruvate (10), malate (10), succinate (10), fumarate (10), proline (10), alanine (10), and glycin (10). Compounds tested but not utilized with sulfate were (mM): acetate (20), glycerol (10), glucose (1 g l^{-1}), fructose (1 g l^{-1}), glutarate (10), serine (10), betaine (10), cholin (10), sorbite (5), nicotinate (1), casein (0.05 g l^{-1}), and yeast extract (0.05 g l^{-1}). Fermentation was observed with (mM): pyruvate (20), but not with lactate (20), malate (20), fumarate (20), glucose (1 g l^{-1}), or fructose (1 g l^{-1}).

The pH optimum of strain 15^T was 7.1-7.5 and growth occurred over pH 6.8-7.5. The strain grew at NaCl concentrations of 0.05-4.5% and best at 1-1.5%, the optimum concentration for $MgCl_2 \cdot 6H_2O$ was 0.4% and the growth range was 0.4-2.5% $MgCl_2$. The optimum temperature of strain 15^T was 44°C and growth was observed between 26 and 46.5°C. The growth rate of strain 15^T with sulfate and lactate at 41°C was 0.046 h^{-1}. Cells of strain 15^T contained, as sole menaquinone, MK7 which was determined by the Deutsche

Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. The G + C content of the DNA was 48.9 mol% (determined by the DSMZ).

The 16S rDNA of strain 15^T was amplified and the sequence analyzed as previously described (Vandieken *et al*., submitted). The phylogenetic position was evaluated by the ARB program package (Ludwig *et al.*, 2004) using the neighbor joining, maximum likelihood and maximum parsimony algorithms with different sets of filters. Positions 109-1387 (*Escherichia coli* numbering) were used for analyses as regions were excluded that either exhibited alignment uncertainties or were not sequenced. Strain 15^T was closely related to *Desulfotomaculum thermosapovorans* (93.5% 16S rDNA sequence similarity), *Desulfotomaculum sapomandens* (93.4%), *Desulfotomaculum gibsoniae* (93.3%), and *Desulfotomaculum geothermicum* (93.2%) (Fig. 2).

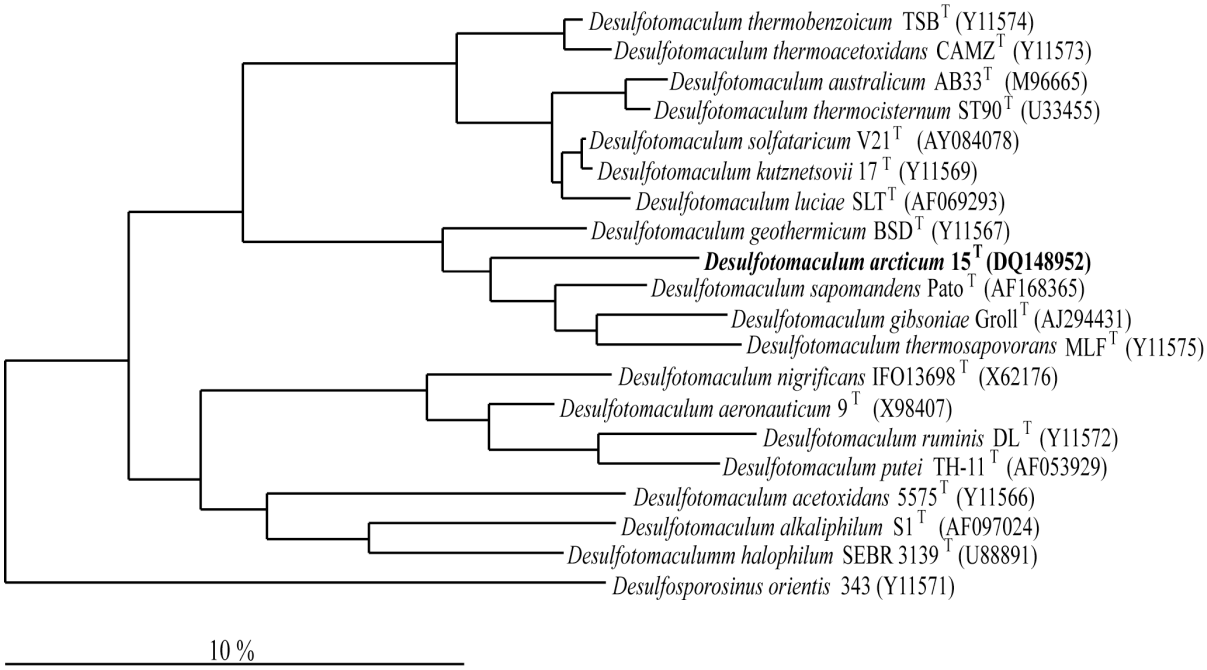

Fig. 2. Phylogenetic tree calculated by maximum-likelihood analysis. The scale bar indicates 10% estimated sequence divergence.

Strain 15^T shares important physiological characteristics with its closest relatives *D. thermosapovorans*, *D. sapomandens*, *D. gibsoniae*, and *D. geothermicum* in that it uses sulfate, sulfite, and thiosulfate as electron acceptors and formate, hydrogen, butyrate, ethanol, butanol, propanol, malate, fumarate, and pyruvate as electron donors (Table 1) (Cord-Ruwisch & Garcia, 1985; Daumas *et al*., 1988; Fardeau *et al*., 1995; Kuever *et al*., 1999). The

species are easily to distinguish by their differences in temperature tolerance and usage of other electron donors and acceptors (Table 1).

Table 1. Characteristic of *Desulfotomaculum arcticum* 15^T and its closest relatives

	15^T	***D. thermo-sapovorans***	***D. sapo-mandens***	***D. gibsoniae***	***D. geo-thermicum***
Temperature optimum (°C)	44	50	38	35-37	54
Temperature range (°C)	26-46.5	35-60	20-43	20-42	37-57
Electron acceptors					
sulfate	+	+	+	+	+
thiosulfate	+	+	+	+	nd
sulfite	+	+	+	+	+
elemental sulfur	-	-	+	-	-
Electron donors					
acetate	-	-	+	-	-
lactate	+	+	-	-	+
propionate	+	-	-	+	+
methanol	+	+	nd	-	-
succinate	+	-	+	+	nd
fructose	-	-	-	-	+
G + C content (mol%)	48.9	51.2	48.0	54.8	50.4

nd: not determined; + = substrate was used for growth, - = substrate was not used for growth; in this table not all electron donors and acceptors used by the species are listed.

Desulfotomaculum antarcticum was isolated from a pond sediment sample of the Antarctic (Iizuka *et al*., 1969). This bacterium was described to have a significantly lower temperature optimum for growth (20-30°C) than our new strain 15^T (44°C). However, the authors did not test for growth at *in situ* temperatures, and today the strain is considered as lost (Stackebrandt *et al*., 1997). Due to the moderately thermophilic growth range, 26-46.5°C, strain 15^T was not able to multiply at *in situ* temperatures of its habitat in permanently cold Arctic sediments, where the temperature never exceeds 4°C. During laboratory experiments with fjord sediments of Svalbard, sulfate reduction in the thermophilic temperature range has been observed (Maren Nickel, unpublished data). This suggests that our isolate belongs to a thermophilic population of sulfate-reducing bacteria present as spores in the sediment. In cold sediments of Aarhus Bay (Denmark) thermophilic populations of sulfate-reducing and aerobic respiring bacteria have been found with optimum temperatures for thermophilic sulfate reduction of 60°C and for aerobic respiration of 55°C (Isaksen *et al*., 1994; Thamdrup

et al., 1998). From MPN enumeration at 60°C with propionate, a population density of thermophilic sulfate reducers of 2.8 10^4 cells cm^{-3} for the Aarhus Bay sediment was estimated and a thermophilic spore-forming bacterium with similarities to *Desulfotomaculum kuznetsovii* was isolated (Isaksen *et al*., 1994). From permanently cold habitats such as deep sea sediments and permafrost soil aerobic and sulfate-reducing, spore-forming bacteria were isolated with minimum growth temperatures considerably above *in situ* temperatures (Bartholomew & Paik, 1966; Barnes *et al*., 1998; Vainshtein *et al.*, 1995). The origin of the different thermophilic populations have been proposed to be geothermal environments or anthropogenic sources from which the spores were dispersed by wind or terrestrial river run-off (Bartholomew & Paik, 1966; Isaksen *et al*., 1994; Thamdrup *et al*., 1998). We do not know the origin, where growth is supported for our isolates.

Description of *Desulfotomaculum arcticum* sp. nov.

Desulfotomaculum arcticum (arc'ti.cum. L. neut. adj. *arcticum* from the Arctic, referring to the place where the strain was isolated). Cells are rod-shaped, endospore-forming, 2-3 x 1 μm, strict anaerobic. No vitamins required for growth. Sulfate, thiosulfate, and sulfite serve as electron acceptors. Oxidation of H_2/CO_2, formate, lactate, propionate, butyrate, hexanoate, methanol, ethanol, propanol, butanol, pyruvate, malate, succinate, fumarate, proline, alanine, and glycin in the presence of sulfate. Fermentative metabolism with pyruvate. pH range of growth 6.8-7.5, optimum pH 7.1-7.5. Temperature growth range 26-46.5°C, optimum temperature 44°C. 48.9 mol% G + C of the DNA. Type strain is 15^T (DSM 17038^T, JCM 12923^T). The 16S rDNA sequence has been deposited in GenBank (DQ148942).

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3 CONCLUSIONS

This present study was carried out in sediments of four stations of Svalbard to assess the contribution of Mn(IV) and Fe(III) reduction to carbon remineralization in permanently cold sediments. Additionally, the role of volatile fatty acids as substrates for Fe- and sulfate-reducing bacteria was investigated in an inhibition experiment of a fjord sediment of the west coast of Svalbard. Furthermore, psychrophilic and psychrotolerant Fe-reducing bacteria were isolated from fjord sediments on the west coast of Svalbard in order to investigate the physiology and adaptation of the organisms that contribute to the process of Fe reduction in permanently cold sediments. The following sections summarize the main conclusions of the project.

3.1 Mn and Fe reduction

This study highlights the importance of dissimilatory Mn and Fe reduction for carbon mineralization in marine sediments. The contribution of microbial Mn and Fe reduction (13% and 0%, respectively) to total carbon oxidation in the uppermost 10 cm of the Smeerenburgfjorden sediments was similar to average values determined for coastal sediments (Thamdrup, 2000). An unexpectedly high contribution of microbial Mn and Fe reduction, ranging from 69% to at least 90% of anaerobic carbon oxidation, was determined for the sediments from the northeast coast of Svalbard. Various investigations have shown that microbial Mn and Fe reduction depend primarily on the presence of Mn and Fe oxides (Thamdrup, 2000). Additionally, the relative contribution of these processes is regulated by the availability of organic carbon, the mixing of the sediment by benthic fauna, and the reactivity of the respective metal oxide. Differences in these parameters among the investigated sites are probably responsible for the various contributions of Mn and Fe reduction to benthic carbon mineralization.

a) Mn and Fe oxide concentrations. The vertical occurrence of microbial Mn and Fe reduction in the sediments of all stations correlated with the distribution of solid Mn and Fe(III). In Smeerenburgfjorden sediments, microbial Fe reduction accounted for 43% of anaerobic carbon oxidation in surface sediments from 0 to 5 cm. The zone of microbial Fe reduction in the top 2 cm sediment overlapped with the zone of sulfate reduction, which was in agreement to a rapid depletion of Fe(III). The Fe-reducing community in this sediment seemed to be limited mainly by the availability of Fe(III) in the sediment.

Although the solid total Fe (Fe(II) + Fe(III)) content in Smeerenburgfjorden and the stations at the northeast coast of Svalbard was similar, concentrations of solid Fe(III) in the

surfaces were higher and Fe(III) penetrated deeper into the sediments of the northeast coast. Additionally, the Mn content was one to two orders of magnitude higher at the northeast coast than at Smeerenburgfjorden. In two of the three sediments at the northeast coast of Svalbard, solid Mn and Fe(III) was abundant at depths of 0-10 cm and accordingly microbial Mn and Fe reduction were the only significant anaerobic carbon oxidation pathways. At the third station, the depletion of Mn at 3 cm sediment depth restricted microbial Mn reduction to this surface interval. In the 3-5 cm interval, a zone with simultaneous microbial Fe and sulfate reduction occurred, where the contribution of sulfate reduction to carbon mineralization increased with decreasing Fe(III) concentrations and became the sole electron accepting process at 5-10 cm.

b) Organic carbon availability. The flux of organic material to the sediment is derived mainly by primary and secondary productivity in the overlying water column. Primary productivity in the ocean around Svalbard is restricted to the ice-free periods, which are controlled by the different water masses around Svalbard. The fjords along the west coast are influenced by relatively warm, nutrient-rich Atlantic water of the West Spitsbergen Current and are ice covered for 5-10 months of the year. The primary productivity in these fjords is similar to that of more southern latitudes, which are not significantly ice covered (Eilertsen *et al.*, 1989). In contrast, the east coast of Svalbard is influenced by cold Arctic water of the East Spitsbergen Current. The melting of the ice proceeds from the south along the east coast as well as from the west along the north coast so that the ice coverage in general persists longest at the northeast coast of Nordaustlandet (Falk-Petersen *et al*., 2000). Primary production was found to be significantly lower in this area compared to areas influenced by Atlantic water (Wassmann & Slagstad, 1993; Sakshaug, 1997).

Modeling studies showed that the contribution of microbial Mn and Fe reduction to carbon oxidation is highest when the supply with organic carbon is intermediate (Van Cappellen & Wang, 1996; Wang & Van Cappellen, 1996; Wijsman *et al*., 2002). In low organic carbon sediments, oxic respiration dominates, while in environments with high organic carbon deposition, the oxic zones become smaller and more organic carbon is diverted into anaerobic carbon oxidation pathways. Additionally, bacterial processes may have to compete with reduced compounds for electron acceptors: at high microbial Fe reduction rates, Mn(IV) is mainly reduced by Fe(II), whereas at high sulfate reduction rates, Fe is consumed by sulfide. In Smeerenburgfjorden sediment where sulfate reduction was active in the surface sediment, Fe was consumed by sulfide and, thus, less Fe was available for the Fe-reducing community. The sediments at the northeast coast of Svalbard were characterized by a lower carbon deposition compared to Smeerenburgfjorden due to the

shorter periods of primary productivity. Thus, less organic carbon was available to the microbial community and in particular to bacteria using electron acceptors with lower energy yield such as sulfate-reducing bacteria.

c) Mixing processes. Mixing of the sediment by bioturbation can counteract the rapid depletion of Mn(IV) and Fe(III) by a fast recycling of Mn and Fe oxides, thus, allowing a faster turnover of both. Efficient reoxidation of Mn and Fe by bioturbation in all sediment was indicated by abundant polychaetes. Especially in Smeerenburgfjorden where the Fe-reducing bacteria seemed to be limited by Fe(III) availability, bioturbation is probably an important factor for the maintenance of high rates of dissimilatory Fe-reduction.

d) Additional factors. The reactivity of Mn and Fe oxides in the sediments influences the rate of microbial Mn and Fe reduction. As mineral reactivity is complex and its determination neither simple nor easy to interpret, it was not measured for this study.

Most fjords have actively calving tidal glaciers in their inner parts, which create a very dynamic aquatic environment often characterized by extreme gradients of temperature, salinity, turbidity, and sedimentation. At the glacial front, growth of phytoplankton and zooplankton is restricted and also the diversity of benthic organisms is reduced due to high turbidity of the water and high sedimentation as well as low salinity and temperature (Hop *et al*., 2002). Because biomass production of pelagic organisms as well as mixing of the sediment by benthic organisms influence microbial Fe reduction, the relative contribution of Fe reduction to carbon degradation might differ spatially from the glacier front towards the outer fjord. The influence of glaciers may contribute to the differences in the importance of Fe reduction between fjords of the west coast of Svalbard. While Fe reduction accounted for 10-26% to carbon oxidation in three fjord sediments (Kostka *et al*., 1999; this study), the insignificance of Fe reduction in another fjord (Hornsund) was suggested to result from high sedimentation rates and a shallow mixing zone (Kostka *et al.*, 1999).

In summary, the environmental settings at the northeast coast of Svalbard seemed to be more favorable for dissimilatory Mn- and Fe-reducing bacteria compared to Smeerenburgfjorden sediment. The differences between the sites were obvious in lower supply of organic carbon and higher concentrations of particulate Mn and Fe(III) at the northeast coast than in Smeerenburgfjorden. Thus, the Mn- and Fe-reducing community in surface sediments at the northeast coast was probably limited by the supply of organic carbon to the sediment, while the Fe-reducing population in Smeerenburgfjorden seemed to be limited primarily by the availability of Fe(III). However, the relative contributions of microbial Mn and Fe reduction to carbon mineralization might vary temporally and spatially dependent on ice coverage,

sedimentation of organic carbon, mixing processes and calving of tidal glaciers. Thus, it is necessary to study more habitats to understand the influence of physical, chemical, and biological factors on the regulation of dissimilatory Mn- and Fe-reducing bacteria.

The results of the sediment incubations were combined with the distribution of potential oxidants available for bacterial respiration to define zones of dominating microbial processes. Especially in surface sediments with insignificant sulfate reduction, anoxic bag incubations permit a good estimate of the importance of carbon degradation by other anaerobic pathways. Liberation rates of soluble Mn^{2+} and Fe^{2+} during bag incubations alone could not give realistic rates of microbial reduction, due to adsorption, precipitation, and reoxidation processes. These reactions depend on the sediment and pore water composition and therefore differ between sites and between depths of a single sediment. Although the accumulation of dissolved inorganic carbon (DIC) may be biased by precipitation of $CaCO_3$, more realistic rates of Mn and Fe reduction can be gained when the excess of carbon oxidation determined by subtraction of the sulfate reduction based DIC production from the total anaerobic DIC production during sediment incubations is attributed to microbial Mn and/or Fe reduction.

3.2 Carbon oxidation

Total oxygen uptake and anaerobic carbon oxidation rates of Smeerenburgfjorden were higher than at the northeast coast of Svalbard. The water depths of the sites might contribute to these differences, as with the station in Smeerenburgfjorden was only 212 m, while the stations at the northeast coast ranged from 286 to 503 m water depth. However, it is most likely that the differences reflect mainly the influence of the dominating water masses at the east and west coast of Svalbard. Arctic water at the east coast and Atlantic water at the west coast control the duration of ice coverage and accordingly the production of biomass and its sedimentation.

Mineralization rates in permanently cold sediments from fjords of the west coast of Svalbard, including Smeerenburgfjorden, and also from the east coast of Greenland were similar to rates in temperate sediments such as the coast of Denmark and Chile (see Introduction, Table 2, & Chapter 2.1). This comparison indicates that temperature is not the primary rate controlling factor for the microbial processes in permanently cold sediments and that the microbial community is well adapted to the permanently low temperatures.

Aerobic and anaerobic mineralization rates (calculated for 0-5 cm) at the northeast coast were 1.2- to 10-fold lower compared to Smeerenburgfjorden, which was attributed to a lower carbon supply by primary productivity rather than to temperature, which is similar in sediments along the coast. A semipermanent polynya west of Kvitøya is ice-free for a longer

annual period compared to the surrounding areas, which probably results in enhanced primary productivity (Strass & Nöthig, 1996; Falk-Petersen *et al*., 2000). Accordingly, enhanced deposition of organic carbon to the sediment of the station in the central Kvitøya trench (station XII) seemed to be responsible for relatively high oxygen uptake and anaerobic carbon oxidation rates, which were only 1.2- and 3.1-fold lower than rates at Smeerenburgfjorden. In addition to the primary production of the overlying water column, a transport of sediment due to slopes at the sea floor can supply additional organic material. This was probably responsible for relatively high rates of organic carbon oxidation at the northernmost station at 81°N (station VIII). Therefore the area at the northeast coast of Svalbard seems to be highly influenced by a very dynamic setting of ice coverage and sediment transports, and accordingly benthic mineralization rates are not easy to predict.

3.3 Fe- and sulfate-reducing bacteria

The isolated Fe-reducing bacteria originated from two fjord sediments of the west coast of Svalbard (Tempelfjorden and Smeerenburgfjorden). The isolates were phylogenetically related to *Shewanella* as well as *Desulfuromusa* and *Desulfuromonas* within the family *Geobacteraceae*, which represent the largest taxonomic groups of Fe-reducing bacteria. Additional isolated strains related to species of *Desulfovibrio* showed the ability to reduce Fe but without growth. The bacteria isolated of this study seemed to be well adapted to the environmental settings of their habitat such as to temperature and substrate usage.

a) Temperature adaptation. Strains of the genera *Shewanella*, *Desulfuromusa*, *Desulfuromonas*, and *Desulfovibrio* have been isolated from diverse habitats and are mostly mesophiles. Psychrophilic strains of *Shewanella* were isolated from Arctic and Antarctic sea ice and Antarctic sediments (Bowman *et al*., 1997; Bozal *et al*., 2002; Junge *et al*., 2002; Brinkmeyer *et al*., 2003). Within the family *Geobacteraceae*, the first psychrophilic and psychrotolerant species were recently isolated (Holmes *et al.*, 2004b; Nevin *et al.*, 2005). Our new isolates were able to grow down to temperatures of –2°C, similar to sulfate-reducing and sulfur-oxidizing bacteria previously isolated from fjord sediments of Svalbard (Knoblauch *et al.*, 1999; Knittel *et al.*, 2005), indicating the adaptation of the benthic microbial community to permanently low temperatures of the Arctic Ocean. For psychrophilic sulfate-reducing bacteria, a special adaptation to low temperature was shown by almost constant growth yields between 0°C and their respective optimum temperature for growth (Knoblauch & Jørgensen, 1999). A similar study for psychrophilic Fe-reducing bacteria is, however, complicated by the particulate nature of Fe oxide.

The isolation of a moderately thermophilic sulfate-reducing bacterium from a permanently cold fjord sediment indicated the abundance of a thermophilic population of sulfate reducers in these sediments. As the isolate was spore-forming and not able to grow below 26°C, it was suggested that it was present in the sediment as spores and had to multiply in an unknown habitat, where environmental conditions support growth. The current Ph D thesis by Maren Nickel concerns with the abundance, origin, and phylogenetic composition of the thermophilic sulfate-reducing community in sediments of Svalbard.

b) Substrate usage. The characterization of pure cultures indicates their potential activities and adaptation to environmental settings. All isolates utilized important fermentation products of marine sediments such as hydrogen, formate, acetate, lactate, and propionate. In addition to Fe, one or more other electron acceptor was reduced such as oxygen, Mn oxide, elemental sulfur, or sulfate. Thus, these bacteria *in situ* might be involved in various important processes. The repeated finding that sulfate-reducing bacteria, especially of the genus *Desulfovibrio*, are able to reduce Fe(III) indicates a possible contribution of sulfate-reducing bacteria to benthic Fe reduction (Coleman *et al.*, 1993; Lovley *et al.*, 1993; Li *et al.*, 2004; Chapters 2.1 and 2.3).

In general fermentation products such as hydrogen and volatile fatty acids are believed to be the major electron donors for Fe- and sulfate-reducing bacteria in marine sediments. However, the turnover rates of ^{14}C-acetate and ^{14}C-lactate of slurries of Smeerenburgfjorden sediment accounted only for a maximum of 43 and 6% of sulfate and Fe reduction, respectively. Similar results have been obtained previously from another fjord sediment of Svalbard (Arnosti *et al.*, 2005). Potential alternative electron donors are sugars, amino acids, and alcohols, which are degraded by several Fe- and sulfate-reducing bacteria (Rabus *et al.*, 2000; Lovley, 2004). The role of these substances has to be investigated in future studies.

To link the studies of pure cultures to biogeochemical processes in the natural environment it is important to identify the quantitatively important Mn- and Fe-reducing bacteria *in situ*. The size and composition of bacterial Mn- and Fe-reducing populations for most habitats is virtually unknown, as studies investigating the community by fluorescence *in situ* hybridization (FISH) are rare. For members of the *Geobacteraceae*, the *in situ* abundance is indicated for diverse habitats as sequences related to species of the *Geobacteraceae* have been found in 16S rDNA libraries including fjord sediments of Svalbard (Ravenschlag *et al.*, 1999). This and most other clone libraries of marine sediments lack sequences related to *Shewanella* or *Desulfovibrio*, which leaves open the question of their quantitative importance for carbon degradation in these habitats. The availability of the full genome sequences of

Shewanella oneidensis, *Geobacter sulfurreducens*, and *Desulfovibrio vulgaris* today allows to identify genes involved in Fe reduction and study the gene regulation inside the cell (Coppi *et al.*, 2001; Heidelberg *et al.*, 2002; Methé *et al.*, 2003; Heidelberg *et al.*, 2004; Methé *et al.*, 2005). New approaches to identify members of *Geobacteraceae* by real-time PCR target on a gene involved in Fe reduction, *omcB* (*c*-type cytochrome), but have so far only been tested in a chemostat-grown pure culture (Chin *et al.*, 2004). Another study targeted the gene for nitrogen fixation, *nifD*, of *Geobacteraceae* in a subsurface sediment (Holmes *et al.*, 2004a). However, currently it is not possible to identify the complete Fe-reducing community as Fe-reducing bacteria are interspersed throughout the bacterial domain and a common gene solely abundant in all Fe-reducing bacteria is not known. Thus, more studies on the size and composition of the microbial Mn- and Fe-reducing communities in marine sediments are needed to understand the regulation of Mn and Fe reduction *in situ*.

3.4 Outlook

Our results indicate that dissimilatory Mn and Fe reduction are important processes in permanently cold sediments around Svalbard and that these processes depend on the abundance of Mn and Fe oxides as well as the sedimentation of organic carbon.

Knowledge of the contents of Mn and Fe oxides in sediments around Svalbard is limited. In a forthcoming cruise this spring samples along a transect of the southern part along the east coast of Svalbard will be collect and the importance of microbial Mn and Fe reduction will be further investigated. As the area is characterized by decreasing carbon deposition to the sea floor towards the north due to the annually northwards retreat of the ice coverage, the cruise can give new insights into the influence of carbon supply on benthic carbon mineralization and the contribution of respiratory pathways especially of Fe and Mn reduction.

For most sediments where microbial Mn and/or Fe reduction was determined to be important for carbon oxidation, the bacterial metal-reducing population has not been determined. Studies of Mn-reducing bacteria are particularly lacking. In sediments of the Black Sea, higher numbers of Mn-reducing than Fe-reducing bacteria in most-probable-number (MPN) counts indicated that Mn reduction was not necessary conveyed by Fe-reducing bacteria, and a specialized Mn-reducing community might be abundant (Thamdrup *et al.*, 2000). Particularly the Mn and Fe reduction dominated sediments of the northeast coast provide a good location to analyze the bacterial community. With 16S rDNA clone libraries

sequences of potential Mn- and Fe-reducing bacteria can be identified. Based on these sequences, FISH probes can be designed to quantify a special phylogenetic population.

3.5 References

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