The Role of Volatile Fatty Acids and Hydrogen in the Degradation of Organic Matter in Marine Sediments

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Niko Finke

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1. Gutachter: Prof. Dr. Bo Barker Jørgensen

2. Gutachter: Piv. Doz. Dr. Matthias Zabel

Prüfer:

Prof. Dr. Jörn Peckmann

Dr. Sabine Kasten

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Thesis Abstract

This thesis presents results of experiments addressing the role of volatile fatty acids (VFA) and hydrogen in marine sediments. They are produced by fermenting bacteria and serve as substrates for the terminal oxidizers. Thus, VFA and hydrogen link the fermentation with the terminal oxidation processes in the degradation of organic matter in marine environment.

Despite the fact that more than 90% of the sea floor is permanently colder than 4°C, most studies on biogeochemical processes in marine sediments have been performed in temperate environments. In these temperate environments, biogeochemical processes show strong temperature dependences, with decreasing rates during the cold season. Previous investigations on terminal electron accepting processes revealed, however, that the rates in permanently cold arctic sediments are similar to rates measured in temperate environments at higher temperatures. The availability of organic matter, rather than the permanently low temperature has been suggested to be limiting the microbial activity in arctic fjords at Svalbard, Norway. As the temperature control of the different processes is not well understood, extrapolating rates from temperate sites to the cold habitats, measurements at temperate sites during the cold season, or incubation of temperate sediments at low temperatures does not lead to a better understanding of processes at permanently cold sites.

The primary aim of this thesis was to investigate the role of VFA and hydrogen in the degradation of organic matter in permanently cold environments. In one experiment, the effect of temperature changes on sulfate reduction rates (SRR) and VFA and hydrogen concentrations were studied using a temperature gradient. The responses to temperature changes in sediments from permanently cold and temperate environments were compared. Conclusions about fermentation processes could be drawn by comparing the pool sizes of the intermediates and the rate of the degradation process (i.e. the concentrations of VFA and hydrogen and the SRR). Low concentrations of the intermediates over a broad temperature range revealed a close coupling between fermentation and sulfate reduction that was not disturbed by the temperature change. Only above the optimum temperature for sulfate

reduction (~26 and 33°C for the permanently cold and temperate sites, respectively), the concentrations increased, showing a higher temperature tolerance of the fermenting bacteria compared to the sulfate reducing bacteria (SRB). In the sediments from the temperate site, the SRB were not as efficient in scavenging hydrogen at very low temperatures, resulting in concentrations above thermodynamically predicted values. Thus, the response to temperature changes reflected the different *in situ* temperature of the samples with respect optimum and maximum temperature. The sediments from the permanently cold site, however, are not less responsive to temperature changes than sediments from the temperate site, which experience seasonal temperature changes.

Polar environments encounter a strong seasonality in primary production with high productivity during the short summer period. To investigate the response of the microbial community to a single high input of organic matter, sediments were amended with different substrate types and the response of microbial processes was monitored in the second experiment. The microbial community responded rapidly to the addition of organic matter. The most pronounced effect was the transient increase in VFA and hydrogen concentrations, which reached approximately 100-fold of the initial concentrations. The response in the dissolved inorganic carbon (DIC) pool was delayed. The substrate addition resulted in an increase in DIC only after the VFA concentrations started to decrease again. The VFA and hydrogen concentrations returned to low concentrations, similar to levels measured in whole cores, before all the added carbon was mineralized. This could either be due to recalcitrance of the remaining carbon or an adjustment of the rates of the different steps to new steady state levels. The shift from the fermentation-dominated to the respiration-dominated processes shows that the response of the terminal oxidizers was slower compared to the fermentation. The relatively stable SRR over the first 160 hours despite the increase of VFA indicates that the sulfate reducers were not substrate limited in these experiments. After 48 days, approximately half to 2/3 of the added carbon was degraded in three of the four experimental treatments, in the fourth treatment, only 1/5 was degraded. The carbon degradation rates were similar to rates observed in temperate sediments at higher temperatures, showing that the permanently low temperatures do not rule out high metabolic rates. The difference in the substrate quality of the four

amendments, however, was not obvious from the bulk substrate characteristics.

In a third experiment on the degradation of organic matter in permanently cold sediments, the role of VFA and hydrogen under steady state conditions was addressed. Turnover rates of three different pools at different levels of the microbial food web, hydrolysis, sugar and VFA degradation, were analyzed in addition to terminal oxidation rates. The results of these experiments indicate that the initial and terminal steps of the degradation (i.e. hydrolysis and sulfate reduction) were similar to rates reported for temperate sites. The VFA turnover was lower than usually reported from temperate sites, but similar to rates measured during the cold season. Hence, temperature seems to have different effects on the different steps of the complex degradation pathway. Comparison of the VFA turnover rates with SRR, the dominant terminal electron accepting process, revealed that VFA accounted only for a small portion of the electron flow through to the inorganic electron acceptors. Thus, additional electron donors must account for a larger part of the electron flow as compared to previous studies at temperate sites. Whether this difference is due to different methodology or to the influence of the permanently low temperatures remains to be investigated. Unfortunately, to date, the hydrogen turnover in marine sediments can not be measured. The importance of hydrogen for the electron flow, therefore, remains open. Neither the VFA concentration nor the production rate could be correlated to the sulfate reduction rate. The actual mechanism controlling the SRR, therefore, remains uncertain.

In the last experiments, the role of the hydrogen concentrations in marine sediments was investigated. Hydrogen concentrations at steady state are controlled by the terminal electron accepting process. Microorganisms using the most favorable electron acceptor lower the hydrogen concentration to values that make it inaccessible for other microorganisms using less favorable electron acceptors. This thermodynamic control of the hydrogen concentration explains the spatial separation between the hydrogen oxidation steps, using different electron acceptors. Other substrates for the terminal oxidizing bacteria do not show a similar separation on a thermodynamic basis. Incubation experiments with methylamine and methanol revealed, however, that hydrogen concentrations exert control even on reactions hydrogen is not directly involved in. This is accomplished via hydrogenases that

catalyze the transfer of electrons from the cellular electron acceptors to hydrogen. The leakage of hydrogen is dependent on the external hydrogen concentration. At the lowest incubation temperature, the methylotrophic methanogens leaked hydrogen even under conditions favorable for methanogenesis from CO_2 and H_2. Thus, leaking energy out of the cells under these conditions means a loss of energy for the methylotrophic methanogen. The leakage likely represents a side effect of the presence of the hydrogenase, which is capable of catalyzing this reaction rather than an energy yielding reaction.

Zusammenfassung

Diese Doktorarbeit präsentiert die Ergebnisse von Untersuchungen zur Rolle von kurzkettigen Karbonsäuren und Wasserstoff in marinen Sedimenten. Sie werden von den fermentierenden Bakterien produziert und dienen als Substrat für die Bakterien, die die terminiale Oxidation durchführen. Auf diese Weise schaffen sie eine Verbindung zwischen der Fermentation und der terminalen Oxidation im komplexen Abbauweg von organischem Material.

Mehr als 90% des Meeresbodens sind permanent kälter als 4°C. Dennoch wurden die meisten Untersuchungen zu biogeochemischen Prozessen in temperierten Sedimenten durchgeführt. In diesen Sedimenten zeigen die untersuchten Prozesse eine starke Temperaturabhängigkeit mit niedrigeren Raten während der kalten Jahreszeit. Frühere Untersuchung zu terminalen Oxidationsprozessen zeigten aber, dass die Raten in permanent kalten Sedimenten ähnlich denen in temperierten Sedimenten bei höherer Temperatur sind. Als limitierender Faktor wurde die Verfügbarkeit von organischem Material eher als die niedrige Temperatur vorgeschlagen. Da die Temperaturabhängigkeit von den verschiedenen Prozessen noch nicht gut untersucht ist, ist es schwierig, die Messungen von wärmeren Standorten zu den niedrigen Temperaturen zu extrapolieren. Messungen während der kalten Jahreszeit oder Inkubationen von temperierten Sedimenten bei niedrigen Temperaturen können somit nicht zu einem besseren Verständnis von Prozessen an permanent kalten Standorten führen.

Das Hauptziel dieser Dissertation war es, die Rolle von kurzkettigen Karbonsäuren und Wasserstoff beim Abbau von organischem Material in permanent kalten Sedimenten zu untersuchen. Im ersten Experiment wurde der Effekt von Temperaturänderungen auf die Sulfatreduktionsraten, Karbonsäure- und Wasserstoffkonzentrationen in einem Temperatur-gradienten untersucht. Die Reaktion auf die Temperaturänderungen in permanent kalten und temperierten Sedimenten wurden verglichen. Rückschlüsse auf die Fermentationsprozesse konnte durch Vergleich von der Konzentration der Intermediate mit ihrer Abbaugeschwindigkeit (d.h. den Konzentrationen der Karbonsäuren und Wasserstoff und

der Sulfatreduktionsrate) geschlossen werden. Geringe Intermediatkonzentrationen über eine große Temperaturspanne zeigten eine enge Kopplung zwischen Fermentation und Sulfat-reduktion, die durch die Temperaturänderungen nicht gestört wurde. Nur bei Temperaturen über den Optimaltemperaturen für die Sulfatreduktion (~26 und 33°C für den permanent kalten, beziehungsweise den temperierten Standort), stiegen die Konzentrationen aufgrund einer höheren Temperaturtoleranz der Fermentierer an. In den Sedimenten des temperierten Standortes waren die Sulfatreduzierer auch bei geringen Temperaturen nicht Effizient bei der Aufnahme von Wasserstoff. Die Konzentrationen lagen über denen, die aufgrund von themodynamischen Berechnungen zu erwarten wären.. In der Reaktion der Sedimente auf die Temperaturänderungen spiegelt sich die unterschiedliche *in situ* Temperatur wieder. Die Reaktion der permanent kalten Sedimente auf Temperaturänderungen unterscheidet sich nicht grundlegend von der der temperierten Sedimente, die saisonalen Temperaturschwankungen ausgesetzt sind.

Polargebiete zeichnen sich durch eine ausgeprägte Saisonalität der Primärproduktion mit hoher Produktivität in der kurzen Sommerperiode aus. Um die Reaktion der Bakterien-gemeinschaft auf einen pulsartigen Eintrag von organischem Material zu untersuchen, wurden im zweiten Experiment Sedimentproben mit unterschiedlichen Substraten versetzt und die mikrobiellen Prozesse verfolgt. Die Bakteriengemeinschaft reagierte schnell auf die Zugabe von organischem Material. Der ausgeprägteste Effekt war der vorrübergehende starke Anstieg der Karbonsäure- und Wasserstoffkonzentrationen auf etwa das 100-fache der Ursprungskonzentrationen. Eine Veränderung im gelösten Karbonat trat erst verspätet ein. Die Konzentrationen nahmen erst zu, als die Konzentrationen der Karbonsäuren schon wieder abnahmen. Die Karbonsäure- und Wasserstoffkonzentrationen erreichten Werte, ähnlich zu Messungen in Sedimentkernen, bevor das zugegebene Substrat komplett abgebaut war. Der Grund hierfür kann entweder die hohe Refraktivität des verbliebenen Substrats oder aber ein neu eingestelltes Gleichgewicht zwischen Fermentation und Sulfatreduktion sein. Der Wechsel von fermentationsdominierten zu respirationsdominierten Prozessen zeigt, dass die Bakterien, die für die terminale Oxidation verantwortlich sind, langsamer auf die Substratgabe reagieren, als die Fermentierer. Die Sulfatreduktionsraten bleiben während der ersten 160 Stunden der Inkubation trotz der ansteigenden

Karbonsäurekonzentration relativ stabil. Dies zeigt, dass die Sulfatreduzierer in den Ansätzen nicht substratlimitiert sind. Nach 48 Stunden Inkubation war in drei der vier Ansätze die Hälfte bis 3/4 des zugegebenen Substrat abgebaut. Im vierten Ansatz war nur 1/5 des Substrats abgebaut. Die Mineralisationsraten waren ähnlich zu Messungen von temperierten Sedimenten bei höherer Temperatur. Dies zeigt, dass die permanent niedrigen Temperaturen hohe Stoffwechselraten nicht ausschließen. Die Unterschiede der Qualität der zugegebenen Substrate war den generellen Substrateigenschaften nicht anzusehen.

Im dritten Experiment zum Abbau von organischem Material in permanent kalten Sedimenten sollte die Rolle kurzkettiger Karbonsäuren und Wasserstoff unter Steady State Bedingungen untersucht werden. Die Umsatzraten von drei Kohlenstoffreservoiren auf unterschiedlichen Ebenen der mikrobiellen Nahrungskette, die Hydrolyse von Polysachariden und der Umsatz von Zuckern und kurzkettigen Karbonsäuren, wurde neben den Raten der terminalen Oxidation gemessen. Die Raten des ersten und letzten Schrittes, der Hydrolyse und der Sulfatreduktion, waren ähnlich zu früheren Messungen in temperierten Sedimenten. Der Umsatz der kurzkettigen Karbonsäuren war langsamer als in Messungen in temperierten Sedimenten bei höherer Temperatur, aber ähnlich zu Messungen in Wintermonaten. Die Temperatur scheint die unterschiedlichen Schritte im komplexen Abbauweg verschieden zu beeinflussen. Ein Vergleich der Karbonsäureumsatzraten mit den Sulfatreduktionsraten, dem dominierenden terminalen Oxidationsprozess, zeigte, dass die Karbonsäuren nur für einen geringen Teil des Elektronenflusses zum anorganischen Elektronenakzeptor verantwortlich waren. Alternative Substrate für die Sulfatreduzierer müssen für einen größeren Anteil am Elektronenfluss verantwortlich sein, als in Untersuchungen von temperierten Sedimenten berichtet wurde. Ob dieser Unterschied auf die unterschiedlichen Messprinzipien oder auf den Einfluss der permanent niedrigen Temperatur auf die Abbauprozesse zurückzuführen ist, muss noch genauer untersucht werden. Weder die Konzentrationen noch die Umsatzraten der kurzkettigen Karbonsäuren konnte mit den Sulfatereduktionsraten korreliert werden. Der kontrollierende Faktor für die Sulfatredutionsraten bleibt also weiter ungewiss.

Im letzten Experiment wurde die Rolle von Wasserstoffkonzentrationen in marinen

Sedimenten untersucht. Die Wasserstoffkonzentrationen werden unter Steady State Bedingungen vom terminalen Oxidationsprozess bestimmt. Die Bakterien, die den Elektronenakzeptor mit dem höchsten Energiegewinn nutzen, können die Wasserstoffkonzentrationen auf Werte senken, die andere Wasserstoffoxidationsschritte zur Energiegewinnung energetisch ausschließen. Diese Kontrolle der Wasserstoffkonzentration, basiert auf thermodynamischen Prinzipien, ist der Grund für die räumliche Trennung der Wasserstoffoxidationsprozesse mit unterschiedlichen Elektronenakzeptoren in aquatischen Systemen. Für andere Substrate zeigt sich eine solche thermodynamisch basierte Trennung nicht. Die Inkubationsexperimente mit radioaktiv markiertem Methylamin und Methanol zeigen, dass die Wasserstoffkonzentrationen im Sediment auch Reaktionen beeinflussen, an denen Wasserstoff nicht direkt beteiligt ist. Dies geschieht über Hydrogenasen, die die Übertragung von Elektronen von den zelleigenen Elektronenüberträgern auf Wasserstoff katalysieren. Der Verlust von Elektronen ist abhängig von der externen Wasserstoffkonzentration. Bei den niedrigen Inkubationstemperaturen verloren die metylotrophen Methanogenen Elektronen in Form von Wasserstoff auch bei Bedingungen, unter denen die Methanogenese von Wasserstoff und Kohlendioxid energetisch begünstigt ist. Die methylotrophen Methanogenen verlieren mit den Elektronen somit Energie. Der Transport von Elektronen aus der Zelle ist somit sicherlich nur ein Nebeneffekt, bedingt durch den Besitz einer Hydrogenase, die diese Reaktion katalysieren kann und keine energiebringende Reaktion.

Introduction

1 Carbon Cycle

The biogeochemical carbon cycle describes the path of carbon through different pools over geological time. The different pools are connected and each pool represents a source or sink for some of the other pools. By far the biggest pool is the rocks that contain 99% of the global carbon (80% as carbonates and 19% as reduced organic carbon) (Hedges 1992, Falkowsky et al. 2000). These pools are replenished by sedimentation and preservation of organic and carbonate material mainly in the oceans. The major sink for these pools is the weathering of exposed carbonate and reduced carbon on the continents. The present-day burning of fossil fuels and cement production has introduced an additional sink to these pools. Due to the very large pool sizes and relatively small inputs and outputs, the turnover of these two pools is in the order of several million years. Cycling, therefore, does not influence the carbon budget of the other pools on biological and human, or short term geological time scales. The remaining pools are more dynamic due to the much smaller pool sizes and higher flux rates. Of these, the ocean accounts for 93% of the "remaining" carbon (91% as inorganic carbon, 2% as organic carbon), while the terrestrial biosphere, atmosphere and humus account for 1.3 - 3.8% (Hedges 1992, Falkowsky et al. 2000). Turnover times of these pools are a few to a few thousand years.

Due to anthropogenic activity (burning of fossil fuels, cement production, change in land use) the input of organic carbon (C_{org}) to the atmosphere has increased, resulting in an increased CO_2 concentration. However, the concentration increase does not equal the predicted increase based on the elevated input rates (Falkowsky et al. 2000). Further study shows that the oceans and terrestrial soils have temporarily fixed increasing amounts of carbon, diminishing the increase of the atmospheric carbon (Falkowsky et al. 2000).

Understanding the exchange processes between the different pools is important for understanding the short term global cycles (Fig. 1). The ocean, the biggest of these dynamic reservoirs, is in contact to the atmosphere via exchange processes at the ocean surface.

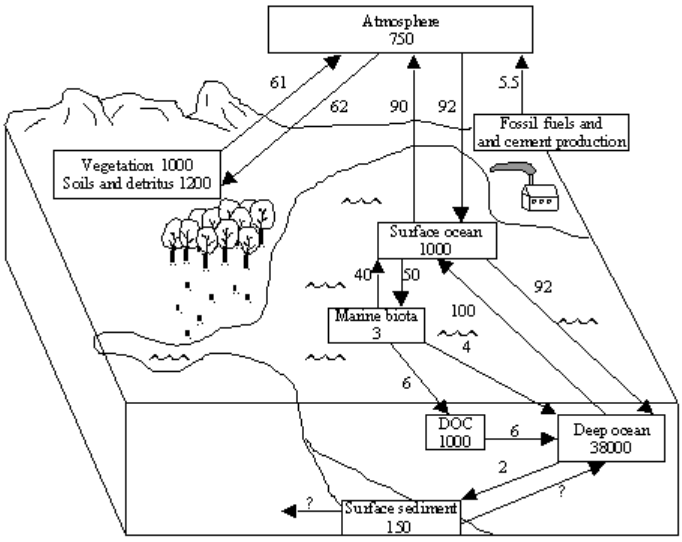

Figure 1: Simplified scheme of the global carbon cycle. The biggest reservoirs, the rocks are not shown. The pool sizes are in Pg C, the fluxes in Pg C year^{-1}. (Modified after Schmidt 1995, Falkowsky et al. 2000)

Depending on the physical and chemical conditions of the ocean water, the atmosphere acts as source or sink to the ocean. At the poles atmospheric CO_2 is sequestered by the cold, saline waters, due to the high solubility, and is exported to the deep sea (Sigmann & Boyle 2000, Ittekot et al. 2003). Photosynthesis in the photic zone consumes CO_2 and therefore decreases the partial pressure of CO_2 in the seawater. Biogenic carbonate precipitation in the upper water column, leads to an increase in the partial pressure. CO_2 fixed in organic matter via primary production and in carbonates is exported from the surface ocean through particle sinking into the deeper waters. The terrestrial photosynthesis also sequesters CO_2 from the atmosphere. Dead plant material is either rapidly remineralized or transferred into humus that is only slowly degraded. Degradation of organic matter in soils and weathering of silicate rocks are important sources of CO_2 to the ocean by riverine transport (Falkowsky et al. 2000). Besides the degassing to the atmosphere, the burial of carbonate or C_{org} is the only sink for carbon in the ocean. Most of the primary production is remineralized within the water column. The fraction of C_{org} that reaches the sediment is largely dependent on the water depth (Suess 1980, Wenzhöfer & Glud 2002, Zabel & Hensen 2002). In deep regions of the ocean, most of the primary production is already remineralized in the water column, whereas in shallow regions 10 - 50% of the primary production lands in the sediment (Jørgensen 1983, Canfield 1993). Even though the shallow shelf regions account for only

7.5 % of the ocean surface (Wollast 2002), their contribution to the total primary production is 18 - 33 % (Wollast 1991). Thus, the shelf sediments are important potential sinks for the C_{org} (Zabel & Hensen 2002). Most of the C_{org} is remineralized in the sediment, releasing CO_2 and nutrients into the pore water and the overlying water column (Canfield 1993). Only a small fraction of the C_{org} is ultimately buried. Understanding the processes that control degradation of organic matter in marine sediments is, therefore, important to quantify the carbon cycling in the oceans.

2 Degradation of organic matter

Organic matter in marine sediment derives mainly from photosynthetic primary production in the upper water column, and is either fully degraded to CO_2, mainly by microorganisms, or buried into the sediment. The degradation processes in the sediments can be differentiated into two zones. The oxic zone, where the organic matter is degraded with oxygen as electron acceptor, and the anoxic zone, where other electron acceptors are used.

With oxygen as an electron acceptor, complex organic matter can be degraded all the way to CO_2 by a single organism (e.g. Capone & Kiene 1988). The oxygen concentration in seawater is relatively low. In high productivity regions the oxygen is, therefore, usually consumed within millimeters, long before all C_{org} is remineralized (Canfield 1993). The rest of the C_{org} remains for anoxic degradation and burial.

In anoxic environments, several organisms work together in degrading high molecular weight organic matter, forming an anaerobic food web (e.g. Capone & Kiene 1988). The breakdown is performed in a series of steps (Fig. 2) that are performed by various bacteria. High molecular weight organic matter is too big to be taken up by bacteria and degraded intracellularly. The first step in the degradation of polymers is, therefore, the extracellular hydrolysis by exoenzymes, excreted by heterotrophic bacteria. The products of hydrolysis are mono-, di- and small oligomers, which are taken up by fermenting bacteria. The fermentation of the organic matter only allows for partial oxidation of the substrates. The substrates are either disproportionated or electrons are used to form molecular hydrogen

(e.g. Gottschalk 1985). The fermentation products are volatile fatty acids (VFA), small alcohols, small amines, CO_2 and hydrogen. Under steady state conditions, short-chain VFA and hydrogen were found to be the most important intermediates, with longer VFA and alcohols only formed as response to high input of organic matter (Schink 1988). The fermentation products are the substrates for the bacteria performing the terminal oxidation.

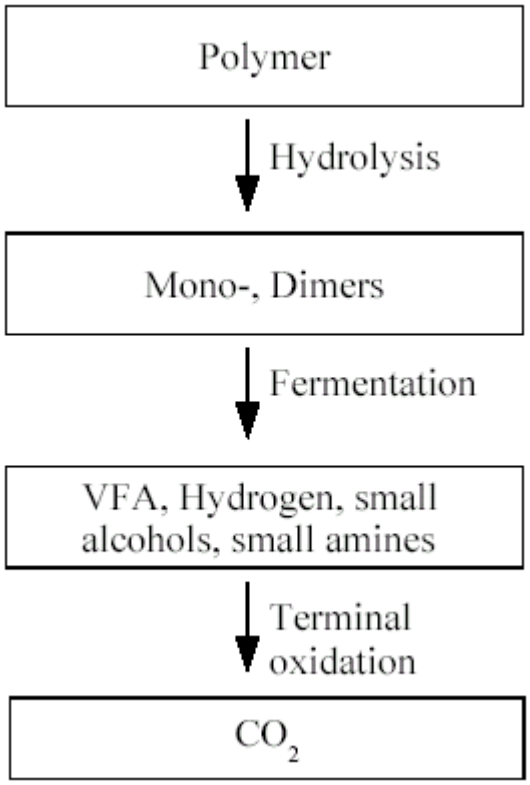

Figure 2: Simplified scheme of the degradation of organic matter in anoxic marine sediments.

Terminal oxidation is accomplished by the transfer of electrons to an inorganic electron acceptor (Froehlich et al. 1979, Table 1). The source of all of these electron acceptors (other than CO_2) is the overlying water column. Therefore, in fine-grained sediments, the concentrations in pore water are controlled by diffusion of the electron acceptor into the sediment, bioturbation and the degradation rate of C_{org} in the sediment. The different electron acceptors have varying energy yield for the oxidation of organic matter. The ones giving the highest energy yield are preferentially used, when available. Electron acceptors

therefore are sequentially depleted with sediment depth (Froehlich et al. 1979).

In the deep ocean, oxygen is the most important electron acceptor. In sediments with a relatively high load of organic matter, the oxygen is rapidly depleted and alternative electron acceptors are used (Canfield 1993). The concentration of nitrate is usually only

Table 1: List of inorganic electron acceptors used in marine sediments and their standard free energy yields, ΔG°, per mol organic carbon (from Jørgensen 2000).

Reaction	ΔG° (kJ mol-1)
$CH_2O + O_2 \rightarrow CO_2 + H_2O$	-479
$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O$	-453
$CH_2O + 3CO_2 + H_2O + 2MnO_2 \rightarrow 2MN^{2+} + 4HCO_3^-$	-349
$CH_2O + 7CO_2 + 4Fe(OH)_3 \rightarrow 4Fe^{2+} + 8HCO_3^- 3H_2O$	-114
$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$	-77
$4H_2 + CHO\ H^+ \rightarrow CH_4 + 3H_2O$	-136

very low in marine systems and manganese and iron reduction are limited by the low solubility of the oxidized minerals. Sulfate reduction is the most important electron acceptor in anoxic marine environments, accounting for up to 50% of the carbon mineralization (Jørgensen 1982). Once sulfate is depleted, the terminal oxidation is performed by methanogenic archea, producing methane and/or CO_2, depending on the substrate.

3 Volatile fatty acids and hydrogen as important intermediates in the marine carbon cycle

Volatile fatty acids (VFA) and hydrogen are important intermediates in the anaerobic food chain. Formed by fermenting bacteria, they serve as electron donors for microorganisms

carrying out terminal oxidation.

Turnover rates of VFA have been measured in a number of environments (e.g. Christensen & Blackburn 1982, Sansone & Martens 1982, Wellsbury & Parkes 1995, Wu & Scranton 1994). Two main methods have been used for these studies. The first method is the inhibition of sulfate reduction with molybdate (Oremland & Capone 1988) and measurement of the accumulation of the intermediates (Sørensen et al. 1981, Christensen 1984). The main problem with this method is that fermentation pathways are greatly influenced by the concentration of these intermediates (Gottschalk 1985). Increased concentrations will ultimately lead to a change in the fermentation pathway and the formation of different intermediates. In addition, molybdate inhibits only sulfate reducing bacteria. The method is therefore very specific for this single step, but the electrons channeled through bacteria using alternative electron acceptors are not included. The increased concentration of the intermediates due to the inhibition might even result in an increased consumption by these bacteria. Thus, the measured relative importance of the intermediates as substrates for the terminal oxidation do not necessarily reflect steady state conditions. In addition, molybdate complexes VFA and interferes with some of the HPLC methods for the VFA measurement (Finke 1999).

The second method is incubation with radio-labeled VFA (Ansbæk & Blackburn 1980). The decrease in radio-labeled VFA or the increase in $^{14}CO_2$ in the sediment gives the turnover rate constant. The turnover rate is calculated by multiplying the turnover rate constant with the concentration. Turnover rate measurements with radiotracer techniques have the advantage of not changing concentrations of the intermediates under investigation significantly. Several investigations revealed problems with the tracer technique (Christensen & Blackburn 1982, Parkes & Jorck-Ramberg 1984, Wellsbury & Parkes 1995), as the VFA are present in different pools with different biological availability. The concentration of freely-available acetate in the pore water is smaller than the measured concentrations. The added tracer is in the bio-available form of acetate, which is turned over more rapidly than the total acetate pool. The measurement of the total acetate pool is used for the calculation of the turnover rate, thus resulting in an overestimation of the *in situ* rate.

This was reflected in studies comparing the acetate turnover rate with sulfate reduction rates as well as comparison with total carbon mineralization rate (e.g. Christensen & Blackburn 1982, Wellsbury & Parkes 1995). In some of the studies the VFA turnover rate exceeded the sulfate reduction rate, even though acetate is only one of the potential substrates for sulfate reducing bacteria. With the radiotracer technique, the total turnover of a substrate will be measured, providing no information about the pathway or the electron acceptor. *As described in chapter 3, VFA turnover measurements with the radiotracer technique were performed in a permanently cold environment and compared to the sulfate and iron reduction rates. Long incubations were used for the VFA turnover measurements to minimize the problems associated with the different pools of VFA in the pore water.*

Hydrogen is also an important substrate for terminal oxidation processes. Under steady state conditions, concentrations are controlled by thermodynamics (see below) and reflect the terminal electron accepting process. The concentrations of hydrogen in aquatic environments are typically about 1000-fold lower than VFA concentrations. Direct turnover rate measurements of hydrogen are not yet possible. Molybdate inhibition experiments, as used for VFA turnover rate measurements, are even more problematic for hydrogen. Due to the higher stoichiometry, the hydrogen concentrations have a stronger influence on the fermentation pathways.

As important intermediates, the VFA and hydrogen concentrations reflect the coupling between fermentation and terminal oxidation. Under steady state conditions, the concentrations are low, reflecting a close coupling of the two steps. Changes in the environmental parameters can cause an imbalance in this coupling, resulting in elevated concentrations. Hoehler et al. (1998) reported increased acetate and hydrogen concentrations after sulfate depletion in coastal marine sediments. Due to the loss of the dominant electron acceptor, the fermentation products were degraded at a much lower rate and therefore accumulated in the sediments. The microorganisms that used a different terminal electron acceptor became active enough to restore steady state concentrations of the intermediates only after a significant time lag. Homogenization of marine sediments also resulted in increased pore water VFA concentrations (Finke 1999). The effect was enhanced

with elevated incubation temperatures. The relative concentrations of the acids was similar to results from molybdate inhibition (Sørensen et al. 1981), indicating that the increase was due to an uncoupling of fermentation and terminal oxidation. *In the second chapter, studies on the response of the potential hydrolysis, VFA concentrations and terminal oxidation to sediment mixing are described. In addition to a pure homogenization experiment, organic matter was added to the sediment to investigate the response of the microbial community to substrate addition.*

Pure culture studies have revealed a high versatility of sulfate reducing bacteria with respect of their energy and carbon sources. In addition to VFA and hydrogen sulfate reducing bacteria degrade fatty acids (C_1-C_{18}) (Widdel 1980), amino acids (Stams et al. 1985), aliphatic hydrocarbons (Aeckersberg et al. 1991), aromatic compounds (Widdel 1980) and simple sugars (Sass et al. 2002). In marine sediments VFA and H_2 have been found to be the most important substrates for sulfate reducing bacteria (Sørensen et al. 1981). The relative importance of substrates other than VFA and H_2 for sulfate reducers in marine sediments is not yet clear. The fact that sulfate reducing bacteria carry the potential to oxidize a wide range of substrates makes it unlikely that they are not using this ability to some degree in the environment. One possible reason for the limited substrate spectrum found with inhibition experiments would be the intense competition between sulfate reducers and fermenting bacteria for fermentable substrates. Even a slight increase in the concentration in intermediates such as monomers due to the inhibition of the sulfate reduction could lead to an increased uptake by the fermentative bacteria. These organisms would ferment the substrates to VFA and hydrogen. An increase in VFA and hydrogen would be observed and would be misinterpreted as the only substrates for the sulfate reducing bacteria. Additionally, VFA are often the only substrate group measured in these experiments (Sørensen et al. 1981, Christensen 1984). Parkes et al. (1989), however, measured increased amino acid concentrations in sediments following inhibition of sulfate reduction with molybdate. A further problem in determining the importance of a broad range of potential subtrates is that investigations with radiotracers lead to turnover rates of specific substrates, yielding no information about the pathway or the involved organisms. The pathways can only be estimated by the comparison of turnover rates and, for example, the rate of terminal

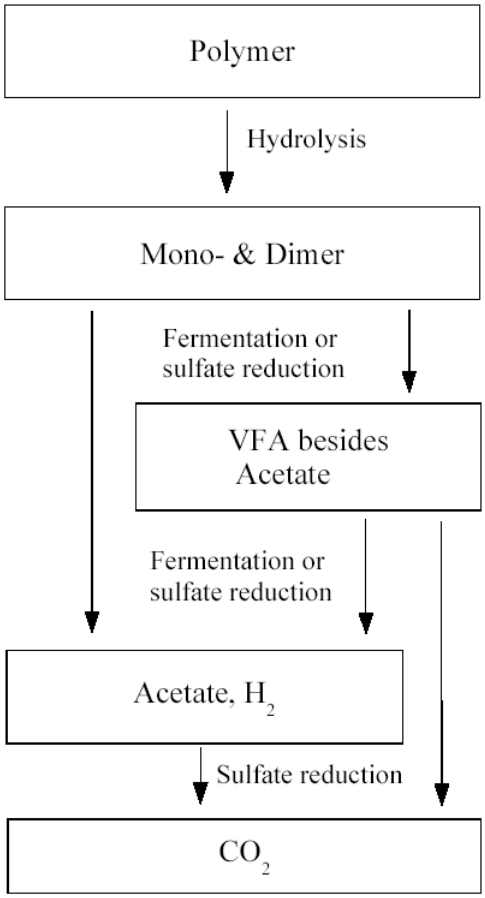

Figure 3: Possible alternative scheme of the degradation of organic matter in sulfate reducing sediments, taken the high versatility of sulfate reducing bacteria into account.

oxidation measured with the inorganic electron acceptor. Due to multiple pools of VFA with different availabilities (Christensen & Blackburn 1982, Parkes & Jorck-Ramberg 1984, Wellsbury & Parkes 1995), the reported rates measured with short incubation times likely overestimate the turnover of the VFA. Lower actual VFA turnover rates might not cover the electron flow for the sulfate reduction, thus demanding other substrates for the sulfate reducers (Fig. 3).

4 Controlling parameters

Rates of biogeochemical processes in marine environments, and concentrations of the chemical species involved in degradation processes span a wide range. For example, SRR

span from ~1nmol cm^{-3} d^{-1} (Knoblauch et al. 1999, Weber et al. 2001) to > 4 µmol cm^{-3} d^{-1} (Ferdelmann et al. 1997, Elsgaard et al. 1994), H_2 concentrations range from 30 pM to 130 nM under steady state conditions (Hoehler et al. 1998) and µM concentrations have been reported under sulfate - methane transition (Hoehler et al. 1999), $S_2O_3^{2-}$ concentrations in marine environments range from 0.1 - >100µM (Zopfi 2000). The regulating parameters for this variability are not understood in all cases. Temperature and thermodynamics, two physical and chemical parameters that influence microbial processes, will be discussed in more detail below.

4.1 Temperature

Temperature affects biogeochemical processes in a number of ways. Probably the most obvious effect is the increase in chemical reactions with increasing temperature according to the Arrhenius law (Arrhenius 1889). This direct effect is also reflected in the activity of enzymes and mircroorganisms for a limited temperature range (Ratkowsky et al. 1982). Process studies of enzymes (Lengeler et al. 1999), pure cultures (Knoblauch & Jørgensen 1999) and sediments (Arnosti et al. 1998, Sagemann et al. 1998, Wellsburry et al. 1997, Fey & Conrad 2000) showed increasing metabolic rates with increasing temperature over a wide temperature range. Accordingly, decreasing rates of microbial degradation processes during the cold season have been reported for temperate sites (e.g. Jørgensen & Sørensen 1985, Crill & Martens 1987, Westrich & Berner 1988). But sediments from permanently cold sites exhibit fermentation and terminal oxidation rates similar to those found at temperate sites at higher temperatures (Glud et al. 1998, Sagemann et al. 1998, Thamdrup & Fleischer 1998, Knoblauch & Jørgensen 1999). These authors concluded that the remineralization processes are limited by the supply of organic matter rather than the temperature. Giardina & Ryan (2000) reported, that decomposition rates of C_{org} in mineral soils does not vary with the mean annual *in situ* temperature. Thus, the bacteria living in different climate regions must be adapted to the *in situ* temperature to overcome the pure chemical effect of varying temperatures. To understand global cycles, process rates from measurements at temperate sites can not be extrapolated to the low temperatures, as the effect of temperature on the complex interaction of the microbial food chain are not understood. *In chapter 3 of this*

thesis, several steps of the degradation of organic matter in permanently cold sediments were measured and compared to the terminal oxidation steps.

Wiebe et al. (1993) found enhanced substrate requirements for heterotrophic bacteria growing in complex media at low temperature. Nedwell & Rutter (1994) concluded that heterotrophic bacteria have a lowered affinity for substrates that are taken up by active transport at low temperatures. Reay et al. (1999) found, that nitrate and ammonia show distinctively different temperature response in pure culture studies. Nitrate taken up by active transport shows higher residual concentrations at low temperatures, while ammonia taken up by channel mediated diffusion, in contrast, shows no temperature dependence. The authors concluded that the effect of lowered temperature on the viscosity of the bacterial membrane is responsible for the effect on affinity (Pomeroy & Wiebe 2001).

Increasing rates at elevated temperatures can indirectly affect biogeochemical processes and pathways. Hoehler et al. (1998) reported a decreased sulfate penetration in summer months in coastal marine sediments. In response to the loss of the dominating electron acceptor, the terminal oxidation shifted to methanogenesis. This shift was delayed, with a transition of high concentrations of fermentation products and predomination of acetogenesis. *Similar observations are reported in chapter 1 of this thesis from permanently cold and temperate sediments that were heated in a temperate gradient block.*

Detailed studies on temperature effects on most intermediates in anaerobic degradation of organic matter are rare. Despite the finding of decreased substrate affinity at lowered temperature by Nedwell & coworkers (Wiebe et al. 1993, Nedwell & Rutter 1994, Reay 1999, Pomeroy & Wiebe 2001), studies on the *in situ* concentrations and turnover rates of organic substrates that are taken up by active transport, such as mono- and disaccharides, are missing. Westermann (1992, 1994) studied the temperature response of steady state VFA and hydrogen concentrations in limnic sediments over a wide temperature range. The hydrogen concentrations showed a strong increase with incubation temperature, whereas the concentrations of VFA slightly decreased. The variation in concentration balanced the temperature-related change in the thermodynamics (see below), thus keeping the free energy

of the degradation reactions constant. Similar observations have been made for hydrogen in marine environments (Lovley & Goodwin 1988, Hoehler et al. 1998), but investigations of VFA were missing. *In chapter 1 of the thesis, the change of VFA and hydrogen concentrations and sulfate reduction rates as response to a temperature change was measured. The combination of the two measurements allowed investigation of the effect of temperature on the coupling between fermentation and sulfate reduction, which is reflected in the concentrations of the intermediates.*

4.2 Thermodynamics

Thermodynamics play a key role in any chemical reactions, as the free energy needs to be negative for the reaction to occur. Standard free energy of a reaction does not reflect the thermodynamics at *in situ* conditions, however, as temperature and the concentrations of the participating compounds play a significant role in defining the non-standard state energy yield. The free energy yield of a reaction at non-standard conditions is defined as:

$$\Delta G_r = \Delta G°(T) + RT \ln\left(\frac{\prod P^y}{\prod E^z}\right) \qquad \textit{equation } 1$$

where ΔG_r is the Gibbs free energy for a reaction under non-standard conditions, $\Delta G°$ is the standard Gibbs free energy, R is the gas constant, and T is the temperature in Kelvin. P represents the reaction products whereas E represents the reactants, y and z are the stoichiometric coefficients for the reaction. To illustrate the importance compound concentration on the energy yield of a reaction under non-standard conditions, one could consider the reaction mediated by some methanogenic archea. Under typical conditions, these microorganisms produce methane from CO_2 and hydrogen (Thauer 1994). It has been postulated that at low hydrogen concentrations, resulting from sulfate reducing activity, the methanogens perform the reverse reaction, anaerobic methane oxidation (Alperin & Reeburgh 1984, Hoehler et al. 1994).

Minimum concentrations of a substrate are set by two important parameters. First, the

affinity of enzymes for a substrate set the minimum concentration at which a substrate can be degraded. Secondly, the concentrations of the additional products and substrates of the reaction influence the minimum concentration at which the reaction is still exergonic. For energy conserving steps, the reaction does not only need to be exergonic, i.e. have a negative ΔG, but must also meet the minimum energy requirements of bacterial cells. ATP formation can be directly coupled to the reduction of organic substrates via substrate level phosphorylation. Under energy limiting conditions, however, ATP is usually formed by an ATPase driven by a proton gradient across the cell membrane. ATP is synthesized by the translocation of 3-5 electrons across the membrane and requires approximately $\Delta G = -45$ kJ mol^{-1} (Thauer & Morris 1984). Thus, the minimum energy requirement for the electron translocation is about $\Delta G = -10$ to -15 kJ mol^{-1} considering a non-ideal energy transfer a value of $\Delta G = -20$ kJ mol^{-1} is usually used as the minimum energy requirement (Schink 1997). Thermodynamic calculations can therefore not only tell whether a reaction is possible, but also if it meets the minimum energy requirements for energy conservation of $\Delta G = -20$ kJ mol^{-1}. Degradation of a substrate in a microbial consortium is only possible if minimum energy requirements all organisms involved are met.

As mentioned above, hydrogen serves as a good example for illustrating the thermodynamic control of the concentration of an intermediate in the degradation of organic matter. Hydrogen concentrations are very dynamic, varying with incubation temperature (Westermann 1992, Hoehler et al. 1998), terminal electron acceptor (Lovley & Goodwin 1988, Hoehler et al. 1998) and the concentration of the terminal electron acceptor (Hoehler et al. 1998). The change in concentration counter balances the change in free energy resulting from the varying parameters. The hydrogen concentration in sediments forms a thermodynamic basis for the spatial separation of redox processes in aquatic environments. The bacteria using the most favorable, available electron donor lower the hydrogen concentration to levels that make the oxidation with less favorable electron donors endergonic or not meet the minimum energy requirement (Lovley & Goodwin 1988, Hoehler et al. 1998). Thus, the hydrogen concentrations are not only controlled by thermodynamics of the terminal electron accepting process, but also exert a thermodynamic control on alternative hydrogen oxidizing reactions. *In chapter 4 of this thesis, the influence*

of hydrogen concentrations on methanogenesis from methylamine and methanol was investigated.

Volatile fatty acid concentrations are not as strongly controlled by thermodynamics. In methanogenic, limnic sediments, the VFA concentration changed with incubation temperature (along with the hydrogen concentration) to counter balance the change in free energy due to the temperature variation (Westermann 1992, 1994), but the terminal electron acceptor does not exert a strong influence on the VFA concentrations. Due to the lower stoichiometry, the VFA concentrations would need to change more drastically, than the hydrogen concentrations. The concentrations in methanogenic sediments would need to be about 1000-fold higher than in sulfate reducing sediments to overcome the thermodynamic difference, but the concentrations usually differ only by a factor of 10 or less (eg. Blair & Carter 1992, Albert & Martens 1997). Thus, the VFA concentrations do not form a thermodynamic basis for spatial separation according to the terminal electron acceptor. *Chapter 4 of this thesis focuses on the potential of hydrogen to influence microbial oxidation of intermediates other than hydrogen. In chapter 1 the influence of the temperature on the thermodynamic control of hydrogen concentrations in permanently cold and temperate sediments was investigated.*

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Aim and outline of the present study

The importance of volatile fatty acids and hydrogen as intermediates in the sedimentary carbon cycle has been recognized for some time. Previous investigations ranged from concentration measurements or turn over rate measurements and comparison to terminal electron accepting processes to investigations of the seasonality of the concentrations and turnover rates. Almost all of these investigations were performed at temperate sites, where the microbial processes exhibit a strong correlation to temperature changes. Potential hydrolysis and sulfate reduction, the initial and terminal step of the degradation of organic matter were measured in permanently cold sediments before. The rates were similar to rates from temperate sites at higher temperatures. It was suggested, that the availability of organic matter, rather than the low *in situ* temperature limited the rates. As the sulfate reducers, the dominating terminal oxidizers in anoxic marine environments, degrade only small molecules, the availability of complex organic matter can not be the rate determining factor. Rather, the intermediates between fermentation and sulfate reduction, VFA and hydrogen, should play a more dominant role in regulating the terminal oxidation. Accordingly, the role of VFA and hydrogen as substrates for sulfate reducing bacteria needed to be investigated in permanently cold environments. Furthermore, potential controlling parameters for VFA and hydrogen concentrations should be investigated. The response of fermentation and sulfate reduction to temperature changes and input of organic matter should be monitored. Low concentrations of these intermediates reflect a close coupling of the fermentation and sulfate reduction.

Additionally, the role of hydrogen in marine sediments in affecting redox reactions that do not involve hydrogen warranted investigation. Culture studies had revealed that lowering the hydrogen concentration in a methanogenic culture by addition of a sulfate reducer resulted in a shift in the ratio between the oxidized and the reduced products of the methanogen. It should be investigated whether this phenomenon is observed only under hydrogen concentration that reflect sulfate reducing conditions. Under these conditions, the leakage of hydrogen might be beneficial for the methanogen in contrast to hydrogen

concentrations typical for methanogenesis.

In the first chapter, the influence of temperature on sulfate reducing and fermenting bacteria was investigated. Sediments from permanently cold and temperate sites were incubated at temperatures ranging from ~0 - 40°C. Changes in sulfate reduction, VFA and hydrogen concentrations were measured. Conclusions on the response of the fermenting community could be drawn from the comparison of the pool size of the fermentation products and their degradation rate (i.e. the VFA and hydrogen concentrations and the SRR)

In chapter 2, the response of the microbial community to a pulse of organic matter was monitored. Homogenized arctic sediment was amended with fresh organic carbon in the form of freeze-dried algae and the extracted, high molecular weight carbohydrates of the two algae in parallel incubations. Potential hydrolysis of polysaccharides, VFA concentrations and turnover, H_2 concentrations and SRR were monitored over 48 days of incubation.

In chapter 3, the sequence of the degradation steps of polysaccharides was investigated at two permanently cold, arctic sites. Potential hydrolysis, monomer turnover and VFA turnover rates were measured in addition to the terminal electron accepting processes. The VFA turnover and the SRR were investigated in detail.

Chapter 4 focuses on the effect of extracellular hydrogen concentrations on microbial degradation pathways, in which hydrogen is not directly involved. The relative importance of the oxidized and reduced products of methanogenesis from methylamine and methanol under changing hydrogen concentrations were quantified, using radiotracer techniques. Methylamine and methanol are uncompetitive substrates for the methanogenic archaea. This means, they can only be degraded by the methanogens, not by the sulfate reducers. The hydrogen concentrations were caused to vary as a function of incubation temperature and sulfate concentration.

Overview of manuscripts

The response of sulfate reduction and fermentation to temperature changes in temperate and permanently cold sediments

Niko Finke, Bo Barker Jørgensen

Anoxic carbon degradation in Arctic sediments: Microbial response to substrate addition

Carol Arnosti, Niko Finke, Ole Larsen, Sherif Ghobrial

Degradation of organic matter in two permanently cold sediments, Svalbard, Norway

Niko Finke, Ole Larsen, Carol Arnosti, Bo Barker Jørgensen

Affect of hydrogen concentrations on methanogenesis from methylamine and methanol

Niko Finke, Tori Michael Hoehler, Bo Barker Jørgensen

Chapter 1

The response of sulfate reduction and fermentation to temperature changes in temperate and permanently cold sediments

(prepared for submission to Biogeochemistry)

Niko Finke*, Bo Barker Jørgensen

Dept. of Biogeochemistry, Max Planck Institute for Marine Microbiology, Celsiusstr. 1, 28359 Bremen, Germany
*Corresponding author: nfinke@mpi-bremen.de

Abstract

Sulfate reduction rates, volatile fatty acids (VFA) and H_2 were analyzed in sediments from temperate and permanently cold environments using a temperature gradient block ranging from 0.3 - 40°C. To study the temperature response of fermenting and sulfate reducing bacteria, measurements were undertaken after multiple incubation periods in the temperature gradient block. In permanently cold Arctic sediment, the optimum temperature of sulfate reduction decreased with prolonged incubation, whereas rates increased. The samples from a temperate North Sea showed a higher optimum temperature that did not change between short and long term incubations. The VFA and H_2 concentrations revealed a close coupling of fermentation and sulfate reduction, reflected in low concentrations at both stations. Up to a critical temperature, the concentrations of VFA remained low (<3 μM for acetate and <1 μM for the other VFA) and the hydrogen concentration stayed close to the thermodynamically predicted value. Thus, the sulfate reduction rate and the fermentation rate changed at the same rate at each temperature. According to the different *in situ* temperatures of the samples, the critical temperature was lower for sediments from the arctic than from the temperate station. In samples above the critical temperature, the concentrations of the VFA and hydrogen increased 100 - 1000 fold. The hydrogen concentrations decreased again upon prolonged incubation to values that are typical for sulfate depleted methanogenic sediments. This shows that fermentative bacteria and methanogenic archea in both sediments tolerated higher temperatures than the sulfate reducing community. Sediments from the temperate station with seasonal temperature variations did not show a higher potential to respond to changes in temperature than the sediment from the permanently cold station. This indicates that different populations of microorganisms are active in the temperate sediment over the course of the year.

Introduction

Degradation of organic matter in marine sediments is controlled by the quality and quantity of the organic matter (Arnosti et al. 1998, Thamdrup & Fleischer 1998). Temperature is also a major regulationg factor (Westrich & Berner 1988, Westermann 1992, Nedwell 1999, Pomeroy & Wiebe 2001). Rates of microbial processes at temperate stations decrease substantially during the cold season (eg. Jørgensen & Sørensen 1985, Crill & Martens 1987, Westrich & Berner 1988), whereas sediments from permanently cold stations may have sulfate reduction and nitrification rates similar to rates at higher temperatures at temperate stations (Sagemann et al. 1998, Thamdrup & Fleischer 1998, Knoblauch & Jørgensen 1999). The response of bacterial processes to changes in temperature vary according *in situ* temperature (Arnosti et al. 1998, Sagemann et al. 1998) and the studied metabolic process (Arnosti et al. 1998, Arnosti & Jørgensen 2003). Additionally, bacteria show a wide range of temperature adaptation (Wiebe et al. 1993, Knoblauch & Jørgensen 1999, Isaksen & Jørgensen 1996, Nedwell 1999). Sulfate reduction rates (SRR), accordingly, shows a strong response to incubation temperature, that is dependent on the *in situ* temperature of the sediment (Arnosti et al. 1998, Sagemann et al. 1998). Carbohydrate hydrolysis has a different temperature response than SRR with a similar optimum temperature but a higher maximum temperature in permanently cold and temperate samples (Arnosti et al. 1998). Acetate production from organic matter in coastal sediment (Wellsburry et al. 1997) showed a temperature response with a higher optimum and maximum temperature than for sulfate reduction from comparable temperate stations (Arnosti et al. 1998).

In anoxic sediments organic matter is degraded through a sequence of steps with different organisms involved (eg. Capone & Kiene 1988). Starting with the extracellular degradation of polymers followed by the fermentation of the mono- and small oligomers to mainly volatile fatty acids (VFA) and H_2 and the terminal oxidation of the VFA and H_2 to CO_2 and water. In anoxic marine shelf sediments sulfate reduction is the most important terminal oxidation step (Jørgensen 1982). Under *in situ* conditions the sulfate reduction and fermentation are usually well balanced resulting in low concentrations of VFA and hydrogen. Under steady state conditions the hydrogen concentration is thermodynamically

controlled by a change in free energy of -15 to -20 kJ mol^{-1} for its oxidation with the respective electron acceptor (Lovley & Goodwin 1988 , Schink 1997, Hoehler et al. 1998). The VFA concentrations are probably not thermodynamically controlled, but the concentrations are usually in the lower µM range (eg. Christensen & Blackburn 1982, Wellsburry & Parkes 1995, Wu & Scranton 1994). The actual mechanism controlling the concentration of VFA in marine pore waters in not yet understood. Spatial heterogeneity and temporal changes within the sediment can, however, influence the balance between fermentation and the terminal oxidation leading to higher concentrations of the fermentation products. Hoehler et al. (1998) reported increased VFA and hydrogen concentrations after sulfate depletion during spring in Cape Lookout Bight during the transition from sulfate reducing to methanogenic metabolism. Mixing of marine sediments also disturbs the close coupling between fermentation and sulfate reduction causing the VFA and hydrogen concentrations to increase (Finke 1999, Arnosti et al., in prep., Chapter 2). The response of the VFA concentration to mixing was enhanced by increased temperatures, but the steady state concentrations were again the same after 8 days, regardless of the incubation temperature (Finke 1999).

The uncoupling between sulfate reduction and fermentation by a temperature increase is not well understood. Under substrate limiting conditions the sulfate reducing bacteria (SRB) depend on increasing substrate production by the fermenting bacteria to increase their metabolism. The addition of substrate to sediment from Guayamas Basin resulted in a decrease of the optimum temperature for sulfate reduction in the sediment (Elsgaard et al. 1994). The authors concluded that the SRB were substrate limited at their optimum temperature and that the fermenting bacteria had a higher optimal temperature. To date it is not clear whether short and long term responses of the SRB are the same and whether the SRB and the fermenters respond similar to temperature changes. A faster response of the fermenting bacteria would lead to a transient increase in fermentation products at high temperatures. A potential faster response of the SRB would be masked by the substrate limitation and not lead to a change in substrate concentration or SRR.

Bacteria from temperate stations encounter seasonal variations in *in situ* temperatures. Bacteria from permanently cold stations on the other hand do not need to adapt to changing

environmental temperatures. Studies of aerobic respiration in sediments from temperate stations during different seasons revealed different temperature responses according to the *in situ* temperature (Thamdrup et al. 1998). This indicates that different populations adapted to different temperatures are active over the course of the year rather than one population well adapted for the whole temperature range. In the absence of temperature changes, it would be plausible to have just one specialized population in a permanently cold environment.

In the present study, we investigated the influence of a temperature shift on the SRR and the VFA and hydrogen concentrations in sediments from permanently cold and temperate environments. To analyse the short term response of fermenting and sulfate reducing sediments, we conducted time course experiments in a temperature gradient block.

Material and Methods

Sampling stations

The investigated sediments were sampled at four different stations. The stations BC and J are located in permanently cold fjords on the west coast of Svalbard, whereas the stations Neuharlinger Siel (NHS) and Weddewarden (WW) are situated on mud flats in the German Wadden Sea.

Station BC is located at 78°31'N, 015°06' E in the Nordfjorden, Svalbard. Sediments were taken with a HAPS corer in September 2001. The bottom water temperature was 1°C and the water depth 100 m. Sediment from the 3-8 cm depth horizon was transferred into brown glass jars, capped without air space, and stored at 0°C. Station J is located at 79°93'N, 11°05'E in the Smeerenburgfjorden, Svalbard. Samples were taken with a HAPS corer in July 1999. The bottom water temperature was 0°C and the water depth 215 m. Sediment from 2-9 cm depth was transferred into brown glass bottles and stored at 0°C.

Station NHS is located at Neuharlinger Siel at 53°42'8,5"N 7°42'33,2"E on an intertidal mud flat about 15 m seawards from the shore break in the German Wadden Sea. The

samples were taken by hand in October 2001 during low tide and sediment from 5-12 cm depth was filled into brown bottles and stored at 16°C. At the time of sampling, the *in situ* temperature ranged from 16°C at the sediment surface to 10,5°C at 15 cm depth. During the course of the year, the sediment surface temperature ranges from -1°C to about 30°C. Station WW is located at Weddewarden at 53°36.1'N 8°31.3'E on an intertidal mud flat on the east coast of the estuary of the German river Weser. Sediments were collected by hand in June 1995. The sediment temperature was 20°C at the sediment surface. The annual temperature ranges from 0 to 30°C. Samples were collected at low tide by hand and transported directly to the laboratory.

Incubation

The sediments from Stations J and WW were homogenized and diluted 1:1 with oxygen-free seawater from the sampling station. A 8-10 ml subsample of the slurry was transferred into N_2-flushed 12 ml Hungate tubes, that were immediately sealed with butyl rubber stoppers. The tubes were preincubated for 1 hour (station WW) or for 0.3, 2.1, 4.2, 6.2, 8.5, 10.8, and 11.5 days (station J) in a temperature gradient block (Sagemann et al. 1998) prior to the addition of 100 µl of $^{35}SO_4^{2-}$-tracer solution (100 kBq). The temperature gradient block consisted of a 200 cm x 15 cm x 15 cm insulated aluminum block, cooled at one end and heated at the other end. For this experiment the temperature range was set from -3.5 to 40°C. Sulfate reduction was terminated after 4-24 h by addition of 1 ml of 20% zinc-acetate solution. Reduced ^{35}S was analyzed by the single step chromium reduction method (Fossing & Jørgensen 1989), and the sulfate reduction rates were calculated per cm^3 of sediment as described by Jørgensen (1978).

The sediment from stations BC and NHS was filled into gas-tight plastic bags (Hansen et al. 2000), homogenized without airspace, and incubated for 3 weeks at 0°C for Station BC and 16°C for Station NHS. About 5 ml of the sediment were than filled into N_2-flushed 12 ml glass centrifuge tubes. The tubes were closed with 1 cm thick butyl rubber stoppers and placed in a temperature gradient block with a temperature range of 0.3 to 40°C. The tubes were placed in three rows of parallel holes in the block at temperature increments of 1.3°C. Headspace gas samples for hydrogen measurements and pore water samples for VFA and

sulfate analysis were taken after different preincubation periods in the temperature gradient block.

Hydrogen measurements

Hydrogen concentrations in the sediment were determined by a headspace equilibration technique (Lovley & Goodwin 1988, Hoehler et al. 1998). A 100 µl gas sample was withdrawn from the headspace of the glas centrifuge tubes with a gastight syringe and replaced with N_2. The H_2 concentration was measured with a reduced gas analyzer (RGA3, Trace Analytical). Hydrogen concentrations were calculated from the hydrogen solubility in seawater (Crozier & Yamamoto 1974). The concentrations were measured after 0.3, 1, 2, 4, 6, 10, and 29 days and 2, 3, 4, and 5 days of preincubation for the permanently cold station BC and the temperate station NHS respectively.

Volatile fatty acid measurements

Volatile fatty acids (VFA) were measured with an HPLC according to Albert and Martens (1997). After 6, 10, and 29 days for the BC samples and 3, 6, and 9 days for the NHS samples one of the three parallel rows of tubes was centrifuged at 2000 g at 4°C for 10 minutes. The supernatant was frozen in precombusted brown borosilicate vials. Before analysis, the acids were derivatized with p-nitrophenyl hydrazine (Albert & Martens, 1997). The derivatives were separated on an HPLC using a LiChrosphere 80/100 (Knauer) column and the concentrations determined from the absorption on a UV/VIS detector (Linear) at 400 nm. The detection limits for the different acids are 0.5 µM for glycolate, lactate, and propionate, 1 µM for acetate, formate, and isobutyrate, 2 µM for butyrate and 5 µM for isovalerate and valerate. Formate could only be measured at relatively low acetate levels, as the two peaks have very similar retention times.

Sulfate measurements

A 50 µl subsample was taken from the pore water and added to the same amount of 20% ZnAc to precipitate sulfide. The samples were frozen until analysis. For the analysis, 10 µl were diluted 1000-fold with distilled H_2O. The sulfate concentration was measured using a

non-supressed ion chromatograph with a Waters IC-Pak anion exchange column (50 x 4.6 mm) and a Waters 430 conductivity detector (Ferdelman et al 1997). The eluant was 1 mM isophthalate buffer in 10% methanol, adjusted to pH 4.5 with saturated sodium borohydrate. The flow rate was 1 ml min^{-1}.

$$\Delta G_r = \Delta G° + RT * \ln\left(\frac{\prod P^z}{\prod E^z}\right) \qquad \textit{equation } 1$$

Calculated H_2 concentrations

Hydrogen concentrations based on thermodynamic predictions (Hoehler et al. 1998) were calculated for the different hydrogen oxidation pathways: sulfate reduction, acetogenesis and methanogenesis (Damgaard & Hanselmann 1996). The calculations are based on the equation:

ΔG_r is the Gibbs free energy for the reaction under non-standard conditions, $\Delta G°$ is the standard Gibbs free energy, R is the gas constant, T the temperature in Kelvin. P are the reaction products whereas E are the educts, and y and z are the stoichiometric coefficients. The free energy was adjusted to temperature changes according to the van't Hoff equation:

$$\Delta G_r(T) = T * \left(\frac{\Delta G_r}{298K} + \left(\frac{\Delta H°}{T} - \frac{\Delta H°}{298K}\right)\right) \qquad \textit{equation } 2$$

where $\Delta H°$ is the enthalpy of the reaction. Temperature changes of the enthalpy were not included in the calculation as the change is relatively small within the tested temperature range (Hoehler 1998). The rearrangement of equation 1 gives the equation for calculation of the hydrogen concentration at steady state conditions. For sulfate reduction this is:

$$[H_2](T) = \left(\frac{[HS^-]}{[SO_4{}^{2-}]} * \exp\left(\frac{\Delta G°_s(T) - \Delta G°}{RT}\right)\right)^{0.25} \qquad \textit{equqtion } 3$$

for acetogenesis:

$$[H_2](T) = \left(\frac{[acetate]}{[CO_2]^2} * \exp\left(\frac{\Delta G^\circ{}_a(T) - \Delta G^\circ}{RT} \right) \right)^{0.25} \qquad \textit{equation } 4$$

and for methanogenesis:

$$[H_2](T) = \left(\frac{[CH_4]}{[CO_2]} * \exp\left(\frac{\Delta G^\circ{}_m(T) - \Delta G^\circ}{RT} \right) \right)^{0.25} \qquad \textit{equation } 5$$

$\Delta G^\circ_s(T)$, $\Delta G^\circ_a(T)$ and $\Delta G^\circ_m(T)$ are the temperature adjusted standard free energies for sulfate reduction, acetogenesis and methanogenesis, respectively.

Results

Sulfate reduction

Svalbard: Sulfate reduction rates (SRR) were measured in slurries of sediment from the permanently cold Station J, Smeerenburgfjorden, Svalbard, in a temperature gradient block with seven different periods of preincubation in the block. The results from 0.3, 2.1, and 8.5 days of preincubation are presented here (Fig. 1a). After 0.3 days of preincubation, SRR increased 10-fold with temperature, from 20 nmol cm^{-3} day^{-1} at –3.6°C to 200 nmol cm^{-3} day^{-1} at 27°C. At higher temperatures SRR decreased again to 40 nmol cm^{-3} day^{-1} at 39°C. Sulfate reduction increased 70-fold after 2.1 days of preincubation, from 7 nmol cm^{-3} day^{-1} at –2.8°C to 480 nmol cm^{-3} day^{-1} at 21°C. The sulfate reduction decreased at higher temperatures to rates less than 9 nmol cm^{-3} day^{-1} at 35°C. After 8.5 days of preincubation the highest SRR was 880 nmol cm^{-3} day^{-1} and the optimum temperature was 18°C. The highest tested temperature for this incubation was 29°C. Optima and temperature response curves of the additional measurements were similar, albeit with more scatter at longer incubations (data not shown).

Pore water sulfate concentrations were measured the sediment from Svalbard Station BC

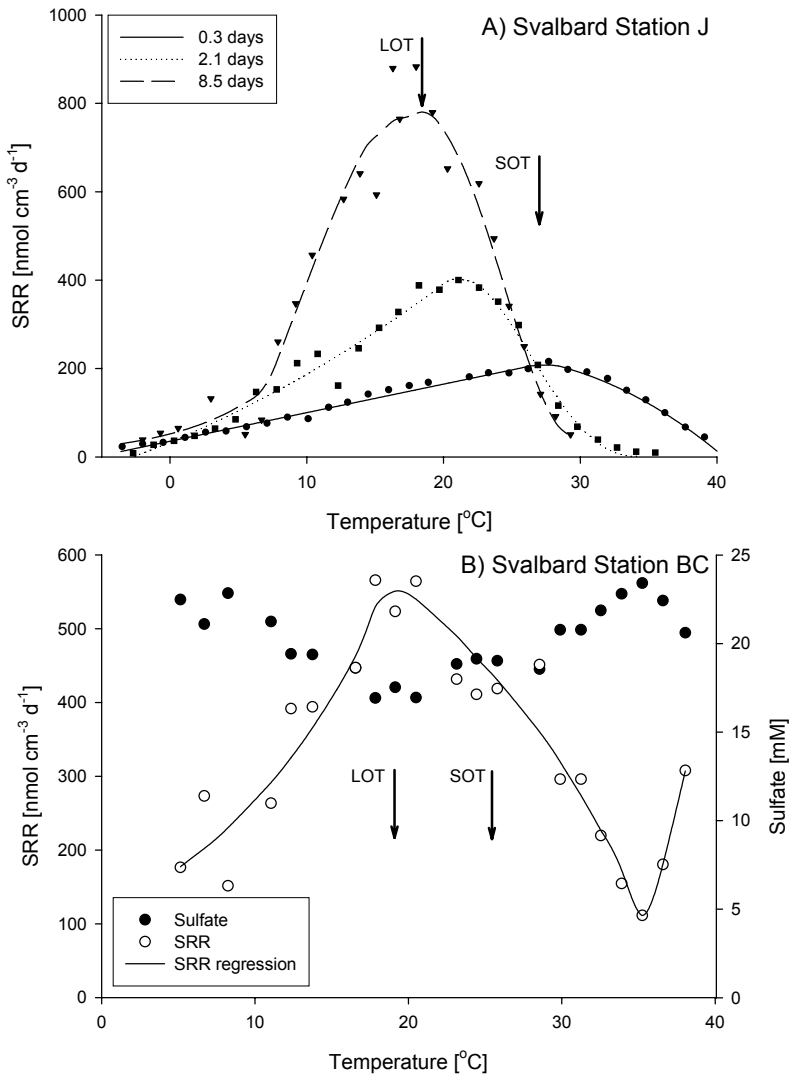

Figure 1: A) SRR from the permanently cold station J, with different times of preincubation. SOT = short incubation optimal temperature, LOT = long incubation optimal temperature
B) Sulfate concentrations and calculated SRR from the permanently cold station BC, measured after 10 days of incubation. SRR were calculated from the decrease in sulfate concentration. LOT, SOT as determined with the radiotracer incubation for station J.
Note the different temperature scales for the two graphs.

after 10 days of incubation (Fig. 1b). The sulfate concentrations in the pore water were about 23 mM at the lower temperature end slowly decreased with increasing temperature to reach a minimum of 17 mM at 18-20°C. At temperatures above 20°C the concentration increased again, reaching 24 mM at 35°C and showed slightly lower values at the maximum temperature. SRR were calculated from the decrease in sulfate concentration. The initial sulfate concentrations of 25 mM was used for the calculation.

Wadden Sea: The SRR in sediment slurries from the temperate tidal flat at Station WW,

are shown in figure 2a. The incubations were carried out after a preincubation of one hour in the temperature gradient block. The rates increased 60-fold from 30 nmol cm^{-3} day^{-1} at –3.5°C to a maximum of 1800 nmol cm^{-3} day^{-1} at 34°C. The rates decreased again to 1100 nmol cm^{-3} day^{-1} at the highest investigated temperature of 40°C.

In the sediments from the Wadden Sea Station NHS, pore water sulfate concentrations were

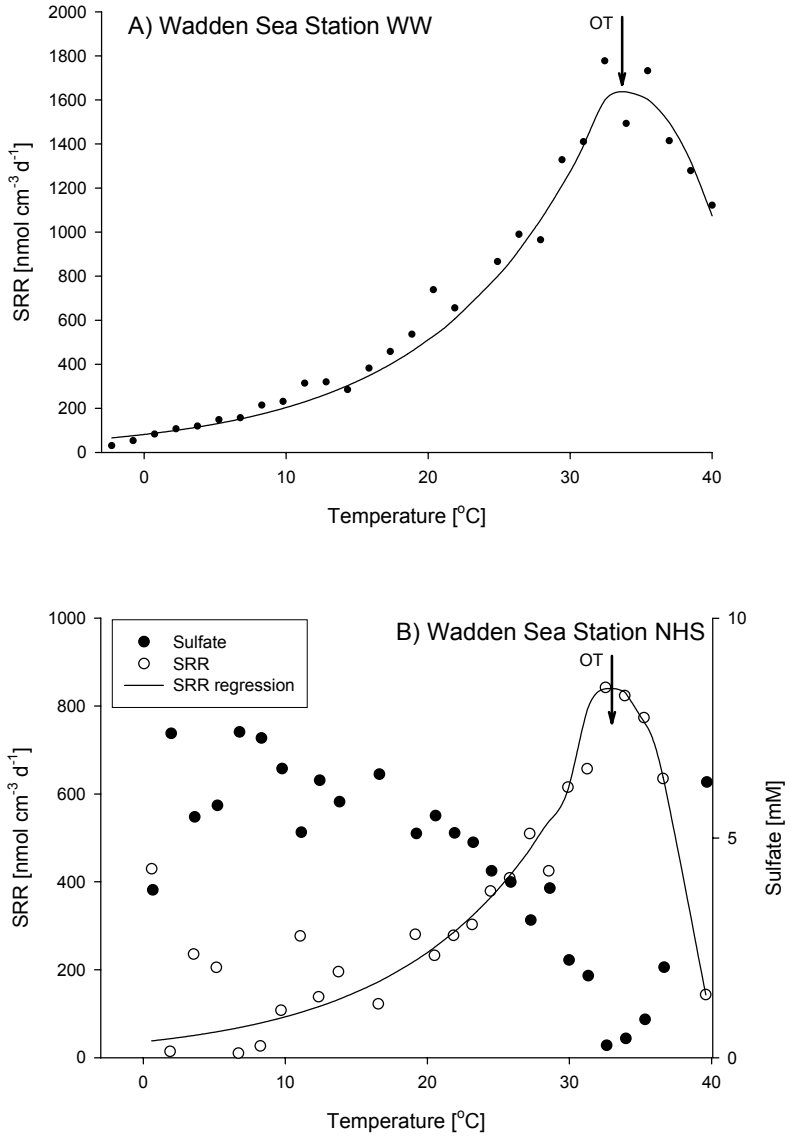

Figure 2: A) SRR from the temperate station WW in the German Wadden Sea, after 1 hour of preincubation. The data were previously published by Arnosti et al. (1998) and are reprinted with kind permission of the authors.
B) Sulfate concentrations and calculated SRR from the temperate station NHS in the Wadden Sea, measured after 6 days of preincubation. SRR were calculated from the decrease in sulfate concentration.
OT = optimal temperature for SRR

measured after 6 days of incubation (Fig. 2b). The concentrations started at about 6.5 mM at 0.3°C, slowly decreasing with increasing temperature, reaching a minimum of 0.28 mM at 32.5°C. At higher temperatures, the concentration increased to about 6.5 mM at 40°C. SRR were calculated from the decrease in sulfate concentration over time. The initial sulfate concentrations of 7.5 mM was used for the calculation.

Hydrogen

Hydrogen concentrations in the tubes with sediment from Svalbard Station BC were measured at seven different time points: after 3 hours, 1, 2, 4, 6, 10 and 29 days (Fig. 3). The hydrogen concentrations are plotted in addition to a theoretical curve based on thermodynamic calculations (Hoehler et al. 1998). The basis for the calculations are a free energy of $\Delta G_r = -20$ kJ mol^{-1} for sulfate reduction with hydrogen as an energy source, a sulfide concentration of 1 µM and a sulfate concentration of 25 mM. The actual sulfide concentration was below the detection limit of 1 µM for all samples.

At the first time point (after 3 hours), the measured hydrogen concentration was consistently 2-3 fold higher than the calculated theoretical values (Fig. 3a). At the later sampling times, the hydrogen concentrations began to deviate from the theoretical curve above a certain temperature. This critical temperature decreased from 34°C after 1 day of incubation to 25°C after 4 days and later. After 1 day, the concentration of hydrogen reached a maximum concentration of 500 nM at 36°C. After two days the maximum concentration had increased to 600 nM at 36°C.

With prolonged incubation the maximum hydrogen concentrations increased to a maximum of 5800 nM at a temperature of 36 °C after 4 days. After longer incubation, however, this peak in hydrogen concentration began to decrease again and was only 220 nM after 29 days of incubation at 30°C (Fig. 3b).

Hydrogen concentrations in the tubes with sediment from Wadden Sea Station NHS, the temperate tidal flat, were measured at four different time points: after 2, 3, 4, and 5 days of incubation (Fig. 4). Hydrogen concentrations are plotted in addition to a theoretical curve based on thermodynamic calculations. The basis for the calculations are a free energy of

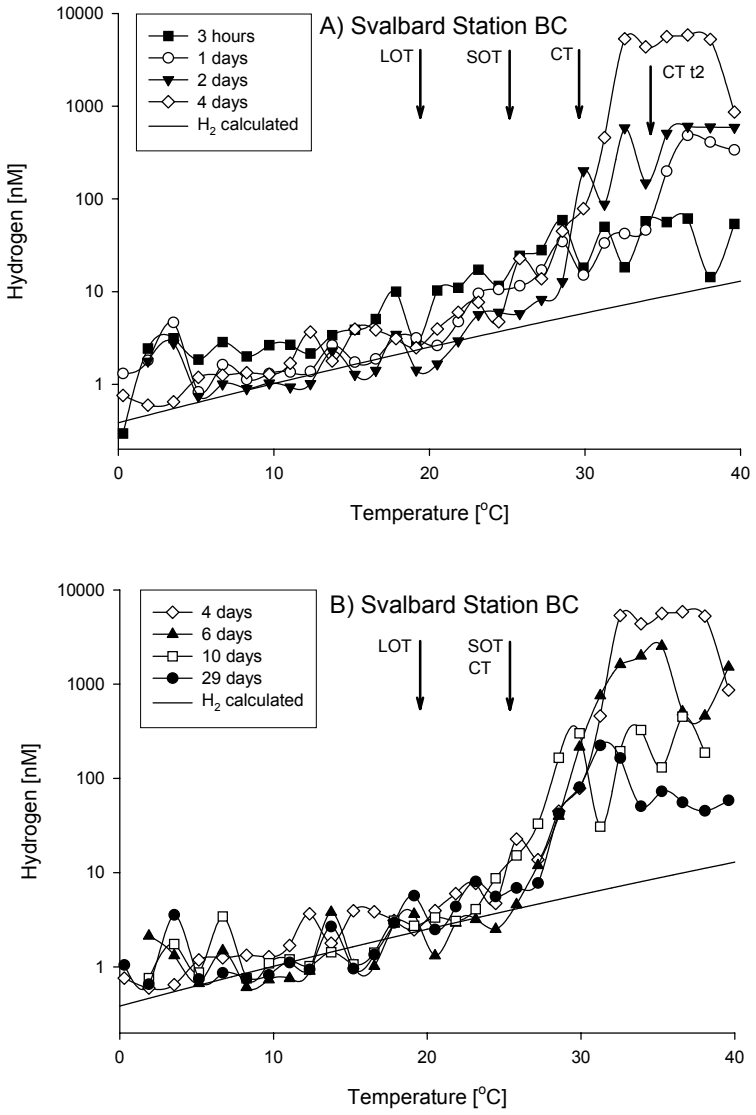

Figure 3: Hydrogen concentrations in the samples from the permanently cold Svalbard Station BC measured after different incubation periods: A) 0.3 to 4 d incubation, B) 4 to 29 d incubation. SOT = short incubation optimal temperature for SRR, LOT = long incubation optimal temperature for SRR, CT critical temperature, CT t2 = critical temperature after 1 day. H_2 calculated based on thermodynamic control of H_2 oxidation by sulfate.
Note the logarithmic scale for hydrogen.

ΔG_r –20 kJ mol^{-1} for sulfate reduction with hydrogen, a sulfide concentration of 1 µM and a sulfate concentration of 7.5 mM.

In the samples from the Wadden Sea Station NHS, the hydrogen measurements did not show such a strong change in concentration with incubation time as the samples from the permanently cold station. This might be due to a faster response of methanogenic archea or the lower number of measurements performed at the Wadden Sea station. Due to the lack of

sampling points at the start of the incubation, the initial increase of the hydrogen concentrations could not be examined. The critical temperature was 31°C, above which the hydrogen concentrations increased to maximum values at 38-40°C, the highest temperature tested. The maximum hydrogen concentration at 40°C was 45 nM for the first two time points decreasing with the following measurements to 16 nM at the last time point. At temperatures below 13°C, the hydrogen concentrations deviate from the theoretical value. The concentrations stayed almost constant around 0.6 nM down to 0.3°C. At the lowest temperature, the concentrations were about five times higher than the theoretical value.

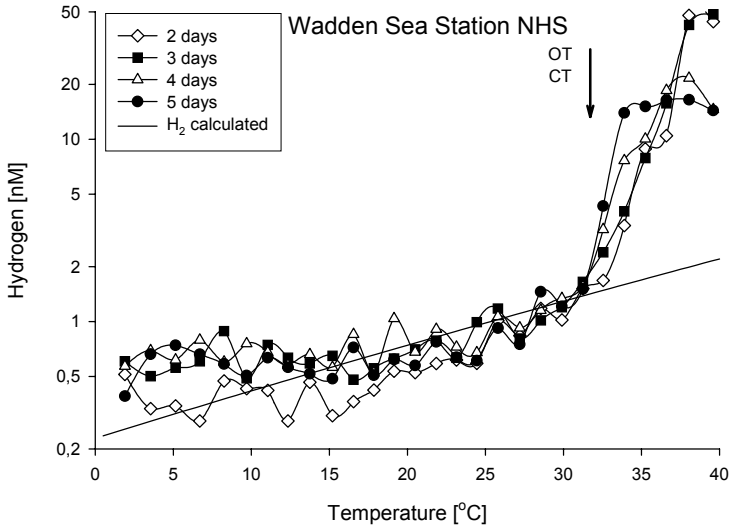

Figure 4: Hydrogen concentrations in the samples from the Wadden Sea station NHS measured after different incubation periods. OT = optimal temperature for SRR, CT critical temperature H_2 calculated based on thermodynamic control of H_2 oxidation by sulfate.
Note the logarithmic scale for hydrogen.

Volatile fatty acids

Volatile fatty acid (VFA) concentrations in the tubes with sediment from the permanently cold Station BC were measured at three different time points during the incubations of the sediment: after 4, 10, and 29 days (Fig. 5). Below the critical temperature (26, 27, and 28°C at the three different time points, respectively), the concentrations of the acids remained relatively constant. The only detectable (>0.5 µM for glycolate, lactate, and propionate, >1 µM for acetate, formate, and isobutyrate, >2 µM for butyrate and >5 µM for isovalerate and

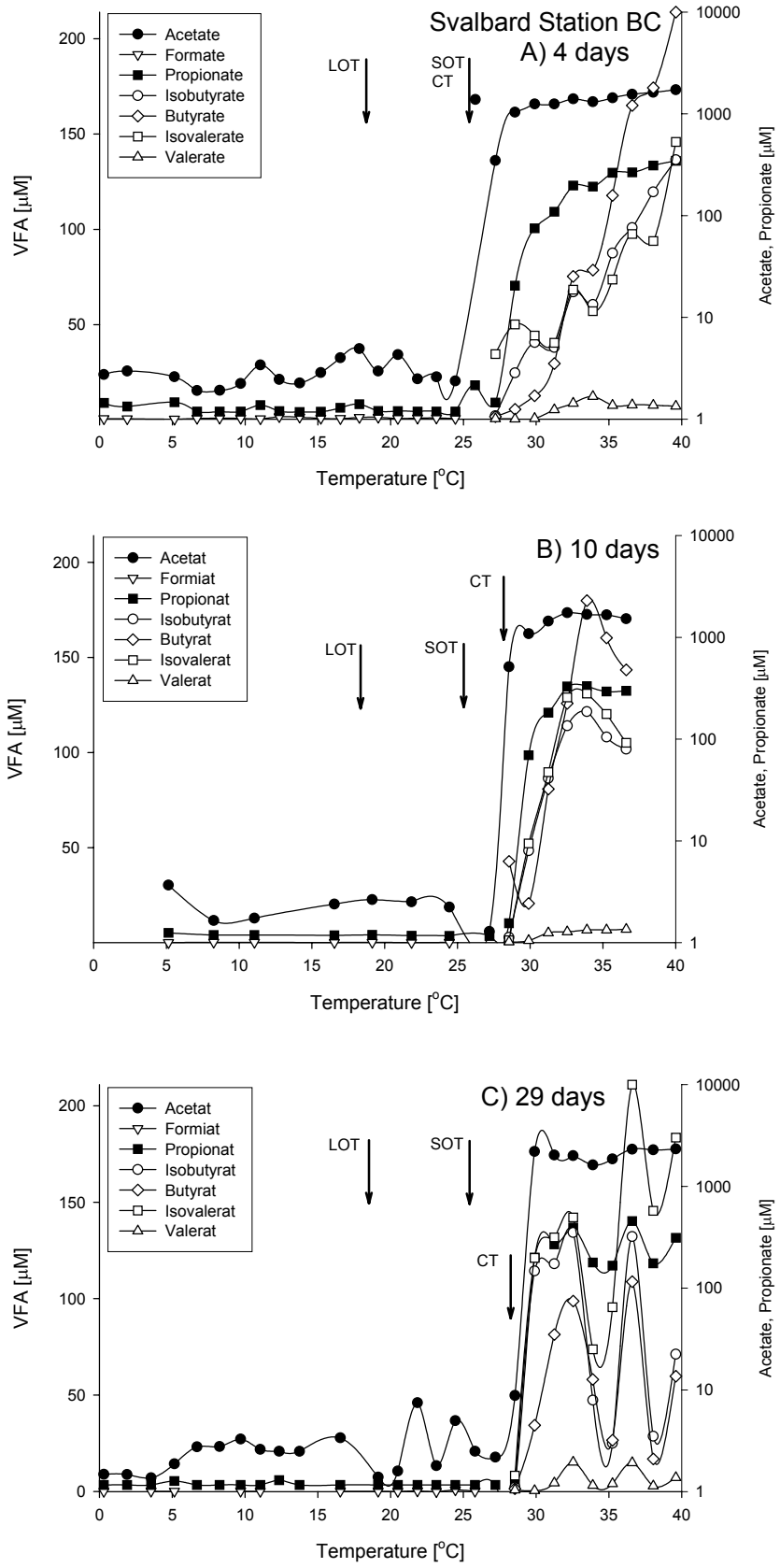

Figure 5: VFA concentrations in the samples from the permanently cold Svalbard Station BC after 4, 10 and 29 days of incubation. SOT = short incubation optimal temperature for SRR, LOT = long incubation optimal temperature for SRR, CT critical temperature for acetate
Note the different scale for the acetate and propionate concentrations.

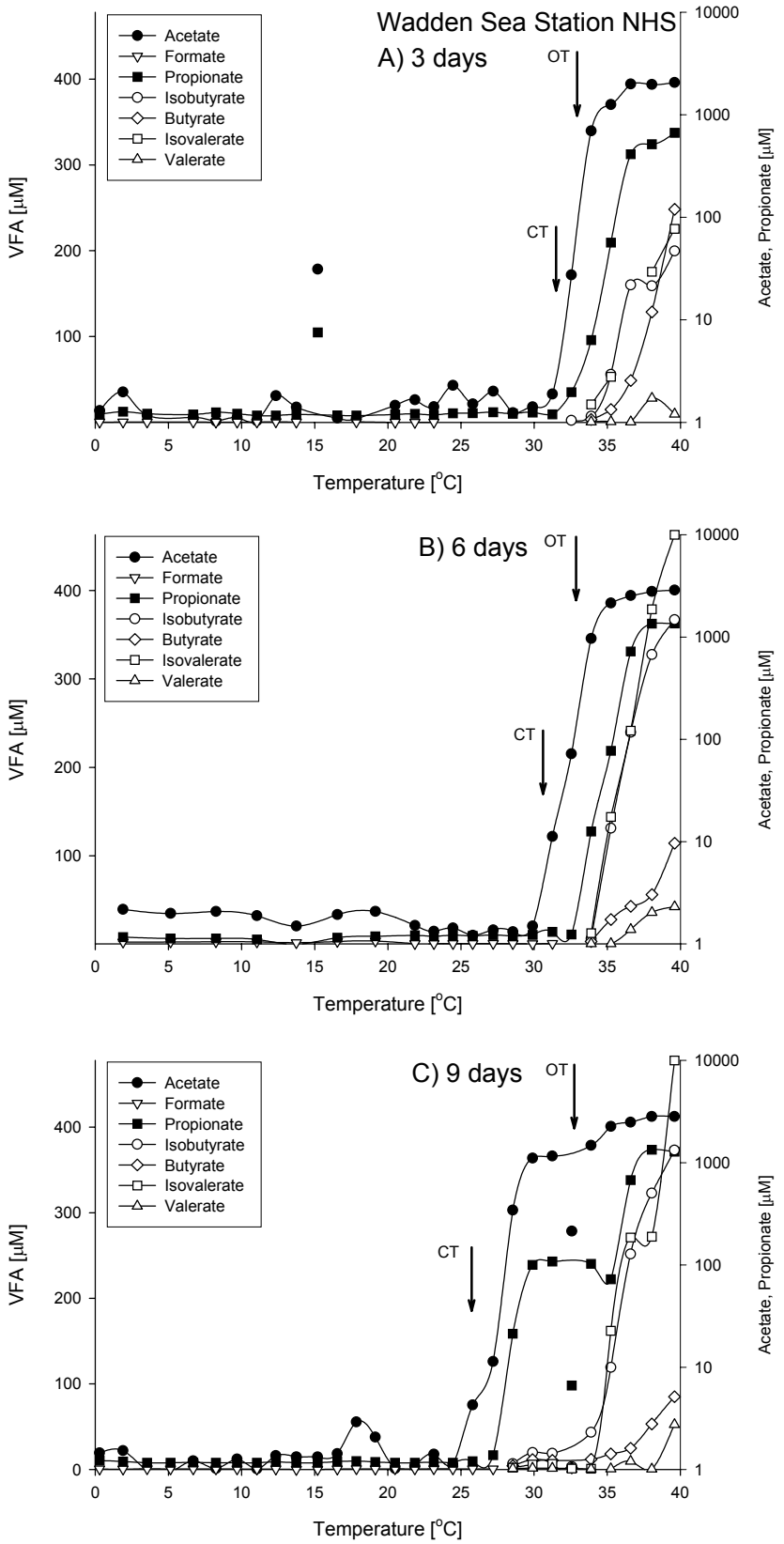

Figure 6: VFA concentrations in the samples from the temperate Station NHS after 3, 6 and 9 days of incubation. OT = optimal temperature for SRR, CT critical temperature for acetate
Note the different scale for the acetate and propionate concentrations.
The measurements from 33°C after 9 days of incubation were excluded from the graph, as they are probably too low due to analytical problems.

valerate) were acetate, propionate and formate. Above the critical temperature, the concentrations of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate increased steeply with temperature. Lactate and glycolate were below or close to the detection limit in all samples at the low temperatures and reached 2-4 μM at the highest temperatures, without a clear temperature dependence (data not shown). The concentration of acetate is in all samples higher than of the other VFA, increasing above the critical temperature at the first sampling time (Fig. 5a). The maximum concentration was 1,750 μM at 40°C. The second most abundant acid was propionate (350 μM) followed by butyrate (220 μM), butyrate and isovalerate (both about 140 μM), and valerate (12 μM). The concentrations of acids with more than 3 carbon atoms increased above 27 to 28°C, whereas acetate and propionate increased above 26°C. The concentrations of the acids showed a steady increase with temperature, except for valerate reaching maximum concentration at 34°C. After 10 days, all VFA concentrations increased above 27°C reaching maximum concentrations at 32-33°C (Fig. 5b). The maximum concentrations were similar to those found after 4 days of incubtion.

After 29 days the concentrations of acetate showed a higher scatter at temperatures below 28°C compared to the previous sampling dates (Fig. 5c). The average acetate concentration was only slightly higher than before. The increase of the VFA concentrations above the critical temperature was more abrupt at the third sampling time compared to the previous sampling dates, reaching a plateau with high scatter in the concentrations. These concentrations were similar to the maximum concentrations of the previous sampling dates.

VFA concentrations in the tubes with sediment from the temperate Wadden Sea Station NHS, were measured at three different time points along the incubations: after 3, 6, and 9 days (Fig. 6). The general picture of the temperature response is very similar to the data from the permanently cold station. The concentrations of the acids stayed low and relatively constant at temperatures below the critical temperature of 31°, 30°, and 25°C, respectively, at the different time points. The only detectable VFA below the critical temperature were acetate, propionate and formate. Above the critical temperature, the concentrations of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate increased. Lactate and glycolate were below or close to the detection limit in all the samples below the critical

temperature reaching 2-5 μM at the highest temperatures (data not shown). The concentration of acetate was the highest in all the samples compared to the other VFA increasing above the critical temperature. The steepness of response (μM/°C) of acetate was always greatest at the two to three temperatures analyzed just above the critical temperature, and leveled off above that. The maximum acetate concentration at the first sampling time was 2,000 μM at 40°C (Fig. 6a). The second most abundant VFA at 40°C was propionate (660 μM) followed by butyrate (250 μM), isovalerate (225 μM), butyrate (200 μM) and valerate (30 μM). The critical temperature for the VFA with more than 3 carbon atoms was 34 to 35°C, compared to 32°C for acetate and propionate. The concentrations of those VFA showed a steady increase with temperature. After 6 days, the acetate concentrations started to increase at 31°C followed by propionate (34°C), isovalerate, butyrate and isobutyrate (35°C) and valerate at (37°C) (Fig. 6b). Maximum concentrations were higher for all VFA, besides butyrate, compared to the first sampling point. Acetate reached a maximum concentration of 2900 μM, followed by propionate (1350 μM), isovalerate (460 μM), isobutyrate (370 μM), butyrate (115 μM), and valerate (42 μM). After 9 days the critical temperature was 26 to 30°C depending on the acid. (Fig. 6c). Acetate showed the lowest critical temperature, followed by propionate and then the longer acids (besides valerate). Between 34-36°C the concentrations of all VFA, besides acetate, showed a second major increase with temperature. The maximum concentrations were similar to those found after 6 days of incubation.

Discussion

Sulfate reduction rates

Sulfate reduction rates (SRR) calculated from the decrease in sulfate concentration in homogenized sediments from the permanently cold station BC were similar to rates measured with the tracer technique in sediment slurries from the permanently cold station J. The time series of SRR measured in sediments from station J showed a shift of the temperature optimum depending on preincubation time (Fig. 1a). With only 0.3 days of preincubation, the temperature optimum of sulfate reduction was 27ºC, similar to the

optimum rates found in a previous investigation with Svalbard sediments using short preincubation (Arnosti et al. 1998, Sagemann et al. 1998). After 8.5 days of preincubation, the optimum temperature had shifted to 17ºC while the rates at the optimum temperature had increased 4-fold. This demonstrates that the sulfate reducers were able to be active at relatively high temperatures for a short time, reflected in the short incubation optimum temperature (SOT), but were not able to keep this high activity with prolonged incubation. Thus, the rate at the SOT decreased with time, whereas at temperatures optimal for the sulfate reducers for longer time periods, the rate increased with prolonged incubation, reflected in the long incubation optimum temperature (LOT). These findings consistent with previous results from experiments with SRB cultures (Knoblauch & Jørgensen 1999) and from other psychrophilic bacteria (Harder & Veldkamp 1967, Christian & Wiebe 1974). Knoblauch & Jørgensen (1999) found higher temperature optima for sulfate reduction than for growth of psychrophilic sulfate reducers in pure cultures. Whether the increasing activity at the optimum temperature (LOT) are due to growth or whether the increased rates are only due to increased metabolic activity of the bacteria is not clear, as bacterial numbers were not quantified. Previous incubation experiments in the temperate gradient block with Arctic sediments showed no bacterial growth within 10 days (Marc Mussmann, unpublished data).

SRR of sediment from the permanently cold Station BC, calculated from the decrease in sulfate concentration after 10 days of incubation, showed an optimal temperature (OT) of 19ºC (Fig. 1b), similar to the LOT found with the tracer studies with prolonged preincubation. The decrease of the sulfate concentration in the pore water reflects an accumulative effect of the sulfate reduction over time. At the LOT, the decrease is highest, as the sulfate reduction rates was highest and occurred over the whole incubation time. At the SOT, the decrease will be lower, as only initially the sulfate was reduced at a relatively high rate. This will result in a slower decrease of the SRR with increasing temperature calculated after 6 days of preincubation, compared to tracer measurements with a similar preincubation time. The low hydrogen and VFA concentrations at temperatures above the LOT demonstrate that the SRB were still active under these conditions, even after 29 days of incubation.

The measurements in sediment from station WW, the temperate tidal flat, showed a higher optimum temperature of 34ºC for the sulfate reduction (Fig. 2a), than found at the permanently cold station. The optimum temperature of 33ºC in the incubation of the sediment from station NHS, the second temperate tidal flat (Fig. 2b), was almost identical to the optimum temperature found in the tracer study. Previous investigations with sediment from a temperate station did not show a strong dependence of the optimum temperature for the SRR on the preincubation, as found for the permanently cold sediments (data not shown). Isaksen & Jørgensen (1996) found similar temperature optima for growth and sulfate reduction in pure culture studies of a mesophilic sulfate reducer.

The studies of the two permanently cold stations J and BC from Svalbard resulted in a similar temperature response curve and so did the studies of the two temperate tidal flat stations WW and NHS. This demonstrates that the results reflect the different nature of permanently cold and temperate sediments.

Hydrogen

The hydrogen concentrations in the sediment reflect the interactions of two different processes involved in the degradation of organic matter. The hydrogen is produced by the fermenting bacteria and serve as an energy source for the terminal oxidizing bacteria. If these two processes are in balance, the hydrogen concentrations are controlled by the terminal electron accepting process (Lovley & Goodwin 1988, Hoehler et al. 1998). The terminal oxidizers can reduce the concentration to a level, at which they gain only 15-20 kJ mol^{-1}, the minimum energy necessary to produce ATP. By lowering the hydrogen concentration to this level the terminal oxidizers can outcompete bacteria of energetically less favorable oxidation reactions (Hoehler et al. 1998).

The hydrogen concentrations in sediments incubated in the temperature gradient block were plotted together with a theoretical concentration curve, based on thermodynamic control of the hydrogen concentration at all temperatures (Hoehler et al. 1998) (Fig. 3,4). In both incubation experiments the hydrogen concentration stayed rather constant over the course of the experiment and close to the theoretical values over a broad range of temperatures up to a

certain critical temperature.

The hydrogen concentrations in sediment from the permanently cold station BC showed increasing hydrogen concentrations over time at temperatures above 25-30°C (Fig. 3). After 3 hours of incubation the hydrogen concentrations were still in rather good agreement with the theoretical value. This can in part be due to the short incubation time, that might not have been long enough to allow the sediment to reach equilibrium with the headspace with respect to hydrogen. After prolonged incubation, the hydrogen concentrations increased at the higher temperatures. The critical temperature, above which the concentrations started to deviate from the theoretical curve, decreased from the second (1 day) to the third (2 days) time point from 35ºC to 30ºC, reflecting that sulfate reducing activity was gradually inhibit with time at the high temperatures, as found in the SRR tracer studies. At temperatures that were above the upper temperature limit of the sulfate reducing community, reflected in SRR of less than 10% of the maximum value (Fig. 1a, 8.5 days), the concentrations increased. The increasing hydrogen concentration at higher temperatures revealed a higher thermotolerance of the fermenting bacteria, that were still active at temperatures above the maximum temperature for the SRB. The hydrogen concentrations increased to a maximum of over 5000nM after 4 days of incubation. With prolonged incubation, the hydrogen concentrations decreased again. Hoehler et al. (1999) found increasing concentrations of hydrogen and acetate in Cape Lookout Bight sediment after sulfate reduction had stopped due to sulfate depletion. After a very rapid increase following sulfate depletion, the hydrogen concentration dropped to a level typical for acetogenesis and dropped again further to methanogenic levels after another five days of incubation. These physiological groups were present in the sediments, but only oxidized H_2, after hydrogen concentrations had increased to levels above their thermodynamic minimum. The maximum hydrogen concentrations of 5,800nM in the Svalbard Station BC samples after 4 days of incubation are close to the concentrations calculated for acetogenesis according to:

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$$

assuming a CO_2 concentration of 0.5 mM, an acetate concentration of 2 mM, and a ΔGr of –20 kJ mol^{-1} for acetogenesis (Fig. 7a). The CO_2 concentration of 0.5 mM corresponds to 5

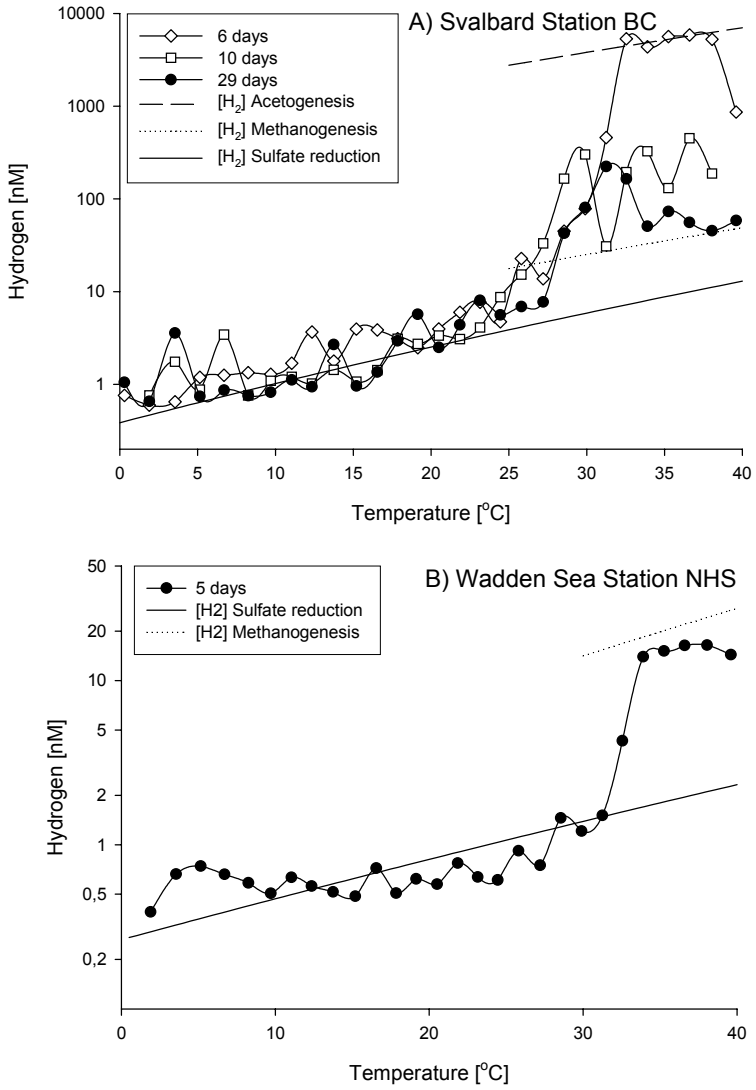

Figure 7: Hydrogen concentrations of samples from the A) permanently cold Svalbard station BC and B) the temperate Wadden Sea station NHS. Hydrogen concentrations for acetogenesis, methanogenesis and sulfate reduction were calculated based on thermodynamical considerations (see text for details).
Note the logarithmic scale for hydrogen.

mM dissolved inorganic carbon at neutral pH, which is a typical value in these sediments (O. Larsen, personal communication). The hydrogen concentrations above 33ºC after 29 days of incubation are close to the concentrations calculated for methanogenesis according to:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

assuming a CO_2 concentration of 0.5 mM, a methane concentration of 0.1 mM, and a ΔGr of –20 kJ mol^{-1} for methanogenesis (Fig. 7a). Methane concentrations in Cape Lookout Bight

sediments increased to about 1 mM within 30 days after sulfate depletion at 22°C (Hoehler et al. 1994). Methanogenesis in Cape Lookout Bight sediments is about 10-fold higher than typically found in methanogenic Wadden Sea sediments (M. Krueger, personal communication). As the investigated sediments do not undergo sulfate depletion on a seasonal basis, the rate of methane increase was assumed to be 1/10 of the rate found in Cape Lookout Bight despite the higher temperature. Fermentation of glucose to acetate, CO_2, and H_2 was still exergonic, even at the highest hydrogen concentrations (Damgaard & Hanselmann 1996). Thus, it seems that the acetogenic bacteria were the first to benefit from the increased hydrogen concentration and that they control its concentration. After prolonged incubation the methanogenic bacteria may have outcompeted the acetogenic bacteria for hydrogen by lowering the concentration to levels thermodynamically unfavorable for acetogenesis, as it was also shown in Cape Lookout Bight sediments. As the chemical species involved in acetogenesis and methanogenesis were not all determined, the calculations can give only a rough estimate of the true thermodynamics.

The sediment from station NHS, the temperate tidal flat sediment, did not show such a strong shift in hydrogen concentrations over time as the sediment from the permanently cold station (Fig. 4). At temperatures between 13 and 33ºC, the hydrogen concentrations were close to a theoretical curve based on thermodynamic considerations. The increased hydrogen concentrations at temperatures above 33ºC did not change over the first two sampling times. After 4 days of incubation, the maximum hydrogen concentrations decreased. After 4-5 days the hydrogen concentration above 34ºC was close to the theoretical value for methanogenesis according to:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

assuming a CO_2 concentration of 0.5 mM, a methane concentration of 0.01 mM, and a ΔGr of –20 kJ mol^{-1} for the methanogenesis (Fig. 7b). The consideration for the CO_2 and the CH_4 concentrations are similar as for the calculations for station BC. Due to the shorter incubation time, the increase in methane concentration was assumed to be lower, in accordance to the increase measured in Cape Lookout Bight Sediments (Hoehler et al. 1994).

In contrast to the permanently cold sediment from Svalbard, the critical temperature of the Wadden Sea sediment corresponded to the optimum temperature for sulfate reduction (Fig. 2a, 4). Sulfate reducing activity decreases above the optimum temperature for the SRB at both stations. At the permanently cold station, the SRB show a high affinity for hydrogen above the LOT, resulting in efficient removal of the hydrogen down to thermodynamically predicted values. The affinity decreases only at temperatures above the SOT, where the rates are substantially lower after prolonged incubation. In the sediments from the temperate site, the hydrogen concentrations increase above the optimum temperature for sulfate reduction, reflecting the decreased efficiency in the oxidation of the fermentation products.

Differing to the samples from Svalbard station BC, the hydrogen concentrations in NHS samples deviated from the theoretical curve also at the low-temperature. At temperatures below 13ºC the hydrogen concentrations stayed constant. Conrad & Wetter (1990) reported upper and lower critical temperatures for methanogenic and acetogenic bacteria with respect to hydrogen consumption. Above and below these temperatures the threshold concentrations for hydrogen deviated from the theoretical curve based on thermodynamics with a constant energy yield. At temperatures beyond the upper and lower critical temperature, the bacteria are not well adapted to efficiently remove the hydrogen from the pore water, resulting in an increased threshold concentration (J. Harder, personal communication).

Volatile Fatty Acids (VFA)

As for hydrogen, the VFA concentrations reflect the balance between fermentation and terminal oxidation. However, in contrast to the hydrogen concentrations, the VFA concentrations are not controlled by thermodynamics. The concentration versus temperature curves of the VFA showed a similar pattern as the curves for hydrogen (Fig. 5, 6). Up to a temperature of 25ºC for BC (Svalbard) and 30ºC for NHS (Wadden sea) the fermenters and sulfate reducers were in balance and the VFA concentrations remained at a constant low level.

The sediments from the permanently cold station BC showed a critical temperature of 26-28ºC (Fig. 5). Above this temperature the concentrations of the acids increased. At the

critical temperature, the SRR of the long term slurry incubations from Svalbard Station J were about 10% of the maximum SRR at the optimum temperature. For the sediment from the temperate station NHS, the critical temperature was 32 and 30ºC for the first two sampling times, respectively, and dropped to 26-27ºC at the last sampling time. In addition to the lower critical temperature, the Wadden Sea Station NHS samples from the 9 day

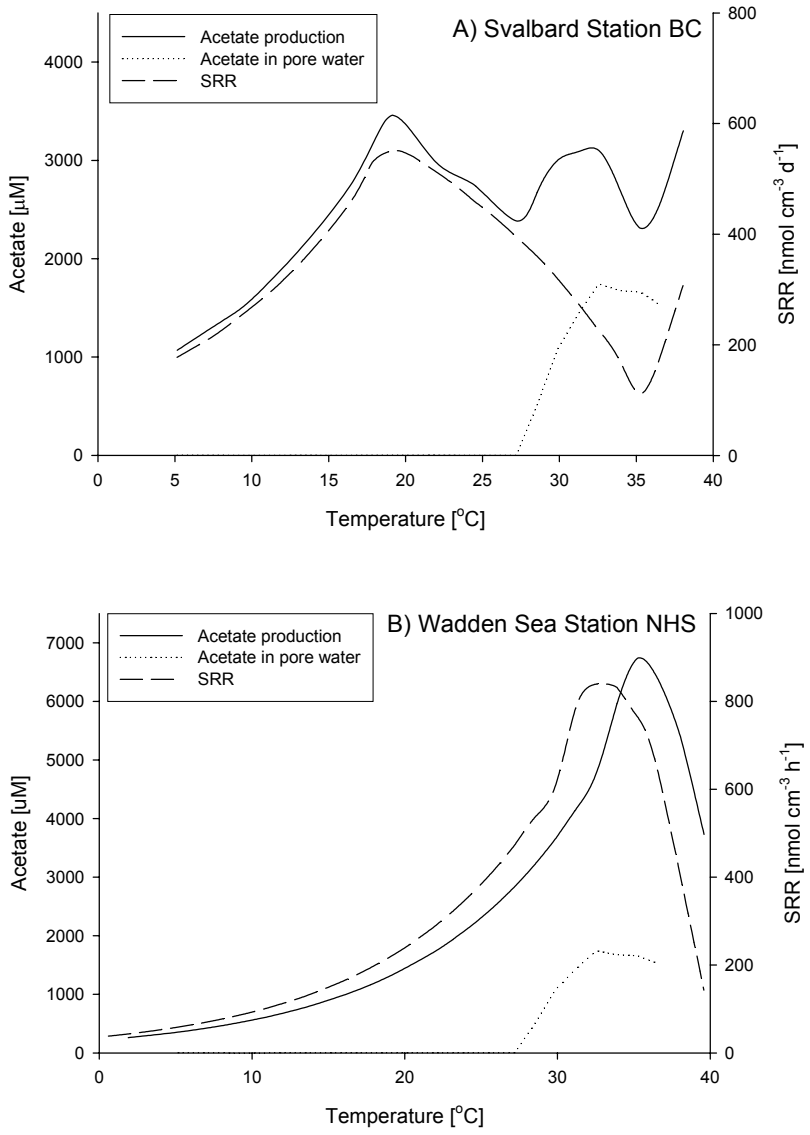

Figure 8: Acetate production in the samples from A) the permanently cold Svalbard station BC and B) the temperate Wadden Sea station NHS. The acetate production was calculated from the measured acetate concentrations and the SRR, based on the assumption, that acetate is the only substrate for the sulfate reduction.

incubation also showed a second increase of the VFA (other than acetate) at even higher temperatures (Fig. 6c). The first increase is probably due to sulfate depletion. Extrapolating the sulfate reduction rates calculated from the sulfate depletion to the last sampling time results in sulfate concentrations below 0.1 mM at temperatures between 28 and 39°C (data not shown). The second increase is close to the critical temperature from the 3 and 6 day incubations and is probably due to the decreasing activity of SRB above their optimal temperature. This is also in good agreement with the fact that mainly acetate and propionate increase already from 25ºC, whereas the acids that can be further fermented, also by the sulfate reducing bacteria, only increase above 32ºC.

The critical temperature is not the same for the different acids. Acetate always increased at the lowest temperature and reached the highest concentrations in all samples. Valerate started to increase only at the highest temperature and remained at the lowest concentrations. Except for Isovalerate, the critical temperature at the first sampling point as well as the maximum concentration seem to be related to the chain length of the acid. The shorter acids have the higher maximum concentration and the lower critical temperature.

Homogenization of sediment has been found to result in a strong increase of the VFA concentrations (Finke 1999, Arnosti et al., in prep., Chapter 2). The acetate concentrations increased faster, reached the highest values, and stayed longer at an elevated level compared to the other VFA. Propionate was the second most abundant VFA, followed by isobutyrate and butyrate. Acetate is the main end product of fermentation under substrate limited conditions (Schink 1988). The other VFA can be fermented further, eventually to acetate, H_2 and CO_2. Only at very low hydrogen concentration can acetate be syntrophically fermented further to CO_2 and hydrogen (Diekert 2002). Organic matter in sediments is fermented mainly to acetate, CO_2 and hydrogen under well balanced conditions (Schink 1988). Increased fermentation rates under substrate unlimited conditions lead to production of longer-chain VFA that accumulate (Arnosti et al., in prep., Chapter 2). The VFA concentrations above the critical temperature were relatively stable after long incubation and were similar at the two stations (Fig. 5, 6). This might be due to inhibition, as high

concentrations of a metabolic product can inhibit its formation. Inhibition by fermentation products has been found to change metabolic pathways in culture experiments. This is in good agreement to the observation that acetate reaches stable concentrations earlier in the experiment and at lower temperatures, whereas it takes longer for the other VFA. At high acetate concentrations, the fermentation is shifted to longer acids. With increasing concentrations of total VFA, the fermentation might have shifted further, possibly to alcohol production. The stable concentrations can also be reflecting a new equilibrium between the fermentation rate and VFA oxidizing metabolism other than sulfate reduction, e.g. acetoclastic methanogenesis.

The VFA did not show a similar pattern with time as the hydrogen concentrations. Hoehler et al. (1998) found the response in the acetate concentration after sulfate depletion to be slower than for hydrogen. This is probably mainly due to the much longer turnover times for the VFA compared to hydrogen. The turnover time for free acetate was found to be 15-20 h at station BC at *in situ* temperature (Finke et al. in prep., Chapter 3), and is in the order of several minutes to an hour in temperate sediments (eg. Christensen & Blackburn 1982, Sansone & Martens 1982, Wu & Scranton 1994, Wellsbury & Parkes 1995). The turnover time of hydrogen at *in situ* concentrations is in the order of seconds (Boone et al. 1989).

Wellsburry et al. (1997) reported increasing acetate production in sediments in a similar heating experiment. Acetate production was calculated from the measured concentrations and sulfate reduction, assuming acetate to be the only substrate for sulfate reduction. Similar curves for the stations NHS and BC calculated for 6 days of incubation are plotted in figure 8. The SRR according to the regression curve shown in figures 1b and 2b were used to calculate the acetate oxidized by sulfate reduction. The acetate production followed the SRR curve for the major part of the experiment, indicating that the sulfate reducers were substrate limited. The acetate production in the BC sediment showed three maxima (Fig. 8a). The minimum between 33 and 36ºC is probably due to thermodynamic inhibition of the acetate production and a shift to other fermentation products, as no decrease in acetate concentration occurred within this temperature window after 3 days. Thus, two populations of fermenters were present in these sediments, a psychrophilic and a mesophilic population. The acetate production in the sediment from the Wadden Sea Station NHS showed one

maximum. The decrease in acetate production at temperatures above 36ºC is probably due to thermodynamic inhibition as discussed for the BC sediment. The high concentrations of the VFA in both experiments at the highest tested temperature already at the first sampling time shows that the fermenters were still active at these temperatures.

The balance between Sulfate Reduction and Fermentation

VFA and hydrogen are produced by the fermenting bacteria and serve as electron donors for the bacteria carrying out the terminal oxidation. Even though, the controlling factors for the VFA concentrations are not completely understood, low concentrations of the electron donors reflect a close coupling between the fermentation and the terminal oxidation. Up to a critical temperature of about 25 and 31ºC for station BC and NHS, respectively, the concentrations of hydrogen and VFA showed a close coupling of the two processes. The SRR changed with temperature, and due to the close coupling, the fermentation must have changed accordingly. The SRB probably were substrate limited in the experiment with the homogenized sediment from station BC and NHS for the most part of the investigated temperature window. Experiments with substrate addition to slurries from permanently cold and temperate sediments did not give clear evidence for a substrate limitation of the sulfate reduction (Sagemann et al. 1998). The concentrations of the VFA in those experiments were, however, probably elevated due to the short preincubation after mixing the sediment, as found in homogenized sediments (Finke 1999, Arnosti et al., in prep., Chapter 2), and therefore not necessary limiting the sulfate reduction. This could also have been the reason for the lower SRR in the samples preincubated at *in situ* temperature for several days (NHS, BC) compared to the slurry incubations (J, WW) that were preincubated only for an hour after mixing.

The VFA and the hydrogen response of both the temperate and the permanently cold station showed a close balance between fermentation and sulfate reduction up to a critical temperature. In accordance with the different *in situ* temperatures, this critical temperature was higher for the temperate station. In the sediments of the permanently cold station, the fermentation and sulfate reduction reveal a close coupling in temperature intervall above the optimum temperature for sulfate reduction, whereas in the sediment from the temperate

station, the fermentation products increased above the optimum temperature for sulfate reduction. This indicates that for the SRB from the temperate sediment the optimum temperature is closer to the maximum temperature compared to the SRB from the permanently cold sediment. Similar results were found in pure culture studies of psychrophilic and mesophilic bacteria (Harder & Veldkamp 1968, Christian & Wiebe 1974, Isaksen & Jørgensen 1996, Knoblauch & Jørgensen 1999). The SRB are active at temperatures above their optimal temperature, keeping the substrate concentrations at low levels. The SRR shows a strong decrease above the optimal temperature at longer incubation times (Fig. 1a). Thus, the fermenting bacteria must also show a decreased activity above the optimum temperature for the SRB up to the critical temperature, where the fermentation exceeds the terminal oxidation. This trend is illustrated by the acetate production curve (Fig. 8a). Higher potential SRR and a higher optimal temperature could be masked by a substrate limitation due to lower fermentation rates as found in Guayamas Basin sediment (Elsgaard et al.1994). Experiments with substrate addition to slurries from Svalbard sediments showed the same optimum temperature for sulfate reduction (Sagemann et al.1999, unpublished data). Thus, the psychrophilic fermenting and sulfate reducing bacteria from the sediments from permanently cold sediments of Svalbard have a very similar optimum temperature.

Fermenting bacteria were active over the whole temperature range in both of the experiments. Acetate production in experiments with heated deep sea sediment was found to proceed at temperatures well above the maximum temperature investigated here (Wellsburry et al. 1997). In the NHS samples the VFA concentrations were higher after 3 days of incubation than in the BC samples after 4 days. In contrast to the BC samples, the concentrations in the NHS samples did not increase substantially with prolonged incubation. Thus, the fermenting bacteria of the permanently cold sediment were less active at higher temperatures than those from the temperate station. This lead to an inhibition for acetate production already after 3 days for the NHS samples and only after longer incubation for the BC samples. The experiments do not give direct information about the activity of the fermenters at temperatures, at which the fermentation is in balance with the sulfate reduction. At these temperatures the response of the sulfate reduction and the fermentation

to the temperature change must have been the same, as the sulfate reduction was substrate limited and could only have changed as fast as the fermentation increased.

Mixing experiments with marine sediments showed, that SRB responded slower than fermenters to the changed conditions, resulting in an accumulation of fermentation products (Finke 1999, Arnosti et al., in prep., Chapter 2). Thus, sediment mixing and temperature change have different effects on the coupling of fermentation and sulfate reduction. The SRB do not respond slower to temperature changes than fermenters. They probably respond faster, as they can keep the VFA and hydrogen concentration low (within their temperature window) being limited by the substrate production. High molecular weight organic carbon is too large to be taken up by bacterial cells. Extracellular enzymes from heterotrophic bacteria hydrolyse the particulate organic matter in sediments, braking it down to molecules that can be transported over the cell membrane. These heterotrophic bacteria depend on a close spatial proximity to the organic matter. Sulfate reducers degrade water soluble substrates only. Initial mixing of sediment samples resulted in an increase in VFA concentration, whereas repeated mixing did not show an effect on the VFA concentrations (Finke 1999, Arnosti et al., in prep., Chapter 2). The mixing probably leads to a more regular distribution of the fermenters in the sediment. This may increase the fermentation as more particulate organic matter is in close contact with the fermenting bacteria. SRB can only react upon the increased fermentation once the substrate concentration is increased. Increased temperatures on the contrary increase metabolic activity of both populations without influencing the spatial distribution of the bacteria. This might be the reason for the different responses of the bacterial communities to homogenization and warming of sediment samples.

The methanogenic bacteria, ultimately controlling the hydrogen concentrations in samples at high temperatures, responded considerably slower than the fermenting bacteria, resulting in a transient increase of the hydrogen concentrations to values, typical for acetogenic conditions (Fig. 7). This is probably due to a change of their metabolism as hydrogen is not a substrate for the methanogenic bacteria at the *in situ* concentrations in these sediments. The methanogenic bacteria of the temperate NHS samples respond faster to the increased

hydrogen concentrations than the methanogens from the permanently cold station.

Conclusions

The sulfate reduction rates as well as the acetate production rates showed a higher optimum temperature with higher maximum rates in samples from the temperate station compared to samples from the permanently cold station. Hence, the rates are reflecting the higher *in situ* temperature of the temperate station. Not only the sulfate reducers, but also the fermenting bacteria from the permanently cold station are not as well adapted to high temperatures compared to the bacteria from the temperate station.

Sediments from the permanently cold sediment showed a different temperature response depending on the preincubation time. The different temperature optima are similar to the optimum temperature for growth and sulfate reduction as determined for psychrophilic sulfate reducing bacteria isolated from Svalbard sediments. Thus, the different temperature optima seem to be reflecting the optimum temperature for the enzymes involved in sulfate reduction and the optimum temperature for the bacteria as a whole.

The sulfate reduction and the fermentation show a close coupling over a broad temperature range, reflected in low concentrations of the VFA and H_2 for both sediments. The sulfate reducing bacteria respond as fast to the changes in temperature as the fermenting bacteria. A potential faster response of the sulfate reducers would be inhibited by the substrate limitation due to lower fermentation rates. At low temperatures, the sulfate reducers from the temperate station are not as well adapted in respect of their hydrogen metabolism as those from the permanently cold station. Hence, the populations from the temperate tidal flat station, experiencing fluctuating temperatures on a seasonal and even daily basis, are not more responsive to changes in temperature than the population from the permanently cold station.

Mesophilic methanogenic archea seem to be present in both sediments, starting to actively oxidize H_2, once the concentration increases as result of the temperature induced inhibition of the sulfate reduction.

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Chapter 2

Anoxic carbon degradation in Arctic sediments: Microbial response to substrate addition

(prepared for submission to Geochimica et Cosmochimica Acta)

Carol Arnosti[1*], Niko Finke[2], Ole Larsen[2], Sherif Ghobrial[1]

[1]Dept. of Marine Sciences, 12-7 Venable Hall, Univ. North Carolina, Chapel Hill NC 27599-3300, USA
[2]Dept. of Biogeochemistry, Max Planck Institute for Marine Microbiology, Celsiusstr. 1, 28359 Bremen, Germany
*Corresponding author: arnosti@email.unc.edu

Abstract

Complex substrates are degraded in anoxic sediments by the concerted activities of diverse microbial communities. In high latitude environments, such communities may receive episodic inputs of organic matter that settles quickly from the upper ocean and is mixed rapidly into deeper sediment layers by the activities of macrofauna. In order to explore the effects of rapid carbon addition and of substrate complexity on carbon flow in anoxic sediments, four complex substrates—*Spirulina* cells, *Isochrysis* cells, and soluble high molecular weight carbohydrate-rich extracts of these cells (Spir-Ex and Iso-Ex)—were added to permanently cold anoxic sediments collected from Svalbard. The sediments were homogenized and incubated in gas-tight bags at 0 °C, and the dynamics of enzyme activities, fermentation, and terminal respiration were monitored over a time course of 1134 h. All substrate additions yielded a fraction of carbon that was rapidly metabolized, with 7-13% of added carbon respired to CO_2 over the first 384 hours' incubation. The timecourse of VFA production and consumption, as well as the suite of VFA produced, was similar for all substrate additions during this time period. The timecourse and quantities of VFA produced were similar for *Spirulina*, *Isochrysis*, and Spir-Ex, despite their dissimilar chemical and bulk composition; Iso-Ex yielded 82% of these concentrations. Pathways of carbon degradation diverged after this initial phase, with an additional 43%, 32%, 33%, and 8% of *Isochrysis*, Iso-Ex, *Spirulina*, and Spir-Ex respired to CO_2 over the next 750 hours' incubation. Although *Spirulina* and Iso-Ex differed in physical and chemical characteristics (solid/soluble, C/N ration, lipid and carbohydrate content), essentially the same quantities of carbon were respired to CO_2, incorporated into biomass, and calculated as residual carbon, with half of the total carbon ultimately used by the sedimentary microbial community. In contrast, only an estimated 18% of Spir-Ex carbon was accessible to the sedimentary microbial community over 1134 h, despite the initial burst of activity that it fueled, its soluble nature, and relatively high (50%) carbohydrate content. The microbial community in these permanently cold anoxic sediments clearly has the capacity to react rapidly to carbon input; the extent and timecourse of remineralization of added carbon is comparable observations made at much higher temperatures in temperate sediments. The extent of carbon remineralization from these specific substrates, however, would not have been

predictable solely on the basis of bulk substrate characteristics.

Introduction

The degradation of complex organic substrates in anoxic marine sediments requires the concerted action of sedimentary microbial communities. These communities may receive fresh organic matter that has settled quickly from the upper ocean and is mixed rapidly by the action of macrofauna into the sediments, resulting in close coupling of pelagic and benthic production (e.g. Blair et al. 1996, Witte et al. 2003). Complex substrates are then degraded in a sequential process, resulting in new biomass and in respiration of carbon to CO_2, with a fraction of the organic carbon resisting remineralization and being buried in the sediments, locked away from further active participation in the carbon cycle.

The general processes by which sedimentary carbon is cycled by microbial communities and the chemical changes accompanying degradation of phytoplankton have been investigated in a number of laboratory experiments. Harvey et al (1995) compared the oxic and anoxic degradation of diatoms and cyanobacteria in flow-through water tanks, and measured changes in biochemical components of particulate organic carbon (POC) and in microbial activity with time. The extent of degradation of ^{14}C-labeled diatoms added to the surface of sediment cores incubated under oxic and anoxic conditions has been followed by measuring the distribution of dissolved and respired ^{14}C label with time (Andersen 1996). Comparative changes in fermentation and respiration in anoxic mesocosms that had received addition of seagrass or phytoplankton have also been studied (Hee et al. 2001). These studies have demonstrated differences in the reaction rates of specific biochemical pools (Harvey et al. 1995) and of total carbon (Andersen 1996, Hee et al. 2001) depending on the nature of the terminal respiration process, as well as differences in the extent of carbon respiration between seagrass and phytoplankton (Hee et al. 2001). Harvey et al. (1995), however, found that differences in the extent of degradation of diatom and cyanobacterial particulate organic carbon (POC) were relatively small, and that differences among biochemical classes were more significant than compositional differences between specific biochemicals.

We wished to take a closer look at degradation rates and patterns of complex organic substrates in anoxic sediments, focusing in particular on the extent to which the nature and structural complexity of the added substrate may affect the rates and extent of carbon remineralization. Although Harvey et al. (1995) were able to characterize bulk biochemical changes in the POC pool, their experimental approach did not permit them to establish a carbon budget, or to distinguish between carbon lost from their system as dissolved organic carbon (DOC) vs. carbon remineralized to CO_2. Furthermore, their anaerobic microbial innoculum was obtained from anoxic Chesapeake Bay waters, which may be dominated by different organisms than are active in organic-rich anoxic sediments. We added whole freeze-dried plankton cells (Spirulina, a cyanobacterial mixture, and Isochrysis), as well as soluble high molecular weight extracts of these cells that contained a high fraction of carbohydrates (S-extract and I-extract) to replicate portions of anoxic sediments. By comparing whole cells and extracts, we sought to assess the relative effects of adding soluble and solid substrates, as well as to determine whether substrates with a relatively high carbohydrate would be metabolized more rapidly under anoxic conditions than the bulk cells from which they were derived. In order to follow carbon flow quantitatively from complex substrate all the way to terminal remineralization, the incubations were carried out in gas-tight bags, and production and consumption of fermentation products (VFA, volatile fatty acids) as well as respiration of CO_2 were quantified. Homogenization of sediments also permitted us to focus on robust differences among different sediment amendments and between amended and unamended sediments, without the additional complication caused by sediment variability in core incubations. The study was carried out using anoxic sediments collected from a fjord off the west coast of Svalbard, an archipelago in the high Arctic. Episodic carbon input is particularly characteristic of high Arctic latitudes, where primary production varies strongly on a seasonal basis (Hop et al. 2002). Furthermore, the permanently cold temperatures of Svalbard sediments are also characteristic of deep sea environments that receive episodic influx of fresh organic carbon. Although the general means by which microbial communities remineralize complex substrates to CO_2 is understood, the rates at which carbon derived from specific complex substrates flows along degradation pathways, and the factors ultimately controlling the fate of organic carbon in

anoxic sediments, are still unclear.

Methods

Sediment collection: Sediments were collected from several Haps cores taken at Nordfjorden (Station BC; 78°31'N, 015°06' E), along the west coast of Svalbard. Water depth was 100 m, bottom water temperature was 1ºC. The 2-7 cm depth interval of the cores was collected directly from the Haps corer, transferred to a dark brown glass jar which was filled to the top with no headspace, and stored at *in situ* temperature in the dark.

Substrate preparation

Four substrates were used: Spirulina (S), Spirulina extract (SE), Isochrysis (I), and Isochrysis extract (IE). The substrates were prepared and characterized as follows:

Spirulina: (mixed cyanobacterial species, freeze-dried powder) was used as obtained from Sigma.The Spirulina powder was 42.3% TOC and 9.1% TON. The 500 mg substrate addition (see below) therefore represents 211.5 mg organic carbon (C_{org}), 45.5 mg organic nitrogen (N_{org}) added to 1.5 liter sediments.

Isochrysis: A culture of Isochrysis sp. was obtained from Reed Mariculture, Inc. (Instant AlgaePremium 3600, www.instant-algae.com), as whole (dead) cells. The cells were frozen and lyophilized prior to CHN analysis, which yielded 40.3% TOC and 5.5% TON (C. Siegel, pers comm.). The 500 mg substrate addition therefore represents 201.5 mg C_{org} and 27.5 mg N_{org} to 1.5 liter sediments.

Extract preparation: Initial preparation of Isochrysis: Isochrysis cells were pre-treated in order to obtain a homogenous mixture. The cells were thawed and centrifuged, the aqueous supernatant was decanted, and the bulk material was lyophilized. After lyophilization, the cells (1 g portions) were vortexed with 10 ml milli-Q H_2O, and transferred to a grinding tube along with 10 ml acetone. The cell slurry was ground using a variable speed drill fitted with Teflon grinder. After grinding, the cells were centrifuged at 4500 x g for 10 min to condense pellet material, and this material was then subject to the same procedure as

Spirulina.

Extraction procedure: 1.0 g portions of cells were ground (Teflon grinder) with 30 ml acetone, vortexted, and sonicated for 20 min. The cell slurry was centrifuged 10 min at 4500x g, and the supernatant was decanted. This procedure was repeated 3 times. A second extraction step was carried out in the same manner, using 2:4:1 chloroform:methanol: milli-Q H_2O, until the supernatant was clear (typically 3 treatments). Cell material was then transferred to a boiling flask, and 30 ml of 0.05% SDS (sodium dodecyl sulfate) in milli-Q-H_2O was added, and the solution was refluxed for 1 h. After centrifugation, the supernatant was carefully collected and dialyzed in 1 kd, 5 kd, and/or 10 kd Spectra/Por membrane vs 2700 ml milli-Q H_2O. The dialysis solution was changed twice per day for 4 days. The retentate in the bag was lyophilized and characterized. CHN analysis of the Spirulina extract yielded 36.7% TOC and 8.43% TON. Addition of 500 mg of SE therefore added 184 mg C_{org} and 42 mg Norg to 1.5 liters of sediment. CHN analysis of the Isochrysis extract yielded 40.1% TOC and 4.6% TON. Addition of 500 mg of IE therefore added 201 mg Corg, 18.5 mg N_{org} to 1.5 liters of sediment.

Table 1: Composition of added substrates

	Spirulina	Spirulina Extract	Isochrysis	Isochrysis Extract
molecular weight	Variable; solid	> 10 kd	Variable; solid	> 5 kd
%C_{org}	42.2	36.7	40.3	40.1
% N	9.4	8.4	5.5	4.6
Carbohydrate content	~20% of cell mass	~50% of extract mass	~13% of cell mass	~65% of extract mass
Carbohydrate structure		Complex, branched		Some branches
Carbohydrate composition		Rhamnose (44%) Glurcuronic acid (13%) fucose (10%) glucose, xylose, mannose, N-acetyl residues		Glucose (82%), arabinose, galactose, mannose (4-7% each)
Neutral lipids (mg/g)	23.3	0.6	32.1	0.3
fatty acids(mg/g)	8.6	0.7	15.2	0.5

Extract characterization: In addition to CHN analysis, lipid analyses and total carbohydrate measurements were carried out on extracts and whole cells. Carbohydrate composition and linkage analysis was carried out on the extracts by the Complex Carbohydrate Research Center (Univ. Georgia) (Table 1).

Preparation of sediments:

Approximately 10 liters of sediment were homogenized with a power mixer under N_2. Volumes of 1.5 liters were measured, substrate solutions added, and the sediments were again thoroughly homogenized. For substrate solution preparation, 500 mg of each substrate were added to 20 ml 0.2 μm sterile-filtered seawater. Ten ml sediment was added to the solution, and the slurry was homogenized thoroughly, vortexted, and placed in the incubator the sediments were ready for substrate addition. The unamended sediments received 20 ml filtered water plus 10 ml sediment, without substrate addition. Each 30 ml solution was then homogenized thoroughly with 1.5 liter sediments under N2 , and the entire volume was sealed in a laminated plastic bag (NEN/PE 80/100, Danisco Flexible, Denmark). The bags were composed of four layers: nylon (for the strength), ethylene vinylalcohol (gas impermeable), nylon, and polyethylene (for sealing). The bags were made with a heat sealer (Elwis-Pack, Andertech International, Denmark), and each bag contained a spigot (sealed tightly with a butyl rubber stopper) for periodic sampling. The oxygen flux through such bags is approximately 5 μmol m^{-2} d^{-1} (Hansen et al. 2000). The bags were stored in an incubator at 0°C; bottom water temperature at the time of sediment collection was 1°C. Note that the 0-h sample was collected approximately 90 minutes after substrate addition. Samples were collected at the following times: 0, 10, 21, 46, 68, 118, 166, 214, 384, and 1134 hours. At each timepoint, sediment was extruded through the spout of the bag and dispensed for individual analyses. The bags were kneaded to re-homogenize the sediments, and returned to the incubator.

Concentration and rate measurements:

Total inorganic carbon: Pore water samples were filled into glass vials excluding any gas bubbles and stored cold until analysis (within 24 h) in the laboratory. Dissolved inorganic carbon (DIC) was measured via flow injection analysis with a conductivity detector (Hall & Aller 1992). 50 µl of sample were injected into a stream of 10 mM HCl. The CO_2 diffuses across a teflon membrane into a stream of 10 mM NaOH. The electrical conductivity change of the NaOH solution is measured on a specific conductivity meter (VWR scientific, model 1054).

Volatile fatty acids (VFA): The sediment was extruded into Spinex (Phenomenex) centrifugal filter units, centrifuged at 2000 g for 10 min and the pore water frozen immediately in brown borosilicate glass vials that were precombusted at 450°C for a minimum of 3 hours. The samples were derivatized with 2-nitrophenylhydrazine, separated on an HPLC with a LiChrosphere RP8 (5µm, 20*4mm ID, Knauer) column and the concentrations measured at 400 nm (Albert & Martens 1997). Differing from the original method, in solvent A the concentration of butanol was reduced to 1,25%, and the concentration of tetrabutylammonium hydroxide to 1 mM and in solvent B the concentration of tetradecyltrimethylammonium bromide was reduced to 25 mM. Detection limits are 0.2 µM for glycolate and lactate, 1 µM for acetate and formate (formate is substantially higher at high acetate concentrations due to coelution, therefore no formate was detected in later samples), 0.5 µM for propionate and isobutyrate, and 2 µM for butyrate, valerate and isovalerate. Standard deviation for replicate analyses is less than 3% for concentrations above 5 µM. Blank values are approximately 0.5 µM for acetate and formate.

VFA turnover: VFA turnover rates were determined using radiotracer incubations. Sediment was extruded into 10ml syringes with cut off ends. Radioactive acetate (ca. 100 kBq) or lactate (ca. 100 kBq) was injected along a line in the middle of the syringe and mixed into the sediment. The syringes were sealed with butyl rubber stoppers and incubated at *in situ* temperature for a total of 60 or 75 min for acetate and lactate, respectively. Subsamples were taken at 8 time points for the acetate turnover measurements (1.5, 3, 5, 8,

15, 30, 45, 60 min.) and at 5 time points for the lactate measurements (13, 30, 45, 60, 75 min) At each sample point, ca. 1 and 1.5 ml (for acetate and lactate, respectively) sediment was extruded from a syringe into 5 ml 2.5% NaOH. The sample was then frozen. Radioactive CO_2 and VFA in the samples were separated by the diffusion method (Treude et al., in prep.) and measured in a scintillation counter. Rate constants were determined from the slope of the increase in fraction of $^{14}CO_2$ of the total ^{14}C in the sample. Turnover rates were calculated by multiplication of the turnover rate constant with the concentration.

Hydrogen concentrations: Hydrogen concentrations in the sediment were determined via the headspace technique (Lovley & Goodwin 1988, Hoehler et al. 1998). 3 ml of sediment were transferred into 10ml glass vials, flushed with N_2. The vials were stored at 0°C. Three parallel vials from each bag were analyzed for hydrogen concentrations. Subsamples for the concentration measurements were taken after 6 timepoints. For the analysis, 100µl sample were withdrawn from the headspace of the vials with a gastight syringe and replaced with N_2. The gas was analyzed with a reduced gas analyzer (RGA3, Trace Analytical). Hydrogen concentration were calculated according to the hydrogen solubility in seawater (Crozier & Yamamoto 1974).

Enzymatic hydrolysis rates: Potential activities of extracellular enzymes were measured by adding fluorescently labeled (FLA) polysaccharides to 2 ml portions of sediments incubated typically for 24-48 h in N_2-filled bags in the incubator. The FLA-polysaccharides were prepared as described in Arnosti (1995). After incubation, the tubes were centrifuged, pore water was removed and filtered through a 0.2 µm pore-sized filter, and stored frozen until analysis. Samples were analyzed using a HPLC-GPC system with a fluorescence detector set to excitation and emission wavelengths of 490 and 530 nm, respectively. Hydrolysis rates were calculated as described in Arnosti (2000).

Sulfate reduction rates: SRR were measured in a manner analogous to the whole core $^{35}SO_4^{2-}$ incubation method (Jørgensen, 1978). The sediment was extruded into 5ml glass tubes closed with rubber stoppers at both ends. Approximately 120 kBq $^{35}SO_4^{2-}$ was injected into the sediment and the samples were incubated at *in situ* temperature, typically for 6 hours. The reaction was terminated by extruding the sediment into 20 ml of 20% (W/V) zinc

acetate. Reduced ^{35}S was analyzed by the single step chromium reduction method (Fossing & Jørgensen 1989), and the sulfate reduction rates were calculated per cm^3 of sediment as described by Jørgensen (1978).

Results

Microbial community response to carbon input was assessed by homogenizing anoxic sediments and adding lyophilized cyanobacterial cells (S), soluble extract of the lyophilized

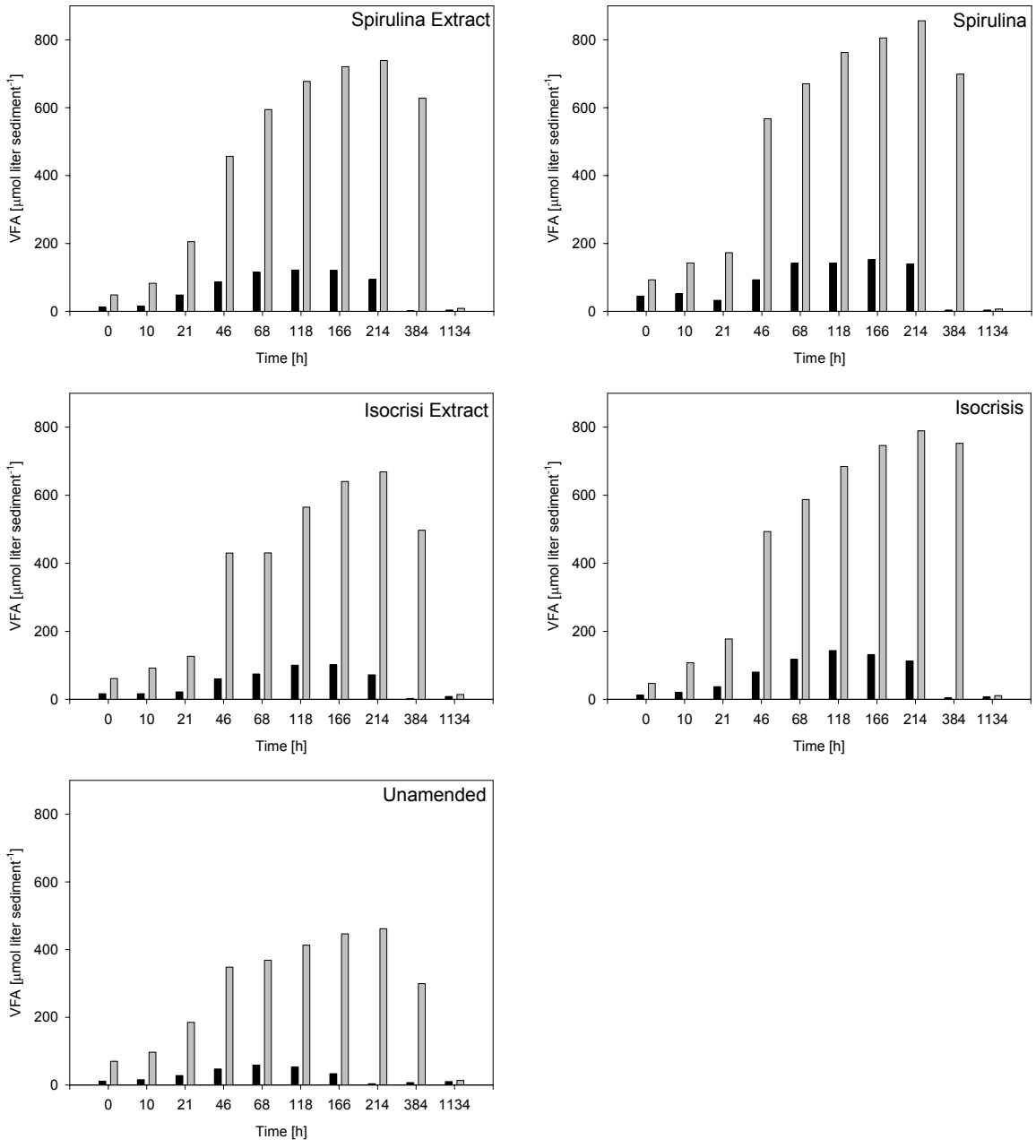

Figure 1: Volatile fatty acids (VFA) concentrations in the different bags over time. Other VFA include butyrate, formate, glycolate, isobutyrate, lactate and propionate.

cyanobacterial cells (SE), lyophilized Isochrysis sp. (I), or soluble extract of the lyophilized Isochrysis (IE) to replicate sediments. Unamended sediments (U) were used to determine community response to homogenization. Changes in the activities of the sedimentary microbial communities were most immediately evident in the VFA concentrations, which increased with time and reached peak concentrations at 214 h before decreasing to concentrations below initial after 1134 h incubation (Fig. 1). The same general pattern of VFA concentrations was observed for all sediments. Concentrations of VFA, however, were considerably higher in sediments to which organic matter had been added than in the U sediment.

Calculated on a molar-C basis and normalized for carbon addition levels, total VFA concentrations were initially 130-175 µM C for all except SE sediments, which had initial VFA concentrations close to 320 µM C (Fig. 2). Throughout the initial 214 h of incubation, total VFA concentrations increased to maxima 7-15 times initial concentrations. When normalized for the quantity of substrate carbon added, after the first 21 h of incubation Se and S sediments showed virtually identical VFA profiles. S and I sediments likewise were nearly identical. IE sediments averaged slightly more than 82% of the VFA concentrations

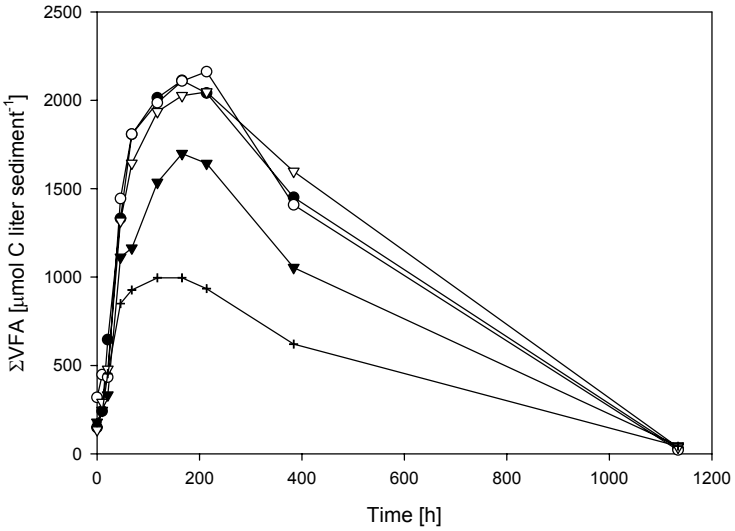

Figure 2: Total volatile fatty acids (VFA) concentrations in the different bags over time. VFA include acetate, formate, glycolate, lactate, propionate, isobutyrate and butyrate. The values were normalized for the carbon addition. This was done by multiplication of the value with the amount of carbon added devided by the amount added to the spirulina bag.

in I sediments, while U sediments averaged 57% of the VFA concentrations detected in S sediments.

Initial VFA concentrations and compositions were similar among sediments, with the

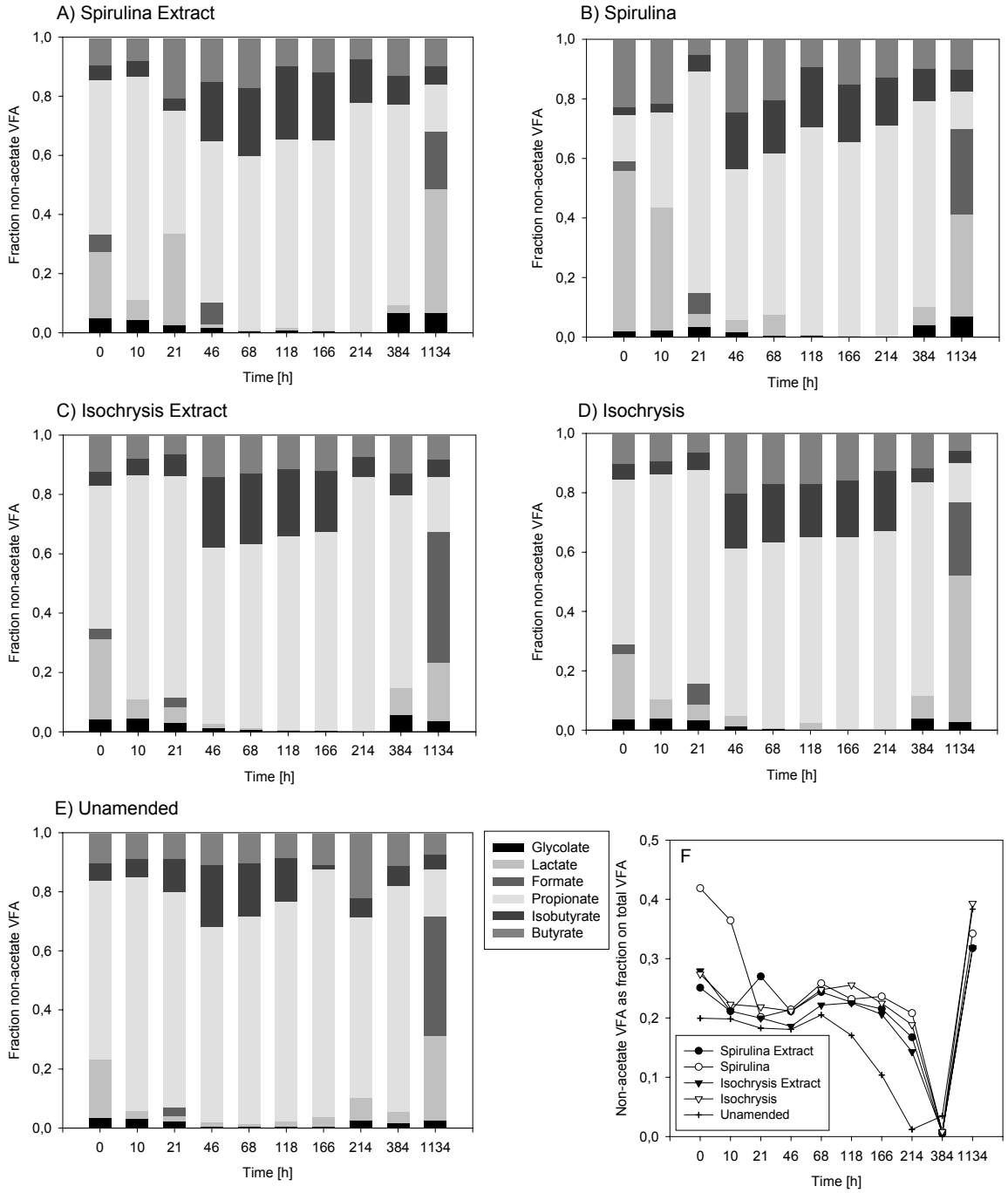

Figure 3: Fractoin of the volatile fatty acids (VFA) other than VFA in the different bags over time. Other VFA include butyrate, formate, glycolate, isobutyrate, lactate and propionate. a - e) Relative abundance of the different acids. f) Sum of non-acetate VFA as a fraction of total VFA.

exception of somewhat higher acetate and much higher lactate in S sediments relative to all other treatments (Figs. 1, 3). Acetate was always quantitatively the most significant VFA,

with maximum concentrations of 670-850 μM and 462 μmol acetate/liter of sediments for amended and unamended bags, respectively. In addition to acetate, propionate, isobutyrate, butyrate, lactate, and glycolate (in descending order of concentration) were present. Formate was detected at several time points (Fig. 3); detection of formate was dependent in part on acetate concentrations (see Methods). During incubation, relative contributions of VFA other than acetate developed in a similar fashion among the sediments (Fig. 3f). As a group, non-acetate VFA typically comprised 20-25% of total VFA, a proportion that decreased greatly at 384 h and increased at 1134 h. Both changes were driven principally by changes in acetate concentrations. As a group, non-acetate VFA in the U sediments changed in a slightly different pattern, with relative contributions dropping below 20% at 118 h, well before the acetate concentration peaked and declined.

In order to monitor carbon flow through the microbial food chain, rates of specific steps in the carbon degradation pathway were also measured. Degradation of high molecular weight organic matter is initiated by the activities of extracellular enzymes that hydrolyze substrates to sizes sufficiently small to be taken into microbial cells. Potential hydrolysis rates of five high molecular weight polysaccharides [pullulan (pull), xylan (xyl), fucoidan (fu), chondroitin sulfate (chon), and Spirulina extract (spir)] were measured throughout the experiment as an indication of possible substrate-, amendment-, or time-related changes in hydrolytic activities. In general, potential hydrolysis rates varied strongly by substrate, rather than by sediment treatment. Rates were most rapid for pull, followed in decreasing order by xyl, chon and spir, and fu. Potential hydrolysis rates of spir, xyl, and fu increased slightly from the beginning to the end of the incubation period, while pull and chon hydrolysis showed no specific temporal patterns (Fig. 4). Although there was no systematic difference between whole cells (S, I), extracts (SE, IE), and unamended sediments (U), some of the sediments showed discernable patterns with specific substrates. Hydrolysis rates of pullulan in I sediments remained low throughout the incubation, while hydrolysis rates in U sediments started high and decreased through 214 h, increased again at 384 h, and decreased at 1134 h. S, SE, and I sediments showed variable levels of activity, with S at

1134 h reaching a maximum level of pullulanase activity. IE sediments showed initially low rates of chondroitin sulfate hydrolysis, which increased progressively for the last three timepoints.

Hydrolysis of high molecular weight substrates such as polysaccharides yields low molecular weight sugars that can be fermented to produce VFA, CO_2, and cellular biomass. The concentrations of VFA measured in sediments therefore reflect the net outcome of production via fermentation, and consumption of VFA by terminal members of the food chain that remineralize the VFA to CO_2. In order to assess VFA turnover rates, turnover rate constants were measured twice for acetate and for lactate. At 0 h, turnover rate constants ranged from 0.013 – 0.22 x 10^{-3}min^{-1} and 0.078-1.2 x 10^{-3}min^{-1} for acetate and lactate, respectively (Table 2), with no discernable pattern among amended and unamended sediments. After 118 h, acetate turnover rate constants increased for all but IE sediments, with a range of 0.13-0.23 x 10^{-3}min^{-1}, and lactate turnover rate constants had increased for all sediments, with a range of 1.0-2.9 x 10^{-3}min^{-1}. At 118h, acetate turnover rate constants increased by factors of 3-16 relative to 0 h for all but IE sediments, and lactate rate constants were higher by factors of 2-30.

Table 2: Turnover rate constants for acetate and lactate

	turnover rate constant (x 10^{-3}); min^{-1}			
	0 h Acetate	118 h Acetate	0 h Lactate	118 h Lactate
Spirulina Extract	0.034	0.23	0.57	1.0
Spirulina	0.070	0.20	0.078	2.3
Isochrysis Extract	0.22	0.14	1.2	2.9
Isochrysis	0.12	0.13	0.72	2.1
Unamended	0.013	0.21	0.71	2.9

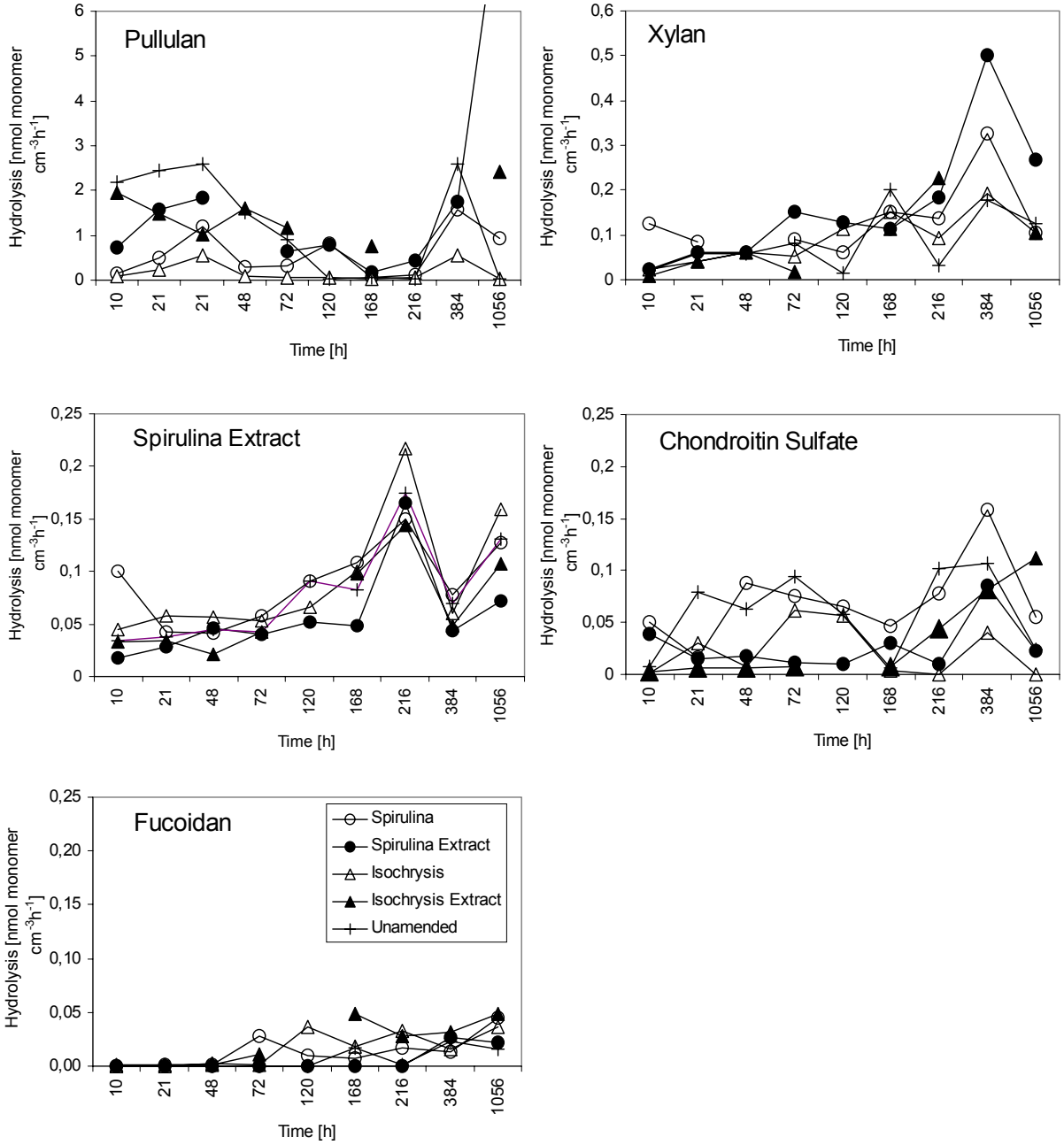

Figure 4: Potential hydrolysis rates of pullulan, spirulina extract, chondroitin sulfate, xylan and fucoidan over time. Note the different scales for the different polycsaccarides.

Hydrogen concentrations were also measured at 6 timepoints during the incubation (Fig. 5). Scatter was quite large, especially for S and SE samples during the first two timepoints. Maximal concentrations were measured at 67 h, and amended sediments generally reached higher concentration levels than unamended sediments. Hydrogen concentrations at 92 h were substantially lower and declined further after 92 h, remaining at very low concentrations for the remaining three timepoints at 186, 235, and 746 h.

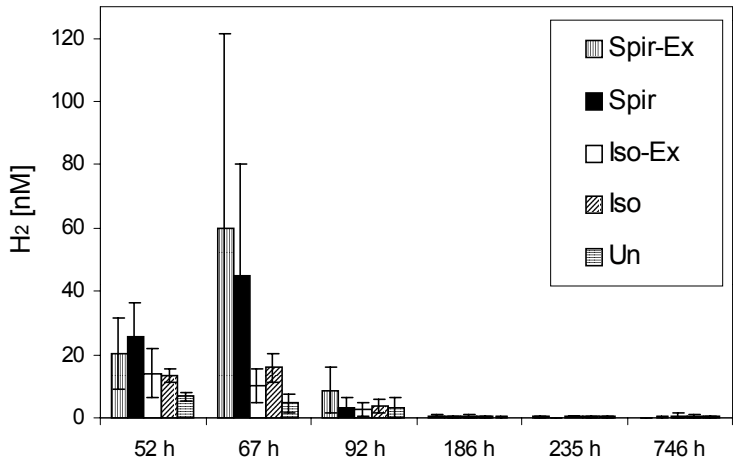

Figure 5: Hydrogen concentrations measured in the bags at 6 time points during the incubation. The error bars represent the standard deviation from single measurements in triplicate samples.

Terminal members of the microbial food chain are responsible for consumption of H_2, as well as of VFA, which are oxidized to CO_2 with concurrent reduction of sulfate to sulfide. Sulfate reduction rates (SRR) were measured periodically during the incubation (Fig. 6). Aside from a possible temporary enhancement in the earliest time points at 10 and 21 h, these rates changed relatively little through the initial 166 h of incubation. No systematic difference was detected among different sediment amendments. At 214 and 384 h, SRR in the amended sediments were ca. 94 nmol SO_4^{-2} cm^{-3} h^{-1}, close to double the rate measured at earlier sample times and more than 150% of the rate measured in the unamended sediments at the same timepoints. At the end of the incubation, 1134 h, rates in amended and

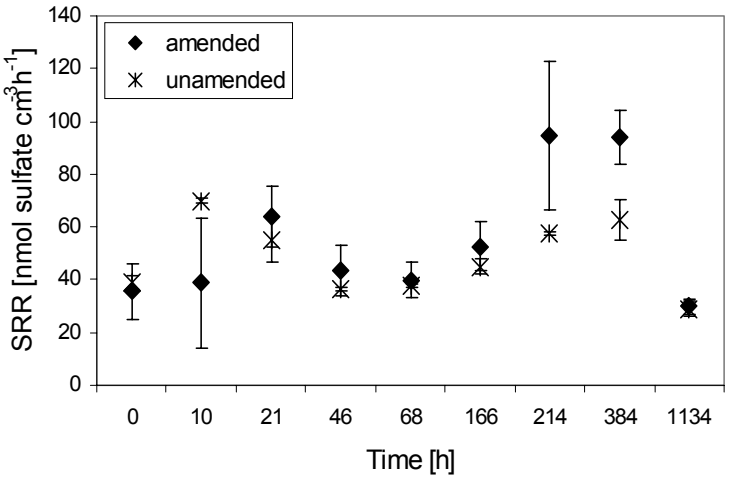

Figure 6: Sulfate reduction rates (SRR) were measured in the bags. The rates measured in the different amendments showed no significant difference. The error bars represent measurements in triplicate samples, in the amended all four substrates were combined

unamended sediments were again comparable to rates at the start of the incubation.

Fermentation of low molecular weight hydrolysis products as well as remineralization of VFA contribute to the production of CO_2, which was measured as DIC (dissolved inorganic carbon). DIC concentrations increased throughout the course of the incubations, with relatively small increases during the first 68 h of incubation, and much larger cumulative increases throughout the remainder of the incubation (Fig. 7a (not C addition normalized)). At early time points, differences among amendment types as well as between amended and

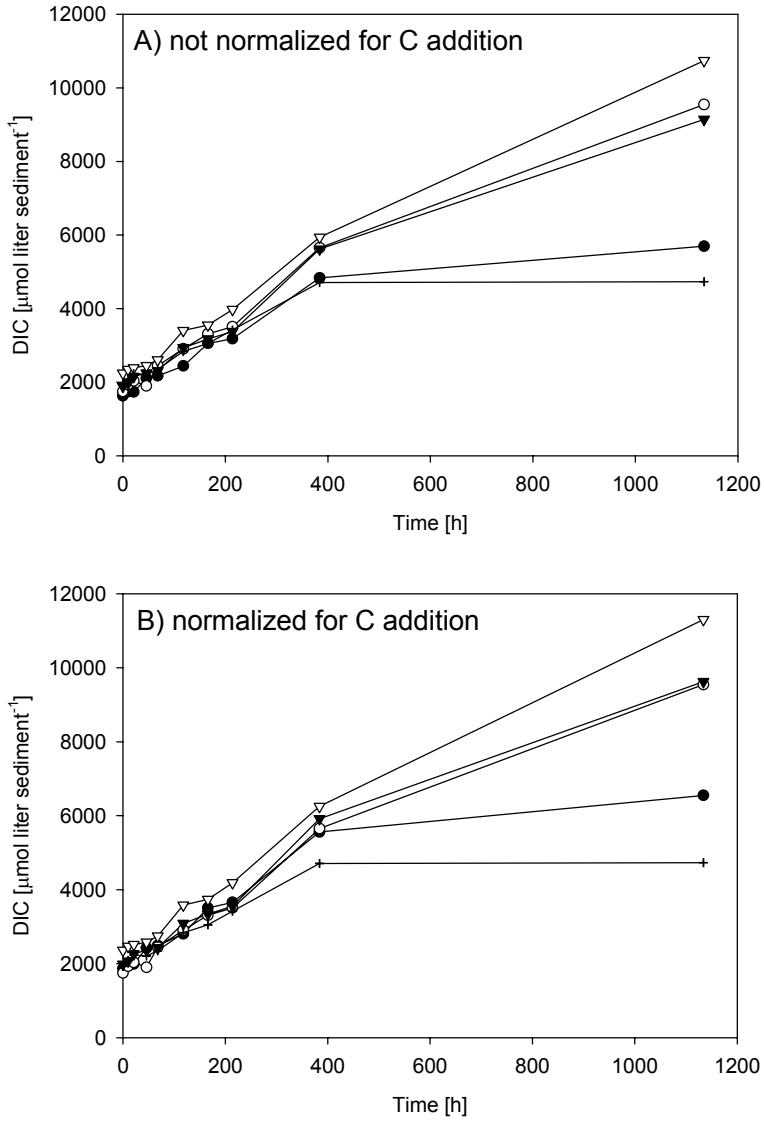

Figure 7: Dissolved inorganic carbon (DIC) a) Concentrations as measured in the samples. b) Concentrations normalized to carbon addition as described for figure 2.

unamended sediments were small. Beginning in the time interval of 214-384 h, however, when sulfate reduction in amended sediments also increased, DIC accumulation was also greater in amended than unamended sediments. DIC concentrations increased between 384 and 1134 h by factors of 1.2 for SE and approximately 1.7 for S, I, and IE sediments. Normalized to C input (Fig. 7b (C addition normalized)), the amended sediments reached 5560-6250 μmol C liter^{-1} sediment at 384 h, while DIC in the unamended sediments reached 4710 μmol C liter^{-1} sediment. Final DIC accumulations at 1134 h differed among amendments, with DIC concentrations greatest for I, S, and IE sediments, considerably lower for SE sediments, and lowest for U sediments.

Discussion

Carbon degradation in anoxic sediments is accomplished through the activities of diverse microbial communities acting in concert, producing new biomass and remineralizing complex organic substrates to CO_2. Although the general sequence of processes by which carbon is degraded - hydrolysis, fermentation, respiration - is known, the factors controlling the rates and specific pathways by which these processes are accomplished are less well understood. This lack of knowledge reflects the difficulty of studying the details of degradation pathways directly in sediments: changes in inventories of specific products are difficult to measure against bulk POC and DOC backgrounds. Furthermore, the size of a given carbon pool may not reflect its relative importance in degradation pathways. Although methods have been developed to measure rates of specific processes such as sulfate reduction (Jørgensen, 1978), rates and relative importance of many other processes can only be assessed indirectly (e.g. Canfield et al. 1993, Thamdrup 2000). By combining rate and inventory measurements with addition of substrates to sediments, we sought to constrain the responses of microbial communities to complex substrates.

Four substrates were added to replicate portions of homogenized anoxic sediments. The four substrates were two different types of freeze dried cells, plus a high molecular weight soluble extract obtained from each cell type. By using these substrates, the relative importance of factors such as solubility and chemical composition on rates and pathways of

degradation could also be investigated. Carbon addition levels were a further important consideration. We sought to add sufficient carbon to cause a measurable change in carbon inventories and carbon degradation processes relative to unamended sediments, but to avoid adding quantities of carbon that could cause unrealistic changes in sediment conditions or sequences of reactions. Additions of complex organic matter for the four substrates therefore ranged from 120-140 mg C liter^{-1} sediment, a level of carbon addition that proved sufficient to stimulate microbial activity. Microbial response was stimulated simply by homogenizing the sediments, as is clear from the VFA and DIC profiles (Fig. 1, 7), but the addition of complex substrates stimulated activity significantly above the levels observed with unamended sediments. The added substrates produced a suite of VFA very similar to those observed in the unamended sediments (Fig. 3), suggesting that the added carbon was degraded via the same pathways as organic carbon naturally present in the sediments. Furthermore, the suite of VFA produced, and the concentrations measured in amended and unamended sediments are similar to those that have been reported in an annual survey of carbon cycling in temperate sediments (Sansone & Martens 1982). The carbon addition levels used here therefore are likely within a 'reasonable' range for a sedimentary system.

The suite of fermentation products measured in all sediments was dominated by acetate. This dominance could due to acetate production from incomplete oxidation of other VFA, or to direct production of acetate via fermentation. The similarities of initial SRR in amended and unamended sediments, despite their different VFA concentrations (Fig. 6, 1), suggest that the principle pathway of acetate production was not incomplete oxidation of propionate, butyrate, isobutyrate, and lactate. Furthermore, SR increased in amended sediments only after 214 h incubation, when VFA concentrations began to decline (Fig. 6, 1), suggesting that much of the acetate was produced directly via fermentation (Sansone 1986)

The observation that VFA and H_2 production were stimulated in unamended as well as amended sediments shows that homogenization of sediments is sufficient to provide access to substrates (as POC or DOC) that otherwise are not available in the same timeframe to the sedimentary microbial community. Increased microbial activity due to homogenization of sediment samples in bag incubation has been reported before (Kostka et al. 1999).

Stimulation of activity through homogenization is also evident in the sulfate reduction rate measurements. Sulfate reduction rates in intact cores at other stations from Svalbard ranged up to ca. 25 nmol SO_4^{2-} $cm^{-3}d^{-1}$ (Jørgensen, unpubl. data), a factor of ca. 50 lower than rates measured in both amended and unamended sediments prior to 214 h (Fig. 6).

Hydrogen concentrations also reflected response to substrate addition. Hydrogen headspace concentrations increased faster and reached maximum levels considerably before maximum VFA concentrations were reached (Fig. 5), perhaps due to higher turnover rates, lower concentrations, and faster diffusion of H_2 relative to VFA. Similar observations have been made in temperate sediments (Hoehler et al. 1999).

In the initial response, the nature of the added substrate had little effect on either timecourse or extent of reaction. VFA concentrations (normalized to C addition levels, Fig. 2) were virtually identical for SE, S, and I amendments, and VFA in the IE amendment were ca. 80% of these concentrations. The timecourse of VFA production and consumption was identical for the different amendments, as was the suite of VFA produced. Turnover rate constants of VFA additionally showed no systematic differences among amendments (Table 2). The somewhat lower concentrations of VFA in IE sediments relative to other amended sediments might have been affected by differences in initial turnover rates, since initial turnover rate constants of acetate and lactate in IE sediments were higher than for other amended and for unamended sediments (Table 2). At 118 h, however, the turnover rate constants in IE sediments were comparable to those observed in other sediments.

A plot of carbon inventories (DIC and VFA), normalized to carbon addition levels, shows that the response of the microbial community to carbon addition was dominated by fermentation through the first 214 h of incubation (Fig. 8). Only relatively modest increases in DIC are observed at 214 h, whereas the flow of added substrate carbon into the VFA pool can clearly be distinguished between amended and unamended sediments. At 384 h, the community response had shifted so that carbon oxidation began to dominate: the VFA pools began to decrease, and VFA carbon was oxidized to DIC, a process that evident at the last measurement point at 1134 h. (Note that given the long time interval between the last two sampling points; these levels of DIC may have been achieved at a much earlier time, and

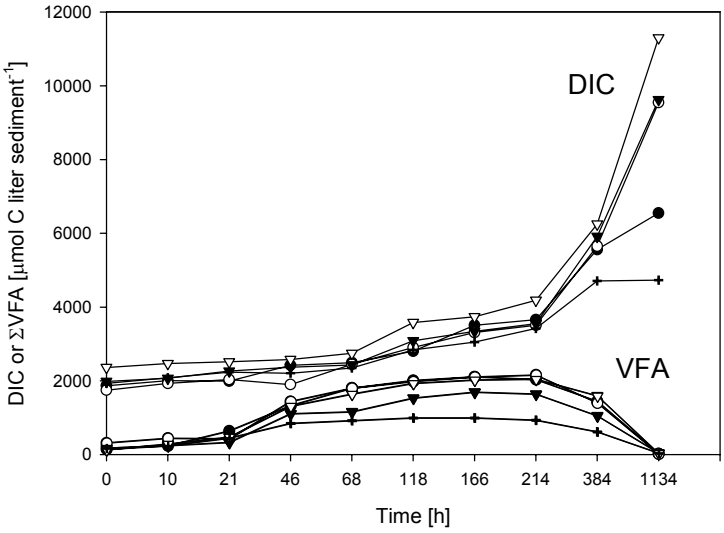

Figure 8: Volatile fatty acids (VFA) and dissolved inorganic carbon (DIC) in the different bags. Concentrations were normalized to carbon addition as described for figure 2

only detected at 1134 h.).

The shift from fermentation-dominated to respiration-dominated processes shows that members of the sedimentary microbial community reacted on different timescales to the influx of organic carbon. Hydrolysis of added substrate must have happened quickly, since increases in VFA concentrations were measured within a short period of time. This impression is supported by the fact that although some differences in enzyme activities and specificities could be observed between amendments, for the most part these differences were quite subtle (Fig. 4). The enzymatic capabilities of the sedimentary community therefore were likely sufficient to respond rapidly to the most reactive fraction of the added organic matter. Rates measured in this experiment are also similar to those measured in slurried sediments from Svalbard fjords (Finke et al., in prep., Chapter 3). Fermentation then followed rapidly, with a large increase in VFA inventories between 21 and 214 h (Fig. 1). The similarities in timecourse of VFA production in amended and unamended sediments over the first 384 h of incubation also suggest that the absolute quantity of VFA produced did not control the timescale of VFA consumption. Consumption processes reacted only after a lag time to the increased concentrations of VFA. The response of terminal members of the food chain, in this case sulfate reducers, lagged the buildup of fermentation products

by a considerable amount of time. Although VFA concentrations increased three- to five-fold during the first 46 h of incubation, turnover rate constants of acetate did not change in a consistent fashion between 0 h and 118 h, and sulfate reduction rates in amended sediments were generally unchanged until 214 h incubation. This lagged response of sulfate reducers to substrate input is consistent with a flow-cell investigation of the degradation of chondroitin sulfate, a high molecular weight polysaccharide, in Svalbard sediments (Brüchert & Arnosti 2003). The flow-cell experiments also demonstrated that hydrolysis was more rapid than fermentation of the resulting low molecular-weight carbohydrates, and that fermentation in turn proceeded far more rapidly than oxidation of the VFA with concurrent reduction of sulfate to sulfide.

The differences in final DIC concentrations among the amended bags at 1134 h demonstrate

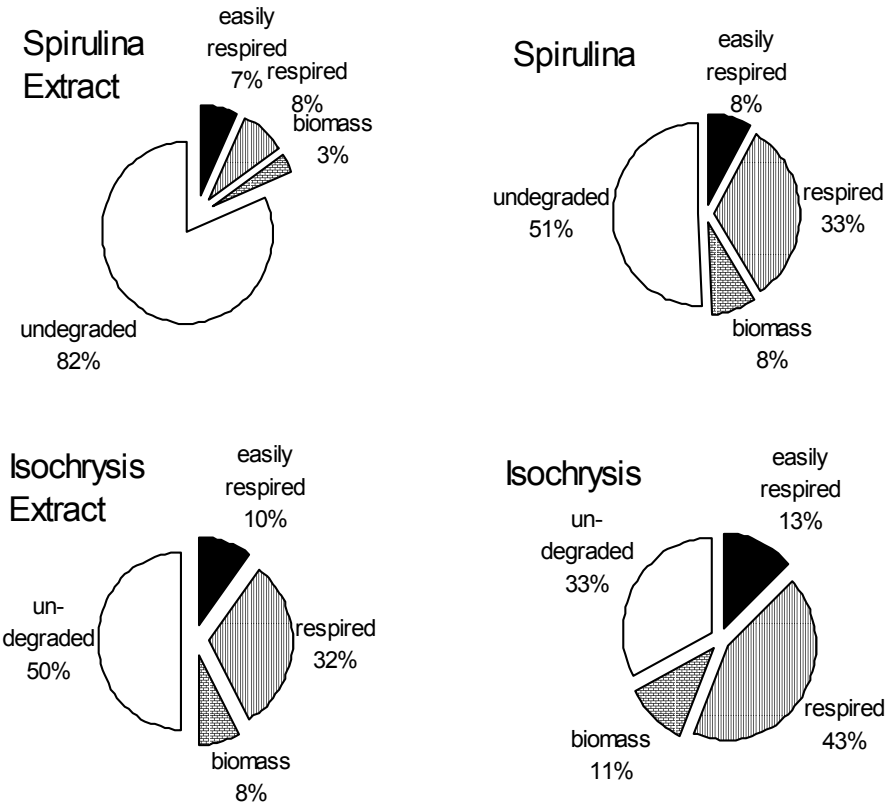

Figure 9: Fate of the carbon from the different additions. Easily respired and respired were calculated from the DIC increase relative to the unamended bag after 384 and 1134 h, respectively. Incorporation into biomass was estimated assuming a carbon incorporation efficiency of 20%.

that the nature of the added substrate ultimately did affect the extent of reaction. The quantity of added carbon ultimately respired to CO_2 can be estimated by comparing DIC concentrations of amended and unamended sediments at 384 h and at 1134 h (Fig. 7). The DIC concentrations reached in the unamended sediments presumably represent carbon oxidation due to stimulation of the microbial community caused by sediment homogenization. The DIC concentrations above this level (above 4710 μmol C liter^{-1} sediment at 384 h; above 4730 μmol C liter^{-1} sediment at 1134 h) are presumably a reflection of the added substrate carbon ultimately oxidized to CO_2. By this calculation, 8% of S, 7% of SE, 13% of I, and 10% of IE amendments were 'easily respired' (measurable as DIC at 384 h). At the final timepoint, 1134 h, DIC concentrations (normalized for C-addition levels) had increased to the equivalent of 41% of the total added substrate for the S amendment, 15% of the total SE amendment, 56% of the I amendment, and 42% of the IE amendment. The fraction of 'respired' carbon (measurable as DIC in the interval between the 384 and 1134 h timepoints) is then 33% of S, 8% of SE, 43% of I, and 32% of IE. The fraction of carbon used by the microbial communities that was incorporated into biomass can be estimated by assuming that carbon incorporation efficiency was 20%. Using this assumption, 8% of S, 3% of SE, 11% of I, and 9% of IE amendments were incorporated into cellular biomass (Fig. 9). This calculation yields a total of 49% of S, 18% of SE, 67% of I, and 50% of IE carbon utilized (respired or incorporated into cellular biomass) by the sedimentary microbial community within 1134 hours' incubation.

Although the quantity of carbon flowing through the VFA pool between the 384 and 1134 h time points cannot be assessed directly, a comparison of the VFA and DIC profiles (C-normalized; Fig. 2, 7) shows a significant divergence among sediment amendments. By comparing C-inventories as VFA at 384 h with the increase in DIC between 384 and 1134 h, the fraction of VFA measured at 384 h that ultimately was respired to CO_2, and by implication, the fraction that was channeled through other pathways and perhaps incorporated into biomass can be estimated. At one extreme, it is clear that most of the VFA carbon measured in unamended sediments at 384 was not respired. Total VFA constituted 620 μmol C liter^{-1} sediment at 384 h and 43 μmol C liter^{-1} sediment at 1134 h. Since DIC increased by only 20 μmol C liter^{-1} sediment between these timepoints, approximately 557

μmol C liter^{-1} sediment must have followed another path. By similar reasoning, approximately 2/3 of the SE VFA at 384 h were respired to DIC; the other third must have followed another path. For S, I, and IE amendments, in contrast, fermentation must have continued on through timepoints beyond 384 h. More than double the quantity of carbon measured as VFA at 384 h for I and IE amendments was measurable as DIC at 1134 h; for the S amendment, nearly double the 384 h VFA-C concentration was respired by 1134 h. This comparison suggests that all of the amendments had a fraction of carbon that was rapidly and easily metabolized. Since the SE and IE amendments had been dialyzed, this easily metabolized fraction was not necessarily low molecular weight, but was nonetheless rapidly degraded. The IE, I, and S amendments additionally contained substrates that provided something of a sustained 'push' for the microbial community, since fermentation likely continued at a significant level for some time beyond 384 h.

The remineralization in Arctic sediments of a substantial fraction of plankton and extract carbon over a timecourse of 1134 h (48 days) demonstrates that the microbial communities in these sediments have the capability to react relatively rapidly and comprehensively to an influx of organic carbon. To the best of our knowledge, no similar studies following carbon flow in anoxic sediments have been carried out in permanently cold sediments. The extent of carbon remineralized and the timecourse of reaction observed in the present study are at least as rapid and extensive as has been observed in experiments carried out under temperate conditions. Anoxic incubation of Cape Lookout Bight sediment at 23°C with carbon added as phytoplankton or seagrass resulted in ca. 70% remineralization of phytoplankton carbon and 15% of seagrass carbon over a timecourse of 180 days (Hee et al. 2001). An investigation of the degradation of ^{14}C-labeled diatoms (which contained 11.5% of the ^{14}C label as a DOC fraction) added to the surface of cores showed that 42% of the added carbon was respired to $^{14}CO_2$ and 13% recovered as DO^{14}C after 80 days' incubation under anoxic conditions at 15°C. A different experimental approach was used by Harvey et al. (1995), who measured changes in particulate constitutes and concentrations of diatom and cyanobacterial biomass under oxic and anoxic conditions. Since the experiment was carried out using a flow-through system, mass balances could not be established. After 153 days' incubation under anoxic conditions, however, 36% of cyanobacterial POC and 22% of

diatom POC remained in the system. (Note that the fraction of POC transformed to DOC and washed out of the system could not be constrained.) By comparison, 49% of Spirulina (a cyanobacterial mixture) and 67% of Isochrysis were respired or transformed to biomass after 48 days' incubation in permanently cold sediments.

A comparison of substrate types and carbon fate demonstrates that the extent of carbon remineralization and incorporation is not necessarily apparent when comparing bulk chemical characteristics of complex substrates. Although S and IE substrates differed in essentially all measured characteristics - solid vs. soluble, C/N ratio, lipid and carbohydrate content (Table 1) - the quantity of carbon respired to CO_2, incorporated into biomass, and calculated as residual after 1134 h incubation was the same (Fig. 9). The contrasting behavior of the S and SE substrates was also notable; despite the soluble nature of SE, and its relatively high carbohydrate content, only an estimated 18% of this substrate was available to the sedimentary microbial community over an 1134 h incubation. Furthermore, the initial burst of activity fueled by the SE substrate provided no indication of the recalcitrance of the remainder of the organic carbon. Clearly, a great deal of work will be needed to understand the factors that control bioavailability of substrates on microbially-significant scales.

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Chapter 3

Degradation of organic matter in two permanently cold sediments, Svalbard, Norway

(prepared for submission to Aquatic Geochemistry)

Niko Finke[1*], Ole Larsen[1], Carol Arnosti[2], Bo Barker Jørgensen[1]

[1]Dept. of Biogeochemistry, Max Planck Institute for Marine Microbiology, Celsiusstr. 1, 28359 Bremen, Germany
[2]Dept. of Marine Sciences, 12-7 Venable Hall, Univ. North Carolina, Chapel Hill NC 27599-3300, USA
[*]Corresponding author: nfinke@mpi-bremen.de

Abstract

Three important steps along the degradation pathway of complex polysaccharides and the anaerobic terminal electron accepting processes were quantified and compared in two Arctic, permanently cold sediments, Svalbard, Norway. Potential hydrolysis, sugar turnover and volatile fatty acid (VFA) turnover were measured and compared to rates of total carbon oxidation, sulfate reduction rates (SRR) and iron reduction rates (FeRR) in three depths at both stations. The two stations exhibited similar biogeochemical characteristics, suggesting that the results might reflect a more general pattern for sediments found in the arctic fjords of Svalbard. Measurements of the net and gross SRR revealed that at least 50-90 % of the sulfide produced by dissimilatory sulfate reduction is reoxidized with reactive iron. Dissimilatory iron reduction accounted for only 1-16% of the terminal oxidation. The high rates of chemical and dissimilatory iron reduction compared to the relatively small pool size of reactive iron suggested that bioturbation plays an important role in resupplying the deeper parts of the sediment with oxidized iron. Rates of the initial and terminal steps of organic matter degradation in the Svalbard sediments - hydrolysis and the sulfate reduction - were similar to values reported from temperate sites at higher temperatures. VFA turnover rates, however, were similar to rates reported from temperate sites at low temperatures. The sugar turnover rates were higher than previous reports from limnic sediments, but probably did not reflect *in situ* conditions. Thus, the *in situ* temperature appeared not to have an effect on the overall rate, but rather on the pathways of degradation.

Introduction

Degradation of organic matter in anaerobic environments involves several distinct steps (e.g. Capone & Kiene 1988) mediated by different organisms. The first step is to hydrolyze high molecular weight organic matter that is too large to be taken up by the bacteria. This step is performed by enzymes excreted by heterotrophic bacteria. These enzymes degrade polymers to mono- and small oligomers that can be taken up by fermentative bacteria, which ferment substrates to volatile fatty acids (VFA), H_2 and low molecular weight alcohols. The fermentation products in turn serve as electron donors for the bacteria carrying out the terminal oxidation step. Depending on the availability of electron acceptors, the terminal oxidation is carried out by nitrate-, Fe or Mn-, or sulfate reducing bacteria, or methanogenic archea.

For degradation of many compounds, different fermentation or terminal oxidation steps and pathways are possible (e.g. Gottschalk 1986 and references therein). Little is known about the relative importance of the different steps in the environment that (on the basis of culture experiments and thermodynamic considerations) could potentially occur. Furthermore, laboratory investigations of the potential substrates of a bacterium do not reveal the role of that particular substrate in the complex organic carbon (C_{org}) degradation pathways in sediments.

Several factors are important in controlling the metabolic pathways that are actually significant in sediments. First, the compound needs to be present or excreted in pore water in significant quantities. For example acetate is formed under anaerobic conditions in significant amounts. On the other hand, acetate does not play an important role as an intermediate in oxic marine sediments, as organic matter is not fermented but rather fully oxidized to CO_2 under these conditions (Capone & Kiene 1988). Secondly, competition for substrates plays a role. Bacteria with higher affinity for a specific substrate will outcompete those with a lower affinity (e.g. Schönheit et al. 1982, Lovley & Klug 1983, Krumholz et al. 1999). In addition, bacteria may not take up all available substrates, but rather degrade the energetically most favorable ones. Even these substrates may not be oxidized all the way to

CO_2. An example would be the incomplete oxidation of lactate to acetate by non-substrate limited sulfate reducers that are capable of complete oxidation of acetate to CO_2 (Widdel 1988).

Another important consideration are the thermodynamics that control whether degradation of a potential substrates via a certain pathway will be energetically favorable. For example, the hydrogen concentrations in marine sediments under steady state conditions are controlled by thermodynamics. Field investigations (Lovley & Goodwin1988, Hoehler et al. 1998) have shown that bacteria performing the oxidation with the electron acceptor giving the highest energy yield lower H_2 concentrations to levels that make the reaction thermodynamically unfavorable for the other bacteria. Thus, pure culture studies and thermodynamic considerations are meaningful to understand the potential contributions of different microorganisms, but field studies are important to test these potential roles.

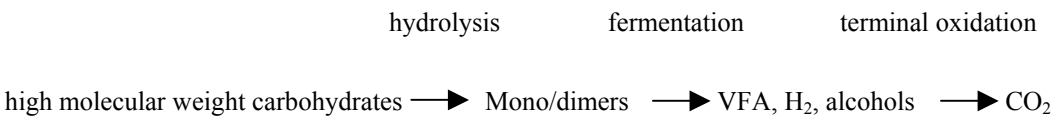
Degradation of organic carbon in marine sediments has been studied in numerous investigations. In some investigations, the focus was mainly on concentrations of potential intermediates (e.g. Mueller-Harvey & Parkes 1987, Finke 1999a, Burdige et al. 2000). Other investigations have focussed on turnover processes of one or more intermediates, or have compared the C turnover rates of one of the steps with the terminal electron accepting process (e.g. Christensen & Blackburn 1982, Wu & Scanton 1994, Albert et al. 1995, Wellsburry & Parkes 1995, Arnosti 2000). The concentration of a specific substrate does not reveal its importance as an intermediate, however. For example, the hydrogen concentration is usually 3 orders of magnitude lower than VFA concentrations in sediments, although the importance of the two for electron flow is similar. Rates measured at different sites may vary substantially, however, without revealing the factor controlling this variation. Therefore it is difficult to get a detailed picture of the importance of the different steps and potential controlling factors from comparing rates measured in different investigations. To understand the pathway of degradation of organic matter in anoxic marine sediments, the knowledge of the different involved steps is important. A combination of concentration and turnover measurement of potential substrates furthermore may reveal the importance of possible intermediates and pathways in sediments.

The oxidation of organic matter to CO_2 in anoxic environment is a stepwise transfer of electrons from the reduced carbon finally to inorganic electron acceptors. In the fermentation steps, the electrons are transferred from one carbon to another or to hydrogen. During terminal oxidation, the electrons are finally transferred to the inorganic electron acceptor and the carbon is oxidized to CO_2. No electrons can be lost along the way, and the rates must balance each other at steady state conditions. Complete measurement of any of the steps (hydrolysis, fermentation or terminal oxidation) should therefore yield the carbon oxidation rate of a sample. Measurements of the first step are only possible as potential rates, and only for specific polymers, accounting only for a fraction of the organic matter. Such measurements thus do not give an actual C_{org} degradation rate, but the maximum rate for the specific enzymes, if the substrate is not limiting. Potential rates can be measured for the turnover of specific carbohydrates (Arnosti 1995, 2001) and proteins (Pantoja et al. 1997, Pantoja & Lee 1999), although little is known about the actual abundance of these specific polymers as components of marine organic matter. Measurements of terminal oxidation are mostly performed as an indirect measurement. Terminal oxidation is measured as reduction of the terminal electron acceptor. This measurement integrates the oxidation of all different electron donors along the degradation pathway. Knowledge about the specific pathways by which the electrons are derived and the substrates for the terminal oxidation is unimportant for this measurement, and cannot in any case be derived from these experiments. For these measurements, however, it is important to know all contributing terminal electron accepting processes, in addition to other reactions affecting the involved species. Oxidation of reduced sulfur, for example, can cause underestimation of sulfate reduction rates (SRR) (Moeslund et al. 1994, Fossing 1995).

Quantitative measurement of the oxidation of an electron donor requires coverage of all the important substrates. Comparing measurements of the electron donating and electron accepting steps can indicate the extent to which the substrates for the TEAP (terminal electron-accepting process) have been quantified. Solely measuring fermentation is exceptionally difficult, as a variety of reactions and pathways are possible. This makes it difficult to comprise enough reactions to actually quantify the total rate. In addition, many intermediates such as sugars and VFA longer than acetate can either be fermented or

directly oxidized. The factors controlling the competition between these two steps are not yet understood. For a conservative estimate of total carbon mineralization, measuring the increase of DIC (dissolved inorganic carbon) or NH^{4+} is useful. DIC measurements can also result in an underestimation of the carbon oxidation rate, however, as carbonates might precipitate with prolonged incubation, and NH_4^+ adsorbs to clays.

The best way to investigate the relative importance of potential intermediates in the degradation of complex organic polymers such as polysaccharides is the measurement of the most important contributors for every step. Polysaccharides were chosen as a focal point because carbohydrates make up about 3-30 % of the organic matter in marine sediments (e.g. Bergamaschi et al. 1997, Hedges et al. 2000, Hernes et al. 1996, Keil et al. 1998, Wakeham et al. 1997). Hydrolysis mainly leads to mono- & disaccharides that are taken up by heterotrophic bacteria. The classical scheme for the degradation of carbohydrates is:



Sulfate reduction is the most important terminal oxidation step in anoxic marine sediments, accounting for up to 50% of C_{org} oxidation (Jørgensen 1982). The main substrates for sulfate reducing bacteria (SRB) are VFA, hydrogen, and small alcohols according to this scheme. Pure culture studies showed that these are potential substrates for SRB and sediment analysis indicate that short VFA and hydrogen are the most important substrates in marine environments (Christensen 1984, Skyring 1988, Parkes et al. 1989).

The sediments used in this study were collected from two fjords, located on the west coast of Svalbard. Arctic sediments were used for this study as most process studies have been performed in temperate sediments, even though more than 90% of the ocean floor is permanently colder than 4°C (Levitus and Boyer 1994) At temperate sites, the metabolic rates of marine organisms decrease substantially during the cold season (Westrich & Berner 1988, Wu et al. 1997). Psychrophilic bacteria are adapted to cold temperatures. Sulfate reducers isolated from permanently cold arctic sediments show higher metabolic rates than

mesophilic bacteria at low temperatures (Knoblauch & Jørgensen 1999). Consequently, polysaccharide hydrolysis, SRR and nitrate reduction rates in permanently cold sediments of Svalbard are similar to rates measured in temperate sites at higher temperatures (Arnosti et al. 1998, Thamdrup & Fleischer 1999). Arnosti et al. (1998) found very similar hydrolysis rates at the *in situ* temperatures in temperature gradient block incubations of samples from permanently cold and temperate environments. Thus, the total electron flow is not necessarily smaller at lower temperatures. The temperature control of the different processes is not well understood. Therefore, extrapolating rates from temperate sites to the cold habitats, measurements at temperate sites during the cold season, or incubation of temperate sediments at low temperatures does not lead to a better understanding of processes at permanently cold sites. For the understanding of global cycles, it is thus important to study the marine processes at sites with permanently low temperatures.

Focus of this study

In this study, we measured potential hydrolysis rates, sugar and VFA turnover rates, and sulfate and iron reduction rates, in permanently cold marine sediments. Some of the source rocks for the sediments along the west coast of Svalbard are rich in oxidized iron (Hjelle 1993). Therefore we performed detailed studies of sulfide oxidation with these iron-rich samples to understand the importance of sulfide oxidation in these sediments and help interpreting the SRR data. SRR were measured at two different sites and three sediment horizons to obtain a picture of the spatial variability of processes in sediments. These measurements make a direct comparison of different rates possible, and enabled us to estimate whether the studied intermediates and proposed pathways reflect the important steps in carbon degradation, or whether there are additional important steps that we missed. Measuring turnover of several pools of organic carbon in sediments and comparing these rates with data from temperate sites may reveal the factors controlling organic carbon degradation, as well as different temperature responses among these processes. Since each step in the degradation pathway may be differentially affected by multiple parameters, investigation of multiple steps at the same site is necessary.

Previous studies in whole cores of Svalbard sediment showed high lateral variability of

VFA concentrations (Finke 1999b) and SRR (Jørgensen, unpublished data). We therefore conducted slurry experiments to minimize effects of horizontal heterogeneities in sediments to determine if microbial activities varied in a fundamental fashion with depth or station.

Methods:

Sample location

Samples were taken from two fjords off the west coast of Spitzbergen, the main island of Svalbard. Station AB is located in Bellsund close to the mouth of Van Keulenfjorden at 77°35,1' N, 015°05,7' E. Station BC is located in the Nordfjorden at 78°31'N, 015°6' E (Fig. 1). The water depth was 100 m at both sites and the bottom water temperature was 0.2 and –0.4°C at station AB and BC, respectively.

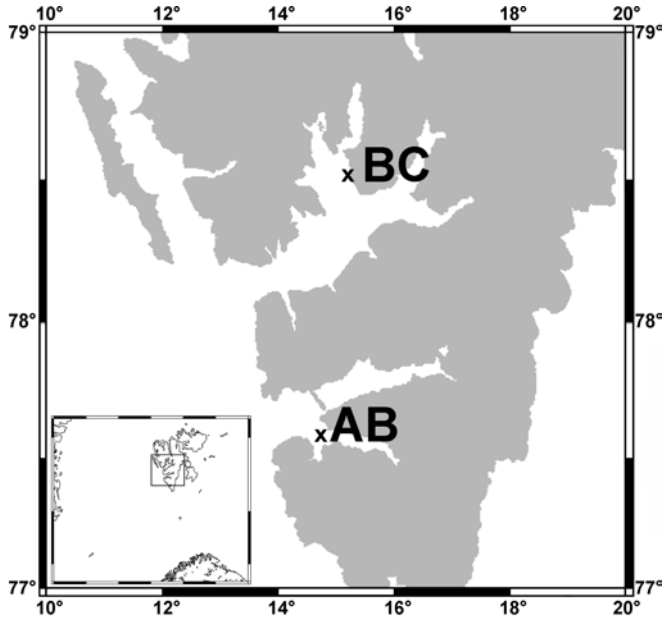


Figure 1: Samples were collected at two locations at 100m water depth from fjords along the west coast of Spitzbergen, the main island of Svalbard. Station AB is located at 77°35,13' N, 015°05,7' E, Station BC at 78°32,94'N, 015°08,3' E.

The sediment of station AB was dark grayish-brown, very dense and had a porosity of aproximately 0.6. The sediment from station BC was red-brown and had a similar texture and porosity as the sediment from station AB. Single drop stones of up to a few cm diameter

were found throughout all depths. Worm tubes were found up to 20 cm sediment depth at both stations. The tubes consisted either of organic sheaths or cemented sediment surrounding the worm.

Sediment sampling

Sediment samples were taken with a Haps corer and sub-sampled aboard the ship. The sediment for the slurry experiments was immediately divided into three different depths (top: 0-2cm, middle: 4-6cm, deep: 8-12cm). The sediment was filled into brown glass bottles and stored at 0°C. For the slurry, the sediment was mixed with equal amounts of anoxic seawater in 2.5 l glass bottles with a glass joint and butyl rubber outflow tube at the bottom. The homogenized slurry was transferred into 20 and 50 ml serum vials via the outflow tube. The vials were incubated at *in situ* temperature of 0°C. All preparations were carried out under N_2.

The vials were pre-incubated for 10 days before concentration and turnover measurements were conducted.

Inorganic electron acceptors

SRR measurement: Sulfate reduction rates (SRR) in the slurries and whole cores were measured using the radiotracer technique described by Jørgensen (1978). For the slurry incubations the incubations were performed in a time series. Two samples of 50 mL slurry were sampled from a 250 mL slurry vial into a 60 mL syringes equipped with valves. One syringe was amended with 80 MB radio-labeled sulfate ($^{35}SO_4^{2-}$) through the tip and shaken thoroughly. Within 0.5-10 min ten 5 mL samples were transferred into 20 ml of 10% zinc acetate. The other syringe was amended with 30 MBq and sampled from 10 – 30 min. Finally, 5 MBq $^{35}SO_4^{2-}$ was added to the remaining 100 mL slurry that was sampled from 1 h – 2 days. All samples were frozen immediately on dry ice to terminate the sulfate reduction.

Reduced ^{35}S was analyzed by the single step chromium reduction method (Fossing & Jørgensen 1989). In brief, first acid volatile sufide (AVS) was degassed as H_2S through

addition of 6 M HCl to the sediment preserved in 10% Zn-acetate. Acidic Cr(II) was used to reduce chromium reducable sulfur (CRS = FeS_2 and S^o) to H_2S. Evolved H_2S was carried with a stream of N_2 and trapped in 10 ml 5% zinc-acetate . The amount of ^{35}S in the SO_4^{2-} and the reduced S-pools were determined on a Canberra-Packard 2400 liquid scintillation counter. The total concentration of SO_4^{2-} in pore water samples was determined by ion chromatography using high performance liquid chromatography (HPLC) with a Waters IC-Pak anion exchange column (50*4.6mm), and a Waters 431 conductivity detector. The SRR were calculated per cm^3 of sediment or slurry as described by Jørgensen (1978).

Iron: For determination of dissolved Fe^{2+} 100-2000 µl of pore water was added to 100 µl of ferrozine solution (5.14 g/l ferrozine in 3.4 M sodium acetate buffer) and filled with distilled H_2O to a total volume of 2.2 ml. The concentration was measured spectrophotometrically at 562 nm (Stookey 1970). The pool of reactive iron oxides was described by extracting iron with 1 M HCl or NH_4-oxalate (pH 3) (Schwertmann 1964) and dithionite-bicarbonate-citrate (Mehra & Jackson, 1960) of crushed air-dried (oxidized) samples. Oxalate is widely used for the determination of the amorphous Fe(III) fraction in soils (McKeague& Day 1966) but it also dissolves magnetite (Chao & Zhou 1983). Allowing the samples to oxidize has the result that FeS and maybe also $FeCO_3$ will be transferred into amorphous iron oxides. Extraction with dithionite was used as a measure for the total content of iron oxides due to the strong reducing power of dithionite. With this method $FeCO_3$ and FeS is also extracted but not magnetite (Thamdrup et al. 1994). Furthermore, the iron pool was characterized by extracting Fe(II) and Fe(III) from fresh sediment with 1 M HCl and anoxic NH_4-oxalate (pH 3) (Schwertmann 1964).

Electron donors

Hydrolysis: The slurry was prepared as described above and dispensed into 50 ml serum vials. Slurry volumes were targeted at 20 ml; slurry volume was determined visually by comparing slurry level with a parallel vial showing volume markings.

Four different fluorescently-labeled polysaccharide substrates (pullulan, xylan, chondroitin sulfate, and fucoidan) were synthesized and characterized as previously described (Arnosti,

1995; 2000). Each substrate was added to two replicate vials from each depth and station. Substrate addition levels per vial were as follows:

Pull: 500 µl of a 20 mM monomer-equivalent solution

Chon: 300 µl of a 16.4 mM monomer-equivalent solution

Xyl: 300 µl of a 6.4 mM monomer-equiv. solution

Fu: 300 µl of a 15.8 mM monomer-equiv solution

Vials were shaken prior to substrate addition to resuspend the sediment, and the substrates were injected via syringe directly through the stoppers. After shaking the vials again, approximately 1 ml slurry was removed, filtered directly through a 0.2 µm pore-size disposable filter, and stored frozen until analysis as a time-zero sample. Vials were incubated at *in situ* temperature for a total of 147 h, and subsampled at 29 h; 47, 48, or 49 h; 74, 75, or 78 h; and 146 or 147 h. At each sampling point, the vial was shaken to re-suspend the slurry, and approximately 1 ml was removed by syringe (needle inserted through stopper). After the 0 h sample, all subsequent samples were processed by first centrifuging the sample in an Eppendorf 2 ml microcentrifuge tube, and then filtering the supernatant through a 0.2 µm pore-size filter.

Frozen samples were thawed and then analyzed by gel permeation chromatography using fluorescence detection (ex. 490 nm, em. 530 nm) in order to determine the molecular weight range of the hydrolyzed polysaccharides and to derive the hydrolysis rate, as described in detail in Arnosti (1995, 2000).

Sugar concentration: For the sugar concentration measurements, two 25 mL vials from each sample were emptied into centrifugation tubes, centrifuged, and filtered through a 0.2 µm pore-size disposable filter, and stored frozen until analysis. The sugar concentration was measured adapting the method from Borch and Kirchman (1997). In brief, the concentrations of xylose, glucose, maltose and lactose in pore water samples were determined by HPLC with Pulsed Amperometric Detection. The samples were desalted in a mixed ion exchanger (Biorad AG2-X8 and AG50-X8) preconditioned with H^+ (HCl) and

HCO_3^- ($NaHCO_3$), prior to the measurement. Standards were prepared in seawater, filtered and desalted as the samples. The sugars were separated on an anion-exchange column (PA-10, Dionex for monosaccharides and PA-100, Dionex for oligosaccharides) by gradient elution with 0.1 M NaOH (0-50%).

VFA concentration: The concentration of volatile fatty acids (VFA) was measured in 3-5 samples. The sample was centrifuged on Spinex™ (Phenomenex) centrifugal filter units. The filter units were washed with 0.5 M HCl followed by a rinse with destilled H_2O prior to filtration. The pore water was frozen immediately in brown borosilicate glass vials that were pre-combusted at 450°C for 3 hours. VFA were analyzed using the method of Albert and Martens (1997). In brief, the samples were derivatized with 2-nitrophenylhydrazine, separated on a LiChrosphere RP8 (5µm, 20*4mm ID, Knauer) column and absorbance was measured at 400 nm. Differing from the original method, in solvent A the concentration of butanol was reduced to 1,25%, and the concentration of tetrabutylammonium hydroxide to 1 mM and in solvent B the concentration of tetradecyltrimethylammonium bromide was reduced to 25 mM.

Sugar and VFA turnover: The turnover of xylose, glucose, maltose, lactose, acetate, propionate, lactate was studied using ^{14}C-labelled substrates in incubation experiments. After preincubation, 100 kBq of each substrate was injected into two parallel 50 ml serum vials containing approximately 20 ml of sediment. The vials were shaken and incubated at *in situ* temperature for a total of 400 min. Subsamples were taken at 6 time points: after approximately 0, 20, 40, 60, 200 and 400 min. At each sample point, the vial was shaken to resuspend the slurry. Approximately 1 ml was removed by syringe, added to 0.1 ml 0.02 M NaOH and frozen immediately. Radioactive carbon dioxide and ^{14}C-substrate the samples were separated by the diffusion method (Treude et al., in prep.) and measured in a scintillation counter. The rate constants were determined from the slope of the increase in fraction of $^{14}CO_2$ of the total ^{14}C in the sample. The turnover rates were calculated by multiplication of the turnover rate constant with the concentration. The equation used for calculating the turnover of sugar was developed to take the large fraction of tracer that was turned over into account. 25% turnover of the glucose tracer was often observed. It cannot be assumed that the concentration of tracer in the samples were constant during the

incubations. It is therefore necessary to use a rate expression, where an exponential decrease in the concentrations of unreacted tracer over time is assumed, as would be the case if the rates and unlabeled sugar concentrations were constant over time (Hansen et al. 2001).

$$turnover\,rate = \frac{\alpha * sugar\,concentration}{time} * \ln\left(\frac{^{14}C - sugar + ^{14}CO_2}{^{14}C - sugar}\right)$$

Accordingly, the sugar turnover rates were calculated from :

α = fractionation factor (1.08)

Hydrogen measurements: Hydrogen concentrations in the sediment were determined via the headspace technique (Lovley & Goodwin 1988, Hoehler et al. 1998). From each station three cores and three parallel vials from each depth were analyzed for hydrogen concentrations. The cores were sliced and the sediment samples were incubated in 10 ml glass vials with a N_2 atmosphere, closed with a thick butyl stopper. The vials with the core samples and the sediment slurry were incubated at *in situ* temperature. Headspace samples from the vials were taken at multiple time points along the incubation. For the analysis, 250 µl sample were withdrawn from the headspace of the vials with a gastight syringe and replaced with N_2. The gas was analyzed with a reduced gas analyzer (RGA3, Trace Analytical) amended with a 100 µl sample loop. Hydrogen concentrations were calculated according to the hydrogen solubility in seawater (Crozier & Yamamoto 1974).

DIC and alkalinity

Pore water samples from centrifugation were filled into glass vials excluding any gas bubbles and stored cold until analysis (within 24 h) in the laboratory. Dissolved inorganic carbon (DIC) was measured via flow injection analysis with a conductivity detector (Hall & Aller 1992). 50 µl of sample were injected into a stream of 10 mM HCl. The CO_2 diffuses across a teflon membrane into a stream of 10 mM NaOH. The electrical conductivity change of the NaOH solution is measured on a VWR scintific specific conductivity meter, model 1054.

Alkalinity was measured by Gran titration using 0.05 M HCl, a standard pH-meter, and a burette.

Total sedimentary carbon (TC) and nitrogen were measured by combustion of 10 mg crushed sample on a Fisons Eager 200. Total sedimentary inorganic carbon (TIC) was determined as CO_2 evolved upon acidification with 20% HNO_3 measured on a CM 5012 CO_2 Coulometer (UIC inc. Coulometric) Coulometer. Total organic carbon (TOC) was calculated as the difference between TC and TIC.

Results

Different carbon pools were characterized by pool size and turnover rate measurement to investigate the early diagenesis of C_{org} in sediments. Pool sizes were measured for representatives of two important substrate groups, sugars and volatile fatty acids (VFA). The concentrations of glucose, maltose, lactose and galactose decreased with depth; only the concentration of xylose increased. The VFA concentrations showed no consistent trends with depth, either in the slurries, or in the whole core measurements. Previous investigations of whole cores from Svalbard also showed no clear, consistent profile for the VFA (Finke 1999b, see below). In addition to pool sizes, turnover rates were measured for three important intermediates in the degradation of carbohydrates. We measured potential

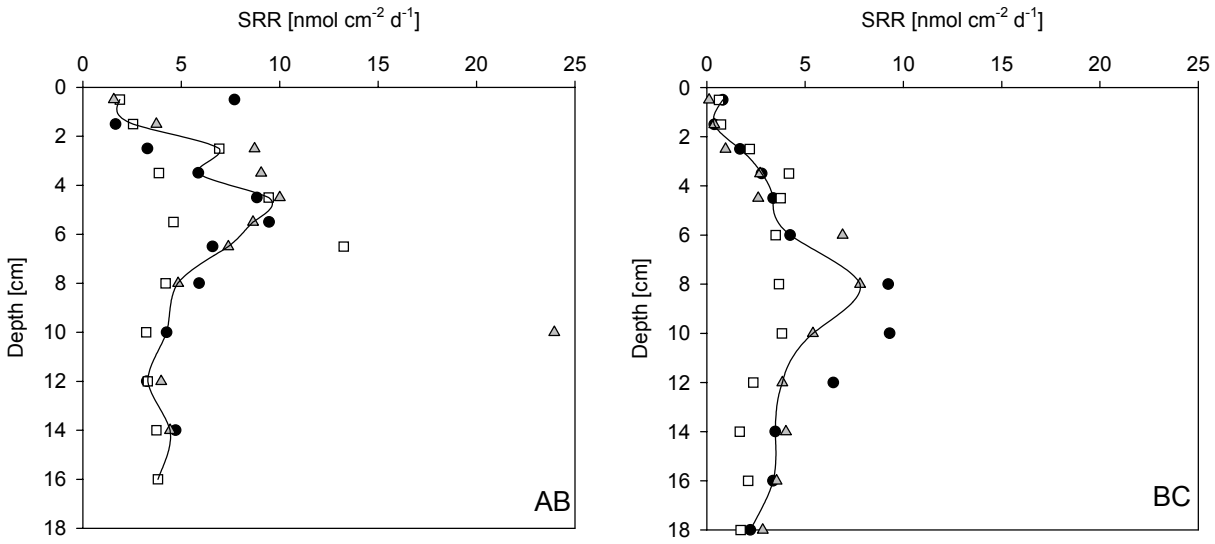


Figure 2: Sulfate reduction rates measured in three parallel cores. The three different symbols represent samples from three different cores, respectively. The solid line represents the middle value of the three measurements.

hydrolysis rates of polysaccharides, turnover rates of mono- and disaccharides, as well as VFA turnover. Turnover rates of most of these carbon pools showed a decrease with depth. This trend was especially obvious for the sugar turnover rates, but was also apparent for most of the hydrolysis rates of the polymers, a result consistent with the sulfate reduction rates (SRR) measured in whole cores. The total organic carbon (TOC) content did not change with depth at either site.

Terminal electron accepting processes

SRR and H_2S - Oxidation: The SRR measured in whole cores for station BC showed a clear maximum at around 8 cm, which corresponds to the deepest layer from the slurry incubations (Fig. 2). The uppermost 2 cm of BC displayed low SRR, indicating that other electron acceptors prevail in this zone. In contrast, station AB has a less pronounced peak and the SRR was high throughout the core. The triplicate cores from station AB displayed a higher scatter than for station BC. At station BC and AB the depth integrated (0-15 cm) SRR were 0.6 and 1 mmol $m^{-2}d^{-1}$ respectively. Taking the dilution of the sediment into account, the rates measured in the whole core incubations were similar to the (net) rates

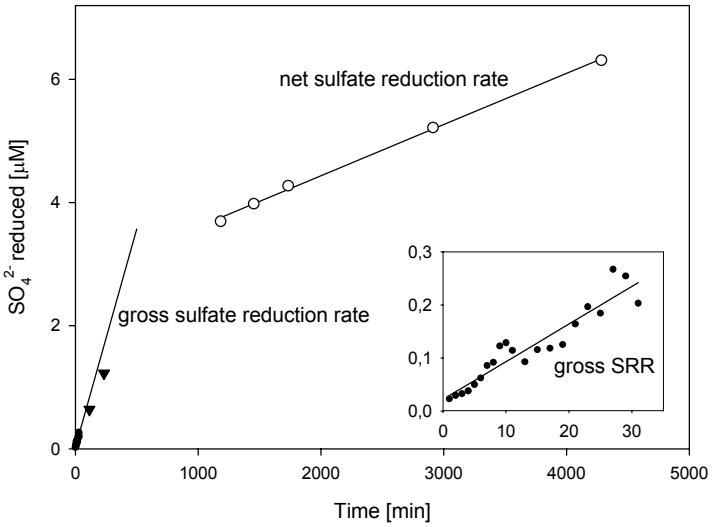


Figure 3: Net and gross SRR were determined with rediotracers in a time series experiment. The insert shows an enlargement of the first 30 minutes. In short-term incubations (=gross SRR), the fraction of the tracer in the reduced pool is extremely small, so the effect of reoxidation of reduced sulfur should be negligible. The gross SRR was calculated from the regression through the data points of the first 30 minute. The net SRR was calculated from long time incubations (several hours to days). The data marked with triangles represent the transition between gross and net rate and were not included in the regressions.

Table 1: Net and gross SRR, determined according to Figure 10. Minimum and maximum values for the gross SRR were calculated from the regression through the first 30 data points and the first measurement respectively.

	net SRR nmol ml^{-1} h^{-1}	gross SRR nmol ml^{-1} h^{-1}
AB		
Top	0.18	1.2 - 3
Middle	0.3	1.4 - 1.8
Deep	0.16	0.33 - 1.5
BC		
Top	0.06	0.38 - 1
Middle	0.1	0.44 - 2.4
Deep	0.045	0.43 - 2.7

measured in the slurry incubations (see below) .

The process of sulfate reduction was further investigated in detailed time course experiments of $^{35}SO_4^{2-}$ turnover (Fig. 3). These experiments showed that the SRR decrease when the incubation time is increased. This allows us to differentiate between net and gross rate (Fossing 1995). The gross SRR is measured shortly after tracer addition. The fraction of the reactive sulfide in the reduced pool is still very small, so effects of oxidation of reduced sulfur are negligible. The net rate represents the rate measured with long incubation times. The sulfide oxidation rate was calculated from the difference in net and gross SRR. The rates for the gross SRR were calculated from samples taken after 0.5-30 minutes (Fig. 3, Table 1).The upper and lower limit for the gross rate shown in table 1 were calculated using the first data point for the upper and the regression line for the lower limit. For the comparisons with other measured rates the upper limit was used if not stated otherwise. The rates for the net SRR were calculated using the data from the samples taken after 12-48 hours.

The net SRR was about 3 times higher at station AB than at Station BC (Fig. 4, Table 1). The highest rate at both stations was measured in the middle depth. The net rate accounts for 6 to 50 % of the gross rate (Table 1), which in turn means that at least 50-94% of the

sulfide produced through sulfate reduction, was reoxidized to sulfate. For station AB the percentage of net to gross decreased with depth. At station BC no trend was seen.

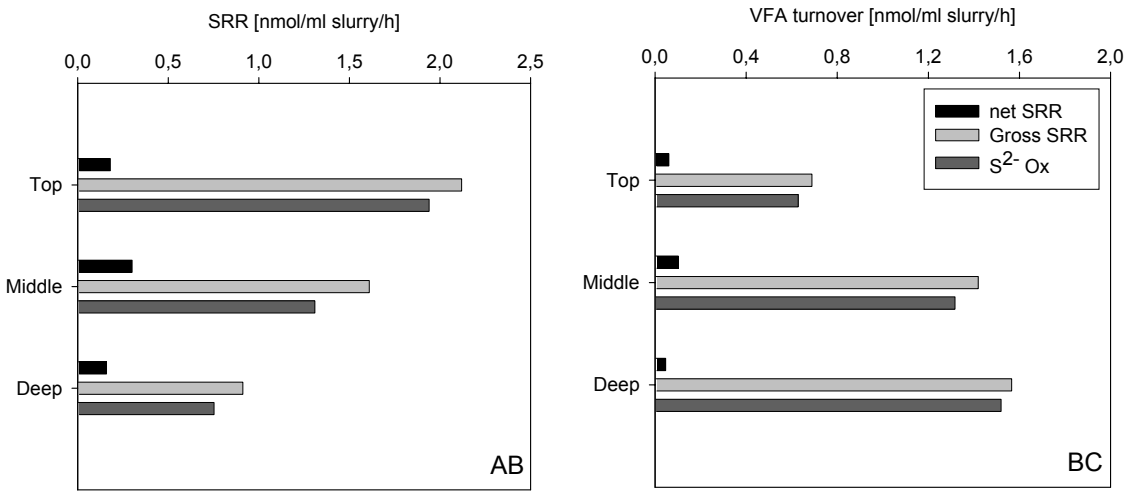


Figure 4: Net and gross sulfate reduction rates (SRR) measured in time series experiments (Figure 11). The sulfide oxidation is calculated as the difference in net and gross SRR. Note the different scales.

Iron reduction: Data from the selective iron oxide extraction methods showed that all sediment samples contained reactive iron(III) oxides, as determined with HCL extraction (Fig. 5). The concentrations from the oxalate extraction method showed a similar trend with somewhat lower values (data not shown). The total concentration of iron (DCB) was 70-80 mM in all samples, but in the deepest samples only about 1% of the DCB iron consisted of "reactive" Fe(III) (Table 2). Iron(III) oxide reduction was evident from the increase of Fe(II) over sediment depth. As often experienced in incubation experiments, the concentration of dissolved Fe^{2+} decreased during incubation, probably as result of the formation of siderite, magnetite and Fe-sulfides. However, the relative changes in the large sedimentary Fe(II) pool were too small to detect. Assuming that iron(III) was the dominant electron acceptor for sulfide oxidation, we quantified the chemical Fe(III) reduction rate using the increase in Fe(II). The dissimilatory iron(III) reduction rate was estimated by the difference between alkalinity production and gross SRR.

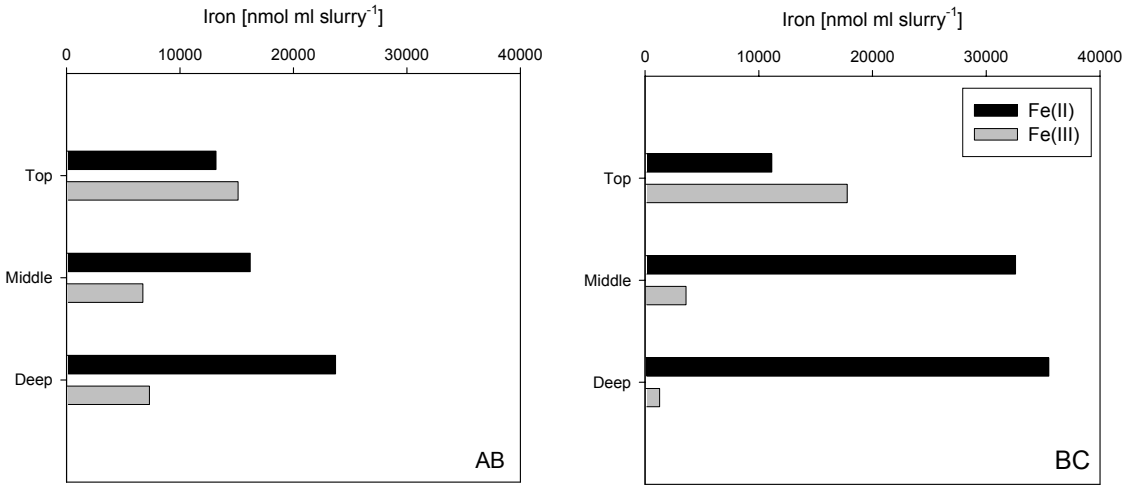


Figure 5: HCL extractable Fe(II) and Fe(III) in the slurries. For extraction methods see text.

Table 2: Iron (II) and Iron(III) extracted with various methods from the sediment. The concentrations are given in µmol Fe ml slurry^{-1}. See text for the extraction techniques.

	Ferric (III) Iron, HCl extraction	Total Iron, HCl extraction	Ferrous (II) Iron, HCl extraction	Total Iron DCB extraction
AB				
Top	15	28	13	78
Middle	7	23	16	83
Deep	7	31	24	88
BC				
Top	18	29	11	61
Middle	4	36	32	72
Deep	1	37	36	70

Organic carbon turnover

Hydrolysis: The potential hydrolysis rates of four high molecular weight carbohydrates (pullulan, xylan, chondroitan sulfate, and fucoidan) were measured in slurries (Fig. 6). Hydrolysis rates measured in the samples are not the actual but potential rates. The rates reflect the maximum rates for the enzymes if they are not substrate limited. The differences in hydrolysis rates were greater between the different substrates than between the stations or

depths. Pullulan was hydrolysed most rapidly, followed by xylan and chondroitan sulfate. Fucoidan was most slowly hydrolyzed. This is in good agreement with previous findings from Arctic and temperate sediments (Arnosti 2000). The hydrolysis rates of pullulan were 2.7 - 9 times higher than for xylan and chondroitan sulfate and 30 - 100 times higher than for fucoidan.

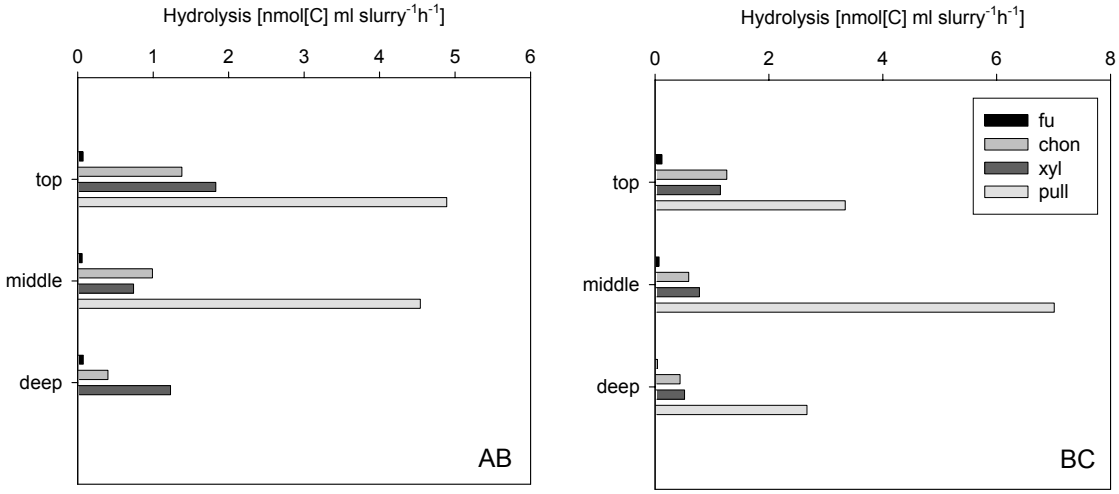


Figure 6: Potential hydrolysis rates measured for 4 different polysaccharides. (fu = fucoidan, chon = chondroitin sulfate, xyl = xylan, pul = pullulan) The hydrolysis rates were normalized to mol C turned over.

The hydrolysis rates for pullulan showed no clear difference between the different sites and depth. Xylan was hydrolyzed slightly faster at station AB compared to BC. Both sites show the highest potential hydrolysis rates of xylan in the samples from the shallowest horizon. At station BC, the rates showed a clear decrease with depth. The hydrolysis rates for chondroitan sulfate showed no difference between the two sites. At both sites the rates decreased with depth. Hydrolysis rates for chondroitan sulfate increased at later time points for several samples. This increase was most likely due to enzyme induction as observed in with previous studies (Arnosti, 2000). Fucoidan was hydrolyzed at a very slow rates, close to the detection limit with this method. No difference between the sites or depths is apparent.

Sugar: The concentration of three dissolved monosacharides (glucose, galactose and xylose) and two dissolved disaccharides (maltose and lactose) measured in the slurries

ranged from 1 to 250 μM (Fig. 7) Both stations had almost similar composition in terms of relative concentration, although the total concentration was almost twice as high at station AB compared to station BC. At both sites, glucose was the most abundant sugar with concentrations almost one order of magnitude higher than the other sugars, and showing a strong decrease with depth. Only xylose and mannose (measured as sum) exhibited the opposite trend, with concentration increasing with depth and being about twice as high at station BC compared to station AB.

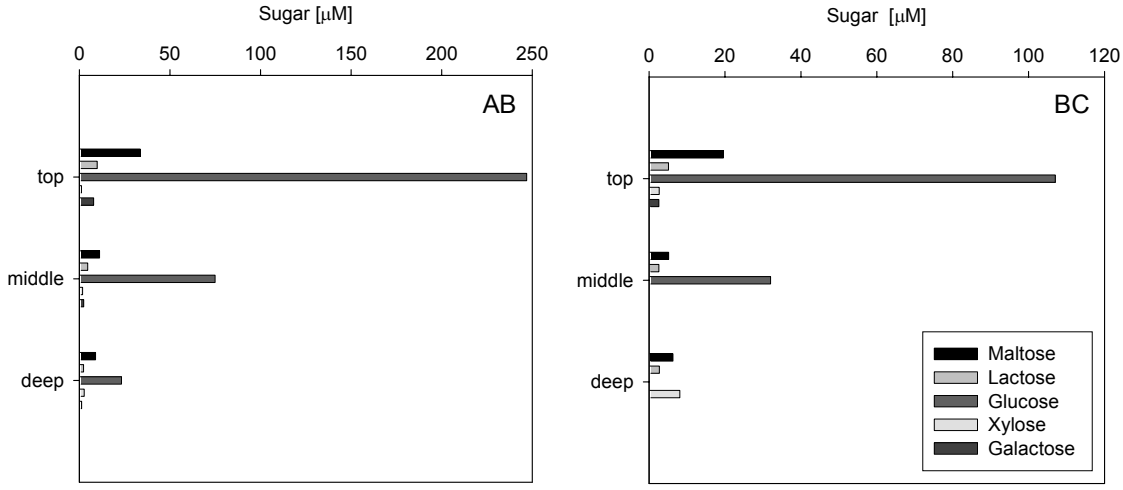


Figure 7: Sugar concentrations measured in the slurries. Note the different scales.

Sugar turnover rates were similar at the two sites and sugar concentrations decreased with depth (Fig. 8). Glucose and maltose were turned-over much faster than the other studied

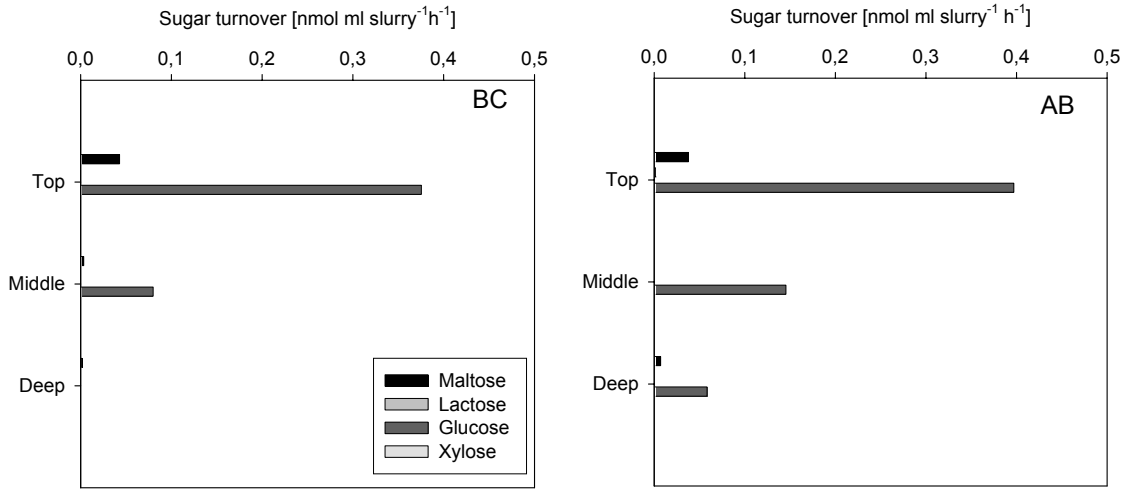


Figure 8: Sugar tunover rates calculated from the measured turnover rate constant and the concentrations.

sugars with rates being similar to the rates of volatile fatty acid (VFA) turnover.

Volatile fatty acids: The VFA concentration data are shown as depth profiles from three cores from every station (Fig. 9) and measurements from the slurry incubations (Fig. 10). All VFA, other than formate, exhibited higher values, more scatter, and high extreme values towards the sediment surface in the cores at station AB. At station BC, only lactate showed the highest scatter and values towards the surface. For the other acids, the highest values appeared in 8-14 cm. The highest concentrations were found for acetate (260 µM), followed by lactate (24 µM), propionate (16 µM), and formate (5 µM). The average concentrations were 2-10 µM for acetate, 2-8 µM for lactate, 0.2-2 µM for propionate, and 0.5-2 µM for

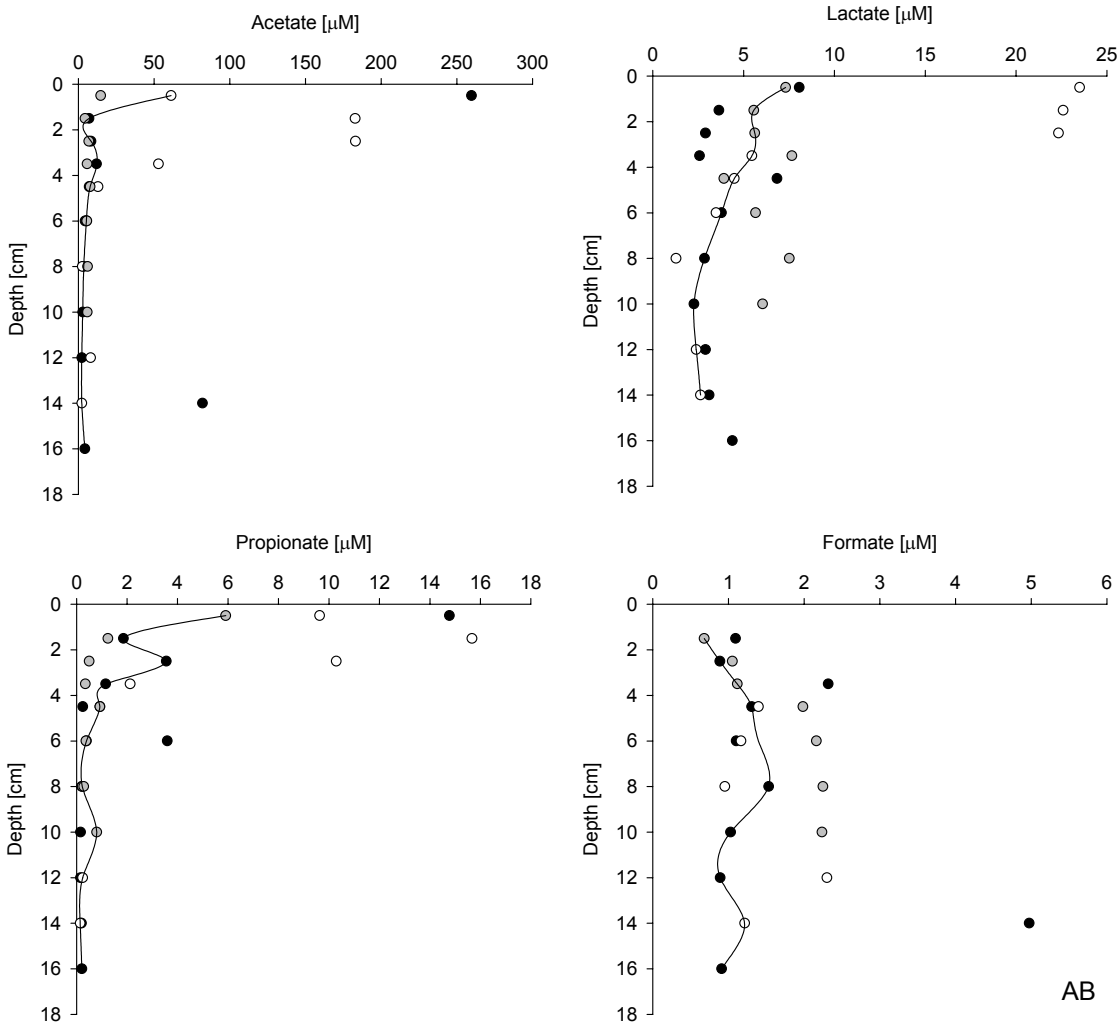


Figure 9: Volatile Fatty acid (VFA) concentrations measured in three parallel cores from A) station AB and B) station BC. The three different symbols represent samples from three different cores, respectively. The solid line represents the middle value of the three measurements.

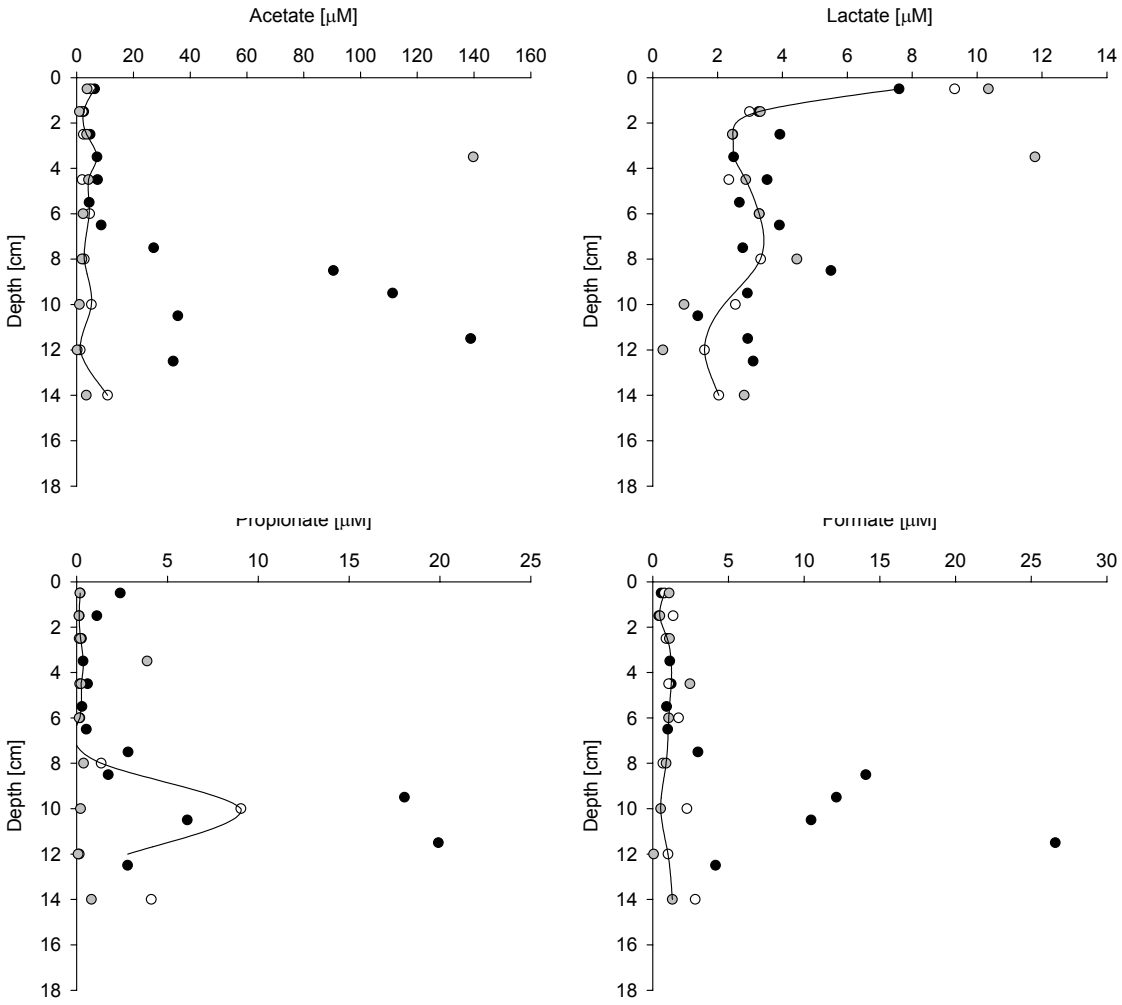


Figure 9: continued

formate. The acids did not show consistent profile shapes between the different cores. The extremely high concentrations of acetate in some of the samples might be due to the presence of worms that were cut while sampling the sediments.

The concentrations in the slurries were slightly lower, ranging from 2.4 to 7.4 µM for acetate, 0.3 to 1.5 µM for lactate, and 0.2-0.3 µM for propionate. The lactate concentrations in the slurries from station BC were 3 times higher than in the slurries from station AB. The VFA turnover rates were highest for acetate, followed by lactate. Propionate did not play an important role in these sediments. The rates for acetate varied from 0.1-0.4 nmol $ml^{-1}h^{-1}$, for lactate from 0.03-0.15 nmol $ml^{-1}h^{-1}$, and for propionate from 0.002-0.01 nmol $ml^{-1}h^{-1}$ (Fig. 11). The turnover rates decreased with depth for most of the samples. At station BC, the turnover of lactate and acetate was very similar, whereas at station AB the turnover for acetate is higher at all depths. The VFA turnover rates determined in these experiments are

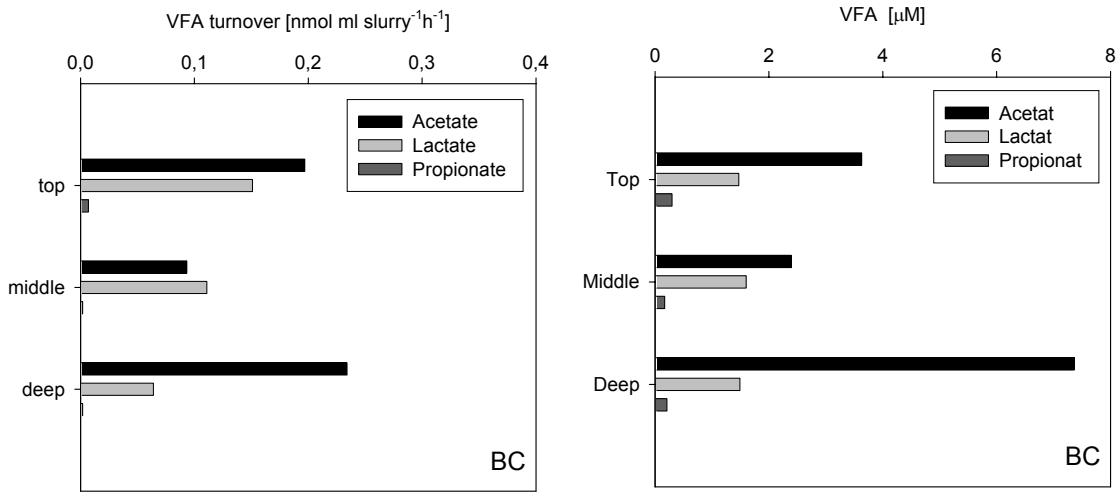


Figure 10: Volatile fatty acid (VFA) concentrations measured in the sediments.

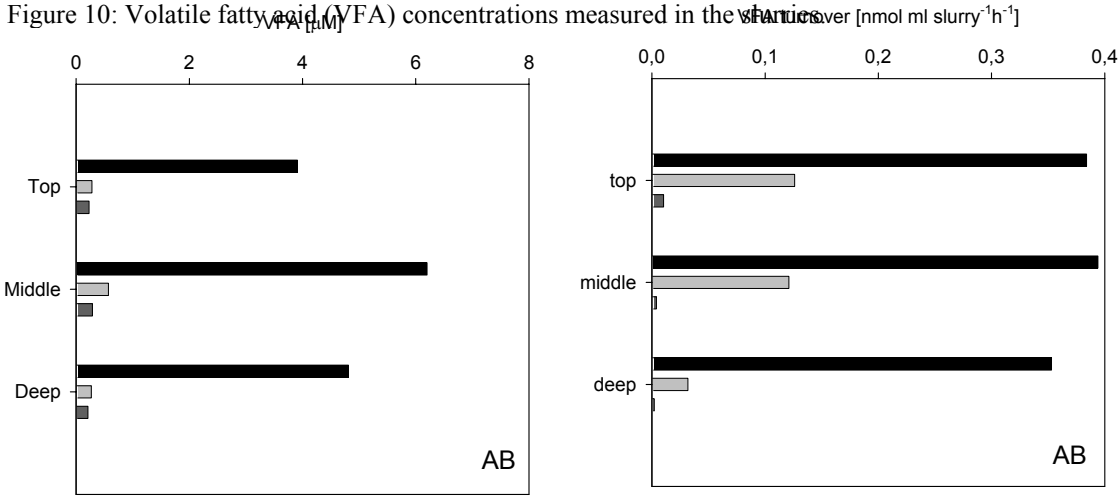


Figure 11: Volatile fatty acid (VFA) turnover tunover rates calculated from the measured turnover rate constant and the concentrations.

minimum rates, as degradation products other than CO_2 were measured as VFA, and therefore as not turned over.

Hydrogen: The hydrogen concentrations were measured in two - three parallel cores (Fig. 12) and three slurries from all depths and both sites (Fig. 13). The measurements in the cores showed high scatter with some extreme values in the profiles. The depth profiles measured in the three replicate cores were not reproducible, and even the shape of the profiles were dissimilar. The concentrations in the slurries also showed high variation with time and between the different vials. The average concentration decreased with depth for both stations

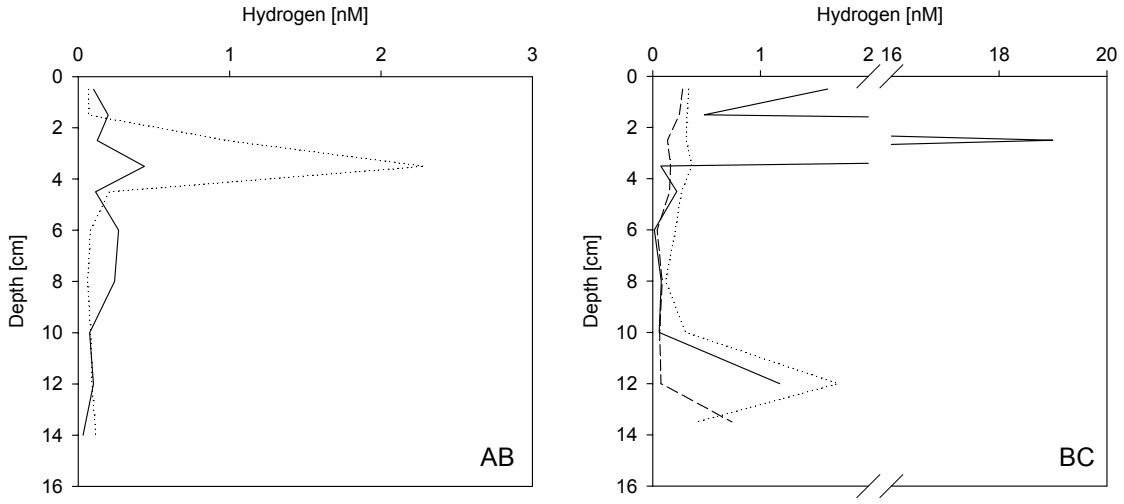


Figure 12: Hydrogen concentrations in cores. A) Hydrogen concentrations measured in two different cores from station AB. The values represent the average of three measurements. B) Concentrations measured in three different cores from station BC. The solid line represents the average of 8, the broken lines the average of three measurements. Note the different scales.

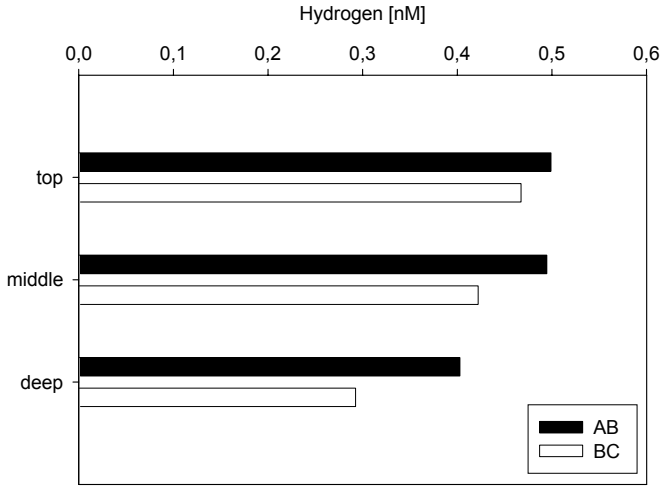


Figure 13: Hydrogen concentrations measured in the slurries

Carbon content

Total carbon (TC), total organic carbon (TOC), and total inorganic carbon (TIC) were determined in subsamples from the slurries (Table 3). Concentrations of all three showed no differences between the stations and the depths. During the incubations, the concentration of DIC and alkalinity was followed. The alkalinity showed an increase during the incubation with a rate of 0.05 to 1.3 mM/day (Table 3). The alkalinity production for station AB showed little variation over depth, whereas station BC displayed a higher alkalinity

production in the upper sediment sample. The increase in alkalinity was expected to originate from the anoxic oxidation of organic matter. Due to increased saturation in the samples (2-3.5 mM alkalinity) the DIC decreased probably as result of the precipitation of solid carbonates. From the alkalinity production, we estimated the gross carbon oxidation rate, assuming that the carbonate species were controlled by sulfate reduction, sulfide oxidation (coupled to iron(III) reduction) and dissimilatory iron(III) reduction.

Table 3: Carbon pools measured from bulk sediments, dissolved inorganic carbon and alkalinity production in the pore water. The total organic carbon was calculated from the difference between total and inorganic carbon.

	Total Carbon [%]	Total organic Carbon [%]	Total inorganic Carbon [%]	Dissolved inorganic Carbon [mM]	Alkalinity production [nmol ml^{-1} h^{-1}]
AB					
Top	2.5	2.0	0.48	3.2	5.4
Middle	2.4	2.0	0.45	2.8	3.2
Deep	2.4	1.9	0.45	2.6	2.1
BC					
Top	2.4	2.0	0.45	2.9	4.0
Middle	2.4	2.0	0.45	2.3	3.2
Deep	2.6	2.0	0.55	2.1	4.0

Discussion

The sediments around Svalbard typically contain about 1.5-2.5% organic carbon (C_{org}) (Kostka et al. 1999, Table 3). Generally, organic matter arrives on the seafloor as a complex mixture of polymers, proteins, lipids and uncharacterized particulate organic carbon. Most of these compounds have been partially decomposed during the settling through the water column. Large polymers must be hydrolyzed by extracellular enzymes to soluble monomers in order to be taken up by the bacteria. Previous studies in Svalbard sediments have predominately focused on benthic mineralization rates addressed from only from one side: that being the electron accepting processes (Glud et al1998, Thamdrup & Fleischer 1998,

Kostka et al. 1999). Only few studies have focused on the turnover of organic matter by studies of specific compound classes (Arnosti et al. 1998, Brüchert & Arnosti 2003).

In this study, turnover of C_{org} was measured in two arctic sediments at three different depths. Carbon turnover was measured as potential hydrolysis of polysaccharides, turnover of mono- & disaccharides and turnover of volatile fatty acids (VFA). The rates were compared to the two most important terminal electron accepting processes - sulfate reduction and iron reduction - and the total C_{org} degradation measured as increase in alkalinity.

The rates measured for carbon turnover generally decreased for both sites and most of the substrates with depth. This trend was particularly evident for station AB, at station BC the data showed a higher scatter. The sugar concentration data showed the same trend at both stations, only the values at station AB were twice as high as at station BC for almost all depths (Fig. 7). The alkalinity production as an indicator for C_{org} mineralization decreased for station AB, but does not show a trend at station BC (Table 3). The sulfate reduction rates (SRR) showed different trends for the net and the gross SRR (Fig. 4). The net SRR showed the highest activity in the middle layer for both sites. For the gross SRR, the rates decreased with depth at station AB but increased at station BC. The sulfide oxidation, which seem to be triggered by the sulfide production (see below) follow the gross SRR data. The relative importance of the net versus the gross SRR shows a clear increase with depth and is generally higher at station AB than at station BC, where the middle layer showed the highest percentage. The dissimilatory iron reduction rates (FeRR) did not correlate to the iron content, the SRR or the C_{org} turnover data at both sites and showed no difference between the stations (Fig. 14). The absolute turnover rates did not vary between the two stations. Although the net SRR was 3 fold higher at station AB compared to station BC, the gross SRR was similar (Fig. 4).

Measurements of VFA and hydrogen concentrations and SRR in whole cores revealed a high lateral heterogeneity in the sediments. The data from the parallel cores show no consistent profiles for the two sites. A similar observation has been made with previous VFA measurements in different fjords along the west coast of Svalbard (Finke 1999b). The

VFA profiles for station AB showed the highest scatter and the highest values in the upper layers (Fig. 9). The concentrations in the deeper samples were more uniform. Station BC did not show any trend with depth, showing the maximum values for the different VFA at different depths. The SRR measurements were more homogeneous for station BC, showing a clear maximum, which corresponds with the deepest layer from the slurry incubations (Fig. 2, 4). In agreement with the gross SRR measurements from the slurry incubations, the rates increase up to this depth, though these samples were incubated for several hours, like the slurry samples for the net SRR measurements. The core data for station AB show a higher scatter than for station BC (Fig. 2). The total rates are about twice as high as for station BC, with a maximum that corresponds to the middle layer for the slurry incubations. The shape of the curve reflects the slurry data of the net SRR, which showed the highest rates for the middle layer.

The total organic carbon (TOC), total inorganic carbon (TIC) and total carbon (TC) data of the two stations were almost identical for the two stations (Table 3), the iron data did not show any substantial difference (Table 2). Thus, the only recognizable difference between the two stations is a 2 - 3 fold higher net sulfate reduction rate that is not reflected in the gross SRR or the C_{org} turnover.

In general the sediments show no significant different with respect to the measured parameters. The results might, therefore, reflect a more general pattern of biogeochemical processes in Svalbard sediment rather than local specifics of these sites.

Terminal electron acceptors

SRR and H_2S-Oxidation: Incubation of whole cores (Fig. 2) showed that in respect to SRR the sediment at BC is more homogenous than at AB. The highest SRR were measured in the upper centimeters at AB which might indicate that the surface layer at the time of sampling had received fresh organic matter that is being mineralized. At a previous cruise later in the summer, the depth integrated SRR at station AB was three times higher. This shows that there is a large seasonal change in SRR at the sites and that our cruise in 2000 probably took place just in the beginning of the growth season. Station BC is situated at a larger distance

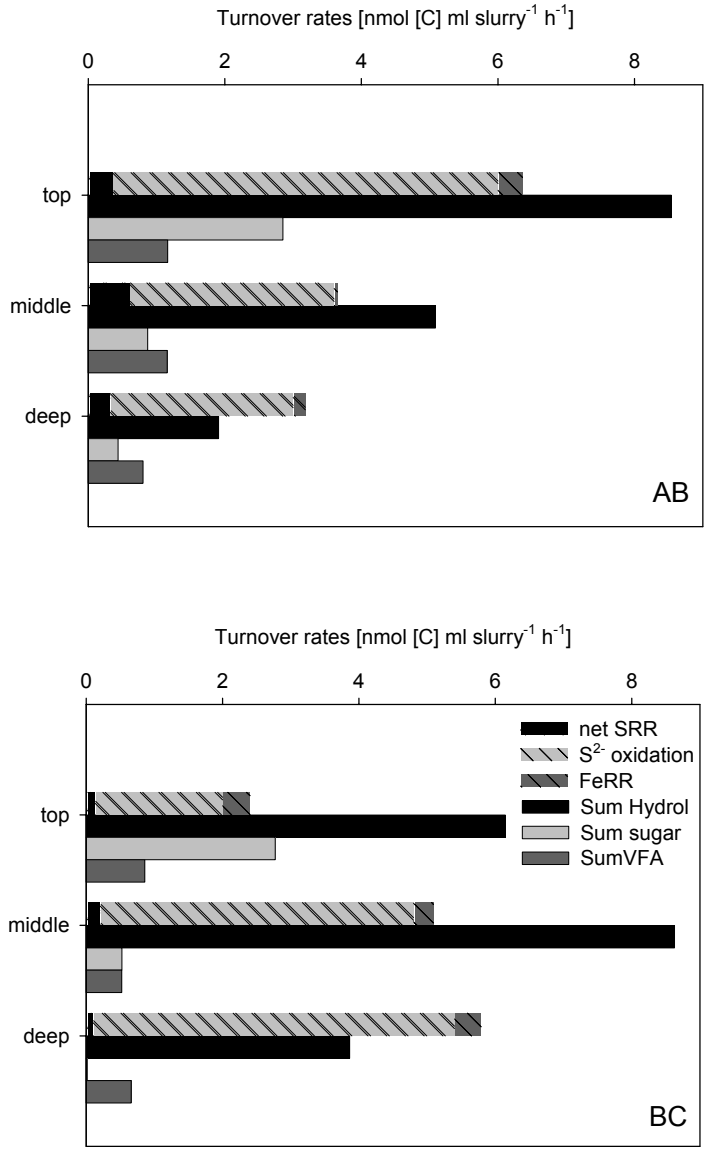


Figure 14: Comparison of the terminal electron accepting processes and the Corg turnover rates as measured for the different pools. The sum of the net SRR and the sulfide oxidation gives the gross SRR. Note that the hydrolysis rates are potential rates. All rates are normalized to mol C oxidized to allow comparison of the different steps.

from the Atlantic. The lower observed rates might reflect the fact that this site is ice-covered for a longer time (Falk-Petersen et al. 2000) and that the spring bloom has not yet had an impact on the sediment processes at the time of sampling.

Detailed analysis of SRR in a time series revealed that the incubation time had a strong influence on the measured rate as has previously been shown by Moeslund et al. (1994) and Fossing (1995). Very short incubation times of only a few minutes yielded SRR that were

substantially higher than rates measured after hours of incubation. This difference is ascribed to the chemical oxidation of some of the sulfide formed by dissimilatory sulfate reducing bacteria.

The detailed time course of this experiment allows us to estimate a "true" bacterial SRR being the rate with which the sulfate is reduced and described as the gross sulfate reduction rate. The main methodological problem is that the gross rate is less well determined by the first time point in comparison to the net rate. A lower gross rate would consequently imply a higher importance of additional electron acceptors i.e. iron, as the total C_{org} oxidation rate was determined independently through the increase in alkalinity. In the Svalbard sediments 50-94% of the sulfide is oxidized, demonstrating the importance of other electron acceptors in the sediments. As long as reactive oxidized iron and manganese are present, microbially-generated sulfide will be partly oxidized. In long term incubations studies, this fast internal cycling between of the sulfur back to sulfate can not be detected, resulting in underestimation of the SRR in iron rich sediments. The net rates measured in these slurry incubations are similar to rates found in this and previous studies in arctic fjords with whole core incubations (Jørgensen, unpublished data), whereas the maximum gross rates are about 10-fold higher.

Both Moeslund et al. (1994) and Fossing and Ferdelman (unpublished data) observed decreasing importance of the sulfide oxidation with depth. This was due to the consumption of the oxidized iron resulting in a less pronounced oxidative path. The sediments from Svalbard are in comparison to "normal" marine sediments rich in hematite being relatively inaccessible to bacteria but reactive towards sulfide (Canfield, 1992). The iron concentrations are similar at the two stations and it is evident that the pool of Fe(II) is increasing with depth (Table 2). In contrast, the sulfide oxidation shows no decrease with depth, but is proportional to the gross SRR. Thus, the sulfide oxidation is not limited by reactive iron (III) but is controlled by the production rate of sulfide. Due to the high prevalence of sedimentary rocks containing oxidized iron surrounding the fjords along the west coast of Svalbard (Hjelle 1993), sulfide oxidation plays an important role in these sediments, and measured rates so far likely underestimate sulfate reduction.

Iron content and iron reduction rates: Iron(III) play an important role as electron acceptor in most sediments (Thamdrup 2000). The importance of iron(III) for sulfide oxidation has already been discussed but iron(III) is also important as electron acceptor for carbon oxidation (Thamdrup 2000). Due to the short transport distances, the mineralogy of the iron oxides in sediments off Svalbard are dominated by the iron oxides delivered in the glacial outwash. These oxides were characterized using selective extraction techniques, (see method section). In comparison to other marine sediments, these sediments contain a large fraction of Fe that only can be extracted with dithionite-bicarbonate-citrate (DCB). Station AB contains about 10-20% more total iron oxide than station BC in the slurries. The reactive Fe(III) concentrations, however show no significant difference between the two stations (Table 2). Direct measurement of the FeRR is not yet technically possible. FeRR can be calculated balancing the DIC and alkalinity production in the incubations, taking the SRR into account. The rates calculated for these sediments account for 2-30% and 6-50% of the gross SRR for station AB and BC respectively, showing no trend with depth.

The reactive Fe(III) pool measured in the top layer of these sediment is sufficient to support up to 1,5 years of dissimilatory iron reduction and up to 40 or 160 days of sulfide oxidation to sulfate or sulfur, respectively, calculated from the iron reduction rates and the concentration of reactive iron. The sediments contained reactive iron (III) at least down to 12cm. With a sedimentation rate of 0.25-0.3 cm (^{137}Cs data, not shown) per year, the sediments in the deepest layer were certainly older, but still showed high rates of iron reduction and sulfide oxidation. High rates of bioturbation as observed in the sediments in Svalbard can resupply the pool of oxidized iron by introducing more oxidized sediments in deeper layers and mixing reduced iron into the upper, more oxidized zone.

Electron donors

Potential hydrolysis of polysaccharides: Hydrolysis is the initial step in the degradation of high molecular weight organic matter. Extracellular enzymes degrade the polymers to molecules small enough to be transported across bacterial membranes. Our investigations of potential hydrolysis of polysaccharides in the sediment slurries showed large variation between the different substrates. Pullulan hydrolysis was consistently the most rapid of the

four enzyme activities measured. Since extracellular enzymes are selective for specific structures, the span in rates suggest differential capabilities in the microbial community to react to high molecular weight substrates. Note, however, that potential rate measurements do not differentiate between enzyme numbers (quantities of enzymes) and enzyme turnover (cycling of enzymes.) Low fucoidinase activities could therefore be due to low enzyme numbers, or slow turnover times, or to a combination of factors; the same considerations apply to the other activities. Furthermore, we cannot directly assess the relative importance of specific polysaccharides in marine sediments, since the technical capabilities to extract and structurally characterize specific polysaccharides from sediments have yet to be developed. The potential rates measured here, however, suggest that the microbial community potentially possess the capability to rapidly hydrolyze high molecular weight polysaccharides. Furthermore, the potential hydrolysis rates measured in this investigation are similar to rates measured in previous investigations in permanently cold and in temperate environments (Arnosti 1998, Arnosti & Holmer 1999, Arnosti 2000).

Oxidation of di- and monosaccharides: Sugar mono- and dimers are the hydrolysis products from the degradation of polysaccharides. They serve as substrates for the fermenting bacteria. The turnover rates for the tested sugars span three orders of magnitude. Maltose and glucose are the di- and monosaccharide with the highest turnover rates, respectively. With our technique we were not able to separate mannose from xylose, however, the sum of concentrations of these two sugars were low, indicating a limited importance for both mannose and xylose in these sediments.

In general, the sugar concentrations in the Svalbard sediments were higher than those reported for temperate environments (King & Klug 1982, Burdige et al. 2000). Furthermore, the high glucose concentrations measured at station AB are similar to total hydrolyzable carbohydrates measured in pore water samples from whole cores (Arnosti, unpublished data). As neutral sugars usually only account for only a small fraction of total carbohydrates, the concentrations measured in the slurries cannot reflect the *in situ* conditions. The possible causes of these high measured concentrations are being investigated. To verify these results, time course measurements of sugar concentrations after sediment mixing are necessary. The turnover rates should be considered minimum rates, as

degradation products other than CO_2 are counted as sugars, but are probably higher than under *in situ* conditions, due to the elevated concentrations. The uncertainty of the measurements is due to the elevated concentrations, and also to the potential lower availability of the sugars for bacteria as described by King and Klug (1982). To minimize the affect of different sugar pools, relatively long incubation times were used for the turnover measurements, thus allowing the tracer to distribute itself between the pools. As the sugar concentrations in the incubations were high, precautions with measuring uptake rather than turnover rates due to increased concentrations after tracer addition, as reported by King and Klug (1982), were not necessary.

Oxidation of volatile fatty acids: VFA are intermediates in marine sediments that link the fermentation steps and the terminal oxidation. As endproducts of the fermentation reactions, they are the substrates for the terminal oxidation processes. VFA can be the fermentation product regardless of the degraded polymer. In pure culture studies, fermentation of sugars yield different VFA hydrogen and alcohols in varying quantities according to the organism and culture conditions (Gottschalk 1985). In substrate limited environments, VFA and hydrogen are likely the main fermentation products, with alcohols only playing a minor role (Schink 1988).

According to the classical scheme a high percentage of the C_{org} is funneled through VFA, accounting for the major part of the electron flow derived from complex organic matter. Previous investigations found that VFA and hydrogen are the most important substrates for sulfate reducing bacteria in marine environments, accounting for 66-91 % of the sulfate reduction (Sørensen et al. 1981, Christensen 1984, Parkes et al. 1989). In this investigation, VFA accounted for only 9-36% of the electron flow to the inorganic electron acceptors (Fig. 14). Additionally, many sulfate reducing bacteria oxidize their substrates incompletely to acetate (Widdel 1988). Some of the lactate in the samples might therefore have been degraded via acetate. The total VFA turnover rate presented in figure 11 is a simple addition of the single rates, not taking flow of carbon through more than one of the VFA pools into account. The importance of VFA for the carbon turnover might therefore be overestimated.

The VFA concentrations in the Svalbard sediments were similar to the lower range of

concentrations found in temperate sites at higher temperatures (Table 4) and in whole cores in previous investigations (Finke 1999b). The turnover rates in the permanently cold Svalbard sediments are similar to rates found in winter at temperate sites (Table 4).

Oxidation of hydrogen: Hydrogen is a potential additional electron donor for the terminal electron accepting processes. As with VFA, hydrogen is formed by the fermentative bacteria and consumed by terminal oxidizers. At steady state, the concentrations of hydrogen in marine sediments are controlled by thermodynamics (Lovley & Goodwin 1988, Hoehler 1998). The concentrations found in the slurry incubations (Fig. 13) and the low concentrations in the cores (Fig. 12) are close to the theoretically predictable values. The turnover of the pool, and therefor the significance for the electron flow is not known and can only be assumed by predicting how much of the terminal oxidation is due to hydrogen oxidation.

Balance of organic carbon turnover and terminal electron accepting processes

The sum of the potential hydrolysis rates is higher than the sum of the terminal electron accepting processes (TEAP) for the upper two layers (note that for the BC deep samples, the rate of pullulan turnover, which is the highest among the measured rates in the other samples, is missing). This indicates that the exoenzymes are likely substrate limited. This is consistent with results from Brüchert & Arnosti (2003) and Arnosti & Jørgensen (2003), who found that at *in situ* temperature the potential hydrolysis of even one macromolecule potentially could account for the electron flow to the terminal oxidation. The potential rates measurements, however, provide no information on the quantity of suitable carbon actually present. Addition of fresh organic matter can overcome the substrate limitation of the hydrolyzing enzymes, resulting in an accumulation of intermediates (Arnosti et al., in prep., Chapter 2).

The total sugar turnover calculated based on the elevated concentrations was about 2 and 6 fold higher in the top layer than the total VFA turnover, when both were normalized to mol C (Fig. 14). The glucose turnover alone was more than twice and three times the VFA turnover for station AB and BC respectively, and can alone account for the whole electron

flow to the inorganic electron acceptors. The sugar concentrations were elevated in the incubations compared to the *in situ* values. The VFA concentrations, in contrast, were close to the minimum values measured in the cores and the steady state concentrations previously measured in bag incubations (Finke 1999a, Finke 1999b), indicating that these were steady state values. At steady state, the input and output for the VFA pool, i.e. the sugar turnover to VFA and the VFA turnover respectively should balance. The sugar turnover to VFA was not measured, only the total turnover to CO_2. Fermentation of glucose to VFA can potentially yield a maximum of 8 H_2, if all glucose is fermented exclusively to acetate, H_2 and CO_2. In this case, half the electrons from glucose are going through hydrogen and not through VFA, allowing the sugar turnover to be twice as high as the VFA turnover if only sugars are contributing to the VFA pool. If the VFA concentrations were not at steady state, they were certainly decreasing, as the increase of the VFA concentrations after mixing is rapid (Finke 1999a, Arnosti et al., in prep., Chapter 2) and the concentrations in the slurries are rather low. For the VFA pool size to be decreasing, the input (i.e. the sugar oxidation) must be smaller than the output (i.e. VFA oxidation), thus, the sugar turnover should be less than twice the VFA turnover. Therefore, assuming the concentration measurements were correct, a portion of the sugars might be directly oxidized, rather than fermented. Sulfate reducers that can oxidize sugar with sulfate as electron acceptor have been isolated (e.g. Sass et al. 2002), but these organisms oxidize sugars only incompletely to acetate and CO_2, thus, half of the electrons would still be funneled through the VFA pool. Even though our sugar concentrations and turnover do not reflect the *in situ* concentrations, the measurements may indicate that there might be an additional oxidation pathway for sugars not involving VFA. One possible explanation would be sulfate reducing bacteria that can actually completely oxidize the sugar to CO_2. Further considerations await confirmation of the elevated sugar concentrations measured in the slurries.

VFA turnover measurements have previously been performed at a number of temperate sites (Table 4). In some of these investigations the VFA turnover rates have been compared to related rates of the carbon cycle, most of the times with SRR. The comparison of the two rates usually showed a higher VFA oxidation rate than the SRR. As sulfate reduction was the most important anaerobic electron accepting process in these investigations and VFA

are not the only possible substrates for sulfate reducing bacteria (SRB), the VFA turnover rate should be similar or lower than the SRR. This discrepancy between two steps has been previously explained by the existence of several pools of acetate with different biological availabilities that cannot be differentiated with the radiotracer technique. Christensen & Blackburn (1982) found VFA oxidation rates that were higher than calculated C-oxidation rates based on the ammonia production. For the VFA turnover rate measurements they performed time course experiments only using the short incubation times, as longer incubation times gave lower turnover rates. Incubating sediment samples amended with radiolabeled acetate longer will allow the tracer to be equally distributed between different pools of acetate with different biological availability, resulting in differing turnover rates. Rate measurements with long incubation times should therefore give the turnover rate for the total acetate pool, and thus, the production and consumption rate. Multiple pools with varying variability were also reported for sugars in limnic pore waters (King & Klug 1982). In our investigation, long incubation times between 10 and 400 minutes were used for the VFA and sugar turnover measurements to minimize the problem of varying availabilities of different pools.

In this investigation, volatile fatty acids account only for 9-36 % of the terminal oxidation (Fig. 14). Thus, additional substrates must play an important role for the terminal oxidizers. SRB are very versatile, degrading a wide spectrum of substrates. In addition to VFA and hydrogen, fatty acids (C1-18) (Widdel 1980), amino acids (Stams et al. 1985), aliphatic hydrocarbons (Aeckersberg et al. 1991), aromatic compounds (Widdel 1980) and sugars (Sass et al. 2002) are potential substrates for SRB.

SRR are usually measured with long incubation times of several hours. This net SRR can be significantly smaller than the actual gross SRR. In the previous experiments (Table 4), the VFA turnover rates were compared with net SRR. Underestimation of the SRR due to measurement of net SRR, not taking the sulfide oxidation into account, can also be a reason for the discrepancy between VFA turnover and the overall terminal oxidation step. VFA turnover rates measured here are 1.8 to 6.7 fold higher than the net SRR, but account for only 9-36% of gross SRR. Thus measuring the VFA turnover with long incubation times and the gross SRR can eliminate discrepancies between these two rates. Rates measured

with these methods likely reflect the role the VFA play in sedimentary carbon cycle more closely, than previously reported rates. Underestimation of SRR or overestimation of VFA turnover measurements in previous investigations might also be the reason of the lack of finding important contributions of electron donors other than VFA to sulfate reduction in marine sediments.

Conclusions

The two, permanently cold, Svalbard sediments investigated here behaved similarly with respect to the measured parameters. Thus, the results might reflect more general patterns of Svalbard sediment biogeochemistry rather than local specifics of these sites. Comparison of the C_{org} turnover and TEAP reveals some interesting points. Consistent with previous findings (Brüchert & Arnosti 2003, Arnosti et al., in prep., Chapter 2), the potential hydrolysis accounts for more than the total electron flow, showing that the enzymes are likely substrate limited under *in situ* conditions. The sugar turnover certainly does not reflect *in situ* conditions. At the elevated sugar concentrations, sugar turnover accounts for a large fraction of the total turnover. The VFA, in contrast, play only a smaller role as substrates for terminal oxidation, as compared to previous investigations in temperate environments. Whether this is due to the different methods of measurement, or to a decreased importance of VFA at this site compared to temperate sites, remains to be investigated. In Svalbard sediments, additional substrates for terminal oxidation must play an important role.

Sulfate reduction is the most important anaerobic terminal oxidation step in these sediments. But measurements with the standard technique likely underestimates the rates, as 50 - 94% of the sulfide produced through sulfate reduction is rapidly oxidized by Fe(III). Previous investigations, therefore, have likely underestimated the role of SRR in these sediments. FeRR, as the second most important TEAP, accounts for a considerably smaller fraction of the electron flow. The pools of oxidized iron are not sufficient to cover the iron reduction, considering the age of the sediment. High bioturbation must therefore resupply the oxidized pool. Chemical reduction of the oxidized iron is more important than microbially mediated

iron reduction. Turnover rate measurements of VFA with radiotracer are usually complicated by problems of different pools of VFA that can not be differentiated. Giving the radiotracer time to equilibrate with the different pools can reduce these problems, resulting in rates that are consistent with total mineralization rates.

The effects of low temperature on the microbial processes seem to vary. The initial and terminal step of carbon turnover, seem not to be affected by the lower *in situ* temperature, because we found rates that were similar to measurements in temperate sites at higher temperatures. VFA turnover rates, in contrast, resembled VFA turnover rates in temperate sites at low temperatures, which are substantially lower than the rates at higher temperatures. Thus, the pathways of the carbon degradation, rather than the absolute rates seem to vary with different *in situ* temperatures.

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Chapter 4

Affect of hydrogen concentrations on methanogenesis from methylamine and methanol

(prepared for submission to Microbial Ecology)

Niko Finke[1*], Tori Michael Hoehler[2], Bo Barker Jørgensen[1]

[1]Dept. of Biogeochemistry, Max Planck Institute for Marine Microbiology, Celsiusstr. 1, 28359 Bremen, Germany
[2]Dept. of Astrobiology, Nasa Ames Research Center, Moffet Field, Ca 94035, USA
[*]Corresponding author: nfinke@mpi-bremen.de

Abstract

Methanogenesis from methylated substrates (e.g. methanol, methylamine) yields CO_2 and CH_4. In pure cultures, the ratio of the products is 1:3 for CO_2 and CH_4 respectively. This ratio change when sulfate reducing activity lowers the H_2 concentrations. Under such conditions, the methanogens transfer part of the electrons to protons, which yields H_2 instead of methane. In the experiments described here, H_2 concentrations controlled the ratio between CO_2 and CH_4, even under methanogenic conditions. At low temperatures, protons represented a more important electron acceptor than the methyl carbon for the methylotrophic mehtanogenesis. During methanogenic control of the H_2 concentration, hydrogenotrophic methanogenesis is exergonic and meets the minimum energy requirements of the cells. Therefore, the electron transfer to protons instead of methyl carbon means a loss of energy by the methylotrophs. Thus, the leakage of electrons to molecular H_2 is not a mechanism for the methylotrophic methanogen to conserve energy but may be a consequence of possessing a hydrogenase that is capable of catalyzing this reaction.

Introduction

Our understanding of the regulatory role of H_2 concentrations on the rates and pathways of carbon cycling in aquatic environments has improved over the past decades (e.g. Abram & Nedwell 1978, Conrad et al. 1986, Westermann 1992, Hoehler et al. 1998, Valentine 2001). H_2 is an important intermediate in a number of metabolic reactions that are key steps in the microbially mediated degradation of organic matter. H_2 is produced by fermenting bacteria and subsequently serves as an important electron donor for a variety of terminal oxidations. Changed in the H_2 concentration can alter the energetics and stoichiometry of fermentation reactions (e.g. Gottschalk 1985 and references therein) and in some cases may inhibit or even reverse specific reactions. For example, some acetogenic bacteria reduce CO_2 with H_2 to form acetate at high H_2 concentrations but oxidize acetate and produce H_2 and CO_2 at low H_2 concentrations (Lee and Zinder 1988). A similar reversal of methanogenesis has been proposed for anaerobic methane oxidation, accomplished by a consortium of archaea oxidizing the methane and sulfate reducers oxidizing an electron shuttle, that could be H_2 (Alperin & Reeburgh 1984, Hoehler et al. 1994).

As a potential electron shuttle for fermentation reactions, molecular hydrogen can carry 2 electrons. As most fermentation reactions yield 6 - 8 electrons per molecule substrate, H_2 has a 3 - 4 fold higher stoichiometry compared to the fermented substrates (Equation 1) or the terminal electron acceptor (Equations 2 & 3):

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \qquad \textit{equation } 1$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \textit{equation } 2$$

$$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O \qquad \textit{equation } 3$$

The free energy of a reaction under *in situ* conditions, ΔG_r, is dependent on the temperature-corrected standard free energy $\Delta G°(T)$, the temperature, and the concentrations of the involved products and educts, P and E, raised to the power of their stoichiometric coefficients, y and z:

$$\Delta G_r = \Delta G°(T) + RT * \ln\left(\frac{\prod P^y}{\prod E^z}\right) \qquad \textit{equation } 4$$

The higher stoichiometric coefficient for H_2 means that the free energy of a reaction including H_2 is more dependent on the H_2 concentration than on the concentration of the other involved species (that have lower stoichiometric coefficients). The examples shown above, i.e. the fermentation of propionate to acetate (Equation 1), the reduction of CO_2 to methane (equation 2), or the reduction of sulfate to sulfide (equation 3) show that a two-fold change in the H_2 concentration produces an 8- to 16-fold change in the free energy yield. The H_2 concentration, thus, exerts a strong control on microbial processes in the environment.

Steady state H_2 concentrations in aquatic systems are extremely low: <20nM for sulfate reducing systems and <70nM for methanogenic systems.The turnover is accordingly rapid, on the order of seconds (Hoehler et al. 1994, Hoehler et al. 1998). Therefore, the energetics of H_2-dependent reactions are not only very responsive to changes in H_2 concentrations, but the concentrations can also change very rapidly, quickly adjusting to shifting conditions. In addition to their lower stoichiometry, the other species shown in Equations 1 through 3 are usually present in much higher concentrations than H_2 (e.g., µM to mM instead of nM). Previous work with sediment from Cape Lookout Bight (CLB), North Carolina, USA, showed, that, following sulfate depletion, the H_2 concentrations adjusted much faster to the new geochemical regime than did the acetate concentrations (Hoehler et al. 1998).

In aquatic systems, the steady state H_2 concentrations are controlled by thermodynamics and vary according to the dominating terminal electron accepting process (Lovley & Goodwin 1988, Hoehler et al. 1998) and to the temperature (Westermann 1992, Hoehler et al. 1998, Valentine 2001). The *in situ* H_2 concentrations reflect the threshold concentrations at which the different bacterial groups using the most favorable electron acceptor can derive energy from the H_2 oxidation. The oxidation of H_2 is performed by hydrogenases that transfer electrons to an internal electron carrier (e.g. Ferredoxin, NAD, FAD, F_{420}). H_2 oxidation is, in most cases, reversible (Vignais et al. 2001), meaning that at low H_2 concentrations (or at high ratios of reduced to oxidized electron carriers) protons can be reduced to H_2. The dynamic between H_2 production or oxidation is reflected in the reversibility of acetogenesis (Lee & Zinder 1988), but also by the fact that pure cultures often respond to substrate addition (which generates a high ratio of reduced to oxidized electron carriers) with a rapid

increase in headspace H_2 (eg. Pohorelic et al. 2002). In addition to the direct effects of the H_2 concentration described above, the H_2 concentration also exerts indirect effects on biogeochemical processes. For example, methanogenic archaea oxidize a greater portion of methyl carbon derived from methanol or methylamine to CO_2 if they are co-cultured with a sulfate reducer capable of keeping the H_2 concentration low (Phelps et al. 1985).

In the work presented here, we hypothesized that the H_2 concentration in aquatic environments would influence the ratio of the products of methylotrophic methanogenesis even under steady state methanogenic conditions. These reactions represent a model for redox reactions that do not directly involve H_2, but that are controlled by the H_2 concentrations due to electron transfer via a hydrogenase. In a broad sense, our results suggest that the reactions recognized to be associated with H_2 concentration to date probably represent only a fraction of those that are influenced by the H_2 concentrations *in situ*.

Material and Methods

Sediment

Sediment was collected at two different coastal marine sites. Station CLB (Cape Lookout Bight) is a 10 m deep back barrier island lagoon on the Atlantic coast of North Carolina, USA. The system is characterized by en extremely high sedimentation rate and organic carbon loading (Chanton et al. 1983, Martens et al. 1992). The sediment is anoxic below 2 mm (Canuel 1992) and organic remineralization is dominated by sulfate reduction and methanogenesis (Martens & Klump, 1984). The sediment temperature ranges from <6 C in winter to >28 C in summer, causing the sulfate depletion depth to vary between 10 cm in summer to more than 25 cm in winter (Crill & Martens 1987). The sediment was collected from a sediment depth of 20-40 cm in late fall and had been stored for 6 months at 20°C prior to incubation.

Station DA (Dangast) is situated on a tidal flat in the German Wadden Sea. The sediment contains sulfate to at least 1 m depth throughout the year and the organic remineralization is

dominated by sulfate reduction (M. Boettcher, personal communication). The sediment temperature ranges from 0 to 30°C at the sediment surface over the course of the year and was about 10-15°C between 5 and 15 cm sediment depth at the time of sampling. The upper 20 cm sediment were sampled by hand in late fall and stored at 16°C for 12 month prior to the slurry preparation. The sulfate concentration had dropped to <0.1 mM during this period and methane was present in the pore water (data not shown).

Incubations

Preparation of the CLB sediment was performed in a polyethylene glove bag under N_2 while preparation of the DA sediment was performed in a glove box under N_2. Sediment from the two sites was incubated at different temperatures and different sulfate concentrations, and was amended with ~500 μM lactate in order to increase the H_2 concentration. Sediment was diluted with an equal amount of anoxic artificial seawater without sulfate. Slurries were incubated anaerobically in the dark at 22ºC for 5 weeks. Methylamine or methanol (to give approximately 100 μM) was added twice a week for the first four weeks of incubation to two parallel slurries to establish well developed methanogenic communities.

The temperature experiment was carried out using sediment from CLB or DA. The sediment from CLB was incubated at 9.3, 14, 25 and 30°C while the sediment from DA was incubated at 20 and 30°C. The samples were incubated in a constant temperature bath at the desired temperature for one week before tracer addition to allow the microbial community to adapt to the temperature regime provided. Three replicates were prepared for every temperature, sulfate and lactate concentration. The sulfate and lactate addition experiments were carried out with sediment from Station DA. Up to 200 μl of a 100 mM sulfate solution was added to 20 ml of slurry to give final concentrations of ~ 100, 200 and 1000 μM. The actual concentrations were confirmed by ion chromatography. In the 100 and 200 μM sulfate slurries, the concentrations were checked just before the tracer addition and after the termination of the tracer incubation. For the 1000 μM sulfate samples, the concentrations were checked only after the termination of the tracer incubation. Incubations were performed at 20°C. For the lactate addition experiment, 10 μl of a 1 M lactate solution was added to 20 ml of slurry to give a final concentration of approximately 500 μM. Incubations

were performed at 30°C.

For the radiotracer incubations and methane concentration measurements, 4 ml of sediment was transferred into a cut-off 5 ml glass syringe, taking care to exclude gas bubbles. For the measurements of additional parameters, approximately 5 ml of sediment was transferred into 10 ml serum vials. The syringes and serum vials were sealed using thick butyl rubber stoppers.

Tracer incubations

A 10 µl solution of ^{14}C-labeled methanol (2 kBq) or methylamine (2 kBq) tracer solution (dissolved in sulfate-free, anoxic artificial seawater) was injected into each syringe. After 12 h of incubation, the sediment was transferred quickly into 20 ml glass scintillation vials containing 10 ml of 0.5 N NaOH, and sealed with a butyl rubber stopper. The NaOH served to terminate biological activity within the sample and to "fix" any $^{14}CO_2$ produced during incubation in the aqueous phase.

Quantification of $^{14}CH_4$ and $^{14}CO_2$

The radio activity of $^{14}CH_4$ and $^{14}CO_2$ was quantified after termination of the incubation using a headspace flushing technique (Alperin et al. 1988). Flushing was performed >24 h after the termination of the incubation to assure that all methane had been transferred to the headspace.

To determine the $^{14}CH_4$ activity, the headspace was flushed with air for 30 minutes (which accounts for approximately 30 headspace volumes of gas flushed through the vial). Under alkaline sample conditions, methane and any residual methanol or methylamine are labile, but CO_2 is not. Radioactive methanol or methylamine was removed from the gas phase with an efficiency of more than 99% in trap containing 6 ml 0.1 M HCl followed by a trap containing 6 ml distilled H_2O to remove any HCl from the gas stream. After passage through the traps, the stripped gas was passed over cupric oxide heated to 850ºC, which served to oxidize the methane to CO_2. The gas stream was then passed through two consecutive traps filled with 10 ml of 50% ScintiVerse II (Fisher Scientific), 25% methanol

and 25% phenylethylamine which collectively sequestered >99% of the CO_2. After flushing the radiolabeled methane from the sample, the heated cupric oxide was disconnected from the gas line and the sediment slurry was acidified with 3 ml of 6 M HCl to liberate any $^{14}CO_2$ produced during substrate oxidation. The stripping procedure was repeated for 60 minutes, after exchanging the distilled H_2O trap with another HCl trap. During this procedure, both the sediment and the headspace were sparged with N_2. The radioactivity of the traps was measured on a Packard 1900CA scintillation counter. The recovery of known standards with these techniques was > 95%.

Pore water gas analysis

Methane: Methane concentrations in sediment pore water were measured in the headspace of the fixed radiotracer incubations before flushing (Alperin 1988). 200 µl of the headspace was injected into a Shimadzu GC-14A gas chromatograph with a FID detector (injector 120°C, column 40°C, detector 125°C, carrier flow 100 ml min^{-1}, column Porapak Q (80/100 mesh) 6' x 1/8'' stainless steel (Alltech Asociates)).

Hydrogen: The H_2 concentrations in sediment pore water were determined using a headspace equilibrium technique in the serum vials (Lovley and Goodwin 1988). Vials were incubated until the headspace concentration of H_2 was in equilibrium with the steady state pore water concentration, indicated by stable concentrations. 100 µl of the headspace was injected into a reduction gas analyzer (RGA3, Trace analytical). The pore water H_2 concentrations were calculated using as a function of temperature and salt concentration tabulated solubility coefficients (Croizer and Yamamoto 1974).

Pore water salt and pH analysis

Pore water for the determination of sulfide, sulfate, and $\sum CO_2$ concentration and pH was obtained by centrifuging (2500*g*) sediment samples in the serum vials under an N_2 atmosphere for 10 minutes. The supernatant was subsampled for the various analyses and pH was measured directly in a subsample using a pH-electrode standardized against NBS buffers.

Sulfide: Dissolved sulfide was measured in Zn-preserved pore water samples using the methylene blue method (Cline 1969). 0.2 ml of the samples were fixed with 0.2 ml of 2% zinc acetate solution. The samples were diluted with 1.1 ml of distilled H_2O before 140 µl of Cline's reagent (0.17 M n,n-dimethyl-p-phenylenediamine sulfate, 0.37 M ferric chloride in 6 M HCL) was added. The resulting adsorbance was measured at 670 nm.

Sulfate: For sulfate analysis, 0.5 ml of pore water was amended with 0.1 ml of 1 M HCl and sparged with N_2 for 10 minutes to strip out any sulfide present. Sulfate concentrations were determined by ion chromatography using a Dionex DX120 ion chromatograph (IC). Samples were diluted 1:10 with distilled H_2O and a 25 µl sub-sample was then injected into the IC. A Dionex IonPac CS12A column operated at room temperature was used to separate the anions. The eluent was 1 mM $NaHCO_3$ and 3.5 mM Na_2CO_3 and the flow rate was 1.2 ml min^{-1}.

CO_2: CO_2 was quantified by flow injection analysis using a conductivity detector (Hall and Aller 1992). A 100 µl sub-sample was injected into a stream of 10 mM HCl. The CO_2 diffuses across a Teflon membrane into a stream of 10 mM NaOH. The conductivity change of the NaOH solution was measured on a VWR Scientific EC meter, Model 1054.

Results

H_2 concentrations

Varying geochemical and temperature regimes were used to alter H_2 concentrations in the different experimental treatments. The H_2 concentrations were strongly influenced by the incubation temperature (Fig. 1). The sediment from Station CLB (Cape Lookout Bight, North Carolina, USA) was incubated at four different temperatures (9.3, 14, 25 and 30°C) and the sediment from Station DA (Dangast, German Wadden Sea) was incubated at two different temperatures (20 and 30°C). The H_2 concentrations increased with increasing temperature, consistent with thermodynamic predictions (Hoehler et al 1998).

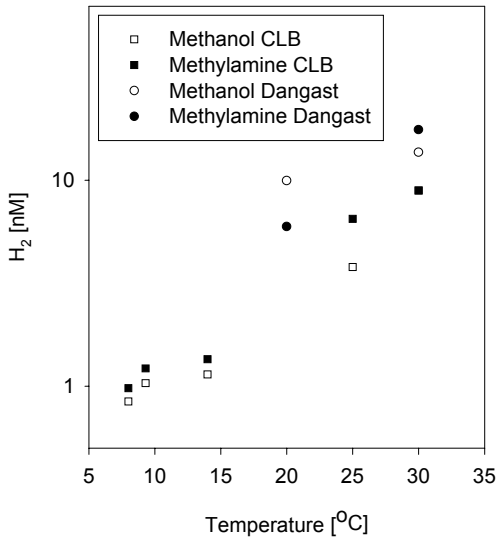

Figure 1: Hydrogen concentration as a function of the incubation temperature. Samples were taken at Station CLB (Cape Lookout Bight, NC, USA) and Station DA (Dangast, Germany). Note the logarithmic scale for the H_2 concentrations.

In a second approach, the H_2 concentrations were controlled by addition of different amounts of sulfate to the methanogenic sediment (Fig. 2). Methanogenic sediment from Station DA was incubated with four different sulfate concentrations (approximately 15, 100, 200 and 1000 µM). The H_2 concentration decreased with increasing sulfate concentration as expected based on previous work (Hoehler et al. 1998), again consistent with a thermodynamic control. Sulfate reduction decreased the sulfate concentrations during the incubation. This was most pronounced in the 100 µM samples, in which the sulfate concentration decreased from 100 to about 35 µM. This decrease in sulfate concentration was reflected in the H_2 concentration, which increased from 3.5 to 7.5 and from 1.8 to 5.7 nM during the incubation for the methanol and the methylamine incubations, respectively. Due to the higher initial sulfate concentration, this effect was not observed in the 200 and 1000 µM treatments. 15 µM was the threshold level for sulfate uptake in these sediments, measured after several days of incubation under methanogenic conditions. No decrease in sulfate concentrations was observed in these samples. It is important to note that the methanogenic substrates used in this investigation are non-competitive substrates for methanogens and sulfate reducers, as they are not degraded by sulfate reducing bacteria. To date, sulfate reducing bacteria are not known to oxidize methylamine and only a few sulfate reducers oxidize methanol (J. Kuever, personal communication). Furthermore, the known methanol oxidizing sulfate reducers are no marine species, but were isolated from limnic

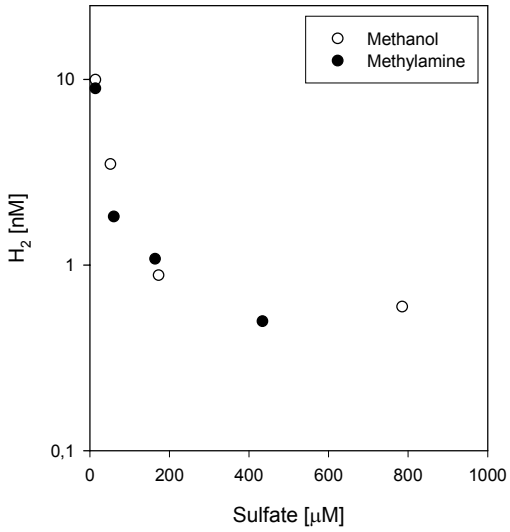

Figure 2: Hydrogen concentration as a function of the sulfate concentration. Samples were taken at Station DA (Dangast, Germany).
Note the logarithmic scale for the H_2 concentrations.

habitats.

In a third experiment, 0.5 mM lactate was added to stimulate fermentative H_2 production. The H_2 concentration in lactate-amended samples increased 270-fold compared to the non-amended controls (Table 1).

Table 1: H_2 concentrations and CO_2 production during ^{14}C-methanol oxidation in response to 500 µM lactate addition.

	H_2 [nM]	CO_2 production [%CO_2]
Control	14 ±2,3	44 ±2,2
500 µM Lactate	3800 ±150	37 ±1,4

$^{14}CO_2$ versus $^{14}CH_4$

In all the experiments presented here, the $^{14}CO_2$:$^{14}CH_4$ ratio produced from ^{14}C-labeled methanol or methylamine increased with decreasing H_2 concentration. The percentage of $^{14}CO_2$ (%$^{14}CO_2$=[$^{14}CO_2$/($^{14}CH_4$+$^{14}CO_2$)]*100) (referred to as %CO_2) decreased at temperatures >20°C (Fig. 3). Thermodynamic calculations indicate that, with increasing temperature, the oxidation of the methyl carbon is favored over the reduction, assuming constant H_2 concentration (Damgaard & Hanselmann 1996, data not shown). Including the changing H_2 concentrations, as observed in the sediments, results the same trends for the two reactions with changing temperature, not favoring one over the other. Thus, increased

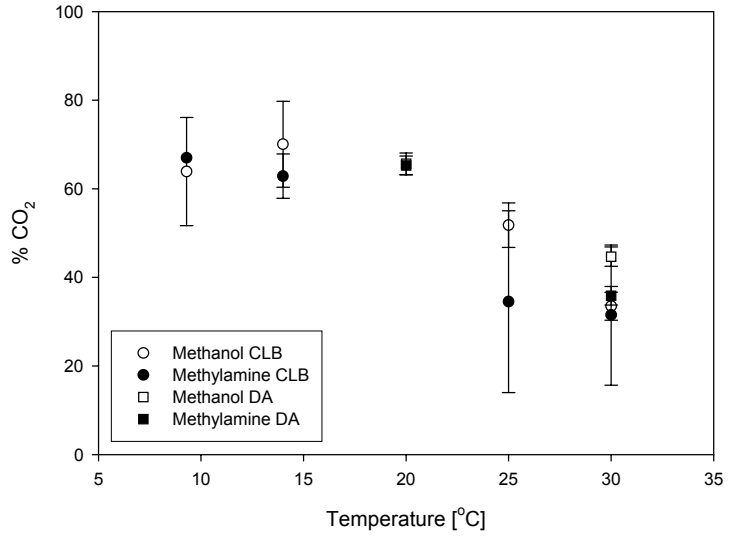

Figure 3: %CO_2 ($^{14}CO_2$ as fraction of the sum of $^{14}CO_2$ and $^{14}CH_4$) as a function of the incubation temperature. Samples were taken at Station CLB (Cape Lookout Bight, NC, USA) and Station DA (Dangast, Germany). The error bars represent the standard deviation of triplicate measurements.

%CO_2 with increased temperature would be expected based on a thermodynamic dependence of the ratio between the oxidative and the reductive part of the reaction. The observation of the opposite trend (i.e. decreasing %CO_2 with increasing temperature) reveals that the H_2 concentrations primarily control the ratio between oxidized and reduced products. In the sulfate addition experiment, the %CO_2 increased with increasing sulfate concentrations (Fig. 4). For the 100 and 250 µM sulfate samples, the average concentrations from measurements at the beginning and the end of the incubations were used to estimate

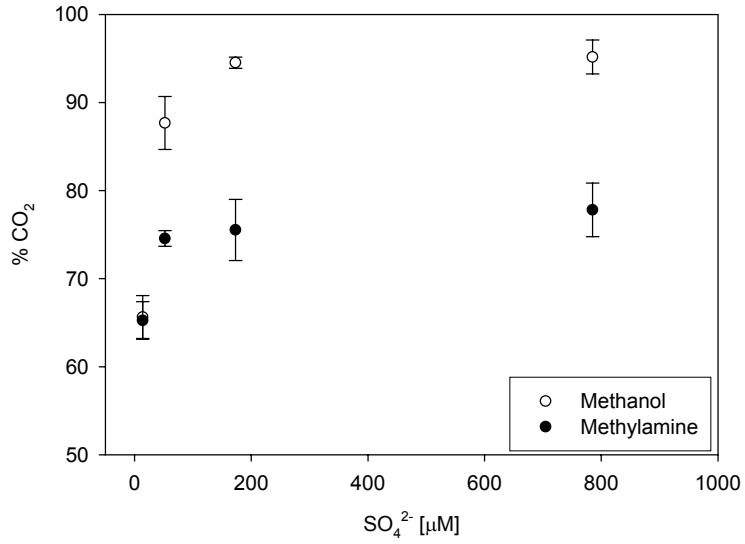

Figure 4: %CO_2 ($^{14}CO_2$ as fraction of the sum of $^{14}CO_2$ and $^{14}CH_4$) as a function of the incubation sulfate concentration. Samples were taken at Station DA (Dangast, Germany). The error bars represent the standard deviation of triplicate measurements.

average sulfate and H_2 concentrations. Addition of lactate to the methanogenic sediment decreased the %CO_2 (Table 1). The decrease was less pronounced than the increase in the H_2 concentration.

When plotted versus the H_2 concentration, the %$^{14}CO_2$ from the temperature and the sulfate experiment for both stations followed a linear trend for each of the amended tracers (Fig. 5).

Pore water gas and salt concentrations

In all the samples a variety of geochemical parameters, including dissolved inorganic carbon, methane, sulfide, and sulfate concentrations, and the pH, was measured, thus encompassing the parameters that have been found to influence the H_2 concentration under methanogenic and sulfate reducing conditions (Hoehler et al. 1998) (data not shown). Only sulfate, sulfide and methane changed systematically in the sulfate addition experiment. Sulfide concentrations increased with increased sulfate concentration, reflecting the increased sulfate reduction activity. Only samples with the highest sulfate concentration did not show an increase in sulfide concentration. The methane concentration decreased with increasing sulfate concentration, probably due to inhibition of hydrogenotrophic

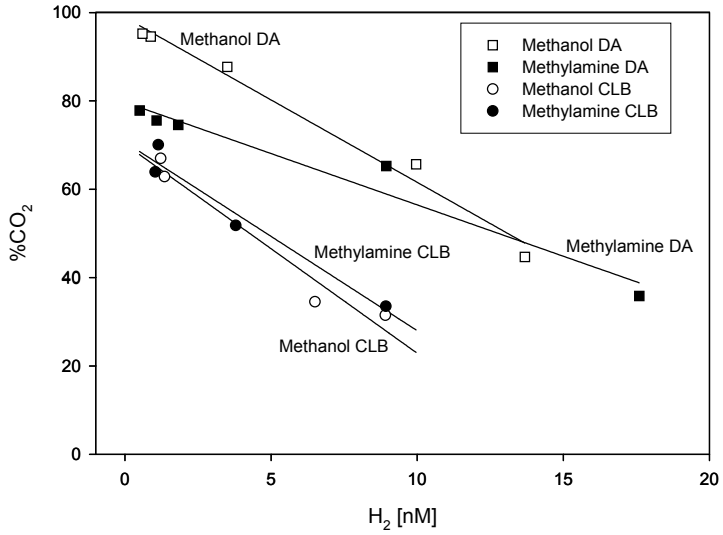

Figure 5: $\%CO_2$ versus hydrogen concentrations from the temperature and the sulfate addition experiments. Samples were taken at Station CLB (Cape Lookout Bight, NC, USA) and Station DA (Dangast, Germany). The solid lines are the regression lines for the data sets as labeled, with $r^2 > 0.98$ for the methanol DAand $r^2 > 0.95$ for the other samples.

methanogenesis due to the low H_2 concentration as a result of the sulfate reducing conditions.

Discussion

Methanogenesis from methylated substrates such as methylamine or methanol yields CO_2 and CH_4. In pure cultures the product ratio is about 1:3 for CO_2 and CH_4, respectively, according to the redox state of the carbon in the substrates (Kjeltens & Vogels 1993). Methanogenesis from methyl compounds can be separated into an oxidative and a reductive part (Thauer 1998). The biochemical pathway starts with the formation of methyl-coenzyme M. In the next step, the methyl carbon is either oxidized or reduced. During oxidation, electrons from the methyl group are transferred stepwise to the electron carrier F_{420}. The reductive part receives these electrons from F_{420} via an electron transport chain and transfers them to the methyl carbon. The oxidation of the methyl group to CO_2 releases 6 electrons while reduction to CH_4 consumes 2 electrons. If the electrons are not transferred elsewhere the ratio of oxidation to reduction is 1:3 (Fig. 6).

The experiments performed here reveal that this stoichiometry changes under low H_2

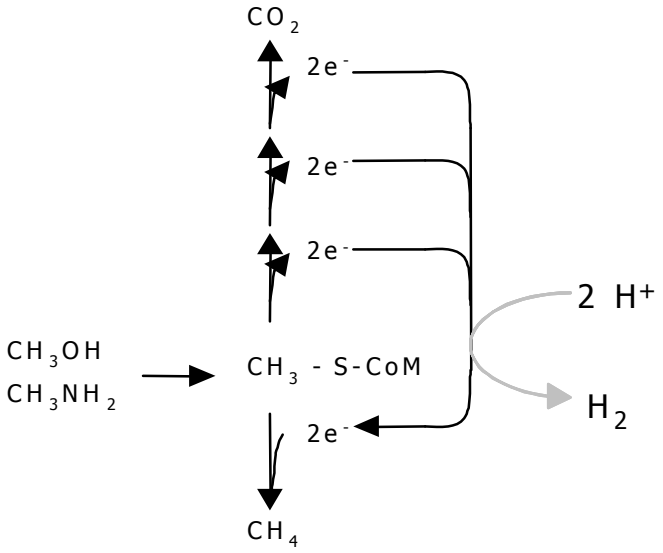

Figure 6: Pathway of methanogenesis from methanol and methylamine. Dependent on the surrounding hydrogen concentration, methylotropic methanogens transfer more or less electrons to hydrogen instead of methane.

concentration because electrons are transferred from the electron carrier to protons rather than to the methyl carbon. In the temperature experiment, hydrogenotrophic archaea controlled H_2 concentrations over the whole temperature range. Thus, methanogenesis from CO_2 and H_2 was exergonic and met the minimum energy requirements for the microorganisms. The leakage of reducing equivalents by the methylotropic archaea to form H_2 therefore reflects a loss of potential energy that is utilized by the hydrogenotrophic archaea. This suggests that the H_2 leakage is not a mechanism for the methylotrophic methanogen to conserve energy but is rather a consequence of possessing a hydrogenase that is capable of catalyzing this reaction. A possible way of scavenging the produced hydrogen and to prevent the loss of energy would be a simultaneous methylotrophic and hydrogenotrophic activity in the same organism. It is known, that some methanogens carry the potential for both pathways (Madigan et al. 2000). Thus, culture experiments to test for the simultaneous activity of both pathways in one organism are necessary.

Hydrogenases catalyze the electron transfer between H_2 and protons and are divided into two functional groups according to their biochemical function (Vignais et al. 2001). Hydrogenases are involved either in H_2 uptake or in H_2 production and most of the hydrogenases can catalyze the reaction in either direction (Vignais et al. 2001). The actual

direction of electron flow depends on the concentrations of the reactants and products. In addition to protons and H_2, the substrates for hydrogenases are electron carriers in the form of the cytoplasmatic F_{420}, NAD(P) or FAD (Blaut 1994, Nandi & Sengupta 1998) or membrane located ferrodoxin, flavodoxin or cytochroms (Fitzgerald et al. 1980, Deppenmeier et al. 1996). The electron carriers are usually involved in energy yielding redox reactions.

In aquatic sediments, microorganisms are usually energy limited. Energy yielding redox reactions for the oxidation of organic matter usually involve several distinct steps, of which only one or a few are actually coupled to energy conservation. The molecules involved in the transfer of the electrons from the reduced (H_2, organic carbon) to the oxidized (terminal electron acceptor, e.g. sulfate) species include electron carriers such as ferredoxin, F_{420} or NAD (e.g. Lengeler et al. 1999). To maximize the energy gain of the overall reactions, the steps that are not coupled to energy conservation are kept close to equilibrium ($\Delta G = 0$). Hydrogenases can transfer electron between electron carriers and H_2. Since hydrogenases are capable of catalyzing the transfer of electrons between H_2 and the electron carriers in either direction, the concentrations of the involved substrates will control the direction of hydrogenase activity under a given set of environmental conditions. H_2 concentrations will therefore have a strong influence on the activity of hydrogenases and on the fate of electrons that are passed through the electron carriers if the appropriate hydrogenases are present.

According to the dominating electron acceptor used for terminal oxidation, terminal metabolic processes in marine sediments are horizontally stratified. Methane is usually found only in horizons where sulfate is depleted. The different energy yields of the terminal oxidation with different electron acceptors has been used to explain spatial separation of redox processes in aquatic environments (Froehlich et al. 1979). The energy yield itself, however, cannot account for an exclusion of certain processes, only the chemical conditions in the environment can. With respect to H_2 as an electron donor, spatial separation is based on thermodynamic control of the H_2 concentration (Lovley & Goodwin 1988, Hoehler et al. 1998). Sulfate reducers lower the H_2 concentrations to levels that make it thermodynamically inaccessible for those methanogens that use H_2 as electron donor in their energy metabolism (Lovley & Goodwin 1988, Hoehler et al. 1998). For alternative

competitive substrates such as acetate, a similar concentration control does not exist. Due to the lower stoichiometric coefficient (acetate oxidation yields 8 electrons while H_2 oxidation yields 2 electrons), acetate concentrations in the sulfate reducing zone would need to be about 1000-fold lower than in the methanogenic zone to account for a similar thermodynamic effect. The concentrations usually observed in the methanogenic zone are only 2 to 10 fold higher than in the corresponding sulfate reducing zone (Sansone & Martens 1981, Sansone & Martens 1982, Blair & Carter 1992, Albert & Martens 1997). However, even though acetate concentrations are not substantially different in the sulfate reducing and methanogenic zones, also acetoclastic methanogenesis is inhibited at high sulfate concentrations.

Thus, another mechanism must account for the inhibition of methanogenesis from competitive substrates other than H_2 and from non-competitive substrates in the sulfate reducing sediments. As demonstrated in our experiments, external H_2 concentrations control the ratio between oxidized and reduced carbon produced from methanogenic archaea. At low temperatures, methylotrophic methanogens leak H_2 at conditions that are suitable for hydrogenotrophic methanogenesis. This means that methylotrophic methanogens lose energy that is used by hydrogenotrophic methanogens to reduce CO_2. In the sulfate reducing zone, this energy goes to the sulfate reducers that are more efficient in scavenging hydrogen. Support for this hypothesis comes from the observation that in microbial mats methanogenesis occurs even in the presence of 40 mM sulfate, provided that H_2 concentrations are high (Hoehler et al. 2002).

Electron carriers are involved in a broad range of microbial redox processes, and hydrogenases are widely distributed among anaerobic bacteria and archaea (Vignais et al. 2001). Molecular H_2 is a small non-polar molecule that easily diffuses across biological membranes. The exchange between intracellular and extracellular H_2 pools should therefore be rapid and efficient. Leakage of electrons through hydrogenases in the form of H_2 influences a wide range of microbial processes whose dependence on H_2 concentrations is not obvious from their general reaction scheme. In sulfate reducing bacteria, for example, the electrons from reduced carbon are passed on to sulfate via an electron transport chain, involving a hydrogenase that is also responsible for the electron uptake from H_2 (Madigan

et al. 2000). At very low H_2 concentrations under, for example, nitrate or O_2 reducing conditions, a sulfate reducer might lose electrons to H_2, thus inhibiting sulfate reduction. The occurrence of sulfate reduction in oxygen containing environments might be due to microenvironments, depleted in oxygen and with higher H_2 concentrations. Thus, the leakage of reducing power through H_2 probably is not only found in methanogenic pathways, but may be widespread among microorganisms having hydrogenases.

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Conclusions and Perspectives

The first three chapters of the thesis addressed questions on the degradation of organic matter in permanently cold sediments. The studies revealed that the response to temperature changes was similar in permanently cold and temperate sediments. The optimum and maximum temperature for sulfate reduction was higher in the temperate sediments, reflecting the higher *in situ* temperature. In both sediment types, however, the close coupling of fermentation and sulfate reduction was preserved over a broad temperature range despite the temperature changes. The increase in intermediate concentrations at temperatures above the optimum temperature for sulfate reduction revealed a higher thermostability of the fermenting bacteria.

Despite the unchanged close coupling of the two steps in the temperature gradient block experiment, comparison of the turnover rates measured in permanently cold sediments with previous results from temperate sites suggests that different steps in the complex degradation pathway are affected differently by the *in situ* temperature. The hydrogen concentrations were controlled by thermodynamics of the terminal oxidation process, but the measured VFA concentrations show no strong dependence on the *in situ* temperature. The turnover of organic carbon, reflected in the terminal electron accepting process, was similar to measurements from temperate sites at higher temperatures, as were the potential hydrolysis rates. Carbon addition as well as sediment mixing resulted in a transient increase in fermentation products, reflecting a disturbance of the close coupling. The fermenting bacteria responded faster to the changed conditions than the sulfate reducing bacteria. The response to carbon addition in terms of degradation rate was similar to reports from temperate sites. The VFA turnover rates, however, were lower than values reported from temperate sites at higher temperatures, but similar to measurements during the cold season. Thus, the degradation pathway rather than the turnover rate of the organic carbon seems to be affected by the permanently cold temperatures.

To verify this finding, more detailed studies on potential alternative substrates for the

sulfate reducing bacteria and measurements with the same method at temperate sites are necessary. Simultaneous measurement of ^{14}C-VFA turnover and $^{35}SO_4^{2-}$-reduction in the same sample might help to understand the role of VFA for the sulfate reduction. However, more detailed studies on the bioavailability of the VFA and the consequences for turnover measurements with radiotracers are necessary for such investigations.

Hydrogen concentrations seem to have a broader influence on biogeochemical processes than previously thought. In the incubations with radiolabeled methylamine and methanol as substrates for methylotrophic methanogens, the external hydrogen concentrations controlled the ratio of oxidized to reduced products. In the temperature experiment, hydrogen concentrations were controlled by hydrogenotrophic methanogenes, and methanogenesis from CO_2 and H_2 is exergonic. Under these conditions, the leakage of hydrogen by the methylotrophic methanogens is likely not a mechanism for energy conservation, but the side effect of the presence of the hydrogenase capable of catalyzing the reaction. Leakage of reducing equivalents in the form of hydrogen might be a common phenomenon for bacteria possessing hydrogenases.

Studies with a purely hydrogenotrophic methanogen in coculture with a methanogen capable of methylotrophic and hydrogenotrophic activity would be needed. These studies would reveal, if scavenging the leaked hydrogen by hydrogenotrophic actity in the methylotrophic methanogen is a potential way of preventing the energy loss due to the hydrogen leakage. Incubations with other metabolic groups will help to test the validity of this hypothesis as a common phenomenon. A first approach could be culture studies with, for example, nitrate reducing and sulfate reducing bacteria with an organic substrate, degraded only by the sulfate reducer.