Geochemical Perspectives

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BO BARKER JØRGENSEN

Sulfur Biogeochemical Cycle of Marine Sediments

Each issue of *Geochemical Perspectives* presents a single article with an in-depth view on the past, present and future of a field of geochemistry, seen through the eyes of highly respected members of our community. The articles combine research and history of the field's development and the scientist's opinions about future directions. We welcome personal glimpses into the author's scientific life, how ideas were generated and pitfalls along the way. *Perspectives* articles are intended to appeal to the entire geochemical community, not only to experts. They are not reviews or monographs; they go beyond the current state of the art, providing opinions about future directions and impact in the field.

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About the cover

The seafloor during summer in a Danish eutrophic fjord. The benthic animals suffer from oxygen depletion in the bottom water, while the sediment turns black from iron sulfides and is over-grown by mats of colorless sulfur bacteria.

PHOTO CREDIT: NANNA RASK

CONTENTS

Preface	IV
Acknowledgements	V
Abstract	145
1. Introduction	147
1.1 Experimental Approach to the Sulfur Cycle	147
1.2 The Sulfur Cycle of Marine Sediments	148
2. My Personal and Scientific History	150
1.2 How it Began	150
2.2 Exploring the Ecology of the Sulfur Cycle	154
2.3 “The Mud Group” in Aarhus	158
2.4 The Max Planck Institute for Marine Microbiology	161
2.5 Back in Denmark	166
3. Sulfate Reduction	169
3.1 ^{35}S Sulfate Reduction Rate (SRR) Measurements	169
3.2 Problems with the ^{35}S Method	172
3.3 Reactive Transport Modelling	174

3.4	Reconciling Modelled and ^{35}S Measured Sulfate Reduction Rates	176
3.4.1	Aarhus Bay coastal sediment	176
3.4.2	SE Atlantic continental slope	178
3.4.3	Measured versus modelled SRR	180
3.5	Cryptic Sulfur Cycling	181
3.6	Substrate Kinetics of Sulfate Reduction	182
3.7	Temperature Control of Sulfate Reduction	186
4.	Sulfide Oxidation	191
4.1	The Dynamic Surface Sediment	191
4.2	Sulfide Oxidation Experiments	193
4.3	Sulfide Oxidation Mechanisms	198
4.4	Big Sulfur Bacteria	199
4.5	Cable Bacteria	203
4.6	Chemoautotrophy and Dark CO_2 Fixation	206
5.	Dynamics of Intermediate Sulfur Species	210
5.1	Elemental Sulfur and Polysulfides	211
5.2	Sulfur Oxyanions	213
5.3	Disproportionation of Thiosulfate	215
5.4	Disproportionation of Elemental Sulfur	219
5.5	Pathways of Pyrite Formation	221
6.	Sediment Mineralisation Processes	223
6.1	Age Control of Turnover Rates	223
6.2	Pathways of Anaerobic Mineralisation	226
6.3	The “Redox Cascade”	231
7.	Interactions with the Methane Cycle	236
7.1	The Methanic Zone	237
7.2	Controls on Methane Production	239
7.2.1	The local scale	239
7.2.2	The regional scale	242
7.2.3	The global scale	244
7.3	Quantitative Role of Methane for Sulfate Reduction	245
7.4	Processes at the Sulfate-Methane Transition (SMT)	246
7.5	Anaerobic Oxidation of Methane (AOM)	249
8.	Stable Sulfur Isotopes	251
8.1	The Mechanisms of Isotope Fractionation	251
8.2	Controls on Isotope Fractionation	252
8.3	Stable Isotopes and Open System Diagenesis	255
8.4	The Sulfidisation Front	257

9.	The Sulfur Cycle – From Coast to Deep Sea	262
9.1	Organic Carbon Cycle of the Global Seabed	262
9.2	The Different Depth Regions	264
9.3	Role of Methane.....	267
9.4	Global Sulfur Budget of Marine Sediments	269
10.	Future Perspectives	272
References.....		276
Index.....		304

PREFACE

Every young scientist, who starts in a research field, builds her or his work on the experience of previous generations of scientists. We try to absorb the wisdom of those earlier generations, but with time we start to question the validity of their interpretations. Then we venture into new and unknown regions of the field, and over the years we may ourselves generate some valuable new understanding – for the young generations to absorb and to question. It is especially the early experiences as scientists that steer the way we think about science later in life. We start with a knowledge and a set of tools that may be state of the art at the time but that are outdated years later. When we look back at the work done in the past it may appear simple or even naive. However, we must remember what was the knowledge at the time and what were the tools. Only then can we appreciate how original and important the discoveries were and sense the excitement when they were made.

This article presents my personal perspective on the science that I have enjoyed throughout my professional life as a marine biogeochemist, with a long term focus on the sulfur cycle of marine sediments. The text is not meant to be only retrospective, or even historical, yet I will try to place my own progress in sulfur biogeochemistry in the context of the research field at the time. This is seldom done in reviews and not in original research articles, but I think it adds an interesting perspective to the evolution of our science. I hope it will also inspire you.

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SULFUR BIOGEOCHEMICAL CYCLE OF MARINE SEDIMENTS

ABSTRACT

Complex interactions between microbial communities and geochemical processes drive the major element cycles and control the function of marine sediments as a dynamic reservoir of organic matter. Sulfate reduction is globally the dominant pathway of anaerobic mineralisation and is the main source of sulfide. The effective re-oxidation of this sulfide at the direct or indirect expense of oxygen is a prerequisite for aerobic life on our planet. Although largely hidden beneath the oxic sediment surface, the sulfur cycle is therefore critical for Earth's redox state.

This *Geochemical Perspectives* begins with a brief primer on the sulfur cycle of marine sediments and a description of my own scientific journey through nearly fifty years of studies of sulfur geochemistry and microbiology. Among the main objectives of these studies were to quantify the main processes of the sulfur cycle and to identify the microbial communities behind them. Radiotracers in combination with chemical analyses have thereby been used extensively for laboratory experiments, supported by diverse molecular microbiological methods. The following sections discuss the main processes of sulfate reduction, sulfide oxidation and disproportionation of the inorganic sulfur intermediates, especially of elemental sulfur and thiosulfate. The experimental approaches used enable

the analysis of how environmental factors such as substrate concentration or temperature affect process rates and how concurrent processes of sulfate reduction and sulfide oxidation drive a cryptic sulfur cycle. The chemical energy of sulfide is used by chemolithotrophic bacteria, including fascinating communities of big sulfur bacteria and cable bacteria, and supports their dark CO₂ fixation, which produces new microbial biomass.

During the burial and aging of marine sediments, the predominant mineralisation processes change through a cascade of redox reactions, and the rate of organic matter degradation drops continuously over many orders of magnitude. The main pathways of anaerobic mineralisation and the age control of the organic matter turnover are discussed. In the deep methanic zone, only a few percent of the entire degradation process remains, which provides a small boost of substrate for sulfate reduction through the process of anaerobic methane oxidation. The stable isotopes of sulfur provide an additional tool to understand these diagenetic processes, whereby the combination of microbial isotope fractionation and open system diagenesis generate a differential diffusion flux of the isotopes.

In relation to the organic carbon cycle of the seabed and the contribution of methane, the paper discusses the global sulfur budget and the role of sulfate reduction for organic matter mineralisation in different depth regions of the ocean - from coast to deep sea. The published estimates of these parameters are evaluated and compared. Finally, the paper looks at future perspectives with respect to gaps in our current understanding and the need for further studies.

1.1 Experimental Approach to the Sulfur Cycle

My view of the sulfur cycle

There are many ways to present the sulfur cycle of marine sediments, with emphasis on the geochemistry or the microbiology, the global scale or the microscale, the present or the past. Here is my version. It is based on my own experience and what I have found interesting in this research field. The text is not a balanced review of the literature, and many important aspects and publications are not included. The examples used and presented in graphs are mostly from my own work and work of my students and colleagues. The study sites are therefore strongly biased towards the coastal zone around Denmark. This has the advantage that the discussion of the sulfur cycle becomes consistent for these types of sediments. It has the disadvantage that many other important sedimentary systems are neglected. I realise, of course, that results from Danish coastal waters cannot simply be extrapolated to the world ocean. Yet, most of the microbial and geochemical processes are the same in different marine sediments, although their mutual balance and significance may differ strongly.

With this disclaimer, I am ready to attack the dynamic sulfur cycle of the seabed and its exploration over the past half century. There are many questions to address. Which were the tools that we used and what were the ideas? How did we arrive at the present state of understanding? What are we still missing and what do we still fail to understand?

My primary approach to the study of sulfur cycling has been experimental, based on the use of radiotracers and other methods to determine the rates and pathways of individual processes. The results were integrated with geochemical data and checked by simple transport reaction modelling. Often, the experimental and the modelled rates did not compare well. In an attempt to understand why, I learned to distinguish between net and gross processes and developed the concept of cryptic cycling. The results were also compared with microbiological data to better understand how the physiological potential and constraints of microorganisms control the biogeochemical processes. While the microbiology is a fast moving field today due to the rapid development of molecular biology and nucleic acid sequencing, it also makes us realise how much we still have to learn. Our view of the sulfur cycle continues to change as new discoveries are made and new important results are published. I can hardly wait.

1.2 The Sulfur Cycle of Marine Sediments

A brief primer

The biogeochemical sulfur cycle of marine sediments is discussed in the following sections and is briefly introduced here in Figure 1.1. The figure shows in green the organic carbon (C_{org}) that drives the cycle. The main oxidants (O_2 , NO_3^- , $Mn(IV)$, $Fe(III)$ and SO_4^{2-}) are shown in purple. The main sulfur species involved in the cycle within the sediment are shown in yellow. Arrows and their names symbolise microbial and geochemical processes.

Figure 1.1

The sulfur cycle in the seabed and its interactions with predominant oxidants (purple) and organic carbon (green). The main microbial and geochemical processes are shown, including the surface-bound cable bacteria and sulfur bacteria, *Beggiatoa*. The figure is very sulfur-centric, and diverse reactions of manganese, iron and nitrogen are not shown. From Jørgensen et al. (2019a).

The sulfur cycle is primarily fueled by buried organic matter and its oxidation by sulfate reducing microorganisms (Fig. 1.1). A part of the organic matter becomes buried beneath the depth of sulfate penetration and is partly degraded to methane, which also serves as a substrate for sulfate reduction. The produced H_2S is very

reactive. If the H_2S reaches within centimetres of the sediment surface, it may be used by specialised sulfide oxidising microorganisms, such as cable bacteria and filamentous sulfur bacteria (*Beggiatoa*). Most of the sulfide is gradually oxidised back to sulfate within the sediment by a depth sequence of oxidants including oxygen (O_2), nitrate (NO_3^-), manganese oxides (Mn(IV)) and iron oxides (Fe(III)). The initial oxidation products are diverse intermediate sulfur species such as elemental sulfur (S^0), thiosulfate ($\text{S}_2\text{O}_3^{2-}$), tetrathionate ($\text{S}_4\text{O}_6^{2-}$) and sulfite (SO_3^{2-}). These intermediates may be further oxidised to sulfate or reduced back to sulfide, or they may be disproportionated, whereby they are simultaneously oxidised and reduced with no net change in oxidation state. Sulfide reaction with iron leads to amorphous iron sulfides (FeS) that are gradually converted to the more stable pyrite (FeS_2) by reaction: (1) with polysulfide (S_x^{2-}), or (2) with H_2S . Together with the burial of organic sulfur in sediments and of sulfate in evaporites (not shown in Fig. 1.1), the burial of pyrite is a main sink in the marine sulfur cycle.

In the next sections these microbial and geochemical processes will be discussed in detail. The aim is thereby to provide a general description of the sedimentary sulfur cycle with focus on the inner shelf and coastal zone. The examples used will mostly be taken from the transition between the Baltic Sea and the North Sea. Locations highlighted on the map in Figure 1.2 include the Bornholm Basin, Arkona Basin, Aarhus Bay, Kattegat, Skagerrak and Limfjorden. The sediments are mostly organic-rich marine mud at water depths ranging from 10-15 m in Limfjorden, 15-40 m in Aarhus Bay, 90 m in Bornholm Basin, to 100-700 m in Skagerrak.

Figure 1.2

Map of the sea around Denmark, from the North Sea to the Baltic Sea. The main location names discussed in this article are indicated. The colour scale shows water depths in metres. Map courtesy of Jiarui Liu (GeoMapApp).

This section is a personal account of my time in science and the science of my time over the past fifty years. In retrospect, I could try to describe my scientific career as well planned and structured. Yet, the truth is that major shifts in my life, both personal and professional, were directed by opportunities that showed up unexpectedly or developments that I had not foreseen. That was how I came to study sediment biogeochemistry in Aarhus, and how I went to work in Germany for fifteen years and then come back to Aarhus. What really created scientific continuity through all those years was my fascination of the seabed with all its creatures and processes, and my curiosity for the life of its microbial inhabitants.

1.2 How it Began

The choice of a research topic

I was born 1946 as the third out of four children and grew up in a suburb of Copenhagen. During the school years, I enjoyed bird watching, botany and nature in general. I spent much of my free time outdoors with boots and binoculars together with friends who had similar interests. It therefore became an obvious choice to study biology at the University of Copenhagen. During my studies, I became an enthusiastic SCUBA diver and discovered the wonderful underwater world of marine life. From then on my goal was to become a marine ecologist and preferably to study benthic animals. In that respect, I was inspired by my father, Carl Christian Barker Jørgensen, who was an animal physiologist and had worked on suspension feeding marine invertebrates in his younger years (Jørgensen, 1966).

When it became time to think about a master's project, I contacted Tom Fenchel, who was a young scientist at the Marine Biology Laboratory, University of Copenhagen. I had read some of his excellent work on protozoa and the microbiology of sulfidic sediments (Fenchel, 1969) and had heard him talk very enthusiastically about this fascinating microbial world. We made an appointment to meet in his office one morning in 1970 to talk about ideas for my project. The meeting became a decisive moment for the rest of my scientific life. That same morning, Tom had received a letter stating that he was appointed Professor of Ecology at the University of Aarhus, the second largest university in Denmark. At the age of 30, he would become the youngest full professor in Denmark. During our conversation, he invited me to join him to Aarhus and start as his first student, which I did. The project should deal with sulfidic sediments with focus on the processes of sulfur cycling rather than on the microbiota, which he had studied himself. I quickly added a course on "*Radiotracers in Biology*" to my graduate studies in Copenhagen to be prepared for the coming experiments. A year later, I moved to Aarhus together with my wife, Inga.

My studies of the sulfur cycle started at Aarhus University in 1971. Everything was new in the labs in Aarhus. The research group initially consisted

of Tom Fenchel plus two young marine biologists and a technician. It was the primary goal of my project to measure bacterial sulfate reduction in marine sediments using the $^{35}\text{SO}_4^{2-}$ radiotracer technique. I had read about this method from the pioneering work of the Russian microbiologists, Yuri I. Sorokin (Sorokin, 1962; Fig. 2.1) and Michail V. Ivanov (Ivanov, 1956, 1968; Fig. 2.2). However, there was no equipment for this purpose in Aarhus and there was no previous experience with any of the relevant methods. To start with, I learned how to acidify and drive off sulfide from a sediment sample under N_2 after a radiotracer experiment and to recover the ^{35}S -labelled H_2S in a bubble trap with a heavy metal ion. I chose cadmium because CdS was easier to filter than the less toxic, but very flocculent ZnS . For sulfate analysis there was no ion chromatograph, so I extracted the pore-water, precipitated the sulfate with barium, and weighed the dried BaSO_4 precipitate. For radioactivity measurements of ^{35}S -labelled sulfate and sulfide there was no scintillation counter but only an old Geiger-Müller tube in another department, which I could use to measure the soft beta radiation from the precipitates. This required cumbersome calibrations for self-absorption in the dried precipitates. I aimed for minimal disturbance of the sediment and therefore injected only a few μL of radiotracer into whole sediment cores before incubating these for several hours. In this way, the original chemical and microbial zonation in the sediment could, hopefully, be maintained (Jørgensen, 1978a).

Figure 2.1 Yuri I. Sorokin with an orphaned baby owl at Gelendzhik Marine Station, Shirshov Institute of Oceanology at the Black Sea. Photo: Bo Barker Jørgensen.

Figure 2.2 Michail V. Ivanov lecturing in Moscow on pyrite and sulfur oxidation. Photo courtesy of Alla Lein.

Once all these steps plus some other methods worked, I was ready to attack the sulfur cycle of marine sediments. I was at the time 25 years and rather insecure about the whole thing because it had taken so long to learn and develop the methods. Yet, I remained optimistic and determined to carry through the master's project. Then in the middle of the project, four months before the sharp deadline for submission of my thesis, my wife gave birth to our twin boys. From then on, the focus was shared between family and science, and life became rather hectic and sleep-deprived. The thesis was submitted hours before the deadline, under strong time pressure.

My master's project was literally made in a sand box. This box of marine sand, set up at Aarhus University's field station in Rønbjerg at Limfjorden, was supposed to resemble the shallow sandy seabed. A combination of the ^{35}S radio-tracer technique and some simple sulfur geochemistry at different times and depths over half a year enabled the calculation of the main processes of the sulfur cycle in the sand. A few cm thick layer of seawater flowed over the sediment, covered by a floating glass plate to prevent gas exchange with air. I could thereby measure oxygen in inflowing and outflowing water and calculate the sediment oxygen uptake. It dawned to me that this was the first time that aerobic and anaerobic mineralisation rates of organic matter had been measured simultaneously in a marine sediment. The results showed, surprisingly, that **sulfate reduction was quantitatively just as important as aerobic respiration for the oxidation of the organic matter and up to half of the oxygen uptake was directly or indirectly used to re-oxidise sulfide** (Jørgensen and Fenchel, 1974).

The next step was to try a similar combination of measurements in nature. The chance came in 1973 with a three year PhD funding through a new environmental programme, aimed to study eutrophication in Limfjorden. The Limfjorden is a complex system of broads and belts cutting through northern Denmark, with narrow connections to the North Sea in the west and to the Kattegat in the east (Fig. 1.2). In a great hurry, I planned a two year programme during which I would take cores from the seabed at regular time intervals at nine stations and determine the role of sulfate reduction in the sediment relative to the benthic oxygen consumption and organic carbon turnover. Since our laboratory had no sediment coring device, since the water depths were only 5-15 m, and since the project was planned during a warm summer period, I decided to do all the sampling by SCUBA diving using my own wet suit. As winter came, however, and the water temperature approached the freezing point, I experienced serious problems with hypothermia during multiple dives in murky and ice cold water. I realised that this was not a good idea and finally managed to acquire a Haps corer (Fig. 2.3) (Kannevorff and Nicolaisen, 1973), with which sediment could be sampled from a small fishing vessel in a much safer manner. It was a great improvement to sample sediment with a corer at the end of a wire rather than with me at the end of a rope.

To my great satisfaction, the data from two years of field-work in Limfjorden largely confirmed the results from the sand box (Jørgensen, 1977a). Suddenly, this was not just an observation in an artificial laboratory system. The results revealed functional and quantitative aspects of the sulfur cycle that were of much more general, and partly controversial, significance. I learned that the **sediment in Limfjorden functioned as a very open system**

to sulfur cycling, retaining only 7 % of the produced sulfide as pyrite in the top 0-10 cm and recycling 93 % of the sulfide back to sulfate near the sediment surface. Sulfate reduction in the top 0-10 cm accounted for 65 % of sulfate reduction in the entire sediment column, the remaining 35 % occurring from 10 cm and down to the bottom of the sulfatic zone (*i.e.* the zone dominated by sulfate reduction). As in the sand box, **sulfate reduction was equally important as oxygen respiration for the direct oxidation of organic matter to CO₂, and sulfide re-oxidation was, directly or indirectly, responsible for half of the entire oxygen consumption of the sediment.**

These results were in contrast to published knowledge at the time. Much of the earlier geochemical literature treated processes in marine sediments as closed system diagenesis, and according to the microbiological literature dissimilatory sulfate reducers could not oxidise their substrates completely to CO₂. The known sulfate reducing bacteria lacked a complete tricarboxylic acid cycle and could therefore oxidise organic substrates only to acetate (Selwyn and Postgate, 1959). Selwyn and Postgate suggested that a complete oxidation might perhaps be possible through an unknown syntrophy with acetate-fermenting bacteria but they had no suggestions as to how this might work. John R. Postgate from the University of Sussex in England was one of the reviewers of my Limfjorden manuscript and reminded me in some critical remarks about the physiological constraints of sulfate reducing bacteria. Yet, **I remained convinced that acetate could not be the end product of anaerobic oxidation of organic matter by sulfate reduction. The seabed was not pickled like sauerkraut.**

Figure 2.3 Bo Barker Jørgensen (with beard) and Preben G. Sørensen operating the Haps corer, 1979. Photo: Niels Peter Revsbech. From Jørgensen (2019) with permission from John Wiley and Sons.

During the revision of the Limfjorden manuscript, a new microbiology paper came to my rescue. The publication was not unexpected to me, but the timing was just right. During my PhD project, the German microbiologist, Norbert Pfennig, from the University of Göttingen in Germany had read about the results from my sand box and heard about the Limfjorden work. He came to visit me in Aarhus together with his new Master's student, Friedrich (Fritz) Widdel. We discussed the evidence for complete substrate oxidation by sulfate reducing bacteria and they went back to Göttingen with the aim to search for such bacteria. Within a year, Fritz Widdel had successfully isolated several pure cultures of spore-forming sulfate reducers, *Desulfotomaculum acetoxidans*, that could perform complete acetate oxidation (Widdel and Pfennig, 1977).

With an impressive skill and patience, Fritz Widdel went on to isolate a large collection of sulfate reducers with novel physiological capabilities. Among them was a sulfate reducer that was completely specialised on the oxidation of acetate to CO₂. With friendly irony, they named the new organism *Desulfobacter postgatei* (Widdel and Pfennig, 1981). Interestingly, Fritz Widdel observed by this culture that the bacteria tended to grow on the glass wall of the culture tubes. This probably explained why Selwyn and Postgate had missed them as they routinely transferred cultures only from the liquid phase of the medium.

The microbiological work of Fritz Widdel had far-reaching consequences for the understanding of sulfate reducing bacteria and for my scientific career. Firstly, it was a true revelation in my early years to experience **how biogeochemical process studies could inspire and direct microbial cultivation and physiological studies in the laboratory and vice versa**. The discovery of complete substrate oxidation during sulfate reduction in the sea bed and by sulfate reducers in pure culture was a convincing example. Secondly, fifteen years later Fritz Widdel and I were invited to found and build up a new Max Planck Institute for Marine Microbiology in Bremen, Germany, where he established the Department of Microbiology and I the Department of Biogeochemistry. It was our vision to inspire interdisciplinary research between the fields of microbial physiology and sediment biogeochemistry.

2.2 Exploring the Ecology of the Sulfur Cycle

During my PhD studies in 1973-77, I was interested in the pathways and rates of sulfur cycling in sediments and in the microbiology behind the processes. This was long before DNA-based methods were developed to identify and quantify bacteria, so I used the available method of counting bacterial colonies that would grow in shake agar tubes (Jørgensen, 1978c). I could then compare the number of viable sulfate reducers with the measured sulfate reduction rates to calculate a mean sulfate reduction rate *per* colony. The mean rate was 2,500 fmol colony⁻¹ d⁻¹ (1 femtomol = 10⁻¹⁵ mol). I compared this number to the cell specific sulfate reduction rates of exponentially growing pure cultures of sulfate reducers at 25-35 °C, which were only 0.2-10 fmol cell⁻¹ d⁻¹. I was sure that sulfate reducers

in marine sediments could not have thousand fold higher metabolic rates than pure cultures. I trusted my rate measurements, so the colony counts (about 10^4 colonies cm^{-3}) had to be at least 1,000 fold too low relative to the real cell abundance (Jørgensen, 1978c).

This was apparently the first time that such a calculation had been made for anaerobic bacteria in the marine environment, and it was discouraging to realise the vast underestimation of the true bacterial numbers. I therefore decided to abandon viable counts. However, twenty years later we picked up the theme again and made similar quantifications of cell numbers and sulfate reduction rates, now using modern DNA-based methods. Today, we know from our quantification of SRB by slot blot hybridisation (Sahm *et al.*, 1999), by fluorescence *in situ* hybridisation (Ravenschlag *et al.*, 2000), and by quantitative PCR of *dsrAB* genes from similar Danish sediments (Leloup *et al.*, 2009; Petro *et al.*, 2019) that **the old viable counts had indeed underestimated the real numbers of sulfate reducers by 10,000 to 100,000 fold**. The viable counts had revealed only the fastest growing organisms and not the organisms of real quantitative importance.

Instead of doing viable counts during my PhD, I turned my interest to sediment bacteria that I could directly recognise and quantify under the light microscope, namely the filamentous, colourless sulfur bacteria, *Beggiatoa* spp. I had become fascinated during SCUBA diving by these *Beggiatoa* communities that formed white sheets on the sediment surface. When the water was oxic, the seabed was light brown, and *Beggiatoa* were not visible (Fig. 2.4). When the bottom water approached anoxia, the sediment surface turned black and sulfidic. It was then quite dramatic to observe how the benthic fauna crept out of the sediment to escape sulfide and reach the last traces of oxygen, and a white coating of *Beggiatoa* started to spread over the seabed (Fig. 2.4).

Figure 2.4

(a) The shallow oxic seabed with eelgrass vegetation and algae. **(b)** During seasonal anoxia in fjords and coastal basins, the seabed turns black from iron sulfide and is overgrown by colourless sulfur bacteria, *Beggiatoa*. Photo: (a) Peter Bondo Christensen, (b) Nanna Rask.

The choice of *Beggiatoa* was inspired by Tom Fenchel, who had studied the conspicuous bacterial communities of coastal sulfureta and had observed the different types of sulfur bacteria in the microscope (Fenchel, 1969). I had

also read the pioneering work of Sergei Winogradsky (1887) who was the first to recognise a chemolithotrophic type of metabolism in the sulfide oxidising *Beggiatoa* (Dworkin, 2011). I discovered that the filaments were quite common but only rarely occurred as visible sheets on the surface. Instead, **they were scattered in the upper few cm of the sediment, often in large abundance and with a biovolume approaching that of all other bacteria combined** (Jørgensen, 1977b).

The *Beggiatoa* lived in the few cm deep, brown and oxidised surface zone, which had a redox potential (Eh) > 0 mV. Sulfide concentrations were here below my detection limit of 1 μM . The question was then, what was their source of sulfide? Using the ^{35}S method, I could show that sulfate reduction indeed occurred throughout the oxidised sediment, possibly within mm-sized, reduced micro-niches (Jørgensen, 1977c). The chemistry of those microniches could not be determined at the time due to insufficient spatial resolution of available techniques. We needed microsensors for that.

Figure 2.5

Niels Peter Revsbech by the first field study of oxygen in the seabed using microelectrodes. From Jørgensen (2019) with permission from John Wiley and Sons; photo: Bo Barker Jørgensen.

A few years later, Niels Peter Revsbech started in our group and became my first PhD student. Already during his master's project, he constructed **micro-electrodes with which oxygen and sulfide could be measured in sediments at sub-mm resolution for the first time**. When used in coastal marine sediments, he discovered that only the upper few mm was oxic (Revsbech *et al.*, 1980). This comprised only the upper tenth of the brown, oxidised surface layer. During the 1980's, Niels Peter Revsbech refined the microelectrode techniques and broadened the spectrum of sensors. Microelectrodes soon became a powerful tool with many applications in ecology and microbiology (Revsbech and Jørgensen, 1986) (Fig. 2.5).

During the 1960's, the geochemist, Robert A. Berner, at Yale University had developed diffusion reaction models to calculate rates of sulfate reduction and other mineralisation processes

in marine sediments (Berner, 1964). His kinetic models were a breakthrough in sediment geochemistry and showed how porewater solutes and diffusion laws could be combined to understand open system diagenesis in the seabed. In 1971, Bob Berner published the book *"Principles of Chemical Sedimentology"* (Berner, 1971), which compiled his impressive knowledge of dynamic sediment diagenesis. Bob Berner's papers and book became a great inspiration for me and **I compared the results of his diffusion reaction model with my ^{35}S sulfate reduction rate data. They did not compare very well.** For one thing, I found that the decrease in sulfate reduction rates with depth in the seabed could be described by a power law. Bob Berner's model assumed an exponential relation, corresponding to the degradation kinetics of a single organic carbon pool. If the initial organic material consisted of several pools with different degradation rate constants, however, the Berner model could match the experimental rate data (Jørgensen, 1978b). This led to the development of a multi-G model (Westrich and Berner, 1984), which was later generalised into a reactive continuum model (Boudreau and Ruddick, 1991). Still today, however, the modelling approach and the experimental rate measurements of sulfate reduction often yield diverging results, as will be discussed in Section 3.

Towards the end of my PhD in 1977, I learned that stable sulfur isotopes could be used as an independent tool to assess sulfur transformations in the seabed and perhaps even to test the validity of the ^{35}S method. Inspiration came from several key papers at the time, including Kaplan *et al.* (1963), Hartmann and Nielsen (1969), Trudinger and Chambers (1973), and Goldhaber and Kaplan (1975). I visited Heimo Nielsen at the University of Göttingen, Germany, to learn how to prepare sulfate and sulfides for isotope analysis. In Denmark there was only one isotope ratio mass spectrometer available for environmental samples, namely that of Willy Dansgaard at the University of Copenhagen. Dansgaard and his group were running large series of ^{18}O analyses from Greenland ice cores for his seminal studies of arctic climate variability (*e.g.*, Dansgaard *et al.*, 1982). With some hesitation, his kind engineer let me have my samples analysed for sulfur isotopes on his machine. The mass spectrometer was out of function for two weeks after that. Dansgaard was very upset and forbade me to come back with further samples. Thus, my career as an isotope geochemist ended before it had really started.

In despair, I decided instead to develop a theoretical model of sulfur isotopes in the seabed, using what I knew about the sulfur cycle from my PhD work, combined with the diffusion reaction model of Berner (1971), and using isotope data of Hartmann and Nielsen (1969) from the Baltic Sea to test the model. As I lacked practical experience in isotope geochemistry, I contacted Martin Goldhaber at UCLA for advice. Marty Goldhaber was very helpful and had new isotope data and novel ideas about the dynamics of sulfur diagenesis. We exchanged manuscripts, both of which showed how an open system sulfur cycle in diffusion exchange with the overlying seawater could generate the sulfur isotope distributions observed in marine sediments (Jørgensen, 1979; Goldhaber and Kaplan, 1980). As the seabed had previously been considered a rather

closed system, it was very reassuring to find that the open system diagenesis I had learned from my PhD work could indeed be reconciled with sulfur isotope geochemistry.

2.3 “The Mud Group” in Aarhus

During my PhD work, the group of Tom Fenchel was strengthened by a research professorship for the microbiologist, T. Henry Blackburn. Henry Blackburn established a very successful group of young PhD students and postdocs who developed new methods to study nitrogen cycling in marine sediments. Jan Sørensen and Niels Peter Revsbech were hired as associate professors. Together, we now constituted a collaborating research group focused on marine sediments, which inspired Tom Fenchel’s nickname, “The Mud Group”. Teaching of classes and organising lab courses for a growing number of interested biology students was intensive and took much of our time, but there was still time to pursue research and supervise highly motivated MSc and PhD students.

Throughout the 1980’s we benefitted from our diverse expertise in the group, which enabled integrated studies of sediment microbiology and element cycling, including carbon, nitrogen, sulfur and oxygen. Our early studies included the first comparisons of oxygen, nitrate and sulfate respiration in marine sediment (Sørensen *et al.*, 1979; Jørgensen and Sørensen, 1985), experimental identification of the fermentation products that fed the sulfate reducing bacteria (Sørensen *et al.*, 1981), and determination of the general role of sulfate reduction for the mineralisation of organic matter in the seabed (Jørgensen, 1982). The microelectrodes were a key tool in much of our work and revealed how aerobic processes are controlled at a microscale dominated by diffusion transport and low Reynolds number fluid flow. We discovered the extremely steep oxygen and sulfide gradients in which mat-forming *Beggiatoa* live (Jørgensen and Revsbech, 1983) and identified the diffusive boundary layer, which drapes all solid surfaces but is only a few hundred μm thick (Jørgensen and Revsbech, 1985). We also used the microelectrodes to study photosynthesis.

The Solar Lake in Sinai

Back in 1973, Tom Fenchel had organised a study tour to the Red Sea, which I joined as a master’s student. Here we met Yehuda Cohen from the Hebrew University, Jerusalem, who had just started his PhD project on the microbiology and limnology of the small, hypersaline Solar Lake located on the Sinai coast of the Gulf of Aqaba (Cohen *et al.*, 1977). The lake had an extraordinary stratification in winter with cool seawater on top and up to 65 °C hot brine below one metre depth. Along the shore, the bottom was covered by up to 1 metre thick, massive cyanobacterial mats that had been growing for several thousand years (Fig. 2.6). I was deeply fascinated by this lake, and the study tour became the start of many years of collaboration between Yehuda Cohen and our group.

Figure 2.6 The Solar Lake at the Gulf of Aqaba, Red Sea. Colourful mats of cyanobacteria grow along the shore in the lake. Photo: Bo Barker Jørgensen.

The cyanobacterial mats of the Solar Lake became a testbed for many of our microsensor techniques. We discovered extremely dynamic diel cycles of oxygen and sulfide in the chemocline, both in the water column and in the cyanobacterial mats (Jørgensen *et al.*, 1979a,b). Microsensors became the main tool to analyse respiration and photosynthesis in the mats. We discovered that the **cyanobacteria switch daily between anoxygenic photosynthesis in the morning, when the sulfide level is high at the mat surface, to oxygenic photosynthesis in the afternoon, when the sulfide has been depleted** (Jørgensen *et al.*, 1983; Revsbech *et al.*, 1983). Hot spring mats that grow under very sulfidic water may even express cryptic oxygen production in the light where the oxygen does not reach outside the mat but is recycled within the mat within seconds (Jørgensen and Nelson, 1988). This observation was interesting in relation to the earliest photosynthetic O_2 production in benthic cyanobacterial communities, some 2.5 billion years ago.

The studies of microscale photosynthesis were for several years limited by our lack of light microsensors. In 1984-85 I went to NASA Ames Research Center in California for a sabbatical to work with David J. DesMarais, and optical properties of benthic photosynthetic communities became my new research theme. Dave DesMarais organised expeditions to the great salt ponds on the Pacific coast of Baja California in Mexico where cyanobacterial mats grew over tens of km^2 . NASA had an interest in such mat systems as potential analogues of early life on our planet. I developed fibre optic microsensors and combined these with microelectrodes to understand the controls on oxygenic and anoxygenic photosynthesis in cyanobacterial mats (Jørgensen *et al.*, 1987). Several years later, my

master's student in Aarhus, Carsten Lassen, constructed optical microsensors to measure irradiance and scalar irradiance (Lassen and Jørgensen, 1994) and benthic photosynthesis became an important research theme for several of my students, including Helle Ploug and Michael Kühl (*e.g.*, Ploug *et al.*, 1993; Kühl *et al.*, 1993).

In 1987, I was granted a 5 year professorship from the Danish Science Research Council, which for the first time secured funding of my work for a longer period and enabled me to concentrate on research. I decided to focus on the oxidative pathways of the sulfur cycle in marine sediments. My student, Henrik Fossing, and I tried to use ^{35}S -labelled sulfide in an approach similar to the use of ^{35}S -labelled sulfate. We soon realised that **non-biological isotope exchange reactions happen quickly among several of the reduced sulfur species, such as H_2S , polysulfides, elemental sulfur and iron sulfides**, which made it difficult or even impossible to distinguish the ongoing sulfide oxidation (Fossing *et al.*, 1992).

A few years earlier, however, Friedhelm Bak and Heribert Cypionka at the University of Konstanz, Germany, had discovered a novel type of sulfur metabolism in sulfate reducing bacteria, the disproportionation of thiosulfate to sulfide and sulfate (Bak and Cypionka, 1987). I expected that thiosulfate would not undergo fast isotope exchange and purchased, at a very high price, thiosulfate with the inner (sulfonate) or with the outer (sulfane) sulfur atom ^{35}S -labelled. Although thiosulfate and sulfite generally occur at only μM concentration in marine sediments (Thamdrup *et al.*, 1994b), I discovered that **thiosulfate functions as an important shunt in the sulfur cycle and can concurrently be oxidised, reduced and disproportionated by different sediment bacteria** (Jørgensen, 1990).

Donald E. Canfield joined my group as a young visiting scientist in 1990-91 (Fig. 2.7). He was a former PhD student of Bob Berner, and this became the start of a fruitful and enjoyable collaboration. Don Canfield joined our expeditions to study the pathways of organic carbon oxidation in marine sediments. He and my PhD student, Bo Thamdrup, used a new type of anaerobic bag incubation to measure concurrently iron, manganese and sulfate reduction in marine sediments under quasi-natural conditions (Canfield *et al.*, 1993a,b; Thamdrup *et al.*, 1994a). The bag technique was developed by our master's student, Jens Würgler Hansen (Hansen *et al.*, 2000), and we therefore called them "Würgler bags".

Figure 2.7 Donald E. Canfield as visiting scientist in Aarhus, 1990. Photo: Bo Barker Jørgensen.

In order to study chemical microgradients and sediment-water fluxes *in situ* we constructed benthic landers equipped with either micro-electrodes or a flux chamber to perform

measurements directly on the sea floor. The microsensor instrument started as a highly ambitious, yet successful master's project for Jens K. Gundersen (Gundersen and Jørgensen, 1990), while the flux chamber instrument was a similarly ambitious PhD project for Ronnie N. Glud (Glud *et al.*, 1994). The establishment of this new and advanced technology was supported by a very helpful exchange of know-how with Clare Reimers and Ken Smith at Scripps Institution of Oceanography in California.

In the early 1990's we started to apply the emerging DNA-based techniques to study the diversity and abundance of specific microbial communities using approaches that had been developed within the preceding few years (Stahl *et al.*, 1988; Amann *et al.*, 1990). Niels Birger Ramsing combined for the first time fluorescent oligonucleotides and microsensors to detect the microscale distribution and respiration of sulfate reducing bacteria in a biofilm (Ramsing *et al.*, 1993; Teske *et al.*, 1998). Ever since that early start, we have combined molecular microbial ecology and biogeochemistry in our research.

An opportunity to join my first international ocean expedition came in 1988 when Holger W. Jannasch of the Woods Hole Oceanographic Institution invited me to join a research cruise with the RV "Knorr" in the Black Sea. Henrik Fossing and I made detailed studies of sulfide oxidation in the chemocline of the water column. In contrast to the existing literature, we discovered that the oxic and the sulfidic zones were separated by a 20-30 m deep intermediate layer with no detectable H_2S or O_2 (Murray *et al.*, 1989; Jørgensen *et al.*, 1991). Little did I know during that Black Sea cruise that Holger Jannasch would soon have a decisive influence on my career, an influence that changed both my professional life and my family life for the next decades (Fig. 2.8).

Figure 2.8

Holger W. Jannasch, cruise leader on board the RV "Atlantis II" during an ALVIN diving expedition in the Gulf of California, 1988. Photo: Bo Barker Jørgensen.

2.4 The Max Planck Institute for Marine Microbiology

It was a great surprise when in June 1989 a letter arrived in my mailbox from Klaus Kühn of the Max Planck Institute for Biochemistry in Martinsried, Germany. He was the head of a search committee of the Max Planck Society with the goal to establish a new institute for marine science in the city of Bremen. Klaus Kühn invited me "to assume a leading role in the establishment of the institute". As I learned

only later, Holger Jannasch was an external expert to the committee and had suggested me for this challenging task. My immediate thought was to decline politely. I was 42 years old, my wife and our three children had a happy life in Aarhus, and I enjoyed working with my group of ten young and enthusiastic students and postdocs. I had no ambition at the time to become the head of an entire institute, especially not if that would prevent me from doing my own hands-on research. Yet, my wife thought that I should at least find out what it was all about. So I did. It turned out to be a scientist's dream come true.

Through later negotiations with the Max Planck committee, I developed a concept for the institute together with Fritz Widdel. Our idea was to **establish an institute where biogeochemical studies of processes in the marine environment could inspire and direct cultivation and physiological studies in the laboratory, and vice versa**. We would each lead our own department and take turns in being the managing director. We would also have research groups working specifically with microensors and with the emerging molecular biological techniques. The Max Planck Society offered positions for scientists and other staff members as well as PhD students. They also offered a new institute building and an annual budget until my retirement. It became clear to me that from now on the only limitation to the research of my group would be my own abilities as a scientist and a leader – an intimidating thought. Within a short time, I had to learn to write and speak German. My German language skills were soon tested by a press conference on the occasion of the opening of our new institute (Fig. 2.9).

Figure 2.9

Press conference at the opening of the Max Planck Institute for Marine Microbiology in Bremen, 1992. Left to right: Max Planck president Hans F. Zacher, Bo Barker Jørgensen and Friedrich Widdel. Photo courtesy of the Max Planck Society.

In summer 1992, I moved to Bremen with my family to start the Max Planck Institute for Marine Microbiology. My group from Aarhus joined me together with the group of Fritz Widdel from the University of Munich, and these young people became the founders of the institute. Over the following years, we built

up a permanent group for microsensor research headed by Michael Kühl and later by Dirk de Beer, and we added a third Department for Molecular Ecology with Rudi Amann as the director. Today, a fourth Department for Symbiosis research, headed by Nicole Dubilier, and a number of independent working groups have been established (see www.mpi-bremen.de). From the very start, the institute developed collaborations with colleagues at the University of Bremen, where I was appointed Professor in the Geology Department. I now had to give lectures in German to geoscience students, which in the beginning was a challenge for both me and the students.

As Director and Head of Department, I became responsible for a large group of people and for many different projects. One of the hallmarks of the Max Planck Society, however, is that they expect and encourage their directors to remain active research scientists. So, as we now had the staff and the funding secured, I could plan scientific expeditions that had not been possible before. Our first expedition in 1994 went to the oxygen minimum zone off the coast of Chile where we explored the extensive benthic communities of filamentous sulfur bacteria, *Thioploca* spp., that had been discovered by Victor Ariel Gallardo from the University of Concepción (Gallardo, 1977).

An unexpected highlight from our Chile expedition was the discovery that **these fascinating sulfide oxidisers store nitrate in internal vacuoles and perform anaerobic sulfide oxidation while commuting up and down in their gelatinous tunnels** (Fossing *et al.*, 1995; Jørgensen and Gallardo, 1999) (see Section 4). We later organised an expedition to the oxygen minimum zone off Namibia to search for similar *Thioploca* communities. My PhD student, Heide N. Schulz, found no *Thioploca* there but instead discovered the biggest bacteria yet recorded (Schulz *et al.*, 1999). She named them *Thiomargarita namibiensis*, the “sulfur pearls of Namibia” (Fig. 2.10a). They are a similar kind of sulfide oxidising bacteria as *Thioploca* and *Beggiatoa*, can reach $\frac{3}{4}$ mm in diameter, and can use their large internal nitrate storage to respire for months (Schulz and Jørgensen, 2001). Since that time, many other fascinating types of large sulfur bacteria have been discovered with a similar type of chemolithotrophic metabolism (Salman *et al.*, 2011; Bailey *et al.*, 2011). One of them was kindly named after me (Fig. 2.10b).

Another of my long term ambitions was to study the microbiology and biogeochemistry of arctic sediments. We chartered a Norwegian research vessel, RV “Jan Mayen” of the University of Tromsø, and with Don Canfield as the chief scientist we sailed along the coast of Spitsbergen in the archipelago of Svalbard up to 78 degrees northern latitude. This expedition became the start of a long term research programme at Svalbard, organised by the Max Planck Institute, where a group of 4-8 people would come to Svalbard each summer for a couple of weeks to perform studies that built on results from previous years. Participants included many international colleagues, who also introduced their students and colleagues to this fascinating polar environment. The studies included very diverse aspects of microorganisms and biogeochemical processes

of the sediments, which in more than fifty publications provide an insight into the life and function of psychrophilic microbial communities in the arctic seabed (reviewed by Jørgensen *et al.*, 2020a).

Figure 2.10 (a) A 0.5 mm large sulfur bacterium, *Thiomargarita namibiensis*, next to a fruit fly. (b) *Thiomargarita joergensenii* were found in empty silica frustules of centric diatoms. (a) From Schulz *et al.* (1999) with permission from the American Association for the Advancement of Science; photo courtesy of Heide N. Schulz-Vogt; (b) from Salman *et al.* (2011) with permission from Elsevier.

Another highlight during those years was the discovery by our postdoc, Antje Boetius, of **conspicuous consortia of methane oxidising archaea and sulfate reducing bacteria that in syntrophy catalyse the anaerobic oxidation of methane in the seabed** (Boetius *et al.*, 2000). This discovery triggered an avalanche of new research on microbial methane cycling in the seabed, both at our institute and internationally. Syntrophy and interspecies electron transfer among archaea and bacteria are now well established research fields that continue to deliver a profound and unexpected understanding of microbial life.

In my department, we continued to study the sulfur cycle, and Tim Ferdelman, Don Canfield, Bo Thamdrup, Volker Brüchert and Michael Böttcher were among the scientists leading the way. We acquired our first isotope ratio mass spectrometer (IRMS) with the aim to study sulfur isotope geochemistry. The IRMS became an important tool in our work and it was very satisfying for me to experience, for the first time by our own data, how the combination of stable isotopes and diffusion diagenesis modelling could explain dynamic aspects of the sulfur cycle in the Black Sea (Jørgensen *et al.*, 2004) and in many other sedimentary environments.

In 2002, Steven D'Hondt and I were co-chiefs on ODP Leg 201 in the eastern tropical Pacific with the DV "JOIDES Resolution" (ODP ; Ocean Drilling Program, today the International Ocean Discovery Program, IODP). This was the first drilling expedition devoted to deep biosphere research and we had a large scientific crew on board dominated by microbiologists and geochemists. After

having started my research taking 10 cm deep sediment cores in the shallow waters of Limfjorden, it was truly fascinating to core several hundred metres down into the seabed at a water depth of 5,000 m and recover 40 million year old sediments (Fig. 2.11). Among the microbiologists were R. John Parkes and Barry Cragg who had already long experience in sampling and analyses of ODP cores. The expedition became a breakthrough in deep biosphere research (D'Hondt *et al.*, 2004). Living microorganisms of great diversity were found to the greatest depths and ages cored, and the biogeochemical data provided rich information on their physiological activity and interactions with the environment. Since then, many IODP expeditions have expanded our knowledge of the depth and age limits of deep subsurface microorganisms and their identity and potential functions (e.g., D'Hondt *et al.*, 2009; Inagaki *et al.*, 2015; Hoshino *et al.*, 2020). The experience from Leg 201 and the interest it generated in understanding the deep biosphere led to a new turning point in my scientific career five years later.

Figure 2.11 "Core on deck" on the DV "JOIDES Resolution" drill ship during ODP Leg 201 in the tropical eastern Pacific. Photo courtesy of the IODP.

We also organised expeditions in the Black Sea and gained new experience with stratified and anoxic water masses. Through some stormy Christmas days in December 2001, I led a cruise with the German RV "Meteor" during which we studied the chemistry and microbiology of the water column chemocline at high depth resolution. For the first time we used an on line nutrient analyser coupled to a pump-CTD, developed in collaboration with Siegfried Krüger and other colleagues at the Institute for Baltic Sea Research in Warnemünde, Germany.

Marcel Kuypers, Gaute Lavik and other young colleagues performed experiments with ^{15}N tracers and discovered that ammonium was oxidised anaerobically by anammox bacteria (Kuypers *et al.*, 2003). This inspired us to do similar studies at continental margins in the South Atlantic and Pacific Oceans where anammox was indeed also found to be a major sink for nitrogen in the oxygen minimum zones (Kuypers *et al.*, 2005; Lam *et al.*, 2009). Anammox and the nitrogen cycle in the ocean developed into a very successful research theme of Marcel Kuypers and enabled him to establish his own junior research group at the Max Planck Institute in 2005. A few years later we installed a high resolution secondary ion mass spectrometer (nanoSIMS) which enabled the study of microbial physiology at the single cell level (Musat *et al.*, 2008).

On the background of wonderful colleagues, excellent students, and exciting science in Bremen, it was a very painful and difficult decision in 2007, for family reasons, to leave the Max Planck Institute and move back to Denmark. The Max Planck Society and the Danish National Research Foundation (DNRF) provided outstanding support and co-financed a joint Center of Excellence in Aarhus. I thereby became the head of a new Danish Center for Geomicrobiology while remaining a director at the MPI in Germany. Marcel Kuypers followed me as a director at the institute and head of the Department of Biogeochemistry. I retired from the MPI in 2011 and became a professor at the University of Aarhus, where I continue to work.

2.5 Back in Denmark

At the age of 60, it was quite a challenge to switch country again in 2007 and start a new research group. The only remaining Dane at the MPI in Bremen was Hans Røy who joined me back to Aarhus (Fig. 2.12). We started the search for young scientists and PhD students and gradually established a very enthusiastic and international group.

Based on my experience from the ODP Leg 201 drilling expedition, the objectives of the Center for Geomicrobiology were to explore the deep, sub-seafloor biosphere with respect to the phylogenetic and functional diversity of the microbial communities, their low energy mode of life, and their main biosphere-geosphere interactions. What drove our motivation was, among other things, that the marine deep subsurface is the largest coherent ecosystem on Earth and harbors the largest number of living organisms (Whitman *et al.*, 1998). That was at least the status when we started, until my former PhD student, Jens Kallmeyer, to my disappointment downscaled the estimated number of deep biosphere cells (Kallmeyer *et al.*, 2012). This, however, did not change our most burning question: How was it possible for more than 10^{29} microorganisms to persist in up to hundred million year old marine deposits with hardly any food or energy available.

Figure 2.12 Hans Røy (to the left) operates a gravity corer on the Aarhus University vessel, RV "Aurora", in Aarhus Bay. Photo: Bo Barker Jørgensen.

It was clear right from our start in 2007 that most of the research would be based on growth independent techniques. Sequencing and analysis of extracted DNA and RNA became an important part of the repertoire. However, we also wanted to understand the genetic potential and the physiology of individual organisms in the subsurface and therefore planned to work also with single cell techniques. Our postdoc, Karen G. Lloyd, sent samples to Ramunas Stepanauskas at the recently established facility for fluorescence assisted cell sorting (FACS) at the Bigelow Laboratory for Ocean Sciences in Maine, USA. This led to the first single cell genomes of bacteria and archaea from marine sediment and to the discovery that **some of the most abundant archaea in the seabed are protein degraders** (Lloyd *et al.*, 2013).

Only five years later, it had become rather routine to sequence single amplified genomes (SAG's) of microbial cells from the environment. DNA sequencing technology continued to advance at a breathtaking pace and microbial genomes could now be obtained also by deep metagenomic sequencing of extracted DNA, supported by new binning algorithms. This made it possible to discover novel clades of microorganisms with unknown physiology and unusual phylogeny, such as the fascinating Asgaard archaea that are affiliated with eukaryotes (Zaremba-Niedzwiedzka *et al.*, 2017). It also made it possible to analyse how and where the subsurface microbial communities assemble and to what extent evolution is a prerequisite for their adaptation to life in the energy starved subsurface (Starnawski *et al.*, 2017; Marshall *et al.*, 2018).

It was important not only to identify and quantify the microbial cells but also to understand their activity *in situ*. We used a combination of diffusion reaction modelling of porewater solutes and sensitive radiotracer experiments to quantify processes such as sulfate reduction. Comparison with the total number of cells or the number of potentially sulfate reducing microorganisms then enabled rough calculations of the mean rate of carbon metabolism *per cell*. By assuming a certain low growth yield of the organisms, also their mean growth rate could be calculated. For sulfate reducing microorganisms, this led to estimated generation times of years to thousands of years (Hoehler and Jørgensen, 2013). It was possible to test the estimates by independent calculations of microbial biomass turnover. For this, we used the **built in molecular clock in dead microbial biomass (necromass) based on the spontaneous, but extremely slow racemisation between the L- and D-forms of amino acids**, such as aspartic acid (Lomstein *et al.*, 2012).

The buried microbial communities thus appear to be turning over and not just to persist in a dormant or inactive state. They live in balance with the traces of food available to the cells. Even the main fermentation products, such as volatile fatty acids at micromolar concentration, can take a year or longer to turn over (Glombitza *et al.*, 2015). The turnover of cells may be driven by virus induced mortality, as both lytic and lysogenic viruses are abundant and active in the subsurface (Engelhardt *et al.*, 2015; Cai *et al.*, 2019). Bacterial endospores are also abundant (Wörmer *et al.*, 2019), yet they are not permanent resting stages but very slowly decay (Hubert *et al.*, 2009; Rosa de Rezende *et al.*, 2012). These and many other interesting aspects of life in the sub-seafloor were the topic of a series of international workshops, which we have organised together with international colleagues, Jan P. Amend, Tori M. Hoehler, Mark A. Lever and Victoria J. Orphan. The title of these inspiring meetings was “*Microbial Life under Extreme Energy Limitation*”, and they resulted in several publications, including the reviews by Hoehler and Jørgensen (2013) and Lever *et al.* (2015).

Through all the studies of the sulfur cycle, I now realise that I have myself completed a full cycle over the half century since I started as a master's student in this field. I now again try to understand process rates and pathways of the sulfur cycle and ask questions that should have been resolved decades ago. How do physical isotope exchange between sulfur species or back reaction in biochemical pathways affect our interpretation of radioisotope experiments and stable isotope fractionation? How do these processes affect the ^{35}S method by the measurement of sulfate reduction rates? How important is concurrent sulfate reduction and sulfide oxidation for the apparent discrepancy between net and gross sulfate reduction? How do chemical and biochemical processes interact to catalyse sulfide oxidation all the way to sulfate in anoxic sediments? What are the most important pathways and intermediates during this sulfide oxidation? *Etc., etc.* So many important questions still remain open and I wish I could start all over again, knowing what we know today.

3. SULFATE REDUCTION

The seabed is the largest anaerobic bioreactor on Earth. Ocean water contains 28 mM sulfate, which can penetrate metres down into the seabed. Dissimilatory sulfate reduction to sulfide is therefore globally the most important terminal pathway of organic matter mineralisation in the anoxic seabed. The sulfate reducing microorganisms feed on small organic molecules and H_2 from which they transfer electrons to reduce sulfate to sulfide. The process of dissimilatory sulfate reduction thereby transforms the sulfur to its most reduced form, H_2S , which drives the sulfur cycle in marine sediments.

Sulfate-reducing microorganisms

The diversity and distribution of sulfate reducing microorganisms (SRM) in marine sediments have been analysed from the 16S rRNA of predominantly sulfate reducing groups (e.g., Amann *et al.*, 1990), from functional marker genes encoding for key enzymes of microbial sulfate reduction (Wagner *et al.*, 2005), and from metagenomes and single cell amplified genomes (Wasmund *et al.*, 2016). In particular, genes encoding for dissimilatory (bi)sulfite reductase (*dsrAB*) are used for this purpose, based on a comprehensive sequence database and phylogeny (Müller *et al.*, 2014a). The SRM comprise a diverse phylogenetic group with the capacity to catabolise a wide spectrum of fermentation products (e.g., Rabus *et al.*, 2015). Many of the predominant SRM belong to the deltaproteobacteria, *Desulfobacteraceae* and *Desulfobulbaceae*, while in subsurface sediments other taxa such as the phyla Firmicutes, Chloroflexi and Atribacteria are more common (Wasmund *et al.*, 2017). Genomic data have revealed that many other bacterial and archaeal phyla have the capacity for dissimilatory sulfate or sulfite reduction and that these mostly belong to uncultured groups only distantly related to the SRM studied in laboratory culture (Anantharaman *et al.*, 2018).

This is the reason why I start this sulfur cycle story with sulfate reduction. Another reason is that this is also where I started myself. As a young student I thought, or at least hoped, it would be straightforward to measure this well known microbial process in the laboratory using ^{35}S -radiolabelled sulfate as a sensitive tracer. As it turned out, the main problem was not to perform the experiments but to do so without affecting the activity of the bacteria. When it came to the interpretation of the results there were further complications and pitfalls. There still are. That is partly what this section is about.

3.1 ^{35}S Sulfate Reduction Rate (SRR) Measurements

Radiolabelled sulfate, $^{35}SO_4^{2-}$, is ideally suited to determine the rate of sulfate reduction in marine sediments. The ^{35}S is a soft beta emitter with a half-life of 87 days and decays into the stable, harmless isotope, ^{35}Cl . To minimise disturbance of the sediment, I developed a “whole core injection technique” by which a few μL ^{35}S sulfate solution was injected through small, sealed holes in the core

liner into intact sediment cores that were then incubated at *in situ* temperature for some hours. In this way, the original chemical and microbial zonation in the sediment could, hopefully, be maintained (Jørgensen, 1978a) (Fig. 3.1).

Advantage of the ^{35}S method

The main advantage of using ^{35}S sulfate instead of measuring a change in sulfate concentration is the much higher sensitivity of the radiotracer technique. For each measurement, a few hundred kBq ^{35}S sulfate is typically injected (1 Bq (Becquerel) = 1 radioactive decay *per second*). A minute fraction of this ^{35}S sulfate is converted into ^{35}S sulfide by the sulfate reducing bacteria. It is possible in the scintillation counter to detect 6 decays *per minute*, *i.e.* 1/10 of a Bq (Røy *et al.*, 2014). This is 1 millionth of the injected ^{35}S and, thereby, 1 millionth of the porewater sulfate. If the decrease in sulfate concentration were determined instead by ion chromatography, the detection limit would typically be 1 %, *i.e.* a hundredth of the sulfate concentration. Thus, **the radiotracer can enhance the sensitivity of experimental sulfate reduction rate measurements by 10,000 fold.**

Figure 3.1 The whole core $^{35}\text{S}\text{-SO}_4^{2-}$ injection method to measure microbial sulfate reduction in marine sediments. The drawing illustrates how one of my students conceived the principle of this technique. From anonymous.

Some improvements of the ^{35}S sulfate reduction method have been necessary over the years and some complications are still not fully resolved (*e.g.*, Fossing and Jørgensen, 1989; King, 2001; Kallmeyer *et al.*, 2004; Røy *et al.*, 2014). Originally, the ^{35}S sulfide was driven off by HCl, which dissolves acid volatile sulfides (AVS) but does not attack pyrite or elemental sulfur. Robert W. Howarth used aqua regia to extract ^{35}S from pyrite and carbon disulfide to extract ^{35}S from elemental sulfur (Howarth, 1979). He found the pyrite to hold much more ^{35}S than AVS in salt marsh sediments. An acidic chromium reduction technique for pyrite, developed by Zhabina and Volkov (1978), was used by the PhD project of Joseph T. Westrich for the extraction of non-acid volatile, reduced ^{35}S (Chromium Reducible Sulfur; CRS) (Westrich, 1983). Following Don Canfield's careful test of the method, it became standard for the extraction of reduced, inorganic sulfur from sediments (Canfield *et al.*, 1986). The use of aqua regia extraction was abandoned as it led to an overestimate

of reduced ^{35}S , possibly due to contamination from remaining traces of radiolabelled sulfate.

Bob Howarth visited me in Aarhus and we tested the improved CRS method of pyrite reduction. Indeed, the pyrite and the elemental sulfur fractions were also radioactive after a few hours of incubation with ^{35}S sulfate (Fig. 3.2). We concluded that my earlier measurements had missed on average 19 % of the sulfate reduction activity in Limfjorden sediments (Howarth and Jørgensen, 1984). King *et al.* (1985) made a similar comparison in salt marsh sediments and concluded that 50 % of the reduced ^{35}S was recovered in the elemental sulfur and pyrite fractions. In other sediments, this ^{35}S fraction in the CRS varied between 15 % and 70 %, lowest in sediments with high sulfate reduction rates and high sulfide concentration (Thode-Andersen and Jørgensen, 1989). Thus, ^{35}S -SRR measurements made up until the early 1980's had missed a fraction of the reduced ^{35}S and thereby systematically underestimated ^{35}S sulfate reduction.

Figure 3.2

^{35}S sulfate reduction rate measurement in Limfjorden sediment with separate extraction of acid volatile sulfide ($\text{H}_2\text{S} + \text{FeS}$), elemental sulfur (S^0), and pyrite (FeS_2). In this sulfidic sediment only a very small part of the reduced ^{35}S was recovered in the pyrite fraction. Redrawn from Howarth and Jørgensen (1984).

While these early studies of ^{35}S incorporation into pyrite during short term incubation experiments concluded that this was due to rapid pyrite formation (Fig. 3.2), later studies were more cautious about this conclusion (e.g., Thode-Andersen and Jørgensen, 1989). We began to realise that physical isotope exchange between the reduced sulfur pools modifies the ^{35}S distribution among the reduced sulfur species, independent of their rate of formation (see Section 4.4).

3.2 Problems with the ^{35}S Method

It is critical by the ^{35}S method to remember that **each measurement is an experiment with living microorganisms that respond immediately, and perhaps irreversibly, to changes in their environment**, e.g., by transitional warming of cold sediment, incidental introduction of oxygen, loss of sulfide, or contamination by surface sediment when coring deeper subsurface sediments (Røy *et al.*, 2014). Another problem is that data often scatter strongly, which is mainly due to natural heterogeneity of the sediment and not due to the method (Jørgensen, 1978a). One solution to natural heterogeneity is to do more measurements (Jørgensen *et al.*, 2019b).

In order to overcome potential artifacts due to core retrieval and sediment manipulation, we developed a free-falling benthic lander equipped to perform pre-programmed sediment coring, ^{35}S sulfate injection, *in situ* incubation, core retrieval, and finally ascent and onboard collection of incubated cores (Greeff *et al.*, 1998). We compared *in situ* and on board ^{35}S -SRR measurements in Black Sea sediments from the shelf to 2,000 m water depth. The results showed similar *in situ* and on board rates beneath the deep anoxic water column, meaning that **hydrostatic pressure up to 200 bar did not have a detectable effect on the metabolic rate** (Weber *et al.*, 2001). This is in contrast to the oxygen uptake of marine sediments by which shipboard data exceed *in situ* data already at depths beneath 600 m (Glud, 2008).

The sulfate reducing microorganisms discriminate slightly against the radioactive isotope, ^{35}S , relative to the natural, light isotope, ^{32}S . Based on a published 4 % isotope fractionation between ^{34}S and ^{32}S in Baltic Sea sediments (Hartmann and Nielsen, 1969), I originally suggested a 6 % isotope fractionation between ^{35}S and ^{32}S in Danish coastal sediments (Jørgensen, 1978a). The $^{34}\text{S}/^{32}\text{S}$ fractionation factor has a theoretical range of 0-7 % (Brunner and Bernasconi, 2005; Wing and Halevy, 2014), due to a **partial back reaction during individual steps of the sulfate reduction pathway** (see Section 8). A potential back reaction of ^{35}S -labelled sulfide was first suggested by Trudinger and Chambers (1973) who spiked a pure culture of sulfate reducing bacteria with ^{35}S sulfide and observed a slow formation of ^{35}S sulfate. A similar experiment by Eckert *et al.* (2011) suggested the back flux of ^{35}S sulfide to ^{35}S sulfate to be 4-10 % of the forward reaction. The highest back reaction is expected by sulfate reduction close to thermodynamic equilibrium (Wing and Halevy, 2014), e.g., by the anaerobic oxidation of methane (AOM) by sulfate. In an AOM enrichment culture, Holler

et al. (2011) calculated a ^{35}S back flux of 5-13 %. Considering that SRR in marine sediments vary over many orders of magnitude, a maximum error of 5-13 % is a small offset. Furthermore, the back reaction of ^{35}S is relatively smaller than of ^{34}S in sediments because the produced ^{35}S sulfide undergoes rapid isotope exchange with reduced sulfur pools in the sediment and is thereby protected against back reaction.

A stronger effect is caused by a **concurrent re-oxidation of the formed ^{35}S sulfide back to sulfate during the incubation experiment**. Since the re-oxidation takes place from a sulfide pool in which the ^{35}S is diluted out, relatively less of the ^{35}S sulfide is re-oxidised than of the bulk sulfide. This is particularly the case by short incubations, which may therefore give a more correct picture of the true, gross rate of sulfate reduction. With prolonged incubation time, the calculated rate drops towards a lower net SRR as a balance between sulfate reduction and sulfide oxidation (Fossing, 1995) (Fig. 3.3). This effect is largest in oxidised surface sediment with a very small free H_2S pool, as shown in Figure 3.3. It is of little importance in the sulfidic sediment below (Moeslund *et al.*, 1994; Findlay *et al.*, 2020). The re-oxidation is not an artifact of the ^{35}S -SRR method but is a real process that remains undetected by the modelling of porewater sulfate.

Figure 3.3

Experimental measurement of sulfate reduction rates (SRR) in oxidised surface sediment (top 0-2 cm) from Skagerrak using ^{35}S sulfate and very short incubation times of 1 to 30 minutes. The calculated initial rate corresponds to the gross SRR while rates at later time points decrease due to concurrent re-oxidation of the produced ^{35}S sulfide. During the first 10 min, the calculated SRR therefore approaches a lower net SRR that reflects the steady state balance between sulfate reduction and sulfide oxidation. Redrawn from Fossing (1995).

3.3 Reactive Transport Modelling

Robert A. Berner developed the first mathematical models to determine sulfate reduction rates. Such models became widely used after Bob Berner moved to Yale University and established a young and dynamic geochemistry group known as FOAM (Friends of Anoxic Mud) (Goldhaber *et al.*, 1977). More complex, non-steady state radial diffusion modelling was developed to describe the ventilation by tube-dwelling deposit feeders living in the sediment (*e.g.*, Aller, 1978). The focus on sediment reworking and bioirrigation by macrofauna was critical for the understanding of mass transfer in the upper sediment layers where this non-local transport of solutes strongly exceeds molecular diffusion. In their review, Goldhaber and Kaplan (1975) pointed out that **bioirrigation and high sulfate reduction rates near the sediment surface are critical for the proper modelling of this process.**

Due to sediment mixing and bioirrigation in the uppermost 10-20 cm of inner shelf sediments, high sulfate reduction rates in this zone are hardly detectable from the porewater profiles of sulfate or of key products of anaerobic mineralisation, such as ammonium or bicarbonate. The problem is illustrated by the cumulative ^{35}S -SRR depth distributions in Figure 3.4. Water depths here range from 15 to 200 m and sedimentation rates from 0.3 to 3 mm yr⁻¹. A depth of 15 cm depth indicates the zone of bioturbation in these sediments (*e.g.*, Chen *et al.*, 2017; Deng *et al.*, 2020). Thus, **between 10 % and 90 % (mostly >50 %) of the entire sulfate reduction in each sediment column is located within the bioturbated zone.** Of the two stations where less than half of the sulfate reduction took place in the top 0-15 cm, the deep Station K12 in central Skagerrak had predominance of Fe(III) reduction, while Station K9 had very deep bioirrigation and no drop in sulfate concentration down to 60 cm.

The FOAM group combined diagenetic modelling with sediment incubation experiments by which the gradual depletion of sulfate was monitored (Goldhaber *et al.*, 1977). Highly inspired by this work, and with helpful advice from Bob Berner, I combined ^{35}S -SRR experiments with modelling to see whether I could reconcile the two approaches. Berner (1964) assumed first order kinetics by the degradation of a bulk pool of organic matter, G, which led to an exponential decrease in SRR with depth in the sediment. I found from my ^{35}S -SRR data that the rate changed with depth in Limfjorden sediments according to a power law rather than to an exponential function but that the ^{35}S -SRR data could also be modelled as the sum of several exponential functions, corresponding to several organic matter pools, each with its own decay constant (Jørgensen, 1978b). One of Bob Berner's PhD students, Joe Westrich, performed ^{35}S -SRR measurements in Long Island Sound sediments and described these by a multi-G model (Westrich and Berner, 1984). The ^{35}S -SRR data of Joe Westrich (Berner and Westrich, 1985) were later shown by Middelburg (1989) to also fit perfectly to a power law relationship with depth.

Figure 3.4

Relative cumulative rates of sulfate reduction (SRR) in marine shelf sediments calculated from the sediment surface and down to the bottom of the sulfatic zone. Stations are located on the Baltic Sea – North Sea transition: K3 – Langeland Belt, K2 – Great Belt, K12 – Skagerrak, K9 – Kattegat, M5 and M27 – Aarhus Bay. Note logarithmic depth scale. Redrawn from Jørgensen *et al.* (1990, 2019b).

In order to provide a more realistic representation of organic matter mineralisation, Boudreau and Ruddick (1991) developed a reactive continuum model by which the composite reactivity of organic matter was described by a Gamma distribution. The reactive continuum model had two free parameters and provided an equally good fit to the experimental organic matter decay experiments of Joe Westrich as did a 3-G model. In 1995, Bernard P. Boudreau spent a sabbatical with me in Bremen, where he wrote the monography on “*Diagenetic Models and Their Implementation*” (Boudreau, 1997). A common quality of these models is that they are based on a mechanistic principle of organic matter degradation: the degradation rates of all the components, being just a few or an infinite number, are proportional to their concentration. The power law, in contrast, has no *a priori* mechanistic underpinning.

Since these early studies, one might expect that one of the models had become the preferred choice in sediment biogeochemistry, but that is not the case. Diverse 1-G, multi-G, reactive continuum and power law models are used by different researchers to estimate the depth and age dependent rate of organic matter mineralisation in marine sediments (reviewed in Arndt *et al.*, 2013; Stolpovsky *et al.*, 2018). It is thereby assumed that **the rate of organic carbon mineralisation decreases monotonously with age of the sediment, regardless of**

whether the terminal step is metal reduction, sulfate reduction or methanogenesis. While this assumption has been taken for granted in many diagenetic modelling studies (*e.g.*, Berner, 1980; Soetaert *et al.*, 1996; Van Cappellen and Wang, 1996; Burdige *et al.*, 2016), it was only recently confirmed directly by a combination of ^{35}S and ^{14}C radiotracer experiments (Beulig *et al.*, 2018).

3.4 Reconciling Modelled and ^{35}S Measured Sulfate Reduction Rates

During the past half century, both ^{35}S experiments and reaction transport modelling have been widely used, but mostly without comparing the two approaches. It is therefore relevant to ask, which method provides the more accurate rates of sulfate reduction. This is particularly important considering that sulfate reduction accounts for the major part of anaerobic organic carbon degradation in the global seabed (Canfield *et al.*, 2005). The answer is not simple, however, as the following two examples will show.

3.4.1 Aarhus Bay coastal sediment

Our most detailed comparison of ^{35}S measurements and modelling of sulfate reduction were done on our doorstep, in Aarhus Bay (Fig. 1.2). We studied a 7–10 m thick, organic-rich, Holocene mud that has experienced a constant sedimentation rate since the last glaciation, over the past 9,000 years (Flury *et al.*, 2016). Twelve individual gravity cores were taken along a 400 m long seismo-acoustic transect. A typical example of the sulfate gradients is shown in Figure 3.5a. The sulfate gradient showed a slight concave down curvature, reaching a sulfate methane transition (SMT) at 300 cm depth. Using the diffusion reaction modelling tool, PROFILE, of Berg *et al.* (1998), we calculated a theoretical distribution of sulfate reduction rates that provided an optimal fit to the sulfate data (Fig. 3.5a). The modelled rates were very low and were focused towards the sediment surface and the bottom of the sulfatic zone (Fig. 3.5b) (Dale *et al.*, 2019). In the same cores, a total of 125 measurements of ^{35}S sulfate reduction rates were done. In a double log plot, these rates showed a linear depth relationship, *i.e.* a power law distribution (Fig. 3.6a). The data showed significant scatter, but due to the large number of measurements the general trend and the 95 % confidence limits were well defined.

The power law function is not intuitive. The data show that sulfate reduction is active throughout the sulfatic zone, but at rates that drop 10,000 fold with depth from 10 cm to the SMT at 300 cm (Fig. 3.6a). This means that rates in the middle and lower sulfatic zone are extremely low, almost negligible, compared to the activity near the top. Only at the SMT is there a small addition of organic substrate (methane). Since this methane kicks in at the bottom of the sulfatic zone, where the organoclastic sulfate reduction is very low, it has a relatively strong effect and tends to generate quasi-linear sulfate profiles. Methane thereby appears to contribute strongly to the overall sulfate reduction, yet it accounted for only 1 % (Flury *et al.*, 2016) (Figs. 3.5, 3.6; see also Section 7).

Figure 3.5

Data from Aarhus Bay, Station M27. **(a)** Sulfate concentration in the pore-water (data points) and sulfate distribution simulated by the PROFILE model (curve). **(b)** Sulfate reduction rates (SRR) calculated using PROFILE. **(c)** Depth distribution of the bioirrigation coefficient that could reconcile measured and modelled sulfate reduction rates. Redrawn from Jørgensen *et al.* (2019b) and Dale *et al.* (2019).

Figure 3.6

Data from Aarhus Bay, Station M27. **(a)** Sulfate reduction rates (SRR), measured by the ³⁵S method shown in a double logarithmic plot *versus* depth. The data are fitted by a skewness-corrected linear regression (red line) that indicates a power law relationship. The surrounding dashed lines show the 95 % confidence interval for the regression. **(b)** The power law of SRR *versus* depth is used here to calculate the cumulative SRR from the sediment surface to different depths (green curve). The SRR in the top 0-10 cm was calculated from rate data specific for that interval. Redrawn from Jørgensen *et al.* (2019b).

The ^{35}S -SRR exceeded the diffusion modelled rates by more than 100 fold at 10 cm depth in the sediment (compare Figs. 3.5b and 3.6a). If sulfate transport down into the sediment had been by diffusion only, the sulfate would have been gone at 20 cm depth. To reconcile the measured and modelled rates, excess solute transport due to the pumping activity of burrowing fauna (bioirrigation) had to be introduced in a more elaborate reactive transport model. Figure 3.5c shows the bioirrigation coefficients required to make the Berg modelling match the ^{35}S measured SRR. The sulfate flux by bioirrigation across the sediment-water interface was 20 times the diffusive flux (Dale *et al.*, 2019), and bioirrigation was required down to 10-20 cm depth. Although bioirrigation was not directly quantified, this depth matched the distribution of benthic macrofauna and their burrow structures (Chen *et al.*, 2017). Bioirrigation introduced not only sulfate but also oxygen, which caused a re-oxidation of produced sulfide back to sulfate in the upper 10 cm (Findlay *et al.*, 2020). This provided an additional source of sulfate, which reduced the bioirrigation needed to balance ^{35}S rates and modelled rates.

A similar comparison between ^{35}S measured and modelled SRR data in Limfjorden sediments indicated that bioirrigation was significant down to 10-20 cm depth and that it finally tapered out only at 50-75 cm depth (Jørgensen and Parkes, 2010). Such deep bioirrigation is difficult to check without careful studies of the burrowing fauna. However, a deep and intensive bioirrigation down to 60-70 cm depth, probably due to large king ragworms (polychaete *Alitta virens*), was documented in sediments of the nearby northern Kattegat (Jørgensen *et al.*, 2019b; Station K9 in Fig. 3.4). In Aarhus Bay, sediment reworking by benthic macrofauna was strong down to 6 cm and weaker down to 14 cm depth according to the distribution of the natural radionuclides, ^{210}Pb and ^{137}Cs (Chen *et al.*, 2017). These examples show that there is delivery of both particulate organic carbon and sulfate to feed sulfate reduction in the upper sulfatic zone where most sulfate reduction takes place. A cumulative plot of sulfate reduction rates in Aarhus Bay shows that 50 % of the entire sulfate reduction takes place in the uppermost 10 cm and 90 % takes place in the uppermost 30 cm (Fig. 3.6b).

3.4.2 SE Atlantic continental slope

An earlier attempt to reconcile ^{35}S measured and modelled SRR was made for the upper continental slope off Southwest Africa. Detailed porewater profiles of sulfate and other key ions were made in both surface cores and deep gravity cores. Some sulfate profiles were linear from near the sediment surface to the SMT. Diffusion reaction modelling of these profiles concluded that all sulfate reduction was due to methane oxidation at the SMT (Niewöhner *et al.*, 1998). Yet, experimental ^{35}S -SRR measurements by Fossing *et al.* (2000) indicated that most sulfate reduction took place in the upper 30 cm where there was little or no drop in sulfate concentration (Fig. 3.7a). Only 15 % of the entire sulfate reduction was measured at the SMT (Fig. 3.7b). Importantly, sulfate reduction was detected throughout the sulfatic zone with a linear sulfate gradient, at gradually lower rates with depth. Thus, the relatively **high SRR near the sediment surface had little**

impact on the sulfate gradient due to bioirrigation and the short diffusion distance, while SRR at the SMT had a strong impact on the entire, quasi-linear sulfate profile due to the long diffusion distance.

Figure 3.7

Sulfate reduction in sediment from the tropical east Atlantic off Namibia (Station GeoB 3703, 1373 m water depth). **(a)** Sulfate and methane distributions. Shallow sulfate data are from a multicorer (light blue) while deep data are from a gravity corer (dark blue). Methane data (red) that are expectedly compromised due to outgassing are marked (+). **(b)** Cumulative sulfate reduction rates from the sediment surface down to the SMT as % of the total depth integrated SRR. Redrawn from Fossing *et al.* (2000).

There are many examples of such an apparent discrepancy between modelled and ^{35}S measured SRR. Yet, under long term steady state conditions, the degradation rate of buried organic matter should decrease with depth throughout the sulfatic and the methanic zones according to the same reactive continuum or power law (Beulig *et al.*, 2018). If a linear sulfate gradient is interpreted as no degradation in the sulfatic zone, it is difficult to explain why degradation should start in the methanic zone.

Other sulfate profiles from the South Atlantic study showed a striking S-shape with little or no drop in sulfate concentration down to several metres depth and then a steep drop that reached background levels at an SMT at 7–15 m depth (Schulz *et al.*, 1994). Fossing *et al.* (2000) suggested that this might be due to deep bioirrigation. They found open tubes with faecal pellets of burrowing fauna

down to two metres depth in the sediment and suggested that active bioirrigation or passive ventilation of abandoned tubes might take place to this depth. While such deep bioirrigation could possibly reconcile ^{35}S measured and modelled SRR, it is doubtful whether bioirrigation could explain sulfate profiles in other cores taken in the region with no drop in sulfate concentration down to 3–8 m depth and with a steep drop in sulfate below that depth (Schulz *et al.*, 1994).

As an alternative explanation, Zabel and Schulz (2001) suggested that the S-shape is the result of down slope transport several hundred years ago of sliding sediment blocks with retained stratigraphy. This interpretation was supported by a recent study from the same deep sea fan off the Zaire River by Croguennec *et al.* (2017) who found a sulfate-free, methane-rich sediment section sandwiched in between two sulfatic zones. Similar observations of down slope mass transport within the past hundreds of years have been made in other deep sea fans with high sedimentation rates, *e.g.*, in the Bay of Bengal (Hong *et al.*, 2014).

3.4.3 Measured versus modelled SRR

There are several reasons for the discrepancies between the ^{35}S method and modelling. Net consumption of sulfate down through the sulfatic zone should cause sulfate profiles to be concave down, according to the classical model by Berner (1980). By the analysis of a comprehensive database of porewater sulfate distributions at 740 sites of the global seabed (Egger *et al.*, 2018), it was striking that **only about one third of all sulfate profiles are concave down, while one third are linear and one third are concave up**. It would be interesting to understand why this is the case, especially because the modelling literature tends to have a preference for the concave down examples (*e.g.*, Bowles *et al.*, 2014).

Modelling of sulfate reduction generally assumes steady state, which may not be reached, in particular not in the bioturbated surface zone. Bioirrigation masks the highest rates of sulfate reduction in the upper sulfatic zone. Sediment compaction during burial drives porewater advection and causes decreasing porosity, increasing tortuosity, and decreasing diffusion coefficients with depth. These mechanisms counteract the concave down shape and enhance the linearity of sulfate profiles (Berner, 1975; Jørgensen, 1978b; Dickens, 2001). So does the re-oxidation of the produced sulfide back to sulfate, the role of which depends on the introduction of oxygen by bioirrigation and the balance between the reactivity of buried organic matter and of buried iron(III) (see Section 4.2). A (cryptic) re-oxidation of sulfide by iron(III) is most pronounced near the sediment surface and most obvious below the sulfatic zone, but it takes place throughout the sulfatic zone (Pellerin *et al.*, 2018; Findlay *et al.*, 2020). In conclusion, these **transport and reaction processes need to be constrained accurately before sulfate reduction rates can be inferred correctly by modelling of sulfate profiles**.

The power law trend of organic matter mineralisation was found to continue from the sulfatic zone and into the methanic zone (Beulig *et al.*, 2018). **Extrapolation of the depth trend in measured ^{35}S sulfate reduction rates down into**

the methanic zone should therefore provide a means to check the ^{35}S -SRR data. Since most of the produced methane diffuses up to the SMT (Jørgensen *et al.*, 2019b) the extrapolated mineralisation rate beneath the SMT should largely match the methane flux. **Several studies have confirmed such a match** within the statistical uncertainty given by the extrapolation of a power law (Jørgensen and Parkes, 2010; Flury *et al.*, 2016; Jørgensen *et al.*, 2019b; Section 7.1).

3.5 Cryptic Sulfur Cycling

Cryptic element cycling has become a widely studied concept during the past decade. Canfield *et al.* (2010) used the term to describe sulfate reduction at barely detectable rates in the oxygen minimum zone off the coast of Chile. In sediment biogeochemistry, “cryptic cycling” was used to indicate processes that are not evident from the porewater chemistry and are not detected by reactive transport modelling (Jørgensen *et al.*, 2019a). **Cryptic cycling may reflect the difference between gross and net processes, for example between total sulfate reduction and sulfate reduction minus sulfide re-oxidation to sulfate** (as discussed in Section 3.3).

The term “cryptic sulfur cycling” in marine sediments was first used to describe sulfate turnover in the methanic zone where sulfate had supposedly been depleted to an insignificant background concentration (Holmkvist *et al.*, 2011). Since this sulfate could not have diffused down from the sulfatic zone, it was apparently produced within the methanic zone through sulfide oxidation by deeply buried iron(III). The extremely low rates of sulfate reduction in this zone detected by the ^{35}S method indicated a sulfate turnover time of a few hundred years. Although the main, terminal reaction product between sulfide and iron(III) is pyrite, the redox stoichiometry of the net process invariably generates excess reducing power, with or without the concurrent production of sulfate (Holmkvist *et al.*, 2011). We suggested that the net reaction without sulfate formation could be:

A reaction with sulfate formation could be:

In both examples, the excess reducing power was assumed to be H_2 . Within the methanic zone, this H_2 could be used by methanogens to reduce CO_2 to CH_4 , which then diffuses up to the SMT and becomes oxidised with sulfate. In that way, excess reducing power would be exported and the reaction of sulfide and iron(III) to pyrite could maintain redox balance, even with a small production of sulfate:

It remained a nagging question for us whether the low sulfate concentrations measured in the methanic zone could be an artifact, either from sulfide oxidation during sediment handling (Brunner *et al.*, 2016) or from seawater contamination during coring and sampling of the sediment (Pellerin *et al.*, 2018). The latter turned out to be the main risk. Pellerin *et al.* (2018) found that the *in situ* sulfate concentrations in the methanic zone were only around 10 μM , which appears to be a background concentration, balanced between slow sulfate production and consumption. They calculated that the Gibbs free energy, ΔG_r , for sulfate reduction was here only -12 to -18 kJ mol^{-1} sulfate, which is near the suggested minimum free energy yield of -19 kJ mol^{-1} required for microbial sulfate reduction (Hoehler *et al.*, 2001). Thus, **a residual sulfate concentration of ca. 10 μM is probably energetically controlled by the sulfate reducing microorganisms.**

3.6 Substrate Kinetics of Sulfate Reduction

The ^{35}S -SRR method enables many studies of the environmental parameters that control the rates of organic matter degradation with sulfate reduction as the terminal step. I will here discuss the control by sulfate concentration and, in Section 3.7, the control by temperature.

During 1979-80, I ran experiments on the sulfate-dependent kinetics of sulfate reduction in sediments from Aarhus Bay. I first removed most of the sulfate with sulfate-free, anoxic, artificial seawater and then packed the sediment gas tight to let the bacteria deplete the remaining sulfate. After a couple of months, sulfate was down to a non-detectable background concentration, and I performed two types of kinetic experiments: a) “initial velocity” experiments with addition of different concentrations of sulfate to individual subsamples followed by short term ^{35}S -SRR measurements, and b) “progress curve” experiments whereby a continuous time course of ^{35}S sulfate depletion was monitored. The initial velocity experiments had sulfate concentrations in the 0.2-24 mM range and could be fitted with a Michaelis-Menten saturation curve: $\text{SRR} = 4.6 \times (\text{S}/(\text{S} + 1200)) \mu\text{M SO}_4^{2-} \text{ hr}^{-1}$, where S is the sulfate concentration in μM (Fig. 3.8a). The apparent half-saturation constant, K_m , by which the rate is half of the maximum rate, was 1.2 mM SO_4^{2-} .

I then spiked the sulfate depleted sediment with a known concentration of sulfate, including $^{35}\text{SO}_4^{2-}$, and followed the gradual depletion of ^{35}S sulfate over time. From the slope of the depletion curve, the rate of sulfate reduction could be calculated as a function of the remaining sulfate concentration. Repeated experiments with spikes ranging from 10 to 50 μM also showed Michaelis-Menten kinetics, this time with an apparent K_m value of 1.3 μM (Fig. 3.8c,d). Thus, the apparent K_m dropped by 1,000 fold at very low sulfate concentration. By reproducing these results several times, I concluded that, at very low sulfate concentration either sulfate reducing bacteria with high SO_4^{2-} affinity became active or predominant, or the existing community of sulfate reducers induced a high affinity SO_4^{2-} uptake. The results clearly showed that high affinity uptake would

only be detected in experiments when very low sulfate concentrations were used. Unfortunately, the manuscript was rejected and the data were never published. The reviewers argued that the low μM to sub- μM concentrations of sulfate had not been measured directly, a requirement that we were unable to fulfil at the time. Instead, I had used the ^{35}S kinetic experiments to indirectly determine the sulfate concentration.

Figure 3.8

Kinetic experiments with sulfate-dependent ^{35}S -SRR in coastal marine sediments. (a) and (b) two initial velocity experiments; (c) and (d) a progress curve experiment. (a), (c) and (d) show my unpublished data; (b) is redrawn from Boudreau and Westrich (1984).

A few years later, Boudreau and Westrich (1984) published results very similar to my low affinity data (Fig. 3.8b). They used the same initial velocity method with sulfate concentrations in the mM range. They found an apparent K_m of 1.6 mM SO_4^{2-} and, rightly, concluded that they reported the first systematic investigation of how SRR in marine sediment is controlled by sulfate concentration. Since then, many other studies have been done of the sulfate-dependent kinetics of SRR in marine sediments, finding apparent K_m values generally ranging from 0.1 to 2 mM (e.g., Roychoudhury *et al.*, 2003; Pallud and Van Cappellen, 2006). The studies have not included experiments in the very low concentration range, however. Furthermore, the experiments were generally done with sediments that had always been exposed to high sulfate in nature so that the microbial communities were not naturally adapted to low sulfate. Marine

sediments with very low sulfate concentration occur universally at and below the SMT, where the sulfate concentration drops to *ca.* 10 μM , but the very low microbial activity here would make kinetic experiments in the laboratory difficult.

It took many years before I returned to the study of sulfate reduction kinetics. Then, Hans Røy and I motivated a PhD student, Irene H. Tarpgaard, to repeat and improve my “initial velocity” and “progress curve” experiments with Aarhus Bay sediments. Irene’s results were nearly identical to mine from nearly thirty years earlier (Tarpgaard *et al.*, 2011), but this time using a highly sensitive ion chromatographic sulfate analysis with a detection limit of 0.15 μM SO_4^{2-} in marine porewater. Tarpgaard discovered **both low affinity and high affinity SRR within the same batch of sediment, the result depending on the type of experiment and the working range of sulfate concentrations**. The kinetic data could be described by dual Michaelis-Menten kinetics:

$$V_1 = V_{\max 1} (C/(K_{m1} + C)) \quad (3.4a)$$

$$V_2 = V_{\max 2} (C/(K_{m2} + C)) \quad (3.4b)$$

where V is the rate of sulfate reduction, C is the concentration of sulfate, and K_m is the apparent half-saturation concentration. In the studied sediment, the maximum rate, $V_{\max 1}$, was 0.9 $\mu\text{M hr}^{-1}$, and the affinity, K_{m1} , was 300 μM , for the low affinity sulfate reduction. For the high affinity sulfate reduction, the $V_{\max 2}$ was 0.5 $\mu\text{M hr}^{-1}$ and K_{m2} was 2.5 μM . Thus, the sulfate affinity increased 100 fold (*i.e.* the K_m dropped 100 fold) when shifting from a high to a low concentration range.

We did not know at the time whether the dual sulfate affinities were due to different populations of sulfate reducers or due to regulation of the sulfate uptake in individual bacteria. To answer this question we studied a pure culture of the marine sulfate reducer, *Desulfobacterium autotrophicum*, and, indeed, this organism could switch from low affinity ($K_m = 150 \mu\text{M}$) to high affinity ($K_m = 10 \mu\text{M}$) sulfate uptake when the ambient sulfate concentration dropped below 500 μM (Tarpgaard *et al.*, 2017) (Fig. 3.9):

$$\text{Low affinity SRR} = 19 (C/(150 + C)) \text{ fmol } \text{SO}_4^{2-} \text{ cell}^{-1} \text{ d}^{-1} \quad (3.5a)$$

$$\text{High affinity SRR} = 14 (C/(10 + C)) \text{ fmol } \text{SO}_4^{2-} \text{ cell}^{-1} \text{ d}^{-1} \quad (3.5b)$$

By this switch, the organism also switched the expression of genes for different sulfate transporters. When offered an excess of organic substrate, the marine sulfate reducers in Aarhus Bay sediment could even deplete the sulfate concentration below our detection limit of 0.15 μM SO_4^{2-} (Tarpgaard *et al.*, 2017). Calculations of the energetic control of sulfate reduction in sediment amended with organic substrate showed that the theoretical threshold concentration, below which sulfate reduction changed from kinetic to thermodynamic limitation, was only about 1 μM SO_4^{2-} . This is 10 fold below the *in situ* residual sulfate concentration of *ca.* 10 μM (Section 3.5).

Thus, **sulfate reducing communities in marine sediments may turn on their high affinity sulfate transporters, but only by low sulfate concentrations**. Some marine sulfate reducers isolated under high sulfate concentrations,

such as *Desulfovibrio salexigens* or *Desulfobacter postgatei*, apparently do not regulate their sulfate transporters in response to low external sulfate concentration (Ingvorsen and Jørgensen, 1984; Ingvorsen *et al.*, 1984). In contrast, *Desulfobacterium autotrophicum* does regulate its sulfate transporters (Marietou *et al.*, 2021). A recent survey of sulfate reducer genomes detected a hitherto overlooked group of CysZ-type sulfate transporters that may only be expressed at low sulfate concentration (Marietou *et al.*, 2018). Chemostat cultures of *Desulfobacterium autotrophicum* were shown to maintain a background concentration of 3–5 μM sulfate (Marietou *et al.*, 2021), comparable to the background sulfate concentration of *ca.* 10 μM in the methanic zone of marine sediments (Pellerin *et al.*, 2018). When excess sulfate was provided in the chemostat cultures, the steady state electron donor concentration of lactate or acetate was $<0.2 \mu\text{M}$. This is 10–100 fold below their concentration in the sulfatic zone of coastal marine sediments (Glombitza *et al.*, 2019) and shows that **we have still not identified what controls these substrate concentrations in the environment.**

Figure 3.9

Kinetic experiments with *Desulfobacterium autotrophicum* showing how the mean sulfate reduction rates *per cell* depend on the sulfate concentration. The graph combines results of initial velocity (blue) and progress curve (red) experiments. The data were fitted with two Michaelis-Menten curves (see text). The sulfate scale is logarithmic to cover the large concentration range, which makes the Michaelis-Menten curves look atypical. Redrawn from Tarpgaard *et al.* (2017).

As is often the case, microorganisms are more complex and adaptable than immediately expected from experiments such as these. When sulfate reducing bacteria are starved of sulfate and then offered different sulfate concentrations they immediately take up an excess of sulfate and concentrate it intracellularly. Heribert Cypionka and his group at the University of Oldenburg did detailed

physiological studies on pure cultures to unravel these mechanisms. They found that marine sulfate reducing bacteria actively take up sulfate by a symport with Na^+ ions. At very low external sulfate concentrations they may accumulate sulfate to a concentration more than 1,000 fold higher inside the cell than outside, whereas at high external sulfate only little accumulation takes place (Warthmann and Cypionka, 1990; Stahlmann *et al.*, 1991).

3.7 Temperature Control of Sulfate Reduction

Temperature regulation of microorganisms

Temperature has a fundamental impact on the catabolic rates of microorganisms in the environment. Temperature is also a major controlling variable for the physiological selection of microbial communities and for their differentiation and evolution. Microorganisms can be generally characterised according to the minimum ($T_{\min}$), optimum (T_{opt}) and maximum ($T_{\max}$) temperatures of their growth rate or metabolic rate. A general, yet somewhat arbitrary, classification is thereby made for psychrophilic ($T_{\min} < 0\text{ }^{\circ}\text{C}$, $T_{\text{opt}} \leq 15\text{ }^{\circ}\text{C}$, $T_{\max} \leq 20\text{ }^{\circ}\text{C}$), psychrotolerant ($T_{\min} \leq 0\text{ }^{\circ}\text{C}$, $T_{\text{opt}} \leq 25\text{ }^{\circ}\text{C}$, $T_{\max} \leq 35\text{ }^{\circ}\text{C}$), mesophilic ($T_{\text{opt}} \sim 25\text{--}40\text{ }^{\circ}\text{C}$, $T_{\max} \sim 35\text{--}45\text{ }^{\circ}\text{C}$), and thermophilic ($T_{\text{opt}} > 45\text{ }^{\circ}\text{C}$) microorganisms (Morita, 1975). Temperature is easy to measure, and it is straightforward to perform experiments on the temperature regulation of processes such as sulfate reduction. It is therefore tempting to extrapolate the results of such experiments to geographic regions or to long term climate developments. Such extrapolations have a high degree of uncertainty, however, and may be quite misleading, as we and others have gradually learned to appreciate.

Short term experiments on the temperature dependence of sulfate reduction in marine sediment provide one kind of information on how the community is adapted to the ambient temperature regime. Figure 3.10 shows examples from coastal marine sediments from three different latitudes. In each case, the “optimum temperature”, T_{opt} , *i.e.* the temperature of highest respiration rate, is well above the seasonal temperature range of the site. It is striking from these and many other such temperature curves that the T_{opt} is about $10\text{ }^{\circ}\text{C}$ above the highest summer temperature in the temperate and tropical regions, whereas the T_{opt} is more than $20\text{ }^{\circ}\text{C}$ higher than the constantly low seasonal temperature in polar regions (Fig. 3.10) (*e.g.*, Robador *et al.*, 2015). A further property of arctic sulfate reducing communities is their ability to endure freezing during winter, as was shown experimentally by repeated freezing and thawing of sediment (Sawicka *et al.*, 2010).

The temperature curves for arctic sediments (Fig. 3.10a) are distinguished by a relatively high activity at low temperature. The SRR at $0\text{ }^{\circ}\text{C}$ was 10–20 % of the SRR at T_{opt} in different arctic sediments while it was only 2–4 % in temperate sediments (Robador *et al.*, 2015). The authors concluded that the arctic communities of sulfate reducers might be a mixture of psychrophilic, psychrotolerant and mesophilic organisms. The question remained, however, why psychrophilic

sulfate reducing bacteria were not more predominant in the permanently cold sediments, and why the community was apparently not more specialised to the low ambient temperature?

Figure 3.10 Temperature control of sulfate reduction rates (SRR) in coastal marine sediments from three climate regions **(a)** arctic in the Smeerenburgfjorden, Svalbard, **(b)** temperate in the Wadden Sea, and **(c)** tropical in the South China Sea. The optimum temperature of SRR (red) is compared to the seasonal temperature range at the sampling site (green). Redrawn from Robador *et al.* (2015).

We addressed these questions in the mid-1990's during our first expedition to Svalbard (see Section 2.4). It was a challenging project for my PhD student, Christian Knoblauch, to isolate psychrophilic sulfate reducers for the first time and to study their physiology (Knoblauch and Jørgensen, 1999; Knoblauch *et al.*, 1999). The most extreme of the psychrophiles had growth optimum at 7 $^{\circ}\text{C}$ and died already at 10 $^{\circ}\text{C}$. Growth rates at 0 $^{\circ}\text{C}$ were 25-40 % of the optimum growth rates (Knoblauch *et al.*, 1999). This showed that **there are indeed psychrophilic sulfate reducers in the arctic sediments but they are not detected by short term SRR experiments.**

When the incubation experiments with arctic sediments were continued for several days, the apparent T_{opt} for sulfate reduction dropped by up to 10 $^{\circ}\text{C}$ (Finke and Jørgensen, 2008; Scholze *et al.*, 2021). Figure 3.11a shows temperature curves for short term SRR experiments with different sediments from Svalbard and Greenland, which had apparent T_{opt} of 20-30 $^{\circ}\text{C}$. Scholze *et al.* (2021) made longer term experiments with the same sediments and showed that the T_{opt} for growth was only 10-20 $^{\circ}\text{C}$ (Fig. 3.11b). None of the communities survived above 25 $^{\circ}\text{C}$ after several days of incubation. Even more striking, the T_{opt} for growth yield was 0-10 $^{\circ}\text{C}$ (Fig. 3.11c). The growth yield shows how much the cells have grown in g biomass carbon *per* mol of sulfate respired. A high growth yield is expectedly a more important competitive factor under stable marine sediment conditions than the potential for fast growth. Thus, in spite of the high T_{opt} for short term SRR, the experiments revealed a truly psychrophilic physiology of the entire arctic sediment community (Scholze *et al.*, 2021).

Figure 3.11 Temperature regulation of sulfate reducing communities in arctic sediments from Svalbard (Krossfjorden, Kongsfjorden and Smeerenburgfjorden) and Northeast Greenland (Young Sound). (a) Short term sulfate reduction rates, (b) specific growth rates, (c) growth yields. Redrawn from Scholze *et al.* (2021).

It is generally observed in temperate climates that the microbial degradation of organic matter is relatively fast in summer and slows down in winter. This, however, does not imply that degradation rates in cold polar seas are relatively

lower than in warmer regions. We have compared SRR data from comparable coastal sediments around Svalbard and around Denmark and found rather similar rates of anaerobic degradation. There is also no indication that the community size of sulfate reducing microorganisms is higher in the Arctic as a compensation for lower cell specific catabolic rates at low temperature. The psychrophilic bacteria that predominate in cold sediments have higher cell specific catabolic rates at low temperature than the mesophiles that predominate in temperate regions have at the same low temperature (Knoblauch *et al.*, 1999).

While sulfate reduction is the terminal step in the microbial food chain of anaerobic degradation, the microorganisms responsible for the upstream steps have similar temperature regulations of their catabolic rates. Extracellular enzymes involved in the hydrolytic degradation of polysaccharides are clearly cold adapted in arctic sediments with T_{opt} in the range of 15–20 °C and with relatively high activity at 0 °C (Arnosti and Jørgensen, 2003). In this respect, hydrolytic degradation and the terminal steps are closely coupled with a relatively short turnover time of the intermediate, dissolved polysaccharides of a few days to weeks (Arnosti and Jørgensen, 2006).

When performing the temperature experiments shown in Figure 3.10, it was striking that the SRR dropped to near zero at 40–45 °C and then increased again at 50 °C. We showed that the increase was due to thermophilic sulfate reducing bacteria that grew up rapidly with a T_{opt} of 55–60 °C (Fig. 3.12a). A pasteurisation of the sediment for one hour at 80 °C before the temperature experiment showed that all psychrophilic and mesophilic SRM were killed (Fig. 3.12b). Since the thermophiles survived pasteurisation, we concluded that **thermophiles were present as endospores in the sediment**. Such “thermospores” belonging to the Firmicutes (*e.g.*, *Desulfotomaculum* spp.) occur widespread in the seabed, even in the high Arctic where the *in situ* temperature is always near 0 °C (Isaksen *et al.*, 1994; Hubert *et al.*, 2009). The thermophiles appear unable to grow at the low *in situ* temperature and their spores gradually disappeared with depth in the sediment with a half-life of several hundred years or more, probably due to spontaneous germination (Rosa de Rezende *et al.*, 2012). We suspect that they originate from hot environments, *e.g.*, deep subsurface sediments or hydrothermal systems, from which they are transported widely by ocean currents. As they do not grow and multiply in the cold ocean, they are not affected by environmental selection. They can therefore be used as **tracers of passive microbial dispersal by ocean currents** (Müller *et al.*, 2014b) and even as tracers of historical changes in source strengths and dispersal patterns (Hanson *et al.*, 2019).

Sulfate reducing microorganisms thrive in hydrothermal sediments and in the hot deep subsurface. During several expeditions to the hydrothermal vent fields in the Guaymas Basin, Gulf of California, we studied the temperature regulation of sulfate reduction using ^{35}S . The temperature gradient was very steep and could range from 3 °C at the sediment surface to >120 °C at 30 cm depth (Weber *et al.*, 2001). Communities within this gradient included mesophiles with T_{opt} of around 35 °C, thermophiles with T_{opt} of around 60 °C, and hyperthermophiles

with T_{opt} at 80–90 °C. In a study published in 1992, we detected sulfate reduction in these sediments also at 105 °C (Jørgensen *et al.*, 1992). Unfortunately, this temperature record could not be reproduced in our later studies. Indeed, we tried to reproduce the results during two subsequent expeditions to the Guaymas Basin without success and are therefore uncertain about those earlier results (Weber and Jørgensen, 2002).

Figure 3.12 Temperature dependence of ^{35}S -SRR in Svalbard sediment incubated for 24 hours. **(a)** Both psychrophilic-mesophilic sulfate reducers and germinated endospores of thermophilic sulfate reducers are active. **(b)** Pasteurisation of the sediment killed the vegetative cells of psychrophiles and mesophiles but left the endospores of thermophiles unaffected. Redrawn from Hubert *et al.* (2009).

The oxidation of sulfide is equally important for the sulfur cycle as the reduction of sulfate. It is particularly important in coastal sediments because of the potential toxic effect of H_2S to the benthic fauna or to fish. Sulfide inhibits cytochrome c oxidase in the mitochondrial respiratory chain and thereby blocks the production of ATP. Sulfide is therefore a serious environmental threat to the marine biota in coastal upwelling systems with high phytoplankton productivity or in basins with reduced water circulation where oxygen depletion does not ensure complete sulfide oxidation in the seabed (Diaz and Rosenberg, 1995; Vaquer-Sunyer and Duarte, 2008). By seasonal anoxia, iron oxides in the surface sediment may buffer against sulfide release for a certain period (Seitaj *et al.*, 2015), but with prolonged anoxia sulfide may be released to the water column as a diffusive flux or facilitated by methane gas ebullition and may result in extensive fish kills (Levin *et al.*, 2009).

In bioturbated near surface sediments with an influx of oxygen and nitrate, and with the presence of metal oxides, sulfide oxidation goes all the way to sulfate, thereby closing the sulfur cycle. In subsurface sediments the oxidant is mostly buried Fe(III) and the oxidation may be only partial and lead to iron monosulfide (FeS), pyrite (FeS_2), elemental sulfur (S^0) and other intermediates. This section will discuss some of the experimental approaches used to study sulfide oxidation in the seabed and highlight differences between microbial and geochemical processes in surface and subsurface sediments.

4.1 The Dynamic Surface Sediment

I will start with the organic-rich coastal surface sediments where the sulfur cycle is most dynamic and where sulfide oxidation can be studied experimentally. This is indeed where much of the global marine sulfide oxidation takes place. The chemical gradients may indicate which processes predominate at which depth, yet they reflect only the net outcome of concurrent reduction, oxidation and disproportionation. In order to unravel the individual processes, experimental approaches are needed to supplement biogeochemical profiling and modelling.

During my PhD studies of Limfjorden sediments, I found that much of the sulfate reduction occurred in the top 0–5 cm where the sediment appeared brown and oxidised due to predominance of iron oxides (see Figs. 6.7a, 6.8). Those early studies were done before the introduction of microelectrodes in aquatic sciences and I therefore measured redox potentials, E_h , using a naked platinum electrode as an indication of oxidised *versus* reduced conditions (*cf.* Fenchel, 1969). These measurements showed that sulfate reduction took place also at positive E_h , where I had expected conditions to be too oxidised for sulfate reducers to be active (Jørgensen, 1977b).

Five years later, Niels Peter Revsbech had constructed microsensors for the measurement of oxygen, sulfide and pH, which enabled measurements of the chemical zonation in sediments at 50-100 μm depth resolution (Revsbech *et al.*, 1983; Revsbech and Jørgensen, 1986). We learned that the brown and oxidised surface zone was anoxic and that the oxic zone was only a mm thin skin draping the sediment topography (Jørgensen and Revsbech, 1985). This absence of O_2 in most of the oxidised zone explained why 100 μm size sulfidic sediment pellets could exist as reduced microniches with active sulfate reduction in the oxidised sediment. My model calculations had shown that this should not be possible if the sediment had been oxic (Jørgensen, 1977b). Rather than being an oxic environment with reduced microniches, the oxidised surface sediment is an anoxic environment with occasional oxic conditions along the burrow walls of ventilating fauna (Jørgensen *et al.*, 2005; Meysman *et al.*, 2006). The reduced microniches with active sulfate reduction are here of mm to cm size rather than of sub-mm size. This was clearly illustrated for a salt marsh sediment where the 2 dimensional distribution of free sulfide was mapped using a planar H_2S sensor (Yin *et al.*, 2017). As shown in Figure 4.1, free H_2S at up to $>100 \mu\text{M}$ concentration occurred in cm-sized zones away from the oxidised walls of worm tubes.

Figure 4.1

The upper 10 cm of a salt marsh sediment contained in a box core with burrowing ragworms (polychaete *Nereis succinea*). (a) Photograph through the transparent wall showing worms and worm tubes with lighter gray, oxidised walls. (b) Distribution of free sulfide in the same sediment detected by a 2 D H_2S sensor. From Yin *et al.* (2017) with permission from Elsevier; courtesy of Robert C. Aller.

The bioirrigated surface sediment is thus a heterogeneous and variable zone where multiple mineralisation processes co-occur, thereby maintaining a general biogeochemical zonation but without apparent thermodynamic control of the vertical distribution of the processes. The predominant oxidants in the anoxic but oxidised sediment are, in the general sequence of their consumption, nitrate, manganese(IV) and iron(III). Based on detailed geochemical data in deep sea sediments, Froelich *et al.* (1979) combined these

processes in the term “suboxic” in order to distinguish the zone from the “oxic” zone above and the “anoxic” zone below where sulfate reduction and methanogenesis predominate. In coastal marine sediments, the suboxic zone corresponds roughly to the brown oxidised surface sediment beneath the thin oxic zone and above the grayish black sulfidic zone (*cf.*, Fig. 6.7a).

Diagenetic terminology revisited

Whereas the term “suboxic” continues to be used, Canfield and Thamdrup (2009) rightly argued that the terminology is not logical or systematic and that it should rather be abandoned. They suggested a more consistent nomenclature for the biogeochemical zonation of sediments based on the distribution of the predominant electron acceptor and the predominant respiration process (see Section 6.3). They divided the suboxic zone into the “nitrogenous”, “manganous” and “ferruginous” zones according to the predominant electron acceptor. While such a specification of the nomenclature is very useful, I think we still need a combined name for the “suboxic” zone, which includes the processes of nitrate, manganese(IV) and iron(III) reduction. In lack of a good name, I will here call it the “anoxic, oxidised zone”.

For the deeper zones, Canfield and Thamdrup (2009) used the term “sulfidic zone” for the sediment interval where sulfate reduction dominates and “methanic zone” for the sediment below where methanogenesis dominates. However, since the sediment generally continues to be sulfide-rich deep down into the methanic zone, “sulfidic” is not an unequivocal term to indicate only the sulfate-containing zone. I therefore suggest the term “sulfatic zone” instead (see Fig. 6.3).

4.2 Sulfide Oxidation Experiments

In bioturbated sediments with burrowing fauna, particle reworking and burrow ventilation provide important mechanisms of oxidant transport and subsurface sulfide oxidation. It is therefore a true experimental challenge to determine the rates and pathways of sulfide oxidation under such conditions of heterogeneity and variability. Incubation experiments amended with oxidants such as manganese oxide or iron oxides have, in the past, shown their potential role in sulfide oxidation (*e.g.*, Aller and Rude, 1988), but not the *in situ* rates or pathways of oxidation. It was therefore tempting to use ^{35}S -labelled sulfide as a tracer to determine the rate of sulfide oxidation. This turned out to be more difficult than we had anticipated.

Starting in 1986, Henrik Fossing worked with me as a PhD student. It was our ambition to use ^{35}S -labelled sulfide as a tracer to determine the complex sulfide oxidation pathway and its rate in marine sediment. The early results were very confusing, until we realised the importance of physical isotope exchange between several of the reduced sulfur species. To be honest, what we discovered were isotope exchange reactions that had been studied and described in chemical systems already in the 1930's, when radioisotopes first became available to science (*e.g.*, Voge, 1939), but it was apparently the first time that sulfur isotope

exchange was considered in studies of marine sediments. A reason for this was probably that the different sulfur species, such as H_2S , S^0 , FeS , FeS_2 and SO_4^{2-} , have distinct isotope compositions, which indicates that an effective isotope equilibration does not occur between them.

By a physical isotope exchange, individual atoms of sulfur move randomly between the analytically defined pools of sulfide, polysulfide, elemental sulfur and iron sulfide. This exchange of atoms is purely statistical and may approach an equilibrium distribution of the isotopes. The exchange is independent of which biogeochemical reactions take place at the same time and is therefore not detectable by chemical analysis. However, **if ^{35}S sulfide is introduced, the ^{35}S will soon move from sulfide to other reduced sulfur pools, and the change in radioactivity distribution will deceptively look like a chemical or biological transformation.** The exchange between sulfide and elemental sulfur is particularly fast and operates through polysulfides within minutes (Fossing and Jørgensen, 1990a).

In marine sediment with low sulfide concentrations, we found that an added radiotracer of elemental sulfur, $^{35}\text{S}^0$, was exchanged into FeS_2 and FeS , whereas in sediment with high sulfide concentrations it was exchanged into H_2S and FeS , both within a few tens of minutes (Fossing *et al.*, 1992). An example of this is shown in Figure 4.2, where the distribution of ^{35}S in different sulfur pools is followed for three days. The radiotracer rapidly exchanged from S^0 to H_2S and FeS with a small transitional exchange in and out of FeS_2 . It should be stressed that there is practically no net reaction taking place between the sulfur pools. The concentrations of H_2S , S^0 , FeS and FeS_2 remain nearly constant within the first five hours while the ^{35}S isotope moves around. The information from the radioisotope distribution is therefore very deceiving.

During the prolonged experiment, it was evident that sulfate slowly became radioactive (Fig. 4.2). Although a slight exchange between sulfate and sulfide may be possible due to the reversibility of the enzymatic pathway of sulfate reduction (Wing and Halevy, 2014), it is known from stable isotope geochemistry of marine sediments that sulfate does not readily exchange sulfur isotopes with the reduced sulfur species. The formation of ^{35}S sulfate in our experiment at the expense of ^{35}S sulfide is therefore a true sulfide oxidation to sulfate. By calculating the specific radioactivity of H_2S and the rate of radioactive sulfate formation from 6 hours and onwards, when the fast, initial isotope exchange had relaxed, the rate of sulfate formation can indeed be estimated (Findlay *et al.*, 2020). The rate, $270 \text{ nmol cm}^{-3} \text{ d}^{-1}$, corresponds to half of the ongoing net sulfate reduction to sulfide, $530 \text{ nmol cm}^{-3} \text{ d}^{-1}$, calculated from the rate of increase in total H_2S (data not shown). Thus, the net rate of sulfate reduction underestimated the gross sulfate reduction rate of $800 \text{ nmol cm}^{-3} \text{ d}^{-1}$ by about a third due to the concurrent re-oxidation of sulfide back to sulfate in the anoxic sediment. As discussed in Section 3.4.1, concurrent sulfate reduction and sulfide oxidation cause a discrepancy between the gross rates (only sulfate reduction) and the net rates (sulfate reduction minus sulfide oxidation to sulfate) of sulfate reduction. In sulfidic sediments, the concurrent

sulfide oxidation has only a small effect on short term ^{35}S radiotracer measurements of sulfate reduction, whereas in surface sediments without free sulfide it can have a large effect (Section 3.2).

Figure 4.2 Experiment with ^{35}S sulfide oxidation in a highly sulfidic marine sediment from Kysing Fjord. At time zero, ^{35}S -labelled S^0 was added and the distribution of ^{35}S in the different sulfur pools was traced for three days. Note the break in the time axis between 4 and 6 hours. The slope of the blue line was used to calculate the rate ^{35}S sulfate formation after 6 hours. Redrawn from Fossing *et al.* (1992).

The possibility to quantify ^{35}S sulfide oxidation in spite of fast isotope exchange motivated us to repeat the earlier experiments of Fossing *et al.* (1992), with careful avoidance of air exposure, sediment dilution, or other potential artifacts (Findlay *et al.*, 2020). Parallel sediment incubations with ^{35}S sulfate and ^{35}S sulfide as radiotracers showed how sulfide oxidation in the uppermost 0-4 cm balanced the high sulfate reduction rates but then dropped with depth to gradually become undetectable (Fig. 4.3). These results show that **sulfide is effectively recycled in the anoxic surface sediment where mineralisation rates are highest and pyrite formation is fastest**. Similar radiotracer studies with ^{35}S -labelled elemental sulfur would be more difficult, as the added $^{35}\text{S}^0$ does not fully equilibrate with the natural S^0 pool.

Looking back, I wonder how many similar experiments have been done in different laboratories over the years using radiolabelled sulfide as a tracer, experiments that have remained unpublished because they were compromised by the rapid (and extremely annoying) isotopic exchange between free sulfide

and the different reduced sulfur species. New ideas and approaches are clearly needed to experimentally determine the mechanisms and *in situ* rates of sulfide oxidation and, in particular, the interactions between the involved chemical and microbiological processes in marine sediments.

Figure 4.3 Rates of concurrent sulfate reduction to sulfide and sulfide oxidation to sulfate in a sulfidic coastal marine sediment. Experiments were done using ³⁵S sulfate and ³⁵S sulfide, respectively. (a) Two cm depth resolution. (b) 5-10 cm depth resolution. Redrawn from Findlay *et al.* (2020).

Concurrent sulfate reduction and sulfide oxidation are generally not detectable by geochemical analyses or modelling of porewater chemistry and therefore constitute a poorly constrained, but potentially very active sulfur cycle. The formation of sulfate by sulfide re-oxidation is hardly detectable by sulfate analyses in short term experiments with normal marine sediments where sulfate concentrations are several tens of mM. However, in experiments with sulfate depleted marine sediment, for example sediment used to determine the half-saturation constant for sulfate reduction, it may become evident.

We performed such a time course experiment with ³⁵S-labelled sulfate (Fig. 4.4) (Tarpgaard *et al.*, 2011). Sulfate was consumed at a rate fitted by a Michaelis-Menten curve with a maximum rate of 0.4 μM SO₄²⁻ hr⁻¹ and an apparent half-saturation constant of 2 μM SO₄²⁻. After 70 hours, sulfate was depleted and all the added ³⁵S sulfate was reduced to ³⁵S sulfide. We then spiked

the sediment with non-radioactive sulfate and saw how this sulfate pool started to become radioactive. The results show that, concurrent with the rapid net consumption of sulfate, there was also a cryptic re-oxidation of sulfide to sulfate. Thus, the total gross sulfate reduction rate was indeed higher than the net rate of sulfate depletion. As the specific radioactivity of the oxidising sulfide was not determined, we could not calculate how much higher.

Figure 4.4 Time course experiment showing the reduction kinetics of ^{35}S -labelled sulfate in a marine sediment. Data points (yellow) are fitted by a Michaelis-Menten kinetic equation. After complete sulfate depletion at 70 hr, a pool of sulfate was added at 90 hr, which revealed subsequent formation of radiolabelled sulfate and, thereby, concurrent sulfate reduction to sulfide and sulfide oxidation to sulfate. Redrawn from Tarpgaard *et al.* (2011).

In conclusion, **due to a cryptic re-oxidation of produced sulfide back to sulfate, there is a clear discrepancy between gross and net sulfate reduction rates.** In near surface marine sediments, where rates are often highest, the gross rates may be 3 to 5 fold higher than the net rates (Moeslund *et al.*, 1994; Fossing, 1995). In the top several centimetres, net rates may even be negligible in spite of high gross rates (Findlay *et al.*, 2020). There may be a similar discrepancy deeper down in the sediment, but rates are too slow to perform similar laboratory experiments. The reactivity of buried Fe(III) drops strongly with depth, but so does the reactivity of buried organic matter and thereby the rate of sulfate reduction. Depending on which reactivity drops faster, the relative role of cryptic re-oxidation of sulfide to sulfate may decrease or increase with depth. As an example, the SRR dropped 1,000 fold from the sediment surface to 3 m depth in Aarhus Bay sediment. A similar drop in reactive Fe(III) would hardly be detectable from

the analysis of iron geochemistry. However, a very slow, but continued reactivity of deeply buried Fe(III) is apparent in many sediments from the drop in sulfide concentration once sulfate has been consumed and sulfide production stops. Down in the methanic zone, free sulfide may be depleted and Fe^{2+} start to accumulate as a sign of continued Fe(III) reduction (Fig. 5.2).

4.3 Sulfide Oxidation Mechanisms

Oxidised manganese (Mn(IV)) and iron (Fe(III)) minerals are quantitatively the main oxidants for sulfide in subsurface sediments (Aller, 1994; Raiswell and Canfield, 1996; Findlay *et al.*, 2020). Manganese oxides are stronger oxidants than iron oxides and react correspondingly faster with sulfide (Yao and Millero, 1993, 1996). Also iron sulfide and pyrite are oxidised to sulfate or to intermediate oxidation states by Mn(IV) (Schippers and Jørgensen, 2001, 2002). Sulfide oxidation by Mn(IV) involves a two electron transfer with S^0 as the immediate oxidation product, but the microbial oxidation of S^0 in sediment may go completely to sulfate (Aller and Rude, 1988; Böttcher and Thamdrup, 2001). Bacteria of the genus *Sulfurimonas* have been shown to grow autotrophically while oxidising sulfide to sulfate with MnO_2 (Henkel *et al.*, 2019).

Fe(III) is a less effective oxidant of sulfide. Products are S^0 and thiosulfate, while formation of sulfate was not observed in experiments by Pyzik and Sommer (1981) or King (1990). Iron minerals such as ferrihydrite, goethite and hematite react with sulfide on timescales ranging from minutes to many thousands of years (Canfield, 1989; Canfield *et al.*, 1992). The iron speciation in marine sediments is very heterogeneous and even the “reactive iron” fractions have a reactivity that ranges over a few orders of magnitude (Poulton *et al.*, 2004). The chemical reaction mechanisms between sulfide and iron minerals are complex and have been the objective of many studies that will not be discussed here (*e.g.*, Rickard and Luther, 2007; Rickard, 2012a).

Chemical reaction rates of sulfide in anoxic marine sediments depend very much on the mineral form and the reactive surface area of the metal oxides. Poulton *et al.* (2004) therefore developed an operational scheme for the extraction and analysis of iron species that could be related to the reactivity of iron (oxy) hydroxide minerals towards dissolved sulfide. It is not clear from the scheme to what extent the sulfide reactivity depends on the presence of bacteria. Bonneville *et al.* (2004), Hyacinthe *et al.* (2006) and Laufer *et al.* (2020) compared experimental bioassays for the microbiological extraction efficiency of iron minerals in marine sediments with chemical extraction techniques. Microbial extractions of coastal marine sediments amended with a culture of iron reducing *Shewanella* bacteria were compared to chemical extractions using a buffered ascorbate-citrate solution (pH 7.5). The two approaches provided similar trends in the distribution of reactivity, with extraction efficiencies decreasing in the sequence of ferrihydrite – lepidocrocite – goethite – hematite. Less iron was extracted using the bioassay, probably due to different iron dissolution mechanisms and different targeted iron fractions.

4.4 Big Sulfur Bacteria

The large colourless sulfide oxidising bacteria that live at the surface of very sulfidic sediments are highly complex and deeply fascinating microorganisms. They are generally characterised by abundant light refracting sulfur globules in the cells, which reveal their sulfide oxidising activity. *Beggiatoa* are best known from the white biofilms that drape the surface of highly sulfidic mud and decaying macroalgae or seagrasses. They live at the sharp interface between oxygen, penetrating down through the diffusive boundary layer, and sulfide, penetrating up from the sulfidic sediment below (Jørgensen and Revsbech, 1983) (Fig. 4.5a). In this way the filaments maximise the diffusive fluxes of both oxygen and sulfide needed for their energy metabolism. The organisms are microaerophilic and tolerate neither high oxygen nor high sulfide but live here in a microzone of very low oxygen and sulfide concentration. Such *Beggiatoa* biofilms occur worldwide. The largest species found at hydrothermal vents reach a diameter of 120 μm and a length of 1 cm (Nelson *et al.*, 1989; McKay *et al.*, 2012).

Figure 4.5

Distribution of filamentous sulfur bacteria, *Beggiatoa* spp., and of oxygen, sulfide and pH in two coastal marine sediments. (a) Due to a high sulfide flux, the *Beggiatoa* form a white sheet on the sediment surface where O_2 and H_2S

meet. **(b)** With a lower sulfide flux, the *Beggiatoa* live in the oxidised zone where oxygen and sulfide are not detectable. In both cases, the oxic-anoxic interface is characterised by a pH minimum due to acid formation during sulfide or sulfur oxidation. Redrawn from **(a)** Jørgensen and Revsbech (1983); **(b)** Preisler *et al.* (2007).

The discovery of the diffusive O₂ and H₂S gradients in which *Beggiatoa* live inspired Douglas C. Nelson, then a postdoc with Holger W. Jannasch in Woods Hole, to successfully grow marine *Beggiatoa* in artificial O₂-H₂S gradients in agar tubes (Nelson and Jannasch, 1983). Using microelectrodes he could calculate the sulfide oxidation and growth rates of the bacteria and show that they were chemolithotrophs and grew purely autotrophically with CO₂ at a high growth yield of 8.4 g dry biomass *per* mol H₂S oxidised (Nelson *et al.*, 1986).

Surprisingly, **most *Beggiatoa* in marine sediments occur scattered in the oxidised, anoxic zone where neither O₂ nor H₂S is detectable** (Jørgensen, 1977c; Musmann *et al.*, 2003; Preisler *et al.*, 2007) (Fig. 4.5b). When I first observed this, the distribution could not be explained from the known physiology of *Beggiatoa*. The explanation emerged unexpectedly during a German-Chilean research expedition in 1994. Our aim was to study the benthic, filamentous *Thioploca* in the oxygen minimum zone on the Chilean shelf. Massive communities of these *Beggiatoa*-related sulfur bacteria were known under the popular name “spaghetti bacteria” (Gallardo, 1977). The several cm long filaments live in gelatinous sheaths and glide vertically to either stretch up into the overlying, oxygen-free, but nitrate-rich seawater or to hide in the sulfidic mud below (Huettel *et al.*, 1996; Schulz *et al.*, 1996) (Fig. 4.6). When squeezing pore fluid out of the *Thioploca* inhabited sediment, it became clear that these microorganisms are full of nitrate and that this holds the secret to their physiology (Thamdrup and Canfield, 1996). From the anoxic seawater, they take up nitrate and store it in intracellular vacuoles at a concentration up to several hundred mM (Fossing *et al.*, 1995). Down in the sulfidic mud they oxidise the ambient sulfide to sulfate and store elemental sulfur as an intermediate. They commute up and down and bring with them both the electron donor (S⁰) and electron acceptor (NO₃⁻) for their energy metabolism (Jørgensen and Gallardo, 1999). By their chemolithotrophic metabolism, the stored sulfur is oxidised to sulfate while the nitrate is reduced to ammonium (Otte *et al.*, 1999).

In 1997 we organised an expedition to the oxygen minimum zone off Namibia where Heide N. Schulz discovered large communities of *Thiomargarita namibiensis*. These sulfide oxidising bacteria form chains of spherical cells up to 0.7 mm in diameter and appeared as shiny little strings of pearls in the black mud (Schulz *et al.*, 1999) (Fig. 4.7a). This discovery of “the world’s largest bacteria” attracted much attention and they were even presented on a Namibian stamp (Fig. 4.7b). About 98 % of the cell volume consists of a nitrate-filled vacuole surrounded by a thin film of cytoplasm. They are non-motile and can endure nitrate starvation for months (Winkel *et al.*, 2016). Similar to *Beggiatoa*, *Thiomargarita* are able to respire both with the internally stored nitrate and with ambient oxygen (Schulz and de Beer, 2002).

Figure 4.6

Filamentous sulfur bacteria, *Thioploca* spp., from anoxic shelf sediment off central Chile. **(a)** A dense community of several cm long, protruding filaments make the sediment surface look like the fur coat of a sheep. **(b)** Photomicrograph of a bundle of *Thioploca* stretching out of their common sheath. Photos: Bo Barker Jørgensen.

Figure 4.7 *Thiomargarita* spp., giant sulfur bacteria that grow in sulfidic sediment as a string of pearls surrounded by a common gelatinous sheath. (a) String of 300 µm wide cells positioned next to a human hair for comparison of size. Photo courtesy of Heide N. Schulz-Vogt. (b) Namibian stamp displaying *Thiomargarita namibiensis*, the “sulfur pearls of Namibia”.

Soon after this discovery, similarly **high nitrate accumulation to more than 100 mM was also detected in marine *Beggiatoa*** (McHatton *et al.*, 1996). Sulfur globules and nitrate vacuoles are thus a general characteristic of the large marine *Beggiatoaceae* (Teske and Salman, 2014). The elemental sulfur is a combination of cyclooctasulfur, S_8 , and polysulfides, S_n^{2-} , the latter being more available to enzymatic oxidation or reduction (Berg *et al.*, 2014). The different nitrate-storing sulfur bacteria perform either denitrification or dissimilatory nitrate reduction to ammonium (DNRA) and may even alternate between these pathways depending on the environment (Schutte *et al.*, 2018). The bacteria may gain 60 % more energy *per mol* sulfide oxidised by denitrification than by DNRA, but about equal energy *per mol* nitrate by the two pathways (Jørgensen and Nelson, 2004). In practice, however, the denitrification pathway appears to be less energy efficient (Strohm *et al.*, 2007).

Beggiatoa are often abundant in the intermediate oxidised sediment zone where neither oxygen nor sulfide is detectable (Fig. 4.5b; Jørgensen, 1977c; Mussmann *et al.*, 2003). Instead of oxygen, they bring along their electron acceptor within the nitrate vacuole, and they utilise the sulfide produced in the surrounding

sediment so efficiently that the sulfide concentration remains below our detection limit of 1 μM (Preisler *et al.*, 2007). They commute in a random, diffusion-like manner and reach the nitrate or the sulfide zone with a statistical frequency that ensures that they do not run out of stored nitrate or sulfur (Dunker *et al.*, 2010).

4.5 Cable Bacteria

Whereas gliding *Beggiatoa* filaments might explain the frequent gap between oxygen and sulfide in coastal marine sediments, they could not explain laboratory observations in such sediments by Lars Peter Nielsen and his colleagues (Nielsen *et al.*, 2010). They performed experiments with alternating oxic and anoxic seawater flowing over marine sediment and observed a response to sulfide at 1-2 cm depth that was too fast to be driven by molecular diffusion. A **distinct peak of pH at the oxic-anoxic interface and a broad pH minimum in the oxidised but anoxic sediment** could also not be explained from known biogeochemical oxidation processes that all tend to produce a pH minimum at the oxic-anoxic interface (Fig. 4.8; compare to Fig. 4.5). The authors made the bold hypothesis that subsurface sulfide oxidation drove an electric current upwards *via* bacterial nanowires and that outer membrane cytochromes, perhaps combined with pyrite and other conductive electron carriers, ultimately led to an electrochemical oxygen reduction at the sediment surface. Such an electric current could account for 40 % of the entire oxygen uptake of the sediment (Nielsen *et al.*, 2010; Risgaard-Petersen *et al.*, 2012). **The two half reactions of sulfide oxidation to sulfate with oxygen would thereby be physically separated by a long distance electron transport:**

Two years later the cable bacteria were discovered as long chains of cells up to several cm in length that bridge the gap between O_2 and H_2S (Fig. 4.9; Pfeiffer *et al.*, 2012). They have gliding motility of about $0.5 \mu\text{m s}^{-1}$ and continuously adjust chemotactically to the variable chemical gradients (Bjerg *et al.*, 2016). The lower part of the filament oxidises free sulfide and iron sulfide down to 2-3 cm depth (Eq. 4.1). The electrons are conducted up through the filament to the top at the oxic sediment surface. Here, the second half reaction takes place as the electrons are transferred to oxygen (Eq. 4.2). Nitrate may function as an alternative electron acceptor and the bacteria may also use organic electron donors and grow as heterotrophs. Yet, fermentation products such as propionate are rather converted to CO_2 in the sediment before assimilation (Marcocchi *et al.*, 2014; Vasquez-Cardenas *et al.*, 2015; Geerlings *et al.*, 2020). If the oxygen level in the overlying seawater drops, the cables stretch up into the overlying water to reach more oxygen, just like the *Beggiatoa* (Burdorf *et al.*, 2018). Thus, the cable bacteria have a similar ecological niche as *Beggiatoa*, with which they compete and may alternate in community size through the year (Lipsewiers *et al.*, 2017).

Figure 4.8 Distribution of oxygen, sulfide and pH in a sediment densely populated by cable bacteria. The unique pH peak at the oxic-anoxic interface combined with the separation of O₂ and H₂S are diagnostic for the presence of cable bacteria. Redrawn from Nielsen *et al.* (2010).

Cable bacteria are now being detected worldwide in both marine and freshwater sediments (Malkin *et al.*, 2014; Burdorf *et al.*, 2017). The pH peak at the oxic-anoxic transition and the broad pH minimum below result from their electrogenic sulfide oxidation (Risgaard-Petersen *et al.*, 2012; Burdorf *et al.*, 2017) (Fig. 4.8). Their abundance can be very high, up to 10^9 cells cm⁻³, corresponding to 2 km of cables *per* cm² or 25 % of all microbial cells (Schauer *et al.*, 2014). They control sediment geochemistry by dissolving iron sulfides and carbonates in the slightly acidified subsurface and form an amorphous crust of iron and manganese oxides and calcium carbonate at the alkaline pH peak (Pfeffer *et al.*, 2012). **This electron-driven element cycling has a seasonal effect in eutrophic, coastal waters with periodically low oxygen** (Seitaj *et al.*, 2015). In summer, such sediments build up an oxygen debt by the accumulation of FeS, which enhances the oxygen uptake in fall and winter. The large pool of iron oxides that builds up during winter and spring buffers against sulfide emission from the sediment in summer.

Cable bacteria form two distinct, monophyletic clades related to *Desulfobulbus* with the proposed genus “*Candidatus Electrothrix*” in marine sediments and the genus “*Candidatus Electronema*” in freshwater sediments (Trojan *et al.*, 2016; Kjeldsen *et al.*, 2019). The biophysics of the electric current is not yet clear but is associated with a conductive fibre network (Meysman *et al.*, 2019). The long bacterial filaments have multiple, continuous periplasmic fibres that run beneath the cell surface within distinct ridge structures and that bridge the transition between adjacent cells in the chain (Cornelissen *et al.*, 2018; Jiang *et al.*, 2018) (Figs. 4.9 and 4.10).

The electric wiring provided by the cable bacteria appears to be exploited by a diversity of other microorganisms that swarm around the cables as long as they conduct electrons (Vasquez-Cardenas *et al.*, 2015). The swarming microorganisms include aerobic sulfide oxidisers and methylotrophs that apparently utilise the cables as electron acceptors in the anoxic sediment. Dark CO₂ assimilation in the few cm deep electrogenic zone was shown to depend directly on the presence of intact cable bacteria (Meysman, 2018).

Figure 4.9

Cable bacteria from marine sediment. **(a)** Cross section in the transmission electron microscope. **(b)** Filament in the scanning electron microscope. Both images show multiple ridges with electron conducting properties that run through the periplasmic space along the chains of cells. **(a)** Courtesy of C. Bortolini, K. Thomsen and L.P. Nielsen; **(b)** from Teske (2019).

Figure 4.10

(a) Artist's version of cable bacteria with electron conducting fibres running through the periplasmic space and connecting at each cell-cell junction. (b) Function of the cable with the two half reactions of sulfide oxidation by oxygen separated by multiple cells. Electrons from sulfide are taken up at the anodic part of the filament and conducted through the fibres to the cathodic part where the electrons are transferred to oxygen. (a) From Eachambadi *et al.* (2020); (b) from Meysman (2018); courtesy of Filip Meysman and Ragha T. Eachambadi.

4.6 Chemoautotrophy and Dark CO_2 Fixation

The earliest recognised genera of marine sulfide oxidising bacteria, such as the marine *Thiobacillus* and *Thiomicrospira*, were isolated from coastal surface sediments using defined inorganic media, sometimes supplemented with an organic carbon source (e.g., Kuenen and Veldkamp, 1972; Brinkhoff *et al.*, 1999). While these microorganisms were all chemolithotrophs, conserving energy from the

oxidation of reduced sulfur compounds, only some were autotrophs, growing with CO₂ as sole carbon source. Others were heterotrophs and generated ATP from the oxidation of reduced inorganic sulfur species but developed biomass from assimilated organic substrates. Others again were more flexible and could grow as either autotrophs, heterotrophs or mixotrophs.

Genes of sulfide oxidising microorganisms

In contrast to the sulfate reducing bacteria, there are currently no specific molecular marker genes known that are both universal and unique among the sulfide oxidising microorganisms. The most widespread functional marker genes are sulfide-quinone oxidoreductase (Sqr) and the thioesterase subunit, SoxB, of the thiosulfate oxidising multi-enzyme complex (SOX-pathway) (Wasmund *et al.*, 2017). Yet, surveys of these genes most likely underestimate the abundance and diversity of sulfide oxidisers in marine sediments. According to metagenomic studies or to 16S rRNA gene amplicon sequencing, their diversity is large and includes representatives of alpha-, gamma-, and epsilon-proteobacteria (Pham *et al.*, 2008; Lenk *et al.*, 2011; Thomas *et al.*, 2014).

Interestingly, none of the cultivated genera of *Thiobacillus* or *Thiomicrospira* appear to be important sulfide oxidisers in marine sediments (Brinkhoff *et al.*, 1998). Instead, uncultured *Gammaproteobacteria* have been found to be the most abundant. The total abundance of sulfide oxidising *Gammaproteobacteria* may reach 10⁸ cells cm⁻³ in organic-rich marine sediments, corresponding to about 5 % of all cells (Lenk *et al.*, 2011). They are the most active bacteria with respect to chemoautotrophic dark CO₂ assimilation, which is most likely based on the oxidation of reduced sulfur compounds (Lenk *et al.*, 2011; Dykstra *et al.*, 2016).

The growth efficiency of autotrophic sulfur bacteria in pure culture varies depending on the sulfur source (H₂S or S₂O₃²⁻), the electron acceptor (O₂ or NO₃⁻), and the microbial species. I here **define the autotrophic growth efficiency as the fraction of reducing equivalents from sulfur that is channeled into the reduction of CO₂ to organic carbon instead of being used to generate ATP**. Kelly (1982) predicted a theoretical growth yield of autotrophic bacteria growing on S₂O₃²⁻ and O₂ to be 6.7 g dry weight *per* mol S₂O₃²⁻. This corresponds to a 14 % growth efficiency. Nelson *et al.* (1986) determined a 17 % growth efficiency of autotrophic *Beggiatoa* in gradient cultures.

Estimates of dark CO₂ fixation by chemoautotrophic sulfide oxidation in marine sediments vary depending on the applied model assumptions or experimental approach. Howarth (1984) used pure culture data from Kelly (1982) and estimated a dark CO₂ fixation corresponding to 7-13 % of the total sediment respiration in estuarine sediment and 3-6 % in continental shelf sediment. Dale *et al.* (2010) and Middelburg (2011) assumed *a priori* a very high growth yield of 0.5 mol CO₂ fixed *per* mol H₂S oxidised to calculate dark CO₂ fixation. As CO₂ reduction to organic carbon requires 4 equivalents while the oxidation of H₂S to SO₄²⁻ delivers 8 equivalents, this corresponds to a growth efficiency of 25 %, which exceeds pure culture efficiencies. Experimental measurements of dark CO₂ fixation have been done by stable isotope probing, using ¹³C-CO₂ as a tracer

by sediment incubations, followed by isotope analysis of extracted phospholipid-derived fatty acid biomarkers of sediment bacteria. From such experiments, Vasques-Cardenas *et al.* (2020) calculated an **autotrophic growth efficiency in coastal marine sediment equivalent of 1-11 % of the total oxygen uptake, with a mean value of 4 %**. They also made a literature survey of such measurements and found a range of 1-22 %, with a mean value of 7 %.

This 7 % average is similar to the maximum autotrophic growth efficiency estimated by Jørgensen and Nelson (2004). Our rationale for the estimate is illustrated in Figure 4.11. Based on my data from coastal sediments, we assumed that half of the mineralisation of organic carbon is due to aerobic respiration and half to sulfate reduction. An estimated 10 % of the sulfide is bound by iron and gradually buried as FeS₂, while 90 % is ultimately oxidised back to sulfate at the expense of oxygen. If we assume that all sulfide oxidation is by microorganisms with a high growth efficiency of 15 %, then the autotrophic growth efficiency could theoretically reach $(0.5 \times 0.9 \times 0.15 \times 100 =)$ 7 %. If autotrophy is not running at this high efficiency, the number will be smaller than 7 %. It will expectedly also be smaller if Fe and Mn reduction contribute more to the anaerobic mineralisation.

Figure 4.11

Coupling of carbon and sulfur cycles in marine sediment with sulfide oxidising microorganisms. The numbers show the allocation of electrons from organic carbon via H₂S and back to organic carbon by CO₂ assimilation in chemoautotrophic sulfur bacteria. Redrawn from Jørgensen and Nelson (2004).

In conclusion, chemoautotrophic biomass production contributes to the organic carbon turnover in coastal sediments by recycling some of the mineralised organic matter, *via* H₂S production and dark CO₂ fixation, to microbial necromass (Middelburg, 2011). Yet, this is only a small fraction relative to the total influx of organic matter and may hardly be detectable in budgets of carbon cycling in marine sediments (Bradley *et al.*, 2018).

5.

DYNAMICS OF INTERMEDIATE SULFUR SPECIES

The inorganic sulfur species, with an oxidation state intermediate between sulfide (-2) and sulfate (+6), play an important role in the sulfur cycle of marine sediments. **They tend to be chemically reactive and can be utilised for the energy metabolism of a large diversity of microorganisms.** They may be reduced back to sulfide in the presence of H_2 or organic substrates that serve as electron donors and carbon sources. They may be oxidised in the presence of suitable electron acceptors, such as oxygen, nitrate, Mn(IV) or Fe(III) that enable the conservation of energy for the microorganisms. If neither electron donor nor electron acceptor is available, the intermediate sulfur species may be disproportionated, whereby they are simultaneously reduced to H_2S and oxidised to SO_4^{2-} . This provides energy for microorganisms that grow either heterotrophically, assimilating volatile fatty acids and other small organic molecules, or autotrophically, assimilating CO_2 .

Figure 5.1

Inorganic sulfur species arranged according to the oxidation state (red numbers) of their sulfur atoms. Elemental sulfur is represented by one of its forms, cyclic octasulfur, while polysulfide, which has variable chain lengths of mostly 4-7 sulfur atoms, is represented by one of the abundant forms, S_6^{2-} .

Most sulfide oxidation in marine sediments takes place below the oxic zone. The primary oxidants are Fe(III) and Mn(IV), whereby the concentration of oxidised iron is often an order of magnitude or more higher than of oxidised manganese. Both the chemical and biological reaction between sulfide and Fe(III) may be fast and compete with iron reduction by heterotrophic bacteria (Hansel *et al.*, 2015). The oxidation leads to a redox cascade of inorganic sulfur species with intermediate oxidation states: polysulfides (S_x^{2-} , $x = 4-7$), elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), tetrathionate ($S_4O_6^{2-}$), and sulfite (SO_3^{2-}) (Fig. 5.1). I will first discuss the distribution and dynamics of these intermediates.

5.1 Elemental Sulfur and Polysulfides

Elemental sulfur is the predominant immediate product in experiments by which sulfide reacts with iron oxides in seawater under neutral pH (*e.g.*, Yao and Millero, 1993, 1996; Pyzik and Sommer, 1981). Elemental sulfur is also an important product in marine sediments where it occurs at concentrations about three orders of magnitude higher than the oxyanions, thiosulfate or sulfite (*e.g.*, Zopfi *et al.*, 2004). However, the pool size of an intermediate does not necessarily indicate its relative role in the sulfur cycle. Elemental sulfur is of particular interest for sulfur isotope geochemistry because its concentration is high enough to determine its isotopic composition, whereas the pool sizes of thiosulfate and sulfite have so far been too small for isotope analysis (see Section 8).

The solubility of cyclooctasulfur, S_8 , in seawater at 12 °C is 8 nM (Kamyshny, 2009). Thus, only a minute fraction of the S^0 is dissolved in the porewater, partly in the form of nanoparticulate elemental sulfur with a particle size $<0.2 \mu m$ (Findlay *et al.*, 2014). Elemental sulfur produced freshly from microbial sulfide oxidation has a different chemical form than the coarser bulk α - S_8 in sediments. It is less hydrophobic and is therefore more reactive towards microbial metabolism (Kleinjan *et al.*, 2003; Garcia and Druschel, 2014).

The elemental sulfur concentration in coastal marine sediments generally peaks in the upper few cm to dm with concentrations of $0.5-10 \mu mol cm^{-3}$ (Troelsen and Jørgensen, 1982; Zopfi *et al.*, 2004; Pjevac *et al.*, 2014). Troelsen and Jørgensen (1982) found that the S^0 pool varied seasonally with the highest concentrations in winter when the sediment was most oxidised and sulfate reduction rates were lowest. In off shore sediments, the S^0 distribution can be very different, being more constant or increasing at several metres depth where the sediment becomes sulfidic and a sulfidisation front may occur (*e.g.*, Kaplan *et al.*, 1963; Riedinger *et al.*, 2017; Liu *et al.*, 2020a).

Elemental sulfur and sulfide form reactive polysulfides: $xS^0 + HS^- \leftrightarrow S_{(x+1)}^{2-} + H^+$, where x is generally 3–6 in coastal marine sediments (Findlay and Kamyshny, 2017). The polysulfides may approach thermodynamic equilibrium with sulfide with respect to concentration and polysulfide chain length distribution, depending on the speciation of the elemental sulfur and the rate of turnover

(Kamyshny and Ferdelman, 2010; Lichtschlag *et al.*, 2013; Holmkvist *et al.*, 2014). As S^0 is often in excess, polysulfide concentrations tend to follow closely the distribution of free sulfide.

This is shown for a coastal marine sediment, in which the chemical zonation of sulfur species is somewhat unusual, but very distinct, and which therefore illustrates the processes well. A minimum in sulfate concentration at 20–30 cm is here wedged in between sulfate diffusing down from the overlying seawater and sulfate diffusing up from a deep sulfate reservoir (Fig. 5.2a). Such a sulfate minimum is not uncommon in coastal seas that were land locked during the last glaciation, as explained in Section 8.4 (Figs. 8.4, 8.5). The sulfate minimum coincided with a peak in porewater H_2S (Fig. 5.2c). Elemental sulfur was most abundant near the sediment surface and at a sulfidisation front at 70 cm depth (Fig. 5.2b), where the H_2S met dissolved Fe^{2+} diffusing up from below (Fig. 5.2c). This Fe^{2+} was formed by Fe(III) reduction in deep, iron-rich glacial clay beneath the Holocene mud. A peak in total polysulfide of 60 μM coincided with the peak in H_2S and a minimum in S^0 concentration, indicating that polysulfide formation consumed S^0 and was in balance with the 20 fold higher sulfide concentration. The individual polysulfides had chain lengths with decreasing concentration in the order of: S_5^{2-} , S_6^{2-} , S_4^{2-} , and S_7^{2-} (Fig. 5.2d).

Elemental sulfur is generally very heterogeneous and may include cyclooctasulfur (S_8), polymeric sulfur (S_n), dispersed colloidal sulfur, dissolved sulfur, organically bound sulfur, and polysulfides (S_n^{2-}) (*e.g.*, Luther *et al.*, 1985; Kamyshny *et al.*, 2009; Lichtschlag *et al.*, 2013). In marine sediments, these sulfur forms may be oxidised, reduced or disproportionated, mostly driven by reactions between the highly reactive polysulfides and iron minerals (Findlay and Kamyshny, 2017). It is not known whether microorganisms contribute to these processes, but S^0 metabolising bacteria are abundant and diverse in coastal sediments (Wasmund *et al.*, 2017). As an example, studies by Pjevac *et al.* (2014) in tidal flat sediments amended with S^0 showed a high enrichment in the oxidised surface sediment of the epsilonproteobacterial *Sulfurimonas/Sulfurovum* group, which comprises many strains known to be sulfur oxidising. In the reducing sediment below, deltaproteobacterial *Desulfocapsa/Desulfobulbus*-related sequences indicated the abundance of S^0 disproportionating microorganisms, while diverse *Desulfurimonadales* sequences were rather related to S^0 reducing strains. Thus, although the processes of S^0 transformation were not determined under *in situ* conditions, the microbial communities in enrichment experiments corresponded well to the expected pathways and zonation of microbial S^0 metabolism.

Figure 5.2 Sulfur geochemistry of a Baltic Sea sediment (Arkona Basin). (a) Sulfate, (b) total elemental sulfur, (c) free sulfide and dissolved iron(II), (d) polysulfides. Redrawn from Holmkvist *et al.* (2014).

5.2 Sulfur Oxyanions

The oxidation of H_2S and S^0 produces diverse sulfur oxyanions such as thio-sulfate, sulfite and tetrathionate. I learned about the discovery of thiosulfate disproportionating bacteria at the end of the 1980's (Bak and Cypionka, 1987; Bak and Pfennig, 1987) and began to work on the turnover of thiosulfate in limnic, brackish and marine sediments. We used the sensitive UV-HPLC technique of Vairavamurthy and Mopper (1990), by which sulfur oxyanions are derivatised with DTNP (2,2'-dithiobis(5-nitropyridine)), but later changed to derivatisation

with monobromobimane (Fahey and Newton, 1987). We found that thiosulfate and sulfite in coastal marine sediments generally occur at low micromolar or sub-micromolar concentrations, controlled by their rapid turnover (Thamdrup *et al.*, 1994b) (Fig. 5.3). The subsurface concentrations of H_2S or S^0 may be 100–1,000 fold higher. Thiosulfate is chemically stable in anoxic sediments but is a favourable energy source for dissimilatory sulfur metabolism. Sulfite, in contrast, is highly reactive and may react with iron(III) or with sulfide or S^0 to form thiosulfate.

Figure 5.3 Distribution in Aarhus Bay sediment of, (a) thiosulfate and sulfite (b) redox potential and porewater sulfide. Redrawn from Thamdrup *et al.* (1994b).

A comparison with the literature at the time showed a very large scatter in published thiosulfate concentrations in marine sediment porewater ranging over four orders of magnitude, with the highest values of 300–2500 μM obtained by cyanolysis technique and polarography (*e.g.*, Howarth *et al.*, 1983; Lein, 1984; Luther *et al.*, 1985). It was not clear to us whether the large span was related to the different sediment environments or whether the analytical techniques affected the results. Later data have generally confirmed the low micromolar concentrations of thiosulfate and sulfite in marine sediments (Zopfi *et al.*, 2004, 2008; Findlay and Kamysny, 2017).

Tetrathionate ($\text{S}_4\text{O}_6^{2-}$) was detected at concentrations up to 20 μM in Baltic Sea sediments (Podgorssek and Imhoff, 1999) and up to 300 μM by polarographic analysis in salt marsh sediments (Luther *et al.*, 1985). Other studies found very low tetrathionate concentrations of ≤ 1 μM (see references in Zopfi *et al.*, 2004).

In anoxic sediments, tetrathionate is reduced microbially to thiosulfate, or it reacts chemically with sulfide to form thiosulfate and elemental sulfur (Bak *et al.*, 1993; Sorokin, 1996; Podgorsek and Imhoff, 1999; Zopfi *et al.*, 2004). Under more oxidising sediment conditions, tetrathionate may be produced by the microbial oxidation of thiosulfate with Fe(III) or MnO₂ and may be further oxidised to sulfate (Schippers and Jørgensen, 2001).

5.3 Disproportionation of Thiosulfate

Over the years, marine sulfur research has shown many examples of cross fertilisation between the discovery of biogeochemical processes in marine sediments and of microbial physiology in laboratory cultures. An example going from physiology to biogeochemistry was the **discovery of thiosulfate disproportionation in a pure culture of sulfate reducing bacteria and the subsequent demonstration that disproportionation drives key processes in the sulfur cycle of marine sediments** (Bak and Cypionka, 1987; Jørgensen, 1990; Canfield and Thamdrup, 1996).

The first isolated, disproportionating bacteria, *Desulfovibrio sulfodismutans*, became the type strain for this type of physiology, whereby thiosulfate or sulfite are disproportionated to sulfide and sulfate:

Mistakes can be valuable

Scientific discoveries, such as the discovery of thiosulfate disproportionation, are sometimes incidental, maybe even due to a mistake. What turns the mistake into a breakthrough is the talent and imagination of the researcher. This was the case when Friedhelm Bak studied pure cultures of sulfate reducing bacteria and tested their capability to use thiosulfate as an alternative electron acceptor. By mistake, he forgot to add the organic carbon source as electron donor to some cultures, yet the cultures produced sulfide from thiosulfate. He concluded that the bacteria disproportionated the thiosulfate to sulfate and sulfide (Bak and Cypionka, 1987; Bak and Pfennig, 1987). A novel physiology had been discovered, which was similar to a fermentation reaction, but which for the first time was found to use an inorganic compound. Disproportionation reactions are now known to occur among many anaerobic bacteria and to constitute key pathways in the sulfur cycle.

Disproportionating bacteria were soon found in both freshwater, brackish and marine sediments (Bak and Pfennig, 1987). It is now recognised that a large diversity of microorganisms can disproportionate inorganic sulfur compounds. Most of these belong to the deltaproteobacteria, including the genera *Desulfocapsa*, *Desulfovibrio* and *Desulfomonile*. Many are able to switch between dissimilatory reduction and disproportionation of sulfur compounds, whereas others are fully specialised on disproportionation (Finster *et al.*, 1998; Wasmund *et al.*,

2017; Slobodkin and Slobodkina, 2019). About half of the isolated strains can grow autotrophically. Unfortunately, no unique diagnostic genes are known for sulfur disproportionation and their community size and diversity therefore remain difficult to determine by current DNA-based techniques.

In 1990, Friedhelm Bak joined me as a postdoc and we studied the microbiology and pathways of sulfate reduction and thiosulfate disproportionation in marine sediments (Jørgensen and Bak, 1991). He cultivated a broad range of sulfate reducing and disproportionating microorganism. After returning to Germany, he continued this research but was tragically stopped, as he died from cancer at the age of 34.

I started in 1988 to work on thiosulfate disproportionation in marine sediments. By analogy to sulfate, I initially expected the oxidation states to be +6 for the inner (sulfonate) sulfur atom and -2 for the outer (sulfane) sulfur atom (Jørgensen, 1990). This motivated Vairavamurthy *et al.* (1993) to check the oxidation states by XANES spectroscopy. They concluded that the charge densities rather correspond to the oxidation states +5 and -1 and that this explains why disproportionating microorganisms can conserve energy when they perform a concurrent oxidation to sulfate (from +5 to +6) and reduction to sulfide (from -1 to -2). Yet, there still appears to be some ambiguity in the literature about the correct interpretation of oxidation states (*e.g.*, Karen *et al.*, 2014).

By using inner and outer ^{35}S -labelled thiosulfate in parallel experiments with homogenised sediment, I could distinguish the pathways of thiosulfate transformation. By parallel experiments with ^{35}S -labelled sulfate and sulfide, I could differentiate disproportionation from concurrent sulfate reduction, sulfide oxidation and isotope exchange (Jørgensen, 1990). The experiments showed that disproportionation competes with both oxidation and reduction of thiosulfate down through the sediment column, and that **thiosulfate constitutes an important shunt in the sulfur cycle of both marine, brackish and freshwater sediments** (Jørgensen, 1990; Fossing and Jørgensen, 1990b; Jørgensen and Bak, 1991; Elsgaard and Jørgensen, 1992).

Our results from a marine sediment from the Kattegat (see map Fig. 1.2), are illustrated in Figure 5.4. The figure shows for the top 0-1 cm of the sediment how the two sulfur atoms in thiosulfate may be either oxidised to sulfate, reduced to sulfide, or disproportionated to both sulfate and sulfide. In the sediment incubation with inner ^{35}S -labelled thiosulfate, 16 % of the ^{35}S -label was recovered in the reduced sulfur pool and 84 % in the sulfate pool (Fig. 5.4c). Since a conversion of the inner (oxidised) sulfur atom of thiosulfate to sulfide represents a net reduction (Fig. 5.4b), 16 % of the thiosulfate was reduced. The remaining 84 % was either oxidised or disproportionated to sulfate, but the conversion of the inner sulfur atom cannot be used to distinguish between these two pathways. In the experiment with outer ^{35}S -labelled thiosulfate, 76 % of the label was recovered in the reduced sulfur pool and 24 % in the sulfate pool (Fig. 5.4d). Since a conversion of the outer (reduced) sulfur atom of thiosulfate to sulfate represents a net oxidation (Fig. 5.4b), 24 % of the thiosulfate was oxidised. The remaining

76 % was either reduced or disproportionated to sulfide, but the conversion of the outer sulfur atom cannot be used to distinguish between the two pathways. Since the remaining $(100 - 16 - 24 =)$ 60 % of the converted ^{35}S thiosulfate was converted to sulfide and sulfate, but neither by net reduction nor net oxidation, this major percentage was disproportionated (Fig. 5.4a).

Figure 5.4

Thiosulfate transformations in marine sediment from Kattegat. **(a)** Thiosulfate disproportionation to sulfate and sulfide. Oxidation states of the inner (+5) and outer (-1) sulfur atoms in thiosulfate are shown. **(b)** Thiosulfate reduction to sulfide or oxidation to sulfate, **(c)** and **(d)** sediment experiments by which the inner (sulfonate) or the outer (sulfane) sulfur atom of thiosulfate was ^{35}S -labelled. Redrawn from Jørgensen and Bak (1991).

Similar time course experiments were done for Kattegat sediment at intervals down to 10 cm depth. In addition, parallel experiments were done with the reduction of ^{35}S -labelled sulfate and the oxidation of ^{35}S -labelled sulfide. The data showed how the fraction of transformed thiosulfate that became reduced increased with depth, while the fraction that became oxidised decreased with depth (Fig. 5.5a). This was in agreement with the general redox zonation of the sediment where the upper 0-2 cm was brown and oxidised while the sediment below was black and reduced, but with no free sulfide. Disproportionation was

overall the most important pathway of thiosulfate transformation. Similar to thiosulfate reduction, sulfate reduction was lowest in the oxidised surface layer but high below 2 cm (Fig. 5.5b). The immediate products of ^{35}S sulfide oxidation showed a predominance of ^{35}S thiosulfate, which accounted for 75 % in the reducing sediment (Fig. 5.5c). This thiosulfate was then further oxidised to sulfate or reduced to sulfide. The terminal products of sulfide oxidation in the oxidised sediment were thus a 70 % complete oxidation to sulfate and a 30 % recycling *via* thiosulfate back to sulfide (Fig. 5.5d). In the reduced sediment below, half of the oxidised sulfide was recycled *via* thiosulfate back to sulfide.

Figure 5.5

Transformation of thiosulfate, sulfide and sulfate in marine sediment from Kattegat. (a) Relative contributions of reduction, disproportionation and oxidation of thiosulfate, (b) sulfate reduction, (c) products of sulfide oxidation, (d) pathways of sulfide oxidation after complete transformation of thio-sulfate. Redrawn from Jørgensen (1990).

These processes of the sulfur cycle, which I called the “thiosulfate shunt”, are illustrated in Figure 5.6, where process rates are calculated for a specific sediment interval in percent of the rate of sulfate reduction. Of the produced sulfide, 10 % was bound in the solid phase as FeS , FeS_2 and S^0 . The remaining 90 % was re-oxidised, but only 20 % was oxidised directly to sulfate while 70 % was first oxidised to thiosulfate. Of these 70 % thiosulfate, 6 % was oxidised to sulfate, 28 % was reduced to sulfide, while $2 \times 18 = 36$ % was disproportionated to both sulfate and sulfide.

This key role of thiosulfate was very surprising to us, but was confirmed by a number of other experiments with coastal marine sediments. Based on the combined use of ^{35}S -labelled sulfate, thiosulfate, elemental sulfur and sulfide, we concluded that **half the oxidation of sulfide and elemental sulfur to sulfate proceeds through thiosulfate in the reduced sediment** (Fossing and Jørgensen, 1990b). In some of the oxidation experiments with ^{35}S -labelled sulfide, we “froze” the ^{35}S thiosulfate pool from further conversion by spiking it with non-radioactive thiosulfate. The large pool size thereby slowed down its turnover. Such experiments showed that the oxidation products of sulfide were 29 % thiosulfate and 71 % sulfate in the oxidised zone and 92 % thiosulfate and 8 % sulfate in the reduced zone (Fossing and Jørgensen, 1990b).

Figure 5.6

Summary of the sulfur cycle in anoxic marine sediment (4-6 cm depth in Fig. 5.5) with emphasis on the thiosulfate shunt. Redrawn from Jørgensen (1990).

5.4 Disproportionation of Elemental Sulfur

In this thiosulfate-centric view of the sulfur cycle, the role of elemental sulfur was not considered. Elemental sulfur is also a key intermediate in the sulfur cycle and may be concurrently reduced, disproportionated and oxidised. Much of the sulfide oxidation may indeed pass through elemental sulfur on the way to thiosulfate and sulfate. This cannot be distinguished by the ^{35}S radiotracer experiments alone due to isotope exchange between sulfide and elemental sulfur (Section 4.2).

Disproportionation of elemental sulfur was discovered in enrichment cultures of marine, chemolithotrophic bacteria grown on S^0 and amorphous ferric hydroxide (FeOOH) (Thamdrup *et al.*, 1993):

Bacteria that disproportionate S^0 were soon found to be common and widespread in coastal marine sediments. The reaction in Equation 5.3 is endergonic at pH 7 under standard conditions ($\Delta G^{0'} = +10.3 \text{ kJ mol}^{-1} S^0$) and is sensitive to the concentration of sulfide (Slobodkin and Slobodkina, 2019). Growth of the culture was therefore dependent on the addition of an effective sulfide scavenger such as MnO_2 , FeOOH or $FeCO_3$ to remove sulfide and make the process exergonic (Thamdrup *et al.*, 1993; Finster *et al.*, 1998; Slobodkin and Slobodkina, 2019):

Sulfide scavengers are present in oxidised surface sediments where elemental sulfur disproportionation takes place (Canfield and Thamdrup, 1996). In very sulfidic sediments, S^0 disproportionation may occur *via* polysulfides, whereby the process is less sensitive to the sulfide concentration (Milucka *et al.*, 2012; Poser *et al.*, 2013).

Due to isotope exchange, it is difficult to determine the turnover rate of $^{35}S^0$ in marine sediments. Experiments have therefore depended on spiking with non-radioactive S^0 and following the change in different sulfur pools over time (Canfield and Thamdrup, 1996). This approach shows potential rates of S^0 turnover, but results cannot be accurately related to *in situ* conditions. As an alternative, Zopfi *et al.* (2004) determined the total sulfide oxidation rate from ^{35}S sulfate reduction measurements. The authors then assumed that all sulfide oxidation passed through S^0 and compared this with the bulk concentration of S^0 . The calculated turnover times of S^0 ranged from a few weeks to a few months.

I made a similar, rough estimate of the turnover time of thiosulfate in a coastal marine sediment (Jørgensen, 1990) (Figs. 5.5, 5.6). At 4–6 cm depth, the sulfate reduction rate was $18 \text{ nmol cm}^{-3} \text{ d}^{-1}$ corresponding to $23 \text{ } \mu\text{M SO}_4^{2-} \text{ d}^{-1}$. With 90 % of the produced sulfide ultimately being oxidised to sulfate, and with 70 % of this oxidation going through thiosulfate, the rate of thiosulfate oxidation was $15 \text{ } \mu\text{M d}^{-1}$ (Thamdrup *et al.*, 1994b) (Fig. 5.2). With a pool size $0.2 \text{ } \mu\text{M}$, the thiosulfate turnover time was only 20 minutes. This illustrates how **the sulfur oxyanions constitute very dynamic intermediates in the sulfur cycle with fast turnover of a small pool, in contrast to elemental sulfur with a relatively slow turnover of a large pool** (Fig. 5.2 *vs.* Fig. 5.3).

In conclusion, sulfate may be formed by direct chemical or microbial sulfide oxidation with reactive iron(III) and manganese(IV) (Section 4) or it may be formed through microbial disproportionation of the intermediate species, elemental sulfur, thiosulfate or sulfite. Manganese oxides (Mn(IV)) are stronger oxidants for sulfide than Fe(III) and reaction rates are higher than for iron,

depending on the manganese speciation (Yao and Millero, 1993, 1996). Both in biotic and abiotic experiments, oxidation with MnO_2 formed products of higher oxidation state, such as thiosulfate and sulfate, whereas sulfate was not produced in experiments with Fe(III) addition (Aller and Rude, 1988; Yao and Millero, 1996; Böttcher and Thamdrup, 2001). MnO_2 can also chemically oxidise FeS and FeS_2 to sulfate in marine sediments with concurrent formation of thiosulfate, tetrathionate and sulfate (Schipper and Jørgensen, 2001, 2002; Schippers, 2004). Pyrite oxidation in these experiments was confirmed by the release of ^{55}Fe iron from radioactively labelled pyrite ($^{55}\text{FeS}_2$) and its binding in HCl soluble minerals. It was suggested that the pyrite oxidation with MnO_2 was indirect, *via* a Fe(II)/Fe(III) -shuttle (Schipper and Jørgensen, 2001).

5.5 Pathways of Pyrite Formation

The oxidation of sulfide with Fe(III) competes with its precipitation as amorphous iron sulfides and its gradual conversion to pyrite. In microbial culture experiments with sulfate reducing bacteria and Fe(II) , the initially formed iron-sulfur minerals are amorphous and poorly crystalline mackinawite (FeS) and greigite (Fe_3S_4) (Picard *et al.*, 2016). In the presence of hematite (Fe_2O_3), also pyrrhotite ($\text{Fe}_{(1-x)}\text{S}$) is formed (Neal *et al.*, 2001), while the presence of Fe(III) phosphate led to a rapid formation of pyrite (Berg *et al.*, 2020). Similar iron-sulfur species are formed also by a purely chemical reaction between sulfide and Fe(II) , yet with a different morphology (*e.g.*, Gramp *et al.*, 2010).

The detailed mechanisms of FeS and FeS_2 formation have mostly been studied under abiotic conditions. Experiments with biotic systems have shown that bacteria and archaea may play important roles by the formation and transformation of inorganic iron-sulfur minerals. For example, sulfate reducing bacteria can provide templates for the nucleation of mackinawite (FeS) and accelerate the formation of greigite (Fe_3S_4) (Picard *et al.*, 2018). A mixed community of bacteria and methanogenic archaea can accelerate the formation of pyrite through the H_2S pathway because the methanogens consume H_2 , using CO_2 as an oxidant (Thiel *et al.*, 2019):

Pathways of pyrite formation

The formation of pyrite can follow different pathways, depending on the intermediate formation of ferrous iron minerals, of elemental sulfur, and of polysulfides (Kamyshny and Ferdelman, 2010; Findlay, 2016). Two different reactions appear to be predominant in marine sediments. One is the reaction between FeS and H_2S , by which H_2 (or $2\text{H}^+ + 2\text{e}^-$) is produced (Rickard, 1997; Rickard and Luther, 1997; Thiel *et al.*, 2019):

The other is the reaction between FeS and polysulfide (Rickard and Luther, 2007):

where x is generally between 4 and 7. This reaction is reminiscent of the direct reaction between FeS and S^0 to form FeS_2 , proposed by Berner (1970). It remains poorly constrained which of the two pathways predominates in marine sediments. Rickard and Luther (2007) concluded from general reaction kinetics that **the H_2S pathway is the fastest under normal sediment conditions**. The reaction mechanism appears to depend on the $\text{Fe(III)}:\text{sulfide}$ ratio, on the availability of intermediate sulfur species, and on the pH and temperature. At a low sulfide:iron(III) ratio, which is typical of lacustrine sediments, a “ferric hydroxide-surface” pathway was suggested (Wan *et al.*, 2017). In more sulfidic sediments, the highest rate of pyrite formation is often found near the sediment surface where FeS and S^0 are available (Liu *et al.*, 2020a).

A similar enhancement of the H_2S pathway of pyrite formation may be driven by sulfate reducing bacteria that even more effectively consume the H_2 (Burdige, 2006). Pyrite constitutes the main sink in the sulfur cycle of marine sediments. The intermediate sulfur species contribute only little to the accumulation of reduced sulfur, whereas organic sulfur can be significant (*e.g.*, Werne *et al.*, 2004). Pyrite formation generally starts at the sediment surface in the oxidised but anoxic zone. This is a highly variable environment with a heterogeneous redox structure, which favours the conversion of iron sulfides to pyrite. This was proposed already by Emery and Rittenberg (1952) as an explanation for the occurrence of pyrite in oxidising near surface sediments off southern California. It was repeatedly shown for different Baltic Sea sediments where the gradient in pyrite concentration is generally steepest at the top of the sediment column (Thode-Andersen and Jørgensen, 1989; Böttcher and Lepland, 2000; Liu *et al.*, 2020a). The product of this gradient and the sediment accumulation rate (or the bioturbation coefficient) provide an estimate of the net rate of pyrite formation. However, as for the other sulfur species, this does not show how many times pyrite is turned over due to bioturbation, re-oxidation and other mechanisms before it is buried.

6.1 Age Control of Turnover Rates

When viewed over a very broad time interval, from days to millions of years, the degradation rate of sediment organic matter is primarily a function of its age. This was shown clearly in an analysis by Middelburg (1989, 2019) in which the reactivity, *i.e.* the specific degradation rate constant, of marine organic matter was shown to be a log-log linear function (power law function) of its “initial age” over a time span of eight orders of magnitude (Fig. 6.1). The exponent of the power law is close to -1, which means that **the degradation rate constant is inversely proportional to the age of the organic matter**.

Figure 6.1

Degradation rate constants (“reactivity”) of organic matter as a function of its age. Data sources are from sediment trap material, laboratory experiments, and sediment cores. The data plot is double logarithmic and is linearly correlated by a power law function. Redrawn from Middelburg (2019; data courtesy of Jack Middelburg).

Our own high resolution measurements of sulfate reduction rates in marine sediments have shown a power law exponent of -1.2 to -2.5 for the rate (not the rate constant) of organic matter degradation (see Section 3.4.1; Dale *et al.*, 2019; Jørgensen *et al.*, 2019b). Such differences in exponents result in large differences in calculated degradation rates between surface and depth in the sediment, and **it is therefore important to obtain further high quality data over a broad time span for a thorough analysis of degradation kinetics**.

Benthic communities of bacteria and archaea carry out the subsurface degradation of organic matter and its final oxidation to CO₂. The size of the microbial community is primarily controlled by the rate of degradation, which provides organic substrate and energy for the cells (Jørgensen and Marshall, 2016). This was shown for sulfate reduction by comparison of sulfate reduction rates with the abundance of total microbial cells or of sulfate reducing microorganisms. If sediments from the extremely low productive ocean gyres with an organic carbon content <0.1 % are omitted, there is a rough relationship between cells *per* cm³ and age in years: cell number = $10^{11} \times \text{age}^{-0.8}$, (Jørgensen and Marshall, 2016). Parkes *et al.* (2014) found a similar relationship of cell number *per* cm³ and depth in metres of, cell number = $10^{8.05} \times \text{depth}^{-0.68}$. These relationships show that the power law exponent for total cell numbers is numerically smaller than the exponent for organic matter mineralisation rate. Thus, the substrate and energy supply for the subsurface microbial communities drops more steeply with depth and age of the sediment than the community size. This implies that **the mean cell specific catabolic rates drop with increasing age of the seabed.**

We have made several comparisons between the rate of sulfate reduction and the abundance of sulfate reducing bacteria (Fig. 6.2). Near the sediment surface, mean cell specific rates are up to 0.07 fmol cell⁻¹ d⁻¹ (1 femtomol = 10⁻¹⁵ mol). Rates drop 100 fold to about 0.0003 fmol cell⁻¹ d⁻¹ with depth down to 300 cm, where the sediment is about 5,000 years old. If we assume a growth yield of the subsurface bacteria of 2 g cell carbon assimilated *per* mol substrate (acetate) consumed (Jørgensen and Marshall, 2016; D'Hondt *et al.*, 2014) and a biomass of 20 fg C *per* cell (Braun *et al.*, 2016), their mean turnover time (generation time) is on the order of a year at the sediment surface and 100 years at 3 metres depth in Aarhus Bay (Hoehler and Jørgensen, 2013). Langerhuus *et al.* (2012) used a D:L amino acid racemisation model to calculate microbial biomass turnover in the same subsurface sediments of Aarhus Bay and reached a similar magnitude of about 100 years at depth. It was estimated that these subsurface microbial communities turned over about 200 times during the 500 years of burial from the sediment surface to 0.5 m depth, with most of the turnover near the sediment surface (Jørgensen *et al.*, 2019a). For comparison, microbial biomass turnover times in 10 million year old sediments of the eastern, tropical Pacific were about 1,000 years at depth (Lomstein *et al.*, 2012).

The growth yield by such calculations is clearly difficult to determine, but the agreement with the D:L amino acid racemisation approach provides independent support for the growth calculations. In the very deep biosphere, it has been questioned whether growth and cell division occur at all (Bradley *et al.*, 2020). However, a significant fraction of the cells appear microscopically to be in different stages of division (Parkes *et al.*, 2014), and there is evidence of **mortality due to virus attack that must be balanced by formation of new cells** (Engelhardt *et al.*, 2015; Cai *et al.*, 2019). Thus, bacterial cells in 6,000 year old Baltic Sea sediments were shown directly by electron microscopy to be infected by virus particles (Cai *et al.*, 2019).

Figure 6.2

Mean sulfate reduction rates *per* sulfate reducing bacteria (cell specific SRR) vs. depth in sediment. Data from Aarhus Bay based on ^{35}S -SRR measurements and qPCR quantification of *dsrAB* genes. Data from Sahm *et al.* (1999) (yellow symbols), Leloup *et al.* (2009) (red symbols), and Petro *et al.* (2019) (blue symbols).

Life under these conditions requires the ability to minimise energy consumption to the extreme, to utilise recalcitrant substrates, to protect nucleic acids and proteins from decay, and to repair random mismatches in DNA or damaged amino acids in proteins (Mhatre *et al.*, 2019; Jørgensen *et al.*, 2020b). Yet, the mean cell specific catabolic rate must remain above a minimum energy turnover required to sustain life, a minimum called the **“basal power requirement”** by Hoehler and Jørgensen (2013). Based on the data cited above, and assuming a required minimum Gibbs free energy yield ΔG_r , of -10 kJ mol^{-1} sulfate, Lever *et al.* (2015) estimated that the energy turnover of sulfate reducers beneath 10 m depth in cold subsurface sediments approached a minimum power of around $10^{-15} \text{ kJ cell}^{-1} \text{ yr}^{-1}$, corresponding to 30 zW cell^{-1} ($1 \text{ zeptoWatt} = 10^{-21} \text{ Watt}$) or $3 \times 10^{-4} \text{ fmol SO}_4^{2-} \text{ cell}^{-1} \text{ d}^{-1}$.

Bradley *et al.* (2020) estimated a somewhat larger ΔG_r by sulfate reduction of $-2.68 \text{ kJ g}^{-1} \text{ C}$, corresponding to 64 kJ mol^{-1} of sulfate, resulting in a median power utilisation of sulfate reducers in the sub-seafloor of 108 zW cell^{-1} . From theoretical considerations, LaRowe and Amend (2015a) estimated the minimum power required for cells to remain viable to be $1\text{--}10 \text{ zW cell}^{-1}$, which by a ΔG_r

of 64 kJ mol^{-1} of sulfate would correspond to 10^{-5} to $10^{-4} \text{ fmol SO}_4^{2-} \text{ cell}^{-1} \text{ d}^{-1}$. Further studies are required to understand whether this indeed represents an ultimate basal power requirement for anaerobic, respiring microorganisms and whether fermenting microorganism may potentially subsist at even lower energy turnover. In this respect, a compilation of cell specific maintenance power showed large variations but no systematic trend related to the catabolic pathway of the organisms (LaRowe and Amend, 2015b).

In comparison, the cell specific rates in pure cultures of sulfate reducing bacteria in a similar temperature range of $0\text{-}15^\circ\text{C}$ and with diverse organic carbon sources fall mostly in the range of $1\text{-}100 \text{ fmol cell}^{-1} \text{ d}^{-1}$ (Fig. 8.2a; Knoblauch and Jørgensen, 1999; Knoblauch *et al.*, 1999; Brüchert, 2004; Tarpgaard *et al.*, 2006). Thus, the cell specific catabolic rates at the sediment surface are two to three orders of magnitude lower than those in pure culture, and already a few metres deeper they are four to six orders of magnitude lower. This indicates that it may be **risky to extrapolate the physiological properties of pure cultures to deep subsurface communities** (Jørgensen and Marshall, 2016).

6.2 Pathways of Anaerobic Mineralisation

Both bacteria and archaea are engaged in the anaerobic degradation of complex organic matter derived from plants, algae, and marine zooplankton. Their extracellular enzymes initiate the microbial degradation of bulk organic matter by hydrolysing macromolecular structures to oligomeric or monomeric molecules that are sufficiently small to be taken up by heterotrophic microorganisms. Some marine bacteria may also take larger sized polysaccharides into the periplasmic space and thereby avoid the subsequent diffusion loss of dissolved hydrolysis products (Reintjes *et al.*, 2017). There is a large diversity and abundance of genes and gene transcripts in coastal marine sediments for the hydrolytic degradation and fermentation of proteins and carbohydrates (Zinke *et al.*, 2019).

Overall, the hydrolytic breakdown of organic detritus is extremely efficient, as only about 0.1 % of the organic matter produced by planktonic microalgae ultimately becomes buried in sediments (Hedges, 1992). Some substrates that are not hydrolysed while sinking through the water column are readily hydrolysed in the sediment where the microbial communities may have a different spectrum of hydrolytic enzymes (Arnosti, 2008). In the photic zone of the water column, phytoplankton blooms are known to trigger blooms of heterotrophic bacteria and archaea that produce hydrolytic enzymes with relevant specificities to degrade the microalgal biomass (*e.g.*, Needham and Fuhrman, 2016; Avci *et al.*, 2020). Such short lived blooms of heterotrophic, hydrolytic activity are not known to the same extent from sediments where the bulk organic matter has a slower turnover and the residence time of extracellular, hydrolytic enzymes may be longer.

An overview of the pathways of organic matter degradation by microorganisms in marine sediment is shown in Figure 6.3. The graph originates from a review I wrote during my PhD together with my supervisor, Tom Fenchel (Fenchel and Jørgensen, 1977). It was revised for a textbook on *Marine Geochemistry* (Jørgensen, 2006) and is presented here in an updated version with the classification of redox zones largely as suggested by Canfield and Thamdrup (2009). I introduced the term “redox cascade” for the depth sequence of oxidants and electron carriers.

Figure 6.3 Organic matter degradation pathways and terminal oxidation processes in marine sediment. Modified from Jørgensen (2006).

The hydrolysis of bulk organic matter by extracellular enzymes releases oligomers and monomers of sugars, amino acids, nucleic acids and lipids (Fig. 6.3). These are assimilated and respired directly by the sediment microbial communities or are used for fermentation. Fermentative microorganisms disproportionate the organic molecules into a narrow range of volatile organic acids, such as acetate, formate, propionate, and butyrate, plus H_2 and CO_2 . The products of hydrolysis and fermentation provide substrates for respiring microorganisms that utilise a depth sequence of electron acceptors, largely in accordance with their decreasing energy yield: O_2 , NO_3^- , $Mn(IV)$, $Fe(III)$, SO_4^{2-} and CO_2 . The decreasing energy yield is accompanied by a narrowing substrate spectrum utilised by the respiring microorganisms. Thus, in the methanic zone the terminal process largely depends on acetate and H_2 as energy sources and electron donors. The reduced products of all the respiration processes include: N_2 (and NH_4^+), Mn^{2+} , Fe^{2+} , H_2S and CH_4 ,

plus CO₂ and inorganic nutrients. Due to the resulting concentration gradients, these products tend to diffuse upwards. Most of them are oxidised again through a redox cascade of gradually increasing redox potential. A small fraction of the organic matter may be released from the sediment in dissolved form (Burdige *et al.*, 1992), while another small fraction resists degradation and is buried deeply into the sediment.

The schematic presentation of degradation pathways in Figure 6.3 is obviously highly simplified and the microbial food web in marine sediments is much more complex and diverse. The vertical zonation of the different terminal oxidation processes is often not distinct due to bioturbation and sediment heterogeneity. The individual processes tend to overlap because they are energetically not completely exclusive and because they include both dissolved and solid phase electron acceptors.

Our studies of organic matter degradation over the years have focused mostly on the production and turnover of the predominant fermentation products and their consumption by the terminal oxidation processes. The two main approaches to determine the *in situ* turnover rate of organic substrates were by ¹⁴C radiotracer experiments and by the use of specific inhibitors for either sulfate reduction or methanogenesis. It was a problem by the ¹⁴C tracer experiments with the quantitatively most important fermentation product, acetate, that a significant fraction was adsorbed to clay minerals in the sediment (Wang and Lee, 1993). The experiments therefore showed dual kinetics with a free pool of acetate that turned over fast and an adsorbed pool that turned over more slowly, probably in exchange with the free pool (Gibson *et al.*, 1989). This strongly complicated the calculation of total acetate turnover in the sediment (Christensen and Blackburn, 1982). Similar problems of differentiating a small free and a large adsorbed pool of amino acids or of methylamines in sediments (Wang and Lee, 1993; Zhuang *et al.*, 2017) have frustrated experimental studies of their turnover.

An approach to detect the role of different energy and carbon sources for sulfate reducing microorganisms has been the use of molybdate as a specific inhibitor of sulfate reduction (Oremland and Capone, 1988). Addition of 10–20 mM molybdate to a marine sediment from the sulfatic zone immediately stops sulfate reduction and consumption of the main electron donors (Gibson *et al.*, 1989). The fermentation processes continue, however, as they are not directly affected by molybdate. As a result, the fermentation products accumulate at a rate that initially reflects their steady state turnover before inhibition. We used this approach to show that acetate, propionate, butyrate and H₂ accounted for more than two thirds of all electron donors for the sulfate reducing bacteria in a coastal sediment (Sørensen *et al.*, 1981).

These ¹⁴C radiotracer and inhibition experiments have been used repeatedly in different modifications. Finke *et al.* (2007a) used selenate as inhibitor of volatile fatty acid (VFA) turnover and concurrently measured the turnover of ¹⁴C-labelled acetate and lactate. Christensen (1984) and Shaw and McIntosh (1990) injected molybdate directly into intact sediment cores to avoid mixing and dilution effects and followed the gradual accumulation of volatile fatty acids in the

porewater of parallel cores. **While the relative contributions of the different VFA's to sulfate reduction vary between different sediments and experiments, a general trend appears to be 40-50 % acetate, 10-20 % propionate, 10-20 % butyrate, and 5-10 % H₂** (Jørgensen *et al.*, 2019a). The accumulation of H₂ in such inhibition experiments potentially makes the fermentation of propionate and butyrate to acetate energetically unfavourable, which means that the relative role of acetate may be underestimated (Sørensen *et al.*, 1981).

The VFA's generally occur in low μM or sub- μM concentrations and therefore require high analytical sensitivity. Different HPLC methods are currently used in configurations that may include also isotope ratio mass spectrometry to detect their $\delta^{13}\text{C}$ composition, *e.g.*, of acetate. A survey in Baltic Sea sediments, from the surface to 100 m depth, showed that the VFAs are controlled at uniform and relatively low concentration by their consumers, both in the zones of sulfate reduction and methanogenesis, with only a slightly increasing trend with depth (Glombitza *et al.*, 2019) (Fig. 6.4). Concentrations were mostly 2-30 μM acetate, 0.3-3 μM propionate, 0.1-1 μM butyrate, and 1-10 μM formate.

Figure 6.4 Depth distributions of volatile fatty acids (VFA) in Baltic Sea sediments, from 1 cm to 100 m depth. Redrawn from Glombitza *et al.* (2019).

Figure 6.5 shows, as an example, data from Aarhus Bay sediment that has relatively high organic matter turnover. Acetate and formate concentrations increased from 2-3 μM near the sediment surface to 5-7 μM at 1-2 m depth (Fig. 6.5a,b). Propionate similarly increased from 0.5 μM to 1-3 μM (Fig. 6.5c). Butyrate was mostly 0.1-0.2 μM in the upper 0.6 m and dropped below detection in the deeper sediment (data not shown). The concentrations of these VFA's did not change systematically with the geochemical transition at 150 cm depth from

sulfatic to methanic sediment (Fig. 6.5d). The calculated Gibbs free energy by sulfate reduction from acetate showed a relatively large energy yield at the sediment surface of -70 kJ mol^{-1} acetate and a lower energy yield of -30 kJ mol^{-1} acetate subsurface (Fig. 6.5e). The indicated -20 kJ mol^{-1} corresponds to the expected minimum energy yield required for sulfate reduction (Hoehler *et al.*, 2001). The Gibbs free energy by acetoclastic methanogenesis was -30 kJ mol^{-1} acetate at the sediment surface and dropped to -10 to -20 kJ mol^{-1} acetate below, probably near the minimum required for this process (Fig. 6.5f).

Figure 6.5

Depth distributions in Aarhus Bay Station M1 sediments: (a) acetate, (b) formate, (c) propionate, (d) sulfate and methane, (e) Gibbs free energy by acetate-dependent sulfate reduction, (f) Gibbs free energy by acetate-dependent methanogenesis. The vertical, broken lines in frames (e) and (f) indicate the expected minimum free energy required for sulfate reduction and methanogenesis, respectively. Redrawn from Glombitza *et al.* (2019).

The idea that the **VFA concentrations are controlled by the minimum required energy yield** is supported by the observation that their concentration is independent of their turnover time. This was shown in a sediment from the continental shelf off southwest Greenland where the acetate concentration remained very constant at 4-9 μM with depth while its turnover time increased from 10 hr at the sediment surface to 4 years at 6 m depth (Glombitza *et al.*, 2015). It thus appeared that the acetate concentrations were controlled by the sulfate reducers at near their thermodynamic limit of 2-10 μM , just like H_2 concentrations are controlled at about 1-2 nM (Hoehler *et al.*, 1998).

6.3 The “Redox Cascade”

By the end of the 1970's, our small research group in Aarhus started the ambitious long term goal to study and quantify the main biogeochemical processes of the redox cascade in Danish coastal sediments. An important aim of the studies was to develop and apply methods that, as well as we could, allowed the determination of *in situ* rates of the processes.

Oxygen microelectrodes were developed by which the O_2 distribution could be measured for the first time (Revsbech *et al.*, 1980). The oxygen penetration into Aarhus Bay sediment was found to vary seasonally with shallowest penetration to 0.5-1 mm in summer and deepest penetration to 4-5 mm in winter (Fig. 6.6). Due to the high depth resolution of 50-100 μm , we detected a thin diffusive boundary layer (DBL), only a few hundred μm thick. The oxygen gradient could often be determined directly in the DBL and the oxygen flux calculated through a simple diffusion equation, thereby avoiding the problem of determining porosity and tortuosity in the sediment (Jørgensen and Revsbech, 1985):

$$\text{O}_2 \text{ flux} = D \times dC/dz \quad (6.1)$$

where D is the molecular diffusion coefficient of oxygen in seawater at the relevant temperature and dC/dz is the vertical O_2 gradient in the DBL. In the example shown in Figure 6.6, the O_2 gradient was 100 $\mu\text{M mm}^{-1}$, D was $1.23 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ (Schulz and Zabel, 2006) and the resulting O_2 flux was 11 $\text{mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$. Due to the topography of the sediment surface, the 3 dimensional diffusion flux is somewhat higher than this (Gundersen and Jørgensen, 1990; Røy *et al.*, 2002). In addition, the benthic macrofauna consumes oxygen and enhances the oxygen penetration by ventilating the burrows.

We added nitrate reduction measurements to the studies of oxygen respiration and sulfate reduction for the first time and found that, although important for the nitrogen cycle, it accounted for only a small fraction of the organic carbon mineralisation, even in a eutrophic fjord where the contributions were 65 % oxygen, 8 % nitrate and 27 % sulfate (Sørensen *et al.*, 1979; Jørgensen and Sørensen, 1985). Of the 8 % nitrate reduction, 40 % was due to denitrification to N_2 while 60 % was due to dissimilatory nitrate reduction to ammonium (DNRA). Later studies have shown that anammox also plays an important role

for the nitrogen cycling in these coastal marine sediments (Risgaard-Petersen *et al.*, 2003; Dalsgaard *et al.*, 2005). A large part of the nitrate does not originate from the water column but from nitrification of ammonium within the sediment (Seitzinger *et al.*, 2006). It was interesting that nitrate reduction showed a distinct peak in spring due to high nitrate concentration in the fjord water, while oxygen and sulfate reduction rather followed the seasonal temperature. This showed the **need for seasonal studies to catch the main activity of the different mineralisation processes.**

Figure 6.6 Oxygen in Aarhus Bay sediment measured by microelectrode. (a) O_2 profile measured during winter with O_2 penetration to 4–5 mm. (b) The same O_2 profile with expanded scales to show the gradient in the diffusive boundary layer (DBL). (c) Seasonal variation in O_2 penetration. From Jørgensen *et al.* (2001) with permission from John Wiley and Sons.

Metal oxide reduction was more difficult to measure correctly. We developed an incubation technique by which sediment samples were enclosed in gas tight plastic bags (Hansen *et al.*, 2000) and performed time course experiments with or without molybdate inhibitor to measure manganese, iron and sulfate reduction. **The direct role of manganese and iron respiration is difficult to determine due to chemical reactions between manganese, iron and sulfide.** Even in low sulfate sediments, iron reduction may be dominated by reaction with sulfide rather than by a microbial iron reduction (Hansel *et al.*, 2015). A possible approach is therefore to combine measurements of the production of DIC or ammonium, and of sulfate reduction, during anaerobic bag incubations. The part of DIC or ammonium production that cannot be attributed to sulfate respiration is then assumed to be due to metal reduction (Canfield *et al.*, 1993b). The use of this approach in the upper 0–10 cm of Danish coastal sediments showed how

strongly the relative contribution of the anaerobic processes to total mineralisation changed with sediment depth and water depth (Canfield *et al.*, 1993a,b; Thamdrup *et al.*, 1994a).

In the organic-rich shallow water sediment of Aarhus Bay, sulfate reduction was the dominant electron acceptor for the anaerobic oxidation of organic matter. Manganese reduction corresponded to about 25 % of the sulfate reduction on an areal basis while iron reduction was only 12 %, of which two thirds of the produced iron(II) precipitated with sulfide (Thamdrup *et al.*, 1994a). At intermediate water depths of 200-400 m in the Skagerrak, manganese plus iron reduction accounted for 21-78 % of the anaerobic carbon oxidation in the top 0-10 cm, mostly due to iron reduction (Canfield *et al.*, 1993a,b). A deep station at 700 m in the Skagerrak functioned as an effective trap for manganese from surrounding waters and had an extremely high manganese content of 3.5 % dry weight. The anaerobic mineralisation was here dominated by manganese reduction. Manganese and iron in these shelf sediments function as electron shuttles between the reduction by organic matter or sulfide oxidation and the oxidation by oxygen or, for iron(II), by manganese(IV). Due to bioturbation, each manganese or iron atom was in average recycled between the oxidised and reduced phase 100-300 times before being buried down into the sulfidic sediment (Canfield *et al.*, 1993a).

Iron reducers and sulfate reducers compete for similar organic substrates and, although the iron reducers may be favoured by the higher energy yield of their respiratory metabolism, they are limited by the availability of their solid phase electron acceptor (diverse Fe(III) minerals), which the sulfate reducers are not. Thus, sulfate reduction can be detected at relatively high rates in oxidised surface sediments rich in Fe(III), such as that shown in Figure 6.7a. Even in an arctic fjord of sediment that is bright red from a high content of hematite, Fe_2O_3 , originating from a local glacier (Fig. 6.7b,c), we measured sulfate reduction rates of 2-10 $\text{nmol cm}^{-3} \text{ d}^{-1}$, with a peak rate of 25 $\text{nmol cm}^{-3} \text{ d}^{-1}$ in the top 0-1 cm (Jørgensen *et al.*, 2020a). This shows that **hematite, like other crystalline iron minerals, is not very reactive towards microbial reduction** (Michaud *et al.*, 2020). Other arctic fjord sediments of Svalbard showed that oxygen respiration accounted for 53 % of the carbon oxidation, sulfate reduction for 34 %, and iron reduction for 13 %, while manganese reduction was not detectable (Vandieken *et al.*, 2006).

Sulfate reduction in the manganous and ferruginous zones has little effect on the sulfate gradient as the produced sulfide is effectively oxidised back to sulfate by the metal oxides (Findlay *et al.*, 2020). The co-occurrence of processes in this zone is illustrated in Figure 6.8 with data obtained from Aarhus Bay (Thamdrup *et al.*, 1994a). Manganese reduction took place in the upper 0-1 cm (Fig. 6.8a), below which most of the reactive Mn(IV) had been depleted (Fig. 6.8b). Reduction of reactive Fe(III) created a peak of Fe^{2+} at 3-4 cm depth (Fig. 6.8a). This was also the depth of highest sulfate reduction rates (Fig. 6.8c). The concurrent iron and sulfate reduction led to the rapid formation of FeS and FeS_2 and to free sulfide (H_2S) accumulation below 4 cm depth (Fig. 6.8a).

Figure 6.7

(a) Sediment core from Kattegat showing zonation with 4-5 cm brown, oxidised sediment on top and black, sulfidic sediment below. Oxygen penetrated only 4 mm. (b) Aerial photo of a proglacial landscape in Svalbard with hematite-rich silt flowing into the adjacent fjord, Kongsfjorden. (c) Sediment core from Kongsfjorden, coloured bright red from hematite. (a) and (c) From Jørgensen *et al.* (2020a) with permission from John Wiley and Sons; (b) photo: Bo Barker Jørgensen.

Figure 6.8

Chemical profiles from Aarhus Bay. (a) Porewater concentrations of dissolved manganese, iron and sulfide. (b) Solid phase manganese(IV), iron(III) and pyrite (FeS_2). (c) Depth distribution of sulfate reduction rates. Redrawn from Thamdrup *et al.* (1994a).

Thus, iron reduction and sulfate reduction took place concurrently in the oxidised zone at 0-4 cm depth where the sulfide concentration remained below our detection limit of 1 μM . From the rate of H_2S production, it could be calculated that free sulfide was turning over within less than 30 min (Thamdrup *et al.*, 1994a). The reactivity of Fe(III) is stimulated by bioturbation, which maintains a more reactive iron pool relative to the sulfidic sediment below (Canfield *et al.*, 1993b; Antler *et al.*, 2019). As a result, this zone is inhabited by a large physiological diversity of iron oxidising and iron reducing bacteria (Laufer *et al.*, 2016).

Methane is produced in the sulfate depleted subsurface sediments through two main pathways: a) reduction of CO₂ with H₂ as the electron donor (hydrogenotrophic methanogenesis; Eq. 7.1), and b) dismutation of acetate (aceticlastic methanogenesis; Eq. 7.2) (e.g., Rudd and Taylor, 1980; Conrad, 2005):

In lake sediments, methane production has generally been found to be dominated by aceticlastic methanogenesis, in particular at low *in situ* temperature (e.g., Schulz and Conrad, 1996; Glissmann *et al.*, 2004). With depth in lake sediments, where the buried organic matter becomes less degradable and the microbial communities are more strongly energy limited, hydrogenotrophic methanogenesis becomes relatively more important (Chan *et al.*, 2005; Liu *et al.*, 2017). Parallel to this depth trend, the communities of methanogenic archaea change from mainly aceticlastic Methanosaetaceae to hydrogenotrophic Methanomicrobiales phenotypes. In addition to these well established phyla, metagenome-based genomes show that the ability to produce methane is more broadly represented among the archaea than previously recognised (Evans *et al.*, 2015; Lever, 2016).

In marine sediments, hydrogenotrophic methanogenesis predominates (Oremland and Taylor, 1978; Whiticar *et al.*, 1986; Crill and Martens, 1986; Burdige *et al.*, 2016; Beulig *et al.*, 2018). Due to the deep sulfatic zone, methanogenesis starts only at a subsurface depth where the buried organic matter is already quite refractory to microbial degradation. This might explain the difference in terminal methanogenic pathways in freshwater and marine sediments. Interestingly, acetate is just as important a fermentation product in marine sediments as in freshwater sediments, accounting for about 40 % of the overall fermentation pathway (*cf.* Beulig *et al.*, 2019), but **acetate seems not to be used directly by methanogens in marine sediments**. Instead, ¹⁴C-labelled acetate is oxidised to CO₂ by bacteria and only the electrons, but not the carbon, are transferred to the methanogens (Beulig *et al.*, 2019). Such an interspecies electron transfer has been detected among a number of syntrophic bacteria and archaea. Rotaru *et al.* (2018) discovered in Baltic Sea sediments that the syntrophy between acetate oxidising bacteria and autotrophic methanogens was not a direct electron transfer from cell to cell. The electrons from acetate were transferred from the bacteria to conducting mineral particles, such as black carbon, magnetite or pyrite, and from there taken up by the archaea and used to reduce CO₂ to CH₄.

7.1 The Methanic Zone

Methane accumulates in marine sediments beneath the main sulfatic zone, as methanogens are not able to compete effectively with sulfate reducing bacteria for common substrates, in particular not for acetate and H_2 . The available free energy by sulfate reduction is larger than by methane production in the sulfatic zone (e.g., Fig. 6.6). Methanogenesis therefore takes over as the predominant terminal process only when the organic matter has been buried beneath the depth of sulfate penetration.

Methane production rates are generally quantified by transport reaction modelling or by experimental rate measurements using ^{14}C -radiotracer. About 80 % of the global methane production takes place in shelf sediments (Egger *et al.*, 2018), well below the bioturbated zone, and most of the methane is lost again by molecular diffusion along the concentration gradient up to the sulfate-methane transition (SMT). A simple calculation of the methane flux is (e.g., Boudreau, 1997):

$$\text{Flux} = \varphi D_s dC/dz \quad (7.3)$$

where φ is the porosity, D_s is the whole sediment diffusion coefficient at *in situ* temperature (corrected for tortuosity), and dC/dz is the vertical concentration gradient of methane in the porewater. We determined diffusion coefficients of methane in marine sediments experimentally using ^{14}C -labelled methane (Iversen and Jørgensen, 1993).

Figure 7.1a shows an example of sulfate and methane profiles from the Bornholm Basin in the Baltic Sea. The sediment core was taken at a water depth of 96 m and has a very shallow SMT depth of 0.38 m (Beulig *et al.*, 2018). A near linear methane profile was observed below the SMT and used to calculate an upwards diffusion flux of $140 \text{ nmol CH}_4 \text{ cm}^{-2} \text{ d}^{-1}$. Already at 0.65 m depth the methane concentration exceeded 5–6 mM. At the *in situ* temperature of 7°C and salinity of 17, the methane solubility at 1 bar was only 1.87 mM (Yamamoto *et al.*, 1976). The data started to become unreliable at 3–5 bar methane partial pressure. Methane data from beneath 0.65 m depth that fall below the linear trend are thus affected by outgassing when the sediment cores were retrieved and subsampled. The porosity and the viscoelastic properties of the sediment affect the rate of bubble growth upon core retrieval and thereby interfere with how much methane escapes through gas bubbles and cracks (e.g., Boudreau, 2012; Egger *et al.*, 2017; Hilligsøe *et al.*, 2018). To **avoid false interpretation of compromised methane data**, I used crosses as symbols to mark data that are expectedly incorrect due to degassing (Fig. 7.1a; cf. Jørgensen *et al.*, 2020b).

Measured rates of methanogenesis using ^{14}C radiotracer were very low, but detectable, throughout the sulfatic zone and peaked in the SMT (Fig. 7.1b). Below the SMT, rates dropped steeply with depth as fitted by the power law: methanogenesis rate = $0.7 \times z^{-2.0} \text{ nmol CH}_4 \text{ cm}^{-3} \text{ d}^{-1}$, where z is the sediment depth in metres. When calculated as a cumulative, areal rate in $\text{nmol CH}_4 \text{ cm}^{-2}$

d^{-1} from the SMT and down to 5 m depth, most of the methanogenesis was found to take place in the uppermost part of the methanic zone (Fig. 7.1c). The total thickness of the methane-producing Holocene mud was 9 m, but the additional methanogenesis below 5 m was minimal. Thus, the methane was largely produced within the upwards-directed gradient, which forced the methane to diffuse up to the SMT. The calculated diffusive flux is therefore representative of most of the methane production in the entire sediment column. The cumulative rate of methanogenesis calculated from the ^{14}C experimental rate data was $150 \text{ nmol CH}_4 \text{ cm}^{-2} \text{ d}^{-1}$, which is very close to the calculated diffusive flux to the SMT of $140 \text{ nmol CH}_4 \text{ cm}^{-2} \text{ d}^{-1}$. Given the high quality of methane data used for the diffusive flux calculations to the SMT, this agreement supports the validity of the ^{14}C experimental rate measurements (cf. Hølligsøe *et al.*, 2018).

Figure 7.1

Methane turnover in Baltic Sea sediment from Station BB03 in the Bornholm Basin. (a) Sulfate and methane profiles. The sulfate-methane transition (SMT) is shown by a gray shade. Methane data that are compromised due to degassing are distinguished by "x" symbols. The calculated diffusion flux of methane is shown. (b) Rates of methanogenesis measured by ^{14}C experiments using radiolabelled DIC (dissolved inorganic carbon). Rates in the methanic zone are fitted by a power law (black line) in this double logarithmic plot. (c) Methanogenesis rates per unit area accumulated with depth beneath the SMT. Redrawn from Beulig *et al.* (2018) and Jørgensen *et al.* (2019a).

An important observation from the Bornholm Basin was a log-log linear relation between depth and rate of organic matter mineralisation, by which the same power law continued throughout the sulfatic zone and the methanic zone (Beulig *et al.*, 2018). Such a continuity suggests that **the intrinsic rate of organic matter mineralisation is independent of whether the terminal process is sulfate reduction or methanogenesis**. The actual step in the degradation pathway that controls the mineralisation rates has been questioned in the literature and may still be open to discussion based on different types of experiments and transport reaction modelling (Burdige and Gardner, 1998; Arnosti,

2004). However, the assumption of continuity in the power law of organic matter degradation with sediment depth and age has been used also in our earlier studies, where we extrapolated measured sulfate reduction rates down through the methanic zone and compared the estimated, cumulative rate of methanogenesis with the methane flux coming back up to the SMT. The general observation from these comparisons has been that the extrapolated rate data and the upwards diffusion flux of methane roughly match (Jørgensen and Parkes, 2010; Jørgensen *et al.*, 2019a). I consider this an **independent validation of the ^{35}S -SRR rate measurements**.

7.2 Controls on Methane Production

Due to the steep power law decrease in mineralisation rates with depth and age in the sediment, the overall rate of methane production is very sensitive to small variations in the depth of the SMT. Only from the SMT and downwards are the methanogens relieved of competition from the sulfate reducers and can dominate the terminal degradation step. For this reason, small changes in the SMT position will strongly affect the overall methanogenesis rate. This affects the geographic distribution of methane production, both on local, regional and global scales. In the following, I will give examples of the three scales.

7.2.1 The local scale

Hotspots of methane production in inner shelf sediments are often revealed by the occurrence of free gas bubbles in the upper sediment column. This “shallow gas” causes a strong reflectance of sound waves and “acoustic blanking” during seismo-acoustic surveys for stratigraphic mapping of the seabed. While this is a nuisance for the geophysicists, it can be a blessing for the geochemists. The distribution and depth of shallow gas, combined with information on water depth, temperature and salinity, can be used to map where and how deep the methane partial pressure exceeds the local hydrostatic pressure and, thereby, what is the methane concentration at that depth. Such data have been used to map the geographic distribution of shallow gas, which occurs globally along the continental margins (Fleischer *et al.*, 2001). Shallow gas is also present in many areas of the Baltic Sea - North Sea transition, primarily in local basins where organic-rich mud has accumulated during the Holocene and is now producing abundant methane beneath a relatively shallow SMT (Laier and Jensen, 2007) (Fig. 7.2).

Aarhus Bay is such a sedimentation basin and has several areas of shallow gas (Fig. 7.2). The sediment has a rather even depth contour in the central bay, while the buried landscape of underlying sand and glacial till has much more irregular depth contours, shaped by glaciers more than 10,000 years ago. In order to understand the relationship between gas distribution and the controls on methane production, we studied a 900 m long transect crossing from non-gassy into gassy sediment (Flury *et al.*, 2016). Acoustic data were used to describe the

stratigraphy of the sediment, while multiple gravity cores were taken to analyse sulfate, methane, sulfate reduction rates and other parameters. Figure 7.3a shows that the sediment surface is uniform with hardly any slope along the transect. The marine mud, however, drapes a glacial landscape and hides its topography by a mud layer of varying thickness. This mud layer is organic-rich and supports intensive sulfate reduction and methanogenesis.

Figure 7.2 Occurrence of shallow gas (red signature) in marine sediments from the western Baltic Sea to the North Sea. The map is based on a dense network of seismo-acoustic transects that record the occurrence and depth of the gas. From Laier and Jensen (2007).

Where the marine mud exceeds a thickness of 8 m, gas bubbles appear. Data from three out of twelve 6 m long gravity cores are presented in Figure 7.3b. They show how the SMT becomes shallower over a short distance as the thickness of the marine mud layer increases and we transit from non-gassy into gassy sediment. Detailed analyses of sediment stratigraphy, geochemical profiles, and sulfate reduction rates show that the relative shift from low to high methane production was triggered by the deepening of the mud layer. This is schematically shown in Figure 7.3b where the methanogenic sediment zone (from 4.2 to 7.4 m depth) at Station M24 produced $2.0 \text{ nmol CH}_4 \text{ cm}^{-2} \text{ d}^{-1}$. Going into the gassy sediment, that zone had the same overall methanogenesis rate, but due to the

Figure 7.3

(a) Acoustic (Chirp) transect from Aarhus Bay going from gas-free to gas-loaded sediment. The sediment depth is calculated relative to the sediment surface to the extreme left. Holocene marine mud overlays post-glacial sand and glacial till, shifting at the broken line. (b) Three 6 m deep gravity cores were analysed for sulfate and methane. The sulfate-methane transition (gray shading) and the sand and till deposit (light olive shading) beneath the marine mud are shown. Blue, black and green numbers show the methane production rate in each of the three depth intervals bounded by broken lines. For further explanation, see text. Redrawn from Flury *et al.* (2016).

increasing mud thickness the deeper sediment column now produced additional methane at a rate growing to $+0.2 \text{ nmol CH}_4 \text{ cm}^{-2} \text{ d}^{-1}$ at M27 and $+0.3 \text{ nmol CH}_4 \text{ cm}^{-2} \text{ d}^{-1}$ at M29. Since the deepest sediment was the oldest and had the lowest organic carbon reactivity, this contribution was small compared to the $2.0 \text{ nmol CH}_4 \text{ cm}^{-2} \text{ d}^{-1}$. However, it caused a small increase in the upward flux of methane, which pushed the SMT upwards. As the methanogenic zone thereby expanded up into younger sediment, it strongly stimulated methane production by $+0.8 \text{ nmol CH}_4 \text{ cm}^{-2} \text{ d}^{-1}$ at M27 and $+4.0 \text{ nmol CH}_4 \text{ cm}^{-2} \text{ d}^{-1}$ at M29, which

thereby enhanced the upwards shift in SMT depth. As a result, the upwards expansion of the methanogenic sediment became the main additional source of methane in the sediment column. This **positive feedback**, triggered by a deepening of the methanogenic mud layer, stimulated methane accumulation to the extent that gas bubbles formed. The high sensitivity of the feedback was demonstrated by a 3 fold increase in overall methanogenesis rate (from 2.0 to 6.3 nmol CH₄ cm⁻² d⁻¹) by only a 0.2 fold increase in overall mud thickness (from 7.6 to 9.2 m depth). This conclusion was supported by 15 additional sediment cores along the same transect (Flury *et al.*, 2016).

By these calculations it is interesting that the upwards shift of the SMT position, which reduced the sulfatic zone from 4.2 to 2.2 m thickness between Station M24 and M29, had little effect on the overall, areal sulfate reduction rates because the change occurred at the bottom of the sulfatic zone where the organic matter reactivity was anyway low. Exactly how much sulfate reduction changed could be calculated from multiple ³⁵S-SRR measurements (Flury *et al.*, 2016; Jørgensen *et al.*, 2019b) (Fig. 3.5). The upwards shift of the SMT depth between M24 and M29 (Fig. 7.3b) resulted in a drop in the areal rate of organo-clastic sulfate reduction by only 1 %. This small change was accompanied by an increase in the relative contribution of anaerobic methane oxidation to the total sulfate reduction from 0.5 % to 1.7 %, corresponding to a 3 fold increase in the overall methane production.

In conclusion, **although sulfate and methane profiles may indicate that methane oxidation controls a large part of the sulfate consumption in gassy sediment, the overall contribution of methane was only 1-2 %.**

7.2.2 The regional scale

The combination of acoustic transects with sediment cores for geochemical analyses and microbial process measurements is a powerful approach to understand controls on both methane production and sulfate reduction rates. Without such a combination, we would not have understood why the sulfate and methane zonation could vary so strongly within a few hundred metres of an apparently homogenous seabed in a flat sedimentation basin (Fig. 7.3). In fact, I wish I had realised this much earlier during our many years of biogeochemical studies in marine sediments. It is now clear that the overall methane production is highly sensitive to the thickness of marine mud deposited on the continental shelf during the Holocene.

Sediment coring combined with chemical and microbiological analyses is a very time consuming and costly approach to map sediment biogeochemistry. Seismo-acoustic surveys, on the other hand, are ideally suited to map the depth of shallow gas and the thickness of the Holocene mud layer. In combination, the sediment cores provide ground truth to the stratigraphy deduced from the geophysical data and can relate these data to the methane distribution and other parameters. We used such a combination in the 90-100 m deep Bornholm Basin in the southwestern Baltic Sea (Fig. 7.4a) (Hilligsøe *et al.*, 2018). The transition

from post-glacial Ice Lake clay to marine mud was distinct in the echograms and showed, like in Aarhus Bay, how the flat Holocene mud draped a glacial landscape, with marine mud thicknesses varying from <2 m to >20 m (Fig. 7.4b). Based on multiple sediment cores, we developed an algorithm for the relationship between Holocene mud layer thickness, SMT depth, and methane flux. With this Holocene mud layer model we mapped the distribution of methane fluxes in the basin and showed how strongly the high fluxes were focused into hotspots compared to surrounding areas devoid of methane (Fig. 7.4c). Another model was developed for the relationship between free gas depth, SMT depth, and methane flux. A map constructed from this model showed a rather similar distribution of methane fluxes, yet even more focused towards hotspots of methane production (Fig. 7.4d). The total methane fluxes calculated from the two independent models were very similar, 17×10^6 and 15×10^6 g C_{org} per day. The 13 % lower estimate by the free gas depth model was explained by the peripheral areas, which had low methane fluxes, but no free gas, and were therefore included in the Holocene mud layer model but not in the free gas depth model.

Figure 7.4

Hotspots of methanogenesis in the seabed. (a) Bathymetric map of the SW Baltic Sea and the gateway to the North Sea. The studied area in the Bornholm Basin is framed. (b) Thickness of the organic-rich, Holocene mud layer. (c) Distribution of methane fluxes to the SMT modelled from the Holocene

mud layer thickness. **(d)** Distribution of methane fluxes to the SMT modelled from the depth of free gas. From Hilligsøe *et al.* (2018) with permission from Elsevier.

Further studies have shown how **seismo-acoustic mapping, combined with ground truthing by sediment cores, provides a powerful tool for the remote quantification and mapping of methane fluxes in the coastal seabed** (Dale *et al.*, 2009; Mogollón *et al.*, 2013). The approach is also efficient for the mapping of sulfate fluxes to the SMT and, thus, of the methane-dependent sulfate reduction. However, since most sulfate reduction takes place in the upper few dm of the rather uniform sediments, and since methane provides only a very small fraction of the substrate for sulfate reduction, the distribution of total sulfate reduction in the basin shows no clear dependence on the methane flux. In contrast, **methane production is very sensitive to the sedimentation rate, and a positive feedback mechanism focuses methane production into hotspots.**

7.2.3 The global scale

These mechanisms that focus methane production to hotspots of high sedimentation rate on the continental shelf also operate on a global scale where **seafloor methane fluxes are strongly skewed towards the ocean margins**. This is illustrated by the global map of SMT depth distribution in Figure 7.5 (Egger *et al.*, 2018). On the continental shelf the SMT depths range from <1 m to several tens of metres. Down along the continental slope and rise, the SMT becomes gradually deeper and ultimately exceeds 100 m, except for ocean areas with high primary productivity in the photic water column. For two thirds of the abyssal plains, there is no SMT because sulfate is not depleted but reaches down to the ocean crust and prevents the formation of a methanic zone. D'Hondt *et al.* (2015) estimated that even oxygen reaches down to the ocean crust in 9-37 % of the global sea floor.

Shallow SMT depths of one to a few metres, and high fluxes of methane, occur on the inner continental shelf where sedimentation rates are high during the present interglacial period of high sea level stand. Of the total methane flux in the global seabed, 80 % occurs on the continental shelf at 0-200 m water depth, which covers only 7 % of the total ocean area (Egger *et al.*, 2018). It follows that the methane-dependent sulfate reduction is similarly skewed towards the continental margins, even more skewed than the overall sulfate reduction (Section 8).

In a double logarithmic plot, the sulfate fluxes (J_{SO_4} , $\text{mmol m}^{-2} \text{d}^{-1}$) and the methane fluxes (J_{CH_4} , $\text{mmol m}^{-2} \text{d}^{-1}$) to the SMT are inversely proportional to the SMT depth (Egger *et al.*, 2018). Thus, shallow SMT depths along the ocean margins correspond to high methane fluxes and high methane-dependent sulfate reduction rates. The global fluxes of SO_4^{2-} and CH_4 to the SMT range over 10,000 fold, from 0.001 to 10 $\text{mmol m}^{-2} \text{d}^{-1}$.

Figure 7.5 Global distribution of the sulfate-methane transition (SMT) depth in the seabed. Depths are given as mbsf (metres below sea floor). Light blue indicates absence of methane. From Egger *et al.* (2018).

7.3 Quantitative Role of Methane for Sulfate Reduction

As discussed before, the degradation rate constant of organic matter drops according to a power law with increasing age and depth in the sediment (Fig. 6.1; Middelburg, 1989). The reactivity of sediment organic matter is therefore strongly reduced once it is buried beneath the sulfatic zone. Based on measured ^{35}S -SRR and modelled CH_4 fluxes, **areal rates of methanogenesis generally correspond to only 1-10 % of the total sulfate reduction rate** (Jørgensen and Parkes, 2010; Iversen and Jørgensen, 1985; Fossing *et al.*, 2000). Such a small contribution of methane to the total sulfate reduction in the sediment column is in stark contrast to the frequently observed quasi-linear diffusion gradients of sulfate, from near the sediment surface and down to the SMT. Such gradients seem to imply that sulfate reduction is primarily driven by methane oxidation (Borowski *et al.*, 1996; Berelson *et al.*, 2005; Meister *et al.*, 2013; Burdige *et al.*, 2016). In Section 3.4.3 I argued that this conclusion is generally not correct and discussed the reasons for the discrepancy between measured organoclastic SRR and modelled methane-based SRR. Thus, even a slightly steeper sulfate gradient near the sediment surface reveals that rates of reduction are here higher than indicated by a linear sulfate gradient (Burdige *et al.*, 2016).

Not all methane production takes place in the main methanogenic zone. Experiments using a ^{13}C CH_4 isotope dilution method showed a peak of methylotrophic methanogenesis near the sediment surface where fresh organic detritus, rich in methylated compounds, is deposited (Xiao *et al.*, 2018). Methanogenic archaea related to methylotrophic and autotrophic species, but not to acetoclastic species, were also abundant near the sediment surface. Although the surface peak of methane production was distinct, it was still less than 1 % of the total sulfate

reduction (Iversen and Blackburn, 1981; Xiao *et al.*, 2017). The produced methane is rapidly oxidised, either aerobically or anaerobically, which drives a cryptic methane cycle in the upper few cm of marine sediments (Riedinger *et al.*, 2010; Xiao *et al.*, 2017, 2018).

Although sulfate reducers effectively outcompete methanogens for H₂ (Hoehler *et al.*, 1998, 2001), acetate may in some sediments occur at sufficiently high concentration to make acetoclastic methanogenesis energetically favourable (Glombitza *et al.*, 2019). Methanogenic archaea are also observed at low abundance throughout the sulfatic zone. The co-existence of methanogens and sulfate reducers may have the interesting effect that the methanogens produce H₂ instead of CH₄. In the presence of a strong H₂ scavenger, the conversion of acetate to CO₂ and H₂ is energetically more favourable for the methanogens than its conversion to CO₂ and CH₄ (Ozuolmez *et al.*, 2015). By non-competitive substrates, such as methanol or methylamine, H₂ scavenging by sulfate reducers did indeed induce the methanogens to convert the substrates to CO₂ and H₂ instead of to CH₄ (Finke *et al.*, 2007b).

7.4 Processes at the Sulfate-Methane Transition (SMT)

Methane that diffuses up into the SMT is quantitatively oxidised with sulfate to CO₂:

The stoichiometry of the reaction is 1:1, and in many cases this ratio has been confirmed by modelling of sulfate and methane profiles and calculating their fluxes. Yet, the flux ratio of sulfate and methane into the SMT often shows an excess of sulfate reduction relative to methane oxidation. This was shown by a compilation of 740 combined sulfate and methane datasets from the global seabed. The diffusion fluxes of sulfate and methane to the SMT showed significant scatter with a mean ratio for all data of 1.4:1, *i.e.* a **40 % excess of sulfate relative to methane** (Fig. 7.6). The ratio is not dependent on the overall fluxes or on the depth of the SMT. It is therefore not clear whether the scatter is due to non-steady state, to non-optimal data quality, or to other causes (Egger *et al.*, 2018).

There is, however, an explanation why the sulfate flux should exceed the methane flux, because the organic matter buried down into the SMT continues to serve as substrate for sulfate reduction. This **organoclastic sulfate reduction in the SMT** has been found by transport reaction modelling to be 35-45 %, or even more than half, of the total sulfate reduction in the SMT in sediment basins off southern California (Berelson *et al.*, 2005; Komada *et al.*, 2016). In order to test this, we used an extrapolation down through the SMT of the power law for sulfate reduction rates determined from ³⁵S tracer experiments.

Figure 7.6

(a) Sulfate flux versus methane flux to the SMT. Data are from ODP/IODP cores (orange symbols) and from other cores (green symbols). The theoretical 1:1 ratio for anaerobic oxidation of methane is shown (red line). (b) Ratio between sulfate flux and methane flux relative to the methane flux. The theoretical 1:1 ratio (red line) and the mean 1.4:1 ratio (black line) are shown together with the standard deviation of the ratios (shading). Redrawn from Egger *et al.* (2018).

An example is shown in Figure 7.7 where the SMT is located at 300–340 cm depth in the sediment. The power law distribution of ^{35}S measured organoclastic SRR was taken from Figure 3.5a, while the anaerobic oxidation of methane (AOM) was calculated by diffusion reaction modelling of the methane profile (Jørgensen *et al.*, 2019b) (Fig. 7.b). Out of the total SRR in the SMT ($10.0 \text{ nmol cm}^{-2} \text{d}^{-1}$), calculated from the diffusion flux of SO_4^{2-} down into the SMT, only $5.8 \text{ nmol cm}^{-2} \text{d}^{-1}$ or 58 % was used for methane oxidation. As shown by the ^{35}S -SRR data, the remaining 42 % was organoclastic sulfate reduction, driven by the oxidation of buried organic matter. We made similar calculations for different sediments in the Baltic Sea – North Sea transition and found that organoclastic sulfate reduction accounted for 14–59 % of the total sulfate reduction in the SMT (Jørgensen *et al.*, 2019b). A similar estimate by Berelson *et al.* (2005) yielded even 40–80 % organoclastic sulfate reduction in the SMT.

The SMT is the zone where the net oxidation of methane takes place. However, detailed radiotracer experiments have revealed that there is also **a cryptic methane cycle within the SMT**. Organoclastic sulfate reduction in the SMT is different from that in the overlying sulfatic zone because it competes with methanogenesis at the low sulfate concentration. In Baltic Sea sediments we found that up to 60 % of the organic matter that was oxidised within the SMT was first converted to methane (Beulig *et al.*, 2019). All this methane was concurrently oxidised to CO_2 by sulfate reduction and was therefore not detectable by modelling of the porewater gradients. The general importance of such a cryptic methane cycle in the SMT remains to be determined, but in the Baltic Sea, it accounted for the highest rates of methanogenesis in the entire sediment column (Fig. 7.1b).

Figure 7.7

(a) The sulfate and methane profiles define the sulfate-methane transition (SMT) at 300–340 cm depth in Aarhus Bay, Station M27. (b) A comparison of ^{35}S measured sulfate reduction rates (SRR) and modelled anaerobic oxidation of methane (AOM) shows how the organoclastic sulfate reduction continues down into the SMT. Redrawn from Jørgensen *et al.* (2019; SRR data from Fig 3.6a).

It is interesting that **methanogenesis and methane oxidation may occur concurrently in the same sediment**. Thermodynamic calculations in Baltic Sea sediments suggested that both processes were exergonic, but close to thermodynamic equilibrium, with Gibbs free energy of -10 kJ mol^{-1} for acetoclastic methanogenesis and -15 to -20 kJ mol^{-1} CH_4 for AOM (Beulig *et al.*, 2019). The depth range where AOM is exergonic is broad and includes both the SMT and the lower sulfatic zone. The depth of peak AOM rates, in contrast, generally falls in the lower part of the SMT and coincides with the maximum kinetic drive of the process, rather than with the maximum energetic drive (Knab *et al.*, 2008).

Anaerobic oxidation of methane may also be driven by oxidised forms of iron or manganese. This has been shown in laboratory experiments with marine and freshwater sediments in which ANME-2 archaea (ANME = anaerobic methane oxidisers) oxidised methane using soluble Fe(III) complexes and nanoparticles or magnetite (Beal *et al.*, 2009; Ettwig *et al.*, 2016; Aromokeye *et al.*, 2020). Continuous bioreactor cultivation of freshwater sediment ultimately enriched archaea of the family *Methanoperedenaceae* (ANME-2d), able to couple methane oxidation to the reduction of Mn(IV) oxides (Leu *et al.*, 2020). Iron-dependent methane oxidation has also been inferred from biogeochemical modelling of freshwater sediments (Sivan *et al.*, 2011) and of marine sediments from

the Argentine Basin and the Baltic Sea (Riedinger *et al.*, 2014; Egger *et al.*, 2017). Those marine sediments are characterised by iron-rich layers beneath the sulfatic zone where the co-existence of high Fe(III) and high methane concentrations could support the process. The potential free energy yield calculated by Riedinger *et al.* (2014) was surprisingly high in the Argentine Basin, -150 to -170 kJ mol⁻¹ CH₄, by the reaction:

The actual Fe minerals involved are not known but are important for the energy yield and reactivity. Considering that the Fe(III) and methane co-exist over many thousands of years in the sediment, which should provide sufficient time for the relevant microbial communities to develop and grow, the **iron-based methane oxidation is extremely sluggish**. It may be that the process rates are constrained by a low reactivity of the buried iron minerals towards microbial reduction. The Fe(III) reactivity should therefore be tested using a bioassay for potential bacterial iron reduction in marine sediments. Methane may also be oxidised by nitrate or nitrite reducing denitrifying bacteria by a unique oxygen generating pathway (Raghoebarsing *et al.*, 2006). That process has been documented in freshwater sediments (*e.g.*, Nordin and Thamdrup, 2014), but it expectedly plays little role in marine sediments due to the deeply seated methanic zone.

7.5 Anaerobic Oxidation of Methane (AOM)

Although the general zonation of sulfate and methane has been known to marine geochemists since the late 1960's (Reeburgh, 2007), many microbiologists remained skeptical about the existence of anaerobic oxidation of methane as it required an unknown activation of the methane and an unknown pathway for its anaerobic oxidation. This changed when Antje Boetius discovered **consortia of bacteria and archaea that were apparently responsible for the ongoing AOM** (Boetius *et al.*, 2000). She stained microbial aggregates from gas hydrate-rich sediments with two different 16S rRNA targeted oligonucleotide probes for fluorescence *in situ* hybridisation (FISH), with a red fluorescent probe targeting archaea and a green fluorescent probe targeting sulfate reducing bacteria. The resulting images of red and green fluorescent aggregates have since been detected worldwide and have become iconic for the AOM process. The consortia have diverse morphologies and occur in highly diverse, methane-rich environments (Knittel and Boetius, 2009; Ruff *et al.*, 2015). Distinct AOM cell aggregates are most abundant where methane-rich fluid or gas mixes with sulfate-rich seawater. This is the case by many hot springs, cold seeps, mud volcanoes and other advective systems in the seabed (*e.g.*, Michaelis *et al.*, 2002; Treude *et al.*, 2005a,b; Knittel *et al.*, 2005; Niemann *et al.*, 2006).

Soon after their discovery, it was suggested that the archaea attack the methane and oxidise it to CO₂ while the sulfate reducers receive some form of reducing equivalents from the archaea and transfer them to sulfate (Knittel

and Boetius, 2009). It turned out that a modification of the key enzyme, methyl coenzyme-M reductase (mcr), responsible for the terminal step in methanogenesis from CO₂ and H₂, activated methane and initiated the opposite process of methane oxidation (Hallam *et al.*, 2003, 2004; Thauer, 2011). Experimental evidence has later shown that the syntrophy between archaea and sulfate reducers may function by an extracellular electron transfer between the two partner organisms and not by a transfer of carbon (McGlynn *et al.*, 2015; Wegener *et al.*, 2015; Scheller *et al.*, 2016).

Most AOM in the seabed takes place in the subsurface SMT where these bacterial-archaeal aggregates are generally not detected. The archaea rather occur as pure ANME aggregates or as free cells (Treude *et al.*, 2005b; Lloyd *et al.*, 2011). This raises the question whether these ANME live only in loose association with sulfate reducing bacteria or whether they perform the entire AOM process within their cells (Milucka *et al.*, 2012). In several coastal marine sediments, archaea of the ANME-1 group were found to be highly dominant both in the SMT and in the methanic zone below. This led to the conclusion that ANME-1 archaea may function as both methanogens and methane oxidisers in these sediments (Lloyd *et al.*, 2011; Beulig *et al.*, 2019; Kevorkian *et al.*, 2021). Similar indications of a dual role of the predominant ANME-1 were found in a metagenome assembled genome in Baltic Sea sediments that supported the complete reversibility of a methane oxidising and methanogenic pathway (Beulig *et al.*, 2019). Among the open questions is now **whether and how free-living ANME-1 archaea exchange electrons with sulfate reducing bacteria when they oxidise methane, and whether they may possibly use the same mechanism to receive electrons from bacteria and thereby produce methane from acetate.**

The stable isotopes of sulfur include ^{32}S (95.02 %), ^{33}S (0.75 %), ^{34}S (4.21 %) and ^{36}S (0.02 %). Most sulfur isotope studies have focused on ^{32}S and ^{34}S , while ^{33}S has been included later as the analytical techniques and the know-how were developed (e.g., Farquhar *et al.*, 2003; Johnston *et al.*, 2007). Stable isotopes provide an important tool to interpret the sulfur cycle of marine sediments in the present and in the geological past. Originally, the literature on sulfur isotope fractionation in marine sediments mostly assumed closed system sulfate reduction, *i.e.* limited exchange between subsurface sediment and the sulfate pool in the overlying seawater (e.g., Sweeney and Kaplan, 1980). My early studies of marine sediments using ^{35}S radiotracer experiments showed a sulfur cycle that was very open to diffusional exchange. This provoked me to take a closer look at how diffusion might affect the sulfur isotope distribution in the modern seabed. As it turned out, **the degree of openness is very important, not only for the correct understanding of the sulfur cycle, but also for the correct interpretation of sulfur isotope data** (Jørgensen, 1979; Goldhaber and Kaplan, 1980).

As mentioned in Section 2, my first attempts at sulfur isotope analysis in 1978 were unsuccessful. Fifteen years later, Don Canfield, Bo Thamdrup, Henrik Fossing, Tim Ferdelman and Kirsten Habicht joined me as young pioneering scientists at the new Max Planck Institute for Marine Microbiology in Bremen. We purchased our first IRMS instrument and established a sulfur isotope laboratory at the institute. I was later able to hire Volker Brüchert and Michael Böttcher, and sulfur isotopes became an important part of our toolbox to study sulfur transformations in the seabed.

In the following, I will describe some examples of what we learned from the study of sulfur isotopes. For a more general discussion, I refer to several key papers and reviews (e.g., Habicht and Canfield, 2001; Wing and Halevy, 2014; Fike *et al.*, 2015; Sim *et al.*, 2017).

8.1 The Mechanisms of Isotope Fractionation

The relative difference in molecular mass between ^{32}S sulfate and ^{34}S sulfate is small, only 2 %. This, however, suffices to generate a kinetic difference between the net uptake and reduction rates of the two isotopes by sulfate reducing bacteria. The light isotope, ^{32}S , is reduced slightly faster than the heavy isotope, ^{34}S , during dissimilatory sulfate reduction. This causes a fractionation between the two isotopes whereby the produced sulfide is enriched in the light isotope while the remaining sulfate becomes progressively enriched in the heavy isotope. The individual enzymatic steps by the sulfate reduction pathway are to some extent reversible, in particular when the energy yield is low due to low substrate concentrations and/or high product concentrations (Wing and Halevy, 2014).

When both forward and backward reactions occur during sulfate reduction, the fractionation of the two isotopes between sulfate and sulfide may approach thermodynamic equilibrium (Farquhar *et al.*, 2003).

The re-distribution of isotopes during sulfur cycling in marine sediments result from a combination of kinetic and equilibrium fractionations. The magnitude of the fractionation depends on environmental conditions in the sediment, in particular conditions that affect the individual sulfate reducing bacteria. With high sulfate and electron donor concentrations, the change in free energy is high, and so is the thermodynamic drive for sulfate respiration. The cell specific sulfate reduction rate therefore tends to be high and the resulting sulfur isotope fraction low. With low electron donor concentration the energy yield is low and so are sulfate reduction rates (Jin and Bethke, 2009). During the slow degradation of recalcitrant organic substrates in the seabed the highest isotope fractionation may be reached (Hoek *et al.*, 2006; Sim *et al.*, 2011; Leavitt *et al.*, 2013; Deusner *et al.*, 2014; Pellerin *et al.*, 2015a). The isotope ratios of sulfate and sulfide may thereby carry information on environmental conditions, both in the present and in the geological past (*e.g.*, Habicht *et al.*, 2002; Johnston *et al.*, 2008).

In modern sediments, the isotopic composition of sulfate and sulfide can be analysed in the porewater and in solid phase fractions such as acid volatile sulfide (AVS), chromium reducible sulfide (CRS), elemental sulfur, and organic sulfur. This enables dynamic diffusion diagenesis modelling of the ongoing processes. In sedimentary rocks, only the solid phase fractions, in particular pyrite, are generally available for analysis of the past processes. The interpretation of the data therefore relies heavily on lessons learned from modern sediments. These lessons show that the correct interpretation of bulk pyrite isotopes may depend on whether the pyrite formed near the sediment surface or at depth in the sediment column (Liu *et al.*, 2020a). In spite of this uncertainty, the interpretation may provide important information on the geochemical zonation of the past sediment and its microbial processes, for example on the position of the sulfate-methane transition and the anaerobic oxidation of methane (Meister *et al.*, 2019).

8.2 Controls on Isotope Fractionation

Several experimental studies with pure cultures of sulfate reducers indicate that the magnitude of the dynamic sulfur isotope fractionation, ϵ , is a function of the cell -specific sulfate reduction rate, csSRR (Fig. 8.1). The data shows relatively low fractionation of <20 ‰ at the highest csSRR of 100-1,000 fmol cell⁻¹ day⁻¹. With low csSRR of 0.1-1 fmol cell⁻¹ day⁻¹ fractionations are highly scattered, reaching up to the theoretical maximum of 70 ‰.

Sulfur isotope description

The ³²S and ³⁴S isotopic composition of the different sulfur species is formally described by the delta notation, $\delta^{34}\text{S}$, where:

$$\delta^{34}\text{S} = (([^{34}\text{S}/^{32}\text{S}]_{\text{sample}}/[^{34}\text{S}/^{32}\text{S}]_{\text{standard}} - 1) \times 1000 \text{ ‰} \quad (8.1)$$

In other words, the $\delta^{34}\text{S}$ shows how many permille the $^{34}\text{S}/^{32}\text{S}$ ratio of a sample deviates from the similar ratio of a standard [the Vienna-Canyon Diablo Troilite international reference scale (V-CDT)].

The change in sulfur isotope composition, $R = ^{34}\text{S}/^{32}\text{S}$, between produced sulfide, and other reduced sulfur species, and consumed sulfate is driven primarily by microbial sulfate reduction. This change is described by the fractionation factor, $^{34}\alpha = R_{\text{sulfide}}/R_{\text{sulfate}}$. The dynamic fractionation of the sulfur isotopes during sulfate reduction is also expressed in permille:

$$^{34}\epsilon = (^{34}\alpha - 1) \times 1000 \text{ ‰} \quad (8.2)$$

The $^{34}\epsilon$ may theoretically vary between 0 and 70 ‰, where the latter is near the thermodynamic equilibrium distribution between sulfide and sulfate under marine sediment conditions (Tudge and Thode, 1950; Farquhar *et al.*, 2003).

Figure 8.1 Dynamic $^{34}\text{S}/^{32}\text{S}$ isotope fractionation, $^{34}\epsilon$, by different mean cell specific sulfate reduction rates in pure cultures. The theoretical maximum, *i.e.* the equilibrium fractionation, is indicated by the broken line. Redrawn from Jørgensen *et al.* (2019a).

The csSRR in pure culture studies covers a range of 0.1–1,000 $\text{fmol SO}_4^{2-} \text{ cell}^{-1} \text{ d}^{-1}$ (Fig. 8.2a). The rates are generally much higher than the estimated csSRR in marine sediments, which range over at least six orders of magnitude, from the highest values of up to 1 $\text{fmol SO}_4^{2-} \text{ cell}^{-1} \text{ d}^{-1}$ to the lowest values of $<10^{-5} \text{ fmol SO}_4^{2-} \text{ cell}^{-1} \text{ d}^{-1}$ (Fig. 8.2b) (*e.g.*, Biddle *et al.*, 2006; Hoehler and Jørgensen, 2013; Beulig *et al.*, 2018). The high values of 0.01–1 $\text{fmol SO}_4^{2-} \text{ cell}^{-1} \text{ d}^{-1}$ originate from the bioturbated surface zone of coastal sediments, while values of below 0.01 $\text{fmol SO}_4^{2-} \text{ cell}^{-1} \text{ d}^{-1}$ are from subsurface sediments (*cf.* Hoehler and Jørgensen,

2013). Most of this *in situ* csSRR range is below what can practically be studied in pure cultures or even in laboratory experiments with marine sediments. **The extrapolation of sulfur isotope fractionation data from pure cultures to the *in situ* conditions in subsurface marine sediments is therefore subject to considerable uncertainty.**

Figure 8.2

(a) Studies of sulfur isotope fractionation during microbial reduction by different mean cell specific sulfate reduction rates (csSRR) in pure cultures. (b) Range of csSRR in marine sediments. Redrawn from Figure 9 in Jørgensen *et al.* (2019a) (in which a much broader range of sediment csSRR was erroneously indicated).

Whereas the availability of electron donors generally controls sulfate reduction rates in marine sediments, the availability of sulfate is also important for the isotopic fractionation. Several studies in pure cultures and in marine sediments have shown that low sulfate concentrations lead to small ³⁴ε values. Such a trend has been used to estimate sulfate concentrations in the Archaean ocean (*e.g.*, Habicht *et al.*, 2002; Gomes and Hurtgen, 2013). The effect of low sulfate concentrations on isotope fractionation depends on the concurrent availability of electron donors, however, and thus on the csSRR and the extent of reversibility during sulfate reduction (Wing and Halevy, 2014). The sulfate reducing microorganisms are able to regulate their uptake of sulfate with an affinity, K_m, that depends on the ambient sulfate concentration (Tarpgaard *et al.*, 2011, 2017; Section 3.6). This explains how the ³⁴ε can remain high far down into the sulfate-methane transition where the sulfate concentration becomes very low (Pellerin *et al.*, 2018). Values of ³⁴ε > 20 ‰ may be maintained even down to a

few μM sulfate (Crowe *et al.*, 2014). In conclusion, **the controls on sulfur isotope fractionation are complex and depend on a combination of factors that are poorly constrained in the geological record.**

The microbial or chemical oxidation of sulfide and other reduced sulfur species to sulfate is generally associated with small or insignificant isotope effects (*e.g.*, Fry *et al.*, 1986; Böttcher and Thamdrup, 2001; Zerkle *et al.*, 2009; Balci *et al.*, 2012). Yet, a large inverse isotope fractionation, by which the produced sulfate was enriched $\geq 12\text{‰}$ in ^{34}S relative to sulfide, was observed by the sulfide oxidising microorganism, *Desulfurivibrio alkaliphilus* (Pellerin *et al.*, 2019). Since the pathway for sulfide oxidation in these organisms is ubiquitous in marine sediments, and since the re-oxidation of sulfide to sulfate is an integral part of the sulfur cycle in sediments, the isotope effect of sulfide oxidation may turn out to influence the net sulfur isotope distribution and, thus, to be important for its correct interpretation.

Sulfide oxidation in anoxic marine sediment leads to intermediate sulfur species such as elemental sulfur, thiosulfate and sulfite (see Sections 4 and 5). These intermediates can be further metabolised by microbial disproportionation reactions that are associated with large fractionations of up to 30 ‰ (Canfield and Thamdrup, 1994; Habicht *et al.*, 1998). In pure cultures of S^0 disproportionating bacteria, grown under excess iron(III) to bind the H_2S and make the process exergonic, the produced sulfide was depleted in ^{34}S by 5 ‰ relative to the disproportionated S^0 , while the sulfate was enriched by up to 18 ‰ relative to the S^0 (Böttcher *et al.*, 2001). This yielded an isotopic difference between the two disproportionation products of up to 23 ‰. Since sulfate reduction and sulfur disproportionation operate concurrently in marine sediments, it is difficult to differentiate the two processes from the ^{32}S and ^{34}S isotopes alone. However, **by also including a third sulfur isotope, ^{33}S , there is a possibility to distinguish kinetic and equilibrium fractionation and to tease apart the individual processes of the sedimentary sulfur cycle** (Farquhar *et al.*, 2003; Johnston *et al.*, 2005; Pellerin *et al.*, 2015b; Lin *et al.*, 2018; Liu *et al.*, 2020b).

8.3 Stable Isotopes and Open System Diagenesis

The sulfur isotopic composition of sulfate in the open ocean and in coastal waters is very constant with a $\delta^{34}\text{S}$ of +21 ‰. As sulfate is gradually consumed with depth in the seabed, the lighter isotope is preferentially reduced and the $\delta^{34}\text{S}$ of the remaining sulfate increases. Figure 8.3 shows an example from Station M24 in Aarhus Bay where sulfate was depleted at the sulfate-methane transition at 380 cm depth. The $\delta^{34}\text{S}$ of the sulfate increased from +21 ‰ at the sediment surface to +100 ‰ in the SMT (Fig. 8.3b). The $\delta^{34}\text{S}$ of porewater sulfide increased parallel to sulfate with an offset of about -70 ‰ throughout most of the sulfatic zone (Fig. 8.3b). The isotopic difference was 15-20 ‰ smaller at the sediment surface where the influx of fresh organic matter combined with bioturbation enhanced the substrate supply for the sulfate reducing bacteria and probably diminished

the isotope fractionation. It is striking that the sulfide at depth approached a $\delta^{34}\text{S}$ of +21 ‰, *i.e.* exactly the same $\delta^{34}\text{S}$ as seawater sulfate. Sulfide peaked at the SMT and diffused further down into the underlying, methanic sediment with an unaltered $\delta^{34}\text{S}$ of +21 ‰.

Figure 8.3 Depth distribution in Aarhus Bay, Station M24 of (a) sulfate and (b) sulfur isotope composition of sulfate and sulfide. The isotopic difference between sulfate and sulfide is also shown (red crosses). Data from 2015 shown in lighter symbols, from 2016 in darker symbols. Red arrows show direction of total concentration diffusion fluxes. The sulfate-methane transition (SMT) is indicated by a shaded area. Redrawn from Pellerin *et al.* (2018).

The fact that the isotopic composition of sulfate changes as it diffuses from the sediment surface and down into the sediment has interesting consequences for the isotope mass balance. The gradient of porewater sulfate shown in Figure 8.3a comprises the sum of the isotopes ^{32}S and ^{34}S . However, since the $^{34}\text{S}/^{32}\text{S}$ ratio in the sulfate increases with depth, the two isotopes do not have the same, relative gradient. ^{32}S is consumed relatively faster than ^{34}S and the ^{32}S sulfate gradient is therefore relatively slightly steeper than the ^{34}S sulfate gradient. This means that the diffusion flux of ^{32}S sulfate, is slightly enhanced over the ^{34}S sulfate flux when compared to their isotopic ratio at any depth in the sulfatic zone. In other words, the $\delta^{34}\text{S}$ of the downward sulfate flux is more negative than the $\delta^{34}\text{S}$ of the sulfate pool at the same depth. This may appear counter intuitive but is a simple consequence of the gradient in $\delta^{34}\text{S}$. As a result, **sulfate that diffuses down into the sediment is relatively enriched in ^{32}S** . This is indeed a prerequisite for the isotope mass balance. It explains how sulfate, in a steady state system open to diffusion, can be quantitatively converted to sulfide, which at any depth is enriched in ^{32}S relative to the sulfate (*cf.* Jørgensen, 1979).

The same argument applies to dissolved sulfide that diffuses up towards the sediment surface. The $^{34}\text{S}/^{32}\text{S}$ ratio in the sulfide decreases towards the surface (Fig. 8.3b), which means that the ^{34}S sulfide gradient is relatively slightly steeper than the ^{32}S sulfide gradient. The upward diffusion flux of ^{34}S sulfide is therefore enhanced over the ^{32}S sulfide flux when compared to their isotopic ratio at any depth in the sulfatic zone. In other words, the $\delta^{34}\text{S}$ of the upward sulfide flux is more positive than the $\delta^{34}\text{S}$ of the sulfide pool at the same depth. As a result, **sulfide that diffuses up through the sediment is relatively enriched in ^{34}S .**

The downward transport of ^{32}S -enriched sulfate combined with the upward transport of ^{34}S -enriched sulfide ensures a mass balance of the two isotopes in the seabed. In fact, **the downward flux of sulfate and the upward flux of sulfide have nearly the same isotopic composition at any depth** (Jørgensen, 1979). This mass balance is not perfect, however, because some of the sulfide precipitates as pyrite within the sediment column. The diffusion fluxes of sulfate and sulfide are therefore slightly out of isotopic balance, depending on where the pyrite precipitation takes place, *e.g.*, near the sediment surface (Liu *et al.*, 2020a), at the sulfate-methane transition (Lin *et al.*, 2017), or at a sulfidation front in an underlying, iron-rich sediment (Jørgensen *et al.*, 2004; Holmkvist *et al.*, 2014; Riedinger *et al.*, 2017).

It is important to notice that **this phenomenon is not due to a difference in diffusion coefficients between ^{32}S and ^{34}S .** Sulfate with the light ^{32}S isotope was suggested to have a 0.1–0.9 % higher diffusion coefficient compared to sulfate with the heavy ^{34}S isotope (Donahue, 2008a), but this has been contradicted in the literature (Bourg, 2008; Donahue, 2008b). The diffusion coefficients of ^{32}S and ^{34}S in H_2S were found to be identical within 0.1 % (Baune and Böttcher, 2010). Wortmann and Chernyavsky (2011) concluded that the effect of isotope specific diffusion coefficients is smaller than the error associated with the determination of the fractionation factor.

8.4 The Sulfidation Front

Most sulfide production takes place in the upper part of the sulfatic zone, with an additional, small production peak at the SMT. The sulfide concentration tends to peak at the SMT, and as sulfide is not produced to any significant degree beneath the SMT, some of the SMT sulfide diffuses down into the underlying methanogenic zone. The diffusion gradient may be driven by a deeper sulfide trap and/or by a non-steady state adjustment to past sediment conditions. The Baltic Sea and the Black Sea provide examples of both. Similar to a number of other marginal seas at high latitudes, they were landlocked during the last glaciation and formed large freshwater basins with thick deposits of limnic clay. The post-glacial sea level rise and re-opening of gateways to the ocean turned both into modern brackish-marine water bodies. This happened *ca.* 8,500 years ago in the Baltic Sea (Björck, 1995; Andrén *et al.*, 2011) and *ca.* 7,000 years ago in the Black Sea (Jones and Gagnon, 1994; Ryan *et al.*, 1997), with transient seawater intrusions already some 1,000–2,000 years before that.

The marine transgression in both seas provided nutrients, which resulted in enhanced phytoplankton production and deposition of organic-rich Holocene mud that now overlays the organic-poor, but iron-rich, limnic clay. Today, the marine Holocene mud supports high sulfate reduction rates and a high concentration of free sulfide that diffuses down into the post-glacial clay. Such a transition is shown schematically for a Baltic Sea sediment in Figure 8.4. As the salinity in the Baltic Sea rose during the post-glacial marine transgression, sulfate diffused down into the deep limnic clay deposited in the Baltic Ice Lake (Fig. 8.4a). The seawater salinity increased to about 15 ppt, and on top of the limnic clay, organic-rich mud began to deposit in which sulfate reduction caused a steeper drop in sulfate concentration (Fig. 8.4b,c). After several thousand years, the Holocene mud layer was sufficiently thick to cause an intermediate minimum in sulfate (Figs. 8.4d, 5.2). An intermediate zone of methanogenesis then developed in the marine mud while a deep, relic reservoir of sulfate still remained in the glacial clay (Fig. 8.4e). Ultimately, this deep sulfate reservoir was depleted and a normal sulfate-methane zonation developed as is seen in most of the Baltic Sea today (Fig. 8.4f).

Figure 8.4 Transition of a Baltic Sea (Arkona Basin) sediment over the past 8,000 years from initial post-glacial, limnic clay through a marine transgression to a modern stratification with a growing thickness of Holocene mud (gray shading). The six frames show the transition, first with a gradual deepening of sulfate penetration (a-c), and then a gradual sulfate depletion within the marine mud (d-f). Redrawn from Holmkvist *et al.* (2014).

Figure 8.5 shows the geochemical zonation in a Baltic Sea sediment that corresponds to the transition stage shown in Figure 8.4e (Liu *et al.*, 2020a). Sulfate is just barely depleted in the 300–500 cm depth interval where, surprisingly, methanogenesis has not yet started. Below 500 cm, sulfate from the early marine transgression still remains at up to 1 mM concentration (Fig. 8.5a). The H₂S peaks at 100 cm depth, in the middle of the sulfatic zone, and diffuses down to reach the limnic clay at 400 cm where a sulfidation front is located. Here, the sulfide reacts with iron oxides in the Ice Lake clay and forms a black layer rich in acid volatile sulfides and zero valent sulfur (Fig. 8.5b). Deeply buried iron oxides are slowly reduced deeper down, and dissolved Fe²⁺ diffuses up to meet the H₂S at 400 cm depth where it precipitates as AVS (Fig. 8.5a,b). As the sulfidation front slowly progresses downwards it leaves behind AVS and zero-valent sulfur (ZVS) that are gradually transformed into pyrite. Pyrite is the dominant iron-sulfur pool in the Holocene mud with a concentration 40 fold higher than the AVS peak in the sulfidation front (Fig. 8.5c).

The $\delta^{34}\text{S}$ of sulfate increases to +110 ‰ at the bottom of the sulfatic zone and is even up to +120 ‰ in the relic sulfate pool in the limnic clay below (Fig. 8.5d). The isotopic composition of pyrite, which constitutes the bulk of the chromium reducible sulfur (CRS), should correspond roughly to the $\delta^{34}\text{S}$ of H₂S from which the AVS and ZVS are formed. The $\delta^{34}\text{S}$ of CRS in Figure 8.5d thereby reveals that most pyrite in the Holocene mud formed near the sediment surface where it has similar $\delta^{34}\text{S}$ as the H₂S, *ca.* -35 ‰. Acid volatile sulfide and elemental sulfur also have similar isotopic compositions as the H₂S near the sediment surface (data not shown). In contrast, the additional authigenic pyrite formed at the sulfidation front (CRS in Fig. 8.5d) carries the heavy isotopic signal of H₂S at that depth, 0 ‰ to +20 ‰.

While the difference in $\delta^{34}\text{S}$ between sulfide and sulfate ($\Delta^{34}\text{S}_{(\text{H}_2\text{S}-\text{SO}_4)}$) was about 70 ‰ in the Aarhus Bay core (Fig. 8.3), this difference increased with depth down through the sulfatic zone from 63 ‰ to 83 ‰ in the Bornholm Basin (Fig. 8.5d). This may still reflect a dynamic fractionation factor, $^{34}\epsilon$, of *ca.* 70 ‰, but the diffusion of sulfide and sulfate tends to make the $\Delta^{34}\text{S}_{(\text{H}_2\text{S}-\text{SO}_4)}$ smaller than $^{34}\epsilon$ near the sediment surface and larger than $^{34}\epsilon$ at depth (Jørgensen, 1979).

A rather similar geochemical zonation and sulfur isotopic distribution was observed in the Black Sea where modern brackish-marine mud overlays deep Pleistocene deposits of limnic origin. Also here, H₂S is drawn down from the SMT through a diffusion gradient leading into the limnic clay where a sulfidation front is slowly progressing downwards into deeper layers and precipitates isotopically heavy pyrite (*e.g.*, Calvert *et al.*, 1996; Neretin *et al.*, 2004). Through the formation of iron sulfides (FeS_n, including greigite, Fe₃S₄), elemental sulfur and polysulfides from the reaction between H₂S and iron oxides, the sulfidation front becomes a site of intensive pyrite formation. Due to the strong ^{34}S -enrichment in H₂S beneath the SMT, this pyrite has a $\delta^{34}\text{S}$ of up to +20 ‰ (Jørgensen *et al.*, 2004).

Figure 8.5

Sulfur geochemistry and stable isotope composition in Baltic Sea sediment (Bornholm Basin, Station BB05, 95 m water depth). **(a)** Sulfate, sulfide and dissolved iron(II) (notice different scales). **(b)** Acid volatile sulfide (FeS) and zero valent sulfur (S^0). **(c)** Chromium reducible sulfur (FeS_2). **(d)** Isotopic composition of sulfate, sulfide and CRS; the isotopic difference between sulfate and sulfide is also shown (red crosses). Concentrations in **(b)** and **(c)** are per g wet weight of sediment. The shaded area indicates the sulfidation front. Redrawn from Liu *et al.* (2020a).

These sulfur isotope distributions in the Baltic Sea and the Black Sea result from the combination of a sulfide production peak in the SMT and a sulfidisation front below, which lead to the formation of isotopically very heavy pyrite. There are many other examples where post-glacial marine transgression has shifted the sedimentation from limnic to marine, or where intense sulfide production in marine sapropels drives the downwards migration of a sulfidisation front (Passier *et al.*, 1996). The results presented here therefore have general implications for the interpretation of sulfur isotope data in modern sediments and in sedimentary rocks through Earth's history.

The previous sections have discussed biogeochemical pathways, rates, regulation and microbiology of the sulfur cycle. I will here focus on some quantitative aspects of microbial sulfate reduction in marine sediments, from the regional to the global scale.

9.1 Organic Carbon Cycle of the Global Seabed

The primary control on sulfate reduction in marine sediments is the rain rate of particulate organic matter (POC) from the water column to the seafloor. This flux of organic matter feeds the aerobic respiration by all the benthic fauna and microorganisms. It also feeds the anaerobic processes in the sediment and affects their mutual zonation and rates. By data compilation and modelling of fluxes from different water depths and regions in the ocean, global budgets have been developed for these processes. While such budgets are important for our quantitative understanding of element cycling in the seabed, the numbers represent only our currently best estimates. As more data become available, and as the modelling of these data improves, the numbers will be adjusted and, hopefully, become even more accurate. It is therefore important to consider the limitations of the available database and of the methods used to develop the budgets. The published numbers should therefore be accompanied by a measure of their statistical uncertainty, which is seldom the case. This makes it difficult to evaluate whether new numbers fall within the confidence interval of earlier estimates or whether they are significantly different. It adds to this problem that global budgets are often published with too many significant figures relative to their statistical uncertainty. This said, much of the following discussion will cite published numbers, knowing well that some of these may not be as accurate as they appear.

For a quantitative comparison of the element cycles, it is important to know the *in situ* stoichiometry by the aerobic and anaerobic mineralisation of organic carbon (C_{org}). To facilitate this, I use molar units throughout the following discussion. Jahnke (1996) assumed a rather low $C_{org}:O_2$ molar ratio of 0.6:1 based on a Redfield stoichiometry of $P:N:C:O_2 = 1:16:103:172$, determined by Takahashi *et al.* (1985). This ratio includes the aerobic oxidation of organic nitrogen to nitrate, which accounts for up to 20 % of the oxygen consumption by a C:N ratio of 8-12. Anderson and Sarmiento (1994) and Tanioka and Matsumoto (2020) determined a $C_{org}:O_2$ molar ratio of about 0.7:1 as a global mean for the respiratory quotient of phytoplankton. They extrapolated a similar ratio for aerobic organic matter mineralisation in the deep sea. Hammond *et al.* (1996) used benthic incubation chambers in the deep sea and determined a $CO_2:O_2$ exchange rate across the sediment surface of 0.7:1. Others have found exchange rates closer to 1:1. Since

the relative O_2 consumption by organic N oxidation to nitrate drops from the inner shelf to the deep sea, I estimate that the respiratory quotient, $CO_2:O_2$, drops accordingly from 0.9 to 0.75.

Recent estimates of the global marine primary production are about $4,300 \text{ Tmol } C_{org} \text{ yr}^{-1}$ (Field *et al.*, 1998; Carr *et al.*, 2006; Silsbe *et al.*, 2016). Najjar *et al.* (2007) estimated a global particulate organic carbon (POC) export flux of $1,400 \pm 500$ ($\pm$ s.d.) $\text{Tmol } C_{org} \text{ yr}^{-1}$ from the photic zone, while Dunne *et al.* (2007) estimated 800 ± 300 ($\pm$ 1 s.d.) $\text{Tmol } C_{org} \text{ yr}^{-1}$. Dunne *et al.* (2007) used data of Jahnke (1996) on the global oxygen uptake of marine sediments to calculate that about a fourth of this export reached the seafloor, corresponding to a global benthic oxygen uptake of $320 \pm 125 \text{ Tmol } O_2 \text{ yr}^{-1}$ (1 teramol = 10^{12} mol). There are other estimates in the literature of the global benthic oxygen respiration in the seabed, several of which include only ocean depths >1000 m. I will here use the estimate of Dunne *et al.* (2007). With a $C_{org}:O_2$ molar ratio ranging from 0.9 to 0.75, this is equivalent to the oxidation of $260 \text{ Tmol } C_{org} \text{ yr}^{-1}$.

Large quantities of terrigenous organic material, in the order of $35 \text{ Tmol } C_{org} \text{ yr}^{-1}$, are delivered by rivers to the coastal ocean where about half may be sequestered in inner shelf sediments (Schlesinger and Melack, 1981; Regnier *et al.*, 2013). Coastal vegetated ecosystems, including salt marshes, mangroves, seagrasses, macroalgae and coral reefs, also export particulate organic carbon to the adjacent sea, with $25 \text{ Tmol } C_{org} \text{ yr}^{-1}$ as a conservative estimate and $95\text{--}280 \text{ Tmol } C_{org} \text{ yr}^{-1}$ as a very high estimate by Duarte *et al.* (2005). Macroalgal beds and seagrass meadows alone were estimated to export 50 and $40 \text{ Tmol } C_{org} \text{ yr}^{-1}$, respectively (Krause-Jensen and Duarte, 2016; Duarte and Krause-Jensen, 2017). Regnier *et al.* (2013) estimated that the lateral export of carbon from estuaries to the coastal ocean is $80 \text{ Tmol } C_{org} \text{ yr}^{-1}$. This adds to the flux of POC from planktonic primary production. **A detailed budget of the organic carbon flux to the seabed shows the complexity and uncertainty of these benthic POC flux estimates along the ocean margins.** Such a budget needs to take into account also POC from atmospheric dust, resuspension of shelf sediments, export of dissolved organic carbon from sediments, and possibly other parameters.

Wallmann *et al.* (2012) estimated the global accumulation rate of organic carbon in Holocene sediments to be $11.4 \text{ Tmol } C_{org} \text{ yr}^{-1}$, a number that I will use in the following discussion. This is much less than the uncertainty of the C_{org} flux to the seabed. It is lower than earlier estimates of $16 \text{ Tmol } C_{org} \text{ yr}^{-1}$ by Sarmiento and Gruber (2006), $65 \text{ Tmol } C_{org} \text{ yr}^{-1}$ by Dunne *et al.* (2007), and $40 \text{ Tmol } C_{org} \text{ yr}^{-1}$ by Regnier *et al.* (2013). An estimated 88 % of the global accumulation of organic carbon in marine sediments during the current interglacial period occurs within 500 km from the nearest coast and shallower than 2,000 m water depth (Wallmann *et al.*, 2012; Xie *et al.*, 2019). The sum of total mineralisation and burial of organic carbon in the global seabed is thus about $250 \text{ Tmol } C_{org} \text{ yr}^{-1}$.

In a comparison between oxygen uptake and organic carbon oxidation there are several important, but complex, aspects to consider. The oxygen consumption includes all the aerobic respiration plus most, but not all, of the anaerobic

mineralisation, namely the part of the reduced products of anaerobic respiration that diffuses up towards the sediment surface and is re-oxidised, ultimately at the expense of oxygen. In coastal sediments, that part may be close to 90 % of the produced H_2S (Jørgensen, 1982), while in off shore sediments it decreases with ocean depth to reach zero in the abyss where all the produced sulfide is buried as pyrite (D'Hondt *et al.*, 2019; Section 4).

Apart from organic carbon, a significant reducing power is also buried in the seabed in the form of pyrite, FeS_2 . The burial ratio of organic carbon and pyrite sulfur in marine sediments is about 3:1 by weight (Berner and Raiswell, 1983). This corresponds to a molar ratio of 7:1 and a 2.5:1 ratio of oxidation equivalents, assuming a C_{org} oxidation state of -0.7 (see below). This should be added to the reducing power buried as organic carbon. D'Hondt *et al.* (2019) used the organic carbon burial of 16 and 65 $\text{Tmol C}_{\text{org}} \text{ yr}^{-1}$ published by Sarmiento and Gruber (2006) and Dunne *et al.* (2007), respectively, to estimate that 2-9 Tmol S yr^{-1} is buried as pyrite. The low estimated organic carbon burial of Wallmann *et al.* (2012) would, by a $\text{C}_{\text{org}}:\text{S}_{\text{pyrite}}$ weight ratio of 3:1, correspondingly yield a pyrite burial rate of 1.5 Tmol S yr^{-1} .

The burial ratio of organic carbon to nitrogen is 3:1 in terms of oxidation equivalents (D'Hondt *et al.*, 2019). The nitrogen is released as NH_4^+ during the organic matter degradation and is oxidised when it diffuses up to into the surface sediment. This affects the $\text{C}_{\text{org}}:\text{O}_2$ flux ratio and the apparent respiratory quotient of organic matter mineralisation. Deep sea sediments tend to release the formed nitrate, whereby the $\text{C}_{\text{org}}:\text{O}_2$ ratio may approach that of complete aerobic mineralisation (cf. Li and Katsev, 2014). Coastal sediments tend to be nitrate sinks, whereby nitrification-denitrification or anammox release N_2 rather than NO_3^- (Seitzinger *et al.*, 2006).

9.2 The Different Depth Regions

There is a strong focus of the benthic POC flux towards the ocean margins. Thus, only about 1 % of the export from the photic surface water reaches abyssal sediments (>4,000 m water depth), while a large, but highly variable, fraction reaches the seafloor on the inner shelf and in estuaries and lagoons. As a result, 71 % of the entire POC flux from the photic zone to the seafloor is estimated to take place on the continental shelf (<200 m) (Dunne *et al.*, 2007), which accounts for only 7 % of the ocean area. Half of the POC flux (48 %) is estimated to occur at water depths of less than 50 m, covering only 2 % of the global ocean area.

Canfield *et al.* (2005) compiled data on sulfate reduction rates in the global seabed and integrated these numbers for different categories of ocean margin types and ocean depths. Table 9.1 lists these regions and the total sulfate reduction rate estimated for each region, recalculated to units of $\text{mmol m}^{-2} \text{ d}^{-1}$. The total rates are based on the geographic area covered by each region and their estimated mean areal sulfate reduction rate. The abyssal ocean depths of >3,000 m comprise

the largest area of 77 %. Table 9.1 shows data for the mean areal sulfate reduction rates integrated from the sediment surface to the bottom of the sulfatic zone. The mean areal sulfate reduction rates drop by four orders of magnitude, from 10-80 mmol m⁻² d⁻¹ in different coastal ecosystems to 0.003 mmol m⁻² d⁻¹ in the >5,000 m deep abyss. It should be noted that specific data are not available for the deep sea trenches that reach down to 11,000 m but that the areal flux of organic matter to the bottom of these trenches exceeds the flux to the deep abyssal plains (Glud *et al.*, 2021).

Table 9.1 Total sulfate reduction rates for different categories of sediments and water depths (Tmol SO₄²⁻ yr⁻¹). The table shows the area of each category, its estimated mean sulfate reduction rate (SRR), and the total, global sulfate reduction rate for the category. Data from Canfield *et al.* (2005).

Category	Area 106 km2	Areal SRR mmol SO ₄ ²⁻ m-2 d-1	Total SRR Tmol SO ₄ ²⁻ yr-1
1. Salt marsh	0.4	77	11.2
2. Mangroves	0.1	19.7	0.72
3. Intertidal	0.05	11.5	0.21
4. Seagrass beds	1.1	20.3	8.14
5. Estuaries and embayments	2	7.4	5.2
6. Upwelling	0.13	7.4	0.35
7. Shelf-depositional	11	4.7	18.7
8. Shelf-non depositional	14	1.1	5.6
9. Upper slope (200-1000 m)	16	2.0	11.5
10. Lower slope (1000-2000 m)	15	0.41	2.25
11. Rise (2000-3000 m)	22	0.055	0.44
12. Abyss (3000-4000)	71	0.027	0.71
13. Abyss (4000-5000)	120	0.004	0.18
14. Abyss (>5000 m)	88	0.003	0.11
Sum	361	---	65

Table 9.1 includes coastal marine ecosystems such as salt marshes, mangroves, seagrass beds and intertidal sediments. These ecosystems are special in that the organic matter source is primarily vascular plants and benthic microalgae, in addition phytoplankton from the water column. All these ecosystems are extremely productive, and the sediments have correspondingly high

rates of both aerobic and anaerobic respiration. Among the intertidal sediments, some are highly sulfidic, organic-rich mud while others are sandy and have relatively low organic carbon content. Yet, both types tend to have high rates of sulfate reduction (Al-Raei *et al.*, 2009).

The geographic areas covered by the different sediment types and zones shown in Table 9.1 differ by up to four orders of magnitude. The total sulfate reduction taking place within each zone is therefore a product of area and mean areal rates for each sediment category. Salt marshes, seagrass beds, and estuaries and embayments account for more than a third of the entire global marine sulfate reduction, while other environments on the inner and outer shelf account for more than a third. **This leaves only a quarter of the entire global sulfate reduction to marine sediments beyond the shelf break at >200 m water depth.**

Not only the depth integrated sulfate reduction rates in marine sediments drop with the water depth and distance from the coast, also the relative depth distribution of rates within the sediment changes. In shelf sediments, the highest rates are focused towards the sediment surface and drop off steeply with depth according to a power law (Section 3.4). This implies that **a coring technique that does not preserve the top 10-20 cm and a modelling approach that does not take bioturbation into account may miss half or more of the entire sulfate reduction when working with inner shelf sediments** (Fig. 3.4). In sediments from the continental slope and rise, the anoxic, oxidised zone of predominant nitrate, manganese(IV) and iron(III) reduction gradually expands with increasing water depth, and sulfate reduction becomes the main terminal oxidant only at a depth of several metres (Froelich *et al.*, 1979). In the abyssal plains, iron and manganese reduction gradually take over and sulfate reduction loses significance (D'Hondt *et al.*, 2004). In the central ocean gyres, oxygen even reaches the basaltic basement and totally excludes anaerobic processes (D'Hondt *et al.*, 2015).

Canfield *et al.* (2005) also compiled data on oxygen uptake rates in sediments from the same sediment categories as shown in Table 9.1. A comparison of the oxygen uptake and sulfate reduction in each of these categories is shown in Figure 9.1. (Note: the figure looks very similar to Figure 8.3 in Jørgensen and Kasten (2006) but has now been corrected for an unfortunate error in that earlier graph where all rates were calculated 10 fold too low for both oxygen and sulfate). Among the highest areal rates of organic matter mineralisation are marine ecosystems with a vegetation of vascular plants (salt marshes, mangroves and seagrass beds). These, together with intertidal flats, have very high primary productivity, and the produced organic matter is to a large extent decomposed within the same ecosystem.

In general, the mean mineralisation rate by oxygen respiration in shelf and slope sediments is equivalent to that of sulfate reduction or two fold larger (Fig. 9.1). The data show that sulfate reduction does not exceed the mineralisation by aerobic processes but is the dominant anaerobic process in all these

environments. This trend shifts strongly in sediments of the continental rise and abyss where the mean oxalic mineralisation exceeds the mean sulfate reduction by an order of magnitude or more (Fig. 9.1).

Figure 9.1 Mean areal sulfate reduction rates *versus* oxygen uptake rates. Numbers refer to the different sediment categories listed in Table 9.1. The line represents equal rates of oxygen uptake and sulfate reduction for the mineralisation of organic carbon after correcting for stoichiometry. Data from Canfield *et al.* (2005); redrawn from Jørgensen and Kasten (2006).

9.3 Role of Methane

As discussed in Section 3.4.3, many sulfate profiles in marine sediments show a near linear trend from the sediment surface and down to the sulfate-methane transition (SMT), suggesting that little or no sulfate reduction takes place in the main sulfatic zone and that most sulfate reduction is coupled to the oxidation of methane. The experimental measurements of ^{35}S sulfate reduction show a very different trend with most sulfate reduction taking place in the upper part of the sulfatic zone and only a small contribution of methane at the SMT. Such a trend is in accordance with general knowledge of how mineralisation rates decrease with depth and age of buried organic matter (Fig. 6.1). It is therefore a question, how large a fraction of the POC flux to the seafloor may ultimately reach the methanic zone and feed the process of methanogenesis?

Egger *et al.* (2018) compiled global data on methane and sulfate in marine sediments and calculated methane and sulfate fluxes to the SMT from a large number of coring sites throughout the world oceans. The global distribution of methane fluxes and, thus, of methane production in the seabed, is strongly skewed towards the ocean margins with the highest fluxes exceeding $1 \text{ mmol CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ in the coastal zone (Fig. 9.2). About 80 % of the global methane production in the seabed takes place on the continental shelf at 0-200 m water depth. Based on the mapped methane fluxes, the calculated global methane production in the seabed is $2.8\text{--}3.8 \text{ Tmol CH}_4 \text{ yr}^{-1}$ (Egger *et al.*, 2018). This is higher than the $0.3\text{--}2 \text{ Tmol CH}_4 \text{ yr}^{-1}$ estimate by Wallmann *et al.* (2012) who used a transport reaction model to study the effects of key parameters on gas hydrate accumulation in the seabed. It is lower than the $19 \text{ Tmol CH}_4 \text{ yr}^{-1}$ estimated as total AOM rate by Reeburgh (2007), which was based on fewer data.

Figure 9.2

Global map of methane fluxes ($\text{mmol CH}_4 \text{ m}^{-2} \text{ d}^{-1}$) to the sulfate-methane transition in marine sediments. The map illustrates how strongly methane production in the seabed is skewed towards the ocean margins. From Egger *et al.* (2018).

If we assume the global annual POC flux to the seafloor to be about $260 \text{ Tmol C}_{\text{org}} \text{ yr}^{-1}$, the organic carbon burial rate during the Holocene to be $11.4 \text{ Tmol C}_{\text{org}} \text{ yr}^{-1}$ (Wallmann *et al.*, 2012), and the methane production in the seabed to be $2.8\text{--}3.8 \text{ Tmol CH}_4 \text{ yr}^{-1}$, it means that 5 % of all deposited POC becomes buried beneath the bioturbated zone and that 1–1.5 % of the deposited organic carbon is ultimately converted into methane. Since methanogenesis consumes *ca.* 2 mol C_{org} per 1 mol CH_4 produced ($2\text{CH}_2\text{O} \rightarrow \text{CH}_4 + \text{CO}_2$), it means that **2–3 % of the deposited organic carbon is mineralised in the methanic zone**. This is a similarly small fraction as we have found in studies of sulfate reduction and methane production in a number of coastal marine sediments (Section 7.3). Relative to the organic carbon burial beneath the bioturbated zone, the fraction converted to methane is much higher, one quarter to one third. Although the Holocene accumulation rate of methane in marine sediments is not well constrained, only

a marginal fraction of the produced methane is currently trapped as gas hydrate (Burwicz *et al.*, 2011) or as shallow gas (Fleischer *et al.*, 2001), while the vast majority of the produced methane is oxidised to CO₂.

9.4 Global Sulfur Budget of Marine Sediments

Sulfate reduction in anoxic sediments produces large amounts of sulfide that is effectively recycled back to sulfate or accumulated as pyrite and organic sulfur. Only 3 % of the global sulfide production is due to thermochemical processes and is released from volcanoes and deep sea hydrothermal vents, while the remaining 97 % is produced by microbial sulfate reduction (Elderfield and Schultz, 1996; Andres and Kasgnoc, 1998; Rickard, 2012b; Picard *et al.*, 2016).

The total volume of the ocean is 1.37 billion km³ (Garrison, 1997). With a mean sulfate concentration of 29 mM, the total sulfate pool in the modern ocean is 4.0×10^{19} mol SO₄²⁻. If we assume a global sulfate reduction in the seabed of 65 Tmol SO₄²⁻ yr⁻¹, including salt marshes, mangroves, seagrass beds and intertidal sediments, the overall turnover time of the global marine sulfate pool is $(4.0 \times 10^{19} / 65 \times 10^{12}) \approx$ ca. 0.6 million years. In addition to this microbial sulfate reduction there is also a thermochemical sulfate reduction in seawater that circulates through the hot ocean crust at a rate of $3\text{--}6 \times 10^{13}$ litre yr⁻¹ (Elderfield and Schultz, 1996). Even if the sulfate were thereby quantitatively reduced to sulfide, this would correspond to only 1-3 % of the microbial sulfate reduction (Elderfield and Schultz, 1996; Andres and Kasgnoc, 1998; Rickard, 2012a; Picard *et al.*, 2016). Thus, it is primarily the microbial sulfate reduction in the seabed that drives the global marine sulfur cycle.

The main anaerobic mineralisation process in the global seabed is sulfate reduction, whereby sulfate has exactly twice the oxidation capacity *per* mole compared to oxygen. A commonly used stoichiometry has been a simple C_{org}:SO₄²⁻ ratio of 2:1, where C_{org} is the bulk organic carbon with an assumed composition of CH₂O:

Burdige (2006) argued that the mean oxidation state of organic carbon in sediments is not 0.0 as in CH₂O but rather -0.7 to -0.5. The more reduced C_{org} yields a C_{org}:SO₄²⁻ ratio of 1.70-1.78:1 rather than 2:1. In incubation experiments with Cape Lookout Bight sediment, Alperin *et al.* (1994) found a ratio between DIC production and SO₄²⁻ consumption of 1.7. Archer *et al.* (2002) assumed a C_{org}:SO₄²⁻ ratio of 1.8:1 in their diagenetic model, whereas Arning *et al.* (2016) concluded that a C_{org}:SO₄²⁻ ratio of 2:1 provided an acceptable representation of the anaerobic mineralisation. I have used a ratio of 2:1 in earlier publications without seriously considering the actual oxidation state of the mineralised organic carbon. For the present discussion, however, I will assume a C_{org}:SO₄²⁻ ratio of 1.7:1. This ratio differs from the aerobic stoichiometry of C_{org}:O₂ in that it does

not include the oxidation of organic N to NO_3^- . This oxidation is separated from the sulfate reduction and generally takes place only when the released NH_4^+ diffuses up to the oxic zone.

Several studies have estimated the total sulfate reduction in the global seabed. Canfield *et al.* (2005) combined data from both transport reaction modelling and from experimental ^{35}S sulfate reduction studies (Table 9.1). They multiplied the mean areal SRR for each setting with the geographic area they represented to derive a total global rate of $65 \text{ Tmol SO}_4^{2-} \text{ yr}^{-1}$. (In the textbook chapter by Jørgensen and Kasten (2006) we used these data directly but erroneously cited the number as $75 \text{ Tmol SO}_4^{2-} \text{ yr}^{-1}$ due to a very annoying spreadsheet typo). **When excluding salt marshes, mangroves, seagrass beds and intertidal sediments, the global sulfate reduction rate of marine sediments is ca. $45 \text{ Tmol SO}_4^{2-} \text{ yr}^{-1}$** (Table 9.1). About 75 % of this global sulfate reduction takes place in continental shelf sediments at $<200 \text{ m}$ water depth.

With a $\text{C}_{\text{org}}:\text{SO}_4^{2-}$ mineralisation ratio of 1.7:1, the $45 \text{ Tmol SO}_4^{2-} \text{ yr}^{-1}$ corresponds to the oxidation of $77 \text{ Tmol C}_{\text{org}} \text{ yr}^{-1}$. Compared to the $237 \text{ Tmol C}_{\text{org}} \text{ yr}^{-1}$ of aerobic mineralisation, sulfate reduction accounts for a third of the direct mineralisation of organic carbon in the global seabed. This fraction is in good agreement with our data from individual sites in coastal and shelf sediments and corresponds to the early estimate by Jørgensen (1982). In the deep sea, the fraction is much lower, but abyssal sediments contribute only 2 % to the global sulfate reduction, while shelf sediments account for two thirds (Table 9.1).

For comparison, Thullner *et al.* (2009) applied a numerical modelling approach with ocean depth as the master variable and estimated a higher global sulfate reduction of $80 \text{ Tmol SO}_4^{2-} \text{ yr}^{-1}$. They concluded that this accounts for 76 % of the total sedimentary C_{org} oxidation, while aerobic respiration by direct organic carbon oxidation accounted for only 15 % globally. Such a high relative contribution of sulfate reduction exceeds what we have found in a number of site specific studies, even in organic-rich coastal sediments, and is probably an over estimate. The numbers are derived from 3 fold lower sulfate reduction and 11 fold lower oxygen uptake of the global seafloor than estimated here.

Bowles *et al.* (2014) applied an artificial neural network approach with modelled sulfate profiles from the IODP database to estimate a global sulfate reduction of $11.3 \text{ Tmol SO}_4^{2-} \text{ yr}^{-1}$. This is only about twice the calculated global sulfate flux of $5.3 \text{ Tmol SO}_4^{2-} \text{ yr}^{-1}$ to the SMT (Egger *et al.*, 2018). As discussed in Section 7, our studies of sulfate reduction in shelf sediments, where most sulfate reduction and methanogenesis take place, show that methane generally accounts for less than 10 % of the total sulfate reduction at individual sites, so that the sulfate flux should be at least ten fold higher than the methane flux. I conclude that the most likely global sulfate reduction falls somewhere in between the estimates by Thullner *et al.* (2009) and Bowles *et al.* (2014), as indicated by the estimate by Canfield *et al.* (2005).

In conclusion, **the marine cycling of organic matter in the seabed is strongly focused towards the continental shelf at <200 m water depth, which globally receives 71 % of the particulate organic carbon flux from planktonic primary production (Dunne *et al.*, 2007), accounts for 75 % of the sulfate reduction (Canfield *et al.*, 2005), and accounts for 80 % of the methane production (Egger *et al.*, 2018).** The estimated global seabed budget of oxygen uptake was 288 Tmol O₂ yr⁻¹ and of sulfate reduction 45 Tmol SO₄²⁻ yr⁻¹. It was estimated that about 90 % of the sulfide produced from sulfate reduction, *i.e.* 40 Tmol H₂S yr⁻¹, is re-oxidized to sulfate at the direct or indirect expense of oxygen. With a stoichiometry of 2:1 by complete sulfide oxidation with O₂, this will consume 80 Tmol O₂ yr⁻¹ or about 25 % of the global sediment oxygen consumption. This corresponds to the early estimate by Jørgensen (1982) of a 50 % contribution of sulfide re-oxidation to the total sediment O₂ uptake in coastal sediments and 25 % contribution in the main shelf sediments of 20-200 m water depth.

10. FUTURE PERSPECTIVES

Now that the marine sulfur cycle has been studied for many decades, we are tempted to think that we understand the most important aspects and only need to wrap up the details. Then we are surprised by unexpected and refreshing discoveries, such as the electron conductance through cable bacteria, or electron transfer between bacteria and archaea and solid minerals such as magnetite or greigite. At the interface between geochemistry and microbiology we realise how microorganisms are involved in many geochemical processes, such as pyrite formation, and thereby affect the pathways of sulfur transformation and the sulfur isotope signals they leave in the rock record.

In the following, I will return to some open questions that were raised in the preceding sections, where the relevant references are given. These questions are only some of my own ideas of where further understanding is needed. More exciting questions will be those that we have not even thought of yet. That is where the real discoveries are waiting.

Sulfate reduction. I have discussed at length the apparent discrepancy between sulfate reduction rates determined by the ^{35}S radiotracer method and by reaction transport modelling. Both approaches have their limitations and potential errors, and further comparative studies are needed to find their optimal combination. This will require a higher density of ^{35}S -SRR measurements than is generally seen in the literature in order to reconcile the two approaches. It will require further controlled experiments to check how accurately the ^{35}S method reflects *in situ* SRR. It will also require an analysis of why only about a third of published sulfate profiles are concave down, indicating sulfate reduction throughout the sulfate zone, while a third are linear and a third are concave up. Linearity has been interpreted as sulfate reduction driven mainly by methane oxidation at the deep sulfate-methane transition rather than by organoclastic sulfate reduction. However, such a suppressed mineralisation of organic matter during burial down through the sulfatic zone and then a revived mineralisation in the methanic zone contradicts a large literature showing how degradation rates decrease continuously with age. **We need to understand better what controls the shape of seabed sulfate profiles, in particular with respect to the role of bioturbation and anaerobic methane oxidation.**

Sulfide oxidation. Several studies using the ^{35}S radiotracer have indicated that overall about 90 % of the sulfide produced from sulfate reduction is re-oxidised back to sulfate. While such a number is often cited, further studies are needed to check its accuracy and range in different sediments. Most sulfide oxidation in marine sediments takes place beneath the oxidised surface sediment, probably with iron as the predominant oxidant. It remains poorly understood how geochemical and microbial processes interact to catalyse a nearly quantitative sulfide oxidation to sulfate in anoxic sediments. What are the main pathways and intermediates during this sulfide oxidation? To what extent do

bacteria performing extracellular electron transfer interact with minerals to drive the diagenetic reactions? Does elemental sulfur or thiosulfate provide the main shunt in the sulfur cycle during these processes? New ideas and experiments are needed to determine the mechanisms and *in situ* rates of sulfide oxidation. **New approaches will need to unravel the concurrent processes of sulfate reduction, sulfide oxidation and disproportionation of sulfur intermediates.** Such studies may also help constrain the potential for dark CO₂ fixation by chemolithotrophic bacteria, which recycles a fraction of the mineralised organic matter into new microbial biomass and necromass.

Cryptic sulfur cycling. The term “cryptic sulfur cycling” has been used for marine sediments to describe a concurrent sulfate reduction and sulfide oxidation, which prevents sulfate reduction from being fully expressed as a corresponding drop in sulfate concentration. Such a cryptic cycling has been recognised in the bioturbated surface sediment, where process rates are high, and in the deep methanic sediment, where the sulfate concentrations are low. It remains to be demonstrated and quantified in the main sulfatic zone, where I expect it to be a general phenomenon and cause a discrepancy between net and gross sulfate reduction rates. It may be argued that there is little reactive Fe(III) left in this zone to re-oxidise the sulfide, but there is also little reactive organic carbon left to drive sulfate reduction, and it is the balance between the two that controls the degree of cryptic cycling. Future studies should analyse to what extent deep sulfide re-oxidation counteracts the theoretically expected concavity of sulfate profiles. **Diagenetic models need to focus more on quantifying sulfide oxidation to sulfate. This will help to address the discrepancy between gross rates of sulfate reduction, as determined by radiotracers, and net rates of sulfate reduction, as determined by transport reaction models.**

Mineralisation processes. It has been questioned in the literature which step in the degradation pathway of sediment organic matter controls the overall mineralisation rates. The answer apparently depends on the type of sediment, type of experiment, and type of diagenetic modelling. With increasing depth and age of the buried organic matter, the initial hydrolytic attack appears to limit degradation more strongly. Yet, given the chemical and physical complexity of the buried organic matter and the difficulty of determining the specific molecular structures being attacked, this remains a difficult but important field for future studies. The results will have important implications for our understanding of the degree of preservation of organic matter and of the life of the deep sub-sea-floor biosphere. Also the dissolved substrates, such as volatile fatty acids, used by respiring microorganisms, continue to provoke open questions and show that **we have still not identified what controls these substrate concentrations in the seabed.**

Methane. Some microbial processes in the seabed appear to be extremely sluggish and limited by poorly defined factors. One example is the anaerobic oxidation of methane, by which co-existing methane and sulfate may have turnover times of many years and co-existing methane and iron(III) have turnover

times of thousands of years. By the latter, the availability of the iron minerals towards microbial reduction and the potential role of extracellular electron transfer should be studied. The finding of a cryptic methane cycle with highest rates of methanogenesis in the sulfate-methane transition opens interesting questions about concurrent methane production and anaerobic methane oxidation in subsurface sediments. Are some ANME archaea able to shift the direction of their catabolic metabolism depending on whether they receive electrons from fermenting bacteria or they transfer electrons to sulfate reducing bacteria? **What is the role of solid minerals as electron capacitors by such extracellular exchange?**

Isotope fractionation. The sulfur isotope fractionation during microbial sulfate reduction has for many years been studied experimentally in pure cultures or by sediment incubations and have been determined from the isotopic composition of relevant sulfur species in sediment cores. The assumed cell specific sulfate reduction rates by the different approaches differ strongly, which complicates the conclusions from laboratory experiments to be transferred to *in situ* fractionation. It remains a question as to how close the isotope fractionation during sulfate reduction in subsurface sediments is to the theoretical maximum of 70 ‰ and, as a result, to what extent the fractionation factor is regulated by environmental factors. It also remains a question how physical isotope exchange between sulfur species affects stable isotope distributions in sediments. Experiments using ^{35}S have demonstrated such an isotope exchange, but a more quantitative understanding calls for similar experiments using ^{34}S . **Back reaction during sulfate reduction affects the interpretation of both radioisotope experiments and stable isotope fractionation and needs to be better understood.**

Global sulfur cycle. Published efforts to determine the role of sulfate reduction and sulfur cycling relative to other major element cycles in the seabed show numbers that diverge considerably, partly due to the different approaches used. Some studies are based only on diffusion reaction modelling of sulfate profiles, but this approach tends to miss the highest rates of sulfate reduction in near surface sediments of the continental shelf, where most of the global marine sulfate reduction takes place. Further studies are needed to reconcile measured and modelled sulfate reduction rates and to understand their quantitative relationship as a function of sedimentation rate and other environmental factors. This will also help to better understand how methane production in subsurface sediments is regulated relative to the deposition of organic matter and the extent of sulfate reduction in the seabed. While the respiratory quotient of phytoplankton and the $\text{C}_{\text{org}}:\text{O}_2$ ratio by the aerobic mineralisation of export production from the photic zone have been studied extensively, **the $\text{C}_{\text{org}}:\text{SO}_4^{2-}$ stoichiometry by sulfate reduction in marine sediments is still poorly constrained and calls for further study.**

Microbiology. Our understanding of the diversity of microorganisms and their expressed genes in the seabed has been growing at an impressive pace in recent years due to the rapidly increasing sequencing capacity for DNA and RNA.

It is important to also focus on the targeted cultivation and experimental studies of organisms with specific functions in the sulfur cycle and in other biogeochemical processes, functions that cannot be predicted from genetic information alone. An example are the cable bacteria, which are apparently normal sulfate reducers that have a unique property: long distance electron transfer. Other examples are the syntrophic associations among bacteria and archaea that provide the partners with a physiological potential that is not realised in the individual organisms. **Here is a field open to new exciting discoveries and surprises.**

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INDEX

A

Aarhus Bay 149, 167, 175-178, 182, 184,
197, 214, 224, 225, 229-233, 239, 241,
243, 248, 255, 256, 259
acetate 153, 154, 185, 224, 227-231, 236,
237, 246, 250
acid volatile sulfide (AVS) 252
amino acid racemisation 224

B

back-reaction 168, 172, 173, 274
Bak, Friedhelm 160, 213, 215-217
Baltic Sea 149, 157, 165, 172, 175, 213,
214, 222, 224, 229, 236-240, 242, 243,
247-250, 257-261
basal power requirement 225, 226
Beggiatoa 149, 155, 156, 158, 163, 199,
200, 202, 203, 207
benthic oxygen consumption 152
Berner, Robert A. 156, 157, 160, 174, 176,
180, 222, 264
bioirrigation 174, 177-180
bioturbation 174, 222, 228, 233, 235, 255,
266, 272

Black Sea 151, 161, 164, 165, 172, 257, 259,
261
Boetius, Antje 164, 249, 250
Bornholm Basin 149, 237, 238, 242, 243,
259, 260
Boudreau, Bernard P. 157, 175, 183, 237
butyrate 227, 228, 229

C

cable bacteria 146, 149, 203-206, 272, 275
Canfield, Donald E. V. 160, 163, 164, 170,
176, 181, 193, 198, 200, 215, 220, 227,
232, 233, 235, 251, 255, 264-267, 270,
271
carbon dioxide (CO₂) 146, 153, 154, 181,
200, 203, 205-210, 221, 224, 227, 228,
236, 246, 247, 249, 250, 263, 268, 269,
273
chromium reducible sulfur (CRS) 259
Cohen, Yehuda 158
colony counts of bacteria 155
cryptic methane cycle 246, 247, 274
cryptic sulfur cycle 146
cyanobacteria 158, 159
Cypionka, Heribert 160, 185, 186, 213, 215

D

dark CO₂ assimilation 205, 207
deep biosphere 164, 165, 166, 224
denitrification 202, 231, 264
DesMarais, David J. 159
Desulfobacterium autotrophicum 184, 185
Desulfobacter postgatei 154, 185
Desulfotomaculum 154, 189
Desulfovibrio 185, 215
diffusion coefficient 180, 231, 237, 257
diffusive boundary layer (DBL) 231, 232
disproportionation 145, 160, 191, 215-219,
220, 255, 273
dissimilatory nitrate reduction to
ammonium (DNRA) 202, 231
dsrAB genes 155, 225

E

electron acceptor 193, 200, 202, 203, 205,
207, 210, 215, 227, 228, 233
endospores 168, 189, 190

F

Fenchel, Tom 150-152, 155, 158, 191, 227
fermentation 158, 168, 169, 203, 215,
226-229, 236
Fossing, Henrik V. 160, 161, 163, 170, 173,
178, 179, 193-195, 197, 200, 216, 219,
245, 251

G

Gibbs free energy 182, 225, 230, 248
global budget 262
global POC flux 262-264, 267, 268
Goldhaber, Martin 157, 174, 251
greigite (Fe₃S₄) 221
growth efficiency 207, 208
growth yield 168, 187, 188, 200, 207, 224
Guaymas Basin 189, 190

H

Haps corer 152, 153
hematite (Fe₂O₃) 221
Holocene mud 176, 212, 238, 242, 243,
258, 259
hydrogen (H₂) 181, 203, 215, 246
hydrolytic degradation 189, 226

I

iron Fe(III) 148, 149, 174, 191, 197, 198,
210-212, 215, 220-222, 227, 233, 235,
248, 249, 273
iron reduction 211, 232, 233, 235, 249
iron sulfide (FeS) 155, 194, 198, 203, 221
isotope:
diffusion 146, 156, 157, 158, 164, 168,
174, 176, 178, 179
exchange 152, 157, 160, 161, 168, 172,
173, 193-195, 216, 219, 220, 228,
250, 251, 262, 274
fractionation 146, 168, 172, 251-257,
259, 274
Ivanov, Michail V. 151

J

Jannasch, Holger W. 161, 162, 200
Jørgensen, Carl Christian Barker 150

K

Kattegat 149, 152, 175, 178, 216-218, 234
Knoblauch, Christian 187, 189, 226

L

lactate 185, 228, 283
Limfjorden 149, 152-154, 165, 171, 174,
178, 191

M

mackinawite (FeS) 221
manganese Mn(IV) 192, 193, 220, 232,
233, 234, 266
manganese reduction 233, 266
Max Planck Institute for Marine
Microbiology VI, 154, 161, 162, 251
methane (CH₄):
anaerobic oxidation (AOM) 153, 164,
172, 233, 247-249, 252, 273
methanic zone 146, 179-182, 185, 193,
198, 227, 237-239, 244, 245, 249,
250, 257, 267, 268, 272
methanogenesis 176, 193, 228-230,
236-240, 250, 258, 259, 267, 268,
270, 274
methanogenic sediment 240, 242
methanogens 181, 221, 236, 237, 239,
246, 250

shallow gas 239, 240, 242, 269
 microelectrode 156, 232
 mineralisation 145, 146, 152, 156, 158, 169,
 174, 175, 180, 181, 192, 195, 208, 223,
 224, 226, 231-233, 238, 239, 262-264,
 266, 267, 269, 270, 272-274
 model:
 multi-G 157, 174, 175
 power law 157, 174-177, 179-181, 223,
 224, 237-239, 245, 246, 247, 266
 reactive continuum 157, 175, 179
 stable sulfur isotopes 157, 251
 molybdate (MoO_4^{2-}) 228, 232

N

Nelson, Douglas C. 159, 199, 200, 202,
 207, 208
 nitrate (NO_3^-) 149
 North Sea 149, 152, 175, 239, 240, 243, 247

O

optimum temperature (T_{opt}) 186, 187

P

pH 192, 198-200, 203, 204, 211, 220, 222
 polysulfide (S_x^{2-}) 149
 Postgate, John R. 153, 154
 propionate 203, 227-230
 pyrite (FeS_2) 149, 171, 191, 234

R

radiotracer (^{14}C) 151, 152, 168, 170, 176,
 194, 195, 219, 228, 237, 247, 251, 272
 radiotracer (^{35}S) 152, 246, 251, 272
 reactive transport model 178
 redox cascade 211, 227, 228, 231
 reduced microniches 156, 192
 respiratory quotient 262-264, 274
 Revsbech, Niels Peter V. 156, 158, 159,
 192, 199, 200, 231

S

Schulz-Vogt, Heide N. V. 164, 202
 sediment oxygen uptake 152
 seismo-acoustic 176, 239, 240, 242, 244
 Shewanella 198
 Solar Lake 158, 159
 Sorokin, Yuri I. 151, 215

stable sulfur isotopes (^{32}S , ^{33}S , ^{34}S) 157,
 251
 suboxic zone 193
 sulfate (SO_4^{2-}) 148, 181-184, 194, 196, 203,
 207, 210, 215, 220, 225-227, 244, 246,
 247, 253, 265, 269-271, 274
 sulfate-methane transition (SMT) 237,
 238, 245, 246, 248, 256, 267
 sulfate-reducing bacteria (SRB) 155
 psychrophilic 164, 186, 187, 189, 190
 thermophilic 186, 189, 190
 sulfate-reducing microorganisms
 (SRM) 169
 sulfate reduction:
 cell-specific (csSRR) 254
 global 265, 266, 269, 270
 kinetics 157, 174, 182-184, 197, 222,
 223, 228
 rate (SRR) 169, 265
 temperature 186-189
 thermochemical 269
 sulfatic zone 153, 175, 176, 178, 180, 181,
 185, 193, 228, 236-238, 242, 245-249,
 255-257, 259, 265, 267, 272, 273
 sulfide (H_2S) 233
 sulfide oxidation 145, 146, 160, 161, 163,
 168, 173, 181, 182, 191, 193-198, 200,
 203, 204, 206-208, 211, 216, 218-220,
 233, 255, 271-273
 sulfide oxidising bacteria 163, 199, 200,
 206
 sulfidisation front 211, 212, 257, 259-261
 sulfite (SO_3^{2-}) 149, 211
 sulfur:
 elemental (S^0) 145, 149, 160, 170, 171,
 191, 194, 195, 200, 202, 210, 211,
 213, 215, 219-221, 252, 255, 259, 273
 sulfur bacteria 146, 149, 155, 163, 199-201,
 202, 207, 208
 Sulfurimonas 198, 212
 Svalbard 163, 187-189, 190, 233, 234
 syntrophy 153, 164, 236

T

tetrathionate ($\text{S}_4\text{O}_6^{2-}$) 149, 214
 Thamdrup, Bo 160, 164, 193, 198, 200,
 214, 215, 220, 221, 227, 233-235, 249,
 251, 255
Thiobacillus 206, 207

Thiomargarita 163, 164, 200, 202
Thiomicrospira 206, 207
Thioploca 163, 200, 201
thiosulfate ($\text{S}_2\text{O}_3^{2-}$) 149, 211

V

viable counts 155
virus 168, 224
volatile fatty acid (VFA) 228

W

Wadden Sea 187
Widdel, Friedrich (Fritz) 154, 162

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