Doctoral Thesis

The sulfur cycle: from bacterial microenvironment to global biogeochemical cycles

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THE SULFUR CYCLE:
FROM BACTERIAL MICROENVIRONMENT TO
GLOBAL BIOGEOCHEMICAL CYCLES

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH
for the degree of
Doctor of Natural Sciences

presented by
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2003
PhD theses become more and more collections of papers, which can be read elsewhere, and one might therefore conclude that there is no longer any need to read a thesis. However, having been in the audience at many PhD defences, I noticed that most people read only the acknowledgements of the thesis while it is being circulated. The order in the appearance (or non-appearance) of names is noted, and is then interpreted on a popularity- and importance scale, even when the author did not intend this at all. I have been thinking of many ways to avoid the embarrassment of placing the names of the acknowledged people in an order but I did not find a solution. At least, I did not want to place just one on the top and therefore, I first of all say “thank you” to the sulfur isotope curve below:

This isotope curve was the first data set I measured after some months of lab work full of failures and it was this that I brought along to a crisis meeting with my two supervisors – now come names – Dr. Stefano Bernasconi and Prof. Helmut Weissert. Based on this curve, showing a huge negative sulfur isotope excursion during the “Selli” oceanic anoxic event (OAE 1a), we decided that my thesis was not going to be stopped. Thanks to this isotope curve, I can write today the acknowledgements of my PhD thesis which otherwise would not have been written at all. Later, we found that the sulfur isotope data of this curve did not reflect environmental signals but the darkness of the rock sample color. After a while, the curve turned into a positive sulfur isotope excursion, but in the end, the final result was again a negative sulfur isotope shift. During these four years of stagnation and rapid change, Stefano and Helmi patiently accompanied me on my way through the labyrinth of scientific success and failure. The unfading optimism of Helmi in face of new puzzling results coupled with the
calmness of Stefano being confronted with endless calculations of isotope mass balances always encouraged me to keep going (and to make another calculation – sorry Stefano). Thank you both for giving me the freedom to explore the sulfur-jungle and to secure me of being rescued in case I got lost!

It would not have been possible to carry out this thesis without somebody in the background, paying for this study. To me, the financial support by Prof. Judy McKenzie was not only a salary, but also a great gift: Trust. One needs a lot of trust to invest into a PhD project with a working title not far from “let’s look a bit at the sulfur cycle”. Judy, I am very grateful for the trust you paid in me and I hope the results of my work will also encourage other professors to invest from time to time into a “high-risk thesis”.

And there are two other people who paid a lot of trust in me: Dr. Jutta Kleikemper and Dr. Martin H. Schroth from the Institute of Terrestrial Ecology ETHZ. They were willing to let us participate in their work with sulfate reducing bacteria, on the risk that we geologists would spoil their results. I am thankful for this outstanding interlaboratory partnership!

Many people gave me important input, helped me to new ideas and opened my eyes and mind to new perspectives; without their contribution I would not have achieved any of the results presented in this thesis: Dr. Michael Böttcher tempted me to consider oxygen isotopes in sulfate as a tool to investigate sulfate reduction rates, he invited me to an exiting sulfur-workshop in Bremen together with the post-docs of the Cenomanian-Turonian network and he agreed to do the job as external referent. Thank you Michael for your help and for the interest in my PhD! I thank Dr. Lukas Wissler for introducing me to the modeling of isotope mass balances and the complex interplay of alkalinity and carbon reservoirs. I thank Prof. Ulrich G. Wortmann for his enthusiastic interest in modeling biogeochemical processes in interstitial waters and his unbeatable ability to listen openly to my weird ideas about the sulfur-world. Uli Heimhofer always was willing to update my knowledge about black shales, Dr. Daniela Schmidt saved me from stumbling into biostratigraphic traps and Prof. Hans Thierstein encouraged me to investigate the sulfur isotopes at the Cretaceous-Tertiary boundary.

Further, I would like to thank Dr. Peter Hochuli for allowing me to use lab space in the Paly- nology laboratory, I would like to thank the Petrology group for letting me use their second rock mill right after destroying their first one, I would like to thank Dr. Karsten Kunze for the hours helping me searching marine barite with the electron microscope in samples where no barite occurred and I would like to thank the NO-library staff, especially Mrs. C. Niemz, for helping me find unfindable papers and books.
It’s wonderful to have a family, for being cheered up when things are not going well and for celebrating parties when they fall. In my case, in the years studying Earth sciences and doing a PhD, I have had the luxury of being part of three families, the “geological” family, my “biological” family and the “girl-friend” family. From all of them, I got invaluable support and friendship. I am very grateful for that.

My greatest thanks go to my life partner Anita Weber: She prevented my brain from growing anoxic during the writing-up of my thesis…

Benjamin Brunner, June 2003
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ABSTRACT

The biogeochemical cycle of sulfur plays an active role in the biosphere, hydrosphere, atmosphere and lithosphere. This is mainly due to the large abundance and the properties of sulfur to attain a broad range of oxidation stages (+VI to –II) and to be present in ionic (e.g. as sulfate), elemental and gaseous forms (e.g. as SO$_2$ and H$_2$S). Biologically mediated reduction of sulfate in low oxygen environments is the most dominant process in sulfur cycling. The processes in the cycling of sulfur have a strong impact on the environment, from the small scale (e.g. the degradation of organic matter in a contaminated aquifer) to a large scale (e.g. the influence of the sulfur cycle on the atmospheric oxygen content on geologic time scales). Understanding the sulfur cycle, therefore, is a key to the understanding of environmental changes. An important tool for the investigation of fluxes in the sulfur system is the measurement of stable isotope ratios.

This study investigates four important topics concerning the cycling of sulfur, covering the spatial and temporal span from bacterial sulfate reduction in a microenvironment to Phanerozoic global biogeochemical cycles.

The results of this study are:

• contribution to the basic understanding of microbial sulfate and nitrate reduction and the related isotope effects,
• improvement of the data quality of sulfur and oxygen isotope measurements of sulfate extracted from bulk carbonate rocks,
• demonstration that imbalances in the sulfur cycle are likely to have contributed to the environmental perturbations in the Aptian (Early Cretaceous),
• evidence that hydrothermal and volcanic sulfur fluxes are needed to balance Earth’s oxygen budget during the Phanerozoic.

The addressed topic, the used approach and the results of each chapter are briefly summarized below:
Chapter 1

Combined measurements of δ³⁴S and δ¹⁸O of sulfate: A tool to determine microbial sulfate reduction rates?

Dissimilatory reduction of sulfate causes changes in the sulfur and oxygen isotope composition (δ³⁴S, δ¹⁸O) of the remaining sulfate pool. The sulfur isotope effects are interpreted as result of a cascade of kinetic isotope fractionation steps within sulfate reducing bacteria. The mechanisms by which sulfate reducing bacteria cause oxygen isotope effects are less constrained. There is strong evidence that these effects are dominated by equilibrium oxygen isotope exchange between sulfur-oxygen compounds and ambient water within the cells of sulfate reducing bacteria. The mathematical relation between the amount and the sulfur isotope composition of residual sulfate in a closed system affected by sulfate reducers is a commonly used tool to determine sulfur isotope fractionation factors, which in turn have been used as indicators for sulfate reduction rates, however with limited success. A similar mathematical tool describing the relation of the amount and the oxygen isotope composition of residual sulfate has not been available. Therefore, and based on the assumption that equilibrium oxygen isotope exchange is the dominating process controlling the δ¹⁸O of residual sulfate, a mathematical model was developed. It describes the relationship between the amount, the δ³⁴S and the δ¹⁸O of residual sulfate for bacterial sulfate reduction in a closed system. The equations can be used as a tool for the investigation of biochemical sulfate reduction pathways and have the potential to be the long-sought-for key to determine cell-specific sulfate reduction rates. Furthermore, it is speculated that the changes in the δ¹⁸O of residual nitrate during denitrification are the result of an equilibrium isotope exchange between ambient water and cell-internal nitrogen-oxygen compounds. Thus, the presented equations can also be used for the analysis of processes related to dissimilatory nitrate reduction.

Chapter 2

Sulfur isotopes from structural substituted sulfate in bulk carbonates: Improved method and application to the K-T boundary

The reconstruction of the sulfur isotopic composition of seawater through time is based on measurements of sulfur-containing minerals in sediments, mainly evaporites, pyrite and marine barite. A further method, the extraction of structural substituted sulfate (SSS) from cal-
cites, has successfully been applied to fossil shells, e.g. belemnites and brachiopod shells and also on bulk carbonates. The use of this method on bulk rock samples (limestones and marls), however, has many uncertainties. Therefore, an improved extraction treatment focusing on the removal of sulfides contaminating SSS was developed. It is demonstrated that the measurement of the oxygen isotopic composition of the extracted sulfate is crucial to identify SSS-sulfur that has been altered in its isotopic composition by early diagenetic processes within interstitial waters. The improved method is applied to the reconstruction of the sulfur isotopic composition of seawater around the K-T boundary. The results indicate that the sulfur and oxygen isotopic composition of seawater sulfate did not change over the Cretaceous-Tertiary boundary.

Chapter 3
Towards a better understanding of major perturbations in the biogeochemical cycles of the Early Cretaceous (Valanginian and Aptian): Evidence from sulfur isotope analysis and combined modeling of the carbon and sulfur cycle

Understanding the behavior of the “system Earth” during times of rapid environmental change is a major concern in earth science. The Early Cretaceous is marked by two periods of turnover in the biogeochemical cycles: The Valanginian and the Aptian. These time intervals can be used as archives that contain information how the “system Earth” behaves in times of change. Processes that affect the alkalinity of the ocean and the carbonate equilibrium in seawater play a major role in these perturbations. Imbalances in the sulfur cycle strongly affect the oceanic alkalinity, but precise sulfur isotope data to detect such imbalances have been missing for the investigated time slices. Therefore, sulfur and oxygen isotope data of structural substituted sulfate in carbonates from bulk rocks samples of the Valanginian and Aptian were determined. The Valanginian $\delta^{34}$S values remain constant around 19‰ whereas the corresponding $\delta^{18}$O values are around 16‰. The Aptian-Albian time slice is marked by a constant $\delta^{18}$O value of around 15.4‰. The sulfur isotope data for the Early Aptian show a decrease from 18.2‰ to 17.3‰ within roughly three million years. With the onset of the Albian, the $\delta^{34}$S value increases again to values around 19‰. In order to interpret these results, a numerical model was implemented. This model combines information from the sulfur and carbon cycle and investigates the impact of changes on the carbonate equilibrium in seawater. The results of the model runs imply that increased volcanic and hydrothermal degassing of
carbon dioxide, hydrogen sulfide and sulfur dioxide is likely to be the initial cause for the environmental perturbations in the Valanginian and Aptian.

Chapter 4

Hydrothermal and volcanic sulfur fluxes are needed to balance Earth’s oxygen budget during the Phanerozoic

Throughout the Phanerozoic, the amount of atmospheric oxygen has remained relatively constant. The biogeochemical cycles of carbon and sulfur play a major role in this balance. The estimates for the size of the fluxes in the sulfur cycle vary significantly, especially for fluxes related to seafloor hydrothermal systems. This has led to a major disagreement in the scientific community: Some researchers postulate the presence of significant degassing of hydrogen sulfide from seafloor hydrothermal systems on geologic time scales, whereas others claim that such fluxes are incompatible with the sulfur and carbon isotope record and a stable atmospheric oxygen budget. In order to shed light on this issue, an oxygen budget was calculated that includes a steady state mass balance for the carbon and sulfur cycle and considers the mantle-degassing of hydrogen sulfide and carbon dioxide and further oxygen sinks. The somewhat surprising result of this calculation is that hydrothermal and volcanic sulfur fluxes are needed to balance earth’s oxygen budget during the Phanerozoic.
ZUSAMMENFASSUNG


Die vorliegende Arbeit untersucht vier wichtige Themen bezüglich des Schwefelkreislaufes, welche die räumliche und zeitliche Spanne von der bakteriellen Sulfatreduktion in einem Mikromilieu bis zu globalen biogeochemischen Kreisläufen während des Phanerozoikums abdecken.

Die Resultate dieser Studie
• tragen zum grundsätzlichen Verständnis von mikrobieller Sulfat- und Nitratreduktion und den involvierten Isotopeneffekten bei,
• verbessern die Datenqualität von Schwefel- und Sauerstoffisotopenmessungen an Sulfat, welches aus karbonatischen Gesteinen extrahiert wurde,
• zeigen, dass Ungleichgewichte im Schwefelkreislauf wahrscheinlicherweise zu den Umweltstörungen im Apt (Frühe Kreide) beitrugen,
• bezeugen, dass hydrothermale und vulkanische Schwefelfluxe für die Aufrechterhaltung des Gleichgewichts im atmosphärischen Sauerstoffgehalt während des Phanerozoikums notwendig sind.

Im Folgenden werden das untersuchte Thema, der benutzte Lösungsansatz und die Resultate der Kapitel kurz zusammengefasst.
Kapitel 2

Schwefelisotopen von strukturell substituiertem Sulfat in Gesamtgesteinskarbonaten: Verbesserte Methodik und Anwendung an der K–T Grenze


Kapitel 3


Kapitel 4

*Hydrothermale und vulkanische Schwefelfluxe sind notwendig um die Sauerstoffmassenbilanz der Erde während des Phanerozoikums auszubalancieren*

massenbilanz während des Phanerozoikums hydrothermale und vulkanische Schwefelfluxe notwendig sind.
INTRODUCTION

Overview

Sulfur is the fourteenth most abundant element in the Earth’s crust. It occurs in several oxidation states. In its reduced state, sulfur is a key nutrient for life, providing structural integrity to protein-containing tissues. In its fully oxidized state, sulfur exists as sulfate. Sulfate is the second most abundant anion in rivers and seawater (after bicarbonate in rivers and after chloride in seawater). In both natural and polluted rainwater, sulfate is the major cause of acidity (acid rain). In the atmosphere, sulfate serves as condensation nuclei in clouds. High atmospheric concentrations of sulfate, therefore, enhance the formation of clouds. This, in turn, influences the Earth’s greenhouse radiation-budget. In anoxic environments, sulfate replaces oxygen as the available electron acceptor. Microbial sulfate reduction is quantitatively the most important process of organic matter degradation under low oxygen conditions in the oceans. Together with other major elemental cycles (i.e. C, N, O, P), the sulfur cycle is heavily perturbed by anthropogenic emissions of sulfur, mainly by the burning of fossil fuel. (CHARLSON et al., 2000)

Sulfur and oxygen isotopes as tracers for biogeochemical transformations in the sulfur cycle

Processes in the sulfur cycle can be traced by the measurement of concentrations of different sulfur containing compounds and by the analysis of their respective sulfur isotope composition. The investigation of the sulfur cycle on geologic time scales, however, mainly relies on isotope studies because other quantitative information is missing or very imprecise. An example for the lack of information is the concentration of sulfur gases in the atmosphere, whereas an example for imprecise information is the concentration of seawater sulfate (the concentration / presence of sulfate in seawater of past oceans can only be estimated by the composition of evaporite rocks). Thus, isotope studies are the key to the investigation of recent and past sulfur cycling.
There are four stable isotopes of sulfur:

<table>
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<th>Isotope</th>
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<tr>
<td>$^{32}\text{S}$</td>
<td>95.0</td>
</tr>
<tr>
<td>$^{33}\text{S}$</td>
<td>0.76</td>
</tr>
<tr>
<td>$^{34}\text{S}$</td>
<td>4.22</td>
</tr>
<tr>
<td>$^{36}\text{S}$</td>
<td>0.014</td>
</tr>
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</table>

Chemical and biogeochemical transformations of sulfur compounds fractionate the sulfur isotopes. The second most abundant stable sulfur isotope $^{34}\text{S}$ is commonly used to trace these processes. In order to investigate mass independent isotope fractionation processes, the isotopes $^{33}\text{S}$ and $^{36}\text{S}$ have recently gained much interest (Farquhar et al., 2000; Farquhar et al., 2002; Wiechert, 2002). Additionally, the radioactive isotope $^{35}\text{S}$ is used as a tracer in studies investigating the sulfur metabolism of microbes (e.g. the determination of sulfate reduction rates). Due to the stability of sulfate ions towards isotopic exchange of oxygen with ambient seawater, oxygen isotopes can also be used for tracing pathways in the sulfur cycle. The isotope values are reported relative to international standards according to a conventional δ-notation (‰):

$$
\delta^{34}\text{S}(‰) = \left( \frac{R_{\text{sample}}}{R_{\text{CDT}}} - 1 \right) \cdot 1000 = \left( \frac{^{34}\text{S}_{\text{sample}}}{^{32}\text{S}_{\text{sample}}} \right) \left( \frac{^{34}\text{S}_{\text{CDT}}}{^{32}\text{S}_{\text{CDT}}} \right) - 1 \cdot 1000
$$

$$
\delta^{18}\text{O}(‰) = \left( \frac{R_{\text{sample}}}{R_{\text{SMOW}}} - 1 \right) \cdot 1000 = \left( \frac{^{18}\text{O}_{\text{sample}}}{^{16}\text{O}_{\text{sample}}} \right) \left( \frac{^{18}\text{O}_{\text{SMOW}}}{^{16}\text{O}_{\text{SMOW}}} \right) - 1 \cdot 1000
$$

$R$ equals the ratio between the heavy (less abundant) isotope and the light (more abundant) isotope. CDT refers to the $^{34}\text{S}/^{32}\text{S}$-ratio of the standard troilite, an iron monosulfide from the Canyon Diablo Meteorite. SMOW refers to the $^{18}\text{O}/^{16}\text{O}$-ratio of Standard Mean Ocean Water. Isotope effects by biogeochemical processes are described by the relation of the isotope composition of the source material (substrate) to its instantaneous reaction product. There are two commonly used notations for this relation, the fractionation factor $\alpha$ and the isotope enrichment factor $\varepsilon$, also designated $\Delta$. Using sulfate reduction as example, the relation between $\alpha$, $\Delta$, $\varepsilon$ and $\delta$ is introduced below.
The chemical equation for sulfate reduction is written as follows:

\[ \text{SO}_4^{2-} + 2\text{CH}_2\text{O} \rightarrow \text{H}_2\text{S} + 2\text{HCO}_3^- \]

The isotope fractionation factor \( \alpha \) of the above reaction is defined as:

\[
\alpha_{\text{H}_2\text{S}-\text{SO}_4^{2-}} = \frac{R_{\text{product}}}{R_{\text{source}}} = \frac{^{34}\text{S}_{\text{H}_2\text{S}}}{^{32}\text{S}_{\text{H}_2\text{S}}} \frac{^{34}\text{S}_{\text{SO}_4^{2-}}}{^{32}\text{S}_{\text{SO}_4^{2-}}}
\]

The isotope enrichment factor (\( \epsilon \) or \( \Delta \)) illustrates the relation between the source (substrate) and the product in a more intuitive way. Negative %e-values indicate that the product is depleted in the heavy isotope \(^{34}\text{S}\), positive %e-values indicate that the product of the reaction is enriched in the heavy isotope \(^{34}\text{S}\) compared to the isotopic composition of the source sulfur compound.

\[
\Delta^{34}\text{S}_{\text{H}_2\text{S}-\text{SO}_4^{2-}}(\%e) \approx \epsilon_{\text{H}_2\text{S}-\text{SO}_4^{2-}}(\%e) = (\alpha_{\text{H}_2\text{S}-\text{SO}_4^{2-}} - 1) \cdot 1000
\]

The \( \delta^{34}\text{S} \) values of the source and the product in a sulfate reduction experiment can be used for the determination of the enrichment factor. The equations for the isotope enrichment factor and for the \( \delta \)-notation are combined:

\[
\Delta^{34}\text{S}_{\text{H}_2\text{S}-\text{SO}_4^{2-}}(\%e) \approx \epsilon_{\text{H}_2\text{S}-\text{SO}_4^{2-}}(\%e) = \left( \frac{\delta^{34}\text{S}_{\text{H}_2\text{S}}(\%e) - \delta^{34}\text{S}_{\text{SO}_4^{2-}}(\%e)}{\delta^{34}\text{S}_{\text{SO}_4^{2-}}(\%e) + 1000} \right) \cdot 1000
\]

As long as the \( \delta \)-values of the source material is relatively small, the above equation can be approximated as:

\[
\Delta^{34}\text{S}_{\text{H}_2\text{S}-\text{SO}_4^{2-}}(\%e) \approx \epsilon_{\text{H}_2\text{S}-\text{SO}_4^{2-}}(\%e) = \delta^{34}\text{S}_{\text{H}_2\text{S}}(\%e) - \delta^{34}\text{S}_{\text{SO}_4^{2-}}(\%e)
\]

The error by this approximation is calculated in the following example: The \( \delta^{34}\text{S} \) of today’s seawater sulfate is around 21‰. Assuming that the instantaneous \( \delta^{34}\text{S} \) of produced H\(_2\)S by sulfate reduction is around –39‰, an approximated \( \Delta^{34}\text{S} \) of –60‰ results. Without approximation, the \( \Delta^{34}\text{S} \) would be –58.8‰. The reproducibility of sulfur isotope measurements is around ±0.2‰. Consequently, the error by the above approximation is larger than the reproducibility of the measurement. This demonstrates that the above approximation is not always valid. However, in the course of this thesis, where enrichment factors are notated as \( \Delta^{34}\text{S}(\%e) \) values, this problem was not encountered.
**The biogeochemical sulfur cycle**

The sulfur cycle consists of four subcycles: (1) weathering and burial of sulfate (e.g. evaporites), (2) weathering and burial of sulfides (e.g. pyrite), (3) metamorphic processes and (4) fluxes from and to the mantle reservoir (Figure 1) (HOLSER et al., 1988).

![Figure 1](image)

**Figure 1** The biogeochemical cycle of sulfur

The sulfur cycle consists of four subcycles:
1) Weathering and burial of sulfate (e.g. evaporites, marine barite, sulfate bound in carbonates)
2) Weathering and burial of sulfides (e.g. as sulfides, formation within sediments due to bacterial sulfate reduction)
3) Meatmorphic processes (e.g. degassing of SO$_2$ and H$_2$S due to heating of sulfate and sulfides)
4) Fluxes from and to the mantle reservoir (e.g. degassing of H$_2$S, SO$_2$ and incorporation of anhydrite and sulfides precipitated in oceanic crust at seafloor hydrothermal systems or subduction sulfides and sulfate)

These four subcycles control the sulfur and oxygen isotope composition and mass balance of seawater sulfate. The chemical reactions related to the fluxes affect the alkalinity and amount of oxygen in the atmosphere-ocean reservoir. Together with the carbon cycle, the sulfur cycle, therefore, plays an import role in controlling the ocean carbonate equilibrium, atmospheric pCO$_2$ and the amount of oxygen in the atmosphere-ocean reservoir (Figure 2).
The alkalinity, the carbonate equilibrium in seawater and the amount of oxygen in the ocean-atmosphere reservoir are controlled by the fluxes from and to the carbon and sulfur rock reservoirs. The stars on the red line mark chemical transformations where oxygen is consumed or produced. Due to the different isotopic composition of the sources and sinks in the carbon and sulfur cycle, imbalances in the fluxes can be traced. In this illustration, the precipitation and leaching of anhydrite has been included into the weathering and burial fluxes of sulfate (evaporites).

Figure 2 shows that the fluxes in the sulfur and carbon cycle control the carbonate equilibrium in seawater, the alkalinity and the amount of oxygen in the ocean-atmosphere reservoir. The subcycles of burial and weathering of reduced compounds (organic matter and sulfides) and the degassing of CO₂, SO₂ and H₂S are not fully independent. The burial and, therefore, also the weathering of organic matter is coupled to the burial of sulfides (increased burial of organic matter induces increased burial of sulfides), the degassing of volatiles (CO₂, SO₂ and H₂S) is related to the activity of volcanoes and hydrothermal systems. The carbon and sulfur isotope composition of degassed CO₂, SO₂ and H₂S deviates from that of inorganic carbon in seawater and seawater sulfate both being a few per mil lower. Sulfides and organic matter are marked by distinctly lower concentrations in the heavy isotopes ³⁴S and ¹³C, respectively. This is due to biochemical kinetic isotope fractionation effects. Compared to the above isotope differences, the isotope effects related to carbonate and sulfate crystallization from dissolved species are relatively small. To investigate imbalances in the weathering- and burial fluxes of carbonates and sulfates, the Δ¹³C and Δ³⁴S of carbonates and sulfates, therefore, have very limited use. However, due to the constant small fractionation during precipitation, the Δ¹³C of...
carbonates and the $\delta^{34}\text{S}$ of sulfates can be used as proxies for the isotopic composition of dissolved inorganic carbon and sulfate in the ocean at the time of their deposition. Carbon and sulfur isotope versus age curves derived from the analysis of carbonates and sulfates consequently mirror the balance between the input of volatiles and the burial and weathering fluxes of reduced sulfur and carbon compounds. As shown in Figure 2, the balance between these fluxes is a major controlling factor of the amount of atmospheric oxygen, the alkalinity and the carbonate equilibrium in seawater. Trends in the $\delta^{34}\text{S}$ and $\delta^{13}\text{C}$ curves, therefore, record the history of these reservoirs. However, one must be aware that this history is only a fragmentary record: Other biogeochemical cycles that also influence the ocean-atmosphere reservoirs, e.g. the consumption of oxygen due to oxidation of iron-containing minerals, are not traced by carbon and sulfur isotopes. The sensitivity of the carbon and sulfur reservoir to changes in their input and output fluxes is strongly different: This is mainly due to the different reservoir size of inorganic carbon in the ocean-atmosphere reservoir (about $3.3 \times 10^{18}$ mol C) and the amount of sulfate in seawater (about $40 \times 10^{18}$ mol sulfate; HOLSER et al. (1988)). In addition to this, the fluxes in the sulfur cycle are a ten to a hundred times smaller than the fluxes in the carbon cycle. These circumstances lead to totally different sensitivities towards changes in the concentration or isotopic composition for the carbon and sulfur cycle. This is expressed in the different residence time for the carbon and sulfur cycle (a few 10 ka to 100 ka for carbon, about 20 Ma to 40 Ma for sulfur; HOLSER et al. (1988)). Sulfur isotope changes in seawater, therefore, are likely to occur slowly, whereby a rapid shift would be indicative of a strongly perturbed sulfur cycle.

Open questions in the sulfur cycle: From Earth’s biogeochemical cycles to the microenvironment of bacterial sulfate reducers

For many reasons, the extraction of relevant information about past and ongoing environmental change from the sulfur isotope record is difficult. The major problems encountered cover the span from Earth’s biogeochemical cycles to the microenvironment of bacterial sulfate reducers:

- The size of most fluxes in the sulfur cycle is not well defined. This is due to the long residence time of sulfur, the anthropogenic overprint of today’s sulfur fluxes, the highly episodic deposition of evaporites and the relative inaccessibility of seafloor hydrothermal systems.
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• On a global long-term scale, the investigation of the impact of changing sulfur fluxes on the environment has mainly focused on the amount of atmospheric oxygen. The influence of sulfur fluxes on the seawater carbonate equilibrium has not been investigated. This is likely to be due to the (wrong?) impression that the fluxes in the carbon system play an overwhelming role in balancing the seawater carbonate equilibrium.

• Some of the methods to determine the sulfur isotope composition of seawaters from the rock record are not reliable. Other more reliable methods seem not to be applicable to the whole geologic record.

• Probably the most frustrating fact about the investigation of the sulfur cycle is that one half of the potential isotopic trace to study processes is almost unused, i.e., the use of oxygen isotopes is in its infancy. This becomes especially evident in the study of microbial sulfate reduction.

A PhD thesis provides the ideal environment to tackle some of the above problems. The broad field of study allows applying and learning different scientific approaches and reduces the risk of complete failure of the project. One could say that the general objective of this thesis was to select the most interesting major open questions in the sulfur cycle. Below, the problems addressed are introduced.

Addressed problems

Chapter 1

Combined measurements of $\delta^{34}S$ and $\delta^{18}O$ of sulfate: A tool to determine microbial sulfate reduction rates

Microbial sulfate reduction is the major process degrading organic matter in low oxygen environments. Understanding this process is one of the key questions about sulfur cycling and of utmost importance in the study of the processes related to degradation of organic matter in anoxic water columns, sediments and soils. The role of sulfate reducers is a key issue in the monitoring of contaminated soils and waste deposits.
Figure 3  Sulfate reduction in a closed system with an isotope enrichment factor of -40‰

The sulfur isotope composition of residual sulfate, produced sulfide and accumulated sulfide was calculated according to the Rayleigh equation (MARIOTTI et al., 1981).

Figure 3 illustrates an example the evolution of the isotope composition of residual sulfate (blue line) and produced (accumulated) sulfide (green line) in a closed system. Due to the constant kinetic isotope enrichment factor (red arrows), the difference between the $\delta^{34}$S of sulfate and the $\delta^{34}$S of the instantaneous reaction product remains constant. In this example, an isotope enrichment factor of 40‰ was used. The remaining (residual) sulfate is constantly enriched in $^{34}$S, but this is also the case for the accumulated sulfide. When all sulfate is consumed, the $\delta^{34}$S of the accumulated sulfide equals the initial sulfur isotope composition of sulfate. The relationship between the sulfur isotope enrichment factor, the sulfur isotope composition and the amount of sulfate can be expressed as a mathematical equation (Rayleigh equation; MARIOTTI et al. (1981)). Using this relation, the sulfur isotope enrichment factor by a specific population of bacteria under specific conditions can be determined in laboratory experiments. Thus, natural conditions can be simulated in the laboratory and the resulting fractionation factors can be applied to field studies, where other parameters (e.g. the amount of sulfate in a waste deposit) cannot be measured precisely.

The sulfur isotope enrichment factor by a bacterium is the result of a cascade of transformations of sulfur compounds within the cell. Each of the transformation steps fractionates sulfur isotopes with a constant isotope fractionation factor. The reasons for varying bacterial isotope enrichment factors are different exchange rates between cell-internal sulfur compounds (REES, 1973). Because the cell specific sulfate reduction rate is a controlling factor in this isotopic exchange, it has been postulated that enrichment factors could be used to determine
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sulfate reduction rates (HABICH and CANFIELD, 1997). However, there is little or no correlation between cell specific sulfate reduction rates and isotope enrichment factors when different sulfate reducing bacteria are compared (CANFIELD et al., 2000; DETMERS et al., 2001).

Much less is known about the isotope effects in the oxygen isotope composition of sulfate that are caused by sulfate reducing bacteria:

Fritz et al. (1989) and Mizutani and Rafter (1973) have shown that sulfate reducing bacteria enable equilibrium oxygen isotope exchange between cell-internal sulfur compounds and ambient water, whereas sulfate does not exchange oxygen isotopes in natural waters. Bacterial oxygen isotope exchange seems to dominate potential kinetic oxygen isotope fractionation during sulfate reduction. The oxygen isotope exchange rate and the cell specific sulfate reduction rates therefore interact, that is, rapid sulfate reduction rates shorten the time for oxygen isotope exchange. This should be reflected in the measurements of \( \delta^{18}O \) of residual sulfate and could allow determining cell specific sulfate reduction rates. However, this interesting potential has not been explored until recent. We propel a first step to address this topic by calculating the cell-internal oxygen isotope mass balance and integrated these results into a “Rayleigh-type” equation for oxygen isotopes in sulfate reduction processes. Chapter 1 describes the derivation of the equations. The promising potential for the study of processes related to sulfate reduction and the implication for other biochemical processes (i.e. the denitrification) are presented.

Chapter 2

Sulfur isotopes from structural substituted sulfate in bulk carbonates: Improved method and application to the K-T boundary

At the initiation of this thesis, there were two publications which stimulated interest in the sulfur cycle: In 1998, Paytan et al. (1998) published a sulfur isotope curve for the Cenozoic based on marine barite. This data set is much more reliable than the known data from evaporites (Strauss, 1999). The curve shows a 5‰ increase in the \( \delta^{34}S \) value of marine sulfate from the Early- to the Mid-Eocene, within a time span of 10 Ma, which has not been explained satisfactorily. In 1999, Ohkouchi et al. (1999) published sulfur isotope data for sediments deposited around the Cenomanian-Turonian boundary, known as oceanic anoxic event (“Livello Bonarelli”, OAE 2). These data show a rapid \( \delta^{34}S \) increase of around 16‰ to 22‰ within a time period of 1 Ma. Such a fast change in the sulfur isotopic composition of seawater sulfate can only be achieved by dramatic changes in the sulfur fluxes (e.g. the flux of
pyrite removal would have to increase by a factor of 10; OHKOUCHI et al. (1999)). However, the scatter in the Cenomanian-Turonian boundary data (±2‰) clearly demonstrates that the method used by (OHKOUCHI et al., 1999), the extraction of sulfate from bulk carbonates, is less reliable than the extraction of marine barite.

Figure 4 The concept of incorporation of structural substituted sulfate into carbonate rocks

Figure 4 demonstrates that seawater sulfate is incorporated into carbonate particles. These particles form a major part of the sediment and contain the primary sulfur and oxygen isotope signal of seawater sulfate. However, sulfate from interstitial water is also incorporated into cements. This sulfate does not reflect the primary isotope composition of seawater sulfate. Extraction and analysis of structural substituted sulfate (SSS) from bulk carbonates therefore represents a mixed isotope signal. This is a major disadvantage of the isotope analysis of SSS from bulk carbonate rocks. Nevertheless, the extraction of SSS from bulk carbonates has also advantages: Carbonate rocks are abundant and relatively easy to place in a stratigraphic framework, whereas marine barite, on the other hand, is difficult to extract from rocks. A major goal of this thesis was to improve the SSS-extraction technique from bulk carbonate rocks in order to achieve better data quality. The improved method was then tested with analysis of the sulfur isotopic composition of SSS from carbonate rocks around the Cretaceous-Tertiary boundary. The results were compared to the SSS-sulfur isotope record of (KAIHO et al., 1999), who report a positive shift of roughly 4‰ over the Cretaceous-Tertiary boundary.
Chapter 3
Towards a better understanding of major perturbations in the biogeochemical cycles of the Early Cretaceous (Valanginian and Aptian): Evidence from sulfur isotope analysis and combined modeling of the carbon and sulfur cycle

The Early Cretaceous is marked by two periods of major turnover in the biogeochemical cycles: The Valanginian and the Aptian. There is evidence for an increase in the ocean crust production rates (LARSON, 1991) and the emplacement of large igneous provinces (WIGNALL, 2001). We hypothesize that these events initiated the observed geochemical and sedimentological patterns of environmental change in the Valanginian and the Aptian. These changes involve the disappearance of carbonate producing organisms, a “drowning” of carbonate platforms and positive excursions in the carbon isotope composition of marine carbonates. Additionally, the Aptian is marked by a shift in the strontium isotope curve, by an oceanic anoxic event (OAE 1a, “Livello Selli”) and by a rapid negative $\delta^{13}$C shift at the base of the interval corresponding to the OAE 1a. It is likely that these perturbations also affected the sulfur cycle. Detailed information about the sulfur isotope trends of seawater in the Valanginian and Aptian is, however, lacking. Thus, we determined the SSS from carbonate rocks, in order to evaluate the influence of sulfur fluxes on the Aptian seawater carbonate equilibrium and implement a numerical box model. This model then was extended to incorporate and simulate the observed environmental changes (Figure 5).

Figure 5  A numerical model to investigate environmental changes related to external forcing (e.g. the volcanic degassing of carbon dioxide)
The implemented model (Figure 5) calculates iteratively the systems response to external forcing mechanisms and is able to simulate the balance between burial and weathering fluxes in order to reproduce carbon isotope shifts determined from the geologic archive. The results of the different model runs allow us to test the hypothesis that volcanic and hydrothermal activity caused the environmental perturbations in the Early Cretaceous.

Chapter 4

Hydrothermal and volcanic sulfur fluxes are needed to balance Earth’s oxygen budget during the Phanerozoic

Throughout the Phanerozoic, the amount of oxygen in the atmosphere has remained approximately constant (BERNER and CANFIELD, 1989). This is attributed to feedback mechanisms in the biogeochemical cycles of carbon, sulfur and oxygen (PETSCH and BERNER, 1998). Imbalances in the weathering and burial fluxes of organic matter were balanced by imbalances in the weathering and burial fluxes of sulfides and seem to have efficiently balanced the production and consumption of atmospheric oxygen (BERNER, 1999). As presented above, imbalances in the fluxes from and to the reduced sulfur and carbon reservoirs induce changes in the isotopic composition of inorganic carbon and seawater sulfate. The compensation of imbalance in the fluxes of organic matter by imbalances in the fluxes of sulfides, therefore, should be manifested in the Phanerozoic sulfur and carbon age curves by inverse isotope trends (HOLSER et al., 1988). However, the amount of oxygen in the atmosphere depends also on other fluxes, such as the degassing of hydrogen sulfide and sulfur dioxide from seafloor hydrothermal systems and volcanoes. The size and importance of these fluxes is controversial: CARPENTER and LOHMANN (1997) postulated the presence of significant degassing of hydrogen sulfide from seafloor hydrothermal systems on geologic time scales. PETSCH (1999) questioned this and claimed, “A mantle-derived sulfide flux would be expected to generate secular trends in the sulfur isotopic composition of seawater if atmospheric oxygen is assumed to remain approximately constant over the Phanerozoic”. By using the approach of PETSCH (1999), we constructed a steady state model over the Phanerozoic and included estimates for mantle-derived carbon and sulfur fluxes in order to test if reasonable atmospheric oxygen partial pressures can be maintained. Chapter 4 presents the used estimates and the somewhat surprising result that hydrothermal and volcanic sulfur fluxes are needed to balance Earth’s oxygen budget during the Phanerozoic.
Relevance of this study

The results of this thesis contribute to the study and understanding of sulfur cycling by:

1. Providing a completely new approach for the investigation and understanding of oxygen isotope fractionation processes in sulfate reduction and denitrification.
2. Improving an unreliable sulfate extraction method to be a valid tool for the reconstruction of the sulfur and oxygen isotope composition of seawater sulfate in earth’s history.
3. Demonstrating the relevance of sulfur fluxes for the oceanic carbonate equilibrium and by shedding light on environmental perturbations in the Valanginian and Aptian (Early Cre-taceous).
4. Contributing evidence that hydrothermal and volcanic sulfur fluxes are needed to balance Earth’s oxygen budget during the Phanerozoic.

References


A MODIFIED SCHEME FOR DISSIMILATORY SULFATE REDUCTION AND
COMBINED MEASUREMENTS OF $\delta^{34}S$ AND $\delta^{18}O$ OF SULFATE:
A TOOL TO DETERMINE MICROBIAL SULFATE REDUCTION RATES?*

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Abstract

Dissimilatory reduction of sulfate causes changes in the sulfur and oxygen isotope composition of the remaining sulfate pool. The sulfur and oxygen isotope effects are related to the stepwise reduction of sulfate to sulfide within the cells of sulfate reducing bacteria. Here, we address three unresolved problems concerning the isotope effects by dissimilatory sulfate reduction.

1. In natural environments, sulfides are commonly depleted in $^{34}S$ by $-45\%$ to $-70\%$ relative to seawater sulfate. Based on laboratory culture experiments, theoretical models for microbial dissimilatory sulfate reduction predict a maximum sulfur isotope fractionation effect around $-50\%$. The apparent discrepancy between the maximum isotope fractionation value by sulfate reducing bacteria and the observation from natural environments is commonly explained by an additional sulfur isotope fractionation process, the disproportionation of elemental sulfur to sulfide and sulfate. However, from hypersulfidic environments, where disproportionation of elemental sulfur does not occur, there is evidence for sulfur isotope fractionation up to $-72\%$. This implies that the maximum sulfur isotope fractionation by dissimilatory sulfate reduction can exceed the theoretical maximum value of $-50\%$.

2. The relation between sulfate reduction rates and corresponding kinetic sulfur isotope fractionation factors is controversial. There seems to be an inverse relationship between cell-specific sulfate reduction rates (sSRR) and sulfur isotope fractionation ($\Delta^{34}S$) factors. However, this correlation of sSRR and $\Delta^{34}S$ is not observed when different sulfate reducing bacteria are compared.

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3. During bacterial sulfate reduction, the changes in the $\delta^{18}O$ of residual sulfate depend on $\delta^{18}O$ of ambient water. The mechanism of this oxygen isotope exchange process is poorly understood. Based on observations from natural environments, it has been suggested that, under certain environmental conditions, kinetic oxygen isotope fractionation is driving the oxygen isotope composition of residual sulfate instead of oxygen isotope exchange with ambient water.

Reviewing the commonly accepted model for the isotope effects by dissimilatory sulfate reduction, we come to the conclusion, that the sulfur isotope effects related to the reduction of sulfite to sulfide are not explained by the hitherto existing model. We come to the conclusion, that the sulfur isotope effects related to this step depend on environmental and cell-specific parameters. Therefore, it is impossible to assign a kinetic sulfur isotope fractionation factor to the reduction of sulfite to sulfide. We emphasize that the sulfur isotope fractionation in this step is in a broad range, from 0‰ to values exceeding the equilibrium isotope effect between sulfite and sulfide around $-50‰$. Consequently, sulfur isotope fractionation by dissimilatory sulfate reduction can reach values above $-70‰$. Considering that the sulfur isotope fractionation factor for the reduction of sulfite to sulfide depends on environmental and cell-specific properties, and not alone on the sulfate reduction rate, it becomes evident that the relationship between $sSRR$ and $\Delta^{34}S$ is also cell-specific. $\Delta^{34}S – sSRR$ relations of different sulfate reducing bacteria, therefore, do not correlate. However, with equations describing the relation between $\delta^{34}S$, $\delta^{18}O$ and the amount of residual sulfate, we present a potential tool to determine $sSRR$, oxygen isotope exchange rates and equilibrium oxygen isotope exchange factors. The developed equations show that equilibrium oxygen isotope exchange between sulfate and ambient water dominates other potential oxygen isotope fractionation processes, a “typical” $\delta^{34}S – \delta^{18}O$ slope does not exist. With culture experiments, we investigate the range of sulfur isotope fractionation factors in which a simplified set of equations can be used and demonstrate the application of the presented equations with numerical examples. We speculate that, during dissimilatory reduction of nitrate (denitrification), the oxygen isotope effects in residual nitrate are the result of oxygen isotope exchange with ambient water. Consequently, the developed equations for the relation between $\delta^{34}S$, $\delta^{18}O$ and the amount of residual sulfate could be modified and used to calculate the relation between $\delta^{15}N$, $\delta^{18}O$ and the amount of residual nitrate during denitrification.
**Introduction**

Dissimilatory reduction of sulfate by microbes causes changes in the sulfur and oxygen isotope composition of the remaining sulfate pool. Despite intense field and laboratory studies, major questions concerning sulfur and oxygen isotope fractionation effects caused by bacterial sulfate reduction remain unresolved. Here, we address three related questions.

1. In natural environments, sulfides are commonly depleted in $^{34}\text{S}$ by $-45\%$ to $-70\%$ relative to seawater sulfate (OHMOTO et al., 1990). Laboratory culture experiments with *Desulfovibrio desulfuricans* yield a maximum sulfur isotope difference between produced sulfides and residual sulfate of around $-46\%$ (see THODE (1991) and references therein) and theoretical models for microbial dissimilatory sulfate reduction predict a maximum sulfur isotope fractionation effect around $-50\%$ (REESE, 1973). An explanation for the apparent discrepancy between the maximum isotope fractionation value by sulfate reducing bacteria and the observation from natural environments has been presented by CANFIELD and THAMDRUP (1994): Sulfides produced by sulfate reduction are oxidized to elemental sulfur, which in turn is disproportionated to sulfide and sulfate. This process can be repeated several times. A consequence of this cycling is a multi-step sulfur isotope fractionation leading to the strongly depleted sulfur isotope composition of sulfides. In hypersulfidic environments, disproportionation of elemental sulfur to sulfide and sulfate is inhibited (CANFIELD and THAMDRUP, 1994). Consequently, a maximum isotope fractionation effect of around $-50\%$ should be observed. However, from hypersulfidic environments, there is evidence for sulfur isotope fractionation up to $-72\%$ (WERNE et al., 2003; WORTMANN et al., 2001). These observations contradict the theoretical maximum isotope fractionation value for microbial sulfate reduction of around $-50\%$.

2. The relation between sulfate reduction rates and corresponding kinetic sulfur isotope fractionation factors is controversial. There seems to be an inverse relationship between cell-specific sulfate reduction rates ($sSRR$) and sulfur isotope fractionation factors ($\Delta^{34}\text{S}_{\text{cell}}$); high $sSRR$ are related to small isotope fractionation factors (THODE, 1991; HABICHT and CANFIELD, 1997; BOLLIGER et al. (2001); KLEIKEMPER et al. (2003)). However, there is little or no correlation of $sSRR$ and $\Delta^{34}\text{S}$ when different sulfate reducing bacteria are compared (CANFIELD et al. (2000); DETMERS et al. (2001); KLEIKEMPER et al. (2003)).

3. The mechanisms by which sulfate reducing bacteria cause oxygen isotope effects are a matter of debate. In natural waters at low temperatures, oxygen isotope exchange between
sulfate and ambient water is extremely slow. Laboratory experiments with sulfate reducing bacteria have shown that the oxygen isotope composition of residual sulfate depends on the oxygen isotope composition of ambient water and that the δ^{18}O of residual sulfate approaches an equilibrium steady state value. Thus, sulfate reducing bacteria apparently enable oxygen isotope exchange between sulfur compounds and ambient water. From natural environments, a linear relation of the sulfur and oxygen composition in residual sulfate has been observed. This seems to contradict the notion that δ^{18}O of residual sulfate approaches an equilibrium steady state value. Based on this observation MANDERNACK et al. (2003) conclude that oxygen isotope effects by bacterial sulfate reduction are not always dominated by oxygen isotope exchange with ambient water.

In the first part of this paper, we review the commonly accepted reaction-scheme for the stepwise bacterial reduction of sulfate (model by REES (1973)). We demonstrate, that in a revised model, a maximum sulfur isotope fractionation of −70‰ is possible and emphasize that it is likely that such an extreme fractionation occurs in hypersulfidic environments. In the second part, we discuss the relation between cell-specific sulfate reduction rates (sSRR) and sulfur isotope fractionation factors (Δ^{34}S_{cell}). It is shown that Δ^{34}S are unlikely to be unequivocal indicators of sSRR. In a third part, we introduce the oxygen isotope effects by bacterial sulfate reducers. We describe experiments with sulfate reducing bacteria that were performed in order to learn more about the cell-internal oxygen and sulfur isotope effects. We then develop mathematical equations that describe the relation between the amount of residual sulfate and its sulfur- and oxygen isotope composition in a closed system. The potential use of these equations is demonstrated by numerical examples. In the fourth part, we compare the oxygen isotope effects caused in residual sulfate by bacterial sulfate reduction bacteria to the corresponding oxygen isotope effects in (residual) nitrate affected by dissimilatory nitrate reduction (denitrification).
Sulfur isotope effects caused by dissimilatory sulfate reduction

Theoretical background

The sulfur isotope effects caused by dissimilatory sulfate reduction are interpreted as a result of a cascade of kinetic isotope fractionation steps within sulfate reducing bacteria (HABICHT and CANFIELD, 1997; REES, 1973).

Figure 1 Pathway of dissimilatory sulfate reduction (modified after FRITZ et al. (1989); REES, (1973))

Sulfate is transformed to sulfide by enzyme catalyzed steps within the cell of the sulfate reducing organism. Forward- (f_i) and backward-fluxes (b_i) connect pools (P_i) of intermediate sulfur compounds (X_i, equal the b_i:f_i-ratios). Each forward- and backward reaction is associated with kinetic sulfur isotope fractionation effects ($\Delta^{34}S_{f_i}$, $\Delta^{34}S_{b_i}$).

Figure 1 illustrates the general concept for the sulfur isotope effects caused by stepwise reduction of sulfate to sulfide by a sulfate reducing bacterium. Each step is characterized

- by backward- and forward fluxes, respectively by the ratio between these two fluxes (b_i, f_i and X_i; X_i becomes also zero where b_i = 0).
- by kinetic sulfur isotope fractionation factors related to the forward and backward reactions ($\Delta^{34}S_{f_i}$, $\Delta^{34}S_{b_i}$).

Under steady state conditions, the cell specific sulfate reduction rate (sSRR) equals the difference between the forward- and backward fluxes (sSRR = f_i – b_i) and the total sulfur isotope fractionation effect caused by a cell ($\Delta^{34}S_{\text{cell}}$) can be calculated ((REES, 1973), for a generalized derivation see Appendix 1):
COMBINED MEASUREMENTS OF $\delta^{34}S$ AND $\delta^{18}O$

\[
\Delta^{34}S_{\text{cell}} = \\
\Delta^{34}S_{f,1} + X_1 \cdot (\Delta^{34}S_{f,2} - \Delta^{34}S_{b,1}) + X_1 \cdot X_2 \cdot (\Delta^{34}S_{f,3} - \Delta^{34}S_{b,2}) + ... \\
+ X_1 \cdot ... \cdot X_{(i-1)} \cdot (\Delta^{34}S_{f,i} - \Delta^{34}S_{b,(i-1)}) + ... + X_1 \cdot ... \cdot X_{(\text{last_STEP-1})} \cdot \left(\Delta^{34}S_{f,\text{last_STEP}} - \Delta^{34}S_{b,(\text{last_STEP-1})}\right) \\
- X_1 \cdot ... \cdot X_{\text{last_STEP}} \cdot \Delta^{34}S_{b,\text{last_STEP}} \\
\]

where:

$\Delta^{34}S_{\text{cell}}$ = total sulfur isotope effect by cell

$\Delta^{34}S_{f,i}$ = kinetic sulfur isotope fractionation in a forward step “i”

$\Delta^{34}S_{b,i}$ = kinetic sulfur isotope fractionation in a backward step “i”

$X_i$ = ratio between backward- and forward flux in step “i”

The Rees-model

**Figure 2** Pathway of dissimilatory sulfate reduction (modified after Fritz et al. (1989); Rees (1973))

Sulfate is transformed to sulfide by enzyme catalyzed steps within the cell of the sulfate reducing organism. Forward- ($f_i$) and backward-fluxes ($b_i$) connect pools of intermediate sulfur compounds ($X_i$, equal the $b_i:f_i$-ratios). Sulfur isotope fractionation is caused by uptake of sulfate into the cell (step 1) and splitting of S-O bonds (steps 3 and 4). It is assumed that backward fluxes do not cause sulfur isotope fractionation. The theoretical maximal sulfur isotope effect by the cell ($\Delta^{34}S_{\text{cell}}$) equals the sum of the isotope fractionation steps.

Based on work by Kemp and Thode (1968), Peck (1959), Peck (1961) and Peck (1962), Rees (1973) developed a reaction and isotope fractionation scheme (in the following called “Rees-model” for the pathway of dissimilatory sulfate reduction (Figure 2). The reaction pathway for sulfate reduction consists of four principal enzyme catalyzed steps (steps 1 to 4):
Sulfate is transferred into the cell in a first step, reacted with ATP (adenosine triphosphate) to APS (adenosine-5’-phosphosulfate) in the second step, in the third step APS is reduced to sulfite and in step four sulfite is reduced to sulfide by the enzyme sulfite reductase. For the assignment of kinetic sulfur isotope fractionation factors, Rees considered work of Harrison and Thode (1958), Kaplan and Rittenberg (1964) and Kemp and Thode (1968). Here, we review these considerations.

Reversibility of flows

Rees (1973) assumed that under normal conditions the final reaction, the reduction of sulfite to hydrogen sulfide, is probably fast. Consequently, the production of hydrogen sulfide external to the bacterium from internal sulfite is shown as a single step without backward reaction. All other flows were assumed to be reversible.

Assignment of kinetic isotope fractionation factors

Rees (1973) postulated that, with the exception of the breakage of sulfur-oxygen bonds and the uptake of sulfate into the cell, the isotope effects of the forward and backward steps are small since they are associated with either sulfur oxidation or reactions where the oxidation state of sulfur is not altered. Therefore, all isotope fractionation factors except $\Delta^{34}S_{f_1}$, $\Delta^{34}S_{f_3}$, $\Delta^{34}S_{f_4}$ were set equal to zero.

These assumptions result into a simplified equation for the total sulfur isotope effect by a sulfate reducing bacterium:

$$\Delta^{34}S_{\text{cell}} = \Delta^{34}S_{f_1} + X_1 \cdot X_2 \cdot \Delta^{34}S_{f_3} + X_1 \cdot X_2 \cdot X_3 \cdot \Delta^{34}S_{f_4}$$

Two extreme cases can be identified. In the first case, no backward fluxes occur and the values for $X_1$, $X_2$ and $X_3$ are all zero. The total sulfur isotope effect by a cell becomes

$$\Delta^{34}S_{\text{cell}} = \Delta^{34}S_{f_1}.$$  

Rees (1973) hypothesized that at very low sulfate concentrations ($10^{-5}$ M) the forward reactions proceed as fast as sulfur is supplied and that, therefore, no backward flows are established. Under such conditions, in laboratory experiments, a overall isotope effect of $+3\%$ was found (Harrison and Thode, 1958). Consequently, the value of $+3\%$ was assigned to the uptake of sulfate into the cell of the bacterium ($\Delta^{34}S_{f_1} = +3\%$). The other extreme case is when the backward fluxes equal the forward fluxes and the values for $X_1$, $X_2$ and $X_3$ are close to unity and the total sulfur isotope effect by a cell equals the total of the sulfur isotope fractionation steps:

$$\Delta^{34}S_{\text{cell}} = \Delta^{34}S_{f_1} + \Delta^{34}S_{f_3} + \Delta^{34}S_{f_4}$$
The estimates for the values of $\Delta^{34}S_{f_3}$ and $\Delta^{34}S_{f_4}$ by Rees (1973) were mainly based on two observations from bacterial sulfate reduction experiments, (1) the largest isotope fractionation effect is below $-50\%e$, (2) isotope fractionation factors larger than $-25\%e$ are reported from a minority of experiments and on an assumption concerning the reaction kinetics. That is, under normal conditions, the reduction of sulfite to sulfide (step 4) is fast compared to the other steps.

Based on the assumption that the transformation of sulfite to sulfide is rapid, Rees (1973) concluded that the sulfite pool was depleted immediately, in most cases, after the supply of sulfite from the reduction of APS. Therefore, no backward flux to APS could occur ($b_3 = 0$, $X_3 = 0$). Consequently, the fractionation effect caused by step 4 would contribute to the measured total fractionation effect by a cell only in a few exceptions, most likely the few cases in which fractionation factors were more negative than $-25\%e$. Rees (1973), therefore, assigned a fractionation factor of $-25\%e$ to step 3 ($\Delta^{34}S_{f_3}$) to match the “normal” conditions and a fractionation factor of $-25\%e$ to step 4 ($\Delta^{34}S_{f_4}$) to match the few exceptional cases in which the fractionation factors reach the maximal experimental value of $-50\%e$. Using the assumptions of Rees (1973) the sulfur isotope fractionation factor by a cell can be written as follows:

$$\Delta^{34}S_{cell} = +3\%e - X_1 \cdot X_2 \cdot 25\%e - X_1 \cdot X_2 \cdot X_3 \cdot 25\%e$$

Under “normal” conditions, $X_3$ equals zero and under “exceptional” conditions, $X_3$ is smaller than one and larger than zero. However, from the discussion above, it is evident that the estimates for the fractionation factors only represent “best” guesses.

Revision of the Rees-model

In the literature, the Rees-model is commonly accepted. However, there is evidence that the step describing the reduction of sulfite to sulfide should be revised.

The Rees-model describes the reduction of sulfite to sulfide as unidirectional reaction catalyzed by the enzyme sulfite reductase (dissimilatory sulfite reductase). A kinetic sulfur isotope fractionation effect of $-25\%e$ was attributed to this step. According to the reaction scheme, this value has to be identical for all dissimilatory sulfite reduction processes (no smaller or larger isotope fractionation factors for the sulfite reduction step). However, pure culture studies have shown that the sulfur isotope fractionation factors for the reduction of sulfite to sulfide are in a range of $0\%e$ to maximum values of $-25\%e$ to $-33\%e$ (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968). Even larger sulfur isotope fractionation effects between $-31\%e$ and $-37\%e$ have been observed during sulfite disproportionation experiments.
with *Dv. Sulfodismutans* (HABICHT and CANFIELD, 2001). These results show that a one-step kinetic sulfur isotope fractionation effect, as has been used in the Rees-model, cannot explain the observed isotope effects and that sulfur isotope fractionation factors larger than the proposed 25‰ exist. Two approaches can be used to explain the large range of sulfur isotope effects related to the reduction of sulfite to sulfide. Either, there must be a backward flux from sulfide to sulfite including a strong kinetic sulfur isotope fractionation effect or there is no constant kinetic sulfur isotope fractionation factor related to the reduction of sulfite to sulfide. Sulfur isotope effects related to the oxidation of sulfur compounds are commonly small and this also likely to be the case for sulfide oxidation (HABICHT and CANFIELD, 2001). Thus, it is likely that there is no constant kinetic sulfur isotope effect related to the reduction of sulfite to sulfide. Below, we propose a mechanism explaining the observed sulfur isotope fractionations.

**A hypothetic model for the reduction of sulfite to sulfide**

*Chemical reaction pathway of sulfite reduction*

The pathway of the reaction of sulfite to sulfide is controlled by the enzyme sulfite reductase: First, sulfite is bound to the enzyme, and then sulfite is reacted to sulfide by three catalyzed reduction steps ($3 \times 2e^–$). It is likely that these reduction steps are reversible. Finally, sulfide is released. This step is followed by the excretion of the cell-internal sulfide to ambient water. Isotope effects related to the reduction of sulfite (Figure 3)

The bounding of sulfite to sulfite reductase is not related to a splitting of S–O bonds

Therefore, the involved isotope effect is likely to be close to zero. The following steps include the splitting of S–O bonds, but since the reaction is catalyzed by an enzyme bound to sulfite (no intermediate sulfur pools) no fractionation effect can occur. However, the activation energy needed for the splitting of the S–O bonds is higher for $^{34}$S–O bonds than for $^{32}$S–O bonds. Under favorable conditions, the energy supply will be high enough to enable breakage of both $^{32}$S–O and $^{34}$S–O bonds. Under less favorable conditions, the energy supply is low. Statistically, reactions that involve the splitting of $^{34}$S–O bonds, which has to be repeated three times until sulfide is generated, are more likely to fail than the ones that involve the breakage of and $^{32}$S–O bonds. Consequently, the ratio of successful $^{34}$S–sulfite reduction reactions to successful $^{32}$S–sulfite reductions reactions decreases with decreasing energy supply. This results into enrichment in $^{34}$S of the sulfite pool. It is difficult to estimate the maximum sulfur isotope effect by this process.
Hypothetically, the energy supply could be that low that only $^{32}\text{S}–\text{O}$ bonds are broken while the splitting of $^{34}\text{S}–\text{O}$ bonds fails. This would result into an extreme sulfur isotope fractionation. Consequently, we assume that the maximum kinetic sulfur isotope effect related to the reduction of sulfite to sulfide can exceed the equilibrium sulfur isotope fractionation between sulfite and sulfide, which is around $-50\%$ at temperatures between 0°C and 100°C (Farquhar et al., 2000). Correctly, in a “Rees-model”-type scheme, an additional flux would have to be created for the “failed” reduction steps. This flux has an individual isotope composition of 0% to >50%. The isotope effect depends on the supplied energy for the splitting of the S–O bonds and, therefore, reflects environmental conditions relevant for the energy supply (i.e. availability and reactivity of substrate, appearance of compounds inhibiting sulfite-reduction and cell-internal energy distribution). The consequences of these findings for the relation between cell specific sulfate reduction rates and sulfur isotope effects discussed below in more detail.

Mathematical calculation of the sulfur isotope effect related to the reduction of sulfite

Physically, reduction of sulfite is a chemical reaction catalyzed by an enzyme. The total amount of active sulfite reductase enzymes in a cell can be considered as an “intermediate
pool” of sulfur compounds. Mathematically, this “pool” can be fitted into the schematic description of stepwise sulfate reduction (Figure 4).

![Diagram of "Sulfite reductase pool"

Additionally to the major revision of sulfite-sulfide step above, we propose three further minor revisions:

Backward-flux of sulfide to sulfite

Probably, a backward flux of sulfide to sulfite can take place. The involved kinetic isotope fractionation effect is assumed to be small (HABICHT and CANFIELD, 2001) and a value of zero is assigned to this flux.

Sulfide excretion

The Rees-model does not describe the excretion of cell-internal hydrogen sulfide to ambient water. We include this reversal step and assume that the involved sulfur isotope fractionation is very small.

Uptake of sulfate into the cell of a bacterium

Rees assigned a +3‰ isotope fractionation to the uptake of sulfate into the cell of a bacterium. This assignment was based on an experimental setup, where the flux of sulfate was unidirectional. Experimental data for the reverse step is not available. However, if the uptake of sulfate into the cell creates an isotope effect, and this is likely to occur by passing the cell membrane, it has to be expected that a flux in the opposite direction would also create a similar isotope effect. Consequently, we assign a +3‰ isotope fractionation to the release of sulfate from the cell to ambient water. This modification of the Rees-model was also made by (FARQUHAR et al., 2000).
Now, according to the fluxes and values depicted in Figure 5, the sulfur isotope fractionation caused by a sulfate reducing bacterium can be recalculated for the modified Rees-model.

\[
\Delta^{34}\text{S}_{\text{cell}} = 3\% + X_1 \cdot (0\% - 3\%) + X_1 \cdot X_2 \cdot (-25\% - 0\%) + X_1 \cdot X_2 \cdot X_3 \cdot (0\% - 0\%) + X_1 \cdot X_2 \cdot X_3 \cdot X_4 \cdot \left(\{ > -50\%e...0\%e \} - 0\%e \right) + X_1 \cdot X_2 \cdot X_3 \cdot X_4 \cdot X_5 \cdot (0\% - 0\%) + X_1 \cdot X_2 \cdot X_3 \cdot X_4 \cdot X_5 \cdot X_6 \cdot 0\%e
\]

This results in:

\[
\Delta^{34}\text{S}_{\text{cell}} = 3\% + X_1 \cdot (0\% - 3\%) + X_1 \cdot X_2 \cdot (-25\% - 0\%) + X_1 \cdot X_2 \cdot X_3 \cdot X_4 \cdot \left(\{ > -50\%e...0\%e \} - 0\%e \right)
\]

In a case, where sulfate reduction is extremely efficient (no backward fluxes), the total sulfur isotope effect by a sulfate reducing bacterium becomes:

\[
\Delta^{34}\text{S}_{\text{cell}} = 3\%
\]

The opposite extreme, where sulfate reduction is extremely inefficient (backward fluxes equal forward fluxes), the total sulfur isotope effect by a sulfate reducing bacterium becomes:

\[
\Delta^{34}\text{S}_{\text{cell}} = 3\% - 25\% + \{ > -50\%e...0\%e \}
\]

\[
\Delta^{34}\text{S}_{\text{cell}} = -25\% + \{ > -50\%e...0\%e \}
\]

This result demonstrates, that sulfur isotope fractionation by dissimilatory sulfate reduction can exceed −70‰ (roughly the experimental and calculated sulfate-sulfide equilibrium sulfur
fractionation factor at temperatures between 0°C and 100°C (Farquhar et al., 2000; Ohmoto and Lasaga, 1982)).

**Evidence from natural environments for large maximum sulfur isotope effects by sulfate reducing bacteria**

From natural environments, there is evidence for such extreme sulfur isotope fractionation factors for sulfate reducers: In hypersulfidic interstitial waters from the Great Australian Bight sediments environments sulfur isotope fractionation factors up to –70‰ have been observed (Wortmann et al., 2001) and from hypersulfidic pore-waters in the Cariaco Basin Weine et al. (2003) report a offset between pore-water sulfate and pore-water sulfide of –55‰ to –65‰. This is most interesting because under hypersulfidic conditions, disproportionation as additional sulfur isotope fractionation process is inhibited and, therefore, sulfur isotope fractionation by sulfate reducers is likely to be the cause for these large fractionations. From deep ocean sediments at elevated temperatures, Rudnicki et al. (2001) report sulfur isotope fractionation factors around –77‰ ±7‰. They conclude that sulfur isotope fractionation by disproportionation cannot have been the cause for this isotope fractionation and attribute the observed isotope effect to bacterial sulfate reduction. Rudnicki et al. (2001) speculate that this extreme isotope fractionation factor might be related to the very low sulfate reduction rates recorded (about two orders of magnitude lower than literature values that are in the range of µmol cm⁻³ d⁻¹ to tens of nmol cm⁻³ d⁻¹).

**Reasons for extreme sulfur isotope fractionation at the sulfite-sulfide step**

As pointed out above, the sulfur isotope fractionation effect during the reduction of sulfite to sulfide depends on the supplied energy for the splitting of the S–O bonds. “Harsh” environmental conditions, such as low supply of substrate or high sulfide contents (hypersulfidic environments) are likely to cause a minimal supply of energy for the reduction of sulfite. This leads to an increase in the “failure” rate of enzymatically catalyzed sulfite reduction reactions (decrease in the sulfate reduction rate). Because of the higher energy need of the heavy ³⁴S–O bonds, the failure rate in the breakage of these bonds increases over proportionally, creating large isotope effects. Thus, “harsh” environmental conditions, such as low substrate supply or hypersulfidic conditions, cause both high sulfur isotope fractionation and low sulfate reduction rates. However, we emphasize that large sulfur isotope fractionation factors by sulfate reducers might not be restricted to extreme environments. In an environment with moderate
sulfide contents, low to moderate substrate supply and abundant bacteria, the energy supply for the reduction of sulfite is limited and large sulfur isotope effects would be expected. In marine environments, such conditions probably are abundant. In contrast to this, laboratory studies are usually carried out at high substrate conditions and rather low sulfide concentrations. This could be an explanation for the “low” laboratory maximum sulfur isotope fractionation observed (around –46‰). However, DETMERS et al. (2001) demonstrated that environmental conditions not alone control sulfur isotope fractionation by sulfate reducing bacteria, the species-specific physiology of each sulfate reducer needs to be taken into account. DETMERS et al. (2001) carried out laboratory experiments with 32 different sulfate reducing prokaryotes to explore the diversity in the sulfur isotope fractionation during dissimilatory sulfate reduction by pure cultures. Under optimized growth conditions, the determined fractionation factors ranged from –2‰ to –41‰. Sulfate reducers that oxidized the carbon source completely to carbon dioxide (energetically less favorable) showed greater fractionations than sulfate reducers that released acetate as the final product of carbon oxidation (energetically more favorable). This observation confirms that the energy supply controls sulfur isotope fractionation and demonstrates that the cell-specific physiology strongly influences “cell-specific” sulfur isotope fractionation factors. The importance of the cell-specific physiology becomes even more evident when a further observation of DETMERS et al. (2001) is considered: Within the groups of complete oxidizing respectively incomplete oxidizing microorganisms, there was no correlation between cell-specific sulfate reduction rates (sSRR) and isotope fractionation factors. We speculate that differences in the size of the cell-internal enzyme pools of different species create this uneven sulfur isotope fractionation pattern. In bacteria that have large enzyme pools (a large number of enzymes) the supplied energy is distributed. The number of running reduction steps at a time is increased, but energy supply for each reaction is small. This will lead to a preferential failure of $^{34}$S–O breakage and large sulfur isotope effects. Bacteria with small enzyme pools distribute the energy to a smaller number of reactions. Thus, only a small fraction of reduction steps fails and sulfur isotope fractionation is small.
Relation between cell-specific sulfate reduction rates (sSRR) and sulfur isotope fractionation factors

From the discussion above, it is evident that there is no correlation between isotope fractionation and sSRR when different species are compared (DETMERS et al., 2001). However, for individual sulfate reducing species, such a relation might be observed. This relation can be calculated as follows:

At steady state, the difference between backward- and forward fluxes equals the cell-specific sulfate reduction rate (sSRR) and the $X_i$-ratios can be expressed as function of the corresponding backward flux $b_i$ and the sSRR:

\[
sSRR = f_1 - b_1 = f_2 - b_2 = f_3 - b_3 = f_4 = f_5
\]

\[
f_i = b_i + sSRR
\]

\[
X_i = \frac{b_i}{sSRR + b_i}
\]

The equation for the sulfur isotope fractionation therefore becomes

\[
\Delta^{34}S_{cell} = \frac{3}{1000} + \frac{b_1}{sSRR + b_1} \cdot -3_{/1000} + \frac{b_1 \cdot b_2}{(sSRR + b_1) \cdot (sSRR + b_2)} \cdot -25_{/1000}
\]

\[
+ \frac{b_1 \cdot b_2 \cdot b_3 \cdot b_4}{(sSRR + b_1) \cdot (sSRR + b_2) \cdot (sSRR + b_3) \cdot (sSRR + b_4)} \cdot \{-50_{/1000}...0_{/1000}\}
\]

The equation shows that the sSRR and sulfur isotope fractionation factors are related. High sSRR cause small $X_i$-ratios and small $\Delta^{34}S_{cell}$. Based on this observation, high sSRR are expected to be related to small sulfur isotope fractionations and low sSRR are expected to be related to large sulfur isotope fractionations (THODE, 1991). This has been partly confirmed by field and pure culture studies (HABICHT and CANFIELD, 1997). Pure culture studies by BOLLIGER et al. (2001) and KLEIEMPER et al. (2003) also indicate a weak inverse relationship between sulfur isotope fractionation and sSRR. However, at identical sSRR and different ratios between forward- and backward fluxes (these ratios might be to a part also species-specific) different $\Delta^{34}S_{cell}$ result. This is additional evidence that, in general, sSRR and $\Delta^{34}S_{cell}$ do not correlate when different species of sulfate reducing microorganisms are compared. These
results demonstrate that $\Delta^{34}S_{\text{cell}}$ are of limited use for determination of cell-specific sulfate reduction rates. Probably, the combined investigation of the influence of SRB on the sulfur- and oxygen isotopic composition of residual sulfate could be the key to a better understanding of sSSR. Such an approach has been suggested by BÖTTCHER et al. (1999) and BÖTTCHER et al. (1998) who proposed that $\delta^{18}O - \delta^{34}S$ relations of residual sulfate in interstitial waters directly reflect sulfate reduction rates in marine sediments. Below, we investigate the oxygen isotope effects caused by dissimilatory sulfate reduction.

Oxygen isotope effects caused by dissimilatory sulfate reduction

Under natural conditions (intermediate pH and temperatures below 50°C), oxygen isotope exchange of sulfate with ambient water is extremely slow (CHIBA and SAKAI, 1985) and does not directly control the oxygen isotope composition of sulfate. In interstitial waters, changes in the $\delta^{18}O$ of residual sulfate up to $+17‰$ (starting from a seawater sulfate value around 9.6‰) have been observed (AHARON and FU, 2000; AHARON and FU, 2003; BÖTTCHER et al., 1999; BÖTTCHER et al., 1998; KU et al., 1999) and from an anoxic marine basin, the Framvaren Fjord, Norway, MANDERNACK et al. (2003) report an enrichment in $\delta^{18}O$ of sulfate from 10.4‰ to 15.5‰. In natural environments, the $\delta^{18}O$ of sulfate is strongly influenced by microbial sulfate reduction, reoxidation of sulfides (AHARON and FU, 2003; BÖTTCHER and THAMDRUP, 2001; KU et al., 1999; LU et al., 2001) and anaerobic bacterial disproportionation and oxidation of elemental sulfur and sulfides (BÖTTCHER and THAMDRUP, 2001; BÖTTCHER et al., 2001).

Despite the commonly accepted scheme for the pathway of dissimilatory sulfate reduction, that is introduced above, the mechanisms causing the oxygen isotope effects by sulfate reducing bacteria are a matter of debate. The uncertainties concerning the oxygen isotope effects by bacterial sulfate reduction root in the observation that changes in the $\delta^{18}O$ of residual sulfate depend on the $\delta^{18}O$ of ambient water (FRITZ et al., 1989; MIZUTANI and RAFTER, 1973). Taking into account that oxygen exchange of sulfate with ambient water is extremely slow, FRITZ et al. (1989) and MIZUTANI and RAFTER (1973) concluded that, during bacterial sulfate reduction, intermediate sulfate compounds do exchange oxygen with ambient water. Consequently, the overall oxygen isotope effect by a single sulfate reducing bacteria is a combination of (Figure 6):
Kinetic isotope fractionation steps ($\Delta^{18}\text{O}$):

- Equilibrium exchange of oxygen between intermediate sulfate compounds with ambient water ($\text{ex}_i$)
- Oxygen isotope effects during backward reactions (i.e. flux $b_i$: oxidation of sulfite)
- Ratios between backward- and forward fluxes ($X_i$)

**Figure 6** Pathway of dissimilatory sulfate reduction (modified after Fritz et al. (1989), Rees /1973)

Sulfate is transformed to sulfide by enzyme catalyzed steps within the cell of the sulfate reducing organism. Forward- ($f_i$) and backward-fluxes ($b_i$) connect pools of intermediate sulfur compounds ($X_i$, equal the $b_i/f_i$-ratios). The oxygen isotope composition of the residual sulfate fraction is influenced by oxygen isotope exchange with ambient water, by kinetic isotope fractionation steps, by the oxygen isotope composition of the oxygen source for sulfite oxidation and by the $X_i$-ratios.

Fritz et al. (1989) proposed that the equilibrium oxygen isotope exchange is the main driving factor of the oxygen isotope effect by sulfate reducing bacteria. However, based on data sets from natural environments, other authors (Aharon and Fu, 2000; Mandernack et al., 2003) identify kinetic isotope fractionation as major factor controlling the $\delta^{18}\text{O}$ of residual sulfate. This implies that either both mechanisms dominate at different circumstances (e.g. different sSSR, $X_i$-ratios or bacteria) or that the isotope equilibrium dominated process produces $\delta^{18}\text{O} – \delta^{34}\text{S}$ relations that mimic kinetic isotope fractionation. In order to clarify if in fact two different oxygen isotope fractionation processes dominate at different circumstances, we discuss the $\delta^{18}\text{O} – \delta^{34}\text{S}$ patterns in residual sulfate that are derived by “equilibrium oxygen isotope exchange dominated process” respectively “kinetic oxygen isotope fractionation process”.

- $\delta^{18}\text{O} – \delta^{34}\text{S}$ pattern with an equilibrium dominated mechanism:

Fritz et al. (1989) carried out laboratory sulfate reduction experiments with Desulfovibrio desulfuricans in water with different oxygen isotope composition. They found that the oxygen composition of residual sulfate approached asymptotically a steady state value equal to the
oxygen isotope composition of ambient water plus a temperature dependent equilibrium fractionation factor (+25‰ at 30°C, +27‰ at 17°C and, extrapolated, +29‰ at 5°C). In the experiments of (FRITZ et al., 1989), the sulfur isotope composition of residual sulfate followed a Rayleigh distillation process with kinetic isotope enrichment factors of −9‰ to −22‰. In interstitial waters from the Mediterranean the δ¹⁸O values of sulfate tend to reach a steady value, while δ³⁴S values continue to increase (BÖTTCHER et al., 1999; BÖTTCHER et al., 1998). The measured oxygen isotope values are about 3‰ below the proposed equilibrium values of FRITZ et al. (1989).

- δ¹⁸O – δ³⁴S pattern with a mechanism dominated by kinetic oxygen isotope fractionation: Basically, a kinetic oxygen isotope fractionation process is equal to the one of kinetic sulfur isotope fractionation. The only differences are different values for the involved kinetic isotope fractionation steps. However, it can be expected that the most relevant kinetic oxygen isotope effects occur where also large kinetic sulfur isotope fractionation is observed. Thus, the total kinetic oxygen isotope effect by a cell is written analogue to the total sulfur isotope effect:

\[
\Delta ^{18}O_{\text{cell}} = \Delta ^{18}O_{f_{1}} + X_{1} \cdot \Delta ^{18}O_{b_{1}} + X_{1} \cdot X_{2} \cdot \Delta ^{18}O_{f_{3}} + X_{1} \cdot X_{2} \cdot X_{3} \cdot X_{4} \cdot \Delta ^{18}O_{b_{4}} \\
\Delta ^{34}S_{\text{cell}} = \Delta ^{34}S_{f_{1}} + X_{1} \cdot \Delta ^{34}S_{b_{1}} + X_{1} \cdot X_{2} \cdot \Delta ^{34}S_{f_{3}} + X_{1} \cdot X_{2} \cdot X_{3} \cdot X_{4} \cdot \Delta ^{34}S_{b_{4}}
\]

Since all backward- and forward reactions \(X_{i}\) are identical for sulfur- and oxygen isotope fractionation, the enrichments of both isotopes in the residual sulfate follow a linear relation. Theoretically, in a closed system, both isotope compositions would become enriched approaching infinity. However, the complete consumption of sulfate halts this trend. The slope of the described δ¹⁸O – δ³⁴S relation equals the ratio between the total isotope effects \(\Delta ^{18}O_{\text{cell}} : \Delta ^{34}S_{\text{cell}}\). Originally, a constant ratio of 1:4 for this slope (the oxygen isotope effect is one fourth of the sulfur isotope effect) has been proposed (MIZUTANI and RAFTER, 1969). Ever since, this value has been used in the literature as diagnostic for sulfate modified by microbial sulfate reduction (for a discussion see AHARON and Fu (2000)). However, as pointed out by AHARON and Fu (2000), δ¹⁸O – δ³⁴S relations of sulfate modified by microbial sulfate reduction have slopes in a range of 1:3.5 (0.29) to 7:10 (0.71). From the “kinetic oxygen isotope fractionation” point of view, this observation would have to be explained by different individual ratios between single isotope fractionation steps, e.g. \(\Delta ^{18}O_{3} : \Delta ^{34}S_{1}\) equals a ratio of 1:3 and \(\Delta ^{18}O_{4} : \Delta ^{34}S_{4}\) equals a ratio of 1:5. The different combinations of these ratios would then result in the observed variety of δ¹⁸O – δ³⁴S slopes. From an “equilibrium oxygen isotope exchange” point of view, the variation in the slopes of the δ¹⁸O – δ³⁴S relations is explained
by differences in the oxygen isotope exchange rates, by different equilibrium values due to variations in the temperature and by different oxygen isotope compositions of ambient waters.

• Discussion and Interpretation

The $\delta^{18}O$ – $\delta^{34}S$ patterns for “equilibrium oxygen isotope exchange” versus “kinetic oxygen isotope fractionation” can be summarized as follows: If the sulfur isotope effect caused by bacterial sulfate reduction is dominated by equilibrium oxygen isotope exchange, the $\delta^{18}O$ of residual sulfate approaches a steady equilibrium value. If the sulfur isotope effect caused by bacterial sulfate reduction is dominated by kinetic oxygen isotope fractionation, the $\delta^{18}O$ – $\delta^{34}S$ relation is linear and the $\delta^{18}O$ of residual sulfate should be enriched over the postulated steady equilibrium value. However, to our best knowledge, there is no report of oxygen isotope enrichment in sulfate over the postulated equilibrium values. This is strong evidence against a kinetic dominated oxygen isotope fractionation process. A linear $\delta^{18}O$ – $\delta^{34}S$ relation is also observed at an initial stage of equilibrium isotope exchange and is not sufficient to postulate a dominance of kinetic oxygen isotope fractionation by microbial sulfate reducers.

A model integrating sSRR, sulfur and oxygen isotope fractionation and amount of residual sulfate

To our best knowledge, no attempt has been made to put the oxygen isotope effect by a single sulfate reducing bacterium on residual sulfate into a mathematical framework. Such a model is the precondition for the investigation of oxygen isotope effects in residual sulfate and their relation to sulfate reduction rates. We therefore present here a model integrating the following major parameters controlled by sulfate reducing bacteria:

• Cell-specific sulfate reduction rate (sSRR)
• $\delta^{18}O$ and $\delta^{34}S$ of residual sulfate
• Amount of residual sulfate
• Cell-internal backward- and forward fluxes

Assuming that kinetic oxygen isotope fractionation is not important, we first develop a mathematical model for the oxygen isotope effect caused by a single sulfate reducing bacterium. This model distinguishes a “special case” and a “general case”. In order to investigate if this distinction is valid, we describe laboratory experiments that were performed close to the border between “special case” and “general case”. In a second step, the developed model is used to calculate $\delta^{18}O$ – $\delta^{34}S$ relations of residual sulfate during bacterial sulfate reduction in a
closed system with a variable number of bacteria. Our results will show that the oxygen equilibrium dominated model is compatible to the observations of Aharon and Fu (2000) and Mandernack et al. (2003), as well as to the observations of Fritz et al. (1989) and Mizutani and Rafter (1973). We will point out that our model is a promising tool to tackle the unresolved questions discussed above and that it might be the key to the understanding of \( \delta^{18}O - \delta^{15}N \) effects caused by the activity of denitrifying microbes in residual nitrate.

### A scheme for the oxygen isotope effects by sulfate reducing bacteria

There is strong evidence that equilibrium isotope exchange between cell-internal sulfur compounds and ambient water dominates the oxygen isotope effects caused by microbial sulfate reduction in residual sulfate. Either the kinetic isotope fractionation of oxygen is too small or it is erased by equilibrium oxygen isotope exchange between sulfur compounds and ambient water. This allows us to establish a simplified model of the oxygen isotope effect by a sulfate reducing bacterium (Figure 7):

(Fritz et al., 1989) discussed the relative importance of oxygen isotope exchange between the sulfate-enzyme complex and ambient water compared to the oxygen isotope exchange between sulfite and ambient water. They concluded that the backward reaction of sulfite to sulfate (flux \( b_3 \)) incorporates the use of water oxygen and thus the breakage of H–O bonds.
hypothesized that this breakage would cause a kinetic oxygen isotope fractionation, being not compatible to their laboratory results. Therefore, Fritz et al. (1989) concluded that the oxygen isotope exchange of enzymatically complexed sulfate with ambient water is the significant process. Probably, laboratory investigations of anaerobic bacterial disproportionation of elemental sulfur by Böttcher et al. (2001) can be used as an analogue for the oxygen isotope effect by reoxidation of sulfite to sulfate. The overall reaction of this process is written as

$$4H_2O + 4S^0 \Rightarrow 3H_2S + SO_4^{2-} + 2H^+.$$  

This chemical equation shows that the oxygen in sulfate is derived from water. Under laboratory conditions, other oxygen sources are absent. In natural environments, oxygen could also be derived from sources with oxygen isotope composition different from ambient water, such as nitrate or iron oxides. However, in the laboratory experiment, the resulting sulfate was enriched in $^{18}O$ by $+16.6\%$ to $+17.4\%$ (at 28°C) compared to the $\delta^{18}O$ of water (Böttcher et al., 2001). This value is probably the result of oxygen isotope exchange of sulfite with water and subsequent oxidation of sulfite to sulfate with a kinetic isotope fractionation. Using the equilibrium value of Fritz et al. (1989), the oxygen isotope composition of sulfite would be calculated as: $\delta^{18}O_{\text{sulfite}} = \delta^{18}O_{\text{water}} + 25\%$ (at 28°C). By adding one oxygen atom from water (with an enrichment factor of $+4\%$ (Taylor et al., 1984)) sulfite is oxidized to sulfate. The resulting oxygen isotope composition of sulfate could then be approximated as:

$$\delta^{18}O_{\text{sulfate}} = (3 \times (\delta^{18}O_{\text{water}} + 25\%)) + 1 \times (\delta^{18}O_{\text{water}} + 4\%)) / 4 = \delta^{18}O_{\text{water}} + 19.8\%.$$  

This value is not far above the laboratory results of Böttcher et al. (2001) and implies that, in their experiments, the equilibrium exchange between water and sulfite almost reached completeness. However, due to the direct incorporation of oxygen, sulfite derived sulfate can not have been the source of the high oxygen equilibrium value observed by Fritz et al. (1989). Consequently, as postulated, oxygen isotope exchange of enzymatically complexed sulfate with ambient water is likely to be the significant process controlling their experiments. Probably, the assumption of Rees (1973), that is, the sulfur isotope fractionation values less negative than $-25\%$ are typical for a “normal” case where no reoxidation of sulfite occurs ($b_3$ and $X_3$ equal zero), can be applied to the experiments of Fritz et al. (1989). The kinetic sulfur isotope enrichment factors in their experiments were in a range of $-9\%$ to $-22\%$. This explains why $^{18}O$-depleted sulfite derived sulfate did not contribute to equilibrium value of Fritz et al. (1989). It also indicates that the steady values oxygen isotope values of sulfate in interstitial waters from the Mediterranean, which are about $3\%$ below of the predicted equilibrium value was derived by the contribution of oxygen from sulfide oxidation. Thus, we
distinguish between a case in which sulfite oxidation plays an important role and a case in which sulfite oxidation does not influence the oxygen isotope fractionation process. The “border” between these cases is defined by the lack of a backward-flux from sulfite to sulfate and is close to the sulfur isotope fractionation factor of −25‰. For sulfur isotope fractionations larger than −25‰, an oxidative flux from the sulfite pool to sulfate is needed and this flux is expected to create an offset between the equilibrium oxygen isotope value and the observed values. We tested this hypothesis by performing sulfate reduction experiments with bacteria that create sulfur isotope fractionations larger than −25‰. These experiments and the results are described below.

**Experimental**

**Objective**

The goal of the experimental work was to figure out if the oxygen isotope effects during sulfate reduction can be separated in two groups, one in which sulfite reoxidation does not take place and another in which sulfite reoxidation takes place. Our hypothesis is that sulfur isotope fractionations larger than −25‰ involve significant reoxidation of sulfite and that, therefore, the effects of this flux should be detectable in the oxygen isotope pattern of residual sulfate.

**Methods: Culture experiments and isotope analysis**

**Materials, Methods and Microcosm Experiments**

For a detailed description of the materials, methods and microcosm experiments, we refer to KLEIKEMPER et al. (2003) (included in the Appendix of this thesis).

For our experiment, cultures of the SO\(_4^{2-}\)-reducing bacterium PRTOL1 were used. For cultivation, SO\(_4^{2-}\) was added as FeSO\(_4\) and for subsequent microcosm experiments, SO\(_4^{2-}\) was added as NaSO\(_4\). Organic acids were added from anaerobic, autoclaved stock solutions to give final concentrations of 5 mM (acetate, pyruvate) or 1.0 mM (benzoate, 3-phenylpropionate). The addition of carbon sources in concentrations that would not be depleted ensured that carbon sources were non-limiting in our microcosm experiments. The bacteria were cultured in 120 ml serum bottles with a headspace of approximately 17 ml (90% N\(_2\), 10% CO\(_2\)) at 28°C inverted in the dark. The initial SO\(_4^{2-}\) concentration was approximately 1 mM in all microcosms. Two independent control experiments were performed for each set of microcosms. For the first control experiment we prepared microcosms as described above except that the carbon source was omitted. In the second control experiment we prepared microcosms as de-
scribed above except that culture inoculation was omitted. Sulfate concentrations were periodically monitored during the experiments, and at certain intervals sets of three microcosms per culture and employed carbon source were sacrificed and analyzed. Experiments were terminated when the initially supplied SO$_4^{2-}$ was consumed. One third of the experiments with acetate and benzoate, was initially labeled with water strongly enriched in $^{18}$O (95% $^{18}$O). Therefore, in two thirds of the experiments, the $\delta^{18}$O of water was at $-10.2‰$ and in one third at $84.2‰$ respectively at $84.6‰$ (SMOW).

Isotope Analysis

For isotope measurements S(-II) was precipitated as ZnS by addition of 5 ml 1M zinc acetate solution to the microcosms. Serum bottles were vigorously shaken before 1 ml of 2 M NaOH was added. The contents of the microcosms were then filtered using a 0.45 µm HVLP membrane filter (Millipore). Sulfate was subsequently precipitated as BaSO$_4$ by first adding 2 ml 2 M HCl (pH at ~3) and, after maximal 30 minutes, by adding 5 ml 1.2 M BaCl$_2$ solutions to the filtrate. The precipitate was recovered on a separate 0.45 µm HVLP membrane filter (Millipore). Both filters were dried at 60°C over night. For stable sulfur isotope ratio measurements, approximately 400-600 µg of BaSO$_4$ were weighted in tin cups. Vanadium pentoxide was added as catalyst in the amount of about twice the weight of the sample. Sulfur isotopes were subsequently measured on a Fisons OPTIMA mass spectrometer (Fisons, Middlewich, Cheshire, UK) coupled in continuous flow with a Carlo Erba elemental analyzer (CE Instruments, Milan, Italy). Data are reported in the conventional $\delta$-notation relative to the Vienna-Canyon Diablo Troilite (V-CDT) standard according to:

$$\delta^{34}S (‰) = \{(^{34}S/^{32}S)_{\text{sample}} / (^{34}S/^{32}S)_{\text{V-CDT}} - 1\} \times 1000$$

The system was calibrated using the international standards IAEA-S1 ($\delta^{34}S = -0.3‰$) and IAEA-S2 ($\delta^{34}S = 21.7‰$). The mean $\delta^{34}S$ value obtained for the international standard NBS127 was 20.4‰. Analytical reproducibility of the measurements was $\pm 0.3‰$.

The oxygen isotope measurements were carried out at the Environmental Isotope Laboratory (University of Waterloo, Canada). For oxygen isotope analysis, the BaSO$_4$ samples were weighed at 0.2 mg and then stored in a dynamic vacuum for at least 24 hours to remove water. The samples were then dropped individually onto carbon at about 1290°C (Eurovector high temperature Elemental Analyzer). The combustion product, carbon monoxide (CO) was carried to the mass spectrometer (Micromass IsoPRIME) by a helium-stream. The measurements were carried out in duplicate; the reproducibility is $\pm 0.8‰$. The values are reported relative to the Standard Mean Ocean Water (SMOW) standard according to:
\[ \delta^{18}O \, (\text{‰}) = \left\{ \frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}}}{(^{18}\text{O}/^{16}\text{O})_{\text{SMOW}}} - 1 \right\} \times 1000 \]

Results

The sulfur isotope fractionation factors in our experiments were in a range of \(-28\%e\) to \(-34\%e\). In water with a highly positive \(\delta^{18}O\) of about +84.4\%e, the \(\delta^{18}O\) of residual sulfate rises from +17\%e to values higher than +50\%e (filled arrow in Figure 8). In water with a negative \(\delta^{18}O\) of \(-10.2\%e\), the \(\delta^{18}O\) of residual sulfate slowly rises from +12\%e to +14\%e (dashed arrows in Figure 8).

![Figure 8](image)

**Figure 8** \(\delta^{18}O - \delta^{34}S\) trends of residual sulfate in pure culture sulfate reduction experiments with different oxygen isotope composition of water and different substrates

The experiments demonstrate the strong influence of the oxygen isotope composition of ambient water on the oxygen isotope composition of residual sulfate.

Pure culture: PRTOL1, closed system, PA: substrate = acetate, PBT: substrate = benzoate, \(T=28^\circ\text{C}\).

A compilation of four batch culture experiments (Figure 9), which were carried out in water with a \(\delta^{18}O\) of at \(-10.2\%e\), shows a general trend to oxygen isotopes enriched in \(^{18}\text{O}\) (9\%e to 15\%e). A large scatter in the oxygen isotope data is observed.
COMBINED MEASUREMENTS OF $\delta^{34}S$ AND $\delta^{18}O$

CHAPTER 1

Figure 9  $\delta^{18}O - \delta^{34}S$ trends of residual sulfate in pure culture experiments (PRTOL1) with a $\delta^{18}O$ of water at $-10.2‰$ at a temperature of $28°C$ in a closed system

The oxygen isotope equilibrium factor at $28°C$ is close to $+25‰$ (Fritz et al., 1989). This results into an equilibrium value of $+15‰$. Sulfate is strongly enriched in $^{34}S$. The values for $\delta^{18}O$ approach the equilibrium line but do not cross it.

For methods see Kleikemper et al. (2003).

Interpretation

The experiments demonstrate the strong influence of the oxygen isotope composition of ambient water on the oxygen isotope composition of residual sulfate (Figure 9). The oxygen isotope equilibrium factor at $28°C$ is close to $+25‰$ (Fritz et al., 1989). This results into an equilibrium value of $+15‰$ for water at $-10.2‰$. The experiments carried out in water with a $\delta^{18}O$ of $-10.2‰$ show that sulfate is strongly enriched in $^{34}S$ with ongoing sulfate reduction. The $\delta^{18}O$ of residual sulfate approach the equilibrium line around $+15‰$ but do not cross it (Figure 9). The scatter in the oxygen isotope data is striking. The most likely explanation for this scatter is that in some of the experiments, isotopically “light” oxygen was contributed by sulfite reoxidation. This confirms the above hypothesis, that sulfite is reoxidized when sulfur isotope fractionation factors exceed the value of $-25‰$. Additionally, the scatter in the oxygen isotope data implies that there is a strong competition between the equilibrium oxygen isotope exchange process and the contribution of oxygen by sulfite oxidation leading to the large range of measured data.
Calculation of the relation between the oxygen and sulfur isotope composition and the amount of residual sulfate

In the following, we distinguish between two cases:

- The (mathematical) “general case” describes the case in which sulfite derived sulfate is taken into account. Thus, the backward flux $b_3$ and consequently the backward to forward flux ratio $X_3$ are larger than zero. The “general case” corresponds to the “exceptional case” of REES (1973).
- The (mathematical) “special case” describes the situation where no sulfite reoxidation takes place ($b_3$ and $X_3$ equal zero). The “special case” corresponds to the “normal case” of REES (1973).

The expressions “general” and “special” are used to highlight the mathematical relation between the two cases and do not imply the statistical occurrence of the described processes in the natural environment. This is in contrast to the expressions “exceptional case” and “normal case”. By introducing these mathematical expressions, we emphasize that our calculations do not make use of any of the (numerical) assumptions needed to define what is “exceptional” and what is “normal”.

Unlike the overall sulfur isotope effect, the overall isotope effect cannot be expressed as an enrichment factor. This is due to the different isotope fractionation type (equilibrium fractionation instead of kinetic isotope fractionation). Here, we have to use a mathematical description of the time-dependent oxygen-isotope composition of residual sulfate (for calculation see Appendix 3).

General case:

$$\frac{d}{dt} \delta^{18}O_{SO_4_{resid}} = s_{SRR} \cdot k_1 \cdot \left( \delta D \cdot k_2 + (\delta H_2O + \delta ex) \cdot k_3 - \delta^{18}O_{SO_4_{resid}} \cdot k_4 \right)$$

with:

- $k_1 = \frac{X_1}{(1 - X_1) \cdot (1 - X_1 \cdot X_2 \cdot X_3 + (1 - X_1 \cdot X_2 + X_1 \cdot X_2 \cdot X_3) \cdot \alpha)}$
- $k_2 = X_1 \cdot X_2 - X_1 \cdot X_2 \cdot X_3$
- $k_3 = \alpha \cdot (X_2 - X_1 \cdot X_3 + X_1 \cdot X_2 \cdot X_3)$
- $k_4 = X_2 \cdot X_3 + \alpha \cdot (X_2 - X_1 \cdot X_3 + X_1 \cdot X_2 \cdot X_3)$

where:

- $\frac{d}{dt}$ Derivative after time.
- $SO_4_{resid}$ Amount of residual sulfate.
- $\delta^{18}O_{SO_4_{resid}}$ Oxygen isotope composition of residual sulfate.
- $\delta H_2O$ Oxygen isotope composition of ambient water.
**δD’** Oxygen isotope composition of sulfate derived from sulfite-oxidation. As discussed above, under laboratory conditions, the source of oxygen for the oxidation of sulfite to sulfate is derived from water. Under natural conditions, it is possible that other sources contribute oxygen with oxygen isotope composition different from ambient water. However, the value of δD’ is a result of equilibrium isotope exchange between sulfite and ambient water combined with the isotopic composition of oxygen used to oxidize sulfite.

**δex** Oxygen isotope equilibrium fractionation factor.

**sSRR** The cell-specific sulfate reduction rate is assumed to be constant.

**α** ex/sSRR: Ratio between constant water-sulfate oxygen isotope exchange rate and cell-specific sulfate reduction rate.

**X_i** Ratio between backward- and forward-fluxes.

Special case:

When reoxidation of sulfite to sulfate is small, X_3 becomes zero and the time-dependent oxygen-isotope composition is written as follows (see Appendix 4):

\[
SO_{4-\text{resid}} \cdot \frac{d}{dt} \delta^{18}O_{SO_{4-\text{resid}}} = sSRR \cdot \beta \cdot \left( \left( \delta H_2O + \delta ex \right) - \delta^{18}O_{SO_{4-\text{resid}}} \right)
\]

with:

\[
\beta = \frac{x_i}{(1-x_i)} \cdot \frac{\alpha \cdot (x_i - x_i \cdot x_i)}{1 + \alpha \cdot x_i \cdot x_i}
\]

\[
\alpha = \frac{1}{x_i \cdot x_i \cdot \left(1+\frac{1}{\beta}\right) - 1}
\]

The time dependent sulfur isotope composition is written as follows (see Appendix 2):

\[
SO_{4-\text{resid}} \cdot \frac{d}{dt} \delta^{34}S_{SO_{4-\text{resid}}} = -sSRR \cdot \Delta^{34}S_{\text{cell}}
\]

with:

\[
\Delta^{34}S_{\text{resid}} = \Delta^{34}S_i + x_i \cdot \Delta^{34}S_j + x_i \cdot x_j \cdot \Delta^{34}S_k + x_i \cdot x_j \cdot x_k \cdot \Delta^{34}S_l
\]

where:

**d/dt** Derivative after time.

**SO_{4,\text{resid}}** Amount of residual sulfate.

**δ^{34}S_{SO_{4,\text{resid}}}** Sulfur isotope composition of residual sulfate.

**Δ^{34}S_{\text{cell}}** Total sulfur isotope fractionation factor by a single bacterium.
A model to investigate $\delta^{18}O$-$\delta^{34}S$ relations of residual sulfate in a closed system

The calculations above consider the sulfur- and oxygen isotope effects of a single bacterium. The isotope effect of a bacteria population changing in size (but each bacterium with the same isotope effect) is the product of a single cell effect with the time dependent number of bacteria. We therefore introduce four new expressions: $SO_4$, $\delta^{18}O_{SO_4}$ and $\delta^{34}S_{SO_4}$ describe the isotopic composition and total amount of residual sulfate and $cell_{Nr}$ describes the time dependent number of bacteria.

- **Oxygen isotope composition of total residual sulfate:**
  General case:
  $$SO_4 \cdot \frac{d}{dt} \delta^{18}O_{SO_4} = cell_{Nr} \cdot sSRR \cdot \frac{k_1 \cdot (\delta D^* \cdot k_2 + (\delta H_2O + \delta ex) \cdot k_3 - \delta^{18}O_{SO_4} \cdot k_4)}{k_3}$$
  Special case ($X_3 = 0$):
  $$SO_4 \cdot \frac{d}{dt} \delta^{18}O_{SO_4} = cell_{Nr} \cdot sSRR \cdot \beta \cdot \left((\delta H_2O + \delta ex) - \delta^{18}O_{SO_4}\right)$$

- **Sulfur isotope composition of total residual sulfate:**
  $$SO_4 \cdot \frac{d}{dt} \delta^{34}S_{SO_4} = cell_{Nr} \cdot -sSRR \cdot \Delta^{34}S_{cell}$$

The equations can be reformulated in the following way:

General case:

$$\frac{-cell_{Nr} \cdot sSRR}{SO_4} = \frac{d}{dt} \delta^{34}S_{SO_4}$$

$$= k_1 \cdot (-\delta D^* \cdot k_2 - (\delta H_2O + \delta ex) \cdot k_3 + \delta^{34}O_{SO_4} \cdot k_4)$$

Special case ($X_3 = 0$):

$$\frac{-cell_{Nr} \cdot sSRR}{SO_4} = \beta \cdot \left(-(\delta H_2O + \delta ex) + \delta^{34}O_{SO_4}\right)$$

and:

$$\frac{cell_{Nr} \cdot -sSRR}{SO_4} = \frac{d}{dt} \delta^{34}S_{SO_4}$$

$$= \Delta^{34}S_{cell}$$

We can now combine the equation for the oxygen and the sulfur isotope composition of residual sulfate:
General case:
\[
\frac{-\text{cell}_{\text{w}} \cdot \text{sSRR}}{SO_4} = k_1 \left[ -\delta D \cdot k_2 - (\delta H_2O + \delta ex) \cdot k_4 + \delta^{34}SO_4 \cdot k_2 \right] = \frac{d}{dt} \delta^{34}SO_4
\]

Special case (X₃=0):
\[
\frac{-\text{cell}_{\text{w}} \cdot \text{sSRR}}{SO_4} = \beta \left[ - (\delta H_2O + \delta ex) + \delta^{34}SO_4 \right] = \frac{d}{dt} \delta^{34}SO_4
\]

After the integration of the equations (see Appendix 5), we get the following results:

General case:
\[
\ln \frac{SO_4(t)}{SO_4(0)} = k_1 \cdot k_4 \cdot \frac{\delta^{34}SO_4(t) - \delta^{34}SO_4(0)}{\Delta^{34}S_{cell}}
\]

Special case (X₃=0):
\[
\ln \frac{SO_4(t)}{SO_4(0)} = \beta \cdot \frac{\delta^{34}SO_4(t) - \delta^{34}SO_4(0)}{\Delta^{34}S_{cell}}
\]

The expression \( \frac{\delta^{34}SO_4(t) - \delta^{34}SO_4(0)}{\Delta^{34}S_{cell}} \) is well known from the Rayleigh-equation:
\[
\ln \frac{SO_4(t)}{SO_4(0)} = \frac{\delta^{34}SO_4(t) - \delta^{34}SO_4(0)}{\Delta^{34}S_{cell}}
\]

The Rayleigh-equation describes the relation between the sulfur isotope composition and the amount of residual sulfate controlled by bacterial sulfate reduction in a closed system (for derivation see MARIOTTI et al. (1981). This equation is used to determine the \( \Delta^{34}S_{cell} \).
We summarize the equations as follows:

**General case:**

\[
\ln \left( \frac{-\delta D' \cdot k_2 - (\delta H_2O + \delta ex) \cdot k_3 + \delta^{18}O_{SO_4}(t) \cdot k_4}{-\delta D' \cdot k_2 - (\delta H_2O + \delta ex) \cdot k_3 + \delta^{18}O_{SO_4}(0) \cdot k_4} \right) = k_1 \cdot k_4 \cdot \frac{\delta^{34}S_{SO_4}(t) - \delta^{34}S_{SO_4}(0)}{\Delta^{34}S_{cell}}
\]

and:

\[
\ln \left( \frac{-\delta D' \cdot k_2 - (\delta H_2O + \delta ex) \cdot k_3 + \delta^{18}O_{SO_4}(t) \cdot k_4}{-\delta D' \cdot k_2 - (\delta H_2O + \delta ex) \cdot k_3 + \delta^{18}O_{SO_4}(0) \cdot k_4} \right) = k_1 \cdot k_4 \cdot \ln \frac{SO_4(t)}{SO_4(0)}
\]

with:

\[
k_i = \frac{X_i}{(1-X_i) \cdot (1-X_i \cdot X_i \cdot X_i \cdot (1-X_i \cdot X_i \cdot X_i \cdot X_i)) \cdot \alpha}
\]
\[
k_2 = X_i \cdot X_i \cdot X_i \cdot X_i\]
\[
k_3 = \alpha \cdot (X_2 \cdot X_2 \cdot X_2 \cdot X_2 \cdot X_2 \cdot X_2)
\]
\[
k_4 = X_2 \cdot X_2 \cdot X_2 \cdot X_2 + \alpha \cdot (X_2 \cdot X_2 \cdot X_2 \cdot X_2 \cdot X_2 \cdot X_2)
\]
\[
\Delta^{34}S_{cell} = \Delta^{34}S_{f_{-1}} + X_1 \cdot -\Delta^{34}S_{b_{-1}} + X_1 \cdot X_2 \cdot \Delta^{34}S_{f_{-3}} + X_1 \cdot X_1 \cdot X_1 \cdot X_2 \cdot -\Delta^{34}S_{k_{-4}}
\]

**Special case (X_3=0):**

\[
\ln \left( \frac{-\delta H_2O + \delta ex + \delta^{18}O_{SO_4}(t)}{-\delta H_2O + \delta ex + \delta^{18}O_{SO_4}(0)} \right) = \beta \cdot \frac{\delta^{34}S_{SO_4}(t) - \delta^{34}S_{SO_4}(0)}{\Delta^{34}S_{cell}}
\]

and:

\[
\ln \left( \frac{-\delta H_2O + \delta ex + \delta^{18}O_{SO_4}(t)}{-\delta H_2O + \delta ex + \delta^{18}O_{SO_4}(0)} \right) = \beta \cdot \ln \frac{SO_4(t)}{SO_4(0)}
\]

with:

\[
\beta = \frac{X_i \cdot \alpha \cdot (X_2 \cdot X_2 \cdot X_2)}{(1-X_i) \cdot 1 + \alpha - \alpha \cdot X_i \cdot X_i}
\]
\[
\alpha = \frac{1}{X_2 \cdot X_2 \cdot \left( 1 + \frac{1}{\beta} \right)^{-1}}
\]
\[
\Delta^{34}S_{cell} = \Delta^{34}S_{f_{-1}} + X_1 \cdot -\Delta^{34}S_{b_{-1}} + X_1 \cdot X_2 \cdot \Delta^{34}S_{f_{-3}}
\]

where:

SO_4(t) Amount of residual sulfate at time t.
SO_4(t) Initial amount of sulfate.
\( \delta^{18} \text{O}_{\text{SO}_4}(t) \) Oxygen isotope composition of residual sulfate at time \( t \).

\( \delta^{18} \text{O}_{\text{SO}_4}(0) \) Initial oxygen isotope composition of sulfate.

\( \delta^{34} \text{S}_{\text{SO}_4}(t) \) Sulfur isotope composition of residual sulfate at time \( t \).

\( \delta^{34} \text{S}_{\text{SO}_4}(0) \) Initial sulfur isotope composition of sulfate.

\( \delta \text{H}_2\text{O} \) Oxygen isotope composition of ambient water.

\( \delta \text{D}' \) Oxygen isotope composition of sulfate derived from sulfite-oxidation.

\( \delta \text{ex} \) Oxygen isotope equilibrium fractionation factor.

\( \text{sSRR} \) Constant cell-specific sulfate reduction rate.

\( \alpha \) \( \text{ex/sSRR} \): Ratio between constant water-sulfate oxygen isotope exchange rate and cell-specific sulfate reduction rate.

\( X_i \) Ratio between backward- and forward-fluxes.

**Discussion and interpretation**

The presented equations describe the relationship between the amount, the oxygen isotope composition, and the sulfur isotope composition of residual sulfate for bacterial sulfate reduction. They are based on the following assumptions:

- Sulfate reduction takes place in a closed system (i.e. no external in- and output and no re-oxidation of produced hydrogen sulfide).
- The cell-specific sulfate reduction rates (sSRR) have to be constant.
- The number of bacteria can change in time.
- The oxygen isotope equilibrium between cell internal sulfur-compounds and ambient water dominates kinetic oxygen isotope fractionation steps.

A “general case” includes the oxygen isotope effects of cell internal reoxidation of sulfite \((X_i>0)\). Also, a “special case”, where reoxidation of sulfite is neglected \((X_i=0)\), has been calculated. In the following, we divide the discussion and interpretation of the model into four topics; each topic addresses one of the questions below:

1) Does the presented model, which is based on an “equilibrium oxygen isotope assumption”, explain linear \( \delta^{18} \text{O} - \delta^{34} \text{S} \) relationships that point to a “kinetic oxygen isotope fractionation”?

2) How can this model be falsified?
3) If this model passes falsification, what is its use? In an outlook, we will present how the presented equations could be used as a tool to unravel major questions concerning microbial sulfate reduction.

4) Do other biochemical pathways follow a similar isotope pattern? We will propose that denitrification is a process that is likely to be dominated by equilibrium oxygen isotope exchange.

1) **Kinetic oxygen isotope effects versus equilibrium oxygen isotope fractionation**

The developed model is based on the assumption that equilibrium exchange of oxygen isotopes between cell-internal sulfur compounds and ambient water dominates over kinetic oxygen isotope fractionation. Other authors (Aharon and Fu, 2000; Mandernack et al., 2003) observe a linear relationship between the oxygen and sulfur isotope composition of residual sulfate. Based on these observations, they conclude that kinetic isotope fractionation controls the sulfur and oxygen isotope composition of residual sulfate. This implies that either both oxygen isotope fractionation types do occur at different circumstances (e.g. different sSSR, X-ratios or bacteria) or that the isotope equilibrium dominated process cannot be easily distinguished from a process dominated by kinetic isotope fractionation. However, a linear relation between $\delta^{18}O$ and $\delta^{34}S$ of residual sulfate seems to contradict the developed model, which predicts an exponential relation between oxygen- and sulfur isotope values. We, therefore, compare our model with linear $\delta^{18}O - \delta^{34}S$ relations. For this comparison, we use the “special case”, where reoxidation of sulfite can be neglected because this model is mathematically easier to solve. If the “special case” can generate linear relationships between oxygen- and sulfur isotopes in residual sulfate, this is also the case for the general model.

- **Observation:** Linear relation between $\delta^{18}O$ and $\delta^{34}S$ of residual sulfate.

$$
\left( \delta^{18}O_{SO_4} (t) - \delta^{18}O_{SO_4} (0) \right) = \text{slope} \cdot \left( \delta^{34}S_{SO_4} (t) - \delta^{34}S_{SO_4} (0) \right)
$$

$$
\Rightarrow \delta^{18}O_{SO_4} (t) = \text{slope} \cdot \left( \delta^{34}S_{SO_4} (t) - \delta^{34}S_{SO_4} (0) \right) + \delta^{18}O_{SO_4} (0)
$$

Aharon and Fu (2000) report slopes of 0.29 to 0.71 and Mandernack et al. (2003) report a slope of 0.23.

- **Theoretical model:** Exponential relation between $\delta^{18}O$ and $\delta^{34}S$ of residual sulfate.

$$
\ln \left( \frac{-\delta^{18}O_{SO_4} (t) - \delta^{18}O_{SO_4} (0)}{-\delta^{18}O_{SO_4} (t) + \delta^{18}O_{SO_4} (0)} \right) = \beta \cdot \frac{\delta^{34}S_{SO_4} (t) - \delta^{34}S_{SO_4} (0)}{\Delta^{34}S_{cell}}
$$

$$
\Rightarrow \delta^{18}O_{SO_4} (t) = \left( \delta^{18}O_{SO_4} (0) - (\Delta H + \delta ex) \right) \cdot \exp \left( \beta \cdot \frac{\delta^{34}S_{SO_4} (t) - \delta^{34}S_{SO_4} (0)}{\Delta^{34}S_{cell}} \right) + (\Delta H + \delta ex)
$$
For a comparison, we calculate linear $\delta^{18}O - \delta^{34}S$ relations with different slopes and compare these data to the data from our exponential equation (Figure 5). The following values have been used to generate the comparison below:

- $\delta^{18}O_{SO_4}(0) = +9.8\%e$ (initial oxygen isotope composition of sulfate)
- $\delta^{34}S_{SO_4}(0) = +21\%e$ (initial sulfur isotope composition of sulfate)
- $\delta^{18}H_2O = 0\%e$ (oxygen isotope composition of ambient water)
- $\delta^{18}ex = 29\%e$ (oxygen isotope equilibrium fractionation factor)
- $\text{slope}_1 = 0.23$
- $\beta_1 = 0.23$ (fits best linear relation at initial stage)
- $\text{slope}_2 = 0.47$
- $\beta_2 = 0.5$ (fits best linear relation at initial stage)
- $\text{slope}_3 = 0.71$
- $\beta_3 = 0.7$ (fits best linear relation at initial stage)
- $\Delta^{34}S_{cell} = -17.25\%e$ (The value of $\Delta^{34}S_{cell}$ has no influence on the shape of the calculated $\delta^{18}O - \delta^{34}S$ relation as long as the ratio $\beta : \Delta^{34}S_{cell}$ is constant. Because $\beta$ is freely chosen to fit best with the linear relation at an initial stage, any value for $\Delta^{34}S_{cell}$ can be compensated.)

Figure 10 demonstrates that there is a strong discrepancy between a linear behavior and the $\delta^{18}O - \delta^{34}S$ relations calculated by our model. However, this discrepancy becomes only detectable at high enrichments in $^{18}O$ and $^{34}S$. The calculated non-linear $\delta^{18}O$ values do not reach...
a plateau at the equilibrium value (here chosen to be 29‰). The argument of (AHARON and 
FU, 2000) that a oxygen isotope equilibrium fractionation dominated process should reach a 
plateau at the theoretical equilibrium value is, therefore, not valid. Additionally, the linear 
δ¹⁸O – δ³⁴S ratios presented by AHARON and FU (2000) and MANDERNACK et al. (2003) fall in 
a data range (shaded areas in Figure 10) where a linear relation can hardly be distinguished 
from the non-linear model. This implies that there is no evidence for the dominance of kinetic 
oxygen isotope fractionation during bacterial sulfate reduction.

2) Falsification of the presented model

The mathematical description of the relations between δ¹⁸O, δ³⁴S and the amount of residual 
sulfate allows falsification of the developed model (i.e. the taken assumptions). Unfortu-
nately, the equation for the “general case” is rather complex and consists of a large number of 
unknown parameters making falsification difficult. The “special case” however is more suit-
able because the only unknown expression (β) represents the slope in a linear relation:

Special case (X₁=0):

\[
\ln\frac{-(δH₂O + δex) + δ¹⁸Oₘ₀(t)}{-(δH₂O + δex) + δ¹⁸Oₘ₀(0)} = β \cdot \frac{δ³⁴Sₘ(t) - δ³⁴Sₘ(0)}{Δ³⁴S_{cell}} = β \cdot \ln \frac{SO₄(t)}{SO₄(0)}
\]

with:

\[
β = \frac{X_1 \cdot α \cdot (X_1 - X_1 \cdot X_2)}{(1-X_1)} \cdot \frac{1 + α - α \cdot X_1 \cdot X_2}{X_1 \cdot X_2 \cdot (1 + \frac{1}{β}) - 1}
\]

\[
Δ³⁴S_{cell} = Δ³⁴S_1 + X_1 \cdot Δ³⁴S_2 + X_1 \cdot X_2 \cdot Δ³⁴S_3
\]

where:

SO₄(t) Amount of residual sulfate at time t.
SO₄(t) Initial amount of sulfate.
δ¹⁸O₈₅(t) Oxygen isotope composition of residual sulfate at time t.
δ¹⁸O₈₅(0) Initial oxygen isotope composition of sulfate.
δ³⁴S₈₅(t) Sulfur isotope composition of residual sulfate at time t.
δ³⁴S₈₅(0) Initial sulfur isotope composition of sulfate.
δH₂O Oxygen isotope composition of ambient water.
δex Oxygen isotope equilibrium fractionation factor.
sSRR Constant cell-specific sulfate reduction rate.
\( \alpha \) ex/sSRR: Ratio between constant water-sulfate oxygen isotope exchange rate and cell-specific sulfate reduction rate.

\( X_i \) Ratio between backward- and forward-fluxes.

\( \Delta^{34}S_i \) Cell internal kinetic sulfur isotope fractionation steps.

With the exception of \( \alpha \) and \( X_i \), all variables can be determined or estimated from laboratory experiments. Therefore, experiments can be designed for the falsification of the developed model. An example for such a falsification is the use of water with different oxygen isotope composition in a set of identical sulfate reduction experiments (Figure 11).

![Synthetic \( \delta^{18}O - \delta^{34}S \) plots](image)

**Figure 11** Synthetic \( \delta^{18}O - \delta^{34}S \) plots where ambient water has different oxygen isotopic compositions

For the calculation of the \( \delta^{18}O - \delta^{34}S \) relations we used the following values:

- \( \delta H_2O = -50‰ / 0‰ / +50‰ / +100‰ \)
- \( \delta ex = 29‰ \) (oxygen isotope equilibrium fractionation factor)
- \( \beta = 0.9 \)
- \( \Delta^{34}S_{cell} = -17.25‰ \)
- \( X_3 = 0 \)
According to the equation
\[ \ln \left( \frac{-\delta H_2O + \delta ex(i) + \delta^{18}O_{SO_4}(i)}{-\delta H_2O + \delta ex(i) + \delta^{18}O_{SO_4}(0)} \right) = \beta \frac{\Delta^{34}S_{SO_4}(i) - \Delta^{34}S_{SO_4}(0)}{SO_4(i)} = \beta \ln \frac{SO_4(i)}{SO_4(0)} \]
the measured data can then be plotted in the following way (Figure 8):

![Figure 12](image)

**Figure 12**  Plot of corresponding to mathematical relation between $\delta^{18}O$ and $\delta^{34}S$, respectively the amount of residual sulfate

The slope corresponds to the value of $\beta$.

The values of the experiments with different $\delta^{18}O$ of ambient water should fall on an identical line. If the data from the different experiments fall together, the model describes the $\delta^{18}O - \delta^{34}S$ reasonably well, if not, the model has to be reconsidered. However, the used equations apply only for the special case where $X_3$ equals zero. Therefore, for the design of the experiments, it is crucial to know cases where no reoxidation of sulfite takes place. From the above discussion of the total sulfur isotope fractionation by a bacterium, it is evident that there is no absolute criterion to ensure that no sulfite is reoxidized. The results of FRITZ et al. (1989) only indicate that the assumption of REES (1973), that is, $X_3$ equals zero in experiments where $\Delta^{34}S_{cell}$ is less negative than $-25‰$, is not entirely wrong. Therefore, we suggest the use of bacteria culture experiments, where $\Delta^{34}S_{cell}$ is distinctly less negative than $-25‰$, for falsification of the presented “special case”. However, there could be exceptions where $X_1$ or $X_2$ are small and $X_3$ is large; Table 1 demonstrates that $\Delta^{34}S_{cell}$ less negative than $-25‰$ could also occur when $X_3$ is larger than zero.
Table 1  \( X_i \)-ratios and corresponding \( \Delta^{34}S_{\text{cell}} \)-values

\( \Delta^{34}S_{\text{cell}} \)-values more positive than \(-25\%e\) can also be achieved with \( X_3 \) larger than zero (shaded cells). The probability of the occurrence of such an exception depends on the assumed value for \( \Delta^{34}S_3 \) (\(-25\%e\) or \(-50\%e\)). The values in Table 1 are calculated from the equation

\[
\Delta^{34}S_{\text{cell}} = \Delta^{34}S_1 + X_1 \Delta^{34}S_2 + X_1 X_2 \Delta^{34}S_3 + X_1 X_2 X_3 \Delta^{34}S_4,
\]

where \( \Delta^{34}S_1 = +3\%e \), \( \Delta^{34}S_2 = 0\%e \), \( \Delta^{34}S_3 \) = \(-25\%e\), \( \Delta^{34}S_4 \) = \(-25\%e\) or \(-50\%e\).

In absence of a better tool for the identification of sulfate reduction processes without reoxidation of sulfite, we propose, as a “rough” rule of thumb, to use experimental setups where \( \Delta^{34}S_{\text{cell}} \)-values are distinctly less negative than \(-25\%e\) to ensure that \( X_3 \) equals zero. It has to be expected that at least some experiments (where \( X_3=0 \)) should produce the predicted relationship between \( \delta^{18}O \), \( \delta^{34}S \) and the amount of residual sulfate. Unfortunately, there are no data sets available to test our model, and further laboratory experiments are needed.

3) Outlook

The falsification of the mathematical model will increase the knowledge about sulfate reducing bacteria dramatically. If the model fails to reproduce laboratory experiments, the proposed mechanisms for the oxygen isotope effects caused by bacterial sulfate reduction have to be reconsidered. If the model passes the test, a complete new tool for the investigation of sulfate reduction is available. Here, we would like to present an outlook of the possibilities arising:

- Determination of the precise equilibrium fractionation factor between enzyme-sulfate and ambient water: The temperature-dependent fractionation values proposed by FRITZ et al., (1989) are based on the isotope composition that was approached during the depletion of sulfate due to bacterial sulfate reduction. With a set of equal sulfate reduction experiments, where only the oxygen isotope composition of water is held at different values, a set of \( \delta^{18}O \)-\( \delta^{34}S \) relations will result (Figure 11). By assuming a value for the equilibrium fractionation factor (\( \delta_{\text{ex}} \)) the relations can be plotted according to the equation below (Figure 13):
\[ \ln \left( \frac{\delta^{13}O + \delta^{18}O_{SO_4}(t)}{\delta^{13}O + \delta^{18}O_{SO_4}(0)} \right) = \beta \cdot \frac{\delta^{34}S_{SO_4}(t) - \delta^{34}S_{SO_4}(0)}{\Delta S_{cell}} = \beta \cdot \ln \frac{SO_4(t)}{SO_4(0)} \]

The slope \( \beta \) is only equal for each experiment with different \( \delta^{18}O \) of water when the correct equilibrium factor (\( \delta^{18}O_{ex} \)) was chosen. \( \delta^{18}O_{ex} \) therefore can be changed until the slopes in all experiments are equal.

Figure 13 Different assumptions for \( \delta^{18}O_{ex} \) (27‰ to 30‰) lead to different plots corresponding to the mathematical relation between \( \delta^{18}O \) and \( \delta^{34}S \), respectively the amount of residual sulfate below

\[ \ln \left( \frac{\delta^{13}O + \delta^{18}O_{SO_4}(t)}{\delta^{13}O + \delta^{18}O_{SO_4}(0)} \right) = \beta \cdot \frac{\delta^{34}S_{SO_4}(t) - \delta^{34}S_{SO_4}(0)}{\Delta S_{cell}} = \beta \cdot \ln \frac{SO_4(t)}{SO_4(0)} \]

The values of the experiments with different \( \delta^{18}O \) of ambient water fall on an identical line when the appropriate value for \( \delta^{18}O_{ex} \) was chosen.
• Determination of $\alpha$, exchange rate (ex) and sSRR: $\alpha$ is the ratio between the equilibrium oxygen isotope exchange rate (ex) and the cell-specific sulfate reduction rate (sSRR). $\alpha$ is a function of $\beta$, which has been determined above.

\[
\alpha = \frac{1}{X_1 \cdot X_2 \cdot \left(1 + \frac{1}{\beta}\right) - 1}
\]

The unknown product of $X_1$ and $X_2$ is derived from the equation for $\Delta^{34}S_{\text{cell}}$:

\[
\Delta^{34}S_{\text{cell}} = \Delta^{34}S_1 + X_1 \cdot X_2 \cdot \Delta^{34}S_3
\]

\[
\Rightarrow X_1 \cdot X_2 = \frac{\Delta^{34}S_{\text{cell}} - \Delta^{34}S_1}{\Delta^{34}S_3}
\]

The ratio between the oxygen isotope exchange rate and sSRR therefore can be calculated using an assumption for the value of $\Delta^{34}S_3$. The exchange rate is calculated as the product of $\alpha$ and the sSRR. Probably, the exchange rate is a constant not as yet determined. In this case, the equation shows that “slow” cell-specific sulfate reduction rates (sSRR) correspond to large $\alpha$ values, which correspond, in turn, to large values for $\beta$ (Figure 11). If this is the case, the exchange rate could be used for the calculation of sSRR. This would partly confirm the relation between sulfate reduction rates and the shape of $\delta^{18}O - \delta^{34}S$ plots postulated by BÖTTCHER et al. (1999) and BÖTTCHER et al. (1998). However, bulk sulfate reduction rates cannot be derived from $\delta^{18}O - \delta^{34}S$ relations.

*Figure 14 Synthetic $\delta^{18}O - \delta^{34}S$ plots with different values for $\beta$*

For the calculation of the $\delta^{18}O - \delta^{34}S$ relations we used the following values:

- $\beta =$ 0.3 (fast sSRR), 0.9 (intermediate sSRR) and 2 (slow sSRR)
- $\delta H_2O = 0\%e$
- $\delta ex = 29\%e$ (oxygen isotope equilibrium fractionation factor)
- $\Delta^{34}S_{\text{cell}} = -17.25\%e$
- $X_3 = 0$
Figure 15  The plots correspond to the mathematical relation between δ¹⁸O and δ³⁴S, respectively the amount of residual sulfate below

\[ \delta_{34}^{S_{\text{cell}}} - \delta_{34}^{S_{\text{cell}}(0)} = \beta \cdot \ln \frac{\delta_{18}^{O_{\text{final}}}}{\delta_{18}^{O_{\text{initial}}}} \]

The data can be plotted in two different ways: If the value for \( \Delta^{34} S_{\text{cell}} \) is unknown and no data for the amount of residual sulfate are available, the slope equals the product between \( \beta \) and \( \Delta^{34} S_{\text{cell}} \) (Figure on the left). When \( \Delta^{34} S_{\text{cell}} \) or the amount of residual sulfate are known, \( \beta \) can be directly determined from the plot (Figure on the right).

Figure 12 demonstrates the determination of \( \beta \), respectively the product of \( \beta \) and \( \Delta^{34} S_{\text{cell}} \). Probably, the value of \( \beta \) can be used to determine sSRR. If sSRR could be calculated from oxygen and sulfur isotope measurements, this would permit the investigation of sSRR where a direct determination is not possible. Information could be gained from waters in waste deposits, from interstitial waters from drill holes (e.g. deep biosphere) and from recent and ancient sediments (e.g. about sulfate reduction processes associated with dolomite formation or “early life”-sulfate reducers).

4) Denitrification – an example of another biochemical pathways with a similar isotope pattern?

It is likely that other biochemical pathways have similar isotope fractionation patterns as the microbial sulfate reduction. Here we present evidence that this could be the case for the dis-
similatory reduction of nitrate (denitrification). As dissimilatory sulfate reduction, denitrification is a stepwise transformation of compounds, catalyzed by enzymes. The chemical transformation can be written as follows:

\[ 2\text{NO}_3^- \rightarrow 2\text{NO}_2^- \rightarrow 2\text{NO} \rightarrow \text{N}_2 \text{O} \rightarrow \text{N}_2 \]

As for microbial sulfate reduction, where the $\delta^{34}S$ of residual sulfate is affected by kinetic sulfur isotope fractionation, denitrification causes changes in the nitrogen isotope composition of residual nitrate. Similar to $\Delta^{34}S$, measured nitrogen enrichment factors ($\Delta^{15}N$) in culture, marine and freshwater studies show a wide range from 0‰ to $-40‰$. The small isotope effects have been attributed by SIGMAN et al. (2003) to situations where the rate of nitrate supply to denitrifying bacteria is limited (i.e. the concentration of NO$_3^-$ is low). HABICHET al. (2002) have shown that the same effect occurs for microbial sulfate reduction: kinetic sulfur isotope fractionation is small where the concentration of sulfate is low. Due to the similarities between the stepwise reaction path and the similar isotope effects of nitrogen and sulfur, we hypothesize that the reaction path of denitrification is an analogue of the one of dissimilatory sulfate reduction (Figure 16).

![Figure 16](image)

**Figure 16** Hypothetical pathway of dissimilatory nitrate reduction (denitrification) analogue to the pathway of dissimilatory sulfate reduction (modified after (F RITZ et al., 1989; REES, 1973))

Nitrates are reduced by enzyme catalyzed steps within the cell of the denitrifying bacterium (dashed line = cell wall). Forward- ($f_i$) and backward-fluxes ($b_i$) connect pools of intermediate nitrogen compounds ($X_i$ equal the $b_i:f_i$-ratios). The total kinetic nitrogen isotope fractionation by the cell is the result of a combination of the $X_i$-ratios and $\Delta^{15}N$-fractionation steps. The total oxygen isotope effect is the result from a combination of $X_i$-ratios, $\Delta^{18}O$-fractionation steps, reoxidation-steps of nitrogen-compounds and oxygen isotope exchange with ambient water.
According to the presented hypothetical denitrification pathway (Figure 16), it is likely that the nitrogen isotope fractionation in the first and second step are rather small (no breakage of N–O bonds), while the further steps (3 to 6) are likely to be associated with large nitrogen isotope fractionation effects. At low nitrate concentrations, the backward fluxes (b) are small and, therefore, the large isotope fractionation effect by the latter steps (e.g. $\Delta^{15}N_3$) does not affect the $\delta^{15}N$ of residual nitrate. This would explain the low kinetic isotope effects observed at low nitrate concentrations. The experiments of MARIOTTI et al. (1981) indicate that the transformation of nitrate to nitrite might be a rate limiting step: During the experiment, where an enrichment factor of $-24.6\%$ to $-29.4\%$ was determined, nitrite was not detectable since it was reduced as rapidly as it was formed. This could be an analogue to the reduction of sulfate to sulfite ($X_3=0$, “normal case” according to REES (1973), respectively “special case” in our calculations). As demonstrated in Figure 13, the oxygen isotope effect by denitrification is probably a complex combination of kinetic oxygen isotope fractionation steps, oxidation steps of intermediate nitrogen compounds, equilibrium exchange of oxygen isotopes of nitrogen compounds with ambient water and backward- to forward flux ratios. CASCIOSSI et al. (2002) investigated the oxygen isotope effects associated with the denitrifier method for $^{15}N/^{14}N$ analysis described by SIGMAN et al. (2001). They found a strong $^{18}O$-enrichment of $N_2O$ compared to the oxygen isotope composition of initial nitrate ($40\%$). This is probably due to a preferential loss of $^{16}O$ during the reduction of nitrate; an effect on the $\delta^{18}O$ of residual nitrate might be involved ($\Delta^{18}O_3$). CASCIOSSI et al. (2002) further demonstrate that the exchange of oxygen isotopes of water with nitrite ($NO_2^-$) and nitric oxide (NO) depends on the involved bacterial strains. However, if the transformation of nitrate to nitrite is a rate-limiting step ($X_3$ equals zero), these exchange reactions do not affect the oxygen isotope composition of nitrate. Assuming that the transformation of nitrate to nitrite is rate limiting ($X_3=0$), the $\delta^{15}N$ of residual nitrate would depend on the first three kinetic nitrogen isotope fractionation steps. In addition to the first three kinetic oxygen isotope fractionation steps, the $\delta^{18}O$ of residual nitrate might be influenced by equilibrium oxygen exchange between enzymatically bound nitrate and ambient water. As no N–O bond is broken in the first two steps; the oxygen and nitrogen isotope fractionation is likely to be small. Therefore, the nitrogen- and oxygen isotope effect in residual nitrate would be mainly controlled by kinetic oxygen and nitrogen isotope fractionation during the reduction of nitrate to nitrite ($\Delta^{15}N_3$, $\Delta^{18}O_3$) and the proposed equilibrium oxygen isotope exchange with ambient water. In case there is no equilibrium exchange, the ratio between the kinetic isotope enrichment factors ($\Delta^{15}N_3 : \Delta^{18}O_3$) would control
the $\delta^{15}N$ and $\delta^{18}O$ of residual nitrate: In a plot, a linear relationship between $\delta^{15}N - \delta^{18}O$ should result, and the slope should be equal to the ratio $\Delta^{15}N : \Delta^{18}O$. In freshwater studies, a linear relationship of 1 : 0.6 (LEHMANN et al., 2003) has been observed; from the Santa Barbara Basin, SIGMAN et al. (2003) report a relationship of 1 : 1 ($\Delta^{15}N : \Delta^{18}O$). Under the assumption that $X_3$ equals zero during denitrification, these observations can be interpreted as follows: Either, the relation of $\Delta^{15}N : \Delta^{18}O$ is not identical for seawater and freshwater (different bacteria?), or, as has been demonstrated above for dissimilatory sulfate reduction, equilibrium oxygen isotope exchange with ambient water plays an important role. The latter could explain the difference in the observed slopes: The $\delta^{18}O$ of freshwater is usually depleted in $^{18}O$ (e.g. around $-7‰$ for the 1 : 0.57 relation reported by LEHMANN et al. (2003), while the $\delta^{18}O$ of seawater is around $0‰$. A potential oxygen isotope equilibrium value between enzyme-nitrate and ambient water for seawater would therefore be around $7‰$ higher than for freshwater. Starting with roughly the same initial oxygen isotope composition of nitrate (around $5‰$ for freshwater in LEHMANN et al. (2003) and around $3‰$ for seawater in SIGMAN et al. (2003)), a steeper $\Delta^{15}N : \Delta^{18}O$-slope for seawater than for freshwater would result. Figure 14 illustrates the proposed relation between $\delta^{18}O$ of ambient water and the $\delta^{18}O - \delta^{15}N$ relation.

Figure 14 Hypothetical $\delta^{18}O - \delta^{15}N$ relation in residual nitrate dependent on the $\delta^{18}O$ of ambient water

The different slopes are caused by the different oxygen composition of ambient water.

We emphasize that our denitrification model is a hypothesis based on the observation of strong similarities between dissimilatory reduction of nitrate and sulfate. However, the
existence of a strong influence of equilibrium (or other) oxygen isotope exchange between nitrate and ambient of water can be tested with similar experimental setups as proposed for the falsification of the sulfate reduction model: Already a single denitrification experiment in water strongly depleted in $^{18}$O could be a sufficient test. If the $\delta^{18}$O of residual nitrate still increases, equilibrium oxygen isotope exchange is not important; if the $\delta^{18}$O of residual nitrate decreases, oxygen isotopes in the residual nitrate must have been derived from water.

**Conclusions**

The hitherto existing, and commonly accepted model for the isotope effects by dissimilatory sulfate reduction (REES, 1973) cannot explain the sulfur isotope effects related to the reduction of sulfite to sulfide. The sulfur isotope effects related to this step depend on environmental and cell-specific parameters. Therefore, it is impossible to assign a kinetic sulfur isotope fractionation factor to the reduction of sulfite to sulfide. We emphasize that the sulfur isotope fractionation in this step is in a broad range, from 0‰ to values exceeding the equilibrium isotope effect between sulfite and sulfide around –50‰. Consequently, sulfur isotope fractionation by dissimilatory sulfate reduction can reach values above –70‰. This confirms observations from natural environments, where fractionation factors up to –77‰ have been observed (RUDNICKI et al., 2001; WERNE et al., 2003; WORTMANN et al., 2001). Considering that the sulfur isotope fractionation factor for the reduction of sulfite to sulfide depends on environmental and cell-specific properties, and not alone on the cell specific sulfate reduction rate ($sSRR$), it becomes evident that the relationship between $sSRR$ and $\Delta^{34}S$ is also cell-specific. $\Delta^{34}S$ – $sSRR$ relations of different sulfate reducing bacteria, therefore, do not correlate.

We present mathematical equations describing the relationship between the amount, the oxygen isotope composition, and the sulfur isotope composition of residual sulfate for bacterial sulfate reduction. The equations are based on the following assumptions:

- Sulfate reduction takes place in a closed system (no external in- and output and no reoxidation of produced hydrogen sulfide).
- Cell-specific sulfate reduction rates ($sSRR$) from individual bacteria are constant.
- The number of bacteria can change in time.
- The oxygen isotope equilibrium between cell internal sulfur-compounds and ambient water dominates over kinetic oxygen isotope fractionation steps.
One equation describes a “general case”, where the oxygen isotope effects of cell internal re-oxidation of sulfite are taken into account. A second equation is derived for a “special case”, where reoxidation of sulfite is neglected.

We demonstrate that, in despite of the non-linear relation between $\delta^{18}O$ and $\delta^{34}S$ at an initial stage, $\delta^{18}O – \delta^{34}S$ plots mimic a linear relationship. The $\delta^{18}O$ values approach a constant equilibrium value only when sulfate reduction reaches a high degree of sulfate depletion. Linear relations between $\delta^{18}O$ and $\delta^{34}S$, therefore, can be caused by equilibrium oxygen isotope exchange and not only by kinetic isotope fractionation. Based on these observations, we question whether kinetic oxygen isotope fractionation influences the oxygen isotope composition of residual sulfate. Consequently, slopes derived from linear regression lines in $\delta^{18}O – \delta^{34}S$ plots do not reflect the relation between the kinetic sulfur and oxygen isotope fractionation during microbial sulfate reduction. A “typical” constant 1:4 ratio for sulfate reduction, as is sometimes used in the literature, does not exist. The reason why ratios close to 1:4 have often been observed is due to the fact that most analyzed samples were derived from marine environments with a constant $\delta^{18}O$ of seawater at 0‰. The presented mathematical equations can be used to design experiments for the testing and falsification of the model and the taken assumptions. However, the proposed experimental work must first be carried out. If the model passes falsification, the equations will serve as a tool for the determination of precise oxygen isotope equilibrium fractionation factors and exchange rates. Further, $\delta^{18}O – \delta^{34}S$ measurements potentially can be used to determine cell-specific sulfate reduction rates, leading to completely new approaches in the investigation of processes related to microbial sulfate reduction.

We hypothesize that equilibrium oxygen isotope exchange with ambient water is also important in other biochemical pathways. Dissimilatory nitrate reduction (denitrification) causes $^{18}O – ^{15}N$ isotope patterns similar to the observed $^{18}O – ^{34}S$ isotope relations in residual sulfate. Thus, we speculate that changes in the $\delta^{18}O$ of residual nitrate are caused by equilibrium oxygen isotope fractionation between cell-internal nitrogen-compounds and ambient water during denitrification. As in the case of dissimilatory sulfate reduction, a constant slope for $^{18}O – ^{15}N$ relations (e.g. a slope of 1:0.6 for $\delta^{15}N:\delta^{18}O$) can not exist. However, if denitrification follows a similar pathway as dissimilatory sulfate reduction, the presented equations can be used for the analysis of processes related to dissimilatory nitrate reduction.
References


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Appendix 1 (for a similar calculation see Rees (1973))

Calculation of the total sulfur isotope fractionation by a sulfate reducing bacterium

In a laboratory steady state setup, where the $\delta^{34}\text{S}$ of sulfate, sulfate- and nutrient-concentration and physical and chemical parameters (e.g. temperature and pH) are kept constant, the sulfur isotopic composition of the produced sulfides is also constant. The total sulfur isotope fractionation by the sulfate reduction equals the difference between $\delta^{34}\text{S}_{\text{sulfide}}$ and $\delta^{34}\text{S}_{\text{sulfate}}$. This value is the result of the combination of different forward- and backward fluxes in the cascade of enzymatic transformations of sulfur compounds within the cell and can be calculated by steady state mass balances for each sulfur pool.

**Figure 18** Pathway of dissimilatory sulfate reduction (modified after Fritz et al. 1989, Rees 1973)

Sulfate is transformed to sulfide by enzyme catalyzed steps within the cell of the sulfate reducing organism (dashed line = cell wall). Forward- ($f_i$) and backward-fluxes ($b_i$) connect pools ($P_i$) of intermediate sulfur compounds ($X_i$ equal the $b_i:f_i$ ratios). The cell-specific sulfate reduction rate ($s\text{SRR}$) equals the difference between forward- and backward-fluxes. For simplicity, the sulfur pools and their isotope composition are abbreviated, e.g. external sulfate = $P_1$ and isotope composition of external sulfate = $\delta P_1$. 

- $\Delta^{34}\text{S}_{\text{P}_1}$: External sulfate
- $\Delta^{34}\text{S}_{\text{P}_2}$: Internal sulfate
- $\Delta^{34}\text{S}_{\text{P}_3}$: APS
- $\Delta^{34}\text{S}_{\text{P}_4}$: Sulfate
- $\Delta^{34}\text{S}_{\text{P}_5}$: "Sulfate reduction pool" 
- $\Delta^{34}\text{S}_{\text{P}_6}$: Hydrogen sulfide
- $\Delta^{34}\text{S}_{\text{P}_7}$: External sulfide

$X_1 = b_1/f_1$, $X_2 = b_2/f_2$, $X_3 = b_3/f_3$, $X_4 = b_4/f_4$, $X_5 = b_5/f_5$, $X_6 = b_6/f_6$
The following equations are derived from the steady state assumption above:

Step 7 = last step (external sulfide):
\[
\frac{d}{dt} P_7 = + f_s - b_s = sSRR
\]
\[
\frac{d}{dt} (P_7 \cdot \delta P_7) = \frac{d}{dt} P_7 \cdot \delta P_7 + \frac{d}{dt} \delta P_7 \cdot P_7 = + f_s \cdot (\delta P_7 + \Delta \delta) - b_s \cdot (\delta P_7 + \Delta \delta)
\]

steady state condition:
\[
\frac{d}{dt} \delta P_7 = 0
\]

it follows:
\[
\frac{d}{dt} P_7 \cdot \delta P_7 = sSRR \cdot \delta P_7 = + f_s \cdot (\delta P_7 + \Delta \delta) - b_s \cdot (\delta P_7 + \Delta \delta)
\]
\[
0 = + f_s \cdot (\delta P_7 + \Delta \delta) - b_s \cdot (\delta P_7 + \Delta \delta) - sSRR \cdot \delta P_7
\]
\[
\Rightarrow sSRR \cdot \delta P_7 = + f_s \cdot (\delta P_7 + \Delta \delta) - b_s \cdot (\delta P_7 + \Delta \delta)
\]
\[
\left(\delta P_7 + \Delta \delta\right) = \frac{b_s}{f_s} \cdot \left(\delta P_7 + \Delta \delta\right) + sSRR \cdot \delta P_7
\]

Step 6 (internal sulfide):
\[
\frac{d}{dt} (P_s \cdot \delta P_s) = 0 = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta) - \left(\delta P_s + \Delta \delta\right) - \left(\delta P_s + \Delta \delta\right)
\]
\[
0 = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta) - sSRR \cdot \delta P_s
\]
\[
\Rightarrow sSRR \cdot \delta P_s = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta)
\]
\[
\left(\delta P_s + \Delta \delta\right) = \frac{b_s}{f_s} \cdot \left(\delta P_s + \Delta \delta\right) + sSRR \cdot \delta P_s
\]

Step 5 (“sulfite reductase pool”):
\[
\frac{d}{dt} (P_s \cdot \delta P_s) = 0 = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta) - \left(\delta P_s + \Delta \delta\right) - \left(\delta P_s + \Delta \delta\right)
\]
\[
0 = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta) - sSRR \cdot \delta P_s
\]
\[
\Rightarrow sSRR \cdot \delta P_s = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta)
\]
\[
\left(\delta P_s + \Delta \delta\right) = \frac{b_s}{f_s} \cdot \left(\delta P_s + \Delta \delta\right) + sSRR \cdot \delta P_s
\]

Step 4 (sulfite):
\[
\frac{d}{dt} (P_s \cdot \delta P_s) = 0 = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta) - \left(\delta P_s + \Delta \delta\right) - \left(\delta P_s + \Delta \delta\right)
\]
\[
0 = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta) - sSRR \cdot \delta P_s
\]
\[
\Rightarrow sSRR \cdot \delta P_s = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta)
\]
\[
\left(\delta P_s + \Delta \delta\right) = \frac{b_s}{f_s} \cdot \left(\delta P_s + \Delta \delta\right) + sSRR \cdot \delta P_s
\]

Step 3 (APS_sulfate):
\[
\frac{d}{dt} (P_s \cdot \delta P_s) = 0 = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta) - \left(\delta P_s + \Delta \delta\right) - \left(\delta P_s + \Delta \delta\right)
\]
\[
0 = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta) - sSRR \cdot \delta P_s
\]
\[
\Rightarrow sSRR \cdot \delta P_s = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta)
\]
\[
\left(\delta P_s + \Delta \delta\right) = \frac{b_s}{f_s} \cdot \left(\delta P_s + \Delta \delta\right) + sSRR \cdot \delta P_s
\]

Step 2 (internal sulfate):
\[
\frac{d}{dt} (P_s \cdot \delta P_s) = 0 = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta) - \left(\delta P_s + \Delta \delta\right) - \left(\delta P_s + \Delta \delta\right)
\]
\[
0 = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta) - sSRR \cdot \delta P_s
\]
\[
\Rightarrow sSRR \cdot \delta P_s = + f_s \cdot (\delta P_s + \Delta \delta) - b_s \cdot (\delta P_s + \Delta \delta)
\]
\[
\left(\delta P_s + \Delta \delta\right) = \frac{b_s}{f_s} \cdot \left(\delta P_s + \Delta \delta\right) + sSRR \cdot \delta P_s
\]
Summary:
\[
(\delta P_i + \Delta f_{i,x}) = \frac{b_i}{f_i} (\delta P_i + \Delta f_{i,x}) + \frac{s_{SRR}}{f_i} \delta P_i
\]

Model considerations:
\[
s_{SRR} = f_j - b_j = f_2 - b_2 = f_3 - b_3 = f_4 - b_4 = f_5 - b_5 = f_6 - b_6
\]

When \(X_i = \frac{b_i}{f_i}\)
\[
s_{SRR} = \frac{f_j - b_j}{f_j} = 1 - \frac{b_j}{f_j} = 1 - X_i
\]

It follows:
\[
(\delta P_i + \Delta f_{i,x}) = X_i (\delta P_i + \Delta f_{i,x}) + (1 - X_i) \cdot \delta P_i = X_i \cdot \Delta f_{i,x} + \delta P_i
\]
\[
(\delta P_i + \Delta f_{i,x}) = X_i \cdot \Delta f_{i,x} + \delta P_i \Rightarrow \delta P_i = -\Delta f_{i,x} + X_i \cdot \Delta f_{i,x} + \delta P_i
\]
\[
(\delta P_i + \Delta f_{i,x}) = X_i (\delta P_i + \Delta f_{i,x}) + (1 - X_i) \cdot \delta P_i
\]
\[
(\delta P_i + \Delta f_{i,x}) = X_i (\delta P_i + \Delta f_{i,x}) + (1 - X_i) \cdot \delta P_i
\]
\[
(\delta P_i + \Delta f_{i,x}) = X_i \cdot \Delta f_{i,x} + (1 - X_i) \cdot \delta P_i
\]
\[
\delta P_i = -\Delta f_{i,x} + X_i \cdot \Delta f_{i,x} + \delta P_i
\]
\[
\delta P_i = -\Delta f_{i,x} + X_i \cdot (\delta P_i + \Delta f_{i,x}) + (1 - X_i) \cdot \delta P_i
\]
\[
\delta P_i = -\Delta f_{i,x} + X_i \cdot (\delta P_i + \Delta f_{i,x}) + (1 - X_i) \cdot \delta P_i
\]
\[
\delta P_i = -\Delta f_{i,x} + X_i \cdot (\delta P_i + \Delta f_{i,x}) + (1 - X_i) \cdot \delta P_i
\]
\[
\delta P_i = -\Delta f_{i,x} + X_i \cdot (\delta P_i + \Delta f_{i,x}) + (1 - X_i) \cdot \delta P_i
\]
\[
\delta P_i = -\Delta f_{i,x} + X_i \cdot (\delta P_i + \Delta f_{i,x}) + (1 - X_i) \cdot \delta P_i
\]
\[
\delta P_i = -\Delta f_{i,x} + X_i \cdot (\delta P_i + \Delta f_{i,x}) + (1 - X_i) \cdot \delta P_i
\]
\[
\delta P_i = -\Delta f_{i,x} + X_i \cdot (\delta P_i + \Delta f_{i,x}) + (1 - X_i) \cdot \delta P_i
\]
COMBINED MEASUREMENTS OF $\delta^{34}S$ AND $\delta^{18}O$

APPENDIX 1 – CHAPTER 1

\[ \delta P_2 = -\Delta f_{-2} + X_5 \cdot (\delta P_2 + \Delta b_{-5}) - X_6 \cdot (\Delta b_{-6} + X_6 \cdot \Delta b_{-6}) \]

\[ \delta P_7 = -\Delta f_{-7} + X_2 \cdot (\delta P_7 + \Delta b_{-2}) - X_3 \cdot (\Delta b_{-3} + X_3 \cdot \Delta b_{-3}) \]

The sulfur isotope difference between isotope composition of hydrogen sulfide produced and sulfate consumed equals:

\[ -\Delta_{cell} = \delta P_1 - \delta P_2 = -\Delta f_{-1} + X_1 \cdot (\delta P_1 + \Delta b_{-1}) - X_2 \cdot (\Delta b_{-2} + X_2 \cdot \Delta b_{-2}) \]

The overall isotope effect caused by a single bacterium is equal to the isotope difference above:

\[ \Delta_{cell} = \Delta f_{-1} + X_1 \cdot (\delta P_1 + \Delta b_{-1}) - X_2 \cdot (\Delta b_{-2} + X_2 \cdot \Delta b_{-2}) \]
Appendix 2

Calculation of the time dependent sulfur isotope composition

Figure 19  Sulfate reduction as a black box model

The output of H$_2$S, with an output rate equal to the cell-specific sulfate reduction rate has a different sulfur isotope composition as the consumed sulfate. The sulfur output is derived by an equal uptake of sulfate with the same sulfur isotope composition. The time dependent composition of residual sulfate can now be calculated. For $\Delta S_{\text{cell}}$ see Appendix 1.

\[
\frac{d}{dt} \text{SO}_4^{-\text{resid}} = -sSRR
\]

\[
\frac{d}{dt} \left( \text{SO}_4^{-\text{resid}} \cdot \delta^{34} \text{S}_{\text{SO}_4^{-\text{resid}}} \right) = \text{SO}_4^{-\text{resid}} \cdot \frac{d}{dt} \delta^{34} \text{S}_{\text{SO}_4^{-\text{resid}}} + \delta^{34} \text{S}_{\text{SO}_4^{-\text{resid}}} \cdot \frac{d}{dt} \text{SO}_4^{-\text{resid}}
\]

\[
\frac{d}{dt} \left( \text{SO}_4^{-\text{resid}} \cdot \delta^{34} \text{S}_{\text{SO}_4^{-\text{resid}}} \right) = -sSRR \left( \delta^{34} \text{S}_{\text{SO}_4^{-\text{resid}}} + \Delta^{34} S_{\text{cell}} \right)
\]

\[
= \text{SO}_4^{-\text{resid}} \cdot \frac{d}{dt} \delta^{34} \text{S}_{\text{SO}_4^{-\text{resid}}} + \delta^{34} \text{S}_{\text{SO}_4^{-\text{resid}}} \cdot -sSRR = -sSRR \cdot \delta^{34} \text{S}_{\text{SO}_4^{-\text{resid}}} - sSRR \cdot \Delta^{34} S_{\text{cell}}
\]

\[
\Rightarrow \text{SO}_4^{-\text{resid}} \cdot \frac{d}{dt} \delta^{34} \text{S}_{\text{SO}_4^{-\text{resid}}} = -sSRR \cdot \Delta^{34} S_{\text{cell}}
\]

where:

$\Delta^{34} S_{\text{cell}} = \Delta^{34} S_{f^{-1}} + X_1 \cdot -\Delta^{34} S_{b^{-1}} + X_1 \cdot X_2 \cdot \Delta^{34} S_{f^{-3}} + X_1 \cdot X_2 \cdot X_3 \cdot X_4 \cdot -\Delta^{34} S_{b^{-4}}$
Appendix 3

Calculation of the total oxygen isotope fractionation by a sulfate reducing bacterium

A steady state laboratory setup scenario is not suitable to calculate this effect because oxygen is exchanged with water and no “oxygen-output” value can be determined: \( \text{H}_2\text{S} \) does not contain oxygen and the amount of ambient water is much larger than the exchanged sulfate-oxygen, therefore a oxygen isotope effect in water cannot be measured. Consequently, we calculate the time-dependent oxygen isotope evolution of residual sulfate. We assume that the amount of sulfur in the different enzymatic steps is constant. Further, we assume that the external sulfate reservoir is large compared to the cell internal sulfur reservoirs and the oxygen isotope composition of the external reservoir does change slowly. Therefore, on short timescales, the oxygen isotope composition of the internal sulfur pools is at steady state. This steady state is governed by the result of the in- and output fluxes. The time dependent oxygen isotope composition of the external reservoir can now be calculated as a function of short-time internal steady states. Further, we assume that the exchange rate and the cell-specific sulfate reduction rate are constant. In this case, the exchange rate can be expressed as:

\[
\text{ex} = \alpha \times \text{sSRR}.
\]

Figure 20 Pathway of dissimilatory sulfate reduction (modified after FRITZ et al. (1989), REES (1973))

Sulfate is transformed to sulfide by enzyme catalyzed steps within the cell of the sulfate reducing organism (dashed line = cell wall). Forward- (\( f_i \)) and backward-fluxes (\( b_i \)) connect pools of intermediate sulfur compounds (\( X_i \) equal the \( b_i:f_i \)-ratios). The cell-specific sulfate reduction rate (\( \text{sSRR} \)) equals the difference between forward- and backward-fluxes. Kinetic oxygen isotope fractionation is either low or erased by oxygen exchange with ambient water (all \( \Delta^{18}\text{O} = 0 \)). For simplicity, the sulfur pools and their isotope composition are abbreviated, e.g. external sulfate = \( A \) and isotope composition of external sulfate = \( \delta A \). The oxygen isotope composition of sulfate, which is derived from oxidation of sulfite, is a combination of the isotope composition of sulfite (\( \delta D \)) and the isotope composition of the oxygen which is needed to oxidize sulfite and abbreviated as \( \delta D' \) (see text).
• General consideration about backward-forward flux ratios and cell-specific sulfate reduction rates (sSRR):

\[ X_i = \frac{b_i}{f_i} \]

\[ sSRR = f_i - b_i \]

\[ \Rightarrow f_i = sSRR + b_i \]

\[ \Rightarrow X_i = \frac{b_i}{sSRR + b_i} \]

\[ \Rightarrow X_i \cdot sSRR + X_i \cdot b_i = b_i \]

\[ \Rightarrow b_i - b_i \cdot X_i = X_i \cdot sSRR \]

\[ \Rightarrow b_i = \frac{X_i \cdot sSRR}{1 - X_i} = \frac{X_i}{1 - X_i} \cdot sSRR \]

\[ \Rightarrow f_i = sSRR + b_i = sSRR + \frac{X_i}{1 - X_i} \cdot sSRR \]

\[ \Rightarrow f_i = sSRR \cdot \left( 1 + \frac{X_i}{1 - X_i} \right) = \frac{1 - X_i + X_i}{1 - X_i} \cdot sSRR = \frac{1}{1 - X_i} \cdot sSRR \]

• External sulfate:

\[ \frac{d}{dt} A = -sSRR = -(f_i - b_i) = -(f_i - b_i) \]

\[ \frac{d}{dt} (A \cdot \delta A) = A \cdot \frac{d}{dt} \delta A + \delta A \cdot \frac{d}{dt} A = -f_i \cdot \delta A + \delta A \cdot (\delta B - \delta A) \]

\[ \Rightarrow A \cdot \frac{d}{dt} \delta A = \delta A (\delta B - \delta A) \]
• Internal sulfate:
\[ \frac{d}{dt} B = 0 \]
\[ \frac{d}{dt} \delta B = 0 \]
\[ \frac{d}{dt} (B \cdot \delta B) = 0 = f_1 \cdot \delta A - b_1 \cdot \delta B - f_2 \cdot \delta B + b_2 \cdot \delta C \]
\[ \Rightarrow \]
\[ b_1 \cdot \delta B + f_2 \cdot \delta B = f_1 \cdot \delta A + b_2 \cdot \delta C \]
\[ \Rightarrow \]
\[ \delta B \cdot (b_1 + f_2) = f_1 \cdot \delta A + b_2 \cdot \delta C \]
\[ \Rightarrow \]
\[ \delta B = \frac{f_1 \cdot \delta A + b_2 \cdot \delta C}{b_1 + f_2} \]

• Sulfate-enzyme:
The $\delta H_2O$ equals the oxygen isotopic composition of ambient water; the $\delta ex$ equals the equilibrium fractionation factor between ambient water and sulfate-enzyme.

\[ \frac{d}{dt} C = 0 \]
\[ \frac{d}{dt} \delta C = 0 \]
\[ \frac{d}{dt} (C \cdot \delta C) = 0 = f_2 \cdot \delta B - b_2 \cdot \delta C = f_3 \cdot \delta C + b_3 \cdot \delta D' - ex \cdot \delta C + ex \cdot (\delta H_2O + \delta ex) \]
\[ \Rightarrow \]
\[ b_2 \cdot \delta C + f_3 \cdot \delta C + ex \cdot \delta C = f_2 \cdot \delta B + b_3 \cdot \delta D' + ex \cdot (\delta H_2O + \delta ex) \]
\[ \Rightarrow \]
\[ \delta C \cdot (b_2 + f_3 + ex) = f_2 \cdot \delta B + b_3 \cdot \delta D' + ex \cdot (\delta H_2O + \delta ex) \]
\[ \Rightarrow \]
\[ \delta C = \frac{f_2 \cdot \delta B + b_3 \cdot \delta D' + ex \cdot (\delta H_2O + \delta ex)}{b_2 + f_3 + ex} \]

summary:

\[ A \cdot \frac{d}{dt} \delta A = b_1 \cdot (\delta B - \delta A) \]

\[ \delta B = \frac{f_1 \cdot \delta A + b_2 \cdot \delta C}{b_1 + f_2} \]

\[ \delta C = \frac{f_2 \cdot \delta B + b_3 \cdot \delta D' + ex \cdot (\delta H_2O + \delta ex)}{b_2 + f_3 + ex} \]


and:

\[
X_i = \frac{b_i}{f_i}
\]

\[sSRR = f_i - b_i\]

\[b_i = \frac{X_i}{1 - X_i} \cdot sSRR\]

\[f_i = \frac{1}{1 - X_i} \cdot sSRR\]

\[ex = \alpha \cdot sSRR\]

• Calculation of \(\delta B\) (we remove \(\delta C\) from the equation):

\[\delta B = \frac{f_1 \cdot \delta A + b_2 \cdot \delta B + b_1 \cdot \delta D + ex \cdot (\delta H_2O + \delta ex)}{b_1 + f_2}
\]

\[= \]

\[\delta B \cdot (b_1 + f_2) \cdot (b_2 + f_2 + ex) = f_1 \cdot \delta A \cdot (b_2 + f_2 + ex) + b_2 \cdot (f_3 \cdot \delta B + b_1 \cdot \delta D + ex \cdot (\delta H_2O + \delta ex))\]

\[= \]

\[\delta B \cdot (b_1 + f_2) \cdot (b_2 + f_2 + ex) - b_1 \cdot (f_2 \cdot \delta B) = f_1 \cdot \delta A \cdot (b_2 + f_2 + ex) + b_2 \cdot (b_3 \cdot \delta D + ex \cdot (\delta H_2O + \delta ex))\]

\[= \]

\[\delta B \cdot ((b_1 + f_2) \cdot (b_2 + f_2 + ex) - b_2 \cdot f_2) = f_1 \cdot \delta A \cdot (b_2 + f_2 + ex) + b_2 \cdot (b_1 \cdot \delta D + ex \cdot (\delta H_2O + \delta ex))\]

\[= \]

\[\delta B = \frac{f_1 \cdot \delta A \cdot (b_2 + f_2 + ex) + b_2 \cdot (b_1 \cdot \delta D + ex \cdot (\delta H_2O + \delta ex))}{(b_1 + f_2) \cdot (b_2 + f_2 + ex) - b_2 \cdot f_2}\]
• Calculation of A x d/dt (δA) (we remove δB from the equation):

\[ A \cdot \frac{d}{dt} \Delta A = b_1 \cdot (\delta B - \Delta A) \]

and:

\[ \delta B = f_1 \cdot \Delta A \cdot \left( \frac{(b_1 + f_1 + ex)}{b_1 + f_1 + ex} \right) + b_2 \cdot \left( \frac{\delta D + ex \cdot (\delta H_1 O + \delta ex)}{b_1 + f_1 + ex} \right) - b_2 \cdot f_2 \]

⇒

\[ A \cdot \frac{d}{dt} \Delta A = b_1 \cdot \left( \frac{f_1 \cdot \Delta A \cdot (b_1 + f_1 + ex) + b_2 \cdot \left( \frac{\delta D + ex \cdot (\delta H_1 O + \delta ex)}{b_1 + f_1 + ex} \right) - b_2 \cdot f_2}{(b_1 + f_1) \cdot (b_1 + f_1 + ex) - b_2 \cdot f_2} \right) \]

⇒

\[ A \cdot \frac{d}{dt} \Delta A = b_1 \cdot \left( \frac{f_1 \cdot \Delta A \cdot (b_1 + f_1 + ex) + b_2 \cdot \left( \frac{\delta D + ex \cdot (\delta H_1 O + \delta ex)}{b_1 + f_1 + ex} \right) - b_2 \cdot f_2}{(b_1 + f_1) \cdot (b_1 + f_1 + ex) - b_2 \cdot f_2} \right) + b_1 \cdot \left( \frac{b_1 \cdot \left( \frac{\delta D + ex \cdot (\delta H_1 O + \delta ex)}{b_1 + f_1 + ex} \right) - b_2 \cdot f_2}{(b_1 + f_1) \cdot (b_1 + f_1 + ex) - b_2 \cdot f_2} \right) \]

⇒

\[ A \cdot \frac{d}{dt} \Delta A = b_1 \cdot \Delta A \cdot \left( \frac{f_1 \cdot \left( b_1 + f_1 + ex \right) - \left( b_1 + f_1 \right) \cdot \left( b_1 + f_1 + ex \right) - b_2 \cdot f_2}{(b_1 + f_1) \cdot (b_1 + f_1 + ex) - b_2 \cdot f_2} \right) + b_1 \cdot \left( \frac{b_1 \cdot \left( \frac{\delta D + ex \cdot (\delta H_1 O + \delta ex)}{b_1 + f_1 + ex} \right) - b_2 \cdot f_2}{(b_1 + f_1) \cdot (b_1 + f_1 + ex) - b_2 \cdot f_2} \right) \]

⇒

\[ A \cdot \frac{d}{dt} \Delta A = b_1 \cdot \Delta A \cdot \left( \frac{f_1 \cdot \left( b_1 + f_1 + ex \right) - \left( b_1 + f_1 \right) \cdot \left( b_1 + f_1 + ex \right) - b_2 \cdot f_2}{(b_1 + f_1) \cdot (b_1 + f_1 + ex) - b_2 \cdot f_2} \right) + b_1 \cdot \left( \frac{b_1 \cdot \left( \frac{\delta D + ex \cdot (\delta H_1 O + \delta ex)}{b_1 + f_1 + ex} \right) - b_2 \cdot f_2}{(b_1 + f_1) \cdot (b_1 + f_1 + ex) - b_2 \cdot f_2} \right) \]

⇒

\[ A \cdot \frac{d}{dt} \Delta A = b_1 \cdot \Delta A \cdot \left( \frac{f_1 \cdot \left( b_1 + f_1 + ex \right) - \left( b_1 + f_1 \right) \cdot \left( b_1 + f_1 + ex \right) - b_2 \cdot f_2}{(b_1 + f_1) \cdot (b_1 + f_1 + ex) - b_2 \cdot f_2} \right) + b_1 \cdot \left( \frac{b_1 \cdot \left( \frac{\delta D + ex \cdot (\delta H_1 O + \delta ex)}{b_1 + f_1 + ex} \right) - b_2 \cdot f_2}{(b_1 + f_1) \cdot (b_1 + f_1 + ex) - b_2 \cdot f_2} \right) \]
Replacement of fluxes by ratios in the first expression:

\[
A \frac{d\Delta}{dt} = s\text{SRR} \cdot \frac{X_1}{1 - X_1} \cdot \Delta \cdot \left( \frac{s\text{SRR} \cdot \left( \frac{X_2}{1 - X_2} + \frac{1}{1 - X_3} + \alpha \right) \cdot \frac{1}{1 - X_3} \cdot \left( \frac{1}{1 - X_3} + \alpha \right)}{s\text{SRR} \cdot \left( \frac{X_1}{1 - X_1} \right) \cdot s\text{SRR} \cdot \left( \frac{1}{1 - X_2} \right) + s\text{SRR} \cdot \left( \frac{1}{1 - X_3} + \alpha \right)} \right) + b_1 \left( \frac{b_2 \cdot \left( b_2 \cdot \delta D + ex \cdot (\delta H_O + \delta ex) \right)}{b_1 \cdot \left( b_2 + f_2 + ex \right) + f_2 \cdot (f_2 + ex)} \right)
\]

Calculation of the denominator:

\[
\frac{(1 - X_1) \cdot (1 - X_2) \cdot (1 - X_3)}{(1 - X_1) \cdot (1 - X_2) \cdot (1 - X_3)} \cdot \left( \frac{X_1}{1 - X_1} \cdot \frac{X_2}{1 - X_2} + \frac{1}{1 - X_3} + \alpha \right) + \frac{1}{1 - X_1} \cdot \left( \frac{1}{1 - X_3} + \alpha \right)
\]

\[
= \frac{1}{(1 - X_1) \cdot (1 - X_2) \cdot (1 - X_3)} \cdot \left( X_1 \cdot (1 - X_3) \cdot X_1 + (1 - X_3) \cdot X_1 + (1 - X_2) \cdot X_1 \cdot \alpha + (1 - X_2) \cdot (1 - X_1) \cdot X_1 \cdot \alpha + ((1 - X_3) + (1 - X_1) \cdot (1 - X_1) \cdot \alpha) \right)
\]

\[
= \frac{X_1 \cdot X_1 \cdot X_1 + (1 - X_3) \cdot X_1 \cdot X_1 + (1 - X_3) \cdot X_1 \cdot X_1 \cdot \alpha + (1 - X_2) \cdot X_1 \cdot \alpha + (1 - X_2) \cdot (1 - X_1) \cdot X_1 \cdot \alpha}{(1 - X_1) \cdot (1 - X_2) \cdot (1 - X_3)}
\]

\[
= \frac{1 - X_1 \cdot X_1 \cdot X_1 \cdot (1 - X_3) \cdot X_1 \cdot (1 - X_1) \cdot \alpha}{(1 - X_1) \cdot (1 - X_2) \cdot (1 - X_3)}
\]

\[
= \frac{1 - X_1 \cdot X_1 \cdot X_1 \cdot (1 - X_3) \cdot X_1 \cdot (1 - X_1) \cdot \alpha}{(1 - X_1) \cdot (1 - X_2) \cdot (1 - X_3)}
\]

\[
= \frac{1 - X_1 \cdot X_1 \cdot X_1 \cdot (1 - X_3) \cdot X_1 \cdot (1 - X_1) \cdot \alpha}{(1 - X_1) \cdot (1 - X_2) \cdot (1 - X_3)}
\]

\[
= \frac{1 - X_1 \cdot X_1 \cdot X_1 \cdot (1 - X_3) \cdot X_1 \cdot (1 - X_1) \cdot \alpha}{(1 - X_1) \cdot (1 - X_2) \cdot (1 - X_3)}
\]
COMBINED MEASUREMENTS OF $\delta^{13}C$ AND $\delta^{18}O$

APPENDIX 3 – CHAPTER 1

Calculation of the counter:

$$
\frac{(1-X_1) \cdot (1-X_2) \cdot (1-X_3)}{(1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4)} \left( \left( \frac{X_2}{1-X_2} + \frac{1}{1-X_3} + \alpha \right) - \frac{1}{1-X_3} \cdot \left( \frac{1}{1-X_3} + \alpha \right) \right) = \\
= \frac{1}{(1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4)} \left( \frac{(1-X_1) \cdot (1-X_2) \cdot X_4}{1-X_3} + \frac{(1-X_1) \cdot (1-X_3)^2 \cdot (1-X_4)}{1-X_3} \right) \\
= \frac{1}{(1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4)} \left( (1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4) \cdot \alpha \right) \\
= \frac{1}{(1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4)} \left( (1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4) \cdot \alpha \right) \\
= \frac{-X_1 \cdot X_2 \cdot X_3 - X_1 \cdot X_2 \cdot X_3 \cdot X_4}{(1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4)} \\
= \frac{-X_1 \cdot X_2 \cdot X_3 - X_1 \cdot X_2 \cdot X_3 \cdot X_4 \cdot \alpha}{(1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4)} \\

We introduce the denominator and the counter into the first and second expression:

$$
A \cdot \frac{d}{dt} \Delta t = \frac{sSRR \cdot X_1}{1-X_1} \cdot \Delta t \cdot \left( \frac{-X_2 \cdot X_3 - X_1 \cdot X_2 \cdot X_3 \cdot X_4}{(1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4) \cdot \alpha} \right) + b_2 \cdot \left( \frac{b_2 \cdot (b_2 \cdot \delta D + \delta ex \cdot (\delta H_2 + \delta ex))}{b_2 \cdot (b_2 + f_1 + ex) + f_2 \cdot (f_1 + ex)} \right) \\
= \frac{sSRR \cdot X_1}{1-X_1} \cdot \Delta t \cdot \left( \frac{-X_2 \cdot X_3 - X_1 \cdot X_2 \cdot X_3 \cdot X_4}{(1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4) \cdot \alpha} \right) + b_2 \cdot \left( \frac{b_2 \cdot (b_2 \cdot \delta D + \delta ex \cdot (\delta H_2 + \delta ex))}{b_2 \cdot (b_2 + f_1 + ex) + f_2 \cdot (f_1 + ex)} \right) \\
= \frac{sSRR \cdot X_1}{1-X_1} \cdot \Delta t \cdot \left( \frac{-X_2 \cdot X_3 - X_1 \cdot X_2 \cdot X_3 \cdot X_4}{(1-X_1) \cdot (1-X_2) \cdot (1-X_3) \cdot (1-X_4) \cdot \alpha} \right) + b_2 \cdot \left( \frac{b_2 \cdot (b_2 \cdot \delta D + \delta ex \cdot (\delta H_2 + \delta ex))}{b_2 \cdot (b_2 + f_1 + ex) + f_2 \cdot (f_1 + ex)} \right)
This equation can be rewritten as:

\[
SO_{\text{eq, resid}} \cdot \frac{d}{dt} \delta^{18}O_{SO_{\text{eq, resid}}} = sRR \cdot \frac{X_i}{(1 - X_i \cdot X_2 \cdot X_3 + (1 - X_i - X_3) \cdot X_2 \cdot X_1) \cdot \alpha} \cdot (1 - X_i)
\]

\[
\left\{ \begin{align*}
\delta Y \cdot \left( X_i \cdot X_2 \cdot X_3 - \alpha \cdot (X_i - X_2 \cdot X_3) \right) + \\
-\delta^{18}O_{SO_{\text{eq, resid}}} \cdot (X_i \cdot X_2 \cdot X_3 - \alpha \cdot (X_i - X_2 \cdot X_3))
\end{align*} \right\}
\]

\[
\Rightarrow SO_{\text{eq, resid}} \cdot \frac{d}{dt} \delta^{18}O_{SO_{\text{eq, resid}}} = sRR \cdot k_1 \cdot \left( \delta Y \cdot k_2 + (\delta H_{SO_{\text{eq, resid}}} + \delta ex) \cdot k_1 - \delta^{18}O_{SO_{\text{eq, resid}}} \cdot k_1 \right)
\]

with:

\[
\begin{align*}
k_1 &= \frac{1}{(1 - X_i) \cdot (1 - X_i \cdot X_2 \cdot X_3 + (1 - X_i - X_3) \cdot X_2 \cdot X_1) \cdot \alpha} \\
k_2 &= X_i \cdot X_2 \cdot X_3 \cdot X_3 \\
k_3 &= \alpha \cdot (X_i - X_2 \cdot X_3 \cdot X_3) \\
k_4 &= X_i \cdot X_2 \cdot X_3 \cdot X_3 + \alpha \cdot (X_i - X_2 \cdot X_3 \cdot X_3) + X_3 \cdot X_3 \cdot X_3
\end{align*}
\]
Appendix 4

Calculation of the total oxygen isotope fractionation by a sulfate reducing bacterium assuming that the reoxidation of sulfite can be neglected

Assumption:

$X_3 \approx 0$

From Appendix 2:

$SO_{\text{red, tot}} \cdot \frac{d}{dt} \delta^{18}O_{SO_{\text{red}}} = s_{\text{SRR}} \cdot k_i \cdot (\delta D \cdot k_2 + (\delta H_2O + \delta ex) \cdot k_i - \delta^{18}O_{SO_{\text{red}}})$

$k_i = \frac{X_i}{(1 - X_i) \cdot (1 - X_i \cdot X_2 \cdot X_4 + (1 - X_i - X_i \cdot X_2 \cdot X_4) \cdot \alpha)}$

$k_2 = X_i \cdot X_3 - X_i \cdot X_2 \cdot X_3$

$k_i = \alpha \cdot (X_3 - X_2 \cdot X_4 + X_2 \cdot X_3)$

$k_i = X_i \cdot X_2 - X_i \cdot X_2 \cdot X_3 + \alpha \cdot (X_3 - X_2 \cdot X_4 + X_2 \cdot X_3)$

Calculation:

$s_{\text{SRR}} \cdot k_i \cdot (\delta D \cdot k_2 + (\delta H_2O + \delta ex) \cdot k_i - \delta^{18}O_{SO_{\text{red}}})$

$\Rightarrow SO_{\text{red, tot}} \cdot \frac{d}{dt} \delta^{18}O_{SO_{\text{red}}} = s_{\text{SRR}} \cdot \frac{X_i}{(1 - X_i) \cdot (1 + (1 - X_i \cdot X_2) \cdot \alpha)} \cdot (\delta D \cdot 0 + (\delta H_2O + \delta ex) \cdot k_i - \delta^{18}O_{SO_{\text{red}}})$

$s_{\text{SRR}} \cdot \frac{X_i}{(1 - X_i) \cdot (1 + (1 - X_i \cdot X_2) \cdot \alpha)} \cdot \left( (\delta H_2O + \delta ex) \cdot k_i - \delta^{18}O_{SO_{\text{red}}} \right)$
This equation can be rewritten as:

\[
SO_{\text{resid}} \frac{d}{dt} \delta^{13}O_{SO_{\text{resid}}} = SRR \cdot \frac{X_i}{(1 - X_i)} \cdot \frac{\alpha \cdot (X_2 - X_i \cdot X_3)}{1 + (1 - X_i \cdot X_2) \cdot \alpha} \cdot ((\delta H_2 O + \delta e x) - \delta^{13}O_{SO_{\text{resid}}})
\]

\[
SO_{\text{resid}} \frac{d}{dt} \delta^{13}O_{SO_{\text{resid}}} = SRR \cdot \beta \cdot (\delta H_2 O + \delta e x) - \delta^{13}O_{SO_{\text{resid}}})
\]

with:

\[
\beta = \frac{X_i}{(1 - X_i)} \cdot \frac{\alpha \cdot (X_2 - X_i \cdot X_3)}{1 + (1 - X_i \cdot X_2) \cdot \alpha} = \frac{X_i}{(1 - X_i)} \cdot \frac{\alpha \cdot (X_2 - X_i \cdot X_3)}{1 + \alpha - \alpha \cdot X_i \cdot X_2}
\]

Calculation of \( \alpha \):

\[
\beta = \frac{X_i}{(1 - X_i)} \cdot \frac{\alpha \cdot (X_2 - X_i \cdot X_3)}{1 + \alpha - \alpha \cdot X_i \cdot X_2}
\]

\[
\Rightarrow \frac{(1 - X_i)} \beta = \frac{\alpha \cdot (X_2 - X_i \cdot X_3)}{1 + \alpha - \alpha \cdot X_i \cdot X_2}
\]

\[
\Rightarrow (1 + \alpha \cdot (1 - X_i \cdot X_3)) \cdot \frac{(1 - X_i)}{X_i} \beta = \alpha \cdot (X_2 - X_i \cdot X_3)
\]

\[
\Rightarrow (1 - X_i) \cdot \beta \cdot (1 - X_i \cdot X_3) \cdot \frac{(1 - X_i)}{X_i} \beta = \alpha \cdot (X_2 - X_i \cdot X_3)
\]

\[
\Rightarrow \frac{(1 - X_i)}{X_i} \beta = \alpha \cdot (X_2 - X_i \cdot X_3) \cdot (1 - X_i \cdot X_3) \cdot \frac{(1 - X_i)}{X_i} \beta
\]

\[
\Rightarrow \frac{(1 - X_i)}{X_i} \beta = \alpha \cdot \left( X_2 - X_i \cdot X_3 - (1 - X_i \cdot X_3) \cdot \frac{(1 - X_i)}{X_i} \beta \right)
\]

\[
\Rightarrow \alpha = \frac{(1 - X_i)}{X_i \cdot \left( X_2 - X_i \cdot X_3 - (1 - X_i \cdot X_3) \cdot \frac{(1 - X_i)}{X_i} \beta \right) \beta}
\]

\[
\alpha = \frac{(1 - X_i)}{(X_i \cdot X_2 - X_i \cdot X_3 - (1 - X_i \cdot X_3) \cdot (1 - X_i) \beta) \beta}
\]

\[
\alpha = \frac{(1 - X_i) \beta}{(X_i \cdot X_2 - (1 - X_i \cdot X_3) \cdot (1 - X_i) \beta)}
\]

\[
\alpha = \frac{\beta}{(X_i \cdot X_2 - (\beta - X_i \cdot X_3) \cdot (1 + \beta ^{-1}) - 1}
\]

\[
\alpha = \frac{1}{X_i \cdot X_2 \cdot (1 + \beta ^{-1}) - 1}
\]
Appendix 5
Integration of the differential equation for the oxygen- and sulfur isotope composition of residual sulfate

We simplify the equations for the general and the special case in the same manner:

\[-\text{cell}_p \cdot \text{sRR} \ \frac{\text{d} \delta^{18} \text{O}_{\text{SO}_4}}{\text{d}t} = \frac{\text{d} \delta^{34} \text{S}_{\text{SO}_4}}{\text{d}t} = \frac{\text{d} \delta^{18} \text{O}_{\text{SO}_4}}{\text{d}t} \]

\[\Rightarrow -\frac{k_{\text{red}} \text{SO}_4}{\text{SO}_4} = \frac{\text{d} \delta^{18} \text{O}_{\text{SO}_4}}{\text{d}t} = a \cdot \left( B + \delta^{18} \text{O}_{\text{SO}_4} \cdot b \right) \frac{\text{d} \delta^{14} \text{S}_{\text{SO}_4}}{\text{d}t} \]

where:
\[a = k_1\]
\[B = -\delta^D \cdot k_2 - \left( \delta^H \text{O} + \delta^\text{ex} \right) \cdot k_3\]
\[b = k_4\]

for \(X_3=0\):

\[-\text{cell}_p \cdot \text{sRR} \ \frac{\text{d} \delta^{18} \text{O}_{\text{SO}_4}}{\text{d}t} = \left( \beta \left( -\delta^H \text{O} + \delta^\text{ex} \right) + \delta^{18} \text{O}_{\text{SO}_4} \right) \frac{\text{d} \delta^{14} \text{S}_{\text{SO}_4}}{\text{d}t} \]

\[\Rightarrow -\frac{k_{\text{red}} \text{SO}_4}{\text{SO}_4} = \frac{\text{d} \delta^{18} \text{O}_{\text{SO}_4}}{\text{d}t} = \frac{\text{d} \delta^{14} \text{S}_{\text{SO}_4}}{\text{d}t} \]

where:
\[a = \beta\]
\[B = \left( -\delta^H \text{O} + \delta^\text{ex} \right)\]
\[b = 1\]

Now, both equations have the same form:

\[\frac{\text{d} \delta^{18} \text{O}_{\text{SO}_4}}{\text{d}t} = \frac{\text{d} \delta^{14} \text{S}_{\text{SO}_4}}{\text{d}t} \]

\[\Rightarrow \]

\[\frac{\text{d} \delta^{18} \text{O}_{\text{SO}_4}}{B + \delta^{18} \text{O}_{\text{SO}_4} \cdot b} = \frac{\text{d} \delta^{14} \text{S}_{\text{SO}_4}}{\text{d}t} \]
The left side of the equation is the derivative after time of a logarithmic function:

\[
\frac{d}{dt}\left[\ln(B + \delta^{34}SSO_4 \cdot b)\right] = \frac{d}{dt}\left[\frac{B + \delta^{34}SSO_4 \cdot b}{b}\right] = \frac{d}{dt}\left[B + \delta^{34}SSO_4 \cdot b\right] = \frac{d}{dt}\left[B + \delta^{34}SSO_4 \cdot b\right] = \frac{d}{dt}\left[B + \delta^{34}SSO_4 \cdot b\right]
\]

Therefore, the equation can be transformed into:

\[
\frac{d}{dt}\delta^{34}SSO_4 = \frac{d}{dt}\ln\left[B + \delta^{34}SSO_4 \cdot b\right] \cdot b = \frac{d}{dt}\delta^{34}SSO_4 \cdot \frac{a \cdot b}{\Delta^34SSO_4}
\]

Both sides of the equation can be easily integrated:

\[
\ln\left[B + \delta^{34}SSO_4 \cdot b\right] = \frac{d}{dt}\delta^{34}SSO_4 = \frac{a \cdot b}{\Delta^34SSO_4} + \text{Const}_1
\]

For the two constants, we can choose a boundary condition, e.g. for the starting point of the sulfur isotope measurements, \(t=0\). Both sides of the equations need to have the same value, which is achieved by setting both expressions equal to zero.

\[
\ln\left[B + \delta^{34}SSO_4 (0) \cdot b\right] + \text{Const}_1 = 0
\]

\[
\Rightarrow \text{Const}_1 = -\ln\left[B + \delta^{34}SSO_4 (0) \cdot b\right]
\]

and:

\[
\delta^{34}SSO_4 (0) \cdot \frac{a \cdot b}{\Delta^34SSO_4} + \text{Const}_2 = 0
\]

\[
\Rightarrow \text{Const}_2 = -\delta^{34}SSO_4 (0) \cdot \frac{a \cdot b}{\Delta^34SSO_4}
\]

The equation is now rewritten as:

\[
\ln\left[B + \delta^{34}SSO_4 (0) \cdot b\right] - \ln\left[B + \delta^{34}SSO_4 (0) \cdot b\right] = \delta^{34}SSO_4 (0) \cdot \frac{a \cdot b}{\Delta^34SSO_4} - \delta^{34}SSO_4 (0) \cdot \frac{a \cdot b}{\Delta^34SSO_4}
\]

\[
\Rightarrow \ln\frac{B + \delta^{34}SSO_4 (0) \cdot b}{B + \delta^{34}SSO_4 (0) \cdot b} = \frac{\delta^{34}SSO_4 (0) \cdot \delta^{34}SSO_4 (0) \cdot \frac{a \cdot b}{\Delta^34SSO_4}}{\Delta^34SSO_4}
\]

\[
\Rightarrow \ln\frac{B + \delta^{34}SSO_4 (0) \cdot b}{B + \delta^{34}SSO_4 (0) \cdot b} = \frac{\delta^{34}SSO_4 (0) \cdot \delta^{34}SSO_4 (0) \cdot \frac{a \cdot b}{\Delta^34SSO_4}}{\Delta^34SSO_4}
\]
Now, the values for a, A and b can be resubstituted:

General case:
\[ a = k_1 \]
\[ B = -\Delta D' k_2 - (\Delta H_2 O + \Delta ex) k_3 \]
\[ b = k_4 \]
\[ \Rightarrow \ln \frac{-\Delta D' k_2 - (\Delta H_2 O + \Delta ex) k_3 + \delta^{18}O_{SO_4} (t) k_4}{-\Delta D' k_2 - (\Delta H_2 O + \Delta ex) k_3} = k_1 k_4 \frac{\delta^{14}S_{SO_4} (t) - \delta^{14}S_{SO_4} (0)}{\Delta^{34}S_{cell}} \]

for \( X_3 = 0 \):
\[ a = \beta \]
\[ B = -\Delta H_2 O + \Delta ex \]
\[ b = 1 \]
\[ \Rightarrow \ln \frac{-\Delta H_2 O + \Delta ex + \delta^{18}O_{SO_4} (t)}{-\Delta H_2 O + \delta^{18}O_{SO_4} (0)} = \beta \frac{\delta^{14}S_{SO_4} (t) - \delta^{14}S_{SO_4} (0)}{\Delta^{34}S_{cell}} \]
SULFUR ISOTOPES FROM STRUCTURAL SUBSTITUTED SULFATE IN BULK CARBONATES: IMPROVED METHOD AND APPLICATION TO THE K-T BOUNDARY

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Abstract

The reconstruction of the sulfur isotopic composition of seawater through time is based on measurements of sulfur-containing minerals in sediments, mainly evaporites, pyrite and marine barite. A further method, the extraction of structural substituted sulfate (SSS) from calcites has successfully been applied to fossil shells, e.g. belemnites and brachiopod shells, and also on bulk carbonates. The use of this method on bulk rock samples (limestones and marls) has many uncertainties. Here, we present an improved extraction treatment focusing on the removal of sulfides contaminating SSS. Additionally, we show that the measurement of the oxygen isotopic composition of the extracted sulfate is crucial to identify SSS-sulfur isotope being altered by early diagenetic processes within interstitial waters. Further, we stress that SSS-sulfur isotope data of bulk samples can be shifted to more positive values during lithification and recrystallization of carbonates by an unidentified process. The improved method is applied to the reconstruction of the sulfur isotopic composition of seawater around the K-T boundary. Our results confirm that SSS extracted from bulk rock samples can be used to reconstruct sulfur isotope trends of seawater, but it does not provide absolute values.

Introduction

The biogeochemical cycles of carbon and sulfur are closely linked to the oxygen cycle and balance the amount of oxygen in the atmosphere (BERNER and PETSC, 1998). The recon-
struction of the sulfur cycle is a prerequisite to unravel the history of atmospheric oxygen levels. Sulfate reducing bacteria produce sulfides enriched in $^{32}$S leaving the residual sulfate enriched in $^{34}$S ($\Delta^{34}S$ up to $-70\%$; CANFIELD (2001); CANFIELD ET AL. (1998); WORTMANN ET AL. (2001)). Consequently, reduced sulfur reservoirs (sulfides) and oxidized reservoirs (sulfates) have different isotopic compositions, and sulfur isotopes can be used to reconstruct the history of the balance between the weathering- and burial-fluxes of reduced and oxidized sulfur reservoirs. Due to the large amount of sulfate in seawater ($40 \times 10^{18}$ mol sulfate; HOLSER et al. (1988)), the residence time of sulfate in the ocean is around 20-40 Ma (depending on the estimates of in- and output fluxes, e.g. $0.89 \times 10^{12}$ to $1.53 \times 10^{12}$ mol $SO_4$ per year (HOLSER et al., 1988; PAYTAN and ARRIGO, 2000). As a consequence, isotopic changes in the composition of seawater-sulfate through geologic time take place only gradually. Different sulfur-containing minerals in sediments, such as evaporites (CLAYPOOL et al., 1980), pyrites (STRAUSS, 1997) and marine barite (PAYTAN et al., 1998), have been used to reconstruct the sulfur isotope curve of seawater sulfate over Earth’s history. As carriers of the seawater sulfate signal, all of these minerals have specific advantages and disadvantages (for a review, see STRAUSS (1997)). A supplementary approach for the reconstruction of seawater $\delta^{34}S$ through time utilizes structurally substituted sulfate (SSS) in marine carbonates (e.g. BURDETT et al. (1989); HURTGEN et al. (2002); KAIHO et al. (2001); KAIJWARA et al. (1997); KAMPSCHULTE et al. (2001); OHKOUCHI et al. (1999); STRAUSS et al. (2001)). KAMPSCHULTE et al. (2001) showed that $\delta^{34}S_{SSS}$ from shells of modern biota reflect seawater $\delta^{34}S$ reasonably well. $\delta^{34}S_{SSS}$ from fossil shells, such as foraminifera (BURDETT et al., 1989), belemnites and brachiopods (KAMPSCHULTE et al., 2001; STRAUSS, 1999), has been successfully applied for the reconstruction of ancient seawater $\delta^{34}S$. In contrast to shells, SSS in bulk rock is prone to alteration, either during diagenesis and lithification or during sample preparation. Consequently, the use of $\delta^{34}S_{SSS}$ from bulk carbonates is hampered by many uncertainties. Despite these uncertainties, $\delta^{34}S_{SSS}$ from bulk carbonates is a valuable alternative to other methods because carbonates are abundant and usually easier to place in a stratigraphic framework. These properties permit investigating the sulfur isotope curve in a high temporal resolution. For example, $\delta^{34}S_{SSS}$ can be used to investigate the reaction of the sulfur system to global perturbations, such as at time boundaries (e.g. Permian-Triassic boundary (KAIHO et al., 2001) and Cretaceous-Tertiary boundary (KAIHO et al., 1999)) or during oceanic anoxic events (Cenomanian-Turonian boundary, “Livello Bonarelli” (OHKOUCHI et al., 1999)). In time slices of Earth’s history where other carriers of $\delta^{34}S$-signals of seawater sulfate are missing (e.g. the Neopro-
terozoic (Hurtgen et al., 2002)), SSS is a valuable source of sulfur isotope data. An evaluation and improvement of the use of SSS from bulk carbonates for sulfur isotope determinations can help to refine the sulfur isotope age curve of seawater sulfate and allows for the study of the sulfur cycle at a high resolution.

First, we review the processes that can alter primary $\delta^{34}S_{\text{SSS (bulk)}}$ and $\delta^{18}O_{\text{SSS (bulk)}}$ during diagenesis and lithification and discuss an improvement of the SSS leaching technique. We then present a case study, the Cretaceous-Tertiary boundary, and propose oxygen-isotope measurements on sulfate as a tool to improve data quality.

**Processes influencing $\delta^{34}S_{\text{SSS}}$ and $\delta^{18}O_{\text{SSS}}$ of bulk carbonate samples during diagenesis and lithification**

A bulk carbonate rock mainly consists of carbonate-particles and -precipitates derived by diagenesis and lithification (Figure 1). For simplicity, the latter are designated “cements”. Primary carbonate particles, if not recrystallized, have $\delta^{34}S_{\text{SSS}}$ and $\delta^{18}O_{\text{SSS}}$ reflecting the sulfur and oxygen isotope composition of seawater sulfate, while $\delta^{34}S_{\text{SSS}}$ and $\delta^{18}O_{\text{SSS}}$ from cements reflect the sulfur isotope composition of the interstitial water during their precipitation. Under natural conditions, sulfate does not exchange oxygen with seawater (Chiba and Sakai, 1985; Holser et al., 1979; Loyd, 1968). The influence of the cement-$\delta^{34}S_{\text{SSS}}$ and -$\delta^{18}O_{\text{SSS}}$ on the $\delta^{34}S_{\text{SSS (bulk)}}$ and $\delta^{18}O_{\text{SSS (bulk)}}$ depends on the relative amount of cement-SSS present and its isotopic composition.

![Figure 1](image.png)  
**Figure 1**  Incorporation of diagenetic $\delta^{34}S$- and $\delta^{18}O$-signals into bulk carbonate rocks
Seawater-sulfate (open circles) is incorporated into carbonate particles within the water column or at the sediment-water interface, while sulfate (filled circles) from interstitial water is incorporated into cements. Sulfate reduction, sulfide reoxidation or advection of brines with different composition influence the oxygen and sulfur isotope composition of pore-water sulfate.

The sulfur and oxygen isotope composition of sulfate in interstitial waters depends on its initial composition (usually close to the $\delta^{34}$S and $\delta^{18}$O of seawater) and on the three following major processes:

- **Microbial sulfate reduction**: Isotopically light dissolved sulfide is precipitated as iron sulfide or lost by diffusion. The remaining sulfate is enriched in heavy sulfur isotopes. Microbial sulfate reduction also changes the oxygen isotope composition of sulfate. Obviously, sulfate entering the cells of sulfate reducers is not entirely consumed and can be released back into seawater. Within the cell, sulfate is transformed into chemical compounds which exchange oxygen with water with a temperature-dependent isotope fractionation (25‰ at 30°C and 29‰ at 5°C; Fritz et al. (1989)). Consequently, when sulfate reducers are active, the oxygen isotope composition of sulfate in a closed system at 5° and with an oxygen isotope composition of seawater around 0‰ will approach a value of +29‰. This enrichment trend is confirmed by $\delta^{18}$O data from interstitial waters from ODP drill sites in the Mediterranean (Böttcher et al., 1999; Böttcher et al., 1998).

- **During late diagenesis and lithification**: dissolved and solid sulfides (pyrite) can be reoxidized within the sediment, leading to pore water sulfate with a light sulfur isotope composition. The oxygen isotope composition of the resulting sulfate is close to the oxygen isotope composition of the interstitial water (Claypool et al., 1980; Ku et al., 1999; van Stempvoort and Krouse, 1994).

- **Brines with different sulfur contents and isotopic compositions**: can be advected through the sediment column leading to changes in the concentration and isotopic composition of sulfate in interstitial waters (e.g. Mediterranean Sea, Bernasconi (1999); Great Australian Bight, Wortmann et al. (2001)).

The sulfate content of carbonate sediments progressively decreases during diagenesis and lithification (Staudt and Schoonen, 1995). It is unknown if this loss of sulfate has an influence on the sulfur and oxygen isotope composition of the remaining SSS. A diffusion-process would probably remove isotopic light sulfate (see below).
Experimental and leaching technique

Leaching techniques for the extraction of SSS from bulk rock try to avoid contamination of SSS by sulfate derived from other rock-sulfur-species, such as sulfides (mainly pyrite), evaporites, barite, elemental sulfur and sulfur bound in organic matter. Previous studies (e.g. Duan et al., 1997; Hall et al., 1988; Kajiwara et al., 1997; Rice et al., 1993; Westgate and Anderson, 1982) propose sequential leaching steps to remove contaminating sulfur-phases from a ground sample prior to a dissolution-step of the carbonates, where SSS is liberated and afterwards extracted. Usually, the following leaching steps are applied: evaporites are dissolved with distilled water, organic matter and sulfides are destroyed and removed with oxidizers and elemental sulfur, if abundant, is leached with organic solvents. Barite is not affected by the dissolution of carbonates with acids and, therefore, does not contaminate SSS. The dissolution of carbonates is carried out under a nitrogen atmosphere, preventing remaining sulfides from being oxidized to sulfate. Nevertheless, this reaction is not completely inhibited. During the dissolution of carbonates, iron oxides are liberated and provide oxygen, which reacts with sulfides to produce sulfate. Dissolved oxygen in the leaching acid could be a further cause of oxidation. Therefore, the most critical part in the leaching process is the elimination of sulfides (pyrite). As they are abundant in rocks and due to their light isotopic composition, only a minor amount of contamination can strongly affect $\delta^{34}$S_{SSS}.

To test the efficiency of the oxidation of sulfides, we carried out the following experiments:

In a first experiment, we oxidized pyrite of different grain sizes with two oxidizers, NaOCl (sodium hypochlorite “bleach”) and H$_2$O$_2$ (hydrogen peroxide). Both reactions depend strongly on the pyrite grain size. With grain sizes < 63 μ and 1 g of sample, the reaction with hydrogen peroxide lasted less than 2 hours, while the reaction with NaOCl after 12 hours was still not complete. In a second experiment, we performed the same treatment with a powdered (< 63 μ) mixture of pyrite and calcite. Within 12 hours, both reactions did not completely oxidize pyrite. After placing the reaction tubes into an ultrasonic bath, oxidation with hydrogen peroxide reached completeness within 2 hours, while the reaction performed with NaOCl did not reach completeness. The presence of carbonates obviously inhibits the oxidation of pyrite, most likely by coating its surface with calcium sulfate. This coating is removed by using an ultrasonic bath. A complete oxidation of sulfides requires:

- Grain sizes smaller than 63 μ
- A strong oxidizing agent (e.g. hydrogen peroxide)
• A repeated use of ultrasonic bath
Under the precondition of a complete removal of sulfide prior to the dissolution of carbonate with acid, the use of a nitrogen atmosphere during the latter is not required and serves only as additional precaution.

Based on these findings, we use the following treatment-scheme for the extraction of SSS from bulk carbonate samples (Figure 2):

<table>
<thead>
<tr>
<th>Treatment-scheme for the extraction of SSS from bulk carbonate samples</th>
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<tbody>
<tr>
<td><strong>Preparation:</strong></td>
</tr>
<tr>
<td>Crushing and grinding of the sample (20-60g): &lt;63µm</td>
</tr>
<tr>
<td><strong>Washing:</strong></td>
</tr>
<tr>
<td>Distilled water &amp; Ultrasonic bath (&gt;30 Min)</td>
</tr>
<tr>
<td><strong>Oxidation:</strong></td>
</tr>
<tr>
<td>The sample is treated with hydrogen peroxide (H₂O₂) for at least 3 days, then follows again a washing step with distilled water and ultrasonic bath. The oxidation step is 3 times repeated.</td>
</tr>
<tr>
<td><strong>Dissolution of carbonate:</strong></td>
</tr>
<tr>
<td>The sample is reacted with concentrated hydrochloric acid (HCl) under a nitrogen atmosphere (removes acid soluble sulfides if present). The residual is centrifuged off.</td>
</tr>
<tr>
<td><strong>Residual:</strong></td>
</tr>
<tr>
<td>The residual might contain barite. This can be checked by XRD analysis.</td>
</tr>
</tbody>
</table>

**Figure 2**  Treatment-scheme for the extraction of SSS from bulk carbonate samples
Washing step: After settling of the carbonate particles from suspension, the overstanding water is decanted. This step is repeated three times.

**Measurement on mass spectrometer**

For stable sulfur isotope analysis, 400 to 500 µg of BaSO₄ were weighed into tin cups. To enhance combustion efficiency, vanadium pentoxide with about twice the weight of the sample was added. The samples were analyzed on a FISONS OPTIMA mass spectrometer (Fisons, Middlewich, Chesire, UK) connected by continuous flow to a Carlo Erba elemental analyzer.
(CEW Instruments, Milan, Italy). The sulfur isotope data are reported in the conventional δ-notation relative to the Vienna-Canyon Diablo Troilite (V-CDT) standard according to:

\[ \delta^{34}S (\text{‰}) = \left\{ \frac{^{34}S/^{32}S_{\text{sample}}}{^{34}S/^{32}S_{\text{V-CDT}}} - 1 \right\} \times 1000 \]

The system was calibrated using the international standards IAEA-S1 (\(\delta^{34}S = -0.3\text{‰}\)) and IAEA-S2 (\(\delta^{34}S = 21.7\text{‰}\)) (GONFIANTINI et al., 1995). The mean \(\delta^{34}S\) value obtained for the international standard NBS127 was 20.4‰. Analytical reproducibility of the measurements was ±0.25‰.

For stable oxygen isotope analysis, we modified the on-line pyrolysis method of WERNER et al. (1996). We use an automated elemental analyzer (Carlo Erba elemental analyzer, CE-Instruments, Milan, Italy). 430 to 460 µg of BaSO\(_4\) were weighed into tin cups. In order to improve pyrolysis efficiency and provide an additional carbon source, a small amount of nickelized carbon was added to each sample. Pyrolysis was carried out at 1080°C in a modified reaction tube filled with 1.5 cm\(^3\) glassy carbon. The smaller amount of glassy carbon compared to the Werner et al. (1996) method was found to strongly decrease memory effects. The pyrolysis products in the He stream were passed through a water trap and a 1-m GC column packed with 5 Å zeolite to separate carbon monoxide from N\(_2\), which would produce an isobaric interference at Masses 28 and 29 and 30. The oxygen isotope composition was subsequently measured on a FIONS OPTIMA mass spectrometer (Fisons, Middlewich, Chesire, UK). The oxygen isotope data are reported in the conventional δ-notation relative to the Standard Mean Ocean Water (SMOW) standard according to:

\[ \delta^{18}O (\text{‰}) = \left\{ \frac{^{18}O/^{16}O_{\text{sample}}}{^{18}O/^{16}O_{\text{V-SMOW}}} - 1 \right\} \times 1000 \]

In the absence of a set of internationally accepted reference sulfate standards, the method was calibrated using a set of different substances, including carbonates and one sulfate sample (NBS127), and checked with nitrates of known composition (Table 1, Figure 3).

<table>
<thead>
<tr>
<th></th>
<th>Measured (\delta^{18}O)</th>
<th>Standard (\delta^{18}O) (V-SMOW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS2, Carbonate</td>
<td>8.8</td>
<td>29.0</td>
</tr>
<tr>
<td>NBS18, Carbonate</td>
<td>–8.0</td>
<td>7.1</td>
</tr>
<tr>
<td>LSVEC, Carbonate (LiCarb)</td>
<td>–13.3</td>
<td>3.54</td>
</tr>
<tr>
<td>NBS127, BaSO(_4)</td>
<td>–8.0</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Table 1 Measured vs. published oxygen isotope values
The data were corrected relative to a linear regression line ($\delta^{18}\text{O}_{\text{corrected}} = \delta^{18}\text{O}_{\text{measured}} \times 1.1831 + 18.3$). To estimate the accuracy of the barium sulfate measurement, NBS127-data were also corrected using a regression line without taking into account the NBS 127-standard ($\delta^{18}\text{O}_{\text{corrected}} = \delta^{18}\text{O}_{\text{measured}} \times 1.1894 + 18.2$).

Using the calibration including the BaSO$_4$-standard (NBS 127), the corrected average value of barium sulfate is 0.3‰ lighter than the published reference value. Calibrating only with the carbonate standards, the corrected average value of barium sulfate is 0.6‰ lighter than the published reference value (see Table 2) and, in a second run, 0.7‰ lighter than the published reference value. We can use these values to estimate that the accuracy of barium sulfate measurements is ±0.7‰. The real accuracy of the data is better because of the use of the NBS127-standard for calibration (e.g. ±0.3‰). The analytical reproducibility of the measurements was ±1‰.

<table>
<thead>
<tr>
<th></th>
<th>Measured $\delta^{18}\text{O}$</th>
<th>Standard $\delta^{18}\text{O}$ (V-SMOW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS2, Carbonate</td>
<td>29.1</td>
<td>29.0</td>
</tr>
<tr>
<td>NBS18, Carbonate</td>
<td>8.42</td>
<td>7.1</td>
</tr>
<tr>
<td>LiCarb, Carbonate</td>
<td>2.27</td>
<td>3.54</td>
</tr>
<tr>
<td>NBS127, BaSO4</td>
<td>8.7</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Table 2 Corrected vs. published oxygen isotope values

To estimate the accuracy of the barium sulfate measurement, NBS127-data were corrected using a regression line without taking into account the NBS 127-standard ($\delta^{18}\text{O}_{\text{corrected}} = \delta^{18}\text{O}_{\text{measured}} \times 1.1894 + 18.2$).
Due to the large seawater sulfate reservoir, rapid shifts (< $10^5$ years) in its sulfur and oxygen isotope composition are difficult to interpret. Only enormous changes in sulfur-fluxes coupled with large isotope fractionations can lead to fast changes in the sulfur isotope composition of seawater. Thus, rapid shifts in the $\delta^{34}$S$_{\text{seawater}}$ imply that catastrophic perturbations of Earth’s geochemical cycles must have occurred. Such a catastrophe has been proposed by KAIHO et al. (2001) to explain a negative shift in the sulfur isotope composition of SSS$_{\text{bulk}}$ from 20‰ to 5‰ at the Permian-Triassic boundary. They propose that the impact of an asteroid or comet could have caused a rapid release of sulfur from the mantle with a $\delta^{34}$S of –10‰ to 0‰ to the ocean-atmosphere system.

At the Cretaceous-Tertiary (K-T) boundary, there is evidence for an extraterrestrial impact. In K-T boundary samples from the Caravaca-section (Spain), KAJIWARA et al. (1997) and KAIHO et al. (1999) report a rapid shift of about +5‰ in the sulfur isotope composition of SSS$_{\text{bulk}}$ within a few thousand years (Figure 4).

![Figure 4](image)

**Figure 4** SSS-sulfur isotope data from the Cretaceous-Tertiary boundary from the Caravaca-section in Spain (KAIHO et al., 1999; KAJIWARA et al., 1997)

Note the rapid shift in the sulfur isotopic composition around the K-T boundary of about +5‰. (Shaded area = Cretaceous, blank area = Tertiary).

It has to be questioned if this shift represents a global or local seawater-signal or if it was derived by diagenetic alteration of the primary signal after sediment deposition. The K-T boundary is an ideal time slice to analyze $\delta^{34}$S$_{\text{bulk}}$ from different sections and trace $\delta^{34}$S across...
the time of the reported change. We proposed to reconfirm the presence of the sulfur isotope shift, and to investigate if $\delta^{34}S_{\text{bulk}}$ data for the reconstruction of the primary seawater sulfate isotope composition are reliable. We investigated three additional boundary-sections, one from Tunisia (Elles), one from Italy (Contessa-section) and one from Kazachstan.

**Samples**

The samples from Elles were mainly greenish marly limestones (about 60-80% carbonate content), the samples from the Contessa-sections were reddish limestones (> 85% carbonate content) strongly recrystallized and the samples from Kazachstan are white limestones (> 90% carbonate content).

**Results**

![SSS-sulfur and oxygen isotope data from three Cretaceous-Tertiary boundary sections: Elles (Tunisia), Contessa (Italy) and Kazachstan](image)

The data are plotted relative to the distance above and below the K-T boundary level. Note the different axis scales. (Shaded area = Cretaceous, blank area = Tertiary).

The sulfur isotope values from the Elles-section (Figure 5) start with values between 19.2‰ and 20.2‰ and decrease to a single data-point with a value of 17‰ just below the K-T boundary and increase again to single data-point of 20.7‰, which lies 3 meters above the K-T boundary. Between 10 and 15 meters above the K-T boundary, sulfur isotope values increase from around 26‰ to more than 31‰. A subsequent decrease to 13‰ is followed by an increase to 20‰. The oxygen isotope values are relatively constant between 11‰ and 13.5‰.

The sulfur isotope values for the Contessa-section (Figure 5) show an increase from 23.5‰ to 24‰ below the K-T boundary followed by a decrease to 22‰ above the K-T boundary.
Correspondingly, the oxygen isotope values decrease from about 18.5‰ to 17.5‰ across the K-T boundary.

The sulfur isotope values for the samples from Kazachstan (Figure 5) are approximately 20.1‰ below the K-T boundary and scatter around 20‰ above the boundary. The oxygen isotope values increase from 11.8‰ to 14.1‰ just below the boundary and then to 15.2‰ above the boundary.

**Discussion and Interpretation**

As a reference for our values, we can estimate the sulfur and oxygen isotope composition of seawater from long-term sulfur isotope trends derived from isotope analysis of evaporites (CLAYPOOL et al., 1980) The sulfur isotope composition of seawater sulfate at the K-T boundary would be 18‰ ±2‰ and the oxygen isotope composition 12‰ ±2‰. More precise sulfur isotope data from marine barite point to a value of 19‰ ±0.5 for the early Paleocene (PAYTAN et al., 1998). The isotope values from the three different studied sections do not show a common trend, neither for sulfur nor for oxygen isotopes. Obviously in most of the samples, the primary seawater-isotope signal has been altered or destroyed.

The greenish marls of the Elles-section contain pyrite probably causing the rapid drop in the sulfur isotope composition just below the K-T boundary, as well as the drop on the level at 19 meters above of the boundary. The strongly positive values of 26‰ and 31‰ either indicate a rapid rise of sulfur isotope values in seawater-sulfate to extremely high values or, much more likely, that sulfate enriched in $^{34}$S by microbial sulfate reduction was incorporated into the carbonates during diagenesis. The latter is supported by the slightly enriched oxygen-isotope value of 13.5‰. Primary signals, if any, from the Elles-section point to values around 20‰ below and above the K-T boundary, which are slightly more positive than the reference value of 18‰ to 19‰.

The sulfur isotope values from the strongly recrystallized marly limestones of the Contessa-section show a much more defined trend with increasing values from 23.5‰ to 24‰ below and decreasing to 22‰ above the K-T boundary. The shift to lower values is paralleled by a drop in the oxygen isotope values. If these sulfur isotope compositions were primary, they would be more than 2‰ greater than the primary signal in the Elles-section, and oxygen isotope values are more than 5‰ greater. We speculate that the whole section was altered by sulfate enriched in $^{34}$S during diagenesis and recrystallization of the rocks.
The sulfur isotope values from the light-colored, pure carbonate samples from Kazakhstan are close to a value of 20.1‰ below the K-T boundary and, above the boundary they scatter around 20‰. These values are in the same range as the primary data from the Elles-section. The increase in the oxygen isotope composition from 11.8‰ to 14.1‰ could point to a possible incorporation of sulfate enriched in $^{34}$S by sulfate during diagenesis. Our data set demonstrates that the diagenetic overprint of SSS can be very important, creating both shifts to higher and lower sulfur and oxygen isotope values. In addition, our data show that a combined analysis of sulfur- and oxygen-isotopic composition of SSS is needed to identify artifacts.

The oxygen isotopic composition of seawater sulfate over Phanerozoic time varies in a relatively narrow range of 8‰ to 15‰ (Claypool et al., 1980). This balanced composition is the result of two competing processes:

- Sulfate reducing bacteria enable oxygen exchange between sulfate and seawater (Fritz et al., 1989; Mizutani and Rafter, 1973). Oxygen isotope fractionation causes sulfate to be enriched compared to seawater by 29‰ at 5°C and 27‰ at 30°C (Fritz et al., 1989). In general, during sulfate reduction the remaining sulfate pool becomes enriched in $^{18}$O.

- Oxidation of sulfides produces sulfate with $\delta^{18}$O close to ambient water (Claypool et al., 1980; Ku et al., 1999; Van Stempvoort and Krouse, 1994).

Because 90% of sulfide produced by microbial sulfate reduction is reoxidized (Jørgensen, 1982), this balance is very effective in surface sediments. In anoxic interstitial waters, however, where sulfides produced by microbial sulfate reduction are not reoxidized but precipitated as iron sulfides or removed by diffusion processes, the oxygen isotopic composition of sulfate is not balanced and therefore enriched in $^{16}$O and $^{34}$S. Thus, unusually high oxygen isotope values indicate alterations of SSS due to sulfate reduction within interstitial waters. In Figure 6, we schematically depict the expected trends in $\delta^{18}$O and $\delta^{34}$S for different diagenetic alteration processes.

The data set from the Contessa-section is an example for an enrichment due to microbial sulfate reduction in interstitial waters, that is, an increase in oxygen isotope values is found in combination with an increase in sulfur isotope values (Figure 6). The opposite process, the oxidation of sulfides within interstitial waters, adds sulfate with an oxygen isotope composition closer to the $\delta^{18}$O of ambient water. This effect is rather small compared to the effect caused in the sulfur isotope composition by the incorporation of strongly $^{34}$S-depleted sulfate derived from the sulfides. Consequently, the oxidation of sulfides cannot be as easily detected.
by combined measurements of the oxygen and sulfur isotope composition of SSS. However, the amount of strongly $^{34}$S-depleted sulfate contributed by oxidation is likely to be concentrated locally where sulfides are abundant. Therefore, the sulfur isotope imprint by oxidation is not homogeneous over a rock section. Consequently, rapid negative shifts in a sulfur-isotope data set are a characteristic sign of such a contamination. However, this pattern does not necessarily occur. In fact, the data set from Caravaca probably is, in part, the result of an alteration by the reoxidation of sulfides, either during diagenesis or sample preparation. The strong anticorrelation between sulfur isotopic composition of SSS and sulfide content in the section below the K-T boundary (Figure 7) suggests that this is a likely explanation. The remaining sulfur isotope data above the K-T boundary show no correlation with respect to sulfide content and have values close to the ones recorded from Kazachstan, indicating that these values are unaltered by sulfide oxidation.

![Figure 6](image.png)

**Figure 6** Changes in the sulfur and oxygen isotope composition of SSS$_{bulk}$ due to incorporation of SSS$_{cements}$ being altered due to microbial sulfate reduction or oxidation of sulfides within interstitial waters

The comparison of the three studied sections indicates that, in order to avoid contaminations with sulfides oxidized during diagenesis, only light colored pure carbonates (> 85% carbonate content) should be analyzed. Such samples potentially allow the recovery of a primary signal.
Other bulk samples, such as marls or marly limestones, can only serve as additional data sets because they are much more prone to diagenetic alteration and the creation of artifacts during sample processing. In addition, the analysis of the oxygen isotope composition of SSS is crucial to detect alterations by sulfate reduction within pore waters.

**Figure 7**  
Correlation between sulfide content and sulfur isotope values of SSS in the data set from Caravaca (KAIHO et al., 1999)

- Circles: Values of sulfide content and sulfur isotope composition below the K-T boundary.
- Star: Single value for sulfide content and sulfur isotope composition just below the K-T boundary.
- Squares: Values for sulfide content and sulfur isotope composition above the K-T boundary.

Below the K-T boundary, increasing amounts of sulfide in the samples correlate well with the sulfur isotope values (circles in Figure 7b). The data point (star in Figure 7b) just below of the K-T boundary does neither correlate with the data below nor the data above of the K-T boundary. The latter show no clear correlation (squares in Figure 7b).

Based on these considerations, we conclude that most of the collected sulfur isotope data around the K-T contain a questionable primary signal. The whole data set from the Contessa-section is discarded, as well as the part of the Caravaca-section below the K-T boundary. The
most reliable data are derived from the Kazakhstan section, but two of the oxygen-measurements point to a possible enrichment in the sulfur isotope composition of the reported data. Taking the data from the Elles-section and the Caravaca-section into account, we conclude that the sulfur- and oxygen isotope composition of primary SSS above and below the K-T boundary remained close to 20‰ showing no rapid changes. This value is about 2‰ higher than those inferred from evaporite-data (Claypool et al., 1980) and about 1‰ higher than sulfur isotope data from marine barite (early Paleocene, Paytan et al. 1998). Possibly, the lithification and recrystallization of the carbonate rocks, which is accompanied by a decline of the amount of SSS, can also change the sulfur- and oxygen isotope values. Data from Kampschulte et al. (2001) seem to confirm this interpretation. They compared δ34S values of SSS from Carboniferous brachiopods to contemporaneous SSS from bulk carbonate rocks and found that SSS bulk is enriched in 34S by about 0.5‰. In the same study, Kampschulte et al. (2001) determined δ34S values of SSS from modern brachiopods and found a mean value of 21.55‰, slightly enriched in 34S compared to an average value of seawater sulfate of 20.9‰. Compared to seawater sulfate, SSS bulk would be enriched in 34S by 1‰. The enrichment in 34S and 18O of SSS bulk during lithification and recrystallization of carbonate rocks might be caused by loss of isotopically light sulfate due to diffusion. Theoretically, the diffusion coefficient of 32S16O4− is larger than the diffusion coefficient of 34S18O4− and would lead to a preferential loss of lighter isotopic species. However, such a difference has not yet been observed in laboratory diffusion experiments (personal communication by Trudinger in Jørgensen (1979)). Figure 8 summarizes the processes controlling δ34S and δ18O of SSS from bulk carbonates and the consequences for the interpretation of such data sets.
Figure 8  Summary of the processes controlling $\delta^{34}S$ and $\delta^{18}O$ of SSS from bulk carbonates

A: The primary signal of seawater sulfate is incorporated into carbonate particles.

B: Cements incorporate sulfate with the sulfur and oxygen isotopic composition of sulfate from interstitial waters. The primary seawater sulfate signal is partly overprinted by “early diagenetic artifacts”: Reoxidation of sulfides lead to negative shifts in the sulfur isotope composition of SSSbulk while the oxygen isotope composition does not change dramatically. Sulfate reduction within the pore water leads to positive oxygen and sulfur isotope excursions.

C: During lithification and recrystallization of the carbonates, sulfate depleted in $^{18}O$ and $^{34}S$ is preferentially lost. The whole isotope curves are shifted to more positive values (+1‰ to +2‰ for $\delta^{34}S$).

D: The abundance of carbonates allows to sample sections at high resolution providing detailed $\delta^{18}O$ and $\delta^{34}S$ records.

E: Interpretation of the data:
Data points with unusual high $\delta^{18}O$ indicate that the $\delta^{34}S$ values have been altered. The $\delta^{34}S$ are more positive than the primary seawater sulfate signal. These can only be used as an upper limit for the sulfur isotope composition of seawater sulfate. Rapid negative shifts in the $\delta^{34}S$ composition of SSS indicate contamination with sulfur from sulfides. These values are excluded from the data set.

F: Comparison with the interpreted sulfur and oxygen isotope curve of seawater with the primary signal: The isotope trends are reproduced, but the absolute isotope values are higher than the primary isotope composition.
Conclusions

The application of structural substituted sulfate (SSS) on bulk rock samples for the reconstruction of temporal and spatial sulfur isotope changes in seawater reveals many uncertainties. The removal of sulfides with strong oxidizers prior to the dissolution carbonates is required to avoid contamination. Because SSS from bulk rocks are partly derived from cements having precipitated in equilibrium with pore waters, an alteration of the bulk signal is still possible. Contamination with isotopically light sulfate derived from oxidation of sulfides during diagenesis can only be detected by a strong scatter in the data set and is best avoided by the use of light colored pure carbonate samples (> 85% carbonate content). In contrast, contamination with isotopically heavy sulfate derived from sulfate reduction within the pore water can be identified with the measurement of the oxygen isotope composition of the sulfate, which is also enriched in the heavier oxygen isotopes. Oxygen isotope measurements of the extracted sulfate, therefore, must be carried out to obtain a reliable data set. Nevertheless, isotope data based on SSS from bulk rocks do not provide absolute sulfur isotope values; they can be shifted to more positive values during lithification and recrystallization of carbonates by a yet unidentified process (diffusion out of $^{32}$S). To test the reproducibility of the sulfur isotope trends, samples from different sections have to be studied. The application of this methodology to the K-T boundary leads to the conclusion that – in contrast to what was previously reported – no strong change in the sulfur isotope composition of seawater occurred across this critical interval.

References


dissolved oxygen levels at the Cretaceous/Tertiary boundary: Their decrease, subsequent warming, and recovery. *Paleoceanography* 14(4), 511-524.


TOWARDS A BETTER UNDERSTANDING OF MAJOR PERTURBATIONS IN THE BIOGEOCHEMICAL CYCLES OF THE EARLY CRETACEOUS (VALANGINIAN AND APTIAN):
EVIDENCE FROM SULFUR ISOTOPE ANALYSIS AND COMBINED MODELING OF THE CARBON AND SULFUR CYCLE*

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a) Geological Institute ETH Zurich
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Abstract

Understanding the behavior of the “system Earth” during times of rapid environmental change is a major objective in earth science. The Early Cretaceous is marked by two periods of perturbation in the biogeochemical cycles, the Valanginian and the Aptian. These time intervals can be used as archives that contain information on how the system Earth behaves in times of change. Processes that affect the alkalinity of the ocean and the carbonate equilibrium in seawater play a major role in these perturbations. Imbalances in the sulfur cycle strongly affect the oceanic alkalinity, but precise sulfur isotope data to detect such imbalances are missing for the investigated time slices. Thus, to fill this gap, we analyzed sulfur and oxygen isotope data of structural substituted sulfate in carbonates from bulk rocks samples of Valanginian and Aptian age. The Valanginian $\delta^{34}S$ values are stable around 19‰, the corresponding $\delta^{18}O$ values are stable around 16‰. The Aptian-Albian time slice is marked by a constant $\delta^{18}O$ value around 15.4‰. The sulfur isotope data for the Early Aptian show a decrease from 18.2‰ to 17.3‰ within roughly three million years. With the onset of the Albian, the $\delta^{34}S$ increases again to values around 19‰. We emphasize that the $\delta^{34}S$ of structural substituted sulfate is likely to be enriched by +1‰ compared to seawater sulfate.

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We integrated these results and data from the literature into a numerical model for the carbon and sulfur cycle to investigate the impact of environmental perturbations on the oceanic carbonate equilibrium and on pCO$_2$ feedback mechanisms, such as the burial flux of carbonates and silicate weathering.

- The model results show that in order to cause a negative shift of 1‰ in the $\delta^{34}$S of seawater sulfate within 3 million years, the weathering flux of sulfides (pyrite) would have to increase by about 25% to 30%. A 25% to 30% decrease in the burial flux of sulfides or a multiplication of the degassing flux of hydrogen sulfide and sulfur dioxide by a factor of two to four would also cause a negative shift of 1‰ in the $\delta^{34}$S of seawater sulfate within 3 million years. Our calculations and measurements further show that it is unlikely that a change in the fractionation factor of sulfide could have caused the Aptian isotope shift.

- The Valanginian and Aptian are time slices with increased volcanic activity (Paraná and Etendeka flood basalts, respectively Ontong Java Plateau) and ocean crust production rates. Therefore, the most likely cause for the decrease in the sulfur isotope composition of Aptian seawater sulfate is an increase in the degassing flux of hydrogen sulfide and sulfur dioxide from volcanic and hydrothermal activity. In the Valanginian, we do not observe a similar isotope shift. This indicates that the degassing rate of volatiles (CO$_2$, H$_2$S and SO$_2$) in the Valanginian was not as large as in the Early Aptian.

- The enhanced degassing of CO$_2$, H$_2$S and SO$_2$ due to volcanic and hydrothermal activity in the Valanginian and Aptian is likely to have generated two responses in the carbon cycle: (1) A drop in the burial rate of carbonates (crisis of marine carbonate producers and rise in the CCD) due to the immediate imbalance in the carbonate equilibrium and (2) a rise in the burial rate of organic matter due to an indirect pCO$_2$ – greenhouse – weathering – nutrient feedback (positive $\delta^{13}$C excursion).

- The results of our numerical model indicate that the Early Aptian oceanic anoxic event (OAE 1a) started under strong greenhouse conditions, which prevailed over the OAE 1a and its termination. The burial flux of organic matter during the OAE 1a was quantitatively out-competed by the burial flux subsequent to the OAE 1a, indicating the deposition of organic matter under anoxic conditions did not efficiently reduce the amount of carbon dioxide in the ocean-atmosphere reservoir.
Introduction

The Early Aptian is a time of major perturbation in Earth's biogeochemical cycles. In the geologic rock record, the most distinct witnesses of these perturbations are the widespread black shale deposition due to an oceanic anoxic event (OAE 1a, "Livello Selli", approximately 120 Ma with a duration of 0.8-1.2 Ma (HERBERT, 1992; WISSLER, 2001)) and a crisis of pelagic and platform carbonate producers preceding the OAE 1a. This crisis is manifested in the partial disappearance of calcareous pelagic nanofossils (nanoconids) and the cessation of the accumulation of bioclast dominated carbonate sediments (carbonate platform “drowning”). From a geochemical perspective, the most striking indicators for major perturbations in the biogeochemical cycles come from distinct changes in the stable carbon isotope composition ($\delta^{13}C$) of organic matter and carbonates. A rapid negative carbon isotope excursion occurs at the base of the OAE 1a. During the oceanic anoxic event, the $\delta^{13}C$-values reach a plateau. After the OAE 1a, a positive carbon isotope excursion follows. The fluctuations in the $\delta^{13}C$ are not the only geochemical signatures that point to a major change: The strontium isotope curve records a decrease in the isotope composition of seawater, the start of which coincides with the OAE 1a (JONES and JENKYNs, 2001). The Aptian time slice (121-112 Ma) is not the only stage in the Early Cretaceous marked by environmental change. Earlier, in the Valanginian (137-133 Ma, GRADSTEIN et al. (1995)), part of the Aptian pattern is also found. Pelagic calcareous nanofossils and carbonate platforms partly disappear. This “carbonate crisis” is followed by a positive carbon isotope excursion. At the base of this positive excursion, even a small excursion to more negative $\delta^{13}C$ values is observed (HENNING, 2003). Another part of the Aptian perturbation pattern is absent in the Valanginian: There is no indication for an oceanic anoxic event, no carbon isotope “plateau” and a distinct drop in the strontium isotope composition of seawater has not been observed.

With regard to the various biogeochemical changes observed in the Valanginian and the Early Aptian, the following basic questions arise:

- Is each individual biogeochemical pattern (e.g. carbonate crisis, $\delta^{13}C$-excursion, OAE 1a) triggered by a different cause or is there a single cause for the observed changes?
- If there is a single cause, what is the mechanism and is this mechanism the same type of process for both the Valanginian and the Early Aptian?

A potential candidate for a common single cause is volcanism and increased ocean crust production. The Valanginian is associated with the emplacement of a major continental flood ba-
salt province, the Paraná and Etendeka flood basalts with a peak around 133 Ma (Stewart et al., 1996; Wignall, 2001) and by moderately increased ocean crust production rates (Larson, 1991). The Aptian was a time of strongly increased ocean crust production rates starting around 120 Ma (Larson, 1991) and in the latest Barremian to Early Aptian, the largest single volcanic province on Earth, the Ontong Java Plateau was emplaced (around 122 Ma, Wignall (2001)). However, if volcanism is the common single cause for the perturbations in the Valanginian and Aptian, we should be able to explain how this cause initiated the observed environmental changes (e.g. “carbonate crisis”) and why some characteristics (e.g. “oceanic anoxic event”) occur only in one case. Practically, one could imagine the common cause “volcanism” as the first stone thrown in a domino game, starting several lines of falling stones; e.g. a carbon production crisis line, an oceanic anoxia line, a strontium isotope line. However, not each of the lines will reach completeness. Probably, in some lines stones are missing and other lines are stopped or restarted by the interaction with stones from different domino paths. Transferred to the Valanginian and Aptian, the pattern “carbonate production crisis followed by a positive $\delta^{13}C$ excursion” probably represents one line of stones. The Aptian oceanic anoxic event, which has no counterpart in the Valanginian, would be interpreted as another line. The “postponement” of the positive $\delta^{13}C$ excursion by the OAE 1a in the Aptian would then be the example of an interaction between two different lines paths. We can only understand the interactions between different mechanisms when we know their respective direct and indirect links. Therefore, to understand the processes affecting the Valanginian and Aptian biogeochemical cycles, it is necessary to have an overview as complete as possible. As shown above, detailed information about volcanism, sediment types, and the strontium isotope and stable carbon isotope record are available for these time slices. However, there is almost no information about the biogeochemical cycling of sulfur in the Early Cretaceous. There are several reasons why we think that such information is urgently needed:

- The perturbations in the Valanginian and Early Aptian obviously strongly affect the carbon cycle. Probably, atmospheric carbon dioxide pressures rose due to the degassing of carbon dioxide from volcanoes and mid ocean ridges and caused greenhouse conditions (Larson and Erba, 1999). Atmospheric $pCO_2$ is controlled by the amount of inorganic carbon in the atmosphere-ocean system and the alkalinity of the ocean. The alkalinity is not only controlled by the carbon cycle, the fluxes in sulfur cycle (i.e. the weathering and burial of sulfides and the volcanic degassing of $SO_2$ and $H_2S$) strongly affect oceanic alkalinity. Perturbations in the
sulfur balance also affect the oceanic carbonate equilibrium and consequently also pCO₂. In addition, the carbonate equilibrium also controls the saturation state of seawater with respect to carbonate and consequently the carbonate compensation depth and calcite supersaturation in surface seawater.

- The sulfur cycle is closely linked to the carbon cycle by anaerobic degradation of organic matter by sulfate reducing bacteria. About 90% of the produced sulfide is reoxidized at the anoxic-oxic interface in the sediment or the water column. The remaining 10% are buried in the sediment, mainly as iron sulfides (pyrite). Consequently, the burial flux of sulfides is related to anoxic conditions and availability of degradable organic matter. The carbon isotope excursions point to changes in the fluxes of organic matter and therefore could also have an imprint on the sulfur cycle.

- OHKOUCHI et al. (1999) report a strong shift in the sulfur isotope composition of seawater sulfate from the OAE 2 (“Livello Bonarelli”, Cenomanian-Turonian boundary). If this signal is real, one would expect to observe a similar trend at the OAE 1a.

- WALKER (1986) suggested that hydrogen sulfide degassing from seafloor hydrothermal systems could cause oceanic anoxia. The sulfur cycle therefore could even be the trigger for environmental change.

Figure 1  The biogeochemical cycles of carbon and sulfur and the effects on the alkalinity and the amount of inorganic carbon and oxygen in the atmosphere-ocean system
Figure 1 illustrates the relation between the carbon and sulfur cycle. The fluxes from and to the reduced reservoirs not only affect the alkalinity respectively the amount of inorganic carbon, but also the amount of oxygen in the atmosphere-ocean system (stars indicate where oxidation respectively reduction occurs). Information about the sulfur cycling can be gained from the analysis of stable sulfur isotopes. Due to kinetic isotope fractionation during sulfate reduction, sulfides are strongly depleted in the heavy sulfur isotope $^{34}$S (enriched in the light isotope $^{32}$S). Consequently, the remaining sulfate pool is isotopically enriched in $^{34}$S. Imbalances between the burial and weathering fluxes of sulfides, therefore, can be detected as changes in the sulfur isotope composition of seawater sulfate. A further process affecting the $\delta^{34}$S of seawater sulfate is the input of isotopic light sulfur dioxide and hydrogen sulfide from volcanic and hydrothermal activity. The burial and weathering fluxes of evaporites mainly affect the amount of sulfate in the ocean but have little effect on its sulfur isotope composition because there is little sulfur isotope fractionation related to the precipitation of sulfate. The amount of sulfate in the ocean is large (today around 40 x $10^{18}$ mol sulfate, HOLSER et al. (1988)) and the fluxes in the sulfur system are small compared to the fluxes in the carbon cycle (about one tenth). This results in a long residence time for the sulfur cycle (about 17 to 40 million years, calculated from BERNER and BERNER (1996); HOLSER et al. (1988) and PAYTAN and ARRIGO (2000)). Due to the large reservoir, the $\delta^{34}$S of seawater sulfate is a rather insensitive tracer. Only “smooth” changes in the $\delta^{34}$S of seawater can be expected to occur. However, if a rapid change in the $\delta^{34}$S of seawater sulfate is observed, one can conclude that a dramatic perturbation in the sulfur cycle took place, e.g. the perturbation reported from the OAE 2 (OHKOUCHI et al., 1999).

In the following, we present sulfur isotope data from the Early Cretaceous. A major finding is a decrease in the sulfur isotope composition of seawater sulfate during the Aptian. Using a numerical model for the sulfur cycle, and taking the evidence from the strontium isotope record into account, we interpret this shift as a result of volcanic or hydrothermal input of sulfur dioxide or hydrogen sulfide. The numerical model includes the carbon and sulfur cycle and considers the weathering flux of silicates. We investigate the impact of an increase in the input of carbon dioxide and hydrogen sulfide on the carbon and alkalinity reservoir of the ocean in the Aptian. Finally, we compare these results to the Valanginian time slice.
Structural substituted sulfate from bulk carbonate rocks as carrier of $\delta^{34}$S-signals from seawater sulfate

To compare the trends in the seawater sulfur isotope composition with the observed patterns of change, a tight stratigraphic framework is required. This was achieved by the extraction of the sulfur isotope signal from sections well controlled by stable carbon isotope stratigraphy. Seawater sulfate is conserved in sedimentary rocks in two different phases, as marine barite and structural substituted sulfate (SSS) in carbonates. Of these two carriers, marine barite is more reliable than SSS from bulk rocks. However, we did not succeed to extract marine barite from several investigated sections. Therefore, we chose the analysis of SSS as a tool to determine the sulfur isotope trends in the Valanginian and Aptian. Structural substituted sulfate is incorporated into the crystal lattice of carbonate minerals. This incorporation apparently occurs without sulfur isotope fractionation. Therefore, SSS has been used for the reconstruction of seawater $\delta^{34}$S by many authors (BRUNNER et al., 2003b: Chapter 2; BURDETT et al., 1989; HURTGEN et al., 2002; KAIHO et al., 2001; KAJIWARA et al., 1997; KAMPSCHULTE et al., 2001; OHKOUCHI et al., 1999; STRAUSS et al., 2001). KAMPSCHULTE et al. (2001) showed that $\delta^{34}$S$_{SSS}$ from shells of modern biota reflect seawater $\delta^{34}$S reasonably well. $\delta^{34}$S$_{SSS}$ from fossil shells, such as foraminifera (BURDETT et al., 1989), belemnites and brachiopods (KAMPSCHULTE et al., 2001; STRAUSS, 1999) has been successfully applied for the reconstruction of ancient seawater $\delta^{34}$S. In contrast to shells, SSS in bulk rock is prone to alteration, either during diagenesis and lithification or during sample preparation (BRUNNER et al., 2003b: Chapter 2). However, seawater sulfate isotope trends from SSS$_{bulk}$ can be determined when the following preconditions are accomplished:

- The chosen rock samples should be pure carbonates with a white color. Other samples, such as marly limestones, can only be used for a confirmation of values determined from pure carbonates.
- The extraction of SSS from the rock samples has to ensure that no contamination of sulfate with sulfate derived from oxidation of sulfides can take place.
- The $\delta^{18}$O of the extracted SSS has to be analyzed and compared to the corresponding $\delta^{34}$S values. Sulfur isotope alterations due to bacterial sulfate reduction in pore waters often are accompanied by enrichments in the $\delta^{18}$O of sulfate. Therefore, the measurement of the $\delta^{18}$O$_{SSS}$ is used to identify and exclude artifacts.
• A sulfur isotope data set is best compiled from different sections, in order to disentangle artifacts from the general sulfur isotope trend.

**Samples**

For the determination of the Valanginian sulfur isotope curve, samples from three different pelagic limestone sections were taken:

Val de Mis section:
The Val de Mis section is located 10 km West of Belluno (Northern Italy). The sampled part of the section covers the latest Berriasian to Hauterivian. Palaeogeographically, these pelagic carbonates were deposited in the Belluno Basin. The rock samples are pure limestones with a white colour. For a detailed description of the section and stable carbon isotope stratigraphy, we refer to Lini (1994).

Capriolo section:
The Capriolo section is located in an abandoned quarry near the village of Capriolo at the southwest tip of the Lago d’Iseo (Northern Italy). The sampled part of the section covers the Valanginian to the Aptian. The sediments were deposited in a pelagic environment (Lombardian Basin). The rock samples are pure limestones with a slightly brownish colour. For a detailed description of the section and stable carbon isotope stratigraphy, we refer to Lini (1994).

Val Aviana section:
The Val Aviana section is located 4 km NW of the Village of Avio (Northern Italy). The sampled part of the section covers an interval corresponding to the peak of the Valanginian positive carbon isotope excursion. The depositional environment of the sediments is interpreted as pelagic environment on a submarine plateau (Trento Platform). The rock samples have a yellowish colour. For a detailed description of the section and stable carbon isotope stratigraphy, we refer to Lini (1994).

It is difficult to find suitable sections for the determination of the Aptian sulfur isotope curve. Most sections well controlled by stable carbon isotope stratigraphy consist of marly limestones. The samples often have dark colors, especially in the part corresponding to the OAE 1a. Pure white limestones are mostly absent. The Monte Raggetto section, a platform
section well correlated with basinal sections by stable carbon isotope stratigraphy, was used to determine the δ³⁴S of structural substituted sulfate in carbonates. Additionally, samples from two basinal sections, the Cismon section and the Gorgo a Cerbara section were analyzed for the δ³⁴S composition.

Monte Raggetto section:
The Monte Raggetto section is located in the southern part of the Maggiore Mountain about 35 km North of Naples (Italy). The sampled interval of the section covers the Aptian and a part of the Albian. The samples are pure white limestones which have been deposited in a carbonate platform environment. On the level time equivalent to the deposition of the basinal black shales (“Livello Selli”) the limestones are thinner bedded and the rocks get a slightly brownish color. For a detailed description of the section and stable carbon isotope stratigraphy, we refer to Ferreri et al. (1997) and Wissler (2001).

Cismon section:
The Cismon section is located in the Venetian Alps (Northern Italy), 15 km West of Feltre in the valley of the Cismon river. The sampled part of this basinal section mainly covers the Early Aptian. Marly limestones (40%-70% carbon content) with mostly brownish to greenish color were taken as samples. On the level corresponding to the OAE 1a, dark marls (8%-60% carbonate content) have been used. For a detailed description of the section and stable carbon isotope stratigraphy, we refer to Menegatti (1999).

Gorgo a Cerbara section:
The Gorgo a Cerbara section is located in the Umbro-Marchean Apennines (Central Italy), 3 km East of Piobbico. The sampled interval of the section covers the latest Barremian and the Early Aptian. The sediments were deposited in a pelagic realm. The investigated samples are marly limestones (40%-70% carbon content) of brownish to greenish color. On the level corresponding to the OAE 1a, samples with dark green-brown colors were analyzed (8%-60% carbonate content). For a detailed description of the section and stable carbon isotope stratigraphy, we refer to Menegatti (1999).
Methods

Extraction of structural substituted sulfate (SSS) from carbonate rocks for sulfur and oxygen isotope analysis

SSS was extracted following the treatment-scheme proposed by Brunner et al. (2003b: Chapter 2). This method focuses on the removal of sulfides by oxidizing agents prior to the dissolution of the carbonates with hydrochloric acid (Figure 2).

<table>
<thead>
<tr>
<th>Treatment-scheme for the extraction of SSS from bulk carbonate samples</th>
</tr>
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<tbody>
<tr>
<td><strong>Preparation:</strong> Crushing and grinding of the sample (20-60g): &lt;63μm</td>
</tr>
<tr>
<td><strong>Washing:</strong> Distilled water &amp; Ultrasonic bath (&gt;30 Min)</td>
</tr>
<tr>
<td><strong>Oxidation:</strong> The sample is treated with hydrogen peroxide (H₂O₂) for at least 3 days, then follows again a washing step with distilled water and ultrasonic bath. The oxidation step is 3 times repeated.</td>
</tr>
<tr>
<td><strong>Dissolution of carbonate:</strong> The sample is reacted with concentrated hydrochloric acid (HCl) under a nitrogen atmosphere (removes acid soluble sulfides if present). The residual is centrifuged off.</td>
</tr>
<tr>
<td><strong>Residual:</strong> The residual might contain barite. This can be checked by XRD analysis.</td>
</tr>
<tr>
<td><strong>Solution (SSS):</strong> The solution contains acid soluble sulfate (SSS). By addition of BaCl₂, SSS is precipitated as BaSO₄.</td>
</tr>
<tr>
<td><strong>Effect:</strong></td>
</tr>
<tr>
<td>Water soluble sulfate is removed.</td>
</tr>
<tr>
<td>Sulphides and organic compounds are destroyed.</td>
</tr>
<tr>
<td>Remaining sulfides are removed by the nitrogen stream.</td>
</tr>
</tbody>
</table>

Figure 2  Treatment-scheme for the extraction of SSS from bulk carbonate samples

Washing step: After settling of the carbonate particles from suspension, the overstanding water is decanted. This step is repeated three times.

For the extraction of SSS from the marly limestones of the Gorgo a Cerbara and Cismon section the treatment was modified: Instead of cold hydrochloric acid for the dissolution of carbonates, we used hot hydrochloric acid. The modification was made in order to increase the speed of the reaction. This should reduce the time in which disseminated pyrite released by the dissolution of carbonates could be oxidized by reactive iron. However, as presented in the results, this modification did not lead to SSS free of contaminations by oxidized sulfides. A disadvantage of this modification is that hot acids accelerate the oxygen isotope exchange rate between sulfate and water. We therefore did not measure the oxygen isotope composition of SSS extracted by the modified method.
Extraction of sulfides from bulk rock samples for sulfur isotope analysis

In order for a qualitative determination of the isotope fractionation between sulfides and sulfate, sulfides were extracted from a few samples. The samples were crushed and powdered (\(<63\mu\)). Carbonate was removed by treatment with hydrochloric acid (HCl), silicates were leached with hydrofluoric acid (HF). In order to separate sulfide (pyrite) from organic matter, the residual was treated with hot chromium-II-chloride solution under a nitrogen atmosphere (for method see CANFIELD et al. (1986) and NEWTON et al. (1995)). Chromium-II-chloride reduces pyrite to hydrogen sulfide according to the following chemical reaction:

\[
4H^+ + 2\text{Cr}^{2+} + \text{FeS}_2 \rightarrow 2\text{H}_2\text{S} + 2\text{Cr}^{3+} + \text{Fe}^{2+}
\]

The produced hydrogen sulfide was carried by a nitrogen stream into a silver-nitrate trap and collected as silver sulfide (AgS). The reaction usually terminated after about two hours; the experiments were kept running for four hours. The residual from the chromium-II-treatment might contain sulfur bound in organic compounds and barite. However, only one of the sample-residuals contained enough sulfur for sulfur isotope analysis. This finding demonstrates that the chromium-II-treatment quantitatively reduces pyrite to hydrogen sulfide.

Measurement on mass spectrometer

Sulfur isotope analysis

For stable sulfur isotope analysis, 400 to 500 µg of BaSO_4, respectively 200 to 250 µg of AgS were weighed into tin cups. To enhance combustion efficiency, vanadium pentoxide with about twice the weight of the sample was added. The sample size for sulfur isotope analysis of sulfides from bulk samples was adjusted to match the (mass spectrometer) \(^{34}\text{S}\) peak size of pure sulfide samples. The needed sample weight was in the range of 0.2 mg to 14 mg. The samples were analyzed on a FISON OPTIMA mass spectrometer (Fisons, Middlewich, Cheshire, UK) connected by continuous flow to a Carlo Erba elemental analyzer (CEW Instruments, Milan, Italy). The sulfur isotope data are reported in the conventional \(\delta\)-notation relative to the Vienna-Canyon Diabolo Troilite (V-CDT) standard according to:

\[
\delta^{34}\text{S} (\text{‰}) = \left\{ \left( \frac{^{34}\text{S}}{^{32}\text{S}} \right)_{\text{sample}} / \left( \frac{^{34}\text{S}}{^{32}\text{S}} \right)_{\text{V-CDT}} - 1 \right\} \times 1000
\]

The system was calibrated using the international standards IAEA-S1 (\(\delta^{34}\text{S} = -0.3\text{‰}\)) and IAEA-S2 (\(\delta^{34}\text{S} = 21.7\text{‰}\)) (GONFIANTINI et al., 1995). The mean \(\delta^{34}\text{S}\) value obtained for the international standard NBS127 was 20.4‰. Analytical reproducibility of the measurements was ±0.25‰. The error in the sulfur isotope measurements of bulk sulfide samples is esti-
mated to be ±3‰. This is due to a strong tailing in the sulfur isotope measurements caused by the large amount of gas produced by the combustion of the bulk samples.

**Oxygen isotope analysis**

For stable oxygen isotope analysis of the samples from the Monte Raggeto, we modified the on-line pyrolysis method of (Werner et al., 1996). We use an automated elemental analyzer (Carlo Erba elemental analyzer, CE-Instruments, Milan, Italy). 430 to 460 µg of BaSO₄ were weighed in tin cups. In order to improve pyrolysis efficiency and provide an additional carbon source, a small amount of nickelized carbon was added to each sample. Pyrolysis was carried out at 1080°C in a modified reaction tube filled with 1.5 cm³ glassy carbon. The smaller amount of glassy carbon compared to the (Werner et al., 1996) method was found to strongly decrease memory effects. The pyrolysis products in the He stream were passed through a water trap and a 1-m GC column packed with 5 Å zeolite to separate carbon monoxide from N₂ which would produce an isobaric interference at Masses 28 and 29 and 30. The oxygen isotope composition was subsequently measured on a Fisons Optima mass spectrometer (Fisons, Middlewich, Chesire, UK). The oxygen isotope data are reported in the conventional δ-notation relative to the Standard Mean Ocean Water (SMOW) standard according to:

\[ \delta^{18}O (‰) = \left\{ \frac{(^{18}O/^{16}O)_{\text{sample}}}{(^{18}O/^{16}O)_{\text{V-SMOW}}} - 1 \right\} \times 1000 \]

In the absence of a set of internationally accepted reference sulfate standards, the method was calibrated using a set of different substances, including carbonates and one sulfate sample, (Table 1) and checked with nitrates of known composition.

<table>
<thead>
<tr>
<th></th>
<th>Measured δ¹⁸O</th>
<th>Standard δ¹⁸O (V-SMOW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS2, Carbonate</td>
<td>10.6</td>
<td>29.0</td>
</tr>
<tr>
<td>NBS18, Carbonate</td>
<td>-6.6</td>
<td>7.1</td>
</tr>
<tr>
<td>LSVEC, Carbonate (LiCarb)</td>
<td>-10.9</td>
<td>3.54</td>
</tr>
<tr>
<td>NBS127, BaSO₄</td>
<td>-6.1</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Table 1  Measured vs. published oxygen isotope values

We estimate of the accuracy of barium sulfate measurements to be ±0.3‰. The analytical reproducibility of the measurements was ±1‰.
The oxygen isotope analysis of the samples from the Val Aviana, Capriolo and Val Mis sections was carried out at the Environmental Isotope Laboratory (University of Waterloo, Canada). For oxygen isotope analysis, the BaSO₄ samples were weighed at 0.2 mg and then stored in a dynamic vacuum for at least 24 hours to remove water. The samples were then dropped individually onto carbon at about 1290°C (Eurovector high temperature Elemental Analyzer). The combustion product, carbon monoxide (CO) was carried to the mass spectrometer (Micromass IsoPRIME) by a helium-stream. The measurements were carried out in duplicate; the reproducibility is better than ±0.8‰. The values are reported relative to the Standard Mean Ocean Water (SMOW) standard according to:

\[ \delta^{18}O \ (\%e) = \left\{ \frac{(^{18}O/^{16}O)_{\text{sample}}}{(^{18}O/^{16}O)_{\text{SMOW}}} - 1 \right\} \times 1000 \]
Results

Val de Mis section

Figure 3  Sulfur and oxygen isotope data of SSS extracted from samples of the Val de Mis section

The lithological profile and carbon isotope data are derived from LINI (1994).

The $\delta^{18}$O and $\delta^{34}$S data from the Val de Mis section show an increase in the isotope composition from the Late Berriasian to the Valanginian (Figure 3). In the Valanginian, the oxygen isotope values are close to 15.8‰. The sulfur isotope values scatter around a value of 19‰.
Figure 4  Sulfur and oxygen isotope data of SSS extracted from samples of the Capriolo section

The lithological profile and carbon isotope data are derived from LINI (1994).

Figure 4 shows a strong scatter in the $\delta^{18}$O and $\delta^{34}$S data from the Capriolo section. The scatter in the oxygen isotope data is less distinct (from 15‰ to 18.2‰) than the scatter in the sulfur isotope data set (from 14.3‰ to 21‰). Most of the oxygen isotope data are close to 16‰.
Val Aviana section

**Figure 5** Sulfur and oxygen isotope data of SSS extracted from samples of the Val Aviana section

The lithological profile and carbon isotope data are derived from LINI (1994).

The δ¹⁸O and δ³⁴S data from the Val Aviana section cover only a short part of the positive carbon isotope excursion (Figure 5). The sulfur and oxygen isotope data correlate and show a trend to values enriched in $^{18}$O and $^{34}$S towards the peak of the carbon isotope excursion (from 11.2‰ to 16.5‰ for δ¹⁸O and from 16‰ to 19‰ for δ³⁴S).
The δ¹³C and δ³⁴S data from the Monte Raggetto section cover the Aptian and the Early Albian (Figure 6). A strong scatter in the sulfur and oxygen isotope values is observed at the level equivalent to the “Livello Selli”. Figure 7 illustrates this part in more detail: The δ¹³C-curve of the Monte Raggetto section is correlated to the δ¹³C-curve of the Cismon section. The correlation lines demonstrate that the δ¹³C values abruptly rise from 3‰ to 5‰. The sulfur isotope data from the Monte Raggetto-section are in a range of 17‰ to 20‰ and scatter strongly on the level time equivalent to the OAE 1a. The oxygen isotope of sulfate fall in a range of 14.5‰ to 20‰. δ¹⁸O values higher than 16.5‰ only occur at and just below of the stratigraphic level equivalent to the basinal “Livello Selli”.

Figure 6  Sulfur and oxygen isotope data of SSS extracted from samples of the Monte Raggetto section

The sulfur isotope data are correlated with a carbon isotope curve (lower part Cismon section, upper part Monte Raggetto section) compiled by Wissler (2001).
Figure 7  “Close-up” of the carbon, sulfur and oxygen isotope data from the Monte Raggeto section

The data are correlated by stable carbon isotope stratigraphy (Wissler, 2001). Compared to the Cismon section, the carbon isotope values from the Monte Raggeto section rise rapidly after the termination of the “Livello Selli” equivalent.
Cismon section

Figure 8  Sulfur isotope data of SSS extracted from samples of the Cismon section

The lithological profile and carbon isotope data are derived from Menegatti (1999). The grey shaded area represents the “Livello Selli”.

The δ³⁴S record from the Cismon section starts at the base of the “Livello Selli” and ends at the top of the positive carbon isotope excursion (Figure 8). A single data point from the Albian was measured. The sulfur isotope data are in a range of 7‰ to 20‰. The values below 16‰ fall in level corresponding to the OAE 1a, values higher than 16‰ are derived from samples above of the “Livello Selli”.
Gorgo a Cerbara section

Figure 9  Sulfur isotope data of SSS extracted from samples of the Gorgo a Cerbara section

The lithological profile and carbon isotope data are derived from Menegatti (1999). The grey shaded area highlights a pure black shale part of the section where no stable carbon isotope data in carbonates could be measured. The “Livello Selli” covers the interval from C3 (negative carbon isotope excursion) to the end of C5.

The $\delta^{34}$S data from the Gorgo a Cerbara section cover the time from Barremian-Aptian boundary to the end of the positive carbon isotope excursion (Figure 9). We measured $\delta^{34}$S values from $-3^{\circ}$ to $+11^{\circ}$ within the “Livello Selli”. Above of the OAE 1a, the values are in a range $13^{\circ}$ to $18^{\circ}$ and below the OAE 1a the values are in a range of $16^{\circ}$ to $22^{\circ}$. 
Discussion of the data and identification of artifacts

The sulfur isotope signal in SSS from carbonates is prone to alteration by incorporation of sulfate from interstitial waters (for a discussion see BRUNNER et al. (2003b: Chapter 2)). This sulfate has often a different isotope composition than seawater (e.g. BÖTTCHER et al. (1998); WORTMANN et al. (2001)). This is due to various processes in interstitial waters: Microbial sulfate reduction leads to an enrichment in $^{34}$S, oxidation of sulfides leads to sulfate depleted in $^{34}$S. The sulfur isotope signal can also be altered by advection of brines containing sulfate with different sulfur isotope composition or even in the outcrop, when pyrite is oxidized. In order to minimize the potential of contamination by oxidized sulfides, we concentrated on sections where pure carbonate samples were available. However, the data of some of these sections strongly scatter, indicating alteration of the primary sulfur isotope signal. The difference between sections where we observe a strong scatter (e.g. the Capriolo section) and a section where the data are more consistent (e.g. the Val de Mis section) is subtle: We observe that the colour of the rocks in the “better” section are white, while “not ideal” sections are slightly stained, e.g. with a brown colour in the case of the Capriolo section. For the interpretation of the data, we strongly rely on the oxygen isotope composition of the extracted sulfate. In seawater, the oxygen isotope composition of sulfate is buffered by a sulfate reduction-reoxidation loop: Sulfide produced by sulfate reducers is to about 90% reoxidized (JØRGENSEN, 1982). During sulfate reduction, residual sulfate is enriched in the $\delta^{18}$O, while sulfide oxidation contributes oxygen with low $\delta^{18}$O. Thanks to this balancing process, the $\delta^{18}$O of seawater sulfate is expected to change slowly. In interstitial waters, where biochemical processes change the sulfur and oxygen isotope composition of sulfate, $\delta^{18}$O values are not balanced. The combined analysis of the $\delta^{18}$O and $\delta^{34}$S of SSS therefore allows us to identify artifacts more precisely. In a not entirely altered section, we expect to find a majority of $\delta^{18}$O in a similar range, reflecting the primary oxygen isotope composition of seawater. The corresponding sulfur isotope value can then be expected to be unaltered as well. By combining the results from different sections, these findings can be checked.
Valanginian time slice

Figure 10  Sulfur and oxygen isotope composition of SSS from different sections covering the Valanginian and Hauterivian

The grey bars indicate the “primary” $\delta^{18}O$ composition of seawater sulfate, the dashed grey bars indicate the “primary” $\delta^{34}S$ composition of seawater sulfate. The dotted circles mark values where the measured oxygen isotope composition does not correspond to the estimate for the “primary” $\delta^{18}O$ value.

Figure 10 shows a compilation of the Valanginian $\delta^{18}O – \delta^{34}S$ data sets. The data were correlated with stable carbon isotope stratigraphy (illustrated by the carbon isotope curve to the left of Figure 10). Most of the $\delta^{18}O$ values of the Val Mis section plot around a value of 16‰ ±0.5‰. This is also the case for the data set from the Capriolo section. A part of the data from the Val Aviana section also fall into this data range. We therefore estimate the “primary” $\delta^{18}O$ composition of Valanginian seawater to be at 16‰. Correspondingly, the $\delta^{34}S$ is around 19‰.

The data points for the Barremian and Early Aptian from the Capriolo Section can not be judged on this base, we do not have comparable $\delta^{18}O$ values from the other sections. The data point from the Early Aptian will be discussed in the interpretation of the Aptian time slice below. The remarkable correlation between the sulfur and oxygen isotope values in the data set of the Val Aviana section can be explained by the incorporation of sulfate derived from oxidation of pyrite. The oxygen isotope value of sulfate derived from oxidation of sulfides is close to the one of ambient water. The steep slope in the oxygen-sulfur isotope relation would then point to a meteoric water source with a lower oxygen isotope composition (e.g. $< -5‰$).
than seawater (= 0‰). However, we still have to expect that the oxidized pyrite had a rather positive sulfur isotope value close to –5‰. Figure 11 shows the interpreted and compiled Valanginian data set: δ³⁴S-δ¹⁸O couples deviating from the estimated primary δ¹⁸O value were discarded, values not checked by δ¹⁸O measurements are dotted. We estimate the value of seawater sulfate to be close to 19‰ for δ³⁴S and 16‰ for δ¹⁸O (dashed grey and grey bar).

Figure 11  Interpreted and compiled Valanginian / Hauterivian δ¹⁸O – δ³⁴S data set

The dashed grey and grey bares indicate the estimated sulfur and oxygen isotopic composition of seawater.
**Aptian time slice**

The data basis for the interpretation of the Aptian sulfur isotope composition is not as good as the one from the Valanginian. We mainly rely on the data from the Monte Raggeto section. The two basinal sections, the Cismon section and the Gorgo a Cerbara section are less reliable and serve only as additional source of information. We do not have oxygen isotope measurements of these two sections because of the use of a modified method in order to improve sulfur isotope data quality (see extraction of SSS above). The low sulfur isotope values at the level of the OAE 1a in the basinal section are caused by contamination with sulfate derived from the reoxidation of sulfides. We do not know if this contamination occurred during sample preparation or earlier. However, the measured sulfur isotope values from the “Livello Selli” are artifacts and the values from the basinal sections that are left have to be used with caution. Unfortunately, the situation in the platform equivalent of the “Livello Selli” is not better. The positive excursion in the oxygen isotope data and the strong scatter in the sulfur isotope data show that the whole interval is diagenetically altered. Therefore, no reliable sulfur isotope data from the “Livello Selli” time slice are available. Figure 12 shows a compilation of the \( \delta^{34}S \) and \( \delta^{18}O \) measurements of the different sections correlated by stable carbon isotope stratigraphy. Eight oxygen isotope measurements remain for the interpretation of the data from the Monte Raggeto section (Figure 13). These values are in the range of $14.6\%_{oo}$ to $16.2\%_{oo}$ with one exception, where the oxygen isotope value is at $16.8\%_{oo}$. Compared to adjacent values, the latter coincides with an enriched sulfur isotope measurement. We therefore assume that the value of $16.8\%_{oo}$ is a diagenetic artifact (crossed squares in Figure 13). The remaining \( \delta^{18}O \) values point to a “primary” oxygen isotope composition of seawater at $15.4\%_{oo}$. The oxygen isotope data scatter around this value in a range of ±0.8. Taking the reproducibility of the oxygen measurements of ±1‰ into account this scatter does not surprise. The only available oxygen isotope measurement (star in Figure 13) from a different section is derived from the Capriolo section and falls within the determined data range ($\delta^{18}O = 15.9\%_{oo}$). By compiling the remaining data, we find a sulfur isotope trend from around $18.2\%_{oo}$ at the Barremian-Aptian boundary to values around $17.3\%_{oo}$ at the level of the peak of the positive carbon isotope excursion, and a turn to more positive values around $19\%_{oo}$ in the Early Albian (Figure 13).
On the level corresponding to the OAE 1a, no reliable sulfur isotope data are available.
Figure 13  Interpreted and compiled Aptian $\delta^{18}$O – $\delta^{34}$S data set

The grey dashed and grey bars indicate the estimated sulfur and oxygen isotopic composition of seawater.
Comparison to other available sulfur isotope data

The trend recorded in the sulfur isotope data from the Aptian to the Albian has been observed by (CLAYPOOL et al., 1980) based on evaporite data, but it is pinned down by our data set more precisely. The decrease in the sulfur isotope data in the Early Aptian is confirmed by unpublished data from marine barites (A. PAYTAN, pers. communication). However, the barite data are generally about 1‰ below the sulfur isotope data presented here. We speculate that this difference might be due to a isotope fractionation effect associated with the incorporation of sulfate into the crystal lattice of carbonate minerals.

Interpretation of the Valanginian and Aptian SSS-sulfur isotope record

We emphasize that it is possible that the primary sulfur isotope values are 1‰ below the measured value due to a sulfur isotope fractionation effect related to the incorporation of sulfate in the lattice of carbonate minerals. It is possible that a similar isotope effect also affects the oxygen isotope values. However, this does not affect the observed isotope trends.

The Valanginian-Hauterivian time slice is marked by constant oxygen and sulfur isotope values. We estimate a $\delta^{18}O$ of 16‰ and a $\delta^{34}S$ of 19‰ for seawater sulfate. The Aptian-Albian time slice is marked by a constant $\delta^{18}O$ of 15.4‰ and a decrease in the sulfur isotope trend from 18.2‰ to 17.3‰ in the Early Aptian and a turnover to values around 19‰ in the Early Albian. As consequence, the oxygen and sulfur isotope composition of seawater sulfate should drop by 0.6‰ ($\delta^{18}O$) and 0.8‰ ($\delta^{34}S$) during the Barremian time slice (duration of about 6 Myr). Compared to this, the drop in the sulfur isotope values of 0.9‰ in the Early Aptian is fast. Assuming constant sediment accumulation rates for the Monte Raggeto and the Cismon section (data from WISSLER (2001)) and considering that the duration of the “Livello Selli” is between 0.8 Myr and 1.3 Myr (HERBERT, 1992; WISSLER, 2001), we estimate the duration of the time slice from the Barremian-Aptian boundary to the peak of the positive carbon isotope curve to be less than 3 Myr. With respect to the large oceanic sulfate reservoir, this is a rapid shift.

In order to be consistent with the unpublished sulfur isotope data of barites (see above), we ran our numerical model from below with sulfur isotope values corrected by −1‰ (sulfur isotope shift from 17‰ to 16‰). However, this has very little effect on the calculated fluxes.
In the second part of this paper, we will focus on the causes and effects of this sulfur isotope shift. These questions are addressed by the implementation of a numerical model. However, a important parameter for such a model is an estimate for the sulfur isotope fractionation factor. Therefore, we first present sulfur isotope data from sulfides.

**Sulfide (pyrite) sulfur data: results and interpretation**

![Figure 14 Sulfur isotope date from sulfides for different Aptian time intervals](image)

In order to determine the sulfur isotope fractionation between pyrite (sulfides) and SSS for the Aptian time slice, we analyzed HCl and HF leached samples for their sulfur isotope composition (Figure 14). Samples were taken from the Cismon and the Gorgo a Cerbara section. We measured bulk HCl–HF-residual samples but also silver-sulfide samples (AgS) derived from pyrite reduction by the reaction with chromium-II-chloride solution (Cr$^2+$Cl$_2$). Different sulfide-forms (e.g. euhedral pyrite vs. framboidal pyrite) might have different $\delta^{34}$S signatures.

These sulfide forms are probably attacked by chromium-II (Cr$^{2+}$) at different reaction speeds. In a few experiments, we tested this by using two different silver-nitrate traps. The first trap was used in an early stage (first 15 minutes) of the reaction, the second one in a late stage (start after 2 hours). The residuals after the Cr$^{2+}$-treatment were also analyzed for the sulfur
isotope composition. Possible sulfur-containing compounds in the Cr\(^{2+}\)-residual are barite and organic matter. However, only one residual contained enough sulfur to determine a sulfur isotope composition.

For the sulfur isotope analysis of bulk residual samples (HCl–HF-residuals and HCl–HF–Cr\(^{2+}\)-residuals), large sample sizes have to be combusted. The weight is up to a hundred times larger than the weight of pure sulfide samples. Consequently, a large amount of gas is released by the combustion, diluting the produced SO\(_2\). This causes a tailing in the continuous flow sulfur isotope analysis leading to a less precise sulfur isotope ratio determination by the mass spectrometer. The data from the bulk samples therefore are not as reliable as the data from pure sulfide samples (±0.25‰). We estimate the error to be around ±3‰.

The sulfide data scatter in a range of –15‰ to –52‰ with an average around –35‰. A distinct sulfur isotope trend does not occur. Probably the values around –45‰ in the lower part of the “Livello Selli” point to a larger sulfur isotope fractionation between sulfides and sulfate or to a different “mixture” of sulfide-forms with different δ\(^{34}\)S values. There is little difference in the δ\(^{34}\)S data of “initial” and “late” AgS samples. This indicates that the hot chromium-II treatment attacks different sulfide forms with a the same reaction speed or that different sulfide forms have similar δ\(^{34}\)S.

At the first sight, the differences in the sulfur isotope composition between the AgS and the bulk HCl–HF-residuals are rather surprising: In two cases, the δ\(^{34}\)S values of the bulk samples are more negative than the corresponding AgS measurements. This would indicate that in the bulk sample, a non-sulfide compound containing sulfur strongly depleted in \(^{34}\)S is present. However, the error in the bulk measurement is large (±3‰) and it is possible that the “mixture” of different sulfide forms was not equal for the bulk and the sulfide sample. The latter points to different sulfur isotope compositions for different sulfide forms (e.g. framboidal vs. euhedral pyrite) and implies that the hot chromium-II solution attacks different sulfide forms at the same reaction speed (see above). The efficiency in pyrite reduction by Cr\(^{2+}\) is confirmed by the fact that only one HCl–HF-Cr\(^{2+}\)-residual contained enough sulfur to determine a sulfur isotope composition. The δ\(^{34}\)S of the HCl–HF-Cr\(^{2+}\)-residual was at –13‰ (±3‰). It is possible the analyzed sulfur was bound to organic matter. During diagenesis, hydrogen sulfide is incorporated into organic matter. This is likely to happen when no reactive iron for the formation of iron sulfides is left and sulfide values are enriched due to Raleigh distillation processes in a closed pore water space. This would explain the enrichment in the sulfur isotope value of the HCl–HF-Cr\(^{2+}\)-residual compared to the sulfide data.
KUHN (1996) determined the sulfur isotope composition of Valanginian sulfides. The measured range for $\delta^{34}S$ was from $-13\text{‰}$ to $-43\text{‰}$ with an average around $-33\text{‰}$. Compared to the average from the Aptian time slice (around $-35\text{‰}$), there is little evidence for a change in the sulfur isotope fractionation from the Valanginian to the Aptian.

A numerical model combining the biogeochemical cycles of carbon and sulfur and silicate weathering fluxes

The Valanginian and Aptian are time slices marked by coinciding patterns of change in different geologic archives, such as changes in the type of sedimentary rocks but also changes in their carbon, sulfur and strontium isotope composition. At the onset and during the perturbations, an increase in the volcanic activity has been reported (Paraná and Etendeka continental flood basalts in the Valanginian and Ontong Java Plateau submarine large igneous province in the Aptian (WIGNALL, 2001)). Additionally, an increase in the ocean crust production rates has been observed (LARSON, 1991). This leads to the speculation, that volcanic and hydrothermal activity initialized the observed reorganization of Earth’s biogeochemical cycles in the Valanginian and Aptian. However, the complex interaction between the different biogeochemical processes (e.g. climate, weathering, primary production of organic matter), where positive and negative feedback mechanisms play a decisive role, prevents observation of a direct link between cause and effect. Therefore, any reasonable hypothesis explaining how a mechanism could have caused the observed changes might be the “right one”. The here presented numerical model does not tell if a hypothesis is right, it only helps to estimate the size of the parameters required to achieve the desired effect by a proposed cause. This adds a quantitative aspect to the mechanistic reasoning and gives a “feeling” if the proposed scenario is possible, and if, how probable. In the following, we first present the developed numerical model. In a second part, we discuss different model runs for chosen scenarios and interpret the results.

Model description

Our model is designed to investigate how changing fluxes in the carbon and sulfur cycle affect the carbonate equilibrium. This equilibrium controls the atmospheric $pCO_2$ pressure and the burial flux of carbonates (e.g. CCD). As perturbations, we consider the volcanic degassing of carbon dioxide, changes in the burial of organic matter, as recorded in the $\delta^{13}$C-curve and
changes in the sulfide fluxes as recorded in the $\delta^{34}\text{S}$-curve. As feedback mechanisms, we involve the burial of carbonates and the silicate weathering flux. During a specified time frame, the model iteratively calculates the size of all involved fluxes and reservoirs as a result of the applied forcing and feedback mechanisms. Figure 15 illustrates the main features of the presented numerical model.

Figure 15 Numerical model for the investigation of the effects of changing fluxes of organic matter, sulfides and volcanic carbon dioxide

The model includes two feedback-mechanisms: Burial of carbonates and silicate weathering.

Below, we describe how such a model is implemented.
Natural processes are transformed into an abstract box model consisting of reservoirs for chemical compounds and fluxes, which connect these pools. The box model is described by mathematical equations. To investigate different scenarios in model runs, the parameters are set to fulfill the required conditions. The numerical results are then interpreted in context with the taken assumptions.

Figure 16 visualizes the used concept to develop the numerical model. We focus on the biogeochemical cycles of carbon and sulfur. But we also consider the weathering of silicate rocks because this is a process removing carbon dioxide from the atmosphere (equation sil1 in Table 2). The natural system that we used for the development of the box model is presented in Figure 17, the box model is depicted in Figure 18 and the involved biogeochemical reactions are listed in Table 2. We postulate the presence of two feedback mechanisms: Silicate weathering and burial of carbonates. These two mechanisms will be discussed in more detail below, together with the required model-conditions. These requirements are divided in two groups: The first group consists of estimates based on the geologic record. These include values for the size of different reservoirs, fluxes and their respective isotopic composition. The second group considers chosen boundary conditions (artificial requirements): We postulate an initial steady state for the model runs and include the following forcing mechanisms (perturbations): Burial of organic matter, volcanic degassing of carbon dioxide and sulfide-fluxes. The assumptions then are combined with the mathematical equations to a numerical model.
Figure 17 Carbon, sulfur and silicate fluxes from and to the ocean-atmosphere system

The arrows represent fluxes that affect the alkalinity, the reservoir of inorganic carbon and the sulfate reservoir of the ocean-atmosphere reservoir.

Figure 17 illustrates the exogenic fluxes of carbon and sulfur. Additionally the weathering flux of silicate rocks is considered. Below, we introduce the involved fluxes. The abbreviations (e.g. c1) refer to the box model (Figure 18) and the chemical equations (Table 2).

- **Burial and weathering of carbonates:** The burial and weathering fluxes of carbonate have a major effect on the concentration of $\text{CO}_3^{2-}$. These fluxes therefore affect the alkalinity of the ocean and the amount of total inorganic carbon (weathering = c1; burial = c2).

- **Burial and weathering of organic matter:** These fluxes influence the amount of carbon dioxide (weathering = c3; burial = c4).

- **A flux not depicted in Figure 17 and Figure 18 is the release of methane from gas hydrates. It can be considered as a special case of weathering of organic matter. Due to the light carbon isotope composition, a massive release of methane can cause a rapid negative shift in the marine carbon isotope record ("negative spike", methane-flux = c5).**

- **Degassing of carbon dioxide from volcanoes and seafloor hydrothermal systems:** CO$_2$ is released by volcanic and hydrothermal activity (c6).

- **Burial and weathering of sulfate (mainly evaporites):** The burial- and weathering fluxes of sulfate affect the oceanic calcium and sulfate reservoirs but do not change seawater alkalinity (weathering = s1, burial = s2).

- **Burial and weathering of sulfides (mainly pyrite):** These fluxes deliver or remove hydrogen ions and therefore strongly affect the alkalinity of seawater (weathering = s3, burial = s4).
• Hydrogen sulfide is degassed from seafloor hydrothermal systems. This flux has a similar effect as the weathering flux of sulfides: Due to the input of hydrogen ions, the alkalinity declines (s5).

• Precipitation and leaching of anhydrite in seafloor hydrothermal systems: Due to the heating of seawater in hydrothermal systems, anhydrite is precipitated. When cold seawater penetrates into inactive (abandoned) hydrothermal systems, the reverse process takes place; anhydrite is leached. Similar to the weathering and burial of evaporites, this process affects the calcium and sulfate pool of the ocean without causing changes in seawater alkalinity.

• Weathering of silicate rocks: This process transforms carbon dioxide to bicarbonate and delivers Ca$^{2+}$ and Mg$^{2+}$ ions to the ocean reservoir (sil1).

The descriptive picture of Figure 17 can be transformed into a more schematic diagram (descriptive box model), which illustrates the flow-paths of elements (as chemical compounds, Figure 18). Each of these fluxes has a specific effect on the investigated reservoirs. These effects are described by chemical equations (Table 2).
tems. The abbreviations (e.g. c6 for volcanic degassing of carbon dioxide) refer to the chemical equations in Table 2.

The fluxes illustrated in Figure 18 are combined with the chemical equations for the involved processes. Considering that the fluxes in the carbon and sulfur system also involve a transfer of isotopes, mass balances and isotope mass balances can be calculated for each involved reservoir. We calculated the equations for the carbon, sulfur and alkalinity reservoir. In Table 2, we also listed the effect of each flux on the amount of oxygen in the atmosphere. For completeness, we also calculated the corresponding oxygen mass balance, however, we did not consider the oxygen reservoir further.

<table>
<thead>
<tr>
<th>Flux</th>
<th>Chemical reactions</th>
<th>Alk</th>
<th>C</th>
<th>S</th>
<th>O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>c1) wea carbonate</td>
<td>CaCO₃ → Ca²⁺ + CO₂⁻</td>
<td>+2</td>
<td>+1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>c2) buf carbonate</td>
<td>Ca²⁺ + CO₂⁻ → CaCO₃</td>
<td>-2</td>
<td>-1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>c3) wea corg</td>
<td>CH₃O + O₂ → CO₂ + H₂O</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-1</td>
</tr>
<tr>
<td>c4) buf corg</td>
<td>CO₂ + H₂O → CH₃O + O₂</td>
<td>-</td>
<td>-1</td>
<td>-</td>
<td>+1</td>
</tr>
<tr>
<td>c5) methane</td>
<td>CH₄ + 2O₂ → CO₂ + 2H₂O</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-2</td>
</tr>
<tr>
<td>c6) vaLCO₂</td>
<td>CO₂</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>s1) wea sulfate</td>
<td>Ca SO₄ → Ca²⁺ + SO₄²⁻</td>
<td>-</td>
<td>-</td>
<td>+1</td>
<td>-</td>
</tr>
<tr>
<td>s2) buR sulfate</td>
<td>Ca²⁺ + SO₄²⁻ → Ca SO₄</td>
<td>-</td>
<td>-</td>
<td>-1</td>
<td>-</td>
</tr>
<tr>
<td>s3) wea sulfide</td>
<td>4FeS₂ + 15O₂ + 8H₂O → 2Fe₂O₃ + 8SO₄²⁻ + 16H⁺</td>
<td>-2</td>
<td>-</td>
<td>+1</td>
<td>-15 8</td>
</tr>
<tr>
<td>s4) buR sulfide</td>
<td>2Fe₂O₃ + 8SO₄²⁻ + 16H⁺ → 4FeS₂ + 15O₂ + 8H₂O</td>
<td>+2</td>
<td>-</td>
<td>-1</td>
<td>+15 8</td>
</tr>
<tr>
<td>s5) hydro₃,5</td>
<td>H₂S + 2O₂ → 2H⁺ + SO₄²⁻</td>
<td>-2</td>
<td>-</td>
<td>+1</td>
<td>-2</td>
</tr>
<tr>
<td>s6) hydro sulfate (leach)</td>
<td>Ca SO₄ → Ca²⁺ + SO₄²⁻</td>
<td>-</td>
<td>-</td>
<td>+1</td>
<td>-</td>
</tr>
<tr>
<td>s7) hydro sulfate (precip)</td>
<td>Ca²⁺ + SO₄²⁻ → Ca SO₄</td>
<td>-</td>
<td>-</td>
<td>-1</td>
<td>-</td>
</tr>
<tr>
<td>sll1) wea silicate</td>
<td>2CO₂ + H₂O + CaSiO₃ → Ca²⁺ + 2HCO₃⁻ + SiO₂</td>
<td>+2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Alkalinity:
Alk = HCO₃⁻ + 2CO₃²⁻ - H⁺ + OH⁻ = Na⁺ + 2Mg²⁺ + 2Ca²⁺ - Cl⁻ - 2SO₄²⁻

Total inorganic carbon:
C = CO₂ + HCO₃⁻ + CO₃²⁻

| Reservoir |

Table 2 Chemical reactions of the considered fluxes and their effect on alkalinity, the reservoir of inorganic carbon, sulfate and oxygen

The listed effects can be combined to mass balance equations.

Below, we present the developed isotope and mass balance equations for the carbon and sulfur system and the mass balance equations for alkalinity and oxygen. The fluxes correspond to Table 2 respectively Figure 18. The mass balances are calculated as differential equations,
reflecting the temporal changes in the alkalinity, the amount of oxygen, the carbon isotope composition and the amount of inorganic carbon, respectively the sulfur isotope composition and the amount of sulfate.

Isotope and mass balance of inorganic carbon in atmosphere-ocean system:

\[
\frac{dM_C}{dt} = \text{wea}_{\text{carbonate}} - \text{bur}_{\text{carbonate}} + \text{wea}_{\text{corg}} - \text{bur}_{\text{corg}} + \text{methane} + \text{volc}_{\text{CO}_2},
\]

\[
\frac{d(\delta^{13}C_C \cdot M_C)}{dt} = \text{wea}_{\text{carbonate}} \cdot \delta^{13}C_{\text{carbonate}} - \text{bur}_{\text{carbonate}} \cdot \left( \delta^{13}C_C + \Delta_{\text{carbonate}} \right) + \text{wea}_{\text{corg}} \cdot \delta^{13}C_{\text{corg}} + \text{methane} \cdot \delta^{13}C_{\text{methane}} - \text{bur}_{\text{corg}} \cdot \left( \delta^{13}C_C + \Delta_{\text{corg}} \right) + \text{volc}_{\text{CO}_2} \cdot \delta^{13}C_{\text{volc}}
\]

where:

\[
\frac{d}{dt} \quad \text{derivative after time}
\]

\[M_C \quad \text{amount of total inorganic carbon}
\]

\[\delta^{13}C_C \quad \text{carbon isotope composition of inorganic carbon in seawater}
\]

\[\delta^{13}C_{\text{carbonate}} \quad \text{carbon isotope composition of weathered carbonates}
\]

\[\Delta^{13}C_{\text{carbonate}} \quad \text{carbon isotope enrichment factor for carbonates}
\]

\[\delta^{13}C_{\text{corg}} \quad \text{carbon isotope composition of weathered organic matter}
\]

\[\Delta^{13}C_{\text{corg}} \quad \text{carbon isotope enrichment factor for organic matter}
\]

\[\delta^{13}C_{\text{volc,CO}_2} \quad \text{carbon isotope composition of carbon dioxide degassed by volcanoes}
\]

Isotope and mass balance of seawater-sulfate:

\[
\frac{dM_{SO_4}}{dt} = \text{wea}_{\text{sulfur}} - \text{bur}_{\text{sulfur}} + \text{wea}_{\text{sulfide}} - \text{bur}_{\text{sulfide}} + \text{hydro}_{\text{H}_2\text{S}} + \text{hydro}_{\text{sulfate leach}} - \text{hydro}_{\text{sulfate precip}}
\]

\[
\frac{d(\delta^{34}S_{SO_4} \cdot M_{SO_4})}{dt} = \text{wea}_{\text{sulfur}} \cdot \delta^{34}S_{\text{sulfur}} - \text{bur}_{\text{sulfur}} \cdot \left( \delta^{34}S_{SO_4} + \Delta_{\text{sulfur}} \right) + \text{wea}_{\text{sulfide}} \cdot \delta^{34}S_{\text{sulfide}} - \text{bur}_{\text{sulfide}} \cdot \left( \delta^{34}S_{SO_4} + \Delta_{\text{sulfide}} \right) + \text{hydro}_{\text{sulfate leach}} \cdot \delta^{34}S_{\text{sulfate leach}} - \text{hydro}_{\text{sulfate precip}} \cdot \delta^{34}S_{SO_4} + \text{hydro}_{\text{H}_2\text{S}} \cdot \delta^{34}S_{\text{H}_2\text{S}}
\]
where:

\[ \frac{d}{dt} \]

derivative after time

\[ M_{SO4} \]

amount of sulfate in ocean

\[ \delta^{34}S_{SO4} \]

sulfur isotope composition of inorganic carbon in seawater

\[ \delta^{34}S_{sulfate} \]

sulfur isotope composition of weathered sulfate (evaporites)

\[ \Delta^{34}S_{sulfate} \]

sulfur isotope enrichment factor for sulfates

\[ \delta^{34}S_{sulfide} \]

sulfur isotope composition of weathered sulfides (pyrite)

\[ \Delta^{34}S_{sulfide} \]

sulfur isotope enrichment factor for sulfides

\[ \delta^{34}S_{sulfate(ether)} \]

sulfur isotope composition of leached anhydrite

\[ \delta^{34}S_{H2S} \]

sulfur isotope composition of degassed hydrogen sulfide

Alkalinity mass balance:

\[ \frac{dAlk}{dt} = -2 \left( bur_{carbonate} - wea_{carbonate} \right) + 2 \left( bur_{sulfide} - wea_{sulfide} \right) - 2 \cdot hydro_{H_2S} + 2 \cdot wea_{silicate} \]

Oxygen mass balance for atmosphere-ocean system (not used in the model calculations):

\[ \frac{dO_2}{dt} = bur_{org} - wea_{org} + \frac{15}{8} \cdot bur_{sulfide} - \frac{15}{8} \cdot wea_{sulfide} - 2 \cdot hydro_{H_2S} - further_{oxy} \]

where:

\[ further_{oxy} \]

In a model investigating the response of the amount of oxygen in the atmosphere, besides of the fluxes in the sulfur and carbon system, further oxygen sinks would have to be considered.
Required parameters and initial values

To develop a numerical model, the values of the involved parameters (e.g. size, rate and isotope composition) have to be estimated. This is not trivial, because for many values, the geologic record does not provide precise information. This has to be considered in the interpretation of the model results. For the derivation and the discussion of the estimated and calculated values see Appendix 1. The marked values (*) for fluxes have been determined from the calculation of an initial steady state below. These fluxes are subject to change when different estimates for the initial values are used (i.e. the isotope fractionation factors or the initial isotope compositions are changed).

<table>
<thead>
<tr>
<th>Reservoir</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_C$ (total inorganic carbon)</td>
<td>$3.8 \times 10^{18}$ mol</td>
</tr>
<tr>
<td>$M_{SO_4}$ (amount of sulfate)</td>
<td>$40 \times 10^{18}$ mol</td>
</tr>
<tr>
<td>$Ca^{2+}$ (amount of calcium)</td>
<td>$15 \times 10^{18}$ mol</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>$3.9 \times 10^{18}$ mol</td>
</tr>
<tr>
<td>$CO_3^{2-}$</td>
<td>$15 \times 10^{16}$ mol</td>
</tr>
<tr>
<td>$H_2CO_3$</td>
<td>$5 \times 10^{16}$ mol</td>
</tr>
</tbody>
</table>

$\alpha$ = 10:1

Isotope compositions

| $\delta^{13}C_{volc,CO2}$   | $-5\%$                     |
| $\delta^{13}C_{methane}$    | $-60\%$                    |
| $\delta^{34}S_{fluvial,IN}$ | $7\%$                      |
| $\delta^{34}S_{H2S}$        | $2.5\%$                    |

Isotope enrichment factors

| $\Delta^{13}C_{carbonate}$  | $0\%$                      |
| $\Delta^{13}C_{corg}$       | $-28\%$                    |
| $\Delta^{34}S_{sulfate}$    | $0\%$                      |
| $\Delta^{34}S_{sulfide}$    | $-40\%$                    |


Fluxes

\[
\begin{align*}
\text{volc}_{\text{CO}_2} & = 6 \times 10^{12} \text{ mol/a} \\
\text{hydro}_{\text{H}_2\text{S}} & = 0.41 \times 10^{12} \text{ mol/a} \\
\text{methane} & = 0 \text{ mol/a} \\
\text{bur}_{\text{carbonate}} & = 40 \times 10^{12} \text{ mol/a} \\
\text{wea}_{\text{carbonate}} & = 35.5 \times 10^{12} \text{ mol/a} \\
\text{bur}_{\text{org}} & = 10 \times 10^{12} \text{ mol/a} \\
\text{wea}_{\text{org}} & = 8.5 \times 10^{12} \text{ mol/a} \\
\text{bur}_{\text{sulfate}} & = 2.55 \times 10^{12} \text{ mol/a} \\
\text{wea}_{\text{sulfate}} & = 2.55 \times 10^{12} \text{ mol/a} \\
\text{bur}_{\text{sulfide}} & = 1 \times 10^{12} \text{ mol/a} \\
\text{wea}_{\text{sulfide}} & = 0.85 \times 10^{12} \text{ mol/a} \\
\text{hydro}_{\text{sulfate (precip)}} & = 0.5 \times 10^{12} \text{ mol/a} \\
\text{hydro}_{\text{sulfate (leach)}} & = 0.24 \times 10^{12} \text{ mol/a} \\
\text{wea}_{\text{silicate}} & = 4.76 \times 10^{12} \text{ mol/a}
\end{align*}
\]

(*)

Chosen boundary conditions

Definition of a model-steady state:

The cycles of carbon and sulfur are hardly ever at a steady state. Both systems would reach a steady state only if the involved fluxes would not change for several residence times (a few ten thousands to hundred thousands of years for inorganic carbon, probably about twenty million years for sulfate). However, the reactions of these cycles on a perturbation in a numerical model are best studied when a steady state is simulated: A change in the fluxes and reservoirs is caused by the perturbation and not by a “background” trend due to a non-steady state system. Some of the fluxes (above marked with (*)) in our model therefore are calculated from the assumptions taken for other fluxes and isotopic compositions in order to match steady state conditions. The corresponding equations are described in Appendix 2.

The determination of a steady state for the sulfur and calcium reservoir is problematic. This is due to our poor knowledge concerning the following three fluxes in the sulfur cycle: the precipitation and leaching fluxes of anhydrite in seafloor hydrothermal systems and the deposition of sulfate as evaporites. The deposition of evaporites is typically highly episodic and the mechanisms of leaching- and precipitation of anhydrite in seafloor hydrothermal systems is poorly understood (BRUNNER et al., 2003a: Chapter 4). However, these processes are likely to
have a certain buffering effect on the amount of sulfate in seawater. High concentrations of sulfate will increase the potential to precipitate large amounts of evaporites. In seafloor hydrothermal systems, where sulfate is entirely removed from seawater, high concentrations of sulfate will cause an increase in the deposition of anhydrite. We therefore assume, for the sake of a steady state, that the burial flux of evaporites equals the corresponding weathering flux, while the difference in the fluxes for the precipitation and leaching of anhydrite has to compensate for other potential imbalances in the sulfur cycle. These fluxes neither affect the budget of total inorganic carbon nor seawater alkalinity and their influence on the sulfur isotope composition of seawater is small compared to other fluxes. Consequently, this arbitrary decision has little direct impact on the results of our model. However, there is a severe consequence for the calculation of the calcium reservoir: This reservoir depends on the discussed sulfate-fluxes and the model results for the calcium reservoir would hardly be useful if these arbitrarily chosen fluxes were included. Consequently, the deposition and weathering of evaporites and the precipitation and leaching of anhydrite have not been included into the calculation of the calcium reservoir. This solution has a disadvantage: The calculated size of the calcium reservoir has to be interpreted with precaution since not all fluxes are included into the mass balance. The reported Ca$^{2+}$-values only reflect trends.

Sediment recycling:

A hardly known parameter is how fast weathering processes recycle deposited sediments. Therefore, the isotopic composition of the weathered sediments is not known. A slow recycling model preserves a constant isotope value for the weathered sediments; the other extreme, a rapid recycling model, would set the value for the isotopic composition of the weathered sediments equal to the value of simultaneously deposited sediments. We decided to implement a slow (non-) recycling algorithm because of the problematic definition of sulfate-steady state fluxes: If we would implement a rapid recycling model, the large fluxes for precipitation and leaching of anhydrite as well as the burial and weathering fluxes of evaporites would drive the sulfur system rapidly towards a steady state. Since we calculated a relatively short residence time of 10 Ma for the sulfur cycle (see Appendix 1), this is likely to be too fast. For consistency reasons, we also implemented a slow recycling for the carbon cycle. A consequence of this type of sediment recycling is an overestimation of the fluxes that are calculated as forcing mechanisms (burial of organic matter and sulfide fluxes); the other extreme, a rapid recycling model would tend to underestimate the forcing mechanisms.
Forcing mechanisms:
We implement three different “forcing” mechanisms (Figure 15):

- **Input of carbon dioxide from volcanic activity:**
The input of carbon dioxide can be changed to simulate increased volcanic and hydrothermal activity.

- **Burial of organic matter:**
The Valanginian and Aptian carbon isotope records indicate that strong imbalances (positive carbon isotope excursion) between the weathering and burial fluxes of organic matter occurred. Our model therefore calculates the burial flux needed to match an idealized carbon isotope curve.

- **Changes in sulfide fluxes (burial of sulfide, weathering of sulfide, input of hydrogen sulfide from seafloor hydrothermal systems, change in the sulfur isotope enrichment factor of sulfides):**
The Aptian sulfur isotope curve is marked by a 1‰ decrease from about 17‰ to 16‰ (measured $\delta^{34}\text{S}$ of SSS minus 1‰ in order to match barite record, see above). In order to identify the cause for this shift, the model separately calculates the time-dependent size of the fluxes potentially triggering this perturbation: The burial and weathering flux of sulfides and the degassing of hydrogen sulfide from seafloor hydrothermal systems. Additionally, the model calculates the change in the sulfur isotope enrichment factor of sulfide that would be needed to cause the sulfur isotope shift.

The forcing mechanisms are recalculated in each iteration step. Therefore, the fluxes of organic matter and sulfides are simultaneously in- and output parameters. For the mathematical calculation of these fluxes we refer to Appendix 4.

Feedback mechanisms:
We consider two different “feedback” mechanisms: Silicate weathering and burial of carbonates. We assume that the weathering-intensity of silicates correlates to the turnover rates in the global water cycle, which in turn is temperature-driven. Since carbon dioxide acts as greenhouse gas, high pCO$_2$ will induce an increase in the weathering flux of silicates. Silicate weathering removes carbon dioxide and therefore provides a negative (dampening) pCO$_2$-feedback. For a calculation of this weathering feedback, we need a proxy for pCO$_2$. From the size of the alkalinity and carbon reservoir, we can calculate the concentrations of the dissolved carbon species $\text{H}_2\text{CO}_3$ (dissolved carbon dioxide), $\text{HCO}_3^-$ (bicarbonate ion) and $\text{CO}_3^{2-}$ (carbonate ion). In surface waters, dissolved carbon dioxide is exchanged with atmospheric
carbon dioxide, atmospheric pCO$_2$ and dissolved carbon dioxide concentrations therefore are closely related. However, this relation is not constant: The equilibrium concentration of carbon dioxide with seawater strongly depends on the water temperature (at low temperatures water contains more dissolved carbon dioxide). A further complication is the distribution of dissolved carbon dioxide in seawater: deep water masses, which do not exchange CO$_2$ with the atmosphere, contain more dissolved carbon dioxide than surface waters. Finally, the dependency of the turnover rates in the water cycle on CO$_2$ as a greenhouse gas is not linear. Because of these restrictions we have to use an arbitrary assumption for the relation between the weathering of silicates and the concentration of dissolved carbon dioxide. We use the following equation:

$$\text{wea}_{\text{silicate}}(t) = \text{wea}_{\text{silicate}}(\text{initial}) \cdot \left(1 + 0.1 \cdot \left( \frac{[\text{H}_2\text{CO}_3](t)}{[\text{H}_2\text{CO}_3](\text{initial})} - 1 \right) \right)$$

The equation is formulated in order to keep the time dependent silicate weathering flux close to the initial steady state flux: The change in the pCO$_2$ is approximated as the ratio between the time dependent concentration of dissolved carbon dioxide and its initial counterpart. We assume that a rise in the pCO$_2$ causes a buffered reaction in the water cycle and therefore, we multiply the calculated change by an arbitrary value of 0.1: A doubling in the concentration of dissolved carbon dioxide (a 100% increase) causes a doubling in the pCO$_2$ and an increase in the weathering flux of silicates of 10%. This might be a reasonable estimate. The calculation of the concentration of dissolved carbon dioxide from the amount of total inorganic carbon and alkalinity is derived in Appendix 3.

The second implemented feedback mechanism is the burial of carbonates. For two reasons, this flux depends to a great extent on the carbonate solubility product ([CO$_3^{2-}$] x [Ca$^{2+}$]):

- In the ocean, this product controls the position of the lysocline (depth where seawater is undersaturated with respect to calcium carbonate) and consequently the global carbonate burial flux.
- Surface waters are supersaturated with respect to aragonite and calcite. In today’s surface waters, the product of the concentrations of carbonate ions and calcium ions is roughly 6-7 times larger than the theoretical carbonate solubility product. A lowering of this supersaturation leads to a decrease in the calcification rate of biocalcifying organisms, such as corals, green algae and calcareous nanoplankton (GATTUSO and BUDDEMEIER, 2000; GATTUSO et al., 1998; KLEYPAS et al., 1999; RIEBESELL et al., 2000).
As discussed above, the calculated values for the amount of calcium only tell us if this reservoir is growing or diminishing. However, the calcium reservoir is two magnitudes larger than the reservoir of \( \text{CO}_3^{2-} \). A small change in the amount of \( \text{CO}_3^{2-} \) therefore has a strong impact on the product of the concentrations of \( \text{CO}_3^{2-} \) and \( \text{Ca}^{2+} \); a large change in the amount of \( \text{Ca}^{2+} \) has only a minor effect. Consequently, the concentration of the carbonate ion mainly controls the burial flux of carbonates. We therefore assumed a linear relationship between the burial flux of carbonates and the concentration of \( \text{CO}_3^{2-} \):

\[
\text{bur}_{\text{carbonate}}(t) = \text{bur}_{\text{carbonate}}(\text{initial}) \cdot \left( \frac{[\text{CO}_3^{2-}](t)}{[\text{CO}_3^{2-}](\text{initial})} \right)
\]

If the concentration of \( \text{CO}_3^{2-} \) is half the initial value, the burial flux of carbonate is equal to one half of its initial flux. We postulate the 1:1 relation for this strong feedback because otherwise, with a weak relation, the concentration of \( \text{CO}_3^{2-} \) could drop to values where the whole ocean would be undersaturated with respect to carbonate while carbonates would still be buried. This would be an unrealistic scenario. For the calculation of the concentration of \( \text{CO}_3^{2-} \), see Appendix 3.
**Model Runs: Results and Interpretation**

With the developed numerical model, we proceed as follows:

- We investigate the possible reasons for the observed change in the sulfur isotope composition of seawater sulfate in the Aptian (Figure 19, Run 1).
- We check how these different scenarios influence the oceanic alkalinity and carbon reservoir, i.e. the effect on the carbonate equilibrium (Figure 20, Run 2-4).
- After an evaluation of the possible scenarios, we will conclude that the most likely scenario is the degassing of hydrogen sulfide and sulfur dioxide from seafloor hydrothermal systems and volcanoes. We investigate the effect of such a flux on the carbonate equilibrium and on the involved feedback mechanisms, the weathering of silicates and the burial of carbonates (Figure 21, Run 5-7).
- It is likely that the degassing of carbon dioxide from volcanic activity strongly increased in the Aptian (and Valanginian). We therefore investigate the impact of an increase in the input of CO₂ on the Aptian carbon system (Figure 22, Run 8-10).
- In a next step, we combine the degassing of carbon dioxide and hydrogen sulfide.
- The Aptian (and Valanginian) are marked by positive carbon isotope excursions. These excursions are most likely due to an imbalance in the weathering- and burial fluxes of organic matter. We express this imbalance by a change in the burial flux of organic matter and test the effect of this additional parameter to the degassing fluxes of hydrogen sulfide and carbon dioxide on the carbonate equilibrium and the involved feedback mechanisms (Figure 23, Run 11).
- To test if the above system would balance itself without a strong carbonate burial feedback, we exclude this feedback mechanism in an additional run (Figure 23, Run 12).
- In a last model run for the Aptian time slice, we include the massive degassing of methane from gas hydrates to simulate the observed negative spike in the carbon isotope record. We investigate in how far this additional perturbation affects the carbonate equilibrium and summarize the model results in a perturbation-scenario or the Early Aptian (Figure 24, Run 13).
- Finally, we present a model run for the Valanginian, where we investigate the effect of increased carbon dioxide degassing and enhanced burial of organic matter (Figure 25, Run 14).
Run 1 (Figure 19)

In the Early Aptian, the $\delta^{34}S$ of seawater sulfate changes from $17\%$ to $16\%$ within a time span of about three million years. We therefore tested how such a change can be produced. We identified four different “candidates”: An increase in the weathering flux of sulfides (isotopically light sulfate is delivered to the ocean), a decrease in the burial flux of sulfides (less isotopically light sulfides are removed from seawater), an increase in the input of isotopically light hydrogen sulfides by seafloor hydrothermal systems and a change in the enrichment factor for sulfides.

As discussed above, we implemented a slow sediment recycling model. To exemplify the difference of such an approach to a rapid recycling model, both calculation-types are illustrated in Figure 19.

Figure 19  Run 1: Possible reasons for the observed change in the sulfur isotope composition of seawater sulfate in the Aptian

The estimates for the changes in the sulfide fluxes are larger for a slow recycling model than for a rapid sediment recycling. A further difference is that in a rapid recycling model, the fluxes immediately return to their initial sizes after the end of the perturbation. In a slow recy-
clinging model, the fluxes remain differently from the initial value in order to sustain the new isotope composition of seawater sulfate (16‰ instead of 17‰). The “real” flux value would be somewhere in between of the calculated extremes. However, we will run the following models assuming a slow sediment recycling, being aware that we tend to overestimate the involved fluxes.

The model runs show (upper picture in Figure 19) that the weathering flux of sulfides would have be increased by about 30% to cause the –1‰ sulfur isotope effect. The same effect could also be caused by decrease of about 30% in the burial flux of sulfides. The much smaller initial flux of hydrogen sulfide from seafloor hydrothermal systems has to be increased by a factor of 3 to 4 in order to cause the 1‰ negative shift. The fourth possibility to create an isotope shift is to change the sulfur isotope enrichment factor of sulfides from –40‰ to smaller values. As consequence, the burial flux of sulfides remains at its initial size, but the isotopic composition of the removed sulfides is less negative during the perturbation. The lower picture in Figure 19 shows that the enrichment factor would have to be around –27‰ instead of –40‰ to cause a –1‰ sulfur isotope effect. The last scenario would not at all influence the sizes of the involved sulfide fluxes, therefore no change in the carbonate equilibrium and alkalinity would occur. The other fluxes however influence the oceanic alkalinity and also the carbonate equilibrium. To check the importance of these potential changes, we calculated the response of the alkalinity and the carbon reservoirs affected by the different forcing mechanisms without using feedback mechanisms (Figure 20, Run 2-4).

- **Run 2-4 (Figure 20)**

The results of the runs 2-4 (Figure 20) show that the change in each of the sulfide fluxes causes a rapid drop in the alkalinity. In the case of enhanced degassing of hydrogen sulfide, the alkalinity would drop below zero, the ocean would become acid (arrow in right picture column, Figure 20).
Figure 20
The impact of changing sulfide fluxes on the carbonate equilibrium

In each scenario, the amount of dissolved \( \text{H}_2\text{CO}_3 \) rapidly grows while the amount of \( \text{CO}_3^{2-} \) drops to zero. No feedback mechanisms were involved.

Left picture column: Increase in the flux of sulfide weathering

Middle picture column: Decrease in the burial flux of pyrite

Right picture column: Increase in the input of hydrogen sulfide

Run 2
Increase in pyrite weathering

Run 3
Decrease in pyrite burial

Run 4
Increase in the input of \( \text{H}_2\text{S} \)
A consequence of this drop in the alkalinity is a complete turnover in the carbonate equilibrium: The concentration of carbonate ions drops rapidly to zero while the amount of dissolved carbon dioxide increases (and goes off scale). We therefore conclude that changing sulfide fluxes do strongly influence the alkalinity of seawater and that this change has to be buffered by feedback mechanisms in order to maintain reasonable conditions.

The above results do not exclude any of the proposed scenarios to cause a $-1\%$ drop in the sulfur isotope composition of seawater. However, the calculated size of change needed to match the required isotope shift can be used to discuss the probability of each proposed scenario:

A. Increase in the weathering flux of sulfide by 30%: From the rock record, it is almost impossible to judge if a quantitatively important increase in the weathering flux from the continents took place during the investigated time span. However, an increase in the weathering of sulfides (pyrite) has to go together with an increase in the weathering flux of organic matter, because pyrite is most abundant in organic-rich rocks. The here used sulfide-organic matter burial ratio of 1:10 has to be reflected in a similar weathering ratio. Therefore, the weathering flux of organic matter would also have to be increased by 30%. This would lead to a negative carbon isotope excursion with a duration of three million years. This is not observed. One could argue that this negative trend was compensated by an increase in the burial of organic matter. However, an increase in the burial of organic matter would also enhance the burial of sulfides. This would in turn also buffer a negative sulfur isotope excursion resulting into no sulfur isotope excursion at all. An increase in the weathering flux of sulfides is therefore an unlikely process to explain the Aptian sulfur isotope shift.

B. The above reasoning holds also for a decrease in the burial flux of sulfides: This should go together with a 30% decrease in the burial flux of organic matter, a unrealistic scenario in a time of enhanced deposition of organic matter (positive C-isotope excursion). Additionally, it is unlikely that during the OAE 1a less sulfides were buried than weathered.

C. 3- to 4-fold increase in the flux of hydrogen sulfide from seafloor hydrothermal systems: Evidence for a strong increase in the hydrothermal activity comes from the strontium isotope record, showing rapid negative shift coinciding with the OAE 1a (JONES and JENKYNs, 2001). JONES and JENKYNs (2001) interpret this and other negative shifts in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio as a “short-term burst of hydrothermal activity” at mid ocean ridges. They calculate that for such a shift, hydrothermal activity has to increase between 7% and 104%. The large range is due to the uncertainties in the relative flux of strontium from axial high-temperature hydrothermal
systems and low-temperature off-axis systems. These uncertainties about the flux sizes in the oceanic strontium budget probably are even underestimated: DAVIS et al. (2003) calculated a ocean crust basaltic strontium flux (3.1±0.8 x 10^9 mol/a) five times smaller than the value estimated by JONES and JENKYNs (2001) (15 x 10^9 mol/a) and concluded that the present day strontium budget is not balanced. According to JONES and JENKYNs (2001), a small initial axial hydrothermal Sr flux (3 x 10^9 mol/a) would imply that the increase in the Aptian hydrothermal activity is rather 100% than 7%. However, the decrease in the sulfur isotope record seems to precede the change in the strontium isotope record: Our data show trend to lower values already prior to the OAE 1a. This would indicate that a part of the sulfur isotope trend cannot be correlated with the strontium isotope record: However, the emplacement of the Ontong Java Plateau was prior to the Sr isotope shift and is likely to have been an enormous source for sulfur dioxide. The effect of sulfur dioxide on the sulfur isotope budget is similar to the degassing of hydrogen sulfide because the sulfur isotope composition of degassed SO_2 (δ^{34}S around +3.5‰, BRUNNER et al. (2003a: Chapter 4)) is close to the one of hydrogen sulfide (here assumed to be +2.5‰). The effect of the input of sulfur dioxide and hydrogen sulfide on the alkalinity are equal. Both reactions deliver the same amount of hydrogen ions to the ocean:

\[2H_2S + 4O_2 \Rightarrow 4H^+ + 2SO_4^{2-}\]
\[2SO_2 + 2H_2O+O_2 \Rightarrow 4H^+ + 2SO_4^{2-}\]

The only difference in the two chemical reactions is the amount of consumed oxygen. However, we do not consider the oxygen reservoir in our model and therefore, the input of SO_2 can be added to the flux of hydrogen sulfide. THORDARSON et al. (2001) calculated the release of sulfur dioxide of the largest flood lava eruption in the last millennium, the Eldgjá eruption (Iceland), and report an annual flux of 30 to 70 Mt SO_2 per year (=0.47 to 1.1 x 10^{12} mol/a). It is reasonable to assume that the sulfur dioxide flux from the Ontong Java igneous province was not smaller than this estimate. The initial flux of hydrogen sulfide in the model of 0.41 x 10^{12} mol/a therefore was at least doubled during the submarine emplacement of the Ontong Java Plateau. WIGNALL (2001) speculates that the emitted SO_2 was oxidized in the water column and did not reach the atmosphere. Based on the presented evidence, we conclude that the scenario of a strongly increased flux of hydrogen sulfides (from enhanced Aptian Mid-Ocean seafloor hydrothermal activity) combined with the degassing of sulfur dioxide (from the emplacement of the Ontong Java Plateau) is a reasonable explanation of the postulated 3 to 4 fold increase in the model flux of hydrogen sulfide.
D. Change in the sulfur isotope enrichment factor from $-40\%\text{e}$ to $-27\%\text{e}$: The sulfur isotope composition of the sulfides measured by us show a strong scatter. We do not observe a trend, e.g. less negative $\delta^{34}\text{S}$ values for sulfides formed during the OAE 1a. Our data would rather suggest a larger sulfur isotope fractionation during a part of the OAE 1a. However, a trend to smaller isotope enrichment factors under anoxic conditions has been proposed by other authors (KAIHO et al., 1999; KAJIWARA and KAIHO, 1992). Our sulfur isotope data set is too small to exclude this possibility. However, recent investigations demonstrate that there is no consistent drop in the sulfur isotope enrichment factor in anoxic water bodies (WERNE et al., 2003; WORTMANN et al., 2001). Further, as pointed out above, the decrease in the sulfur isotope composition of seawater sulfate starts prior to the OAE 1a. If the $1\%\text{e}$ negative sulfur isotope shift of seawater sulfate would have to be accomplished during the time span of the OAE 1a with a duration of 0.8 to 1.2 Ma (HERBERT, 1992; WISSLER (2001)) instead of 3 Ma the sulfur isotope enrichment factor would get close to zero, which is unrealistic.

Considering the above discussion, we conclude that the observed $1\%\text{e}$ drop in the $\delta^{34}\text{S}$ of seawater sulfate in the Aptian was due to a strong increase in the degassing of sulfur dioxide and hydrogen sulfide. Because both fluxes have the same effect on the alkalinity, they can be treated as a single flux. For simplicity, we keep the expression “hydrogen sulfide degassing” in the model runs.
Figure 21
The impact of degassing of H₂S and SO₂ on the carbonate equilibrium

The inclusion of feedback mechanisms strongly affects the amount of dissolved H₂CO₃ and CO₃²⁻.

Left picture column: Inclusion of a silicate weathering feedback

Middle picture column: Inclusion of a carbonate burial feedback

Right picture column: Inclusion of a carbonate burial and silicate weathering feedback

Run 5
Inclusion of silicate weathering

Run 6
Inclusion of carbonate burial

Run 7
Carbonate & silicate feedback
• Run 5-7 (Figure 21)
In Figure 21 (Run 5-7), we investigate the effect of the H₂S–SO₂ flux on the carbonate equilibrium and on the implemented feedback mechanisms. The model results show that the carbonate and the weathering feedback mechanisms balance the input of hydrogen sulfide. However, with a pure silicate feedback (Run 5), the concentration of carbonate ions would drop to a third of its initial value. This would create an ocean strongly undersaturated with respect to carbonate; a rise in the CCD would result. The silicate feedback is therefore not sufficient to balance the perturbation and a carbonate feedback has to be included. In Run 6, we tested a pure carbonate feedback. As a response to the input of hydrogen sulfide, the burial of carbonates drops. This leads to a parallel increase of the alkalinity and carbon reservoir. However, the amount of dissolved carbon dioxide rises by a factor of ten. This would create an extreme greenhouse earth. Therefore, a pure carbonate feedback is very unlikely. Run 7 combines both feedback mechanisms. The rise in the amount of dissolved carbon dioxide is now 3-fold and drops right after the end of the perturbation. The alkalinity and carbon reservoir remain in a reasonable range. The system was buffered by changes in the weathering flux of silicates and the burial flux of carbonates: Silicate weathering rises about 10%, the burial of carbonates drops about 3%. The amount of calcium ions rises during the perturbation by 30%. Run 5-7 demonstrate that combined carbonate-silicate feedback mechanisms effectively buffer the input of hydrogen sulfide by significant but not dramatic changes in their respective flux sizes.

• Run 8-10 (Figure 22)
It is likely that the degassing of carbon dioxide strongly increased due to the volcanic and hydrothermal activity in the Aptian (and Valanginian). We therefore investigate the impact of an increase in the input of CO₂ on the Aptian carbon cycle (Figure 22, Run 8-10). We test how the carbonate equilibrium reacts on this perturbation with three different setups: First with no feedback at all (Run 8), secondly with a carbonate burial feedback (Run 9) and in a third run with a combination of the carbonate burial and the silicate weathering feedback (Run 10). As forcing mechanism, we chose a rise in the carbon dioxide degassing of 80% within a time span of 1 Ma (−121 to −120 Ma). Afterwards, the degassing rate remains at the elevated level. This exemplifies that ocean-ridge crust production (and also the large igneous province activity?) went on at high levels during the Aptian (and also during the Valanginian), for a compilation see Jones and Jenkyns (2001).
Feedback mechanisms have to be included in order to maintain reasonable CO$_3^{2-}$ concentrations. However, the silicate weathering and carbonate burial feedback mechanisms inefficiently buffer pCO$_2$.

Left picture column: No feedback implemented

Middle picture column: Inclusion of a carbonate burial feedback

Right picture column: Inclusion of a carbonate burial and silicate weathering feedback

Run 8  
No feedback mechanism

Run 9  
Inclusion of carbonate burial

Run 10  
Carbonate & silicate feedback
The model runs demonstrate that the amount of dissolved carbon dioxide strongly increases with or without feedback mechanisms. The weak silicate weathering feedback does not sufficiently buffer carbon dioxide partial pressures (9 times the initial value in Run 10). Over longer time intervals, either the silicate weathering has to be enhanced, the carbon dioxide degassing has to be lowered or a further feedback mechanism (e.g. burial of organic matter) has to compensate the ongoing outgassing of carbon dioxide. In Run 9 and 10, the carbonate burial is lowered by 5% to 10% at an initial stage of the CO₂-input. Due to the weathering feedback, the burial flux of carbonates later increases over its initial value.

• Run 11 and 12 (Figure 23)

The input of isotopically light carbon dioxide from volcanoes leads to a slight drop in the carbon isotope composition (Figure 22, Run 8 and 9). The carbon isotope record shows a different picture: The Aptian (and Valanginian) are marked by positive carbon isotope excursions. This is due to imbalances in the burial- and weathering fluxes of organic matter. These imbalances do also affect the amount of carbon dioxide an therefore have to be considered in our model. From the carbon isotope record, we calculate the corresponding burial flux of organic matter by assuming a constant burial flux of organic matter. We test the effect of this parameter in addition to the degassing flux of hydrogen sulfide and carbon dioxide (Figure 23, Run 11 and 12). Run 11 includes the both feedback mechanisms, while run 12 investigates if a carbonate burial feedback is still needed to balance the carbonate equilibrium when the burial flux of organic matter is integrated into the numerical model. The Aptian carbon isotope curve roughly rises from a value around 2‰ to 3‰ (at –121 Ma). The values stay on this level until the termination of the OAE 1a (the duration of the OAE 1 was estimated to be –120 to –119 Ma). However, a negative carbon isotope spike “interrupts” this plateau at the onset of the OAE 1a (not depicted). This spike is most likely due to an input of isotopically extremely light methane released from gas hydrates (the effect of this methane release is investigated in Run 13). After the termination of the OAE 1a a positive carbon isotope excursion up to 4‰ follows (peak at –118 Ma). In the following, the carbon isotope values decline.
Figure 23  The effect of burial of organic matter as additional forcing mechanism

The left picture column (Run 11) includes a carbonate burial feedback, the right does not (Run 12).

Figure 23 shows that the changes in the burial flux of organic matter strongly influence the oceanic carbonate equilibrium (Run 11 and 12). High burial rates of organic matter reduce the amount of dissolved carbon dioxide efficiently. However, without the carbonate burial feedback (Figure 23, Run 12), the concentration of carbonate ions would strongly shift. With respect to calcium carbonate, such an ocean would rapidly switch from an undersaturated to an oversaturated mode. This is not realistic. The implemented carbonate burial feedback mechanism therefore is needed to balance the carbonate equilibrium in seawater. Figure 24 (Run 13) summarizes the observations in a scenario for the perturbations in the Aptian.
• Run 13: A scenario for the perturbations in the geochemical cycles in the Early Aptian

The Early Aptian is a time slice marked by an increase in the seafloor spreading rates and by the emplacement of the largest single volcanic province on Earth, the Ontong Java Plateau in the SW Pacific. It is likely that the overall increase in volcanic activity led to a contribution of large amounts of carbon dioxide to the ocean-atmosphere system. From the sulfur isotope record, we conclude that additionally to the enlarged flux of carbon dioxide also the flux of hydrogen sulfide and sulfur dioxide strongly increased. The increase in these fluxes severely affected the alkalinity, the total amount of inorganic carbon and the oceanic carbonate equilibrium.

₁ At an initial stage of the CO₂, H₂S and SO₂ degassing, the oceanic carbonate equilibrium was stabilized by a decrease (≈5%) in the burial of carbonates, either by a rise of the carbonate compensation depth or by a decrease in the production rates of biocalcifying organisms (“carbonate crisis”). The concentration of carbonate ions in seawater therefore was balanced.

₂ However, the concentration of dissolved carbon dioxide and atmospheric pCO₂ constantly rose.

₃ We assume that this rise promoted a temperature rise and caused greenhouse conditions. This in turn induced an increase in the runoff from the continents and enhanced silicate weathering. The input of nutrients into the ocean and greenhouse conditions led to a higher productivity. We therefore observe an increase in burial rates of organic matter and a positive carbon isotope trend.

₄ However, the pCO₂-feedback by increased silicate weathering and burial of organic matter did not sufficiently compensate the rise in the amount of dissolved carbon dioxide. At the level, where the modeled H₂CO₃ content becomes almost three times its initial size we observe a negative spike in the carbon isotope record. A likely cause for this spike is the massive release of methane from gas hydrates. We speculate that this release was due to a “greenhouse induced” reorganization of the ocean circulation patterns, transporting warm water masses in formerly cold water mass regions. This destabilized gas hydrates and methane was released. The subsequent phase of oceanic anoxia (OAE 1a) supports the hypothesis of a change in the oceanic circulation pattern. The impact of the methane-release on the overall carbon and alkalinity budget is minor. However, the flux of carbonate burial probably experienced a short-time drop.
Figure 24  Run 13: A scenario for the perturbations in the geochemical cycles in the Early Aptian
During the OAE 1a (grey area), the burial rates remained high but did not compensate for the increase in dissolved carbon dioxide. From our model calculation, it seems unlikely that that burial of organic matter removed enough carbon dioxide to bring down pCO$_2$ to non-greenhouse conditions. Either, the pCO$_2$ would have to be diminished by a drop in the degassing of hydrogen sulfide and carbon dioxide from the Ontong Java plateau volcanism (not calculated) or the OAE 1a terminated under extreme greenhouse conditions (calculated).

Towards the end of the OAE 1a, the carbon isotope data show a negative trend pointing to a drop in the burial of organic matter (for a composite carbon isotope curve see HEIMHOFER et al. (2003)). This suggests that the “anoxia-type” burial of organic matter was not continuously replaced by an “oxic-type” burial mode. Probably, the “anoxic mode” had to be terminated before an “oxic mode” could be established: It is likely that again a reorganization of the ocean circulation patterns led to the termination of the OAE 1a. This might have been due to an eustatic sea level rise (SAHAGIAN et al., 1996) opening seaways. The cause for the sea level rise in the Early Aptian was an increase in the volume of mid-ocean ridges due to higher ocean crust production rates.

The subsequent positive carbon isotope excursion reaching values of $+4\%$ and more is caused by an increase in the burial flux of organic matter. The increased burial flux of organic matter was promoted by the coincidence of high productivity conditions with increased sediment accumulation rates: Greenhouse conditions and nutrients from increased weathering stimulated productivity, input of detritus from weathering, increased burial rates of carbonates and an enlarged sediment accommodation space due to the sea level rise enhanced sediment accumulation.

In the model calculation, the burial flux of organic matter effectively removes dissolved carbon dioxide. However, if the degassing flux of carbon dioxide from volcanoes is lowered or silicate weathering strongly enhanced, the pCO$_2$ rises again as soon as the burial flux of organic matter decreases below 140% of the initial value.
Figure 25 Run 14: A scenario for the perturbations in the geochemical cycles in the Valanginian

- **Run 14 (Figure 25)**

For a comparison, we calculated a model run for the Valanginian time slice (Figure 25, Run 14). The implemented carbon isotope excursion is not as distinct as the one for the Aptian, the implemented carbon dioxide degassing forcing mechanism is the same as in the previous runs (increase of 80%). A constant steady state input of hydrogen sulfide was implemented. The
model demonstrates that an increased flux of carbon dioxide alone (without in increase in the 
H₂S degassing) also causes a drop in the burial rate of carbonate (i.e. a change in the CCD or
a crisis of calcifying organisms). The major difference in the results of model runs for the
Aptian to the Valanginian scenario is the behavior of dissolved carbon dioxide. For the
Valanginian, the calculated size of the amount of dissolved carbon dioxide rises not as ex-
remely as in the Aptian case. This is due to two reasons: There is no increased input of hy-
drogen sulfide and the burial of organic matter is 10% higher than the burial flux of organic
matter during the Aptian OAE 1a. This shows again that the OAE 1a was a time with an en-
hanced burial flux of organic matter, but still not the most effective mode to bury large
amounts of organic matter.

Conclusions

For the analysis of environmental and geochemical perturbations in the Valanginian and Ap-
tian, information about the biogeochemical processes that have a major influence on the oce-
anic carbonate equilibrium is needed. We have shown that imbalances in the sulfur cycle
strongly affect the oceanic alkalinity and the carbonate equilibrium. Sulfur isotope trends in
the composition of seawater sulfate can be used to trace imbalances in the sulfur cycle. How-
ever, detailed information about the δ³⁴S of Early Cretaceous seawater sulfate was missing.
Thus, we first obtained sulfur and oxygen isotope data of structural substituted sulfate in
carbonates from bulk rocks samples of the Valanginian and Aptian. The Valanginian δ³⁴S
values are stable around 19‰, the corresponding δ¹⁸O values are stable around 16‰. The
Aptian-Albian time slice is marked by a constant δ¹⁸O around 15.4‰. The sulfur isotope data
for the Early Aptian show a drop from 18.2‰ to 17.3‰ within roughly three million years.
With the onset of the Albain, the δ³⁴S rise again to values around 19‰. We emphasize that the
δ³⁴S of structural substituted sulfate is likely to be enriched by +1‰ compared to seawater
sulfate. The sulfur and oxygen isotope composition of structural substituted sulfate (SSS)
from carbonate platform sediments equivalent the basinal “Livello Selli” are strongly altered.
Probably, these sediments originally contained a larger amount of organic matter than the
sediment above and below of this interval. In this case, microbial degradation of organic
matter would have altered the isotopic composition of sulfate by sulfate reduction and sulfide
oxidation processes. The scatter in the sulfur and oxygen isotope composition of SSS from
platform carbonates therefore indicates environmental changes on the platform. Additionally
to the analysis of SSS, the sulfur isotope composition of Aptian sulfides was determined. The values scatter in a range of $-15\%$ to $-52\%$ with an average around $-35\%$. This is similar to sulfide data derived for the Valanginian (KUHN, 1996).

With the isotope data, we next developed a numerical model for the carbon and sulfur cycle to investigate the impact of environmental perturbations on the oceanic carbonate equilibrium and on pCO$_2$ feedback mechanisms, such as the burial flux of carbonates and silicate weathering. The model is based on rough estimates, assumed boundary conditions (e.g. an initial steady state) and feedback mechanisms that are not well constrained. Despite these limitations, general conclusions can be drawn from the model results:

- The shift of $1\%$ in the sulfur isotope composition of seawater sulfate within 3 million years has to be considered as “rapid”: In order to cause such a shift, the weathering flux of sulfides (pyrite) has to increase by about 25% – 30%, respectively the burial flux of sulfides has to decrease by about 25% – 30%. The shift of $1\%$ in the sulfur isotope composition of seawater sulfate within 3 million years could also be caused by the multiplication of the degassing flux of hydrogen sulfide or sulfur dioxide by a factor of two to four (depending on the assumption of the initial flux size). Our calculations and measurements further show that it is unlikely that a change in the fractionation factor of sulfide could have caused the Aptian isotope shift.

- Changes in the range of the above calculated sulfide fluxes strongly affect the alkalinity of the ocean and therefore also the carbonate saturation state of seawater and pCO$_2$. Sulfur isotope shifts of seawater in earth’s history therefore should be considered in the calculation of oceanic alkalinity, pCO$_2$ and pH.

- The concentration of the carbonate ion (CO$_3^{2-}$) in seawater is extremely sensitive with respect to changes in the alkalinity or amount of total inorganic carbon. This in turn has a direct impact on the carbonate saturation state of seawater. A drop in the carbonate supersaturation of surface waters is likely to cause a decrease in the calcification rate of marine carbonate producers. In deep water masses, a decrease in the CO$_3^{2-}$ content initiates carbonate dissolution and a rise in the CCD.

- The Early Aptian oceanic anoxic event (OAE 1a) was a time of enhanced burial of organic matter. However, it was quantitatively outcompeted by the burial flux of organic matter subsequent to the OAE 1a.
• In terms of pCO$_2$, the above observation indicates either that the OAE 1a was terminated under “greenhouse” conditions or that the subsequent enhanced burial of organic matter generated an “icehouse” climate.

For the model runs, we assumed that enhanced degassing of carbon dioxide (for the Valanginian and Aptian) and input of hydrogen sulfide and sulfur dioxide (only for the Aptian) from volcanic and hydrothermal activity originally caused the observed perturbations in the biogeochemical cycles. However, this approach can be questioned for many reasons, e.g. the timing between the perturbations and the volcanic respectively hydrothermal events is not perfect; the estimates for the carbon dioxide, sulfur dioxide and hydrogen sulfide degassing fluxes might be completely wrong and the sulfur and carbon isotopic composition of the weathered rocks are completely unknown. Consequently, the results that are specific for the “volcanism”-scenario are less constrained than the general observations from the numerical model. Nevertheless, being aware of the intrinsic limits of such a numerical model, the results provide a stimulating base for the investigation of the environmental perturbations in the Early Cretaceous:

• Enhanced degassing of carbon dioxide due to volcanic and hydrothermal activity generates two responses in earth’s biogeochemical cycles: A drop in the burial rate of carbonates due to the immediate imbalance in the carbonate equilibrium and a rise in the burial rate of organic matter due to an indirect pCO$_2$ – greenhouse – weathering – nutrient feedback. This in turn leads to an enhanced (or at least recovered) burial rate of carbonates. The pattern “carbonate crisis followed by positive carbon isotope excursion” is caused by the unphasing between the carbon and organic matter feedback mechanisms.

• The most likely cause for the decrease in the sulfur isotope composition of Aptian seawater sulfate is an increase in the degassing flux of hydrogen sulfide and sulfur dioxide from volcanic and hydrothermal activity. In the Valanginian, we do not observe a similar isotope shift. This indicates that the degassing rate of volatiles (CO$_2$, H$_2$S and SO$_2$) in the Valanginian was not as large as in the Early Aptian.

• The implemented pCO$_2$-silicate weathering feedback is not strong enough to buffer pCO$_2$ efficiently. Either, silicate weathering is much more sensitive on pCO$_2$ changes than calculated in our model, or imbalances in the partial pressure of carbon dioxide have to be buffered by imbalances in the burial- and weathering flux of organic matter.
Oceanic anoxic events are likely to be due to special ocean circulation patterns (e.g. a halothermal circulation instead of a thermohaline circulation, different stratification of water bodies). The initiation of such conditions probably goes together with a reorganization of deep water formation. This has the potential to destabilize gas hydrates. The negative carbon-isotope spike prior to the OAE 1a could be the result of a massive release of methane from gas hydrates and a signpost for the reorganization of ocean circulation. Our model calculations indicate that strong greenhouse conditions could be the primary cause for the change in the ocean circulation pattern. The Early Aptian “super-greenhouse” was caused by the combined effect of degassed CO₂, H₂S and SO₂ on the pCO₂. The smaller Valanginian degassing rates were not sufficient to create such a “super greenhouse”. Therefore, the preconditions for an Valanginian oceanic anoxic event were not created.

The above conclusion that a “super greenhouse” initiated the OAE 1a collides with the model output that the OAE 1a also terminated under “super greenhouse” conditions. We speculate that a trigger besides a (decreased) greenhouse-forcing must have caused once more a change in ocean circulation. A possible candidate is a sea level rise (due to expanded ocean ridge volumes).

The calculation of the burial flux of organic matter from the carbon isotope data indicates the burial flux of organic matter during the OAE 1a was quantitatively outcompeted by the burial flux of organic matter subsequent to the OAE 1a. We speculate that this could be due to factors: Higher total sedimentation rates subsequent to the OAE 1a and increased efficiency of organic matter recycling during the OAE 1a. During the OAE 1a the level of the CCD was elevated due to high pCO₂. In pelagic settings the sedimentation rate of carbonate therefore was low, resulting into the observed organic rich sediments (black shales) even when the burial flux of organic matter was not extremely high. Extremely high burial rates of organic matter would rapidly diminish the environmental factors promoting oceanic anoxia, such as high pCO₂ and low oxygen contents in the water column. We therefore speculate that organic matter is effectively recycled by the anoxic microbial loop in order to sustain the extended oxygen poor water masses.

How far the flux of hydrogen sulfide may have lowered the oxygen content of seawater and promoted bottom water anoxia was not addressed here. However, other authors proposed this mechanism as trigger for oceanic anoxia (Carpenter and Lohmann, 1997; Walker, 1986).
References


Appendix 1

Derivation of the estimated values

Reservoir size

Basically, the following effects can be observed when different reservoir sizes are chosen:

- Larger reservoirs have longer residence times (a new steady state is approached more slowly when a reservoir is large).
- The response of a system to a short-lived perturbation (below of the time needed to adopt a new steady state) is less distinct when a reservoir is large (e.g. a carbon isotope excursion of $-1\%$ instead of $-2\%$ for a methane-pulse).

We emphasize that different residence times do not cause different lag times between perturbation and system response. The sometimes observed “delay” due to a longer residence time is only because the system answer becomes detectable later. However, as long as the estimates for the reservoir sizes are in the same order, an over- or underestimate does not dramatically change the results of the model runs.

$M_C$ (total inorganic carbon) $3.8 \times 10^{18}$ mol

Here, we use a value estimated by KUMP and ARTHUR (1999) for the Cretaceous ocean.

$M_{SO_4}$ (amount of sulfate) $40 \times 10^{18}$ mol

This value is an estimate for the preanthropogenic sulfur cycle by HOLSER et al. (1988). As discussed below, the amount of sulfate is held constant during the model runs.

$Ca^{2+}$ (amount of calcium) $15 \times 10^{18}$ mol

This estimate is derived for the recent amount of calcium in the ocean GARRELS and MACKENZIE, (1972). In the model runs, we depict the change in the amount of calcium, however, our model does not involve the entire calcium cycle and therefore, the $Ca^{2+}$-data only indicate trends.

Alkalinity $3.9 \times 10^{18}$ mol

Here, we use the value calculated for the Aptian ocean by WISSLER (2001). The values of $M_C$ (total inorganic carbon) and alkalinity control the amount of the carbon species $H_2CO_3$ and $CO_3^{2-}$. To maintain a natural carbonate equilibrium, the values of alkalinity and $M_C$ have to be close by.
The values for $\text{H}_2\text{CO}_3$ and $\text{CO}_3^{2-}$ are determined from the equations for the carbonate equilibrium (see Appendix 3).

\begin{align*}
\text{CO}_3^{2-} & : 15 \times 10^{16} \text{ mol} \\
\text{H}_2\text{CO}_3 & : 5 \times 10^{16} \text{ mol}
\end{align*}

**bur\text{\_org}:bur\text{\_sulfde} – mol Ratio**

\[ \alpha = 10:1 \]

From this ratio and the estimates for the fluxes in the carbon cycle, we calculate the fluxes in the sulfur system.

The weight ratios of pyrite and organic matter in normal marine shales give an upper limit for the organic matter – pyrite ratio in sediments. The amount of iron in other sediment types, such as carbonates, is limited. These sediments therefore contain organic matter but only a small amount of pyrite. The C/S weight ratio for Quarternary marine shales is about 2.8 (RAISWELL and BERNER, 1986). Assuming that a considerable amount of organic matter is deposited in iron-limited sediments, an upper C/S weight ratio for all sediments might be around 4. We therefore calculated the mol-ratio of the value of Quarternary shales and of the assumed upper limit. The C/S ratio on a mol basis for Quarternary shales is 7.5. For the weight ratio of 4 results a C/S ratio of 10.7. From this range, we chose a value of 10:1.

A different estimate for this ratio would mainly influence the residence time in the sulfur cycle. The residence time of sulfate in our model is about 10 Ma. This is short compared to the residence time estimated by other authors (around 18 Ma by BERNER and BERNER (1996), around 44 Ma by HOLSER et al. (1988)). If we would use a ratio of 7.5:1 instead of 10:1, the residence time would get even shorter. Therefore, and because of the discussion above, a value of 10:1 is more reasonable than a value of 7.5:1.

**Isotope compositions**

The used isotope compositions and isotope enrichment factors strongly influence the model results. However, as long as the used parameters are kept equal from model run to model run, a slightly overestimated or underestimated isotope value will not create inconsistent model results.

\[ \delta^{13}\text{C}_{\text{volc_CO2}} = -5\% \]

We use the value proposed by KUMP and ARTHUR (1999).

\[ \delta^{13}\text{C}_{\text{methane}} = -60\% \]
The isotope composition of methane is highly negative (-60‰, **DICKENS** (2000)). We use this value to create a negative spike in one of the model runs.

\[ \delta^{34}S_{\text{fluvial,IN}} = 7\%e \]

The sulfur isotope composition of riverine sulfate is around 7‰ (**CLAYPOOL** et al., 1980). **OHKOUCHI** et al. (1999) used the same value for sulfur isotope mass balance calculations for the Cenomanian-Turonian boundary.

\[ \delta^{34}S_{\text{H}_2\text{S}} = 2.5\%e \]

The average isotope composition of hydrogen sulfide is around 3.5‰ (for a discussion see **BRUNNER** et al. (2003a: Chapter 4); **SHANKS** et al. (1995)). This value is enriched compared to the mantle sulfur isotope composition of 0‰ and points to a contribution of seawater sulfate. A strongly increased flux of hydrogen sulfide might be related to an intensified flux of hydrogen sulfide from the mantle and would therefore lower the isotope composition. Since we use such a scenario in our model, we chose a value of 2.5‰ instead of 3.5‰. The calculated flux of hydrogen sulfide is somewhat lower than for 3.5‰.

### Isotope enrichment factors

\[ \Delta^{13}C_{\text{carbonate}} = 0\%e \]

According to **KUMP** and **ARTHUR** (1999), the \( \Delta^{13}C_{\text{carbonate}} \) was estimated to equal zero. This value is probably underestimated. However, a test run of the model with an enrichment factor of 2‰ did not change the results considerably.

\[ \Delta^{13}C_{\text{org}} = -28\%e \]

The isotope enrichment factor of organic matter was derived from **LINI** et al. (1992) and **MENEGATTI** et al. (1998).

\[ \Delta^{34}S_{\text{sulfate}} = 0\%e \]

The \( \Delta^{34}S_{\text{sulfate}} \) is low (**CLAYPOOL** et al., 1980). The uncertainties concerning the weathering and burial flux sizes of evaporites are large, an error in the estimate of \( \Delta^{34}S_{\text{sulfate}} \) is therefore not detectable.

\[ \Delta^{34}S_{\text{sulfide}} = -40\%e \]

We estimate the isotope enrichment factor for sulfides (pyrite) as -40‰. The sulfur isotope composition of sulfides (average around -35‰) and the sulfur isotope composition of SSS (+16‰ to +17‰) point to an enrichment factor of -51‰. However, the average sulfur isotope enrichment factor in the Phanerozoic is between -30‰ and -40‰ (**CLAYPOOL** et al., 1980; **STRAUSS**, 1997; **STRAUSS**, 1999). This indicates that the analyzed sulfides reflect spe-
cial diagenetic conditions. We therefore chose an intermediate value of –40‰. With a more negative enrichment factor, a smaller imbalance in the weathering and burial fluxes of pyrite would be required to cause a shift in the sulfur isotope composition of seawater sulfate.

**Fluxes**

\[ \text{volc}_{\text{CO}_2} \quad 6 \times 10^{12} \text{ mol/a} \]

We chose the estimate by FRANÇOIS and GODDÉRIS (1998) for the recent value of volcanic carbon dioxide degassing.

\[ \text{hydro}_{\text{H}_2\text{S}} \quad 0.41 \times 10^{12} \text{ mol/a} \]

The flux of hydrogen sulfide degassed from seafloor hydrothermal system was calculated from steady state assumption. This result equals the average of the value for a long-term steady state flux of 0.8 \times 10^{12} \text{ mol/a} calculated by BRUNNER et al., (2003a: Chapter 4) and the value of 0.8 \times 10^{11} \text{ mol/a} by ALT (1995) for the recent degassing flux of hydrogen sulfide.

\[ \text{methane} \quad 0 \text{ mol/a} \]

The “background” input flux of methane is assumed to be low.

The values for the Cretaceous carbonate and organic matter burial flux are derived from KUMP and ARTHUR (1999), the corresponding weathering fluxes have been calculated from the steady state assumption and are lower than the burial fluxes. This is due to the fact that the degassing of carbon dioxide has to be compensated by imbalances in the burial- and weathering fluxes of organic matter and carbonates.

\[ \text{bur}_{\text{carbonate}} \quad 40 \times 10^{12} \text{ mol/a} \]

\[ \text{wea}_{\text{carbonate}} \quad 35.5 \times 10^{12} \text{ mol/a} \quad \text{calculated from steady state} \]

\[ \text{bur}_{\text{corg}} \quad 10 \times 10^{12} \text{ mol/a} \quad (KUMP \text{ and } ARTHUR, 1999) \]

\[ \text{wea}_{\text{corg}} \quad 8.5 \times 10^{12} \text{ mol/a} \quad \text{calculated from steady state} \]

The flux estimates for burial and weathering of sulfides have been calculated from the C:S ratio and the values for \( \text{bur}_{\text{corg}} \), respectively \( \text{wea}_{\text{corg}} \) (\( \alpha =10:1 \)). The calculated flux of 1 \times 10^{12} \text{ mol/a} for the burial of sulfides is larger than the estimate of the sulfide burial flux for the preantropogenic sulfur cycle of 0.62 \times 10^{12} \text{ mol/a} from HOLSER et al. (1988). This partly explains the different residence times for seawater sulfate discussed above.

\[ \text{bur}_{\text{sulfide}} \quad 1 \times 10^{12} \text{ mol/a} \quad \text{calculated from steady state} \]

\[ \text{wea}_{\text{sulfide}} \quad 0.85 \times 10^{12} \text{ mol/a} \quad \text{calculated from steady state} \]

As pointed out in the discussion of the steady state assumption, the determination of steady state values for the sulfate fluxes is problematic. The weathering of evaporites was calculated
from the steady state assumption and is 2.5 times larger than the sulfide burial flux. This is a ratio in the center of estimates by various workers (for a discussion see BERNER and BERNER (1996)).

\[
\begin{align*}
\text{bur}_{\text{sulfate}} & \quad 2.55 \times 10^{12} \text{ mol/a} \quad \text{calculated from steady state} \\
\text{wea}_{\text{sulfate}} & \quad 2.55 \times 10^{12} \text{ mol/a} \quad \text{assumed to equal bur}_{\text{sulfate}}
\end{align*}
\]

In order to guarantee a steady amount of seawater sulfate, the value for the precipitation of anhydrite in seafloor hydrothermal systems was arbitrarily chosen to be larger than the flux of hydrogen sulfide. The removal of sulfur from seawater (0.5 x 10^{12} mol anhydrite/a) is therefore larger than the contribution from hydro_{H2S} (0.41 x 10^{12} mol hydrogen sulfide/a). The flux for the leaching of anhydrite was then accommodated to match steady state conditions. More relevant than the arbitrarily estimated values for the precipitation and leaching of anhydrite is the difference between these values: The value of 0.26 x 10^{12} mol/a for the flux of sulfate into the oceanic crust are in the range of the estimates for today (for a discussion see (BRUNNER et al., 2003a)).

\[
\begin{align*}
\text{hydro}_{\text{sulfate(precip)}} & \quad 0.5 \times 10^{12} \text{ mol/a} \quad \text{estimate} \\
\text{hydro}_{\text{sulfate(leach)}} & \quad 0.24 \times 10^{12} \text{ mol/a} \quad \text{calculated from steady state}
\end{align*}
\]

From the steady state calculation, the weathering flux of silicates was determined to be 4.76 x 10^{12} mol/a. This flux is below the estimate of BERNER et al. (1983) and BICKLE (1996) who calculated a value of 7 x 10^{12} mol/a for the long-term annual carbon dioxide uptake by silicate weathering.

\[
\begin{align*}
\text{wea}_{\text{silicate}} & \quad 4.76 \times 10^{12} \text{ mol/a} \quad \text{calculated from steady state}
\end{align*}
\]
Appendix 2
Calculation of steady state values

For the calculation of the fluxes below, we use the following values from Appendix 1:

- Isotope fractionation factor for organic matter \( \Delta^{13}C_{\text{org}} \)
- Amount of buried organic matter \( \text{bur}_{\text{org}} \)
- Size of the degassing flux of carbon dioxide \( \text{volc}_{\text{CO}_2} \)
- Isotope composition of degassed carbon dioxide \( \delta^{13}C_{\text{steady}} \)
- Ratio for C:S burial (\( \alpha \), on a mol basis): \( \text{bur}_{\text{org}} = \alpha \times \text{bur}_{\text{sulfide}} \) (consequently the same for \( \text{wea}_{\text{org}}: \text{wea}_{\text{sulfide}} = \alpha \times \text{wea}_{\text{org}} \))
- Isotope fractionation factor for sulfides \( \Delta^{34}S_{\text{sulfide}} \)
- Sulfur isotope composition of riverine sulfate \( \delta^{34}S_{\text{fluvial-DH}} \)
- Isotope composition of degassed hydrogen sulfide \( \delta^{34}S_{\text{SO}_4,\text{steady}} \)
- Value for the hydrothermal uptake of sulfate \( \text{hydro}_{\text{H}_2\text{S}}: \text{precip} \)
- Long term weathering and burial fluxes of evaporites are equal \( \text{wea}_{\text{evap}} = \text{bur}_{\text{evap}} \)

Additionally, the initial isotope values of the carbon and sulfur isotope record were used:

- Steady state composition of dissolved inorganic carbon \( \delta^{13}C_{\text{steady}} \) = +2‰
- Steady state composition of seawater sulfate \( \delta^{34}S_{\text{SO}_4,\text{steady}} \) = +17‰

The following steady state equations were used:

**Steady state mass balance of inorganic carbon in atmosphere-ocean system:**
\[
0 = \text{wea}_{\text{carbonate}} - \text{bur}_{\text{carbonate}} + \text{wea}_{\text{org}} - \text{bur}_{\text{org}} + \text{volc}_{\text{CO}_2}
\]

**Steady state isotope mass balance of inorganic carbon in atmosphere-ocean system:**
\[
0 = \left( \text{wea}_{\text{carbonate}} - \text{bur}_{\text{carbonate}} \right) \times \delta^{13}C_{\text{steady}}
+ \left( \text{wea}_{\text{org}} - \text{bur}_{\text{org}} \right) \times \left( \delta^{13}C_{\text{steady}} + \Delta^{13}C_{\text{org}} \right)
+ \text{volc}_{\text{CO}_2} \times \delta^{13}C_{\text{volc}}
\]

**Steady state mass balance of seawater-sulfate:**
\[
0 = \text{wea}_{\text{sulfate}} - \text{bur}_{\text{sulfate}} + \text{wea}_{\text{sulfide}} - \text{bur}_{\text{sulfide}} + \text{hydro}_{\text{H}_2\text{S}} + \text{hydro}_{\text{sulfate-precip}} - \text{hydro}_{\text{sulfate-precip}}
\]
Steady state isotope mass balance of sulfur:

\[ 0 = \left( wea_{\text{sis}} - bur_{\text{sis}} \right) \cdot \delta^{34} S_{\text{H}_{2}S} + \left( wea_{\text{sis}} - bur_{\text{sis}} \right) \cdot \left( \delta^{34} S_{\text{SO}_{4}^{2-}} + \Delta_{\text{H}_{2}S_{\text{iso}}} \right) + \left( \text{hydro}_{\text{sis}} - \text{hydro}_{\text{lor}} \right) \cdot \delta^{34} S_{\text{H}_{2}S} + \text{hydro}_{\text{H}_{2}S} \cdot \delta^{34} S_{\text{H}_{2}S} \]

Steady state alkalinity mass balance for ocean:

\[ 0 = \left( bur_{\text{carbonate}} - wea_{\text{carbonate}} \right) + \left( wea_{\text{carbonate}} - bur_{\text{carbonate}} \right) - \text{hydro}_{\text{H}_{2}S} + wea_{\text{acid}} \]

- **Calculation of the degassing flux of hydrogen sulfide**

Mass balance of inorganic carbon in atmosphere-ocean system:

\[ -\text{volcCO}_{2} = wea_{\text{carbonate}} - bur_{\text{carbonate}} + wea_{\text{org}} - bur_{\text{org}} \]

Isotope mass balance of inorganic carbon in atmosphere-ocean system:

\[ -\text{volcCO}_{2} \cdot \delta^{13} C_{\text{volc}} = \left( wea_{\text{carbonate}} - bur_{\text{carbonate}} + wea_{\text{org}} - bur_{\text{org}} \right) \cdot \delta^{13} C_{\text{carbonate}} + \left( wea_{\text{org}} - bur_{\text{org}} \right) \cdot \Delta_{\text{volcorg}} \]

\[ \Rightarrow \text{volcCO}_{2} \cdot \delta^{13} C_{\text{carbonate}} = \left( wea_{\text{org}} - bur_{\text{org}} \right) \cdot \Delta_{\text{volcorg}} \]

\[ \Rightarrow wea_{\text{org}} - bur_{\text{org}} = \text{volcCO}_{2} \cdot \frac{\delta^{13} C_{\text{carbonate}} - \delta^{13} C_{\text{volc}}}{\Delta_{\text{volcorg}}} \]

\[ \Rightarrow wea_{\text{org}} = bur_{\text{org}} + \text{volcCO}_{2} \cdot \frac{\delta^{13} C_{\text{carbonate}} - \delta^{13} C_{\text{volc}}}{\Delta_{\text{volcorg}}} \]

Steady state mass balance of seawater-sulfate:

\[ -\text{hydro}_{\text{H}_{2}S} = 0 + wea_{\text{sis}} - bur_{\text{sis}} + \text{hydro}_{\text{sis}} - \text{hydro}_{\text{lor}} \]

Steady state isotope mass balance of sulfur:

\[ 0 = \left( 0 + \text{hydro}_{\text{sis}} - \text{hydro}_{\text{lor}} \right) \cdot \delta^{34} S_{\text{H}_{2}S} + \left( wea_{\text{sis}} - bur_{\text{sis}} \right) \cdot \left( \delta^{34} S_{\text{SO}_{4}^{2-}} + \Delta_{S_{\text{iso}}} \right) + \text{hydro}_{\text{H}_{2}S} \cdot \delta^{34} S_{\text{H}_{2}S} + \text{hydro}_{\text{H}_{2}S} \cdot \delta^{34} S_{\text{H}_{2}S} \]

\[ = \left( \text{hydro}_{\text{sis}} - \text{hydro}_{\text{lor}} \right) + wea_{\text{sis}} - bur_{\text{sis}} \right) \cdot \delta^{34} S_{\text{SO}_{4}^{2-}} + \Delta_{S_{\text{iso}}} + \text{hydro}_{\text{H}_{2}S} \cdot \delta^{34} S_{\text{H}_{2}S} + \text{hydro}_{\text{H}_{2}S} \cdot \delta^{34} S_{\text{H}_{2}S} \]
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\[ \text{wea}_{\text{sulfide}} - \text{bur}_{\text{sulfide}} = \text{hydro}_{H,5} \cdot \frac{\delta^{34}S_{\text{SO}_4,\text{aq}} - \delta^{34}S_{H_2S}}{\Delta_{\text{H}_2\text{S},\text{sulfide}}} \]

\[ \Rightarrow \text{wea}_{\text{sulfide}} = \text{bur}_{\text{sulfide}} + \text{hydro}_{H,5} \cdot \frac{\delta^{34}S_{\text{SO}_4,\text{aq}} - \delta^{34}S_{H_2S}}{\Delta_{\text{H}_2\text{S},\text{sulfide}}} \]

Combination of sulfur and carbon cycle:

\[ \text{wea}_{\text{org}} - \text{bur}_{\text{org}} = \text{volc}_{C_{\text{org}}} \cdot \frac{\delta^{13}C_{\text{C,\text{aq}}}}{\Delta_{\text{C}_{\text{org}}}} - \frac{\delta^{13}C_{\text{C,\text{aq}}}}{\Delta_{\text{C}_{\text{org}}}} \]

and:

\[ \text{wea}_{\text{org}} = \alpha \cdot \text{wea}_{\text{sulfide}} \]

\[ \text{bur}_{\text{org}} = \alpha \cdot \text{bur}_{\text{sulfide}} \]

\[ \Rightarrow \text{wea}_{\text{org}} - \text{bur}_{\text{org}} = \alpha \cdot (\text{wea}_{\text{sulfide}} - \text{bur}_{\text{sulfide}}) \]

\[ \Rightarrow \text{volc}_{C_{\text{org}}} \cdot \frac{\delta^{13}C_{\text{C,\text{aq}}}}{\Delta_{\text{C}_{\text{org}}}} - \frac{\delta^{13}C_{\text{C,\text{aq}}}}{\Delta_{\text{C}_{\text{org}}}} = \alpha \cdot \text{hydro}_{H,5} \cdot \frac{\delta^{34}S_{\text{SO}_4,\text{aq}} - \delta^{34}S_{H_2S}}{\Delta_{\text{H}_2\text{S},\text{sulfide}}} \]

\[ \Rightarrow \text{hydro}_{H,5} = \frac{\text{volc}_{C_{\text{org}}} \cdot \frac{\delta^{13}C_{\text{C,\text{aq}}}}{\Delta_{\text{C}_{\text{org}}}} - \frac{\delta^{13}C_{\text{C,\text{aq}}}}{\Delta_{\text{C}_{\text{org}}}}}{\alpha \cdot \frac{\delta^{34}S_{\text{SO}_4,\text{aq}} - \delta^{34}S_{H_2S}}{\Delta_{\text{H}_2\text{S},\text{sulfide}}}} \]

• Calculation of leaching flux of anhydrite (\text{hydro}_{\text{sulfate,leach}})

\[ -\text{hydro}_{H,5} = \text{wea}_{\text{sulfide}} - \text{bur}_{\text{sulfide}} + \text{hydro}_{\text{sulfate,leach}} - \text{hydro}_{\text{sulfate,precip}} \]

\[ \Rightarrow \text{hydro}_{\text{sulfate,leach}} = \text{wea}_{\text{sulfide}} + \text{hydro}_{\text{sulfate,precip}} - \text{hydro}_{H,5} \]

\[ \Rightarrow \text{hydro}_{\text{sulfate,leach}} = \text{wea}_{\text{sulfide}} + \text{hydro}_{\text{sulfate,precip}} - \text{hydro}_{H,5} \cdot \left( \frac{\delta^{34}S_{\text{SO}_4,\text{aq}} - \delta^{34}S_{H_2S}}{\Delta_{\text{H}_2\text{S},\text{sulfide}}} \right) \]

• Calculation of sulfate burial and weathering flux (\text{wea}_{\text{sulfate}} = \text{bur}_{\text{sulfate}}) from \delta^{34}S_{\text{fluvial-IN}} and \text{wea}_{\text{sulfide}}

\[ \text{wea}_{\text{sulfate}} \cdot \delta^{34}S_{\text{SO}_4,\text{aq}} + \text{wea}_{\text{sulfide}} \cdot \left( \delta^{34}S_{\text{SO}_4,\text{aq}} + \Delta_{\text{S,\text{sulfide}}} \right) \]

\[ \text{wea}_{\text{sulfate}} + \text{wea}_{\text{sulfide}} = \delta^{34}S_{\text{fluvial-IN}} \]
\[ \text{wea}_{\text{silicate}} = \text{bur}_{\text{carbonate}} - \text{wea}_{\text{carbonate}} - \text{bur}_{\text{sulfide}} + \text{wea}_{\text{sulfide}} + \text{hydro H}_2S \]
Appendix 3

Calculation of equilibrium in inorganic carbon from alkalinity and total inorganic carbon

Carbon / Alkalinity system and equations:

1) \[ C = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \]

2) \[ Alk = [HCO_3^-] + 2 \cdot [CO_3^{2-}] \]

3) \[ H_2CO_3 ⇌ HCO_3^- + H^+ \]
   \[ k_1 = \frac{[HCO_3^-] \cdot [H^+]}{[H_2CO_3]} \]

4) \[ HCO_3^- ⇌ CO_3^{2-} + H^+ \]
   \[ k_2 = \frac{[CO_3^{2-}] \cdot [H^+]}{[HCO_3^-]} \]

Calculations:

(2) \[ [HCO_3^-] = Alk - 2 \cdot [CO_3^{2-}] \] and
   \[ [CO_3^{2-}] = \frac{Alk - [HCO_3^-]}{2} \]

(1) \[ [H_2CO_3] = C - [HCO_3^-] - [CO_3^{2-}] \] and
   \[ [H_2CO_3] = C - [HCO_3^-] - \frac{Alk - [HCO_3^-]}{2} \]
   \[ [H_2CO_3] = \frac{2 \cdot C - 2 \cdot [HCO_3^-] - Alk + [HCO_3^-]}{2} \]

Elimination of hydrogen-ion:

(3,4) \[ K = \frac{k_2}{k_1} = \frac{[CO_3^{2-}] \cdot [H^+]}{[HCO_3^-]} \cdot \frac{[HCO_3^-] \cdot [H_2CO_3]}{[HCO_3^-] \cdot [HCO_3^-] \cdot [HCO_3^-]} \]
   \[ K = \frac{k_2}{k_1} = \frac{[CO_3^{2-}] \cdot [H_2CO_3]}{[HCO_3^-]^2} \]
\[ K = \frac{[\text{CO}_3^2^-][\text{H}_2\text{CO}_3]}{[\text{HCO}_3^-]^2} \]

\[ [\text{CO}_3^2^-] = \frac{\text{Alk} - [\text{HCO}_3^-]}{2} \]

\[ [\text{H}_2\text{CO}_3] = \frac{2 \cdot \text{C} - \text{Alk} - [\text{HCO}_3^-]}{2} \]

\[ K = \frac{\text{Alk} - [\text{HCO}_3^-]}{2} \cdot \frac{2 \cdot \text{C} - \text{Alk} - [\text{HCO}_3^-]}{2} \]

\[ K \cdot 2 \cdot 2 \cdot [\text{HCO}_3^-] = (\text{Alk} - [\text{HCO}_3^-]) \cdot (2 \cdot \text{C} - \text{Alk} - [\text{HCO}_3^-]) \]

\[ = 2 \cdot \text{C} \cdot \text{Alk} - \text{Alk}^2 - 2 \cdot \text{C} \cdot [\text{HCO}_3^-] - \text{Alk} \cdot [\text{HCO}_3^-] + [\text{HCO}_3^-] \cdot \text{Alk} + [\text{HCO}_3^-]^2 \]

\[ K \cdot 4 \cdot [\text{HCO}_3^-]^2 = 2 \cdot \text{C} \cdot \text{Alk} - \text{Alk}^2 - 2 \cdot \text{C} \cdot [\text{HCO}_3^-] + [\text{HCO}_3^-]^2 \]

\[ [\text{HCO}_3^-]^2 \cdot (1 - K \cdot 4) - 2 \cdot \text{C} \cdot [\text{HCO}_3^-] + 2 \cdot \text{C} \cdot \text{Alk} - \text{Alk}^2 = 0 \]

quadratic equation:

\[ [\text{HCO}_3^-] = \frac{-2 \cdot \text{C} \pm \sqrt{4 \cdot \text{C}^2 - 4 \cdot (1 - 4 \cdot K) \cdot 2 \cdot \text{C} \cdot \text{Alk} - \text{Alk}^2}}{2 \cdot (1 - 4 \cdot K)} \]

\[ [\text{HCO}_3^-] = \frac{\text{C} \pm \sqrt{\text{C}^2 - (1 - 4 \cdot K) \cdot 2 \cdot \text{C} \cdot \text{Alk} - \text{Alk}^2}}{(1 - 4 \cdot K)} \]

consequences:

\[ \text{C}^2 - (1 - 4 \cdot K) \cdot (2 \cdot \text{C} \cdot \text{Alk} - \text{Alk}^2) \geq 0 \]

\[ K > 0 \rightarrow 1 > (1 - 4 \cdot K) > 0 \rightarrow \frac{\text{C}}{(1 - 4 \cdot K)} > \text{C} \]

and:

\[ [\text{HCO}_3^-] \leq \text{C} \Rightarrow [\text{HCO}_3^-] = \frac{\text{C} \pm \sqrt{\text{C}^2 - (1 - 4 \cdot K) \cdot 2 \cdot \text{C} \cdot \text{Alk} - \text{Alk}^2}}{(1 - 4 \cdot K)} \]
therefore:

\[
\left[ \text{CO}_3^{2-} \right] = \frac{\text{Alk} - \left[ \text{HCO}_3^- \right]}{2}
\]

\[
\Rightarrow \left[ \text{CO}_3^{2-} \right] = \frac{\text{Alk}}{2} - \frac{\left[ \text{HCO}_3^- \right]}{2} = \frac{\text{Alk}}{2} - \frac{C - \sqrt{C^2 - (1 - 4K) \times (2C \times \text{Alk} - \text{Alk}^2)}}{2 \times (1 - 4K)}
\]

\[
\left[ \text{CO}_3^{2-} \right] = \frac{\text{Alk}}{2} - \frac{C - \sqrt{C^2 - (1 - 4K) \times (2C \times \text{Alk} - \text{Alk}^2)}}{2 \times (1 - 4K)}
\]

and:

\[
\left[ \text{H}_2\text{CO}_3 \right] = \frac{2C - \text{Alk} - \left[ \text{HCO}_3^- \right]}{2}
\]

\[
\Rightarrow \left[ \text{H}_2\text{CO}_3 \right] = \frac{2C - \text{Alk} - \sqrt{C^2 - (1 - 4K) \times (2C \times \text{Alk} - \text{Alk}^2)}}{(1 - 4K)}
\]

where:

\[K = 0.000575 + 0.000006 \times (\text{temperature of water (in Kelvin)} - 278 \text{ Kelvin})\] (Walker, 1991).

278 Kelvin equal 4.85°C. We used this value for the temperature of seawater. It is possible that the average Cretaceous seawater was warmer. However, the potential error caused by this temperature estimate is very low.
Appendix 4
Calculation of sulfide and carbonate burial forcing mechanisms dependent on the $\delta^{34}S$ and $\delta^{13}C$ of seawater

Calculation of sulfur fluxes

Mass balance for seawater sulfate:
\[
\frac{dM_{SO_4}}{dt} = \text{wea}_{\text{sulfate}} - \text{bur}_{\text{sulfate}} + \text{wea}_{\text{sulfide}} - \text{bur}_{\text{sulfide}} + \text{hydro}_{H_2S} - \text{hydro}_{\text{anhydrite precipitate}} + \text{hydro}_{\text{anhydrite leach}}
\]
\[\Rightarrow M_{SO_4}(0) + t \left( \text{wea}_{\text{sulfate}} - \text{bur}_{\text{sulfate}} + \text{wea}_{\text{sulfide}} - \text{bur}_{\text{sulfide}} + \text{hydro}_{H_2S} - \text{hydro}_{\text{anhydrite precipitate}} + \text{hydro}_{\text{anhydrite leach}} \right)\]

Isotope mass balance for sulfur in seawater sulfate:
\[
\frac{d(\delta^{34}S_{SO_4} \cdot M_{SO_4})}{dt} = \text{wea}_{\text{sulfate}} \cdot \delta^{34}S_{\text{sulfate}} - \text{bur}_{\text{sulfate}} \cdot \delta^{34}S_{SO_4} + \text{wea}_{\text{sulfide}} \cdot \delta^{34}S_{\text{sulfide}} - \text{bur}_{\text{sulfide}} \cdot \delta^{34}S_{SO_4} + \text{hydro}_{H_2S} \cdot \delta^{34}S_{H_2S} - \text{hydro}_{\text{anhydrite precipitate}} \cdot \delta^{34}S_{SO_4} + \text{hydro}_{\text{anhydrite leach}} \cdot \delta^{34}S_{\text{anhydrite}}
\]
Calculation of hydro H2S:

\[
\frac{d\delta^{34}S_{\text{so}}}{dt} \cdot M_{\text{so}} = \begin{align*}
&w_{\text{aqw}} \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) - b_{\text{aqw}} \cdot \Delta_{S_{\text{aqw}}} \\
+w_{\text{aqw}} \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) - b_{\text{aqw}} \cdot \Delta_{S_{\text{aqw}}} \\
+ \text{hydro}_{aq} \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) \\
+ \text{hydro}_{aq \text{aqw} - \text{aqw}} \cdot (\delta^{34}S_{\text{aqw} - \text{aqw}} - \delta^{34}S_{\text{so}})
\end{align*}
\]

\[
\Delta^{34}S_{\text{sulfate}} = 0
\]

\[
\frac{d\delta^{34}S_{\text{so}}}{dt} \cdot M_{\text{so}} = \begin{align*}
&w_{\text{aqw}} \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) \\
+w_{\text{aqw}} \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) - b_{\text{aqw}} \cdot \Delta_{S_{\text{aqw}}} \\
+ \text{hydro}_{aq} \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) \\
+ \text{hydro}_{aq \text{aqw} - \text{aqw}} \cdot (\delta^{34}S_{\text{aqw} - \text{aqw}} - \delta^{34}S_{\text{so}})
\end{align*}
\]

\[
\frac{d\delta^{34}S_{\text{so}}}{dt} = \frac{d\delta^{34}S_{\text{so}}(t + \Delta t) - d\delta^{34}S_{\text{so}}(t)}{\Delta t} = \begin{align*}
&w_{\text{aqw}}(t) \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) \\
+w_{\text{aqw}}(t) \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) - b_{\text{aqw}}(t) \cdot \Delta_{S_{\text{aqw}}} \\
+ \text{hydro}_{aq}(t) \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) \\
+ \text{hydro}_{aq \text{aqw} - \text{aqw}}(t) \cdot (\delta^{34}S_{\text{aqw} - \text{aqw}} - \delta^{34}S_{\text{so}})
\end{align*}
\]

Calculation of hydro H2S:

\[
\frac{d^{34}S_{\text{aqw}}(t + \Delta t) - d^{34}S_{\text{aqw}}(t)}{\Delta t} = \begin{align*}
&w_{\text{aqw}}(t) \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) \\
+w_{\text{aqw}}(t) \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) - b_{\text{aqw}}(t) \cdot \Delta_{S_{\text{aqw}}} \\
+ \text{hydro}_{aq}(t) \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) \\
+ \text{hydro}_{aq \text{aqw} - \text{aqw}}(t) \cdot (\delta^{34}S_{\text{aqw} - \text{aqw}} - \delta^{34}S_{\text{so}})
\end{align*}
\]

\[
0 = \begin{align*}
&w_{\text{aqw}}(t) \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) \\
+w_{\text{aqw}}(t) \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) - b_{\text{aqw}}(t) \cdot \Delta_{S_{\text{aqw}}} \\
+ \text{hydro}_{aq}(t) \cdot (\delta^{34}S_{\text{aqw}} - \delta^{34}S_{\text{so}}) \\
+ \text{hydro}_{aq \text{aqw} - \text{aqw}}(t) \cdot (\delta^{34}S_{\text{aqw} - \text{aqw}} - \delta^{34}S_{\text{so}})
\end{align*}
\]
Calculation of \( \Delta^{34}S_{\text{sulfide}} \):

\[
\begin{align*}
\text{Calculation of bur}_{\text{sulfide}}: \\
\text{bur}_{\text{sulfide}}(t) & = \frac{\left( \text{wea}_{\text{sulfide}}(t) \cdot (\Delta^{34}S_{\text{sulfide}} - \Delta^{34}S_{\text{SO}_4}) \right) + \left( \text{wea}_{\text{sulfide}}(t) \cdot (\Delta^{34}S_{\text{sulfide}} - \Delta^{34}S_{\text{SO}_4}) - \text{bur}_{\text{sulfide}}(t) \cdot \Delta^{34}S_{\text{sulfide}} \right) + \left( \text{hydro}_{\text{anhydrite leach}}(t) \cdot (\Delta^{34}S_{\text{anhydrite}} - \Delta^{34}S_{\text{SO}_4}) \right) - \frac{\Delta^{34}S_{\text{SO}_4}(t + \Delta t) - \Delta^{34}S_{\text{SO}_4}(t)}{\Delta t} \cdot M_{\text{SO}_4}(t)}{\Delta^{34}S_{\text{SO}_4} - \Delta^{34}S_{\text{SO}_4}}
\end{align*}
\]

\[
\begin{align*}
\text{Calculation of wea}_{\text{sulfide}}: \\
\text{wea}_{\text{sulfide}}(t) & = \frac{\left( \text{wea}_{\text{sulfide}}(t) \cdot (\Delta^{34}S_{\text{sulfide}} - \Delta^{34}S_{\text{SO}_4}) \right) - \text{bur}_{\text{sulfide}}(t) \cdot \Delta^{34}S_{\text{sulfide}} + \left( \text{hydro}_{\text{anhydrite leach}}(t) \cdot (\Delta^{34}S_{\text{anhydrite}} - \Delta^{34}S_{\text{SO}_4}) \right) - \frac{\Delta^{34}S_{\text{SO}_4}(t + \Delta t) - \Delta^{34}S_{\text{SO}_4}(t)}{\Delta t} \cdot M_{\text{SO}_4}(t)}{\Delta^{34}S_{\text{SO}_4} - \Delta^{34}S_{\text{SO}_4}}
\end{align*}
\]

\[
\begin{align*}
\text{Calculation of } \Delta^{34}S_{\text{sulfide}}: \\
\Delta^{34}S_{\text{sulfide}}(t) & = \frac{\left( \text{wea}_{\text{sulfide}}(t) \cdot (\Delta^{34}S_{\text{sulfide}} - \Delta^{34}S_{\text{SO}_4}) \right) + \left( \text{wea}_{\text{sulfide}}(t) \cdot (\Delta^{34}S_{\text{sulfide}} - \Delta^{34}S_{\text{SO}_4}) - \text{bur}_{\text{sulfide}}(t) \cdot \Delta^{34}S_{\text{sulfide}} \right) + \left( \text{hydro}_{\text{anhydrite leach}}(t) \cdot (\Delta^{34}S_{\text{anhydrite}} - \Delta^{34}S_{\text{SO}_4}) \right) - \frac{\Delta^{34}S_{\text{SO}_4}(t + \Delta t) - \Delta^{34}S_{\text{SO}_4}(t)}{\Delta t} \cdot M_{\text{SO}_4}(t)}{\Delta^{34}S_{\text{SO}_4} - \Delta^{34}S_{\text{SO}_4}}
\end{align*}
\]
Calculation of carbon flux

Mass balance for carbon:

\[
\frac{dM_C}{dt} = \text{wea}_{\text{carbonate}} - \text{bur}_{\text{carbonate}} + \text{wea}_{\text{org}} - \text{bur}_{\text{org}} + \text{methane} + \text{volc}_{\text{CO}_2}
\]

Isotope mass balance for Carbon in atmosphere/ocean system:

\[
\frac{d(\delta^{13}C \cdot M_C)}{dt} = \text{wea}_{\text{carbonate}} \cdot \delta^{13}C_{\text{carbonate}} - \text{bur}_{\text{carbonate}} \cdot (\delta^{13}C_c + \Delta_{\text{carbonate}}) + \text{wea}_{\text{org}} \cdot \delta^{13}C_{\text{org}} - \text{bur}_{\text{org}} \cdot (\delta^{13}C_c + \Delta_{\text{org}}) + \text{methane} \cdot \delta^{13}C_{\text{methane}} + \text{volc}_{\text{CO}_2} \cdot \delta^{13}C_{\text{volc}}
\]

\[
\frac{d\delta^{13}C_c \cdot M_c}{dt} = \frac{d(\delta^{13}C \cdot M_c)}{dt} - \text{wea}_{\text{carbonate}} \cdot (\delta^{13}C_c - \delta^{13}C_{\text{carbonate}}) - \text{bur}_{\text{carbonate}} \cdot (\delta^{13}C_c + \Delta_{\text{carbonate}} - \delta^{13}C_c)
\]

\[
\frac{d\delta^{13}C_c \cdot M_c}{dt} = \frac{d(\delta^{13}C \cdot M_c)}{dt} - \text{wea}_{\text{org}} \cdot (\delta^{13}C_{\text{org}} - \delta^{13}C_c) - \text{bur}_{\text{org}} \cdot (\delta^{13}C_c + \Delta_{\text{org}} - \delta^{13}C_c) + \text{methane} \cdot \delta^{13}C_{\text{methane}} - \delta^{13}C_{\text{volc}} + \text{volc}_{\text{CO}_2} \cdot \delta^{13}C_{\text{volc}} - \delta^{13}C_c
\]
\[
\Delta (\delta^13C(t), \delta^13C(t + \Delta t)) \cdot M = \delta^13C(t + \Delta t) - \delta^13C(t) \cdot M = \\
\begin{align*}
\text{wea}_{\text{carbonate}} \cdot (\delta^13C_{\text{carbonate}} - \delta^13C_C) - \text{bur}_{\text{carbonate}} \cdot \Delta_{\text{carbonate}} \\
+ \text{wea}_{\text{org}} \cdot (\delta^13C_{\text{org}} - \delta^13C_C) - \text{bur}_{\text{org}} \cdot \Delta_{\text{org}} \\
+ \text{methane} \cdot (\delta^13C_{\text{methane}} - \delta^13C_C) + \text{volc}_{\text{CO}_2} \cdot (\delta^13C_{\text{volc}} - \delta^13C_C)
\end{align*}
\]

\[
\Rightarrow \\
\delta^13C(t + \Delta t) - \delta^13C(t) = \frac{\Delta \delta^13C_C}{\Delta t} - \delta^13C_C(t) \\
\begin{align*}
\text{wea}_{\text{carbonate}} \cdot (\delta^13C_{\text{carbonate}} - \delta^13C_C) - \text{bur}_{\text{carbonate}} \cdot \Delta_{\text{carbonate}} \\
+ \text{wea}_{\text{org}} \cdot (\delta^13C_{\text{org}} - \delta^13C_C) - \text{bur}_{\text{org}} \cdot \Delta_{\text{org}} \\
+ \text{methane} \cdot (\delta^13C_{\text{methane}} - \delta^13C_C) + \text{volc}_{\text{CO}_2} \cdot (\delta^13C_{\text{volc}} - \delta^13C_C)
\end{align*}
\]

\[
\Rightarrow \\
0 = \frac{\Delta \delta^13C_C}{\Delta t} - \delta^13C_C(t) \\
\begin{align*}
\text{wea}_{\text{carbonate}} \cdot (\delta^13C_{\text{carbonate}} - \delta^13C_C) - \text{bur}_{\text{carbonate}} \cdot \Delta_{\text{carbonate}} \\
+ \text{wea}_{\text{org}} \cdot (\delta^13C_{\text{org}} - \delta^13C_C) - \text{bur}_{\text{org}} \cdot \Delta_{\text{org}} \\
+ \text{methane} \cdot (\delta^13C_{\text{methane}} - \delta^13C_C) + \text{volc}_{\text{CO}_2} \cdot (\delta^13C_{\text{volc}} - \delta^13C_C)
\end{align*}
\]

\[
\Rightarrow \\
\text{bur}_{\text{org}} \cdot \Delta_{\text{org}} = \frac{\Delta \delta^13C_C}{\Delta t} - \delta^13C_C(t) \\
\begin{align*}
\text{wea}_{\text{carbonate}} \cdot (\delta^13C_{\text{carbonate}} - \delta^13C_C) - \text{bur}_{\text{carbonate}} \cdot \Delta_{\text{carbonate}} \\
+ \text{wea}_{\text{org}} \cdot (\delta^13C_{\text{org}} - \delta^13C_C) \\
+ \text{methane} \cdot (\delta^13C_{\text{methane}} - \delta^13C_C) + \text{volc}_{\text{CO}_2} \cdot (\delta^13C_{\text{volc}} - \delta^13C_C)
\end{align*}
\]

\[
\Rightarrow \\
\text{bur}_{\text{org}} = \frac{\Delta \delta^13C_C}{\Delta t} - \delta^13C_C(t) \\
\begin{align*}
\text{wea}_{\text{carbonate}} \cdot (\delta^13C_{\text{carbonate}} - \delta^13C_C) - \text{bur}_{\text{carbonate}} \cdot \Delta_{\text{carbonate}} \\
+ \text{wea}_{\text{org}} \cdot (\delta^13C_{\text{org}} - \delta^13C_C) \\
+ \text{methane} \cdot (\delta^13C_{\text{methane}} - \delta^13C_C) + \text{volc}_{\text{CO}_2} \cdot (\delta^13C_{\text{volc}} - \delta^13C_C)
\end{align*}
\]
HYDROTHERMAL AND VOLCANIC SULFUR FLUXES ARE NEEDED TO BALANCE EARTH’S OXYGEN BUDGET DURING THE PHANEROZOIC

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Abstract

The exogenic cycles of carbon and sulfur play a major role in the balance of the amount of oxygen in the atmosphere. The exogenic and endogenic carbon cycles are connected through volcanic degassing of carbon dioxide and subduction of carbonates and organic matter. The carbon isotope composition of mantle-degassed CO₂ (–5‰ to –7‰) is distinctly lower than the carbon isotope composition of carbonates deposited during the Phanerozoic (-2‰ up to +6‰). In order to compensate for the isotopic light carbon dioxide input, organic matter (Δ₁³C around –28‰) must have been formed and removed from the exogenic carbon cycle, either by storage in the sediment reservoir or by subduction. A consequence of this compensation is an excess production of oxygen during the Phanerozoic. Based on a steady state carbon mass balance and estimates of long-term averages of carbon-fluxes and isotope compositions, the excess production of oxygen is in the order of 1 x 10¹² mol O₂ per year. To balance the amount of oxygen in the atmosphere a sink for this flux is needed. Volcanic and hydrothermal hydrogen- and methane degassing and the oxidation of reduced iron from silicate rocks compensate up to 20% of the excess oxygen flux. In absence of other important oxygen sinks, the sulfur cycle has to be the missing sink for about 8 x 10¹¹ mol O₂ per year. The excess oxygen is mainly consumed by the oxidation of hydrogen sulfide from seafloor hydrothermal systems. Using a steady state sulfur-cycle mass balance and estimates of long-term averages of sulfur-fluxes and -isotope compositions, we estimate this flux to be in the order of 8 x 10¹¹ mol H₂S per year.

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Introduction

Over the Phanerozoic, the amount of atmospheric oxygen has remained approximately constant (Berner and Canfield, 1989). This is probably due to feedback mechanisms in the biogeochemical cycles of carbon, sulfur and oxygen (Petsch and Berner, 1998). Imbalances in the weathering and burial fluxes of organic matter were balanced by imbalances in the weathering and burial fluxes of sulfides and seem to have effectively balanced the production and consumption of atmospheric oxygen (Berner, 1999). Due to biological isotope fractionation processes, organic matter and sulfides are strongly enriched in the light isotopes $^{12}\text{C}$ and $^{32}\text{S}$ compared to bicarbonate and sulfate in seawater. Imbalances in weathering and burial fluxes of organic matter and sulfides, therefore, cause shifts in the carbon and sulfur isotope composition of bicarbonate and sulfate in seawater. Carbonates and sulfates precipitate in isotopic equilibrium with the dissolved species in seawater and record the balance between the cycling of organic matter and sulfides controlling atmospheric oxygen partial pressures (Berner et al., 2000; Holser et al., 1988; Paytan and Arrigo, 2000; Strauss, 1999). The exogenic biogeochemical cycles of oxygen, carbon and sulfur are linked to their endogenic counterparts by weathering of igneous and metamorphic rocks, by subduction and by degassing of volatiles from volcanoes and hydrothermal systems. The fluxes of volatiles from the mantle and oceanic crust are smaller than the ones within the exogenic cycles, e.g. carbon dioxide degassing from mid-ocean ridges is in the order of $6 \times 10^{12}$ mol CO$_2$ per year while burial of organic matter is in the order of $10 \times 10^{12}$ mol CH$_2$O per year (Holser et al., 1988). Nevertheless, over long time scales (million of years), fluxes from and to the mantle and oceanic crust could cause significant changes in the amount of atmospheric oxygen and secular trends in the isotope- and mass balances of carbon and sulfur. Since such changes have not been observed in the Phanerozoic rock record, significant degassing of hydrogen sulfide from seafloor hydrothermal systems on geologic time scales, as proposed by Carpenter and Lohmann (1997), were questioned by Petsch (1999) claiming, “A mantle-derived sulfide flux would be expected to generate secular trends in the sulfur isotopic composition of seawater if atmospheric oxygen is assumed to remain approximately constant over the Phanerozoic”. However, degassing of volatiles (e.g. CO$_2$, SO$_2$ and H$_2$S) from seafloor hydrothermal systems and volcanoes are well known phenomena and cannot be negated just because of their potential to contradict observations from the rock record. Rather, a model combining and explaining both observations, namely a long-term steady state (i.e. no secular trends) for the
Phanerozoic cycles of oxygen, carbon and sulfur and the degassing of volatiles from mantle sources has to be developed. We, therefore, consider isotope and mass balances for the carbon- and sulfur-cycle as well as mass balances for oxygen and alkalinity at long-term (Phanerozoic) steady state. These balances are combined with estimates of mantle-derived fluxes, e.g. volcanic degassing of CO₂, methane, hydrogen, hydrogen sulfide and sulfur dioxide. We will show that at steady state, the carbon cycle produces excess oxygen, which has to be consumed by other geochemical processes: imbalances in the sulfur cycle, mantle degassing of hydrogen and weathering and oxidation of reduced iron in silicates. Based on a long-term steady state mass balance of oxygen including these sources and sinks for oxygen, we calculate the flux of degassed hydrogen sulfide needed to compensate for the oxygen excess production of the carbon cycle. This result is compared with published estimates of "mantle"-sulfur fluxes. The consequences of mantle derived sulfur- and carbon fluxes for the amount of oxygen in the atmosphere, for the size of the continental crust reservoirs of sulfur and carbon and possible long-term feedback mechanisms are discussed. The derivation of the formulae for the steady state carbon cycle is described in the text. The analogue calculations for the sulfur cycle are listed in Appendix 1.

Defining a long-term (Phanerozoic) steady state in the cycles of oxygen, carbon and sulfur

During the Phanerozoic, the geochemical cycles of oxygen, carbon and sulfur most likely have passed through a sequence of different steady states or were not at steady state. A "Phanerozoic steady state" does not exist, but, over long time scales, the different states of the geochemical cycles of oxygen, carbon and sulfur have oscillated around average values that could be considered as "Phanerozoic steady state". By using the concept of a long-term average steady state, we postulate that there is no secular trend in the cycling of oxygen, carbon and sulfur during the Phanerozoic. The definition of a (not existing) long-term Phanerozoic steady state is used as tool to calculate the consequences of this assumption.

The amount of oxygen in the atmosphere-ocean system is controlled by the consumption of oxygen due to oxidation of reduced compounds (e.g. organic matter, sulfides and silicates containing reduced iron) and the production of oxygen by burial of organic matter and sulfides. Over the long-term Phanerozoic average, the consumption of oxygen should be compensated by an equal production of oxygen. This requires that the sum of oxygen-fluxes from and to all involved geochemical cycles (e.g. carbon-, sulfur- and silicate-cycle) is balanced, but each involved cycle does not need to be balanced with respect to oxygen in itself. Conse-
quently, the burial fluxes of organic matter or sulfides do not need to be matched by the corresponding weathering fluxes. However, over the long-term Phanerozoic average, the amount and isotopic composition of carbon and sulfur in the atmosphere-ocean system, and probably also in the crust, are assumed to be balanced. Based on isotope- and mass balance calculations and long-term steady state assumptions for isotope values and flux sizes, we can calculate the average annual oxygen-production or -consumption by the carbon- and sulfur biogeochemical cycles separately. These results are integrated into mass balance calculations of atmospheric oxygen.

Figure 1  The geochemical cycles of carbon, sulfur and oxygen

The amount of oxygen in the atmosphere is controlled by imbalances in the carbon- and sulfur cycle, but also affected by weathering (oxidation of mantle rocks and metamorphic rocks) and degassing of hydrogen. It is obvious, that over the long-term, the net sum of the imbalances in the carbon- and sulfur cycle should be a contribution of oxygen to the atmosphere to compensate the oxygen removal by oxidation of Fe$^{2+}$-silicates and hydrogen.

filled arrows: fluxes affecting the sulfur cycle
dashed arrows: fluxes affecting the carbon cycle
dotted arrows: fluxes affecting the oxygen cycle

The fluxes of oxygen, carbon, sulfur and alkalinity between the rock reservoirs and the ocean-atmosphere reservoirs can be described as chemical reactions (see tables 1, 2, 3 and 5).
We define the sizes of the atmosphere-ocean reservoirs as follows:

- **Oxygen reservoir (O<sub>2</sub>)** amount of O<sub>2</sub> in the atmosphere-ocean system
- **Sulfur reservoir (S)** amount of SO<sub>4</sub><sup>2-</sup> in the ocean
- **Carbon reservoir (C)** amount of CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> in the atmosphere-ocean system
  \[ C = CO_2 + HCO_3^- + CO_3^{2-} \]
- **Alkalinity (Alk)** buffering capacity of the ocean:
  \[ HCO_3^- + 2CO_3^{2-} - H^+ + OH^- = Na^+ + 2Mg^{2+} + 2Ca^{2+} - Cl^- - SO_4^{2-} \]

**Carbon isotope and mass balance of inorganic carbon in atmosphere-ocean system**

The carbon cycle consists of four subcycles: weathering and burial of carbonates, weathering and burial of organic matter, metamorphic processes and fluxes from and to the mantle reservoir.

**Figure 2** The geochemical cycle of carbon

The carbon cycle consists of four subcycles:
1) Weathering and burial of carbonates
2) Weathering and burial of organic matter (including formation and release of gas hydrates by biogenic methanogenesis)
3) Metamorphic processes (e.g. degassing of CO<sub>2</sub> and methane due to breakdown of organic matter and carbonates)
4) Fluxes from and to the mantle reservoir (e.g. degassing of CO<sub>2</sub> and methane at seafloor hydrothermal systems or subduction of carbonates and organic matter)
These four subcycles control the carbon isotope composition and mass balance of inorganic carbon in atmosphere-ocean system. The effect of each flux on the atmosphere-ocean reservoirs can be described by a generalized chemical equation (Table 1).

<table>
<thead>
<tr>
<th>Flux</th>
<th>Chemical reactions</th>
<th>Reservoir</th>
<th>Alk</th>
<th>C</th>
<th>S</th>
<th>O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>c1) wee(carbonate)</td>
<td>CaCO₃ → Ca²⁺ + CO₃²⁻</td>
<td>+2</td>
<td>+1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>c2) bur(carbonate)</td>
<td>Ca²⁺ + CO₃²⁻ → CaCO₃</td>
<td>-2</td>
<td>-1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>c3) wee(corg)</td>
<td>CH₂O + O₂ → CO₂ + H₂O</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-</td>
<td>-1</td>
</tr>
<tr>
<td>c4) bur(corg)</td>
<td>CO₂ + H₂O → CH₂O + O₂</td>
<td>-</td>
<td>-1</td>
<td>-</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td>c5) wee(methane)</td>
<td>CH₄ + 2O₂ → CO₂ + 2H₂O</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>c6) bur(methane)</td>
<td>CO₂ + 2H₂O → CH₄ + 2O₂</td>
<td>-</td>
<td>-1</td>
<td>-</td>
<td>+2</td>
<td></td>
</tr>
<tr>
<td>c7) metamorph.CO₂-carbonate</td>
<td>CO₂</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>c8) metamorph.CO₂-*corg</td>
<td>CO₂</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>c9) metamorph.methane</td>
<td>CH₄ + 2O₂ → CO₂ + 2H₂O</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>c10) mantle.CO₂</td>
<td>CO₂</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>c11) mantle.methane</td>
<td>CH₄ + 2O₂ → CO₂ + 2H₂O</td>
<td>-</td>
<td>+1</td>
<td>-</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>c12) subduct(carbonate)</td>
<td>Ca²⁺ + CO₃²⁻ → CaCO₃</td>
<td>-2</td>
<td>-1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>c13) subduct(corg)</td>
<td>CO₂ + H₂O → CH₂O + O₂</td>
<td>-</td>
<td>-1</td>
<td>-</td>
<td>+1</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Fluxes in the carbon cycle and their corresponding chemical equations and effect on the atmosphere-ocean reservoirs of alkalinity, carbon, sulfur and oxygen:

- c1 and c2: Weathering and burial of carbonates.
- c3 to c6: Weathering and burial of organic matter. The chemical equation for the methanogenesis combines the production and transformation of organic matter to methane:
  \[ 2CO₂ + 2H₂O \rightarrow 2CH₄ + 2O₂ \text{ and } 2CH₃O \rightarrow CH₄ + CO₂ \]
  therefore: \[ 2CO₂ + 2H₂O \rightarrow CH₄ + CO₂ + 2O₂ \]
- c7 to c9: Degassing of carbon dioxide and methane due to metamorphic transformations of organic matter and carbonates, e.g.:
  \[ CaCO₃ + SiO₂ → CaSiO₃ + CO₂ \] (silicate formation)
  or
  \[ 2Fe₂O₃ + 4SiO₂ + CH₂O \rightarrow 4FeSiO₃ + H₂O + CO₂ \text{ (Fe}^{2+}-\text{silicate formation)} \]
- c10 and c11: Degassing of carbon dioxide and methane from mantle or oceanic crust sources.
- c12 and c13: Subduction of carbonates and organic matter: We treat this process as a direct flux from the ocean-atmosphere system. This enables us to consider the mass balance of the continental crust reservoir of carbon (see discussion).
The mass balance of inorganic carbon in the atmosphere-ocean system is described by the following equation: 

\[
\frac{dM_C}{dt} = \text{wea}_{\text{carbonate}} - \text{bur}_{\text{carbonate}} + \text{wea}_{\text{corg}} - \text{bur}_{\text{corg}} + \text{wea}_{\text{methane}} - \text{bur}_{\text{methane}} + \text{metamorph}_{\text{CO}_2 - \text{carbonate}} + \text{metamorph}_{\text{methane} - \text{corg}} + \text{mantle}_{\text{CO}_2} + \text{mantle}_{\text{methane}} - \text{subduct}_{\text{carbonate}} - \text{subduct}_{\text{corg}}
\]

The isotope mass balance of inorganic carbon in the atmosphere-ocean system is described by the following equation:

\[
\frac{d(\delta^{13}C_{\text{Ocean}} \cdot M_C)}{dt} = \text{wea}_{\text{carbonate}} \cdot \delta^{13}C_{\text{carbonate}} - \text{bur}_{\text{carbonate}} \cdot (\delta^{13}C_{\text{Ocean}} + \Delta^{13}_{\text{carbonate}}) + \text{wea}_{\text{corg}} \cdot \delta^{13}C_{\text{corg}} - \text{bur}_{\text{corg}} \cdot (\delta^{13}C_{\text{Ocean}} + \Delta^{13}_{\text{corg}}) + \text{wea}_{\text{methane}} \cdot \delta^{13}C_{\text{methane}} - \text{bur}_{\text{methane}} \cdot (\delta^{13}C_{\text{Ocean}} + \Delta^{13}_{\text{methane}}) + \text{metamorph}_{\text{CO}_2 - \text{carbonate}} \cdot \delta^{13}C_{\text{carbonate}} + \text{metamorph}_{\text{methane} - \text{corg}} \cdot \delta^{13}C_{\text{corg}} + \text{mantle}_{\text{CO}_2} \cdot \delta^{13}C_{\text{mantle}} - \text{CO}_2 + \text{mantle}_{\text{methane}} \cdot \delta^{13}C_{\text{mantle}} - \text{methane} - \text{subduct}_{\text{carbonate}} \cdot \delta^{13}C_{\text{carbonate}} - \text{subduct}_{\text{corg}} \cdot \delta^{13}C_{\text{corg}}
\]

where the symbols represent (see also Table 1):

- \(M_C\) \text{ amount of carbon in the atmosphere-ocean system}
- \(\delta^{13}C_{\text{Ocean}}\) \text{ carbon isotope composition of inorganic carbon in ocean}
- \(\text{wea}_{\text{carbonate}}\) \text{ weathering flux of carbonates (c1)}
- \(\delta^{13}C_{\text{carbonate}}\) \text{ carbon isotope composition of carbonates}
- \(\text{bur}_{\text{carbonate}}\) \text{ burial flux of carbonates (c2)}
- \(\Delta^{13}_{\text{carbonate}}\) \text{ fractionation between precipitated carbonate and dissolved inorganic carbon}
- \(\text{wea}_{\text{corg}}\) \text{ weathering flux of organic matter (c3)}
- \(\delta^{13}C_{\text{corg}}\) \text{ carbon isotope composition of organic matter}
- \(\text{bur}_{\text{corg}}\) \text{ burial flux of organic matter (c4)}
- \(\Delta^{13}_{\text{corg}}\) \text{ fractionation between organic matter and dissolved inorganic carbon}
- \(\text{wea}_{\text{methane}}\) \text{ release of methane formed by microbial activity (c5)}
Because we assume a steady state for the Phanerozoic, the derivative is equal to zero. This leads to a constant mass of carbon in the atmosphere-ocean system with a constant carbon isotope composition. Further, we neglect the degassing and storage of microbial methane because this reservoir is rapidly recycled (no long-term storage in the earth’s crust) and therefore has no net effect on the amount of oxygen in the atmosphere. The fractionation factor for carbonates is rather small and can be neglected (Kump and Arthur, 1999), consequently, the carbonates have the same carbon isotope composition as the inorganic carbon reservoir in the ocean, whereas the organic matter differs from this value by the fractionation factor of organic matter. A fractionation factor of $+1\%$ for carbonates would not change the results dramatically (see Eq 10_C and Eq 11_C).

From the long-term steady state assumptions follows:

\[
M_C(t) = M_{C\text{-steady}} \Rightarrow \frac{dM_C}{dt} = 0
\]

\[
\delta_{13}^C_{\text{Ocean}}(t) = \delta_{13}^C_{C\text{-steady}} \Rightarrow \frac{d\delta_{13}^C_{\text{Ocean}}}{dt} = 0
\]

\[
bur_{\text{methane}} - wea_{\text{methane}} = 0
\]

\[
\Delta_{13}^{\text{carbonate}} = 0\%e
\]

\[
\Rightarrow \delta_{13}^{\text{carbonate}} = \delta_{13}^{C\text{-steady}}
\]

\[
\Rightarrow \delta_{13}^{\text{corg}} = \delta_{13}^{C\text{-steady}} + \Delta_{13}^{\text{corg}}
\]
Now, the mass balance equation can be rewritten:

\[
\text{mantle}_{\text{CO}_2} + \text{mantle}_{\text{methane}} = \]

\[
b_{\text{bur, carbonate}} - w_{\text{wea, carbonate}} + b_{\text{corg, wea}} - w_{\text{corg, b}} - \text{metamorph}_{\text{CO}_2, - \text{carbonate}} - \text{metamorph}_{\text{CO}_2, - \text{corg}} - \text{metamorph}_{\text{methane, - corg}}
\]

\[
+ s_{\text{subduct, carbonate}} + s_{\text{subduct, corg}}
\]

\[
\Rightarrow
\text{mantle}_{\text{CO}_2} + \text{mantle}_{\text{methane}} =
\]

\[
b_{\text{bur, carbonate}} + s_{\text{subduct, carbonate}} - w_{\text{wea, carbonate}} - \text{metamorph}_{\text{CO}_2, - \text{carbonate}}
\]

\[
+ b_{\text{corg, wea}} + s_{\text{subduct, corg}} - w_{\text{corg, b}} - \text{metamorph}_{\text{CO}_2, - \text{corg}} - \text{metamorph}_{\text{methane, - corg}}
\]

This equation highlights that an input of carbon dioxide or methane from the mantle or oceanic crust has to be compensated by an increased removal of carbonates or organic matter. We introduce two new expressions: \(\text{Diff}_{\text{carbonate}}\) and \(\text{Diff}_{\text{corg}}\). These expressions stand for the difference between the removal- and input-fluxes of carbonate respectively organic matter. In this way, the equation is simplified:

\[
\text{Diff}_{\text{carbonate}} = b_{\text{bur, carbonate}} + s_{\text{subduct, carbonate}} - w_{\text{wea, carbonate}} - \text{metamorph}_{\text{CO}_2, - \text{carbonate}}
\]

\[
\text{Diff}_{\text{corg}} = b_{\text{corg, wea}} + s_{\text{subduct, corg}} - w_{\text{corg, b}} - \text{metamorph}_{\text{CO}_2, - \text{corg}} - \text{metamorph}_{\text{methane, - corg}}
\]

\[
\Rightarrow
\text{mantle}_{\text{CO}_2} + \text{mantle}_{\text{methane}} = \text{Diff}_{\text{carbonate}} + \text{Diff}_{\text{corg}}
\]

The steady state isotope balance is simplified as follows:

\[
\frac{d(\delta^{13}C_{\text{Ocean}} \cdot M_C)}{dt} = 0 =
\]

\[
(w_{\text{wea, carbonate}} - b_{\text{bur, carbonate}}) \cdot \delta^{13}C_{\text{C, steady}} + (w_{\text{wea, corg}} - b_{\text{corg, wea}}) \cdot \left(\delta^{13}C_{\text{C, steady}} + \Delta_{13\text{corg}}\right)
\]

\[
+ \text{metamorph}_{\text{CO}_2, - \text{carbonate}} \cdot \delta^{13}C_{\text{C, steady}} +
\]

\[
(\text{metamorph}_{\text{CO}_2, - \text{corg}} + \text{metamorph}_{\text{methane, - corg}}) \cdot \left(\delta^{13}C_{\text{C, steady}} + \Delta_{13\text{corg}}\right)
\]

\[
+ \text{mantle}_{\text{CO}_2} \cdot \delta^{13}C_{\text{mantle-CO}_2} + \text{mantle}_{\text{methane}} \cdot \delta^{13}C_{\text{mantle-methane}}
\]

\[
- s_{\text{subduct, carbonate}} \cdot \delta^{13}C_{\text{C, steady}} - s_{\text{subduct, corg}} \cdot \left(\delta^{13}C_{\text{C, steady}} + \Delta_{13\text{corg}}\right)
\]
mantle\textsubscript{CO}_2 \cdot \delta^{13}C_{\text{mantle-CO}_2} + \text{mantle}_{\text{methane}} \cdot \delta^{13}C_{\text{mantle-methane}} = \\
- \left( \text{wea}_{\text{carbonate}} + \text{metamorph}_{\text{CO}_2 \_\text{carbonate}} - \text{bur}_{\text{carbonate}} - \text{subduct}_{\text{carbonate}} \right) \cdot \delta^{13}C_{C - \text{steady}} \\
- \left( \text{wea}_{\text{org}} + \text{metamorph}_{\text{CO}_2 \_\text{org}} + \text{metamorph}_{\text{methane}_\text{org}} - \text{bur}_{\text{org}} - \text{subduct}_{\text{org}} \right) \cdot \left( \delta^{13}C_{C - \text{steady}} + \Delta_{13c_{\text{org}}} \right) \\
\Rightarrow \\
mantle\textsubscript{CO}_2 \cdot \delta^{13}C_{\text{mantle-CO}_2} + \text{mantle}_{\text{methane}} \cdot \delta^{13}C_{\text{mantle-methane}} = \\
\text{Diff}_{\text{carbonate}} \cdot \delta^{13}C_{C - \text{steady}} + \text{Diff}_{\text{org}} \cdot \left( \delta^{13}C_{C - \text{steady}} + \Delta_{13c_{\text{org}}} \right)

This equation highlights that the observation for the mass balance also holds true for the isotope balance: The isotope effect caused by outgassed volcanic and hydrothermal carbon dioxide and methane has to be compensated by the balance between removal and input of carbonates and organic matter.

The equations for the steady state mass- and isotope balance can now be combined and allow to investigate the effect of hydrothermal and volcanic degassing of methane and carbon dioxide:

\[ \text{Diff}_{\text{carbonate}} = \text{mantle}_{\text{CO}_2} + \text{mantle}_{\text{methane}} - \text{Diff}_{\text{org}} \]

\[ \Rightarrow \]

\[ \text{mantle}_{\text{CO}_2} \cdot \delta^{13}C_{\text{mantle-CO}_2} + \text{mantle}_{\text{methane}} \cdot \delta^{13}C_{\text{mantle-methane}} = \\
(\text{mantle}_{\text{CO}_2} + \text{mantle}_{\text{methane}} - \text{Diff}_{\text{org}}) \cdot \delta^{13}C_{C - \text{steady}} + \text{Diff}_{\text{org}} \cdot \left( \delta^{13}C_{C - \text{steady}} + \Delta_{13c_{\text{org}}} \right) \]

\[ \Rightarrow \]

\[ \text{mantle}_{\text{CO}_2} \cdot \delta^{13}C_{\text{mantle-CO}_2} + \text{mantle}_{\text{methane}} \cdot \delta^{13}C_{\text{mantle-methane}} - (\text{mantle}_{\text{CO}_2} + \text{mantle}_{\text{methane}}) \cdot \delta^{13}C_{C - \text{steady}} = \\
- \text{Diff}_{\text{org}} \cdot \delta^{13}C_{C - \text{steady}} + \text{Diff}_{\text{org}} \cdot \left( \delta^{13}C_{C - \text{steady}} + \Delta_{13c_{\text{org}}} \right) \]

\[ \Rightarrow \]

\[ \text{Diff}_{\text{org}} \cdot \Delta_{13c_{\text{org}}} = \\
\text{mantle}_{\text{CO}_2} \cdot \left( \delta^{13}C_{\text{mantle-CO}_2} - \delta^{13}C_{C - \text{steady}} \right) + \text{mantle}_{\text{methane}} \cdot \left( \delta^{13}C_{\text{mantle-methane}} - \delta^{13}C_{C - \text{steady}} \right) \]

It follows:

\[ \text{Diff}_{\text{org}} = \text{bur}_{\text{org}} + \text{subduct}_{\text{org}} - \text{wea}_{\text{org}} - \text{metamorph}_{\text{CO}_2 \_\text{org}} - \text{metamorph}_{\text{methane}_\text{org}} \]

\[ = \text{mantle}_{\text{CO}_2} \cdot \frac{\delta^{13}C_{\text{mantle-CO}_2} - \delta^{13}C_{C - \text{steady}}}{\Delta_{13c_{\text{org}}}} + \text{mantle}_{\text{methane}} \cdot \frac{\delta^{13}C_{\text{mantle-methane}} - \delta^{13}C_{C - \text{steady}}}{\Delta_{13c_{\text{org}}}} \]

(Eq 7_C)

(Eq 8_C)
and:

$$\text{Diff}_{\text{carbonate}} = \text{bur}_{\text{carbonate}} + \text{subduct}_{\text{carbonate}} - \text{wea}_{\text{carbonate}} - \text{metamorph}_{\text{CO}_2-\text{carbonate}}$$

$$= \text{mantle}_{\text{CO}_2} \cdot \left( 1 - \frac{\delta^{13}C_{\text{mantle-}\text{CO}_2} - \delta^{13}C_{\text{-steady}}}{\Delta C_{\text{org}}} \right) + \text{mantle}_{\text{methane}} \cdot \left( 1 - \frac{\delta^{13}C_{\text{mantle-}\text{methane}} - \delta^{13}C_{\text{-steady}}}{\Delta C_{\text{org}}} \right)$$

These equations show that a volcanic or hydrothermal “mantle”-flux of carbon dioxide and methane might have consequences on the balance between removal and input of organic matter. Such an imbalance would also affect the production of oxygen in the atmosphere. We therefore consider the average amount and isotope composition of carbon and methane degassed from hydrothermal systems and volcanoes.

MARTY and TOLSTIKHIN (1998) estimate annual carbon dioxide fluxes from mid-ocean ridges, arcs and plumes as 6 x 10^{12} mol/a with a range of 4-10 x 10^{12} mol/a. The average volcanic and hydrothermal carbon dioxide flux during the Phanerozoic is likely to be in the same range. However, since metamorphic carbon dioxide and methane fluxes are not considered as volcanic input but as additional weathering fluxes, arc volcanism should not be taken completely into account either: According to MARTY and TOLSTIKHIN (1998), 80% of the degassed carbon dioxide is derived from the subducting plate. Therefore, we subtract 80% of the carbon dioxide contribution of 2.5 x 10^{12} mol/a by arcs (MARTY and TOLSTIKHIN, 1998) from the total volcanic and hydrothermal carbon dioxide flux, resulting in a value of 4 x 10^{12} mol carbon dioxide per year. The carbon isotope value of metamorphic and volcanic carbon dioxide is around –5‰ (KUMP and ARTHUR, 1999). Because of the subtraction of contributions by metamorphism and arc volcanoes, we use a carbon isotope value of –7‰ which is similar to carbon dioxide from mid-ocean ridge basalts (SHANKS et al., 1995). Only rough estimates for volcanic and hydrothermal methane fluxes exist. According to MÖRNER and ETIOPE (2002) the estimate for the global volcanic methane flux of 0.34 Tg methane per year (= 2.13 x 10^{10} mol/a) by CADLE (1980) is probably too low. The estimate of the total methane flux from the world-ocean ridge system is in the order of 0.1Mt (=6.25 x 10^{9} mol) per year (WELHAN and CRAIG, 1979). Assuming that a large part of the volcanic methane flux could be derived from sediments and therefore is added to the metamorphic methane flux, we estimate that the global flux of methane by hydrothermal systems and volcanoes is around 3 x 10^{10} mol/a. With these estimates, the ratio between carbon dioxide flux (4 x 10^{12} mol CO_2/a) and methane flux (3 x 10^{10} mol CH_4/a) degassing is 133:1. This ratio is comparable to the vent fluid data from unsedimented volcanic ocean ridges compiled by SHANKS et al.
(1995): The carbon dioxide content in the vent fluids is in the range of 3.7-18.4 mmol/kg, the corresponding methane content is in the range of 0.05-0.12 mmol/kg resulting in a ratio of 74:1 to 153:1. As carbon isotope composition for methane we use data from volcanic ocean ridges not covered by sediments. This methane is unlikely to be affected by microbial methanogenesis and its isotopic composition is in the range of –20.8‰ to –15‰ (SHANKS et al., 1995). We use the average value of –18‰. The carbon isotope values of Carbonates in the Phanerozoic is in the range of –2‰ to +6‰ (VEIZER et al., 1999) with an average around +1‰ and the fractionation factor of organic matter is in a range of 23‰ to 34‰ (HAYES et al., 1999) with an average of –28‰ (KUMP and ARTHUR, 1999).

Based on the estimates below, we calculate the difference between burial and organic matter:

\[ \text{mantle}_{CO_2} = 4 \times 10^{12} \text{ mol carbon dioxide per year} \]
\[ \text{mantle}_{methane} = 3 \times 10^{10} \text{ mol methane per year} \]
\[ \delta^{13}C_{mantle,CO_2} = -7‰ \]
\[ \delta^{13}C_{mantle,methane} = -18‰ \]
\[ \delta^{13}C_{C-steady} = +1‰ \]
\[ \Delta^{13}c_{org} = -28‰ \]

(Eq 9_C)

\[ \text{mantle}_{CO_2} \cdot \frac{\delta^{13}C_{volc} - \delta^{13}C_{C-steady}}{\Delta^{13}c_{org}} = 4 \times 10^{12} \cdot \frac{-7‰ - 1‰}{-28‰} = 1.14 \times 10^{12} \text{ mol/a} \]
\[ \text{mantle}_{methane} \cdot \frac{\delta^{13}C_{volc,methane} - \delta^{13}C_{C-steady}}{\Delta^{13}c_{org}} = 3 \times 10^{10} \cdot \frac{-18‰ - 1‰}{-28‰} = 2 \times 10^{10} \text{ mol/a} \]

If we used a fractionation factor of +1‰ instead of 0‰ for carbonate, the results would not change dramatically: The steady-state value of seawater would become 0‰ (1‰ lower than the carbonates) and equation 10_C would be reformulated as follows:

(Eq 11_C)

\[ \text{mantle}_{CO_2} \cdot \frac{\delta^{13}C_{volc} - \delta^{13}C_{C-steady}}{\Delta^{13}c_{org}} = 4 \times 10^{12} \cdot \frac{-7‰ - 0‰}{-28‰} = 1 \times 10^{12} \text{ mol/a} \]
\[ \text{mantle}_{methane} \cdot \frac{\delta^{13}C_{volc,methane} - \delta^{13}C_{C-steady}}{\Delta^{13}c_{org}} = 3 \times 10^{10} \cdot \frac{-18‰ - 0‰}{-28‰} = 1.9 \times 10^{10} \text{ mol/a} \]

Using the results of Eq 10_C, we get:

(Eq 12_C)

\[ \text{Diff}_{corg} = \text{bur}_{corg} + \text{subduct}_{corg} - \text{wea}_{corg} - \text{metamorph}_{CO_2}_{corg} - \text{metamorph}_{methane}_{corg} = \text{mantle}_{CO_2} \cdot \frac{\delta^{13}C_{mantle,CO_2} - \delta^{13}C_{C-steady}}{\Delta^{13}c_{org}} + \text{mantle}_{methane} \cdot \frac{\delta^{13}C_{mantle,methane} - \delta^{13}C_{C-steady}}{\Delta^{13}c_{org}} \]
\[ = 1.14 \times 10^{12} \text{ mol/a} + 2 \times 10^{10} \text{ mol/a} = 1.16 \times 10^{12} \text{ mol/a} \]
The annual difference between removal and input of organic matter is in the range of $1 \times 10^{12}$ mol/a. We can use this difference to calculate the amount of oxygen contributed to the atmosphere to maintain a steady state (Table 1).

Oxygen produced by imbalance between weathering and burial of organic matter:

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} + \text{O}_2 = 1 \times 10^{12} \text{ mol/a}$$

Oxygen consumed by input of volcanic and hydrothermal methane degassing:

$$\text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O} = 2 \times 2 \times 10^{10} \text{ mol/a} = 4 \times 10^{10} \text{ mol/a}$$

As consequence, the oxygen mass balance gets input from the carbon cycle:

(Eq 13_C)

$$\frac{d\text{O}_2}{dt} = \text{Diff}_{\text{org}} - 2 \cdot \text{mantle}_{\text{methane}} = 1 \times 10^{12} \frac{\text{mol O}_2}{\text{a}}$$

To keep the carbon isotope values of carbonates during the Phanerozoic at +1‰, the input of isotopic light (-7‰) “mantle” carbon dioxide and methane is compensated by an “overproduction” of organic matter (-27‰), leading to an average annual excess production of oxygen in the range of $1 \times 10^{12}$ mol O$_2$ per year. The hydrothermal flux of methane is at least one magnitude too small to compensate for this flux. To keep the amount of oxygen in the atmosphere at reasonable values oxygen sinks other than the carbon system have to be identified.
Sulfur isotope and mass balance of seawater-sulfate

The sulfur cycle consists of four subcycles: weathering and burial of sulfate (e.g. evaporites), weathering and burial of sulfides (e.g. pyrite), metamorphic processes and fluxes from and to the mantle reservoir.

Figure 3  The geochemical cycle of sulfur

The sulfur cycle consists of four subcycles:
1) Weathering and burial of sulfate (e.g. evaporites, barite, sulfate bound in carbonates)
2) Weathering and burial of sulfides (e.g. as sulfides, formation within sediments due to bacterial sulfate reduction)
3) Metamorphic processes (e.g. degassing of SO\textsubscript{2} and H\textsubscript{2}S due to heating of sulfate and sulfides)
4) Fluxes from and to the mantle reservoir (e.g. degassing of H\textsubscript{2}S, SO\textsubscript{2} and incorporation of anhydrite and sulfides precipitated in oceanic crust at seafloor hydrothermal systems or subduction sulfides and sulfate)

These four subcycles control the sulfur isotope composition and mass balance of seawater-sulfate. The effect of each flux on the atmosphere-ocean reservoirs can be described by a generalized chemical equation (Table 2).
<table>
<thead>
<tr>
<th>Flux</th>
<th>Chemical reactions</th>
<th>Reservoir</th>
<th>Alk</th>
<th>C</th>
<th>S</th>
<th>O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>s1) weather sulfate</td>
<td>Ca SO₄ → Ca²⁺ + SO₄²⁻</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+1</td>
<td>-</td>
</tr>
<tr>
<td>s2) burial sulfate</td>
<td>Ca²⁺ + SO₄²⁻ → Ca SO₄</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-1</td>
<td>-</td>
</tr>
<tr>
<td>s3) weather sulfide</td>
<td>4FeS₂ + 15O₂ + 8H₂O ⇌ 2Fe₂O₃ + 8SO₄²⁻ + 16H⁺</td>
<td>-2</td>
<td>-</td>
<td>+1</td>
<td>15</td>
<td>8/8</td>
</tr>
<tr>
<td>s4) burial sulfide</td>
<td>2Fe₂O₃ + 8SO₄²⁻ + 16H⁺ → 4FeS₂ + 15O₂ + 8H₂O</td>
<td>+2</td>
<td>-1</td>
<td>-</td>
<td>15</td>
<td>8/8</td>
</tr>
<tr>
<td>s5) metamorph SO₂-sulfate</td>
<td>2SO₂ + 2H₂O + O₂ → 4H⁺ + 2SO₄²⁻</td>
<td>-2</td>
<td>-</td>
<td>+1</td>
<td>1/2</td>
<td>-</td>
</tr>
<tr>
<td>s6) metamorph H₂-sulfate</td>
<td>H₂S + 2O₂ → 2H⁺ + SO₄²⁻</td>
<td>-2</td>
<td>-</td>
<td>+1</td>
<td>-2</td>
<td>-</td>
</tr>
<tr>
<td>s7) metamorph SO₂-sulfide</td>
<td>2SO₂ + 2H₂O + O₂ → 4H⁺ + 2SO₄²⁻</td>
<td>-2</td>
<td>-</td>
<td>+1</td>
<td>1/2</td>
<td>-</td>
</tr>
<tr>
<td>s8) metamorph H₂-sulfide</td>
<td>H₂S + 2O₂ → 2H⁺ + SO₄²⁻</td>
<td>-2</td>
<td>-</td>
<td>+1</td>
<td>-2</td>
<td>-</td>
</tr>
<tr>
<td>s9) hydro_mantle-H₂S</td>
<td>H₂S + 2O₂ → 2H⁺ + SO₄²⁻</td>
<td>-2</td>
<td>-</td>
<td>+1</td>
<td>-2</td>
<td>-</td>
</tr>
<tr>
<td>s10) vol_mantle-SO₂</td>
<td>2SO₂ + 2H₂O + O₂ → 4H⁺ + 2SO₄²⁻</td>
<td>-2</td>
<td>-</td>
<td>+1</td>
<td>1/2</td>
<td>-</td>
</tr>
<tr>
<td>s11) hydro sulfate(leach)</td>
<td>Ca SO₄ → Ca²⁺ + SO₄²⁻</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+1</td>
<td>-</td>
</tr>
<tr>
<td>s12) hydro sulfate(precip)</td>
<td>Ca²⁺ + SO₄²⁻ → Ca SO₄</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-1</td>
<td>-</td>
</tr>
<tr>
<td>s13) subduct sulfate</td>
<td>2Fe₂O₃ + 8SO₄²⁻ + 16H⁺ → 4FeS₂ + 15O₂ + 8H₂O</td>
<td>+2</td>
<td>-1</td>
<td>-</td>
<td>15</td>
<td>8/8</td>
</tr>
</tbody>
</table>

Table 2  Fluxes in the sulfur cycle and their corresponding chemical equations and effect on the atmosphere-ocean reservoirs of alkalinity, carbon, sulfur and oxygen

s1 and s2: Weathering and burial of sulfate.
s3 and s4: Weathering and burial of sulfide (i.e. pyrite).
s5 to s8: Degassing of sulfur dioxide and hydrogen sulfide due to metamorphic transformations of sulfate and sulfides.
s9 and s10: Degassing of hydrogen sulfide and sulfur dioxide from mantle or oceanic crust sources.
s11: Leaching of anhydrite from oceanic crust.
s12: Precipitation of anhydrite in oceanic crust due to heating of seawater in seafloor hydrothermal systems: The difference between s12 and s11 could be considered as “subducted” sulfate. A part of s12 is reacted with the fayalite component of basaltic rocks:

\[
11\text{Fe}_2\text{SiO}_4 + 2\text{SO}_4^{2-} + 4\text{H}^+ \rightarrow \text{FeS}_2 + 7\text{Fe}_2\text{O}_3 + 11\text{SiO}_2 + 2\text{H}_2\text{O}
\]

s13: Subduction of sulfides: We treat this process as a direct flux from the ocean-atmosphere system.
The mass balance of seawater sulfate is described by the following equation:

\[
\frac{dM_{SO_4}}{dt} = \text{wea}_{\text{sulfate}} - \text{bur}_{\text{sulfate}} \]
\[
+ \text{wea}_{\text{sulfide}} - \text{bur}_{\text{sulfide}} \]
\[
+ \text{metamorph}_{\text{SO}_2_{-sulfate}} + \text{metamorph}_{\text{H}_2\text{S}_{-sulfate}} + \text{metamorph}_{\text{SO}_2_{-sulfide}} + \text{metamorph}_{\text{H}_2\text{S}_{-sulfide}} \]
\[
+ \text{hydro}_{\text{mantle}_{-H_2S}} + \text{volc}_{\text{mantle}_{-SO_2}} + \text{hydro}_{\text{sulfate leach}} - \text{hydro}_{\text{sulfate precip}} - \text{subduct}_{\text{sulfide}} \]

The isotope mass balance of seawater-sulfate is described by the following equation:

\[
\frac{d\delta^{34}S_{SO_4-\text{Ocean}} \cdot M_{SO_4}}{dt} = \]
\[
\text{wea}_{\text{sulfate}} \cdot \delta^{34}S_{\text{sulfate}} - \text{bur}_{\text{sulfate}} \cdot \left( \delta^{34}S_{SO_4-\text{Ocean}} + \Delta^{34}S_{\text{sulfate}} \right) \]
\[
+ \text{wea}_{\text{sulfide}} \cdot \delta^{34}S_{\text{sulfide}} - \text{bur}_{\text{sulfide}} \cdot \left( \delta^{34}S_{SO_4-\text{Ocean}} + \Delta^{34}S_{\text{sulfide}} \right) \]
\[
+ \left( \text{metamorph}_{\text{SO}_2_{-sulfate}} + \text{metamorph}_{\text{H}_2\text{S}_{-sulfate}} \right) \cdot \delta^{34}S_{\text{sulfate}} \]
\[
+ \left( \text{metamorph}_{\text{SO}_2_{-sulfide}} + \text{metamorph}_{\text{H}_2\text{S}_{-sulfide}} \right) \cdot \delta^{34}S_{\text{sulfide}} \]
\[
+ \text{hydro}_{\text{mantle}_{-H_2S}} \cdot \delta^{34}S_{\text{mantle}_{-H_2S}} + \text{volc}_{\text{mantle}_{-SO_2}} \cdot \delta^{34}S_{\text{mantle}_{-SO_2}} \]
\[
+ \text{hydro}_{\text{sulfate leach}} \cdot \delta^{34}S_{\text{sulfate leach}} - \text{hydro}_{\text{sulfate precip}} \cdot \delta^{34}S_{SO_4-\text{Ocean}} \]
\[
- \text{subduct}_{\text{sulfide}} \cdot \delta^{34}S_{\text{sulfide}} \]

where the symbols represent (see also Table 2):

- \(\frac{d}{dt}\) derivative after time
- \(M_{SO_4}\) amount of sulfate in seawater
- \(\delta^{34}S_{SO_4-\text{Ocean}}\) sulfur isotope composition of seawater sulfate
- \(\text{wea}_{\text{sulfate}}\) weathering flux of sulfate (e.g. evaporites) (s1)
- \(\delta^{34}S_{\text{sulfate}}\) sulfur isotope composition of sulfate
- \(\text{bur}_{\text{sulfate}}\) burial flux of sulfate (s2)
- \(\Delta^{34}S_{\text{sulfate}}\) fractionation between precipitated sulfate and seawater sulfate
- \(\text{wea}_{\text{sulfide}}\) weathering flux of sulfide (e.g. pyrite) (s3)
- \(\delta^{34}S_{\text{sulfide}}\) sulfur isotope composition of sulfides
- \(\text{bur}_{\text{sulfide}}\) burial flux of sulfides (s4)
- \(\Delta^{34}S_{\text{sulfide}}\) isotope fractionation between microbial sulfides and seawater
- \(\text{metamorph}_{\text{SO}_2_{-sulfate}}\) metamorphic degassing of sulfur dioxide from sulfates (s5)
- \(\text{metamorph}_{\text{H}_2\text{S}_{-sulfate}}\) metamorphic degassing of hydrogen sulfide from sulfides (s6)
Using the assumption of a steady state for the Phanerozoic, we set the derivatives equal to zero. This leads to a constant mass and sulfur isotopic composition of seawater sulfate. The fractionation factor for sulfate precipitation from seawater is small (CLAYPOOL et al., 1980) and therefore neglected.

From the long-term steady state assumptions follows:

\[
M_{SO_4}(t) = M_{SO_4 - steady} \Rightarrow \frac{dM_{SO_4}}{dt} = 0
\]

\[
\delta^{34}S_{SO_4 - Ocean}(t) = \delta^{34}S_{SO_4 - steady} \Rightarrow \frac{d\delta^{34}S_{SO_4}}{dt} = 0
\]

\[
\Delta^{34}S_{sulfate} = 0\%
\]

\[
\Rightarrow \delta^{34}S_{sulfate} = \delta^{34}S_{SO_4 - steady}
\]

\[
\Rightarrow \delta^{34}S_{sulfide} = \delta^{34}S_{SO_4 - steady} + \Delta^{34}S_{sulfide}
\]

\[
\Rightarrow \delta^{34}S_{sulfate leach} = \delta^{34}S_{SO_4 - steady}
\]

The steady state mass balance and isotope mass balance can be reformulated (see Appendix 1):

\[
(Eq 3_S)
\]

\[
(Eq 4_S)
\]

This equation highlights that an input of sulfur dioxide or hydrogen sulfide from the mantle or oceanic crust has to be compensated by an increased removal of sulfates or sulfides. We in-
introduce two new expressions: Diff$_{\text{sulfate}}$ and Diff$_{\text{sulfide}}$. These expressions stand for the difference between the removal- and input-fluxes of sulfate respectively sulfides. In this way, the equation is simplified:

\[
\text{Diff$_{\text{sulfate}}$} = \text{bur$_{\text{sulfate}}$} + \text{hydro$_{\text{sulfate precip}}$} - \text{hydro$_{\text{sulfate leach}}$} - \text{wea$_{\text{sulfate}}$} - \text{metamorph$_{SO_2}$ - sulfate} - \text{metamorph$_{H_2S}$ - sulfate}
\]

\[
\text{Diff$_{\text{sulfide}}$} = \text{bur$_{\text{sulfide}}$} + \text{subduct$_{\text{sulfide}}$} - \text{wea$_{\text{sulfide}}$} - \text{metamorph$_{SO_2}$ - sulfide} - \text{metamorph$_{H_2S}$ - sulfide}
\]

\[
\Rightarrow \text{hydro$_{\text{mantle} - H_2S}$} + \text{volc$_{\text{mantle} - SO_2}$} = \text{Diff$_{\text{sulfate}}$} + \text{Diff$_{\text{sulfide}}$}
\]

The steady state isotope balance of seawater sulfate is simplified (see Appendix 1):

\[
\text{hydro$_{\text{mantle} - H_2S}$} \cdot \delta^{34}S_{\text{mantle - H}_2\text{S}} + \text{volc$_{\text{mantle} - SO_2}$} \cdot \delta^{34}S_{\text{mantle - SO}_2} = \text{Diff$_{\text{sulfate}}$} \cdot \delta^{34}S_{\text{SO}_4 - \text{steady}} + \text{Diff$_{\text{sulfide}}$} \left( \delta^{34}S_{\text{SO}_4 - \text{steady}} + \Delta^{34}S_{\text{sulfide}} \right)
\]

This equation shows that the isotope effect caused by the “mantle” input has to be compensated by imbalances between input and removal of sulfate respectively sulfides. To investigate the effect of the hydrothermal and volcanic input of mantle-$H_2S$ and -$SO_2$, the equations for steady state mass and isotope balance of seawater sulfate are combined (see Eq 7_S, Appendix 1) and solved for Diff$_{\text{sulfate}}$ and Diff$_{\text{sulfide}}$:

\[
\text{Diff$_{\text{sulfate}}$} = \text{bur$_{\text{sulfate}}$} + \text{hydro$_{\text{sulfate precip}}$} - \text{hydro$_{\text{sulfate leach}}$} - \text{wea$_{\text{sulfate}}$} - \text{metamorph$_{SO_2}$ - sulfate} - \text{metamorph$_{H_2S}$ - sulfate}
\]

\[
\Rightarrow \text{hydro$_{\text{mantle} - H_2S}$} \cdot \delta^{34}S_{\text{mantle - H}_2\text{S}} - \delta^{34}S_{\text{SO}_4 - \text{steady}} = \text{volc$_{\text{mantle} - SO_2}$} \cdot \delta^{34}S_{\text{mantle - SO}_2} - \delta^{34}S_{\text{SO}_4 - \text{steady}} + \Delta^{34}S_{\text{sulfide}}
\]

\[
\text{Diff$_{\text{sulfide}}$} = \text{bur$_{\text{sulfide}}$} + \text{subduct$_{\text{sulfide}}$} - \text{wea$_{\text{sulfide}}$} - \text{metamorph$_{SO_2}$ - sulfide} - \text{metamorph$_{H_2S}$ - sulfide}
\]

\[
\Rightarrow \text{hydro$_{\text{mantle} - H_2S}$} \cdot \delta^{34}S_{\text{mantle - H}_2\text{S}} - \delta^{34}S_{\text{SO}_4 - \text{steady}} = \text{volc$_{\text{mantle} - SO_2}$} \cdot \delta^{34}S_{\text{mantle - SO}_2} - \delta^{34}S_{\text{SO}_4 - \text{steady}} + \Delta^{34}S_{\text{sulfide}}
\]
To maintain the isotope and mass steady state of seawater sulfate, the input of "mantle"-H$_2$S and SO$_2$ is compensated by an increased removal of sulfides and sulfate. The difference between removal and input of sulfides and the input of sulfur dioxide and hydrogen sulfide affect the oxygen mass balance. Based on estimates of long-term steady state sulfur isotope compositions, we can calculate the influence of the sulfur cycle on the atmospheric oxygen budget. The increased burial flux of sulfides (to balance sulfur isotopic steady state) results in a production of oxygen, the degassed sulfur dioxide and hydrogen sulfide consume oxygen (for chemical reactions see Table 2, for calculations see Appendix 1):

\[
\frac{dO_2}{dt} = \frac{15}{8} \cdot \text{Diff}_{\text{sulfide}} - 2 \cdot \text{hydro mantl}_{\text{H}_2\text{S}} - \frac{1}{2} \cdot \text{volc mantl}_{\text{SO}_2}
\]

\[\Rightarrow \]

\[
\frac{dO_2}{dt} = -\text{hydro mantl}_{\text{H}_2\text{S}} \cdot \left(2 - \frac{15}{8} \cdot \frac{\delta^{34}S_{\text{mantl}_{\text{H}_2\text{S}}} - \delta^{34}S_{\text{SO}_2 \text{steady}}}{\Delta^{34}S_{\text{sulfide}}} \right) + \text{volc mantl}_{\text{SO}_2} \cdot \left(\frac{15}{8} \cdot \frac{\delta^{34}S_{\text{mantl}_{\text{SO}_2}} - \delta^{34}S_{\text{SO}_2 \text{steady}}}{\Delta^{34}S_{\text{sulfide}}} - \frac{1}{2} \right)
\]

Mantle sulfur has a mean $\delta^{34}S$ composition of 0‰ to +1‰ (White, 2000). Vent fluid hydrogen sulfide isotope values are in a range from −3‰ to 8‰ (Shanks et al., 1995) with an average value around +3.5‰. This value is also used by other authors (e.g. Carpenter and Lohmann (1997); Petsch (1999)) and indicates that a part of the degassed hydrogen sulfide originates from seawater sulfate (high $\delta^{34}S$) reduced within seafloor hydrothermal systems. The equilibrium fractionation between mantle H$_2$S and -SO$_2$ at high (volcanic) temperatures is rather small (at 800°C +3.58‰ and at 1000°C 2.40‰, (Ohmoto and Rye, 1979)). A value around +3.5‰ for mantle degassed sulfur dioxide is therefore a reasonable estimate. Phanerozoic sulfur isotope values of sulfides differ from contemporaneous sulfates in a range of −15‰ to −50‰; fractionation factors are in a range of −30‰ to −40‰ and sulfur isotope values of seawater are in a range of 11‰ to 35‰ (Claypool et al., 1980; Strauss, 1997; Strauss, 1999) probably with an average in the range of 17‰ to 24‰. As steady state value, we choose an average value of 20‰ for seawater sulfate. As fractionation factor, we choose −30‰, a value attributed to sulfate reducers with intermediate cell specific sulfate reduction
rates (Rees, 1973) which might be quantitatively more important than sulfate reducers with slow cell specific sulfate reduction rates.

\[ \delta^{34} S_{\text{SO}_4,\text{steady}} = 20\%e \]
\[ \delta^{34} S_{\text{mantle}_H_2S} = +3.5\%e \]
\[ \delta^{34} S_{\text{mantle}_SO_2} = +3.5\%e \]
\[ \Delta^{34} S_{\text{sulfide}} = -30\%e \]

Based on these assumptions, we calculate:

\[ \frac{dO_{2,\text{sulfur-cycle}}}{dt} = \]
\[ -\text{hydro}_{\text{mantle}_H_2S} \cdot \left( 2 - \frac{15}{8} \cdot \frac{3.5\%e - 20\%e}{-30\%e} \right) + \text{volc}_{\text{mantle}_SO_2} \cdot \left( \frac{15}{8} \cdot \frac{3.5\%e - 20\%e}{-30\%e} - \frac{1}{2} \right) \]
\[ \approx -\text{hydro}_{\text{mantle}_H_2S} \cdot 0.97 + \text{volc}_{\text{mantle}_SO_2} \cdot 0.53 \]

Different steady state assumptions (maxima and minima-values) lead to different results:

\[ \Delta S_{\text{sulfide}} = -30\%e \text{ and } \delta^{34} S_{\text{SO}_4,\text{steady}} = +20\%e : \]
\[ \frac{dO_{2,\text{sulfur-cycle}}}{dt} = -\text{hydro}_{\text{mantle}_H_2S} \cdot 0.97 + \text{volc}_{\text{mantle}_SO_2} \cdot 0.53 \]

\[ \Delta S_{\text{sulfide}} = -40\%e \text{ and } \delta^{34} S_{\text{SO}_4,\text{steady}} = +17\%e : \]
\[ \frac{dO_{2,\text{sulfur-cycle}}}{dt} = -\text{hydro}_{\text{mantle}_H_2S} \cdot 1.37 + \text{volc}_{\text{mantle}_SO_2} \cdot 0.13 \]

\[ \Delta S_{\text{sulfide}} = -30\%e \text{ and } \delta^{34} S_{\text{SO}_4,\text{steady}} = +24\%e : \]
\[ \frac{dO_{2,\text{sulfur-cycle}}}{dt} = -\text{hydro}_{\text{mantle}_H_2S} \cdot 0.72 + \text{volc}_{\text{mantle}_SO_2} \cdot 0.78 \]

In general, the results demonstrate that volcanic and hydrothermal degassing of sulfur dioxide and hydrogen sulfide do not have the same effect on the long term steady state budget of oxygen: The isotopically light input of hydrogen sulfide and sulfur dioxide are compensated by increased burial of sulfides. This leads to a production of oxygen. At the same time, the degassing fluxes of SO\(_2\) and H\(_2\)S also consume oxygen, but not to the same extent. The oxygen consumption by sulfur dioxide is smaller than the amount of oxygen produced to compensate its isotope effect, as consequence, sulfur dioxide emissions result in an oxygen production. In
contrast, hydrothermal degassing of hydrogen sulfide is a sink for oxygen because its oxygen consumption is larger than the oxygen production by the burial flux of sulfides compensating for the light isotopic input. Because of the uncertainties concerning the average steady state values of the sulfur isotope composition of seawater and the sulfur isotope fractionation factor, the coefficient of the hydrogen sulfide flux is in the range of 0.72 to 1.37.

“Mantle” degassing of hydrogen

Volcanoes and hydrothermal systems degass hydrogen in considerable amounts. Oxygen reacts with hydrogen to water. Therefore, the flux of hydrogen from mantle sources acts as an oxygen sink and has to be evaluated (Table 3).

<table>
<thead>
<tr>
<th>Flux</th>
<th>Chemical reaction</th>
<th>Reservoir</th>
</tr>
</thead>
<tbody>
<tr>
<td>mantle hydrogen</td>
<td>2H₂ + O₂ → 2H₂O</td>
<td>A lk C S O₂</td>
</tr>
</tbody>
</table>

Table 3 The effect of mantle-derived hydrogen degassing from seafloor hydrothermal systems and volcanoes on the atmosphere-ocean reservoirs

To estimate the amount of degassed hydrogen, we calculated ratios of methane and hydrogen concentrations in seafloor hydrothermal fluids from a data set (Table 4) compiled by Von Damm (1995).

<table>
<thead>
<tr>
<th>site</th>
<th>CH₄ mmol/kg</th>
<th>H₂ mmol/kg</th>
<th>Ratio H₂:CH₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>11°N, East Pacific Rise</td>
<td>0.067 ⇒ 0.012</td>
<td>0.47</td>
<td>7:1 ⇒ 39:1</td>
</tr>
<tr>
<td>11°N, East Pacific Rise</td>
<td>0.051</td>
<td>0.14</td>
<td>2.7:1</td>
</tr>
<tr>
<td>South Cleft, Juan de Fuca ridge, Plume</td>
<td>0.082 ⇒ &lt; 0.09</td>
<td>0.196 ⇒ 0.436</td>
<td>2.4:1 ⇒ 4.8:1</td>
</tr>
<tr>
<td>South Cleft, Juan de Fuca ridge, Vent 1</td>
<td>0.093 ⇒ 0.12</td>
<td>0.27 ⇒ 0.527</td>
<td>2.9:1 ⇒ 4.4:1</td>
</tr>
</tbody>
</table>

Table 4 Methane and hydrogen concentrations in seafloor hydrothermal fluids

Based on this small data-set, we estimate that the amount of degassed hydrogen is probably five times larger than the one for methane: 1 x 10¹¹ mol H₂/a. Considering the oxygen budget, this flux results in an annual oxygen consumption of 5 x 10¹⁰ mol O₂/a (2H₂+O₂ → 2H₂O), a
similar amount to the one caused by methane degassing from seafloor hydrothermal fluids and volcanoes:

$$\frac{dO_{2,\text{mantle, hydrogen}}}{dt} \approx -5 \times 10^{10} \frac{\text{mol} \ O_2}{\text{a}}$$

Together volcanic and hydrothermal degassing of hydrogen and methane sum up to annual oxygen sink of $1 \times 10^{11}$ mol O$_2$/a, this is a tenth of the annual oxygen excess production by the carbon cycle.

**Oxidation of reduced iron during silicate weathering**

Silicate rocks contain reduced iron (e.g. pyroxenes and amphiboles). When silicates are weathered, the reduced iron is oxidized. The oxidation of reduced iron during silicate weathering therefore is another sink for oxygen (Table 5).

<table>
<thead>
<tr>
<th>Flux</th>
<th>Chemical reactions</th>
<th>Reservoir</th>
</tr>
</thead>
<tbody>
<tr>
<td>sil(1) wea$$_{silicate}$$</td>
<td>$2\text{CO}_2 + 2\text{H}_2\text{O} + \text{CaSiO}_3 \Rightarrow \text{Ca}^{2+} + 2\text{HCO}_3^- + \text{SiO}_2$</td>
<td>+2</td>
</tr>
<tr>
<td>sil(2) wea$$_{Fe^{2+}_silicate}$$</td>
<td>$4\text{FeSiO}_3 + \text{O}_2 \Rightarrow 2\text{Fe}_2\text{O}_3 + 4\text{SiO}_2$</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5 The effect of silicate weathering on the atmosphere-ocean reservoirs

From the long-term steady state assumption for oceanic alkalinity, we can estimate the average annual silicate weathering flux. Combining this flux with an estimate of the content of reduced iron in silicates, we can calculate the amount of oxygen consumed during silicate weathering.

Alkalinity mass balance for ocean for carbon and silicate fluxes (for chemical equations see Tables 1, 2 and 5):

$$\frac{d\text{Alk}}{dt} = 0 =$$

\[-2(\text{bur}_{\text{carbonate}} + \text{subduct}_{\text{carbonate}} - \text{wea}_{\text{carbonate}}) -

\[-2(\text{wea}_{\text{sulfide}} + \text{metamorph}_{\text{SO}_2_{sulfide}} + \text{metamorph}_{\text{H}_2\text{S}_{sulfide}} - \text{bur}_{\text{sulfide}} - \text{subduct}_{\text{sulfide}})

\[-2(\text{hydro}_{\text{mantle, H}_2\text{S}} + \text{volc}_{\text{mantle, SO}_2})

+2 \cdot \text{wea}_{\text{silicate}}$$

(Eq 1_Sil)
and from Eq 5_C and Eq 5_S:
\[
\text{bur}_{\text{carbonate}} + \text{subduct}_{\text{carbonate}} - \text{wea}_{\text{carbonate}} = \text{Diff}_{\text{carbonate}} + \text{metamorph}_{\text{CO}_2-\text{carbonate}}
\]
\[
\text{Diff}_{\text{sulfide}} = \text{bur}_{\text{sulfide}} + \text{subduct}_{\text{sulfide}} - \text{wea}_{\text{sulfide}} - \text{metamorph}_{\text{SO}_2-\text{sulfide}} - \text{metamorph}_{\text{H}_2\text{S}-\text{sulfide}}
\]
\[
0 = -2 \cdot (\text{Diff}_{\text{carbonate}} + \text{metamorph}_{\text{CO}_2-\text{carbonate}}) - 2 \cdot \text{Diff}_{\text{sulfide}} - 2 \cdot (\text{hydro}_{\text{mantle}}_{\text{H}_2\text{S}} + \text{volc}_{\text{mantle}}_{\text{SO}_2}) + 2 \cdot \text{wea}_{\text{silicate}}
\]

and from Eq S_8 and Eq C_8:
\[
\text{Diff}_{\text{sulfide}} = \frac{\delta^{34}S_{\text{mantle}}_{\text{H}_2\text{S}} - \delta^{34}S_{\text{SO}_2-\text{steady}}}{\Delta^{34}_{\text{S-sulfide}}} + \text{volc}_{\text{mantle}}_{\text{SO}_2} \cdot \frac{\delta^{34}S_{\text{mantle}}_{\text{SO}_2} - \delta^{34}S_{\text{SO}_2-\text{steady}}}{\Delta^{34}_{\text{S-sulfide}}}
\]
\[
\text{Diff}_{\text{carbonate}} = \text{mantle}_{\text{CO}_2} \cdot \left( 1 - \frac{\delta^{13}C_{\text{mantle-CO}_2} - \delta^{13}C_{\text{corg- steady}}}{\Delta^{13}_{\text{C-corg}}} \right) + \text{mantle}_{\text{methane}} \cdot \left( 1 - \frac{\delta^{13}C_{\text{mantle-methane}} - \delta^{13}C_{\text{corg- steady}}}{\Delta^{13}_{\text{C-corg}}} \right)
\]
\[
\Rightarrow \text{wea}_{\text{silicate}} = \text{mantle}_{\text{CO}_2} \cdot \left( 1 - \frac{\delta^{13}C_{\text{mantle-CO}_2} - \delta^{13}C_{\text{corg- steady}}}{\Delta^{13}_{\text{C-corg}}} \right) + \text{mantle}_{\text{methane}} \cdot \left( 1 - \frac{\delta^{13}C_{\text{mantle-methane}} - \delta^{13}C_{\text{corg- steady}}}{\Delta^{13}_{\text{C-corg}}} \right) + \text{metamorph}_{\text{CO}_2-\text{carbonate}}
\]
\[
+ (\text{hydro}_{\text{mantle}}_{\text{H}_2\text{S}} + \text{volc}_{\text{mantle}}_{\text{SO}_2}) \cdot \left( 1 - \frac{\delta^{34}S_{\text{mantle}}_{\text{H}_2\text{S}} - \delta^{34}S_{\text{SO}_2-\text{steady}}}{\Delta^{34}_{\text{S-sulfide}}} \right)
\]
The silicate weathering has to balance the effects of mantle degassing of CO₂, methane, H₂S and SO₂, but also the metamorphic degassing of CO₂.

Using the steady-state assumptions for the carbon- and sulfur cycle, we get:
\[
\text{wea}_{\text{silicate}} = 4 \cdot 10^{12} \cdot \left( 1 - \frac{-7\% - 1\%}{-28\%} \right) + 3 \cdot 10^{10} \cdot \left( 1 - \frac{-18\% - 1\%}{-28\%} \right)
\]
\[
+ \text{metamorph}_{\text{CO}_2-\text{carbonate}}
\]
\[
+ (\text{hydro}_{\text{mantle}}_{\text{H}_2\text{S}} + \text{volc}_{\text{mantle}}_{\text{SO}_2}) \cdot \left( 1 - \frac{3.5\% - 20\%}{-30\%} \right)
\]
wea_{silicate} = 2.86 \times 10^{12} \text{ mol/a} + 1 \times 10^0 \text{ mol/a} + \text{metamorph}_{\text{CO}_2\_carbonate} \\
+ \left( \text{hydro}_{\text{mantle\_H}_2\text{S}} + \text{volc}_{\text{mantle\_SO}_2} \right) \times 0.45

We have not yet evaluated the “mantle”-flux of hydrogen sulfide and sulfur dioxide, a lower limit would be no flux at all, an upper limit probably equals to the amount of degassed carbon dioxide (4 \times 10^{12} \text{ mol SO}_2 \text{ and H}_2\text{S per year}).

According to estimates by MÖRNER and ETIOPE (2002), today’s global annual flux of carbon dioxide is at least 600 Mt CO$_2$/a (=13 \times 10^{12} \text{ mol CO}_2/a). This value is well above the long-term estimate for annual CO$_2$ uptake by silicate weathering of 295 Mt CO$_2$/a (=7 \times 10^{12} \text{ mol CO}_2/a) by BERNER et al. (1983) and BICKLE (1996). MÖRNER and ETIOPE (2002) identify two reasons for the discrepancy between these estimates. First, Quaternary metamorphic carbon dioxide degassing fluxes are likely to be higher than the long-term average (KERRICK and CALDEIRA, 1998) and secondly, mantle sources of carbon dioxide were not considered in the carbon cycle examined by BERNER et al. (1983) and BERNER (1991). We therefore add our calculated value for the silicate weathering caused by mantle sources to the estimate of 7 \times 10^{12} \text{ mol CO}_2/a. This results into an average silicate weathering flux of 1 \times 10^{13} \text{ mol per year plus the not yet defined effect of degassed hydrogen sulfide and sulfur dioxide:}

(Eq 4_Sil)

\[
\text{wea}_{silicate} = 2.86 \times 10^{12} \text{ mol/a} + 1 \times 10^0 \text{ mol/a} + 7 \times 10^{12} \text{ mol/a} \\
+ \left( \text{hydro}_{\text{mantle\_H}_2\text{S}} + \text{volc}_{\text{mantle\_SO}_2} \right) \times 0.45 \\
= 1 \times 10^{13} \text{ mol/a} + \left( \text{hydro}_{\text{mantle\_H}_2\text{S}} + \text{volc}_{\text{mantle\_SO}_2} \right) \times 0.45
\]

As value for the average Fe$^{2+}$-content in silica rocks, we use the average crustal composition of (RONOV and YAROSHEVSKY, 1969): SiO$_2$=57.6 weight% and FeO=4.27 weight%. This results in a molar Si : Fe$^{2+}$ ratio of 16:1, a value similar to the composition of diorite or andesite (Table 6).
<table>
<thead>
<tr>
<th>Element composition</th>
<th>Syenite</th>
<th>Granite</th>
<th>Grano–diorite</th>
<th>Tonalite</th>
<th>Diorite</th>
<th>Andesite</th>
<th>Gabbro</th>
<th>Basalt</th>
<th>Pyroxenite</th>
<th>Peridotite</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt% SiO₂</td>
<td>58.58</td>
<td>71.3</td>
<td>66.9</td>
<td>61.52</td>
<td>57.48</td>
<td>57.94</td>
<td>50.14</td>
<td>49.2</td>
<td>46.27</td>
<td>42.26</td>
</tr>
<tr>
<td>wt% FeO</td>
<td>3.04</td>
<td>1.64</td>
<td>2.73</td>
<td>3.82</td>
<td>4.92</td>
<td>4.04</td>
<td>7.62</td>
<td>7.13</td>
<td>7.18</td>
<td>6.58</td>
</tr>
<tr>
<td>Mol ratio Si:Fe²⁺</td>
<td>23:1</td>
<td>52:1</td>
<td>29:1</td>
<td>19:1</td>
<td>14:1</td>
<td>17:1</td>
<td>8:1</td>
<td>8:1</td>
<td>7.7:1</td>
<td>7.7:1</td>
</tr>
</tbody>
</table>

*Table 6 Si : Fe²⁺ ratios of common igneous rock types (data from Cox et al. (1979))*

The amount of Fe²⁺ oxidized during silicate weathering is therefore one sixteenth of the silicate weathering flux. Using the chemical equation for iron-silicate weathering (4FeSiO₃ + O₂ ⇌ 2Fe₂O₃ + 4 SiO₂), we get a value for oxygen consumption:

\[
\frac{dO₂\_{Fe^{2+} silicate}}{dt} = -\frac{1}{16} \cdot \frac{1}{4} \cdot \text{wea silicate} =
\]

\[
-\frac{1}{16} \cdot \frac{1}{4} \cdot \left(1 \cdot 10^{13} \text{mol} / a + \left(\text{hydro mantle}_\text{H}_2\text{S} + \text{volc mantle}_\text{SO}_2\right) \cdot 0.45\right)
\]

\[
= -1.56 \cdot 10^{11} \text{mol} / a - 0.007 \left(\text{hydro mantle}_\text{H}_2\text{S} + \text{volc mantle}_\text{SO}_2\right)
\]

*Phanerozoic steady state for oxygen*

In absence of other important oxygen sinks, the oxygen production and consumption by the carbon- and sulfur cycle and by the oxidation of degassed hydrogen and Fe²⁺-silicates must be balanced. Consequently, we can calculate the amount of hydrogen sulfide and sulfur dioxide degassed by seafloor hydrothermal systems and volcanoes (Eq 1_H₂, Eq 13_C, Eq 5_Sil and Eq 10_S):

\[
\frac{dO₂\_{mantle hydrogen}}{dt} = -5 \cdot 10^{10} \frac{\text{mol} \_ O₂}{a}
\]

\[
\frac{dO₂\_{Carbon cycle}}{dt} = +1 \cdot 10^{12} \frac{\text{mol} \_ O₂}{a}
\]

\[
\frac{dO₂\_{Fe^{2+} silicate}}{dt} = -1.56 \cdot 10^{11} \text{mol} / a - 0.008 \left(\text{hydro mantle}_\text{H}_2\text{S} + \text{volc mantle}_\text{SO}_2\right)
\]

\[
\frac{dO₂\_{sulfur cycle}}{dt} = -\text{hydro mantle}_\text{H}_2\text{S} \cdot 0.97 + \text{volc mantle}_\text{SO}_2 \cdot 0.53
\]
**Oxygen mass balance:**

\[
0 = \frac{dO_2}{dt}_{\text{atmosphere}} + \frac{dO_2}{dt}_{\text{Carbon cycle}} + \frac{dO_2}{dt}_{\text{Sulfur cycle}} + \frac{dO_2}{dt}_{\text{Mantle hydrogen}} + \frac{dO_2}{dt}_{\text{Fe}^{2+} \text{silicate}}
\]

\[
= +1 \times 10^{12} \frac{\text{mol} \cdot O_2}{a} - \text{hydro} \_{\text{H}_2S} \cdot 0.97 + \text{volc} \_{\text{SO}_2} \cdot 0.53 - 5 \times 10^{10} \frac{\text{mol} \cdot O_2}{a} - 1.56 \times 10^{11} \text{mol} \cdot a - 0.007 \left( \text{hydro} \_{\text{H}_2S} + \text{volc} \_{\text{SO}_2} \right)
\]

\[
\Rightarrow \text{hydro} \_{\text{H}_2S} \cdot (0.97 + 0.007) = \text{volc} \_{\text{SO}_2} \cdot (0.53 - 0.007) + \left( 1 \times 10^{12} - 5 \times 10^{10} - 1.56 \times 10^{11} \right) \frac{\text{mol} \cdot O_2}{a}
\]

\[
\Rightarrow 0.97 \cdot \text{hydro} \_{\text{H}_2S} = 0.53 \cdot \text{volc} \_{\text{SO}_2} + 7.9 \times 10^{11} \frac{\text{mol} \cdot O_2}{a}
\]

\[
\text{hydro} \_{\text{H}_2S} = 0.55 \cdot \text{volc} \_{\text{SO}_2} + 8 \times 10^{11} \frac{\text{mol} \cdot O_2}{a}
\]

The oxygen consumption by hydrogen sulfide is in the range of \(8 \times 10^{11}\) mol O\(_2\) per year, a value that equals 80% of the oxygen produced by the imbalance in the steady state of the carbon system.

**Discussion**

Based on a steady state model for the oxygen, carbon and sulfur cycle, a steady state oceanic alkalinity reservoir and estimates of flux sizes and isotope values, we calculated the flux of hydrogen sulfide degassing from mantle and oceanic crust.

Several questions have to be addressed:

1) **Would slightly different estimates of flux sizes or isotope values cause a strong change in our results? And would these results negate significant “mantle”-fluxes of hydrogen sulfide?**

The oxygen consumption by silicate weathering and oxidation of degassed methane and hydrogen is small compared to the oxygen production by the carbon cycle (\(2 \times 10^{11}\) mol/a respectively 20%). As long as these fluxes are not grossly underestimated, they cannot compensate for the excess oxygen production by the carbon cycle. However, the estimates for the CO\(_2\)-degassing flux and for the steady state values of the long-term carbon cycle affect the
resulting oxygen-production strongly: Oxygen production is proportional to the degassing of carbon dioxide and inversely proportional to the carbon isotope fractionation factor (Eq 13_C):
\[
\frac{dO_2 \text{ }_{\text{Carbon cycle}}}{dt} = \text{mantle}_{\text{CO}_2} \cdot \frac{\delta^{13}C_{\text{mantle-CO}_2} - \delta^{13}C_{\text{C-steady}}}{\Delta_{\text{13 corg}}}
\]
Consequently, an overestimated mantle degassing of carbon dioxide would result in higher oxygen production. The following scenario estimates a minimum oxygen production by the long-term steady state imbalance in the carbon cycle (Eq 12_C and Eq 13_C):
• Carbon dioxide fluxes from ridges only, lowest estimate: $2.2 \pm 0.9 \times 10^{12}$ mol per year (MARTY and TOLSTIKHIN, 1998)
• A fractionation factor for the Phanerozoic of $-30\%$ (HAYES et al., 1999)
• A carbon isotope composition of degassed carbon dioxide of $-5\%$ (KUMP and ARTHUR, 1999)
• A steady state carbon isotope value of $0\%$
\[
\frac{dO_2 \text{ }_{\text{Carbon cycle}}}{dt} = \text{Diff}_{\text{corg}} - 2 \cdot \text{mantle}_{\text{methane}} = \text{mantle}_{\text{CO}_2} \cdot \frac{\delta^{13}C_{\text{mantle-CO}_2} - \delta^{13}C_{\text{C-steady}}}{\Delta_{\text{13 corg}}} + \text{mantle}_{\text{methane}} \cdot \frac{\delta^{13}C_{\text{mantle-methane}} - \delta^{13}C_{\text{C-steady}}}{\Delta_{\text{13 corg}}}
\]
\[
-2 \cdot \text{mantle}_{\text{methane}} \Rightarrow \frac{dO_2 \text{ }_{\text{Carbon cycle}}}{dt} = 1.3 \cdot 10^{12} \cdot \frac{-5\% - 0\%}{-30\%} + 3 \cdot 10^{10} \cdot \left(\frac{-18\% - 0\%}{-30\%} - 2\right)
\]
\[
= 1.3 \cdot 10^{12} \cdot \frac{6}{5} - 3 \cdot 10^{10} \cdot \frac{7}{5} = 1.75 \cdot 10^{11}
\]
Even without a contribution of carbon dioxide from plumes, the resulting oxygen production is only slightly lower than the consumption by silicate weathering and oxidation of hydrogen ($2.06 \times 10^{11}$ mol). Therefore, it is unlikely that different estimates of “mantle” carbon dioxide fluxes and steady state isotope assumptions will reduce the oxygen excess production to an amount that could be balanced without degassing fluxes of hydrogen sulfide. But even in such a case, degassing of hydrogen could still be possible as a compensation of the sulfur dioxide degassing from mantle sources:
\[
\text{hydro}_{\text{mantle-H_2S}} \approx 0.55 \cdot \text{volc}_{\text{mantle-SO}_2}
\]
The mantle derived sulfur dioxide flux to volcanoes is in the range of 2.7 Mt SO₂
\((= 4.2 \times 10^{10} \text{ mol SO}_2/\text{a})\) per year (Wallace, 2001) (the total volcanic degassing of SO₂ is about a magnitude larger, e.g. Symonds et al. (1994); Cadle (1980)). The effect of the degassing of mantle sulfur dioxide is small and consequently, a mantle-flux of hydrogen sulfide would be also small if it were only to compensate SO₂ degassing:

\[
\text{hydro}_{\text{mantle}} \cdot \text{H}_2\text{S} \approx 0.55 \cdot \text{volc}_{\text{mantle}} \cdot \text{SO}_2 = 0.55 \cdot 4.2 \cdot 10^{10} = 2.3 \cdot 10^{10} \text{ mol } \text{H}_2\text{S}/\text{a}
\]

2) **Is the calculated value in the range of estimated fluxes?**

Neglecting the minor effect of sulfur dioxide degassing, the flux of hydrogen sulfide is in the order of \(8 \times 10^{11} \text{ mol H}_2\text{S} \text{ per year}\). This value is at the upper limit proposed by Carpenter and Lohmann (1997) of \(5–8 \times 10^{11} \text{ mol H}_2\text{S} \text{ per year}\) and close to the \(\text{H}_2\text{S}\) flux estimate of Edmond et al. (1979) \(10 \times 10^{11} \text{ mol H}_2\text{S} \text{ per year}\). Alt (1995b) estimates the flux of sulfur from the oceanic crust to seawater as \(2.5 \times 10^{12} \text{ g S per year} (=0.78 \times 10^{11} \text{ mol H}_2\text{S per year})\), a flux ten times smaller than proposed by us. The flux sizes calculated by Edmond et al. (1979) and Alt (1995b) are based on measurements of sulfur contents in ocean crust rocks and estimates of the annual production of oceanic crust while the flux sizes calculated by Carpenter and Lohmann (1997) and us is based on estimates of long-term isotope and flux values. The large range of the proposed hydrogen sulfide fluxes is mainly caused by the uncertainties in the corresponding estimates. As the knowledge about seafloor hydrothermal systems is currently growing, we are optimistic that more precise estimates will soon be available, probably leading to a convergence in the estimates of degassed hydrogen sulfide.

3) **What are the consequences of “mantle”-derived fluxes?**

Probably, not only the mass balances of the atmosphere-ocean system should be at steady state, but on time scales of several million years, also the continental crust reservoirs of carbonate, organic matter, sulfate and sulfides. Especially changes in the ratios of reduced to oxidized reservoirs could lead to runaway feedback mechanisms: A growing reservoir of organic matter and a steady reservoir of carbonate would, over the long-term, lead to increased weathering of organic matter over carbonate-weathering. This would have to be buffered by increased burial of organic matter leading to an even larger reservoir of organic matter. As long as the oxidized reservoirs grow in the same range as reduced reservoirs, no runaway feedback will occur. However, we can calculate the differences between removal and input of carbonates, organic matter, sulfate and sulfides:
Organic matter (Eq 8_C):

\[ \text{Diff}_{\text{corg}} = \text{bur}_{\text{corg}} + \text{subduct}_{\text{corg}} - \text{wea}_{\text{corg}} - \text{metamorph}_{\text{CO}_2}_{\text{corg}} - \text{metamorph}_{\text{methane}}_{\text{corg}} \]

\[ = \text{mantle}_{\text{CO}_2} \cdot \frac{\delta^{13} C_{\text{mantle-CO}_2} - \delta^{13} \text{C}_{\text{c-steady}}}{\Delta^{13} \text{corg}} + \text{mantle}_{\text{methane}} \cdot \frac{\delta^{13} C_{\text{mantle-methane}} - \delta^{13} \text{C}_{\text{c-steady}}}{\Delta^{13} \text{corg}} \]

Carbonates (Eq 8_C):

\[ \text{Diff}_{\text{carbonate}} = \text{bur}_{\text{carbonate}} + \text{subduct}_{\text{carbonate}} - \text{wea}_{\text{carbonate}} - \text{metamorph}_{\text{CO}_2}_{\text{carbonate}} \]

\[ = \text{mantle}_{\text{CO}_2} \cdot \left( 1 - \frac{\delta^{13} C_{\text{mantle-CO}_2} - \delta^{13} \text{C}_{\text{c-steady}}}{\Delta^{13} \text{corg}} \right) + \text{mantle}_{\text{methane}} \cdot \left( 1 - \frac{\delta^{13} C_{\text{mantle-methane}} - \delta^{13} \text{C}_{\text{c-steady}}}{\Delta^{13} \text{corg}} \right) \]

Sulfides (Eq 8_S):

\[ \text{Diff}_{\text{sulfide}} = \]

\[ = \text{subduct}_{\text{sulfide}} = \text{hydro}_{\text{mantle}_{\text{H}_2\text{S}}} \cdot \delta^{34} S_{\text{mantle}_{\text{H}_2\text{S}}} - \delta^{34} S_{\text{SO}_4} - \text{steady} \]

\[ + \text{volc}_{\text{mantle}_{\text{SO}_2}} \cdot \delta^{34} S_{\text{mantle}_{\text{SO}_2}} - \delta^{34} S_{\text{SO}_4} - \text{steady} \]

Sulfate (Eq 8_S):

\[ \text{Diff}_{\text{sulfate}} = \]

\[ = \text{subduct}_{\text{sulfate}} + \text{hydro}_{\text{sulfatePrecip}} - \text{hydro}_{\text{sulfateWeather}} - \text{wea}_{\text{sulfate}} - \text{metamorph}_{\text{SO}_2}_{\text{sulfate}} - \text{metamorph}_{\text{H}_2\text{S}_{\text{sulfate}}} \]

\[ = \text{hydro}_{\text{mantle}_{\text{H}_2\text{S}}} \cdot \left( 1 - \frac{\delta^{34} S_{\text{mantle}_{\text{H}_2\text{S}}} - \delta^{34} S_{\text{SO}_4} - \text{steady}}{\Delta^{34} S_{\text{sulfide}}} \right) + \text{volc}_{\text{mantle}_{\text{SO}_2}} \cdot \left( 1 - \frac{\delta^{34} S_{\text{mantle}_{\text{SO}_2}} - \delta^{34} S_{\text{SO}_4} - \text{steady}}{\Delta^{34} S_{\text{sulfide}}} \right) \]

Neglecting the quantitatively unimportant fluxes of “mantle”–methane and –sulfur dioxide, and assuming that subduction keeps the continental crust reservoirs at steady state, we get:

Organic matter:

\[ \text{Diff}_{\text{corg}} = \text{subduct}_{\text{corg}} = \text{mantle}_{\text{CO}_2} \cdot \frac{\delta^{13} C_{\text{mantle-CO}_2} - \delta^{13} \text{C}_{\text{c-steady}}}{\Delta^{13} \text{corg}} \]

Carbonates:

\[ \text{Diff}_{\text{carbonate}} = \text{subduct}_{\text{carbonate}} = \text{mantle}_{\text{CO}_2} \cdot \left( 1 - \frac{\delta^{13} C_{\text{mantle-CO}_2} - \delta^{13} \text{C}_{\text{c-steady}}}{\Delta^{13} \text{corg}} \right) \]

Sulfides:

\[ \text{Diff}_{\text{sulfide}} = \text{subduct}_{\text{sulfide}} = \text{hydro}_{\text{mantle}_{\text{H}_2\text{S}}} \cdot \frac{\delta^{34} S_{\text{mantle}_{\text{H}_2\text{S}}} - \delta^{34} S_{\text{SO}_4} - \text{steady}}{\Delta^{34} S_{\text{sulfide}}} \]
Sulfate:

\[ \text{Diff}_{\text{sulfate}} = \text{hydro}_{\text{sulfate \{precip\}}} - \text{hydro}_{\text{sulfate \{leach\}}} = \text{hydro}_{\text{mantle \_ H}_2S} \cdot \left( 1 - \frac{\delta^{34}S_{\text{mantle \_ H}_2S} - \delta^{34}S_{SO_4 \_ \text{steady}}}{\Delta^{34}S_{\text{sulfide}}} \right) \]

We can calculate now the ratio between carbonates and organic matter removed by subduction:

\[ \frac{\text{subduct}_{\text{carbonate}}}{\text{subduct}_{\text{corg}}} = \frac{1 - \frac{\delta^{13}C_{\text{mantle \_ CO}_2} - \delta^{13}C_{\text{c \_ steady}}}{\Delta^{13}C_{\text{corg}}}}{\frac{\Delta^{13}C_{\text{mantle \_ CO}_2} - \delta^{13}C_{\text{c \_ steady}}}{\Delta^{13}C_{\text{corg}}}} = \frac{\Delta^{13}C_{\text{mantle \_ CO}_2} - \delta^{13}C_{\text{c \_ steady}}}{\Delta^{13}C_{\text{mantle \_ CO}_2} - \delta^{13}C_{\text{c \_ steady}}} \]

\[ = \frac{\Delta^{13}C_{\text{mantle \_ CO}_2} - \delta^{13}C_{\text{c \_ steady}}}{\delta^{13}C_{\text{mantle \_ CO}_2} - \delta^{13}C_{\text{c \_ steady}}} - 1 \approx -28\% - 7\% - 1\% - 1 = 2.5 \]

The ratio between organic matter and carbonates in sediments is between 1:3 and 1:4, the carbonate content of subducted sediments is likely to be lower than this average. A value of 1:2.5 for subducted sediments is therefore possible.

As a ratio between the sulfate and sulfides removed by subduction, we get:

\[ \frac{\text{hydro}_{\text{sulfate \{precip\}}} - \text{hydro}_{\text{sulfate \{leach\}}}{\text{subduct}_{\text{sulfide}}} = \frac{1 - \frac{\delta^{34}S_{\text{mantle \_ H}_2S} - \delta^{34}S_{SO_4 \_ \text{steady}}}{\Delta^{34}S_{\text{sulfide}}}}{\delta^{34}S_{\text{mantle \_ H}_2S} - \delta^{34}S_{SO_4 \_ \text{steady}}} \]

\[ = \frac{\Delta^{34}S_{\text{sulfide}} - (\delta^{34}S_{\text{mantle \_ H}_2S} - \delta^{34}S_{SO_4 \_ \text{steady}})}{\delta^{34}S_{\text{mantle \_ H}_2S} - \delta^{34}S_{SO_4 \_ \text{steady}}} - 1 \approx \frac{-30\%}{3.5\% - 20\%} - 1 \approx 0.82 \]

A larger fraction of pyrite than sulfate has to be subducted to keep the sulfide reservoir of the continental crust balanced. In subduction zones, sulfate is only removed in minor quantities, e.g. as barite and sulfate structural bound in carbonates, while pyrite is abundant. Sulfate should therefore be removed in seafloor hydrothermal systems. We can calculate the amount of sulfate removed by this process for the case of a constant continental sulfate reservoir size:

\[ \frac{\text{hydro}_{\text{sulfate \{precip\}}} - \text{hydro}_{\text{sulfate \{leach\}}}{\text{subduct}_{\text{sulfide}}} = \text{hydro}_{\text{mantle \_ H}_2S} \cdot \left( 1 - \frac{\delta^{34}S_{\text{mantle \_ H}_2S} - \delta^{34}S_{SO_4 \_ \text{steady}}}{\Delta^{34}S_{\text{sulfide}}} \right) \]
\[ \Rightarrow \text{hydro}_{\text{sulfate \text{(precip)}}} - \text{hydro}_{\text{sulfate \text{(leach)}}} = 8 \cdot 10^{11} \cdot \left(1 - \frac{3.5\%e - 20\%e}{-30\%e}\right) = 3.6 \cdot 10^{11} \frac{\text{mol SO}_4^{2-}}{a} \]

In seafloor hydrothermal systems, precipitation of anhydrite starts at 150°C (ALT, 1995a). We can use the amount of sulfate entering mid-ocean ridges \(1 \times 10^{12} \text{ mol SO}_4^{2-} \text{ per year}\) (HOLLAND, 2002) to \(3.8 \times 10^{12} \text{ mol SO}_4^{2-} \text{ per year}\) (EDMOND et al., 1979)) as a maximum value for sulfate precipitation. However, a large part of the entering sulfate is either redissolved or not precipitated (TEAGLE et al., 1998).

ALT (1995b) estimates the flux of sulfate to the oceanic crust as \(2.1 \times 10^{12} \text{ g S per year} \) \(= 0.65 \times 10^{11} \text{ mol SO}_4^{2-} \text{ per year}\) which is probably slightly underestimated (BACH et al., 2003). In addition, peridotites are a sink for 0.4 to 6 \(\times 10^{12} \text{ g S per year}\) (ALT and SHANKS, 2003) \(= 0.13 \text{ to 1.87 x 10}^{11} \text{ mol SO}_4^{2-} \text{ per year}\). These estimates sum up to a value of 0.78 to \(2.52 \times 10^{11} \text{ mol SO}_4^{2-} \text{ per year}\) indicating that our value of \(3.6 \times 10^{11} \text{ mol SO}_4^{2-} \text{ per year}\) is overestimated. From the study of long-term fluxes and budget of ferric iron, LÉCUYER and RICARD, (1999) calculated a hydrothermal alteration of Fe\(^{2+}\) to Fe\(^{3+}\) by reaction with seawater in the order of \(20.8 \times 10^3 \text{ kg Fe per second} \) \(=1.174 \times 10^{13} \text{ mol Fe per year}\) and a subduction of \(12.5 \times 10^3 \text{ kg Fe}^{3+} \text{ per second} \) \(=7.05 \times 10^{12} \text{ mol Fe per year}\). The proposed chemical reaction transfers \(21 \text{ mol Fe}^{2+}\) to \(21 \text{ mol Fe}^{3+}\) under the consumption of \(2 \text{ mol SO}_4^{2-}\):

\[
11\text{Fe}_2\text{SiO}_4 + 2\text{SO}_4^{2-} + 4\text{H}^+ \Rightarrow \text{FeS}_2 + 7\text{Fe}_3\text{O}_4 + 11\text{SiO}_2 + 2\text{H}_2\text{O}
\]

Therefore, the alteration consumes \(1.12 \times 10^{12} \text{ mol SO}_4^{2-} \text{ per year}\). Compared to the estimates above this is likely to be an overestimate. Per year, the subduction-flux of LÉCUYER and RICARD, (1999) transfers \(2.68 \times 10^{12} \text{ mol O}^{2-}\) derived from sulfate to the mantle. However, the consumption of seawater sulfate in seafloor hydrothermal systems removes oxidized sulfur-species from seawater while degassing of hydrogen sulfide brings in reduced sulfur-species. This mechanism probably works as a long-term oxygen-feedback: During times of high atmospheric oxygen pressures, sulfate reduction and sulfide burial could be less efficient. Consequently, the sulfate concentration of seawater would rise, leading to an increased flux of sulfate into hydrothermal systems.
Conclusions

Significant degassing of hydrogen sulfide from seafloor hydrothermal systems on geologic time scales has been questioned because of its potential to generate secular trends in the sulfur isotopic composition of seawater (Petsch, 1999). However, degassing of volatiles (e.g. carbon dioxide, sulfur dioxide and hydrogen sulfide) from seafloor hydrothermal systems and volcanoes are-well known phenomena. Based on long-term (Phanerozoic) steady state mass and isotope balance calculations of the atmospheric and oceanic reservoirs of alkalinity, oxygen, carbon and sulfur, we conclude that an overproduction of oxygen caused by mantle degassing of carbon dioxide is partly compensated by the mantle degassing and subsequent oxidation of hydrogen sulfide.

Estimating a flux of CO₂ in the order of \(4 \times 10^{12}\) mol CO₂ per year, an average overproduction of \(1 \times 10^{12}\) mol O₂ per year results. On the long-term average, volcanic and hydrothermal degassing of hydrogen and methane, and oxidation of reduced iron during silicate weathering act as sinks of \(0.2 \times 10^{12}\) mol O₂ per year while the degassing of hydrogen sulfide consumes about \(0.8 \times 10^{12}\) mol O₂ per year. The calculated value of hydrogen sulfide degassing is proportional to the degassing-flux of CO₂ and therefore the estimated value strongly depends on the estimate of degassed carbon dioxide. A part of the degassed and oxidized hydrogen sulfide is removed as sulfate from seawater entering seafloor hydrothermal systems (\(3.6 \times 10^{11}\) mol SO₄²⁻ per year). Based on investigations of sulfur concentrations and isotope values in oceanic crust rocks and ophiolites, the annual flux of hydrogen sulfide degassed from seafloor hydrothermal systems is in the order of \(0.78 \times 10^{11}\) mol H₂S per year and the flux of sulfate removed into seafloor hydrothermal systems in the range of \(0.13\) to \(1.87 \times 10^{11}\) mol SO₄²⁻ per year (Alt, 1995b; Alt and Shank, 2003). This indicates that our calculated flux of hydrogen sulfide is either an overestimate or that current degassing of H₂S is lower than the long-term average. The same observation holds for the removal of sulfate from seawater. Especially lower estimates of mantle derived CO₂ fluxes, different estimates of the average sulfur isotope fractionation and long-term sulfur isotope composition of seawater as well as further oxygen sinks would reduce our estimate of hydrogen sulfide degassing and sulfate removal. However, based on our findings, we conclude that hydrogen sulfide degassing by seafloor hydrothermal systems is needed to keep the amount of oxygen in the atmosphere balanced on the Phanerozoic timescale. Due to uncertainties in the amount of degassed hydrogen sulfide and removed seawater-sulfate, it can only be speculated how far
seafloor hydrothermal systems could also play a more active role as negative feedback-mechanism lowering high oxygen pressures.

References


Appendix 1

Calculations for the steady-state sulfur cycle

The mass balance of seawater sulfate:

\[
\frac{dM_{\text{SO}_4}}{dt} = \text{wea sulfate} - \text{bur sulfate} + \text{wea sulfide} - \text{bur sulfide} + \text{metamorph SO}_2 \_ \text{ sulfate} + \text{metamorph H}_2\text{S} \_ \text{ sulfide} + \text{hydro mantle} \_ \text{H}_2\text{S} + \text{volc mantle } \_ \text{SO}_2 + \text{hydro sulfate leach} - \text{hydro sulfate precip} - \text{subduct sulfide}
\]

The isotope mass balance of seawater-sulfate:

\[
\frac{d(\delta^{34} \text{SO}_4 \_ \text{Ocean} \cdot M_{\text{SO}_4})}{dt} = \text{wea sulfate} \cdot \delta^{34} \text{S sulfate} - \text{bur sulfate} \cdot \left( \delta^{34} \text{S} \_ \text{Ocean} + \Delta^{34} \text{S sulfate} \right) + \text{wea sulfide} \cdot \delta^{34} \text{S sulfide} - \text{bur sulfide} \cdot \left( \delta^{34} \text{S} \_ \text{Ocean} + \Delta^{34} \text{S sulfide} \right) + \left( \text{metamorph SO}_2 \_ \text{ sulfate} + \text{metamorph H}_2\text{S} \_ \text{ sulfide} \right) \cdot \delta^{34} \text{S sulfate} + \left( \text{metamorph SO}_2 \_ \text{ sulfide} + \text{metamorph H}_2\text{S} \_ \text{ sulfide} \right) \cdot \delta^{34} \text{S sulfide} + \text{hydro mantle} \_ \text{H}_2\text{S} \cdot \delta^{34} \text{S mantle} \_ \text{H}_2\text{S} + \text{volc mantle } \_ \text{SO}_2 \cdot \delta^{34} \text{S mantle } \_ \text{SO}_2 + \text{hydro sulfate leach} \cdot \delta^{34} \text{S sulfate leach} - \text{hydro sulfate precip} \cdot \delta^{34} \text{S} \_ \text{Ocean} - \text{subduct sulfide} \cdot \delta^{34} \text{S sulfide}
\]

Long-term steady state assumptions:

\[
M_{\text{SO}_4}(t) = M_{\text{SO}_4 \_ \text{steady}} \Rightarrow \frac{dM_{\text{SO}_4}}{dt} = 0
\]

\[
\delta^{34} \text{S} \_ \text{Ocean}(t) = \delta^{34} \text{S} \_ \text{steady} \Rightarrow \frac{d\delta^{34} \text{S} \_ \text{Ocean}}{dt} = 0
\]

\[
\Delta^{34} \text{S sulfate} = 0 \%
\]

\[
\Rightarrow \delta^{34} \text{S sulfate} = \delta^{34} \text{S} \_ \text{steady}
\]

\[
\Rightarrow \delta^{34} \text{S sulfide} = \delta^{34} \text{S} \_ \text{steady} + \Delta^{34} \text{S sulfide}
\]

\[
\Rightarrow \delta^{34} \text{S sulfide leach} = \delta^{34} \text{S} \_ \text{steady}
\]
Reformulation of the steady state mass balance and isotope mass balance:  

(Eq 4_S)

\[
\text{hydro}_\text{mantle}_{-H_2S} + \text{volc}_\text{mantle}_{-SO_2} = \\
\text{bur}_{\text{sulfate}} - \text{wea}_{\text{sulfate}} \\
+ \text{bur}_{\text{sulfide}} - \text{wea}_{\text{sulfide}} \\
- \text{metamorph}_{SO_2-\text{sulfate}} - \text{metamorph}_{H_2S-\text{sulfate}} - \text{metamorph}_{H_2S-\text{sulfide}} \\
- \text{hydro}_{\text{sulfate(leach)}} + \text{hydro}_{\text{sulfate(precip)}} + \text{subduct}_{\text{sulfide}} \\
\Rightarrow \\
\text{hydro}_\text{mantle}_{-H_2S} + \text{volc}_\text{mantle}_{-SO_2} = \\
\text{bur}_{\text{sulfate}} + \text{hydro}_{\text{sulfate(precip)}} - \text{hydro}_{\text{sulfate(wea)}} - \text{wea}_{\text{sulfate}} - \text{metamorph}_{SO_2-\text{sulfate}} - \text{metamorph}_{H_2S-\text{sulfate}} \\
+ \text{bur}_{\text{sulfide}} + \text{subduct}_{\text{sulfide}} - \text{wea}_{\text{sulfide}} - \text{metamorph}_{SO_2-\text{sulfide}} - \text{metamorph}_{H_2S-\text{sulfide}} \\
\Rightarrow \\
\text{hydro}_\text{mantle}_{-H_2S} + \text{volc}_\text{mantle}_{-SO_2} = \text{Diff}_{\text{sulfate}} + \text{Diff}_{\text{sulfide}}
\]

Expressions to simplify the equations: \(\text{Diff}_{\text{sulfate}}\) and \(\text{Diff}_{\text{sulfide}}\). These expressions stand for the difference between the removal- and input-fluxes of sulfate respectively sulfides.  

(Eq 5_S)

\[
\text{Diff}_{\text{sulfate}} = \\
\text{bur}_{\text{sulfate}} + \text{hydro}_{\text{sulfate(precip)}} - \text{hydro}_{\text{sulfate(wea)}} - \text{wea}_{\text{sulfate}} - \text{metamorph}_{SO_2-\text{sulfate}} - \text{metamorph}_{H_2S-\text{sulfate}} \\
\text{Diff}_{\text{sulfide}} = \\
\text{bur}_{\text{sulfide}} + \text{subduct}_{\text{sulfide}} - \text{wea}_{\text{sulfide}} - \text{metamorph}_{SO_2-\text{sulfide}} - \text{metamorph}_{H_2S-\text{sulfide}} \\
\Rightarrow \\
\text{hydro}_\text{mantle}_{-H_2S} + \text{volc}_\text{mantle}_{-SO_2} = \text{Diff}_{\text{sulfate}} + \text{Diff}_{\text{sulfide}}
\]

Reformulation of the steady state isotope balance of seawater sulfate:  

(Eq 6_S)

\[
\frac{d}{dt}(\delta^{34}S_{SO_4-Ocean} \cdot M_{SO_4}) = 0 = \\
\text{wea}_{\text{sulfate}} \cdot \delta^{34}S_{SO_4-\text{steady}} - \text{bur}_{\text{sulfate}} \cdot \delta^{34}S_{SO_4-\text{steady}} \\
+ \text{wea}_{\text{sulfate}} - \text{bur}_{\text{sulfate}} \cdot \left(\delta^{34}S_{SO_4-\text{steady}} + \Delta \delta^{34}S_{\text{sulfide}}\right) \\
+ \text{metamorph}_{SO_2-\text{sulfate}} + \text{metamorph}_{H_2S-\text{sulfate}} \cdot \delta^{34}S_{SO_4-\text{steady}} \\
+ \text{metamorph}_{SO_2-\text{sulfide}} + \text{metamorph}_{H_2S-\text{sulfide}} \cdot \left(\delta^{34}S_{SO_4-\text{steady}} + \Delta \delta^{34}S_{\text{sulfide}}\right) \\
+ \text{hydro}_{\text{sulfate(wea)}} - \text{hydro}_{\text{sulfate(precip)}} \cdot \delta^{34}S_{SO_4-\text{steady}} \\
- \text{subduct}_{\text{sulfide}} \cdot \left(\delta^{34}S_{SO_4-\text{steady}} + \Delta \delta^{34}S_{\text{sulfide}}\right)
\]
\[ \text{hydro mantle } _{H_2S} \cdot \delta ^{34} S_{\text{mantle } _{H_2S}} + \text{volc mantle } _{SO_2} \cdot \delta ^{34} S_{\text{mantle } _{SO_2}} = \\
\left( \text{wea sulfate } + \text{metamorph } _{SO_2} \cdot \text{sulfate} + \text{metamorph } _{H_2S} \cdot \text{sulfate} \right) \cdot \delta ^{34} S_{SO_4 - \text{steady}} \\
\left( -\text{bur sulfate } + \text{hydro sulfate } \cdot \text{precip} - \text{hydro sulfate } \cdot \text{precip} \right) \cdot \left( \delta ^{34} S_{SO_4 - \text{steady}} + \Delta ^{34} S_{\text{sulfide}} \right) \]

\[ \text{Diff sulfate } \cdot \delta ^{34} S_{SO_4 - \text{steady}} + \text{Diff sulfide } \cdot \left( \delta ^{34} S_{SO_4 - \text{steady}} + \Delta ^{34} S_{\text{sulfide}} \right) \]

Combination of the equations for steady state mass and isotope balance of seawater sulfate:

\[ (\text{Eq 7-S}) \]

\[ \text{Diff sulfate } = \text{hydro mantle } _{H_2S} + \text{volc mantle } _{SO_2} - \text{Diff sulfide} \]

\[ \Rightarrow \]

\[ \text{hydro mantle } _{H_2S} \cdot \delta ^{34} S_{\text{mantle } _{H_2S}} + \text{volc mantle } _{SO_2} \cdot \delta ^{34} S_{\text{mantle } _{SO_2}} = \\
\left( \text{hydro mantle } _{H_2S} + \text{volc mantle } _{SO_2} - \text{Diff sulfide} \right) \cdot \delta ^{34} S_{SO_4 - \text{steady}} + \text{Diff sulfide } \cdot \left( \delta ^{34} S_{SO_4 - \text{steady}} + \Delta ^{34} S_{\text{sulfide}} \right) \]

\[ \Rightarrow \]

\[ \text{hydro mantle } _{H_2S} \cdot \delta ^{34} S_{\text{mantle } _{H_2S}} + \text{volc mantle } _{SO_2} \cdot \delta ^{34} S_{\text{mantle } _{SO_2}} = \\
\text{Diff sulfide } \cdot \left( \delta ^{34} S_{SO_4 - \text{steady}} + \Delta ^{34} S_{\text{sulfide}} \right) - \text{Diff sulfide } \cdot \delta ^{34} S_{SO_4 - \text{steady}} \]

\[ \text{Diff sulfide } \cdot \Delta ^{34} S_{\text{sulfide}} = \\
\text{hydro mantle } _{H_2S} \cdot \left( \delta ^{34} S_{\text{mantle } _{H_2S}} - \delta ^{34} S_{SO_4 - \text{steady}} \right) \\
+ \text{volc mantle } _{SO_2} \cdot \left( \delta ^{34} S_{\text{mantle } _{SO_2}} - \delta ^{34} S_{SO_4 - \text{steady}} \right) \]

Calculation of \( \text{Diff sulfate} \) and \( \text{Diff sulfide} \):

\[ (\text{Eq 8-S}) \]

\[ \text{Diff sulfate } = \\
\text{bur sulfide } + \text{subduct sulfide } - \text{wea sulfide } - \text{metamorph } _{SO_2} \cdot \text{sulfide } - \text{metamorph } _{H_2S} \cdot \text{sulfide} \]

\[ = \text{hydro mantle } _{H_2S} \cdot \left( \delta ^{34} S_{\text{mantle } _{H_2S}} - \delta ^{34} S_{SO_4 - \text{steady}} \right) \\
\Delta ^{34} S_{\text{sulfide}} \]

\[ + \text{volc mantle } _{SO_2} \cdot \left( \delta ^{34} S_{\text{mantle } _{SO_2}} - \delta ^{34} S_{SO_4 - \text{steady}} \right) \]

\[ \Delta ^{34} S_{\text{sulfide}} \]
\[
\text{Diff sulfate} = \\
\text{bur}_{\text{sulfate}} + \text{hydro}_{\text{sulfate}} \cdot \text{precip} - \text{hydro}_{\text{sulfate}} \cdot \text{wea} - \text{metamorph}_{\text{SO}_2-\text{sulfate}} - \text{metamorph}_{H_2S-\text{sulfate}} \\
= \text{hydro}_{\text{mantle } H_2S} \cdot \left(1 - \frac{\delta^{34} S_{\text{mantle } H_2S} - \delta^{34} S_{\text{SO}_2-\text{steady}}}{\Delta^{34} S_{\text{sulfide}}} \right) \\
+ \text{volc}_{\text{mantle } SO_2} \cdot \left(1 - \frac{\delta^{34} S_{\text{mantle } SO_2} - \delta^{34} S_{\text{SO}_2-\text{steady}}}{\Delta^{34} S_{\text{sulfide}}} \right)
\]

Influence of the sulfur cycle on the atmospheric oxygen budget (for chemical reactions see Table 2):

\[
\frac{dO_2_{-\text{sulfur cycle}}}{dt} = \frac{15}{8} \cdot \text{Diff sulfate} - 2 \cdot \text{hydro}_{\text{mantle } H_2S} - \frac{1}{2} \cdot \text{volc}_{\text{mantle } SO_2}
\]

\[
\Rightarrow \frac{dO_2_{-\text{sulfur cycle}}}{dt} = \\
\frac{15}{8} \cdot \left( \text{hydro}_{\text{mantle } H_2S} - 2 \cdot \frac{\delta^{34} S_{\text{mantle } H_2S} - \delta^{34} S_{\text{SO}_2-\text{steady}}}{\Delta^{34} S_{\text{sulfide}}} + \text{volc}_{\text{mantle } SO_2} \cdot \frac{\delta^{34} S_{\text{mantle } SO_2} - \delta^{34} S_{\text{SO}_2-\text{steady}}}{\Delta^{34} S_{\text{sulfide}}} \right) \\
- 2 \cdot \text{hydro}_{\text{mantle } H_2S} - \frac{1}{2} \cdot \text{volc}_{\text{mantle } SO_2}
\]

\[
\Rightarrow \frac{dO_2_{-\text{sulfur cycle}}}{dt} = \\
- \text{hydro}_{\text{mantle } H_2S} \cdot \left(2 - \frac{15}{8} \cdot \frac{\delta^{34} S_{\text{mantle } H_2S} - \delta^{34} S_{\text{SO}_2-\text{steady}}}{\Delta^{34} S_{\text{sulfide}}} \right) \\
+ \text{volc}_{\text{mantle } SO_2} \cdot \left(\frac{15}{8} \cdot \frac{\delta^{34} S_{\text{mantle } SO_2} - \delta^{34} S_{\text{SO}_2-\text{steady}}}{\Delta^{34} S_{\text{sulfide}}} - \frac{1}{2} \right)
\]
OUTLOOK: EXPLORING THE SULFUR CYCLE FROM BACTERIAL MICROENVIRONMENT TO GLOBAL BIOGEOCHEMICAL CYCLES

From the spatial and temporal viewpoint, this thesis consists of four rather different chapters: Four times a different topic, four times a different approach. Besides the words “sulfur, isotope, measurement and calculation”, these chapters have also something else in common: Each of the chapters contains a concluding message, such as “we have solved the equation for the $\delta^{18}O-\delta^{34}S$ relations”, “we provide a method to determine reliable $\delta^{34}S$-values from bulk carbonates”, “imbalances in the sulfur cycle severely influence the oceanic carbonate equilibrium” and “hydrothermal H$_2$S-fluxes exist in significant quantities over geologic time”. These messages are banner pages, not only telling what has been done during the thesis, but also telling that “something” was achieved, justifying the spent money and time. However, there are also “things” that were not achieved, things that one should have done, one would have done if time was infinite and things that one would like to do in the future. The Outlook is an ideal “sedimentary deposit” to let these “things” rest, in the calming (and possibly wrong) hope that sooner or later someone will come to dig them out. And this is another common feature of the four chapters: Each contributes to this “deposit”.

Chapter 1

Concerning the $\delta^{18}O-\delta^{34}S$ respectively the $\delta^{18}O-\delta^{15}N$ relations in the study of sulfate reduction and denitrification, the most important work that has to be done in the future is the falsification of the developed model and equations. However, this is not only dependent on laboratory and field studies, but also on accurate and comparable measurements of the oxygen isotope composition of sulfate and nitrate. While encouraging news emerge from the oxygen isotope analysis of nitrate (Casciotti et al., 2002), the technique of oxygen isotope analysis of sulfate has not been pushed forward in the last years. However, to be ready for the “hot sulfur topics”, such as the oxygen isotope fractionation processes at extremely low sulfate concentrations as additional information to the fractionation of sulfur isotopes (Habicht et al., 2002), the oxygen isotope measurement technique of sulfate has to be greatly improved.
Chapter 2

The established method to extract SSS from bulk carbonates is very time consuming. In this study, little attempt was made to “industrialize” the method, i.e. to let machines do some of the treatment-steps. It remains unclear if the contamination with sulfides during the sample preparation is a severe problem when absolutely pure white carbonates are used. Probably, the time-consuming sulfide-oxidation step could be skipped in such a case. An additional sin of omission by the author of this thesis is that the sulfur-analyzed rocks were hardly “looked at”. A detailed investigation by light and cathode luminescence would probably have revealed much about the diagenetic history of the samples called “contaminated by various processes in interstitial waters”. The sulfur- and oxygen isotope data of these samples would then not be lying in a data-cemetery, but would contribute to the understanding of microbial and diagenetic processes. And if one look at a thin section could tell if a sample is unlikely to be useful for the extraction of a “primary” seawater sulfate signal, this would reduce the amount of rejected data substantially.

For the author of this thesis it is obvious that the SSS-sulfur isotope data produced for the Cenomanian-Turonian boundary (OHKOUCHI et al., 1999) and the Permian-Triassic boundary (KAIHO et al., 2001) should be checked and revised by the improved SSS-method.

Chapter 3

The sulfur isotope data for the Early Aptian indicate that hydrothermal activity induces a parallel trend in the sulfur- and strontium isotope curves. It is worth to test this in the Toarcian, where JONES and JENKYNs (2001) describe a similar drop in the strontium isotope values as in the Aptian.

The developed numerical model was used for the evaluation of the perturbations in the Early Cretaceous but could be used for any other time-slices as well. This was not done during this thesis. However, an ideal playground would have been and is still available: The remarkable pattern in the sulfur isotope curve of the Paleocene-Eocene time slice (PAYTAN et al., 1998) is waiting for an explanation.
Chapter 4

It is evident that new estimates for the considered fluxes and isotope values will change the numerical result of this chapter. However, it has to be doubted that the message could turn from “H₂S-fluxes are needed” to “H₂S-fluxes must not exist” by new estimates. Therefore, in a way, this specific subject has found a preliminary end. The global biogeochemical cycles, however, remain or even have become a more exciting field. And it turns out that the sulfur cycle could contain a wealth of information not really studied yet: The age curve of the oxygen isotope composition of sulfate is in a regrettable condition (Figure 1)!

Figure 1  The age curves of δ¹⁸O from marine carbonates and marine (?) sulfate

The age curve of δ¹⁸O from marine carbonates is redrawn and simplified after VEIZER et al. (1999) and VEIZER et al. (1997) and the age curve of δ¹⁸O marine sulfate (evaporites) is redrawn and simplified after CLAYPOOL et al. (1980). The arrow indicates the secular trend in the oxygen isotope data of carbonates. The red areas highlight measurements of δ¹⁸O from marine sulfate.

Despite the poor δ¹⁸O data set for sulfate (Figure 1; CLAYPOOL et al., 1980), one could infer that the secular trend in the oxygen isotope data for carbonates (VEIZER et al., 1999; VEIZER et al., 1997) is not reflected in the sulfate data. Throughout this thesis, it was claimed that the δ¹⁸O of sulfate is derived by the balance of oxygen isotope enrichment by sulfate reducers (by an equilibrium oxygen isotope enrichment factor) and the subsequent 90%-reoxidation (JØRGENSEN, 1982) of sulfides adding approximately the δ¹⁸O of seawater. The δ¹⁸O of
seawater sulfate therefore depends on the $\delta^{18}O$ of seawater. One would expect that a secular trend in the $\delta^{18}O$ of seawater therefore should leave its trace in the $\delta^{18}O$-sulfate record.

Questions arise: Is there no secular change in the $\delta^{18}O$ of seawater (in contrast to what is indicated by the $\delta^{18}O$ of carbonates)? Which processes control the $\delta^{18}O$ of seawater sulfate if there is a secular trend in the $\delta^{18}O$ of seawater? Is the $\delta^{18}O$ data set of seawater sulfate so bad that we do not see the secular trend? What is hidden behind the cloud of $\delta^{18}O$-data of seawater sulfate?

– enough questions for more than one thesis! –

References


SULFUR ISOTOPE FRACTIONATION DURING GROWTH OF SULFATE REDUCING BACTERIA ON VARIOUS CARBON SOURCES*

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Abstract

Stable sulfur isotope fractionation during microbial sulfate reduction is a potential tool to estimate sulfate reduction rates. However, little is known about the influence of the utilized carbon source on the magnitude of sulfur isotope fractionation. To investigate this effect, both a pure culture (strain PRTOL1) and enrichment cultures from a PHC-contaminated aquifer were used and grown in microcosms on various carbon sources with an initial sulfate concentration of 1 mM. As sole carbon sources the PHC components naphthalene, 1,3,5-trimethylbenzene, and heating oil (enrichment culture) and the organic acids acetate, pyruvate, benzoate, and 3-phenylpropionate (enrichment culture and PRTOL1) were used. Sulfate reduction rates of all cultures ranged from 6 ± 1 nmol cm⁻³ d⁻¹ (enrichment culture grown on 1,3,5-trimethylbenzene) to 280 ± 6 nmol cm⁻³ d⁻¹ (enrichment culture grown on pyruvate). Cell-specific sulfate reduction rates ranged from 1.1 · 10⁻¹⁴ mol cell⁻¹ d⁻¹ (PRTOL1 grown on pyruvate) to 1.5 · 10⁻¹³ mol cell⁻¹ d⁻¹ (PRTOL1 grown on acetate). Sulfur isotope enrichment factors (ε) for the enrichment culture ranged from 15.4‰ (3-phenylpropionate) to 33.1‰ (1,3,5-trimethylbenzene) and for PRTOL1 from 30.3‰ (acetate) to 34.5 ‰ (pyruvate). The ε values were constant down to sulfate concentrations of 0.2 mM, sometimes even below 0.1 mM. Cultures of PRTOL1 always showed higher ε values than the enrichment culture when grown on the same carbon sources due to culture-specific properties. Higher ε values were obtained when the en-

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richment culture was grown on PHC components than on organic acids, possibly due to lower energy yields (less negative ∆\(G^f\) values) of sulfate reduction with PHC than with organic acids. For strain PRTOL1, a weak inverse relationship between \(\varepsilon\) values and cell-specific sulfate reduction rate could be shown, while no such relationship existed for the enrichment culture or when all data were combined. The average enrichment factor of all enrichment cultures (22.95 ‰) agreed well with \(\varepsilon\) values determined in field experiments at a PHC-contaminated site. Hence, the results of this study support the theory that sulfur isotope fractionation may be a useful tool to quantify microbial sulfate reduction at field sites.

1 Introduction

Microbial \(\text{SO}_4^{2-}\) reduction is an important process in many contaminated aquifers (Wiedemeier et al., 1999). To accurately estimate its contribution to contaminant degradation, in situ quantification of this process is essential (Madsen, 1991). Unfortunately, quantification of microbial \(\text{SO}_4^{2-}\) reduction in contaminated aquifers based on measurements of \(\text{SO}_4^{2-}\) consumption or \(\text{S}(-\text{II})\) production is often obscured by concurrent abiotic transformations, e.g. by dissolution of gypsum (\(\text{CaSO}_4\)) from the aquifer matrix (Stumm and Morgan, 1981) or by precipitation of \(\text{S}(-\text{II})\) in form of iron sulfides (Anderson and Lovley, 2000). Hence, alternative methods are needed to investigate rates of \(\text{SO}_4^{2-}\) reduction. Stable isotope methods were successfully used to characterize and quantify biological processes in the subsurface (Hunkeler et al., 1999; Meckenstock et al., 2002). So far, sulfur isotope fractionation has been used qualitatively to indicate microbial \(\text{SO}_4^{2-}\) reduction in contaminated aquifers (Alewell and Giesemann, 1996; Arneth and Hoefs, 1989; Asmussen and Strauch, 1998; Bottrell et al., 1995; Schroth et al., 2001). However, in order to quantitatively use sulfur isotope fractionation at a field site, the enrichment factor (\(\varepsilon\)) has to be known a priori with reasonable accuracy (Aggarwal et al., 1997).

While a maximum enrichment factor of 46.9‰ has been observed in pure culture studies (Bolliger et al., 2001), the general range of \(\varepsilon\) values reported in the literature for pure and mixed cultures was -3 – 46.9‰ (Bolliger et al., 2001; Canfield, 2001; Detmers et al., 2001; Habicht et al., 2002; Harrison and Thode, 1958). In petroleum hydrocarbon (PHC) -contaminated aquifers, \(\varepsilon\) values of 20 - 23‰ were obtained during single-well push-pull tests (Schroth et al., 2001). These values were in good agreement with data from Bolliger et al. (2001), who investigated sulfur isotope fractionation (average \(\varepsilon = 23.5\%\)) by a toluene-degrading enrichment culture.
However, in a PHC-contaminated aquifer, SRB may not only directly degrade PHC components but also a range of organic acids which are metabolic products of fermenting bacteria. In first field experiments, $\epsilon$ values with organic acids as carbon sources in a PHC-contaminated aquifer ranged from 16-26‰ as determined using push-pull tests (Kleikemper et al., 2002b). These values were similar to those obtained with PHC components as carbon sources (Bolliger et al., 2001; Schroth et al., 2001).

The influence of different carbon sources on isotope fractionation by the same pure culture was investigated only by few early researchers with contradictory results (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968). Detmers et al. (2001) hypothesized that if one species was tested for several carbon sources, then a relationship between $\epsilon$ and cell-specific \( \text{SO}_4^{2-} \) reduction rates might be shown. In previous studies, enrichment factors were found to be inversely related to cell-specific \( \text{SO}_4^{2-} \) reduction rates when pure cultures of different \textit{Desulfovibrio desulfuricans} strains were grown on lactate varying temperature, lactate, and \( \text{SO}_4^{2-} \) concentrations (Chambers et al., 1975; Harrison and Thode, 1958; Kaplan and Rittenberg, 1964). Such a relationship, however, was not found in more recent studies (Brüchert et al., 2001; Detmers et al., 2001). Instead, strain-specific properties, complete or incomplete carbon source degradation (Brüchert et al., 2001; Detmers et al., 2001), and \( \text{SO}_4^{2-} \) concentration (Habicht et al., 2002) were found to be the most important factors influencing the extent of fractionation. Most of the studies published so far were conducted under optimal growth conditions. However, in PHC-contaminated freshwater environments, low \( \text{SO}_4^{2-} \) concentrations, low carbon source concentrations due to low solubility of most heating oil components in water (Wick et al., 2001), and complex carbon sources are frequently encountered. This study presents an extension of the work of Bolliger et al. (2001) who investigated sulfur isotope fractionation of pure and enrichment cultures with toluene as sole carbon source and different \( \text{SO}_4^{2-} \) concentrations. Our objective here was to quantify and compare sulfur isotope fractionation in microcosms amended with various carbon sources under conditions that resemble freshwater field conditions in a PHC-contaminated aquifer (1 mM \( \text{SO}_4^{2-} \)). In particular, enrichment cultures from sediment of a PHC-contaminated aquifer were grown on naphthalene, 1,3,5-trimethylbenzene, heating oil, acetate, pyruvate, benzoate, and 3-phenylpropionate. A pure culture, strain PRTOL1 that had been isolated with toluene as sole carbon source from another PHC-contaminated freshwater aquifer by Beller et al. (1996), was used as model strain to explore the influence of carbon source on sulfur isotope fractionation by a single organism. PRTOL1 was incubated in parallel with the enrichment cultures on the same
carbon sources (except for naphthalene, 1,3,5-trimethylbenzene, and heating oil on which PRTOL1 is not able to grow).

2 Materials and Methods

2.1 Organisms and Cultivation
Active cultures of the SO$_4^{2-}$-reducing bacterium PRTOL1 (Beller et al., 1996) were purchased from the Oregon Collection of Methanogens (Portland, OR). The inocula for the enrichment cultures were obtained from a PHC-contaminated aquifer in Studen, Switzerland (Bolliger et al., 1999) and maintained under SO$_4^{2-}$-reducing conditions with either acetate, pyruvate, benzoate, 3-phenylpropionate, naphthalene, 1,3,5-trimethylbenzene, or weathered heating oil (recovered from the same site) as sole carbon sources.

The enrichment cultures and PRTOL1 were grown in basal media as described by Beller et al. (1996). For cultivation, SO$_4^{2-}$ was added as FeSO$_4$ (MicroSelect, Fluka, Switzerland). For subsequent microcosm experiments, SO$_4^{2-}$ was added as NaSO$_4$ (MicroSelect, Fluka, Switzerland) except of experiments with naphthalene, 1,3,5-trimethylbenzene and heating oil, where FeSO$_4$ had to be employed due to severe growth inhibition by S(-II) when NaSO$_4$ was used. The media were supplemented with non-chelated trace element mixture, selenite-tungstate solution, bicarbonate solution, vitamin mixture, vitamin B$_{12}$ solution, and S(-II) solution as described by Widdel and Bak (Widdel and Bak, 1992).

Aromatic hydrocarbons (naphthalene, 1,3,5-trimethylbenzene; purum, Fluka) were added as dilute solutions in an inert lipophilic solvent used as carrier phase (Mineral oil, MicroSelect; Fluka) to maintain nearly constant hydrocarbon concentrations in the aqueous phase of 0.1 mM during cultivation and microcosm experiments (Rabus et al., 1993). Three ml of carrier phase containing 0.69 mmol naphthalene or 0.79 mmol 1,3,5-trimethylbenzene were added per 100 ml of medium.

One ml of heating oil was added to the respective microcosms. Organic acids were added from anaerobic, autoclaved stock solutions to give final concentrations of 5 mM (acetate, pyruvate) or 1.0 mM (benzoate, 3-phenylpropionate). The addition of carbon sources in a mineral oil reservoir or at concentrations that would not be depleted if 1 mM SO$_4^{2-}$ was used ensured that carbon sources were non-limiting in our microcosm experiments. The enrichment cultures were grown on all carbon sources and strain PRTOL1 on organic acids. The final pH of the media was approximately 7.1. The media were inoculated with 10% (v/v) (naphthalene, 1,3,5-trimethylbenzene, heating oil) or 5% (v/v) (all other carbon sources) pre-grown cultures.
Bacteria were cultured in 120 ml serum bottles with a headspace of approximately 17 ml (90% N\textsubscript{2}, 10% CO\textsubscript{2}) at 28°C inverted in the dark.

### 2.2 Microcosm Experiments

Microcosm experiments were prepared in 120 ml serum bottles from basal media and substrates as described in the previous section. The initial SO\textsubscript{4}\textsuperscript{2-} concentration was approximately 1 mM in all microcosms. Two independent control experiments were performed for each set of microcosms. For the first control experiment we prepared microcosms as described above except that the carbon source was omitted. In the second control experiment we prepared microcosms as described above except that culture inoculation was omitted. All experiments were conducted at 28°C in the dark. Sulfate concentrations were periodically monitored during the experiments, and at certain intervals sets of three microcosms per culture and employed carbon source were sacrificed and analyzed. Experiments were terminated when the initially supplied SO\textsubscript{4}\textsuperscript{2-} was consumed.

### 2.3 Chemical Analyses

After vigorous shaking, 0.2 ml of medium was removed from the microcosms and immediately dispensed in 4.8 ml of 20 mM zinc acetate solution for S(-II) analysis. Cline reagent (0.5 ml) was immediately added and 20 min later absorbance was measured at 670 nm by spectrophotometry (Cline, 1969). An additional 2 ml of medium was withdrawn for SO\textsubscript{4}\textsuperscript{2-} measurement and counting of bacterial cell numbers. After centrifugation (10 min at 13000 rpm), the supernatant was used for anion (SO\textsubscript{4}\textsuperscript{2-}, organic acids) measurement by ion chromatography (IC-320, Dionex) according to Kleikemper (Kleikemper et al., 2002b). The remaining pellet was further treated for counting of bacterial cells (see below). Analytical reproducibility of the anion measurements was approximately ± 5% for SO\textsubscript{4}\textsuperscript{2-} and organic acids and ± 0.02 mM for S(-II).

### 2.4 Bacterial Cell Numbers

Bacterial cell numbers were determined in microcosms sacrificed at all sampling times of the respective experiments, and the arithmetic mean of these data was used to represent average cell numbers for each experiment. In experiments with naphthalene, 1,3,5-trimethylbenzene, and heating oil cell numbers were determined only at the first sampling point since FeS precipitates rendered cell counting under the microscope impossible at later times.
The remaining pellet (see previous section) was fixed over night in 4% paraformaldehyde in phosphate buffered saline solution (PBS, (Sambrook et al., 1989)) and then washed twice with PBS. Cell suspensions were stored at −20°C in 50% (v/v) ethanol / PBS. Before application to slides, the paraformaldehyde-fixed cell suspensions were centrifuged, the supernatant removed, and the pellet was dispersed in sodium pyrophosphate (0.1%) by mild sonication for 3 min in a sonication bath. Ten µl of suspension were subsequently spotted onto slides, dried at room temperature and finally dehydrated in 50, 80, and 100% ethanol for 3 min each. The cells were stained with DAPI (4’,6-diamidino-2-phenylindole, final concentration 500 ng ml⁻¹) at 42°C for 2 h. After staining and washing, slides were mounted with Citifluor solution (Citifluor, Caterbury, UK) and examined with a Zeiss Axiophot microscope fitted for epifluorescence with a high pressure mercury bulb as described in detail by (Zarda et al., 1997). Cells were counted in 40 images per sample.

2.5 Isotope Analyses

For isotope measurements S(-II) was precipitated as ZnS by addition of 5 ml 1M zinc acetate solution to the microcosms. Serum bottles were vigorously shaken before 1 ml of 2 M NaOH was added. The contents of the microcosms were then filtered using a 0.45 µm HVLP membrane filter (Millipore). Sulfate was subsequently precipitated as BaSO₄ by first adding 2 ml 2 M HCl and then 5 ml 1.2 M BaCl₂ solutions to the filtrate and the precipitate was recovered on a separate 0.45 µm HVLP membrane filter (Millipore). Both filters were dried at 60°C over night. Excess mineral or heating oil from the microcosms was removed from filtrates using hexane.

For stable sulfur isotope ratio measurements approximately 400-600 µg of BaSO₄ or 150-200 µg of ZnS were weighted in tin cups. Vanadium pentoxide was added as catalyst in the amount of about twice the weight of the sample. Sulfur isotopes were subsequently measured on a FISONS OPTIMA mass spectrometer (Fisons, Middlewich, Cheshire, UK) coupled in continuous-flow with a Carlo Erba elemental analyzer (CE Instruments, Milan, Italy). Data are reported in the conventional δ–notation relative to the Vienna-Canyon Diabolo Troilite (V-CDT) standard according to:

\[
\delta^{34}S(\text{‰}) = \left( \frac{^{34}S}{^{32}S} \right)_{\text{sample}} / \left( \frac{^{34}S}{^{32}S} \right)_{\text{CDT}} - 1 \times 1000
\]

(1)

The system was calibrated using the international standards IAEA-S1 (δ³⁴S = -0.3‰) and IAEA-S2 (δ³⁴S = 21.7‰) (Gonfiantini et al., 1995). The mean δ³⁴S value obtained for the in-
International standard NBS127 was 20.4‰. Analytical reproducibility of the measurements was ± 0.3 ‰.

2.6 Determination of Sulfate Reduction Rates

We computed \( \text{SO}_4^{2-} \) reduction rates (SRR, units of nmol cm\(^{-3}\) d\(^{-1}\)) for each culture and carbon source based on \( \text{SO}_4^{2-} \) consumption measured during the experiments assuming zero-order kinetics. Thus, values of SRR were obtained from the slope of a straight line fitted to data of remaining \( \text{SO}_4^{2-} \) concentration versus time using linear regression analysis.

For the enrichment culture grown on pyruvate and PRTOL1 incubated with 3-phenylpropionate we also computed first-order \( \text{SO}_4^{2-} \) reduction rate coefficients (\( k \), units of d\(^{-1}\)). For these computations we assumed a first-order-type reaction \( \frac{dC}{dt} = -kC \), where \( C \) is reactant (here \( \text{SO}_4^{2-} \)) concentration and \( t \) is time. Hence, values of \( k \) were obtained from the slope of a straight line fitted to data of \( \ln C \) versus \( t \) using linear regression analysis. Employing a generic linear regression tool we computed standard deviations for both SRR and \( k \), and coefficients of determination \( (R^2) \) were used as a measure of goodness of the respective fits. Finally, we computed values of sSRR (mol cell\(^{-1}\) d\(^{-1}\)) by dividing SRR values by average cell numbers previously determined for the respective cultures and carbon sources.

2.7 Determination of Isotope Enrichment Factors

Stable isotope fractionation during a reaction is commonly quantified in terms of the fractionation factor \( \alpha \) or the isotope enrichment factor \( \varepsilon \) (in ‰, e.g., (Hoefs, 1997)). These two measures of isotope fractionation are directly related by \( \varepsilon = (\alpha - 1) \times 1000 \). Throughout this study we will use values of \( \varepsilon \) to quantify stable sulfur isotope fractionation. In a closed system, values of \( \varepsilon \) can be determined using Rayleigh distillation equations (Mariotti et al., 1981). Using linear regression analysis, values of \( \varepsilon \) were obtained from the slope of a straight line simultaneously fitted to measured \( \delta^{34}\text{S} \) values of remaining, unconsumed \( \text{SO}_4^{2-} \) (\( \delta^{34}\text{S}(\text{SO}_4^{2-})) \) and accumulated S(-II) (\( \delta^{34}\text{S}(\text{S(-II)}) \)) according to (Böttcher et al., 1999):

\[
\delta^{34}\text{S}(\text{SO}_4^{2-}) = \delta^{34}\text{S}(\text{SO}_4^{2-})_0 + \varepsilon \cdot \ln f \\
\delta^{34}\text{S}(\text{S(-II)}) = \delta^{34}\text{S}(\text{SO}_4^{2-})_0 - \varepsilon \cdot (\ln f) / (1-f)
\]

In Eq. 2 and 3, \( f \) denotes the fraction of unconsumed \( \text{SO}_4^{2-} \), and \( \delta^{34}\text{S}(\text{SO}_4^{2-})_0 \) represents the initial isotope composition of dissolved \( \text{SO}_4^{2-} \) (equal to 8.1 ± 1.5 ‰ in our experiments). Measured \( \delta^{34}\text{S}(\text{S(-II)}) \) data were corrected for the initial S(-II) concentration contained in the
microcosms and its isotope composition. For a detailed derivation of Eq. 2 and 3 the reader is referred to (Mariotti et al., 1981).

3 Results

3.1 Microbial Sulfate Reduction
Strain PRTOL1 and the enrichment culture consumed SO\(_4^{2-}\) in all microcosms amended with different substrates as sole carbon source and with SO\(_4^{2-}\) as sole electron acceptor (Fig. 1). Concomitantly, we observed production of S(-II) during these experiments. On the other hand, SO\(_4^{2-}\) concentrations remained unchanged in microcosms of the control microcosms during up to 132 d of incubation (not shown).

The enrichment culture consumed most of the SO\(_4^{2-}\) within ~25 d of incubation in microcosms amended with acetate, benzoate, and 3-phenylpropionate (Fig. 1a, c, d), and within ~6 d of incubation in microcosms amended with pyruvate (Fig. 1b). Conversely, SO\(_4^{2-}\) was consumed only within 100-130 d of incubation in microcosms amended with naphthalene, 1,3,5-trimethylbenzene, and heating oil (Fig. 1i-k). Using data presented in Fig. 1 we performed a mass balance on the sum of SO\(_4^{2-}\) and S(-II) in each sacrificed microcosm (Tab. 1). In microcosms amended with acetate, pyruvate, phenylpropionate, and benzoate close to 100% of the sum of initially added SO\(_4^{2-}\) and S(-II) was recovered, while in naphthalene, 1,3,5-trimethylbenzene, and heating oil microcosms, only 68-76% were recovered.

Strain PRTOL1 consumed only about 50% of SO\(_4^{2-}\) within 85 d of incubation in microcosms amended with acetate (Fig. 1e). Thereafter, SO\(_4^{2-}\) concentrations remained nearly constant for another 60 d (not shown). Conversely, nearly the entire SO\(_4^{2-}\) was consumed by PRTOL1 within 6 d in experiments with pyruvate (Fig. 1f), within 120 d with benzoate (Fig. 1g), and within 45 d with 3-phenylpropionate (Fig. 1h). In all PRTOL1 experiments, slightly more than 100% of the sum of initially added SO\(_4^{2-}\) and S(-II) was recovered in the sacrificed microcosms (Tab. 1).

3.2 Carbon source consumption
Concomitantly to SO\(_4^{2-}\) reduction and S(-II) production, organic acids were consumed in enrichment culture and PRTOL1 microcosms grown on acetate, benzoate, and 3-phenylpropionate, and presumably, also on pyruvate (not shown). Pyruvate concentrations were erratic for unknown reasons. Furthermore, we observed an evolution of acetate in enrichment culture microcosms grown on pyruvate, benzoate, 3-phenylpropionate and in
PRTOL1 microcosms grown on benzoate (not shown). Usually, a large portion, but not all of the substrate degraded during microcosm experiments was accounted for by SO$_4^{2-}$ reduction (Tab. 1). Increasing acetate concentrations to up to 0.46 mM were also observed in enrichment culture microcosms grown on naphthalene and heating oil, but not on 1,3,5-trimethylbenzene (not shown).

### 3.3 Bacterial Cell Numbers

Bacterial cell numbers increased during experiments with the enrichment culture (Fig. 2a). In particular, increases in cell numbers of almost one order of magnitude were observed in enrichment culture experiments with acetate and pyruvate. For enrichment cultures grown on naphthalene, 1,3,5-trimethylbenzene, and heating oil, cell numbers are only available for the first data point (Tab. 2) since FeS precipitates in these microcosms at later time points made cell counts impossible.

An increase of cell numbers in cultures of PRTOL1 was only observed for the pyruvate-amended culture (Fig. 2b). In the experiment with PRTOL1 grown on acetate, cell numbers decreased with time, whereas in all other PRTOL1 experiments, cell numbers decreased with time and then increased again.

Average bacterial cell numbers for the experiments ranged from $6.7 \times 10^4$ to $1.8 \times 10^7$ (Tab. 2), associated with fairly large standard deviations ($\sigma_{\text{cells}}$, often $> 50\%$ of the mean ($\bar{x}$)).

### 3.4 Sulfate Reduction Rates

Sulfate reduction rates, determined from linear regression analyses of SO$_4^{2-}$ concentration versus time (Fig. 1), varied by up to a factor of 47 between different cultures and carbon sources (Tab. 2). In general, PRTOL1 and the enrichment culture degraded SO$_4^{2-}$ with rates in the same order of magnitude. Highest SRR were obtained for cultures grown on pyruvate ($280$ nmol cm$^{-3}$ d$^{-1}$ for the enrichment culture and $143$ nmol cm$^{-3}$ d$^{-1}$ for PRTOL1), lowest values were observed for PRTOL1 grown on benzoate ($8$ nmol cm$^{-3}$ d$^{-1}$) or the enrichment culture grown on 1,3,5-trimethylbenzene ($6$ nmol cm$^{-3}$ d$^{-1}$). Furthermore, values of SRR were characterized by small standard deviations ($\sigma_{\text{SRR}}$, $\leq 10\%$ of SRR in most cases), and coefficients of determination ($R^2$) were $> 0.9$ in all but three cases (Tab. 2).

Sulfate reduction in the enrichment culture incubated with pyruvate and PRTOL1 incubated with 3-phenylpropionate appeared to follow first-order kinetics (Fig. 1b, h) with SO$_4^{2-}$ concentrations declining exponentially. Using linear regression analyses (dashed lines in Fig. 1b,h), we obtained first-order rate coefficients $k = 0.398 \pm 0.019$ d$^{-1}$ for the enrichment cul-
ture, and $k = 0.018 \pm 0.002 \text{d}^{-1}$ for PRTOL1. Thus, values of $k$ were characterized by standard deviations $< 13\%$ of $k$, and coefficients of determination were $R^2 = 0.98$ (number of data points, $n = 15$) for the enrichment culture on pyruvate, and $R^2 = 0.84$ ($n = 15$) for PRTOL1 on 3-phenylpropionate.

Computed values of sSRR ranged from $1.1 \times 10^{-14}$ to $1.5 \times 10^{-13} \text{mol cell}^{-1} \text{d}^{-1}$ and thus varied by a factor of up to 13 between experiments (Tab. 2). Differences in sSRR values between the cultures were smaller than differences in SRR values. Values of sSRR were in the same range for the enrichment cultures and PRTOL1.

### 3.5 Sulfur Isotope Fractionation

Values of $\delta^{34}\text{S(SO}_4^{2-})$ increased from $8.1\%o (\delta^{34}\text{S(SO}_4^{2-})_0)$ to values of up to $85.0\%o$ during the experiments (Fig. 3). Simultaneously to increases in $\delta^{34}\text{S(SO}_4^{2-})$, values of $\delta^{34}\text{S(S(-II))}$ increased and, in general, approached the initial isotope composition of $\text{SO}_4^{2-}$ over the course of the experiments. The latter was not the case in the experiment with PRTOL1 grown on acetate in which $\text{SO}_4^{2-}$ reduction had ceased prior to consumption of most of the supplied $\text{SO}_4^{2-}$ (Fig. 1e). In this experiment, $\delta^{34}\text{S(S(-II))}$ remained substantially more negative than $\delta^{34}\text{S(SO}_4^{2-})_0$ (Fig. 3e).

Combined evaluation of measured sulfur isotope data revealed approximately linear relationships for all experiments when data was plotted as $\delta^{34}\text{S(SO}_4^{2-})$ versus $(-\ln f)$ (Eq. 2) and $\delta^{34}\text{S(S(-II))}$ versus $(f/\ln f)/(1-f)$ (Eq. 3, Fig. 3). Using linear regression analysis, we determined $\epsilon$ values that ranged from $15.4$ to $34.5\%o$ (Tab. 3). Enrichment factors were computed from 21 to 27 independent data points each and were generally characterized by small standard deviations ($\leq 8\%$ of $\epsilon$ in all cases), with $R^2$ values larger than 0.95 in all cases (Tab. 3). Enrichment factors were generally stable down to $\text{SO}_4^{2-}$ concentrations of 0.2 mM, for the enrichment cultures in some cases even down to less than 0.1 mM (Fig. 3, $-\ln f > 2.3$). In a few instances, decreasing fractionation was found when $\text{SO}_4^{2-}$ concentrations were below 0.066 mM (not shown). A weak relationship existed between $\epsilon$ and sSRR for the PRTOL1 experiments ($R^2 = 0.5$), while no such relationship was apparent for the enrichment culture data or when all data were combined (Fig. 4) ($R^2 < 0.5$).
4 Discussion and Conclusions

4.1 Microbial sulfate reduction

In our study SO$_4^{2-}$ was consumed during all but the two control experiments. However, even though acetate and SO$_4^{2-}$ were initially degraded and S(-II) was produced when PRTOL1 was grown on acetate (Figs. 1e), SO$_4^{2-}$ reduction apparently ceased, with about half of the initially added SO$_4^{2-}$ remaining in aqueous solution. Since acetate was still present (not shown), substrate limitation can be excluded as a reason for cessation of SO$_4^{2-}$ reduction. During this experiment, we observed that cell numbers decreased (Fig. 2b) and on day 84, cells were not detectable anymore despite sample concentration. Beller et al. (1996) had observed that PRTOL1 did not show growth on acetate, even though SO$_4^{2-}$ was reduced. In contrast to their results, we observed, at least initially, a decrease of the acetate concentration (not shown).

Mass balances on SO$_4^{2-}$ and S(-II) indicated that consumed SO$_4^{2-}$ was entirely converted to S(-II) during many of our experiments (Fig. 1). However, in enrichment culture microcosms amended with naphthalene, 1,3,5-trimethylbenzene, and heating oil, mass balances indicated that significantly less than 100% of consumed SO$_4^{2-}$ was converted to S(-II) (Tab. 1). Differences between the sum of initially supplied and recovered SO$_4^{2-}$ and S(-II) were too large to be explained by analytical uncertainty alone. Possibly, sulfur may have been used in assimilatory processes during bacterial growth, or SO$_4^{2-}$ was reduced to sulfur species other than S(-II) such as e.g. sulfite (Chambers and Trudinger, 1979; Madigan et al., 2003). Apart from the quantification of produced S(-II), however, no effort was made here to further elucidate the fate of initially supplied SO$_4^{2-}$.

4.2 Carbon source consumption

Acetate production in some of the enrichment culture experiments indicated the presence of SRB that can only incompletely degrade the substrates to acetate. Strain PRTOL1 is known to degrade carbon sources completely to CO$_2$ (Beller et al., 1996). Complete or incomplete degradation of carbon sources by SRB may have an influence on the extent of sulfur isotope fractionation ((Detmers et al., 2001), see below).

In most experiments, SO$_4^{2-}$ reduction did not account for all the carbon source degradation (Tab. 1). Hence, a certain proportion of the carbon was likely incorporated into biomass (Fig. 2) (Widdel, 1988) or possibly degraded to unidentified intermediates (e.g., benzylsuccinate (Beller et al., 1996)). In addition, some of the added acetate or acetate produced during incomplete degradation of the carbon sources in the enrichment culture microcosms may have
been degraded by methanogenic microorganisms (Zinder, 1993). Indeed, CH₄ was detected when headspace gas of some enrichment culture microcosms was sampled and analyzed.

4.3 Sulfate Reduction Rates

A wide range of SRR was obtained for the different experiments (Tab. 2). However, SRR depend to a large extent on the cell density in the respective microcosms. In our experiments, inoculants had different cell densities and initial (Fig. 2) and average bacterial cell numbers (Tab. 2) varied between different experiments, which may explain some of the observed variability in SRR. Interestingly, the first-order rate coefficients for SO₄²⁻ reduction determined in this study (\( k = 0.398 \pm 0.019 \) d⁻¹ for the enrichment culture grown on pyruvate, and \( k = 0.018 \pm 0.002 \) d⁻¹ for PRTOL1 grown on 3-phenylpropionate) mark the upper and lower end of first-order rate coefficients that have been determined for SO₄²⁻ reduction in PHC-contaminated aquifers (0.02 - 0.32 d⁻¹) (Chapelle et al., 1996; Kleikemper et al., 2002b; Schroth et al., 2001).

Our sSRR values (1.1 x 10⁻¹⁴ to 1.5 x 10⁻¹³ mol cell⁻¹ d⁻¹, Tab. 2) are in the upper range of sSRR values obtained in previous studies (e.g., (Chambers et al., 1975; Detmers et al., 2001; Kaplan and Rittenberg, 1964)) and in the same range of sSRR values determined by Bolliger et al. (2001). However, caution is required when comparing sSRR values between various studies as discussed in Bolliger et al. (2001). The accuracy of sSRR values depends on the variability of the underlying parameters, i.e. SRR values and bacterial cell numbers. Cell numbers varied substantially during most of our experiments (Fig. 2). Consequently, computed average bacterial cell numbers, and hence, sSRR values, were associated with fairly large uncertainties (Tab. 2).

4.4 Sulfur Isotope Fractionation

Stable sulfur isotope ratios of unconsumed SO₄²⁻ and produced S(-II) changed during our experiments as expected for a reaction in a closed system (Fig. 4) (Chambers and Trudinger, 1979; Thode, 1991). In general, the range of \( \varepsilon \) values obtained in this study were comparable to those reported by other authors for various strains under different growth conditions (Bolliger et al., 2001; Böttcher et al., 1999; Chambers et al., 1975; Detmers et al., 2001; Kaplan and Rittenberg, 1964). The stable fractionations down to SO₄²⁻ concentrations of sometimes < 0.1 mM (Fig. 3) and decreasing fractionations below this value (not shown) agree with Habicht et al. (2002). Our results corroborate the observation that for freshwater strains as used in our study only a very low SO₄²⁻ concentration limits isotope fractionation
and not, as previously thought, already concentrations below 1 mM (Harrison and Thode, 1958).

We will see that the variability of $\varepsilon$ values in this study may be explained in terms of investigated organism and the influence of carbon source, which in turn is controlled by the variability of cell-specific $\text{SO}_4^{2-}$ reduction rates, the energy yield of the reaction ($\Delta G$ values), and complete or incomplete carbon source degradation.

Influence of culture:
Cultures of PRTOL1 always showed higher $\varepsilon$ values than the enrichment cultures when both were grown on the same carbon source (Tab. 3). This is in agreement with Bolliger et al. (2001) who cultivated PRTOL1 and an enrichment culture derived from the same field site with toluene as sole carbon source. Two reasons may explain this result. Firstly, the enrichment cultures consisted of mixtures of different SRB, and different SRB are known to show a range of individual $\varepsilon$ values (Detmers et al., 2001). Hence the overall $\varepsilon$ of an enrichment culture will be an average value and therefore lower than that of a pure strain that tends to show high fractionation. Secondly, complete or incomplete carbon source degradation may have influenced $\varepsilon$ values as discussed below.

Influence of cell-specific sulfate reduction rate:
Several authors have suggested that sulfur isotope fractionation is controlled by sSRR rather than SRR (Chambers and Trudinger, 1979; Habicht and Canfield, 1997). Thus, $\varepsilon$ values should be related to values of sSRR when comparing results of different studies. A weak inverse relationship between sulfur isotope fractionation and sSRR was found for data from PRTOL1 ($R^2 = 0.49$, Fig. 4), which agrees with data from Bolliger et al. (Bolliger et al., 2001) and tends to support the prediction of Detmers et al. (2001) that a correlation between sSRR and fractionation may be found if various substrates were tested for one organism. When data from all microcosm experiments were combined, there was no obvious correlation between $\varepsilon$ values and sSRR ($R^2 = 0.12$, Fig. 4). This agrees with more recent findings of Canfield et al. (2000) and Detmers et al. (2001), who concluded that there is little or no correlation between $\varepsilon$ and sSRR values for a broad range of SRB.

Influence of energy yield:
This study is the first investigation of sulfur isotope fractionation when the PHC naphthalene, 1,3,5-trimethylbenzene and heating oil served as sole carbon sources. Interestingly, $\varepsilon$ values for these PHC compounds were very similar (27.0-33.1‰) and significantly larger (average:
29.4‰) (p = 0.05) than for enrichment cultures grown on organic acids (average: 18.1‰). Apart from the fact that probably different SRB communities were enriched on the different carbon sources (Kleikemper et al., 2002a; Parkes et al., 1993), the energy yields ($\Delta G_0$) of the respective reactions may influence the extent of isotope fractionation as has been suggested previously by Detmers et al. (2001). Higher energy yields were generally associated with low $\varepsilon$ values. However, we suggest that instead of using standard $\Delta G_0$ values (Detmers et al., 2001), it is be more appropriate to consider actual $\Delta G_f$ values under the conditions the reaction is occurring (Tab. 4). Values of $\Delta G_0$ for the enrichment cultures were not very different between organic acids and PHC compounds (except pyruvate, Tab. 4), but if actual $\Delta G_f$ values were taken into account, growth on the PHC compounds tended to yield less energy than growth on organic acids (Tab. 4). This explains in part the trend towards higher $\varepsilon$ values for the PHC.

Influence of complete or incomplete carbon source degradation:

Several authors observed that completely degrading pure strains tended to show higher $\varepsilon$ values than incompletely degrading strains, due to generally lower energy yields (less negative $\Delta G_0$ values) of complete substrate oxidations (Brüchert et al., 2001; Detmers et al., 2001). While PRTOL1 is a completely degrading strain (Beller et al., 1996), the enrichment cultures grown on organic acids (except for acetate) likely also contained incompletely degrading SRB since acetate was produced in many of our experiments. The lower energy yields ($\Delta G_f$) for the complete oxidations of strain PRTOL1 as compared to incomplete carbon source degradation by the enrichment cultures is the second reason to explain the higher $\varepsilon$ values for strain PRTOL1 (Tab. 4, see above).

In our enrichment cultures all carbon sources except acetate and 1,3,5-trimethylbenzene were - at least to some degree - incompletely degraded (production of acetate). Hence, $\varepsilon$ values for these two substrates may be expected to be higher. Indeed, growth of cultures on 1,3,5-trimethylbenzene was associated with high $\varepsilon$ values (33.08‰), explained by the comparatively low energy yield of the reaction (Tab. 4). However, while the energy yield for acetate was the lowest of all substrates (Tab. 4), $\varepsilon$ was quite low (18.54‰, Tab. 3). Hence, the different SRB communities that developed on different carbon sources likely influenced $\varepsilon$ values to some extent.
Influence of carbon source for strain PRTOL1:
Growth of PRTOL1 on various carbon sources resulted in a narrow range of \( \varepsilon \) values (30.78 ± 2.52‰, average ± standard deviation, Table 3). More variable \( \varepsilon \) values were reported for PRTOL1 when grown on toluene and three different \( \text{SO}_4^{2-} \) concentrations (Bolliger et al., 2001) (\( \varepsilon = 32.1 - 46.9‰ \)). Hence, for strain PRTOL1, \( \text{SO}_4^{2-} \) concentration seemed to have a greater influence on \( \varepsilon \) than carbon source type when the same range of sSRR values was considered. Only few other authors investigated sulfur isotope fractionation by the same strain grown on different carbon sources. For example, Kaplan and Rittenberg (1964) found that \textit{Desulfovibrio desulfuricans} showed a higher fractionation on ethanol than on lactate, but Kemp and Thode (1968) found the reverse effect. The reason for this discrepancy may have been due to the different \textit{D. desulfuricans} strains used in both studies (Kemp and Thode, 1968). Kaplan and Rittenberg (1964) explained their results in terms of the substrate-induced variability in sSRR, which also explains part of the variability in our data (Fig. 4). Nevertheless, experiments with similar sSRR, e.g. PRTOL1 grown on acetate and 3-phenylpropionate on the one hand or on toluene and benzoate on the other hand (Fig. 4) showed different \( \varepsilon \) values, suggesting that factors other than sSRR also influence the extent of sulfur isotope fractionation.

4.5 Relevance to field studies at contaminated sites

To use sulfur isotope fractionation for quantification of microbial sulfate reduction, the enrichment factor for a particular site has to be known (Aggarwal et al., 1997). In this study, the experiments that are probably most relevant for field studies in contaminated aquifers are the enrichment cultures grown on PHC components. However, SRB in the aquifer may not only directly degrade PHC but also a range of organic acids, which are metabolic products of fermenting bacteria. Hence, our enrichment cultures grown on organic acids also carry relevance for field sites. In our experiments, we observed that enrichment cultures grown on PHC tended to show higher \( \varepsilon \) values than on organic acids. However, the average \( \varepsilon \) value of all enrichment cultures (23.0 ± 6.5‰) agreed well with values from field experiments (20.7‰ (Kleikemper et al., 2002b) or 21.5‰ (Schroth et al., 2001)) and with the average \( \varepsilon \) of toluene-degrading enrichment cultures inoculated with material from the same aquifer (23.5 ± 4.3‰)(Bolliger et al., 2001). Hence, SRB in the field may be using both fermentation products and PHC as carbon sources, leading to average \( \varepsilon \) values.
Enrichment cultures grown on heating oil showed surprisingly high \( \varepsilon \) values (28.10\%\) compared to values generated in field experiments (21.5\% \( \pm \) 1.8\% (Schroth et al., 2001)) in the same contaminated aquifer that the inoculum for the enrichment culture was obtained from. This discrepancy may be due to different conditions in field and microcosm experiments, of which temperature is the most eminent. In addition, the population in the field was possibly also growing on other substances (natural organic compounds) and during enrichment probably some specific SRB groups were selected for. Other authors previously observed that increasing temperature lead to increasing \( \varepsilon \) values for natural SRB populations (Brüchert et al., 2001) and ascribed this to different SRB and associated fermenting bacteria being active at different temperatures.

In general, \( \varepsilon \) values at PHC-contaminated sites seem to be higher than in other freshwater aquifer environments. For example, values of 9.7 - 15.5 were determined for uncontaminated aquifers (Robertson and Schiff, 1994; Strebel et al., 1990), while values of 9.5 – 21 were found for aquifers contaminated with landfill leachate, phenol, or with a multicomponent pollutant mixture (Asmussen and Strauch, 1998; Bottrell et al., 1995; Spence et al., 2001).

Our results show that due to the similar extent of sulfur isotope fractionation in microcosm and field studies, a relationship between \( \varepsilon \) and the \( \text{SO}_4^{2-} \) reduction rate coefficient such as proposed by Aggarwal et al. (1997) may be used for estimations of \( \text{SO}_4^{2-} \) reduction rates in field settings. However, our results for growth of SRB on PHC compounds were all generated with microorganisms from the same site, hence, a comparison with other PHC-contaminated sites would be useful.

Acknowledgements

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References


**Tables and Figures**

**Table 1  Stoichiometric data**

Percentage recovery of initially added \( \text{SO}_4^{2-} \) and S(-II) and percentage of degraded carbon source accounted for by \( \text{SO}_4^{2-} \) reduction for microcosm experiments conducted for two different cultures and various carbon sources.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Carbon source</th>
<th>% recovery of initially added ( \text{SO}_4^{2-} ) and S(-II)(^a)</th>
<th>% of degraded carbon source accounted for by ( \text{SO}_4^{2-} ) reduction(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrichment cultures</td>
<td>acetate</td>
<td>109</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>pyruvate</td>
<td>98</td>
<td>106(^c)</td>
</tr>
<tr>
<td></td>
<td>benzoate</td>
<td>105</td>
<td>75(^d)</td>
</tr>
<tr>
<td></td>
<td>3-phenylpropionate</td>
<td>109</td>
<td>79(^e)</td>
</tr>
<tr>
<td></td>
<td>naphthalene</td>
<td>76</td>
<td>n.a.(^f)</td>
</tr>
<tr>
<td></td>
<td>1,3,5-trimethylbenzene</td>
<td>68</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>heating oil</td>
<td>75</td>
<td>n.a.</td>
</tr>
<tr>
<td>PRTOL1</td>
<td>acetate</td>
<td>107</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>pyruvate</td>
<td>101</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>benzoate</td>
<td>109</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>3-phenylpropionate</td>
<td>102</td>
<td>61</td>
</tr>
</tbody>
</table>

\(^a\) calculated by a mass balance of the sum of \( \text{SO}_4^{2-} \) and S(-II) in each microcosm

\(^b\) calculated by taking into account the stoichiometries of the respective reactions

\(^c\) value calculated from measured acetate since pyruvate concentrations were erratic; a theoretical (degraded pyruvate) : (produced acetate) ratio of 1:1 was assumed, resulting in an acetate: \( \text{SO}_4^{2-} \) stoichiometry of 4.00 (see Table 4)

\(^d\) a theoretical (degraded benzoate) : (produced acetate) ratio of 1:2 was assumed, resulting in a benzoate : \( \text{SO}_4^{2-} \) stoichiometry of 0.57 (see Table 4)

\(^e\) a theoretical (degraded 3-phenylpropionate) : (produced acetate) ratio of 1:3 was assumed, resulting in a 3-phenylpropionate : \( \text{SO}_4^{2-} \) stoichiometry of 0.44 (see Table 4)

\(^f\) n.a. = not available
<table>
<thead>
<tr>
<th>Culture</th>
<th>Carbon source</th>
<th>Average bacterial cell numbers</th>
<th>n</th>
<th>SRR ± σ_{SRR}</th>
<th>R^2</th>
<th>sSRR ± σ_{sSRR}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrichment</td>
<td>acetate</td>
<td>$6.2 \times 10^5 \pm 4.6 \times 10^5$</td>
<td>15</td>
<td>35 ± 2</td>
<td>0.971</td>
<td>5.6 x 10^{-14} ± 4.1 x 10^{-14}</td>
</tr>
<tr>
<td></td>
<td>pyruvate</td>
<td>$1.8 \times 10^7 \pm 1.4 \times 10^7$</td>
<td>9</td>
<td>280 ± 6</td>
<td>0.999</td>
<td>1.5 x 10^{-14} ± 1.2 x 10^{-14}</td>
</tr>
<tr>
<td></td>
<td>benzoate</td>
<td>$2.3 \times 10^6 \pm 1.5 \times 10^6$</td>
<td>12</td>
<td>38 ± 3</td>
<td>0.936</td>
<td>1.6 x 10^{-14} ± 1.1 x 10^{-14}</td>
</tr>
<tr>
<td></td>
<td>3-phenylpropionate</td>
<td>$1.9 \times 10^6 \pm 1.3 \times 10^6$</td>
<td>15</td>
<td>61 ± 2</td>
<td>0.989</td>
<td>3.1 x 10^{-14} ± 2.1 x 10^{-14}</td>
</tr>
<tr>
<td></td>
<td>naphthalene</td>
<td>$1.3 \times 10^7 \pm 6.7 \times 10^7$</td>
<td>15</td>
<td>9 ± 1</td>
<td>0.887</td>
<td>7.1 x 10^{-14} ± 8.4 x 10^{-14}</td>
</tr>
<tr>
<td></td>
<td>1,3,5-trimethylbenzene</td>
<td>$1.1 \times 10^7 \pm 9.1 \times 10^7$</td>
<td>15</td>
<td>6 ± 1</td>
<td>0.871</td>
<td>5.6 x 10^{-14} ± 8.0 x 10^{-15}</td>
</tr>
<tr>
<td></td>
<td>heating oil</td>
<td>$1.7 \times 10^5 \pm 3.0 \times 10^4$</td>
<td>15</td>
<td>12 ± 1</td>
<td>0.936</td>
<td>7.0 x 10^{-14} ± 1.3 x 10^{-14}</td>
</tr>
<tr>
<td>PRTOL1</td>
<td>acetate</td>
<td>$1.3 \times 10^7 \pm 1.0 \times 10^7$</td>
<td>9</td>
<td>19 ± 2</td>
<td>0.970</td>
<td>1.5 x 10^{-13} ± 1.1 x 10^{-13}</td>
</tr>
<tr>
<td></td>
<td>pyruvate</td>
<td>$1.3 \times 10^7 \pm 1.1 \times 10^7$</td>
<td>15</td>
<td>143 ± 8</td>
<td>0.965</td>
<td>1.1 x 10^{-14} ± 9.5 x 10^{-15}</td>
</tr>
<tr>
<td></td>
<td>benzoate</td>
<td>$1.3 \times 10^7 \pm 1.2 \times 10^7$</td>
<td>15</td>
<td>8 ± 1</td>
<td>0.900</td>
<td>6.1 x 10^{-14} ± 5.8 x 10^{-14}</td>
</tr>
<tr>
<td></td>
<td>3-phenylpropionate</td>
<td>$2.0 \times 10^7 \pm 1.3 \times 10^7$</td>
<td>9</td>
<td>26 ± 3</td>
<td>0.953</td>
<td>1.3 x 10^{-13} ± 8.3 x 10^{-14}</td>
</tr>
</tbody>
</table>

*a* Standard deviation, *b* Number of data points included in SRR computation.

*c* Coefficient of determination for linear regression analyses performed to obtain SRR.
### Table 3

Calculated sulfur isotope enrichment factors ($\varepsilon$) obtained during microbial $\text{SO}_4^{2-}$ reduction in microcosm experiments with various carbon sources.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Carbon source</th>
<th>$n^a$</th>
<th>$\varepsilon \pm \sigma_{\varepsilon}^{b}$ (%)</th>
<th>$R^2^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enrichment cultures</td>
<td>acetate</td>
<td>27</td>
<td>$18.5 \pm 0.4$</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>pyruvate</td>
<td>27</td>
<td>$21.3 \pm 1.2$</td>
<td>0.950</td>
</tr>
<tr>
<td></td>
<td>benzoate</td>
<td>21</td>
<td>$17.3 \pm 0.7$</td>
<td>0.976</td>
</tr>
<tr>
<td></td>
<td>3-phenylpropionate</td>
<td>24</td>
<td>$15.4 \pm 1.2$</td>
<td>0.986</td>
</tr>
<tr>
<td></td>
<td>naphthalene</td>
<td>24</td>
<td>$27.0 \pm 0.8$</td>
<td>0.981</td>
</tr>
<tr>
<td></td>
<td>1,3,5-trimethylbenzene</td>
<td>24</td>
<td>$33.1 \pm 1.3$</td>
<td>0.970</td>
</tr>
<tr>
<td></td>
<td>heating oil</td>
<td>22</td>
<td>$28.1 \pm 1.1$</td>
<td>0.972</td>
</tr>
<tr>
<td>PRTOL1</td>
<td>acetate</td>
<td>21</td>
<td>$30.3 \pm 0.5$</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>pyruvate</td>
<td>27</td>
<td>$34.5 \pm 0.8$</td>
<td>0.986</td>
</tr>
<tr>
<td></td>
<td>benzoate</td>
<td>21</td>
<td>$28.8 \pm 1.1$</td>
<td>0.975</td>
</tr>
<tr>
<td></td>
<td>3-phenylpropionate</td>
<td>27</td>
<td>$32.0 \pm 0.9$</td>
<td>0.983</td>
</tr>
</tbody>
</table>

---

*a* Number of data points included in computation of $e$ values.

*b* Standard deviation.

*c* Coefficient of determination for linear regression analyses performed to obtain $e$ values.
### Table 4: Stoichiometric equations of the degradation of the carbon sources used in this study under SO$_4^{2-}$-reducing conditions and free-energy changes (kJ / mol degraded SO$_4^{2-}$) under standard conditions ($\Delta\Delta G_0^a$) and actual conditions ($\Delta\Delta G_f^b$).

The stoichiometries of the reactions were derived from the measured SO$_4^{2-}$ and organic acid concentrations.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Complete / incomplete carbon source degradation$^c$</th>
<th>Culture</th>
<th>Stoichiometric reaction</th>
<th>$\Delta\Delta G_0$</th>
<th>$\Delta\Delta G_f$</th>
<th>$\epsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>complete</td>
<td>PRTOL1</td>
<td>$\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} \rightarrow 2\text{HCO}_3^- + \text{HS}^-$</td>
<td>-48</td>
<td>-58</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>enrichment</td>
<td></td>
<td>18.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvate</td>
<td>complete</td>
<td>PRTOL1</td>
<td>$\text{CH}_3\text{COCOO}^- + \text{H}_2\text{O} + 1.25\text{SO}_4^{2-} \rightarrow 3\text{HCO}_3^- + 1.25\text{HS}^- + 0.75\text{H}^-$</td>
<td>-106</td>
<td>-153</td>
<td>34.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>enrichment</td>
<td></td>
<td></td>
<td>18.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Benzoate</td>
<td>incomplete (1)</td>
<td>PRTOL1</td>
<td>$\text{C}_6\text{H}_5\text{CO}^- + 4\text{H}_2\text{O} + 2.75\text{SO}_4^{2-} \rightarrow 5\text{HCO}_3^- + 2.25\text{HS}^- + 2.75\text{H}^-$</td>
<td>-341</td>
<td>-558</td>
<td>21.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>enrichment</td>
<td></td>
<td></td>
<td>18.5</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>incomplete (2)</td>
<td>PRTOL1</td>
<td>$\text{C}_6\text{H}_5\text{CO}^- + 4\text{H}_2\text{O} + 1.75\text{SO}_4^{2-} + 3\text{H}_2\text{O} + 2.75\text{H}^- + 1.75\text{HS}^- + 2\text{CH}_3\text{COO}^-$</td>
<td>-40</td>
<td>-144</td>
<td>17.3</td>
</tr>
<tr>
<td>3-Phenylpropionate$^d$</td>
<td>complete</td>
<td>PRTOL1</td>
<td>$\text{C}_8\text{H}_6\text{O}_2^- + 4\text{H}_2\text{O} + 5.25\text{SO}_4^{2-} \rightarrow 9\text{HCO}_3^- + 2.75\text{H}^- + 5.25\text{HS}^-$</td>
<td>-51$^d$</td>
<td>-98$^d$</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>enrichment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naphthalene$^e$</td>
<td>complete</td>
<td>C$<em>{10}$H$</em>{10}$</td>
<td>$\text{C}_{10}\text{H}_8 + 6\text{H}_2\text{O} + 6\text{SO}_4^{2-} \rightarrow 10\text{HCO}_3^- + 4\text{H}^- + 6\text{HS}^-$</td>
<td>-48</td>
<td>-101</td>
<td>27.0</td>
</tr>
<tr>
<td>1,3,5-Trimethylbenzene</td>
<td>complete</td>
<td>C$<em>{12}$H$</em>{16}$</td>
<td>$\text{C}<em>{12}\text{H}</em>{12} + 3\text{H}_2\text{O} + 6\text{SO}_4^{2-} \rightarrow 9\text{HCO}_3^- + 3\text{H}^- + 6\text{HS}^-$</td>
<td>-45</td>
<td>-85</td>
<td>33.1</td>
</tr>
<tr>
<td>Heating oil$^f$</td>
<td>complete</td>
<td>C$<em>{17}$H$</em>{36}$</td>
<td>$\text{C}<em>{17}\text{H}</em>{36} + 13\text{SO}_4^{2-} \rightarrow 17\text{HCO}_3^- + 4\text{H}^- + 13\text{HS}^- + 17\text{H}_2\text{O}$</td>
<td>-48</td>
<td>-74</td>
<td>28.1</td>
</tr>
</tbody>
</table>

$^a$ values of standard free energy are from (Madigan et al., 2003) and (Yaws, 1999)

$^b$ calculated using the Nernst equation: $\Delta\Delta G_f = \Delta\Delta G_0 + RT \ln (c(\text{products})/c(\text{reactants}))$ (e.g., (Jakobsen and Postma, 1999))

$^c$ numbers in brackets refer to number of acetate molecules produced per carbon source molecules degraded

$^d$ standard free energy of formation for 3-phenylpropionate was not available; a nominal value of -200 kJ/mol was assumed based on values for related compounds (Yaws, 1999). Hence, these values may only be compared with each other and not with the other values.

$^e$ even though some acetate was produced during naphthalene and heating oil degradation, the greater proportion of these carbon sources was degraded by a complete mechanism as calculated using measured SO$_4^{2-}$ and acetate concentrations

$^f$ heptadecane was chosen since it represents the average carbon number of heating oil (Lecomte and Mariotti, 1997)
Figure 1: Concentrations of $\text{SO}_4^{2-}$ and S(-II) during microcosm experiments for (a-d, j-l) the enrichment cultures and (e-i) PRTOL1 grown on (a, e) acetate, (b, f) pyruvate, (c, g) benzoate, (d, h) 3-phenylpropionate, (i) naphthalene, (j) 1,3,5-trimethylbenzene, and (k) heating oil.

Solid lines represent the fit of measured $\text{SO}_4^{2-}$ concentrations versus time used to compute zero-order $\text{SO}_4^{2-}$ reduction rates (SRR). Data of enrichment culture microcosms amended with pyruvate (b) and PRTOL1 microcosms amended with 3-phenylpropionate (h) were additionally fitted assuming first-order kinetics to obtain rate coefficients (k) (dotted lines).
Figure 2: Bacterial cell numbers determined by DAPI-staining/microscopy from sacrificed microcosms during experiments for (a) the enrichment cultures and (b) PRTOL1.

Each data point represents the average of three replicate microcosms. Note that in (b), values on the x-axis (days) for PRTOL1 grown on pyruvate were multiplied by a factor of 20 and values for PRTOL1 grown on 3-phenylpropionate were multiplied by a factor of 2.
Figure 3: Sulfur isotope ratios in $\text{SO}_4^{2-}$ and S(-II) during microcosm experiments for (a-d, j-l) the enrichment cultures, (e-i) PRTOL1 grown on (a, e) acetate, (b, f) pyruvate, (c, g) benzoate, (d, h) 3-phenylpropionate, (i) naphthalene, (j) 1,3,5-trimethylbenzene, and (k) heating oil. Values of $\delta^{34}\text{S}(\text{SO}_4^{2-})$ (closed symbols) are plotted versus $-\ln f$ (Eq. 2), whereas values of $\delta^{34}\text{S}(\text{S(-II)})$ (open symbols) are plotted versus $(f \ln f)/(1-f)$ (Eq. 3). Solid lines represent the linear fit used to compute isotope enrichment factors ($\varepsilon$).
Calculated enrichment factors ($\varepsilon$) for all microcosm experiments as a function of cell-specific $\text{SO}_4^{2-}$ reduction rate (sSRR).

Error bars represent standard deviations in $\varepsilon$ and sSRR values.

* data from Bolliger et al. (Bolliger et al., 2001) were included for comparison.
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