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Sulfur isotope fractionation during bacterial sulfate reduction

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ABSTRACT: The isotopic fractionation during bacterial sulfate reduction was determined in batch culture experiments with new strains of sulfate-reducing bacteria that grow at temperatures between -1.8 and 20°C. The observed isotopic fractionations for the two isolates are significantly smaller than commonly measured isotopic differences between sulfate and sulfide in temperate and cold marine sediments. For all cultures, sulfate reduction rates decrease with decreasing temperature. However, in one strain the isotopic fractionation was independent of the sulfate reduction rates and the temperature at which the bacteria were grown whereas another culture showed an increasing isotopic fractionation with decreasing temperature. Phylogenetic differences between the strains, differences in the cellular electron transport pathways during substrate oxidation, and the formation of sulfur intermediates at very low rates of sulfate reduction are inferred to be the dominating factors controlling the observed isotopic fractionations.

1 INTRODUCTION

Bacterial sulfate reduction is the dominant metabolic process in anoxic marine sediments (Jørgensen 1982). It has long been known that the reduction of sulfate to sulfide is coupled to a pronounced enrichment of sulfide in ^{32}S , yet the factors controlling the isotopic enrichment are still insufficiently understood. Rates of bacterial sulfate reduction and temperature have been suggested to exert the dominating control on variations in isotopic fractionation. Here we report experimental results on sulfur isotopic fractionations by 4 strains of sulfate-reducing bacteria to quantify the effects of temperature and sulfate reduction rates and to determine if additional processes can affect the isotopic fractionation. Sulfate reducers were isolated from arctic marine habitats off the coast of Svalbard, Spitsbergen. This is the first report on isotopic fractionations by sulfate-reducing bacteria that are capable of growing at temperatures of very cold marine habitats and at temperatures that are common to most temperate marine environments worldwide.

2 METHODOLOGY

Pure cultures of the sulfate-reducing strains LSv54, ASv26, LSv514, and ASv20 were grown in batch

culture at temperatures of -1.8, 2, 4, 9, 16, and 20°C using the anaerobic medium described in Widdel and Bak (1992). Organic substrates were lactate (20 mM final concentration) for strains LSv54 and LSv514 and acetate (20 mM final concentration) for strains ASv26 and ASv20. Most cultures were grown to the stationary phase of growth. Samples for the isotopic determination of sulfate and sulfide were taken in the lag phase, the early exponential, the late exponential, and the stationary phase. At each time point, concentrations of sulfate and sulfide were determined. Sulfate was determined by unsuppressed anion chromatography, and sulfide was determined by the methylene blue method (Cline 1969). At each of the above time points, 10 ml aliquots of the cultures were incubated for 6 hours with $^{35}\text{SO}_4$ (100 kBq) radiotracer to determine specific sulfate reduction rates for each growth phase. An additional aliquot of the culture was used for cell counting. Sulfate reduction rates could thus be reported as moles of sulfate reduced per bacterial cell rather than on a volumetric basis. To determine the stable isotopic composition 20 % zinc acetate was added to the remaining aliquot to terminate bacterial activity and to precipitate dissolved sulfide. Dissolved sulfate and precipitated zinc sulfide were separated by acid distillation and trapping of H_2S in AgNO_3 . Dissolved sulfate was recovered as BaSO_4 . The isotopic composition of

dissolved sulfate and sulfide were determined by continuous flow isotope ratio monitoring gas chromatography mass spectrometry according to methods described in Giesemann et al. (1994).

3 RESULTS AND DISCUSSION

All analyzed strains show a characteristic decrease in sulfate reduction rates with temperature. In general, sulfate reduction rates decrease from the exponential to the stationary phase of growth. Strain LSV54, which oxidizes lactate to acetate and is not capable of complete substrate oxidation showed a small isotopic fractionation of 4.6 ‰ at 9°C (Figure 1). To our knowledge this is the smallest isotopic fractionation ever reported for sulfate-reducing bacteria. Strain ASv26, which oxidizes acetate to CO₂ and is thus capable of complete substrate oxidation showed a fractionation of 22 ‰ (Figure 2). Phylogenetic differences based on 16S rRNA sequences indicate that these two strains belong to two different new genera of sulfate-reducing bacteria (Knoblauch et al. in press). The isotopic fractionation in strain ASv26 was constant over the viable temperature span for this bacterial strain. Furthermore, the isotopic fractionation is constant for the different growth stages. In strain LSV54 sulfide had a lower isotopic composition at -1.8°C than at 9°C while the evolution of the isotopic composition of sulfate was consistent with a Rayleigh-type fractionation during reduction of sulfate by 4.6 ‰ (Figure 1). These differences can be reconciled with the formation of intermediate sulfur species that are enriched in ³⁴S relative to sulfide. One of the proposed pathways during dissimilatory sulfate reduction includes the formation of the intermediates thiosulfate and trithionate during the reduction of sulfite (Widdel and Hansen 1992). The turnover time of these intermediates at low rates of sulfate reduction may be significantly longer creating an additional sulfur pool that was not accounted for in the Rayleigh model. Thus the observed isotopic differences at different temperatures of strain LSV54 may reflect the formation of a larger pool of sulfur intermediates in the cellular sulfate reduction process at -1.8°C. The continuous trend in the isotopic composition of sulfate indicates that the intermediate sulfur species are not reoxidized to sulfate.

The observed sulfur isotopic fractionations in these pure culture experiments are significantly smaller than the isotopic differences observed between dissolved sulfide and sulfate in coexisting sediments and in most anoxic sediments from temperate environments. In these sediments the

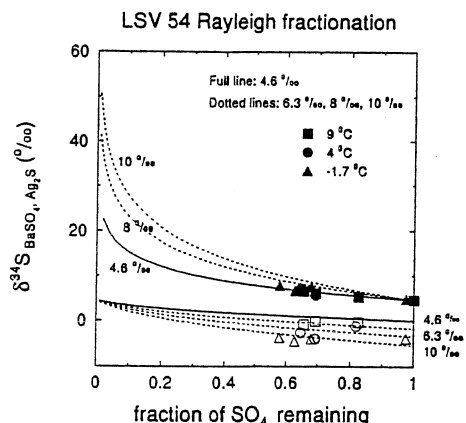


Figure 1. Isotopic fractionation of sulfate and sulfide in batch culture experiments with strain LSV54. Closed symbols represent sulfate and open symbols represent sulfide. Regression lines indicate the different isotopic fractionations.

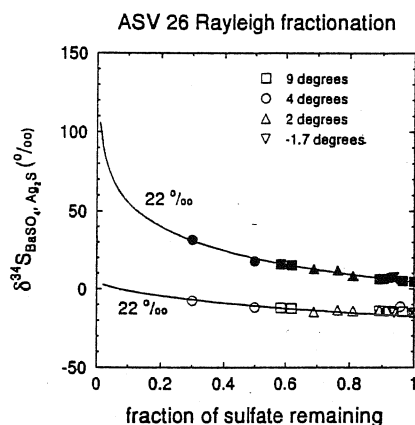


Figure 2. Isotopic fractionation of sulfate and sulfide in batch culture experiments with strain ASV26. Closed symbols represent sulfate and open symbols represent sulfide. Regression lines indicate the isotopic fractionation.

observed isotopic differences between dissolved sulfide and sulfate are generally between 36 and 58 ‰ and imply additional fractionations during the oxidative sulfur cycle.

4 CONCLUSIONS

Our results suggest that naturally observed isotopic differences between sulfate and sulfide may not only

be caused by variations in the rates of bacterial sulfate reduction. Genetic and physiological differences between different genera of sulfate-reducing bacteria may also exert a strong influence on the isotopic fractionation. The electron transport pathways for the oxidation of organic substrates differ significantly between complete- and incomplete-oxidizing sulfate reducers (Widdel and Hansen 1992; Cypionka 1994). Also, two different electron transport pathways have been proposed for the reduction of sulfite to sulfide in sulfate-reducing bacteria. These physiological differences can be reflected in phylogenetic differences. In marine sediments, different genetic populations and populations with different adaptations to temperature will result in different gross isotopic fractionations during sulfate reduction. However, comparison of the experimental results with field data confirms previous findings that additional fractionations must exist in the oxidative part of the sedimentary sulfur cycle. The observed fractionations are too small to explain the naturally occurring isotopic differences of porewater sulfate and sulfide. Therefore additional fractionations must occur either during disproportionation or sulfide oxidation.

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