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GEOCHEMISTRY

Elemental Sulfur and Its Isotopic Composition in the Black Sea Water

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Abstract—As one of the main intermediate products of hydrogen sulfide oxidation, elemental sulfur plays an important indicator role in understanding the oxidative cycle of sulfur in the water of anoxic basins. The distribution of elemental sulfur in the water column of the Black Sea at stations located in the area of the continental slope is considered. For the first time, the concentration distributions of two forms of elemental sulfur depending on the depth in the Black Sea water were obtained: suspended elemental sulfur with a fraction of more than 0.45 μm (S^0) and zero-valency sulfur (ZVS), which includes the sum of elemental sulfur (suspended and colloidal) and polysulfide sulfur. In the upper anoxic waters, the concentration of S^0 noticeably increases (almost 200 times relative to the depth of 400 m) with an increase in the concentration of hydrogen sulfide and the density of water. At depths of more than 250 m, the concentration of both forms of sulfur remains almost constant (ZVS = 0.21 ± 0.03 μmol/kg, S^0 = 0.05 ± 0.01 μmol/kg). A sharp increase in the concentration of S^0 at the depths of 150–250 m is associated with the oxidation of hydrogen sulfide due to bacterial anoxygenic photosynthesis after sampling. The value of $\delta^{34}S$(ZVS) was determined in the waters of two stations Ash-26 and 149 at the depths of 450 and 600 m, respectively, which turned out to be +2.2‰ higher than $\delta^{34}S(H_2S)$ from the same depths, which indicates the bacterial origin of elemental sulfur.

Keywords: zero-valency sulfur, elemental sulfur, hydrogen sulfide, isotopic composition, Black Sea
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INTRODUCTION

Elemental sulfur in meromictic water basins is a product of both chemical and biogenic oxidation of dissolved sulfide. Chemical reactions of hydrogen sulfide with available oxidizing agents such as oxygen, nitrate, MnO_2, or Fe_2O_3, may produce abiogenic elemental sulfur in the upper part of the anoxic zone [11]. The following is an example of an oxidation reaction of sulfide by manganese oxide:

$$MnO_2 + HS^- + 3H^+ \leftrightarrow Mn^{2+} + 2H_2O + S^0.$$

Anoxigenic oxidation of sulfide to elemental sulfur can also occur biogenically with photoautotrophic bacteria consuming dissolved carbon dioxide [3, 6]:

$$CO_2 + 2H_2S \leftrightarrow 2S^0 + C(H_2O) + H_2O.$$

Elemental sulfur in the anoxic zone reacts with sulfide to form soluble polysulfides [7, 9]:

$$HS^- + (n-1)S^0 \leftrightarrow S_n^{2-} + H^+.$$

The phototrophic green and purple sulfur bacteria oxidize sulfides to elemental sulfur (or to sulfate if sulfides are deficient) when absorbing quanta of light [13]. These bacterial communities play an important role in the oxidation cycle of sulfur in the upper part of the anoxic zone in case when redoxycline is located in the photic zone. In the Black Sea, redoxycline is found at minimum depths (80–95 m) in the center of the sea. However, owing to the isopycnals convex to the surface in the center, the onset of hydrogen sulfide (potential water density 16.15 kg/m^3) deepens to 150–160 m on the periphery of the sea, where reactions under the influence of light are practically impossible. A study of the photoautotrophic bacterial activity of *Chlorobium* BS-1 has shown that the species is adapted to very low light conditions and can only be physiologically active in the central part of the sea. Undamaged bacterial cells were found in the peripheral parts of the sea. They retain the ability to grow in suitable light conditions [10].

The isotopic composition of the elemental sulfur probably depends on whether its origin was abiogenic

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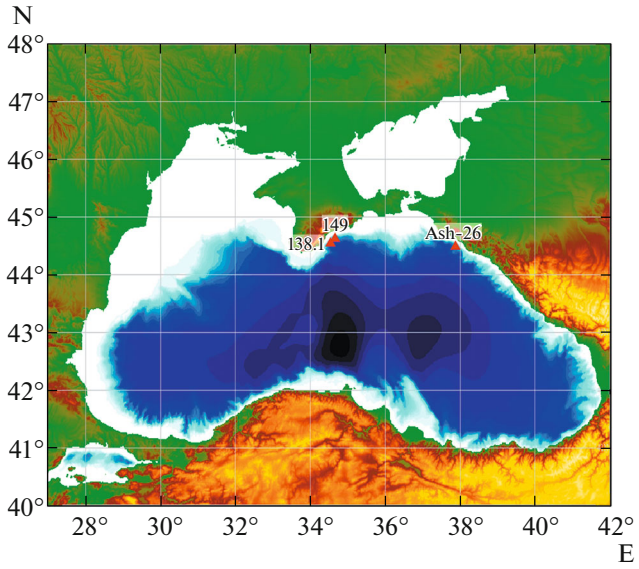

Fig. 1. Location of stations Ash-26 (44.489° N, 37.870° E), 149 (44.628° N, 34.654° E), and 138.1 (44.543° N, 34.533° E).

or biogenic [5]. Abiogenic oxidation of hydrogen sulfide in a model solution of sea water resulted in the fractionation of sulfur isotopes $\varepsilon = \delta_{S0} - \delta_{H_2S} = -4.1 \pm 2.6‰$ [3, 5], while enrichment of the elemental sulfur with ^{34}S during equilibrium isotope exchange with hydrogen sulfide at 25°C is about +3‰ [4, 11]. Experiments with photoautotrophic bacteria have shown that elemental sulfur is enriched in ^{34}S relative to hydrogen sulfide by a small amount ε from 0 to +3‰ [13]. Oxidation of hydrogen sulfide by phototrophic bacteria in the photic zone of the meromictic Green Lake in Fayetteville, NY (United States) results in variable enrichment of elemental sulfur (and sulfur of polysulfides) with ^{34}S depending on the rate of bacterial oxidation [14]. In spring, when the phototrophic oxidation rate is high, fractionation varies in the range of +1.4 to +1.6‰; in autumn, when oxidation rate decreases due to a decrease in daylight hours and lower water transparency, the fractionation increases to +6.5 to +6.8‰. Noticeable enrichment in ^{34}S ($\varepsilon = +3.6 \pm 0.8‰$) was observed in the redoxycline of the meromictic Rogoznica Lake (Croatia) [7].

Dark CO_2 fixation is characteristic of the upper part of the anoxic zone of the Black Sea. It results from the vital functions of a large number of chemolithoautotrophic and heterotrophic bacteria providing anoxygenic oxidation of hydrogen sulfide [6, 12]. The isotopic composition of elemental sulfur obtained from experiments with the *Thiobacillus* chemolithoautotrophic culture turned out to be enriched in ^{32}S relative to initial hydrogen sulfide; fractionation was in the range from 0 to −2.5‰, and the only positive value was +1.2‰ [8].

The isotopic composition of zero-valency sulfur (ZVS) in the Black Sea is known from [11]. It varies from −31.3 to −39.7‰ while the sulfide isotope composition is in the range of −39.4 to −40.3‰. The fractionation was reported to be +0.1‰ at 1660 m and +8.6‰ at 1896 m. ZVS separation in [11] was carried out using a $Zn_2(OH)_2CO_3$ suspension, however the conditions of elemental sulfur filtration were not reported. The difference in the isotopic compositions obtained can be due to the filtration of samples of elemental sulfur in the air, which might have caused partial abiogenic oxidation of sulfur [2]. Based on the data available, it should be noted that the distribution of elemental sulfur in the water of the Black Sea is very poorly known. Data are available only for zero-valency sulfur [2]. The indicator role of elemental sulfur is to localize hydrogen sulfide oxidation in the water column and to determine its mechanism. Oxidation of hydrogen sulfide by phototrophic bacteria produces elemental sulfur, enriched in ^{34}S, while either abiogenic oxidation of hydrogen sulfide or chemolithotrophic bacterial activity gives isotopically light elemental sulfur as a reaction product. Thus, the isotopic composition of elemental sulfur allows the questions about the probable mechanism of hydrogen sulfide oxidation and the appearance of elemental sulfur in the anoxic zone of the sea to be solved.

The purpose of this study is to investigate the distribution of elemental sulfur in the anoxic zone of the Black Sea. In order to assess the genesis of zerovalency sulfur, we obtained the first data on its isotopic composition.

MATERIALS AND METHODS

Water samples were collected in Niskin bottles using the Rosette complex. At stations 138.1 and 149 (Fig. 1) during cruise 142 of the R/V *Professor Vodyanitskii* (October 2022), sampling was carried out with twelve 8 L Niskin bottles of General Oceanic and CTD measurements were performed using the Idronaut Oceanseven 320 PlusM.

At Ash-26 station (Fig. 1), sampling was carried out on July 9, 2022, from the small R/V *Ashamba* using the Rosette complex equipped with six 4 L Niskin bottles. CTD measurements were conducted with the Sea Bird 19+ manufactured by Sea-Bird Electronics, Inc.

All three stations are located on the continental slope; the water depth is 1200 m at Ash-26 station and about 1500 m at stations 138.1 and 149. Water from the bottles was retrieved under low argon pressure. All PET bottles for sample storage were prefilled with argon. For the analysis of elemental sulfur at station 138.1, two separate samples weighing approximately 1 kg each were taken from a certain depth; a suspension $Zn_2(OH)_2CO_3$ was preliminary added to one of the samples. Not more than three days after sampling,

the bottles were stored in a refrigerator at +4°C until the filtration procedure through a 0.45 μm Millipore filter in an argon atmosphere. The filter and precipitate were placed in a polypropylene tube filled with argon and sealed tightly with a lid. The filters were stored at –20°C before analysis. Elemental sulfur was analyzed by distillation of hydrogen sulfide after reduction with the $CrCl_2$ solution [2].

During sampling for isotopic analysis of sulfur at station Ash-26, all six Niskin bottles were closed at a depth of 450 m. We collected 18 samples, each weighing about 1 kg, in the bottles pre-filled with argon and with addition of a $Zn_2(OH)_2CO_3$ suspension. The same was done at station 149 for 20 samples at a depth of 600 m. Three samples from station Ash-26 and five samples from station 149 were used to determine the elemental sulfur concentration. The mean elemental sulfur concentration at station Ash-26 (450 m depth) was 0.21 ± 0.3 μmol/kg (n = 3) and at station 149 (600 m depth) was 0.24 ± 0.02 μmol/kg (n = 5) thus being indistinguishable for each stations within the analysis error. The remaining samples (15 each) were used for conversion of elemental sulfur into silver sulfide and subsequent determination of the isotopic composition. At station Ash-26 two samples (from 7 and 8 kg of seawater) were prepared for determination of the elemental sulfur isotope composition; at station 149 one sample of Ag_2S was prepared after distillation from 15 kg of seawater. The concentration of hydrogen sulfide at station Ash-26 (depth 450 m) obtained by the spectrophotometric method [1] was 128 μM and at station 149 (depth 600 m), 204 μM. Three samples from each series intended for isotopic analysis of elemental sulfur were taken for isotopic analysis of sulfide sulfur.

The sulfur isotope composition of the obtained samples was determined by CF-IRMS on a DELTA V+ mass spectrometer (Finnigan, Germany) after conversion of Ag_2S in a FlashEA HT 1112 elemental analyzer to SO_2 gas. Sample weights for determination of the sulfur isotopic composition ranged from 120 to 400 μg Ag_2S. The international standards IAEA-S-1 and IAEA-S-3 were analyzed in each sample series to normalize the data to the international VCDT (Vienna Cañon Diablo Troilite) scale using generally accepted $\delta^{34}S$ values for IAEA-S-1 and IAEA-S-3 (–0.3‰) and (–32.55‰), respectively. The reproducibility of the results was better than ±0.2‰. The results were calculated relative to VCDT:

$$\delta^{34}S_{sample} = [(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{VCDT} - 1] \times 1000.$$

RESULTS AND DISCUSSION

Elemental sulfur concentrations in the water of the Black Sea. At station 138.1 elemental sulfur was selected in two ways. The elemental sulfur obtained after addition of $Zn_2(OH)_2CO_3$ includes elemental sulfur (colloidal and suspended) and sulfur of polysulfides after their destruction. Further we will call it zero valency sulfur (ZVS). In samples without adding $Zn_2(OH)_2CO_3$ after filtration, only suspended elemental sulfur with a particle size of more than 0.45 μm (hereinafter simply elemental sulfur, S^0) was found. Starting from the interface area (depth 150 m) with the appearance and increase in the hydrogen sulfide concentration (Fig. 2), the ZVS concentration increases from 0.07 to 0.20–0.26 μmol/kg at a depth of 400 m (Fig. 3) further remaining constant. At the same time, the hydrogen sulfide concentration increases from 121 to 336 μM. Above the interface, in the suboxic zone, where oxygen concentrations increase from 1.6 to 7.3 μM, the elemental sulfur and ZVS concentrations gradually fall below the detection limit (0.01 μmol/kg).

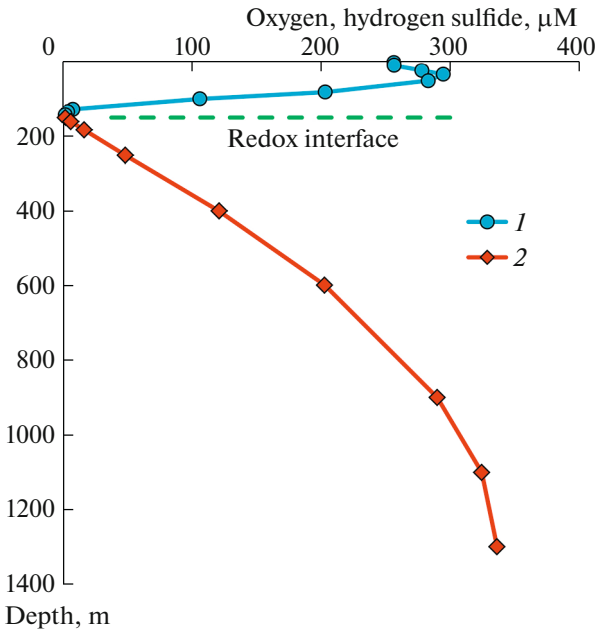

Fig. 2. Distribution of (*1*) dissolved oxygen and (*2*) hydrogen sulfide at station 138.1.

In the distribution of suspended elemental sulfur concentration, there is a noticeable maximum at the 150–250 m depths, where the S^0 concentration reaches a value of 9.33 μmol/kg. Deeper than 250 m, it changes weakly from 0.04 to 0.06 μmol/kg, showing no relation to growth of the hydrogen sulfide concentration. Below 400 m depth, the S^0 fraction is about 23 ± 5% of ZVS. The remaining 77% of sulfur in the form of ZVS appears to be represented by polysulfides and colloidal elemental sulfur.

The ratio of sulfur forms changes abruptly in the upper part of the anoxic zone due to abnormal growth of elemental sulfur (Fig. 3). Obviously, this maximum is not related to the initial distribution of sulfur in seawater because it is not reflected in the distribution of the ZVS concentration: the ZVS maximum is absent

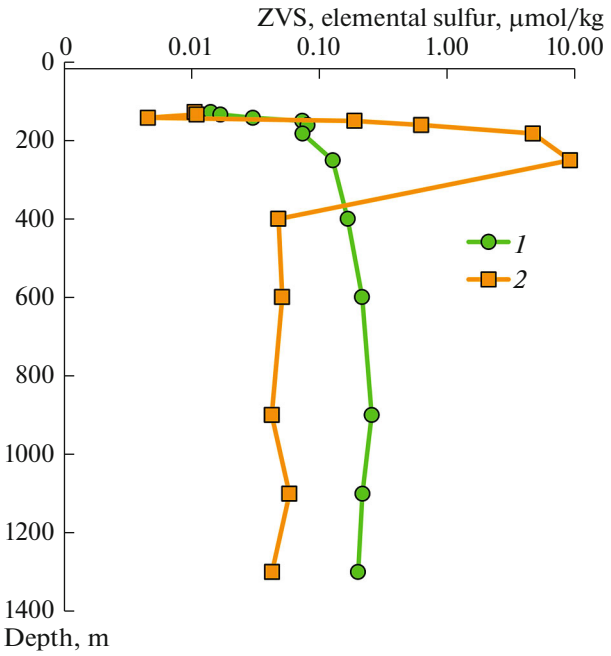

Fig. 3. (*1*) Zero-valency sulfur (ZVS) and (*2*) weighted elemental sulfur (S^0) at station 138.1.

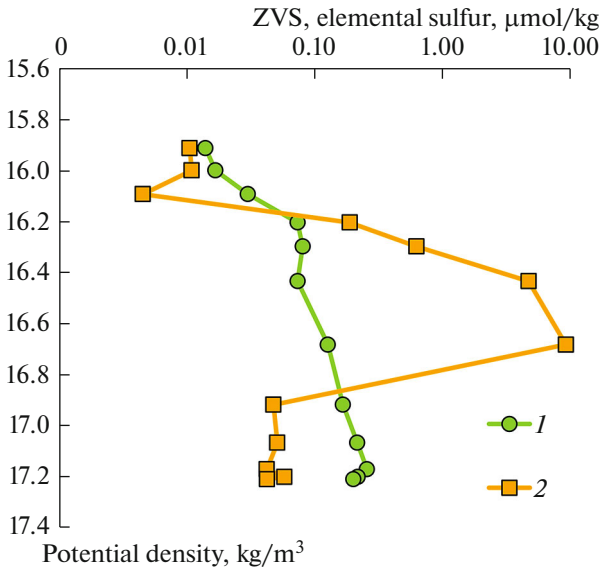

Fig. 4. (*1*) Distribution of zero valency sulfur (ZVS) and (*2*) weighted elemental sulfur (S^0) at station 138.1 with respect to the potential density.

in the area of the S^0 maximum; the ZVS concentration increases monotonically with depth from 0.07 to 0.13 μmol/kg.

The maximum observed can be explained as a consequence of anaerobic oxidation of sulfide by photoautotrophic bacteria after water sampling. As there was no access to air oxygen at all stages of sampling and filtering, light exposure initiating bacterial activity in samples not fixed with $Zn_2(OH)_2CO_3$ can be considered as the main factor. The light exposure was at least four hours during sampling on deck and additionally in the laboratory during filtration. The biogenic origin of the elemental sulfur at 150–250 m is also supported by the coincidence of elevated concentrations of sulfur and suspended organic matter in the upper part of the anoxic zone in both depth and density (Figs. 4, 5).

Isotopic composition of elemental sulfur in the water of the Black Sea. As ZVS concentrations in the Black Sea waters are low, large water samples (15 kg at each station) were taken to ensure sufficient sulfur for isotopic analysis (about 400 μg of Ag_2S). Two samples were taken at depths greater than 450 m at station Ash-26 and one at 600 m at station 149; in addition, three water samples were taken for the same depths to determine $\delta^{34}S$ of dissolved hydrogen sulfide. As a result, the isotopic composition of sulfur was determined for three samples of zero valence sulfur and for six samples of associated sulfides. In order to account for the nonlinearity of the isotopic measurements of sulfur in small samples, one of the hydrogen sulfide samples was analyzed repeatedly with gradual reduction of the amount of Ag_2S. This has allowed us to make corrections to the values of $\delta^{34}S$ during the analyses of zero valence sulfur. The corrected $\delta^{34}S$(ZVS) value was $38.8 \pm 0.4‰$ ($n = 3$), which is 2.2‰ heavier than the average isotope composition ($\delta^{34}S(H_2S) = -41.0 \pm 0.4‰$, $n = 6$), determined in water of both stations.

The origin of elemental sulfur in the Black Sea water. Photoautotrophic sulfide oxidation is limited in the

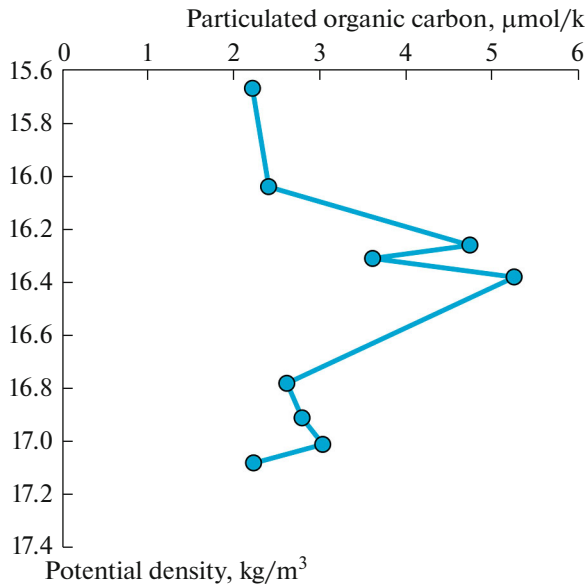

Fig. 5. Distribution of particulated organic carbon at station Ash-21 (sampling date July 20, 2020). Station location is the same as for Ash-26.

deep parts of the Black Sea by a lack of illumination and the amount of bacterioplankton [10], which can be represented as particulated organic carbon (Fig. 5). The upper limit of the anoxic zone at stations 149 and Ash-26 at depths of 150–160 m is very poorly illuminated. In spite of the same illumination of samples during the preparation procedures (sampling and filtering), the amount of elemental sulfur below 250 m remained constant in relation to the amount of zero-valence sulfur. Consequently, the amount of photoautotrophic bacteria at depths greater than 250 m is too low to give a noticeable and rapid increase in elemental sulfur.

However, the sulfur isotopic composition at depths of 450 and 600 m appeared to be enriched in ^{34}S relative to the coexisting sulfide. Such an increase in elemental sulfur is characteristic of anoxic oxidation of sulfide by photoautotrophic bacteria [13]. The value of the isotope effect under photoautotrophic oxidation of sulfide by *Chlorobium tepidum* bacteria for elemental sulfur turned out to be $+1.8 \pm 0.5‰$ and practically coincided with our data $+2.2‰$. When added to the sample, $Zn_2(OH)_2CO_3$ inhibited bacterial activity, removing sulfide as a possible electron donor. This led to the preservation of the original sulfur isotope composition in the form of ZVS.

The enrichment of elemental sulfur with the heavy isotope ^{34}S is typical for anoxic oxidation of sulfide by phototrophic bacteria and differs considerably from the isotope composition of sulfur obtained by abiogenic oxidation of sulfide by oxygen or chemolithotrophic bacteria [3, 5, 8]. The distribution of zero valence sulfur (elemental + polysulfide sulfur) and elemental sulfur with a particle size greater than 0.45 μm in the water column below 250 m shows that their concentrations are almost constant and make up 0.21 ± 0.03 and 0.05 ± 0.01 μmol/kg, respectively. Such constancy could be a result of the sinking of suspended particles of ZVS from the upper part of the anoxic zone after the anoxigenic oxidation of sulfide by photo- and chemoautotrophic bacteria in the presence of an increased amount of bacterial plankton [6, 12]. Thus, although the abundance of photoautotrophic bacteria in the upper part of the anaerobic zone was quite significant, the activity was markedly suppressed by the low illumination on the periphery of the Black Sea basin [10].

CONCLUSIONS

In the framework of this study, the concentration profiles of two forms of elemental sulfur suspended in the Black Sea water were first obtained: elemental sulfur with a particle size larger than 0.45 μm and zero valence sulfur, which includes the sum of elemental sulfur and polysulfide sulfur. It was shown that below a depth of 250 m the concentrations of both forms of sulfur are practically constant and amount to 0.21 ± 0.03 and 0.05 ± 0.01 μmol/kg, respectively. No maximum of zero valence sulfur that would have resulted from oxidation of hydrogen sulfide by either a biogenic or abiogenic way was revealed at the interface of the anoxic zone. Since the elemental sulfur is the oxidation product of hydrogen sulfide, a decrease in its concentrations below the detection limit in the suboxic zone is apparently caused by the absence of the latter. The increase in elemental sulfur concentrations in the upper part of the anoxic zone at depths of 150–250 m is associated with the activity of photoautotrophic bacteria after sampling as a result of their reaction to increased light exposure. High concentrations of elemental sulfur coincide in both depth and density with those containing the maximum amount of bacterial plankton in the water column of the Black Sea. The fact that ZVS concentrations fail to increase in these same depths is caused by the inhibitory effect of the $Zn_2(OH)_2CO_3$ suspension by means of the removal of sulfide as electron donor. The ZVS sulfur isotopic composition was found to be similar at the 450 and 600 m horizons for the stations located on the continental slope of the Black Sea near the coast of Crimea and the Caucasus. The $\delta^{34}S$ value in ZVS sulfur for the three samples was $-38.8 \pm 0.4‰$, which is $2.2‰$ heavier than the average isotopic composition of sulfide sulfur ($-41.0 \pm 0.4‰$). Such an increase in heavier isotopes could be determined by the bacterial anaerobic oxidation of sulfide. As the activity of phototrophic bacteria in the upper part of anaerobic zone is noticeably suppressed because of poor illumination, anoxigenic oxidation of hydrogen sulfide seems to occur in a biogenic way. In this case, it should be assumed that oxidation of hydrogen sulfide by chemolithotrophic microorganisms leads to enrichment of sulfur in the ^{34}S isotope.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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