Relationship of sulfur speciation to hydrographic conditions and chemoautotrophic production in the Cariaco Basin

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Sulfur species are likely to be important microbial substrates at redox transition zones. In this study, we measured thiosulfate (S2O3 2−), sulfite (SO3 2−), particulate elemental sulfur (S0), total zero-valent sulfur (particulate S0 + polysulfides + colloidal S0), hydrogen sulfide (H2S) together with chemoautotrophic production and heterotrophic bacterial production at several locations in the Cariaco Basin as part of the on-going CARIACO (Carbon Retention in a Colored Ocean) time-series project. Sulfite and thiosulfate concentrations always covaried within the redoxcline, but no consistent distribution pattern was observed nor did it correlate with chemoautotrophic production. In contrast, particulate elemental sulfur maxima were consistently found at the interface and were highly correlated with chemoautotrophic production. Higher concentrations of particulate elemental sulfur at stations closer to the basin's margin were associated with higher inventories of hydrogen sulfide. Comparisons of profiles of total zero-valent sulfur and of particulate elemental sulfur suggest that a large fraction of zero-valent sulfur is either colloidal or in the form of polysulfides. Although thiosulfate stimulated bacterial activity in amendment experiments, the role of thiosulfate and sulfite in chemoautotrophic production is not yet clear.

1. Introduction

In anoxic basins, a pronounced peak in the rate of dark CO2 fixation has been repeatedly observed in the redoxcline of the water column (Jørgensen et al., 1991; Fenchel et al., 1995; Taylor et al., 2001). However, the relatively high rates observed for chemoautotrophic production are problematic, since vertical fluxes of reductants (H2S, NH4+, Fe2+, Mn2+) and oxidants (O2, MnO2, Fe2O3, NO3−) calculated from concentration profiles and estimates of vertical eddy diffusion can only support a few percent of the measured carbon fixation (Murray et al., 1995; Zopfi et al., 2001; Taylor et al., 2001). In addition, based on the organic matter flux and stoichiometry of sulfate reduction, Hayes et al. (2006) calculated that the particulate carbon sinking flux at the CARIACO site is too low to support rates of sulfate reduction required to satisfy the putative microbial demand for H2S.

One possible explanation for the apparent discrepancy between vertical fluxes of chemical species and biological demand is that horizontal supply of these species is important (Taylor et al., 2001, 2006). Transient lateral intrusions of oxic water or high wind events that circulate and introduce oxygenated water into the suboxic or anoxic zone may supply electron acceptors (Holmén and Rooth, 1990; Scranton et al., 2001; Astor et al., 2003). Calculations have suggested that enough O2 may be able to enter the basin through intrusions to produce sufficient thiosulfate to support the chemoautotrophic growth of bacteria (Hayes et al., 2006). However, the origin of the required sulfide is still unclear. Taylor et al. (2001) calculated that vertical sulfide flux can account for only 0.7–2.8% of the estimated demand for reductants at Carriaco Station. Percy et al. (2007) pointed out that higher inventories of sulfide (between 250 m and 340 m) were found in shallower, more productive water. However, the observed
variations in sulfide inventory were not large. Even at the more productive, shallower station B (Fig. 1), the inventory of sulfide is only twice that of station A.

Since the maximum dark carbon fixation rate is always slightly deeper than the depth of detectable O₂ and nitrate (Taylor et al., 2001, 2006), our recent microbiological and geochemical studies have explored the cycling of S, Fe and Mn, which have been proposed to play important roles in sediments (Jørgensen, 1990b; Nealson and Myers, 1992; Aller, 1994). Sulfide oxidation results in the formation of sulfur intermediates such as polysulfides (Sₙ⁻), elemental sulfur (S⁰), thiosulfate (S₂O₃⁻²) and sulfite (SO₃⁻²). In recent investigations of thiosulfate and sulfite in the Cariaco (Hayes et al., 2006; Percy et al., 2007), no apparent relationship was found between either thiosulfate or sulfite and chemoautotrophic production within the redoxcline. Percy et al. (2007) suggested that elemental sulfur and polysulfides might be important, although no direct measurement of elemental sulfur had been carried out in the Cariaco Basin since Hastings and Emerson (1988).

In the present study, abundances and distributions of thiosulfate, sulfite and elemental sulfur were investigated during both upwelling and non-upwelling periods at four sites in the Cariaco Basin, and concentrations were compared to rates of chemoautotrophic and heterotrophic microbial production. Our objective is to identify, among the many potential factors, those hydrographic, chemical and microbial processes that can explain the observed distributions of sulfur intermediates in this unique environment. Based on the results to date, we suggest that CO₂ fixation is at least in part a result of efficient cycling of sulfur species by chemoautotrophs in the Cariaco, and that elemental sulfur plays an important role either as a product or as a substrate.

2. Materials and methods

2.1. Field site

The Cariaco Basin (Fig. 1) is a large, marine anoxic system on the continental shelf off the northern coast of Venezuela. A 900 m deep saddle separates the eastern and western basins. The deepest parts of the basin reach 1400 m and are isolated by a shallow (90 to 150 m) sill that restricts the entrance of Caribbean water. Channels to the northeast (La Tortuga channel: about 135 m) and the west (Centinela channel: about 146 m) provide pathways for denser water to penetrate into the deep basin (Richard, 1975). The stability of the basin is controlled by temperature but density is uniform to within 0.1 σθ units below about 250 m (Richards, 1975). The depth of first appearance of hydrogen sulfide has ranged between about 200 and 350 m (Ho et al., 2004), and a suboxic zone in which both oxygen and sulfide concentrations are below 1–2 μmol L⁻¹ has varied in thickness from zero to over 50 m between 1995 and 2007 (Scranton et al., 2006; Percy et al., 2007).

2.2. Sample collection

Water samples were collected in the Cariaco Basin as part of the international CARIACO (Carbon Retention in a Colored Ocean) time-series program. In CARIACO, a single station (station A) has been sampled monthly, and oxygen, temperature, salinity, nutrients, primary production and CO₂ data have been collected since 1995. A general description of the CARIACO program and data links can be found at the web site (http://www.imars.usf.edu/CAR/). In addition to the data available from monthly cruises, we have undertaken two or
three additional cruises annually during which more detailed chemical and microbiological measurements have been made. Some of the data from these cruises are described by Taylor et al. (2001, 2006), Hayes et al. (2006), Scranton et al. (2006), Lin et al. (2006, 2007), and Percy et al. (2007) as well as in other publications.

For the present study, samples were collected at four stations (Fig. 1, Table 1), including the CARIACO time-series station (station A, maximum depth ca. 1400 m, 10°30' N 64°40' W), a station southeast of La Tortuga Channel (station B, maximum depth ca. 600 m, 10°40' N 64°45' W), a station in the western basin (station C, maximum depth ca. 1400 m, 10°40' N 65°35' W) and a station to the northeast of the CARIACO station (station D, maximum depth ca. 500 m, 10°43' N 64°32' W). Dates of sample collection are summarized in Table 1. Water samples were collected in 12 8-L Teflon-lined Niskin bottles mounted on a Seabird rosette system and analyzed with the DTNP method of Vairavamurthy and Mopper (1990) as modified by Hayes et al. (2006). Lin et al. (2006, 2007), and Percy et al. (2007) as well as in other publications. For the present study, samples were collected at four stations (Fig. 1, Table 1), including the CARIACO time-series station (station A, maximum depth ca. 1400 m, 10°30' N 64°40' W), a station southeast of La Tortuga Channel (station B, maximum depth ca. 600 m, 10°40' N 64°45' W), a station in the western basin (station C, maximum depth ca. 1400 m, 10°40' N 65°35' W) and a station to the northeast of the CARIACO station (station D, maximum depth ca. 500 m, 10°43' N 64°32' W). Dates of sample collection are summarized in Table 1. Water samples were collected in 12 8-L Teflon-lined Niskin bottles mounted on a Seabird rosette system equipped with a SBE 25 CTD, a SBE 43 oxygen probe, a WetLab profiling fluorometer for chlorophyll a, and a WetLab c-beam transmissometer (660 nm). The Niskin bottles were slightly pressurized with N2 during sampling to minimize O2 contact. In the Cariaco Basin, peaks in beam attenuation (BAT) from 2.2.1. Oxygen

Seawater samples for dissolved oxygen were drawn into standard oxygen bottles in duplicate and Winkler reagents were added immediately. Details of methods are provided in Astor et al. (2003). Samples were stored in an air-conditioned room with seawater around the caps to minimize oxygen transfer across the stoppers and were run within 48 h of returning to the Fundación La Salle laboratory on Margarita Island. The oxygen probe on the CTD also provided continuous O2 profiles which were corrected for response time and calibrated with Winkler O2 data.

2.2.2. Sulfide, thiosulfate and sulfate

Samples for sulfide, thiosulfate and sulfate analyses were collected as described by Hayes et al. (2006) and Percy et al. (2007) by placing the tip of a 10 mL all-glass Hamilton Gas-Tight syringe below the surface of water flowing upward through a 60 mL plastic syringe barrel which was attached to the Niskin bottle by Tygon tubing. Sampling from flowing seawater was used to minimize contact of samples with O2. Sulfide samples were taken in triplicate and were analyzed using Cline (1969). Thiosulfate and sulfate were collected in triplicate and were analyzed with the DTNP method of Vairavamurthy and Mopper (1990) as modified by Hayes et al. (2006).

Kinetic calculations for a mixing of sea water with 250 μM O2 and 60 μM sulfate (full oxygenation of anoxic bottom water) suggest a maximum production rate of sulfate of 0.017 μmol L−1 min−1 (Zhang and Millero, 1993b), indicating that under the worst conditions during our derivatization about 0.1 μmol L−1 SO4− could be produced by the reaction of sulfide with oxygen during a 5 min reaction time. Since oxygen was carefully excluded during sample collection and the sulfide concentration was always lower than 10 μM above 350 m where we observed sulfite concentration as high as ca. 4 μmol L−1 (see Fig. 3 CAR 122), this calculation suggests that concentrations of sulfite in the upper anoxic water are not a consequence of sample collection artifacts.

2.2.3. Elemental sulfur

Duplicate particulate elemental sulfur samples were acquired by gravity filtering directly from the Niskin bottles as described by Trouwborst (2005) and were analyzed by a modification of the method of Henneke et al. (1997). Filter holders, loaded with 0.2 μm polycarbonate filters, were attached to the Niskin bottle by Tygon® tubing. Filtrate was collected for each filter in a graduated cylinder to determine the filtered volume. The filters were rinsed with de-ionized water, dried by passing argon gas through the filters and stored in 15 mL centrifuge tubes at −20 °C. After return to Stony Brook University, 6 mL methanol was added to each centrifuge tube to extract elemental sulfur from the filter. The centrifuge tubes were shaken for 2.5 h on a mechanical shaker and the S0 concentration of each sample was analyzed on a Shimadzu HPLC consisting of a SCL 10A-VP system controller, two LC-10AT pumps, an SPD-10AV/VP ultraviolet detector, and a SIL-10A auto-injector. We used a ODS hypersil (C18) reverse phase, 250 mm x 4.6 mm, 5 μm column (Supelco Co.) at room temperature. Twenty μL samples were injected into the chromatograph and eluted with 100% methanol at a pump speed of 1 mL/min. Retention time of the elemental sulfur peak was typically about 2.2 min. Elemental sulfur was detected at 226 nm, with a detection limit of about 1 μmol L−1 and a precision of 0.5% relative standard deviation among replicates. Standard solutions, made by dissolving sulfur powder in methanol and serially diluting, are linear in the range of 1–100 μmol L−1.

We also measured total zero-valent sulfur at station A during cruise CAR-132. Duplicate 40 mL sub-samples were taken with a gas tight BD syringe in same manner as sulfide samples and fixed with 1 mL 2% (w/v) zinc acetate (Ramsing et al., 1996; Henneke et al., 1997; Zopfi et al., 2001). With this treatment, the sulfane-S components of polysulfides are transformed to elemental sulfur. Therefore, this method determines the sum of particulate, colloidal and sulfane fractions of the polysulfides. Samples were stored at −20 °C in the dark. After return to Stony Brook University, total elemental sulfur was extracted twice using 1 mL chloroform, and the pooled chloroform extract was diluted 1:1 with methanol to optimize chromatography. The HPLC configuration was basically similar to that used for the measurement of particulate elemental sulfur, but the mobile phase was changed to 90% methanol: 10% DI water at a flow rate of 0.5 mL/min. The retention time of sulfur (0) was around 8.5 min.

2.2.4. Microbiological analysis

Heterotrophic bacterial net production (BNP) and chemosynthetic production (CHEMO) were determined by...
assimilation of $^3$H-leucine into protein and $^{14}$C-bicarbonate into particles, respectively. Details of the methods have been described previously (Taylor et al., 2001).

Amendment experiments with thiosulfate and sulfite were performed during CAR-132 at station A to assess the role of sulfur intermediates on chemosynthetic metabolism at 6–7 depths, spanning the redoxcline. In addition to $^{14}$C-bicarbonate, duplicate samples were spiked with 100 µL of N$_2$-purged thiosulfate or sulfite (final concentration: 50 µmol L$^{-1}$) using a gas tight syringe, and then were incubated in the dark at in situ temperature (17 °C) for 18 h. Samples were processed in the same manner as chemosynthetic samples to test whether addition of thiosulfate or sulfite stimulated dark carbon fixation. Previous incubation experiments with additions of S$^0$ did not stimulate chemosynthetic carbon fixation (Taylor et al., 2001), although this result may have been affected by the low bioavailability of the crystalline S$^0$ used.

2.2.5. Inventory calculation

Concentrations of sulfur species (sulfide, particulate elemental sulfur) were integrated from 250 m to 340 m to calculate their “inventory” for each of the four cruises. We picked this depth range because it brackets the full suboxic and upper sulfidic zones, the depth interval most impacted by temporal variability and in which chemolithotrophic production is typically most active. We also integrated BNP and CHEMO to estimate rates per meter square per day.

3. Results

3.1. Suboxic zone variability

For the Cariaco Basin, we have operationally defined the suboxic zone as lying between the first depth where O$_2$ was determined to be ≤2 µmol L$^{-1}$ by Winkler titration and the first depth where H$_2$S was greater than 1 µmol L$^{-1}$. Therefore, the water column can be classified into three zones (Fig. 2): an oxic zone where O$_2$ > 2 µmol L$^{-1}$, a sulfidic zone where H$_2$S > 1 µmol L$^{-1}$, and a suboxic zone, where sulfide and O$_2$ are both very low (or are both absent) with little perceptible vertical gradient (Murray et al., 1995; Glazer et al., 2006). Because we sampled at ten meter intervals across the interface, in some cases we did not have measurements at these specific concentrations. Under those circumstances, when there was a clear change in the slope of the oxygen or sulfide profile, we used an oxygen value of 2–3 µmol L$^{-1}$ or a sulfide value of 1–2 µmol L$^{-1}$. Estimates of the top and bottom of the suboxic zone are marked using dashed lines in Fig. 2 for each cruise. As found by Scranton et al. (2006) and Percy et al. (2007), depth and position of the suboxic zone varied depending on location and time, exhibiting changes in both the depth of oxygen disappearance and of sulfide appearance. Changes from cruise to cruise were not uniform at all stations. As pointed out by Percy et al. (2007), variations in the suboxic zone demonstrate the dynamic nature of geochemical fluxes.

Fig. 2. Vertical profiles of sulfide and oxygen concentrations during the four reported cruises. The dashed lines indicate the suboxic zone. From top to bottom: CAR-118, CAR-122, CAR-128, and CAR-132. Symbols and error bars represent means and 1 standard deviation of triplicate samples.
within the Cariaco Basin, and indicate that intrusions, other advective processes and episodic events all likely contribute to spatial and temporal variability of chemical properties. Since changes in the suboxic zone thickness caused by intrusions likely result in oxidation of sulfide, sulfide inventories as well as those of sulfur intermediates are expected to vary spatially and temporally.

3.2. Sulfur intermediates

3.2.1. Thiosulfate and sulfite

As found previously by Hayes et al. (2006) and Percy et al. (2007), several distribution patterns were evident for thiosulfate and sulfite in the Cariaco (Fig. 3). During Jan 2006 (CAR-118), thiosulfate and sulfite profiles showed distinct maxima at the bottom of the station A suboxic zone. In May 2006 (CAR-122), thiosulfate and sulfite concentration maxima were observed again at station A, apparently within the suboxic zone. However, the maximum concentration of sulfite was twice that observed in CAR-118. The concentration of thiosulfate during CAR-122 at station A was comparable to the concentration of sulfite, while in all other situations, thiosulfate was typically half of the concentration of sulfite. During Nov 2006 (CAR-128), at station B, maxima in thiosulfate and sulfite concentrations were found at the bottom of the suboxic zone, but the amounts of thiosulfate/sulfite were much lower than seen during CAR-118 and CAR-122.

We also observed minima of thiosulfate and sulfite near the interface on a few occasions. For example, during CAR-118, there was a distinct decrease for thiosulfate and sulfite in the suboxic zone of station B, although this was the only occasion where we found minimum of thiosulfate and sulfite in the suboxic zone compared to concentrations in the oxic and anoxic zone. At station C, during the same cruise, a maximum of sulfite/thiosulfate was found at the top of the suboxic zone which was followed by a minimum at the base of the suboxic zone.

Finally, on several other occasions, no clear pattern was found around the interface. For example, the distribution of thiosulfate and sulfite at stations B and D (CAR-122), station A (CAR-128), stations A, B and D (CAR-132) fell into this category. Under these circumstances, the vertical distribution of sulfite/thiosulfate was irregular, with low concentration near the interface, and a tendency to increase with depth. The concentration of thiosulfate and sulfite for these occasions was always below 1 μmol L⁻¹ for most of the sampling depths.

3.2.2. Particulate elemental sulfur

A particulate elemental sulfur peak was consistently found within the redoxcline at all stations during all four cruises (Fig. 3). Maximum concentrations of elemental sulfur at each station are listed in Table 2 and ranged from a low of 0.22 μmol L⁻¹ at station C during CAR-118 to a high of 1.22 μmol L⁻¹ at station B during CAR-128. Particulate elemental sulfur was measured at stations A and B during all four cruises, and the highest sulfur peak was detected during CAR-128 at both stations (Nov 2006).

Fig. 3. Depth profiles of thiosulfate, sulfite and particulate elemental sulfur concentrations. From top to bottom: CAR-118, CAR-122, CAR-128, and CAR-132. Symbols and error bars represent means and 1 standard deviation of replicate samples. Note the scale change from cruise to cruise.
During CAR-118, 122 and 128, higher particulate elemental sulfur was always found in the suboxic zone of station B than other stations, possibly related to a higher sulfide source at this station (Fig. 4). During April 2007 (CAR-132), the elemental sulfur maximum was highest at station D, then station A, followed by station B. Station B is closer to La Tortuga Channel and more susceptible to effects of oxygen intrusion than station A. The suboxic zone of station B is always shallower and broader than station A (Percy et al., 2007; this work, Fig. 2), suggesting higher particulate organic flux and lateral intrusion of oxic water, respectively. However, in contrast to previous cruises, the upper boundary of sulfide at station B during CAR-132 was deep, even deeper than that of station A (Fig. 2), and the sulfide inventory was lower than the previous three cruises (Fig. 4). Chlorophyll a data derived from SeaWIFS (http://imars.marine.usf.edu/) revealed that in the two weeks before CAR-132, chlorophyll a concentrations at station D were 2–8 times of that at station B, while before CAR-122, the chlorophyll a concentrations at these two stations were similar. This result highlights the dynamic nature of the Cariaco Basin and is supplementary to the results of Percy et al. (2007) who found that higher inventories of sulfide were found in more productive areas.

### 3.2.3. Total zero-valent sulfur and particulate elemental sulfur

A more detailed analysis of the distribution of total zero-valent sulfur compared to particulate elemental sulfur was carried out at station A during CAR 132 (Fig. 5). Particulate elemental sulfur showed a sharp peak of 0.5 μmol L\(^{-1}\) at 290 m, which was the depth of first appearance of sulfide, but was almost undetectable below 400 m. In comparison, the peak in total zero-valent sulfur was broader, with a maximum observed at a shallower depth (270 m). Total zero-valent sulfur (which includes particulate elemental sulfur, the sulfane fraction of polysulfides and colloidal sulfur) was still detectable to 400 m, the deepest depth we sampled for this parameter. Particulate elemental sulfur made up from 2 to 63% of the total zero-valent sulfur. In the water below 305 m, most zero-valent sulfur (70–98%) was present in the form of...
polysulfides or colloidal sulfur since particulate elemental sulfur was almost undetectable (Fig. 5). Unfortunately, we can’t differentiate between polysulfides and colloidal sulfur with the data on hand at present.

3.3. Amendment experiment with thiosulfate and sulfite

Amendment experiments have been done on a number of CARIACO cruises to assess the role of thiosulfate and sulfite on chemosynthetic metabolism (Taylor, unpub. data). The result from CAR-132 station A is shown as an example here to demonstrate the effect of $S_2O_3^{2-}$ and $SO_3^{2-}$ on dark carbon fixation rates (Fig. 6). Thiosulfate stimulation was apparent in the deeper suboxic and upper anoxic zones. In contrast, in the upper suboxic and deeper anoxic zones, the stimulation effect by $S_2O_3^{2-}$ was marginal, suggesting that the organisms at these depths either are not using $S_2O_3^{2-}$ or are limited by other resources, e.g., oxidants. Stimulation of carbon fixation by sulfite at all sampling depths was minimal, suggesting that sulfite may not be an important substrate for chemosynthetic metabolism in the Cariaco Basin.

4. Discussion

4.1. Distributions of sulfur species in the Cariaco Basin

Oxygen in intruding water can oxidize H$_2$S, either directly or in reactions catalyzed by MnO$_2$/Fe$_2$O$_3$ to produce sulfur intermediates such as S$^2-_2$, S$^0$, S$_2$O$_3^{2-}$ and SO$_3^{2-}$ (Chen and Morris, 1972; Millero, 1991). Under situations where mixing was enhanced due to temporarily varying oxygen intrusion, elemental sulfur was by far the most abundant sulfur intermediate in Mariager Fjord (Zopfi et al., 2001). However, in some environments, thiosulfate can be the major intermedium oxidation product and can represent as much as 50% of all sulfur intermediates (in lake sediments, Jørgensen 1990a,b). Biological activity can also cause conversion of one sulfur species to another (Steudel, 1989). During the four cruises reported here, relative abundances of thiosulfate, sulfite and particulate elemental sulfur varied among stations and temporally.

4.1.1. Thiosulfate and sulfite

Thiosulfate/sulfite findings corroborate earlier results of Hayes et al. (2006) and Percy et al. (2007). It remains unclear why profiles of sulfite and thiosulfate do not consistently show either maxima or minima within the redoxcline. Sulfide oxidation is likely to be dependent on vertical mixing and lateral intrusions within the redoxcline and this variability is high both spatially and temporally (Fig. 2). Therefore, sulfur intermediates are expected to be dynamic, and high variability for thiosulfate and sulfite is likely.

The distribution of thiosulfate and sulfite in the Cariaco Basin probably reflects a balance between production (abiotic or biotic oxidation of sulfide) and consumption (chemical transformation or the reaction time for microbial population to access the resource). The extremely low thiosulfate near the suboxic zone at some stations is probably due to its value as substrate for bacteria (Jørgensen, 1990b). Our thiosulfate amendment experiments stimulated both cell growth rates (data not shown) and dark carbon dioxide fixation (Fig. 6).

Thus, the depth distribution of thiosulfate may reflect the high efficiency of its consumption (Thamdrup et al., 1994).

While there was no apparent dependence of thiosulfate/sulfite on sulfide (Table 3), sulfite concentration was highly correlated with that of thiosulfate ($R=0.93$, $n=106$, Table 3). This has been seen previously (Hayes et al., 2006; Percy et al., 2007). Sulfite concentrations were typically 2–3 times higher than thiosulfate (Fig. 3) although this varied somewhat (see CAR-122 station A data in Fig. 3). This strong relationship may suggest coupled production or inter- conversion of the two compounds (Thamdrup et al., 1994). As suggested by Jørgensen (1990b), the two compounds can be produced by sulfide oxidation at a certain ratio which depends on the oxidants and sulfide availability. Similar distribution patterns of thiosulfate and sulfite have been reported for the Black Sea (Vairavamurthy and Mopper, 1990), the sediments of a Danish salt marsh (Thamdrup et al., 1994), and previously in the Cariaco Basin (Zhang and Millero, 1993a; Hayes et al., 2006, Percy et al., 2007). Sulfite was not detected by the DTNP method in Framaren Fjord (where sulfite concentrations are millimolar) while thiosulfate was found to increase with depth (Millero, 1991).

4.1.2. Elemental sulfur

So far, most studies of elemental sulfur have been carried out in the sediments and very few measurements in anoxic water are available (Hastings and Emerson, 1988; Zopfi et al., 2001). In the present study, concentrations of particulate elemental sulfur were similar to previously reported values for the Cariaco Basin, in spite of the different sampling and analytical method used (Hastings and Emerson, 1988). Particulate elemental sulfur maxima near the redoxcline of the Cariaco were similar to the highest values in the Black Sea in spite of lower sulfide concentration (Jørgensen et al., 1991; Konovalov et al., 2003).

The inventory of particulate elemental sulfur is significantly correlated to sulfide inventory below the interface (250–340 m) (Fig. 4). Higher elemental sulfur inventories are always found in the more productive areas where there was higher sulfide, such as in stations B and D. This is consistent with the result of Zopfi et al. (2004), who found that the $S^0$ content was higher in an environment where the sulfate reduction rate and therefore the sulfide production was higher.

Table 3

<table>
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<tr>
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<th>CHEMO</th>
<th>CHEMO</th>
<th>BNP</th>
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<th>S$^0$</th>
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<tr>
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<td>-0.24*</td>
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<td>-0.04</td>
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<td>0.01*</td>
<td>0.02</td>
<td>0.93**</td>
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CHEMO: dark carbon fixation rate; BNP: bacterial net production; BAT: beam attenuation.
** Pearson product–moment correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
Seasonal variability of particulate elemental sulfur also may be due to temporal changes of water circulation patterns in the Cariaco. Over four cruises in different parts of the Basin, maximum particulate sulfur near the interface varied by a factor of 5 (Fig. 3, Table 2), with higher values of particulate elemental sulfur seen in November at both stations A and B. Preliminary interpretation of Acoustic Doppler Current Profiler data suggests that currents are lowest during winter and reach their maximum value during late summer (Charles Flagg, pers. comm.). These currents seem to be associated with a gyre in the Basin which could result in Ekman transport of O2-containing water from the sill into the suboxic zone. If this is the case, then variations in S0 may be explained by greater pumping of oxidants into suboxic zone during summer and fall (CAR 128) (Wang et al., 2008).

Elemental sulfur is an important product of both chemical and biological sulfur oxidation. From the similarity of sulfide oxidation rates in poisoned and unpoisoned water samples, Sorokin (1972) concluded that bacteria played no significant role in the initial step of sulfide oxidation to elemental sulfur. However, kinetics of sulfide oxidation in water samples from the redoxcline of Solar Lake, where sulfide fluxes were high enough to sustain a large population of sulfide-oxidizing bacteria, demonstrate the strong involvement of bacteria in elemental sulfur formation (Jørgensen et al., 1979). Under in situ conditions in the Black Sea, chemical oxidation of sulfide appeared to be more important, but when amended with bacterial isolates with a modest density (ca. 10^4 mL^-1), biological sulfide oxidation could compete successfully with spontaneous chemical oxidation (Jannasch et al., 1991). Intracellular pools of sulfur granules from chemosynthetic sulfur bacteria can also be extracted using our protocol of particulate element sulfur. Steudel (1989) found that elemental sulfur can be produced by sulfide-oxidizing bacteria, and is present as intracellular storage products. However it is not possible with present data to tell whether the observed particulate S fraction represents intracellular granules or extracellular inorganic S (O). Based on the fact that particulate elemental sulfur is only a fraction of total zero-valent sulfur, we speculate that the particulate sulfur pool we quantified is not all intracellular. Another piece of evidence supporting this speculation is that total bacterial abundances in the redoxcline of the four cruises do not correlate with particulate elemental S (data not shown). Further investigation using environmental SEM and elemental sulfur isotope probes would help to clarify this question.

Several oxidants, such as O2, MnO2 or FeO2, could be used to oxidize sulfide (chemically or biologically) near theoxic–anoxic interface to produce elemental sulfur. For example,

\[ \text{MnO}_2 + \text{HS}^- + 3\text{H}^+ \rightarrow \text{Mn}^{2+} + \text{S}^0 + 2\text{H}_2\text{O} \] (1) 

\[ 2\text{FeOOH} + \text{HS}^- + 5\text{H}^+ \rightarrow 2\text{Fe}^{2+} + \text{S}^0 + 4\text{H}_2\text{O} \] (2) 

In the Black Sea, oxidized Mn species have a significant relationship with the sulfur concentration near the interface. Konovalov et al. (2003, 2006) argued that, instead of oxygen, the particulate Mn was the direct oxidant of sulfide in the western Black Sea. Also in the Black Sea, dissolved Mn (III) complexes were shown to account for most of the oxidizing power (Trouwborst et al., 2006; Yakushev et al., 2007). Unfortunately, we do not have particulate Mn/Fe and Mn (III) data to test these hypotheses in the Cariaco Basin. However, estimates of upward fluxes of dissolved Mn and Fe are not strongly correlated with elemental sulfur concentrations (data not shown).

Elemental sulfur has been reported to be the main product of sulfide oxidation with Mn (IV) (Burdige and Nealson, 1986) (Eq. (1)), although at high abundances of Mn (IV) relative to H2S, thiosulfate and even sulfate become the main products (Yao and Millero, 1995). Compared to Mn (IV), Fe (III) is a weaker oxidant and oxidation of sulfide completely to sulfate is marginally thermodynamically favored (Aller and Rude, 1988). During the reaction of sulfide with Fe (III), elemental sulfur is produced first (Eq. (2)) (Luther et al., 1991). In the Cariaco Basin and Black Sea, dissolved Fe concentrations are similar, but dissolved Mn in the Black Sea is an order of magnitude higher than that in the Cariaco (Lewis and Landing, 1991; Percy et al., 2007). Thus the quantity of Mn/Fe oxidant may only be sufficient to oxidize sulfide to elemental sulfur in the Cariaco, possibly resulting in a greater role for this species.

4.1.3. Total zero-valent sulfur and particulate elemental sulfur

As mentioned earlier, measured total zero-valent sulfur includes particulate elemental sulfur, colloidal sulfur and the sulfane part of polysulfides. Quantification of both total zero-valent sulfur and particulate elemental sulfur during CAR 132 allows us to assess the potential significance of colloidal sulfur and polysulfides in the Cariaco.

In sulfidic waters, the solubility of elemental sulfur can be greatly increased by its reaction with sulfide to form polysulfides (Chen and Gupta, 1973):

\[ \text{HS}^- + (n-1)\text{S}^0 \rightarrow \text{S}_n^{2-} + \text{H}^+ \] (3) 

Sulfur speciation data from Rogoznica Lake (Croatia) obtained using a voltammetric technique support the hypothesis that at depths above the oxic–anoxic interface, a part or even all elemental sulfur is in the form of soluble or colloidal sulfur while in deeper layers, when sulfide concentrations are several times higher than that of elemental sulfur, zero-valent sulfur is probably in the form of polysulfides (Ciglenečki et al., 1996). In Solar Lake (Jørgensen et al., 1979), particulate element sulfur peaked near the oxic–anoxic interface while in deeper waters, constant and high polysulfide S0 concentrations (around 100 μmol L^-1) were observed. In the Black Sea, polysulfides were observed at the base of the suboxic zone (Glazer et al., 2006). Zopfi (pers. comm.) examined partitioning of elemental sulfur between particulate and dissolved fractions in the Mariager Fjord’s redoxcline where concentrations of particulate elemental sulfur and total sulfur were highest, and found that particulate elemental sulfur (trapped on 0.2 μm polycarbonate filter) only accounted for 30–40% of the total sulfur, with the rest appearing in the filtrate as either colloids or polysulfides. In the Cariaco redoxcline, particulate sulfur:total sulfur ratios varied from 0.1 to 0.6. In other environments where the sulfide concentration is low, elemental sulfur is typically found in the particulate or colloidal phases (Troelsen and Jørgensen, 1982).

To estimate polysulfide speciation in Cariaco water, we used in situ pH, temperature and sulfide concentrations from CAR 132 to calculate thermodynamically the concentration of...
each polysulfide ($n=2–8$) (Table 4). The calculated concentrations are based on the assumption that our system is saturated with elemental sulfur and therefore the activity of elemental sulfur is assumed to be 1. The equilibrium constant for Eq. (3) thus is given by

$$K_n = \frac{\{S_{n}^{2-}\} \cdot \{H^{+}\}}{\{HS^{-}\}^n}.$$  \hspace{1cm} (4)

Then we can calculate the concentration of each polysulfide as:

$$[S_{n}^{2-}] = [HS^{-}] \cdot K_n / [H^{+}]$$  \hspace{1cm} (5)

We used the thermodynamic constants from Kamyshny et al. (2007). Our calculations indicate that in Cariaco, at all depths below the first appearance of sulfide, sulfide concentration is high enough to support polysulfide formation (Table 4). Consistent with observation, at 290 m where sulfide first appears, approximately 60% of the total elemental sulfur was calculated to be present as particulate elemental sulfur, but at depths of higher sulfide, the particulate S0 fraction decreases and most elemental sulfur should be present in the form of polysulfides.

We can test the assumption that our system is saturated with elemental sulfur by comparing calculated and measured polysulfide. Here, we define potential maximum concentration of polysulfides as the difference between measured total zero-valent sulfur and particulate elemental sulfur. Column J4 in Table 4 summarizes the amount of zero-valent sulfur in calculated polysulfides ($ln S_{n}^{2-}$ there is one S0 atom, in $S_{n}^{2-}$ there are two S0 atoms, and so on). When we compare this value to the potential maximum concentration of polysulfides, we find that, in the upper 290 m, our system is in equilibrium with elemental sulfur, while below 305 m the sum of zero-valent sulfur for calculated polysulfides is more than the measured potential maximum concentration of polysulfides. Therefore, our system is likely not to be saturated with S0 below ca. 305 m.

We need to point out that only limited thermodynamic data are available for polysulfide speciation (Kamyshny et al., 2007). We cannot be certain that constants picked here are representative of the Cariaco Basin. Therefore, the thermodynamic calculations are meant to provide a general idea of the form of sulfur rather than a rigorous quantitative assessment. Direct measurement of specific polysulfides is problematic at this moment since the detection limit is relatively high (ca. 1 μmol L$^{-1}$ when expressed as S0 (Kamyshny et al., 2006)), compared to the potential maximum concentration of polysulfide in the Cariaco (ca. 0.5 μmol L$^{-1}$).

If zero-valent sulfur is present in the deep anoxic waters, it could act as a potential oxidant in the deep water column, or even in the sediment. Based on the sulfur isotope value of organic sulfur compounds in Cariaco sediment, Werne et al. (2003) suggested that organic matter sulfurization might be taking place via sulfur intermediates, such as elemental sulfur. Our data support the possibility of the existence of a water column source of elemental sulfur to the sediment.

4.2. Sulfur species and chemoautotrophic production

We are aware of the fact that the redoxcline of anoxic basins harbors a tremendous diversity of marine microbes and different functional groups of bacteria, archaea, and protists arise from this diversity to dominate various habitats and drive important biogeochemical cycles. Exploration of the distribution of microbial taxa and possible microbial interactions should be encouraged when exploring the relatively high rates of chemoautotrophic production for future research. In this communication, based on the depth distribution and relationship of particulate elemental sulfur with chemoautotrophic production and heterotrophic bacterial production, we want to discuss elemental sulfur form as a potential substrate for chemoautotrophic bacteria, a process which has not been considered in Cariaco previously.

Chemoautotrophic and heterotrophic bacterial production at a particular depth covaried strongly with concentration of particulate elemental sulfur at that depth, but not with thiosulfate or sulfite concentrations (Table 3). The position and the shape of the peak of chemoautotrophic production, heterotrophic bacterial production and particulate sulfur are generally well in CAR-118, CAR-122 and CAR-132 (Fig. 7). However, in CAR-128, the chemoautotrophic production was lower than other dates (note the scale of dark carbon fixation for CAR-128 is three times lower than for other cruises, Fig. 7).

Based on the depth distribution and relationship with chemoautotrophic production and heterotrophic bacterial production, we speculate that elemental sulfur is an important substrate for chemoautotrophic bacteria around the oxic–anoxic interface and upper anoxic zone. One possible reaction coupling chemoautotrophic production and elemental sulfur

<table>
<thead>
<tr>
<th>Measured parameters</th>
<th>Calculated polysulfides (μmol L$^{-1}$)</th>
<th>S(0)$^{\text{a}}$ (μmol L$^{-1}$)</th>
<th>S(0)$^{\text{a}}$ saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (m) Sulfide (μmol L$^{-1}$) Particulate S0 (μmol L$^{-1}$) Total S0 (μmol L$^{-1}$) pH Temperature (°C)</td>
<td>S2 S3 S4 S5 S6 S7 S8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>260 0.0 0.01 0.68 7.65 17.7</td>
<td>0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
<td>0.00</td>
<td>Yes</td>
</tr>
<tr>
<td>270 0.0 0.22 1.24 7.65 17.7</td>
<td>0.00 0.00 0.00 0.00 0.00 0.00 0.00</td>
<td>0.00</td>
<td>Yes</td>
</tr>
<tr>
<td>280 0.0 0.05 0.77 7.65 17.7</td>
<td>0.03 0.02 0.00 0.00 0.00 0.00 0.00</td>
<td>0.34</td>
<td>Yes</td>
</tr>
<tr>
<td>290 1.1 0.51 0.80 7.65 17.7</td>
<td>0.14 0.11 0.09 0.02 0.00 0.00 0.00</td>
<td>1.76</td>
<td>No</td>
</tr>
<tr>
<td>305 5.9 0.16 0.58 7.65 17.7</td>
<td>0.23 0.18 0.26 0.14 0.04 0.01 2.87</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>320 9.7 0.11 0.75 7.65 17.7</td>
<td>0.26 0.20 0.30 0.16 0.04 0.01 3.28</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>340 11.0 0.06 0.60 7.65 17.7</td>
<td>0.46 0.35 0.53 0.29 0.07 0.02 5.82</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>400 19.6 0.01 0.50 7.65 17.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ Maximum theoretically possible S (0) in all polysulfide species.
is sulfur disproportionation. Under standard conditions, the \( \Delta G^0 \) value of elemental sulfur disproportionation is positive, while the \( \Delta G^0 \) value of thiosulfate/sulfite disproportionation is negative (Rabus et al., 2006). However, environmental concentrations are substantially lower than the 1 M standard values. We used pH measured during monthly CARIACO cruises, \([SO_4^{2-}]\) concentration measured gravimetrically (Li, unpublished data), and measured dissolved sulfur intermediate concentrations to recalculate the energy yield of sulfur intermediates disproportionation under in situ conditions based on Eqs. (6)–(8). For simplicity, we assume that the concentrations of \( S_0, S_2O_3^{2-}, SO_3^{2-} \) and \( HS^- \) are all 1 \( \mu \)mol L\(^{-1}\), which is reasonable considering typical conditions near the interface (Figs. 2 and 3). Under these circumstances, the energy yield of sulfur intermediate disproportionation turns out to be:

\[
4SO_2^- + 3H^+ \rightarrow 3SO_3^{2-} + HS^- \quad \Delta G^0 = -29.6 \text{kJ mol sulfite}
\]

\[
S_2O_3^{2-} + H_2O \rightarrow SO_3^{2-} + HS^- + H^+ \quad \Delta G^0 = -72.8 \text{kJ mol thiosulfate}
\]

\[
4S^0 + 4H_2O \rightarrow SO_3^{2-} + 3HS^- + 5H^+ \quad \Delta G^0 = -70.2 \text{kJ mol sulfur}
\]

These calculations show that at the in situ conditions of the Cariaco redoxcline, the energy yield of elemental sulfur disproportionation is similar to that of thiosulfate, but is 2–3 times higher than sulfite, although all three reactions are thermodynamically favorable. The lower yield for sulfite may explain the minimal stimulation observed in enrichment experiment with sulfite. These results suggest that elemental sulfur might be as important a substrate for chemosynthetic production as thiosulfate. Other workers have suggested that sulfur disproportionation should occur preferentially where the sulfide concentration is maintained at low levels (less than 1 \( \mu \)mol L\(^{-1}\)) either by oxidation with \( O_2 \) or by reaction with \( MnO_2 \) and FeOOH (Thamdrup et al., 1993). However, further calculation with the in situ conditions in the Cariaco Basin indicated that even when sulfide concentration is 10 \( \mu \)mol L\(^{-1}\), the energy yield of disproportionation of sulfur intermediates does not change much \((S_2O_3^{2-} \rightarrow S^0 \rightarrow SO_3^{2-})\). Therefore, from the redoxcline to the upper anoxic zone, where we always observe maxima of both elemental sulfur and chemosynthetic production, and occasionally maxima in \( SO_3^{2-} \) and \( S_2O_3^{2-} \), disproportionation of sulfur intermediates is always thermodynamically favored.

We do not yet understand why the elemental sulfur concentrations were more closely related to chemosynthetic production than were concentrations of thiosulfate. Based on the stoichiometry, the measured quantity of particulate \( MnO_2 \) and FeOOH (Percy et al., 2007) is only adequate to oxidize sulfide to zero-valent sulfur, but not further to thiosulfate or sulfite. It is likely that there is a metal/sulfur redox shuttle
operating in our system where dissolved sulfide could diffuse up to oxic/suboxic water and rapidly get oxidized by either O₂ or metal oxidants (MnO₂ and Fe(OH)₃), forming particulate or colloidal zero-valent sulfur, which would then sink across the interface and be utilized by chemautotrophic bacteria. This mechanism permits deeper and faster penetration of potential oxidant into the chemoclone than diffusion alone and could be associated with repetitive cycling of the redox pairs. Direct kinetic measurements at sea with radiotracer technique under controlled conditions might help resolve this possibility.

Although we don’t have direct evidence for sulfur disproportionation in the Cariaco system, microorganisms whose energy metabolism is based on the disproportionation of inorganic sulfur compounds have been found to be numerically abundant in some similar environments (Jørgensen, 1990a). Disproportionation of elemental sulfur in pure cultures was first reported by Bak and Cypionka (1987). In the Mariager Fjord, Ramsing et al. (1996) pointed out that elemental sulfur could be an important sulfur intermediate with a high turnover rate. Based on stable isotope mass balances, Sørensen and Canfield (2004) indicated that half of the sulfide is oxidized to either elemental sulfur or thiosulfate and subsequently disproportionated. To date, enrichment cultures from the Cariaco Basin have yielded microaerophilic thiosulfate-oxidizers, sulfur and thiosulfate disproportionators, thiosulfate-oxidizing manganese reducers and denitrifying thiosulfate-oxidizers (Madrid et al., 2001; Lin, unpub. data). However, there is a variety of pathways involving sulfur, metal oxides and nitrogen species which are possible, and should also be further investigated.

5. Conclusion

The concentrations of several sulfur intermediates have been determined in the Cariaco water column, including particulate elemental sulfur, zero-valent sulfur, thiosulfate and sulfite. Results showed that the concentrations of sulfur intermediates near the interface vary with space and time, and thiosulfate and sulfite profiles near the interface lack a reproducible distribution pattern, likely reflecting the dynamic and complicated nature of the Cariaco Basin. In contrast, a particulate element sulfur peak was consistently observed near the interface. Based on the distribution profile and relationship analysis between sulfur species and chemautotrophic production, we postulate that elemental sulfur is important in supporting the chemoaautotrophic bacterial near the interface and upper anoxic zone.

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References