

Great Lakes Environmental Research Lab in Ann Arbor, MI
Vacant
Contact Scott Mowery for further assistance
301-713-8204 x120
E-Mail: Scott.Mowery@noaa.gov

AREA: Minnesota, Wisconsin, Michigan, Pennsylvania, and New York

Data submitted by MI Scranton and GT Taylor
Marine Sciences Research Center
Stony Brook University
Stony Brook NY 11794-5000
631-632-8735 (MIS; mscranton@notes.cc.sunysb.edu)
631-632-8688 (GTT); gtaylor@notes.cc.sunysb.edu
631-632-8820 (fax)

Methods:

Sampling: All samples are collected in standard 8 or 12-L Niskin bottles. For samples in and below the oxycline, an N₂ line is attached to the upper air vent to prevent air from entering the bottle during sub-sampling. Samples for live analysis are first transferred without headspace to a 1L glass sample bottle with Teflon standard taper stopper. In the ship's lab, sub-samples are transferred to 25 or 40 ml incubation vials, also under N₂. All vials are filled from the bottom with overflow of about 3 vial volumes and then sealed with no headspace.

Fatty acid uptake rate constants: Fatty acid measurements have been discontinued. Contact Mary Scranton for further information.

CH₄: CH₄ is assayed by gas chromatography using the vial equilibration technique of Johnson et al. (1990) an HP 5890GC. The GC was calibrated for each run using two standards, one containing 9.98 ppmv CH₄ in nitrogen and the other containing 999.3 ppmv CH₄ in nitrogen. The two point calibration yields a slightly higher concentration (by about 15%) for deep water values, than data reported prior to CAR 108. For more information contact Scranton. Samples are poisoned by addition of 10N KOH solution at a rate of 200 l per 50 ml vial.

H₂S: Seawater samples for sulfide were collected without bubbles by placing the tip of a gas-tight syringe below the surface of water flowing upward through a 60 ml plastic syringe barrel which had been attached to the Niskin bottle by a 60 cm length of Tygon tubing. Samples are injected into vials containing Zn-acetate or Zn-chloride (50 mM). Samples were chilled on the ship and stored refrigerated in the dark until analysis. Upon return to the laboratory, the ZnS is dissolved and is analyzed spectrophotometrically by the method of Cline (1969). Based on the relative standard deviation of triplicate samples, the precision of the field samples during analysis was $\pm 3.3\%$. The detection limit for sulfide analyses was 0.59 micromolar, calculated as six times the standard deviation of quintuplicate blanks. While standard curves appeared linear over a large concentration range as determined by a high correlation coefficient (greater than 0.98), close inspection showed they were consistently convex over the entire concentration range (from 0 to ~50 micromolar). Therefore, we matched concentrations of standards to those in samples and kept total absorbance low to optimize analysis for the concentration range expected in the Cariaco Basin. We used a linear calibration equation to be consistent with previous sulfide

measurements. We did not subtract a reagent blank from the samples. In some cases, this has lead to small positive values (~1 micromolar) in the suboxic zone.

Sulfite and thiosulfate: Seawater samples for thiosulfate and sulfite analyses were collected as for sulfide in triplicate without bubbles by placing the tip of a gas-tight syringe below the surface of water flowing upward through a 60 ml plastic syringe barrel which had been attached to the Niskin bottle by a 60 cm length of Tygon tubing. Samples were analyzed using the method of Vairavamurthy and Mopper (1990) as modified by Hayes et al. (2006). Ten-milliliter water samples were collected from Niskin bottles as described above and were transferred within seconds into a glass serum vial containing 0.5 ml sodium acetate buffer (0.2 M). All reaction vials were prepared in advance at the shore-based laboratory by adding buffer, flushing with argon and crimp sealing for transport to the field. To minimize oxidation, the derivatizing agent (5 mM 2,2'-dithiobis(5-nitro) pyridine in acetonitrile) was added within seconds of dispensing seawater into serum vials. Derivatization was allowed to proceed for 5 min, after which water was passed through preconditioned Waters SepPak tC18 Solid Phase Extraction (SPE) cartridges. Cartridges were preconditioned immediately before use with 5 ml methanol, 5 ml distilled water, and 5 ml of a mixture of 20 mM sodium acetate and 10 mM tetrabutylammonium hydrogen sulfate (TBAHS). Samples on cartridges were kept in a cooler on deck until the cast was completed. Upon returning to the local laboratory, cartridges were purged with argon and frozen until analysis. Frozen samples are typically thawed for about 10 minutes prior to elution.

Upon return of the samples to Stony Brook, thiosulfate and sulfite derivatives were eluted from cartridges with methanol and 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. Mobile phases for analysis were (A) 100% acetonitrile and (B) a solution of 0.05 M sodium acetate and 7.5 mM TBAHS adjusted to pH 3.5 ± 0.03 . The gradient for this method was 1 min with 10% B followed by a gradient to 34% B at 9 min, to 55% B at 23 min, to 100% B at 28 min, continued elution with 100% B for 2 min, then a gradient back to 10% B at 32 min and to 0% B at 40 min. Absorbance of the derivatives was measured at 320 nm.

Lab blanks (10 ml of distilled water, buffer and 0.05 ml of the derivatizing agent (DTNP)) were concentrated through preconditioned SepPak cartridges and analyzed in the same manner as field samples and were 0.07 ± 0.04 micromolar for sulfite and 0.3 ± 0.1 micromolar for thiosulfate. The analytical detection limit (6x the standard deviation of five laboratory blanks) was 0.3 micromolar for sulfite and 0.6 micromolar for thiosulfate. Field blanks were much higher and are assumed to be lower than the lowest measured sample in a given cast. Upper estimates of the true blanks are the lowest thiosulfate and sulfite value which were measured during a particular cruise and are 0.3 micromolar and 0.5 micromolar, respectively. The precision of analysis (relative standard deviation of 5 replicates of a 10 micromolar standard) for thiosulfate and sulfite was $\pm 2.2\%$ and $\pm 1.6\%$ respectively.

Particulate elemental sulfur: Duplicate particulate elemental sulfur samples were acquired by gravity filtering directly from the Niskin bottles and were analyzed by modification of the method of Trouwborst (2005). Filter holders loaded with 0.2 micrometer pore 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 degrees C until extraction in the lab.

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 hours on a mechanical shaker and the elemental S concentration of the 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 of Supelco. A 20 microliter of sample was injected to the chromatograph using 100% methanol as the mobile phase with a pump speed of 1 ml/min. This resulted in a retention time of the elemental sulfur peak at about 2.2 minutes. Standard solutions were made by dissolving sulfur powder in methanol. Elemental sulfur was detected at 226 nm, with a detection limit of about 1 micromolar, and a precision of 0.5% relative standard deviation. This procedure gave a linear calibration curve in the range 1-100 micromolar.

Microbial census: Abundances of remineralizers (bacteria) and regenerators (flagellates) are determined using microscopic censuses. Preserved samples (2% formaldehyde) are stained with a fluorochrome (DAPI or acridine orange) and captured on the appropriate porosity Nuclepore membrane (0.2 or 0.8 µm). Filter-retained cells are enumerated and sized by epifluorescence microscopy according to Taylor et al. (1986). Larger, less abundant protozoa are enumerated on settled samples using inverted microscopy.

Bacterial production: Bacterial incorporation is measured using 3H-leucine incorporation as described by Kirchman (1993). Triplicate samples are incubated for 10-12 h in gas-tight screw-top vials to minimize alteration of the redox potential. Time course experiments have confirmed that uptake is linear for at least 15 h. Due to the fact that some important anaerobic bacteria appear to not take up exogenous thymidine under anoxic conditions (McDonough et al. 1986; Gilmour et al. 1990), the more common method of Fuhrman and Azam (1982) is inappropriate for this system.

Bibliography

- Cline, J.D. (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanogr.*, 14, 454-458.
- Doddema, H.J. and G.D. Vogels (1978) Improved identification of methanogenic bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.*, 36, 752-754.
- Fuhrman, J.A. and F. Azam (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar. Biol.*, 66, 109-120.
- Gilmour, C.C., M.E. Leavitt and M.P. Shiaris (1990) Evidence against incorporation of exogenous thymidine by sulfate-reducing bacteria. *Limnol. Oceanogr.*, 35, 1401-1409.
- Hayes, M.K., G.T. Taylor, Y. Astor and M.I. Scranton (2006) Vertical distributions of thiosulfate and sulfite in the Cariaco Basin. *Limnol. Oceanogr.* 51, 280-287.
- Johnson, K.M., J.E. Hughes, P.L. Donaghay and J.McN. Sieburth (1990) Bottle calibration static headspace method for the determination of methane dissolved in seawater, *Anal. Chem.*, 62, 2408-2412.
- Kirchman, D.L. (1993) Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp, P.F., B.F. Sherr, E.B. Sherr and J.J. Cole (eds.) *Handbook of Methods in Aquatic Microbial Ecology*, Lewis Publ., Boca Raton, pp., 509-512.

McDonough, R.J., R.W. Sanders, K.G. Porter and D.C. Kirchman (1986) Depth distribution of bacterial production in a stratified lake with an anoxic hypolimnion. *Appl. Environ. Microbiol.*, 52, 992-1000.

Taylor, G.T., D.M. Karl and M.L. Pace (1986) Impact of bacteria and zooflagellates on the composition of sinking particles: an in situ experiment. *Mar. Ecol. Prog. Ser.*, 29, 141-155.

Taylor, G.T., M.I. Scranton, M. Iabichella, T.-Y. Ho, R.C. Thunell, F. Muller-Karger & R. Varela (2001) Chemoautotrophy in the redox transition zone of the Cariaco Basin: A significant midwater source of organic carbon production. *Limnol. Oceanogr.* 46: 148-163.

Trouwborst, R. E. (2005) Geochemistry of Mn and Fe across both stable and dynamic natural oxic-anoxic transition zones. Ph.D. Dissertation, University of Delaware, Delaware, USA.

Tuttle, J.H. and H.W. Jannasch (1973) Sulfide and thiosulfate-oxidizing bacteria in anoxic marine basins. *Mar. Biol.*, 20, 64-70.

Vairavamurthy, A. and K. Mopper (1990) Determination of sulfite and thiosulfate in aqueous samples including anoxic seawater by liquid chromatography after derivatization with 2,2'-Dithiobis(5-nitropyridine). *Environ. Sci. Technol.* 24, 333-336.