Dissolved organic carbon and bacterial during Antares 4 cruise

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Experimental

Field sampling

Discrete seawater samples were collected by using a Seabird SBE 9 rosette sampler equipped with 24 12 dm3 Niskin bottles from the *RV Marion* Dufresnes in Southern Ocean at a series of stations across the frontal areas (Fig. 1, Table 1) which were initially located by temperature and salinity intensive study. At the beginning of the cruise, Niskin bottles were cleaned with HCl 0.2% and distilled water. Plastic o-rings were replaced by Viton material whereas original plastic ribbons were replaced by steel spring and silicone ribbons to avoid organic contamination. Samples were not filtered and were drawn as soon as possible after the rosette sampler was on the deck of the ship (before any other sampling). The 10 ml samples were collected in duplicate in precombusted (450° C, at least 6 hours) glass ampoules (Wheaton®). All ampoules were poisoned by addition of $HgCl_2$ (10 mg l^{-1} final conc.) immediately after filling of ampoules, flame-sealed, and stored in the dark for later analyses at the shore laboratory within 5 months. As particulate organic carbon (POC) usually accounted from less than 10 % in surface waters in oligotrophic waters and probably less than 5 % in deeper waters, DOC comprized most of TOC. Duplicate TOC were averaged for further calculations. Samples for bacterial production (BP) and bacterial counting were collected by using black polycarbonate without external tubing immediately after TOC sampling. Then, samples were immediately processed in the laboratory onboard.

TOC:HTCO analysis

The Shimadzu instrument used in this study was the commercially available Model TOC-5000 Total Carbon Analyzer with quartz combustion column filled with 1.2% Pt on silica pillows with approximate diameter of 2 mm (*Cauwet*, 1994). Several aspects of our modified unit have been already described (Yoro et al., 1997; 1999 ; Dafner et al., 1999; 2000). Briefly, the furnace temperature was maintained at 680^{O}C and the effluents passed through a mercury trap (gold wire) to remove mercury (Ogawa and Ogura, 1992). A magnesium perchlorate water trap has been added to the system located before the halogen scrubber, and an in-line membrane filter and the non-dispersive infrared CO_2 detector. Prior to analysis, sub samples were acidified with 10 µl of 85% H_3PO_4 to a pH ~2 and sparged for 10 min with CO_2-free pure air at a gas flow rate of 40 ml min^{-1} to remove inorganic carbon as CO_2. TOC contamination from the preservation reagent and from H_3PO_4 was below the detection limit. A hundred µl injections were repeated 3-4 times for each sample, the analytical precision of the procedure being within 3%, on average. Some variability in values from two different vials gives rise to a lower overall precision (10 %).

To bring the blank down, the catalyst was pre-treated by washing in 1% HCl and gently rinsed with DW, and dried in a furnace at a temperature of about 450^{O}C for 10-15 min. Prior to analyses of standards and samples, the catalyst bed was 'conditioned' (during 1-3 days) by injecting 100 µl of acidified and sparged water from a high-quality water purifier, a Millipore Milli-Q Plus® System (hereafter DW), until the lowest stable integrated area was obtained. After two seawater sample injections the column was flushed by three injections of 100 µl of Milli-Q water. The catalyst was regenerated by using the TC catalyst function of the instrument once a week. Most of the time and after every two weeks of 25 daily samples injections, top of the catalyst (2 cm) was replaced was fresh material. The catalyst being completely renewed every three weeks.

Calibration of the instrument

Standardisation was carried out every day using potassium hydrogen phthalate (Kanto Chemical Company, Inc.) dissolved in DW prepared just before sample analyses. The instrument response factor, measured as the slope of the standard addition to DW (r^2>0.999 for 19 runs), remained relatively constant and reproducible over the time of analysis. Calibration curves have exhibited little difference in the slope (av. slope: 6022±217 area units, n=19) and intersect (av. intersect: 765±92 area units, n=19).

The accuracy and the system blank of our instrument were determined by analysis of the Deep Atlantic Water (DAW) reference and low carbon water (LCW) from ampoules provided by Denis Hansell. The average DOC concentration in the DAW reference and in the LCW were 44±2 μM C, n=21 and 3±1 μM C, n=21, respectively. Carbon level of LCW ampoules was similar and often higher than DW produced from our laboratory unit. TOC concentration in samples was calculated by averaging all replicate injections of samples, subtracting the average DW value as the total blank and dividing by the slope of the standardisation curve.

Bacterial production (BP) measurements

Bacterial production was estimated by the ^{3}H-Leucine method (Kirchman et al, 1986; Kirchman, 1993). At each depth, duplicate samples and a control were incubated with 3 nM L-[4,5 ^{3}H]-Leucine (specific activity 136 Ci mmol^{-1}, Amersham) + 30 nM non radioactive Leucine. Samples were incubated in the dark, at *in situ* temperature (± 1 °C). Preliminary experiments demonstrated linear uptake of 3H-leucine for at least 2 hours in surface waters, 6 hours between 60 and 200 m and 12 hours below. The incubations were stopped by addition of formalin (1% final concentration). Samples killed with 1% formalin prior to isotope addition were used as control blanks. Following the incubation, the samples were filtered through 0.2 μm cellulose filters (Millipore GSWP), extracted with 10 ml 5% trichloroacetic acid (TCA), rinced three times with 5% TCA and once with 80% ethanol. The filters were dissolved in 1 ml ethyl acetate and after 10 min, 10 ml of scintillation coktail (PCS Amersham) was added.

The radioactivity was analyzed by a Packard Liquid scintillation counter, and corrected in TSIE mode with a quenched standard curve. We performed concentration kinetic experiments to verify that the total concentration of Leucine added (33 nM) was sufficient to saturate incorporation and, consequently, to check that isotopic dilution would be negligible. For this purpose, we added constant concentrations of labelled leucine (1.9 nM) and five different concentrations of non radioactive leucine (0 to 60 nM). The plot of T/f (radioactive Leucine incorporated per unit time) against leucine concentration allows the calculation of an index of isotopic dilution (X intercept) and the Vmax (inverse of the slope, Pollard & Moriarty, 1984, Kirchman et al, 1986). With the above concentration used we found no isotopic dilution in the samples (average ID = 1.1 ± 0.1, n=4). Bacterial production was calculated according to Kirchman (1993) from ^{3}H-leucine incorporation rates with our actual isotopic dilution factor. The coefficient of conversion was thus set at 1.54 ngC per pmole leucine incorporated.

Recent references in relation with TOC dynamic

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