

Changes in major carbon components during a fertilization experiment with surface water from the Central Indian Ocean

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Shipboard iron- and macronutrient-addition experiment was conducted in the Central Indian Ocean, to investigate the variations of carbon in different forms and its records for plankton activities. Experiments were conducted in four 500-L acrylic barrels with the surface seawater near 24°S, 70°E. The concentration of chlorophyll a, dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and particulate organic carbon (POC) were measured. DOC concentration had a dramatic increase when phytoplankton bloom, which is caused by the excretion of blooming phytoplankton. Another maximum of DOC concentration occurred after phytoplankton bloom corresponds to the bacterial degradation of organic matters or the excretion of phytoplankton. The decrease of DOC concentration following the two maxima might result from the absorption of DOC by bacteria. The increasing trends of POC before phytoplankton bloom were consistent with that of DOC and chlorophyll a, indicating that phytoplankton growth dominated the plankton activities. The variation of POC concentration just after bloom was controlled by the decomposition of phytoplankton and the growth of bacteria. However, POC concentration increased at the end of the experiment, which might be caused by the growth of bacteria with the utilization of DOC. The present study infers that the carbon records, particularly POC and DOC, in the seawater are indicative to plankton activities.

[Keywords: fertilization experiment, carbon record, plankton activities, Indian Ocean]

Introduction

Dissolved organic carbon (DOC), particulate organic carbon (POC) and dissolved inorganic carbon (DIC) are the three largest reservoirs of reactive carbon in surface seawater, which play crucial roles in the global geochemical cycle of carbon. The major source for DOC in seawater is biological and related to primary productivity¹⁻⁷. Most of POC exists in the body of plankton and it represents an important food source for planktonic consumers⁸. DIC is one of the most important factors utilized in course of photosynthesis.

Many shipboard fertilization experiments have been carried out in the Indian Ocean, which is an important and effective method to promote plankton growth. Scientists have made great progress in marine biology, geochemistry and ecology in previous studies⁹⁻¹⁶. In the Indian sector of the Southern Ocean, carbon intake by zooplankton⁹, particulate biogenic silica and carbon production rates¹⁰ and quantification

of algal iron requirements¹¹ have been studied as well as the feeding, respiration and egg production of copepods¹² and bacterial degradation of large particles¹³. The nitrogen assimilation was measured using incubation techniques in the northwestern Indian Ocean¹⁴. In the kinetic incubation experiments, Statham *et al*¹⁵ got the dissolved Fe (II) oxidation rates in hydrothermal plumes at the Kairei and Edmond vent sites. Recently, microzooplankton grazing on phytoplankton was investigated in eastern Indian Ocean¹⁶. Most of these shipboard experiments in Indian Ocean lasted no more than four days, and the study on the plankton activities after phytoplankton bloom was scarce. Furthermore, the sampling spots were mainly distributed in sub Antarctic area.

The present study emancipates the hypothesis that the changes in major carbon components in surface seawater record plankton activities. A long-term fertilization experiment was conducted in central

Indian Ocean during the Chinese First Around-world Researching Cruise in December, 2005. The concentration of Chlorophyll a, DIC, DOC and POC were measured.

Materials and Methods

The experimental facility set on the bow consists of four 500-L barrels each with a diameter of ca. 100 cm, a set of cooler and a temperature controller (Fig. 1), which made the fertilization condition be as close to actual surface seawater as possible. The side wall of acrylic barrels is transparent, ensuring enough light into the barrels. Cooling water from the cooler could cycle within the rubber tubes, which are circled through each barrel. Thus, the cooler and temperature controller could adjust the experimental temperature close to that of the actual surface seawater, which varied during the experiment.

The experimental water was taken from the surface seawater in the Central Indian Ocean (Fig. 2) with vacuum pump, which was characterized by relatively low nutrients ($0.25 \mu\text{mol l}^{-1}$ nitrate and $0.06 \mu\text{mol l}^{-1}$ phosphate) and low chlorophyll a (0.03 mg m^{-3}). The tubing, barrels and water pump were washed three times with the surface seawater in study area. After

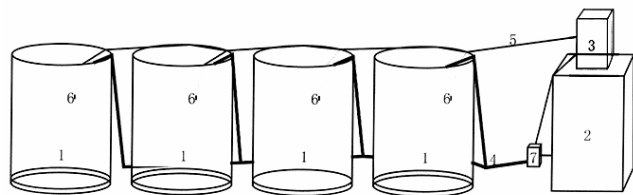


Fig. 1—Diagrammatic sketch of the experimental facility: 1. barrel; 2. cooler; 3. temperature controller; 4. water tubing; 5. conducting wire; 6. temperature detector; 7. tubing branch and water valve.

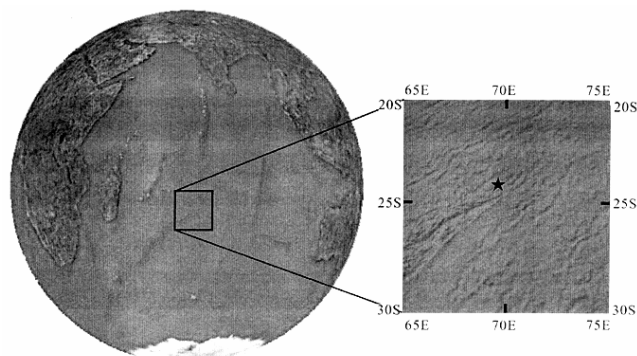


Fig. 2—Location of sampling station in the central Indian Ocean

the experimental water was collected in the four barrels (B1, B2, B3, B4), selected nutrients were added separately (Table 1). Experimental water was mixed well using inorganic glass rod. The nutrients N, Fe, P were KNO_3 , $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and KH_2PO_4 , respectively were added.

The concentration of Chlorophyll a was measured aboard. Two-liter sub-samples were collected from the upper 20 cm using rubber tube in each barrel. They were filtered through Whatman GF/F filters, extracted in 90% acetone, and analyzed before and after acidification on a Turner Fluorometer.

DIC samples were filled into 50-ml plastic sampling bottle with the addition of about 1 ml HgCl_2 (7%), sealed and stored in the dark (4°C) prior to analysis on shore. The DIC was extracted from seawater by adding 100% H_3PO_4 at 45°C , and measured on Gas Bench system (Finnigan MAT), equipped with a fully automated extraction line. The signal intensity of $^{44}\text{CO}_2$ was used to get DIC concentration. This process was performed at State Key Laboratory for Mineral Deposits Research in Nanjing University.

DOC samples were preserved in the same way as DIC samples. The particulates in water samples were removed with refrigerated centrifuge for DOC analysis. About 30 ml water was centrifuged ten minutes with the speed of 7980 rpm at 2°C . DOC was measured by high temperature combustion (HTC) on a Shimadzu TOC 5000 analyzer, equipped with an ASI 502 automatic sample injector. Inorganic carbon was eliminated by adding HCl (2 mol l^{-1}) and injecting. The minimum quantification limit of the analyzer was 0.05 mg/L. Analytical precision was within 1-4%.

The particulate mater was filtered onto GF/F filters ($0.7 \mu\text{m}$) for determination of POC. Samples were wrapped with aluminum foil and stored in a sealed plastic bag at -20°C . About thirty particulate samples were preserved in the experiment. Onshore, a few milliliters of 2 mol l^{-1}

Table 1—Nutrient addition in different barrels in the experiment

Number of barrel	N addition (mol)	Fe addition (mol)	P addition (mol)
B1	0.005	—	—
B2	—	0.001	—
B3	0.005	0.001	—
B4	0.005	0.001	0.0007

HCl were dropped to the surface of thawed GF/F filters to eliminate the inorganic carbon. Then, the filters were rinsed with Milli-Q water and freeze dried. The amount of POC per unit area was measured with elemental analyzer (Vario EL III CHN) in Guangzhou institute of geochemistry, Chinese academy of sciences. The bulk concentration of POC was calculated with the actual filtering area (4 cm^2) of the filters and the volume of filtered water.

Results

Phytoplankton growth

Chlorophyll a concentrations were shown in Fig. 3(A) and Table 2. The time when the first maximum of Chlorophyll concentration appeared had been defined as the blooming spot of phytoplankton (BSP). The increase of Chlorophyll a concentration at BSP (Chl_{BSP}) in B4 was the most obvious, which was 4.11 mg m^{-3} higher than the initial value (Chl_{ini}). The increase in B1 and B3 at BSP was similar (ca. 1.03 mg m^{-3}). However, the Chlorophyll a concentration in B2 only had an increase of 0.14 mg m^{-3} on the 9th day, indicating little variation

of Chlorophyll a concentration at BSP. It was shown that addition of iron had little influence to phytoplankton growth, but nitrogen could promote the bloom. The addition of phosphate with N & Fe proved to be the best to promote plankton growth. This further infers that phosphate was more significant than iron in fertilizing the surface water.

After BSP, the Chlorophyll a concentration in B3 went down slowly, and that in B4 dropped to 0.92 mg m^{-3} on the 12th day. In these two barrels, the Chlorophyll a concentration was decreased to 0.18 mg m^{-3} at the terminal of the experiment. The average Chlorophyll a concentration in B1 (0.84 mg m^{-3}) was relatively high and showed little variation after BSP.

Temperature

The water temperature in the four barrels was measured during sub-sampling. The average was used to represent the water temperature at that time. The variation of temperature in the experiment was shown in Fig. 4. The average temperature during the experiment was ca. 25°C , and it can be seen that the

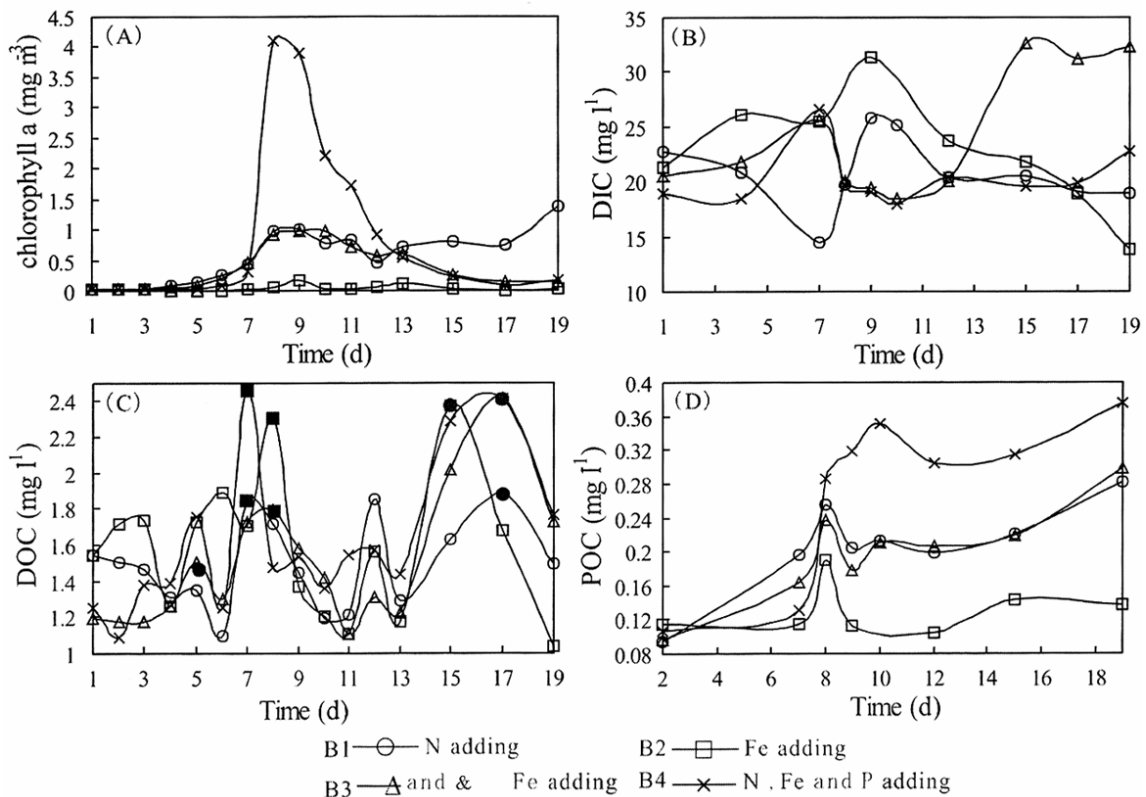


Fig. 3—Trends of chlorophyll a (A), DIC (B), DOC (C) and POC (D) in the four barrels during the experiment. In the trends of DOC concentration (C), the solid squares represent the P1 peaks and the solid circles mark the P2 peaks.

Table 2*—Summary of the concentration and its variation of chlorophyll a, DIC, DOC, and POC in the experiment

Number of barrel	B1	B2	B3	B4
Chl _{ave} (mg m ⁻³)	0.54	0.04	0.38	0.9
Chl _{ini} (mg m ⁻³)	0.03	0.03	0.03	0.03
Chl _{BSP} (mg m ⁻³)	0.98	0.05	0.92	4.11
DIC _{ave} (mg l ⁻¹)	20.77	22.8	24.17	20.29
DIC _{ini} (mg l ⁻¹)	22.8	21.36	20.52	18.96
DOC _{ave} (mg l ⁻¹)	1.49	1.59	1.49	1.62
DOC _{ini} (mg l ⁻¹)	1.54	1.54	1.19	1.26
ΔDOC _{P1} (+) (mg l ⁻¹)	0.3	0.76	0.6	1.2
T _{P1} (d)	7	8	8	7
ΔDOC _{P2} (+) (mg l ⁻¹)	0.33	0.84	1.22	1.15
T _{P2} (d)	17	15	17	17
ΔDOC _{ter} (-) (mg l ⁻¹)	0.38	1.34	0.69	0.64
POC _{ave} (mg l ⁻¹)	0.21	0.13	0.2	0.27
POC _{ini} (mg l ⁻¹)	0.09	0.11	0.1	0.11
POC _{BSP} (mg l ⁻¹)	0.26	0.19	0.24	0.29
ΔPOC _{ter} (+) (mg l ⁻¹)	0.06	Nil	0.08	0.06

*Chl_{ave}, DIC_{ave}, DOC_{ave} and POC_{ave} are the average concentration of chlorophyll a, DIC, DOC and POC in the experiment.

Chl_{ini}, DIC_{ave}, DOC_{ini} and POC_{ini} are the initial concentration of chlorophyll a, DIC, DOC and POC measured in the first day during the experiment.

Chl_{BSP} and POC_{BSP} are the concentration of chlorophyll a and POC at BSP.

ΔDOC_{P1} (+) and ΔDOC_{P2} (+) are the increases of DOC concentration at P1 and P2 relative to the initial value, respectively.

T_{P1} and T_{P2} are the fertilization period in days when DOC peak appears.

ΔDOC_{ter} (-) is the decrease of DOC concentration at the termination of the experiments relative to that at the peaks of P₂.

ΔPOC_{ter} (+) is the increase of POC concentration at the termination of the experiments relative to that on the 15th day in the experiment, being close to the time when P₂ appears.

water temperature increased rapidly from the 6th to 9th day. The temperature remained stable before the 6th day (average temp: 23°C) and after the 9th day (average temp: 27°C).

Dissolved Inorganic Carbon (DIC)

The average DIC concentration (DIC_{ave}) in B2 and B3 was a bit higher than that in B1 and B4 (Table 2). The change of DIC in these four barrels was different as shown in Fig. 3(B). Around BSP, DIC concentrations in B1 and B2 increased whereas those in B3 and B4 decreased. After that, a decrease of DIC concentration in B1 and B2 was observed while an increase occurred in B3. The DIC concentration in B4 had a slight change after BSP.

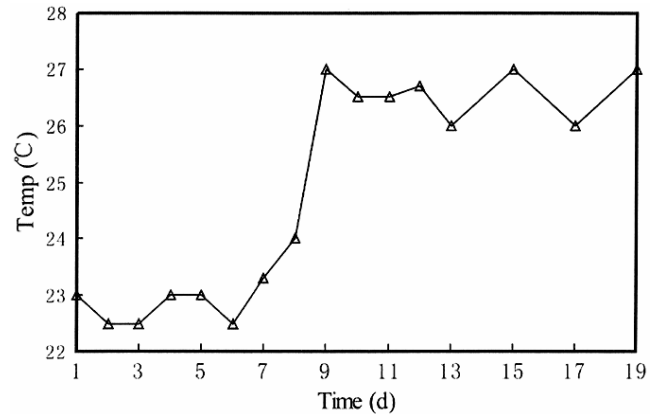


Fig. 4—Temperature variations in the experimental water

Dissolved Organic Carbon (DOC)

The average DOC concentration (DOC_{ave}) in B1 was the lowest in these four barrels (Table 2). The occurrence of two peaks, marked P1 and P2 in Fig. 3(C), reflected the most remarkable changes of DOC concentration in the experiment. P1 appeared around BSP and P2 was near the termination of experiment. Obviously, DOC concentration at P1 and P2 were similar in B2 and B4, which were all much higher than the initial value (DOC_{ini}). In B3, the DOC concentration at P1 was half as much as that in B2 and B4, and the peak at P2 was as high as that in B2 and B4.

Particulate Organic Carbon (POC)

Both the average concentration (POC_{ave}) and the concentration at BSP (POC_{BSP}) in B4 were higher than those in other three barrels. The POC_{ave} in B1 was approximate to that in B3, and the lowest POC_{ave} was observed in B2 (shown in Table 2). The trend curves of POC and chlorophyll a were similar in the four barrels (Fig. 3(A & D)). Especially, the variation trend in B1 was coincident with that in B3. The POC concentration at BSP (POC_{BSP}) has a prominent increase in the four barrels. The highest and lowest POC concentration occurred in B4 and B2, respectively. Right after BSP, the POC concentration dropped down except that in B4 with a continuous increase. The decrease of POC concentration in B4 occurred on the 12th day in the experiment. At the termination of the experiment, the POC values went up and exceeded POC_{BSP} in the barrels except B2 (Table 2).

Discussion

Dissolved Inorganic Carbon (DIC) variation

The DIC concentration in surface seawater was affected by several factors, including photosynthesis,

respiration and remineralization, temperature and air-sea gas exchange¹⁷. The difference of the nutrient addition will result in variations of the species and amount of phytoplankton in the barrels, which will lead to different trend of DIC. However, the different trends (shown in Fig. 3) and poor correlations ($P > 0.05$) between DIC and chlorophyll *a* in each barrel indicated that the amount of phytoplankton did not dominate the DIC concentration in the experiment. In addition, there was no significant correlation ($P > 0.05$) between DIC and temperature, which showed that variations of DIC were not the result of temperature change in the experiment. In consideration of the diversity of influence factors to DIC concentration, we suggest that DIC concentration was not a better parameter to reflect the plankton activities.

Implication of Dissolved Organic Carbon (DOC) for plankton activities

Two obvious peaks (P1 and P2) occurred at the trends of DOC concentration (Fig. 3(C)) in the four barrels, which were indicative to plankton activities.

The DOC concentration all went up to the maximum (P1) around BSP in the four barrels (Fig. 3(C) and Table 2). Phytoplankton could exude organic matter to seawater, which accounted for 30-40 percent of photosynthetic production¹⁸. The release of DOC from photosynthetic production was completed rapidly¹⁹. It was also realized that a significant fraction of carbon fixed by producers was known to flow through DOC²⁰. So it could be proposed that the increase of DOC at BSP related with the phytoplankton bloom. Especially, the good correlation between DOC and chlorophyll *a* concentration at the P1 in B1, B3 and B4 ($R^2 = 0.99$, $P < 0.05$) indicated the significant influence of phytoplankton exudation on DOC variation. In B2, the concentration of chlorophyll *a* remains stable during the experiment, indicating that the increase of DOC concentration might be caused by other factors other than the excretion of phytoplankton.

The occurrence of P2 showed a maximum of DOC concentration after phytoplankton bloom and near the termination of the experiment (Fig. 3(C) and Table 2). Seasonal DOC changes have been observed with increases following the spring bloom by a few weeks^{1,5,7,21}. The production of DOC during the post-bloom conditions has also been identified by experiments²²⁻²⁴. The concentration of Chlorophyll *a*

in B1 kept high after BSP (the average was 0.85 mg m^{-3}), which revealed that large numbers of phytoplankton thrived until the termination of the experiment. DOC concentration at P2 was similar to those at P1 and the peak on the 12th day in this barrel. The intervals among these three peaks were also alike (5 days). It was suggested that DOC increase at the three peaks might result from the periodic exudation and exudation of phytoplankton in B1. Since the concentration of chlorophyll *a* at P2 in B3 and B4 was close to that of actual surface seawater, the exudation of phytoplankton couldn't explain the accretion of DOC. Previous studies demonstrated that processes such as bacterial degradation and cell lysis might all lead to the solubilization of organic carbon⁴, and bacterial populations were reported to reach large abundances after phytoplankton blooms²⁵⁻²⁷. It is presumed that the degradation of organic matters by bacteria played an important role in the DOC increase at P2. The low concentration of chlorophyll *a* implied the death of most of the blooming-produced phytoplankton around P2 in B3 and B4. This could provide enough organic matter for bacterial degradation.

The present study implies that the DOC concentration dropped after P1 and P2. Bacteria had an important influence on the consumption of DOC in the seawater²⁸. The absorption of DOC by bacteria reduced its concentration²⁹⁻³². In addition, the large amount of bacteria appearing after phytoplankton blooms in the coast of Galicia (NW Spain) was likely resulted from the utilization of DOC derived from phytoplankton²⁰. The drop of DOC concentration was likely to be caused by bacterial consumption of DOC.

Implication of Particulate Organic Carbon (POC) for plankton activities

In this experiment, the plankton provided most of POC due to the absence of persistent detrital organic material. Therefore, the POC could reflect the information of plankton activities with the analysis of chlorophyll *a* and DOC.

The POC concentration increased rapidly with chlorophyll *a* in B1, B3 and B4 at BSP (Table 2 and Fig. 3). There was a good correlation between POC and chlorophyll *a* at BSP in these three barrels ($R^2 = 0.86$, $P < 0.05$). This infers that phytoplankton controlled the POC concentration in these three barrels before BSP. However, the concentration of chlorophyll *a* increased only 0.02 mg m^{-3} when P1

appeared in B2, and was not in relative with the POC increase. This suggests that there are other sources for POC besides phytoplankton growth.

The weak correlation between POC and chlorophyll a in the barrels ($P > 0.05$) after BSP showed POM was not mainly composed by phytoplankton at this stage. Previous studies³³⁻³⁶ has shown that, in highly oligotrophic systems, bacterioplankton might contribute the major fraction of POC. This might account from 40 to more than 70% of the organic carbon. It might be the most evident characteristic of the oligotrophic environment. In the experiment, the POC concentration might have a close relationship with bacteria in the oligotrophic seawater after BSP.

Two processes might influence the POC concentration just after BSP. The initial decomposition of old POC by bacteria, mainly from phytoplankton bloom, would reduce the POC concentration in the experimental water. Bacteria would grow up with the utilization of DOC, which could produce new POC and cause the increase of POC concentration. The POC concentration would dependent on complex interactions between the decomposition of phytoplankton and the growth of bacteria. The marginal decrease in concentration of chlorophyll after BSP in B1 and B3 (Fig. 3 (A)), the POC concentration also reduces (Fig. 3 (D)). This infers that during this period, initial decomposition of POC by bacteria played more important role than the bacterial growth in these two barrels. In B2, the low and stable concentration of chlorophyll a showed that the drop of POC had little relationship to the phytoplankton activities. The continuous increase of POC concentration reveals that the bacterial growth dominated the POC variation rather than phytoplankton degradation in this barrel.

The increase of POC concentration was in proportionate with the decrease of DOC in the barrels near the termination of experiment (except in B2). This will establish that the bacterial growth occurs with the consumption of DOC. The decrease of DOC ($\Delta\text{DOC}_{\text{ter}} (-)$) was more apparent than the increase of POC ($\Delta\text{POC}_{\text{ter}} (+)$) in the barrels except in B2 (Table 2) since the decreased DOC was sufficient to be altered to the increased POC. Besides the utilization by bacteria, the DOC could be consumed by other paths, including the absorption of particulates.

The variation of POC, chlorophyll a and DOC concentration in the experiment will presume the information about phytoplankton bloom, bacterial growth and the decomposition of phytoplankton

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