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Chromatographic Separation of Nitrogen, Argon, and Oxygen in Dissolved Air for Determination of Triple Oxygen Isotopes by Dual-Inlet Mass Spectrometry

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A chromatographic system was developed to separate oxygen from nitrogen, argon, carbon dioxide, and water vapor mixture for the determination of precise isotopic ratio measurements of oxygen in dissolved air. This system separates oxygen not only quantitatively but also rapidly as well; typical oxygen separation takes about 30 min. Fractionation of oxygen between liquid and gas phase was found to be similar to that of earlier reports.

The isotopic ratios of oxygen are precise diagnostic tools for understanding chemical kinetic, thermodynamic, geological, biological, climatic, and physical processes in the Earth's surface. A recent discovery of mass independent fractionation of oxygen isotopes (^{16}O, ^{17}O, and ^{18}O) during production of ozone from molecular oxygen suggests that the analysis of these three isotopes in the dissolved oxygen would serve as a unique tracer to estimate global marine oxygen production[1,2] and global paleoproductivity.[3] Such analysis in the atmosphere would help us to understand stratosphere−troposphere mixing.[4,5] The ratio of $^{18}O/^{16}O$ is commonly determined in the chemical form of CO_2 by measuring the m/z 46/44 and 45/44 mass ratios.[6] The analytical method to convert O_2 to CO_2 cannot yield $^{17}O/^{16}O$ directly and simultaneously because $^{13}C^{16}O^{16}O$ overlap the signal from the ionized molecule of $^{12}C^{17}O^{16}O$. Also isotopomers of N_2O (m/z 44, 45, and 46) interfere to determine isotope ratios of CO_2. Epstein[7] developed a technique for ^{17}O determination by converting to CO_2, but this technique requires four times the mass spectrometric measurements and equilibration with water. Nonetheless, it has a poor precision for $\delta^{17}O$ (±0.5‰ in SD) because of the error propagation. To determine isotopic ratios of oxygen at high accuracy, the sample must be introduced as pure O_2 into the mass spectrometer to avoid isobaric interferences and formation of nitrogen oxides in the ionization chamber due to the reaction with N_2. Thiemens and Meagher[8] developed a method of direct introduction of O_2 to the dual-inlet mass spectrometer after quantitative separation from N_2, CO_2, N_2O, and water by the cryogenic method. However, they did not separate Ar from O_2. Nevertheless, the precision of their measurements is ±0.1‰ and ±0.2‰ (in SD), respectively, on $\delta^{18}O$ and $\delta^{17}O$ analysis. Wassenaar and Koehler[9] developed an on-line technique for the determination of $\delta^{18}O$ and $\delta^{17}O$ of gaseous and dissolved O_2 using a continuous flow mass spectrometer (CF−IRMS). In this technique, O_2 and N_2 are separated chromatographically using 5 Å molecular sieves at 35 °C, but again they could not separate Ar from O_2. The reported precision is no better than the earlier methods (±0.17‰ and ±0.5‰ in SD, respectively, for $\delta^{18}O$ and $\delta^{17}O$). Recently a more precise method was developed by Luz et al.[1] based on chromatographic separation of N_2 from the O_2−Ar mixture using a 5 m long stainless steel column packed with 5 Å, 45 × 60 mesh molecular sieve held at 0 °C. They determined triple oxygen isotopic ratios in the O_2−Ar mixture by a dual-inlet mass spectrometer and applied corrections for the presence of Ar. The standard error of mean (SEM) involved in their measurements was reported to be 0.009‰ and 0.003‰ for $\delta^{17}O$ and $\delta^{18}O$, respectively. Recently, Abe and Yoshida[10] analyzed triple oxygen isotopes by injecting dehydrated air sample directly to the dual-inlet mass spectrometer without removing N_2 and Ar. They found partial pressure dependency of isotopic composition of O_2 and demonstrated that the interference by the presence of heavier Ar could be more than N_2.

To examine the influence of Ar on oxygen isotopic analysis, variable concentrations of O_2−Ar mixtures of gases were prepared with the range of 0−100% of Ar in O_2. The $\delta^{17}O$ and $\delta^{18}O$ of these gas mixtures were determined relative to the identical O2 as referenced by the dual inlet mass spectrometer (Delta Plus, Thermo Quest). Figure 1 suggests that the presence of Ar significantly influences oxygen isotope ratios by 0.5‰ and 0.1‰ for a change in 10% of the Ar/O_2 ratio on $\delta^{17}O$ and $\delta^{18}O$, respectively. The magnitude of the correction depends on many

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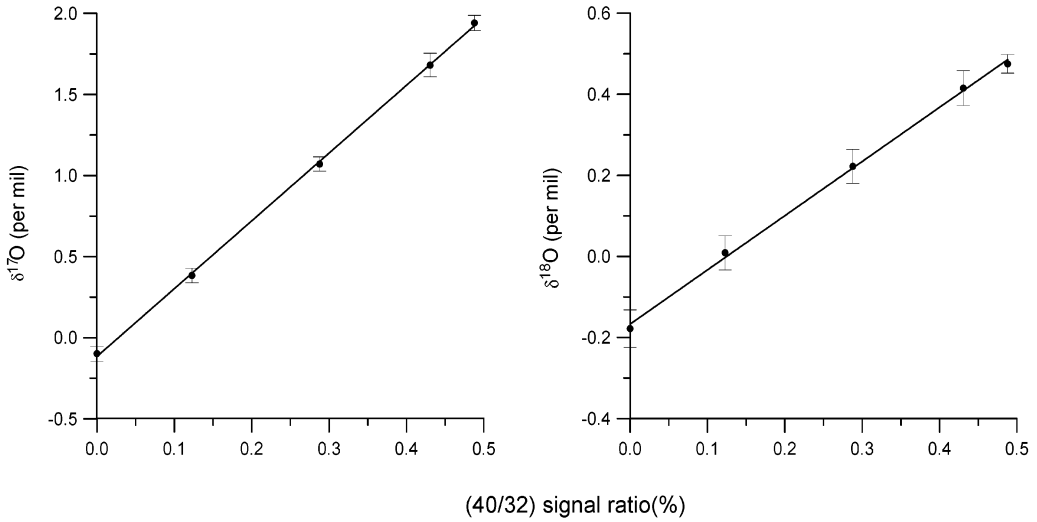

Figure 1. Influence of argon on isotopic ratios of δ^{17}O and δ^{18}O. Isotopic ratios are expressed relative to reference gas.

factors that include the type of instrument and the electrostatic condition of the ionization chamber[10] [Dr. Boaz Luz personal communication]. Since the O_2/Ar ratio varies widely in the oceanic waters (from 3 to 23), due to oxygen supersaturation in the surface waters and undersaturation due to consumption in the intermediate waters, the influence of Ar would be more significant for samples from greater depth with low O_2 concentration. The influence of Ar was found to be linear with respect to concentration (Figure 1); however, the correction applied using a linear regression line would introduce an error to the isotopic values of oxygen ($\pm$0.030‰ on δ^{17}O and $\pm$0.009‰ on δ^{18}O), that results in less accurate determinations. Goyette et al.[11] developed a method to separate N_2, O_2, CO_2, and Ar chromatographically in the controlled air. In their method, a 1.83 m long Porapak (30−80 mesh) column is used to separate CO_2, and a 10.98 m long Haysept-A (80−100 mesh) is used to separate N_2, O_2, and Ar with helium as the carrier gas. If the O_2 concentration levels are higher (>10% of total gas), column inversion was delayed by circulating O_2 and Ar for a second time through a Haysept-A column before being detected by the TCD. The main objective of this study is to develop a method that would allow us to separate interfering gases such as N_2, Ar, CO_2 and water vapor from O_2 at all dissolved oxygen (DO) concentration levels found in the marine waters and determine triple oxygen isotope ratios on pure DO at high precision.

EXPERIMENTAL SECTION

Collection of Sample and Equilibration. Prior to sampling, the 300 mL flask with Louwers Hapert O-ring stopcock (Figure 2) containing 250 μL of $HgCl_2$ saturated solution is well evacuated and closed with a water lock.[1] About 150 mL of water sample was collected in the flask while leaving 150 mL of headspace. Extreme care was taken to avoid the trapping of gas bubbles during sampling. The stopcock is closed, and the port is refilled with distilled water and then sealed with a rubber cap to avoid air contamination. The water and headspace in the sampling flasks were equilibrated for 24 h at room temperature followed by the procedure of Luz et al.[1] After equilibration, the water was sucked

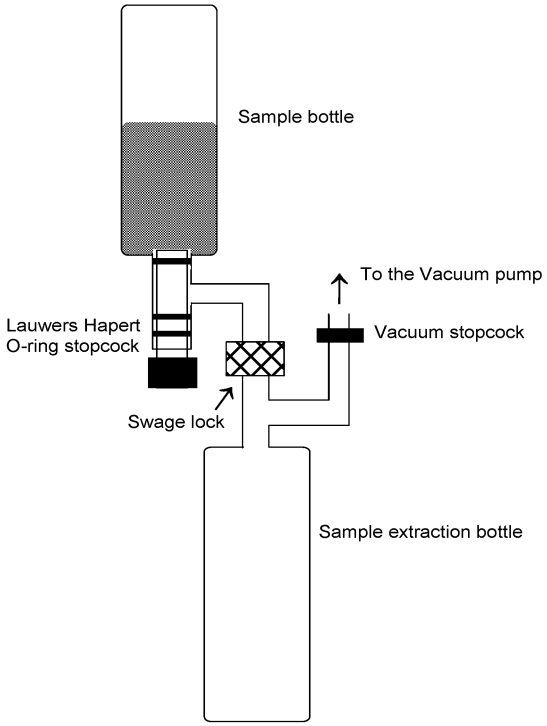

Figure 2. Schematic diagram shows sample extraction unit.

out of the flasks by leaving only headspace gases (Figure 2). The flasks were then connected to the preparation system for separation and purification of O_2. Based on the solubility of gas, it was computed that >99% of the gas is transferred to the gas phase and ~1% remained in the liquid phase. Using the isotopic mass balance, we found that the influence of leftover gas in the liquid phase does not affect the total triple O_2 isotopic ratio within the errors involved in this method.

Separation and Purification of DO. The schematic of the DO separation system is shown in Figure 3. The entire system, except for the GC column, is pumped up to ~10^{-4} atm with a vacuum pump. The in-line separation system consists of a 30 cm long Pyrex U-tube (trap 1) with a molecular sieve (13X, 1/16 in. pellets), a second stainless steel chromatographic column (8 m × 2 mm inside diameter) packed with 45/60 mesh 5 Å molecular

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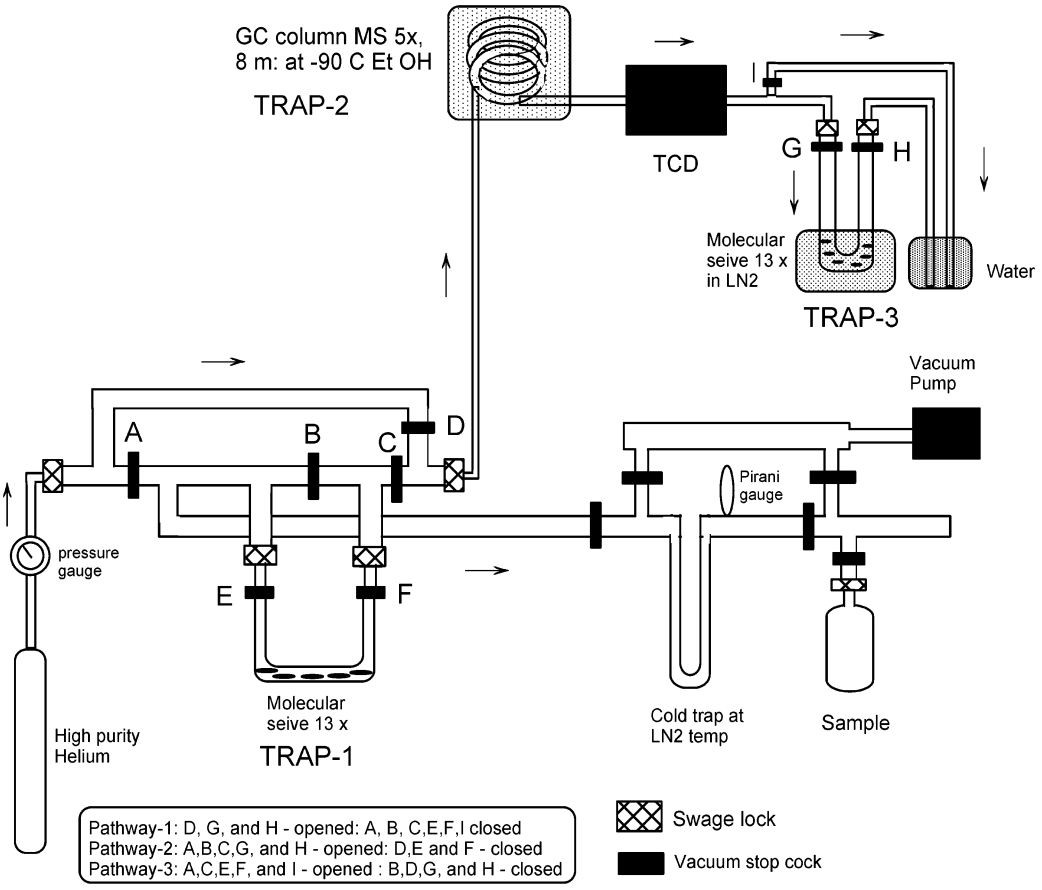

Figure 3. Schematic diagram shows the separation and purification system for dissolved oxygen and air.

sieve (trap 2), and a third Pyrex U-tube (70 cm × 5 mm inside diameter; trap 3) with a molecular sieve 13X, 1/16 in. pellets at liquid nitrogen temperature. Prior to separation, traps 1 and 3 are baked at ~300 °C overnight, while pumping until a pressure of at least 1×10^{-4} atm is reached, whereas trap 2 is baked at ~300 °C in the presence of high purity helium carrier gas. Once traps are baked, they can be used for up to 5−6 samples.

At least 1 h prior to sample injection, the GC column is kept in the ethanol at −90 °C with carrier gas flow via pathway 1 (see Figure 3). The separation is initiated by evacuating the atmosphere between valves A to C and trap 1 using vacuum pump and chilling both the sucked sample flask and the in-line cold trap at liquid nitrogen temperature to freeze CO_2, N_2O, and remaining water and transferred O_2, N_2, and Ar in the trap 1. Then valves E and F are closed, and trap 1 is warmed to room temperature using a hot air blower. Then the flow of carrier gas is changed to pathway 2 by closing D and opening A, B, and C, and it reaches trap 3 via the GC column at a carrier gas flow rate of 20 mL/min. Once trap 3 is completely filled with the He carrier gas (it takes about 3 min), valves G and H are closed and trap 3 is kept at the liquid nitrogen temperature. Now valve I is opened to allow the carrier gas to vent. Both vents (after I and H) are put into the water reservoir to avoid back suction of the atmosphere during valve operation. Then the sample is injected to the line by changing to pathway 3 by closing valve B followed by the opening of valves E and F. Ar elutes at 16 min followed by O_2 at 20 min. N_2 remains trapped by the molecular sieve at −90 °C in trap 2 (GC column) (Figure 4). Once Ar is eluted completely, which is monitored with

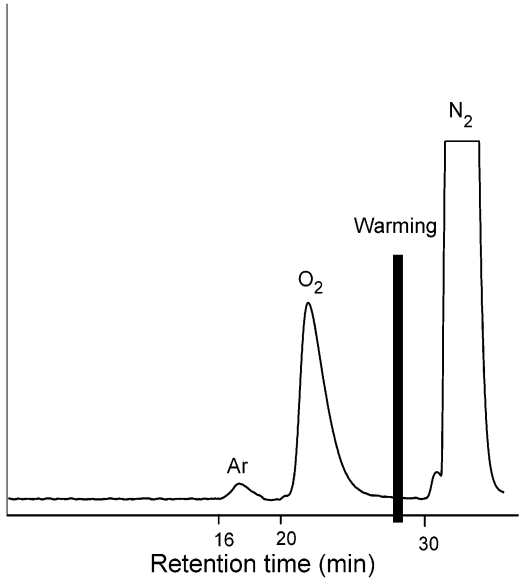

Figure 4. Relative output signal of the detector obtained when 3 mL of air sample is injected to the GC column.

TCD response, valve I is closed and valve G followed by valve H is opened. The molecular sieve at liquid nitrogen temperature traps the eluted O_2 by trap 3. It takes about 8 min to elute oxygen completely (Figure 4). Then valves G and H are closed, and trap 3 is separated from the line. The GC column is warmed to room temperature using a hot air blower to elute N_2. The excess He in the separated trap 3 is pumped out in the vacuum line, and the O_2 is transferred to the 1 mL Pyrex tube which has a vacuum

stopcock and contains molecular sieve (13X) at liquid nitrogen temperature. It is admitted to the mass spectrometer for triple oxygen isotope determinations. Once the sample is prepared they can be stored up to 6 months.

The isotopic composition of oxygen sample was determined relative to the reference gas of O_2 by the dual-inlet mass spectrometer (Delta Plus, Thermo Quest). In the same manner as in the typical dual-inlet mass spectrometric analysis, the output voltages of the major signals (m/z 32) were arranged to be equal to 2.0 V for both sample and reference sides throughout all runs. Prior to the usual changeover from the reference to sample gases, the signals of m/z 18, 28, 32, and 40 were determined by mass jumping to confirm the separation of gases is successful. Each sample was measured for 10 cycles with each cycle having 10 runs to obtain greater precision. All of the samples were determined relative to the reference gas and the isotopic compositions of the reference gas were determined repeatedly relative to the prepared atmosphere. All values are represented as delta values (in ‰) relative to Standard Mean Ocean Water (SMOW) assuming the atmospheric O_2 isotopic ratios of $\delta^{18}O$ and $\delta^{17}O$ to be 23.5‰[12] and 12.2‰[13] with respect to SMOW, respectively.

RESULTS AND DISCUSSION

Repeated injections of 3 mL of air sample collected in the laboratory over a 3-month period yielded a repeatability (in SD) of ±0.065‰ for $\delta^{17}O$ and ±0.020 ‰ for $\delta^{18}O$ (n = 23) and a standard error of 0.008‰ and 0.003‰, respectively. The yield test was carried out by injecting 1 mL of air to the chromatographic separation line and comparing the recovered O_2 with what was expected and finding the recovery was better than 99.8%. To examine the fractionation of oxygen isotopes during the separation procedure, high purity O_2 gas is injected into the separation system and admitted to the mass spectrometer in the sample side and determined relative to the same gas (without passing through the separation procedure) as reference. The differences for both $\delta^{17}O$ and $\delta^{18}O$ are found to be within the errors involved in the measurements ($\delta^{17}O = 0.036 \pm 0.052$ and $\delta^{18}O = 0.011 \pm 0.018$). This suggests that no fractionation takes place within the error of the method during separation of O_2 from the mixture.

To compare our method with that of Luz et al.[1] two of the atmospheric samples were prepared, one was purified to O_2 and the other was purified to the O_2−Ar mixture. Isotope ratios of O_2 gas were then determined by our method, and the O_2−Ar mixture was determined followed by the procedure of Luz et al.[1] with the correction of Ar interference. Both values are comparable within the errors involved in the measurements (see Table 1). In addition to this, triple oxygen isotopes in our laboratory O_2/Ar mixture standard gas was measured by Dr. E. Barkan with reference to HLA (Holy Land Air) at Hebrew University, Jerusalem, Israel using Luz et al.[1] method, and O_2 and Ar in the same gas mixture were separated by our method and the triple isotopes on pure oxygen were measured, and they both agreed well with each other within the error of the measurements.

LABORATORY AND FIELD INVESTIGATIONS

A series of laboratory tests using this technique on air-saturated distilled water confirmed the $\delta^{17}O$ and $\delta^{18}O$ isotopic fractionation between air and water at 22 °C (10 samples), resulting in 0.371 ± 0.048 and 0.691 ± 0.012‰, respectively (Table 2), in accordance with the results of Benson et al.[14]

Table 1. Influence of **Argon on Oxygen Isotope Ratio Measurements**[a]

gases analyzed	$\delta^{17}O$ relative to reference gas	$\delta^{18}O$ relative to reference gas
O_2/Ar mixture	1.289 ± 0.071	2.255 ± 0.028
after Ar correction	1.076 ± 0.083	2.198 ± 0.036
O_2	1.070 ± 0.065	2.204 ± 0.018

[a] $\delta^{17}O$ and $\delta^{18}O$ of air was measured in the presence of argon and pure O_2.

Table 2. **Results of Air-Saturated Waters**[a]

$\delta^{17}O$	difference water−air	$\delta^{18}O$	difference water−air
12.574	0.374	24.202	0.702
12.576	0.376	24.189	0.689
12.589	0.389	24.192	0.692
12.568	0.368	24.178	0.678
12.581	0.381	24.192	0.692
12.552	0.352	24.186	0.682
12.548	0.348	24.189	0.689
12.596	0.396	24.196	0.696
12.572	0.372	24.198	0.698
12.545	0.345	24.184	0.684

[a] All values were expressed relative to SMOW. The fractionation difference between water and air is derived by assuming $\delta^{18}O$ and $\delta^{17}O$ of air to be 23.5 and 12.2‰, respectively.

We present here some preliminary oxygen isotopic results from our ongoing research to illustrate a wide range of DO isotope values found in nature. The DO isotopic data collected in the Sagami Bay, Japan in August 2002 (Figure 5) show a trend of systematic enrichment of $\delta^{17}O$ and $\delta^{18}O$ with decreasing saturation degrees of DO, indicating ^{16}O is preferentially consumed by respiration. On the contrary, photosynthesis in the euphotic zone leads to oxygen supersaturation, hence it imparts a strong depletion of $\delta^{17}O$ and $\delta^{18}O$ signature of DO. An especially large depletion in both $\delta^{17}O$ and $\delta^{18}O$ can be observed at 15 m depth where chlorophyll maximum (by >2 mg m^{-3}) and high saturation of DO (~130%) were found. Photosynthetically produced O_2 in the euphotic zone should have an isotopic composition similar to that of oxygen in seawater,[15] which is close to the value of 0‰ with respect to SMOW. According to Luz et al.,[1] oxygen consumption fractionates mass-dependently such that ^{16}O is more preferable than ^{17}O and ^{18}O. As a result, ^{17}O enrichment is about half of the ^{18}O enrichment relative to ^{16}O. It was found in the laboratory experiment that the changes in $\delta^{17}O$ to $\delta^{18}O$, that have fractionated mass-dependently, follow a line with a slope of about 0.521.[1] The relation between $\delta^{17}O$ and $\delta^{18}O$ in the Sagami Bay yields a slope of 0.518 and suggests changes in isotopic ratios are driven by mass-dependent fractionation. The $\delta^{18}O$ in the mixed layer (20 m) ranges between 21.7 and 22.7‰ (SMOW) which is away from the air-saturated $\delta^{18}O$ (24.2‰) value. It suggests DO in the Sagami

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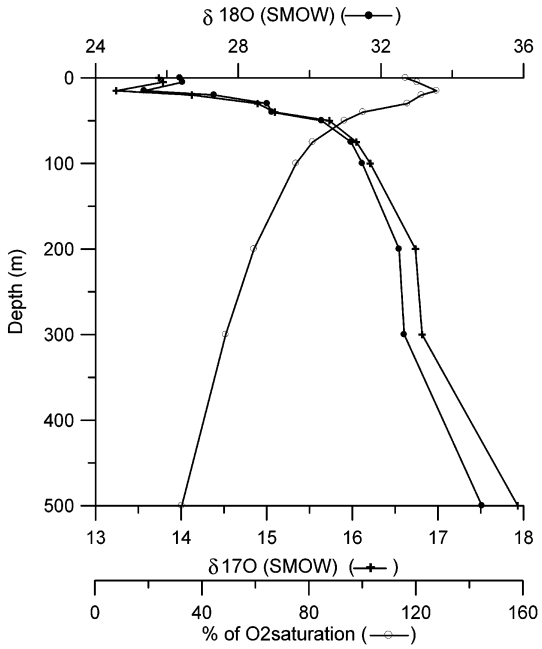

Figure 5. $\delta^{17}O$ (●), $\delta^{18}O$ (‰) (+), and % of oxygen saturation (○) profiles during August 2002 in the Sagami Bay, Japan.

Bay is a mixture of O_2 derived from air, photosynthesis, and oxygen fractionated by respiration.

In the stratosphere, the ozone recombination reaction, $O + O_2 \rightarrow O_3$, causes O_3 to be mass-independently fractionated. The mass-independent fractionation of oxygen isotopes leads to equally lowering of $\delta^{17}O$ and $\delta^{18}O$ in stratospheric O_2. The ultraviolet photolysis of ozone in the stratosphere generates an electronically excited oxygen atom which can undergo isotope exchange with CO_2 or O_2 in a mass-independent way. Finally, this mass-independently fractionated oxygen makes the isotopic content of CO_2 enriched and that of O_2 depleted. The stratosphere−troposphere mixing of air makes the isotope ratio of tropospheric O_2 anomalous. On the contrary, photosynthetic O_2 has a mass-dependent isotope ratio. Hence, the degree of the anomaly in the dissolved oxygen is a measure of gross production. According to Luz et al.,[1] for a given $\delta^{18}O$ of O_2 produced solely by biological production, there is an excess of $\delta^{17}O$ in the water in comparison to air O_2 due to mass-independent and -dependent fractions of O_2 in the stratosphere and troposphere, respectively. This excess O_2 is called a $\Delta^{17}O$ anomaly and is defined as

$$\Delta^{17}O = 1000(\delta^{17}O - 0.521\delta^{18}O)$$

The $\Delta^{17}O$ is computed for the Sagami Bay during August 2002 and is presented in Figure 6. This figure shows three important features: (1) High $\Delta^{17}O$ anomaly is found at 15 m within the mixed layer that corresponds to chlorophyll maximum. Photosynthesis and air−sea exchange mainly contribute the variations of anomaly in the mixed layer. (2) Deeper aphotic zone waters, that have not

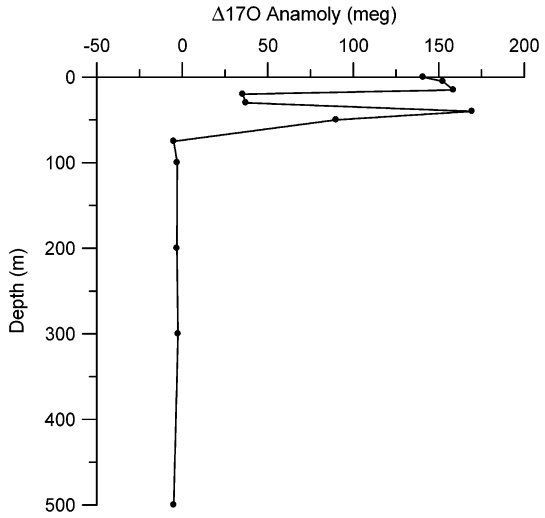

Figure 6. Vertical distribution of ^{17}O anomaly in the Sagami Bay during August 2002.

entered the euphotic zone for a long time, have low dissolved oxygen and high $\delta^{17}O$, $\delta^{18}O$ and low $\Delta^{17}O$, reflecting respiration in the absence of photosynthesis. (3) Waters of the sunlit thermocline where $\delta^{18}O$ is high and O_2 concentration is low, however, have an elevated $\Delta^{17}O$ anomaly which is found which could be due to photosynthesis proceeds in the absence of gas exchange. Gross production of oxygen in the mixed layer computed based on the Luz and Barkan[2] model amounted to 181 mmol m^{-2} d^{-1} which is higher than oxygen incubation in the light and dark bottles (163 mmol m^{-2} d^{-1}).

CONCLUSIONS

Based on the chromatographic separation technique, dissolved O_2 can be separated from the other dissolved gases with no measurable fractionation. This method facilitates the measuring of triple oxygen isotopes on pure oxygen with better precision. Our separation system works at all DO concentration levels found in the marine waters (i.e., from 5 to 300 μmol kg^{-1}), and it is not only quantitative but also rapid as well; typical O_2 separation takes about 30 min. The fractionation of O_2 between liquid and gas phase was found to be 0.371 ± 0.048‰ and 0.691 ± 0.012‰, respectively, for $\delta^{17}O$ and $\delta^{18}O$, and it is in close agreement with the previous estimates.[14]

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