The influence of reactive oxygen species on "‘respiration" isotope effect

Website: https://www.bco-dmo.org/dataset/923859
Data Type: experimental
Version: 1
Version Date: 2024-03-28

Project
» Clumped Oxygen Isotope Signature of Marine Dissolved Oxygen (Microbial isotope effects)

Contributors	Affiliation	Role
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Abstract
The triple-oxygen isotope (17O/16O, 18O/16O) measurement of oxygen-bearing species represents one of the most robust tools to directly trace oxygen cycling in the environment. One particularly consequential application of this isotope system is the analysis of dissolved oxygen (O2) in aquatic environments to determine gross oxygen production. This approach assumes that photosynthesis, microbial respiration, and gas exchange are the main drivers of dissolved O2 isotope compositions, and that each process is described by predictable, consistent triple-oxygen isotope effects. However, there currently exists a large disagreement in the literature on the triple-oxygen isotope effect of respiration, which carries major implications for global primary productivity estimates. Recent work has additionally highlighted the ubiquitous production of extracellular reactive oxygen species (ROS) such as superoxide and hydrogen peroxide by microorganisms; this flux maybe responsible for as much as 20% of net oxygen utilization in the ocean. To examine the influence of ROS-mediated O2 recycling on the oxygen utilization isotope effect, we measured the triple-oxygen isotope fractionations and mass laws of superoxide dismutase, catalase, and iron-mediated H2O2 degradation. We incorporate these constraints into an oxygen isotope flux model to explore the influence of ROS-mediated oxygen cycling on "respiration" isotope effects in previous studies. We find that ROS-mediated oxygen cycling can reconcile the previously reported range of triple-oxygen isotope fractionation factors and that typical marine isotope effects are broadly consistent with independent estimates of superoxide-mediated oxygen loss. These data are described further in the related publication, Sutherland et al., 2022 (doi: 10.1016/j.gca.2022.02.033).

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Methods & Sampling

We have chosen a representative from each enzyme group to offer initial isotopic constraints on O2 evolved from superoxide dismutase (SOD) and catalase. Specifically, we analyzed a representative CuZn SOD (Sigma S5395) and typical catalase (heme-binding, clade 3, small subunit, Sigma C1345). The same experiment apparatus was used to investigate the isotope effects of both enzymes. The reaction vessel consisted of a closed-system glass bulb with an inlet subject to continuous He flow (pre-scrubbed with 5A molecular sieve at liquid nitrogen temperature) through a glass frit, an outlet stream, and an injection port outfitted with a blue butyl rubber septum. The outlet stream was passed through multiple loops of stainless steel tubing held at liquid nitrogen temperature to trap any water vapor that escaped the reactor. KO2 or H2O2 were added for

reactions with SOD and catalase, respectively. Effluent He carrier gas and product O2 were then passed through a 5A molecular sieve trap to isolate O2 gas evolved by the enzyme of interest.

In the case of catalase, 2 milligrams (mg) of enzyme was dissolved in approximately 50 milliliters (mL) of ultra-high purity (18 MX MilliQ) water and bubbled with He for a minimum of one hour to remove O2, at which point 30% H2O2 was introduced to the solution through the injection port to an initial concentration of 3.9 millimolar (mM) in the reaction vessel. The reaction was allowed to proceed for a range of time intervals (a few minutes to several hours) to ensure adequate coverage of reaction progress for use with the Rayleigh equation. The oxygen isotope composition of the starting H2O2 was determined using the same experimental setup (without catalase) by adding a slurry containing MilliQ water and KMnO4 (amounting to a 10X excess of H2O2), which quantitatively oxidizes H2O2 to O2. The average O2 yield of the catalase and H2O2 treatment was 48% (n = 3) that of the KMnO4 treatment, consistent with the expected 2:1 stoichiometry of catalase.

SOD experiments were conducted with the same reaction vessel with some modifications. Given the rapid rate of reaction between superoxide and SOD, we instead characterized the oxygen isotope composition of the starting material (introduced as KO2) and the fully completed reaction to determine how oxygen isotopes are partitions between the oxidized and the reduced product. Methods for both measurements were modified from one previous study investigating the 18O isotope effects of CuZn SOD. To measure the initial ROS oxygen isotope composition, several milligrams of KO2 were introduced into the empty and dry reaction chamber, which was immediately flushed with He. Since water/moisture will cause KO2 to spontaneously disproportionate, the powder was kept dry prior to starting the reaction. Separately, a He-sparged solution containing MilliQ water and an excess of K3[Fe(CN)6] was prepared and added to the chamber to quantitatively oxidize the starting material to O2. The SOD disproportionation reaction was performed similarly but using a solution of CuZn SOD, horse-radish peroxidase (HRP, to reduce product H2O2 quantitatively to water), and K4[Fe(CN)6]•3H2O (reducing equivalents for HRP; Smirnov and Roth, 2006). To further survey ROS reactions in the environment and their impact on the oxygen isotope systematics of dissolved O2, we examined one non-enzymatic ROS decay pathway - Fe-mediated H2O2 degradation. We used the methods outlined in Dole et al. (1952) with the same experimental apparatus described above to explore this Fe-catalyzed pathway. Reaction progress was monitored by comparing the amount of O2 evolved from the reaction vessel relative to that from the KMnO4 treatment.

Instruments:
Following O2 gas collection, the sample volume was sealed with gas-tight valves under He flow and immediately transferred to a gas purification manifold to be analyzed on a Thermo Scientific MAT 253 Plus isotope ratio mass spectrometer (IRMS) at Harvard University (Cowie and Johnston, 2016). Carrier He gas was pumped away from the still-frozen sample before thawing. Oxygen gas should be the only product gas evolved from these reactions; however, to ensure trace atmospheric contaminants were not present, each sample was passed through a 3 m gas chromatography (GC) column packed with 5A molecular sieve maintained at 30 °C with He carrier gas at 15 mL/min. Oxygen was collected from GC effluent on a U-trap containing 5A molecular sieve held at liquid nitrogen temperature. Since all sample O2 was generated from KO2 and H2O2, Ar - which can interfere with TOI analyses - was not present (Yeung et al., 2018; Ash et al., 2020). Finally, effluent O2 gas was cryo-focused and allowed to thaw at room temperature for a minimum of 20 min to ensure no isotope fractionation before introduction into the IRMS for analysis. Each analysis comprises the average value of four acquisition blocks, each consisting of 20 cycles between the reference gas and sample gas (total counting time on sample gas was 400 s per acquisition). Measurements were typically run at 5000 m V signal on the m/z 32 Faraday cup (3 x 10^8 resistor). Total acquisition time for a single analysis is roughly two hours.

Data Processing Description

All values of d17O and d18O were converted to a VSMOW2-SLAP2 scale using a two point calibration of O2 liberated from VSMOW2 and SLAP2 standards using a CoF3 reactor as previously described (Schoenemann et al., 2013; Barkan and Luz, 2005). All slopes (i.e., k values) and slope uncertainties were calculated as linear fits to plots of d17O vs. d18O using the polyfit function in MATLAB (linear least-squares). Typical reproducibility of Ar-free lab air is 0.005‰ for D'17O and 0.02‰ 0.02‰ for d18O. The oxygen isotope results presented in this study are the mean and standard deviation when the number of samples receiving the exact same treatment is greater than one. In situations where replicates were not possible (e.g., measurements along a reaction progress array), uncertainty is assumed to be equal to the reproducibility of lab air reported above.

BCO-DMO Processing Description

- Imported original file "Sutherland GCA table.xlsx" into the BCO-DMO system.
- Renamed fields to comply with BCO-DMO naming conventions.
- Saved the final file as "923859_v1_reactive_o2_species.csv".

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Related Publications

Ash, J. L., Hu, H., & Yeung, L. Y. (2019). What Fractionates Oxygen Isotopes during Respiration? Insights from Multiple Isotopologue Measurements and Theory. ACS Earth and Space Chemistry, 4(1), 50–66. https://doi.org/10.1021/acsearthspacechem.9b00230
Methods

Barkan, E., & Luz, B. (2005). High precision measurements of 17O/16O and 18O/16O ratios in H2O. Rapid Communications in Mass Spectrometry, 19(24), 3737–3742. Portico. https://doi.org/10.1002/rcm.2250
Methods

Cowie, B. R., & Johnston, D. T. (2016). High-precision measurement and standard calibration of triple oxygen isotopic compositions (δ18O, Δ17O) of sulfate by F2 laser fluorination. Chemical Geology, 440, 50–59. https://doi.org/10.1016/j.chemgeo.2016.07.003
Methods

Dole, M., Rudd, D. P., Muchow, G. R., & Comte, C. (1952). Isotopic Composition of Oxygen in the Catalytic Decomposition of Hydrogen Peroxide. The Journal of Chemical Physics, 20(6), 961–968. https://doi.org/10.1063/1.1700657
Methods

Schoenemann, S. W., Schauer, A. J., & Steig, E. J. (2013). Measurement of SLAP2 and GISP δ17O and proposed VSMOW-SLAP normalization for δ17O and 17Oexcess. Rapid Communications in Mass Spectrometry, 27(5), 582–590. Portico. https://doi.org/10.1002/rcm.6486
Methods

Smirnov, V. V., & Roth, J. P. (2006). Mechanisms of Electron Transfer in Catalysis by Copper Zinc Superoxide Dismutase. Journal of the American Chemical Society, 128(51), 16424–16425. https://doi.org/10.1021/ja066369r
Methods

Sutherland, K. M., Hemingway, J. D., & Johnston, D. T. (2022). The influence of reactive oxygen species on “respiration” isotope effects. Geochimica et Cosmochimica Acta, 324, 86–101. https://doi.org/10.1016/j.gca.2022.02.033
Results

Yeung, L. Y., Hayles, J. A., Hu, H., Ash, J. L., & Sun, T. (2018). Scale distortion from pressure baselines as a source of inaccuracy in triple-isotope measurements. Rapid Communications in Mass Spectrometry, 32(20), 1811–1821. doi:10.1002/rcm.8247
Methods

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Parameters

Parameter	Description	Units
Sample	Experimental label	unitless
n	Number of replicates	unitless
d18O_smowslap	The 18O/16O ratio on a SMOW scale	permil
D17O	The capital delta value	permil
d17O_smowslap_log	The 17O/16O ratio on a SMOW scale, log form	unitless
d18O_smowslap_log	The 18O/16O ratio on a SMOW scale, log form	unitless

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Instruments

Dataset-specific Instrument Name	Faraday cup
Generic Instrument Name	Faraday cup
Generic Instrument Description	A Faraday cup is a metal (conductive) cup designed to catch charged particles in a vacuum. The resulting current can be measured and used to determine the number of ions or electrons hitting the cup.

Dataset-specific Instrument Name	3 m gas chromatography (GC) column
Generic Instrument Name	Gas Chromatograph
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Thermo Scientific MAT 253 Plus isotope ratio mass spectrometer (IRMS)
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

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Project Information

Clumped Oxygen Isotope Signature of Marine Dissolved Oxygen (Microbial isotope effects)

NSF Award Abstract:
The balance between photosynthetic production of oxygen and biological consumption of oxygen in the marine environment plays a critical role in regulating the composition of Earth's atmosphere and the long-term stability of Earth's climate. The ability to accurately measure the production and consumption of oxygen in the marine environment is central to building an informed understanding of the past, present, and future of oxygen and Earth's climate. Measurement of the abundance of different oxygen isotopes (i.e. oxygen-16, oxygen-17, and oxygen-18) in dissolved oxygen in seawater is a powerful analytical tool that can be used to determine the magnitude of photosynthesis and biological oxygen consumption. This is possible because biological reactions that produce or consume oxygen tend to preferentially utilize different isotopes of oxygen. This analytical tool has been used for two decades to investigate ocean primary productivity, however, there is still significant uncertainty in how biogeochemical processes such as respiration preferentially select and utilize different oxygen isotopes. To remedy this uncertainty, researchers at Harvard University will perform a lab-based

calibration and sea-going field deployment of emerging oxygen isotope analyses that target molecules of oxygen that contain two rare oxygen isotopes inside of one molecule (a.k.a. “clumps”, i.e. 17O18O and 18O18O). This work will quantify the clumped oxygen isotope signatures of several of the most consequential oxygen-involving reactions that occur in the marine ecosystem. These oxygen isotope signatures will be used to refine current methods and assumptions for the measurement of gross oxygen production in the global ocean. This research will also help train the next generation of Earth scientists through the mentorship of one graduate student and two undergraduate students. This project will also facilitate the participation of researchers in content creation for a national science competition for middle and high school students that reaches thousands of students annually.

The application of clumped O2 isotope measurements to dissolved oxygen in seawater is poised to give a greater mechanistic view of gross primary productivity and marine oxygen cycling than previously attainable. To realize the analytical potential of clumped oxygen isotope methods, these researchers will characterize the clumped oxygen isotope effects associated with enzyme-level reactions, whole organisms, and the marine water column. Enzyme-level studies will include isotope characterization of a terminal-O2 reductase and enzymes that metabolize reactive oxygen species such as superoxide and hydrogen peroxide. Organism studies will target common and numerically abundant phototrophs and heterotrophs to explore the expected breadth of isotope signatures and fractionation factors in the marine water column. Marine water column dissolved oxygen samples will be collected during one of multiple candidate cruises and analyzed using both traditional triple oxygen isotope techniques and newly developed clumped oxygen isotope methods. Lastly, they will employ the results of lab-based study of clumped O2 to build a model for the interpretation of environmental data with the aim of improving the accuracy and precision of field measurements of gross primary production.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2049298

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