Goldschmidt 2025 Abstract
https://doi.org/10.7185/gold2025.31082

Water stable isotope signals in molecules secreted by the marine diatom *Phaeodactylum tricornutum*

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As primary producers in aquatic ecosystems, algae play a crucial role in the provision of energy among food webs and fluxes of the global carbon cycle. Decades of research in algal physiology and paleo-records have established a consensus that water isotopes are systematically incorporated into algal biomass (particularly lipids) in response to environmental and physiological factors, such as temperature, salinity, and growth rate, yet this knowledge has not been extended to algal exudates that feed the microbial loop. In this study, the diatom *Phaeodactylum tricornutum* was cultivated at different temperatures (10, 18, and 22 °C) in YBCII minimal growth medium spiked with ^{2}H- and ^{18}O-labeled water, ranging up to +300 ‰ and +40 ‰, respectively. Cultures were harvested by centrifugation of biomass and solid phase extraction of dissolved organic matter (DOM) in the supernatant, and Uniprep-IRMS was employed to determine the stable isotope composition of their non-exchangeable H and O. These values were used to calculate the traditional isotope fractionation factors ($^{2}\varepsilon$ and $^{18}\varepsilon$) between the accumulating biomass, DOM, and water as well as water assimilation efficiency [1] into both biomass and DOM. Under natural isotope conditions, both $^{2}\varepsilon_{DOM\text{-}H2O}$ and $^{18}\varepsilon_{DOM\text{-}H2O}$ were correlated to cultivation temperature, with DOM becoming more depleted in ^{2}H and enriched in ^{18}O relative to water at higher growth temperatures, where DOC also accumulated to the highest concentrations (up to 0.8 mM C). In contrast, the magnitudes of $^{2}\varepsilon_{BIOMASS\text{-}H2O}$ and $^{18}\varepsilon_{BIOMASS\text{-}H2O}$ were highest at the apparent optimal growth temperature (18 °C). The spiked treatments revealed that diatom assimilation of water-H was stable across growth temperature ($^{2}a_{w}$ = 0.67 ± 0.05), whereas incorporation into DOM was much lower and strongly coupled with temperature. Parallel trends in water-O assimilation represent among the first ever reported, and alternatively to water-H, incorporation of the ^{18}O-label was higher in DOM relative to biomass. These findings will help to interpret $^{2/1}$H and $^{18/16}$O distributions of organic matter accumulating aquatic systems, and thereby our ability to read molecular messages imprinted by production and transformation processes.

[1] S. H. Kopf et al. (2016) Proc. Natl. Acad. Sci. U.S.A. 113, E110–E116.