Year-3 progress report ACS Award# 55430-ND2
Title: "Molecular-level Composition and Cycling of Dissolved Protokerogen: A Nuclear Magnetic Resonance and Carbon Isotopic Study"
September 1, 2017 through August 31, 2018
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The scientific objective of this study is to quantify relationships between dissolved organic carbon (DOC) molecular-level composition (via ^{1}H-NMR spectroscopy) and radiocarbon (^{14}C) age in several marine environments. We are studying the formation, diagenesis and removal mechanisms of recalcitrant DOC (i.e. carboxylic-rich alicyclic molecules; CRAM). Recalcitrant DOC can also be absorbed and transported by sinking particles and preserved in marine sediments. This *dissolved protokerogen* can influence the oceanic kerogen reservoir on geological timescales. We have been granted a second year no-cost-extension of this grant.

To date, we have accomplished approximately 75% of the goals that were proposed. We have measured all of the seawater samples from the North Atlantic and the Gulf of Mexico for DOC Δ^{14}C and δ^{13}C signatures and published these results. We have finished developing the ^{1}H-NMR method at UCI on seawater samples and have analyzed approximately 70% of the proposed samples using this technique. We estimate to have completed all ^{1}H-NMR seawater analysis by mid-2019. Details of our progress in year-3, and plans for year-4 appear below.

We have measured Δ^{14}C and δ^{13}C of water samples from the Mississippi River, river plume/shelf, slope and offshore Loop Current sites in the Gulf of Mexico (Walker et al. 2017, GRL). We found that DOC Δ^{14}C values decrease with distance from the river mouth, and that there are similar Δ^{14}C values found in the deep Gulf of Mexico and the deep Caribbean Sea. In addition, we found an isotopic imprint of mid-water column methane oxidation still persisting in the Gulf of Mexico following the Deepwater Horizon oil spill. This work is also being highlighted in a recently submitted book review chapter co-authored with Jeff Chanton (Springer International Publishing).

Dr. Walker is analyzing seawater samples using ^{1}H-NMR spectroscopy to obtain an understanding of how the molecular structure of DOC changes in its transfer from riverine, to estuarine, to open ocean pools. We have refined the water suppression method at UCI to obtain adequate spectra for our seawater samples. We found that storage of samples >24hrs in NMR tubes allowed for aromatic contamination from the caps to "grow into" our spectra. We also learned that additional 0.2µm filtration of seawater samples, thought to be a requirement for high particulate samples, resulted in gross contamination. An infrequent, and many times insuppressable water signal found within seawater samples has been attributed to the presence of residual particulate organic matter. We have found that centrifugation of sample ampules, and "sipping" carefully the top of samples and not shaking them at all has largely resolved this issue. To date, all of the Gulf of Mexico spectra have been analyzed. These spectra were presented at the 2018 Ocean Sciences Meeting in Portland, OR. We are very excited about the new approach that this opportunity provides for our research on refractory DOC (protokerogen) in riverine and oceanic systems of the Gulf of Mexico and the North Atlantic.

In the final phase of the study, Dr. Walker will be working with Dr. Hussain Abdulla at Texas A&M Corpus Christi to analyze the NMR spectra using novel 2D- and 3D-correlation statistical techniques in order to identify key dissolved protokerogen components.

This study will help answer fundamental questions involving the production, diagenesis and preservation of marine DOC. In particular, we are focusing on understanding the formation and removal mechanisms of recalcitrant DOC (RDOC). RDOC is a contributor of pre-aged, organic matter to sinking particulate organic matter. In this way, RDOC is an important source of protokerogen preserved in marine sediments over geologic timescales. During the grant period, Dr. Walker has also developed a novel graphitization technique for the natural abundance radiocarbon dating of ultra-small samples, including individual compounds in the RDOC pool (Walker and Xu, NIMB 2018). A technique our lab and Keck AMS facility now use routinely for small sample ^{14}C dating.

Most of the funds used during the past year were spent for the advanced education of doctoral student Christian Lewis. Lewis is learning how to conduct DOC isotopic analyses and molecular level characterizations of our seawater samples. He and Dr. Walker have collected more samples on a cruise in the South Pacific in winter 2017, which included a new solid phase extraction technique that are valuable for extracting protokerogen-like material from seawater. DOC extracted using this technique is to be subjected to molecular level characterization using ^{1}H-NMR and incorporated into the results obtained from the ACS PRF study.

Finally, a select subset of these seawater samples have been measured inside and outside of the oxygen minimum zone of the Eastern Tropical North Pacific. These ^{1}H-NMR spectra suggest large compositional changes within the ocean water column concomitant with a succession of microbial metabolism (i.e. oxic heterotrophy, to denitrification, and anaerobic ammonia oxidation).

Peer-reviewed papers

Walker, B.D., E.R.M. Druffel, J. Kolasinski, B.J. Roberts, X. Xu and B.E. Rosenheim (2017), Stable and radiocarbon isotopic composition of dissolved organic matter in the Gulf of Mexico, *Geophysical Research Letters*, 44 DOI:10.1002/2017GL074155.

Walker, B.D., Xu, X., 2018. An improved method for the sealed-tube zinc graphitization of microgram carbon samples and 14C AMS measurement. *Nuclear Inst. and Methods in Physics Research, B* 1–8. doi:10.1016/j.nimb.2018.08.004

Jeff Chanton, Aprami Jaggi, Jagoš R. Radović, Brad Rosenheim, Brett D. Walker, Stephen R. Larter, Kelsey Rogers, Samantha Bosman, and Thomas B.P. Oldenburg. Mapping isotopic and dissolved organic matter baselines in waters and sediments of the Gulf of Mexico. In Scenarios and Responses to Future Deep Oil Spills – Fighting the Next War. In review at *Springer International Publishing*.

Lewis, C.B., B.D. Walker and E.R.M. Druffel. Heterogeneity in dissolved organic carbon from coastal California, *Geophysical Research Letters*, in preparation.