Final Report for Period: 09/2009 - 08/2010 **Submitted on:** 11/01/2010
Principal Investigator: Perdue, E. Michael . **Award ID:** 0728050
Organization: GA Tech Res Corp - GIT
Submitted By:
Perdue, E. Michael - Principal Investigator

Title:
Collaborative Research: Comprehensive Chemical Characterization of Marine Dissolved Organic Matter using Efficient Isolation Coupled to Advanced Analytical Techniques

Project Participants

Senior Personnel

Name: Perdue, E. Michael
Worked for more than 160 Hours: Yes
Contribution to Project:
Dr. Perdue provided overall supervision of Nelson Green's doctoral research. In addition, he wrote several substantial computer programs that he and Nelson Green are using to investigate and improve the algorithms by which FTICR mass data are analyzed.

Post-doc

Graduate Student

Name: Green, Nelson
Worked for more than 160 Hours: Yes
Contribution to Project:
Nelson Green is the Georgia Tech graduate student whose primary research activity is this project. He was largely responsible for fabricating the new RO/ED system that is being used in this project and for all testing and calibration of the system. He is working closely with a graduate student at Old Dominion University (Ms. Hongmei Chen) to help our collaborating institution build and use their own RO/ED system. He participated in our first research cruise in September, 2008. He has developed considerable computational skills in the past year that have been invaluable to our efforts.

Undergraduate Student

Technician, Programmer

Other Participant

Research Experience for Undergraduates

Organizational Partners

Old Dominion University
This is a collaborative project between Georgia Institute of Technology and Old Dominion University.

Other Collaborators or Contacts

We are very grateful to Dr. Wenhao Chen at RSMAS who analyzed several critical samples for DOC and DON at no cost to our project.

Activities and Findings

Research and Education Activities:

In September, 2008, Dr. Perdue and Nelson Green participated in Cruise 449-3 of the R/V Oceanus between the Cape Verde Islands and the Canary Islands. They worked collaboratively with Dr. Aron Stubbins and Ms. Hongmei Chen from Old Dominion University to collect four large samples of seawater (355?473 L) from a deep ocean site (3000 m), from a site at the oxygen minimum layer (400 m), and from two surface sites (5 m) where very high fluorescence intensity in the seawater indicated a very high level of primary production. Each sample was processed in the ship's wet lab using the coupled reverse osmosis-electrodialysis (RO/ED) system. During processing at sea, volumes of samples were reduced by RO to 5?6 L and more than 99.99% of sea salts were removed by ED. Smaller aliquots of the four samples were processed using C18 solid phase extraction to isolate the more hydrophobic components of marine DOM. In 2009, Dr. Perdue and Nelson Green joined Dr. Aron Stubbins and Ms. Hongmei Chen from Old Dominion University to process two samples from deep (3500 m) and surface (5 m) seawater at Station ALOHA. The RO/ED method was used to isolate marine DOM at the Natural Energy Laboratory of Hawaii Authority in Kona, Hawaii. The research team from this project was joined at NELHA by 11 other scientists from four other institutions in a spontaneous, unfunded comparative study of several methods of isolation of marine dissolved organic matter. Our research team isolated DOM from a total of 20 seawater samples during this three-year project, recovering an average of 80 ? 11% of dissolved organic carbon (DOC). For the 14 samples of deep and surface ocean water that were collected and processed at NELHA, the average yield of DOC was 82 ? 6%. The final concentrated, desalted samples are in storage at Old Dominion University, where a variety of advanced chemical and spectroscopic methods are being used to characterize the isolated samples of marine DOM. Our co-investigators at Old Dominion University have received a no-cost extension on this project to complete those analyses.

The Georgia Tech group has devoted a significant effort to the development of computational methods that will enable us to maximize our ability to assign molecular formulae to the peaks that can be observed in FTICR mass spectra while minimizing the number of ambiguous or incorrect assignments. Our analysis of existing computational methods has revealed potential pitfalls that can very easily result in incorrect assignments of molecular formulae. These errors are attributable to chemical moieties of the general form 13Ca12CbHcOdNeSf that have a nominal mass of zero and a very small exact mass. As an example, the moiety 13C012C7H-8O3N-2S-3 has a nominal mass of zero and an exact mass of ?0.2165 mDa. Such moieties function as operators that interconvert two valid molecular formulae. For example,

12C12H24O8N2S3 + 12C7H-8O3N-2S-3  12C19H16O11
420.06948 Da -0.0002165 Da 420.06926 Da
H/C = 2.00 H/C = ?1.14 H/C = 0.84
O/C = 0.67 O/C = 0.43 O/C = 0.58

It may be noted that the two molecular formulae, whose masses are virtually indistinguishable, plot in significantly different regions of van Krevelen space. It is further noted that the moiety plots in one of the negative quadrants of van Krevelen space and that the set of three points lie on a straight line.

Findings:

The new RO/ED system that was constructed for this project functioned properly and has been used successfully at sea and in research laboratories. The yield of DOC is substantially greater than can be achieved by any other known method of isolation of marine DOM, and we anticipate that advanced chemical and spectroscopic analyses of the isolated samples will enable us to much more confidently discuss the chemical properties and reactivity of marine DOM.

We have thus far identified at least 100 moieties whose nominal masses are zero and whose exact masses are less than ?0.2165 mDa. Altogether, we have found at least 234 such moieties that have exact masses of ?0.5 mDa or less. For reference, the mass of an electron is approximately 0.5 mDa. Modern FTICR instruments have amazing capabilities, but some of these tiny masses lie beyond the resolution of such instruments.

Our computational work includes a systematic exploration of these zero-nominal-mass moieties and how they interact with the assumptions made by the user when FTICR-MS data sets are processed to assign molecular formulae. For example, if the user elects to allow at least two N and three S when fitting molecular formulae to FTICR-MS data, then some assigned molecular formulae may contain 'N2S3', even if the actual compound consisted of only C, H, and O. Conversely, a compound that contains 'N2S3' can be incorrectly assigned a molecular formula containing only C, H, and O, if less than two N and/or three S are allowed by the user.

We have found that such errors are deterministic, rather than random. As such, incorrect molecular formulae tend to aggregate in certain well-defined and predictable regions of van Krevelen graphs of H/C versus O/C. Many published results contain molecular formulae in these regions of van Krevelen space, so caution is required when chemical significance is attributed to fitted molecular formulae that plot in these suspect regions of van Krevelen space.

Inasmuch as the interpretation of FTICR-MS data sets is a key component of the collaborative project, our computational results will be shared in their entirety with our colleagues at Old Dominion University, who are primarily responsible for FTICR mass spectrometric analyses of our isolated samples of marine DOM. Chemical and spectroscopic results will be presented in the annual report of our collaborators at Old Dominion University.

Training and Development:

Nelson W. Green has learned all aspects of fabrication, calibration, and use of the RO/ED system. He has taken responsibility for purchasing components and designing some of the electronic subsystems of the RO/ED system. By aiding another student in my research group on a related project and by working closely with the graduate student at our collaborating institution, he has also had some opportunity to share this recently acquired knowledge with his peers. He has now completed two research cruises (one of which is outside this project), during which he was responsible for most of the labor-intensive operations of the RO/ED system. He has also used the RO/ED system in research laboratories to process a large number of seawater samples.

Outreach Activities:

Three presentations have been made at the Humic Science and Technology Conference in March, 2009, for which the Honorary Chair and Dedicatee were Dr. Perdue and Dr. Hatcher (Old Dominion University), respectively. Dr. Perdue gave a lecture at the Humic Science and Technology Conference in March, 2010, an invited lecture at the 2010 American Chemical Society meeting in San Francisco, CA, a lecture at the 2010 meeting of the International Humic Substances Society in Tenerife, and an invited lecture in 2010 at Helmholtz Muenchen. Three posters were presented at the Symposium on Unknown Knowns and Known Unknowns: Chemical Oceanography in a Changing World, organized by Skidaway Institute of Oceanography, Savannah, GA, February 22-24, 2009. Nelson Green has given a lecture in 2009 at the AIChE conference in Nashville, TN.

Journal Publications

Books or Other One-time Publications

Web/Internet Site

Other Specific Products

Contributions

Contributions within Discipline:

Contributions to Other Disciplines:

Contributions to Human Resource Development:

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Conference Proceedings

Categories for which nothing is reported:

Any Journal

Any Book

Any Web/Internet Site
Any Product
Contributions: To Any within Discipline
Contributions: To Any Other Disciplines
Contributions: To Any Human Resource Development
Contributions: To Any Resources for Research and Education
Contributions: To Any Beyond Science and Engineering
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Organizational Partners
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Activities and Findings
Research and Education Activities:
Just prior to the research cruise in September, 2008, our new electrodialysis stack was destroyed during our tests to determine the limiting current density of our system when used to desalt seawater. The manufacturer of the stack was fortunately able to rebuild it (at substantial additional cost) in time to participate in Cruise 449-3 of the R/V Oceanus between the Cape Verde Islands and the Canary Islands. Dr. Perdue and Nelson Green from Georgia Tech worked collaboratively with Dr. Stubbins and Ms. Hongmei Chen from Old Dominion University to collect four large samples of seawater (355–473 L) from a deep ocean site (3000 m), from a site at the oxygen minimum layer (400 m), and from two surface sites (5 m) where very high fluorescence intensity in the seawater indicated a very high level of primary production. Each sample was processed in the ship's wet lab using the coupled reverse osmosis-electrodialysis

(RO/ED) system. During processing at sea, volumes of samples were reduced by RO to 5–6 L and more than 99.99% of sea salts were removed by ED. Smaller aliquots of the four samples were processed using C_{18} solid phase extraction to isolate the more hydrophobic components of marine DOM. Advanced chemical and spectroscopic characterization of the isolated samples of marine DOM are underway at Old Dominion University.

The Georgia Tech group has turned its attention to the development of computational methods that will enable us to maximize our ability to assign molecular formulae to the peaks that can be observed in FTICR mass spectra while minimizing the number of ambiguous or incorrect assignments. Our analysis of existing computational methods has revealed potential pitfalls that can very easily result in incorrect assignments of molecular formulae. These errors are attributable to chemical moieties of the general form $^{13}C_a{}^{12}C_bH_cO_dN_eS_f$ that have a nominal mass of zero and a very small exact mass. As an example, the moiety $^{13}C_0{}^{12}C_7H_{-8}O_3N_{-2}S_{-3}$ has a nominal mass of zero and an exact mass of –0.2165 mDa. Such moieties function as operators that interconvert two valid molecular formulae. For example,

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Findings:

The new RO/ED system is functioning properly and has already been used successfully at sea in September, 2008. Although the equipment functioned properly, the lack of access to a suitable carbon analyzer has complicated the determination of the yield of marine DOM (as organic carbon) at each of the four sample sites. Until very recently, neither the Georgia Tech group nor the Old Dominion group had a low-level carbon analyzer that is suitable for analysis of seawater. The Georgia Tech group has sent a few carefully selected samples (aliquots of initial, final, and rinse solutions) to Dr. Wenhao Chen at RSMAS for TOC measurements. Lacking a TOC instrument, we have not yet analyzed the multitude of samples that were collected during each RO/ED experiment for the purpose of tracking the yield of TOC during the process. Such results will allow us to identify the point(s) in the process when losses of TOC are most substantial. Aliquots of final and rinse solutions were also analyzed by colleagues at Old Dominion University using an older, less sensitive instrument that lacks the sensitivity to analyze the initial solutions. The two sets of TOC measurements for final and rinse samples are in partial agreement, but the uncertainty in TOC concentrations complicates any effort to achieve a mass balance. Based on the available data and pending further measurements of TOC on preserved aliquots of important samples, the yields of marine DOM from the oxygen-minimum sample and the two surface samples are 55, 78, and 74%, respectively. For reference, the average yield of DOM from 16 samples that were collected previously using the RO/ED method is 75 ± 12%.

Dr. Chen found that the aliquot of the initial deep ocean sample was somehow contaminated, most likely with surface seawater, so it has not yet been possible to obtain a direct estimate of the yield of marine DOM for that sample. If we assume an initial TOC concentration of 41 μM for that sample, then the yield of marine DOM from the deep ocean sample is approximately 47%. Although this yield is much less than those obtained in other applications of the RO/ED method, it still compares favorably with yields that have been obtained using most other methods. Average molar C/N ratios for the deep ocean sample, the oxygen-minimum sample, and the two surface samples are 16.4, 15.9, 14.5, and 15.6, respectively. We have frozen, redundant aliquots of all important samples, and a comprehensive set of TOC measurements will be conducted at Old Dominion University as soon as their new TOC instrument is operating within specifications.

We have thus far identified at least 100 moieties whose nominal masses are zero and whose exact masses are less than ±0.2165 mDa. Altogether, we have found at least 234 such moieties that have exact masses of ±0.5 mDa or less. For reference, the mass of an electron is approximately 0.5 mDa. Modern FTICR instruments have amazing capabilities, but some of these tiny masses lie beyond the resolution of such instruments.

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