ULTRAHIGH RESOLUTION MASS SPECTROMETRY OF DISSOLVED ORGANIC MATTER IN ESTUARIES

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Introduction

Electrospray ionization combined with high-field Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) can now identify individual compounds in complex dissolved organic matter (DOM) mixtures. This ultrahigh resolution (UHR) technique generates large databases of chemical formulas for individual samples which can be searched for specific compounds and compound classes or integrated across the entire dataset to summarize the molecular characteristics of DOM (e.g. aromaticity, elemental ratios, degree of unsaturation). Here we demonstrate how UHR MS data can define the molecular changes that accompany terrestrial DOM as it moves from its fresh water sources through estuaries and into the coastal zone. Specific examples included are microbial alteration of riverine DOM as it percolates through coastal sands, and photochemical processes that change fluorescence signatures.

Figure 1: Ultrahigh resolution (9.4 Tesla) FT-ICR Mass Spectrometer at the National High Magnetic Field Laboratory. The use of this instrument to describe the molecular composition of DOM mixtures has been described in several previous publications.

A combination of in-situ measurements in the Gulf of Mexico and laboratory experiments in columns (see Figure 2 below) have demonstrated that filtration through 5 cm permeable shelf sediments promotes the degradation of diatom-derived and riverine DOM. Photochemical effects were identified by irradiating riverine DOM in a solar simulator for 21 hours. Ultrahigh resolution mass spectra data were evaluated for changes in degree of unsaturation, elemental H/C ratios and oxygen-subtracted double bond equivalency (DBE-O).

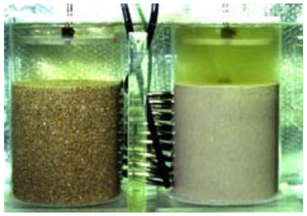

Figure 2: This experiment tested whether filtration of DOM (derived from river water) at realistic flushing velocities through a sediment layer, comparable in thickness to the flushed sediment surface layer at the study site, causes degradation of the DOM. Fifty cm long sediment columns were used to test the hypothesis that a fraction of the DOM is degraded while passing through natural marine sediment. See poster by Huettel et al. for additional information regarding these experiments.

Materials and Methods

Sampling. For irradiation experiments, 2 L water samples were collected from the Black River (salinity 0) and Cape Fear River Estuary (salinity 13) in North Carolina. One liter from each sample was irradiated for 21 h using a 1000 W solar simulator, while one liter of the same water sample was stored in the dark at 4 °C (dark controls).

In the sand filtration experiment, one of two 50 cm columns was filled with sterile sediment (combusted for 8 h at 500 °C) and another was filled with natural sediment. Water collected from the St. Marks river estuary 50 miles east of our St. George Island study site that had a DOC concentration of 615 µM was pumped for 48 h through the columns. Samples were collected in 500 ml glass bottles at the entry, and then the exit of each column (1 natural and 1 sterile). It took approximately 10 h to collect each.

DOM Extraction. Prior to mass spectrometric analyses, sample solutions were acidified to pH 2 and extracted using Varian Mega Bond Elut PPL solid-phase extraction (SPE) cartridges filled with 1 g of the PPL resin. The cartridges were activated with methanol and rinsed with 20 mL acidified Milli-Q water (pH 2). After the sample solution had passed through the SPE cartridge, the remaining salts were washed off with acidified Milli-Q water and the cartridge vacuum dried prior to the elution of the adsorbed dissolved organic matter (DOM) using 10 mL HPLC-grade methanol.

Excitation Emission Matrix Fluorescence (EEM) Spectroscopy. EEM fluorescence measurements were measured using a Jobin Yvon SPEX FluoroMax-3 fluorescence spectrometer. The emission was recorded between 280 and 600 nm for excitation wavelengths at 5 nm intervals ranging from 250-500 nm. Fluorescence intensities are expressed as quinine sulfate equivalents (QSE).

ESI-FT-ICR-MS Analysis. All samples were all analyzed using the 9.4 Tesla FT-ICR mass spectrometer at the National High Magnetic Field Laboratory (NHMFL), Tallahassee, Florida, USA. A spectrum of ions present in one DOM sample can contain several thousand peaks. A particularly useful method to interpret the data is to create van Krevelen diagrams, which are effective for visualizing large numbers of exact molecular formulae. A Van Krevelen plot consists of elemental ratios of hydrogen to carbon (H/C) (y-axis) for each formula identified plotted against the oxygen to carbon (O/C) ratios (x-axis) of the same formula.

Results and Discussion

Photochemically-induced changes in the EEM fluorescence of DOM. An EEM fluorescence spectrum provides a three dimensional plot which can be used to distinguish fluorescent compound groups which show distinct differences in their excitation and emission maxima. Figure 3 suggests that the decrease in specific classes of the EEM fluorescence (humic-like A-peak and fulvic-like C-peak) are quite different in these samples. The marked changes in optical properties after exposure to simulated sunlight appear to be due to the original molecular composition of the DOM (Figure 4).

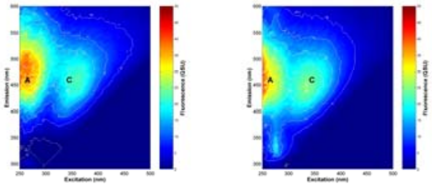

Figure 3. Absolute changes in the EEM fluorescence intensities arising from 21 h solar irradiation in the Black River (left) and Cape Fear Estuary (right) samples.

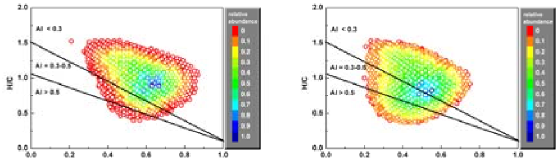

Figure 4. van Krevelen diagrams summarizing molecular formulas of the (a) Black River and (b) Cape Fear Estuary DOM samples.

Effects of Filtration through Sand Columns on DOM Composition. Figure 5 contains three-dimensional van Krevelen diagrams that describe the intensities of molecular formulas before and after passage through the sand columns. There are clearly complex processes involved that result in both loss of some compounds and appearance of others.

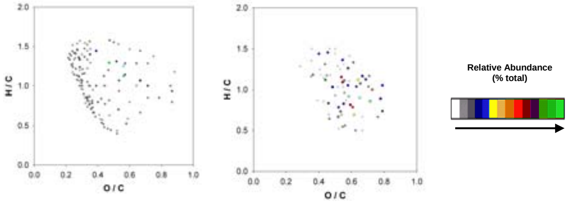

Figure 5. van Krevelen diagrams of DOM before and after passage through sand columns. Left; formulas lost: Right; formulas that appear.

Acknowledgements

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