Sarah HENDRICKSON
Lewis and Clark Fund in Astrobiology Field Scholars
awarded in 2011

Dissolved Organic Carbon Cycling in the Deep Crustal Biosphere

Project Report

Introduction

Situated in central South Africa, the Witwatersrand Basin is nearly 3 billion years old. The formation began when fluvial systems deposited massive amounts of sediment into an inland sea. This initial process, followed by periods of volcanism and metamorphosis, resulted in the deposition, consolidation, of the three major units: the Witwatersrand Supergroup (2.9 Ga), the Ventersdorp Supergroup (2.7 Ga) and the Transvaal Supergroup (2.45 Ga). Approximately 2 Ga, one of the largest meteorites know to have made impact with Earth, struck what is now the center of the basin (Fig. 1). A cross section created using seismic profiling is shown in (Fig 2); note the significant number and varied orientation of the faults.

Faults and fractures allow for distribution of water from hydrothermal sources in the lower crust and meteoric water from the surface. Our samples consist of water that has remained in the subsurface for anywhere between 10,000 and over one hundred million years. This environment may seem inhospitable to life, but over the past decade molecular genomic analyses have shown a ubiquitous presence of microbial communities at depths of over two miles. Our responsibility within this collaboration was to quantify the amount of dissolved organic carbon (DOC) in these water samples in order to better understand the sources and sinks of carbon in this system, particularly to determine whether the carbon is of terrestrial or microbial origin.

Our samples were collected at depths between 1.3 kilometers and over 4 kilometers.

Field work

Over a seven week period between from July to August, 2011, the group visited four sites, collecting bulk water samples at each site and performing one isolation consisting of loading 105 liters of bulk water onto the macroporous resin columns at Goldfield's Beatrix Mine. Water was collected from boreholes fit with a stainless steel manifold, run through acidified Teflon tubing, passed through Whatman PES 0.2 micron filters and stored in acidified and combusted glass bottles with Teflon coated caps. This protocol was used at each site to minimize organic contamination. Samples were refrigerated, as freezing might result in the separation and concentration of salts which might ultimately damage any DOC in the samples.

Filtered, bulk-water samples underwent the following analyses: DOC concentration, total nitrogen concentration, ultra violet-visible spectrum spectroscopy, fluorescence spectroscopy and the creation of excitation-emissions matrices (EEMs), and one sample was sent to Woods Hole Oceanographic Institute for carbon isotope analysis.

A dual column array of macroporous resin was used to isolate carbon from 105 liters of water at the Beatrix, Borehole 2 site (Fig. 3). Water was filtered, acidified with hydrochloric acid and passed over the two columns in series at an average rate of 0.75 L/min. The columns were then eluted back at the microbiology teaching lab at the University of the Free State (UFS), Bloemfontein, South Africa. Eluted water was then lyophilized (partially at UFS and the remainder at New Mexico Institute of Technology in Socorro, New Mexico). This isolate was sent to Dr. Cathy Clewett at West Texas A&M who performed solid state NMR at Sandia National Laboratory, Albuquerque, NM.

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Results

DOC Concentration

It was hypothesized that DOC concentration would decrease with depth. Although all samples contain low concentrations of DOC compared to the majority of surface water sites, there does not appear to be a correlation with depth. In fact, the highest concentration samples collected was from the Kloof Mine borehole, at a depth exceeding 4 km (Table 1). Total nitrogen concentration were not correlated to carbon concentrations; Joel Mine in the Welkom region was one of the more shallow samples, had a low DOC concentration, but had a much higher nitrogen concentration than any of the other samples (Table 1).

UV-Vis Absorbance

All samples were analyzed with a Shimadzu spectrophotometer within the wavelength range of 200 nm to 700 nm. Absorbance intensity is proportional to the concentration of molecules absorbing at that wavelength. The wavelength at which the molecules have the greatest absorbance tells us a bit about the type of carbon molecules in the samples. In general, absorbance near 350 nm is representative of lignin, 254 nm would indicate aromatics, while absorbance in the 210 nm-240 nm range of aliphatics. These are significant characteristics when attempting to elucidate a carbon source; lignin and aromatics are associated with terrestrial plant material while aliphatics are indicative of a microbial source.

Deep subsurface samples collected in 2011, and during following expeditions, appear to have aromatics in concentrations below the detection limit. No samples exhibit absorbance at wavelengths greater than 240 nm (Fig. 4).

Fluorescence and Excitation-Emissions Matrices (EEMs)

In fluorescence analyses, a sample absorbs a photon to move to some excited state and emits a photon to return to the ground state. The intensity of the fluorescence is generally proportional to the concentration of fluorophores in the sample.

The construction of an excitation-emission matrix (EEM) for a particular sample can determine the presence of a variety of carbon compounds such as proteins or humic/fulvic substances associated with plant decomposition. The EEM requires that the fluorometer run a scan of fluorophores at all possible wavelengths. As the excitation wavelength remains constant, emission wavelengths are scanned; as subsequent emission scans are measured at various excitation wavelengths, a 3D image emerges. The contour lines indicate intensity, and the peak location indicate the presence of certain DOC components, with protein-like substances associated with the excitation-emission pair found at 278 nm- 330 nm (310 nm for tyrosine –like and 340 nm for tryptophan-like) and humic-like substances at 380 nm-460 nm (Coble, 1995).

All of our samples show the presence of a protein-like or possibly a polysaccharide peak (Her, et al, 2003) and no peak in the fulvic-humic range (Fig. 5).

High Performance Size Exclusion Chromatography (HP-SEC)

High pressure liquid chromatography can be used to separate molecules based on size. The size distribution can then be used as a proxy for molecular weight distribution. This method passes a mobile phase through a column and

records the retention time based on the size of the molecules. Low molecular weight molecules are slowed by pores within the column material while larger molecules pass more quickly; higher retention times are indicators of low

molecular weight. We compare the retention times in our samples to those of five polymer standards or known molecular weight to estimate the atomic mass of our molecules.

We ran HPLC-SEC at six wavelengths between 205 nm and 230 nm, since absorbance appeared to drop off after 240 nm. At 205 nm nearly every molecule exhibits absorbance, so we chose 210 nm as the best representation of the absorbance in our samples. Figure 6 shows the log molecular weight distribution at this wavelength, with the majority of molecules falling in the range of 100 to 1000 Daltons.

Amino acids and Carbohydrates

One of our goals was to quantify the total carbohydrates in the samples as well as identify the types and concentrations of primary and secondary amino acids. To date, we have not been able to find methods that are sensitive enough for these high sulfur, low DOC concentration, often saline samples. Removing salts was considered, but the complexation of salts with DOC would likely lead to a situation where the carbon would also be removed from the sample.

Nuclear Magnetic Resonance (NMR)

The first sample to be collected on the resin columns, and the only one from the summer of 2011, was contaminated with metals during freeze drying. The canisters in South Africa were decontaminated, but the amount of time required to lyophilize upwards of 8 liters of eluate in an old freeze dryer lead to a long contact time between the sample and the canister. The sample was sent for analysis, and preliminary results, despite likely paramagnetic interference, are shown in Table 2. Despite the interference, it does appear that the carbon in the Beatrix sample is primarily aliphatics with little aromatic content. This is consistent with our bulk water analyses.

Conclusion

All analyses point to a microbial origin of DOC. There is no evidence of input from the surface, which is consistent with the old ages of the water samples and the ancient molecular signatures of the bacterial communities.

Two more resin isolation trips occurred during the summer of 2012, and results are pending.

I have completed my research at New Mexico Tech and am currently living in Albuquerque, New Mexico.