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# Biogeochemical Characterization of Core, Fluids, and Gas at MSEEL Site

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**Summary**

The deposition and genesis of organic-rich shale create complex heterogeneities in the reservoir, which remain poorly understood despite their importance in the unconventional energy revolution. In addition, several questions need to be answered about the complex biogeochemical interactions that take place in the reservoir after injection of hydraulic fracturing fluids. Our interdisciplinary team is utilizing the core, fluids, and gas samples collected from the Marcellus Shale Energy and Environmental Laboratory (MSEEL) to develop a better understanding of these characteristics and processes to ensure that energy is extracted in an economically efficient and environmentally responsible manner.

The availability of well-preserved cores from MSEEL has given us the opportunity to significantly increase the resolution of subsurface stratigraphic characterization. We integrated geochemical, petrographic, molecular, and isotopic techniques to better understand the biogeochemical controls on temporal heterogeneities in quantity and quality of total organic carbon (TOC). The preliminary data from multiple proxies indicate that temporal variations in paleoredox condition, depositional environment, and microbial cycling effect both the type and amount of organic carbon preserved in the core. The composition, molecular structure, porosity and density of kerogen also appears to be affected by the same processes.

The unrestricted access to hydraulic fracturing fluid, drilling additives and produced fluid/gas samples with minimal exposure to the atmosphere has enabled our team to develop preliminary models of fluid-gas-shale and biogeochemical interactions in the reservoir. Our initial results on the produced water chemistry indicate that injection of hydraulic fracturing fluids results in dissolution of pristine biogenic carbonates in the reservoir. The increasing concentrations of redox-sensitive elements over time in produced water is strongly suggestive of the existence of an active anaerobic microbial community in the reservoir involved in fermentation of carbon compounds in hydraulic fracturing fluids, sulfur reduction, and methanogenic production. The molecular composition of produced gases in conjunction with the stable and noble gas isotopic signatures of produced gases and waters is being monitored over time to further constrain the biogeochemical interactions in the reservoir.

**Introduction**

The MSEEL site is located in the heart of the dry-gas area of the Marcellus Shale play in Monongalia County, West Virginia. Northeast Natural Energy LLC (NNE) drilled two previous wells the MIP 4H and MIP 6H in 2011. The two NNE wells are inside the gas "city gate", where the gas is transferred to the local natural gas utility, and are constrained by the natural gas usage of Morgantown. Production from the old wells had declined and two wells (MIP 5H and 3H) drilled as part of the MSEEL project help meet the city demand. A dedicated scientific observation well was drilled in-between MIP 5H and 3H horizontal laterals to collect detailed subsurface data, and to monitor and test new hydraulic fracturing technologies in production wells drilled over the project lifetime. A diverse team of hydrologists, geochemists, health professionals and social scientists are conducting water/air quality and noise monitoring at this site over the five-year period. Researchers will also be given access to produced water and gas samples from the hydraulically fractured horizontal production well before they are disposed in holding tanks or pumped to production/distribution

**Methods**

Core collection and analysis

Complete 111 feet of vertical core and 50 sidewall cores were obtained from well 3H. In addition, 150 sidewall cores were collected from the scientific observation well. The cores were preserved on the site per the required protocols and available to all investigators for a wide variety of microbiological/geochemical/mineralogical/ sedimentological analysis, macro and micro scanning and conducting high-pressure temperature experiments.
The core plugs samples collected from every foot of the vertical core and side wall cores were ground and homogenized and distributed among different investigators for geochemical and mineralogical analysis using ICP-MS, X-ray fluorescence (XRF), X-ray diffraction (XRD), and pyrolysis. Total organic carbon (TOC), stable carbon isotopic composition of bulk organic carbon ($\delta^{13}C_{org}$), and nitrogen ($\delta^{15}N_{org}$) were analyzed using a combustion elemental analyzer (EA), coupled to a Delta Advantage IRMS via a ConFlo IV interface while inorganic carbon ($\delta^{13}C_{carb}$) was measured using a GasBench coupled to a Delta Advantage IRMS. Samples and internal standards were analyzed in replicates to determine the precision of instrument which was better than ± 0.1‰ for $\delta^{13}C$ and ± 0.2‰ for $\delta^{15}N$. The internal laboratory standards and check standards (USGS 40 and USGS 41) were calibrated and reported in per mil (‰) relative to their respective IAEA standards (V-PDB and AIR) for $\delta^{13}C$ and $\delta^{15}N$ respectively.

Kerogen was also extracted from sidewall cores collected from Mahantango Formation and different zones of Marcellus Shale. The kerogen extraction procedure was standardized using both chemical and physical separation techniques, and was performed by extracting the maximum amount of unaltered kerogen with effective removal of soluble organic matter, carbonates, silicates and heavy minerals (Agrawal et al., 2016). Kerogen was analyzed using direct analytical techniques such as XPS (X-ray photoelectron spectroscopy), ATR-FTIR (Attenuated total reflection-Fourier transform infrared spectroscopy), 13C solid state NMR (Nuclear magnetic resonance) and Raman spectroscopy to characterize its molecular structure.

Sidewall cores were collected using recommended practices in Wilkins et al., 2014 from a 360-foot span within the MIP 3H well targeting the Genesseo, Tully, Mahantango, Marcellus and Onondaga formations. Briefly, fluorescent microspheres (0.5 μm, Polysciences Inc.) were added to the drilling mud at a concentration of ~105 beads per mL. After collection, cores were immediately transported to The Ohio State University anaerobically, on ice. Cores were surface sterilized using successive brine washes (sterile 1.5M NaCl solution). Microscopy was used to verify removal of microsphere tracers and cores were ground to a particle size less than 500 μm and homogenized. All ground core material was stored at -80°C until DNA extraction. Low biomass in the subsurface makes it important to develop methods of lipid extractions with the ability to yield higher and persistent recovery of microbial lipid biomarkers. We, therefore, conducted experiments and tested different established microbial lipid extraction procedures to avoid the underestimation of resulting profiles, biomass, and diversity. We tested three extraction methods: (i) modified Bligh and Dyer (mBD), (ii) Folch (FOL), and (iii) microwave assisted extraction (MAE) treatments. Within mBD method we utilized either a phosphate or citrate buffer. We also tested the effect of three different spikes on the extraction performance: (i) magnesium (Mg2+), (ii) Escherichia coli biomass (E.coli), and (iii) intact phospholipid (POPC) to determine if the lipid yield and profile quality of the mBD extraction treatments could be enhanced.

Produced gas and fluid collection and analysis

Produced water samples were collected in 3-5 gallon (~11-18 litres) carboys just after the separator. The samples were transported, filtered and processed in Sharma Laboratory at WVU immediately after sampling. All water samples were collected in different containers using different methods and preservatives specified for different kinds of analysis. The geochemical analysis of water was analyzed via ICP-OES and ICP-MS. Ammonia and phosphate were measured on the Skalar nutrient analyzer. The samples were collected hourly during the initial phase of flowback followed by weekly to monthly sampling during later stages. Currently, samples are being collected every six weeks. The collected fluids are currently being processed for biomass, reactive chemistry, organic acids, and noble gas and stable isotope analysis at different institutes.

Produced gas samples were collected from well heads of the two production wells and transported to the Sharma Lab at WVU and analyzed for molecular composition and C/H isotope composition of methane, ethane, and $CO_2$ using GC Isolink connected to a Finnigan Delta Advantage mass spectrometer. A duplicate set of gas samples were sent to Darrah's lab at OSU for He, Ne, Ar, Kr, and Xe concentration analysis by quadruple mass spectrometry; and helium, neon, and isotopes by noble gas mass spectrometry. Both flow back fluids and produced gas samples have shown low levels of atmospheric gasses, indicating the acquisition of high-quality samples (a known challenge in sampling for noble gasses).

**Results**

The Total Organic Carbon (TOC) concentrations show a general decreasing trend from bottom (8-10%) to top (2-4%) of Marcellus. The $\delta^{13}C_{org}$ was relatively higher (-29‰ V-PDB) in the organic matter (OM) poor zone compared to the organic rich (OR) zone (-31‰ V-PDB) of the lower Marcellus, suggesting more influence of terrestrial OM in OM-poor zone compared to the OM-rich zone. Both $\delta^{13}C_{org}$ and $\delta^{15}N_{org}$ show larger fluctuations in the bottom OR zone indicating fluctuating redox and more nutrient recycling that might have fueled higher productivity and higher OM accumulation in that zone (Chen and Sharma 2016; Sharma et. al, 2016). The analysis of bulk geochemistry and pyrolysis data collected from all the plugs from the vertical core and sidewall cores is currently underway.

Bulk mineralogy of core samples, as determined with powder XRD, of core samples from well MIP 3H shows that Marcellus core is composed mainly of siliciclastic mudstones with interbedded carbonates. There is high content of illite in Mahantango and Marcellus Shale and overall the clay content decreases with depth and is accompanied by increase in TOC (Song et al., in press). Of the four sidewall cores targeted within the Marcellus, three (Marcellus top, depth 7451', middle (7509') and lower (7543') are mainly comprised of phyllosilicates (mostly illitic clay with minor chlorite), quartz, pyrite, alkali feldspar, calcite and dolomite. One (upper Marcellus, depth 7467') is a carbonate mudstone composed mostly of calcite, quartz, and dolomite, but with minor illite and chlorite detected. SEM images of unpolished fragments of this sample show sub-micron scale chlorite platelets and relatively small patches of organic matter (OM) disseminated throughout a dominant calcite matrix.

SEM analysis of unpolished, bedding-parallel cleavage fragments of Marcellus top (7451') show oriented,100-200 micron diameter pods of organic material (OM), interpreted as preserved algal cysts containing large (several 10s of microns in dimension), euhedral dendritic chlorite crystals, as well as euhedral forms of calcite, quartz, and pyrite. Figure 1a shows an example of such an OM-rich feature that was targeted for dual beam FIB/SEM. Subsequent Gallium-ion beam slices of a large (40 x 40 μm) region of interest including one the pods, acquired at the Molecular Foundry (LBNL), also reveal small, porous patches of OM dispersed within the fine-grained illitic clay matrix, as well as pores formed at mineral phase boundaries and between OM and minerals (Figure 1b). In addition, large field BSE image analysis of bedding-perpendicular polished core sections of Mahantango, Marcellus top and lower Marcellus (Figure 2) show that these large algal OM features are common in several targeted depths. These data suggest that more than one type of OM exist within a single core sample, and that also they are distributed among different depths within the Mahantango and Marcellus. Detailed observations suggest, that OM/mineral/pore associations and rock fabric microstructure do vary with depth in the formation.

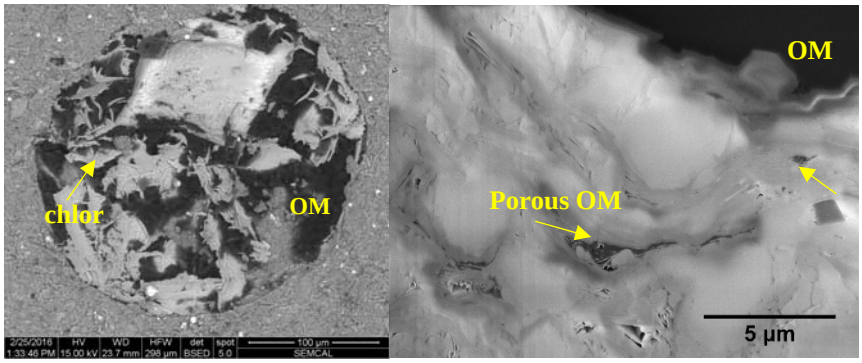


**Figure 1**a (left). Algal material replaced by OM (dark), euhedral dendritic chlorite, and other secondary minerals observed in Marcellus Top, depth 7451'. Figure 1b (right) is a dual beam FIB slice showing porous OM 1s of µm or less in length scale (yellow arrows), in addition to the large OM algal pod (upper right) without obvious pores.

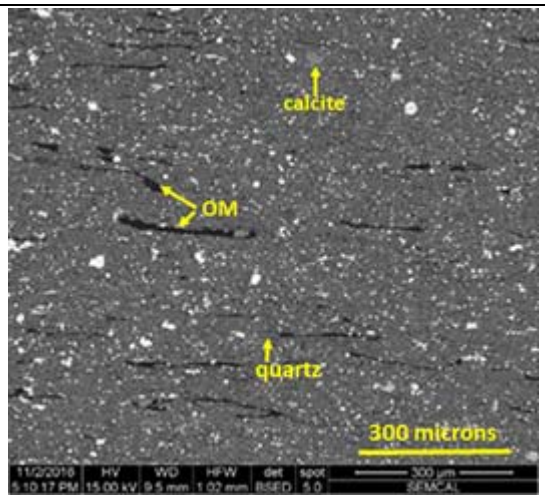


**Figure 2.** Example image of large field BSE scan for Lower Marcellus (depth 7543.0'). OM appears black, quartz and other siliciclastics appear medium gray; carbonates and phosphates appear light gray, and the strongest BSE scattering minerals (mainly pyrite) appear white.

The complex shale matrix, including high concentrations of organic constituents and salts as well as exceedingly low porosities, constitute serious challenges in extracting microbial lipids from shale. After testing different extraction methods and effect of the addition of spikes, we determined that the modified Bligh and Dyer method using a phosphate buffer and phospholipid spike (mBD+Phos+POPC) consistently provided reproducible results and higher recovery of phospholipid fatty acids (PLFAs) and diglyceride fatty acids (DGFA's) over other methods (Figure 3). This suggested that the addition of the POPC spike helped with the extraction of a pool of lipid material that was not accessible with the other methods. This method demonstrates that the extraction solvent mixtures are polar enough to release PLFA from microbial cell membranes and non-polar enough to release DGFA from neutral lipids (Akondi et al., 2016, 2017). This optimized method was used to extract lipid biomarkers from pristine sidewall cores collected from MSEEL site. Little evidence of intact lipid biomarkers was observed in Marcellus cores.

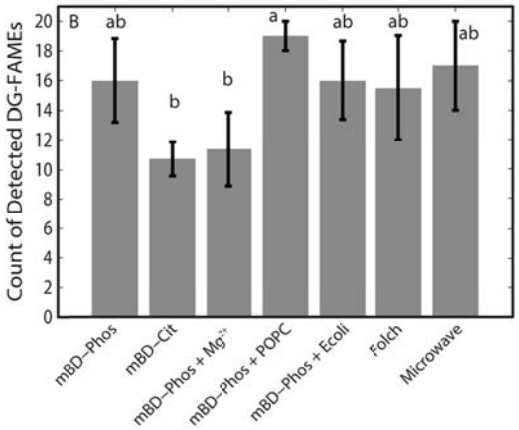


**Figure 3.** Individual DG-FAME detected in each extraction treatment (n = 7). Error bars represent the standard deviation between triplicates. Levels of average means, based on ANOVA and Tukey-HSD tests (α = 0.05), are plotted above each bar.

Preliminary direct kerogen analysis from samples collected at the MSEEL site suggests that majority of carbon chains present in kerogen are mainly aromatic in nature (Figure 4). This indicates that most of the aliphatic chains have been already thermally degraded at this maturity stage. Agrawal and Sharma, 2017 showed that aliphatic biomarkers and pyrolysis proxies at high maturity zones of Marcellus site can be used to determine thermal maturity and sources of organic matter. Aliphatic biomarkers of pristine sidewall cores collected from MSEEL show that contribution of organic matter from marine input increased from Marcellus top to lower Marcellus. However,

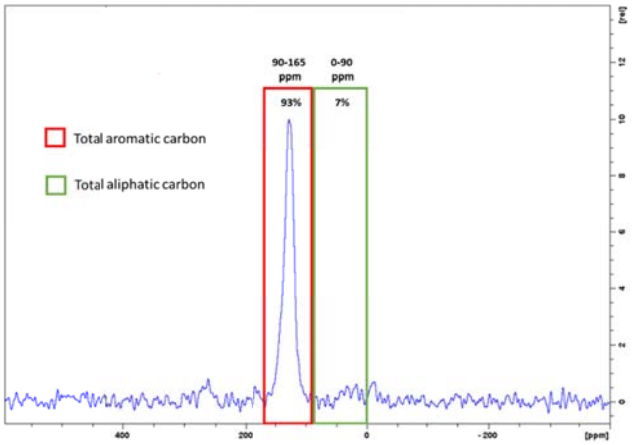


**Figure 4.** Multiple CP (cross polarization) 13C NMR spectra of kerogen sample from lower Marcellus Shale. Peak area distribution shows kerogen contains 7% aliphatic carbon chains and 93% aromatic carbon chains.

kerogen structural parameters determined by 13C solid-state NMR and Raman spectroscopy are very similar throughout Marcellus at MSEEL site. This indicates thermal maturity has the dominant control on kerogen structure (Agrawal et al., 2016, Agrawal et al., 2017). Using the structural parameters determined from direct kerogen analysis and S2 values of SRA (source rock analysis), multiple regression models will be built to determine the HC generative potential of different carbon chains of kerogen.

The microbial community composition in pristine sidewall cores from the MIP3H well and input fluids (e.g. drilling muds) was compared to the microbial communities in produced fluids from MIP3H and MIP5H wells, using a combination of 16S rRNA sequencing and culturing. Fluids were filtered to collect microbial biomass. Comparison

of core material samples to the controls did not show any signature for indigenous microbial life in the MIP3H well. Comparison of the microbial communities in drilling muds and produced fluids show that many of the injected microorganisms thrive in the subsurface, eventually dominating the communities after 250 days. The dominant microorganisms in both wells are *Halanaerobium* spp. (up to 43% relative abundance) and *Orenia* spp. (up to 75% relative abundance), detected in the drilling muds between 4.0 and 8.5% relative abundance (Daly et al, 2016). DNA-based analyses from sidewall core material and produced fluids were complimented by anaerobic culturing enrichments. Although no cultures were obtained from pristine sidewall core material, the produced fluid enrichments resulted in the isolation of multiple *Halanaerobium* spp. and the dominant methanogen in produced fluids, a member of *Methanohalophilus*. Laboratory cultivation techniques confirmed that the *Methanohalophilus* is halotolerant (growing at or above 80 g/L NaCl) and piezotolerant (>3,000 psi). Several *Halanaerobium* spp. were isolated from the MIP3H and MIP5H wells, growing across broad pressure and salinity gradients, including salinities as high as 250 g/L NaCl (Borton et al, 2016). Together these results show that the microorganisms that dominate the produced fluid communities are introduced into the wells during the drilling and hydraulic fracturing process and that these microorganisms are well adapted to growing and persisting in fractured shales. Consistent, visual evidence of cells indicates viable microbial biomass exist in both wells through all stages of completion and production. Concentrations of carbon, nitrogen and phosphorus species confirm the shale system is not oligotrophic or limited for key macronutrients. The increasing concentrations of redox-active elements and reduced electron acceptors strongly suggests an active anaerobic microbial community exists in the well involved in the respiration of iron, manganese, and sulfur species (Booker et al, 2016, Evert et al., 2016).

The average molecular composition of produced gas samples collected from wells MIP3H and 5H indicates that gas its primarily composed of methane (97-98%) with little ethane (2.5%) and propane (.14%). The $\delta^{13}C_{CH4}$ values of methane is around -36.4‰ V-PDB and is heavier than ethane which has value of around -39.2‰ V-PDB. The isotope values are higher than produced gas samples collected from Green County wells, PA (Sharma et al., 2015) but do show the characteristic isotope reversal observed in gas sources from Marcellus Shale. The bulk produced fluid geochemistry data from MIP 3H and 5H wells show that chloride concentrations range from approximately 55,000 to 84,000 mg/L. However, the Cl- concentrations did not increase systematically over time, nor did these two wells exhibit similar changes. Dissolved sulfate was not detected in the flowback samples collected from April through September. Fluoride concentrations were approximately an order of magnitude lower during this time, as compared to the first month of flowback samples. Dissolved NH3 followed a similar trend as Cl-, with concentrations ranging from approximately 80 to 100 mg/L N. Data reduction from trace metal analysis on the ICP-OES and ICP-MS continues. Core flood experiments with fracturing fluid and sidewall cores are being conducted to evaluate fluid-rock reactions in the fractured shale, and to date have shown chemical changes associated with pyrite oxidation and calcite dissolution (Hakala et al., 2017). The carbon isotopic composition of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) in the produced fluids are highly enriched in $^{13}C$ with values ranging from +10‰ to +31‰ V-PDB compared to the injected frac water which had values of -8.7‰ to -8.2‰ V-PDB. The highly enriched $\delta^{13}C_{DIC}$ are consistent with values reported from Marcellus produced waters collected from Greene County, PA (Sharma et al., 2014). The high carbon isotope values are indicative of microbial utilization of lighter carbon by methanogenic bacteria in the reservoir indicating biogenic methanogenesis in the reservoir (Sharma et al., 2014, Wilson and Sharma, 2016, 2017). Genomic analysis reveals that like other shale systems *Methanohalophilus* could be the dominant source of biogenic methane at MSEEL (Borton et al., 2016). The noble gas analysis is being conducted to identify the contribution of microbial methane to the total gas. The evidence of microbial methanogenesis raises the possibility of enhanced gas recovery from these shales using biological amendments.

**Conclusions**

The preliminary characterization of vertical and sidewall cores indicates there are many spatiotemporal biogeochemical heterogeneities in the shale from the molecular, pore to core scales. The highest TOC in the lower part of Marcellus is accompanied by isotopic and geochemical signatures of fluctuating redox and higher nutrient cycling that might have played a key role in higher organic matter production. The access to pristine sidewall cores at this site will enable us to accurately characterize viable and non-viable microbial life in different zones of Marcellus and its interfaces with overlying and underlying formation using lipid biomarkers. The modified Bligh and Dyer method that we developed using a phosphate buffer and phospholipid spike (mBD+Phos+POPC) appears to give the highest yield and reproducibility for PLFA's and DGFA's.

The complete access to water and gas samples from all stages of drilling and production at MSEEL has helped us to infer that variety of water-microbe-rock interactions are initiated in the reservoir after injection of nutrient enriched fracturing fluid. Isotopic and genomic data supports the existence of microbial methanogenesis in the Marcellus shale.

**Acknowledgements**

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