Bioavailability of Aromatic Hydrocarbons and Their Interactions with Natural Organic Matter: Linking Molecular- and Microbial-Scale Interactions

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Accurately measuring the bioavailability of organic contaminants associated with complex environmental matrices is necessary for assessing contaminant risk, as well as for evaluating the effectiveness of proposed and in-place remediation strategies. Due to its importance, the bioavailability of organic contaminants associated with soils and sediments has been widely examined at the macroscopic or bulk-scale and found to be highly controlled by natural organic matter (NOM) as well as the contact time between the contaminant and environmental matrix. Although chemical and physical interactions between natural organic matter and contaminant molecules are expected to influence bioavailability, there has been minimal success identifying how these interactions impact the bioavailability of the contaminants.

The aims of this project are to identify the key physical, chemical, and biological conditions and processes governing bioavailability of aromatic hydrocarbons associated with NOM, such as humic and fulvic acids and humin [1]. Molecular interactions and dynamics of aromatic hydrocarbon molecules associated with NOM are being characterized and quantified using liquid- [2] and solid-state [3] deuterium nuclear magnetic resonance experiments, fluorescence probe experiments [4], and NOM surface area and adsorption capacity characterization. To assess bioavailability, we are measuring the activity of the degradative enzyme naphthalene 1,2-dioxygenase (NDO) [5] as it catalyzes the degradation of aromatic hydrocarbons associated with NOM. NDO activity will be characterized as a function of NOM type and concentration, and NOM-aromatic hydrocarbon aging time. Using the analytical methods noted above to examine NOM-aromatic hydrocarbon samples before and after exposure to the enzyme will permit characterization at a molecular-scale of the effect of NOM-aromatic substrate interactions on enzymatic bioavailability, i.e., molecular interactions will be identified that facilitate or hinder enzymatic bioavailability of aromatic hydrocarbons associated with NOM. Enzymatic-scale bioavailability subsequently will be coupled to the microbial-scale by measuring the bioavailability of aromatic hydrocarbons associated with NOM to reporter microbes that luminesce upon expressing NDO in the presence of aromatic substrates. Describing bioavailability over this continuum of scale, i.e., from molecular to microbial, will provide a basis for extrapolating the environmental consequences of aromatic hydrocarbon contamination from both discrete chemical interactions and microbial-level responses.

NOM-Aromatic Hydrocarbon Interactions

We are using solid state static ^{2}H NMR to observe the motional behavior of benzene-d_6 adsorbed, at near monolayer coverage, to humic substances in order to eventually quantitatively correlate this motional behavior with adsorption-desorption behavior and bioavailability of adsorbed aromatic hydrocarbons. In this case up to three different motional models (large-angle wobble (LAW), small-angle wobble (SAW), and isotropic (ISO) may be detected simultaneously, as already reported for the Ca-montmorillonite/benzene system [3]. ISO motion is liquid-like, and the more restricted SAW motion is indicative of binding on a surface of the substrate. Samples have been prepared for solid state ^{2}H NMR analysis by two methods: (1) by

vapor deposition of benzene-d_6 onto the substrate in an NMR tube on a vacuum system and flame sealing, and (2) by slurry packing the solid phase with the liquid benzene-d_6, drying and sealing with a Teflon cap. Nitrogen BET surface area measurements have been performed to gauge the quantity of benzene required for monolayer coverage.

In Figure 1 we compare spectra taken at 25°C for three samples prepared on the vacuum line. A sample with 25% monolayer coverage of benzene-d_6 on Ca-montmorillonite (surface area 78.5 m^2/g) gave a spectrum closely resembling that observed previously, displaying LAW and ISO motions [3]. Monolayer coverage of benzene on Aldrich humic acid (AHA, surface area 3 m^2/g) results in a spectrum with both SAW and ISO motions. The motion of benzene on Suwannee River humic acid (SRHA, surface area 23m^2/g) appears to be entirely isotropic in a sample loaded with somewhat more than one monolayer. This is surprising because one would expect to see SAW motion as an indication of binding of the benzene to the humic acid. In contrast to the sample of SRHA prepared by vapor deposition, the spectrum of a slurry-packed sample of benzene-d_6 on SRHA (Fig. 2) shows both SAW and ISO motions. The motional differences between these two samples may be due to variations in the hydration state of the humic acid. A systematic study of the effects of hydration on the benzene dynamics is underway. One challenge in extending this work is the low surface area of the humic acids, which results in near-monolayer loading corresponding to small quantities of benzene and low signal-to-noise ratio.

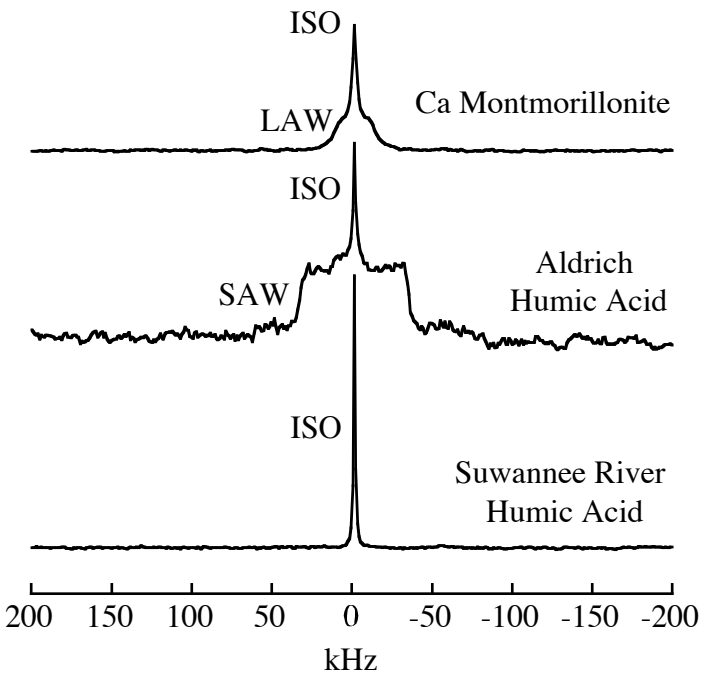

Figure 1. ^{2}H NMR Spectra at 25 °C of benzene-d_6 sorbed to the indicated solids.

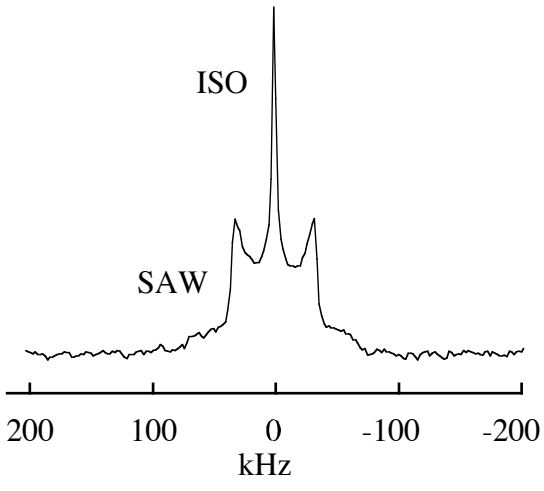

Figure 2. ^{2}H NMR spectrum at 25 °C of benzene-d_6 on Suwannee River Humic Acid.

Dynamic information (such as activation energies of motions) is derived from deuterium NMR echo spectra taken at a series of temperatures by assuming a motional model, quadrupole and acquisition parameters, and calculating spectra with different rates of motion until one that matches each experimental spectrum can be found. Rather than using this trial-and-error method, we are developing an automated fitting program able to fit spectra that are

combinations of spectra due to different motional models (SAW, LAW, ISO), and have demonstrated that the simulated annealing method is effective for this purpose. To make the calculation speedy enough to be practical even in cases where the motional model has a large number of sites, libraries of pre-calculated spectra have been accessed, instead of calculating the spectrum at each annealing step. So far, fitting of calculated spectra has been accomplished. We are working toward fitting of experimental spectra with appropriate consideration for the effect of spectral noise on the goodness of fit.

Bioavailability Studies

This effort has begun with cultivation of NDO-producing microbes and isolation and extraction of NDO in sufficient quantities for experiments. We have revived the NDO-containing *E.coli* strains we received from Dr. Sylvestre (INRS-IAF, Universite du Quebec) and verified that they can produce the histidine-tagged components of NDO [6]. Steps in this process included determining the optimal growth medium and growth phase to induce maximum expression of the NDO polypeptides, verifying the size of the induced polypeptides by gel electrophoresis, confirming that the polypeptides are his-tagged by Western blotting, and varying buffer and other conditions to purify the recombinant proteins.

The NDO subunits have been purified and the reconstituted enzyme tested for its ability to degrade biphenyl and benzene. The enzyme was found to actively degrade biphenyl whereas enzyme inactivation was observed with benzene as the substrate. Hence attempts have been made to isolate enzymes that can degrade benzene from hydrocarbon-contaminated soil samples. Isolates obtained from the Tall Grass Prairie contaminated sites have been tested for their ability to utilize various carbon sources including benzene. All of the isolates were found to grow on naphthalene and biphenyl however only two were able to utilize benzene as the sole carbon source. Studies are also being performed on samples from gas condensate contaminated ground water sediments containing hydrocarbons including benzene. Four bacterial colonies with ability to degrade benzene have been obtained. After further identification and characterization, isolates best able to utilize benzene will be employed in enzyme assays.

References

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