Ecological interactions between metals and microbes that impact bioremediation

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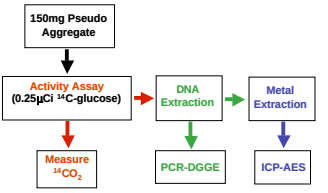
Spatial Heterogeneity of Microbial Activity, Community Structure, and Metal Contamination

Objective

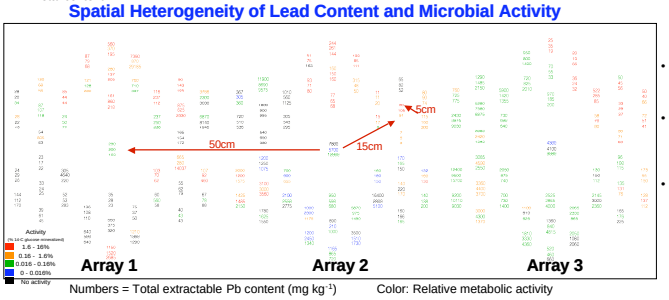
Determine the spatial relationship between microbial community dynamics and local heavy metal contamination from a long-term mixed-waste contaminated site.

Methods

- Soil was collected from the Indiana Department of Transportation (INDOT) property in Seymour, Indiana in nested arrays at distances of <1, 5, 15, and 50 cm along a trench with sample depth ranging from 40 to 80 cm.
- Three 150 mg pseudo-aggregates from each sample underwent the sequential analysis indicated in the flow chart to assess microbial activity, community structure, and extractable metal content.



Spatial Heterogeneity of Lead Content and Microbial Activity

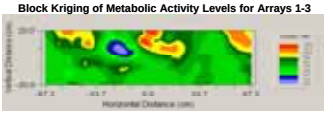


Numbers = Total extractable Pb content (mg kg^{-1}) Color: Relative metabolic activity

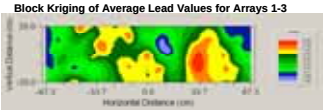
- Activity was spatially heterogeneous within the soil horizon. At one sampling location (3 subsamples), activity levels could vary as much as 100-fold.
- Metal levels also could vary at small spatial scales. Some locations had nearly the same level of metal for all three subsamples, while others varied by as much as a factor of 5.
- Some samples 5 cm distant varied in metal content by 10-fold. Similar variances in metal content were seen with samples separated 15 cm. Samples separated 50 cm also showed large variances in metal content with differences as great as 40-fold.

Geostatistical Analysis

Block Kriging of Metabolic Activity Levels for Arrays 1-3



Block Kriging of Average Lead Values for Arrays 1-3



- Log-log linear plots revealed no correlation between metal content and activity level at any spatial scale.
- Geostatistical analysis showed a correlation between metal content and activity level both isotropically and anisotropically at both the 45° and 135° angles, with the strongest anisotropy at 135°. The data suggest that increasing metal results in localized decreased metabolic activity.
- A periodicity was seen in the isotropic and anisotropic variograms that is echoed in the kriging maps. This periodicity is due to the non-uniform distribution of microbes and metals.

Work in progress…

- Community composition using PCR-DGGE revealed variable community structure with Dice similarity coefficients ranging from 0.29-0.77. Samples from the same sample point (<1 cm apart) exhibited different phylotype banding patterns, indicative of phylogenetic diversity. Samples with comparable levels of activity and metal content varied in their community composition. Analysis of the gels revealed a set of 11 bands that appear to be statistically important within the community. Currently work is underway to sequence those bands.

Biodiversity at the cm scale

Objectives

1. Evaluate patchiness of phylotype richness and distribution at a cm spatial scale
2. Culture novel organisms using newly developed growth chamber

Methods

- Soil was collected from the INDOT property in Seymour, Indiana in a 5 x 5 grid with samples 5 cm apart horizontally and vertically.
- Bacterial diversity was assessed in 5 150 mg aggregates from each sample. Bacteria were removed from the soil matrix, filtered onto 0.45 µm polycarbonate filters, and placed in growth chambers for 2 weeks.
- Microcolony morphotypes were enumerated and streaked onto various media in attempts to culture the organisms.

Preliminary Results

- Viable counts on standard laboratory media resulted in a lower percentage of microbes recovered from the total community compared to growth using the designed growth chamber. (See table)
- 19-24 different morphotypes were observed on filters with both common and unique types present.
- Abundant morphotypes from one filter set were not the same abundant types in samples 5 cm apart. This is indicative of species distribution heterogeneity.

Growth Substrate	% Recovery*
Soil Extract Agar	0.19 ± 0.10
1/10 Nutrient Agar	0.20 ± 0.09
1/50 Nutrient Agar	0.10 ± 0.07
XBM + Polymers**	0.23 ± 0.09
Filters on soil	3.9 ± 3.4

*Numbers are mean ± standard deviation
**Polymers: gelatin, pectin, starch, and xylan (0.025% (w/v))

Work in progress…

- Analysis of isolates obtained from the microcolony streaks is currently underway.
- Isolates will be characterized using ARDRA and FT-IR (Fourier Transformed Infrared Spectroscopy).
- Preliminary results using FT-IR show that strains which look morphologically similar can be segregated into discrete group. This technique will be used to thereby reducing the number of isolates dramatically.

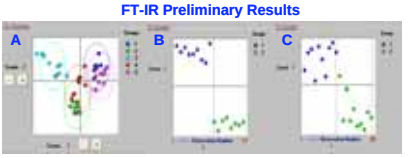
Growth Chambers – A Continuous Flow System

- Inorganic nutrients flow into chamber base at a rate of 5 ml hr^{-1}
- Via capillary action, nutrients within the soil are displaced and rise to the surface
- Sitting on the surface are filters containing the extracted bacterial inoculum
- An outflow valve near the soil surface allows for removal of excess liquid from the system

This approach quickly generates a large number of strains which have limited morphological diversity. We are investigating the use of FT-IR microspectroscopy to rapidly discriminate among different strains and indicate similar ones.

These results are based upon reflectance FT-IR of isolates grown on laboratory media. Our goal is to conduct analyses of biomass derived from microcolonies on filters.

FT-IR Preliminary Results



- Ten FT-IR spectra were collected from each of 5 morphologically similar colonies. The 50 spectra underwent canonical variate analysis
- Initial results indicated 3 groups with isolates 1 and 5, and 2 and 4 grouping apart from isolate 3 (Panel A)
- Additional (finer) spectral analyses were performed. Isolate 1 separated from 5 (Panel B) and isolates 2 and 4 also separated, yet not as distinctly as 1 and 5 (Panel C)

Anaerobic Microcosm Studies

Objectives

- To determine the response of anaerobic soil microbial communities to the addition of energy substrates and Cr(VI)
- To determine if reduction of Cr(VI) is necessary before organic substrate is utilized
- To isolate Cr-resistant (Cr^R) bacterial strains from the microcosms

Microcosm Design

- Electron donors (glucose or gelatin), electron acceptors (amorphous crystalline Fe^{+3} or NO_3^-), and Cr(VI) were added to soil from the INDOT site in Seymour

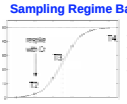
Microcosm Setup

- Soil – 10 g dry weight (3 reps)
- Electron donor – 30 mg g^{-1} soil
- Cr:
  - Low – 140 µg Cr g^{-1} soil
  - Medium – 200 µg Cr g^{-1} soil
  - High – 300 µg Cr g^{-1} soil

Measurements on Sampled Microcosms

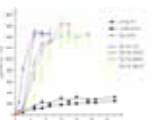
- CO_2 evolution in headspace (GC)
- Remaining Cr(VI) (1,5-Diphenylcarbazide method)
- Isolation of Cr^R strains on various media with 0.25mM Cr(VI)

Sampling Regime Based on CO_2 Evolution

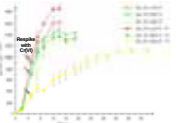


T1 – 48 hrs after start
T2 – beginning of respiration
T3 – at maximum respiration rate
T4 – after respiration plateau is reached

CO_2 Evolution in Microcosms

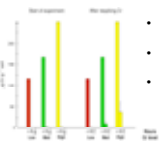


Chromium addition leads to an increase in lag phase and a decrease in total CO_2 production.



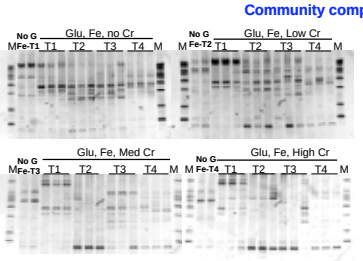
Respiking (-R) low and medium Cr treatments had no effect. The community in the high treatment was severely impacted by respiking, recovering slowly.

Cr(VI) reduction



- Within 24 hrs, Cr(VI) was completely reduced in low Cr treatment.
- In the medium and high Cr treatments, Cr(VI) was completely reduced after 96 hrs.
- After respiking with Cr, the Cr reduction proceeded at a slower rate. At the final sampling (FS) of respiked microcosms, Cr(VI) was completely reduced.

Community composition changes (DGGE)



- The addition of Cr(VI) at any level led to an acute response of the soil microbial community. All 3 treatments had similar dominant phylotypes 48 hrs after Cr addition (T1).
- During sampling times T2 and T3, the microbial communities were very dynamic. There were major differences among the 3 Cr treatments, even though the Cr(VI) was reduced in all.
- At the end of the experiment (T4), when no further microbial activity was observed, the communities were similar in all 3 treatments.
- Future work: sequence dominant DGGE bands; Q-PCR of Geobacteraceae