Virus and bacterial community responses to anaerobic Fe(III) bioreduction vary with down-gradient distance from the point of electron-donor addition

Xiaolong Liang[a], Yusong Wang[a], Jie Zhuang[a], and Mark A. Radosevich[a*]

[a]*Department of Biosystems Engineering and Soil Science, The University of Tennessee, Knoxville, TN 37996*

Introduction

In situ anaerobic bioremediation (AB) has been widely used to degrade or transform subsurface organic and inorganic contaminants. During anaerobic bioremediation, microorganisms are stimulated to degrade/transform contaminants under anaerobic subsurface conditions by introducing a variety of organic substrates that generally stimulate Fe(III)-oxide reducing bacteria (e.g. *Geobacter* species).

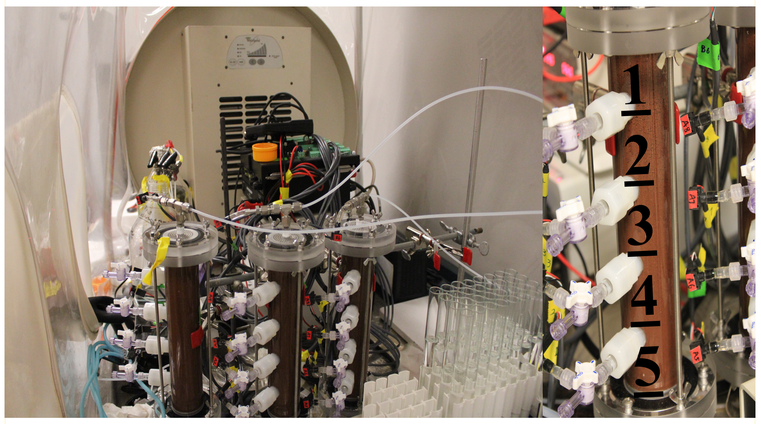
Objectives

- To determine the distribution and diversity of bacterial communities along the flow path of injection.
- Measure viral abundance in active Fe(III) bioreducing sediments.

Materials and Methods

Iron oxide-rich soil was wet sieved through 2000, 250, and 53 μm sieves using the modified method as described in Zhuang et al. (2008) to extract aggregates. Fractions of water-stable soil microaggregates (53~250 mm) were collected and air-dried for subsequent use.

The air-dried water stable soil aggregates were inoculated with *Geobacter. sulfurreducens* prior to packing the columns. The soil aggregates were packed into columns (25 cm in length and 3.8 cm i.d.).



In the course of evaluating the secondary impacts of AB on chemical, physical, and biological properties of porous media, microbial communities and viral assemblages in iron-rich soil aggregates were investigated after 60 d of Fe(III)-bioreduction. Artificial ground water (AGW) containing acetate was pumped through the coulmns A and B (treated columns) while AGW without acetate was pumped through column C (control column).

After 60 d the columns were sectioned and bacterial communities characterized via sequencing of 16S rRNA gene libraries. Sequence data were analyzed using Mothur as described in elsewhere (Kozich et al, 2013). Viruses were enumerated as described by Williamson et al. (2005).

Acknowledgement

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Results and Discussion

- The greatest iron bioreduction and colloid release occurred at the influent sections (A5 and B5) of columns A and B which were flushed with AGW containing acetate (Fig.1). The amount of accumulated iron and colloid significantly decreased from influent to effluent (***P*<0.01), while much more iron and colloid was detected in Column A and B than in the control column (****P*<0.001) which demonstrated more bioreduction was stimulated by the addition as the electron donor.

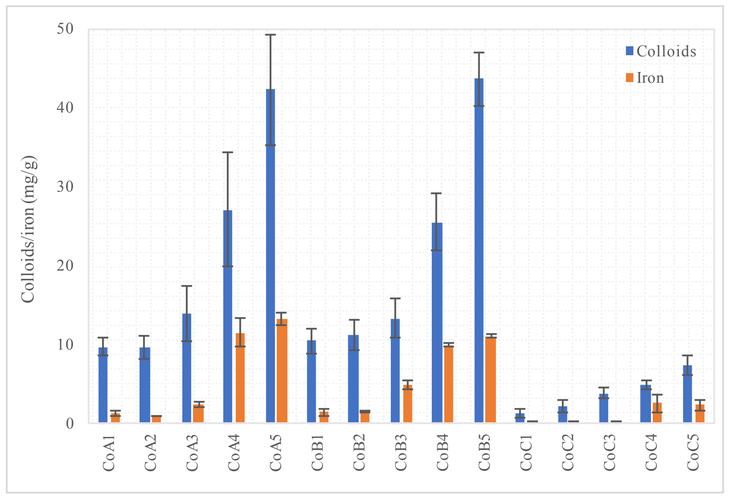

Figure 1. Release of colloids and iron in each section of column soils after 60 d of bioreduction. Soil aggregates were destructed with iron bioreduction releasing colloids and Fe(II). The amount of released colloids and iron depended on the bioreduction level. Data points are the mean values of triplicate samples and error bars are standard deviation.

- The diversity of bacterial communities in the influent fraction of the experimental column is significantly lower than that of the influent fraction of the control column (Shannon's Index) (***P* < 0.01) (Fig. 2).

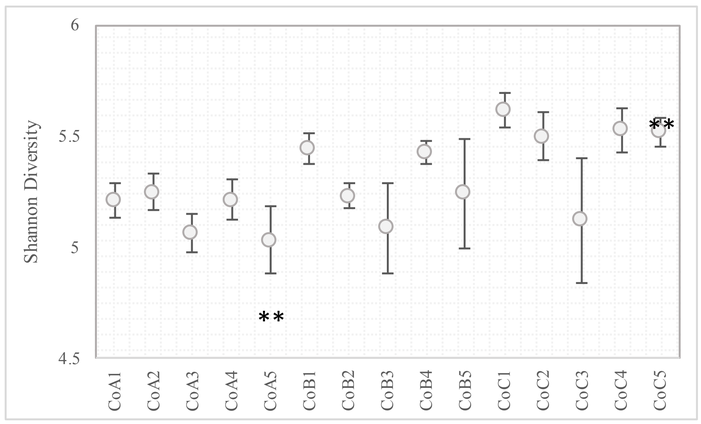

Figure 2. Diversity (Shannon's Index) of bacterial communities under different sections in acetate-treated columns and control column. Error bars are standard deviation.

- Microbial communities in the 3 sections nearest the effluent end of each column were not different based on Simpson's diversity index (*P* > 0.05; Fig.2).
- Bacterial communities from CoA1, CoA2, CoA3, CoB1, CoB2, and CoB3 samples clustered together (Fig.3). In the treated columns (A and B), the communities in 3 sections nearest the effluent end were separated from those in the 2 sections nearest influent. The communities in column A and B were separated from those in column C by axis 1 (Fig.3). All the results of bacterial communities were consistent with the bioreduction effects.

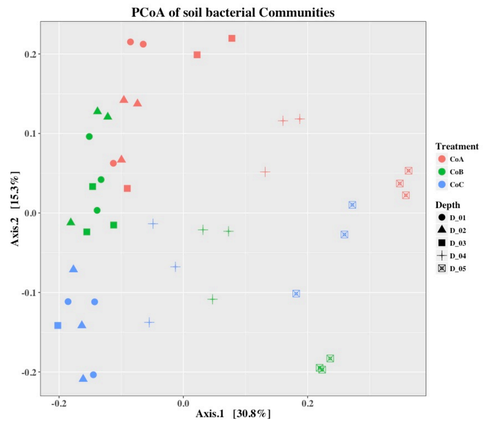

Figure 3. PCoA plots of Bray-Curtis distances of bacterial community structures in soils. Samples are coded by flushing treatment (symbol color) or depth in columns (symbol shape).

- Overall Proteobacteria, Firmicutes, Bacteroidetes, Acidobactria and Actinobacteria were most abundant (Fig.4). The microbial communities in the columns treated with acetate were similarly dominated by Proteobacteria and Firmicutes nearest the influent end of the column A and B. These phyla were reported to include iron reducers (Weber et al., 2006),

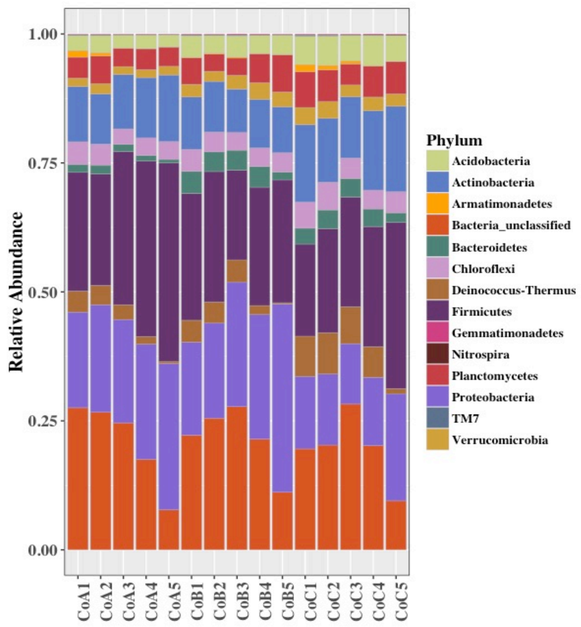

Figure 4. Relative abundance of bacteria phyla through vertical depth in acetate-treated columns (A and B) and control column (C) after 60 d of cultivation in anaerobic chamber.

- The abundance of Proteobacteria decreased gradually from influent factions through whole column soils leading to significant differences in the abundance of Proteobacteria and *Geobacter* between the efluent fraction and the influent fraction in column A and B (Fig.5). Few differences of Proteobacteria abundance among all fractions of column C and the top 3 fractions in column A and B indicated limited nutrient availability.

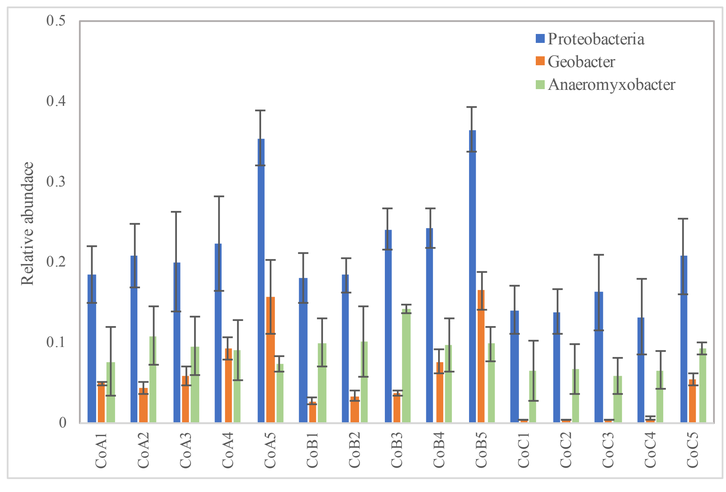

Figure 5. Relative abundance of Proteobacteria, *Geobacter*, and *Anaeromyxobacter* in soils through all sections cultivated under anaerobic conditions.

- The virus abundance of viruses was investigated to explore the biotic factors driving microbial community shifts with abiotic factors. The highest concentration of VLPs were detected at the influent fraction of column A and B where significant shifts in microbial community structure and activities were detected indicating viruses as an important factor of shaping bacterial communities (Zhang et al., 2017).

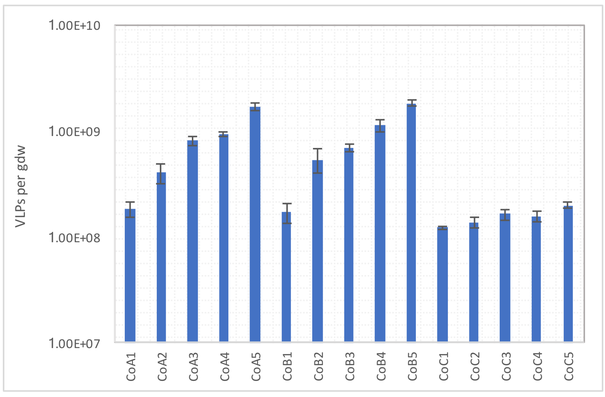

Figure 6. Virus-like particles (VLPs) abundance in column soils by epifluorescence microscopy direct counting. Error bars are standard deviation.