The Geobiology of Microbial Mediated Manganese Oxide Formation in an Environmental Remediation System

NC STATE UNIVERSITY
DEPARTMENT of SOIL SCIENCE

UNIL | Université de Lausanne

Smithsonian

Terrence G. Gardner[1], Megan Y. Andrews[1], Dominique L. Chaput[2], Boris Droz[3], Jasquelin Peña[3], Matthew L. Polizotto[1], Nelson A. Rivera[1], Cara M. Santelli[2] and Owen W. Duckworth[1]

(1) Department of Soil Science, North Carolina State University, Raleigh, NC ; (2) Smithsonian Institution, National Museum of Natural History, Washington, DC; (3) Institute of Earth Sciences (ISTE), Faculté des géosciences et de l'environnement, Université de Lausanne, Lausanne, Switzerland

BACKGROUND

The on-site removal of potentially harmful groundwater contaminants (e.g., inorganic and organic compounds, heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, volatile organic compounds (VOCs), radioactive waste) requires an in-depth inquiry of the biogeochemical transformation processes and metal ion behavior occurring within the remediation treatment system. Therefore, an understanding of the biogeochemical processes including the cycling of potentially toxic compounds in the ecosystem was performed.

An initial evaluation of Farm Lot 86 groundwater extraction and treatment remediation system, in Raleigh, NC, revealed high concentrations of dissolved manganese in the groundwater effluent. Further analysis of the entire system components (Fig. 1.) (i.e., influent, processing, and intermediate tanks, air strippers, activated carbon filters, ion selective resins and the effluent) revealed that the dissolved manganese from the influent was being transformed by the oxidative precipitation of system inhabiting microbes (fungi and bacteria). It was determined that the dense microbially mediated Mn(III/IV) oxide sludge was obstructing the remediation system function.

PRESENT STUDY

Our objective was to evaluate the chemical composition and microbial community diversity within the Mn-bearing sludge using an inclusive approach to understanding the evolution of system chemistry, mineralogy, and biology.

Sludge and water were collected from all components of the treatment system, and samples analyzed using traditional (Sanger method) and high through-put (pyrosequencing) DNA sequencing analyses carried out using 16S rRNA gene and nuclear ribosomal internal transcribed spacer (ITS) region assays to characterize the sludge microbial isolates and community diversity. X-ray absorption spectroscopy, X-ray diffraction, and scanning electron microscopy with energy dispersive spectroscopy were utilized to characterize the mineralogy and morphology of sludge solids.

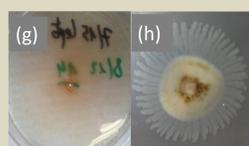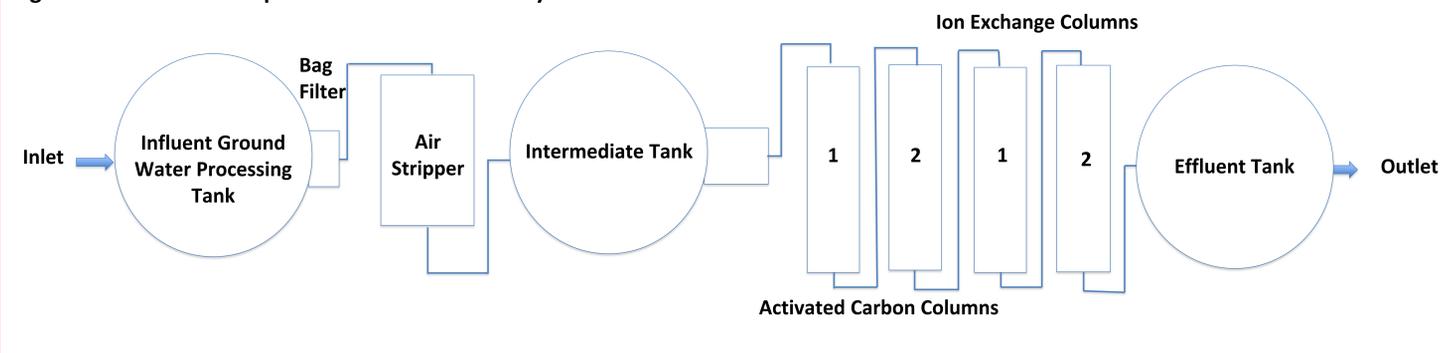### Fig. 1. Schematic of Pump and Treat Remediation System

Bacterial Analysis
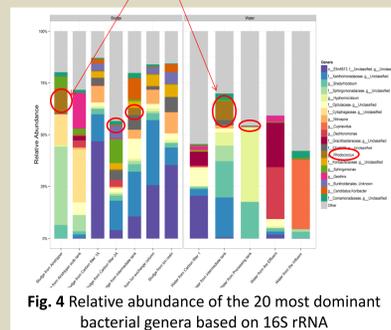

Fig. 3 g. *Rhodococcus sp.* ; h. *Bacillus sp.*

Fig. 4 Relative abundance of the 20 most dominant bacterial genera based on 16S rRNA
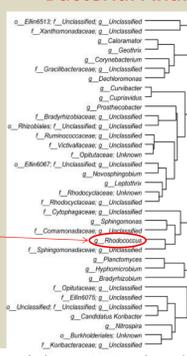
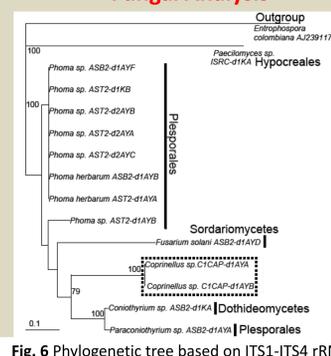**Fig. 5** Phylogenetic tree based on 16S rRNA

Fungal Analysis

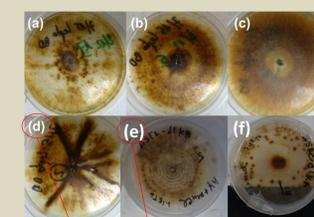**Fig. 6** Phylogenetic tree based on ITS1-ITS4 rRNA

Fig. 7 a. *Coniothyrium sp.*; b. *Paraconithyrium sp.*; c. *Coprinellus sp.*; d. *Phoma sp.*; e. *Fusarium sp.*; f. *Paecilomyces sp.*
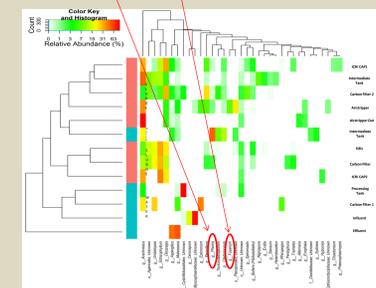
Fig. 8. Double hierarchal dendogram (Heat map) evaluation of fungi distribution as affected by the different components of the remediation system.

Microbiological Analysis and Results

A culture-based assay of samples taken from different locations along the treatment stream resulted in isolation of 14 strains of microorganisms that promote Mn(II) oxidation. Morphological and phylogenetic analysis of all the isolates revealed high sequence similarity (avg. ≥98%, 16S and ITS) with two different genera of bacteria and six different genera of Mn (II) oxidizing fungi (Fig. 3 , 6 and 7, respectively).
Non culture-based assay of bacterial and fungal DNA associated with Mn oxide deposits within the system reveal high diversity of microbial communities, of which, cultured phylotypes represent a minor proportion. Several of the 20 (Fig. 4) and 35 (Fig. 5) most dominant bacterial genera and the 35 most dominant fungal assemblages (Fig. 8) generated from pyrosequencing data (454 Genome Sequencer FLX System) using 16S and ITS1-4 genes are closely related to known metal cycling organisms.

Chemical Analysis and Results

Inductively coupled plasma-optical emission spectrometry (ICP-OES) reveals the bulk chemical composition of the sludge (Table 1.). Manganese, copper, and cobalt identified in sludge collected from the carbon filter have elevated concentrations.

Table 1. Water ICP-OES and ICP-MS values

	ICP-OES								ICP-MS						
Sample	Ba	Ca	Fe	Mn	Na	Zn	K	Ce	Co	Cr	Cu	Ni	Pb
	-mg/L-							-ug/L-					
Influent	0.125	3.760	9.068	4.820	9.070	0.101	2.574	1.600	12.900	<1.00	11.700	3.900	1.100
Processing tank	0.033	5.990	0.035	1.350	14.100	<0.031	2.426	<0.5	4.600	<1.00	5.800	1.500	<0.24
Intermediate Tank	0.077	3.370	0.044	1.210	8.470	0.065	2.473	<0.5	1.000	<1.00	10.600	2.800	0.800
Carbon column	0.031	4.810	<0.017	26.160	8.760	0.080	2.897	<0.5	13.100	<1.00	28.100	5.800	<0.24
Effluent	0.141	2.320	<0.017	3.230	7.270	0.049	2.086	<0.5	2.700	<1.00	<1.00	1.400	<0.24

<value: means less than reporting limit

Microscopic Analysis and Results

Scanning Electron Microscopy reveals major morphologies include the presence of spherical and fragmented carbon particles, platy minerals, puffy biomass, fungal hyphae and filaments. (Fig. 2a)
Energy Dispersive Spectroscopy of the SEM images indicates the presence of aluminosilicates, which is in agreement with XRD measurements. Other areas of the biomass contain carbon and manganese, with lower concentrations of trace metals. (Fig. 2b).

Figs. 2a
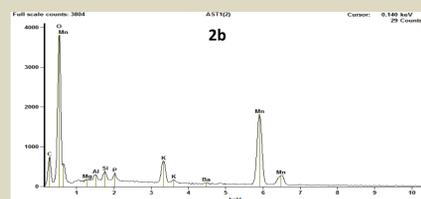
Mn oxide coated clays, particles, platy minerals and fluffy biomass from sludge

Mineralogical Analysis and Results

X-ray diffraction (SR-XRD) analysis patterns of samples throughout the system, indicate that the dominant crystalline phases are clays, micas, iron oxides and species dependent Mn oxide precipitate in birnessite or todorokite phases (Fig. 9).

Mn K-edge X-ray absorption spectroscopy (XAS) suggest that the dominant manganese precipitates in all samples is a poorly crystalline layer-type Mn(IV)-bearing phyllomanganate (Fig. 10).

Mn oxides contain significant concentrations of Co, Zn, Ba and O, which are incorporated into specific binding sites on the oxide surface (Fig. 11). (Data not shown)
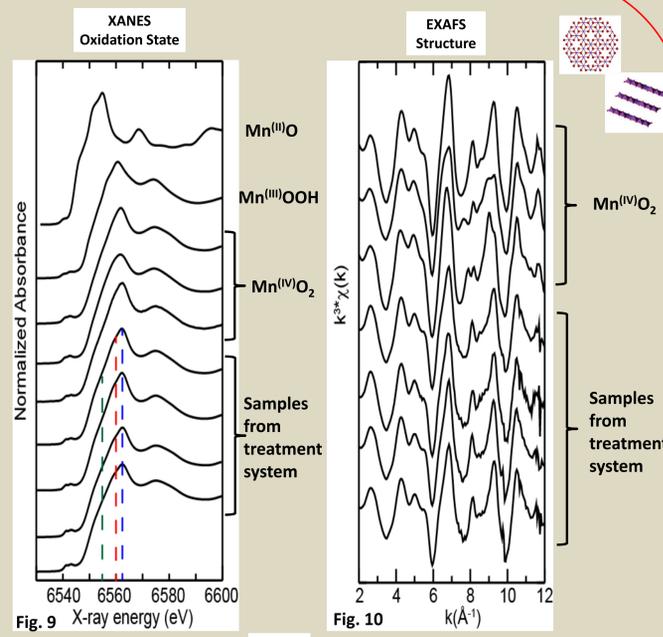

Fig. 9 X-ray energy (eV)

Fig. 10

Fig. 11

Manganese = purple
Cobalt = blue
Zinc, Barium = yellow
Oxygen = red

Spectra collected at Stanford Synchrotron Radiation Lightsource BL 4-1 and 11-2

CONCLUSION

The same Mn layer-type oxide phase is found at all points in the treatment stream. Because these phases are metastable and typically transform to other phases, biological activity in the system may stabilize these minerals.

Culture based analysis yielded a predominance of Mn oxidizing fungi. However, culture independent analysis of the bacterial and fungal community suggests a diverse range of organisms, many of which are related to known metal cyclers.

Mineralogy is dominated by layer-type Mn(IV) oxides, iron oxides, and phyllosilicates. Iron oxides are similar to those produced by known Mn oxidizing organisms.

Mn oxides contain significant concentrations of Zn, Co, and Ba, which are incorporated into specific binding sites on the oxide surface. The sorption of metals to these Mn oxides may help remove metals from the aqueous phase.