Microbially Mediated Formation of Manganese Oxides at a Superfund Site Remediation System

Terrence G. Gardner[1], Cara M. Santelli[2], Tyler Johnston[1], Boris Droz[1,3], Megan Y. Andrews[1], Nelson N. Rivera[1], Matthew Polizzotto[1] and Owen W. Duckworth[1]

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

INTRODUCTION

The Environmental Protection Agency has identified contaminated waste sites designated for clean-up (Superfund sites) as a significant problem throughout the United States. These sites can emit groundwater laden with high concentrations of dissolved metals, resulting in serious environmental contamination. Remediation of contaminated sites requires an in-depth understanding of the identities, growth characteristics, and oxidation mechanisms of the microbial community contributing to the biogeochemical processes that occur in the treatment system. In this study, we sampled a pump and treat remediation system associated with Farm Lot 86 Superfund site. Water and solid samples from the system were collected after a prolonged shut down of the system resulting from the formation of a Mn-bearing sludge. To gain a more informed understanding of the mechanism and composition of this sludge, we implemented a multi-faceted approach of methods to investigate the system (chemical, mineralogical, microscopic, and biological). The objective was to define the Mn(II)-oxidizing microbial community existing in Lot 86 treatment system designed to remove dissolved metals from the site. Because the mechanisms of microbial Mn(II) oxidation are not fully elucidated, we initiated a culture survey to identify microorganisms that catalyze Mn(II) oxidation and precipitate Mn(III/IV) oxide minerals. These results provide the groundwork for future studies identifying the key players in Superfund site remediation and factors impacting their activity. Our results show that at all points in the treatment system, microbes (dominantly fungi) are facilitating the precipitation of elevated concentrations of naturally occurring dissolved Mn, which produces deposits of Mn oxides associated with microbial biomass.

Table 1. Mn(II)-oxidizing fungi isolated from Lot 86 treatment system. Identification based on BLAST search similarity using internal transcribed spacer (ITS) GenBank database for sequences.

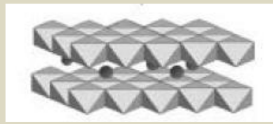
Number of representative isolates	Location of isolate	Phylum identification	Closest Genbank Entry	GenBank accession numbers	% Similarity
1	Air stripper	Ascomycota	Coniothyrium sp.	DQ132816.1	99
2	Air stripper	Ascomycota	Fusarium sp.	JX282604.1	97
1	Ion Exchange Columns	Ascomycota	Paecilomyces sp.	HQ315850.1	93
1	Air stripper	Ascomycota	Paraconiothyrium sp.	HQ999974.1	99
5	Air stripper	Ascomycota	Phoma sp.	FJ903371.1	100
2	Activated Carbon columns	Basidiomycota	Coprinellus sp.	JN159560.1	99

Mineralogical Analysis and Results

X-ray diffraction (XRD) analysis of samples from 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.

Mn K-edge X-ray absorption spectroscopy (XAS) suggests that the dominant manganese precipitates in all samples is a poorly crystalline layer-type Mn(IV)-bearing phyllomanganate (Villalobos, et al., 2003). (Fig. 1)

XRD



Birnessite- found in samples. The black circles are structural interlayer cations.

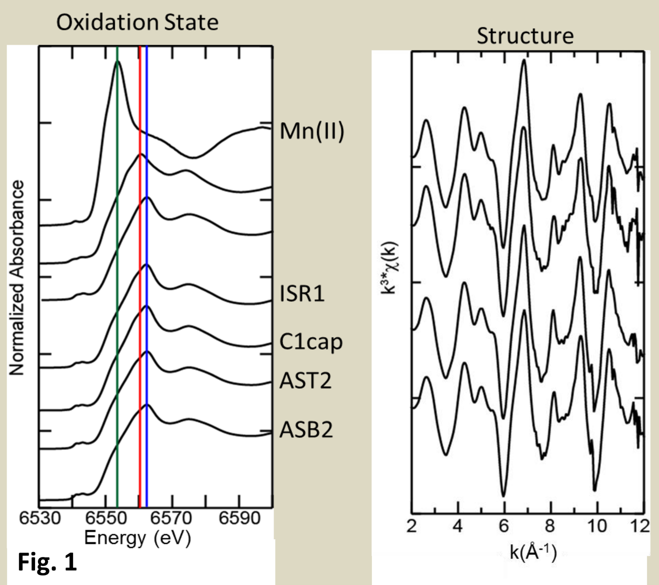


Fig. 1

Microbiological Analysis and Results

A **culture-based assay** of samples taken from different locations along the treatment stream resulted in isolation of 12 strains of microorganisms that promote Mn(II) oxidation (Santelli et al. 2010; 2011). Morphological and phylogenetic analysis of all the fungal isolates revealed high sequence similarity (avg. ≥98%, ITS) with six different genus of Mn (II) oxidizing fungi (Table 1., Fig. 3 and 4).

Non culture-based assay pyrosequencing was performed using a 454 Genome Sequencer FLX System to analyze bacterial 16S rDNA and fungal ITS1-4 genes.

Bioinformatics error reduction, processing, chimera and contaminant removal, and downstream OTU-based and phylogeny-based analysis was performed using open-access software (Mothur and R).

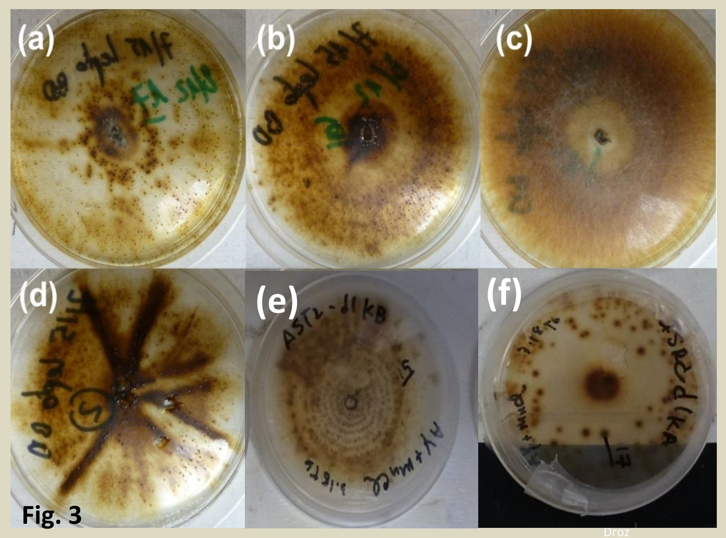


Fig. 3

a. *Coniothyrium sp.*; b. *Paraconithyrium sp.*; c. *Coprinellus sp.*; d. *Fusarium sp.*; e. *Phoma sp.*; f. *Paecilomyces sp.*

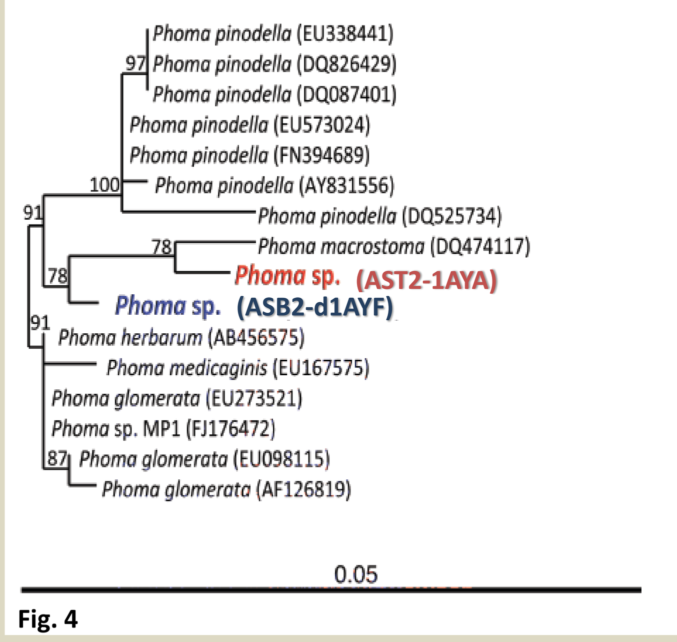
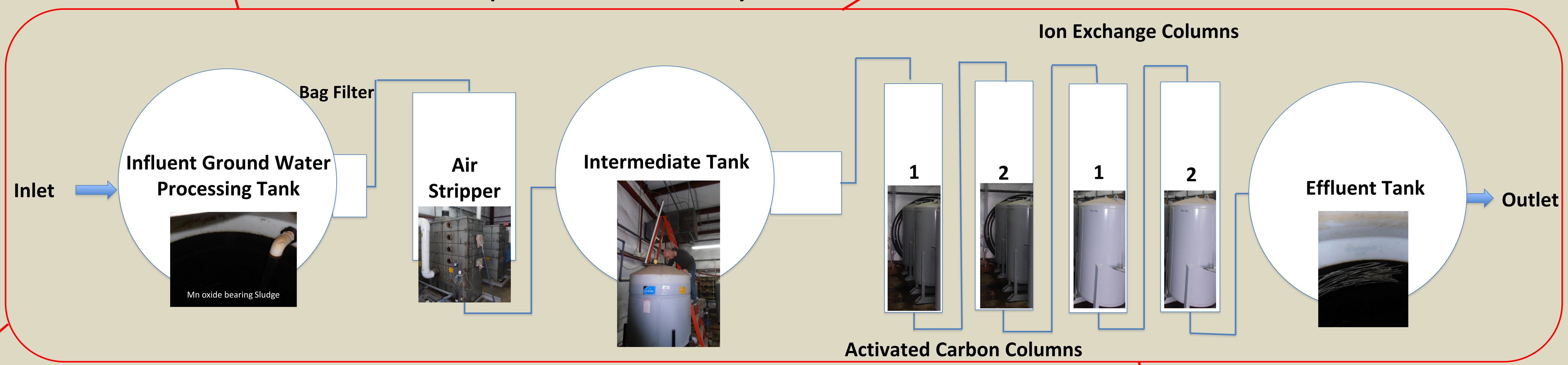


Fig. 4

Schematic of Pump & Treat Remediation System



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.

Energy Dispersive Spectroscopy of the SEM images reveal high percent composition of Mn, Fe, Al, and Si oxides (trace Na, and Ti) (Fig. 2).

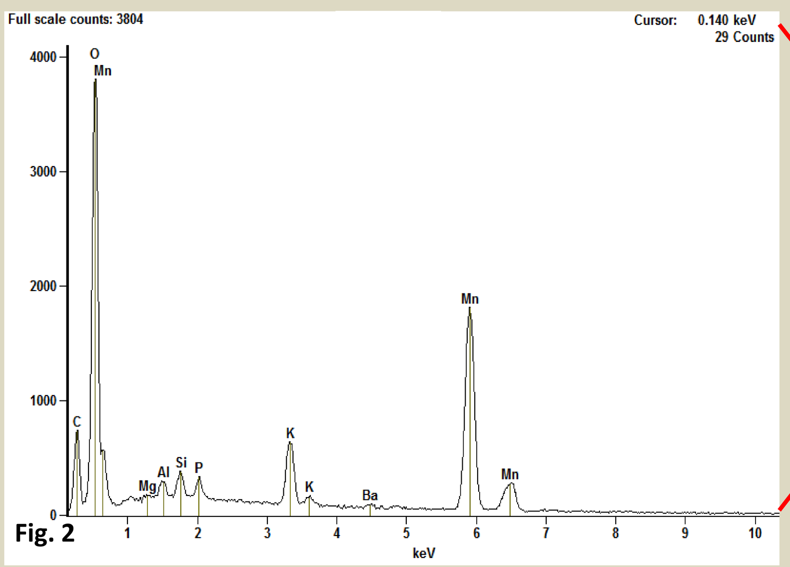
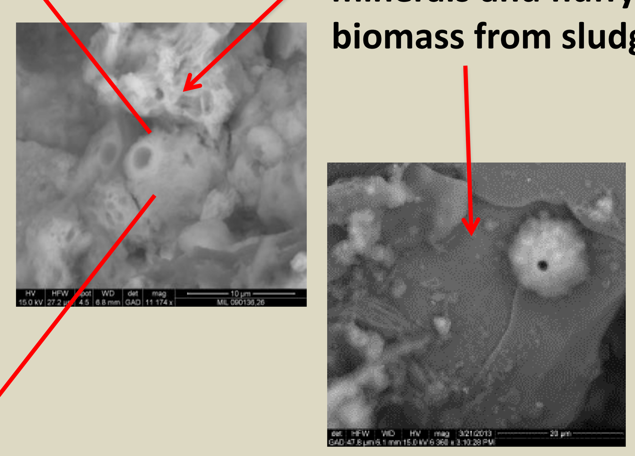


Fig. 2

Mn oxide coated clays, particles, platy minerals and fluffy biomass from sludge



Chemical Analysis and Results

Inductively coupled plasma-optical emission spectrometry (ICP-OES) (Table 2.) reveals the bulk chemical composition of the sludge. Manganese, copper, cobalt, and Nickel identified in sludge collected from the carbon filter have elevated concentrations while K, Ca, and Mg being minor, and Na marginal.

K-edge X-ray absorption spectroscopy (XAS) of sludge suggests Zn, Co, and Ba, which are incorporated into specific binding sites on the Mn oxide surface (preliminary results).

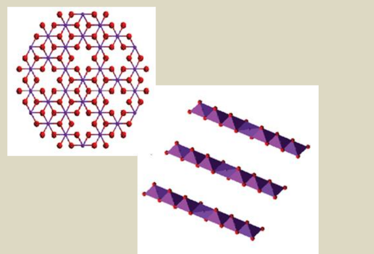


Table 2. Water ICP-OES and ICP-MS values

	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
Intemediate 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

DISCUSSION

- **ICP-OES** reveals fluctuation of solid (mg/g) and aqueous (mg/L) phase chemical concentrations along the treatment stream, reflecting the contaminants of concern listed in NCSU Lot 86 (EPA/ROD/R04-96/277-1996). Dissolved concentrations of Mn (26.16 mg/L) in carbon filter columns are elevated compared to other system components. Sasaki et al., (2008), reported that carbon fiber enhanced *Phoma sp.* oxidation ability of Mn(II), most significantly under more unfavorable conditions such as high Mn concentrations, coexisting inhibitive components, or lacking nutrients.
- **Culture based microbial analysis** reveals approximately 95% are fungi and only 5% are bacteria. Mycogenic birnessites are highly redox active and may facilitate the breakdown of organic molecules. Mn oxides are forming in all stages of the treatment system, likely resulting from biologically mediated processes.
- **Scanning Electron Microscopy** reveals solids recovered from the treatment system to be complex microbial-mineral assemblages (Mn oxides coated fungal hyphae and organism-like spheres) (Fig. 2).
- **Energy Dispersive Spectroscopy** of the SEM suggests that the platy minerals are likely aluminosilicates, in agreement with XRD measurements. Other areas in the biomass contain carbon and manganese, with lower concentrations of trace metals.
- **X-ray diffraction (XRD) analysis** reveals the same Mn layer-type oxide phase (birnessite) 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.
- **Mn K-edge X-ray absorption spectra (XAFS)** of sampled biogenic oxides have similar amplitude, shape, and frequency, meaning that the four oxides have essentially very similar structures (Fig. 1). These minerals, which are often biogenic, are commonly associated with redox transformations of organic contaminants and the sorption of metal ions (Post, 1999). The sorption of metals to these Mn oxides may help remove metals from the aqueous phase. These metals may be remobilized by reductive dissolution of Mn oxides (possibly in Activated Carbon column 2).

CONCLUSION

It is becoming increasingly evident that fungi represent a great potential for the remediation of a wide range of pollutants, including metals. The results in this study suggest fungi may also contribute to the remediation of Mn-contaminated sludge and ground water, warranting continued investigations of the cultivated Mn(II)-oxidizing fungi. Future investigations of these organisms revealing the mechanisms of Mn(II) oxidation and the factors influencing optimal growth and activity will greatly aid the engineering of efficient systems for Superfund site bioremediation.

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