Evaluating the Metal Tolerance Capacity of Microbial Communities Isolated from the Alberta Oil Sands Process Water

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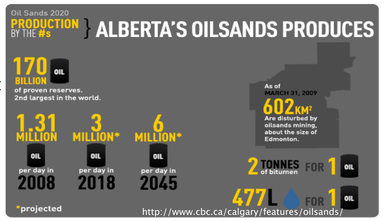
Introduction

The Alberta Oil Sands are one of the largest oil sand deposits in the world. It took until 2003 to be considered economically viable. The oil sands have been known as "tar sands" because the oil is found in a tar-like form mixed with sand, making it more difficult and costly to extract[8].

The Extraction Process

Oil sands are mined via open pit mining. In the 1990s new methods improving efficiency[7]:

- Trucks loaded with the oil sands, sent to extraction plant
- Hot caustic water used to separate crude oil from sand
- Tailings/wastewater sent to tailings ponds to settle, overlying water can be recycled



Tailings Ponds

Tailings consist of[5]:

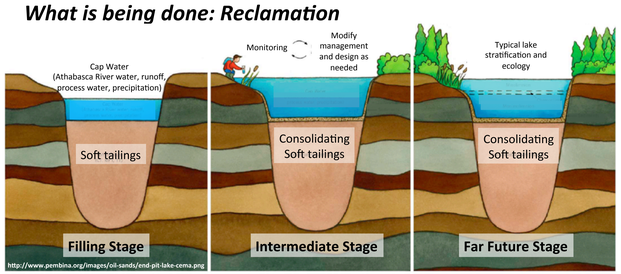
- Water
- Sand
- Clay
- Contaminants[6]
- Unrecovered hydrocarbons

Contaminants[6]:
- Metals (As, Al, Cr, Cu, Pb, Fe, Ni, Zn)
- Naphthenic acids
- Polycyclic aromatic hydrocarbons

What is being done: Reclamation

Goal – to return the landscape to natural, sustainable form

Contaminants still present in underlying tailings

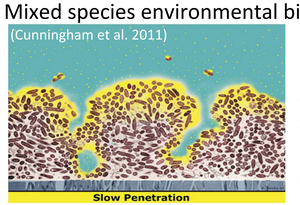


What else can be done: Remediation

We are investigating the microbial communities present in the Oil Sands Process Water (OSPW) for the potential application in bioremediation. Specifically aim to harnessing the microbes in biofilms to use in bioreactors.

Why biofilms?

Mixed species environmental biofilms have a competitive edge[3]:



1. Cooperative community effort to degrade complex organic molecules
2. Protection from metals present in OSPW
3. Diffusion limitations within biofilm create different nutrient tensions
4. Highly adaptive consortia

Methods

- Bacteria grown under metal stresss using the Calgary Biofilm Device and 96-well microtiter plates
- Organisms tested:
  - OSPW mixed species community
  - *C. metallidurans* (model organism)
- Confocal Laser Scanning Microscopy (CLSM)
- Statistical analysis and correlations with physiochemical parameters

Statistical analysis and correlations with physiochemical parameters:
- Metal-sulfide solubility product (pK_{sp})
- Electronegativity (X_m)
- Standard redox potentials (ΔE_0)
- Pearson's softness index (σ_p)
- First ionization energy (I_1)
- Log of the first hydrolysis constants ($|\text{Log } K_{OH}|$)

Results

CLSM – OSPW biofilm growth

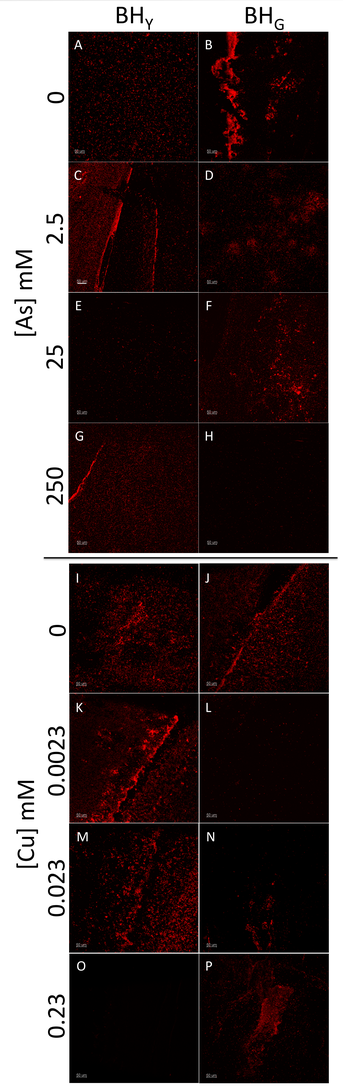


Fig. 1. CLSM 3D images of OSPW biofilms under two metal stressors: one that the consortia exhibited a relatively high tolerance (As, 125 – 250 mM), and another to which a lower tolerance was evident (Cu, 0.4 – 1.6 mM). Contrasts can be seen in biofilm microcolony formation from unstressed to inhibitory concentrations of metal stresses, over several orders of magnitude. Biofilms were grown using the Calgary Biofilm Device (CBD) aerobically (25°C 125 rpm) for six days, with a tailings pond water inoculant, using a minimal salts media (BH) amended with 1 g L^{-1} yeast extract (BH_Y) or glucose (BH_G).

Relative toxicity of metals

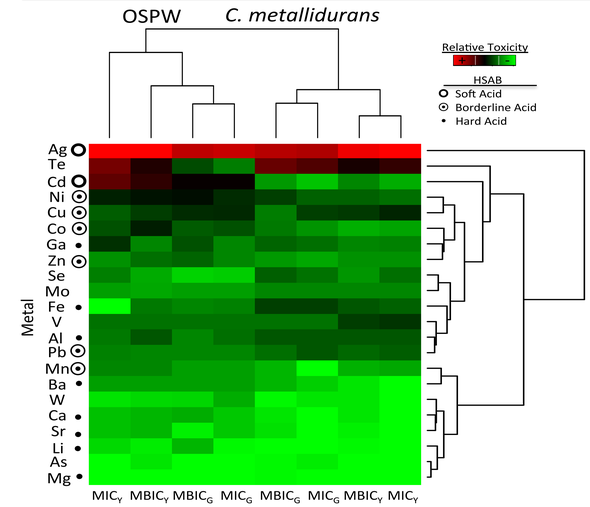


Fig. 2. Heat map showing relative toxicity of metals tested on the OSPW mixed species consortia and *C. metallidurans*. The heat map colors represent averages based on values obtained from two to nine trials, where red reflects the most toxic metals and green represents the least toxic. MIC_G: Minimum inhibitory concentration (MIC) with glucose amendment; MIC_Y: MIC with yeast extract amended; $MBIC_G$: minimum biofilm inhibitory concentration (MBIC), glucose amended; $MBIC_Y$: MBIC, yeast extract amended.

Typical OSPW consortia correlative profile

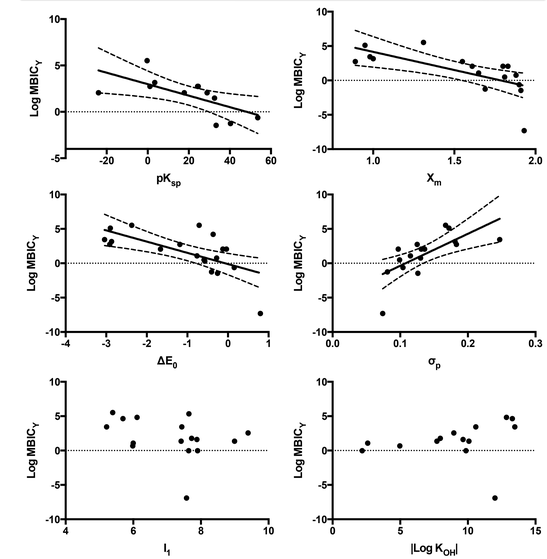


Fig. 3. Typical trends seen between physiochemical parameters and OSPW mixed species community metal susceptibility ($MBIC_Y$ shown). Trend lines and 95% confidence bands (dotted lines) shown on linear regressions that correlate with significance.

Correlational disparity between OSPW and single species cultures

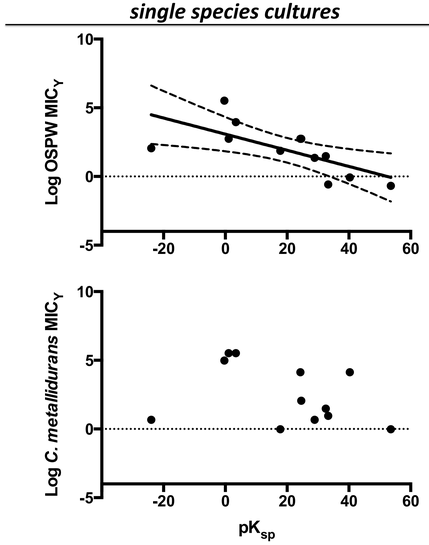


Fig. 4. Comparison of pK_{sp} vs. MIC_Y, where significant correlation is evident with the OSPW community (top), and absent with *C. metallidurans* (bottom).

Conclusions

Metal toxicity
- Soft acids > borderline acids > hard acids

Consistent correlations with ΔE_0, X_m, σ_p, pK_{sp}
- Reflection of a metal's affinity for electrons and ligands

Single versus multi species
- Stronger correlations were observed with the *C. metallidurans* (except pK_{sp})

Tolerance values grouped closer by media augment than growth form
- Highlights importance of growth conditions when investigating metal-microbe interactions (*in vitro* vs *in situ*)

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