Comparison of the Biocatalytic Ability of *Shewenella* used in the Reduction of Cr (VI)

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Introduction

Hexavalent chromium is a common contaminant in groundwater and is a byproduct of the production of dyes, paints, inks, plastics, and metal welding. Highly mobile in aqueous environments, Cr (VI) is a known carcinogen and can enter the body through contact with the skin.

This work explores the bioremediation of hexavalent chromium in microbial fuel cells (MFCs) with strains of the *Shewanella* genus as biocatalysts for the cathodic reaction. MFCs produce electricity from biochemical energy and consist of an anode, cathode, electrolyte, ion exchange membrane, and biocatalyst (generally bacteria). As microbes oxidize substrates at the anode, electrons from this process are directed through an external circuit to the cathode. In the external circuit, electrons are passed through a load or resistor. Finally, electrons accumulate at the cathode and reduce oxidants. Aqueous Cr (VI) served as the oxidant in the MFC and was reduced to a less toxic and insoluble form, Cr (III).

Shewanella have been proven to function in MFCs at the anode and was used in this study as biocatalysts at the cathode. To explore differences occurring on the species/strain level the following strains were tested: *S. oneidensis* MR-1, *S. species* W3-18-1, *S. amazonesis* SB2B, *S. species* ANA-3, *S. loihica* PV-4, and *S. species* MR-4. Evaluations were based on overpotential losses, residual chromium following injection, efficiency ratings, and electron microscopy.

Methods

Shewanella strains were cultured from frozen stock under the conditions and introduced to the fuel cell as shown in the flowchart shown in Figure 2. MR-1 was utilized as the anodic biocatalyst. The fuel cell utilized in these experiments is shown in Figure 1.

During operation, sampling of the cathode for Cr(VI) was performed at regular intervals and analyzed by ion chromatography. Voltages across a 10 ohm external resistance was recorded every minute by a digital multimeter. Nitrogen was purged through each chamber to maintain anaerobic conditions for the duration of operation.

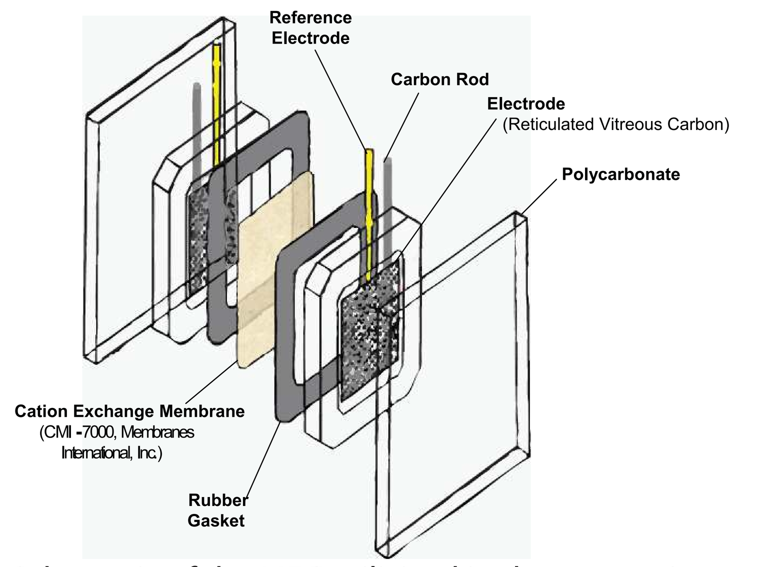

Figure 1. Schematic of the MFC utilized in these experiments.

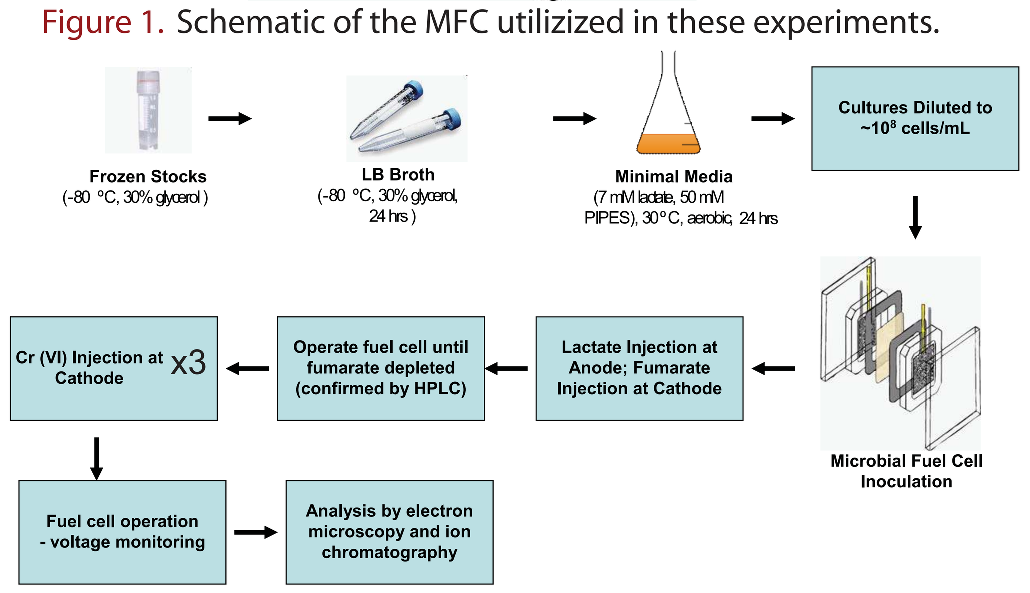

Figure 2. Flow chart describing experimental procedure.

Results

Differences in observed voltage from theoretical voltages from the Nernst equation can be characterized as overpotentials.

$$E_{OCV} = E_{Nernst} - (\sum \eta_{anode} + \sum |\eta_{cathode}|)$$

These losses can be grouped into 3 categories: (1) activation losses, (2) bacterial metabolic losses, and (3) mass transport or concentration losses. Overpotentials under open circuit conditions are displayed in Figure 3.

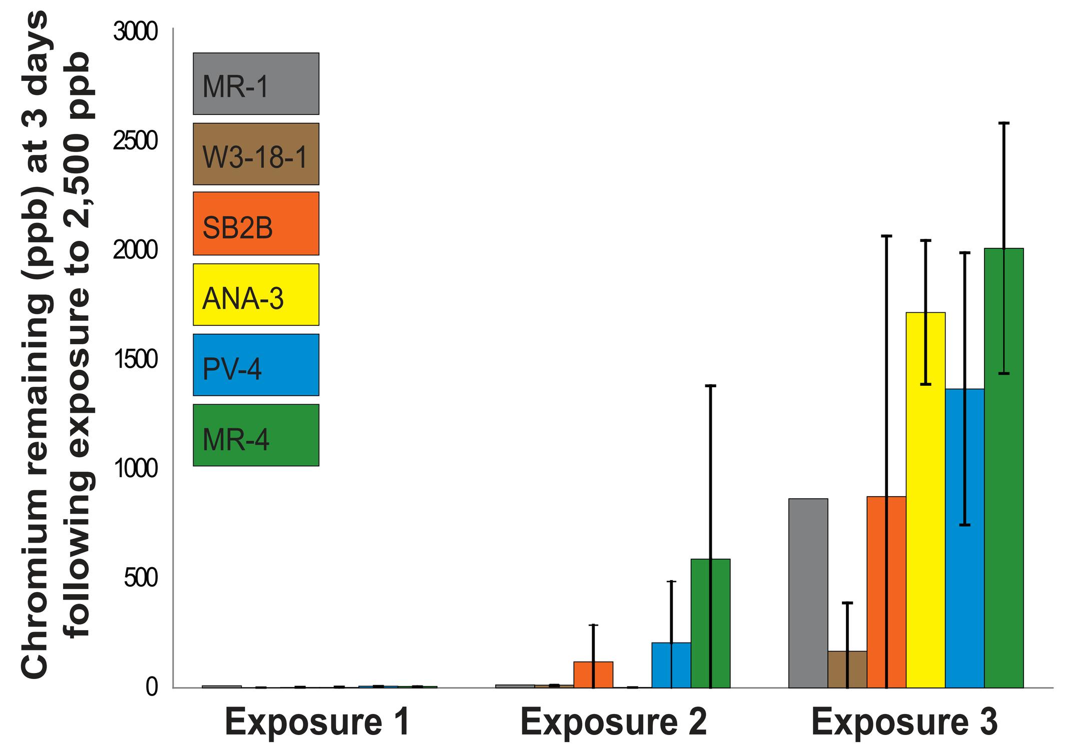

Figure 4. Final Cr (VI) concentrations.

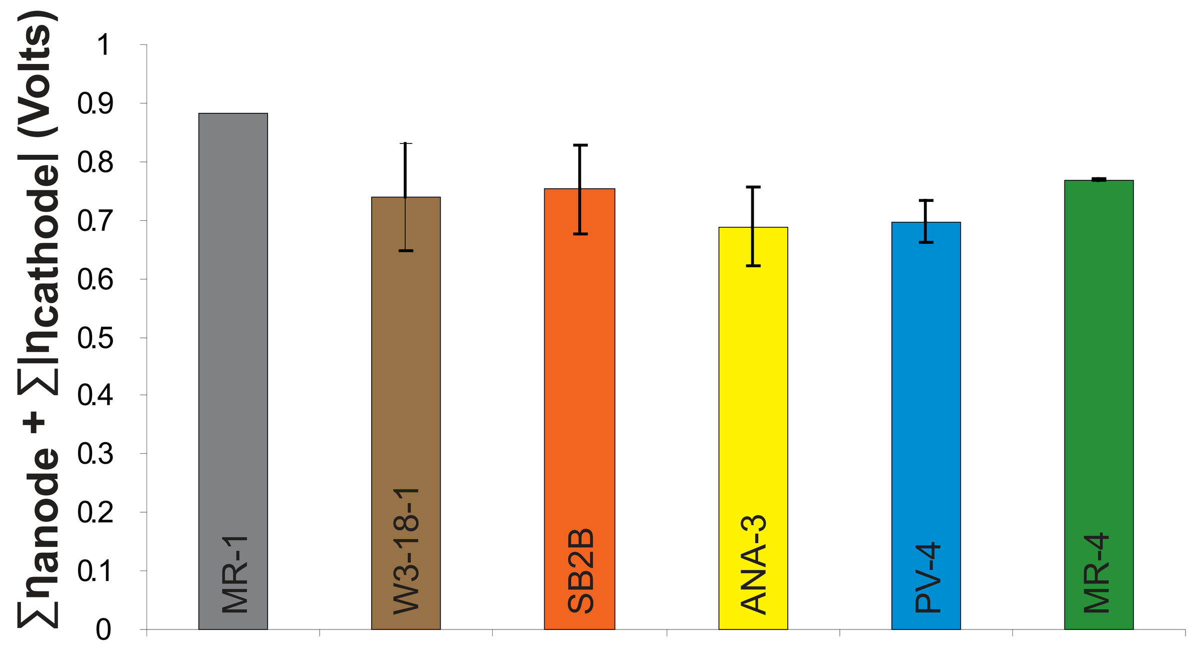

Figure 3. Overpotentials at open circuit. Error bars represent one standard deviation.

Fuel cells were injected with concentrated Cr (VI) to bring cathode Cr (VI) concentrations to approximately 2,500 ppb for each exposure window. Two additional injections were performed every three days after sampling. Chromium remaining after three days of initial exposure was measured and is shown in Figure 4.

For each exposure, an efficiency rating (Figure 5) was calculated by taking the ratio of theoretical electrons used in reducing Cr (VI) to Cr (III) to the amount of electrons transferred across the external resistor of the fuel cell. The amount of electrons transferred over the external resistance was calculated by integration of current vs. time curves.

$$\eta = \frac{\text{\# of electrons required cause observed Cr (VI) reduction}}{\text{\# of electrons transferred over external resistance}}$$

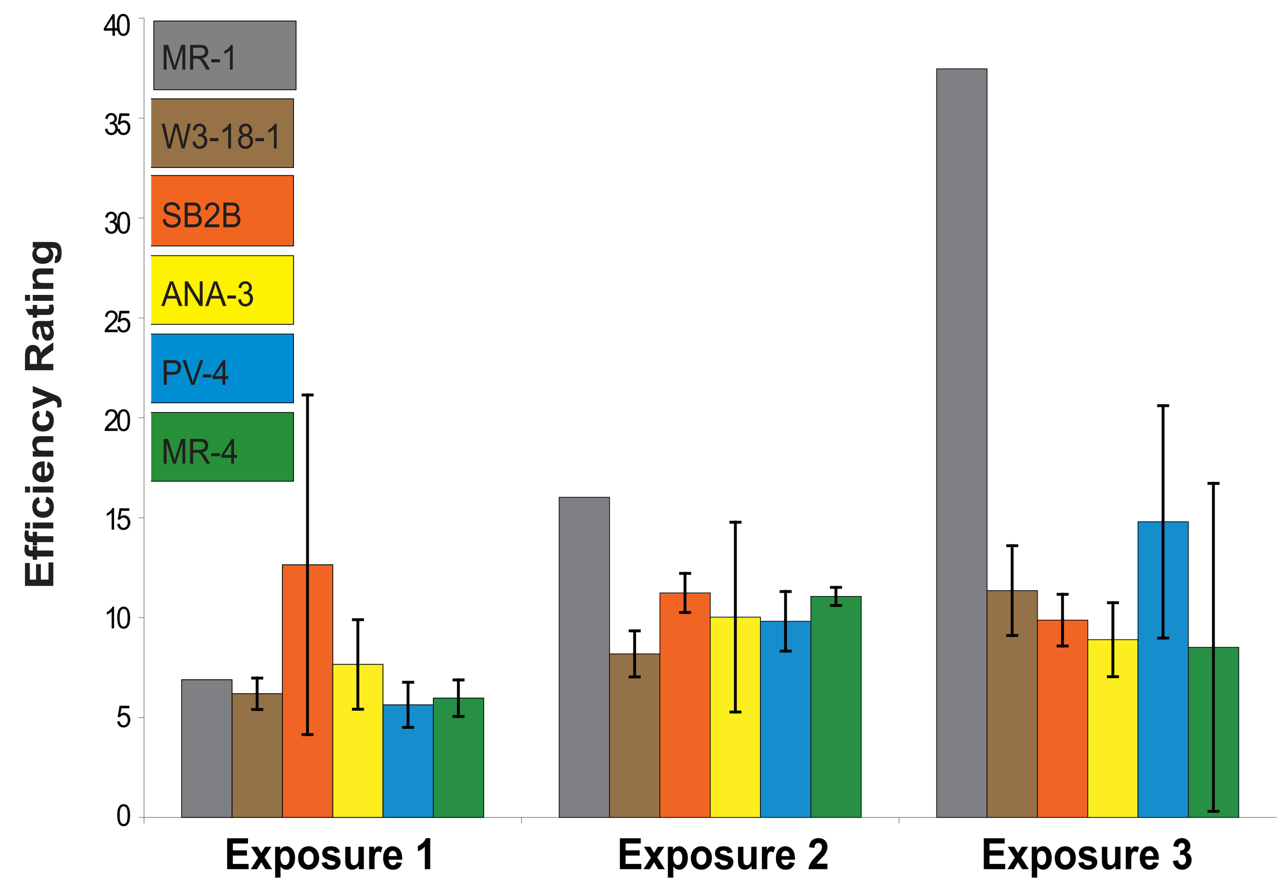

Figure 5. Efficiency ratings for each exposure. Error bars indicate one standard deviation.

Exposed electrodes were imaged by electron microscopy and selective area elemental analysis (by energy dispersive spectroscopy, EDS) performed on areas of interest. Results are shown in Figure 6. Values reported are in total counts between 5 and 6 keV. Chromium Ka emission is tabulated at 5.4 keV

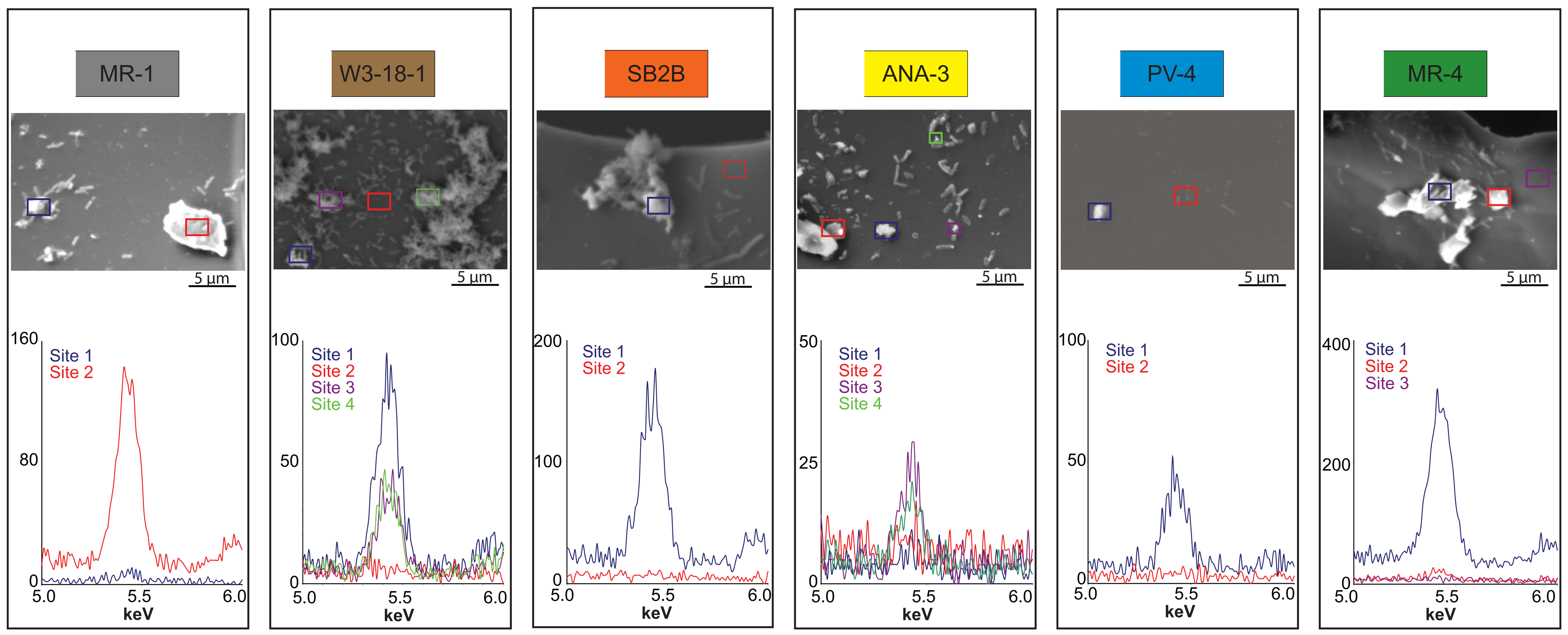

Figure 6. Scanning electron micrographs of electrodes after exposure 3. Elemental analysis results for chromium is shown below with selected areas highlighted on the micrographs. Values reported are total counts from 5 - 6 keV.

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Discussion

Statistically similar overpotentials in Figure 3 suggest similar internal resistances between cells populated with different strains. Any differences in performance can be attributed to differences associated with the strains rather than internal losses.

Figure 4 compares final Cr (VI) concentrations after three consecutive exposures. Consecutive exposures saw a decrease in Cr (VI) reduction for all strains. *S. species* W3-18-1 consistently reduced the most Cr (VI) for all three exposures. For all species, Cr (VI) was reduced to negligible concentrations after the first exposure, however, this was not for true for exposure 2.

Figure 5 compares efficiencies between strains calculated by the ratio of electrons transferred in the reduction of Cr (VI) to the electrons transferred across the external resistance. Surprisingly, all strains produced efficiency ratings greater than one. This implies oxidation and electron production within the cathodic compartment of each MFC. This may occur by several methods including oxidation of inactive biomass and unintentional lactate transport through the cation exchange membrane.

Strains vary in their efficiencies between exposures, however, *S. species* W3-18-1 and *S. loihica* PV-4 show a consistent increase between exposures.

Future Work

Future work will include further analysis of *S. oneidensis* MR-1, *S. species* MR-7, and *S. putrefaciens* CN-32. Data from the eight strains will be compared to determine the greatest Cr (VI) reduction capabilities. The system will be optimized by mixed microbial population studies and the analysis of additional strains at the anode in terms of coulombic effi-

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