

Natural Humics Impact Uranium Bioreduction and Oxidation

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ABSTRACT

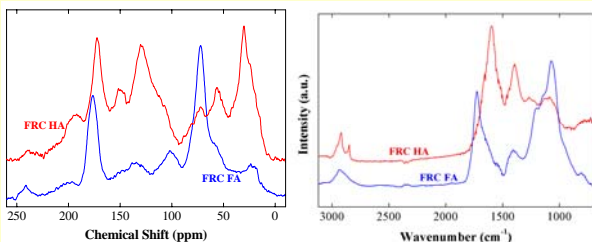
Although humic substances occur ubiquitously in soil and groundwater, their effect on the biological reduction of uranium(VI) and subsequent reoxidation of U(IV) is poorly understood. This study investigated the role of natural humics in enhancing the bioreduction of U(VI) in laboratory kinetic studies, in field push-pull tests, and in the presence or absence of metal ions such as Ca^{2+} and Ni^{2+} , which are known to inhibit the biological reduction of U(VI). Results from laboratory experiments indicate that, under strict anaerobic conditions, the presence of humic materials enhanced the U(VI) reduction rates (up to tenfold) and alleviated the toxicity effect of Ni^{2+} on microorganisms. Humic acid was found to be more effective than fulvic acid in enhancing the reduction of U(VI). Such an enhancement effect is attributed to the ability of these humics in facilitating electron transfer reactions and/or in complexing Ca^{2+} and Ni^{2+} ions. Similarly, field push-pull tests demonstrated a substantially increased rate of U(VI) reduction when humic acid was introduced into the site groundwater. However, humics were also found to form complexes with reduced U(IV) and increased the oxidation of U(IV) (when exposed to oxygen) with an oxidation half-life on the order of a few minutes. Both of these processes render uranium soluble and potentially mobile in groundwater, depending on site-specific and dynamic geochemical conditions. Future studies must address the stability and retention of reduced U(IV) under realistic field conditions (e.g., in the presence of dissolved oxygen and low concentrations of complexing organics).

BACKGROUND

Humic substances are known to contain electron-accepting and donating moieties (e.g., quinonoid C=O and ketonic C=O) and acts as exogenous electron shuttles in mediating microbial reduction or oxidation of metals. Humics are also known to be highly heterogeneous and consists of a mixture of organic compounds (or subcomponents) with different structures and functional groups, which were hypothesized to play different roles in redox reactions such as microbial reduction of uranium and in complexation reactions with metals including reduced U(IV).

The FRC soil and groundwater contain relatively high contents of metal ions such as Ca^{2+} , Ni^{2+} , and others, which inhibit the bioreduction of U(IV). We hypothesize that addition of humic substances could enhance the bioreduction rates of U(VI) because humics could potentially act both as electron shuttles to mediate the bioreduction processes and as complexing agents to alleviate the toxic effect of Ni^{2+} to microorganisms. On the other hand, humics may also form complexes with reduced U(IV), resulting in an increased solubility and mobility of U(IV). The overall goal of this study is to provide an improved understanding of the interactions between microorganisms and humics for immobilizing uranium through bioreduction and the effects of humics on the solubilization and mobilization of uranium through complexation and reoxidation processes.

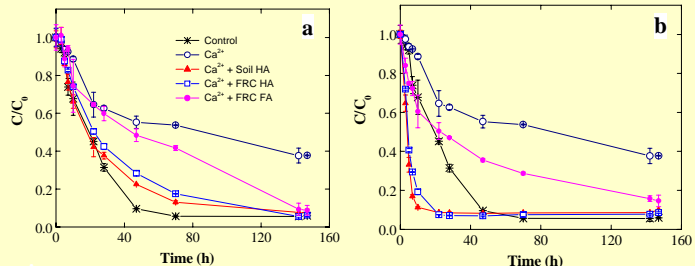
FRC HUMIC CHARACTERIZATION



Both FRC humic (FRC-HA) and fulvic (FRC-FA) acids were isolated and well characterized. ^{13}C -NMR analysis indicates that FRC HA contains relatively high amounts of conjugated aromatic moieties (120-140ppm) but less amounts of carboxyl and hetero-aliphatic C-O and C=O (or ketone) functional groups.

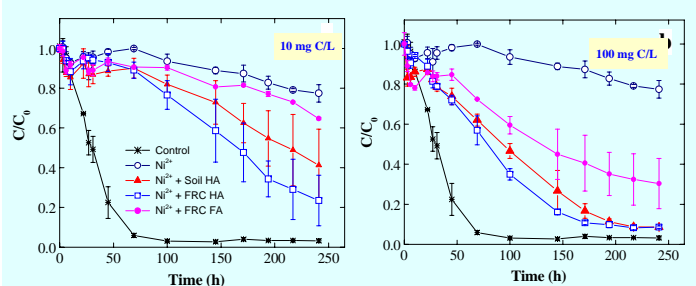
FTIR results are consistent with NMR analysis. The abundance of aromatic C=C functional groups (at ~1600 nm) are in the order of Soil HA > NOM-PP > NOM-CH fractions. However, FRC FA contains a relatively higher amount of C=O and carboxyl functional groups (at ~1730 nm). Additionally, FRC HA contains a relatively higher amount of -CH₂ and -CH₃ functional groups than the FRC FA.

EFFECT OF Ca^{2+} AND HUMICS ON U(VI) BIOREDUCTION



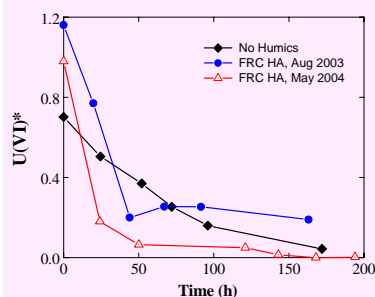
- Recent studies (Brooks et al. 2003) indicate that Ca^{2+} ions (0.5 mM) significantly inhibit the bioreduction of U(VI) (as uranyl acetate). This study found that addition of humics not only alleviated the inhibitory effect of Ca^{2+} but also enhanced the bioreduction rates of U(VI), particularly at a relatively high humic concentrations (100 mg C/L). The added humic concentration was (a) 10 and (b) 100 mg C/L; the initial U(VI) concentration was ~60 mg/L, and *Shewanella* CN-32 cell concentration was $\sim 1.5 \times 10^8 \text{ mL}^{-1}$.
- The FRC HA and IHSS Soil HA are much more effective than the FRC FA in enhancing the bioreduction of U(VI). Results are consistent with our previous findings that different humic materials or fractions play different roles in mediating the bioreduction of U(VI) due to their chemical and structural characteristics (Gu and Chen 2003).

EFFECT OF Ni^{2+} AND HUMICS ON U(VI) BIOREDUCTION



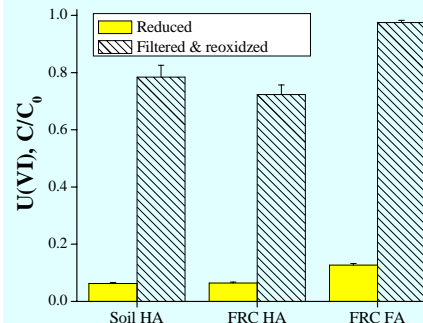
- Ni^{2+} ion (0.2 mM) is known to be toxic and appeared to be much more effective in inhibiting the bioreduction of U(VI) than Ca^{2+} ions. Addition of humics partially alleviated its toxicity but did not completely restore the bioreduction rates as compared with the control (even at the humic concentration of 100 mg C/L). The added U(VI) concentration was ~60 mg/L, and *Shewanella* CN-32 cell concentration was $\sim 1.5 \times 10^8 \text{ mL}^{-1}$.
- Similarly, the FRC HA and IHSS Soil HA were found to be more effective than the FRC FA in enhancing the bioreduction of U(VI), likely due to their relatively high contents of conjugated aromatic compounds.

FIELD PUSH-AND-PULL TEST RESULTS



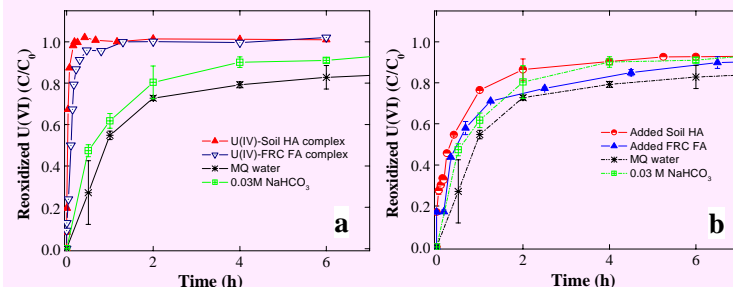
- Field push-pull tests at FRC site confirmed that addition of FRC HA enhances the removal of U(VI), likely due to an increased bioreduction and/or the complexation of humics with Ca^{2+} , Ni^{2+} or other toxic metals. Ethanol was used as an electron donor. Note that U(VI)* is the dilution-adjusted U(VI) concentration normalized by the injection concentration.

COMPLEXATION OF HUMICS WITH BIOREDUCED U(IV)



- Like many organic ligands (e.g., citrate and EDTA), humics were found to form soluble complexes with reduced U(IV) and prevented it from precipitation.
- After the bioreduction, more than 70% of reduced U(IV) in the presence humics readily passed through 0.2- μm membrane filters and was oxidized to U(VI) in open air.
- These observations have important implications on uranium stability and mobilization.

RAPID REOXIDATION OF BIOREDUCED U(IV)



- Figure shows the oxidation kinetics of bioreduced U(IV) in the presence of humics or in 0.03 M NaHCO_3 and water. The oxidation of U(IV) was rapid, on the order of minutes to hours. (a) Reduced U(IV) was complexed with humics (obtained by the reduction of U(VI) in the presence of FRC HA or FRC FA); (b) FRC HA or FRC FA were added to U(IV) precipitates (obtained by the reduction of U(VI) in the absence of humics).
- Addition of Soil HA and FRC FA increased the re-oxidation rates of the bioreduced U(IV) in the presence of these humics [due to the complexation of humics with reduced U(IV)]. However, the reoxidation rates are comparable with those added with bicarbonates when humics were added to the bioreduced U(IV) precipitates. The Soil HA appears to be more effective than FRC FA in enhancing the reoxidation.
- The complexation of humics with reduced U(IV) was validated – the bioreduced U(IV) could not be filtered out by 0.2 μm filters; and the filtrate uranium concentration increased to its original concentration after oxidation of the filtered samples.

SUMMARY

- The presence of humics greatly enhances the bioreduction rates of U(VI), particularly in the presence of metals such as Ca^{2+} and Ni^{2+} in laboratory batch kinetic studies. These studies were validated by the field push-pull tests that addition of humics enhanced the removal of U(VI) in groundwater.
- The soil humic acid (HA) is more effective than the fulvic acid (FA) fraction in enhancing the bioreduction of U(VI), indicating that different humic materials vary greatly in structural features, functional groups, and redox characteristics.
- However, the bioreduced U(IV) could also be rapidly oxidized when it is exposed to air (or oxygen), and the re-oxidation rates are on the order of minutes to hours. In particular, the reduced U(IV) forms complexes with humics, and it renders U(IV) to be more readily oxidized, on the order of a few minutes.
- This research confirms our previous studies that humics are complex heterogeneous materials, which may play dual functional roles in both mediating the bioreduction of U(VI) under low redox conditions but also increase the reoxidation rates of the reduced U(IV) under oxic conditions.
- These observations point out potential challenges for immobilizing uranium in field applications. The reduced U(IV) may rapidly reoxidize and be remobilized, depending on site-specific geochemical conditions such as the fluctuations of groundwater dissolved oxygen, the presence or absence of humic substances, carbonate concentrations, and pH.

REFERENCES AND ACKNOWLEDGEMENT

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