Integrated investigation on the production and fate of organo-Cr(III) complexes from microbial reduction of chromate

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I. ABSTRACT

Chromium contamination exists at 13 of the 18 Department of Energy facilities studied; microbial reduction to form insoluble trivalent Cr, Cr(III), is a potential treatment for such sites. In our prior research on Cr(VI) reduction, we have discovered that soluble organo-Cr(III) complexes are likely formed and then, perhaps, further transformed to insoluble Cr(III) precipitates. The production of organo-Cr(III) complexes from chromate reduction is documented in eukaryotic systems because the stable DNA-Cr adducts are mutagenic. However, the formation of soluble organo-Cr(III) complexes from microbial reduction of chromate has only recently been discovered, bringing up the necessity for investigating the significance of the soluble complexes in Cr bioremediation. The proposed research is aimed at: (1) characterizing the scope and extent of organo-Cr(III) complex formation by chromate reducing microorganisms, (2) evaluating cellular components that can potentially form organo-Cr(III) complexes, (3) addressing the stability and biodegradability of these organo-CR(III) complexes, and (4) assessing the fate and transport of these compounds in soils. The results will provide scientific guidance on whether organo-Cr(III) should be considered during application of Cr bioremediation. The information will also help establish a more complete biogeochemical cycle for Cr, which is currently lacking organo-Cr(III) complexes.

II. BACKGROUND

- Current use of chromium
  Stainless steel, furnace bricks, dyes and pigments, chrome plating, wood preserving, leather tanning, and chemical processes as a powerful oxidizing agent.
- Consumption
  1996-2001 USA, 362 to 540 thousand metric tons per year
- Chromium (III), Cr(III)
  Naturally Occurring as ($FeO*Cr_2O_3$)
  insoluble
  and non-carcinogenic
- Chromium (VI), Cr(VI)
  Oxyanions, chromate, CrO_4^{2-}
  soluble
  and toxic
- Cr(VI) contamination is DOE concern as it is found at multiple sites

III. MICROBIAL REDUCTION AS A MEANS OF BIOREMEDIATION

A. Current model

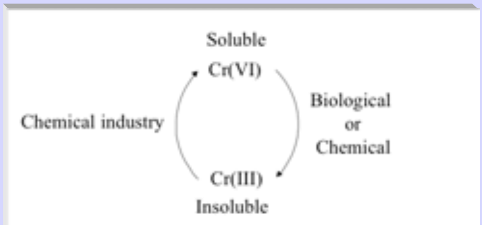


Fig. 1 A simplified model of chromium biogeochemical cycle

B. Evidence for a more complex Cr biogeochemical cycle

The end products of Cr(VI) reduction by microorganisms are often undefined in earlier studies

Recent studies have indicated soluble Cr(III) in the culture supernatants

Soluble organo-Cr(III) complexes have been observed from wastewater treatment effluents

Soluble Cr(III) has been detected in the groundwater of the DOE's Savannah River Site

Enzymatic reduction of Cr(VI) resulted in NAD^+-Cr(III) and cytochrome c-Cr(III) complexes

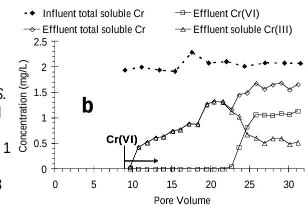
In mammalian systems, Cr(VI) reduction leads to stable organo-Cr(III) complexes with DNA, RNA, protein, and small metabolic intermediates

New concept: Organo-Cr(III) is an important component of the Cr biogeochemical cycle.

C. Supporting Results

1. Production of soluble Cr(III) after Cr(VI) reduction from a soil column

Fig. 2. Production of soluble Cr(III) from Cr(VI) by a soil column. The column was inoculated with *S. oneidensis* MR-1, fed with lactate and chromate (2 mg/L) at 1 mL/h with a column retention time of 22.3 hours.



2. *S. oneidensis* MR-1 converts Cr(VI) to soluble Cr(III) and then slowly to insoluble Cr(III)

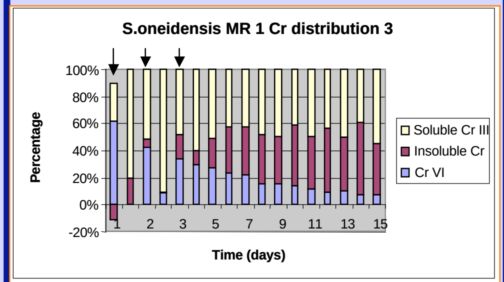


Fig. 3. Transformation and distribution of Cr(VI) by *S. oneidensis* MR1 cell suspensions. Arrows indicate the addition of 2 mg/L of chromate. The produced Cr(III) is initially soluble (passing 0.2-µm membrane filters) and gradually becomes insoluble Cr(III).

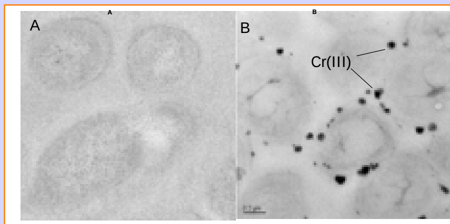


Fig. 4. Transmission electron micrograph showing *S. oneidensis* MR-1. Chromate (2 mg/L) was added to the late log phase culture and was completely reduced in 12 hours. Cells from 1 day (A) and 14 days (B) after chromate reduction. (Images taken by Viamajala/Peyton/Gorby at EMSL). Dark spots are Cr(III) particles shown by EDAX.

3. Production of soluble organo-Cr(III) complex from enzymatic Cr(VI) reduction

A **flavin reductase system** catalyzes:

Cr(VI) + 1.5 NADH

→ Cr(III) + 1.5 NAD^+

NAD⁺-Cr(III) Complexes

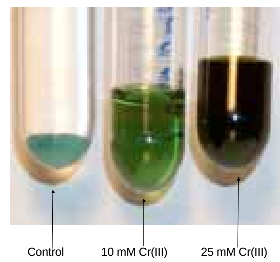
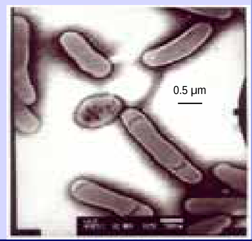


Fig. 5. Soluble Cr(III) end product formed at both low (1 mM) and high (50 mM) Concentrations. The product is characterized as an NAD^+-Cr(III) complex. Control contains 10 mM Cr(III) in phosphate buffer (pH 7) with apparent precipitation.

4. Bacterial degradation of the NAD^+-Cr(III) complex

Fig. 6. TEM picture of bacteria from 1-year old culture on NAD^+-Cr(III). Chemical analysis shows the decrease in both NAD^+ and soluble Cr(III). The dark area sounding the cells represents Cr precipitates. The rod-shaped bacterium is dominant, and the coccus is also present. The picture was taken at WSU by Geoff Puzon.



D. Summary

1. Soluble Cr(III) is produced from bacterial reduction of Cr(VI)
2. An enzyme system produces a soluble NAD^+-Cr(III) complex
3. Bacteria can degrade the NAD^+-Cr(III) complex
4. Cr(III) accumulation is observed on bacterial surfaces after the degradation of the complex
5. Our observations suggest that soluble Cr(III) in the groundwater of the DOE's Savannah River Site is possibly organo-Cr(III)
6. Organo-Cr(III) is likely an integral part of the biogeochemical cycle of Cr

V. REFERENCES

1. Puzon, G. J., J. N. Petersen, A. G. Roberts, D. M. Kramer, and L. Xun. 2002. A bacterial falvin reductase system reduces chromate to a soluble chromium(III)-NAD^+ complex. Biochem. Biophys. Res. Comm. 294:76-81.
2. Alam, M.,DR Yonge, A Hossain, BM Peyton and JN Petersen. 2004. Soil column experiments for the bioremediation of Cr(VI) contaminated groundwater using *Shewanella oneidensis* MR-1. Submitted to Environ. Sci. Technol.