

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

Geoffrey J. Puzon^{1,5}, Ranjeet Tokala^{3,5}, Zhicheng Zhang^{2,5}, Sue Clark^{2,5}, Brent Peyton^{3,5}, David Yonge^{4,5}, and Luying Xun^{1,5}, Departments of Molecular Biosciences¹, Chemistry², Chemical Engineering³, Environmental and Civil Engineering⁴, and Center for Multiphase Environmental Research⁵, Washington State University, Pullman, Washington

I. ABSTRACT

Chromium contamination exists at several Department of Energy facilities; microbial reduction to form insoluble trivalent Cr, Cr(III), is a potential treatment for such sites. We have discovered that some soluble organo-Cr(III) complexes are likely formed and then further transformed to insoluble Cr(III) precipitates. The production of organo-Cr(III) complexes from chromate reduction is well documented in Cr toxicity studies to humans. However, the formation of soluble organo-Cr(III) complexes from microbial reduction of chromate has only recently been discovered. Here we present more evidence on the formation, mineralization, and subsurface transport potential of organo-Cr(III) complexes. First, production of soluble organo-Cr(III) complexes by selected microorganisms has been observed. Second, we have observed the production of organo-Cr(III) complexes during chromate reduction in the presence of microbial cellular components. Third, a bacterium capable of mineralizing an organo-Cr(III) complex has been isolated. Fourth, the transport and fate of organo-Cr(III) complexes in soils have been investigated. Some results have been published. Our data thus far point to a likely extensive formation of various organo-Cr(III) complexes during bioreduction of chromate. Further studies will help establish a more complete biogeochemical cycle for Cr, including organo-Cr(III) complexes as an integral link. The information should also provide guidance on whether organo-Cr(III) complex formation should be considered during application of Cr bioremediation.

II. BACKGROUND

A. Previous findings supporting a more complex Cr biogeochemical cycle

1. Soluble Cr(III) is produced from *Shewanella oneidensis* MR-1 reduction of Cr(VI)
2. An enzyme system produces a soluble NAD⁺-Cr(III) complex
3. Bacteria can degrade the NAD⁺-Cr(III) complex

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

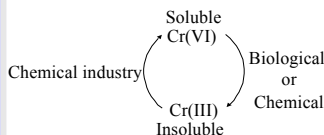


Fig. 1 A simplified model of chromium biogeochemical cycle

III. MICROBIAL PRODUCTION, TRANSPORT AND CHARACTERIZATION SOLUBLE Cr(III)

B. Additional Supporting Results

1. Production of soluble Cr(III) end-products by different microorganisms

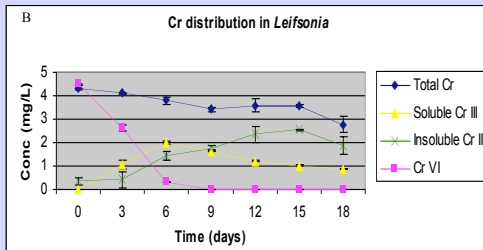
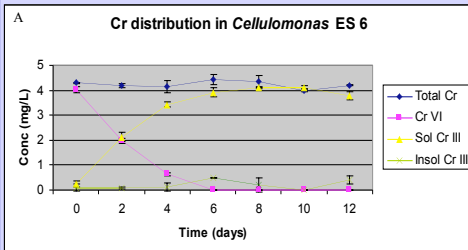


Figure 2A & B. Mass balance analysis of Cr during reduction of Cr(VI) by *Cellulomonas* ES6 and a *Leifsonia* sp. Bacteria were initially grown to an OD_{600nm} of 0.2 and 2.0 respectively. Cultures were then centrifuged and reconstituted in PBS with 5.5 mM lactate as the carbon source and 4 mg/ml Cr(VI). Cr(VI) reduction was monitored by diphenylcarbazide analysis. Total Cr and total soluble Cr were calculated by ICP/MS. Total soluble Cr was analyzed after centrifugation and filtration (0.22 μm) of the sample. Different bacteria appear to produce both soluble Cr(III) and insoluble Cr(III) with different efficiencies.

2. Formation of organo-Cr(III) end-products with cellular organics¹

Complex solubility	Organic ligand	Soluble Cr(III) (mM)	Percent soluble Cr(III)
Highly soluble organo-Cr(III) end products	Histidine	5.01 ± 0.06	100%
	Glutathione	4.76 ± 0.15	95%
	α-ketoglutarate	4.65 ± 0.05	93%
	Citrate	4.30 ± 0.10	86%
	Malate	3.88 ± 0.04	78%
	Serine	3.62 ± 0.14	72%
	Cysteine	3.43 ± 0.10	69%
	Pyruvate	3.25 ± 0.17	65%
	Oxaloacetate	2.86 ± 0.05	57%
	Slightly soluble organo-Cr(III) end products	Leucine	0.71 ± 0.04
Glycine		0.68 ± 0.01	13%
Insoluble organo-Cr(III) end products	Succinate	0.02 ± 0.01	0.4%
	Fumarate	< 0.01	0%
	Lactate	< 0.01	0%
	Tyrosine	< 0.01	0%
	Acetate	< 0.01	0%
	Ethanol	< 0.01	0%
KPI-Cr(III) Control	100 mM KPI pH 7.0	< 0.01	0%

Table 1. Organo-Cr(III) end-products formed by the reduction of Cr(VI) in the presence of cellular organics. Complete Cr(VI) reduction is confirmed by diphenylcarbazide assay. Soluble Cr(III) is measured by ICP/MS after 21 days. End-products were confirmed to be organo-Cr(III) complexes by EPR and absorption spectroscopy

3. Transport and fate of soluble organo-Cr(III) end-products

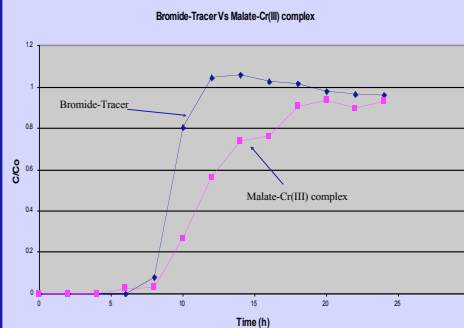


Figure 3. Mobility analysis of a soluble organo-Cr(III) complex, malate-Cr(III), in soil as compared to a bromide-tracer. The shift to the right for the malate-Cr(III) complex curve shows partial sorption of complex the initially, but then the complex is later released and remains in a mobile phase.

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

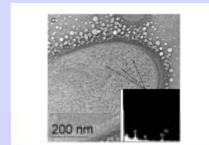


Fig. 4. TEM/EDS picture of bacterial isolate PTX1 after 4 months growth on NAD⁺-Cr(III). Chemical analysis confirmed a decrease in both NAD⁺ and soluble Cr(III). EDS analysis confirms Cr precipitates on the cell surface. Image taken by Alice Dohnalkova at EMSL.

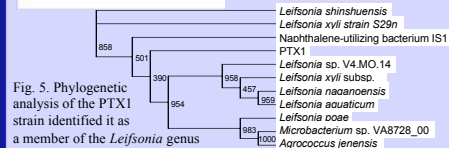


Fig. 5. Phylogenetic analysis of the PTX1 strain identified it as a member of the *Leifsonia* genus

5. Chemical characterization of organo-Cr(III) end-products

Chemical formula	Donor	Ionic charge	Notice
K ₂ [Cr(Ox) ₂]·2H ₂ O	Oxygen	-3	Synthesized
Cr(ACAC) ₃	Oxygen	0	Commercial
Cr(en) ₃ Cl ₃ ·2H ₂ O	Nitrogen	+3	Synthesized
[Cr(OAc) ₂ (H ₂ O) ₂](Cr ⁶⁺) ₄ (OH) ₆	Oxygen	+1	Synthesized

Table 2. Cr(III) complexes for characterization (Ox = Oxalate, ACAC = Acetylacetonate, en = ethylenediamine and Ac = Acetate).

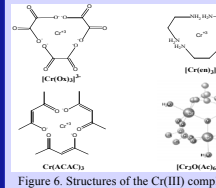


Figure 6. Structures of the Cr(III) complexes

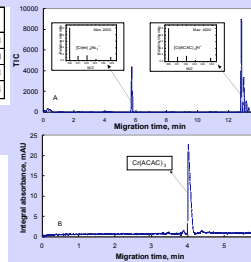


Figure 7. Electropherograms for separation of Cr(III) mixture ([Cr(en)₃]Cl₃ / Cr(ACAC)₃) with capillary electrophoresis (CE). A. MS detector with ESI interface (positive) B. UV detector (wavelength = 250nm).

D. Summary

1. Both gram positive and gram negative bacteria produce significant amounts of soluble Cr(III) upon reduction of Cr(VI)
2. Soluble organo-Cr(III) complexes readily form upon the reduction of Cr(VI) in the presence of cellular organics
3. Organo-Cr(III) complexes are able to move through soil columns
4. Degradation of organo-Cr(III) complexes results in Cr(III) precipitation on the cell surface as confirmed by EDS
5. Organo-Cr(III) consuming bacteria are characterized as a *Leifsonia* sp.
6. Cr(III) complexes with various ligands have been synthesized for internal standards on CE separation and model materials on EXAFS study.
7. Development of CE-ESI-MS characterization method has been started.

V. PUBLICATIONS

1. Puzon, G. J., J. N. Petersen, A. G. Roberts, D. M. Kramer, and L. Xun. 2002. A bacterial flavin reductase system reduces chromate to a soluble chromium(III)-NAD⁺ complex. *Biochem. Biophys. Res. Comm.* 294:76-81.
2. Puzon, G. J.; Roberts, A. G.; Kramer, D. M.; Xun, L. 2005. Formation of Soluble Organo-Chromium(III) Complexes after Chromate Reduction in the Presence of Cellular Organics. *Environ. Sci. Technol.* In Press.

VI. SUPPORT

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