Nitrate Enhanced Chromium Reduction in Three Model Organisms: *Geobacter metallireducens*, *Sulfurospirillum barnesii*, and *Desulfovibrio desulfuricans 27774*

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Chromium contaminates ground water and sediments at many DOE sites (reactor operations, irradiated fuel production, fuel recovery)

Also used in wood preservation (CCA) and industrial applications

D Reactor Building, Hanford
Microbial Cr(VI) reduction:

Several microbes known to readily reduce Cr(VI) to Cr(III)

*Rhodobacter sphaeroides* (Moore and Kaplan, 1992)

*Pseudomonas ambigua* (Susuki et al., 1992)

*Pseudomonas putida* (Park et al., 2000)

*Geobacter metallireducens* (Lovley et al., 1993)

*Shewanella oneidensis* (Viamajala et al., 2002)

*Desulfovibrio vulgaris* (Clark et al., 2008)

Several enzymes shown to have Cr(VI) reductase activity

FADH$_2$-dependent metal reductase

nitroreductase

$c$-type cytochrome

hydrogenase

Field trials using lactate stimulated bioreduction of Cr(VI)
BUT:

Chromium can be inhibitory to growth and activity

Cr(VI) reduction can be inhibited by co-contaminants

NO$_3$

Nitric acid and nitrate salts in nuclear fuel processing and fabrication has resulted in nitrate being the most commonly reported anion

Nitrate reduction to ammonia generates more energy than chromate reduction

So: Need to understand dynamics of Cr(VI) reduction under nitrate reducing conditions
Nitrate Enhanced Chromium Reduction

The central hypothesis of this project is that the presence of nitrate can impact the biotransformation of Cr(VI) in three ways:

1) as a competitive alternative electron acceptor inhibiting transformation
   *Geobacter metallireducens*

2) as a co-metabolite resulting in concomitant reduction, stimulating transformation
   *Sulfurospirillum barnesii*

3) as an inducer of specific proteins and pathways involved in oxidation/reduction reactions stimulating transformation
   *Desulfovibrio desulfuricans* strain 27774
Nitrate Enhanced Chromium Reduction

All three organisms reduce nitrate to ammonia in a two step process involving nitrate reductase and nitrite reductase.

*G. metallireducens* possesses a unique Nar-type nitrate reductase (NarCGHJI).

*S. barnesii* and *D. desulfuricans* have a periplasmic nitrate reductase (NapAGHBFLD, NapCMADGH).

All three have a pentaheme cytochrome c nitrite reductase (Nrf). Preliminary studies have shown Nrf from *G. metallireducens* and *S. barnesii* can be oxidized by chromate. Our hypothesis is that Nrf can function as a chromate reductase and is the link between nitrate and chromium reduction.
Not all nitrate reductases are alike: Size (MW) and surface charge of NapA of *D. desulfuricans* and *S. barnesii* are significantly different.
Nitrate Enhanced Chromium Reduction

**Specific aim 1.** To investigate the affects of chromate on nitrate respiration in *G. metallireducens, S. barnesii,* and *D. desulfuricans.* Preliminary experiments demonstrated that chromate affects growth with nitrate differently in each of the three organisms. The effect of Cr(VI) concentration on the kinetics of both growth and reduction of nitrate, nitrite, and Cr(VI) in these three organisms is to be determined.

**Specific aim 2.** To develop a profile of bacterial enzymes involved in nitrate transformation (e.g., oxidoreductases) using a proteomic approach. The proteome of the three species grown under different growth conditions will be compared to identify proteins involved in nitrate and chromate metabolism. It is possible that several proteins are upregulated/downregulated under these conditions.

**Specific aim 3.** To investigate the function of periplasmic nitrite reductase (Nrf) as a chromate reductase. Nrf from *G. metallireducens, S. barnesii,* and *D. desulfuricans* will be purified and the kinetics (K_m, V_max, K_cat) of chromate reduction and other biochemical characteristics determined.

**Specific aim 4.** To develop a strategy to maximize microbial chromium reduction in the presence of nitrate. The results from specific aims 1-3 will provide insight into possible amendments and manipulations for enhanced in situ remediation. This will be accomplished using both pure culture and natural populations (sediment slurries).
Cr(VI) inhibits growth on nitrate
Nitrate Enhanced Chromium Reduction

Simultaneous reduction of Cr(VI) and nitrate, no growth inhibition
Nitrate Enhanced Chromium Reduction

Cr(VI) reduced first, then growth. Lag phase can be reduced by reinoculum. Initial “die off” also seen in *D. vulgaris*.
Proteomics Investigation:

Cells were harvested during log phase and stationary phase, lysed by French Pressure cell and 2-D gel electrophoresis run on the ETTAN system from GE.

The gels were stained with colloidal coomassie blue or silver. Protein spots of interested were removed from the gels, subject to trypsin digest, and cleaned up using “zip-tips”.

MALDI-TOF was run on an ABI Voyager DE-STR, and the data analyzed with MASCOT (Matrix Science) using a mass tolerance of +/- 0.1

LC-MS-MS was run on a ProteomeX (LC) ThermoFinnigan LCQ Deca XP (MS/MS)
Genome Enabled Proteomics: 
*Alkaliphilus oremlandii* strain OhILAs

Over 200 proteins identified using MALDI-TOF MS
Genome Enabled Proteomics: *Alkalilimnicola ehrlichii*

**Green - anaerobic**
1. Nitrous oxide reductase
10. Nitrate transporter
16. Fructose bisphosphate aldolase
18. Triosephosphate isomerase
21. Phosphoribulose kinase

**Red - aerobic**
4. TonB dependent Cu receptor
23. Oxoglutarate dehydrogenase
24. Superoxide dismutase
**S. barnesii proteome: Nitrate vs Nitrate + Cr(VI)**

Spot 18

Metalloid reductase RarA *S. barnesii*

Spot 41

Ribosome recycling protein *H. hepaticus*
**D. desulfuricans** proteome: Nitrate vs Nitrate + Cr(VI)

Spot 51

Spot 52

Spot 53

Spot 55
**S. barnesii** periplasmic nitrite reductase (Nrf)

Protein matches from LC-MS-MS analysis.

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<th>P (pep)</th>
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<td>Putative cytochrome c nitrite reductase [Sulfurospirillum barnesii]</td>
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<td>A Chain A, Cytochrome C Nitrite Reductase From Wolinella Succinogenes</td>
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S. barnesii periplasmic nitrate reductase (Nap)
gi|169104652 Mass: 105393  Score: 84  Expect: 0.024  Queries matched: 31

periplasmic nitrate reductase [Sulfurospirillum barnesii]

Nominal mass (M_r): 105393; Calculated pI value: 8.77
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 134
Number of mass values matched: 31
Sequence Coverage: 34%
Matched peptides shown in Bold Red

1  MSLSRRDFLK TTAASAAAA VGIGVPAELK AAGEQAEANW KWDKAVCRFC
51  GTGCGIMVAT KEKIVAVKG DPAAPVRGL NCIGGYFNAK IMGYGADRLKQ
101  PLLRMNDKGE FDKKQGQFKPV SWKRAFDEME KHIKAALKVG GPEAIGVFGS
151  GQYTIQEGYA AAKMKKAFGR ANGIDPNAH CMASAVAGFM QTGIDEAPAG
201  CYDDEIEITDT IITWGANMAE MHPILWSRVS DRKLTSFDHV KIVNLSTYTH
251  RCSDLADLEI IFSPSTDLAI WNYIAREIVY NHPEAIDWDF VKKNTIPTTG
301  FANIGYGMT EAAEKKLGS AKELEVIKKE DAKVISEKEA PGLAHLGVKA
351  GDVMKMDKAD AAGHAEISF EDFKKALEPY TLEYVAKISK GNPDEKLED
401  KVLQELANL YIEKNRKVVS FWTMGFPHQG RGTVWNEQSY MVHLGLGKQA
451  KPGDGAFSLT GQPSACGTR EVGTPTFRLP XDMDSIPKQH REVSEKIWKL
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901  MINKVCLDATE CPSKQTDYK KCAVKLYKA
PROGRESS TO DATE

Specific Aim 1

1. *G. metallireducens* is inhibited by Cr(VI) when using nitrate as the terminal electron acceptor.
2. *D. desulfuricans* reduces the Cr(VI) before growing on nitrate.
3. *S. barnesii* could simultaneously reduce Cr(VI) and nitrate.

Specific Aim 2

1. Proteomics analysis revealed differences in the proteomes of both *S. barnesii* and *D. desulfuricans* when exposed to Cr(VI).
2. Protein identification by MALDI-TOF, however, was limited by the lack of genome sequence data.

Specific Aim 3

1. Progress has been made on the purification proteins involved in nitrate respiration in *S. barnesii*
FUTURE DIRECTIONS (Year 2)

Initiate proteomic analyses of nitrate grown *G. metallireducens* in the presence and absence of chromate

Attempt to identify key proteins from *S. barnesii* and *D. desulfuricans* using LC-MS/MS

Characterize purified Nrf from *S. barnesii*

Purify and characterize Nrf from *D. desulfuricans* strain 27774.

Await release of annotated genome of *D. desulfuricans* strain 27774.

Propose sequencing the genome of *S. barnesii*. 
Basu/Stolz lab

Peter Chovanec
Courtney Sparacino

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