Microbial Transformation of Arsenic and Selenium for Bioremediation Strategies

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67% of Superfund sites contaminated with As

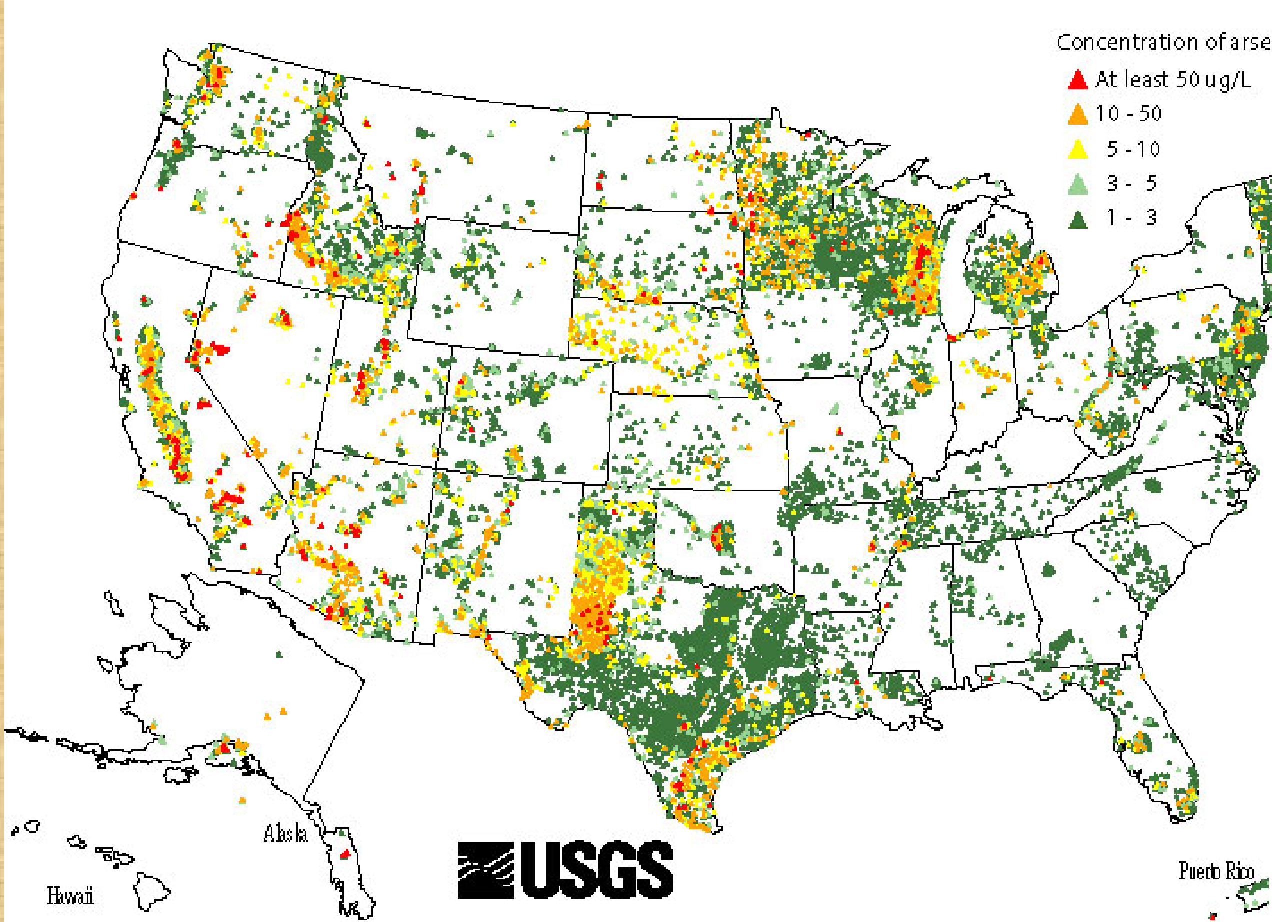
NIEHS has 5 centers
(7 others have projects)

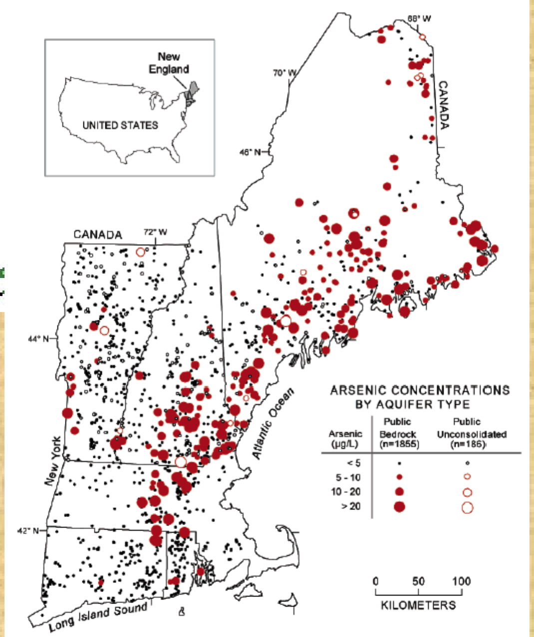
Dartmouth (NH)
Columbia (NY)
Harvard (MA)
New York University (NY)
University of Arizona (AZ)

Hermiston, OR

Vineland, NJ

Occurrence of As in Groundwater (USGS - NAWQA 1973-2001)





EPA standards:

old: 50 ppb

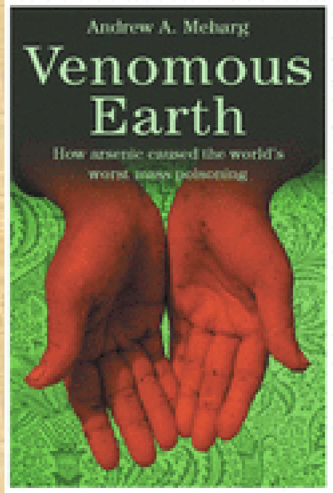
new: 10 ppb

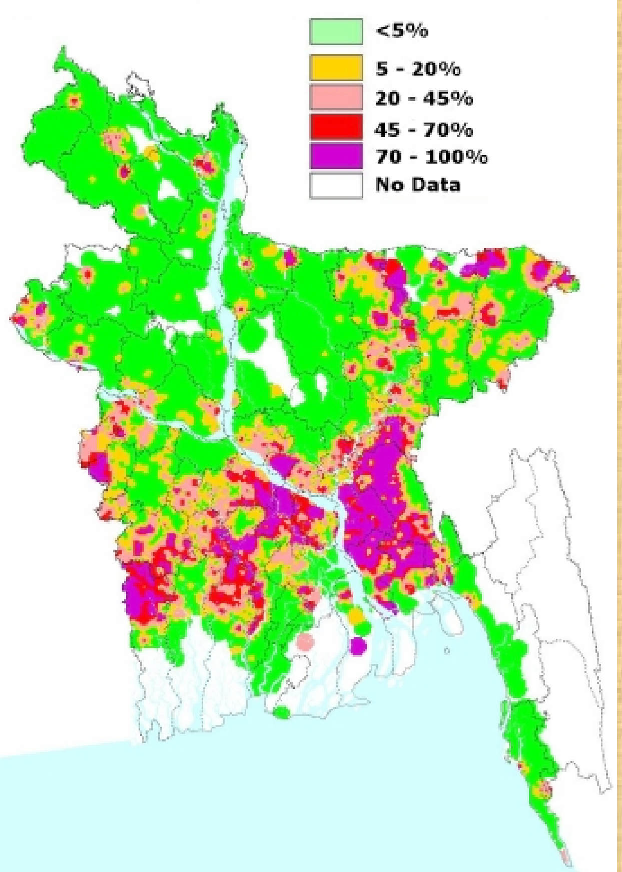
WHO - 10 ppb

"Arsenic crescent"

Ayotte et al., 2003

Bangladesh, West Bengal





Abul Hussam SONO Filter 2007 Gold Grainger Prize

Physical (abiotic) removal:

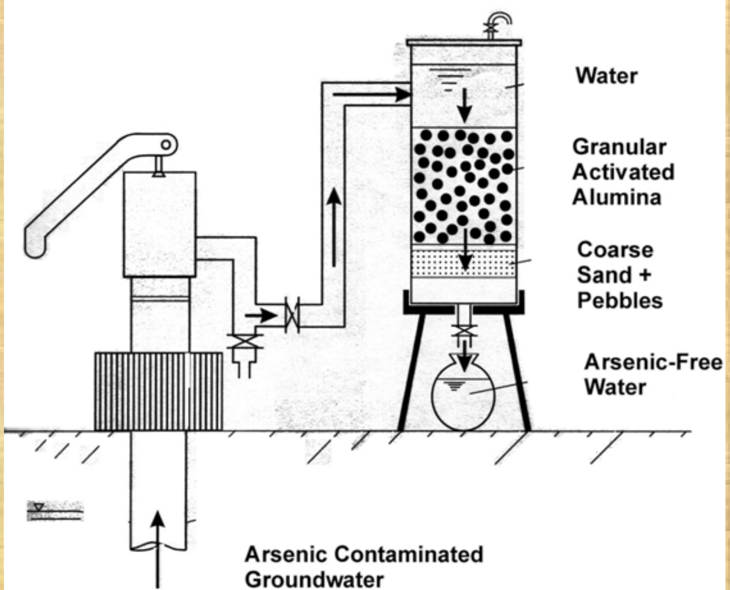
- alumina sorption
- anion exchange
- Fe(0)
- Fe(III) Cl coagulation

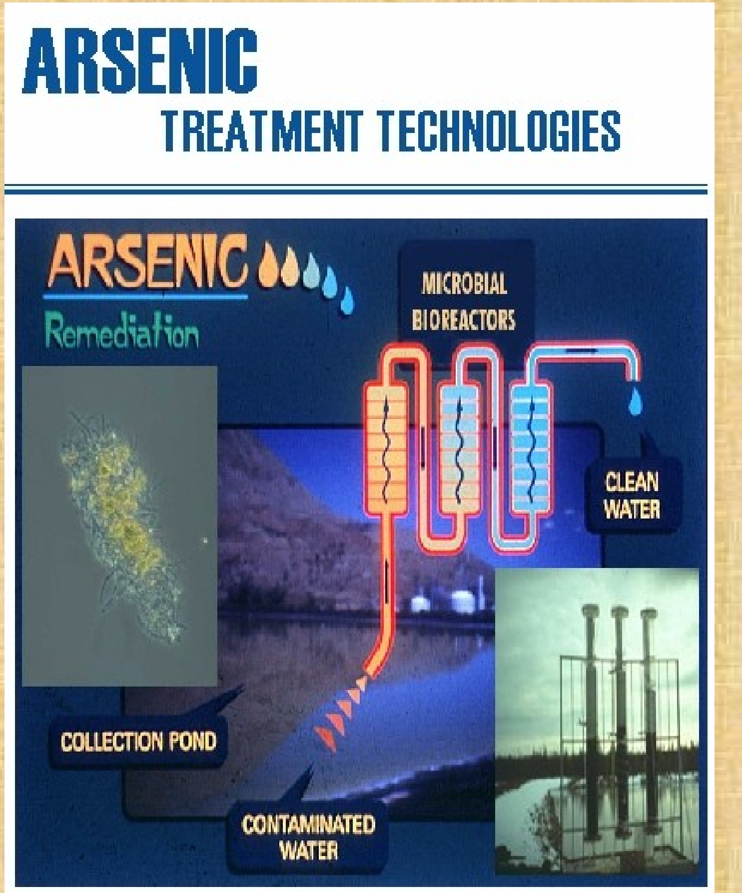
(In)expensive, simple design, functional

However, most work best with As(V)

Arup K. SenGupta 2007 Silver Grainger Prize

Well-Head Arsenic Removal Unit





Bioremediation

Bacterial systems

- arsenite oxidizing bacteria
- arsenate reducing bacteria
- sulfidogenic bacteria

Phytoremediation

- yeast (*Saccharomyces*)
- brake fern (*Pteris* spp.)

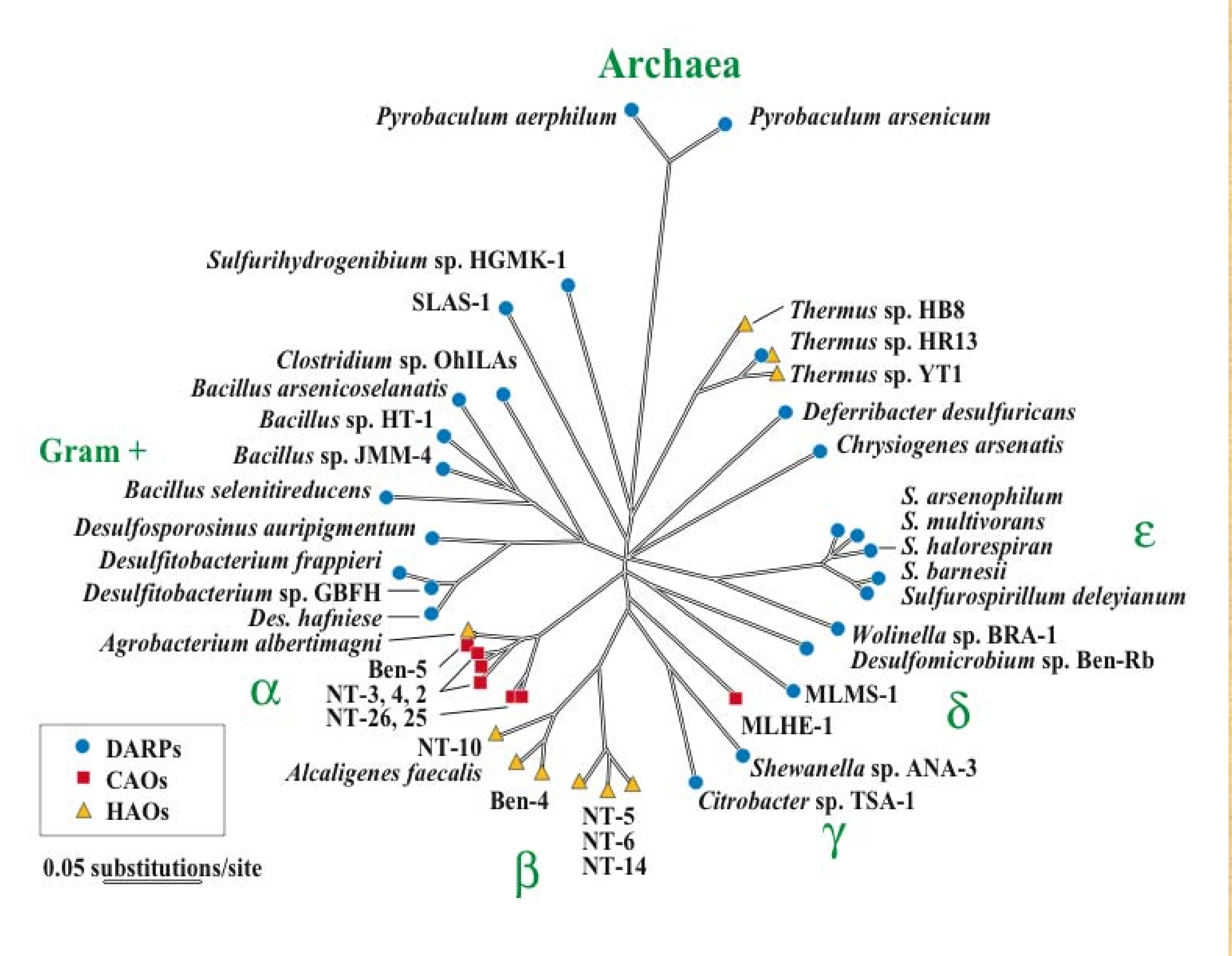
Weber State U. Center for Bioremediation

- Accelerated bioleaching with acid producing bacteria
- H_2S Precipitation and microbial/polymer accumulation

Inorganic Arsenic Metabolism

I. Assimilation
    Organoarsenicals - arsenobetaine, arsenolipids
II. Detoxification
    A. Oxidation - arsenite oxidase
    B. Methylation (MMA, DMA, TMA)
        methyltransferase
        S-adenosylmethionine (SAM)
    C. ArsC - AsV reduction to AsIII
        E. coli - ArsC, glutaredoxin/glutathione, ArsAB
        S. aureus - ArsC, thioredoxin, ArsB
        Sa. cerevisiae - Arr2p, Grx/GSH, Arr3p
III. Energy
    A. AsIII oxidation - arsenite oxidase
    B. AsV reduction - dissimilatory arsenate reductase

Diversity of Arsenic Metabolizing Bacteria



Oremland and Stolz, 2005

Arsenic Metabolizing Bacteria

- Phylogenetically and physiologically diverse.

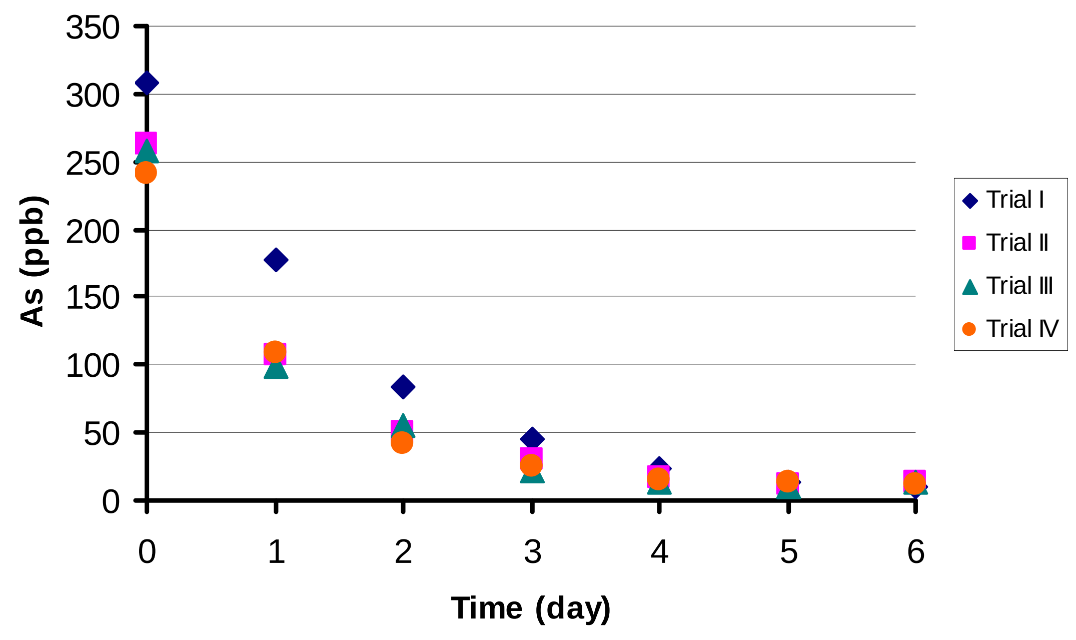
- Heterotrophic and Chemolithoautotrophic As(V) Respirers
  - Are not obligate and may use other electron acceptors
  - May also use a variety of electron donors
  - Can be sensitive to arsenic concentrations

- Heterotrophic and Chemolithoautotrophic As(III) Oxidizers
  - May/or may not gain energy from arsenite oxidation
  - May also possess resistance genes (ars) and express both arsenite oxidizing and arsenate reducing phenotype!

So, are As-metabolizing microbes useful for biomediation?

Pteris spp. for As(V) Removal

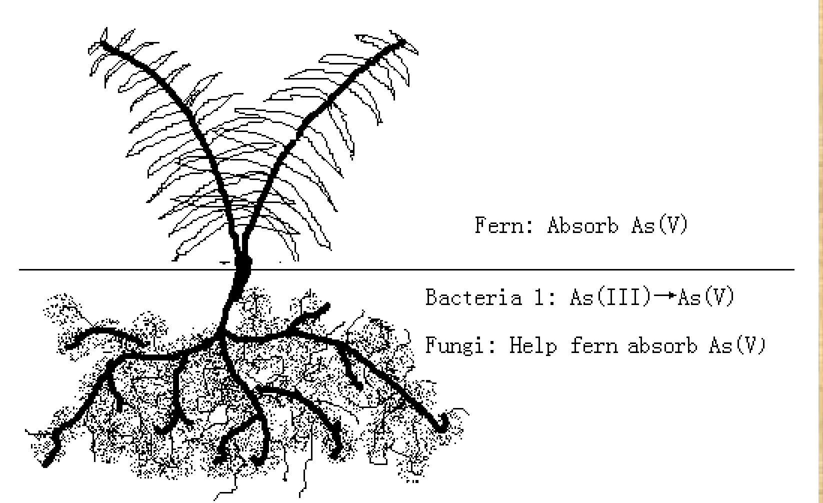
- *Pteris* spp. (e.g. *P vittata, P. cretica*) take up As(V) through phosphate channels in roots
- As(V) is subsequently reduced to As(III) and stored in different tissues (Pickering et al., 2006 Environ Sci &Technol 40:5010-5014)

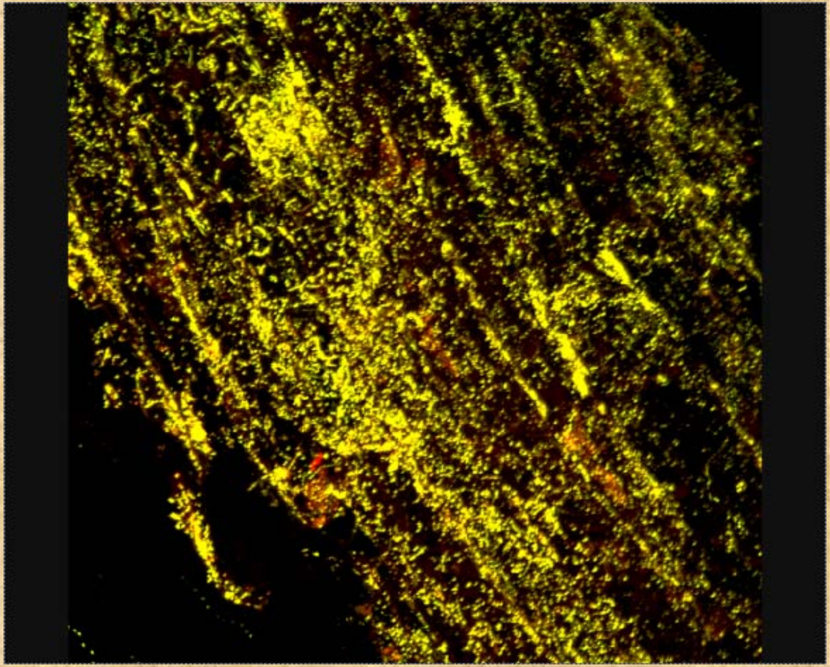


Vidic et al., in prep.

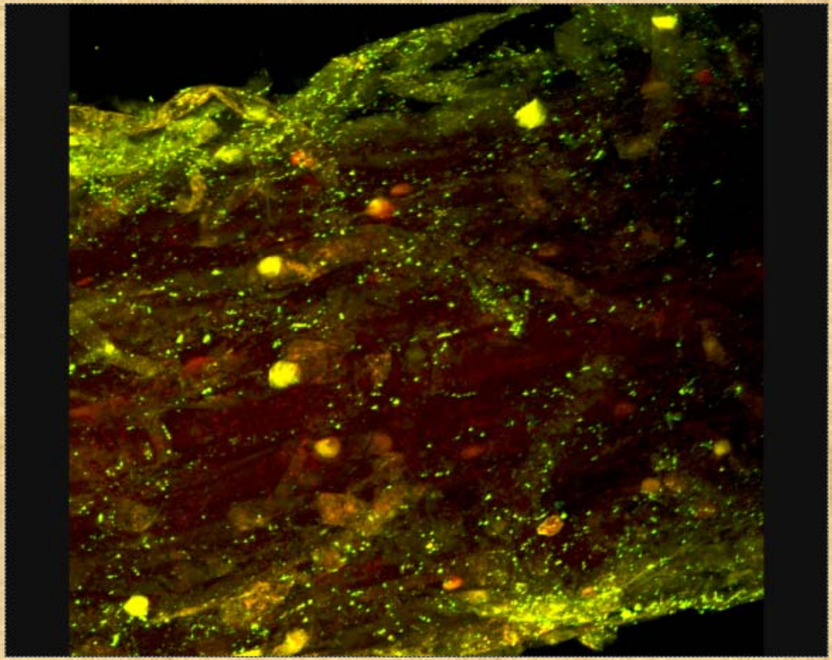
Impact of Antibiotics on Rhizosphere Bacteria

Vidic et al., in prep.



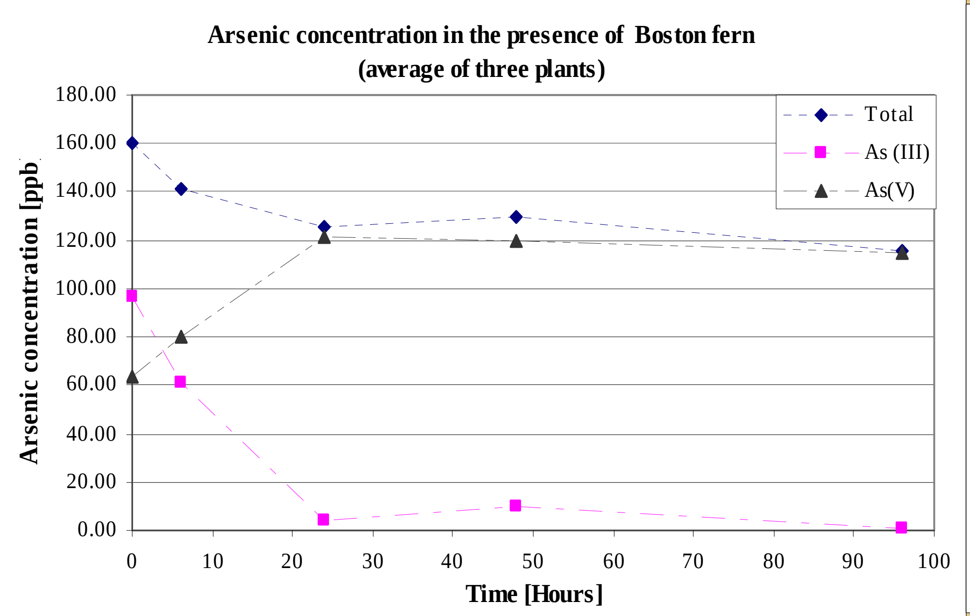


Boston fern control

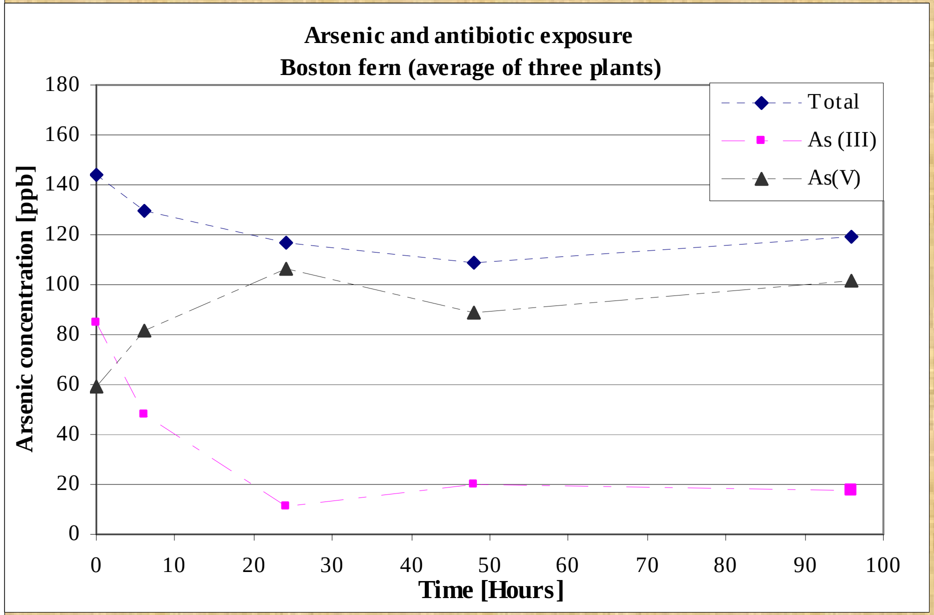


Boston fern antibiotic treatment

Impact of Antibiotics on As(III) Fate with Boston Fern



Control

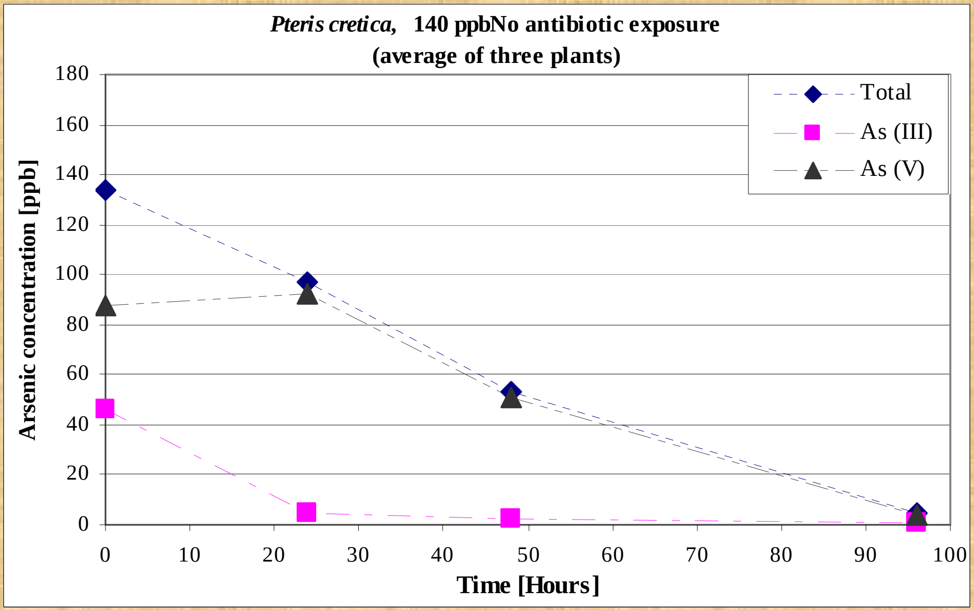


With antibiotics

- 13% of the initially added As(III) remained as arsenite in the presence of antibiotics.
- Suppression of microbial activity in the root zone impacts arsenite oxidation.

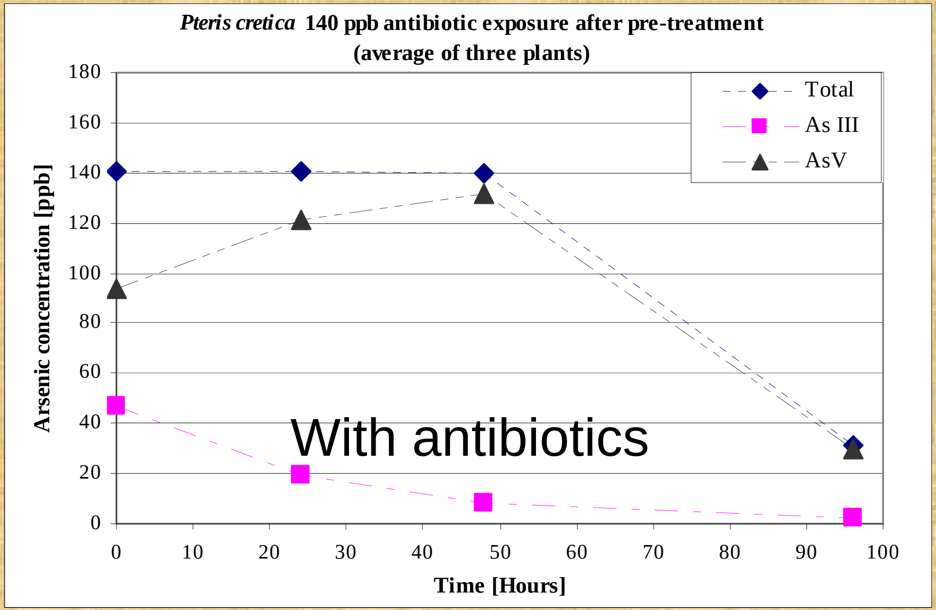
Vidic et al., in prep.

Impact of Antibiotics on As(III) Uptake by *P. cretica*



***Pteris cretica*, 140 ppbNo antibiotic exposure (average of three plants)**

Control

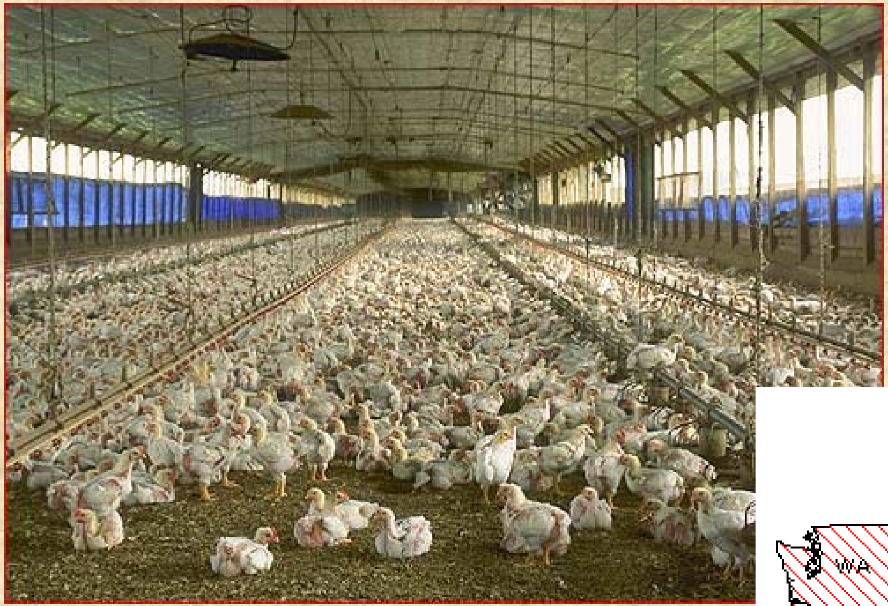


***Pteris cretica* 140 ppb antibiotic exposure after pre-treatment (average of three plants)**

With antibiotics

Vidic et al., in prep.

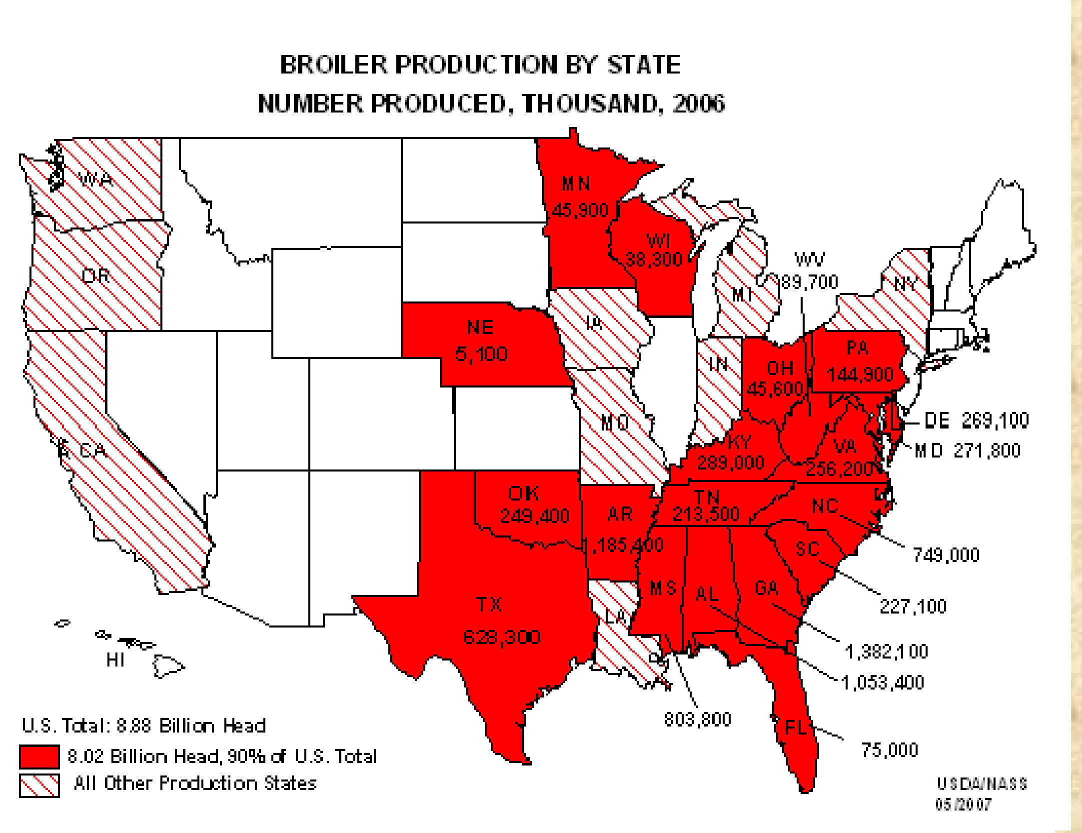
Organoarsenicals in Agriculture: CAFOs



Roxarsone
3-nitro-4-hydroxybenzene
arsonic acid

Farm in Florida with 1 million

Use to treat coccidiosis, but chickens grow faster, larger, and with better color



Organoarsenicals in Agriculture: Fate

Dose: 25-45 mg/kg

Each chickens excretes ~150 mg of roxarsone over its 42 day growth.

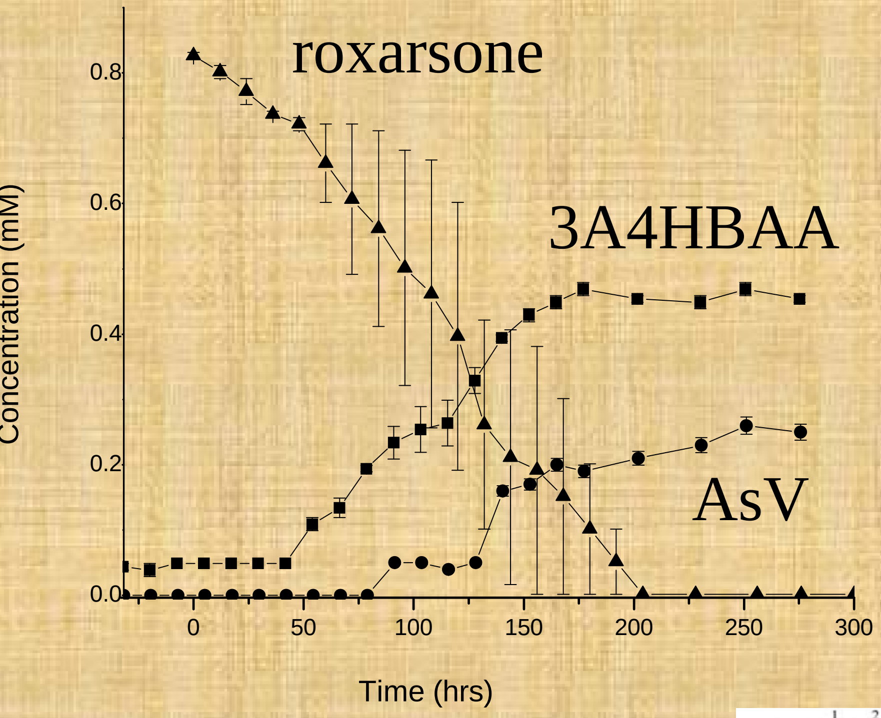
The litter contains ~25-45 mg/kg.

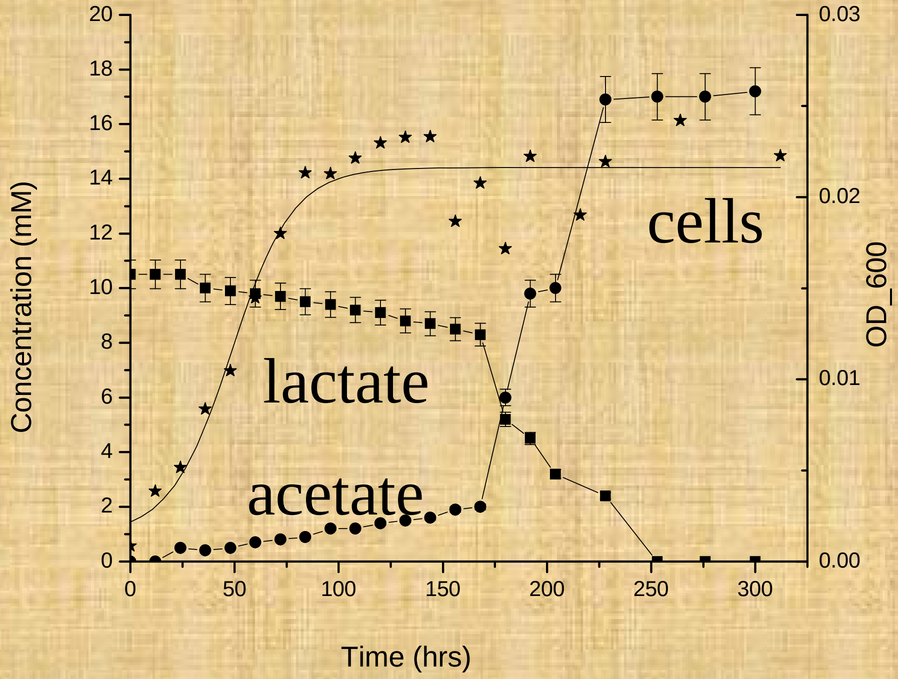
The litter is applied as fertilizer in neighboring farms or sold as "organic fertilizer"

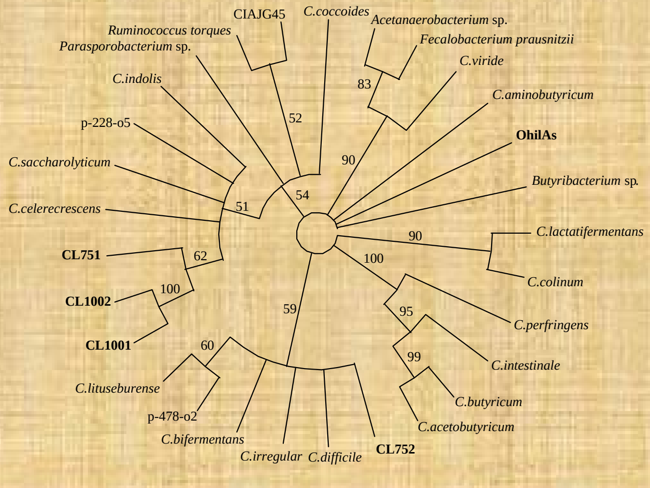
~70% of over 8 billion broilers

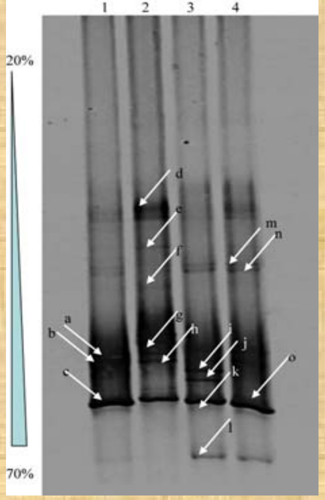
Maryland - 340,000 tons of litter/yr (75% from 4 counties)

Roxarsone Biotransformation in Chicken Litter Enrichments





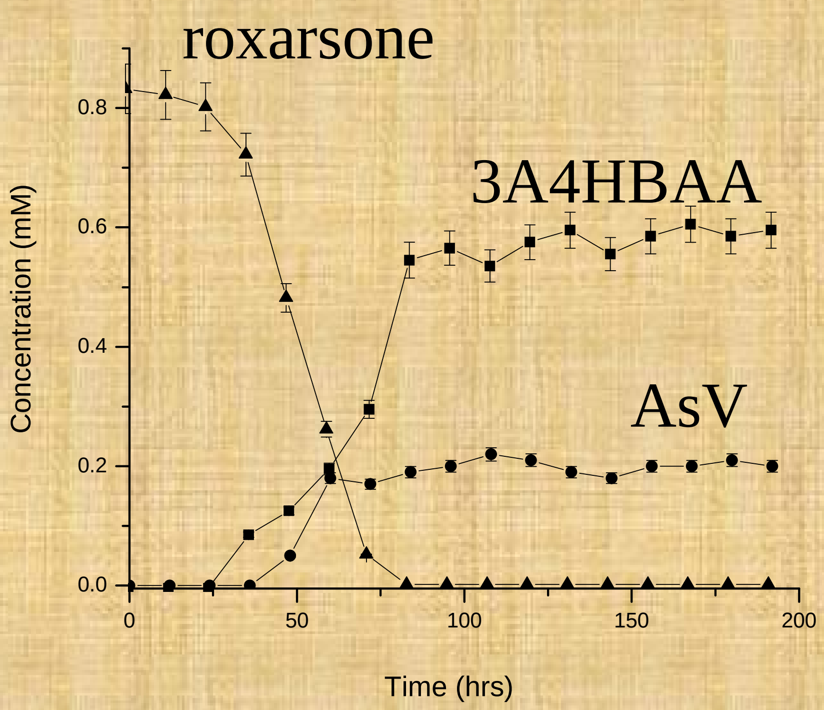


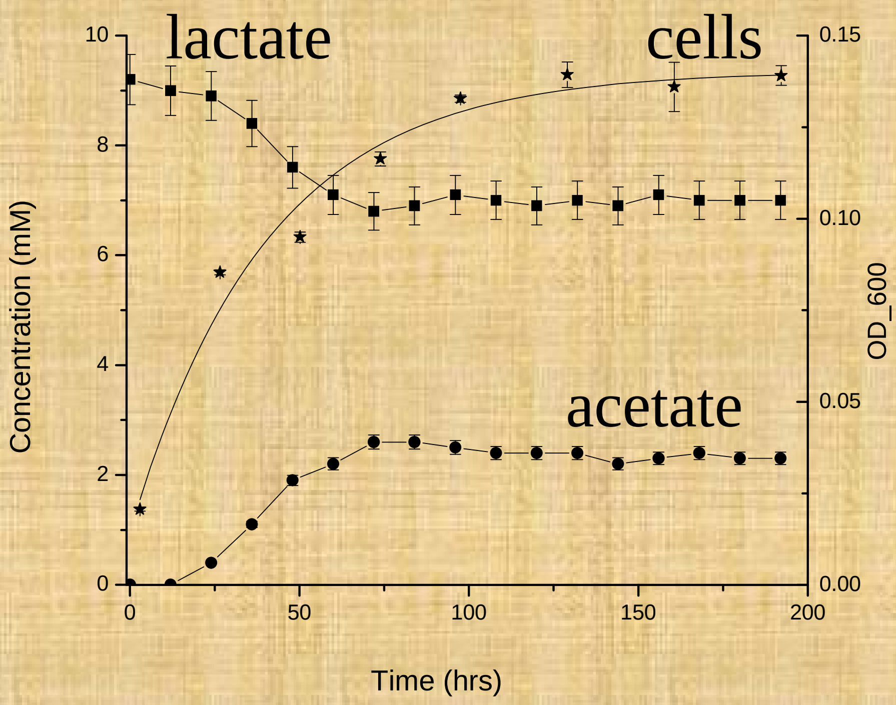


Clostridium and *Desulfotomaculum* spp.

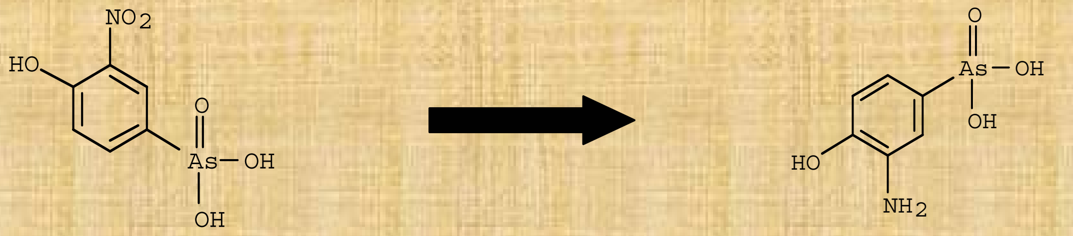
Stolz et al., 2007 Environ Sci &Technol 41:818-823

Roxarsone biotransformation by *A. oremlandii* strain OhILAs





2 rox + 3 lactate --> 2 3A4HBAA + 3 acetate + $3CO_2$ + H_2O



+ As(V) + ?

Stolz et al., 2007 Environ Sci &Technol 41:818-823

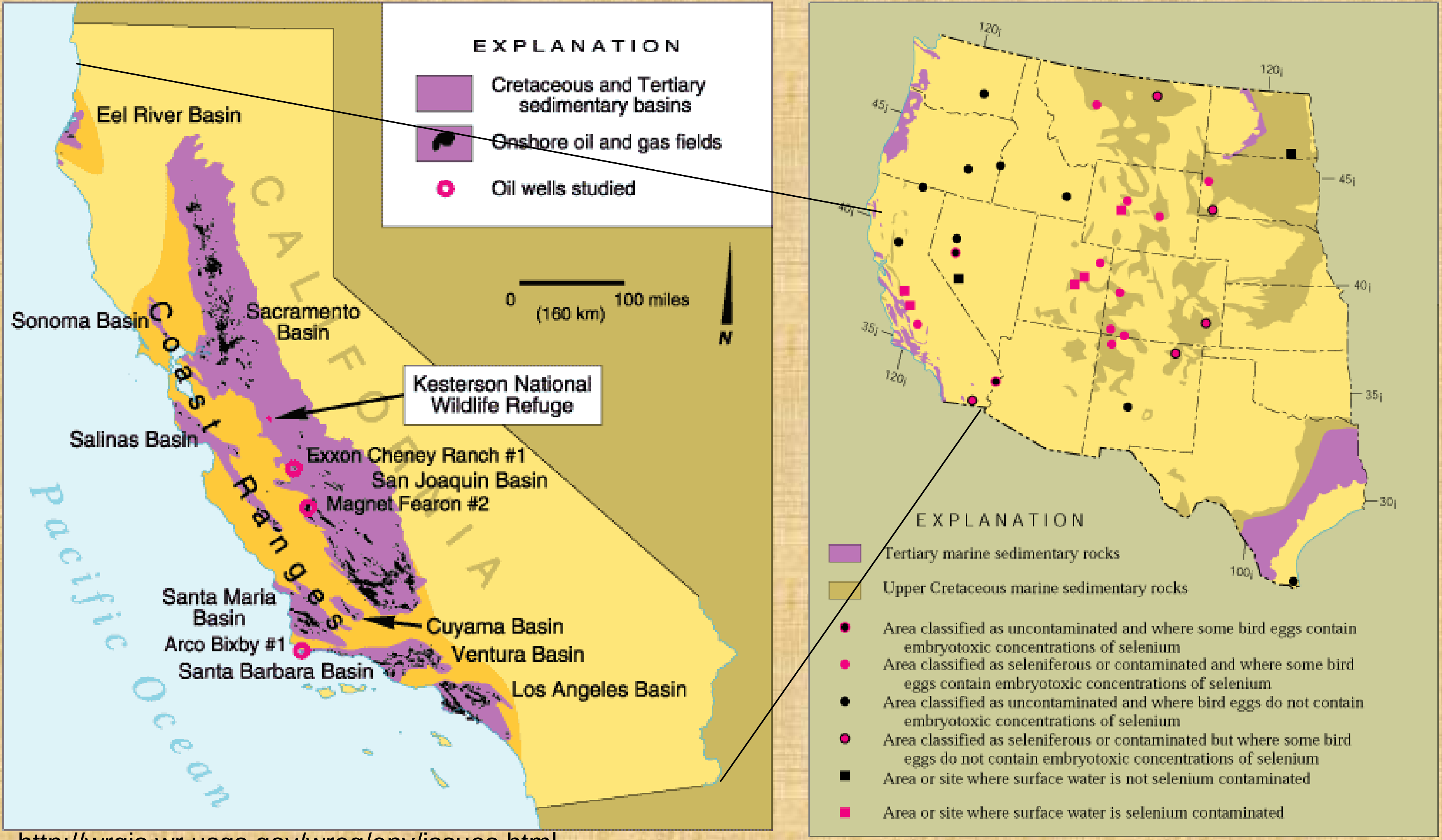
Conclusions

- Arsenite oxidizing bacteria can enhance both chemical methods and phytoremediation technologies for arsenic removal.

- Microbial activity (e.g., arsenate-respiring bacteria) can enhance the mobilization of arsenic from soils and sediments.

- In designing effective remediation technologies, the metabolic versatility of the organism(s) chosen must be considered.

- Microbes can be used to transform organoarsenicals to inorganic arsenic through composting.

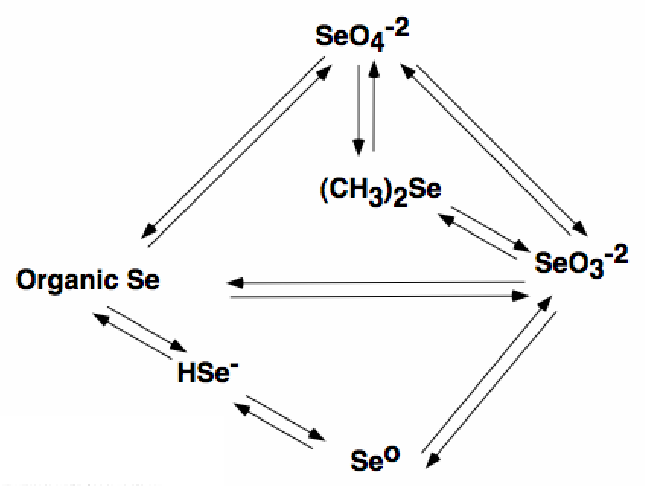


http://wrgis.wr.usgs.gov/wreg/env/issues.html

Selenium is associated with fossil fuel deposits (e.g., Cretaceous mudstones). Erosion and agricultural runoff followed by evaporation can lead to accumulation (e.g., Kesterson Wildlife Refuge)

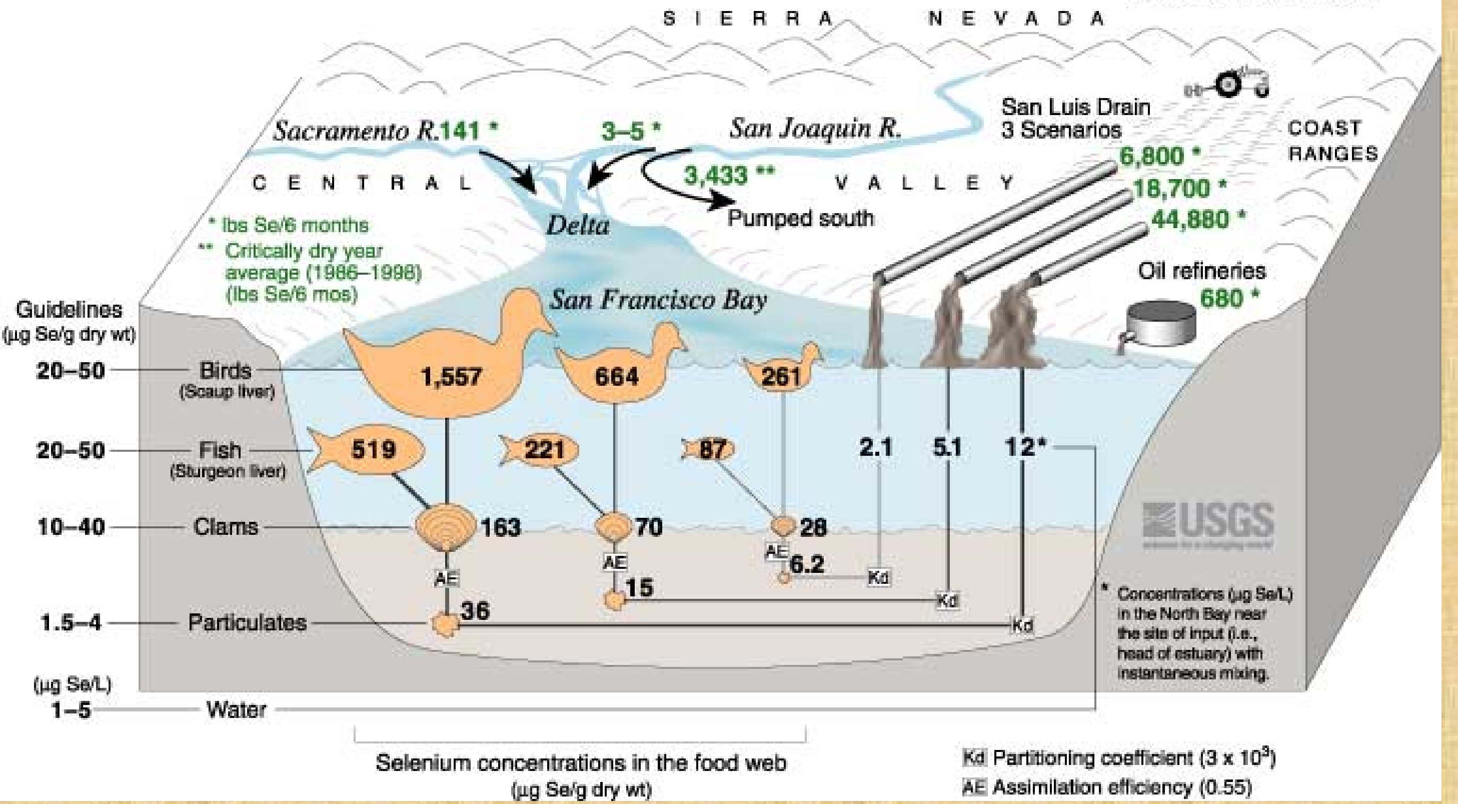
Selenium biogeochemical cycle

Over 20 species of prokaryotes known to respire selenate (only 2 selenite)



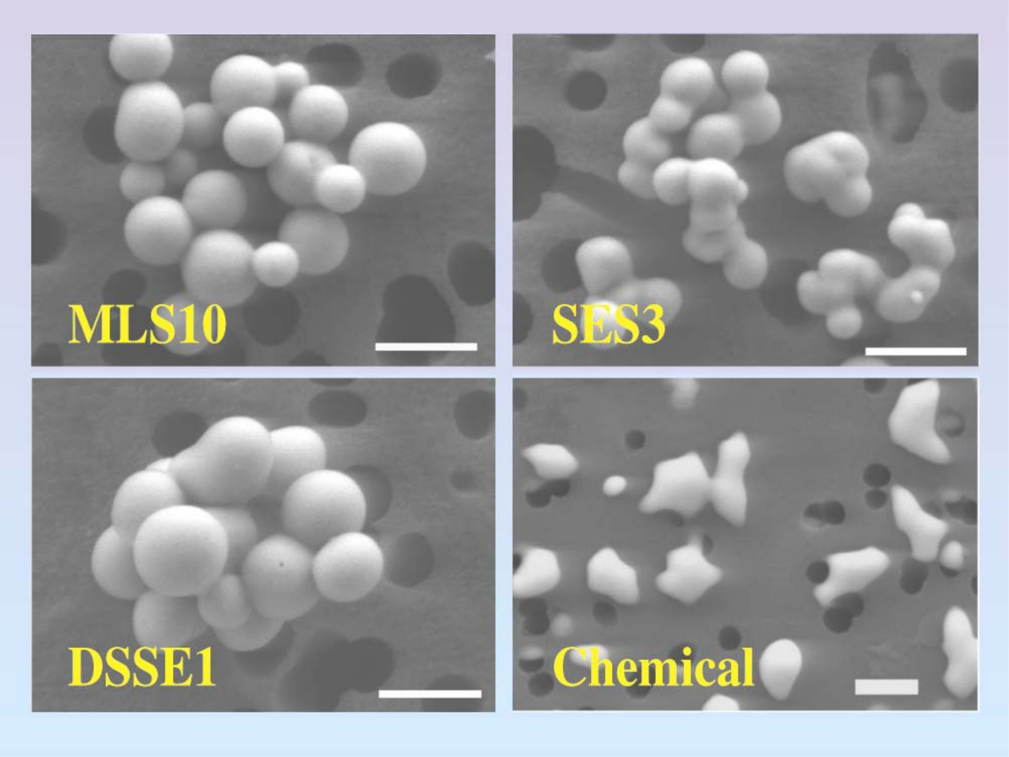
Stolz et al., 2006

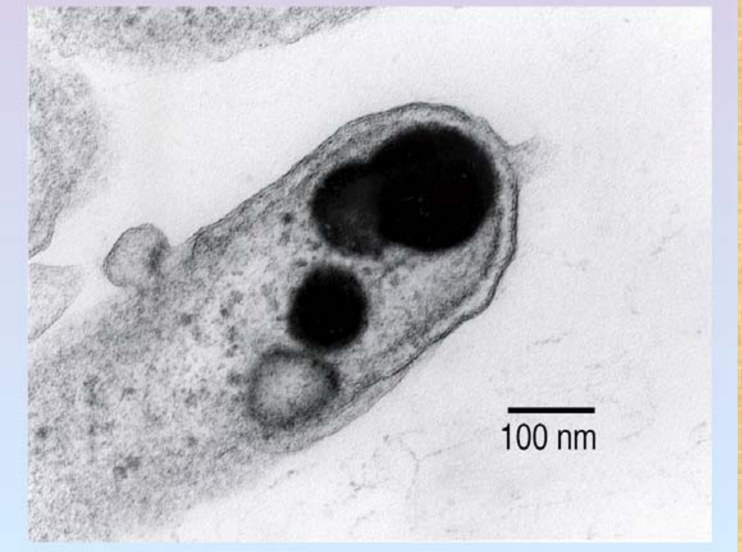
Example Selenium Forecast for the Bay-Delta



Only one selenate reductase characterized to date

http://wwwrcamnl.wr.usgs.gov/tracel/data/se_model/index.htm





Dissimilatory selenate reduction

Se(VI) -> Se(IV) -> Se(0)

Intracellular and extracellular nanospheres

Oremland et al., 2004 Appl Environ Microbiol. 70:52–60

Effect of Co-contaminants on Selenium reduction

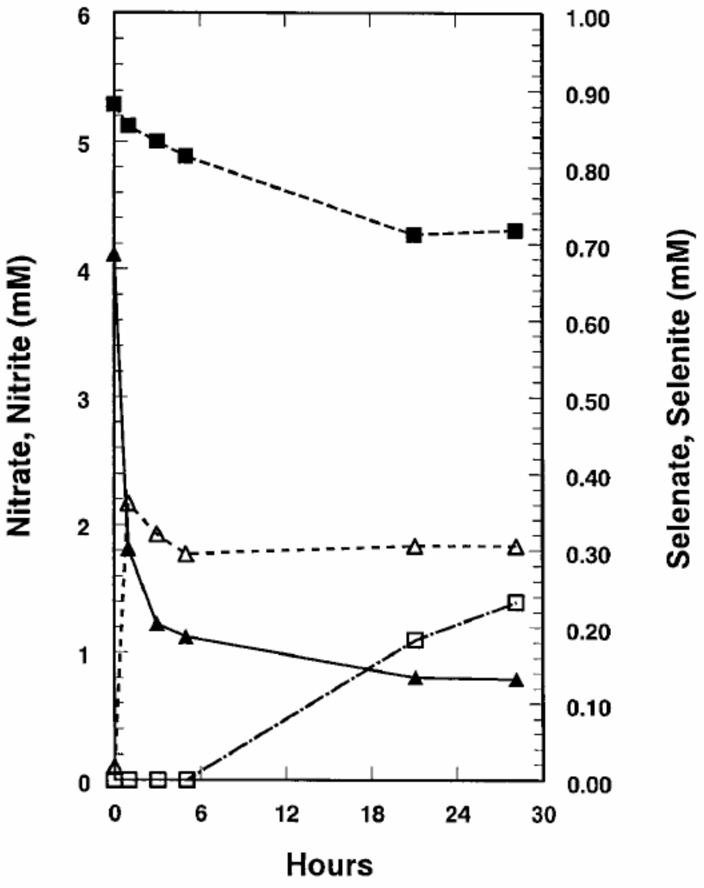


FIG. 2. Reduction of Se(VI) (▲) and nitrate (■) to Se(IV) (△) and nitrite (□) by selenate-grown washed-cell suspensions of *S. barnesii* with 5 mM lactate as electron donor. Cell density = 8.0 × 10^8 cells/ml.

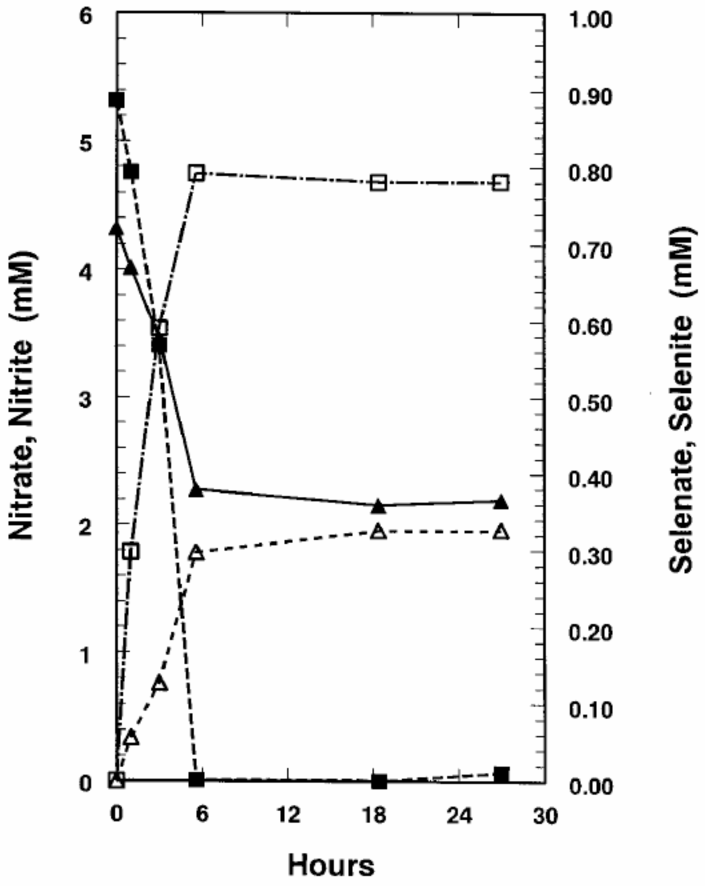


FIG. 3. Reduction of Se(VI) (▲) and nitrate (■) to Se(IV) (△) and nitrite (□) by washed-cell suspensions of nitrate-grown *S. barnesii* with 5 mM lactate as electron donor. Cell density = 1.3 × 10^9 cells/ml.

Simultaneous reduction of nitrate and Se(VI) by *S. barnesii* (Oremland et al., 1999 Appl Environ Microbiol 65:4385-4392

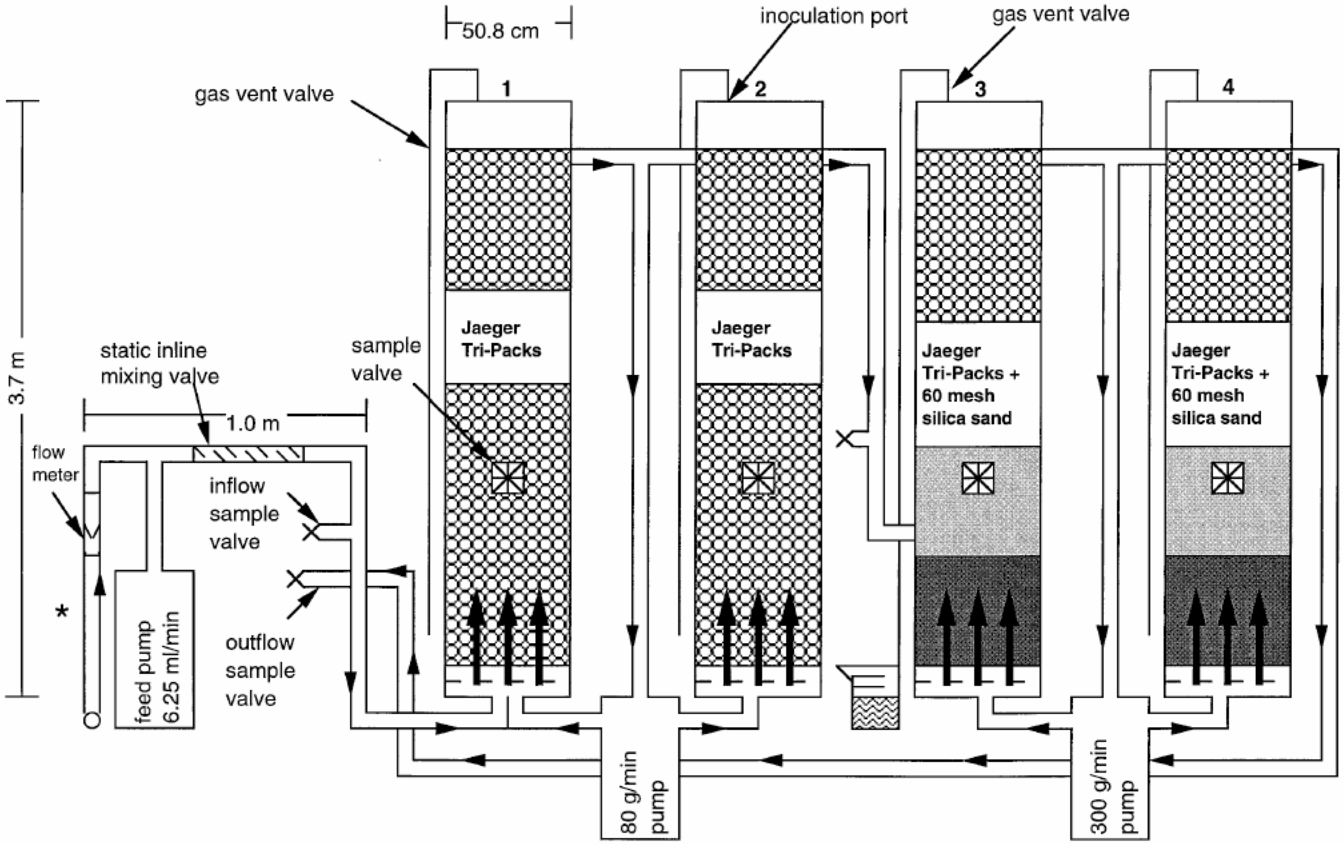


FIG. 1. Schematic representation of the pilot-scale biological reactor used for bioremediation of selenium oxyanions in agricultural drainage water. The gas vent valve represented on tank 3 is present on all tanks. The point at which methylene blue was added for determination of residence times is indicated by an asterisk.

Cantafio et al., 1996 Appl Environ Microbiol. 62:3298–3303

Se Phytoremediation

- **Accumulation - *Neptunia amplexicaulis*** Selenium weed
  Astragalus sp. "loco weed", milk vetch

- **Volatilization - *Brassica juncea* (Indian mustard)**

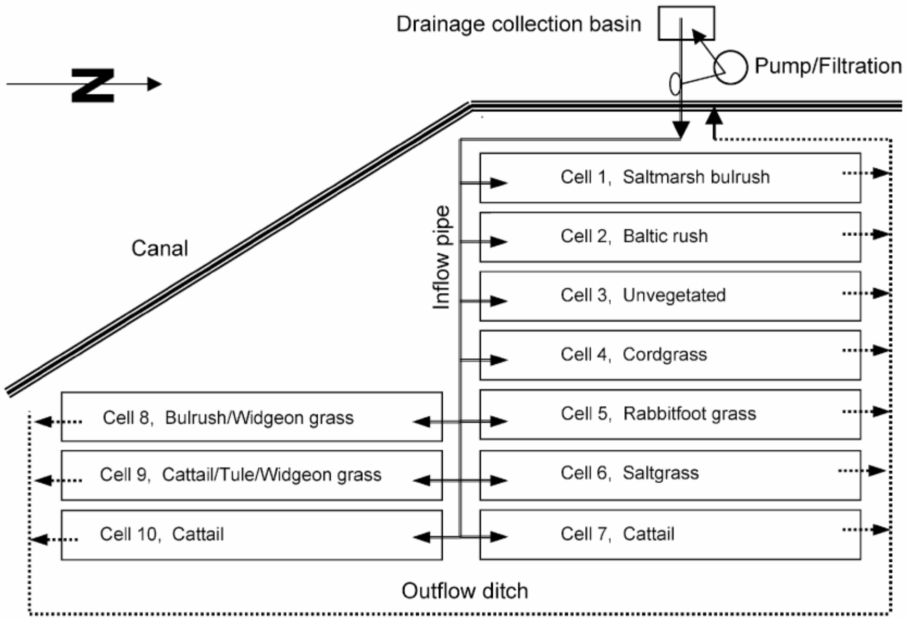


FIGURE 1. Field layout of 10 flow-through constructed wetland cells in Corcoran, California, built in May 1996 and terminated in February 2001.

Lin and Terry, 2003 Environ Sci Technol. 37:606-615

~69% of the Se removed
>95% was retained in soil

Of the <5% retained by plant, some volatilized

Conclusions

•The number of microbes identified that are capable of respiring selenate or selenite has slowly increased, but the biochemistry and physiology lags behind.

•Se(VI) reducing microbes produce unique Se nanomaterials.

•Microbial activity (e.g., selenate-respiring bacteria) can enhance the removal of selenium from impacted surface and subsurface water.

•Volatilization (via phytoremediation) does not appear to be an effective strategy, but removal by precipitation (through microbial reduction) does.

•Again, in designing effective remediation technologies, the metabolic versatility of the organism(s) chosen must be considered.

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