Role of Microorganisms in the Speciation and Mobility of Arsenic

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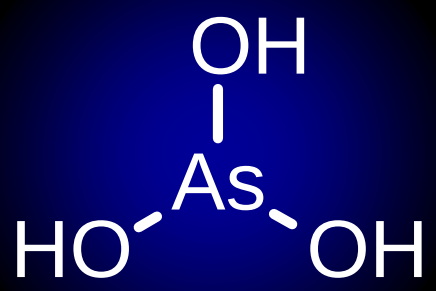
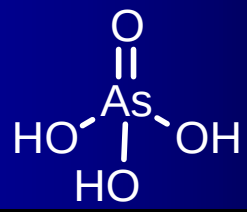


Inorganic Arsenic

Predominant Species of the Biogeochemical Cycle

arsenite
As(III)



arsenate
As(V)

- More toxic than As(V)
- Reacts with R-SH
- Adsorbed by iron oxides
- More mobile

- Analogue of phosphate
- Adsorbed by iron and aluminum oxides
- Less mobile

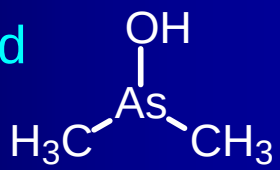
Organoarsenicals

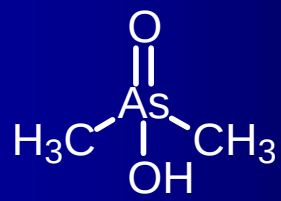
Methylated Forms are Less Predominant Species in the Biogeochemical Cycle

cid

dimethylarsinous acid

DMA(III)





methylarsinic acid

DMA(V)

- Very Toxic
- Metabolite
- Unstable

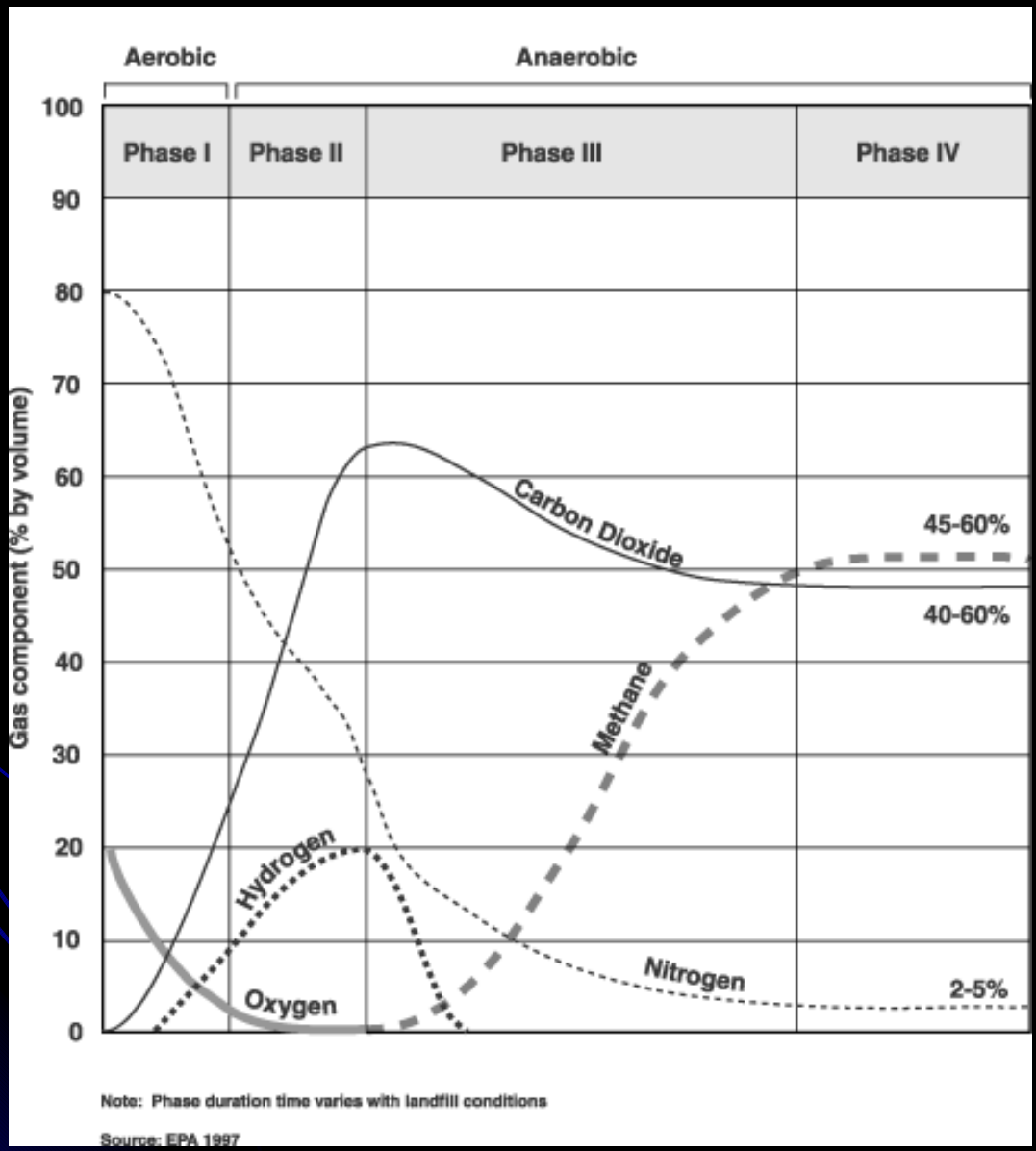
- Pesticides
- Metabolite

Landfill

Methanogenesis in Landfill

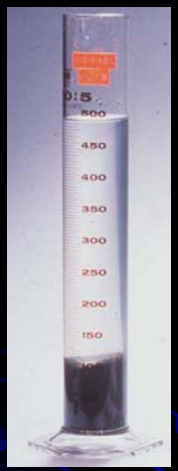
- Phase I: Consumption of O_2
- Phase II: Formation of organic acids, release NH_4^+
- Phase III: Conversion of organic acids to CH_4 and HCO_3^-, continued release NH_4^+, mildly alkaline pH

Landfill

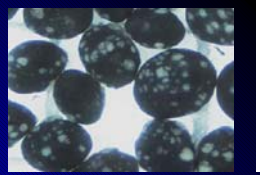

Aerobic
Anaerobic
Phase I
Phase II
Phase III
Phase IV
100
90
80
70
60
50
40
30
20
10
0
Gas component (% by volume)
Carbon Dioxide
45-60%
40-60%
Methane
Hydrogen
Oxygen
Nitrogen
2-5%
Note: Phase duration time varies with landfill conditions
Source: EPA 1997

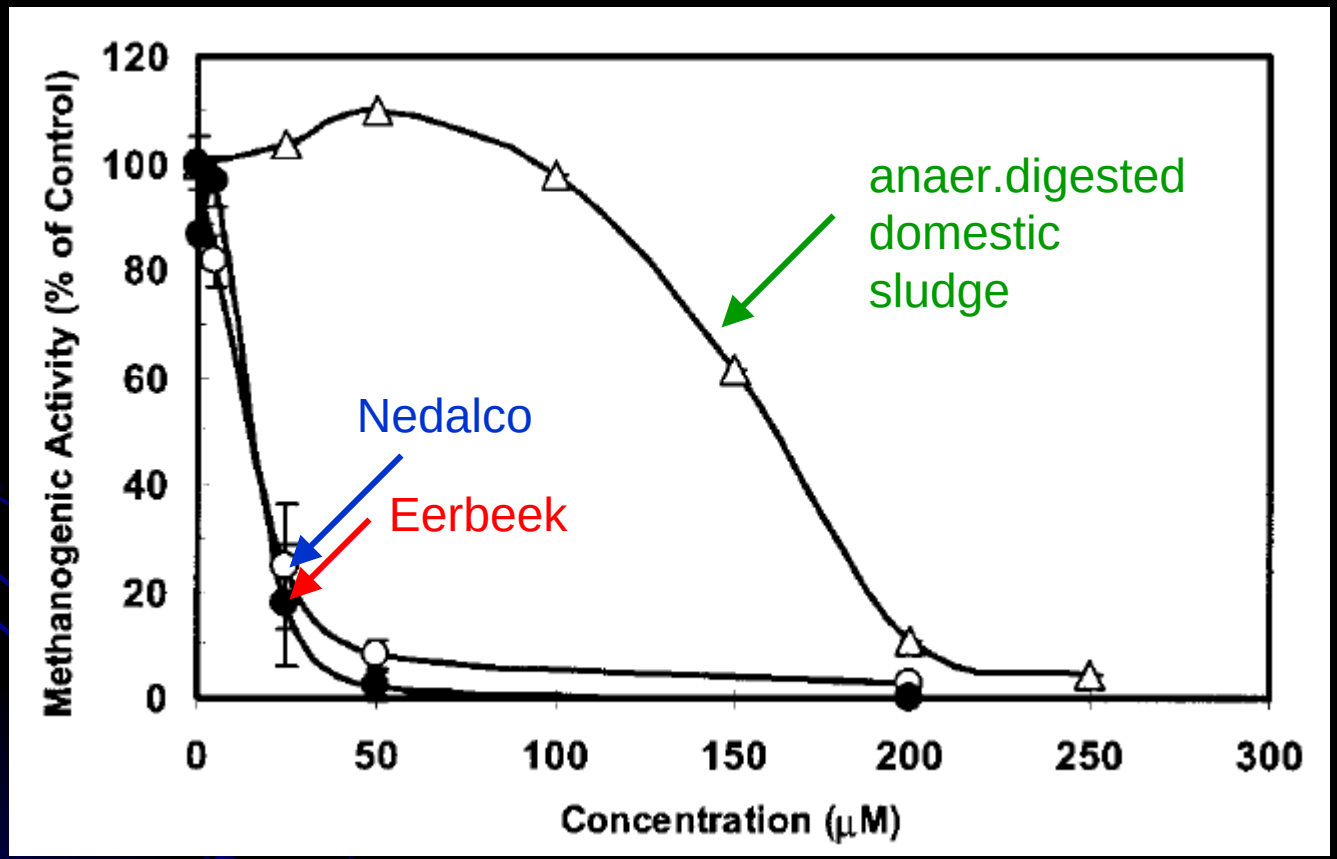
Toxicity Arsenic to Methanogens

Dose Response Curves of As(III) on Acetoclastic Methanogenesis



Nedalco
Eerbeek





ADDS

Sierra-Alvarez et al. 2004 AEM 70:5688

Toxicity Arsenic to Methanogens

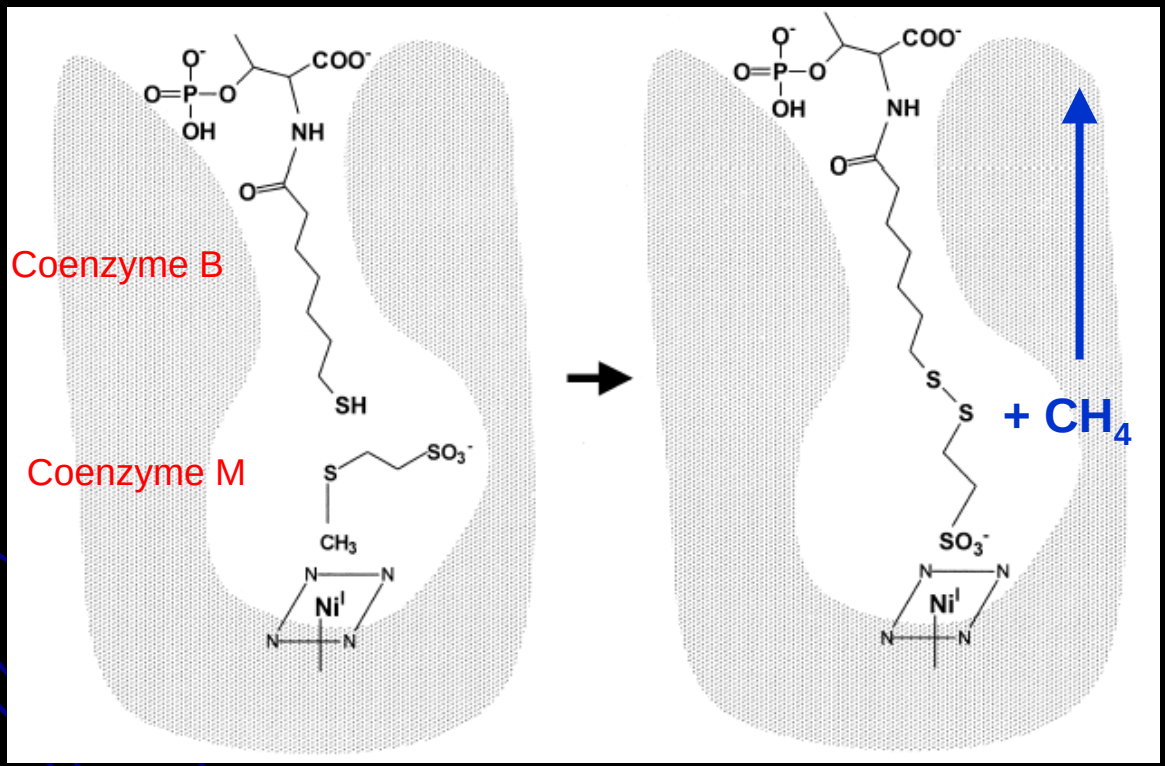
50% Inhibitory Concentrations of Arsenicals on Methanogensis

Compound	Substrate	IC50 (μM)	IC50 (ppm)
As(III)	acetate	15.5	1.16
As(III)	H_2	27.1	2.03
As(III)	lactate	4.4	0.33
As(V)	acetate	>500.0	
As(V)	H_2	>500.0	
MMA(III)	acetate	9.1	0.68
MMA(V)	acetate	>5,000.0	
DMA(V)	acetate	>5,000.0	

Sierra-Alvarez et al. 2004 AEM 70:5688

Toxicity Arsenic to Methanogens

Sulfhydryl Groups are Central to the Biochemistry of Methanogens

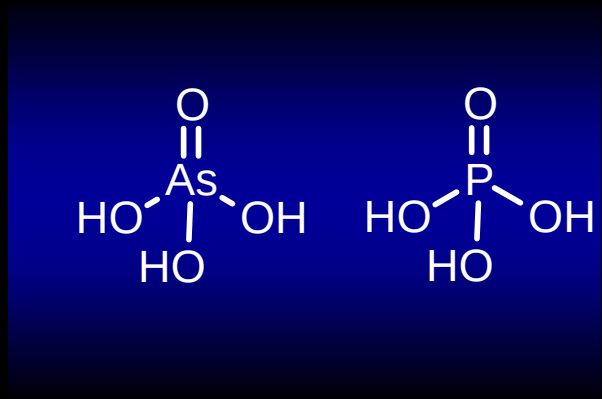


Methyl Reductase: F430 Complex

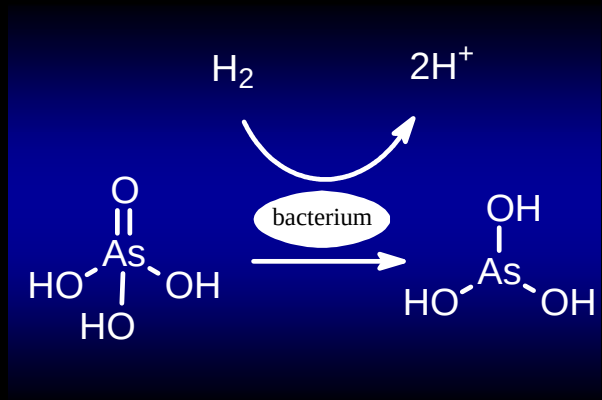
Reduction of Arsenate

Microorganisms use Two Strategies for Arsenate Reduction

- Arsenate Reductase (ArsC) for Detoxification: As(V) is reduced to As(III) to facilitate pumping it from the cell without accidently pumping out phosphate

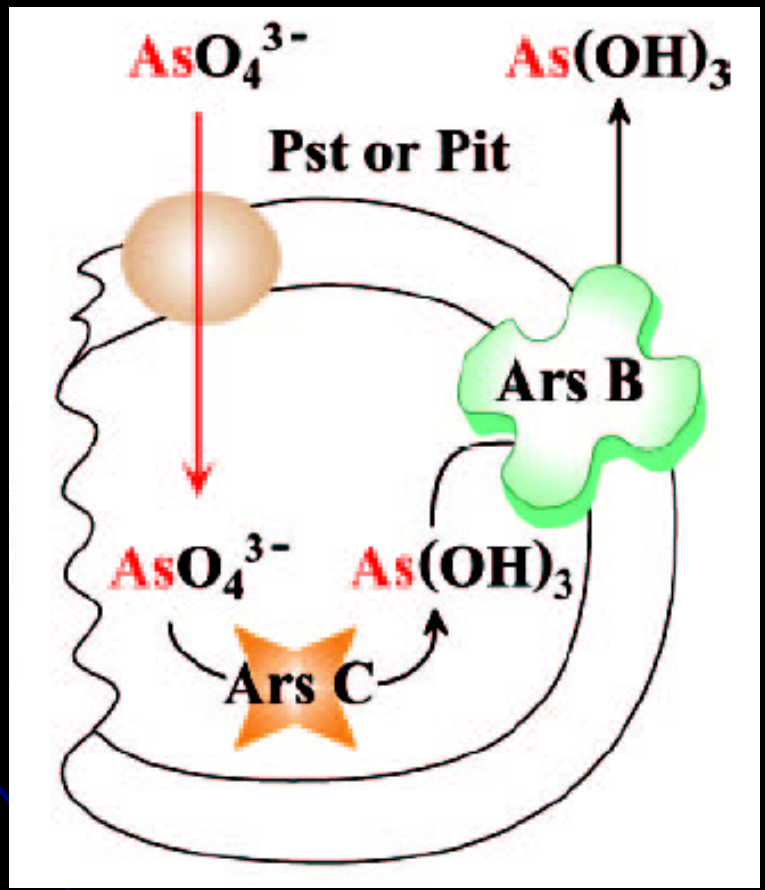


- Dissimilatory Arsenate Reductase (ArrA): Arsenate is reduced as a terminal electron acceptor during the anoxic respiration coupled to the oxidation of simple substrates



Reduction of Arsenate

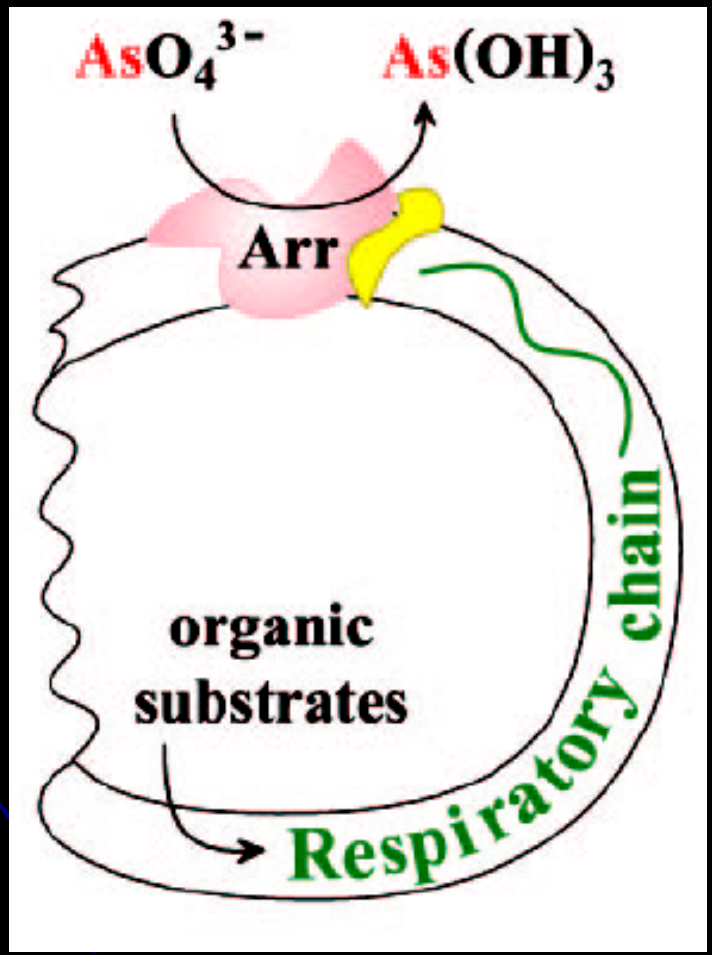
Arsenate Reductase (ArsC): Detoxification



Silver & Phung 2005. AEM 71:599

Reduction of Arsenate

Dissimilatory Arsenate Reductase (ArrA): Respiration



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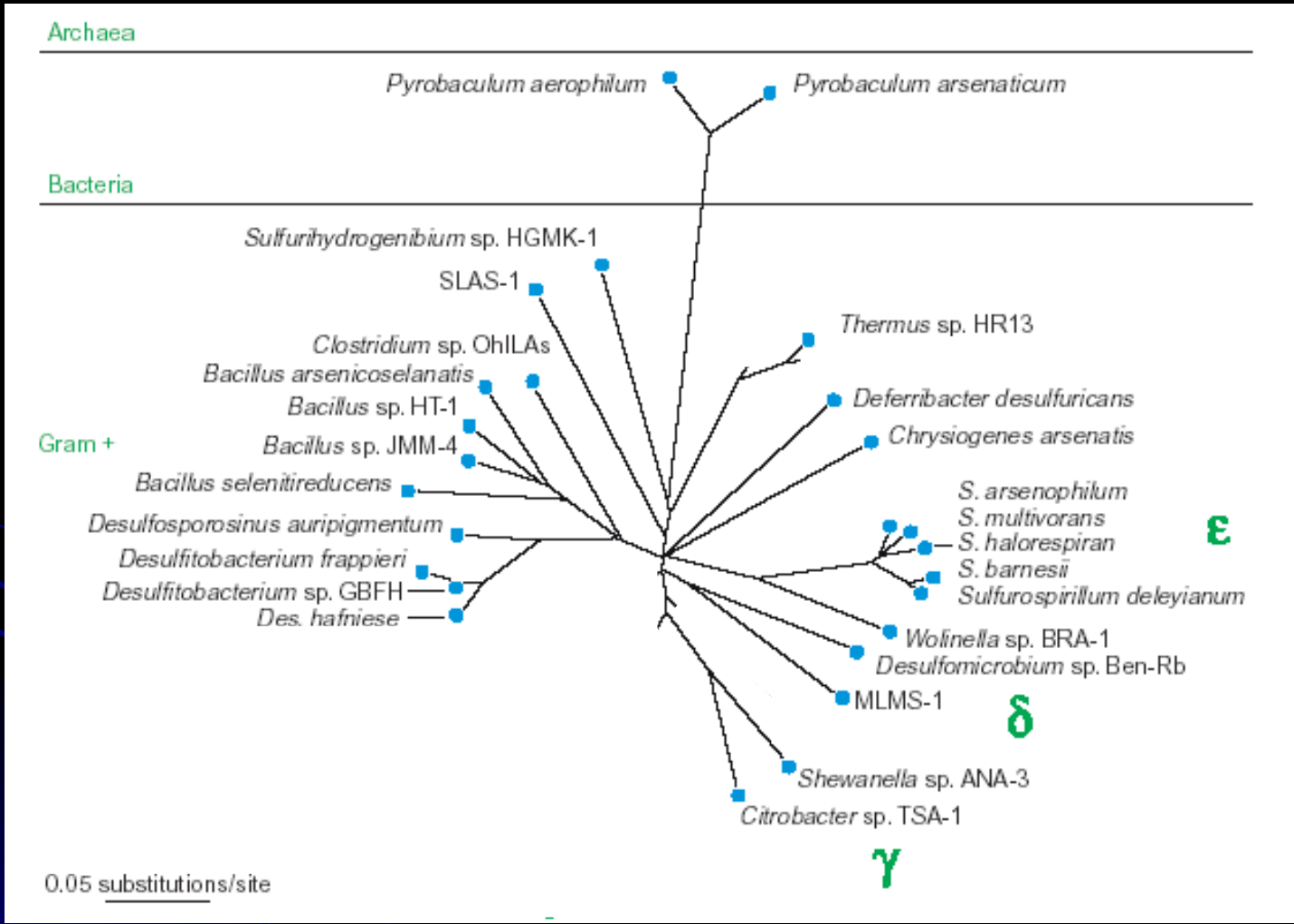
Reduction of Arsenate

Dissimilatory Arsenate Reducing Bacteria



Oremland & Stolz 2005. Trends in Microbiol. 13:45

Reduction of Arsenate

Dissimilatory Arsenate Reducing Bacteria

- Example of Reactions

Complete oxidation organic matter

$$CH_3COOH + 4\ AsO(OH)_3 \rightarrow 2\ CO_2 + 4\ As(OH)_3 + 2\ H_2O$$

acetate As(V) CO_2 As(III)

Partial oxidation organic matter

$$CH_3CHOHCOOH + 2\ AsO(OH)_3 \rightarrow CO_2 + CH_3COOH + 2\ As(OH)_3 + H_2O$$

lactate As(V) CO_2 acetate As(III)

Reduction of Arsenate

Dissimilatory Arsenate Reducing Bacteria

- Electron Donors Known to Support Dissimilatory Arsenate Reducing Microorganisms

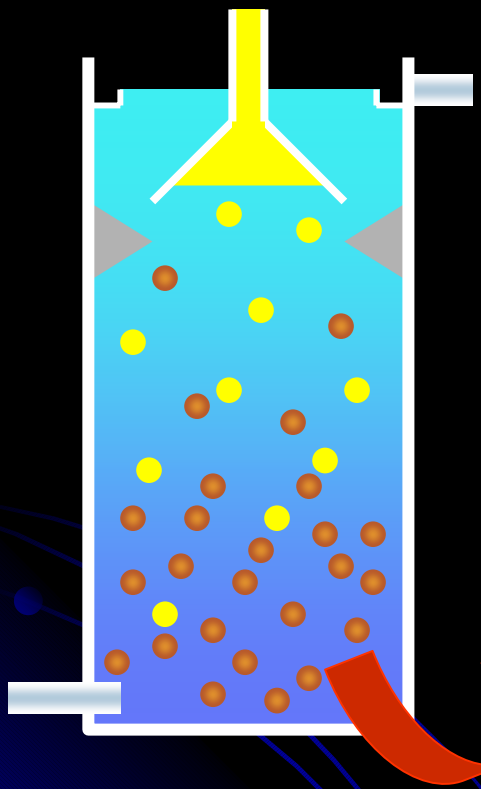
Organic Acids	Alcohol, Sugar	Aromatic	Inorganic
Lactate	Ethanol	Phenol	H_2
Pyruvate	Glycerol	Benzoate	H_2S
Fumurate	Glucose	Syringate	
Malate		Ferulate	
Succinate		Toluene	
Citrate			
Formate			
Acetate			
Butyrate			

Niggemyer et al 2001. AEM 67:5568

Liu et al 2004. FEMS Microbiol. Ecol. 48:323

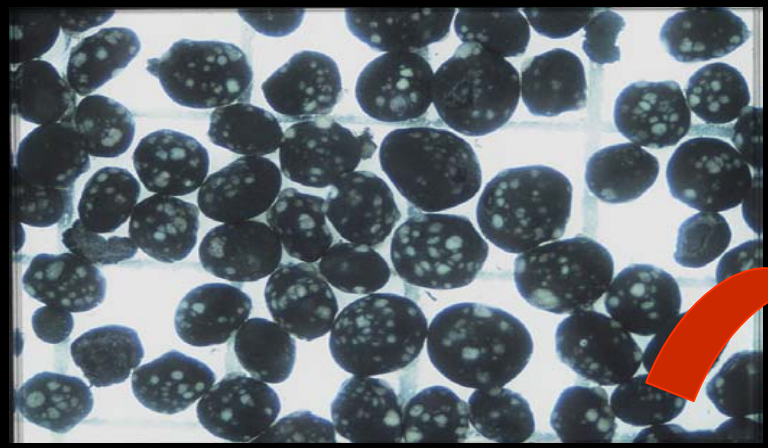
Hoeft et al 2004. AEM 70:2741

Does Arsenate Reduction Occur in a Methanogenic Consortium?

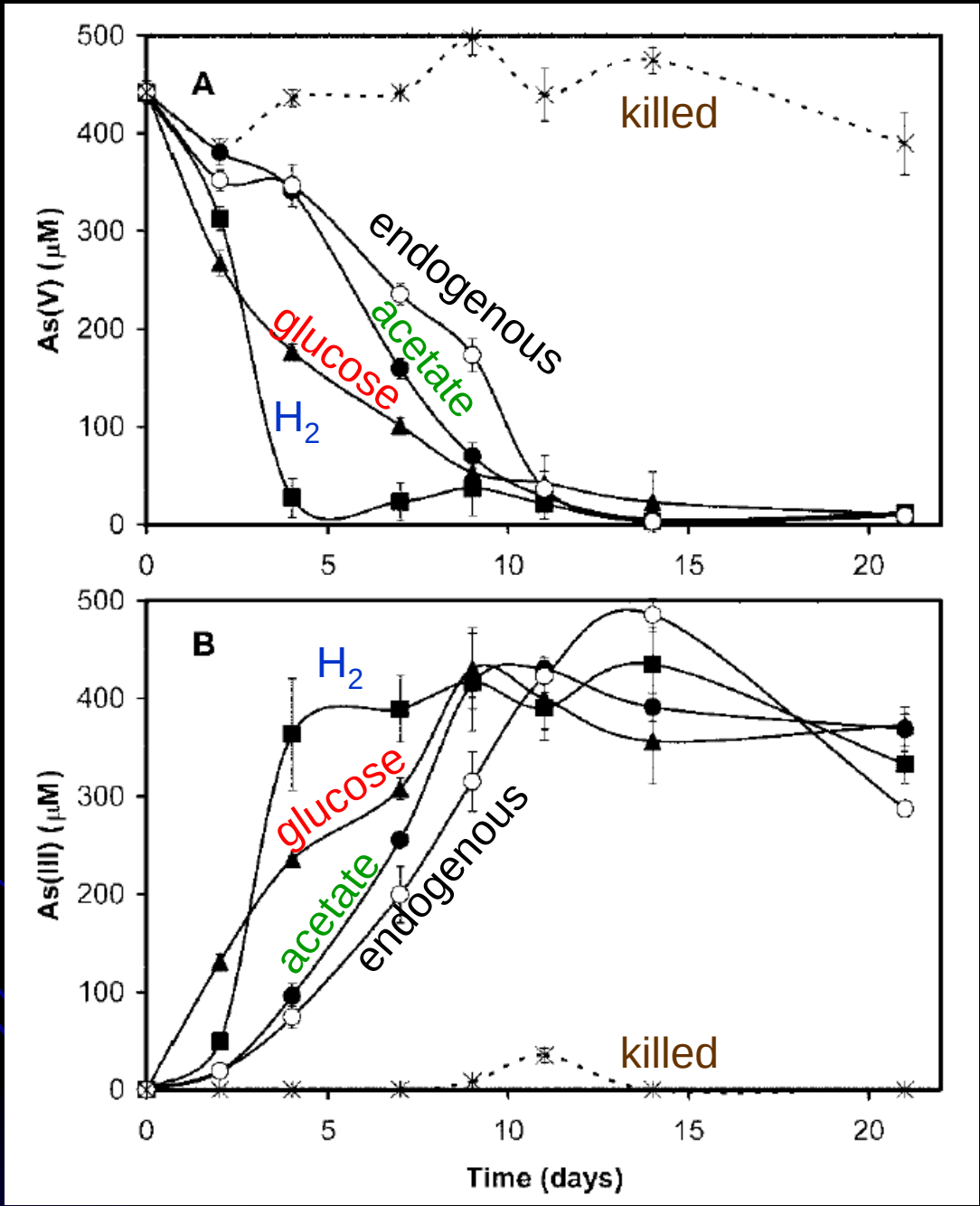


bioreactor

biofilm granules

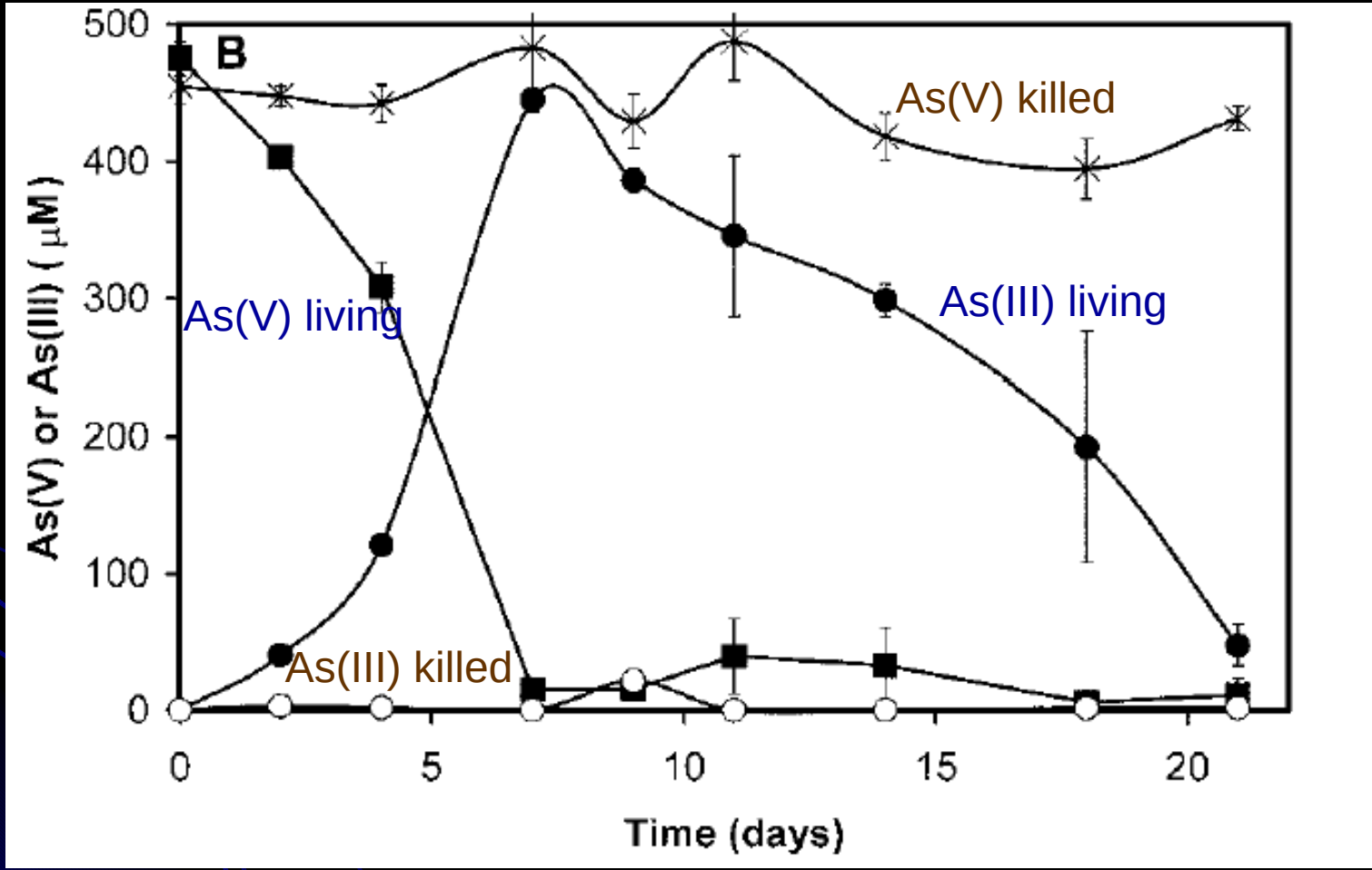


microorganisms



Field et al 2004. Biodegradation 15:185

Arsenate Reduction In a Methanogenic Consortium with Sulfate Added



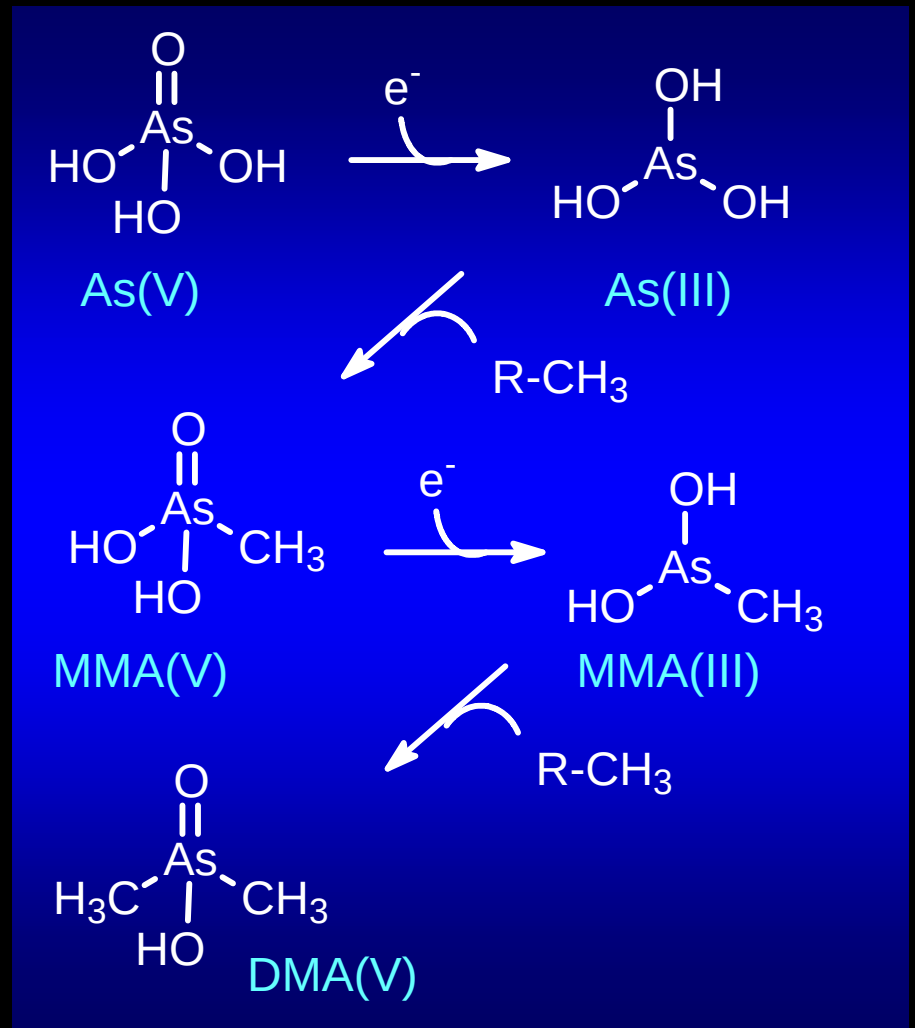
Field et al 2004. Biodegradation 15:185

Methylation of Arsenic

Both Eukaryotes and Prokaryotes Methylate Arsenic

- Challenger Mechanism
- Enzymes
  - Cyt19 in fungi, mammals
  - ArsM in bacteria, archaea
- Methyl Donors
  - S-adenosyl methionine
  - Methyl-Vitamin B12

Bentley& Chasteen 2002. MMBR 66:250



Oxidation of Arsenite

There are two physiologically distinct classes of Arsenite Oxidizers

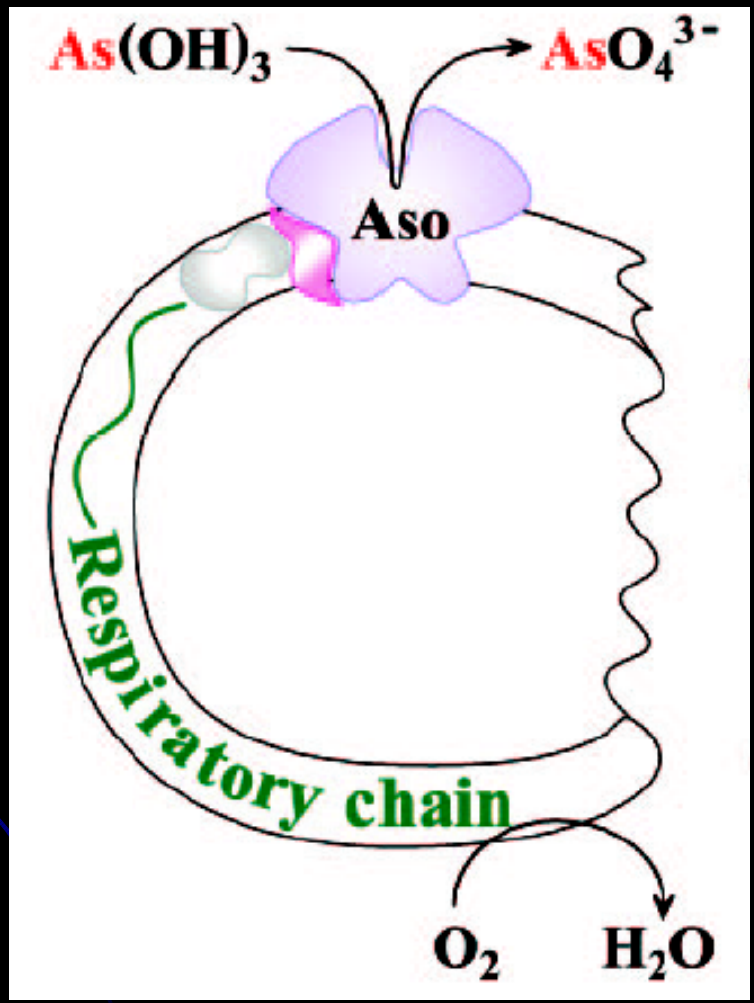
- Chemoorganoheterotrophic Arsenite Oxidizers: Detoxification Mechanism
- Chemolithoautotrophic Arsenite Oxidizers: Arsenite is used as an energy source (electron-donor); CO_2 is fixed

Oxic

Anoxic $NO_3^- + 2\ H^+ + 5\ As(OH)_3 \rightarrow N_2 + 5\ AsO(OH)_3 + H_2O$

Reduction of Arsenate

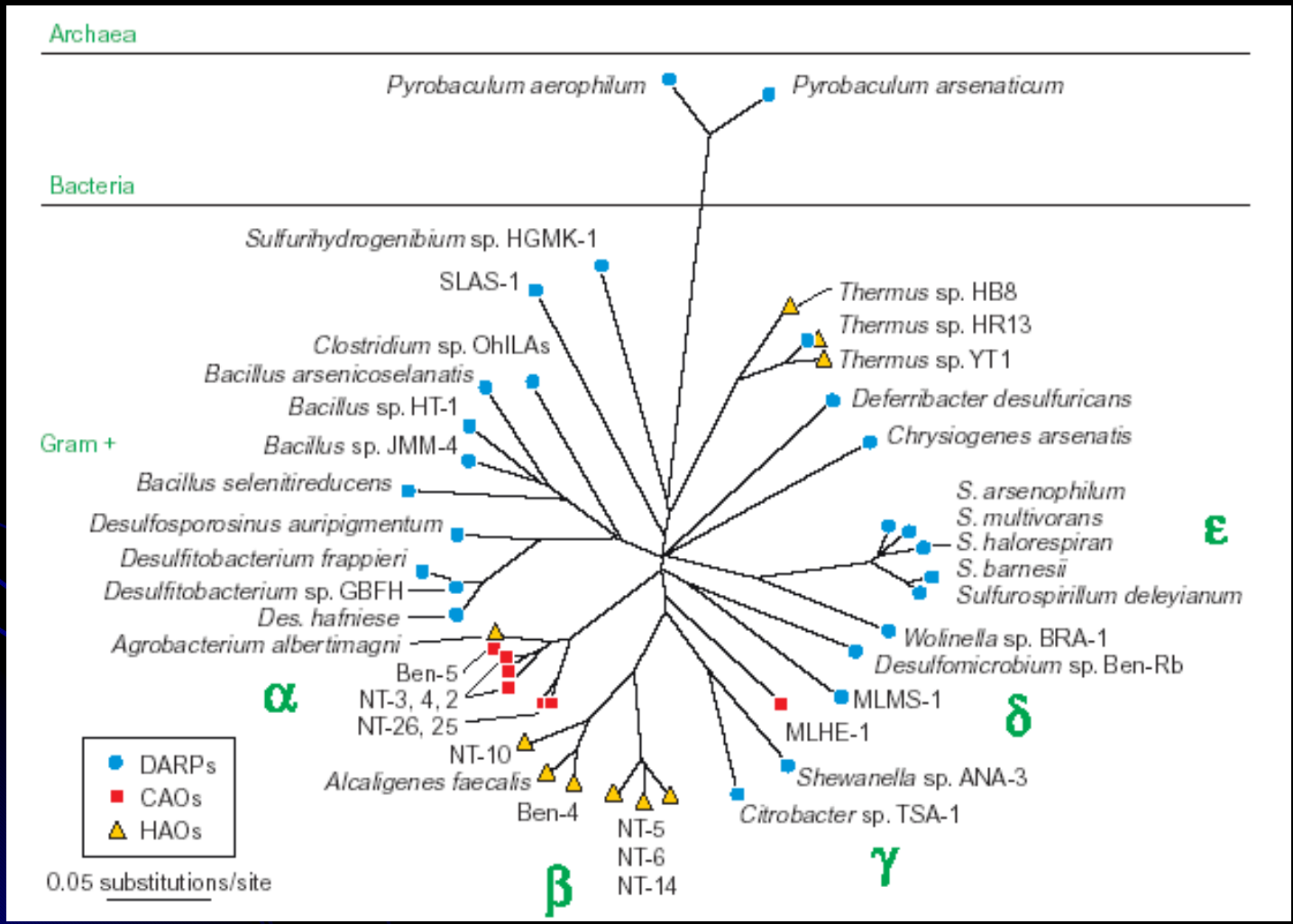
Respiratory Arsenite Oxidase



Silver & Phung 2005. AEM 71:599

Oxidation of Arsenite

Arsenite Oxidizing Bacteria (▲ ■)



Oremland & Stolz 2005. Trends in Microbiol. 13:45

Anoxic Arsenite Oxidation by Municipal Anaerobic Digester Sludge

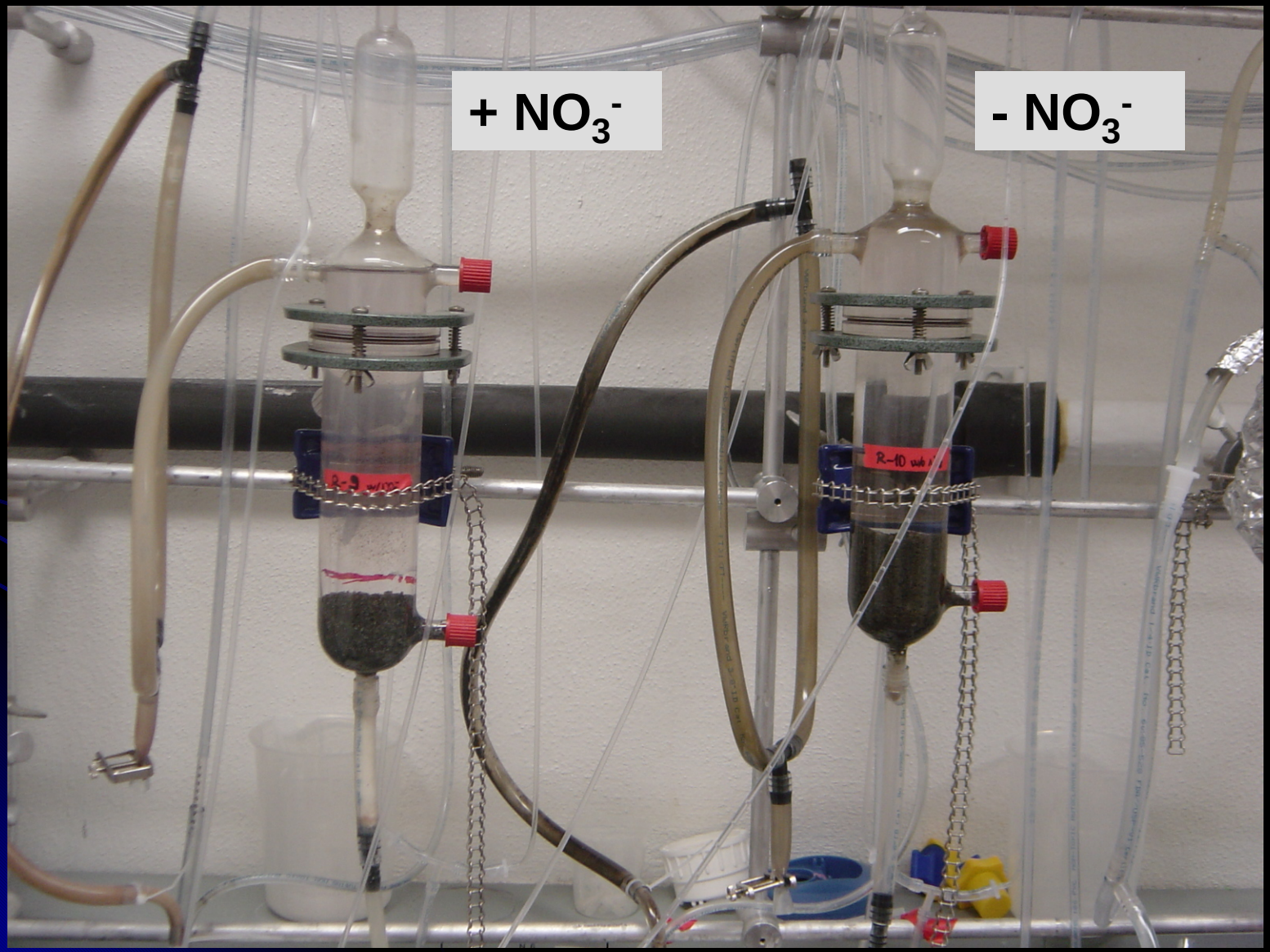
Formation of As(V) from 0.5 mM As(III)

Anoxic Oxidation of Arsenite is Ubiquitous

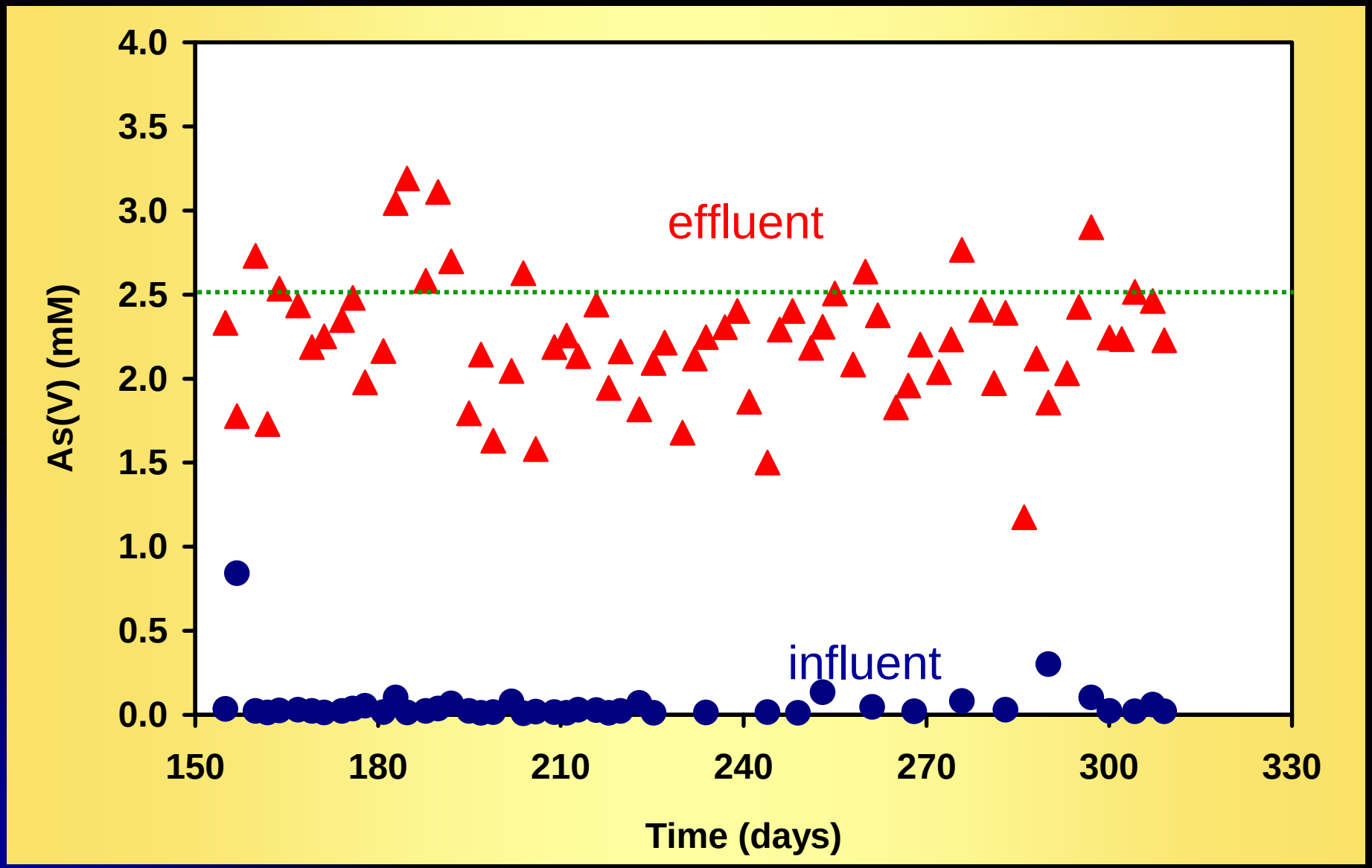
Sample	As(V) formation		Time†
	$+NO_3^-$	$-NO_3^-$	(d)
anaer. bioreactor sludge, distillery	+	-	< 4
anaer. bioreactor sludge, paper	-	-	
municipal anaer. digester sludge	+	-	10
thiosulfate-denitrification enrichment	+	-	10
municipal aerobic active sludge	-	-	
duck pond sediments	+	-	< 5
Winogradsky column sediment	+	-	< 5
Pinal Creek sediments (high Mn)	+	+	
groundwater	-	-	

† time to oxidize 0.5 mM As(III) to As(V) linked to denitrification

Bioreactor Test for Continuous Anoxic Oxidation of Arsenite

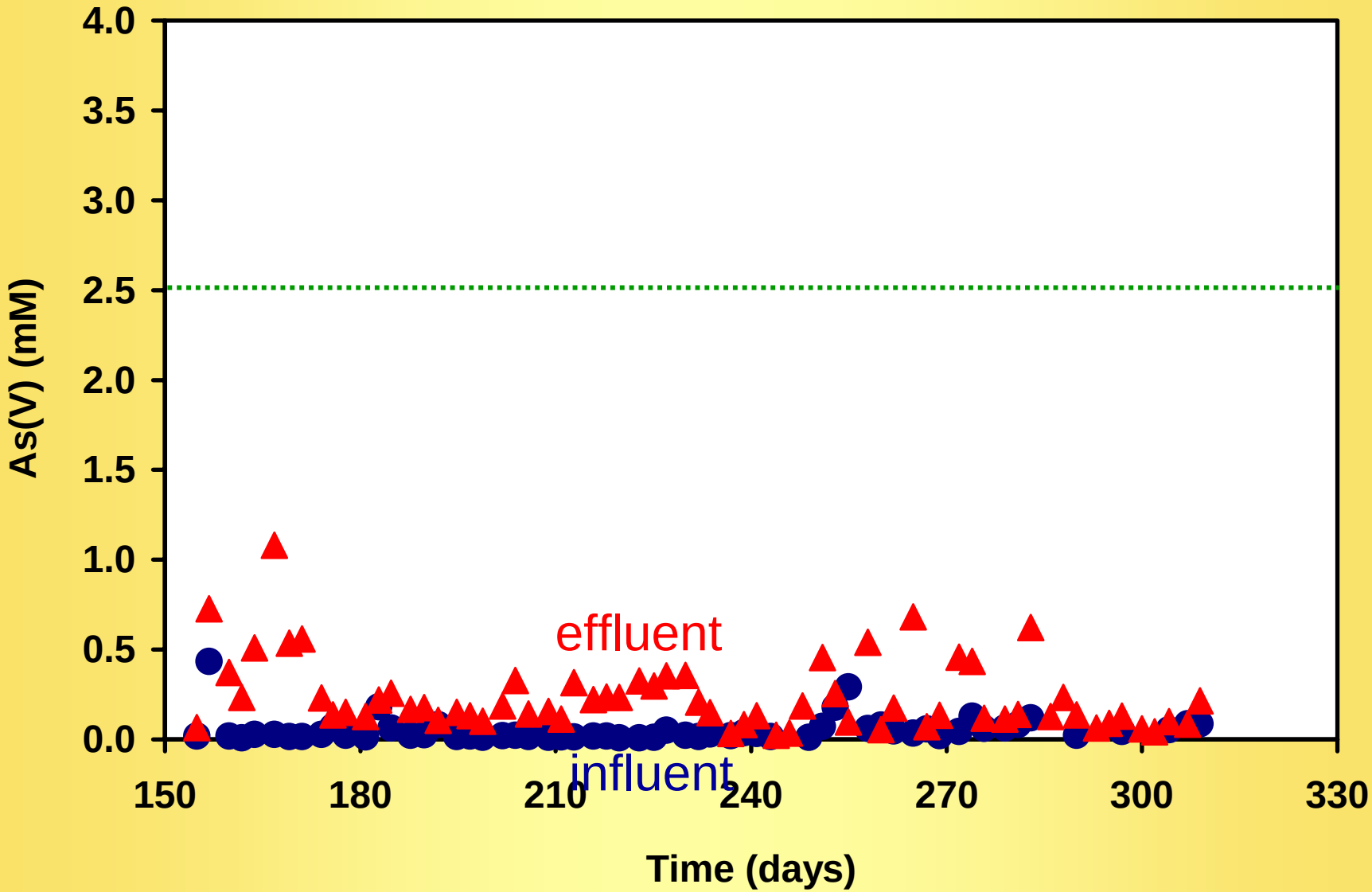


Bioreactor Results

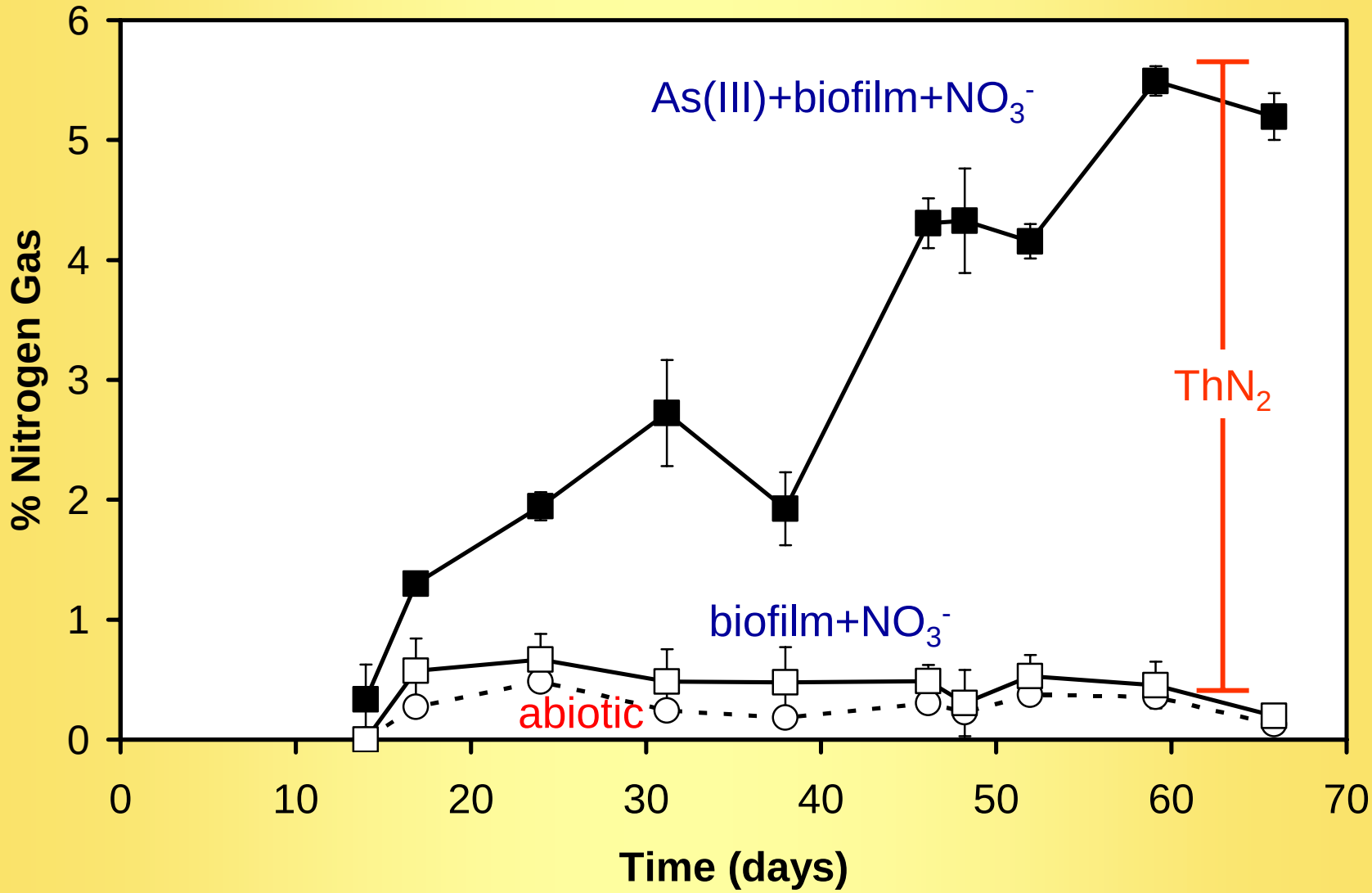


Bioreactor Results

As(V) in Control Reactor not Fed NO_3^-

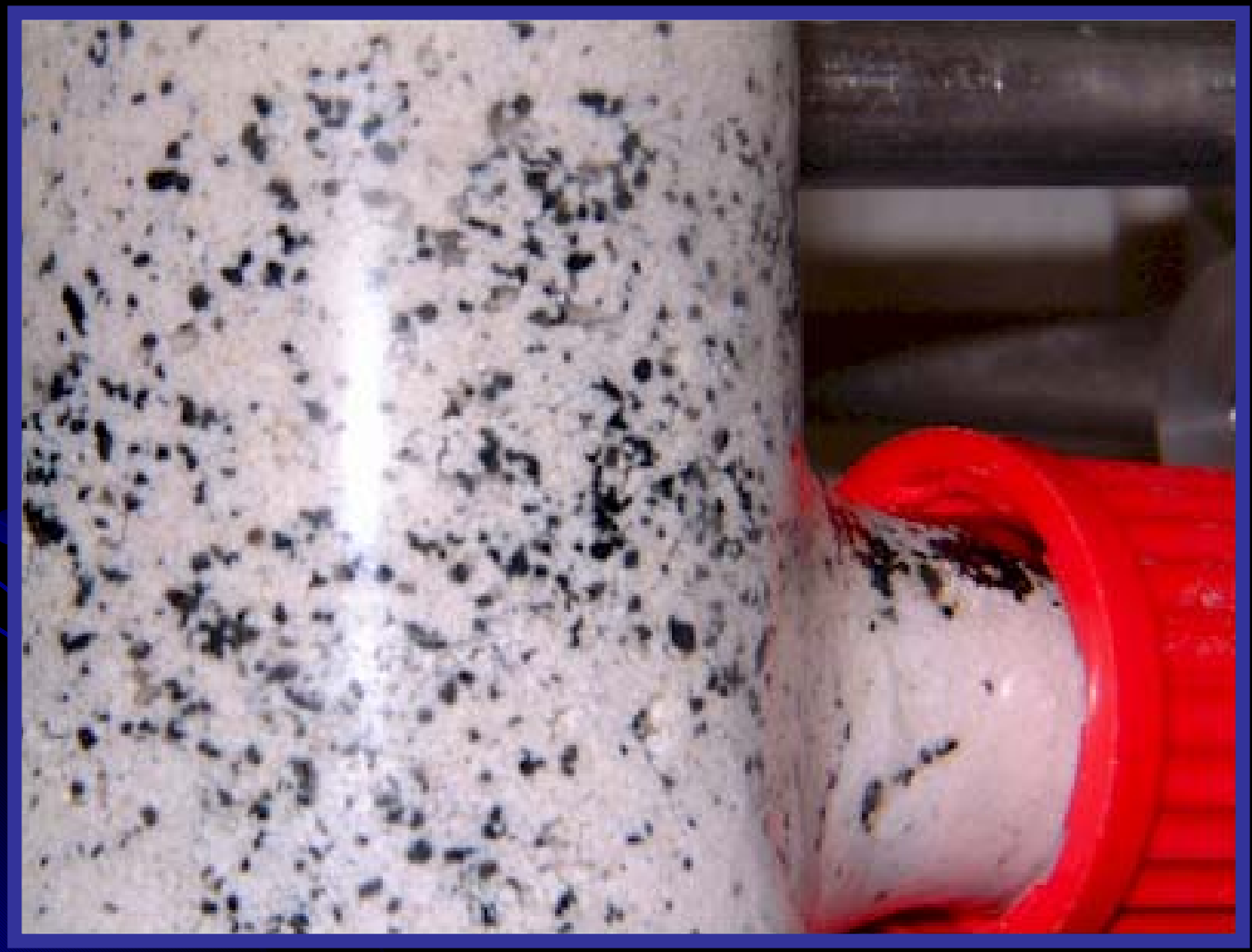


As(III) Oxidation Linked to Complete Denitrification (3.5 mM AsIII)



Microbial Mobilization of Arsenate

Experimental: Continuous Columns



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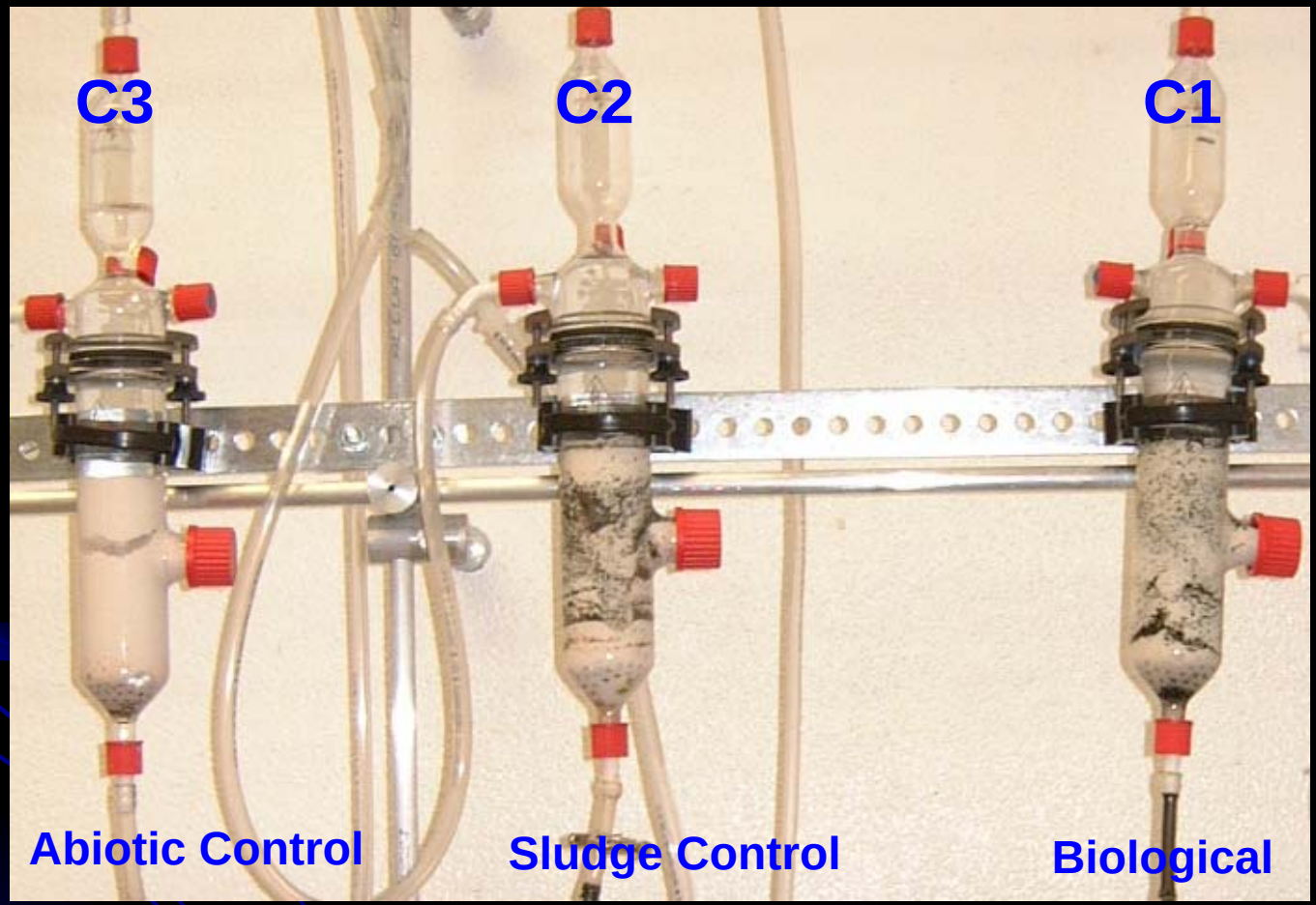


		volatile fatty acids
	sludge	sludge
$NH_3 + NaHCO_3$	$NH_3 + NaHCO_3$	$NH_3 + NaHCO_3$

Microbial Mobilization of Arsenate

Experimental: Continuous Columns

- **Activated Aluminum (AA)** operated for 257d:

  Sierra-Alvarez et al 2005 Water Research 39:199

  Biologically Active Column = 17.4% of arsenic released as freely soluble identified arsenic species; loss of 37.2% from AA

  The predominate species released was As(III), 85% of soluble species

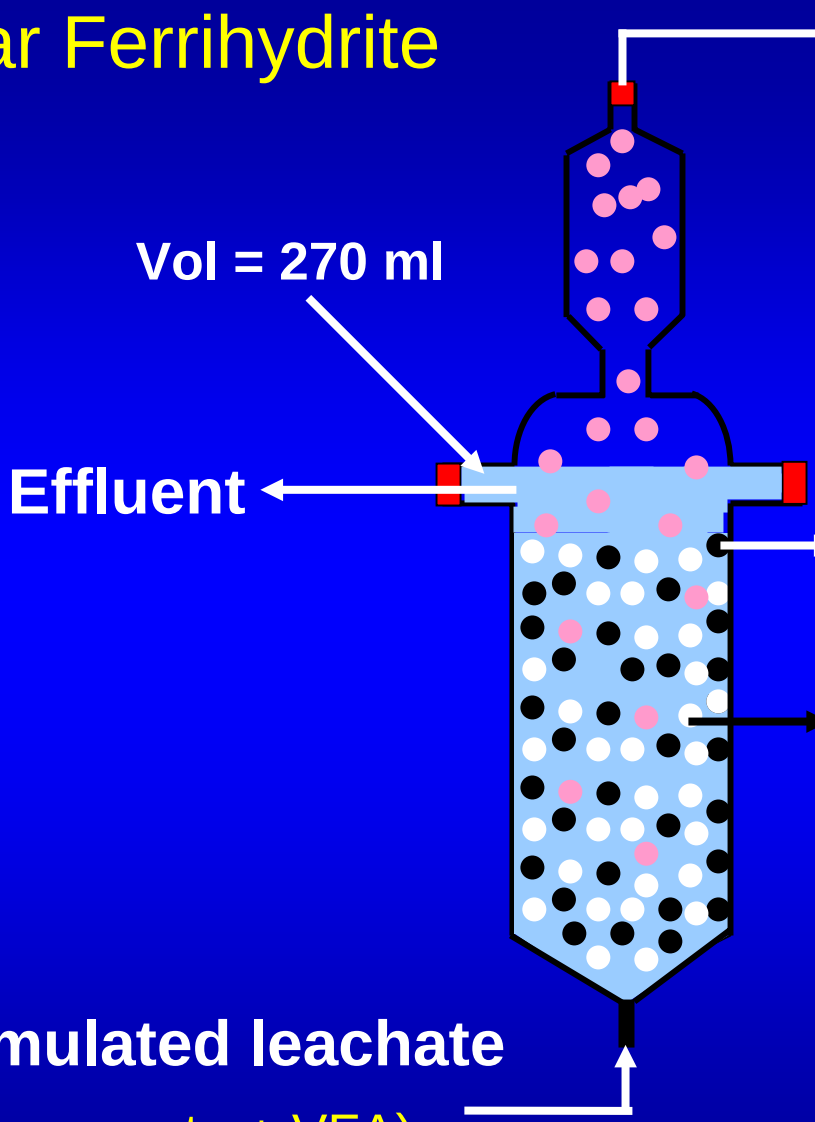
  Abiotic Column = 3.4% of arsenic released as freely soluble identified arsenic species; loss of 7.6% from AA

- **Granular Ferrihydrite (GFH)** operated for 387d:

  Results reported in next slides (Cortinas et al. 2006. In preparation)

Microbial Mobilization of Arsenate

Granular Ferrihydrite



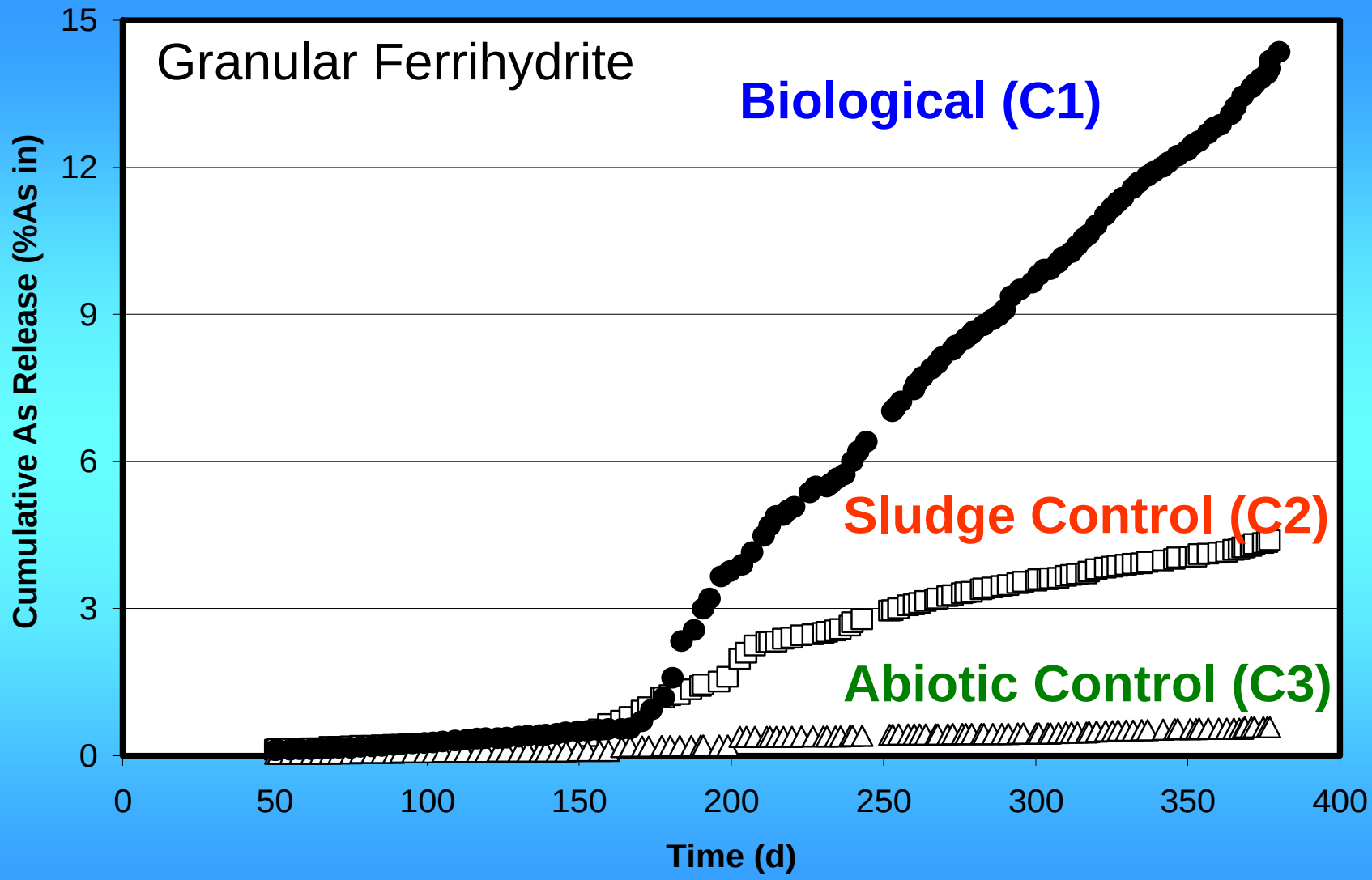
Cortinas et al 2006 in prep.

Microbial Mobilization of Arsenate

Experimental: Continuous Columns

Microbial Mobilization of Arsenate

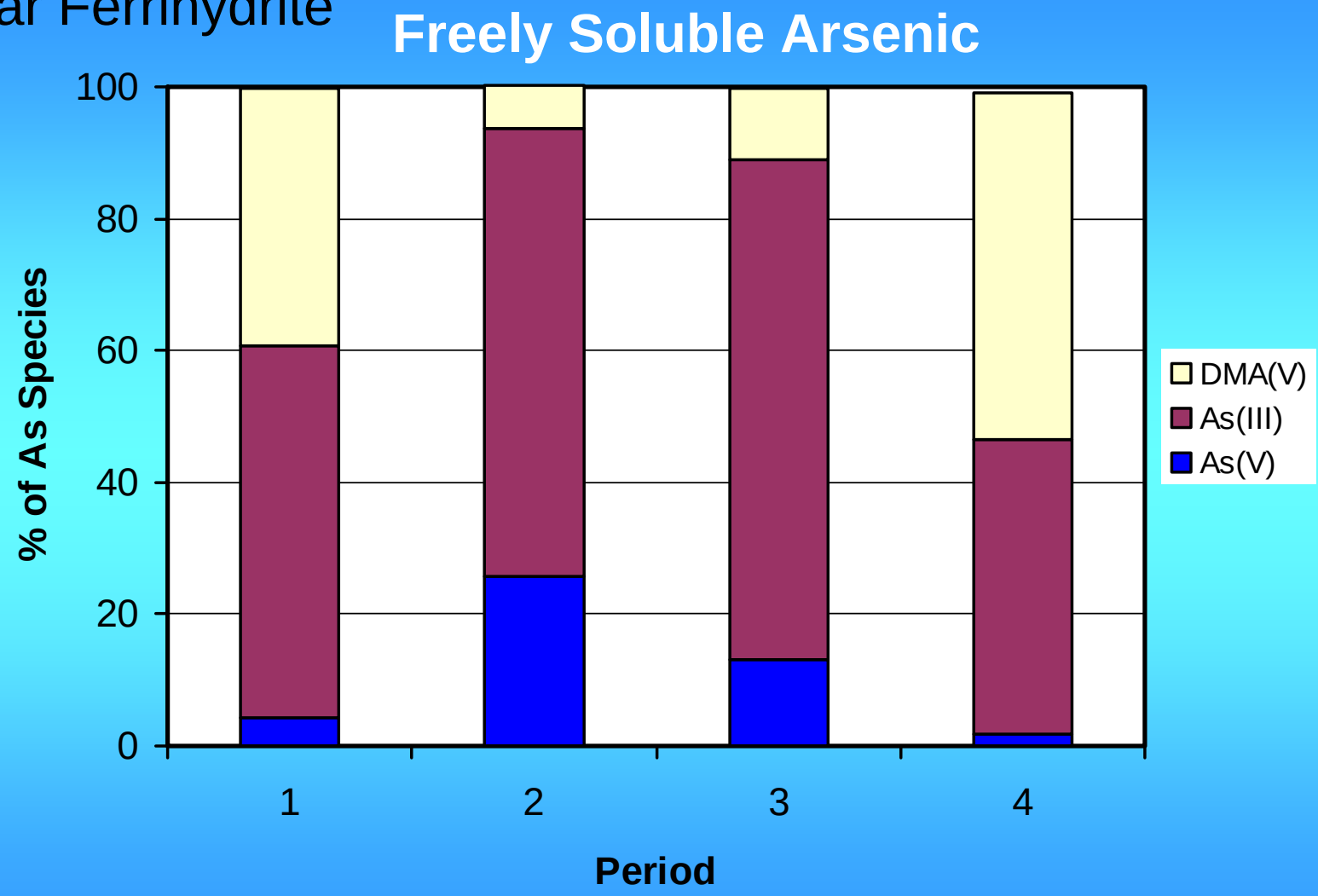
Experimental: Continuous Columns



Microbial Mobilization of Arsenate

Experimental: Continuous Columns

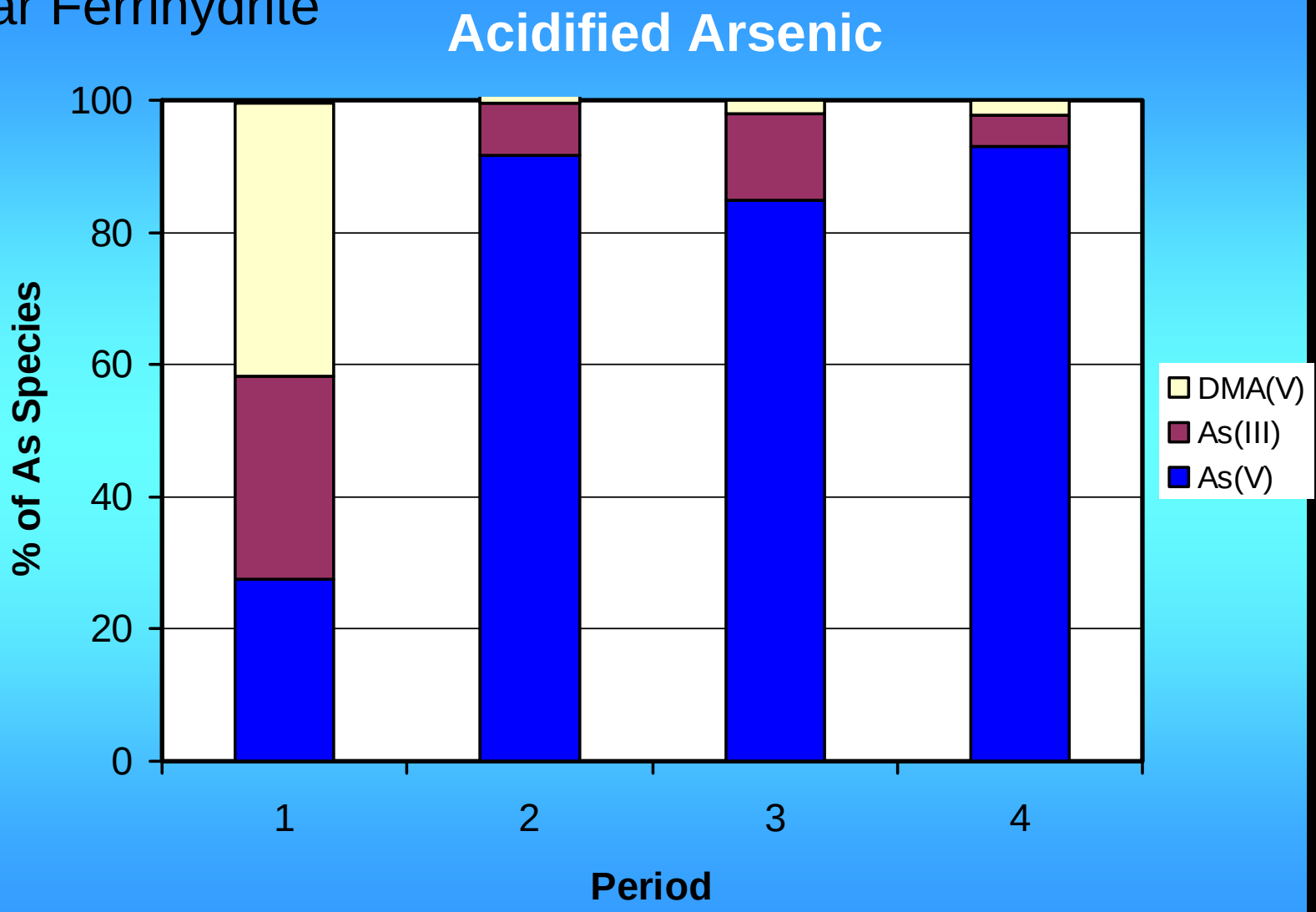
Granular Ferrihydrite



Microbial Mobilization of Arsenate

Experimental: Continuous Columns

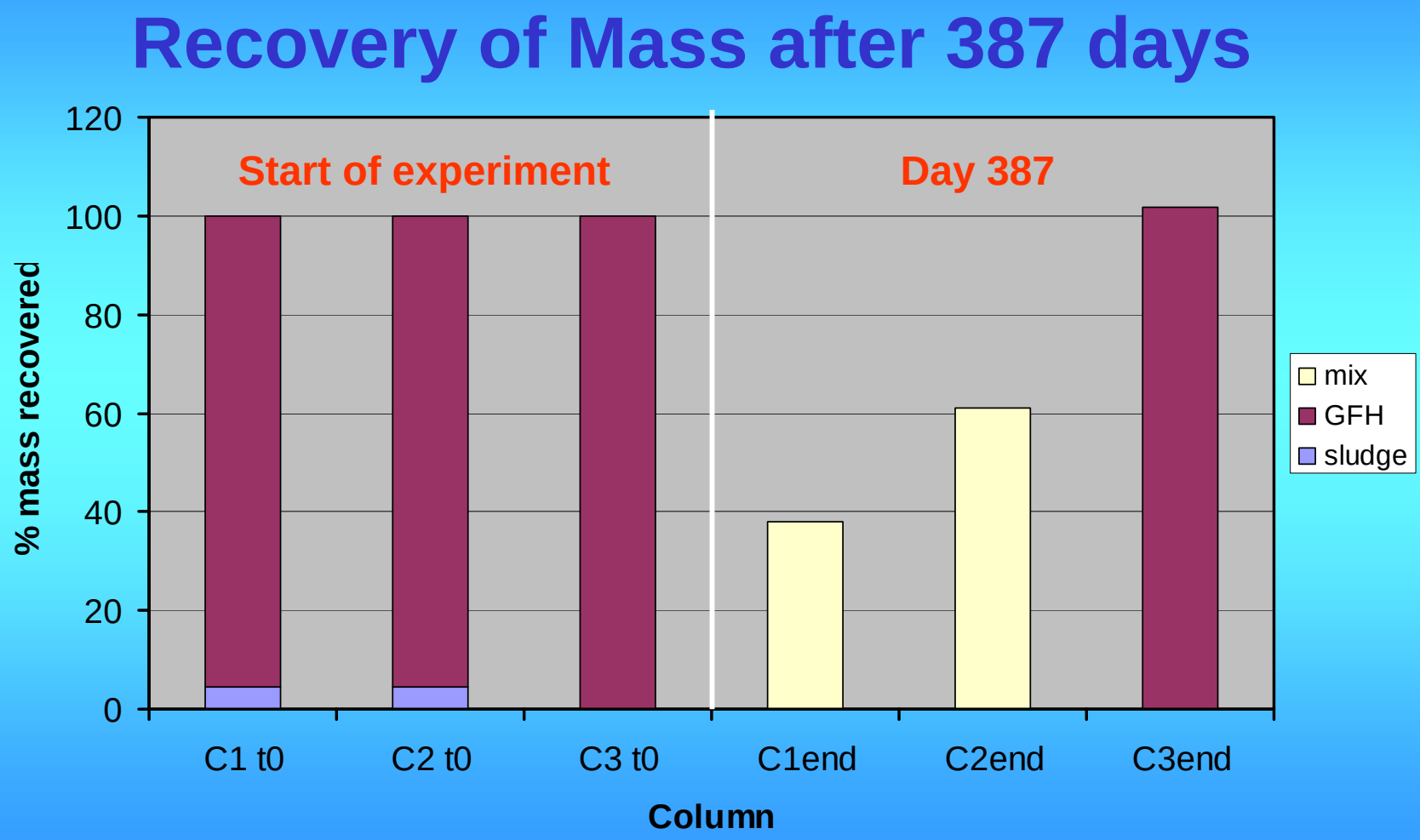
Granular Ferrihydrite



Microbial Mobilization of Arsenate

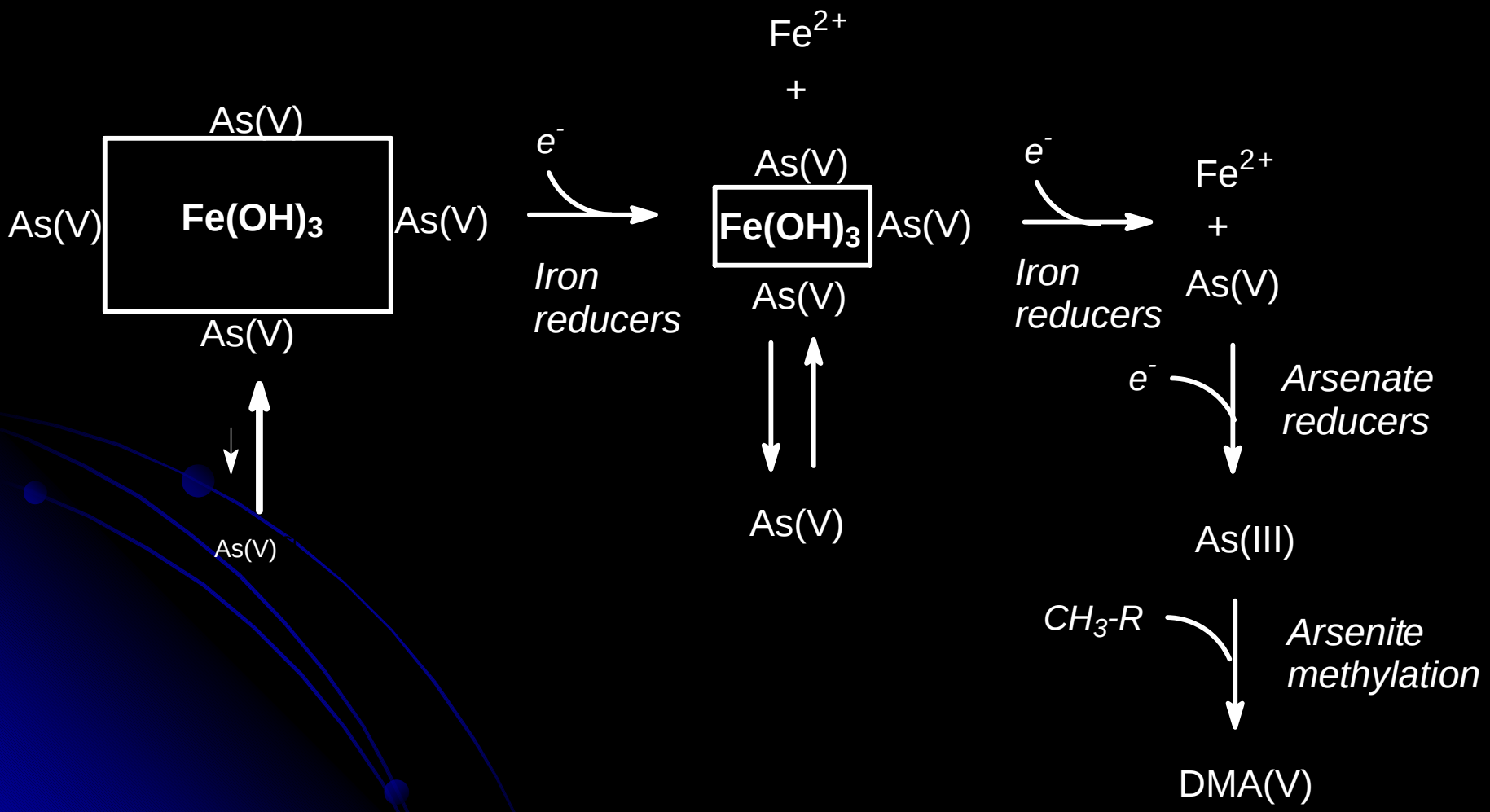
Experimental: Continuous Columns

Granular Ferrihydrite



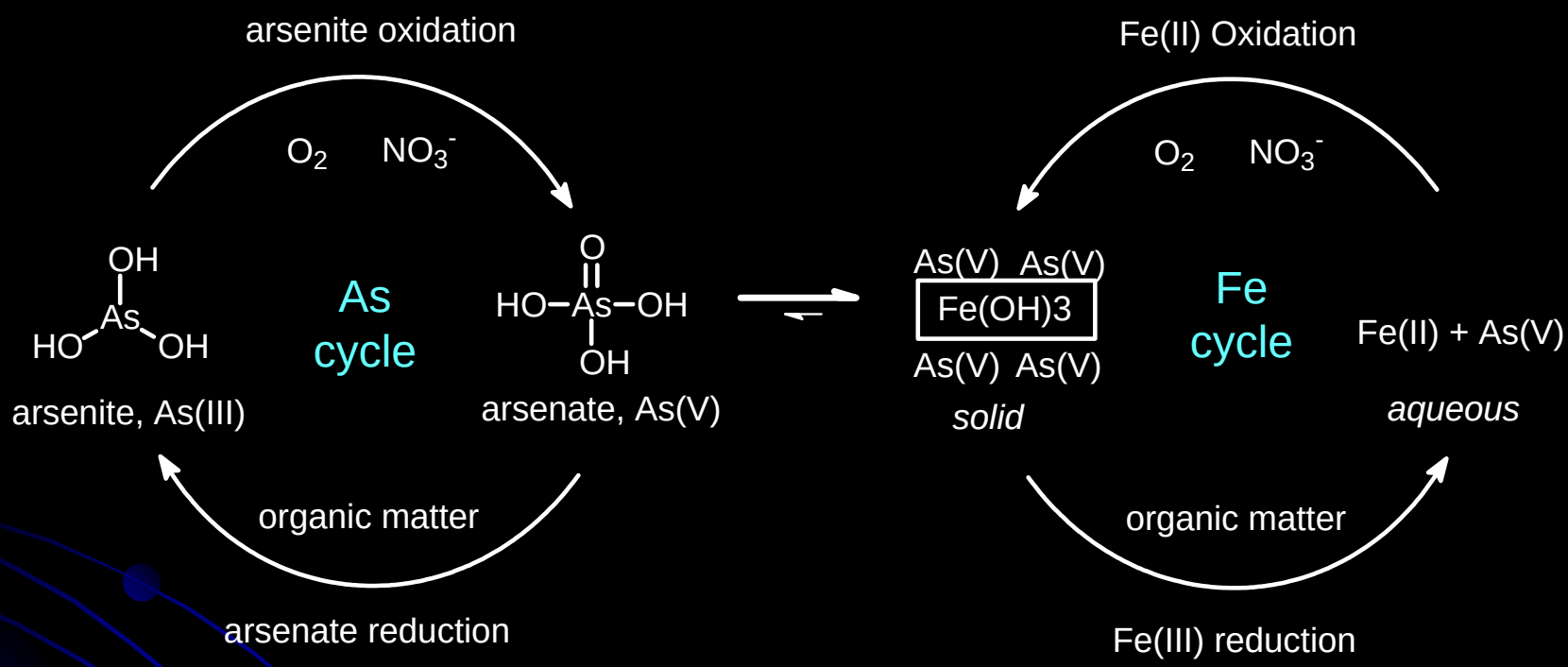
Microbial Mobilization of Arsenate

Schematic Overview GFH Columns



Conclusion 1

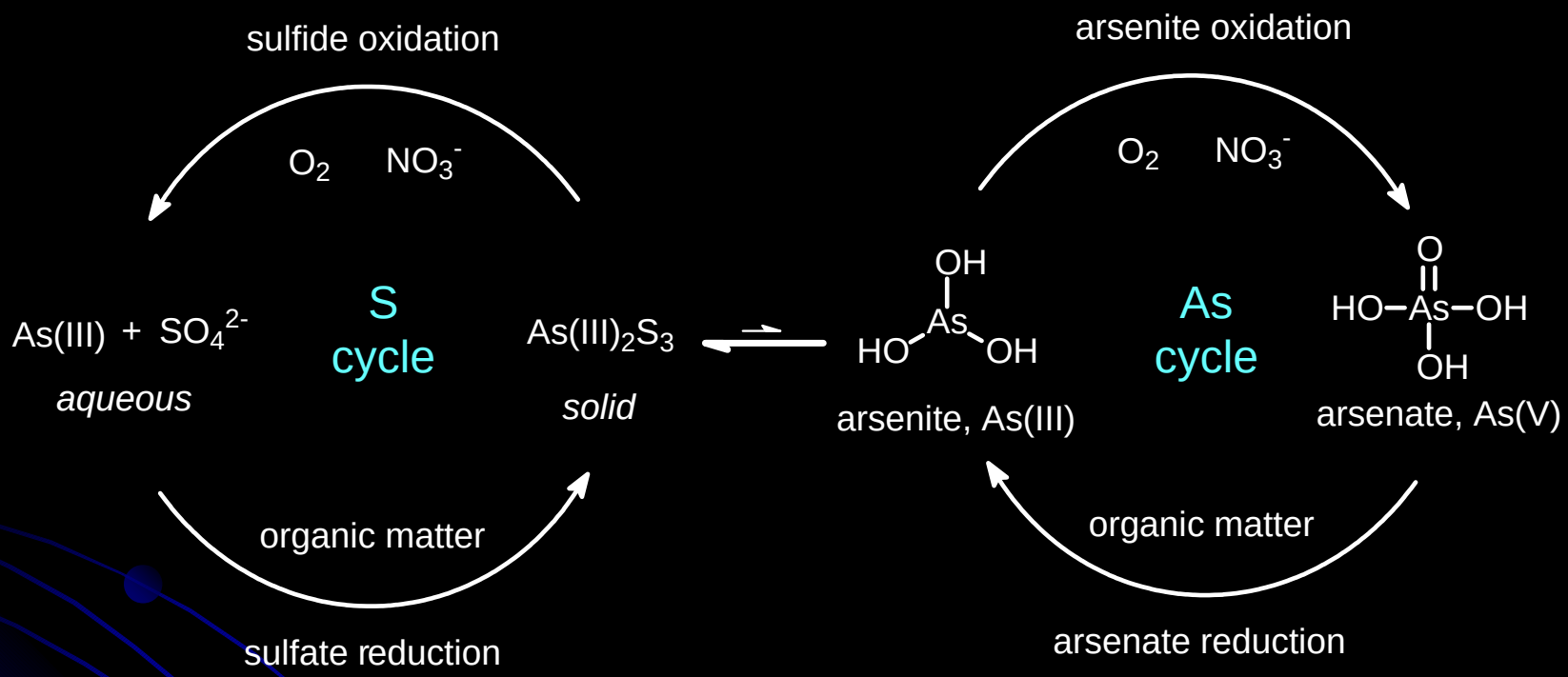
Arsenic in Matrix with Fe/Al Oxide Minerals



- **Aerobic Conditions:** Arsenic immobile
- **Anaerobic Conditions:** Arsenic mobile

Conclusion 2

Arsenic in Matrix with Sulfide Minerals



- **Aerobic Conditions:** Arsenic mobile
- **Anaerobic Conditions:** Arsenic immobile

Graduate Students