Metabolic Characterization of Three Arsenite-Oxidizing Nitrate-Reducing Bacterial Strains

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Superfund Research Program

Arsenic is a carcinogenic compound widely distributed in the groundwater around the world. Long-term exposure to arsenic contaminated water is known to increase the risk of skin-, liver-, bladder- and lung cancers. The fate of arsenic in groundwater depends on the activity of microorganisms which biotransform arsenic either by using arsenite (As^{III}), as an electron donor (arsenite-oxidizing bacteria), or by using the arsenate (As^{V}) as an electron acceptor (dissimilatory arsenate-reducing bacteria). Because of the higher toxicity and mobility of As^{III} compared to As^{V}, a bioremediation strategy has been developed to utilize the microbial-catalyzed oxidation of As^{III} to As^{V}.

OBJECTIVE

The scope of this research was to better understand the physiological role of three isolated pure cultures, *Azoarcus* sp. strain EC1, *Azoarcus* sp. strain EC3 and *Diaphorobacter* sp. strain MC, involved in the anoxic oxidation of As^{III}. The three isolated nitrate dependent-arsenic oxidizing bacteria were characterized by studying the oxidation of As^{III} to As^{V} and other electron donors with different electron acceptors

INTRODUCTION

Realgar

The two most important oxidation states of inorganic arsenic in aqueous environments are:

As^{V} or Arsenate: $H_2AsO_4^-$, $HAsO_4^{2-}$

As^{III} or Arsenite: $H_3AsO_3^0$, $H_2AsO_3^-$

Arsenopyrite

Arsenic standard for drinking water (EPA, 2006)

Arsenic MCL = 10 μg/L

As^{III} is more toxic and mobile than As^{V}

Orpiment

As^{V} is the predominant species in oxidizing/aerobic environments

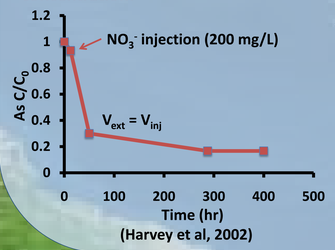
As^{III} is the predominant species in reduced/anaerobic environments.

The Role of Microorganisms in the Arsenic Cycle

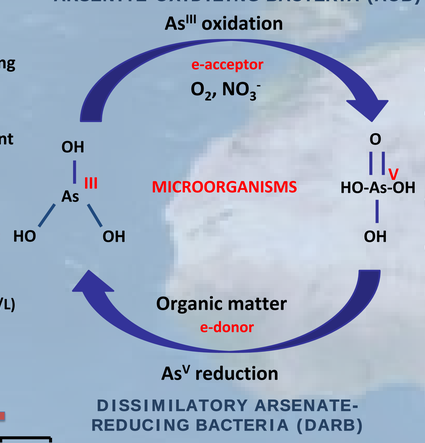
AEROBIC OXIDATION

- Well studied.
- First aerobic arsenite-oxidizing bacteria recognized in 1918 (Green, 1918)
- Widely distributed in different environments (Inskeep et al, 2007)

2002: Injecting NO_3^- into Bangladesh well lowers arsenic concentration:



(Harvey et al, 2002)



ANOXIC OXIDATION

- Isolated MLHE-1 strain from *arsenic-contaminated* lake, CA, linked partial denitrification to As^{III} oxidation (Oremland et al, 2002)

$$H_2AsO_3^- + NO_3^- \rightarrow H_2AsO_4^- + NO_2^-$$

- Strains DAO1 and DAO10 isolated from *arsenic-contained* soil appear to link As^{III} oxidation to complete denitrification (Rhine et al, 2006)

$$5H_2AsO_3^- + 2NO_3^- \rightarrow 5H_2AsO_4^- + N_2 + 8H^+ + H_2O$$

- Strains EC1, EC3 and MC isolated from *pristine sludge and sediments* cause complete denitrification (Sun et al, 2009)

Strains EC1, EC3 & MC

Isolated from pristine, arsenic-free environments (Sun et al, 2009)

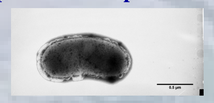
Azoarcus sp. strain EC1

EC isolated from bioreactor: Anoxic oxidation of arsenic linked with denitrification

Azoarcus sp. strain EC3

EC isolated from duck point sediment (Agua Calientes Park)

Diaphorobacter sp. strain MC



MC isolated from duck point sediment (Agua Calientes Park)

MATERIALS & METHODS

Batch Experiments

Gas Phase (40 mL)
- N_2/CO_2 80:20
- He/CO_2 80:20

Liquid Phase (120 mL)
- 100 mL basal medium
- 10 mL e-donor solution
- 10 mL inocula

pH = 7.2. HCO_3^- buffer

- 0.5 mM As^{III}
- 1.5 mM NO_3^-

As^{III} + e-acceptor —AOB→ As^{V}

- Nitrate, NO_3^- → Nitrite, NO_2^-
- Oxygen, O_2 → Chlorate, ClO_3^-

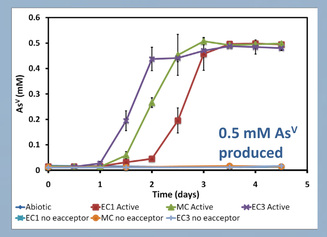
NO_3^- + e-donors —AOB→ products

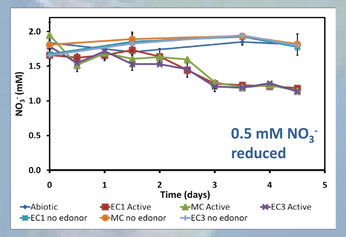
- Arsenite, As^{III} → Ferrous Iron, Fe^{2+}
- Hydrogen, H_2 → Manganese, Mn^{2+}
- Acetate, $C_2H_3O_2^-$ → Sulfide, S^{2-}

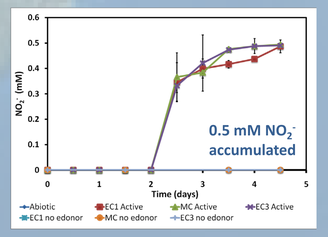
RESULTS

ELECTRON ACCEPTORS: Flexibility – EC1, EC3 & MC oxidize As^{III} with NO_3^- & O_2.

$$H_2AsO_2 + NO_3^- \longrightarrow HAsO_4^{2-} + NO_2^- + 2H^+$$

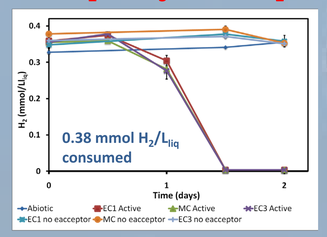


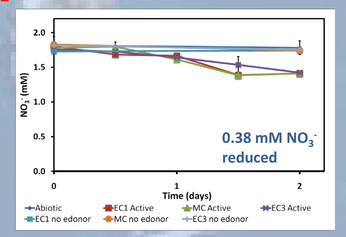


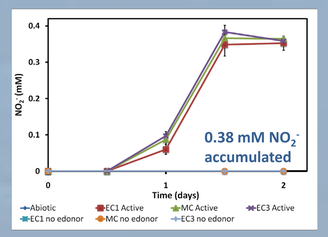


ELECTRON DONORS: Flexibility – EC1, EC3 & MC reduce NO_3^- with H_2 & acetate

$$H_2 + NO_3^- \longrightarrow NO_2^- + H_2O$$







A summary of the results is presented in the table below. Positive results are in red.

ELECTRON DONOR: As^{III} 0.5 mM			
Electron acceptors (mM)	*Azoarcus* sp. EC1	*Diaphorobacter* sp. MC	*Azoarcus* sp. EC3
KNO_3 (1.5 mM)	+	+	+
$NaNO_2$ (0.5 mM)	-	+/-	+/-
O_2 (0.35 mmol/L_{liq})	+	+	+
$NaClO_3$ (0.25 mM)	-	-	-
ELECTRON ACCEPTOR: NO_3^- 1.5 mM			
Electron donors (mM)	*Azoarcus* sp. EC1	*Diaphorobacter* sp. MC	*Azoarcus* sp. EC3
$NaAsO_2$ (0.5 mM)	+	+	+
CH_3CO_2Na (0.35 mM)	+	+	+
H_2 (0.35 mmol/L_{liq})	+	+	+
$FeCl_2.4H_2O$ (1 mM)	-	-	-
$Na_2S.9H_2O$ (0.125 mM)	-	-	-
$MnSO_4.H_2O$ (0.5 mM)	-	-	-

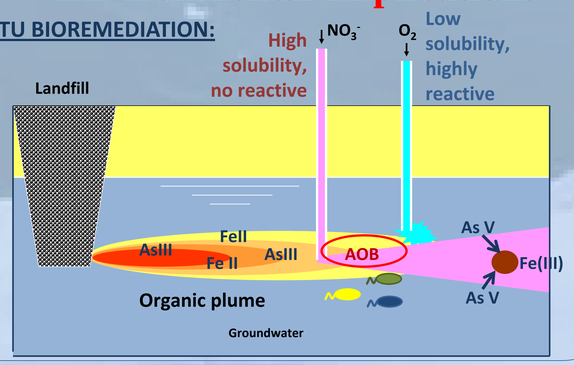
CONCLUSIONS

- *Azoarcus* sp. strain EC1, *Azoarcus* sp. strain EC3 and *Diaphorobacter* sp. strain MC are able to oxidize As^{III} with:
  - NO_3^-
  - O_2
- The three strains were characterized as facultative autotrophs, which can grow with the inorganic substrates, As^{III} and H_2, as well as with acetate
- The three strains linked As^{III} and H_2 oxidation to partial nitrate reduction to nitrite. Nitrite was detected as the final product.
- The bacteria were able to grow with As^{III} even though they were isolated from As^{III} free environments. This was most likely due to their flexibility to grow with more common occurring substrates such as H_2 and acetate.
- These results suggest that AOB can be present in a contaminated aquifer:

  In-Situ Bioremediation

Environmental Implications

IN-SITU BIOREMEDIATION:



ACKNOWLEDGMENTS

The work presented here was funded by a grant of the NIEHS-supported Superfund Research Program (NIHES-04940). Arsenic analyses were performed by the Analytical Section of the Hazard Identification Core (Superfund Basic Research Program grant NIEHS-04940). TEM was conducted at USIF (University Spectroscopy and Imaging Facilities).

REFERENCES

Green, H. H., 1918. S. Afr. J. Sci., 14:465-467
Harvey, C. F. et al., 2002. Sci., 298:1602-1606
Inskeep, W. P. et al., 2007. Env. Micr., 9:934-943
Oremland, R. S. et al., 2002. App. Env. Micr., 68:4795-4802
Rhine, E. D. et al., 2006. Env. Micr. 8:899-908
Sun, W. J.,2009. F. Micr. Ecol. 68:72-85