Towards Living Nanoscale Sorbents: Highly Selective and Sustainable Arsenic Removal

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Sustainable Nanotechnology Organization Conference
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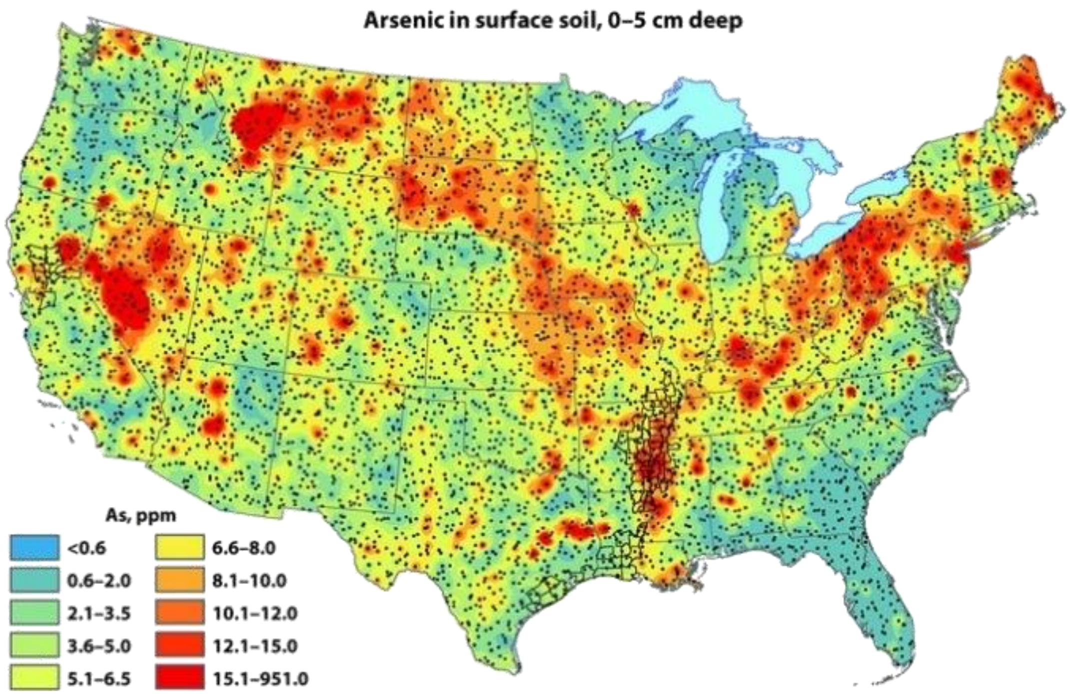
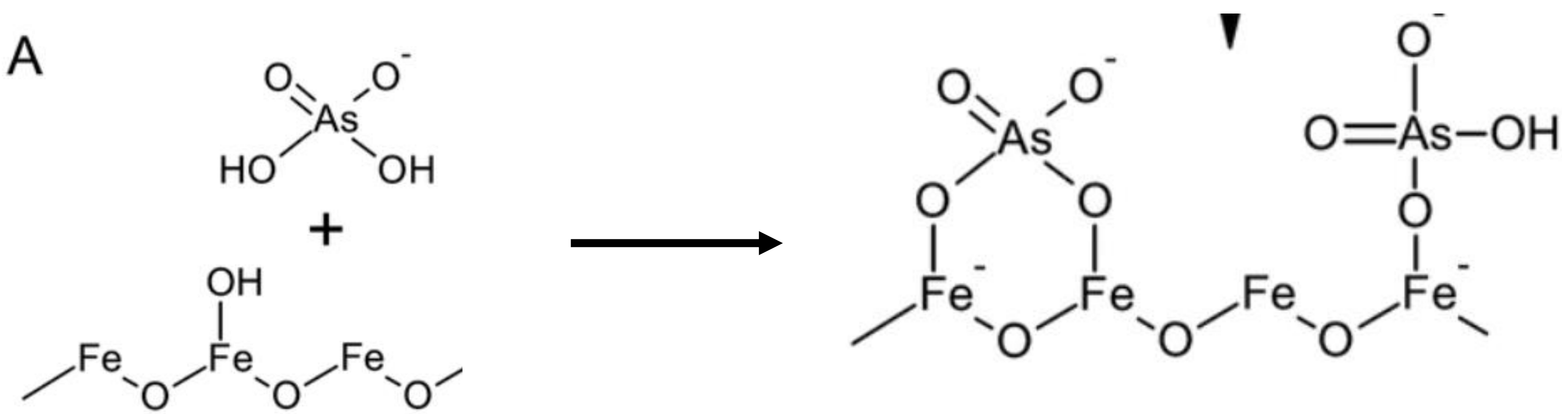
Arsenic Removal Can Exploit Arsenic-Iron Oxide Chemistry



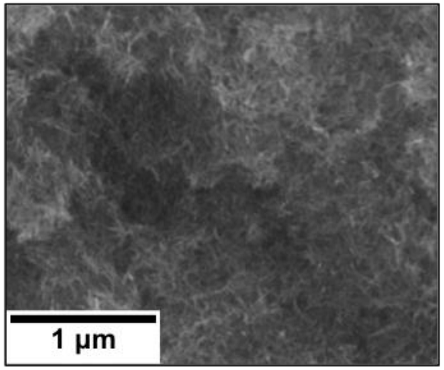
Credit: U.S. Geological Survey

Arsenic in water linked to cancer

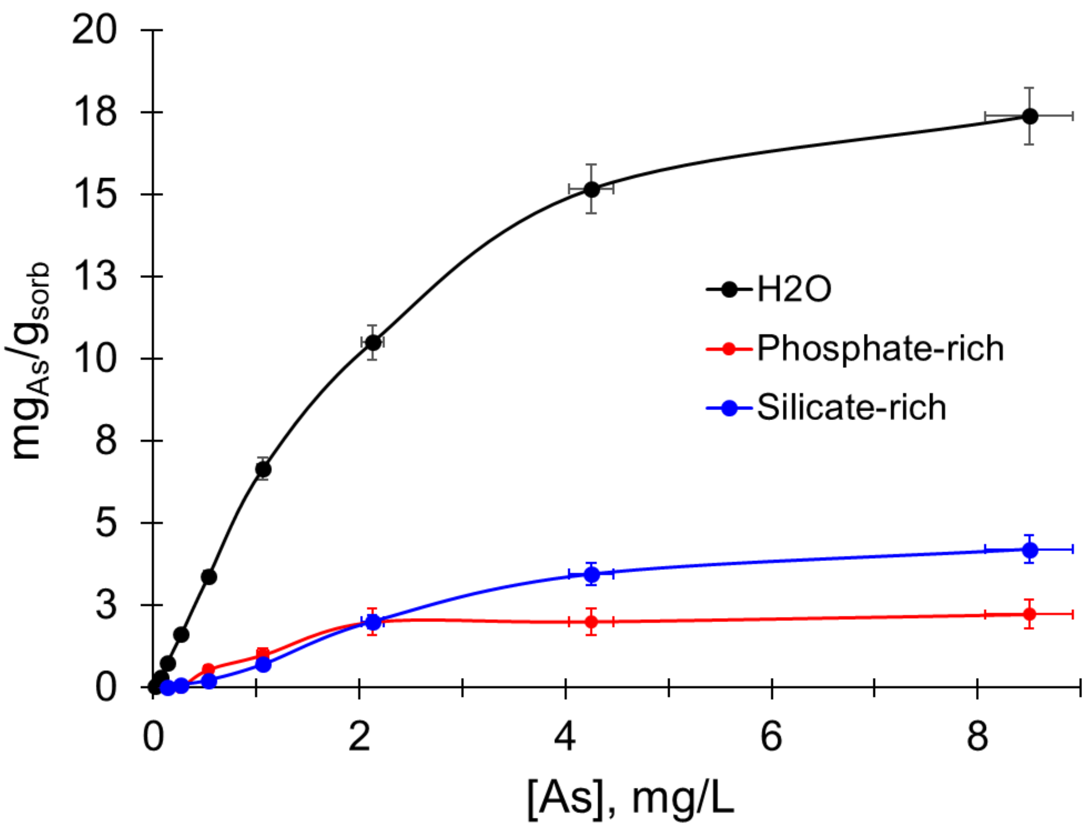
EPA standards (2001):
50 ppb → 10 ppb



Challenges for Inorganic Sorbents: Selectivity, Costs (all kinds)



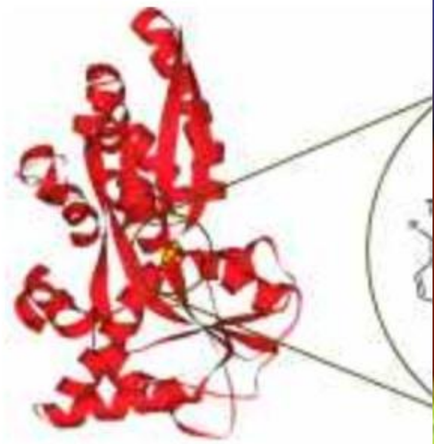
Images of Bayoxide E33
(Mesoporous goethite)
Credit: Dr. N. Gonzalez

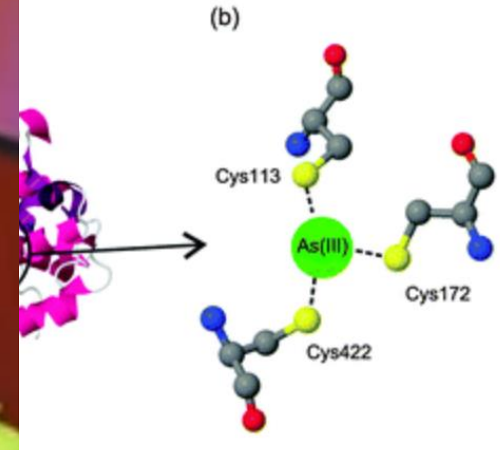


- Media cost to treat 20 MGPD with Bayoxide, annually, > 250,000 USD
- Environmental costs of transportation of media, handling even higher

Life cycle analysis of two Hungarian drinking water arsenic removal technologies; **Jones, CJ; Colvin, VL**
WATER SCIENCE AND TECHNOLOGY-WATER SUPPLY Volume: 14 Issue: 1 Pages: 48-60 Published: 2014

Biological Sorbents – Possible Advantages





Sulfate-binding p

binding protein

The Claw (the movie Toy Story)

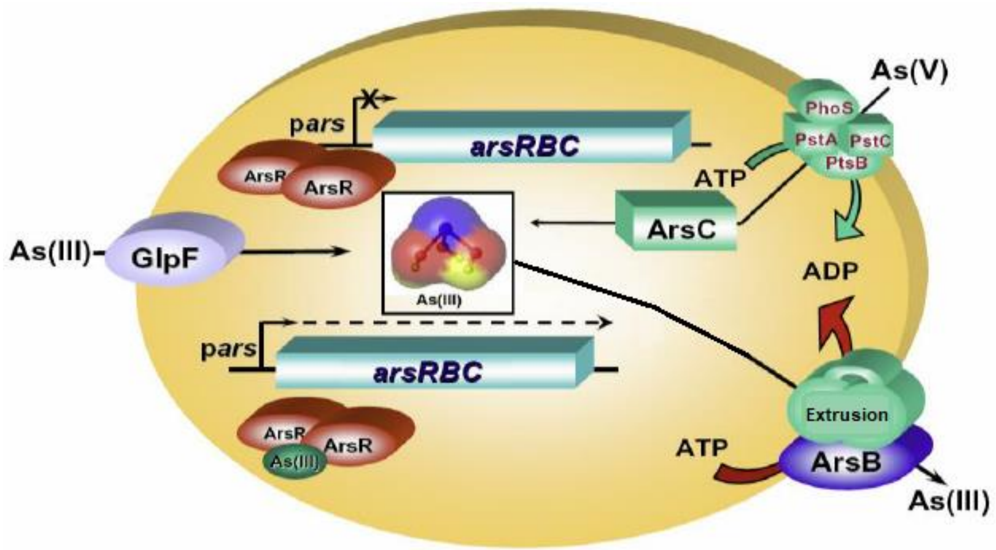
1: Can arsenic-binding proteins be overexpressed in E. Coli?

2: Do these proteins and their hosts remove arsenic selectively?

3: Can arsenic removal occur in diverse samples? (e.g. beverages)

Therapeutic and analytical applications of arsenic binding to proteins Beibei Chen [a], Qingqing Liu [a], Aleksandra Popowich [b], Shengwen Shen [a], Xiaowen Yan [a], Qi Zhang [a], Xing-Fang Li [a], Michael Weinfeld [c], William R. Cullen [d] and X. Chris Le *[ab] Metallomics , 2015, **7**, 39-55

E. coli and arsenic resistance



Adapted from Chen *et al.* Biosensors, *4, 494* (2014)

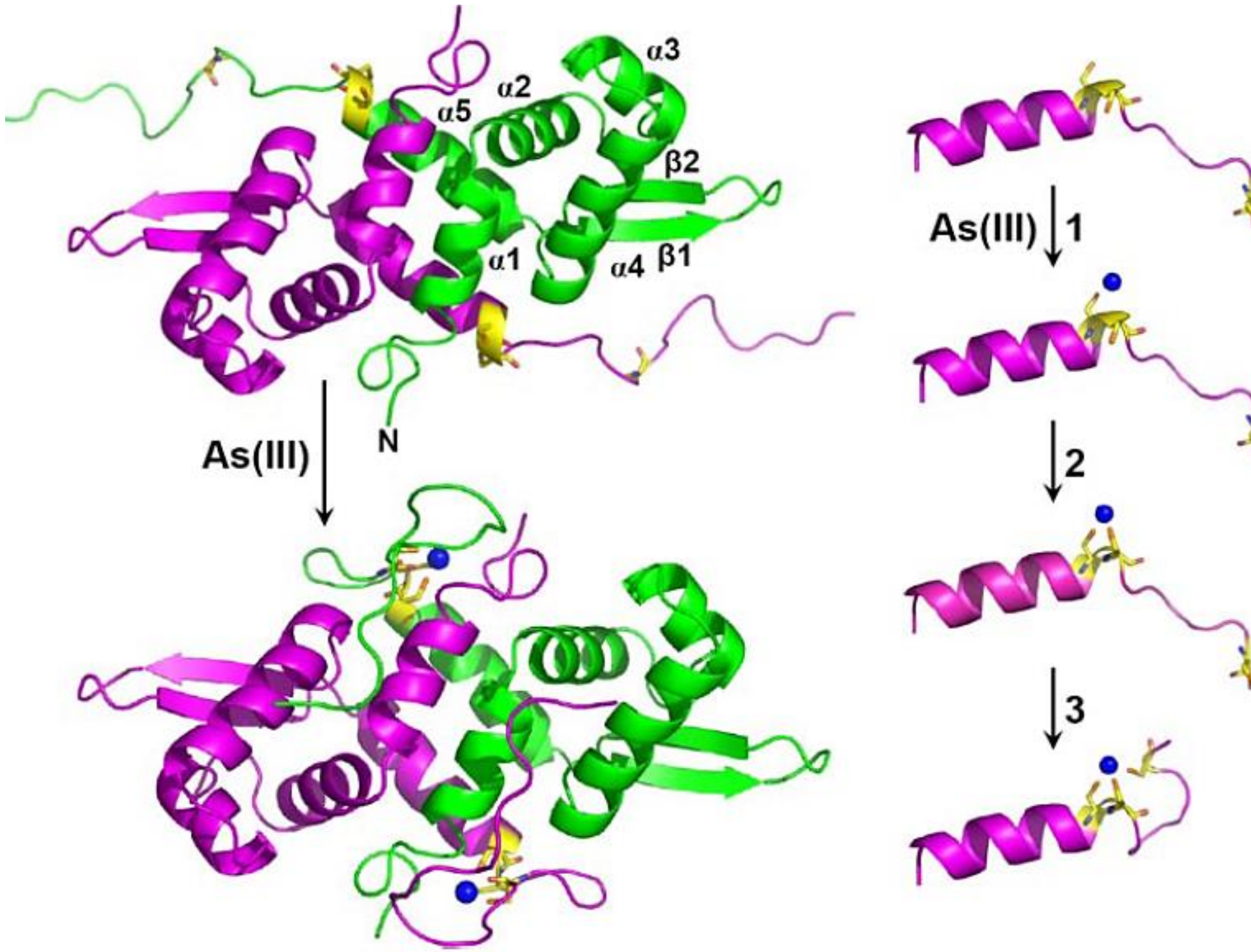
Nearly every organism, from ***E. coli*** to humans, has mechanisms for arsenic detoxification

In *E. coli*, the arsenic resistance (ars) operon has three genes:

- **ArsR**, the As(III)-responsive transcriptional repressor
- **ArsB**, the $As(OH)_3/H^+$ antiporter that extrudes As(III), conferring resistance
- **ArsC**, the arsenate reductase that converts As(V) to As(III), the substrate of ArsB, hence extending the range of resistance to include As(V).

Xu et al., *J. Biochem.* 123, 16-23 (1998); Mukhopadhyay *et al.*, FEMS Microbiology Rev., 26, 311-325 (2002)

E. coli ArsR protein

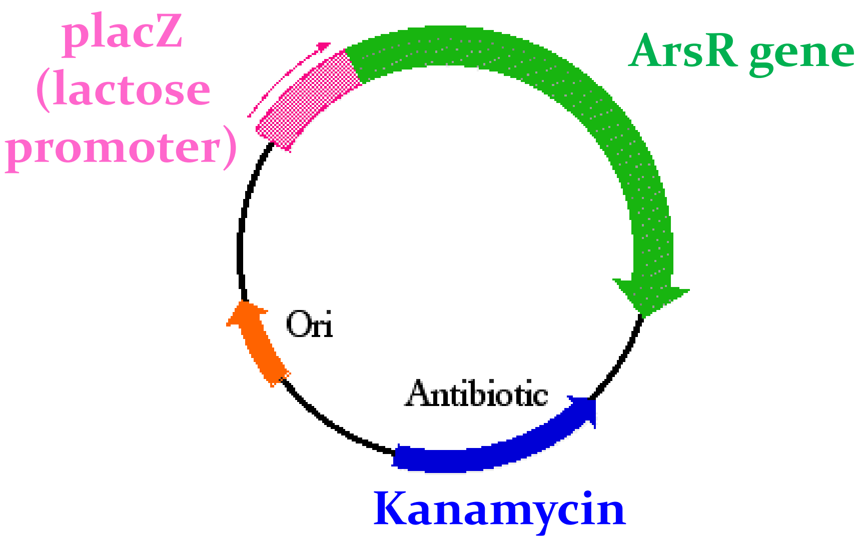


ArsR has a strikingly high affinity, 10^{-15} M As(III) could induce the ars promoter

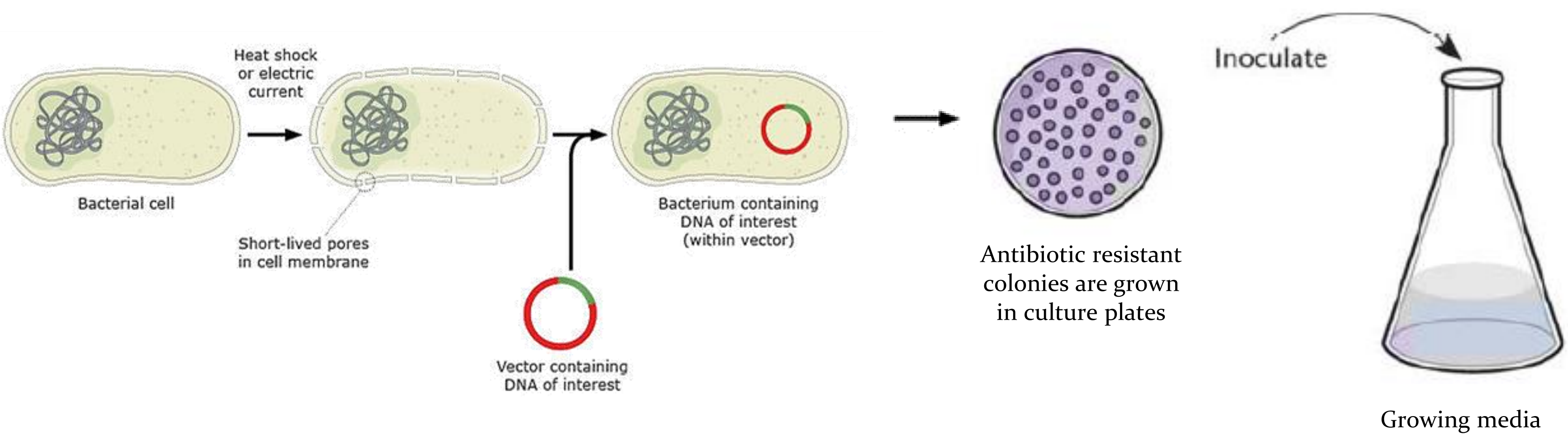
As(III) coordinates via three cysteine thiolate ligands

Busenlehner *et al*, FEMS Microbiology Rev., 27, 131 (2003)

ArsR-expressing plasmid

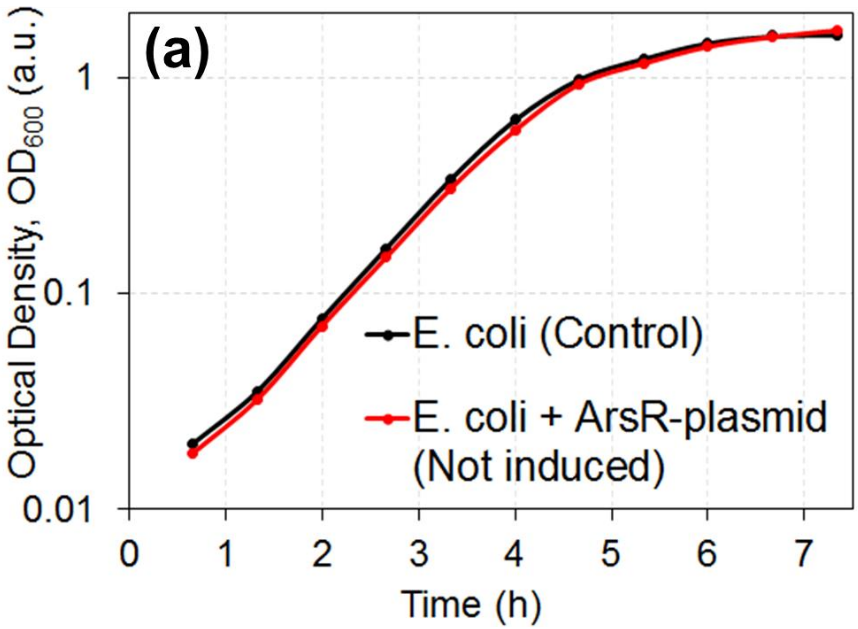


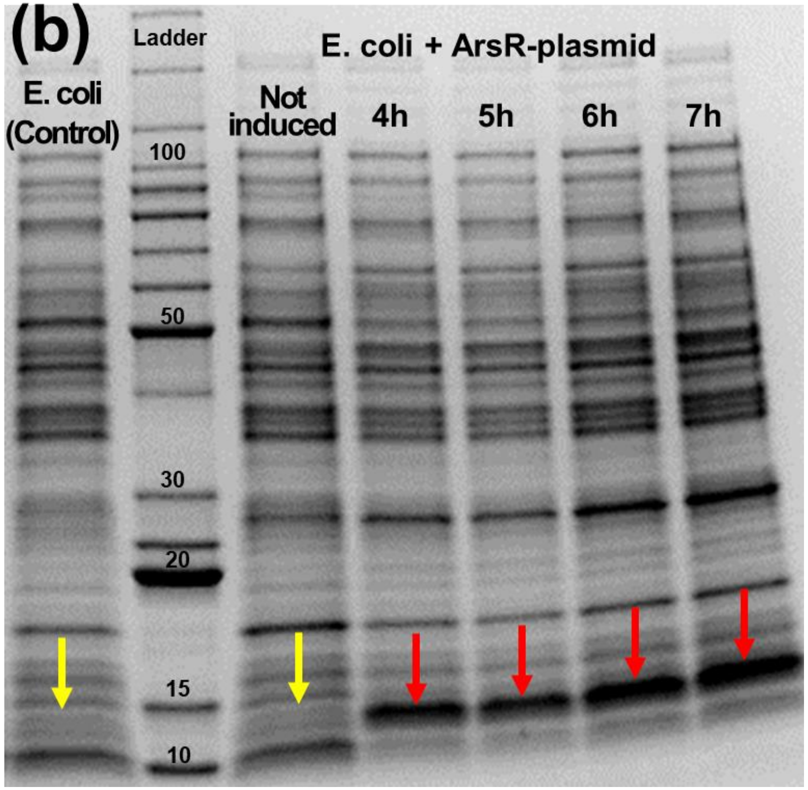
The *arsR* gene is cloned onto a medium-copy-number plasmid.



https://www.sciencelearn.org.nz/resources

Overexpression of ArsR protein in *E. coli*



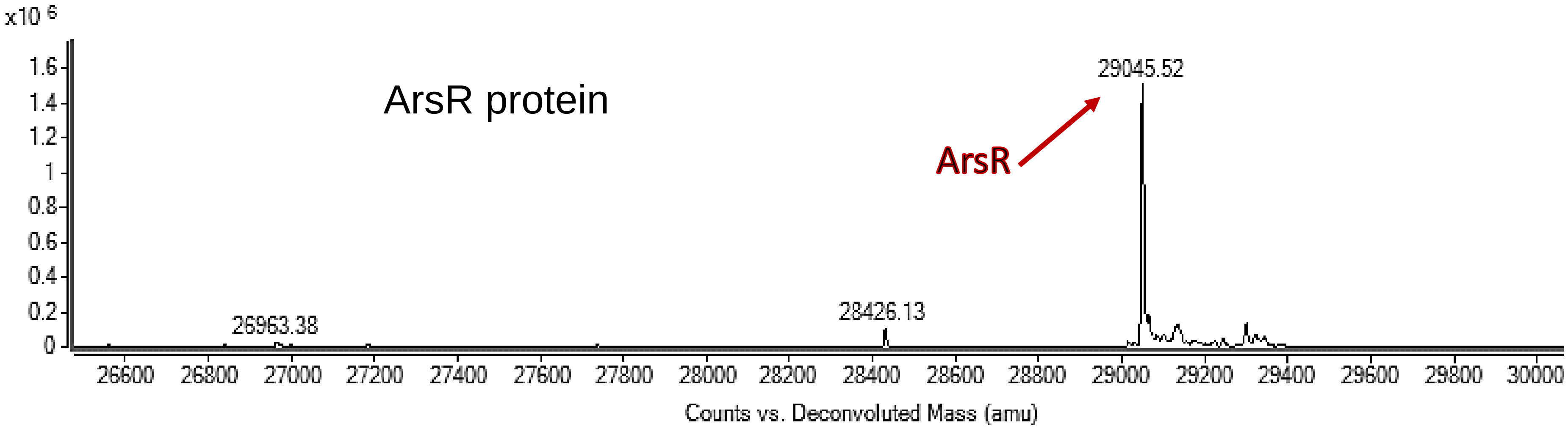


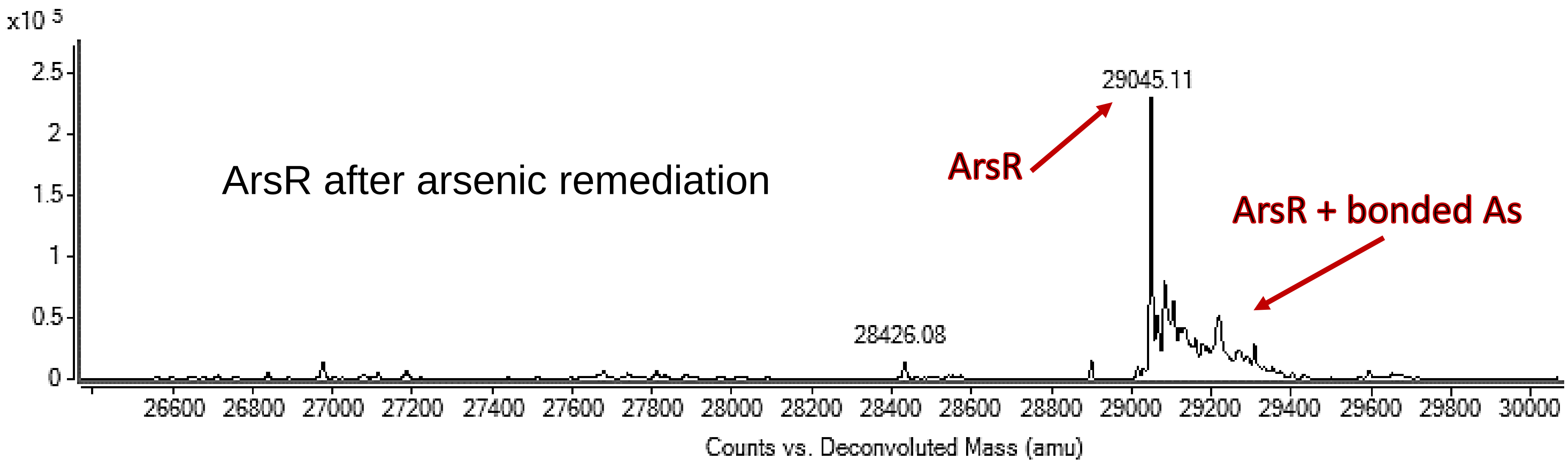
When repressed, ArsR overexpression plasmid does not affect *E. coli* growth rate

Optimization of induction time for ArsR overexpression

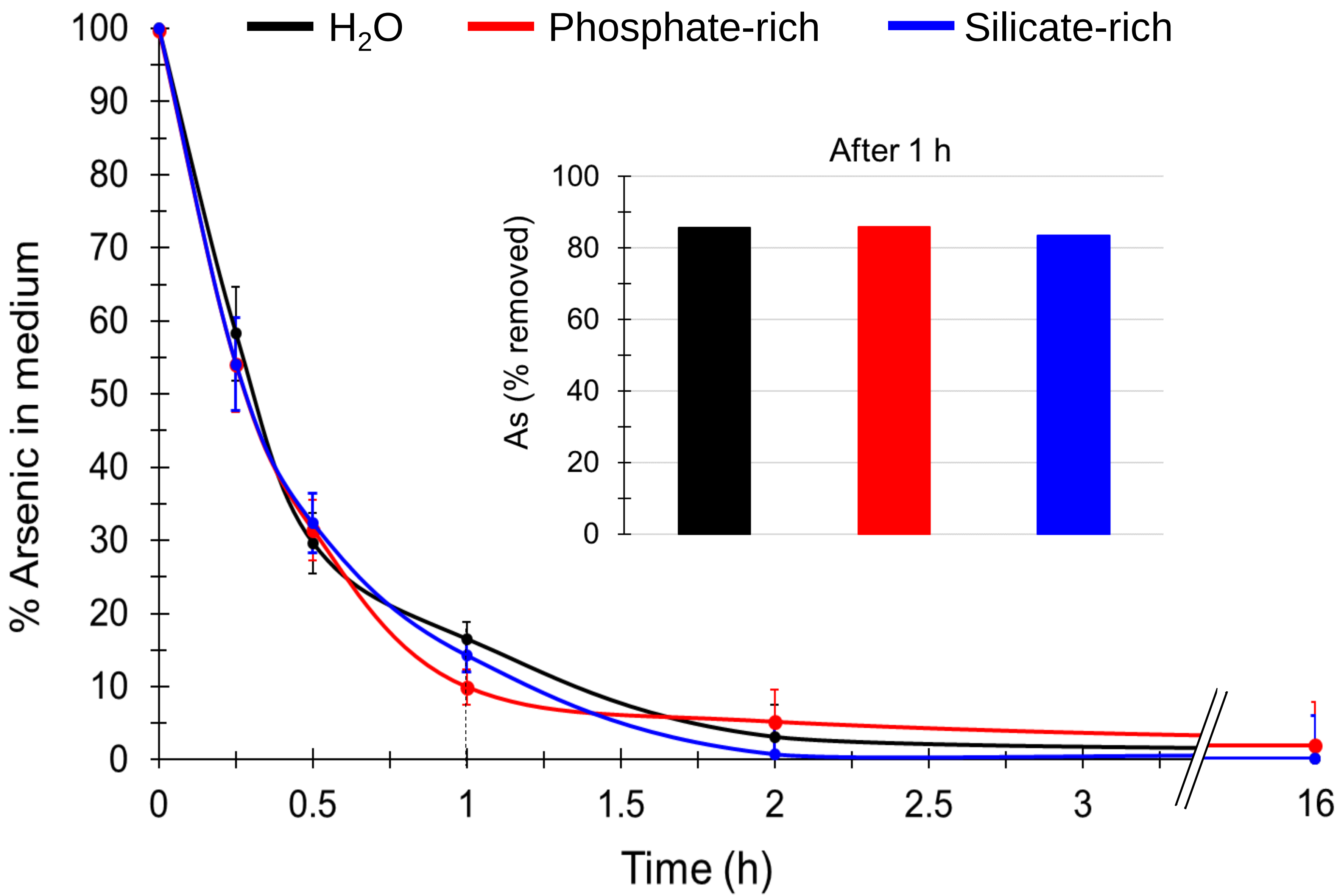
We are able to grow cells in only 7 h with high levels of expression

Preliminary LC-MS results: Protein extraction

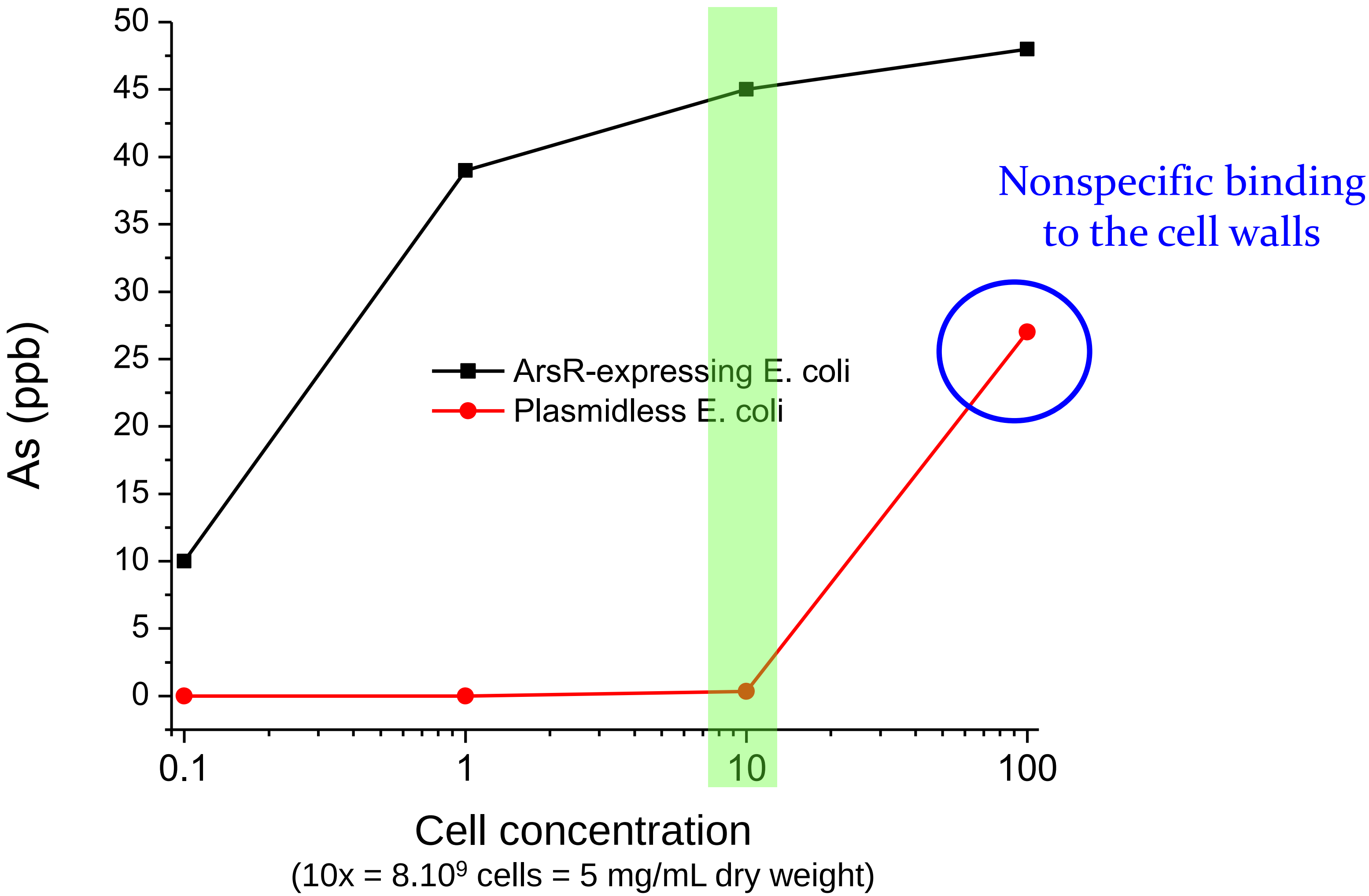


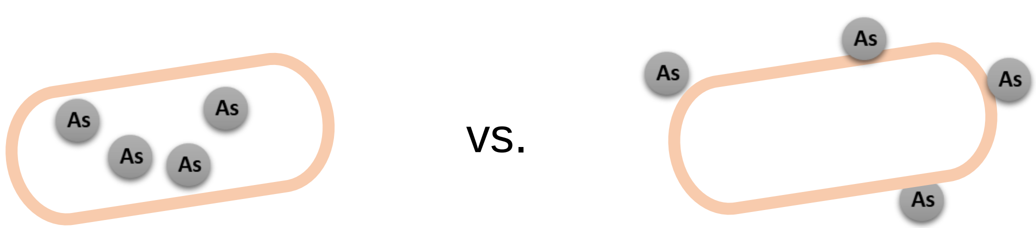


Arsenite accumulation by resting cells (50 ppb, pH 7)

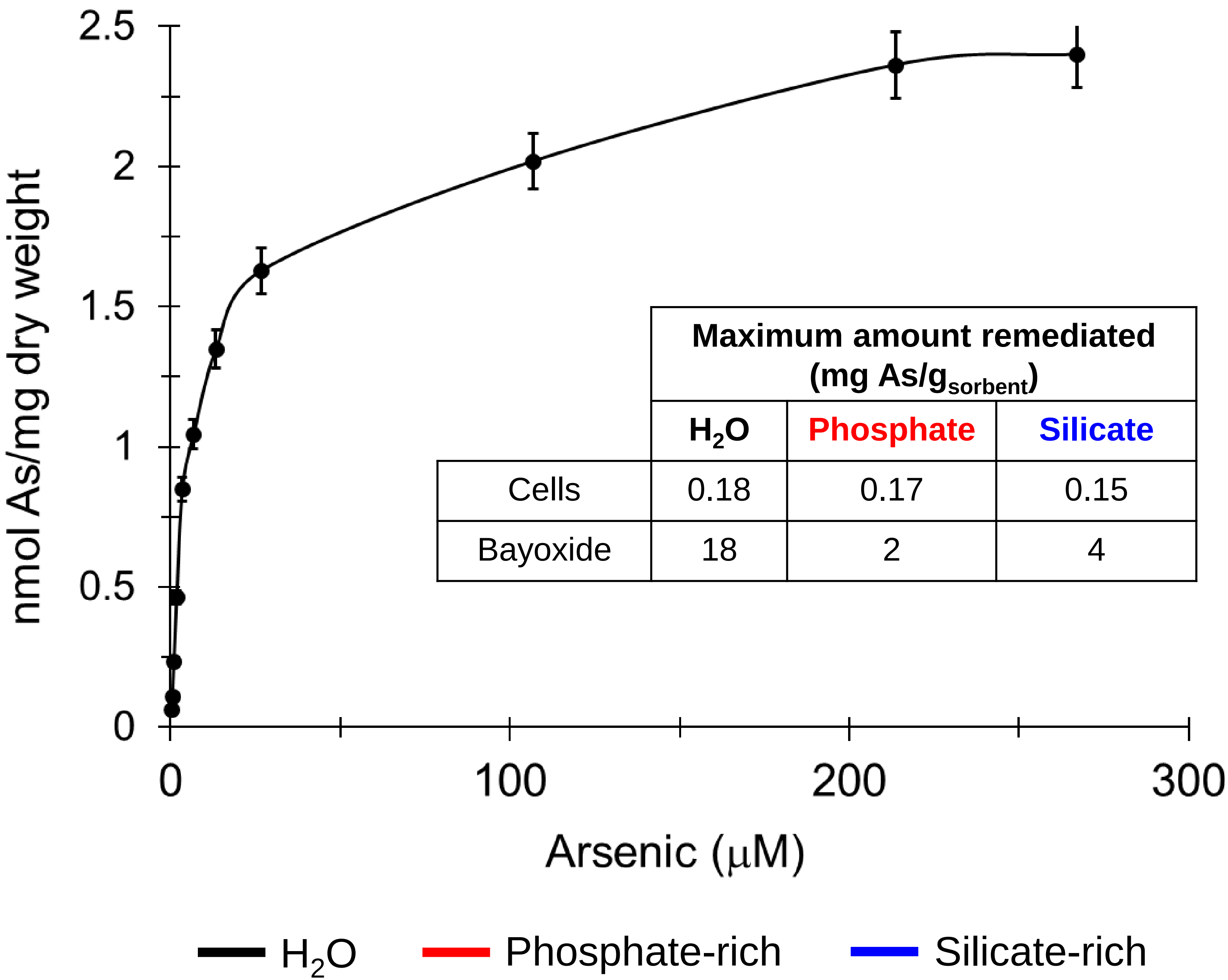


Uptake of As by different cell concentrations

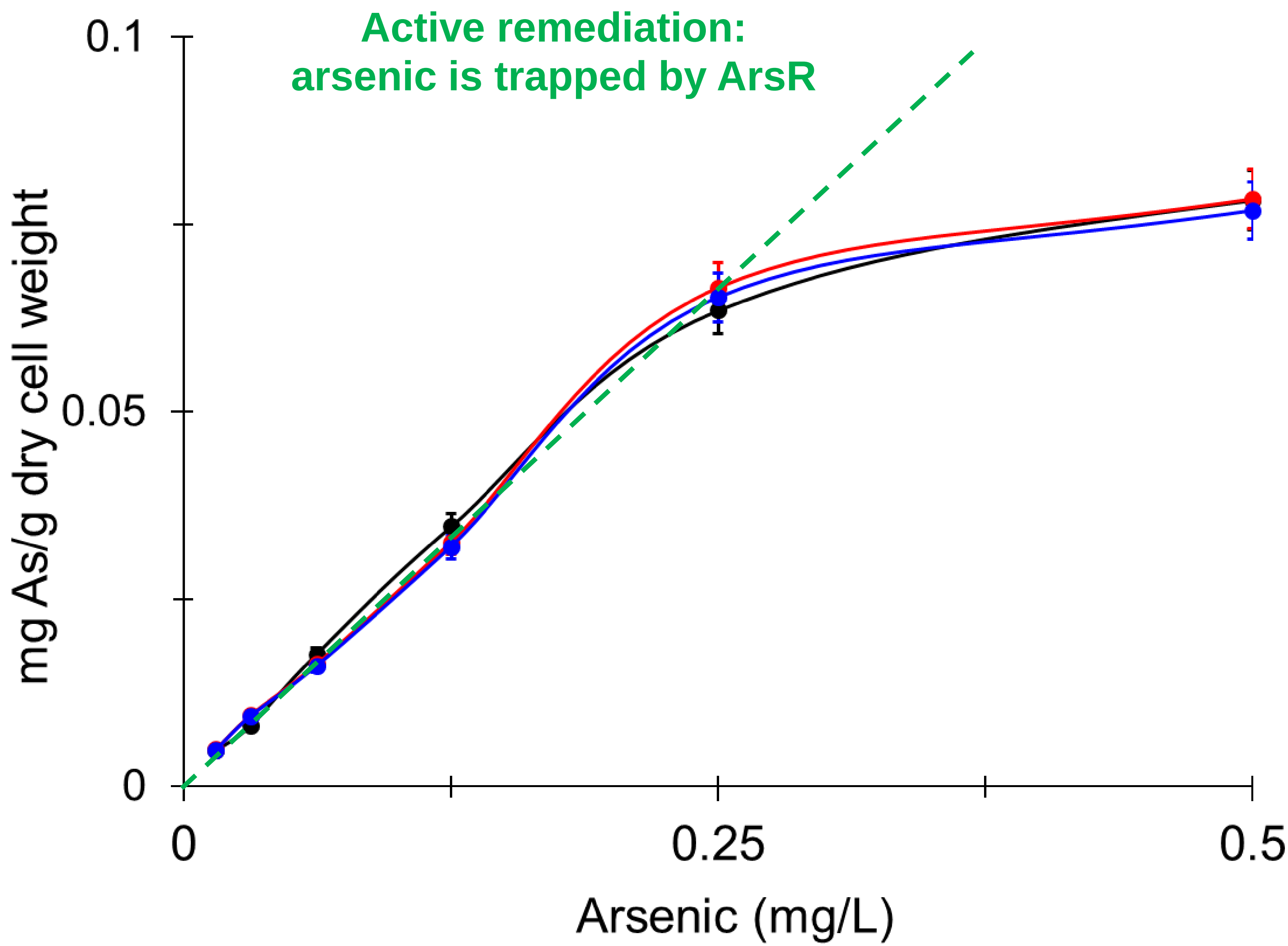




Isotherms: Accumulated arsenic (pH 7)



	Maximum amount remediated ($mg\ As/g_{sorbent}$)		
	H_2O	Phosphate	Silicate
Cells	0.18	0.17	0.15
Bayoxide	18	2	4


Active remediation:
arsenic is trapped by ArsR
0.1
0.05
0
mg As/g dry cell weight
0
0.25
0.5
Arsenic (mg/L)

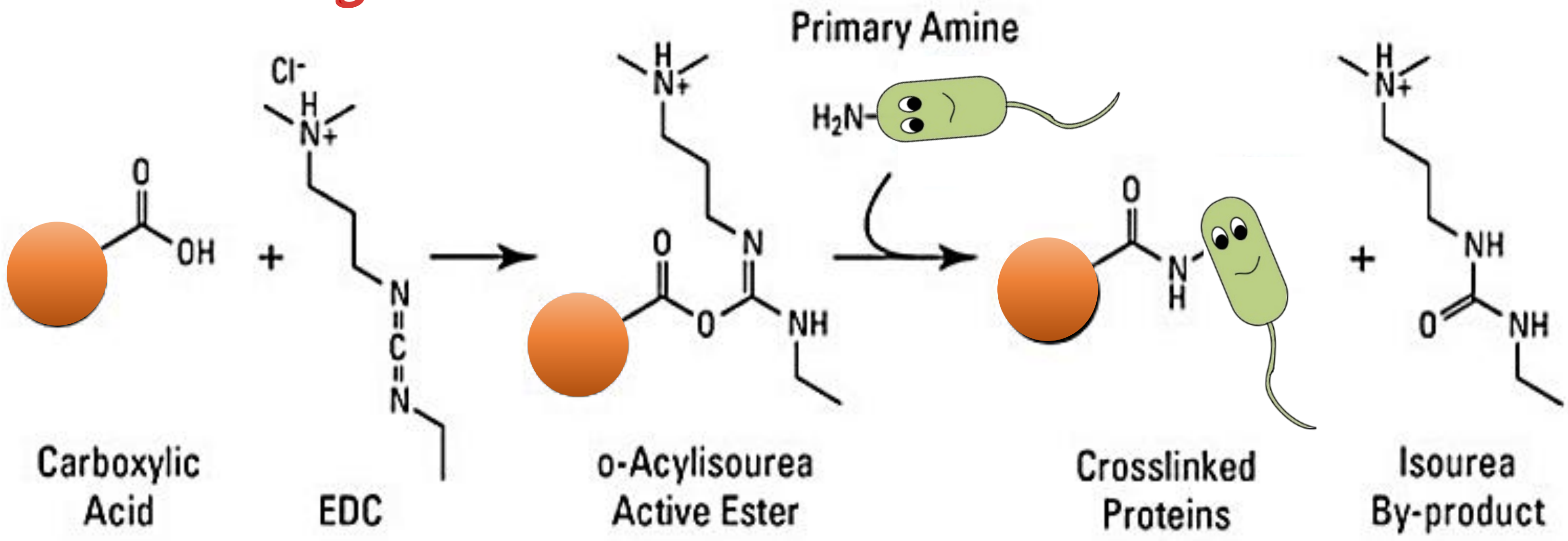
How do you get the sorbent out of the water?

CENTRIFUGATION

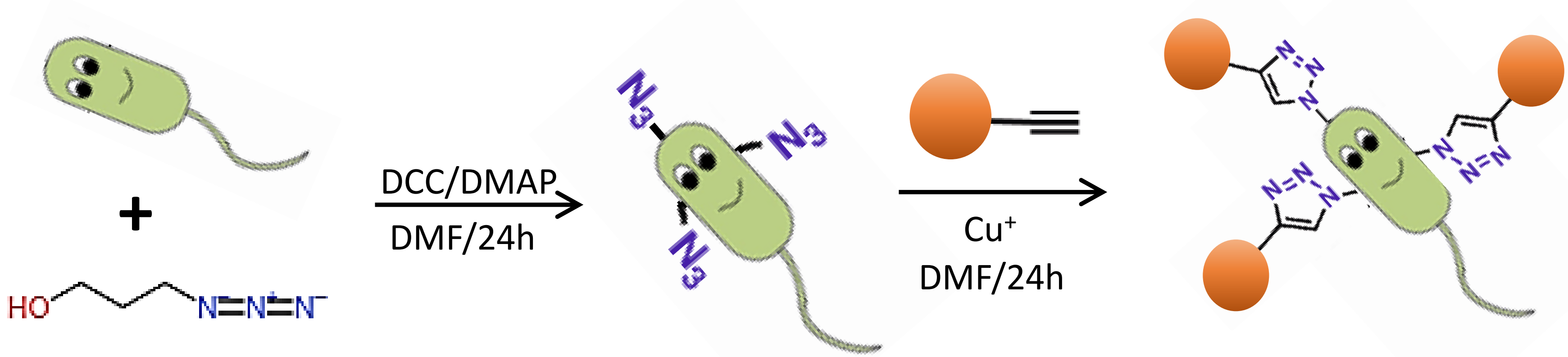
FILTRATION

E. coli removal – attachment of magnetic particles

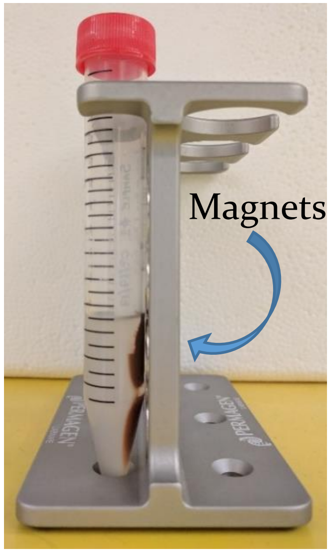
EDC crosslinking

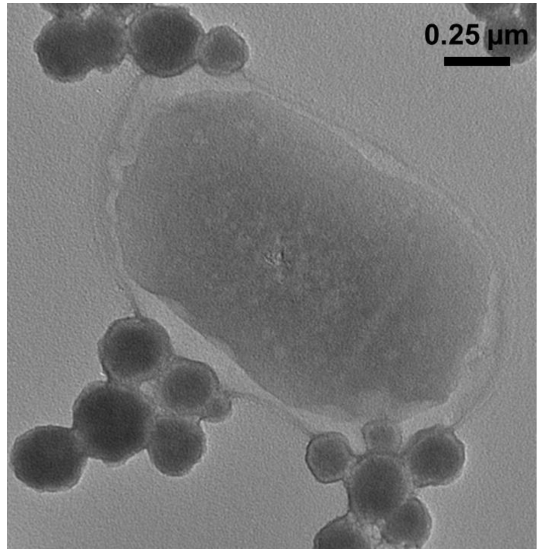


Azide click-chemistry

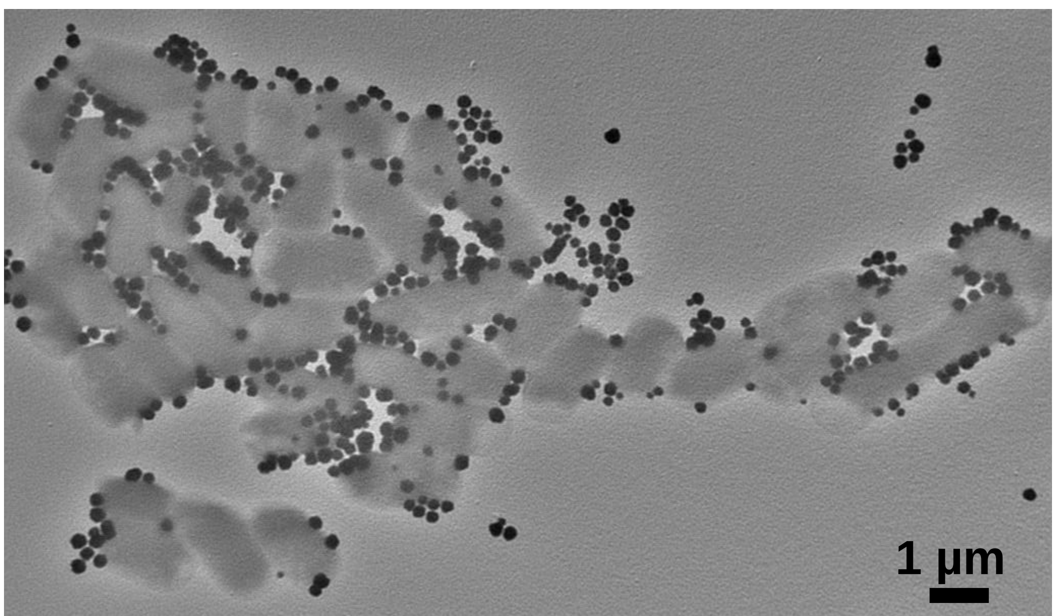


E. coli conjugation to magnetic beads





Carboxyl-functionalized magnetic beads were conjugated to *E. coli* after As remediation



Cells were successfully removed from different media

Remediation of complex media: The case of wine

CBS NEWS / *March 19, 2015, 7:39 AM*

"Very high levels of arsenic" in top-selling wines

A worker stacks cases of Charles Shaw wine at the Bronco Wine Company facility in Napa, California, Tuesday, April 17, 2007. / AP PHOTO/ERIC RISBERG

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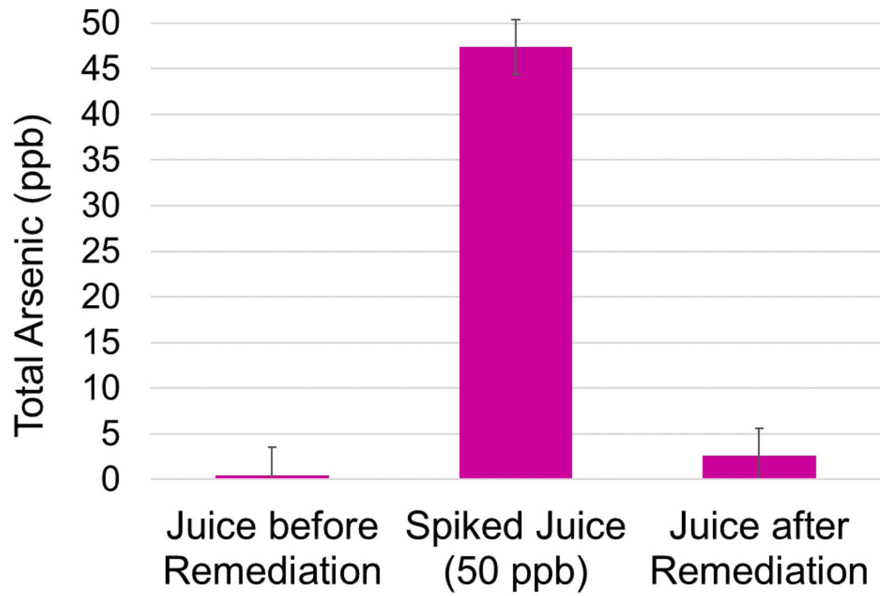
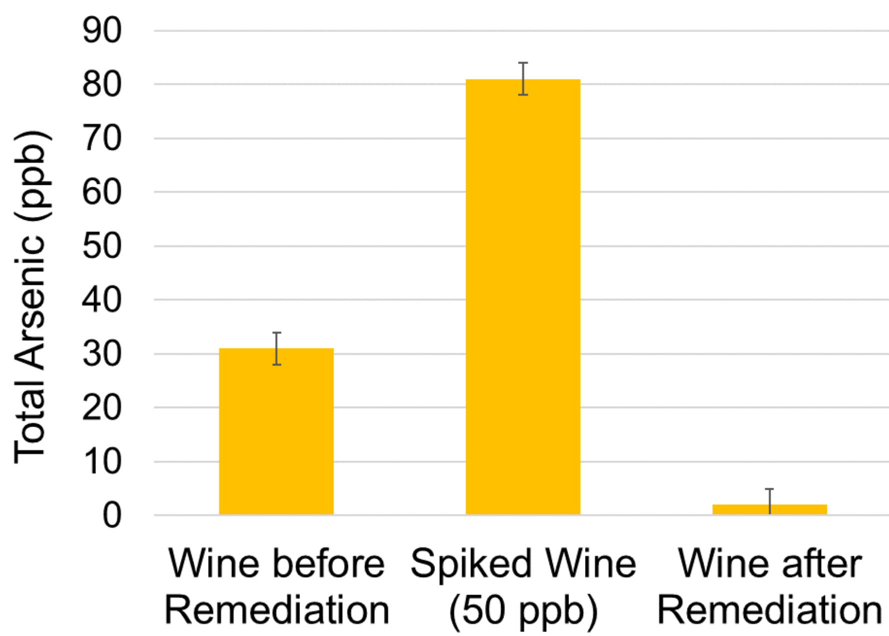
Last Updated Mar 21, 2015 2:54 PM EDT

Preliminary results: Remediation of white wine





Conditions

Cell concentration: 5 mg/mL

4 h remediation

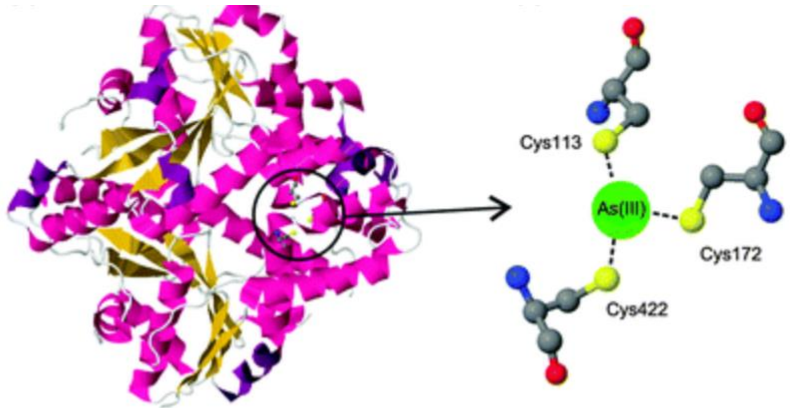
pH juice: 4 / pH wine: 3

Room Temperature

Agitation: 250 rpm

No detectible DNA afterwards

Biological Sorbents – Summary



- We can overexpress ArsR in healthy E.Coli
- Arsenic removal by the cells is highly selective
- These living sorbents can operate in low pH environments

Biological Sorbents – Future Work

Transfecting proteins into more acceptable organisms (e.g. yeast)

Protein extraction/purification for nanoparticle modification

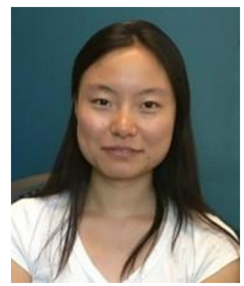
Expanding to sulfate and heavy metal removal

Therapeutic and analytical applications of arsenic binding to proteins Beibei Chen [a], Qingqing Liu [a], Aleksandra Popowich [b], Shengwen Shen [a], Xiaowen Yan [a], Qi Zhang [a], Xing-Fang Li [a], Michael Weinfeld [c], William R. Cullen [d] and X. Chris Le *[ab] Metallomics , 2015, **7**, 39-55

Colvin Research Group

- Postdocs
  - Adriana Mendoza-Garcia
  - Hyewon Kim
  - Qingbo Zhang
- Grad students
  - Yue Hu
  - Daniel Garcia
  - Caitlin Masterson
- Undergrad students
  - Melissa Nakamoto
  - Zahra Ahmed
  - Edward Esposito

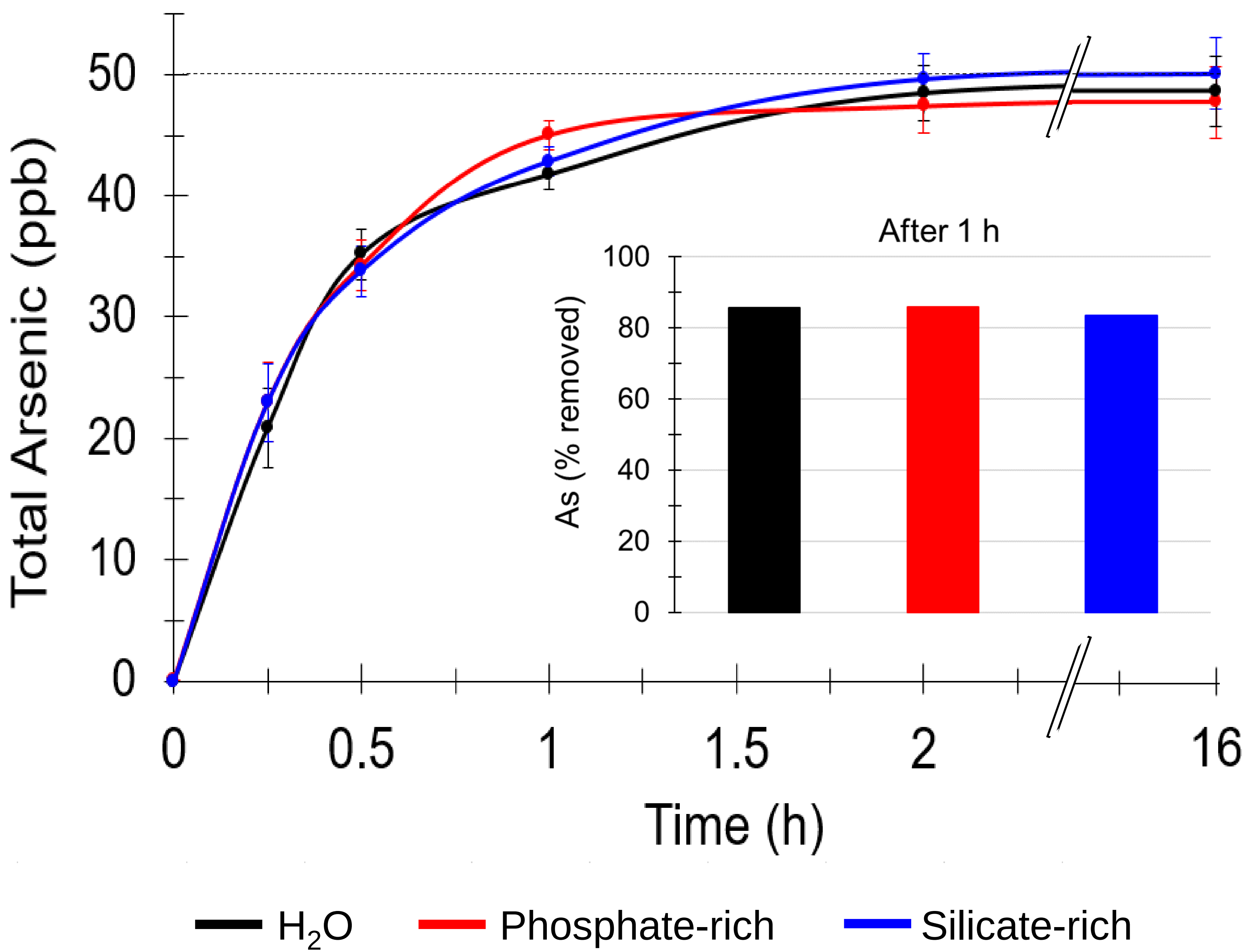


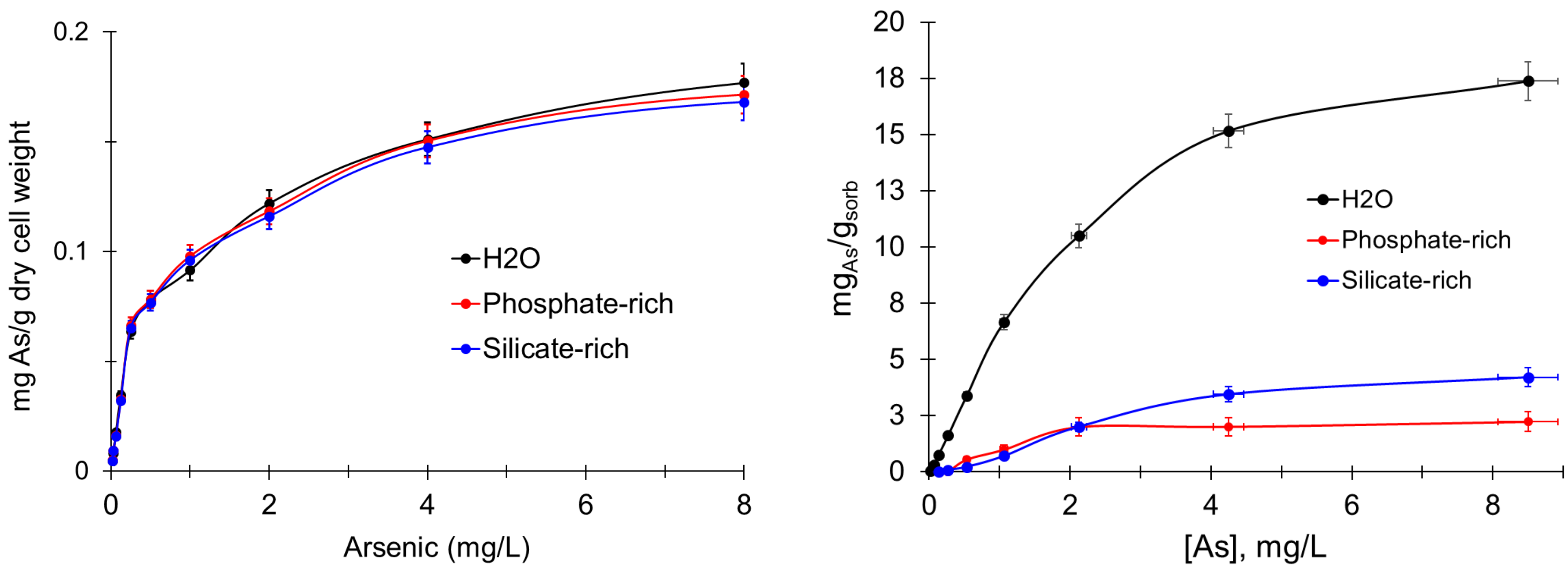



Current members of the Colvin Lab

Arsenite accumulation by resting cells (pH 7)



Bayoxide vs. *E. coli*



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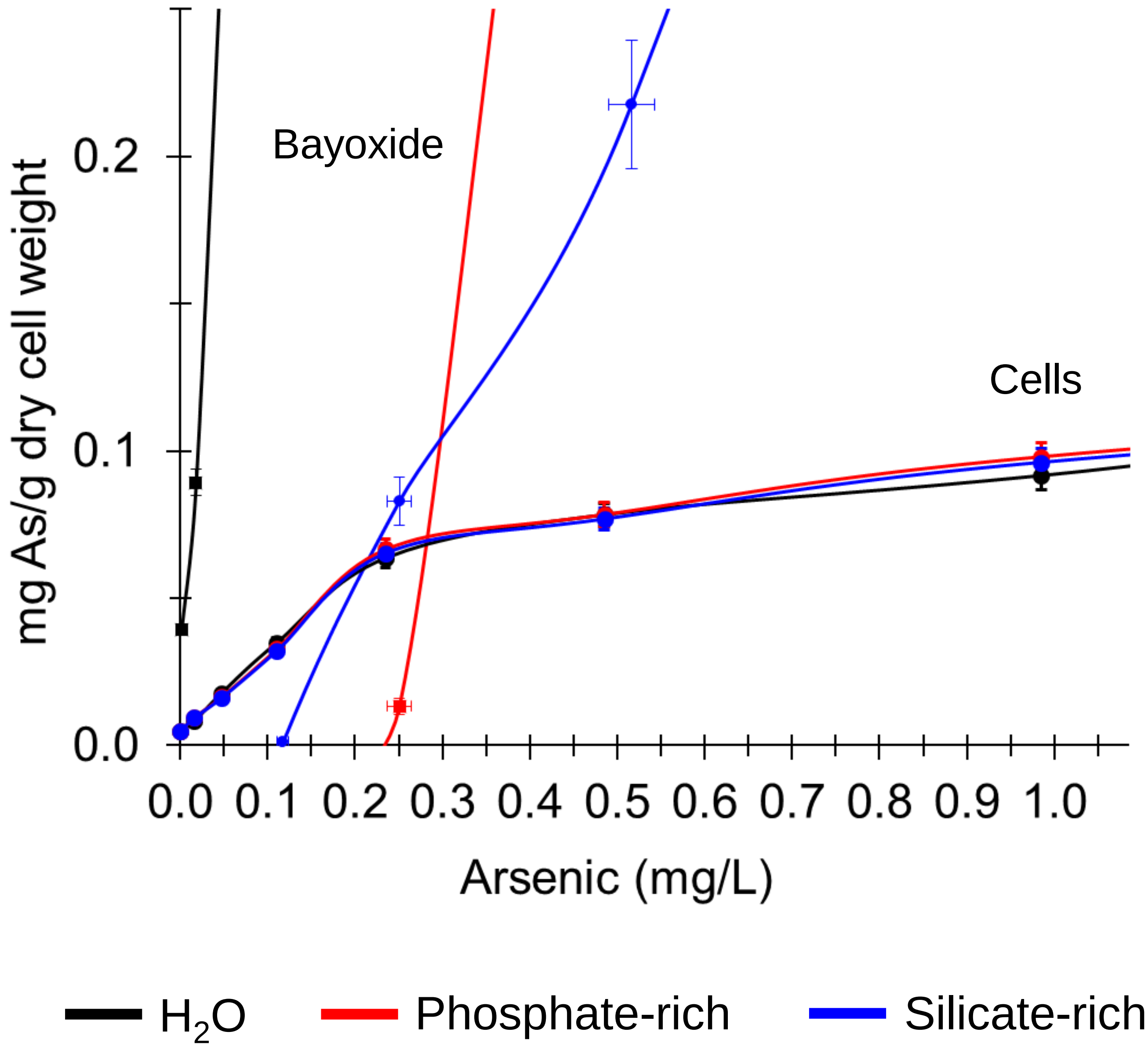
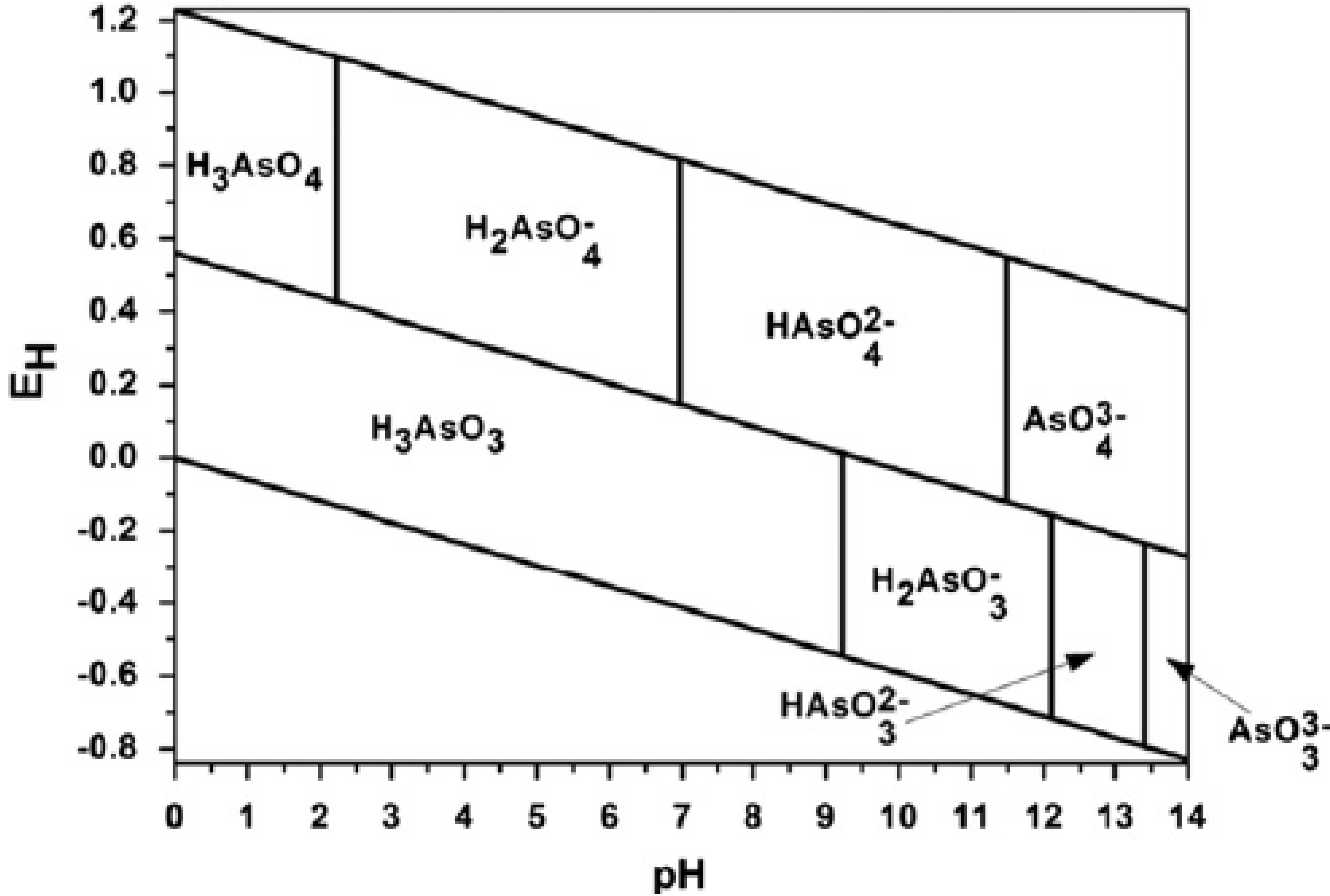
Remediation slope: Comparison



Arsenite/Arsenate forms according to pH



M9 growth media

- M9 salts: 33.9g/L Na_2HPO_4 15g/L KH_2PO_4 5g/L NH_4Cl 2.5g/L NaCl
- Casaminoacids (mixture of amino acids and some very small peptides obtained from acid hydrolysis of casein)
- Glucose
- Thiamine
- $MgSO_4$
- $CaCl_2$

LB broth

- Tryptone
- Yeast extract
- NaCl
- It can be complemented with a source of carbon (glucose or glycerol)

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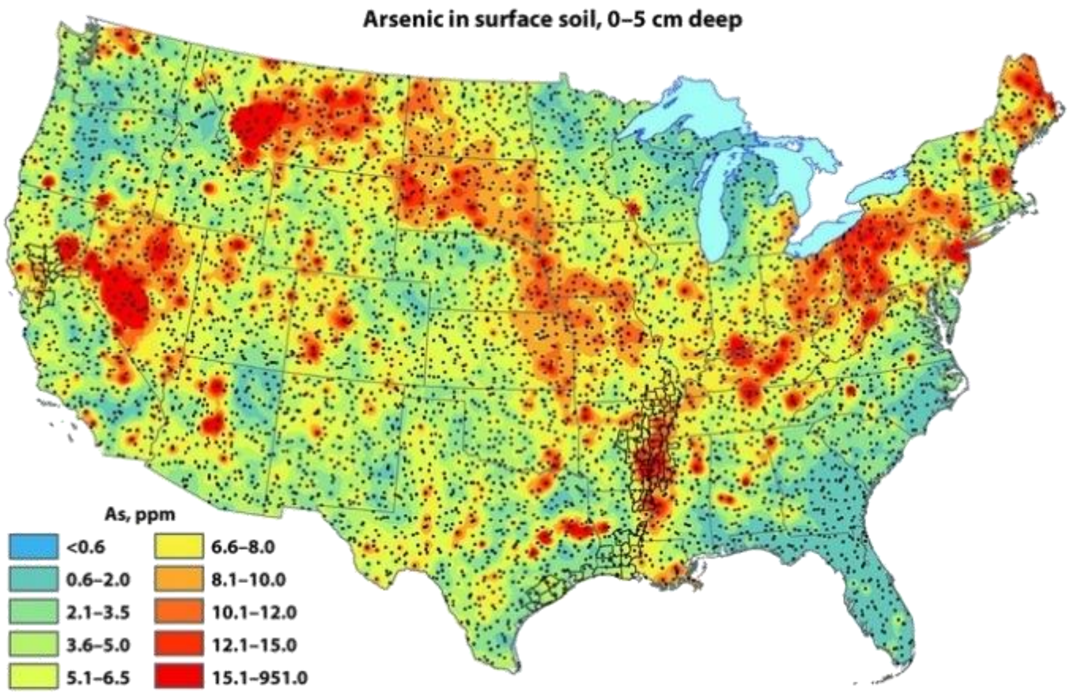
Arsenic Removal Leverages Arsenic-Iron Oxide Chemistry



Credit: U.S. Geological Survey

Arsenic IS a problem

Arsenic in water linked to cancer

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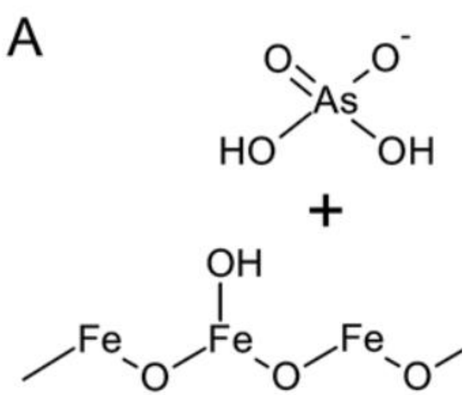

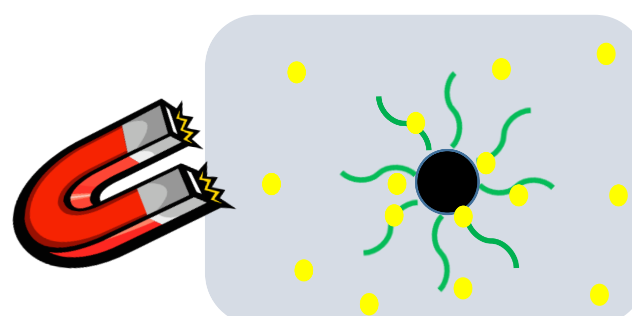

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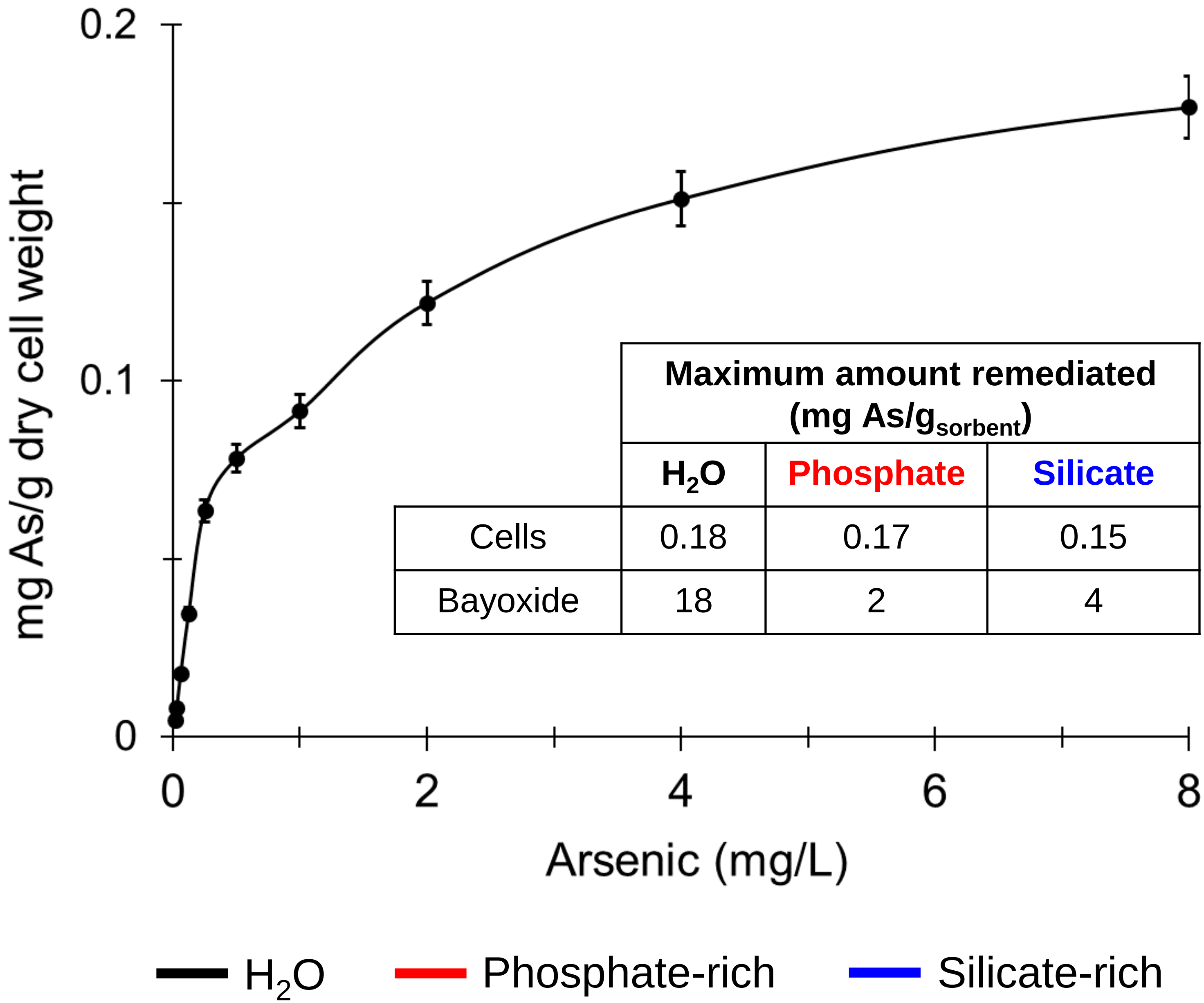

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