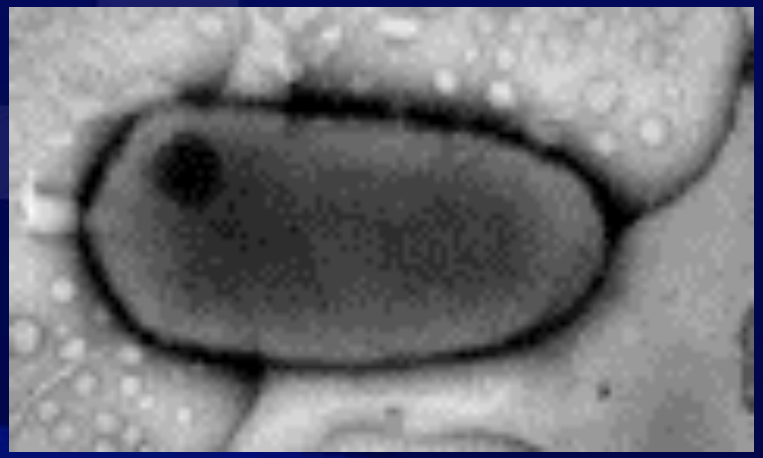
Effects of Fermentative Activity on Fate and Transport of U and Cr

Brent Peyton

Brady Lee, Robin Gerlach, Bill Apel

ERSP PI Meeting 2008



Center for Biofilm Engineering

MONTANA STATE UNIVERSITY

Mountains & Minds

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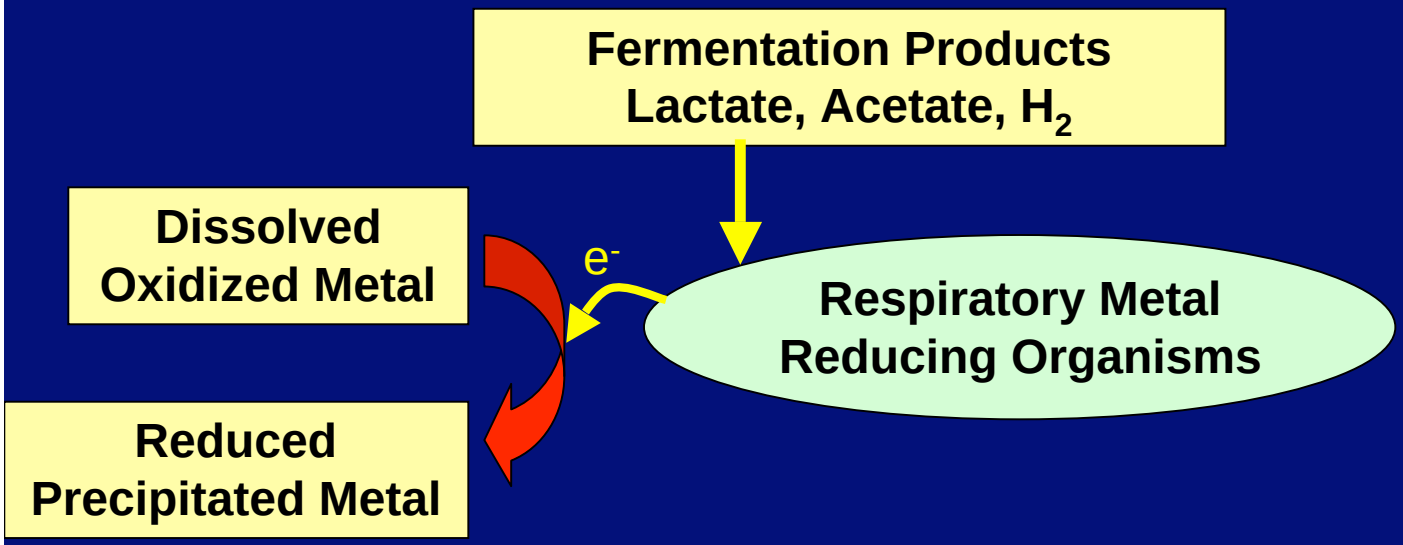
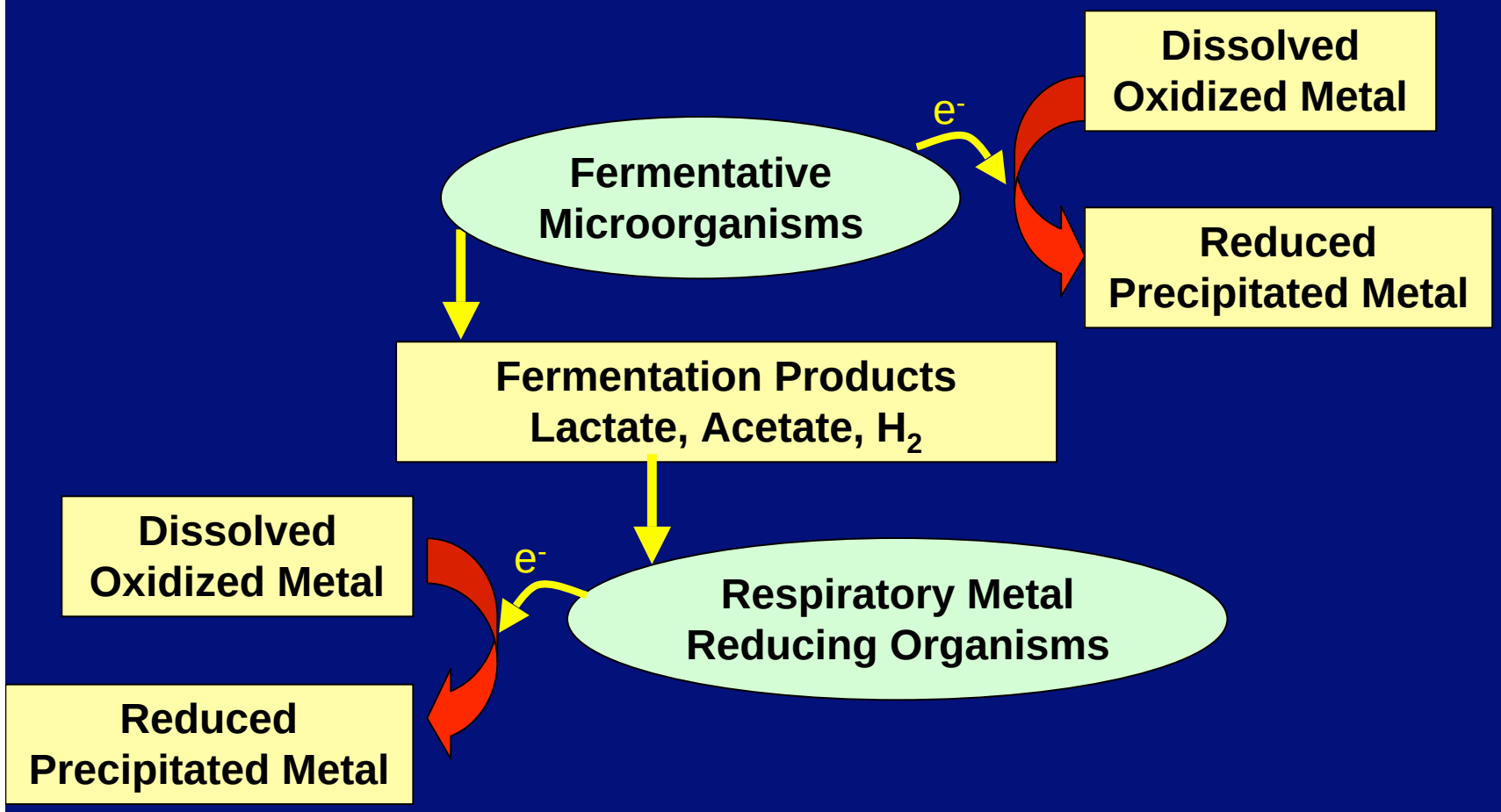
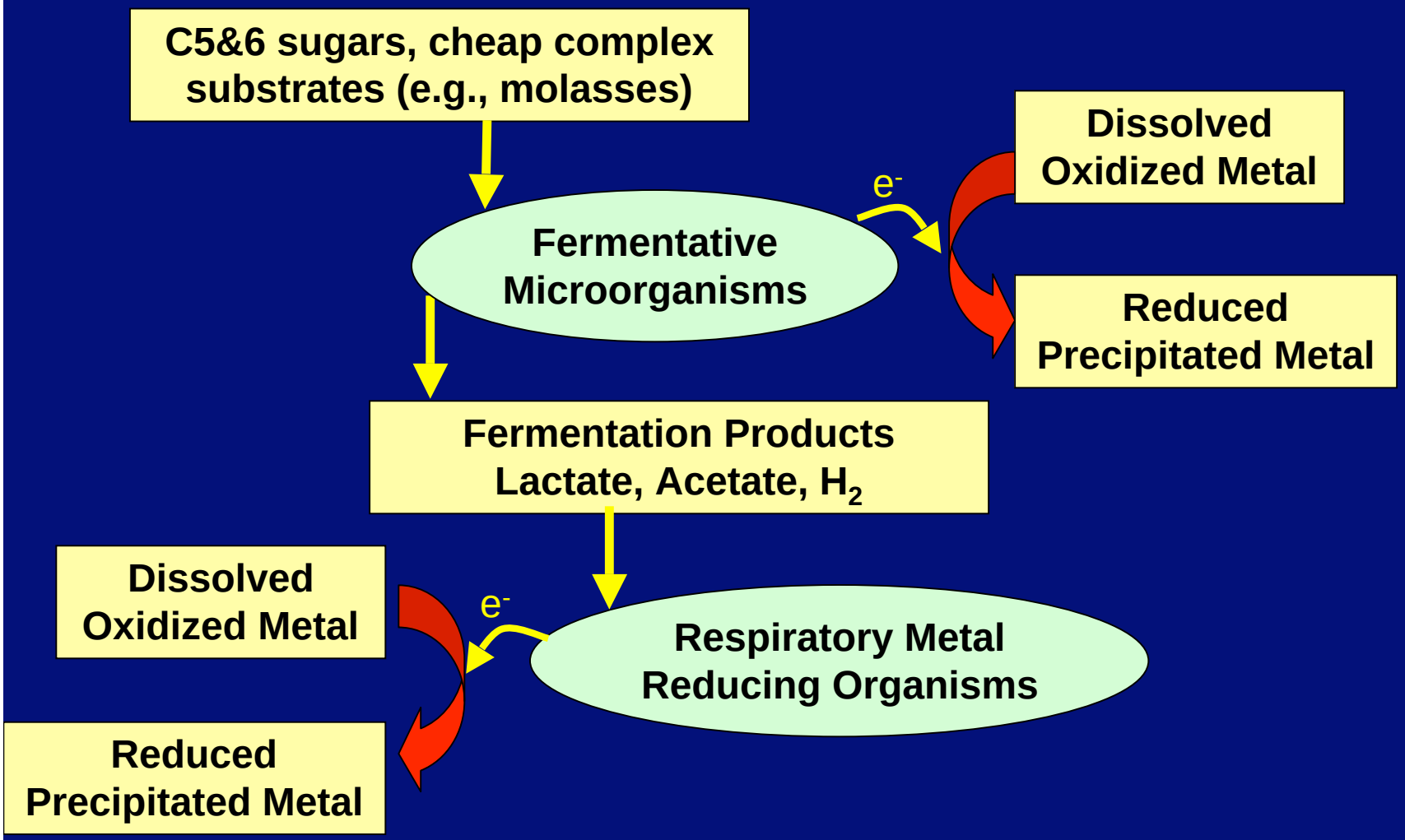
Microbial Metal Transformations



Microbial Metal Transformations

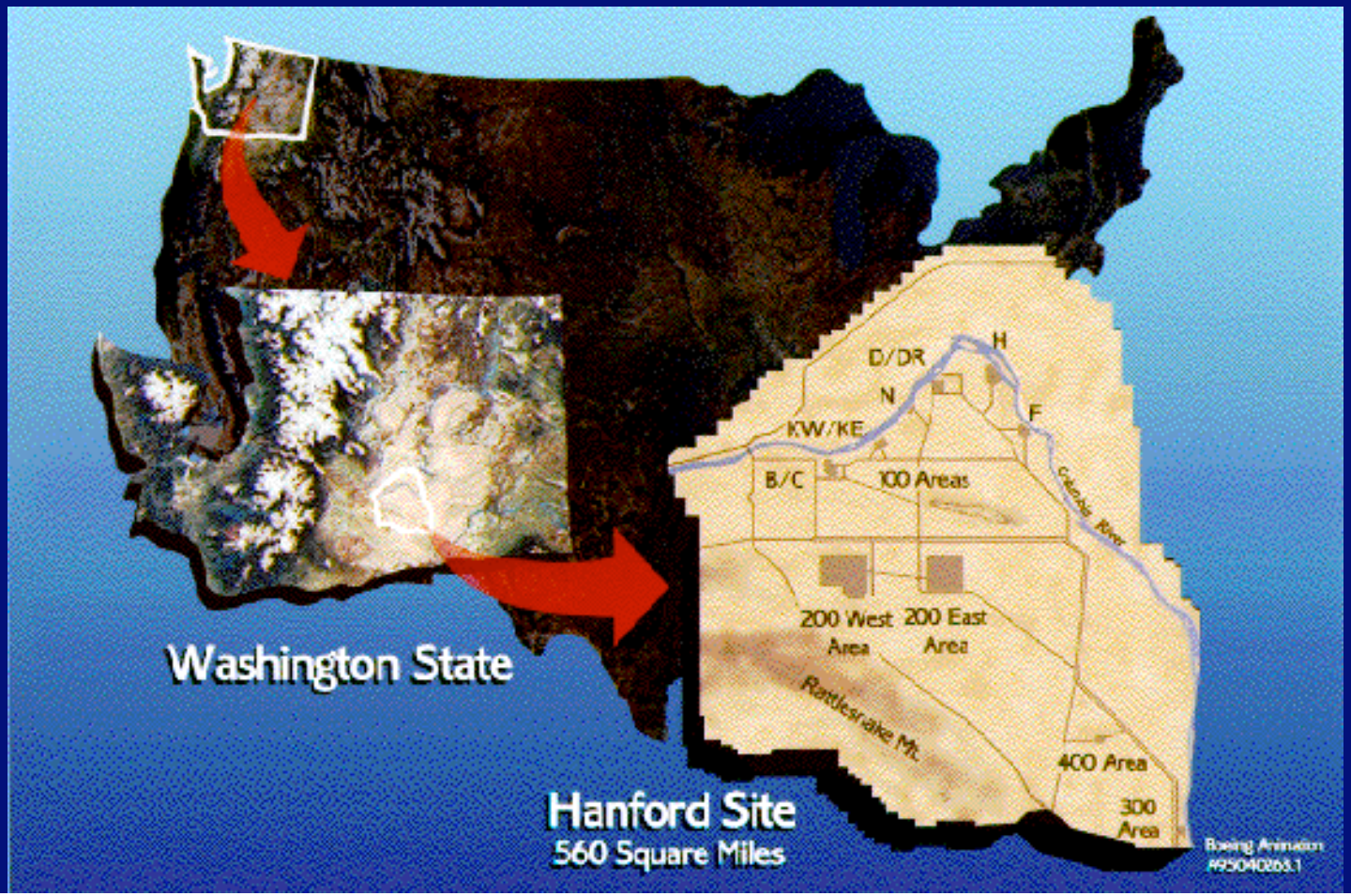


Microbial Metal Transformations



Background

- Cr(VI) impacted core from 100-D area borehole (DOE Hanford facility in southeastern Washington State), designated groundwater monitoring well 199-D2-8



Background

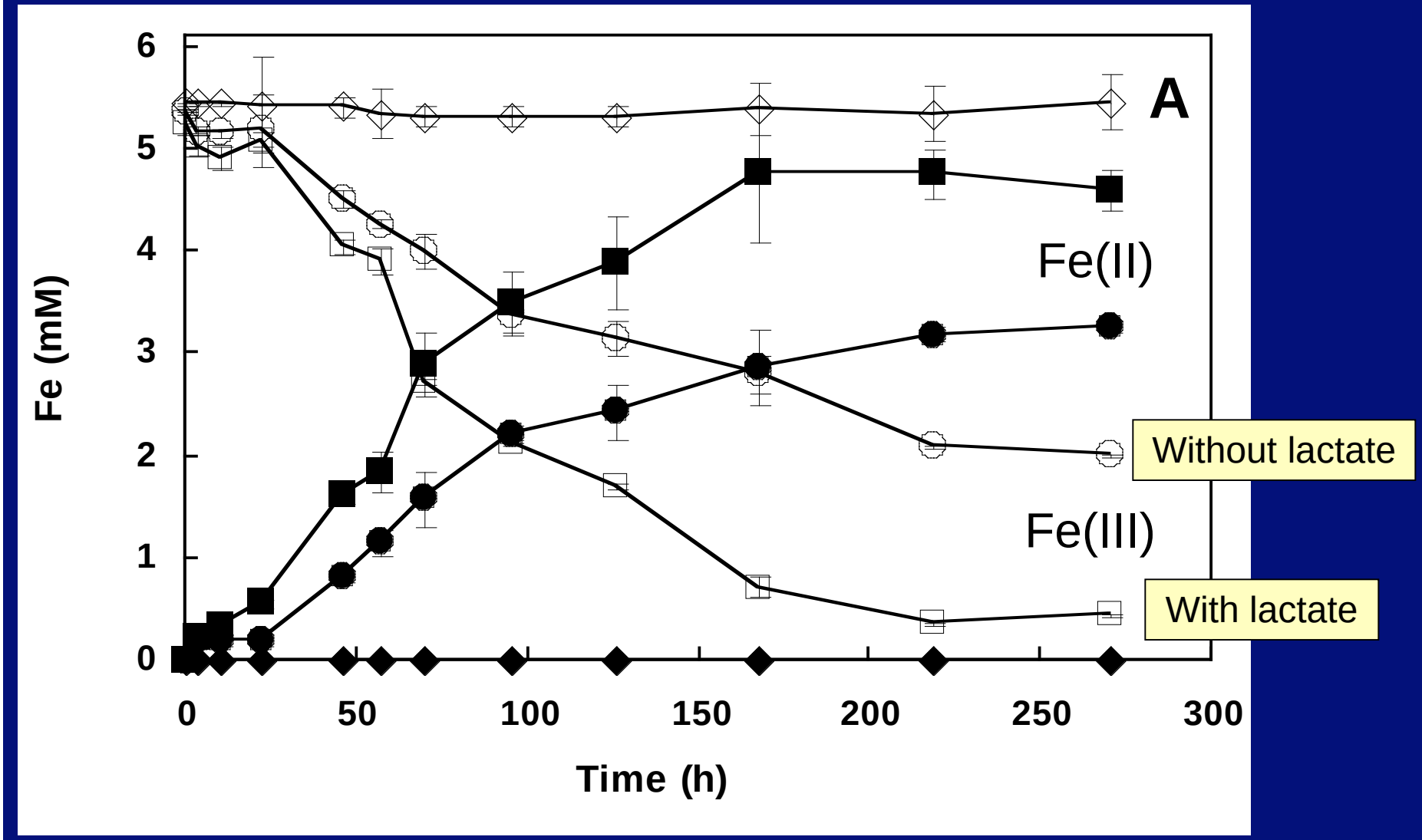
- Cr(VI) impacted core from 100-D area borehole (DOE Hanford facility in southeastern Washington State), designated groundwater monitoring well 199-D2-8
- Sedimentary flood deposits described as loosely consolidated fine-grained sand and silt (Lindsey and Jaeger, 1993)
- The water table was 24.8m bgs. Sediment samples taken from Ringold Formation at 23.3m and 25.9m bgs.
- Enrichments grown in the presence of Cr(VI) and nitrate - with acetate, D-xylose or glycerol as a carbon and energy source.

Rep. Strain1	RDP II2		Isolates found			
	Closest match	Similarity index3	26m-X	23m-X	23m-G	ES
WS01	*Cellulomonas turbata*	0.856	—	—	—	—
WS12	*Cellulomonas* sp. str. 1533	0.954	—	—	—	—
WS18	*Cellulomonas turbata*	0.811	—	—	—	—
ES6	*Cellulomonas hominis*	0.933	—	—	—	—
WS06	*Sanguibacter inulinus*	0.996	—	—	—	—
WS08	1397 clone SB-22	0.972	—	—	—	—
WS10	*Pseudomonas brassicacearum*	0.953	—	—	—	—
WS19	Marine snow bacterium	0.712	—	—	—	—
WS13	*Arthrobacter* sp. str. S2	0.771	—	—	—	—

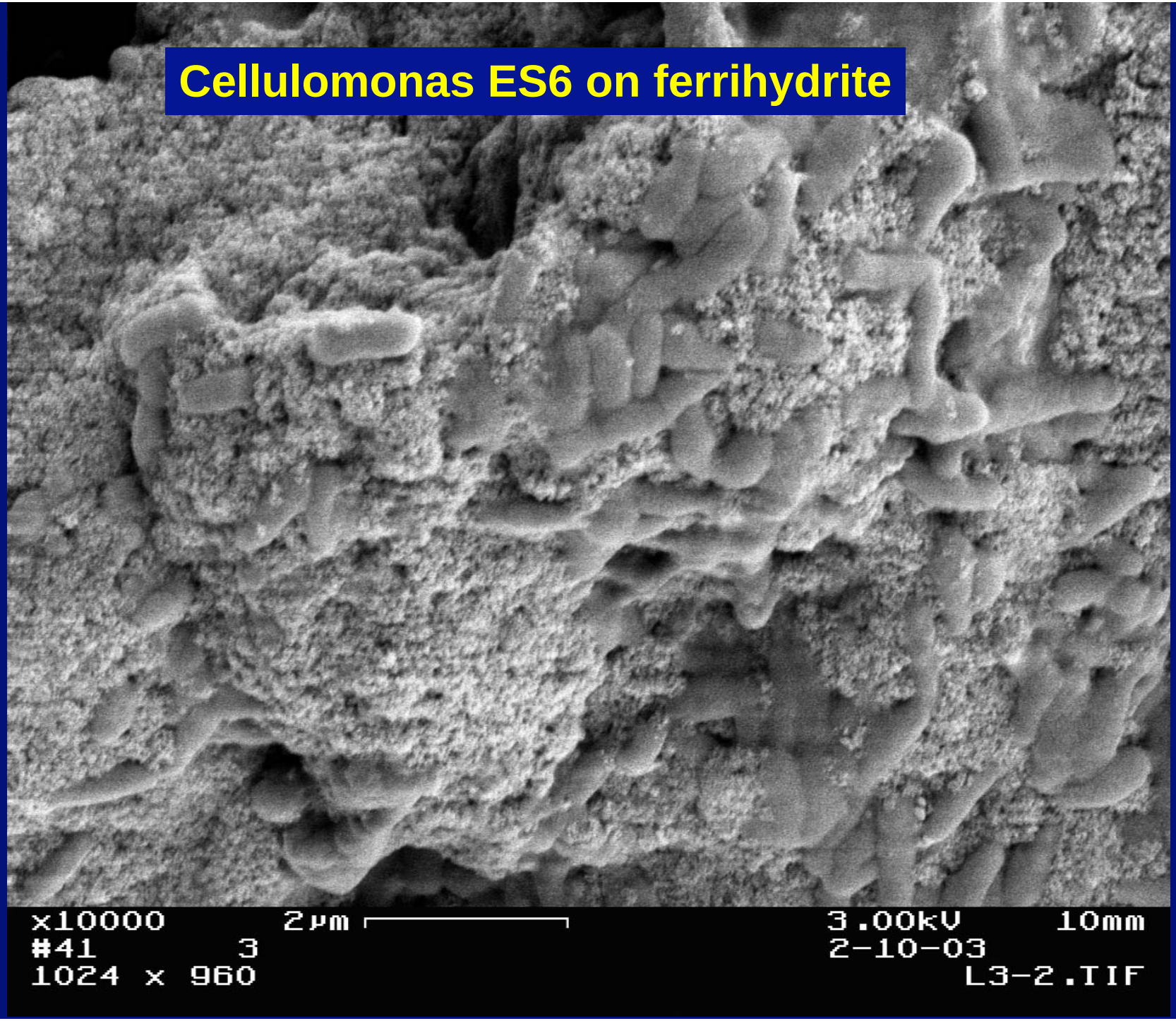
- 8 of 9 isolated strains were Gram positive
- 4 were identified by 16S rRNA sequence and membrane fatty acid composition as belonging to the genus *Cellulomonas*

Smith, W. A.; Apel, W. A.; Petersen, J. N.; Peyton, B. M. Effect of carbon and energy source on bacterial chromate reduction. *Bioremediation Journal* **2002,** *6* (3), 205-215

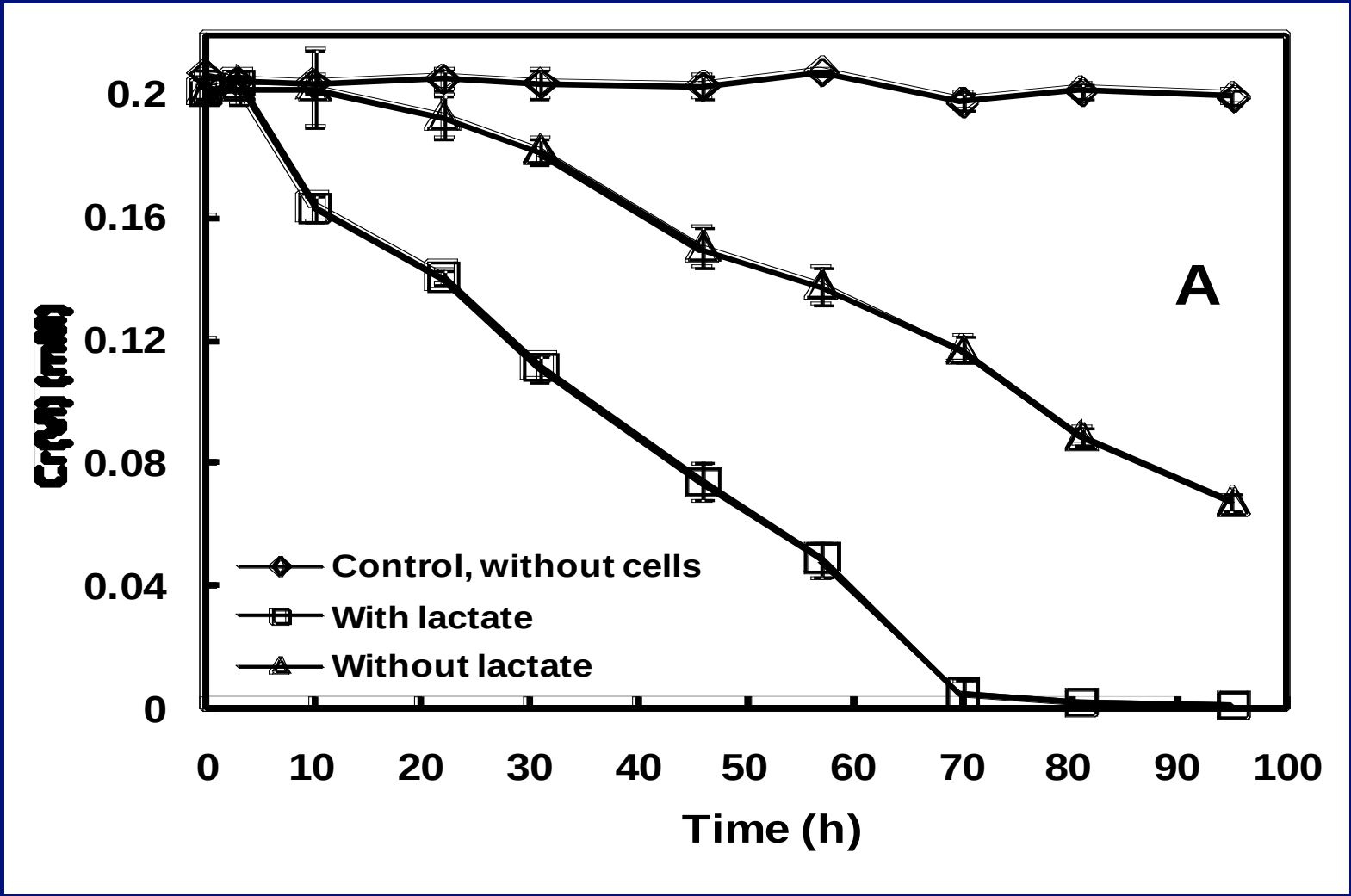
Fe(III) Reduction



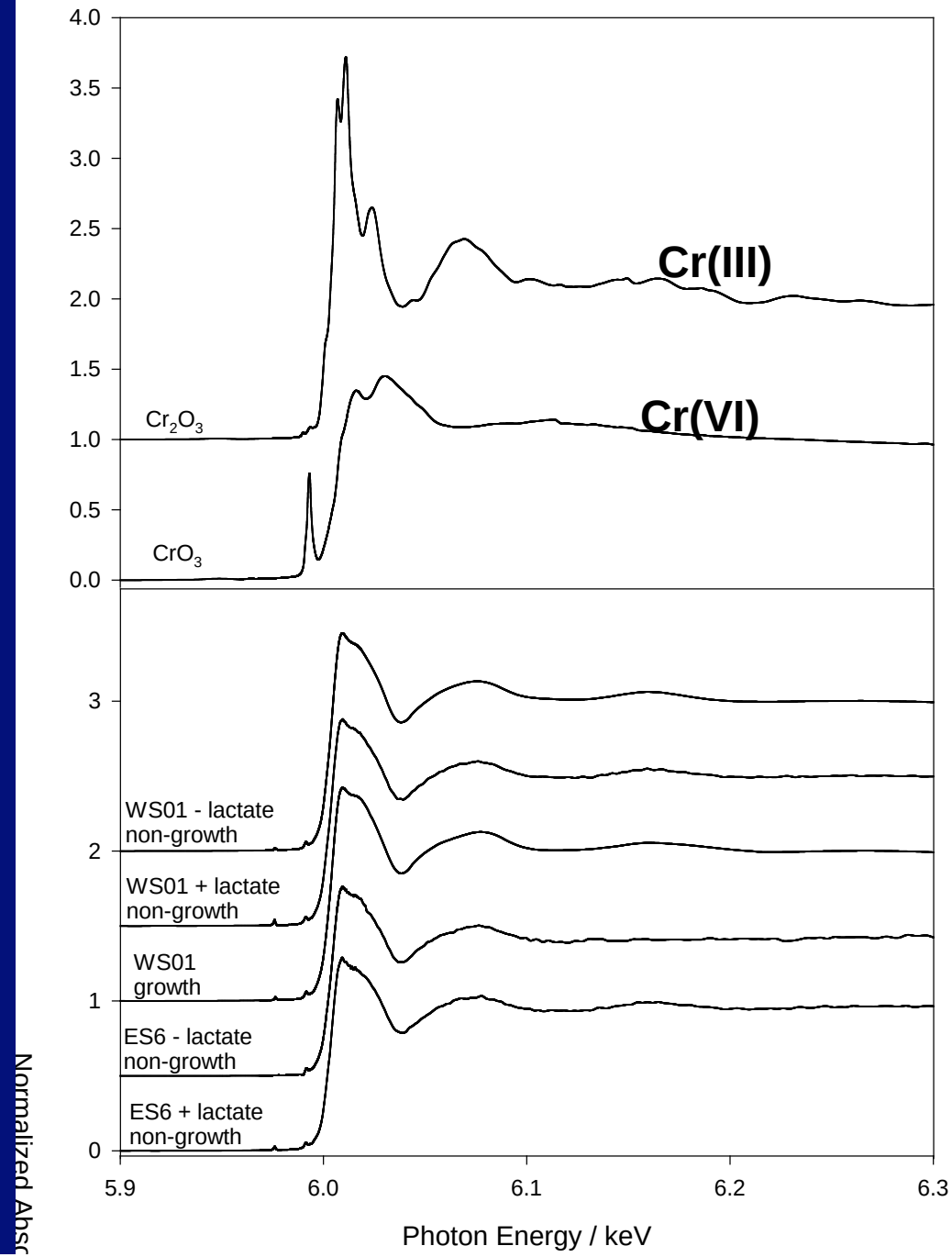
Cellulomonas ES6 on ferrihydrite



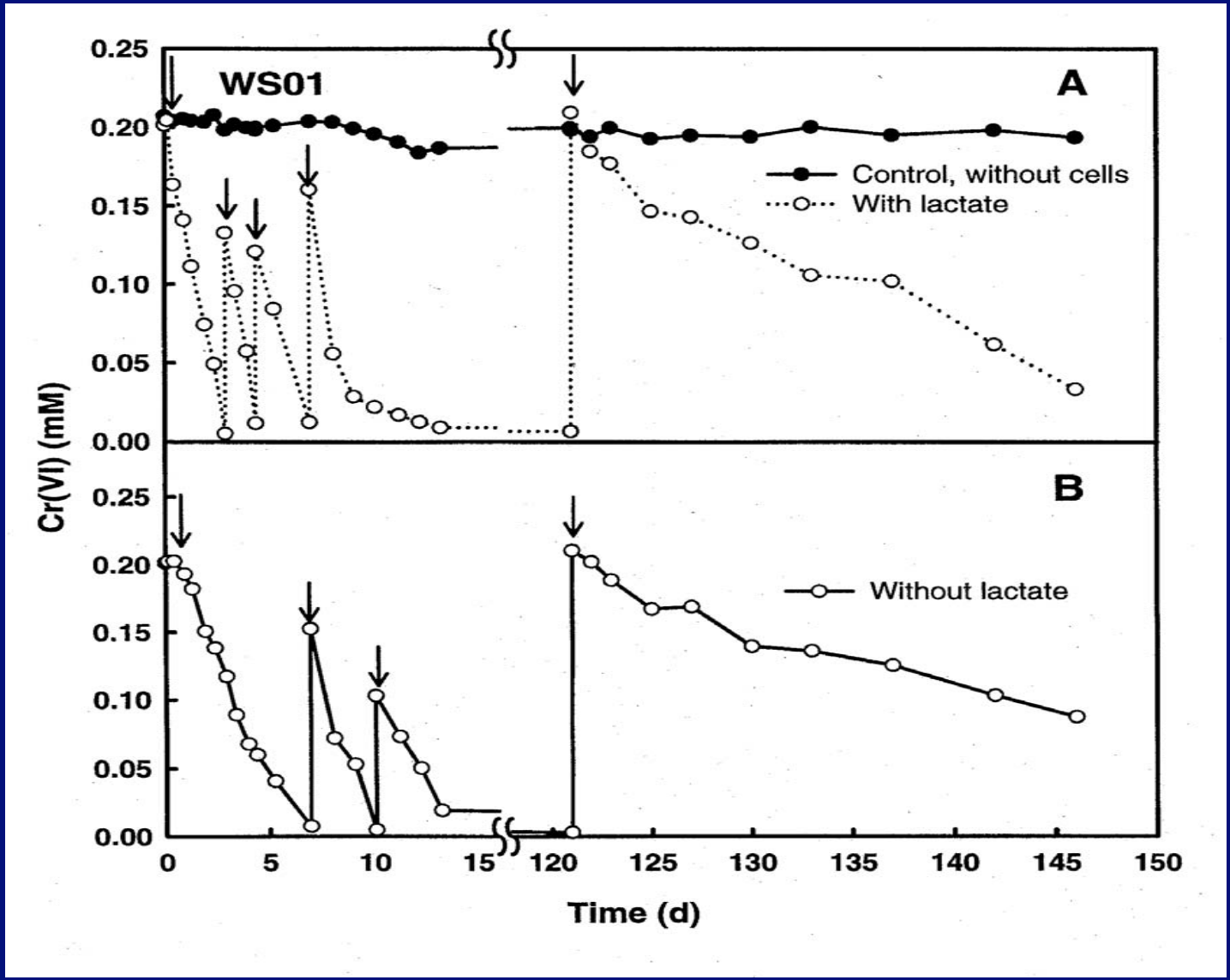
Cr(VI) Reduction



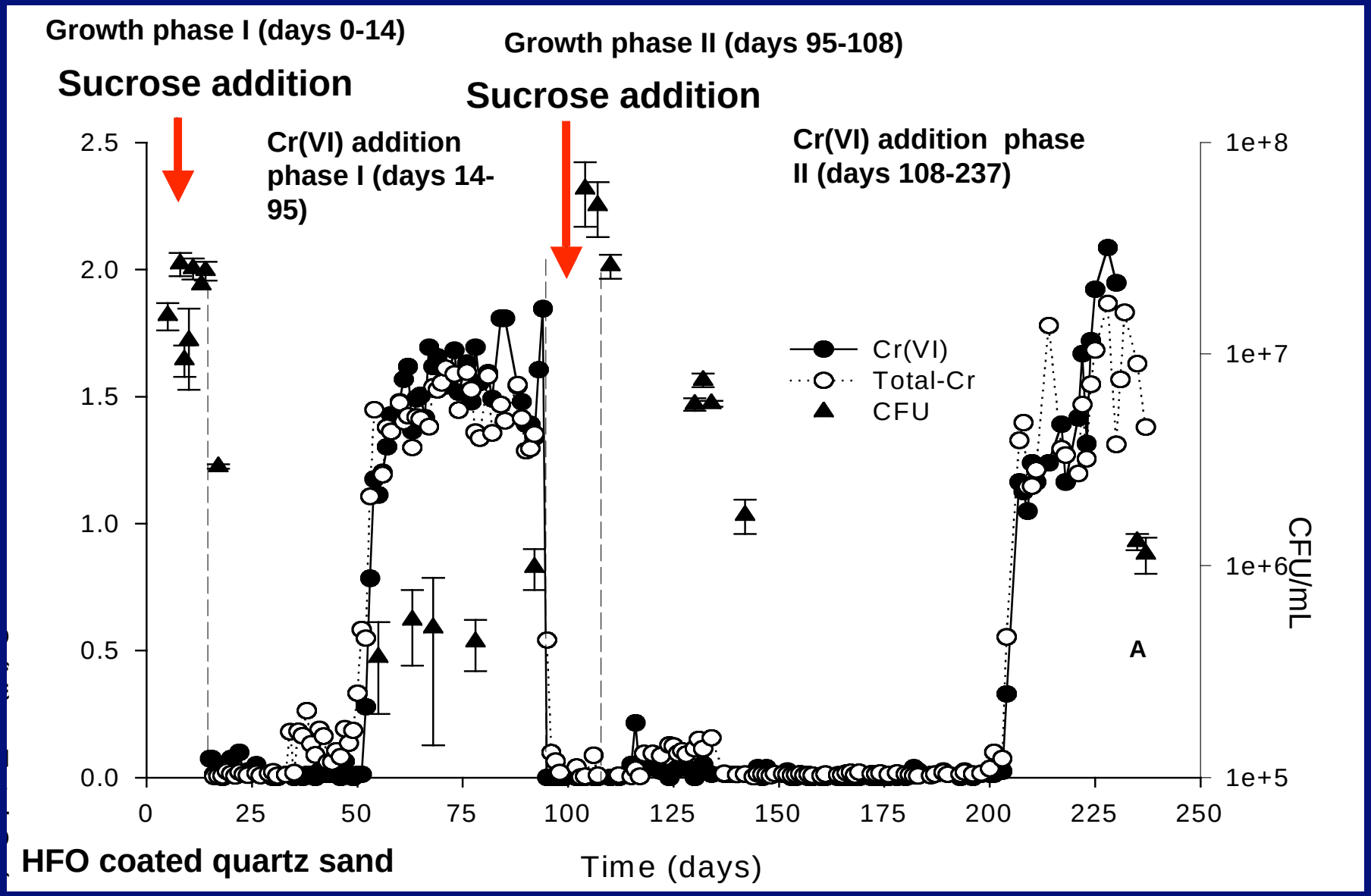
Cr K-edge X-ray absorption near-edge spectroscopy (XANES) spectra indicate that Cr(VI) was reduced to Cr(III) form.



Long Term Cr(VI) Reduction

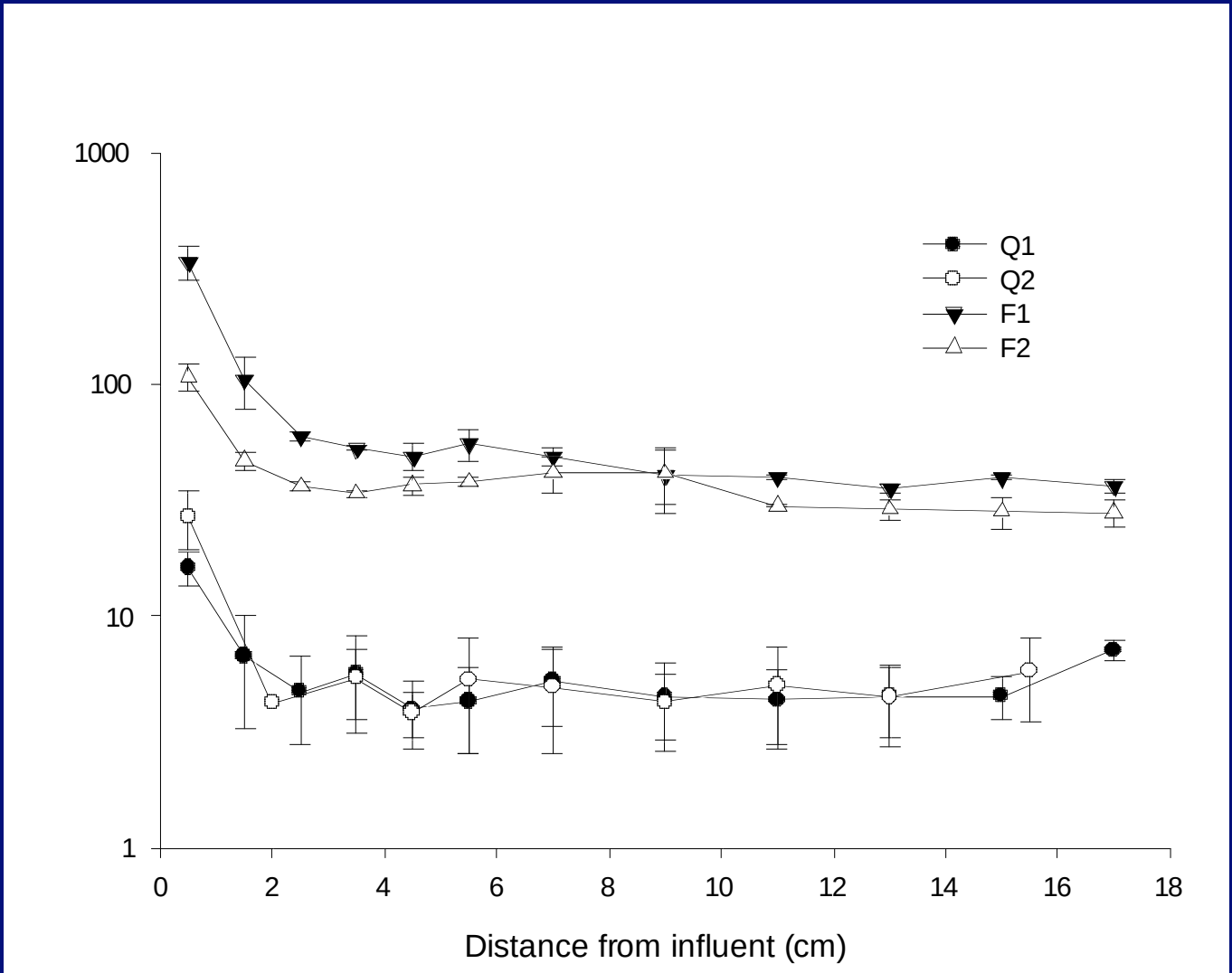


Long Term Flow Studies



Submitted to Biotech. Bioeng., Permeable reactive biobarriers for *in-situ* Cr(VI) reduction: Bench scale tests using *Cellulomonas* sp. strain ES6. S. Viamajala, B.M. Peyton, R. Gerlach, V. Sivaswamy, W.A. Apel, J.N. Petersen

Solid Phase Cr Accumulation



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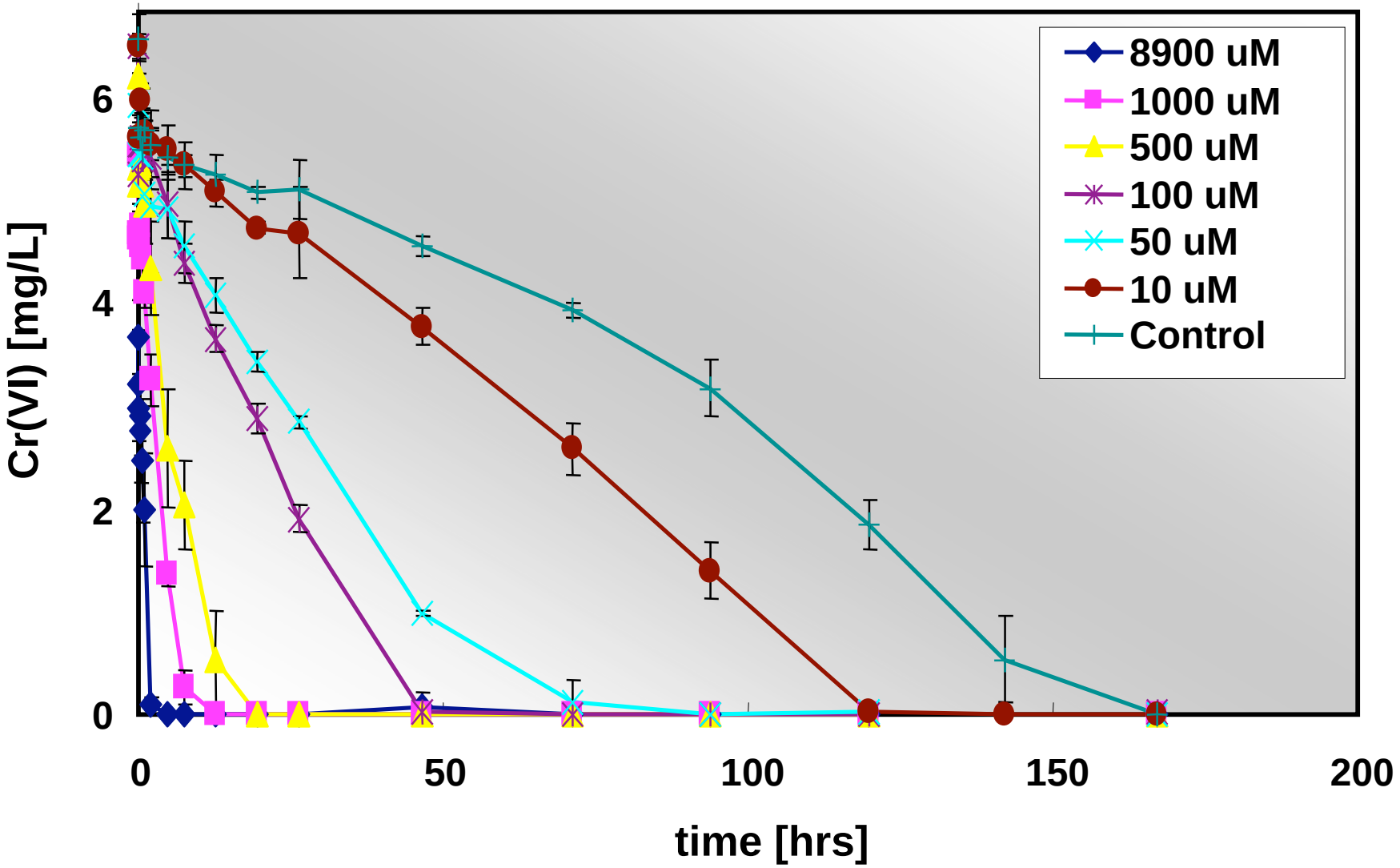
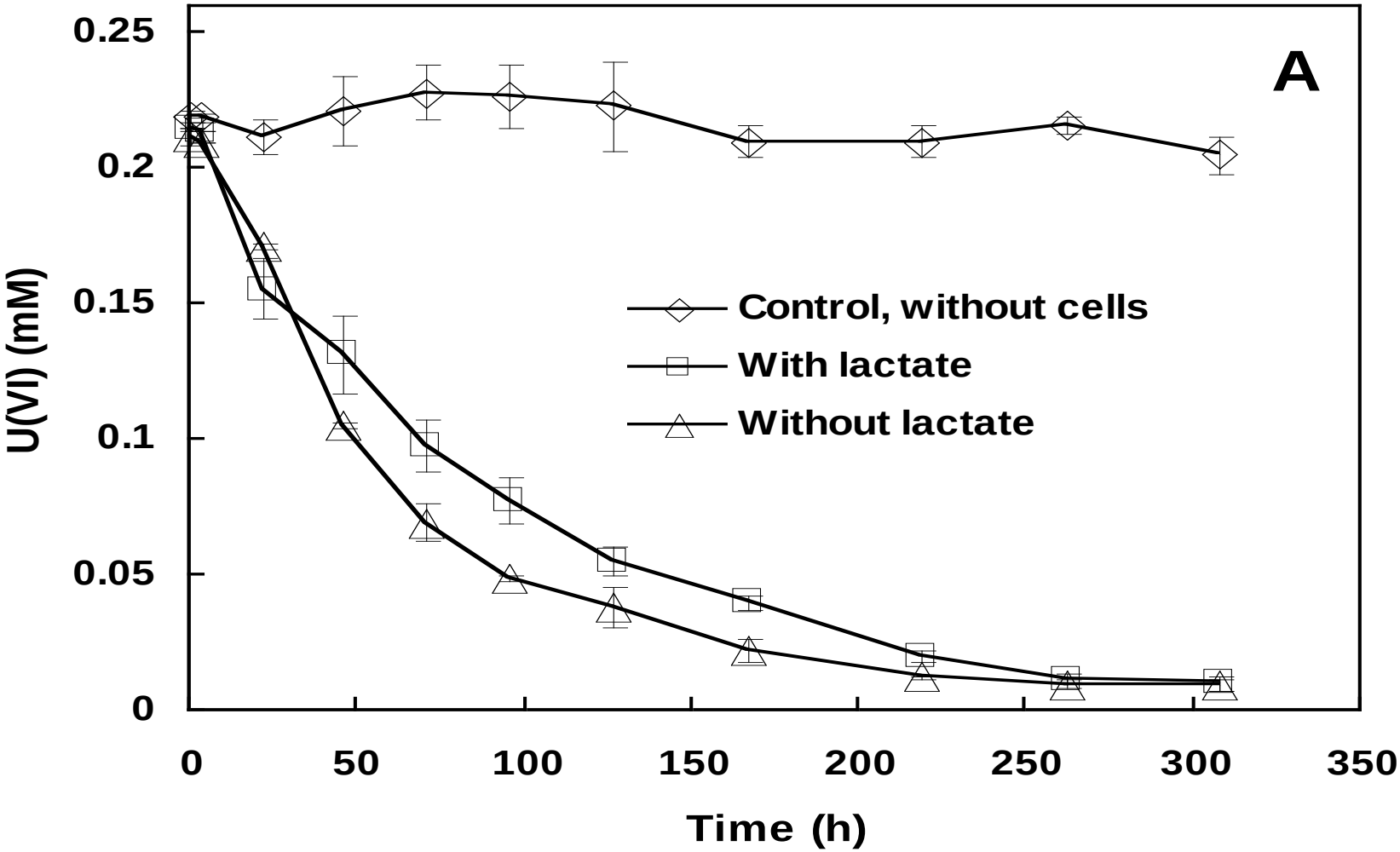
Cr(VI) Reduction - ES6 w/AQDS



e- donor =

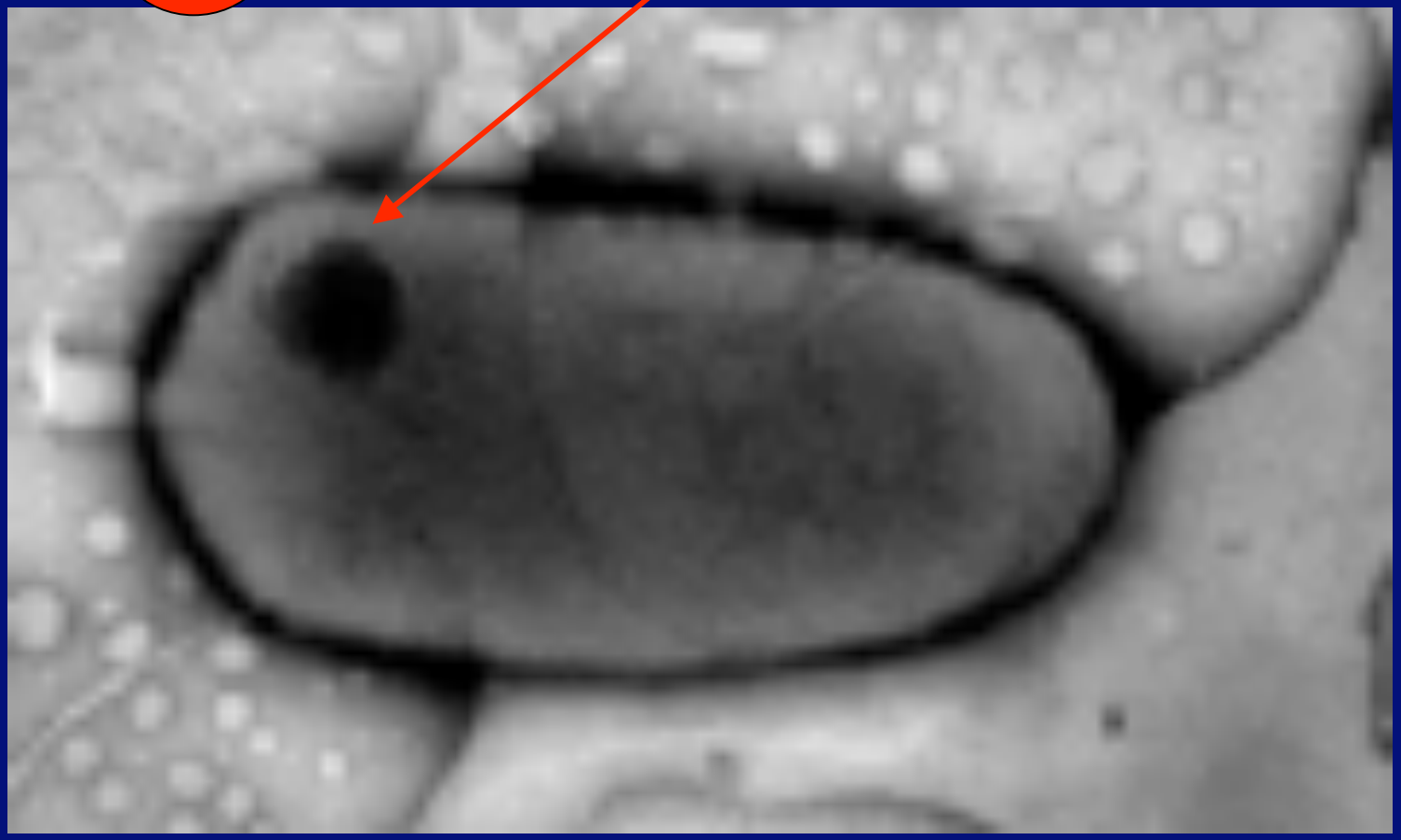
U(VI) reduction ?

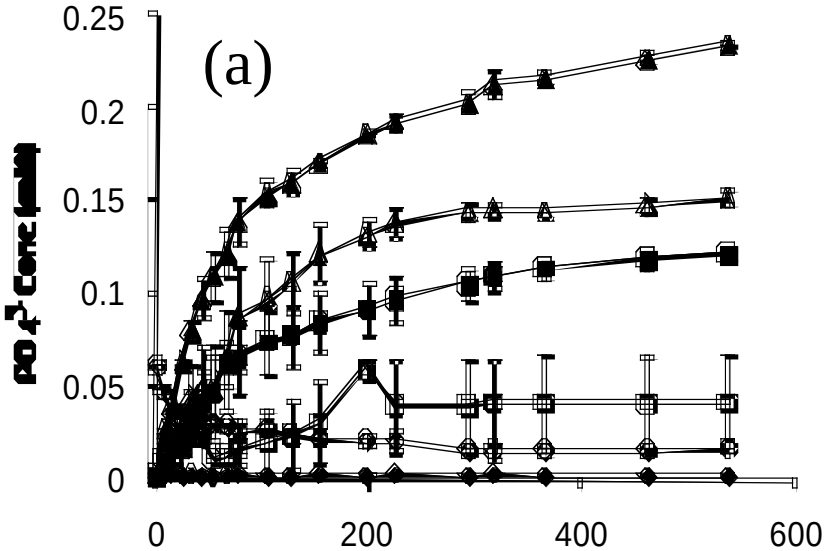


Energy Storage Compounds?

- Polyphosphate

- Polyhydroxybutyrate (PHB)
- Trehalose





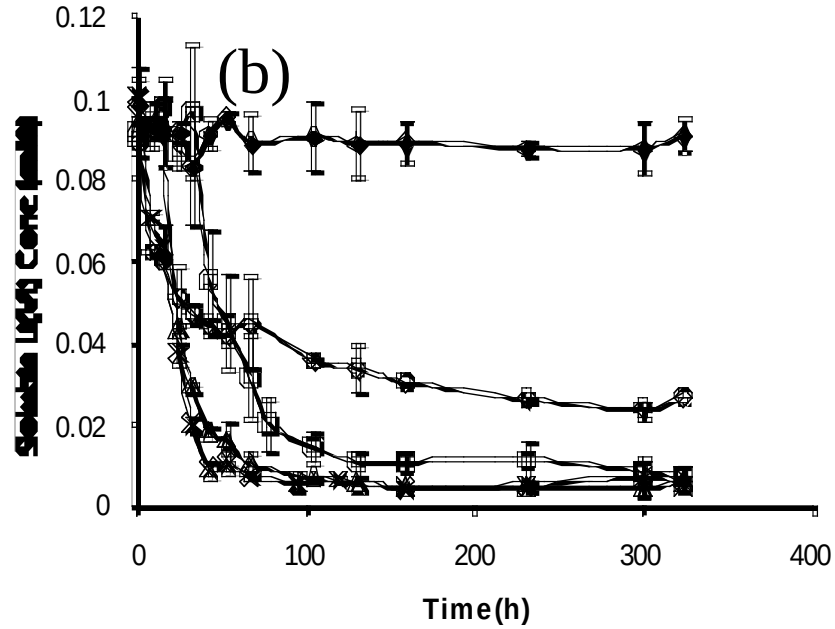
520mg DW cells/L

520mg DW cells/L + 0.1mM U(VI)

260mg DW Cells/L

260mg DW Cells/L + 0.1mM U(VI)

Heat killed cells (equivalent to 520mg DW cells/L) + 0.1mM U(VI)



520mg DW cells/L + 0.1 mM U(VI)

520mg DW cells/L + 0.1 mM AQDS + 0.1 mM U(VI)

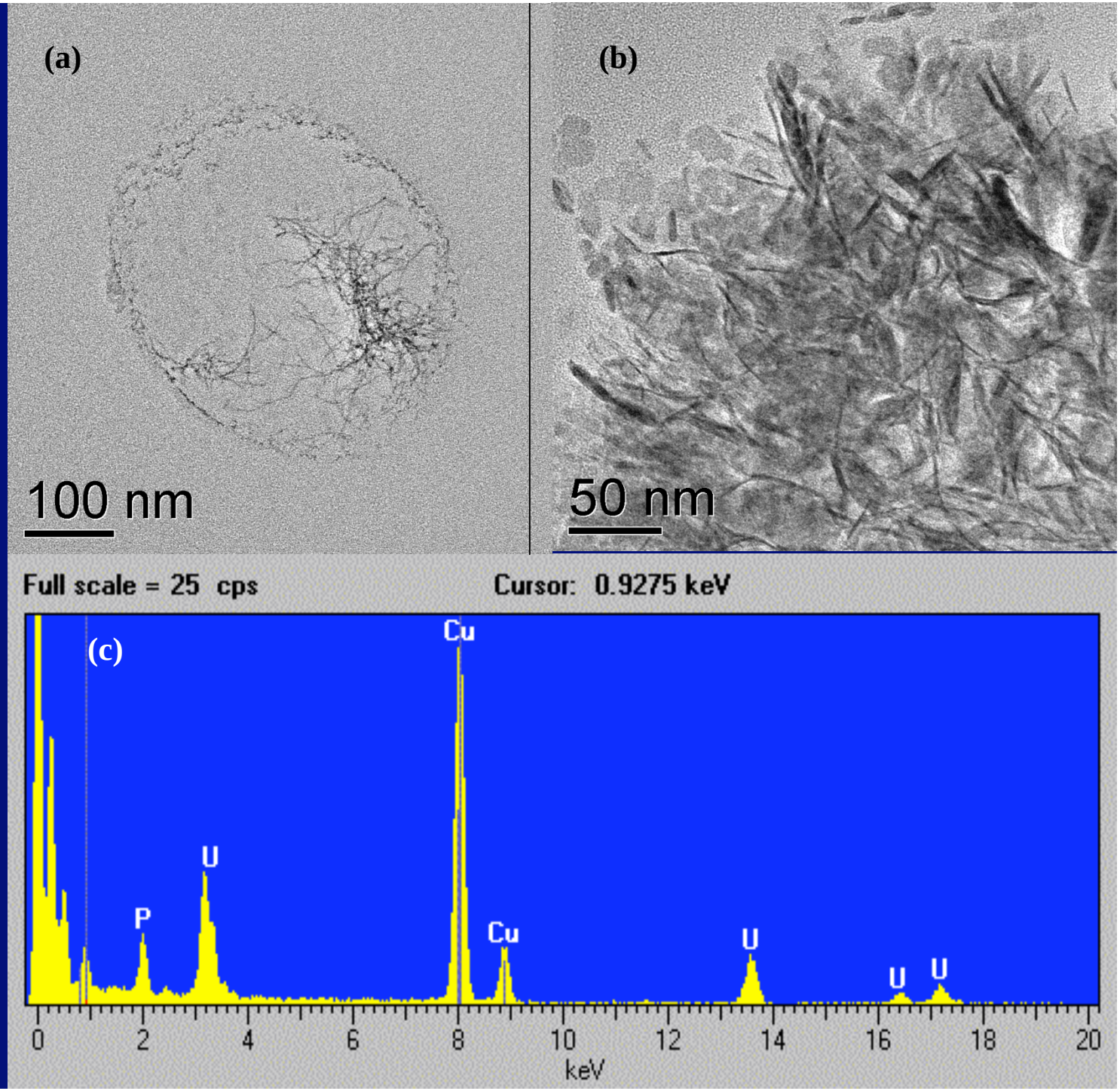
260mg DW cells/L + 0.1 mM U(VI)

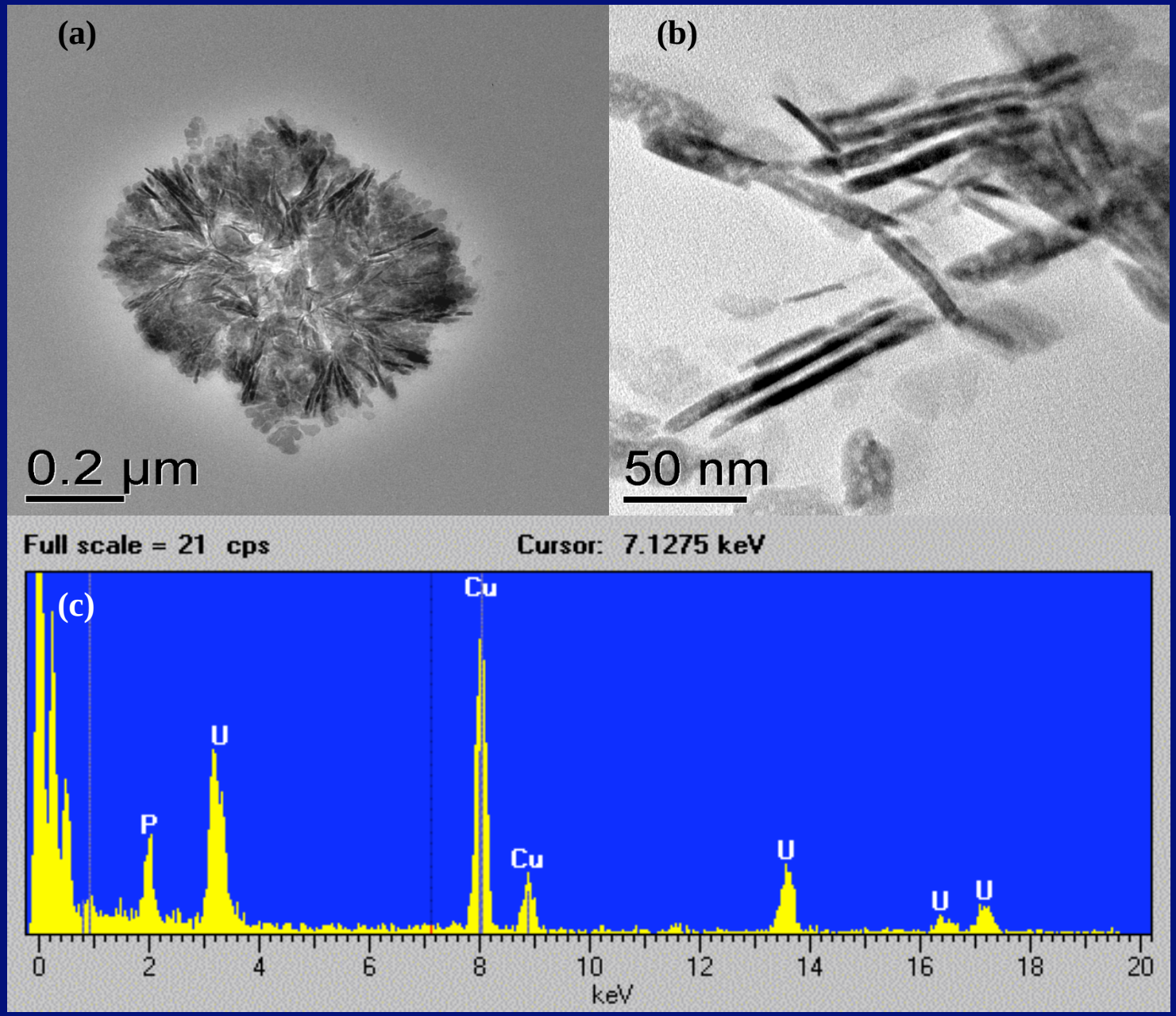
Heat killed cell + 0.1 mM U(VI)

Cell free control

PIPES Buffer (30mM)

HR-TEM w/ elemental analysis indicates that U & phosphate was precipitating both inside and outside the cell.

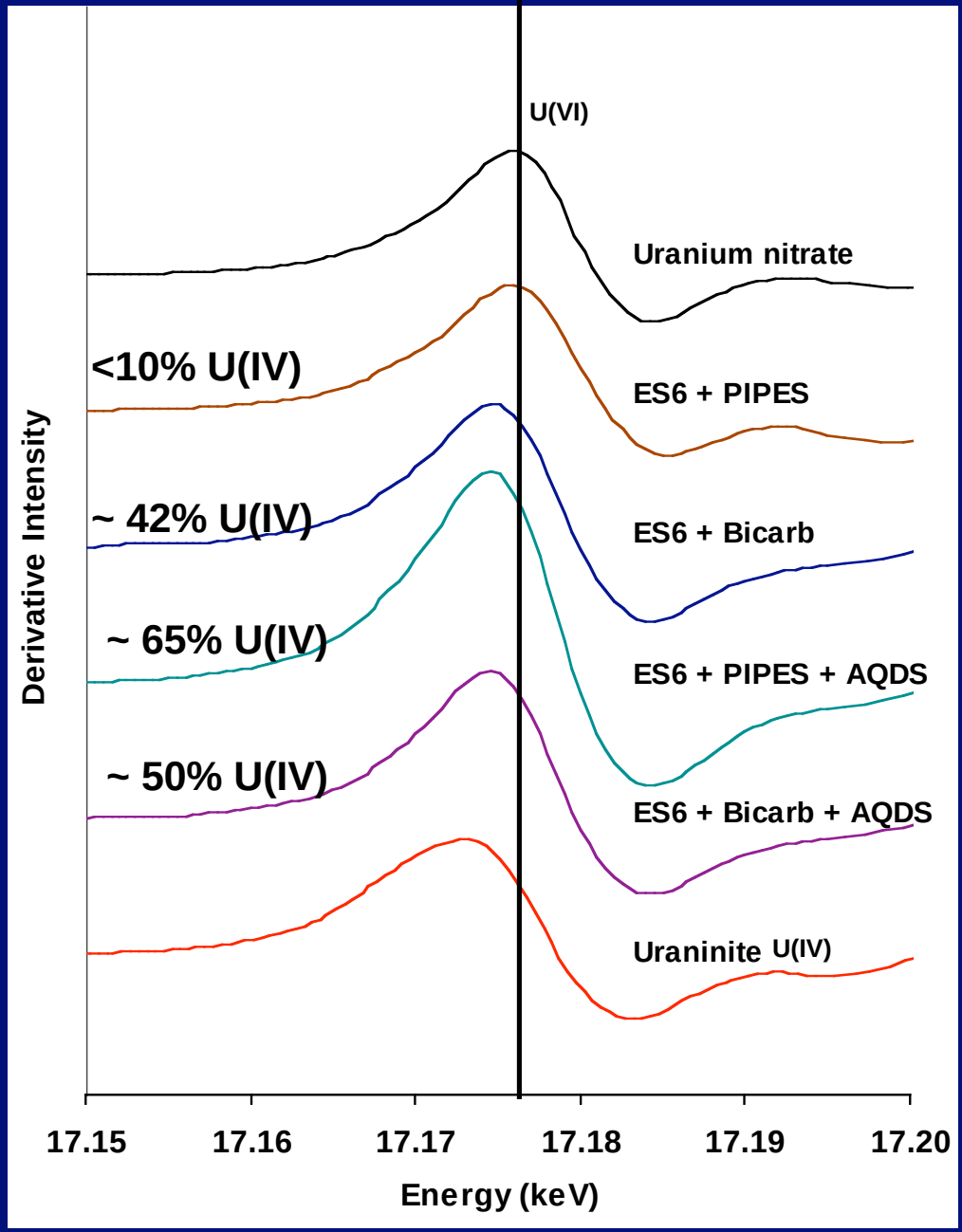



(a)
0.2 µm
(b)
50 nm
Full scale = 21 cps
Cursor: 7.1275 keV
(c)
Cu
U
P
Cu
U
U
U
0
2
4
6
8
10
12
14
16
18
20
keV

Uranium Solids

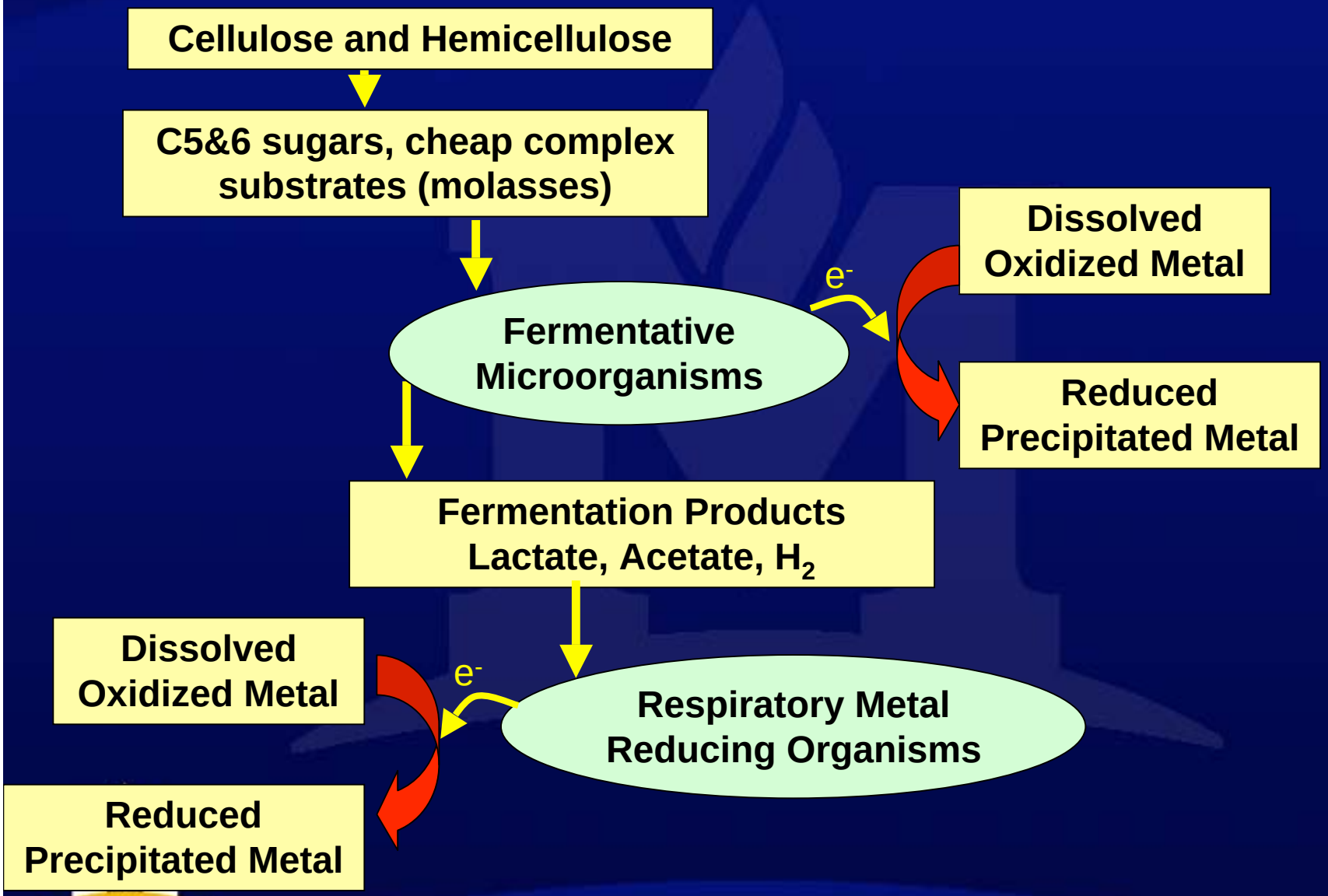
First derivative X-ray absorption near edge structure (XANES) spectra of uranium precipitates.

The buffer and the presence or absence of AQDS affected the U(IV):U(VI) ratio.

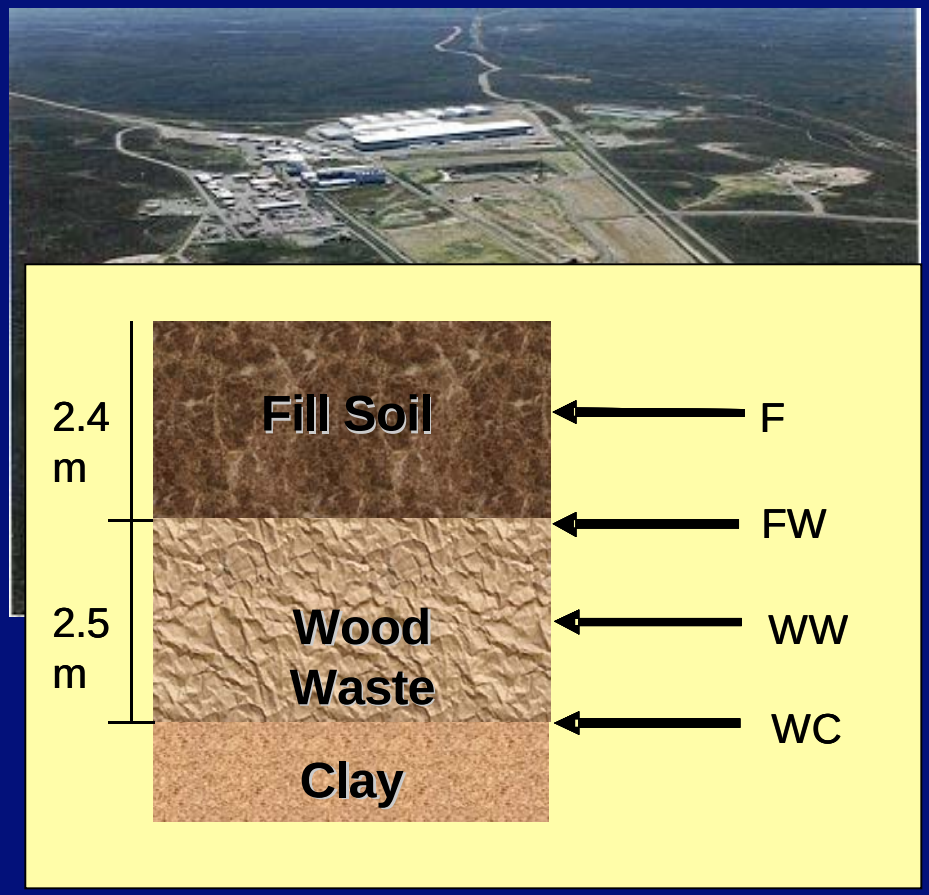


Thomas Borch, CSU

Microbial Metal Transformations

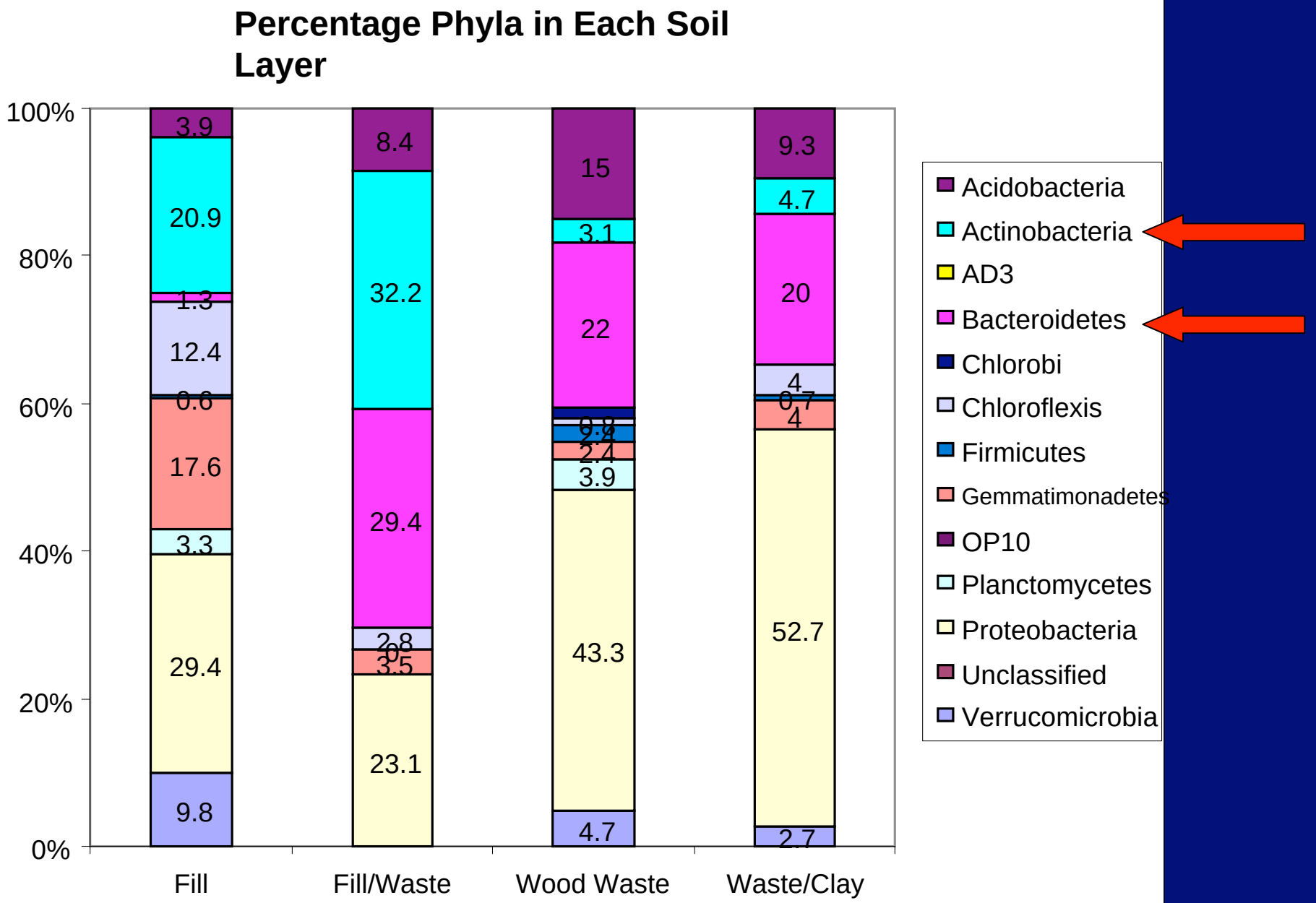


DOE Field Site Description



• DNA was extracted from each of these four layers for microbial community analysis.

- INL Radioactive Waste Management Complex (RWMC) – Cold Test Pit South (CTPS)
- CTPS constructed in 1988, filled with simulated LLW waste that conforms to the historical disposal practices at the site – buried wood and cardboard.
- CTPS provides an environment to test innovative waste characterization and retrieval technology.


Percentage Phyla in Each Soil Layer
100%
80%
60%
40%
20%
0%
Fill
Fill/Waste
Wood Waste
Waste/Clay
3.9
20.9
1.3
12.4
0.6
17.6
3.3
29.4
9.8
8.4
32.2
29.4
2.8
0.5
3.5
23.1
15
3.1
22
0.8
0.4
2.4
3.9
43.3
4.7
9.3
4.7
20
4
0.7
4
52.7
2.7
Acidobacteria
Actinobacteria
AD3
Bacteroidetes
Chlorobi
Chloroflexis
Firmicutes
Gemmatimonadetes
OP10
Planctomycetes
Proteobacteria
Unclassified
Verrucomicrobia

Microbial Community Analysis
G2 Phylochip

Sent subsamples of each DNA extract (F, FW, WW, and WC) to Gary Anderson & Yvette Piceno (LBNL) for G2 Phylochip analysis (Brodie et al. 2006)

Comparison of Clone Library and Phylochip Diversity

Clone Library

	# of Phyla	# of Classes	# of Orders	# of Families	OTUs
Fill	10(2)	18	29	45	151
Fill/Waste	7(0)	13	18	32	143
Wood/Waste	11(1)	19	36	48	127
Waste/Clay	11(1)	17	37	46	150

G2 Phylochip

	# of Phyla	# of Classes	# of Orders	# of Families	OTUs
Fill	30(7)	46	90	210	1630
Fill/Waste	33(9)	61	124	289	2056
Wood/Waste	30(8)	51	98	212	1984
Waste/Clay	28(7)	49	91	198	1925

( ) indicate number of candidate Phyla

	F	FW	WW	WC
Acidobacteria	62	65	24	21
Actinobacteria	157	218	219	175
Aquificae	1	2	2	1
Bacteroidetes	95	143	148	136
BRC1	0	3	0	0
Caldithrix	2	2	2	2
Chlamydiae	1	2	2	2
Chlorobi	13	11	11	11
Chloroflexi	35	35	38	31
Coprothermobacter	1	1	0	0
Cyanobacteria	7	38	49	49
Deferribacter	0	1	1	0
Deinococcus-Thermus	3	4	4	4
Dictyoglomi	1	1	1	1
Firmicutes	264	391	353	308
Gemmatimonadetes	9	13	13	12
Marine Group A	1	2	2	0
Natronoanaerobium	5	5	5	5
NC-10	4	4	4	4
Nitrospira	8	9	9	7
OP-10	7	8	7	15
OP3	3	3	0	0
OP8	2	2	0	0
OP9	0	3	0	0
Planctomycetes	18	23	23	23
Proteobacteria	805	969	970	934
Spirochaetes	34	35	37	36
Synergistes	9	3	9	6
TM-7	6	8	6	8
Thermodesulfobacteria	1	1	1	1
Thermotogae	0	1	1	1
Verrucomicrobia	20	25	28	27
WS3	3	3	3	0

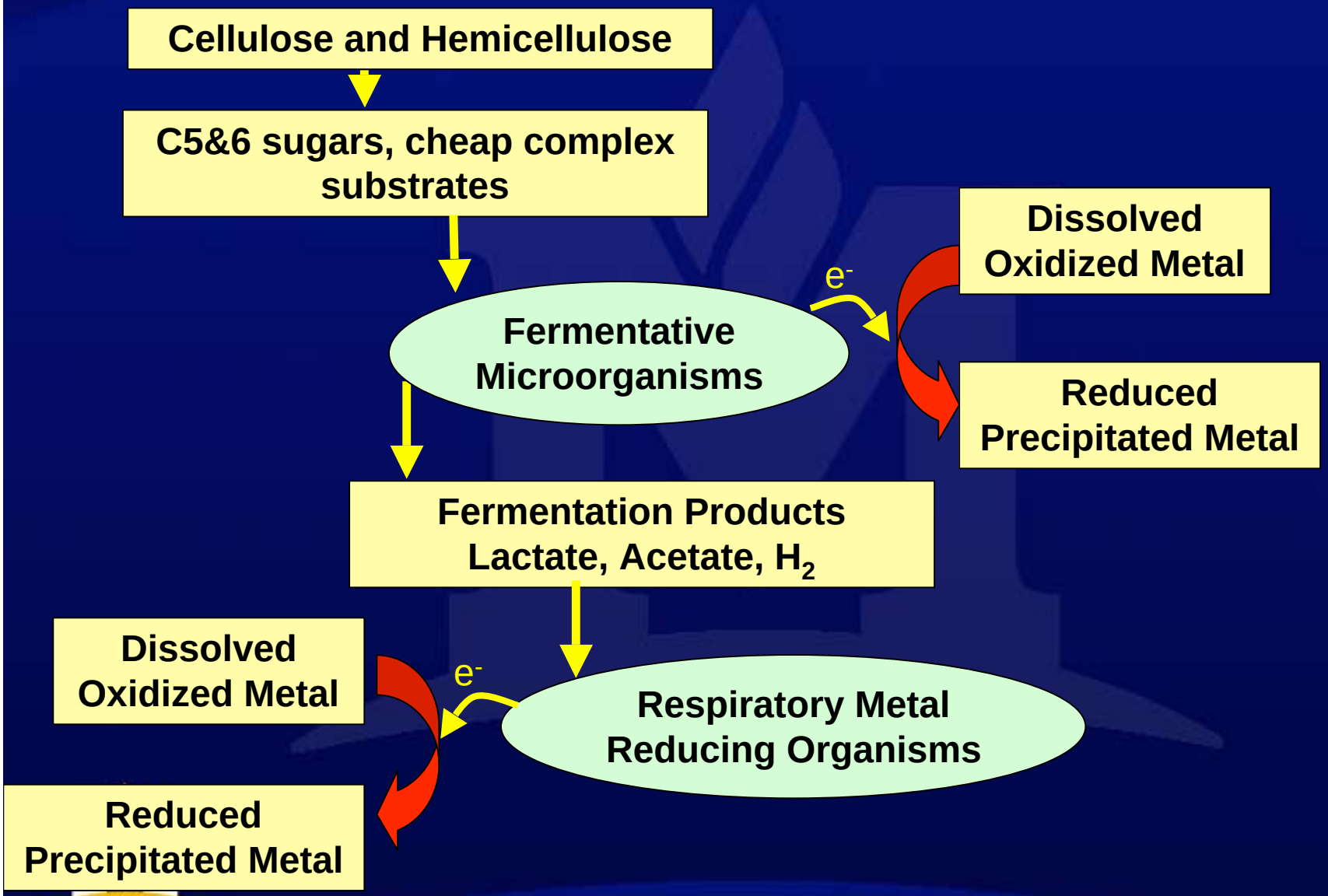
G2 Phylochip Results

- Rolled up to the Phylum level
  - ~~Detailed physiology for each OTU?~~
- Translated into potential physiological capabilities, e.g., e- donor/acceptor combinations

CWTP Isolates

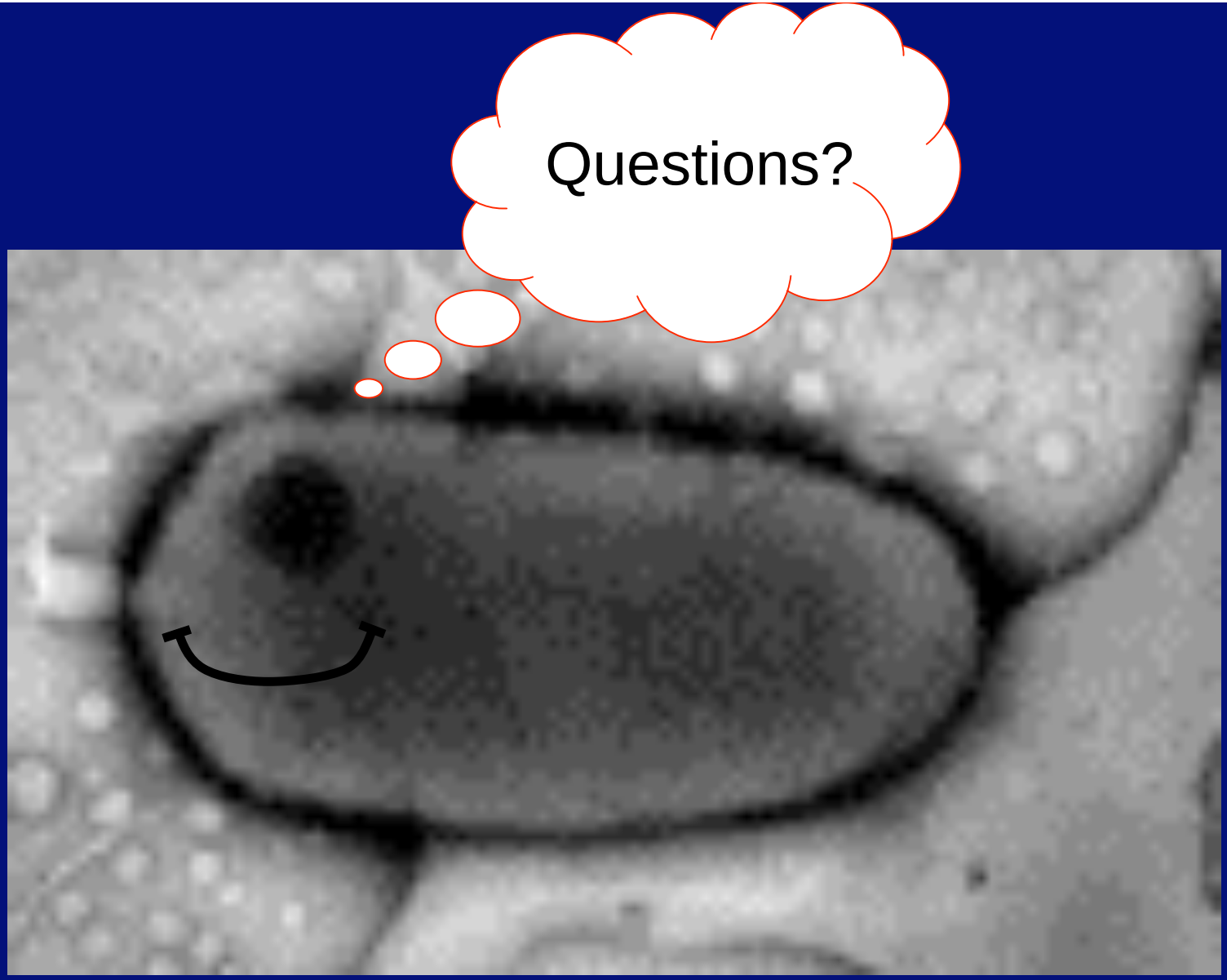
Isolate Designation	Genus of Isolate	Top BLAST Search Result	% Similarity to BLAST Result	O_2 Requirements	Sources of Isolate
A	Pseudomonas	*Pseudomonas sp. 4/11GC3#e*	99	Aerobic	FW, WW, WC
B	Streptomyces	*Streptomyces atratus*	99	Aerobic	FW, WW, WC
C	Flavobacteria	*Flavobacterium sp. WB 3.1 -83*	98	Aerobic	FW, WW, WC
D	Pedobacter	*Pedobacter terrae*	99	Aerobic	WW
E	Serratia	*Serratia proteamaculans*	97	Facultative Anaerobe	FW
F	Cellulomonas	*Cellulomonas parahominis*	99	Facultative Anaerobe	FW, WW, WC
G	Paenibacillus	*Paenibacillus sp. GP26-03*	99	Facultative Anaerobe	WC

Fermentation - Metal Transformations



Summary

- Fermentative organisms have unique metal transformation capabilities and can play a significant role in subsurface Cr and U bio-immobilization.
- *Cellulomonas* sp. strain ES6 was shown to simultaneously immobilize U by reduction to U(IV) and by precipitation through release of PO_4^{3-}, with the ratio dependent on bicarbonate conc.
- ES6 reduced Cr(VI) for long periods of time with little substrate addition.
- Established fermenting communities are complex … requiring modern tools to characterize – But we need to reduce the data to "functional physiologies" for modeling, and for field applications & monitoring.


Questions?