The effect of U(VI) bioreduction rate on subsequent reoxidation of UO_2

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Research Hypothesis

Kinetics of U(VI) reduction will directly control subsequent rate of oxidation of biogenic uraninite solids

Experimental Approach

Manipulate U(VI) bioreduction rate by varying concentration of DMRB and/or incubation temperature

Experimental Methods – CN32

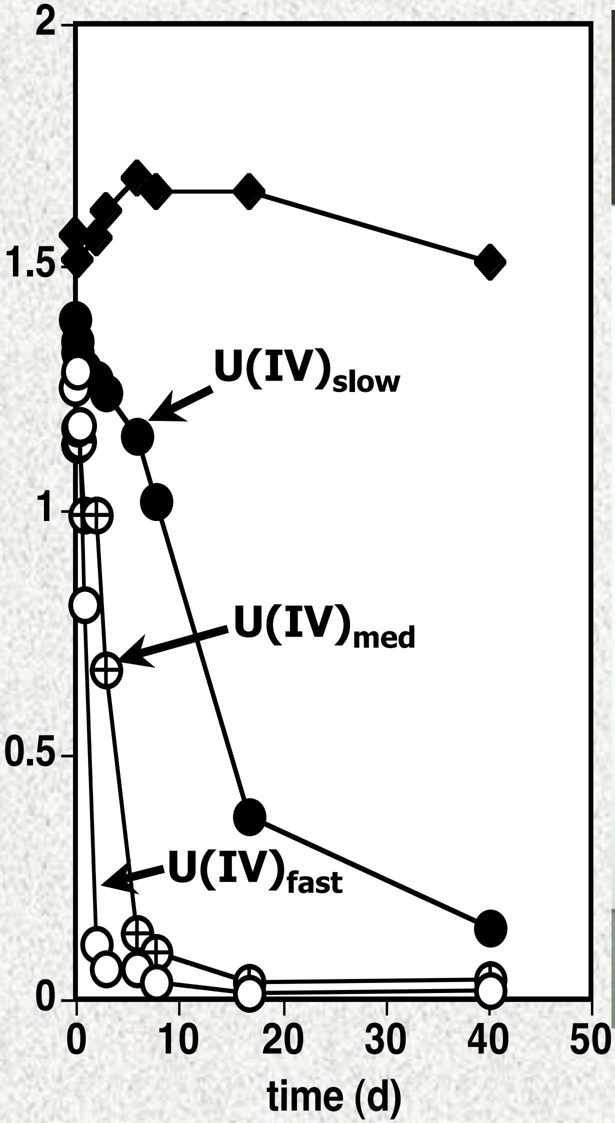
- *Shewanella putrefaciens* CN32 grown on TSB-D in open shake flasks to late-log phase
- 30 mM $NaHCO_3$ background electrolyte (pH 6.8) + 1.2 mM U(VI) acetate

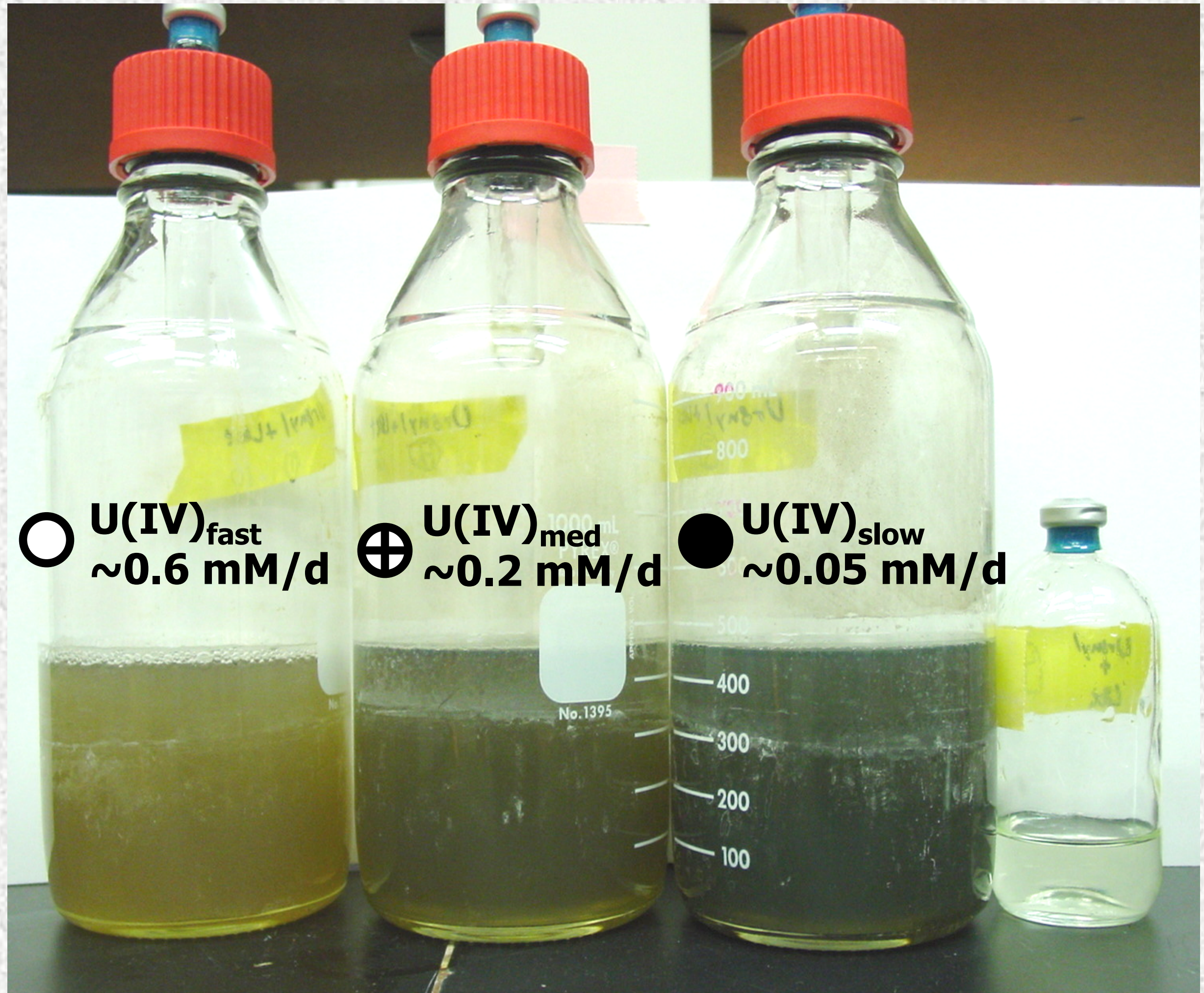
- [soluble U(VI)] determined by KPA after centrifugation
- [total U(VI)] determined by KPA after 5 minute extraction in 1 M anoxic $NaHCO_3$ (pH 8.4)
- [total U] determined by KPA after 24 hr extraction in 10% HNO_3 open to air

Experimental Methods

- cells and uraninite solids examined by Scanning Electron Microscopy, Transmission Electron Microscopy, and X-ray Diffraction
- U oxidation state in cell-solid pellets confirmed by X-ray Absorption Near Edge Spectroscopy (XANES)
- coordination environment of U in cell-solid pellets resolved by Extended X-ray Absorption Fine Structure (EXAFS)

Kinetics of U(VI) reduction by *S. putrefaciens* CN32 in 30 mM $NaHCO_3$





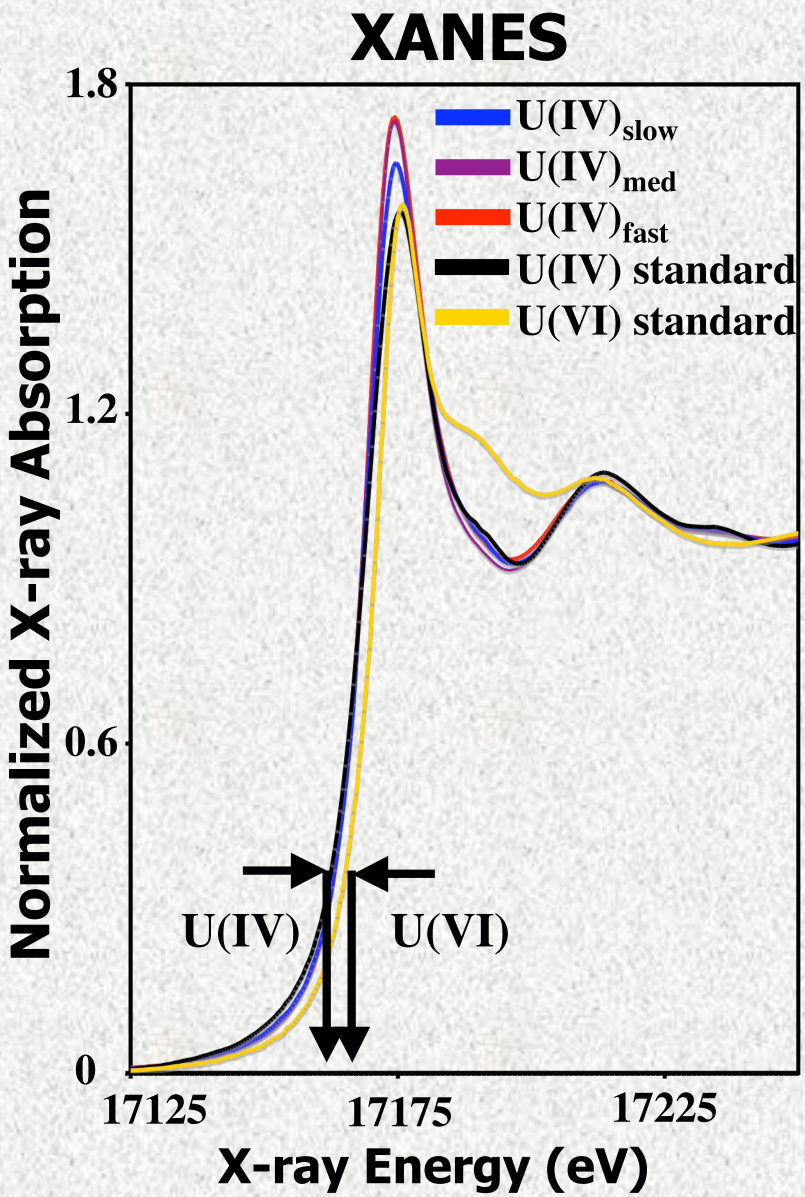
$U(IV)_{fast}$ ~0.6 mM/d

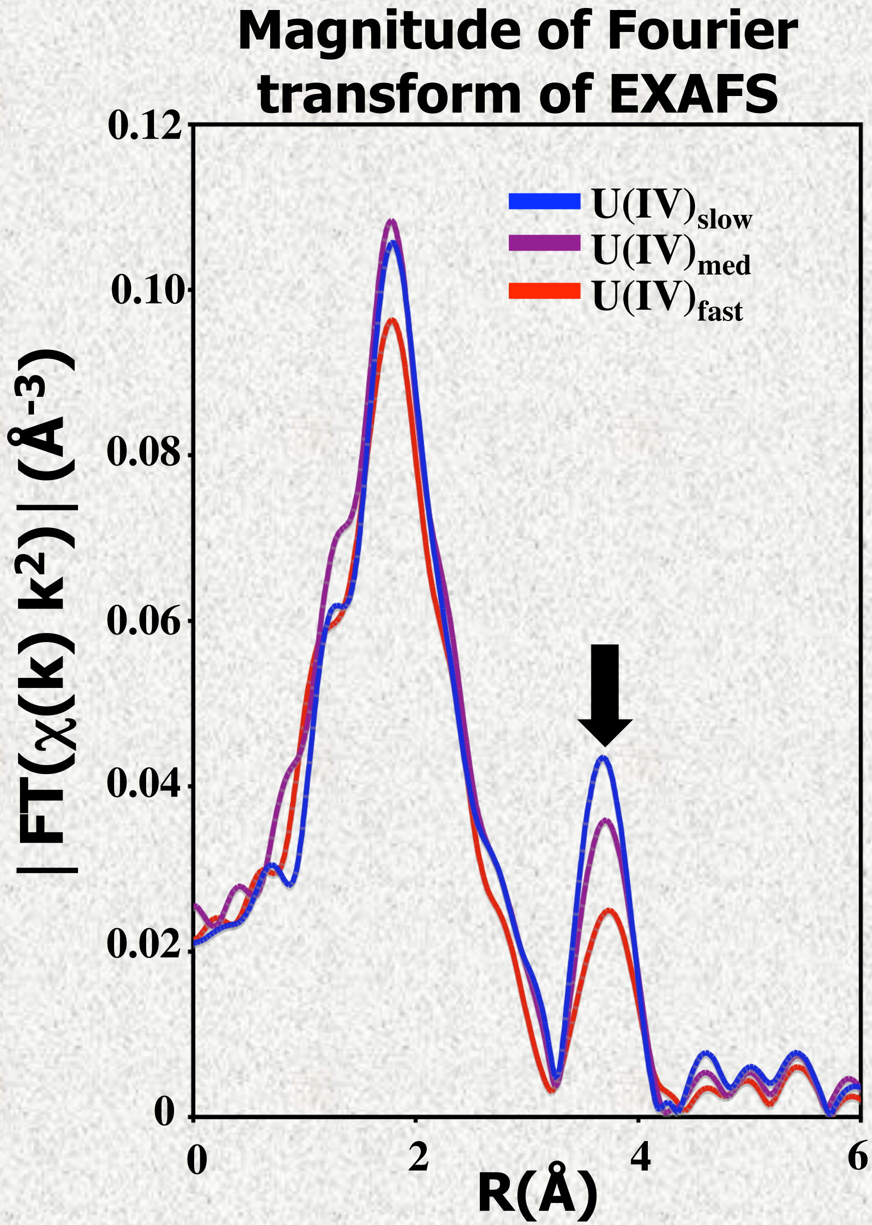
$U(IV)_{med}$ ~0.2 mM/d

$U(IV)_{slow}$ ~0.05 mM/d

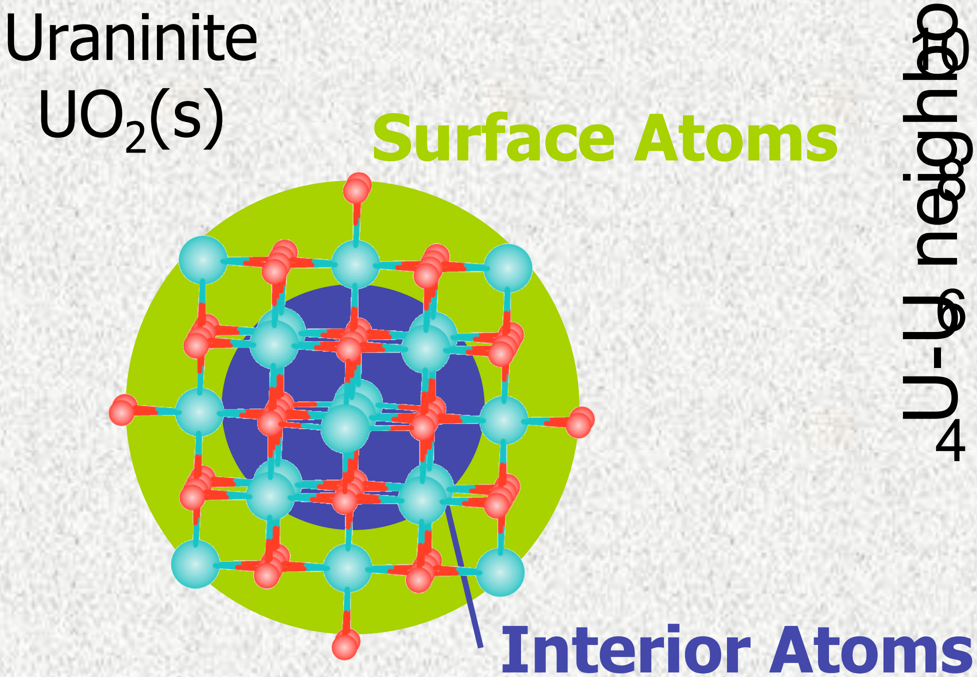
$2.2x10^8$ cell/ml $9.1x10^7$ cell/ml $2.3x10^7$ cell/ml

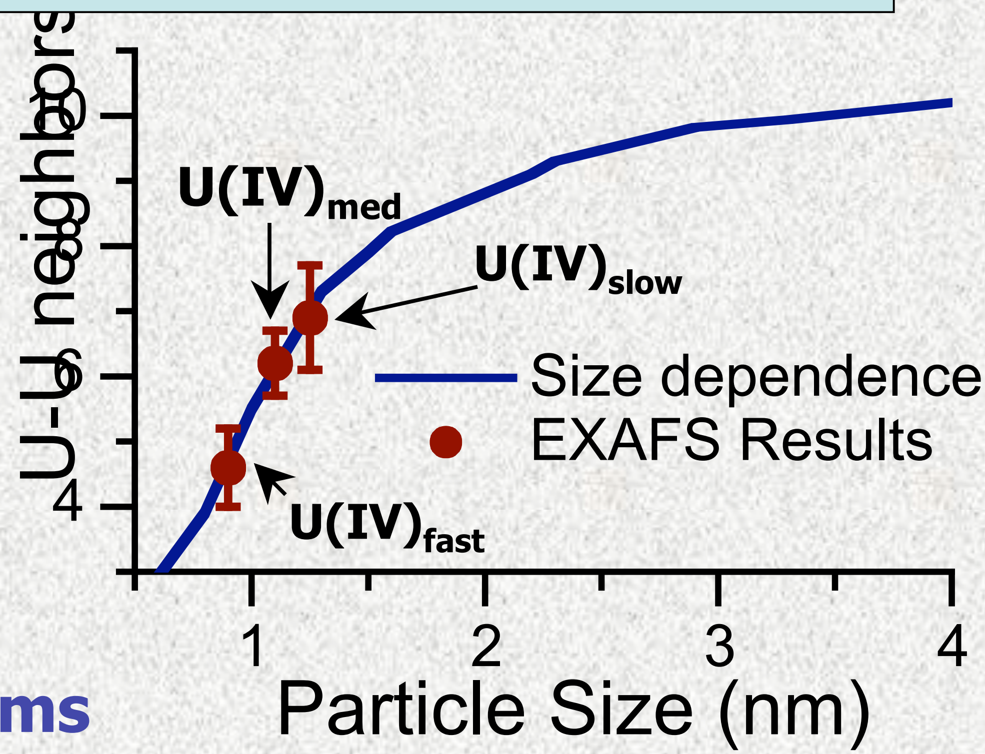
X-ray Absorption Spectroscopy of U(IV)$_{fast}$, U(IV)$_{med}$, and U(IV)$_{slow}$





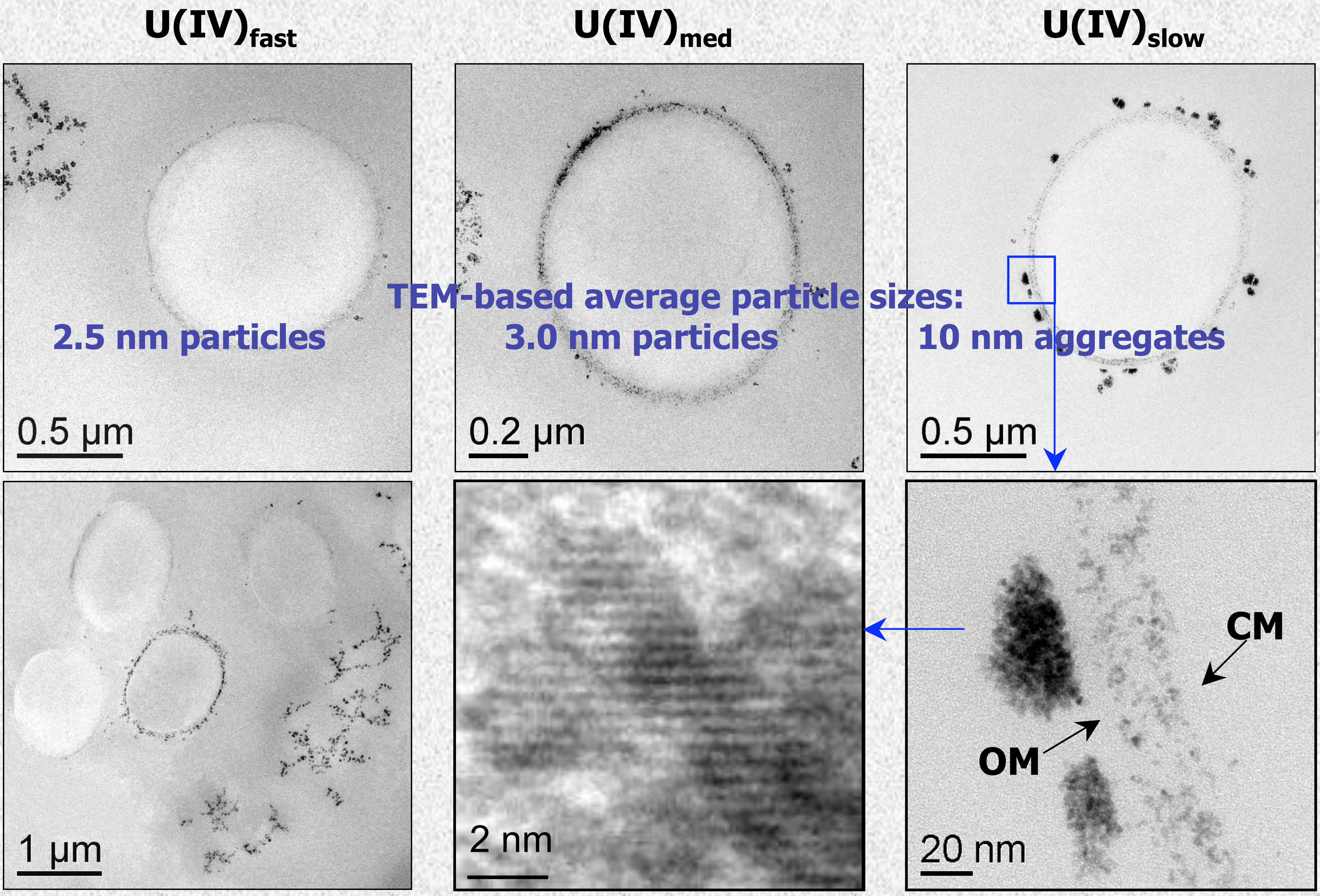
Particle size estimated from EXAFS



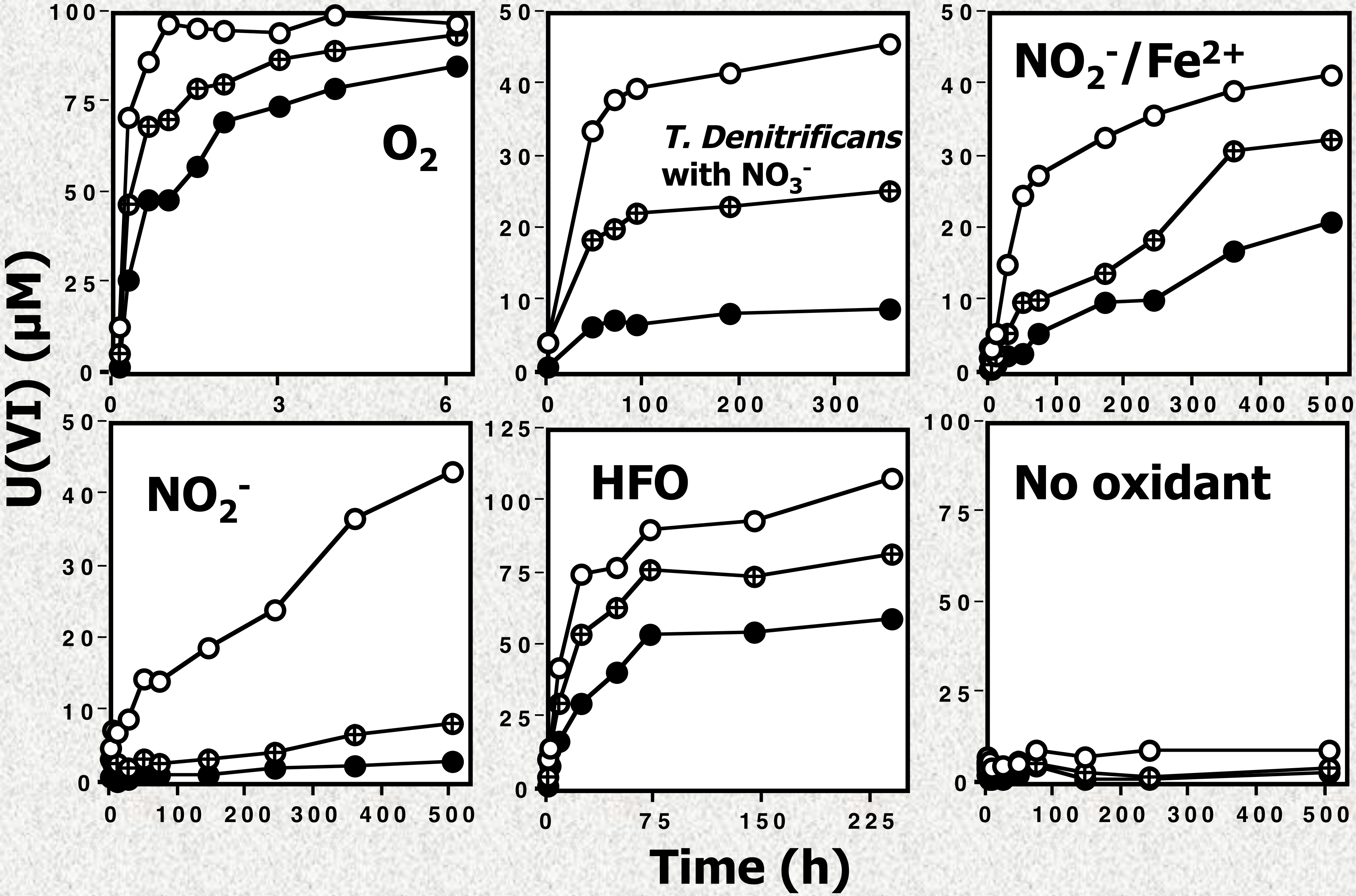


- **Atoms on the surface of uraninite particle have fewer neighbors than those in the interior.**
- **Smaller particles have more surface atoms compared to interior atoms.**
- **Decreasing number of U-U atoms is expected for smaller particles of uraninite.**

Transmission Electron Microscopy



Reoxidation of $U(IV)_{fast}$ (○), $U(IV)_{med}$ (⊕), and $U(IV)_{slow}$ (●)



Experimental Methods – MR-1

- *Shewanella oneidensis* MR-1 grown in defined media with air in sealed flasks to the onset of electron acceptor-limiting conditions (late-log phase)

- 30 mM $NaHCO_3$ background electrolyte (pH 6.8) + 1 mM U(VI) acetate
- 5 mM $NaHCO_3$ + 4.2 mM $CaCl_2$ + 0.01 mM KH_2PO_4 + 10 mM PIPES background electrolyte (pH 6.3) + 1 mM U(VI) acetate (PBAGW)

Careful batch culturing of *Shewanella oneidensis* MR-1

- 2:1 vol/vol air:media
- 50 rpm
- 30 °C
- Inoculum volume/growth state
- Chemically defined media

  60 mM Na-lactate

  0.01 mM PO_4

  0.10 mM NH_4

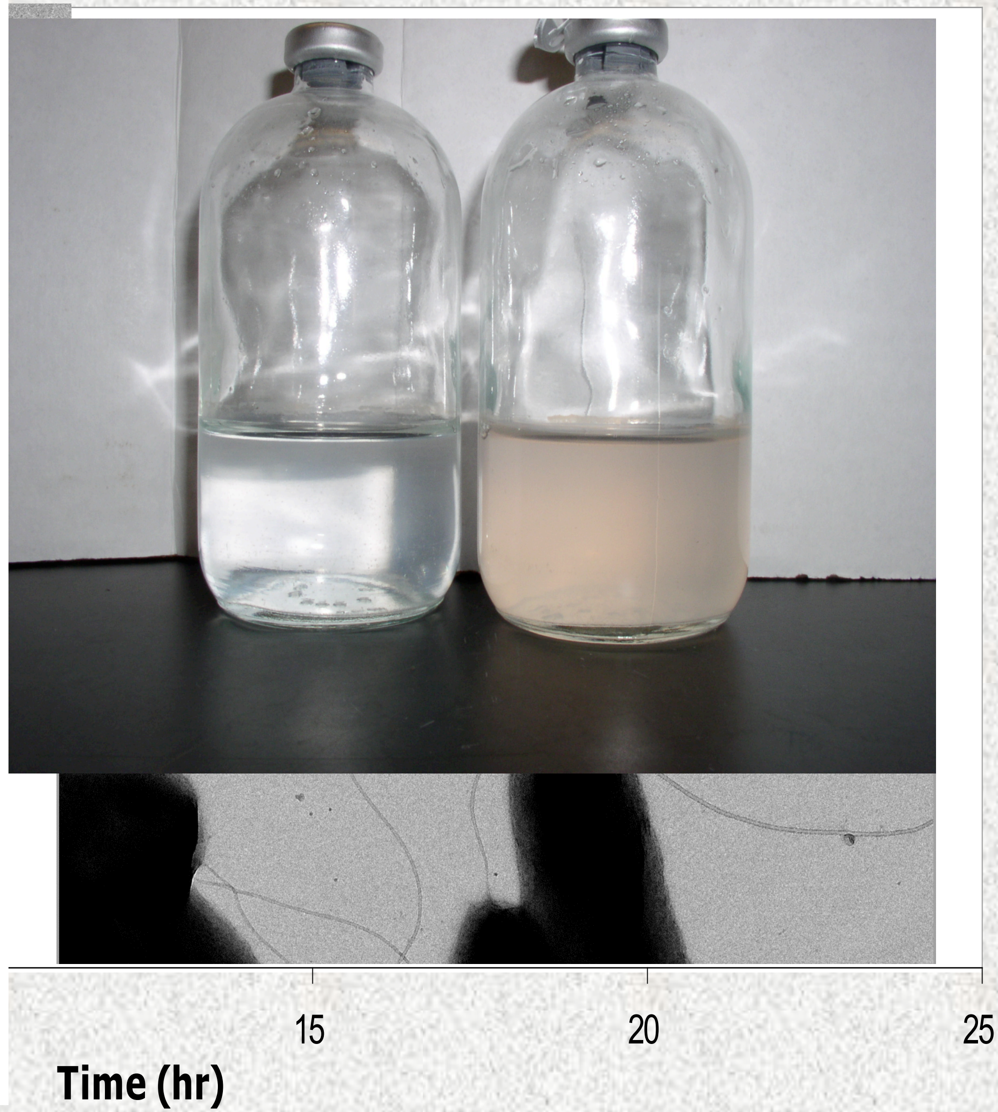
  vitamin mix

  mineral mix

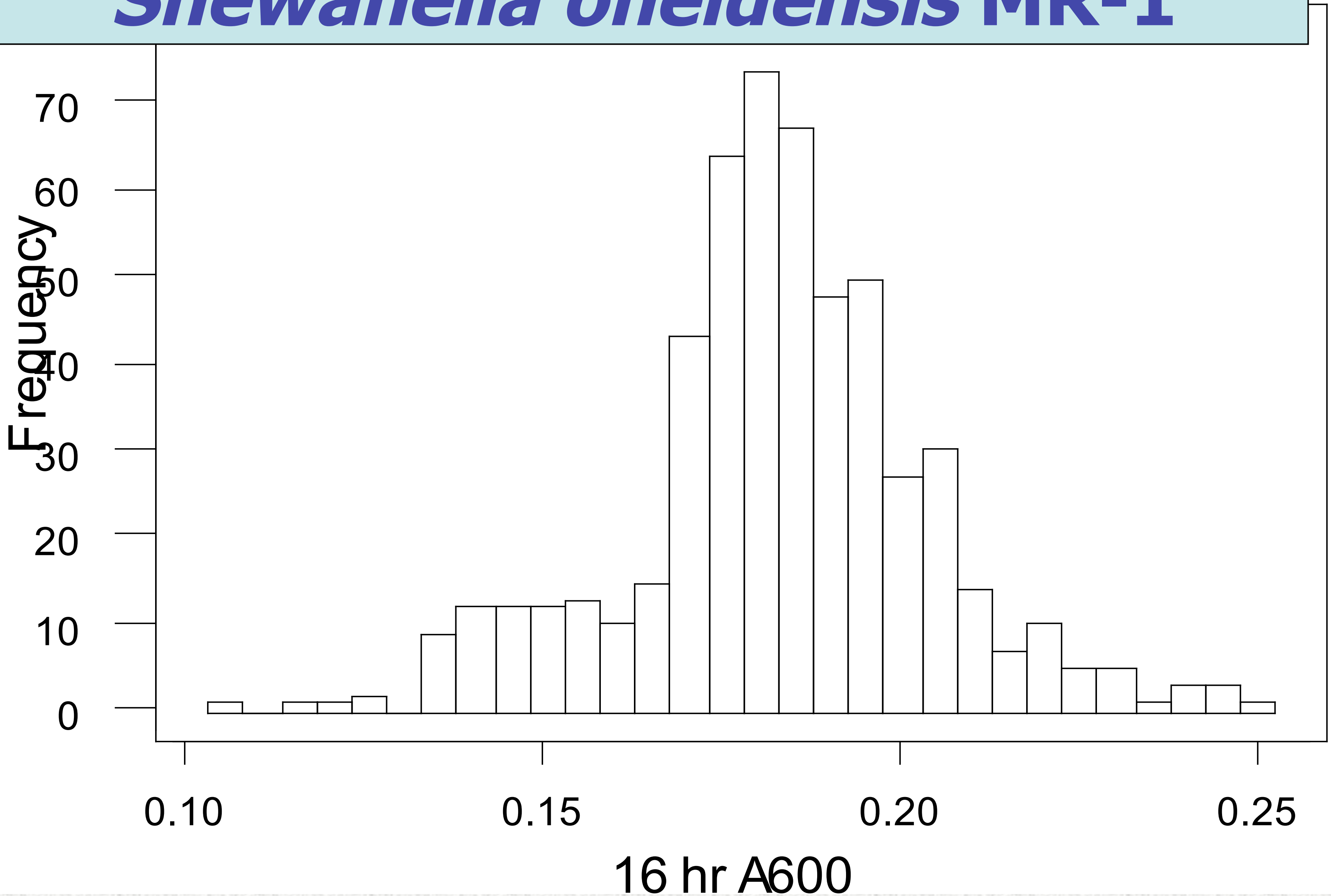
  amino acids

  5 mM Fe(III)-NTA

  10 mM Na-PIPES

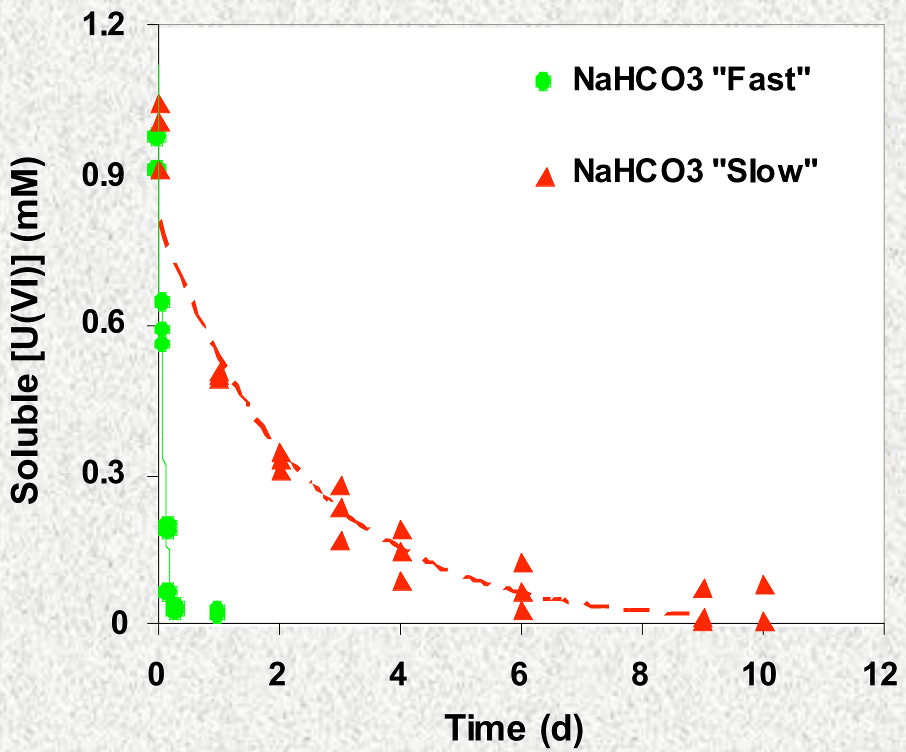


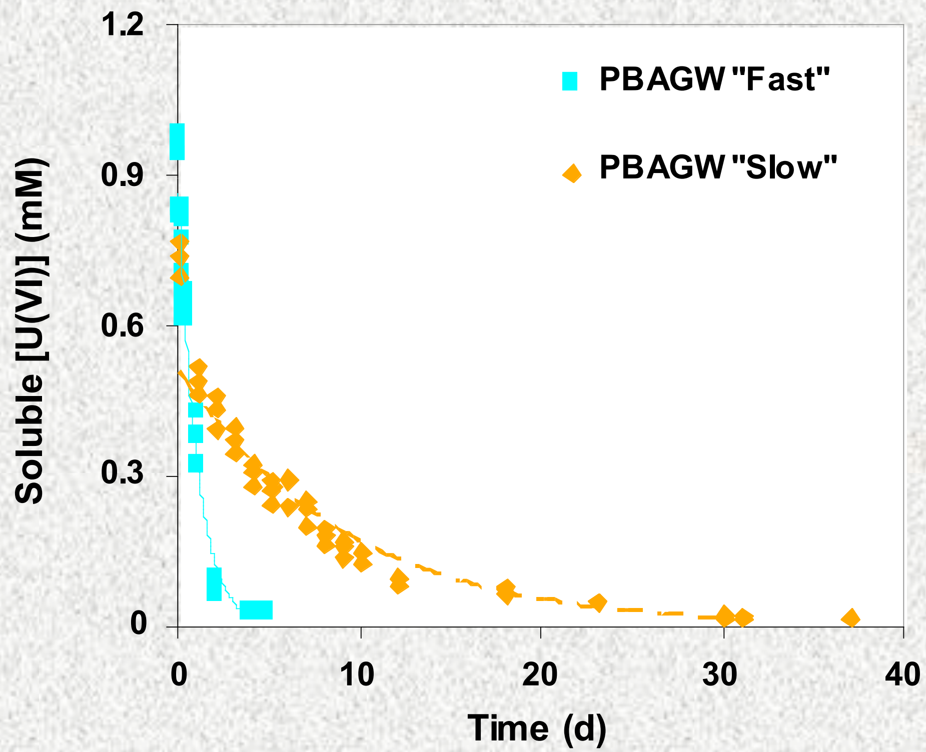
Careful batch culturing of *Shewanella oneidensis* MR-1



Average A600 = **0.182;** Standard Deviation = **0.02;** # of Trials = **508**

Kinetics of U(VI) reduction by *S. oneidensis* MR-1





"Fast" – 2*10^8 cell/mL, 37 °C

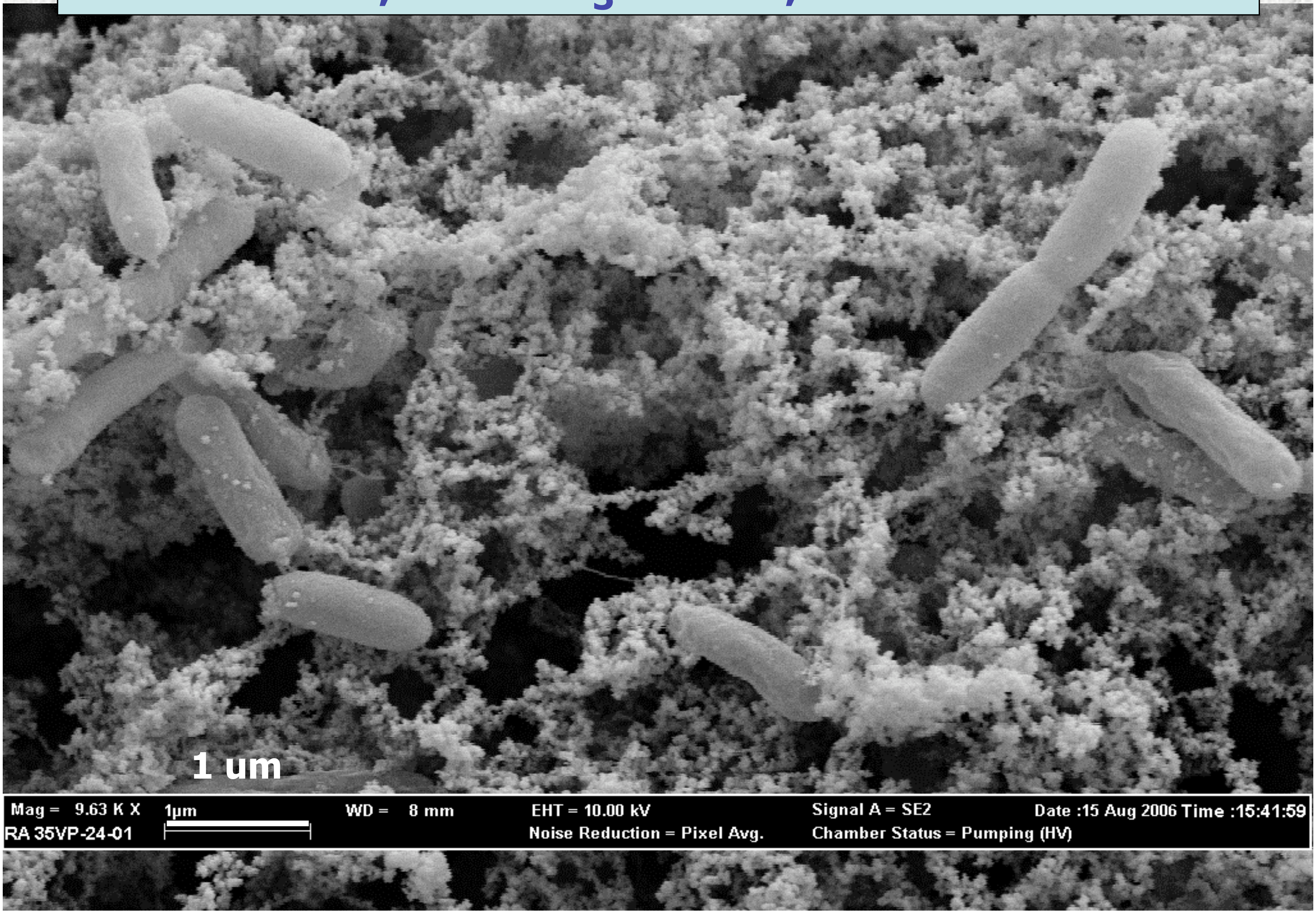
"Slow" – 1*10^8 cell/mL, 20 °C

1^{st} order rate constants

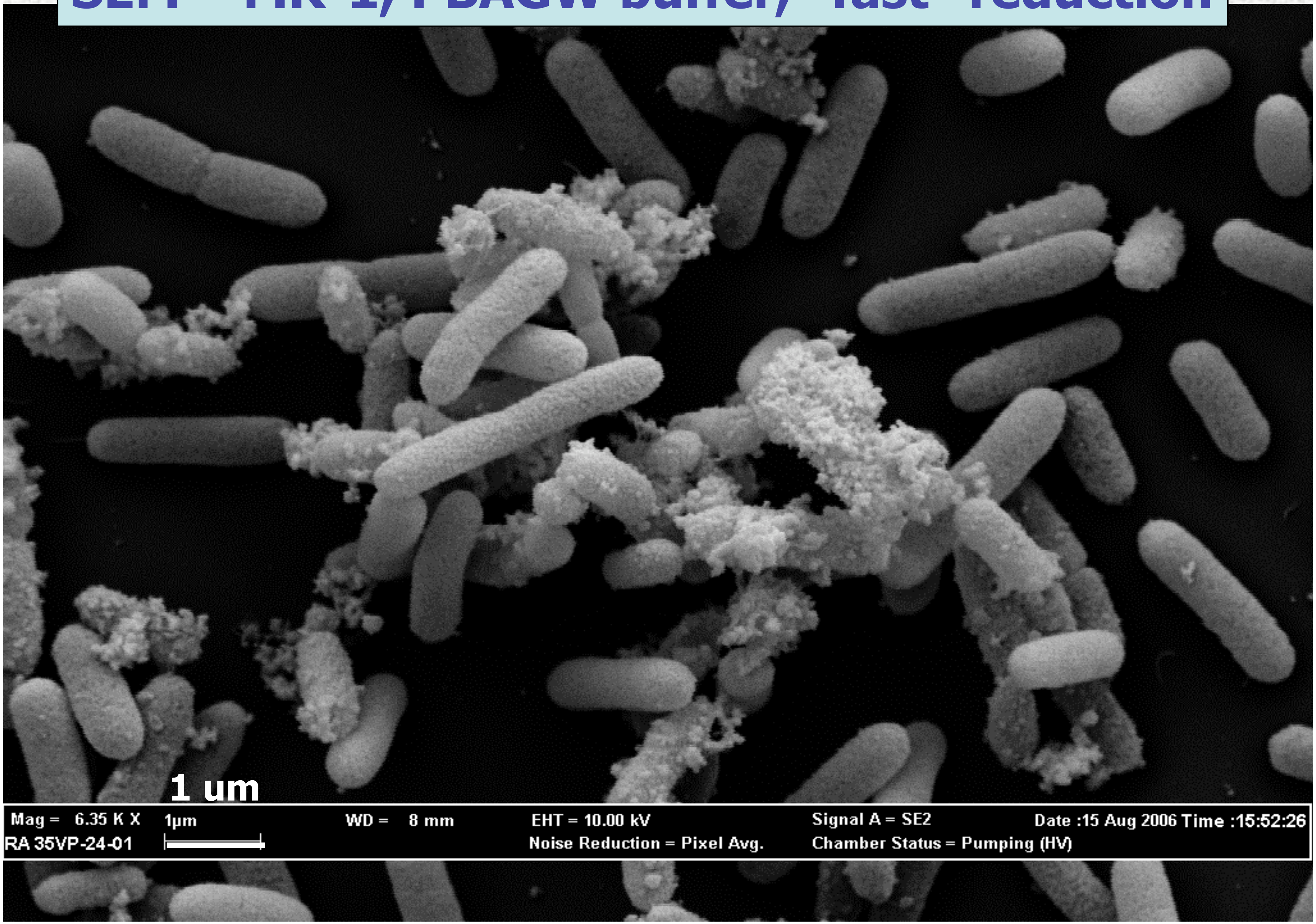
	$NaHCO_3$	PBAGW
"Fast"	11 d^{-1}	0.49 d^{-1}
"Slow"	0.45 d^{-1}	0.050 d^{-1}

SEM – MR-1, $NaHCO_3$ buffer, "fast" reduction

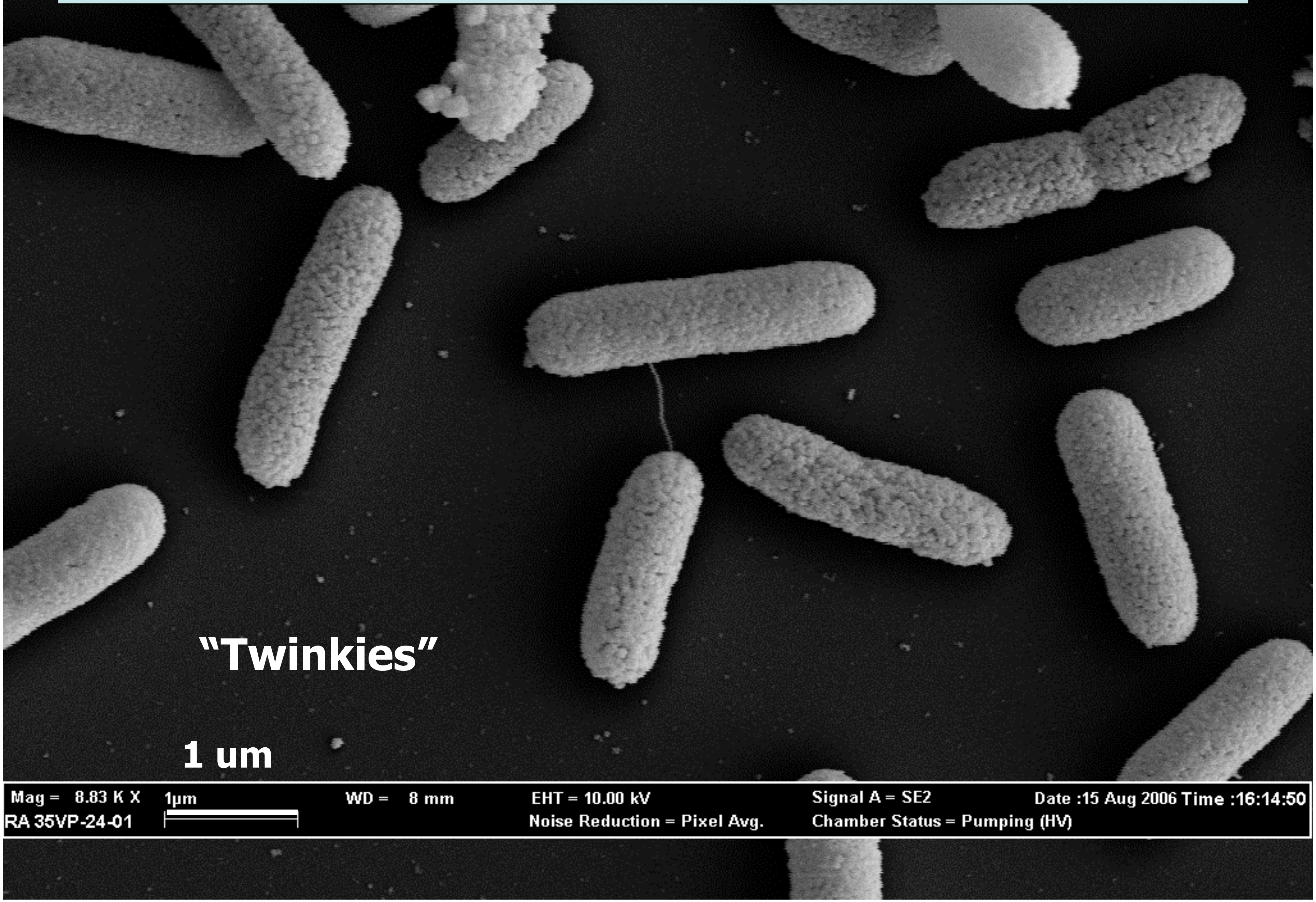
SEM – MR-1, $NaHCO_3$ buffer, “slow” reduction



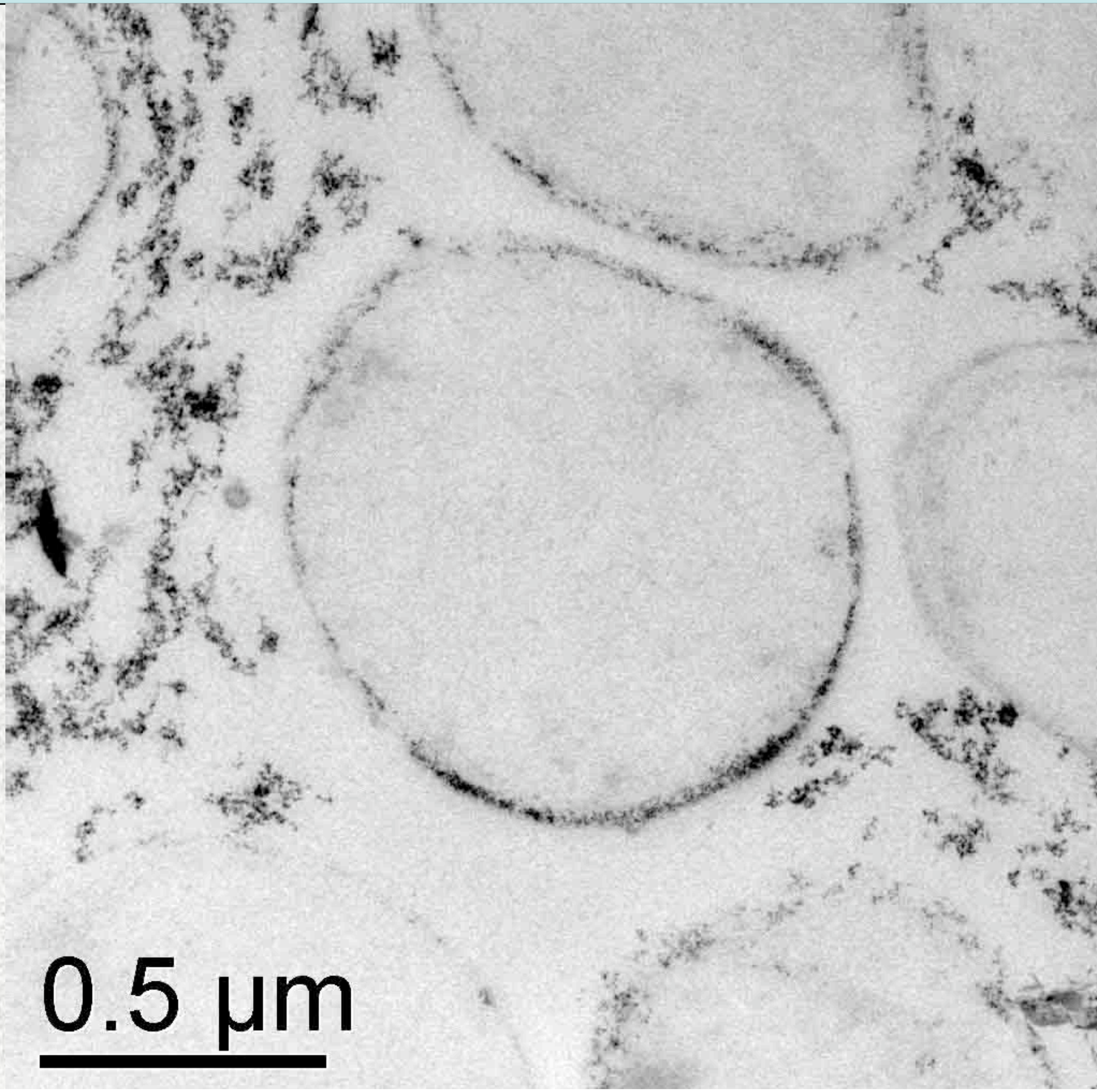
SEM – MR-1, PBAGW buffer, “fast” reduction



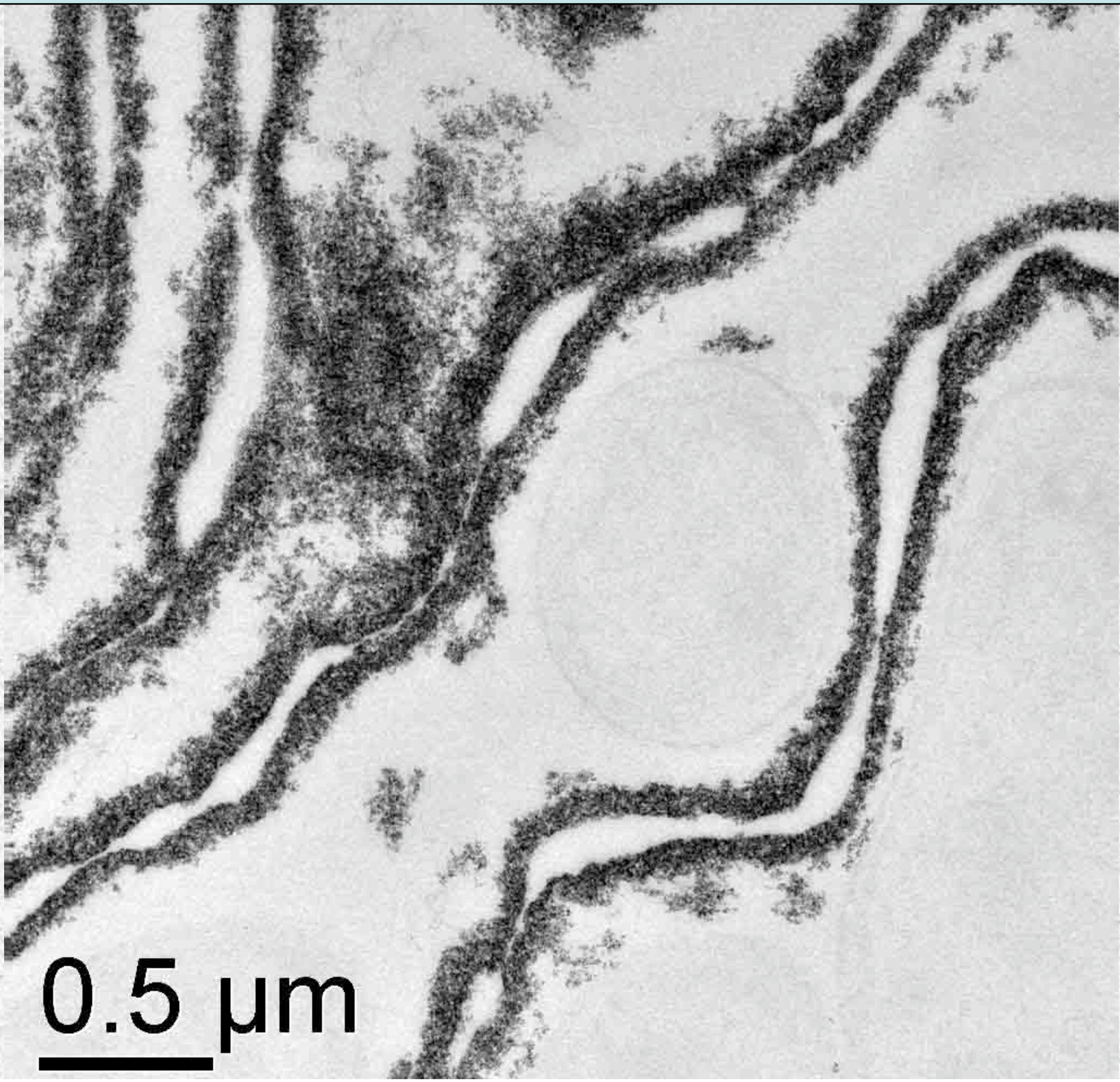
SEM – MR-1, PBAGW buffer, "slow" reduction



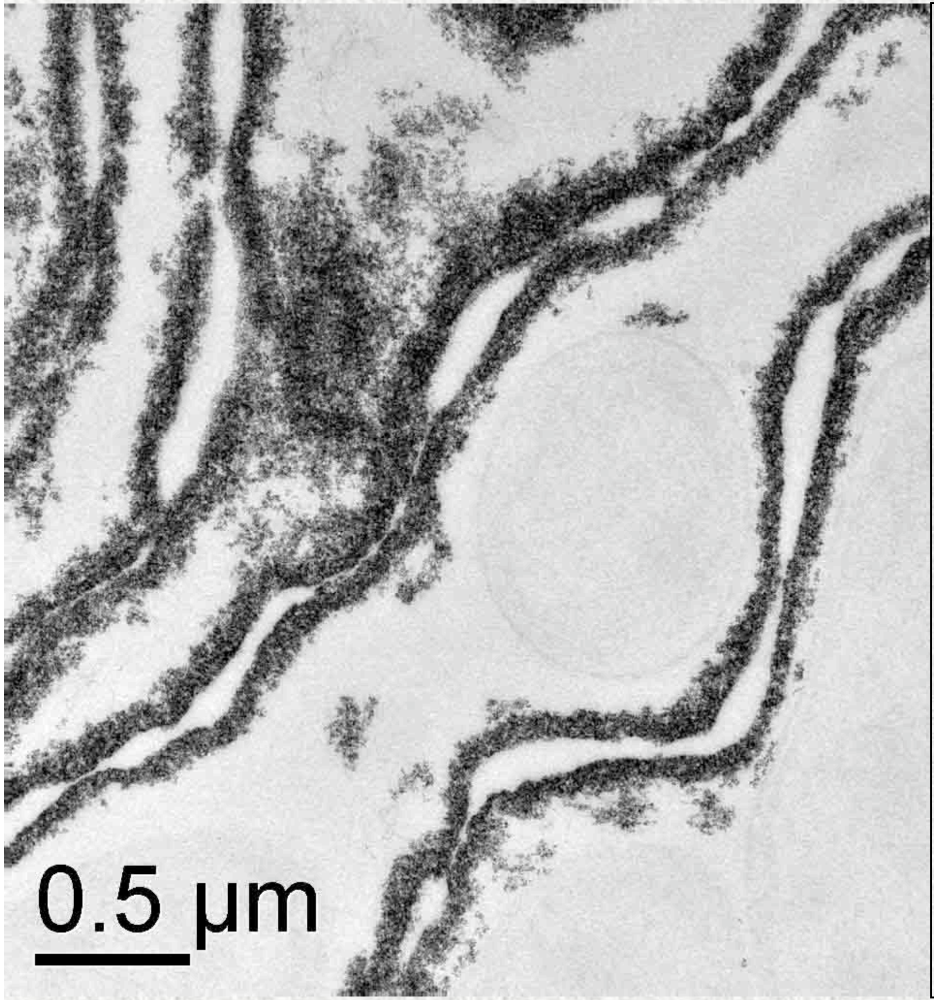
TEM – MR-1, $NaHCO_3$ buffer, “fast” reduction



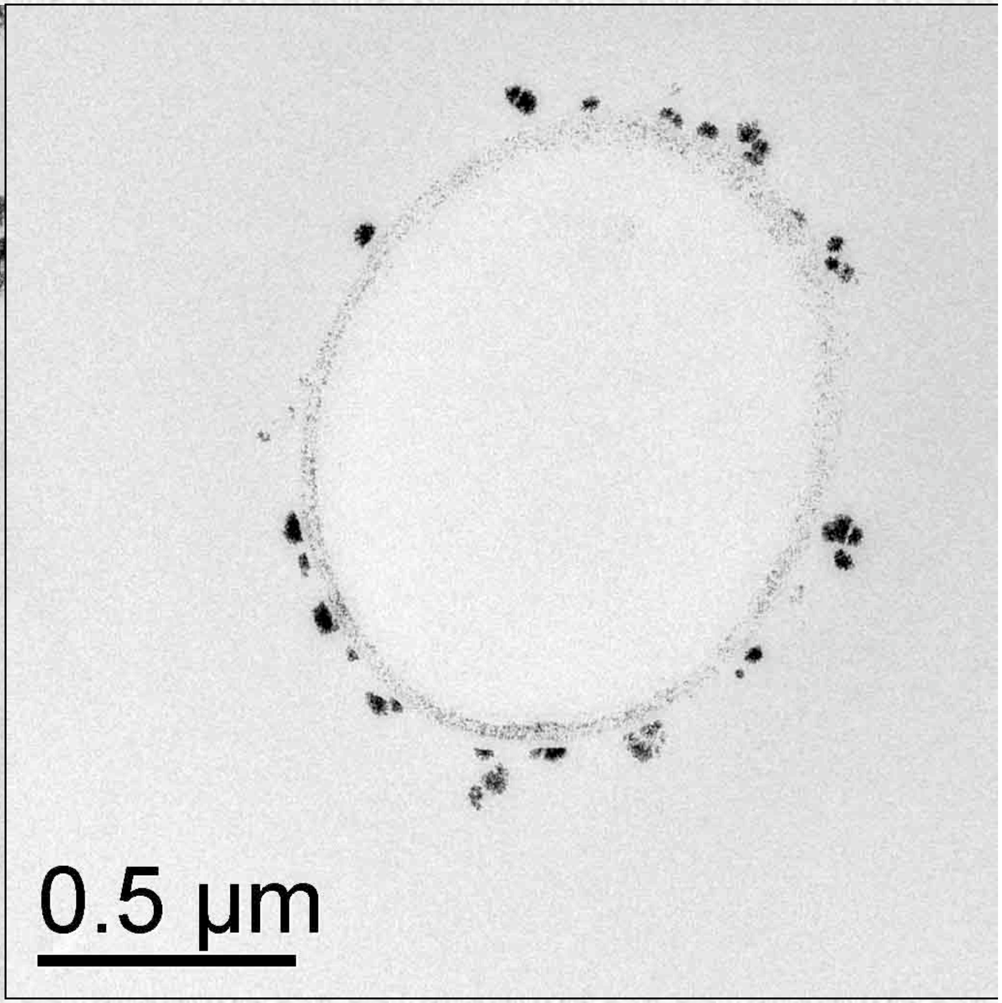
TEM – MR-1, $NaHCO_3$ buffer, "slow" reduction



TEM – $NaHCO_3$, "slow" reduction conditions MR-1 versus CN32

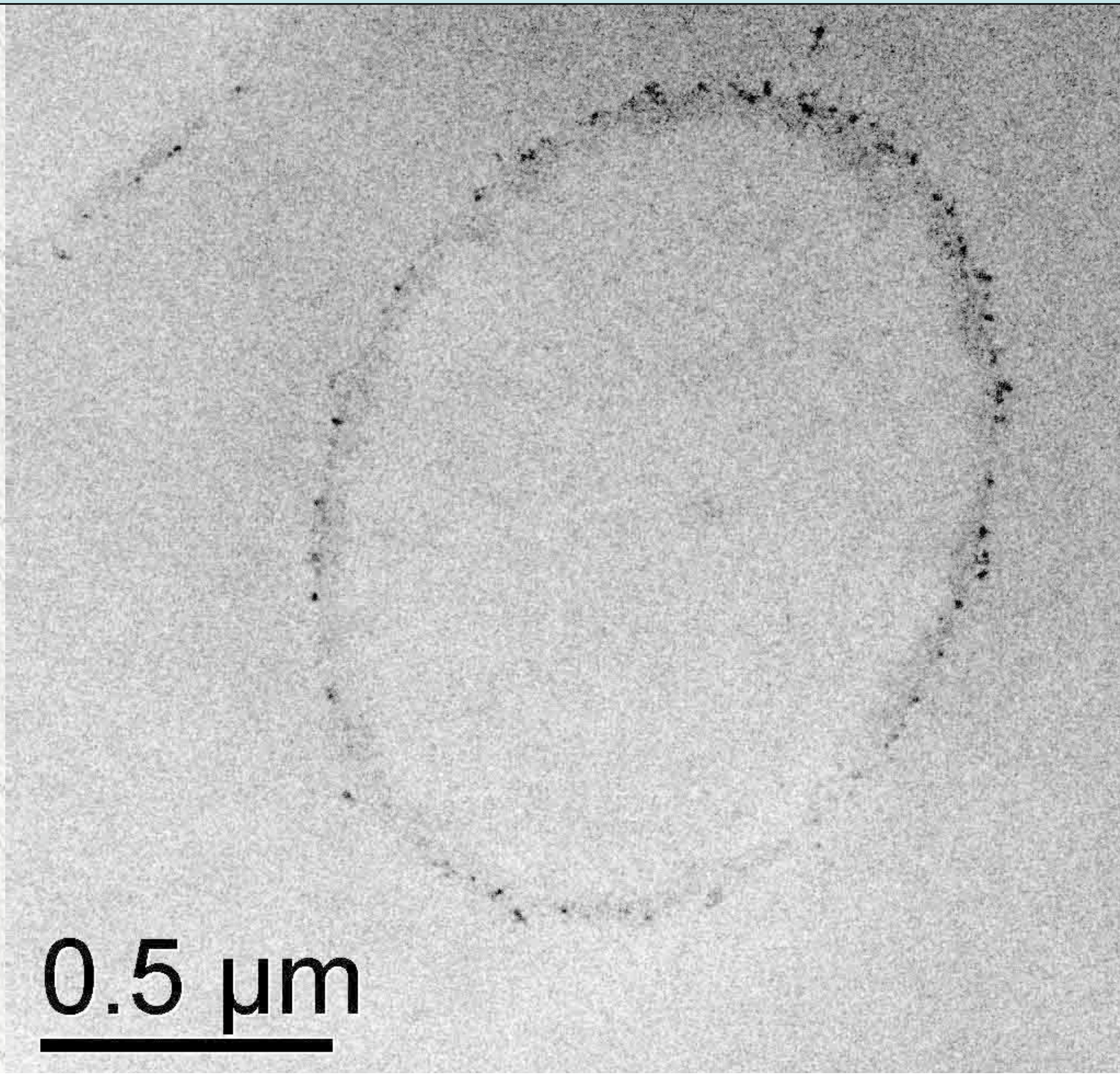


S. oneidensis **MR-1**

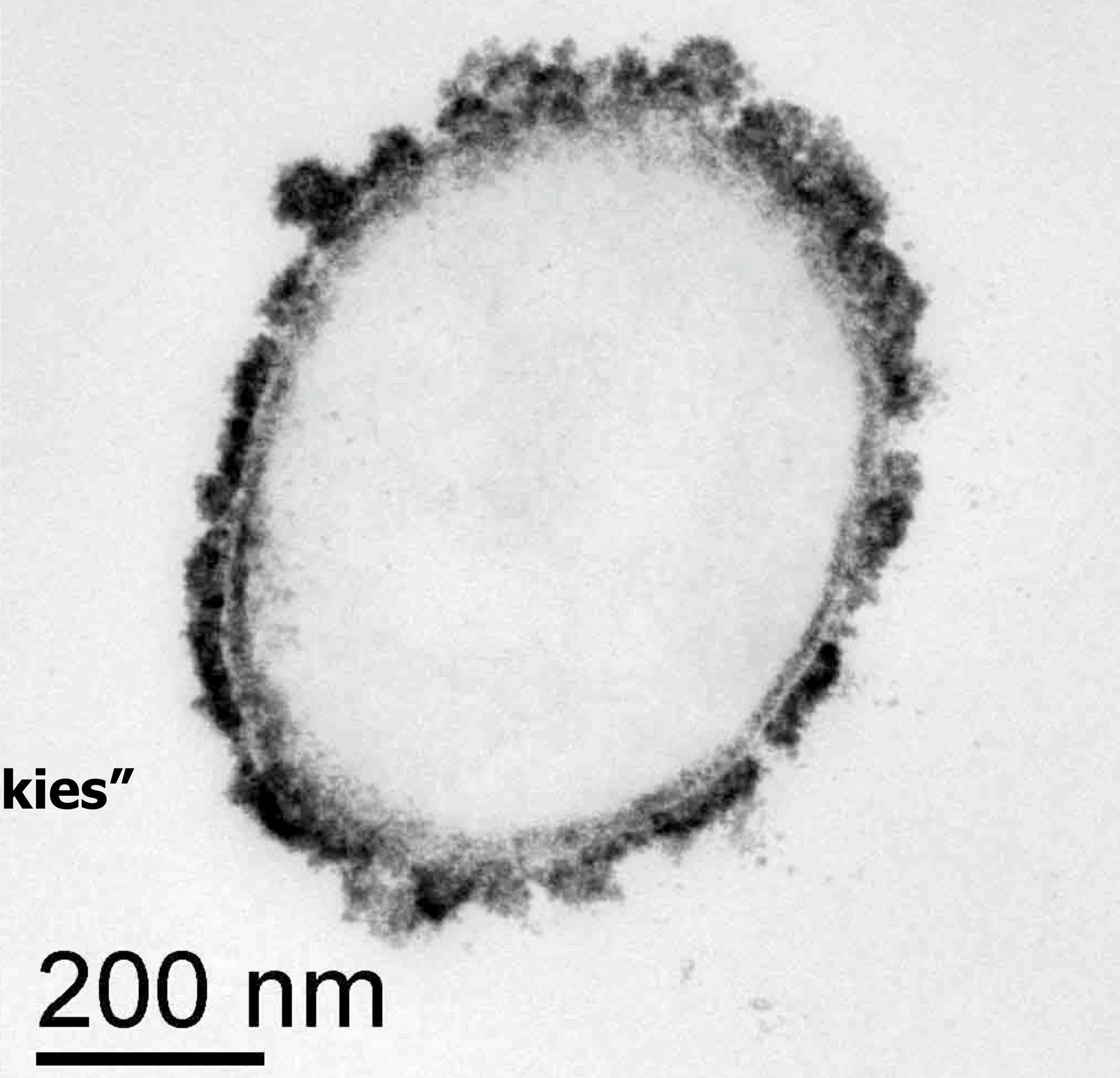


S. putrefaciens **CN32**

TEM – MR-1, PBAGW buffer, “fast” reduction

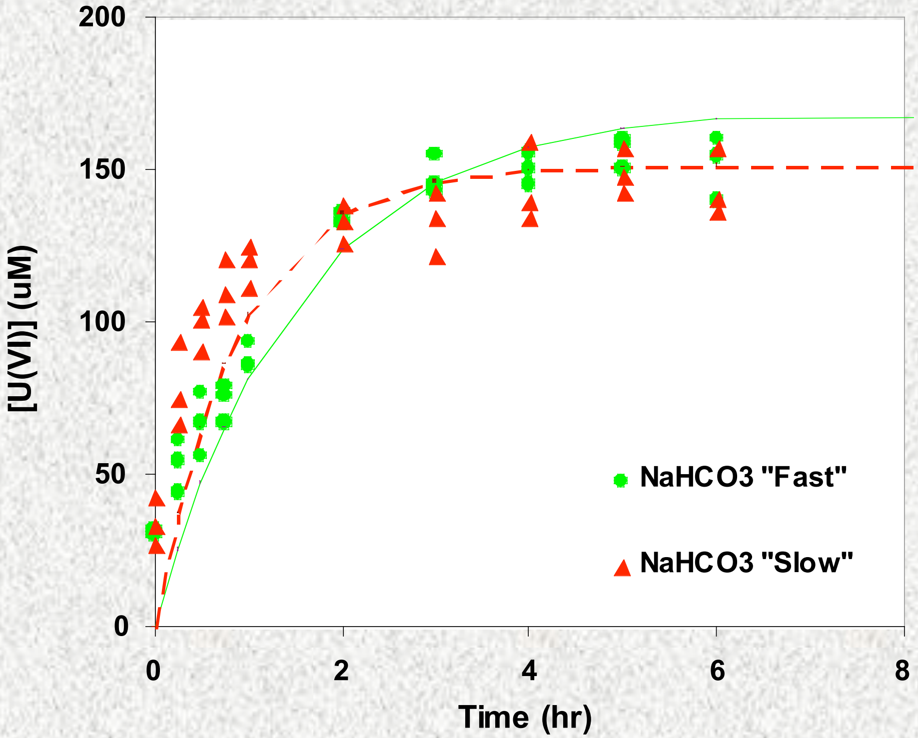


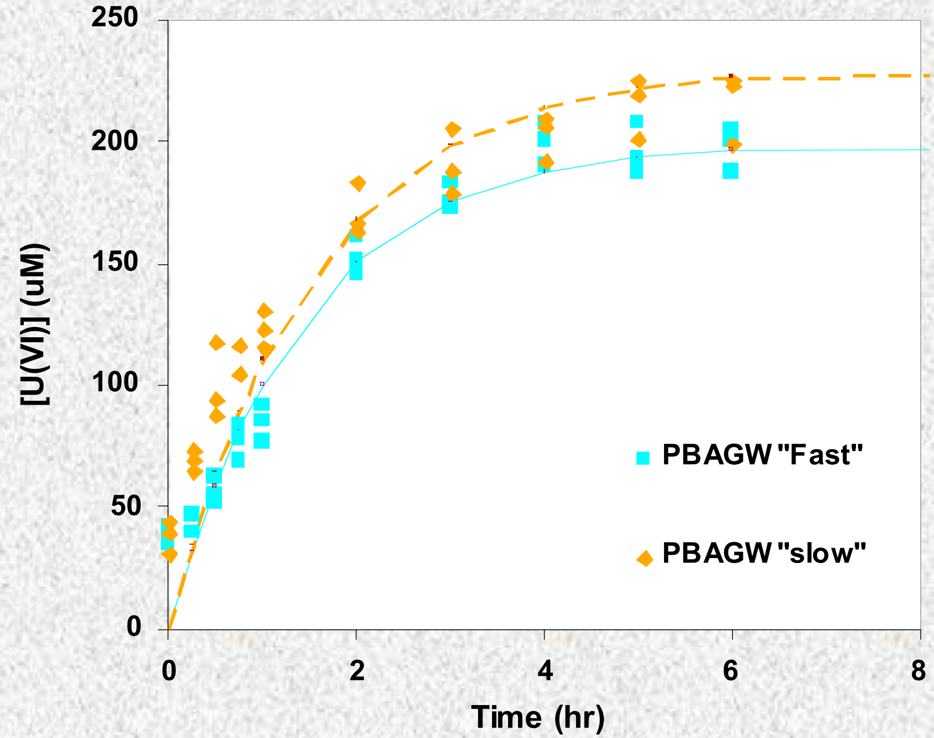
TEM – MR-1, PBAGW buffer, "slow" reduction



"Twinkies"

Air oxidation of biogenic uraninite produced by *S. oneidensis* MR-1





While U(VI) bioreduction rates varied by >10X, rates of $U^{IV}O_2$(s) oxidation by dissolved oxygen were statistically inseparable.

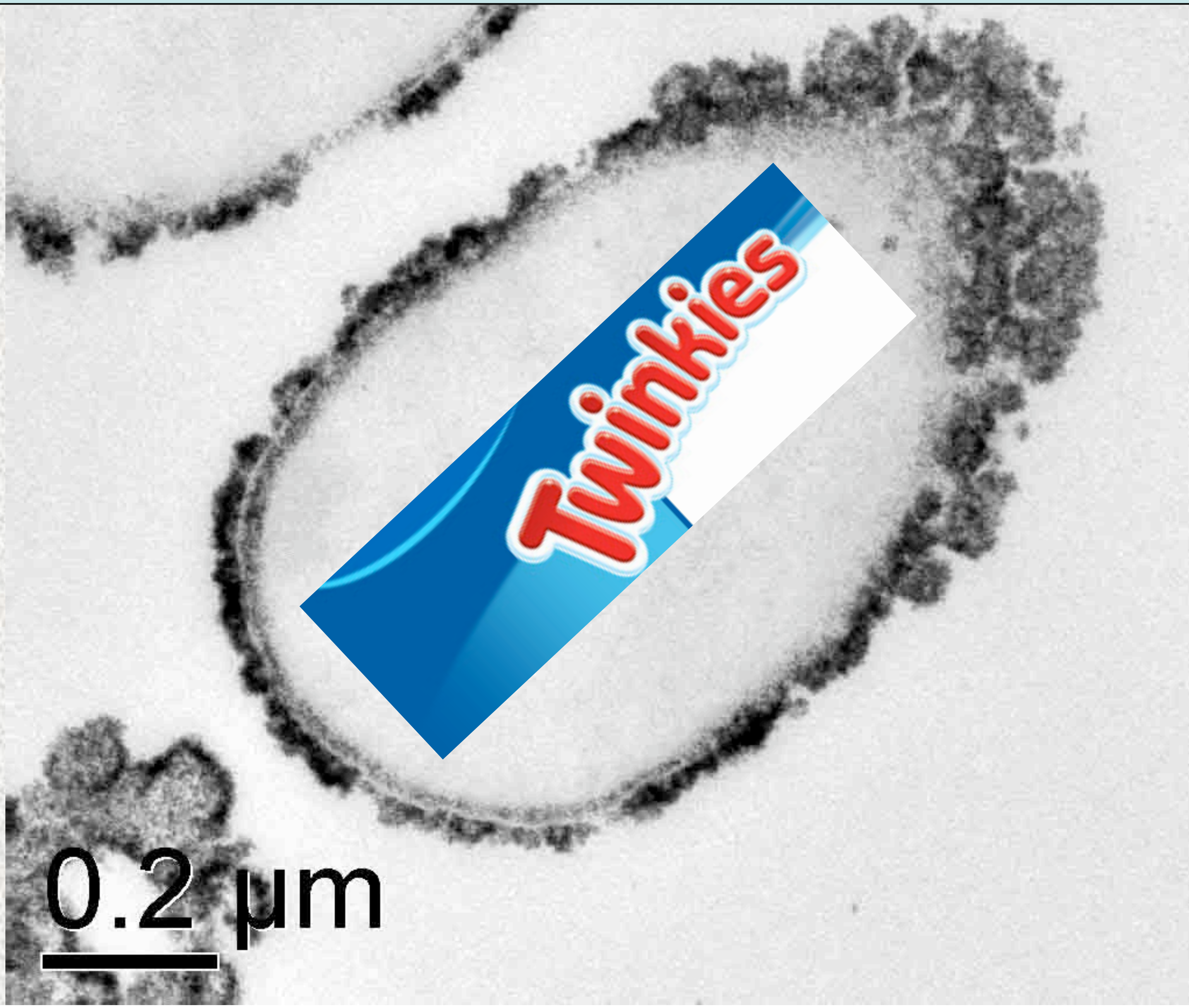
Summary and Conclusions

Hypothesis – Kinetics of U(VI) reduction will directly control subsequent rate of oxidation of biogenic uraninite solids

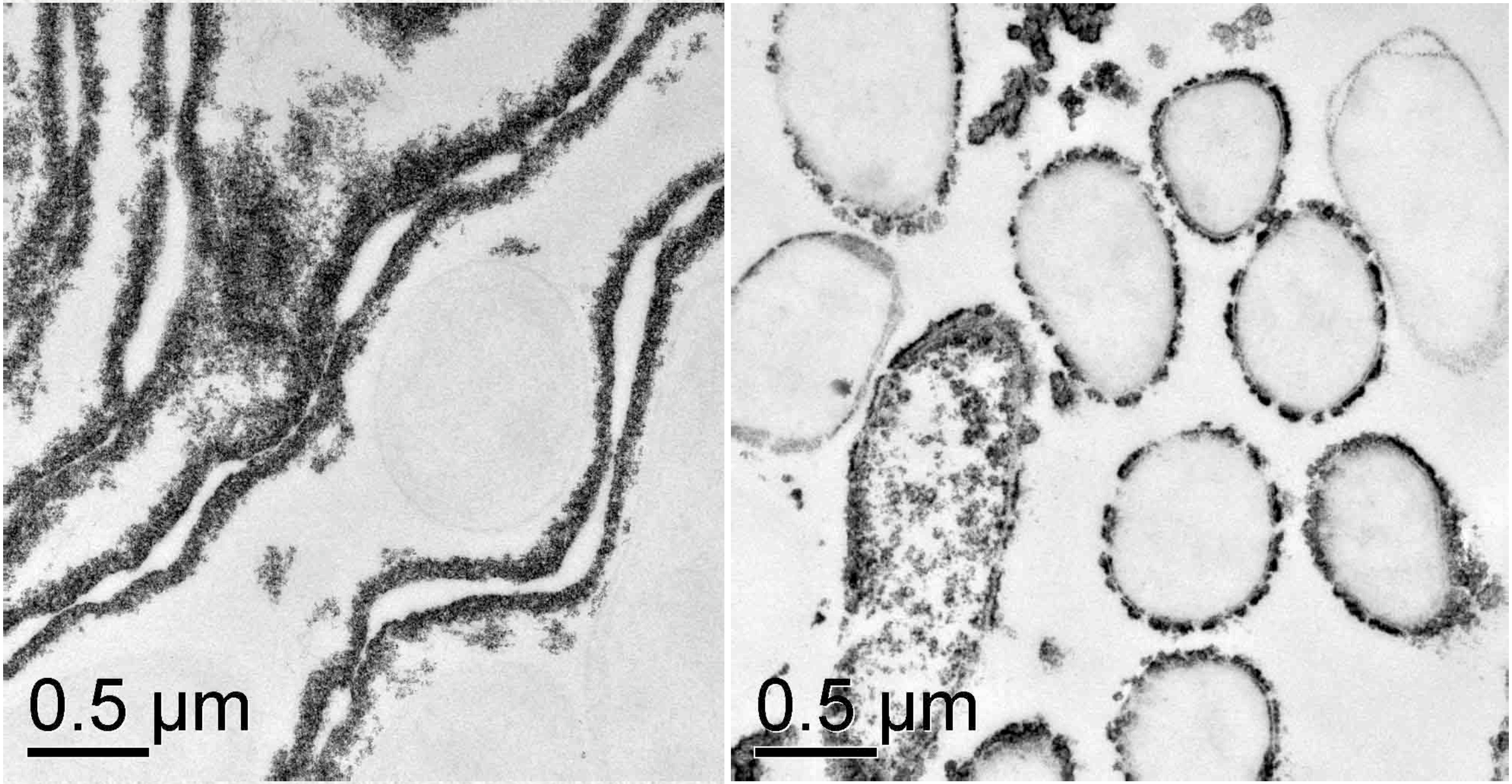
Findings – "Slow" U(VI) reduction rates can produce larger U(IV) particles that are more resistant to oxidation than rapidly formed (and smaller) U(IV) particles. But this was bacteria-specific.

Implication – Molecular-scale mechanisms of U(VI) reduction and UO_2 formation differ between *Shewanella putrefaciens* CN32 and *Shewanella oneidensis* MR-1.

Thanks for your attention.

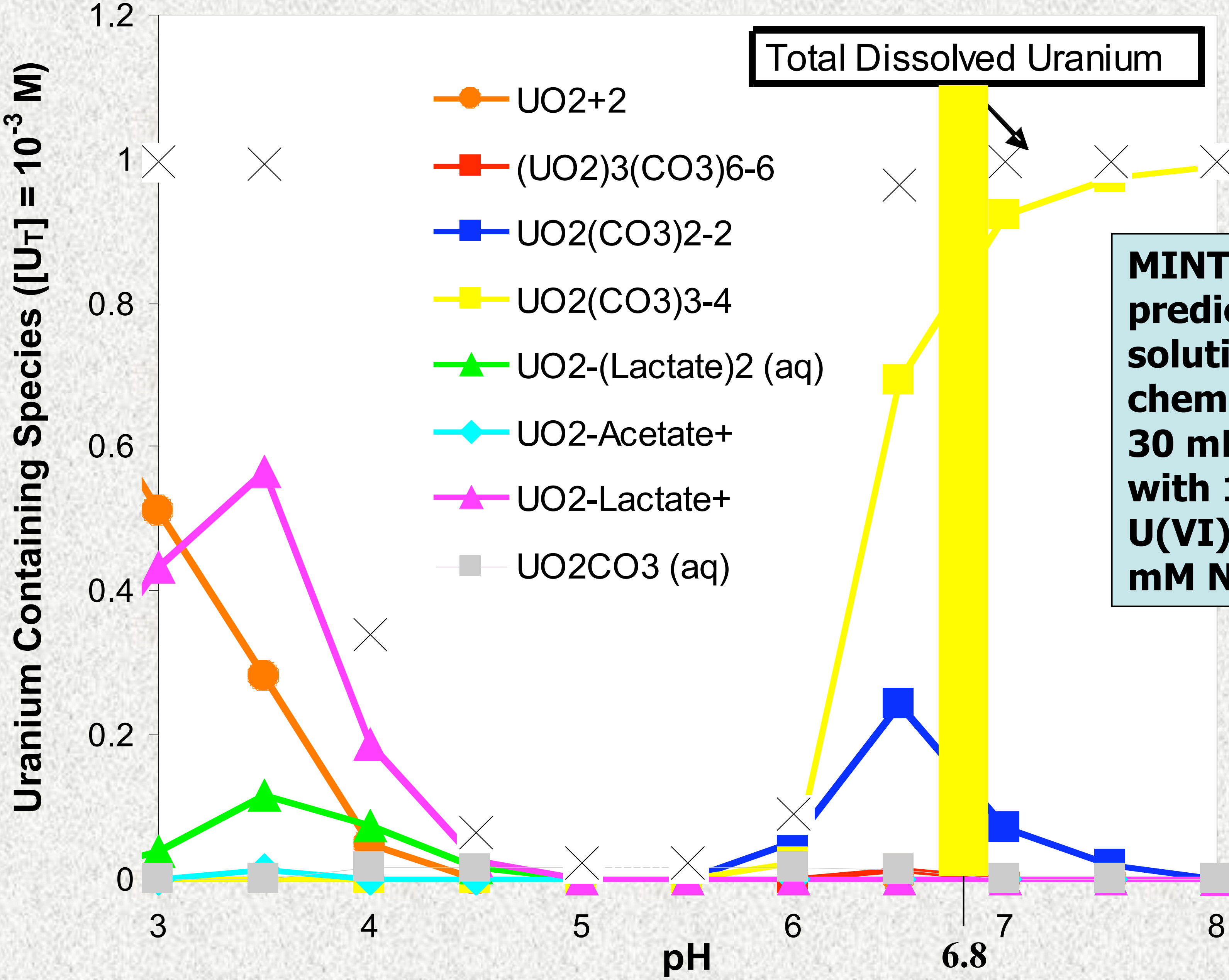


TEM – MR-1, "slow" reduction conditions $NaHCO_3$ versus PBAGW



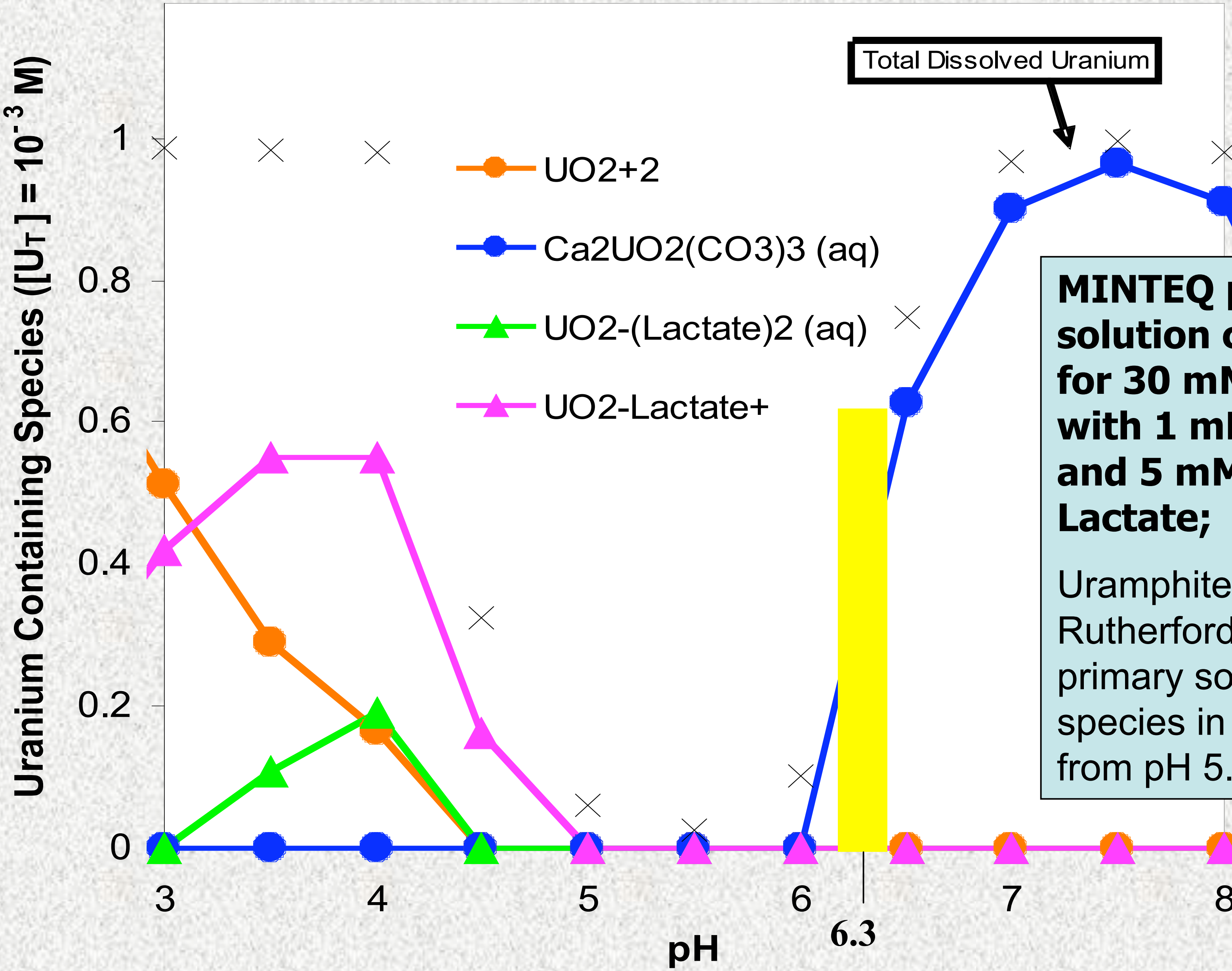
$NaHCO_3$ buffer **PBAGW buffer**

Solution Chemistry – 30 mM $NaHCO_3$



MINTEQ predicted solution chemistry for 30 mM $NaHCO_3$ with 1 mM U(VI) and 5 mM Na-Lactate

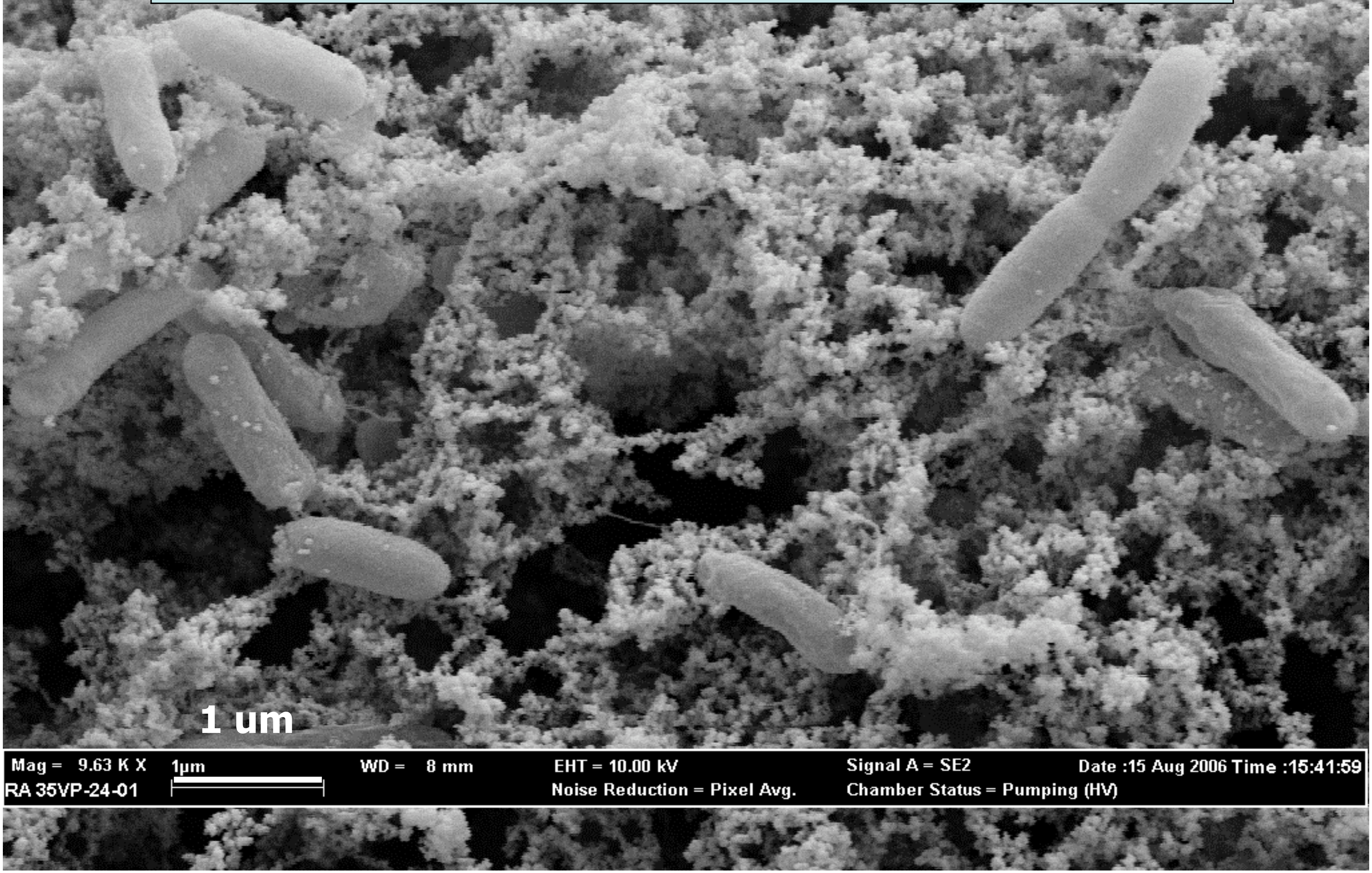
Solution Chemistry – PBAGW



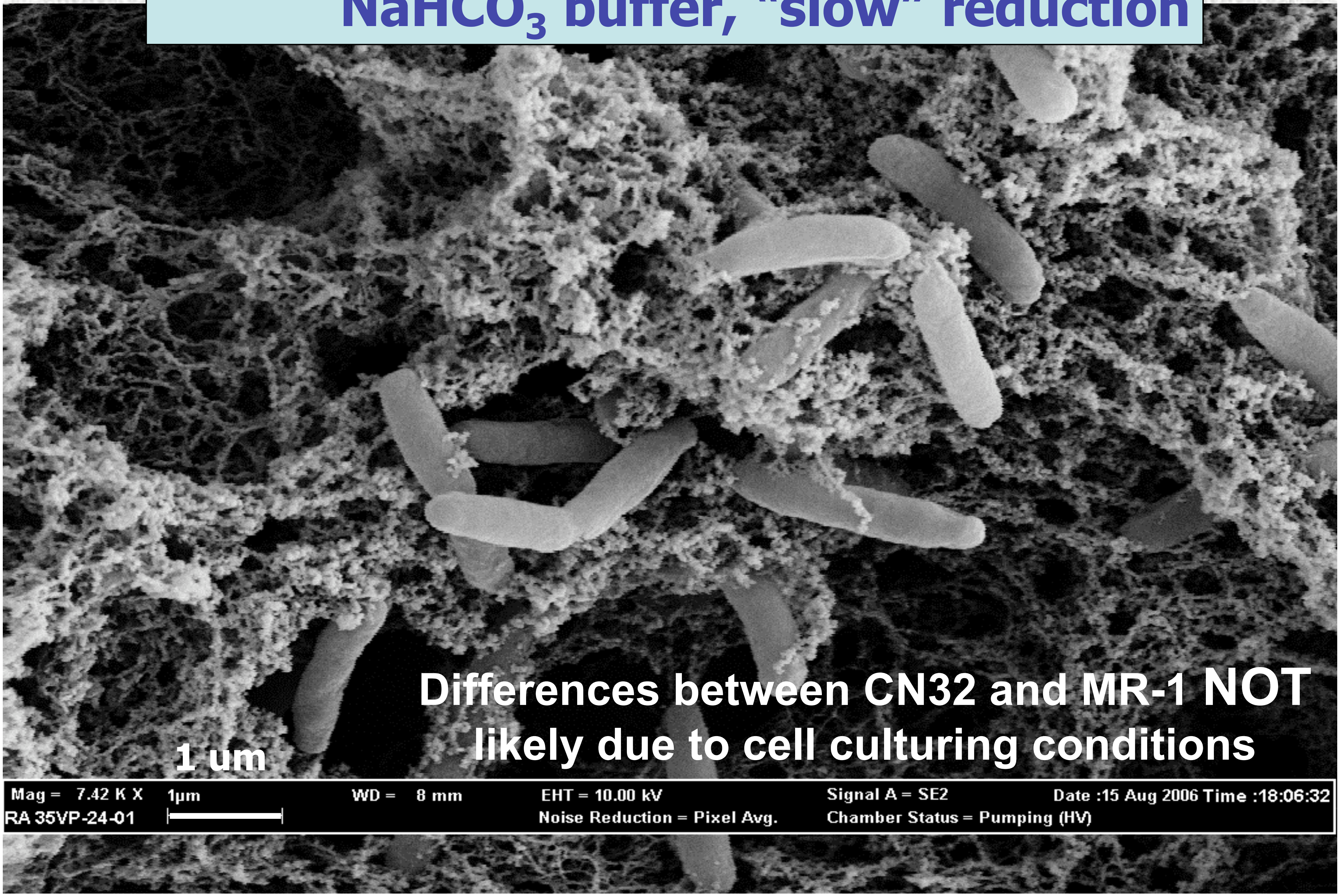
MINTEQ predicted solution chemistry for 30 mM PBAGW with 1 mM U(VI) and 5 mM Na-Lactate;

Uramphite and Rutherfordine are the primary solid phase species in PBAGW from pH 5.0-7.0.

SEM – MR-1 grown in defined media, $NaHCO_3$ buffer, “slow” reduction



SEM – MR-1 grown in TSB-D $NaHCO_3$ buffer, “slow” reduction



Differences between CN32 and MR-1 NOT likely due to cell culturing conditions