IN SITU COMMUNITY CONTROL OF THE STABILITY OF BIOREDUCED URANIUM

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NABIR

UT CBA

We could not do this work without our excellent collaborators!

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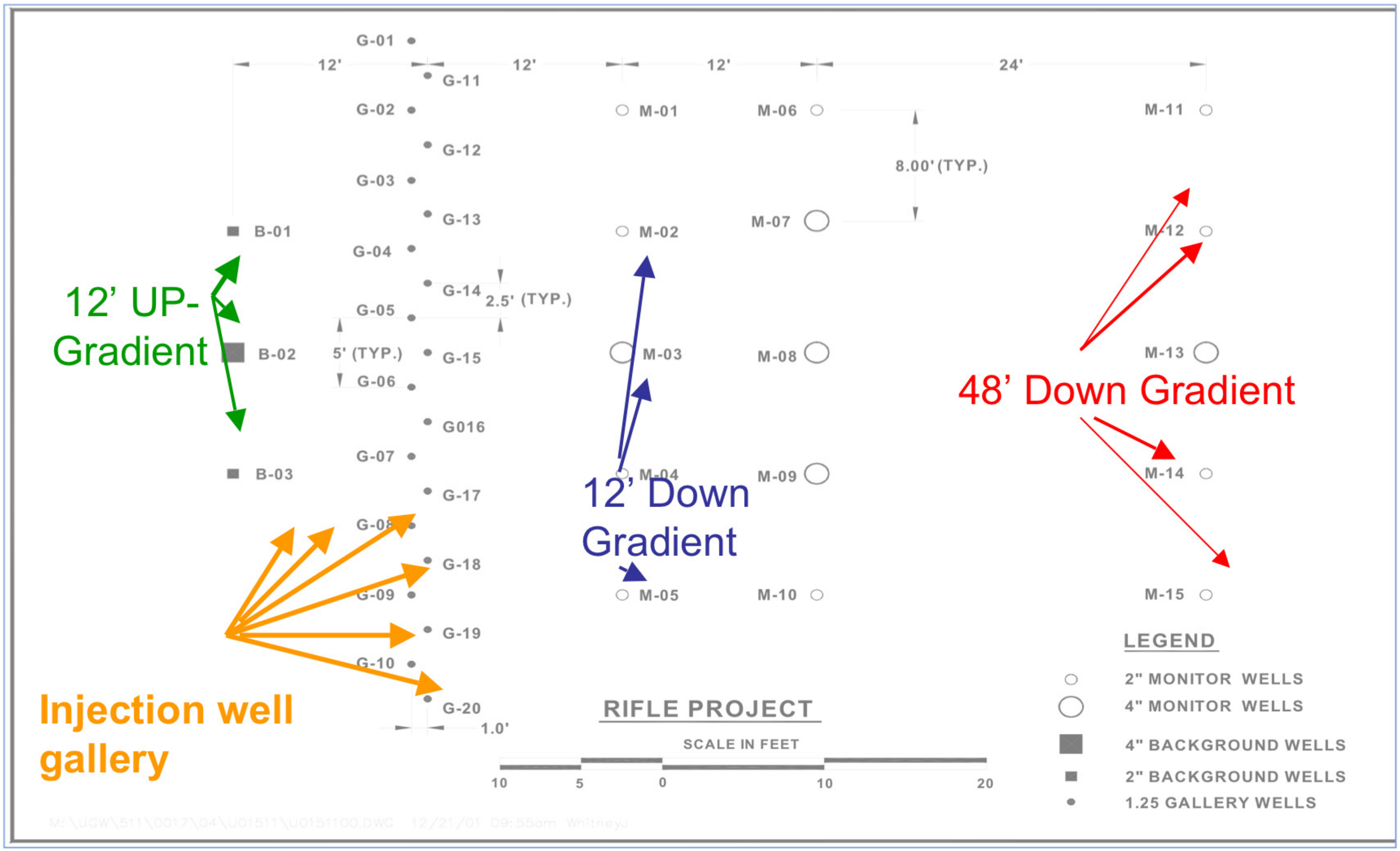
Darrell Chandler

ANL

Shift of viable biomass & community composition by acetate addition to groundwater ~UMTRA site

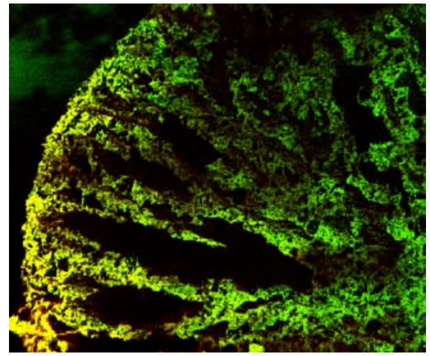
Well field



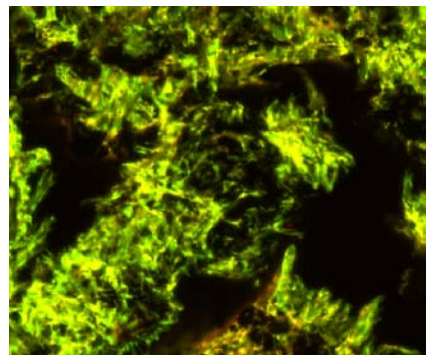
UMTRA Old Rifle site

Sampler with Beads

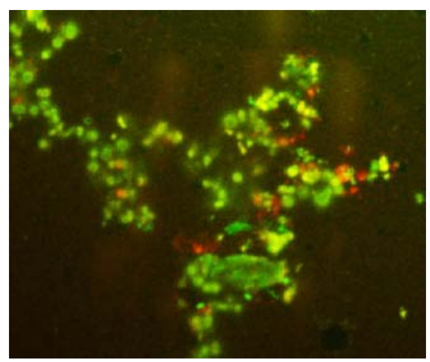
Confocal Laser Scanning Microscopy of beads



CLSM cross section of bead showing surface area for colonization

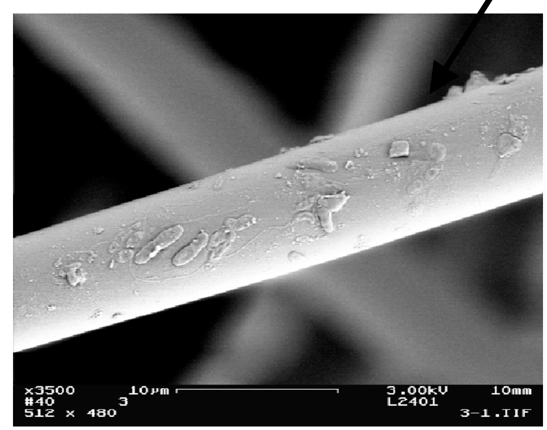


CLSM close-up of cross section



CLSM image of microbial colonization live/dead stain

SEM of microbial colonization of glass wool



Acetate Infusion induced

A. Changes in the mineralogy ⇨ $FeS_{0.9}$

B. Changes in the microbial community

Program is to test hypotheses as to How changes in A & B Maintained the decrease in U(VI) in the groundwater

TEST

Monitor U(VI) loss in-well sediment incubators different mineral & microbiological amendments

Assessing Subsurface Microbiota

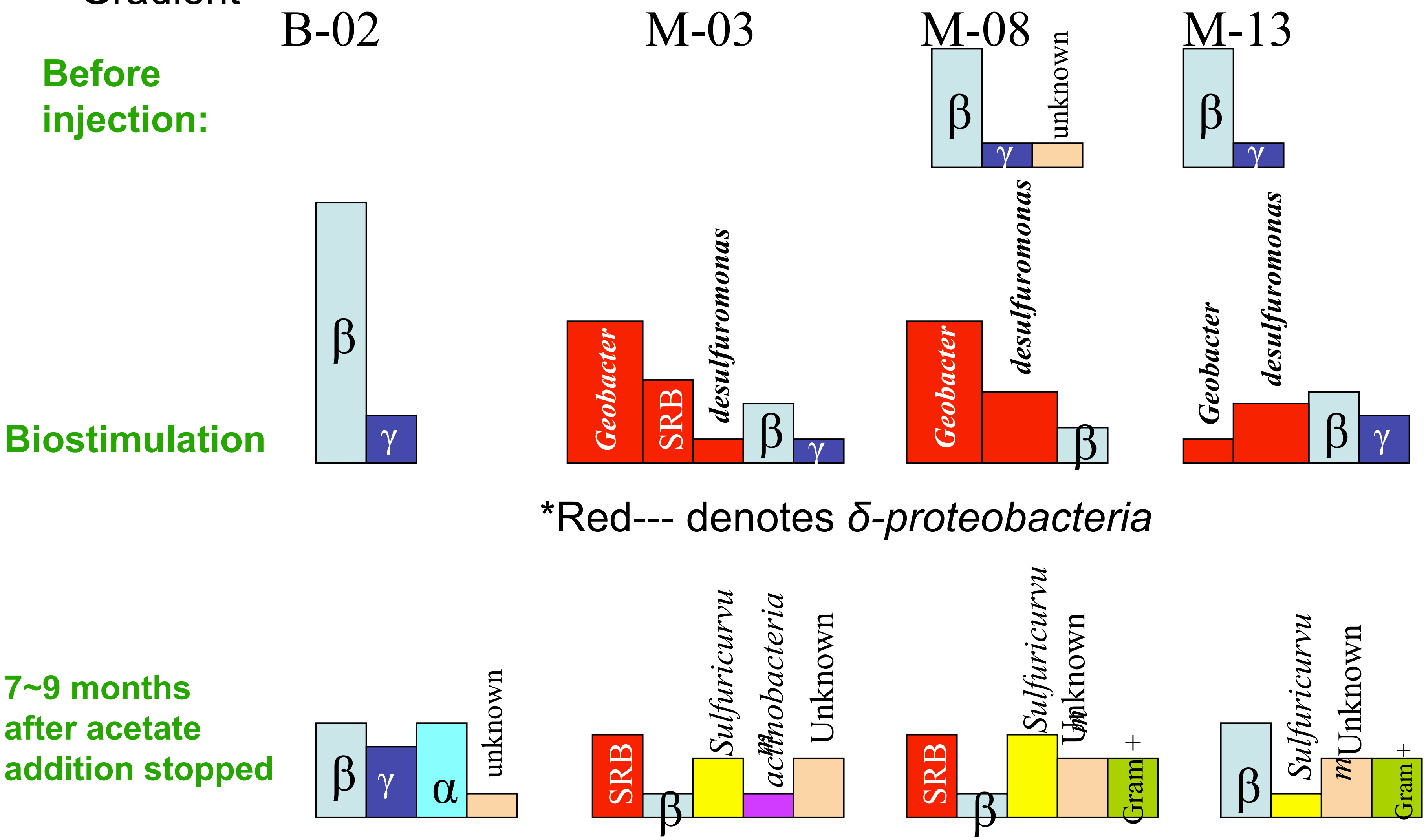
Collecting Microbes

	Bead Coupons	**Groundwater**	**Sediment**
	Must colonize from water	Grab Sample	Disruptive
	Actively Growing	mRNA	Includes slow & non
	^{13}C in DNA & PLFA	+ Non Growing	Growing
Biomass	Intermediate	Lowest	Highest

Complexity Increases →

Why did U(VI) decrease in groundwater persist 7-9 months after acetate infection stopped? (Bead Coupons)

Up-Gradient 12’ Down-Gradient 24’ Down-Gradient 48’ Down-Gradient



*Red--- denotes *δ-proteobacteria*

Preliminary data (DGGE from Bead Coupons) suggests:

Up gradient & before injection stable community dominated by β-protrobacteria (*Hydrogenophaga, Dechloromonas, Rhodoferax, Ferribacterium*, *Rhodocyclus, Methylophilus, Azoarcus,* γ-Proteobacteria *Pseudomonas*
A Gram-negative, heterotrophic, carbon-limited, (high cyclo/monoenoic PLFA), facultative-anaerobic, oxygenated (UQ/MK~2), utilizing refractory organics

Infusion *Geobacter, Desulfuromonas/Pelobacter* DIRB, δ-Proteobacteria *Desulfobacter,* β-protrobacteria *Ferribacterum*, γ-Proteobacteria *Pseudomonos*,

Rapid Biostumulation DIRB,SRB goes anaerobic & growth stimulated

7-9 months later , Sulfuricurvum, β-& γ-Proteobacteria, Gram-positive Clostridia, δ-Proteobacteria SRB

Sulfur oxidizing bacteria use sulfide to ➡ reduce Nitrate/ Oxygen maintain anaerobic status UQ/MK ~06-0.2 & SRB form U(IV)?

Hypotheses:

1) After stimulation Fe(III) terminal electron acceptor ⬇
   non sulfate-reducing DIRB will be leave or be out-competed by more versatile microbes ? SRB

2) SRB & Sulfur oxidizing bacteria play a critical role in the post-treatment maintenance of bio-reduced uranium by directly reducing U(VI), generating H_2S, HS^- and/or $FeS_{0.9}$ ➡oxygen sinks maintaining U(IV).

3) Bioprecipitated amorphous $FeS_{0.9}$ in sediments will maintain low U(IV) reoxidation rates under conditions of low biomass, especially in presence **of sulfur oxidizing bacteria**

   but $FeS_{0.9}$ by itself is not sufficient to remove U(VI) from groundwater by abiotic reduction

Conceptual Model

Start Acetate to C-starved β-proteobacter, oxygenated ➡ Anaerobic ➡ Geobacter + DIRB ➡ reduce Fe(III) ➡ great ⬆ increase biomass ➡ Lo ⬇ cal Fe(III) surfaces all reduced ➡ wave Geobacter (+ DIRB) moves distally

Continued acetate ➡ SRB increase in diversity (DSR) & biomass ➡ produce HS^- ➡ HS^- + Fe(II) ➡ $FeS_{0.9}$

Stop Acetate Biomass ⬇ Cell lysis feeds Heterotrophs

Gram-positive Clostridia + Desulfotomaculum (+ SRB) ➡ reduce U(VI) & produce acid ⬇ carbonate & U(VI) complex

Sulfur Oxidizing (Sulfuricurvum) use HS^- to reduce NO_3 & O_2

Without NO_3 & O_2 Dechloromonas & Geobacter not reoxidize U(IV)

Does Cigar Lake U mine deposit have high $FeS_{0.9}$ + SOB & low UQ/MK?

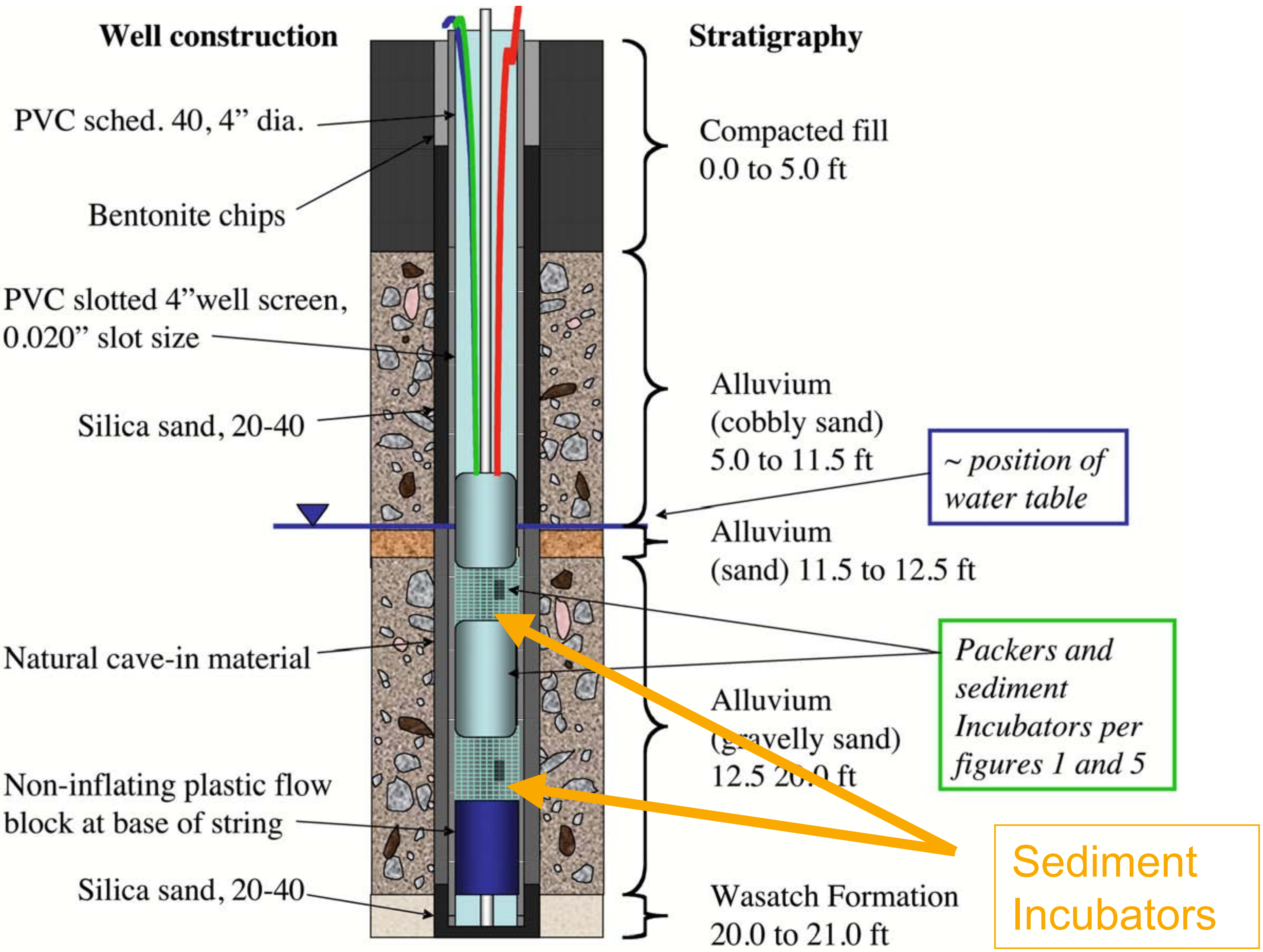


Figure 4. Proposed emplacement of two sediment incubators in a typical 4” well (B-02, Old Rifle UMTRA Site).

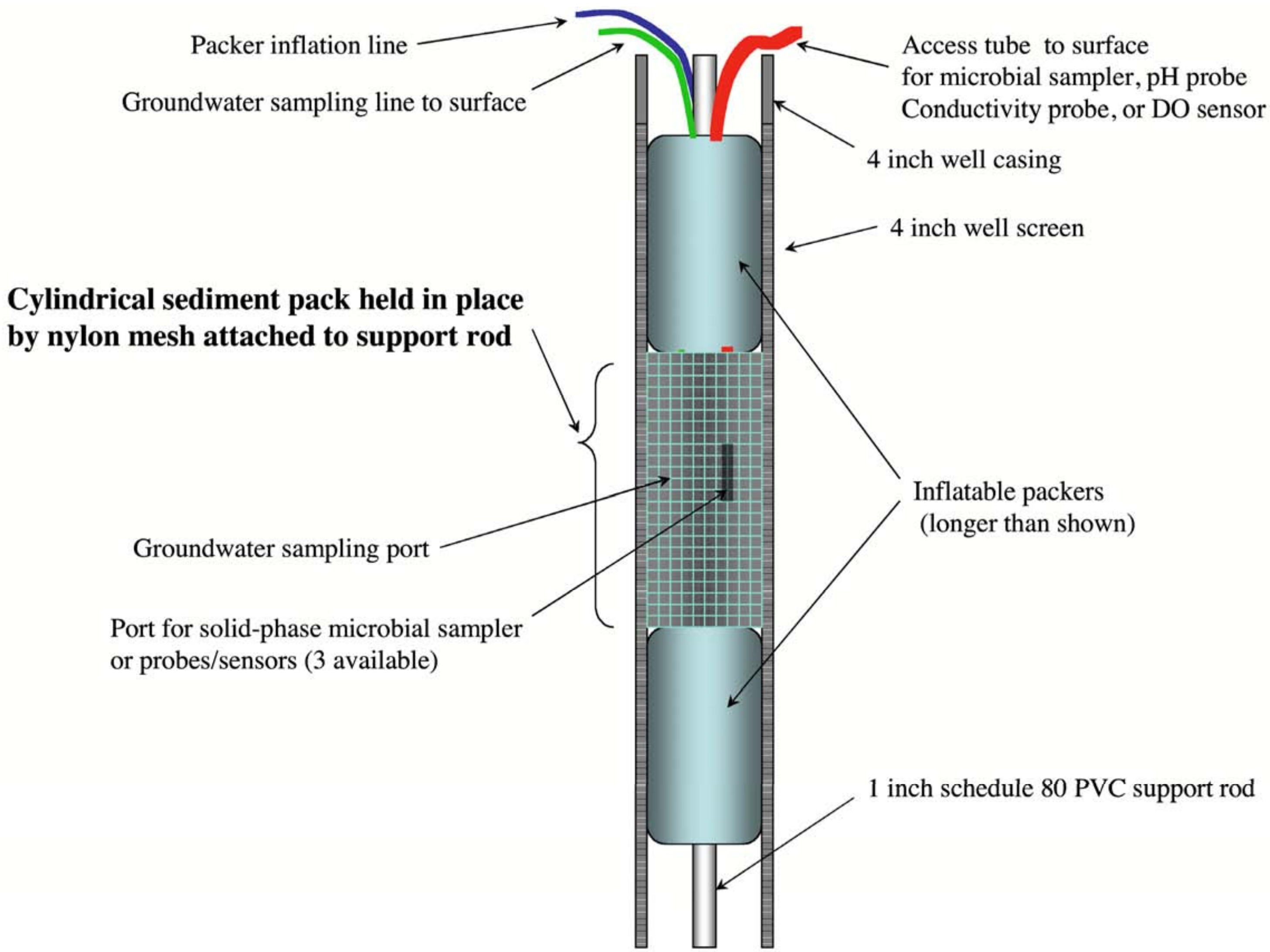


Figure 1. Conceptual design of sediment incubator

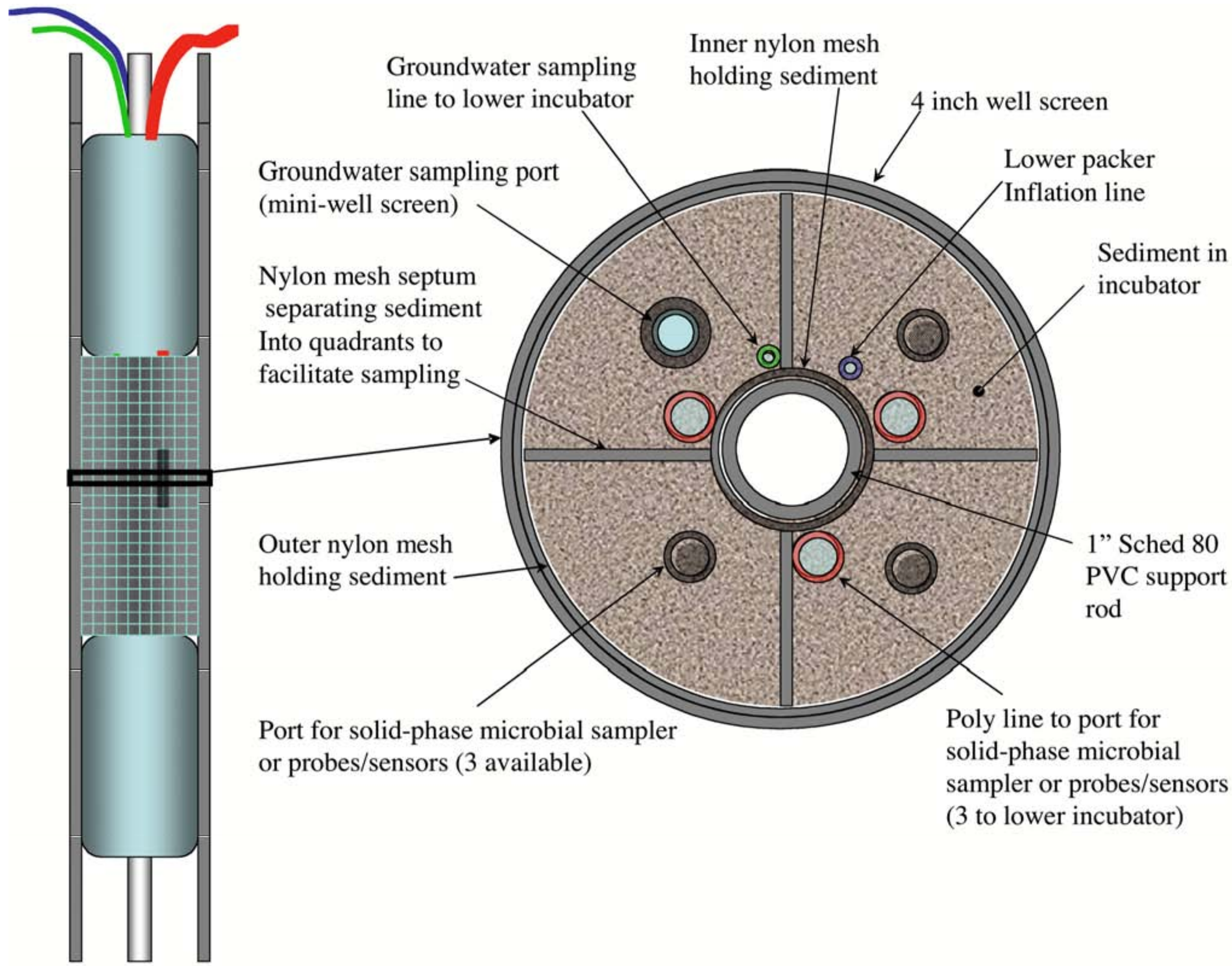


Figure 5. Top view (section) of sediment incubator.

Sediment for the in situ sediment incubators

Rifle sediment Cobles removed & mixed 1-3 mm sieved for higher permeability sand-silt

U(VI) 0.17 mg/L from groundwater during perfusion

Sediment + DIRB + Lactate ➡ SRB⬆ DIRB ⬇ [Hypothesis 1]

Sediment + Lactate + DIRB vs Sediment + Lactate + SRB measure bioreduced U(IV) [Hypothesis 2]

Sediment + $FeS_{0.9}$ Sterile (short time) vs SRB + acetate [Hypothesis 3]

Model: Sediment + FeS_{09} + Lactate SRB + SOB

Assessing subsurface microbiota

From Sediment Samplers

DNA 16S rRNA, rDNA,

Genes

DSR (SRB),

DIRB ? NADPH-iron reductase?

*sox*A sulfite oxidase

by DGGE, Q-PCR, T-RFLP

RNA D. Chandler

Lipids PLFA, Respiratory Quinones, PHA, DMA (Clostridia) ? Spores (DPA)

Better Respiratory Quinone Assessment

Problem present at mmol/mol PLFA

HPLC/electrochemical cell/electrospray/ MS/MS

HPLC separates components so greater duty cycle

Electrochemical cell ➡ Reduces only Quinones at the specific Eo potential : + 112 mV Ubiquinone; + 36 mV Desmethyl Menaquinone; - 74 mV Menaquinone

100% ionized with - 2 charge ➡ ideal for electrospray ionization

Compare to atmospheric pressure chemical ionization (APCI) ~inefficient H^+ charge transfer from activated gas

MS/MS search for progenitors ions at products: UQ m/z – 197, - 98.5
DMK m/z - 173, -86.5; MK m/z -187 -93.5

Greatly increase sensitivity ➡ greatly increase localization