

Genetically Enhanced Rhizoremediation

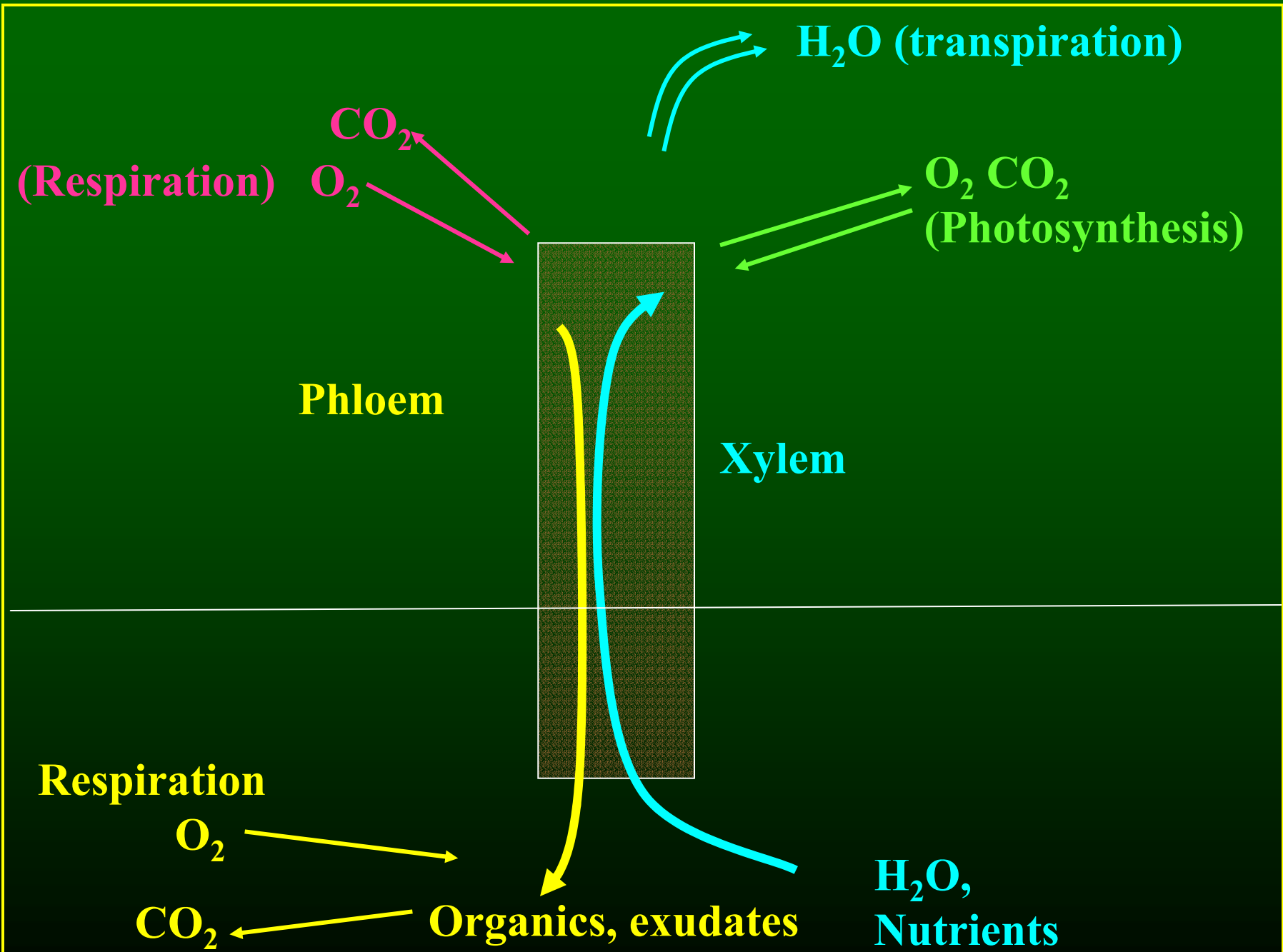
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Phytoremediation Mechanisms

- Stabilization
- Uptake
 - Degradation
 - Volatilization
 - Storage
- Rhizosphere degradation



Technological Approach

- Stabilization
 - Utilizing high transpiration rates to hydraulically isolate contaminated site
- Genetically Enhanced Rhizoremediation
 - Utilize rhizosphere as a selective environment.
 - Promote established symbiotic plant-microbe relationships to promote biodegradation.
 - Select ideal plants for contaminated site.

Plant-Microbe Symbiosis

Genetically engineered microbes (GEMs) languish in uncontrolled environment, flourish in selective lab

Rhizosphere is a selective environment

- Organic substrate: Unique and plentiful
- pH properties: Can vary > 1 unit from bulk soil
- Nutrient availability: Enhanced by disturbance and hydraulic gradient
- Attachment sites: High surface area roots

State of Technology

Proof of concept at bench scale with numerous contaminants

Little on optimizing for application

No known work on Dioxins and Furans

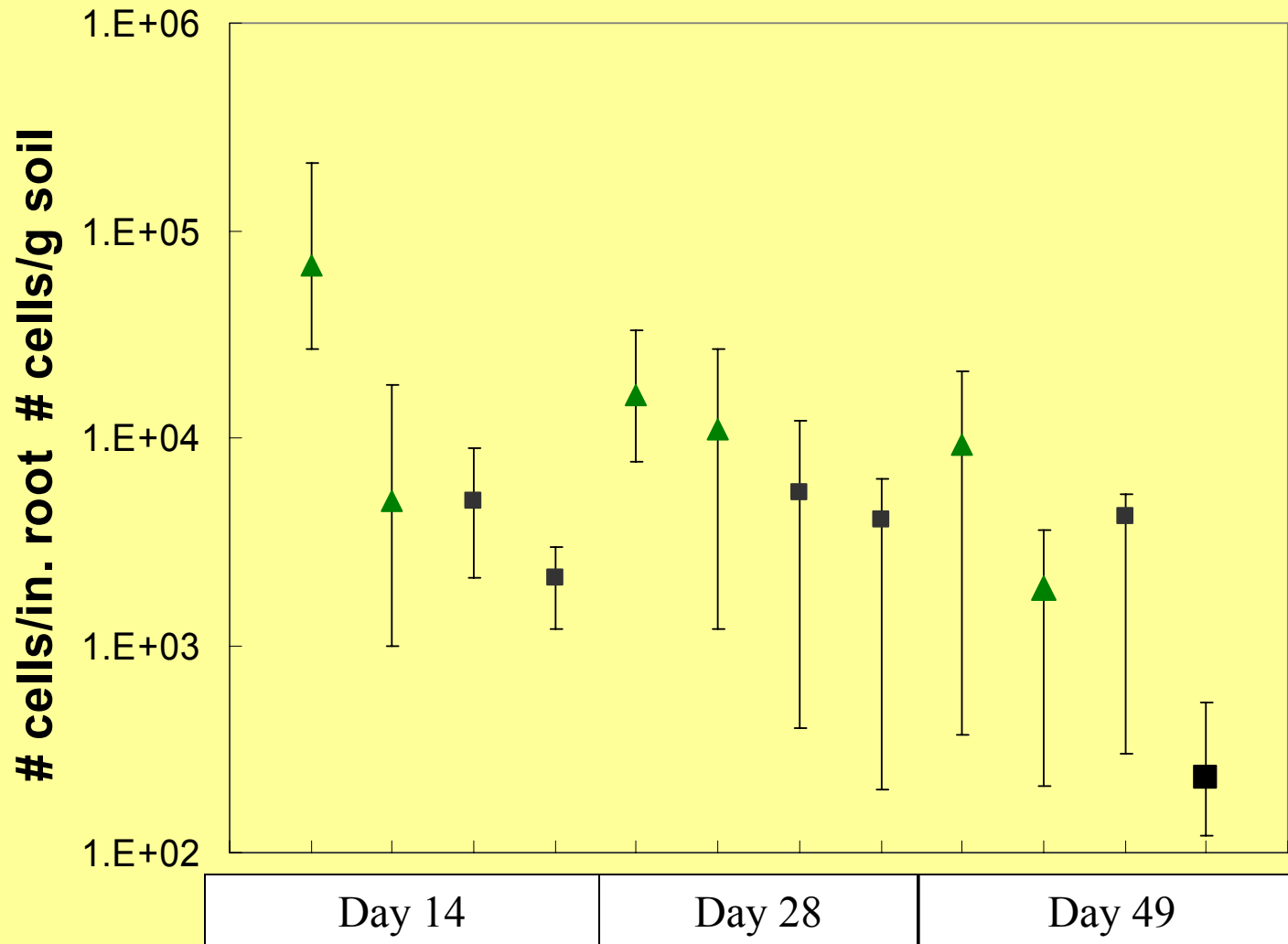
Completed work: TCE

Toluene *o*-monooxygenase incorporated into wheat and poplar colonizing bacteria, working with Tom Wood (U. Connecticut)

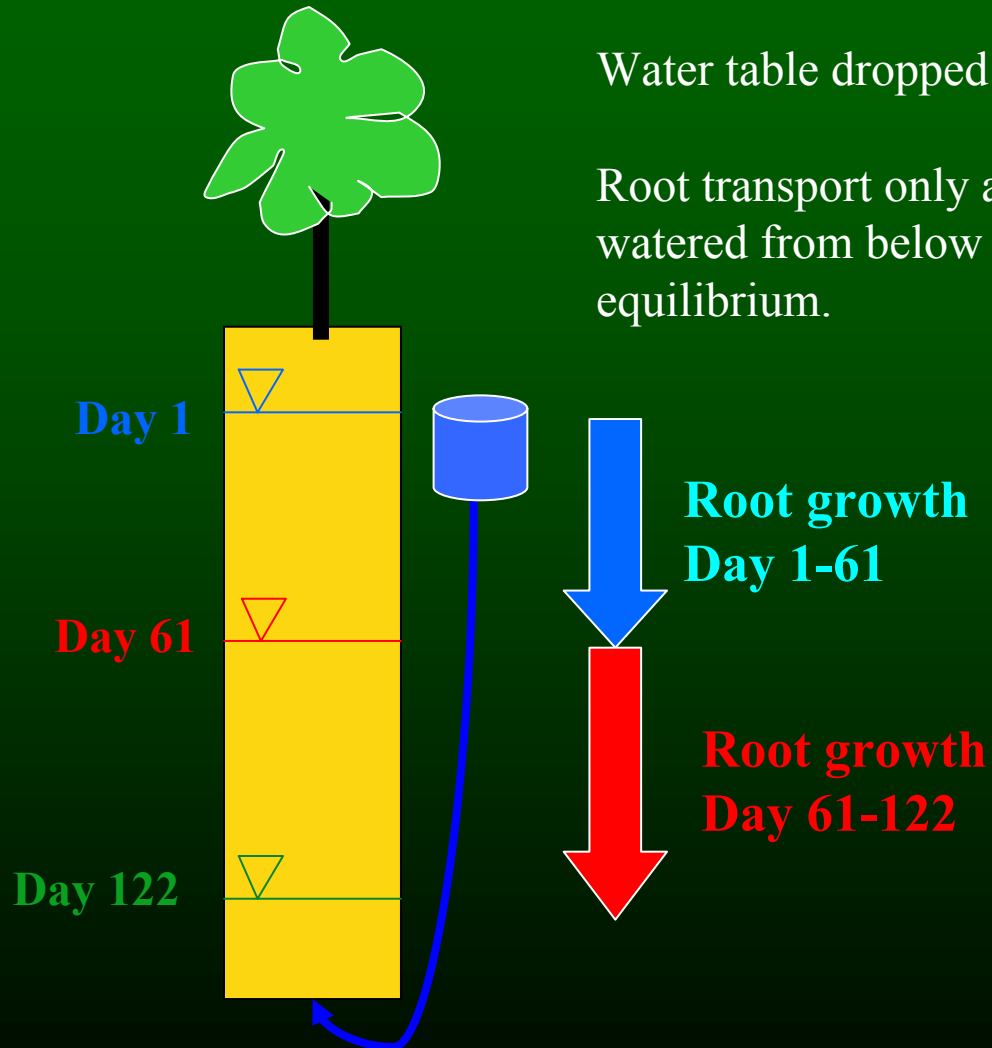
- ✓ Viability
- ✓ Growth rates
- ✓ Selectivity
- ✓ Degradation

Shim, H., *et al.*. 2000. Rhizosphere Competitiveness of Trichloroethylene-Degrading, Poplar-Colonizing Recombinants. *Applied and Environmental Microbiology*. 66: 4673 – 4678

Survival Plate Counts



Transport Experiment

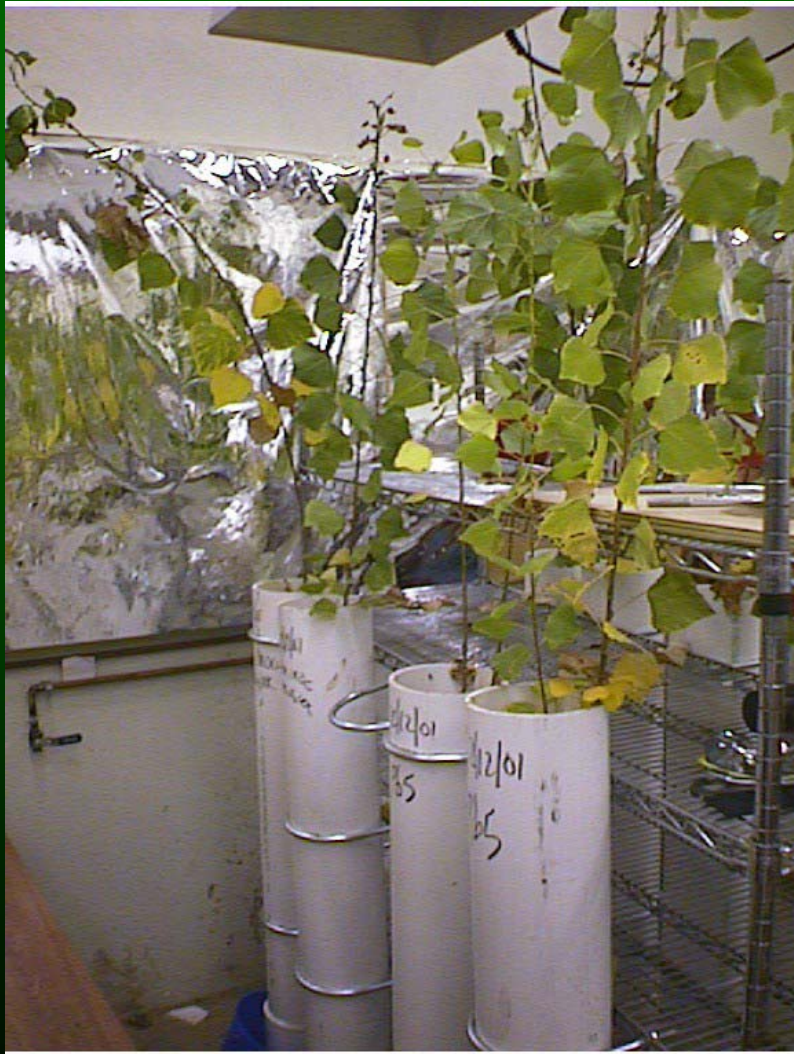


Water table dropped every 60 days.

Root transport only as plants were watered from below by hydrostatic equilibrium.



Transport Experiment; 180 days

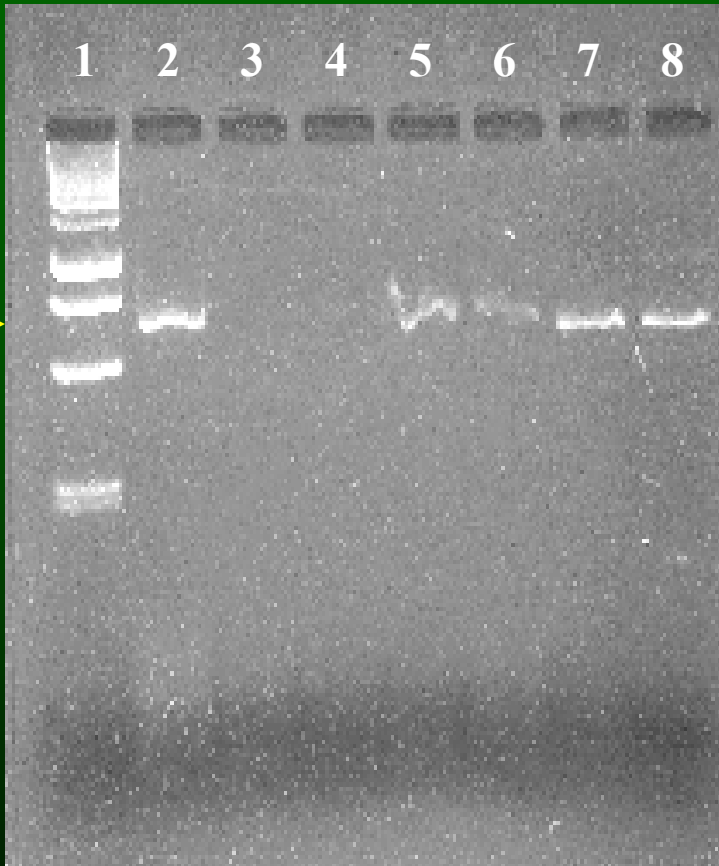


Recent: Visual Detection



Photograph of poplar root from Transfer experiment under an epifluorescent microscope. Visualized using specific GFP filters.

PCR Results



Lane 1 1 kb Ladder

Lane 2 Pb5gfp2-2 (+Control)

Lane 3 Pb5TOM (- Control)

Lane 4 P. f. 2-79 (- Control)

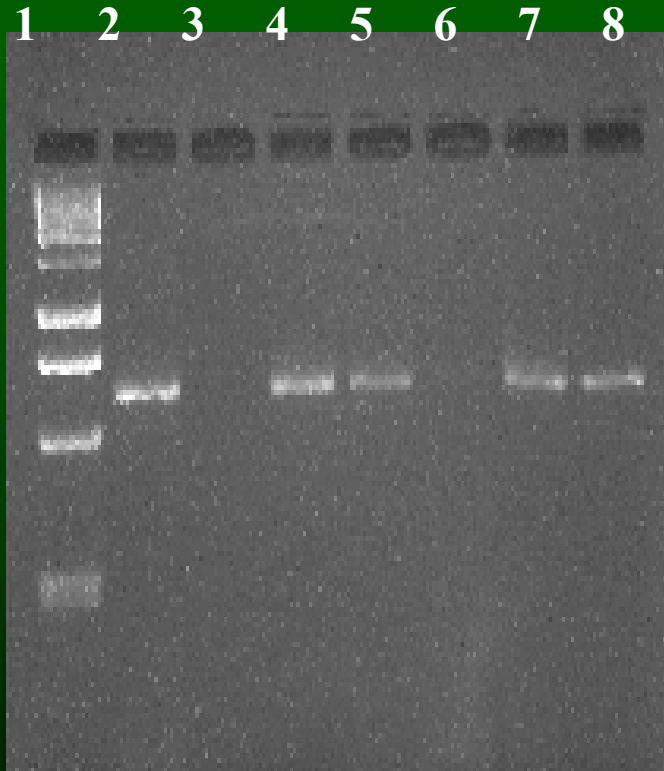
Lane 5 Pb5gfp2-2 Day 14

Lane 6 Pb5gfp2-2 Day 14

Lane 7 Pb5gfp2-2 Day 14

Lane 8 Transfer isolate (150 d)

PCR Results



Lane 1 1 kb Ladder

Lane 2 Pb5gfp2-2 (+ control)

Lane 3 Pb5TOM (- Control)

Lane 4 Pb5 #2/R upper

Lane 5 Pb5 #3/S lower

Lane 6 Pb5 #2/S lower (not gfp)

Lane 7 Rhiz #1/R upper

Lane 8 Rhiz #3/S lower

Findings: GEMs/Rhizosphere

- Survival for up to 180 days
- Increased degradation
- Selective to plant type (wheat and poplar colonizers did not cross inoculate)

Limitations

- Survival populations were not extremely competitive
- Degradation was minor in overall fate, still primarily taken up and volatilized.
- Poplar roots do not have a high specific surface area.

Ongoing efforts

Targeting hydrophobic contaminants

- Higher surface area, more fibrous root systems
- Hearty plants, shallow rooting
- Optimize microbial recipient

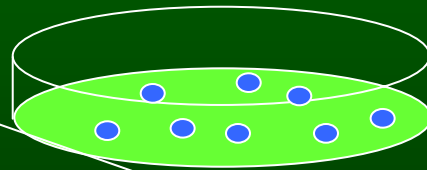
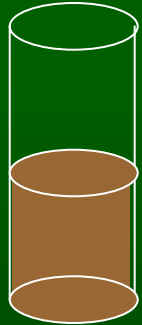
Selecting Better Bacteria

Replicate plating of bacteria from PCB contaminated root zone

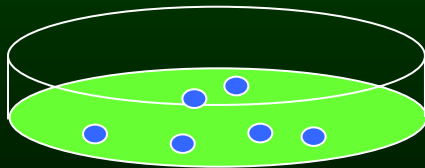
- LB media (culturable)
- Macerated root biomass
- Sterile root exudate
- Biphenyl

Initial Plating

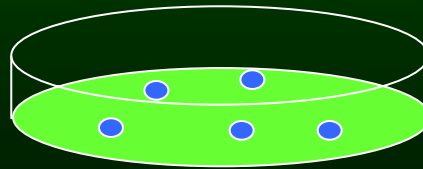
Rhizosphere sample



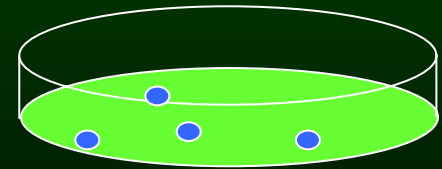
LB media
Master Plate



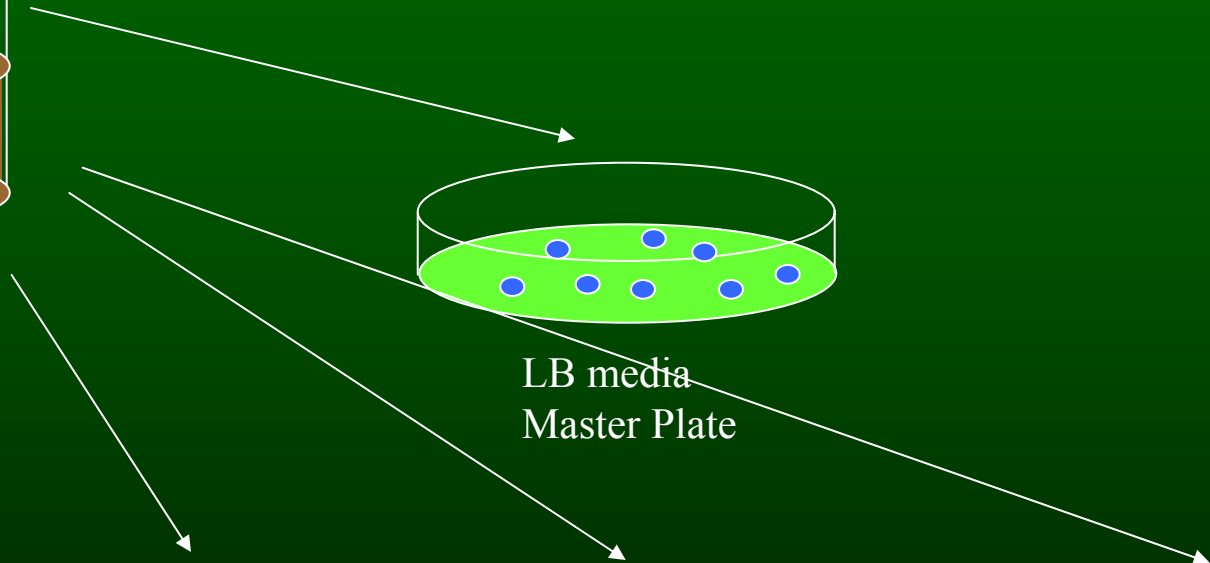
Macerated root biomass



Sterile root exudate

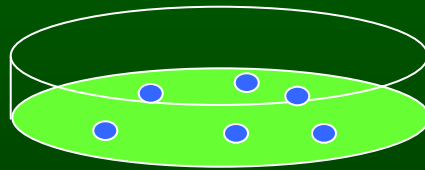
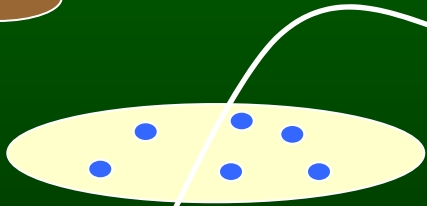
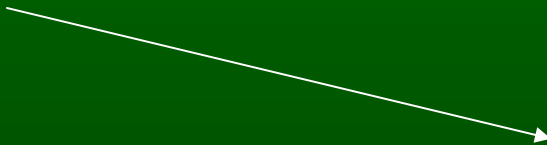
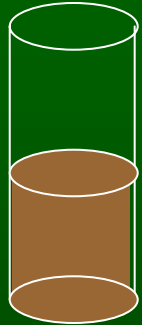


Biphenyl

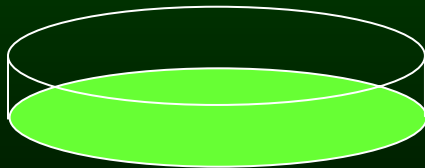
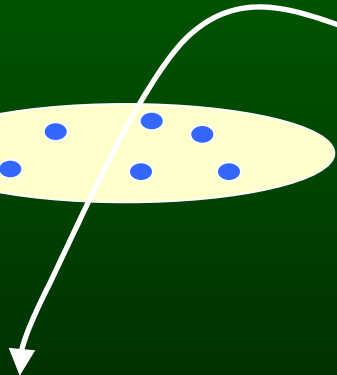


Replica Plating

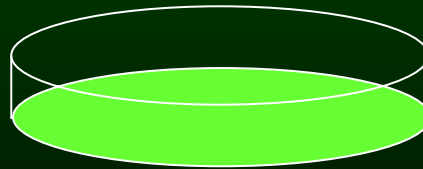
Rhizosphere sample



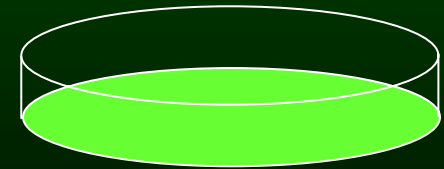
LB media
Master Plate



Macerated root biomass



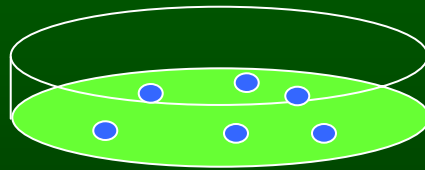
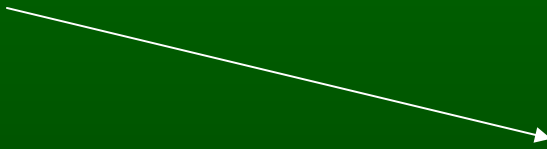
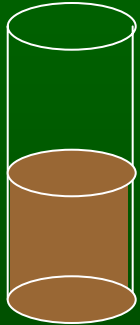
Sterile root exudate



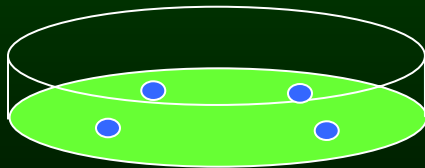
Biphenyl

Replica Plating

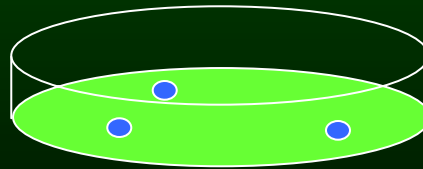
Rhizosphere sample



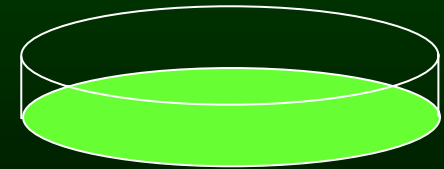
LB media
Master Plate



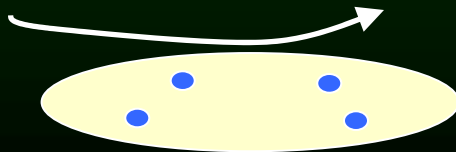
Macerated root biomass



Sterile root exudate

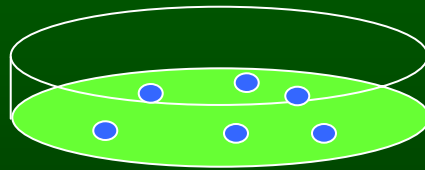
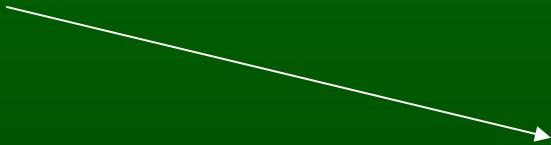
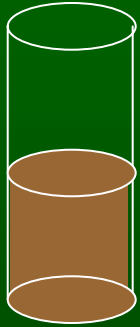


Biphényl

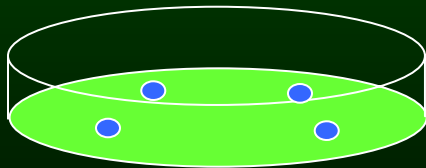


Replica Plating

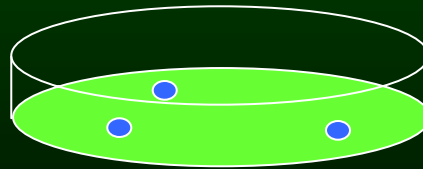
Rhizosphere sample



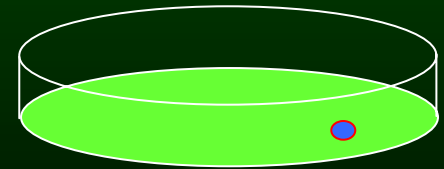
LB media
Master Plate



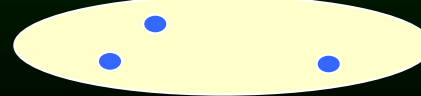
Macerated root biomass



Sterile root exudate

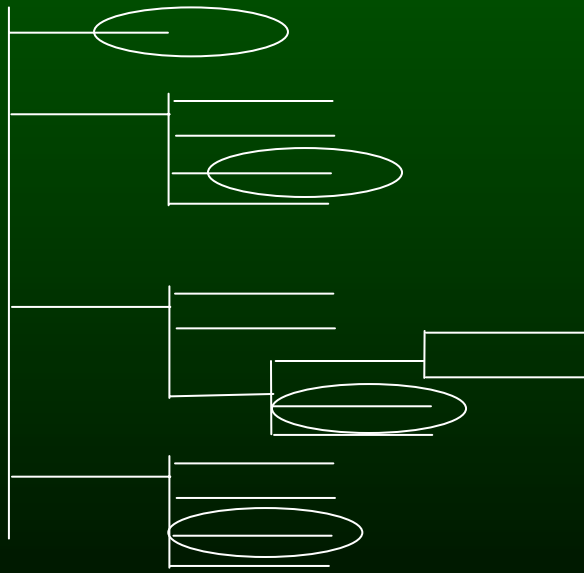


Biphenyl



Engineer selected colonizers

Sequence and Select cultures to represent the diversity present in Rhizosphere



Testing Degradation

- Cultures – track individual congeners (not all expected now)
- Soil microcosm – Planted/Not Planted Sterile/Not Sterile
- Greenhouse Column/Lysimeter –investigate degradation patterns and rooting patterns
- Enhanced survivability

Current progress

Target	Plant/ Microbe	Enzyme/ gene	Engineering	Survival/ thriving	Degradation (planted)
TCE	Poplar/Pb & Rhiz.	<i>tom</i>	Yes	>180 days no	Yes Yes
GFP Marker	Poplar/Pb & Rhiz.	<i>gfp</i>	Yes	>120 days	Visual Marker
PCBs	Alfalfa/S. <i>Mel.</i> Fescue/?	<i>bph</i>	Yes	? ?	Yes
Dioxins & Furans	Fescue/?	<i>dfd</i>	TBA	TBA	TBA

Limitations

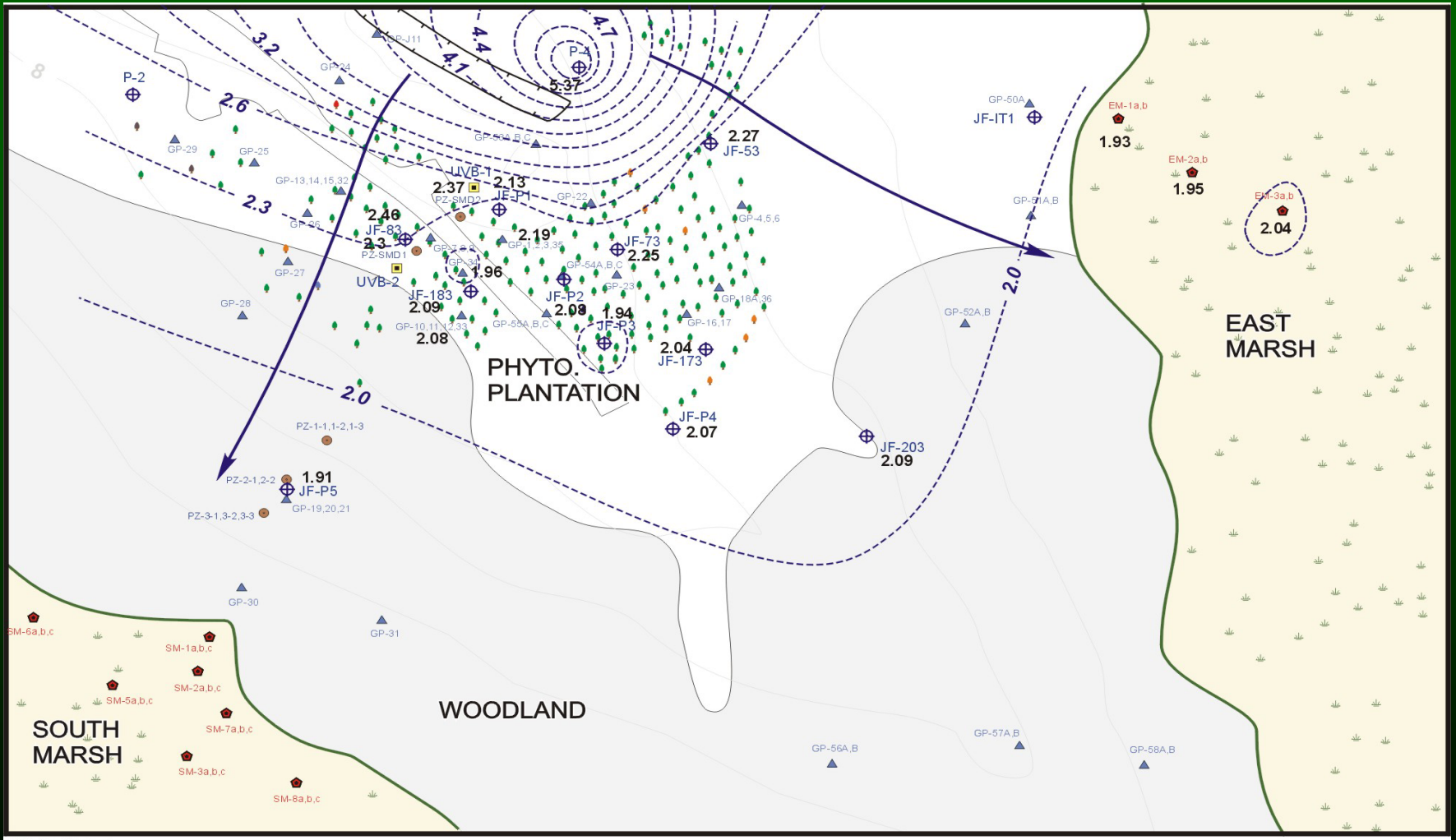
- Bioavailability
- Potential replanting and reinoculation
- Slow: must think years not weeks – months.
- Depth, rooting depth of grasses/vadose zone
- *bph* attacks limited PCB congeners *

* Raschke, H., *et al.* (2001) **Biotransformation of Various Substituted Aromatic Compounds to Chiral Dihydrodihydroxy Derivatives**, *Applied and Environmental Microbiology* **67** (8) 3333 – 3339.

Benefits

- Site may still be useful, limited restrictions and disturbance
- Remediation and restoration at the same time. Leaving site in useful state
- Stabilization, hydraulic containment and remediation – concurrently (poplar – willow)

Hydraulic Control: January 2001 Aberdeen Proving Ground



Conclusion & hypothesis

Engineered systems can alter this fate and enhance degradation mechanisms, and the selective nature of the rhizosphere can foster GEM survival and transport

Proper selection of plant and native bacteria can increase survival and thereby degradation of hydrophobic contaminants in-situ.

Acknowledgments

- National Science Foundation (BES 9984064)
- US EPA National Risk Management Lab (R-82933101) Steve Rock (EPA)
- Dr. Tom Wood – UCONN
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