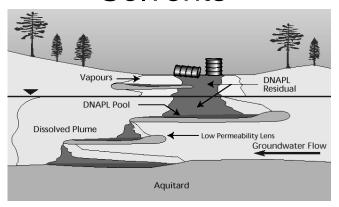
Biodegradation of Chlorinated Solvents



Jim A. Field

Department of Chemical and Environmental Engineering, University of Arizona jimfield@email.arizona.edu

Literature Review

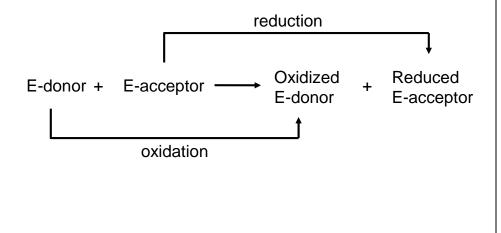
Biordegradability of chlorinated solvents and related chlorinated aliphatic compounds

J.A. Feld' & R. Serrio Alvascu
Department of Chemical and Environment Engineering, Environity of Bioma, P.D. Bio 2001, Turns, Reima (2017) (1987)

Field, J.A. & R. Sierra-Alvarez (2004) Biodegradability of chlorinated solvents and related chlorinated aliphatic compounds. *Reviews in Environmental Science & Bio/Technology* **3**:185-254.

Biodegradation Reaction

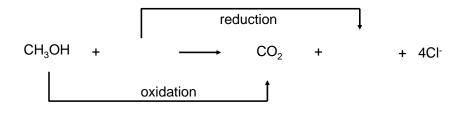
Biodegradation = Redox Reactions



Example: vinyl chloride as electron donor

reduction
$$+ O_2 \longrightarrow CO_2 + Cl^- + H_2O$$
 oxidation

Example: perchloroethylene as electron acceptor



Definitions Biodegradation

- Biodegradation: biologically catalyzed transformation of chemical resulting in simpler forms
- Mineralization: Conversion of organics to mineral products
 CO₂ + Cl⁻
- **Biotransformation:** Transformation of pollutant by a biological process

Definitions Biodegradation

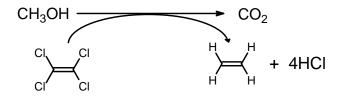
- Growth Substrate, Primary Metabolism: Pollutant (substrate) used as the primary energy and carbon source for microbial growth
 - as pollutant is degraded biocatalyst concentration increases
- Cometabolism: Accidental conversion of pollutant by enzymes and cofactors used for the metabolism of a primary substrate

Definitions Biodegradation

Reductive Dehalogenation: Microbially catalyzed replacement of a halogen atom on an organic compound with a hydrogen atom

$$R-CI + 2e^{-} + 2H^{+} \longrightarrow R-H + HCI$$

- Halorespiration: An organohalogen is used as an electron acceptor in an energy yielding metabolism
 - as pollutant is degraded biocatalyst concentration increases



Mechanisms of Dechlorination

Oxygenolytic:

Hydrolytic:

Mechanisms of Dechlorination

Reductive Hydrogenolysis:

Hydrolytic Reduction:

$$\begin{array}{c|c} CI \\ R-\overset{\bullet}{C}-CI & \xrightarrow{2e^-,\ 2H^+} \\ CI & & \\ \end{array} \begin{array}{c} 2HCI + R-\overset{\bullet}{C} \\ CI & \\ \end{array} \begin{array}{c} H_2O & CO + 2HCI \\ H_2O & COOH + 2HCI \end{array}$$

Mechanisms of Dechlorination

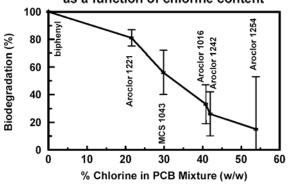
Reductive Dichloroelimination:

Important Trends

Aerobic Degradation

Chlorine # increases Biodegradation decreases

Elimination of PCB's in Aerobic Activated Sludge as a function of chlorine content



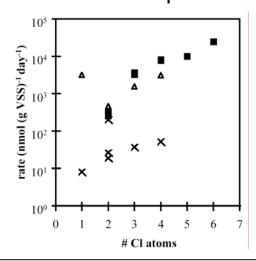
Tucker et al. 1975. Bull. Environ. Contam. Toxicol. 14, 705-713

Important Trends



Chlorine # increases

Biotransformation increases 1



Cometabolism of chlorinated solvents by anaerobic sludge

- chloroethanes
- **▲** chloromethanes
- * chloroethenes

Van Eekert 1999. PhD Dissertation (no. 2638). Wageningen University, The Netherlands, p. 129.

Five Physiological Roles

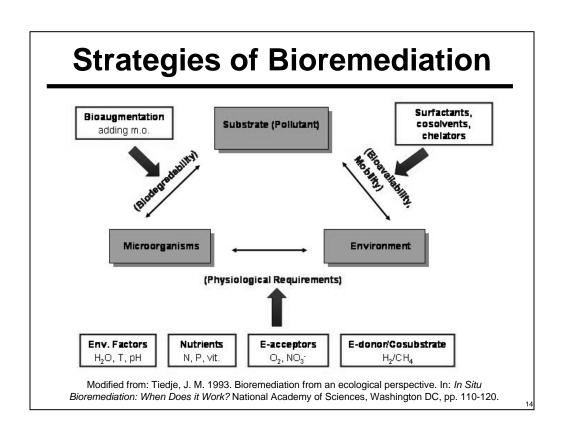
1st: aerobic carbon and energy source
ED-A

2nd: aerobic cometabolism (cooxidation) CoM-A

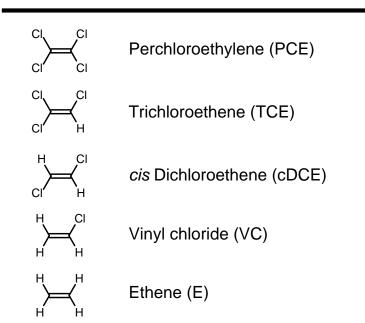
3rd: anaerobic carbon and energy source ED-AN

4th: anaerobic electron acceptor (halorespiration)
EA-AN

5th: anaerobic cometabolism (reduced cofactors) CoM-AN



Abbreviations Chloroethenes



Biodegradation Chloroethenes

ED-A VC; cDCE

faster

- CoM-A VC; cDCE; TCE
- **ED-AN** VC (?), cDCE (?)
- EA-AN VC; cDCE; TCE; PCE
- CoM-AN vc; cDCE; TCE; PCE

slower

Chloroethenes ED-A

Microorganisms Involved: Mycobacterium, Nocardioides, Pseudomonas

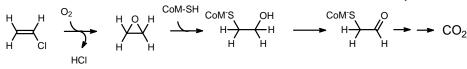
Pathway

 $HS \xrightarrow{H} GSO_3$

monooxygenase

epoxyalkane:CoM transferase

Coenzyme M



Coleman & Spain 2003 JB 185:5536

Kinetics

Growth rates (

0.05 to 0.96 d⁻¹

Activity

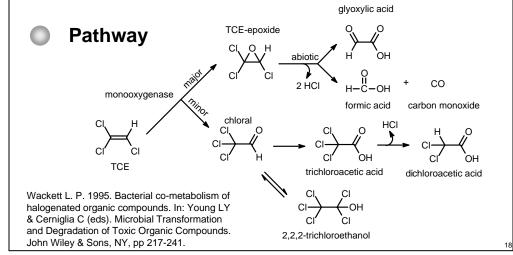
226 to 4950 mg g⁻¹ dwt d⁻¹

 $K_{\rm m}$ or $K_{\rm s}$

 $0.07 \text{ to } 0.70 \text{ mg } I^{-1}$

Chloroethenes CoM-A (cooxidation)

Microorganisms Involved: Methylosinus, Peudomonas, Burkholderia, Nitrosomonas, Mycobacterium, Rhodococcus, Alcaligenes

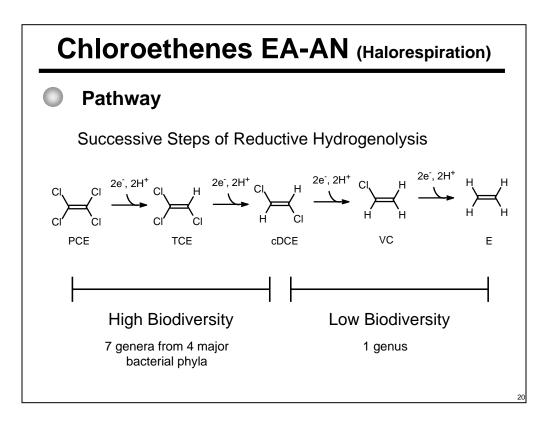


Chloroethenes CoM-A (cooxidation)

- Primary Substrates Supporting Cooxidation: methane, toluene, phenol, ammonium, ethane, ethene, propane *etc*
 - Substrates for which monooxygenases are utilized for biodegradation
- **Kinetics** Activity 57 to 55,000 mg g⁻¹ dwt d⁻¹

 Transformation Capacity $\kappa_{\rm m}$ 86 to 150 mg TCE g⁻¹dwt

 0.4 to 29.6 mg l⁻¹



Microorganisms Involved: PCE to cDCE

Low G+C gram + Desulfitobacterium H₂, lactate, formate, etoh

Clostridium YE, glucose

Dehalobacter H₂

δ Proteobacteria Desulfuromonas acetate,pyruvate

 ϵ Proteobacteria Dehalospirillim H_2 , lacate, formate, etoh

Sulfurospirillum lactate

Green non-sulfur Dehalococcoides H₂

Microorganisms Involved: cDCE to E

Green non-sulfur Dehalococcoides H₂

Biochemistry

Reactions catalyzed by specific reductive dehalogenases
 All contain vitamin B12
 Most are membrane bound enzymes

■ Kinetics: PCE to TCE and/or cDCE

Growth rates 0.23 to 6.65 d⁻¹

Activity 856 to 37,312 mg g⁻¹ dwt d⁻¹

Kinetics: VC to E

Growth rates 0.32 to 0.40 d⁻¹

Activity 3047 to 6030 mg g⁻¹ dwt d⁻¹

 $K_{\rm m}$ or $K_{\rm s}$ 0.16 to 0.31 mg l⁻¹

Hypothetical Example

Assumptions: $t_0 = 1$ bacterium per m³

1 bacterium = 1×10^{-12} g

Ideal conditions for growth

Kinetic data: Dehalosprillium Dehalococcoides multivorans strain VS

Growth rate (d⁻¹) 6.65 0.40
Activity (mg g⁻¹ dwt d⁻¹) 5970 3047

Growth Equation: $C_{xt} = C_{x0}e^{ut}$

 C_{x0} & C_{xt} = cell biomass conc. at time 0 & t (g dwt l⁻¹)

 μ = growth rate (d⁻¹), t = time (d)

Hypothetical Example (continued)

Question: How long will it take for a bioconversion

rate of 10 mg l⁻¹ chloroethenes per day?

Initial Biomass: 1×10^{-15} g dwt I⁻¹

Final Biomass: 10/5970 = 1.675 × 10⁻³ g dwt l⁻¹ Dehalosprillium

 $10/3047 = 3.282 \times 10^{-3}$ g dwt |-1 Dehalococcoides

Time:

$$t = \frac{\ln\left(\frac{C_{xt}}{C_{x0}}\right)}{\mu}$$

4.2 days Dehalosprillium

72.1 days Dehalococcoides

Chloroethenes CoM-AN

- Microorganisms Involved: Methanogens, Acetogens
- Pathway Successive Steps of Reductive Hydrogenolysis
 - Reactions catalyzed by reduced enzyme cofactors
 Cobalt containing vitamin B12; Nickel containing Factor 430
- Kinetics: PCE to TCE and/or cDCE

Activity $0.006 \text{ to } 20 \text{ mg g}^{-1} \text{ dwt d}^{-1}$

Kinetics: cDCE or VC to E

Activity 0.001 to 0.366 mg g⁻¹ dwt d⁻¹

Chloroethenes Bioremediation

Anaerobic - Aerobic

First: Rapid reductive dehalogenation to TCE & cDCE

Second: Rapid cooxidation of TCE and cDCE to CO₂ & Cl⁻

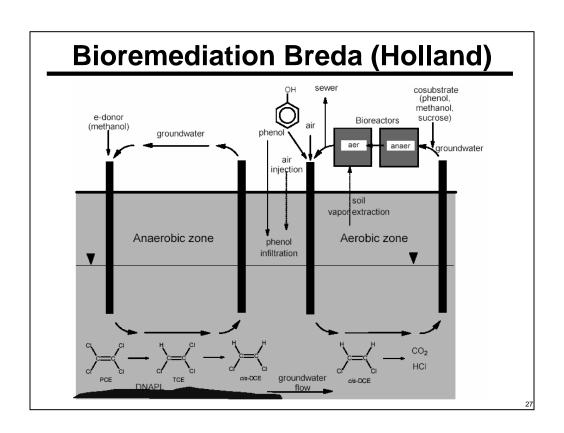
PCE
$$\xrightarrow{\text{anaerobic}}$$
 cDCE $\xrightarrow{\text{aerobic}}$ CO₂, Cl-+ electron donor + primary substrate

Anaerobic with Dehalococcoides

Promote complete halorespiration to ethene

```
anaerobic

PCE → E
+ electron donor
+ Dehalococcoides (if absent)
```

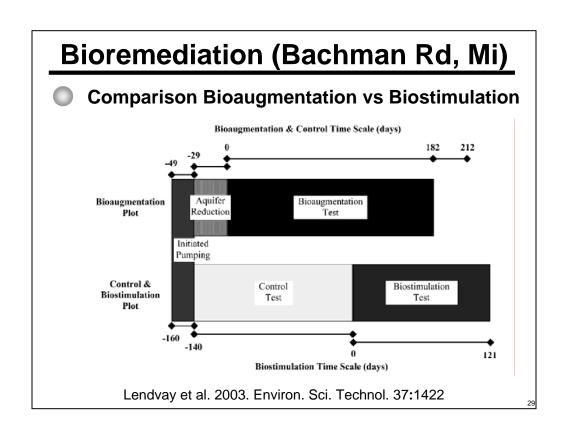


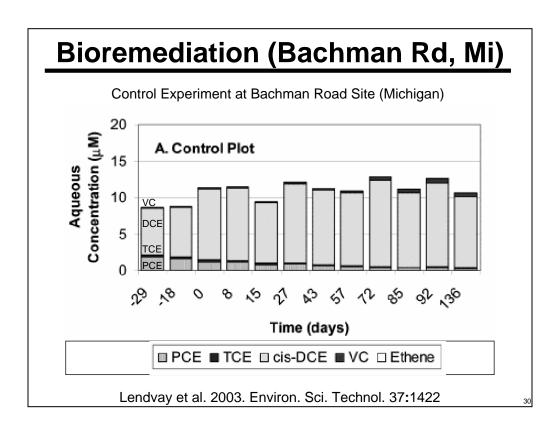
Bioremediation Breda (Holland)

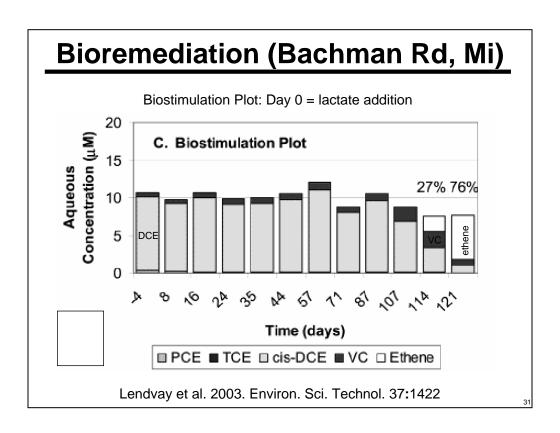
Facts about Full-Scale Bioremediation

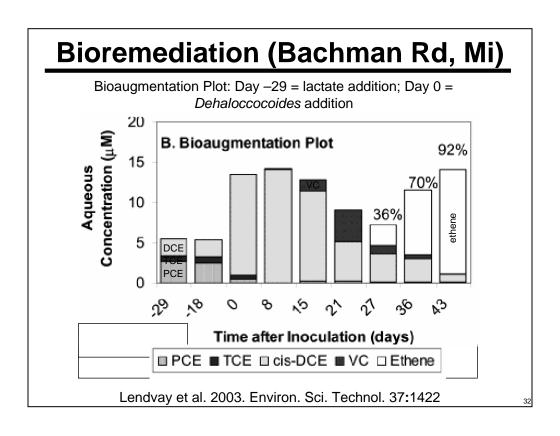
- 85% removal of PCE in situ within 6 months
- Inorganic chloride concentration in anaerobic zone increased from 1 to 6 mM
- In the aerobic zone all of the cDCE and VC as well as injected phenol was removed
- After one year the total mass of chloroethenes decreased from 1500 to 550 mol

Spuij et al. 1997. Full-scale application of in situ bioremediation of PCE-contaminated soil. *4th Int. In Situ and On Site Bioremediation Symp., New Orleans, LA*, Columbus, OH: Battelle Vol 5, pp. 431–37.









Bioenhancement DNAPL Dissolution

Dissolution equation

$$r_{TA} = K_L a(C_s - C_b)$$

 r_{TA} = dissolution rate (mg l⁻¹ s⁻¹)

K_L = mass transfer coefficient m s⁻¹

a = interfacial surface area m² m⁻³

C_s = maximum aqueous solubility

 C_b = actual concentration

Biodegradation can increase (C_s-C_b) and enhance dissolution

Reported enhancements

PCE dehalogenation feasible at saturated concentrations

16 × dissolution enhancement

5 × based on model

Yang and McCarty 2000. EST 34:2979

Cope and Hughes 2001. EST 35:2014

Christ et al. 2005. EHP 113:465

Bioenhancement DNAPL Dissolution

Combine Surfactant/Cosolvent Assisted Dissolution DNAPL with Biodegradtion

Biodegradable surfactants/cosolvents will be used as electron donors

Residual PCE remaining after flushing reductively dehalogenated

Residual surfactant biodegraded

Christ et al. 2005. EHP 113:465

Abbreviations Chloromethanes

Biodegradation Chloromethanes

ED-A CM; DCM

faster

- CoM-A CM, DCM; CF
- **ED-AN** CM, DCM
- EA-AN
- COM-AN DCM, CF, CT

slower

- Microorganisms Involved: Methanogens, acetogens, fermentative bacteria, sulfate reducing bacteria, iron reducing bacteria, denitrifiers
- Pathway Reductive Hydrogenolysis Hydrolytic Reduction
 - Reactions catalyzed by reduced enzyme cofactors, chelating agents, magnetite, quinones

Cobalt containing vitamin B12

Zinc containing porphorinogens

Pyridine-2,6-bis(thiocarboxylic acid)

Quinones, humus

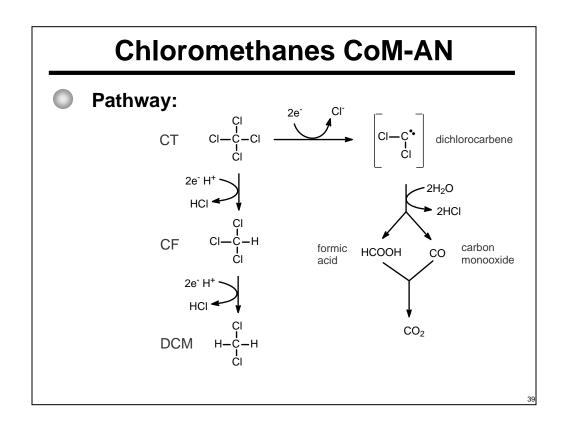
Biogenic magnetite

Kinetics: CF dechlorination

Activity 0.3 to 36 mg g⁻¹ dwt d⁻¹

Kinetics: CT dechlorination

Activity 3 to 1198 mg g⁻¹ dwt d⁻¹





Effect Redox Mediators

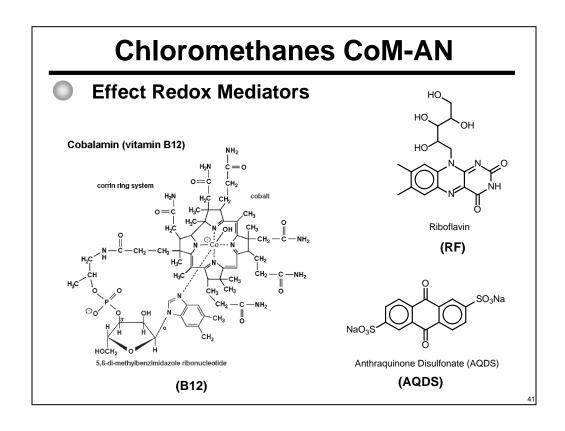
Experimental

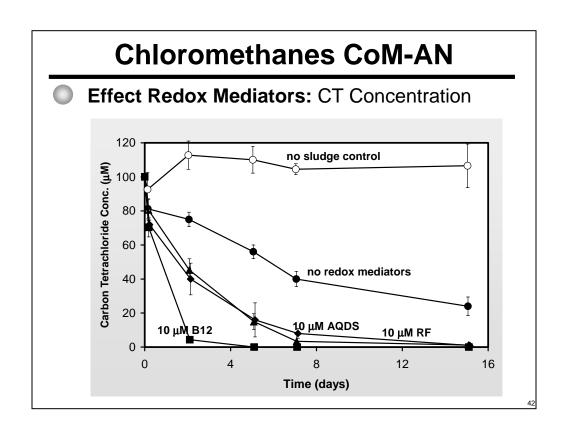
- → Phosphate buffer pH 7.0
- mineral medium (Cl- free)
- methanogenic sludge (0.5 g VSS/L)
- → VFA mixture (0.25 g COD/L)
- → CT or CF (100 uM)
- Redox Mediators (10 uM)

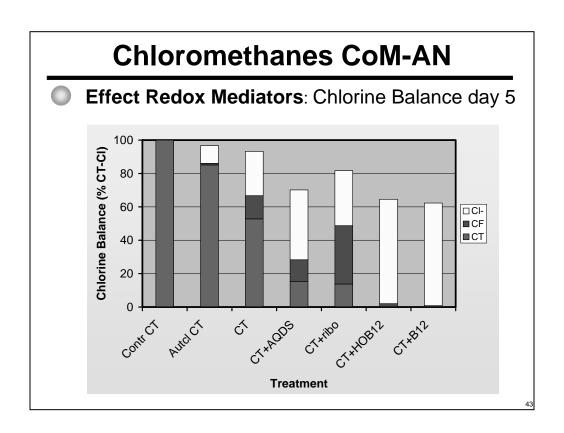
viton septum



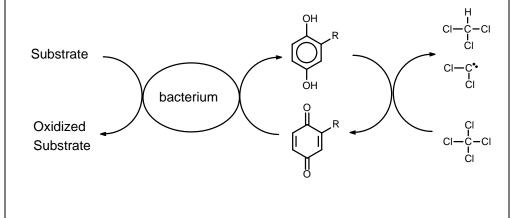
Guerrero-Barrajas & Field. 2005. Enhancement of anaerobic carbon tetrachloride biotransformation in methanogenic sludge with redox active vitamins. Biodegradation 16: 215–228.



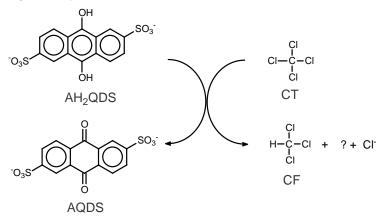




Role Redox Mediators:



Evidence of Direct Dechlorination by Hydroquinone



Curtis & Reinhard. 1994. EST 28:2393

Conclusions 1

Biodegradation Chloroethenes

- High biodiversity for rapid halorespiration PCE to cDCE;
 halorespiration cDCE to E restricted to one genus, *Dehaloccocoides*
- Slow anaerobic cometabolism of PCE, TCE, cDCE and VC (dominant process reductive hydrogenolysis)
- Rapid aerobic cooxidation of VC, cDCE, TCE feasible
- Aerobic biodegradation of VC (and cDCE) as growth substrates feasible with newly discovered bacterial strains

Bioremediation Chloroethenes

- Anaerobic halorespiration (PCE → cDCE) followed by aerobic cooxidation (cDCE → CO₂, Cl⁻)
- Complete reductive dechlorination with Dehaloccocoides

 $(PCE \rightarrow E)$

Conclusions 2

Biodegradation Chloromethanes

- Rapid aerobic or anaerobic biodegradation of CM or DCM as growth substrates
- Aerobic cooxidation CM, DCM and CF feasible
- Slow Anaerobic cometabolism of DCM, CF and CT
 - 1) hydrolytic reduction to CO₂
 - 2) reductive hydrogenolysis to lower chlorinated methanes
- Redox mediators can greatly enhance anaerobic biotransformation CT, CF

Bioremediation

Anaerobic cometabolism for CF and CT

What is Phytoremediation?







What is Phytoremediation?

A solar driven, biological system that is used to Contain, Sequester, Remove, or Degrade Organic and Inorganic Contaminants in Air, Soils, Sediments, Surface Water, and Groundwater

Types of Phytoremediation

Artificial Wetlands
Phytostabilization
Phytoextraction
Rhizofiltration
Rhizosphere
enhancement
Phytovolatilization

Phytodegradation
Air purification
Water and
Wastewater
Management
Landfill caps
Green Roofs
Combination
technologies

Wide Range of Contaminants

Organic: hydrocarbons, chlorinated solvents, phenols, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), agricultural wastes

Inorganic: metals, radionuclides, salinity, nitroaromatics, amines, excess fertilizers, pesticides, CCA (chromium copper arsenic)

Different Types of Impacted Media

Solid phase: soils, sediments, sludges **Liquid phase:** run-off, stormwater, wastewater, groundwater, leachate **Gaseous phase:** greenhouse gases, VOCs, NO_x

Advantages

Safety

Minimized emissions & effluent and low secondary waste volume

Controls erosion, runoff, rain infiltration, and dust emissions

Ecological

Habitat friendly, habitat creation, promotes biodiversity

Sequesters greenhouse gases (carbon dioxide)

Public / Regulatory

Acceptable brownfields applications
Aesthetics, green technology
Increasing regulatory approval and standardization

Limitations or Common Regulatory Issues

Depth

Only effective if within the relative rooting depth of the vegetation

Time

Requires longer periods to become effective (establishment)

May requires longer periods to reach clean up targets

Seasonal effects

Phytotoxicity

Generally considered applicable for low to moderate concentrations

In most cases, the vegetation must survive in order to operate

Media Transfer / Food Chain Impacts

Fate and transport often unclear Air emissions, leaf litter Harvesting, hazardous waste? Toxicity of parent vs. by-products

Web Addresses

http://www.rtdf.org/public/phyto/phyto doc.htm

http://www.itrcweb.org/gd_Phyto.asp

http://www.dsa.unipr.it/phytonet/

http://plants.usda.gov/

http://clu-in.org/techdrct/

http://www.acap.dri.edu/

Books

Phytoremediation (Hardcover) Tsao

Phytoremediation McCutcheon and Schnoor

Phytoremediation of Contaminated Soil and Water (Hardcover)
Terry and Banuelos

Plants That Hyperaccumulate Heavy Metals: Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining (Hardcover) Brooks

Companies

Edenspace – Metals

Applied Natural Sciences – Organics

Phytokinetics – Organics

Applied Phytogenetics – Genetically
 Engineered Plants

Thomas Engineering - Organics

Ecolotree – Landfill caps and riparian
 restoration

Phytoextraction Associates – Metals and
 phytomining

Journals

International Journal of Phytoremediation
Environmental Science and Technology
Environmental Pollution
Plant and Soil
Chemosphere
Journal of Environmental Quality
New Phytologist
Plant Physiology

Conferences

EPA International Applied
Phytotechnologies Conference
Battelle
American Society for Agronomy
American Chemical Society
Association for Environmental Health
and Sciences

Phytodegradation

Using plants to themselves take up and degrade organic contaminants.

Enzyme Systems

Green Liver Concept

P-450's

Peroxidases

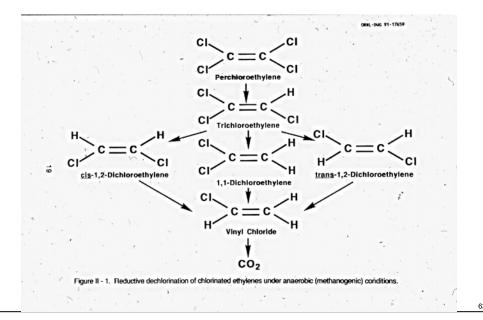
Dehalogenases

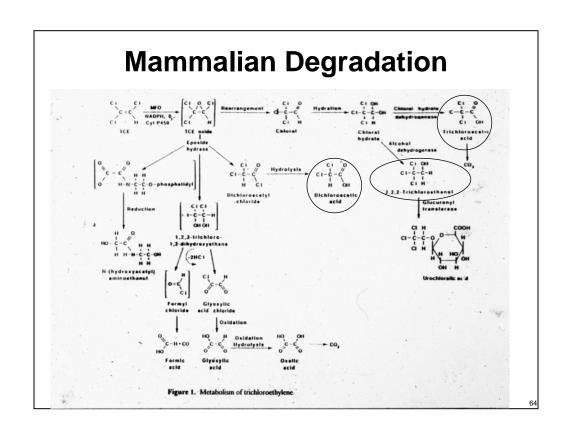
Reductases

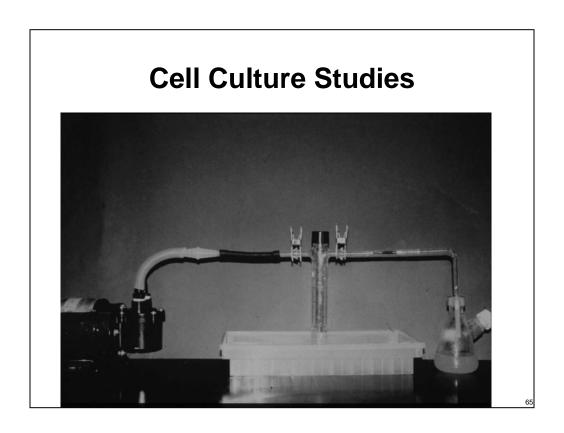
Glutathionone-s-transferases

Conjugation enzymes

Anaerobic Degradation of TCE

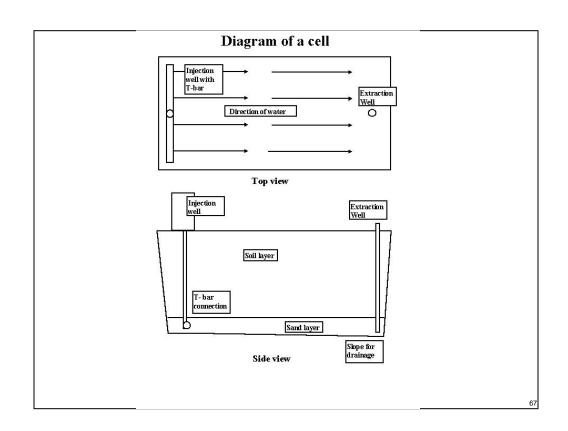


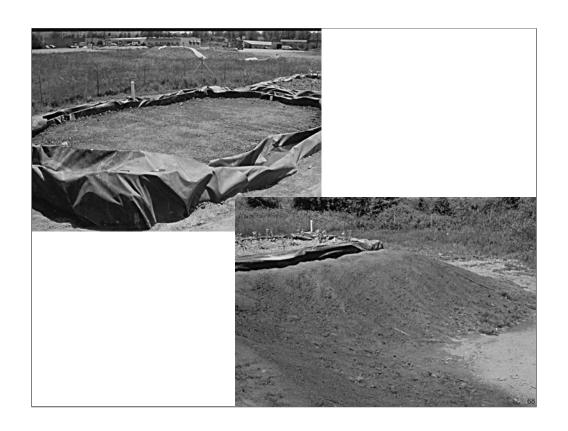




Greenhouse Studies

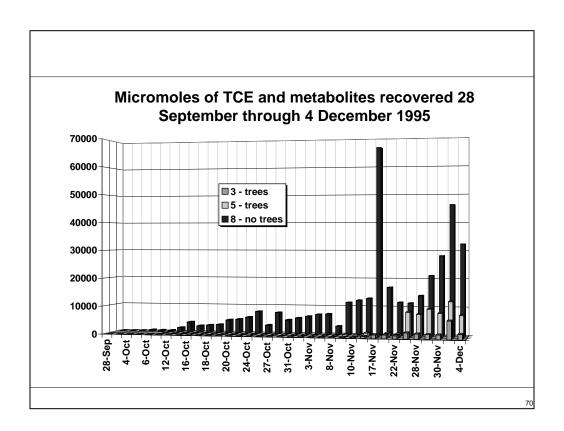




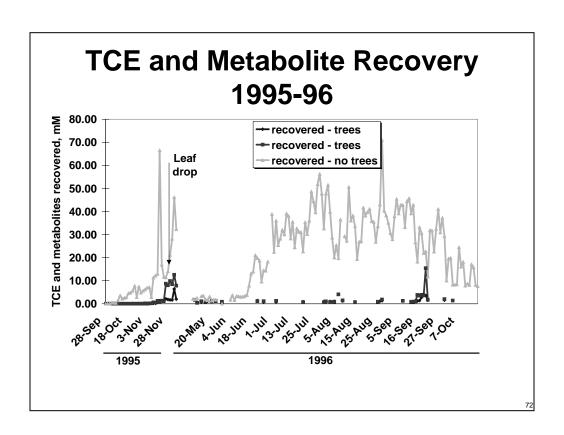






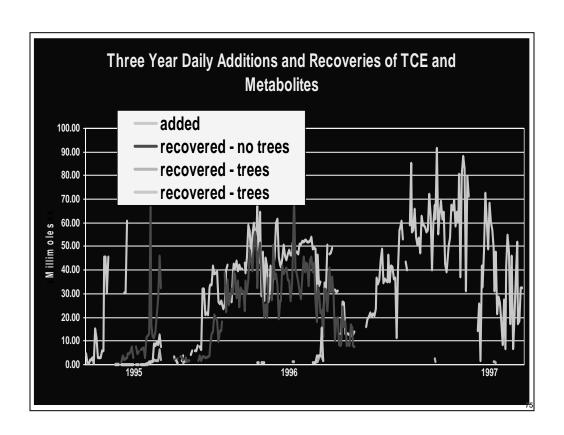


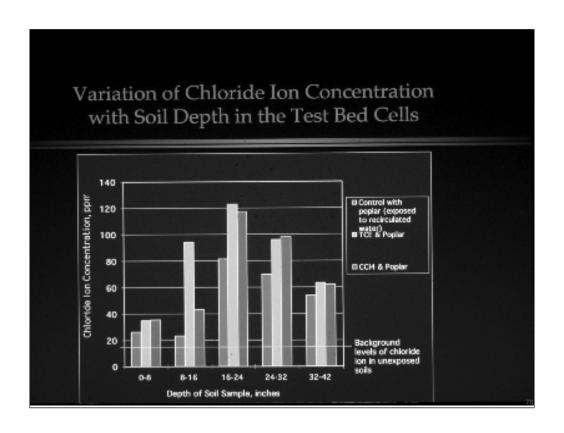


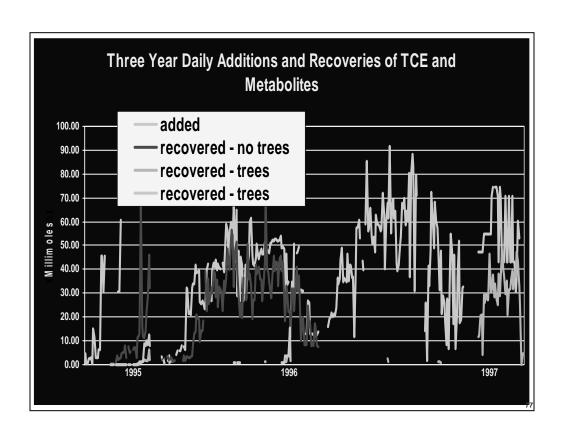












Other Compounds

MtBE, Benzene, other gasoline additives
Pesticides; Ethylene dibromide, lindane
Explosives: TNT, BDY, perchlorate

Explosives; TNT, RDX, perchlorate

Solvents; TCE, CT, PCE

Deicing agents; benzotriazoles

Regulatory Concerns

How to convince the regulators that this is a good idea.

Regulatory Issues

Regulator unfamiliarity with process

Not knowing all the answers to assure
the regulator

Testing and Monitoring

How often

What needs to be tested

Soil

Air

Water

Plant tissues

How do you analyze these crazy samples???

Unusual Monitoring Parameters

Tree health or why are all my plants brown?
Water issues
Nutrient availability
Is the soil itself killing the plants
Fungus, bugs and other munching critters
Convincing the regulators that plant health

is a measurable criteria for success

Where do those roots go?

Weather Impacts on Monitoring

Why doing transpiration measurements in the rain is not a good idea.

Temperature and light intensity have strong effects on plant metabolism and thus your test results.

What do you mean by success?

Do you need different standards for success? What are actually testing?

Plant survival

Root depth

Root penetration

Transpiration

Presence of metabolites

Groundwater depression

Soil analysis

Transpiration rates

Security Issues

Securing a field can be more challenging than securing a building.

Squatters/Vagrants on the site.

Community member access on sites.

Involving the community with site protection.

Problems with radical groups.



Do Contaminants Enter the Food Chain?



Food chain transfer

Insects munching
Animals munching on the plants or insects
Local people taking the plants



And children want to play



Decision making

How to decide if phytoremediation is right for the site.

Site Evaluation

Evaluating a site as a potential phytoremediation site involves some different parameters.

Weather
Water availability
Soil fertility
Toxicity

Site Evaluation

Will phyto work with the contaminant I have on the site?

How will the plants and contaminants interact?

Is this acceptable to the regulators?

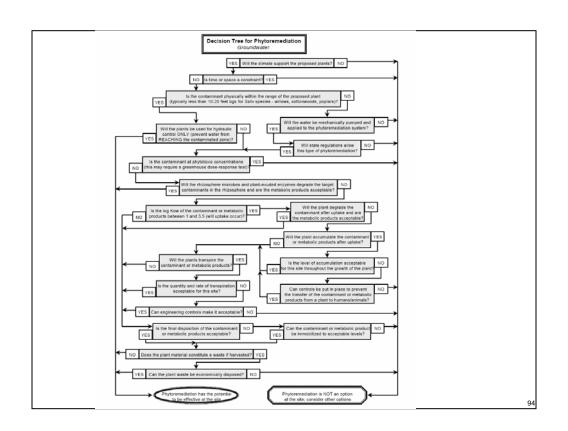
Site Evaluation

Is the site now, or could it be, applicable to plant growth?

Blacktop and plants do not mix

Shade/sun issues

Soil toxicity that is not related to contaminant



Designing a site

How do we make this work?

Unique Site Prep Problems

Concrete is not dependent on soil nutrients, but your plants will be.

Soil compaction can be an issue for planting.

Shades from buildings or other plants can be a major issue.

Security Issues

Securing a field can be more challenging than securing a building.

Squatters/Vagrants on the site.

Problems with radical groups.

Weather

Why doing transpiration measurements in the rain is not a good idea.

Temperature and light intensity have strong effects on plant metabolism and thus your test results.

Working With Mother Nature

Construction can go on anytime of the year, but planting has to be in sync with the seasons.

When dealing with natural systems we have to deal with all of nature (ie. bugs and birds are now a fact of life).

Selecting Plants For Your Site

You need to research and find the plant type that can handle the contaminant you are dealing with on the site

You need to find a plant that will

SURVIVE on your site, or why brown trees don't impress anyone...

Screening Trees







Before You Install, or Why Feasibility Testing is a Good Idea

Better to have a few plants die in the greenhouse, rather than have a field full of dead plants.....

Preparing the Site for Planting, or Why You Need to Learn to Think Like a Farmer

Weather plays an important role
Why plowing mud is a BAD idea...
Plants have their own time schedules,
and you have to meet theirs, not the
other way around

Sometimes That Extra Plowing is NOT a Good Idea...



Department of Energy – Ash Basin

Understanding how your preparations will affect the site



Know How Your Treatment Will Work



Tilled, no compost



No site prep

Consider Combination Technologies

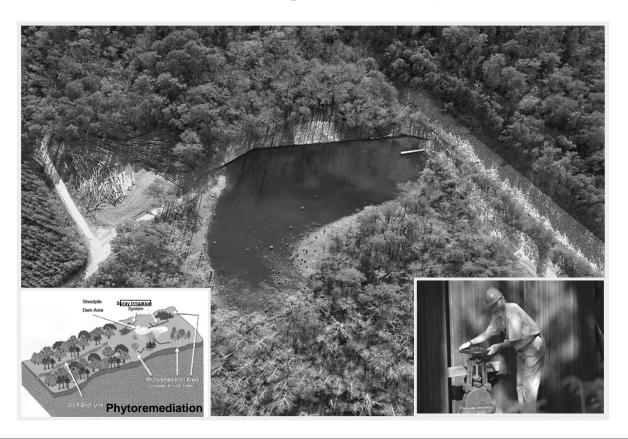
- Pump and irrigate
- Water management behind a plume containment wall
- Reactive barriers and plants
- Combined bioremediation and phytoremediation
- Using plants to minimize recharge zones



Spray systems



Tritium Irrigation System



Medford

Timing of the installation can be critical

Timing the herbicide application to
remove existing vegetation can be vital

Digging up irrigation lines does not help

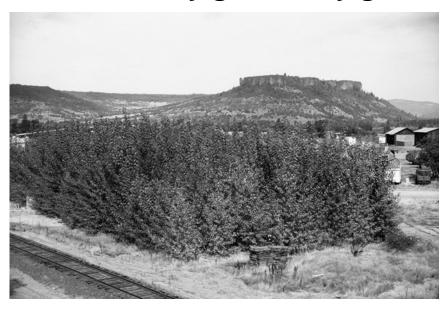
Mud, Anyone?



Or how about rocks?



But when they grow, they grow!

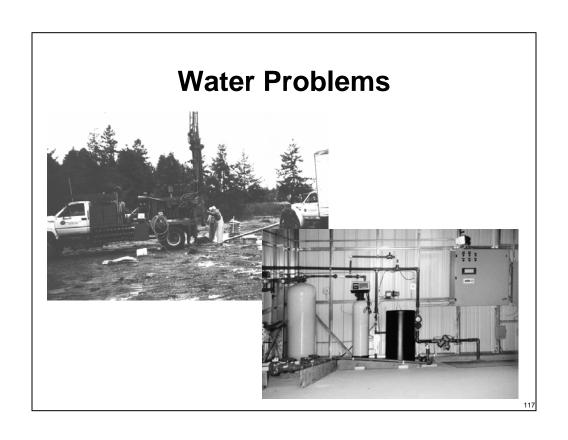


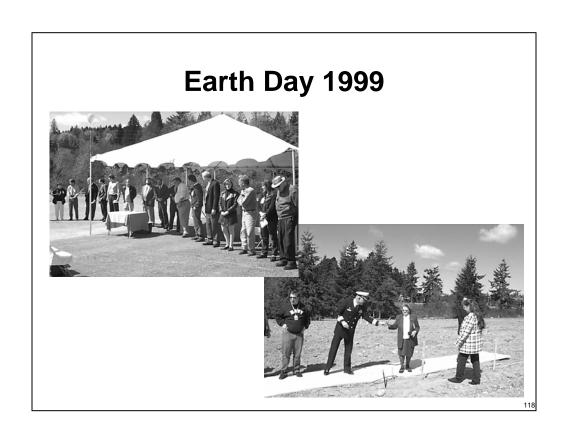
Navy Base I

Plowing and moving mud
Meeting unrealistic time schedules
Why a wood chip road is NOT a good
idea
Hydrologic problems
Poplars don't swim real well

Plowing and Moving Mud







Wood Chips are Not Always a Good Idea



Start of third year

Saginaw Mill

Drainage problems
Trees and deer don't mix well
The problems with weeds
Why community knowledge is a good thing

Lake Saginaw



Surviving the weeds, the deer, the fire...



And finally they grow

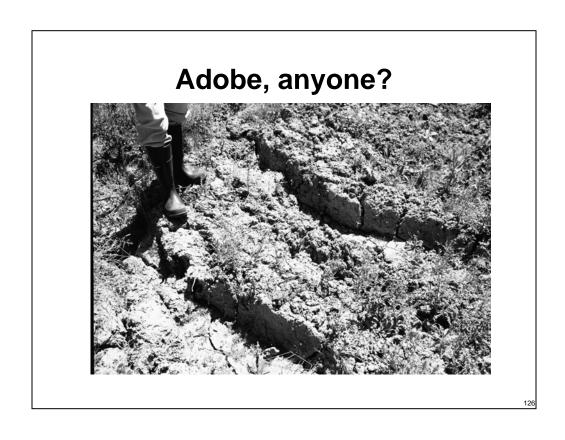




Portland

Why plowing mud is a bad idea or adobe is not a good growing medium

Plant selection can be crucial



Plant Selection + Poor Growing Conditions...





Navy Base II

You need to know what is happening all around you, even if it is not related to what you are doing

A Little Asphalt, Anyone?



Vale

What do you mean there is too much fertilizer or when wood chips ARE a good idea

Didn't you say the pesticides were burned?

Why more mixing is not always better



Wood Chips Just Might be a Good Idea Here!



And Then There Were Pesticides...



Still Not a Good Idea...



Okay, the Plants are Finally in the Ground and Growing. What Can Go Wrong Now???

Mechanical breakdowns
Invaders of the four-legged kind
Don't forget the "Save the Trees Society"
Nutrient needs
Droughts are Mother Nature's way of saying
"Gotcha!"

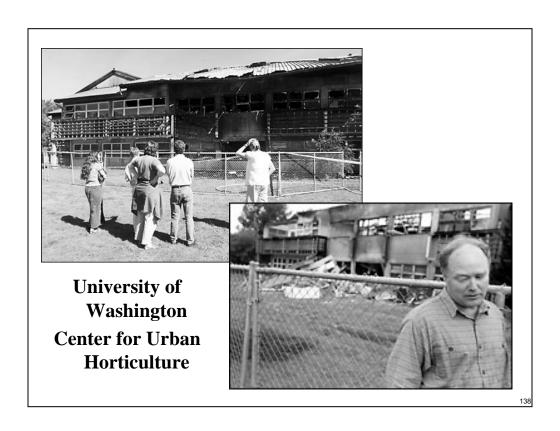
Poplars Can't Swim!



Trees and Deer Don't Mix Well







Sigh.....

Plants are Growing, Everything is Looking Green and Healthy. Time to Start Testing. Now What?

Weather can still be a big issue Sample collection can pose some unique problems (getting liquid nitrogen into the field is NOT fun)

Where do I ship the samples for analysis?

Tell Me Again.....

WHY Do I Want to Put Myself Through This???

Less expensive

Citizens groups LIKE trees

Many regulatory agencies are looking favorable at groups that try innovative technologies

Because it is a great technology that will have you constantly learning as you go

Have faith in the trees!





