Bioresmediation: A Strategy for Addressing Superfund Chemicals

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In the beginning was the mounting fire
That set alight the weathers from a spark,
A three-eyed, red-eyed spark, blunt as a flower;
Life rose and spouted from the rolling seas,
Burst in the roots, pumped from the earth and rock
The secret oils that drive the grass.

—Dylan Thomas
What are the chemicals of concern at contaminated sites?

- Metals (arsenic, cadmium, lead, etc.)
- Organic compounds
  - chlorinated solvents (e.g., trichloroethylene)
  - other chlorinated chemicals (e.g., PCBs, DDT, chlorinated dioxins)
  - aromatic hydrocarbons (e.g., benzene, toluene)
  - polycyclic aromatic hydrocarbons (PAHs)
Types of sites requiring remediation

• Superfund sites
  – subject to cleanup under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA)
  – sites must be placed on the National Priority List (NPL); ~1,300 sites currently (37 in North Carolina)

• Private industrial sites
  – *e.g.*, former manufactured-gas plants (MGP sites)

• Government sites
  – military (bases, contractors)
  – National Labs (Department of Energy sites)
  – other (civilian) government sites
Remediation options

• On-site containment
• Excavate and dispose off site
• On-site treatment
  – soil, groundwater, or both?
  – above ground (ex situ) or in place (in situ)?
• Off-site treatment
• note bioremediation is only one form of treatment
Excavation of MGP site, Greenville, South Carolina (built in early 1900s, abandoned and cleared in 1950s)
MGP site in Greenville, SC (closeup of tar holder)
free-phase tar at soil surface

debris and tar pool

excavated tar-soil mixture

MGP site in Charlotte, NC
excavation and buried pipe leaking tar, MGP site in Salisbury, NC
Figure 1: Actual Remedy Types at Sites on the NPL
(FY 1982 - 2005)*
Total Number of Sites = 1,536

- **Containment and Other (370)** 24%
  - No Action or No Further Action (91) 6%
- **No Decision (204)** 13%
  - No Remedy Decision (204) 13%
- **Treatment (962)** 63%
  - Treatment of a Source Only (178) 12%
  - Treatment of Both a Source and Groundwater (427) 28%
  - Treatment of Groundwater Only (357) 23%

Figure 4: Completed Treatment Projects by Remedy Type (FY 1982 - 2005)*

Total Projects Completed = 687

- Ex Situ Groundwater Treatment (P&T) (73) 11%
- In Situ Source Control and Groundwater Treatment (11) 2%
- In Situ Groundwater Treatment Only (35) 5%
- In Situ Source Control Treatment Only (170) 25%
- Ex Situ Source Control Treatment (398) 57%
Source control treatment projects, 1982-2005

Ex Situ Technologies (515) 53%
- Physical Separation (21) 2%
- Incineration (on-site) (42) 4%
- Bioremediation (60) 6%
- Thermal Desorption (71) 7%
- Incineration (off-site) (105) 11%
- Solidification/Stabilization (173) 18%
- Other Ex Situ (43) 4%

In Situ Technologies (462) 47%
- Soil Vapor Extraction (248) 26%
- Bioremediation (53) 5%
- Multi-Phase Extraction (46) 5%
- Solidification/Stabilization (44) 5%
- Chemical Treatment (20) 2%
- Flushing (17) 2%
- Thermal Treatment (14) 1%
- Other In Situ (20) 2%

Chemical Treatment - 9
Neutralization - 7
Soil Vapor Extraction - 7
Soil Washing - 6
Mechanical Soil Aeration - 4
Open Burn/Open Detonation - 4
Solvent Extraction - 4
Phytoremediation - 1
Vitrification - 1

Neutralization - 8
Phytoremediation - 6
Mechanical Soil Aeration - 3
Vitrification - 2
Electrical Separation - 1
What is bioremediation?

- bioremediation ≡ the application of microbial processes to treat contaminated soil, sediment, or groundwater
- the term “biodegradation” is often used to describe what happens in bioremediation. But what is biodegradation?
  - making a contaminant go away?
  - reducing the impact of the contaminant?
    - on the environment
    - on human health
How do microorganisms make a contaminant “go away”?

- Many contaminants are organic compounds that serve as sources of carbon and energy for growth (i.e., “food”)
  - many are naturally occurring compounds (e.g., hydrocarbons, including PAHs)
- Some contaminants are electron acceptors (oxidants) used for respiration, in the same way that oxygen is used for aerobic respiration (e.g., chlorinated hydrocarbons such as TCE)
  - this process is coupled to growth of the organism on an organic compound as a carbon and energy source
- Some contaminants are neither
  - may be transformed fortuitously (cometabolized) during metabolism of another compound
Outcomes of biodegradation mechanisms

- complete metabolism (generally associated with growth)
  - mineralization of a fraction of the initial compound mass to CO$_2$, H$_2$O, Cl$^-$, SO$_4^{2-}$, etc.; e.g., aerobic metabolism of benzene:
    \[
    \text{C}_6\text{H}_6 + 7.5\text{O}_2 \rightarrow 6\text{CO}_2 + 3\text{H}_2\text{O}
    \]
    mineral (inorganic) products
  - assimilation of a fraction of the initial compound mass into cellular biomass

- incomplete metabolism (fortuitous transformation to dead-end metabolites, unrelated to growth)
  - e.g., transformation of TCE to TCE epoxide by methanotrophic bacteria, catalyzed by the enzyme methane monooxygenase (MMO)
    \[
    \text{C}_2\text{HCl}_3 + \text{O}_2 + \text{NADH} + \text{H}^+ \xrightarrow{\text{MMO}} \text{C}_2\text{HCl}_3\text{O} + \text{NAD}^+ + \text{H}_2\text{O}
    \]
Incomplete metabolism is “biodegradation,” but is it necessarily good?

Consider the anaerobic transformation of DDT

\[
\begin{align*}
\text{Cl-} & \text{C} & \text{Cl} & \text{C} & \text{Cl} & \text{Cl} & \text{C} & \text{Cl} & \text{Cl} & \text{H} & \text{Cl} & \text{2H}^+, 2\text{e}^- & \text{HCl} \\
p,p'-\text{DDT} & \rightarrow & \text{reductive dechlorination} & \rightarrow & p,p'-\text{DDD} \\
\text{dehydrochlorination} & \text{(abiotic?)} & \downarrow & \text{HCl} \\
p,p'-\text{DDD} & \rightarrow & p,p'-\text{DDE}
\end{align*}
\]

EPA regulates the sum of DDT + DDE + DDD (assumes equal risk), so a single-step transformation of DDT does not reduce risk from a regulatory perspective (i.e., would not be considered acceptable treatment).
Incomplete aerobic metabolism of PAHs can form toxic products

Pyrene transformation to a pyrene quinone

pyrene-4,5-dione (pyrene quinone)
Why does incomplete metabolism matter?

- Because microbial enzymes can have broad specificity for related chemicals, incomplete metabolism is inevitable in complex contaminated environments.
- Can products be degraded by other organisms?
- If products accumulate, what is their effect?
  - on organisms that produce them
  - on other organisms
  - on overall toxicity
- Overall toxicity or genotoxicity of soil or sediment after cleanup is usually not taken into account.
Overall responses in complex systems

- naphthalene
- fluorene
- phenanthrene
- anthracene
- fluoranthene
- pyrene
- chrysene
- benzo[a]pyrene

**bug 1**

- **bug 2**
- **bug 3**
- **bug 4**

**pyrene**

- **biomass**
  - **CO₂ + H₂O**
    - **growth**

- **product(s)** of incomplete metabolism
Effects of biological treatment on (geno)toxicity

Use of a bioassay with chicken lymphocyte cells (DT40) and a mutant of DT40 deficient in DNA repair (Rad54) on PAH-contaminated soil from an MGP site treated in a lab-scale bioreactor

From Hu et al. (2012) Environmental Science & Technology 46: 4607-4613
What microorganisms are involved in bioremediation?

- All bioremediation applications rely on naturally occurring microorganisms
  - All involve complex microbial communities
  - Most rely on bacteria, but some may involve archaea or fungi
  - All systems are open (anyone can join the party!)

- For naturally occurring compounds, expect ubiquity and broad diversity

- For xenobiotics, expect high specialization and more restricted distribution of the relevant organisms
Microbial diversity: an under-explored universe

• estimated to be $\approx 5 \times 10^6$ prokaryotic species (bacteria and archaea) on Earth
  – we know *nothing* about most of them
• for example: soil can contain *thousands of species* of prokaryotes per gram
  – at $\approx 3$ Mbp per prokaryotic genome, $>10^{10}$ bp in “metagenome” of a one-gram soil sample
  – human genome $\approx 3 \times 10^9$ bp $\Rightarrow$ one gram of soil is genetically more complex than the human genome
  – abundances range over several orders of magnitude
Underlying principles of microbial ecology

- every organism has a unique range of capabilities, some of which might be useful in bioremediation (or any form of biological treatment of wastes)
- every organism has a unique range of conditions under which it will grow or at least survive
- environmental systems are likely to be characterized by relatively few dominant species and a large number of low-abundance species
- open environments permit the growth of heterogeneous communities

therefore a diverse community of microorganisms can be expected in a given environmental system, each species with its own “niche”
Factors that influence microbial communities

- **environmental conditions** govern which organisms dominate (which organisms are selected)
  - major energy and carbon sources
  - dissolved oxygen availability and concentration
    - aerobes
    - microaerophiles
    - anaerobes
  - presence of electron acceptors other than oxygen (e.g., $\text{NO}_3^-$, $\text{SO}_4^{2-}$, $\text{Fe}^{3+}$, chlorinated hydrocarbons, perchlorate)
  - pH (e.g., acidophiles thrive at low pH)
  - temperature (e.g., thermophiles thrive at high temperature)
  - salinity
  - availability of nutrients (e.g., sorbed to a surface or within a non-aqueous matrix vs. dissolved in water)
Influencing microbial communities

• native organisms are almost always better adapted to local environmental conditions than added organisms would be
  – creates problems for applications of genetically engineered “superbugs” or commercial cultures

• *bioremediation involves control of the microbial community’s immediate environment*
  – dissolved oxygen or other electron acceptors
  – pH
  – temperature (unusual for bioremediation)
  – reactor design to control availability and/or concentration of contaminants and other compounds (*ex situ* treatment)

Therefore we have control, to a large extent, over microbial selection. This is the key to success in bioremediation or other biological processes for waste treatment
Putting microbial ecology into practice

- The science: which organisms do which functions? what conditions do they require to grow and be competitive?

- The art: providing conditions to select for the microorganisms that carry out the desired function

- The engineering
  
  how much?  how fast?  how big?  how good?

  stoichiometry  kinetics  design  analysis
Postulates for biodegradation

- a biochemical mechanism for transformation or complete metabolism of the compound must exist
- organism(s) possessing the relevant gene(s) must be present in the system
  - indigenous organisms
  - organisms inoculated into system (bioaugmentation)
- gene(s) coding for the relevant enzyme(s) must be expressed
  - induced by the compound itself
  - induced by some other means
- the mechanism must be manifested
Why biodegradation mechanisms might not be manifested

- limited bioavailability (generally an issue for hydrophobic chemicals, particularly in the subsurface)
- concentration effects
  - inhibition by the contaminant at high concentration
  - concentration too low to support growth
- inhibition by other chemicals in the system
- other conditions not favorable
  - pH
  - inorganic nutrient (N, P) limitations
  - electron acceptor limitations
Types of bioremediation

- **In situ**
  - monitored natural attenuation
  - biostimulation (injection of nutrients and/or oxygen)
  - reactive barrier

- **Ex situ**
  - pump-and-treat groundwater
  - biofiltration of air removed by soil vapor extraction
  - excavated soil
    - landfarm
    - composting
    - slurry bioreactor
Technical challenges

• challenges are much greater for *in situ* treatment
• if the source is intimately associated with soil, can the soil be excavated?
• if the required process is aerobic, how difficult is it to provide oxygen?
• how difficult is it to provide any other substance needed (e.g., other electron acceptors; inorganic nutrients such as N and P)?
Now I am terrified at the Earth, it is that calm and patient,
It grows such sweet things out of such corruptions,
It turns harmless and stainless on its axis, with such
endless succession of diseas’d corpses,
It distills such exquisite winds out of such infused fetor,
It renews with such unwitting looks its prodigal, annual,
sumptuous crops,
It gives such divine materials to men, and accepts such
leavings from them at last.

—Walt Whitman