Bioremediation and phytoremediation of PAH-polluted soils

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INRA: National Institute for Agronomic Research

- 9500 researchers, engineers, technicians and administrative staff, 1200 students
- Spread over 21 regional research centers, 280 research and 85 experimental units
- Keywords: food, environment, agriculture and society

Phytopharmacy and Semiochemicals Unit
  - improving pesticide uses within an integrated crop protection context
  - studying intoxication processes in target and non-target organisms, pesticide resistance development and the environmental fate of xenobiotics
  - studying chemical communication between organisms as an alternative methods to classical chemical control

http://www.inra.fr
Outcome of the course

I-PAHs: a family of environmental pollutants with hazardous potential (PAH properties and hazard, interactions with soil components)

II-Bacteria, fungi and plants: their potential for PAH biotransformation (principles of PAH transformation, PAH transformation pathways)

III-Bioremediation processes: a way to reduce the environmental hazard of PAHs (mainly used methods, case studies)

IV-Future prospects (improving the bioavailability of PAH, as well as the ability of organisms to transform PAHs, phytoremediation)
I

PAHs:

*a family of environmental pollutants with hazardous potential*

- PAH properties and hazard
- interactions with soil components
Physicochemical properties of PAHs: a basis for recalcitrance

- 2 or more fused benzene rings and/or pentacyclic moieties in linear, angular and cluster arrangement (MW = 128.2 to 278.4)
- thermodynamically stable because of large resonance energy
- very low aqueous solubility (<<1 mg/l), highly hydrophobic (logKow = 3.3 to 6.75)
- neutral
- strongly associated with particle surfaces in the environment (Koc = 2 x 10³ to 3.47 x 10⁶)
- in general, poor volatilization, photolysis and biodegradation
Origin of PAHs

- continuous release into air, soil and water
- contaminate feed and food, and the food chain
- **natural** origin
  - biogenic: components of living organisms
  - geochemical: pyrolysis of organic substances, aromatization of biological compounds during humification processes
  - petrogenic: petroleum, coal, ancient sediments
  - pyrogenic: vegetation fires
- **anthropogenic** origin
  - point sources: spills or mismanaged industrial operations
  - low-level inputs: atmospheric depositions
Biological effects of PAHs on living organisms

- carcinogenicity: increases with ring number and condensation degree
- mutagenicity: form DNA adducts
- teratogenicity
- immunodepressive effects
- high potential to biomagnification
Environmental hazard

- persistent organic pollutants (POP) in the environment
  - long half-lives: phenanthrene: 16-126 d; benzo[a]pyrene: 229-1400 d
  - rate of deposition > rate of degradation → accumulation
- can enter the food chain
- biological adverse effects
  ⇒ persistence + biological effects ⇒ hazard
  ⇒ bio- and phytoremediation of soils may reduce this hazard
determine transport, fate and bioavailability of PAHs

- **sorption/desorption kinetics**
  - mainly due to binding with natural organic matter (humic acids), affinity expressed as $K_{oc}$
  - function of PAH hydrophobicity ($K_{ow}$) and molecular size
  - PAHs also exist as non-aqueous phase liquids (NAPLs)

- **sequestration**
  - time-dependent entrapment within the micropores of soil humic materials
  - mechanisms not fully understood
PAHs/soil interactions (2)

- formation of bound residues
  - complexes formed from parent compounds or derived metabolites with organic constituents of soils, or living organisms
  - attachment of PAHs to reactive sites (amines, phenols, quinones) on the surfaces or the organic colloids, or incorporation of the compound into the structure of humic and fulvic acids, or cells

- characteristics of polluted soils
### Characteristics of PAH-polluted soils

<table>
<thead>
<tr>
<th></th>
<th>Wood-preserving facility</th>
<th>Manufactured gas plant</th>
<th>Oilfield battery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PAH contamination (mg/kg)</strong></td>
<td>&lt;5000</td>
<td>&lt;25000</td>
<td>&lt;5000</td>
</tr>
<tr>
<td><strong>fluoranthene/pyrene ratio</strong></td>
<td>&gt;1</td>
<td>&gt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>3-ring (%)</strong></td>
<td>50-60</td>
<td>10-30</td>
<td>30-45</td>
</tr>
<tr>
<td><strong>4-ring</strong></td>
<td>35-45</td>
<td>40-50</td>
<td>35-45</td>
</tr>
<tr>
<td><strong>5-ring</strong></td>
<td>3-5</td>
<td>15-30</td>
<td>10</td>
</tr>
<tr>
<td><strong>6-ring</strong></td>
<td>&lt;5</td>
<td>5-15</td>
<td>5-15</td>
</tr>
<tr>
<td><strong>Additional chemicals</strong></td>
<td>cresols, phenols, chromated copper arsenate</td>
<td>cyanides, heavy metals, sulfates</td>
<td>methylated PAHs BTEX</td>
</tr>
<tr>
<td><strong>Soil organic carbon content</strong></td>
<td>low</td>
<td>high (&gt;10%)</td>
<td>± high</td>
</tr>
<tr>
<td><strong>Aging</strong></td>
<td>30 years</td>
<td>100 years</td>
<td>±</td>
</tr>
<tr>
<td><strong>Potential for bioremediation</strong></td>
<td>++</td>
<td>--</td>
<td>+</td>
</tr>
</tbody>
</table>
Polluted soils
II

Bacteria, fungi and plants: their potential for PAH biotransformation

- principles of PAH transformation
- PAH transformation pathways
Agents involved in biotransformation reactions

- All living organisms: micro-organisms, higher plants and animals

- The soil micro-organisms

<table>
<thead>
<tr>
<th>Number (/g of soil)</th>
<th>Biomass (ton DM/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>$10^8$</td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>$10^7$</td>
</tr>
<tr>
<td>Fungi</td>
<td>$10^6$</td>
</tr>
</tbody>
</table>

Total biomass: 2 to 4% of carbon, 5 to 8% of nitrogen

- Functions
  - mineralization and humification of organic matter
  - biogeochemical cycles: C, N, P, S (1 to 2 tons of organic carbon mineralized/year/ha)
  - production of toxins or interesting compounds (antibiotics)
  - degradation of xenobiotics
# Soil micro-organisms

<table>
<thead>
<tr>
<th>Prokaryotic organisms</th>
<th>Eukaryotic organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td><strong>Ascomycetes</strong></td>
</tr>
<tr>
<td>The most numerous</td>
<td>Elongated single cells</td>
</tr>
<tr>
<td>Morphology</td>
<td>Elongated single cells</td>
</tr>
<tr>
<td></td>
<td>Branched into filaments</td>
</tr>
<tr>
<td></td>
<td>or hyphae</td>
</tr>
<tr>
<td>Liquid cultures</td>
<td>+, turbidity</td>
</tr>
<tr>
<td>Cellular organization</td>
<td>A few organelles, no cytoskeleton</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Aerobic or anaerobic</td>
</tr>
<tr>
<td>DNA</td>
<td>Cytoplasmic and circular + plasmidic</td>
</tr>
<tr>
<td>RNA and proteins</td>
<td>Synthesized in the same cell compartment</td>
</tr>
<tr>
<td>Reproduction</td>
<td>Division by binary fission</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Competitive pH</td>
<td>6-8</td>
</tr>
<tr>
<td></td>
<td>&lt;5</td>
</tr>
<tr>
<td>Competitiveness</td>
<td>All soils</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Interactions</td>
<td>Symbiotic, pathogenic</td>
</tr>
<tr>
<td>Common genera</td>
<td><em>Arthobacter</em> (40%)</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas</em> (10-20%)</td>
</tr>
<tr>
<td></td>
<td><em>Bacillus</em> (10%)</td>
</tr>
<tr>
<td>Production</td>
<td>Antibiotics: chloramphenicol,...</td>
</tr>
</tbody>
</table>
Biodegradation = mineralization

- Complete breakdown of the xenobiotic which become a substrate
- Carbon and energy are used for cellular growth and division

\[
\text{biomass} + C_6H_{12}O_6 + NH_3 + O_2 \Rightarrow \text{new biomass} + CO_2 + H_2O \\
(C_5H_7NO_2)
\]

- The most interesting process from an environmental viewpoint
Cometabolism

- Partial and fortuitous transformation of the compound
- Carbon and energy are not used for cellular growth and division
- A cosubstrate is needed to supply carbon and energy

\[
\text{C} + \text{\*compound + cosubstrate} \rightarrow \text{\*metabolite or \*CO}_2
\]

- Cometabolism involves enzymes with low substrate specificity
- It induces mainly minor modifications of the compound
- It may lead to the total degradation of the compound during sequential attacks by several organisms

- It represents the main transformation process in the environment, with a possible accumulation of intermediate hazardous products
• Partial transformation leading to metabolites more complex and stable than the parent compound

• Conjugation $\Rightarrow$ linkage of the compound with hydrophilic substrates, followed by excretion or storage

\[
\text{C} + \ast \text{compound} \rightarrow \ast \text{conjugated compound}
\]

• Oligomerization $\Rightarrow$ linkage of the compound with itself, or with residues of xenobiotics or natural products
• incorporation in cellular components or in soil constituents

\[
\text{C} + \ast \text{compound} \rightarrow \ast \text{compound} - \ast \text{compound} \text{ or } \ast \text{C} \text{ or } \ast \text{soil}
\]
Xenobiotic metabolism

**Phase I**
- oxidation
- reduction
- hydrolysis

⇒ Activated metabolites

**Phase II**
- conjugation

**Phase III**
- oligomerization
- secondary conjugation

⇒ Incorporation/stabilization

Final metabolites
### Outcome of the metabolic reactions

<table>
<thead>
<tr>
<th>Initial</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td>xenobiotic</td>
<td>oxidation reduction</td>
<td>conjugation</td>
<td>oligomerisation incorporation</td>
</tr>
<tr>
<td></td>
<td>hydrolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lipophilic</td>
<td>amphiphilic</td>
<td>hydrophilic</td>
<td>hydrophilic insoluble</td>
</tr>
<tr>
<td>mobile</td>
<td>+/- mobile</td>
<td>little mobile</td>
<td>immobile</td>
</tr>
<tr>
<td>toxic</td>
<td>+/- toxic</td>
<td>little or not</td>
<td>not toxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>toxic</td>
<td></td>
</tr>
</tbody>
</table>
PAH phase 1 metabolism

Institut National de la Recherche Agronomique
(from Atlas and Cerniglia, 1995)
PAH phase 2 metabolism

1-Naphthyl sulfate

1-Naphthyl β-D-glucuronide

1-Phenanthryl β-D-glucopyranoside

1-Anthryl β-D-xylopyranoside

(from Sutherland, 1992)
PAH activation

(from Atlas and Cerniglia, 1995)
Actual knowledge on bacterial metabolism of PAHs

David T. Gibson, the pioneer

- *a wide variety of bacteria* metabolize PAHs
- bacteria utilize PAHs as a *sole source of carbon and energy*
- bacteria are able to cleave the aromatic rings and to mineralize them rapidly
- bacteria generally metabolize PAHs to dihydrodiols and to aliphatic chains
- dioxygenases are mainly involved
- the initial ring oxidation reaction is often the rate-limiting step
Actual knowledge on fungal metabolism of PAHs (1)

main data from Carl E. Cerniglia’s work

- a wide variety of fungi among zygomycetes, ascomycetes and basidiomycetes (white rot or brown rot) metabolize PAHs
- they need an additional carbon source to metabolize them
- non-basidiomycetes generally oxidize PAHs without mineralization
- ligninolytic basidiomycetes are able to cleave the aromatic rings and mineralize them
- non-ligninolytic fungi generally metabolize PAHs to:
  - cis-dihydrodiols, phenols $\rightarrow$ conjugated = detoxification
  - quinones, dihydrodiol epoxides $\rightarrow$ bioactive and toxic = activation
• several enzymatic systems are involved
• fungal metabolism is highly regio- and stereoselective and is in some cases similar to mammalian metabolism
• fungal metabolism is important as it produced ring-oxidized products, the rate-limiting step for bacterial degradation
• fungal metabolites have high water solubility and enhanced chemical reactivity
• fungi also detoxify PAHs by forming bound residues during humification
Actual knowledge on higher plant metabolism of PAHs

- our actual knowledge is scarce, but plants may offer a great potential
- whole plants:
  - adsorption onto roots and uptake is significant, but translocation of PAHs with more than 4 rings from roots to leaves is negligible
  - plant germination and growth are strongly inhibited by PAHs with less than 3 rings
  - incorporation of PAHs into cellular components has been reported
- cell suspensions:
  - PAHs are oxidized to phenols or quinones, then conjugated
- actual areas of research:
  - rhizosphere $\Rightarrow$ bacterial metabolism
  - arbuscular mycorrhizal fungi $\Rightarrow$ fungal metabolism
Anaerobic degradation of PAHs

- occurs in anoxic environments
- concerns naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene
- terminal electron acceptors: $CO_2$, $SO_4^{2-}$, $NO_3^-$, $Fe_3^+$
- theoretical equations for naphthalene

(Rockne and Strand, ESET, 1998, 32 3962-3967)

1/48 $C_{10}H_8 + 1/5 NO_3^- + 1/5 H^+ \rightarrow 5/24 CO_2 + 1/10 N_2 + 11/60 H_2O$

1/48 $C_{10}H_8 + 1/8 SO_4^{2-} + 3/16 H^+ \rightarrow 5/24 CO_2 + 1/16 H_2S + 1/160 HS^- + 1/12 H_2O$
Dioxygenases

Equation: \[ R + O_2 + NADH + H^+ \rightarrow R(OH)_2 + NAD^+ \]

Distribution: bacteria

Function: xenobiotic metabolism

Structure: naphthalene dioxygenase
- multi-component system of 3 proteins including a flavoprotein
- terminal dioxygenase: iron-sulfur protein of 158 kDa with two subunits of 20 and 55 kDa

Regulation: ?
- inducible by PAHs
Cytochrome P450 mixed-function oxidases

Equation: \( RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+ \)

Distribution: widely distributed among living organisms

Function: xenobiotic metabolism, cellular metabolism

Structure: multi-enzymatic complex (P450 + reductase), mostly microsomal membrane-bound proteins (ER), exposed to the cytosol
P450: heme-thiolate protein of 400 to 525 aa, 45 to 65 kDa
\( N \)-terminal segment: anchor signal

Regulation: complex
numerous inhibitors (mechanism-based inactivators)
inducible by physiological, physico-chemical and xenobiotic agents

Molecular data: http://drnelson.utmem.edu/homepage.html
superfamily of genes, 500 being cloned
Laccases

Equation: $2RH + \frac{1}{2}O_2 \rightarrow 2R^* + H_2O$

Distribution: fungi, bacteria, higher plants, insects

Function: lignin degradation, morphogenesis, pathogenesis

Structure: dimeric or tetrameric glycoprotein, with 4 Cu, exocellular, 520 to 550 aa, 60 to 80 kDa secreting a N-terminal peptide

Regulation:
- inhibitors: small anions, metals, fatty acids, chelating agents
- inducers: culture medium, lignin and related compounds, xenobiotics
III

Bioresmediation processes:
a way to reduce the environmental hazard of PAHs

-mainly used methods
-case studies
How to select a remediation method?

- pollutant: type, concentration, extent, ageing
- type of soil: hydrogeology, texture, permeability
- local constraints: space, noise, smell, dust (urban areas)
- cost
- time limit
- threshold
Bioremediation

- use of the natural or engineered capacity of micro-organisms to transform PAHs

- incubation conditions are optimized by aeration, agitation, moistening, addition of nutrients

- case studies: Federal Remediation Technologies Roundtable
  http://www.frtr.gov/cost/
Treatments

• Out site

• On site

• In situ
The *ex situ* bioremediation methods

- Many advantages:
  - optimized monitoring ensured by mixing, heating, ventilation and moistening
  - optimized biotransformation due to the supply of electron acceptors and nutrients, (micro-organisms)

→ reduction of the heterogeneous structure of the soil

→ 3 main methods: bioslurry, biopile, landfarming
The bioslurry

organisms

surfactants
electron acceptors
treatment of gaseous phase

bioreactor

liquid / soil phase = slurry

separation

water
treatment

soil
treatment

landfarming

discharge

nutrients

sieved soil

water

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Bioslurry Bioremediation System

Schematic of a bioslurry bioremediation system. Source: Adapted from the U.S. EPA (8).
Bioslurry

- Pollutants: recalcitrant compounds, PAHs (2,500 to 250,000 mg/kg)
- Type of soil: heavy soils, with high clay content
- Duration of the treatment: 1 to 6 months
- Operating conditions: pH 4.5 to 8.8
  - temperature 15 to 35 °C
  - fixed or rotative
  - nutrients and surfactants
- Soil native microflora or engineered strains
- Cost: 50 to 250 $/m³
- Efficiency: 3-ring PAHs 98-99%
  - 4-ring PAHs 85-95%
  - > 4-ring PAHs 55-85%
- Emerging technology: Fenton’s reagent to oxidize heavy PAHs
- http://www.epa.gov/ORD/SITE/reports
The bioslurry reactor: ECOVA
**Bioslurry: case study (1)**

- **Operating conditions**
  - raw materials: 12,630 tons of soil containing up to 10 g PAH/kg
  - after sifting, wettering and centrifuging: 8,925 tons of slurry (<80µm) treated in four bioreactors

- **Parameters of the bioreactors**
  
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>750,000 L</td>
</tr>
<tr>
<td>Shaking</td>
<td>900 rpm</td>
</tr>
<tr>
<td>Air</td>
<td>350 ± 170 m³/h</td>
</tr>
<tr>
<td>Solid particle ratio</td>
<td>20% by weight</td>
</tr>
<tr>
<td>Antifoam</td>
<td>200 mg/L</td>
</tr>
<tr>
<td>Dispersing agent</td>
<td>1000 mg/L</td>
</tr>
<tr>
<td>Temperature</td>
<td>30 ± 10 °C</td>
</tr>
<tr>
<td>pH</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>Dissolved O₂</td>
<td>&gt; 2.0 mg/L</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>60 ± 20 mg/L</td>
</tr>
<tr>
<td>PO₄²</td>
<td>20 ± 10 mg/L</td>
</tr>
</tbody>
</table>

Southeastern Wood Preserving Superfund Site
Canton, Mississippi
## Bioslurry: case study (2)

### Results

<table>
<thead>
<tr>
<th>PAH Type</th>
<th>Initial amount (mg/kg)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-ring PAH&lt;sub&gt;1&lt;/sub&gt;</td>
<td>4613 ± 1785</td>
<td>94</td>
</tr>
<tr>
<td>4-ring PAH&lt;sub&gt;1&lt;/sub&gt;</td>
<td>3432 ± 113</td>
<td>95</td>
</tr>
<tr>
<td>5 and 6 ring PAH&lt;sub&gt;1&lt;/sub&gt;</td>
<td>729 ± 329</td>
<td>62</td>
</tr>
</tbody>
</table>

### Financial Data ($/ton)

<table>
<thead>
<tr>
<th>Category</th>
<th>Slurry  making</th>
<th>Biotreatment drying</th>
<th>Slurry drying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant material and labor</td>
<td>25-30</td>
<td>8-12</td>
<td>4-8</td>
</tr>
<tr>
<td>Supplies, electricity,…</td>
<td>16-20</td>
<td>20-25</td>
<td>12-16</td>
</tr>
<tr>
<td>Analysis</td>
<td>&lt; 4</td>
<td>4-8</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Total</td>
<td>42-50</td>
<td>32-46</td>
<td>16-25</td>
</tr>
</tbody>
</table>

+ treatment of water in wastewater treatment plant
The static biopile

- WATERING SYSTEM: water + nutrients
- POLLUTED SOIL
  - Micro-organisms
- GREENHOUSE or GORE-TEX COVER
- HPDE LINER
- SAND
- DRAINS (air)
- PROTECTING LAYER
Biopile

- **Pollutants:** all organic pollutant, PAHs <5,000 mg/kg
- **Type of soil:** permeable
- **Duration of treatment:** 6 to 24 months
- **Operating conditions:** height: 0.9 to 3 m
  - cover: textile or greenhouse
  - ambient temperature
  - moistening 40 to 75% of MHC
  - soil piled in mounds
  - static (forced venting) or dynamic (soil reversal)
  - nutrients and surfactants
  - collection of leachates
- **Soil native micro-flora or exogenous micro-organisms**
- **Cost:** $40 to 220/ton of soil
- **Efficiency:** 80%
- **Similar method:** composting with addition of fertilizers such as manure
- http://www.epa.gov/OUST/cat/biopiles.htm
Biopile
Biopile: case study (1)

- Operating conditions: laboratory scale study
  sifted sandy loam (15 mm) containing 2800 mg PAH/kg, 220 mg cyanide/kg
  17 g heavy metal/kg, ...
- Parameters of the “biopiles”
  Jars containing 6 kg polluted soil
  Static in the dark
  Air: forced venting 3 L/h
  Temperature: 25 ± 5 °C
  Moistening: 80% of the MHC
  pH: 6.9
  C/N/P ratio: 100/5/1, agricultural fertilizers
  Inoculation: the fungus Trametes versicolor pregrown on wheat bran pellets (0.6g pellets/jar)
  Surfactant: Montanox 80 (non ionic), 2 % by weight
  Duration: 50 weeks

GDF Manufactured Gas Plant
Rouen, France
Biopile: case study (2)
- Results

<table>
<thead>
<tr>
<th>Pellets</th>
<th>T. vers</th>
<th>C/N/P</th>
<th>Surf.</th>
</tr>
</thead>
<tbody>
<tr>
<td>●</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>□</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>●</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>●</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>●</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>●</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

→ a 40% decrease of PAH amounts extracted from the soil after 50 weeks in the presence of the pellets, the fungus, nutrients and surfactant

Rama et al., PAC, 2001, 18(4), 397-414
In land farming, waste or contaminated soil is aerobically treated above ground to biodegrade, transform, and immobilize contaminants. Source: Adapted from the U.S. EPA (8).
Landfarming
Landfarming

- **Pollutants**: all organic pollutant, PAHs <10,000 mg/kg
- **Type of soil**: all
- **Duration of treatment**: 6 to 24 months
- **Operating conditions**: soil layered on a specific area, sometimes incorporated in the top layer of an agricultural soil, soil periodically tilled, ambient temperature, nutrients and moisture may be added, collection of leacheates may be necessary
- **Soil native micro-flora or exogenous micro-organisms**
- **Cost**: $30 to 60 / ton of soil
- **Efficiency**: 90%
Landfarming: case study

Treatek, Blackburn, United Kingdom

- **Operating conditions**
  - 30,000 m$^3$ of soil polluted with tar, phenolics, cyanides, heavy metals, PAHs
  - sifting < 30 mm
  - soil treated in several layers
  - soil moistened by a solution of micro-organisms and nutrients

- **Results**

<table>
<thead>
<tr>
<th></th>
<th>Initial amount (mg/kg)</th>
<th>Removal efficiency (after 30 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAHs</td>
<td>22,050</td>
<td>99</td>
</tr>
<tr>
<td>Phenolics</td>
<td>205</td>
<td>98</td>
</tr>
</tbody>
</table>

- **Cost:** 45 $/m^3
IV

Future prospects

- improving the bioavailability of PAHs
- improving the ability of organisms to transform PAHs
- phytoremediation
Improving the bioavailability of PAHs

- Use of synthetic surfactants and biosurfactants to facilitate the partitioning of PAHs from the solid phase of the soil or from NAPL to the water phase
- A considerable literature has been obtained at a laboratory scale, but a few results are usable in industrial processes

- a variety of ionic and nonionic surfactants enhance the solubilization of PAHs
- the enhancement varies with surfactant structure, concentration in solution, hydrophobicity of the PAHs, degree and age of contamination
  → cationic agents, adsorbed onto soil, solubilize PAHs at supra-CMC levels, but they are toxic for micro-organisms
  → nonionic compounds disperse the soil particles, and also solubilize PAHs, with a reduced toxicity
- limitations: high concentrations of PAHs are required to solubilize low amounts of PAHs, in the presence of a high water-content of the soil
  → most successful results are obtained in bioslurry reactors
Improving the ability of organisms to transform PAHs

The problems
- The complete breakdown of a pollutant requires a sequence of metabolic reactions
- Xenobiotics often include unusual chemical bonds or substitutions
- Organisms have not had enough time to evolve appropriate pathways

The solutions
- Development of a microbial community
- Development of the capabilities of given strains by genetic construction → genetically modified organisms: GMOs
  engineered micro-organisms: GEMs

The limitations
- Ecological problem: risk of rambling genes
- World-wide regulation by legislation

The ways of research
- Improvement of the metabolic properties of the organism (Abstract N°58)
- Development of suicidal elements for biological containment of organisms
Phytodecontamination

- Phytoextraction (harvest and destruction)
- Phytovolatilization
- Phytodegradation (plant metabolism)
- Rhizo- and mycorrhizo-spheric degradation (microbial and fungal metabolism)

Polluted soil
Phytodecontamination ex planta

A chemical present in the soil induces a plant response, which increases or changes its exudation, modifying then rhizospheric microflora composition or activity.

Other effects:
- improvement of physical and chemical soil conditions
- increase of humification and adsorption

→ dissipation of PAHs
  impact of each process not elucidated

Plant species: fescue (Festuca), alfalfa (Medicago), sudangrass (Sorghum), ryegrass (Lolium), corn (Zea), soybean (Glycine),...