

Chemistry of hydrocarbon pollutants

Oil is a complex mixture:

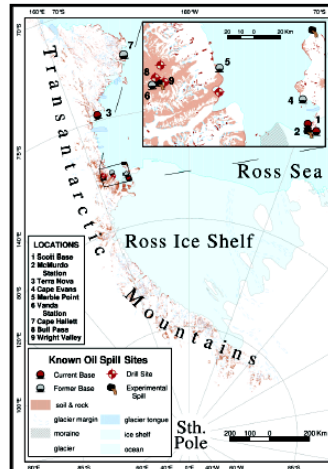
- Alkanes
 - straight chains: easiest to degrade
 - Short-chain (e.g. octane)
 - Long-chain (up to 44 Cs)
 - Terminal oxidation to alcohol, fatty acid, etc
 - Branching decreases biodegradability
 - cyclic alkanes (e.g. cyclohexane)
 - Harder to degrade
- Aromatic rings
 - Simple rings (benzene, toluene, xylene), not too bad
 - polyaromatic HCs (PAHs) >5 rings require co-metabolism
 - monooxygenase, forms diol, ring breakage → dicarboxylic acid
- Resins and asphaltenes
 - Complex, weird, unknown structures, hard to degrade

Limiting factors in bioremediation

- Organisms (toxicity)
 - Pristine environment: <0.1% of microbes degrade HCs*
 - Chronic exposure: up to 100%
 - (*HCs very complex, actually a succession of orgs, including “non-degraders” using breakdown products)
 - “bioaugmentation” adding exogenous microbial population (vs. “biostimulation” – stimulating indigenous community)
 - Commercial mixes sold – OK in lab, bogus in field?
- oxygen/e- acceptors
 - Most HC degraders use monooxygenase, need O₂
 - Anaerobic degradation can occur, given SO₄⁼ or NO₃⁻

Limiting factors in bioremediation

- Nutrients, energy (cometabolism)
 - Redfield ratio: 1 g HC: 0.15 N: 0.03g P
 - Sometimes Fe limiting in seawater
- Other environmental factors: pH, temperature
 - oil degradation usually occurs best at pH 6-9
 - Low temp: high viscosity, lower solubility of high MW HCs, slower rates in general, except in psychrophile communities



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TABLE 2. PAH Contents of Hydrocarbon-Contaminated Soils of the Ross Sea Region

polycyclic aromatic hydrocarbon (ng/g of dried soil)	Scott Base ^a	McMurdo Station ^a	Cape Evans ^a	Marble Point ^a	Vanda Station ^a
soil depths sampled (cm)	0-30	0-5	0-10	0-30	0-5
no. of soils analyzed from each site	5	20	7	4	2
naphthalene	235-6858	5-27 000	<300	<30-127	5.6-244
1-methyl naphthalene	125-2820	na ^b	na	<30	<30-259
2-methyl naphthalene	160-3015	na	na	<30-53	<30-436
acenaaphthylene	<30-43	11-15 700	bd ^c	<30	<30-137
acenaaphthene	<30-203	30-17 800	bd ^c	<30	<30-69
fluorene	29-206	32-1590	1-250	<30	<30-286
phenanthrene	40-232	5-540	11-2460	<30	<30-1052
anthracene	<30	76-5900	2-160	<30	<30-121
fluoranthene	<30-161	2-13 300	18-770	<30	<30-462
pyrene	<30-416	3-132	11-1170	<30	<30-851
benz[a]anthracene	<30-58	4-1420	6-1230	<30	<30-58
chrysene	<30-60	5-1630	6-2950	<30	<30-96
benzo[b]fluoranthene	<30-60	na	6-340	<30	<30
benzo[k]pyrene	<30	na	2-370	<30	<30
indeno[1,2,3-cd]pyrene	<30	na	<70	<30	<30
diacephenanthrene	<30	na	<70	<30	<30
benzo[ghi]perylene	<30	na	<70	<30	<30

^a Data taken from ref. 43. ^b Data presented for McMurdo Station are the range of averages taken from ref. 42. ^c Data taken from ref. 42. ^d First analyzed. ^e Below detection limits.

TABLE 1. Chemical and Microbial Properties and Summer Moisture Contents of Hydrocarbon-Contaminated and Pristine Soils of the Ross Sea Region^a

location depth (cm)	THP (mg/kg dry wt)	MDP of hydrocarbon degraders (g ⁻¹ dry wt)	no. of culturable heterotrophs (g ⁻¹ dry wt)	% moisture	pH (water)	total C (%)	total P (%)	total N (%)	nitrate-N (mg/kg dry wt)	ED ₅₀ (mS/cm)
Scott Base										
pristine ^b										
0-1	<30	33	3.4 × 10 ⁶	1.8	8.9	0.10	0.19	0.01	1.3	0.30
15-30	<30	13	2.5 × 10 ⁵	6.2	8.3	0.06	0.18	0	0	0.07
contaminated ^c										
0-2	33700	1.3 × 10 ⁶	4.2 × 10 ⁶	1.6	7.8	5.14	0.15	0.02	0.8	0.22
20-30	1100	8.3 × 10 ⁴	1.4 × 10 ⁶	7.0	9.8	1.17	0.1	0.00	0.5	0.11
Marble Point										
pristine ^b										
0-3	<20	<10	3.7 × 10 ⁴	2.4	9.6	0.28	0.07	0.02	2.2	0.65
15-32	<20	<10	2.4 × 10 ⁴	5.9	7.9	0.50	0.07	0.01	1.4	0.99
contaminated ^c										
0-3	29100	1.1 × 10 ⁶	5.3 × 10 ⁶	1.9	8.3	5.33	0.06	0.02	0.5	0.18
12-27	200	8.8 × 10 ⁴	6.6 × 10 ⁶	11.4	9.5	0.20	0.06	0	0.5	0.20
Wright Valley										
pristine ^b										
0-2	<20	nd ^d	5.6 × 10 ³	0.2	7.6	0.03	0.02	0.01	0	5.59
15-48	<30	nd	<100	2.0	7.1	0.02	0.03	0.04	103.8	7.25
contaminated ^c										
0-2	<30	nd	<100	0.4	7.4	0.02	0.03	0.01	24.5	0.62
16-35	960	nd	nd	4.8	7.3	0.07	0.02	0.05	116.6	11.96

^a See Table 1a in the Supporting Information for a detailed data. ^b Total polycyclic aromatic hydrocarbons. ^c Most probable number. ^d Electrical conductivity. ^e Unpublished data (Amabile, J.) Data taken from ref. 48. ^f First detected.

Limiting factors in bioremediation

4. solubility: surfactant/emulsifier production by microbes:

Release bound HCs from surfaces, increase surface area, bioavailability

low MW compounds: glycolipids (e.g. rhamnolipid)

high MW compounds: polysaccharides, proteins

prevent oil droplets from coalescing

highly specific, work at ratios of 1:100 - 1:1000

Produced mainly at stationary phase

Release microbes from droplets after useful energy has been used

Acinetobacter calcoaceticus RAG-1 example

exp. phase: emulsan in cell-bound minicapsule

uses up alkanes, releases capsule

freed from droplet

droplet now coated with hydrophilic layer, "marked"

Evolutionary issues:

Cooperation: why produce emulsifiers?

Community interactions (consortium of emulsifier, HC degrader)

Gene transfer (recent example of HGT of capsule polysaccharide in Biofilm)

Applications of microbes in bioremediation

Petroleum processing

Enhanced recovery: mobilize oil from inaccessible areas, viscosity reduction, decrease of surface tension

Deemulsification: surfactants break up oil-in-water emulsions

Desulfurization: bacteria remove organic S, leave HC

Denitrogenation: bacteria remove heterocyclic N compounds (e.g. pyrroles, indoles), otherwise expensive process

Treatment of Contaminated Soils and Sludges

Passive bioremediation processes

Landfarming of oily wastes

Bioreactor-based processes

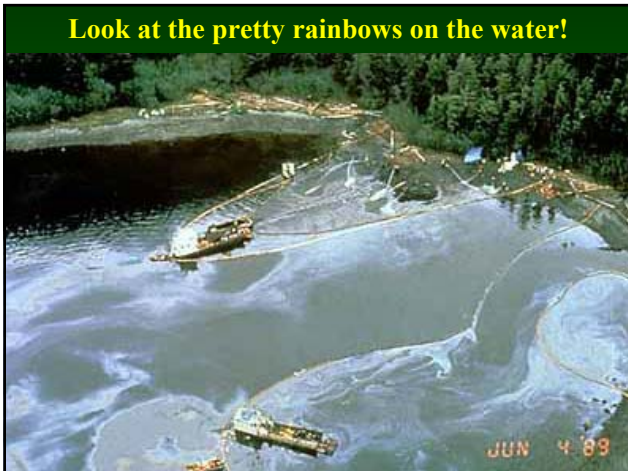
Microbiology and Molecular Biology Reviews 2003, 67: 503–549

TABLE 4. Evaluation of various petroleum sludge treatment technologies

Remediation	Technology	Comments
Bioremediation	Bioreactor	Application of natural and specialized microorganisms in controlled environmental and nutritional conditions, high biodegradation rates, accommodates variety of sludges, nonhazardous residues, on-site operation, cost-effective
	Landfarming	Uses natural microbial population and supplements of mineral nutrients, slow degradation rates, year-round operation difficult, potential to contaminate ground and surface water, cost-effective
	Biopiling	Uses natural microbial population and supplemented nutrients and air, slow degradation rates, year-round operation difficult, potential to contaminate ground and surface water
	Bioventing	A combination of advective soil venting and biodegradation method for in situ treatment of soils, most of the lighter hydrocarbons are volatilized
	Bioaugmentation/bioaugmentation	Application of mineral nutrients/surfactants and/or microorganisms to stimulate or supplement natural microbial population at contaminated site
	Phytoremediation	Uses plants and rhizospheric microorganisms for the treatment of contaminated soil, potential for removal of petroleum contaminants being evaluated, presumably cost-effective
Physicochemical	Incineration	High-temperature treatment, air pollution risks, expensive control equipment, high capital cost
	Thermal desorption	High-temperature oil removal and recovery method from oily solids, high capital and material preparation costs, nonhazardous residues
	Coker	Complicated sludge preparation for coker feed, some oil recovery, high capital and transportation costs
	Cement kiln	Complicated sludge preparation for use of fuel, high material preparation, transportation, and disposal costs
	Solvent extraction	Uses solvents and centrifugation or filtration for the separation of oil from sludges, safety hazard with solvent use, high capital cost



Look at the pretty rainbows on the water!



Bioremediation and metals

Anaerobic respiration and metal reduction

- aerobic respiration: e- acceptor = O₂
- anaerobic respiration:
 - NO₃⁻ (Nitrate) → nitrite (NO₂⁻) → N₂O → N₂
 - SO₄²⁻ → H₂S
 - Fe³⁺ → Fe²⁺
 - Mn⁴⁺ → Mn²⁺
 - Cr⁶⁺ (toxic) → Cr³⁺ (ppt)
 - Uranyl-carbonate complex (sol.) → uraninite (UO₂) (ppt.)
 - Selenate (sol.) → selenite (sol.) → selenium (insol.)
 - Arsenate (bound) → arsenite (soluble)
 - Tc(VII) → Tc(IV) (insol) (technetium)
 - Hg(II) volatile, toxic → Hg(0), inert
 - Cd, Co, Cu, Pd, Zn...

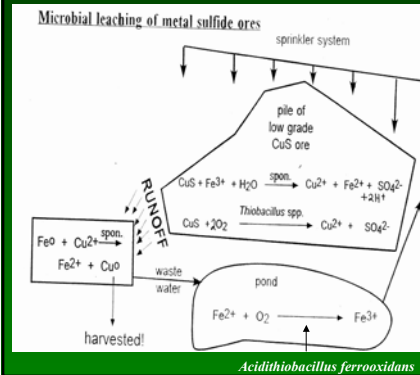
Limiting factors in metal bioremediation

Organisms/toxicity

Energy source: add glucose (or mollasses, e.g. clean up of Cr^{6+} in soil)

Competing electron acceptors (NO_3^- , SO_4^{2-})

Applications: Microbiological ore processing



Applications: bioremediation of hazardous waste

Precipitation of radioactive metals in subsurface environments

Removal of metals in flow-through bioreactors: e.g. chromium waste from nuclear reactors

Selenium contaminated agricultural waste water (even in presence of competing e- acceptor, nitrate)

Perchlorate reduction (munitions dumps)

Constructed wetlands (acid mine drainage, metal-contaminated municipal runoff)

Genetically modified Microorganisms (GEMs) and Bioremediation

Properties of GEMs

High affinity

Rapid growth rate

Resistant to toxicity



e.g. engineered *Deinococcus radiodurans*:

70 million m^3 soil, 3 trillion L groundwater contaminated with mixed waste (radioactive, heavy metals, solvents, acids/bases) during cold war

Pseudomonas toluene deoxygenase gene added

"Superbugs" multiplasmid containing *Pseudomonas* strains



Application of GEMs in the field

Suboptimal conditions

Competition from natural communities

Most experiments show commercial products are useless
(Example: 1 product seems to stimulate, but not better than adding sterilized cell mixture)

Genetic tinkering leads to ecologically crippled organism?

Safety of releasing GEMs

Low risk, high consequences

Gene transfer

Genes can persist in environment: phenol-degrading plasmid found in soil 6 years after addition of GEM

Suicide mechanisms (e.g. hok/sok system)

Cells die in absence of pollutant

Recipients of plasmid die



References

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