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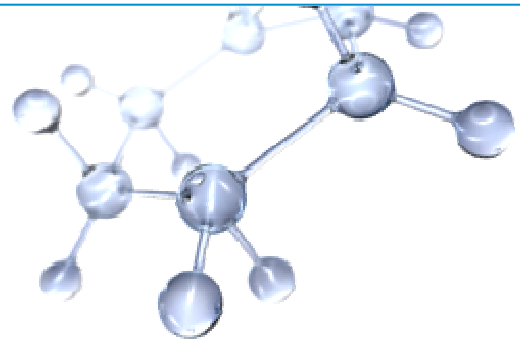
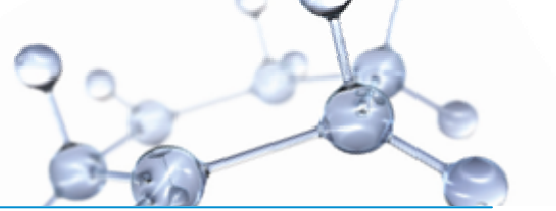


Monitoring oil biodegradation

Roger C. Prince
September, 2011

Fate of spilled oil that is not collected



- Spreading
- Evaporation (20-40%)
- Dissolution (1%)
- Dispersion
- Photochemical oxidation
- Beaching
- Combustion
- Biodegradation

- Only combustion and biodegradation remove oil from the environment.
  - The other processes dilute it, and in the case of photooxidation, can polymerize it.
- Fortunately the biosphere has many oil-degrading microbes.
  - Several hundred genera.

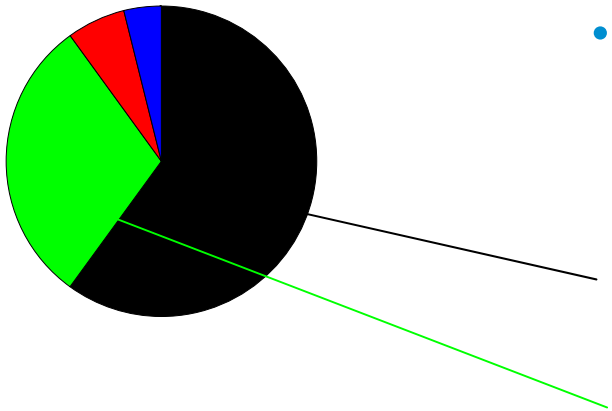
Petroleum

- Petroleum, literally ‘rock oil’, has been part of the biosphere for a very long time
  - The average age of commercially important crude oils is 100 million years.
  - Natural seeps account for about half of the oil getting into the world’s oceans every year, and this has likely occurred for hundreds of millions of years.

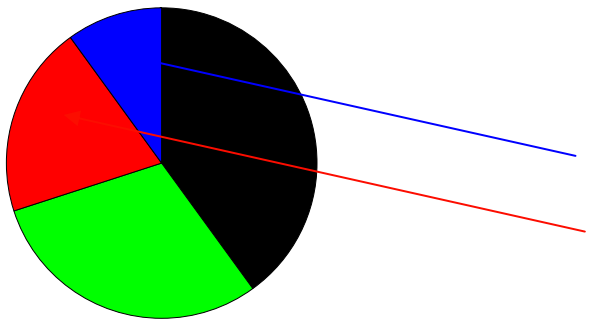
- Crude oils are very complex mixtures – literally hundreds of thousands of discrete chemicals.
  - For convenience the industry divides them into four major classes based on their solubility in sequential solvents :
    + Saturates, including linear, branched and cyclic alkanes
    + Aromatics, including polycyclic aromatics, typically with substantial alkyl substituents
    + Resins and Asphaltenes, which contain heteroatoms in addition to carbon and hydrogen. This group includes the principal colored molecules in petroleum, and is not amenable to gas chromatography.

Composition

Light North Sea Oil



Heavy North Sea Oil

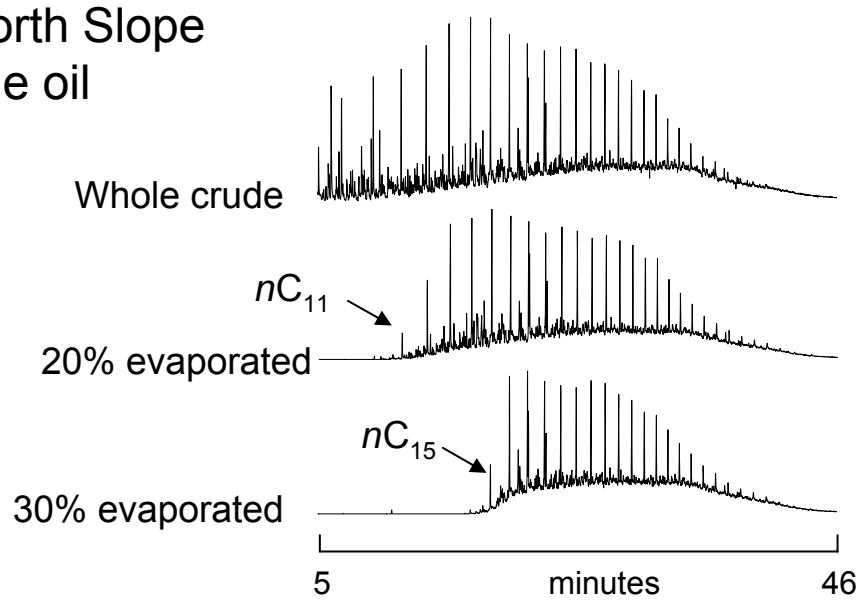


- Crude oils are principally hydrocarbons - and these can be analyzed by Gas chromatography.
  - Saturated molecules
    - + Paraffins
    - + Naphthenes
  - Aromatics
- Also some heteroatom-containing species – asphaltenes and resins - that give the color to crude oils.

Paraffins

- The *n*-alkanes are amongst the most rapidly biodegraded components of crude oils, and they are usually easy to spot in a chromatogram as sharp resolved peaks.

- Although they look so abundant, the discrete peaks in the bottom chromatogram make up only 4.4% of the oil by weight.



GC/MS total ion

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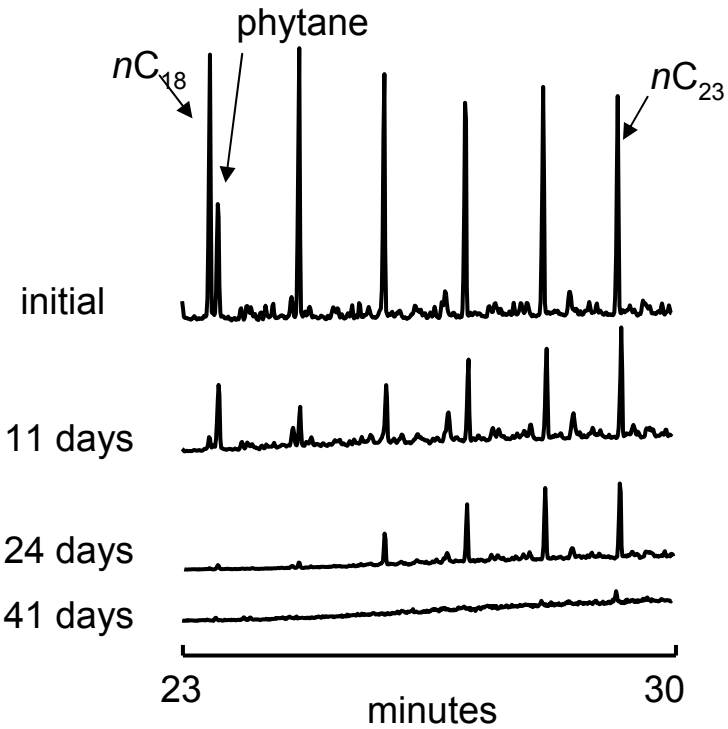
Biodegradation of alkanes

- The *n*-alkanes are usually biodegraded before the branched alkanes pristane and phytane.

- Shorter *n*-alkanes (8-18) are usually biodegraded before longer ones (18+), but all are degraded rapidly.



This is an experiment with New Jersey seawater at 8C

m/z=57

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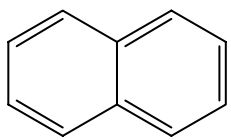
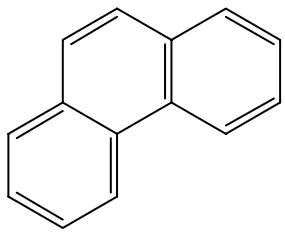
Aromatics

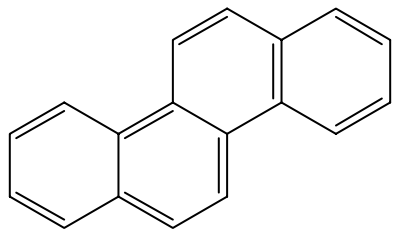
- Aromatic molecules have at least one aromatic (benzenoid – C_6H_6 ring). And very likely many additional carbons (the most abundant resolvable aromatics are three-ring aromatics with about 15 pendant carbons).
- The toxicology of these substituted molecules is poorly known. We do know that the majority of the 16 aromatic hydrocarbons on the USEPA Priority Pollutant list are below the level of detection in most crude oils. Their sum makes up 0.4% of fully evaporated Alaska North Slope crude oil.
- The most abundant polycyclic aromatic hydrocarbons are naphthalene (which readily evaporates), phenanthrene and chrysene. Dibenzothiophenes are also usually present.



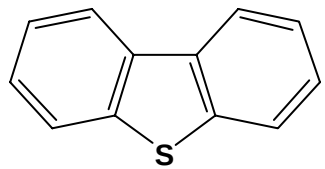
naphthalene



phenanthrene



chrysene



dibenzothiophene

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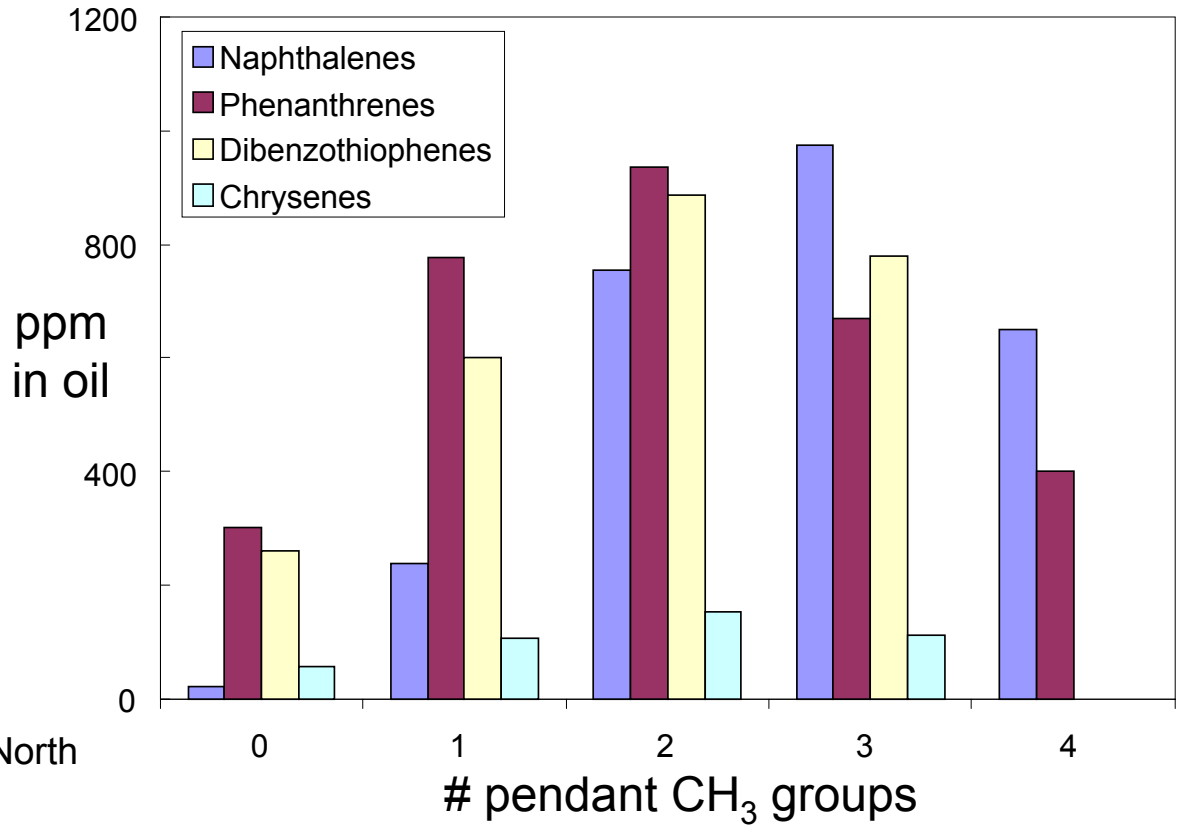
Alkylaromatics

- Petrogenic polycyclic aromatic hydrocarbons show a characteristic distribution of alkyl congeners.

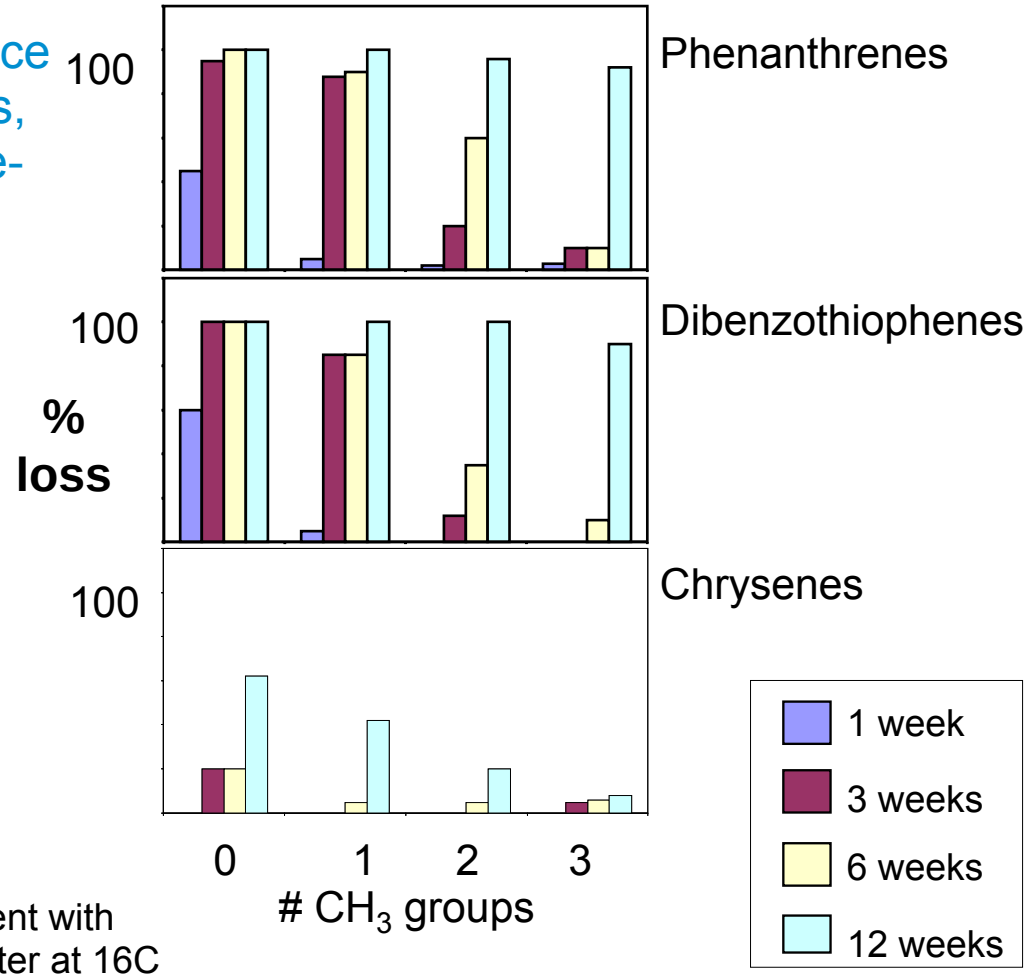
- Pyrogenic polycyclic aromatic hydrocarbons are almost exclusively the parent compound



This is Alaska North Slope crude oil

Biodegradation of aromatics

- There is a well marked preference for smaller over larger aromatics, and for less-alkylated over more-alkylated forms.
- Anaerobic biodegradation not well-studied



This is an experiment with New Jersey seawater at 16C

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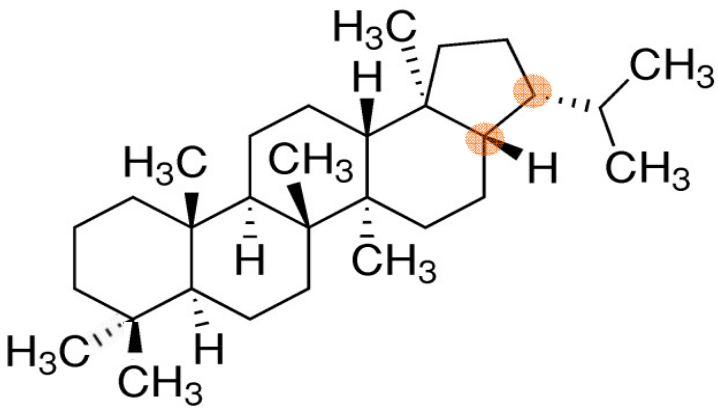
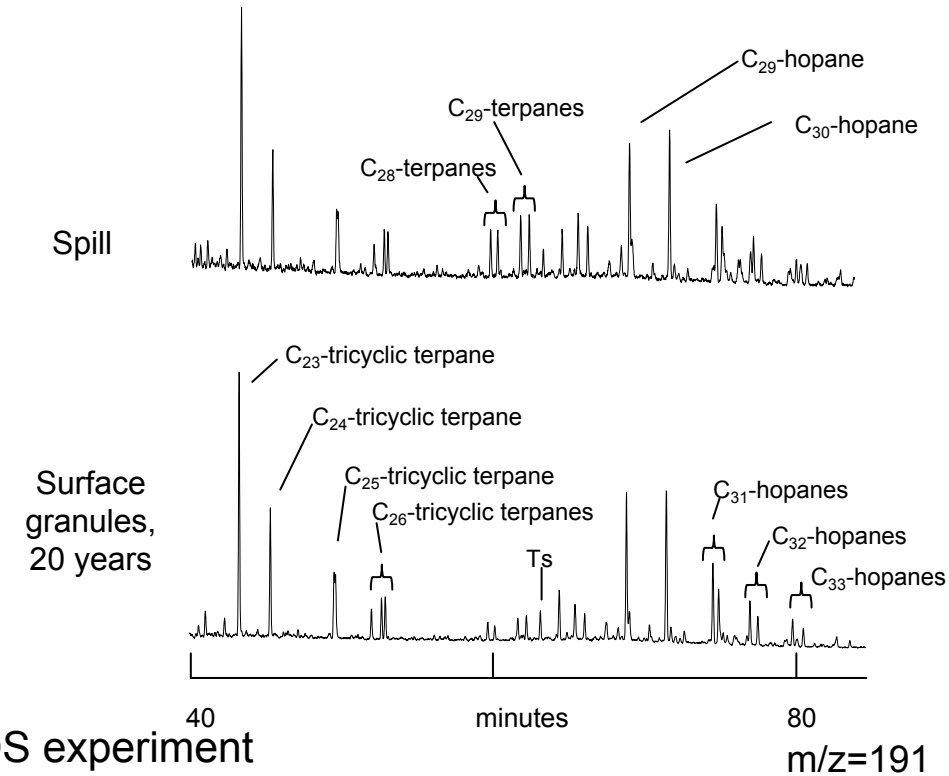
Biomarkers

- Crude oils contain some molecules that are obviously fossil remnants of the initial biomass.
- Reasonably abundant families include the terpanes and hopanes

17α(H),21β(H)-hopane

C_{30}-hopane





BIOS experiment

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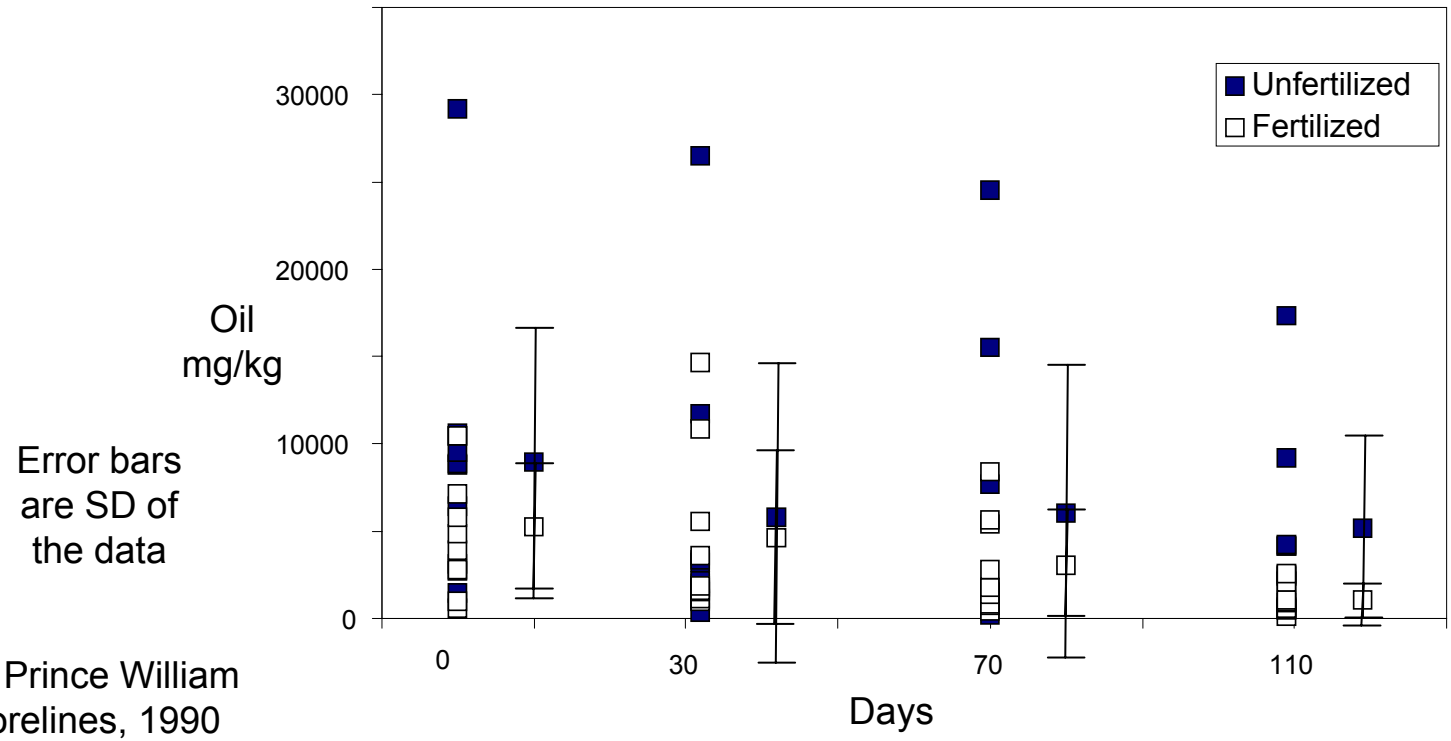
Following biodegradation in the field

- Oil is a very difficult substrate to study – its insolubility means samples are rarely homogeneous.
- Gravimetric estimates can be made with high precision, but with almost no predictive value.



Data from Prince William Sound shorelines, 1990

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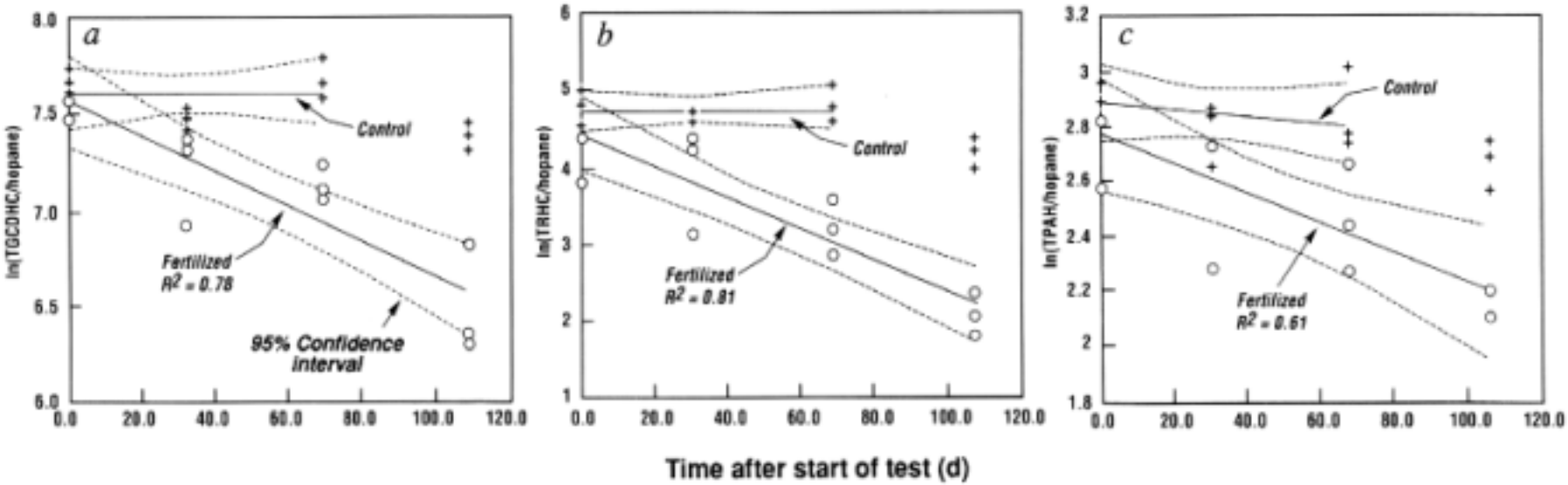
Following biodegradation in the field

- But using a resistant molecule in the oil as a conserved internal marker, and using simple proportion, one can begin to understand and quantify biodegradation.
- Here we used hopane

Total detectable hydrocarbon

Total alkanes

Total polycyclic aromatics



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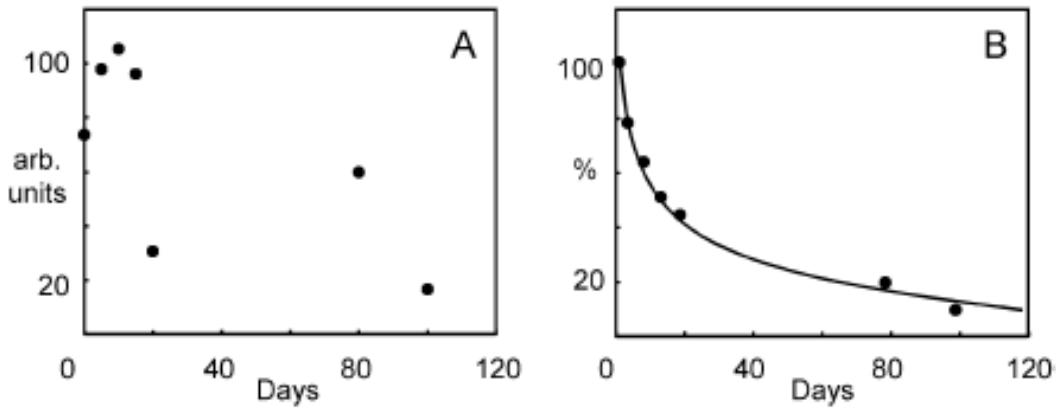
Internal markers aid in lab analyses

- Despite great care, it is difficult to manipulate small amounts of hydrocarbon, especially in a glove-box for anaerobic work.
  - This is the anaerobic biodegradation of 2-methylhexane (in gasoline) under sulfate-reducing conditions. A is raw data, B referred to 1,1,3-trimethylcyclohexane as a conserved internal standard.



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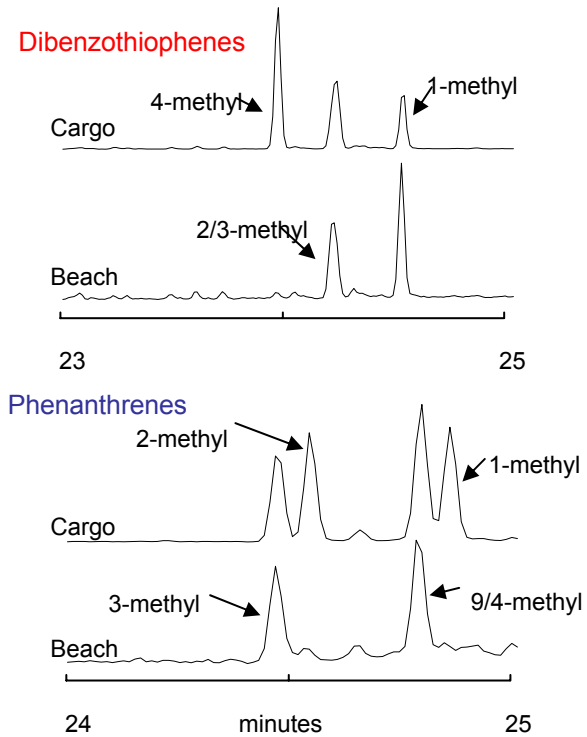
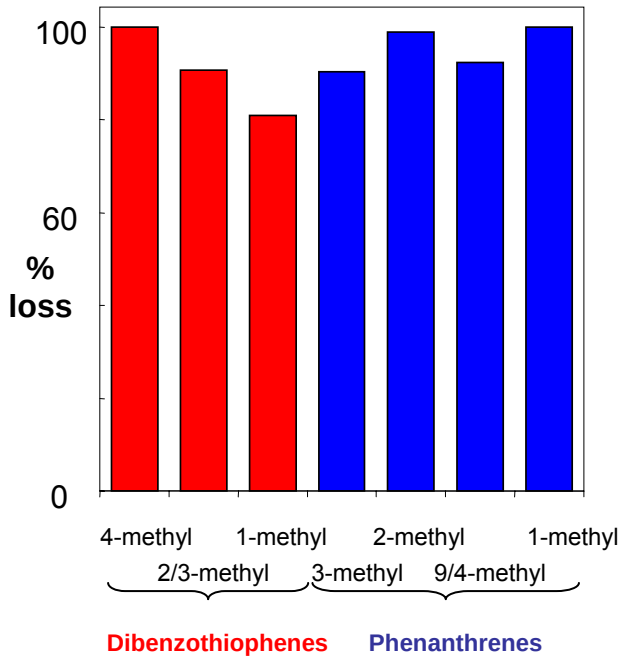
Internal standards allow understanding

- Samples from the *Arrow* spill (1970) collected in 2000 appeared to show substantial isomer specificity of biodegradation of the alkylaromatics.
- In fact, differential biodegradation is minimal.





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Experimental Protocols – nutrients

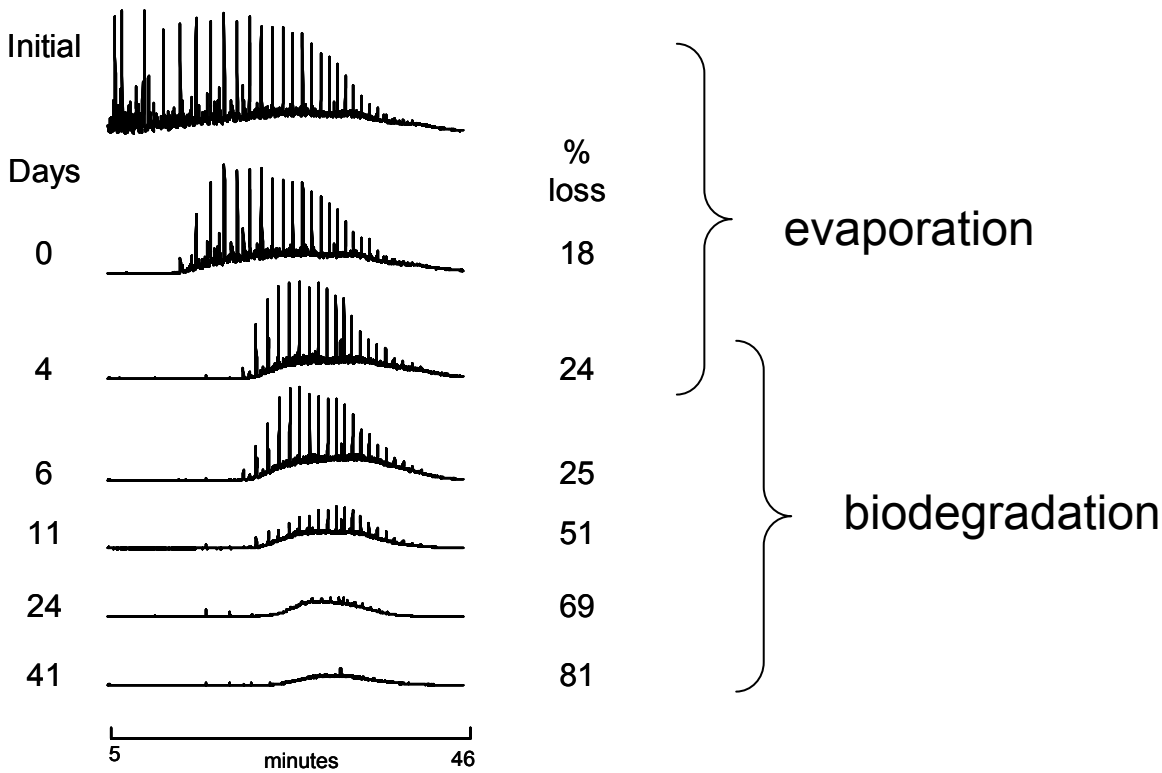
- Microbial Growth
  - Oil is an unusual substrate in providing carbon and energy but no biologically available nitrogen, phosphorus, iron, etc.
  - Many people have used the famous Bushnell-Haas medium (1941) – designed for understanding fuel degradation in World War II.
    + But it contains 25mM N, 15mM P. This is much higher than anything that can ever exist in nature – it would certainly be toxic to fish.
    + We now use 0.5% or 1% of this in some of our experiments – higher than seawater but within range. [Typical seawaters are ~10μM NO_3^-]
  - If we accept the typical Redfield ratio for planktonic algae as a reasonable proxy for hydrocarbon-degrading microbes.

$$C_{106}N_{16}P$$

  - 10μM NO_3^- is enough nitrogen for the complete incorporation of ~1ppm oil into biomass. Of course a lot (50%?) goes to CO_2 .
  - Our experience is that 2.5 ppm oil is essentially completely degraded in natural seawater.

Biodegradation

- The aerobic biodegradation of 2.5ppm Alaska North Slope crude oil at 8C, no added nutrients.



GC/MS total ion

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Experimental Protocols - oxygen

- Oxygen
  - Biodegradation seems fastest under aerobic conditions
  - Complete aerobic biodegradation requires 1.5 O_2 molecules per -CH_2- moiety for complete mineralization of the hydrocarbon to CO_2 and H_2O.
  - Taking account of molecular weights, each ppm of hydrocarbon requires 3.4 ppm of oxygen for complete aerobic biodegradation.
  - Typical surface seawater [O_2] is >6.4ppm – enough for 1.9 ppm hydrocarbon even without mixing.
  - For sealed experiments it is important to consider the hydrocarbon : O_2 ratio.

Experimental Protocols - oil

- Oil concentration
  - We started with aqueous experiments with 1% oil, but this is very high compared to anything likely to be found in nature…We now aim for environmentally relevant concentrations.
    + This would be a few ppm or lower, but we are constrained by analytical sensitivities.
      - For GC/MS we inject 1μl of 10μl/ml oil in methylene chloride – so we need of the order of 10μl of oil in each experiment to allow us to retrieve it. Currently we run 4 liter experiments with 10 μl oil – i.e. 2.5 ppm.
      - The sensitivity of our respirometer is such that we need 50 ppm oil. Currently we add 0.5% Bushnell Haas to natural seawater to provide nutrients.
  - Standard repetitive pipettes with disposable tips are designed for water. They are not calibrated for oil.
  - It is essential to use positive displacement pipettes for small volumes, and they need calibration by weighing dispensed aliquots to ensure precision.

Experimental Protocols – oil extraction

- Oil extraction
  - It is very challenging to take a representative subsample from an experimental flask
    + But analysis using a conserved marker can compensate if necessary.
  - We routinely sacrifice the whole experimental sample, and extract with methylene chloride.
    + Three extractions of aqueous samples work well for us – we do it directly from the flask, removing the solvent with a glass pipette.
    + Other use toluene, but this is not so powerful or complete a solvent.
    + Any solid sample, such as gravel, sand or soil, must be dried before extraction. We use anhydrous sodium sulfate (after decanting any obvious water). Dehydration takes time, and usually several volumes of powdered anhydrous Na_2SO_4. It is important to stir during drying to prevent the mass becoming a solid. Drying is complete (i.e. enough Na_2SO_4 has been added) when you have a free-flowing dry mix.
  - It is essential that no plastic come in contact with the solvents (except Teflon) – it is very easy to pick up silicones and phthalates, which will be obvious in GC traces.

Experimental Protocols - GC

- Gas chromatography
  - GC with FID is best for quantitation (USEPA 8100)
  - But GC with MS is most informative for determining biodegradation (USEPA 8270 with additional analytes).
  - Since we always use GC/MS with internal markers, we do not routinely use the deuterated standards that are in the canonical USEPA methods. We always run a known standard oil with every batch of samples.
  - There are at least three things that must be optimized for successful GC/MS analyses.
    + The MS must be tuned correctly; USEPA recommends perfluorotributylamine.
    + The column must give good resolution (e.g. baseline separation of *n*C_{18} and phytane
    + The injector must not show mass discrimination.
  - We run every sample at least twice – once in full scan mode as recommended in USEPA 5270, the other with selected ion monitoring for compounds of interest – we always include m/z = 191 for hopane, and the small alkyl congeners of the polycyclic aromatic compounds.

Summary

- The overarching goal is to try and mimic the real world
  - We routinely use natural microbial inocula, such as seawater or pondwater.
  - This is not particularly reproducible, but nor is the real world.
  - We try and use natural levels of nutrients, and the levels of oil that would occur in the real world. If we augment nutrients, we use ≤1% Bushnell-Haas.
- To mimic oiled beaches we would use much higher concentrations than for mimicking a dispersed oil slick.
- For dispersed oil we aim for a few ppm oil
  - We note that most previous work has been done at 83-4500 ppm – unrealistically high, with nutrient levels similarly boosted.
  - It is very hard to do dispersant tests on a small scale – edge effects predominate that have no equivalent in the real world.
  - Dispersed oil allowed to ‘mature’ will resurface in the lab – we believe this is an artifact due to the absence of dilution.