Biodegradation and Bioremediation

Dr. Nick Coleman

Biodegradation & Bioremediation

Learning outcomes

Students should be able to…

- **Define the following terms: biodegradation, pollutant, xenobiotic, bioremediation, natural attenuation, biostimulation, bioaugmentation, cometabolism**

- **Describe factors that influence the environmental fate of pollutants, specifically:**

  - intrinsic properties of the pollutant

  - environmental factors - abiotic + biotic

- **Explain how biodegradation potential can be predicted from the chemical structure of a pollutant**

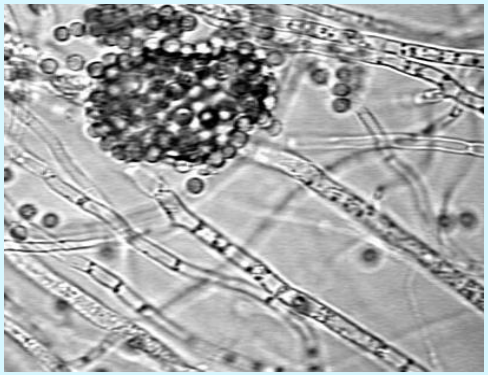
Macro-organisms

Micro-organisms

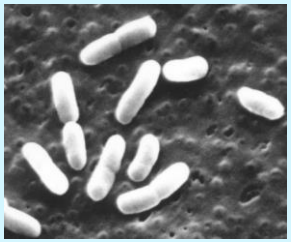
Organic compounds (biomass) $C_{10}H_{20}O_5N_2$

Carnivores

Predators



Decomposers



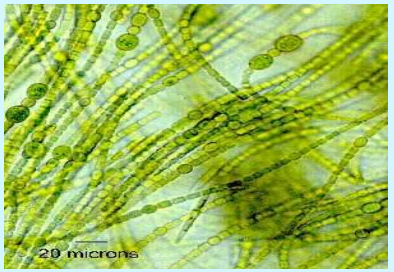
Scavengers

Herbivores

BIODEGRADATION

Plants

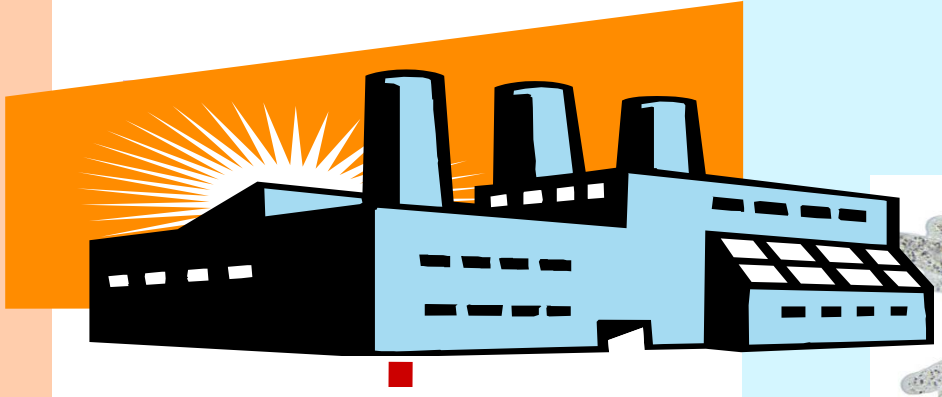
Inorganic compounds CO_2, NH_4^+, SO_4^{2-}, PO_4^{3-}



Various autotrophs

Macro-organisms

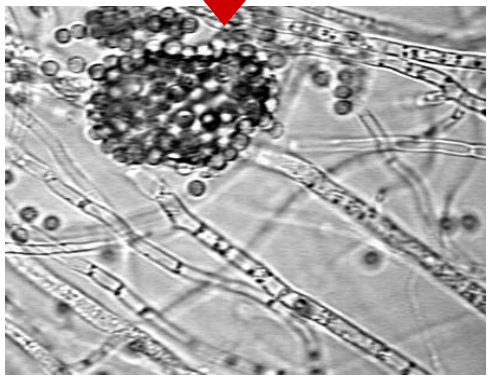
Micro-organisms

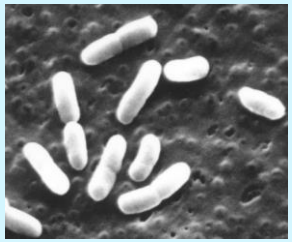


Xenobiotic compounds

Carnivores

Predators



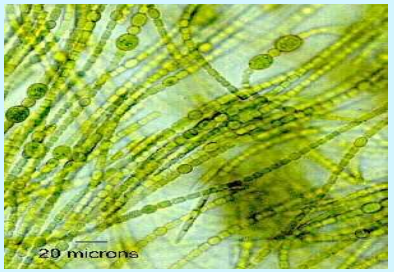


Decomposers

Scavengers

Herbivores

BIODEGRADATION?



Plants

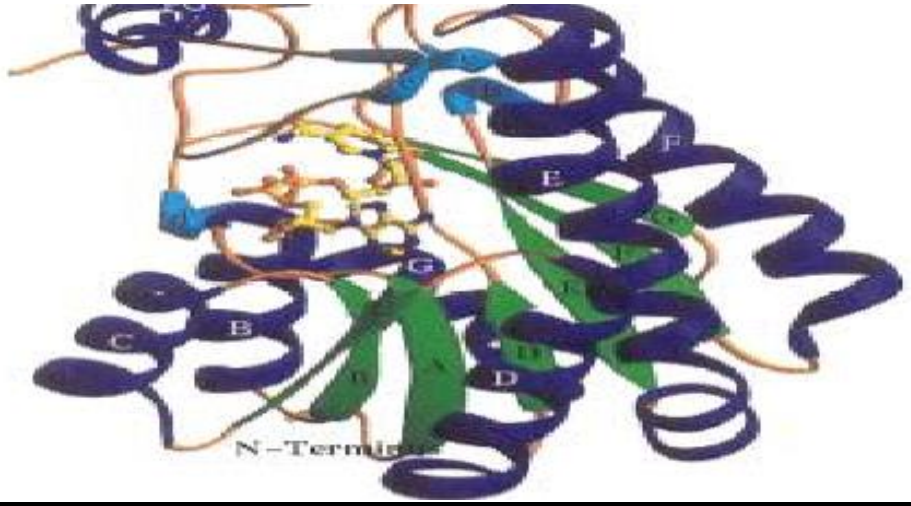
Inorganic compounds
CO_2, NH_4^+, SO_4^{2-}, PO_4^{3-}

Various autotrophs

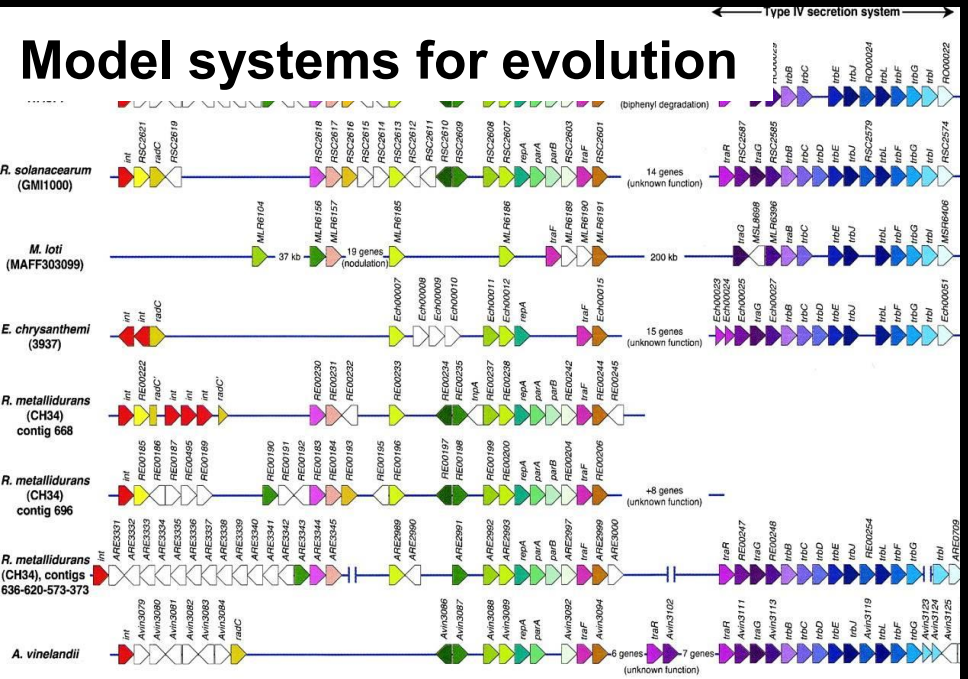
Why is biodegradation research important?

Pollution bioremediation

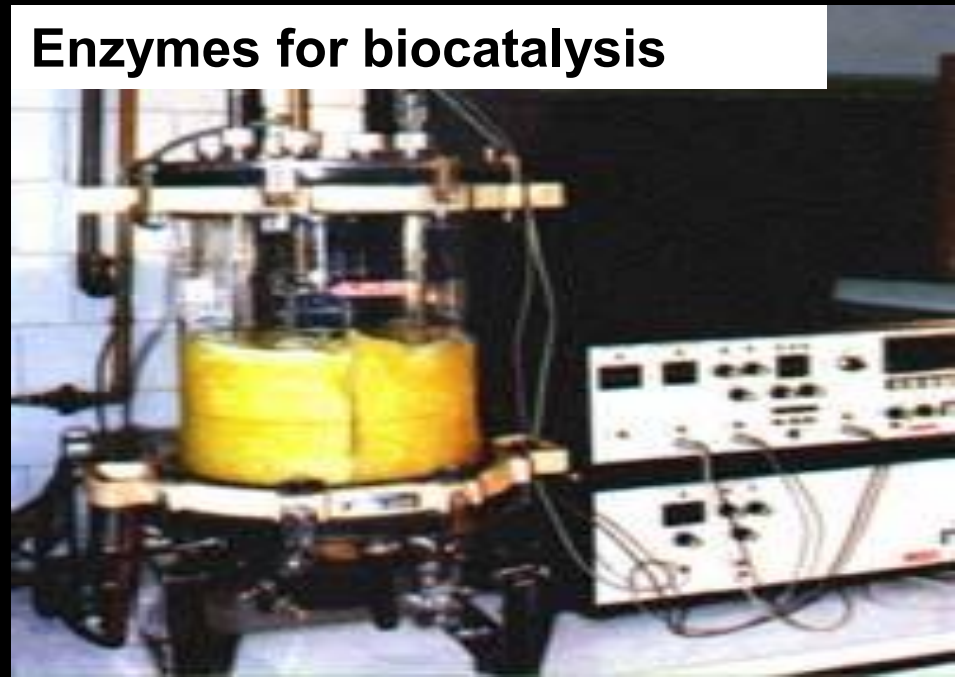
New types of biochemistry



Model systems for evolution



Enzymes for biocatalysis



Local significance of biodegradation research

Botany industrial site

Hexachlorobenzene waste stockpile
Chlorinated alkanes and alkenes in groundwater

Local significance of biodegradation research

Sydney Olympics site (Homebush Bay)

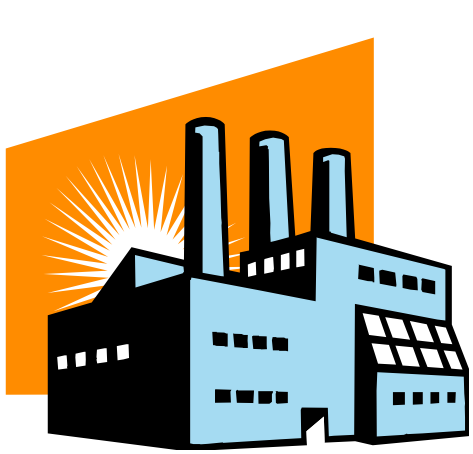
Previously the most contaminated site in the Southern hemisphere
Dioxins, pesticides, explosives, petroleum, heavy metals

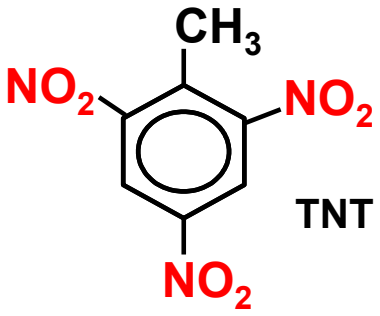
Biodegradation: decomposition of organic compounds by microbes

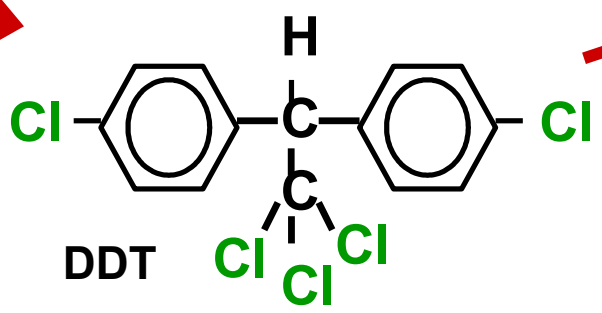
Biodegradation of natural organic compounds (biomass) has been occurring for billions of years –for as long as life has existed.

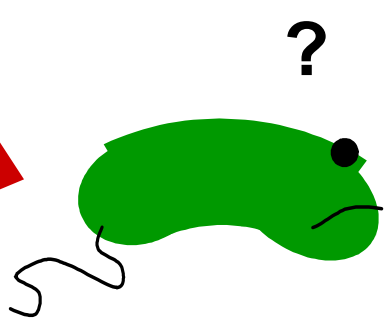
Recycling of dead biomass via the action of microbial decomposers (degraders) is an essential step in maintaining functional ecosystems.

Pollutant chemicals eg. pesticides have only been in the biosphere a short time → present a challenge to microbial degraders.









Pollutant: chemical that causes damage to environment or health

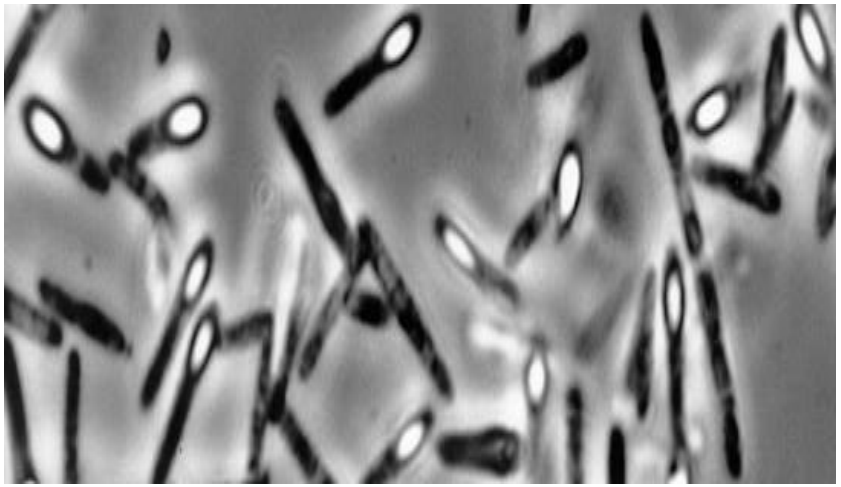
Pollutants may be naturally-occuring or xenobiotic chemicals, which are toxic to living organisms, or damaging to the biosphere.

Toxic effects may be acute or chronic. The level of risk associated with a pollutant depends on many factors.

Comparison of toxicity risks

water

lethal dose : ~ 20 litres



botulism

(*Clostridium botulinum* toxin)

lethal dose : ~ 1 microgram

Xenobiotic: chemical of human manufacture, not from biosynthesis

- present in the environment only ~ 100 years (as long as organic chemists)

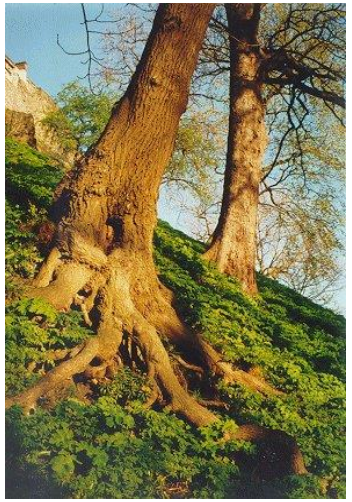
- microbes have not yet adapted to their presence.

- Xenobiotics tend to be recalcitrant to biodegradation.

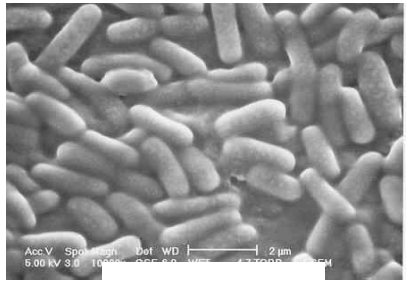
organic chemists

animals

fungi



plants



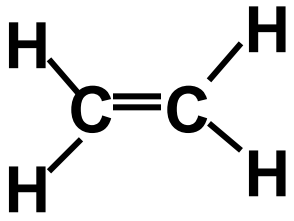
bacteria

Xenobiotic compounds

Natural compounds

The biodegradability of chemicals can sometimes be predicted based on the type and number of xenobiotic groups or structures present.

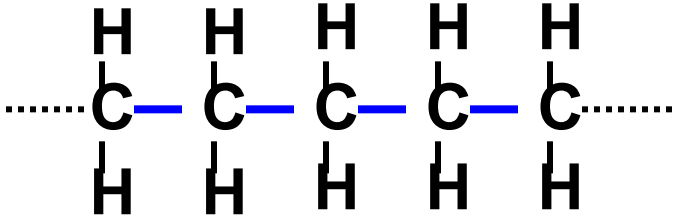
Naturally-occuring



Ethylene (ethene)

Biodegradation: rapid, complete

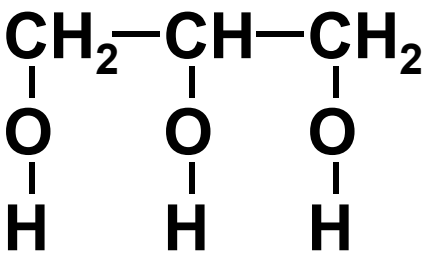
Xenobiotic



Polyethylene (plastic)

Biodegradation: very slow, incomplete

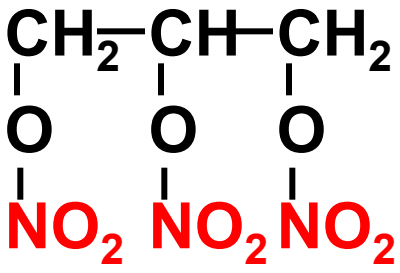
Naturally-occuring



Glycerol

Biodegradation:
very rapid, complete

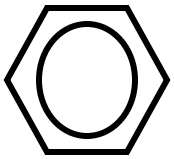
Xenobiotic



Nitroglycerine (explosive)

Biodegradation:
slow, often incomplete

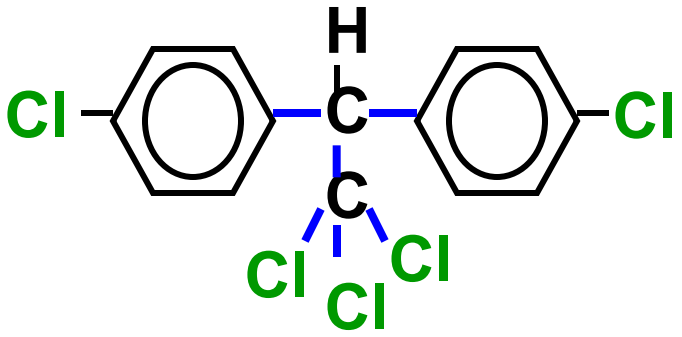
Naturally-occuring



Benzene

Biodegradation:
rapid, complete

Xenobiotic



DDT (insecticide)

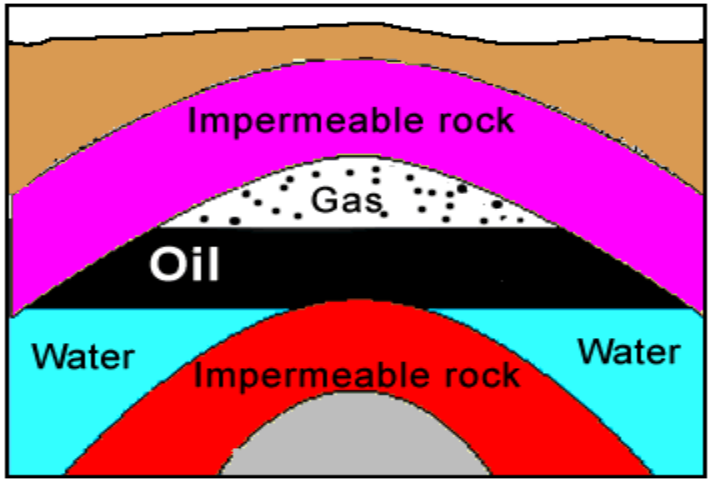
Biodegradation:
very slow, incomplete

Natural compounds can be pollutants when abnormally high local concentrations occur. This is highly context-specific, and could be due to human activity or natural causes.

essential

Salt
NaCl

toxic



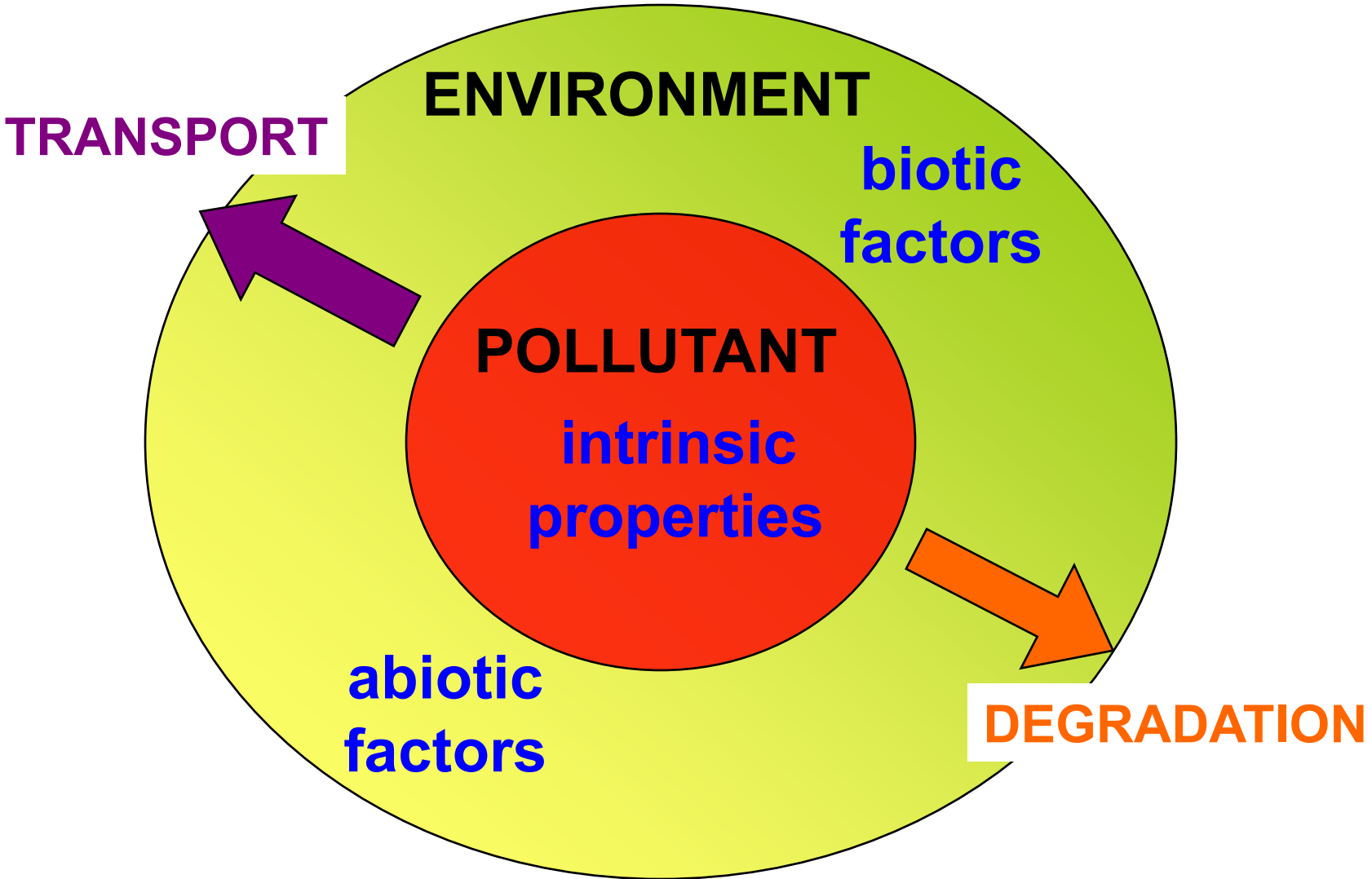
harmless

Crude oil

toxic

Environmental fate of pollutants

The environmental fate of a chemical is determined by transport and degradation processes, which in turn depend on the intrinsic properties of the chemical, & environmental conditions



Intrinsic pollutant properties

- eg. size, molecular weight, charge, solubility, toxicity

  - Large and water-insoluble molecules are not bioavailable, and are slow to biodegrade, also less mobile in groundwater etc.

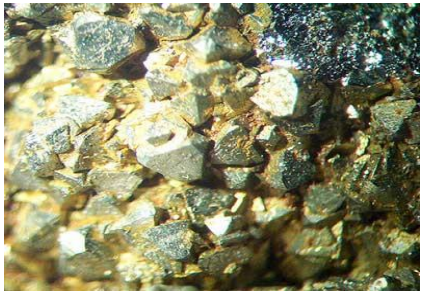
  - Toxic compounds biodegrade slowly due to inhibition of microbes

eg. Cyanide is a small, water soluble molecule that is highly toxic.
- can cause fish kills due to use in mining operations

Abiotic factors – temperature, pH, redox potential (aerobic/anaerobic) water availability, organic matter, inorganic compounds, sunlight.

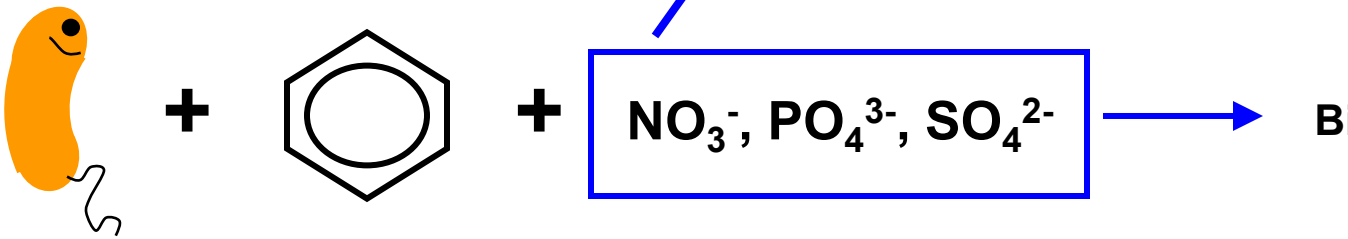
Inorganic compounds have a large influence on biodegradation

eg. breakdown of benzene is accelerated by: O_2 NO_3^- PO_4^{3-} SO_4^{2-}

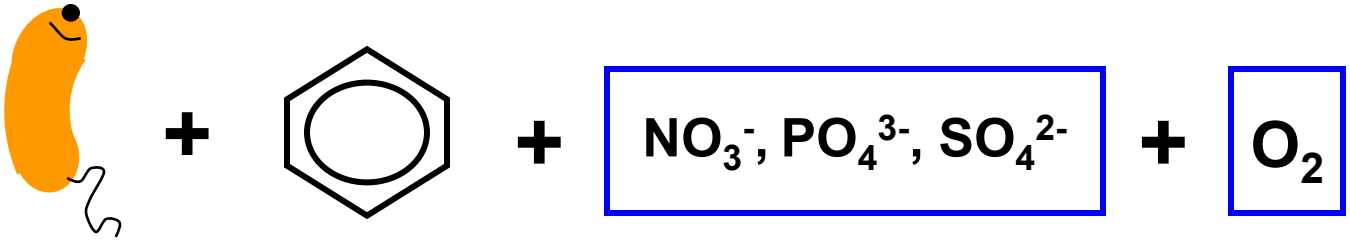


benzene

Note! Inorganics can act as nutrients, electron donors or electron acceptors



NO_3^-, PO_4^{3-}, SO_4^{2-} → Biodegradation



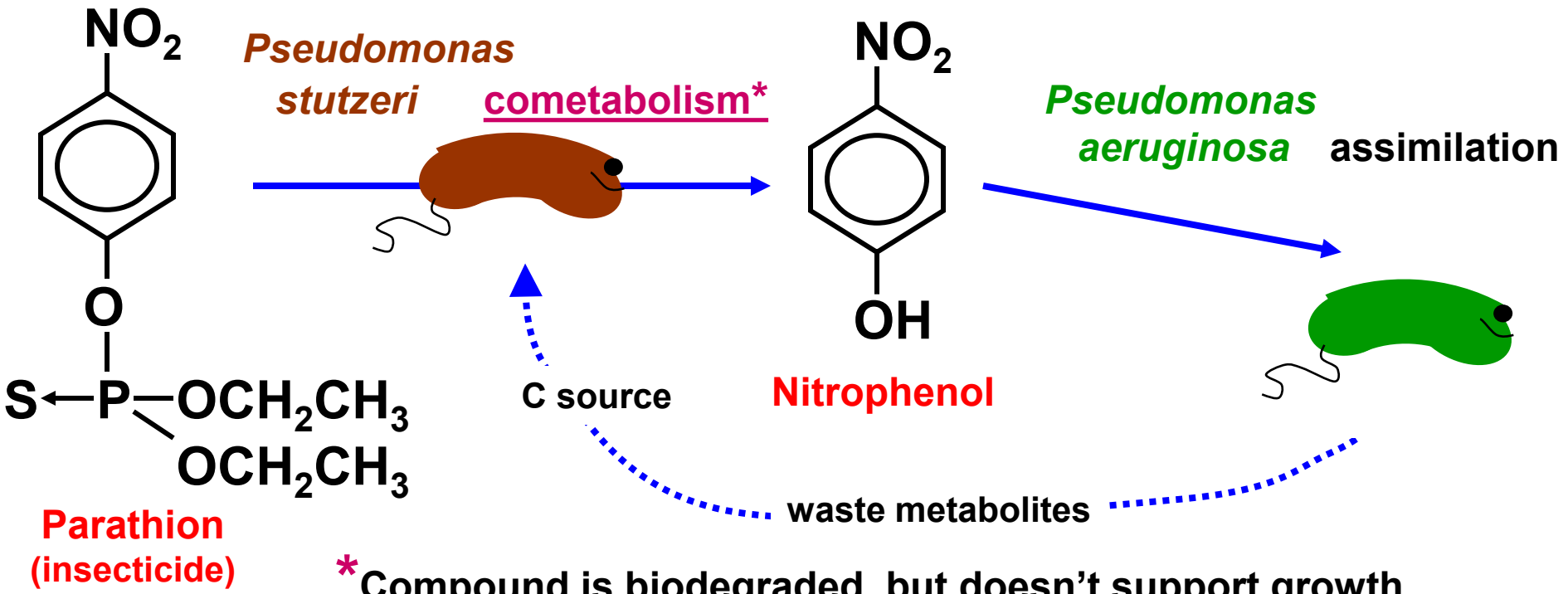
NO_3^-, PO_4^{3-}, SO_4^{2-} + O_2 → Rapid biodegradation

Biotic factors - types, numbers, activities, interactions of microbes

Microbes can mobilize, immobilize, degrade or accumulate pollutants. For effective biodegradation, large numbers of active cells of the 'correct' type of degrader must be present.

Microbial Interactions

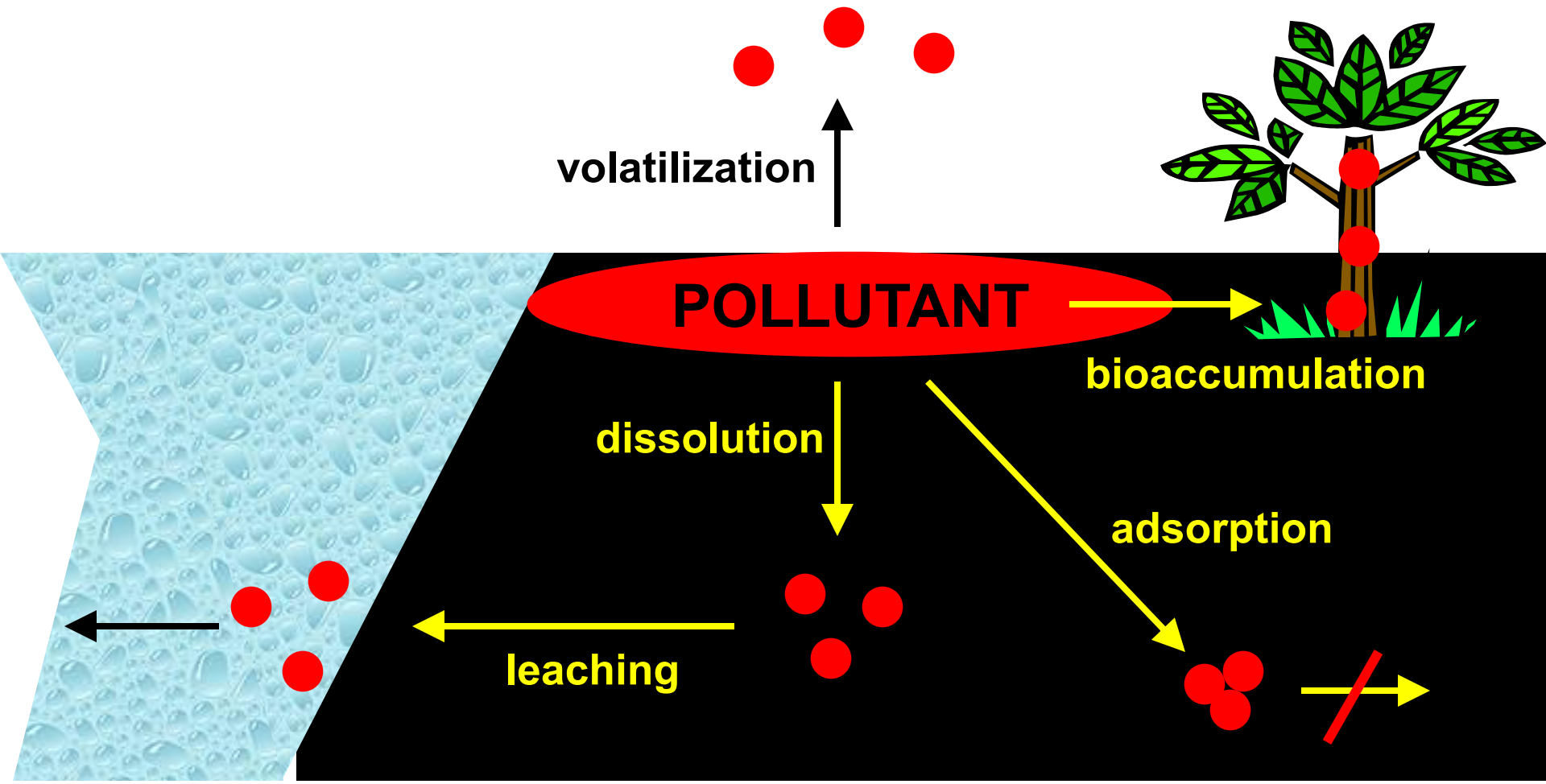
- **Symbiosis** (mutualism): both microbes benefit
- **Competition:** microbes compete for resources
- **Predation:** one microbe is eaten by another



*Compound is biodegraded, but doesn't support growth

Transport: movement of a pollutant within or between environments.

Transport involves a change of location (eg. leaching, adsorption, bioaccumulation), and/or a change of state (eg. dissolution, volatilization).



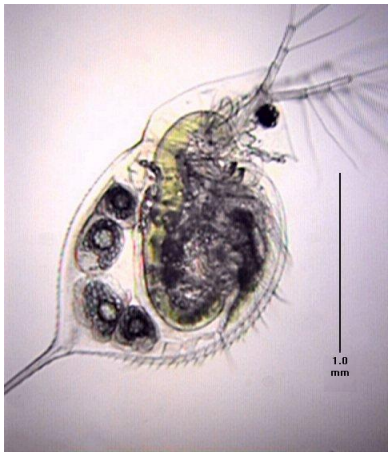
Bioaccumulation: increase in pollutant concentration from environment to organism

Biomagnification: increase in pollutant concentration between one organism and the next in a food chain.

environment (water)
DDT: 0.00005 mg/kg

800 x

Bioaccum.



zooplankton
DDT: 0.04 mg/kg

25 x

Biomag.

fish
DDT: 1 mg/kg

20 x

Biomag.

reproductive effects

predatory birds
DDT: 20 mg/kg

Bioremediation – the application of biodegradation processes to clean up pollution at a contaminated site

Various degrees of human intervention are involved in bioremediation:

Natural attenuation: monitoring only
Bioaugmentation: addition of microbes
Biostimulation: addition of nutrients

Bioremediation may be *in situ* (in the field), or *ex situ* (excavated soil).

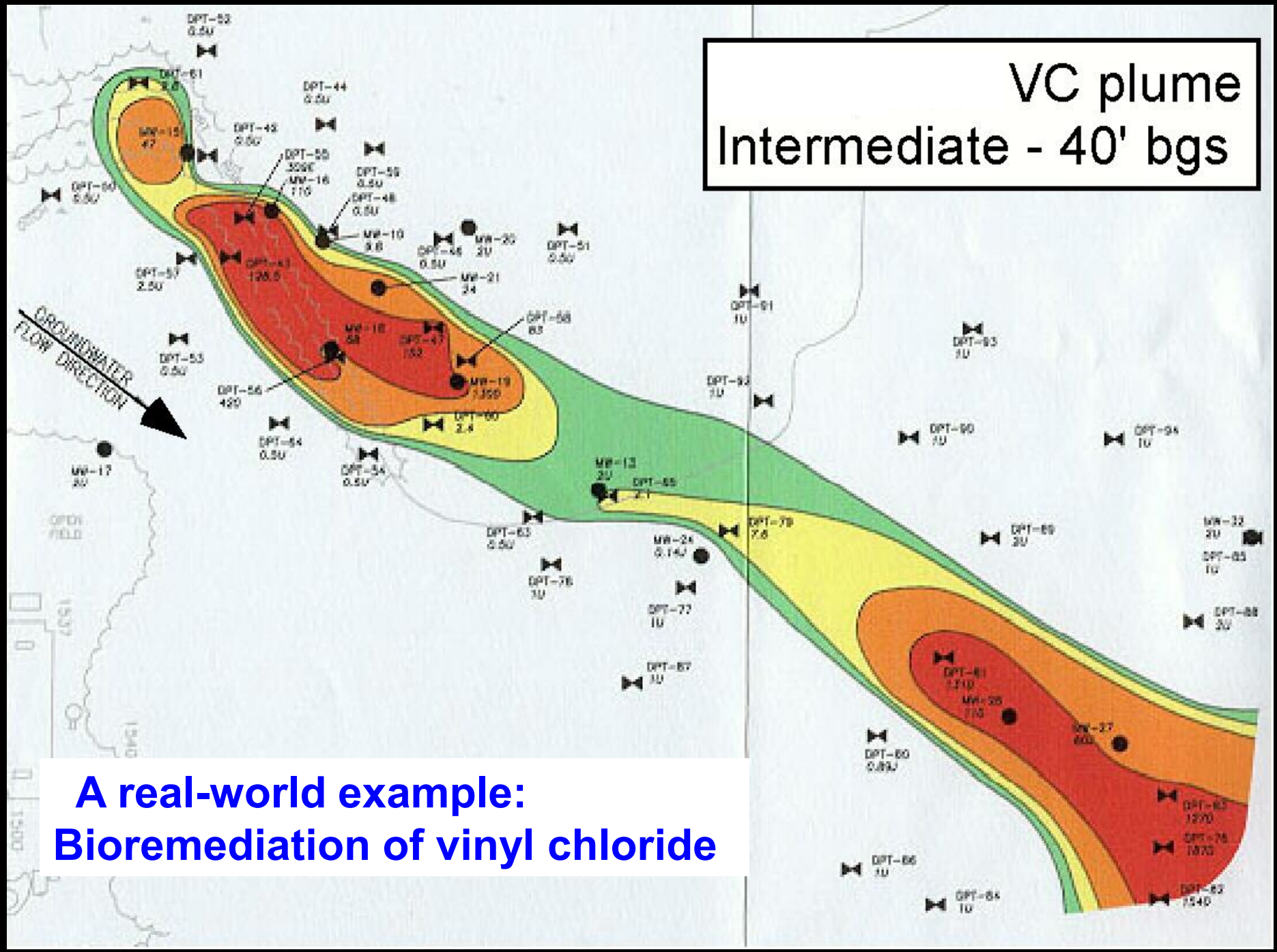
ex situ bioremediation

‘Real world’ bioremediation

Bioremediation can be effective, but its application is not always easy. Complicating factors include:

- **Toxic pollutants**
- **Inhibitory site conditions**
- **Mixtures of contaminants**
- **Low bioavailability (water-insoluble pollutants)**
- **Patchy distribution of degraders.**

***in situ* bioremediation**

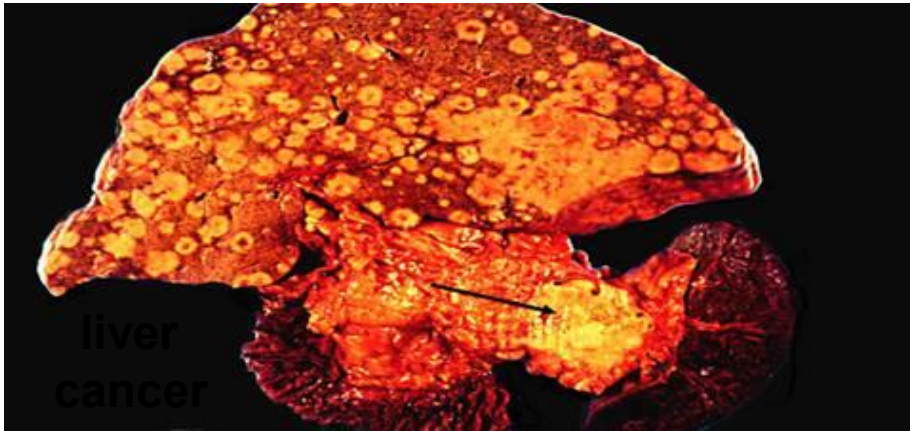

VC plume
Intermediate - 40' bgs
GROUNDWATER FLOW DIRECTION
A real-world example:
Bioremediation of vinyl chloride

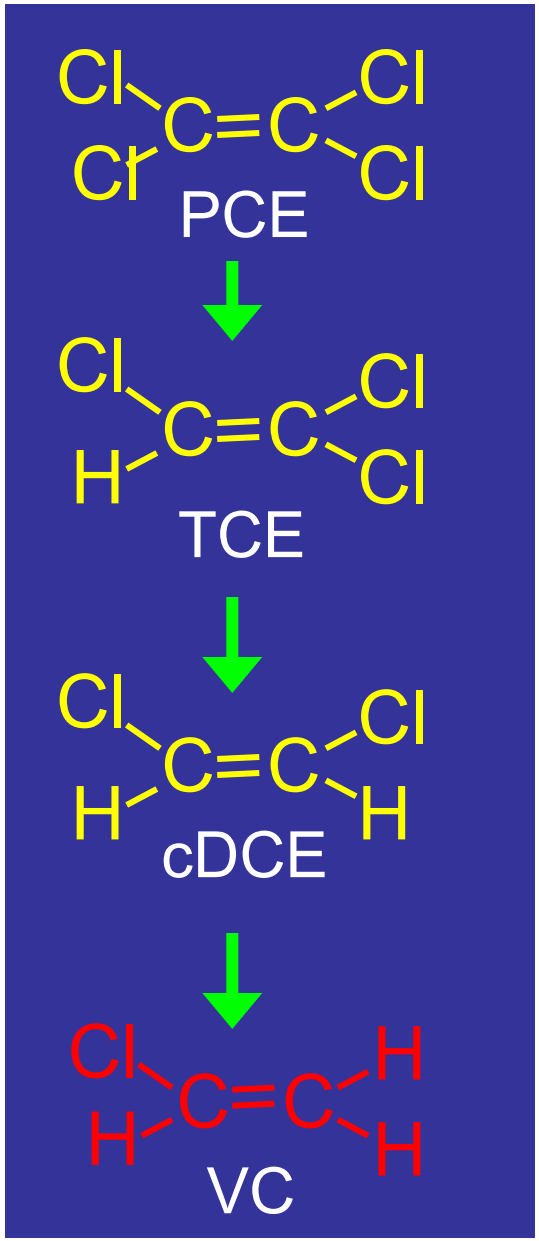
Chlorinated ethene pollutants

Perchloroethene (PCE), trichloroethene (TCE) used as solvents → groundwater contamination

Anaerobic bacteria respire with PCE and TCE, producing cis-dichloroethene (cDCE) and vinyl chloride (VC)

VC is the most dangerous chlorinated ethene; US EPA limit is 1 part per billion

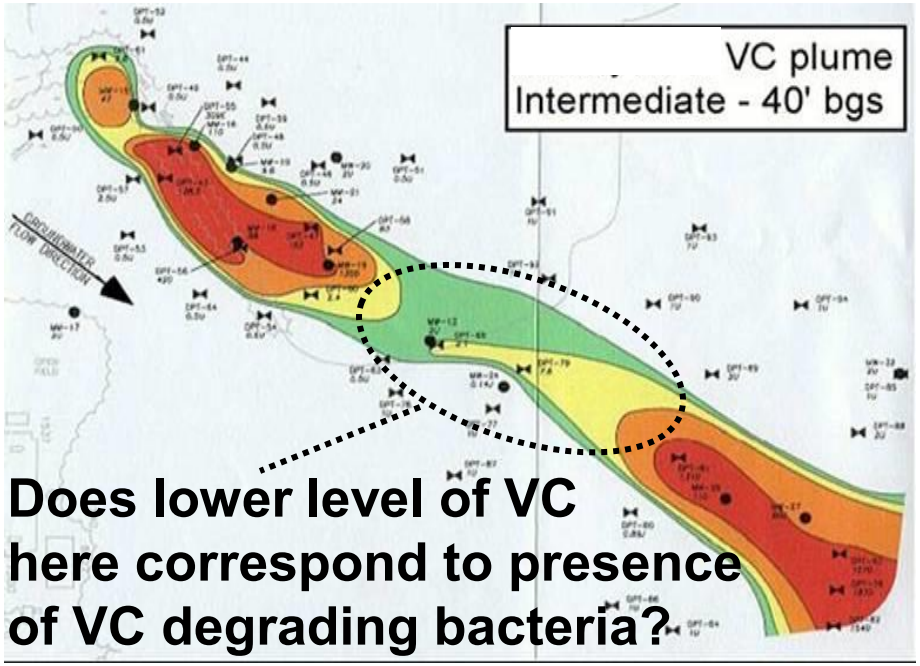




Can we find aerobic bacteria that will use VC as a carbon source?

Vinyl chloride biodegradation: scientific questions

Distribution and diversity of VC degraders ?



Does lower level of VC here correspond to presence of VC degrading bacteria?

Did VC degraders evolve from ethene degraders?

$CH_2=CH_2$

ethene: natural product

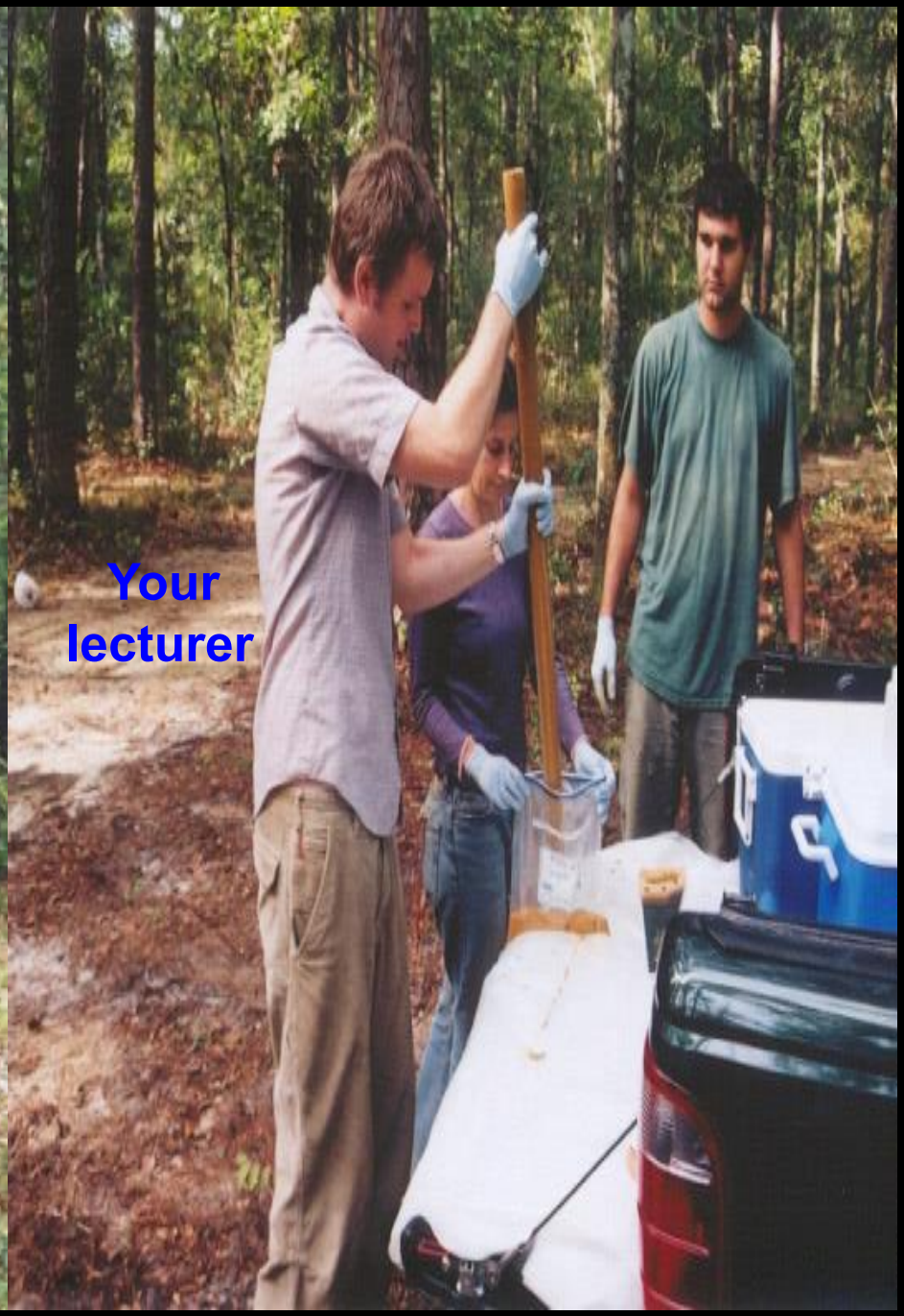
$CH_2=CH\text{-}Cl$

VC: xenobiotic

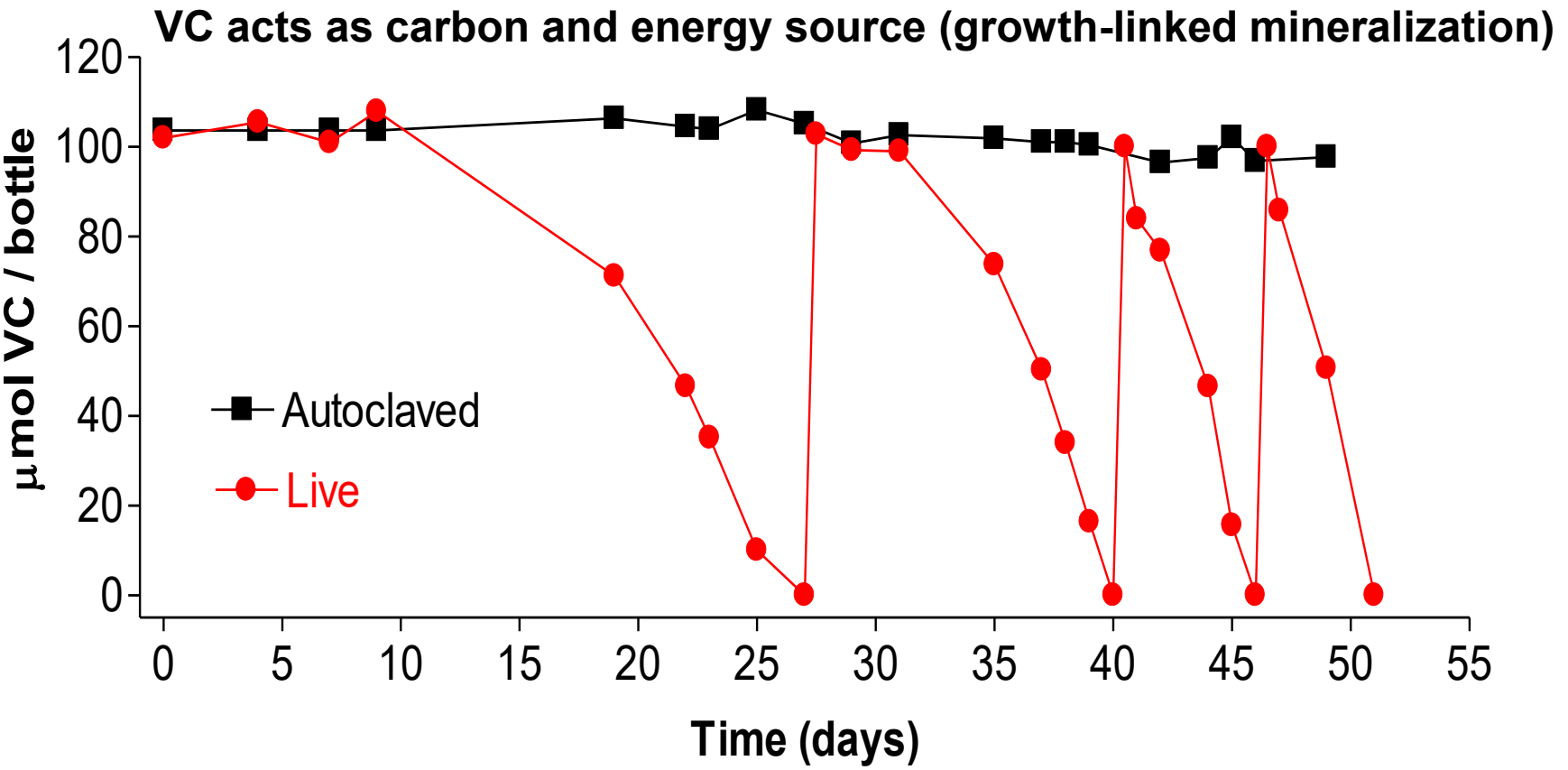
Can we use natural attenuation alone for VC cleanup or do we need to bioaugment?

Metabolic pathway, enzymes and genes involved ?

$CH_2=CH\text{-}Cl$ —(alkene monooxygenase; O_2, NADH)→ $CH_2\text{-}CH\text{-}Cl$ (O) —???→ ???


Your
lecturer

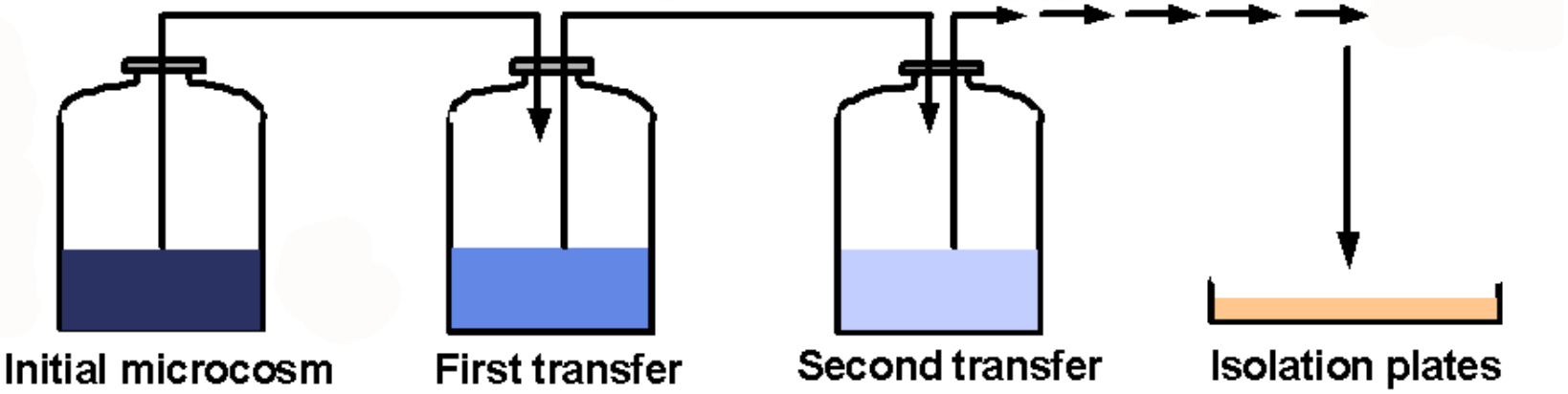
Enrichment of bacteria that grow on VC



Note that rate of degradation increases with each addition of VC to the same bottle – this indicates that the VC-degrading bacteria are increasing in numbers (biodegradation is growth-linked)

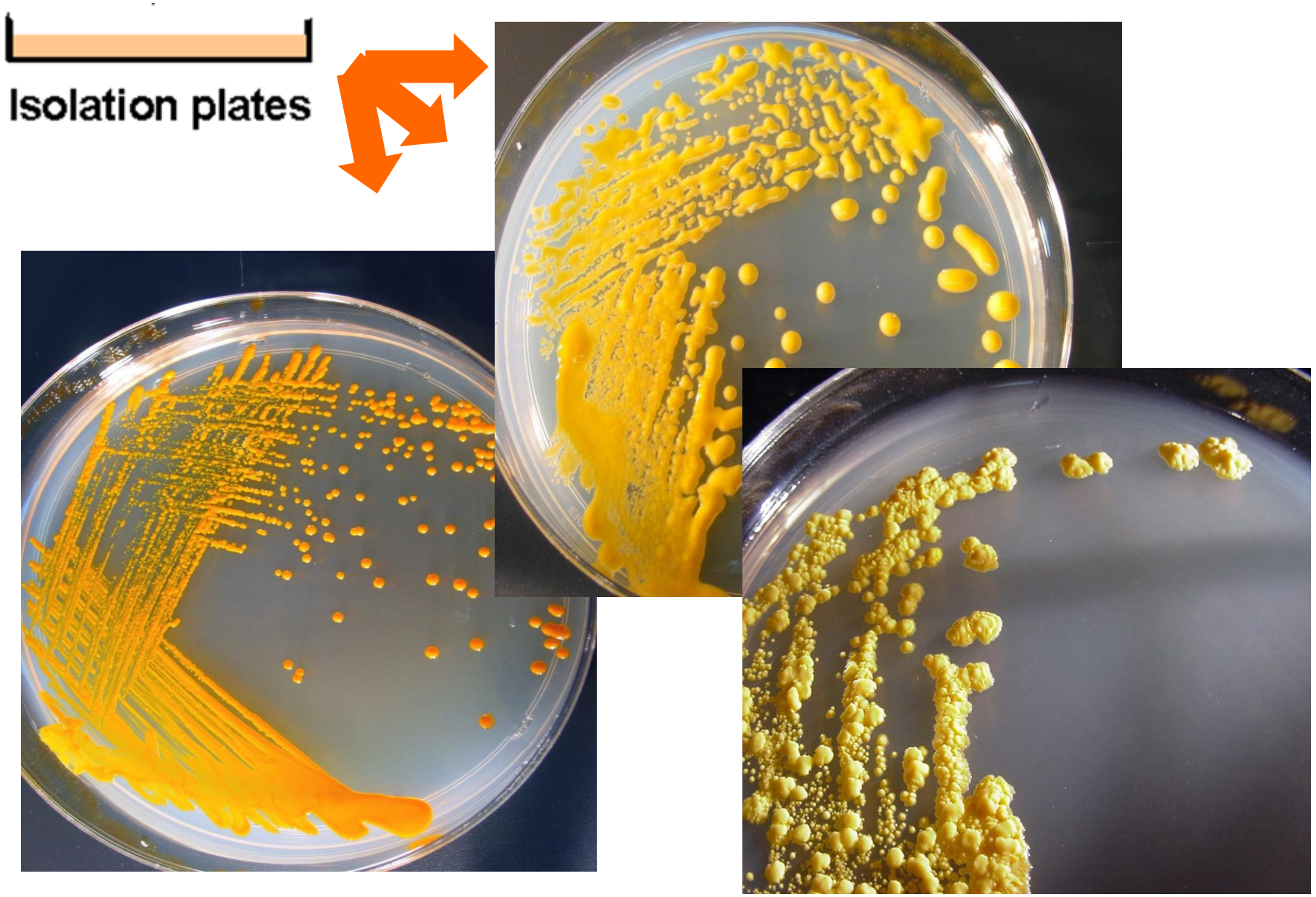
Enrichment and isolation of VC degrading bacteria

Microcosms: mineral salts medium + VC as carbon source
Samples: soil / groundwater from 37 different sites



- All isolates grew on both ethene and VC
- Strains identified by 16S rRNA sequencing
- Physiological tests: growth rate, growth yield, enzyme activity

Enrichment and isolation of VC degrading bacteria

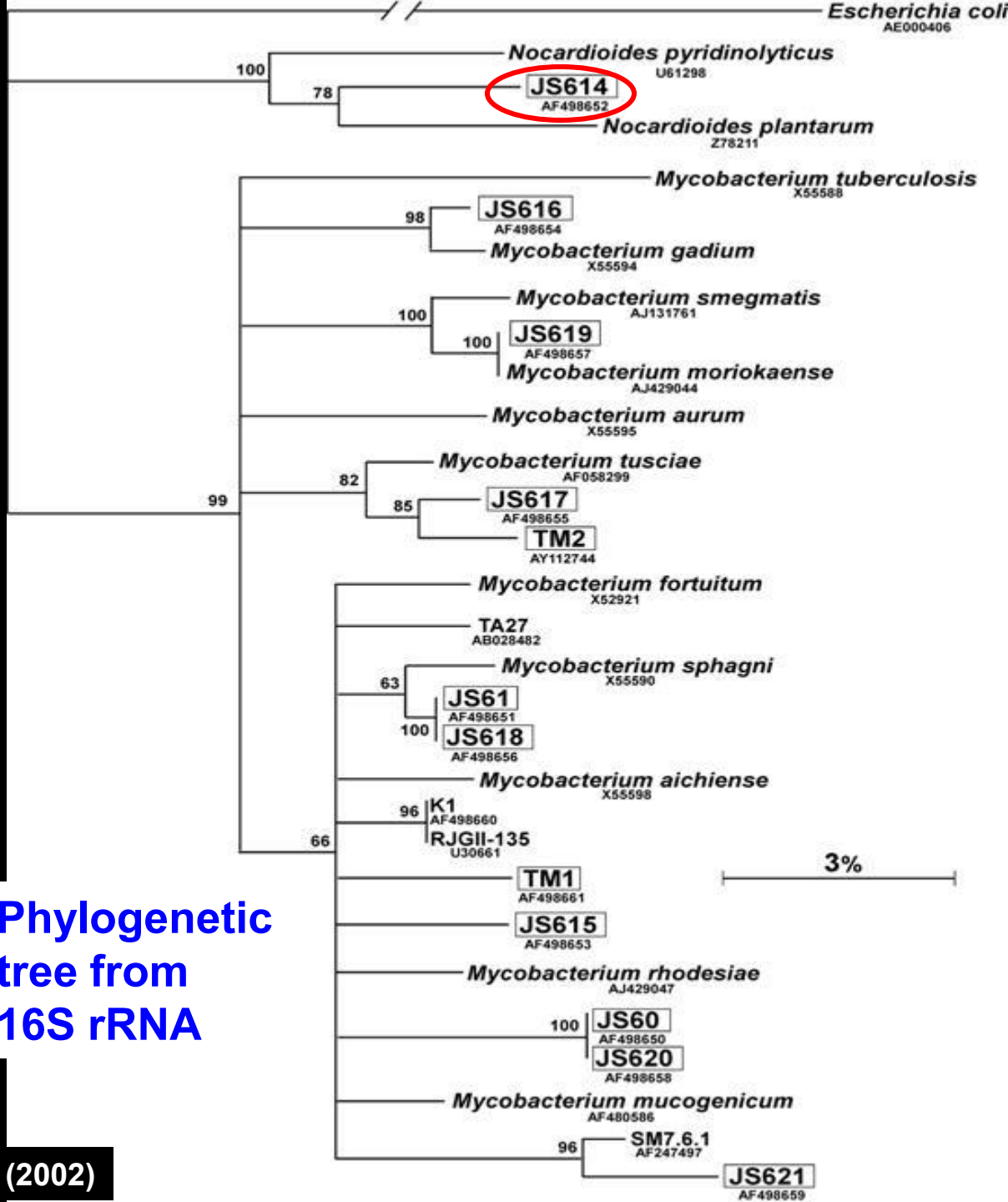


Characterisation of VC degraders

Mycobacterium (11) and *Nocardioides* (1) strains

Actinomycetes

Mycobacterium strains now being genome-sequenced



Phylogenetic tree from 16S rRNA

Coleman et al. Appl. Environ. Microbiol (2002)