IPA 6° gruppo di lezioni

Bioremediation

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BIOTECNOLOGIE AMBIENTALI

Possono essere definite come:

> "l'applicazione delle biotecnologie alla soluzione dei problemi ambientali"

o come:

> "l'uso integrato di scienze naturali e scienze ingegneristiche allo scopo di realizzare l'applicazione di organismi, cellule, parti di essi e analoghi molecolari per uno sviluppo sostenibile" (European Federation of Biotechnology)

OBIETTIVI

- Proteggere le risorse naturali
- Prevenire i danni ambientali
- Trattare rifiuti solidi e liquidi
- Trattare le emissioni gassose
- Biorisanare le aree contaminate
- Sviluppare prodotti e processi che generano meno rifiuti e riducono il consumo energetico

Settori principali delle biotecnologie ambientali

PROBLEMA	PROCESSO BIOTECNOLOGICO
•Rifiuti solidi •Effluenti industriali e acque di scarico •Emissioni gassose	**Processi di biodegradazione, detossificazione,biofiltrazione**
•Suoli contaminati	**Processi di biorisanamento in situ e ex situ**
•Acqua per uso alimentare	**Trattamenti biologici (denitrificazione)**
•Rilevamento e monitoraggio di contaminanti	**Biosensori**
•Monitoraggio di microrganismi per il biorisanamento	**Sonde molecolari**
•Industria chimica	**Processi di chimica pulita**
•Contaminanti di origine industriale e scarti dell'agricoltura	**Processi di bioconversione in prodotti ad alto valore aggiunto**
•Protezione dell'agricoltura	**Bioinsetticidi e Biofertilizzanti**

Types of contamination

- Point source contamination
  - contaminant emanating from a defined source
    - discharge pipe from an industrial operation
- Non-point-source
  - source of contaminant emanating from a large area
    - fertilizers or pesticides applied to agricultural land

Conventional methods of remediation

Dig up and remove it to a landfill

- Risk of excavation, handling and transport of hazardous material
- Very expensive to find another land to finally dispose these materials

Cap and contain

Maintain it in the same land but isolate it

- Only an interim solution
- Requires monitoring and maintenance of isolation barriers for a long time

Products are not converted into harmless products. Stay as a threat!

Better approaches

Destroy them completely, if possible

Transform them in to harmless substances

Methods already in use

- High temperature incineration
- Chemical decomposition like dechlorination, UV oxidation

But, are they effective?

Yes

But only to some extent

Drawbacks

- ➢ Technological complexity
- ➢ The cost for small scale application – expensive
- ➢ Lack of public acceptance – especially in incineration
  - Incineration generates more toxic compounds
  - Materials released from imperfect incineration – cause undesirable imbalance in the atmosphere. Ex. Ozone depletion
  - Fall back on earth and pollute some other environment
  - Dioxin production due to burning of plastics – leads to cancer
- ➢ May increase the exposure to contaminants, for both workers and nearby residents

Bioremediation makes effective better approach possible.

Either by destroying or render them harmless using natural biological activity.

- Relatively low cost
- Low technology techniques
- Generally has general public acceptance
- Can often be carried out on site – no excavation, no transport

Drawbacks

- May not be effective on all contaminants
- Time duration – relatively long
- Expertise required to design and implement – although not technically complex

What is Bioremediation?

Bioremediation

- A technology that encourages growth and reproduction of indigenous microorganisms (bacteria and fungi) to enhance biodegradation of organic constituents in the saturated zone
- Can effectively degrade organic constituents dissolved in groundwater and adsorbed onto the aquifer matrix
- Generally requires a mechanism for stimulating and maintaining the activity of the microorganisms, e.g., addition of an electron acceptor (oxygen, nitrate); nutrients (nitrogen, phosphorus); and an energy source (carbon)

Bioremediation

- is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities

BIOREMEDIATION (biorisanamento):
tecnologia che utilizza il potenziale metabolico dei microrganismi per risanare gli ambienti contaminati.

Viene svolta in ambienti naturali, non sterili che contengono grandi quantità di microrganismi, alcuni dei quali sono in grado di degradare i contaminanti.

Può essere un processo aerobico o anaerobico a seconda del tipo di organismo coinvolto;

Questo concetto comprende la:

BIODEGRADAZIONE
MINERALIZZAZIONE
COMETABOLISMO

Biodegradation

- is a general term referring to the microbially mediated decomposition of paper, paint, textiles, concrete, hydrocarbons
- Superior technique over using chemicals – why?

1. Microorganisms – easy to handle
2. Easy to clear – using antibiotics

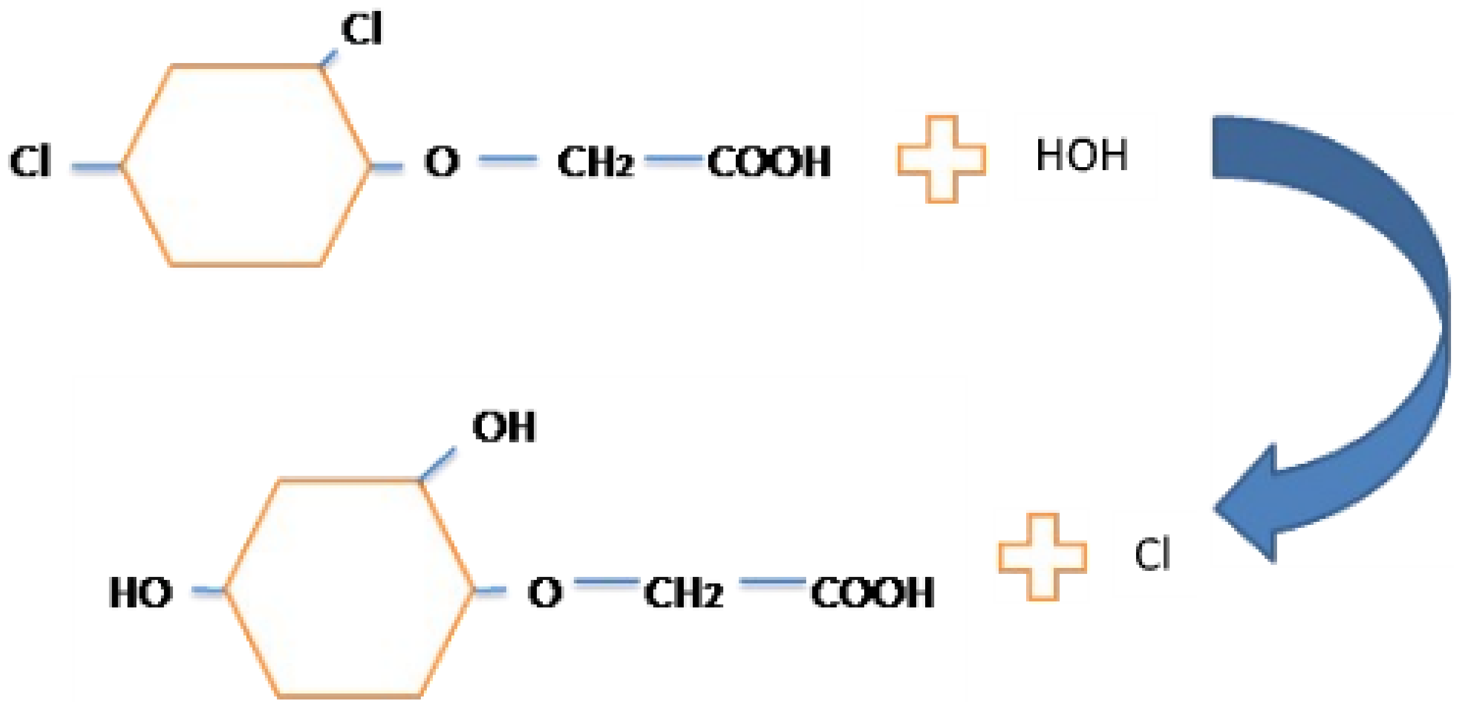
Types of Biodegradation

1. Minor change in a molecule
2. Fragmentation
3. Complete mineralization

Biodegradation

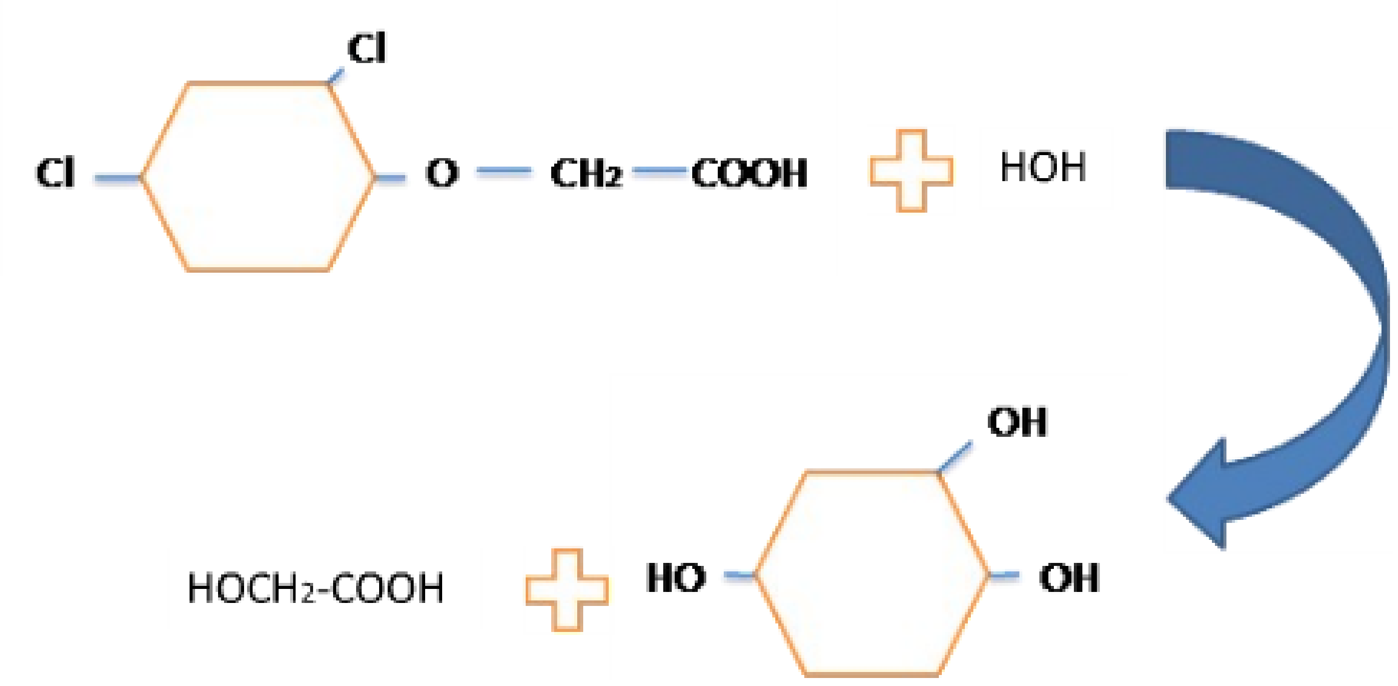
- Biodegradation – microbially catalyzed reduction in complexity of chemicals
- Mineralization - conversion of an organic substrate to inorganic end products
- Growth-linked metabolism - biodegradation provides carbon and energy to support growth
- Maintenance metabolism - biodegradation not linked to multiplication, but to obtaining carbon for respiration to maintain cell viability; take place only when organic carbon concentrations very low

Minor change in a molecule (dehalogenation)



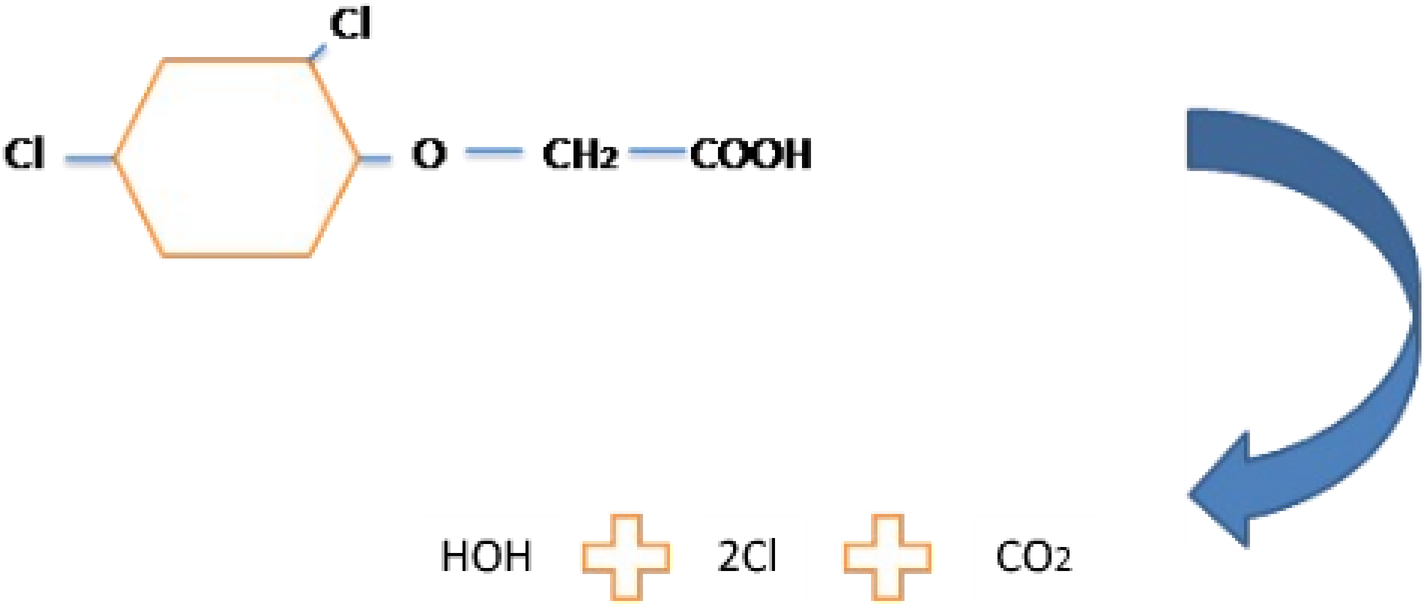
Cl is replaced with OH

Fragmentation



Original structure can still be recognized in these two types. But…

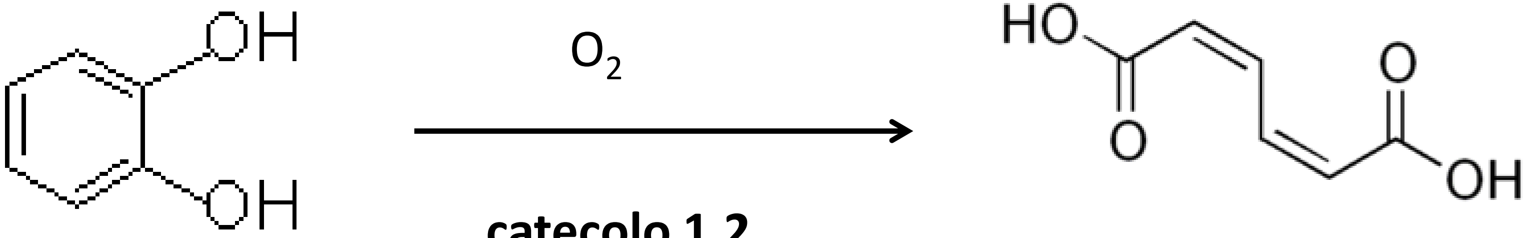
Mineralization



HOH + 2Cl + CO_2

Completely converted into inorganic forms

DEGRADAZIONE DEGLI IPA



catecolo

catecolo 1,2 diossigenasi

acido muconico

intermedi ciclo di Krebs ← **Idrolisi** — **acido dicarbossilico**

IF ANY OF THESE PROCESSES IS TRIGERED /
STIMULATED TO GET A DESIRABLE PRODUCT
THEN IT IS CALLED AS

BIOREMEDIATION

Types of Bioremediation

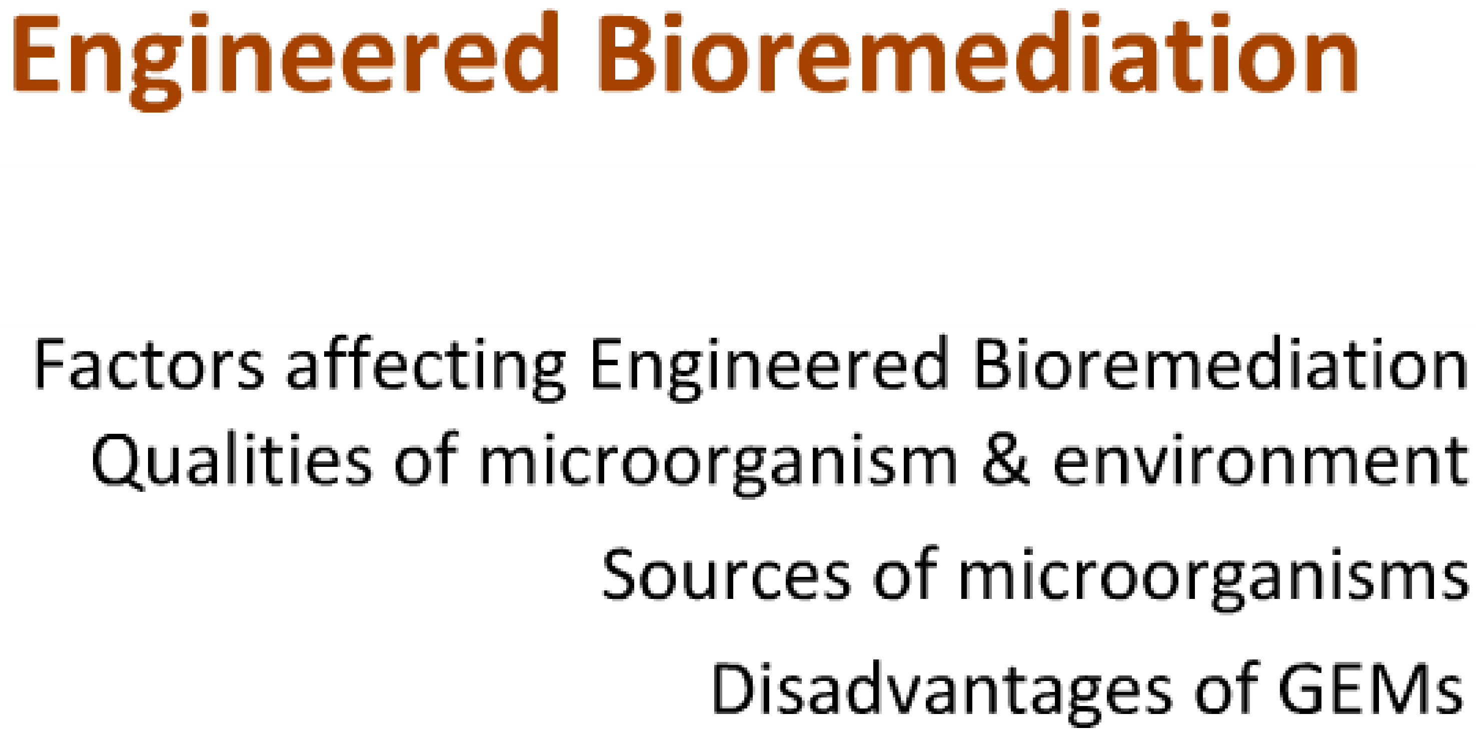
2 types

Engineered Bioremediation

- Intentional changes

Intrinsic Bioremediation

- Simply allow biodegradation to occur under natural conditions



Engineered Bioremediation

Factors affecting Engineered Bioremediation

Qualities of microorganism & environment

Sources of microorganisms

Disadvantages of GEMs

GEM = genetically engineered microorganism

Factors affecting Engineered Bioremediation

1. Contact between the microbes and the substrate
2. Proper physical environment
3. Nutrients
4. Oxygen
5. Absence of toxic compounds

Qualities of

Microorganisms

- Able to degrade hydrocarbons
- Able to fix nitrogen
- No secondary/side effects

Environment

- Presence of accessory nutrients (N P K Fe)
- Absence of heavy metals
- Adequate O_2, Temperature, pH

Sources of microorganisms

- From contaminated field sites

  (with varying environmental conditions -
  subzero temperatures or extreme heat,
  desert conditions or in water,
  with excess of oxygen or in anaerobic conditions,
  with presence of hazardous compounds or on any waste stream)
- From culture collections
- Genetically Engineered Microorganisms (GEMs)

Disadvantages of GEMs

MOSTLY GEMs DO NOT WORK THE WAY WE EXPECT !

Mainly 3 reasons…

1. Lab strains become food source for soil protozoa
2. Inability of GEMs to contact the compounds to be degraded
3. Failure of GEMs to survive/compete indigenous microorganisms. **Mostly due to lack / decreased activity of House Keeping Genes.**

Intrinsic Bioremediation

- a bioremediation under natural conditions

- is a natural attenuation process that leads to the decrease in contaminant levels in a particular environment due to unmanaged physical, chemical and biological processes.

Is it advantageous over engineered bioremed.?

May give unexpected results

Terminologia

- **Biostimolazione**.

Addizione di nutrienti (C,N,P) e accettori finali di elettroni (O_2) alla matrice da biorisanare per stimolare i microrganismi autoctoni in grado di degradare gli inquinanti

- **Bioaugmentation**

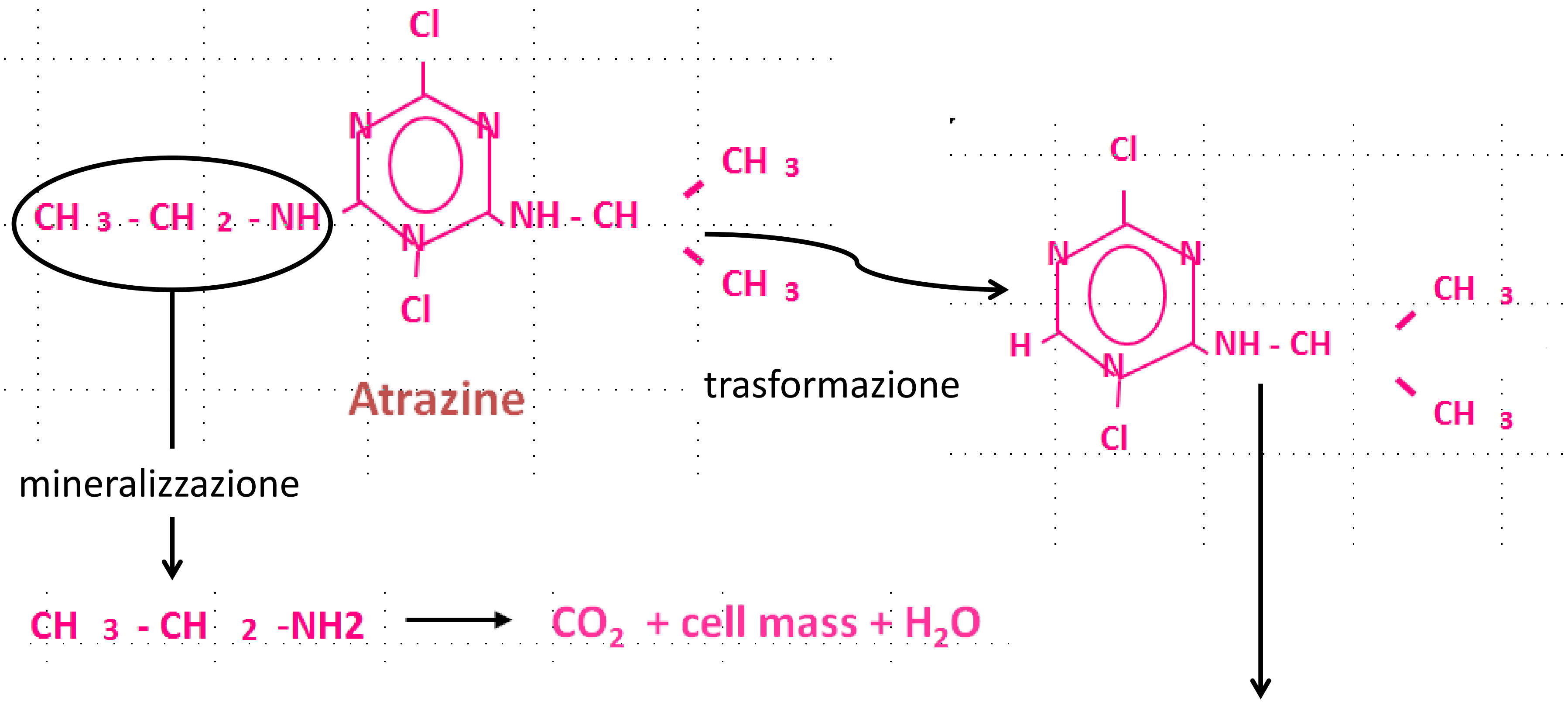
L’addizione alla matrice ambientale di microrganismi con specifiche capacità metaboliche, sotto rappresentati nelle popolazioni autoctone, per stimolare la degradazione di inquinanti

- **Trasformazione**

Qualsiasi stadio della biodegradazione di una molecola è una reazione di trasformazione . Una trasformazione può provocare disintossicazione parziale o totale di un contaminante o può creare un composto ancora più tossico del composto di origine .

- **Mineralizzazione**

Il composto è completamente mineralizzato a CO_2 ed H_2O con liberazione di energia.



Atrazine

trasformazione

mineralizzazione

$CH_3-CH_2-NH_2 \longrightarrow CO_2 + \text{cell mass} + H_2O$

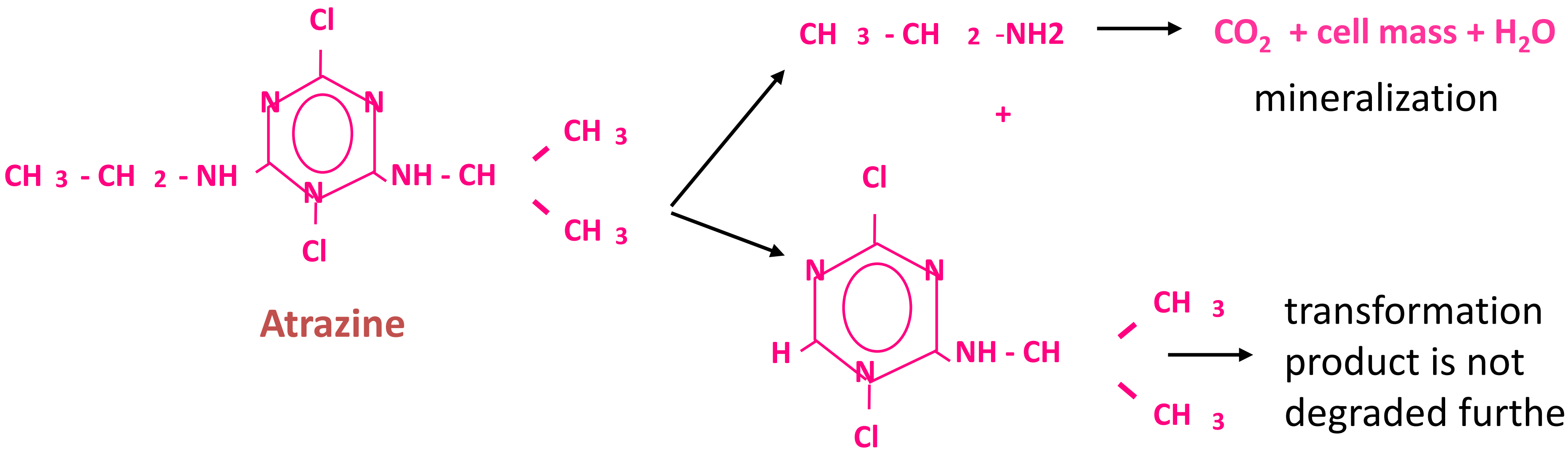
questo prodotto di trasformazione
non può essere più degradato

http://booksite.elsevier.com/9780123705198/casestudies/17~Chapter_20_organics.ppt

Biodegradation terminology

Transformation - any single biodegradation step in a pathway is a transformation reaction. A transformation can result in partial or complete detoxification of a contaminant or can create a compound even more toxic than the parent compound.

Mineralization - the parent compound is completely degraded to CO_2, new cell mass, and water. This is a highly desirable result for toxic contaminants.



Atrazine

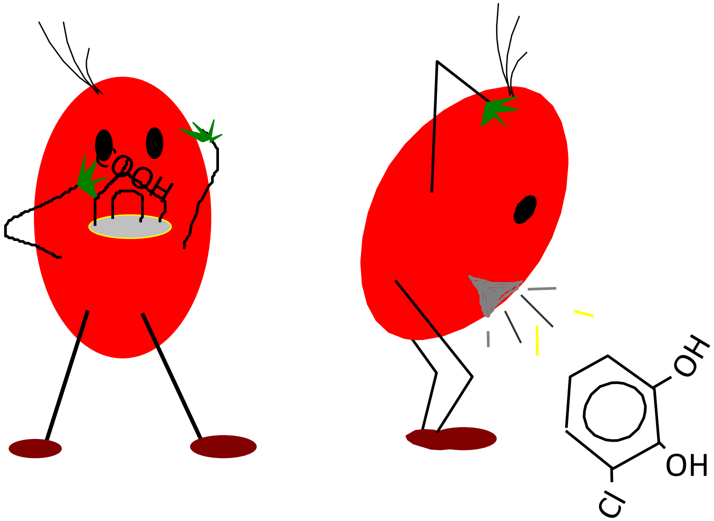
mineralization

transformation product is not degraded further

Biodegradation terminology (cont.)

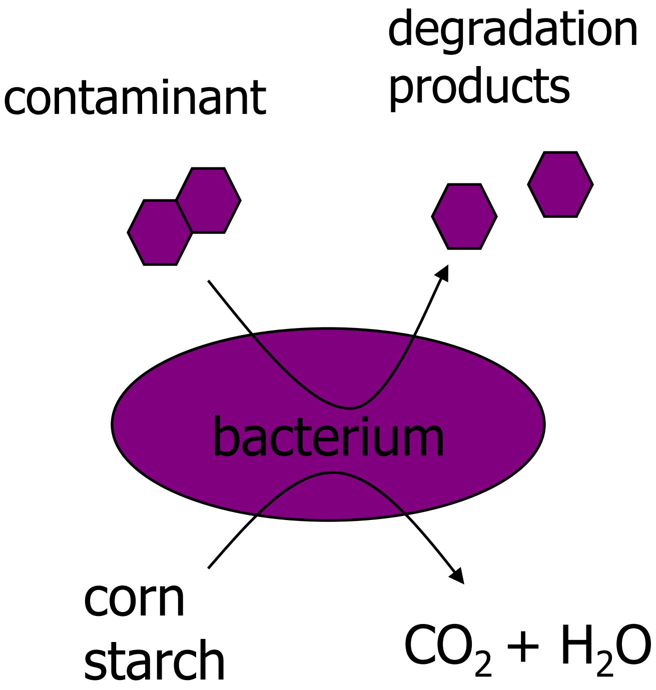
Cometabolism - Sometimes an enzyme can act nonspecifically on a substrate leading to a transformation reaction that does not provide energy to the microbe. A good example is oxidation of TCE by methane-utilizing microbes.

- lack of enzyme specificity
- detoxification



Cometabolism

- Bacterium uses some other carbon and energy source to partially degrade contaminant



contaminant

degradation products

bacterium

corn starch

$CO_2 + H_2O$

COMETABOLISM

- *Cometabolism* - Occurs when compounds are not a source of carbon or energy but are nevertheless metabolized to other compounds. Also referred to as *fortuitous metabolism*. The organism derives no benefit from the reaction.
- One reason for cometabolism may be the presence of non-specific enzymes.
- The product of a cometabolized compound might not be transformed further.
- Cometabolism may be helpful or harmful.

Type I cometabolism

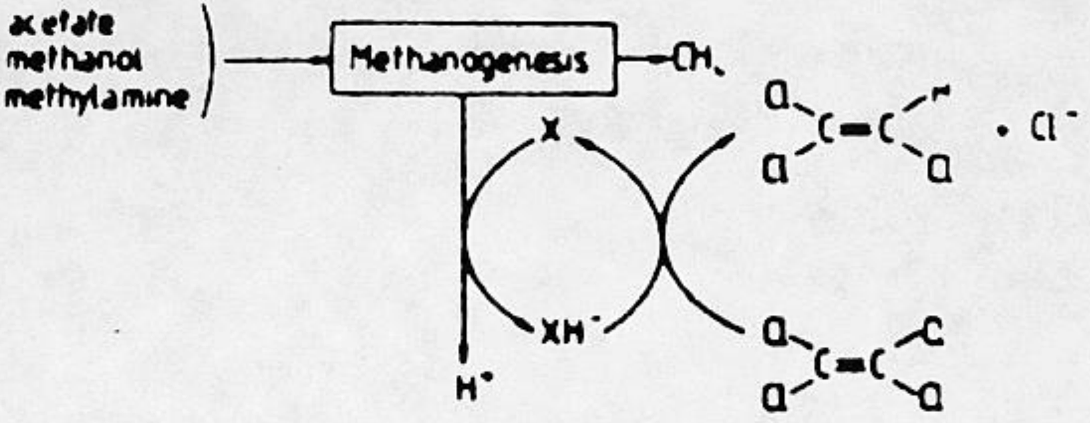
Transformation of cosubstrate is dependent upon the presence of the substrate:

- **acts to induce enzymes mediating transformation**
- **support growth and activity of degraders**

Occurs naturally at low levels (low subtrate levels, population density)

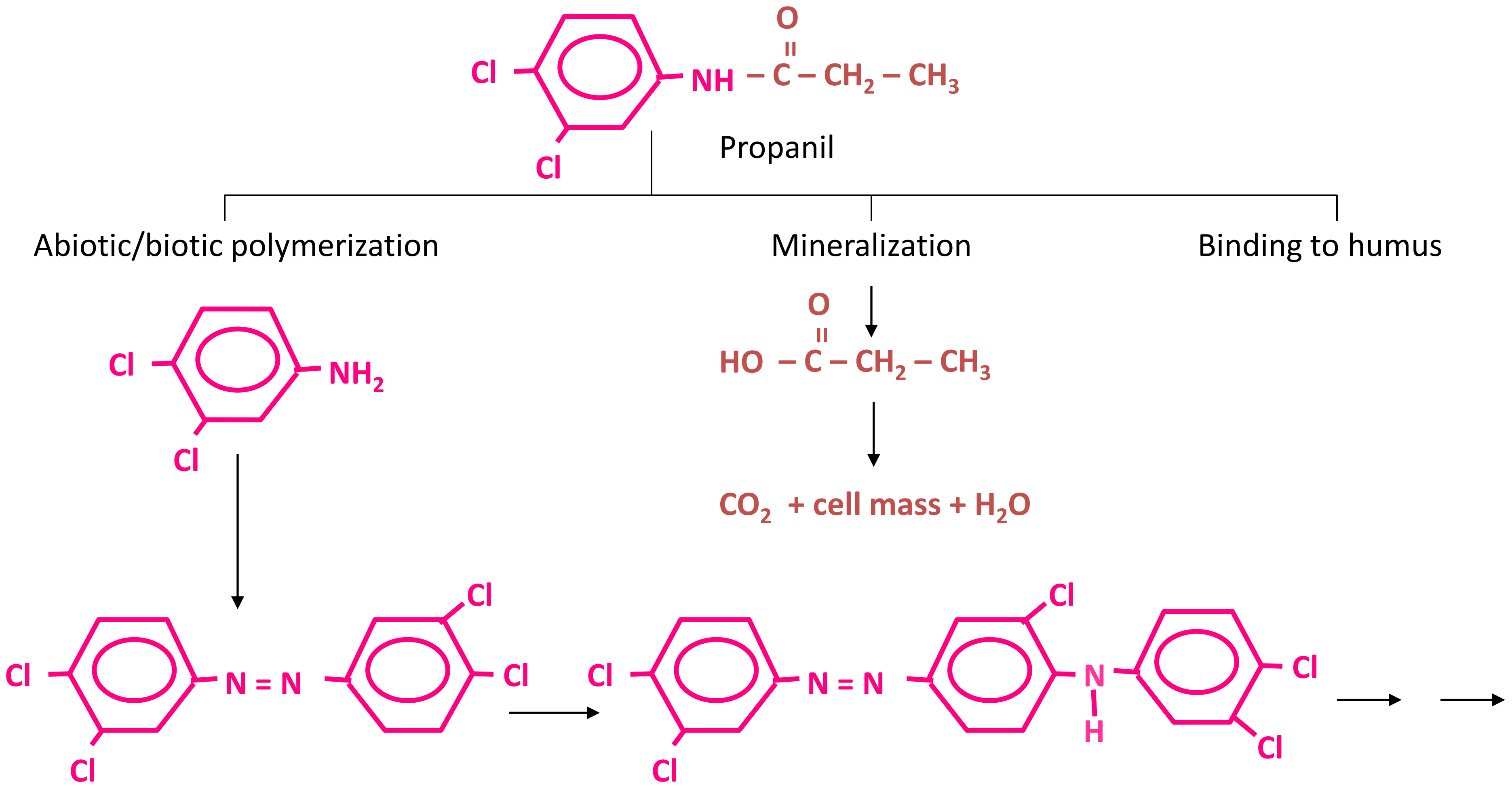
Effective use in bioremediation requires introduction of the substrate (inducer)

Mechanism for Type II cometabolism by methanogens



~~FIG. 12.~~ Hypothetical scheme for the transfer of electrons to tetrachloroethylene during methanogenesis. X is the electron carrier involved in methane production (120).

Biosynthesis - partial or incomplete degradation can also result in polymerization or synthesis of compounds more complex and stable than the parent compound.



Propanil

Abiotic/biotic polymerization

Mineralization

Binding to humus

$HO-\overset{O}{\overset{\|}{C}}-CH_2-CH_3$

CO_2 + cell mass + H_2O

tetrachloroazobenzene

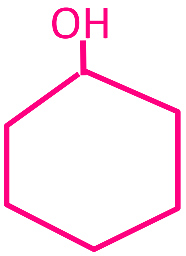
dichloroanilino - trichloroazobenzene

Biodegradation pathways

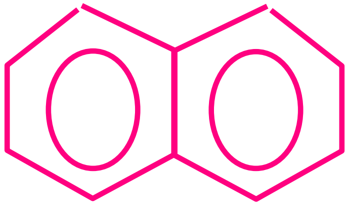
Most contaminants can be categorized into one of three structure types, all commonly found in petroleum products. Some contaminants contain a combination of these structures.

Aliphatics: $CH_3 - (CH_2 - CH_2)_n - CH_3$

Alicyclics:



Aromatics:



Metabolism Modes

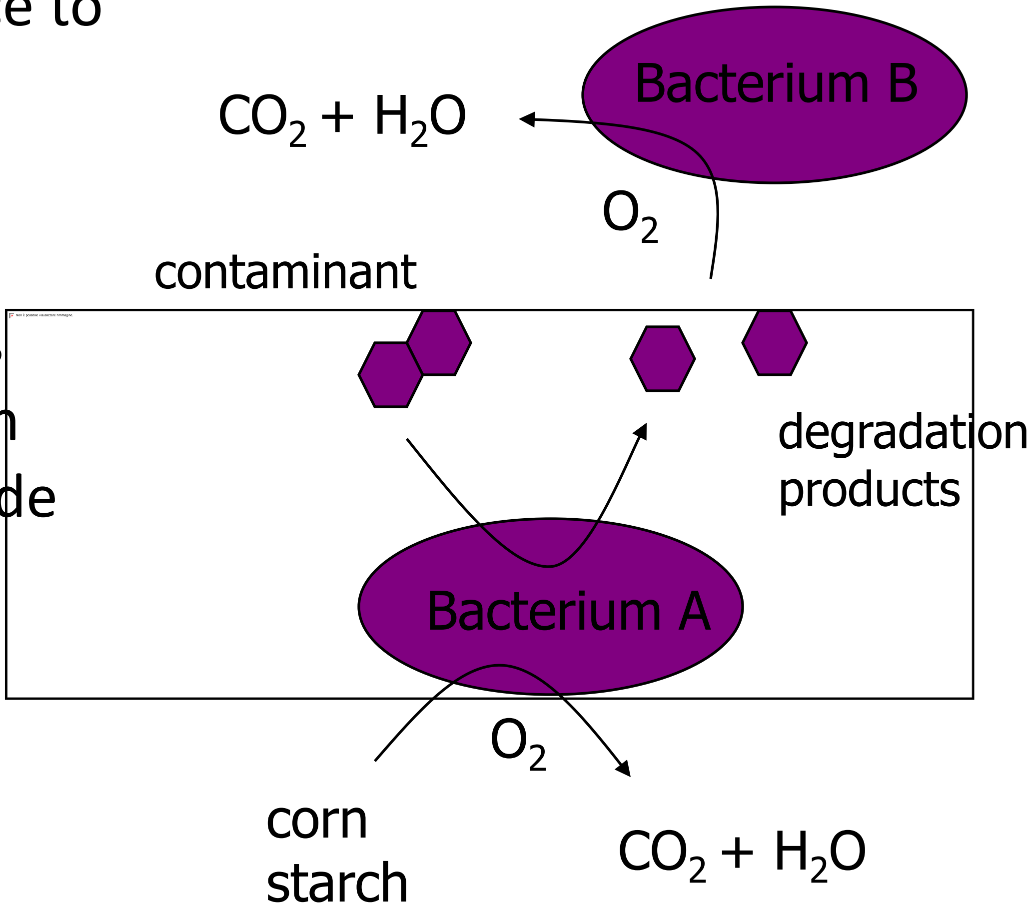
- Cometabolism - transformation of an organic compound by a microorganism that is unable to use the substrate as a source of energy
- Metabolites or transformation products from cometabolism by one organism can typically be used as an energy source by another
- Preferential degradation: higher energy yielding compounds degraded first, e.g., in a petroleum spill under aerobic conditions, benzene → naphthalene →chrysene

CONSORZI MICROBICI

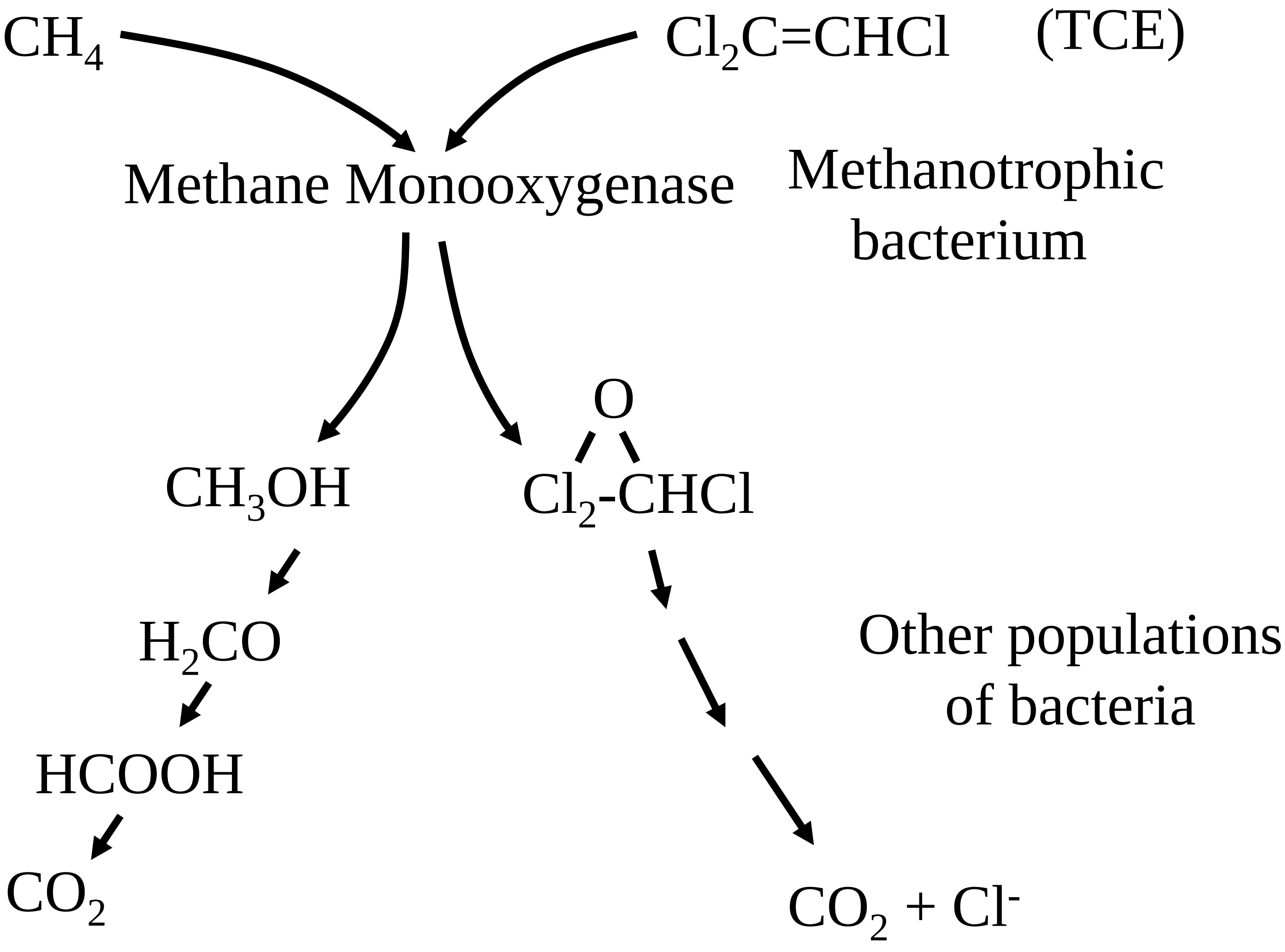
- Interazioni in cui due o più microrganismi determinano la trasformazione di un contaminante che non può essere condotta dalle singole specie
- La biodegradazione condotta da una miscela di più specie è più rapida della somma della velocità delle reazioni condotte dalle specie separatamente
- Può coinvolgere più organismi che traggono energia dal parziale metabolismo del composto oppure una combinazione di cometabolismo e biodegradazione che produce energia

Consortium interactions

- Bacterium A uses some other carbon and energy source to partially degrade contaminant.
- Bacterium B metabolizes contaminant degradation products to carbon dioxide and water.



Combining cometabolism and consortium interactions



CH_4

$Cl_2C=CHCl$ (TCE)

Methane Monooxygenase

Methanotrophic bacterium

CH_3OH

O

Cl_2-CHCl

H_2CO

HCOOH

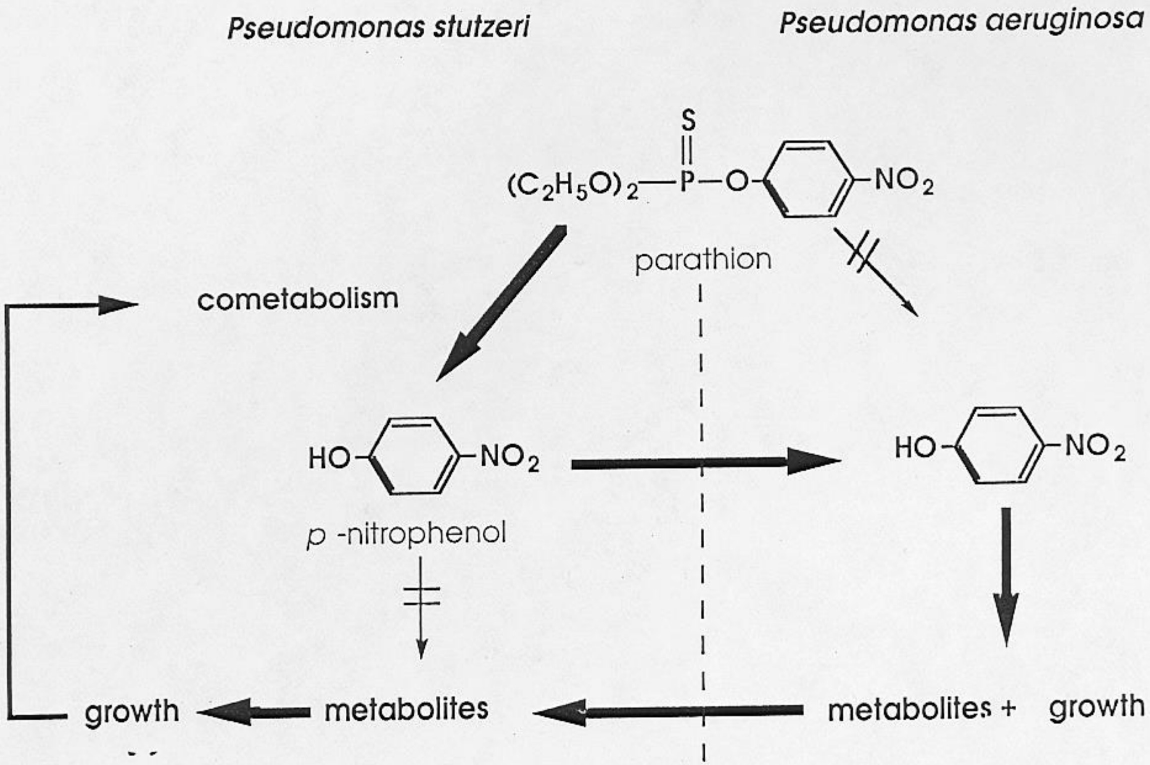
CO_2

Other populations of bacteria

$CO_2 + Cl^-$

Pesticide-degrading consortium

Xenobiotic degradation by a mutualistic consortium



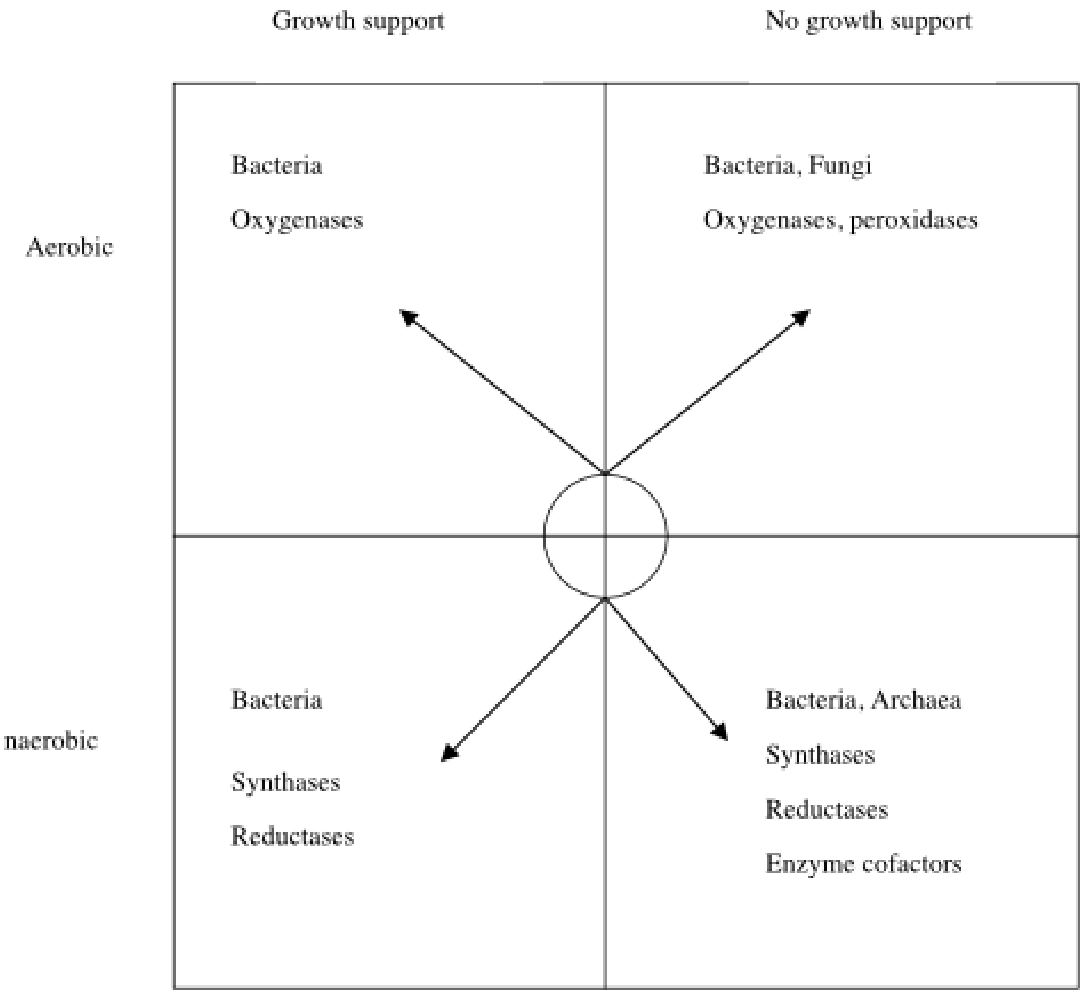
Adapted from Slater and Lovatt (1984)

Biodegradation & biotransformation: Types of processes

Compounds may be degraded by processes that may or may not support growth of the organism effecting the transformation

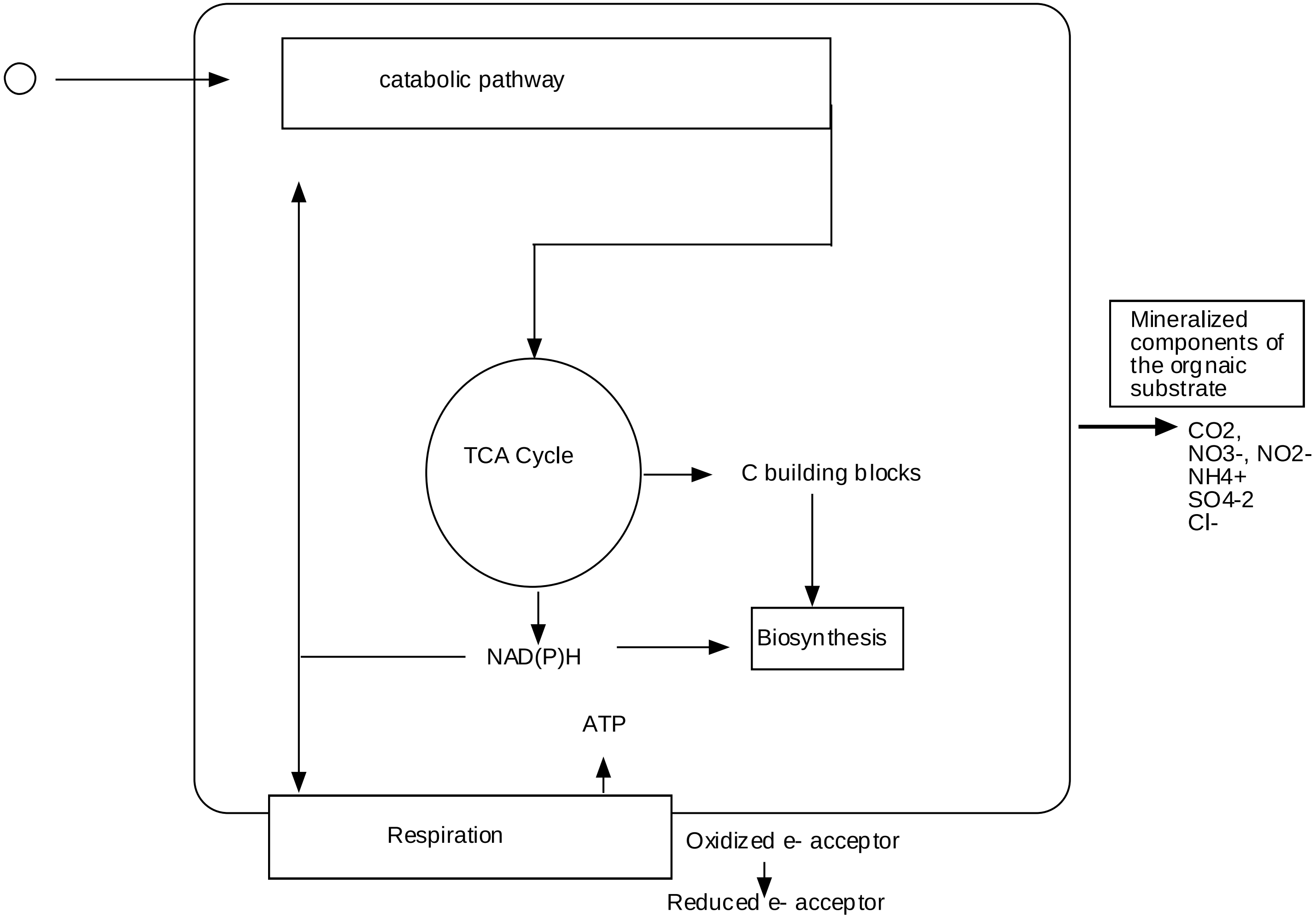
Same for aerobic & anaerobic conditions:

types of enzymes or biomolecules mediating transformations differ

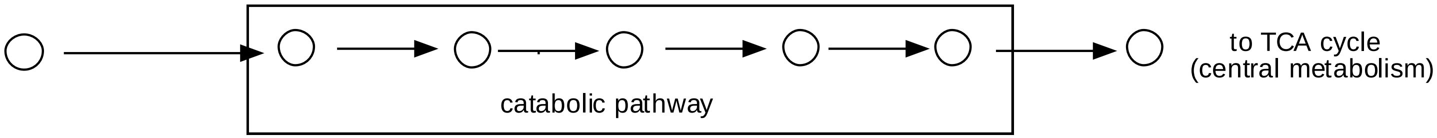


Overview of metabolic processes

Overview of metabolic processes



Catabolic enzymes: Characteristics key to growth support



Characteristics / functions of catabolic pathw:

Series of enzymes that are peripherial to central metabolism, so they are not possessed by all organisms

Product(s) are fed into central metabolism to support growth/activity

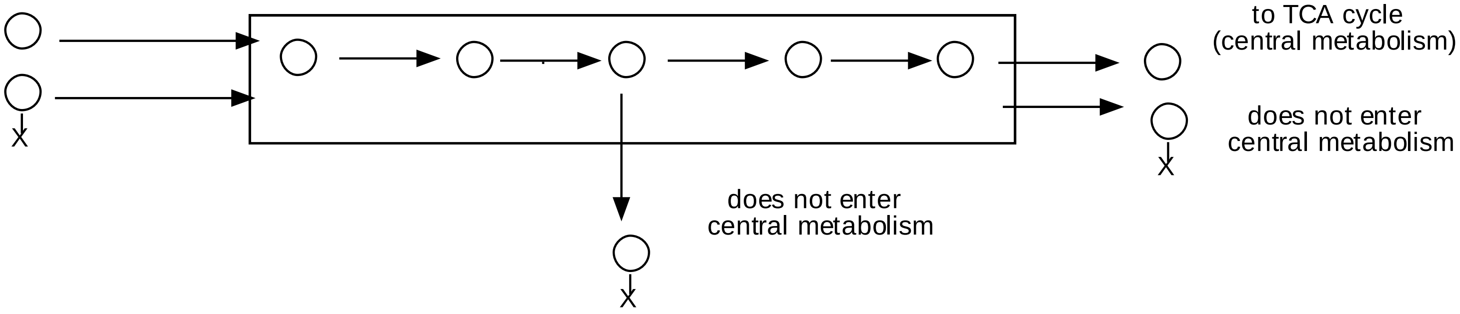
Enzyme production is repressed in the absence of the organic compound (inducer)

Substrate specificty of of catabolic pathway enzymes varies:

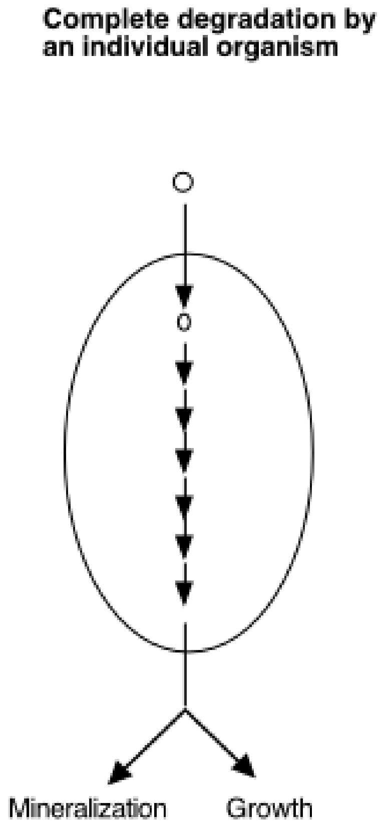
very selective, recognize and transform a single substrate

broad specifity, recognize and transform a variety of substrates (may or may not be structurally similar)

all end products of broad specifity enzymes may not be fed into central metabolism (no growth support)



Degradation mechanisms: Growth Supporting

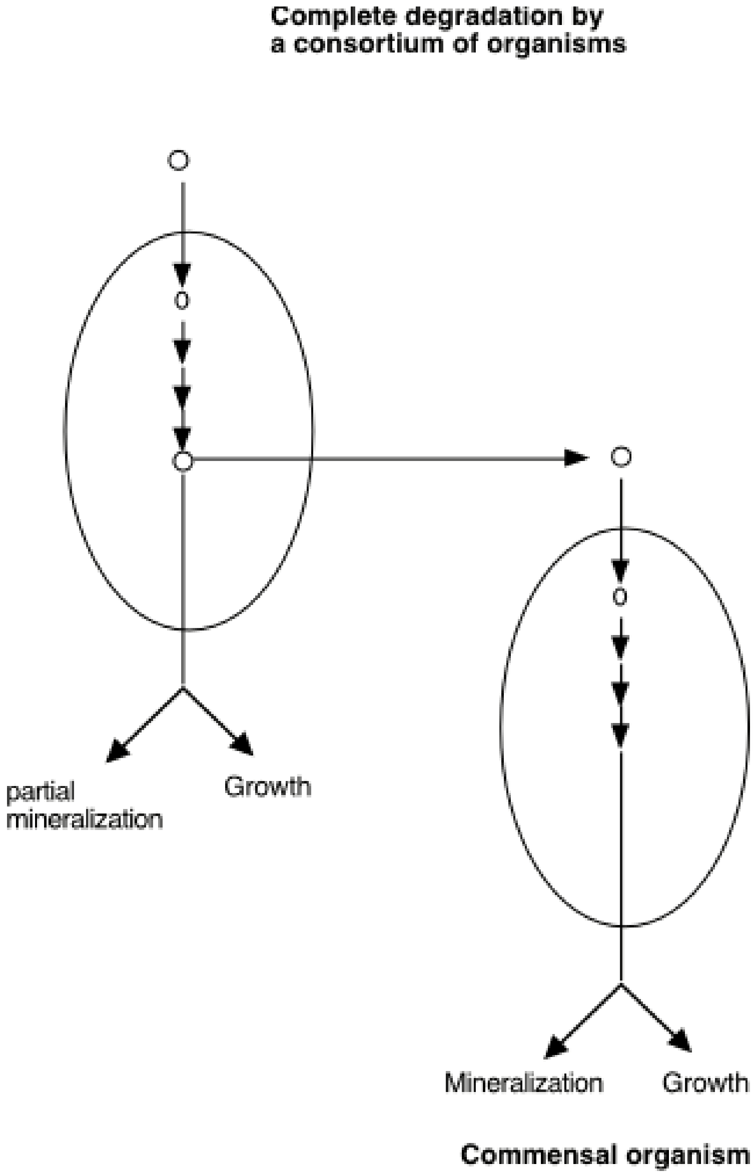


<u>Pure culture model</u>

Good for detailed studies of metabolism and genetics

May not be representative of activity in the environment

Degradation mechanisms: Growth Supporting

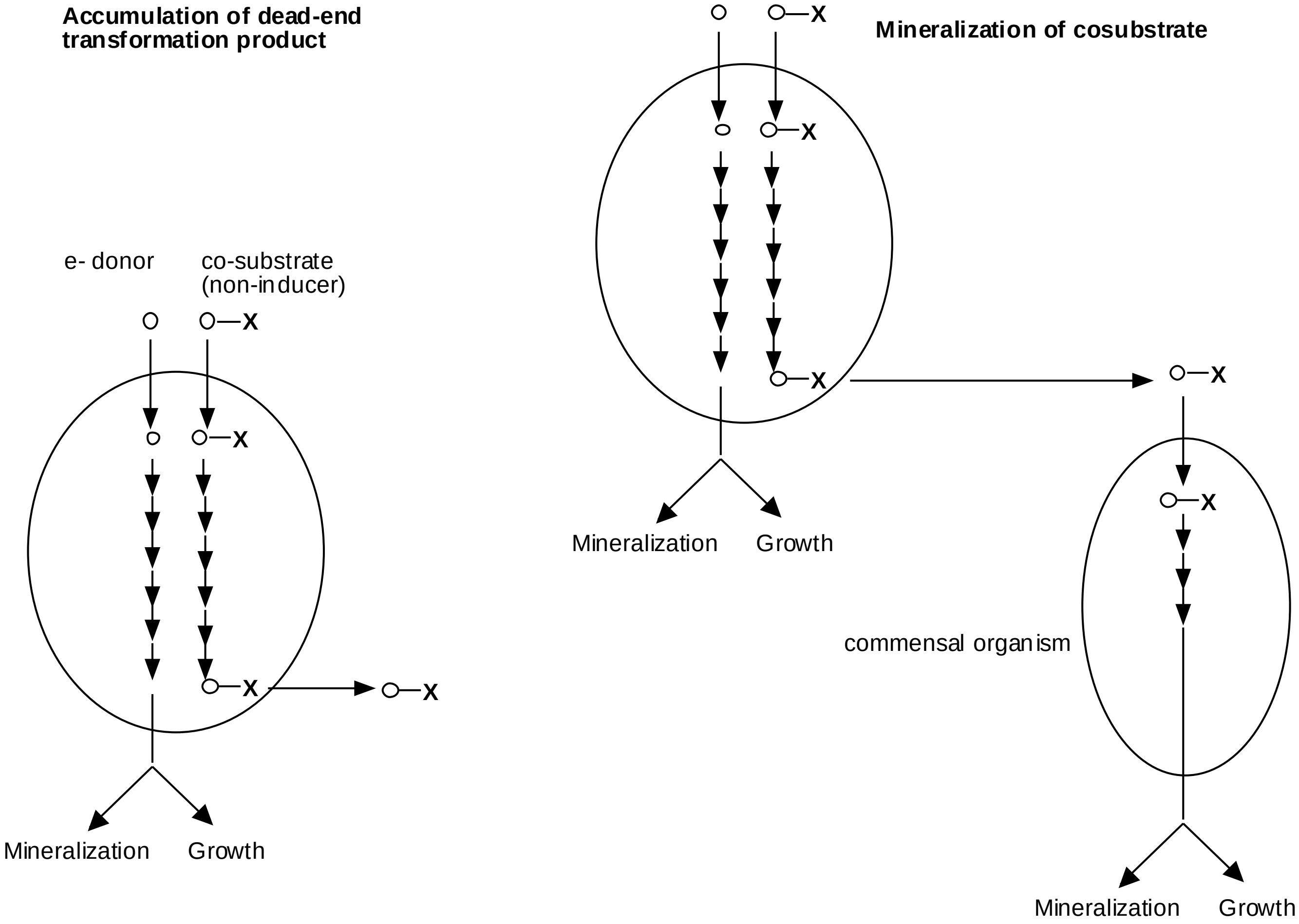


Consortium model
Common for anaerobes

May also be prevelant in aerobic environments, documented for degradation of some pesticides

Complicates detailed studies of metabolism and genetics

Degradation mechanisms: Not growth supporting



Cometabolic transformation by an individual organism:

Accumulation of dead-end transformation product

e- donor

co-substrate
(non-inducer)

X

X

X

X

Mineralization

Growth

Cometabolic transformation coupled to commensal population:

Mineralization of cosubstrate

X

X

X

X

X

commensal organism

Mineralization

Growth

Mineralization

Growth

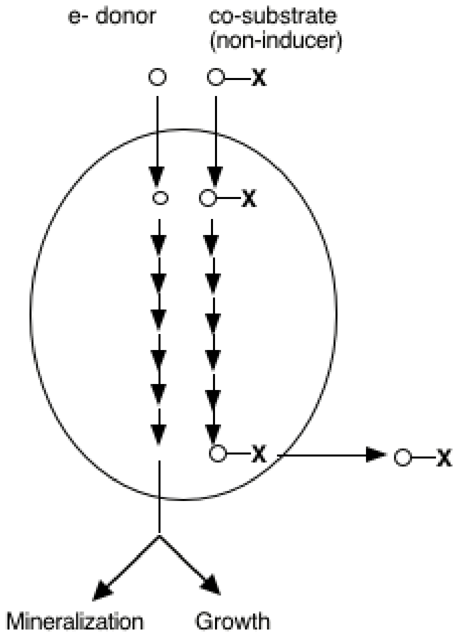
Degradation mechanisms

Type I cometabolism

Transformation of cosubstrate is dependent upon the presence of the substrate:

acts to induce enzymes mediating transformation

supports growth and activity of degraders



Occurs naturally at low levels (low subtrate levels, population density)

Effective use in bioremediation requires introduction of the substrate (inducer)

Example of Type I cometabolism

Biphenyl degraders common in soil

typically cannot grow on PCBs as Cl-products are not utilized

Supplied with biphenyl, enzymes are induced that transform PCBs

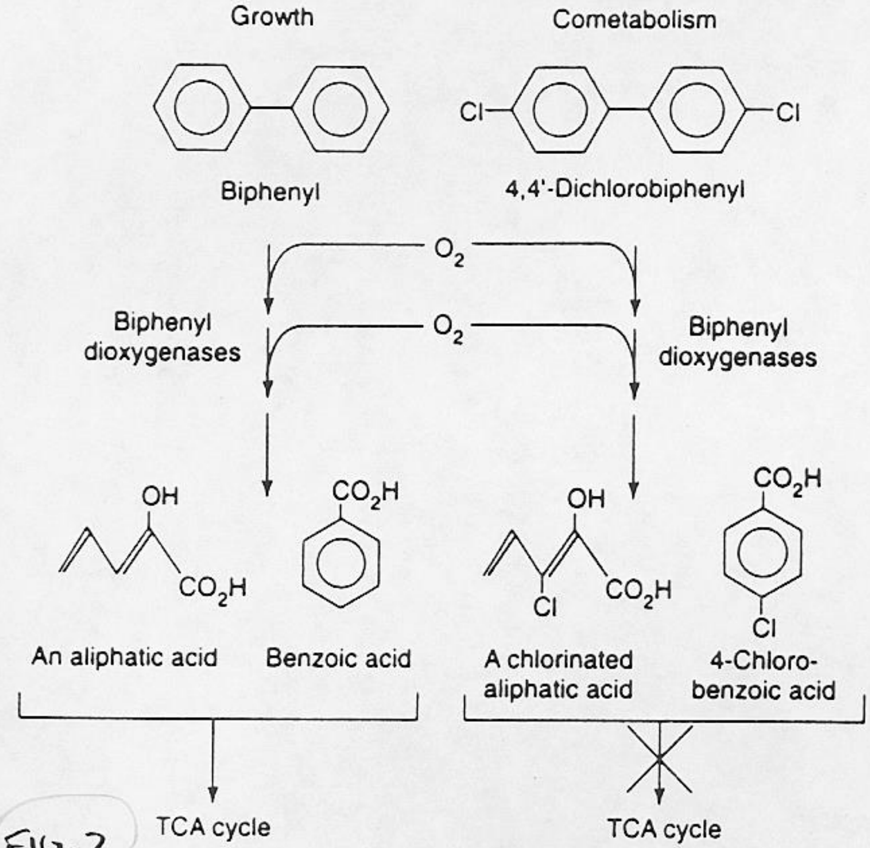
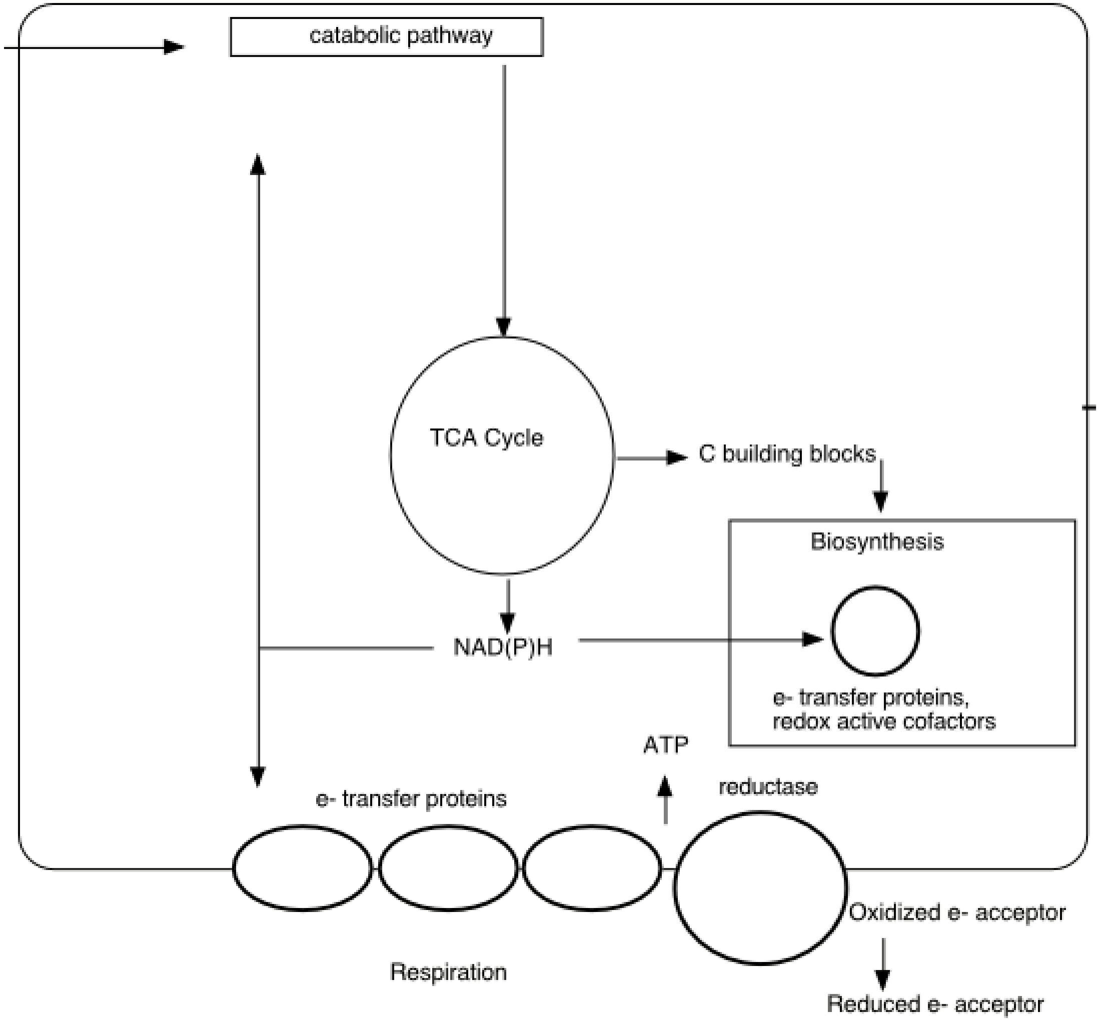


FIG. 2

~~Figure 20~~–3 Growth-supporting metabolism of biphenyl linked to cometabolic degradation of polychlorinated biphenyls (PCBs). Biphenyl induces formation of oxygenases that oxidize and cause ring cleavage of biphenyl and PCBs. Aliphatic and aromatic acids produced from biphenyl are assimilated through the TCA cycle and support microbial growth. In contrast, PCB metabolism is unproductive in that chlorinated aliphatic and aromatic acids are typically not assimilated by the biphenyl degrader.

Degradation linked to use as e- acceptor



Oxidized compounds may be reduced by two kinds of interaction:

1. Redox-active biomolecules involved in biosynthesis

2. Reductases (or other electron carriers) involved in respiration

Degradation via use as e- acceptor: Growth supporting transformations

Dehalorespiration

Halogenated organics used by anaerobes as terminal electron acceptors

Energy from electron transfer is captured

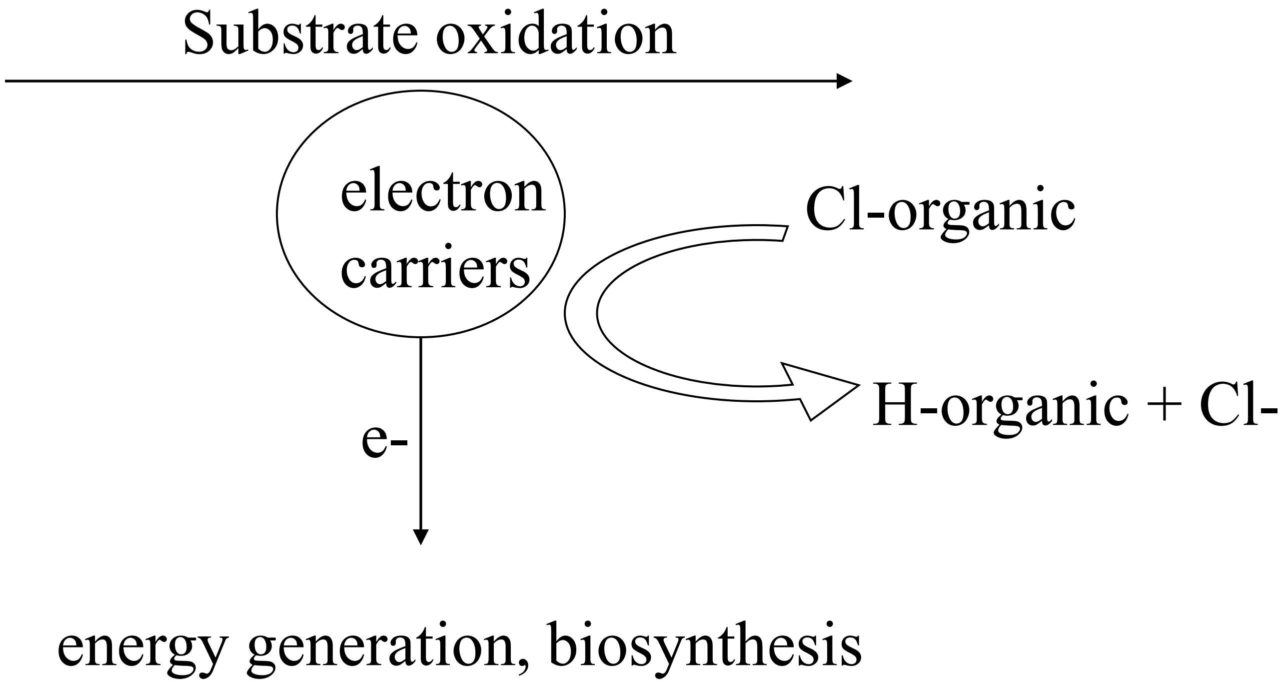
Mediated by a specific reductase induced by growth with Cl-organic as electron acceptor

Substrates include:
chlorinated alkenes (PCE, TCE)

chlorinated aromatics (chlorobenzenes, polychlorinated biphenyls)

Degradation via use as e- acceptor: Transformations not supporting growth

Interaction with redox active cofactors



Substrate oxidation

electron carriers

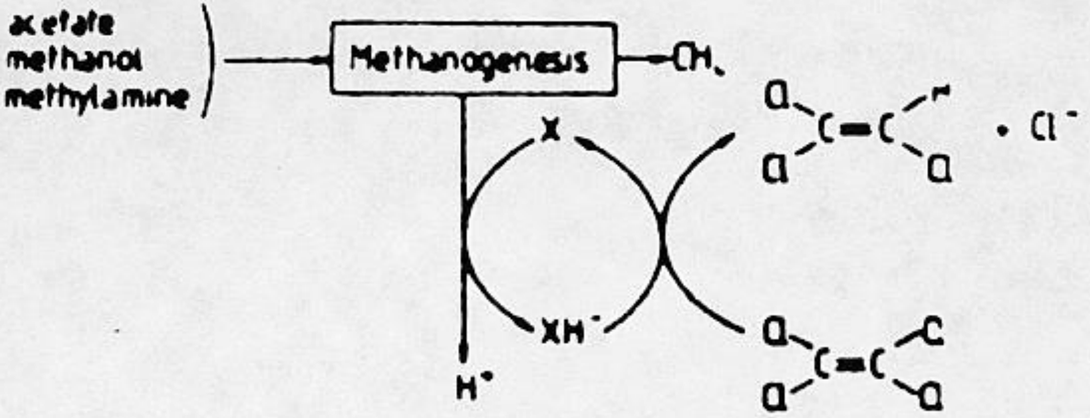
Cl-organic

H-organic + Cl-

e-

energy generation, biosynthesis

Mechanism for Type II cometabolism by methanogens



~~FIG. 12.~~ Hypothetical scheme for the transfer of electrons to tetrachloroethylene during methanogenesis. X is the electron carrier involved in methane production (120).

I principali contaminanti

I principali contaminanti degli ambienti naturali sono:

- Idrocarburi
- Metalli pesanti
- Radionuclidi
- Sostanze xenobiotiche
- **Esplosivi**

Tipicamente si tratta di sostanze chimiche introdotte dall'attività umana e potenzialmente pericolose.

ATTIVITA' INDUSTRIALI

E AGRICOLE

RILASCIO NELL'AMBIENTE

(aria, acqua e suolo)

COMPOSTI DI SINTESI

(Solventi, plastificanti, insetticidi, erbicidi, ecc)

PROBLEMI ECOTOSSICOLOGICI

Valutazione di fattibilità

1 . Monitoraggio dell'attività naturale di degradazione delle molecole contaminanti - risk assesment (Natural Attenuation)

2 . Stimolazione attività degradative del microbiota autoctono mediante aggiunta di nutrienti e/o altri fattori limitanti

3 . Aggiunta di microorganismi degradativi per aumentare l'attività totale di degradazione ?????????

I criteri per considerare la bioremediation una tecnica realizzabile sono:

1) il microrganismo deve poter degradare il contaminante con una efficienza tale da riportare la sua concentrazione a livelli che siano quelli standard;

2) il contaminante target deve essere "biodisponibile";

3) il sito contaminato deve poter consentire la sopravvivenza ed attività enzimatica di microrganismi e piante;

4) i costi della bioremediation devono essere inferiori o non molto più costosi di altre tecnologie.

FATTORI LIMITANTI: BIODISPONIBILITA' possibilità di essere accessibile ad un microrganismo

E' spesso un fattore limitante alla biodegradabilità specie in matrici complesse come il suolo. Molecole apolari vengono adsorbite su superfici apolari con processi lenti di diffusione e adsorbimento. Anche i processi opposti di deadsorbimento e migrazione all'esterno sono lenti

I batteri dispongono di un fattore che può facilitare l'aumento di biodisponibilità di possibili fonti di carbonio: *la sintesi di biosurfattanti*

I biosurfattanti sono polimeri naturali che vengono prodotti direttamente dai batteri attraverso un processo di fermentazione che si sviluppa in presenza di idrocarburi o di altre sostanze (e.g. glucosio) utilizzate come fonte di carbonio. Microrganismi diversi producono molecole di biosurfattanti diverse tra le quali glicolipidi, lipopeptidi, lipopolisaccaridi, fosfolipidi, acidi grassi/lipidi neutri e proteine.

I biosurfattanti, grazie alle loro caratteristiche amfipatiche; aumentano la superficie di interfaccia tra fase organica/acquosa, facilitando la formazione di emulsioni e in generale l'interazione tra batteri e molecole facilmente solubili in fase organica

Fattori che influiscono sulla biodegradabilità

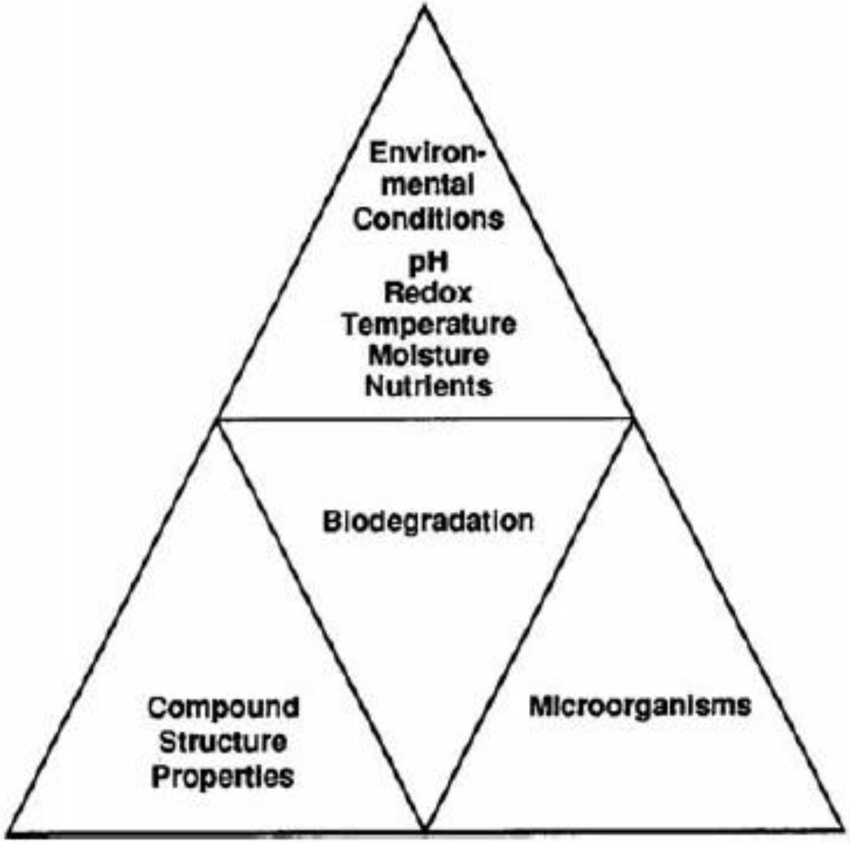
A) Molecole:

- struttura chimica:
  - polimerizzazione e ramificazione
  - presenza di legami resistenti all'idrolisi
  - residui eterociclici, aromatici e policiclici
  - sostituenti cloro e nitro
- concentrazione
- solubilità in acqua
- proprietà fisiche
- tossicità

B) Ambiente:

- biodisponibilità
- pH, pO2, temperatura, potenziale redox
- concentrazione e composizione ionica
- presenza di nutrienti organici e inorganici
- presenza di un'adeguata popolazione microbica

Biodegradation Triangle



Environmental Conditions
pH
Redox
Temperature
Moisture
Nutrients

Biodegradation

Compound Structure Properties

Microorganisms

Environment (biotic and abiotic)

- moisture content (too much limits oxygen availability, too little inhibits microbial activity in general)
- oxygen (required for rapid biodegradation processes)
- pH (extremes limit microbial activity)
- nutrient availability (includes mineral nutrients and organic matter)
- competition (are the microbes of interest active, do added microbes survive?)

All of these need to be with acceptable ranges to allow optimal biodegradation activity.

Metabolismo microbico

Biodegradation Processes

- Conversion of contaminants to mineralized (e.g. CO_2, H_2O, and salts) end-products via biological mechanisms
- Biotransformation refers to a biological process where the end-products are not minerals (e.g., transforming TCE to DCE)
- Involves the process of extracting energy from organic chemicals via oxidation of the organic chemicals

Fundamentals of Biodegradation

- All organics are biodegradable, BUT biodegradation requires specific conditions
- There is no Superbug - not Volkswagon
- Contaminants must be bioavailable
- Biodegradation rate and extent is controlled by a “limiting factor”

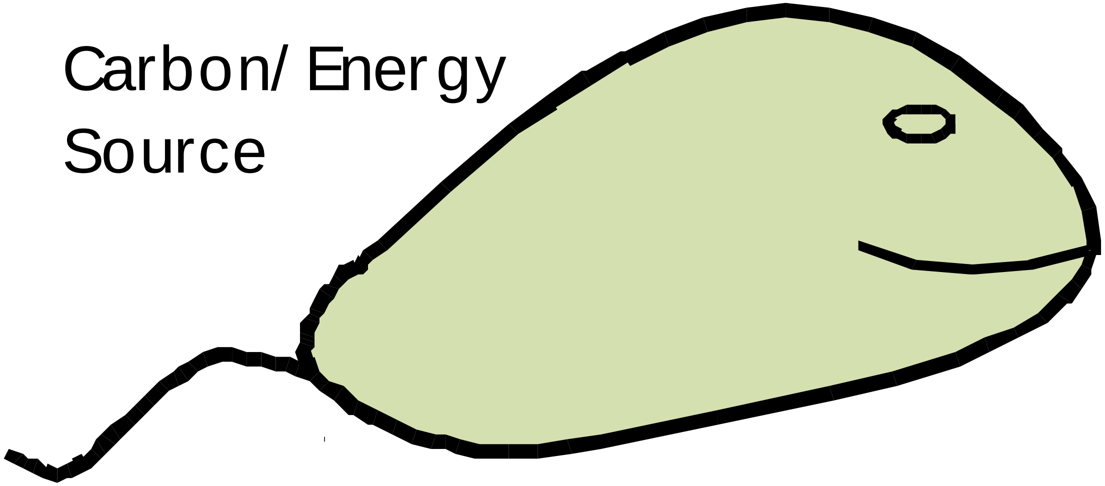
Requirements for Microbial Growth

Toxicants

Electron Acceptor

(O_2, NO_3^-, SO_4^{2-}, etc.)

Carbon/Energy Source



Environmental Conditions

(Temp, pH, Eh)

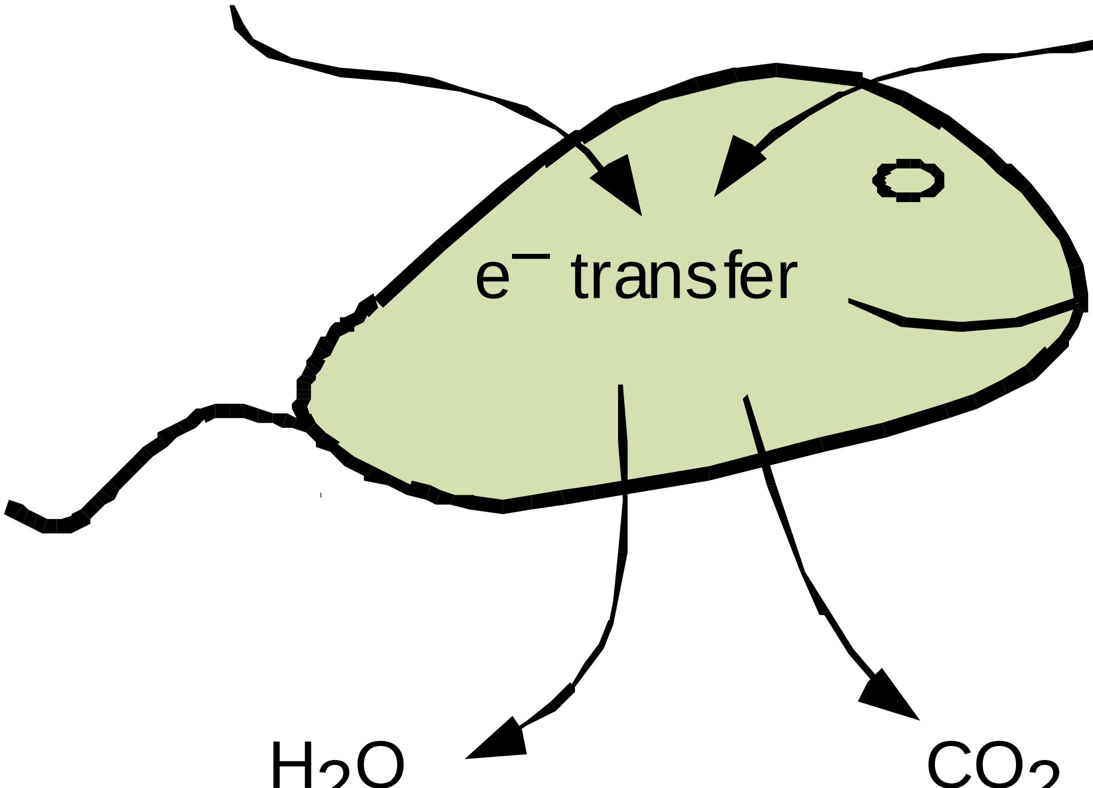
Nutrients (N, P)

Trace Elements

Microbial Metabolism

- Need nitrogen, phosphorus, sulfur, and a variety of trace nutrients other than carbon
- Carbon is often the limiting factor for microbial growth in most natural systems
- Acclimatization period - a period during which no degradation of chemical is evident; also known as adaptation or lag period
- Length of acclimatization period varies from less than 1 h to many months
- Acclimatization of a microbial population to one substrate frequently results in the simultaneous acclimatization to some structurally related molecules

Electron Exchange



Electron Acceptor
(O_2, NO_3^-, SO_4^{2-}, etc.)

Carbon/Energy Source
Electron Donor

e^- transfer

H_2O

CO_2

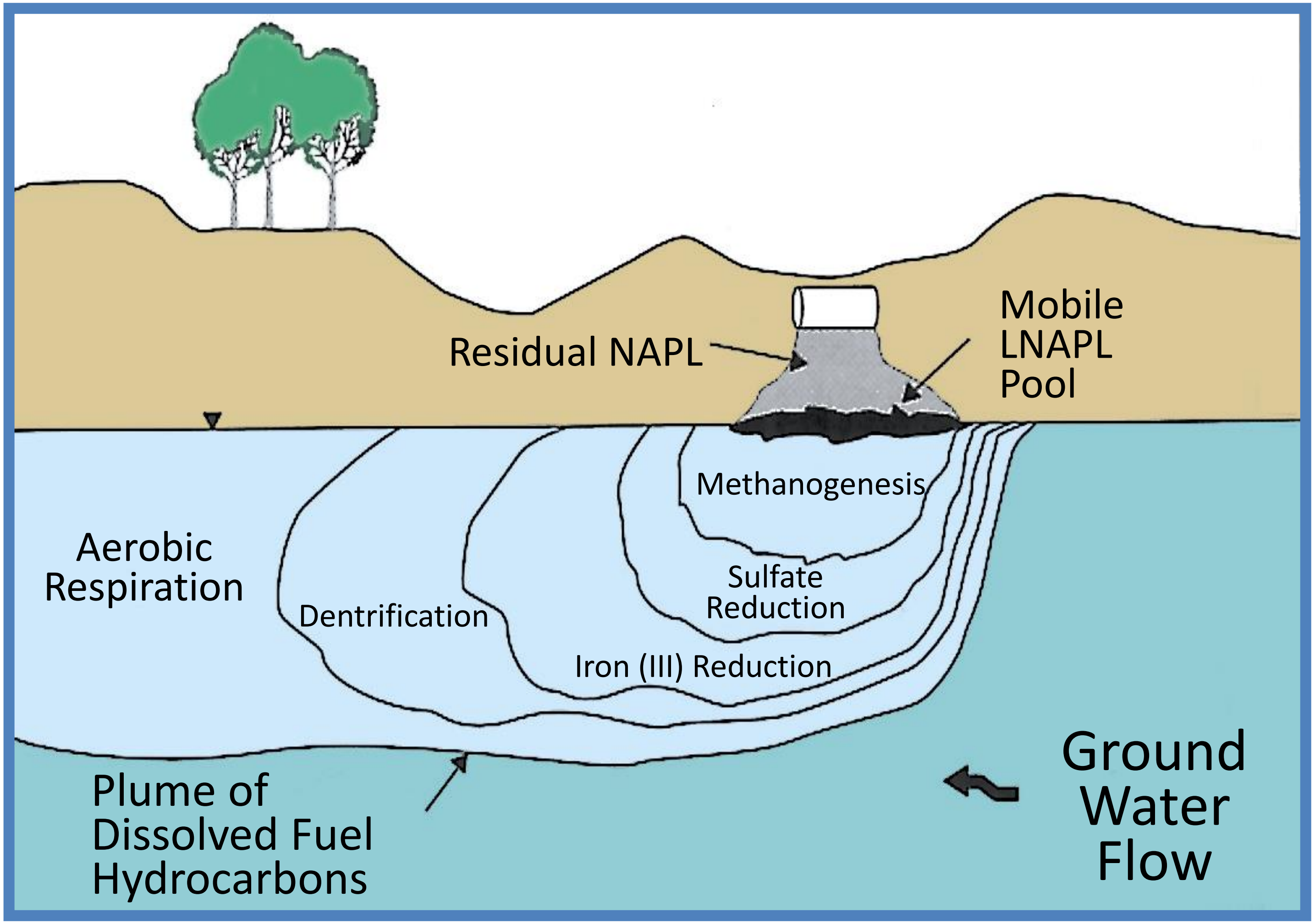
Metabolism Modes

- Aerobic: transformations occur in the presence of molecular oxygen (as electron acceptor), known as aerobic respiration
- Anaerobic: reactions occur only in the absence of molecular oxygen, subdivided into:
  - Anaerobic respiration
  - Fermentation
  - Methane fermentation

Metabolism Modes

- Anaerobic respiration
  - Nitrate as an electron acceptor - denitrifying and nitrate-reducing organisms
  - Sulfate and thiosulfate as electron acceptors - by sulfate-reducing organisms
  - CO_2 as an electron acceptor, by methanogenic organisms
  - Chlorinated organic compounds as electron acceptors
- Fermentation - organic compounds serve as both electron donors and electron acceptors
- Methane fermentation - consecutive biochemical breakdown of organic compounds to CH_4 and CO_2

Electron Acceptor Zone Formation



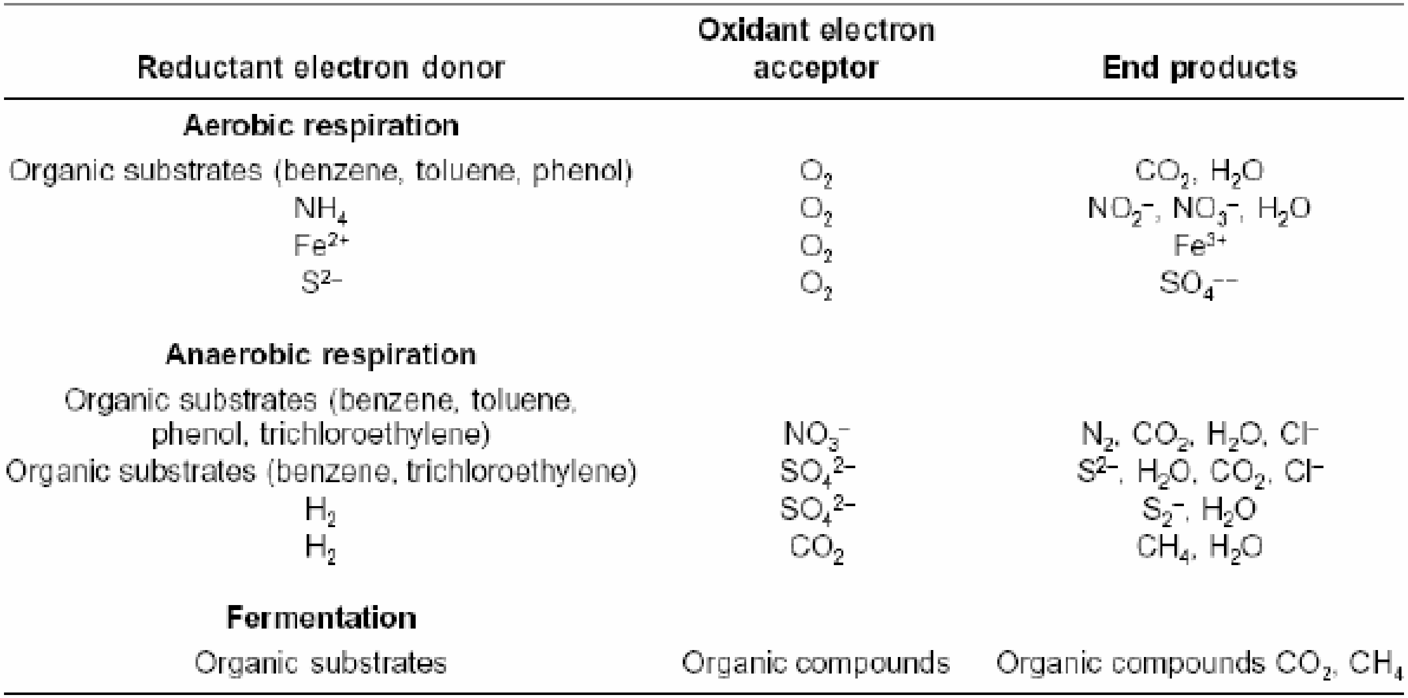
(Source: W,R, N, & W, 1999.) **(Adapted from Lovley et al., 1994b.)**

Dependence on Redox Condition

Compound(s)	Aerobic	Anaerobic
Acetone	1	1
BTEX	1	2 to 4
PAH’s	1	3 to 4
PCB’s		
highly substituted	4	2
minimally substituted	2	4
Chlorinated ethenes		
PCE	4	1 to 2
TCE	3	1 to 2
DCEs	3	2 to 3
Vinyl chloride	1 to 2	3 to 4

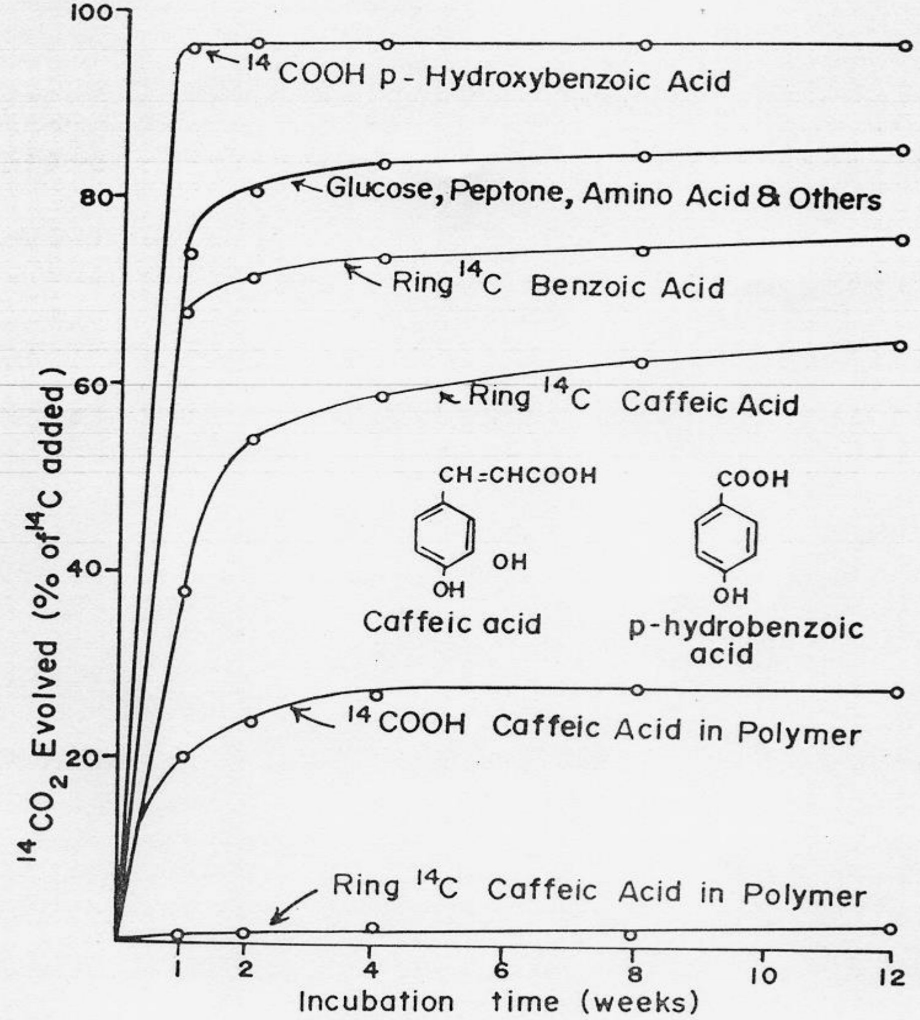
1 Highly biodegradable
2 Moderately biodegradable
3 Slow biodegradation
4 Not biodegraded

Summary of Metabolism Modes

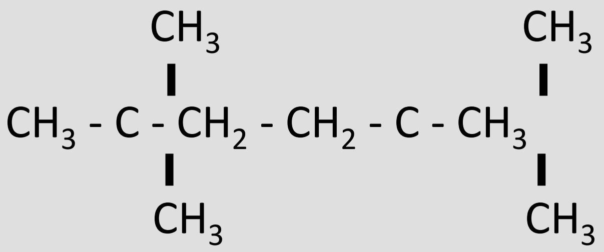


Reductant electron donor	Oxidant electron acceptor	End products
Aerobic respiration		
Organic substrates (benzene, toluene, phenol)	O_2	CO_2, H_2O
NH_4	O_2	NO_2^-, NO_3^-, H_2O
Fe^{2+}	O_2	Fe^{3+}
S^{2-}	O_2	SO_4^{--}
Anaerobic respiration		
Organic substrates (benzene, toluene, phenol, trichloroethylene)	NO_3^-	N_2, CO_2, H_2O, Cl^-
Organic substrates (benzene, trichloroethylene)	SO_4^{2-}	S^{2-}, H_2O, CO_2, Cl^-
H_2	SO_4^{2-}	S_2^-, H_2O
H_2	CO_2	CH_4, H_2O
Fermentation		
Organic substrates	Organic compounds	Organic compounds CO_2, CH_4

Figure 6.5. Decomposition of specifically ^{14}C-labeled benzoic and caffeic acids, caffeic acid linked into phenolic polymers, and several simple organic compounds in Greenfield sandy loam. (From Haider and Martin, 1975).



Given a pair of structures you should be able to predict which of the pair will degrade more rapidly.



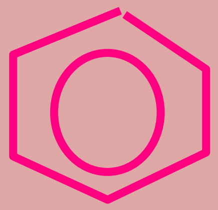
$$CH_3 - \overset{CH_3}{\underset{CH_3}{C}} - CH_2 - CH_2 - \overset{CH_3}{\underset{CH_3}{C}} - CH_3$$

2,2,5,5-tetramethylhexane

vs.

$CH_3 – CH_2 – CH_2 – CH_2 – CH_2 – CH_3$

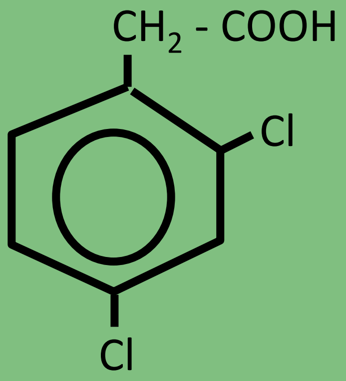
hexane



Benzene

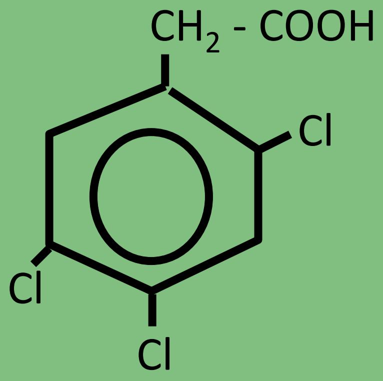
vs.

Benzo(a)pyrene

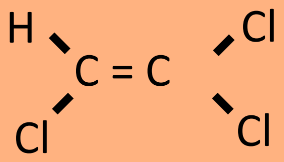


2, 4- D

vs.



2, 4, 5 - T



TCE

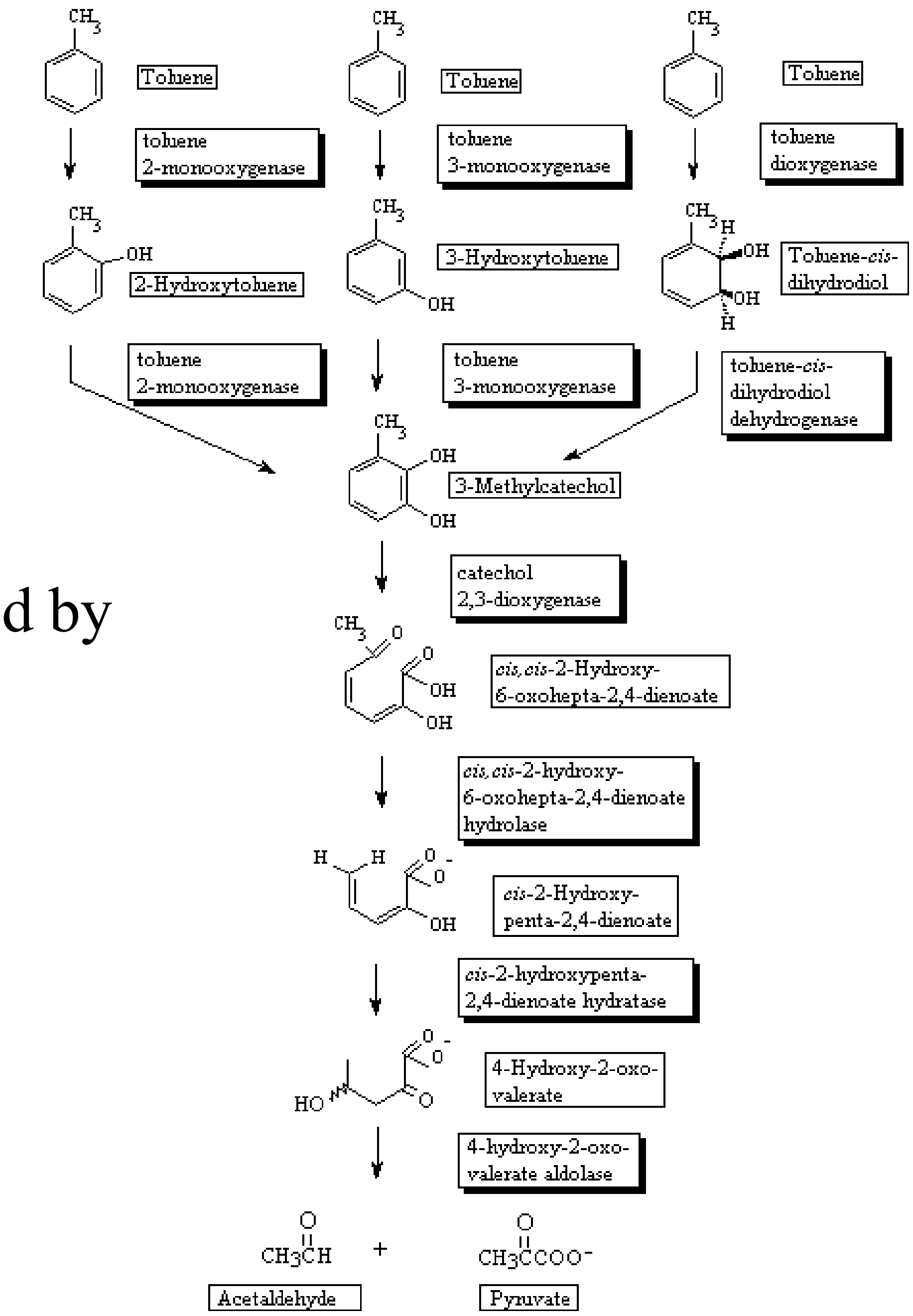
vs.

$CH_3 - CH_2 - OH$

ethanol

Toluene: Aerobic degradation

Degradation is initiated by either a mono- or di-oxygenase.

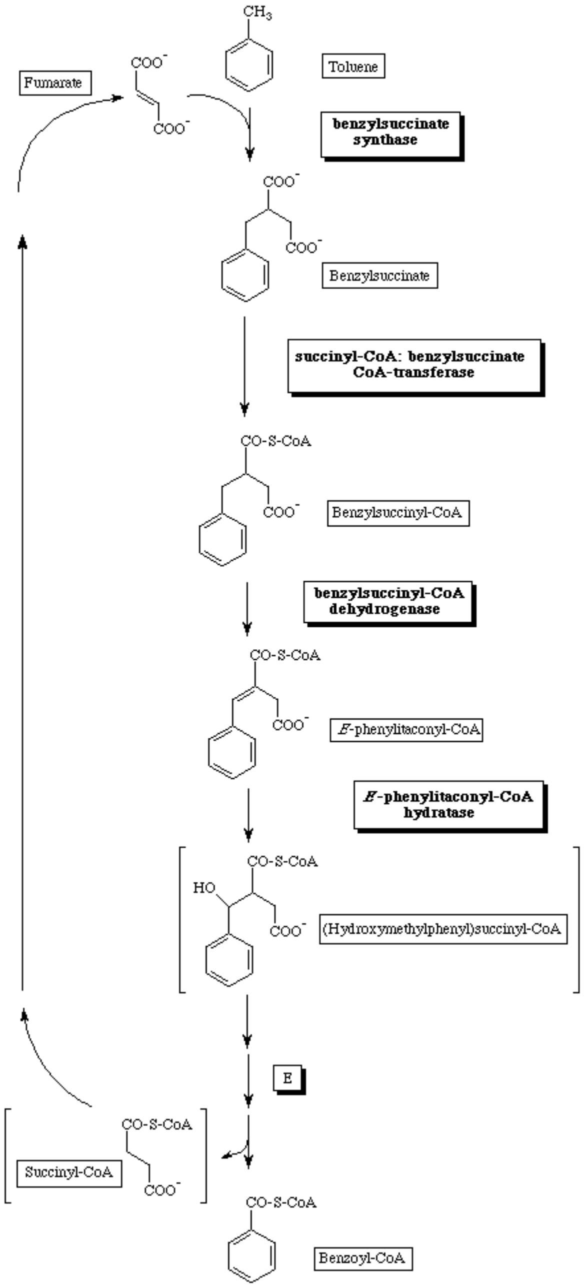


Toluene: Anaerobic degradation

Conjugation of fumarate to toluene gives benzylsuccinate

Benzylsuccinate via B-oxidation-like process to give benzoate (benzyl-CoA).

Benzoate metaboized via B-oxidation-like process



Alfred Spormann and Eva Young

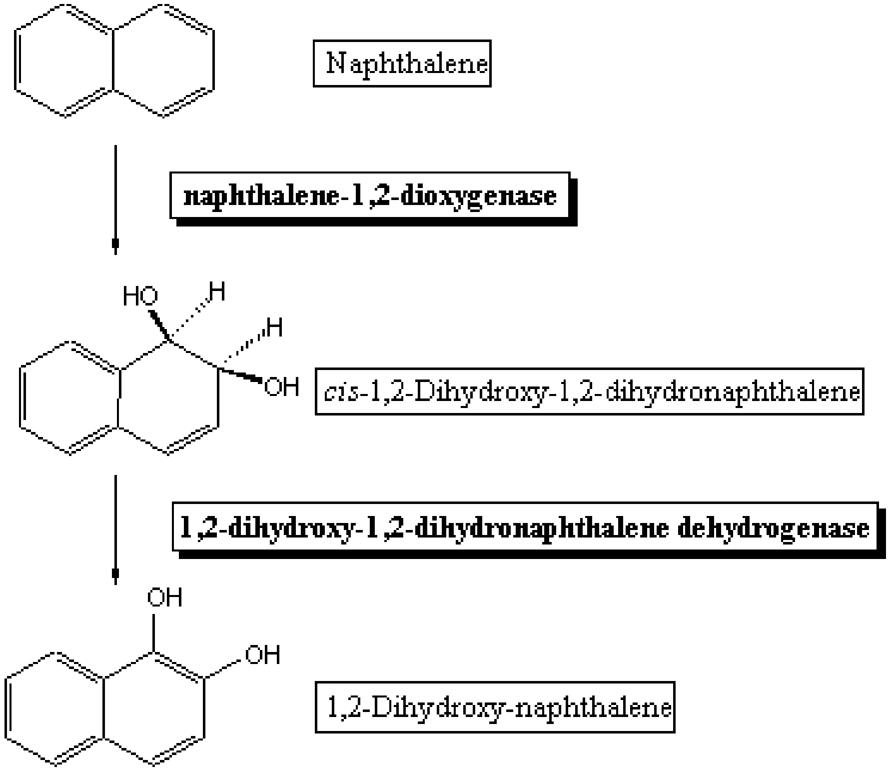
Compounds with differential aerobic vs. anaerobic degradation potential

<u>Benzene, polynuclear aromatic hydrocarbons (PAH)</u>

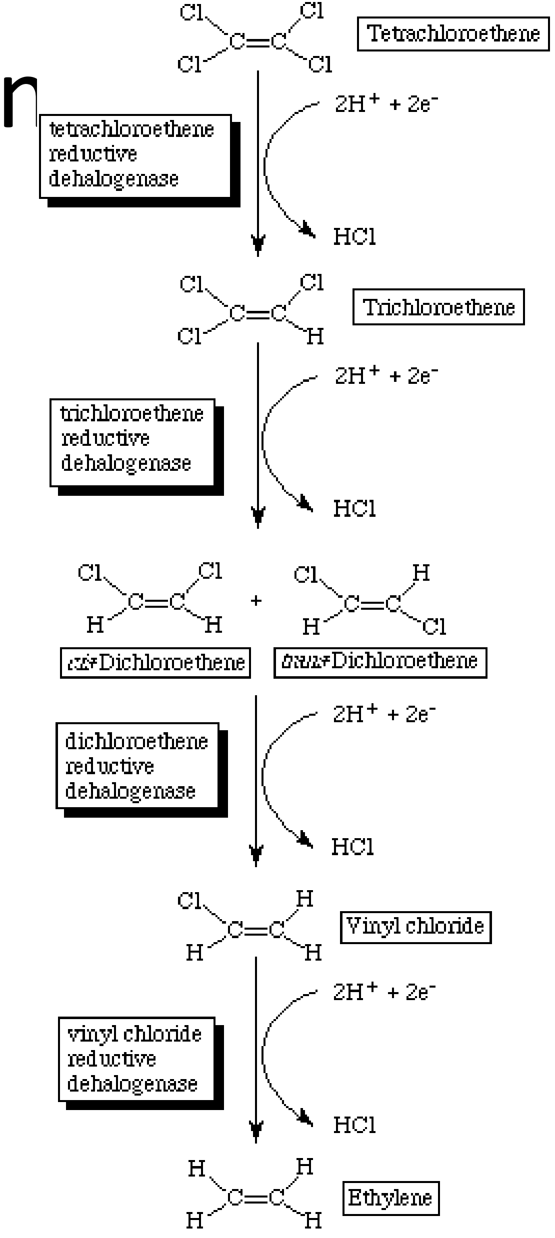
Stable ring structures, aerobes utilize oxidizing power of oxygenases to initiate degradation

Anaerobes lack similarly powerful oxidants

Compounds are readily degraded aerobically, but persistent in anaerobic environments



Compounds with differential aerobic vs. anaerobic degradation



<u>Perchloroethylene (PCE) aka. tetrachloroethene</u>

Highly chlorinated, highly oxidized C atoms are good electron targets for anaerobes but not aerobes

Cl atoms block activity of oxygenases.

Readily degraded anaerobically, but persistent in aerobic environments

Degradation via use as e- acceptor: Growth supporting transformations

Dehalorespiration

Halogenated organics used by anaerobes as terminal electron acceptors

Energy from electron transfer is captured

Mediated by a specific reductase induced by growth with Cl-organic as electron acceptor

Substrates include:
chlorinated alkenes (PCE, TCE)

chlorinated aromatics (chlorobenzenes, polychlorinated biphenyls)

Dehalorespiration: Scheme of electron transfer and energy conservation

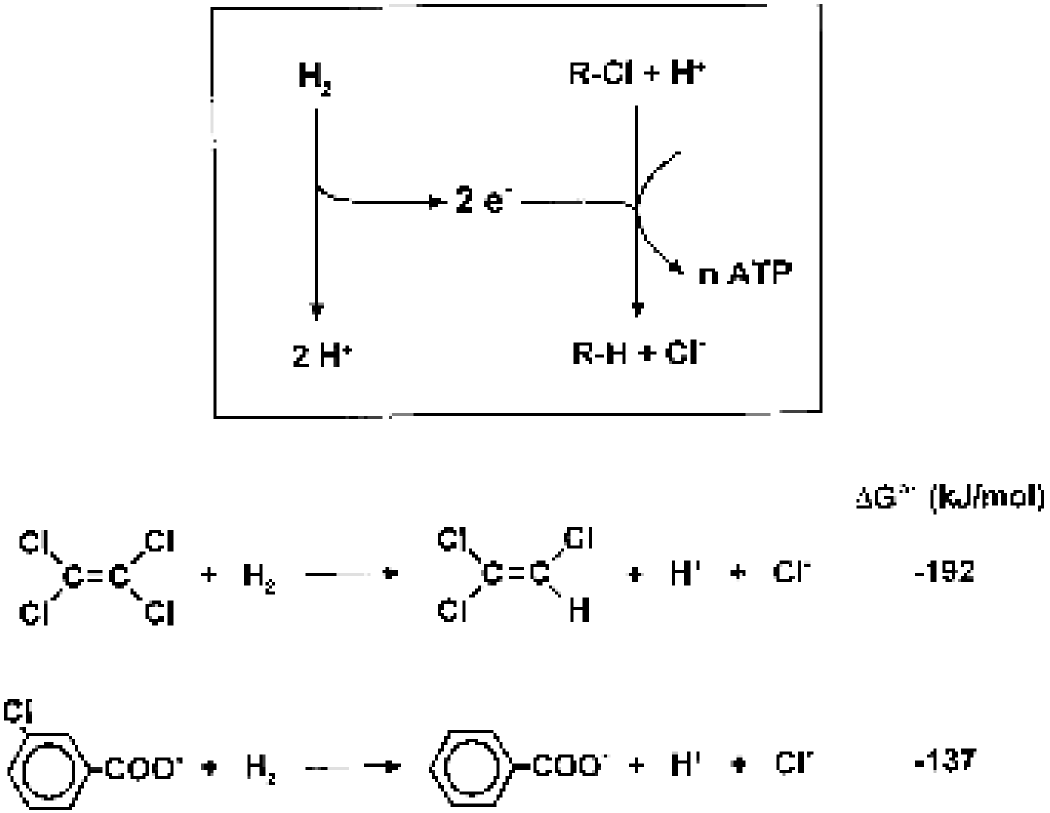


Fig. 1. Scheme of dehalorespiration with H_2 as electron donor and tetrachloroethene or 3-chlorobenzoate as electron acceptor.

Known dehalorespiring organisms

All belong to Bacteria

Many are related to SRB

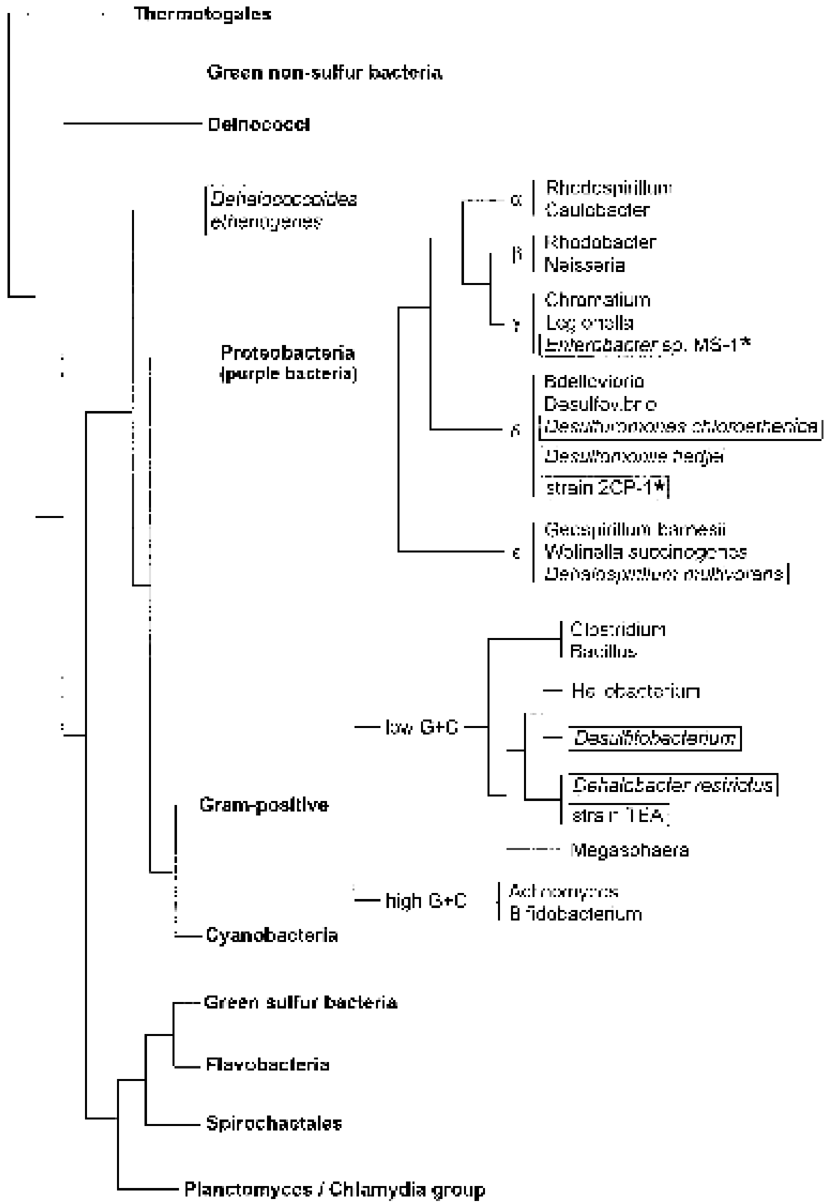
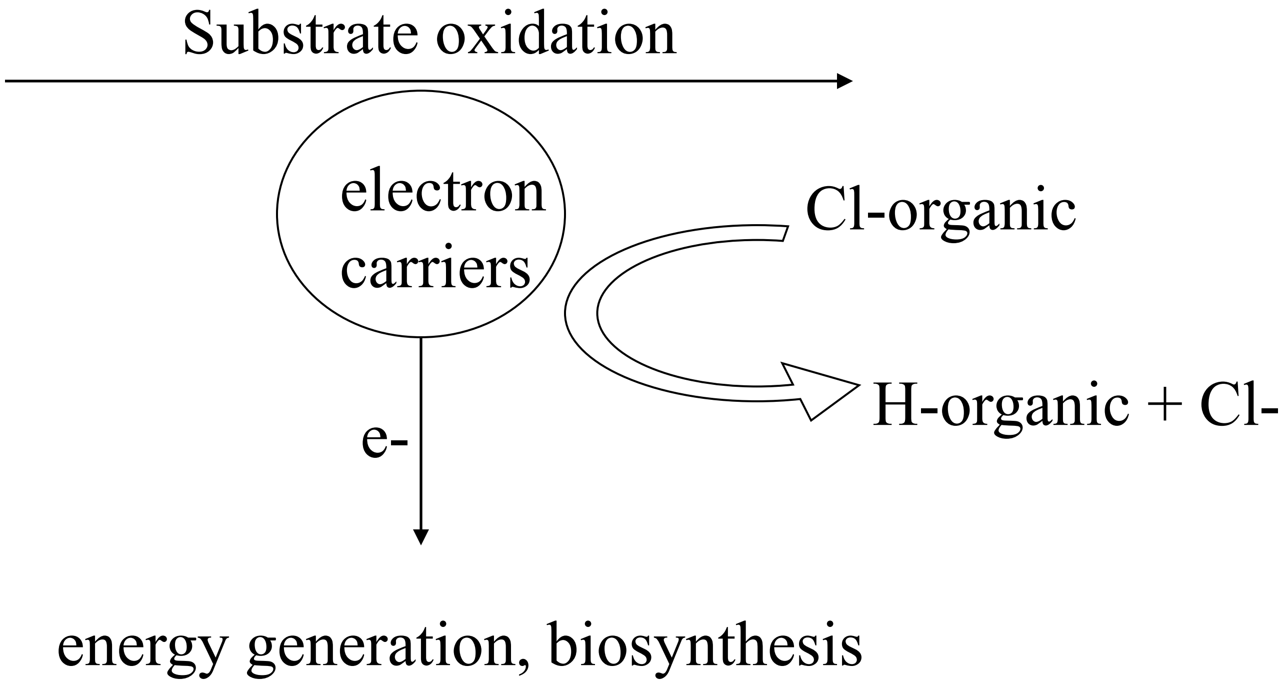


Fig. 2. Phylogenetic affiliations, based on analysis of 16S ribosomal RNA gene sequences, of bacteria capable of reductive dechlorination (framed). The facultative anaerobes are marked with an asterisk.

Degradation via use as e- acceptor: Transformations not supporting growth

Interaction with redox active cofactors



Substrate oxidation

electron carriers

Cl-organic

H-organic + Cl-

e-

energy generation, biosynthesis

Redox active cofactors in Type II cometabolism

Compound serves as electron acceptor

Energy is not conserved, does not support growth

Incidental contact of oxidized Cl-organic with a reduced e- transfer molecule

Growth substrate has no direct affect on the occurance or rate of transformation

No specific inducer is involved = Type II cometabolism

Types of reductive dehalogenation reactions

Two categories: are hydrogenolysis and vicinal reduction

Hydrogenolysis:
displacement of a single chlorine atom by hydrogen.

occurs with both aryl and alkyl compounds.

Vicinal reduction:
displacement of two chlorine atoms from two adjacent carbon atoms and formation of a carbon-carbon double bond.

occurs only with alkyl compounds.

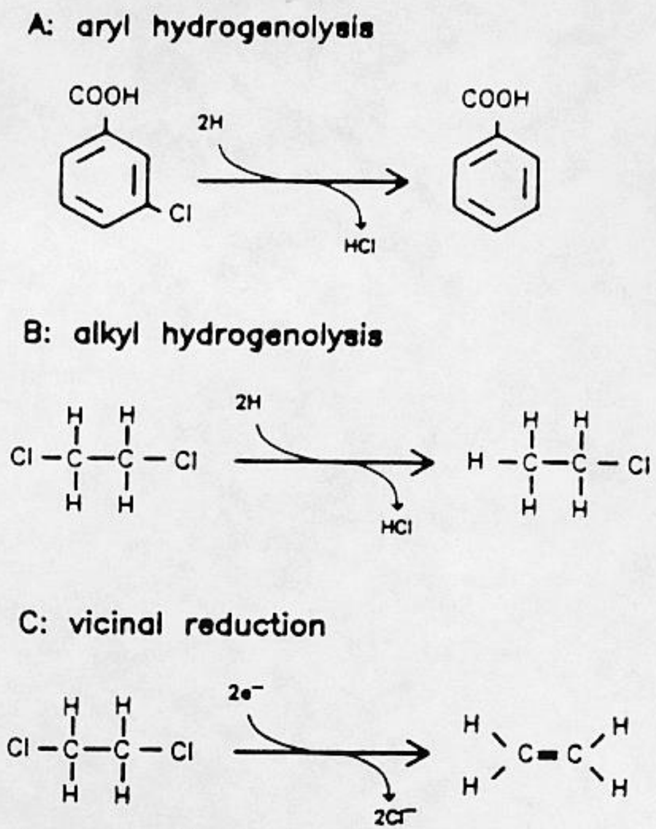


FIG. 1. Examples of reductive dehalogenation. (A) Aryl hydrogenolysis of 3-chlorobenzoate to benzoate; (B) alkyl hydrogenolysis of 1,2-dichloroethane to chloroethane; (C) vicinal reduction of 1,2-dichloroethane to ethene.

Reductases in dehalorespiration

Proteins mediating the final stage of electron transfer specifically induced

May be integrated into membranes or peripherally associated with these

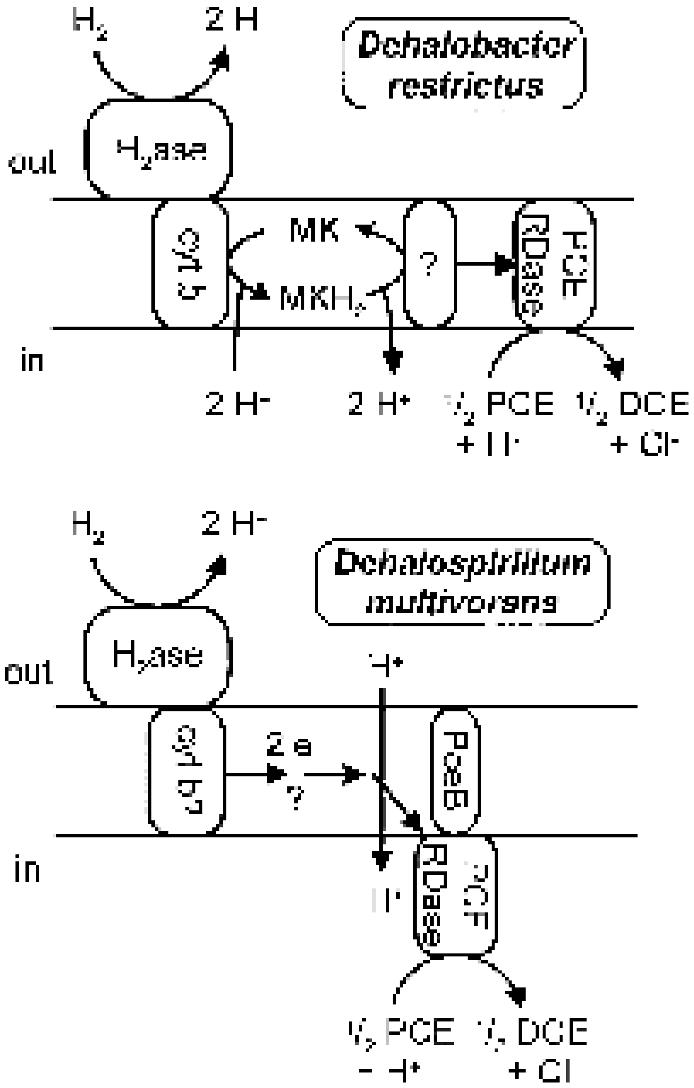


Fig. 4. Tentative scheme of dehalorespiration in *Dehalobacter restrictus* and *Dehalospirillum multivorans*. MK(H_2), menaquinone in the oxidized or reduced state; cyt b, cytochrome *b*; PCE RDase, PCE reductive dehalogenase; PceB, membrane anchor protein.

Types of organisms mediating alkyl reductive dehalogenation

<u>Physiologically diverse</u>

Eucarya, Bacteria, Archaea

aerobes, anaerobes, fac. anaerobes

Activity identified in many culture collection organisms

(isolation not associated with ability to dechlorination)

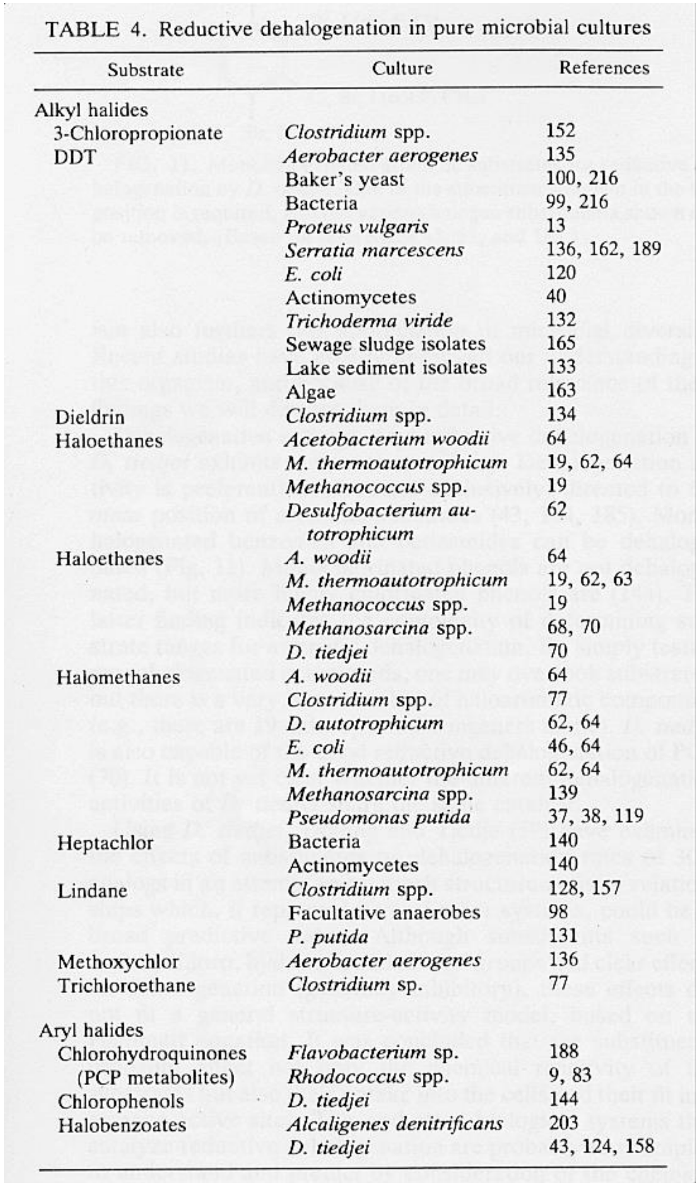


TABLE 4. Reductive dehalogenation in pure microbial cultures

Substrate	Culture	References
Alkyl halides		
3-Chloropropionate	*Clostridium* spp.	152
DDT	*Aerobacter aerogenes*	135
	Baker's yeast	100, 216
	Bacteria	99, 216
	Proteus vulgaris	13
	Serratia marcescens	136, 162, 189
	E. coli	120
	Actinomycetes	40
	Trichoderma viride	132
	Sewage sludge isolates	165
	Lake sediment isolates	133
	Algae	163
Dieldrin	*Clostridium* spp.	134
Haloethanes	*Acetobacterium woodii*	64
	M. thermoautotrophicum	19, 62, 64
	Methanococcus spp.	19
	Desulfobacterium autotrophicum	62
Haloethenes	*A. woodii*	64
	M. thermoautotrophicum	19, 62, 63
	Methanococcus spp.	19
	Methanosarcina spp.	68, 70
	D. tiedjei	70
Halomethanes	*A. woodii*	64
	Clostridium spp.	77
	D. autotrophicum	62, 64
	E. coli	46, 64
	M. thermoautotrophicum	62, 64
	Methanosarcina spp.	139
	Pseudomonas putida	37, 38, 119
Heptachlor	Bacteria	140
	Actinomycetes	140
Lindane	*Clostridium* spp.	128, 157
	Facultative anaerobes	98
	P. putida	131
Methoxychlor	*Aerobacter aerogenes*	136
Trichloroethane	*Clostridium* sp.	77
Aryl halides		
Chlorohydroquinones (PCP metabolites)	*Flavobacterium* sp.	188
	Rhodococcus spp.	9, 83
Chlorophenols	*D. tiedjei*	144
Halobenzoates	*Alcaligenes denitrificans*	203
	D. tiedjei	43, 124, 158

Reductive dechlorination of organochlorine pesticides

Reductive dechlorination affects fate, behavior, toxicity

Occurs commonly in soils usually under anaerobic conditions

Possibly a result of Type II cometabolism

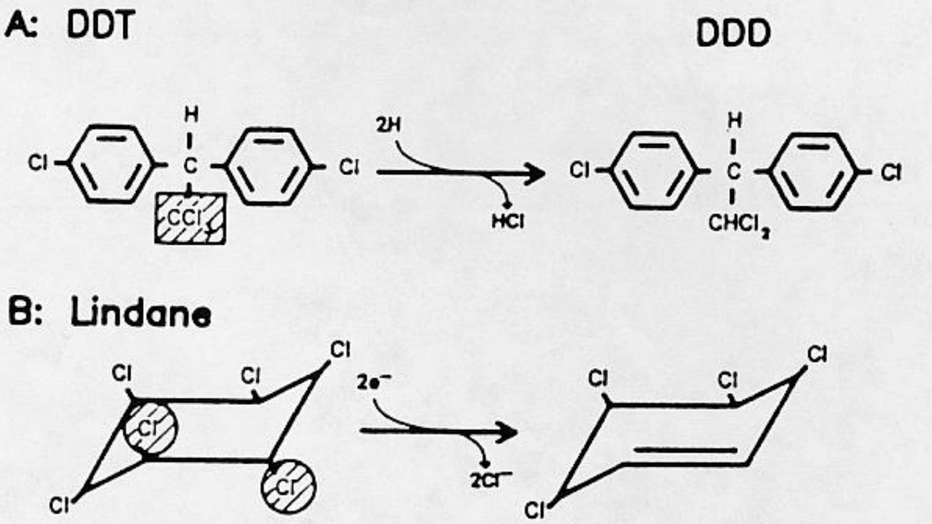


FIG. 2. Alkyl reductive dehalogenation of organochlorine pesticides. (A) Hydrogenolysis of DDT to DDD; (B) Vicinal reduction of lindane to γ-3,4,5,6-tetrachloro-1-cyclohexene. (Based on reference 98.)

BIODEGRADAZIONE

Trasformazione strutturale di un composto organico realizzata da reazioni effettuate da microorganismi

Biodegradazione primaria o funzionale

↓

altri composti organici (in genere"più piccoli")

Biodegradazione totale o mineralizzazione

↓

CO_2, NH_3, PO_4^{--}, H_2O, SO_4^{--}

Detossificazione

1) **La biodegradazione primaria dovrebbe portare direttamente a composti meno tossici di quelli iniziali.**
2) **La biodegradazione primaria può portare a prodotti più tossici, ma meno stabili e più facilmente degradabili.**

Fattori limitanti le biotrasformazioni di xenobiotici:

- permeabilità delle membrane cellulari
- presenza di enzimi in grado di trasformare il composto
- crescita della popolazione di microrganismi in risposta alla presenza della sostanza inorganica

ENZIMI: a) COSTITUTIVI – già presenti
b) INDUCIBILI – si attivano per la sostanza

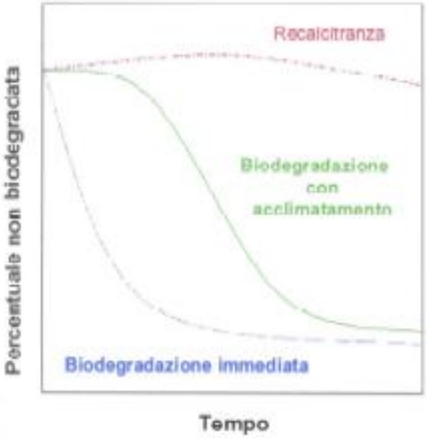
La situazione di inducibilità degli enzimi determina un periodo di LAG (acclimatamento) durante il quale la biodegradazione non avviene o è minima.

3 diverse situazioni di BIODEGRADABILITA':

Tipo di Risposta:

BIODEGRAD.: SI o NO

Velocità di biodegradazione



---- biodegradazione immediata ad opera di enzimi costitutivi

—— biodegradazione con acclimatamento (periodo di lag) per induzione degli enzimi, seguito da degradazione (*)

-.-.- recalcitranza alla biodegradazione. Prodotto pochissimo o per nulla biodegradabile

Principali biotrasformazioni che avvengono nelle biodegradazioni:

- **OSSIDAZIONI** – con forma elettrofila dell'O

- **RIDUZIONI** – con forma nucleofila dell'H (H^-) o cessione diretta di e^-

- **IDROLISI** – con attacco nucleofilo di H_2O

Con queste reazioni lo xenobiotico viene trasformato in composti strutturalmente più simili ai composti usualmente metabolizzati dal microorganismo, il quale poi li degrada nel suo normale ciclo metabolico.

OSSIDAZIONI

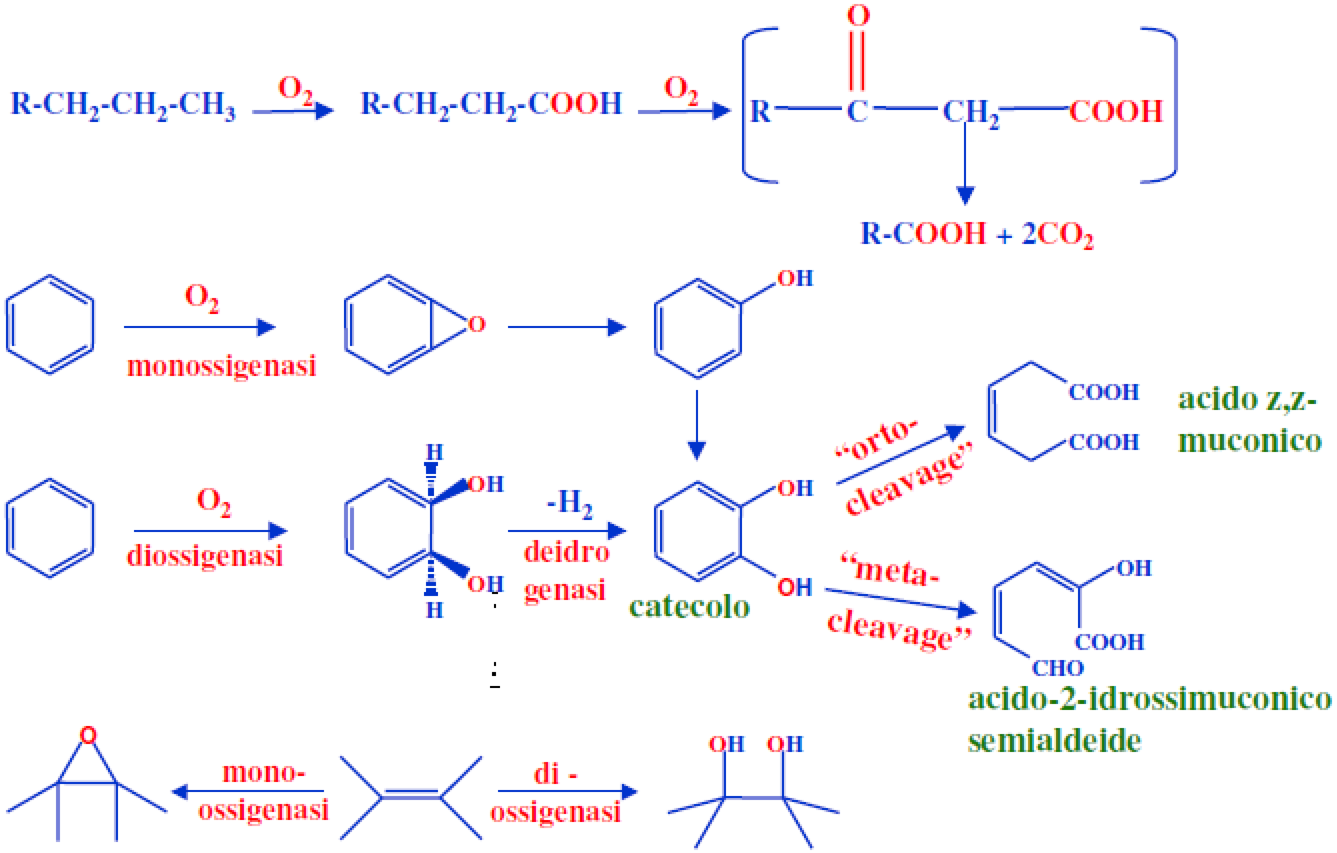
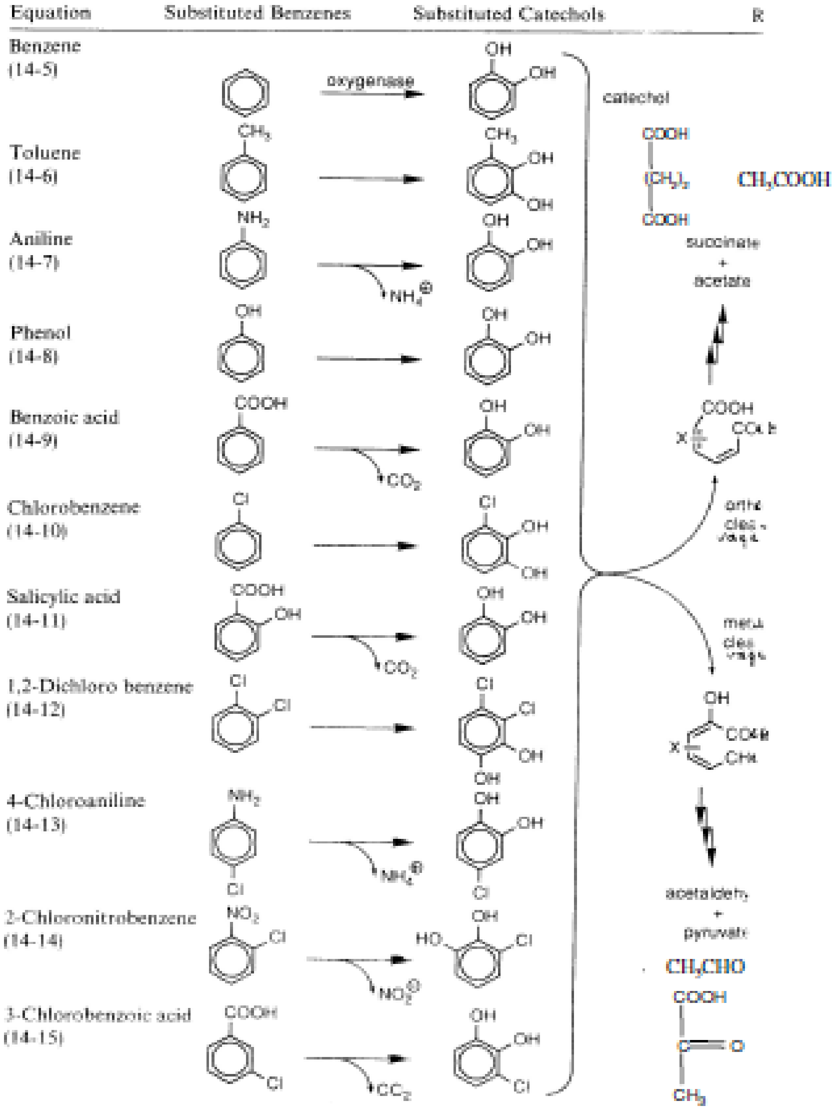
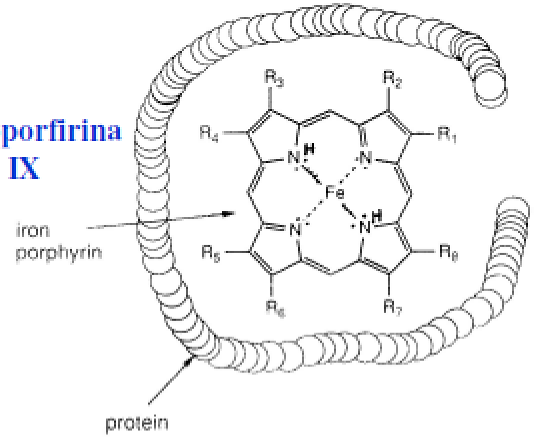


TABLE 14.1 Aerobic Degradation of Substituted Benzenes Proceeding through Ca Catechol-like Intermediates

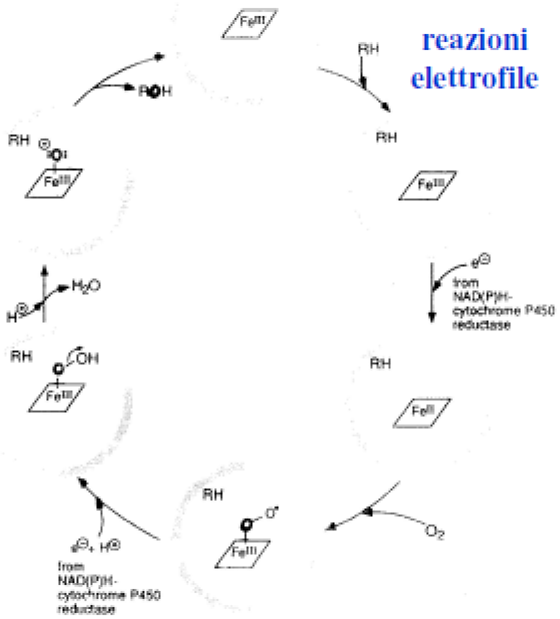


Citocromo P450 monoossigenasi

Protoporfirina IX

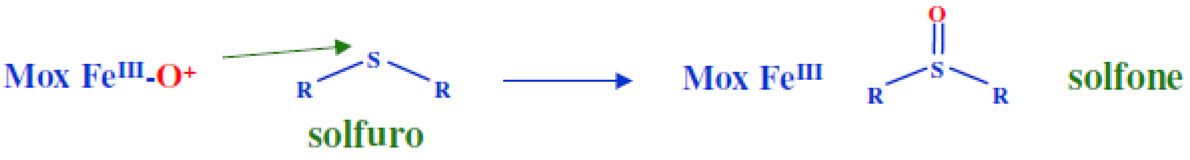


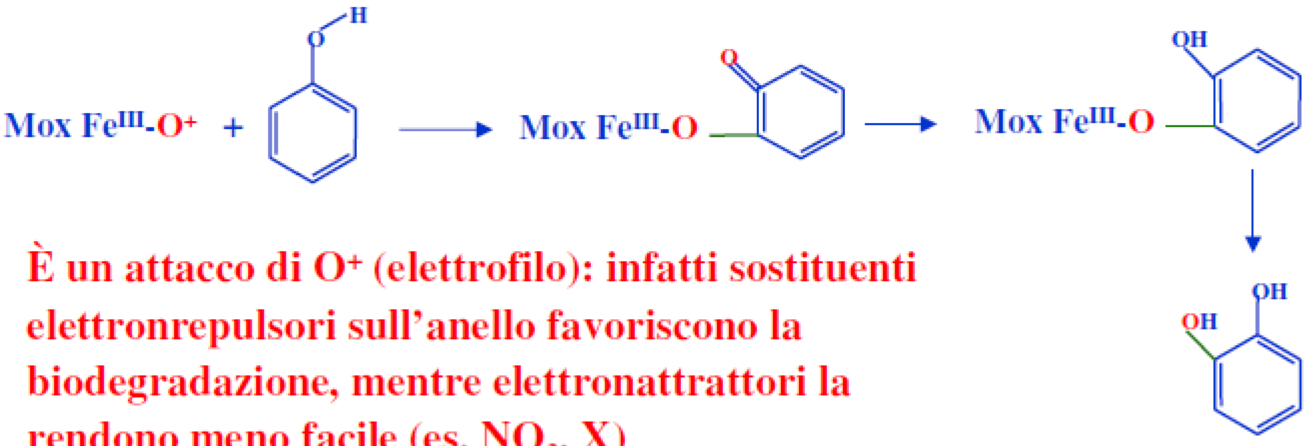
reazioni elettrofile



$$RH + O_2 + NAD(P)H + H^+ \longrightarrow ROH + H_2O + NAD(P)^+$$

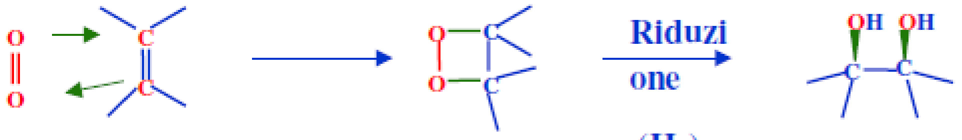
Monoossigenasi





È un attacco di O$^+$ (elettrofilo): infatti sostituenti elettronrepulsori sull'anello favoriscono la biodegradazione, mentre elettronattrattori la rendono meno facile (es. NO_2, X)

Dioossigenasi

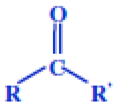


Riduzione

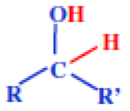
(H_2)

diolo cis

RIDUZIONI

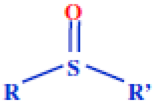


carbonili →



alcoli

$R\text{-}NO_2$ nitrocomposti → $R\text{-}NH_2$ ammine



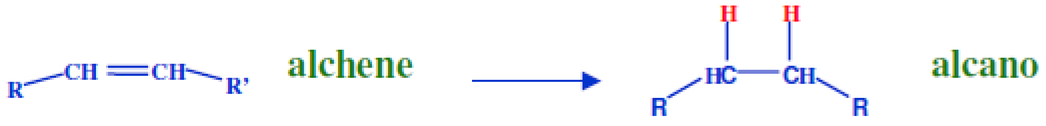
solfone →

solfuro →

RSH
e
R’SH

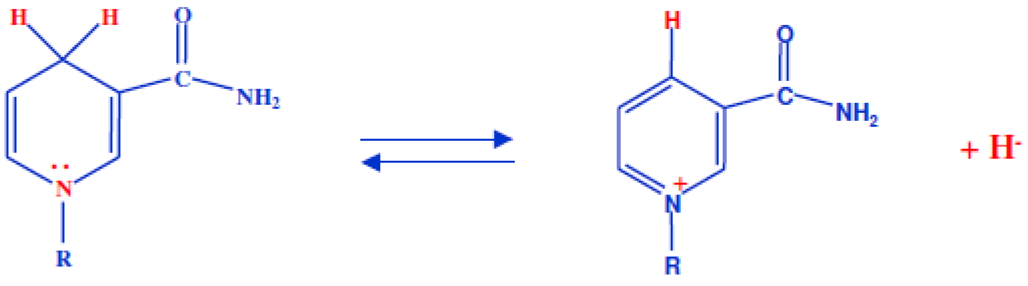
tioli

RIDUZIONI



Enzima: deidrogenasi

Coenzima: NAD(P)H – nicotinammide adenindinucleotide

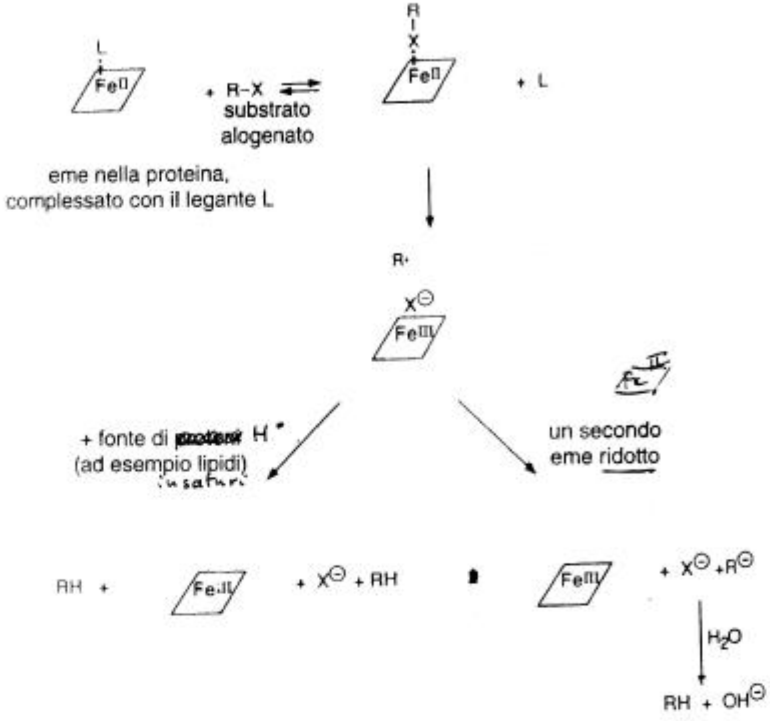


Riduzioni con citocromi (eme-proteine)

Dealogenazioni: $CCl_4 \longrightarrow CHCl_3$

$BrCH_2CH_2Br \rightarrow CH_2{=}CH_2$

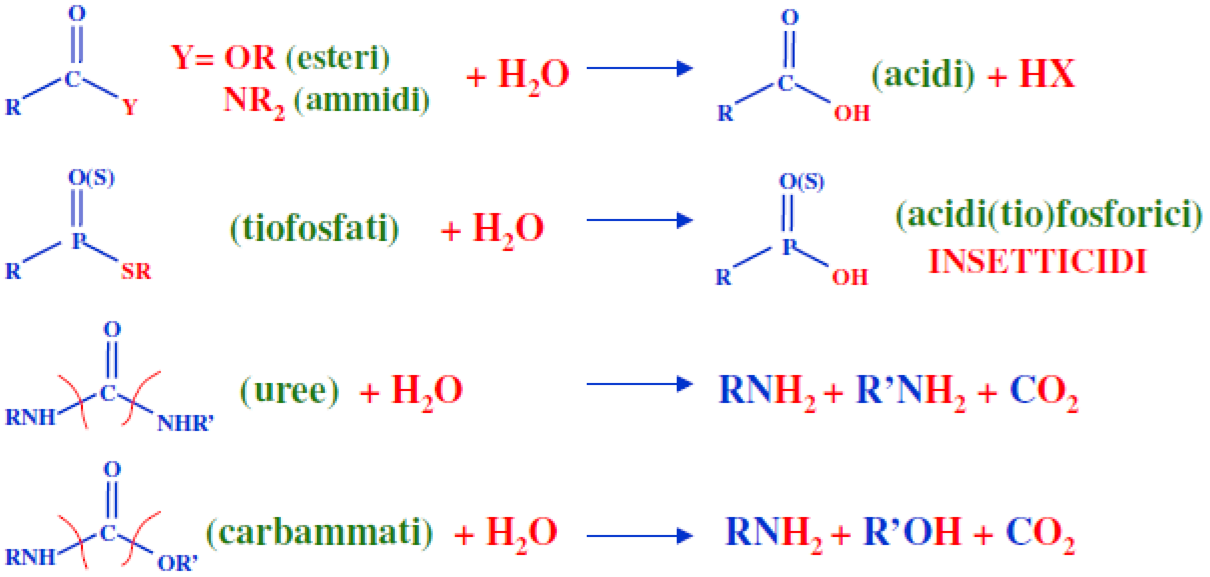
$R\text{-}X \longrightarrow R\text{-}H$



IDROLISI

- Prevalentemente in acqua, ma anche nel suolo
- Le idrolasi sono enzimi costitutivi quindi, in genere, le biodegradazioni idrolitiche sono facili e veloci

R-X (alogenuro alchilico) + H_2O → R-OH (alcool) + HX



Y= OR (esteri)
NR$_2$ (ammidi) + H_2O → (acidi) + HX

(tiofosfati) + H_2O → (acidi(tio)fosforici)
INSETTICIDI

(uree) + H_2O → $RNH_2 + R'NH_2 + CO_2$

(carbammati) + H_2O → $RNH_2 + R'OH + CO_2$

Factors affecting rates of biodegradation in soil

Factors affecting rates of biodegradation in soil

Environmental

- **Temperature, moisture, pH, etc.**

Microbial

- **Acclimation of a population of degraders**

Chemical-Microbial Interactions

- **Levels of chemical (high, toxicity; low, subthreshold)**

- **Chemical structure (aerobic vs. anaerobic degradation)**

Environmental-Chemical Interactions

- **Bioavailability**

Acclimation in “History Soils”

Soils with a history of use of a pesticide may exhibit accelerated degradation of this compound or related compounds

Reflects enrichment of microbes that grow on the pesticide

History effect

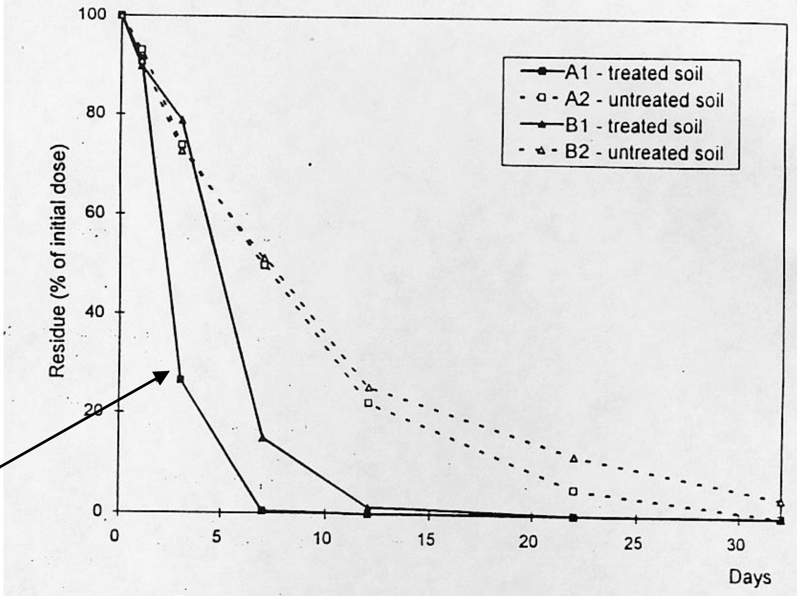


Fig. 1. Rates of carbofuran dissipation in treated and untreated soils.

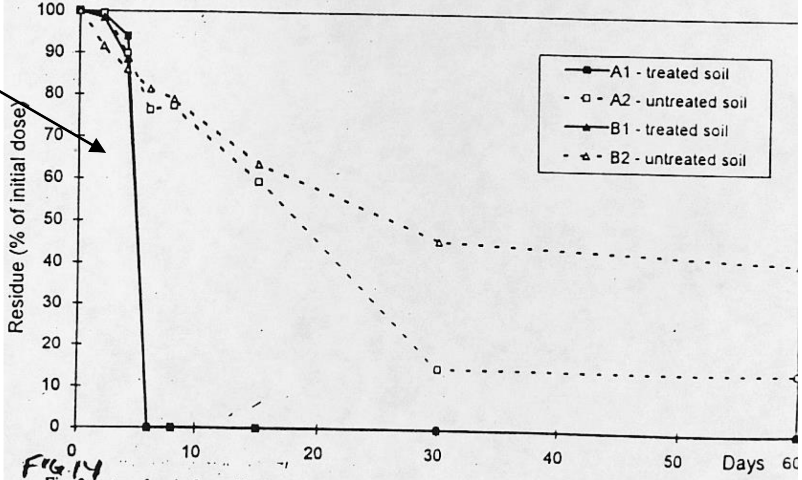


FIG. 14 ~~Fig.~~ 2. rates of carbofuran biodegradation at 30°C in mineral medium M9 inoculated with treated and

Abundance/activity of degraders affects apparent length of acclimation phase

Degrader population initially low and/or inactive (non-induced)

Initial exposure to substrate allows populationgrowth

Supports more rapid degradation of subsequent introduction of substrate

Apparent acclimation phase is shortened or absent

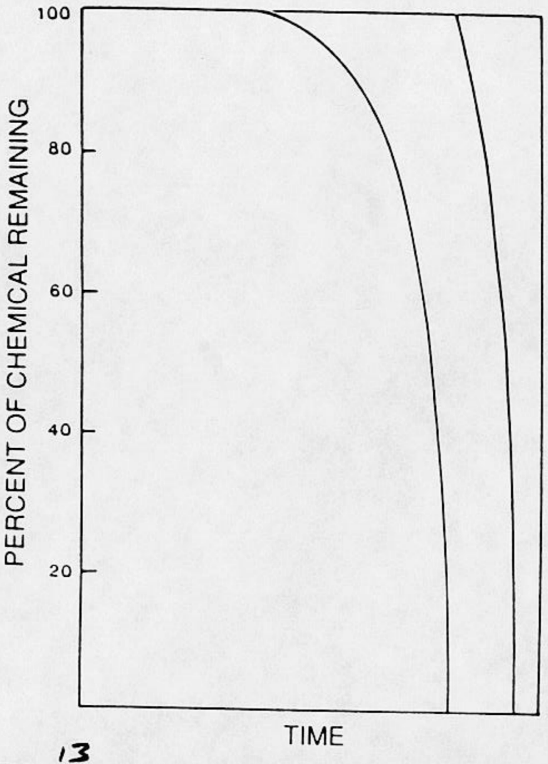


FIG. 13 Acclimation phase preceding the microbial destruction of an organic chemical after its first addition, and the absence of a delay period and rapid metabolism of the chemical following its second addition.

Acclimation of degrader populations in soil

Defination

The period preceding the on-set of biodegradation, which the chemical is not degraded, and after which biodegradation is rapid

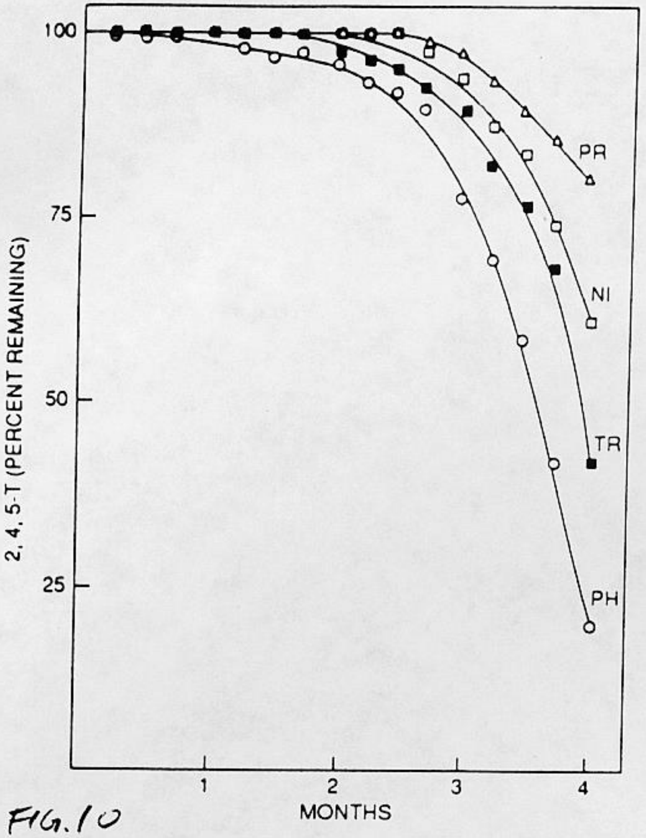


FIG. 10

Disappearance of 2,4,5-T in soil from the Philippines (PH), Trinidad (TR), Nigeria (NI) and Puerto Rico (PR). (From Rosenberg and Alexander, 1980. Reprinted with permission from the American Chemical Society.)

Chemical & microbial factors in apparent acclimation periods

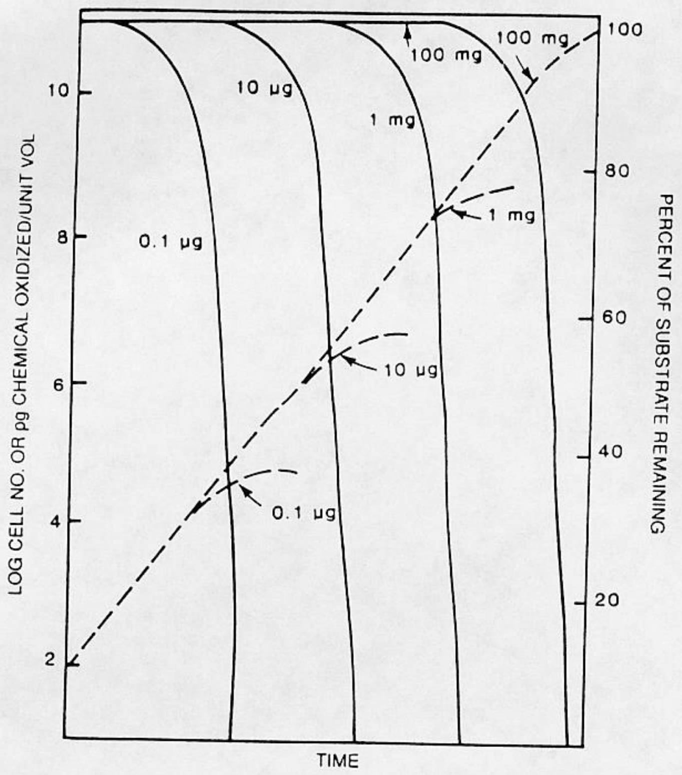
Chemical

Small amounts of degradation are more apparent with low substrate levels

Microbial

Substrate depletion is proportional to growth

At high substrate levels, small amounts of growth may not be detected = apparent acclimation

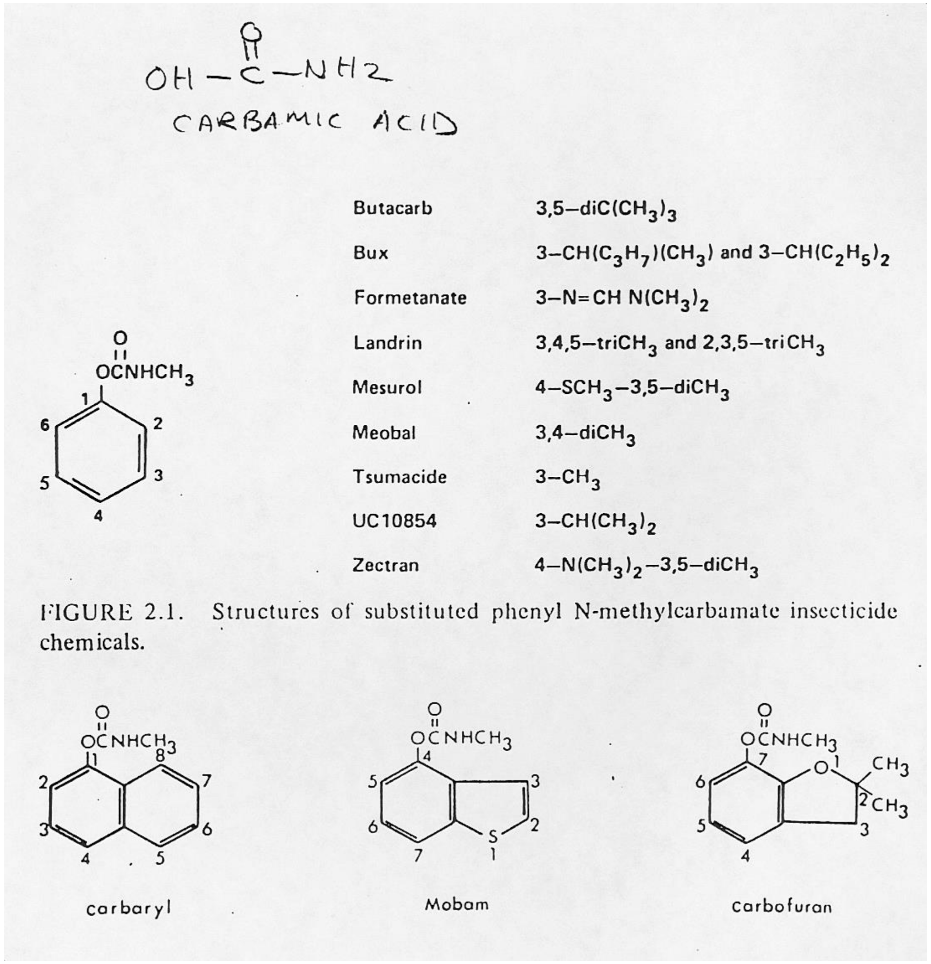


Decline in concentration of organic substrate (solid lines), increase in amount of chemical oxidized (or product formed) on a logarithmic scale (dashed lines), and increase in logarithm of cell density (also dashed lines) per unit volume. The estimates shown are based on the assumptions that the bacteria are growing exponentially by using the substrate as the sole C source, that each cell consumes 1 pg of substrate and forms ca. 1 pg of CO_2 as it multiplies, and that the rate of growth does not change markedly as the substrate concentration declines.

Carbamate History Soils

Carbamates are a widely used class of insecticide

All are variations of a core chemical structure



$$OH-\overset{O}{\overset{\|}{C}}-NH_2$$

CARBAMIC ACID

Butacarb	3,5–di$C(CH_3)_3$
Bux	3–$CH(C_3H_7)(CH_3)$ and 3–$CH(C_2H_5)_2$
Formetanate	3–$N{=}CH\ N(CH_3)_2$
Landrin	3,4,5–triCH_3 and 2,3,5–triCH_3
Mesurol	4–SCH_3–3,5–diCH_3
Meobal	3,4–diCH_3
Tsumacide	3–CH_3
UC10854	3–$CH(CH_3)_2$
Zectran	4–$N(CH_3)_2$–3,5–diCH_3

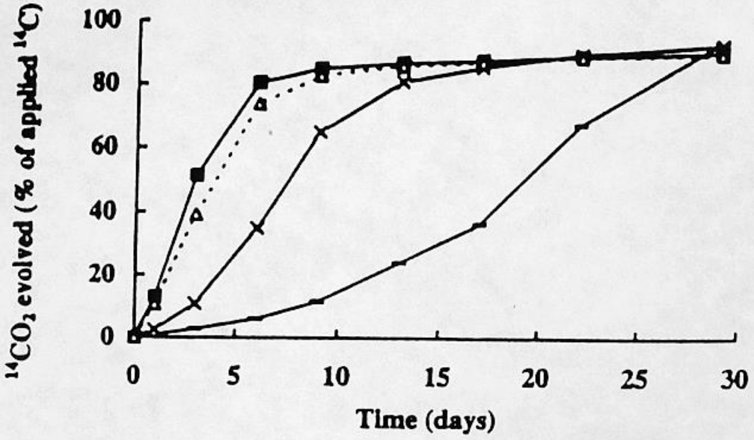
FIGURE 2.1. Structures of substituted phenyl N-methylcarbamate insecticide chemicals.

carbaryl

Mobam

carbofuran

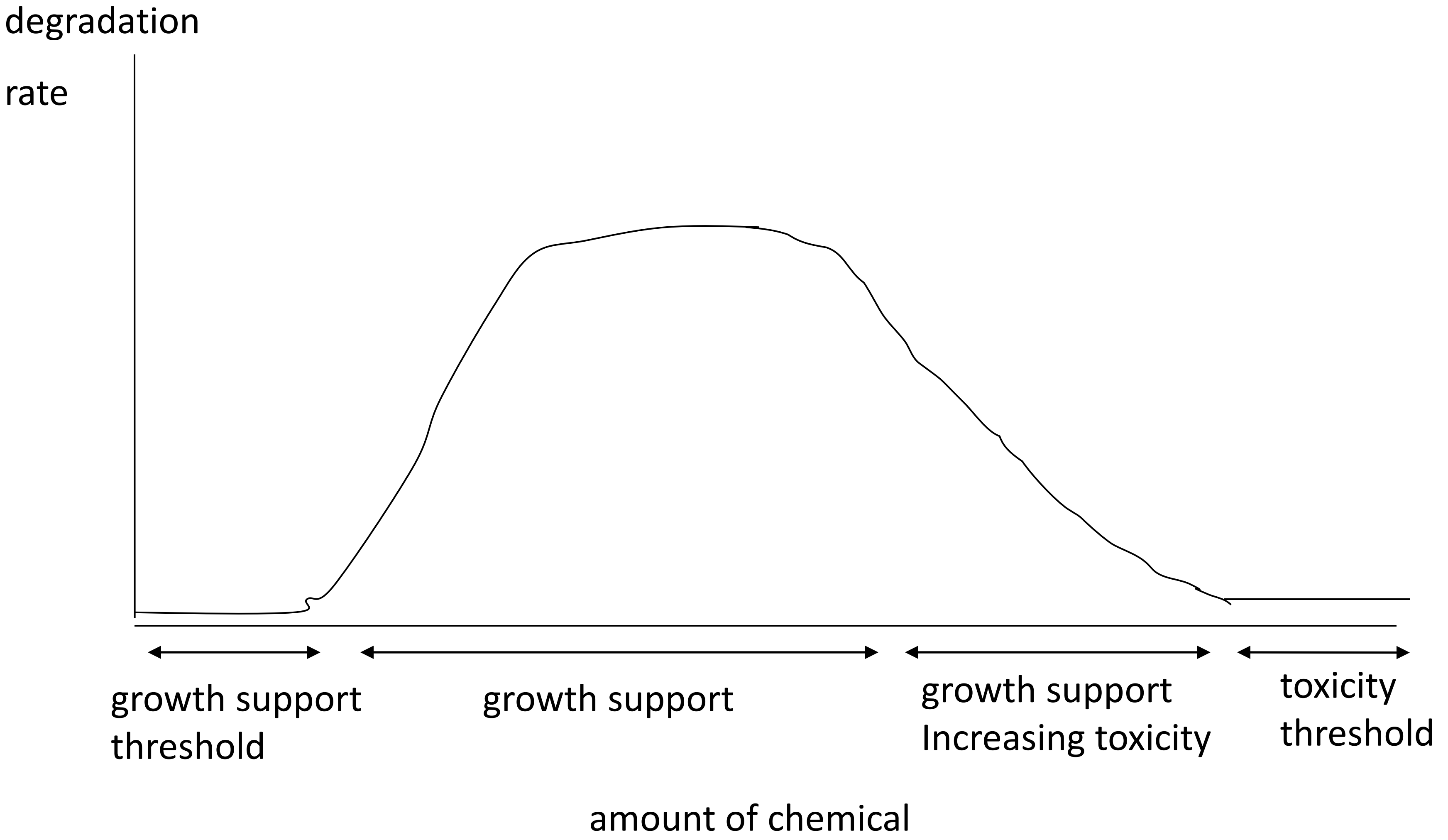
Accelerated degradation of carbamates in history soils



~~Fig.~~ 1. Degradation of [^{14}C-carbonyl]carbofuran in previously untreated Montardon soil (-); Montardon soil treated with carbaryl (×), carbofuran (Δ) or isoprocarb (■). Symbols indicate mean cumulative $^{14}CO_2$ evolved from replicate samples.

Prior exposure to one carbamate acclimates the community to degrade other carbamates

Effects of chemical concentration



Threshold

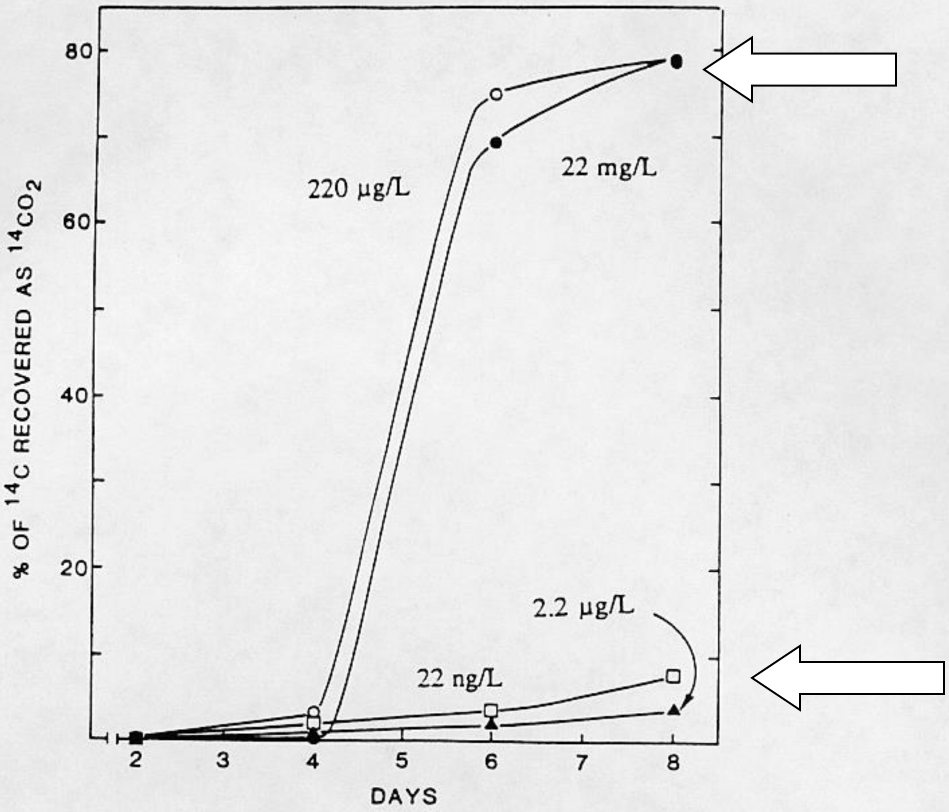


FIG. 7.. Mineralization of 2,4-D added at several concentrations to river water. (From Boethling and Alexander, 1979a. Reprinted with permission from the American Society for Microbiology.)

Growing, increase in biomass,

increasing degradation rate

Non-growing, no change in biomass,

constant degradation rate

Tossicita’

Toxicity of Organic Compounds

Membrane damage: Solvent effect associated with hydrophobic chemicals (hydrocarbons, chlorinated hydrocarbons)

compounds partition into membranes

membranes are disrupted

electrochemical gradients are dissipated

Physiological Disruption: Uncoupling e- transfer from ATP synthesis (chloro- and nitro-phenols)

carry protons across membrane, dissipate ΔpH

Damage to Macromolecules; Enzymes, DNA

Usually associated with reactive intermediates, not the original compound

TCE --Moase--> TCE-epoxide (reactive) ----> Protein/DNA binding

Solvent-effect toxicity: Hydrophobicity and water solubility

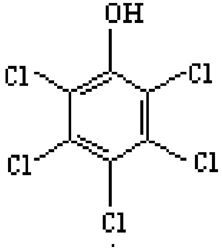
Relative toxicity related to hydrophobicity/water solubility

Increased hydrophob = inc.reased tendency to lodge in membranes, also = decreased water solubility = decreased exposure to microbes

Most toxic are hydrophobic compounds with significant aqueous solubility

Toxicity is dose (concentration)-dependent

PCP: biocidal, a wood treatment to supress microbial degradation

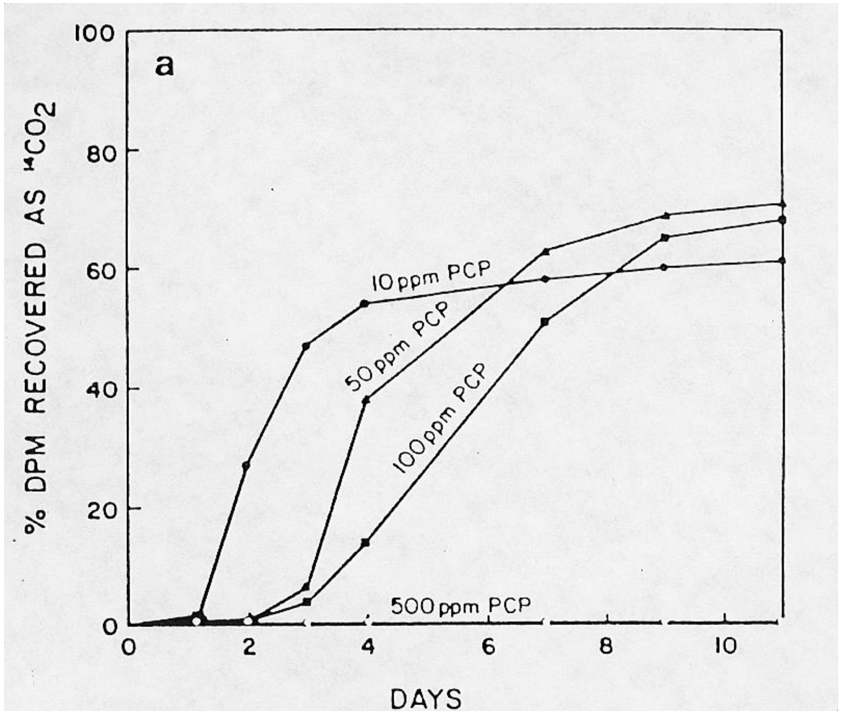


Pentachlorophenol (PCP)

Log(P) = 3.77

Low concentrations:a growth substrate (e- donor) for aerobes

High concentrations: toxicity



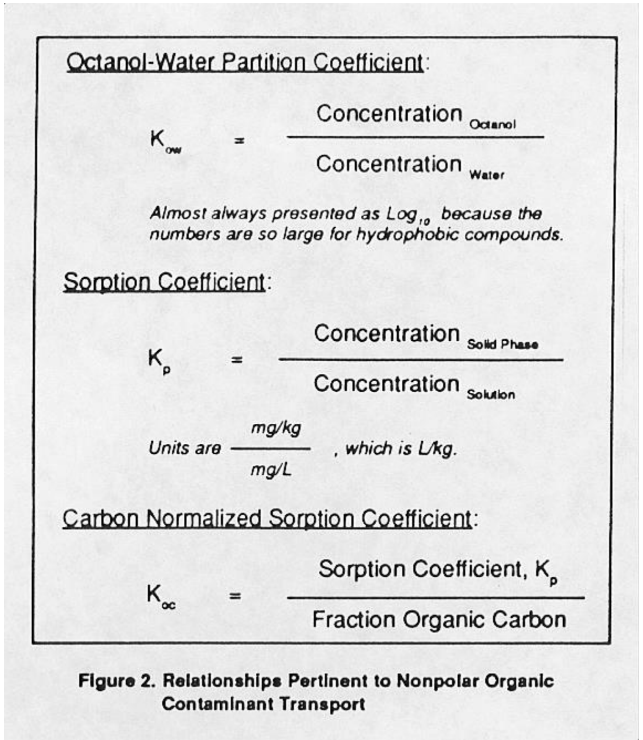
Solvent-effect toxicity: Relation to Kow

Hydrophobicity assayed by octanol-water partitioning (Kow)

Increasing hydrophobicity = increasing Kow

Values of Kow are large, expressed as a log, Log(P) = Log(Kow)

In general: Gm+ more tolerant than Gm-
Toxicity becomes significant at ca. Log(P) < 3



Octanol-Water Partition Coefficient:

$$K_{ow} = \frac{\text{Concentration}_{\text{Octanol}}}{\text{Concentration}_{\text{Water}}}$$

Almost always presented as Log_{10} because the numbers are so large for hydrophobic compounds.

Sorption Coefficient:

$$K_p = \frac{\text{Concentration}_{\text{Solid Phase}}}{\text{Concentration}_{\text{Solution}}}$$

Units are $\frac{mg/kg}{mg/L}$, which is L/kg.

Carbon Normalized Sorption Coefficient:

$$K_{oc} = \frac{\text{Sorption Coefficient, } K_p}{\text{Fraction Organic Carbon}}$$

Figure 2. Relationships Pertinent to Nonpolar Organic Contaminant Transport

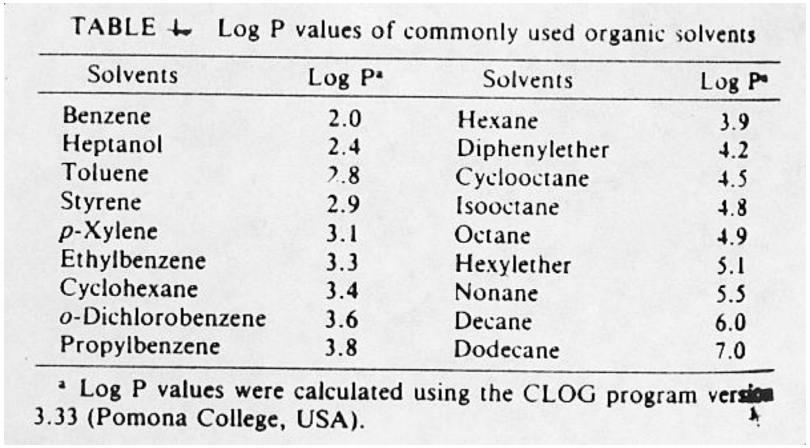


TABLE 1 Log P values of commonly used organic solvents

Solvents	Log P[a]	Solvents	Log P[a]
Benzene	2.0	Hexane	3.9
Heptanol	2.4	Diphenylether	4.2
Toluene	2.8	Cyclooctane	4.5
Styrene	2.9	Isooctane	4.8
p-Xylene	3.1	Octane	4.9
Ethylbenzene	3.3	Hexylether	5.1
Cyclohexane	3.4	Nonane	5.5
o-Dichlorobenzene	3.6	Decane	6.0
Propylbenzene	3.8	Dodecane	7.0

[a] Log P values were calculated using the CLOG program version 3.33 (Pomona College, USA).

Log(P) values

TCE = 2.42

PCE = 3.14

Solvent-effect toxicity in Bacteria

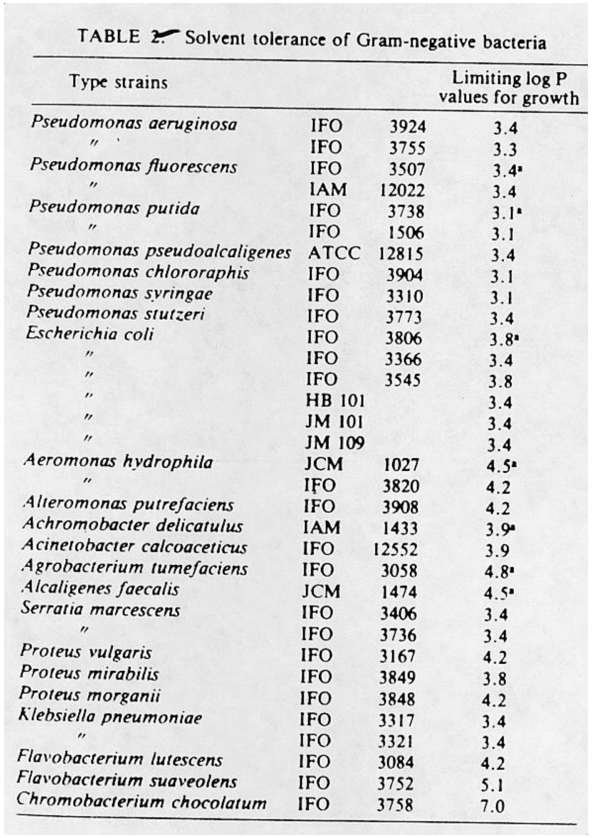


TABLE 2. Solvent tolerance of Gram-negative bacteria

Type strains			Limiting log P values for growth
Pseudomonas aeruginosa	IFO	3924	3.4
〃	IFO	3755	3.3
Pseudomonas fluorescens	IFO	3507	3.4[a]
〃	IAM	12022	3.4
Pseudomonas putida	IFO	3738	3.1[a]
〃	IFO	1506	3.1
Pseudomonas pseudoalcaligenes	ATCC	12815	3.4
Pseudomonas chlororaphis	IFO	3904	3.1
Pseudomonas syringae	IFO	3310	3.1
Pseudomonas stutzeri	IFO	3773	3.4
Escherichia coli	IFO	3806	3.8[a]
〃	IFO	3366	3.4
〃	IFO	3545	3.8
〃	HB 101		3.4
〃	JM 101		3.4
〃	JM 109		3.4
Aeromonas hydrophila	JCM	1027	4.5[a]
〃	IFO	3820	4.2
Alteromonas putrefaciens	IFO	3908	4.2
Achromobacter delicatulus	IAM	1433	3.9[a]
Acinetobacter calcoaceticus	IFO	12552	3.9
Agrobacterium tumefaciens	IFO	3058	4.8[a]
Alcaligenes faecalis	JCM	1474	4.5[a]
Serratia marcescens	IFO	3406	3.4
〃	IFO	3736	3.4
Proteus vulgaris	IFO	3167	4.2
Proteus mirabilis	IFO	3849	3.8
Proteus morganii	IFO	3848	4.2
Klebsiella pneumoniae	IFO	3317	3.4
〃	IFO	3321	3.4
Flavobacterium lutescens	IFO	3084	4.2
Flavobacterium suaveolens	IFO	3752	5.1
Chromobacterium chocolatum	IFO	3758	7.0

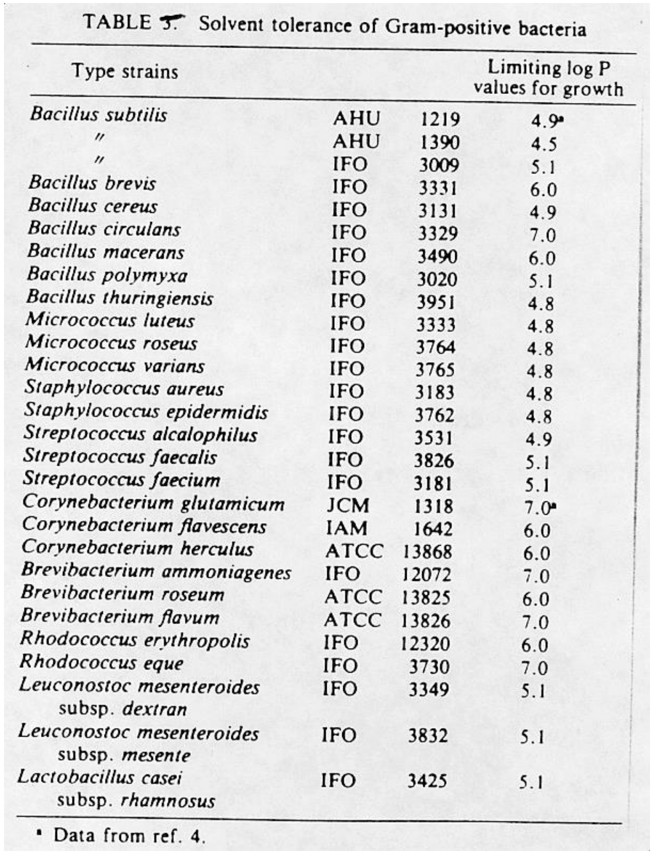


TABLE 3. Solvent tolerance of Gram-positive bacteria

Type strains			Limiting log P values for growth
Bacillus subtilis	AHU	1219	4.9[a]
〃	AHU	1390	4.5
〃	IFO	3009	5.1
Bacillus brevis	IFO	3331	6.0
Bacillus cereus	IFO	3131	4.9
Bacillus circulans	IFO	3329	7.0
Bacillus macerans	IFO	3490	6.0
Bacillus polymyxa	IFO	3020	5.1
Bacillus thuringiensis	IFO	3951	4.8
Micrococcus luteus	IFO	3333	4.8
Micrococcus roseus	IFO	3764	4.8
Micrococcus varians	IFO	3765	4.8
Staphylococcus aureus	IFO	3183	4.8
Staphylococcus epidermidis	IFO	3762	4.8
Streptococcus alcalophilus	IFO	3531	4.9
Streptococcus faecalis	IFO	3826	5.1
Streptococcus faecium	IFO	3181	5.1
Corynebacterium glutamicum	JCM	1318	7.0[a]
Corynebacterium flavescens	IAM	1642	6.0
Corynebacterium herculus	ATCC	13868	6.0
Brevibacterium ammoniagenes	IFO	12072	7.0
Brevibacterium roseum	ATCC	13825	6.0
Brevibacterium flavum	ATCC	13826	7.0
Rhodococcus erythropolis	IFO	12320	6.0
Rhodococcus eque	IFO	3730	7.0
Leuconostoc mesenteroides subsp. *dextran*	IFO	3349	5.1
Leuconostoc mesenteroides subsp. *mesente*	IFO	3832	5.1
Lactobacillus casei subsp. *rhamnosus*	IFO	3425	5.1

[a] Data from ref. 4.

Organisms vary in solvent tolerance, reflecting differences in cell surface or membrane composition

In general Gm+ more tolerant than Gm-

Biodisponibilita’

Bioavailability and residence time in soil

Definition: Occurrence of a compound in a state that is accessible to microbes

Bioavailable = dissolved in aqueous phase

Bioavailability (biodegradation potential) decrease with increasing residence time in soil

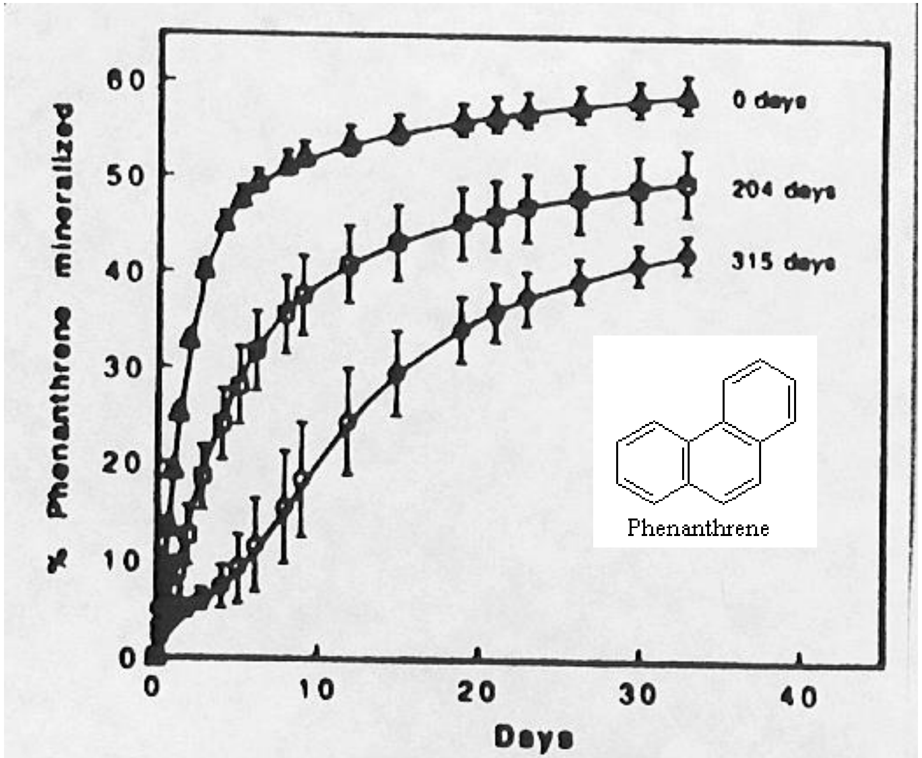
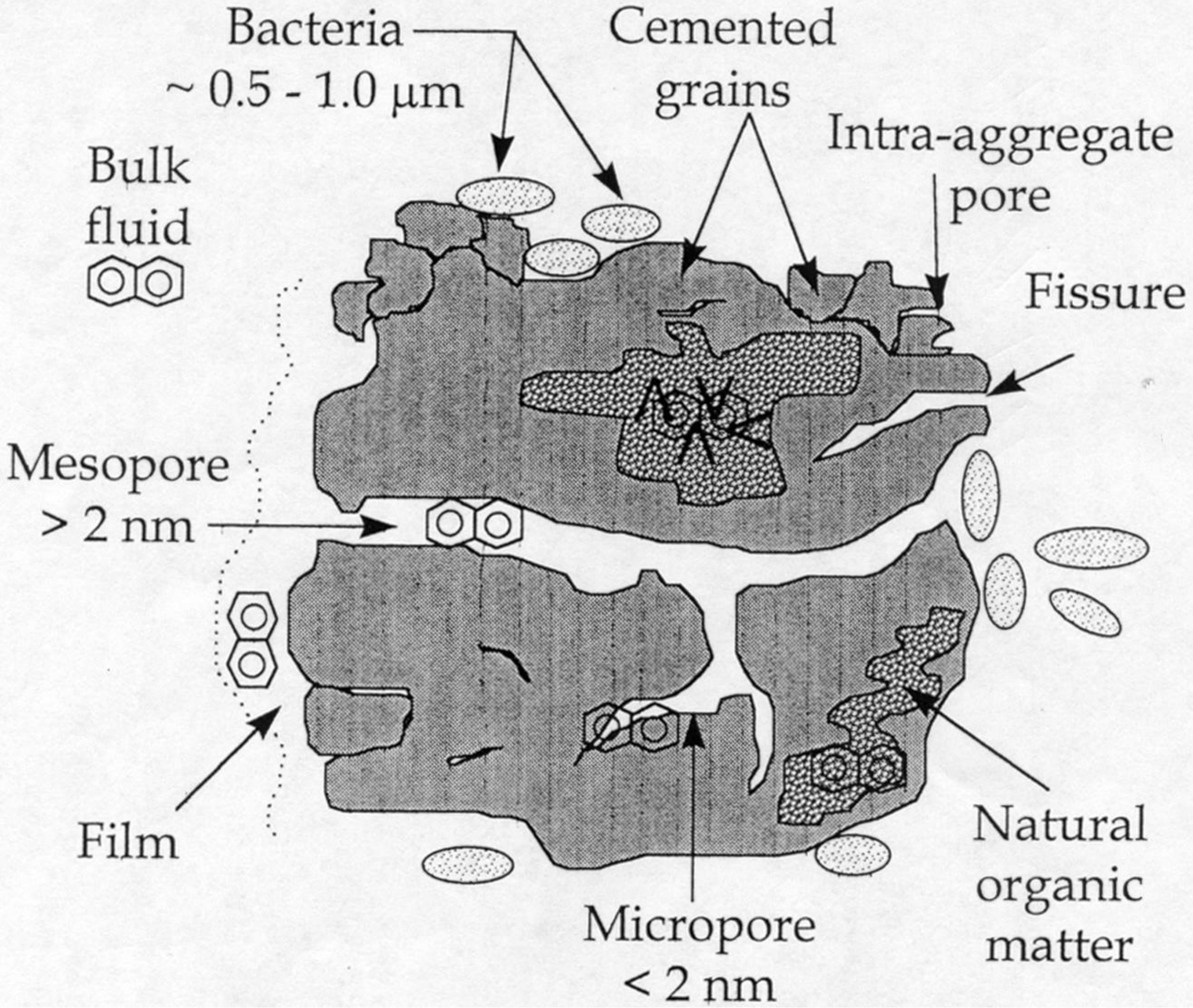
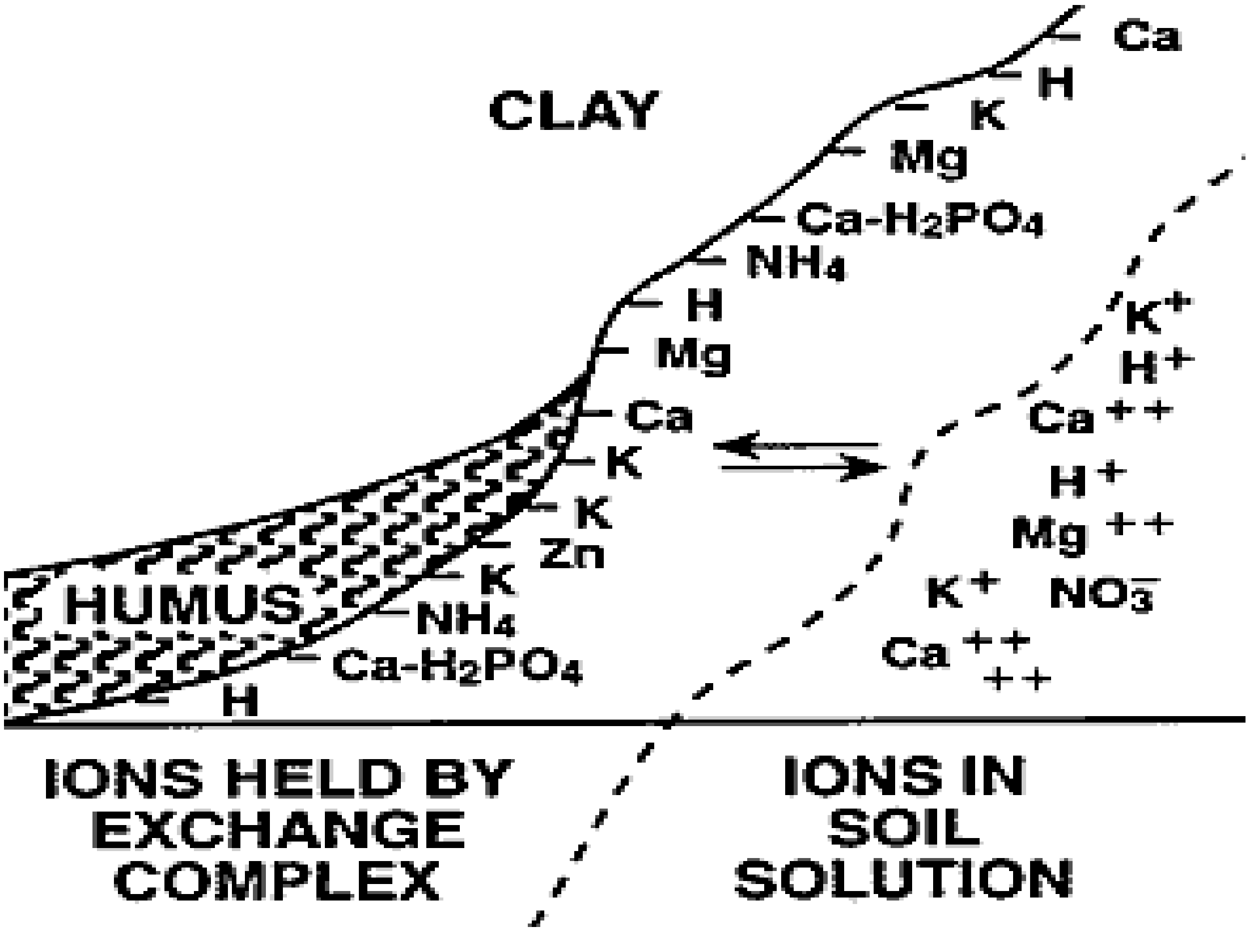


FIGURE 3. Mineralization by *Pseudomonas* strain R of phenanthrene at 10 µg/g aged for 0, 204, or 315 days in Edwards muck. The error bars represent the standard deviations of triplicates.

Soil Grain Sorption




CLAY
Ca
H
K
Mg
Ca-H2PO4
NH4
H
Mg
Ca
K
K
Zn
K
NH4
Ca-H2PO4
H
HUMUS
K+
H+
Ca++
H+
Mg++
K+
NO3−
Ca++
++
IONS HELD BY EXCHANGE COMPLEX
IONS IN SOIL SOLUTION

Terminologia

Assorbimento: indica la ritenzione di una specie chimica senza riferimento al meccanismo di ritenzione.

Adsorbimento: indica la ritenzione di uno ione o di una piccola molecola su di una superficie, con limitata interazione con altre specie chimiche adsorbite.

Absorbimento: indica la ritenzione di una specie chimica all'interno di un materiale (come la ritenzione di acqua da parte di una spugna).

Precipitazione di superficie: precipitazione su di una superficie che determina la crescita dimensionale della specie chimica che precipita. In questo caso c'è interazione tra le specie chimiche che si trovano sulla superficie della particella.

Precipitazione: genesi di una struttura tridimensionale senza interazione con una superficie.

Questa terminologia sottolinea che vi sono molteplici meccanismi capaci di allontanare un soluto dalla soluzione circolante del suolo.

Meccanismi di adsorbimento

Il meccanismo di adsorbimento può essere diviso in tre diverse classi:

1) Reazione chimica

Reazione chimica tra ione e superficie (lo ione perde il suo guscio di idratazione)
Adsorbimento specifico
Legame forte
Scambiabile

2) Reazione elettrostatica

Neutralizzazione di carica localizzata (lo ione non perde il suo guscio di idratazione)
Adsorbimento non specifico
Scambiabile

3) Attrazione elettrostatica

Attrazione elettrostatica delocalizzata (lo ione non perde il suo guscio di idratazione)
Adsorbimento non specifico
Scambiabile

Mechanisms in weathering of organic chemicals

Time-dependent entry of compound into a state or location that is inaccessible to microbes.

Nature of sites or states hypothesized to be
    small pores that restrict entry of cells
    regions within SOM that strongly retain the compound

Microbes can't enter aggregate

Access to compound requires:

1. desorption from sorbed (solid) phase
2. diffusion to reach cells

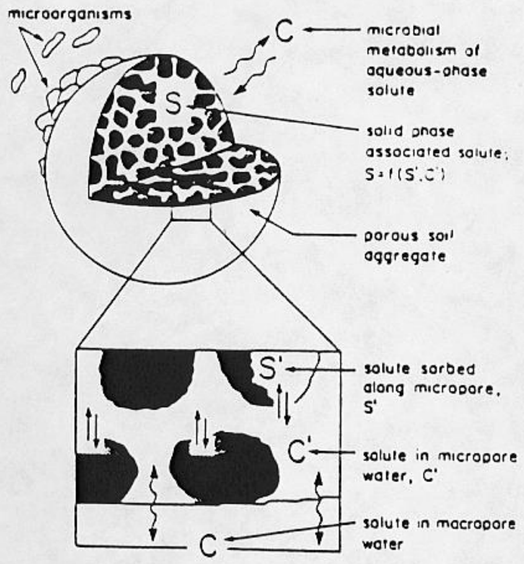
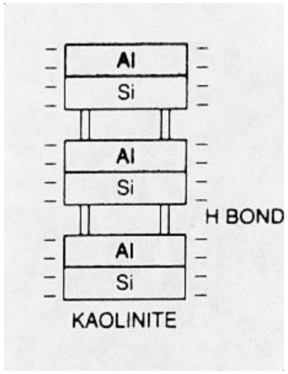


Figure 2.7. Depiction of the physical relationship of microorganisms to the structure of a soil aggregate containing an organic contaminant. Adapted with permission from Mihelcic & Luthy (1991). Copyright 1991, American Chemical Society.

Soil matrix components affecting weathering: Clays

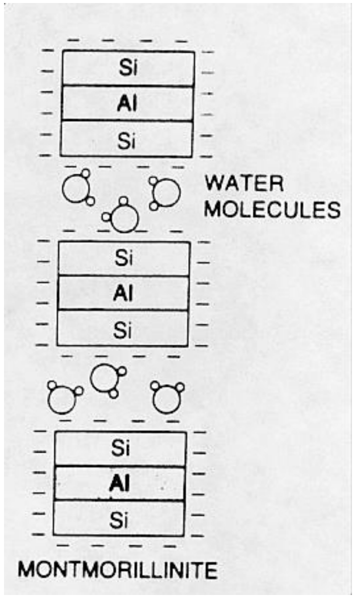
Clays (aluminosilicates); sorb ions, sorption greates by swelling types



Non-swelling clay:

Layers joined

Prevents entry of molecules

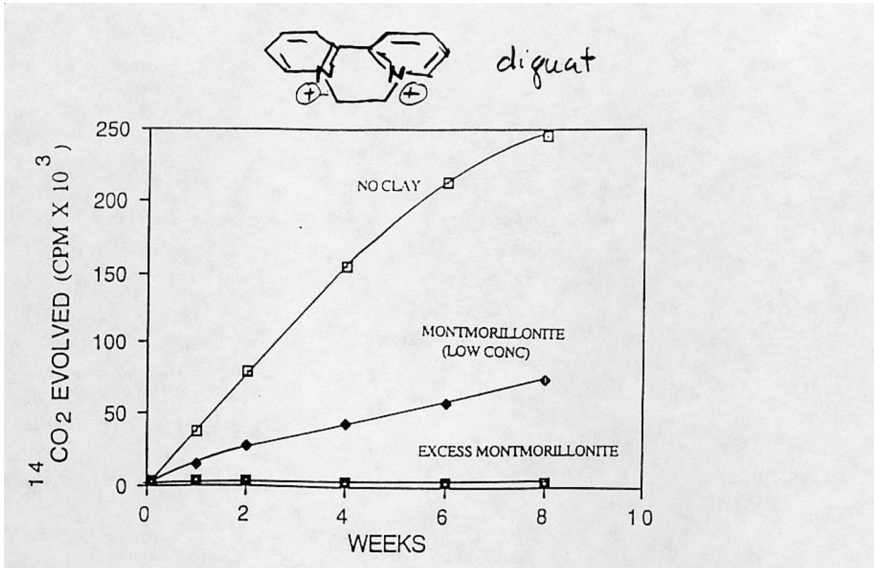


Swelling clay:

Layers not linked

Layers separated by H2O

Interlayers exposed



~~FIG. 8.1~~ Mineralization of ^{14}C-labeled diquat in nutrient solutions with or without clay. (Reprinted with permission from Weber and Coble, 1968.)

Entry and sorbtion to interlayer reduces availability for degradation

Non ionic Organic Contaminants Sorption to SOM

Postulated nature of sorption sites:

1. Hydrophobic clefts & voids within humics?

2. Highly condensed, rigid (glass-like) vs. flexible (rubber-like) regions

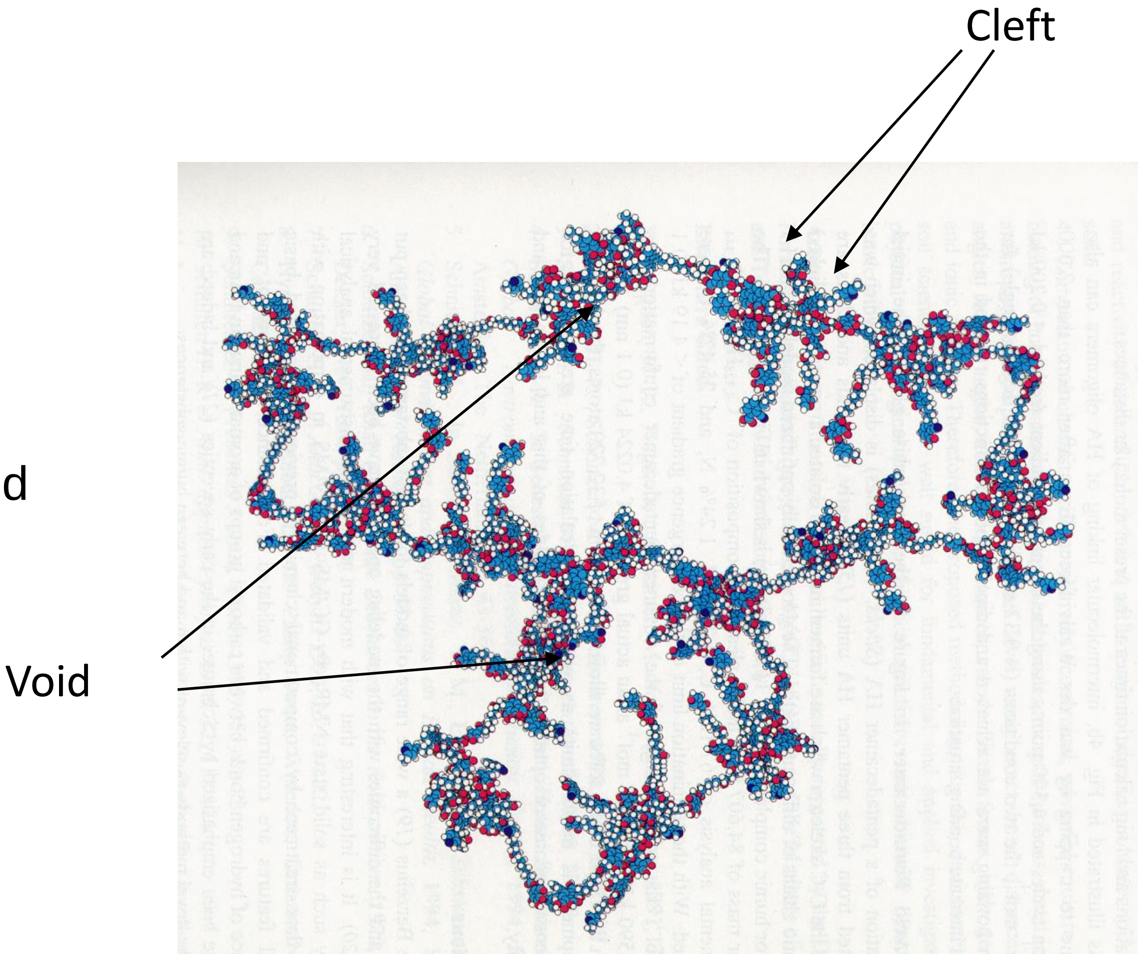


Figure 5 : a) Black/white plot (Sticks); and b) Color 3D structure (Disks) of a HA pentadecamer (n = 15; mol. mass 84,604.73 g mol^{-1} ; 11,370 atoms ; convergence limit < 4.19 kJ (0.1 $nm)^{-1}$ mol^{-1}; yes) following geometry optimization. The element colors are as described in Fig. 1

Table III. Literature Values for Pyrene–Organic Carbon Sorption Coefficients

method	sample	$^{c}K_{oc}$, L kg^{-1}	ref
fluorescence quenching	humic acid	1.7×10^5	4
	fulvic acid	1.2×10^5	
	fulvic acid	5.5×10^4	
	fulvic acid	1.0×10^5	
solvent extraction	bog DOC	3.2×10^4	32
fluorescence quenching	humic acid	1.25×10^5	4
	humic acid	8.8×10^4	
	humic acid	1.61×10^5	
	humic acid	3.20×10^5	
	humic acid	2.40×10^5	
	fulvic acid	6.6×10^4	
	fulvic acid	5.4×10^4	
	fulvic acid	1.04×10^5	
	14 soils/sediments	$6.27 \times 10^{4\,a}$	31

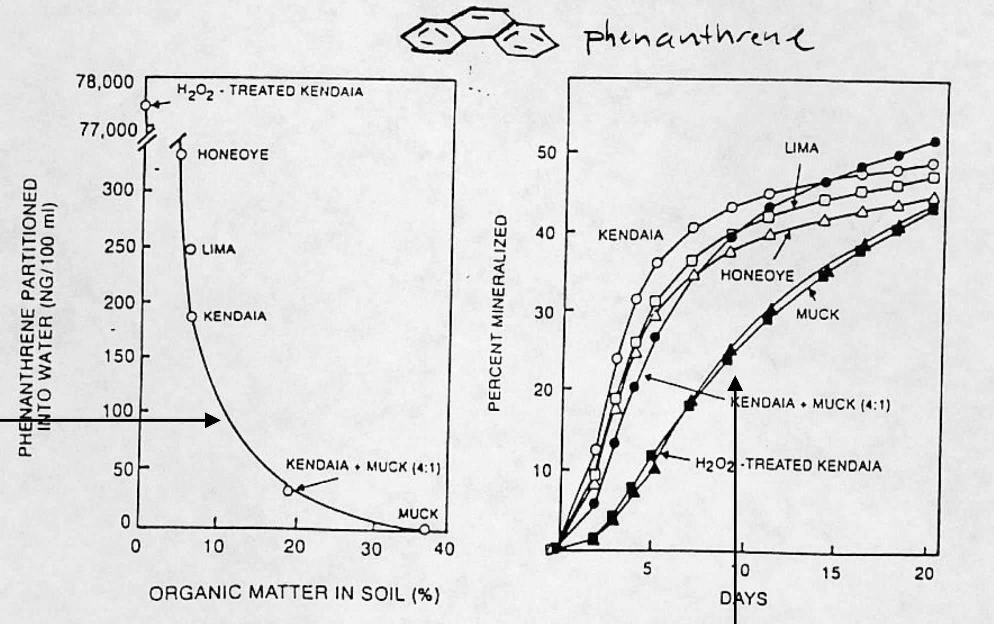
[a] Average $^{c}K_{oc}$ value.

Soil matrix components affecting weathering: SOM

Sorbtion of ions & neutral organic compounds (NOC)

Inc SOM =

Inc. sorption capacity for NOC



~~FIG. 8.2~~ Sorption (left panel) and mineralization (right panel) of phenanthrene in three mineral soils (Honeoye, Lima, and Kendaia), a muck soil, a soil–muck mixture, and soil treated with H_2O_2 to remove organic matter. (From Manilal and Alexander, 1991. Reprinted with permission from Springer-Verlag.)

Reduction of SOM reduces sorption capacity for NOC

Weathering: Combined effects of minerals & SOM

Minerals & SOM combine to form aggregates

NOC sorption to SOM on surface

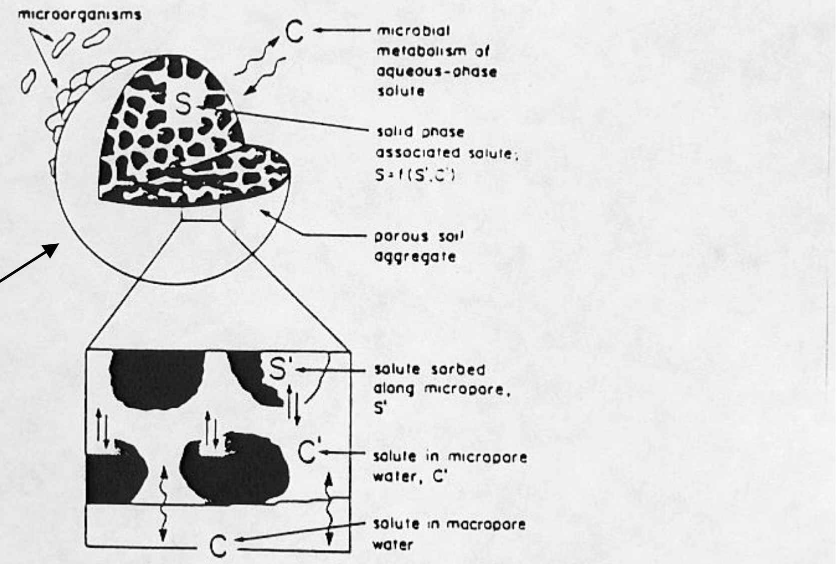


Figure 2.7. Depiction of the physical relationship of microorganisms to the structure of a soil aggregate containing an organic contaminant. Adapted with permission from Mihelcic & Luthy (1991). Copyright 1991, American Chemical Society.

NOC sorption to SOM in aggregate interior

Access to a chemical is limited by diffusion of the compound to the microbial cell

Tecnologie di bioremediation

Types of Bioremediation

Intrinsic

Natural attenuation

“Let nature take its course”

Degradative activities effected by indigenous microbes under ambient conditions

No intervention to alter aspects of the environment affecting microbial activity

Enhanced

Engineered remediation

Biostimulation

Alteration of the environment to enhance activities effected by indigenous microbes

Bioaugmentation

Inoculation of organisms to introduce a type of catalysis not displayed by the indigenous community

Bioremediation

For successful and cost-effective bioremediation, there need to be degrading microbes, adequate bioavailability, and suitable environmental conditions. For petroleum spills, there are normally degrading microbes present so the issues become bioavailability and environmental conditions.

In ocean oil spills, access to the oil is limited to the surface area between the oil-water interface. In general oxygen is not limiting but as shown below, nitrogen and phosphorus are limiting.

From Atlas and Bartha studying degradation constraints in an oil spill:

Treatment in seawater	% biodegradation
1. oil alone	0
2. oil + microorganisms	5
3. oil + micro. + P	5-10
4. oil + micro. + N	5-10
5. oil + micro. + N + P	75

If a more aggressive approach is required, there are several options available:

In situ treatments

- Bioventing
- Air sparging
- Permeable reactive barriers

Ex situ treatments

- Biofiltration
- Soil vapor extraction and treatment
- Groundwater extraction and treatment

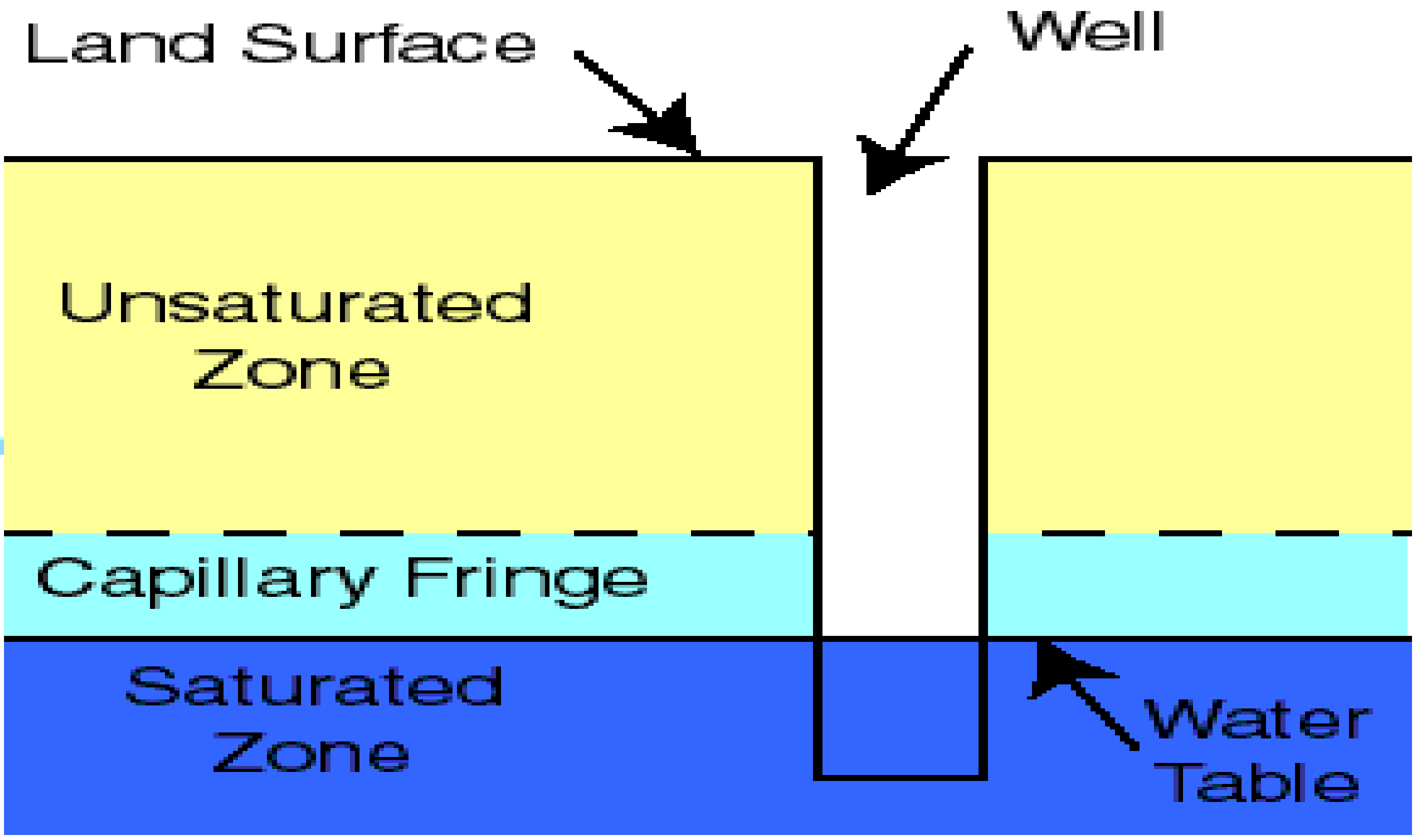
In subsurface terrestrial environments, there are many options. These include both in situ and ex situ treatment.

In the subsurface, the most limiting factor is generally oxygen. Therefore, addition of oxygen is one of the most common approaches to cleanup of subsurface contamination.

In addition, nutrients such as N and P may be added.

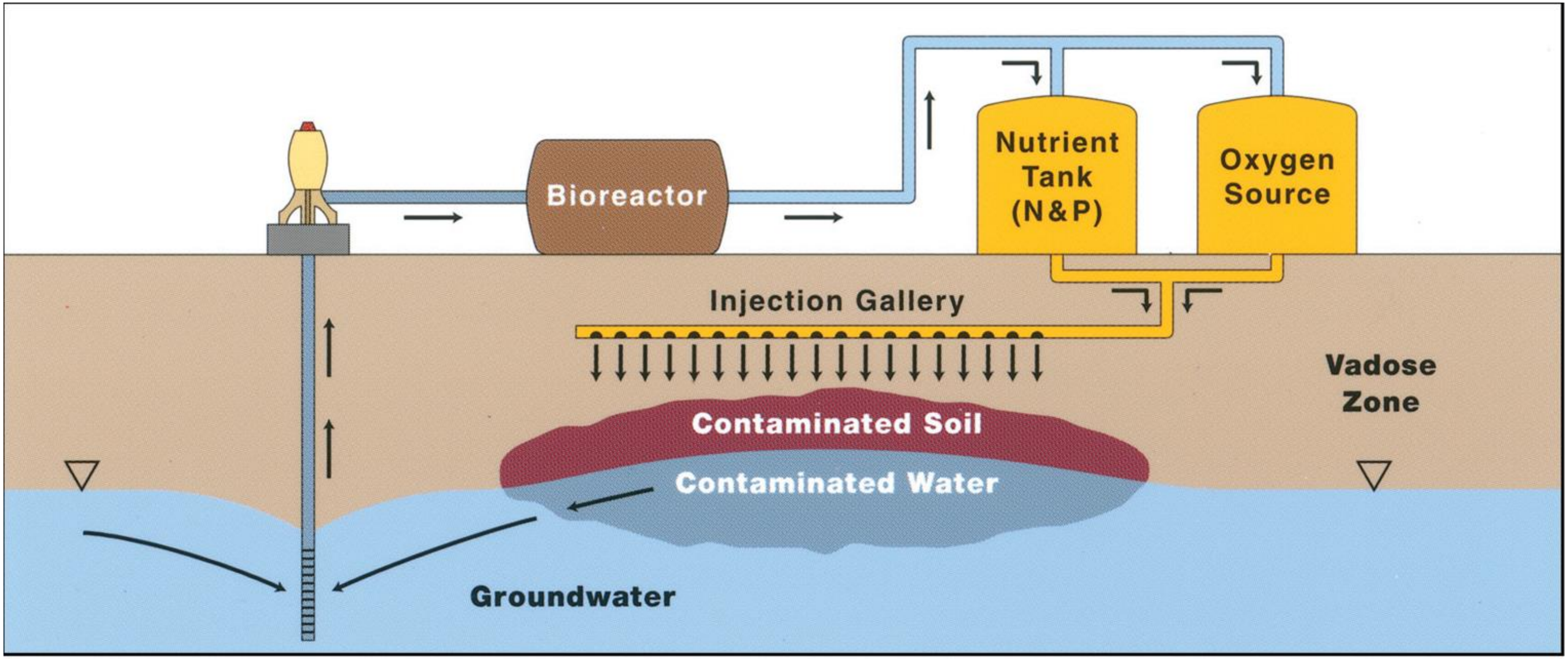
In some cases, natural activities are fast enough to control the contaminant plume. This is called intrinsic bioremediation or natural attenuation. This approach is desirable because it requires only monitoring of the contaminant plume. Must address the questions:

> Is intrinsic activity fast enough?
>
> Will the plume impact human or ecological health?



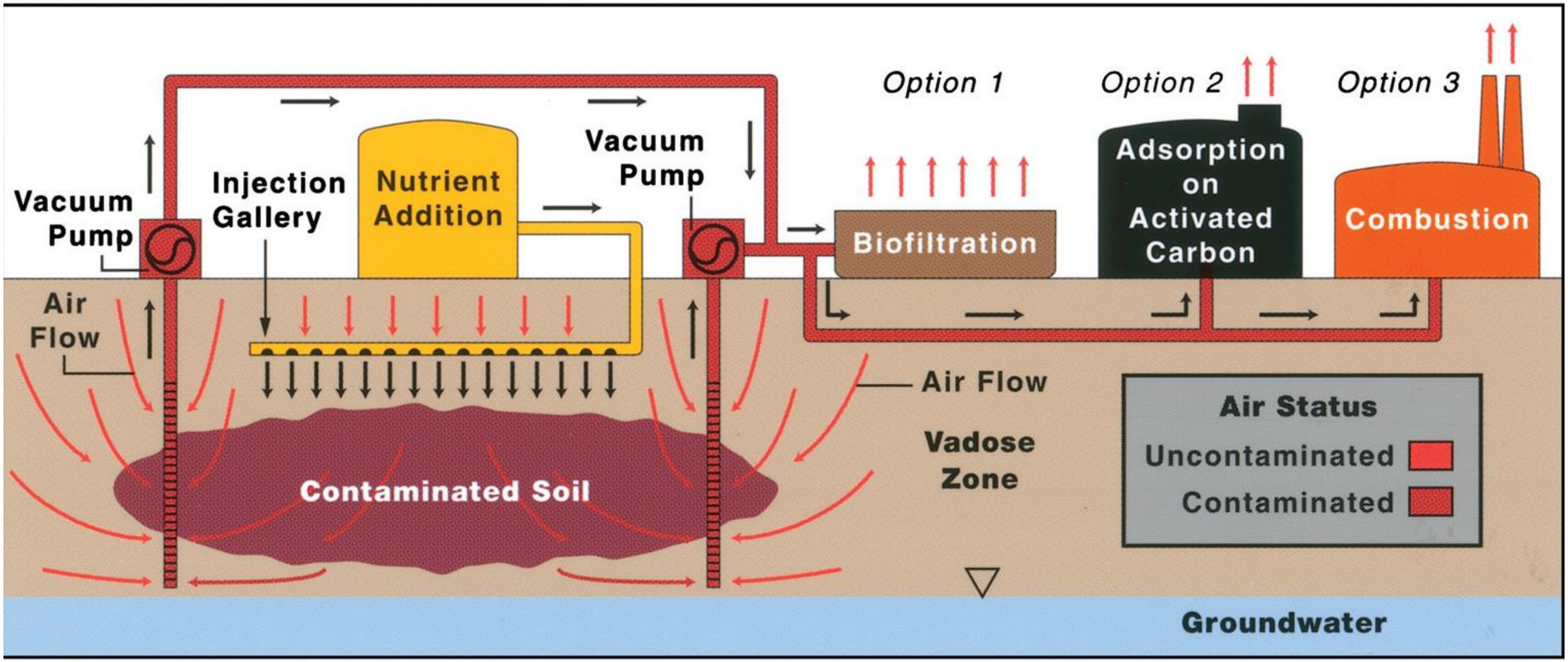
Unsaturated Zone - "The unsaturated zone, which replaces the terms 'zone of aeration' and 'vadose zone,' is the zone between the land surface and the deepest water table. It includes the capillary fringe. Generally, water in this zone is under less than atmospheric pressure, and some of the voids may contain air or other gases at atmospheric pressure. Beneath flooded areas or in perched water bodies the water pressure locally may be greater than atmospheric." - Committee on Redefinition of Ground-Water Terms, 1988

Example 1



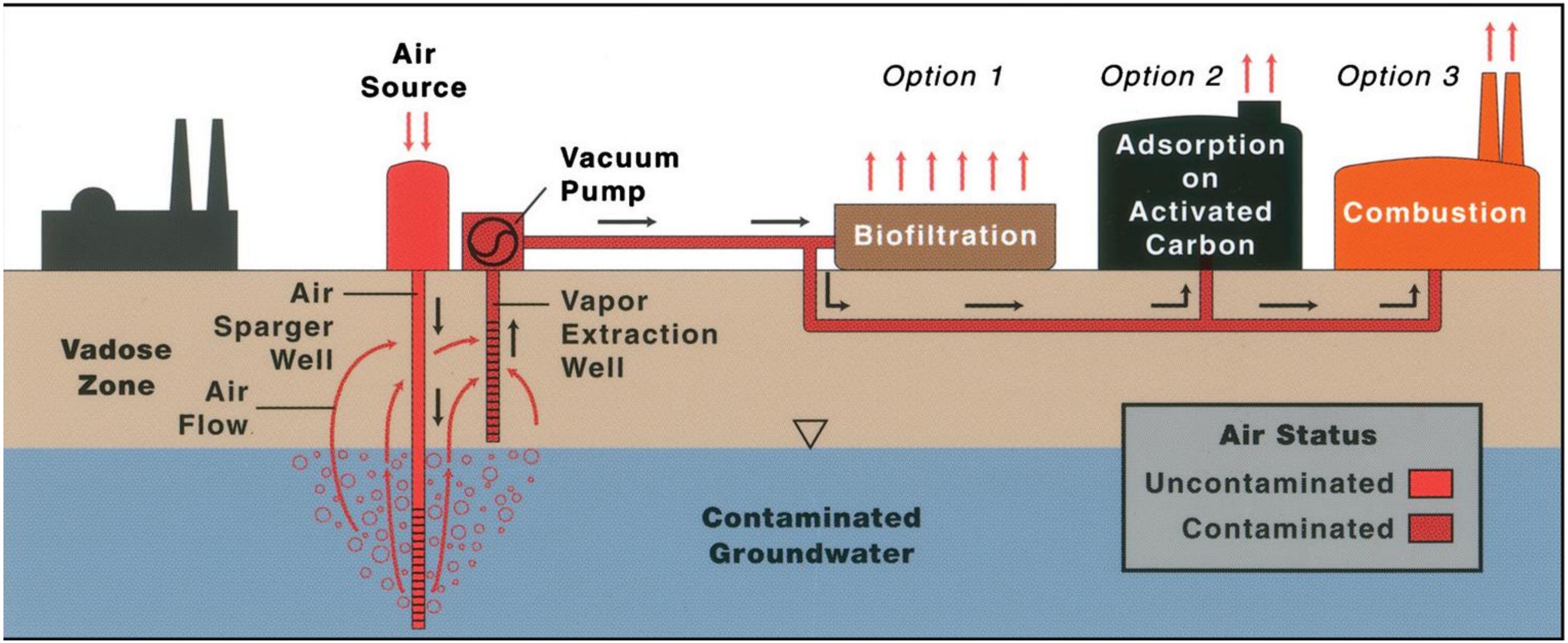
In situ bioremediation in the vadose zone and groundwater. Nutrient and oxygen are being pumped into the contaminated area to promote in situ processes. Water is being pumped to the surface for ex situ treatment in an aboveground bioreactor. Following treatment, an injection well is returning the contaminant-free water to the aquifer.

Example 2



Bioventing and biofiltration in the vadose zone. Air is slowly drawn through the contaminated site (bioventing) which stimulates in situ aerobic degradation. Volatile contaminants removed with the air can be treated biologically using a biofilter as shown or by adsorption on activated carbon, or by combustion.

Example 3



Bioremediation in groundwater by air sparging. Air is pumped into the contaminated site to stimulate aerobic biodegradation Volatile contaminants brought to the surfaced are treated by biofiltration, activated carbon, or combustion.

Il concetto di *carrying capacity*

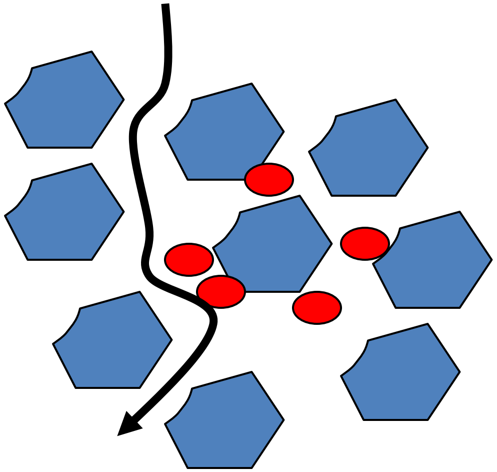
Carrying Capacity

- Although many chemical contaminants in the environment can be readily degraded because of their structural similarity to naturally occurring organic carbon, the amounts added may exceed the carrying capacity of the environment.
- Carrying capacity is the maximum level of microbial activity that can occur under the existing environmental conditions

What limits carrying capacity?

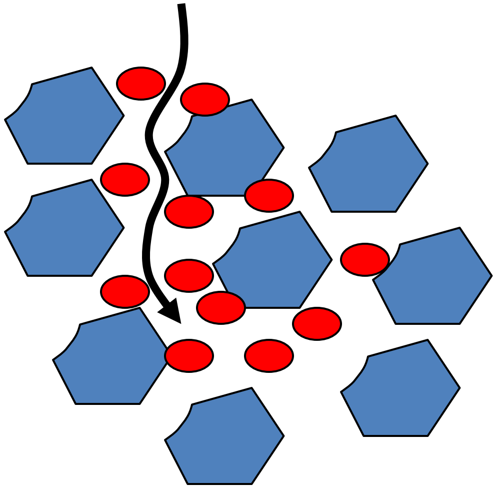
- Physical-chemical factors
  - pH, temperature, nutrients
- types of microbes present and their biomass

Low carrying capacity



Contaminant breakthrough

High carrying capacity



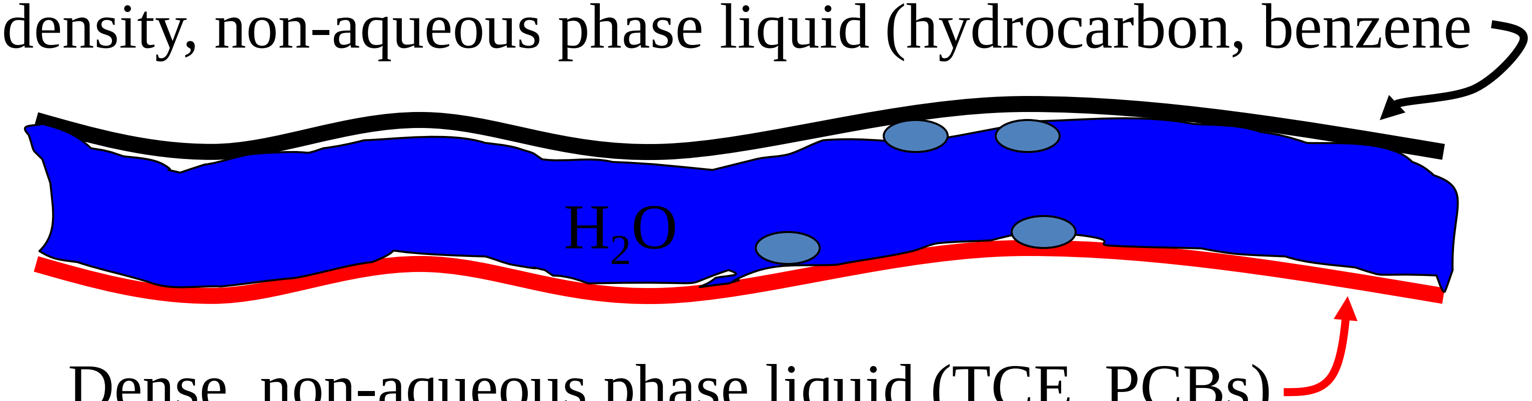
No contaminant left

Determinants of extent and rate of contaminant biodegradation

- Genetic potential of microbes to mutate key genes in such a way that gene product (enzyme) can catalyze step in contaminant degradation
  - This requires period of time for such adaptation to occur (weeks, months, years?)

Determinants of extent and rate of contaminant biodegradation

- Bioavailability
  - First step in biodegradation process is the uptake of the contaminant compound by the cell in order for intracellular enzymes to access the contaminant
  - If contaminant is not water-soluble, it is difficult for cell to access and take up contaminant.

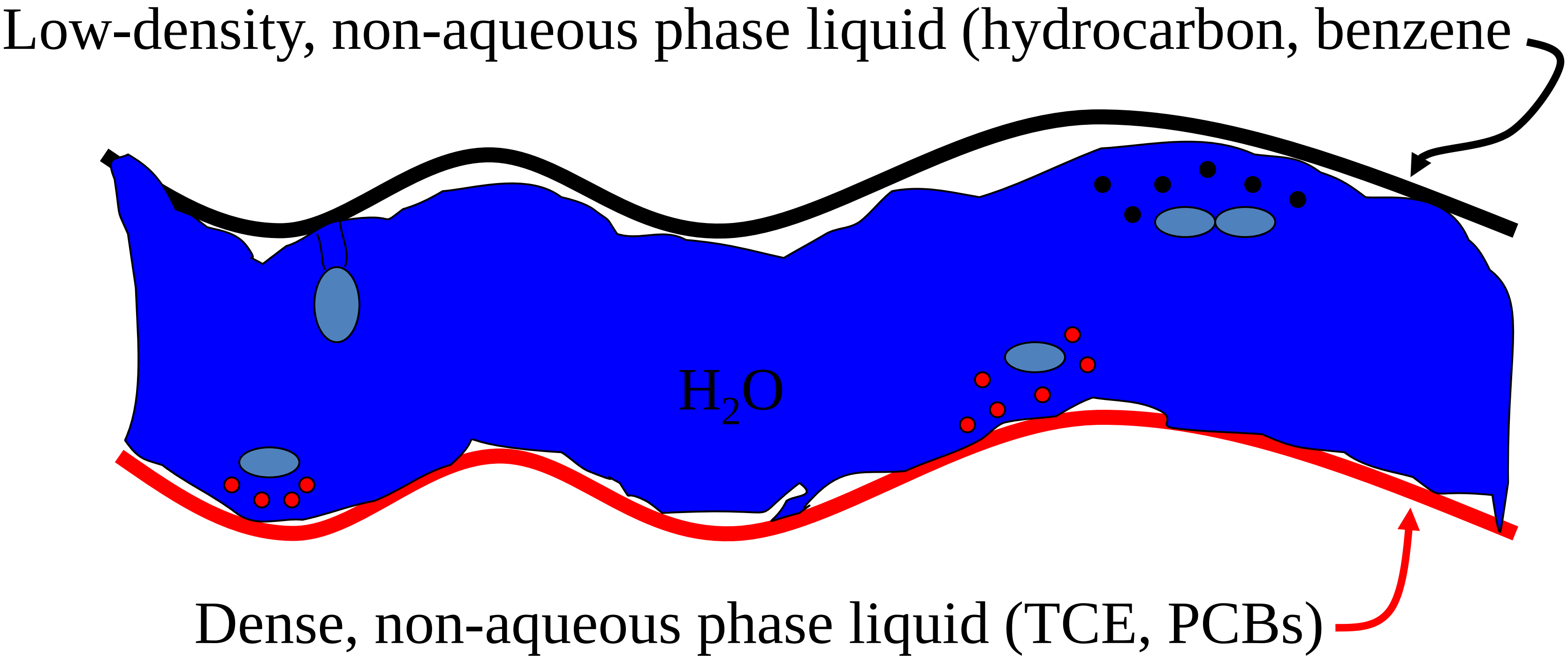


Low-density, non-aqueous phase liquid (hydrocarbon, benzene

Dense, non-aqueous phase liquid (TCE, PCBs)

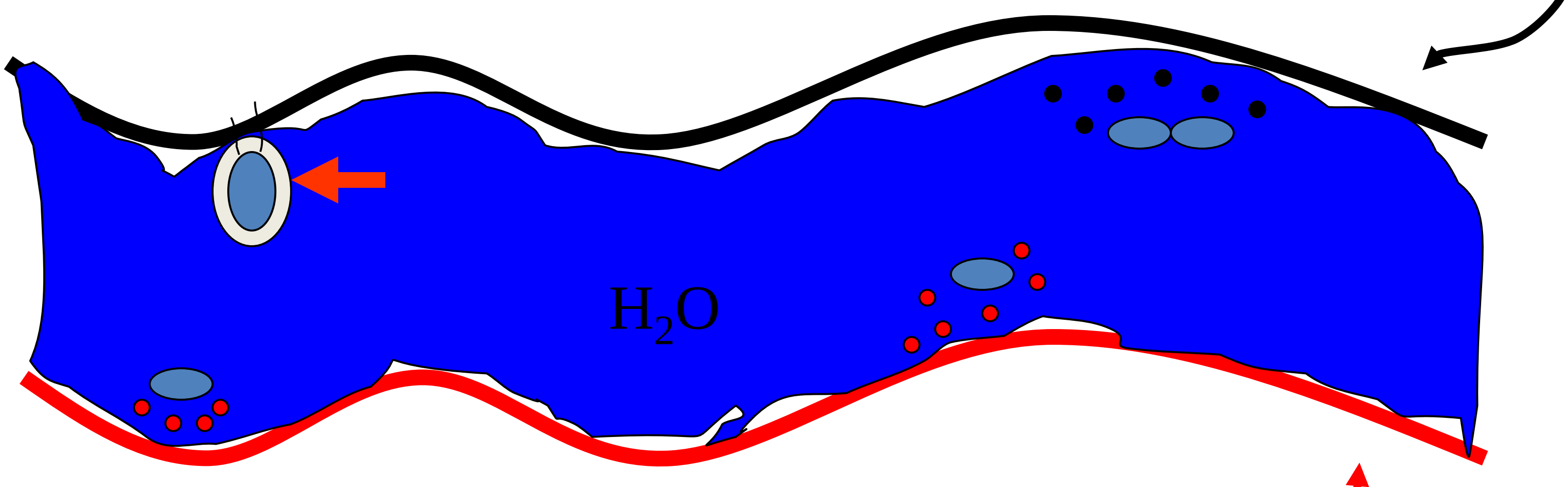
Determinants of extent and rate of contaminant biodegradation

- Bioavailability
  - Production of surfactants
  - Attachment to liquid-liquid interface



Determinants of extent and rate of contaminant biodegradation

- Bioavailability
  - Production of surfactants
  - Attachment to liquid-liquid interface
  - Make cell surface more hydrophobic-nonpolar LPS or EPS

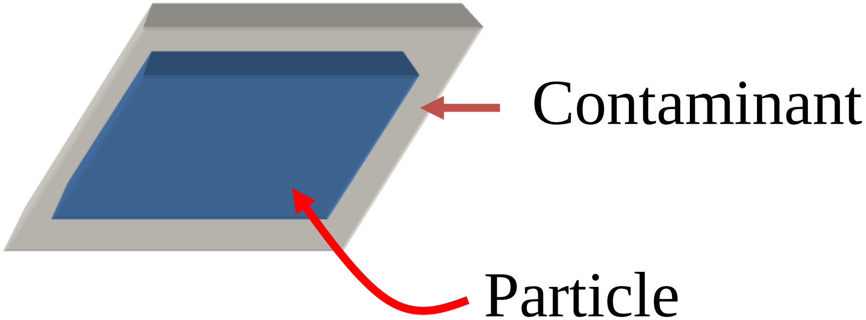


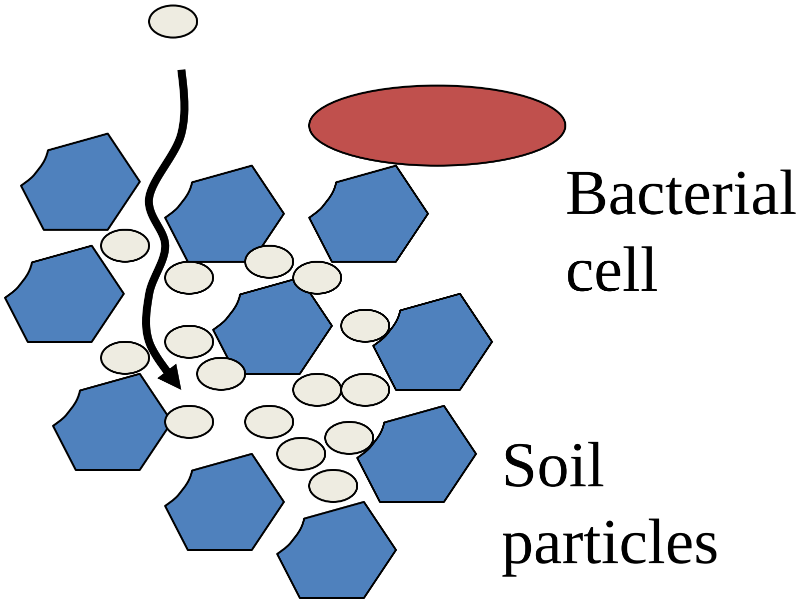
Low-density, non-aqueous phase liquid (hydrocarbon, benzene

Dense, non-aqueous phase liquid (TCE, PCBs)

Determinants of extent and rate of contaminant biodegradation

- Bioavailability
  - Sorption of contaminant to soil particles
  - Diffusion of contaminant into soil matrix





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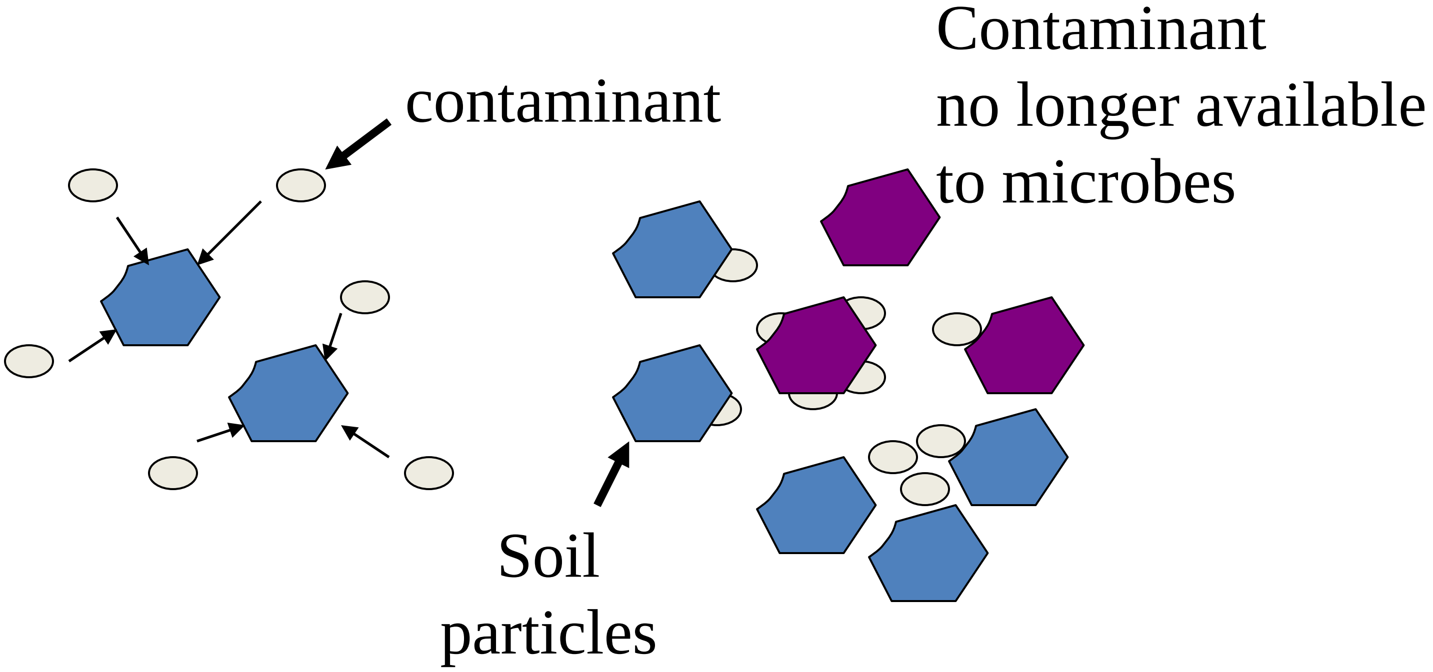

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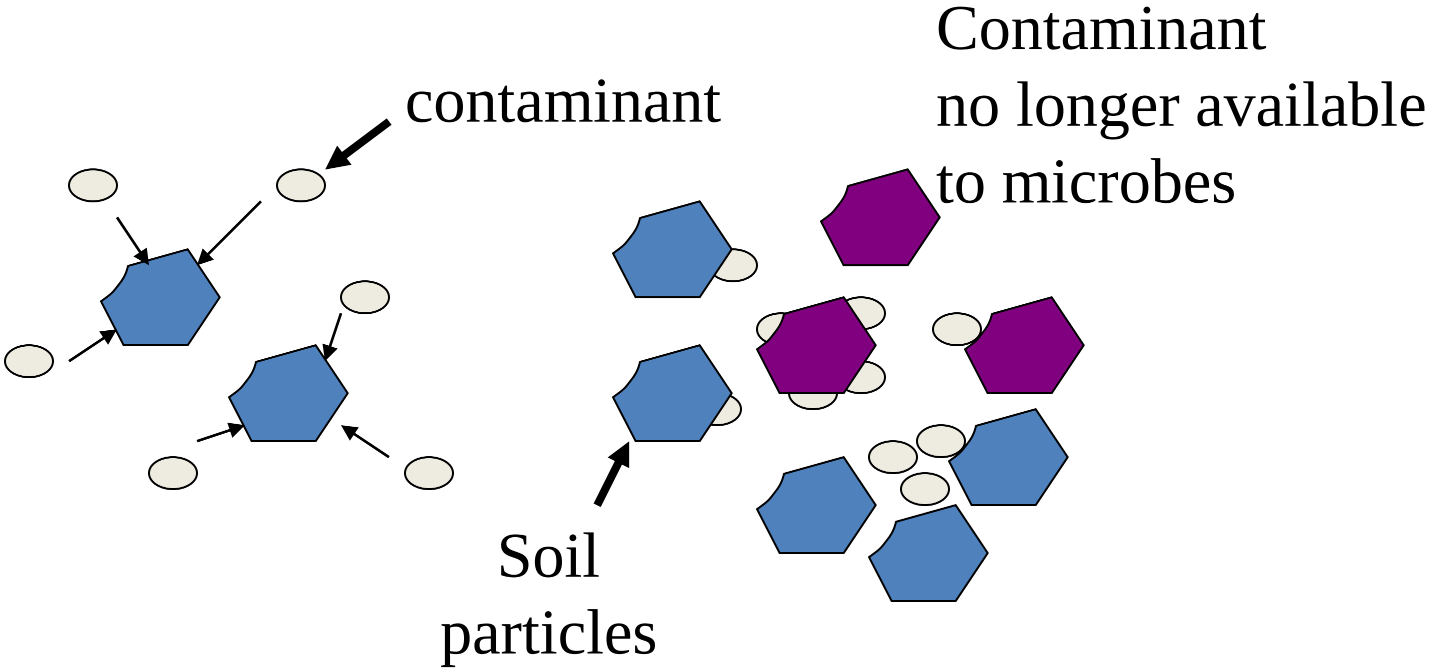
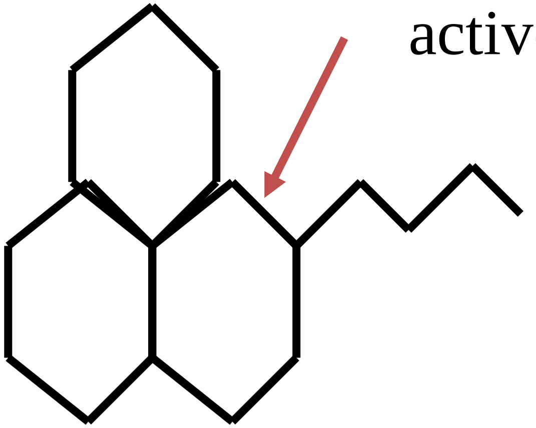


Determinants of extent and rate of contaminant biodegradation

- Contaminant structure
  - Steric effects



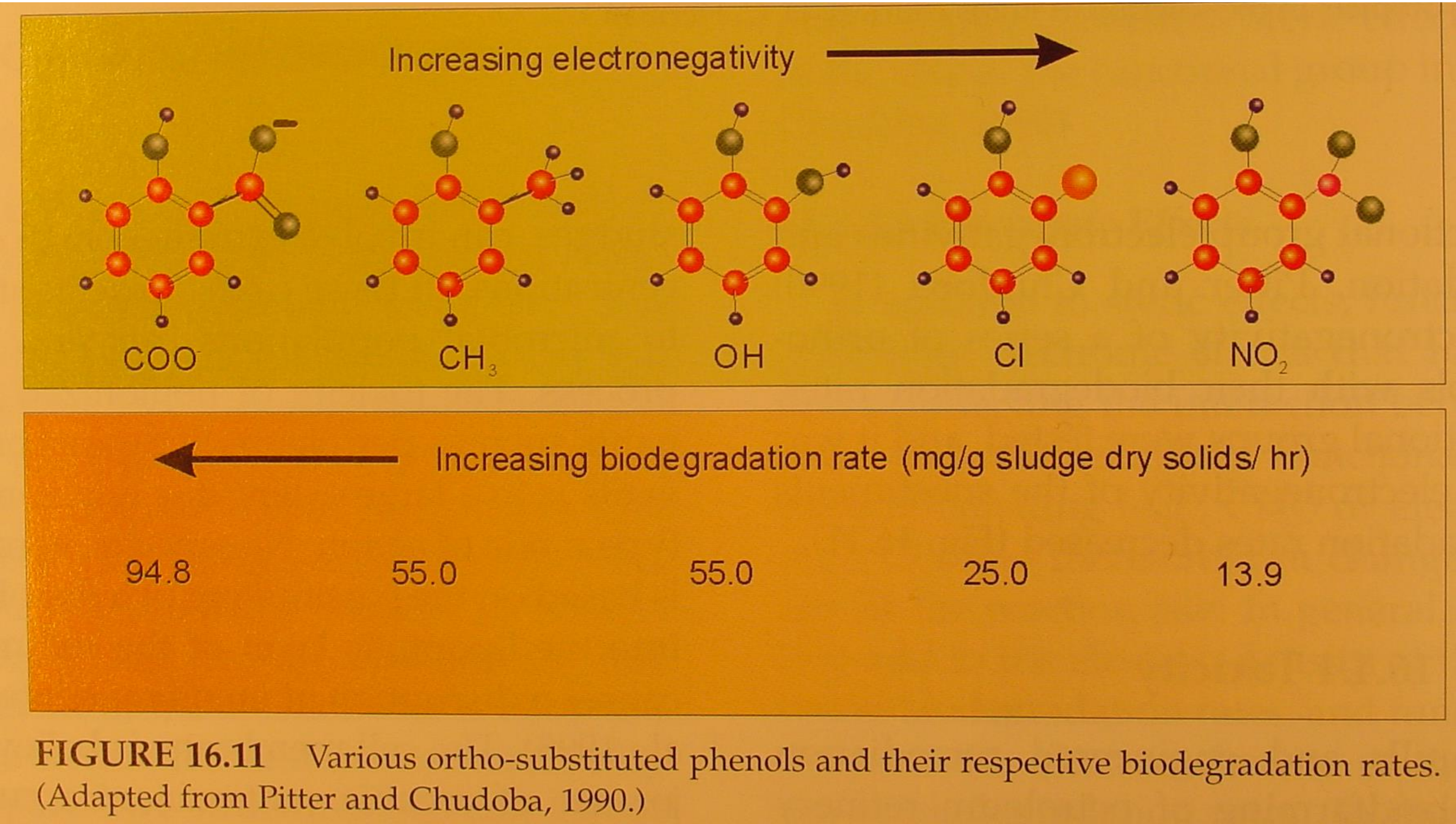
active site for enzyme blocked

Determinants of extent and rate of contaminant biodegradation

- Contaminant structure
  - Electronic effects
    - as electronegativity of substituents increased, biodegradation rates decreased

Electronic Effects

L'elettronegatività è una misura relativa della capacità di un atomo di attrarre elettroni quando prende parte ad un legame chimico.



Increasing electronegativity →

COO^- | CH_3 | OH | Cl | NO_2

← Increasing biodegradation rate (mg/g sludge dry solids/ hr)

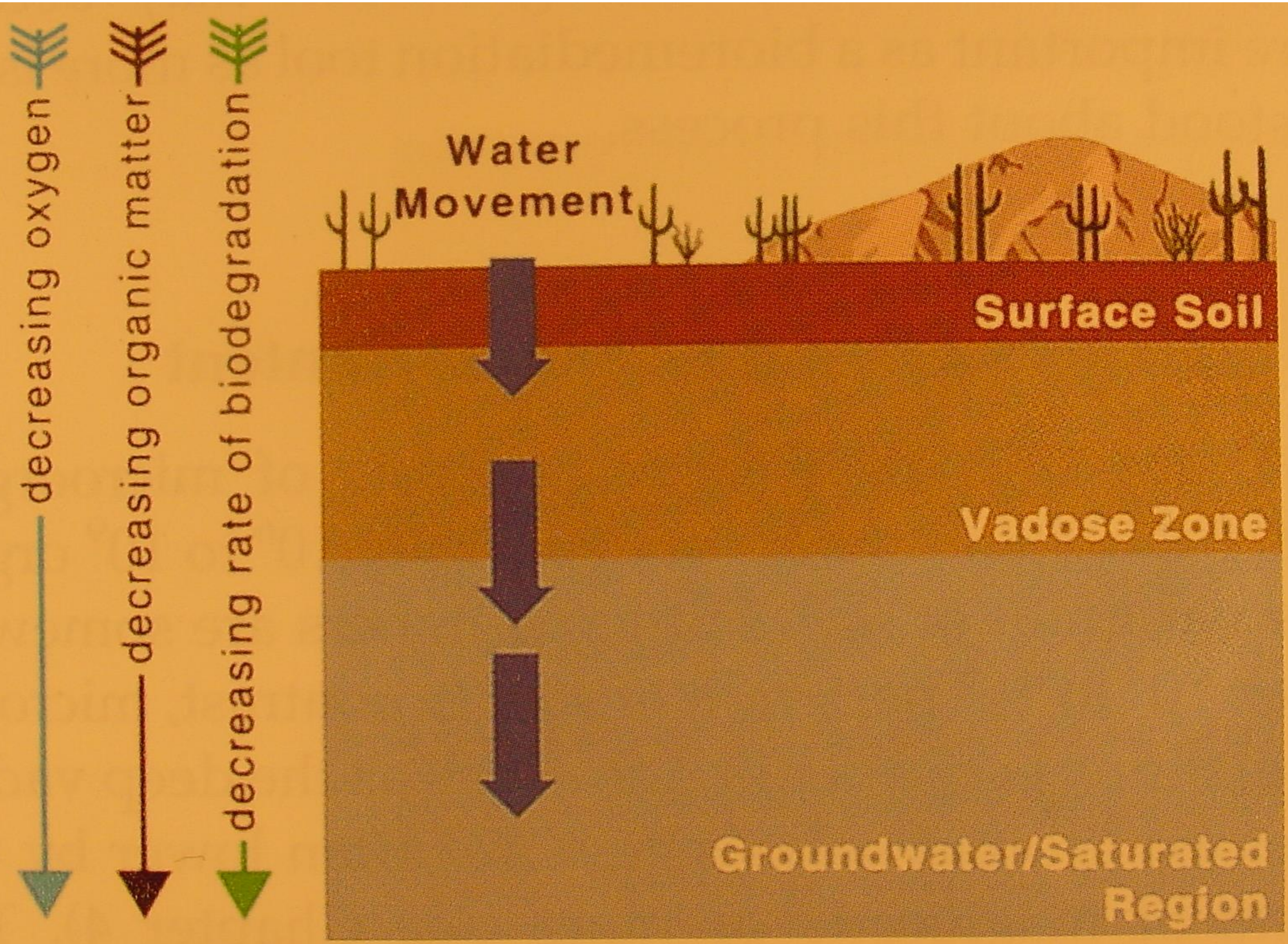
94.8 | 55.0 | 55.0 | 25.0 | 13.9

FIGURE 16.11 Various ortho-substituted phenols and their respective biodegradation rates. (Adapted from Pitter and Chudoba, 1990.)

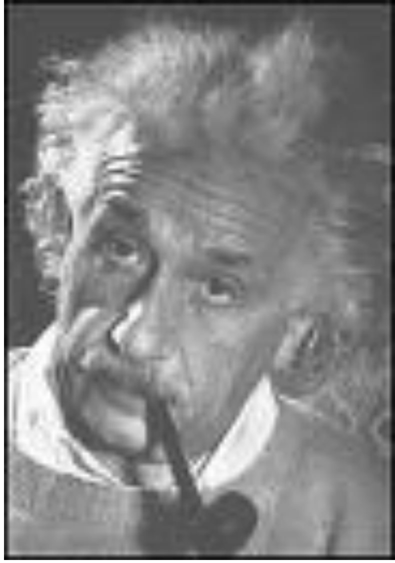
Determinants of extent and rate of contaminant biodegradation

- Environmental factors
  - organic matter (source of carbon and energy)
    - subsurface, unsaturated zones have low organic matter concentrations
  - oxygen availability
  - nutrient (N,S, P) availability
  - temperature
  - pH
  - Eh
  - salinity
  - water activity

Most important factors controlling contaminant biodegradation



Einstein on:
Environmental Monitoring



“Not everything that can be counted counts, and not everything that counts can be counted.“ (oft attributed to Albert Einstein)

corollary for environmental monitoring:

Not everything that can be measured is worth measuring, and not everything worth measuring is measurable.

Slide credit:
Adapted from Christian Daughton, PhD, Environmental Protection Agency

Livelli di sviluppo caratteristici di un processo di biorisanamento e strategia generale con cui se ne valuta l'efficacia

Caratterizzazione dell'inquinante

PARAMETRI CONTROLLATI:

-composizione

-concentrazione

-tossicità

-disponibilità

-solubilità

-volatilizzazione

DOMANDE IMPORTANTI:

-Le sostanze inquinanti sono biodegradabili?

-E' possibile un trattamento ecologico?

-Il processo è cometabolico?

-La biodegradazione può essere migliorata?

Caratterizzazione idrogeochimica

PARAMETRI CONTROLLATI:

-proprietà geologiche

-conduttività idraulica

-direzione del flusso

-entità del flusso

-presenza di NO_3^-, PO_4, ecc.

-presenza di accettori di elettroni

-pH, temperatura

DOMANDE IMPORTANTI:

-Le condizioni idrogeologiche sono favorevoli?

-Le condizioni ambientali sono favorevoli per la crescita e l'attività?

-E' richiesta l'aggiunta di sostanze nutritive?

-L'ambiente può essere modificato (migliorato)?

Caratterizzazione microbiologica

PARAMETRI CONTROLLATI:

-specifica diversità catabolica

-dimensioni della popolazione

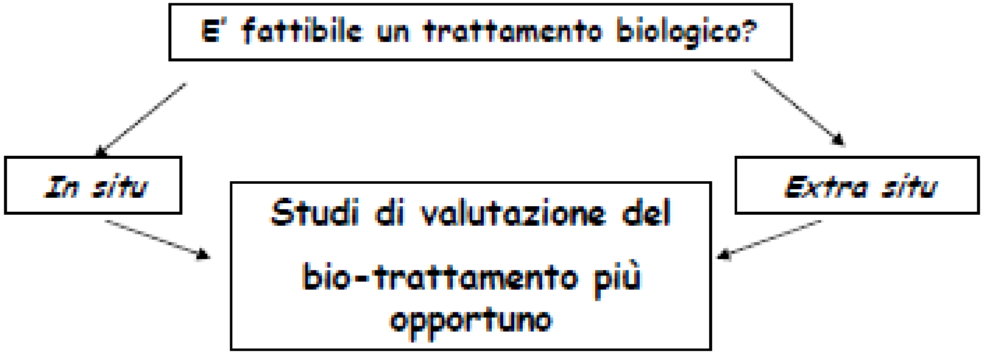
-specifiche attività cataboliche

DOMANDE IMPORTANTI:

-C'è il potenziale biologico per la biodegradazione?

-Si può stimolare la popolazione saprofita?

Valutazione del trattamento



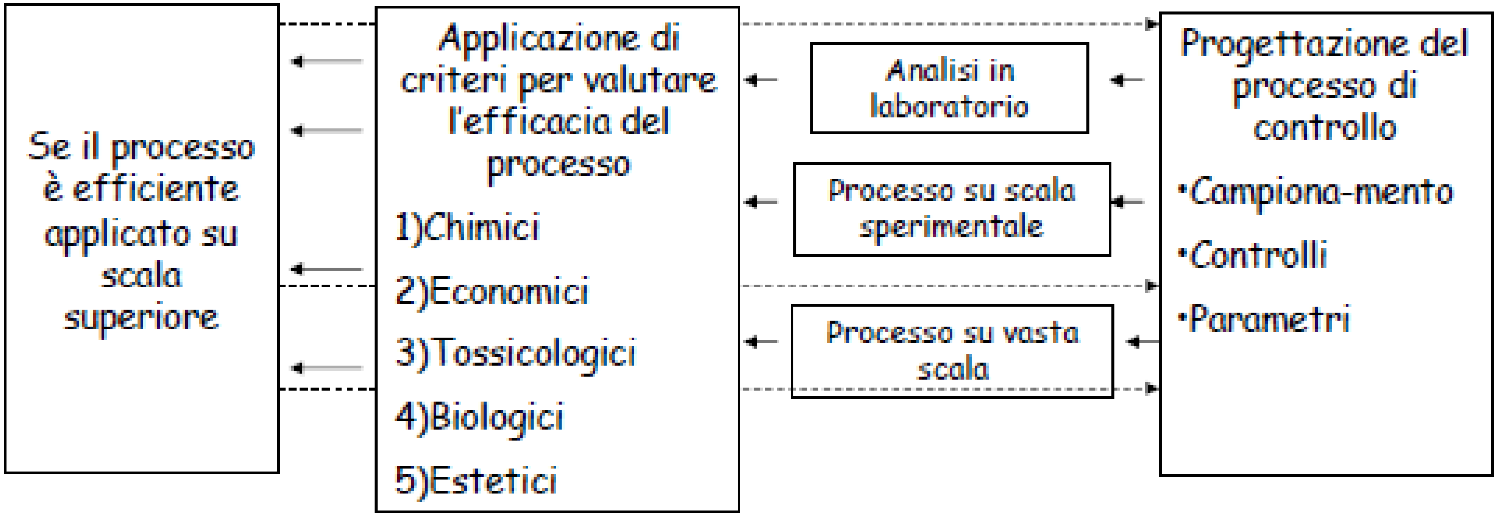
E' fattibile un trattamento biologico?

In situ

Extra situ

Studi di valutazione del bio-trattamento più opportuno

Ampliamento della scala di applicazione del processo



Se il processo è efficiente applicato su scala superiore

Applicazione di criteri per valutare l'efficacia del processo

1)Chimici

2)Economici

3)Tossicologici

4)Biologici

5)Estetici

Analisi in laboratorio

Processo su scala sperimentale

Processo su vasta scala

Progettazione del processo di controllo

- Campiona-mento
- Controlli
- Parametri

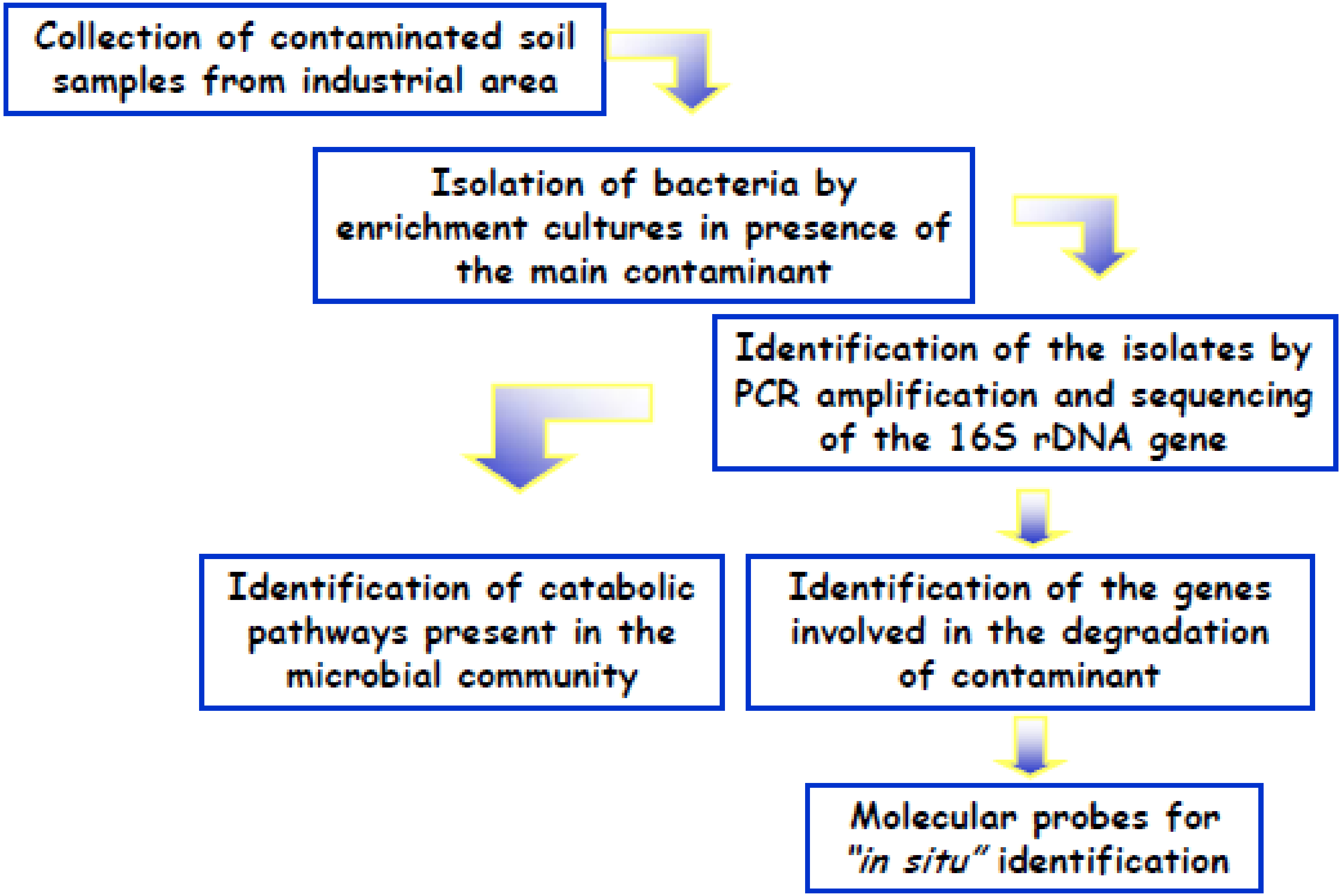
MONITORAGGIO di CONTAMINANTI

biosensori microbici

Sistemi che accoppiano l'elevata specificità e selettività del riconoscimento biomolecolare con un dispositivo elettronico per trasdurre l'evento riconosciuto a livello biomolecolare in un segnale rilevabile

RILEVAMENTO e MONITORAGGIO di MICRORGANISMI per il BIORISANAMENTO

Sistemi molecolari per monitorare la presenza e/o moltiplicazione di microrganismi decontaminanti e il livello del processo di biorisanamento



Collection of contaminated soil samples from industrial area

Isolation of bacteria by enrichment cultures in presence of the main contaminant

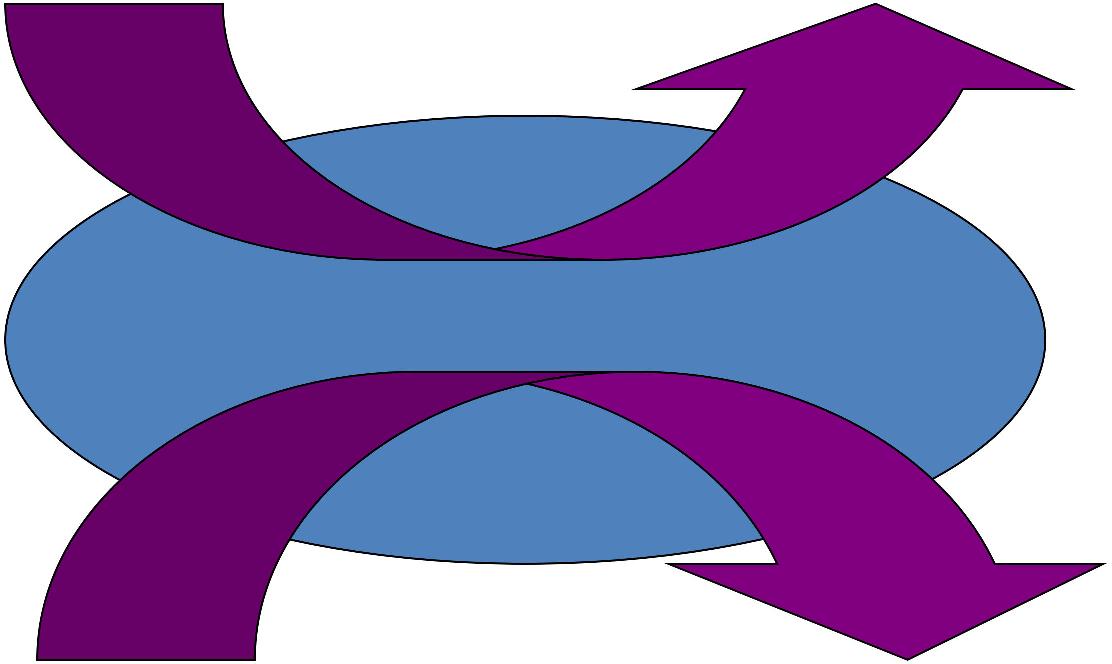
Identification of the isolates by PCR amplification and sequencing of the 16S rDNA gene

Identification of catabolic pathways present in the microbial community

Identification of the genes involved in the degradation of contaminant

Molecular probes for "*in situ*" identification

Overall process of biodegradation



Electron donor

Carbon source

Organic C

Carbon dioxide

H_2O

Electron acceptor

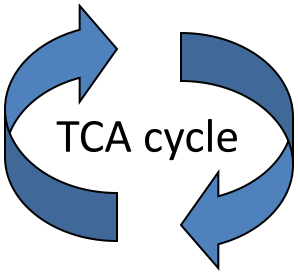
O_2 **aerobic respiration**

NO_3**,** Fe(III), Mn(IV), SO_4, CO_2 **anaerobic respiration**

Complete vs incomplete biodegradation

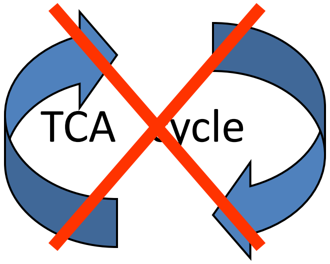
Complete
(mineralization)

$\text{Glucose} \rightarrow CO_2 + H_2O$



Incomplete

$\text{Glucose} \rightarrow \text{pyruvate} \rightarrow \cancel{\rightarrow C}O_2 + H_2O$



Contaminant Fate and Transport Processes

Philip B. Bedient
Environmental Science and Engineering
Rice University, Houston, TX

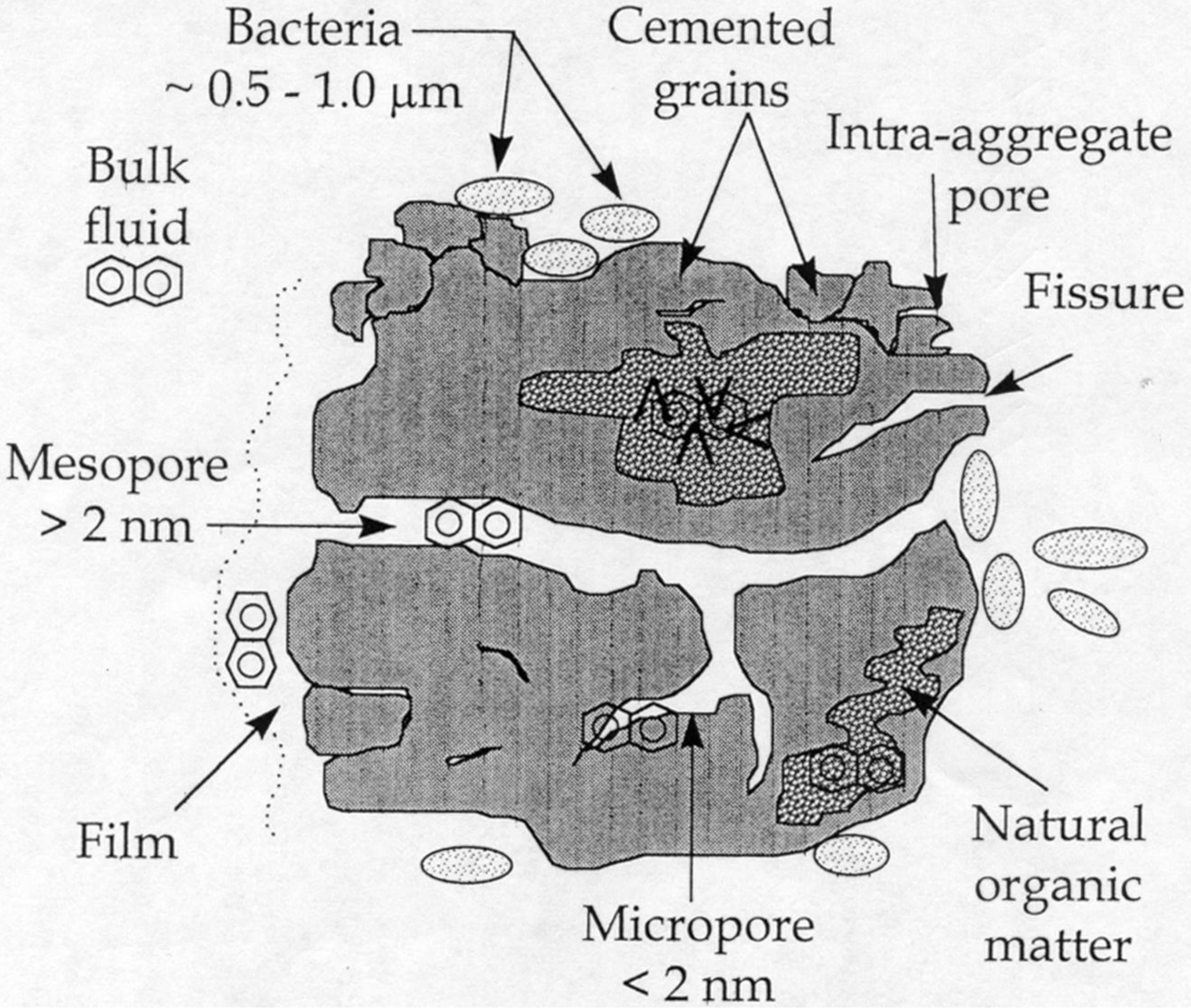
Fate and Transport

- Advection and Dispersion
- Sorption and Retardation
- Chemical/Abiotic processes
- Volatilization
- Biodegradation

Sorption and Retardation

- Sorption – association of dissolved or gaseous contaminant with a solid material
- Adsorption – surface process
- Absorption – internal process
- Leads to retardation of the contaminant front
- Desorption – reverse of either sorption process

Soil Grain Sorption

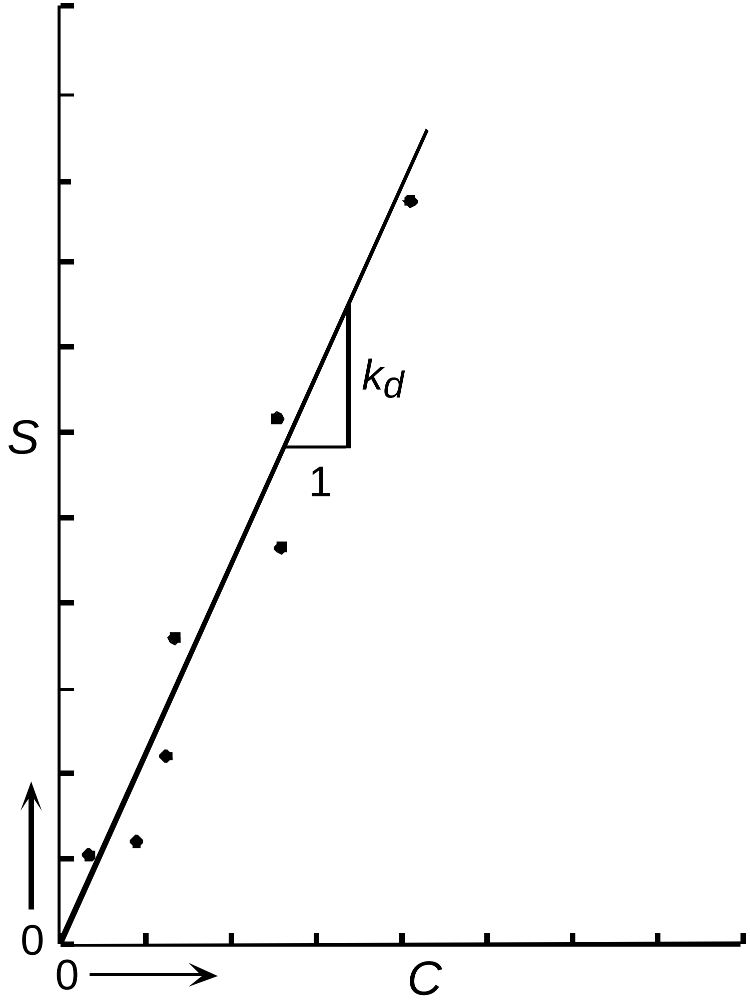


Linear Sorption Isotherm

Sorption linearly related to aqueous concentration.

Partition coefficient is K_d

K_d is related to K_{ow}



Partitioning to Solid Phase

- Octanol water partition coeff.

$$k_{ow} = \frac{[A]_{\text{octanol}}}{[A]_{\text{water}}}$$

- Distribution coeff.

$$k_d = \frac{[A]_{\text{solid}}}{[A]_{\text{aqueous}}}$$

- Fraction in aqueous phase

$$f_w = \frac{1}{1 + \left(\frac{1}{n} - 1\right) k_d}$$

Octanol-Water Partition Coefficient (K_{ow})

The octanol-water partition coefficient is the ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature. Octanol is an organic solvent that is used as a surrogate for natural organic matter. This parameter is used in many environmental studies to help determine the fate of chemicals in the environment. An example would be using the coefficient to predict the extent a contaminant will bioaccumulate in fish. The octanol-water partition coefficient has been correlated to water solubility; therefore, the water solubility of a substance can be used to estimate its octanol-water partition coefficient.

Sorption Processes

The sorption of a contaminant is one of the significant processes that can hinder the remediation of a ground water aquifer system. Sorption is defined as being the attraction of an aqueous species to the surface of a solid.(Alley, 1993). In ground water the sorbing species , usually an organic compound, is called the **sorbate**, and the solid media, usually soil, to which the sorbate is attracted is known as the **sorbent**.

The underlying principle behind this attraction results from some form of bonding between the contaminant and adsorption receptor sites on the solid. The amount of sorption that occurs in groundwater is dependent on particular characteristics of the sorbate and sorbent. The amount of sorption that takes place on organic matter also follows various isotherms or kinetic rates.

Sorption tends to cause contaminants to move more slowly than the groundwater, therefore the effects must be taken into consideration when calculating how far the contaminant has traveled in a given time period.

The following animation sequence displays how sorption can affect two separate particles' velocity. Basically, the animation shows a vertical cut from a soil column, interspersed particles of organic matter, and two contaminants that are moving through the soil.

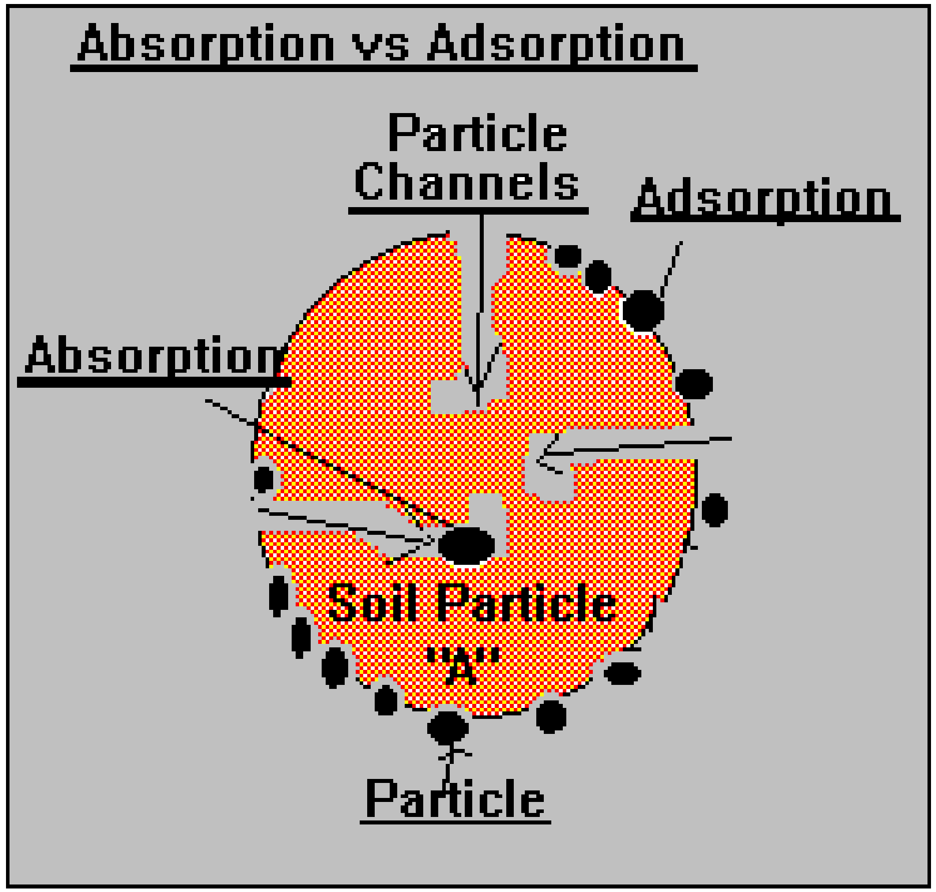
soil1.mov

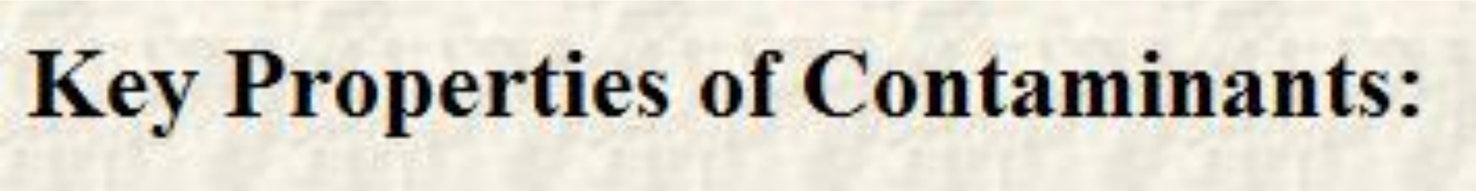
Adsorption vs. Absorption

Sorption reactions generally occur over a short period of time, however if the adsorbed contaminant begins to be incorporated into the structure of the sorbent , a slow occurring reaction, known as absorption, begins to take place. To be more precise , the difference between adsorption and absorption is that adsorption is the attraction between the outer surface of a solid particle and a contaminant, whereas absorption is the uptake of the contaminant into the physical structure of the solid.

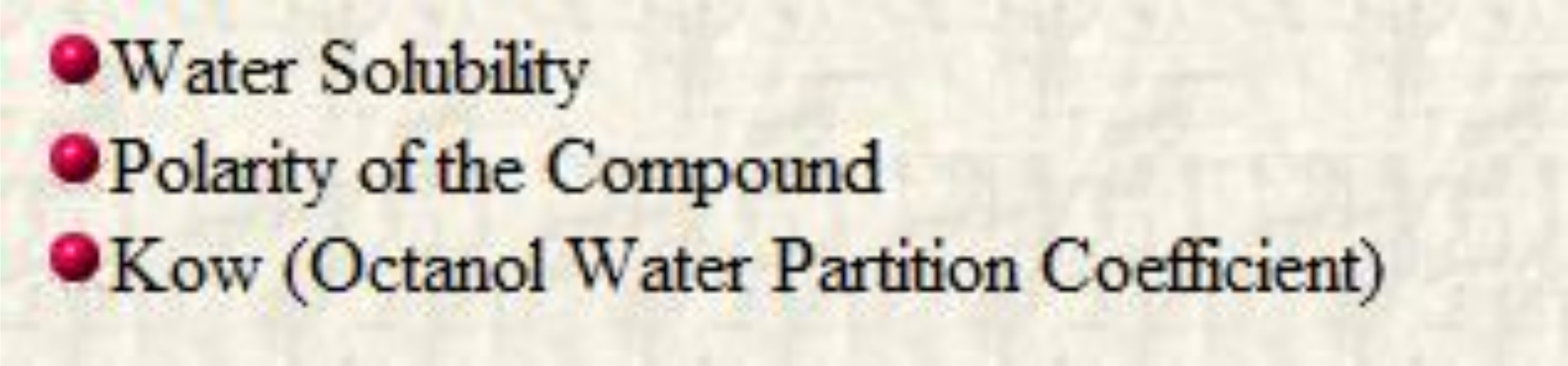
This figure shows the primary differences between intraparticle absorption versus surface adsorption. The main difference being that some contaminant particles are attracted to the outer surface of the soil particle, while another has been actually incorporated into the particle's structure.

Figure 1: Adsorption vs. Absorption





Key Properties of Contaminants:



- Water Solubility
- Polarity of the Compound
- Kow (Octanol Water Partition Coefficient)

ESEMPI DI BIOREMEDIATION

Example - Intrinsic Bioremed.

- Microbes in Hudson river mud developed an ability to partially degrade PCB (Poly Chlorinated Biphenyls)

Process occurs in 2 steps

Partial dehalogenation of PCBs occurs naturally under anaerobic conditions

↓

Less chlorinated residues

↓

Then mud is aerated to promote the complete degradation of these less chlorinated residues

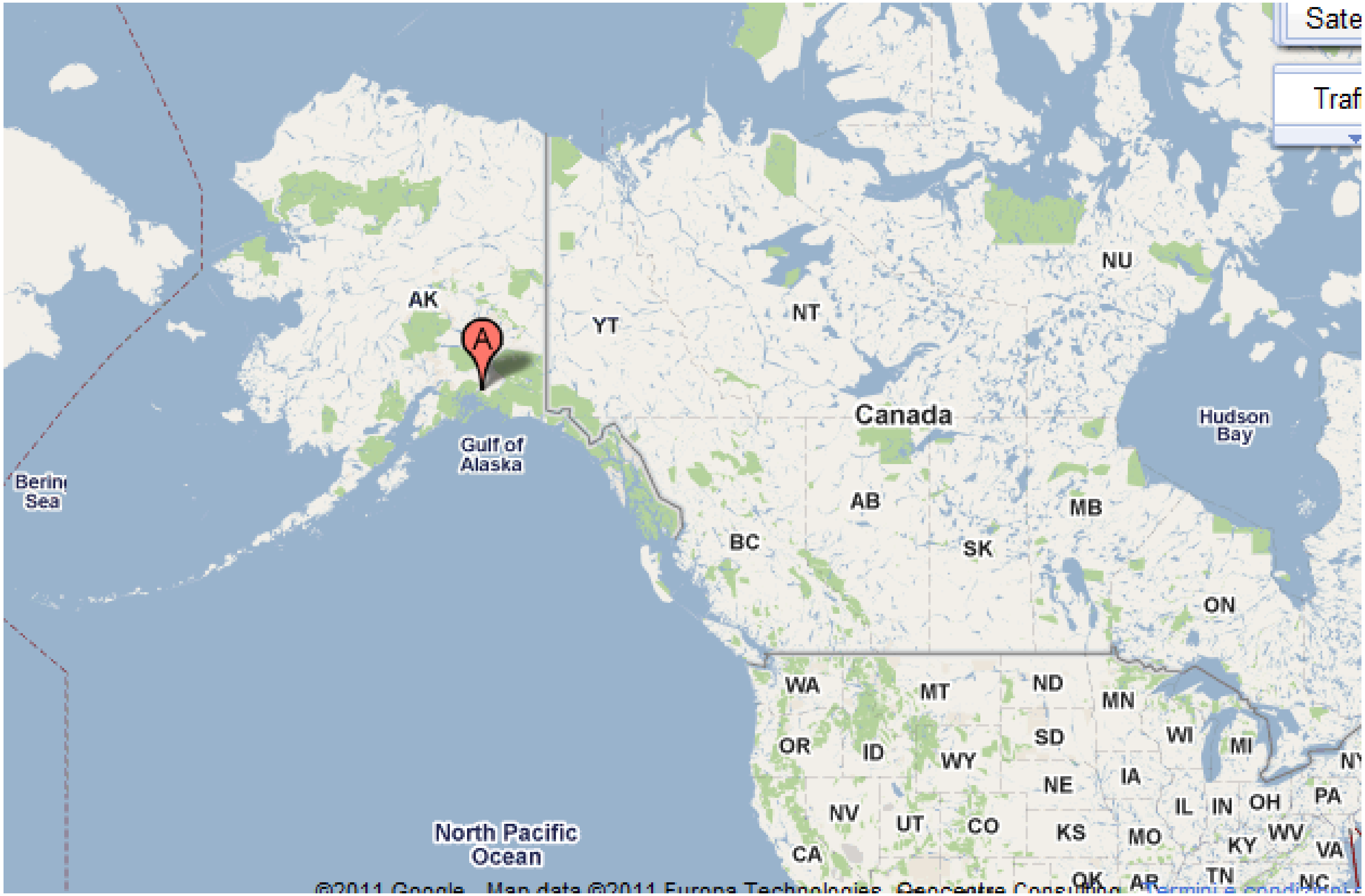
Application of Technology

Bioremediation in
ALASKA OIL SPILL

South Central Alaska

- South Central Alaska is beautiful and unique.
- Prince William Sound, surrounded by land from the Chugach National Forest
- Has many islands, bays, and fjords, giving it more than 2,000 miles of shoreline and making it one of the nation’s largest relatively undeveloped marine ecosystems
- Entire region also has many national Wildlife Refuges.

- People also live in this region. Valdez, population about 4,000
- is about 110 miles from Anchorage, Alaska’s largest city and home to half of the state’s population
- The state of Alaska is heavily dependent on its natural resources, with 80% of the state treasury connected to oil.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NY
NE
IA
IL
IN
OH
PA
NV
UT
CO
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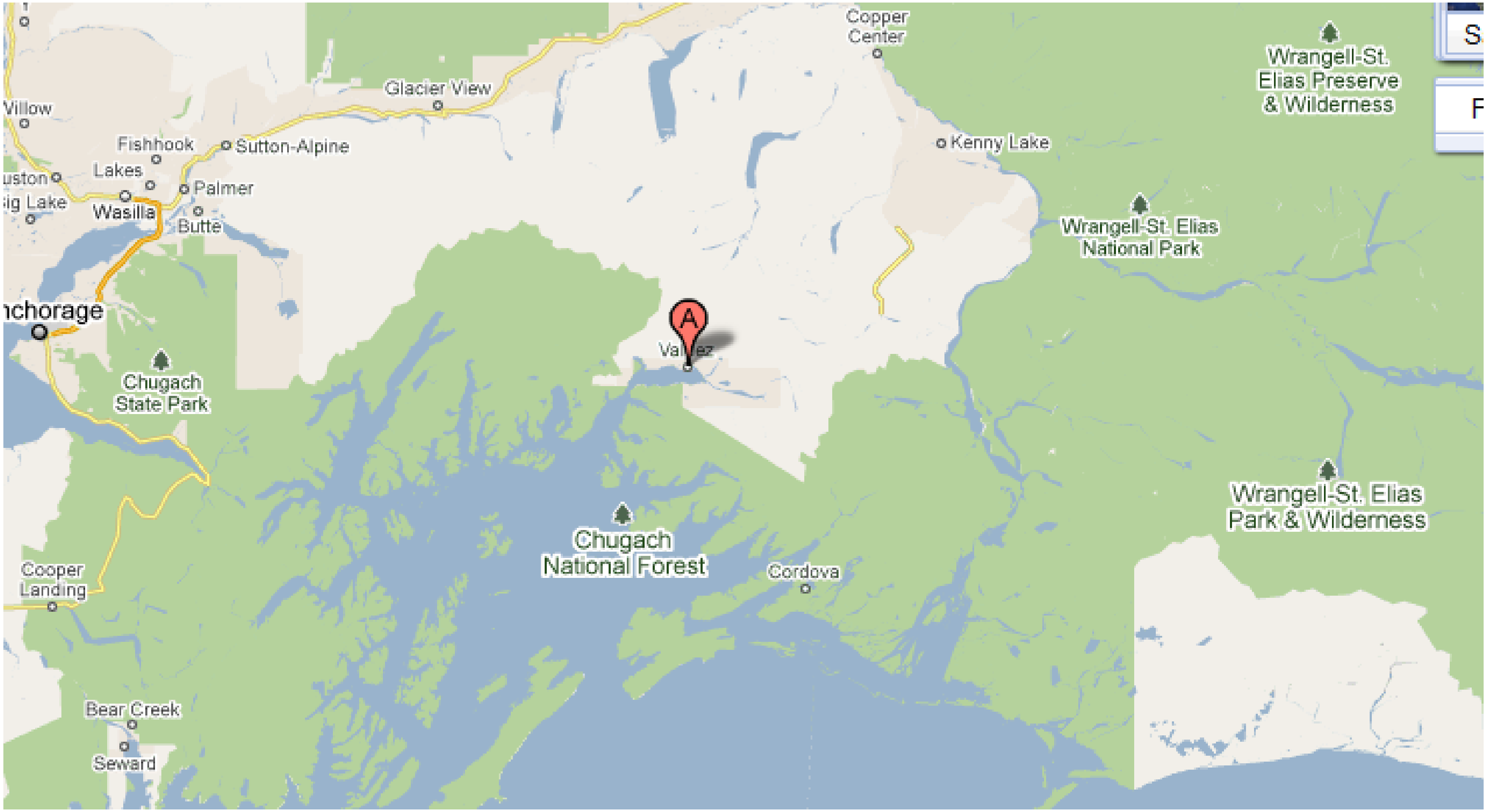
Copper Center
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Villow
Glacier View
Fishhook
Sutton-Alpine
Kenny Lake
Lakes
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Palmer
ig Lake
Wasilla
Butte
Wrangell-St. Elias National Park
nchorage
A
Val ez
Chugach State Park
Wrangell-St. Elias Park & Wilderness
Chugach National Forest
Cooper Landing
Cordova
Bear Creek
Seward

It is here …

a few minutes after midnight

March 24, 1989

The Oil Spill.mp4

la catastrophe de l'Exxon Valdez.mp4

Decisions had to be made!

- The oil was spreading and contaminating more and more beaches every day.
- Clean-up methods had to be decided upon to prevent further spreading of the oil.

Try Bioremediation?

Proceedings

- Panel of experts in this field was assembled on April 17-18, 1989, to discuss the feasibility of using bioremediation in Alaska
- Recommended EPA to apply fertilizer (the addition of nutrients) on small scale plots

Questions raised

- Could the technology be applied to an environment so cold?
- Were there favorable conditions for degradation of petroleum hydrocarbons?
- Is Prudehoe Bay Oil of sufficient quality for biodegradation?

Proceedings (Contd.)

- Scientists knew that this type of oil was degradable from past studies
- Now they needed to know the concentrations of native hydrocarbons degraders.

Is there anything called native hydrocarbon degrader? If so, How?

- Naturally Hydrocarbons have been added to the environment by pine tree droppings and natural seeps for millions of years
- Carbon-hydrogen bonds of hydrocarbons yield very high energy
- This became an excellent energy source that allowed a complex community to evolve over millions of years to degrade these hydrocarbons
- Also, isolated in Prince William Sound that degrade petroleum and it’s products

- So, they expected accumulation of these organisms in the oil spilled areas
- Found **10,000-fold increase** of oil-eating microbes in contaminated areas

What were the organisms found?

Acinetobacter Calcoaceticus
Arthrobacter/Brevibacterium sp.
Oceanospirillum sp.
Pseudomonas putida
Pseudomonas sp.
Trichosporon sp.

Alcaligenes sp.
Flavobacter/Cytophaga sp.
Pseudomonas fluorescens
Pseudomonas stutzeri
Pseudomonas vesicularis
Vibro sp.

This large community of microorganisms made it unnecessary to introduce microbes.

Any other limiting factors?

- Scientists knew that biodegradation was occurring. But to speed up the process…
- Wanted to know the limiting factors
- Nitrogen and Phosphorous in seawater are severely limiting to microbial hydrocarbon degradation (*had already been published*)
- Is Oxygen a limiting factor? – No
- Is Temperature a limiting factor? - Yes

(ranging from 32°F in the winter to as high as 68°F in the summer) – slows down the process

Alaskan Bioremediation Project

- Started on May of 1989 on Knight Island
- This project was designed to determine the feasibility of biodegradation enhancement by adding fertilizers
- Nutrient applications began on June 8, 1989 at Snug Harbor, located on the southeastern side of the island

Selected as the first test site because it had a long length of shoreline with several beach materials

Positive Results?

- Approximately after 10 to 14 days visual reductions in the amount of oil covering the rocks were apparent
- also a visual reduction in the oil-covered sand and gravel beach
- Further tests proved that this reduction is due to BIOREMEDIATION and to prove that this is not due to chemical cleaning.

Application

Based on the *promising results* of the initial field test at Snug Harbor and the *absence of any adverse effects* on the area's ecosystem, EPA recommended in July that the bioremediation efforts be scaled up during summer.

By the end of the summer of 1989, 74 miles of shoreline were treated with nutrient applications

The Exxon Valdez Disaster- 20 Years Later.mp4

Il concetto di *priming effect*

Effects of water-soluble organic substrates on the stabilization and mineralization of lignin and peat

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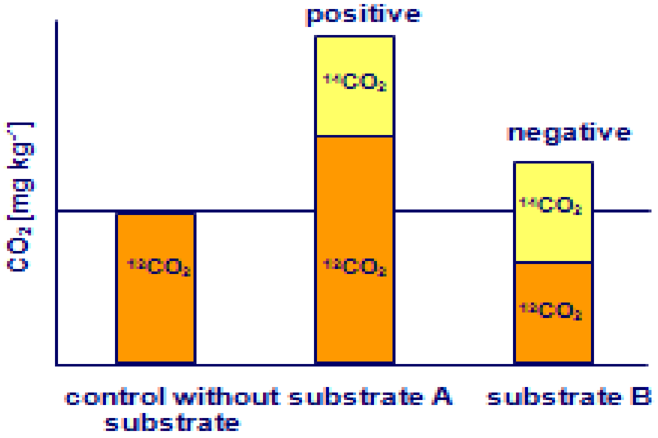
Introduction

Dissolved organic matter continuously enters the soil solution through leaching, root exsudation, desorption or decay processes. It is available for microorganisms and may have a high potential in triggering priming effects. Lignin and peat are organic substances that can be relatively resistant to biodegradation.

priming effects (schematic)



Objective

Identification of DOM-typical substrates that accelerate (positive priming) or retard (negative priming) the decomposition of the model substances.

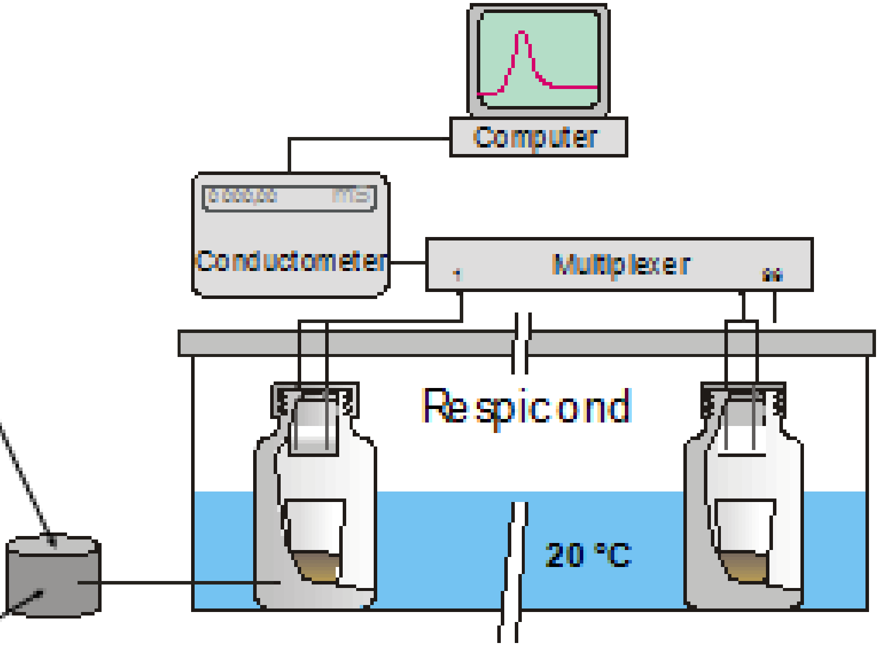
Materials and Methods

DOM-typical substrates (^{14}C-labeled):

- glucose, fructose, glycine, alanine, oxalic acid, acetic acid, catechol
- addition: 80 µg C g^{-1} sand or 400 µg C g^{-1} sand and approx. 3000 Bq

Model system:

- 50 g sand + lignin or peat (3 % C$_{org}$)
- pH: 6.5
- nutrient solution: NPK
- inoculum: batch extract from O-horizon of a forest soil
- water content: 60 % of WHC



Incubation for 26 days in the Respicond
(*Nordgren* 1988)

- measurement of total CO$_2$-evolution hourly
- measurement of ^{14}CO$_2$-evolution at days 4, 6, 12, 19 and 26

Lignin and peat mineralization

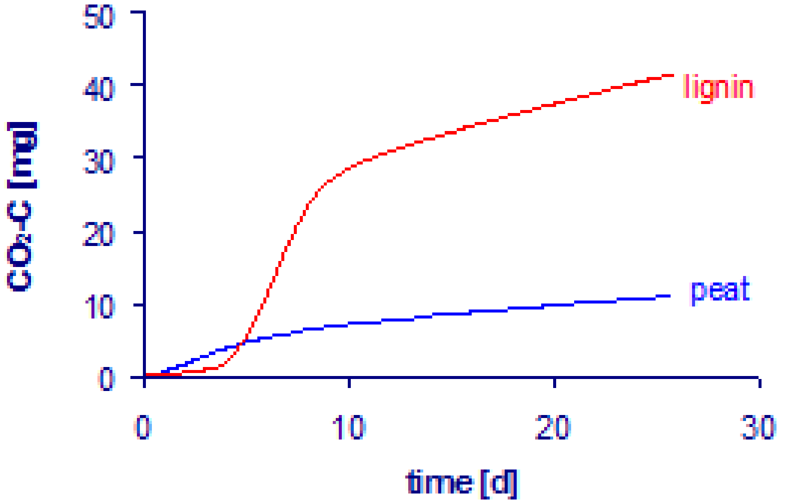


Fig. 1: Cumulative CO_2-C evolution from the unamended peat and lignin system (initial carbon content of 1500 mg C).

- after 26 days of incubation lignin mineralization (2.8 %) was nearly fourfold higher than peat mineralization (0.7 %)
- lignin mineralization started after a lag-phase of 5 days

Dynamics of priming

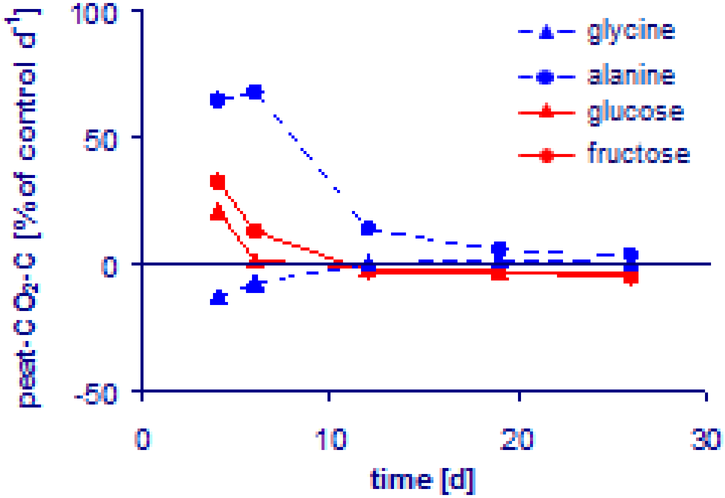


Fig. 2: Dynamics of priming effect intensity after high substrate addition (400 µg C g^{-1}) to the peat system in percent of control.

- strongest priming occurred during the first 4 to 10 days of incubation

Priming effects for peat

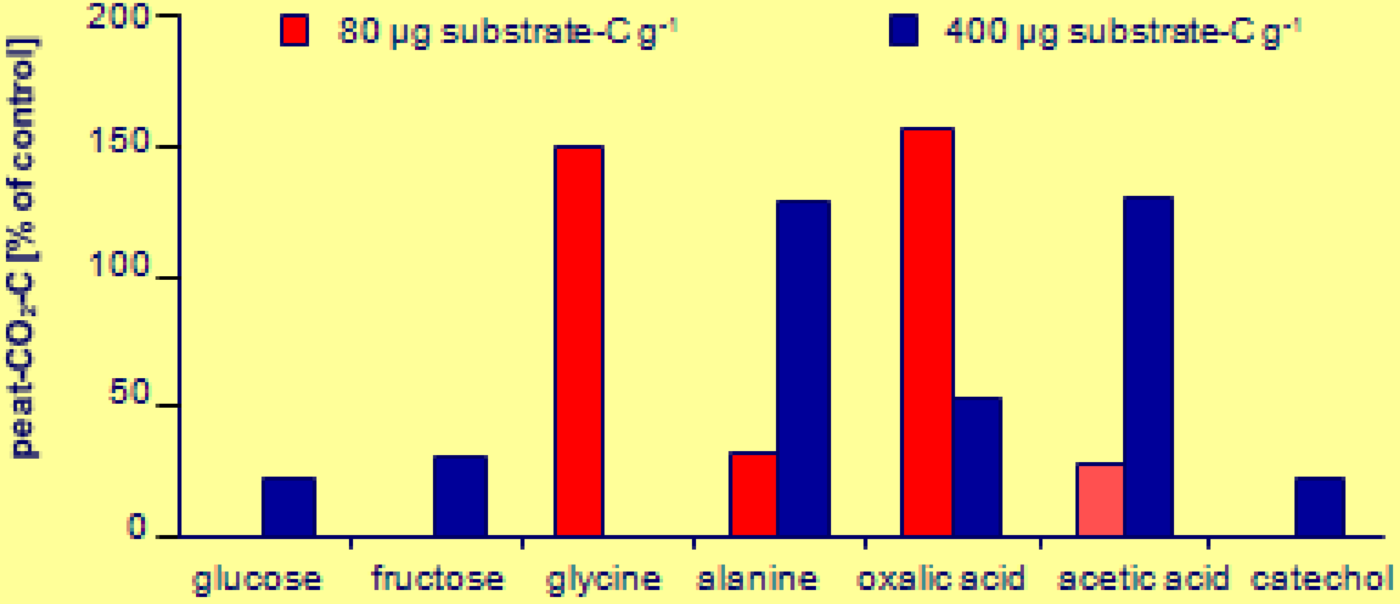


Fig. 3 a: Peat mineralization after substrate addition compared to control after 26 days of incubation.

- only positive priming effects or no priming occurred
- 80 µg oxalic acid-C caused the strongest positive priming effect
- the low addition of glycine and oxalic acid caused stronger effects than the high addition

Priming effects for lignin

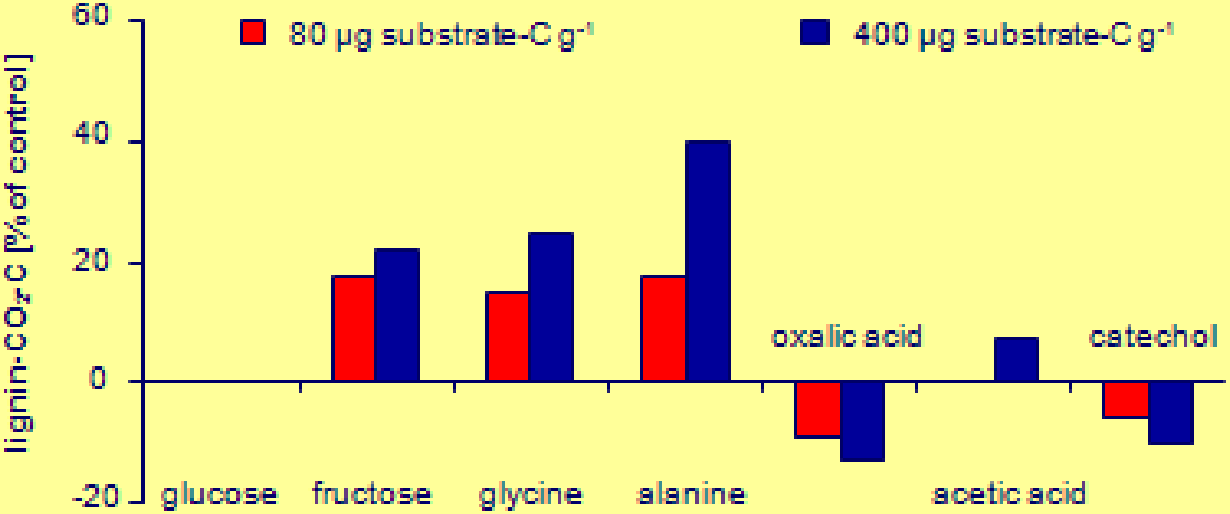


Fig. 3 b: Lignin mineralization after substrate addition compared to control after 26 days of incubation.

- oxalic acid and catechol caused negative priming effects
- 400 µg alanine-C caused the strongest positive priming effect
- compared to high substrate addition the low addition is in most cases more effective

Possible Mechanisms of positive priming

- cometabolism
- degradation of microbial biomass

Possible Mechanisms of negative priming

- inhibition of microbial activity or enzymes
- change of nutrient source from lignin to easier degradable substrates
- rearrangement in the lignin structure to more stable units in the presence of substrate molecules

Future research

- Can priming effects be triggered through repeated substrate addition ?
- Are priming effects influenced by soil organic matter quality ?
- Can priming effects be triggered with "real" DOM-solutions ?
- Are priming effects associated with changes in microbial biomass and population ?

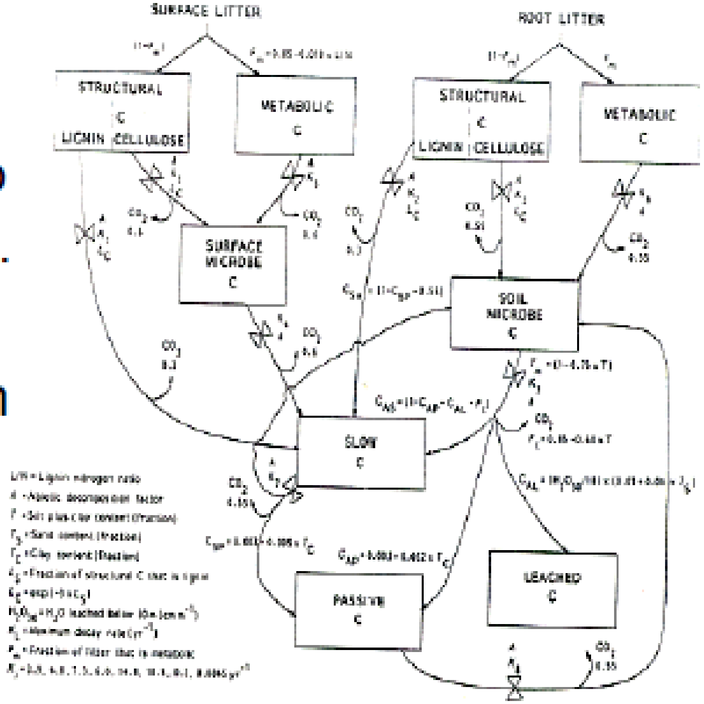
The priming effect and its implication for soil modeling

S. Fontaine *et al.*
INRA Clermont Ferrand

Workshop on ‘Disentangling abiotic and biotic effects on soil respiration’
Innsbruck, 12th - 13th March 2007

Is microbial ecology useful to predict soil C,N cycling in soils?

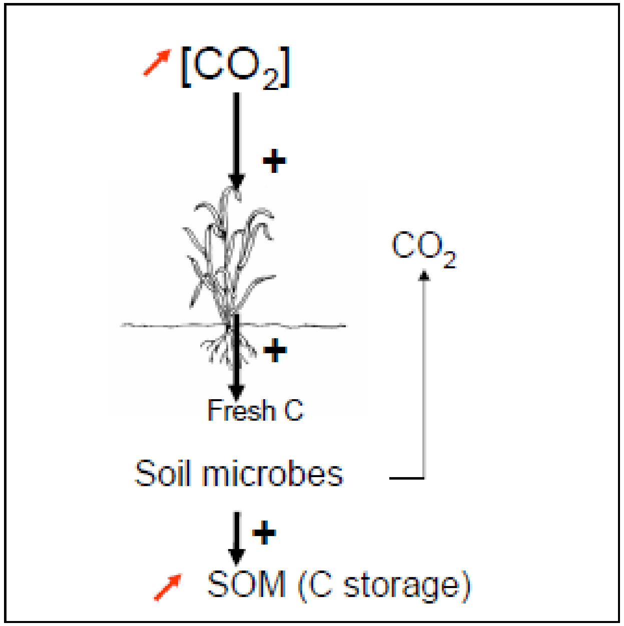
- The microbial component is not (or rarely) taken into account in current models.
- Decomposition process is modeled with the equation dC/dt=-kC.
- Do these models predict soil C cycling efficiently?



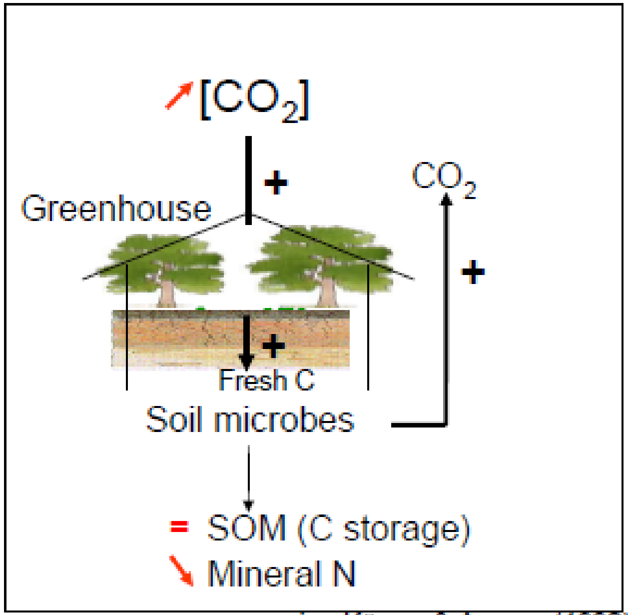
An example of model: Century (Parton)

How will ecosystems respond to an increase in atmospheric CO_2?

Model predictions



Experiments at the ecosystem scale



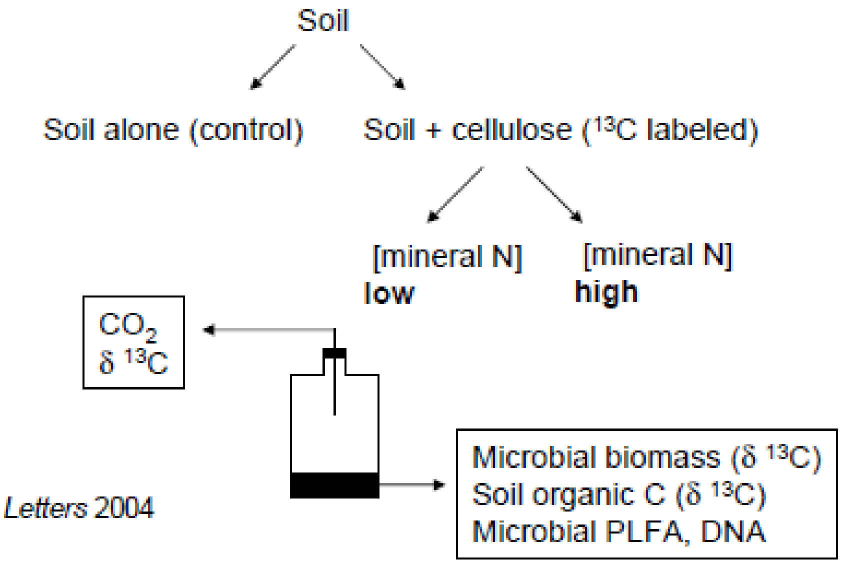
i.e. Körner & Arnone (1992)

An unknown mechanism limits SOM accumulation in ecosystems under elevated CO_2.

Experiment

What are the mechanisms controlling SOM accumulation in soils?

- Laboratory incubation of soil microcosms
  - Objective: to determine the impact of fresh litter input and nitrogen availability on the formation/decomposition of SOM.



Soil

Soil alone (control)

Soil + cellulose (^{13}C labeled)

[mineral N] **low**

[mineral N] **high**

CO_2
$\delta\ ^{13}C$

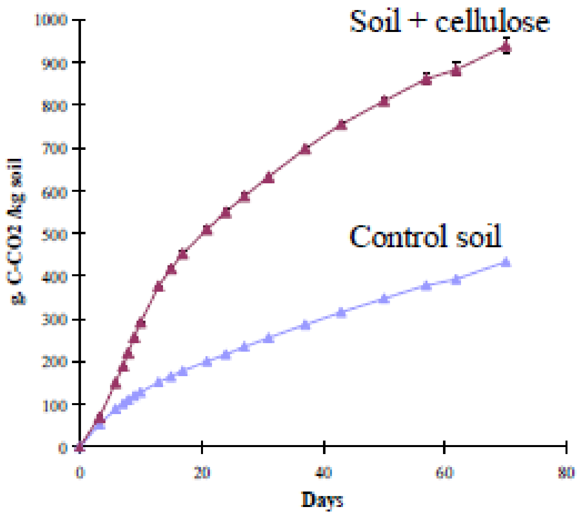
Microbial biomass ($\delta\ ^{13}C$)
Soil organic C ($\delta\ ^{13}C$)
Microbial PLFA, DNA

Fontaine et al., *Ecology Letters* 2004

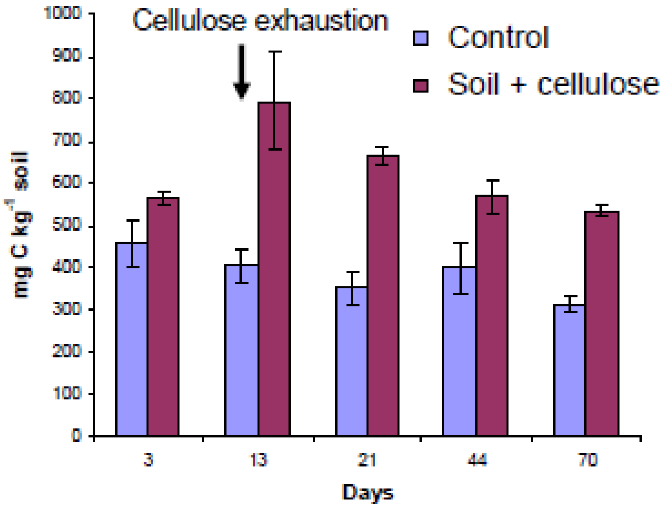
Experiment

The supply of cellulose stimulates microbial respiration and growth.

Total soil respiration (Low N treatment)



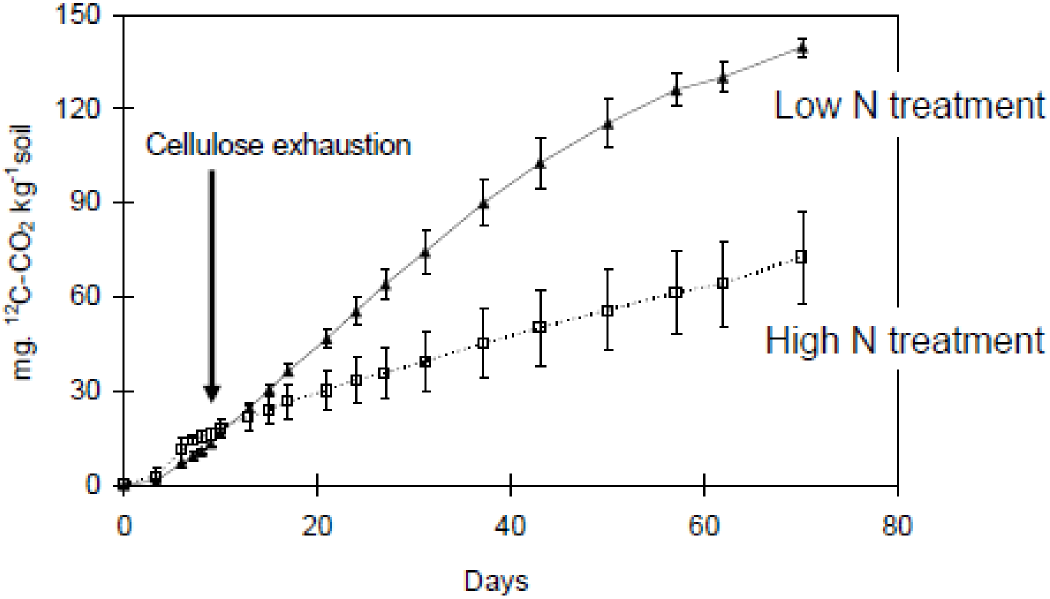
Total microbial biomass (Low N treatment)



✓ **Microbial activity is limited by C despite high SOM content**

Experiment

The stimulation of microbes induces an over-production of unlabeled soil-originated CO_2.



The supply of fresh substrate increases the rate of old soil C decomposition, which characterizes the priming effect.

The priming effect is 2 times higher in the low N treatment compared with the high N treatment.

What is the effect of cellulose supply on soil C storage?

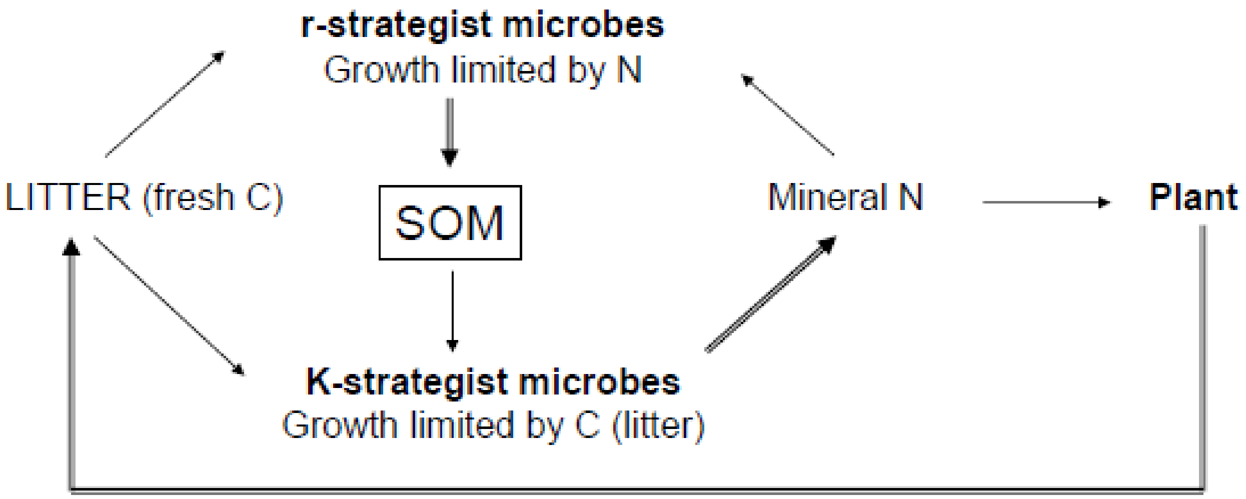
	Nitrogen treatments	
	High N	Low N
New soil C (^{13}C)	140	110
Old soil C (^{12}C) lost by the priming effect	-73	-140
Soil C balance	**+68**	**-30**

in mg C kg^{-1} after the addition of 500 mg cellulose.

✓ Carbon input to soil may decrease soil C content because of the priming effect.

✓ The availability of nitrogen controls the direction of soil C change.

What are the mechanisms controlling the accumulation of soil organic matter?



Alternative theory of SOM dynamics (Fontaine & Barot, *Ecology Letters* 2005)

✓**The theory predicts a chain of interactions between the availability of mineral N, the relative abundance of two microbial functional types and finally the change in the SOM pool.**

✓**The theory shows that plant persistence in ecosystems depends on this chain.**

Conclusions & perspectives

- Several important empirical results on soil C cycling cannot be explained by current theories of SOM dynamics (priming effect, ecosystem response to elevated CO_2, lack of decomposition at depth etc).
- Dynamics, diversity and stoichiometric constraints of microbe populations could explain these results (Fontaine & Barot, 2005).
- A new generation of soil models is needed in which decay rate is controlled by the biology and the ecology of microbes.
- To this end, we have:
  - to find a new mathematical equation describing decomposition
  - to identify the microbial functional types involved in the priming effect (PLFA, DNA and labeling).