Aerobic hydrocarbon degradation

Learning goals:

1) Principle biochemical mechanisms of hydrocarbon degradation

2) Central pathways and metabolites: aerobic, anaerobic

3) Latest research results: role of aerobic organisms?

Organism of the day
Pseudomonas aeruginosa

Systematics

class: Gammaproteobacteria

order: Pseudomonadales

family: Pseudomonadaceae

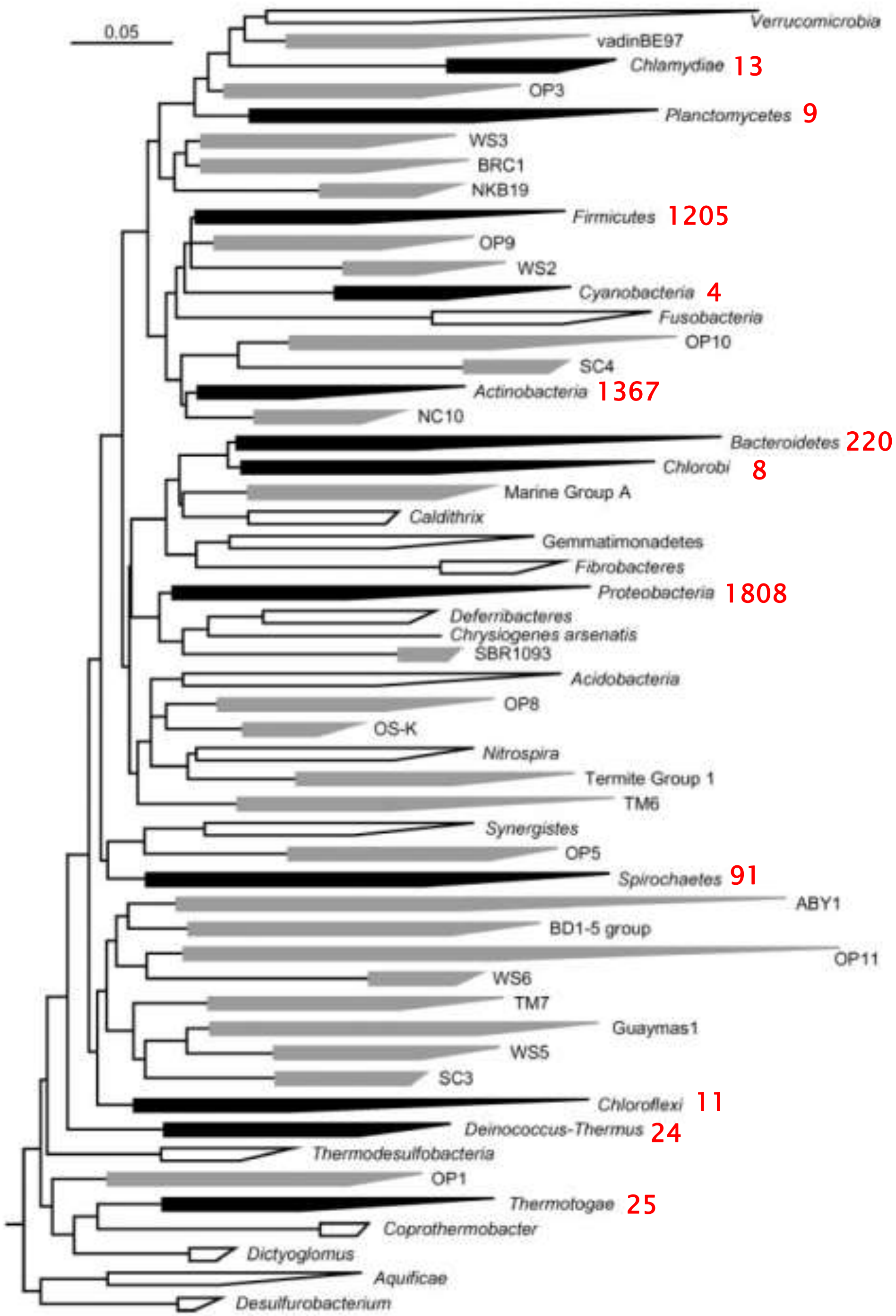
genus: Pseudomonas

species: Pseudomonas aeruginosa

Aerugo: greek Grünspan, (copper oxide)

- metallic-green shine on agar

- species name comes from the blue-green slime in wounds



The uncultured majority

n = published species

- Black: 12 original Phyla
  (Woese 1987)
  many pure cultures
- White: 14 new phyla since 1987
  some isolates
- Gray: 26 candidate phyla
  no isolates

➔ What are they all doing ?

Rappé & Giovannoni (Annu Rev Microbiol, 2003)
Keller & Zengler (Nat Rev Microbiol, 2004)

Features

- Aerobic Bacterium, facultatively anaerob
- An important nosocomial germ.
- Definition: N**osocomial Infections** (hospital infections) are induced by microorganisms within the first 48 h after a hospital stay. „Nosokomia“ were the rooms of the santorium in antique Greece where patients were treated by „health sleeping“ (f.e. in Epidauros)
- Produces infection of wounds, burns
- Pneumonia, especially in immune deficient patients
- and AIDS-Patienten.
- Urinal tract infections, Meningitis.
- Does hemolysis and produces toxins.

P. aeruginosa is often found at Cystic Fibrosis patients

- CF-Patients: Defect in chloride chanels
- Little mucus and lack of efficient coughing out of slime and bacteria
- Chronic infections over decades
  - Protection against antibiotics
  - High resistance and mutator strains

Features

- Degradation of *n*-alkanes in diesel oil by *Pseudomonas aeruginosa* strain WatG (WatG) was verified in soil microcosms.
- bioavailability of phenanthrene (PHE) enhanced in the presence of rhamnolipid biosurfactant and/or a biosurfactant-producing bacterium, *Pseudomonas aeruginosa* ATCC 9027.
- Can grow on detergents like SDS
- others

Biochemistry of aerobic hydrocarbon degradation

- Monooxygenase Alkanes
- Dioxygenase, toluene, PAH
- Ring-cleaving dioxygenases,
- Epoxide-forming monoxygenases (Benzene)

Which contaminants are important?

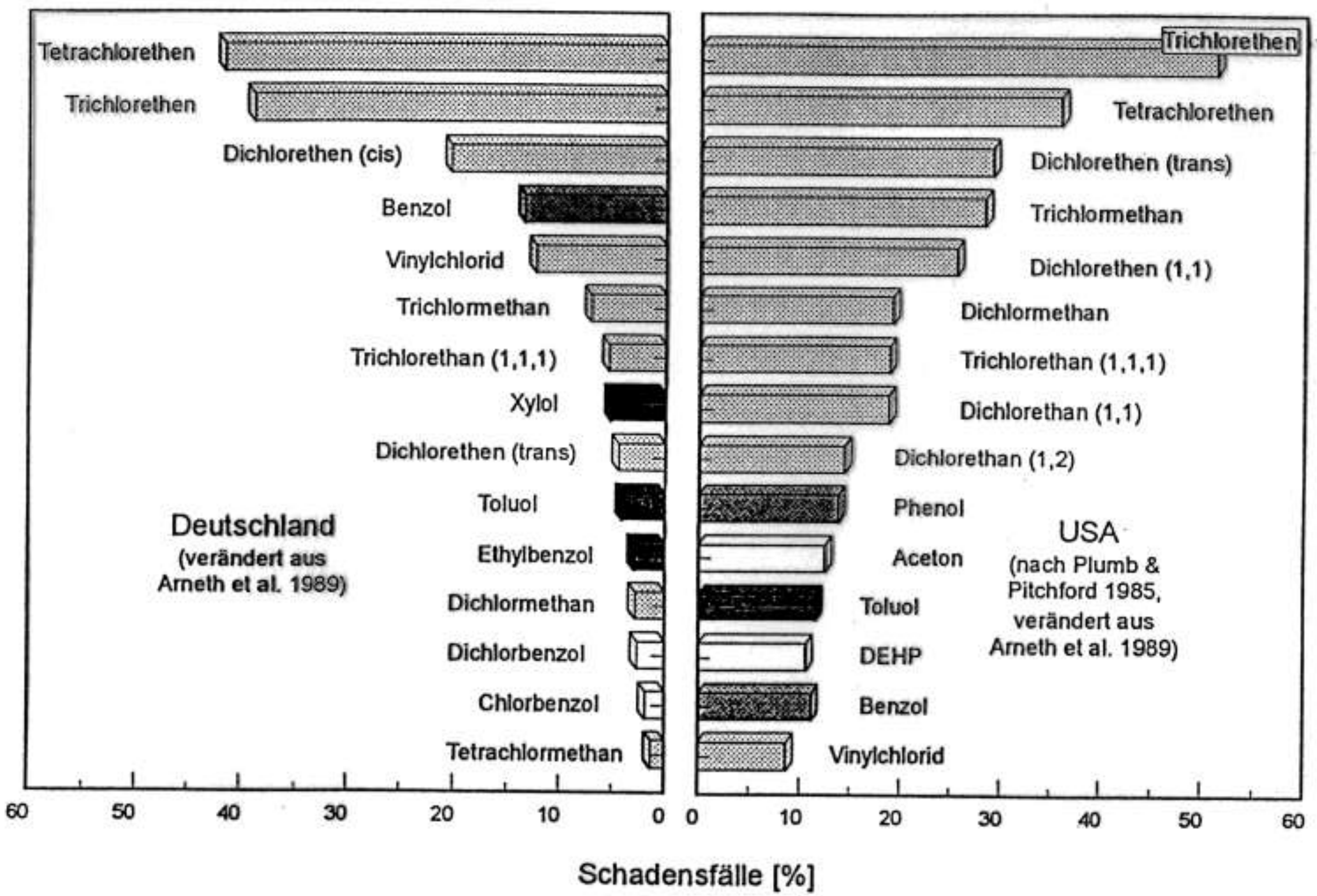
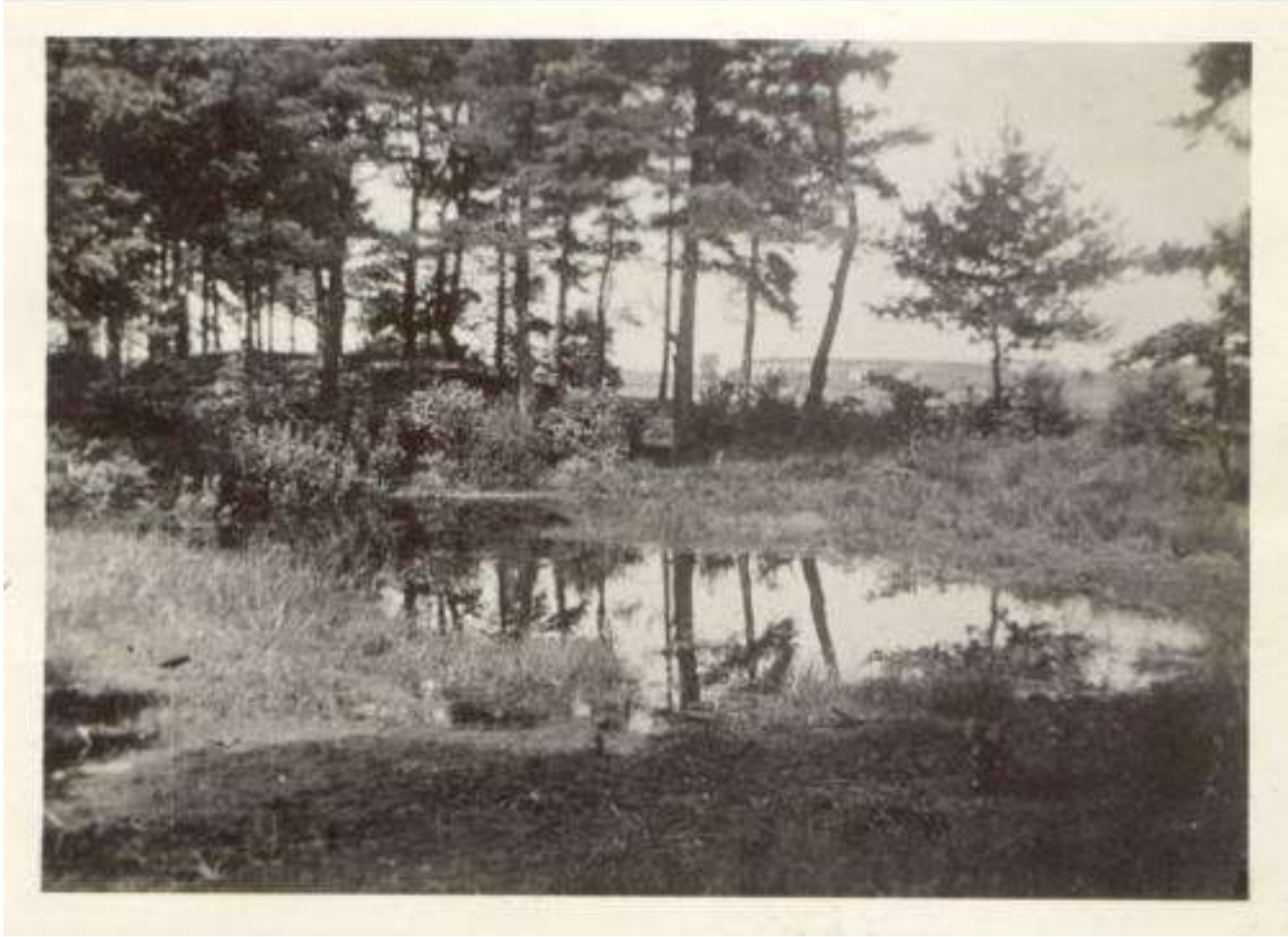


Abbildung 1.2: Häufigkeit einzelner organischer Schadstoffe im Abstrom von Schadensfällen. Vergleich USA - Deutschland

Where has this picture been taken?

Wietze, Lüneburger Heide, around 1900

Natural oil seep in Wietze

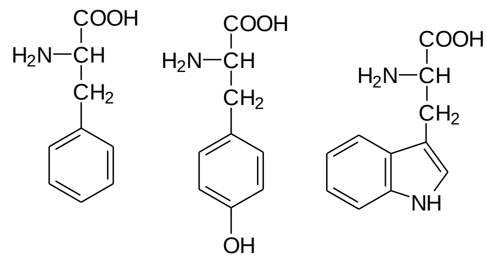


Oil production 400 years ago

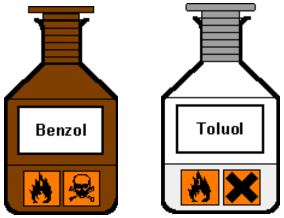
Oil sand production (1950)
height 60 m, 1 Mill m3

Aromatics in nature

Lignin



amino acids



Oil, coal

Degradation by microbes

Flavonoides

Phenols

Tannines

Lignanes

Quinones

+ O_2 → CO_2

- O_2 → CO_2

Biodegradation pathways, aerobic

- Oxygen is special because it is needed as electron acceptor and co-substrate
- Oxygenase reactions for alkanes and aromatic hydrocarbons

Aerobic degradation of alkanes

Figure 14.29 Brock

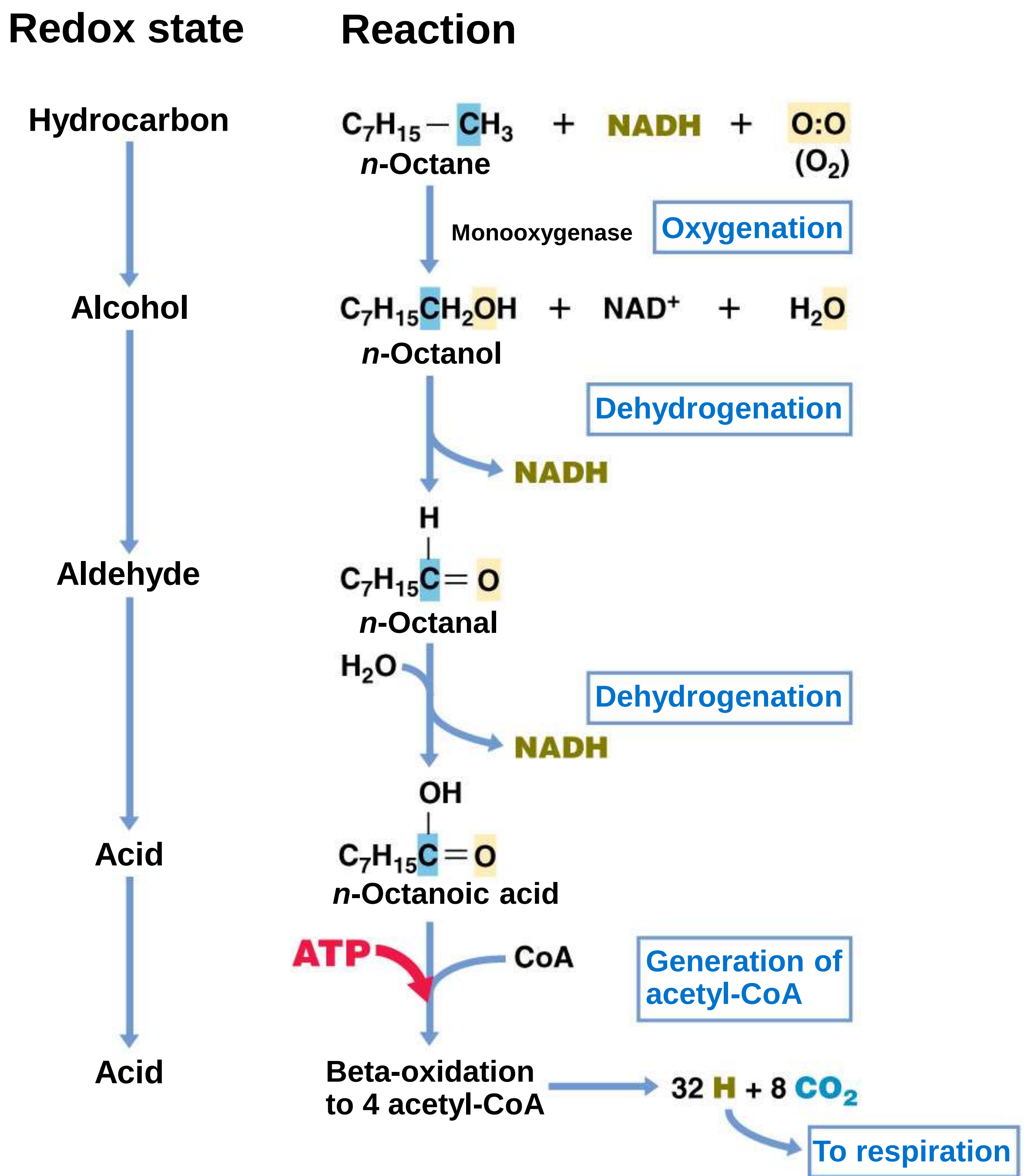
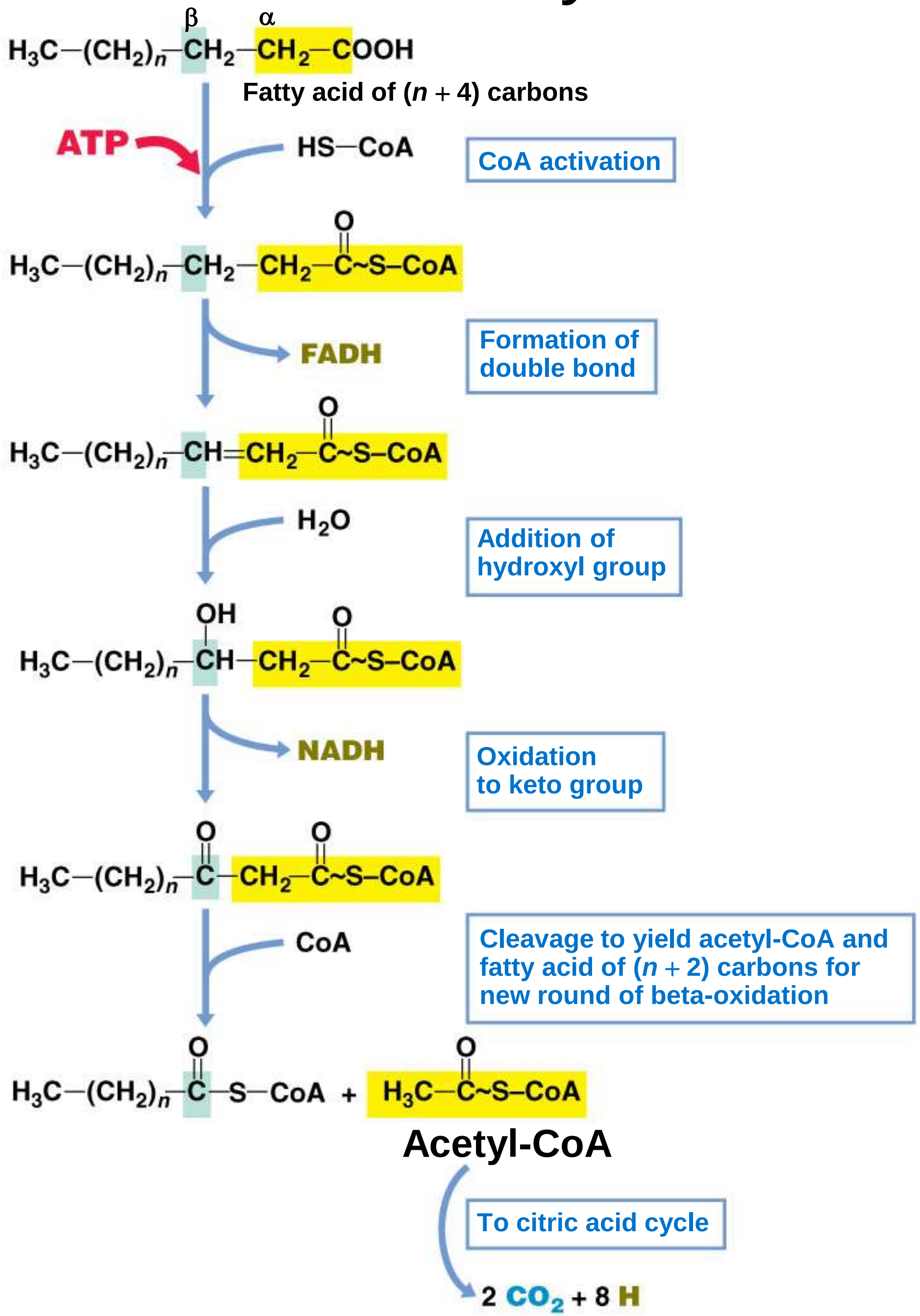


Figure 14.42

Beta-oxidation of fatty acids

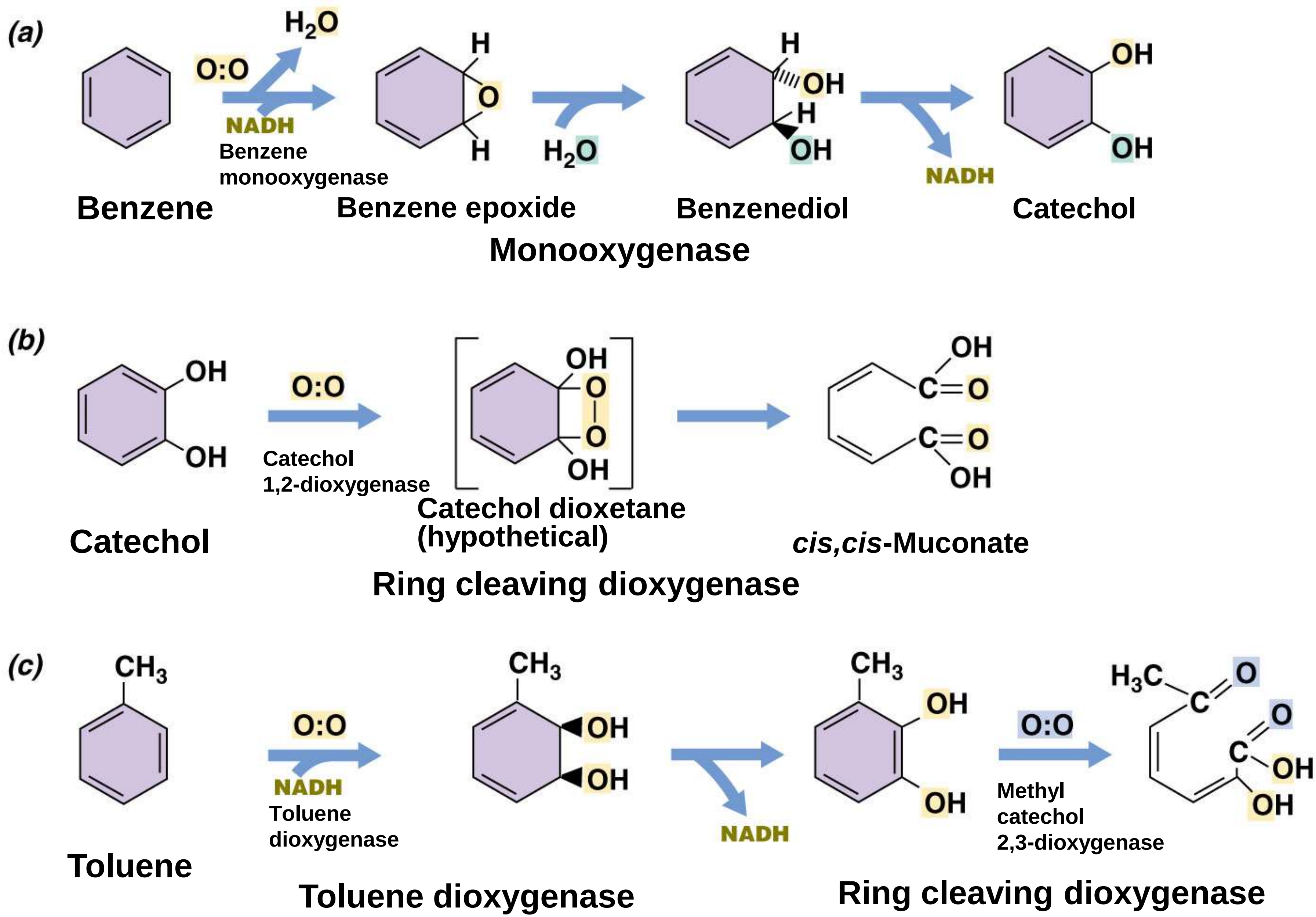


Question

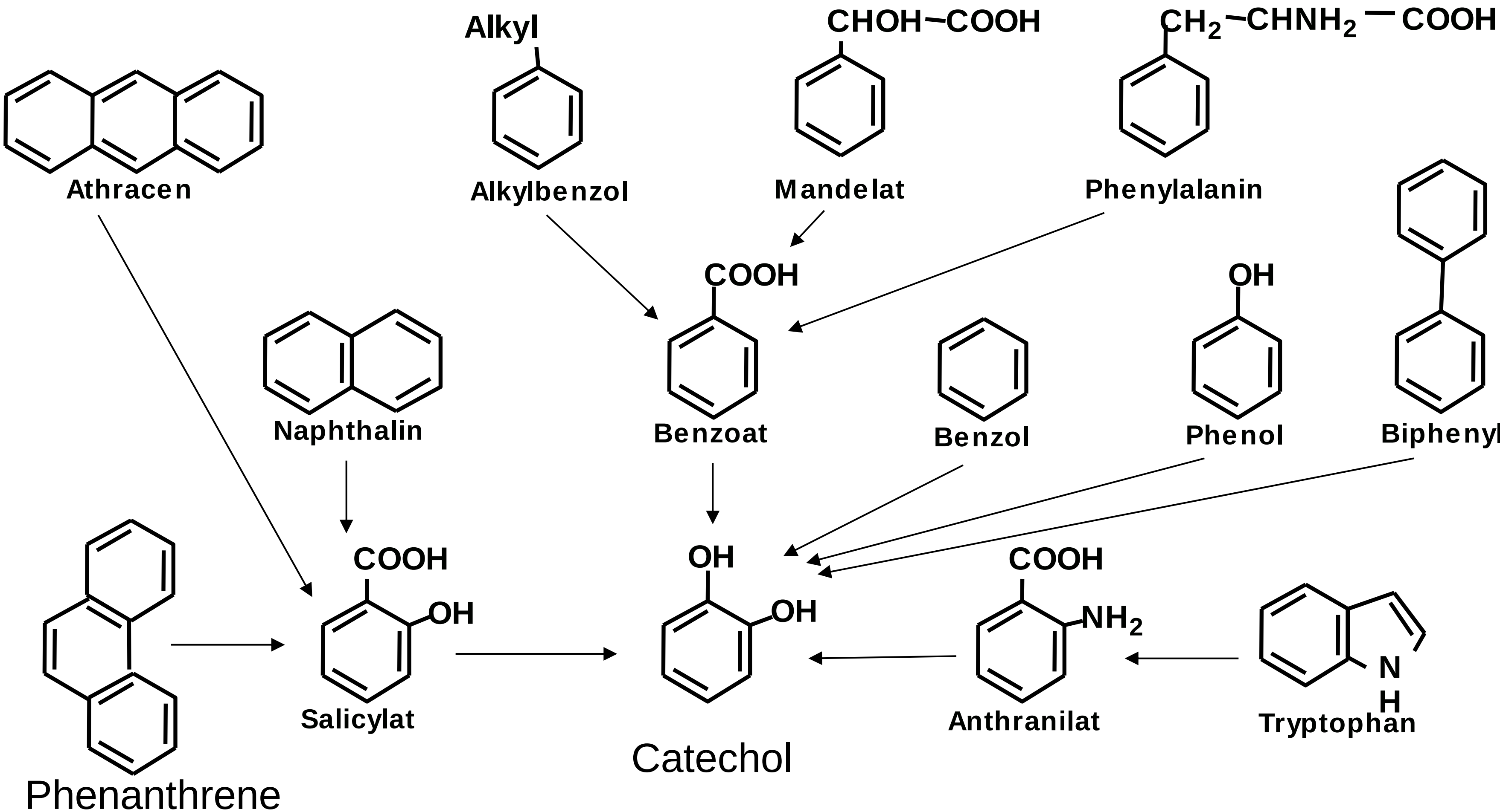
- How would you degrade crotonate?

Figure 14.30

3 types of oxygenases in aromatics degradation



Catechol as central intermediate / converging pathways

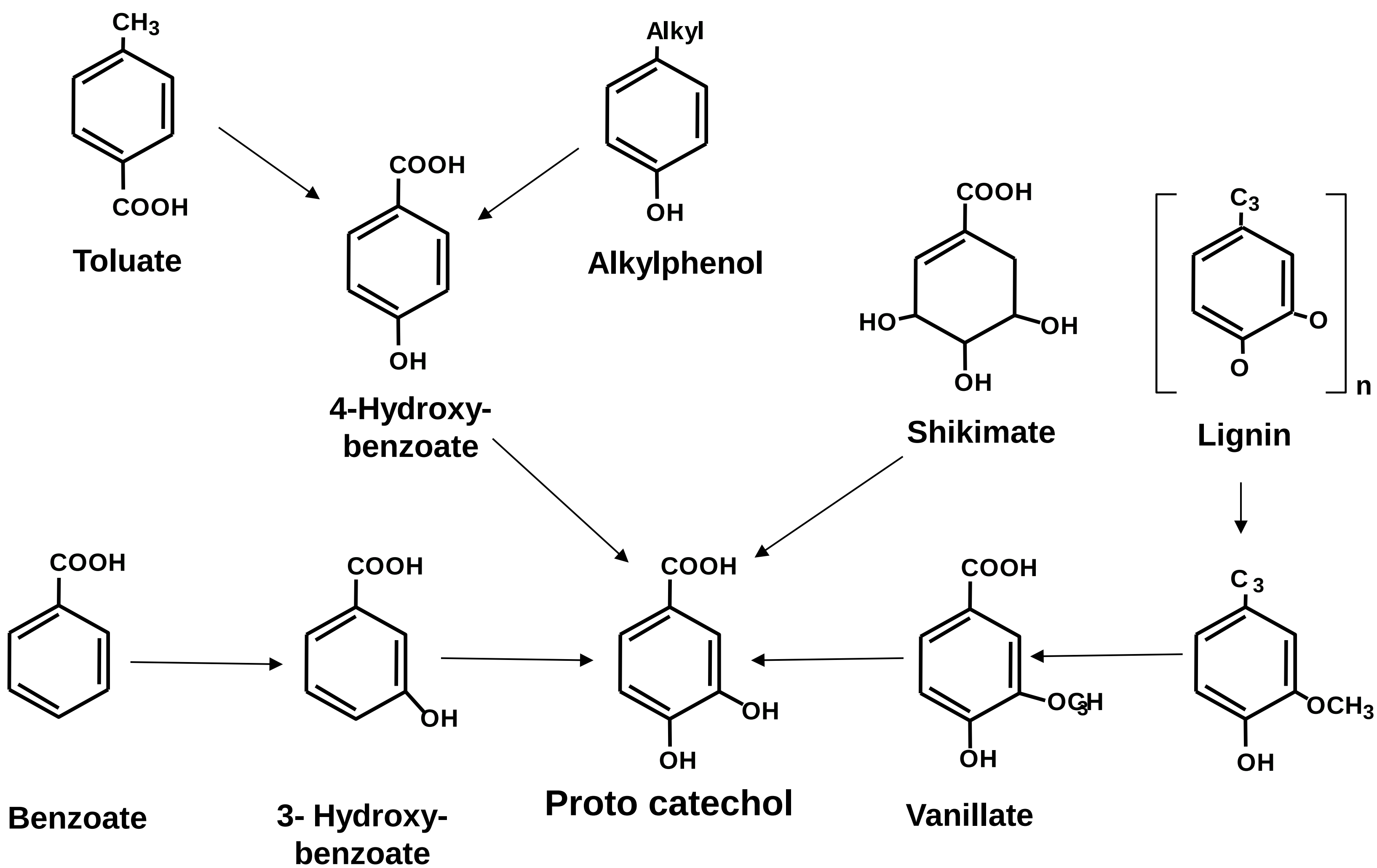


Pathways are ususally composed of genetic cassettes

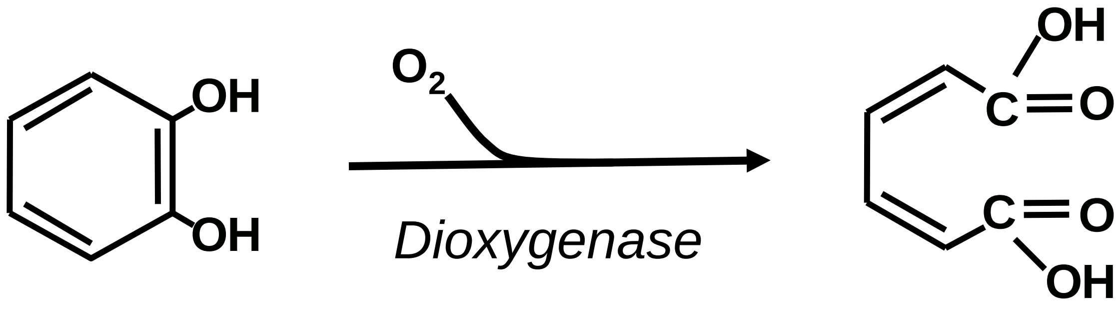
e.g: toluene

- upper pathway from toluene to catechol
- Lower pathway from catechol to muconic acid

proto-catechol as central intermediate

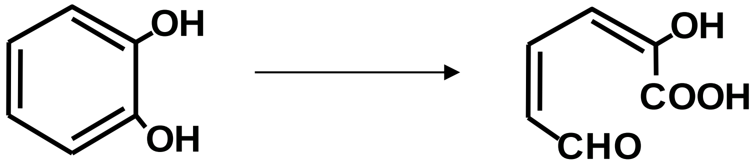


ortho-cleavage of the aromatic ring



Catechol **_cis, cis_- muconate**

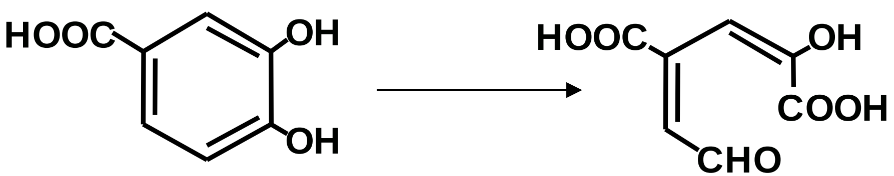
Meta-cleavage of the aromatic ring



Catechol

**2-Hydroxy-
muconat-
semialdehyd**

Metapyrocatechase
(Catechol-2,3-Dioxygenase)

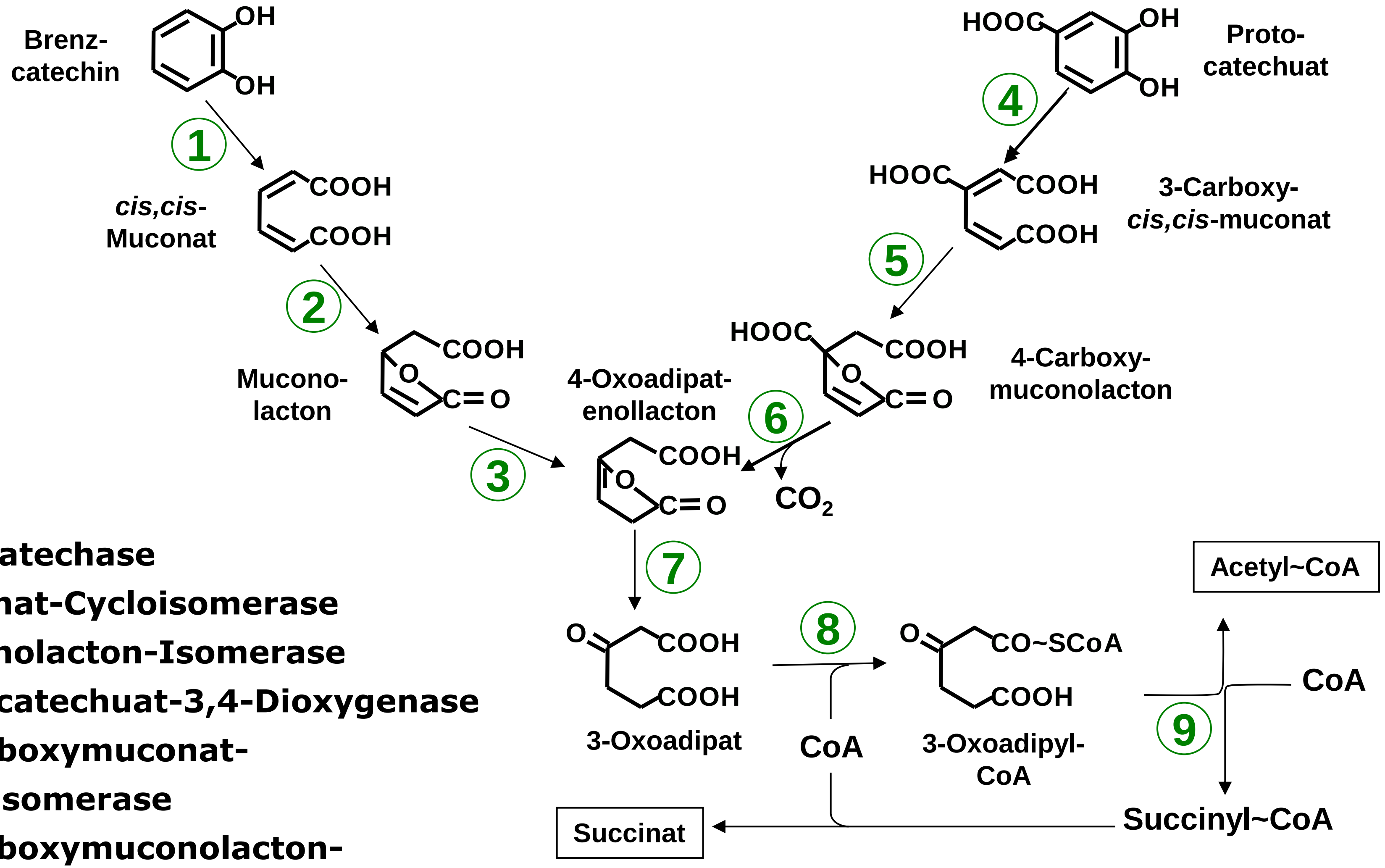


Proto-catechol

**2-Hydroxy-
4-carboxymuconat-
semialdehyd**

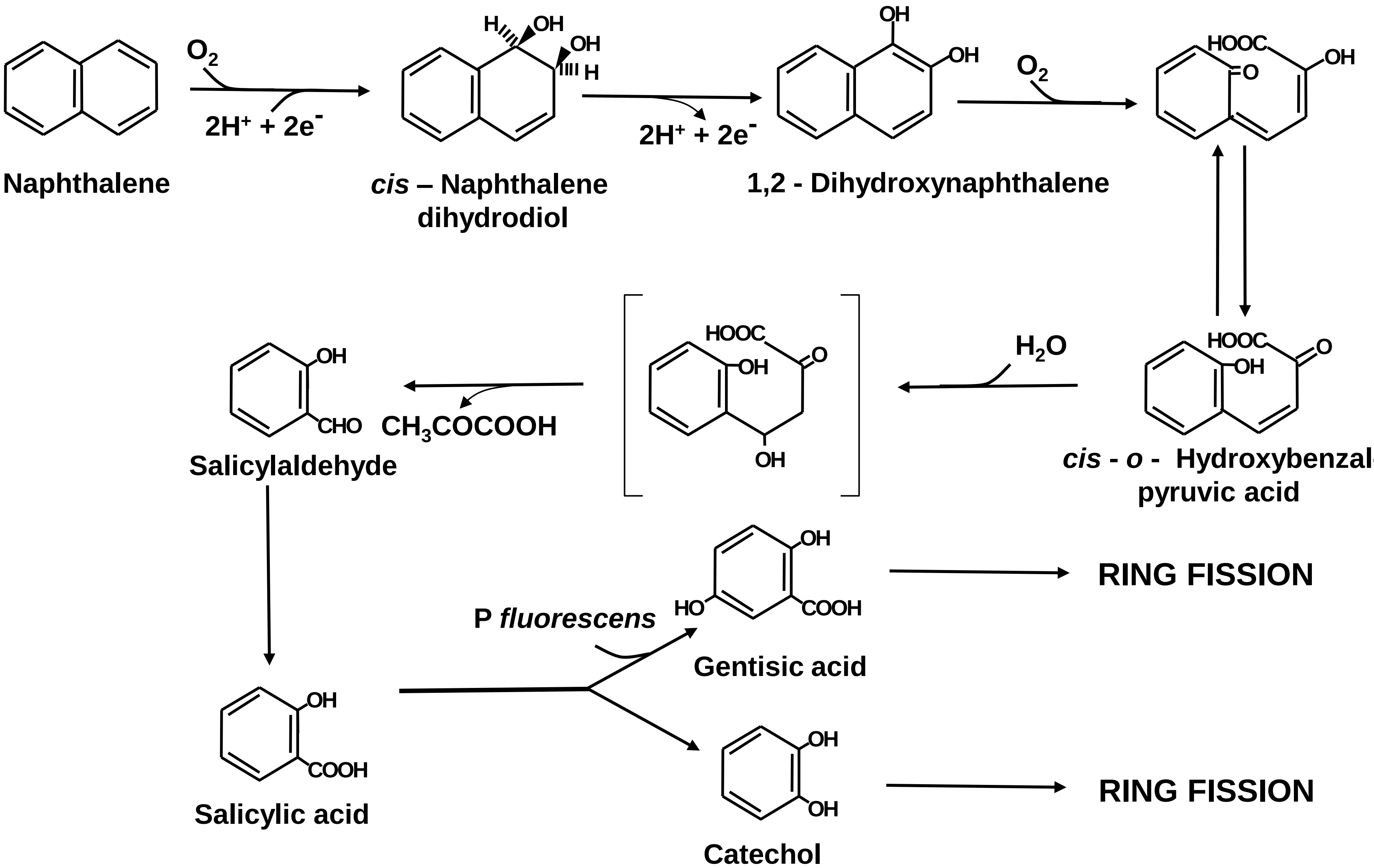
Protocatechuat-
4,5-Dioxygenase

Ortho-cleavage of the aromatic ring in 3-oxoadipate

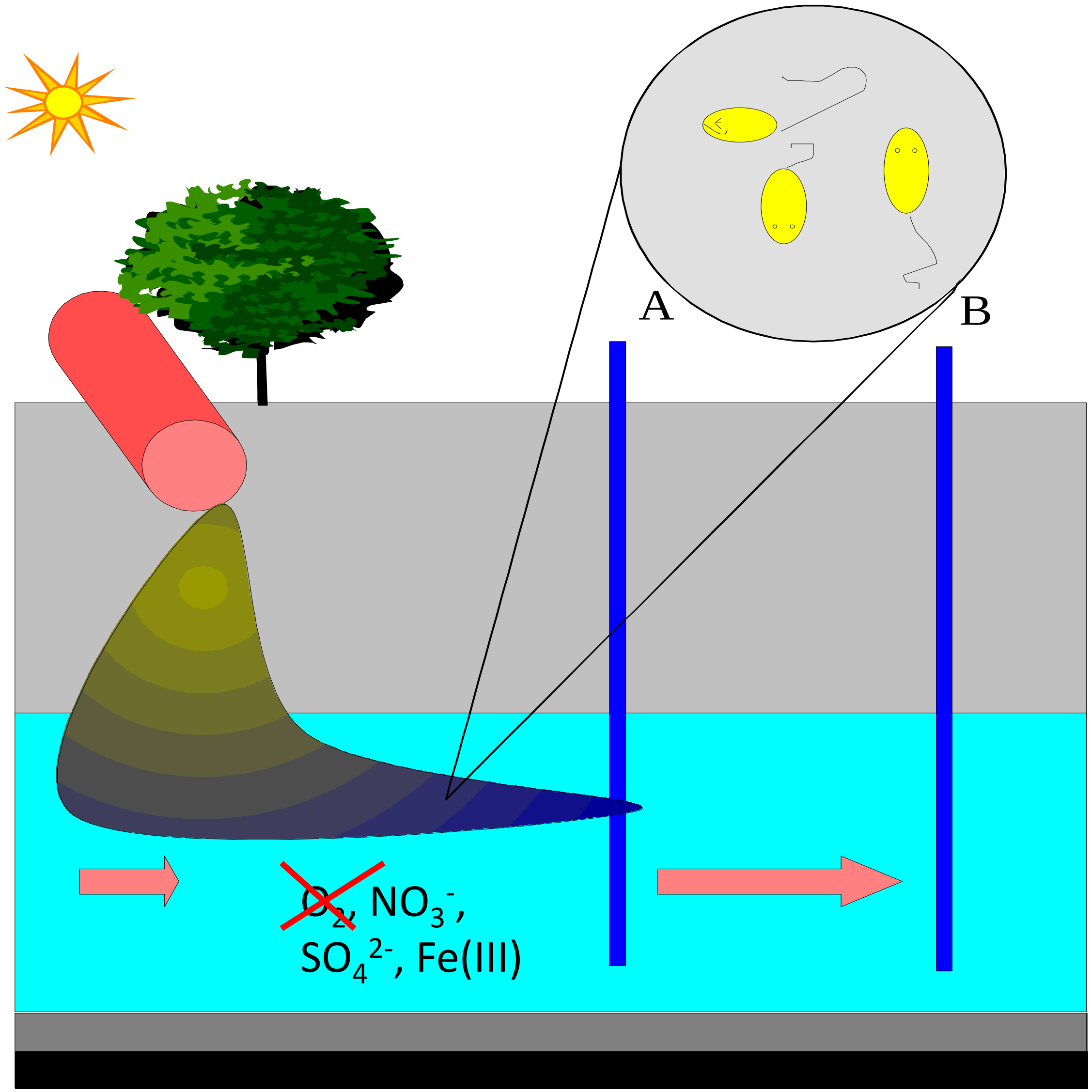


1. Pyrocatechase
2. Muconat-Cycloisomerase
3. Muconolacton-Isomerase
4. Protocatechuat-3,4-Dioxygenase
5. 3-Carboxymuconat-
   Cycloisomerase
6. 4-Carboxymuconolacton-
   Decarboxylase
7. 4-Oxyoadipatenollacton-Hydrolase
8. 3-Oxoadipat-Succinyl-CoA-Transferase
9. 3-Oxoadipyl-CoA-Thiolase

Proposed pathway for the degradation of naphthalene



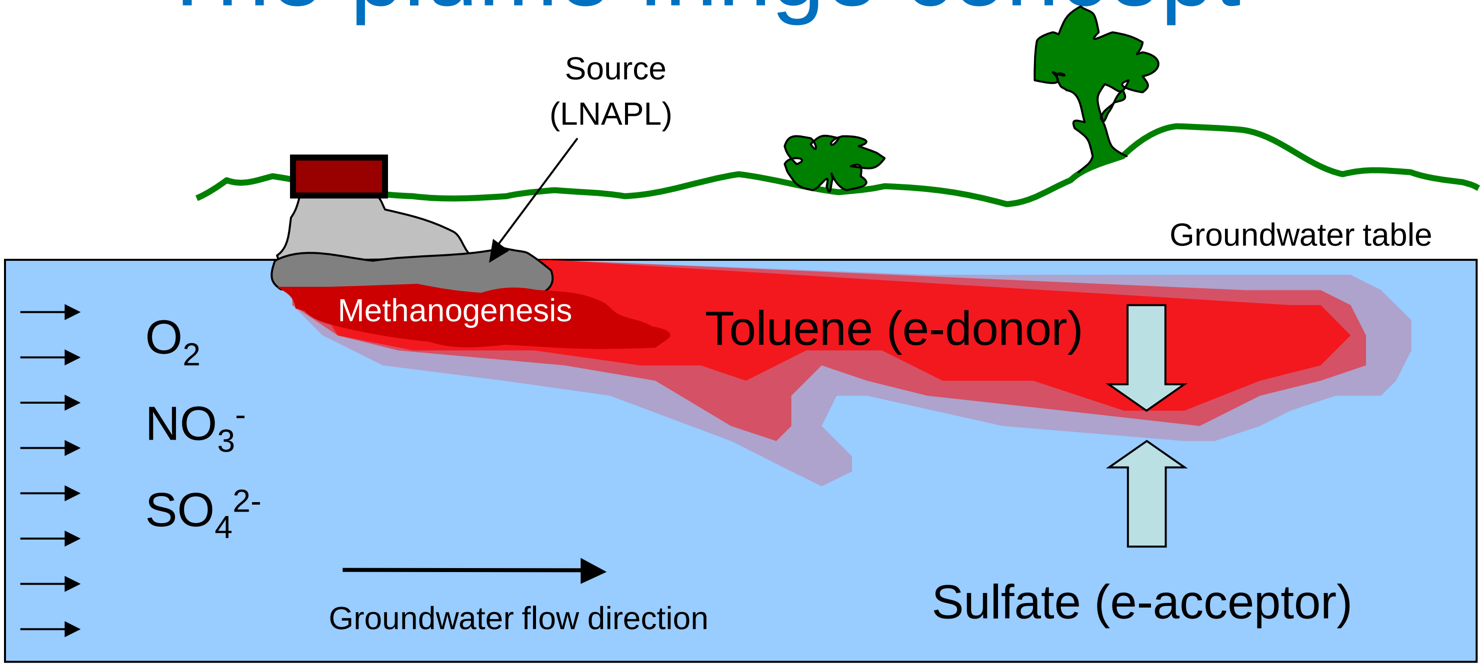
Where are aerobic activities important in contaminated aquifers?



- You find aerobes everywhere!

- But there is no oxygen

The habitat for biodegradation: The plume fringe concept

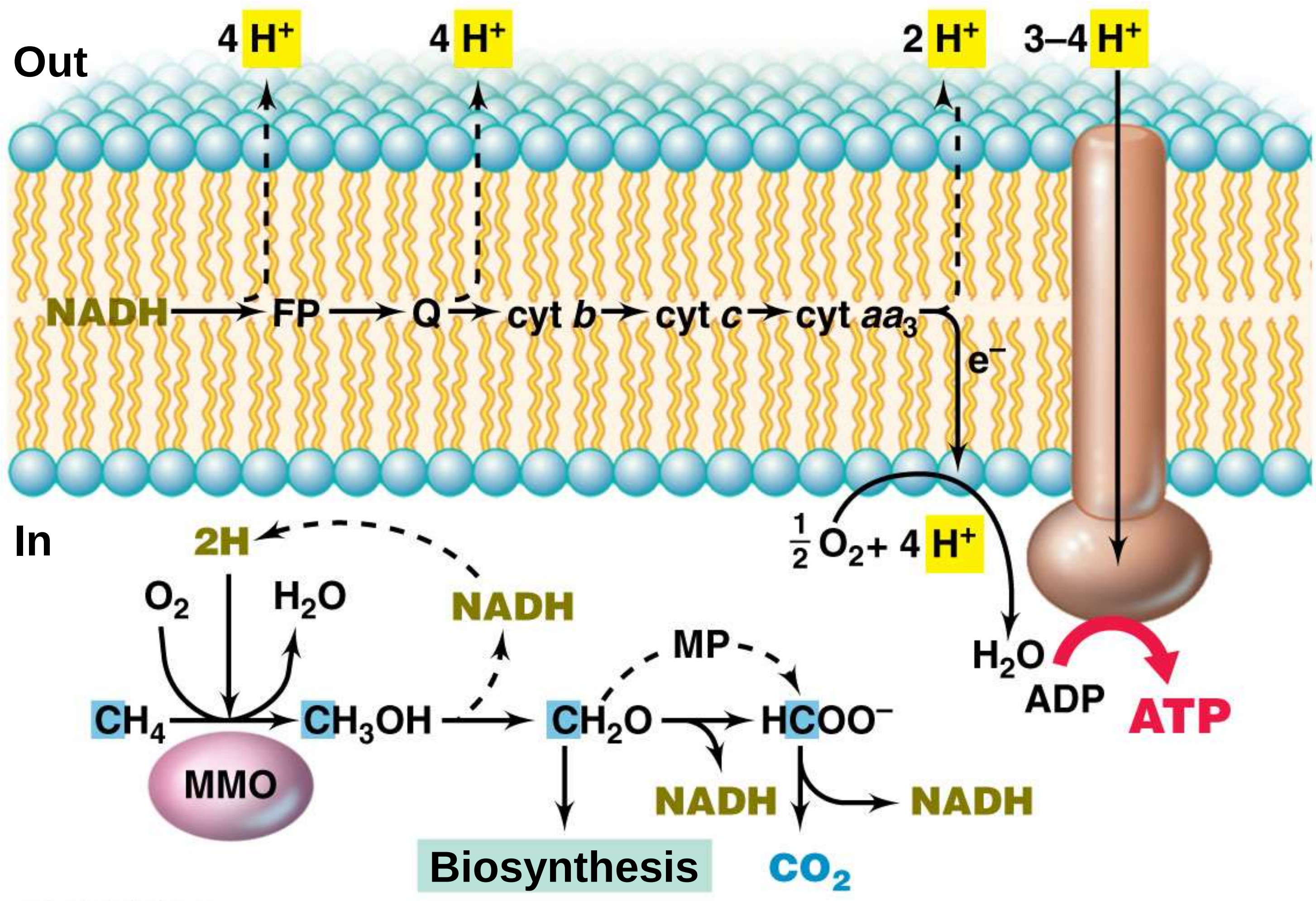


Bauer et al., J. Contam. Hydrol. 2008

Methane!!!

Figure 14.31

Aerobic methane oxidation



„Anaerobic“ methane oxidation → Nitrogen production from nitrite is not denitrification!

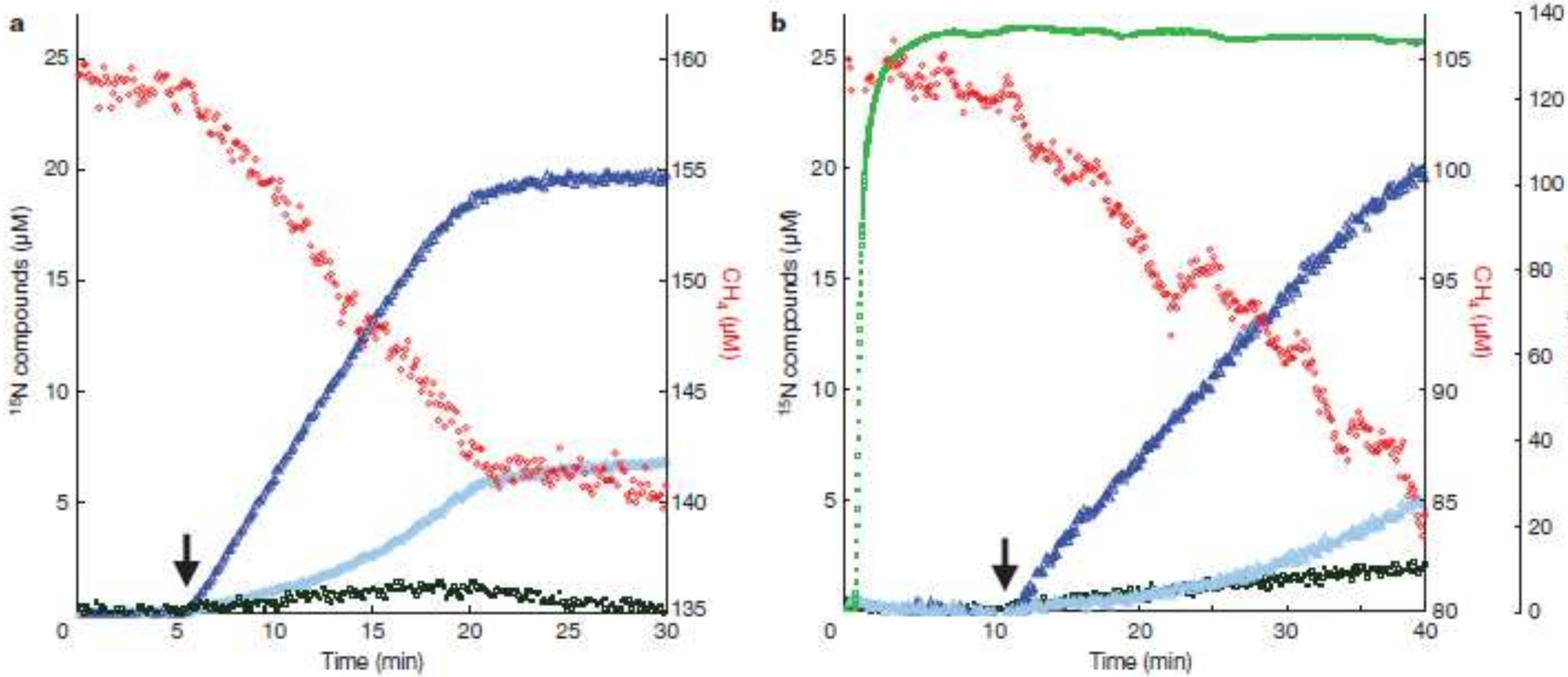


Figure 3 | Coupling of methane oxidation and nitrite reduction in enrichment cultures of '*Methylomirabilis oxyfera*'. Methane is oxidized only after addition of ^{15}N-labelled nitrite (50 µM, arrow), which is converted to ^{15}N-labelled dinitrogen gas in the presence of about 2,000 µM ^{14}N-nitrate (**a**) or 2,000 µM ^{14}N-nitrate and 135 µM ^{14}N-N_2O (**b**). Experiments were performed with 380 ml of anoxic, stirred enrichment culture 'Ooij' (protein content 147 ± 11 mg). Red circles, CH_4; dark blue triangles, $^{15,15}N_2$; light blue triangles, $^{15,14}N_2$; green squares, total N_2O; dark green squares, $^{14,15}N_2O$ and $^{15,15}N_2O$.

Taken from Ettwig et al., Nature (2010)

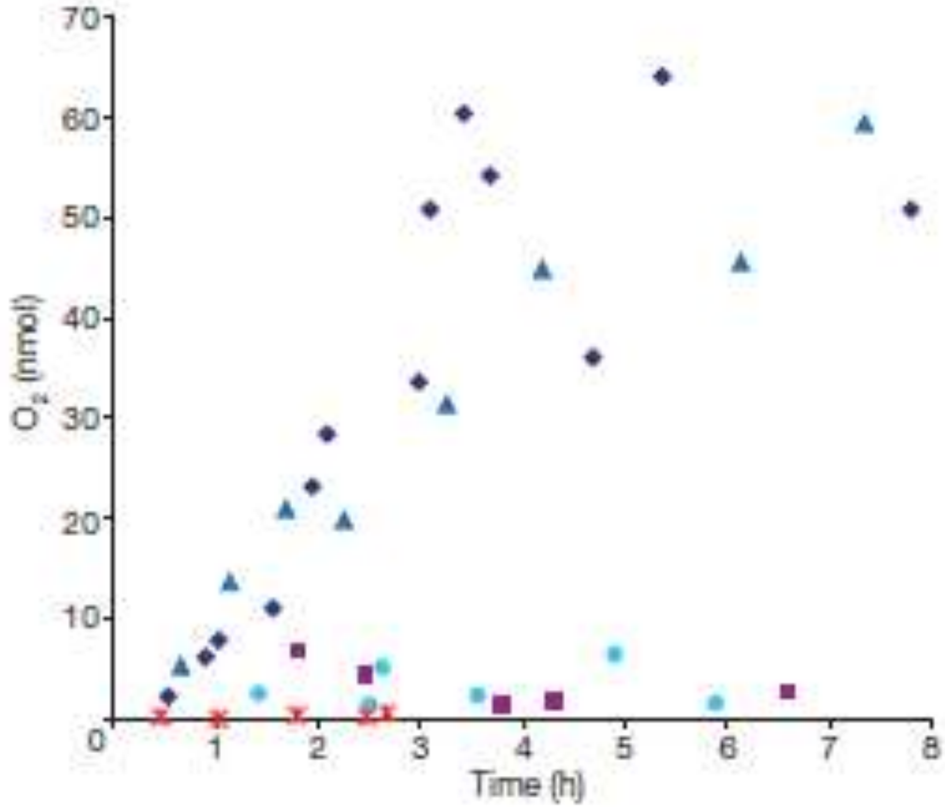
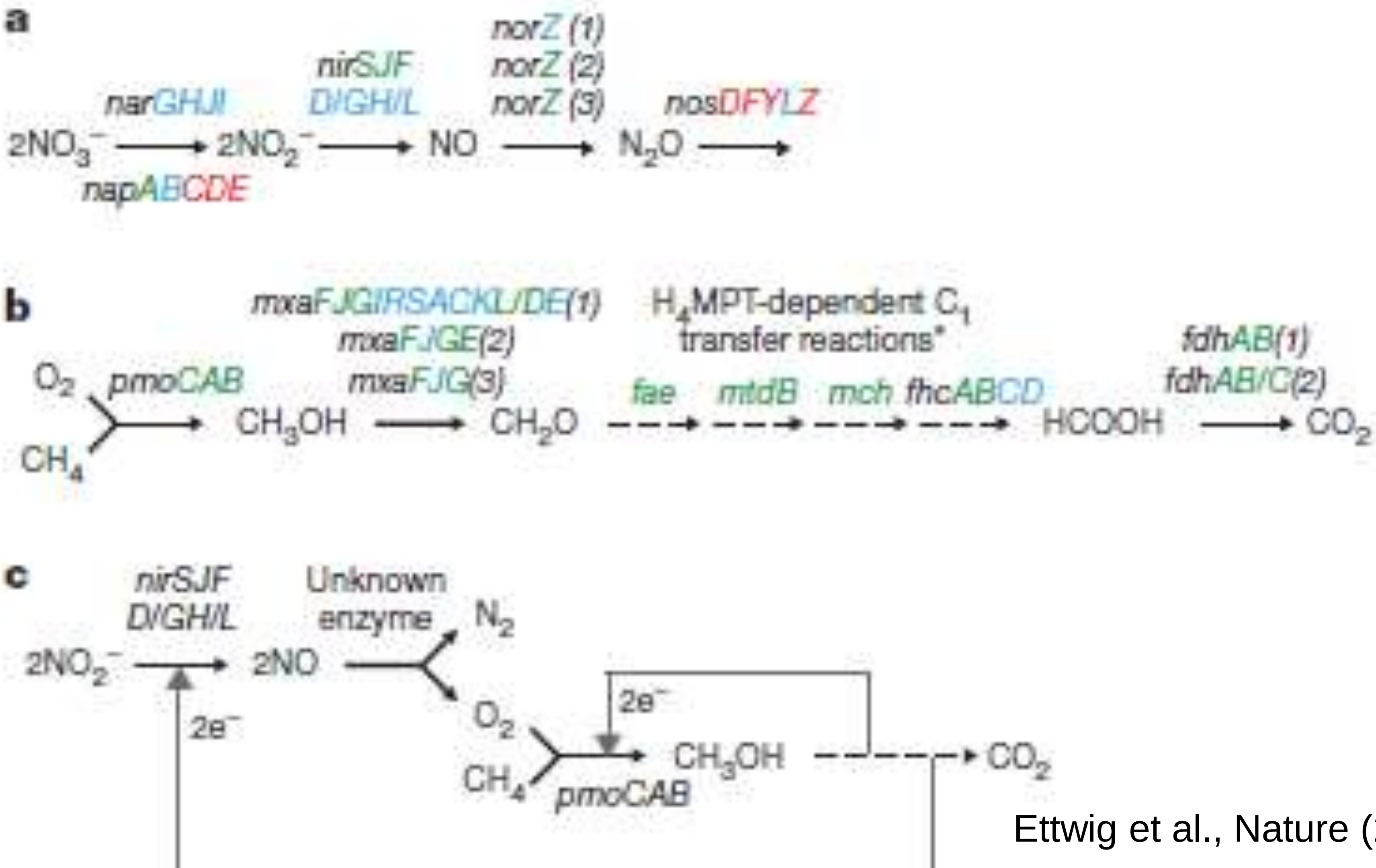


Figure 4 | Oxygen production from nitrite in '*Methylomirabilis oxyfera*'. Whole cells of enrichment culture 'Ooij' were incubated in buffer containing nitrite and 25% ^{18}O-labelled water, leading to 90% O exchange within 30 min. Total oxygen production from this indirectly labelled $N^{18}O_2^-$ was inferred from the measured concentration of $^{16,18}O_2$ and $^{18,18}O_2$ in the helium headspace with the following additions: propylene (dark blue diamonds), propylene and acetylene (blue triangles), methane (purple squares) and oxygen (light blue circles). Anaerobic control incubations of *Methylosinus acidophilus* (red asterisks) with ^{18}O-labelled nitrite did not produce measurable amounts of oxygen. Cells were concentrated to obtain similar maximum rates of propylene oxidation activity; 1.15 nmol min^{-1} (with NO_2^-, 1.22 mg of protein) for '*M. oxyfera*', and 1.68 nmol min^{-1} (with O_2, 0.046 mg of protein) for *M. acidophilus*.

Methylomirabillis spec. produces oxygen which is used by methane mono-oxygenase

Ettwig et al., Nature (2010)

„Anaerobic“ degradation of methane with nitrate



Ettwig et al., Nature (2010)

My Question for the Übungen: Lecture Gradients

- Why does the redox-zonation model only poorly explain the degradation processes in hydrocarbon-contaminated aquifers?

Quiz

1) What can I learn from the detection of catechol in an aquifer?
a) Oxygen got depleted and the degradation stopped at catechol
b) Denitrifying conditions lead to catechol production
c) Aerobic degradation of aromatics takes place
d) An environmental terrorist poored catechol into the groundwater

2) What does the detection of a 16S rRNA gene of *Pseudomonas aeruginosa* in the environment tell us?
a) an infected patient has been spitting into the water
b) There was an oil contamination
c) The 16S rRNA does not tell me about the function of an organism
d) *Pseudomonas* can be detected everywhere

3) How can microorganisms produce molecular oxygen?

a) Cyanobacteria can oxidize water to oxygen in photosynthesis
b) Some bacs can disproportionate NO into oxygen and nitrogen
c) Bacs produce water by reducing oxygen in the respiratory chain
d) Only plants produce oxygen

4) What is special about monooxygenases?

a) Monooxygenase can activate inert compounds such as alkanes and aromatics
b) One oxygen goes to the molecule, one to water
c) They consume NADH
d) They can cleave the aromatic ring of catechol

5) Beta-oxidation

a) Beta-oxidation is the oxidation of an alcohol, which has been produced by a monooxygenase reaction, to an acid

b) Beta-oxidation produces acetyl-CoA units

c) Beta-oxidation is a sequence of reactions oxidizing fatty acids to C2-units

d) Beta-oxidation is the ring-cleaving dioxygenase acting after the primary alpha-oxidation by a dioxygenase

6) Methane can be oxidized by:

a) Methanotrophic aerobic organisms using a methane monooxygenase

b) Methane can only be degraded by methanogenic organisms

c) By denitrifying organisms producing oxygen for a monooxygenase reaction

d) Methane can only be oxidized by a di-oxygenase because two molecules of oxygen are needed according to: $CH_4 + O_2 \rightarrow CO_2 + 2\ H_2O$