What are important features for *Pseudomonas aeruginosa*?

- An important nosocomial germ
- Growth on detergents
- Produces infection of wounds, burns
- Cystic fibrosis and Pneumonia, especially in immune deficient patients and AIDS- patients
- Does hemolyses and produces toxins

You are in a team that has to clean up a toluene contamination aquifer. One of the companies proposes to introduce an *Azorcus tolulylicus* and a *Pseudomonas* to increase biodegradation. What is your comment and why?

- *Azorcus tolulylicus* is able to degrade toluene as well as *Pseudomonas*. *Pseudomonas* is an aerobic bacterium and oxidizes toluene in the aquifer. If there is no oxygen available in the aquifer it is better to use the anaerobe *Azorcus tolulylicus*. However, for hydrocarbon degradation it does not make sense to add microorganisms (bioaugmentation). Usually all the bacs are there on site. If they do not degrade the contaminant, there must be another limitation which of course cannot be overcome by adding bacs.

Which effect has the production of O_2 from NO_2^- to the plume fringe concept?

- Degradation of compounds becomes possible that are not or only extremely slowly degradable without the reactive cosubstrate molecular oxygen.

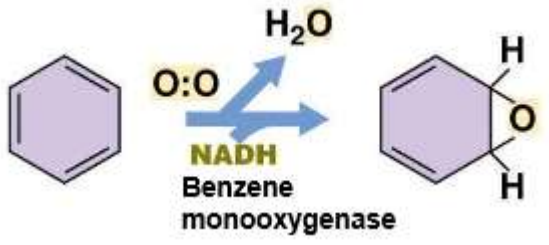
How is an aerobic methane oxidation in an anaerobic environment possible?

Anaerobic degradation of methane with nitrate:

- $2\ NO_3^- \rightarrow 2\ NO_2 \rightarrow NO \rightarrow N_2O$
- $CH_4 + O_2 \rightarrow CH_3OH \rightarrow CH_2 \rightarrow HCOOC \rightarrow CO_2$
- $2\ NO_2^- (+ 4e^-) \rightarrow 2\ NO \rightarrow N_2$
- $NO_2^- \rightarrow NO \rightarrow O_2 (+ CH_4) \rightarrow CH_3OH \rightarrow CO_2$

What is the role of benzene monooxygenase in degradation of benzene?

- Benzene monooxygenase is needed to activate benzene. With O_2 and under use of NADH benzene get oxidized to benzene epoxide.



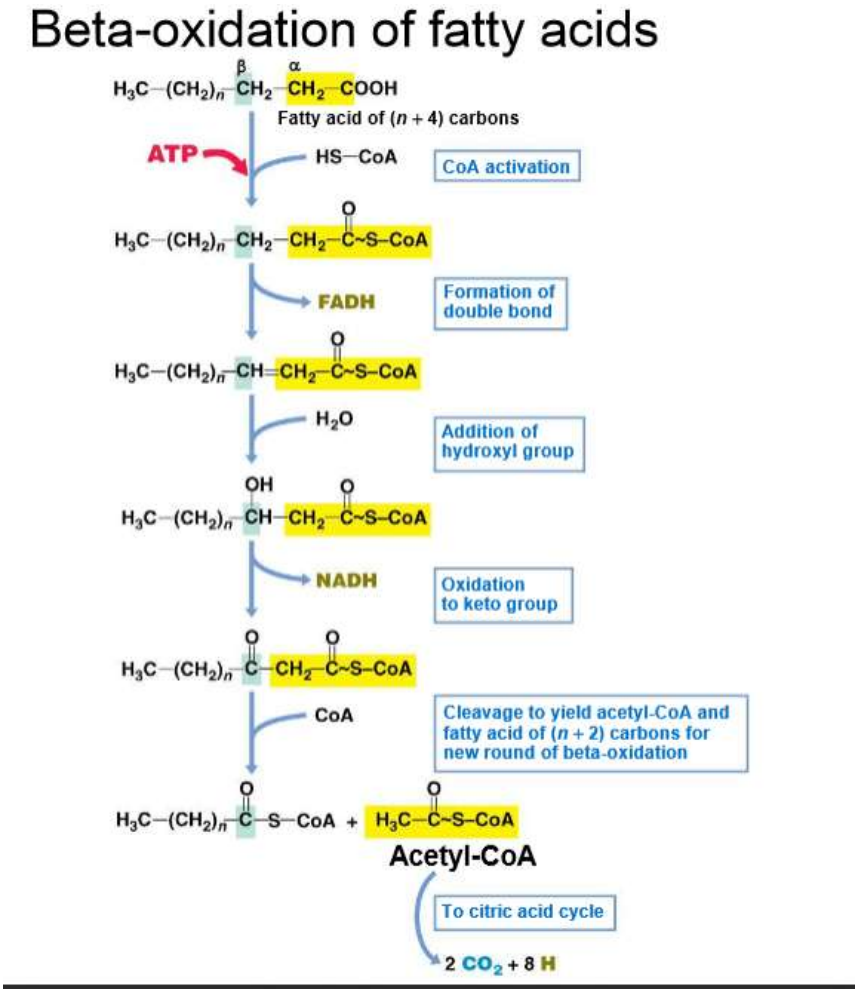
How many types of oxygenase exist in aromatics degradation? What are they used for?

- Monooxygenase is needed to introduce a hydroxyl group or epoxide. O_2 and NADH are needed.
- Toluene gets oxidized by toluene dioxygenase, O_2 and NADH and forms toluenediol.
- Catechol 1,2-dioxygenase belongs to the group of ring-cleaving dioxygenases and builds catechol e.g. cis-cis-muconate. Such dioxygenases can cleave in meta or ortho position.

What is methanogenesis?

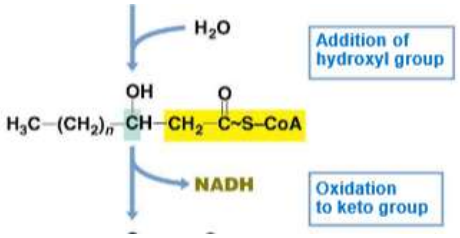
- The formation of methan by methanogens (archaea). Can be either from H2 plus Co2, acetate, or methylgroups.

How does ß-oxidation of fatty acids work? Draw the flow chart, please.



Where does the NADH come from after the addition of the hydroxyl group during the ß-oxidation of fatty acids?

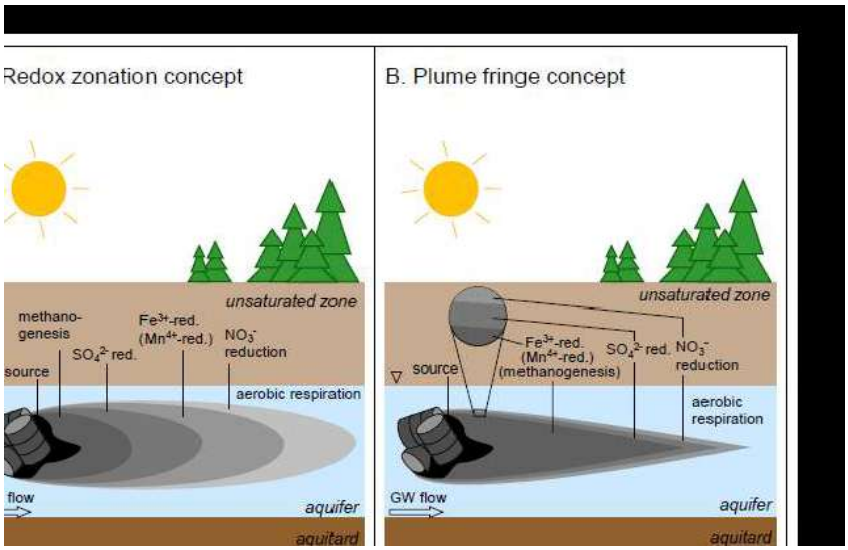
- NADH is produced by the oxidation of the OH-group.



1. What is the Catechol? What is the difference between Catechol and Proto-catechuate?
   Catechol is the central intermediate of aerobic degradation of aromatic hydrocarbons. Proto-Catechol is carboxylated Catechol.

2. Give each step name and enzymes necessary for the aerobic degradation of n-Heptane.
   1. Monooxygenase with NADH and O_2. Activation of the alkane to an alcohol.
   2. Dehydrogenase reaction of the alcohol to an aldehyde and production of NADH
   3. Dehydrogenase reaction: The aldehyde is oxidised to an acid which is
   4. Acid-CoA Ligase: addition of CoA
   5. → beta-oxidation

3. What is the difference between the *plump fringe concept* and the *redox zonation model*?



4. During aerobic degradation of alkanes, oxygenation is a very important step, why?

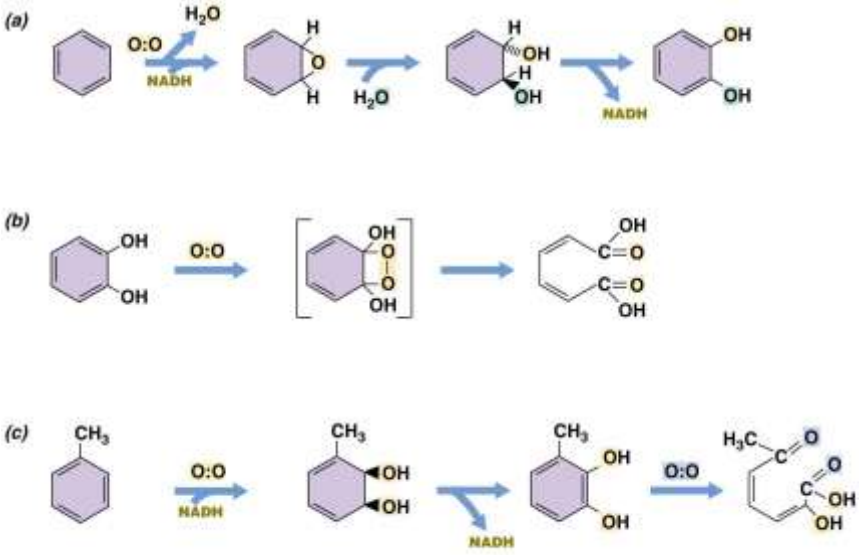
$$C_7H_{15}\text{-}CH_3 + NADH + O{:}O \xrightarrow{\text{Monooxygen}} C_7H_{15}\text{-}CH_2OH + NAD^+ + H_2O$$

Because alkanes are very poorly reactive and they need to be activated with oxygen.

5. How would you be able to assess aerobic degradation of aromatic compounds in a hydrocarbon contaminated aquifer?
   Catechol is a central metabolite of aerobic aromatics degradation. Detection of catechol can be used as an indicator. Attention has to be payed as also natural compounds such as amino acids are degraded via catechol.

6. Name and describe the possible ways to cleave catechol.

   The ring-cleaving dioxygenases convert can cleave in either meta or ortho position with the help of oxygen.



7. Wich characteristic features does *Pseudomona aeruginosa* has and which patients are at risk?
   - It is a a pathogen in infected wounds.
   - Aerobic
   - Can grow on detergents → can live anywhere.
   - Risks: wound infections; nosocomial germ
   - Has resistance against much antibiotics.
   - Some mutants strains are very resistant.
   - Patients at risk: HIV-patients
     - HIV-patients
     - Cystic fibrosis patients.
     - Immunosuppressed patients