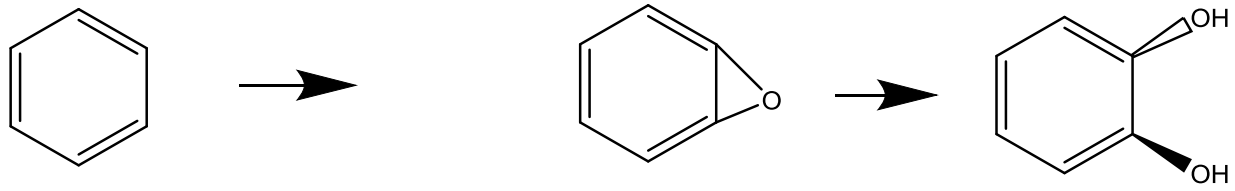
1. Explain the differences between the three types of oxygenase used for aromatic metabolism and how they work.

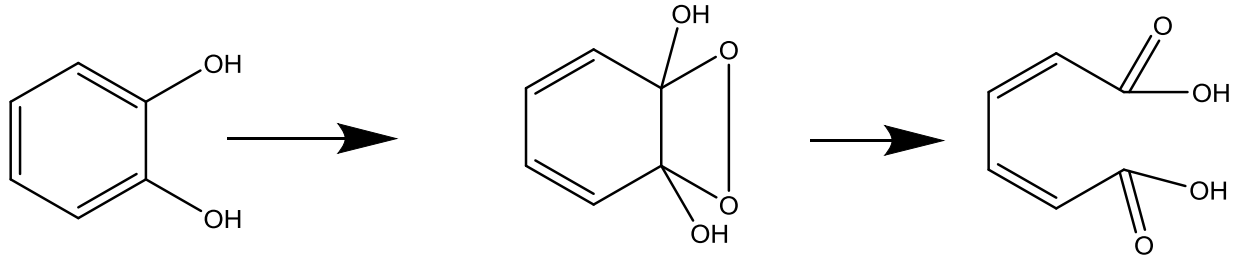
The three types of oxygenases differ by:

- In monooxygenase reactions one oxygen atom is transferred to the substrate. The other atom goes to water.
- In dioxygenase reactions, both oxygen atoms are transferred to the target molecule.
- Both types utilize NADH as electron donor. However, only in the dioxygenase reaction this NADH can be gained back when the aromaticity of the ring is reestablished by in a dehydrogenase reaction.

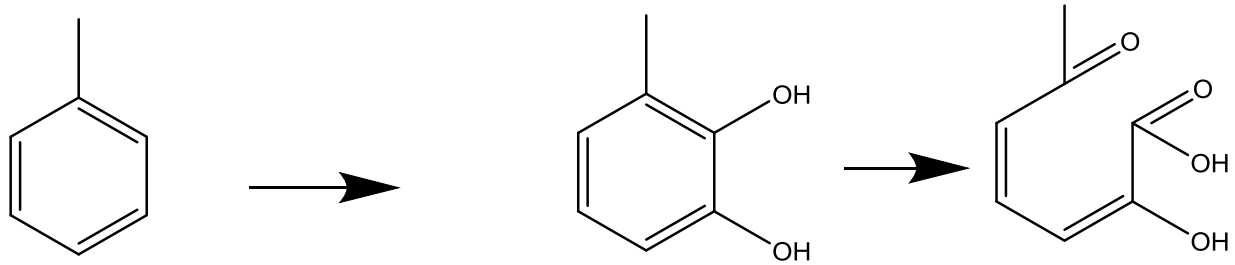
2. Name the 3 types of oxygenases for a) Benzene b) Catechol and c) Toluene:
a) benzene monooxygenase producing an epoxide. (Comment Meckenstock: the catechol depicted here is wrong because it should be a planar molecule. Cosubstrates such as NADH and oxygen missing).



b) Ringcleaving dioxygenase



c) Dioxygenase and Ringcleaving dioxygenase



3. What is the relevance of common intermediates like catechol?

Peripheral pathways lead to a central intermediate such as catechol. From there on, the pathway is the same for all substrates no matter with which substance it started. This way, many organisms share the same genes and enzymes from the catechol onwards, and there is only a pathway from the substrate to catechol needed individually.

5. To which substances is an alkane transformed during aerobic degradation? Is this a reduction or oxidation?
- alcohol → 1. Monoxygenase reaction
- aldehyde → 2. Dehydrogenase
- acid → 3. Dehydrogenation

6. Explain the aerobic degradation of Propane.
Propane is first oxygenated to propanol by monooxygenase.
The alcohol is then dehydrogenated, forming an aldehyde. Then, propanoic acid is formed, which is finally transformed to acetyl-CoA.

7. What role do catechol and proto-catechol play in the degradation of aromatic hydrocarbons?
They are central metabolites. Aromatic compounds are all transformed to one of these two metabolites and then there is only one central pathway needed for the remaining degradation.

8. Describe the redox reactions of *Methylomirabilis*. What is the difference to denitrification?

Nitrite is reduced to NO. The NO is disproportionated to molecular oxygen and nitrogen. The produced O2 is then used in a methane monooxygenase reaction to activate the methane. The difference to denitrification is the disproportionation reaction of NO producing the oxygen.

9. Give the oxidation pathway of pyruvate.

Answer: Pyruvate can be transformed to acetyl-CoA by several reactions, e.g.

- Oxidative decarboxylation: Pyr + HS-CoA + NAD+ → Acetyl-CoA + CO2 + NADH
- Lyase reaction: Pyr + HS-CoA → Acetyl-CoA + HCOOH
- Pyrovate-ferredoxin oxido-reductase: Pyr + HS-CoA + Fd(ox) → Acetyl-CoA + CO2 + Fd(red)

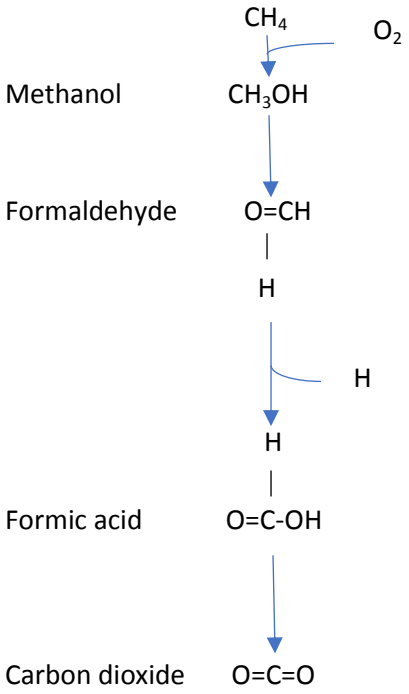
10. Which aromatic compound is often an intermediate of hydrocarbon degradation (aerobic, anaerobic)?
    Answer : Benzoate for anaerobic degradation pathways
    Catechol or protocatechol for aerobic pathways

11. Which are the two principles of destabilizing the aromatic ring for cleavage?
- Oxidation of the aromatic ring (aerobic pathways involving oxygenases)
- Reduction of the aromatic ring (anaerobic pathways involving aryl-CoA reductases

12. What is the reason for common intermediates in nature?

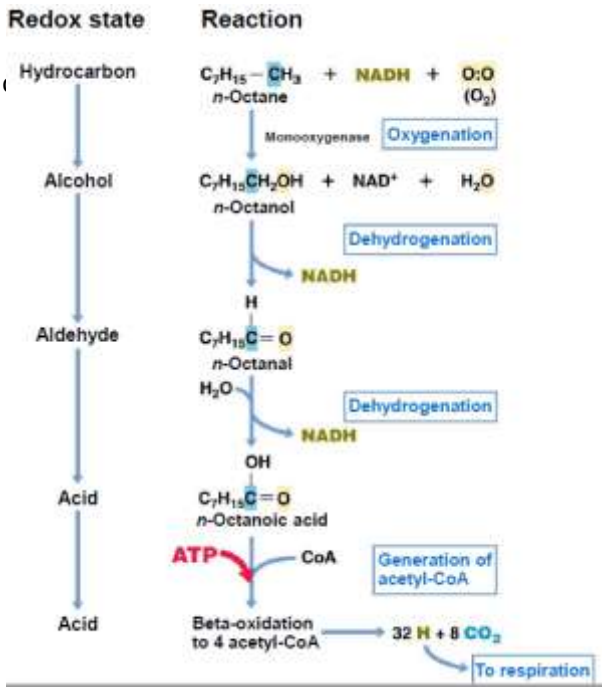
Answer: pathways are organized in gene cassettes for peripheral and central pathways. Thus, the central pathway has only to be in vented ones and can be used for different types of substrates.

13. Explain the pathway of aerobic methane oxidation for energy conservation and carbon assimilation. Where does it take place? What are the mechanism involved?
Answer : Aerobic methane oxidation is initiated by methane monooxygenase. In mostly takes place at the surface of sediments, where methane meets molecular oxygen. A second option is the nitrate dependent generation of molecular oxygen by disproportionation of NO.

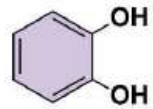


1. What are the general intermediates in the aerobic degration of alkanes?

example for n-octane:

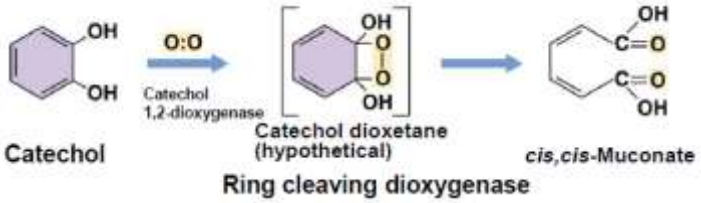


2a) Please draw the stucture of catechol:



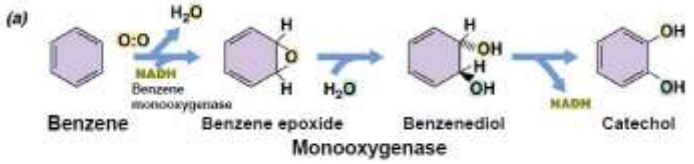
Catechol

2b) Please explain the pathway from catechol to muconic acid. Do you need mono- or Dioxygenase for this reaction?



4. What are possible enzymes for aerobic activation of aromatic hydrocarbons?

Benzene monooxygenase



Toluene dioxygenase