Questions and answers from the 05.12.16

Q1: what are key enzymes in the anaerobic biodegradation of aromatics?

First, the aromatics have to be activated. There are different reactions such as: fumarate addition to the methyl group of toluene or methylnaphthalene producing e.g. benzylsuccinate, ethylbenzene dehydrogenase introducing a hydroxyl group into the benzylcarbon atom of ethylbenzene, carboxylation of benzene. After producing a carboxylic group, there is a Ligase-reaction needed to produce a CoA-thioester. After that there are different reductase reactions reducing the aromatic ring.

Q2: Describe the differences between aerobic and anaerobic degradation of aromatic compounds.

In aerobic degradation, aromatics are degraded by oxidation. For activation and ring cleavage oxgenase reactions are used. This may be either mono or dioxygenases.

In anaerobic degradation, the compounds are reduced to overcome the resonance energy of the aromatic ring.

Q3: Under aerobic conditions, aromatics can simply be oxidized by microorganisms. This is not the case when facing anaerobic conditions. What is the reason for not being able to oxidize aromatics and how do microorganisms degrade them instead?

There is a lack of the reactive molecular oxygen as cosubstrate. Thus, other reactive molecules are needed for activation. Instead several other key reactions are applied as described in Q1.

Q4: What are the reasons some compounds are not utilized by some microorganisms?

First, there is a specific biochemical pathway needed for each compound. If the organisms do not possess that pathway, they cannot degrade the compound. Maybe because some bacteria prefer some compounds over others. For example, in the present of glucose and lactose, bacteria prefer to utilize the glucose first.

Q5: Describe the essential properties of the Ethylbenzene degradation pathway.

Ethylbenzene is activated by the ethylbenzene dehydrogenase introducing a hydroxyl group in the benzylcarbon. This is oxidised to a keto group. This carboxylated producing acetophenone which can be cleaved to benzoyl-CoA and acetyl-CoA.

Q6: With regards to the energetics of benzoate degradation in anoxic environments: which tendency can be seen for the following bacteria classes concerning their energy gain? And why?

-Methanogenic consortia (fermenters and methanogens)
-Iron reducer
-Sulfate reducer
-Denitrifier

Methanogenic consortia < sulfate reducers < iron reducers < denitrifier

because of a lesser energy gain trough the reactions.

1. Why is toluene a hydrocarbon whereas benzoate is not?

Benzoate is an organic acid while toluene is purely composed of hydrogen and carbon atoms.

2. Why are some intermediates of aerobic pathways processed as CoA Thioesters?

CoA bound intermediates cannot diffuse over the membrane and are therefore retained in the cell, whereas free intermediates could diffuse out of the cell. Besides, CoA bound acids can be rapidly recognized and bound to the processing enzymes.

1.) **Q: Describe how the oxidative state of aromatics changes during aerobic/anaerobic biodegradation.**
A: In aerobic degradation the strategy to overcome the resonance energy is to oxidize the ring. In anaerobic degradation, the ring is reduced.

2.) **Q: Explain 3 limitations of biodegradation.**
A: - Plume fringe: spatial seperation of nutrients / donors and e^- acceptors
- shifts of geochemical gradients
- Biological fluctuation: due to hydraulic disturbance MOs are growing never at the optimum conditions

3.) **Q: You are in a team that has to clean up a toluene-contaminated aquifer. One of the companies proposes to introduce *Azoarcus tolulyticus* and a *Pseudomonas* to increase biodegradation. What is your comment and why? (Comment Meckenstock: isn't that a question from my slides?)**
A: Pseudomonas isn't able to degrade aromatics without molecular oxygen. As there is usually no dissolved oxygen in such a contaminated aquifer, the Pseudomonas can never degrade the compounds. Adding Azoarcus would make more sense as long as nitrate is present as electron acceptor. However, such organisms are usually present anyway at such contaminated sites. There addition is therefore not necessary.

4.) **Q: What are the principle problems of anaerobic degradation of hydrocarbons? Which hydrocarbons can be degraded by *Azoarcus tolulyticus*?**
A: The activation of the hydrocarbons is difficult due to the lack of molecular oxygen as cosubstrate. Then, the ring has to be reduced, to overcome the resonsance energy

5) Q: What is electron bifurcation and how does it work? Which organisms use it and what for?

A: It’s the principle to bring one of a pair of electrons from an intermediate electron potential to a more negative potential by coupling this reaction to a second one where one of the two electrons is transferred to a more positive potential. The energy gained by the later reaction is invested to bring the first electron to the more negative state