Bachelor/Master project: Correlation between growth phases and enzyme activity of anaerobic naphthalene degrading cultures

Introduction:

Polycyclic aromatic hydrocarbons (PAHs) are chemically stable compounds consisting of two or more fused aromatic rings which accumulate as environmental pollutants mostly through man made processes. Naphthalene as the smallest PAH is prevalent in coal tar from which it diffuses into water and sediment. Recent studies have now demonstrated the biodegradation of naphthalene under anoxic conditions with alternate electron acceptors. The first reaction in the anaerobic degradation of naphthalene is the carboxylation of naphthalene to 2-naphthoate by a naphthalene carboxylase. A coenzyme A thioester is then formed at the carboxyl group through the 2-naphtoate:CoA ligase. Afterwards the reduction of 2-naphthoyl-CoA to 5,6,7,8-tetrahydro-2-naphthoyl-CoA is catalysed by two oxygen-insensitive reductases, the 2-naphthoyl-CoA reductase and subsequently the 5,6-dihydro-2-naphthoyl-CoA reductase. In contrast, the 5,6,7,8-tetrahydro-2-naphthoyl-CoA reductase that forms hexahydro-2-naphthoyl-CoA is an oxygen-sensitive, ATP-dependent enzyme. From this point, the downstream pathway follows β-oxidation-like reactions leading to the sequential cleavage of both rings. The degradation then proceeds via pimeloyl-CoA to the central metabolism.

Thesis description:

The goal of our working group is the elucidation of the anaerobic naphthalene degradation pathway. We are working with two naphthalene degrading cultures isolated under sulfate-reducing conditions. The freshwater culture N47 and the marine culture NaphS2, both belonging to the *Deltaproteobacteria*. In this thesis, the correlation of growth phase and enzyme activity of these two organisms will be studied. In this context two oxygen sensitive enzymes are of special interest. The naphthalene carboxylase, which initially activates naphthalene for the further degradation and the 5,6,7,8-tetrahydro-2-naphthoyl-CoA reductase. Moreover, the two reductases forming 5,6,7,8-tetrahydro-2-naphthoyl-CoA will be analysed further. For this, a growth curve will be determined by measuring sulfide production with Ion-Chromatography as well as cell counting via flow cytometry. The quantification of enzyme activities and of the gene expression during different growth phases will give further insights into the anaerobic degradation of naphthalene. During this thesis, the student will gain knowledge in working with analytical instruments (IC and LC-MS), molecular biological methods, and the cultivation of anaerobic organisms.

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