Groundwater bacteria - DTU Orbit (18/08/2019)

**Groundwater bacteria: Diversity, activity and physiology of pesticide degradation at low concentrations**

Pesticide contamination of groundwater is a well-documented and extensive pollution problem characterized by low chemical concentrations. As a consequence of widespread and repeated use, pesticide residues are frequently detected in surface- and groundwaters serving as public drinking water resources, in the concentration range of nanogram- to micrograms per liter. Such concentrations may still exceed the European drinking water limits of 0.1 µg L⁻¹ for individual pesticides, and be a threat to drinking water resources.

The behavior, degradation mechanisms and treatment of pollutants occurring at high concentrations (mg L⁻¹) are relatively well understood, however the microbial processes and degradation kinetics of pollutants occurring at low concentrations (µg L⁻¹ to µgL⁻¹) can differ significantly from the processes at higher concentrations. Our knowledge on the processes that cause such bottlenecks is very limited and mostly biased by the fact that the concentrations of organic chemicals used in the laboratory are far higher than those found in the environment.

The overall aim of this PhD study was to improve our understanding of degradation of low pollutant concentrations in oligotrophic aquifers, and to understand the mechanisms and bacterial physiology underlying the occurrence of low pollutant concentrations. This may allow us to develop new and more efficient approaches for remediation of pollutants at low concentrations in situ.

Due to common use of high substrate concentrations in cultivation studies, bacterial populations existing in the same micro-niches which are adapted to metabolize substrates at low concentrations may have been overlooked and they could be more efficient to remediate low levels of organic pollutants in contaminated environments. In order to demonstrate the effect of substrate concentrations in enrichment processes, we have investigated the differences in metabolic activity, community structure and dynamics, population growth, and single cell physiology of two 4-chloro-2-methylphenoxyacetic acid (MCAPA) degrading enrichment cultures. The cultures were obtained by a conventional enrichment approach based on different MCPA concentrations originated from the same aquifer material. We have shown that using low substrate concentrations (100 µg L⁻¹), in contrast to the classical high concentrations (25 mg L⁻¹), provide more efficient and stable bacterial communities in regards metabolic functionality and community structure. Furthermore, we have demonstrated that low nucleic acid (LNA)-content bacteria proliferated in parallel to mineralization activity in cultures selected on low herbicide concentration, suggesting that LNA bacteria may play a role in degradation of low pollutant concentrations in contaminated sources.

One of the major goals of this thesis was to isolate microbial populations that are adapted to metabolize low pollutant concentrations from oligotrophic environments. In this perspective, we have developed a novel cultivation approach which combines long term cultivation with low diffusive fluxes, in order to demonstrate that groundwater sediments contain an unrecognized diversity of pesticide-degraders. This system is called Low Flux Filter (LFF) plate and it provides a constant flux of MCPA to the bacteria allowing them to grow slowly on a membrane placed on an agar surface, which is transferred to fresh MCPA plates periodically. The isolated strains, the first MCPA-degraders isolated directly on MCPA, were most efficient at mineralizing low MCPA concentrations in line with the nutrient scarcity of their natural habitat.

Finally, we investigated the effect of substrate concentrations on the bimodal distribution of low nucleic acid (LNA) and high nucleic acid (HNA) cells of MCPA degrading bacterial strains in order to get a better understanding of the physiological, ecological and functional relevance of LNA-HNA populations. The results showed that bacterial strains obtained with low MCPA levels were dominated by LNA cells and this dominance decreases with increasing MCPA concentrations. Whereas, the strains obtained with high levels of substrate showed strict HNA characteristics regardless of the concentration. The strains showing LNA characteristics were reported to be more efficient in mineralizing low MCPA concentrations and inhibited by high levels of MCPA. This suggests that bacterial populations harbouring LNA-cells could be possible candidates for bioremediation of environments contaminated with low concentrations of pollutants.

Overall this PhD study showed that aquifers contain microbial populations that are adapted to low pesticide concentrations, whose potential can be accessed using specific cultivation approaches and their characteristics could be identified by the use of state-of-the-art methods. The results of this thesis would contribute significantly to our understanding to develop remediation strategies for degradation of pollutants at low concentrations.

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