Real-time PCR quantification of phenoxy acid degraders in aquifer sediment

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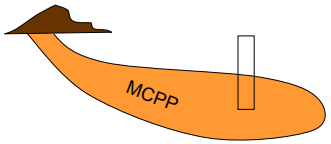
Can the degradation potential of MCPP in the plume from Sjølund landfill be shown by the quantification of the metabolic genes of the microbial degraders?

Need: Tool for quantification of pollution degrading microorganisms *in situ*
There are often uncertainties related to the calculation trace compound flux of and identification of specific degradation indicators (e.g. formation of metabolites, stable isotope fractionation, optical isomer fractionation) at complicated sites. Furthermore, investigation of degradation potential based on laboratory degradation experiments .

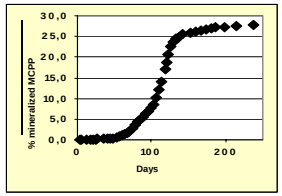
Sjølund landfill



Sediment core collected at transition zone between background and plume. Core divided into 50 sediment samples.

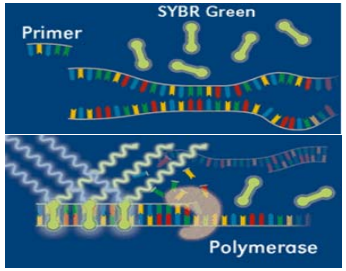
Incubation with ^{14}C-MCPP

Mineralization of MCPP

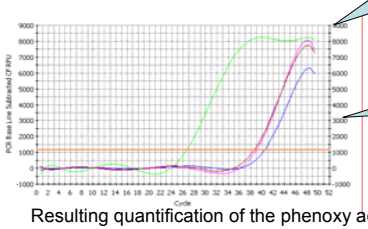


Incubation experiments showed MCPP degradation potential in an active zone at the fringe of the plume

DNA extraction



Real-time PCR with primers specific for genes encoding phenoxy acid degrading enzymes



Resulting quantification of the phenoxy acid metabolic genes (e.g. *tfdA* and *tfdC*)

The detection limit of the real-time PCR assay was low: 10^2 *tfdA* genes/g sediment

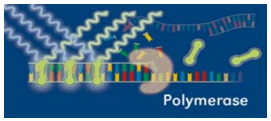
200-2000 *tfdA* and *tfdC*/g was found in 3 samples i.e. no PCR signal in many samples from the active zone

Lacking PCR signal due to PCR inhibiting compounds (e.g. humus) co-extracted from the sediment?

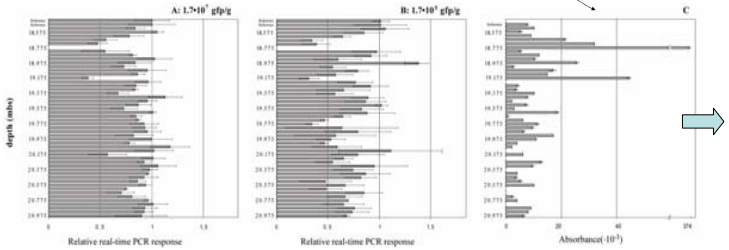
10 µL DNA extract

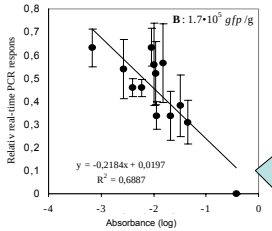
Absorbance at 400 nm measured on DNA extract as indicator of the concentration of humus-like compounds

+ internal PCR control (= *gfp* gene, added at 2 concen-trations)



Real-time PCR with *gfp* specific primers





1) Humus-like compounds contribute to PCR inhibition in many inhibited samples

2) The inhibition is higher at low (<10^5 genes/g) than at high gene concentrations

3) Most sediment samples with degradation potential also inhibited PCR

Advantages and Disadvantages: Real-time PCR vs. incubation methods

+ Faster (Min. 2 days vs. several months)
+ Price ca. 13 €/sample after optimization (≈ price for incubation method with 14C-labelled compound) (price of materials)
+/- Can detect non-cultivable microorganisms
+/- Detection limit (Depending on primers. In our study low: 80 genes/g)
- Inhibition of PCR reaction from compounds co-extracted with DNA
- Primer design (the relevant genes have to be known)

Conclusions

- Genes coding for phenoxy acid degrading enzymes could be detected with a **low detection limit** of 10^2 genes/g sediment => **With suitable primers real-time PCR can be used for quantification of specific degraders** in aquifer sediment despite a low gene concentration
- PCR signal was lacking in many samples from the zone where degradation potential was shown using incubation methods which could be due to 1) The tested **primers do not detect the genes** responsible for the degradation 2) We are **below detection limit** 3) **PCR inhibition**
- Humus-like compounds contributed to PCR inhibition in many of the inhibited samples
- The inhibition is greater at low gene concentrations (<10^5 genes/g) than at higher => Important to **use an internal PCR control (e.g. the *gfp*-gene) at quantification of genes with a low concentration**