Summary

Chlorinated ethenes (CEs) are among the most frequently reported groundwater contaminants. This is much due to irresponsible past practice as well as contemporary accidental spills, resulting in extensive pollution of vast areas. The recognition of the toxicity and persistence of the most highly chlorinated ethene, i.e. tetrachloroethene (perchloroethylene; PCE), and its degradation products, has led to substantial efforts towards deciphering the mechanisms of its degradation and towards developing adequate cleaning techniques for polluted sites. Degradation of chlorinated ethenes through anaerobic reductive dechlorination (RD) by organohalide respiring bacteria (OHRB) is believed to be the most efficient, most environment-friendly and most cost-effective approach towards the remediation of PCE (and its derivatives) pollution. However when the abundances and types of indigenous OHRB in a PCE-polluted site, limit or even thwart the natural attenuation, one may attempt to improve conditions for the organisms already present (a strategy called biostimulation), or to add appropriate exogenous OHRB thereby initiating a process of bioaugmentation.

In this thesis we have used Desulfitobacterium hafniense Y51 as model organohalide respiring bacterium for reductive dechlorination of PCE. Although the selected strain is incapable of performing the complete dechlorination of PCE to ethene (the harmless degradation product) it still offers a potential for bioaugmentation. Its advantages include the availability of its sequenced and annotated genome, its high metabolic versatility, its self-sufficiency for vitamin B₁₂, its motility, its expected chemotaxis toward chlorinated compounds, its relative high growth rate, its easy maintenance in mono-cultures, and its spore-forming capabilities. Its potential for bioaugmentation should be increased by making strain Y51 an integral part of a well-designed bioaugmentation microbial network, formed by a complex set of competent functional guilds. Here further systems ecological research is needed.

The overall aim of this thesis was (i) to understand the environmental factors and interactions (abiotic and biotic) that control the activity of Desulfitobacterium hafniense Y51, (ii) to design a new strategy for PCE-bioremediation using this organohalide respiring bacterium in a new microbial network, and (iii) to then test this strategy experimentally, thereby validating our design methodology.

First, we studied the interaction of the central component of our network, i.e. D. hafniense Y51 with its immediate environment (Chapter 2): D. hafniense Y51 was exposed
in chemostats to environmentally relevant limiting conditions (in terms of carbon donor, and electron-donor and -acceptor limitations), which might resemble its natural habitat. The exploration of transcriptomics and proteomics under limiting conditions revealed that besides its metabolic versatility, *D. hafniense* Y51 possesses an even stronger physiological flexibility. Strain Y51 applies specific strategies, such as inducing alternative metabolic pathways when confronted with limitations, making an arguably optimal use of available sources of carbon and energy, or escaping unfavourable environments by activating sporulation related enzymes.

Dechlorination in the environment does not only depend on metabolic capacities of individual organohalide respiring bacteria, but also on their interactions with other community members such as fermenting organisms, which not only provide electron donors for reductive dechlorination but also (partly) control the chlorinated ethenes-dechlorination rate. Hence, insight in environmental factors affecting the identity and behavior of what thereby emerges as the ‘fermenting guild’ should help when optimizing *in situ* bioremediation. In Chapter 3, the time-courses of glucose fermentation and electron-acceptor reduction obtained in enrichment cultures under each of four different redox conditions were correlated with phylogenetic information derived from 16S rRNA gene-based pyrosequencing analysis. This revealed that redox conditions strongly influenced the identities of the fermenting species as well as the type of fermentation they engaged in. This influence was indirect, i.e. through the cohabiting species that prevented inhibition of further fermentation by consuming the fermentation products. Based on these findings we determined to what extent the community composition, in particular the non-OHRB components of the latter, may affect PCE degradation by strain Y51 (Chapter 4). Metabolite measurements and Illumina sequencing were applied in order to determine degradation performance and composition, respectively, of stable consortia derived (Chapter 3) from the various relevant redox conditions. We did this in co-cultivations with *D. hafniense* Y51 in defined liquid medium, in the presence of PCE and glucose as carbon source. This approach revealed that the redox history of the community co-determined the type of reductive dechlorination of PCE by *D. hafniense* Y51, where fastest degradation rates and highest Y51 cell densities were achieved within consortia obtained under the most favorable redox conditions (i.e. with nitrate and iron as electron acceptors), exceeding those in monoculture with direct carbon source. Presence of acetogens in consortia from sulfate-reducing and methanogenic conditions suggested that their lower dechlorination efficiency was caused by competition for electron sources (hydrogen and formate) between this
functional group and strain Y51.

Further, in order to test our microbial networks in a more realistic situation such as a bioaugmentation situation after a PCE spill, the consortia established during the experiments described in Chapter 4, were inoculated into artificially PCE-polluted river sediments after eliminating the endogenous microbial community in the sediment, by autoclaving the latter (Chapter 5). The bioaugmented Y51-containing consortia behaved comparably to the ones obtained in the defined basal medium (Chapter 4) suggesting a predictable behavior of bioaugmented communities in an in situ bioremediation scenario. However, when the autochthonous ecosystem was kept alive in the sediments by refraining from autoclaving, the results differed. We suggest that the high functional and physiological diversity observed in the natural sediments may have decreased the capacity of colonization by the bioaugmented Y51-containing consortia. Despite this and the presence of other autochthonous Desulfitobacterium sp. in the live sediments, our bioaugmented strain Y51 was still identified as the only organohalide respiring bacterium responsible for the PCE to cis-DCE (cis-dichloroethene) degradation, suggesting the ability of this strain to compete for substrates in environments where all niches are fully filled.

Finally, Chapter 6 synthesizes the results of the preceding experimental chapters into a conceptual framework. Based on this, the chapter discusses the overarching findings of the research with respect to environmental significance and implications for in situ bioremediation of PCE by D. hafniense Y51. We argue that the strain Y51 within an appropriate fermenting guild, in combination with a “downstream bioremediation guild” capable of degrading the intermediate dichloroethene down to the final product ethene, may be the better strategy towards decontaminating PCE polluted sites where autochthonous organohalide respiring bacteria are not fast/active enough. We conclude that, due to the differences between pollution sites, the effectiveness of any static bioremediation design will differ between them. In addition, the concept of a more dynamic “precision bioremediation” strategy is proposed, in which each new pollution site should require a new scientific analysis in order for a site-specific bioremediation strategy to be formulated that has maximal effect. Hereto initial working guidelines should be formulated for implementation at any new site considered for bioremediation. Further data integration and development of ecological systems biology will be the first step towards a complex but rewarding goal: bioremediation optimized with the aid of precise experimentation and mathematical modelling, specific for the pollution site.