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DISSERTATION SUMMARY

# Physiological studies on hydrogen-evolving and diesel-degrading microorganisms

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The physiology of hydrogen-evolving anaerobic bacteria has been studied and their physiology improved by use of additives (Andersson et al. 2000; Halverson et al. 2000). The use of 3% polyethyleneglycol 8000 added to the growth media of *Enterobacter cloaceae* improved the viability and increased the hydrogenase activity 2-fold compared to cells grown in additive-free growth medium. It also exerted a positive effect over the strict anaerobic extremophiles *Termococcus litoralis* (1.4-fold increase in hydrogenase activity), *Fervidobacterium pennivorans* (1.7-fold increase) and *Caldicellulosiruptor saccharolyticus* (1.7-1.9-fold increase). The use of the biodegradable polymer Na-alginate (Ertesvag H and Valla S 1998; Vancov T et al. 2005) has had even higher effect - 4-fold increase of hydrogenase activity for *E. cloaceae*, 2.2-fold for *T.litoralis* and 3.1-3.3-fold for *C. saccharolyticus*. The additives have also had a favourable effect over the cell physiology when immobilized onto solid support matrices (perlite, granulated active carbon, wooden chips) and stored semi-aerobically at room temperature. Additive-treated immobilized stored cells showed a better recoverability and a prolonged activity over time.

A study on soil microbial physiology upon diesel perturbation has been conducted. The dilution method (Griffiths et al. 2001) was implemented. The incubated soil has been contaminated with 2% (v/w) diesel fuel. The diesel degradation rates and the metabolic activity (Biolog GN2) of the soil microbes have been determined. Qualitative and quantitative analysis of diesel - contaminated and non-contaminated microbial and fungal soil communities has been performed on Beckman CEQ 8000 genetic analysis system, using T-RFLP technique based on the 23S bacterial ribosomal DNA and ITS2 fungal ribosomal DNA).

A total of 71 different species were detected. The dilution technique for amending diversity was particularly effective with regard to the fungal component of the community. Diesel-treated soils developed fungi from the genera *Mucor, Geotrichum, Rhitophthora* and *Sporobolomyces*, while non-diesel soils were prevailed by *Gliocladium* and *Verticullum* genera. Diesel amendment reduced diversity (in both dilution treatments) and several bacterial species (*Mycobacterium spp., Rhodobacter spp., Burkolderia spp*.) were only detected in diesel-contaminated soil. Community level catabolic potential was generally unaffected by diesel contamination when all 95 substrates were considered. Oxidation of the simple carbon substrates (e.g. carbohydrates) was unaffected by diesel, but utilisation of more complex substrates (e.g. aromatic compounds) was increased when communities were subjected to diesel contamination. This suggests redundancy of easily degraded substrates and a diesel-mediated selection mechanism leading to more complex degradation capabilities in communities associated with diesel-contaminated soils. Diesel degradation was more rapid in soils with the greatest species-richness than in those with the lower levels of diversity. This was the case for every hydrocarbon measured ($C_{12}$ to $C_{25}$).

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