

Abstract

Twenty seven thermophilic hydrogen oxidizing cultures (pure cultures or co-cultures) were obtained from various hot springs in Grensdalur, Hveragerði in SW-Iceland. The cultures were investigated in detail with respect to phylogenetics, physiology, hydrogen uptake rates and biomass yield.

Enrichments under hydrogen oxidation conditions showed that 10% oxygen gave best results for obtaining stable pure- or co-cultures.

Phylogenetic studies of the enrichments were done with partial- and full 16S rRNA analysis and DGGE analysis. Four main groups of hydrogen oxidizing bacteria were detected in the enrichment cultures obtained. The closest similarities were to species within the genera of *Hydrogenophilus*, *Thiomonas* (moderate thermophiles), *Hydrogenobacter* and *Sulfurihydrogenibium* (true thermophiles). Three enrichments revealed bacteria close to *Hydrogenophilus denitrificans* and *H. thermoluteolus*. Three enrichments resulted in bacteria closely related to several species of *Thiomonas*. Fourteen enrichments gave closest relationship to strict chemolithotrophs belonging to *Hydrogenobacter* and five enrichments showed the closest relationship to *Sulfurihydrogenibium sp.* Phylogenetic studies also showed that heterotrophic bacteria were very persistent. Among contaminants were *Meiothermus*, *Anoxybacillus* and *Geobacillus sp.*

Batch experiments on all enrichments showed great variation in hydrogen oxidation rate and biomass yield. The hydrogen oxidation rate varied from 0,16 to 1,64 mol H₂L⁻¹h⁻¹ and the biomass yield from 0,21 – 3,18 g DCW per mol H₂ consumed. In general, the moderate thermophilic enrichments oxidized hydrogen faster as compared with the true thermophiles and produced more biomass.

Cultivation in fed batch cultures of selected enrichments resulted in higher hydrogen uptake rates and biomass formation compared with batch cultures. The maximum hydrogen uptake rate and biomass yield for *Ralstonia eutropha* was 15,05 H₂L⁻¹h⁻¹ and 4,9 g DCW per mol H₂ utilized. Similar values for the moderate thermophilic enrichments (containing

Hydrogenophilus and *Thiomonas*) 22 and 31 mmol H₂L⁻¹h⁻¹; 4,5 and 3,4 g DWC per mol H₂ utilized, respectively. The true thermophilic enrichments gave less biomass but similar hydrogen uptake rates as compared to the moderate thermophilic enrichments and the mesophilic control.

Finally, four enrichment cultures were tested for oxidation of various sulphur compounds. Two thermophilic enrichments could utilize thiosulphate and produced sulphur or sulphuric acid. Enrichment containing *Thiomonas* sp. could oxidize thiosulphate to sulphuric acid and enrichment containing *Hydrogenophilus* sp. utilized sulphur.

Keywords: Thermophilic; Hydrogen oxidizing; Aerobic; Biomass yield; Hydrogen uptake; Bacteria; Phylogeny