

Table of Contents

1.	Research objective	1
1.1.	Background	2
2.	Intoduction	4
2.1.	Thermophilic prokaryotes and their habitats.....	7
2.1.1.	Geothermal areas.....	10
2.1.2.	Microbial flora in Icelandic hot-springs.....	13
2.2.	Chemolithotrophy	16
2.2.1.	Energeticts of inorganic oxidations.....	19
2.3.	Hydrogen.....	21
2.3.1.	The knallgas reaction	22
2.4.	Hydrogen oxidizing bacteria („knallgas bacteria“).....	25
2.4.1.	History	25
2.4.2.	Physiology	28
2.4.3.	Distribution	29
2.4.4.	Thermophilic hydrogen oxidizing bacteria	30
2.4.5.	Biomass formation	33
2.5.	Single cell protein.....	35
2.5.1.	Alternative protein sources in fish feed.....	36
2.6.	Scale up of microbial processes	37
3.	Materials and methods	40
3.1.	Sampling sites	40
3.2.	Media and growth conditions.....	40
3.3.	Enrichment and isolation.....	41
3.4.	Growth and physiology	42
3.4.1.	Batch cultures.....	42
3.4.2.	Fed batch cultures.....	44
3.5.	Microscopy.....	46
3.6.	Analytical methods - Hydrogen measurements	46
3.7.	Phylogenetic characterization	46

3.7.1.	Extraction of DNA	47
3.7.2.	Amplification	47
3.7.3.	Denaturing Gradient Gel Electrophoresis (DGGE)	49
3.7.4.	Characterization	51
3.7.5.	Estimation of phylogeny	51
4.	Results.....	53
4.1.	Sampling sites and environmental data.....	53
4.2.	Hydrogen oxidizing enrichments	59
4.3.	Growth and physiology in batch cultures.....	65
4.3.1.	Maximum growth rate.....	65
4.4.	Phylogenetic analysis of enrichments	68
4.4.1.	Partial 16S rRNA gene analysis.....	69
4.5.	Denaturing Gradient Gel Electrophoresis (DGGE)	76
4.6.	Biomass yield and hydrogen uptake.....	80
4.6.1.	Selected enrichments for growth yield studies.....	81
4.7.	Fed batch experiments.....	82
4.7.1.	Fed batch experiments on enrichment 16C.....	82
4.7.2.	Fed batch experiments on enrichment 6C.....	83
4.7.3.	Fed batch experiments on enrichment 16A.....	84
4.7.4.	Fed batch experiments on enrichment D10.....	85
4.7.5.	Fed batch experiments on <i>Ralstonia eutropha</i>	87
4.7.6.	Comparison of fed batch enrichments.....	87
4.8.	Determination of growth on sulphur compounds.....	89
5.	Discussion.....	91
5.1.	Phylogeny.....	91
5.1.1.	Phylogeny of 16C, (<i>Hydrogenophilus</i> sp.).....	92
5.1.2.	Phylogeny of 6C (<i>Thiomonas</i> sp.).....	93
5.1.3.	Phylogeny of 16A (<i>Hydrogenobacter</i> sp.).....	95
5.1.4.	Phylogeny of D10 (<i>Sulfurihydrogenibium</i> sp.).....	97
5.1.5.	Summary of phylogenetic analysis	99
5.2.	Hydrogen uptake and biomass yield	99
5.3.	Single cell protein.....	104
6.	Conclusion	106

7. References.....	109
Appendices	a
Appendix 1 HOX sequences obtained from phylogenetic analysis.....	a
Appendix 2 Identity matrices.....	f
Appendix 3 Accession numbers	k
Appendix 4 DGGE gel with 30-70% denaturant.....	l
Appendix 5 BSA standard for protein determination	m

Table of charts and figures

Figure 1. The carbon cycle, natural and anthropogenic fluxes (EIA, 2004)...	5
Figure 2. A spectrum originally put up to describe the spectrum of the four physiological types of the colorless sulphur bacteria, but can be applied to other chemolithotrophic species. (Robertson & Kuenen, 2006).....	18
Figure 3. Different processes of hydrogen utilization by various bacteria in the presence of different electron acceptors (Aragno & Schlegel, 1992).....	23
Figure 4. The oxidation of H ₂ through hydrogenase, resulting in formation of water and cell material (Madigan, Martinko, & Parker, 2003).....	24
Figure 5. Members of the families, <i>Aquificales</i> and β - proteobacteria most of them are known for aerobic hydrogen oxidation, <i>Thiomonas</i> are sulphur oxidizers.....	32
Figure 6. Sampling site of samples 1A and 1B	54
Figure 7. Sampling sites of samples 2A (Fig. A) and 2B (Fig. B)	54
Figure 8. Sampling sites of samples 6A (Fig. A) and 6C (Fig. B)	55
Figure 9. Sampling sites of samples 16A (Fig. A) and 16C (Fig. B)	55
Figure 10. Sampling sites of samples 4A (Fig. A) and 17A (Fig. B)	55
Figure 11. Sampling sites of samples 1 (Fig. A), 2 (Fig. B) and 6 (Fig. C) ..	56
Figure 12. Sampling sites of samples 11 (Fig. A) and 16 (Fig. B).....	56
Figure 13. Sampling sites of samples 20 (Fig. A) and 24 (Fig. B).....	57
Figure 14. Sampling sites of samples 25 (Fig. A), 28 (Fig. B) and 30 (Fig. C)	57
Figure 15. Sampling sites of samples 33 (Fig. A) and 36 (Fig. B).....	57
Figure 16. Sampling sites of samples 41 (Fig. A), 42 (Fig. B) and 44 (Fig. C)	58
Figure 17. Microscopy pictures of enrichment 1A (Fig. A) and 1B (Fig. B)	60
Figure 18. Microscopy pictures of enrichment 2A	61
Figure 19. Colonies obtained from enrichment 6C under aerobic H ₂ /CO ₂ gas phase (Fig. A) and microscopy picture of enrichment 6C (Fig. B)	61
Figure 20. Microscopy pictures of enrichment 16A (Fig. A) and 16C (Fig. B and C).....	62

Figure 21. Microscopy pictures of enrichment 4A	62
Figure 22. Microscopy pictures of enrichment D10	63
Figure 23. Examples of hydrogen uptake rates at 2 and 10% initial oxygen concentrations in selected enrichments.....	64
Figure 24. Maximum growth rates at different temperatures of selected enrichments.....	66
Figure 25. Maximum growth rates at different initial oxygen concentrations of selected enrichments.....	67
Figure 26. Maximum growth rates obtained on enrichment cultures A-E Green columns show cultures enriched and grown on 10% oxygen the red columns show cultures enriched and grown on 2% oxygen.....	67
Figure 27. Maximum biomass yield (absorbance, OD ₆₀₀) on enrichment cultures A-E. Green columns show cultures enriched and grown on 10% oxygen the red columns show cultures enriched on 2% oxygen.	68
Figure 28. Phylogenetic tree obtained from partial 16S rRNA gene analysis of enrichments. Strains marked in bold are from enrichments used for further research. The scale shows 0, 1 substitutions per nucleotide position. Heterotrophic sequences are not shown.....	72
Figure 29. Phylogenetic tree obtained from full 16S rRNA gene analysis (1410 bp) of enrichment cultures. Enrichments marked in bold are enrichments used for further studies. The scale shows 0, 1 substitutions per nucleotide position. The heterotrophic sequences are not shown.....	75
Figure 30. Denaturing gradient gel electrophoresis (DGGE) profiles of partial 16S rRNA genes of fed-batch bioreactor cultures. Sequences showing similarity to HOX bacteria, red, Sequences showing similarity to heterotrophic bacteria, blue.....	77
Figure 31. Phylogenetic tree obtained from 16S rRNA genes of HOX bacteria from DGGE analysis. The scale shows 0, 1 substitutions per nucleotide position. The heterotrophic sequences were not used.	79
Figure 32. Growth curves (red) and hydrogen uptake (blue) of enrichment 16C.....	83
Figure 33. Growth curve (red) and hydrogen uptake (blue) of enrichment 6C in fed batch culture	84

Figure 34. Growth curve (red) and hydrogen uptake (blue) of enrichment 16A.	85
Figure 35. Growth (red) and hydrogen uptake (blue) of enrichment D10, hydrogen leakage in the beginning	86
Figure 36. Growth (red) and hydrogen uptake (blue) of <i>R. eutropha</i>	87
Figure 37. Biomass yields of enrichments grown in fed batch cultures compared to growth in batch cultures.....	89
Figure 38. Enrichments 16A (left) and D10 growing autotrophically on H ₂ supplemented with thiosulphate. Sulphur granules were formed.....	90

Index of tables

Table 1. Gibbs energy yields and estimated numbers of mol ATP per mol substrate of a	20
Table 2. Contents of the master-mix solution prepared for DNA samples, for DGGE analysis	48
Table 3. Steps of the bacterial PCR-program used, steps 2-4 were repeated 30 times.....	49
Table 4. Composition of denaturing solutions used for preparing the two DGGE stacking gels	50
Table 5. Environmental data (T°C, pH) of all sampling sites	53
Table 6. Enrichments from samples showing optical density at 600nm after 3 re-inoculations from the original sample.....	59
Table 7. Results from partial 16S rRNA gene analysis, names and accession numbers of cultured species showing the closest identities.....	70
Table 8. Results from full 16S rRNA gene analysis, names and accession numbers of cultured strains showing the closest identity	74
Table 9. Results of nucleotide-nucleotide BLAST from GenBank. Letters (a-k) denote different operational taxonomic units (OTU's) on the DGGE gel	76
Table 10. Biomass yield g DCW mol ⁻¹ H ₂ ⁻¹ , generation time and hydrogen uptake rates in batch cultures. Cultures marked in bold are the enrichments used for fed batch experiments.	80
Table 11. Hydrogen uptake rates, generation time and biomass yield. Strains 606 and 106 are from Prokaria strain collection, obtained from previous experiments, and used for comparison.	88
Table 12. Different values obtained for the assessment of DCW (gL ⁻¹)	101
Table 13. Values of biomass yields and hydrogen uptake rates for some HOX strains.....	104
Table 14. Composition of emission gases % by volume, from boreholes at two different geothermal sites in Iceland (Ármannson, 2002)	105