

# Contents

<b>1</b>	<b>Introduction</b>	1
1.1	Methane, a Useful Fuel	2
1.1.1	Production Routes	3
1.2	<i>M. maripaludis</i> S2, an Interesting Biocatalyst	4
1.3	In Silico Metabolic Modeling	5
1.4	Scope of the Thesis	6
1.4.1	Objectives	6
1.4.2	Organization	6
	References	7
<b>2</b>	<b>Literature Review</b>	11
2.1	CO <sub>2</sub> Fixing Microbes	11
2.2	<i>M. maripaludis</i> —A CO <sub>2</sub> Fixer	12
2.2.1	Taxonomy, Cell Structure, and Cultivation	13
2.2.2	Metabolic Processes in <i>M. maripaludis</i>	17
2.2.3	Molecular Biology Tools	34
2.2.4	Potential Applications	36
2.3	Genome-Scale Engineering	38
2.3.1	Systems Biology Models: Kinetic Versus Stoichiometric	39
2.3.2	iMM518: A Genome-Scale Model	43
2.4	Microbial Electrolysis Cells (MECs)	44
	References	45
<b>3</b>	<b>A Genome-Scale Metabolic Model of <i>M. maripaludis</i> S2 for CO<sub>2</sub> Capture and Conversion to Methane</b>	57
3.1	Introduction	57
3.2	Materials and Methods	58
3.2.1	Reconstructing Metabolic Network	58
3.2.2	Genome-Scale Metabolic Model	59
3.2.3	Experimental Data	60
3.2.4	Gene Essentiality/Flux Variability Analyses	61

3.3	Results and Discussion . . . . .	61
3.3.1	Reconstructed Metabolic Network. . . . .	61
3.3.2	Model Validation . . . . .	65
3.3.3	Minimal Media . . . . .	68
3.3.4	Gene Essentiality and Flux Variability Analyses. . . . .	69
3.3.5	Formate as Alternate Carbon and Hydrogen Substrate . . . .	69
3.3.6	Effect of Nitrogen Sources . . . . .	70
3.3.7	Novel Strains for Enhanced Methanogenesis. . . . .	71
3.3.8	Comparison with Other Methanogens . . . . .	72
3.4	Conclusions . . . . .	75
	References. . . . .	76
<b>4</b>	<b>Flux Measurements and Maintenance Energy for CO<sub>2</sub></b>	
	<b>Utilization by <i>M. maripaludis</i></b> . . . . .	79
4.1	Introduction . . . . .	79
4.2	Materials and Methods . . . . .	80
4.2.1	Chemicals and Gases . . . . .	80
4.2.2	Strain and Medium . . . . .	80
4.2.3	Batch Cultivation . . . . .	80
4.2.4	Analytical Procedures . . . . .	81
4.2.5	Cell Growth Measurements. . . . .	81
4.2.6	Calculation of Extracellular Fluxes . . . . .	81
4.2.7	Parameter Estimation for <i>i</i> MM518 . . . . .	82
4.3	Results and Discussion. . . . .	84
4.3.1	Cell Growth . . . . .	84
4.3.2	Extracellular Fluxes. . . . .	86
4.3.3	GAM, NGAM, and ATP Gain . . . . .	87
4.3.4	Intracellular Fluxes . . . . .	89
4.3.5	Effects of Amino Acids and Vitamins from <i>i</i> MM518 . . . .	89
4.4	Conclusions . . . . .	91
	References. . . . .	92
<b>5</b>	<b>Diazotrophy Enhances CO<sub>2</sub> to Methane Conversion</b>	
	<b>in <i>M. maripaludis</i></b> . . . . .	95
5.1	Introduction . . . . .	95
5.2	Materials and Methods . . . . .	96
5.2.1	Chemicals and Gases . . . . .	96
5.2.2	Strain and Media. . . . .	96
5.2.3	Reactor Setup . . . . .	96
5.2.4	Analytical Procedures . . . . .	97
5.2.5	Extracellular Fluxes and Maintenance Energy Estimation. . . . .	97

5.3	Results and Discussion . . . . .	98
5.3.1	Growth Under Different Nitrogen Sources . . . . .	98
5.3.2	Comparison with Other Diazotrophic Methanococci . . . . .	100
5.3.3	Growth, Extracellular Fluxes During $N_2$ -Fixation and Maintenance Energy Estimation Using <i>i</i> MM518 . . . . .	101
5.4	Conclusions . . . . .	103
	References. . . . .	104
<b>6</b>	<b>Contributions and Future Recommendations . . . . .</b>	<b>105</b>
6.1	Contributions . . . . .	105
6.1.1	Consolidated Review of <i>M. maripaludis</i> Metabolism . . . . .	105
6.1.2	<i>i</i> MM518, Development of First Constraint-Based Genome-Scale Metabolic Model of <i>M. maripaludis</i> S2. . . . .	106
6.1.3	Experimental Measurement of Fluxes and Estimation of Maintenance Energy Parameters . . . . .	106
6.1.4	Effect of Diazotrophy on Methanogenesis by <i>M. maripaludis</i> . . . . .	106
6.2	Future Recommendations . . . . .	107
6.2.1	Electromethanogenesis . . . . .	107
6.2.2	Model-Driven Approaches for Production of Desired Fuels/Chemicals . . . . .	109
6.2.3	Co-culture Modeling/Experimentation. . . . .	110
	References. . . . .	110
	<b>Summary . . . . .</b>	<b>111</b>
	<b>Appendices. . . . .</b>	<b>113</b>

In silico Modeling and Experimental Validation for  
Improving Methanogenesis from CO<sub>2</sub> via *M. maripaludis*  
Goyal, N.

2016, XXV, 122 p. 32 illus., 5 illus. in color., Hardcover  
ISBN: 978-981-10-2509-9