



## Table of Contents

Summary .....	XI
Zusammenfassung .....	XIII
1 Introduction .....	1
2 Objectives .....	3
3 Theoretical Background .....	4
3.1 Feedstocks for biorefinery applications .....	4
3.1.1 Structure and properties of lignocellulose .....	5
3.1.2 Lignocellulose processing – Extraction and saccharification .....	8
3.1.3 Black liquor – a high potential waste from pulp and paper industry .....	9
3.1.4 Inhibitors from industrial raw materials .....	10
3.2 <i>Corynebacterium glutamicum</i> as industrial working horse .....	11
3.2.1 History – Becoming a platform organism .....	11
3.2.2 Central carbon pathways .....	12
3.2.3 Natural substrate spectrum .....	12
3.2.4 Metabolic engineering for growth on pentoses .....	14
3.3 Bio-based polyamides .....	14
3.4 1,5-Diaminopentane bio-synthesis in <i>C. glutamicum</i> .....	16
4 Material and Methods .....	19
4.1 Strains and Plasmids .....	19
4.2 Chemicals .....	21
4.3 Growth Media .....	21
4.4 Cultivation .....	22
4.4.1 Cultivation in shake flasks .....	22
4.4.2 Fed-batch fermentation .....	23



4.5 Genetic engineering.....	23
4.5.1 Isolation of nucleic acids.....	23
4.5.2 Polymerase chain reaction .....	24
4.5.3 Strain construction .....	24
4.5.4 Codon optimization.....	25
4.6 Analytical techniques.....	25
4.6.1 Quantification of cell concentration.....	25
4.6.2 Substrate and product analysis .....	26
4.6.3 Quantification of total organic carbon .....	27
4.7 Enzymatic activity measurement .....	27
4.7.1 Preparation of cellular extracts .....	27
4.7.2 Quantification of protein concentration .....	27
4.7.3 Xylose isomerase .....	28
4.7.4 Xylulokinase .....	28
4.7.5 Isocitrate dehydrogenase .....	28
4.7.6 Fructose 1,6-bisphosphatase .....	28
4.7.7 Glucose 6-phosphate dehydrogenase.....	29
4.8 Preparation of hemicellulose substrates.....	29
4.8.1 Extraction of hemicellulose from black liquor.....	29
4.8.2 Hydrolysis into bioavailable sugars.....	30
4.9 Metabolomics .....	31
4.9.1 Extraction of intracellular CoA esters.....	31
4.9.2 Intracellular CoA ester analysis .....	31
4.10 Transcriptomics .....	31
4.10.1 RNA sampling and extraction .....	31
4.10.2 Microarray analysis.....	32
4.11 Fluxomics .....	32

## VIII



4.11.1 Computation of elementary flux modes .....	32
4.11.2 Mass isotopomer labeling analysis of all proteins and secreted products.....	33
4.11.3 Metabolic flux calculation .....	34
5 Results and Discussion .....	35
5.1 Systems metabolic engineering of <i>Corynebacterium glutamicum</i> .....	35
5.1.1 Extension of the substrate spectrum of <i>C. glutamicum</i> to xylose.....	35
5.1.2 Growth of <i>C. glutamicum</i> DAP-Xyl1 on xylose and glucose .....	36
5.1.3 Limited xylose uptake can be attributed to inefficient xylose import .....	38
5.1.4 Less efficient DAP production by DAP-Xyl1 on xylose than on glucose .....	39
5.1.5 Intracellular fluxes in xylose and glucose utilizing cells.....	40
5.1.6 Transcript changes in xylose utilizing <i>C. glutamicum</i> .....	44
5.1.7 Transcriptional activation of the methyl citrate cycle on xylose.....	48
5.1.8 Integrated analysis of in vivo and in silico fluxes .....	49
5.1.9 Rational strain design by integrated transcriptome analysis .....	49
5.1.10 Improved DAP production by engineered <i>C. glutamicum</i> strain Xyl2.....	50
5.1.11 The metabolic flux network of <i>C. glutamicum</i> is highly flexible. ....	51
5.1.12 The impact of xylose utilization on the pyruvate metabolism .....	53
5.1.13 Unknown regulatory interactions in <i>C. glutamicum</i> .....	53
5.1.14 The trehalose metabolism seems imbalanced on xylose.....	55
5.1.15 DAP production from xylose by genome integration of xylAB .....	55
5.2 Bioprocess engineering for application of lignocellulosic substrates .....	57
5.2.1 Enzymatic hydrolysis of hemicellulose enables a potent fermentation substrate..	57
5.2.2 Growth and production on hemicellulose hydrolysate.....	59
5.2.3 Hemicellulose is a promising new feedstock in biotechnology.....	60
5.2.4 Extraction of fermentable sugars from black liquor.....	61
5.2.5 Impact of black liquor toxins on growth performance of <i>C. glutamicum</i> .....	62
5.2.6 Bio-production of diamminopentane from black liquor hydrolysate.....	63
5.2.7 The novel strain DAP-Xyl2 performs well during fed-batch fermentation.....	65



6 Conclusions and outlook.....	67
7 Abbreviations and symbols.....	69
8 References .....	72
9 Appendix.....	88