



TABLE OF CONTENTS

SUMMARY	VIII
ZUSAMMENFASSUNG.....	IX
1 INTRODUCTION.....	1
1.1 General Introduction.....	1
1.2 Objectives.....	2
2 THEORETICAL BACKGROUND	3
2.1 Vitamin B ₂ : discovery and pioneering chemical synthesis	3
2.2 <i>Ashbya gossypii</i> – a fungal riboflavin overproducer	4
2.3 Riboflavin biosynthesis – pathways and regulations	6
2.3.1 Terminal biosynthesis.....	7
2.3.2 Precursor supply.....	8
2.3.3 Regulation.....	9
2.3.4 Biosynthesis in <i>B. subtilis</i>	10
2.4 Biotechnology and industrial production of riboflavin	10
2.4.1 Metabolic engineering of <i>A. gossypii</i>	10
2.4.2 Metabolic engineering of other microorganisms	13
2.4.3 Bioprocess engineering and industrial production of riboflavin	16
2.5 Concept of ¹³ C isotope experiments	19
2.5.1 Conventional ¹³ C metabolic flux analysis	19
2.5.2 Metabolic flux studies under complex conditions.....	20
2.5.3 ¹³ C Labeling analysis.....	21
2.5.4 Flux analysis combining different analytical techniques	23
2.5.5 Concept of ¹³ C isotope tracer studies with <i>A. gossypii</i>	23
3 MATERIAL AND METHODS	26
3.1 Strains	26



3.2	Chemicals and media.....	26
3.2.1	Chemicals	26
3.2.2	Medium composition.....	26
3.3	Cultivation.....	27
3.3.1	Shake flask cultivation	27
3.3.2	Cultivations with ^{13}C tracer compounds	28
3.3.3	Supplementation studies	29
3.3.4	Sampling of biomass and supernatant.....	29
3.3.5	Sampling for intracellular metabolites	29
3.4	Analytical methods	30
3.4.1	Cell concentration	30
3.4.2	Quantification of substrates and products.....	30
3.4.3	Mass isotopomer distributions.....	31
3.4.4	Positional isotopomers	35
3.5	Correction of labeling data	35
3.5.1	Correction of ^{13}C labeling data from hydrolyzed cell protein and glycogen.....	35
3.5.2	Correction of LC/MS and ^{13}C NMR data of riboflavin	37
3.5.3	Calculating the contribution of a ^{13}C -labeled tracer to a target molecule	39
3.6	Transmembrane formate flux simulation	40
4	RESULTS AND DISCUSSION	42
4.1	The benchmark process: growth and riboflavin production on vegetable oil	42
4.2	Glucose as main carbon source for growth and riboflavin production	44
4.2.1	First insights into the metabolism with ^{13}C -labeled glucose	47
4.2.2	LC/MS measurements reveal first insights into riboflavin metabolism	51
4.3	Replacing oil with its building blocks.....	53
4.3.1	Riboflavin production on a mixture of acetate and glycerol	53
4.3.2	Acetate plays a major role in the lower part of the carbon core metabolism.....	55
4.3.3	Glycerol contributes to glycolytic intermediates	58
4.3.4	<i>A. gossypii</i> B2 has a reduced glycolytic flux on glycerol and acetate.....	61
4.4	Characterization of growth physiology on vegetable oil via GC/MS analysis	62



4.4.1	Metabolic origin of amino acids reveals interconversion of riboflavin precursors...	62
4.4.2	Glycine is efficiently taken up to fuel the intracellular pool	66
4.4.3	Serine degradation pathways cause undesired loss of riboflavin precursors.....	69
4.4.4	The amino acid <i>de novo</i> biosynthesis is tightly regulated with a few exceptions...	69
4.4.5	Carbon fluxes of <i>A. gossypii</i> B2 during growth on vegetable oil.....	73
4.5	Unraveling the building blocks of riboflavin using LC/MS and ^{13}C NMR.....	80
4.5.1	LC/MS measurements confirm glycine and formate as riboflavin building blocks..	80
4.5.2	NMR analyses yield higher resolution of glycine and formate as precursors.....	81
4.5.3	The dual role of serine in riboflavin biosynthesis	84
4.5.4	Yeast extract and glutamate contribute globally to the riboflavin carbon skeleton.	86
4.6	Increasing production performance via time-resolved precursor feeding	90
4.6.1	Extracellular formate ignites initial riboflavin overproduction	90
4.6.2	The C_1 metabolism displays a bottleneck of initial riboflavin overproduction	94
4.7	The complete picture: riboflavin biosynthesis on vegetable oil	98
5	CONCLUSION AND OUTLOOK.....	105
6	APPENDIX	107
6.1	Abbreviations.....	107
6.2	Symbols.....	111
6.3	Isotope experiments conducted in this study.....	112
6.4	Data from GC/MS analyses	112
6.5	Biomass composition of <i>A. gossypii</i> for flux calculations	125
6.6	Determination of measured fluxes and metabolite balances for flux calculations during growth of <i>A. gossypii</i>	127
6.7	Data from LC/MS analyses	130
6.8	Data from NMR analyses	133
6.9	Data from ^{13}C formate flux simulations	135
6.10	Determination of measured carbon fluxes and carbon balances for carbon flux calculations during the riboflavin biosynthetic phase of <i>A. gossypii</i>	136
7	REFERENCES.....	148