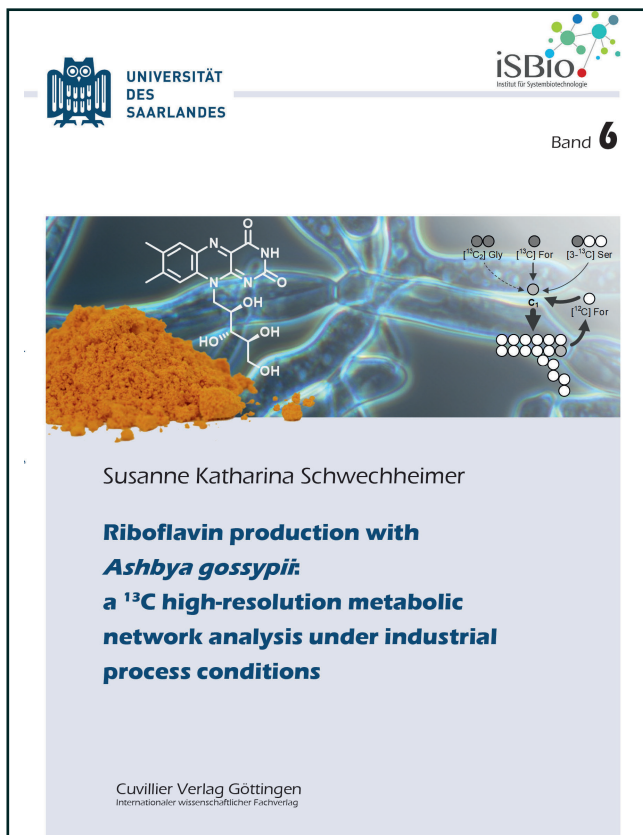




Susanne Katharina Schwechheimer (Autor)  
**Riboflavin production with *Ashbya gossypii***  
A  $^{13}\text{C}$  high-resolution metabolic network analysis under industrial process conditions



<https://cuvillier.de/de/shop/publications/7914>

Copyright:  
Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen,  
Germany  
Telefon: +49 (0)551 54724-0, E-Mail: [info@cuvillier.de](mailto:info@cuvillier.de), Website: <https://cuvillier.de>



---

# TABLE OF CONTENTS

<b>SUMMARY .....</b>	<b>VIII</b>
<b>ZUSAMMENFASSUNG.....</b>	<b>IX</b>
<b>1 INTRODUCTION.....</b>	<b>1</b>
1.1 General Introduction.....	1
1.2 Objectives.....	2
<b>2 THEORETICAL BACKGROUND .....</b>	<b>3</b>
2.1 Vitamin B <sub>2</sub> : 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 <sup>13</sup> C isotope experiments .....	19
2.5.1 Conventional <sup>13</sup> C metabolic flux analysis.....	19
2.5.2 Metabolic flux studies under complex conditions.....	20
2.5.3 <sup>13</sup> C Labeling analysis.....	21
2.5.4 Flux analysis combining different analytical techniques .....	23
2.5.5 Concept of <sup>13</sup> C isotope tracer studies with <i>A. gossypii</i> .....	23
<b>3 MATERIAL AND METHODS .....</b>	<b>26</b>
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 $^{13}\text{C}$ 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 $^{13}\text{C}$ labeling data from hydrolyzed cell protein and glycogen.....	35
3.5.2	Correction of LC/MS and $^{13}\text{C}$ NMR data of riboflavin.....	37
3.5.3	Calculating the contribution of a $^{13}\text{C}$ -labeled tracer to a target molecule.....	39
3.6	Transmembrane formate flux simulation.....	40
<b>4</b>	<b>RESULTS AND DISCUSSION.....</b>	<b>42</b>
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 $^{13}\text{C}$ -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 <sup>13</sup> 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 <sub>1</sub> metabolism displays a bottleneck of initial riboflavin overproduction .....	94
4.7	The complete picture: riboflavin biosynthesis on vegetable oil .....	98
<b>5</b>	<b>CONCLUSION AND OUTLOOK.....</b>	<b>105</b>
<b>6</b>	<b>APPENDIX .....</b>	<b>107</b>
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 <sup>13</sup> 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
<b>7</b>	<b>REFERENCES.....</b>	<b>148</b>