

SCHRIFTENREIHE DES
LEHRSTUHLS FÜR
SYSTEMDYNAMIK UND PROZESSFÜHRUNG

Band 3/2021

Tobias Claus Neymann

**Modeling, Simulation, and Control
of Budding Yeast Cultures**



Tobias Claus Neymann

**MODELING, SIMULATION, AND CONTROL
OF BUDDING YEAST CULTURES**

Schriftenreihe des Lehrstuhls für
Systemdynamik und Prozessführung
herausgegeben von Prof. Dr.-Ing. Sebastian Engell

Band 3/2021

Tobias Claus Neymann

**Modeling, Simulation, and Control
of Budding Yeast Cultures**

Shaker Verlag
Düren 2021

Bibliographic information published by the Deutsche Nationalbibliothek

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at <http://dnb.d-nb.de>.

Copyright Shaker Verlag 2021

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the publishers.

Printed in Germany.

ISBN 978-3-8440-8077-3

ISSN 1867-9498

Shaker Verlag GmbH • Am Langen Graben 15a • 52353 Düren

Phone: 0049/2421/99011-0 • Telefax: 0049/2421/99011-9

Internet: www.shaker.de • e-mail: info@shaker.de



Tobias Claus Neymann

22.6.1977 – 19.7.2018

Preface

This monograph was written by Dipl.-Ing Tobias Claus Neymann as a dissertation in partial fulfillment of the requirements for the degree of Dr.-Ing. of the Faculty of Biochemical and Chemical Engineering of TU Dortmund. Tobias Neymann joined my group on October 1, 2004. His mission statement was to be our scout for the application of system theoretic methods in the field of biotechnology, with the aim to find new, innovative and promising applications of system theory in biotechnology. The general approach was defined as not treating biotechnological production similar to chemical reactors with just slightly different kinetic expressions, but to employ the available biological knowledge to gain a deeper understanding of the processes and mechanisms and to have a real impact beyond the formal application of mathematical tools.

On the basis of Tobias Neymann's literature review in the first year of his project, we agreed to focus the work on the synchronization of the cell cycles of yeast cultures. He quickly developed a first simulation model of such cultures and first ideas how the cell cycle could be synchronized by an externally triggered overexpression of cell proteins. A major challenge for his work was to extend the available models of cell cultures such that the experimental observations are matched by representing the aging processes in the model. Towards this goal, he collected a huge amount of knowledge on the aging process which is documented extensively in this volume, and used this knowledge to extend the model on a sound biochemical basis.

The simulation of the yeast cultures and the optimization of the synchronization are the central results that are presented in this monograph.

Tobias Neymann joined Bayer AG in 2010 and continued to work on his dissertation besides his regular work where among many other projects he initiated and led a joint project with our group on bioreactor modelling and control that ultimately led to the realization of a model-predictive control strategy. Unfortunately, he suffered from a severe illness that dramatically slowed down and interrupted at times the process of the finalization of the thesis, and sadly he died on July 19, 2018 before submitting it to the faculty. He was an outstanding scientist and creative engineer, and a wonderful person, always supportive, kind, optimistic, and energetic.

This monograph is a major independent, monolithic contribution at the interface between biotechnology on the one hand and mathematical modelling, simulation, and optimization on the other. I am extremely happy to give it to print after minor editing, not only as a tribute to a great person, coworker, and engineer, but in particular to make the scientific community aware of his deep and innovative ideas and their validation in computer simulations, and to provide a point of reference for others, so that he lives on in his scientific contributions.

Sebastian Engell, Wetter (Ruhr), April 2021

Zusammenfassung

Die Synchronisation von Kulturen der Bäckerhefe im Hinblick auf Zellzyklusphasen ermöglicht eine Steigerung der Ausbeute von heterolog produzierten Proteinen. Beispiele zeigen, dass synchronisierte Kulturen von *S. cerevisiae* phasenabhängige maximale Produktions- und Sekretionsraten aufweisen. Zudem ermöglichen synchronisierte Kulturen Einblicke in die dynamischen Abläufe wachsender Kulturen. Bisherige Ansätze zur Synchronisation basierend auf toxischen Chemikalien oder induzierten Hungerzuständen und führen zu energieintensivem, zellulärem Stress. Erstere erschweren zudem die Aufreinigung. In dieser Arbeit wird eine optimierungsbasierte Synchronisationsstrategie an einer in-silico Kultur von *S. cerevisiae* entwickelt und getestet. Dabei werden innerzelluläre Proteine als Stellgrößen verwendet. Die ungleiche Teilung und daraus resultierende unterschiedliche Zellzykluslängen von Mutter- und Tochterzellen stellen eine große Herausforderung dar. Die Strategie eignet sich für eine nachhaltige Synchronisation von Fed-Batch und Chemostat Fermentationen.

Die in-silico Kultur setzt sich aus Instanzen eines Einzelzellmodells des Zellzyklus aus der Literatur zusammen. Das Modell besteht aus einem System von Differentialgleichungen erster Ordnung mit hybrider Dynamik. Es wird erweitert, um den experimentell beobachteten Größenzuwachs bei der Alterung von *S. cerevisiae* abzubilden. Zelluläre Alterungsprozesse führen zu einer Schädigung der DNA und einer Stressantwort, die zu einer transienten Verzögerung des Zellzyklus und somit zu dem Größenzuwachs führt. Mathematisch wird dies durch eine diskontinuierliche Änderung von zwei Modellparametern realisiert, die nach jeder Teilung der Zelle neu gesetzt werden und Funktionen der Zellgröße und der Wachstumsrate sind.

Um das Verhalten von Kulturen richtig wiederzugeben, werden Diversitätsparameter eingeführt. Diese werden für jede Zelle zufällig aus Verteilungen gezogen und bilden Variationen zwischen den Zellen ab. Zwei Parameter sind ausreichend, um das beobachtete stochastische Verhalten von Einzelzellen zu beschreiben. Das Kulturmodell bildet die Evolution von Tochterzellkulturen und ausgewählten Kulturen von Zellzyklusmutanten richtig ab.

Für die Synchronisation werden zwei am Zellzyklus beteiligte Proteine ausgewählt. Deren Konzentrationen werden durch eine Änderung zweier Modellparameter, die die Expressionsraten bestimmen, beeinflusst. Um Synchronität zu erreichen und zu erhalten, müssen die optimalen Zeitreihen der Parameter bestimmt werden. Beide Parameter werden auf unterschiedlichen diskreten Niveaus variiert und für unterschiedliche Dauern konstant gehalten. Die Suche nach den optimalen Zeitreihen führt auf ein globales Optimierungsproblem mit einer nichtkonvexen, diskontinuierlichen und berechnungsintensiven Kostenfunktion. Ein neuentwickelter memetischer Algorithmus kombiniert einen evolutionären Algorithmus für die globale Suche mit einer kontinuierlichen Ameisensuche und einer Gradientenmethode zur weiteren Verfeinerung für die lokale Suche zur Lösung des Optimierungsproblems. Zyklische Zeitreihen für Fed-Batch und Chemostat Fermentationen werden berechnet und auf Kulturen mit hohen Zellzahlen angewandt. Die

Zeitreihen führen zu einer anhaltenden Synchronität von mehr als 90 % aller Zellen. Abschließend werden mögliche technische Realisierungen im Labormaßstab skizziert.

Abstract

Synchronized cultures of baker's yeast cells with respect to the phases of the cell cycle are a strategy to optimize the yield of heterologous produced proteins. Examples of synchronized cultures of *S. cerevisiae* show phase dependent maxima of production and secretion rates. Additionally, the availability of synchronized cultures can provide detailed insights into the dynamics of growing cultures. The shortcomings of current synchronization strategies are the use of toxic chemicals or the induction of states of starvation. Both strategies elicit cellular stress responses which re-direct cellular energy. The use of chemicals additionally poses challenges to downstream purification. In this work, an optimization based synchronization strategy using internally available proteins is developed and tested with an in silico representation of *S. cerevisiae* cultures. The unequal division and the different resulting cycle lengths of daughter and mother cells are a major challenge to synchronization. The developed strategy can be applied for persistent synchronization of fed-batch and chemostat cultures.

The in silico cultures are composed of instances of a single cell cycle model from the literature consisting of an ordinary differential equations model with hybrid dynamics. In order to capture the experimentally observed generational size increase of *S. cerevisiae* cells, the model is extended. Cellular aging processes lead to DNA damage and elicit a stress response which transiently delays the cell cycle. This delay leads to the observed increase in cell size. Mathematically the delay is integrated into the model by discontinuously resetting two parameters after cell division. The modified parameter values are functions of cell size and growth rate. The functions approximate several single cell optimizations.

To capture the behavior of real cell cultures, the introduction of diversity parameters is necessary. The parameters are drawn from distributions for each cell individually in order to capture cell-to-cell variations. Two parameters are sufficient to describe the experimentally observed stochastic behavior of single cells. The culture model correctly describes the evolution of elutriated daughter cell and selected cell cycle mutant populations.

For the synchronization, two cell cycle participating proteins are selected as manipulated variables. Their concentrations are influenced by changing two parameters of the cell cycle model which determine their expression rates. In order to achieve and maintain synchrony, optimal time trajectories of these parameters have to be determined. Both parameters can only be varied on different discrete levels and are kept constant for variable periods of time. The search for the optimal time trajectories leads to a global optimization problem with a non-convex, non-smooth, and highly costly to evaluate objective function. A newly developed memetic algorithm that is composed of an evolutionary algorithm for the global search and a continuous ant colony optimization refined by hill climbing for the local search is used for the solution of the optimization problem. Cyclic input patterns for fed-batch and chemostat cultures are determined and applied to cell cultures that are represented by a large number of model instances. These input patterns lead to persistent fractions of synchronized cells well above 90 %. Finally, possible technical realizations on the laboratory scale are outlined.

Table of Contents

List of Figures	XI
List of Tables	XVII
Nomenclature.....	XIX
1 Introduction.....	1
1.1 Motivation	1
1.2 Problem statement	4
1.3 Outline	4
2 Background information	9
2.1 Cell cycle.....	9
2.2 Cell cycle and cell growth.....	13
2.3 Culture models	15
2.4 Common synchronization methods.....	18
3 Model of the aging process	23
3.1 Oxidative damage and aging.....	23
3.1.1 Definition of aging.....	23
3.1.2 Aging mechanisms and their cause.....	24
3.1.3 Reactive oxygen species and reactive oxygen species signaling.....	27
3.1.4 Aging and cell death	29
3.1.5 Longevity factors	32
3.2 Stress responses.....	33
3.2.1 DNA damage repair mechanisms	33
3.2.2 The excision repair pathways and their influence on the cell cycle	36
3.2.3 Checkpoints.....	45
3.2.4 Other stress responses and their influence on the cell cycle.....	46
3.3 Incorporation of the aging process into the cell cycle model.....	50
3.3.1 Overview of the original cell cycle model.....	51
3.3.2 Shortcomings of the original cell cycle model	63
3.3.3 DNA damage response model	67
3.3.4 Objective function & estimation strategy	70

3.3.5	Results.....	74
3.3.5.1	Estimation.....	74
3.3.5.2	Simulation.....	74
3.3.5.3	Analysis.....	82
3.3.6	Other aging models.....	90
4	Culture model.....	93
4.1	Experimental data.....	93
4.1.1	Daughter cell populations.....	93
4.1.2	Cell cycle mutant populations.....	97
4.2	Setup of the simulation environment.....	101
4.2.1	Technical simulation issues.....	101
4.2.2	Diversity parameters & culture characterization.....	105
4.3	Simulation results.....	113
4.3.1	Daughter cell populations.....	113
4.3.2	Cell cycle mutant populations.....	120
5	Culture control.....	129
5.1	Introduction.....	129
5.1.1	Outline.....	129
5.1.2	The global optimization problem and possible solution approaches.....	129
5.1.3	Motivation for optimization approach of choice.....	132
5.1.4	Theory based approaches of culture synchronization.....	133
5.2	Manipulated variables.....	135
5.2.1	Culture model.....	135
5.2.2	Possible realizations of cyclin concentration manipulations.....	139
5.3	Objective function.....	140
5.4	The memetic algorithm.....	146
5.4.1	Structure and realization.....	146
5.4.2	Performance evaluation.....	150
5.5	Optimization results.....	154
5.5.1	Overview.....	154
5.5.2	Fed-Batch cultures.....	155
5.5.2.1	Single initial cell based culture.....	155
5.5.2.2	Multiple initial cells based culture.....	165

5.5.3	Chemostat cultures.....	170
5.5.3.1	Small scale culture.....	170
5.5.3.2	Transfer of small scale results to a large scale culture	178
6	Conclusions and Outlook.....	185
6.1	Summary and conclusions.....	185
6.2	Outlook.....	187
A	List of References	191
B	Additional information on the aging process.....	241
B.1	More information on oxidative damage and aging	241
B.1.1	Definition of aging.....	241
B.1.2	Aging mechanisms and their causes	243
B.1.3	Sources of reactive oxygen species.....	249
B.1.3.1	Intracellular sources.....	249
B.1.3.2	Extracellular sources.....	251
B.1.4	Reactive oxygen species signaling.....	252
B.1.5	Aging and cell death	254
B.1.5.1	Reactive oxygen species in aging cells.....	254
B.1.5.2	Reactive oxygen species induced programmed cell death	256
B.1.5.3	Cell cycle arrest and programmed cell death.....	259
B.1.6	Longevity factors	261
B.2	More information on stress responses.....	263
B.2.1	DNA damage repair mechanisms	263
B.2.2	The excision repair pathways and their influence on the cell cycle	271
B.2.2.1	The base excision and nucleotide excision repair pathways	272
B.2.2.2	BER / NER based DNA damage signaling and the activation of the DNA damage repair response.....	280
B.2.2.3	DNA damage transduction cascade Mec1p-Rad9p-Rad53p	284
B.2.2.4	Cell cycle delay and arrest by Rad53p	289
B.2.2.5	Other downstream effects of the DNA damage transduction cascade	292
B.2.2.6	DNA damage response recovery and adaptation.....	293
B.2.3	Checkpoints.....	294
B.2.4	Other stress responses and their influence on the cell cycle.....	298
B.2.4.1	Environmental stress response	298
B.2.4.2	Cell wall integrity pathway.....	300

B.2.4.3	Heat shock response	305
B.2.4.4	Osmotic stress response.....	307
B.2.4.5	pH and ion homeostasis.....	312
B.2.4.6	Oxidative stress response.....	314
B.2.4.7	Calcium signaling response.....	323
B.2.4.8	Heavy metal response.....	326
B.2.4.9	Unfolded protein response.....	329
B.2.4.10	Nutrient starvation response.....	331
B.2.4.11	Retrograde response	340
B.2.4.12	Ethanol response.....	342
B.3	Integration of senescence into the cell cycle model.....	344
C	Additional information on the cell cycle model	355
C.1	Visualization of the cell cycle network.....	355
C.2	Mother and daughter cell generations	358
C.3	Extended cell cycle model: parameters.....	360
C.4	Extended cell cycle model: data of pedigree.....	361
D	Additional information on the culture model.....	365
D.1	Cell cycle mutant populations – additional information.....	365
D.2	Initial mutant culture distributions	367
D.3	Time series of mutant culture characteristics.....	368
E	Additional information on culture control	371
E.1	Single initial cell based culture	371
E.2	Small culture	373
F	Gene descriptions.....	377