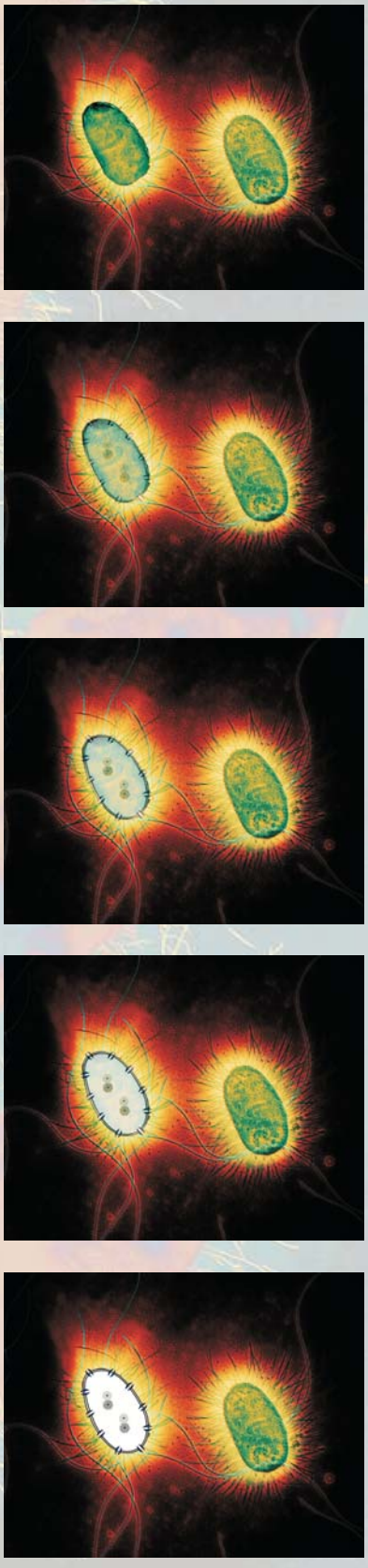
Individual-based Modelling of bacterial cultures in the study of the lag phase



Clara Prats Soler

Universitat Politècnica de Catalunya

Departament de Física i Enginyeria Nuclear

Programa de Doctorat

de Física Aplicada i Simulació en Ciències

Individual-based Modelling of bacterial cultures in the study of the lag phase

Memòria presentada per

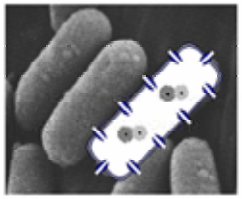
Clara Prats Soler

per optar al grau de Doctora

per la Universitat Politècnica de Catalunya

Co-directors: **Daniel López Codina** i **Antoni Giró Roca**

Castelldefels, abril de 2008



Sistemes complexos. Modelització i simulació discreta de sistemes biològics
Dept. de Física i Enginyeria Nuclear
Escola Superior d'Agricultura de Barcelona
Universitat Politècnica de Catalunya

Contents

1 Introduction **1**

1.1 Basic microbiological concepts 2

1.1.1 Microorganisms and microbiology 2

1.1.2 Bacteria and bacterial cultures 2

1.1.3 The lag phase definitions 5

1.2 Levels of description of a microbiological system 8

1.2.1 Molecular level of description 8

1.2.2 Cellular level of description 9

1.2.3 Population level of description 9

1.2.4 The *mesoscopic* approach 10

1.3 Predictive microbiology 10

1.3.1 Food microbiology 10

1.3.2 Modelling microbial systems in food 11

1.3.3 Modelling the lag phase 12

1.3.4 From science to industry 16

1.4 Aim, approach and outline of the thesis 16

2 Individual-based Models in microbiology **21**

2.1 Interest and background 21

2.1.1 Why Individual-based Modelling in microbiology? 21

2.1.2 Individual-based Modelling of microbial systems: some examples . 22

2.2 INDISIM 28

2.2.1 Background and history 28

2.2.2 General outline of INDISIM in bacterial systems 30

2.2.3 Modelling the bacteria 31

2.2.4 Modelling the environment 37

2.2.5 Simulation program setup 38
2.3 INDISIM for studying the lag phase 40
2.3.1 General outline 41
2.3.2 Modelling the bacteria 41
2.3.3 Modelling the enzyme effects 41
2.3.4 Modelling the environment 44
2.3.5 Mathematical methods 44
2.3.6 Simulation program setup 45
2.3.7 Overview of a simulation 46

3 Study of the microscopic causes of the lag phase 51
3.1 Introduction 51
3.2 Biomass evolution and the lag phase 52
3.2.1 The *distances*: a mathematical tool for assessing the evolution of the biomass distribution 53
3.2.2 Analyzing the biomass distribution of a simulated growth through the *distances* 55
3.3 Enzyme generation and the lag phase 56
3.3.1 Evolution of the biomass distribution during the lag phase 56
3.3.2 Enzyme synthesis rate and lag phase 58
3.4 Variability of the lag phase with different factors 61
3.4.1 Temperature 61
3.4.2 Initial cellular mean mass 63
3.4.3 Maintenance energy 64
3.5 Effects of the inoculum size 64
3.5.1 Inoculum size and the lag phase 66
3.5.2 Inoculum size and mean generation times 68
3.6 Discussion 72

4 Theoretical analysis of the stages in bacterial lag phase 75
4.1 Introduction 75
4.2 Concepts, variables and simulation setup 76
4.2.1 The bacterial lag phase duration 76
4.2.2 Relevant variables: bacterial mean mass, biomass distribution and maximum growth rate 77
4.2.3 INDISIM simulations 79
4.3 Theoretical analysis of the stages in bacterial lag phase 79

4.3.1 Growth rates calculated through total biomass and cell density . . 79
4.3.2 Lag phase assessed through cell density 80
4.3.3 A mathematical model for the transition phase in cell density . . . 82
4.3.4 Lag phase according to total biomass criteria 84
4.3.5 The lag phase as a result of the biomass and cell density interaction 85
4.4 Simulation results on the stages in bacterial lag phase 86
4.4.1 Transition between lag and exponential phases in cell density . . . 86
4.4.2 On the duration of the transition phase in cell density 90
4.4.3 Transition between lag and exponential phases both in cell density and total biomass 92
4.5 On the lag and exponential phase definition 94
4.5.1 The lag parameter 95
4.5.2 Exponential phase and balanced growth 98
4.6 Discussion 100

5 Experimental study of the biomass distribution dynamics 103
5.1 Introduction 103
5.2 Experimental setup 105
5.2.1 Flow cytometry 105
5.2.2 Particle size analysis 107
5.2.3 Comparative performance 108
5.2.4 Bacterial strain and growth conditions 109
5.2.5 Details of the performed experiments 110
5.3 Results 113
5.3.1 Processing the experimental data 113
5.3.2 Results of the series T20: culturing at 20°C 117
5.3.3 Results of the series T35: culturing at 35°C 121
5.4 Discussion 127

6 Optimization methods for IbM parameter estimation 131
6.1 Introduction 131
6.1.1 Input indexes and parameters of an IbM simulation 133
6.1.2 Basis of the IbM parameter estimation 134
6.2 Parameter estimation with INDISIM 135
6.2.1 Experimental dataset 135
6.2.2 Objective function 135
6.2.3 INDISIM adaptation 136

6.3 Parameter estimation methods 140
6.3.1 Classical method: grid search 140
6.3.2 Nelder-Mead Threshold Accepting 142
6.3.3 The NEWUOA method 144
6.4 Results 147
6.4.1 Parameter estimation with exponential phase experimental dataset 147
6.4.2 Parameter estimation with lag and exponential phases experimental dataset 151
6.5 Discussion 157

7 Conclusions **159**
7.1 Synthesis of results and specific conclusions 159
7.2 General conclusions 169
7.3 Perspectives and further work 171

A Glossary **173**

B List of publications **179**

Bibliography **182**

Agraïments **193**