

# RESEARCHSPACE@AUCKLAND

## http://researchspace.auckland.ac.nz

#### ResearchSpace@Auckland

### **Copyright Statement**

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage. <a href="http://researchspace.auckland.ac.nz/feedback">http://researchspace.auckland.ac.nz/feedback</a>

# General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

# Mathematical and Numerical Modelling of Bacterial Colony Growth on High Nutrient Surfaces

Leonie Zandra Pipe

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

University of Auckland 2009

## **ABSTRACT**

Traditionally, growth and morphology studies of bacteria have focused on individual cells. When microbiologists subsequently became interested in bacterial colony growth, they investigated the dynamics of its height as well as those of its diameter. The models they proposed, however, were little more than empirical. In recent decades there has been a shift in focus, as researchers became interested instead in the morphology changes that colonies undergo when exposed to stressful environments such as nutrient and moisture limitation. As a result of this shift, models of colony growth in three dimensions have remained underdeveloped and rudimentary.

My first task in this thesis was to assemble a sufficiently comprehensive data set from which diameter, height and cell-number growth trends in colonies could be properly analysed. This was achieved by studying the growth of many colonies of two bacterial species, *Serratia marcescens* and *Esherichia coli*, on high-nutrient non-selective agar, over a range of incubation temperatures, over periods ranging from two hours to one week.

When graphed and analysed, colony diameter and colony height growth turned out to be most economically described as power-law in time, with exponent < 1. This contrasts with the claims of previous researchers, who had described both growth trends as linear, with diameter switching to a slower yet still linear growth after a certain time, and height growth ceasing altogether.

From my results, I proposed a simple conceptual model, an extension of a model developed by Pirt in 1967. My hypothesis was that, in colonies growing on high-nutrient surfaces, diffusion was the dominant factor in colony growth. Ron Keam transformed my conceptual model into a mathematical one, from which I have developed one-dimensional and two-dimensional numerical simulations. In all simulations to date, in both one and two dimensions, a power-law growth phase emerges as a consequence of nutrient-controlled growth, preceded by an "accelerating" phase during which colony growth overtakes diffusive processes, and succeeded by a slow transition towards growth cessation as nutrient becomes exhausted.

In addition to successful demonstration of the power laws, the model in its final form yields realistic colony profiles and exhibits other features consistent with experimental results reported in the literature.

#### **ACKNOWLEDGEMENTS**

My supervisors, Malcolm Grimson and Ron Keam, have been pivotal in helping me acquire the skills and the confidence to take this project to completion. They have been unfailingly available when needed and I much value their expertise and guidance. Thanks are due also to Ruth Lyons, my supervisor in the early stages of this project, for her support in the experimental aspects of the work, and for the interesting discussions.

Gillian Lewis, of the School of Biological Sciences (SBS), allowed me access to her laboratory facilities, without which I could never have collected the large amounts of data that have made this project so successful. She also encouraged me to join the New Zealand Microbiology Society, of which she was one-time President. Through the NZMS, I obtained funding to present my early work at a conference in Cairns. Gillian, thanks a million!

Tony Roberton, who occupied the other end of the lab, took a kindly interest in my research and made a number of interesting suggestions. Pity the penicillin experiment failed!

Thanks are due to SBS photographers Jae Gae and especially to Iain MacDonald, who gave his time so willingly. I acknowledge the help and friendly support of Yimin Dong, the main technician in Gillian's lab. Thanks also to all the students in the lab who welcomed a stray physicist onto their home base; you guys (and girls) were great.

I am indebted to Francie Norman, head secretary in the Department of Physics, and to Edwin Rogers, computer technician in the same department, both of whom took time out from their very busy schedules to help with document layout and with scanning photographs, respectively. Their help was very much appreciated.

I must thank Peter Crossley, of the Geography Department, for his generous loan of the temperature-sensing device, and the firm Redwood Engineering, who sold one of their precious new digital calipers for no more than the cost to them, so that I could obtain accurate measurements of colony diameters. By the way, Dad, thanks for buying it for me. Thanks, too, for your undying support.

Thanks to the rest of my family; Mum, Vanessa, Rebecca, Isobel; you were always there for me. Special thanks are awarded to my nieces Rebecca and Isobel, for the time they invested in scanning images for me so that I could pepper my thesis with pretty pictures. I know teenage girls have better things to do... Love you heaps.

The last of my acknowledgements must go to the little guys without whom this thesis really could not have been completed; *Serratia marcescens* and *Escherichia coli*. You little guys made beautiful colonies!

# **CONTENTS**

| Ab                           | stract       | i   |
|------------------------------|--------------|---|
| Acl                          | knowledger   | nentsii   |
| Tab                          | ole of Conte | ntsiv   |
|                              |              |   |
| Ch                           | apter One: 9 | Setting Bacterial Colony Growth Modelling in Context                  |
| 1.1.                         | Why M        | Iodel the Growth and Development of Bacterial Colonies?               |
| 1.2.                         | Some I       | Essential Background Information                                      |
|                              | 1.2.1.       | Bacterial Cell Structure  |
|                              | 1.2.2        | Bacterial Growth and Environmental Influences                         |
|                              | 1.2.3        | Chemotaxis and Cell Movement  |
| 1.3                          | Bacteri      | al Colony Dynamics - Lessons from Experiment                          |
| 1.4                          | Bacteri      | al Colony Development from a Modelling Perspective                    |
|                              | 1.4.1.       | Empirical and Curve-Fitting Models Applied to Liquid Broth Cultures15 |
|                              | 1.4.2        | Classification and Comparison of Quantitative Models                  |
|                              | 1.4.3.       | Morphology and Phase Transition in Non-Living Systems20               |
|                              | 1.4.4        | Modelling of Bacterial Colony Dynamics - A Chronology24               |
|                              | 1.4.5        | Applications to Specialised Colony Forms                              |
|                              |              | 1.4.5.1 Interacting Colonies  |
|                              |              | 1.4.5.2 Chiral Morphologies   |
|                              |              | 1.4.5.3. Vortex Morphologies  |
|                              |              | 1.4.5.4. The Myxobacteria   |
|                              | 1.4.6        | Growth of Tumours in Tissues - A Close Analogue56                     |
|                              | _            |   |
| Ch                           | apter Two:   | Experimental Design and Implementation                                |
| 2.1.                         | Choice       | of Organisms60  |
| 2.2.                         | Liquid       | or Solid Inoculum - An Initial Dilemma                                |
| 2.3.                         | Prepar       | ation of Agar and Samples63   |
| 2.4. Measurement of Colony I |              | rement of Colony Diameter   |
| 2.5.                         | Measu        | rement of Colony Height   |
|                              | 2.5.1.       | Conversion of Fine Focussing Units to Millimetres                     |
|                              | 2.5.2        | Correction for Agar Slope and Desiccation in Agar and Colony69        |
|                              | 2.5.3.       | Estimating Statistical Variability in Colony Height                   |
| 2.6.                         | Estima       | tion of Number of Cells in the Colony                                 |

|        | 2.6.1.   | Choice of Method   |  |  |
|--------|--|--|--|--|
|        | 2.6.2.   | The Bacterial Counting Chamber and the Consequences of Cell Clumping.73      |  |  |
|        | 2.6.3.   | Calculation of Cell Number and its Error75                                   |  |  |
| Chap   | oter Three   | e: Experimental Results and Analysis   |  |  |
| 3.1.   | Dynamics of Diameter Growth                              |  |  |  |
|        | 3.1.1.   | Analysis of Diameter Growth over a range of Incubation Temperatures78        |  |  |
|        | 3.1.2.   | The Effect of Plate Size on Diameter Growth80                                |  |  |
|        | 3.1.3.   | The Effect of Plate Drying on Diameter Growth83                              |  |  |
| 3.2.   | 2. Dynamics of Height Growth                             |  |  |  |
| 3.3.   | Increase in Total Cell Number87                          |  |  |  |
| 3.4.   | Colony Profiles  |  |  |  |
|        | 3.4.1.   | Radial-Height Development of Colonies over 7 Days90                          |  |  |
|        | 3.4.2.   | Effect of the Inoculation Technique on the Radial-Height Profiles95          |  |  |
| 3.5.   | Effect   | of Cycling the Temperature between 37° C and Room Temperature96              |  |  |
| 3.6.   | The D  | evelopment of Irregular Forms in <i>E. coli.</i>                             |  |  |
| 3.7.   | Summ   | nary of Results104   |  |  |
| 4.1.   | How s  | general are the Power Laws? Are they Consistent with Results Reported in the |  |  |
| Litera | ature?   |  |  |  |
| 4.2.   | Analysis of Some Existing Bacterial Colony Growth Models |  |  |  |
|        | 4.2.1.   | The Model of Pirt (1967)   |  |  |
|        | 4.2.2.   | The Model of Kamath and Bungay (1988)116                                     |  |  |
|        | 4.2.3.   | The Model of Grimson (1993)  |  |  |
|        | 4.2.4.   | The Conceptual Model of Pipe (2000)133                                       |  |  |
|        | 4.2.5.   | The Model of Lega and Passot (2003)134                                       |  |  |
| Chap   | oter Five:   | Formulation of a New Model and Estimation of Parameters from Biological      |  |  |
| _      | wledge   |  |  |  |
| 5.1.   | A Ne   | w Model Based on Fundamental Physical Principles                             |  |  |
| 5.2.   | A Numerical Plan for the New Model                       |  |  |  |
| 5.3.   | Estimation of Model Parameters                           |  |  |  |
|        |  |  |  |  |
|        | 5.3.1.   | Determination of $n_b$   |  |  |

|       | 5.3.3.  | Determination of N  | 149 |  |  |
|-------|---|---|-----|--|--|
|       | 5.3.4.  | Determination of $k_0$                                      | 152 |  |  |
|       | 5.3.5.  | Determination of Diffusion Coefficients                     | 154 |  |  |
|       | 5.3.6.  | Summary of the Model Parameters                             | 155 |  |  |
| Chapt | er Six: T                                     | he New Model in One Dimension                               |     |  |  |
| 6.1.  | Reduc   | tion of the Model to 1D                                     | 156 |  |  |
| 6.2.  | Resul   | Results of Simulations                                      |     |  |  |
| 6.3.  | Paran   | Parameter Trials  |     |  |  |
| 6.4.  | Nutrie  | ent Density Consistency Check                               | 177 |  |  |
| Chapt | er Sevei                                      | n: The New Model in Two Dimensions - Implementation         |     |  |  |
| 7.1.  | The N   | The Numerical Scheme  |     |  |  |
|       | 7.1.1.  | Derivation of First and Second Order Derivatives for        |     |  |  |
|       |   | Unequal Grid Spacings                                       | 179 |  |  |
|       | 7.1.2.  | Method Selection - Implicit versus Explicit Schemes         | 186 |  |  |
|       | 7.1.3.  | Stability Analysis of an Explicit Numerical Scheme          | 198 |  |  |
|       | 7.1.4.  | Black Box Solvers   | 204 |  |  |
| 7.2.  | Construction of the Colony Grid               |   |     |  |  |
|       | 7.2.1.  | Preliminaries   | 206 |  |  |
|       | 7.2.2.  | Centralising the Boundary Points                            | 207 |  |  |
|       | 7.2.3.  | Determination of Boundary Point Coordinates                 | 213 |  |  |
| 7.3.  | Update of the Velocity Field in the Colony    |   |     |  |  |
|       | 7.3.1.  | Numerical Implementation of the Velocity Field              | 217 |  |  |
|       | 7.3.2.  | The Local Contribution to a Representative Point's Velocity | 219 |  |  |
|       | 7.3.3.  | The "No-Flow" Model   | 222 |  |  |
|       | 7.3.4.  | Error in Radial Velocity                                    | 228 |  |  |
| 7.4.  | Update of Nutrient Density in Colony and Agar |   |     |  |  |
|       | 7.4.1.  | Update of Nutrient in the Colony                            | 234 |  |  |
|       | 7.4.2.  | Update of Nutrient along the Agar/Colony Interface          | 238 |  |  |
|       | 7.4.3.  | Generalisation of the Flux Condition to $f \neq 1$          | 241 |  |  |
| Chapt | er Eight                                      | : The New Model in Two Dimensions - Minimal Version         |     |  |  |
| 8.1.  | Result  | s of Simulations  | 244 |  |  |
| 8.2.  | Nutrient Density Consistency Check            |   |     |  |  |

| Chapter Nine: The New Model in Two Dimensions - Final Version |   |   |  |  |
|---|---|---|--|--|
| 9.1.  | Incorporation of Saturation and Maintenance Modes into the 2D Model |   |  |  |
|   | 9.1.1.  | Estimation of Suitable Values of $n_m$ and $n_s$                |  |  |
|   | 9.1.2.  | Numerical Implementation of Saturation and Maintenance Modes273 |  |  |
| 9.2.  | Results of Simulations  |   |  |  |
| 9.3.  | Radia   | Velocity Convergence Test                                       |  |  |
| Chapt   | er Ten:   | Concluding Remarks  |  |  |
| Appen   | dices   |   |  |  |
| Appen   | dix 1.  | Colony Growth Statistics  |  |  |
|   | A 1.1.  | Dependence of Growth Parameters on Temperature311               |  |  |
|   | A 1.2.  | Coefficient of Dispersion in Cell Numbers319                    |  |  |
| Appen   | dix 2.  | Experimental Colony Growth Trends                               |  |  |
|   | A 2.1.  | Establishing the Empirical Growth Laws                          |  |  |
|   |   | A 2.1.1. Diameter and Area Growth                               |  |  |
|   |   | A 2.1.2. Height Growth  |  |  |
|   |   | A 2.1.3. Cell Number Growth                                     |  |  |
|   | A 2.2.  | Graphical Representation of the Power Laws                      |  |  |
|   |   | A 2.2.1. Diameter Growth  |  |  |
|   |   | A 2.2.2. Height Growth  |  |  |
|   |   | A 2.2.3. Increase in Cell Number                                |  |  |
| Appen   | dix 3.  | The Mathematical Model  |  |  |
|   | A 3.1.  | Author's Note   |  |  |
|   | A 3.2.  | Towards a Fundamental Model of Bacterial Colony Growth          |  |  |
| Appen   | dix 4. R  | adial Velocity Text File  |  |  |
| Refere  | nces  |   |  |  |