Modelling the Growth Curve

0) Introduction

From Literature: 4 phases (more or less distinct)

- Lag Phase
- Exponential Phase
- Stationary Phase
- Death Phase

Lag Phase

- Lag in growth has several causes
- Main Cause: ***Uptake of nutrients in medium by bacteria***
- Other reasons: switching replication machinery on
- As more and more nutrients are available, growth picks up
- Growth in volume then in number

Exponential Phase

- Growth of colony in number – and volume – is almost exponential
- ***Constant amount of nutrients inside cells***

Stationary Phase

- Growth of colony in number – and volume – has stopped
- Cause1: ***cells have run out of nutrients***
- Other Cause (relevance to be checked): death and division in balance

Death Phase

- Unlikely to be observed so we may be able to overlook the death mechanism
- Bibliographical research needed though
- Death term may indeed curb growth in previous three phases
- Its influence might even be noticeable in first three phases…

1) Suggested Modelling Approach

Overview

- Nutrients = Main Driver of Growth
- Let's Build an ODE Model Accordingly!!!!
- ODE/time should be precise enough
- Stochastic would be better but it would be very complex
- ***First Variable:*** Volume occupied by bacteria,
- ***Second Variable:*** Internal Concentration of Nutrients
- ***Third Variable:*** External Concentration of Nutrients

How to Build a Simple Model

- ***Simple Model = Combination of Two Sub-Models***
- ***First Sub-Model:***
  - Models the growth of the total bacterial volume against the concentration of available nutrients
  - Concentration of available nutrients = internal concentration
  - Growth rate function of internal concentration
- ***Second Sub-Model:***
  - Models the dynamic exchange of nutrients between the external medium and the interior of bacteria
  - Variables: internal and external concentrations
  - Process to model: diffusion of nutrients through a membrane

How to Build a More Complex Model

- ***Simple Model = Combination of Two Sub-Models***
- ***More Complex Model = Combination of Three Sub-Models***
- ***Third Sub-Model:***
  - Models the way the replication machinery switches on
  - And link between internal concentration of nutrients and the growth rate
  - We need the pathway(s) that control cell division
  - And we need to know how they consume the nutrients
- ***Not sure we need to build something that complex .. We will see***

Idea behind these models

- **Exponential Phase+ Stationary Phase**
  - Internal and External Concentrations of Nutrients almost equal (dynamic equilibrium)
  - Growth Rate Driven by Internal Nutrient Concentration
- **Lag Phase**
  - Internal and External Concentrations of Nutrients not in dynamic equilibrium =>delay
  - Cell Machinery Switches on Gradually also

2) The First Sub-Model

General Growth Model

Let us first assume that the concentration of nutrient does not have any influence on the bacterial growth.

- Write the ODE model for the Growth of volume depending on a ***constant growth rate***
- Simulate with Matlab

Curbed Growth Model

Unfortunately it seems that the internal concentration plays a role – a curbing role. We model the relationship between the growth rate and the internal concentration with a ***Hill function***

- What is the Hill function?
- What are its properties? Why are they relevant to our problem?
- *Bonus:* Can you think of another suitable way to model the link between the growth rate and the internal concentration?
- Write the New ODE System

We can now investigate the effect of the new term on bacterial growth. First we assume the ***internal concentration remains constant***

- Simulate with Matlab for various values of the internal concentration
- What are your conclusions?

Now let us assume that the internal concentration is controlled by an process in such a way that ***the internal concentration is a given function f(t)***

- Write the new ODE system
- For the function f(t) use a piecewise linear function
  - $f(t)=0$ for $t<t_0$
  - $f(t)$ is affine over $[t_0,t_1]$
  - $f(t)=C$ for $[t_1,t_2]$
  - $f(t)$ is affine over $[t_2,t_3]$
  - $f(t)=0$ for $t>t_3$
- Simulate with Matlab (use different values of C)
- What are your conclusions?

Conclusions

- What conclusions can you draw from your simulations?
- How can you obtain a stationary phase with such models?
- How can you obtain a lag phase with such models?
- Can you suggest a possible improvement?

From a Data Analysis Point of View

- Can these simulations help you analyse your Data?
- If so, How?

3) The Second Sub-Model

Creating a growth model that is controlled by the internal concentration of nutrients is easy. Creating a model for the evolution of the internal concentration of nutrients is not.
In this section we are going to build an acceptable model for the diffusion of nutrients through the membrane of a bacterium like b-subtilis – which we assume is the main process at work. As far as we are concerned, in this section, the nutrients are not consumed by the metabolism of the bacterium.

A Geometric Model for B-Subtilis

A bacterium like b-subtilis can be likened to ***a bag whose membrane separates an external medium from an internal medium***.

- Discuss these assumptions

We can assume that the bacteria in a colony share the same shape and thus that their surface and their volume are linked by a relation of the kind $S=\alpha V^{2/3}$ - α is a function of the shape.

- If the bacteria are spheres, what is α?
- Discuss these assumptions. Can some more assumptions be made?
- Does the thickness of the membrane grow with the bacterium?
- If so how are the volume and the thickness linked?

The Diffusion model

The process controlling the movement of the nutrients in and out of the bacteria is the ***diffusion process***.

- What is diffusion?
- Find a simple model for the exchange of nutrients through a permeable wall (in one dimension)
- Adapt the model to a bag with permeable membrane
- Did you need to make some further assumptions?

Let us assume the bag is spherical and initially empty. The external medium keeps its concentration constant. First, let us assume ***the bag keeps its volume constant*** (say to V=1).

- Write the ODE system controlling the internal concentration of nutrients
- Simulate with Matlab and Comment
- What do your results say about the uptake of the growth curve??

Now let us assume ***the bag keeps being pumped*** such as V(t)=1+t. The external medium still keeps its concentration constant.

- Write the ODE system controlling the internal concentration of nutrients
- Simulate with Matlab and Comment

One last word…

A bacterial colony can be likened to a collection of bags. To use the model you have just built, we need to assimilate this collection of bags to a single bag (of increasing volume). What errors do you think we make by assimilating these collection of bags to a single bag ???

4) Combining the Sub-Models

All that is left to do is to combine the two sub-models. There is still a little work left to do however. Indeed two important phenomena, which we have not addressed yet, link nutrients and growth:

1. Nutrients need to be consumed for the volume to increase
2. There is a finite amount of nutrients available in the culture medium

Incorporating the Consumption of Nutrients

Just like any metabolic function, the increase in volume consumes energy and nutrients. This needs to be incorporated into our model since it means that there will always be movement of nutrients through the membrane in order to make for the nutrients consumed by the growth.
We can assume that the amount of nutrients required to increase the volume by one unit is independent of the volume and constant.

- Comment on this assumption

Let us assume that the ***external concentration of nutrients remains constant*** (which is at least justified for very rich media)

- Write the ODE system
- Simulate with Matlab
- Can we get a lag phase?
- Can we get a stationary phase?

Modelling the Finite Amount of Nutrients

The culture medium has a finite volume and therefore contains ***a finite amount of nutrients***. In classic experiments the medium is not refilled and therefore will run out at some point – leading to the stationary phase of the growth curve.

- Modify your model accordingly
- Simulate with Matlab
- Can we get a lag phase?
- Can we get a stationary phase?
- Give a qualitative interpretation of your results

From a Data Analysis Point of View

We would like to know if our data are explained by the combination of the two sub-models or if the sub-model accounting for the switching-on of the machinery needs to be incorporated

- How can your simulations help you analyse your Data?
- Could a nutrient refill help us separate the influence of diffusion and of the switching on of the cell-division machinery?

That's it… for the moment!!!