

## Modelling in microbiology

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### ABSTRACT

Mathematical models represent one end of a spectrum of activities designed to investigate natural phenomena. They attempt to simplify systems to uncover relationships which yield a consistent pattern when compared with *in situ* behaviour. Models can be *continuous* or *discrete*. Continuous models use families of differential or partial differential equations and are generally applied to large scale events. These can include global or oceanic cycles, the spread of microbial pathogens or genetically engineered species through an ecosystem or pattern formation like fairy rings or periodic bands generated by bacteria and fungi. Discrete models, for example cellular automata or the Swarm modelling system, apply most appropriately to micro-scale phenomena. These have been applied to bacterial colony formation and biofilm structures.

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### Introduction

Routes to investigating problems in microbial ecology form a hierarchy whose highest level is the natural ecosystem itself. Below this level is the microcosm which in the words of Pritchard and Bourquin [25] is defined as: '...an attempt to bring an intact minimally disturbed piece of an ecosystem into the laboratory for study in its natural state'. The microcosm is a homologue of the system itself, but it may also be manipulated under controlled conditions in the laboratory. At the third level is the experimental model system. This aims to simplify the natural system in order to investigate one or a small number of parameters ignoring or holding other factors constant. A good model should reveal the common properties of a class of system it aims to reproduce. Model systems in biology are *synthetic*. For example they may be communities reconstructed from pure cultures. However complex the mixture, the model can never *become* the natural system since it is impossible to prove that some unidentified, unculturable organism is absent though contributing to the natural community.

The most abstract level in this hierarchy is the mathematical model. Here the behaviour of a system is reproduced as a series of mathematical relationships which reflect and often predict its behaviour. Mathematical models try to incorporate the most important aspects of a system but are often extreme simplifications of the *in situ* phenomenon as far as all else is concerned.

Like an experimental model, a mathematical model is an analogue of reality. It takes characteristic properties of the system and translates these into mathematical equations. Each equation attempts to define relationships within the model according to the perceived behaviour of the natural phenomenon. Changes in the latter should be reflected by the behaviour of the mathematical model. A key function of the model therefore is to predict behaviour. A model is only validated if such predictions are confirmed. Where they are not, the model, like any scientific theory (to which models are of course related!), must be

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altered. There is a huge range of different approaches to modelling. Some of these have been reviewed by Characklis [3].

Microbes and their activities bridge the macroscopic and the microscopic worlds. This has a number of important implications. The macro-world is well represented by systems of differential equations. Thus, the Fick laws of diffusion, the Navier-Stokes equation and the Schrodinger equations all apply reliably in the macro world and are called *continuous* models.

In the micro world we are no longer dealing with a continuum of many processes. Thus pH and hence the activity of microbes can be determined by a very small number of protons within or around a single cell. What applies to protons will also probably apply to intracellular pools of substrates, products, messenger and signal molecules. Continuous models in the macro world can be deterministic though they may not. For example, certain coupled non-linear equations can generate catastrophe from what are smoothly changing solutions. Chaos, which is defined as a rate of loss of information to zero, is a property of some functions, for example models as simple as the logistic equation. On the other hand deterministic equations entail zero loss of information.

In the micro world an appropriate approach is *discrete* modelling. Here any or all of space, time and state may be discrete. For example, if space and time are discrete but state is continuous, the system is referred to as a coupled map lattice.

It is possible to use both continuous and discrete modelling to interpret microbial behaviour. For example, periodic growth bands (related to Liesegang ring formation in chemical reaction diffusion systems) can be modeled using continuous partial differential equations. On the other hand, growth of bacterial colonies showing pattern formation related to substrate concentration, is best modeled using cellular automata (CA) models which are *par excellence* examples of discrete systems. One of the more interesting properties of CA and related models is that they can generate complex structures having emergent properties. These cannot be predicted any more quickly than by running the simulation itself. Just to muddy the waters slightly, it *is* possible to construct discrete models, which are deterministic. Finally, difference equations used in finite difference and finite element analytical models are discrete in time and space!

## **Continuous Models**

### *Large Scale Systems*

Very large-scale models apply to oceanic microbial ecology. For example, Thingstad and Lignell [31] have established a group of related models to predict the control of bacterial growth rate, abundance, diversity and carbon demand. They stress the importance of bacterial virus particles, not in controlling growth rate or abundance of bacteria, but in altering the diversity of these populations.

Belov and Giles [1] have investigated the dynamics of cyanobacterial growth and behaviour, in particular the role of buoyancy changes in these organisms. The model consisted of seven differential and integro-differential equations and allowed the authors to investigate several scenarios of population growth.

### *The Spread of Microbes Through Natural Habitats*

An important element in microbial ecology is the way in which disease causing or other microbes and genetic elements spread through natural populations. Scott *et al* [29] have

established a model which predicts the movement of inoculant bacteria and substrates through the rhizosphere of wheat plants. Such a model has obvious value in assessing the risks involved in the release of genetically engineered organisms.

#### *Microbial Growth Dynamics in vitro and in situ*

By far the commonest application of time-continuous modelling has been with microbial growth dynamics (see for example Pirt [24]). More recently Panikov [20] has produced an extensive text on the subject, *Microbial Growth Kinetics*. Panikov includes numerous examples of growth models in closed and open, homogeneous or heterogeneous systems, both in the laboratory and from the environment. Most recently Panikov [21] has applied families of differential equations to model microbial growth in bioreactors and in natural soils.

#### *Continuous Biofilm Models*

Until recently microbial films were assumed to be smooth planar homogeneous structures and this led to the generation of continuous mathematical models such as *Biosim* [34]. However the realization that many biofilms could not be regarded as homogeneous layered systems led Wanner and colleagues to formulate a newer model called *Aquasim* [26, 33, 34, 35, 36]. This version of the model allowed the attachment and detachment of cells at the substratum surface but also incorporated sufficient spatial heterogeneity that transport through pores and water channels could be investigated.

An excellent example of the application of continuous models in biofilm research is the work of Dibdin who set out to model pH changes occurring in dental plaque. Shellis and Dibdin [30] reported on the high buffering capacity of this biofilm. This led Dibdin to formulate a numerical model of acid fluxes in dental plaque [6, 7, 8]. The model incorporated fixed charges associated with the chemical structures in plaque, as well as the neutralisation of acid groups by the flux of carbonate and phosphate from saliva into the plaque. It was a good mechanistic model with clear predictive value. It modeled only the diffusive processes associated with the mature plaque and said nothing about the microbial population present.

#### *Periodic Structures in Microbial Ecology*

Continuous time models can be used to investigate periodic structures such as fungal infections on rotting fruit, concentric ring structures in developing microbial colonies, fairy rings or bands of growth of *Gallionella* in aquatic sediments.

Hoppensteadt and Jager, [12] and Hoppensteadt *et al* [13] explained periodic ring formation in agar plate cultures of *Escherichia coli* having glucose diffusing outwards from the centre using a hysteresis model. *Bacillus cereus* growing on a casamino acid-containing medium in soft agar with counter-gradients of glucose and oxygen developed a series of regular growth bands. The latter (a) depended on oxygen and glucose; (b) were not due to motility; (c) were altered by increasing the buffering capacity of the medium and; (d) could be reproduced by replacing oxygen with a gel layer containing alkali [4, 37]. The system was modeled using a system of partial differential equations. In both systems large pH changes were identified as the main cause of the bands. The only way in which periodic bands or concentric rings could be produced in both of these model systems was by invoking an asymmetric activation threshold. It was considered that the asymmetry was

due to changes in the time constants between stopping growth (fast) due to low pH, and growth recommencing (slow) as conditions became favorable.

### **Discrete Models**

There are many different types of discrete model, however the systems of interest here are all related to cellular automata (CA) derived from Turing's original conceptual automaton model, which lies at the heart of every computer constructed since. The first CA model to capture the public imagination was Conway's Game of Life which consisted of a grid on which cells were placed in particular patterns. The computer scanned every point on the array. If it was occupied, application of very simple rules allowed the cell to survive, die or reproduce. As the game proceeded a range of more or less elaborate patterns could be generated depending on the original placement of the cells. It should be noted that in CA's, rules act locally but apply globally.

There have been a number of applications of cellular automata. According to Ermentrout and Edelstein-Keshet [9] CA's can be divided into three main classes:

1. *Deterministic or Eulerian automata:* These resemble the solution to partial differential equations and can model oscillations in excitable media, cardiac function and predator prey dynamics. The Game of Life is a deterministic automaton.
2. *Lattice gas models:* Particles on a discrete spatial grid are free to move around. Such movements are driven by random events. Lattice gas systems include fibroblast aggregation, ant trail organization and topographical neural maps.
3. *Solidification models:* Particles become bound and cannot move again. Models include phase change (solidification), precipitation, fungal growth patterns, and growth of non-motile bacterial colonies.

### *Biofilm Modelling*

An important question in microbial ecology is the relationship between pattern forming processes and stochastic and chaotic events on the organisation of microbial communities. For example, the anaerobic digester granule is a spherically stratified community with different physiological types located in different zones. Biofilm systems are much more variable and because of this there has not been a sensible consensus model of biofilm in general. As we will see there are at least three categories of biofilm model possible. These are broadly: (i) separate stacked structures consisting of microcolonies well spaced from their neighbours [14, 15]; (ii) stalked mushroom shaped structures penetrated by water channels [5, 17]; (iii) dense homogeneous biofilm containing microcolonies but lacking obvious or extensive water channels [19].

Thus the type of biofilm that forms on the inside of potable water pipes is an example of the first category and it seems unlikely that such complex assemblages could be modeled by using families of differential equations. Here the method of choice is to use discrete models such as CA's.

Van Loosdrecht et al [32] suggested that shear and substrate concentration influenced biofilm structure. Wimpenny and Colasanti [38] considered that the division of biofilm into the three separate classes described above required some kind of unifying explanation. They examined the substrate concentration in a variety of habitats and found that it varied over at least six orders of magnitude. The available nutrient in potable water systems (around 1 mg/l or less) at one end of the scale, rose to at least 100 g/l and probably higher

for short periods in the mouth when a human was consuming chocolate bars or proprietary bottles of cola. It did not seem unreasonable to consider that, over such a range, biofilm structure might vary enormously. A simple cellular automaton was used to investigate this in more detail. Space, time and state were discrete in this system. Substrate was distributed randomly as individual units across the array at the start. The latter were given an arbitrary diffusion coefficient, which determined how far substrate units would move at each iteration of the model. Cells were 'inoculated' onto the substratum at the start of each simulation. Cells had a 'yield coefficient' expressed as the number of units of substrate each cell had to accumulate before it divided. For a simple model the results resembled the natural system in an uncannily convincing manner. With small amounts of substrate, 'growth' led to the slow formation of branched stacks of cells. Many of the inoculum did not start to grow at all since substrate was completely depleted near the substratum surface: this corresponded to a thin layer of apparently non-growing cells seen in pictures of actual biofilm growing in low nutrient water systems. At higher concentrations of nutrient an irregular structure appeared with some pores within it. Modifying the yield and diffusivity of substrate generated convincing mushroom like structures. At the highest concentration a dense smooth structure appeared in the model.

Examination of the model revealed (crudely but effectively) the stochastic nature of film growth. Because of the 'graininess' of the substrate and its random distribution, different cells would start to grow and generally only a few of these would 'survive'. The picture in identical replicates of the simulation shows this clearly. Each of the structures is entirely different in detail but virtually identical in general form. This is true even if the pedigree of each inoculating cell is compared at low or at high substrate concentrations.

Since this work was published, a much more detailed and faithful simulation of biofilm growth has been produced by Picioreanu and his colleagues [22, 23 and this Proceedings]. The model used was a hybrid continuous-discrete system. Here the solutes diffused across the field as a continuum according to reaction-diffusion partial differential equations. The cells or cellular aggregates were modeled as discrete entities according to a cellular automaton. These workers generated two- and three-dimensional results and emphasized the importance of both shear and substrate concentration on biofilm structure. Hermanowicz [11] has also produced a CA model of biofilm development following similar lines to the work described above.

### *Bacterial Colony Morphology*

Bacterial colonial growth is one specific area of microbiology that has at least some links with microbial ecology. It also represents an interface between the use of continuous or discrete models. Where there is evidence of concentric ring formation, especially in swarming organisms such as *Proteus mirabilis*, continuous time models seem appropriate [10, J. Shapiro, E. Budrene personal communication). There are many examples of bacteria which form complex, often beautiful growth patterns. Most attention has been paid to organism of the *Bacillus* genus, in particular *Bacillus subtilis*. A number of different discrete models have been produced which reproduce some of the characteristics of these organisms. Schindler and Rataj [27] and Schindler and Rovinsky [28] used simple models associated with pattern formation, for example the diffusion limited aggregation (DLA) model and the ballistic aggregation model (BAM), to model growth of *B. licheniformis*. Matsushita and Fujikawa [18] mapped the response of a strain of *B. subtilis* to substrate and agar concentration. These workers applied a DLA model to explain their

results. The most sophisticated results used a CA to model the growth of *Bacillus subtilis* in a substrate gradient field [2]. The CA model was modified to include changes in growth pattern due to signaling between groups of cells.

### *Autonomous Agent Models*

Another example of a discrete modelling system is the Swarm system developed by the Santa Fe Institute in New Mexico, USA. Swarm was developed as a system for modelling the behaviour of hundreds or thousands of autonomous agents interacting within a dynamically changing environment. Each agent is an 'individual' object, or piece of computer code which obeys a set of rules governing its behaviour. The Swarm system has been applied in a number of quite disparate areas, for example in ant colony and other ecological models. It has recently been used by us to develop a model of bacterial colony growth [16, and the paper by Kreft *et al* in this Proceedings]. This model, called BactSim, incorporates nutrient uptake, physiology, energy and maintenance reactions, growth and cell division and hence aims to reproduce, albeit simply, the physiology of an individual cell. Swarm is intellectually more satisfying than the simpler CA model since the behaviour of individual agents is closer to the real behaviour of living organisms than are the 'cells' of a CA model. The main problem with Swarm agents is that they require much larger computer resources to model natural communities than does the simpler CA model.

### **Discussion**

There are a great many different approaches to mathematically modelling microbial ecosystems. Choice is determined largely by the types of questions asked, and particularly by the scale under consideration. Continuous models susceptible to analysis using differential and partial differential equations have a huge part to play in the investigation of large scale systems, or where regular phenomena like periodic structures form. As spatial scale is reduced so discrete models can become more important. There is naturally a degree of overlap between the two. For example growth of *Proteus mirabilis* on an agar plate may generate regular radial growth bands which can be modeled with continuous systems of partial-differential equations, whilst the colonial growth of a species like *Bacillus subtilis* is best examined by a discrete system such as a CA or Swarm model.

I believe that there is an exciting future for discrete models, especially as they become more sophisticated and are able to incorporate some of the physiological and genetic properties of interacting species into the structures they predict. In addition (and vitally), they must be able to respond to environmental characteristics in an appropriate fashion. It must be stressed again and again, that discrete modelling systems like CA and Swarm models have emergent properties. Thus it is not possible to predict the composition of a structure which the model generates any more quickly than by running the model itself.

Finally, it is clear that no one modelling system is ideal. Different problems require different solutions. However I am convinced that it is possible to use the best elements of each system to generate hybrid models that will contribute significantly to our understanding of the processes that determine the structure and function of microbial communities.

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