## 3.4 Ordinary differential equations and individual-based simulations to deal with the modelling of bacterial growth for use in classroom activities

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**Résumé** : L'étude de la croissance bactérienne offre d'excellentes possibilités de combiner des exercices de laboratoire, la modélisation mathématique et l'analyse de données basé sur un modèle. L'objectif des tâches conçues axée sur la représentation, l'identification et l'analyse des différentes phases (variations de taux de croissance) dans une croissance bactérienne (décalage, accélération, exponentielle, retard, fixe et baisse) au moyen de deux méthodes de modélisation, ordinaire équations différentielles et simulations individuelles. Les élèves ont eu l'occasion d'étudier la croissance d'une population bactérienne à partir de deux points de vue différents, un modèle continu et déterministe par rapport à un modèle discret et stochastique, qui ont enrichi le processus de connexion des mathématiques à l'étude des systèmes de vie.

**Abstract** : Investigation of bacterial growth provides excellent possibilities to combine laboratory exercises, mathematical modeling and model-based data analysis. The aim of the tasks designed focused on the representation, identification and analyses of the different phases (variations of the growth rate) in a bacterial growth (lag, acceleration, exponential, retardation, stationary and decline) by means of two modeling methodologies, ordinary differential equations and individual-based simulations. The students had the opportunity to investigate the growth of a bacterial population from two different perspectives, a continuous and deterministic model versus a discrete and stochastic model, which enriched the process of connecting mathematics with the study of life systems.

## Introduction

Application of mathematics in the microbial field is very fruitful in obtaining a deeper insight into the process of microbial growth. And vice versa, using this microbial system as an example of where to apply the concepts and tools developed in mathematical subjects provides good opportunities to asses and compare modeling methodologies. There is an interesting review on the growth of bacterial cultures written 65 years ago by J. Monod (1949) showing some fundamental and basic ideas in an attempt to characterize, by means of parameters and continuous functions, microbial populations "living" in a liquid medium. The first variables used for the description of the temporal evolution of a bacterial system can be "cell concentration", as the number of individual cells, or "bacterial density" or "bacterial biomass", as the dry weight of cells, per unit volume of a culture. With these variables the concepts of division rate and growth rate can be taken into account.

Let $x(t)$ be a time-dependent variable describing the variation of a certain substance. The instantaneous rate of the process is the derivative of $x(t)$, $\frac{dx(t)}{dt}$, and the specific rate of this variation, $\mu(t)$, is defined as $\mu(t) = \frac{\frac{dx(t)}{dt}}{x(t)}$. If $x(t)$ denotes the cell concentration in a bacterial culture then the growth rate is defined as the increase in cell concentration per unit time, while the specific growth rate is the increase in cell concentration per unit time per cell. A simple calculation shows that if $x(t)$ is positive then $\mu(t) = \frac{d}{dt} \ln x(t)$. Only when the average size of the bacterial cells does not change in the time interval considered, the increase in bacterial density or bacterial biomass is proportional to the increase in cell concentration. However, it is known that the average size of the cells may differ from one phase to another of a growth cycle or depend on the environmental conditions where the bacteria are growing. Thus, the two variables, cell concentration and bacterial density, are not always equivalent in the description of the system. This distinction has sometimes been ignored, causing confusion or misunderstanding in certain contexts. In fact, one variable or the other may be more important depending on the topic to be studied. For instance, cell concentration is necessary in problems where division is concerned, when only few microbes are involved, where the discrimination of the different types of microbes is crucial, or if the information regarding the composition of the population is significant. On the other hand, in some issues involving microbial chemistry, metabolism and nutrition, the noteworthy variable can be biomass or microbial density. The use of the variable total biomass (as a real variable) or the variable total number of cells (as an integer variable) for the formulation of models to represent these bacterial systems is linked to the perspective of the system that we have and the modeling methodology used for its study. Thus, taking into account the purpose of the study, the election of the one or the other can be justified. Although the two variables are not equivalent, it is convenient to express growth rates in the same units (i.e., number of doublings per hour) in both cases. These definitions involve the implicit assumption that in a growing culture all the bacteria are viable (capable

of division). Nevertheless, these assumptions appear to be fairly good assumptions when homogeneous populations are considered in homogeneous environmental conditions, but not in other situations where there appear gradients of nutrient, clusters or aggregations of microorganisms, or other type of heterogeneities in space or time.

In the growth of a bacterial culture, a succession of phases may be conveniently distinguished, characterized by variations of the growth rate :

 i Lag phase (growth rate null),

 ii Acceleration phase (increases),

 iii Exponential phase (constant),

 iv Retardation phase (decreases),

 v Stationary phase (null),

 vi Phase of decline (negative).

This is a general and somewhat combined representation of the growth of a microbial culture (Monod, 1949), although any one or several of these phases may be absent. Under suitable conditions, the lag and acceleration phases may often be suppressed, the retardation phase is frequently so short as to be almost imperceptible, and the stationary phase can exit for short or very long periods of time, depending on the strategies of the microbes when faced with stress or non-optimal conditions. On the other hand, more complex bacterial growth cycles can be observed in diverse environmental conditions.

# Differential equations and computational models

Each mathematical model is configured by taking into account a set of assumptions (not always explicitly stated). For instance, differential equations necessarily incorporate a set of assumptions about the system to be modelled, and when the discrepancies between those assumptions and the reality are too big they become unsuitable for the purpose for which they were formulated. In biology, in general, and in microbiology, in particular, the real systems are extremely complex, so the models for studying them must inevitably include simplifying idealizations. In addition, if the design of the differential equations is not simple enough their resolutions can become very tricky, or maybe only numerical resolutions can be carried out. Some of the procedures involved in those cases are not available for students in certain academic levels. In order to tackle more realistic approximations of complex systems and to take advantage of computers, in the 1990s a new method of modeling appeared with both a philosophy and perspective different from the classic modeling used up to then, the so-called individual-based models (IBMs), known as well as biological agent-based models. IBMs are models in which the individuals that make up the system are treated as autonomous and discrete entities, focusing on the characterization of these entities by means of rules of behaviour, which allow these elements to interact among themselves and with the environment in which they exist. This type of computational model requires different simplifications from those assumed by continuous models, and is a good modeling alternative for dealing with the study of complex systems (Jacobson & Wilensky, 2006),specific aspects connected with biosystems in general (Grimm & Railsback 2005), and with microbes in particular (Ginovart, López & Valls, 2002; Gomez-Mourelo &Ginovart, 2009; Hellweger & Bucci, 2009). Arguments for microbial IBMs include the ability to resolve population heterogeneity (intra-population variability), complete life cycles, and individual behaviour adapted to internal and external conditions, to link mechanisms at individual level (bottom) to population level (up) behaviour. The behaviour exhibited by bacterial populations, their statistical indicators and the cause-effect relationship with respect to the environment are properties that must emerge from the aggregation of the activities and interactions of all the microorganisms (individuals).

# Aim of the study

Experiences in the design and use of these computational models allows us to identify guidelines to work successfully within the framework of an IBM project in academia (Railsback & Grimm, 2011;Ginovart, Portell, Ferrer-Closas & Blanco, 2011, 2012), and to facilitate progressive incorporation of IBMs into curricula, complementing other existing modeling strategies more frequently used in classroom (such as differential equations). For instance, a set of diverse modeling activities for the study of a predator-prey system for a mathematics classroom in the first year of an undergraduate program in biosystem engineering have been recently implemented with success (Ginovart, 2013).

Continuing in the line of combining different modeling approaches in teaching and learning in a life science context, a set of modeling activities has been designed to deal with bacterial systems, specifically to tackle microbial growth in an environment where a population of bacteria can use a sole nutrient. The aim of the tasks designed for this study focused on the representation, identification and analyses of the different phases in the bacterial growth (variations of the growth rate) by means of two modeling methodologies. Firstly, various ordinary differential equations were proposed to deal with the bacterial growth, some of which were solved by hand and others with the help of the mathematical software Maple. Secondly, a very simple IBM was designed and implemented in the specific multi-agent programmable modeling environment NetLogo (Wilensky, 1999) in order to achieve a set of individual-based simulations of the behaviour of the bacterial population developing in a medium with nutrient. The results accomplished with these two modeling approaches and the possibilities offered for each model to characterize the different bacterial phases were assessed and compared.

# Material and methods

The participants in this study were a group of 30, third-year students of a Bachelor's degree in the field of Biosystems Engineering at the Universitat Politècnica de Catalunya, in the Barcelona School of Agricultural Engineering (Spain). The prior coursework for these students was related with the following compulsory subjects of 6 ECTS each : Mathematics I and II, Physics I and II, Chemistry I and II, General Biology, Microbiology, Statistics, among others, which guarantee a good knowledge of the bacterial system to be modelled and the basic mathematical tools. The activities planned took place in the context of the compulsory subject 'Programming and problem solving for engineering' in the sixth semester. In the context of Mathematics II (second semester) this group of students were previously familiarized in the resolution of ordinary differential equations and in the recognition, creation and description of IBMs, carrying out different modeling activities designed for the study of predator-prey systems (Ginovart, 2013). In 'Programming and problem solving for engineering', the students were training in the use of the multi-agent programmable modeling environment, NetLogo, a free tool accessible on the Web. They were able to pass from the level of being users of simulators already prepared in this framework to the level of developing and implementing their own simple models in this platform (constructing their own simulators).With the designed teaching material and with a computer to perform the resolutions of the ordinary differential equations with Maple and the individual-based simulations with NetLogo, the students were capable of answering questions and completing exercises to achieve the learning goals. Graphical representations of the growth curves obtained and their derivatives, jointly with the calculation of the corresponding specific growth rates, configured the set of results that they analysed and discussed. Students' responses regarding the modeling of bacterial populations and the two distinct methodologies applied for the study of the different growth phases, were collected via open-ended questionnaires in a final written report and face-to face dialogues during the development of the sessions in the computer lab.

# Discussion

Bacterial systems can be studied by formulating simplified representations of these systems using mathematical equations. The assumptions of the models must be substantial enough to generate simple formulations and to keep the mathematical equations analytically or numerically resolvable. The assumptions are symbolized through variables characterizing the state of the whole system and assuming typical behaviours of the microorganisms. In this sense, continuous models are more like a macro perspective of a system limited to descriptions of properties at this level, and not always able to represent heterogeneity. Some classical models used frequently in academia fail in the representation of the diverse bacterial growth phases. As real bacterial life is characterized by the presence of non-continuous events, IBMs have been shown suitable for its representation, using the mathematics of discrete events rather than rates or velocities, and taking into account the intrinsic variability of the system (in composition, space and time). This peculiarity of IBMs was exploited and illustrated when dealing with the growth of a bacterial population, enabling the representations of the different growth phases. Although IBMs are quite complex to develop because computer-programming abilities are required, the use of platforms already prepared and designed specifically for their implementation, such as NetLogo, helped with the programming, implementation and execution of the bacterial models created by the students. From the responses collected during the activities, it can be said that the students recognised the two viewpoints as complementary to each other and convenient for the study of this microbial system. They had the opportunity to investigate the growth of a bacterial population from two different perspectives, a continuous and deterministic model

versus a discrete and stochastic model, which enriched the process of connecting mathematics with the investigation of life systems.

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