

Chapter 1

Introduction

For the past several decades, many scientists have experienced great interest in crossing the frontiers among classic academic disciplines. At present these interdisciplinary domains still have many unexplored areas that, when studied, provide fruitful and exciting results. This thesis gathers four years of research carried out by a physicist in the framework of biology. Thus, this is a biology study from the perspective of classic physics: a mechanistic approach to the study of complex systems.

This project was carried out with the Modelling and Computer Simulation of Biological Systems (MOSIMBIO) research group of the *Universitat Politècnica de Catalunya* (UPC). This is an interdisciplinary group formed by physicists, mathematicians, agronomist engineers and biologists that have been working in Individual-based Modelling (IbM) and simulation of biological systems for a couple of decades. The developed methodology has been implemented in the simulator INDISIM (INDividual DIScrete SIMulation), which was the main tool for carrying out the research presented in this thesis.

We hope that the present study will arouse the interest of physicists and biologists, as well as engineers and scientists of nearby disciplines. Nevertheless, this thesis is basically in the framework of theoretical microbiology. In order to make its reading easier for any scientist or engineer that does not have a biology background, we will begin with an introduction to some basic microbiology concepts. Then, several concepts with regard to the modelling of microbial systems and predictive microbiology will also be introduced prior to the aims statement and outline of the thesis.

1.1 Basic microbiological concepts

1.1.1 Microorganisms and microbiology

Microorganisms are microscopic organisms consisting of a single cell or cell cluster, and they include viruses (Madigan and Martinko, 2006). Despite their small size, microorganisms are an essential part of the earth. They play a central role in every ecosystem and in the global biomass cycle. The bacteria represent 50% of the carbon and 90% of the nitrogen and phosphorus of our planet's biomass. The photosynthetic marine microorganisms produce, at a minimum, as much oxygen as the land plants (Maloy and Schaechter, 2006).

The actions of the microorganisms in nature are important and varied. Microbiology, or the science that studies the microorganisms, deals not only with the basic biology behind them, but also with many important practical problems in medicine, agriculture and industry that are related to microorganisms (Madigan and Martinko, 2006) (Fig. 1.1).

Microbial communities are complex systems hardly explained by reduction. Laboratory and field experiments with microorganisms are costly and often unfeasible. Nearly 99% of the known microbial species have not yet been successfully cultured *in vitro* (Sharma et al., 2005). Moreover, the fast evolution of microorganisms ensures that there will be no permanent solution to agricultural, medical, and environmental problems caused by microbes (Maloy and Schaechter, 2006). For these reasons, it is necessary to introduce new approaches and tools to improve our understanding of the microbial physiology, genetics and ecology.

1.1.2 Bacteria and bacterial cultures

The bacteria are unicellular microorganisms (Fig. 1.2). They are prokaryotic cells; that is, they lack nuclei, mitochondria and chloroplasts (Schaechter et al., 2006). The prokaryotic cells constitute the largest portion of the biomass on Earth. Most of them live underground in the oceanic and terrestrial subsurfaces, but they are also found in the Earth's surface (Madigan and Martinko, 2006).

We call bacterial cultures those growths of these microorganisms in a culture medium (i.e., nutrient solution). These growths are carried out in a laboratory under controlled conditions. The batch culture is a specific kind of culturing in which the bacteria grow in a system of a fixed volume without the addition or removal of the medium (Madigan and Martinko, 2006). Bacteria divide through binary fission. If the growing conditions

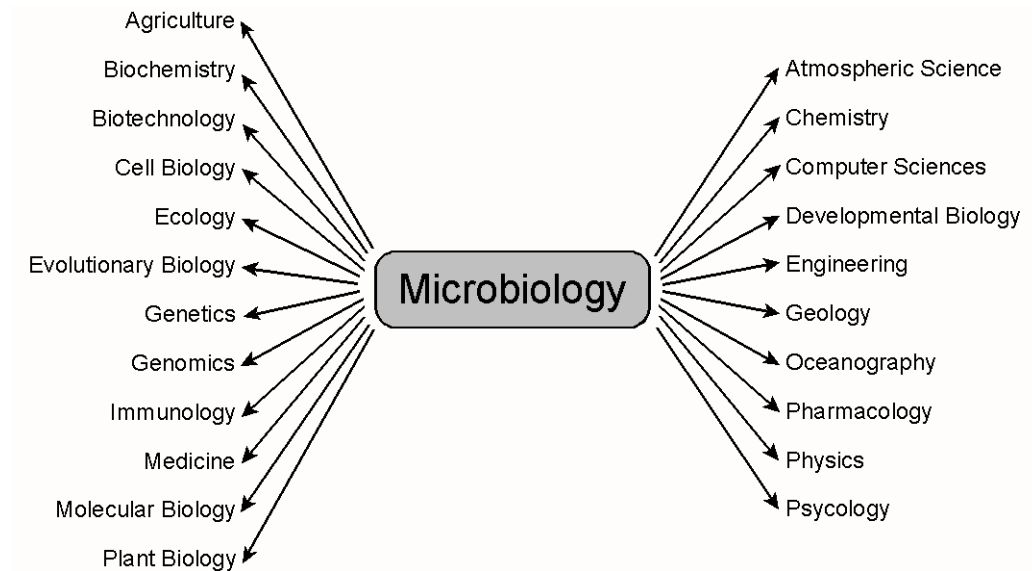


Figure 1.1: *For many years microbiology has had a major impact on the disciplines shown on the left side of the figure. However, as we have learned more about the impact of microbes on the environment, their roles in causing diseases previously attributed to genetics or environment, and their applications in nanotechnology, microbiology has assumed an important role in essentially all disciplines of science, including the disciplines shown on the right side of the figure. Reproduced with permission from Maloy and Schaechter (2006).*

are proper, at each division one bacterium becomes two, then two become four, and so on (Schaechter et al., 2006). This results in exponential growth; that is, the cell number of a population doubles within a fixed time period (Madigan and Martinko, 2006).

Growth curve and phases of growth

The growth cycle of a bacterial culture is commonly represented through the growth curve, which is the plot of the bacterial concentration over time. It is usually represented on a semilogarithmic plot, in order to identify the exponential growth. We find different phases in the growth curve, before and after the exponential phase, depending on the bacteria characteristics and the growth conditions. We may define four phases (Madigan and Martinko, 2006), as is shown in Figure 1.3.

Lag phase: when an inoculum is taken from a previous culture and added into a fresh medium, it may undergo a delay before it starts growing, which is known as the lag phase. During this stage of growth the cells carry out an adaptation that may be at different

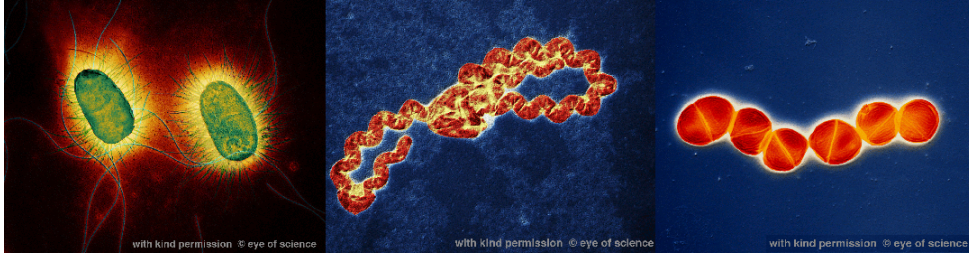


Figure 1.2: *Three examples of the wide variety of bacteria. The images have been obtained through electron microscopy and correspond (from left to right) to Escherichia coli, Leptospira bacterium and Streptococcus pneumoniae (© Eye of science, www.eyeofscience.com).*

levels such as dynamic, metabolic or genetic, among others. This adaptation depends on the history and state of the inoculum, as well as the conditions of the current environment in relation to the previous one.

The exact definition of lag phase and, above all, of its temporal limits, is not clear. We will discuss this in the next subsection.

Exponential phase: when the medium conditions are proper, the cells are in exponential growth. The maximum slope of the curve (in the semilogarithmic plot) is the specific growth rate of the organism in that particular environment, namely μ_{max} . While the environmental conditions do not change, this slope characterizes the main part of the exponential phase. In general, we use μ_{exp} or μ_{max} indistinctly.

Stationary phase: the stationary phase is characterized by the interruption of the divisions due to a lack of nutrient sources or to an increase in inhibitory substances. During this phase, the bacteria try to adapt to the medium to survive and they may even grow, but not divide.

Death phase: if the environmental conditions are too hard for the bacteria to adapt, or if these conditions persist over time, the culture can enter the death phase, in which a decrease in the viable cells is observed.

The transitions from lag to exponential and from exponential to stationary phases may be included in the main phases in different ways, depending on the definition used.

In some cases, we can also find intermediate lags that appear when the environmental conditions change but the bacteria are capable of adapting to the new conditions and start growing again.

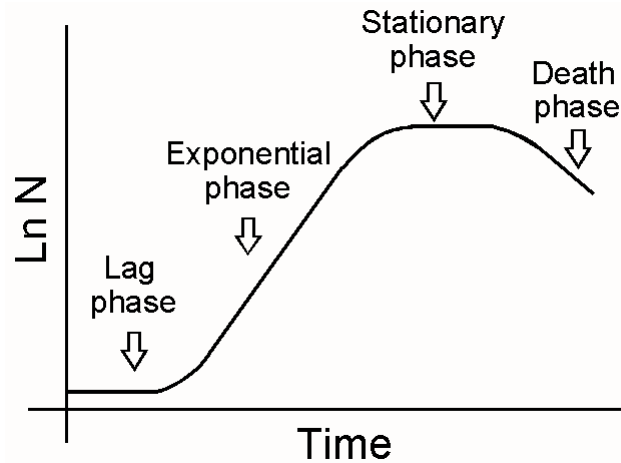


Figure 1.3: Overview of the phases of a bacterial population growth curve.

First division time and generation time

Two important parameters of a culture growth cycle are the first division time and the generation time. The first division time of a culture, t_{FD} , is the time that goes by before the first division of a bacterium takes place. The generation time or doubling time, t_G , is the time needed for a population to double, and it is related with the maximum growth rate (Eq. 1.1):

$$t_G = \frac{\ln 2}{\mu_{max}} \quad (1.1)$$

Population, biomass and biomass distribution

We call population the entirety of the bacteria of a culture. Usually, the term population is used to refer to the total number of cells or the cell density. The biomass is the mass of the bacteria, and the total biomass is the mass of the bacteria's population. We can also classify the bacteria into different *classes of biomass*: if we define some biomass intervals covering the whole possible range and we count the bacteria in each interval, we obtain the biomass distribution (Fig. 1.4).

1.1.3 The lag phase definitions

If we review the literature, we find different definitions for the lag phase. We provide here some examples to illustrate this lack of an exact and precise definition:

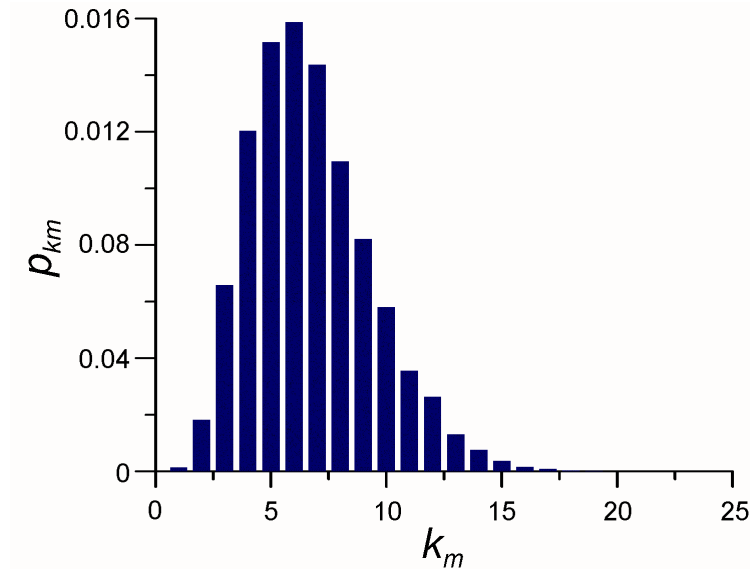


Figure 1.4: Typical biomass distribution of an exponentially growing culture (INDISIM simulation). The horizontal axis gives the intervals of biomass defined for counting the bacteria in each one (classes of biomass, k_m), and the vertical axis gives the frequency of bacteria in each interval (p_{k_m}). For experimental examples of biomass distributions see figures in Chapter 5.

- Classic geometric definition (Lodge and Hinshelwood, 1943): the lag phase duration is characterized by the lag parameter, λ , which is the intersection between the inoculation level and the prolongation of the exponential phase line in a semilogarithmic representation of the growth curve (Fig. 1.5). This point is between the first division and the exponential phase. This definition is a particular case of the one that states: 'if a growth curve converges to a $g_1(t)$ attractor and another one converges to a $g_2(t)$ attractor in such a way that $g_2(t) = g_1(t - \lambda)$, then we say that the second growth curve is lagging behind the first one by the λ interval'. In our case, $g_1(t)$ would be the pure exponential growth, and $g_2(t)$ the observed growth curve. This definition for the lag parameter is more universal than the geometric one because it can be used in different growth patterns and in non-semilogarithmic plots. In our case, the lag parameter calculated by means of the geometric definition and the lag assessed with this definition provide the same result.
- Buchanan and Solberg (1972): the lag phase (adjustment phase) is the 'time required for the 0-time inoculum to undergo a twofold increase in number'. This

definition sets a point between the first division and the exponential phase, but it does not necessarily agree with the lag parameter λ .

- Pirt (1975): the lag phase is a 'transition period during which the specific growth rate increases to the maximum value characteristic of the culture environment'. This definition includes in the lag period everything before the exponential phase.
- Buchanan and Cygnarowicz (1990): the lag phase duration may be calculated through 'the use of the maximum associated with the second derivative of the growth curve'. Thus, this definition sets the end of the lag phase at a point between the first division and the exponential phase. In many cases this is equivalent to the geometric definition.
- Madigan and Martinko (2006): the lag phase is 'a period preceding the exponential growth phase when cells may be metabolizing but are not yet growing'. It specifies that 'growth' is defined as 'an increase in number of cells', so the lag phase is identified with the lack of divisions.
- Schaechter et al. (2006): the lag phase is 'a delay in growth for a period of time when a culture that has been in the stationary phase for some time is transferred to fresh medium'. From the context we may deduce that the growth refers to the culture, so this definition, like the previous one, assumes a lack of divisions.

As been seen , there is no single definition for the lag phase. Usually, the lag parameter λ is accepted as the most suitable parameter because it facilitates comparison with values from the literature. Nevertheless, in Chapter 4 we will see that, although this definition is the easiest to use in most cases, it is not at all appropriate as a universal definition.

Pirt (1975) states five possible causes for the existence of an initial lag phase in a bacterial culture:

1. change in nutrition;
2. change in physical environment;
3. presence of an inhibitor;
4. spore germination; and
5. state of the inoculum culture.

Two of these causes will be tackled in the next chapters: the state of the inoculum culture, and the change in nutrition.

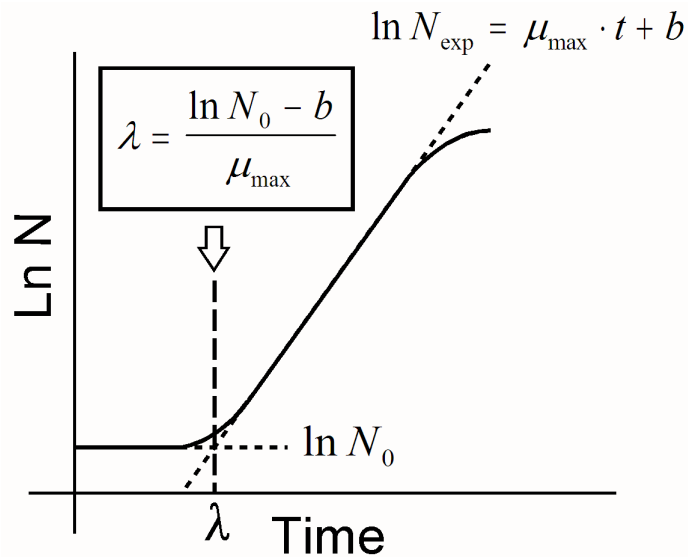


Figure 1.5: *Lag parameter calculation with the classic geometric definition (Lodge and Hinshelwood, 1943).*

1.2 Levels of description of a microbiological system

A theoretical approach to observed phenomena consists in building coherent models with descriptive and predictive capacity. Models are theoretical accounts, and they are always simplifications of the systems they represent. Modelling is an attempt to capture the essence of a system well enough to address specific questions about the system (Grimm, 1999). Therefore, models are abstractions of reality.

Models in biology tackle the systems under study through approaches at different levels of description. We roughly distinguish three different levels of description, and propose an intermediate approach to microbial communities.

1.2.1 Molecular level of description

Molecular models are addressed to the study of the structure of biomolecules and the interactions among them and with other molecules, such as water. They typically deal with 10^3 to 10^9 atoms, and are spatially explicit. The temporal scale for the studied phenomena ranges from 10^{-5} to 10^{-3} seconds, but an acceptable accuracy of models requires describing interactions at scales that may be as short as 10^{-9} seconds (Chu et

al., 2006). These types of models suggest rules for the atomic interactions and then point to study of the comprehensive behaviour of molecules and molecular aggregates. In this group we include studies that range from biochemical kinetics to structural biology. Some of the current topics under study are sequence analysis of macromolecules, study of their structure and related folding phenomena (Finkelstein and Galzitskaya, 2004), molecular interactions and docking (Zhu et al., 2003), and transport of substances through the cellular membrane (Hedrich and Marten, 2006), among others.

1.2.2 Cellular level of description

An average cell contains 10^{13} atoms. Many metabolic processes inside the cell, such as enzymatic limiting reactions or diffusion of substances across the cell, have a duration on the order of 10^{-3} seconds (Jou, 1985). Cellular approaches are focused on the interactions among biochemical kinetics, cellular structure and global regulatory mechanisms.

The study of phenomena at the whole-cell level of description requires a less exhaustive depiction of the involved processes than that provided by molecular models. This implies the use of phenomenological laws describing biochemical interactions, and the compilation of experimental data obtained from thoroughgoing molecular analysis. Cellular models are addressed to explain cell physiology and deal with the problem of reducing a great amount of experimental data in order to obtain a functional description of the cellular processes as a whole. In this group of models we include the fields of bioinformatics (Ashburner et al., 2000) and systems biology (Ishi et al., 2004).

In this thesis we call *microscopic scale* the level of description of the cell as a whole, as a basic unit.

1.2.3 Population level of description

Experimental microbial cultures deal with populations of up to 10^9 cells and last several hours or days. Models at a population level of description account for microbial communities as a whole and are designed to study their structure and evolution.

They offer a dynamic description of the total population by means of differential equations and stochastic treatments. Population models are often built for predictive purposes and by means of phenomenological laws for the behaviour of the whole system. Some of the applications of these models are predictive microbiology in foods and control of fermentation processes (Swinnen et al., 2004), optimization of microbial cultures and antibiotics production in the pharmaceutical industry (Raaijmakers et al., 2002), waste control and water treatment (Seviour et al., 2003), and the study of microbial ecology

and evolution of population diversity in wild or artificial ecosystems (Horner-Devine et al., 2003). Population models are based on assumptions about the individual behaviour of microbes, and they therefore also raise new questions regarding microbial physiology and cellular models (Wood et al., 2004).

In this thesis we use the term *macroscopic scale* to refer to this level of description.

1.2.4 The *mesoscopic* approach

In this thesis we want to focus on a particular subgroup of the population models: *mesoscopic* approaches, centered on the interactions among individual cells and with their local environment. These approaches treat microorganisms individually and may deal with up to 10^8 cells. The study of microbial systems at a mesoscopic level of description builds bridges between the approaches at the cellular and population levels, and thus improves the understanding of both. Its applications range from the testing of different microbial cellular physiology models to the study of how interactions among individuals build the laws of microbial ecology, population dynamics (Dens et al., 2005b), and genetic evolution of microbial strains (Paton et al., 2006).

1.3 Predictive microbiology

1.3.1 Food microbiology

We have seen that bacteria are an essential part of the earth's life. They live in almost every habitat, generally forming biological communities with strong interactions, and they can survive in strongly adverse environments. Specifically, bacteria produce substances that may be toxic for human bodies after their consumption, may modify food quality or, on the contrary, may be essential in food production and processing. Therefore, food microbiology (or the study of the microorganisms in foods) has become an important specific scope of microbiology.

Food microbiology deals with food quality assurance, predictive modelling and risk analysis (Fleet, 1999). Since microbiological analysis techniques are often costly and sometimes technically difficult, predictive modelling in food microbiology has become an essential discipline to provide a suitable complement to experimental assays. The aim of predictive microbiology is to build mathematical models that describe and predict the dynamics of bacterial growth in food (Standaert, 2007).

1.3.2 Modelling microbial systems in food

Baranyi and Roberts (1994) describe the reasons why predictive food microbiology needs to be developed on its own independently of biotechnology and chemical engineering. They state that:

1. the aim of food microbiology is normally to prevent microbial growth, while biotechnologists usually look to optimize it;
2. food microbiology usually deals with low bacterial concentrations, with the methods being validated at high concentrations (biotechnology) not always directly applicable to low concentration situations;
3. the typical growth conditions in food are a high nutrient concentration and a low bacteria concentration, while the growth in bioreactors is usually characterized by a high bacterial concentration while the substrate limitations may be very important;
4. biotechnology experiments are usually carried out in bioreactors, under controlled conditions. In contrast, food storage conditions are not always so controlled.

Consequently, the corresponding particular interests of predictive food microbiology are (Baranyi and Roberts, 1994):

1. to investigate the effects of inhibitory environmental factors;
2. to study the specific characteristics of the low cell concentration;
3. to specifically deal with the initial phases of growth (from lag phase to exponential);
and
4. to use simplifying and empirical elements in mathematical modelling.

Classic food microbiology modelling follows a two-step approach: primary models and secondary models (Swinnen et al., 2004). *Primary models* are designed to describe the bacterial growth curve, defining with precision the different phases of the curve (lag, exponential, stationary and death). For instance, the typical expression for describing the exponential phase is (Eqs. 1.2 and 1.3):

$$\frac{dN}{dt} = \mu_{max} \cdot N \quad (1.2)$$

$$N = N_0 \cdot e^{\mu_{max} \cdot t} \quad (1.3)$$

where μ_{max} is the maximum growth rate and N the cell concentration.

The primary models aim to use as few parameters as possible while still describing the curve with sufficient accuracy (McKellar and Lu, 2003). These models are used to obtain two kinetic parameters that characterize the first phases of the growth; the lag parameter, λ , and the maximum growth rate, μ_{max} (Swinnen et al., 2004). Thus, the primary models may describe the response of microorganisms to a single set of controlled conditions (Ross and Dalgaard, 2004). These growth models are usually continuous. The classic sigmoid growth functions and the mechanistic Baranyi model are two examples of the wide catalogue of growth models (McKellar and Lu, 2003).

Secondary models deal with the effects of environmental conditions on the values of the parameters of a primary model (Ross and Dalgaard, 2004). In general they establish the dependence of growth rate and lag parameter on different variables such as the temperature or the pH, among others. For instance, Ratkowsky et al. (1983) proposed an empirical non-linear regression model for the relationship between the maximum growth rate and the temperature (Eq. 1.4):

$$\sqrt{\mu_{max}} = b \cdot (T - T_{min}) \cdot (1 - e^{c \cdot (T - T_{max})}) \quad (1.4)$$

where T_{min} and T_{max} are the minimum and maximum temperatures at which the growth rate is zero, and b and c are two fitting parameters.

1.3.3 Modelling the lag phase

The lag phase of bacterial cultures has been widely studied for years in an effort to improve knowledge of the basic microbial cycle and kinetics, as well as for applied purposes such as biotechnology, microbial ecology and, above all, food safety (Swinnen et al., 2004; Schaechter et al., 2006). It is generally accepted that the lag phase is a complex process affected by environmental factors, pre-inoculation culture conditions and the genetic events that take place in this particular phase (Baranyi and Roberts, 1994; Schaechter et al., 2006). It is usually defined as the initial phase of a culture that precedes exponential growth whenever the conditions of the culture medium differ from those of the pre-inoculation. During the lag phase, the number of individuals remains approximately constant or increases slowly.

Research on the bacterial lag phase traditionally consists of two main approaches. Experimental approaches using cultured bacterial communities have generated a great deal of data for analysis and discussion (Augustin et al., 1999; Elfving et al., 2004). However, experimental approaches are specific in essence, while biological systems are

very complex and show a wide range of behaviours, so it is difficult to build up a general framework to explain the processes that regulate the lag phase using experimental data alone.

Mathematical modelling of the behaviour of the whole system has been used to look for a general insight into the lag process with predictive purposes (Swinnen et al., 2004). Increasing the holistic understanding of the system would allow a more general scope of the bacterial lag and provide new and deeper tools to study and compare different experimental results.

Primary models describing the evolution of cell number with time and, in particular, describing the lag phase, may be divided into deterministic and stochastic models (Swinnen et al., 2004). We present here a sample of some mathematical models that are found in the specialized literature (Swinnen et al., 2004).

Deterministic population models

In this section we examine a sampling of the published deterministic population models.

A commonly accepted growth model is the one from Baranyi and Roberts (1994). This model shapes the lag phase by means of an adjustment function $\alpha(t)$ (Eq. 1.5) that enables the transition from the lag to the exponential phase. This function depends on the physiological state of the cells, $Q(t)$, $Q(0)$ being the initial physiological state of the inoculum cells. $Q(t)$ grows exponentially (Eq. 1.6) according to the maximum growth rate, μ_{max} . Then, $\alpha(t)$ evolves from a small value towards one (Eq. 1.5).

$$0 < \alpha(t) = \frac{Q(t)}{1 + Q(t)} < 1 \quad (1.5)$$

$$\frac{dQ}{dt} = \mu_{max} \cdot Q(t) \quad (1.6)$$

The evolution of the cell concentration, N , is shaped by the adjustment function during the lag phase. When $\alpha(t) = 1$ it has no more influence on the growth. The cell concentration is limited by a threshold given by N_{max} , which is the maximum bacterial concentration. Thus, the growth curve is described by Equation 1.7.

$$\frac{dN}{dt} = \alpha(t) \cdot \mu_{max} \cdot \left[1 - \frac{N(t)}{N_{max}} \right] \cdot N(t) \quad (1.7)$$

In this model, the relationship between the lag parameter λ and the maximum growth

rate can be written as $\lambda \cdot \mu_{max} = \ln\left(1 + \frac{1}{Q(0)}\right)$. Since $Q(0)$ depends on the pre-culture state of the cells, the product $\lambda \cdot \mu_{max}$ should be constant for different growth curves of inocula taken from the same pre-culture (e.g., growths at different temperatures).

Another deterministic model is the one by Hills and Wright (1994), which builds a *mass-number model* that distinguishes between the growth of cell biomass M and the increase in viable cell number N . The total biomass per cell, s^* , is the sum of the minimum biomass per cell, s_{min} , and the excess biomass per cell, s . They take the minimum biomass per cell as the unit of biomass (Eq. 1.8).

$$M = N s^* = N \cdot (1 + s) \quad (1.8)$$

If no lag phase is assumed in biomass growth, M grows exponentially from the beginning at the maximum growth rate, $dM/dt = \mu_{max} \cdot M(t)$. Then, the lag phase in N is related to the excess of biomass per cell, $s(t)$ (Eq. 1.9).

$$\frac{dN}{dt} = k_n \cdot s(t) \cdot N(t) \quad (1.9)$$

The constant k_n is related with the DNA synthesis rate per cell and depends on the environment. Then, the evolution of the excess of biomass per cell is set by Equation 1.10.

$$\frac{ds}{dt} = (\mu_{max} - k_n \cdot s) \cdot (1 + s) \quad (1.10)$$

In this model, the relationship between λ and μ_{max} is given by Equation 1.11.

$$\lambda \cdot \mu_{max} = \ln\left[1 + (\mu_{max}/k_n)\right] \quad (1.11)$$

Therefore, it is not constant under temperature changes.

Both models (Baranyi and Roberts, 1994 and Hills and Wright, 1994) share the same philosophy: the lag phase is described by an increasing function ($\alpha(t)$ and $s(t)$, respectively) with a maximum value (1 and μ_{max}/k_n , respectively). Once the maximum value is achieved, the culture grows at the maximum growth rate, μ_{max} (Swinnen et al., 2004).

Finally, a third example of deterministic model is the *heterogeneous population model* (HPM) by McKellar (1997), which assumes that the lag phase is caused by the non-growing fraction of the inoculum. That is, the inoculum has two sub-populations: the growing fraction (G) shows no lag phase (Eq. 1.12), and the non-growing fraction (NG)

has an infinite lag (Eq. 1.13). Transitions between the two sub-populations are not considered.

$$\frac{dG}{dt} = \mu_{max} \cdot \left[1 - \frac{G(t)}{N_{max}} \right] \cdot G(t) \quad (1.12)$$

$$\frac{dNG}{dt} = 0 \quad (1.13)$$

In this case, the product $\lambda \cdot \mu_{max}$ is given by $\lambda \cdot \mu_{max} = \ln N_0 - \ln G_0$. McKellar (2001) develops this model to take into account transitions between NG and G compartments.

Stochastic population models

Several stochastic models have been build around the lag phase. A clear example is the *three-phase model* from Buchanan et al. (1997), which describes the lag phase by assigning an individual lag time, t_{LAG_i} , and a certain generation time, t_{m_i} , to each individual cell of the inoculum. The lag time is subdivided into an adjustment period, t_{a_i} , and the generation time (Eq. 1.14).

$$t_{LAG_i} = t_{a_i} + t_{m_i} \quad (1.14)$$

Baranyi (1998) reports the relationship between the individual cell's adjustment periods, τ_i (t_{a_i} in the previous model), and the population lag, λ (Swinnen et al., 2004). Actually, Baranyi (1998) identifies τ_i as the individual lag, with the time to the first division being the sum of the lag time and the generation time.

Individual-based Modelling and simulation

Simulation-oriented research is a rapidly growing approach to studying biological systems. It is usually defined as the *third way* to study complex systems, and is used as a bridge between the theoretical and experimental approaches. It can be used to partially test new theories when the experimental proof is too erratic, time consuming, tedious or expensive, as well as to design and delimit the relevant experiments to be carried out. Individual-based Modelling (IbM) and simulation is a useful bottom-up approach to the systems under study (McKellar and Knight, 2000; Swinnen et al., 2004; Grimm and Railsback, 2005). IbM can isolate the individual-cell level mechanisms behind the lag phase, and study their effects altogether or separately: IbM simulation is also a bridge between the description of individuals and the study of population dynamics.

Individual-based Models (IbMs) are conceptually easy to understand, but practically difficult to use. Although IbM simulations can be accurately parameterized in order to obtain quantitative information about specific cases, the required work to carry out the parameter estimation, calibration and sensitivity analysis is time-consuming.

As we will see in the following chapters, the strong point of IbM is its use to improve understanding of systems. The need for modelling the individual microorganism's behaviour when building an IbM requires an understanding of the processes that take place at an individual level. Moreover, the simulations allow control of the phenomena and resulting processes that emerge from these individual rules, both at the mesoscopic and the population levels.

In this thesis we will use the INDISIM methodology, which is a specific IbM simulation developed by our research group. Therefore, the next chapter will delve deeper into the utility of IbM simulations in the framework of predictive microbiology, as well as in INDISIM outline and involved models.

1.3.4 From science to industry

The aim of fundamental sciences is to make an abstraction of reality in order to explain the observed phenomena through models. Models are abstractions of reality; they aim to improve understanding of the system, but they may be far from practical applications. The step from fundamental science to technology in order to cover this gap is essential: this is the aim of applied sciences such as medicine or engineering.

Biological systems are really complex, which is what makes passing from biology to technology so difficult. Biotechnology is, nowadays, one of the big challenges in science and industry. In fact, most of the published studies regarding the bacterial lag phase are, in a simplified way, on side or the other: (i) they are abstract analyses that may not be directly or easily applicable to productive industry (for instance, IbM); or (ii) they are empirical fits with a direct application in industry.

One of the challenges in order to make progress is to improve abstract understanding of biological systems and, at the same time, to develop methodologies with technical applications.

1.4 Aim, approach and outline of the thesis

The aim of this thesis is to improve our understanding of the lag phase in bacterial cultures. In order to achieve this objective, we have to tackle the problem with three different approaches:

1. Conceptual approach: this approach aims to improve comprehension of the different intrinsic phenomena behind the lag phase.
2. Methodological approach: the modelling and simulation methods must be improved and developed in order to achieve the conceptual objectives. At the same time, these advances must be useful in the study of other transient processes that may be tackled with INDISIM.
3. Experimental approach: it is essential to experimentally verify the simulation results in order to validate the suitability and soundness of INDISIM.

Thus, we can define specific objectives for each approach that must be achieved throughout this work.

A) Conceptual approach

1. To identify the important variables for studying the lag phase. These variables should be appropriate to undertake the study of other transient processes.
2. To distinguish and analyze two of the five causes of the lag phase mentioned in Section 1.1.3.
3. To analyze the relationship of the lag phase duration with different variables such as temperature or the inoculum state.
4. To study the relationship between the individual lag and the population lag.
5. To analyze the temporal behaviour of the system during the lag phase.
6. From the obtained results, to carry out a critical revision of the lag phase concept.

B) Methodological approach

1. To improve INDISIM in order to study the bacterial lag phase:
 - (a) to improve the model with the required adaptations,
 - (b) to implement the new improvements in the simulator, and
 - (c) to incorporate the specific calculus and the appropriate outputs in the simulator.

2. To verify the suitability of the IbM approach and, specifically, the suitability of INDISIM in the study of transient processes in bacterial growth such as the lag phase.
3. To develop mathematical tools for assessing the dynamics of the transient processes.
4. To develop methods for parameter estimation in IbMs.

C) Experimental approach

1. To select appropriate experimental methods for measuring the evolution of the biomass distribution of a culture throughout the growth cycle.
2. To adapt the experimental protocols in order to obtain the necessary measurements.
3. To analyze the experimental results and to develop the necessary mathematical methods for doing this.
4. To interpret the obtained results and to compare them with the previous simulation outputs in order to validate the suitability and soundness of INDISIM simulations.

The aim of this thesis is always tackled from a very specific point of view: Individual-based Modelling. The three approaches described above are understood within an IbM framework; even the experimental results must be interpreted under this specific framework. Therefore, in Chapter 2 we start presenting an overview of the use of IbMs in microbiology. This review comprises some examples of specific problems that have been tackled with different IbMs. Then, a general outline of INDISIM is presented. This outline is made up of an initial history to describe the current context and an overview of the specific models behind the simulator. Finally, a specific section describing the model improvements and specific calculations that have been implemented with INDISIM in order to study the bacterial lag phase is presented. A part of this chapter has been submitted for publication at an international journal (Ferrer et al., 2008).

In Chapter 3 we make the first approach to studying two microscopic causes of the lag phase: the lag caused by the initial state of the inoculum (coming from a stationary phase of a pre-inoculum), and the lag caused by a specific change in the environment (regarding the nutrient source). Then, the lag phase duration is tackled through studying its relationship with the temperature, the initial cellular mean mass, the maintenance energy and the inoculum size. Thus, this chapter basically comprises different series of simulations in order to qualitatively reproduce the available experimental information

from specialized literature. Furthermore, a mathematical tool to assess the evolution of the biomass distribution during the growth cycle is introduced: the *mean mass distance*, the *mass distribution distance* and the *product distance*, with the last one being the most appropriate for describing the above-mentioned evolution. This chapter has been partially published in Prats et al. (2006).

INDISIM simulations are used as an experimental test in Chapter 4. In this chapter, a theoretical approach to culture evolution during the lag phase is made. The evolution of the cell density and the evolution of the total biomass are distinguished. The evolution of the cell density growth rate and the evolution of the total biomass growth rate are studied and used to split the lag phase into an initial stage and a transition stage. A simple mathematical model is built to describe the transition phase in cell density, and INDISIM simulations are used to test the soundness of the model. Finally, an approach to the lag phase definition is carried out, and the *distances* are used to distinguish balanced from non-balanced growth. The main part of this chapter has already been published in Prats et al. (2008).

An experimental study of the biomass distribution dynamics through flow cytometry and electric size analysis is introduced in Chapter 5. First, a description of the experimental instruments and the performed experiments is offered, and the mathematical *distances* are adapted in order to study the experimental results. Then, we present the results of two series of experiments: *Escherichia coli* growth in M9 medium at 20°C and at 35°C. In each series we follow the temporal evolution of the size distribution during the growth cycle, as well as the mean diameter evolution. These results are compared with the previous simulation outputs. A part of these results has been gathered in Prats et al. (2007), and we are currently working on a complete manuscript to be submitted to an international journal.

In Chapter 6 we present a study on optimization methods to estimate parameters in IbMs. First, an introduction about the input indexes and parameters of an IbM simulation is presented, as well as some basis for performing the parameter estimation. Then, INDISIM is adapted (simplified) for carrying out this specific study. Three methods are adapted, implemented and tested to estimate one, two and three parameters with INDISIM. These methods are the *classic Grid Search*, the *Nelder-Mead Threshold Accepting* (NMTA) and the *NEWUOA*. The work behind this chapter was carried out at BioTeC (Bioprocess Technology and Control research group, Katholieke Universiteit Leuven), during a stay from January to April 2007. It was a collaboration with Dr. Arnout Standaert and Dr. Kristel Bernaerts, with the supervision of Dr. Jan Van Impe. We are working on a complete manuscript with the main results reported in this chapter to be

submitted to an international journal.

The last chapter collects a synthesis of the main results, and presents the general conclusions of this work, as well as an overview of the perspectives and further work.

Any research project must be carried out, to a greater or lesser extent, by a group of scientists that work together towards the same aim. They must discuss and collaborate in order to make progress and obtain consistent results. This thesis is not an exception, and several people have done their part, from the scientific discussions around the theoretical models and the results analysis to the correction of the text, as well as the practical execution of the cytometry experiments. These have been essential parts where the collaboration of other scientists has been indispensable.

The lion's share of the research reported in this text was carried out by the author. We should emphasize as the most important contributions of the author the following: the adaptation of the simulation program, the design and programming of the simulations as well as the output analysis of the whole thesis, the development of the specific theoretical models presented in Chapter 4, the design of the general guidelines for the experiments performed, as well as the data processing and results analysis reported in Chapter 5, and the slight adaptation of the NMTA method for INDISIM parameter estimation as well as the entire adaptation of the NEWUOA method to estimate INDISIM parameters reported in Chapter 6. These tasks have been carried out under the supervision and with the collaboration of MOSIMBIO research group (Chapters 1 to 5), who have also supervised the writing of this text, and BioTeC research group (Chapter 6). However, while the author is extremely grateful for the help received in the research process and in the writing of this manuscript, she is solely responsible for its content and, therefore, for the errors or inaccuracies that it may contain.