Chapter 2

Individual-based Models in microbiology

2.1 Interest and background

Grimm (1999) defines Individual-based Modelling (IbM) as 'simulation models that treat individuals as unique and discrete entities which have at least one property in addition to age that changes during the life cycle'. IbM has been used in ecology since the 1970s, and during the last decade it has also come to be used in microbiology (Ginovart et al., 2002a; Kreft et al., 1998).

2.1.1 Why Individual-based Modelling in microbiology?

IbMs are bottom-up approaches. Several rules are applied to individuals (microorganisms) and environment, and the outcoming behaviour of statistical systems is studied. Although IbM is sometimes used in microbiology for its predictive scope, its strong point is its use to improve understanding of systems. Continuous mathematical modelling at a population level is usually a good way to extract relationships among different parameters, so that the predictions are immediately given by the mathematical equations. These models are sometimes too general or, on the contrary, too specific. The improvement in the understanding of systems, in which IbM has an important role to play, produces progress in the mathematical models.

Experiments are essential for proving new theories or detecting unexpected situations. Nevertheless, they are often time-consuming and expensive. Sometimes IbM simulations

can contribute to the experimental approach by means of virtual experiments (Hilker et al., 2006). For instance, IbM simulations may help in experiment design by testing the possible configurations, or they can be used for large-scale experimental conditions that can not be assessed in the laboratory.

In this section we are going to present a small review of some IbM applications in the study of microbial systems to review their evolution in the past decade. This review will include some examples of the use of INDISIM in different areas. INDISIM is the modelling and simulation technique that will be used in subsequent chapters to tackle the bacterial lag phase. Therefore, in Section 2.2 an outline of INDISIM, including a small review of its historical evolution and an explanation of the generic model for bacteria and space, will be presented. Then, the adaptation of INDISIM to the study of the bacterial lag phase, which is one of the aims of this thesis, will be introduced in greater detail in Section 2.3.

2.1.2 Individual-based Modelling of microbial systems: some examples

IbM of microbial systems has been applied to the study of different microorganisms and situations. The fundamental unit is always the cell, which may be prokaryotic or eukaryotic. In this section we present several examples of IbMs applied to the study of microbial systems.

IbM of prokaryotic cells

INDISIM (Ginovart et al., 2002a) and BacSim (Kreft et al., 1998) are two IbMs designed to simulate the growth and behaviour of prokaryotic cells. Both of them take bacteria as the fundamental unit, and simulate their growth in a culture medium. The major rules that govern the bacteria refer to their motion or shoving, nutrient uptake, metabolism and maintenance, reproduction cycle and death or lysis.

Ginovart et al. (2002a) presented two nice examples of emergent behaviours. First, the use of Blackman kinetics at an individual level resulted in the well-known experimental relationship between the culture's growth rate and the nutrient concentration (Bermúdez et al., 1989) (Fig. 2.1). Then, a mechanistic and local definition of the temperature at an individual level resulted in a global behaviour that reproduced Ratkowstky's experimental observations (Ratkowsky et al., 1982; Ratkowsky et al., 1983).

Another example of IbM of prokariotic cells is the paper by Kreft et al. (1998). It showed that, in BacSim simulations, the use of the Donachie (1968) model for the cellular

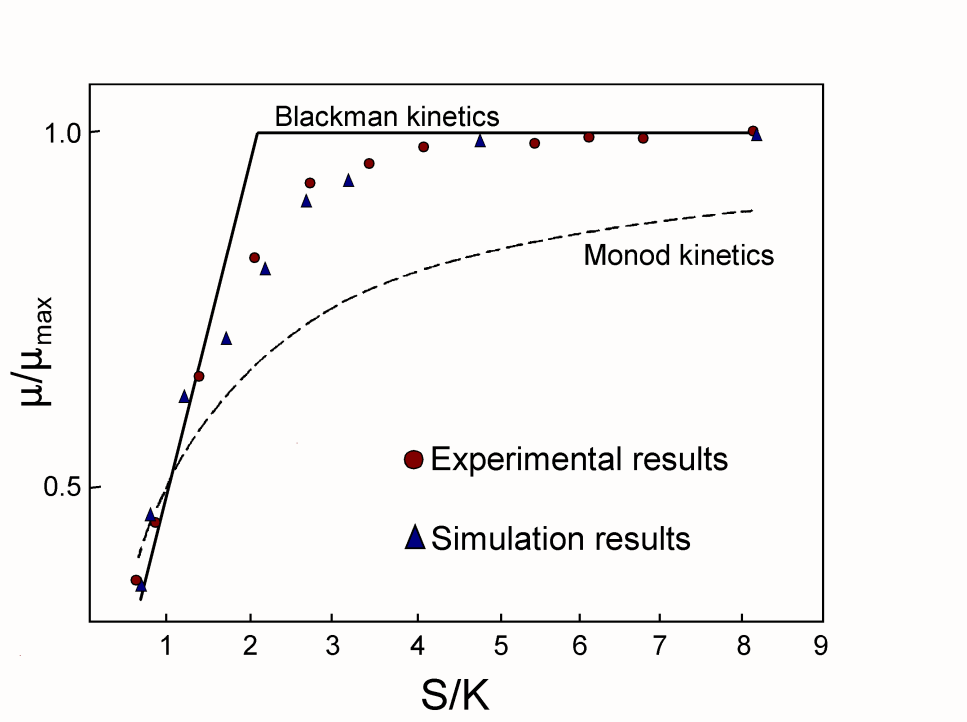

Figure 2.1: *The assumption of Blackman kinetics at the individual level (solid line) provides good results for the IbM simulations (triangles) that fit the experimental data (circles) better than Monod kinetics does (dashed line) (Bermúdez et al., 1989).*

cycle at the individual level reproduced the cell size dependence on growth rate.

IbM of eukaryotic cells

In the framework of the eukaryotic cells, we find several adaptations of INDISIM to study different individuals. INDISIM-YEAST was designed to simulate the growth of yeast populations in batch culture (Ginovart et al., 2007). The basic unit are the yeast cells (*Saccharomyces cerevisiae*), which are governed by specific rules that model their uptake, metabolism, budding reproduction and viability. The growth takes place in a liquid medium, modelled as a three dimensional closed spatial grid with two kinds of particles (glucose and ethanol). The results of the simulations are in good qualitative agreement with established experimental trends.

We also find an INDISIM adaptation called INDISIM-RBC (Ferrer et al., 2007), which

took the red blood cells (RBC) as the fundamental unit for the model and simulations. It is an adaptation of INDISIM to simulate *in vitro* cultures of *Plasmodium falciparum* (the parasite of malaria) infected red blood cells. The major rules governed the RBC behaviour (motion, uptake and metabolism, infection process and viability), and the nutrient diffusion and the parasites spread were also modelled to study their effect on the *in vitro* culture. The study concluded that the spreading of the parasites and susceptibility to invasion are thresholds for the evolution of the infection in the culture.

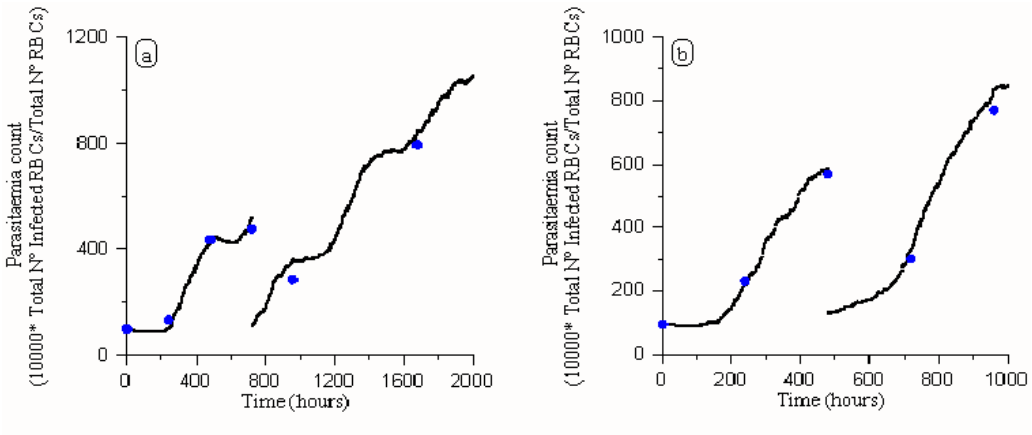

Figure 2.2: *Example of INDISIM-RBC simulations for studying* in vitro *cultures of* Plasmodium falciparum *infected red blood cells (Ferrer et al., 2007). The plot shows the simulation output data compared to experimental results obtained from two different commercial culture mediums, performed according to the MR4 protocol Week culture. Large dots represent experimental results. Small dots represent simulation results. (a) Sample of a synchronous culture. (b) Sample of an asynchronous culture.*

Knudsen et al. (2006) presented a third example of IbM of eukaryotic cells. It modelled the hyphal growth of a biocontrol fungus in soil. The basic units were the fungal segments. This study was a step towards the prediction of fungal growth in natural habitats.

There are some IbMs that model the behaviour of phytoplankton (El Saadi and Bah, 2006; Hellweger and Kianirad, 2007). The term phytoplankton encompasses all photoautotrophic microorganisms in aquatic foodwebs, and it incorporates both eukaryotic and prokaryotic cells. The IbM simulator iAlgae was used by Hellweger and Kianirad (2007). The phytoplankton cells are the fundamental unit, and the rules model their mass balances, photosynthesis, respiration, reproduction, death and motion. Their growth in a batch culture and in a river (real system) were simulated, and the use of the IbM instead of

lumped-system (population level) modelling in different situations was commented upon.

The different IbMs and the associated simulators usually evolve from the study of specific cases. That is, their complexity is increased step by step when required by the characteristics of the system to be studied. At the same time, the strategies that are developed for tackling specific processes or systems are incorporated for later use in cases. We may consider several cases that have forced IbMs to improve their capacity for studying systems with temporal, spatial or structural complexity.

Temporal complexity

The temporal evolution of a bacterial culture or any other microbial system is not linear or constant along a time period. Usually, the temporal evolution is characterized by fluctuations or by different phases that are related to the changes in the environment.

An example of an IbM used for tackling a system with a specific temporal complexity was presented by Ginovart et al. (2002a). They used INDISIM to study the metabolic oscillations in batch bacterial colonies. They found an explanation at an individual level that reproduced the experimental results about the heat dissipation and the pressure evolution of a population of *Escherichia coli* in a batch colony. The oscillations in heat and pressure that appear in atypical regimes were reproduced by the simulations.

A second example is the study of the intermediate lag phase due to changes in temperature that was undertaken by Dens et al. (2005a and 2005b). The I+C+D theory of cell division (Képès, 1986) was explored to study the cellular adaptation to medium and temperature shifts. Several simulations were performed with BacSim, assuming different models for the adaptation at an individual level. They found an unexpected result: theory predicted no intermediate lag due to temperature shifts, while experiments showed that this lag exists. Two explanations were proposed: (i) the product $\mu \cdot (C + D)$ is not constant, because it decreases at lower temperatures; and (ii) a lag in biomass growth appears for shifts from low temperatures. BacSim simulations assuming each model produced results consistent with the experimental data, but they could not elucidate which was the correct model. Thus, the conclusion was drawn that further research was needed in order to distinguish between the two proposed mechanisms.

Spatial complexity

The spatial effects in real systems are often essential. For instance, if the spatial properties are not homogeneous or some fluxes or priviliged directions exist, the spatial effects acquire a special importance.

An interesting IbM approach for examening a spatially complex system was the study of bacterial growth on agar plates by Ginovart et al. (2002c). The bacteria were again the fundamental unit, but in this case they were fixed in the environment (agar plate). The spatial growth of the colony was only due to the bacterial reproductions. INDISIM simulations reproduced different colony patterns by changing the inoculation conditions and the nutrient distribution (Fig. 2.3).

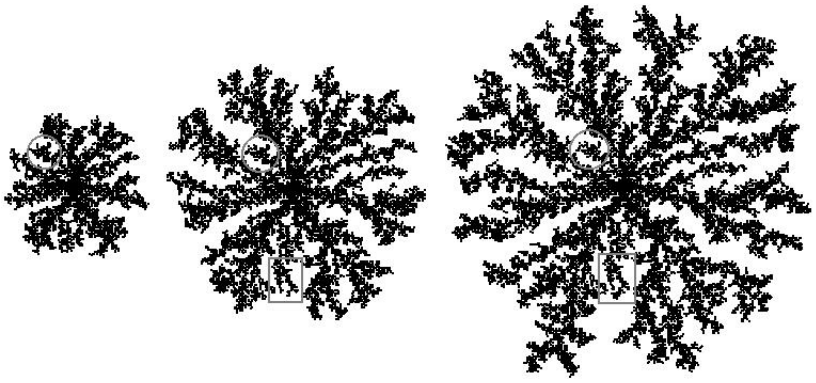

Figure 2.3: *Three different stages in the simulated growth of the bacterial colony on agar plates. The squares and circles identify branches which have stopped growing (Ginovart et el., 2002c).*

BacSim was also used to study a spatially complex system. It was used to simulate bacterial growth in biofilms (Kreft et al., 2001). It considered spherical bacteria in a continuous space, and the spreading occured by shoving of cells to minimize overlap between them. The substrate and product diffusion and reaction were modelled. The bacteria of the inoculum were put on a solid substratum layer with surrounding liquid where the nutrient transport took place. Spreading and diffusion-reaction processes produced a high heterogeneity of substrate concentrations in the biofilm.

Structural complexity

Real systems are usually very complex in many senses. We have already talked about the temporal and the spatial complexities, but often there is a third kind of complexity. We talk about structural complexity, for instance, when different microbial species coexist and interact in a culture.

A nice example of this is the INDISIM-SOM model (Ginovart et al., 2005; Gras, 2004). It was developed for studying the mineralization of C and N and nitrification

processes in soil. Due to the high complexity of the real system, two different prototypes of microbial cells were considered: nitrifier and ammonifier bacteria. Their modelled behaviour took into account motion, uptake, metabolism, reproduction, death and lysis. Then, nine different types of substrate were considered: five groups of organic compounds and four groups of mineral compounds. Several mass transfer processes among them were modelled. An outline of this complex model is shown in Figure 2.4. After the scaling process and calibration, the simulations were in agreement with the experimental data obtained from laboratory incubations of three different Mediterranean soils.

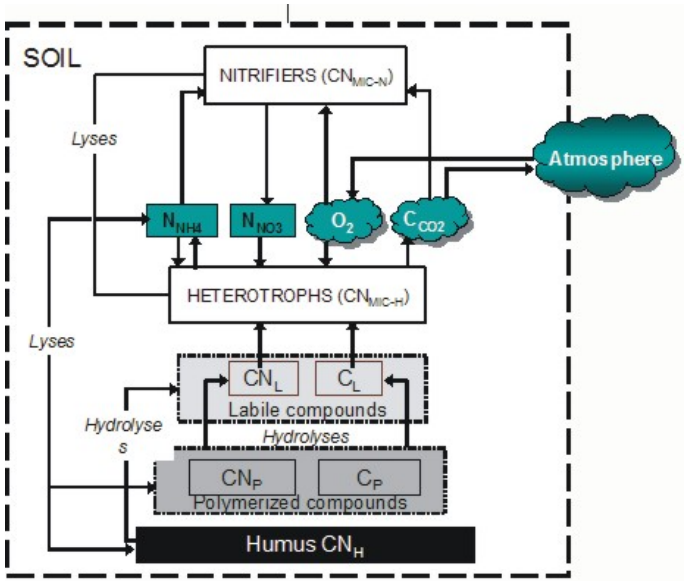

Figure 2.4: *Sketch of the mineralization and immobilization of C and N model and the nitrification process due to the microbial activity in soils, implemented with INDISIM-SOM (Ginovart et al., 2005).*

A similar example is the INDISIM adaptation for studying the composting process. INDISIM-COMP is in its firsts stages, but an overview of the biological model is given by Gras et al. (2006). In this case, three groups of microorganisms were considered, namely mesophilic bacteria, thermophilic actinomycets and mesophilic fungi. Six different types of organic substrates were considered, as well as five groups of gases and two mineral compounds in their liquid phase (water and ammonium). The model took into account several processes such as input and output flows to and from the system, diffusion of the labile compounds, mass transfer and heat transfer. The simulations reproduced the

succession of the different microbial prototypes, as well as the cumulative CO_2 evolution. Prats et al. (2006) presented a preliminary version of the spatial model developed for inclusion in an improved version of INDISIM-COMP.

In the framework of eukaryotic cells, INDISIM-FLOC was used to study flocculation in brewing yeasts (Ginovart et al., 2006). The model was used to compare two published theoretical mechanisms for flocculation of brewing yeasts at an individual level. The simulation results allowed the authors to discriminate which was the best mechanism, according to experimental data.

2.2 INDISIM

2.2.1 Background and history

The acronym INDISIM stands for INDividual DIScrete SIMulation. The origin of this methodology dates back to the 80s. Scientific discussions among Dr. Margalef, one of the fathers of modern ecology, Dr. Wagensberg, professor of Thermodynamics of Irreversible Processes (TIP) at the *Universitat de Barcelona* (UB), and Dr. Giró and Dr. Padró, from the Molecular Dynamics research group at the UB, suggested the idea of applying simulation techniques typically used by physics in solving N-bodies problems (Monte Carlo and Molecular Dynamics) to theoretical ecology. The convergence of scientists coming from different scientific disciplines was the key point for the subsequent development of this approach and the philosophy behind it, which was assembled by Giró et al. (1985). This initial process culminated in the appearence of the simulator Barcelonagram (Giró et al., 1986; Valls, 1986).

This methodology was adapted to simulate bacterial growth. Interesting results in the framework of microbiology were obtained, as well as succeessful results in general ecology. Wagensberg et al. (1988b) matched the mathematical theory of information with the biological adaptation through the Maximum Entropy principle. This conceptual work succeeded in explaining the typical biomass distribution of a bacterial culture in exponential growth conditions: experimental measurements, Monte Carlo simulations and theory predictions were in agreement (Wagensberg et al., 1988a). Bermúdez et al. (1989) succeeded in simulating the growth of *Serratia marcesens* and *Escherichia coli* in different situations, in accord with experimental data. In the framework of complex systems, Solé et al. (1992) studied the existence of self-organized criticality in ecosystems by means of Monte Carlo simulations of a simple ecosystem. The Monte Carlo simulations of predator-prey populations showed the chaotic dynamics that reported in Solé and Valls

(1992).

López (1992) ventured fully into the discrete simulation of bacterial cultures. He presented a study of the dynamics of a bacterial culture, as well as a thermodynamic approach to this concern. The simulator was improved by Ginovart (1996), where it began to be applied to the study of non-bacterial processes such as yeast flocculation and fermentation and the growth of filamentous fungi.

Ginovart et al. (2002a) presented, for the first time, a simulation methodology INDISIM as such. In this paper, the general methodology is explained in detail. From there, INDISIM evolved by studying specific cases of interest, as noted in the previous section. INDISIM simulations have succeeded in topics as varied as bacterial growth in agar plates (Ginovart et el., 2002c) and the study of the influence of bacteria size and shape in yoghurt processing (Ginovart et al., 2002b), in which the interaction between two bacterial species (*S. thermophilus* and *L. bulgaricus*) was tackled by means of the study of axenic and mixed cultures.

At that point, INDISIM was ready to take on more complex microorganisms, processes and systems. This marked the birth of several adaptations such as INDISIM-YEAST (Ginovart et al., 2006; Ginovart et al., 2007), which simulates flocculation in brewing yeasts, INDISIM-RBC (Ferrer et al., 2007), for studying the spread of the malaria parasite in *in vitro* red blood cell cultures, INDISIM-SOM (Ginovart et al., 2005; Gras, 2004), which considers organic matter dynamics in soil, and INDISIM-COMP (Gras et al., 2006; Prats et al., 2006), which focuses on the modelling and simulation of the composting process (see Section 2.1.2).

The INDISIM methodology has improved tremendously since its beginnings. Every application to the study of a specific system has required development of new strategies that have then been used by other studies. For instance, INDISIM-SOM required a great effort in modelling different kinds of microorganisms and substrate particles that were used when building the INDISIM-COMP biological model. The spatial complexity was tackled in INDISIM-COMP, and the strategies developed were useful in building the INDISIM-RBC spatial model.

In some specialized reviews the authors have shown their interest in INDISIM methodology. For instance, O'Donnell et al. (2007) says, in his review of modelling and prediction in soil microbiology published in *Nature reviews*, that Ginovart et al. (2005) were 'the first to use an approach similar to this to model the dynamics of carbon and nitrogen in soil and included the spatio-temporal dynamics of nine different resource components in a simulated soil sample that contained $1\,g$ of soil and 10^7 individual bacterial cells'.

2.2.2 General outline of INDISIM in bacterial systems

INDISIM is designed to simulate the growth and behaviour of microbial cultures (Ginovart et al., 2002a). In its basic and simplest version, it simulates bacterial growth in a certain culture medium. The growth takes place in a two-dimensional space, but it can be extended to three dimensions when this is required by the system under study.

The basic unit is the bacterial cell, each one being defined by means of a vector $\overrightarrow{B_i}$. This vector contains the bacterium label (integer number i), its spatial position, and its characteristics (instantaneous mass, mass to initiate the reproduction cycle, or reproduction cycle status, among others). Some of these properties may change throughout the bacterial life, which is governed by the 'model of bacteria' (Section 2.2.3).

INDISIM is discrete in space and time. The environment is divided into spatial cells that contain the bacteria and the nutrient particles (Fig. 2.5). Each spatial cell is labeled with its coordinates *(x,y)*, and its properties are also gathered in a vector $\overrightarrow{E_{xy}}$ (nutrient content or temperature, among others). The processes affecting the environment also constitute a set of rules (Section 2.2.4).

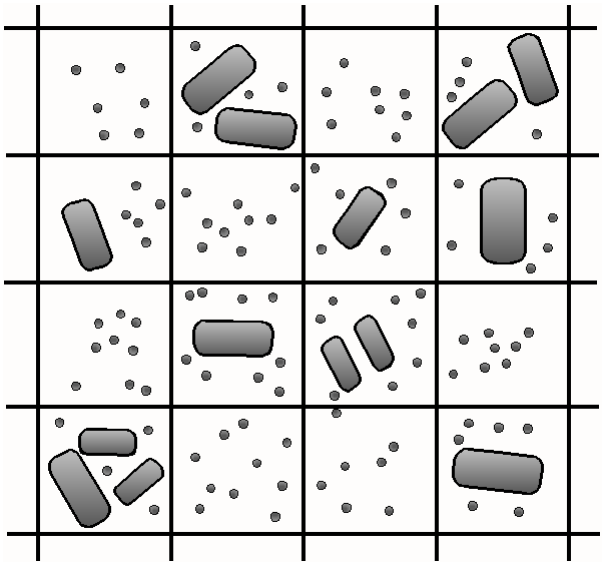

Figure 2.5: *The environment is discretized into spatial cells that contain the bacteria and nutrient particles.*

The time is split into discrete time steps. At each time step, every bacterium of the system acts sequentially and, after that, actions on the environment are carried out.

Figure 2.6 shows a certain moment of a time step when a specific bacterium is chosen to act by following the rules. It has a series of particular properties that can be modified at the end of the time step, according to the actions carried out (Section 2.2.5).

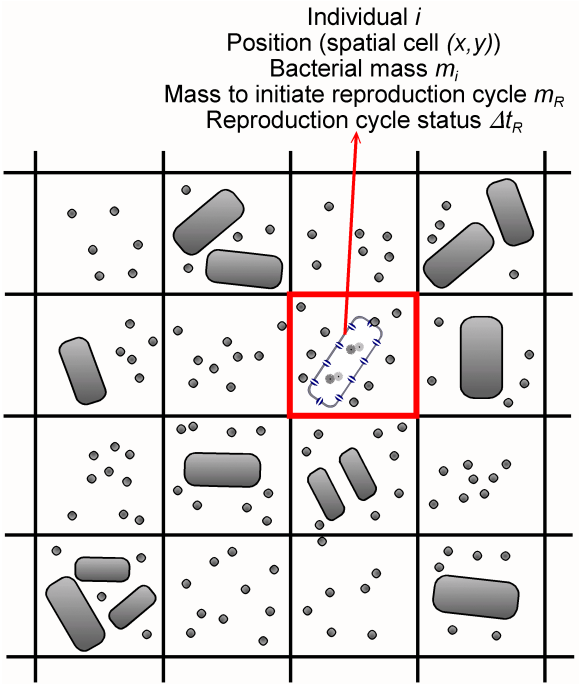

Figure 2.6: *At each time step, all the bacteria of the system act consecutively. The vector that defines each acting bacterium contains its label, which is an integer* i*, its spatial position, which is the spatial cell coordinates* (x,y)*, and its characteristics such as the instantaneous mass, the mass to initiate the reproduction cycle or the reproduction cycle status. This vector is updated after the actions are carried out.*

2.2.3 Modelling the bacteria

The basic unit of INDISIM is the bacterial cell. Each bacterium has its own properties, and it is subject to a set of rules that defines its evolution and, therefore, that may modify its individual properties throughout its life (Fig. 2.6).

The major rules for bacteria that are growing in a culture medium are detailed below. This is the basic model for defining bacterial behaviour in INDISIM simulations. In each application, this model is adapted to the system under study by taking into account the kind of microorganism, the environmental conditions and the specific features.

Motion

The position of a bacterial cell i may change to a new position in space, according to a given probability p_{mov}. The new position is randomly chosen from the available sites within a radius d_{max}. The motion is considered from one spatial cell to another, since it is the basic spatial unit. Therefore, small displacements inside the spatial cells are not considered.

Nutrient uptake

The nutrient particles are spread in the medium. A mechanistic model for the nutrient uptake is considered. The amount of nutrient that can be taken up by the cell is limited by two factors: the nutrient's capacity to reach the cellular surface, and the cellular capacity to consume the nutrient particles.

The nutrient particles are considered to be in random movement caused by essential Brownian motion. These particles are not considered one-by-one, but together. During a certain time interval (in the simulations, fixed by the time step), the nutrient particles in a certain radius around the bacterium may potentially reach the cellular surface. From these available particles, only a percentage will reach one cellular *entrance* in the surface. An outline of this mechanistic model is shown in Figure 2.7.

A summary of the involved parameters in the nutrient uptake model is detailed below:

1. $\overline{U}_{max}$ is the mean maximum number of nutrient particles that can be consumed per unit of time and per unit of cellular surface.

2. $U_{max} = Z_1 \cdot (c \cdot m^{\alpha})$ is the maximum number of nutrient particles that can be taken up by the bacteria. Z_1 is a random variable with mean $\overline{U}_{max}$ and standard deviation σ, m is the mass of the cell, α is a parameter related to its geometry and c is a normalization constant whose value depends on the value of α. For a spherical bacterial cell, $\alpha = 2/3$ if the uptake is considered to be proportional to the bacterial surface.

3. D_{max} determines the maximum uptake range. From the position of a given bacterium, D_{max} defines the number of sites, or spatial cells, that may be reached for the purpose of nutrient particle consumption.

4. k is a given percentage of the $n(D_{max})$ available nutrient particles within the given range D_{max}. This takes into account probabilistic considerations regarding the entry of nutrient particles into the bacterial cell through the cellular membrane. Therefore, the number of available nutrient particles is $k \cdot n(D_{max})$.

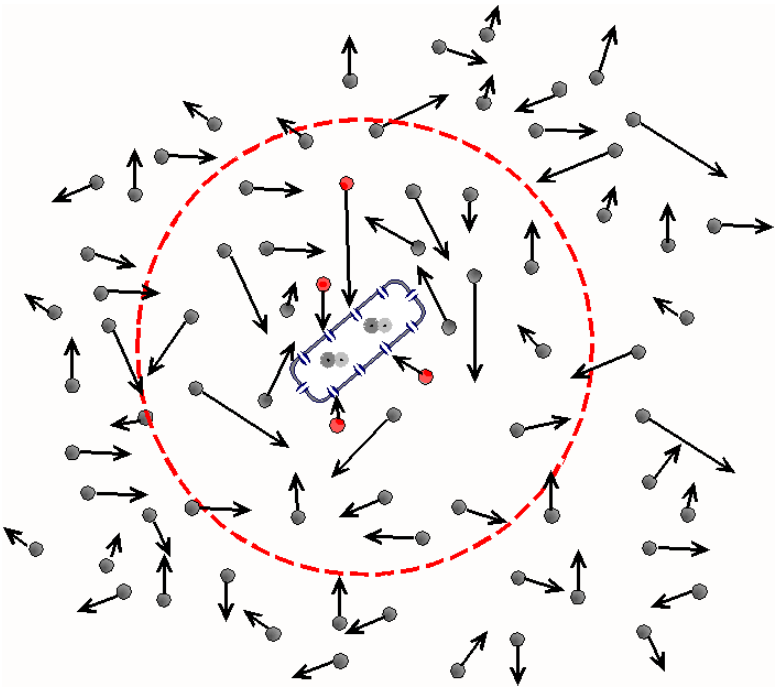

Figure 2.7: *Outline of the uptake model. The small circles represent the nutrient particles, which are in Brownian motion. The dashed circumference contains the nutrient particles that may potentially reach the cell surface during the time interval considered. Some nutrient particles may bump into the cell entrances and, therefore, they are taken up by the cell.*

5. At each time step, every bacterium takes up the minimum quantity between U_{max} and $k \cdot n(D_{max})$, which we denote U.

It is important to emphasize the use of random values around a mean, given by a gaussian function with a certain standard deviation: this is an essential part of the simulation program, since it reproduces the diversity of the real systems. In this case the randomness affects the maximum number of nutrient particles that can be consumed per unit of time and per unit of cellular surface, as was seen in the summary of the uptake model detailed above.

This model is based on Blackman kinetics for its simplicity, in spite of known limitations. It is important to stress that the chosen kinetics applies to each individual. When each individual follows Blackman kinetics, the culture behaviour fits well with the experimental results (Fig. 2.1), allowing the study of the effect of temperature (Ginovart et al., 2002a; Bermúdez et al., 1989).

The widely used Michaelis-Menten's (or Monod's) kinetics does not fit correctly with the experimental results. Other kinetics with 3 or more parameters have been suggested, developed and tested over the years, but they are case-specific and require re-fitting of these parameters to the system under study (Koch, 2005; Kóvarová-Kovar and Egli, 1998; Button, 1998; Dabes et al., 1973).

Metabolism

Bacterial cells need to obtain energy and structural matter in order to maintain and repair their structure, and increase their biomass. These processes are regulated by the metabolism (catabolism and anabolism). The primary source for energy and structural compounds is the uptaken nutrient. Nevertheless, the cells have some reservoirs to be used when they are under stress conditions, resulting sometimes in a decrease in their biomass.

In the INDISIM model for catabolism and anabolism, the following parameters are introduced:

1. I denotes a prescribed number of nutrient particles per unit of biomass or surface (or biomass+surface) that a bacterial cell needs in order to maintain its optimum state. It depends on the medium conditions.

2. Y denotes the metabolic efficiency that accounts for the synthesized biomass units per metabolized nutrient particle, and depends on the chemical reactions. We consider Y as a constant.

By using the above parameters, and recalling the meaning of U (uptaken nutrient particles), we set the following control rules (Fig. 2.8):

1. Maintenance energy for the viability of a bacterial cell, mI.

2. A control relation to check whether the nutrient particles absorbed by a bacterial cell are sufficient for its maintenance, $U \geq mI$?

3. If there is no possibility of covering the maintenance requirements of a bacterial cell, check the possibility of its lysis or inactivation.

4. Once the viability of the bacterial cell is achieved, allow for the increase of its mass from m to $(m + B)$, where $B = (U - mI)Y$.

5. Allow for biomass reserves within each bacterial cell, which can be used up in the above processes whenever the local and external level of nutrient particles is too low for the supply of sufficient maintenance requirements (Nyström, 2004). In such situations, the bacterial biomass can be degraded to cover the maintenance requirements, mI, only if this biomass is higher than a minimum boundary m_{min} (that is, $mI > (m - m_{min})$).

Although Y is constant, variations in I result in different yields when the nutrient is catabolized to synthesize biomass.

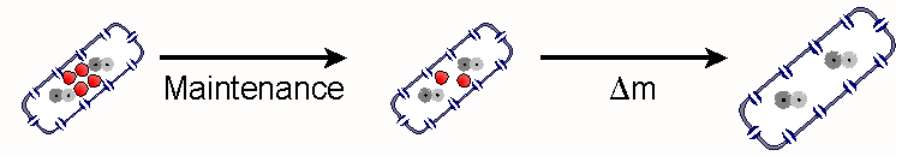

Figure 2.8: *Outline of the metabolism model in proper conditions (nutrient availability in excess). The circles symbolize the uptaken nutrient, which is spent in maintenance and biomass increase.*

Reproduction

The model for the reproduction cycle is based on the I+C+D model (Képès, 1986; Cooper, 2004). Our reproduction model considers a threshold in mass in order to initiate the division process, which has a fixed duration. Therefore, two important parameters regulate the reproduction cycle: the mass to initiate the reproduction cycle, m_R, and the reproduction cycle duration, t_R.

When a bacterium is in a culture medium, it grows at a certain rate (μ). If the conditions are proper, it reaches the mass to initiate the reproduction cycle after a while (depending on the growth rate, μ). At that point, the reproduction cycle starts with no possibility of return. While the DNA replication and the physical division take place, the bacterial cell keeps uptaking nutrients and growing. Since the reproduction process has a fixed duration, the growth rate determines the bacterial mass at the moment of division and, therefore, the masses of the new cells. Thus, the growth rate μ determines the mean mass of the culture. An outline of the cell cycle is depicted in Figure 2.9.

The steps that are followed by the cells in INDISIM simulations are described below:

1. To initiate the reproduction cycle, the mass of the bacterial cell must reach a specific mass $m_R > m_{R,min}$, where $m_{R,min}$ is an absolute minimum mass for beginning the reproduction cycle, and m_R is the individual mass to initiate the reproduction cycle,

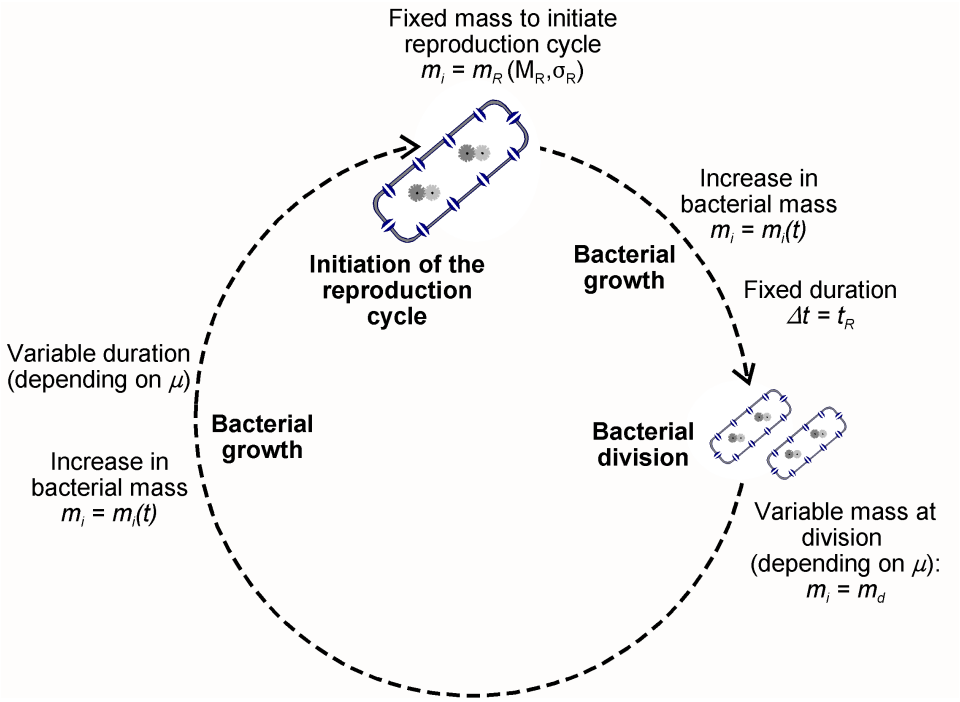

Figure 2.9: *Outline of the cell cycle, including the reproduction model: a threshold mass, m_R, sets the initiation of the reproduction cycle. It is given as a mean value, M_R, with a deviation σ_R. The bacteria keep growing during the process, which has a fixed duration t_R, so that the final mass at division m_d depends on growing conditions.*

which is obtained from the normal random distribution with mean mass M_R and standard deviation σ_R. Again, this randomness is essential for the soundness of the simulation results; it is inseparable from the real bacteria due to the inherent diversity.

2. When the microorganism reaches the mass m_R, the bacterial cell must wait for a fixed period of time, t_R, before the physical separation into two new bacterial cells takes place. At the moment of the division, the mass of the bacterium is m_d. This mass depends on the growth rate during the period t_R.

3. When the duplication is completed, two new bacteria appear in the medium with characteristics similar to those of the original bacterial cell. These new cells have birth masses m_1 and m_2 that are equal to half of the original bacterial cell with a certain deviation, keeping the relationship $m_2 = m_d - m_1$.

4. The new bacterial cells are allowed to take up new neighbour positions in the physical lattice, or one may remain in the original spatial cell.

Viability

Whenever the environmental conditions become unfavourable (for instance, the nutrient runs out), bacteria may lose their viability. As a result, when the bacterial mass drops below a certain value (a fraction of its m_R), either the inactivation or the lysis of the bacterium take place.

2.2.4 Modelling the environment

The basic spatial unit is the spatial cell. The properties of each spatial cell are defined and controlled. The variables that characterize the environment may be related, for instance, to single or multicomponent nutrient particles, to residual or end products arising from the cellular activity, or to extracellular enzyme particles. They can also take into account some physical properties such as the temperature or the porosity. Every specific study determines which variables must be defined, and in all cases they are assumed to be time dependent. The bacterial activity, the external manipulations such as agitation or medium renewals, and the diffusion processes are the major activities that may modify the environment properties and their spatial distribution.

Bacterial activity

The bacterial activity can modify the environment properties in different ways. For instance, the nutrient uptake changes the medium composition. If the bacterial heat production is modelled, the environment temperature changes according to that. Usually, the effect of bacterial activity is defined by the biological model, so it is not specifically modelled for the environment.

External manipulations

The external manipulations are a characteristic of the specific systems that are studied. Therefore, they have to be specifically modelled for each case and there is no need for a generic model of the external manipulations.

Diffusion

The nutrient particles (or product particles) diffusion is used in almost all INDISIM simulations. The diffusion model is based on discrete Fick's law (Bormann et al., 2002), and it is considered between neighbouring spatial cells (Fig. 2.10). Let us study the flux of a certain substance between two neighbouring cells. If we denote Δc_s as the gradient (difference) in the concentration of the substance and d as the mean distance between them, the flux of this substance, J_s, will be (Eq. 2.1):

$$J_s = -D_s \cdot \frac{\Delta c_s}{d} \tag{2.1}$$

where D_s is the diffusion coefficient of the mentioned substance.

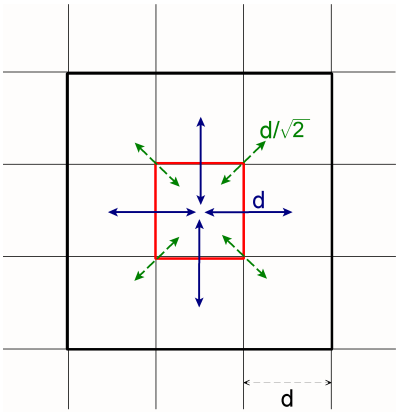

Figure 2.10: *Matter transport: diffusion between neighbouring cells. A factor* $1/\sqrt{2}$ *is considered in the diagonals (dotted arrows).*

2.2.5 Simulation program setup

The bacteria and environment models are implemented in Compaq Visual Fortran Professional Edition 6.1.0. This consists of three parts: (i) the initialization of the system, with the reading of the input data, which ends with the initial configuration of the whole system; (ii) the main loop (time step), where the actions over the bacteria and the medium are carried out and repeated until the simulation ends; and (iii) the final calculations (at the end of each time step or at the and of the simulation) to obtain the simulation results (output data). Figure 2.14, in the next section, shows a depiction of the basic INDISIM flow chart.

Together with the detailed models, some specific simulation strategies must be implemented: periodic boundary conditions and statistical arguments.

Boundary conditions

The conditions for the boundaries of the systems must be fixed: is it an open or a closed system? Are we simulating the entire system or a small part of it?

Since computer capacity is still limited, it is usually impossible to simulate the growth of an entire real system. There is a specific strategy that is widely used in molecular dynamics: the periodic boundary conditions (PBC) (Allen and Tildesley, 1987). These are a set of boundary conditions that are used to simulate an effectively infinitely tiled system.

PBC can be used in the simulation of bacterial cultures when the space is homogeneous and isotropic. It consists of simulating a small part of the entire system that is large enough to be representative of the whole system. Then, the simulated part is assumed to be surrounded by several replicas. That is, what is happening in a certain point at a certain time interval is probably being happening in a similar way in several points of the real system. Figure 2.11 depicts a simple example of the PBC in a bacterial system. When a bacterium or a nutrient particle goes out of the main (simulated) system, an identical bacterium or nutrient particle (its image) enters through the symmetric point.

Statistical arguments

Statistical arguments must be introduced at two different stages in the implementation of INDISIM. First, random variables must be used in setting the individual properties and rules, as was seen in previous sections. Every individual variable, such as the mass to initiate the reproduction cycle or mass at birth, is set following a Gaussian distribution centered at the experimentally observed mean value, and truncated beyond the $\pm 1.96\,\sigma$ range in order to prevent the existence of unlikely cells. This is an indispensable strategy in order to reproduce the diversity of the real cultures, and it is also useful to cover non-explicit parts of the model.

The second stage is at the end of the process, when the 'microscopic' description of the bacterial colony is related to its 'macroscopic', or global, description, namely the properties observed by experiment. Hence, once the single-cell variables are obtained we take a simple arithmetic average to obtain the population-averaged properties.

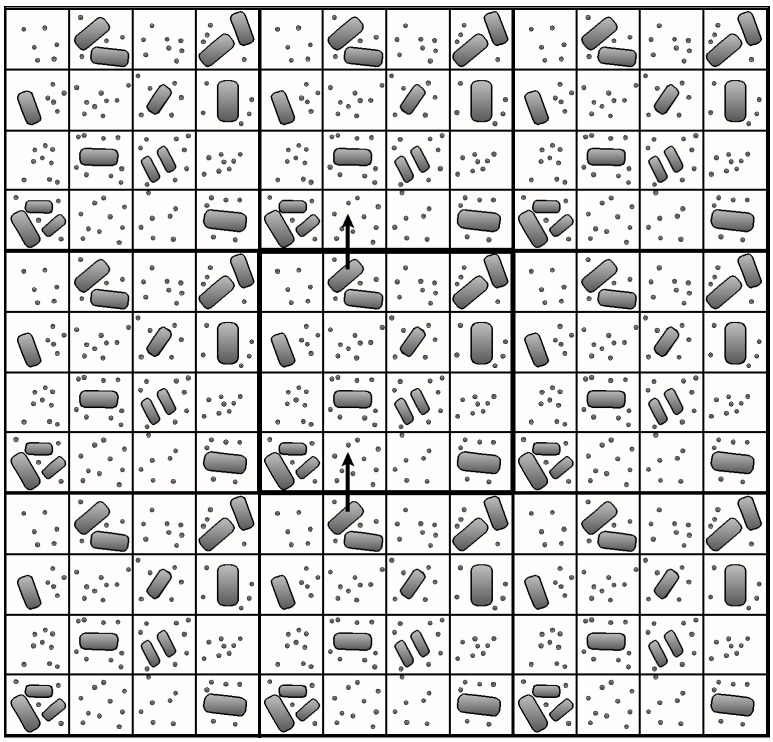

Figure 2.11: *Outline of the periodic boundary conditions. The large central square is the main system, and it is surrounded by the eight replicas. If a bacterium goes out of the main system from an upper spatial cell, an identical bacterium (image) enters the main system through the symmetric lower spatial cell. The same process occurs with all the system compounds.*

2.3 INDISIM for studying the lag phase

The modelling and simulation of bacterial cultures to study the lag phase has been carried out with essentially the basic version of INDISIM (Section 2.2). It is important to point out that the culture's lag phase is a phenomenon that takes place during the first stages of its growth, when there is no nutrient limitation. Moreover, we are simulating the growth of a culture under spatially homogeneous conditions (batch culture and agitated medium). Therefore, the basic INDISIM model for bacterial behaviour is valid under these conditions.

Only a few modifications have been made in order to adjust the simulator for the case under study. They refer to the specific conditions of the performed simulations and to some small new parts of the bacterial model. Furthermore, some mathematical

calculations have been implemented in the simulation program in order to obtain the required results. These are detailed below.

2.3.1 General outline

The simulator deals with the bacterial lag phase in batch agitated cultures. The culture's growth simulations take place in a two-dimensional space; because we consider an agitated and spatially homogeneous culture, the results in a three-dimensional space would be the same. Periodic boundary conditions are used, as the simulated system is homogeneous and isotropic.

2.3.2 Modelling the bacteria

The rules that govern bacterial behaviour are essentially the same as those explained in Section 2.2, with these particularities:

- Motion: when a non-agitated culture is simulated, the bacteria follow the rules explained above. If the growth of an agitated culture is simulated, the individual bacteria motion is not taken into account because it is performed by the external agitation process.
- Metabolism: the maintenance constant is proportional to the individual mass.
- Viability: the cell inactivation when the bacterial mass falls below the minimum is considered (thus, no lysis is modelled).

An important part of this study of the bacterial lag phase is the metabolic adaptation of the bacteria to a new nutrient source. In this way, a specific model of the enzyme effects has been developed. The amount of enzyme particles, as well as their synthesis rate and cost, will be monitored when this limiting factor is considered.

2.3.3 Modelling the enzyme effects

In some simulations, a metabolic adaptation to a new nutrient source is introduced. It is modelled as an enzymatic synthesis (Pirt, 1975), so the enzyme presence and activity must be controlled. Two different situations are considered: first, a culture in which the bacteria need to synthesize intracellular enzymes to catabolise the uptaken nutrient; second, a culture medium with polymers that are not assimilable by the bacteria, so extracellular enzymes must be synthesized by the cells and dropped into the medium to hydrolyze the polymers into assimilable monomers.

Intracellular enzyme

As the enzyme limits nutrient use, it will indirectly restrict the cellular uptake rate. Therefore, in our model the intracellular enzyme will limit the maximum number of nutrient particles that can be consumed per unit of time and per unit of cellular surface (U_{max}).

Four new parameters are introduced at the individual level:

1. a mean maximum enzyme quantity per cell per unit of mass, $\overline{E}nz_{max}$, and its typical deviation, $\sigma(\overline{E}nz_{max})$;
2. an initial quantity of enzyme per bacterial cell, Enz_0;
3. the mean synthesis rate per unit of mass and time step, $\overline{V}_{enz}$, and its typical deviation, $\sigma(\overline{V}_{enz})$; and
4. the energy cost of this synthesis per simulation unit of generated enzyme, Y_{enz}.

If the bacterial cell does not have any intracellular enzyme particle, it has to synthesize some enzymes before being able to take up nutrients from the medium.

In our model, at each time step each bacterial cell can synthesize a certain quantity of enzyme. This synthesis takes place while the intracellular enzyme quantity is under the maximum and once the cellular maintenance requirements are satisfied, according to the bacterial mass, the synthesis rate and the bacterial energy availability. That is:

1. The number of enzyme particles to be synthesized by the cell at the current time step, n_{enz}, is chosen as $n_{enz} = V_{enz} \cdot m$.
2. After satisfying the maintenance requirements, $m \cdot I$, check if the enzyme synthesis can be carried out with the uptaken particles $(U - mI) > Y_{enz}n_{enz}$ or with the biomass resources, $(m - mI - m_{min}) > Y_{enz}n_{enz}$.
3. Once the viability of the bacterial cell is achieved and the enzyme particles synthesized, allow for the increase of its mass from m to $(m + B)$, where $B = (U - mI - Y_{enz}n_{enz})Y$.

The intracellular enzyme quantity limits the cellular uptake with a factor $Enz\,(t)/Enz_{max}$. That is:

1. Evaluate the maximum number of nutrient particles that can be taken up by the bacteria, U_{max}, and the avaliable nutrient particles, $k \cdot n(D_{max})$, as detailed in Section 2.2.3.

2. Transform the maximum particles that can be taken up as $U'_{max} = (Enz\,(t)/Enz_{max}) \cdot U_{max}$.

3. Uptake U, which is the minimum number between U'_{max} and $k \cdot n(D_{max})$.

Once the intracellular enzyme quantity reaches the fixed maximum value, the bacterial metabolism is adjusted to the available nutrient and uptake goes on as usual. When reproduction takes place, the enzyme quantity is distributed proportionally to the new cells' masses.

Extracellular enzyme

The nutrient particles are considered to be polymers that can not be incorporated through the cellular membrane. Therefore, extracellular enzymatic activity is necessary. Enzymes must be synthesized by the bacteria, hydrolyzing the polymers into assimilable monomers. In this situation the monomer concentration is the limiting factor for the growth rate of the culture.

The parameters for describing this process are:

1. the mean synthesis rate per unit of mass and time step, $\overline{V}_{enz}$, and its typical deviation, $\sigma(\overline{V}_{enz})$;

2. the energy cost of this synthesis per simulated unit of generated enzyme, Y_{enz};

3. the number of monomers contained in a polymer, N_{mon};

4. the initial enzyme quantity per spatial cell, Enz_0; and

5. the mean time duration of the simulated extracellular enzyme particles, $\bar{t}_{enz}$, in time steps.

At each time step and for each individual cell, if there are polymers in the culture medium and a lack of monomers around the bacterium, the cell synthesizes enzymes according to its own mass, the synthesis rate and its energy availability. These enzymes are dumped into the spatial cell where the bacterium is located. At each time step, each simulated enzyme particle breaks down a certain number of polymers into the fixed number of monomers.

In summary:

1. During the uptake process, if the medium limits the nutrient uptake and there are polymers to be hydrolyzed, an index I_{gen} is put to 1.

2. During the metabolism, if $I_{gen} = 1$ the bacteria synthesizes n_e enzyme particles according to the model described in previous section (intracellular enzyme). The index is changed again into $I_{gen} = 0$.

3. The n_{enz} generated enzyme particles are dumped into the medium.

4. Extracellular enzymes are diffused within the medium, following the diffusion model. At the end of each time step and at each spatial cell, each enzyme particle hydrolyzes a polimer into N_{mon} monomers. A certain fraction of the enzyme particles ($1/\bar{t}_{enz}$) of each spatial cell is denaturized.

2.3.4 Modelling the environment

The simulation space is divided into 300×300 square spatial cells. Each one is identified by its coordinates x and y, and its nutrient particle content is controlled. Periodic boundary conditions are used.

The simulations reproduce the bacterial growth in agitated liquid medium. We work with batch cultures; that is, the initial nutrient is fixed and no input or output is programmed. At each time step, and after the bacterial activity, the nutrient particles are redistributed, either uniformly (if agitation exists) or by means of diffusion. It should be noted that, since during the lag and exponential phases there is no nutrient limitation, for the purposes of this study the effect of redistribution is the same as the effect of diffusion; it has no effect on the results whether one or the other is chosen.

In the case of extracellular enzymes, the enzyme motion is significant for the simulated culture growth. In this case discrete enzyme diffusion is also considered. The extracellular enzyme particle diffusion takes place after the bacterial activity.

2.3.5 Mathematical methods

Lag time calculation

We take the classic lag parameter definition (see Section 1.1.3). At the end of the simulation, we make a logarithmic regression of an interval in the exponential growth to obtain the straight line $lnN = \mu \cdot t + b$. We must be sure that the used interval is part of the exponential phase. In general, if N_0 is the initial number of bacteria and N_f the final one, we take the interval between $[lnN_0 + \rho_{inf} \cdot (lnN_f - lnN_0)]$ and $[lnN_0 + \rho_{sup} \cdot (lnN_f - lnN_0)]$, being $\rho_{inf} \in [0.5\ 0.6]$ and $\rho_{sup} \in [0.8\ 0.9]$. This means that we take the upper part of the curve, where the synchronism that sometimes affects the first part of the growth has disappeared.

The intersection of the prolongation of this straight line with the lnN_0 line gives the lag time (Eq. 2.2):

$$\lambda = \frac{lnN_0 - b}{\mu} \tag{2.2}$$

Biomass distribution

In order to study the distribution of cell biomass, 25 discrete intervals of biomass with the same width are considered and identified with k_m; the number of bacteria in each interval is counted to obtain the relative frequency (p_{k_m}). In Table 2.1 the values for establishing these intervals are specified. In this study we will focus on the study of the evolution of the biomass distribution during the culture's growth.

Table 2.1: *Values for setting the biomass distribution (s.u. = simulation unit).*

Parameter	*Value*
Minimum mass (s.u.)	1000
Maximum mass (s.u.)	31000
Mass interval (s.u.)	1200

2.3.6 Simulation program setup

We usually work with inocula of 100 bacteria, except when we want to study the growth of small inocula or the effect of inoculum size. The initial mean mass and biomass distribution vary with each simulation. We speak of homogeneous inoculum if the bacteria have an initial mass equal to a fixed value, and we call it heterogeneous inoculum when we use a sample population from a previous simulation, taken from the exponential or the final phase, among which bacteria have different initial individual masses (Fig. 2.12). The former case is useful to perfectly control the initial conditions and their relationship with the growth, but the second one is more realistic.

In this study, a standard Pentium IV computer (minimum 512 Mb) is used, with a maximum number of 2.5×10^5 simulated cells. This number of cells is large enough, as periodic boundary conditions are used. The simulations run for periods of between 10 and $30\,min$. A more detailed flow chart of the used simulation program is outlined in Figure 2.14.

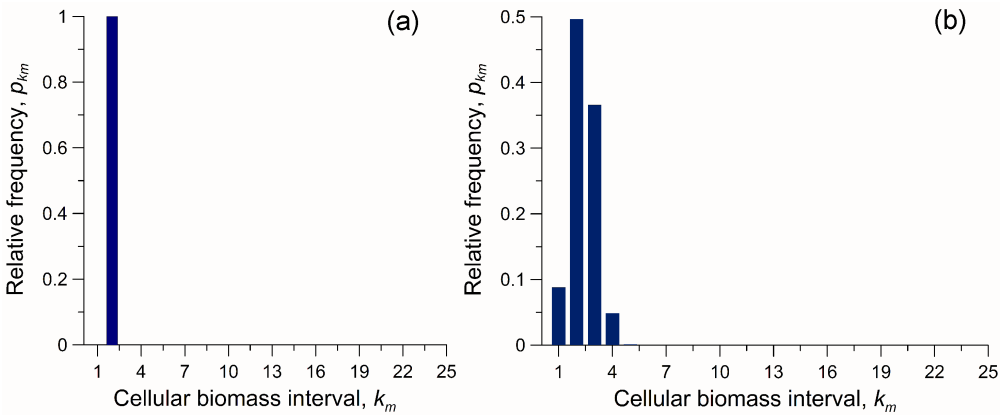

Figure 2.12: *Initial cellular biomass distribution of a homogeneous (a) and a heterogeneous (b) inoculum.*

2.3.7 Overview of a simulation

We present an example of an INDISIM simulation that has been fitted to an experimental dataset to illustrate the general operation of the simulator, as well as to quantify some of the above-mentioned parameters. The experimental data and the corresponding simulated curve are plotted in Figure 2.13.

In Table 2.2, the physical parameters (regarding space and time equivalences) are specified. In Table 2.3 the different parameters in simulation units (s.u.) are shown. In order to provide an understanding of these s.u., the values of three parameters in a specific point of the exponential growth ($t = 202.12h$) are specified in Table 2.4. Finally the obtained results of this simulation (lag phase and maximum growth rate) are presented in Table 2.5.

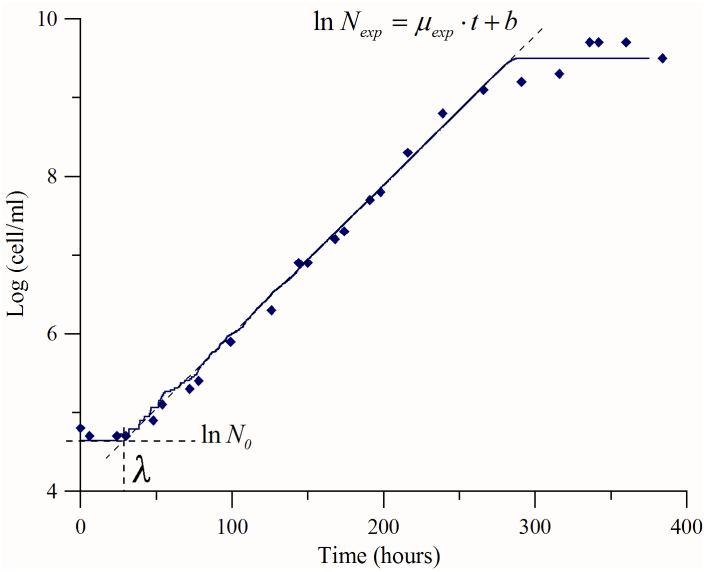

Figure 2.13: *Simulated growth curve (straight line) that fits experimental points from McKellar and Lu (2003). The calculation of the lag time by means of the geometrical method is also shown (dotted lines).*

Table 2.2: *Physical parameters of the simulation for the specific case of Figure 2.13.*

Parameter	*Value*
Simulated system volume, $V_S(10^{-10}m^3)$	1.13
Spatial cell volume, $V_C(10^{-15}m^3)$	1.26
Maximum number of bacteria per spatial cell, I_C(bacterial cells)	4
Time step equivalence, $t_{S_{eq}}$(min)	4.19
Initial bacterial cell concentration, $C_0(10^4 cell/ml)$	4.42
Maximum movement radium[1], $d_{max}(10^{-5}m)$	2.1
Maximum uptake range, $D_{max}(10^{-5}m)$	1.0
Fixed period of time for the reproduction, t_R(min)	41.9

[1]In this case, it does not represent the real bacterial motion. It is used to represent the culture agitation.

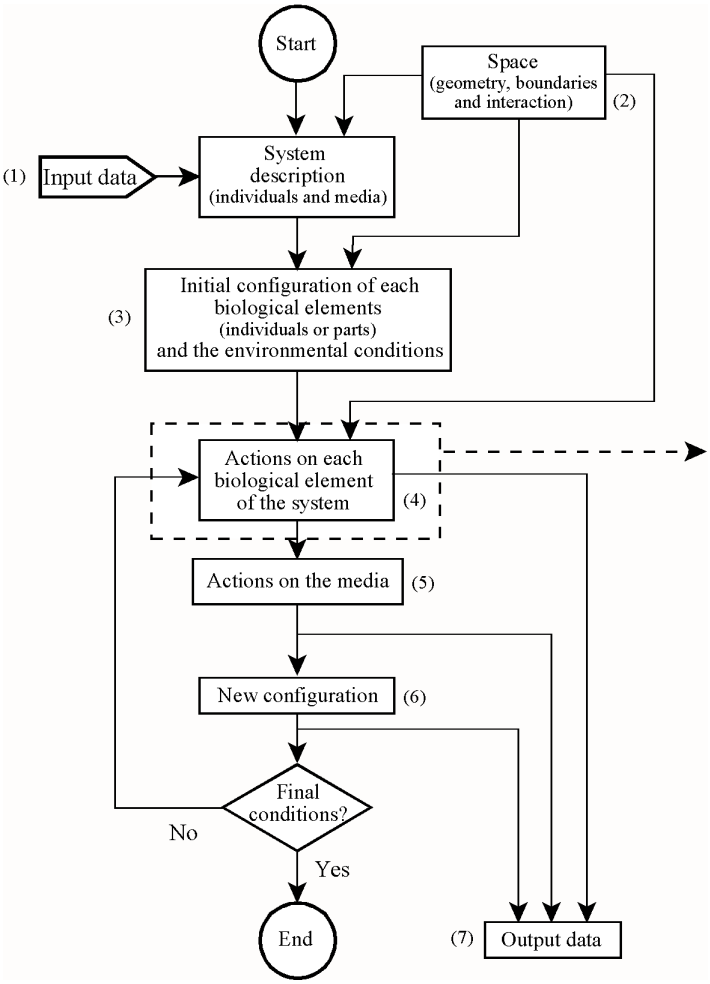

Figure 2.14: *Flow chart of the computer code (INDISIM for studying the lag phase). Note that there are three main parts: a) the initialization, where the input parameters are read (1), the spatial structure is defined according to the cellular method and periodic boundary conditions (2) and the environment and initial population are configured (3); b) the main loop, where at each time step the actions described in Figure 2.15 are taken for every bacteria of the medium (4), the actions over the medium (nutrient diffusion or redistribution, enzyme action over polymers, extracellular enzyme diffusion, etc.) are taken (5) and a new configuration is obtained (6); and c) the final part of the code, where the necessary calculations are made to obtain the results (7).*

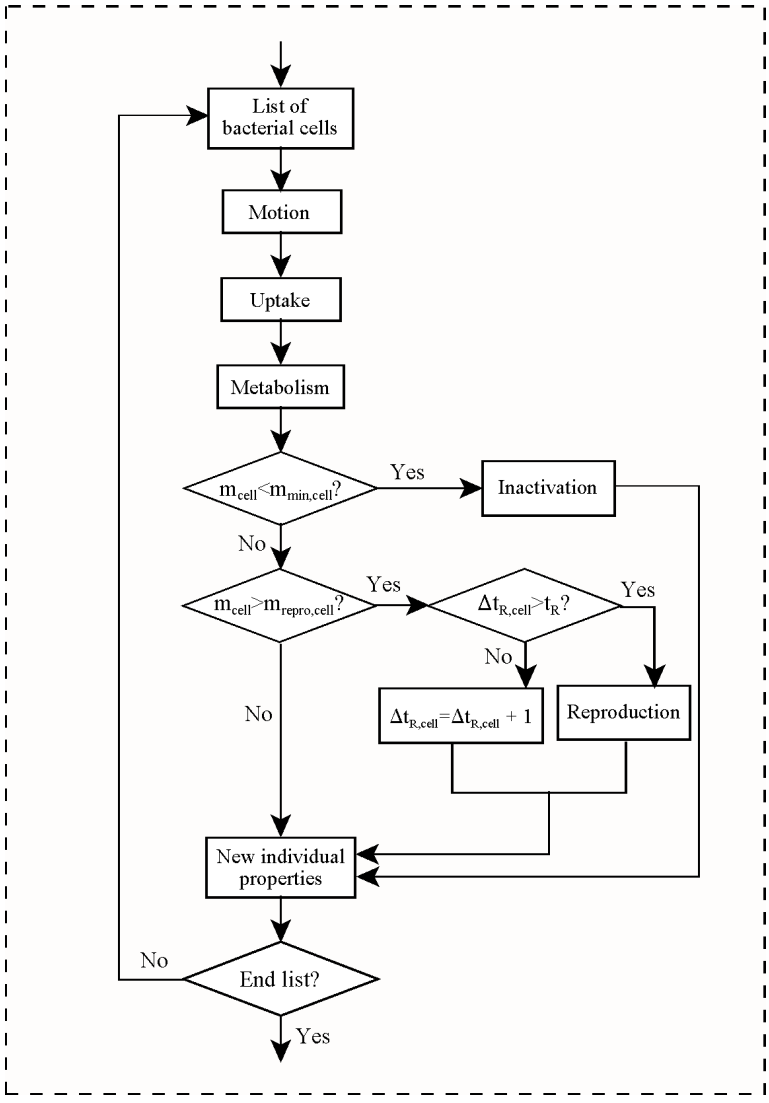

Figure 2.15: *Main loop of the computer code (INDISIM for studying the lag phase), where actions on each biological element of the system are carried out (Section 2.2.3).*

Table 2.3: *Simulation parameters in simulation units (s.u.) for the specific case of Figure 2.13.*

Parameter	*Value*
Initial mean cellular mass, $\overline{m}_0$(s.u.)	2663.4
Maximum uptake constant, U_{max}(s.u.)	315
Standard deviation in U_{max}, $\sigma(U_{max})$	0.3
Mean mass to begin the reproduction cycle, M_R(s.u.)	15000
Standard deviation in M_R, $\sigma(M_R)$	0.4
Minimum mass to initiate the reproduction cycle, $m_{R,min}$(s.u.)	5000
Initial nutrient particles per spatial cell[2], $Nut_{xy,0}$(s.u.)	145000
Nutrient particles per unit of biomass for maintenance, I (s.u.)	0.0070
Biomass synthesis efficiency, Y	1
Maximum enzyme per unit of mass, $_{max}$(s.u.)	10
Standard deviation in Enz_{max}, $\sigma(Enz_{max})$	0.5
Enzyme generation rate per unit of mass and time step, V_{enz}(s.u.)	1

[2]One nutrient particle of the simulation is not equivalent to one real nutrient particle.

Table 2.4: *Some values at a specific point during the exponential growth ($t_1 = 202.12h$) for the example of Figure 2.13 (s.u. = simulation units).*

Variable	*Value*
Mean mass, $\overline{m}(t_1)$ (s.u.)	9731.6
Mean energy for the cellular maintenance, $\overline{m}\Delta I(t_1)$ (s.u.)	67.41
Mean number of uptaken particles[3], $\overline{U}(t_1)$ (s.u.)	96.98

[3]One nutrient s.u. can be used to generate a biomass s.u. or an energy s.u. for the cellular maintenance.

Table 2.5: *Results (lag phase and maximum growth rate) of the simulation example (Fig. 2.13).*

Result	*Value*
Lag-parameter, λ (h)	30.12
Maximum growth rate (exponential phase), $\mu_{max}(10^{-2}h^{-1})$	4.39