A Bacterial Individual-Based Virtual Bioreactor to Test Handling Protocols in a Netlogo Platform

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Abstract: The choice of a basic modeling approach, either population-level or individual-based (IbM), is an important decision to be addressed according to the problem at hand. We present an IbM that simulates the dynamics of a bacterial bioreactor under different handling protocols. The main objective is to provide a simulator that will be easily understood, adjusted and used by non-experts. The model has been designed on the same basis as the IbM simulator INDISIM, and it is described using the ODD protocol. It has been implemented in NetLogo, so that it can be easily shared among the scientific community.

Keywords: Bacterial bioreactor, Individual-based model, INDISIM, NetLogo, Simulation

1. INTRODUCTION

1.1 Individual-based models

The choice of a basic modelling approach to study a bacterial system, either population-level (top-down, usually continuous with differential equations) or individual-based (bottom-up, discrete and computational model) is an important decision to be taken depending on the project's specific aspects, the characteristics of the system, the problem to be studied and the questions to be answered (Ferrer et al., 2009). Population-level models deal with population variables and fix a set of governing laws (equations) which are based on, or at least consistent with, an assemblage of assumptions about the individual behaviour of microbes. Individual-based models (IbMs) deal with individuals as discrete entities. These individuals have particular characteristics which change according to the rules that model their own behaviour and the interactions with other individuals and with the surrounding environment.

The two approaches are not incompatible or exclusive, but are complementary. Population-level approaches are mostly used for predictive purposes, due to their simplicity and computational efficiency. Moreover, they have been widely tested and, nowadays, many modelling frameworks exist. IbMs have also been used for some predictive purposes, but their strength lies in the means they offer to disentangle and understand the dynamics of biosystems. They allow us to deal with intra-population variability, with emergence of population behaviour from actions and interactions at an individual level and with those systems to which the continuum hypothesis is not applicable (Hellweger and Bucci, 2009). They also permit us to simulate different experimental protocols in labs and handling protocols in bioreactors in a simple manner, as IbMs are intrinsically discrete and take into account the individual behaviour adapted to internal and external conditions.

IbMs have been widely used and tested in the framework of ecology for more than 20 years (Grimm, 1999), but their use in microbial systems began during the last decade. In spite of the advantages they offer, IbMs are still far from being as readily accepted and widespread as continuous models in microbiology. Some of the reasons for this are: (i) the difficulty in determining the appropriate degree of complexity of the model, since models that become too complex fail to distinguish properly between causes and effects; and (ii) the difficulty in building, implementing, handling and analysing these computational models in a standard way, which complicates communication between specialists and non-specialists. In recent years the scientific community has developed several strategies to overcome such difficulties, for instance, working with pattern-oriented modelling, establishing a standard protocol (ODD protocol) to describe IbMs (Grimm et al., 2006, 2010) and developing specific open simulation environments such as the NetLogo platform (Wilensky, 1999) to work with them.

1.2 NetLogo

NetLogo is a computing framework which enables us to run individual-based simulations of natural and social phenomena (Wilensky, 1999), and which is continually being updated by the Center for Connected Learning and Computer-Based Modeling in the United States. The NetLogo software, the models library and the NetLogo programming documentation are distributed free of charge. This software provides a user-friendly interface that can be managed by non-experts, and at the same time includes code that can be modified by any advanced user. Therefore, NetLogo can be freely used by the

public in order to explore (test, use and modify) existing models, as well as to implement and use new models (http://ccl.northwestern.edu/netlogo). It allows the implementation of IbMs and the simulation of complex systems that develop and evolve over time. There is no pre-established maximum number of individuals or rules for the model, but the simulation can be faster or slower depending on the number of individuals and rules controlled, and on the technical features of the computer being used. NetLogo can coordinate all the instructions given to a set of individuals so that they all operate independently among themselves and with the environment.

1.3 Objectives and outline

We present an IbM that simulates the dynamics of a bacterial bioreactor under different handling protocols, in an attempt to overcome the stated disadvantages with the above-mentioned strategies. The main objective is to provide an IbM simulator of a generic bacterial bioreactor that will be easily understood, to make its adaptation to specific applications possible and its use by non-experts in the microbial arena.

The model has been designed on the same basis as INDISIM (Ferrer et al., 2008; Ginovart et al., 2002; Prats et al., 2010), and it is described using the ODD protocol in the following Section. It has been implemented in NetLogo, so that it can be shared without difficulty among the scientific community. As will be shown in the Results Section, this simulator allows the user to choose between three operating protocols (batch, fed-batch and continuous culture), to change some of the parameters involved, and to optionally take into account the inhibitory effect of an end product. The output of the simulation shows the evolution of bacterial population, nutrient and end product, and bacterial biomass distribution.

2. ODD DESCRIPTION OF THE SIMULATOR

The ODD protocol was designed by several IbM experts (Grimm et al., 2006) and updated a few years later (Grimm et al., 2010) in order to provide a standard way to communicate IbMs. This protocol allows the scientific community to identify the purpose and the basic features of a model, as well as to interpret and understand the whole model. This presentation is independent of its posterior implementation with a specific programming language in a simulation platform. We have adopted this ODD (Overview, Design concepts and Details) protocol for describing the model behind this virtual bioreactor.

2.1 Overview

Purpose. The purpose of this model is to provide a virtual bacterial bioreactor with three possible operating protocols (batch, fed-batch, and continuous cultures) so that the resulting dynamics can be studied and compared. At this moment it allows a qualitative study, but in the near future it will be parameterized in order to be used for quantitative purposes in an adaption to specific applications.

Entities, state variables, and scales. Basic entities are bacteria and spatial cells. Bacterial variables include for each microorganism, an identity number, a location (i.e., the spatial cell where it is), a mass, the required energy for maintenance, the mass at which it will enter the reproduction cycle, and the viability. Spatial cell variables include the x and y integer coordinates for each site, the nutrient content and end product content. It can simulate a population of up to 10^5 bacterial cells in a 30×30 spatial cell domain. This is a non-parameterized version so there is not a real units correspondence yet.

Process overview and scheduling. The sets of rules governing the behaviour of each bacterium are in the following categories or sub-models: (i) motion (randomly), (ii) uptake and maintenance (i.e., uptake of nutrient particles to achieve cellular maintenance first and then, if there is enough nutrient, increase bacterial biomass, with excretion of the end product to the spatial cell), (iii) reproduction by bipartition (when certain conditions are satisfied) and (iv) cell viability (and death as appropriate). The damage that an excreted end product can produce in bacterial cells may be considered. At each time step of the simulation, bacterial cells act or perform a set of actions. Sub-models regarding spatial cells include the culture stirring, the entrance of fresh medium, and output of medium and bacteria according to the corresponding operating protocol. These actions are performed once individuals have acted.

The global scheduling of the simulation model is made up of various elements: (1) initialization of the system with the input data chosen by the user, where initial configuration of the bacterial population and the spatial environment are set up, as well as the parameters for the chosen operating protocol; (2) the main loop (time step), in which all the rules for each bacterium and the medium are implemented and repeated, and the external actions on the system are applied, until reaching the end of the simulation; and (3) the output of results at the end of each time step.

2.2 Design concepts

Basic principles.: The general concepts and basic principles of this model are taken from INDISIM (Ginovart et al., 2002).

Emergence. The chosen operating protocol of the bioreactor is decisive for the dynamics of the system and structure of the bacterial population. These features arise from the reproduction and viability of the individuals which are conditioned by local nutrient availability and inhibition of the end product (affected by the entrance or not of fresh medium, and/or the output of the growth medium). Therefore, the individual activity is highly affected by the operating protocol of the culture.

Adaptation. An adaptation for individuals is the consumption of their own biomass when there is a nutrient scarcity, but the bacterial biomass has a lower limit under which bacteria just die. Also, in the presence of an end product that has an

inhibitory effect, the microorganism needs to spend more energy to compensate this effect.

Sensing. The bacteria are sensitive to their environmental conditions: nutrient availability and the existence of end product in their local surroundings.

Interaction. Interactions between bacteria are indirect; they are conducted by the interactions with surrounding medium. There may be competence for nutrient when availability is not sufficient, and when the end product may be chosen as inhibitory for the bacterial activity.

Stochasticity. It is introduced into the model when setting some characteristics of individuals using a Gaussian distribution around an expected mean value. This distribution remains the most commonly encountered distribution in nature and statistics, and reflects range in the population. Randomness is also considered when the rules are applied to individuals and to spatial cells by using probabilistic distributions to deal with or manage individual events. This represents the uncertainty in these processes and reflects the high variety of mechanisms that underlie the irregularity observed in natural processes and biological materials. At each time step the order of the individuals to perform the different actions changed randomly, in order to avoid privileging first-acting bacterial cells (Wilensky, 1999).

Observation. Observation data can be divided into macroscopic and mesoscopic (Fig. 1). The former includes the evolution of several population-level variables such as number of individuals, total biomass, amount of nutrient, end product concentration, and rates of change in the size of the bacterial population and its total biomass. The latter refers to variables that connect individuals to population; in this case the mesoscopic variable is the bacterial biomass distribution, which is a reflection of the population structure and its evolution.

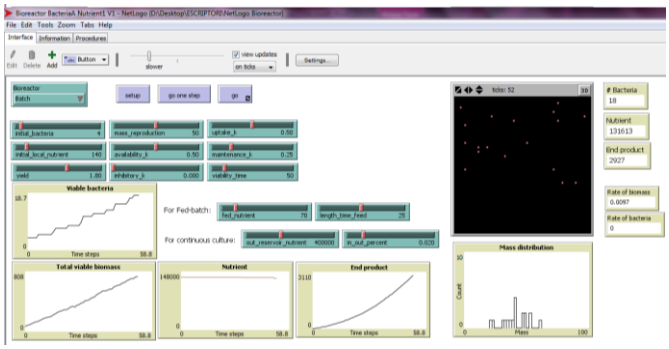

Fig. 1. Screenshot of the NetLogo virtual bioreactor interface. Handling protocol can be chosen by user, as well as the initial conditions and some of the model's parameters. Output is observed in several graphics and numerical boxes.

2.3 Details

Initialization. This is explicitly done by the user by pressing the "setup" button at the interface (Fig. 1). Then the simulator reads all the input data and builds the system in its initial state, generating the "world" with spatial characteristics (a homogeneous distribution of nutrient) and a population with initial individual characteristics for each bacterium. Then, it is ready to start the simulation by pressing the button "go one step" or "go". The operating protocol and model options, as well as the input values for the parameters of the model are chosen by the user at the interface within the allowed range by using "sliders" (Fig. 1). The current version is non-parameterized, so these data are in simulation units. The inputs that have to be set up are: initial number of bacteria, initial amount of nutrient, mean mass to initiate the reproduction cycle, availability of the nutrient to be uptaken for the bacterium, mean time that a bacterium remains viable without its energetic requirements, and the constants to determine the maximum amount of nutrient that a bacterium can uptake, the energetic requirements for cellular maintenance, the metabolic efficiency that accounts for the synthesised biomass per metabolised nutrient (yield), and the extra energy necessary to compensate the damaging effect that the presence of the end product acting as an inhibitor has on a bacterium. Possible operating protocols are a batch culture (no nutrient entrance), a fed-batch culture and a continuous culture. If fed-batch is chosen, the handling characteristics, amount and periodicity of nutrient input, must be fixed. If continuous protocol is chosen, the amount of the external nutrient reservoir and the percentage of medium renewal have to be fixed. The model options are chosen by means of some the above-mentioned parameters: e.g., if the inhibitory constant is fixed to 0, inhibitory effects will not be taken into account.

Input data. The operating protocols chosen by the user determine the external input data during the simulation.

Submodels:

At each time step the model considers the following individual actions:

i) Motion. A bacterium can move from its spatial cell to a one of the 8 adjacent spatial cells chosen at random.

ii) Uptake, maintenance and growth are included in a single sub-model. The quantity of nutrient that a bacterium may absorb is proportional to its cellular surface (assuming a spherical geometry for the microorganism) and it is limited by the available nutrient of its own spatial cell. The maintenance energy required is proportional to its biomass and the concentration of the end product on the spatial cell in the case of assuming an inhibitory effect. Otherwise, if the nutrient achieved is not sufficient to cover the energetic requirements for its maintenance, the bacterium can degrade its own biomass until it achieves a minimum size. If enough nutrient remains, the cell can increase its own biomass according to this and depending on the yield assumed.

iii) Reproduction is done by bipartition. Each individual cell has a particular mass to initiate this process, which is given by the mean value chosen by the user and a Gaussian around it. Once this mass is achieved, the bacterium splits, and two new bacteria appear in the same location, each one having half of the progenitor biomass. New start-reproduction masses will be assigned to each one.

iv) Cell viability is given by a maximum time that a bacterium can survive when the maintenance requirements are not satisfied. Once this time has passed, the bacterium becomes non-viable and dies.

Stirring the culture permits the exclusion of local diffusion limitations. A periodically very high diffusion of nutrient and end product is performed in the medium.

The entrance of fresh medium identified as the entrance of nutrient is carried out according to the handling instructions given by the user at the beginning of the simulation. When a specific amount of nutrient has to be introduced, it is homogenously distributed among the spatial cells. When a percentage of the medium is taken out of the system, this is done with the extraction of an amount of nutrient and end product, as well as a set of bacterial cells chosen randomly.

3. RESULTS

The IbM detailed above was implemented in a NetLogo environment. As it is an initial non-parameterized version, it was used to qualitatively test different bioreactor performances. Specifically, the batch-culture, the fed-batch culture and the continuous-culture operations were explored. This simulator can be used by interested readers in order to execute it to deal with diverse scenarios, as well as to be modified or adapted. The only previous requisite is to download the program from the NetLogo site (http://ccl.northwestern.edu/netlogo/download.shtml). The simulator can be requested directly from the authors.

3.1.1 Batch culture: a closed system with no entry or exit of individual cells and/or substrate particles

This case mimics the classic batch culture where bacteria grow on substrate medium under suitable conditions. They usually grow and reproduce until one necessary growth factor becomes exhausted (nutrient) and it becomes growth limiting or the accumulation of an end product with inhibitory effect is excessive. If no additional nutrient is added or the inhibitors are removed, no further growth will take place. Growth in such a closed loop system is called batch culture, and the resulting population growth curve can be divided into four distinct phases - these are lag, exponential or logarithmic phase, stationary phase, and death phase.

Figures 2 and 3 are generic outputs of the simulations performed under these conditions. In the first case (Fig. 2), no inhibitory effect was considered, while in the second case (Fig. 3) the inhibitory effect of an end product was taken into account. As can be seen, in both cases the lag, exponential, stationary and death phases are present. The basic difference between them is that, while in the first case the growth ends when the nutrient is exhausted, in the second case it ends when the inhibitory product reaches a certain concentration (i.e., there is still nutrient in the medium, but the inhibitory effects of the end product stop the bacterial growth). Besides, it can be noted that the evolutions of the number of viable bacteria and the total biomass of the population show different profiles in such phases where the growth conditions are not optimal. For instance, in Fig. 2, when the nutrient is exhausted and the culture enters the stationary phase, the energetic requirements are satisfied with the use of the bacterial own biomass.

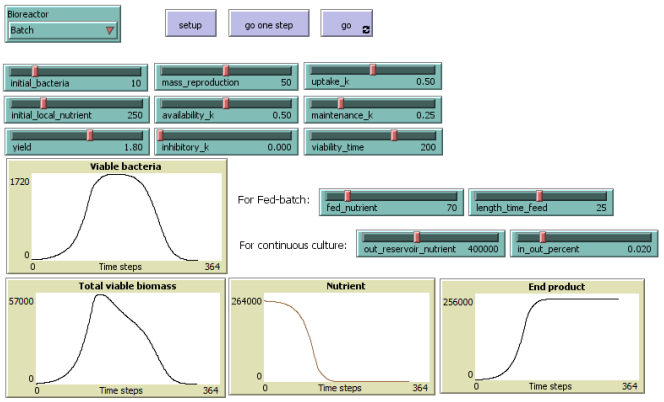

Fig. 2. Screenshot of the NetLogo virtual bioreactor for an evolution of a batch culture without considering inhibitory effect of the end product.

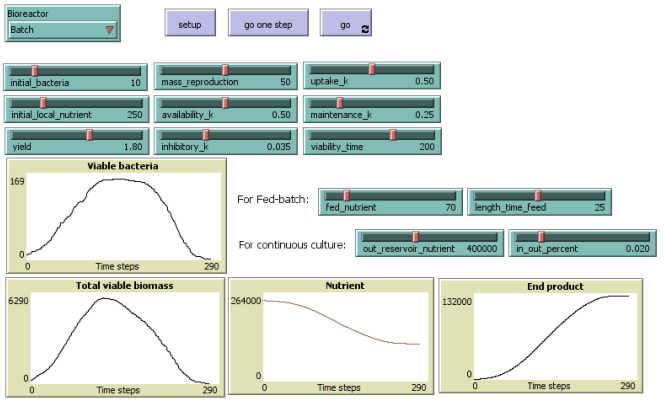

Fig. 3. Screenshot of the NetLogo virtual bioreactor for an evolution of a batch culture considering inhibitory effect of the end product.

3.1.2 A fed-batch culture: an open system where external fluxes are represented as the entry of a substrate (and/or other kind of particles into the system) at certain times

A fed-batch operating protocol implies that there is a periodic renewal of part of the medium. The amount of input nutrient (fed_nutrient) and the frequency of this renewal (length_time_feed) affect the evolution of the bacterial population. As it is an open system, the four classical phases of growth are not observed, and the growth curve is a direct consequence of such renewal size and frequency. Different cases were checked: high and low frequencies of renewal, each one with high and low amount of the input nutrient.

For instance, Figures 4 and 5 show the evolution of a fed-batch bioreactor with a small amount of input nutrient at each renewal, without considering the inhibitory effect of the end product. The first one shows a fed-batch operating with a high renewal frequency, while the second one shows a

bioreactor with a low renewal frequency. In both cases we can distinguish an initial lag phase, a subsequent pseudo-exponential phase and a final phase where the bacterial load tends to synchronize with the renewal characteristics: the population initially decreases until it reaches a kind of stationary state with oscillations, where there is a kind of equilibrium between nutrient entrance and bacterial load. These oscillations are directly related with renewal frequency, and their size is related with the amount of nutrient that enters each time.

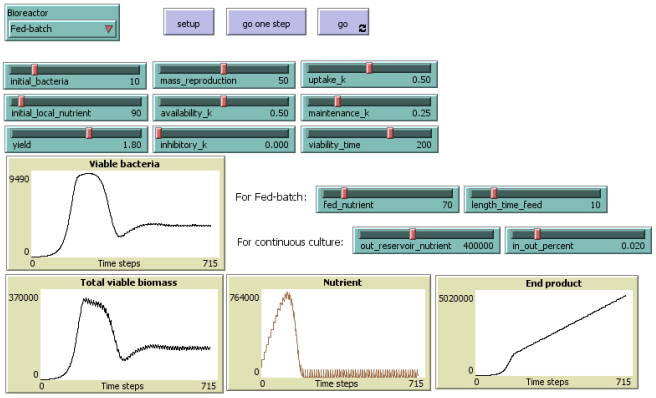

Fig. 4. Screenshot of the NetLogo virtual bioreactor for an evolution of a fed-batch culture with a high renewal frequency, a low amount of input nutrient at each renewal, and without considering inhibitory effect of the end product.

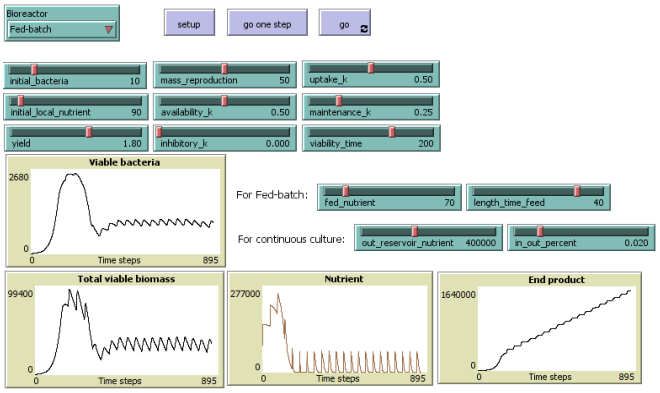

Fig. 5. Screenshot of the NetLogo virtual bioreactor for an evolution of a fed-batch culture with a low renewal frequency, low input nutrient at each renewal, and without considering inhibitory effect of the end product.

3.1.3 Continuous culture: an open system where external fluxes are represented as the entry of a nutrient into the system and the exit of a volume of the medium (exit of nutrient, end product and bacteria) at each time step

For many applications in industry it is desirable to have maintenance of viable bacteria over extended periods of time. In this case, the nutrient concentration and other conditions must remain constant, so that individual cells grow at a constant rate, fully acclimatised exponential rate. In practice, this is achieved by constant addition of fresh effluent to the growing bacteria and concomitant withdrawal of equal volumes of the growing bacterial culture.

We simulated the operation of a bioreactor with a continuous medium renewal, considering different percentages of culture removing (in_out_percent), and nutrient entrance from an external reservoir (out_reservoir_nutrient). Figure 6 shows the evolution of a bioreactor with a low percentage of medium renewal (in concordance with the range of this parameter), while Figure 7 shows the evolution of a bioreactor with a higher percentage of medium renewal. In both cases, inhibitory effect of the end product has not been considered. From these two figures it can be seen that, in spite of showing different transient phases, a constant bacterial load is achieved in both cases according to the renewal level. In particular, it has been observed that in some cases the medium renewal hinders the increase in population (Fig.7).

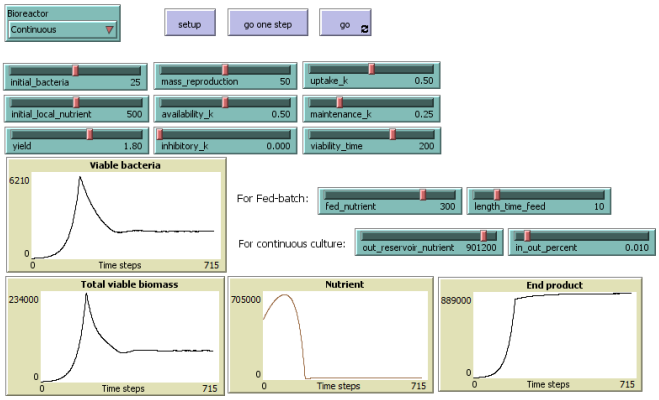

Fig. 6. Screenshot of the NetLogo virtual bioreactor for an evolution of a continuous culture with a low percentage of medium renewal, without considering inhibitory effect of the end product.

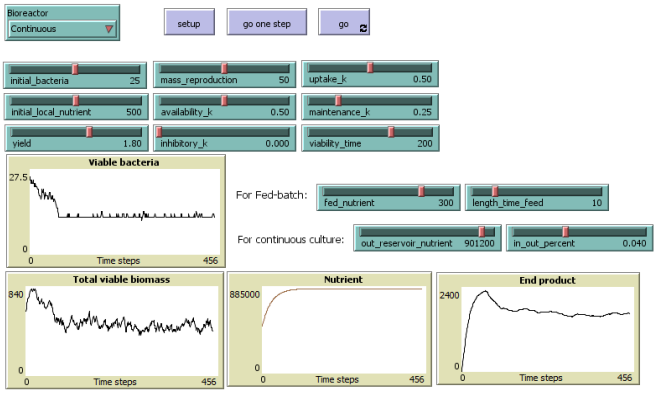

Fig. 7. Evolution of a continuous culture with a higher percentage of medium renewal respect to the simulation of Fig. 6, without considering inhibitory effect of the end product.

4. CONCLUSIONS

Different profiles in the outputs of our NetLogo bacterial bioreactor simulator have been clearly observed when the

user changes the operating options and the parameters involved. This is a first version and needs to be parameterized for a specific application. Nevertheless, it has been demonstrated to be useful for observing the different patterns that a bacterial population shows in a bioreactor depending on the handling protocol. The evolution of a bacterial population as the result of the individual activity of each bacterium strongly depends on the nutrient availability (and inhibitory product concentration), and this relationship can be observed in the simulations presented. When we simulate the growth of bacteria in a batch culture, the classic phases of growth are observed (lag, exponential, stationary and death). However, when these bacteria grow in an open system such a fed-batch culture or a continuous culture, different patterns can emerge from the same individual bacterial model. Although the characteristics or rules that are assigned to bacteria at an individual level are simple and comprehensible in an isolated way, when we incorporate and integrate everything the degree of complexity that the complete model reaches is considerable. With all these features, this simulator seems to be a good tool to be used by modellers and non-modellers alike.

The next step in this research project is to choose a specific application in order to adapt this IbM to the selected bacterium and parameterize it under certain experimental conditions. The resulting simulator will be useful for investigation of the handling protocol appropriate for obtaining certain population evolutions or behaviour patterns, besides fixing the parameters involved in this protocol.

Complete understanding of the model and its implementation in a computer code, along with the corresponding parameterization for a specific application, are neither immediate nor rapidly accomplished tasks. Therefore, the use of the NetLogo platform makes this simulator accessible to non-experts for experimentation, and at the same time, guarantees that any modeller can adjust or change parts of the simulation model to adapt the virtual bioreactor to their own context of research.

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ACKNOWLEDGEMENTS

The financial support of the Spanish Government (MICINN, CGL2010-20160) is gratefully acknowledged. The first author, M. G., is very grateful to the School of Computing Sciences, University of East Anglia, for inviting her to be a Visiting Fellow in Autumn 2011, during which time this project was initiated.