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ORIGINAL PAPER

# INDISIM-YEAST: an individual-based simulator on a website for experimenting and investigating diverse dynamics of yeast populations in liquid media

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**Abstract** Simulation modelling can be used to capture and mimic real-world microbial systems that, unlike the real-world, can then be experimented upon as a new kind of experimental milieu. Individual-based models, in which individuals interact dynamically with each other as structural elements in the model world, exemplify this view of simulation modelling. These models are more difficult to analyze, understand and communicate than traditional analytical models. It is good practice to provide executable versions that perform simulation results. INDISIM-YEAST, developed to deal with yeast populations in liquid media, models the evolution of a set of yeasts by setting up rules of behavior for each individual cell according to its own biological regulations and characteristics. The aim of this work is to develop and present a website from which INDISIM-YEAST is accessible, and how to carry out yeast simulations to further the skills associated with the use of this individual-based simulator. A good and useful way to analyze this yeast simulator is to experiment and explore the manner in which it reacts to changes in parameter values, initial conditions or assumptions. The application results in a very versatile program that could be used in controlled simulation experiments via the Internet.

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## Introduction

With the coming of the computer, the possibility of experimenting with diverse kinds of mathematical models arose. Simulation modelling has become vital for studying a range of complex systems and it has been applied in various ways. Three features of simulation can be identified: (i) it is used to solve analytical equations and exploring statistical properties; (ii) it is a new science somewhere between experimental methods and purely theoretical analytic models, where new methods must be developed and new ways of looking at models must be considered; and (iii) it is used to capture and mimic real-world systems that, unlike real-world systems, can then be experimented upon [1]. These views are not mutually exclusive, but each gives insight into how simulations can be used. We are interested in this last type of simulation. Agent-based Models or Individual-based Models (IbMs), in which individuals interact dynamically with each other as structural elements in the model world, exemplify this view of simulation modelling [2]. This type of modelling has become the sine qua non for understanding complex systems and has been used successfully in microbiology [3–6].

The micro world is complex and we need all the tools that we can muster to understand it. Whole-cell modelling, which was thought difficult until recently, has suddenly become realistic [7]. The set of tools for computational approaches to cell biology is increasing. Some of the relevant software tools applied to cell biology are, for instance, Virtual Cell, E-CELL, BioSpice, StochSim, Mcell

[8], and simulators for biochemical metabolic pathways have been applied to studies of yeast kinetics [9, 10]. Nevertheless, the study of the cell will never be total unless its dynamic behavior is understood. Moreover, whole-cell models integrate subcellular processes into a single cell model. This differs from the IbM described below, whose purpose, while losing details of the single cell processes is capable of integrating cellular processes into population models [5].

IbMs allow researchers to study how system level properties emerge from the adaptive behavior of individuals, as well as how the system affects individuals. IbMs are important both for theory and management because they allow researchers to consider aspects usually ignored in analytical models [2]: (i) variability among individuals, (ii) local interactions, (iii) complete life cycles and, in particular, (iv) individual behavior adapting to the individual's changing internal and external environment. However, the great potential of IbMs comes at a cost; IbMs are necessarily more intricate in structure than analytical models. They have to be implemented and run on computers. IbMs are more difficult to analyze, understand and communicate than traditional analytical models [2]. This is due to the fact that IbMs are often described verbally without a clear indication of the equations, algorithms and schedules that are used in the model. Consequently, the results obtained from an IbM are not easily reproduced.

Grimm and co-authors [11] proposed the basic idea of a standard protocol that could be used for describing IbMs. This protocol has been designed as a tool to facilitate the communication and replication of IbMs. It has been considered as a first step for establishing a more detailed common format of the description of IbMs. They suggested that it would be good practice to provide an executable version of the program capable of performing all or the most important simulation experiments described in the paper explaining IbM. In spite of the growing number and quality of software platforms for Individual-based Simulations, software development remains an obstacle to the use of IbMs for many researchers [12]. This problem results mainly from an absence of training in software skills and programming needed for developing an Individual-based Simulator (IbS) in fields where IbMs are used.

We are interested in exploring the role of simulation as a type of experimental system, and for this purpose we will use the IbS called INDISIM-YEAST, developed to deal with a set of yeast cells growing in liquid media [3, 13]. INDISIM-YEAST is a simulation model that describes an individual organism, a unicellular fungi, being the yeast cell used as a reference from the genera *Saccharomyces* and species *cerevisiae*. INDISIM-YEAST models the evolution of a virtual yeast population by setting up rules of behavior for each individual cell according to its own biological regulations (uptake, metabolism, budding reproduction, viability, etc.) and characteristics (biomass, genealogical age, state in the cellular reproduction cycle, etc.) The microorganisms grow in an environment that contains nutrient particles and metabolites. When we constructed INDISIM-YEAST, we created a world in which we have access to all of the laws and elements of that world, and the relationships among its abiotic and biotic components, which we can also manipulate. To the extent that we can match our simulations to the yeast real world, we should be able to read items of the simulated world that tell us something about reality [1]. We think that this is a skill that must be developed over a lengthy period of trial, error and comparison with both theory and known results from physical experiments. A great number of simulations need to be performed. Equipping INDISIM-YEAST with graphical user interfaces makes it easy for others to run, understand and experiment. An interesting and useful way to analyze this yeast simulator is to explore the way in which it reacts to changes in parameter values, initial conditions or assumptions. The aim of this work is to develop and present a website from which INDISIM-YEAST is accessible, and the way to carry out yeast simulations, virtual experiments, in order to promote the skills associated with the use of this IbS.

## Materials and methods

### The individual-based simulator INDISIM-YEAST

The INDISIM methodology developed by the CS-SIMBIO Research Group of the Technical University of Catalonia (Spain) has advanced very much since its beginnings. Numerous applications for the study of specific microbial systems have meant developing new modelling strategies and engendering new simulators (http://mie.esab.upc.es/mosimbio/english/english.htm). The description of INDISIM and the principal concepts of yeast cell modelling, plus the different elements to assemble the structure of the system for the virtual process of glucose fermentation, can be found in the works of Ginovart and co-authors [3, 13].

INDISIM-YEAST is discrete in space and time. The environment is divided into spatial cells that contain yeast cells, nutrient particles and metabolites. The time evolution of the yeast population is divided into equal intervals associated with computer steps (time steps). Figure 1 shows the basic INDISIM-YEAST flow chart of the simulator with different sections: (i) initialization of the system, with the entrance of input data that fixes the initial configuration of the whole system; (ii) the main loop (time step), where actions on each individual yeast cell and on

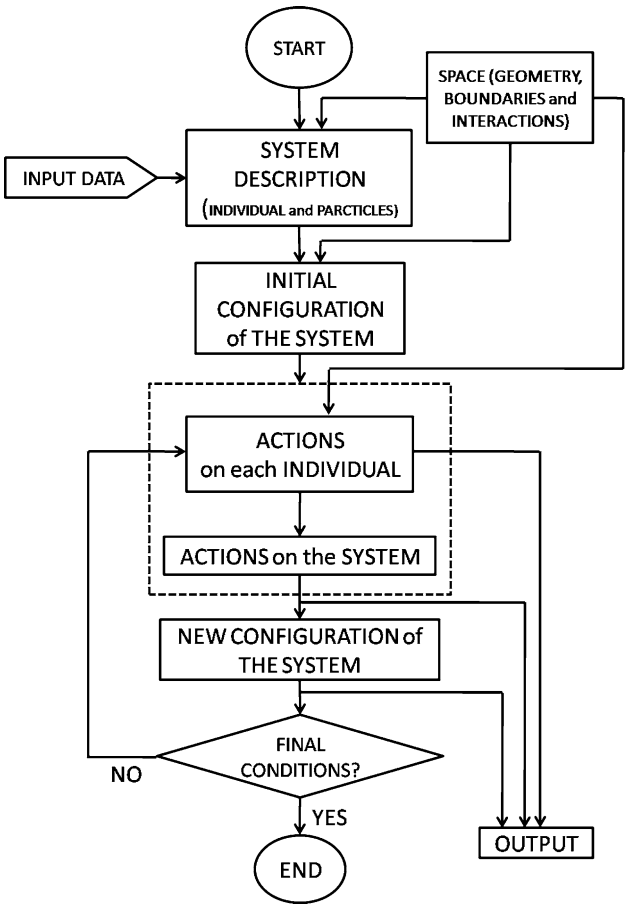


**Fig. 1** The general basic flow chart of the simulator INDISIM-YEAST

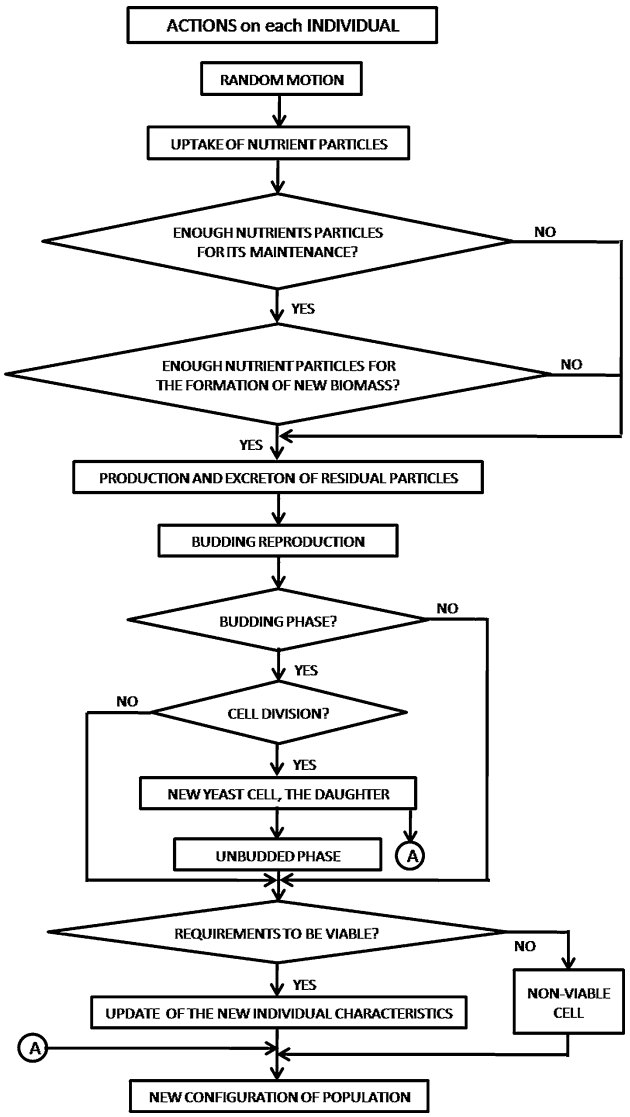


**Fig. 2** *Flow chart* of the time step of the simulator INDISIM-YEAST with the detailed individual actions performed over each yeast cell of the population

the system are carried out and repeated until the simulation ends (these actions originate new configurations of the yeast system), and (iii) output of data at the end of each time step or at the end of the simulation to obtain the results. Figure 2 shows the flow chart with detailed individual actions for every yeast cell (motion, uptake, metabolism, reproduction and viability), and Fig. 3 shows possible actions that give us control over the system in order to manage different strategies of the culture.

We assume in INDISIM-YEAST that the yeast population grows in the bulk of a liquid medium where we consider variables that are space and time dependent. These variables, in each spatial cell of the virtual domain, control the amount of abiotic components, identified as glucose (nutrient particles) and ethanol (metabolites or end product particles) arising from the yeast cellular activity and excreted to the environment through the time. In this work, the system is not altered by further nutrient addition or removal of medium, this means that a yeast batch culture is considered. However, the environment is continuously changing because glucose particles are consumed and ethanol particles are produced and accumulated in the medium. An individual yeast cell is defined by a set of time-dependent variables that describe and control its individual properties. For each microorganism and at each time step, INDISIM-YEAST implements a set of rules for the following actions: motion, uptake of nutrient particles and their metabolism, budding reproduction and cellular viability [13]. Each yeast cell is characterized by: (i) its biomass $m$ which enables us to obtain its cellular surface; (ii) genealogical age $a$, the number of scars that the budding reproduction leaves on its cellular surface; (iii) states in the cellular reproduction cycle, distinguishing between unbudded or budding phase, and control of the remaining times and masses related to these two phases; and (iv) survival time without satisfying the metabolic requirements for its maintenance. Random variables and/or random

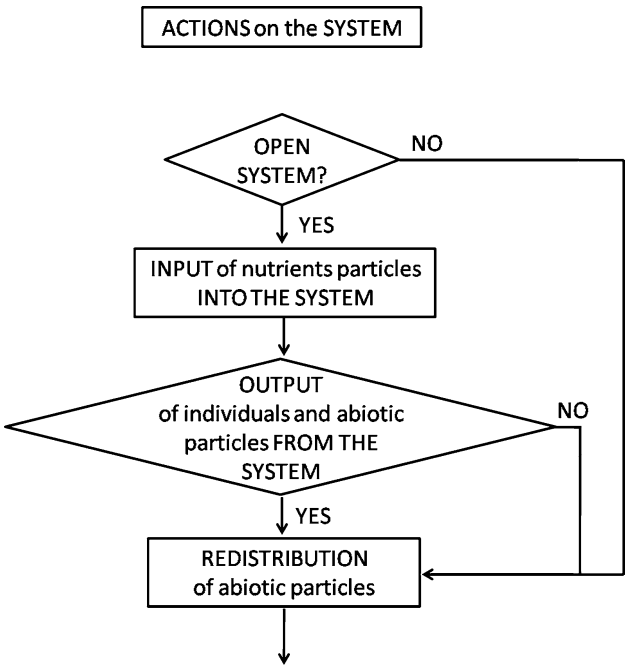


**Fig. 3** *Flow chart* of the time step of the simulator INDISIM-YEAST with possible actions that may be carried out with the system

numbers are used to characterize the individual yeast cell and the individual actions for the update of the set of rules.

The yeast model implemented in the simulator takes into account the effects arising from: (a) the bud scars, as these affect the cellular membrane, (b) the excess of nutrient concentration, as it may also induce inhibition in its uptake, (c) the growth arrest as a consequence of the metabolic final product [14]. These factors are mainly focused on the version developed for the designed website.

## Specific features of INDISIM-YEAST

For some specific details involved in the use of this website, it is important to draw attention to two of the parts that configure the yeast cell model: the uptake model and the metabolism model [13].

Yeast absorbs the nutrients through the cell membrane as low molecular weight compounds dissolved in water [14]. At each time step, the uptake of nutrient particles by a yeast cell will be determined by its own size and by the accessible nutrient particles in its surrounding environment. Assuming spherical shape and constant cellular density for yeast cell, the value $m^{2/3}$ is proportional to its cellular surface which is in contact with the medium. At the same time, uptake will be restricted by the following factors: (i) the genealogical age of the cell, say $a$, due to the fact that bud scars on the cell's surface affect the conditions of the cellular membrane and, (ii) the number $s_1$ of nutrient particles per spatial cell (local glucose concentration) in order to take into account the fact that an excess of glucose induces inhibition in growth [14]. Hence, if $U_{\max}$ is the maximum number of nutrient particles that may be consumed per unit time and per unit of cellular surface, the maximum number of nutrient particles that one yeast cell may absorb, $U$, is given by Eq. (1)

$$U = (U_{\max} + z_1)m^{2/3}[1 - K_1 a - (K_2 + z_2)s_1] \quad (1)$$

where $K_1$ is the constant to represent the effect of the scars on the cellular surface, $K_2$ the constant to emphasize the inhibition by glucose excess, and $z_1$ and $z_2$ are random draws from normal distributions. Evidently the final and actual uptake is determined by the number of accessible nutrient particles of the spatial cell where the yeast cell is located.

A yeast cell requires energy for its cellular maintenance and it is assumed that this energy is proportional to its biomass. Additionally, when ethanol accumulates during fermentation it acts as a potent chemical stress on yeast cells, so various physiological adaptations which are thought to confer protection against ethanol, occur in the cell [14]. Hence, if $e$ is a prescribed amount of translocated glucose per unit of biomass that a yeast cell needs to remain viable, we assume that the total energy $E$ required to maintain viability is given by Eq. (2),

$$E = em + (K_3 + z_3)s_2 m^{2/3} \quad (2)$$

where $K_3$ is the constant to represent the added energy to counteract the ethanol presence, $s_2$ is the number of end product particles per spatial cell (local ethanol concentration), and $z_3$ a random draw from a normal distribution. Whenever a yeast cell does not achieve enough glucose particles to satisfy its metabolic requirements after some time steps, the cell will lose cellular viability.

Heat production also takes place in the system and this is modelled to be proportional to the nutrient particles uptaken by the yeast cells.

## The INDISIM-YEAST's website

A website containing a version of the INDISIM-YEAST simulator has been designed to deal with the behavior of a set of yeast cells growing in a closed liquid medium with glucose. This website is located at https://aneto.upc.es/simulacio/hoja-portada.html. The achieved application is simple and intuitive and it was developed with G95, a free portable open-source Fortran 95 compiler. The operating systems Mandriva Linux (in a server computer) and openMosix with a free cluster management system (in a computer cluster) were used. The server uses Cascading Style Sheets, JavaScript and HyperText Markup Language as languages to construct the web pages. Calculations are made using the simulation program in Fortran 95 which is

located on the computer cluster. The Java language is the system application server and JFreeChart is an open-source framework for the programming language Java that allows the reading of data and creation of graphic representations. The network between the server and the cluster is carried out through the Hypertext Preprocessor (PHP), designed for producing dynamic web pages, and Samba, a set of tools to share resources on a TCP/IP network. All used programs are released under General Public License.

This website is composed of the following: (i) a brief theoretical content with three sketches to show the structure of the general model, (ii) a demonstration of the simulator with graphical outputs for some variables related to the yeast system (*Demo* option), and (iii) an access to execute the simulator allowing changes in the values of some parameters (*Log in* option).

The group of input data offered for modifications, together with graphical outputs, makes it possible to configure different virtual yeast systems and observe their evolutions through the simulator. The simulation process returns a number of output variables which are displayed in a set of graphs (Fig. 4). The graphical summary shown allows the user to visualize the way that the yeast population and fermentation evolve. It can be used to support the process of learning about IbMs and, in experimental simulations reminiscent of virtual laboratory practices. The outputs are structured in different groups: some orientated towards the presentation of global properties of the system (population properties or medium characteristics), and others towards those properties that pertain to individual yeast cells (distributions of microbial properties). The simulation results of the temporal evolutions of global magnitudes of the yeast culture are: number of nutrient particles (glucose), number of residual product particles (ethanol), average nutrient uptake (number of metabolized nutrient particles during one time step divided by total number of viable cells), number of viable cells, number of non-viable cells, viable yeast biomass, non-viable yeast biomass, heat dissipation of the system, and maintenance energy of the population (defined as the number of

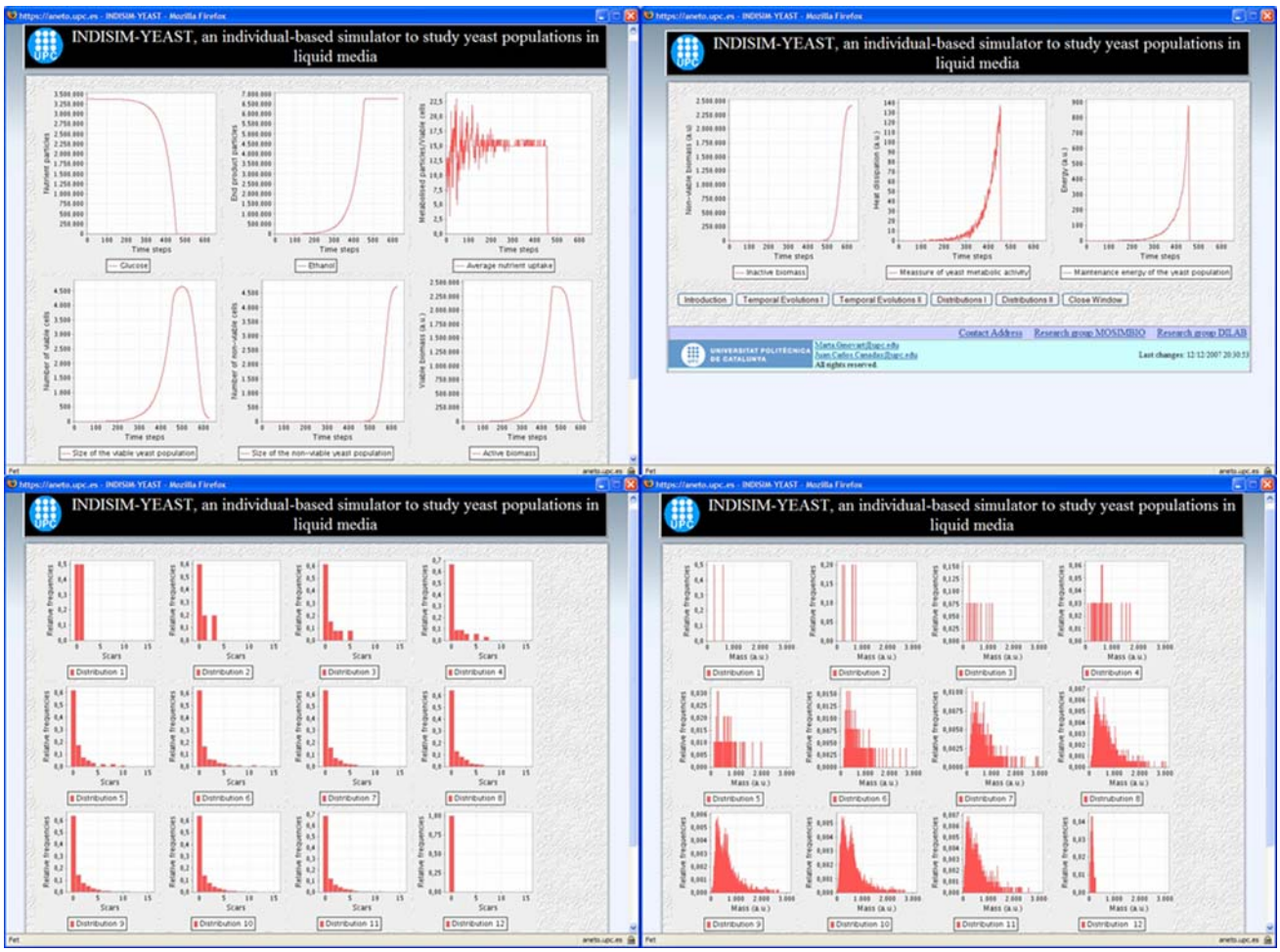


**Fig. 4** *Screenshots* of INDISIM-YEAST in action from the website https://aneto.upc.es/simulacio/hoja-portada.html: temporal evolutions of variables of the system and distributions of genealogical ages and masses of the cells configuring the yeast population

metabolized nutrient particles not used in the production of new biomass). These outputs are presented in the two windows of the first row of Fig. 4. The simulation results concerned with microscopic population parameters are mainly distributions of certain variables controlled at individual level. The graphics shown in the second row of the Fig. 4 are 12 bar charts for the distributions of genealogical ages (left) and 12 histograms for the distributions of masses (right) of the yeast cells of the population, belonging to different stages or times steps of the culture evolution.

Contacting the authors and designers of the website is possible so that microbiologists can send their critical observations, specific contributions and suggest further utilities for this simulator. The implementation of this microbial IbM has been made efficiently with a structured programming language, and the flexibility and versatility of the version produced makes it easy to deal with changes and extensions to simulation model in order to adapt it for specialized studies.

## Results

There are certain types of simulation experiments often used in conducting research with IbMs [2, 11, 12]. Simulation experiments such as uncertainty and sensitivity analyses require multiple model runs, including (a) "replicates", which only vary the pseudorandom number generator seed, and (b) "scenarios" varying inputs such as parameter values. These two kinds of experiments have been designed from the INDISIM-YEAST's website.

5Random variables are used in setting individual properties and rules. This is a way to reproduce the diversity of real cultures and it is also helpful to cover parts of the biological processes non-explicitly introduced in the model. The sequences of random numbers used by the simulator are determined by a first number, the seed. The *Demo* option uses a different seed, a random seed, every time so each time the simulator is executed different realizations of the virtual system appear, which means "replicates" can be immediately achieved. In this way, every execution of the program makes use of a new series of random numbers following certain probability distributions.

The *Log in* option to allow execution of the simulator is designed to deal with the second kind of experiment. In this study, the various "scenarios" for working with this website are limited to changes of some values of the system's initial configuration and some individual model parameters. The values of the constants of both uptake and metabolism models of a yeast cell can be modified, $K_1$ for the bud scars affecting the cellular membrane, $K_2$ for glucose inhibition and $K_3$ for ethanol inhibition. The possibility to change the initial number of glucose particles and ethanol particles at the beginning of the simulation allows the user to configure different cultures and examine various types of evolution with the same initial population. The growth of the yeast cells is determined by the initial conditions of the medium and by its eventual composition which is the consequence of yeast activity. The number of time steps used in the representation of the simulated evolution is another available parameter to be fixed by users. This allows them to show the degree of detail for the scale of representations of temporal evolutions, and also to obtain histograms and bar charts of the distributions of masses and genealogical ages, respectively, for different time steps of the population's evolution.

Diverse simulations can be carried out using the same set of individual yeast model parameters and different initial amounts of glucose or ethanol particles distributed in the domain, or vice versa. For each of these simulations, batch growth curves representing the viable populations of cells against time can be obtained, and these comprises characteristic phases: lag phase, exponential growth, linear phase, metabolic slow down, and the final stage that signals a total stop in the activity of cells. Figure 5 shows an example of one of these temporal evolutions, where distributions of masses and genealogical ages can be obtained at different time steps in order to relate the growth of the population with its structure. The composition of the population, individual characteristics of yeast cells that configure the set of cells, changes according to growth stage of the culture [14].

## Discussion

The IbM methodology can offer an explanation of microbiological phenomena, and the terms used mean something specific about the microbiology of the yeast system. This tactic attempts to offer a theory as to how the abiotic and biotic components of the system work together to produce a given outcome. Sugars are translocated into yeast cells by mechanisms controlled at the plasma membrane and which depend on the sugar, yeast species and growth conditions. Understanding the regulation of how yeasts acquire sugar and other nutrient solutes is important in biotechnology. The control of growth of yeast cell populations in liquid culture is crucial to the performance of industrial processes that exploit yeasts, and this growth is strongly influenced by physical, chemical and biological factors in the growth environment. Thus, understanding the basis of stress effects and the adaptative responses of the individual cells is very important [14] and the use of IbM can contribute to this understanding.

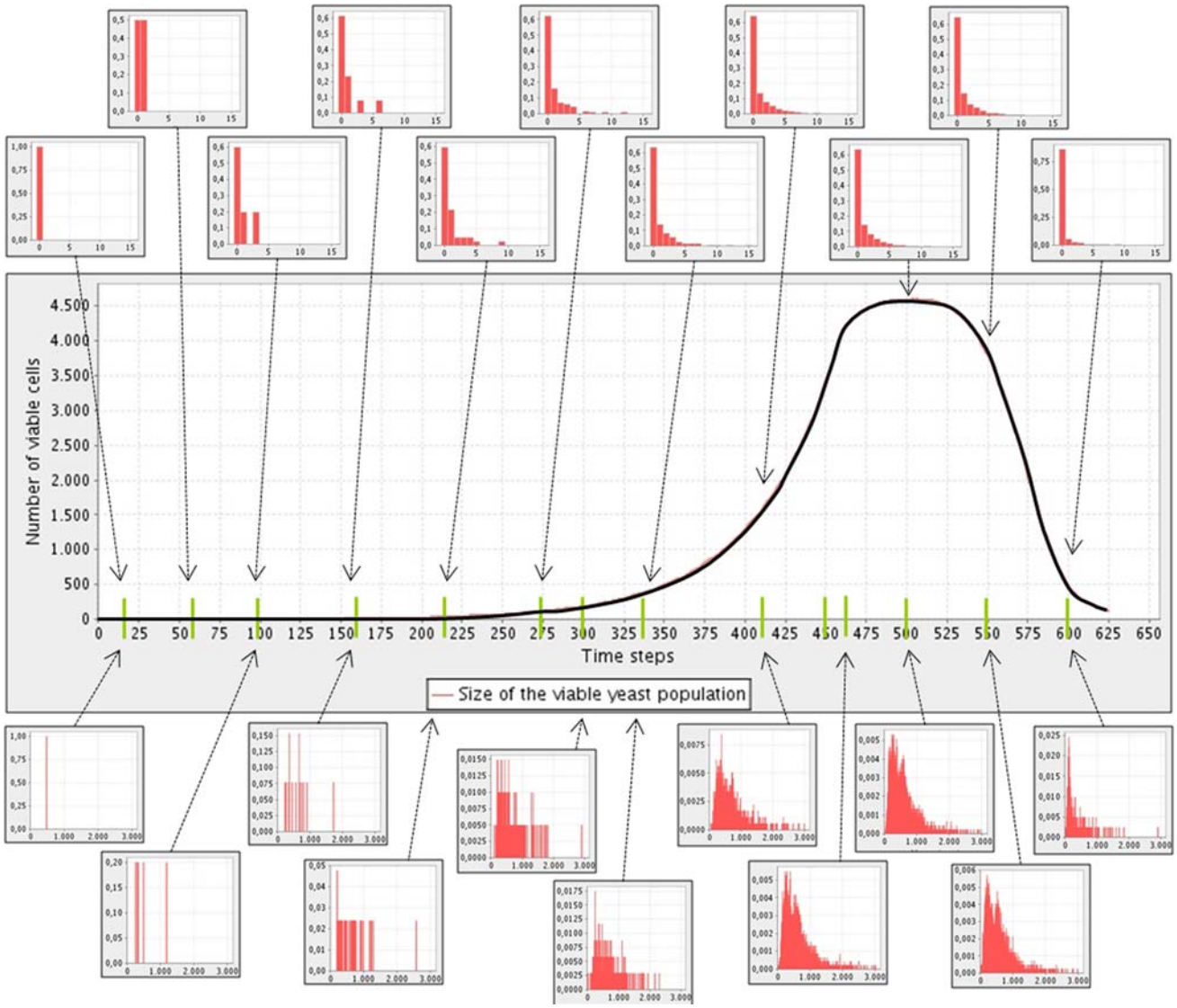


**Fig. 5** Temporal evolution of the number of yeast cells of the simulated population that evolves from one daughter yeast cell at the initial stage. For different time steps of this evolution, the *upper position* shows *bar charts* of the genealogical ages and the *lower position* shows *histograms* for the distributions of masses, corresponding to the set of yeast cells that configure the population

For instance, during industrial yeast fermentations, individual cells may be subject to diverse stresses, which may impair normal growth and metabolism. Ethyl alcohol is a major metabolic product of yeast fermentation. It is known that chemical stress may arise from compounds pre-existing in the growth environment or from toxic metabolites produced by the yeasts themselves. For example, when ethanol accumulates during fermentation, it acts as a potent inhibitor towards yeast cells. Increasing concentrations of ethanol will be initially inhibitory and later lethal to yeast. Ethanol-induced toxicity and ethanol tolerance in yeast has been widely studied [15, 16]. At the same time, it has been reported in several studies that high sugar concentrations also exercise inhibitory effects on the growth of yeast [17, 18]. The use of this INDISIM-YEAST enables comparison of the evolutions of yeast batch cultures from different initial medium conditions. The influence of the initial substrate and ethanol concentration on these yeast population evolutions, as well as the influence of some stoichiometric and kinetic parameters can be visualized from both the global properties of the population (state variables of the system) and the structure or composition of this population (microscopic variables).

INDISIM-YEAST provides insights into the global properties of the yeast batch culture from assumptions made on the properties of the individual cell. At the same time, the modular structure of the model allows exploration of different features in the system under study. The implementation of this microbial IbM has been performed efficiently with a structured programming language, and

the flexibility of the version produced makes it possible to deal with modifications and extensions to simulation models according to particular objectives.

## Conclusions

INDISIM-YEAST can be viewed as a tool to gain a deeper understanding of the yeast microbiological processes that may be explored. If simulationists want to discover functional dependencies in a yeast system, then they must also run a barrage of trials, examining the results across a wide range of parameters. The modeller has complete control of the virtual yeast system. Thus, the advantage that simulation gives to scientific exploration is that the yeast model system is highly manageable. If you have a good representation of a yeast culture, then you have created a world over which you have complete control. Simulation can be visualized as another yeast experimental system with which to explore theories about how the real yeast system works, using an artificial world with virtual yeast cells that we can control. The application presented in this website is simple and intuitive, and results in a very versatile program that can be used to execute controlled virtual experiments. The graphical outputs of the INDISIM-YEAST's website allow the user to examine possible consequences of any decisions made with the input of values for the parameters on the virtual yeast system.

A simulation model can be used as is a new kind of experimental system. The same sorts of experiments and manipulation that may be done in a real system can also be done by computer, the difference being that these experimental systems can be manipulated with ease, whereas real systems cannot. The simulator offers the possibility of dealing with different culture system methods used to study yeast cell populations: classical static batch fermenter, incremental nutrient feed to batch fermenter, and open system to maintain a steady state. These possibilities are interesting cultivation strategies in yeast biotechnology, and the simulator can allow a profound study of the dynamics and structures of these yeast populations.

An important idea is to convince the reader that IbM and yeast populations can be used to set up and simulate sophisticated, applicable models useful for microbiologists, and that INDISIM-YEAST and its website are a tangible opportunity for work in this direction.

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