INDISIM-YEAST, an individual-based model to study yeast population in batch cultures

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INDISIM-YEAST, an individual-based simulator, models the evolution of a yeast population by setting up rules of behaviour for each individual cell according to their own biological rules and characteristics. It takes into account the uptake, metabolism, budding reproduction and viability of the yeast cells, over a period of time in the bulk of a liquid medium, occupying a three dimensional closed spatial grid with two kinds of particles (glucose and ethanol). Each microorganism is characterized by its biomass, genealogical age, states in the budding cellular reproduction cycle and position in the space among others. Simulations are carried out for population properties (global properties), as well as for those properties that pertain to individual yeast cells (microscopic properties). The results of the simulations are in good qualitative agreement with established experimental trends.

Keywords Individual-based Modelling; Yeast batch culture

1. Introduction

This work is concerned with the first application of an individual-based model (IbM), INDISIM [1], to study the observed macroscopic and microscopic behaviour of yeast populations. We specifically use INDISIM to model the biological and metabolic activity of *Saccharomyces cerevisiae* under batch conditions. In *S. cerevisiae* budding leads to the formation of bud scars and cells divide unequally. The daughter cell, the cell produced at division by the bud of the previous cycle, is smaller and has a longer cycle than the parent cell, which produced it. It is important to note that mean cell size of *S. cerevisiae* also increase with cell age [2]. There are a number of models put forward to study different aspects of the cellular cycle and the behaviour in *S. cerevisiae*. Some of them attempt to fit experimental data through a chosen parameterised statistical distribution or statistical approach [3]. Other models are based on differential equations, namely continuous approaches [2]; these may be classified into structured and non-structured models [4, 5]. The latter ignore the differences present between the yeast cells in the culture and analyse these cultures by using variables that define the macroscopic environment in the yeast growth process. Recent approaches to non-structured models are based on neural networks [6, 7]. The minimal level that has independent existence and can be considered to be alive is the cell. The goal of developing realistic "in silico" models of cells is one of the recent research themes, and the eukaryote *S. cerevisiae* is one of the most intensely studied of all organisms [8]. Whole-cell modelling, which was thought intractable until recently, has suddenly become realistic [9]. The collection of tools for computational approaches to cell biology is growing rapidly. Some of the relevant software tools applied to cell biology are, for instance, Virtual Cell, E-CELL, BioSpice, StochSim, Mcell [10], and simulators for biochemical metabolic pathways like GEPASI which has been recently applied for studies of the kinetics in yeast [11, 12]. Most of the microbial population models in use are of the type Grimm refers to

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as “top down” models. The last decade has witnessed the emergence of Individual-based Models (IbM), referred to by Grimm as “bottom up” approaches [13]. IbM stipulates that populations of organisms are modelled in terms of discrete individuals that are unique only in terms of their individual properties. The characteristics of each of these individuals are tracked through space and time. The aim of the IbM approach is the derivation of the properties of biological populations from the properties of the individuals that make up these systems. Moreover, the whole-cell models integrate subcellular processes into a single cell model, and this differs from the microbial IbMs, whose purpose, while losing details of the single cell processes, is the integration of cellular processes into microbial population models [14]. All IbMs acknowledge naturally: (i) the importance of local spatio-temporal interactions between individual organisms, and (ii) that individuals within a population are different. The IbMs study bacterial populations describing bacterial properties including substrate uptake, metabolism, maintenance, cell division and death at the individual cell level. To our knowledge most of the bacterial IbMs have evolved from ecological models. For instance BacSim [14] is an extension of Gecko [15], an ecosystem dynamics model that uses the Swarm toolkit [16]. On the other hand INDISIM, the bacterial IbM we have developed [1], is an extension of another ecological model, the Barcelonagrama [17]. So far BacSim has been applied to the study of biofilms [18]. INDISIM has already been used to study of bacterial growth in yoghurt and on agar plates [19, 20]. INDISIM is a stochastic model, discrete in space and time that simulates the behaviour of microbial populations such that the global properties of the system emerge from the rule-following behaviour of individual organisms. The state of each microorganism is determined by a set of random, time-dependent variables related to spatial location, biomass, cellular cycle and other individual properties. INDISIM has been developed with Compaq Visual Fortran Professional Edition 6.1. The impact of programming paradigms on the efficiency of an individual-based simulation model has been studied by Barnes and Hopkins [21]. The implementation of this microbial IbM has been made efficiently with an structured programming language, and the flexibility of the Fortran version produced allows to deal with changes and extensions to simulation model [1, 19, 20, 22, 23]. In this work we have adapted INDISIM to study the main features of the cell cycle in yeast and the evolution of the yeast population over a period of time in the bulk of a liquid medium. The version INDISIM-YEAST will be used to study diverse properties and characteristics of a system constituted by yeast cells.

2. The simulator

There is an extensive bibliography on the biology of *S. cerevisiae*. Here we have made use of, and benefited from, the books by Walker [2] and Berry [24], the review articles by Kreger-van Rij [25] and Hartwell [26]; and the diverse papers [2, 27-33] related to the cell cycle, nutrition, and metabolism in order to formulate the IbM of INDISIM-YEAST. We will discuss those features of the simulator that are specific to yeast growth. These pertain mainly to the inhibition, reproduction processes and lysis of the yeast cells. The generic aspects of INDISIM are discussed in the work of Ginovart and co-authors [1] to which readers is referred.

2.1. Modelling the spatial cells and the abiotic components of the medium

We assume the yeast population grows in the bulk of a liquid medium where we consider variables that are space and time dependent. These variables control the amount of abiotic components, and are identified as glucose (the nutrient particles) and ethanol (the metabolites or end product particles) arising from the yeast cellular activity and excreted to the environment. The time evolution of the population is divided into equal intervals associated to computer steps or time steps. The spatial domain where the system evolves is a three-dimensional grid made up of cubic cells, the spatial cells. Each is identified by cartesian coordinates. In batch conditions the medium is not altered by further nutrient addition or removal. The environment is continuously changing because glucose particles are consumed and ethanol is accumulating in the medium. The grid is defined at each time step by $G(t) = \{S_{xyz}[s_1(t),s_2(t)]\}_{x,y,z=1,\ldots,Q}$ where Q denotes the size of the spatial domain of the grid; S_{xyz} each of the spatial cells defined within

this domain; and $s_1(t)$, $s_2(t)$ the number of glucose and ethanol particles respectively in each cell and at each time step.

2.2. Modelling a single yeast cell

At each time step, a single organism, an individual yeast cell I_i, is defined by a set of time-dependent variables, which describes and controls its individual properties. For each microorganism INDISIM-YEAST implements a set of rules for the following actions: motion, uptake and metabolism of nutrient particles, reproduction and viability, described below. The set of I_i conforms the yeast population with $N=N(t)$ individuals defined at each time step by $P(t) = \{I_i[v_1(t), v_2(t),..., v_{10}(t)]\}_{i=1,...,N}$ where, for each yeast cell I_i, $v_1(t)$, $v_2(t)$ and $v_3(t)$, identify its position in the spatial domain; $v_4(t)$, its biomass; $v_5(t)$, its genealogical age as a number of bud scars on the cellular membrane; $v_6(t)$, the reproduction phase in the cellular cycle where it is, namely the unbudded or budding phase; $v_7(t)$, its "start mass", the mass to change from the unbudded to budding phase; $v_8(t)$, the minimum growth of its biomass for the budding phase; $v_9(t)$, the minimum time required to complete the budding phase; $v_{10}(t)$, its survival time without satisfying its metabolic requirements. Random variables and/or random numbers are used to characterize the yeast cell and the individual actions to update the set of rules. During the course of this work we denote these variables by adding to the mean values the values z_j, which are random draws from a normal distribution with mean equal to 0 and standard deviation σ_i, $Z(0, \sigma_i)$. The value σ_i allows introducing variability in the individual behaviour. The choice of normal distribution is arbitrary, although it is widely used in biology and it is easy to implement.

The initial configuration of the yeast cells is chosen randomly from all the spatial cells in the domain. Once this is done we change the position of each yeast cell randomly at each time step, within a distance which, in the present simulations, has to be an adjacent spatial cell. Each yeast cell may take up nutrient particles (glucose) from the medium and be capable of metabolising them. We assume that the uptake of nutrient particles satisfies Blackman kinetics [34]; namely, as the concentration of the nutrient particles increases, the number of nutrient particles entering the yeast cell increases linearly and the individual uptake rate also increases linearly until saturation [1]. The number of nutrient particles a cell metabolises is assumed to be, at each time step, proportional to the cell's surface and the number of nutrient particles within a specific range around it. We also assume that the cell surface is proportional to $v_4(t)^{1.1}$ [32, 35]. The following parameters are introduced to determine for each yeast cell the uptake of nutrient particles: U_{max}, is the maximum number of nutrient particles that may be consumed per unit time and per unit of cellular surface; D_{max} defines the number of sites or spatial cells that may be reached by the microorganism for nutrient particles uptake; k is a given percentage, per unit of cellular surface, of the amount of nutrient particles that the yeast cell will actually translocate within the given range (to take into account the probability of nutrient particles translocating into the cell through the cellular membrane). At each time step and within the range D_{max} , the number of nutrient particles a cell may absorb is given by $U_1(t) = (U_{max} + z_1)\, v_4(t)^{1.1}$. The uptake of nutrients is assumed to be limited by the following factors: (i) the genealogical age of the cell, say $v_5(t)$, is defined by the number of bud scars on the cell's surface [33]; (ii) the number $s_1(t)$ of nutrient particles per spatial cell or local glucose concentration [36]; (iii) the number $s_2(t)$ of final product particles per spatial cell, or local ethanol concentration [37]. Whence the maximum number of nutrient particles that one yeast cell may absorb at time step t, is assumed to be $U = U(t) = U_1(t)\, [1\text{-}K_1\, v_5(t) – (K_2 + z_2)\, s_1(t) - (K_3 + z_3)\, s_2(t)]$ where K_1, K_2 are K_3 constants. In order to model the metabolisation of translocated glucose in a yeast cell, we introduce the following parameters for each yeast and glucose particle: I is a prescribed amount of translocated glucose per unit of biomass that a yeast cell needs to remain viable; Y is a constant modelling the metabolic efficiency that accounts for the synthesised biomass units per metabolised glucose particle; K is an enthalpic constant that accounts for the units of energy dissipated (heat dissipation) per metabolised glucose particle; E is a residual constant that accounts for the amount of residual product (ethanol) per unit of metabolised glucose particle. Using the above parameters, and recalling the meaning of U, we set the following control rules: i) a maintenance energy for the viability of a yeast cell, $I\, v_4(t)$; ii) a control

relation to check whether the glucose particles absorbed by a yeast cell are enough for its maintenance, $(U - I\ v_4(t)) \geq 0$; iii) if $(U - I\ v_4(t)) < 0$, evaluate the possibility that the cell remains viable without requiring external energy supply. The longest time that the cell remains viable until the onset of its death or lysis is defined by the value t_H; iv) if the viability of the yeast cell is achieved, allow for the increase of its mass from $v_4(t)$ to $v_4(t)+\Delta m$, where $\Delta m = Y\ (U - I\ v_4(t))$; v) allow for heat dissipation Q to take place, such that $Q = K\ U$; vi) allow for the excretion of R particles of residual product, ethanol, into the spatial cell where the given yeast cell is located, such that $R = E\ U$.

The simulator simplifies the yeast cell cycle by assuming that a new cell cycle is allowed to begin only after the preceding cycle is completed and that the model for the cellular cycle involves only two clearly differentiated phases. Phase 1 or unbudded phase ($v_6(t) = 1$) covers most of phase G1 and a very small fraction of phase S in the traditional division of the cell cycle [38]. Phase 2 or budding phase ($v_6(t) = 2$) covers a small fraction of G1, most of S and all of G2 and M. We assume that, in the unbudded phase the yeast cell is getting ready for budding. The changes into the budding phase takes place only if, at the end of Phase 1, the following conditions are satisfied: (i) the cell has attained a minimum stochastic cellular mass m_S, the start mass, related to the constant m_C, the critical mass; (ii) the cell has achieved a minimum growth of its biomass, Δm_{B1}. When Phase 1 begins, a value $v_7(t) = m_S$ is randomly chosen for each cell in the manner described below. m_S is the minimum mass the cell must attain during this first phase for the process of the phase change to take place and its value is also a function of the individual cell properties. We denote by m_{in} the value of the mass of a yeast cell at the beginning of Phase 1. The following checks are made at each time step and for each yeast cell. If $m_{in} \leq m_C - \Delta m_{B1}$ then the start mass assigned to the cell is $m_S = m_C + z_4$;otherwise, if $m_{in} > m_C - \Delta m_{B1}$ then the start mass assigned to the cell is $m_S = m_{in} + \Delta m_{B1} + z_4$. Hence whenever the mass of a yeast cell is bigger than m_S, the cell experiences the transition to Phase 2. Note that, within our model, Phase 1 does not need to be completed in a given time interval. The check in our model is whether an individual cell has reached a start mass, irrespective of its original value and growth rate. The budding phase is the least flexible in the cellular cycle as it requires both temporal and growth checks. Within our model two conditions, involving two parameters, must be satisfied for the initiation of cell division. These are: a) a minimum growth of biomass $v_8(t) = \Delta m_{B2}$; and b) a minimum time interval $v_9(t) = \Delta T_2$. As in Phase 1, we appeal to random variables. A yeast cell will complete its cellular cycle when: (i) it reaches a minimum growth Δm_2 of its biomass, given by $\Delta m_2 = \Delta m_{B2} + z_5$ and (ii) it has remained in Phase 2 for a minimum time interval given by $\Delta t = \Delta T_2 + z_6$. The first condition is necessary because a yeast cell must have a minimum number of molecules and satisfy minimum structural requirements in order to function as an independent entity. In a culture starved of glucose, or subjected to other inhibitory effects, the growth rate will be slower. On the other hand, the growth rate, even under optimal growth conditions, has to be completed within a minimum time interval; this is what the second condition requires. The budding phase is completed with the cell division, a daughter cell and a parent cell, with a total combined mass $m_T = m_1 + \Delta m_2$. The mass of the daughter cell is given by $m_D = q\ \Delta m_2 + z_7$, where q denotes a percentatge that ensures both that the parent cell will experience growth during this Phase and that the daughter cell is a fraction of Δm_2. The mass of the parent cell is given by $m_P = m_T - m_D$. We note that the reproduction rules in INDISIM-YEAST are implemented every time a new yeast cell appears. Hence different yeast cells in the culture need not have the same mass when the reproduction process begins. Moreover, the yeast cells involved in the reproduction process remain active. The local environmental conditions, in turn, indirectly affect the overall yeast growth rate. The preceding rules are intended for viable yeast cells. We introduce now the rules for when the cells are no longer viable. Whenever a cell does not find enough nutrient particles to satisfy its metabolic requirements, the simulator assigns to this cell an index $v_{10}(t) = t_M$, the mortality index, satisfying the following rules: i) if, after one time step, the cell is still unable to satisfy its metabolic requirements, the index increases by one, otherwise the index is reduced by one; ii) the simulator checks whether t_M exceeds the time $t_L = t_H + z_8$, where t_H denotes an average time interval beyond which the cell cannot survive. Thus, if $t_M > t_L$, the cell is no longer viable. As it stands, the model ignores lysis due to causes external to the yeast culture. In our model the individual cells are no longer viable, either directly or indirectly, for the following reasons: ethanol excess; low glucose concentration; diminishing surface to volume ratio; and genealogical age. All of these

reduce the ability of the yeast to use nutrients in order to achieve enough maintenance energy and increase biomass. The magnitude of these unfavourable conditions will determine the vitality and viability of the individual yeast cell.

3. Results

The simulations are carried out in a three dimensional grid containing 15^3 cubic spatial cells. The simulator assumes that: i) glucose fermentation takes place in a closed environment, namely there is no entry or exit of yeast cells or nutrient and end particles (batch culture); ii) there is an initial amount of 7,726 glucose particles in each spatial cell of the grid, namely a total number of 2.6×10^7 glucose particles, distributed uniformly over the spatial cells. Moreover, by the end of the simulated process, the model requires that there are approximately 10% of the initial concentration of glucose particles left as non-fermented residual particles; namely we assume that the fermentation process has not used up all the glucose particles. In all the work discussed below we use dimensionless units. We assume that units of length are given in terms of the length of a spatial cell, units of time in terms of program steps, and units of mass in terms of the critical mass of reproduction. Actually, one unit of simulated mass, usm, has been chosen to be 0.2% of the value of the critical mass. It is possible to scale our units to physical units, but it is not necessary for the purposes of this work. Consequently, comparison with experimental data is only qualitative at the present level. Nevertheless the following order of magnitude argument suggests that our results do relate to observed values. Choosing a typical yeast growth curve, for instance that shown in figure 4.27 of Walker [2], we find that the duration of the experimental lag phase shown there equals 2.55 h; the value of the maximum specific growth, μ_{max}, equals 0.26 h^{-1} with a doubling time of the population $t_D = \ln 2/\mu_{max} = 2.62$ h. The ratio of these two time periods, lag phase and doubling time, gives 0.97. In our case the corresponding times are 40.0 and 51.3 time steps, with a ratio 0.78. Although different, the experimental and simulated values are of the same order of magnitude. The input data required to implement our simulator, in the dimensionless units referred to above, are as follows: $U_{max} = 0.038$; $D_{max} = 1$; $k = 3\ 10^{-6}$; $K_1 = 0.03$; $K_2 = 2\ 10^{-5}$; $K_3 = 6.5\ 10^{-5}$; $I = 1.46\ 10^{-3}$; $Y = 0.333$; $K = 0.15$; $E = 0.23$, $t_H = 20$; $m_C = 500$; $\Delta m_{B1} = 80$; $\Delta m_{B2} = 400$; $\Delta T_2 = 10$; and $q = 0.8$. The standard deviations of the normal probability distributions introduced in the preceding section are: $\sigma_1 = 9.5\ 10^{-3}$, $\sigma_2 = 10^{-5}$, $\sigma_3 = 3.25\ 10^{-4}$, $\sigma_4 = 25$, $\sigma_5 = 100$, $\sigma_6 = 5$, $\sigma_7 = 0.2$, $\sigma_8 = 5$.

Global properties involve population properties parameters, like the change in concentrations of glucose, of ethanol, and of the biomass. Figure 1 presents the simulation results of these global properties over the same time span. The trends exhibited in these figures reproduce the experimental trends reported by Moresi [39] and by Lafon-Lafourade and co-authors [40], which do not cover oxidative ethanol consumption. The shapes of Figure 1 suggest we may identify five stages in the temporal evolution of the yeast culture: (1) The Lag Phase spans, approximately, the initial 40 time steps, and represents a period of zero growth. In this phase the innoculum cells adapt to their new environment. (2) The Exponential Growth covers the next 360 time steps. This phase represents a period of maximum specific growth rate. The simulations suggest growth without any important limitations. However, depending on the initial glucose concentration, growth inhibition may already be present due to excess of nutrient [36]. (3) The Linear Phase lasts from, approximately, the 400th to the 600th time step. During this phase yeast growth experiences increasing limitations. These are mainly due to the progressive increase of ethanol in the medium. Nonetheless the global metabolism of the system is still at work and the culture continues to grow. (4) The Metabolic Slow Down spans the next 400 time steps. The limiting factors that started to show up in the preceding phase act now in a dramatic fashion. Ethanol concentration is becoming so high that prevent cells their normal nutrient uptake. Under these conditions cells loose vitality and some are no longer viable. We see a metabolic slowdown due to nutrient depletion and an increase in cell mortality. (5) The Final Stage covers the period starting from, approximately, the 1000th time step. This phase signals a total stop in the activity of the culture. There are still a few viable cells left, but these continue to metabolise nutrients mainly to preserve their biomass and reproduction becomes very difficult. We note the fall in viable biomass during this period, first linearly, becoming exponential during the final stages of evolution. Flow cytometric light scattering experiments are capable to probe the properties of individual

yeast cells [31, 41]. In our simulations we are concerned with both time evolving and population parameters, some of which will become directly comparable with experiment when we overcome the question of scaling to real times and energies. This simulation allow to study the following time evolving microscopic parameters: (a) the mean mass of the cell population, defined as the viable biomass in the culture divided by the number of viable cells; (b) the average nutrient uptake, namely the number of metabolised nutrient particles during one time step divided by the number of viable cells; (c) the global maintenance energy of the culture, defined as the number of metabolised nutrient particles not used in the production of new biomass; and (d) the maintenance energy per unit of viable biomass and per viable cell (data not shown).

The simulator saves information about every cell at each time step so it is also possible to deal with distributions of variables controlled at individual level. These are mainly related to the cellular cycle and reflect the state of the yeast population at given times in the fermentation process. Figure 2 presents the distribution of masses resulting from our simulations corresponding to different periods of development in the fermentation process. The histograms correspond to the exponential growth phase show distributions of masses bell-shaped with a positive slant, namely with a longer tail for larger masses. The histograms of steps 500 and 600 correspond to the mass distributions during the linear phase when we may assume that the culture is almost at the peak of its metabolic activity. These distributions remain bell-shaped. However, the magnitudes of the most frequent masses shift towards smaller values as the metabolic slow down phase is approached. Growth inhibition, due to an increasing concentration of ethanol, reduces the value of the most probable mass. Our results are in qualitative agreement with the distributions found by Johnston and co-authors in *S. Cerevisiae* [28]. During the last stages of evolution a bimodal distribution appears. This leads to a progressive splitting up of two sets of yeast cells: those pertaining to daughter cells, with smaller masses; and those pertaining to parent cells with larger masses that reach reasonable values for cells with small genealogical ages, and an increasingly narrow distribution. The latter implies less variability, and is a feature that points to the relatively small number of cells that reach the end of the simulated evolution process. Figure 3 shows the distributions of genealogical ages for the simulated yeast population. Budding produces two non symmetrical cells and the duration of the cellular cycle for daughter cells (age 0) is, on average, longer than that of parent cells (age $\geq$ 1). This results in an asymmetrical population and complex distributions of genealogical ages which are affected by the intrinsic variation in the duration of the cellular cycle of each cell that also depends on the conditions of the culture. Our simulations are comparable to the results obtained using continuous culture models [29]. The last bar charts corresponding to the metabolic slowdown and final phase, show that under conditions of ethanol inhibition both the number of daughter and parent cells experience important changes.

From the simulated yeast population is also possible to obtain: i) durations of the unbudded and the budding phases as a function of the genealogical ages of the yeast cells at different time steps, ii) temporal evolutions of the mean durations of these two cellular phases for parent and daughter cells, iii) final masses at the end of the unbudded phase as a function of the genealogical ages of the yeast cells at different time steps, and iv) the same for parent and daughter yeast cells at the end of the budding phase. These kinds of simulated results are not shown in this manuscript, but they are consistent with observations inferred from experiments [32] and in agreement with experimental results [35].

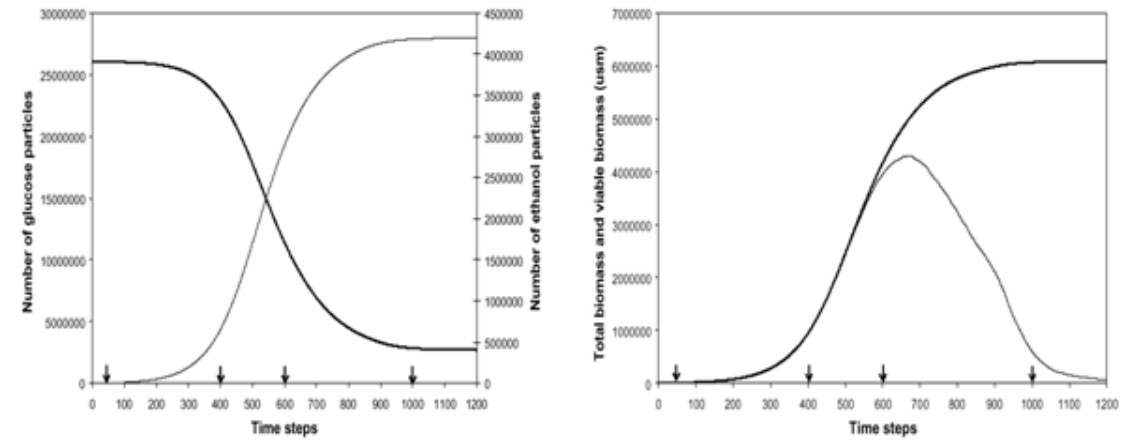

Fig. 1 a) Time dependence of nutrient (number of glucose particles) and metabolites (number of ethanol particles) in the simulated yeast culture. b) Thin line: viable biomass. Thick line: total biomass. The biomass is given in units of simulated mass, usm. Time is given in units of time steps. Different phases of the temporal evolution of the yeast population are shown by arrows.

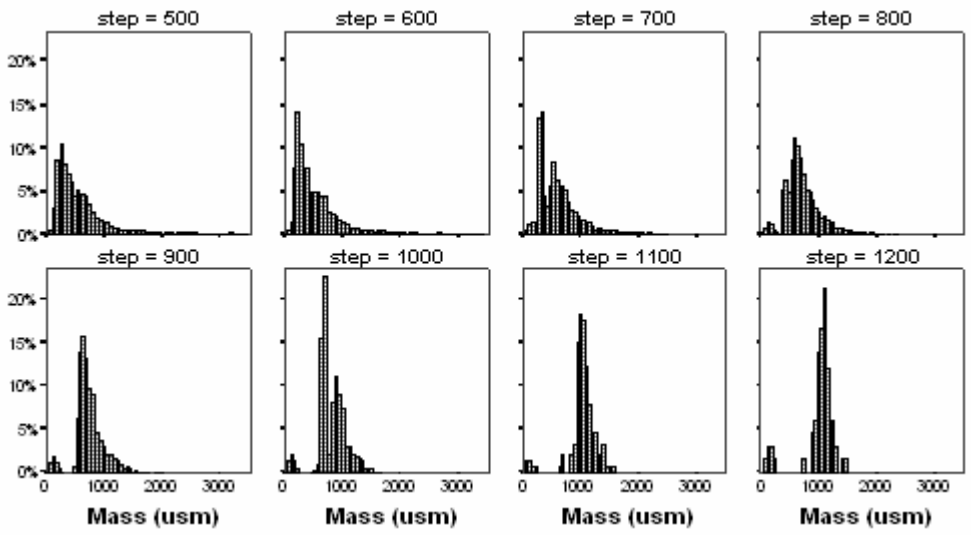

Fig. 2 Histograms of the distributions of masses in the simulated yeast culture at different steps of the simulated evolution. The masses are given in units of the simulated mass, usm.

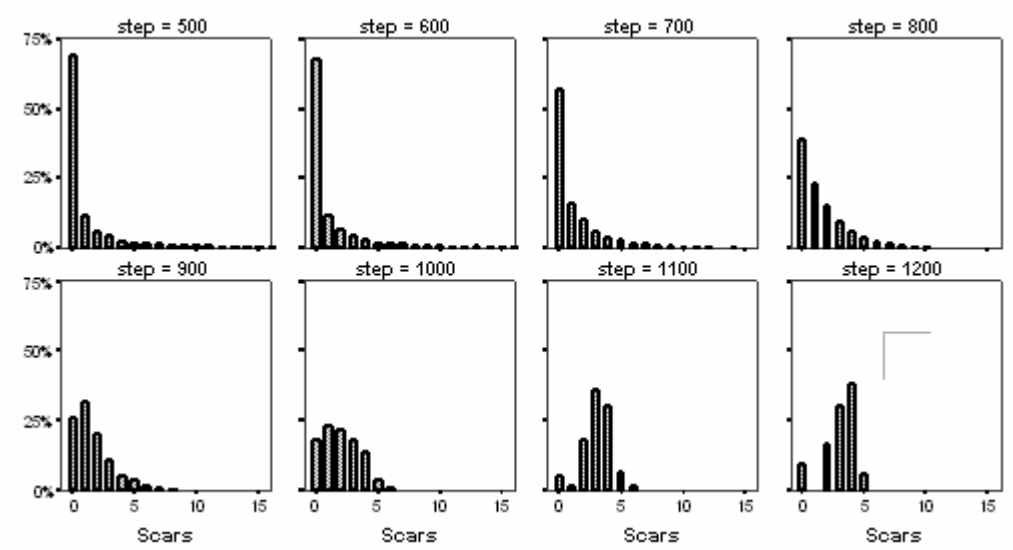

Fig. 3 Histograms of the distributions of genealogical ages of yeast cells in the simulated yeast culture at different steps of the simulated evolution.

5. Discussion and conclusion

In the last years, many researchers have attempted to model yeast fermentation and different approaches have been considered [30]. However, even segregated and structured models do not consider cell cycle dependent, physiological properties of different cells as model variables. A direct consequence is that these kinds of models are only valuable to describe and predict cultures with homogeneous and constant population composition rather than transient behaviour [31]. To our knowledge this is the first work that models the processes involved in yeast population growth using IbM simulations. We have adapted INDISIM to model the growth and behaviour of *S. cerevisiae* under batch conditions. The simulator INDISIM-YEAST models the spatio-temporal evolution and distributions of parameters of the yeast culture by setting rules of behaviour for each cell, such as its uptake, metabolism, budding reproduction and viability. Practicable possibilities of this yeast IbM with some simulated results have been presented. An important advantage of the modular structure of the simulator is that it allows to explore, or modify, different features of the system under study, as well as to examine diverse experimental trends. Various simulation results of INDISIM-YEAST, and other versions of INDISIM, are shown in our web page http://mie.esab.upc.es/mosimbio/english/english.htm. A version of INDISIM was used to examine the predictions of two different models of yeast flocculation [23]. The distinctive contribution of INDISIM-YEAST lies in its ability to deal with each yeast cell, and to investigate mechanisms at individual level, interacting with the medium, to associate these mechanisms with the phenomena in question. One of the outstanding problems, and a recurrent theme with all IbMs, is how to deal with the scaling of time and energy. We do not have ready made answers to this yeast system problem, but hope to overcome at least some of the difficulties which prevent us to present direct quantitative comparison with experiment. Some progress is done in this direction. INDISIM-SOM was developed to study microbial activity in Soil Organic Matter, and its calibration made use of data from laboratory incubation experiments [22].

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