The use of flow cytometry and particle size analysis in the individual-based model INDISIM-YEAST, a simulator of yeast populations

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INTRODUCTION

The sizes and growth rates of individual cells, for instance in *Saccharomyces cerevisiae* cultures, are highly heterogeneous, mainly due to the asymmetric cell division of budding yeast and to ageing.

Flow cytometry and particle size analysis by electric counters are valuable and advanced tools that combine direct and rapid assays to determine numbers, cell size distributions and even biochemical and physiological characteristics of individual cells (Vives-Rego et al., 2000). Experimental data are obtained by flow cytometry and particle analyzer of the temporal evolutions of the *S. cerevisiae* populations in two batch cultures, one in active oxygenation and the other in strict anaerobic conditions.

The growth of *Saccharomyces sp.* under batch conditions was modelled using INDISIM-YEAST, an adapted version of the individual-based simulator INDISIM (Ginovart et al., 2002, 2007).

The analysis and interpretation of this kind of experimental data, and the preliminary simulated results corresponding to the stage after the exponential growth of the aerobic culture, from the slow down metabolic period to the stationary phase, will contribute to the development of INDISIM-YEAST.

The combination of flow cytometry, particle analysis and an individual-based model in our hands is established as an opportunity to deal with the study of yeast population dynamics.

Individual-based Models (IbM) or "agent-based" models are a bottom-up approach which starts with the 'parts' of a system and then tries to understand how the system's properties emerge from the interaction among these'parts' (Grimm and Railsback, 2005)).

Four criteria that distinguish what we consider IbM
(1) The degree to which the complexity of the individual's life cycle is reflected in the model
(2) The extent to which variability among individuals of the same age, size or stage is considered
(3) Whether or not the spatial and temporal dynamics of resources used by individuals are explicitly represented
(4) Whether real or integer number are used to represent the size of a population (IbM are built using the mathematics of discrete events)

MATERIALS AND METHODS

INDISIM (INDividual DIScrete SIMulations)
a model that stands on individual-based methodology to study microbial systems.

INDISIM-YEAST, an adaptation from INDISIM to study yeast populations in batch cultures.

YEAST POPULATION MODEL

➢ The set of $N(t)$ yeast cells conforms the population, defined by

$$P(t) = \{Y_i(v_1(t), v_2(t), \ldots, v_{10}(t)\}_{i=1,2,\ldots,N(t)}$$

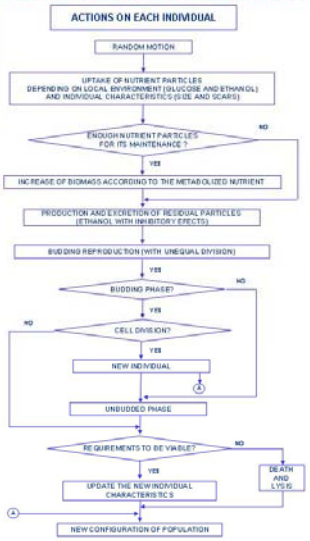
Y_i is a yeast cell with the following individual characteristics:

- v_1 : cell biomass
- v_2 : genealogical age as a number of bud scars on the cellular membrane
- (v_3, v_4, v_5) : position in the spatial domain
- v_6 : the reproduction phase in the cellular cycle, namely the unbudded or budding phase
- v_7 : "start mass", i.e., mass required to change from unbudded to budding phase
- v_8: minimum bud biomass to complete budding reproduction
- v_9: minimum time required to complete the budding phase
- v_{10}: survival time without satisfying the metabolic requirements

➢ The set of Q^3 spatial cubic cells configures the grid, defined by

$$G(t)=\{S_{xyz}[s_1(t), s_2(t)]\}_{x,y,z=1,\ldots,Q}$$

S_{xyz} is a spatial cell, being $s_1(t)$ and $s_2(t)$ the number of glucose and ethanol particles respectively.



Experimental assays

S. Cerevisiae were examinned in two culture conditions in order to study the final exponential growth and entrance to the stationary phase. One using active oxygenation, achieved by magnetic shaking (300 rpm). The other using static screw capped tubes with the medium supplemented with sodium thioglycolate and resazurine. Cultures were incubated at 27ºC and after 20 h of incubation the samples were analyzed by flow cytometer and electronic particle analyzer.

Flow cytometric experiments were carried out using a Beckman coulter FC citomics 500 MPL flow cytometer (ref.: 20061121, Coulter Corporation, Miami, Florida). A standard 488nm air-cooled argon-ion laser at 15mW power was used. The forward scatter (FS) and side scatter (SS) distributions were obtained for each measurement.

Number of cells and cell size distribution were determined with a Multisizer II electronic particle analyser.

Model of the budding reproduction at individual yeast cell level

The cellular cycle model involves two differentiated phases, the unbudded phase and the budding phase (Hartwell and Unger, 1977).

The reproduction rules are implemented with the use of random variables every time that a new yeast cell appears. Hence, different yeast cells do not have the same mass when the reproduction process starts, and the daughters can have different masses at the end of the process.

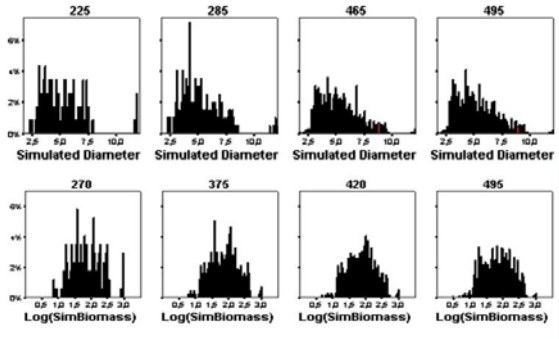
Different subpopulations are in the yeast system: daughter cells without bud scars; single cells with one or more bud scars; and double cells with one or more bud scars.

RESULTS AND DISCUSSION

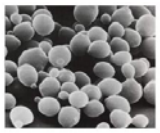
Changes in the population distribution during the entrance into the steady state in our cultures can be explained in the light of these observations (Munch et al., 1992).

These preliminary results for the simulated aerobic culture are in qualitative agreement with the dynamics found by experimental work.

The metabolic model for the anaerobic growth must be revised in the light of these results.

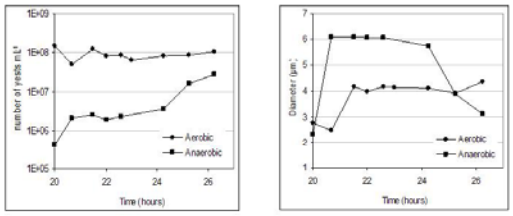


Distributions of the diameters and logarithm of the biomasses of the simulated yeast in aerobic culture at certain time steps of the evolution after the exponential growth.

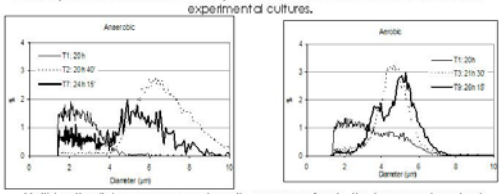


FS is normally assumed to be proportional to yeast size although the relationship between particle size and FS is not monotonic, as it is also affected by cell structure and chemical composition.

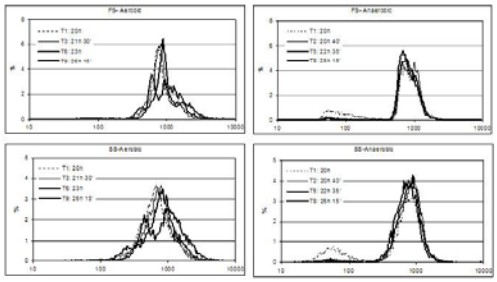
Interpretation of the flow cytometric SS values is complex, due to the fact that these values are dependent on cell granularity, which in turn could be associated with the metabolic activity.



Time dependence of the number of yeast cells and their mean diameter in the two experimental cultures.



Multisizer II cell-size measurement results corresponding to the two experiments at certain times of the stage after exponential growth.



FS and SS results corresponding to the two experiments at certain times of the stage after exponential growth.

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