# INDISIM-YEAST, a simulator for individual-based modelling of yeast metabolism and process dynamics in asynchronous batch fermentations

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## INTRODUCTION

Batch fermentation profiles and interaction between product formation, substrate consumption and biomass growth rate have been studied extensively through the construction of kinetic models.

Complexity has increased from unstructured models to structured models (Bernaerts et al., 2004).

Recent years have seen the emergence of Individual-based Models.

We present a model of the growth of *Saccharomyces cerevisiae* under fermentative batch conditions using an adapted version of the individual-based simulator INDISIM (Ginovart et al., 2002, 2007), called INDISIM-YEAST. This simulator is used to study fermentative yeast populations in asynchronous batch cultures, and to discuss the role of the individual cell variables in determining the yeast batch fermentation profiles, using media with different initial glucose concentrations.

Four experimental fermentations were carried out with the determinations of glucose, ethanol, total yeast cells, viable cells, and ratios of budded and unbudded cells.

Experimental and simulated results are compared and discussed in order to identify the role of the main factors in INDISIM-YEAST and to progress in its parameterisation and calibration.

Individual-based Models (IbM) or "agent-based" models are a bottom-up approach which starts with the 'parts' 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

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

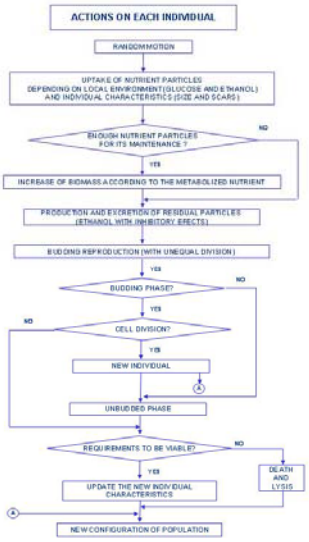
$Y_i$ is a yeast cell with the following individual characteristics:

- $v_1$: 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

Four experiments in triplicate were carried out inoculating about $1 \times 10^4$ CFU $mL^{-1}$, of *S. cerevisie* in flasks with the same medium but with different concentration of glucose (150, 200, 250 or 300 g $L^{-1}$ of glucose) depending of the experiment. The experiment took place along 20 days and the cultures were incubated at 27ºC in aerobic conditions without agitation.

Samples were taken periodically and were performed Total yeast cell counts, viable cells, budded and unbudded cells using a microscopic Neubauer cell counter. Moreover, the number of viable cells was determined by counting the CFU on Sabouraud Dextrose Agar. The plates were incubated at 27ºC for 72 h. Glucose was determined by an enzymatic kit (Boehringer Mannheim). Ethanol was determined by GC (Hewlet Packard 5890 series II) with FID detector.

### Model of the metabolism at individual yeast cell level

The number of substrate particles that a cell may uptake is proportional to the number of nutrient particles within a specific range around it.

We also assume yeast cell as approximately spherical, so the maximum individual uptake is proportional to cell surface, and it is arrested by its genealogical ages, as the bud scars affect the cellular membrane .

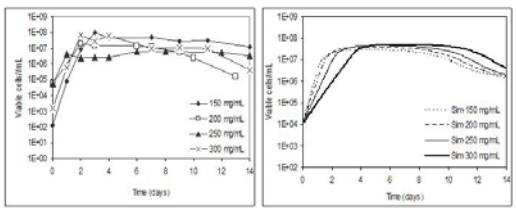
The individual uptake model is assumed to be also limited by the excess of glucose concentration as it may also induce inhibition in this entrance of particles (glucose repression).

We introduce different constants related to the amount of glucose per unit of biomass that a yeast cell needs to remain viable , the metabolic yield that accounts for the biomass production, the residual products (ethanol and carbon dioxide) per unit of metabolised glucose particle among others.

Extra individual energy for viability is assumed to be proportional to ethanol concentration in the medium (ethanol toxicity).

We assume that oxygen and nitrogen are not limiting.

## RESULTS AND DISCUSSION



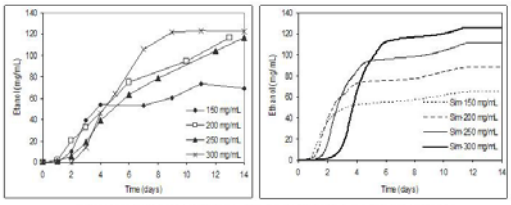

Temporal evolution of the mean of the three replicates of viable cells for the four different initial glucose concentrations of the experiments and simulated results (Sim).

An important advance has been made in the parameterization and calibration of INDISIM-YEAST.

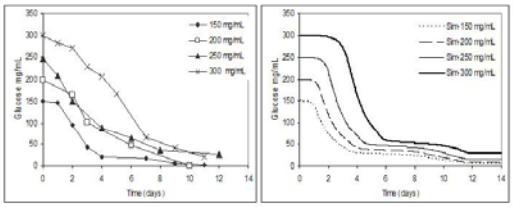
In our model the individual cells are no longer viable, either directly or indirectly, for the following reasons: ethanol excess; high or low glucose concentration; diminishing surface to volume ratio and the genealogical age.

The magnitude of these unfavourable conditions will determine the vitality of the individual yeast cell.

Nevertheless, an extra effort must be made to improve some parts of the metabolic model to study the different stages of the complete evolution of fermentation with more detail.




Temporal evolution of the mean of the three replicates of ethanol concentration for the four different initial experimental conditions and simulated results (Sim).




Temporal evolution of the mean of the three replicates of glucose concentration for the four different initial experimental conditions and simulated results (Sim).

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