REPITCHING OF YEAST IN BEER FERMENTATIONS: INDIVIDUAL-BASED MODEL SIMULATIONS

M. Ginovart[1], X. Portell[1], M. Silbert[2]
[1]Technical University of Catalonia, Barcelona, Spain; [2]Institute of Food Research, Norwich, UK

Corresponding Author: M. Ginovart, Technical University of Catalonia, Department of Applied Mathematics III ESAB, Avda. Canal Olímpic 15, 08860-Castelldefels (Barcelona) Spain; marta.ginovart@upc.edu

Abstract. The industrial production of beer reuses yeast cropped at the end of fermentation in subsequent fermentation, a process unique to brewery fermentations. The fermentation performance of brewer's yeast strains is dependent on their ability to adapt to changes, particularly during batch brewery fermentation .The characterization of the replicative age is decided by the number of birth scars each yeast exhibits on its cellular membrane. It has recently been suggested that the distribution of the cells' age on cropping may affect both the immediate and the long term fermentation performance. This paper is concerned with the effects of age selection on yeast fermentation, studied by carrying out a number of individual-based model simulations of microbial growth of the yeast *Sacccharomyces cerevisae*. Here we specifically "crop" one cell from each of several ages as the initial inoculums in subsequent simulations, and follow their cycle. The simulations are performed by using the individual based simulator INDISIM-YEAST, based on the generic simulator INDISIM. The results of the simulations show that, in agreement with experiments, the initial age of the yeast cell not only influences the population growth, but also the rate of nutrient uptake and ethanol production.

1 Introduction

Industrial fermentation performed to produce beer is unique within the alcoholic beverage industry in that the yeast is not discarded after use but is maintained and reused a number of times in a process termed 'serial repitching'. Serial repitching, whereby yeast cropped at the end of fermentation is reused in subsequent fermentations, is a process ripe for study. The industrial production of beer uses repetitive repitching by suitably cropping yeasts from the flocs. The attempt to both ensure the production of quality beer and maintain yeast vitality, exposes the yeast cells to a number of stresses [2]. In this paper we are only concerned with studying the effects of age selection on yeast fermentation performance. The yeast *Saccharomyces cerevisae* has a limited replicative lifespan. Each cell within a population is only capable of a finite number of divisions prior to senescence and death. Replicative ageing depends on the number of divisions experienced by each cell, and can be determined by counting the number of bud scars on the wall of the mother cell. As a consequence of senescence, yeast cells are subject to morphological, metabolic and genetic modifications [6,7]. Towards the end of fermentation, yeast begins to form large clumps of cells or "flocs" and subsequently sediments and collects within the fermenter cone. The rate at which each cell sediments is believed to vary according to its replicative age. Older cells tend to sediment faster, and accumulate at the bottom of the cone (although their precise location may depend on their strain) leading to stratification by genealogical ages. Sedimentation results in the formation of zones enriched with cells of a particular age. At the end of fermentation a portion of the yeast is removed ('cropped') from the fermentation vessel for serial repitching. Typically this is the centre-top portion of the yeast crop, theoretically comprising middle-aged and virgin cells. However, increasingly yeast is removed early to decrease process time via a 'warm' or 'early' cropping regime and this facilitates removal of the lower portion of the crop, comprising a greater proportion of aged cells. Harvesting yeast may therefore select for a population with an imbalance of young and aged individuals, depending on the cropping mechanism employed [7].

Individual-based Models (IbMs) explicitly simulate individuals, and the population level behaviour emerges from their cumulative behaviour and interaction. In ecological modelling, IbM constitutes a well-established alternative to the traditional population-level approach, in which population parameters are modified directly using model equations. Most applications of IbM have been geared to higher trophic levels. However, advances in microbiology and biochemistry have stimulated an increase in the application of IbM to microbes as well. An IbM to deal with yeast populations has been developed, and the simulator INDISIM-YEAST has been build up [4,5] from the generic bacterial simulator INDISIM [3]. The aim of this study is to present the preliminary simulation results derived from exploring the influence of cell ageing on the fermentation process, carried out with this individual-based simulator INDISIM-YEAST. The study is developed by following the cell cycle of a virtual population of the yeast *S. cerevisiae*, up to the point when the production of ethanol has flattened out. Cells of different genealogical ages are then cropped. For instance, one yeast cell which has not undergone cell division is cropped, a second showing 1 scar, a third with 2 scars, and so on up to 10 or 12 scars. Then individual-based model simulations with INDISIM-YEAST are performed for each case, in order to study the influence of the initial age of each cell on the increase of population, the rate of glucose uptake and ethanol production.

2 The simulator INDISIM-YEAST

We used INDISIM-YEAST as the individual based simulator for this study [4,5]. This, in turn, is based on the generic simulator INDISIM, developed by our group in Barcelona [3]. A short review and selected applications of INDISIM can be found in Ref. [1]. The simulator is discrete in space and time and can be used for modelling yeast populations under different environmental conditions. It is rule-based, using stochastic variables, and subject to the appropriate boundary conditions. The physical domain where the virtual fermentation takes place is divided into spatial cubes, a microcosm of the system: it includes the liquid medium with yeast cells, glucose particles, as the main nutrient, and ethanol particles, the excreted product, as the only metabolite. The temporal evolution of the population is divided into equal intervals associated with computer or time steps. The simulator is made up of several elements: 1) Initialization of the system. Here the input data are entered. They determine the initial configuration of the population; and the environment where its evolution takes place. 2) Main loop (time step), where all the rules for each yeast cell and the medium are implemented and repeated until the end of the simulations. 3) Output data. The simulator stores information obtained from each yeast cell at the end of each time step, which makes it possible to obtain the results of the simulations both at the level of individual cells and at that of the yeast population at the end of the evolutions. The virtual system models the behaviour of each yeast cell in the following vategories: a) motion; b) glucose uptake; c) maintenance energy; d) new biomass production; e) ethanol excretion; f) budding reproduction, assuming two differentiated phases, phase 1 or the unbudded phase, when the cell gets ready to create a new cell (the bud), and phase 2 or the budding phase, in which the daughter cell-genealogical age 0 (virgin cell)-grows until it separates from the parent cell, leaving behind another scar; g) cell viability. Each yeast cell is characterized by its: i) biomass, which we relate to spherical geometry in order to evaluate its cellular surface; ii) genealogical age measured by the number of scars on the cellular membrane; iii) state of the reproductive cycle, involving the time spans of both parent and daughter cells in each of the two phases; and iv) survival times of the yeast cells not satisfying the metabolic requirements for their maintenance. During the implementation of the different parts of the simulator we take into account, amongst other things, the following consequences are: the scars left on the parent cells, as they affect the cellular membrane; the cell's chemical stresses caused by the presence of ethanol in the medium, as it slows down the production of new biomass (it increases the energy requirements for cellular maintenance); the increase in volume, or biomass, of parent cells, as they increase their genealogical age; the varying duration of the reproduction cycles (unbudded and budding phases), since these phases require a minimum time interval of stasis as well as minimum growth of biomasses in order to move from one phase into the other.

The simulation output may be separated into data related to the global properties of the system and data pertaining to the properties of individual yeast cells. The former includes information on temporal evolution of the number of nutrient particles (glucose); the number of metabolites (ethanol particles); the average nutrient consumption (defined as the number of nutrient particles metabolized during a time step, divided by the number of viable cells); the number of viable yeast cells; the number of non-viable yeast cells; viable yeast biomass; non-viable yeast biomass; heat dissipation of the system; and maintenance energy of the yeast population (defined as the number of metabolized nutrient particles not used in the production of new biomass). The latter provides information on the distributions of genealogical age and of the mass of the populations, which allows us to say something about the structure of the population throughout the fermentation process. We note that the preceding separation mirrors the experimental techniques used to study these properties. The web page htpps://aneto.upc.es/simulacio/hoja-portada presents a basic version of INDISIM-YEAST which permits carrying out the simulation of fermentation processes, and represents graphically a few of the variables controlled by the simulator [5].

We carry out individual-based simulations of the yeast *S. cerevisae* in which we attempt to model some aspects of the influence of cell ageing on the fermentation processes. We present below a preliminary study of the influence of the initial inoculums on fermentation performance.

3 Results and discussion

We have performed several simulations using INDISIM-YEAST, in which the only parameter that was changed was the genealogical age of the initial inoculums. We actually follow the population up to the point when the production of ethanol flattens out. We then crop one cell, at each stage, with different genealogical ages. Specifically we show the simulation results corresponding to the crop of one yeast cell which has not undergone cell division with genealogical age 0 (virgin cell), a second showing 3 scars and, finally a third with 10 scars. We then carry out with INDISIM-YEAST simulations for each case, SimA, SimB and SimC, respectively, in order to learn the influence of the initial age of each cell on the increase in population, the rate of glucose usage and ethanol production. We did carry out simulations for other genealogical ages, but these are not distinguishable at the level of the figures presented here. This type of study can only be done with individual-based simulations, and it is designed to complement the experimental studies being done on the subject. Figure 1 shows the time evolution of the number of viable cells. Fig 1(a) shows details of the initial growth, while Fig 1(b) shows the full time evolution of the system. Fig 1(a) shows that in the case of the virgin cell, the lag phase is much longer than in the other cases (as it needs to reach a "critical" biomass in order to start reproducing). The differences be-

tween the other two cases are relatively small in the first steps as compared with this one. Fig 1(b) also shows how maximum number of viable cells are displaced towards a larger number of time steps with lower genealogical ages. A similar picture emerges in Figure 2, where we depict the time evolution of the metabolites (ethanol particles). These evolutions have similar shape but the amount of ethanol produced is not exactly the same, and the rate of production is also different. From the oldest cell (SimC) the originated population has a superior ethanol production, but it stops earlier than in the other cases. The population from the youngest cell (SimA) has a slower ethanol production but for a longer time; consequently a similar level of ethanol is achieved. Our results show that the initial age of the seed yeast cell influences not only the population growth, but also the rate of nutrient uptake and ethanol production. In other words, the individual features of the initial cell influence the evolution of the resulting population. The maximum specific growth rates for the population corresponding to the exponential phases of the three evolutions have been calculated. In order to compare these values among the three simulations, we took into account the same common range of values for the corresponding exponential phases to perform the statistical analyses. The results attained for the maximum specific growth rates were 0.0034 for SimA (R^2=0.996), 0.0031 for SimB (R^2=0.997), and 0.0029 for SimC (R^2=0.997). The growth in the exponential phase achieved from the inoculums of a virgin cell was faster than that achieved from the older cells as inoculums. Our findings are in broad agreement with the experimental results presented in Refs. [6,7], and support the authors' view that artificial selection for a population enriched with young or aged individuals influences yeast fermentation performance. The initial virtual seeds were obtained by carrying out a simulation of yeast fermentation, as described in Refs. [4,5], which produced a heterogeneous population of yeast cells with different genealogical ages and biomasses. From this we chose the inoculums for the ensuing simulations, of which we only present three cases. This type of study highlights one of the benefits of the IbMs. It is possible with our simulator to generate a virtual yeast population starting with one yeast cell that differs in age from the following simulation. In this way we can compare and contrast both the individual and global properties of the yeast cells and the populations generated in each simulation. We believe we will be able to carry out similar studies using populations whose age distributions differ as initial inoculums. We are currently working in this direction, and in addition, some examination of the internal structures of the yeast populations will be done in order to provide more information about this macroscopic behaviour.

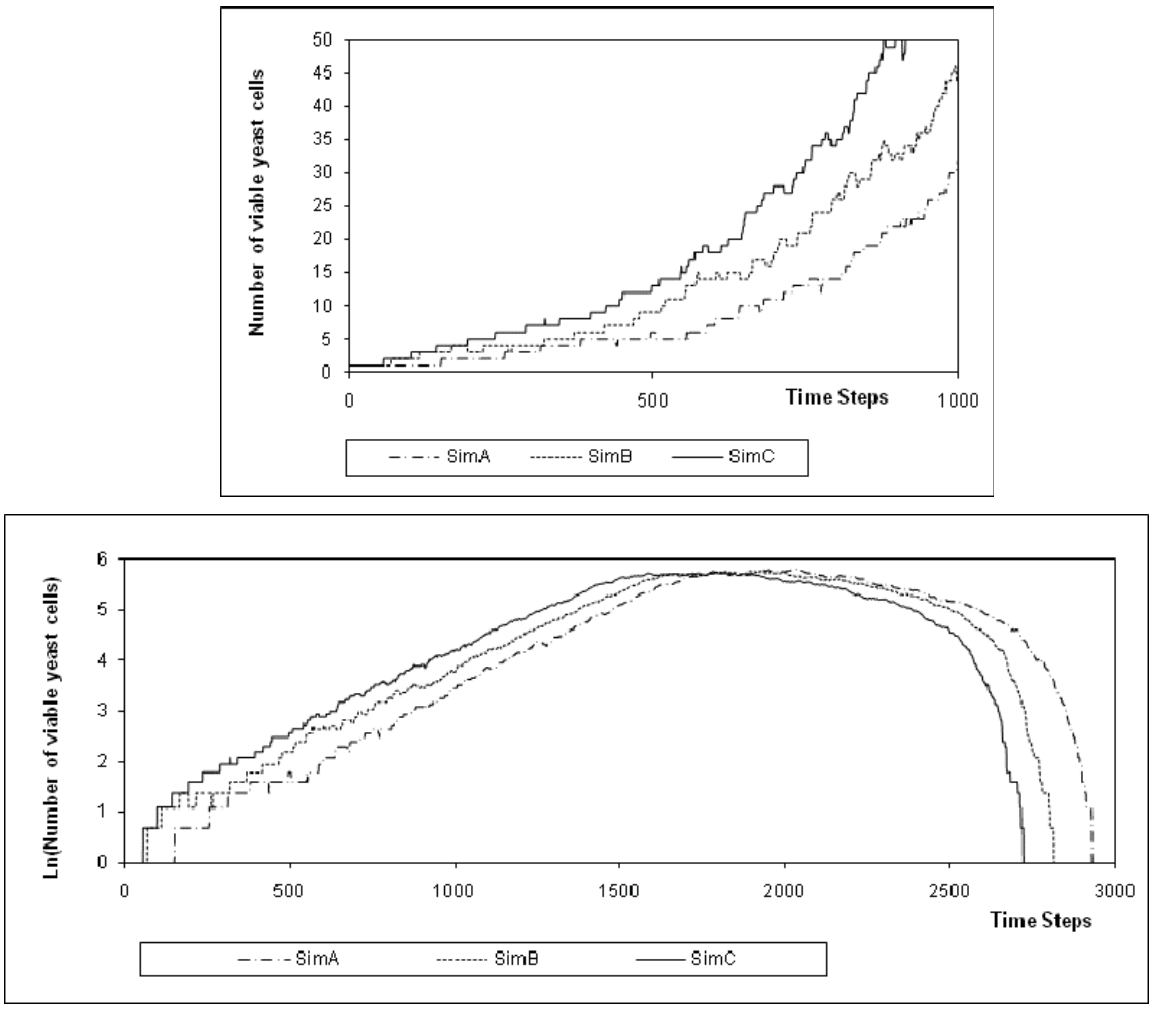

Figure 1. Time evolution of the number of viable yeast cells from the virtual yeast fermentations obtained in the INDISIM-YEAST simulations. Initial cell with genealogical age: 0 for SimA, 3 for SimB, and 10 for SimC. (a) Initial growth; (b) Full evolution (natural logarithm).

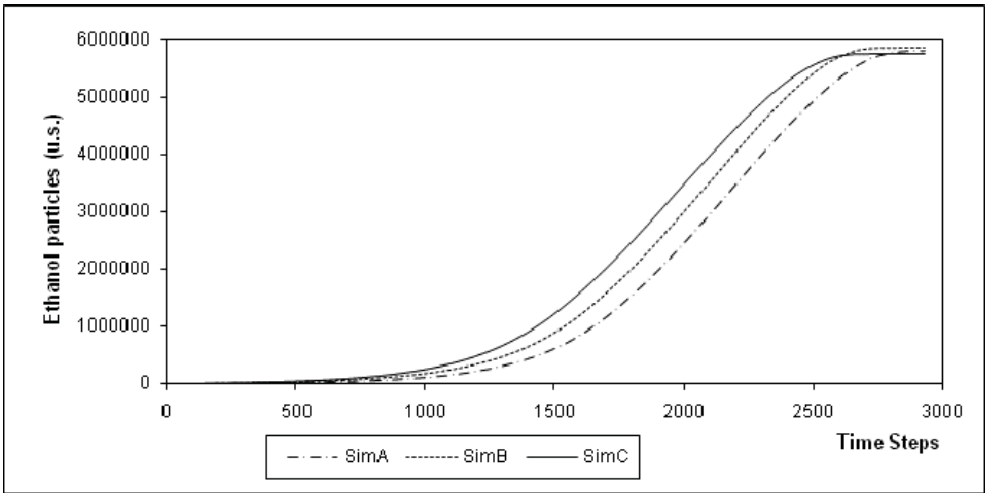

Figure 2. Time evolution of the number of ethanol particles produced by the virtual yeast fermentations obtained in the INDISIM-YEAST simulations. Initial cell with genealogical age: 0 for SimA, 3 for SimB, and 10 for SimC.

The choice of basic modelling approach, population-level or individual-based, is an important decision that needs to be addressed at the beginning of a modelling project. There are arguments for and against either approach, and the right choice depends on project-specific aspects, the characteristics of the system to be simulated and the questions to be asked of the model. In general, arguments for the population-level approach are its simplicity, computational efficiency, the fact that it has been used and tested widely, and the availability of established and peer-reviewed modelling frameworks. Arguments for the individual-based approach include the ability to simulate intra-population variability, complete life cycles and behaviour adapted to internal and external conditions. For our microbe IbM, or yeast IbM, the main motivations have been the ability to resolve intra-population variability, the ability to link mechanisms at the individual level to population level behaviour, and the inapplicability of the continuum hypothesis. The use of the IbM presented, INDISIM-YEAST, offers diverse and attractive possibilities to continue to explore the fermentation process, and specifically all the protocol related with the "repitching" of yeast in the brewing industry.

4 Acknowledgments

We thank Rosa Carbó for several comments and suggestions. We gratefully acknowledge the financial support received from the Ministry of Education and Science (Plan Nacional I+D+i) through Grant CGL2007-65142/BOS, as well as the European Social Fund and AGAUR- Generalitat de Catalunya through Grant 2008FIA-00307. One of us (MS) gratefully acknowledges for hospitality during a research visit at the UPC, during which this study was completed.

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