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Exploring the lag phase and growth initiation of a yeast culture by means of an individual-based model

Marta Ginovart [a,*], Clara Prats [b], Xavier Portell [c], Moises Silbert [d]

[a] Department of Applied Mathematics III, Universitat Politècnica de Catalunya, Campus Baix Llobregat, Esteve Terradas 8, 08860 Castelldefels, Barcelona, Spain
[b] Department of Physics and Nuclear Engineering, Universitat Politècnica de Catalunya, Campus Baix Llobregat, Esteve Terradas 8, 08860 Castelldefels, Barcelona, Spain
[c] Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya, Campus Baix Llobregat, Esteve Terradas 8, 08860 Castelldefels, Barcelona, Spain
[d] Institute of Food Research, Norwich Research Park Colney, Norwich NR4 7UA, UK

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ABSTRACT

The performance of fermentation processes is greatly influenced by the size and quality of inocula. The characterization of the replicative age is decided by the number of birth scars each yeast exhibits on its cellular membrane. Yeast ageing and inoculum size are factors that affect industrial fermentation, particularly those processes in which the yeast cells are reused such as the production of beer. This process reuses yeast cropped at the end of one fermentation in the following one, in a process called "serial repitching". The aim of this study was to explore the effects of inoculum size and ageing on the first stages of the dynamics of yeast population growth. However, only Individual-based Models (IbMs) allow the study of small, well-characterized, microbial inocula. We used INDISIM-YEAST, based on the generic IbM simulator INDISIM, to carry out these studies. Several simulations were performed to analyze the effect of the inoculum size and genealogical age of the cells that made it up on the lag phase, first division time and specific growth rate. The shortest lag phase and time to the first division were obtained with largest inocula and with the youngest inoculated parent cells.

1. Introduction

Biochemical factors have long been recognized as the most effective elements for determining the yield and productivity of industrial fermentation. Since these factors are carried forward from the inocula to the production stages, an accurate characterization of these inocula, in terms of size and quality, is of importance in order to improve fermentation consistency and thus increase the efficiency and/or yield of the biotechnological process (Birol and Özergin-Ülgen, 1995, Gibson et al., 2007).

The yeast *Saccharomyces cerevisiae* has a limited replicative lifespan (Walker, 1998). We are concerned here with yeast budding reproduction, which leads to scar formation and to cells dividing unequally. Ageing in budding yeast is not determined by chronological lifespan (measured as the ability of non-dividing cells to maintain viability over time) but by the number of times an individual cell is capable of dividing (Maskell et al., 2003). Each cell within a population is only capable of a finite number of divisions prior to senescence and death. The number of bud scars on the wall of the mother cell is directly related to the number of times a cell has divided, and so this number constitutes a biomarker of replicative cell age. In addition, it has been demonstrated that there is an increase in cell size with age (Walker, 1998). However, this increase is not considered constant between successive ages, as it may decrease in older individuals in accordance with the diminution of the metabolic rate for aged cells. In fact, although aged cells are known to be larger than younger cells, mixed age yeast populations can exhibit a greater average size than older populations, depending on the environmental conditions and other unidentified factors (Porro et al., 2009). Variety is an intrinsic property of microbial life.

As a consequence of senescence, yeast cells are subject to morphological, metabolic and genetic modifications (Powell et al., 2000, 2003). In industrial applications yeast is usually propagated in a number of steps before being inoculated into the final fermentation medium. Despite the fact that the physiological condition of the yeast cells may greatly affect the fermentation duration and outcome the inoculum is not often well-defined (Walker, 1998).

By way of example, the production of beer reuses yeast cropped at the end of fermentation in subsequent fermentation, so yeast is maintained and reused a number of times in a process called 'serial repitching'. Toward the end of beer fermentation, the yeast sediments and collects within the fermenter cone (Powell et al., 2003). The rate at which each cell sediments is believed to vary according

* Corresponding author.
E-mail address: marta.ginovart@upc.edu (M. Ginovart).

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to its replicative age. Older cells tend toward faster sedimentation, and accumulate at the bottom of the cone (although their precise location may depend on the strain) leading to stratification by genealogical age. Sedimentation results in the formation of zones enriched with cells of a particular age (Kurec et al., 2009). 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 (Powell et al., 2003). However, yeast is increasingly removed early to decrease process time by means of a 'warm' or 'early' cropping regime, which facilitates removal of the lower portion of the crop that comprises a greater proportion of aged cells (Powell et al., 2004). Harvesting yeast may therefore select for a population with an imbalance of young and aged individuals, depending on the cropping mechanism employed (Powell et al., 2003). Although a yeast crop may be considered by the brewer to be a homogenous culture in terms of vitality and viability, it has been demonstrated that it usually comprises a variety of individual cells which may differ in respect to their fermentative capabilities (Walker, 1998, Gibson et al., 2007).

These processes highlight and justify the interest in studying the main features of a yeast inoculum, obtained from one fermentation that is going to be used in the following one.

When yeast cells are inoculated into a fresh growth medium, they enter a brief lag phase in which they are biochemically active but still do not divide (Gibson et al., 2007). After this lag phase, cells enter their cell cycle and start dividing. The microbial lag phase has usually been investigated with continuous population models using rather high inoculum levels or homogeneous inocula (Baranyi and Roberts, 1994; Baranyi, 2002; Swinnen et al., 2004), but lately more and more studies have concentrated on single cell lag times (McKellar, 2001; Devlieghere et al., 2005; Kutalik et al., 2005; Metris et al., 2005; Koutsoumanis, 2008; Malakar and Barker, 2008; Baranyi et al., 2009). Nevertheless, when microbial growth starts from a few accurately characterized cells, the study of this evolution demands an individual-based approach.

Individual-based Models (IbMs) explicitly simulate individuals, and the population behavior emerges from their cumulative behavior and interaction (Grimm and Railsback, 2005). In ecological modeling, IbM constitutes a well-established alternative to the more traditional population-level approach, in which population parameters are modified directly using model equations. Most applications of IbMs have been geared to higher trophic levels. However, advances in microbiology and biochemistry have stimulated an increase in the application of IbMs to microbes as well (see Hellweger and Bucci, 2009 for a recent review). A model in this context that is individual-based means that the basic entities are the individual cells. Thus, every cell is considered as an entity and has its own properties that change during its life according to the set of rules declared. Unlike traditional top-down approaches, IbMs are bottom-up approaches. Sometimes, under certain assumptions, the two approaches converge in yielding similar results as the size of the population increases (Gómez-Mourelo and Ginovart, 2009).

Out of the several microbial IbMs that are available nowadays (Kreft et al., 1998; Dens et al., 2005; Hellweger and Bucci, 2009) we used INDISIM, the simulator developed by our group (Ginovart et al., 2002; Ferrer et al., 2008), which has already been used to study different features of bacterial lag phase yielding an ample pool of interesting results (Prats et al., 2006, 2008). INDISIM-YEAST is the adaptation of INDISIM to the study of specific features of the yeast cell cycle in yeast populations growing in a liquid medium (Ginovart et al., 2007; Ginovart and Cañadas, 2008; Prats et al., in press). This simulator has also recently been used in a preliminary study of some aspects of the influence of cell ageing on fermentation processes (Ginovart et al., 2009).

The aim of this study was to explore the effects of specific characteristics of the initial inocula on the dynamics of a virtual yeast culture in liquid medium during the first stages of growth by means of the individual-based simulator INDISIM-YEAST.

2. Materials and methods

We used INDISIM-YEAST as the individual-based simulator for this study, which is based on the generic simulator INDISIM (Ginovart et al., 2002, 2007; Ginovart and Cañadas, 2008; Prats et al., in press). A short review and selected applications of INDISIM can be found in Ferrer et al. (2008).

A general presentation and schematic outline of the simulator INDISIM-YEAST is as follows. It is discrete in space and time and can be used for modeling yeast populations under different environmental conditions. It is rule-based, using stochastic variables, and subject to 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 excreted ethanol particles, as the only end product. 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) the initialization of the system, in which the input data are entered and the initial configuration of the population and environment is determined; 2) the main loop (time step), in which all the rules for each yeast cell and the medium are implemented and repeated until the end of the simulations; and 3) the output of results, including the information obtained from each yeast cell at the end of each time step stored by the simulation. This makes it possible to obtain the results of the simulations both at the level of individual cells and at that of the yeast population throughout the evolutions.

The virtual system models the behavior of each yeast cell in the following categories: a) random motion; b) glucose uptake; c) cellular maintenance; d) new biomass production; e) ethanol excretion; f) budding reproduction (detailed below); and g) cell viability. The budding reproduction model assumes 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 (Fig. 1). Each yeast cell is characterized by its: i) biomass, which is related by the model to spherical geometry in order to evaluate its cellular surface; ii) genealogical age measured as the number of scars on the cellular membrane; iii) state of the reproductive cycle,

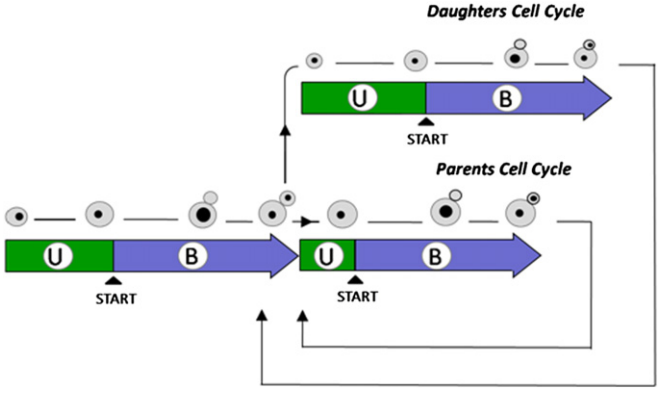

Fig. 1. Scheme of the budding reproduction model implemented at individual level yeast cell. U: phase 1 or the unbudded phase, B: phase 2 or the budding phase. Adapted from Hatzis and Porro (2006).