The effect of bioreactor compartmentalization on yeast population dynamics during continuous cultivation

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Keywords: Population Balance Models; *Saccharomyces cerevisiae*; oscillations; compartment model approach

Introduction

Continuous cultivations (chemostats) are frequently used in fermentation process research, and sometimes also in industrial scale production. Oscillatory behaviors have been reported for certain combinations of the dilution rate (D) and the dissolved oxygen concentration (Porro et al., 1988). Such oscillations have been observed for several variables, e.g. substrate (glucose), metabolites (e.g. ethanol) and budding index (a measure of the distribution of cells over the different cell cycle phases). This type of oscillations is partly related to the cell cycle (Porro et al., 1988; Richard et al., 2003) and the alternation between respiro-fermentative and respiratory metabolism.

Mixing in large-scale bioreactors is not ideal, and thus the development of zones, representing different extracellular environments, can occur. For example, the use of Rushton turbines in industrial fermentors (one of the most commonly used impellers in industrial bioreactors due to their claimed effective gas dispersion) has been observed to generate compartments within the reactor due to the axial flow barriers created by the turbines (Vrebel et al., 2000; Schmalzriedt et al., 2003). The existence of compartmentalization, and thereby implying the presence of different conditions for different reactor locations, may thus influence the performance of the cultivation and, for example, the appearance of oscillations.

A compartment model approach allows for a crude and simple way of taking spatial heterogeneity in a bioreactor into account, and assessing its impact on the biological phenomena (Vrebel et al., 2000; Delafosse et al., 2012). Additionally, the translation of a compartment model to a laboratory experimental set-up can be easily achieved by using scale-down reactors (George et al., 1998; Neubauer et al., 2010). In addition to that, various experimental techniques for measuring single-cell properties are nowadays available (Lencastre Fernandes et al., 2011; Fritzsch et al., 2012)

In the work presented here, a population balance model (PBM) coupled to an unstructured model has been used to describe aerobic growth of a population of *Saccharomyces cerevisiae* cells in a continuous cultivation. A two compartment model approach was used to describe a continuous cultivation in a spatially heterogeneous stirred tank reactor where a highly concentrated glucose feed is used. The population dynamics predicted for a spatially homogeneous (ideally mixed) bioreactor and for a two compartment reactor are compared, and the impact of considering reactor compartmentalization as well as population dynamics (rather than considering an average description of cell behavior, the standard approach in most modeling studies of fermentation processes) to describe the cultivation is discussed.

Model description: two compartment system

A previous study reporting a CFD model for a 22 m^3 bioreactor (Larsson et al., 1996), showed clearly that a zone with high substrate concentration was formed in the top of the reactor where the substrate feed was added (between the overhead space and the first impeller). Analogously, a smaller compartment, with a volume V1, corresponding to the feeding zone is considered in this study. A larger compartment, with a volume V2, then corresponds to the remaining volume of the reactor. The substrate feed (F), an inflow to compartment V1, consists of a glucose (substrate) rich medium. The internal flows F1 and F2 describe the transport from the compartment V1 to V2, and from compartment V2 to V1, respectively. The outlet of the reactor is located in V2. The oxygen supply is assumed to take place exclusively in compartment V2. Continuous mode (chemostat) operation is assumed, implying that the flow F into V1 is equal to the outlet flow from V2. Assuming constant volumes V1 and V2, the following condition applies: F1= F2+F. For all simulations, V1 was assumed as 1/6 of the total working volume.

Aiming at both describing the distributions of single-cell sizes for the two cell cycle stages (non-budding and budding cells), a PBM was coupled to an unstructured model describing the extracellular environment. The PBM developed for this study is based on a multi-stage model reported in the literature (Hatzis and Porro, 2006) that was further developed for describing growth of a yeast population and variation of the extracellular environment during batch cultivation (Lencastre Fernandes et al., 2013). In this work regarding a two compartment model, four (2 stages x 2 compartments) population balance equations are necessary, and the dilution terms taking into account the transport between compartments and outlet are included as well. As an example, the population balance equation for the non-budding and budding stages for compartment V1 are defined as Equations 1 and 2, respectively. Here $N_{Vi}^{NB}(m,t)dm$ and $N_{Vi}^{B}(m,t)dm$ represent the number of cells in the cell size interval *[m, m+dm]* for the non-budding and budding stages, respectively, in the compartment V_i. *m* corresponds to the cell total protein content or cell size (in arbitrary units, due to the fact that these are measured via flow cytometry), and *Z* designates the extracellular environment. The PBM kernel functions, as well as boundary and initial conditions, were defined as previously reported for a batch system (Lencastre Fernandes et al., 2013), and a similar discretization and solution procedure were also used here.

$$\frac{\partial N_{V1}^{NB}(m,t)}{\partial t}+\frac{\partial}{\partial m}\left[r_m(m,Z)N_{V1}^{NB}\right]=-\Gamma_B(m|Z)N_{V1}^{NB}(m,t)+2\int_m^{m_f}\Gamma_D(m'|Z)P(m,m'\Big|Z)N_{V1}^{NB}(m,t)+\frac{F2}{V1}N_{V2}^{NB}(m,t)-\frac{F1}{V1}N_{V1}^{NB}(m,t) \quad (1)$$

$$\frac{\partial N_{V1}^{B}(m,t)}{\partial t}+\frac{\partial}{\partial m}\left[r_m(m,Z)N_{V1}^{B}\right]=-\Gamma_D(m|Z)N_{V1}^{B}(m,t)+\Gamma_B(m|Z)N_{V1}^{NB}(m,t)+\frac{F2}{V1}N_{V2}^{B}(m,t)-\frac{F1}{V1}N_{V1}^{B}(m,t) \quad (2)$$

Critical transition sizes as functions of the substrate availability

The critical budding and division sizes (μ_B and μ_D), defining the budding and division rates (Γ_B and Γ_D, respectively) were, in this work, considered as continuous functions of the concentrations of glucose or ethanol in each compartment, according to the following assumptions:

- If the concentration of glucose, in a given compartment, is equal to or above 0.1 g/l, growth on glucose is assumed for that compartment, and the critical budding (μ_B) and division sizes (μ_D) are calculated based on the glucose concentration, using Monod type expressions (Eq 3). The critical budding and division size maxima are 500 and 950 ch no., and minima of 300 and 550 ch. no., respectively;

$$\mu_i=(\mu_i^{\max}-\mu_i^{\min})\frac{G}{G+K_\mu}+\mu_i^{\min} \quad (3)$$

- If the concentration of glucose, in a given compartment, is below 0.1 g/l, growth on ethanol is assumed for that compartment, and the critical budding and division sizes are calculated using Monod type expressions (similarly to Eq. 3, using ethanol instead of glucose), corresponding to maxima of 300 and 550 ch no., and minima of 180 and 300 ch. no., respectively;
- If the concentrations of glucose and ethanol, in a given compartment, are under 1e-6 g/l, growth in that compartment is assumed to be equal to zero.

The minimum and maximum values for the critical transitions sizes were defined based on the corresponding trajectories along a batch cultivation (Lencastre Fernandes et al., 2013). The same saturation constant in the Monod type expressions was used for all cases, and a value of 0.5 g/l was assumed.

Cell division and birth: partitioning

A mother and a daughter cell are generated upon division, where the ratio of the mother cell size to the daughter cell size is defined by the partitioning function, *P(m,m'|Z)*. This function consists of a symmetrical beta probability density function. The partition shape parameters (defining the beta distribution) were defined as $\alpha=\beta=50$ when growth on glucose was considered. Alternatively, $\alpha=30$ and, $\beta=60$ were considered when growth on ethanol was assumed for a given compartment.

Results

The impact of both the overall dilution rate (*D=F/V*) and the substrate concentration in the feed flow on the cell size and cell cycle distributions of the budding yeast population was investigated considering both a single compartment reactor (the reference case) and a two compartment reactor. In the latter case, the effect of the recirculation flow (F2) was also investigated. The simulated scenarios correspond to a full 2-level, 2 or 3 factor (for the single and two compartment models respectively) factorial design and the center point (Figure 1).

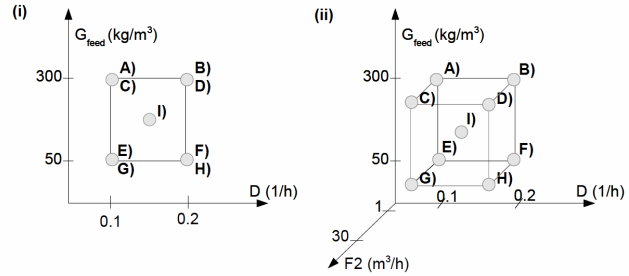

Figure 1: Two level factorial design used for evaluating the effect of the overall dilution rate (D) and glucose concentration in the feed flow (G_{Feed}), as well as recirculation flow (F2), on yeast population dynamics: (i) 2-factor design for the single compartment reactor), (ii) 3-factor design for the two compartment reactor

Single compartment model

Considering an ideally mixed CSTR (i.e. single compartment), steady state is achieved for all scenarios, except for scenario F)-H) where sustained oscillations are observed (Figure 2). These oscillations are particularly visible in the budding index (BI) profiles and oxygen profiles. When the glucose concentration decreases below 0.1 g/l, the partition coefficients change (switch from glucose growth to ethanol growth) leading to an abrupt increase of the number of newly originated non-budding cells (reflected by the steep fall of the budding index). These small new cells grow slower than larger cells, and thus an accumulation of the glucose and oxygen is observed. When the glucose concentration increases to values above 0.1 g/l, the partitioning parameters are re-set to the glucose growth mode values, yielding an increase of the budding index.

When comparing various operation conditions, Porro et al. (1988) suggested that the occurrence of oscillations is observed in a defined range of dilution rates (and thus glucose residual concentrations) and dissolved oxygen concentrations. Such observations are in good agreement with the simulation results reported here. As a result of the residual concentrations of both glucose and ethanol observed at steady state for scenario E)-G), the steady-state cell size distributions are substantially shifted towards smaller cell sizes. Oppositely, the steady state cell size distribution predicted for scenarios B)-D) includes larger cells than observed for other scenarios. This reflects the higher glucose concentration observed at steady state for this scenario.

Two compartment model

When considering a compartmentalized reactor, significant differences, in comparison to the single compartment model, have been observed. Generally, scenarios where a low recirculation flow rate (F2) is imposed (A), B), E) and F)) show the largest differences between compartments in terms of concentrations, but also with regard to predicted budding index and cell size distributions. This is not surprising, as a low recirculation flow rate implies that the liquid exchange between compartments is limited.

Indeed, oscillatory pseudo-steady states are observed for scenarios A), H) and I), characterized by simultaneous oscillations of the extracellular environment variables. In the case of scenarios A) and I), oscillations were not predicted by the corresponding single compartment model.

Oppositely, for scenarios F) and H), oscillations are predicted by both single and two compartment models. For these scenarios, the variation along time of glucose, ethanol, oxygen, and overall biomass concentration, as well as the cell size distribution for the non-budding and budding populations at the simulation end time is presented in Figure 2. These results suggest that the existence of sustained oscillations may be scale dependent: oscillatory behaviors observed in laboratory scale experiments may not be transferred to large scale and vice-versa. It seems that the degree of compartmentalization is also a key operational parameter affecting the occurrence of sustained oscillations. While in the case of low dilution rate and high glucose feed concentration (scenario A), a high degree of compartmentalization (low recirculation flow F2) results in oscillatory pseudo-steady state, a lower degree of compartmentalization (high recirculation flow) is necessary for the occurrence of oscillations in the case of higher dilution rate and lower glucose feed concentration (scenario H).

When comparing scenarios A) and C) (corresponding to the same dilution rate and glucose feed concentration, but different levels for the recirculation flow), a higher biomass concentration is observed at the outlet (i.e. in V2) for the low recirculation scenario (27 g/l for scenario A) and 21 g/l for scenario C)). The corresponding single compartment model predicted a lower biomass concentration (17 g/l). This is rather surprising as it could be expected that higher titers are to be achieved in an ideally mixed reactor (i.e. not compartmentalized). In this case, however, a higher biomass concentration is predicted for the highly compartmentalized scenario A). The oscillatory behavior predicted for this scenario implies that for each oscillation period a number of non-budding cells are generated (as discussed above). As the mass concentration is assumed to be proportional to the number of cells (see Lencastre Fernandes et al., 2013), the predicted biomass concentration increases with the described increase in the number of cells, although these cells belong to smaller size classes. Moreover, a budding index

fraction of budding cells in the population of approx. 40 % is estimated for compartment V2 for scenario A) (as well as C)) whereas, a budding index of 50 % is predicted by the single compartment model.

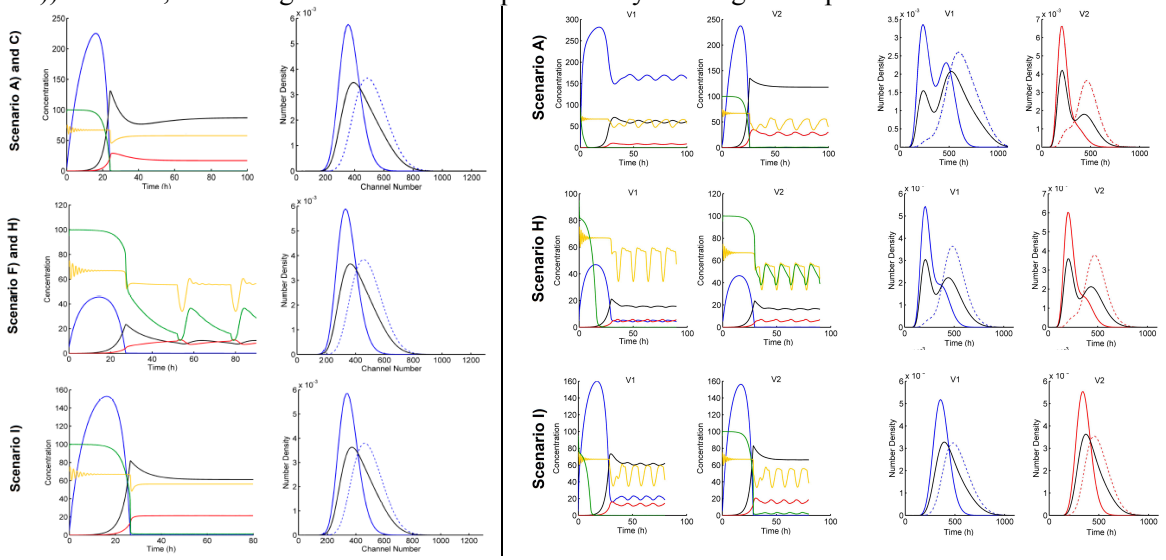

Figure 2: Simulation results for scenarios A)-C), F)-H) and I) for the single compartment model (to the left) and for scenarios A), H) and I) for the two compartment model (to the right): the variation of the concentrations of glucose (blue), ethanol (black), total biomass (red) in g/l, as well as DO (green) and BI (yellow) in percentage, is presented on the subplots on the left-hand side; the normalized cell size distributions for non-budding (full blue line) and budding (dashed blue line) cells, as well as for total cell population (full black line), for the final simulation time, are presented on the subplots on the right-hand side.

Discussion and Conclusions

The work presented in this contribution extends the description of a continuous cultivation by predicting the development of cell size distributions for two subpopulations (non-budding and budding yeast cells), as well as by considering the existence of two compartments within the bioreactor. Cell size and cell cycle position have been used in this work as cell descriptors following the model framework that had been developed for a batch system. These cell properties are easily measured and are deeply connected to growth (Lencastre Fernandes et al., 2013). The proposed extension of the population model framework to a continuous cultivation allowed for reproducing respiratory oscillations that have been reported in the literature for both laboratory and industrial set-ups. Such oscillatory pseudo-steady states could not be reproduced using exclusively a simpler ODE based unstructured model as for example the model proposed by Sonnleitner and Käppeli (1986).

A compartment model approach as the one proposed in this chapter has been proven to be useful for assessing the consequences of reactor compartmentalization due to non-homogeneous mixing patterns. From the study presented in this chapter, it can be concluded that the existence of zones (due to substrate gradients) where cells experience different environments may significantly affect the system behavior in terms of achieving a steady state operation or an oscillatory pseudo steady state, as well as in terms of the predicted overall biomass productivity and yields on glucose. The predicted variations in distributions in the cell size and cell cycle position for various operating conditions, and degrees of compartmentalization, may be perceived as an indicator for other cell properties including the existence of distributions of mRNA levels of specific genes. A previous study (Schweder et al., 1999) on large scale fed-batch cultivation (30 m^3) has shown that, indeed, significant differences in mRNA level of stress genes were observed in different zones in the bioreactor, specially for higher OD levels (i.e. longer cultivation times).

In conclusion, in this work a compartment model approach has been used to understand the effect of the spatial heterogeneity (i.e. the presence of defined spatial zones corresponding to different extracellular environments) in a bioreactor on the dynamics of a microbial population as well as on the overall system behavior. One of the advantages of using a compartment model approach is that it is relatively easy to translate the results to a laboratory set-up. In the case of the work presented in this chapter, the two compartment system can be implemented in lab-scale by connecting two stirred tank reactors. It is, to our knowledge, the first time that such analysis is made based on a population balance model for describing the growth of a microbial population. Analyses as the one presented here and the understanding that can be generated by testing various scenarios *in silico* can be of great value as a complementary tool to the experimental scale-down studies.

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