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Research review paper

Experimental methods and modeling techniques for description of cell population heterogeneity

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ABSTRACT

With the continuous development, in the last decades, of analytical techniques providing complex information at single cell level, the study of cell heterogeneity has been the focus of several research projects within analytical biotechnology. Nonetheless, the complex interplay between environmental changes and cellular responses is yet not fully understood, and the integration of this new knowledge into the strategies for design, operation and control of bioprocesses is far from being an established reality. Indeed, the impact of cell heterogeneity on productivity of large scale cultivations is acknowledged but seldom accounted for. In order to include population heterogeneity mechanisms in the development of novel bioprocess control strategies, a reliable mathematical description of such phenomena has to be developed. With this review, we search to summarize the potential of currently available methods for monitoring cell population heterogeneity as well as model frameworks suitable for describing dynamic heterogeneous cell populations. We will furthermore underline the highly important coordination between experimental and modeling efforts necessary to attain a reliable quantitative description of cell heterogeneity, which is a necessity if such models are to contribute to the development of improved control of bioprocesses.

Contents

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1. Introduction

Bioprocessing operations use the activity of living cells for the production of biomass or other products resulting from the cellular metabolism such as proteins, antibiotics or antibodies. In a typical bioreactor operation, cells are kept viable and at a desired state of metabolic activity by adjusting the nutrient levels and reactor variables (e.g. pH and temperature).

As the microbial cultures used in the bioprocess are, most commonly, isogenic, cell heterogeneity would not be expected if the environment was well controlled at adequate conditions. Nonetheless, population heterogeneity has been observed at large scale. In fact, the scale up of bioprocesses from bench-scale to large scale may lead to lower yields and productivities and an increased by-product formation (Bylund et al., 1998; Enfors et al., 2001). This is caused by a decreased capability to maintain a homogeneous environment in large scale bioreactors as compared to well-mixed bench-scale bioreactors used for process development.

Therefore, while the assumption of a perfectly mixed reactor might be realistic for bench-scale reactors, it certainly is not for large scale bioreactors. Due to limited mixing and mass transfer, gradients of, for example, substrate, oxygen and pH are observed in larger reactors (George et al., 1998). In fact, substrate concentrations may range from high concentrations close to the feed port to residual concentrations in zones more distant to this port, the latter caused by different rates of mixing and biological reaction (Larsson et al., 1996). Cells circulating in the reactor are subjected to successively changing conditions, which, by inducing genetic, metabolic and physiological responses, are held responsible for the development of heterogeneous populations. In the past, such populations were shown to present lower productivity than homogeneous ones (Enfors et al., 2001).

The monitoring and control of bioprocesses, found in industry today, does not account for the heterogeneity in microbial populations. The cell properties, determined using on-line, at-line or off-line monitoring methods, correspond to averaged values and, thus, camouflage valuable information on the dynamics of the population (cf. Fig. 1).

Due to the observed decrease in performance at large scale, heterogeneity in bioprocesses was felt to be undesirable. Nonetheless, it might be the key to cell population robustness as observed in tumors (Kitano, 2004) or in cases of bacterial persistence (Balaban et al., 2004). Similar to mechanical stress, which can be exploited to control fungal morphologies to increase overall productivity (Papagianni, 2004), it might also be possible to take advantage of heterogeneity in a microbial population for process optimization. In fact, to understand and harness cell heterogeneity may show us a new path for achieving improved robustness in bioprocesses.

The study of cell heterogeneity in its many aspects has been the focus of the experimental work of many researchers in the recent years, as the number of experimental methods available for single-cell analysis has boomed (Schmid et al., 2010). However, this knowledge has not yet been integrated into a generally accepted modeling framework that is able to account for distributed properties within a cell population, and thus can be used in the design and control of bioprocesses (Müller et al., 2010).

In this contribution, we aim at a) presenting the concept of heterogeneous microbial populations as well as briefly discussing the main factors causing this heterogeneity; b) presenting experimental methods used for studying of cell heterogeneity, in addition to discussing the information within the data sets that these methods yield; c) discussing the design of experiments on microbial cultivations that can deliver information on the development of heterogeneous populations as a result of variations in the extracellular environment;