# DYNAMICS OF *ESCHERICHIA COLI* POPULATION EXPRESSING HETEROLOGOUS PROTEINS

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*Expression of heterologous proteins in Escherichia coli is an important and highly used system for obtaining large quantities of recombinant proteins for a wide range of applications in biotechnologies, medicine etc. However, the ability for delivering high quantities of recombinant proteins at desired quality and the cost effectiveness of the process is under serious scrutiny. Most improvements on bioprocesses for recombinant proteins production are focused on downstream processing, like primary recovery and purification. The present work relies on monitoring the bacterial population development and dynamics in the bioreactor, and presents the premises for modelling and optimization.*

**Keywords**: *Escherichia coli*, population dynamics, batch reactor

## 1. Introduction

The recombinant protein technology has registered an impressive progress over the past decades [1]. Old limitations like the idea that bacteria are not able to make glycosilated proteins, or the dogma that *Escherichia coli* is not appropriate for expressing proteins, were challenged and proved wrong. The technology is constantly improving and hundreds of therapeutic proteins are entering the market.

Of particular interest is obtaining recombinant proteins (extremozymes) originated from extremophilic microorganisms, in particular from (hyper)thermophiles, for various applications, because of their high intrinsic stability to different stress conditions characteristic of their niche environment [6, 7].

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Extremophiles have adapted to extreme environmental conditions, such as temperatures around 100ºC in volcanic habitats, at freezing temperatures in the cold polar seas, under high hydrostatic pressures (300-500 MPa) in deep-sea environments, at very low and high pH values (pH 0-1 or pH 10-11), or at very high salt concentrations (35 %).

The constitutive biomolecules of extremophiles exhibit unique features, and their structural and functional studies give insight into molecular adaptation mechanisms to extreme conditions.

Among these, surface layers (S-layers) protein is remarkable for its ability to crystallize in monolayers [8, 9]. This simple biomembrane, resulted during evolution, has many applications in biotechnology, nanotechnology and biomimetics, such as development of ultrafiltration membranes, immobilized functional molecules (e.g. enzymes, antibodies, ligands), dipsticks used for diagnosis of allergies, biosensors, conjugated vaccines and matrices for controlled biomineralization [10-12].

Functionalization of S-layers by attaching other molecules to this structured surface opens new perspectives for supramolecular engineering and nanotechnology applications. Nanotechnological applications of S-layers could offer new insights for the surfaces chemical studies, biological models, formation and organization of metal arrays with applications in optics and nanoelectronics.

## 2. Bioprocess control

The overall process performance depends upon the bioprocesses developed in the bioreactor, consisting in the recombinant protein expression linked to the development and multiplication of microorganisms [13]. Therefore, it is important to understand, monitor, model and optimize the bacterial population development and dynamics of this system.

The advent of molecular microbiology and associated molecular technologies makes possible high-resolution single cell analysis, which can provide quantitative information on the dynamic behaviour of microbial communities in bioreactors, the large scale production units [14, 15].

Insights into bioprocesses at single-cell level contribute to the development of new strategies for microbial fermentations, increasing global efficiency, but also helping to develop more accurate kinetic models to be applied to the bioprocesses' prediction and control.

Consequently, the determined cell-cycle dynamics of a genetically engineered microbial population could be used in correlation with the production of recombinant protein, for more effective large scale biotechnological processes.

## 3. Growth kinetics

The productivity and economic efficiency of bioprocesses is strongly influenced by the operating conditions (ambient temperature, growth media, oxygen and nutrient supply, type of bioreactor utilized, relative circulation of phases etc.) which affect the growth process of the bacterial culture. Thus, both the understanding and accurate modelling of these interactions are of paramount importance to optimize the bioreactor-bioprocess system, and to increase the effectiveness of the recombinant protein technology.

The first mathematical models describing microbial growth have been developed as early as 1912. However, the breakthrough came in the early 1950's, when Monod proposed a mathematical model, which connected the microbial growth rate to the concentration of a single limiting substrate, introducing two parameters – the maximum growth rate and a growth affinity constant.

The Monod model has achieved large scale acceptance, despite early criticism, and remains one of the most used mathematical models for describing cellular growth kinetics. However, experimentally it was observed that, as microbial cells proceed through their cellular cycle, the changes in their internal states lead to different values for the growth rates corresponding to population members of different ages, sizes, etc.

These observations led to the development of structured kinetics for modelling the growth of microorganisms in bioreactors. A structured kinetic assumes a conceptual splitting of the cell into functional parts, between which there are interactions seen as condensed metabolic pathways. Also, some of these internal lumped constituents are able to exchange mass with the cell's surroundings. This way it becomes available a quantitative evaluation of how individual cells change in time and how they interact with the environment [14].

Local measurements at single cell level are required for collecting all the information necessary for an efficient upgrade of the mathematical model, adjusting its parameters to obtain a suitable fit. Such measurements, however, are representative if the behaviour of the population can be reduced to the behaviour of a single cell or a group of cells, lumped together in a cell-class, true when synchronization of the population occurs [14]. This way, synchronicity/collective oscillations were induced in microbial populations in different ways, like starvation [17], feast and famine [18], or managing the amount of the limiting substrate [19].

Our previous studies, both experimental [20, 22] and theoretical [21], suggested that there is an intrinsic synchronicity in the population of *E. coli*. The cells growth in a chemostat displayed a periodical variation in the optical density, which was in accordance with the oxygen partial pressure variance (Figure 1 is an exemple for hydraulic retention time of 3.5h). The chemostat, operated both batch

and continuous, was equipped with *in situ* probes that monitored pH, temperature, and oxygen partial pressure ($pO_2$). The feeding media used for bacterial population contained (per liter): 0.1% wt/wt glucose, 6 g $Na_2HPO_4$, 3 g $KH_2PO_4$, 0.5 g NaCl, 1.0 g $NH_4Cl$, 0.24 g $MgSO_4$, 0.05 g EDTA, 0.008 g $FeCl_3$, 0.0005 $ZnCl_2$, 0.0001 g $CuCl_2$, 0.0001 g $CoCl_2$, 0.0001 g $H_3BO_3$, and 0.016 g $MnCl_2$ at pH 7.0 [20, 22]. As only one carbon source (glucose) was used, the metabolic switching was minimized.

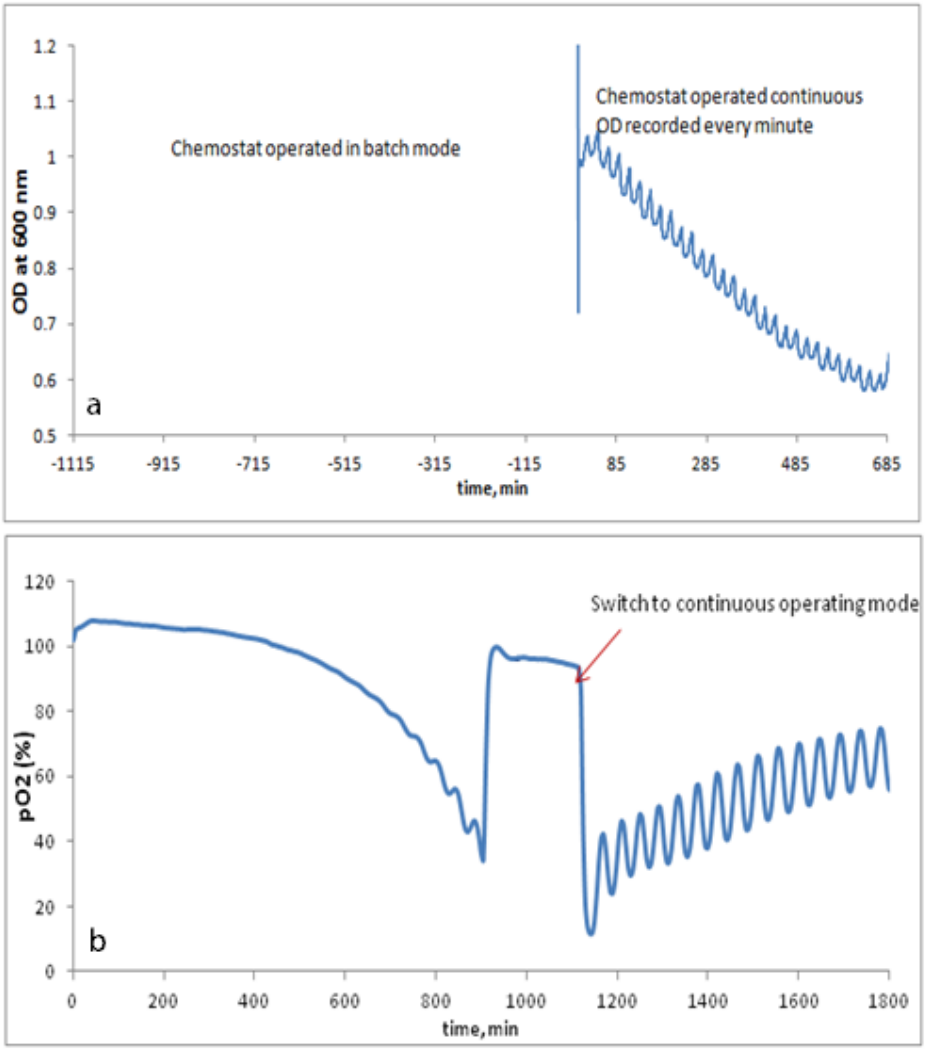


Fig. 1. OD 600 (a) and $pO_2$ (b) time profiles for HRT = 3.5 h

Optical density values are indicative of the number and state of clusters of suspended cells in the liquid phase, as dead cells have different physiological properties from live ones, suffering shrinking and lysis. For the same cumulative number of individuals in both clusters (living and dead) the higher the number of living cells, the larger the values of the optical density [20]. Analyzing Figure 1a we observe the synchronicity installed into the bacterial population due to the intrinsic phenomena of growth, division and death. The values for optical density witnessing the sudden variation of living cells' concentration (measured only for the continuous operation of the chemostat), due to the synchronous death of a large number of cells are backed up by the variation in oxygen consumption which follows the same general path: A steady increase with oscillations around a mean and a period corresponding to that of the optical density. The latter decreases with dampened oscillations, due to the aforementioned phenomena of growth, division and death. In both cases, the oscillations show an almost constant wavelength but varying amplitude, especially in the case of the oxygen partial pressure.

Based on these results, we have concluded that living bacterial populations innately oscillate under apparently steady growth and habitat conditions, and that the confined oscillatory dynamics is related to "group birth and death" events within the population.

Nevertheless, very little has known by now about innate synchronicity in systems for heterologous proteins expression in *E. coli*, if and how this synchronization can influence the recombinant protein production. Even less is known about the timing of expression, wheather it is continuous during the entire cell-cycle, or it is at a certain phase, fact which has important implications upon the operating costs.

## 4. Aging in bacterial populations

Until recently, it was believed that aging does not occur in bacteria in the presence of a sufficient amount of nutrients, and that the bacterial populations are not age-structured [23]. However, recent investigations have rebutted this theory, producing evidence for aging in both symmetrically and non-symmetrically dividing microorganisms.

Aging in bacteria, also denoted as senescence, is defined as the accumulation of defects in the cell, which results in a decrease in the functionality of the cell, its reproduction and survival rate [22, 23].

Firstly, it was theorized that asymmetrically dividing organisms such as *Saccharomyces cerevisiae* and *Caulobacter crescentus* [24] produce upon division an 'aging parent' and a 'rejuvenated offspring'. It was experimentally observed that the time intervals between cell divisions significantly increased over the

course of several generations, as cells divided with a lower rate or stopped dividing altogether with increasing age [25].

These experimental results support the theory that aging promotes significant cell-to-cell heterogeneity, implying that individuals in a bacterial population behave differently, depending on the moment they were born and the amount of cellular damage they have inherited from their parent.

The aging process in symmetrically dividing microorganisms, specifically *E. coli*, has also been evaluated [26], starting from the assumption that apparently symmetrically dividing bacteria exhibit a 'functionally' asymmetrical division process [27, 28].The fact that *E. coli* exhibits lineage – specific replicative aging, determines a variation among individuals in a population, and there is growing evidence showing that this is one of the most important factors in determining cell state [29, 30].

## 5. Conclusions

Since the recombinant protein production in *E. coli* results in increased stress response, different cell morphology, cell viability, filamentation, and growth cessation, this study points toward optimizing the cell cultivation, so that the intrinsic synchronization occur in correlation with a better expression of the useful product.

The reported results opened new fundamental questions regarding controls of fine-scale population dynamics in chemostats, and self-organization in bacterial systems, but more importantly, how such dynamics influence the process stability and control in biotechnology and engineering applications.

Further work will focus on determining if the transformed bacterial population preserves the intrinsic general dynamics of the original population of microorganisms *E. coli* (subspecies BL21/DE3), though the synchronicity characteristics alter due to the presence of the plasmid.

Based on these future experiments, a mathematical model will be developed to quantify the dynamics of bacterial growth and recombinant expression, in view of optimizing/enhancing the industrial production of this recombinant protein.

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