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Mini Review

External, extrinsic and intrinsic noise in cellular systems: analogies andimplications for protein synthesis

Pratap R. Patnaik

Institute of Microbial Technology Sector 39-A, Chandigarh-160036, India. IMTECH Communication no.034/2006
E-mail: pratap@imtech.res.in

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Multicellular systems, typically in bioreactors with one or more feed streams, are under the influences of intrinsic (intra-cellular), extrinsic (inter-cellular) and external (environmental) noise. Of these, intrinsic noise is relatively less important in determining protein synthesis and reactor behavior. Although extrinsic noise and external noise have different origins and controls, they have similarities and interactions. The interactions make it important to control both kinds of noise optimally to enhance the gene expression of a desired protein, and the similarities enable this to be done. These aspects are discussed to evolve a comprehensive noise filtering and control strategy for large bioreactors operated in realistic (noisy) environments.

Key words: cellular noise sources, analogies, interactions, protein synthesis

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1.0 INTRODUCTION

Both molecular level and macroscopic (bioreactor level) studies have shown that noise is a ubiquitous feature of microbial processes. On a macroscopic level, noise enters a cultivation vessel (or bioreactor) mainly through one or more inlet streams. Its presence is seen as fluctuations in the flow rates (Chen and Rollins, 2000; Liden, 2001; Rohner and Meyer, 1995). These fluctuations usually increase with the size of the reactor. Scale-up rules lead to the corollary inference that the influx of noise increases with the flow rate of an inlet stream.

Noise at the molecular level is linked more intimately, but not exclusively, with gene expression and coding for useful proteins. Stochasticity in gene expression is a prime cause of phenotypic variations in a population of cells (Blake et al., 2003; Elowitz et al., 2002; Rao et al., 2002; Thattai and van Oudenaarden, 2004), which in turn confers robustness to environmental disturbances and thus enhances the survival of viable cells for protein synthesis (Kaern et al., 2005; Stelling et al., 2004).

Although noise at the cellular level and at a process level arise from different sources and have different causes, one significant similarity between them is that uncontrolled noise is detrimental whereas judiciously controlled noise can be beneficial. Apart from inducing phenotypic diversity, controlled genetic noise may also favor resistance to certain diseases (Kaern et al., 2005; Seldman and Seldman, 2002) and induce cooperative inter-cellular dynamics that enhances the dominance of favorable phenotypes in adverse conditions (Chen et al., 2005). Likewise, controlled inflow of noise in the feed streams entering a bioreactor containing a microbial cult-

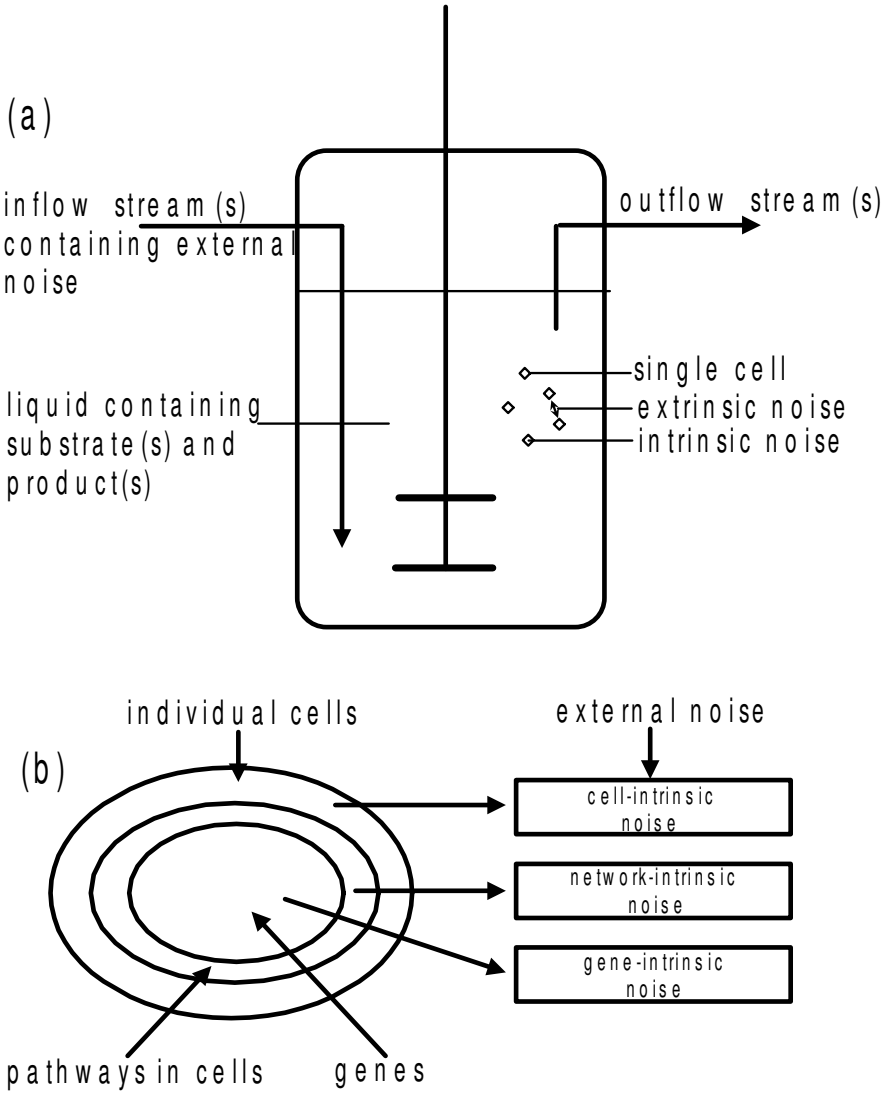

Figure 1. Schematic representations of (a) the noise sources associated with a microbial culture in a continuous flow bioreactor and (b) the locations and interactions among different sources, both within and outside the cells. Part (b) is adapted from Kaern et al. (2005) with permission from Macmillan Publishers Ltd, © 2005.

ure has been shown to increase product formation in a variety of fermentations (Patnaik, 1999, 2003a, 2004, 2006a). Interestingly, stochastic resonance seems to be at the heart of both forms of induced improvements (Chen et al., 2005; Patnaik, 2003b, 2006b), underlining a possible coherence between intra-cellular and extra-cultural fluctuations.

Recent studies (Kiss et al., 2003; Patnaik, 2006b) have demonstrated that optimal filtering of feed stream noise in continuous cultures of *Saccharomyces cerevisiae* helps to restore stable oscillations from chaotic behavior. Even if the noise is not strong enough to create chaos, a change in the variance can drive a culture from a nonoscillating (monotonic) state to an oscillating state or vice versa. These phenomena have remarkable similarities with observations for gene-intrinsic noise. For instance, the galactose-utilization network in *S. cerevi-siae* has two positive and one negative feedback loops. The positive-feedback loops contribute to the establish-ment of two stable expression states, and negative feed-back controls the rate at which the cells switch between these states in a randomly changing environment (Acar et al. 2005; Thattai and van Oudenaarden, 2004).

An active population of cells in a bioreactor experiences noise from within the cells and from the outer environment. Because substrates enter the cells for further processing, any noise present in the inflow streams also penetrates and interacts with intra-cellular noise sources (Figure 1a, b). This possibility, the similarities between the two major sources of noise, and their effects

on the dynamics of a multi-cellular system deserve careful analysis to devise methods to harness the noise in a manner that best promotes the desired protein synthesis functions of a target genetic network. Presently, the effects of feed stream noise on bioreactor performance have been studied by biochemical engineers (Chen and Rollins, 2000; Patnaik, 2004; Schmidt, 2005) while noise in genetic and metabolic networks has engaged the attention of biologists and biochemists (Blake et al., 2003; Kaern et al., 2005; Thattai and van Oudenaarden, 2004). The present overview seeks to unify these two streams of research by deriving similarities and compatibilities between external, extrinsic and intrinsic noises so as to evolve a comprehensive method to harness them to maximize the expression of desired proteins by a genetic cascade or network. The three kinds of noise are illustrated in Figure 1 and are described briefly in the next section.

2.0. Noise sources in microbial cultures

Microbial cells cultivated in a bioreactor are subject to noise within the cells as well as that from the environment. Here we designate noise from the environment as external noise, and this enters a culture medium mainly as fluctuations in the flow rates of inlet (or feed) streams. Carbon and nitrogen substrates as solutions are common feed streams; the noise they carry usually increases with the flow rates, largely because economic, practical and technological constraints place limits on the extent of control and filtering that may be employed. Data from both experimental (Montague and Morris, 1994; Rohner and Meyer, 1995; Schmidt, 2005) and simulated (Patnaik, 2003a, 2004; Riascos and Pinto, 2004; Zhang et al., 2004) fermentations indicate that feed stream noise may be characterized by a set of Gaussian distributions with time-dependent mean values and different variances. This noise has auto-correlation times from several minutes to about an hour.

If allowed to enter without any modulation, noise in the inlet streams can cause serious changes in the performance of a cellular system. Noise may displace a culture from a monotonic stable state to an unstable state or to an oscillating state or vice versa (Liden, 2001; Patnaik, 2005; Zamamiri et al., 2001; Zhang et al., 2004). Even if the displacement is to a second stable state, the latter may not retain all the relevant functional features of the original state, referred to as a loss of robustness (Kitano, 2004). Moreover, during an inter-state transition the cell culture may digress far away from both states, with consequent damage to the cells. These risks underline the importance of proper filtering of the inflow of environmental noise.

Noise entering through feed streams permeates the broth and impinges on the cells and the organelles inside, where it encounters intra-cellular noise (Figure 1a). The latter may be of either of two types, and their difference may be explained with reference to their experimental measurement. This is done by using two green fluorescence protein (GFP) reporter genes under the control of promoters regulated by the Lac repressor (Elowitz et al., 2002). The genes encode the cyan and yellow forms of GFP, which are quantified by the fluorescence intensity of their respective emission peaks. Differences between the expressions of the two genes are indicative of *intrinsic noise*, i.e. noise inside a cell. The other kind of noise, *extrinsic noise*, affects both reporter genes equally within a given cell but generates differences between cells. These differences are attributed to variations in other proteins that affect GFP gene expression.

Intrinsic noise itself may have one or more of three locations (Kaern et al., 2005). Gene-intrinsic noise pertains to molecular level fluctuations in the reaction steps associated with gene expression. Network-intrinsic noise is generated by fluctuations in signal transduction. Both these contribute to cell-intrinsic noise; other factors include metabolite concentrations, cell size and cell age. As Figure 1b shows, all these sources of noise, including noise from the environment, may interact, thereby complicating cellular behavior.

Apart from their nature and sources, one significant difference between external noise, on the one hand, and extrinsic or intrinsic noise is that the former increases with system size where as the latter two decrease. This means external noise is greater for large bioreactors than for small ones but extrinsic (or intrinsic) noise is more pronounced for systems with small numbers of (large) molecules (Kaern et al., 2005; Raser and O'Shea, 2004). Experimental data with *Bacillus subtilis* (Ozbudak et al., 2002), *Escherichia coli* (Rosenfeld et al., 2005) and *S. cerevisiae* (Blake et al., 2003) show that extrinsic noise is the dominant cause of variability in gene expression. Cultures of these organisms are also subject to external noise (Liden, 2001; Rohner and Meyer, 1995; Schmidt 2005), thereby emphasizing the importance of understandding cellular behavior under the simultaneous effects of both kinds of noise.

3.0. Effects of noise on protein synthesis

Some key observations emerge from a comparison of external noise and intra-cellular noise. Of the two main kinds of intra-cellular noise, extrinsic noise is the major contributor to stochasticity in gene expression (Blake et al., 2003; Kaern et al., 2005; Ozbudak, et al. 2002; Stelling et al., 2004). For *E. coli*, the auto-correlation time for extrinsic noise is ~40 min (Rosenfeld et al., 2005), which is four times that for intrinsic noise and approximately equal to that of external noise (Montague and Morris, 1994; Patnaik, 2003a). Preliminary analysis of the time-domain profiles of cell, product and substrate concentra-

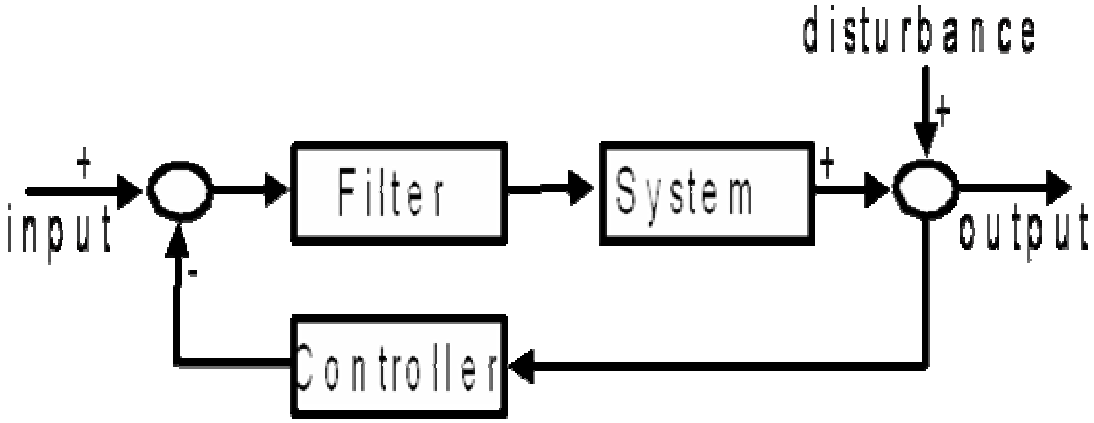

Figure 2. Information flow diagram for noise filtering and control, applicable to both external and extrinsic noise. The noisy inputs pass through a noise filter, typically a software device, and the filtered signals enter the biological system. External disturbances may impinge on this system. The outputs are fed back to a controller, which in turn regulates the operation of the filter.

trations suggest that the comparability of auto-correlation times for *E. coli* are also sustained for *B. subtilis* and *S. cerevisiae*. Variations in cellular output are symptomatic of different kinds of noise (Raser and O'Shea, 2005).

Feedback is a critical aspect of both extrinsic and external noise. A feedback stream may be either an inherent component of a reaction network or introduced as an external arrangement. Figure 2 depicts an information flow diagram common to both. The system box may be a genetic or metabolic network or a pair of cells or a multicellular fermentation broth. Complex systems may have more than one feedback loop and, correspondingly, many controllers and noise filters. Such systems tend to be robust to the impact of noise but may also be fragile and difficult to design (Kaern et al., 2005; Kitano, 2004; Stelling et al., 2004). For genetic networks, negative feedback generally provides a mechanism to reduce noise and increase stability (Becskei and Serrano, 2000; Rao et al., 2002). Similarly, negative feedback of output signals through a noise filter located upstream of a fermentation vessel improves filtering efficiency and reactor stability (Patnaik, 2004, 2006a; Dochain and Perrier, 1997).

Negative auto-regulation also minimizes fluctuations in downstream processes. By functioning effectively as a low pass filter, negative feedback allows the slower downstream processes to perceive only a time-averaged, less fluctuating signal (Kaern et al., 2005; Simpson et al., 2003). However, negative feedback can also destabilize and generate oscillations if it involves a time delay. By anology, positive feedback creates phenotypically distinct populations of cells, bistability and stochastic transitions between these states (Becskei et al., 2001; Ozbudak et al., 2004).

These effects have interesting similarities with control policies for bioreactors. Control theory teaches that negative feedback helps to return a perturbed system to its original state in a decaying oscillatory manner (Dochain and Perrier, 1997), whereas positive feedback has the opposite effect. Kitano (2004) invokes this concept in his explanations of robustness of cellular systems, thereby strengthening the correspondence between microscopic feedback in genetic or metabolic networks and macroscopic feedback in bioreactor operations. A robust (cellular) system, according to Kitano, either returns to its current attractor or moves to a new attractor that presserves the system's functions. An attractor may be either static or periodic.

Continuous cultures of *S. cerevisiae* provide a lucid example of both kinds of attractors at the genetic as well as reactor levels. Isaacs et al.(2003) and Becskei et al. (2001) studies with a single-gene autocatalytic network illustrate bistability arising through positive feedback regulation. One of these stables may be oscillatory, and gene-level fluctuations can drive transitions between the states at a rate governed by a negative feedback loop (Acar et al., 2005). Analogously, a continuous flow bioreactor may exhibit either oscillating or non-oscillating (monotonic) outputs according to the dilution rate, i.e. the flow rate per unit volume of the fermentation broth (Beuse et al., 1998; Jones and Kompala, 1999).

Like the bistability in a genetic network with positive feedback, two oscillatory and one monotonic state are possible in a bioreactor with recycle (Figure 3) (Zamamiri et al., 2001) with the intermediate state being unstable. However, just as genetic networks may be strongly sensitive to certain small perturbations that persist long enough but less sensitive to frequently occurring large fluctuations (Kitano, 2004; Rosenfeld et al., 2005), the three states in a bioreactor may have different sensitivities under different conditions. Therefore, in a highly sensitive region, noise in a feed stream may propel a culture from one state to another, and even cause chaotic behavior (Patnaik, 2005). Such undesirable transitions are avoided by using noise filters. Whereas negative auto

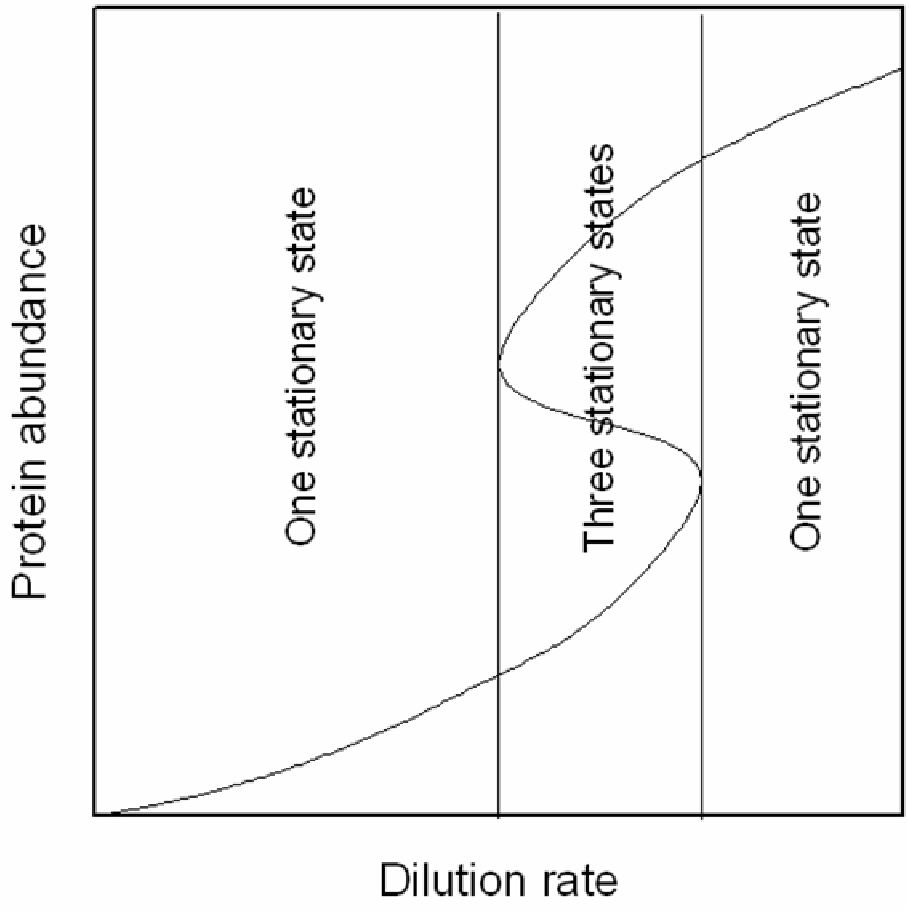

Figure. 3. Multiplicity pattern for a continuous flow bioreactor for different dilution rates. Depending on the starting conditions and the dilution rate, a continuous flow microbial bioreactor may have one or three stationary states. In a regime of three states, sufficiently strong noise may displace a culture from an existing state to another state, the latter usually less desirable. Noise filtering and control are employed to avoid this.

-regulation has an inherent filtering effect at a genetic level (Kaern et al., 2005; Simpson et al., 2003), specific filtering devices are needed for bioreactors (Patnaik, 1999, 2003a, 2003b). Interestingly, a low pass filter is one common device that functionally resembles the filtering by a negative genetic feedback.

In microbial cultures it often becomes necessary to seek a trade-off between sensitivity and productivity. This means a stationary state at which the cells synthesize a desired protein very efficiently may be vulnerable even to short duration disturbances of low intensity whereas another state that is somewhat less productive may offer a better combination of fragility and robustness. Then in a realistic (noisy) environment it may be prudent to operate at a less productive state with mild noise filtering and control.

Finally, we note that genetic buffering, through either chaperones or networks (Kitano, 2004), is a fundamental mechanism to provide robustness at a cellular level.

Buffering by the network topology is particularly effective when it is robust against external perturbances. This feature and its origin in the modularity of gene regulation are attractively similar to the buffering effect explained by certain compartment models of microbial cells. For instance, Nielsen et al. (1991) proposed the four-compartment model shown in Figure 4 for recombinant *E. coli*. Compartment A contains mRNA, tRNA and ribosomes, P contains the plasmid DNA, the recombinant protein is in compartment E, and the rest of the cell mass, comprising mainly genomic DNA and structural material, is lumped into another compartment (G). Since A accounts for about 60% of the cell mass, it buffers any noise that enters through the substrate stream.

4.0. Concluding remarks

Noise in cellular systems is generally perceived as undesirable but unavoidable. However, if the mechanisms and processes underlying the noise are properly understood, it may be possible to harness noise intelligently with beneficial results.

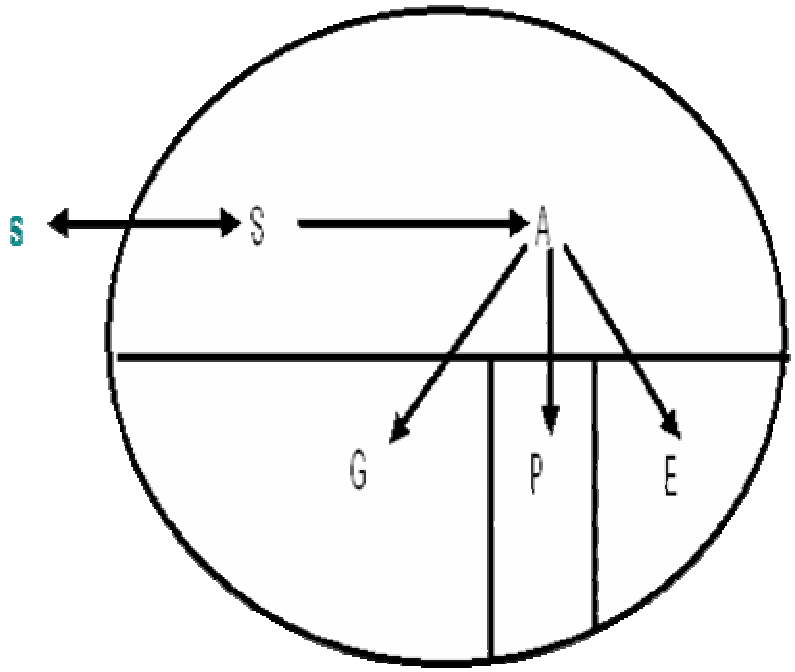

Figure. 4. Four compartment of a cell, according to Nielsen et al. (1999) s=substrate in the fluid around the cells; S=substrate inside the cells. A, E, G and P are explained in the text. Note the interactions between the substrate outside and inside the cells, and between intra-cellular substrate and the key components of a cell. The model is thus conceptually consistent with the noise sources in Figure 1 and their description in section 2.

At the cellular level, extrinsic (intra-cellular) noise is more significant than intrinsic (intra-cellular) noise. Like the external noise that enters a cell culture through a feed stream, the effects of extrinsic noise are governed by feedback loops, which may create two or three expression states. In both cases, negative feedback has a stabilizing and noise-reducing effect. Both kinds of noise have similar auto-correlation times and both may displace a culture from one state to another with different features.

These similarities and the likelihood of interactions between extrinsic and external noise, since the latter permeates the culture broth, emphasize the importance and the feasibility of noise filtering and process control strategies that, unlike most current methods, account for both kinds of noise. However, such a strategy is yet to be evolved. This is understandable since a detailed understanding of the mechanisms of biological noise generation and their effects has itself come recently. The few models proposed so far have focussed on either single cells or groups of cells (Chichigina et al., 2005; Kiehl et al., 2004), or the effects of external noise coupled with the hydrodynamics in the bioreactor (Tian et al., 2002; Patnaik, 2003a, 2004), ignoring fluctuations inside the cells. However, the similarities of techniques among these separate studies and the analogies outlined above lead to the expectation that a model encompassing both aspects is possible.

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