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Integrated modeling to capture the interaction of physiology and fluid dynamics in biopharmaceutical bioreactors

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ABSTRACT

The performance of a bioreactor is sensitive to local gradients of chemical and physical stimuli. Thus, this work presents a model, which captures spatial heterogeneity and interactions of biotic and abiotic phases in animal cell cultures. A computational fluid dynamics simulation that includes gas-liquid mass transfer and kinetics of carbon dioxide dissolution is developed to capture the variations of environmental parameters. Unstructured modeling is implemented to integrate growth, viability and productivity of cells. While predictive accuracy is valuable, it is important to balance it with computational feasibility. In this work, evolutions of hydrodynamics and cell population are obtained sequentially. The outcome is a deterministic model with extended integration between physical and biological phenomena which is computationally tractable. The model calculates the bioreactor performance as a function of time and process parameters such as impeller rotation speed and gas sparging flow rate, which makes it useful for bioprocess design and scheduling.

1. Introduction

Industrialization of mammalian cell culture has been achieved by integration of knowledge from several core concepts of chemical engineering, cellular and molecular biology, and biochemistry. Mammalian cells currently represent the platform of choice for production of therapeutic protein, molecules used in diagnostic tests and vaccines. Protein produced in mammalian cell culture represents half of the annual revenue generated by the biotechnology industry (Davidson and Farid, 2014).

Biopharmaceuticals generate global revenue of $163 billion which is around one fifth of the market of pharmaceutical industry (Otto et al., 2014). The revenue grows at more than 8% annually which is double that of conventional pharma. The number of biotech patents applied for every year has been growing at 25% annually and the percentage of drugs which make it to the market is over twice that of small-molecule products. The growing demand for therapeutic proteins from mammalian cells and increasing focus from regulatory bodies on product quality have driven the requirement to improve in manufacturing capacity and efficiency. The capacities of manufacturing sites has increased up to 200,000 L due to utilization of large scale bioreactors (as large as 25,000 L) (Farid, 2007).

Moreover the average commercial scale titer for mammalian-expressed products has increased from 0.2–0.5 g/L in early 90's to 2.5 g/L in the recent years (Rader and Langer, 2015; Li et al., 2010). This has been achieved via improving vectors, host cell engineering, clone selection, gene amplification and cell line screening (Wurm, 2004) and optimum design of medium, feeding strategy and process engineering (Xie and Wang, 1996). Advancements in biology have outpaced development of mathematical modeling and analysis in biochemical engineering. In most cases cell cultures have been modeled assuming homogeneity for either environment (Dorka et al., 2009; Sidoli et al., 2006) or cell population (Schmalzriedt et al., 2003). Coupling of environmental parameters and cellular metabolism rarely includes more than concentrations of few metabolites (Mantzaris et al., 1999; Meshram et al., 2013). Scale-up methodologies have been mainly based upon characterization of mass transfer phenomena or agitation system.

Currently, research has focused on development of mechanistic tools for process scale up to further understand some of the challenges of scale-up including reduction in productivity and increase in byproduct formation (Pigou and Morchain, 2015). Although the necessity of integration of systems biology and process development has been considered and partially addressed (Meshram et al., 2013; Song et al., 2013; Fernandes et al., 2011) a robust framework for development of mechanistic unit operation models has yet to be

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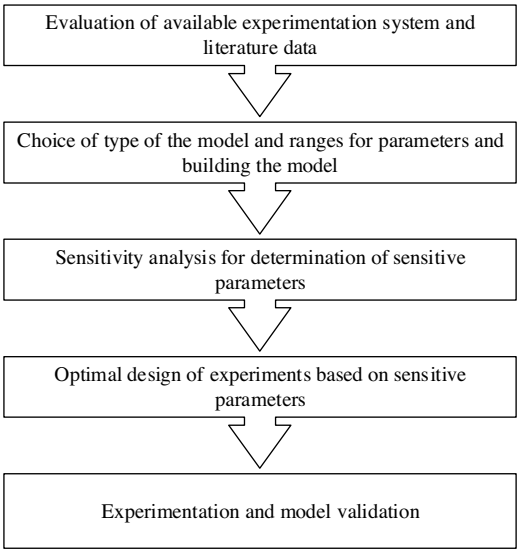

Fig. 1. Systematic development of predictive mathematical models for animal cell cultures.

established (Chen et al., 2016). An instance of systematic approach toward modeling cell cultures is the strategy devised by Pistikopoulos and his colleagues (Fig. 1) (Kontoravdi et al., 2010; Kiparissides et al., 2011).

The model discussed here has been developed following the first two steps of this framework. Model parameters have been chosen based on availability of experimental data and model structure has been designed with the mindset of capturing spatial heterogeneity inside the reactor and integration of environmental mechanical and chemical stimuli with cellular metabolism. The state and input variables of a bioreactor model can be divided into four groups. The first group includes operational parameters such as agitation and aeration rates and temperature; the second group consists of environmental parameters such as pH, concentrations of chemicals inside the bioreactor (i.e. constituents of cultivation medium, dissolved oxygen and carbon dioxide); and the third group comprises of biochemical (intracellular) parameters that can be used to describe the metabolic state of cells including cell mass composition; enzymes and proteins. Finally the fourth group includes macro-biological parameters to take into account contamination, degeneration, aggregation, and/or mutation (Nicoletti et al., 2009).

Fig. 2 explains interactions between the first three groups of these parameters (Li et al., 2010). The desirable operating condition is maintained through control of pH, dissolved oxygen concentration, temperature and pressure. The elements which together define cellular environment and are directly or indirectly affected by selection of control strategy are shown in Fig. 2. The features incorporated in this simulation model make it possible to study effects of impeller rotation speed, CO_2 and O_2 sparging flow rates and pH on growth, viability and productivity of cells in a batch-mode operated bioreactor. Those are shown underlined in Fig. 2. In the next section, the components of the model and their integration are explained. Section 3 includes results for different combinations of aeration and agitation rates whereas Section 4 offers some discussion on challenges and potentials in the area of bioreactor modeling.

2. Model development

To capture effects of agitation and aeration on growth, viability and productivity of cells, a model of a large-scale bioreactor should take into account fluid dynamics, gas-liquid interaction, interphase mass transfer, cell source variability and metabolism. In the rest of this section each of the components of the model is explained then the strategy used for running the dynamic simulation is explained.

2.1. Hydrodynamics

Inefficient mixing creates spatial gradients in pH, dissolved oxygen (DO), carbon dioxide and metabolites concentrations, shear and temperature. As cells travel inside the reactor they are exposed to fluctuating environmental conditions which affect metabolism, yield and quality of product (Lara et al., 2006). Most of the models developed for bioreactors have assumed homogenous environment and been validated with data extracted from laboratory scale bioreactors (Dorka et al., 2009; Sidoli et al., 2006; Meshram et al., 2013; Mantzaris and Daoutidis, 2004; Mantzaris, 2006; Fadda et al., 2012; Jandt et al., 2015; Craven et al., 2014; Sbarciog et al., 2014; Amribt et al., 2013). So further improvements of these models are necessary before using them for scale up analysis (Farzan et al., 2016).

Spatial and temporal variations of environmental parameters are captured by modeling hydrodynamics of the reactor through implementation of computational fluid dynamics (CFD) using ANSYS® Fluent® 15.0.7. The environment refers to physical and chemical stimuli acting on cells. In the proposed model physical stimuli include gas volume fraction, superficial gas velocity and dissipation rate of energy. Chemical stimuli are limited to concentrations of metabolites (glucose, glutamine, lactate and ammonium) and dissolved oxygen; and pH. A more comprehensive model may include concentrations of other supplements which have functions such as species transport enhancement, growth stimulation, shear protection and surface charge modification. The addition of a component imposes extra computational burden on solver, thus it is important to balance computational requirements of various components of the model. The flow of a single phase, gas or liquid, is described by conservation laws of mass, momentum, energy, charge, etc. If thermodynamic, transport and chemical properties of a component needs to be specified these field equations may be accompanied by the constitutive equations of state, stress, chemical reactions, etc. Due to low solubility in water, high densities of cells quickly consume all the oxygen in a saturated culture and produce enough carbon dioxide that it can have inhibitory effects. As a result addition and removal of gases are inevitable parts of operation of a large-scale fermenter.

In multi-phase flows the presence of interfacial surface makes mathematical formulation of the problem much more difficult. To derive the field and constitutive equations of multi-phase flow local characteristics have to be considered which is not straight forward. This difficulty is the result of unknown motions of multiple deformable interfaces, variables' fluctuations due to turbulence and moving interfaces and discontinuity of properties at the interface. It has been concluded that by obtaining mean values of flow properties through proper averaging local instantaneous fluctuations are eliminated. Three methodologies have been introduced for averaging: Eulerian, Lagrangian and Boltzmann statistical averaging. The Eulerian approach takes time and space coordinates as independent variables and other variables are expressed with respect to them. In the Lagrangian description, particle coordinates replace spatial coordinates which gives clear advantage to this method if the behavior of individual particles is of interest. On the other hand if the focus is group behavior of particles the Eulerian approach is preferred (Ishii and Hibiki, 2011). Tracking individual bubbles imposes extra computational cost and would improve model's predictive power only if there was sufficient knowledge on interactions between individual bubbles and the liquid phase; i.e. growth, breakage and agglomeration of bubbles and energy dissipation due to bubble rupture. Therefore gas and liquid phases are considered as continuums and Eulerian averaging is used in this