UNIFICATION OF MIXING AND CELLULAR METABOLISM IN BIOREACTOR MODELING: A MULTI-SCALE APPROACH

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Keywords: bioreactor; CFD; mixing; multi-scale modeling

ABSTRACT

In this paper, we address the progress, challenges and prospect of modeling of mixing in bioreactor. We aim to briefly review the progress in the integration of the mixing behaviors into bioreactor modeling and simulation as well as the obstacles faced towards this integrative modeling effort. Efficient operation of bioreactor is crucial in the biotechnological process, not only to ensure good yield but also to maintain the consistent product quality. Process modeling technique has frequently been adopted in the bioreactor design, optimization and control as well as in scale-up process. Transport phenomena problems have generally posed challenging tasks in the bioreactor scale-up. In this respect, the integration of mixing phenomena into the bioreactor modeling is considered as a vital aspect in the design and scale-up of bioreactor.

INTRODUCTION

Bioreactor has been recognized as the heart of biotechnological processes which provides the central link between the raw materials and products (Cooney 1983). Its use in the biotechnological applications requires proper design that can meet the specifications of the cell-culture environments by addressing parameters such as temperature, oxygen, pH, nutrients, metabolites and biologically active molecules.

The bioreactor operation is multi-scale, whereby cellular level consists of numerous biochemical reactions catalyzed by thousands of enzymes (Schügerl & Bellgardt 2000). The system consists of multiple steady states and it will respond to rapidly changing environment but which will produce sluggish response in output (Leib et al. 2001). This type of response is one of the challenges that need

to be addressed in research as it has important implication on the process control performance.

The design, mixing phenomena and nature of biocatalysts are the factors which will influence the overall bioreactor performance. The proper design of the bioreactor is crucial to the entire production process, whereby it is vital to ensure that optimal conditions for growth and product formation of the microorganisms are achieved. To ensure optimal conditions can be maintained throughout the bioreactor, it is crucial to ensure good mixing (i.e. to maintain homogeneity). The key objectives of mixing are to overcome transport phenomena limitations and to homogenize the conditions inside the bioreactor, i.e. to avoid dead zones. Bioreactors typically operate well in a specified condition of temperature, dissolved oxygen, pH and biomass concentration (Hutmacher & Singh 2008).

Various kinds and sizes of impellers are applied for agitator depending on the properties of the biological system, the size of the reactor and the cultivation medium (Schügerl & Bellgardt 2000). Evaluation of mixing phenomena in bioreactors is of great practical interest in the chemical process industry. In this respects, the Computational Fluid Dynamics (CFD) has proven to serve in the engineering of fluid flow systems, where more comprehensive picture of fluid mechanics and nutrient transport within process equipment can be accomplished (Bode 1994).

But it is interesting to note that good mixing relies also on the design of the bioreactors. Thus different types of bioreactors are developed to optimize the homogeneity of the liquid phase, gas dispersion, transport properties as well as mechanical stress (Schügerl & Bellgardt 2000). Besides, different design of bioreactor is required to overcome certain transport phenomena limitation. On the other hand, mixing will increase the mass transfer in cell cultures, nutrients supply and oxygen to the living cells in the bioreactor (Menisher et al. 2000). Different biocatalysts require different operating conditions, for example mammalian cells use airlift bioreactor instead of mechanical mixing bioreactors since the cells are fragile (Luo & Al-Dahhan 2008).

Typical scale-up problems result from inadequate inter-phase mass transfer or removal of heat from the reactor, and from non-uniform temperature and concentration profiles in the reactor. Low dissolved oxygen content and poor distribution of nutrients will harm the biomass and modify the microbiological metabolism (Moilanen et al. 2006). Mixing of high concentration substrate feed and unexpected behaviors of the biomass, i.e., flocculation, foaming or growth on the reactor wall above the liquid due to splashing, are other typical problems. Therefore, efficient mixing is vital to obtain profitable yields or to eliminate process problems.

The aim of this paper is to briefly review the progress and challenges in modeling and simulation of bioreactor mixing. Additionally, this paper highlights some of the research gaps in the current modeling approaches of mixing in bioreactor.

PROGRESS

The approaches to studying mixing in bioreactor can be broadly categorized into (1) modeling and simulation approach, (2) experimental approach, and (3) combination of both. The key reason for adopting modeling and simulation approach is to provide quick insights into the key behavior of system being studied. Thus, this provides directions for generating hypotheses and clues in the planning of further experimental works. In the latter case even fairly crude modeling and simulation study could significantly reduce the number of experimental runs and time.

Bioreactor Modeling

The Figure 1 shows the 'overall picture' of the role of modeling in bioreactor which is essentially to help the engineers finding the best strategy to meet the specified bioreactor performance. This bioreactors performance can be broadly divided into two categories, which are (1) steady-state performance, and (2) dynamic operability performance. Examples of the steady-state performance commonly encountered in biotechnological processes are yield, conversion and productivity. The dynamic operability performance is normally measured based on how easy to control the system of interest and this is generally depends upon the design of the system i.e. bioreactor.

Furthermore, there are two generalized techniques of modeling. The prevalent approach is called formal approach (Moser 1984) where the unstructured kinetics of the microorganisms are used to develop bioreactor model i.e. macro-scale approach. The key idea of this approach is treat the cells as black box – ignore the complex numerous enzymatic biochemical reactions inside the cells or microbe. The main limitations of this approach are (1) limited range of application of the resulting bioreactor model, (2) lack of prediction of cellular physiology (e.g. shift from respiration to respirofermentative), and (3) unable to predict the micro-scale conditions drift from normal operation (e.g. at branched point the switching from desirable pathway to another desirable pathway might occur unnoticed).

Therefore, the recent efforts attempt to overcome the limitation of this macro-scale approach through the use of multi-scale modelling technique, which incorporates into the bioreactor modeling the details of cellular metabolism – from macro- to meso- and micro-scale. Using this technique it is possible to identify the bottleneck steps in the cellular metabolism and how this is affected by the macro-scale conditions. Thus this enable the timely correction of the macro-scale conditions before, for example, the switching of the pathway reaction to undesirable one in the event of external disturbance occurrence.

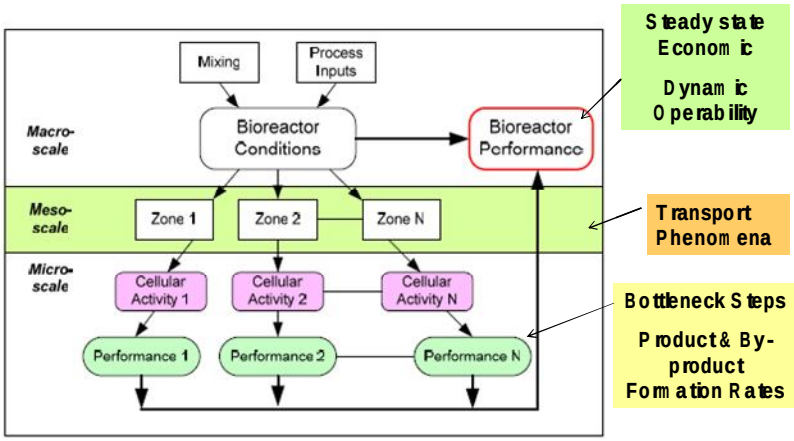

FIGURE 1: Generalized framework of multi-scale modeling of biosystem (Nandong et al. 2008)

But up to date the study at the meso-scale level is still very limited. The key significant at this level is to capture the transport phenomena problems i.e. mass and heat transfer processes. Very often, in the multi-scale modeling, the meso-scale effects are ignored – ideal mixing assumption is made. Thus it is the focus of this paper to highlight what are the current approaches to studying this meso-scale effects and how the related challenges have been addressed.

Modeling of Mixing in Bioreactor

Mixing models are generally based on the concepts of macro-mixing, characterized by the residence time distribution, as well as micro-mixing, which is characterized by the degree of segregation intermediate between the state of complete segregation and ideal mixing (Rao & Edwards 1972). The mathematical modeling of biotechnological processes is an extremely wide field that covers all important kinds of processes with many different microorganisms or cells of plants and animals.

In the CFD study of bioreactor, the k- ε turbulence model is normally utilized to describe the mixing behavior and to compute turbulence in the bioreactor. However, these studies are mainly used to modeling small-scale experimental systems so that the computational results can be compared to measured values. Many of these studies have been reduced to a 2D flow due to the excessive computational cost of 3D calculations (Smith 1996). One of the techniques to reduce the excessive computational burden is multizonal approach – i.e. divide the equipment volume into a network of interconnected zones. But the primary weakness of this representation is the difficulty of characterizing the mass and

energy fluxes between adjacent zones. Thus, one way to overcome this weakness is to adopt the so-called hybrid multizonal/CFD approach (Bezzo et. al. 2003).

CFD has been used for modeling mixing problems in recent years (Marchisio & Barresi 2003). CFD modeling is one of the most effective methods utilized in characterizing flow fields, provided that the models are corroborated by experimental velocimetry methods, such as laser Doppler anemometry (LDA). Furthermore, CFD codes normally facilitate in the visualization of flow phenomena which is beneficial when it is impractical to position probes within the fluid domains for the measurement of parameters such as pressure and velocity (Hutmacher & Singh 2008). Time savings are involved to solve the problem depending on the complexity of the problem and its set-up.

Several models have been suggested by various authors such as The Monte Carlo Coalescence Model of Spielman and Levenspiel (Rao & Edwards 1972). The Two-Environment Model of Ng and Rippin and the Monte Carlo residual life time model of Kattan and Adler Levenspiel (Rao & Edwards 1972), which is developed for studying the effect of arbitrary residence time distribution and intermediate degrees of segregation on chemical reactor performance. It is stated by the author that the model of Ng and Rippin is the simplest to use and should be applied for reactors with mixed feed and the Monte Carlo model of Kattan and Adler, which is a more general model than that of Spielman and Levenspiel, be used for simulating reactors with unmixed feed (Rao & Edwards 1972).

Experimental Approach to Studying Mixing in Bioreactor

Most of the experimental work reported in the literature is based on the Laser Doppler Velocimetry (LDV) techniques to investigate the flow characteristics (Dhainaut et al. 2005). In this work, Cui also showed that Particle Image Velocimetry (PIV) can has a good agreement with LDV. Experimental studies based on PIV and LDV are reliable for mixing studies, thus it would be a good approach to utilize these studies for further improvement to be made (Hutmacher & Singh 2008).

It is vital to justify parameters which are to be utilized in experimental studies of the mixing bioreactor. Mixing time (θ) is the most commonly used parameter to quantify mixing behaviours in a system. This parameter is a function of several numbers of variables such as rotational speed of the agitator (n), direction of rotation, and for propeller only, location of the propeller and inclination of the shaft, presence of baffles and type of rotor. A formula for mixing time as a function of the tank geometry, size of Ruston impellers, number of impellers and operating condition is given in (Cui et al.1996):

$$\frac{\theta\varepsilon^{1/3}}{T^{2/3}} = C\left(\frac{D_h}{T}\right)^{-4/3}\left(\frac{L_s}{H}\right)^{2}\left(\frac{H}{T}\right)^{2} \qquad (1)$$

Where C = 0.11 for 95% homogeneity mixing time, L_s = H+N_1T, T is the diameter of tank, D_h height of the blade, ε the power input per mass, and N_1 number of impeller. As θ has only been arbitrarily defined, it is interesting to identify whether the general results depend in some way on the chosen conditions of measurement (Kramers et al. 1952). Apart from the operating conditions, the size and location of the measuring cells, salt concentration of liquid in the tank and injected liquid into the tank as well as the criterion for sufficient mixing have also an influence on the measured mixing time.

CHALLENGES

The challenge in bio-reaction engineering research is to develop the potential of the organism to transfer different substrates to a range of metabolic products, including desired heterologous proteins. The scale-up of substrate admixing has caused many dissatisfaction in bio-processing of bulk chemicals even though bench-scale experiments were performed. There are several patents which describe optimal construction of feed ports and of mechanical agitators. There are also many academic studies of mixing in bioreactors, whereby both experimental studies and theoretical papers based on more or less realistic compartments in the reactor, and recently also using CFD, but still only with water-like media (Leib et al. 2001).

Lack of suitable models and the complexity of the bioreactor is another obstruction in simulations (Moilanen et al. 2006). The complicated structure of the metabolism and the mechanisms of its regulation are still not fully understood. The exact description of the fluid movement by a simple model is not possible due to the main liquid flow caused by the stirrer is overlapped by turbulence fluctuations. The situation is made more complex by the presence of two or more phases. The accurate description of the biological, chemical and physical processes and their interrelation in stirred tank (ST) reactors is impossible and therefore considerable abstraction is necessary.

The operations of complex processes such as mixing require massive computational time. For example in the case of population balance over the range of possible cell sizes in terms of tens or hundreds of scalar quantities, requires both computational and memory overhead burden. Thus, it is a challenge to discover a way to solve this problem in order to lessen the burden (Bezzo et al. 2003).

The rates measured at steady state can be in principle be used to calculate a reaction rate expression for the overall conversion of substrates to products. However, it is proven to be difficult due to large number of substrates and products makes it virtually impossible to include all inputs in the rate expression as well as the stoichiometry of the postulated single reaction, which will in most cases change with the reaction environment. Most of the mixing models used by CFD were assumed to be well-mixed and limited to simple geometries. Thus it

would be a challenge not to assume a well-mixed bioreactor tank with complicated geometries, such as 3-dimensional property variations (Bezzo et al. 2003).

CONCLUSION

CFD modeling provides a powerful means for enabling the full characterization of 3D flow fields in bioreactors with simple and complex geometries, as well as to allow prediction and possible design optimization, flow, nutritional and metabolic requirements of cells, without having to perform numerous and expensive bioreactor experiments, potentially saving time and resources. Experimental and computational simulations will complement with each other and will therefore lead to improved bioreactor characteristics in the future.

ACKNOWLEDGEMENT

This paper is part of the work supported by MOSTI (grant no. 02-02-07-SF0001).

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