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Modeling a Cell-Culture Process Between Shake Flask and Stirred-Tank Bioreactor: Shear Considerations on Early Stage Scale-up

Abstract

The growth and titer performance of mammalian cells is known to be greatly affected by the cell environment. In addition to other process parameters, the physical fluid environment, or shear, that the cells experience can also affect their performance.

When a cell line, selected for high productivity and growth in a fed-batch shake-flask environment, is to be translated to a stirred-tank bioreactor, divergence in cell-line performance between the two systems may be attributed to many differences in the physical systems, including the shear experienced by the cells. However, computational fluid dynamics (CFD) can guide operations of both shake-flask scaled-down models and conventional stirred-tank bioreactors to more closely mimic each other according to the hydrodynamic forces experienced by the cells.

Benefits of more closely mimicking the fluid environment between these models is discussed in terms of:

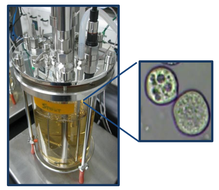
1) **how shear affects productivity of the cell line;**
2) **how scale-down models may be improved to more closely mimic the fluid environment within a stirred tank bioreactor, and**
3) **how predicting the cell's reaction to shear earlier in cell line development may lower development costs by minimizing delays due to scale-up issues.**

Introduction

Physical Situation

Macro Environment
- *Fluid Models*
- *Process Analytical Techniques (PAT)*

Provides impact of reactor heterogeneity/operation



Micro Environment
- *Fluid Models*
- *PAT*

Provides experimental data on rate-limiting steps that impact growth and productivity

Physical Situation: Define a "Control Volume"
Understand the Smallest Volume that Contains all of the Key Physics

Defining Shear

Mammalian cells can be greatly affected by their environment. In addition to other factors, the hydrodynamic forces experienced by cells have been observed to affect cell behavior. In actively mixed systems—like cell cultivation systems—the main hydrodynamic force that may potentially damage cells comes from spatial differences in fluid velocity throughout the vessel. These spatial velocity differences strain entrained particles, and this is quantified by the strain-rate or shear.

For Fluids:

$$\tau(y) = \mu \frac{\partial u}{\partial y}$$

Where:
τ(y) = shear stress (=strain rate)
y = distance perpendicular to flow
u = velocity
μ = dynamic viscosity

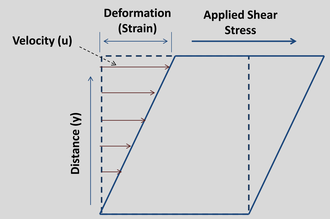


Figure 1. Schematic Illustration of Fluid Shear

Background

Differences in growth and titer are seen between different cultivation systems

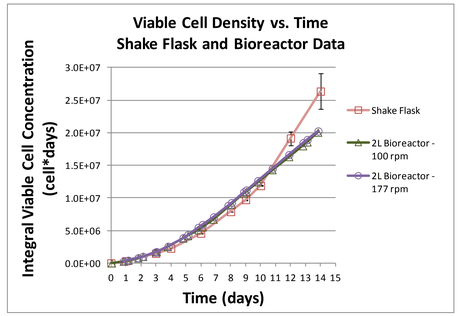


Figure 2. Integrated Viable Cell Concentration Versus Time for Different Cultivation Systems

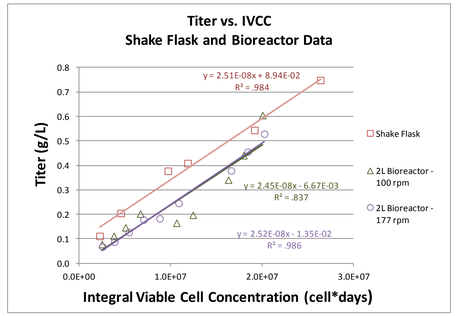


Figure 3. Product Concentration Versus Integrated Viable Cell Concentration for Different Cultivation Systems

Hypothesis: ***Shear distributions in different cultivation systems contribute to the observed growth and titer differences between systems.***

Methods

A clone was cultivated in fed-batch culture in three model systems:

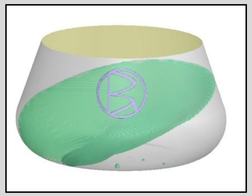
1. **Shake Flask – 120 rpm agitation;**
2. **2 L Stirred-tank bioreactor – 100 rpm agitation; and**
3. **2 L Stirred tank bioreactor – 177 rpm agitation**

Systems were modeled using CFD to produce a strain-rate distribution.

Actual Shake Flask

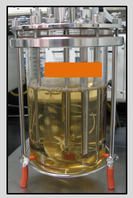
Shake Flask Operating Conditions:
- **DO ~ 80% air sat. (uncontrolled)**
- **pH = 6.9**
- **Temp = 37° C**
- **Rotation = 120 rpm**
- **Throw = 25 mm**
- **Volume = 50 mL**
- **Flask = 250 mL**

Modeled Shake Flask



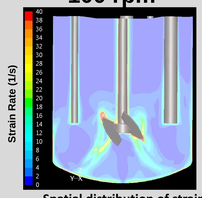
Predicted air-liquid interface in 2-phase shake flask model.

Actual Bioreactor

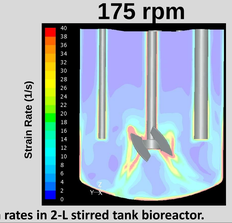


Bioreactor Operating Conditions:
- **DO = 30% air sat.**
- **pH = 6.9**
- **Temp = 37° C**
- **Impeller = pitched blade**

Modeled Bioreactor 100 rpm



Modeled Bioreactor 175 rpm



Spatial distribution of strain rates in 2-L stirred tank bioreactor.

Results and Discussion

As shown in Figures 2 and 3, significant differences in cell growth and mAb production were observed between shake flask and bioreactor systems. However, no significant differences were observed between bioreactors with different impeller speeds.

CFD models were created of the different cultivation systems. The volume distribution of strain rate was calculated for liquid volume of the shake flask and for the region adjacent the impeller in the bioreactor. The results are shown in Figure 4.

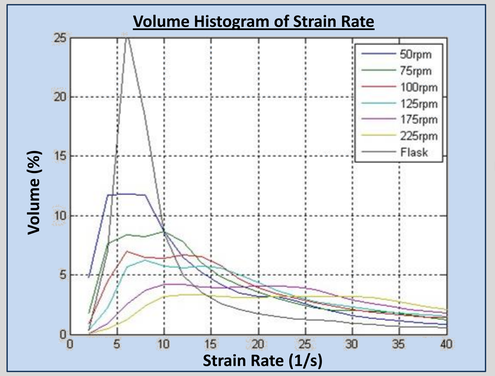


Figure 4. Volume Histogram of Strain for Different Cultivation Systems

In the shake-flask system, the shape of the strain rate distribution has a single peak. This contrasts with even the lowest-energy input bioreactor system, where a second peak at a higher strain rate forms in addition to a primary peak that is similar to that shown by the shake flask. As energy input (rpm) increases in the bioreactor, the lower shear primary peak becomes less important, and the secondary higher shear peak begins becomes increasingly significant.

Variations in the shape of the strain-rate volume distribution may explain some of the differences observed between the shake flask and bioreactor growth and titer curves shown in Figure 4. In general, it can be observed that the distributions for the bioreactor conditions (100 and 175 rpm) are more similar in shape and relative shear to each other than they are to the shake flask.

Conclusions

Differences were observed in growth and titer production in fed-batch culture between cultivation systems. When the hydrodynamic forces of each system were investigated by CFD, it was seen that the differences might be explained by the shape of the strain-rate volume distributions in the systems.

Further work is needed to validate this hypothesis. Fed-batch cultures in baffled shake flasks may more closely mimic shear conditions in stirred-tank bioreactors, and these studies are on-going. Other relevant scale-down models will also be evaluated, including using TPP tubes and 96- and 24-well micro-titer plates.

Predictive cell culture scale-down models are essential to improve throughput for cell-line selection and process development. Continuous improvements in our understanding of scale-down systems, and how cells are affected by these systems, may enable us to be more predictive earlier in development, and therefore aid in our ability to efficiently and effectively develop quality, robust processes.

Authors: Brandon Downey, Lynn Davis, Rick Falk, Ben Brinich, Brian Russell, Jeff Breit, and Lisa Graham

Bend Research Inc., 64550 Research Road, Bend, OR 97701 USA•1-800-706-8665•©Bend Research 2012

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