Design and Scale-Up of Bioreactors Using Computer Simulations

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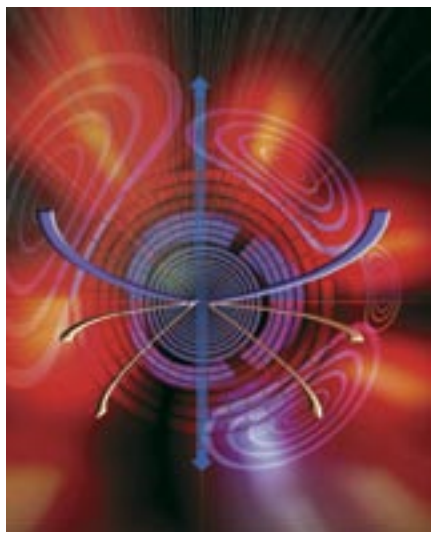
Bioreactors are increasingly used to make a variety of products across several industries. For example, they are used in the manufacture of antibiotics such as penicillin. About 70% of ingredients for the food industry are made through fermentation. Bioethanol is gaining importance as a viable alternative fuel, and it is made from fermented agricultural waste corn. Cell-culture bioreactors lie at the heart of the processes used to produce large-molecule, protein-based therapeutics.

As a result, it is becoming ever more important to scale up bioreactors for meeting higher demands of quantity and efficiency of production. Design, construction, and evaluation of bioreactors for large-scale production are costly and time consuming endeavors. Some critical limiting factors are fluid mechanics effects such as nonideal mixing, nutrient and oxygen distribution, and mass transfer. Distribution of gases such as oxygen, carbon dioxide, and nitrogen in a bioreactor used for production of therapeutic biomolecules can affect product titers.

Yield of biomolecules in an aerobic bioreactor varies greatly with overall oxygen mass transfer (KLa). The usual practice in bioreactor operation to improve yield is an increase of oxygen intake. However, increasing gas flow rates causes two problems. First, it imparts excessive shear force on cells and biomolecules, thus potentially damaging them, although there is some lack of experimental data to support that theory. Second, excessive oxygen causes foaming, which affects reaction volumes—thereby affecting productivity. Bioreactor operating and process conditions are often established by experimental work, which not only increases project costs, but also delays product launch. (Biomolecular yields also depend on biochemistry and cell biologies that are outside of scope of this article.)

Computational approaches based on computational fluid dynamics (CFD) can be used to simulate and optimize mixing, gas hold-up and mass-transfer coefficients, and distribution of gases within bioreactors. In addition, CFD can be used simulate upstream process steps such as clean-in-place (CIP) activities, sterilization cycles, and the location and rates for adding feed, nutrients, and buffers. CFD can help optimize a bioreactor process by quantifying shear stresses, flow fields, and mass transfer characteristics. Downstream processes such as scale-up of chromatography columns can be studied using CFD. Here I focus on hydrodynamic and mixing effects during scale-up of bioreactors and coupled multiphase gas–liquid hydrodynamics and oxygen transfer in airlift and stirred-tank bioreactors.



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KEYWORDS: FERMENTATION, SCALE-UP, CELL CULTURE, STIRRED-TANK, AIRLIFT, COMPUTER MODELING

LEVEL: INTERMEDIATE

IMPORTANCE OF MIXING

Most bioreactors used in industry are stirred-tank reactors that involve fluid mixing. Vessel configurations and impellers can influence product quality, yield, and purity. Typically, the stirred tank design offers nonideal mixing.

Analyses based on CFD and mixing theories can provide significant insight into bioreactor scale-up. CFD can characterize mixing effects including prediction of blend times, power numbers, turbulence quantities, and shear quantities. CFD also predicts the

time-history of shear and turbulence quantities experienced by cells in a reactor. Using such statistics can be a powerful mechanism for scaling up bioreactors along with the traditional power number or tip-speed rules.

For example, during scale-up or scale-down analysis, using those predictions can help in specifying impeller rpm speed, location(s), and type(s). Most companies don't have the flexibility for changing impellers or purchasing new equipment, so this translates to choosing the right reactor vessel from a set of available reactors within the facility. Figure 1 provides an example of a mixing time analysis.

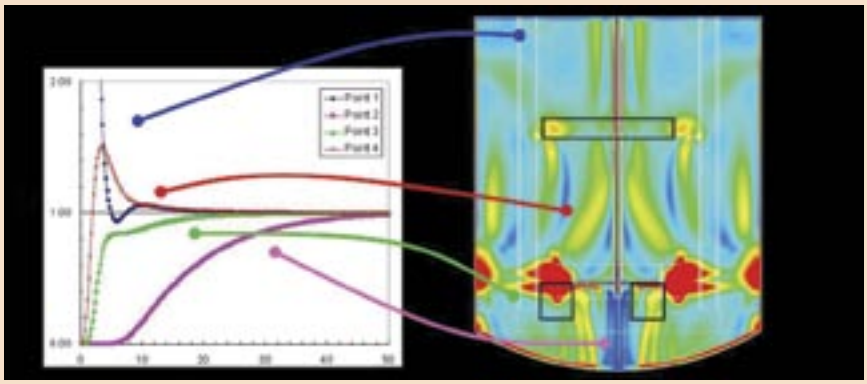
CFD predictions often can be easily validated using small-scale experiments such as scale-down runs. Because the fundamental principles do not change with scale, such models can apply across multiple scales.

Importance of Gas–Liquid Mass Transfer

In large-scale aerobic bioreactors, oxygen is usually a limiting nutrient due to its low solubility in culture media. Dissolved oxygen (DO) concentrations need to meet critical values for oxygen demand by microorganisms. The rate of respiration falls off below a DO concentration of 0.005–0.02 mM/L for most organisms. This aspect of the environment also must be predictable over a range of scales that can span four orders of magnitude between laboratory research and production levels. In mammalian cell-culture reactors, CO_2 production and resulting mass transfer are also of interest. The modeling approach described below for oxygen transfer is general enough to apply in studying CO_2 evolution.

Blending uniformity is essential for oxygen distribution in a bioreactor, but other factors influencing mass transfer include bubble size distribution, DO concentration, and fluid pressure. Bubble size dictates available interfacial areas for gas–liquid mass transfer and is influenced by parameters such as shear rate, turbulence, and buoyancy. Bubbles break up and coalesce as a result of their interactions with turbulent eddies, which gives rise to a distribution of bubble sizes. When bioreactors are scaled up from laboratory to production size, their design must meet both oxygen distribution and oxygen mass transfer requirements. So accurate predication of bubble size distribution is needed for predicting flow characteristics and interfacial areas for heat and mass-transfer calculations.

Figure 1: (LEFT) Dimensionless tracer concentration monitored at different locations as a function of time—the missing time (that taken to reach 99% uniformity) is spatially dependent as indicated by the different curves approaching the dimensionless tracer concentration of 1.00 at different times; (RIGHT) velocity contours in tank show regions of high velocity in red and low velocity in blue.



An Introduction to Computational Fluid Dynamics

Computational fluid dynamics (CFD) refers to solving fundamental conservation (transport) equations for fluid flow, heat and mass transfer and related phenomena like chemical reactions using numerical methods. The approach builds a three-dimensional model of any unit operation and fills the fluid flow region with large number of control volumes that are all connected to each other. On each one, the basic conservation of mass, momentum and heat transfer is solved, so a global conservation of those quantities is automatically satisfied.

In simple terms, CFD models complex unit operations as a network of well-mixed compartments that conform to the boundaries of flow geometry. So it works on any arbitrary geometry, including moving parts on a very fundamental level. The number of well-mixed compartments can be as many as several million, providing fine spatial resolution of flow features and related processes occurring within the unit operation. The basic benefit of this modeling approach is increased process understanding and insight into a unit operation.

CFD technology is well established in the aerospace and automotive industries and entered the chemical industries over the past decade. Wherever "design it right the first time" is encouraged, modeling fits in as a virtual laboratory before prototypes are built. Recently, CFD has seen increased interest in the bioprocessing arena, where insight into fluid flow and related phenomena (e.g., heat and mass transfer) can help manage risks associated with scale-up of bioreactors and downstream processes. Find more information at www.cfd-online.com/books.

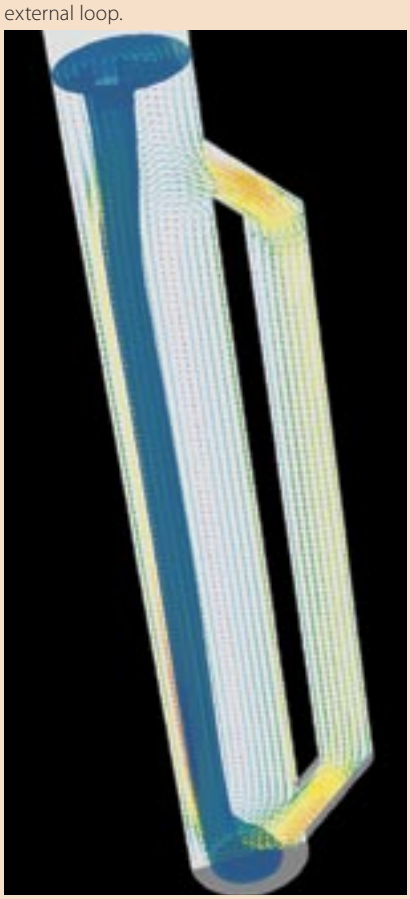
For Further Reading

Wilkes JO. *Fluid Mechanics for Chemical Engineers with Microfluidics and CFD,* 2nd Edition (International Series in the Physical and Chemical Engineering Sciences). Prentice-Hall: Upper Saddle River, NJ, 2005.

CFD Bioreactor Models

Two case studies are presented here focusing on multiphase hydrodynamics and bubble size distribution in stirred-tank and airlift bioreactors. Drag for the bubble phase is based on a Sauter mean diameter (D_{32}) calculated from the bubble size distribution. Gas holdup and volumetric mass-transfer coefficient thus can be predicted in good agreement with experiment for both

Figure 2: Isovalues of gas volume fraction and velocity vectors of water; the picture shows that gas primarily leaves at the top of the riser while water is recycled through the external loop.



types of bioreactors. An air–water system is used for these bioreactors and simulated using the Eulerian multiphase model in the FLUENT CFD code. Coupling between phases comes through interphase exchange terms. A mixture k-ϵ model is used to account for turbulence.

Airlift Example: An airlift bioreactor has been modeled to include a gas-sparged section (the riser, diameter 0.155 m) and an external circulation loop (the down-comer, diameter 0.07 m). The reactor volume is 0.023 m^3. Five CFD runs were conducted for superficial gas velocities of 0.01, 0.02, 0.03, 0.04, and 0.05 m/s. Riser gas holdup was calculated as a ratio of the volume of gas in the riser section divided by the initial ungassed riser volume. A volumetric mass-transfer coefficient is calculated as the product of the liquid-phase mass-transfer coefficient and specific surface area. I compared predictions from these models with published experimental results of Kawase and Hashimoto (**1**).

Figure 2 shows an isosurface of air volume fraction equal to 0.1 for a superficial velocity of 0.03 m/s. Most air in the reactor is contained in the riser section. Contours of the air volume fraction along the symmetry plane for the same conditions show that most of the air rises through the riser section and leaves that domain at the top. One air pocket is trapped in the bend of the down-comer section because of a balance between buoyant forces trying to make the gas rise and liquid velocities circulating downward.

A closer look at the air velocities indicates the presence of recirculation patterns that confirm the above argument. Recirculation is also observed in the riser section, where air is pushed toward the side—away from the down-comer. In a well-designed external loop bioreactor, the flow usually rises in the riser section and moves downward in the down-comer section. However, that desired behavior is exhibited only at an optimum ratio of down-comer diameter to riser diameter. In this example, this ratio is not optimized for the superficial velocities, so undesirable recirculation patterns occur.

For computing a range of bubble sizes during the course of this calculation, discretized population balance equations are solved with birth and death terms due to breakup and coalescence. Contours of the average bubble size show that the larger bubbles rise with liquid in the riser section. On the other hand, smaller bubbles are carried downward by liquid circulation in both the riser and down-comer sections (Figure 3). However, the actual amount of air that circulates through the down-comer is quite small. DO contours show that cells could be oxygen-starved at the bottom of the down-comer section, where O_2 concentration is lower than the critical value for most microorganisms (Figure 4).

Figure 5 compares the predicted riser gas hold-up with experimental data from Kawase and Hashimoto (**1**). Gas hold-up values are well predicted for smaller superficial velocities compared with the values at larger superficial velocities. But the overall predictions are in the same order of magnitude, with a maximum error of only about 13%.

Figure 6 compares predicted volumetric mass-transfer coefficient with the experimental data (**1**). The simulations overpredict by about 25% in the worst case. Typical CFD simulations can capture gas hold-up correctly, but the prediction of mass-transfer coefficient is more difficult due to limitations in accurately

Figure 3: Bubble size (*m*) distribution in a column; larger bubbles leave at the top of the riser, smaller bubbles may be entrained in the down-comer section—but the actual amount of gas entrained is very small.

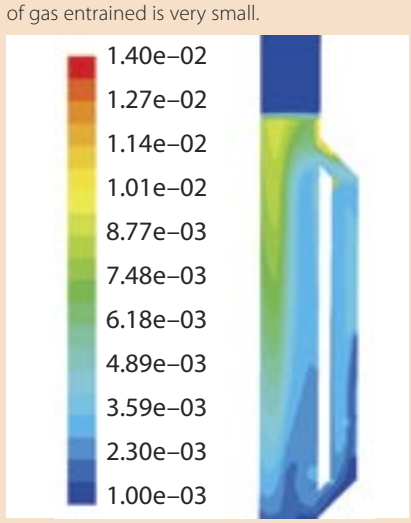


Figure 4: Dissolved oxygen (DO) concentrations in mM/L; the down-comer section toward the bottom is oxygen starved.

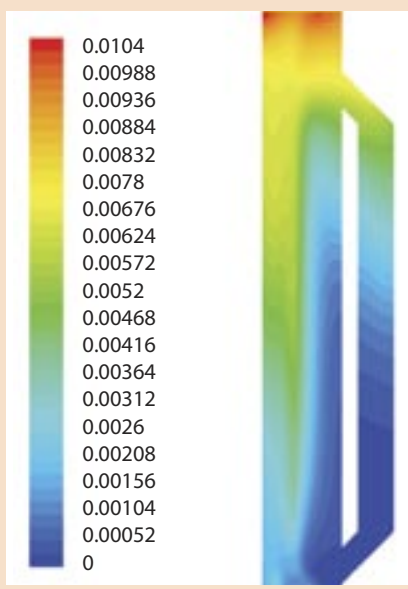


Figure 5: Comparing experimental data with modeling results for gas holdup

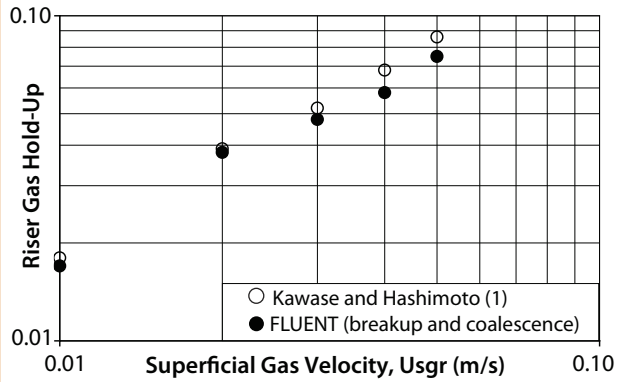


Figure 6: Comparing experimental data with modeling results for mass transfer

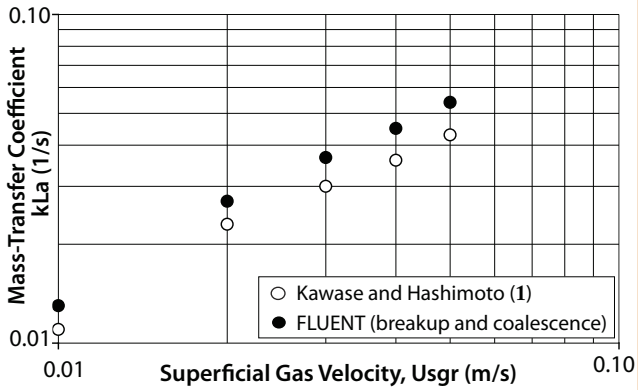


Figure 7: Volumetric mass transfer coefficient (KLa) in (1/s) also shows nonuniform distribution; mass transfer is high near the impellers.

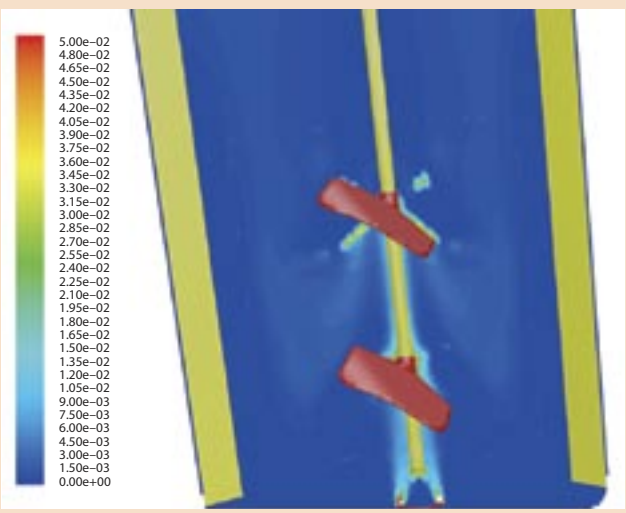
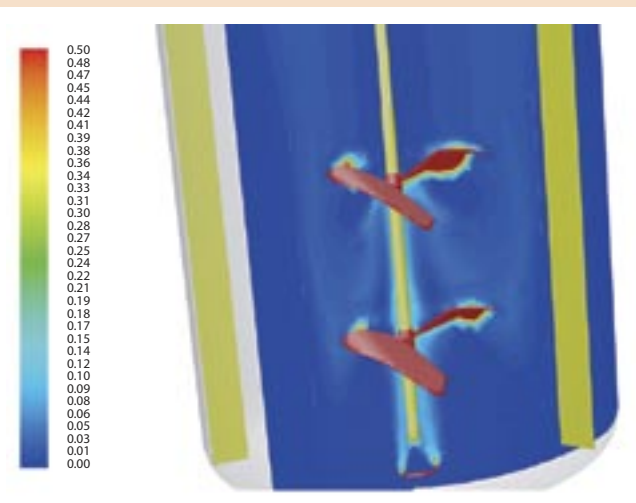


Figure 8: Gas hold-up in the reactor (higher behind the blades); it is nonuniform, which shows a moderate level of gas distribution.



capturing bubble number density distributions. Accuracy can be improved by refining discretization of the population balance equation for bubble number density at increased computational costs. The assumptions of break-up coalescence models may also have to be revisited and modified. Nevertheless, my results compare favorably with experimental data for both gas hold-up and volumetric mass-transfer coefficient.

Stirred-Tank Example: A dual-impeller, 96-in. diameter stirred-tank bioreactor typical of those used for mammalian cell cultures was also simulated using CFD. The stirred tank operates at low mixer power, with low-flow oxygen injected through a sparger into a water-based solution. Velocity vectors show a downward flow produced by two large Lightnin A320 impellers from SPX Process Equipment (www.gowcb.com).

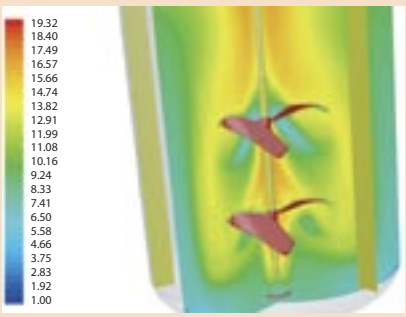
The average bubble size results show smaller bubbles due to breakup near the impellers, where shear is high, and larger ones due to coalescence as they rise along the vessel's outer wall. The largest bubbles are near the center, above and between the impellers where shear is lowest. The spatial variation of turbulence and bubble size cause a nonuniform mass-transfer coefficient distribution. Although in this particular reactor that variation is slight, the potential is extremely important in assessing reactors during scale-up (Figure 7). Figures 8 and 9 show gas distributions and bubble size distributions.

For this example, the experimental results for mass-transfer coefficient follow a commonly used correlation well, forming a reliable baseline for comparison with CFD results. The correlation depends on mixer power per unit volume and superficial gas-rise velocity. Whereas that correlation offers a single mass-transfer coefficient for the entire bioreactor and is specifically developed for this system, the CFD results offer a spatially dependent function derived from flow variables that are system independent. The volumetric average of the resulting mass-transfer coefficient was found to be within an order of magnitude of an experimentally determined value for the same operating conditions.

Planning Ahead

Bioreactor design and scale-up understanding can be gained through systematic modeling studies that begin with mixing analyses using CFD and mixing theories. In a staged modeling approach, you can study mixing and

Figure 9: Bubble size (mm) distribution in a mammalian cell culture bioreactor; the reactor has two A320 impellers and a ring sparger (smaller bubbles are near the impeller, where turbulence is higher and they tend to coalesce as they rise through.



later incorporate multiphase flow dynamics with bubble interactions and size distribution to predict gas–liquid mass transfer. A generalized approach to predict oxygen transfer for bioreactors is demonstrated here, with experimental validation for two case studies. The solution of a population-balance equation for bubble number density
is coupled with CFD calculations to predict bubble size distribution. For the two cases considered here, gas hold-up and liquid volumetric mass-transfer coefficient were both found to be in good agreement with experimental results. The benfits of modeling include managing risk during scale-up and reducing down-time with proper design.

Acknowlegments

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