Fermentation and Bioreactor Industry Spotlight

Introduction-historical significance

Use of fermentation or bioprocessing predates the profession of chemical engineering by hundreds or thousands of years. Ancient civilizations learned how to use bioprocessing to create such useful and tasty items as beer, wine, sauerkraut, kim-chi and cheese. Until the discovery and commercialization of Penicillin in 1943, bioprocessing was not normally done on an industrial scale with equipment resembling that in a chemical processing plant. Since then, the number of products has grown exponentially, as the bioprocessing route generally uses less energy than other routes, usually uses inexpensive raw materials, and sometimes makes products that cannot be made any other way. Modern products include antibiotics, amino acids, enzymes, monomers, proteins, food cultures, biopolymers, soluble gums, ethanol, isopropanol, isobutanol, flavorings, perfume chemicals and a whole host of other organic chemicals.

Though both aerobic and anaerobic processes are used in bioreactors, this industry spotlight focuses on aerobic processes, as these are the most challenging from an agitation viewpoint. We will use the terms *fermenter* and *bioreactor* interchangeably. Though oxygen is the usual gas for which mass transfer is desired, newer biotechnologies include cases where carbon dioxide, carbon monoxide, methane and other gasses may be transferred. The same principles presented here apply to any gas.

General process description

The bioreactor process generally goes through several steps before it gets to the production fermenter. The first step is cultivation of the organisms in the lab. This is often done in shaker flasks, under the direct supervision of the responsible microbiologist. A portion of high cell density broth from the shaker flask will be transferred to a vessel called a seed tank, which is a small fermenter designed to increase the quantity of organisms (“bugs”). From here, it is often transferred to another, larger fermenter called the inoculum tank. Finally, the contents of the inoculum tank are transferred to the production fermenter. The conditions in the shaker flask, seed tank and inoculum tank are optimized for production of more bugs. The conditions in the production fermenter may be different, depending on the product produced. There are sometimes fewer than three steps from shaker flask to production, and sometimes there are more, depending on the scale of the production fermenter. These steps are illustrated below, in Figure 1.

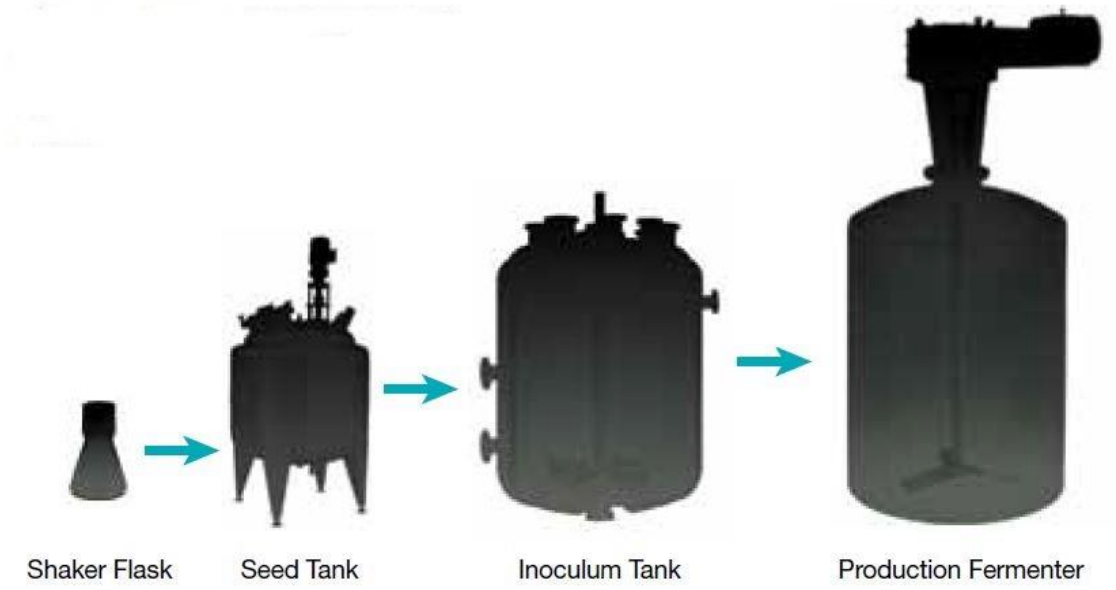

Figure 1, Fermenter sequence

Most production fermenters follow a generic growth curve, as in figure 2. After inoculation, the organisms take some time to get used to the new environment before they begin growing. The cell population density remains almost constant for awhile. This is called the lag phase. After acclimatizing, the bugs enter a period or rapid, exponential growth, called the growth phase. When competition for nutrients or oxygen becomes limiting, the growth stops, leading to a stationary phase. During this phase, production of products excreted by the organism or produced inside it may continue. If the end product is the organism itself, as in yeast production or single cell protein production, the process is normally stopped after the growth phase ends, as it is a waste of nutrients and oxygen to continue. Finally, organisms begin to die off. This is the decline or death phase.

After fermentation, the broth is sent to a harvest tank, awaiting downstream processing. The downstream processing may involve separating cells from the broth and purifying the product, concentrating the cells if they are the product, or rupturing the cells if the product is inside them.

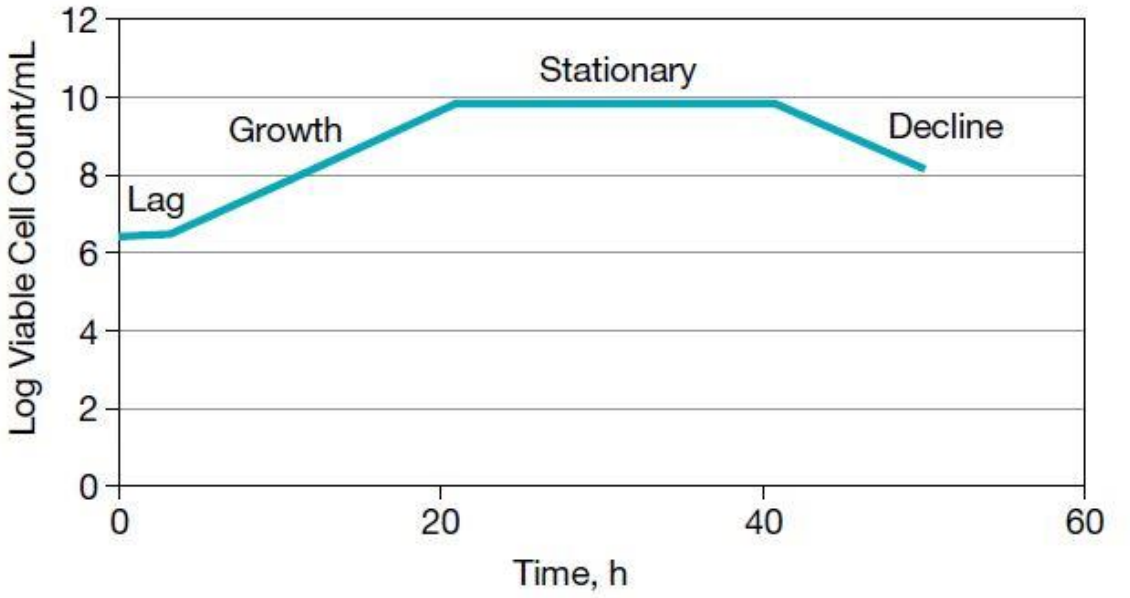

Figure 2, bioreactor profile

How is a bioreactor different from a chemical reactor?

- Chemicals will react the same way each time, dependent only on composition and temperature. No past history of chemicals will affect their reactions. There are no different “strains” of the same chemical.
- Different strains of the “same” organism may have different yields, preferred metabolic pathways, productivity and product distribution. For example, some yeast strains are better at making ethanol, whereas others are better at producing CO_2 for making bread rise. The common bacterium, e. coli, has been modified to make a wide variety of different products.
- Living organisms can die or go into shock. Dead organisms will not “react”, and ones in shock may have different productivity or create different products. For example, if an aerobic organism is briefly exposed to an anaerobic region in the reactor, it may protectively slow down its metabolism. It may not return to its normal metabolic activity for minutes or even hours after returning to an aerobic region. Thus, small regions of sub-optimal conditions can have a drastic effect on the productivity of the entire bioreactor. This is sometimes called “cycling.”
- Nutrient feed and aeration strategy affect not only rate but yield and product distribution.
- Microbiologists normally control “bug” issues and strategies.

How does Benz Technology International, Inc. aid in fermenter design?

We add expertise in the following areas, which complement the knowledge of microbiologists and process engineers:

- Mass transfer
- Mixing
- Heat transfer
- Process optimization
- Design of experiments

We will detail these in the next several sections.

Mass transfer

Mass transfer is crucial in aerobic fermentation. In fact, promoting mass transfer is usually the main purpose for the agitator, as mass transfer is often the rate limiting step in the process. Without sufficient transfer of oxygen from air to liquid, organisms may die, go into shock or make the wrong product.

Aerobic processes demand a certain Oxygen Uptake Rate (OUR). In quasi steady-state conditions, this is matched by the Oxygen Transfer Rate (OTR). Though this varies throughout the process, engineers must design for the peak rate. Traditional processes have rates ranging from about 100 mmol/l-h (easy) through about 150-200 (average) to more than 300 (difficult). Some newer processes include micro aerobic conditions (typically less than 5 mmol/l-h). Some modified e. coli fermentations may require up to 500, which may require oxygen-enriched air as a feed gas.

The basic, simplified equation is:

1) $OTR = k_l a\ (C_{sat}-C)$

The term in brackets is the driving force. It can be expanded to a log mean driving force for more accuracy, since both the local concentration and the saturation concentration are different in the top and bottom of a bioreactor. It is affected considerably by the value of C_{sat}, which depends on temperature, gas concentration, and most importantly, absolute pressure. Engineers might be tempted to increase the saturation value by increasing backpressure. But there is a limitation. High back pressures impede the release of CO_2, which must be kept below reasonable partial pressures to maintain healthy organisms. In practice, fermentations usually have a back pressure of less than 1 bar.

The other term, $k_l a$, is usually referred to the overall mass transfer coefficient. It is treated as a single variable in most cases due to the difficulty of separating out

the interfacial area from the film coefficient. It is a function primarily of the broth type, superficial gas velocity and agitator power input. A common form of correlation is:

2) $k_l a = A\ (P/V)^B (U_S)^C$

The constants A, B and C are broth-specific. Considerable experimental work is often required to develop such correlations. Benz Technology International can aid in designing proper experimental protocol and in interpreting the results.

Mixing

The first bioreactors used multiple Rushton turbines, which created a staged mixing pattern not conducive to uniformity of oxygen or nutrients. Almost all new fermenters have a combination of axial and radial impellers to improve mixing. Some even have all-axial impellers.
When nutrients or dissolved oxygen are non-uniform, it can affect productivity, yield and product distribution. Some processes and organisms are more sensitive than others. Unfortunately, there is presently no good way to model actual concentration distribution in a fermenter. Various researchers are attempting to use numerical methods to combine mixing, mass transfer and reaction kinetics to plot DO distribution. This is a very complex process, and there are no models on the market to do this in a user-friendly manner. Nonetheless, there have been some attempts made to simulate DO distribution numerically, as shown in figure 3.

There are several strategies to improve mixing in a fermenter at a given OTR. These include:

- Using a combination of axial and radial impellers, with optimal power distribution (e.g., 54/23/23, bottom to top, in a 3-impeller, axial/radial system, with the radial impeller on bottom.)
- When in doubt, add an extra impeller
- Upper axial impellers should be up-pumping. (This seems counter intuitive, but such an arrangement has a shorter *gassed* blend time than a down-pumping system)
- Use a larger D/T (> 0.33) if viscosity exceeds about 250 cP. (D/T = ratio of impeller to tank diameter).

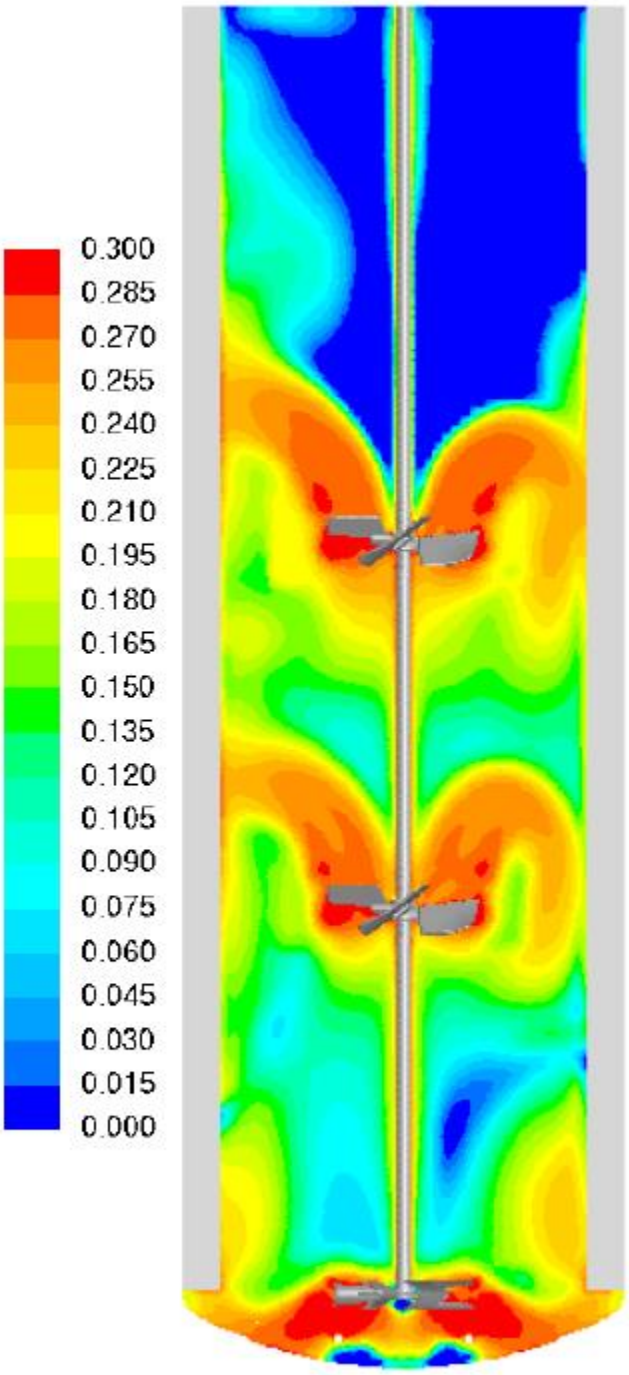

Figure 3, Dissolved Oxygen (DO) spatial distribution simulation

Heat Transfer

There is a wide range of heat transfer requirements in fermenters and bioreactors. The rate usually correlates well to oxygen consumption. A good

estimate is 4.6E5 J/mole of oxygen consumed, or about 110 Kcal/mole. At a mid-range OUR of 200 mmol/l-h, this translates to a heat generation term of 22 kcal/l-h. To this figure, the heat of agitation must be added, plus the air expansion power. The heat of water evaporating by air flow is subtracted. It is reasonable to assume that the gas enters dry and leaves saturated with water.

Even though these heat loads are lower than in many chemical reactions, the removal of this heat is made more difficult by the mild operating temperatures, typically between 30 and 40C. As a result, cooling tower water at 30C usually won't work. Instead, chilled water is often used.

Heat may be removed with an external heat exchange loop or internal vessel surfaces. The external loop can be made in whatever capacity is needed, but may expose organisms to thermal shock and oxygen deprivation. Most fermenters, therefore, use internal surfaces. Examples of these include tank jackets, helical coils and vertical tube bundles, which double as tank baffles. Large fermenters may need to use multiple bundles with multiple rows of tubes per bundle (fig. 4)

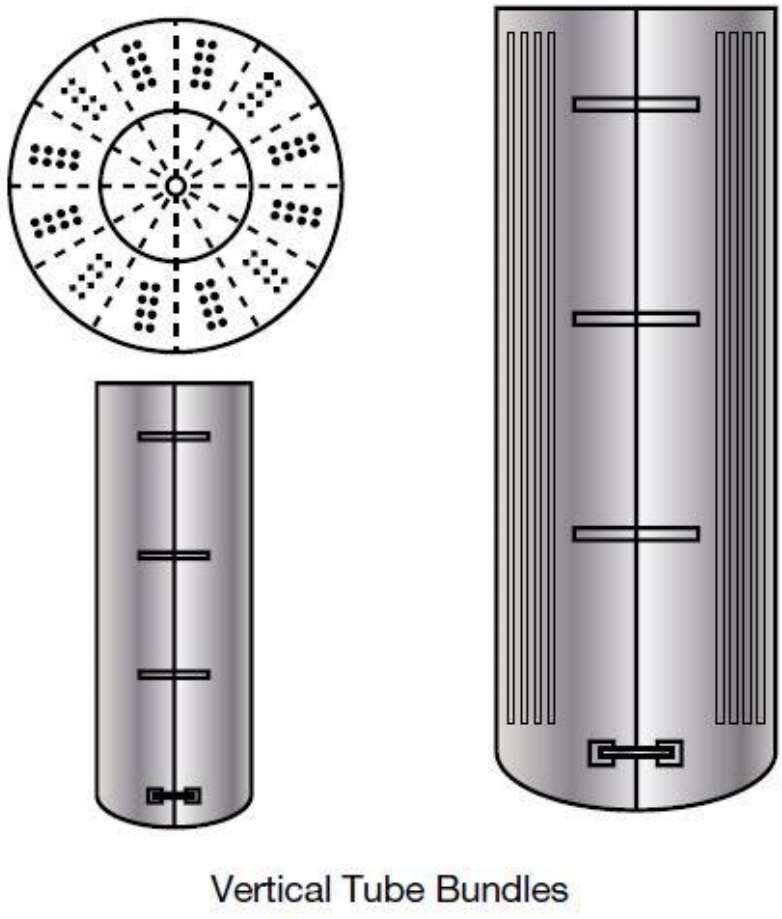

Figure 4 Large fermenter tube bundle groups

Benz Technology International frequently analyzes the heat transfer requirements for fermenters.

One caveat applies. Agitation equipment should be chosen for mixing and mass transfer, not for heat transfer. The exponent for agitator power input on the process side coefficient is only about 0.22, so little additional heat transfer is gained by increasing power. Instead, if there is a problem, add area or change the temperature of the cooling medium.

In low viscosity fermentations, sometimes the resistance inside the pipe is higher than on the agitated side. In such cases, the overall heat transfer coefficient may be improved by using internally finned pipe, or a pipe within a pipe. If the vessel is jacketed, a dimple jacket or half pipe jacket may produce better results than a simple jacket.

Process optimization opportunities

There are many opportunities for engineers to optimize bioreactor design. Among these are:

- Vessel aspect ratio. In aerobic fermentations, tall, thin vessels have a higher absolute pressure at the bottom, producing a higher mass transfer driving force. They also have a higher superficial gas velocity for a given airflow rate. The result is that they may require less total power for a given OTR. They also are easier to equip with sufficient heat transfer surface area. On the other hand, the vessels may be more expensive, and the degree of uniformity of DO and nutrients within the vessel is less. There is no simple rule of thumb to determine the optimum aspect ratio. Optimization consists of doing fairly detailed calculations on a series of aspect ratios to determine the lowest present worth of capital and operating costs for designs that are technically feasible.
- Economics of feed strategy vs. product yield, time in batch. For some processes, the rate of production is influenced by nutrient concentration. The batch time may be less with a high nutrient concentration. But sometimes the yield is less. The cost of nutrients versus the cost of vessel volume and power costs can be optimized. Product distribution may also be affected by nutrient concentration.
- Minimizing energy costs: agitation vs. aeration, back pressure. Within a given vessel geometry, there are many combinations of airflow and agitation that will give the same OTR, bounded by minimum stoichiometry on the low airflow end and excessive broth carryover to the vent at the high end. Such combinations are illustrated in figure 5. Back pressure is another variable which can influence energy cost. Figure 5 denotes power

minimization at peak OTR. Figure 6 indicates a “typical” OTR profile, and Figure 7 indicates the power consumption for 3 cases: running “flat out” (full speed and max airflow), fixed airflow with variable agitator speed, and variable airflow plus variable agitator speed, varied optimally. The latter case can save as much as 50% of the energy per batch.

- Working with CFD providers to fine tune design. Though there are no user-friendly codes on the market to predict such things as DO and nutrient profiles, CFD experts should be able to model this for a price. They would combine mixing, mass transfer and oxygen consumption models. Plan on spending at least $100,000 for 2-3 simulations of the semi-final design. This may be money well spent if it avoids yield or productivity problems by detecting low DO areas. For relatively insensitive fermentations, it may not be worth the time and expense. Benztech works closely with CFD providers to aid in developing appropriate systems to model.
- Judicious application of ASME Bioprocessing Equipment guidelines. These guidelines cover vessel and agitator construction details designed to make cleaning and sterilizing vessels easy, effective and quick. They also add considerably to the cost of the equipment. Robust cultures, such as some used to make antibiotics, may not need such construction, as they will tend to kill contaminating organisms. Standard construction, with steam sterilization between each batch, may be sufficient. On the other hand, sensitive cultures, such as mammalian cell culture, almost certainly require the highest degree of sanitary construction available. Judgment and experience are helpful to decide what level of sanitary construction is needed for intermediate cultures.
- Process control. A well-controlled and monitored process has the potential to consistently make the desired product quickly and at high yields. Key variables to be monitored and controlled must be assessed, and a control strategy developed.

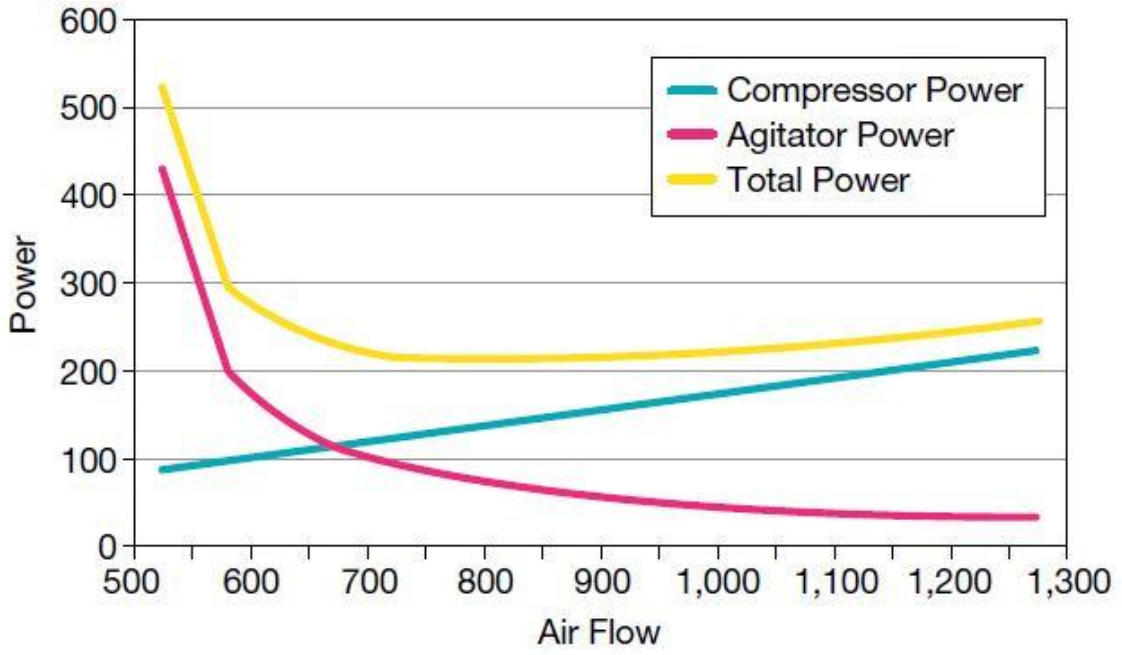

Figure 5, Design power consumption as function of air flow at peak OTR

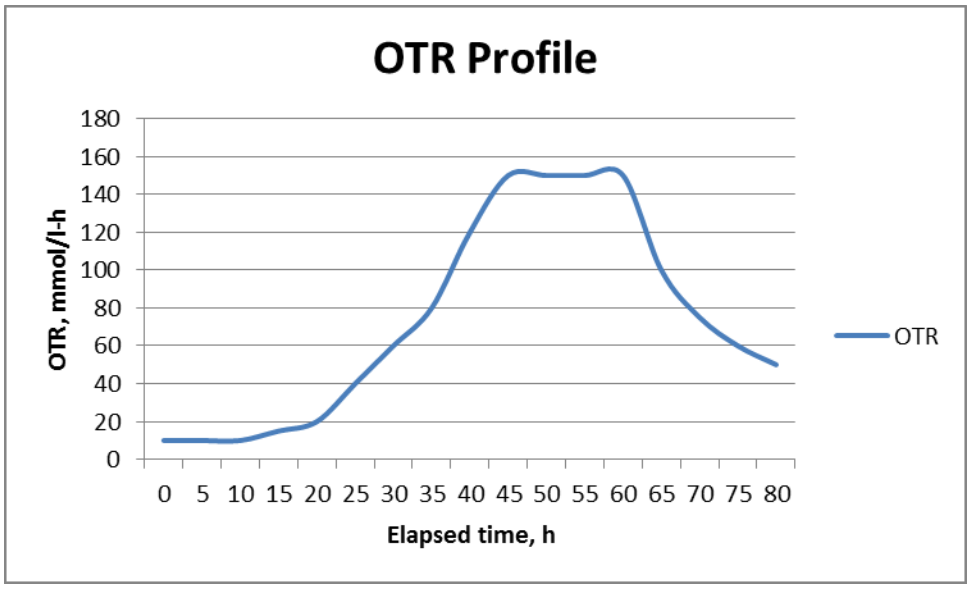

Figure 6 “Typical” OTR profile of a fermenter

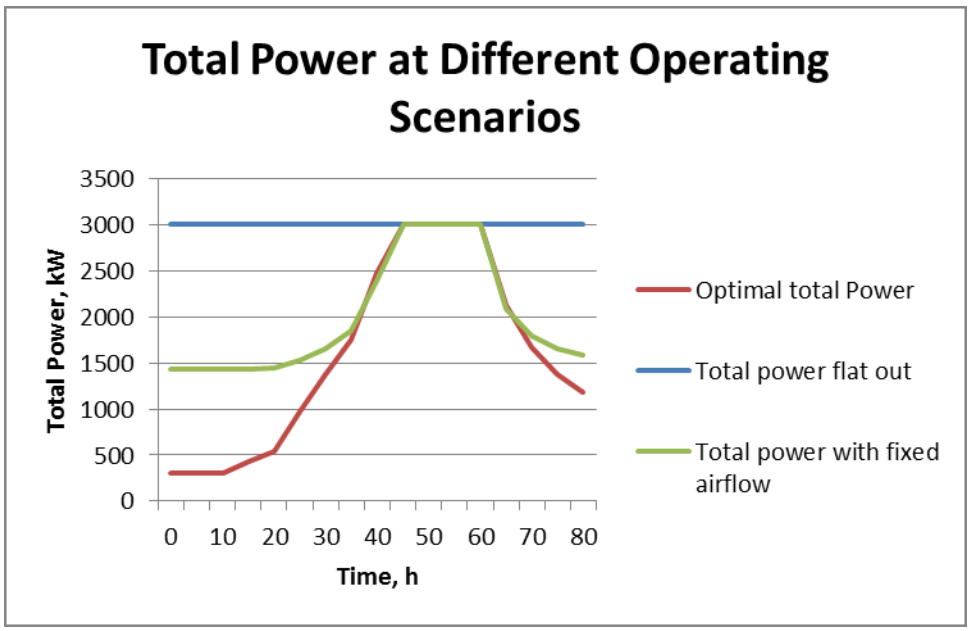

Figure 7 Possible energy savings with variable speed, airflow cases

Design of Experiments

As mentioned previously, the constants in the mass transfer correlation are broth-specific. Conditions in a typical bench-top or small pilot fermenter are often quite different from those in a production fermenter. Specifically, power per volume is usually higher in the small scale, and superficial gas velocity is lower. Benztech frequently designs experimental protocols to cover the full scale range of variables in the small scale, checking for flooding and other problems, and fits the data to correlations so as to scale-up the results. Figure 8 illustrates a typical range of P/V and superficial gas velocity for an OTR of 200 mmol/l-h. The pilot scale would have to be run in a similar range of variables to the planned full scale to develop a reliable mass transfer correlation, so the required air flow rate in the small scale would need to be much higher than normal.

Scale	Volume, L	U_S, m/s	*P/V*, W/L	*VVM*
Pilot	1	0.0065	9.8	3
	5	0.009	7.1	2.5
	20	0.011	6.1	2
	100	0.015	4.5	1.6
	300	0.019	3.9	1.4
Production	5,000	0.032	2.4	0.96
	20,000	0.044	1.8	0.85
	150,000	0.064	1.3	0.68
	350,000	0.074	1.1	0.62
	1,000,000	0.091	0.88	0.57

Figure 8, typical range of variables OTR~200 mmol/l-h

Benz Technology International, Inc. Experience

The principal of Benztech, Mr. Gregory Benz, has decades of experience designing agitation systems for aerobic fermenters, ranging in size from small specialty fermenters to large commodity fermenters with working volumes of almost 2 million liters. These cover processes from micro aerobic to very “hot” e. coli fermentations, and products from low viscosity amino acid or antibiotic solutions to viscous gums. A partial installation list is available on request.

Acknowledgement

Much of this content was taken from two articles by Gregory Benz, “Bioreactor Design for Chemical Engineers”, which was published in Chemical Engineering Progress, August, 2011, plus “Optimize Fermenter Operation”, published in Chemical Engineering Progress, March, 2016.