

Bioreactor Design for Chemical Engineers

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Fermentation processes are based on chemical engineering principles. Understand these concepts to achieve optimal bioreactor design.

Fermentation — the essence of bioprocessing — predates the discipline of chemical engineering by thousands of years. Ancient civilizations used bioprocessing to create such useful and tasty items as beer, wine, sauerkraut, kim-chi, and cheese. Until the discovery of penicillin and its commercialization in 1943, bioprocessing was not performed on an industrial scale with equipment resembling that in a chemical processing plant. Since then, the number of products made by fermentation has grown, because bioprocessing 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, ethanol, isopropanol, isobutanol, flavorings, perfume chemicals, and many other organic chemicals.

This article will familiarize engineers who do not work in the bioprocessing industry, as well as those who are just beginning their bioprocessing careers, with the basics of bioreactor design from a chemical engineering viewpoint. Details of the biological processes are beyond the scope of this article and are not discussed.

Kinds of bioreactors

Here, the terms *fermenter* and *bioreactor* are used interchangeably. In general, there are two main classes of bioreactors: anaerobic and aerobic.

In anaerobic fermentation, the “bugs” (as chemical engineers often call microorganisms) grow and create product in the absence or near-absence of oxygen. Examples of anaerobic fermenters include most fuel processes (such as ethanol or isobutanol) and the manufacture of some organic acids.

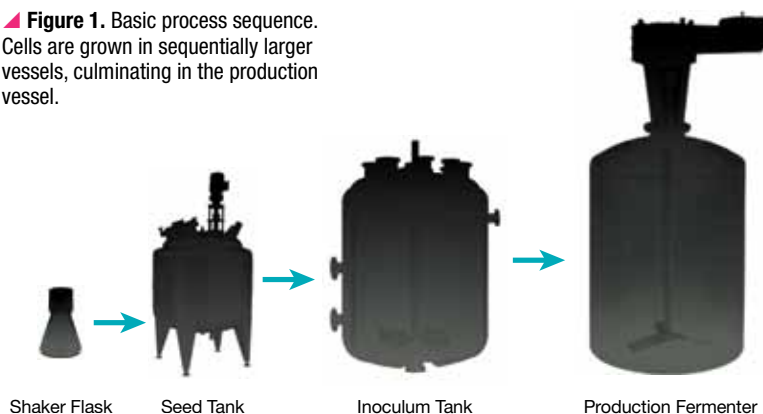
Aerobic fermentation takes place in the presence of oxygen, which the organisms consume in order to grow and/or make the desired product. Examples of aerobic processes include the production of yeast, antibiotics, enzymes, and amino acids. The amount of oxygen required varies from none (anaerobic processes) to a small amount (microaerobic) to a large amount (heavily aerobic).

The basic process

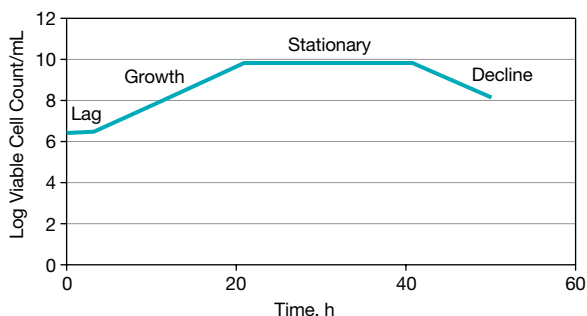
Regardless of whether the process is aerobic or anaerobic, it generally involves several steps before it reaches the production fermenter. The first step is cultivation of the organisms in the lab. This is often done in shaker flasks under the direct supervision of a microbiologist. A portion of high-cell-density broth is then transferred from the shaker flask to a seed tank, which is a small fermenter designed to increase the quantity of organisms. From there, it is usually transferred to a larger fermenter called the inoculum tank. Finally, the contents of the inoculum tank are transferred to the production fermenter (Figure 1).

The conditions in the shaker flask, seed tank, and

▲ **Figure 1.** Basic process sequence. Cells are grown in sequentially larger vessels, culminating in the production vessel.



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▲ **Figure 2.** A generic growth curve illustrates the exponential growth of cells after they are acclimated to the environment. After they reach their maximum density, the cells begin to die.

inoculum tank are optimized for the production of more bugs. The conditions in the production fermenter may be different, depending on the product. 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.

The organisms in most production fermenters follow the generic growth curve in Figure 2. After inoculation, they require some time to adjust to the new environment before they begin growing, and the cell density remains almost constant for a while. This is called the lag phase. After acclimatizing, the bugs enter a period of rapid, exponential growth, called the growth phase.

When competition for nutrients or oxygen becomes limiting, growth stops and the organisms enter the stationary phase. During this phase, they may continue to make the desired product. If the end product is the organism itself, as in the production of yeast or single-cell proteins, the process is normally stopped after the growth phase ends, as it would be 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 to await 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.

Bioreactor vs. chemical reactor

Bioreactors and chemical reactors differ in several important respects.

Chemicals react the same way each time, and the reaction depends 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 metabolic pathways, productivities, yields, and product distributions. For example, some yeast strains are more effi-

cient at making ethanol, whereas others are better at producing CO₂ to make bread rise. The common bacterium *E. coli* can be modified to make a wide variety of different products.

Living organisms can die or go into shock. Dead organisms will not “react,” and organisms in shock may have a different productivity or create different products. For example, if an aerobic organism is briefly exposed to an anaerobic region in the reactor, it might slow down its metabolism as a means of protection, and it might not return to its normal metabolic activity for minutes or even hours after returning to an aerobic region. Thus, small regions of suboptimal conditions can have a drastic effect on the productivity of the entire bioreactor. This is sometimes called cycling.

Nutrient feed and aeration strategies affect not only rate but also yield and product distribution in a bioreactor. Unlike chemical reactors, different reaction paths, or metabolic pathways, may be used by the organism depending on conditions.

Although microbiologists normally control issues and strategies related to the organisms, chemical engineers complement their knowledge with expertise in mass transfer, mixing, heat transfer, reaction engineering, and process optimization.

Mass transfer

Although not a major factor in anaerobic fermentation, mass transfer is crucial in aerobic fermentation. Without sufficient transfer of oxygen from the air to the liquid, organisms may die, go into shock, or make the wrong product.

Aerobic processes demand a certain oxygen uptake rate (OUR). Under quasi steady-state conditions, this equals the oxygen transfer rate (OTR). Although OUR 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 mmol/L-h (average) to more than 300 mmol/L-h (difficult). Some newer processes occur at microaerobic conditions (typically less than 5 mmol/L-h). Some modified *E. coli* fermentations may consume up to 500 mmol/L-h, which may require oxygen-enriched air as a feed gas.

The basic mass transfer equation is:

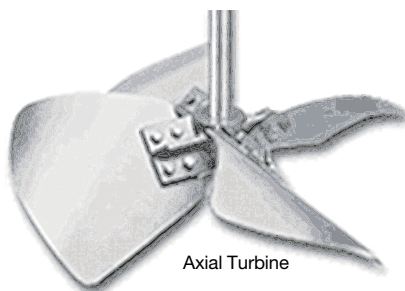
$$OTR = k_L a (C_{sat} - C) \quad (1a)$$

$$OTR = k_L a \left\{ \frac{(C_{sat} - C)_{out} - (C_{sat} - C)_{in}}{\ln \left[\frac{(C_{sat} - C)_{out}}{(C_{sat} - C)_{in}} \right]} \right\} \quad (1b)$$

The term in parentheses is the driving force. It can be expressed in a simple form (Eq. 1a) or in a log-mean form (Eq. 1b). In small vessels (<1 m tall) where both liquid concentration and saturation are almost constant, the simple form is adequate. In tall vessels, the log-mean driving force



▲ **Figure 3.** Rushton turbines, which have six flat blades mounted vertically on a disk, were used in early bioreactors.



▲ **Figure 4.** Axial impellers improve mixing in fermenters. They are often combined with radial impellers on a single shaft, which typically has the axial blades above the radial ones.

should be used for more accuracy, since both the local concentration and the saturation concentration are different in the top and bottom of a bioreactor. The driving force is affected considerably by the value of C_{sat} , which depends on temperature, gas concentration, and, most importantly, absolute pressure. Generally, Henry's Law can be used:

$$C_{sat} = P_{O_2}/H \quad (2)$$

where H is the Henry's law constant, which is a function of temperature. Values for Henry's constant may be found in standard reference handbooks.

It might be tempting to increase the saturation value by increasing backpressure. However, there is a limit to how high backpressure can be raised. High backpressures impede the release of CO_2 , which must be kept below reasonable partial pressures to maintain healthy organisms. In practice, fermenters usually have a backpressure of less than 1 bar.

The other term in Eq. 1, $k_L a$, is the overall mass-transfer coefficient. It is usually treated as a single variable because it is difficult to separate the interfacial area per unit volume (a) from the film coefficient (k_L). It is a function primarily of the broth type, superficial gas velocity, and agitator power input, and is often expressed as:

$$k_L a = A(P/V)^B (U_s)^C \quad (3)$$

References 1–3 provide more detailed discussions of agitation, airflow, mass balances, and agitator impeller systems for aerobic fermenters.

Mixing

The first bioreactors used multiple Rushton turbines (Figure 3), which create a staged mixing pattern that does not promote oxygen or nutrient uniformity. Almost all new fermenters have a combination of axial (Figure 4) and radial impellers, or all-axial impellers, to improve mixing.

When nutrients or dissolved oxygen are not uniformly

distributed, productivity, yield, and product distribution can be affected. Some processes and organisms are more sensitive than others. Unfortunately, there is presently no good way to model the actual concentration distribution in a fermenter. Various researchers are attempting to use numerical methods to combine mixing, mass transfer, and reaction kinetics to plot dissolved oxygen (DO) distribution, but this is a very complex process and there are no models on the market to do this in a user-friendly manner.

There are, however, several strategies to improve mixing in a fermenter at a given OTR:

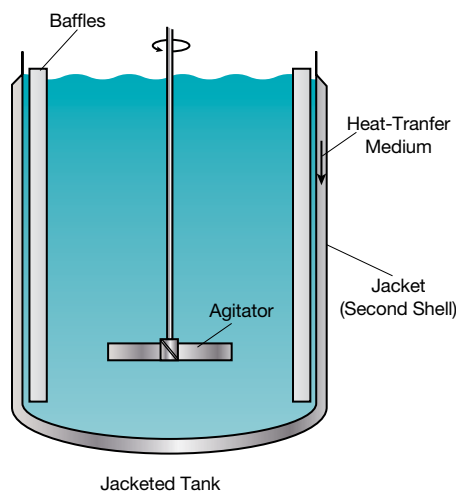
- use a combination of axial and radial impellers with optimal power distribution (for example, a 3-impeller system with a radial impeller on the bottom and two upper axial impellers having a 54/23/23 (bottom to top) power distribution)
- when in doubt, add an extra axial impeller
- specify upper axial impellers to be up-pumping (which seems counter intuitive, but such an arrangement has a shorter blend time when air is introduced than a down-pumping system)
- use a larger impeller-diameter-to-tank-diameter ratio ($D/T > 0.33$) if viscosity exceeds about 250 cP.

One might think that, in anaerobic bioreactors, mixing would not be an issue, since reaction times are always very long compared to blend times, and there seem to be no significant mass-transfer issues. However, agitation in a simple ethanol fermenter was found to affect productivity, yield, and maximum product concentration at specific power inputs an order of magnitude or more higher than needed to mix nutrients or keep solids in suspension (4). Unfortunately, the mechanism responsible for these effects is not clear. Process engineers may wish to study these effects in specific fermentations on more than one scale to determine how to scale up the bioreactor.

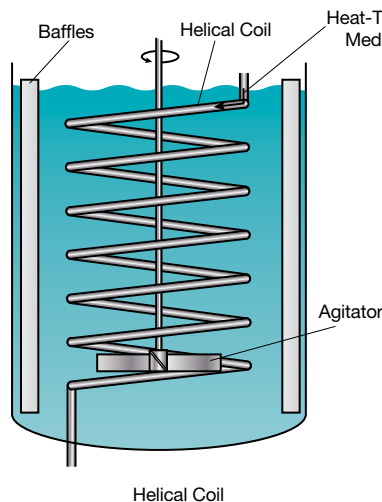
Heat transfer

Heat-transfer requirements for bioreactors vary. Anaerobic fermenters and cell culture reactors typically have very low rates of heat production, whereas aerobic fermenters can have quite high heat-production rates. The rate usually correlates well to oxygen consumption. A good estimate is 460,000 kJ/mol of oxygen consumed (5), or about 110 kcal/mol. A mid-range OUR of 200 mmol/L-h translates to a heat-transfer rate of 22 kcal/L-h. The heat of agitation and the air expansion power must be added to this, and the heat of water evaporating by airflow is

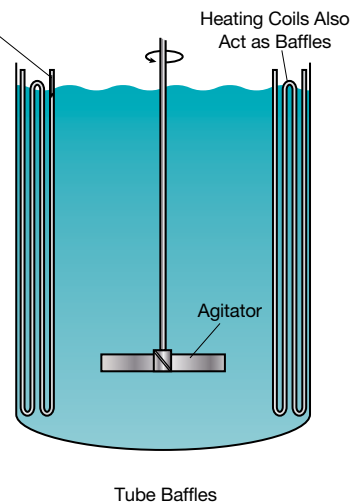
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▲ **Figure 5.** Many fermenters remove heat using tank jackets.



▲ **Figure 6.** Helical coils are often installed in fermenters to remove heat.



▲ **Figure 7.** Vertical tube bundles, which double as tank baffles, are another type of internal structure that can remove heat from fermenters.

subtracted (since it is assumed 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°C and 40°C for aerobic fermenters. As a result, cooling tower water at 30°C is usually inadequate, so chilled water is often used instead.

Heat may be removed by an external heat exchange loop or internal vessel surface. 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 (Figure 5), helical coils (Figure 6), and vertical tube bundles, which double as tank baffles (Figure 7). Large fermenters may require multiple bundles with multiple rows of tubes per bundle (Figure 8).

The following equations may be useful for calculating the process-side convective heat-transfer coefficients (h and h_c), which are incorporated into the Nusselt number (N_{Nu}). The Nusselt number is used in all standard convective heat-transfer correlations to relate the convective heat-transfer coefficient to physical dimensions, thermal conductivity, turbulence, and other fluid property and geometry effects.

For bottom jackets (as in Figure 5):

$$N_{Nu} = K(N_{Re})^{2/3}(N_{Pr})^{1/3}(\mu/\mu_w)^{0.14} \quad (4)$$

where $K = 0.5$ for Rushton turbines, $K = 1.08$ for pitched-blade turbines, and $K = 0.9$ for common hydrofoils. For jackets, the Nusselt number is $N_{Nu} = hT/k$.

For jacket sidewalls (Figure 5):

$$N_{Nu} = K(N_{Re})^{2/3}(N_{Pr})^{1/3}(\mu/\mu_w)^{0.14}(T/Z)^{0.15} \quad (5)$$

where $K = 0.74$ for Rushton turbines, $K = 0.45$ for pitched-blade turbines, and $K = 0.31$ for common hydrofoils.

For a single helical coil, with the Nusselt number defined as $N_{Nu} = h_c d_i/k$:

$$N_{Nu} = K(N_{Re})^{0.67}(N_{Pr})^{0.37}(D/T)^{0.1}(d_i/T)^{0.5} \quad (6)$$

where $K = 0.17$ for pitched-blade or radial turbines and $K = 0.14$ for common hydrofoils.

For a single bank of vertical tube baffles and the same Nusselt number definition ($N_{Nu} = h_c d_i/k$):

$$N_{Nu} = K(N_{Re})^{0.65}(N_{Pr})^{0.3}(D/T)^{0.33}(2/n_b)^{0.2}(\mu/\mu_w)^{0.14} \quad (7)$$

where $K = 0.09$ for pitched-blade or radial turbines and $K = 0.074$ for common hydrofoils.

For multiple banks of helical coils, or multiple rows of tubes within a bundle, the masking effect on the process-side coefficient can be estimated by using a factor of 0.8 for each additional row beyond 1:

$$K_{(multiple)} = K(0.8)^{(n-1)} \quad (8)$$

where n is the number of rows in each bundle.

Agitation equipment should be chosen based on mixing and mass-transfer requirements, not on heat transfer. The exponent on agitator power input on the process-side coefficient is only about 0.22 (which can be derived from the above equations and agitator power proportional to $N^3 D^5$), 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 heat-transfer resistance on the cooling media side of the heat exchange system is higher than on the agitated side (*i.e.*, the process fluid side). In such cases, the overall heat-transfer coefficient may be improved by using internally finned pipe or tubing, or an annular double-pipe arrangement. If the vessel is jacketed, a dimpled jacket or half-pipe jacket may be more effective than a plain jacket.

Reaction engineering

As long as conditions are suitable for the microorganisms inside the bioreactor, the bioreactions may be treated as any other chemical reaction, although the kinetic expression may be more complicated than simple first or second order, and allowances must be made for variations in the biosystem.

For a batch reactor, the process design procedure may be mostly empirical and specified by the microbiologists. For a continuous-flow bioreactor system, good knowledge of the kinetics plus sound reaction engineering will allow the appropriate number of stages and retention time to be chosen.

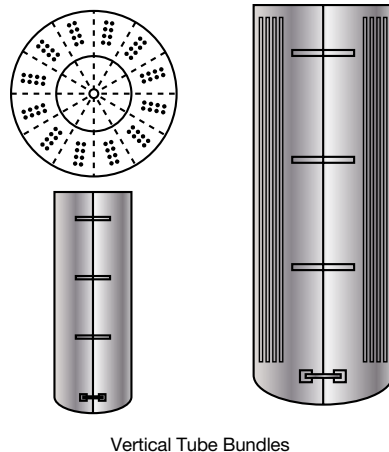
A continuous-flow reactor system requires more total retention time than a batch reactor, and sound chemical engineering reactor-design principles are very important to the success of the system. Sometimes, the kinetics are too complex to calculate, and the continuous-flow system must be modeled in a pilot plant. The pilot plant must have the same general reactor scheme (plug flow vs. mixed flow), number of stages, volumetric mass-transfer rate, and feeding strategies as the proposed production system. This makes it unnecessary to know the kinetics exactly, and standard scale-up techniques should assure reasonably equivalent results.

An example of this is simultaneous saccharification and fermentation (SSF) in a cellulosic ethanol plant. In such a system, enzymes break down cellulose and hemicellulose of various molecular sizes into sugars such as glucose and pentose, which are metabolized by one or more organisms into ethanol while the sugars are being produced. The overall system kinetics are based on a combination of many reactions occurring in series and in parallel. Since this is very difficult to evaluate analytically, a pilot plant is required for the design and scaleup of the continuous-flow system.

Process optimization

There are many opportunities for chemical engineers to optimize bioreactor design.

Vessel aspect ratio (height/diameter, Z/T). In aerobic fermentations, tall, thin vessels have a higher absolute pressure at the bottom, which provides a higher mass-transfer driving force. They also have a higher superficial gas velocity for a given airflow rate. The overall result is that they may require



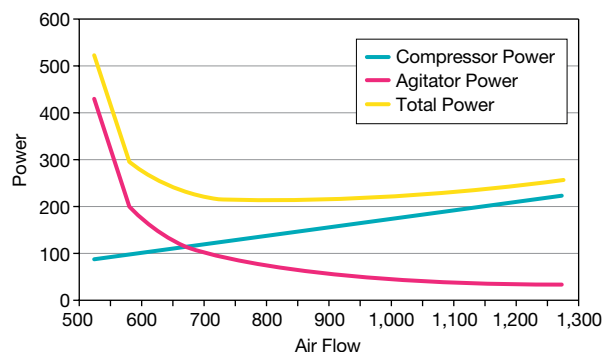
◀ **Figure 8.** Large fermenters may need multiple bundles with multiple rows of tubes per bundle to remove the heat produced by the process.

less total power for a given OTR. In addition, they are easier to equip with sufficient heat-transfer surface area. On the other hand, they may be more expensive, and the distribution of dissolved oxygen and nutrients within the vessel is less uniform.

There is no simple rule of thumb to determine the optimum aspect ratio. Optimization consists of performing fairly detailed calculations for a series of aspect ratios to determine the lowest present worth of capital and operating costs for designs that are technically feasible. For anaerobic fermentations, the optimum aspect ratio is usually 1 from a mixing viewpoint, although vessel cost may dictate other geometries.

Economics of feed strategy vs. product yield and retention/batch time. For some processes, the production rate is influenced by nutrient concentration. For instance, a high nutrient concentration may shorten the batch time, or it may reduce the yield. Product distribution may also be affected by nutrient concentration. Nutrient costs must be balanced against the capital cost of the vessel and the operating (power) costs.

Minimizing energy costs: agitation vs. aeration, back-pressure. Within a particular vessel geometry, there are many combinations of airflow and agitation that will produce the



▲ **Figure 9.** Fermenter agitator power decreases with air flow and the compressor power increases with air flow.

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same OTR, bounded by minimum stoichiometry on the low-airflow end and excessive broth carryover to the vent at the high end, as illustrated in Figure 9 and discussed in detail in Ref. 3. Backpressure also influences energy cost.

Working with CFD providers to fine-tune the design.

Although there are no user-friendly codes on the market to predict such things as DO and nutrient profiles, computational fluid dynamics (CFD) experts should be able to model these by combining mixing, mass-transfer, and oxygen consumption models. This work is expensive — plan to spend at least \$100,000 for two or three simulations of the semi-final design. But 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.

Judicious application of ASME guidelines. The American Society of Mechanical Engineers (ASME) bioprocessing equipment standard (BPE 2009) provides guidelines on vessel and agitator construction details that are designed to

make cleaning and sterilizing vessels easy, effective, and quick. However, complying with the standard adds 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. For these processes, standard construction with steam sterilization between each batch may be sufficient. On the other hand, sensitive cultures, such as mammalian cell culture, almost always require the highest degree of sanitary construction available. Judgment and experience are helpful in deciding 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.

Closing thoughts

Although fermenter/bioreactor design may seem to be primarily the province of microbiologists, by gaining a basic understanding of such systems and combining it with chemical engineering knowledge, chemical engineers can contribute to the overall success of facilities using such processes, and aid in optimizing the economics.

CEP

Nomenclature

A, B, C	= empirical correlation constants for mass-transfer coefficients, Eq. 3, dimensionless
C_{O_2}	= dissolved oxygen concentration, mg/L
C_p	= heat capacity at constant pressure, J/kg-K
C_{sat}	= dissolved oxygen concentration at saturation, mg/L
D	= impeller diameter, m
DO	= dissolved oxygen concentration, mg/L
d_i	= coil or tube diameter, m
h	= convective heat-transfer coefficient, J/m ² -s
h_c	= convective heat-transfer coefficient to coil, J/m ² -s
k	= thermal conductivity, J/m-K
$k_L a$	= overall mass transfer coefficient, 1/s
K	= correlation constant, Eqs. 4–7, dimensionless
n	= number of rows in each bundle
n_b	= number of vertical tube bundles
N	= agitator shaft speed, 1/s
N_{Nu}	= Nusselt number (dimensionless), hT/k or $h_c d_i/k$
N_{Pr}	= Prandtl number (dimensionless), $C_p \mu/k$
N_{Re}	= impeller Reynolds number (dimensionless), $D^2 N \rho/\mu$
OTR	= oxygen transfer rate, mg/L-h
OUR	= oxygen uptake rate, mmol/L-h
p_{O_2}	= partial pressure of oxygen, Pa or kg-m/s ²
P	= agitator power, W
P/V	= specific power, W/m ³
T	= tank diameter, m
U_s	= superficial gas velocity, m/s
V	= vessel volume, m ³
Z	= vessel height, m
Greek Letters	
μ	= viscosity, kg/m-s
μ_w	= viscosity at wall or heat-transfer surface, kg/m-s
ρ	= liquid density, kg/m ³

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