1

Modeling Principles

1.1 Fundamentals of Modeling

1.1.1 Use of Models for Understanding, Design, and Optimization of Bioreactors

Any system used for carrying out bioreactions, from single enzymatic reactions to complex multicellular reaction systems, requires a containment called a bioreactor. The performance of a bioreactor may conventionally be investigated almost entirely empirically. In this approach, the bioreactor behavior would be studied under practically all combinations of possible operating conditions. The results would then be expressed as a series of input–output correlations, from which the performance could be determined. This empirical procedure can be carried out in a routine manner and requires relatively little thought concerning the actual details of the process. While this might seem to be rather convenient, the procedure has actually disadvantages, since very little real understanding of the process would be obtained. Also very many costly experiments would be required in order to obtain correlations that would cover every process eventuality.

Compared to this, the modeling approach attempts to describe both actual and probable bioreactor performances, by means of a well-established theory. Described in mathematical terms, a working model for the process is established. In carrying out a modeling task, the modeler is forced to consider the nature of all the important parameters of the process, their effect on the process, and how each parameter must be defined in quantitative terms. The modeler must identify the important variables and their separate effects, which may have a highly interactive combined effect on the overall process performance. Thus, the very act of modeling is one that forces a better understanding of the process, since all the relevant theory must be critically assessed. In addition, the task of formulating theory into terms of mathematical equations is also very beneficial in that it forces a clear formulation of basic concepts.

Once formulated, the model can be solved, and the predicted behavior can be compared with experimental data. Any differences in performance may then be used to further redefine or refine the model until a good agreement is obtained. Once the model is established, it can then be used, with reasonable confidence, to predict

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performance under differing process conditions. It can also be used for purposes as process design, optimization, and control. An input of plant or experimental data is of course required in order to establish or validate the model, but the quantity of experimental data required, as compared to that of the empirical approach, is considerably reduced. The major advantage of modeling based on the underlying physical and biological principles is the increased understanding of the process that will be linked to an increased power of resulting models, particularly with respect to their prediction power even outside the experimentally studied parameter space. It also serves as an unambiguous basis for communication.

These ideas are summarized below:

Empirical approach: Measure productivity for all combinations of reactor operating conditions, and make correlations.

- Advantage: Little thought is necessary.
- Disadvantages: Many experiments are required. Poor predictivity outside range of experimental observation.

Modeling approach: Establish a model and design experiments to determine the model parameters. Compare the model behavior with the experimental measurements. Use the model for rational design, control, and optimization.

- Advantages: Fewer experiments are required, and greater understanding is obtained. Good predictive power even outside experimental space used for setting up and tuning the model.

1.1.2 General Aspects of the Modeling Approach

An essential stage in the development of any model is the formulation of the appropriate mass and energy balance equations (Russell and Denn 1972). Though synonymous, often the term "material balance" is preferred than "mass balance." Both rely on the conservation of mass in any closed system, on which energy balances are based. To these balances must be added appropriate kinetic equations for rates of cell growth, substrate consumption, and product formation; equations representing rates of heat and mass transfer; and equations representing system property changes, equilibrium relationships, and process control (Blanch and Dunn 1973). Additionally, the conservation of momentum is the basis for corresponding balances, for example, Navier–Stokes equations, that are used for building fluid dynamic models. These are, however, more complex and are not used in this book. The combination of these relationships provides a basis for the quantitative description of the process and comprises the basic mathematical model. The resulting model can range from a very simple case of relatively few equations to models of very great complexity.

Simple models are often very useful, since they can be used to determine the numerical values for many important process parameters. For example, a model based on a simple Monod kinetics can be used to determine basic parameter values such as the specific growth rate (μ), saturation constant (K_S), biomass yield

coefficient ($Y_{X/S}$), and maintenance coefficient (m). This basic kinetic data can be supplemented by additional kinetic factors such as oxygen transfer rate (*OTR*), carbon dioxide production rate (*CPR*), and respiration quotient (*RQ*) based on off-gas analysis. Related quantities such as specific oxygen uptake rate (q_{O_2}) and specific *CPR* (q_{CO_2}) or the specific heat production rate (q_Q) may also be derived and used to provide a complete kinetic description of, say, a simple batch fermentation.

For complex fermentations, involving product formation, the specific product formation rate (q_P) is often correlated as a complex function of fermentation conditions, e.g. stirrer speed, air flow rate, pH, dissolved oxygen content, and substrate concentration. In other cases, simple kinetic models can also be used to describe the functional dependence of productivity on cell density, cell growth rate, and environmental parameters as concentrations of substrates, intermediates, and products.

A more detailed "structured kinetic model" may be required to give an adequate description of the process, since cell composition may change in response to changes in the local environment within the bioreactor. Even whole-genome based stoichiometric network models can be directly incorporated. This kind of modeling can be rationalized by applying modules in a systematic way (Garcia and Trinh 2019). The greater the complexity of the model, however, the greater then the difficulty in identifying the numerical values for the increased number of model parameters, and one of the skills of modeling is to derive the simplest possible model that is capable of a realistic representation of the process.

The basic uses of a process model is thus to analyze experimental data and to use them to characterize the process, by assigning numerical values to the important process variables. The model can then also be solved with appropriate numerical data values, and the model predictions are compared with actual practical results. This procedure is known as simulation and may be used to confirm that the model and the appropriate parameter values are "correct". Simulations, however, can also be used in a predictive manner to test probable behavior under varying conditions; this leads on to the use of models for process optimization and their use in advanced control strategies.

The application of a combined modeling and simulation approach leads to the following advantages:

1) *Modeling improves understanding:* and it is through understanding that progress is made. In formulating a mathematical model, the modeler is forced to consider the complex cause-and-effect sequences of the process in detail, together with all the complex interrelationships that may be involved in the process. The comparison of a model prediction with actual behavior usually leads to an increased understanding of the process, simply by having to consider the ways in which the model might be in error. The results of a simulation can also often suggest reasons why certain observed, and apparently inexplicable, phenomena occur in practice.
2) *Modeling clarifies communication:* Modeling has proven to be an excellent platform for unambiguous communication between experts. This is essential in any modern teamwork in industry and academia.

3) *Models help in experimental design:* It is important that experiments be designed in such a way that the model can be properly tested. Often the model itself will suggest the need for data for certain parameters, which might otherwise be neglected, and hence the need for a particular type of experiment to provide the required data. Conversely, sensitivity tests on the model may indicate that certain parameters may have a negligible effect and hence that these effects therefore can be neglected both from the model and from the experimental program.
4) *Models may be used predictively for design and control:* Once the model has been established, it should be capable of predicting performance under differing sets of process conditions. Mathematical models can also be used for the design of relatively sophisticated control algorithms, and the model itself can often form an integral part of the control algorithm. Both mathematical and knowledge-based models can be used in designing and optimizing new processes.
5) *Models may be used in training and education:* Many important aspects of bioreactor operation can be simulated using very simple models such as linear growth, double substrate limitation, changeover from batch to fed-batch operation dynamics, fed-batch feeding strategies, aeration dynamics, measurement probe dynamics, cell retention systems, microbial interactions, biofilm diffusion, and bioreactor control. Such effects are very easily demonstrated by computer, as shown in the accompanying simulation examples, but are often difficult and expensive to demonstrate in practice.
6) *Models may be used for process optimization:* Optimization usually involves considering the influence of two or more operational variables, related to profits and to costs. For example, the objective might be to run a reactor to produce product at a maximum rate while leaving a minimum amount of unreacted substrate.

1.1.3 General Modeling Procedure

One of the more important features of modeling is the frequent need to reassess both the basic theory (physical model) and the mathematical equations, representing the physical model (mathematical model), in order to achieve the required degree of agreement, between the model prediction and actual process performance (experimental data).

As shown in Figure 1.1, the following stages in the modeling procedure can be identified:

(i) The first stage involves the proper **definition of the problem** and hence the goals and objectives of the study. These may include process analysis, improvement, optimization, design, and control, and it is important that the aims of the modeling procedure are properly defined. All the relevant theory must then be assessed in combination with any practical experience with the process, and perhaps alternative physical models for the process need to be developed and examined. At this stage, it is often helpful to start with the simplest possible conception of the process and to introduce complexities as the development

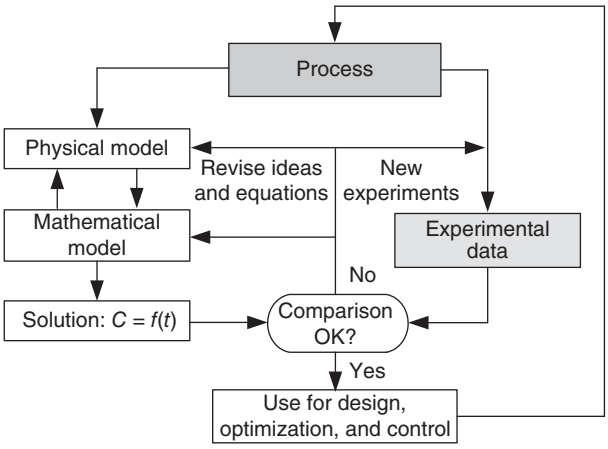

Figure 1.1 Information flow diagram for model building.

proceeds, rather than trying to formulate the full model with all its complexities at the beginning of the modeling procedure.

(ii) The available theory must then be **formulated in mathematical terms**. Most bioreactor operations involve quite a large number of variables (cell, substrate, and product concentrations; rates of growth, consumption, and production) and many of these vary as functions of time (batch, fed-batch operation). For these reasons, the resulting mathematical relationships often consist of quite large sets of differential equations.

(iii) After a model has been developed, the model equations must then be solved. Mathematical models of biological systems are usually quite complex and highly nonlinear, such that the mathematical complexity of the equations is usually sufficient to prohibit the use of an analytical means of solution. Numerical methods of solution must therefore be employed, preferably digital simulation in which the solution of very complex models is accomplished with relative ease.

Digital simulation languages are designed especially for the solution of sets of simultaneous differential equations using numerical integration. Many fast and efficient numerical integration routines are now available and implemented within the structure of the languages, such that many digital simulation languages are able to offer options for integration routine. Sorting algorithms within the structure of the language enable very simple programs to be written, having an almost one-to-one correspondence with the way in which the basic model equations were originally formulated. The resulting simulation programs are therefore very easy to understand and also to write. A further major advantage is a convenient output of results, in both tabulated and graphical forms that can be obtained via very simple program commands.

(iv) The validity of the computer prediction must be checked and steps (i)–(iii) will often need to be revised at frequent intervals during the modeling procedure.

The validity of the model depends on the correct choice of the available theory (physical and mathematical model), the ability to identify the model parameters correctly, and the accuracy of the numerical solution method.

(v) In many cases, owing to the complexity and very interactive nature of biological processes, the system will not be fully understood, thus leaving large areas of uncertainty in the model. Also, in cases where the relevant theory may be very difficult to apply, it is then often necessary to make rather gross simplifying assumptions, which may subsequently be eliminated or improved as a better understanding is obtained. Care must be taken when making judgments to avoid a model from becoming overly complex and hence not defined in terms of too many immeasurable parameters. Often an incorrect choice of parameter values can result in a disagreement between the model and practice and different trends in the variation of parameters during the simulation.

It should be noted, however, that often the results of a simulation model do not have to give an exact fit to the experimental data, and often it is sufficient to simply have a qualitative agreement. Thus, a very useful qualitative understanding of the process and its natural cause-and-effect relationships is obtained.

1.1.4 Simulation Tools

Many different digital simulation software packages are available on the market for PC and Mac applications. Modern tools are numerically powerful and highly interactive and allow sophisticated types of graphical and numerical output. Most packages also allow optimization and parameter estimation. In this book, we have chosen BERKELEY MADONNA (https://berkeley-madonna.myshopify.com) because it is very user-friendly and very fast (details can be found in the Appendix). With it, data fitting and optimization can be done very easily. MATLAB-SIMULINK (https://de.mathworks.com) is a popular and very powerful software for dynamic simulation and includes many powerful algorithms for nonlinear optimization, which can also be applied for parameter estimation. It also provides a direct link to all the powerful computational tools contained in MATLAB.

1.1.5 Teaching Applications

For effective teaching, the introduction of computer simulation methods into modeling courses can be achieved in various ways, and the method chosen will depend largely on how much time can be devoted, both inside and outside the classroom. The most time-consuming method for the students is to assign modeling problems to be solved outside the classroom. If scheduling time allows, computer laboratory sessions could be extended, so the students could work either alone or in groups of up to three on each monitor or computer. In this way, preprogrammed examples, as found in this text, can be used to emphasize particular points related to a previous theoretical presentation. This method has been found to be particularly effective when used for short, continuing-education, professional courses. Using the computer examples,

the students may vary parameters interactively and make program alterations, as well as work through the suggested exercises at his or her own pace. Demonstration of a particular simulation problem via a single personal computer and video projector is also an effective way of conveying the basic ideas in a short period of time, since students can still be very active in suggesting parametric changes and in anticipating the results. The best approach is probably to combine all three methods.

1.2 Development and Meaning of Dynamic Differential Balances

As indicated in Section 1.1, many models for biological systems are expressed in terms of sets of differential equations, which arise mainly as a result of the predominantly time-dependent nature of the process phenomena concerned.

For many people and especially for many students of life sciences, the mention of differential equations can cause considerable aversion. This section is therefore intended, hopefully, to bring the question of differential equations into perspective. The differential equations arise in the model formulation, simply by having to express rates of change of material, due to flow effects or chemical and biological reaction effects. The method for solution of the differential equations will be handled automatically by the computer. It is hoped that much of the difficulty can be overcome by considering the following case. In this section a simple example, based on the filling of a tank of water, is used to develop the derivation of a mass balance equation from the basic physical model and thereby to give meaning to the terms in the equations. Following the detailed derivation, a short-cut method based on rates is given to derive the dynamic balance equations.

Simple introductory examples are provided in the second part of the book starting with filling tanks and later addition of more and more complexity (Section 10.1.4).

Consider a tank into which water is flowing at a constant rate F (m^3/s), as shown in Figure 1.2. At any time t, the volume of water in the tank is V (m^3) and the density of water is ρ (kg/m^3).

During the time interval Δt (s), a mass of water $\rho F \Delta t$ (kg) flows into the tank. As long as no water leaves the tank, the mass of water in the tank will increase by a quantity $\rho F \Delta t$, causing a corresponding increase in volume, ΔV.

Equating the accumulation of mass in the tank to the mass that entered the tank during the time interval Δt gives

$$\rho \Delta V = \rho F \Delta t$$

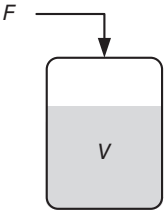

Figure 1.2 Tank of water being filled by stream with flow rate F.

If ρ is constant,

$$\frac{\Delta V}{\Delta t} = F$$

Applying this to very small differential time intervals ($\Delta t \rightarrow dt$) and replacing the Δ signs by the differential operator "d" gives the following simple first order differential equation, to describe the tank filling operation:

$$\frac{dV}{dt} = F$$

What do we know about the solution of this equation? That is, how does the volume change with time or in model terms, how does the dependent variable, V, change with respect to the independent variable, t? To answer this, we can rearrange the equation and integrate it between appropriate limits to give

$$V_1 - V_0 = \int_{t_0}^{t_1} F dt$$

or for constant F,

$$V_1 - V_0 = F \int_{t_0}^{t_1} dt = F(t_1 - t_0)$$

Integration is equivalent to summing all the contributions, such that the total change of volume is equal to the total volume of water added to the tank:

$$\sum V = \sum F \Delta t$$

For the case of constant F, it is clear that the analytical solution to the differential equation is

$$V = F\,t + \text{constant}$$

In this case, as shown in Figure 1.3, the constant of integration is the initial volume of water in the tank, V_0, at time $t = 0$.

Note that the slope in the variation of V with respect to t, dV/dt, is constant and that from the differential equation it can be seen that the slope is equal to F.

Suppose F is not constant but varies linearly with time (Figure 1.4).

$$F = F_0 - kt$$

The above model equation applies also to this situation.

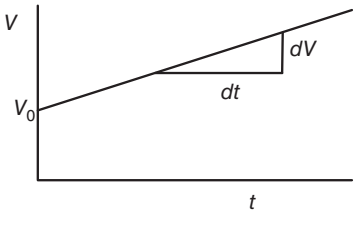

Figure 1.3 Volume change with time for constant flow rate.

Solving the model equation to obtain the functional dependence of V with respect to t,

$$\int dV = \int F dt = \int (F_0 - kt) dt = F_0 \int dt - k \int t dt$$

Integrating analytically,

$$V = F_0 t - \frac{kt^2}{2} + \text{constant}$$

The solution is

$$V = F_0 t - \frac{kt^2}{2} + V_0$$

Note that the dependent variable starts at the initial condition, V_0, and that the slope is always F. When F becomes zero and remains at this value, the slope of the curve relating V and t also becomes zero. In other words, the volume in the tank remains constant and does not change as long as the value of F remains zero.

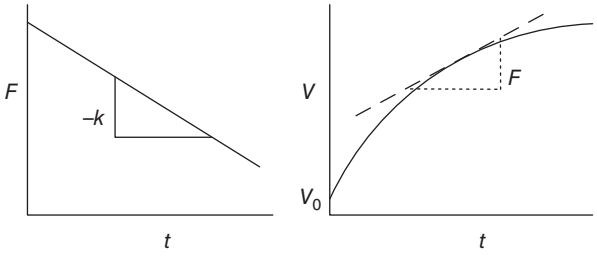

Figure 1.4 Variation of F and V for the tank-filling problem.

1.2.1 Derivation of a Balance Equation Using Rates

A differential balance can best be derived directly in terms of rates of change. For the above example, the balance can then be expressed as

$$\begin{pmatrix}\text{The rate of accumulation}\\ \text{of mass within the tank}\end{pmatrix} = \begin{pmatrix}\text{The flow rate of mass}\\ \text{entering the tank}\end{pmatrix}$$

Thus, the rate of accumulation of mass within the tank can be written directly as dM/dt where the mass M is equal to ρV. The rate of mass entering the tank is given by ρF, where both sides of the equation have quantities of mass per unit time with units of, e.g. kg/s.

$$\frac{dM}{dt} = \rho F$$

and

$$\frac{d(\rho V)}{dt} = \rho F$$

Thus, this approach leads directly to a differential equation model, which is the desired form for dynamic simulation. Note that both terms in the above relationship are expressed in quantities of mass per unit time or units of, e.g. kg/s.

At constant density, the equation again reduces to

$$\frac{dV}{dt} = F$$

which is to be solved for the initial condition, that at time $t = 0$, $V = V_0$. Consider the same situation of a variable flow rate, which is linearly decreasing with time, as given by

$$F = F_0 - kt$$

which is valid until $F = 0$.

These two equations, plus the initial condition, form the mathematical representation or the mathematical model of the physical model, represented by the tank filling with an entering flow of water that is decreasing linearly with time. This approach can be applied not only to the total mass but also to the mass of any component.

As shown above, this model is simple enough to obtain an analytical solution to give $V = f(t)$. However, for more complex cases, for example, when F varies according to a complex function, it is necessary to obtain a computer solution by a numerical integration of the model equations. This is important to understand, since analytical integration is seldom possible in the case of real complex problems.

1.2.2 Computer Solution

In principle, the numerical integration can be performed using the relations:

$$\frac{dV}{dt} = \frac{\Delta V}{\Delta t} = \frac{V_{i+1} - V_i}{t_{i+1} - t_i}$$

where $t_{i+1} - t_1$ represents a very short time interval and $V_{i+1} - V_i$ is the resulting change in volume of the water in the tank. As before, the flow is assumed to decrease with time according to $F = F_0 - kt$.

This integration procedure is equivalent to the following steps:

(1) Set the integration time interval.
(2) Assign a value to the inlet water flow rate at the initial value, time $t = t_0$.
(3) The term involving the water flow rate, $F_0 - kt$, is equal to the derivative value, (dV/dt), at time $t = t_0$.
(4) Knowing the initial value of V, V_0, and the slope dV/dt, a new value of V_1 can be calculated over the small interval of time, equivalent to the integration time interval or integration step length.
(5) At the end of the integration time interval, the value of V will have changed to a new value, representing the change of V with respect to time from its original value. The new value of V can thus be calculated.
(6) Using this new value of V, a next value for the rate of change of V with respect to time (dV/dt) at the end of the integration time interval can now be calculated.
(7) Knowing the value of V and the value of dV/dt at the end of the integration time interval, a further value of V can be estimated over a further integration step forward in time.

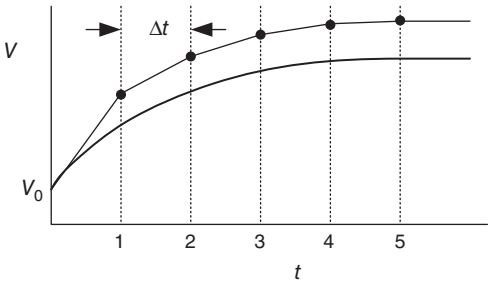

Figure 1.5 Graphical portrayal of numerical integration, showing slopes and approximated values of V at each time interval. The solid line represents the exact solution, and the dots show the values resulting from numerical integration. This method was actually already published in the eighteenth century by Swiss scientist Leonhard Euler.

(8) The entire procedure, as represented by steps (2)–(7) in Figure 1.5, is then repeated with the calculation moving forward with respect to time, until the value of F reaches zero. At this point the volume no longer increases, and the resulting final steady-state value of V is obtained, as well as all the intermediate values of V and F, which were determined during the course of the calculation.

Using such a numerical integration procedure, the computer can thus be used to generate data concerning the time variations of both F and V. In practice, more complex and more powerful numerical procedures are employed in digital simulation languages to give ismproved accuracy and speed of solution (Rasmuson et al. 2014).

1.3 Formulation of Mass Balance Equations

Here we describe mass and energy balances that are based on the conservation of mass and energy in any closed system.

1.3.1 Types of Mass Balance Equations

Dynamic Total Mass Balances

Based on the principle of conservation of mass, the general total mass balance for a system is defined as

$$\begin{pmatrix}\text{Rate of accumulation of}\\ \text{mass in the system}\end{pmatrix} = \begin{pmatrix}\text{Rate of mass flow}\\ \text{into the system}\end{pmatrix} - \begin{pmatrix}\text{Rate of mass flow}\\ \text{out of the system}\end{pmatrix}$$

Here the rate of accumulation term represents the rate of change in the total mass of the system, with respect to time.

Steady-state Total Mass Balance

At steady state, i.e. if all variables of a system do not change with time, the rate of accumulation is zero, and the balance simplifies to

$$\begin{pmatrix}\text{Rate of mass flow}\\ \text{into the system}\end{pmatrix} = \begin{pmatrix}\text{Rate of mass flow}\\ \text{out of the system}\end{pmatrix}$$

This equation can be applied to total mass and also to individual components as long as no conversion by reaction is occurring.

Component Balances

The previous discussion has been in terms of the total mass of the system, but most fluid streams, encountered in practice, contain more than one chemical or biological species. Provided no chemical change occurs, the generalized dynamic equation for the conservation of mass can also be applied to each component. Thus for any particular component:

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of mass}\\ \text{of component}\\ \text{in the system}\end{pmatrix} = \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{component}\\ \text{into}\\ \text{the system}\end{pmatrix} - \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{component}\\ \text{out of}\\ \text{the system}\end{pmatrix}$$

Component Balances with Reaction

Where chemical or biological reactions occur, this can be taken into account by the addition of a further term, the reaction rate, into the generalized component balance. Thus in the case of material produced by the reaction,

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of mass}\\ \text{of component}\\ \text{in the system}\end{pmatrix} = \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{component}\\ \text{into}\\ \text{the system}\end{pmatrix} - \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{component}\\ \text{out of}\\ \text{the system}\end{pmatrix} + \begin{pmatrix}\mathbf{Rate\ of}\\ \mathbf{production}\\ \mathbf{of\ the}\\ \mathbf{component}\\ \mathbf{by\ the\ reaction}\end{pmatrix}$$

and in the case of material consumed by the reaction the value of the rate of production would be negative.

Elemental Balances

The principle of mass balancing can also be extended to the atomic level and applied to particular chemical elements. Thus in the case of bioreactor operation, the general mass balance equation can also be applied to the four main elements, carbon, hydrogen, oxygen, and nitrogen and also to other elements if relevant to the particular problem. Thus for the case of carbon,

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of carbon in}\\ \text{the system}\end{pmatrix} = \begin{pmatrix}\text{Mass flow rate}\\ \text{of carbon}\\ \text{into}\\ \text{the system}\end{pmatrix} - \begin{pmatrix}\text{Mass flow rate}\\ \text{of carbon}\\ \text{out of}\\ \text{the system}\end{pmatrix}$$

Note the elemental balances do not involve reaction terms since the elements do not change by reaction.

PENFERM is based on the use of elemental mass balance equations for C, H, O, and N, which when combined with other empirical rate data, provide a working model for a penicillin production process.

While the principle of the mass balance is very simple, its application can often be quite difficult. It is important therefore to have a clear understanding of both the nature of the system (physical model), which is to be modeled using the mass balance equations and also of the methodology of modeling.

1.3.2 Balancing Procedure

The methodology described below outlines six steps to establish the model balances. The first task is to define the system by choosing the balance or control region.

Step I. *Choose the balance region such that the variables are constant or change little within the system. Draw boundaries around the balance region.*

The balance region may be a reactor, a reactor region, a single phase within a reactor, a single cell, or a region within a cell, but it will always be based on a region of assumed constant composition. Generally, the modeling exercises will involve some prior simplification. Often the system being modeled is usually considered to be composed of either systems of tanks (stagewise or lumped parameter systems) or systems of tubes (differential systems), or even combinations of tanks and tubes, as used in Case C.

In a first view, this step of modeling is usually considered the simplest one but turns out to be probably the most demanding one in more complex real cases. Therefore, we try to provide a flavor of this step in Chapter 9 where we provide a few cases of increasing complexity that are modeled with increasing consideration of details. In this first step, drawing a well-arranged sketch is most important and helps tremendously in creating good models that might be modular and thus expandable. This step requires the ability to abstract from an often confusing, real physical situation, e.g. an incompletely mixed reactor or a tissue. This process of abstraction requires a good knowledge of such physical systems and at the same time the ability to distinguish between important and less important issues.

1.3.2.1 Case A: Continuous Stirred Tank Bioreactor

If the tank is well mixed, the concentrations and density of the tank contents are uniform throughout. This means that the outlet stream properties are identical with the properties within the tank, in this case C_A and ρ. The balance region can therefore be taken around the whole tank (Figure 1.6).

The total mass in the system is given by the product of the volume of the tank contents V (m^3) multiplied by the density ρ (kg/m^3), thus $V\rho$ (kg). The mass of any

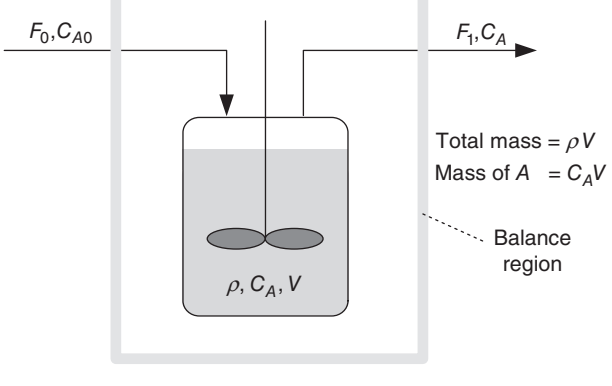

Figure 1.6 The balance region around the well-mixed continuous reactor.

component A in the tank is given as the product of V times the concentration of A, C_A (kg of A/m^3 or kmol of A/m^3), thus $V\,C_A$ (kg or kmol). The mass flow is the product of volumetric flow F (m^3/s) times the density ρ (kg/m^3), thus $F\rho$ (kg/s). Correspondingly the mass flow of component A is $F\,C_A$ (kg/s or kmol/s).

1.3.2.2 Case B: Tubular Reactor

In the case of tubular reactors, the concentrations of the products and reactants will vary continuously along the length of the reactor, even when the reactor is operating at steady state. This variation can be regarded as being equivalent to that of the time of passage of material as it flows along the reactor and is equivalent to the time available for reaction to occur. Under steady-state conditions, the concentration at any position along the reactor will be constant with respect to time, though not with position. This type of behavior can be approximated by choosing the balance regions sufficiently small so that the concentration of any component within a region can be assumed to be approximately uniform. Thus, in this case, many uniform property subsystems (well-stirred tanks or increments of volume elements with each of uniform concentration) comprise the total reactor volume (Figure 1.7).

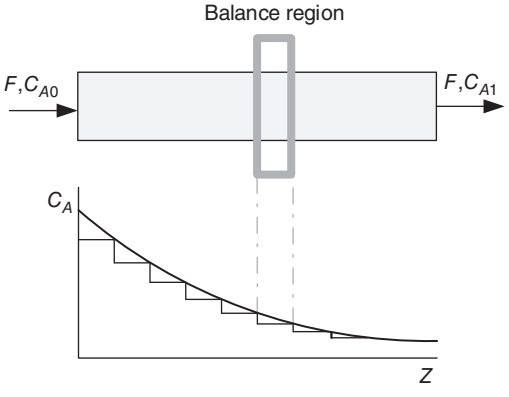

Figure 1.7 The tubular reactor axial concentration gradient and approximation by segmented balance regions.

1.3.2.3 Case C: River with Eddy Current

For this example, the combined principles of both the stirred tank and differential tubular modeling approaches need to be applied. As shown in Figure 1.8, the main flow along the river is very analogous to that of a column or tubular process, whereas the eddy region can be approximated by the behavior of a well-mixed tank. The interaction between the main flow of the river and the eddy, with flow into the eddy from the river and flow out from the eddy back into the river's main flow, must be included in a realistic model.

The real-life and rather complex behavior of the eddying flow of the river might thus be represented, by a series of many well-mixed subsystems (or tanks) representing the main flow of the river. This interacts at some particular stage of the river with a single well-mixed tank, representing the turbulent eddy. In modeling this system by means of mass balance equations, it would be necessary

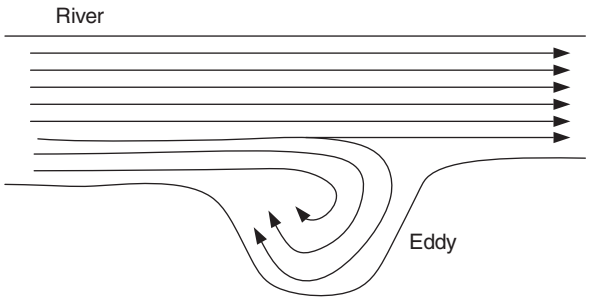

Figure 1.8 A complex river flow system.

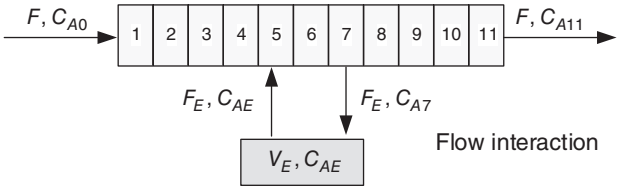

Figure 1.9 A multi-tank model for the complex river flow system.

to draw boundary regions around each of the individual subsystems representing the main river flow (sections 1–11 in Figure 1.9) and also around the tank system representing the eddy indicated by E. This would lead to 12 balance equations being required for each component. The resulting model could be used, for example, to describe the flow of a pollutant down the river in rather simple terms.

Step II. *Identify the transport streams that flow across the system.*

Having defined the balance regions, the next task is to identify all the relevant inputs and outputs to the system. These may be well-defined physical flow rates (convective streams), diffusive fluxes, and also interphase transfer rates. As seen in Figure 1.10, it is important to assume a direction of transfer and to specify this by means of an arrow. This direction might reverse itself but will be accommodated by a reversal in sign.

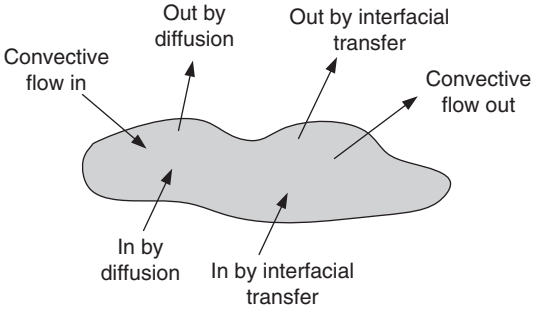

Figure 1.10 Balance region depicting convective, diffusive, and interfacial transfer flows in and out.

Step III. *Write the mass balance in word form.*

This is an important step because it helps to ensure that the resulting mathematical equation will have an understandable physical meaning. Just starting off by writing down equations is often liable to lead to fundamental errors, at least on the part of the beginner. All balance equations have a basic logic as expressed by the generalized statement of the component balance given below, and it is very important that the mathematical equations should retain this. Thus:

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of mass}\\ \text{of component}\\ \text{in the system}\end{pmatrix} = \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{component}\\ \text{into}\\ \text{the system}\end{pmatrix} - \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{component}\\ \text{out of}\\ \text{the system}\end{pmatrix} + \begin{pmatrix}\text{Rate of}\\ \text{production}\\ \text{of the}\\ \text{component}\\ \text{by reaction}\end{pmatrix}$$

This can be abbreviated as

$$\text{Accumulation} = \text{In} - \text{Out} + \text{Production}$$

Step IV. *Express each balance term in mathematical form with measurable variables*

A. Rate of Accumulation Term

This is given by the derivative of the mass of the system with respect to time. For the total mass of the system:

$$\frac{dM}{dt} = \frac{d(\rho V)}{dt}$$

with units

$$\frac{\text{kg}}{\text{s}} = \frac{\text{kg}}{\text{m}^3}\,\frac{\text{m}^3}{\text{s}}$$

For the mass of some component i within the system, we get

$$\begin{pmatrix}\text{Rate of accumulation of mass}\\ \text{of component i within the system}\end{pmatrix} = \frac{dM_i}{dt}$$

where mass, M, is in kg or mol and time, t, is in h, min, or s.

Volume, concentration, and, in the case of gaseous systems, partial pressure are usually the measured variables. Thus for any component i,

$$\frac{dM_i}{dt} = \frac{d(C_i V)}{dt}$$

where, C_i is the concentration of i (kmol/m^3 or kg/m^3). In the case of gases, the Ideal Gas Law can be used to relate concentrations to partial pressures and mol fractions.

Thus,

$$p_i V = n_i RT$$

where p_i is the partial pressure of i within the gas phase system and n_i is the number of moles of i. The gas constant, R ((m^3 Pa)/(K mol)), is in units compatible with p (Pa), V (m^3), n (mol), and T (K).

In terms of concentration,

$$C_i = \frac{n_i}{V} = \frac{p_i}{RT} = \frac{y_i p}{RT}$$

with units

$$\frac{\text{mol}}{\text{m}^3} = \frac{\text{Pa K mol}}{\text{m}^3\ \text{Pa K}}$$

where y_i is the mole fraction of the component in the gas phase and p is the total pressure.

The accumulation term for the gas phase can be written as

$$\frac{dM_i}{dt} = \frac{d(C_i V)}{dt} = \frac{d\left(\frac{p_i V}{RT}\right)}{dt} = \frac{d\left(\frac{y_i p V}{RT}\right)}{dt}$$

B. Convective Flow Terms

Total mass flow rates are given by the product of volumetric flow multiplied by density, and component mass flows by volumetric flow rates multiplied by concentrations.

$$\begin{pmatrix}\text{Convective}\\ \text{mass flow rate}\end{pmatrix} = \begin{pmatrix}\text{Volumetric}\\ \text{flow rate}\end{pmatrix}\ \left(\frac{\text{Mass}}{\text{Volume}}\right)$$

$$\frac{\text{kg}}{s} = \frac{\text{m}^3}{\text{s}}\ \frac{\text{kg}}{\text{m}^3}$$

$$\begin{pmatrix}\text{Total mass}\\ \text{flow rate}\end{pmatrix} = \dot{M} = F\,\rho$$

$$\begin{pmatrix}\text{Component}\\ \text{mass flow rate}\end{pmatrix} = \dot{M}_i = F\,C_i$$

A stream leaving a well-mixed region, such as a well stirred tank, has the same properties as the system volume as a whole, since for perfect mixing the contents of the tank will have uniform properties, identical to the properties of the fluid leaving at the outlet. Thus, the concentrations of component i, both within the tank and in the tank effluent, are equal to C_{i1}, as shown in Figure 1.11.

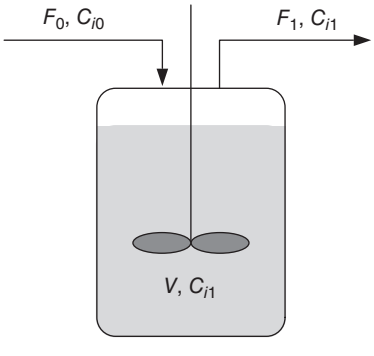

Figure 1.11 Convective flow terms for a well-mixed tank bioreactor.

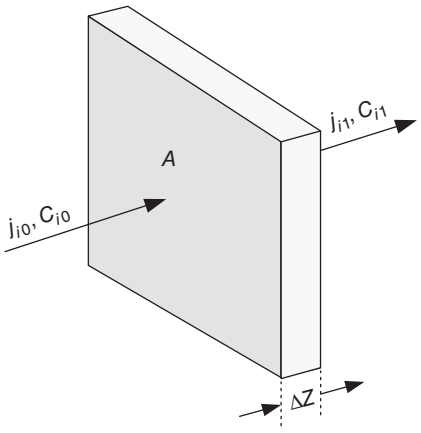

Figure 1.12 Diffusion flux j_i driven by concentration gradient $(C_{i0} - C_{i1})/\Delta Z$ through surface area A.

C. Diffusion of Components

Figure 1.12 depicts the diffusion of a component i through a slab of area A thickness ΔZ. Diffusional flow contributions are then expressed by Fick's law for molecular diffusion

$$j_i = -D_i \frac{dC_i}{dZ}$$

where j_i is the flux of any component i flowing across an interface (kmol/m^2 s or kg/m^2 s) and dC_i/dZ (kmol/m^4) is the concentration gradient.

In accordance with Fick's law, diffusive flow always occurs in the direction of decreasing concentration and at a rate proportional to the concentration gradient. Under true conditions of molecular diffusion, the constant of proportionality is equal to the molecular diffusivity for the system, D_i (m^2/h). For other cases, such as diffusion in porous matrices and turbulent diffusion, an effective diffusivity value is used, which must be determined experimentally. The concentration gradient may have to be approximated in finite-difference terms. Finite-differencing techniques are described in more detail in Section 6.2. Calculating the total diffusive mass rate, J_i, requires the area, through which diffusive transfer occurs.

$$\begin{pmatrix} \text{Mass rate of} \\ \text{component } i \end{pmatrix} = \begin{pmatrix} \text{Diffusivity of} \\ \text{component } i \end{pmatrix} \begin{pmatrix} \text{Area} \\ \text{perpendicular} \\ \text{to transport} \end{pmatrix} \begin{pmatrix} \text{Concentration} \\ \text{gradient of } i \end{pmatrix}$$

$$J_i = -D_i A \left(\frac{dC_i}{dZ} \right)$$

In terms of the concentration differences, the flux is

$$J_i = -D_i A \left(\frac{\Delta C_i}{\Delta Z} \right)$$

$$\frac{\text{kg}}{\text{s}} = \frac{\text{m}^2}{\text{s}} \text{m}^2 \frac{\text{kg}}{\text{m}^3 \text{m}}$$

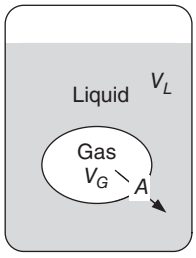

Figure 1.13 Transfer of oxygen from gas phase with volume V_G across a gas–liquid interface of area A into a liquid phase of volume V_L.

D. Interphase Transport

Interphase mass transport also represents a possible flow into or out of a system or balance region and may occur between gas, liquid, and solid phases (G/L, L/L, L/S, or G/S). In bioreactor modeling applications, this is most frequently represented by the case of oxygen transfer from air to the liquid medium, followed by oxygen taken up by the cells during respiration. In this case, the transfer of oxygen occurs across the gas liquid interface, which exists between the surface of the air bubbles and the surrounding liquid medium, as shown in Figure 1.13.

Other applications may involve the supply of oxygen to the bioreactor by transfer from the air, across a membrane and then into the bulk liquid. Where there is interfacial transfer from one phase to another, the component balance equations will need appropriate modification to consider this. Thus, an oxygen balance for the well-mixed gas phase, with transfer from the gas to the liquid, can be written as

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of the}\\ \text{mass of oxygen}\\ \text{in the}\\ \text{gas phase}\end{pmatrix} = \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{oxygen}\\ \text{into the}\\ \text{gas phase}\end{pmatrix} - \begin{pmatrix}\text{Mass flow}\\ \text{of the}\\ \text{oxygen}\\ \text{out the}\\ \text{gas phase}\end{pmatrix} - \begin{pmatrix}\textbf{Rate of}\\ \textbf{interfacial}\\ \textbf{mass transfer}\\ \textbf{from the}\\ \textbf{gas phase}\\ \textbf{into the}\\ \textbf{liquid phase}\end{pmatrix}$$

This form of transfer rate equation will be examined in much more detail in Chapter 5. Suffice it to say here that the rate of transfer can be expressed in the form shown below:

$$\begin{pmatrix}\text{Rate of}\\ \text{mass}\\ \text{transfer}\end{pmatrix} = \begin{pmatrix}\text{Mass}\\ \text{transport}\\ \text{coefficient}\end{pmatrix}\begin{pmatrix}\text{Interfacial}\\ \text{area per}\\ \text{liquid volume}\end{pmatrix}\begin{pmatrix}\text{Concentration}\\ \text{driving force}\end{pmatrix}\begin{pmatrix}\text{Liquid}\\ \text{volume}\end{pmatrix}$$

$$r_{\text{MTR}} = K\, a\, \Delta C\, V_L$$

where r_{MTR} is the rate of mass transfer, a is a specific area for mass transfer, A/V (m^2/m^3), A is the total interfacial area for mass transfer (m^2), V is the liquid phase volume (m^3), ΔC is the concentration driving force (kmol/m^3 or kg/m^3), and K is the overall mass transfer coefficient (m/s). Mass transfer rate expressions are usually expressed in terms of kmol/s and can be converted to mass flows (kg/s), if desired.

The units of the terms in the equation (with appropriate mass quantity units) are

$$\frac{\text{kg}}{\text{s}} = \frac{\text{m}}{\text{s}}\,\frac{\text{m}^2}{\text{m}^3}\,\frac{\text{kg}}{\text{m}^3}\,\text{m}^3$$

E. Production Rate

This term in the component balance equation allows for the production or consumption of material by reaction and is incorporated into the component balance equation. Thus,

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of mass}\\ \text{of component}\\ \text{in the system}\end{pmatrix} = \begin{pmatrix}\text{Rate of}\\ \text{mass flow of}\\ \text{the component}\\ \text{into the}\\ \text{system}\end{pmatrix} - \begin{pmatrix}\text{Rate of}\\ \text{mass flow of}\\ \text{the component}\\ \text{out of the}\\ \text{system}\end{pmatrix} + \begin{pmatrix}\textbf{Rate of}\\ \textbf{production}\\ \textbf{of the}\\ \textbf{component}\\ \textbf{by reaction}\end{pmatrix}$$

Chemical production rates are often expressed on a molar basis, e.g. (mol/m^{3}s), and, as in the case of the interfacial mass transfer rate expressions, can be easily converted to mass flow quantities (kg/s). The mass balance equation can then be expressed as

$$\begin{pmatrix}\text{Mass rate}\\ \text{production of}\\ \text{component } A\end{pmatrix} = r_A V = \begin{pmatrix}\text{Reaction rate}\\ \text{per unit volume}\end{pmatrix}\begin{pmatrix}\text{Volume of}\\ \text{the system}\end{pmatrix}$$

$$\frac{\text{kg A}}{\text{s}} = \frac{\text{kg A}}{\text{s m}^3}\,\text{m}^3$$

Equivalent molar quantities may also be used. The quantity r_A is positive when A is formed as product, and r_A is negative when a reactant A is consumed.

The growth rate for cells can be expressed in the same manner, using the symbol r_X. Thus,

$$\begin{pmatrix}\text{Mass rate of}\\ \text{biomass production}\end{pmatrix} = r_X V = \begin{pmatrix}\text{Growth rate}\\ \text{per volume}\end{pmatrix}\begin{pmatrix}\text{Volume}\\ \text{of system}\end{pmatrix}$$

$$\frac{\text{kg}}{\text{s}} = \frac{\text{kg}}{\text{s m}^3}\,\text{m}^3$$

The consumption rate of substrate, r_S, is often directly related to the cell growth rate by means of a constant yield coefficient $Y_{X/S}$, which has the units of kg biomass produced per kg substrate consumed. Thus,

$$\begin{pmatrix}\text{Mass rate}\\ \text{of substrate}\\ \text{consumption}\end{pmatrix} = \frac{\begin{pmatrix}\text{Growth rate}\\ \text{per volume}\end{pmatrix}}{\begin{pmatrix}\text{Biomass yield}\\ \text{on substrate}\end{pmatrix}}\begin{pmatrix}\text{Volume}\\ \text{of system}\end{pmatrix}$$

$$r_S V = -\frac{r_X}{Y_{X/S}}V$$

$$\frac{\text{kg}}{\text{m}^3\,\text{s}}\text{m}^3 = \frac{\text{kg biomass}}{\text{m}^3\,\text{s}}\,\frac{\text{kg substrate}}{\text{kg biomass}}\,\text{m}^3$$

Note that the value of r_S will have the opposite sign of r_X.

Step V. *Introduce other relationships and balances such that the number of equations equals the number of dependent variables*

The system mass balance equations are often the most important elements of any modeling exercise, but are themselves rarely sufficient to completely formulate

the model. Other relationships are therefore needed to supplement the material balance relations, both to complete the model in terms of other important aspects of behavior and to satisfy the mathematical rigor of the modeling, such that the number of unknown variables must be equal to the number of defining equations.

Examples of this type of relationships that are not based on balances, but nevertheless form an important, usually essential part of any model are as follows:

- Stoichiometric or yield relationships for relation between reaction rates or metabolic fluxes
- Reaction rates as functions of concentration, temperature, and pH
- Ideal gas law behavior
- Physical property correlations as functions of concentration
- Pressure variations as a function of flow rate
- Dynamics of measurement instruments as a function of the instrument response time
- Equilibrium relationships (e.g. Henry's law)
- Controller equations
- Correlations of mass transfer coefficient, gas holdup volume, and interfacial area, as functions of system physical properties and degree of agitation or flow velocity
- Flow as function of pressure

How these and other relationships are incorporated within the development of particular modeling instances are shown later in the cases given throughout the text and in the simulation examples.

Step VI. *For additional insight with complex problems, draw an information flow diagram*

Information flow diagrams can be useful in understanding complex interactions (Franks 1967). They help to identify missing relationships and provide a graphical aid to a full understanding of the interactive nature of systems. Such a diagram is given in the simulation example BATFERM.

1.3.3 Total Mass Balances

In this section the application of the total mass balance principles will be presented. Consider some arbitrary balance region, represented by the gray area in Figure 1.14. Mass accumulates within the system at a rate dM/dt, owing to the competing effects of a convective flow input (mass flow rate in) and an output stream (mass flow rate out).

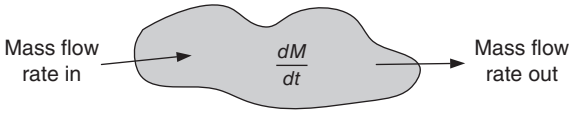

Figure 1.14 Balancing the total mass of an arbitrary system.

The total mass balance is expressed by

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation of mass}\\ \text{in the system}\end{pmatrix} = \begin{pmatrix}\text{Mass flow rate}\\ \text{into the system}\end{pmatrix} - \begin{pmatrix}\text{Mass flow rate}\\ \text{out of the system}\end{pmatrix}$$

$$\frac{dM}{dt} = (\text{Mass rate in}) - (\text{Mass rate out})$$

or in terms of volumetric flow rates, F, densities, ρ, and volume, V:

$$\frac{dM}{dt} = \frac{d(V\rho_1)}{dt} = F_0\rho_0 - F_1\rho_1$$

Indices 0 and 1 represent flow in and flow out. When densities are equal, as in the case of water flowing in and out of a tank,

$$\frac{dV}{dt} = F_0 - F_1$$

The steady-state condition of constant volume in the tank ($dV/dt = 0$) occurs when the volumetric flow in, F_0, is exactly balanced by the volumetric flow out, F_1. Total mass balances therefore are mostly important for those bioreactor-modeling situations in which volumes are subject to change.

1.3.4 Component Balances for Reacting Systems

Each chemical species can be described with a component balance around an arbitrary, well-mixed, balance region, as shown in Figure 1.15.

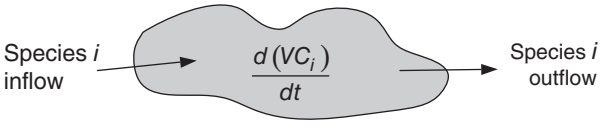

Figure 1.15 Component balancing for species *i*.

Thus for any species *i*, involved in the system, the component mass balance is given by

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of mass}\\ \text{of component } i\\ \text{in the system}\end{pmatrix} = \begin{pmatrix}\text{Mass flow}\\ \text{rate of}\\ \text{component } i\\ \text{into the}\\ \text{system}\end{pmatrix} - \begin{pmatrix}\text{Mass flow}\\ \text{rate of}\\ \text{component } i\\ \text{out of the}\\ \text{system}\end{pmatrix} + \begin{pmatrix}\textbf{Rate of}\\ \textbf{production}\\ \textbf{of}\\ \textbf{component } \boldsymbol{i}\\ \textbf{by reaction}\end{pmatrix}$$

Expressed in terms of volume, volumetric flow rate and concentration, this is equivalent to

$$\frac{d(VC_i)}{dt} = F_{\text{inflow}}C_{i,\text{inflow}} - F_{\text{outflow}}C_i + r_{\text{outflow}}V$$

with units of mass/time:

$$\frac{\text{m}^3\,\frac{\text{kg}}{\text{m}^3}}{\text{s}} = \frac{\text{m}^3}{\text{s}}\frac{\text{kg}}{\text{m}^3} - \frac{\text{m}^3}{\text{s}}\frac{\text{kg}}{\text{m}^3} + \frac{\text{kg}}{\text{m}^3\text{s}}\text{m}^3 = \frac{\text{kg}}{\text{s}}$$

1.3.4.1 Case A: Constant Volume Continuous Stirred Tank Reactor

A constant volume, continuous, tank reactor with reaction $A \rightarrow 2B$ is considered here, as shown in Figure 1.16.

Component A is converted to component B in a 1–2 M ratio.

The total mass balance is

$$\frac{d(V\rho_1)}{dt} = F_0\rho_0 - F_1\rho_1$$

The component balances for A and B are

$$\frac{d(VC_{A1})}{dt} = F_0C_{A0} - F_1C_{A1} + r_{A1}V$$

$$\frac{d(VC_{B1})}{dt} = F_0C_{B0} - F_1C_{B1} + r_{B1}V$$

Here it is convenient to use molar amounts, such that each term has the units of kmol/s.

Since rules of differentiation of products yield

$$d(VC_A) = VdC_A + C_AdV$$

we get under constant volume conditions, $dV = 0$:

$$d(VC_A) = VdC_A$$

$$d(VC_B) = VdC_B$$

and from the total mass balance with constant density, we additionally get $F_0 = F_1$. Thus the two model equations of A and B then simplify to give

$$\frac{dC_{A1}}{dt} = \frac{F}{V}(C_{A0} - C_{A1}) + r_{A1}$$

and

$$\frac{dC_{B1}}{dt} = \frac{F}{V}(C_{B0} - C_{B1}) + r_{B1}$$

In these two balances, there are four unknowns: C_{A1}, C_{B1}, r_{A1}, and r_{B1}. The kinetics are assumed to be first order, as often found in biological systems at low concentration. Then,

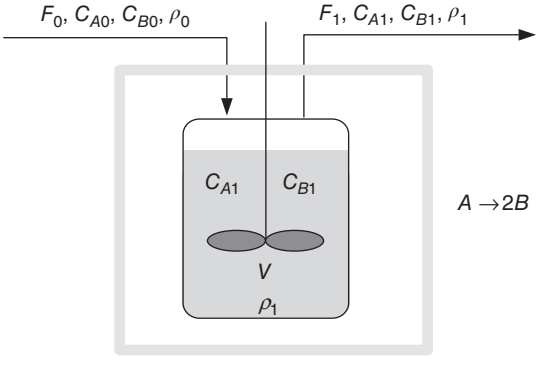

Figure 1.16 Continuous stirred, well-mixed tank reactor with reaction A → 2B.

$$r_{A1} = -k\,C_{A1}$$

According to the molar stoichiometry,

$$r_{B1} = -2\,r_{A1} = +2\,k\,C_{A1}$$

Together with the kinetic relations, there are four equations and four unknowns, thus satisfying the conditions necessary for the model solution. With the initial conditions, C_{A1} and C_{B1} at time $t = 0$, specified, the solution to these two simultaneous equations, combined with the two kinetic relations, will give the resulting changes of concentrations C_{A1} and C_{B1} as functions of time. The simulation example ENZCON is similar to this situation.

1.3.4.2 Case B: Semicontinuous Reactor with Volume Change

The reaction and reaction rate data are the same as in the preceding example, but now the reactor has no effluent stream (Figure 1.17). The operation of the reactor is therefore semicontinuous or fed batch.

The total mass balance with constant density is

$$\frac{dV}{dt} = F_0$$

The component balances with no flow of material leaving the reactor are now

$$\frac{d(VC_A)}{dt} = F_0 C_{A0} + r_A V$$

$$\frac{d(VC_B)}{dt} = r_B V$$

Note that V must remain within the differential, because the volume of the reactor contents is now also a variable and is determined by the total mass balance specified above. The kinetics is as before

$$r_A = -k\,C_A$$

$$\frac{\text{mol}}{\text{m}^3\,\text{s}} = \frac{1}{s}\,\frac{mol}{m^3}$$

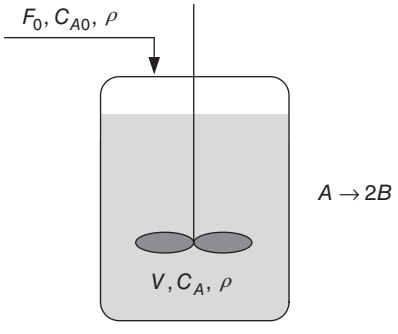

Figure 1.17 A semicontinuous, well-mixed reactor example.

In terms of moles, the stoichiometry gives,

$$r_B = -2\, r_A = +2\, k\, C_A$$

With initial conditions for the initial molar quantities of A and B, (VC_A, VC_B) and the initial volume of the contents, V, at time $t = 0$ specified, the resulting system of equations can be solved to obtain the time-varying quantities $VC_A(t)$, $VC_B(t)$, $V(t)$ and hence also concentrations C_A and C_B as functions of time. Similar variable volume situations are found in examples FEDBAT, VARVOL, and various others. There are also examples with repeated feeding and emptying, e.g. REPFED and REPLCUL.

1.3.4.3 Case C: Steady-state Oxygen Balancing in Fermentation

Calculation of the oxygen uptake rate, *OUR*, by means of a steady-state oxygen balance is an important application of component balancing for fermentation. In the reactor in Figure 1.18, the entering air stream flow rate, oxygen concentration, here expressed using the molar fraction y, temperature, and pressure conditions are designated by the subscript 0 and the exit conditions by the subscript 1.

Writing a balance for O_2 around the combined gas and liquid phases in the reactor gives

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of } O_2\end{pmatrix} = \begin{pmatrix}\text{Flow rate}\\ \text{of } O_2 \text{ in}\end{pmatrix} - \begin{pmatrix}\text{Flow rate}\\ \text{of } O_2 \text{ out}\end{pmatrix} - \begin{pmatrix}\text{Rate of } O_2\\ \text{uptake}\\ \text{by the cells}\end{pmatrix}$$

At steady state, the accumulation terms for both phases are zero and

$$\begin{pmatrix}\text{Rate of } O_2\\ \text{uptake}\\ \text{by the cells}\end{pmatrix} = \begin{pmatrix}\text{Flow rate}\\ \text{of } O_2 \text{ in}\end{pmatrix} - \begin{pmatrix}\text{Flow rate}\\ \text{of } O_2 \text{ out}\end{pmatrix}$$

The assumption of steady-state is usually well justified because of the very low solubility of O_2. For gaseous systems, the quantities are often expressed in terms of molar quantities.

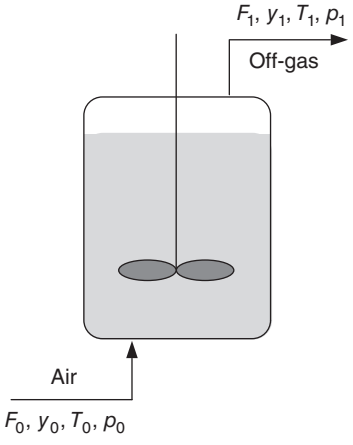

Figure 1.18 Entering air and exit gas during the continuous aeration of a bioreactor.

Often only the inlet air flow rate, F_0, and the mole fraction of O_2 in the outlet gas, y_1, are measured. It is often assumed that the total molar flow rate of gas is constant. This is a valid assumption as long as the number of carbon dioxide moles produced is nearly equal to the number of oxygen moles consumed.

Converting to molar quantities, using the Ideal Gas Law,

$$p\,V = n\,R\,T$$

or in flow terms

$$p\,F = N\,R\,T$$

where N is the molar flow rate (mol/s), R is the gas constant ((m^3 Pa)/(K mol)), and F is the volumetric flow rate (m^3/s). Thus, for the inlet gas flow,

$$N_0 = \frac{p_0}{R\,T_0} F_0$$

where N_0 is molar flow rate of the oxygen entering. Note that the pressure, p_0, and temperature, T_0, are measured at the point of flow measurement.

Assuming $N_0 = N_1$, then measurement of N_0 gives enough information to calculate oxygen uptake rate, *OUR*, from the steady-state balance. Thus,

$$0 = y_0 N_0 - y_1 N_1 + r_{O_2} V_L$$

$$\text{OUR} = -r_{O_2} V_L = y_0 N_0 - y_1 N_1$$

If N_0 is not equal to N_1, then this equation will give large errors in oxygen uptake rate, and N_1 must be measured, or determined indirectly by an inert balance. This is explained in detail below.

1.3.4.4 Case D: Inert Gas Balance to Calculate Flow Rates

Differences in the inlet and outlet-gas flow rates of a tank fermenter can be calculated by measuring one gas flow rate and the mole fraction of an inert gas in the gas streams. Since inert gases, such as nitrogen or argon, are not consumed or produced within the system ($r_{\text{inert}} = 0$), their mass rates must therefore be equal at the inlet and outlet streams of the reactor, assuming steady-state conditions apply. Then for nitrogen,

$$\begin{pmatrix}\text{Molar flow of}\\ \text{nitrogen in}\end{pmatrix} = \begin{pmatrix}\text{Molar flow of}\\ \text{nitrogen out}\end{pmatrix}$$

and in terms of mole fractions,

$$N_1 y_{\text{inert1}} = N_0 y_{\text{inert0}}$$

From this balance, calculation of N_1 can be made on the basis of a combination of measurements of N_0 and the inert gas partial pressures y_{inert}, at both inlet and outlet conditions.

$$N_1 = N_0 \frac{y_{\text{inert0}}}{y_{\text{inert1}}}$$

Since the inlet mole fraction for nitrogen in air is known, the outlet mole fraction, y_{inert1}, must be measured. This is often done by difference, having measured the mole fraction of oxygen and carbon dioxide concentration in the exit gas.

1.4 Additional Relationships

1.4.1 Stoichiometry and Metabolite and Elemental Balancing

Stoichiometry is the basis for any quantitative treatment of chemical and biochemical reactions. In biochemical processes, it is a necessary basis for building kinetic models.

1.4.1.1 Simple Stoichiometry

The stoichiometry of chemical reactions is used to relate the relative quantities of the different materials, which react with one another and also the relative quantities of product that are formed. Most chemical and biochemical reactions are relatively simple in terms of their molar relationship or stoichiometry. For single reactions, stoichiometric coefficients are clearly defined and may usually be determined easily. Some examples are given below:

$$\underset{\text{Pyruvic acid}}{C_3H_4O_3} + NADH + H^+ \rightarrow \underset{\text{Lactic acid}}{C_3H_6O_3} + NAD^+$$

This relation indicates that 1 mol of pyruvic acid reacts with 1 mol of NADH to produce 1 mol of lactic acid.

Another example of stoichiometry is that of the more complex oxidative decarboxylation of pyruvic acid that yields acetyl-CoA:

$$\underset{\text{Pyruvic acid}}{C_3H_4O_3} + \text{CoA-SH} + NAD^+ \rightarrow \underset{\text{Acetyl-CoA}}{CH_3\text{CO-S-CoA}} + CO_2 + NADH + H^+$$

Stoichiometry relations also describe even more complex pathways and can be written with exact molar relationships, like the pentose-phosphate pathway operating in a fully cyclic mode as shown below.

$$\text{Glucose} + 12NADP^+ + ATP + 7H_2O \rightarrow 6CO_2 + 12\left(NADPH + H^+\right) + ADP + P_i$$

where 1 mol of glucose reacted consumes 7 mol of water and produces 6 mol of carbon dioxide. Here the molar quantities of NADPH and ATP produced and consumed, respectively, are shown.

For many complex biological reactions, however, not all the elementary reactions and their contributions to the overall observed reaction stoichiometry are known (Roels 1983; Bailey and Ollis 1986; Moser 1988; Villadsen et al. 2011).

Thus the case of a general fermentation is usually approximated by an overall reaction equation, where

$$\text{Substrate} + \text{Nitrogen source} + O_2 \rightarrow \text{Product} + CO_2 + H_2O$$

$$C_{SC}H_{SH}O_{SO} + \nu_{NH_3}(t)NH_3 + \nu_{O_2}(t)O_2 \rightarrow \nu_{P_i}(t)C_{P_iC}H_{P_iH}O_{P_iO}N_{P_iN} + \nu_{CO_2}(t)CO_2 + \nu_{H_2O}(t)H_2O$$

where the i^{th} product, such as metabolites or biomass, is given by a general formula.

In the case above, the generalized elemental formulae are used for substrate, biomass, and products, but the nitrogen source is given simply as ammonia. The stoichiometric coefficients, ν, for each component are taken relative to that of substrate and their coefficients may vary with time, as a result of changing fermentation conditions. Some indication as to the relative magnitudes of the stoichiometric coefficients can be obtained from elemental balancing techniques or by using the stoichiometry of a large, even genome-scale network. However, particularly in fermentations using complex media, the problem is so complex that other concepts, such as the more approximate yield coefficient concept, are used to relate the relative proportions of materials undergoing conversion during the fermentation in a more empirical way.

1.4.1.2 Metabolic Network Stoichiometry: Metabolite Balancing

A metabolic network of a cell is basically composed of simple reactions as exemplified above. Whole genome data are presently made publicly available on a dramatically increasing number of organisms, e.g. on KEGG (Kyoto Encyclopedia of genes and genomes; Kanehisa et al. 2012). This greatly supports metabolite balancing of whole cells. For practical reasons these networks are usually simplified and can be made part of a dynamic model.

In Chapter 8, we will derive material balances for whole cells leading to metabolite balancing. The result for intracellular steady state, with neglecting cell volume increase, is best shown with an example network as given below (Figure 1.19).

This network can be written in matrix form as

$$0 = \begin{bmatrix} 1 & -1 & 0 & 0 & -1 & 0 & 0 \\ 0 & 2 & -1 & 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & -1 \end{bmatrix} \begin{bmatrix} r_1 \\ r_2 \\ r_3 \\ r_4 \\ r_5 \\ r_6 \\ r_7 \end{bmatrix} = \mathbf{Sr}$$

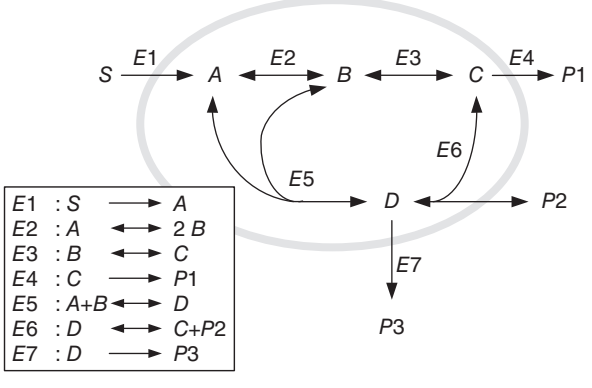

Figure 1.19 Example metabolic network with components. E1 to E7 are enzymes catalyzing the corresponding reactions r_1 to r_7.

This system has seven unknowns and four equations and is therefore underdetermined. Three measurements would make the system fully determined, e.g. S, $P1$, and $P2$, allowing the determination of r_1, r_4, and r_7 in a batch or continuous culture. Splitting the system into measured variables, $\boldsymbol{S_m}$ and $\boldsymbol{r_m}$, and in calculated ones, $\boldsymbol{S_c}$ and $\boldsymbol{r_c}$ results in

$$0 = \begin{bmatrix} -1 & 0 & -1 & 0 \\ 2 & -1 & -1 & 0 \\ 0 & 1 & 0 & 1 \\ 0 & 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} r_2 \\ r_3 \\ r_5 \\ r_6 \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} r_1 \\ r_4 \\ r_7 \end{bmatrix} = \boldsymbol{S_c r_c} + \boldsymbol{S_m r_m}$$

and allows the estimation of nonmeasurable fluxes or rates, $\boldsymbol{r_c}$, after inversion of matrix $\boldsymbol{S_c}$

$$\boldsymbol{r}_c = -(\boldsymbol{S}_c)^{-1} \boldsymbol{S}_m \boldsymbol{r}_m$$

This can be used to calculate intracellular metabolic fluxes if sufficient measurements are available. This method has been used in mammalian cell cultivation (Niklas et al. 2011; Niklas and Heinzle 2012). Stoichiometric network calculations can also be coupled to dynamic models if, e.g. the rates of $\boldsymbol{r_m}$ are determined by corresponding kinetics (Dorka et al. 2009). The network stoichiometry can also be decomposed into elementary modes and then connected to kinetics of, e.g. growth and substrate uptake (Provost et al. 2006; Zamorano et al. 2013). Experimentally established but incomplete metabolite balances can also be applied in kinetic models, e.g. to describe the production of antibodies in mammalian cell culture (Ben Yahia et al. 2017). See the simulation example on such production using CHO cells, CHOMAB.

1.4.1.3 Elemental Balancing

The technique of elemental balancing can be represented as follows:

Take the general case of

$$\underset{\text{Substrate}}{CH_mO_1 + aNH_3 + bO_2} \rightarrow \underset{\text{Biomass}}{cCH_pO_nN_q} + \underset{\text{Product}}{dCH_rO_sN_t} + eH_2O + fCO_2$$

where c, d, and f are the fractions of carbon converted to biomass, product, and CO_2, respectively.

Elemental balances for C, H, O, and N give

$$\begin{array}{lll} \text{C} & 1 & = c + d + f \\ \text{H} & m + 3a & = cp + dr + 2e \\ \text{O} & 1 + 2b & = cn + ds + e + 2f \\ \text{N} & a & = cq + dt \end{array}$$

In this general problem, there are too many unknowns for the solution method to be taken further, since the elemental balances provide only four equations and hence can be solved for only four unknowns. Assuming that the elemental formulae for substrate, biomass, and product and hence l, m, n, p, q, r, s, and t are defined, there still remain six unknown stoichiometric coefficients a, b, c, d, e, and f and only four

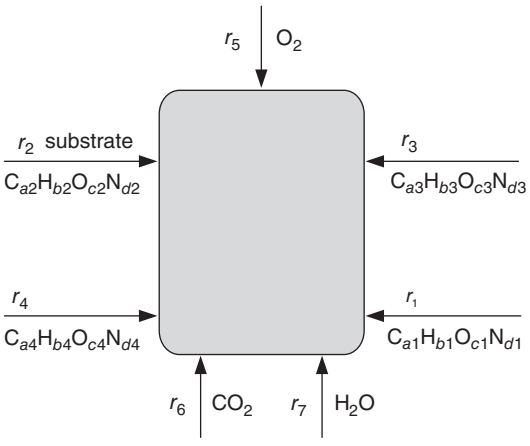

Figure 1.20 Flow inputs into a system. r_i are the fluxes or reaction rates feeding into the system.

elemental balance equations. Thus the elemental balances need supplementation by other measurable quantities such as substrate, oxygen, and ammonia consumption rates (assuming controlled pH conditions) and carbon dioxide or biomass production rates, to satisfy the condition that the number of unknowns is equal to the number of defining equations. In principle, the problem then becomes solvable. In practice, there can be considerable difficulties and inaccuracies involved, although the technique of elemental balancing can still provide useful data. The application of so-called macroscopic principles (Roels 1983; Heijnen and Roels 1981) introduces a more strict systematic system of analysis (depicted in Figure 1.20).

The system is represented here in terms of the various flow inputs, where **r** is the corresponding flow vector

$$\mathbf{r} = \begin{bmatrix} r_1 & r_2 & r_3 & r_4 & r_5 & r_6 & r_7 \end{bmatrix}$$

The steady-state balance for the system is then represented by:

$$\mathbf{E} \times \mathbf{r}' = 0$$

where **E** is the elemental composition matrix

$$\mathbf{E} = \begin{bmatrix} a_1 & a_2 & a_3 & a_4 & 0 & 1 & 0 \\ b_1 & b_2 & b_3 & b_4 & 0 & 0 & 2 \\ c_1 & c_2 & c_3 & c_4 & 2 & 2 & 1 \\ d_1 & d_2 & d_3 & d_4 & 0 & 0 & 0 \end{bmatrix} \begin{matrix} \text{(C)} \\ \text{(H)} \\ \text{(O)} \\ \text{(N)} \end{matrix}$$

The combination of seven unknown quantities and four elemental balance equations leaves three independent quantities. Thus assuming fluxes r_1 (biomass), r_2 (substrate), and r_3 (product) are known, the unknown fluxes r_4, r_5, r_6, and r_7 can be obtained by methods of linear algebra given in detail by Roels (1983) and later Villadsen et al. (2011) using the term "black box" stoichiometry. The system is also completely determined if three kinetic expressions are available, e.g. for r_1, r_2, and r_3.

In more complex cases with growth and product formation, more information is needed. The introduction of the concept of the degree of reduction is useful (Erickson et al. 1978). For organic compounds, this is defined as the number of equivalent available electrons per gram atom C that would be transferred to CO_2, H_2O, and NH_3 upon oxidation. Taking charge numbers C = 4, H = 1, O = −2, and N = −3, reductance degrees, γ, can be defined for

$$\begin{aligned} &\text{Substrate}(S) \quad \gamma_S = 4 + m - 2l \\ &\text{Biomass}(X) \quad \gamma_X = 4 + p - 2n - 3q \\ &\text{Product}(P) \quad \gamma_P = 4 + r - 2s - 3t \end{aligned}$$

The reductances for NH_3, H_2O, and CO_2 are of course zero.

Often the elemental composition of the substrate is not known, and then the reductance method may be supplemented by the following regularities, which apply to a wide variety of organic molecules.

$Q_{O_2} = 27$ J/g equivalent of available electrons transferred to oxygen
$\gamma_X = 4.29$ g equivalent of available electrons per equivalent 1 g atom C in biomass
$\sigma_X = 0.462$ g carbon/g dry biomass

1.4.2 Yield Coefficients

1.4.2.1 Mass Yield Coefficients

Yield coefficients are biological variables resulting from a certain metabolic network activity, which are used to relate the ratio between various consumption and production rates or fluxes of mass and energy. Thus they are representing the lumped stoichiometry of a complex system. They are typically assumed to be time-independent and are calculated on an overall basis. This concept should not be confused with the overall yield of a reaction or a process. The biomass yield coefficient on substrate, $Y_{X/S}$, is defined as

$$Y_{X/S} = \frac{r_X}{r_S}$$

In batch systems, reaction rates are equal to accumulation rates, and therefore

$$Y_{X/S} = -\frac{\left(\frac{dC_X}{dt}\right)}{\left(\frac{dC_S}{dt}\right)} = -\frac{dC_X}{dC_S}$$

After integration from time 0 to time t, the integral value is obtained:

$$Y_{X/S} = \frac{\text{Amount of biomass produced}}{\text{Total amount of substrate consumed}}$$

$$Y_{X/S} = \frac{C_X(t) - C_X(t=0)}{C_S(t=0) - C_S(t)}$$

For a steady-state continuous system, the mass balances give

$$Y_{X/S} = \frac{r_X}{r_S} = \frac{C_{X1} - C_{X0}}{C_{S0} - C_{S1}}$$

where index 0 and 1 indicate feed and effluent values, respectively.

Table 1.1 Typical mass and energy yield values.

Type of yield coefficient	Unit	Value
$Y_{X/S,\text{aer}}$	C-mol/C-mol	0.4–0.7
$Y_{X/S,\text{anaer}}$	C-mol/C-mol	0.1–0.2
Y_{X/O_2} (glucose)	C-mol/mol	1–2
$Y_{X/\text{ATP}}$	C-mol/mol	0.35
Y_{Q/O_2}	kJ/mol	380–490
Y_{Q/CO_2}	kJ/mol	460
$Y_{Q/X,\text{aer}}$ (glucose)	kJ/C-mol	325–500
$Y_{Q/X,\text{anaer}}$	kJ/C-mol	120–190

Note: The molecular weight of biomass is taken here as 24.6 g/C-mol where C-mol denotes the number of moles of carbon.
Source: Data from Roels (1983); Moser (1988); Atkinson and Mavituna (1991) and Villadsen et al. (2011).

In the literature, yield coefficients for biomass with respect to nutrients are most often used (Mou and Cooney 1983; Roels 1983; Moser 1988; Villadsen et al. 2011). In many cases, this is very useful because the biomass composition is quite uniform and often product selectivity does not change very much during an experiment involving exponential growth and associated production. Some useful typical values are given in Table 1.1.

The yield coefficients are usually determined as a result of a large number of elementary biochemical reactions, and it can easily be understood that their values might vary depending on environmental and operating conditions leading, e.g. to different growth phases. A detailed description of some of these dependencies is given in the literature. Despite their variability though within often small ranges, measured yield coefficients are often very useful for practical purposes of process description and modeling. Such yield coefficients are used in almost all simulation examples comprising growth of microorganisms or animal cells.

1.4.2.2 Selectivity

Selectivity ($S_{i,j}$) describes ratios of rates as well but usually the ratio of a desired product to the total production rate or to the production rate of one specific product.

$$S_{i,j} = \frac{r_i}{r_j}$$

1.4.2.3 Energy Yield Coefficients

Energy yield coefficients may be defined similar to mass yield coefficients.

In terms of oxygen uptake,

$$Y_{Q/O_2} = \frac{r_Q}{r_{O_2}} = \frac{\text{Amount of heat released}}{\text{Amount of oxygen consumed}}$$

In terms of carbon substrate consumed,

$$Y_{Q/S} = \frac{r_Q}{r_S} = \frac{\text{Amount of heat released}}{\text{Amount of carbon consumed}}$$

1.5 Thermodynamics and Equilibrium Relationships

1.5.1 Reaction Enthalpy

All chemical reactions are inherently related to changes in enthalpy, ΔH_r, the enthalpy of reaction that can be calculated from heats of formation or heats of combustion

$$\Delta H_r = \sum_{n=1}^{n} v_i \Delta H_{Fi} = \sum_{n=1}^{n} v_i \Delta H_{Ci}$$

where ΔH_{Fi} is the heat of formation of component i, ΔH_{Ci} is the heat of combustion of component i, and v_i is the stoichiometric coefficient for component i. Usually $\Delta H_r < 0$ and therefore heat is released to the environment during reaction (Ingham et al. 2000; Villadsen et al. 2011). Enthalpies of reaction are essential parts of energy balances of biochemical processes.

1.5.2 Chemical Equilibrium

The second most important relationship describes chemical equilibrium. For a typical biochemical reaction

$$A + B \rightleftharpoons C + D$$

the equilibrium constant, K_{eq}, is defined as

$$K_{\text{eq}} = \frac{C_C C_D}{C_A C_B}$$

for low aqueous concentrations C_i that are specified as molar concentrations. The equilibrium constant is related to the free enthalpy, ΔG_r

$$\Delta G_r = \Delta G_r^0 + RT \ln K_{\text{eq}}$$

where R is the universal gas constant and T the absolute temperature. $\Delta G_r{}^0$ is the standard free enthalpy, usually at $T = 25\,^{\circ}\text{C}$, all reacting species are 1 M concentration, and in biochemical reactions pH = 7. Equilibrium is reached if $\Delta G_r = 0$. An exergonic reaction is characterized by $\Delta G_r < 0$ and can proceed spontaneously or catalytically accelerated by an enzyme. For a more detailed discussion, see, e.g. Villadsen et al. (2011).

1.5.3 Receptor Binding

A typical equilibrium reaction is the dissociation of a receptor-protein ligand complex, LP, into the free ligand protein, L, and the receptor protein, P:

$$LP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} L + P$$

This reaction is characterized by the corresponding dissociation equilibrium constant K_D

$$K_D = \frac{C_L\, C_P}{C_{LP}} = \frac{k_1}{k_{-1}}$$

Such relationships can be used to express the concentration of free protein P in an explicit function of concentration of the total concentration, $C_{P\text{tot}}$, and the free ligand concentration, C_L, by adding a protein balance.

$$C_{P\text{tot}} = C_P + C_{LP}$$

$$C_P = C_{P\text{tot}} \frac{K_D}{C_L + K_D}$$

Such type of equilibrium interactions exist for many biological component pairs, e.g. metabolite/protein, drug/transporter protein, protein/DNA, etc.

In more complex cases with interactions of various receptors or with a buffer system containing several components, it is not possible to express the concentrations in explicit forms and a nonlinear algebraic equation has to be solved during the simulation. The implementation of such problems into Madonna is shown below with the example of pH calculation. See also simulation examples ANAMEAS and DCMDEG.

1.5.4 Case A: Calculation of pH with an Ion Charge Balance

Modeling systems with variable pH requires modeling of acid–base equilibria, whose reactions are almost instantaneous. Production of acids or bases causes a variation of pH, which depends on the buffer capacity of the system. pH also influences the biological kinetics. It has been shown that only the non-dissociated acid forms are kinetically important substrates in anaerobic systems. The concentration of these species is a function of the pH as can be seen in the equilibrium equation

$$\text{Acid} \rightleftharpoons \text{Base}^- + \text{H}^+$$

with dissociation constant

$$K_D = \frac{C_{\text{Base}^-}\, C_{\text{H}^+}}{C_{\text{Acid}}}$$

where C_{Acid} is the concentration of the non-dissociated acid and C_{Base^-} is the concentration of the corresponding base (salt).

An ion charge balance can be written as

$$\sum (\text{Cations} \times \text{Charge}) = \sum (\text{Anions} \times \text{Charge})$$

The conservation of charge is actually a special form of mass balance considering the mass of electrons. In the pH range of interest (usually around pH = 7), all strong acids and strong bases are completely dissociated. Moderately strong acids and bases exist in both the dissociated and non-dissociated forms.

In the usual pH range, the sum of the cations are much larger than the H^+ ions.

$$\sum C_{K^+} >> C_{H^+}$$

where $\sum C_{K^+}$ is the total cation concentration.

Negative ions not only originate mainly from strong acids (e.g. Cl^-, SO_4^{2-}) but also arise from weak acids, e.g. acetic, propionic, butyric, and carbonic acids (Ac^-, Pr^-, Bu^-, HCO_3^-). In biological systems, the concentration of CO_3^{2-} is always much smaller than that of HCO_3^-.

The ion balance reduces to

$$\sum \frac{K_{Bi}}{K_{Bi} + \frac{K_W}{C_{H^+}}} C_{B\text{tot},i} + \sum C_{K^+} = \sum \frac{K_{Ai}}{K_{Ai} + C_{H^+}} C_{A\text{tot},i} + \sum C_{A^-}$$

where K_{Ai} are the acid dissociation constants (e.g. K_{Ac}); K_{Bi} are the base dissociation constants (e.g. K_{NH_3}); K_W is the dissociation constant of water; $C_{B\text{tot},i}$ are the total concentrations of base i; $\sum C_{An^-}$ is the sum of the cation concentrations; $C_{A\text{tot},i}$ are the total concentrations of acid i; and $\sum C_{A^-}$ is the sum of the anion concentrations.

The pH can be estimated from the above equation for any situation by solving the resulting nonlinear implicit algebraic equation, provided the total concentrations of the weak acids, $C_{A\text{tot},i}$, weak bases, $C_{B\text{tot},i}$, cations of strong bases, C_{K^+}, and anions of strong acids, C_{A^-}, are known.

It is convenient to use only the difference between cations and anions, C_Z:

$$C_Z = \sum C_{K^+} - \sum C_{A^-}$$

After neglecting any ammonia buffering effect, it is useful to rearrange the above equations in the form,

$$\delta = \sum \frac{K_i}{K_i + C_{H^+}} C_{\text{tot},i} - C_Z$$

To satisfy equilibrium conditions, δ should be zero. The example ANAMEAS includes this ion balance for pH calculation. This equation represents an algebraic loop in a dynamic simulation, which is solved by iteration at each time interval until δ approaches zero. This is accomplished with the root-finding function of BERKELEY MADONNA.

For pH control, a strong base or acid is usually added. The addition of strong alkali for pH control would cause an increase in $\sum C_{K^+}$, which in accordance with the above equation would result in a decrease of C_{H^+}.

An alternative approach, which avoids an algebraic loop, is to treat the instantaneous equilibrium reactions as reactions with finite forward and backward rates. These rates must be adjusted with their kinetic constants to maintain the equilibrium for the particular system; that is, these rates must be very fast compared with the other rates of the model. This approach replaces the algebraic loop iteration with a stiffer and larger set of differential equations, which could be an advantage in some cases.

In simple cases, only one buffer system is present, e.g. bicarbonate. Then an explicit solution for the pH can be obtained as shown in the simulation example

DCMDEG. In MADONNA this is very helpful for staged systems, since root finding with arrays is not supported there.

1.6 Energy Balancing for Bioreactors

Energy balances are needed whenever temperature changes are important, as caused by reaction heating effects or by cooling and heating for temperature control. For example, such a balance is needed when the heat of fermentation causes a variation in bioreactor temperature. Energy balances are essential for modeling heat sterilization processes. Energy balances are written following the same set of rules as given above for mass balances in Section 1.3. Thus the general form is as follows:

$$\begin{pmatrix}\text{Accumulation}\\ \text{rate of}\\ \text{energy}\end{pmatrix} = \begin{pmatrix}\text{Rate}\\ \text{of}\\ \text{energy}\\ \text{in by}\\ \text{flow}\end{pmatrix} - \begin{pmatrix}\text{Rate}\\ \text{of}\\ \text{energy}\\ \text{out by}\\ \text{flow}\end{pmatrix} - \begin{pmatrix}\text{Rate of}\\ \text{energy}\\ \text{out by}\\ \text{transfer}\end{pmatrix} + \begin{pmatrix}\text{Rate of}\\ \text{energy}\\ \text{generated}\\ \text{by}\\ \text{reaction}\end{pmatrix} + \begin{pmatrix}\text{Rate of}\\ \text{energy}\\ \text{added}\\ \text{by}\\ \text{agitation}\end{pmatrix}$$

In a general case it includes all forms of energy. The above balance in word form is now applied to the case of a continuous reactor as shown in Figure 1.21.

Considering only those forms of energy that are relevant in biological reaction systems, we can get an easily applicable form of the energy balance equation. An exact derivation of this was given by Aris (1989) and results in

$$\sum_{i=1}^{S}(n_{i1}c_{pi1})\frac{dT_1}{dt} = F_0\sum(C_{i0}(h_{i0}-h_{i1})) + UA(T_a - T_1) + r_Q V$$

where n_i is the number of moles of component i, c_{pi} is the partial molar heat capacities, and h_i is the partial molar enthalpies. In this equation, the rate of heat production, r_Q, changes at temperature T_1. If the heat capacities, c_{pi}, are independent of temperature, the enthalpies at T_1 can be expressed in terms of heat capacities as

$$h_{i1} = h_{i0} + c_{pi}(T_1 - T_0)$$

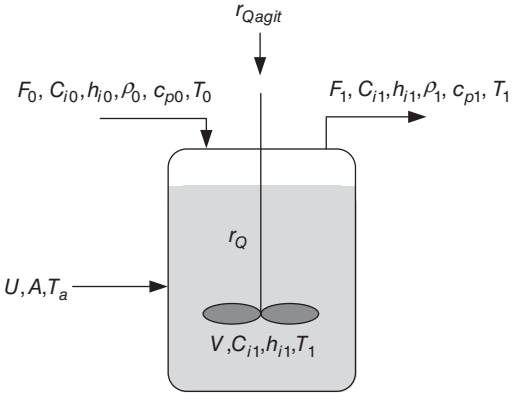

Figure 1.21 A continuous tank bioreactor giving flows with energy-related variables.

and with

$$\sum_{i=1}^{S}(n_{i0}c_{pi0}) \approx \sum_{i=1}^{S}(n_i c_{pi}) = V\rho c_p$$

We eventually get the energy balance form as provided below:

$$V\rho c_p \frac{dT_1}{dt} = F_0\rho c_p(T_0 - T_1) + UA(T_a - T_1) + r_Q V$$

The units of each term of the equation are energy per time (kJ/s or kcal/s). This form is usually applied after neglecting agitation heat effects.

In highly agitated reactors, the heat dissipated by the stirrer may become important and is characterized by $r_{Q\text{agit}}$. This term may be particularly important in slowly growing and viscous cultures. In aerated bioreactors, $r_{Q\text{agit}}$ usually has values between 1 and 10 kW/m^3.

In aerated bioreactors, an additional heat flow term related to the evaporation of water may also be significant. Gas introduced into the reactor is usually dry and does therefore not contain any water vapor. Evaporation of water changes its enthalpy causing heat removal from the reactor liquid at a rate $r_{Q\text{evap}}$.

The resulting modified energy balance is

$$V\rho c_p \frac{dT_1}{dt} = F_0\rho c_p(T_0 - T_1) + UA(T_a - T_1) + r_Q V - r_{Q\text{evap}}V + r_{Q\text{agit}}$$

1.6.1 Accumulation Term

Densities and heat capacities of liquids can be taken as essentially constant. The term

$$V\rho c_p \frac{dT}{dt}$$

has units of

$$\text{m}^3 \frac{\text{kg}}{\text{m}^3} \frac{\text{kJ}}{\text{kg K}} \frac{\text{K}}{\text{s}} = \frac{\text{kJ}}{\text{s}}$$

Here ($\rho\ c_p\ T$) is an energy "concentration" term and has quantities of

$$\left(\frac{\text{Mass}}{\text{Volume}}\right)\left(\frac{\text{Energy}}{\text{Mass Degree}}\right)(\text{Degree}) = \left(\frac{\text{Energy}}{\text{Volume}}\right)$$

Thus the accumulation term has the quantities of energy/time and units of kJ/s. This term is actually describing the accumulation of energy in the form of heat and is therefore often called heat accumulation.

1.6.2 Flow Term

The liquid flow term is

$$(\text{Heat flow term}) = F_0\rho c_p(T_0 - T_1)$$

which comprises the quantities

$$\left(\frac{\text{Energy}}{\text{Time}}\right) = \left(\frac{\text{Volume}}{\text{Time}}\right)\left(\frac{\text{Energy}}{\text{Volume}}\right)$$

and has units

$$\frac{\text{kJ}}{\text{s}} = \frac{\text{m}^3}{\text{s}} \frac{\text{kg}}{\text{m}^3} \frac{\text{kJ}}{\text{kg K}} \text{K}$$

This term actually describes heating of the stream entering the system with T_0 to the reaction temperature T_1. It is important to note here that this term is exactly the same for a continuous reactor as for a fed-batch system.

1.6.3 Water Evaporation Term

An additional flow term is related to the heat of evaporation of volatiles, primarily water, from the fermentation fluid as shown in Figure 1.22.

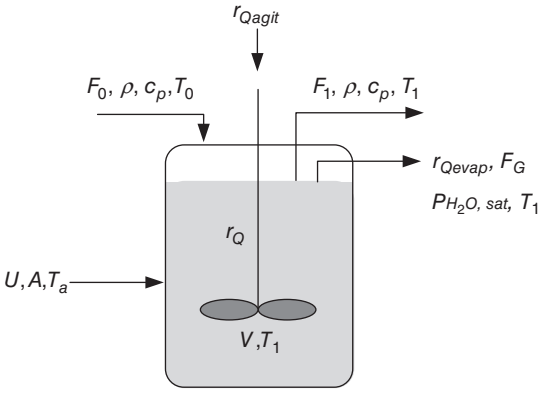

Figure 1.22 A continuous tank fermenter giving flows with energy-related variables.

In most cases the incoming gas, i.e. air, is dry because it was compressed leading to condensation of water. The gas leaving the reactor will be saturated with water at typical operating conditions (Oeggerli and Heinzle 1994). The resulting molar flow of water out of the reactor, $N_{\text{H}_2\text{O}}$, is

$$N_{\text{H}_2\text{O}} = \frac{p_{\text{H}_2\text{O,sat}} F_G}{V_L RT}$$

and has units of

$$\frac{\text{mol}}{\text{m}^3\text{s}} = \frac{\text{kPa} \frac{\text{m}^3}{\text{s}}}{\text{m}^3 \frac{\text{m}^3\text{kPa}}{\text{K mol}} \text{K}}$$

Together with the molar heat of evaporation, we get the respective heat flow, $r_{Q\text{evap}}$

$$r_{Q\text{evap}} = N_{\text{H}_2\text{O}} \Delta H_{\text{evap}}$$

with units of

$$\frac{\text{kJ}}{\text{m}^3\text{s}} = \frac{\text{mol}}{\text{m}^3\text{s}} \frac{\text{kJ}}{\text{mol}}$$

With $T = 303$ K, $p_{\text{H}_2\text{O,sat}} = 4.25$ kPa, $R = 8.315$ m^3 Pa/K mol, and $\Delta H_{\text{evap}} = 40.8$ kJ/mol, the heat removal rate has values of $r_{Q\text{evap}}$ of 0.122 and 1.22 kJ/m^3 s at gas flow rates of 0.1 and 1 vvm (volume gas [volume liquid]$^{-1}$/min). This is usually significantly

smaller than the reaction heat term but creates a constant bias at constant flow rate and temperature. Reducing the gas flow rate will proportionally reduce r_{Qevap}. In this way one can estimate whether inclusion of an evaporation term in the energy balance is required or not.

1.6.4 Heat Transfer Term

The heat transfer term is

$$\text{(Heat transfer rate)} = UA(T_a - T_1)$$

The important quantities in this term are the heat transfer area A, the temperature driving force or difference $(T_a - T_1)$, where T_a is the temperature of the heating or cooling source, and the overall heat transfer coefficient, U. The heat transfer coefficient, U, has the quantities of energy/(time area degree) with units kJ/(s m^2 K).

The quantities for $UA\Delta T$ are thus

$$\left(\frac{\text{Energy}}{\text{Time}}\right) = \left(\frac{\text{Energy}}{\text{Area Time Degree}}\right)\text{(Area)(Degree)}$$

with units

$$\frac{\text{kJ}}{\text{s}} = \frac{\text{kJ}}{\text{Km}^2\text{s}}\text{m}^2\text{K}$$

The sign of the temperature difference determines the direction of heat flow. Here if $T_a > T_1$ heat flows into the reactor.

1.6.5 Reaction Heat Term

The reaction heat term is defined as

$$\text{(Reaction heat term)} = r_Q V$$

$r_Q V$ gives the rate of heat released by the bioreaction and has quantities of

$$\left(\frac{\text{Energy}}{\text{Time}}\right) = \left(\frac{\text{Energy}}{\text{Volume Time}}\right)\text{(Volume)}$$

and units

$$\frac{\text{kJ}}{\text{s}} = \frac{\text{kJ}}{\text{m}^3\text{s}}\text{m}^3$$

The rate term r_Q can alternatively be written in various ways as follows.

In terms of substrate uptake and a substrate-related heat yield,

$$r_Q = r_S Y_{Q/S}$$

In terms of oxygen uptake and an oxygen-related heat yield,

$$r_Q = r_{O_2} Y_{Q/O_2}$$

In terms of a heat of reaction per mole of substrate and a substrate uptake rate,

$$r_Q = \Delta H_{r,S} r_S$$

Here r_S is the substrate uptake rate and $\Delta H_{r,S}$ is the heat of reaction for the substrate, for example, kJ/mol or kcal/kg. The $\Delta H_{r,S}\, r_S$ term therefore has quantities of energy/(time volume) and is equal to r_Q.

Simulation examples using energy balances are TEMPCONT, FERMTEMP, BATSTER, and PENFERM.

1.6.6 Case B: Determining Heat Production Rate of a Batch Fermentation

For a constant-volume batch reactor with no agitation heat effects, as depicted in Figure 1.23, the reactor energy balance is

$$\begin{pmatrix}\text{Accumulation rate}\\ \text{of heat}\end{pmatrix} = -\begin{pmatrix}\text{Energy out}\\ \text{by transfer}\end{pmatrix} + \begin{pmatrix}\text{Heat generated}\\ \text{by reaction}\end{pmatrix}$$

$$V_1\rho_1 c_p \frac{dT_1}{dt} = -UA(T_1 - T_a) + r_Q V_1$$

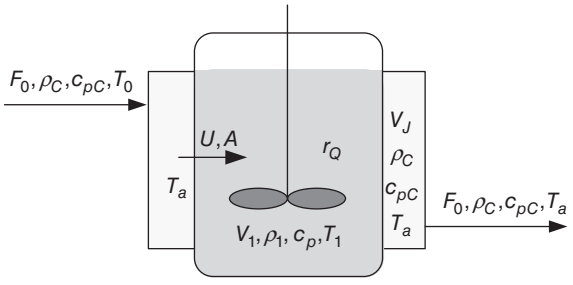

Figure 1.23 A batch-stirred tank bioreactor with cooling jacket.

Combined with the energy balance of a cooling jacket with the assumption of well-mixing,

$$V_J\rho_C c_{pC}\frac{dT_a}{dt} = F_0\rho_C c_{pC}(T_0 - T_a) + UA(T_1 - T_a)$$

where F_0 is the coolant stream with entering temperature T_0. At constant temperatures ($dT_i/dt = 0$), and after combining both equations, we get for specific rate of heat production

$$r_Q = \frac{F_0\rho_C c_{pC}}{V_1}(T_0 - T_a).$$

1.6.7 Case C: Determining Heat Transfer Area or Cooling Water Temperature

If we want to determine the heat transfer area of the reactor depicted in Figure 1.23, we may also use known respiration data of the organism of interest. For aerobic fermentation, the rate of heat production per unit volume of reactor is usually directly related to the oxygen uptake rate, r_{O_2}, as described above (see also Table 1.1).

$$r_Q = r_{O_2} Y_{Q/O_2}$$

After rearrangement of the reactor balance equation of Case A, we get

$$\frac{dT_1}{dt} = \frac{UA}{V\rho c_p}(T_a - T_1) + r_{O_2} Y_{Q/O_2} \frac{1}{\rho c_p}$$

If the temperature is kept constant ($dT_1/dt = 0$),

$$UA(T_a - T_1) = r_{O_2} Y_{Q/O_2} V$$

$$\left(\text{Heat transfer rate}\right) = (\text{Rate of heat release})$$

Using this steady-state energy balance, one can calculate the required heat transfer area for a given cooling water temperature and heat production rate.

$$A = \frac{r_{O_2} Y_{Q/O_2} V}{U(T_a - T_1)}$$

To determine the heat transfer coefficient, U, electrical heating can be applied replacing the biological heat formation term.

$$A = \frac{Q_{el}}{U(T_a - T_1)}$$

Q_{el} is the heat production rate by the electrical heating system (kJ/s). Using this steady-state energy balance, it is possible to calculate the needed cooling water temperature (T_a) for a given oxygen uptake rate and cooling device after rearrangement of the equation:

$$T_a = \frac{r_{O_2} Y_{Q/O_2} V + UAT_1}{UA}$$

In this way one can check whether a certain heat production rate can be reached while keeping the reactor temperature at T_1 if cooling water at T_a is available. Higher heat production rate would require much more expensive cooling systems.

Alternatively this same relation can be used to calculate the biomass concentration allowable for a given cooling system and available cooling water temperature, knowing the specific oxygen uptake rate (kg O_2/kg biomass h).

1.7 Time Constants

Time constants are characteristic parameters that describe the response of a first-order system to a step input. In the context of this book, first-order systems are, e.g. first-order chemical/biochemical reactions, dynamic response of a sensor, mixing in a well-mixed tank. In systems only comprising processes of first order, time constants can be used to compare the time behavior of these processes in a strict sense. Time constants can, however, also be defined for process of orders different from one but only in an approximate way. This is very useful to set up dynamic processes. We can e.g. identify that for growth of microorganisms the time constant is roughly of the order of hours, whereas e.g. evolution has a time constant that is much longer and the dissociation of an enzyme–substrate complex is much

faster than growth. Therefore, we do not have to consider the latter processes for setting up a growth model. Time constants can be used to discover whether a change of regime occurs during scale up. Generally, they can be applied for an order-of-magnitude analysis. Time constants can also be used to determine whether the overall rate of a process is limited by a particular rate process, e.g. by kinetics, mass transfer, mixing, or diffusion.

In many biological systems, processes with large ranges of time constants have to be. Usually it is important to start with a simplification of a system focusing on the most important time constant or rate. For example, if the growth of an organism is to be modeled with a time constant of the order of hours, it is very useful to ignore all aspects of biological evolution with time constants of years. Also fast equilibrium reactions or conformational changes of proteins having time constants below milliseconds should be ignored. Fast reactions can, however, be very important when considering allosteric activation or deactivation of proteins or simply pH changes during biochemical reactions. pH changes can have dramatic effects on the enzyme and microbial activity but can also strongly influence absorption and desorption of carbon dioxide (Section 1.5.4).

Time constants are used in many simulation examples, e.g. OXDYN, KLADYN, ELECTFIT, KLAFIT, TITERDYN, TITERBIO, BIOFILM, CELLDIFFBEAD, CELLDIFFCYL, TURBCON, ADAPTOXCONT, and PLASMID.

1.7.1 Derivation from Differential Equations

Ideally, time constants are derived from their governing differential equations. A general first-order process is described by

$$\tau \frac{dy}{dt} + y = y_0$$

Here, τ is the time constant (s). In this book, we often derive and use the same equation in this form

$$\frac{dy}{dt} = \frac{y_0 - y}{\tau}$$

Integration of this equation yields

$$y = y_0(1 - e^{-t/\tau})$$

For $t = \tau$, we get

$$y_\tau = y_0(1 - e^{-1}) = 0.632 y_0$$

The first-order response to a step change in an incoming signal is depicted in Figure 1.24. It also shows a straightforward determination of τ by just taking the time at a response of 0.632 of the final value. This procedure may be very useful for a quick determination of a sensor time constant that may be applied in a control circuit, as described in Sections 7.2.1 and 7.3.3, and related simulation examples. It may also be applied for the dynamic determination of mass transfer using e.g. an oxygen probe (Section 5.3.1.3)

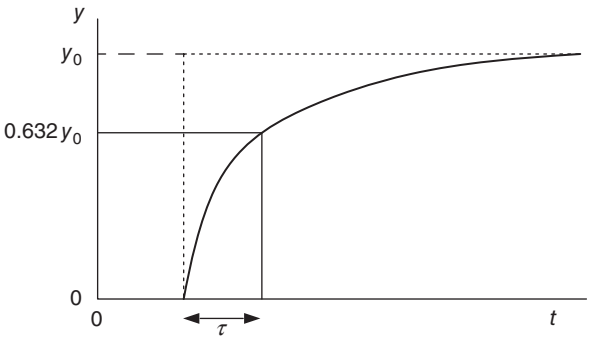

Figure 1.24 First-order response of a process after a step change.

As described in Section 4.1.2, the time constant for pure mixing a well-mixed reactor, τ_{mix} is V/F. The time constant of a reaction of first order is $\tau_r = 1/k$. A diffusion time constant is derived from Fick's Law as shown in Section 6.2.3, $\tau_{\text{mix}} = L^2/D$, where L (m) is the diffusion length and D (m^2/s) the diffusion coefficient.

1.7.2 Derivation from Capacity and Rate

An alternative definition of time constants uses capacity and rate (Table 1.2). The time constant is then

$$\tau = \text{time constant} = \frac{\text{capacity}}{\text{rate}}$$

These and other time constants are used throughout this book and particularly in simulation examples.

Table 1.2 Time constants defined by capacity and rate.

Description	Symbol	Capacity symbol	Units	Rate symbol	Units
Traveling time	τ	L	m	v	m/s
Residence time	τ	V	m^3	F	m^3/s
Mass transfer	τ_{ht}	$V\,C$	kmol	$V\,k_L a\,C$	kmol/s
Reaction time	τ_r	$V\,C$	kmol	$V\,r_C$	kmol/s
Heat transfer	τ_{ht}	$V\,\rho\,c_p\,dT$	J	$U\,A\,dT$	J/s
Heat production	τ_{hp}	$V\,\rho\,c_p\,dT$	J	$V\,r_Q$	J/s
Diffusion time	τ_D	$V\,C$	kmol	$(A\,D\,C)/L$	kmol/s