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CHAPTER 3

Microbial Systems

3.1 Introduction

Microorganisms play an important role in many biological systems. Wetlands, compost piles, fermentation vessels, wastewater treatment reactors, anaerobic digesters, bioprocess operations, bioremediation of contaminated soils, and many other biological systems all require the effective use of microorganisms. Biological systems engineers must understand the function of microorganisms in diverse settings and be able to apply the tools of engineering to the analysis and design of systems containing microorganisms.

Many textbooks are available that discuss various aspects of microorganisms and their functions within specific applications. However, each discussion very quickly takes the reader into the specific processes and applications of that particular field. A wastewater treatment text discusses microbiological function in an aqueous environment where microbial populations are large and well distributed, and energy sources and nutrients are readily available. At the other extreme, a soil microbiology text discusses applications where microorganisms function within an unsaturated soil environment where carbon and nutrient transport and availability are limiting resources for microbial growth. Bioprocess engineers often are interested in the growth and yields associated with microbiological production of a desired product, whereas compost engineers are interested in substrate utilization and the death of pathogenic microorganisms. Aerobic processes (e.g., composting, wastewater treatment) have much different considerations than anaerobic processes (e.g., constructed wetlands, fermentation).

Because biological systems engineers may work with microorganisms in any of their application environments, this chapter must establish the fundamentals of microbial processes and provide the science and engineering that is of general applicability to all biological systems.

To meet this goal, a review will be provided of many basic concepts of microbiology and biochemistry that are of general applicability in almost all analyses of biological systems. Basic concepts of chemistry and engineering sciences will be applied to microbiological systems. Finally, these principles will be applied to several basic types of microbial systems, and several analytical tools will be introduced that are important for biological systems engineers to master and use in their designs and analyses. Of course, the reader is encouraged to continue the study of biological systems of particular interest to her/his field through additional coursework and readings (see selected references at the back of this chapter).

3.2 Microbial Growth

3.2.1 Fundamentals of Microbial Growth

Growth is defined simplistically as an increase in the size of an organism or in the number of members of a population of organisms. Depending on the organism, this definition takes different forms and can be measured in different ways: for example, growth may be measured as a change in height of a plant or change in weight of an infant. For microbes we can measure the growth (or decline) of a population by measuring change in the number of cells or the mass of those cells. Growth rate, then, is simply growth expressed per unit time.

Within a closed environment having environmental conditions that, at least initially, do not limit growth, microbial growth proceeds through a number of phases (Figure 3.1). The organism initially goes through a lag phase (a), where it becomes adjusted to its new environment. Depending on the history and condition of the organism and its new environment, the lag phase may be brief (or nonexistent), or it may be long, if cells are damaged during the transfer, new microbial enzymes must be produced, or the cells must otherwise adapt to the new (richer or poorer) environment. Following this adjustment period, the organisms enter a growth phase (b), which will be discussed further below. Eventually, a nutrient and/or an energy source becomes limiting, or other environmental conditions change, causing the growth to slow and finally cease (c). The subsequent stationary phase (d), which may represent balanced cellular growth and death or simply a "hibernation state" for the cells, continues until the conditions change to allow further growth, or toxic products accumulate that lead to cell population death, identified as the death phase (e).

Generalized units—The units of some equations in this text will be presented in terms of generalized units, indicating that any consistent set of units may be used. M=mass, L=length, T=time.

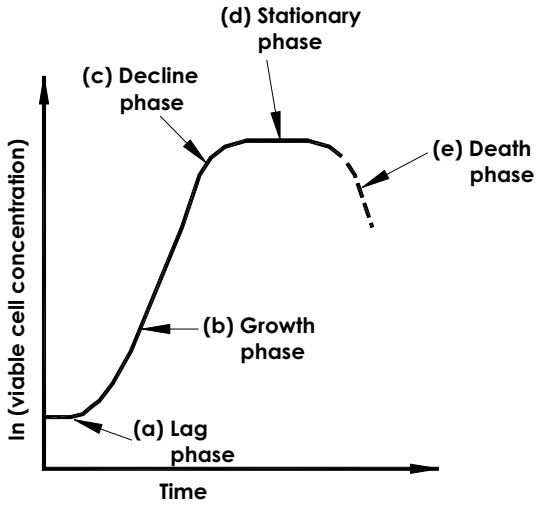


Figure 3.1—Growth of a microbial population.

During the growth and death phases, the rate of change in the population is a function of the size of the current population as described by the following equation:

$$r_x = \mu X \tag{3-1}$$

where r_x = volumetric growth rate ($M L^{-3} T^{-1}$),
μ = specific growth rate (T^{-1}), and
X = viable cell concentration ($M L^{-3}$).

If the organism is in a closed system, and growth is the only way to change the size (or concentration) of the population, then $r_x = dX/dt$. Substituting this into Eq. (3-1), rearranging, and integrating both sides.

$$\int \frac{dX}{X} = \int \mu \, dt \tag{3-2}$$

If μ is constant, integrating Eq. (3-2) with the initial conditions of $X=X_0$ at $t=0$ yields

$$X = X_0 \, e^{\mu t} \tag{3-3}$$

Eq. (3-3) describes a process in an exponential growth phase. Taking natural logarithms of both sides gives

$$\ln X = \ln X_0 + \mu t \tag{3-4}$$

Doubling time—The time required for a population to double in size or number.

The form of this equation dictates that a plot of ln X versus t gives a straight line with slope μ and intercept ln X_0. It often is convenient to describe the growth rate of a population using the term doubling time, the time required for the population of organisms to double or for X to equal $2X_0$. Substituting this relationship into Eq. (3-3),

$$\begin{aligned} 2 X_0 &= X_0 \, e^{\mu t_d} \\ \text{or} \ldots \quad 2 &= e^{\mu t_d} \end{aligned} \tag{3-5}$$

where t_d = doubling time (T).

Taking the natural logarithm of both sides,

$$\begin{aligned} \ln 2 &= \mu t_d \\ \text{or} \ldots \quad t_d &= \frac{\ln 2}{\mu} = \frac{0.693}{\mu} \end{aligned} \tag{3-6}$$

EXAMPLE 3.1

The algae *Lemna* is being grown on the surface of a wetland used for wastewater treatment. This organism has a doubling time of 36 hours under present conditions. Starting with a mass of 20 kg, how long would it take to attain a population of 200 kg?

Solution

Equations:

$$t_d = 0.693/\mu \tag{3-6}$$

$$X = X_0\, e^{\mu t} \tag{3-3}$$

where $t_d = 36$ h
$X_0 = 20$ kg
$X = 200$ kg

Calculations:

$$\mu = 0.693/t_d = 0.693 / 36 \text{ h} = 0.01925 \text{ h}^{-1}$$

$$t = \frac{\ln\left(\frac{X}{X_0}\right)}{\mu} = \frac{\ln\left(\frac{200 \text{ kg}}{20 \text{ kg}}\right)}{0.01925 \text{ h}^{-1}} = \frac{12.3026}{0.01925 \text{ h}^{-1}} = 119.6 \text{ h} \approx 120 \text{ h}$$

It will take 120 h for the initial population mass of 20 kg to reach 200 kg, as long as the environmental conditions do not become limiting.

PROBLEM 3.1

A culture of *E. coli* bacteria was found to have a doubling time of 1.5 hours under current conditions. A sample was held at these conditions for 24 hours before analysis, at which time the bacterial count was found to be 1×10^8 colonies/100 mL. What was the count of the initial *E. coli* sample?

3.2.2 Measuring Microbial Growth

The doubling time, or generation time, can be measured by counting the number of cells (individual organisms) initially and after a period of exponential growth. Doubling time varies among organisms and is dependent on the health of the population and the condition of its surrounding environment. Many common methods are available to measure population numbers (APHA, 1995).

Cells in unsaturated media (such as soil or compost) first must be diluted within a buffer solution to place the microbes in solution. A solution containing a microbial population may then require a series of dilutions to create a microbial density in the proper range for the

given technique, or may require extraction with a solvent to separate the microbes from other constituents. Direct microscopic enumerations may then be used to count the *total cell numbers* visible under magnification using a light microscope.

The dilution plate-count method measures *viable cells* on a selective culture medium after serial dilutions. Centrifugation and drying provides a measure of *total cell mass*. In solutions where extraneous suspended solids are minimal or remain constant and can be factored out, *optical turbidity* of a solution of microbial cells may be measured and correlated to cell mass or numbers. More recently, *molecular methods* are becoming available for quantifying bacterial communities (Pepper and Josephson, 1998). Each of these techniques is described fully in the references stated above as well as elsewhere; a list of references is included in the back of this chapter.

3.2.3 Requirements for Microbial Growth

Every organism has certain requirements for growth. In order for cells to grow, they need nutrients to provide the building blocks for the processes of cellular growth and metabolism, energy to fuel these processes, and an appropriate environment within which to conduct these biochemical reactions. Optimal growth or product yields typically occur only when every requirement is met within a specific range.

Nutrients

Macronutrients are nutrients needed in the greatest quantities for organism growth. The backbone of all organic compounds is comprised of carbon (C), oxygen (O), and hydrogen (H). Oxygen and hydrogen are available to cells from such widely available sources as water (H_2O), free oxygen (O_2), and carbohydrates (represented as CH_2O). Organisms are classified as to how they obtain their carbon. Autotrophs get their carbon directly from CO_2 in the atmosphere whereas heterotrophs obtain carbon from the carbohydrates previously assimilated by other organisms.

Other nutrients also are needed in great quantities for various purposes. Nitrogen (N), needed for production of proteins and amino acids, is most commonly taken into cells in the NH_4^+ or NO_3^- form, though nitrogen-fixing bacteria can utilize N_2 directly from the atmosphere. Phosphorous (P) is used for the synthesis of nucleic acids, phospholipids, and energy carriers (such as adenosine triphosphate, ATP) and is available in the environment for cellular uptake in the organic or inorganic phosphate forms. The ultimate source of inorganic P is weathered rock, which releases orthophosphate, PO_4^{3-}, primarily as $H_2PO_4^-$ at pH < 7.2 and HPO_4^{2-} at pH > 7.2. Potassium (K), sulfur (S), calcium (Ca), and sodium (Na) also are needed for important growth, enzymatic, and regulatory processes and must be obtained from the environment in relatively large quantities.

Micronutrients are the molecules that are necessary for cellular growth but in smaller quantities. These substances play critical roles

Autotroph—An organism that obtains carbon from CO_2.

Heterotroph—An organism that obtains carbon from organic compounds.

Macronutrient—A nutrient present at higher concentrations in an organism (e.g., concentrations greater than 1 mg g^{-1} dry mass in plants). For example, in higher plants, the macronutrients include (in order of decreasing concentration) C, O, H, N, K, Ca, Mg, P, and S.

Micronutrient—A nutrient present at lower concentrations in an organism (e.g., concentrations less than 100 μg g^{-1} dry mass in plants and just a few ng g^{-1} dry mass in soil microbes). In higher plants, the micronutrients include (in order of decreasing concentration) Cl, Fe, B, Mn, Zn, Cu, Ni, and Mo.

in proper cell function and must be available for uptake. Natural soils typically have an ample supply. However, in artificial environments (such as hydroponic plant production in a greenhouse, or tissue culture studies in a growth chamber), it may be necessary to add micronutrients specifically to the culture media. Some bacteria can synthesize all the compounds necessary for growth using just a carbon source and a few nutrients, though most bacteria and other organisms have more complex nutritional requirements.

Energy

Phototroph—An organism that obtains energy from solar radiation.

Chemotroph—An organism that obtains energy from organic or inorganic substances.

Organisms are classified according to how they obtain energy. Phototrophs are able to harness the sun's energy (or equivalent artificial lighting) directly. Green plants, algae, and some bacteria use photosynthesis to translate radiant energy into chemical energy, which provides them with energy for their growth and metabolism. *The chemical energy so encumbered provides energy for most of the other life forms on earth.* These other life forms are called chemotrophs, defined as organisms that must obtain energy for their growth and metabolism from *chemical* sources. Humans are chemotrophs as are the animals we eat. The yeast used in beer or wine making and the bacteria that degrade organic compounds, as well as a multitude of other animals and microorganisms, are all chemotrophs.

Temperature

Thermophile—An organism with optimum growth temperature between 45 and 80°C. Maximum growth temperature can exceed 100°C in some organisms.

Mesophile—An organism that grows best at moderate temperatures, typically ranging from 15 to 45°C.

Psychrophile—An organism with an optimum growth temperature of 15°C or lower and a maximum growth temperature below 20°C. Minimum growth temperatures can be below freezing (0°C).

Every organism has specific requirements for the temperature of its surroundings. Organisms typically are categorized according to these requirements as thermophilic, mesophilic, or psychrophilic. Each type of organism has a minimum temperature, below which it cannot survive (e.g., due to freezing in mesophilic organisms), and a maximum temperature, above which growth is not possible because its proteins and other cellular constituents become denatured or otherwise irreversibly damaged. Between these two extremes, but closer to the maximum temperature, lies an optimal temperature at which growth rate is a maximum. In many biological systems, an engineer controls the rate of a process by utilizing the known effect of temperature on organism growth rate.

Water

Potential—The state of an environmental parameter in one part of a system that, when compared with the state of that parameter in another part of the system or surroundings, establishes a tendency for movement of that parameter.

Gradient—A difference in potential between one volume of space and another.

All life requires water to grow. Water hydrates inter- and intracellular spaces, is incorporated into cellular materials, transports dissolved solutes to and through organisms, affects gas exchange in and around cells and organisms, provides thermal stability with its high heat capacity, and cools organisms and their surroundings by absorbing heat during evaporation. Aerobic biological systems within porous media (such as soils, compost, or hydroponic gravel cultures) typically have optimal water content between 50 and 60% of fillable porosity. This provides adequate moisture while still leaving adequate air space for diffusion of oxygen to organisms or plant roots.

Water moves (into living cells, through media, from liquid to gas, etc.) according to differences in water potential, a term that combines osmotic, matric, and gravitational forces. Water potential is used to express a system's ability to do work. Water flows from high water potential to low water potential (or from high to low energy) according to the 2nd law of thermodynamics, and the rate of movement is determined by the magnitude of the water-potential gradient. There is a complex interaction between osmotic, matric, and gravitational forces that makes it difficult to model the relative contributions of each force to water potential. At this point in our study, it is sufficient to realize that all three contribute to water availability and movement in biological systems. A brief discussion of water potential in plants is given in Chapter 6.

***Water potential* (ψ)**—A measure of the potential energy of water in a system relative to that of pure, free water at atmospheric pressure. For example, water potential drives the movement of water through a plant from soil ($\psi \approx 0$ to -1.5 MPa) to plant cells ($\psi \approx -0.4$ to -4.0 MPa) to air (at 20°C, $\psi = -2.72$ MPa at 98% rh, -14.2 MPa at 90% rh, and -311 MPa at 10% rh).

***Osmotic potential* (ψ_s)**—The pressure caused by the attraction of solute ions for water molecules. Using 0 for the osmotic potential of pure water, all water solutions have negative osmotic potential. Water moves from higher to lower osmotic potential, as when water moves across a membrane in the direction from deionzed water (high ψ_s) to salt water (low ψ_s).

***Matric potential* (ψ_m)**—The pressure caused by adsorption of water to hydrophilic surfaces. Like osmotic potential, it is always negative. Water moves from higher to lower matric potential, as when water (high ψ_m) fills the pores of a floating sponge (low ψ_m) by capillary rise.

***Pressure potential* (ψ_P)**—The pressure caused by external forces exerted on a system. Using 0 for atmospheric pressure, pressure potential can have either a positive or negative value. (A negative pressure is called *tension* or *suction*.) Water moves from higher to lower pressure, as when it moves from a reservoir (positive ψ_P) out a valve to the surroundings (atmospheric P or 0 ψ_P).

Oxygen

Organisms have specific requirements for oxygen in their environments. Aerobic organisms have metabolic systems that require the availability of oxygen in their environment. Most plants, animals, and microorganisms that grow in open air or near the open-air interface of soil or water are aerobic. Anaerobic organisms lack the ability to utilize oxygen and may be sensitive or even intolerant to its presence. For example, obligate anaerobes cannot survive in the presence of oxygen, which may result because of their inability to decompose toxic byproducts of aerobic metabolism, such as hydrogen peroxide. Anaerobic organisms live in deeper waters, saturated soil, or other systems where diffusion of oxygen from the atmosphere is too slow to maintain an adequate supply. Aeration must be provided to achieve optimum growth when a process has an unfavorable balance between oxygen uptake rates of aerobic organisms and oxygen replenishment rates in water or through a porous media.

pH

Organisms have specific pH ranges within which growth is possible, and tighter ranges within which growth is optimal. Most organisms grow best with a near-neutral pH between 5 and 9. Acidophiles grow at pH values less than 2, whereas alkaliphiles grow in pH conditions greater than 10. However, regardless of the external environment, all cells must maintain an intracellular pH near neutral to survive.

3.2.4 Analysis of Microbial Growth and Product Formation

The following techniques are used extensively in analysis of bioprocesses utilizing microorganisms. These concepts are described in detail in the excellent text by Doran (1995), whose nomenclature formed the basis for the discussion in this section. However, all the techniques are completely general and can be applied readily to many different biological systems that involve stoichiometric reactions (e.g., plant photosynthesis and animal metabolism). Many of these topics will be addressed in later chapters of this text.

Aerobic—An organism or process that requires oxygen.

Anaerobic—An organism or process that does not require free oxygen.

Acidophile—An organism that grows well in a low-pH environment. They might be described as "acid loving."

Alkaliphile—An organism that grows well in a high-pH environment. They might be described as "base loving."

Batch process—A process in which all materials are added to a closed system at the beginning of the process and products removed only at the termination of the process.

Semi-batch process—A batch process in which materials may be either added or removed during the process (not both).

Fed-batch process—A semi-batch process in which material is added during the process (but not removed).

Continuous process—A process in which material may flow in and out of the system during the process.

Material Mass Balance

Microbial systems often are analyzed using one of several simplified process descriptions. The most common of these are batch process, semi-batch process, fed-batch process, and continuous process. Each describes a specific set of assumptions used to analyze mass flow into and through the system.

Describing the mass flows into, through, and out of a system during a process is important for quantifying reactant utilization; product yields; nutrient, water or oxygen requirements; or may be important for understanding the impacts of other environmental control measures. A mass balance of the following form can be applied to each of the process types described above:

$$\text{mass in} + \text{mass generated} = \text{mass out} + \text{mass consumed} \quad (3\text{-}7)$$

This balance applies to each constituent of the process as well as to the overall process itself. If the constituent for which the mass balance is being written is not involved in a reaction during the process, Eq. (3-7) can be simplified:

$$\text{mass in} = \text{mass out} \quad (3\text{-}8)$$

Material balances are essential tools for analyzing biological systems. As with all engineering analyses, it is important that mass balances are well organized so that anyone checking your work may follow the steps, methods, and assumptions used. The following structure may help organize material balances used in analyses throughout this text.

1. Determine the goals of the analysis. What are you trying to find? How will the solution be used?
2. Draw a diagram of the system. All mass flows should be shown using directional arrows into or out of the system, as appropriate, and labeled with known values. Convert all values (e.g., values known in volumetric terms) to a common set of mass and mass flow rate units (e.g., kg and kg h^{-1}).
3. Clearly state all assumptions. This may be the most critical step of engineering analyses, but one that too often is overlooked by engineering students during their coursework. Rarely are all the important facts about a real-life problem known, perhaps even more so with biological systems analyses because of the inherent variability of biological material properties and complexity of biological processes. Many of the problems in this textbook are representative of the types of analyses you are likely to see as biological systems engineers; all the information necessary to solve the problem may not be given. You should begin to cultivate your engineering judgment by making and justifying the assumptions necessary to allow you to proceed with the problem solution. These assumptions must be carefully described and included at the beginning of the analysis. This

will allow others to understand the conditions under which your analysis is applicable, assess whether the assumptions are appropriate, or even determine how the assumptions could be improved.

4. Write the appropriate mass balance equation for each constituent. This equation should reflect the assumptions described in steps 2 and 3. Depending upon whether the constituent being studied undergoes a reaction or not will determine whether Eq. (3-7) or the simplified Eq. (3-8) is appropriate.

Stoichiometric Balance

For mass balances involving reactions, the stoichiometry must be known before the mass balance terms can be determined. For example, new cells and products are synthesized during the microbial growth process and must be described quantitatively using stoichiometry before a mass balance can be conducted. Even though the growth process is complex, the mass (or number of moles) of each element must balance between reactants and products. Taking a macroscopic view of the process of aerobic cell growth, we can write a general stoichiometric equation that focuses on the major reactants and products.

$$C_wH_xO_yN_z + a\ O_2 + b\ H_gO_hN_i \rightarrow c\ CH_\alpha O_\beta N_\delta + d\ CO_2 + e\ H_2O \qquad (3\text{-}9)$$

where $C_wH_xO_yN_z$ = substrate (carbon source),
$H_gO_hN_i$ = nitrogen source,
$CH_\alpha O_\beta N_\delta$ = product biomass, and
a, b, c, d, e = stoichiometric coefficients.

The subscripts in Eq. (3-9) are dependent on the species involved in the reaction. For example, glucose as a substrate would have w=6, x=12, y=6, and z=0; ammonium as a nitrogen source would have g=4, h=0, and i=1; *E. coli* as a biomass growth product would have α=1.77, β=0.49, and δ=0.24; and yeast as a biomass growth product would have α=1.75, β=0.5, and δ=0.15 (Roels, 1980; Atkinson and Mavituna, 1983). It is interesting to note that biomass of a variety of forms have remarkably similar composition; thus a general formula of $CH_{1.8}O_{0.5}N_{0.2}$ may be reasonable in lieu of more specific information about a particular species. Because the stoichiometric coefficient of the substrate is 1 in Eq. (3-9), it is said to be written on the basis of one mole of substrate. Thus, the coefficients a, b, c, d, and e represent the moles of each substance that are required or produced per mole of substrate reacted. Other substrates and products can be added to Eq. (3-9) if appropriate to the analysis, though it should be noted that the macronutrients C, H, O, and N comprise 90 to 95% of most biomass (as discussed above), and thus often constitute the primary constituents of concern.

Mass balance for each constituent of Eq. (3-9) can be written (using normal procedures of a stoichiometric balance) as follows:

$$\text{C balance}: \quad w = c + d \tag{3-10}$$

$$\text{H balance}: \quad x + b\,g = c\,\alpha + 2\,e \tag{3-11}$$

$$\text{O balance}: \quad y + 2\,a + b\,h = c\,\beta + 2\,d + e \tag{3-12}$$

$$\text{N balance}: \quad z + b\,i = c\,\delta \tag{3-13}$$

All subscripts are known for the given reactants and products of a reaction, but the coefficients are not known. However, because there are 5 unknown coefficients (a, b, c, d, and e) in Eq. (3-9) and only 4 equations with which to solve for them [Eqs. (3-10) through (3-13)], there must be additional information about the relationships between terms of this equation. A common solution is to experimentally determine a respiratory quotient (RQ). The RQ is a stoichiometric ratio of CO_2 produced to the O_2 consumed during a reaction.

$$RQ = \frac{\text{moles } CO_2 \text{ produced}}{\text{moles } O_2 \text{ consumed}} = \frac{d}{a} \tag{3-14}$$

For example, in the complete oxidation reaction of glucose and oxygen to carbon dioxide and water ($C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$), d = 6, a = 6, and the RQ = 1.0. Now, all the coefficients of Eq. (3-9) can be determined.

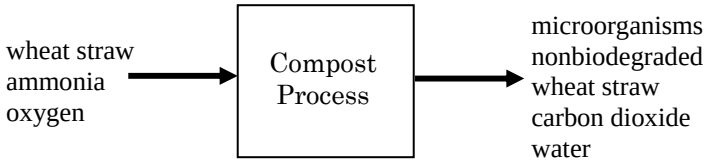
EXAMPLE 3.2

Chemoheterotroph—An organism that obtains carbon and energy from organic compounds.

Consider an in-vessel composter using a population of thermophilic chemoheterotrophic microorganisms to degrade biomass. How many kg of ammonia must be added to break down the biodegradable fraction of 1 kg of wheat-straw substrate to CO_2 and H_2O? The respiratory quotient for wheat straw is assumed to be 1.1. (Note: In practice, the N often would be applied as $NH_4^+Cl^-$ or $NH_4^+SO_4^-$, but for stoichiometric analysis the base state of NH_3 often is used.)

Solution

Diagram:



Assumptions:

- Aerobic conditions
- Microbial composition is $CH_{1.8}O_{0.5}N_{0.2}$
- Ammonia (NH_3) is sole nitrogen source
- Wheat straw has 30% biodegradable substrate

- The biodegradable fraction of wheat straw has composition similar to glucose: $C_6H_{12}O_6$

Equations:

$$C_wH_xO_y + a\ O_2 + b\ H_gO_hN_i \rightarrow c\ CH_\alpha O_\beta N_\delta + d\ CO_2 + e\ H_2O \qquad (3\text{-}9)$$

$$C_6H_{12}O_6 + a\ O_2 + b\ NH_3 \rightarrow c\ CH_{1.8}O_{0.5}N_{0.2} + d\ CO_2 + e\ H_2O$$

N balance:	$b = 0.2\ c$	$\rightarrow b = 0.2\ c$
C balance:	$6 = c + d$	$\rightarrow d = 6 - c$
RQ:	$1.1 = d/a$	$\rightarrow a = d/1.1 = (6 - c)/1.1$
H balance:	$12 + 3\ b = 1.8\ c + 2\ e$	$\rightarrow e = \frac{1}{2}[12 + 3\ (0.2\ c) - 1.8\ c]$
O balance:	$6 + 2\ a = 0.5\ c + 2\ d + e$	$\rightarrow c = 2\ (6 + 2\ a - 2\ d - e)$

Calculations:

Solving the 5 independent equations (i.e., solving first for c and then for each other coefficient based on c) gives:

$a = 1.94$, $b = 0.77$, $c = 3.88$, $d = 2.13$, $e = 3.68$

Thus, the balanced stoichiometric equation is

$$C_6H_{12}O_6 + 1.94\ O_2 + 0.77\ NH_3 \rightarrow 3.88\ CH_{1.8}O_{0.5}N_{0.2} + 2.13\ CO_2 + 3.68\ H_2O$$

and 0.77 moles of NH_3 are used per mole of substrate consumed.

Converting into mass units:

$$MW_{substrate} = 6(12.0\ kg/mol)_C + 12(1.0)_H + 6(16.0)_O = 180\ (kg/mol)_{substrate}$$
$$MW_{NH3} = 1(14.0\ kg/mol)_N + 3(1.0)_H = 17.0\ (kg/mol)_{NH3}$$

$$1\ kg_{straw} \times 30\% = 0.30\ kg_{substrate} \times \frac{mol_{substrate}}{180\ kg_{substrate}} = 0.001667\ mol_{substrate}$$

$$0.001667\ mol_{substrate} \times \frac{0.77\ mol_{NH_3}}{mol_{substrate}} \times \frac{17.0\ kg_{NH_3}}{mol_{NH_3}} = 0.022\ kg_{NH_3}$$

Thus, 22 g of NH_3 are required to compost the biodegradable fraction of 1 kg wheat straw.

PROBLEM 3.2

The algae *Lemna* in Example 3.1 is a photoautotroph, and uses ammonium (NH_4^+) in the water as its primary nitrogen source. Write a bioprocess reaction equation for a photoautotroph that is analogous to Eq. (3.9). How much NH_4^+ must be provided per kg of biomass produced to sustain growth?

Photoautotroph— An organism that obtains carbon from CO_2 and energy from solar radiation.
Chemoautotroph— An organism that obtains carbon from organic compounds and energy from solar radiation.

Electron Balance

Available electrons—The number of electrons available for transfer to oxygen during a biological process.

Degree of reduction—The available electrons per unit carbon of a substance.

One problem with the application of Eq. (3-9) is that it is difficult to measure accurately the amount of water produced during biological reactions because the quantity is so small relative to the total amount of water in the system. This makes it difficult to determine the e coefficient needed to complete the H and O balances [Eqs. (3-11) and (3-12)]. Instead of mass balances in this case, it is more convenient to use the principle of "conservation of reducing power (or available electrons)" to quantify an electron balance. This method tracks how the electrons are transferred from the substrate to the products during a reaction.

The number of available electrons is determined from the valance of an element (from the periodic table), which describes the number of electrons available on its outer orbital shell. The available electrons for the most common elements in biological processes are C (+4), H (+1), O (–2), P (+5), and S (+6). The available electrons for N vary according to the N source being used in the reaction, which typically is called the reference state: N has (–3) available electrons for a reference state of ammonia (NH_3) and ammonium (NH_4^+), (0) for molecular nitrogen (N_2), (+3) for nitrite (NO_2^-), and (+5) for nitrate (NO_3^-). *The number of available electrons per unit of carbon in a material is referred to as its degree of reduction,* γ. Degree of reduction uses consistent units of electrons per molecule or moles of electrons per mole of substance.

EXAMPLE 3.3

For Example 3.2, determine the number of available electrons for each substance, and the degree of reduction for both the substrate and the biomass product.

Solution

Equations:

Use the balanced stoichiometric equation from Example 3.2.

Calculations:

Available electrons (using ammonia as the reference state for N):

$C_wH_xO_yN_z$:	$w(+4)+x(+1)+y(-2)+z(-3) = 4w+x-2y-3z$
H_2O:	$2(+1)+1(-2) = 0$
CO_2:	$1(+4)+2(-2) = 0$
NH_3:	$1(-3)+3(+1) = 0$
$CH_\alpha O_\beta N_\delta$:	$1(+4)+\alpha(+1)+\beta(-2)+\delta(-3) = 4+\alpha-2\beta-3\delta$

Degree of reduction, γ (number of electrons per unit carbon):

Substrate:	$\gamma_S = [4w+x-2y-3z]/w$
Biomass:	$\gamma_B = [4+\alpha-2\beta-3\delta]/1 = 4+\alpha-2\beta-3\delta$

Thus, for wheat straw (glucose) substrate and generalized microbial biomass:

γ_S: $[4(6)+12-2(6)-3(0)]/6 = 4$ $mol_{electrons}$ $mol^{-1}_{substrate}$
γ_B: $4+1.8-2(0.5)-3(0.2) = 4.2$ $mol_{electrons}$ $mol^{-1}_{biomass}$

PROBLEM 3.3

For Problem 3.2.1, determine the number of available electrons for each substance, and the degree of reduction for both the substrate and the biomass product.

Because the number of each molecule is conserved in a stoichiometrically balanced growth reaction, so is the number of available electrons. Applying this concept to Eq. (3-9) and using the available electron calculations from Example 3.3, the available-electron balance is

$$w\,\gamma_S + a\,(-4) + b\,(0) = c\,\gamma_B + d\,(0) + e\,(0) \qquad (3\text{-}15)$$

where γ_S = degree of reduction of substrate, and
γ_B = degree of reduction of biomass.

Rearranging Eq. (3-15), we can find the fraction of electrons in the substrate that are donated to each receptor during the reaction.

$$1 = \frac{4\,a}{w\,\gamma_S} + \frac{c\,\gamma_B}{w\,\gamma_S} = \varepsilon + \eta \qquad (3\text{-}16)$$

where $4\,a/(w\,\gamma_S) = \varepsilon$ = fraction of electrons in substrate donated to oxygen (as it is reduced to H_2O and CO_2), and
$c\,\gamma_B/(w\,\gamma_S) = \eta$ = fraction of electrons in substrate donated to biomass.

EXAMPLE 3.4

For the composting system in Examples 3.2 and 3.3, use an electron balance instead of an O balance [Eq. (3-12)] to recalculate the a coefficient of the stoichiometric balance and to determine the number of kg of O_2 required to oxidize the biodegradable fraction of the wheat straw. How many m^3 of air would be required to satisfy this requirement?

Solution

Equation:

$$w\,\gamma_S + a\,(-4) + b\,(0) = c\,\gamma_B + d\,(0) + e\,(0) \qquad (3\text{-}15)$$

Calculations:

Using degrees of reduction from Example 3.3:

$$6\,(4) - 4\,a = c\,(4.2)$$

and rearranging,

$$a = (24 - 4.2\,c) / 4$$

Recall from the stoichiometrics [Eq. (3-9)]:

N balance:	$b = 0.2\,c$	$\rightarrow b = 0.2\,c$
C balance:	$6 = c + d$	$\rightarrow c = 6 - d$
RQ:	$1.1 = d/a$	$\rightarrow d = 1.1\,a$
H balance:	$12 + 3\,b = 1.8\,c + 2\,e$	$\rightarrow e = ½[12 + 3\,(0.2\,c) - 1.8\,c]$

Solving for the coefficients a through e:

$$a = 1.935,\ d = 2.129,\ c = 3.871,\ b = 0.774,\ e = 3.677$$

Thus, 1.935 moles of O_2 are required for each mole of wheat straw degraded. However, if only 30% of the straw is biodegradable, then

$$1\ \text{kg}_{\text{straw}} \times 0.3 = 0.3\ \text{kg}_{\text{straw}}$$

$$0.3\ \text{kg}_{\text{straw}} \times \frac{1.935\ \text{mol}_{O_2}}{\text{mol}_{\text{straw}}} \times \frac{16.0\ \text{kg}_{O_2}}{\text{mol}_{O_2}} \times \frac{\text{mol}_{\text{straw}}}{180.0\ \text{kg}_{\text{straw}}} = 0.0516\ \text{kg}_{O_2}$$

Using an oxygen content of air of approximately 21% by volume (and density of oxygen of 1.32 kg m^{-3} at 20°C and 1 atm, from a reference table having standard thermophysical properties of matter)

$$0.0516\ \text{kg}_{O_2} \times \frac{\text{m}^3{}_{O_2}}{1.32\ \text{kg}_{O_2}} \times \frac{\text{m}^3{}_{\text{air}}}{0.21\ \text{m}^3{}_{O_2}} = 0.186\ \text{m}^3{}_{\text{air}}$$

Thus, 0.186 m^3 (186 L) of air would need to be available to degrade 30% of the wheat straw. Note, however, that this does not account for the fact that not all supplied air is available to the microbes (delivery is not 100% efficient) and microbes do not use all the available air (they are not 100% efficient users). Thus, this amount represents a theoretical minimum amount of air required for the process.

Biomass and Product Yields

Equation (3-16) also can be used to determine an expression for the theoretical maximum biomass yield, i.e., the yield if all available

electrons were used in biomass formation. In this case, setting ε=0 (which forces η to be 1) and rearranging gives

$$c_{max} = \frac{w\ \gamma_S}{\gamma_B} \tag{3-17}$$

The c_{max} coefficient determines the maximum number of moles of biomass produced per mole of substrate, and is used to calculate the theoretical maximum biomass yield. Actual biomass yield must be determined experimentally for specific environmental conditions and nutrient sources, and can be expressed using the c coefficient from Eq. (3-9):

$$Y_{X/S} = \frac{\text{kg biomass produced}}{\text{kg substrate consumed}} = \frac{c\ (MW_{cells})}{MW_{substrate}} \tag{3-18}$$

where $Y_{X/S}$ = yield of biomass (X) per unit substrate (S) $kg_B\ kg_S^{-1}$), and
MW = molecular weight of a substance ($kg\ mol^{-1}$).

Thus, Eq. (3-18) can be used with c_{max} from Eq. (3-17) to determine the theoretical maximum biomass yield for a balanced reaction.

All the same mass and electron balance concepts presented above can be extended to formation of a product in a biological reaction. Adding a product term to Eq. (3-9) gives:

$$\begin{gathered} C_wH_xO_yN_z + a\ O_2 + b\ H_gO_hN_i \rightarrow \\ c\ CH_\alpha O_\beta N_\delta + d\ CO_2 + e\ H_2O + f\ C_jH_kO_lN_m \end{gathered} \tag{3-19}$$

where $C_jH_kO_lN_m$ = product, and
f = stoichiometric coefficient for the product.

The degree of reduction of the product, γ_P, is calculated similar to the other reduction terms, as shown in Example 3.3. One additional coefficient requires one additional equation to solve the system of mass balance equations. Analogous to biomass yield, the product yield for a specific reaction and environment can be experimentally determined:

$$Y_{P/S} = \frac{\text{kg product produced}}{\text{kg substrate consumed}} = \frac{f\ (MW_{product})}{MW_{substrate}} \tag{3-20}$$

where $Y_{P/S}$ = yield of product (P) per unit substrate (S) ($kg_P\ kg_S^{-1}$).

It is important to note that the product must be produced in association with the growth process, not as a secondary product of metabolism; i.e., the reaction must conform to Eq. (3-19).

Product yield **($Y_{P/S}$)**—The mass of product (P) produced per mass of substrate (S) consumed.

Biomass yield **($Y_{X/S}$)**—The mass of biomass (X) produced per mass of substrate (S) consumed.

EXAMPLE 3.5

Determine the actual and theoretical maximum biomass yields for the compost system in Example 3.2.

Solution

Equations:

$$c_{max} = \frac{w\,\gamma_S}{\gamma_B} \tag{3-17}$$

$$Y_{X/S} = \frac{c\,(MW_{cells})}{MW_{substrate}} \tag{3-18}$$

Calculations:

$MW_{cells} = 1(12.0\ kg/mol)_C + 1.8(1.0)_H + 0.5(16.0)_O + 0.2(14.0)_N$
$= 24.6\ (kg/mol)_{cells}$

$MW_{substrate} = 6(12.0\ kg/mol)_C + 12(1.0)_H + 6(16.0)_O$
$= 180\ (kg/mol)_{substrate}$

Using the c coefficient from Example 3.2 along with Eq. (3-18), actual biomass yield is

$$Y_{X/S} = \frac{3.88\left[24.6\ (kg/mol)_{cells}\right]}{180\ (kg/mol)_{substrate}} = 0.530\,\frac{kg_{biomass}}{kg_{substrate}}$$

Using the degrees of reduction from Example 3.3 along with Eq. (3-17), c_{max} is

$$c_{max} = \frac{6\,(4.0)}{4.2} = 5.714$$

and using Eq. (3-18), theoretical maximum biomass yield is

$$Y_{X/S} = \frac{5.174\left[24.6\,(kg/mol)\,cells\right]}{180\,(kg/mol)_{substrate}} = 0.781\,\frac{kg_{biomass}}{kg_{substrate}}$$

Thus, for the conditions under which the respiratory quotient was determined, 0.53 kg of microbial cells are produced per kg of wheat-straw substrate consumed. For the stated stoichiometric process reaction, a maximum of 0.78 kg of microbial cells could be produced from a kg of wheat straw.

Exergonic—A chemical reaction that releases energy.

Endergonic—A chemical reaction that consumes energy.

3.3 Microbial Metabolism

Cellular metabolism has the goal of obtaining energy and carbon, the two essential components for maintenance and growth of all organic molecules. The metabolic process includes both the exergonic reactions of catabolism and the endergonic reactions of anabolism.

This means that the energy and carbon that result from the breakdown of organic substances (i.e., catabolism) are used for the reactions of cell maintenance, growth (biomass production), and reproduction (i.e., anabolism). The two types of processes are complementary; both must occur for cellular processes to proceed.

Endergonic processes harness the energy released when organic substances are catabolized. Cells do not use the liberated energy immediately, but rather store the energy for future use. (This is a key point that will be emphasized several times throughout this text. Detail on how this works is given in Chapter 5). The two most common forms of storage are as high-energy phosphate compounds, such as adenosine triphosphate (ATP), and electron carriers, such as nicotinamide adenine dinucleotide (NAD^+/NADH), NAD phosphate ($NADP^+$/NADPH), and flavin adenine dinucleotide (FAD/$FADH_2$). Electrons released during the exergonic reactions are captured by the oxidized forms of the carrier (listed first in parentheses above, for example NAD^+) and transform them to the energy-rich reduced form (listed second, for example NADH). Electrons stored in these compounds are often referred to as containing *reducing power*, because the electrons can be used to help reduce compounds in subsequent reactions.

3.3.1 Redox reactions

One way of describing energy transfers during biochemical processes, including those involved with metabolism, is by describing the oxidation-reduction (redox) reactions associated with those processes. Breaking bonds (oxidation) releases electrons whereas creating bonds (reduction) uses electrons. A substance that is oxidized is transformed into one with *lower* internal energy. Associated with every oxidation reaction is a reduction reaction, in which the energy liberated during the oxidation reaction is used to *increase* the internal energy of the reduced substance. Hydrogen often accompanies the electron transferred to the reduced compound during a redox reaction. The concept of "conservation of reducing power" presented in Sec. 3.2.4 is another way of stating the fact that oxidation and reduction reactions are coupled and must balance.

Redox reactions change the oxidation state of a compound. The oxidation state describes changes in electron availability of specific molecules during reactions, but it does not indicate the direction of the reaction. Reactions proceed in the direction that yields a net release of energy (i.e., exergonic), which depends on how readily molecules donate or accept electrons. Is there a way to determine which direction a reaction will occur or to calculate how much energy is released in the process?

3.3.2 Electron Tower

Answering these questions will require an understanding of two concepts: the electron tower, which we will introduce, and free energy, which we will review and apply from Chapter 1.

Oxidation—Loss (or donation) of electrons by a substance. The oxidized form of nicotinamide adenine dinucleotide is NAD^+. (The "+" indicates that a hydrogen is missing and the structure has a + charge.)

Reduction—Gain (or acceptance) of electrons by a substance. The reduced form of nicotinamide adenine dinucleotide is NADH.

Oxidation state—Valence of an element, or the number of free electrons. The sum of oxidation states of elements in a compound equals the overall net charge of the compound. The reduced form of nicotinamide adenine dinucleotide is NADH.

Having trouble remembering the direction of electron transfer during redox reactions? See if this saying helps:

LEO the lion says GER!

Loss of Electrons = Oxidation
Gain of Electrons = Reduction

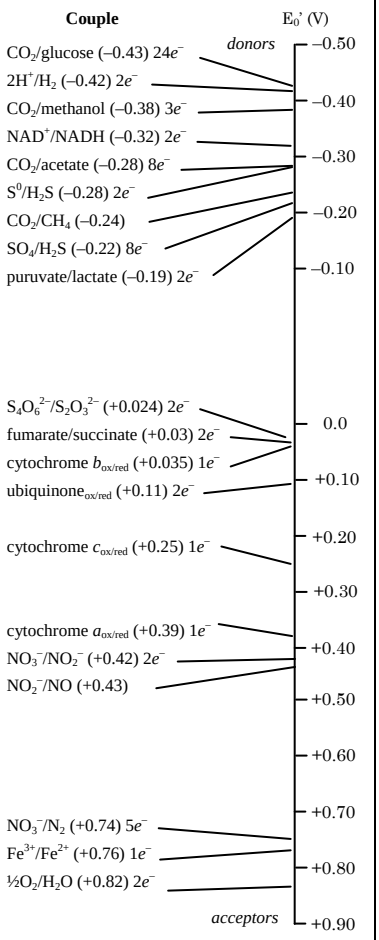


Figure 3.2—The electron tower.

A complete redox reaction can be separated into two half-reactions, an oxidation and a reduction. For example,

$$H_2 + ½O_2 \rightarrow H_2O \tag{3-21}$$

can be separated into the two half-reactions

$$H_2 \rightarrow 2H^+ + 2e^- \tag{3-22}$$

$$½O_2 + 2H^+ + 2e^- \rightarrow H_2O \tag{3-23}$$

Equation (3-22) is an oxidation reaction (electrons are lost or donated by H_2) whereas Eq. (3-23) is a reduction reaction (electrons are gained or accepted by H_2O). Each half-reaction represents an oxidation-reduction (O-R) couple. Each O-R couple has a tendency to proceed that can be measured by its reduction potential (E_0'). The E_0 is the electrical potential generated by a half reaction relative to a reference molecule (H_2) under standardized conditions of temperature, acidity, and pressure (25°C, pH = 0, and 1 atm). The E_0 is converted to E_0' by correcting to pH = 7, to reflect neutral conditions typical of biological reactions. The electron tower further standardizes the E_0' by representing each O-R couple as a reduction reaction, and adjusting the sign of E_0' accordingly. An electron tower is shown in Figure 3.2.

The electron tower is a convenient way to display the reduction potential of O-R couples common to biological systems. The O-R couples with the greatest reducing potential (most negative E_0') are shown at the top of the electron tower. Highly oxidized O-R couples (most positive E_0') are shown at the bottom. Electrons are donated from O-R couples at the top of the electron tower and "caught" by those with lower reduction potential (more positive E_0').

3.3.3 Relating Redox Potential to Free Energy

The E_0' can be related to the Gibb's free energy, defined as the energy available for useful work, of a compound. The difference in reduction potential (ΔE_0') between two O-R couples (or half reactions) determines the amount of energy released in a complete redox reaction. This ΔE_0' is related to the change in Gibb's free energy:

$$\Delta G^{0'} = – n F \Delta E_0' \tag{3-24}$$

where $\Delta G^{0'}$ = Gibb's free energy (kJ),
n = number of electrons transferred (moles of electrons),
F = Faraday's constant (96.5 kJ V^{-1} mol_{elec}^{-1}), and
ΔE_0' = difference in reduction potentials between two O-R couples (V).

Because Gibb's free energy is a measure of the energy available for useful work, the $\Delta G^{0'}$ for a given biochemical reaction indicates the amount of energy released or required for that reaction. A positive $\Delta G^{0'}$ indicates an endergonic (energy-requiring) reaction

whereas a negative $\Delta G^{0'}$ indicates an exergonic (energy-releasing) reaction. It follows that $\Delta G^{0'} = 0$ indicates an equilibrium state.

EXAMPLE 3.6

Determine the change in Gibb's free energy in the reaction: $H_2 + \frac{1}{2}O_2 \rightarrow H_2O$ [Eq. (3-21)].

Solution

Known:

From the electron tower (Figure 3.2), the number of electrons transferred in the reaction is 2 (noted as $2e^-$).

For O-R couple in Eq. (3-22): $E_0' = -0.42$ V

For O-R couple in Eq. (3-23): $E_0' = +0.82$ V

Equations:

$$\Delta G^{0'} = -n\ F\ \Delta E_0' \qquad (3\text{-}24)$$

Calculations:

Because the electron tower represents all reactions as reductions, the E_0' of the oxidation reaction of Eq. (3-22) must be multiplied by –1 before adding it to the E_0' of the reduction reaction of Eq. (3-23). Thus, combining the two half reactions Eqs. (3-22) and (3-23):

$$\begin{aligned}\Delta E_0' &= E_{0\,\text{oxidation}}' + E_{0\,\text{reduction}}' = E_{0\,[\text{Eq. (3-22)}]}' + E_{0\,[\text{Eq. (3-23)}]}' \\ &= -(-0.42\text{ V}) + 0.82\text{ V} = +1.24\text{ V}\end{aligned}$$

Solving for Gibb's free energy:

$$\begin{aligned}\Delta G^{0'} &= -n\ F\ \Delta E_0' = -(2\ \text{mol}_{e^-})\ (96.5\ \text{kJ V}^{-1}\ \text{mol}^{-1}{}_{e^-})\ (+1.24\text{ V}) \\ &= -239\text{ kJ}\end{aligned}$$

A negative $\Delta G^{0'}$ indicates that the reaction releases energy (exergonic), and thus will proceed in the direction as written (from H_2 and O_2 to H_2O). That is, the reaction can proceed spontaneously and release 239 kJ of free energy available for work per mole of water produced.

PROBLEM 3.6.1

Determine the change in Gibb's free energy in the bioprocess of converting glucose to CO_2 under aerobic conditions by a chemoheterotroph. The reaction is given as

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

which can be separated into the two balanced half-reactions

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 6H_2 + 24e^-$$
$$3O_2 + 6H_2 + 24e^- \rightarrow 6H_2O$$

PROBLEM 3.6.2

Determine the change in Gibb's free energy in the bioprocess of converting CO_2 to methane (CH_4) under anaerobic conditions by a chemoheterotroph. The reaction is given as

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

which can be separated into the two balanced half-reactions

$$4H_2 \rightarrow 8H^+ + 8e^-$$
$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$$

3.4 Microbial Enzyme Production

To this point, we have been concerned with determining the mass and energy requirements for microbial growth. We now have the tools to determine how much energy, carbon, or nutrients we must add or remove from a given biological system during the course of a process to keep it operating at a steady state. However, just because a reaction *can* occur does not mean that it *does* occur. In this section, we will study how to determine if a reaction will, or will not, occur, and, importantly for biological systems analysis and design, to develop a method for describing the rate at which a given reaction will proceed.

3.4.1 Role of Enzymes

Even if the free energy of a reaction [Eq. (3-24)] indicates that it should proceed spontaneously, a reaction still may not occur. For example, there is a large, negative free energy difference between the cellulose in paper and its lower energy state of ash, but this does not mean that the pages in this book will immediately and completely oxidize (release electrons and energy, i.e., go up in flames!). Oxidation of paper does occur, but at a very slow rate; there is a relatively large amount of energy needed to allow the free energy to be released (Figure 3.3). The brown edges on old books attest to the slow "burning" of paper. The edges of the paper are exposed to the oxygen needed for the oxidation to occur, and occasionally these exposed edges lose electrons to oxidation. In order for energy to be released more rapidly in a flame, many electrons must release their energy simultaneously. When this occurs, a chain reaction begins in which the energy released by the first batch of electrons provides the

energy needed for the next batch to oxidize, and so on. To initiate this sequence of reactions, a match could be added to one corner of a page (don't try this on your textbook!). The additional energy provided by the match flame allows a great many electrons to oxidize simultaneously and begins a sequence of reactions that (with adequate oxygen) allows the rest of the pages to burn as well.

The match in the above conceptual example provides the activation energy needed to initiate the exergonic process of converting cellulose to ash. In the conversion of H_2 and O_2 to H_2O in the laboratory, a spark often is used. Addition of energy to a system is one way to overcome the activation energy needed for a reaction to occur. Another way is the use of a catalyst; a catalyst reduces the required energy of activation *without itself being altered by the reaction* (Figure 3.3). In biological reactions, an enzyme most commonly fills the role of catalyst.

Activation energy—Energy input required in a chemical process to bring reactants to a reactive state by breaking the chemical bonds that exist between each reactant.

Catalyst—Substance that encourages a reaction to proceed by reducing the activation energy.

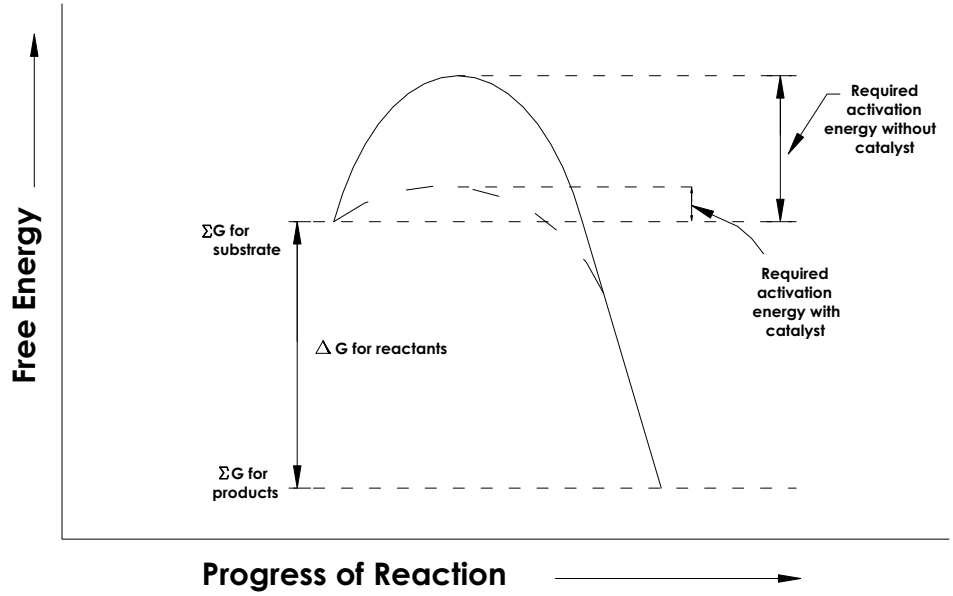


Figure 3.3—The effect of a catalyst on the activation energy of an exergonic reaction.

3.4.2 Function of Enzymes

Enzymes are specialized proteins that catalyze reactions in living cells. Unlike catalysts used in chemical reactions, enzymes are highly specific; an enzyme typically catalyzes only one biochemical reaction. An enzyme functions by binding to a reactant at the enzyme's active site. The presence of the enzyme realigns the forces (hydrogen-bonding, van der Waals, and hydrophobic forces) that normally hold the reactant in a particular configuration (and give the molecule a particular shape). The configuration that results can accept a specific substrate more readily in the position and

Enzyme—Specialized protein that catalyzes a specific biological reaction.

Active site—The small portion of an enzyme that temporarily binds to a reactant.

orientation needed for the reaction to occur. A schematic representation of the enzymatic process is shown in Figure 3.4.

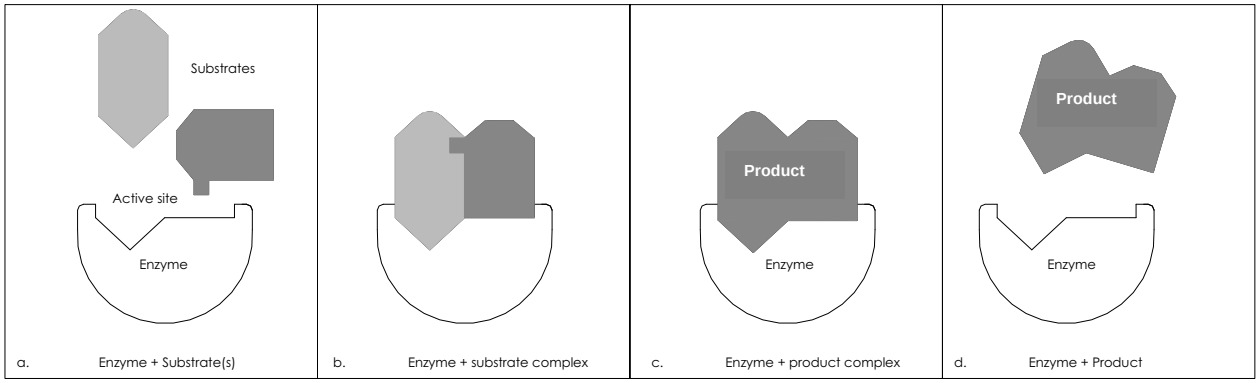


Figure 3.4—The enzymatic reaction process (Fuhrmann, 1998).

In an enzyme-catalyzed reaction, an enzyme is *temporarily* associated with a reactant or substrate to form an enzyme-substrate complex. The reaction proceeds and results in formation of a new product and reclamation of the enzyme in its original form. [Eq. (3-25) is a model of this process].

$$E + S \leftrightarrows ES \leftrightarrows E + P \tag{3-25}$$

where E = enzyme,
S = substrate, and
P = product.

Enzymes can speed the rate of biochemical reactions by as much as 10^3 to 10^{20} times. Some enzymes can even catalyze endergonic reactions.

Enzymes are named by adding the suffix, -ase, to the name of the substrate that the enzyme changes into a new molecule. Examples are:

lactase:	lactose → glucose + galactose
lipase:	lipid → fatty acid + glycerol
maltase:	maltose → glucose
urease:	urea + H_2O → $2NH_3$ + CO_2
cellobiase:	cellobiose → glucose
α-amylase:	amylose (starch) → glucose + maltose + oligosaccharides

Another naming convention is to name the enzyme after the reaction catalyzed. Examples are:

glucose oxidase:	D-glucose + O_2 + H_2O → gluconic acid
glucose isomerase:	glucose ⇆ fructose

alcohol dehydrogenase: ethanol + NAD^+ ⇆ acetaldehyde + $NADH_2$

Enzymatic reactions often require the assistance of coenzymes. Coenzymes may be prosthetic groups that bind covalently to the enzyme or cosubstrates that bind transiently and noncovalently to the enzyme. Carrier molecules supply electrons or small molecules required for the reaction.

Coenzyme—Non-protein substance that facilitates enzymatic reactions. Common coenzymes are metal ions (e.g., Fe, Mo, Mg) and carrier molecules (e.g., NADH, $FADH_2$, vitamins).

Enzymatic reactions also can be inhibited by reactions that compete with a substrate for access to an enzyme. Reactions in which a non-targeted substrate competes directly with the reactant for the active site demonstrate competitive inhibition. Inhibition also can occur when a substrate attaches to an allosteric site on an enzyme, altering the active site into a configuration that cannot bind with the reactant. This differs from competitive inhibition because the allosteric site is separate and distinct from the active site.

Allosteric site—Site, other than the active site, on an enzyme to which a non-substrate compound attaches. This may change the shape of the enzyme such that the target substrate cannot bind to it.

3.5 Modeling Enzyme Reaction Kinetics

Biochemical reactions are commonly analyzed using reaction kinetics. Reaction kinetics describes the relationship between the reaction rate and reaction conditions, such as substrate concentration or temperature.

3.5.1 Reaction Orders

The kinetics of biological processes typically are classified as either zero-order, first-order, or second-order. Fractional-order reactions are possible, but rate analyses of most biological systems assume an integer value for reaction order. Higher order processes are also possible but not common in biological systems. A combination of zero- and first-order reactions also is possible, as demonstrated by the Michaelis-Menten equation discussed later in this section.

Reaction order—The relationship between reaction rate and a reaction constituent, such as substrate concentration, described by the general equation: rate = (substrate concentration)^n, where n is the order.

Zero-order Kinetics

Zero-order reactions occur at a rate independent of the concentration of any reactant. The following kinetic expression describes the rate of disappearance of substrate A:

$$r_A = -\frac{dC_A}{dt} = k_0[C_A]^0 = k_0 \qquad (3\text{-}26)$$

where r_A = rate of reaction with respect to substrate A (M L^{-3} T^{-1}),
C_A = concentration of substrate A (M L^{-3}), and
k_0 = zero-order rate constant (T^{-1}).

The order of this reaction is 0 with respect to C_A. The rate constant includes the effects of both the amount of enzyme present as well as

the rate of reaction specific to that enzyme. Integrating Eq. (3-26) gives

$$C_A = -k_0\ t + \text{constant of integration} \tag{3-27}$$

The constant of integration can be solved by letting $C_A = C_{A0}$ at t = 0. Thus,

$$C_A = C_{A0} - k_0\ t \tag{3-28}$$

The form of this equation is linear (y = a + b x), indicating that for zero-order reactions a plot of C_A vs. t would produce a line with slope $-k_0$ and intercept C_{A0}.

First-order Kinetics

The rate of first-order reactions increases linearly with reactant concentration. The relationship between reaction rate and reactant concentration is

$$r_A = -\frac{dC_A}{dt} = k_1(C_A)^1 = k_1\ C_A \tag{3-29}$$

where k_1 = first-order rate constant (T^{-1}).

The order of this reaction is 1 with respect to C_A. Again, integrating Eq. (3-29) and letting $C_A = C_{A0}$ at t = 0 gives

$$C_A = C_{A0}\ e^{-k_1 t} \tag{3-30}$$

or, taking natural logarithms of both sides,

$$\ln C_A = \ln C_{A0} - k_1\ t \tag{3-31}$$

Again, the form of this equation is linear (y = a + b x), indicating that for first-order reactions a plot of ln C_A vs. t would produce a line with slope $-k_1$ and intercept ln C_{A0}.

3.5.2 Michaelis-Menten Kinetics

Enzyme kinetics deals with the rate at which an enzyme-catalyzed reaction proceeds. Rate equations developed from kinetic studies can be applied in calculating reaction times, yields, and operating conditions to maximize economic return. All these factors are important in the design of a bioreactor.

Conceptually, an enzyme-catalyzed reaction is represented as follows:

$$S \xrightarrow{E} P \tag{3-32}$$

The substrate (S) is converted to a product (P) with the help of an enzyme (E). This is a similar, but condensed, representation of Eq. (3-25). If the concentrations of substrate and product are measured with respect to time, the product concentration will increase and reach a maximum value, whereas the substrate concentration will decrease until it is completely consumed (Figure 3.5). *The enzyme concentration remains constant.* The rate of reaction can be expressed in terms of either the change in substrate concentration C_S or the product concentration C_P.

$$r_S = -\frac{dC_S}{dt} \tag{3-33}$$

$$r_P = \frac{dC_P}{dt} \tag{3-34}$$

These rates are the slopes of the curves shown in Figure 3.5. The rates change continuously as the reaction proceeds.

It is important to understand that reaction conditions, such as substrate, product, and enzyme concentrations, influence reaction rate. If we plot the *initial* reaction rate vs. substrate concentration for a range of substrate concentrations and a given enzyme concentration, we obtain a curve like that in Figure 3.6. A different but similarly shaped curve would be obtained for a different enzyme concentration.

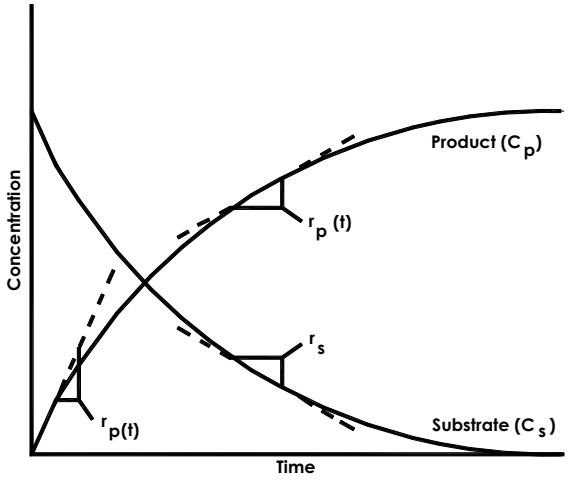


Figure 3.5—Changes in product (C_P) and substrate (C_S) concentrations with respect to time in a typical bioreaction.

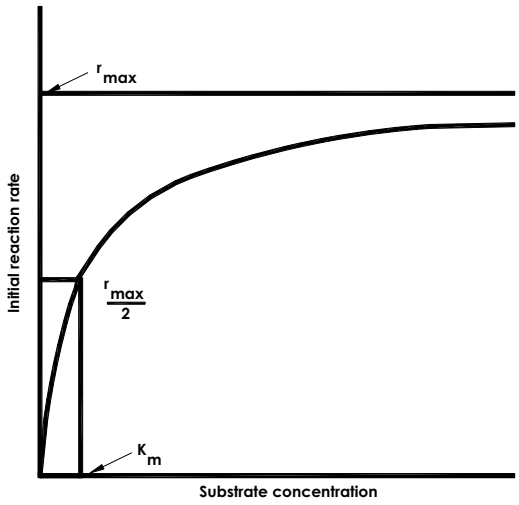


Figure 3.6—Initial reaction rate (r_P) as a function of substrate concentration (C_S) for a given enzyme concentration.

The maximum rate r_{max} is proportional to the enzyme concentration. Higher enzyme concentrations give higher values of r_{max}. As shown in Figure 3.6, K_m is defined as the substrate concentration when the reaction rate is $r_p = r_{max}/2$.

In 1902, Henri (Bailey and Ollis, 1986, p. 100) proposed the following rate equation:

$$r_P = \frac{r_{max} C_S}{K_m + C_S} \tag{3-35}$$

The following observations can be made from Eq. (3-35):

1. When the substrate concentration (C_S) is low (much less than K_m), the reaction rate is proportional to C_S. The reaction is first order.
2. When the substrate concentration (C_S) is high (much greater than K_m), the reaction rate approaches r_{max} and does not depend on C_S. The reaction is zero order.

This equation is a fairly good representation of the curve in Figure 3.6.

Brown (1902) proposed that an enzyme forms a complex with its substrate. This complex (ES) then dissociates to yield the product and regenerate the enzyme. In equation form,

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \tag{3-36}$$

$$ES \xrightarrow{k_3} P + E \tag{3-37}$$

These two equations break Eq. (3-25) into two reactions, one reversible and the other irreversible. The variables k_1, k_2, and k_3 are the rate constants. Rate constants indicate the speed at which a reaction proceeds.

The following assumptions are made before deriving a rate equation for the reaction represented by Eqs. (3-36) and (3-37).

1. Total enzyme concentration remains constant during the reaction.

$$C_{E0} = C_{ES} + C_E \tag{3-38}$$

where C_{E0} = enzyme concentration at beginning of reaction ($t = 0$),
C_{ES} = concentration of enzyme bound with substrate, and
C_E = concentration of free enzyme.

2. The amount of enzyme is very small compared to the amount of substrate. Only a small portion of the substrate is bound in the ES complex at any time t.
3. Initial product concentration is so low that product inhibition is neglected.

In this section, we will discuss two approaches used to derive the rate equation: the Michaelis-Menten method and a numerical solution.

Michaelis-Menten Model

Michaelis and Menten (1913) assumed that the product-releasing step, Eq. (3-37), was much slower than the complex-forming step, Eq. (3-36). Formation of the ES complex is based on very weak interactions. The product-releasing step involves more significant chemical changes; therefore, it is reasonable to expect this step to be slower.

If the reaction shown in Eq. (3-37) determines the overall rate of reaction, the rates of product formation dC_P/dt and substrate consumption dC_S/dt are proportional to the concentration of the enzyme-substrate complex.

$$\frac{dC_P}{dt} = -\frac{dC_S}{dt} = k_3 C_{ES} \tag{3-39}$$

Since the Eq. (3-36) reaction is reversible, the forward reaction is equal to the reverse reaction.

$$k_1 C_S C_E = k_2 C_{ES} \tag{3-40}$$

The concentrations are expressed in molar units such as kmol m^{-3} or mol L^{-1}.

Note that Eq. (3-40) follows from Eq. (3-36). Remember that Eq. (3-36) is only a "model" to show how the reaction proceeds; it is not a

computational equation. Eq. (3-40) demonstrates a standard procedure for translating Eq. (3-36) into a form that allows computation of reaction rates. In this case, the rate constant k_1 times the concentration of substance 1 (C_S) times the concentration of substance 2 (C_E) equals k_2 times the concentration of the enzyme-substrate complex (C_{ES}). It will be important for you to become familiar with this notation.

Recall that we assume that the total amount of enzyme is conserved [Eq. (3-38)]. Substituting Eq. (3-38) into Eq. (3-40),

$$k_1 C_S (C_{E0} - C_{ES}) = k_2 C_{ES} \tag{3-41}$$

Rearranging, we get

$$k_1 C_{E0} C_S = (k_2 + k_1 C_S) C_{ES} \tag{3-42}$$

and finally, solving for C_{ES},

$$C_{ES} = \frac{k_1 C_{E0} C_S}{k_2 + k_1 C_S} = \frac{C_{E0} C_S}{\frac{k_2}{k_1} + C_S} \tag{3-43}$$

Substitution of Eq. (3-43) into Eq. (3-39) gives

$$r_P = \frac{dC_P}{dt} = -\frac{dC_S}{dt} = \frac{k_3 C_{E0} C_S}{\frac{k_2}{k_1} + C_S} \tag{3-44}$$

When the concentration of substrate is very high, the rate of product formation is a maximum, $r_p = r_{max}$, and is dependent primarily upon the amount of enzyme. Therefore,

$$r_{max} = k_3 C_{E0} \tag{3-45}$$

(Note that the initial amount of enzyme C_{E0} also represents the total amount of enzyme in the system.) Eq. (3-44) can now be rewritten as

$$r_P = \frac{r_{max} C_S}{K_m + C_S} \tag{3-46}$$

This equation is known as the Michaelis-Menten Equation, named after Leonor Michaelis and Maude Menten. The Michaelis-Menten constant K_m is defined by

$$K_m = \frac{k_2}{k_1} = \frac{C_S C_E}{C_{ES}} \tag{3-47}$$

K_m has the same units as C_S. To better understand the meaning of K_m, suppose that K_m equals C_S. This happens when $C_E = C_{ES}$, that is, when exactly half of the total enzyme has been transformed into

the ES complex [Eq. (3-38)]. Substitution of $K_m = C_S$ into Eq. (3-46) gives

$$r_P = \frac{r_{max}\, C_S}{C_S + C_S} = \frac{r_{max}}{2} \tag{3-48}$$

Thus, K_m is the concentration of substrate when the initial reaction rate is half the maximum rate. This demonstrates the common definition of K_m (the concentration at half-maximal reaction rate).

At this point it is important to note that the Michaelis-Menten derivation of Eq. (3-46) is not the only one in common use. Briggs and Haldane used a slightly different set of assumptions with the net result that the K_m in their derivation was defined by $(k_2 + k_3)/k_1$. If $k_2 >> k_3$, then the Briggs-Haldane definition is approximately equal to the Michaelis-Menten definition [Eq. (3-47)]. This occurs when the product-releasing step is much slower than the enzyme-substrate dissociation step, the assumption used by Michaelis-Menten.

Numerical Solution

If reaction rate constants k_1, k_2, and k_3 are known, the following set of differential equations can be solved simultaneously to define the change in C_P, C_{ES}, and C_S with time.

$$\frac{dC_P}{dt} = k_3\, C_{ES} \tag{3-49}$$

$$\frac{dC_{ES}}{dt} = k_1\, C_S\, C_E - k_2\, C_{ES} - k_3\, C_{ES} \tag{3-50}$$

$$\frac{dC_S}{dt} = -k_1\, C_S\, C_E + k_2\, C_{ES} \tag{3-51}$$

An analytical solution is not possible; therefore, the equations must be solved numerically. Several software packages are available to solve these equations.

Solution of the three differential equations requires values for k_1, k_2, and k_3. These values are obtained from experimental data.

Modeling Example Problem 3.1—Michaelis-Menten Equation Solution

This problem was taken from Lee (1992). For this problem, the initial substrate concentration is taken to be $C_{S0} = 0.1$ mol L^{-1}, and the initial enzyme concentration is $C_{E0} = 0.01$ mol L^{-1}. Product concentration at time t = 0 is $C_{P0} = 0.0$. The rate constants are $k_1 = 40$ L mol^{-1} s^{-1}, $k_2 = 5$ L s^{-1}, and $k_3 = 0.5$ L s^{-1}.

Two methods are shown for solving Eqs. (3-49) through (3-51). Using the DESIRE software results in a very concise program (Table 3.1).

Table 3.1. DESIRE Program for Modeling Example Problem 3.1.

```
K1=40.    |  K2=5.    |  K3=0.5    |  CEO=0.01
CS=0.1    |  CP=0.0   |  CES=0.0
TMAX=130  | DT=0.1    |  NN=14     |  scale=0.1
disconnect 1  | connect 'd:\chap3\prob31.out' as output 1
drun
disconnect 1  | connect 'con' as output 1
write 'Hurrah  Its done'
DYNAMIC
CE=CEO–CES
d/dt CS=–K1*CS*CE+K2*CES
d/dt CES=K1*CS*CE–K2*CES–K3*CES
d/dt CS=K3*CES
type CP,CS
/--
```

COMMENTS:
Enter the needed parameters:
CS=0.1 sets the value of C_S at t=0
CP=0.0 sets the value of C_P at t=0
CES=0.0 sets the value of C_{ES} at t=0
Write the output to the designated file:
disconnect 1 | connect 'd:\chap3\prob31.out' as output 1
Run the program:
drun
Switch back and now write to the monitor screen:
disconnect 1 | connect 'con' as output 1
On-screen message to indicate the program is finished:
write 'Hurrah Its done'
The program statements:
DYNAMIC
d/dt CS=–K1*CS*CE+K2*CES
d/dt CES=K1*CS*CE–K2*CES–K3*CES
d/dt CS=K3*CES
List the variables you want (time is automatically written with the listed variables):
type CP,CS

A MATLAB program to solve Eqs. (3-49) through (3-51) is written as follows. Each of the three variables C_S , C_{ES}, and C_P must have an initial value. These are:

Variable		**MATLAB Variable Name**	
C_S	(t = 0)	= 0.1 mol L^{-1}	Cso = 0.1
C_{ES}	(t = 0)	= 0.0 mol L^{-1}	Ces = 0.0
C_P	(t = 0)	= 0.0 mol L^{-1}	Cpo = 0.0

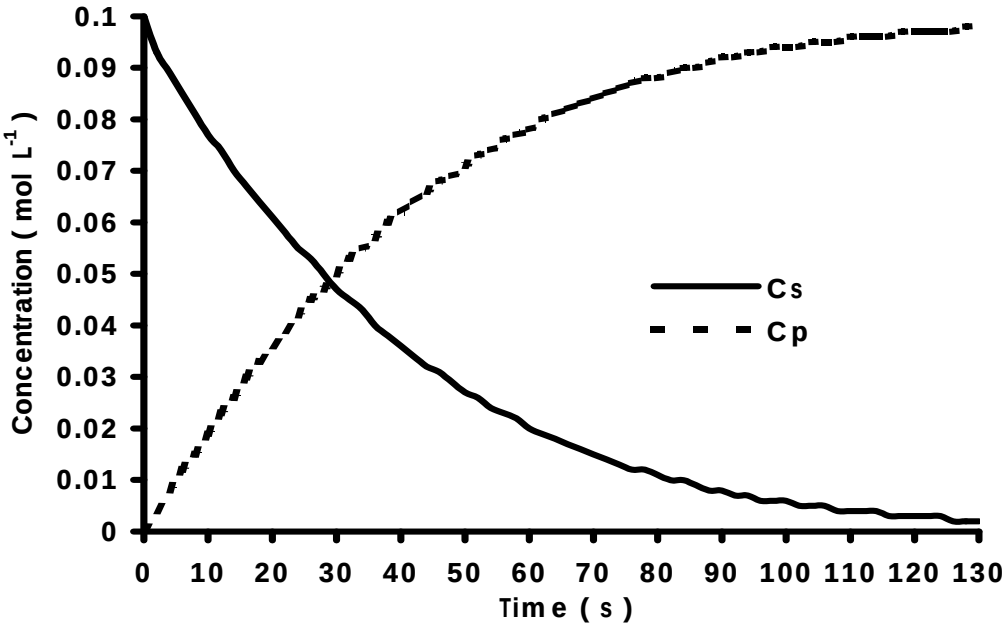


Figure 3.7—Solution to Modeling Example Problem 3.1.

The other input required is the total amount of enzyme.

$$C_{E0} = 0.01 \text{ mol L}^{-1} \qquad \text{Ceo} = 0.01$$

Throughout this text, the MATLAB variable names emulate the variable names in the equations to the maximum degree possible.

The rate constants are:

$k_1 = 40$ L mol^{-1} s^{-1}	k1 = 40
$k_2 = 5$ L mol^{-1} s^{-1}	k2 = 5
$k_3 = 0.5$ L mol^{-1} s^{-1}	k3 = 0.5

It is acceptable to use rectangular integration if you choose the integration interval small enough. Choosing $\Delta t = 0.2$ gives acceptable accuracy. The math procedure is then simply

$$\text{New value} = \text{old value} + \text{rate} \times \Delta t$$

For example, the integration to obtain substrate concentration is

$$C_{S_i} = C_{S_{i-1}} + \frac{dC_S}{dt} dt$$

The $C_{S\,i}$ notation means the substrate concentration at the end of the i^{th} interval. $C_{S\,i-1}$ is the substrate concentration at the end of the i–1 interval (beginning of i^{th} interval). dC_S/dt is given by Eq. (3-51). Written as a finite difference equation, Eq. (3-51) becomes

$$\frac{dC_S}{dt} = -k_1\ C_{S_{i-1}}\ C_E + k_2\ C_{ES_{i-1}}$$

Note, $C_E = C_{E0}$ (the initial value) – C_{ES} (the amount of enzyme tied up in the enzyme-substrate complex). With these hints, the writing of the MATLAB program given in Table 3.2 is straight forward.

Table 3.2. MATLAB Program for Modeling Example Problem 3.1.

```
DEFINITION OF VARIABLES
      Cso = CONCENTRATION OF SUBSTRATE AT t = 0 (mol/L)
      Ceo = CONCENTRATION OF ENZYME AT t = 0 (mol/L)
      Ceso = CONCENTRATION OF ENZYME-SUBSTRATE
      COMPLEX AT t = 0 (mol/L)
      k1, k2, k3 = RATE CONSTANTS (L/mol/s)

      Cso=0.1; Ceo=0.01; Cpo=0.0; Ceso=0.0;
      k1=40.0; k2=5; k3=0.5;

      delt=0.2;

DYNAMIC SECTION WHERE SET OF DIFFERENTIAL
EQUATIONS ARE SOLVED

SET INITIAL VALUES
      t(1)=0;
      Cs(1)=Cso;
      Ces(1)=Ceso;
      Cp(1)=Cpo;

TIME LOOP for I=2:131;

SET VALUES AT BEGINNING OF INTERVAL
      t(I)=t(I–1)+1;
      Cesim1=Ces(I–1);
      Csim1=Cs(I–1);
      Cpim1=Cp(I–1);

DELT LOOP (THE INTERVAL IS 0.2s SO THE NUMBER OF
ITERATIONS FOR EACH SECOND IS 5)
      for IDT=1:5

      Ce=Ceo–Cesim1;
      delCsdt=–k1*Csim1*Ce+k2*Cesim1;
      Csi=Csim1+delCsdt*delt;
      delCesdt=k1*Csim1*Ce–k2*Cesim1–k3*Cesim1;
      Cesi=Cesim1+delCesdt*delt;
      delCpdt=k3*Cesim1;
      Cpi=Cpim1+delCpdt*delt;
```

```
SET VALUES FOR NEXT PASS THROUGH DELT LOOP—(THE
VALUE AT THE BEGINING OF THE NEXT STEP EQUALS THE
VALUE AT THE END OF THE CURRENT STEP)
        Csim1=Csi;
        Cesim1=Cesi;
        Cpim1=Cpi;

end %Ends delt loop
        Cs(I)=Csi;
        Ces(I)=Cesi;
        Cp(I)=Cpi;

end % Ends second loop
PUT VALUES IN A DATA FILE.
        y=[t;Cs;Cp];
        fid=fopen('Prob31.dat','w');
        fprintf(fid,'\n %.1f  %.3f  %.3f',y);
        fclose(fid);
```

Modeling Problem 3.1—Michaelis-Menten Equation Solution

This problem was taken from Lee (1992). A carbohydrate (S) decomposes in the presence of an enzyme (E). The Michaelis-Menten kinetic parameters were determined from experimental data.

$$K_m = 200 \text{ mol m}^{-3}$$
$$r_{max} = 100 \text{ mol m}^{-3} \text{ min}^{-1}$$

1. Write a MATLAB program that uses rectangular integration to calculate substrate concentration over time in a batch reactor. Assume that the initial concentration C_{S0} = 300 mol m^{-3}. Plot C_S vs. t. Solve the equation

$$-\frac{dC_S}{dt} = \frac{r_{max} C_S}{K_m + C_S}$$

   analytically. Verify that the solution is

$$K_m \ln \frac{C_{S0}}{C_S} + (C_{S0} - C_S) = r_{max}\, t$$

   Plot this C_S vs. t curve on the same graph as the curve obtained with rectangular integration.

2. Repeat Part (1) using your MATLAB program to obtain the numerical solution to the differential equation.

Use

$\Delta t = 0.1$
$\Delta t = 0.5$
$\Delta t = 1.0$

Give your results in a table. Compare your data with the analytical solution in Part (1).

3.5.3 Inhibition of Enzyme Reactions

An inhibitor is a substance that decreases enzyme activity. It can decrease the reaction rate either competitively, noncompetitively, or uncompetitively.

Competitive Inhibition

Since a competitive inhibitor has a strong structural resemblance to the substrate, both the inhibitor (I) and the substrate (S) compete for the active site on the enzyme. The formation of an enzyme-inhibitor complex (EI) reduces the amount of enzyme available for interaction with the substrate, thus the reaction rate decreases. Normally, the inhibitor combines reversibly with the enzyme. Its effect can be minimized by increasing the substrate concentration. This technique can be applied up to the point where the concentration of the substrate itself begins to inhibit the reaction.

The mechanism of competitive inhibition can be represented as follows:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \tag{3-52}$$

$$E + I \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EI \tag{3-53}$$

$$ES \xrightarrow{k_5} P + E \tag{3-54}$$

If the product formation step is the slower reaction, as assumed for the derivation of the Michaelis-Menten equation, the rate of product formation can be expressed as

$$r_p = k_5 C_{ES} \tag{3-55}$$

Enzyme is conserved, thus

$$C_{E0} = C_E + C_{ES} + C_{EI} \tag{3-56}$$

where C_{EI} is the amount of enzyme bound in the inhibitor-enzyme complex. The two equilibrium reactions give

$$\frac{C_E\ C_S}{C_{ES}} = \frac{k_2}{k_1} = K_S \tag{3-57}$$

$$\frac{C_E\ \ C_I}{C_{EI}} = \frac{k_4}{k_3} = K_I \tag{3-58}$$

Assigning the variables $k_2/k_1 = K_S$ and $k_4/k_3 = K_I$ simplifies algebraic manipulation. Eqs. (3-55), (3-56), (3-57), and (3-58) will now be manipulated to eliminate the concentrations C_E, C_{ES}, and C_{EI}.

Solving Eq. (3-58) for C_{EI},

$$C_{EI} = \frac{C_E\ C_I}{K_I} \tag{3-59}$$

Substituting into Eq. (3-56) gives

$$C_{E0} = C_E + \frac{C_E\ C_I}{K_I} + C_{ES} = C_E\left(1 + \frac{C_I}{K_I}\right) + C_{ES} \tag{3-60}$$

Solving Eq. (3-57) for C_E and substituting into Eq. (3-60) gives

$$C_{E0} = \frac{K_S\ C_{ES}}{C_S}\left(1 + \frac{C_I}{K_I}\right) + C_{ES} = \left[\frac{K_S}{C_S}\left(1 + \frac{C_I}{K_I}\right) + 1\right] C_{ES} \tag{3-61}$$

Solving Eq. (3-55) for C_{ES} and substituting into Eq. (3-61) gives

$$C_{E0} = \left[\frac{K_S}{C_S}\left(1 + \frac{C_I}{K_I}\right) + 1\right]\frac{r_P}{k_5} \tag{3-62}$$

or

$$r_P = \frac{k_5\ C_{E0}\ C_S}{C_S + K_S\left(1 + \frac{C_I}{K_I}\right)} \tag{3-63}$$

If we define $k_5\ C_{E0} = r_{max}$ and $K_S\left(1 + \frac{C_I}{K_I}\right) = K_{MI}$, then Eq. (3-62) reduces to

$$r_P = \frac{r_{max}\ C_S}{C_S + K_{MI}} \tag{3-64}$$

Since K_{MI} is larger than K_S by definition, the reaction rate decreases due to the presence of the inhibitor. The maximum rate is not affected by the presence of a competitive inhibitor. It does take a higher concentration of substrate before the maximum rate will occur.

Noncompetitive Inhibition

Noncompetitive inhibitors interact with enzymes in many different ways. The end result is that the enzyme-inhibitor complexes (EI, EIS, and ESI) are not available to contribute to product creation. A model for noncompetitive inhibition is shown below.

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \tag{3-65}$$

$$E + I \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EI \tag{3-66}$$

$$EI + S \underset{k_6}{\overset{k_5}{\rightleftharpoons}} EIS \tag{3-67}$$

$$ES + I \underset{k_8}{\overset{k_7}{\rightleftharpoons}} ESI \tag{3-68}$$

$$ES \xrightarrow{k_9} E + P \tag{3-69}$$

The assumption for noncompetitive inhibition is that the substrate and inhibitor do not compete for the *same* site for formation of an enzyme-substrate complex or enzyme-inhibitor complex. It is appropriate then to assume the dissociation constants for the first and third equilibrium reactions are equal.

$$\frac{k_2}{k_1} = K_S = \frac{k_6}{k_5} \tag{3-70}$$

If we also assume that the disassociation constants for the second and fourth reactions are equal,

$$\frac{k_4}{k_3} = K_I = \frac{k_8}{k_7} \tag{3-71}$$

The following equations can now be written.

$$\frac{C_E\ C_S}{C_{ES}} = K_S \tag{3-72}$$

$$\frac{C_E\ C_I}{C_{EI}} = K_I \tag{3-73}$$

$$\frac{C_{EI}\ C_S}{C_{EIS}} = K_S \tag{3-74}$$

$$\frac{C_{ES}\ C_I}{C_{ESI}} = K_I \tag{3-75}$$

$$r_P = k_9\ C_{ES} \tag{3-76}$$

Again, we assume that total enzyme remains constant.

$$C_{E0} = C_E + C_{ES} + C_{EI} + C_{EIS} + C_{ESI} \tag{3-77}$$

These six equations will now be used to derive a rate equation for product formation using the Michaelis-Menten approach. Solving Eqs. (3-73) and (3-74) and substituting into Eq. (3-77), we obtain

$$\begin{aligned} C_{E0} &= C_E + C_{ES} + C_{EI} + \frac{C_{EI}\ C_S}{K_S} + \frac{C_{ES}\ C_I}{K_I} \\ &= C_E + \left(1 + \frac{C_I}{K_I}\right) C_{ES} + \left(1 + \frac{C_S}{K_S}\right) C_{EI} \end{aligned} \tag{3-78}$$

Solving Eq. (3-73) for C_{EI}, substituting into Eq. (3-78) and rearranging, we obtain

$$\begin{aligned} C_{E0} &= C_E + \left(1 + \frac{C_I}{K_I}\right) C_{ES} + \left(1 + \frac{C_S}{K_S}\right) \frac{C_E\ C_I}{K_I} \\ &= \left[1 + \left(1 + \frac{C_S}{K_S}\right) \frac{C_I}{K_I}\right] C_E + \left(1 + \frac{C_I}{K_I}\right) C_{ES} \end{aligned} \tag{3-79}$$

Solving Eq. (3-72) for C_E and substituting into Eq. (3-79) gives

$$\begin{aligned} C_{E0} &= \left[1 + \left(1 + \frac{C_S}{K_S}\right) \frac{C_I}{K_I}\right] \frac{K_S}{C_S} C_{ES} + \left(1 + \frac{C_I}{K_I}\right) C_{ES} \\ &= \left\{\left[1 + \left(1 + \frac{C_S}{K_S}\right) \frac{C_I}{K_I}\right] \frac{K_S}{C_S} + \left(1 + \frac{C_I}{K_I}\right)\right\} C_{ES} \end{aligned} \tag{3-80}$$

Solving Eq. (3-76) for C_{ES}, substituting into Eq. (3-80), and solving for r_p, we obtain

$$r_P = \frac{k_9 C_{E0}}{\left[1 + \left(1 + \frac{C_S}{K_S}\right)\frac{C_I}{K_I}\right]\frac{K_S}{C_S} + \left(1 + \frac{C_I}{K_I}\right)} = \frac{k_9 C_{E0}}{\left(\frac{K_S}{C_S} + 1\right)\left(1 + \frac{C_I}{K_I}\right) + \frac{C_I}{K_I}} \quad (3\text{-}81)$$

$$= \frac{\frac{k_9}{\left(1 + \frac{C_I}{K_I}\right)} C_{E0}}{\frac{K_S}{C_S} + 1 + \frac{C_I / K_I}{\left(1 + \frac{C_I}{K_I}\right)}} = \frac{\frac{k_9}{\left(1 + \frac{C_I}{K_I}\right)} C_{E0} C_S}{C_S\left(1 + \frac{C_I}{K_I + C_I}\right) + K_S}$$

Defining $r_{I,max} = r_{max}/(1 + C_I/K_I)$, where $r_{max} = k_9 C_{E0}$, Eq. (3-81) simplifies to the Michaelis-Menten form.

$$r_P = \frac{r_{I,max} C_S}{C_S\left(\frac{K_I + 2C_I}{K_I + C_I}\right) + K_S} \quad (3\text{-}82)$$

Since $(K_I + 2 C_I)/(K_I + C_I)$ is approximately equal to 1, Eq. (3-82) is often further simplified to

$$r_P = \frac{r_{I,max} C_S}{C_S + K_S} \quad (3\text{-}83)$$

Since $r_{I,max} = r_{max}/(1 + C_I/K_I)$, the maximum reaction rate is decreased by the presence of a noncompetitive inhibitor. However, the inhibitor does not affect the constant K_S.

3.5.4 Influences on Enzyme Activity

The influence of factors such as concentration of substrate, product, enzyme, and inhibitor on an enzyme reaction have been discussed. Temperature and pH are two other important factors.

Effect of pH

Typically, there is a pH range within which an enzyme will react at an optimal rate. This optimum range is different for each enzyme. For example, pepsin from the stomach has an optimum pH in the range 2 to 3.3, whereas the optimum for amylase from saliva is 6.8. Chymotrypsin from the pancreas will react most effectively in the pH range 7 to 8.

The tendency of some amino acids to ionize changes with pH. (Remember that enzymes are protein molecules and proteins are chains of amino acids.) Ionization of an amino acid at a nonessential location can change with pH, and the result will have little effect on

the enzyme's activity. However, ionization of an amino acid at an active site may profoundly affect the enzyme's activity. Experiments must be done to determine the optimum pH for each enzyme and substrate combination.

Typical data are shown in Figure 3.8. In general, the curve is "bell-shaped", and sometimes there is a broader range of maximal activity as shown by the dotted curve.

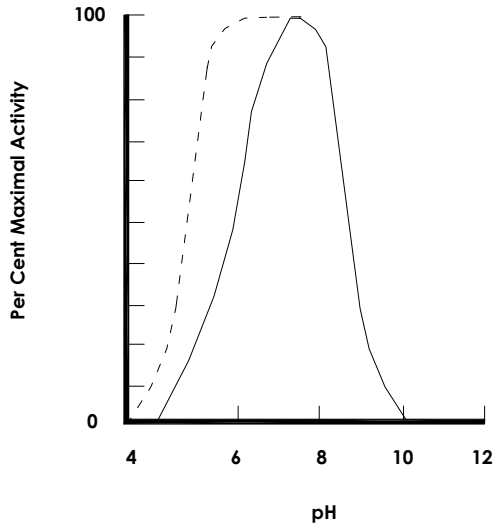


Figure 3.8—Typical pH activity profile for an enzyme. (The dotted curve shows an enzyme with broader range of activity.)

Effect of Temperature

An increase in temperature increases the rate of reaction because the atoms have greater energies and therefore a greater tendency to move. The temperature dependence of many enzyme-catalyzed reactions can be described by the Arrhenius equation.

$$k = A_o \, e^{-E_a/(RT)} \tag{3-84}$$

where k = reaction constant (s^{-1}),
A_o = frequency factor (s^{-1}),
E_a = activation energy (J mol^{-1}),
R = universal gas constant (J $mol^{-1}K^{-1}$), and
T = temperature (K).

The Arrhenius equation is an empirical equation that has been found to represent many chemical and biological processes. The reaction *rate* is given by

$$r = k \, C_E \tag{3-85}$$

where r = reaction rate (mol m^{-3} s^{-1}),
k = reaction constant (s^{-1}) [Eq. (3-84)], and
C_E = enzyme concentration (mol m^{-3}),

Temperature rise for a biological process is limited to the usual biological range. As temperature increases above this range, denaturation processes progressively destroy the activity of the enzyme. Excessive heating causes the protein chain to unfold, thus changing the geometry of the active site. For most enzymes, denaturation begins to occur at 45 to 50°C. Some enzymes isolated from thermophilic organisms collected from hot environments can continue to function at temperatures above 50°C.

3.5.5 Effect of Mass-Transfer Resistance in Immobilized Enzyme Systems

Cost of the enzyme is a significant part of the total cost of product production. Immobilization holds the enzyme in place in a continuous stirred-tank reactor (CSTR). Thus, immobilization avoids the problem of recovering the enzyme from the mixture remaining at the end of a batch reaction. This recovery is often difficult and expensive to achieve. For a continuous process, immobilization retains the enzyme in the reactor.

External Mass Transfer Resistance

If the enzyme is held in position on the surface of an insoluble particle, the substrate has to overcome the resistance shown by steps 1 and 2 in Figure 3.9. These two steps are collectively referred to as the *external* mass transfer resistance.

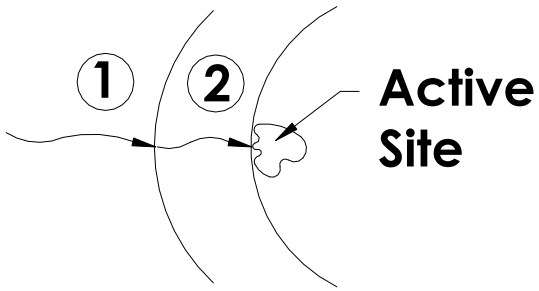


Figure 3.9—Mass transfer resistance encountered by substrate molecule to reach active site on immobilized enzyme.

Step 1 — Transfer from the bulk liquid circulating past the surface into the relatively static layer (unmixed boundary layer) adjacent to the surface.
Step 2 — Diffusion through the relatively static layer to the active site on the surface.

How does a substrate molecule move through a static layer? Concentration difference is the driving force for this mass transfer. A substrate molecule will diffuse from a region of high concentration to a region of low concentration. As substrate is consumed at the active site, new substrate diffuses through the static layer.

Diffusion is a fundamental concept that is part of the understanding of many important events in biological systems. The general equation for mass transfer by diffusion is

Mass Transfer Rate = Mass Transfer Coef. × Area × Conc. Gradient

The equation for the transfer studied here is

$$N_S = k_S\, A\,(C_{Sb} - C_S) \tag{3-86}$$

where N_S = rate of mass transfer ($M\,T^{-1}$),
k_S = mass-transfer coefficient ($L\,T^{-1}$),
A = surface area (L^2), and
C_{Sb} = concentration of substrate in the bulk liquid circulating past the surface ($M\,L^{-3}$).

Surface area, A, is the area associated with a given active site. For example, if there are 100 active sites per mm^2, then A = 0.01 mm^2.

Mass transfer occurs at the rate substrate is consumed. Unless substrate is consumed at the active site, there is no gradient to cause new substrate molecules to diffuse across the static layer. Thus, the rate of substrate transfer equals the rate of substrate consumption, and the rate of substrate consumption equals the rate of product formation. We assume this rate is described by Michaelis-Menten kinetics. Substituting N_S from Eq. (3-86) for the reaction rate, r_p, in Eq. (3-46) results in

$$k_S\, A(C_{Sb} - C_S) = \frac{r_{max}\, C_S}{K_m + C_S} \tag{3-87}$$

It is appropriate to normalize this equation using the following definitions:

$$C'_S = \frac{C_S}{C_{Sb}} \tag{3-88}$$

$$\beta = \frac{C_{Sb}}{K_m} \tag{3-89}$$

Eq. (3-87) can now be rewritten,

$$k_S\, A\, C_{Sb}\,(1 - C'_S) = \frac{r_{max}\,\beta\, C'_S}{1 + \beta\, C'_S}$$

or

$$\frac{1 - C'_S}{N_{Da}} = \frac{\beta\, C'_S}{1 + \beta\, C'_S} \tag{3-90}$$

where $N_{Da} = r_{max}/(k_S\, A\, C_{Sb})$.

This new constant is called the *Damköhler* number. It is

$$N_{Da} = \frac{\text{maximum enzyme–catalyzed reaction rate}}{\text{maximum mass–transfer rate}}$$

If the mass-transfer rate is high (substrate readily diffuses through the static layer, i.e., $N_{Da} << 1$), then mass transfer has a negligible effect on the achieved reaction rate. Reaction occurs at the rate predicted by the Michaelis-Menten model. Product-production rate is given by Eq. (3-46) as

$$r_P = \frac{r_{max}\, C_S}{K_m + C_S}$$

If the mass-transfer rate is low (substrate diffuses through the static layer very slowly, i.e., $N_{Da} >> 1$), then mass transfer is the limiting factor, and the reaction rate is approximately equal to the mass-transfer rate.

$$r_P = k_S\, A\, C_{Sb}$$

The effectiveness factor, η, quantifies the influence of mass transfer. It is defined by

$$\eta = \frac{\text{achieved reaction rate}}{\text{rate if not slowed by mass transfer}} \tag{3-91}$$

where η = effectiveness factor (0 to 1).

The rate that would be achieved if no mass-transfer limitation existed is the rate associated with the concentration in the bulk liquid. The achieved rate is the rate associated with the concentration at the active site on the surface. Substituting the respective reaction rates, we obtain

$$\eta = \frac{\left(\dfrac{r_{max}\, C_S}{K_m + C_S}\right)}{\left(\dfrac{r_{max}\, C_{Sb}}{K_m + C_{Sb}}\right)} \tag{3-92}$$

Again using the normalizing equations,

$$\eta = \frac{\left(\frac{C'_S}{1+\beta C'_S}\right)}{\left(\frac{1}{1+\beta}\right)} \tag{3-93}$$

The η values can range from 0 to 1. Suppose $C'_S = 1$ meaning $C_S = C_{Sb}$. This occurs when there is no concentration gradient across the static layer. No mass-transfer limitation exists. Under these conditions, $\eta = 1$. As mass-transfer resistance increases, $C'_S \rightarrow 0$ and $\eta \rightarrow 0$.

Internal Mass Transfer Resistance

If enzymes are immobilized *within* a structure, the active site may be located some distance below the surface of the structure. It is appropriate to think of the enzyme molecules encapsulated in a 3-dimensional matrix (Figure 3.10). Two techniques used to achieve this encapsulation are copolymerization and microencapsulation. Substrate molecules must diffuse through the matrix to reach the active site. The resulting mass-transfer resistance is known as *internal* resistance.

Copolymerization—With this technique, enzyme solution is mixed with polymer solution. The polymerized gel (containing the enzyme) is either extruded or a template is used to shape the desired particles.

Microencapsulation—Microscopic hollow spheres are formed with the enzyme solution inside. These spheres are then enclosed within a porous membrane which holds them in place.

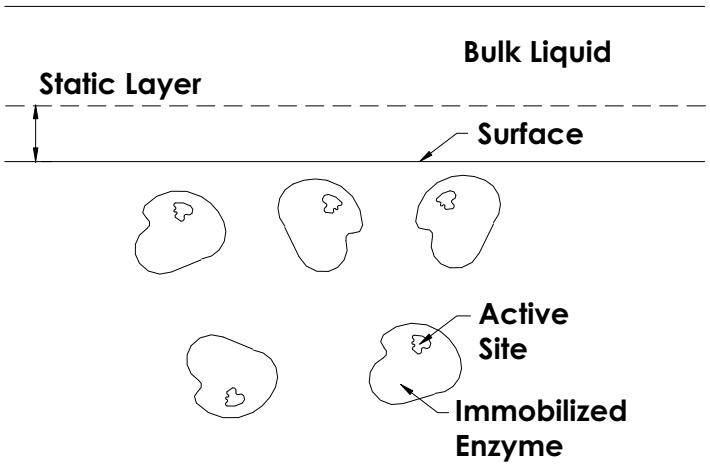


Figure 3.10—Enzyme molecules immobilized in a 3-dimensional matrix.

The following assumptions are used to create an idealized model, which will then be used to study the influence of internal mass-transfer resistance.

- The reaction occurs at every position within the matrix. In actual fact, the active sites are randomly distributed in the matrix. Reactions occur at these specific locations, not at every point within the matrix. The model being developed is a distributed model and thus is an idealization of the actual reaction site geometry.

- Mass transfer through the matrix occurs via molecular diffusion.
- No mass-transfer limitation occurs at the surface.
- The structure holding the immobilized enzyme molecules is a spherical particle.

A spherical shell is defined within the spherical particle as shown in Figure 3.11. This derivation considers internal resistance only, thus any substrate concentration gradient resulting from steps 1 and 2 in Figure 3.9 is neglected. The concentration at the particle surface (r = R) is taken to be C_{Sb}, the concentration in the bulk liquid.

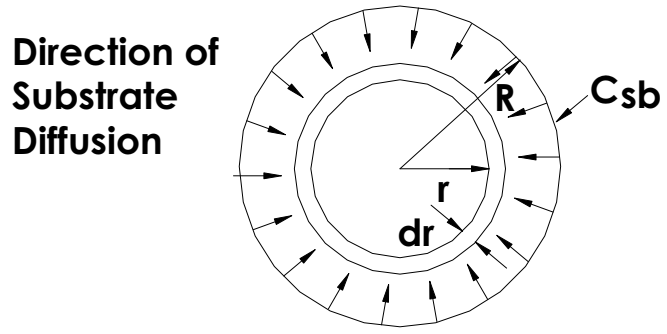


Figure 3.11—Model for diffusion of substrate into a spherical particle.

The mass balance for the spherical shell is

$$\text{Input} - \text{Output} + \text{Generation} = \text{Accumulation}$$

Surface area of a sphere with radius r is $4\pi r^2$, and the volume is $4\pi r^3/3$. Each of the terms in the mass balance will now be considered separately.

Input:

$$4\pi(r + dr)^2 D_S \left[\frac{dC_S}{dr} + \frac{d}{dr}\left(\frac{dC_S}{dr} \right) dr \right] \qquad (3\text{-}94)$$

where D_S = diffusivity, and
r = inner radius of spherical shell.

The term $4\pi (r + dr)^2$ is the surface area of the outer surface of the spherical shell. dC_S/dr is the concentration gradient at the inner surface of the spherical shell. The rate of change of this gradient with respect to radial distance times the radial distance (thickness of the shell) is the increase in concentration gradient between the inner and outer surfaces. Consequently, the term below is the concentration gradient at the outer surface.

$$\left[\frac{dC_S}{dr} + \frac{d}{dr}\left(\frac{dC_S}{dr}\right)dr\right]$$

Output:

The output is the substrate that exits the inner surface of the spherical shell and continues toward the origin.

$$4\pi r^2 D_S \frac{dC_S}{dr}$$

Generation:

Generation of substrate within the spherical shell is given by

$$4\pi r^2 dr\, r_S$$

where $4\pi r^2 dr$ = an approximation of the shell volume, and
r_S = rate of substrate consumption per unit volume.

When substrate is being consumed, r_S is negative, and the generation term represents a decrease in substrate concentration within the shell.

Accumulation:

$$4\pi r^2 dr \frac{dC_S}{dt}$$

As before, $4\pi r^2 dr$ is an approximation of the shell volume. Note, if dC_S/dt is negative, the whole term is negative, which means that mass is disappearing rather than accumulating.

For steady-state conditions, $dC_S/dt = 0$, and the mass balance becomes

$$\begin{aligned} &4\pi\left[r^2 + 2r\,dr + (dr)^2\right]D_S\frac{dC_S}{dr} \\ &+ 4\pi\left[r^2 + 2r\,dr + (dr)^2\right]D_S\frac{d}{dr}\left(\frac{dC_S}{dr}\right)dr \\ &- 4\pi r^2 dr\, D_S\frac{dC_S}{dr} + 4\pi r^2 dr\, r_S = 0 \end{aligned} \qquad (3\text{-}95)$$

Neglecting all terms with $(dr)^2$ and $(dr)^3$ and dividing through by $4\pi r^2$, we obtain

$$D_S\left(\frac{d^2\ C_S}{dr^2}+\frac{2}{r}\frac{dC_S}{dr}\right)+r_S=0 \tag{3-96}$$

We assume substrate is depleted according to Michaelis-Menten kinetics, thus

$$r_S=\frac{-r_{max}\ C_S}{K_m+C_S}$$

and Eq. (3-96) becomes,

$$D_S\left(\frac{d^2\ C_S}{dr^2}+\frac{2}{r}\frac{dC_S}{dr}\right)-\frac{r_{max}\ C_S}{K_m+C_S}=0 \tag{3-97}$$

Dimensionless relations are defined as follows:

$$C'_S=\frac{C_S}{C_{Sb}}$$

$$r'=\frac{r}{R}$$

where R = outer radius of spherical particle.

Using these relations, Eq. (3-97) becomes

$$\frac{C_{Sb}}{R^2}\frac{d^2C'_S}{dr'^2}+\frac{2\,C_{Sb}}{R^2\,r'}\frac{dC'_S}{dr'}-\frac{1}{D_S}\frac{r_{max}\ C_{Sb}\ C'_S}{K_m+C_{Sb}\ C'_S}=0 \tag{3-98}$$

Multiplying through by R^2/C_{Sb}, we obtain

$$\frac{d^2C'_S}{dr'^2}+\frac{2}{r'}\frac{dC'_S}{dr'}-\frac{R^2\ r_{max}}{D_S\ K_m}\frac{C'_S}{\left(1+\frac{C_{Sb}}{K_m}C'_S\right)}=0 \tag{3-99}$$

Eq. (3-99) can be simplified by applying Thiele's modulus, defined by

$$\phi=\frac{R}{3}\sqrt{\frac{r_{max}}{D_s\ K_m}} \tag{3-100}$$

where ϕ = Thiele's modulus,

and Eq. (3-89), which is restated as follows:

$$\beta=\frac{C_{Sb}}{K_m}$$

Eq. (3-99) now becomes

$$\frac{d^2C'_S}{dr'^2} + \frac{2}{r'}\frac{dC'_S}{dr'} - \frac{9\phi^2 C'_S}{(1+\beta C'_S)} = 0 \qquad (3\text{-}101)$$

This equation is a second-order differential equation with a spatial variable r' as the independent variable. Previously, the differential equations solved in this text have all been first-order and the independent variable has been time, t.

Eq. (3-101) is rewritten as a set of first-order equations.

$$\frac{dY}{dr'} = -\frac{2}{r'}Y + 9\phi^2\frac{C'_S}{1+\beta C'_S} \qquad (3\text{-}102)$$

$$Y = \frac{dC'_S}{dr'} \qquad (3\text{-}103)$$

The critical radius is defined as the radius where the substrate has been totally consumed ($C'_S = 0$). Physical and chemical constraints require the following boundary conditions:

At r'	=	R_c/R,	Y	= 0; substrate concentration gradient is zero at the critical radius (R_c).
At r'	=	1,	C'_S	= 1; substrate concentration equals C_{Sb} at the boundary of the sphere

We also must choose initial conditions for the solution of the set of equations [Eqs. (3-102) and (3-103)] such that the boundary conditions are met. These initial conditions are:

$$Y = 0 \text{ at } r' = R_c/R$$
$$C'_S = C'_{So} \text{ at } r' = R_c/R$$

C'_{So} is the substrate concentration at the critical radius. If all the substrate is not consumed before reaching the center of the sphere ($R_c = 0$), then $C'_{so} > 1$. Both C'_{So} and R_c are unknown. How can we obtain solutions to Eqs. (3-102) and (3-103)? We can choose a value of C'_{So} and calculate the companion value of R_c that satisfies the initial conditions. Or we can choose a value of R_c and calculate the C'_{So} that satisfies the initial conditions.

Solutions will be developed for both cases.

Case 1 Slow enzyme reaction rate

Rate of enzyme reaction is slow compared to rate of mass transfer (value of ϕ is low). As a result, the substrate reaches the center of the

immobilized enzyme particle. C'_S has some value greater than zero at r' = 0.

$$C'_{So} > 0 \text{ at } r' = 0 \ (R_c = 0)$$

In this case, $R_c = 0$. We make an initial guess of $C'_{So} = 0$ and integrate from r' = 0.001 to r' = 1. If the value of C'_S at the boundary is less than 1,C'_{So} is increased and the integration is performed again. This "trial and error" technique is continued until we choose a C'_{So} that gives $C'_S = 1$ at r' = 1.

A method for increasing C'_{So} (Lee, 1992) is

$$C'_{So}(\text{new}) = C'_{So}(\text{old}) - 0.5\,(C'_S - 1)$$

C'_S is the calculated substrate concentration at r' = 1. If $C'_{So} < 1$, then C'_{So}(new) will be greater than C'_{So}(old). The integration will be repeated with a larger initial value for the integration,

$$C'_S = C'_{So} + \int \frac{dC'_S}{dr'}\,dr'.$$

Case 2 Fast enzyme reaction rate

Rate of enzyme reaction is fast compared to rate of mass transfer. Substrate is consumed before it reaches the center of the sphere. At some critical radius (R_c), $C'_{So} = 0$. We do not know this critical radius. We choose a value for r' < 1, integrate beginning with $C'_{So} = 0$ and determine if C'_S= 1 at r' = 1. If $C'_S > 1$ at r' = 1, we increase the initial value of r' (point at which $C'_{So} = 0$), and repeat the integration until $C'_S = 1$ at r' = 1.

Modeling Example Problem 3.2—Immobilized Enzyme System, Solution for Case 1

Advanced Continuous Simulation Language (ACSL) is another software package for solving sets of differential equations. ACSL is written in Fortran, so those familiar with this language will be able to quickly learn how to use ACSL.

The ACSL program written to solve Case 1 is given in Tables 3.3 through 3.5.

Table 3.3. ACSL Program for Solution of Eqs. (3-102) and (3-103, Case 1.

```
PROGRAM PROB30 ACSL
      INTEGER          N
      CONSTANT         BETA=5. ,ERR=0.001, CSO=0.001
      CINTERVAL        CINT=0.001
      VARIABLE         R=0.001
INITIAL                        $  'NOTE N IS AN INTEGER'
   N=0
   10..N=N+1                   $'INCREMENT RUN COUNT'
```

```
END                                    $ 'END OF INITIAL'
DERIVATIVE
        CS=INTEG(DCSDR,CSO)
        DCSDR1=–2.*DCSDR/R+9.*PHI**2*CS/(1.+BETA*CS)
        DCSDR=INTEG(DCSDR1,0.0)
    TERMT(R.GE.1)
END                                    $ 'END OF DERIVATIVE'
TERMINAL
    IF(ABS(CS–1.).LT.ERR.OR.N.GT.10) GO TO 20
        CSO=CSO–0.5*(CS–1)
        GO TO 10
20..CONTINUE
        ETA=DCSDR/((3*PHI*PHI)/(1+BETA))
        WRITE(6,50) PHI,ETA,DCSDR,CSO,CS
50..FORMAT(_PHI=',F10.2,'ETA=',F10.3,'DCSDR=',F10.3,...
        'CSO=',F10.3,  ' CS=_,F10.3)
END                                    $ 'END OF TERMINAL'
END                                    $ 'END OF PROGRAM'
```

Table 3.4. Command file for ACSL Program for solution of Eqs. (3-102) and (3-103), Case 1.

```
    SET PHI=1.  —sets parameter values for this run
    PREPAR R,CS,  —these are the variables to be printed in the
listing file
    SET CMD =5  —returns control to the keyboard
```

If program is given in file PRB30A ACSL A, the listing file will be PRB30A LISTING A.

Table 3.5. EXEC file for ACSL Program for Solution of Eqs. (3-102) and (3-103), Case 1.

```
    EXEC ACSL CLG PRB30A
    (CLG = Means Compile, Load, Go)
    (PRB30A = Name of file that contains ACSL Program)
```

(After the program has been run once, this can be changed to LG meaning Load, Go. The compile time is saved.)

Variable names are as follows:

ϕ	=	PHI
β	=	BETA
C'_{So}	=	CSO
C'_{S}	=	CS
r'	=	R
dC'_S/dr'	=	DCSDR
η	=	ETA

In the ACSL program, note the statement VARIABLE R = 0.001. This statement informs the program that the integration is with

respect to R and that the initial condition (point at which integration begins) is at 0.001. The statement CINTERVAL CINT = 0.001 tells the program to use an integration step of 0.001.

The statement CS = INTEG(DCSDR, CSO) corresponds to

$$C'_S = C'_{So} + \int \frac{dC'_S}{dr'} dr'$$

Remembering that Y = dC'S/dr', the statement

DCSDR = INTEG(DCSDR1, 0.0)

corresponds to

$$= \int \left(-\frac{2}{r'} Y + 9\phi^2 \frac{C'_S}{1 + \beta C'_S} \right) dr'$$

The statement TERMT(R.GE.1) means stop the integration when $R \geq 1$. In the TERMINAL section, we check to see if $|C'_S - 1| < 0.001$ or if N>10. If not, we increment the initial value, C'_{So}, and return to the INITIAL section to repeat the integration. The statement WRITE(6,50) writes to the terminal screen.

If you run this program with $\phi = 1$, $\beta = 5$, you will find that it takes nine trials to obtain $C'_S = 1$ at $r' = 1$. The initial condition for the solution is $C'_{So} = 0.757$.

Modeling Problem 3.2—Immobilized Enzyme System, Case 1

This problem requires a MATLAB program to solve Eqs. (3-102) and (3-103). It is analogous to Modeling Example Problem 3.2. The same parameters are used.

$\beta = 5$
$\phi = 1$

It is appropriate to use $C'_{So} = 0.5$ as the initial guess for the first trial. Your program must be written to increase C'_{So} until the integration from $r' = 0$ to $r' = 1$ gives $C'_S = 1$ as a final value.

The simulation cannot be started at $r' = 0$ because Eq. (3-102) has division by r'. It is appropriate to begin at $r' = 0.001$.

It is recommended that $\Delta r' = 0.001$ be used, therefore, it takes 999 steps (intervals) to get to the boundary ($r' = 1$). The final C's (at $r' = 1$) should be within some error bound of $C'_S = 1$. For example, $(C'_S - 1) < err$ where err = 0.001.

1. For your simulation with $\phi = 1$, collect your results for Trials 1, 2, 3, 4, and "final" and plot C'_S vs. r' for these five trials on the same graph. If you use the $C'_{So}(new) = C'_{So}(old) - 0.5\ (C'_S$

– 1) procedure, you should guess the correct C'_{So} in less than 10 trials. Your graph should look like Figure 3.12.

2. Repeat the simulations using ϕ = 0.1, 0.5, and 2.0. Create a table as follows:

ϕ	η	C'_{So}(guess)	C'_{So}(actual)
0.1			
0.5			
1.0			
2.0			

Modeling Problem 3.3—Immobilized Enzyme System, Case 2

This problem requires a MATLAB program to solve Eqs. (3-102) and (3-103). For Case 2, the reaction rate is so fast that the substrate is consumed before it diffuses to the center of the sphere. We know $C'_{So} = 0$ at some critical radius, R_c. We have to guess the correct value of R_c.

The parameters are:

$\beta = 5$
$\phi = 5.0$

It is appropriate to use the following initial values

$C'_{So} = 0.001$
$r' = R_c/R = 0.32$

(Higher values of R_c will need to be used for the initial guess for higher values of ϕ.)

It is recommended that an integration interval of $\Delta r' = 0.001$ be used initially. The error bound for $(C'_S - 1) <$ err should be err = 0.02. Again, this error bound may need to be changed up or down for different values of ϕ.

1. Run your program for the following values ϕ = 5, 10, 20, and 50. Create a table as follows.

ϕ	η	R_c (guess)	R_c (actual)
5			
10			
20			
50			

2. Create a log-log plot of the effectiveness ratio (η) vs. Thiele's

Modulus (ϕ) using the Case 1 and Case 2 data (ϕ = 0.1 to ϕ = 50). Your plot should look like Figure 3.13. Discuss your results.

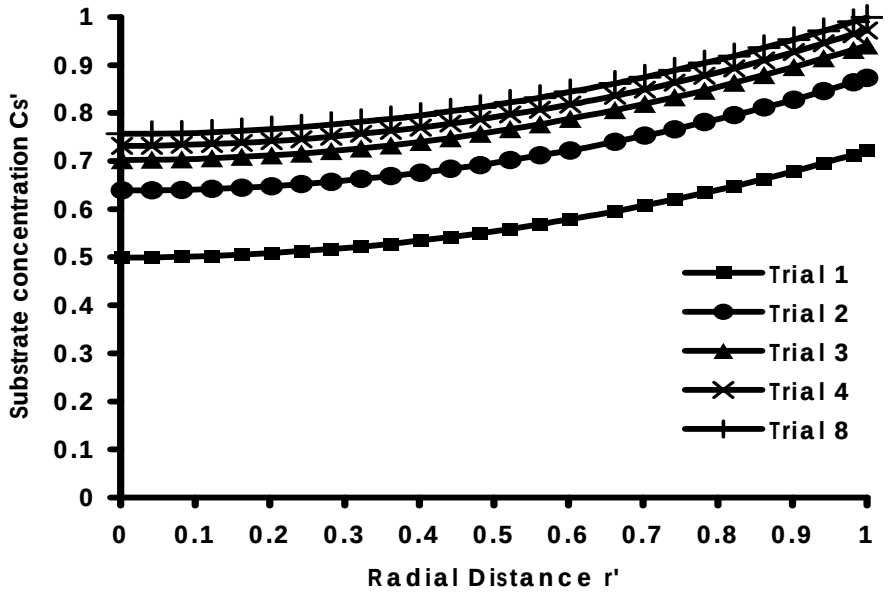


Figure 3.12—Solution for Modeling Problem 3.2 Case 1.

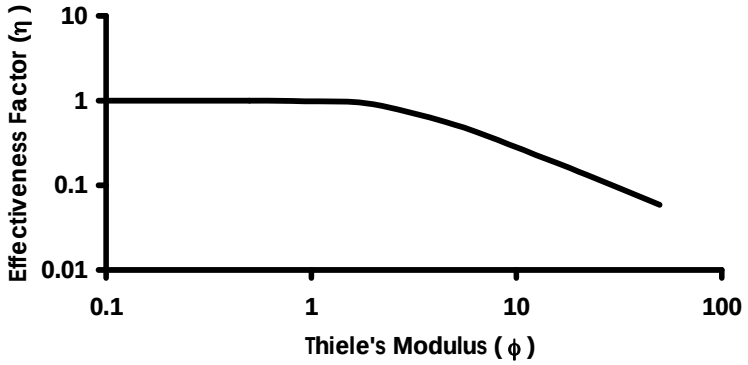


Figure 3.13—Plot of effectiveness factor (η) vs. Thiele's Modulus (ϕ) for β = 5, obtained using Case 1 and Case 2 solutions.

3.6 Using Monod Kinetics to Fit Biological Data

Analogous to the Michaelis-Menten equation for enzyme kinetics is the Monod equation, which is used extensively to model substrate-limited growth in both batch and continuous cultures. The Monod equation (Monod, 1949) is probably the most commonly applied model

of microbial growth, and has been widely used for modeling the bacterial systems used for wastewater treatment.

3.6.1 Monod Kinetic Model

The Monod equation has the same form as the Michaelis-Menten equation with slightly different nomenclature, as follows

$$\mu = \frac{\mu_{max}\ C_S}{C_S + K_S} \tag{3-104}$$

where μ = specific growth rate (T^{-1}),
μ_{max} = maximum specific growth rate (T^{-1}),
C_S = concentration of limiting substrate ($M\ L^{-3}$), and
K_S = Monod half-velocity constant (analogous to Michaelis-Menten constant) ($M\ L^{-3}$).

This equation generally holds only under conditions of balanced growth, not during lag or stationary phases (Figure 3.1), and should not be used when growth conditions are changing rapidly. Also, there may be difficulty applying the equation under conditions of very low substrate concentration.

Values of K_S often are low in many biological processes (Table 3.6). If substrate concentrations are more than 10 times K_S, which is often the case, then the reaction essentially is zero-order and independent of substrate concentration. It is only when substrate concentration in the batch process is depleted to values closer to K_S that specific growth rate μ declines below μ_{max}. At this point, large cellular mass and low substrate concentration may lead to a rapid decline in growth rate, which may lead to a stationary phase.

Table 3.6. K_S values for several organisms (Pirt, 1975; Wang et al., 1979).

Microorganism	Limiting Substrate	K_S (mg L^{-1})
Aspergillus	Glucose	5.0
Escherichia	Glucose	4.0
	Lactose	20
	Phosphate	1.6
Pseudomonas	Methanol	0.7
	Methane	0.4
Saccharomyces	Glucose	25

3.6.2 Determining Kinetic Rate Constants

In order to use the Michaelis-Menten or Monod equations, it is necessary to determine the K_m and r_{max} constants for a given reaction. Methods exist for measuring the maximum overall rate of reactions, r_{max}. This can be done by measuring rate of depletion of substrate or

rate of production of a product or a byproduct during the course of a reaction. For example, rate of organic substrate reduction can be measured in a wetland, rate of pharmaceutical production can be measured in a bioprocess system, or rate of CO_2 production can be measured in a fermentation tank. Production rates of the various constituents in a reaction can be related by the stoichiometry of the reaction, as discussed in Section 3.2.4, to yield the rate of product formation necessary for Michaelis-Menten or Monod analyses.

K_m must be determined as well. One method is to use experiments to determine k_1, k_2, and k_3, and then use Eq. (3-47) to determine K_m. To do this, it is necessary to measure fluxes between reactant phases in both directions, which is outside the realm of this text.

Another method commonly used in biological systems engineering is to determine K_m directly from experimental data. Product formation rates (dC_p/dt or r_p) and substrate concentrations (C_S) are measured immediately after the reaction begins, before any substrate limitation or product inhibition can occur. The initial substrate concentration (C_{S0}) is taken to be the C_S, and r_p is measured for the shortest practical time-step at the beginning of the reaction. This is repeated for a number of C_{S0} conditions. A curve of r_p vs. C_S is then created, as shown in Figure 3.14. The general rectangular hyperbola curve shape can be seen, but determining K_m and r_{max} directly from this curve typically is difficult or impossible.

Several methods have been developed for estimating these important parameters. Each method uses a different derivation of the basic Michaelis-Menten equation [Eq. (3-46)] to create a linear solution to this equation. With the linear transformation, a standard linear curve-fitting method can be used (such as those found on most spreadsheet programs) to determine the K_m and r_{max} parameters.

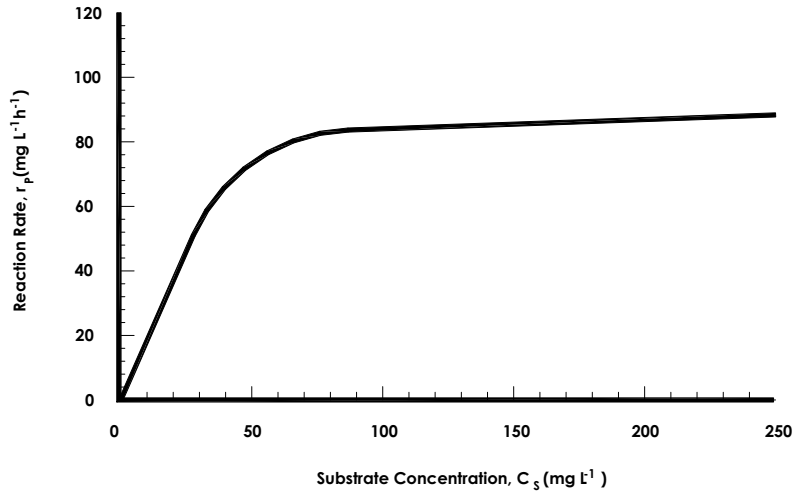


Figure 3.14—Michaelis-Menten (rectangular hyperbola) relationship between reaction rate and substrate concentration.

Lineweaver-Burke Method

The Lineweaver-Burke method transforms Eq. (3-46) into the following form:

$$\frac{1}{r_P} = \frac{K_m + C_S}{r_{max}\ C_S} = \frac{K_m}{r_{max}}\frac{1}{C_S} + \frac{1}{r_{max}} \qquad (3\text{-}105)$$

This equation has the form y = a x + b, where a = K_m/r_{max} and b = $1/r_{max}$. Thus, a plot of $1/r_p$ vs. $1/C_S$ would have y-intercept $1/r_{max}$ and slope K_m/r_{max}. In this equation, low concentrations of S influence the line more than high concentrations. However, you should also realize that reaction rates are highest at high substrate concentrations. With most measurement methods, it is easier and more accurate to measure higher reaction rates. Typically, instruments have a given measurement error, so a higher measured value translates into a smaller error expressed as a percentage of the measured value. Because the inverse of C_S is plotted against the inverse of r_p, the most-accurately known rate values (at high C_S) are clustered near the origin. This can be seen in Example 3.7.

Eadie-Hofstee Method

The Eadie-Hofstee method transforms Eq. (3-46) into the following form:

$$r_P\left(K_m + C_S\right) = r_{max}\ C_S$$

$$K_m\frac{r_P}{C_S} + r_P = r_{max}$$

$$r_P = r_{max} - K_m\frac{r_P}{C_S} \qquad (3\text{-}106)$$

Again, this linear equation greatly simplifies determination of K_m and r_{max}. A linear best-fit line through the data points of a plot of r_p vs. r_p/C_S yields a y-intercept of r_{max} and a slope of $-K_m$. This method has less bias toward points at low C_S. However, both x and y coordinates contain the parameter r_p, which is more difficult to measure than C_S; thus, this method hinges on accurate measurement of r_p.

Hanes-Woolf Method

The Hanes-Woolf method transforms Eq. (3-46) into the following form:

$$\frac{C_S}{r_P} = \frac{K_m}{r_{max}} + \frac{1}{r_{max}}C_S \qquad (3\text{-}107)$$

So, a linear fit of an x-y plot of C_S/r_p vs. C_S for a given data set produces a line with intercept of K_m/r_{max} and slope $1/r_{max}$. This method has more bias toward the more-accurately measured high C_S values. Because of this, some researchers consider the Hanes-Woolf determination of r_{max} (from the slope) to be the most accurate. Differences between the three methods can be seen in Example 3.7.

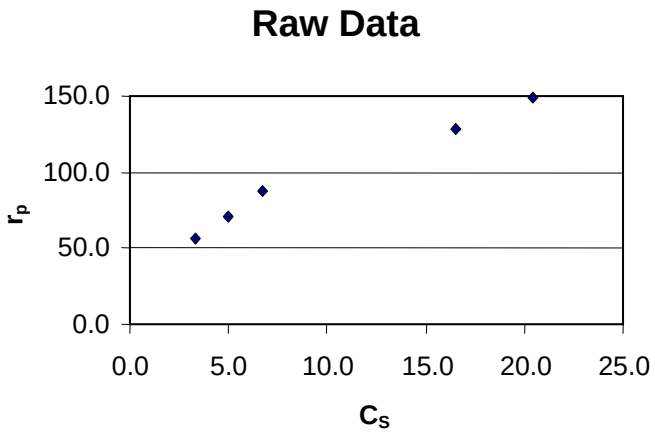
EXAMPLE 3.7

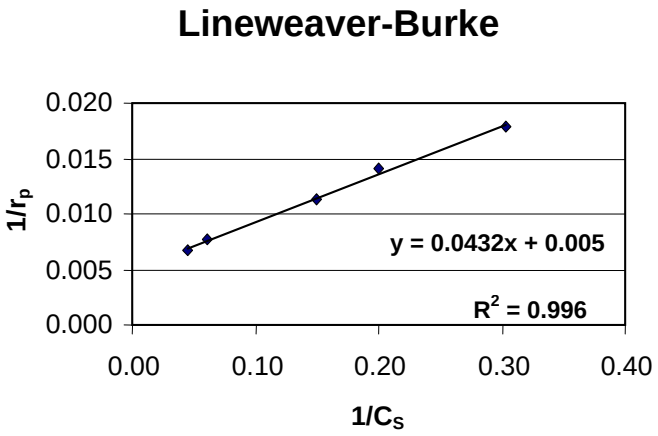
Find r_{max} and K_m using each of the three methods presented in Section 3.6.2. Use the following data of initial reaction rates for various substrate concentrations.

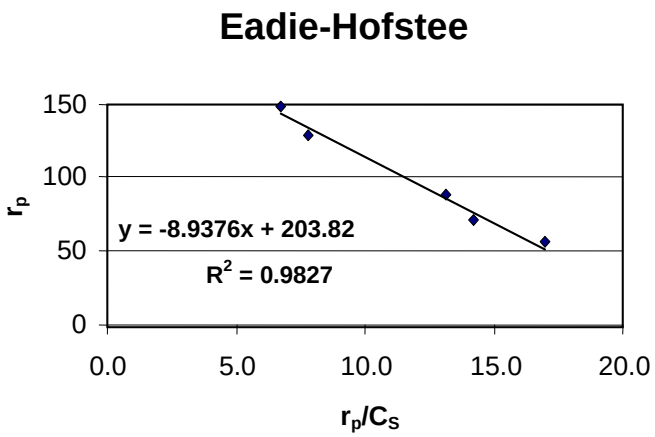
C_S (mg L^{-1})	r_p (mg L^{-1} min^{-1})
3.3	56
5	71
6.7	88
16.5	129
22.1	149

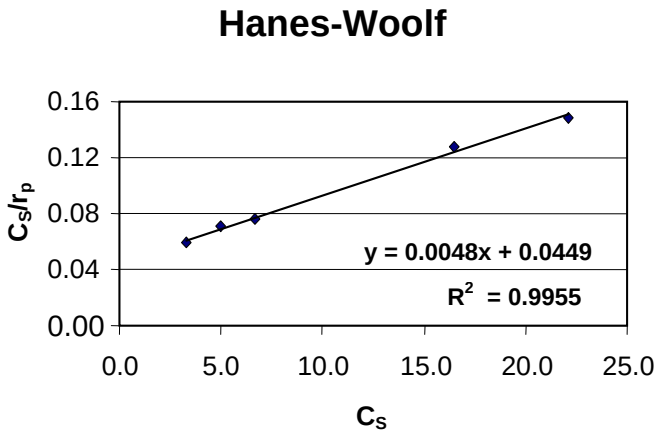
Solution

Raw Data		Lineweaver-Burke		Eadie-Hofstee		Hanes-Woolf	
C_S	r_p	$1/C_S$	$1/r_p$	r_p/C_S	r_p	C_S	C_S/r_p
3.3	56	0.3030	0.0179	16.9697	56.0	3.3	0.06
5	71	0.2000	0.0141	14.2000	71.0	5.0	0.07
6.7	88	0.1493	0.0114	13.1343	88.0	6.7	0.08
16.5	129	0.0606	0.0078	7.8182	129.0	16.5	0.13
22.1	149	0.0452	0.0067	6.7421	149.0	22.1	0.15









Lineweaver-Burke Method

y-intercept: $1/r_{max} = 0.005 \rightarrow r_{max} = 200$ mg L^{-1} min^{-1}
slope: $K_m/r_{max} = 0.0432 \rightarrow K_m = 8.64$ mg L^{-1}

Eadie-Hofstee Method

y-intercept: $r_{max} = 203.82 \rightarrow r_{max} = 204$ mg L^{-1} min^{-1}
slope: $-K_m = -8.9376 \rightarrow K_m = 8.94$ mg L^{-1}

Hanes-Woolf Method

slope: $1/r_{max} = 0.0048 \rightarrow r_{max} = 208$ mg L^{-1} min^{-1}
y-intercept: $K_m/r_{max} = 0.0449 \rightarrow K_m = 9.35$ mg L^{-1}

PROBLEM 3.7

Find r_{max} and K_m using each of the three methods presented in Section 3.6.2. Use the following data of initial rates of an enzyme-catalyzed reaction for various substrate concentrations.

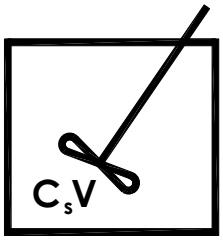
C_S (mol $L^{-1} \times 10^{-5}$)	r_p (mol L^{-1} $min^{-1} \times 10^{-4}$)
410	1.77
95.0	1.73
52.0	1.25
10.3	1.06
4.90	0.80
1.06	0.67
0.51	0.43

3.7 Microbial System Reactors

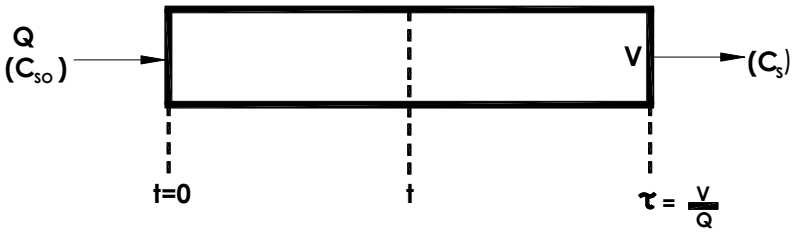
3.7.1 Batch Reactor

An ideal batch reactor is so well mixed that the contents can be assumed to be uniform at all times. The pH is maintained by introducing a buffer solution using a pH controller. Once the reaction has consumed the substrate or reached some predefined end-point, the reactor is emptied.

A schematic of a batch reactor is shown in Figure 3.15(a). The batch reactor has a detention time t equal to the time the contents remain in the reactor.



(a)



(b)

Figure 3.15—Schematic diagrams for (a) batch and (b) steady-state plug-flow reactors.

If the reaction in a batch reactor is described by the Michaelis-Menten equation [Eq. (3-46)],

$$r_P = \frac{dC_P}{dt} = -\frac{dC_S}{dt} = \frac{r_{max}\ C_S}{K_m + C_S}$$

then it can be solved analytically as follows. Rearranging Eq. (3-46) yields

$$-K_m \frac{dC_S}{C_S} - dC_S = r_{max}\, dt \tag{3-108}$$

Integrating and rearranging gives the solution

$$t = \left(\frac{C_{S0} - C_S}{r_{max}}\right) + \left(\frac{K_m}{r_{max}}\right) \ln \frac{C_{S0}}{C_S} \tag{3-109}$$

where C_{S0} = C_S at time t = 0.

The form of this equation is linear (y = a + b x), indicating that a plot of $\ln(C_{S0}/C_S)$ vs. t would produce a line with slope K_m/r_{max} and intercept $(C_{S0}–C_S)/r_{max}$. This form of the Michaelis-Menten equation is applicable for analyzing substrate concentration data taken at a number of times during a batch-reactor process, as long as the accumulation of product is small and does not inhibit the reaction.

3.7.2 Steady-state Plug-flow Reactor

In an enzyme-catalyzed plug-flow reactor, the substrate enters one end of a cylindrical tube that often is packed with immobilized enzyme, and a combination of unused substrate and product leaves the other end. Because there is no stirring device, the properties of the flowing stream vary in both the radial and longitudinal direction. However, variation of the radial direction is much smaller than variation in the longitudinal direction. If a plug-flow reactor is operated at steady state, the properties at any point within the reactor are constant with respect to time.

A schematic of a plug-flow reactor is shown in Figure 3.15b. In the plug-flow reactor, the reaction time is the dwell time τ.

$$\tau = \frac{V}{Q} \tag{3-110}$$

where τ = dwell time (T),
V = volume of the reactor (L^3), and
Q = volume flow rate ($L^3\ T^{-1}$).

Continuous operation of a reactor can increase reactor productivity significantly. The reactor does not have to be shut down, emptied, and sterilized between batches. As with the stirred batch reactor, analysis is based on the assumption that the contents are well mixed.

The substrate balance of a steady-state plug-flow reactor is

$$\text{Input} - \text{Output} + \text{Generation} = \text{Accumulation}$$

In equation form,

$$Q\,C_{S0} - Q\,C_S + r_S\,V = \frac{V\,dC_S}{dt} \tag{3-111}$$

where Q = volume flow rate ($L^3\,T^{-1}$),
V = volume of reactor (L^3),
r_S = rate of substrate consumption for the enzymatic reaction ($M\,L^{-3}\,T^{-1}$), and
dC_S/dt = rate of change of substrate concentration in the reactor ($M\,L^{-3}\,T^{-1}$).

When Q is zero, r_S is equal to dC_S/dt, which is the case for batch operation.

If the plug-flow reactor is operating at steady state, the substrate concentration in the reactor is constant. Therefore, $dC_S/dt = 0$. If the Michaelis-Menten equation is substituted for r_S, then Eq. (3-111) becomes

$$Q\,C_{S0} - Q\,C_S - \frac{r_{max}\,C_S\,V}{K_m + C_S} = 0$$

or

$$\frac{Q}{V} = \frac{r_{max}\,C_S}{(C_{S0} - C_S)(K_m + C_S)} \tag{3-112}$$

The dwell time τ is related to flow and volume as shown in Eq. (110):

$$V = Q\,\tau \quad \ldots\text{or}\ldots \quad \tau = \frac{V}{Q}$$

The reciprocal of the dwell time (or residence time) is known as the dilution rate.

$$D = \frac{1}{\tau} \tag{3-113}$$

where D = dilution rate (T^{-1}).

Substituting $\frac{1}{\tau} = \frac{Q}{V}$ into Eq. (3-112) and rearranging,

$$C_S = -K_m + \frac{r_{max}\, C_S\, \tau}{C_{S0} - C_S} \qquad (3\text{-}114)$$

The Michaelis-Menten parameters, r_{max} and K_m, can be estimated by running a series of steady-state plug-flow runs with different flow rates and plotting C_S vs. $C_S\,\tau/(C_{S0}-C_S)$. However, it is more difficult to run steady-state plug-flow runs than batch runs, so the initial rate approach, previously described, is a better method.

Modeling Example Problem 3.3—Michaelis-Menten Equation Numerical Solutions

This problem was taken from Lee (1992). A carbohydrate (S) decomposes in the presence of an enzyme (E). Using experimental data, the Michaelis-Menten kinetic parameters were determined to be $K_m = 200$ mol m^{-3} and $r_{max} = 100$ mol m^{-3} min^{-1}.

1. a) Write a MATLAB program that uses rectangular integration to calculate substrate concentration over time in a batch reactor. Assume that the initial concentration is $C_{S0} = 300$ mol m^{-3}.
   b) Use Eq. (3-109), the analytical solution of Eq. (3-46), to obtain data to plot a C_S vs. t curve.
   c) Plot this C_S vs. t curve on the same graph as the curve obtained with rectangular integration.
2. Repeat Step 1 using DESIRE to obtain the solution to the differential equation.
3. Assume that the C_S vs. t curve from Step 1 was obtained experimentally. Estimate K_m and r_{max} by plotting the $(C_{S0}-C_S)/\ln(C_{S0}-C_S)$ vs. $t/\ln(C_{S0}-C_S)$ curve. Is this approach reliable for the estimation of K_m and r_{max}?

The solution to Step 1 is plotted in Figure 3.16 and the tabular data given in Table 3.7. The time step used for the rectangular integration in the MATLAB program was 1 sec.

The solution to (Step 2) is given in Table 3.8. The DESIRE program using Runge-Kutta fourth-order numerical integration gives a very accurate solution. Comparison with Table 3.7 shows that the accuracy is equivalent to the analytical solution.

The student should now have confidence in the use of DESIRE to solve a non-linear differential equation. Writing the short MATLAB program to solve the differential equation and then comparing the results should have expanded understanding of the DESIRE program.

The plot of $(C_{S0}-C_S)/\ln(C_{S0}/C_S)$ vs. $t/\ln(C_{S0}/C_S)$ is given in Figure 3.17. The slope is 99.47, which compares with $r_{max} = 100$. The intercept is 200, which compares with $K_m = 200$.

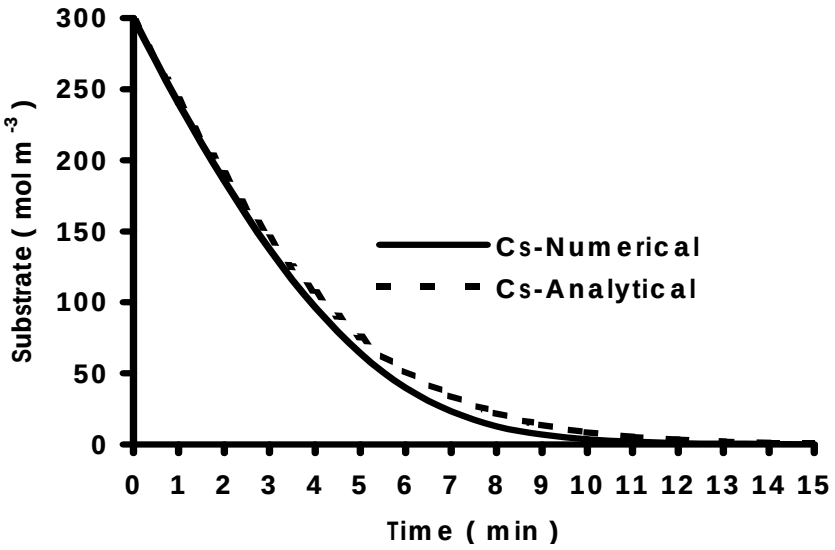


Figure 3.16—Graphical comparison of analytical and numerical solution for Modeling Example Problem 3.3 (Step 1).

Table 3.7. Solution to Modeling Example Problem 3.3 (Step 1).

Time (sec)	Cs Obtained with Numerical Integration (mol m^{-3})	Cs Analytical (mol m^{-3})
0	300.0	300.0
1	240.0	242.5
2	185.5	190.7
3	137.3	145.2
4	96.6	106.7
5	64.1	75.6
6	39.8	51.7
7	23.2	34.2
8	12.8	22.1
9	6.8	13.9
10	3.5	8.7
11	1.8	5.3
12	0.9	3.3
13	0.5	2.0
14	0.2	1.2
15	0.1	0.7

Table 3.8. Solution to Modeling Example Problem 3.3 (Step 2).

Time (sec)	C_S (mol m^{-3}) using MATLAB			C_S (mol m^{-3})
	$\Delta t = 0.1$	$\Delta t = 0.5$	$\Delta t = 1.0$	DESIRE
0	300.0	300.0	300.0	300.0
1	242.3	241.3	240.0	242.5
2	190.2	188.1	185.5	190.6
3	144.4	141.3	137.3	145.1
4	105.8	101.8	96.6	106.7
5	74.5	70.6	64.1	75.6
6	51.6	46.0	39.8	51.7
7	33.2	28.9	23.2	34.2
8	21.2	17.5	12.8	22.0
9	13.2	10.3	6.8	13.9
10	8.1	6.0	3.5	8.6
11	4.9	3.4	1.8	5.3
12	3.0	1.9	0.9	3.2
13	1.8	1.1	0.5	2.0
14	1.1	0.6	0.2	1.2
15	0.7	0.3	0.1	0.7

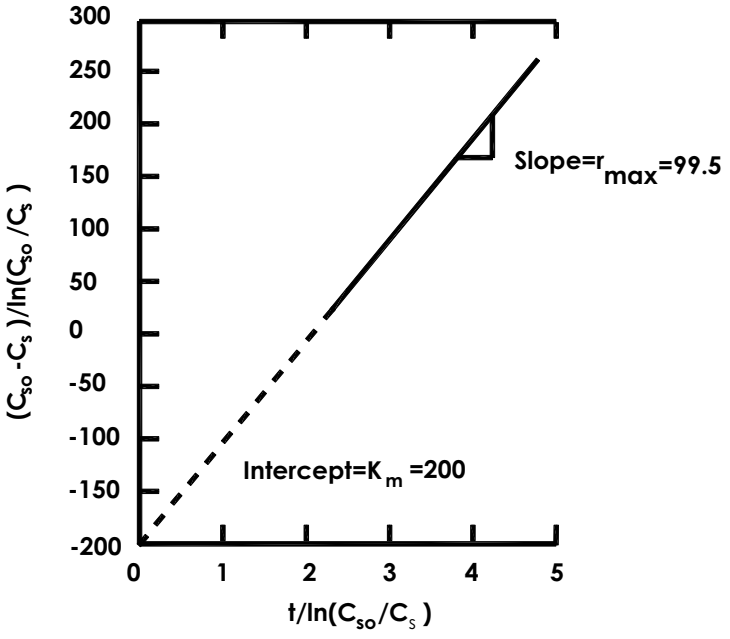


Figure 3.17—Plot to obtain slope and intercept of concentration data obtained for Modeling Example Problem 3.3 (Step 3).

3.8 Summary

Wetlands, compost piles, fermentation vessels, wastewater treatment reactors, anaerobic digesters, bioprocess operations, bioremediation of contaminated soils, and many other (in fact most) biological systems require the effective use of microorganisms. These organisms are critical to the chain of reactions that interconnect plants, animals, soil, water, and air throughout the biosphere. Principles learned in this chapter will be reinforced in Chapter 4 for microbial compost systems, Chapter 5 for plants, and Chapter 7 for animals.

Flow of Gibbs energy throughout the biosphere is enormous. Because this energy is diffuse, its magnitude is not appreciated in the same way as, say, a nuclear reaction. *When we as human beings learn to organize our activities such that we benefit from, rather than struggle against, the enormous biological potential around us, we become a positive force in the biosphere.* The basic premise of biological systems engineering as a discipline is the quantification of the biological potential (energy) in our surroundings. Applying mathematics to analyze the activity of microorganisms is a key first step to the analysis of higher-order organisms presented in later chapters of this text.

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Suggested Additional Reading

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