# Mathematical modeling of bacterial growth curve and fermentation

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Chapter 3

# Bacterial Growth

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Bacterial growth is a complex process involving numerous anabolic (synthesis of cell constituents and metabolites) and catabolic (breakdown of cell constituents and metabolites) reactions. Ultimately, these biosynthetic reactions result in cell division as shown in Figure 3.1. In a homogeneous rich culture medium, under ideal conditions, a cell can divide in as little as 10 minutes. In contrast, it has been suggested that cell division may occur as slowly as once every 100 years in some subsurface terrestrial environments. Such slow growth is the result of a combination of factors including the fact that most subsurface environments are both nutrient poor and heterogeneous. As a result, cells are likely to be isolated, cannot share nutrients or protection mechanisms, and therefore never achieve a metabolic state that is efficient enough to allow exponential growth.

Most information available concerning the growth of microorganisms is the result of controlled laboratory studies using pure cultures of microorganisms. There are two approaches to the study of growth under such controlled conditions: batch culture and continuous culture. In a batch culture the growth of a single organism or a group of organisms, called a consortium, is evaluated using a defined medium to which a fixed amount of substrate (food) is added at the outset. In continuous culture there is a steady influx of growth medium and substrate such that the amount of available substrate remains the same. Growth under both batch and continuous culture conditions has been well characterized physiologically and also described mathematically. This information has been used to optimize the commercial production of a variety of microbial products including antibiotics, vitamins, amino acids, enzymes, yeast, vinegar, and alcoholic beverages. These materials are often produced in large batches (up to 500,000 liters) also called large-scale fermentations.

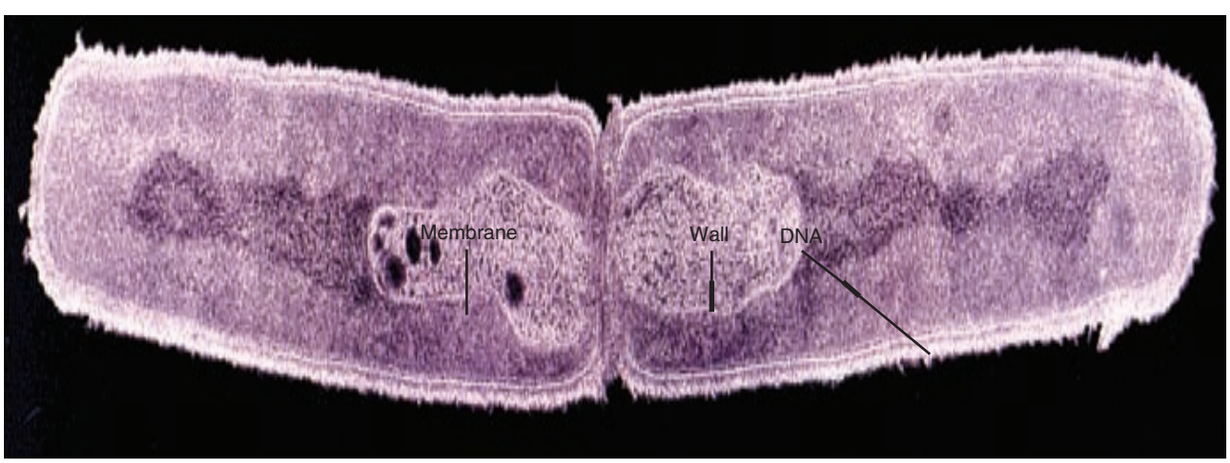



**FIGURE 3.1** Electron micrograph of *Bacillus subtilis*, a gram-positive bacterium, dividing. Magnification 31,200×. Reprinted with permission from Madigan *et al*., 1997.

Unfortunately, it is difficult to extend our knowledge of growth under controlled laboratory conditions to an understanding of growth in natural soil or water environments, where enhanced levels of complexity are encountered (Fig. 3.2). This complexity arises from a number of factors, including an array of different types of solid surfaces, microenvironments that have altered physical and chemical properties, a limited nutrient status, and consortia of different microorganisms all competing for the same limited nutrient supply (see Chapter 4). Thus, the current challenge facing environmental microbiologists is to understand microbial growth in natural environments. Such an understanding would facilitate our ability to predict rates of nutrient cycling (Chapter 14), microbial response to anthropogenic perturbation of the environment (Chapter 17), microbial interaction with organic and metal contaminants (Chapters 20 and 21), and survival and growth of pathogens in the environment (Chapters 22 and 27). In this chapter, we begin with a review of growth under pure culture conditions and then discuss how this is related to growth in the environment.

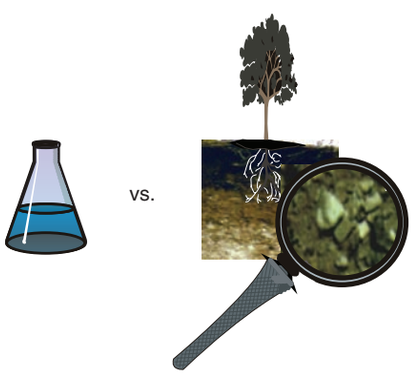



**FIGURE 3.2** Compare the complexity of growth in a flask and growth in a soil environment. Although we understand growth in a flask quite well, we still cannot always predict growth in the environment.

## 3.1 GROWTH IN PURE CULTURE IN A FLASK

Typically, to understand and define the growth of a particular microbial isolate, cells are placed in a liquid medium in which the nutrients and environmental conditions are controlled. If the medium supplies all nutrients required for growth and environmental parameters are optimal, the increase in numbers or bacterial mass can be measured as a function of time to obtain a growth curve. Several distinct growth phases can be observed within a growth curve (Fig. 3.3). These include the lag phase, the exponential or log phase, the stationary phase, and the death phase. Each of these phases represents a distinct period of growth that is associated with typical physiological changes in the cell culture. As will be seen in the following sections, the rates of growth associated with each phase are quite different.

### 3.1.1 The Lag Phase

The first phase observed under batch conditions is the lag phase in which the growth rate is essentially zero. When an inoculum is placed into fresh medium, growth begins after a period of time called the lag phase. The lag phase is defined to transition to the exponential phase after the initial population has doubled (Yates and Smotzer, 2007). The lag phase is thought to be due to the physiological adaptation of the cell to the culture conditions. This may involve a time requirement for induction of specific messenger RNA (mRNA) and protein synthesis to meet new culture requirements. The lag phase may also be due to low initial densities of organisms that result in dilution of exoenzymes (enzymes released from the cell) and of nutrients that leak from growing cells. Normally, such materials are shared by cells in close proximity. But when cell density is low, these materials are diluted and not as easily taken up. As a result, initiation of cell growth and division and the transition to exponential phase may be slowed.

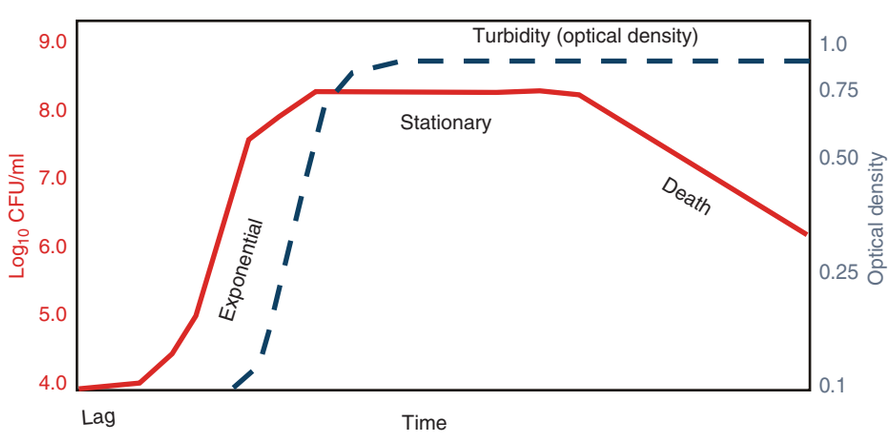



**FIGURE 3.3** A typical growth curve for a bacterial population. Compare the difference in the shape of the curves in the death phase (colony-forming units versus optical density).

The lag phase usually lasts from minutes to several hours. The length of the lag phase can be controlled to some extent because it is dependent on the type of medium as well as on the initial inoculum size. For example, if an inoculum is taken from an exponential phase culture in trypticase soy broth (TSB) and is placed into fresh TSB medium at a concentration of $10^6$ cells/ml under the same growth conditions (temperature, shaking speed), there will be no noticeable lag phase. If the inoculum is taken from a stationary phase culture, however, there will be a lag phase as the stationary phase cells adjust to the new conditions and shift physiologically from stationary phase cells to exponential phase cells. Similarly, if the inoculum is placed into a medium other than TSB, for example, a mineral salts medium with glucose as the sole carbon source, a lag phase will be observed while the cells reorganize and shift physiologically to synthesize the appropriate enzymes for glucose catabolism.

Finally, if the inoculum size is small, for example, $10^4$ cells/ml, and one is measuring activity, such as disappearance of substrate, a lag phase will be observed until the population reaches approximately $10^6$ cells/ml. This is illustrated in Figure 3.4, which compares the degradation of phenanthrene in cultures inoculated with $10^7$ and with $10^4$ colony-forming units (CFU) per milliliter. Although the degradation rate achieved is similar in both cases (compare the slope of each curve), the lag phase was 1.5 days when a low inoculum size was used ($10^4$ CFU/ml) in contrast to only 0.5 day when the higher inoculum was used ($10^7$ CFU/ml).

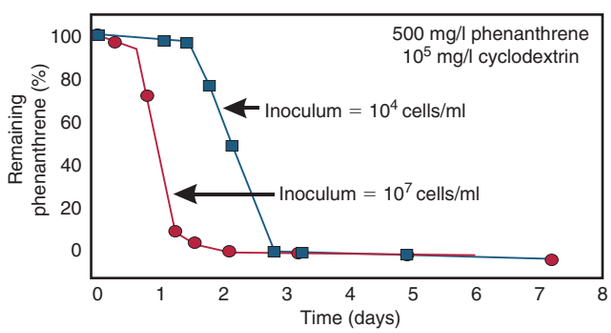



**FIGURE 3.4** Effect of inoculum size on the lag phase during degradation of a polyaromatic hydrocarbon, phenanthrene. Because phenanthrene is only slightly soluble in water and is therefore not readily available for cell uptake and degradation, a solubilizing agent called cyclodextrin was added to the system. The microbes in this study were not able to utilize cyclodextrin as a source of carbon or energy. Courtesy E. M. Marlowe.

## 3.1.2 The Exponential Phase

The second phase of growth observed in a batch system is the exponential phase. The exponential phase is characterized by a period of the exponential growth—the most rapid growth possible under the conditions present in the batch system. During exponential growth the rate of increase of cells in the culture is proportional to the number of cells present at any particular time. There are several ways to express this concept both theoretically and mathematically. One way is to imagine that during exponential growth the number of cells increases in the geometric progression $2^0$, $2^1$, $2^2$, $2^3$ until, after $n$ divisions, the number of cells is $2^n$ (Fig. 3.5).

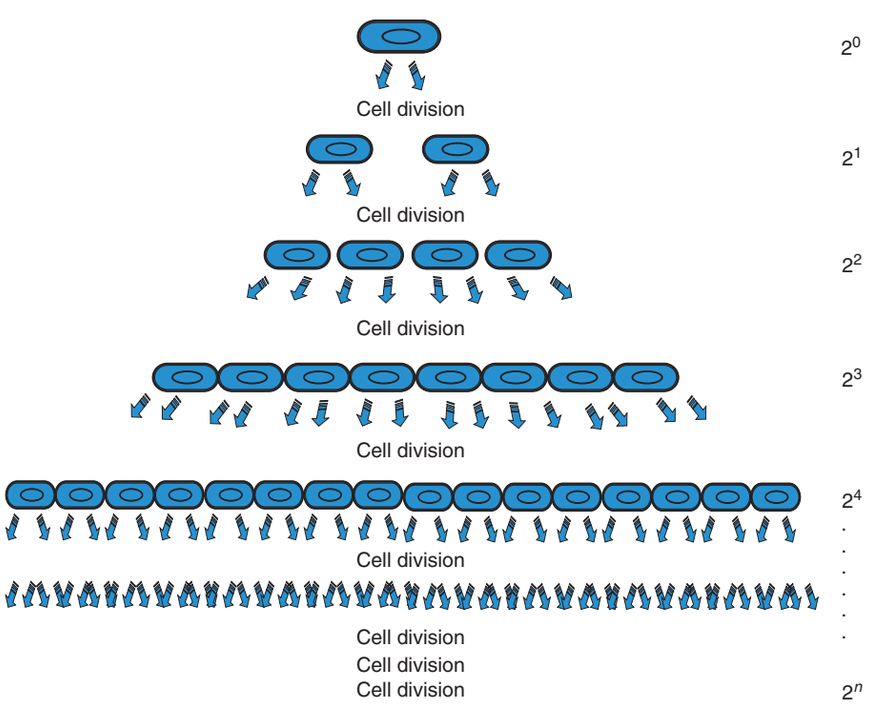



**FIGURE 3.5** Exponential cell division. Each cell division results in a doubling of the cell number. At low cell numbers the increase is not very large; however, after a few generations, cell numbers increase explosively.

**Example Calculation 3.1 Generation Time**

*Problem*: If one starts with 10,000 ($10^4$) cells in a culture that has a generation time of 2 h, how many cells will be in the culture after 4, 24, and 48 h?

Use the equation $X = 2^n X_0$, where $X_0$ is the initial number of cells, $n$ is the number of generations, and X is the number of cells after $n$ generations.

After 4 h, $n$ = 4 h/2 h per generation = 2 generations:

$$X = 2^2(10^4) = 4.0 \times 10^4 \text{ cells}$$

After 24 h, $n$ = 12 generations:

$$X = 2^{12}(10^4) = 4.1 \times 10^7 \text{ cells}$$

After 48 h, $n$ = 24 generations:

$$X = 2^{24}(10^4) = 1.7 \times 10^{11}$$

This represents an increase of less than one order of magnitude for the 4-h culture, four orders of magnitude for the 24-h culture, and seven orders of magnitude for the 48-h culture!

This can be expressed in a quantitative manner; for example, if the initial cell number is $X_0$, the number of cells after $n$ doublings is $2^n X_0$ (see Example Calculation 3.1). As can be seen from this example, if one starts with a low number of cells exponential growth does not initially produce large numbers of new cells. However, as cells accumulate after several generations, the number of new cells with each division begins to increase explosively.

In the example just given, $X_0$ was used to represent cell number. However, $X_0$ can also be used to represent cell mass, which is often more convenient to measure than cell number (see Chapters 10 and 11). Whether one expresses $X_0$ in terms of cell number or in terms of cell mass, one can mathematically describe cell growth during the exponential phase using the following equation:

$$\frac{dX}{dt} = \mu X \qquad \text{(Eq. 3.1)}$$

where $X$ is the number or mass of cells (mass/volume), $t$ is time, and $\mu$ is the specific growth rate constant (1/time). The time it takes for a cell division to occur is called the generation time or the doubling time. Equation 3.1 can be used to calculate the generation time as well as the specific growth rate using data generated from a growth curve such as that shown in Figure 3.3.

The generation time for a microorganism is calculated from the linear portion of a semilog plot of growth versus time. The mathematical expression for this portion of the growth curve is given by Eq. 3.1, which can be rearranged and solved as shown in Eqs. 3.2 to 3.6 to determine the generation time (see Example Calculation 3.2):

$$\frac{dX}{dt} = \mu X \qquad \text{(Eq. 3.1)}$$

Rearrange:

$$\frac{dX}{X} = \mu dt \qquad \text{(Eq. 3.2)}$$

Integrate:

$$\int_{X_0}^{X} \frac{dX}{X} = \mu \int_0^t dt \qquad \text{(Eq. 3.3)}$$

$$\ln X = \mu t + \ln X_0 \quad \text{or} \quad X = X_0 e^{\mu t} \qquad \text{(Eq. 3.4)}$$

For $X$ to be doubled:

$$\frac{X}{X_0} = 2 \qquad \text{(Eq. 3.5)}$$

Therefore:

$$2 = e^{\mu t} \qquad \text{(Eq. 3.6)}$$

where $t$ = generation time.

### 3.1.3 The Stationary Phase

The third phase of growth is the stationary phase. The stationary phase in a batch culture can be defined as a state of no net growth, which can be expressed by the following equation:

$$\frac{dX}{dt} = 0 \qquad \text{(Eq. 3.7)}$$

Although there is no net growth in stationary phase, cells still grow and divide. Growth is simply balanced by an equal number of cells dying.

There are several reasons why a batch culture may reach stationary phase. One common reason is that the carbon and energy source or an essential nutrient becomes completely used up. When a carbon source is used up it does not necessarily mean that all growth stops. This is because dying cells can lyse and provide a source of nutrients. Growth on dead cells is called endogenous metabolism. Endogenous metabolism occurs throughout the growth cycle, but it can be best observed during stationary phase when growth is measured in terms of oxygen uptake or evolution of carbon dioxide. Thus, in many growth curves such as that shown in Figure 3.6, the stationary phase actually shows a small amount of growth. Again, this growth

**Example Calculation 3.2 Specific Growth Rate**

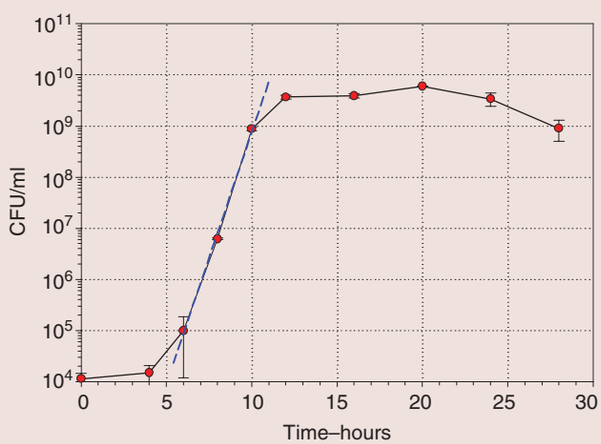
*Problem*: The following data were collected using a culture of P*seudomonas* during growth in a minimal medium containing salicylate as a sole source of carbon and energy. Using these data, calculate the specific growth rate for the exponential phase.

| Time (h) | Culturable cell count (CFU/ml) |
|---|---|
| 0 | $1.2 \times 10^4$ |
| 4 | $1.5 \times 10^4$ |
| 6 | $1.0 \times 10^5$ |
| 8 | $6.2 \times 10^6$ |
| 10 | $8.8 \times 10^8$ |
| 12 | $3.7 \times 10^9$ |
| 16 | $3.9 \times 10^9$ |
| 20 | $6.1 \times 10^9$ |
| 24 | $3.4 \times 10^9$ |
| 28 | $9.2 \times 10^8$ |

The times to be used to determine the specific growth rate can be chosen by visual examination of a semilog plot of the data (see figure). Examination of the graph shows that the exponential phase is from approximately 6 to 8 hours. Using Eq. 3.4, which describes the exponential phase of the graph, one can determine the specific growth rate for this P*seudomonas*. (Note that Eq. 3.4 describes a line, the slope of which is $\mu$, the specific growth rate.) From the data given, the slope of the graph from time 6 to 10 hours is:

$$\mu = (\ln 1 \times 10^9 - \ln 1 \times 10^5)/(10 - 6) = 2.31/\text{h}$$

It should be noted that the specific growth rate and generation time calculated for growth of the P*seudomonas* on salicylate are valid only under the experimental conditions used. For example, if the experiment were performed at a higher temperature, one would expect the specific growth rate to increase. At a lower temperature, the specific growth rate would be expected to decrease.




occurs after the substrate has been utilized and reflects the use of dead cells as a source of carbon and energy. A second reason that stationary phase may be observed is that waste products build up to a point where they begin to inhibit cell growth or are toxic to cells. This generally occurs only in cultures with high cell density. Regardless of the reason why cells enter stationary phase, growth in the stationary phase is unbalanced because it is easier for the cells to synthesize some components than others. As some components become more and more limiting, cells will still keep growing and dividing as long as possible. As a result of this nutrient stress, stationary phase cells are generally smaller and rounder than cells in the exponential phase (see Section 2.2.2).

### 3.1.4 The Death Phase

The final phase of the growth curve is the death phase, which is characterized by a net loss of culturable cells. Even in the death phase there may be individual cells that are metabolizing and dividing, but more viable cells are

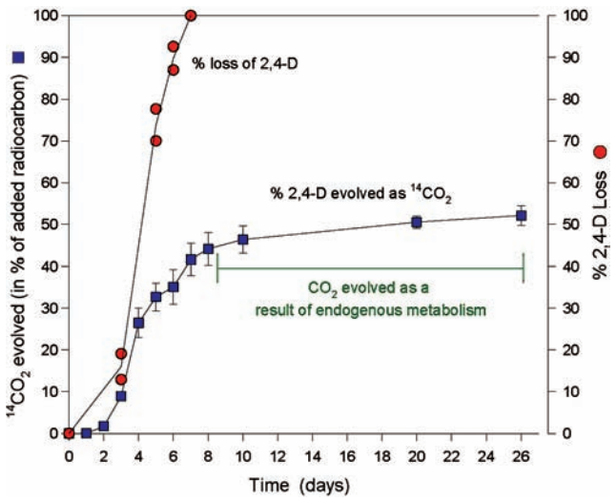



**FIGURE 3.6** Mineralization of the broadleaf herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) in a soil slurry under batch conditions. Note that the 2,4-D is completely utilized after 6 days but the $CO_2$ evolved continues to rise slowly. This is a result of endogenous metabolism. From Estrella *et al*., 1993.

lost than are gained so there is a net loss of viable cells. The death phase is often exponential, although the rate of cell death is usually slower than the rate of growth during the exponential phase. The death phase can be described by the following equation:

$$\frac{dX}{dt} = -k_d X \qquad \text{(Eq. 3.8)}$$

where $k_d$ is the specific death rate.

It should be noted that the way in which cell growth is measured can influence the shape of the growth curve. For example, if growth is measured by optical density instead of by plate counts (compare the two curves in Fig. 3.3), the onset of the death phase is not readily apparent. Similarly, if one examines the growth curve measured in terms of carbon dioxide evolution shown in Figure 3.6, again it is not possible to discern the death phase. Still, these are commonly used approaches to measurement of growth because normally the growth phases of most interest to environmental microbiologists are the lag phase, the exponential phase, and the time to onset of the stationary phase.

### 3.1.5 Effect of Substrate Concentration on Growth

So far we have discussed each of the growth phases and have shown that each phase can be described mathematically (see Eqs. 3.1, 3.7, and 3.8). One can also write equations to allow description of the entire growth curve. Such equations become increasingly complex. For example, one of the first and simplest descriptions is the Monod equation, which was developed by Jacques Monod in the 1940s:

$$\mu = \frac{\mu_{max} S}{K_s + S} \qquad \text{(Eq. 3.9)}$$

where $\mu$ is the specific growth rate (1/time), $\mu_{max}$ is the maximum specific growth rate (1/time) for the culture, $S$ is the substrate concentration (mass/volume), and $K_s$ is the half-saturation constant (mass/volume) also known as the affinity constant.

Equation 3.9 was developed from a series of experiments performed by Monod. The results of these experiments showed that at low substrate concentrations, growth rate becomes a function of the substrate concentration (note that Eqs. 3.1 to 3.8 are independent of substrate concentration). Thus, Monod designed Eq. 3.9 to describe the relationship between the specific growth rate and the substrate concentration. There are two constants in this equation, $\mu_{max}$, the maximum specific growth rate, and $K_s$, the half-saturation constant, which is defined as the substrate concentration at which growth occurs at one half the value of $\mu_{max}$. Both $\mu_{max}$ and $K_s$ reflect intrinsic physiological properties of a particular type of microorganism. They also depend on the substrate being utilized and on the temperature of growth (see Information Box 3.1). Monod assumed in writing Eq. 3.9 that no nutrients other than the substrate are limiting and that no toxic by-products of metabolism build up.

As shown in Eq. 3.10, the Monod equation can be expressed in terms of cell number or cell mass ($X$) by equating it with Eq. 3.1:

$$\frac{dX}{dt} = \frac{\mu_{max} SX}{K_s + S} \qquad \text{(Eq. 3.10)}$$

The Monod equation has two limiting cases (see Fig. 3.7). The first case is at high substrate concentration where $S >> K_s$. In this case, as shown in Eq. 3.11, the specific growth rate $\mu$ is essentially equal to $\mu_{max}$. This simplifies the equation and the resulting relationship is zero order or independent of substrate concentration:

$$\text{For } S >> K_s: \quad \frac{dX}{dt} = \mu_{max} X \qquad \text{(Eq. 3.11)}$$

Under these conditions, growth will occur at the maximum growth rate. There are relatively few instances in which ideal growth as described by Eq. 3.11 can occur. One such instance is under the initial conditions found in pure culture in a batch flask when substrate and nutrient levels are high. Another is under continuous culture conditions, which are discussed further in Section 3.2. It must be emphasized that this type of growth is unlikely to be found under natural conditions in a soil or water environment, where either substrate or other nutrients are commonly limiting.

**Information Box 3.1 The Monod Growth Constants**

Both μmax and $K_s$ are constants that reflect:

- The intrinsic properties of the degrading microorganism
- The limiting substrate
- The temperature of growth

The following table provides representative values of $\mu_{max}$ and $K_s$ for growth of different microorganisms on a variety of substrates at different temperatures and for oligotrophs and copiotrophs in soil.

| Organism | Growth temperature (°C) | Limiting nutrient | $\mu_{max}$ (1/h) | $K_s$ (mg/l) |
|---|---|---|---|---|
| *Escherichia coli* | 37 | Glucose | 0.8–1.4 | 2–4 |
| *Escherichia coli* | 37 | Lactose | 0.8 | 20 |
| *Saccharomyces cerevisiae* | 30 | Glucose | 0.5–0.6 | 25 |
| *Pseudomonas* sp. | 25 | Succinate | 0.38 | 80 |
| *Pseudomonas* sp. | 34 | Succinate | 0.47 | 13 |
| Oligotrophs in soil | | | 0.01 | 0.01 |
| Copiotrophs in soil | | | 0.045 | 3 |

*Source*: Adapted from Blanch and Clark (1996), Miller and Bartha (1989), Zelenev *et al.* (2005).

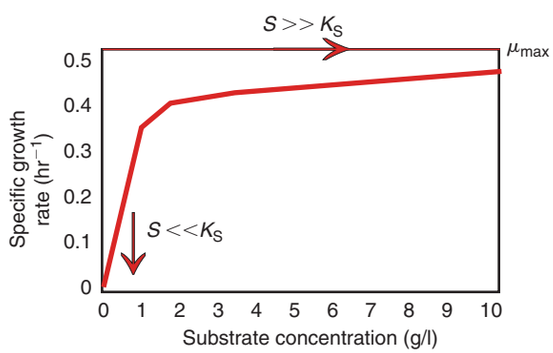



**FIGURE 3.7** Dependence of the specific growth rate, $\mu$, on the substrate concentration. The maximal growth rate $\mu_{max} = 0.5$ 1/h and $K_s = 0.5$ g/l. Note that $\mu$ approaches $\mu_{max}$ when $S \gg K_s$ and becomes independent of substrate concentration. When $S \ll K_s$, the specific growth rate is very sensitive to the substrate concentration, exhibiting a first-order dependence.

The second limiting case occurs at low substrate concentrations where $S \ll K_s$ as shown in Eq. 3.12. In this case there is a first order dependence on substrate concentration (Fig. 3.7):

$$\text{For } S \ll K_s: \quad \frac{dX}{dt} = \frac{\mu_{max} SX}{K_s} \qquad \text{(Eq. 3.12)}$$

As shown in Eq. 3.12, when the substrate concentration is low, growth ($dX/dt$) is dependent on the substrate concentration. Since the substrate concentration is in the numerator, as the substrate concentration decreases, the rate of growth will also decrease. This type of growth is typically found in batch flask systems at the end of the growth curve as the substrate is nearly all consumed. This is also the type of growth that would be more typically expected under conditions in a natural environment where substrate and nutrients are limiting.

The Monod equation can also be expressed as a function of substrate utilization given that growth is related to substrate utilization by a constant called the cell yield (Eq. 3.13):

$$\frac{dS}{dt} = \frac{1}{Y}\frac{dX}{dt} \qquad \text{(Eq. 3.13)}$$

where $Y$ is the cell yield (mass/mass). The cell yield coefficient is defined as the unit amount of cell mass produced per unit amount of substrate consumed. Thus, the more efficiently a substrate is degraded, the higher the value of the cell yield coefficient (see Section 3.3 for more detail). The cell yield coefficient is dependent on both the structure of the substrate being utilized and the intrinsic physiological properties of the degrading microorganism. As shown in Eq. 3.14, Eqs. 3.10 and 3.13 can be combined to express microbial growth in terms of substrate disappearance:

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{\mu_{max} SX}{K_s + S} \qquad \text{(Eq. 3.14)}$$

Figure 3.8 shows a set of growth curves constructed from a fixed set of constants. The growth data used to generate this figure were collected by determining protein as a measure of the increase in cell growth (see Chapter 11). The growth data were then used to estimate the growth constants $\mu_{max}$, $K_s$, and $Y$. Both $Y$ and $\mu_{max}$ were estimated directly from the data. $K_s$ was estimated using a mathematical model that performs a nonlinear regression analysis of the simultaneous solutions to the Monod equations for cell mass (Eq. 3.10) and substrate (Eq. 3.13). This set of constants was then used to model or simulate growth curves that express growth in terms of $CO_2$ evolution and substrate disappearance. Such models are useful because they can help one to: (1) estimate growth constants such

as $K_s$ that are difficult to determine experimentally; and (2) quickly understand how changes in any of the experimental parameters affect growth without performing a long and tedious set of experiments.

## 3.2 CONTINUOUS CULTURE

Thus far, we have focused on theoretical and mathematical descriptions of batch culture growth, which is currently of great economic importance in terms of the production of a wide variety of microbial products. In contrast to batch culture, continuous culture is a system that is designed for long-term operation. Continuous culture can be operated over the long term because it is an open system (Fig. 3.9) with a continuous feed of influent solution that contains nutrients and substrate, as well as a continuous drain of effluent solution that contains cells, metabolites, waste products, and any unused nutrients and substrate. The vessel that is used as a growth container in continuous culture is called a bioreactor or a chemostat. In a chemostat one can control the flow rate, maintain a constant substrate concentration, as well as provide continuous control of pH, temperature, and oxygen levels. As will be discussed further, this allows control of the rate of growth, which can be used to optimize the production of specific microbial products. For example, primary metabolites or growth-associated products, such as ethanol, are produced at high flow or dilution rates, which stimulate cell growth. In contrast, a secondary metabolite or non-growth-associated product such as an antibiotic is produced at low flow or dilution rates, which maintains high cell numbers. Chemostat cultures are also being used to aid in study of the functional genomics of growth, nutrient limitation, and stress responses at the whole-organism level. The advantage of the chemostat in such studies lies in the constant removal of metabolites, including signal molecules (see Chapter 16) or secondary metabolites that may mask or subtly alter physiological conditions under batch culture conditions (Hoskisson and Hobbs, 2005).

Dilution rate and influent substrate concentration are the two parameters controlled in a chemostat to study microbial growth or to optimize metabolite production. The dynamics of these two parameters are shown in Figure 3.10. By controlling the dilution rate, one can control the growth rate ($\mu$) in the chemostat, represented in this graph as doubling time (recall that during exponential phase the growth rate is proportional to the number of cells present). By controlling the influent substrate concentration, one can

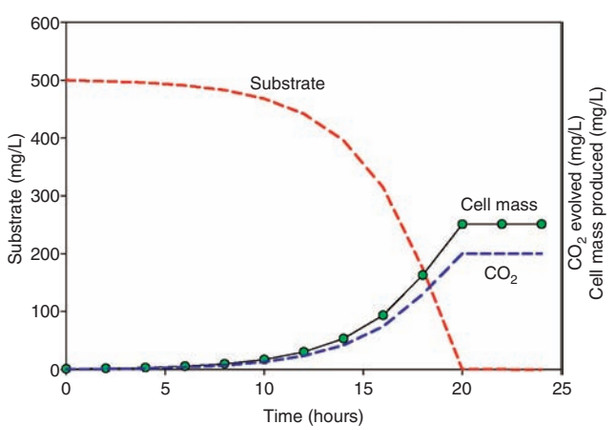



**FIGURE 3.8** This figure shows the same growth curve expressed three different ways: in terms of substrate loss, in terms of $CO_2$ evolution, and in terms of increasing cell mass. The parameters used to generate the curves were as follows: $\mu_{max} = 0.29$ 1/h, $K_s = 10$ mg/1, $Y = 0.5$, initial substrate concentration = 500 mg/1, and initial cell mass = 1 mg/1. In this experiment, cell mass was measured and so the data points are shown. The data for $CO_2$ evolution and substrate loss were simulated using a model and so those data are shown using dashed lines.

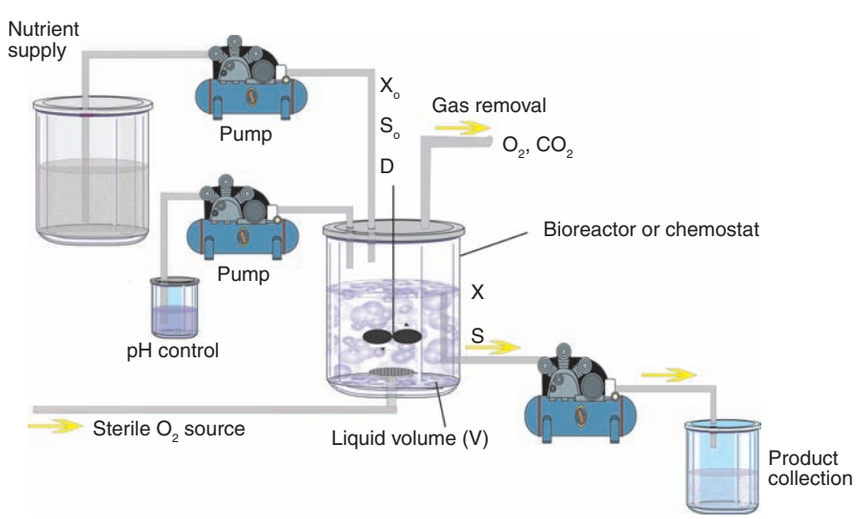



**FIGURE 3.9** Schematic representation of a continuously stirred bioreactor. Indicated are some of the variables used in modeling bioreactor systems. $X_0$ is the dry cell weight, $S_0$ is the substrate concentration, and $D$ is the flow rate of nutrients into the vessel.

control the number of cells produced or the cell yield in the chemostat (the number of cells produced will be directly proportional to the amount of substrate provided). Because the growth rate and the cell number can be controlled independently, chemostats have been an important tool in studying the physiology of microbial growth and also in the long-term development of cultures and consortia that are acclimated to organic contaminants that are toxic and difficult to degrade. Chemostats can also produce microbial products more efficiently than batch fermentations. This is because a chemostat can essentially hold a culture in the exponential phase of growth for extended periods. Despite these advantages, chemostats are not yet widely used to produce commercial products because it is often difficult to maintain sterile conditions over time.

In a chemostat, the growth medium undergoes constant dilution with respect to cells due to the influx of nutrient solution (Fig. 3.9). The combination of growth and dilution within the chemostat will ultimately determine growth. Thus, in a chemostat, the change in biomass with time is

$$\frac{dX}{dt} = \mu X - DX \tag{3.15}$$

where $X$ is the cell mass (mass/volume), $\mu$ is the specific growth rate (1/time), and $D$ is the dilution rate (1/time).

Examination of Eq. 3.15 shows that a steady state (no increase or decrease in biomass) will be reached when $\mu = D$. If $\mu > D$, the utilization of substrate will exceed the supply of substrate, causing the growth rate to slow until it is equal to the dilution rate. If $\mu < D$, the amount of substrate added will exceed the amount utilized. Therefore the growth rate will increase until it is equal to the dilution rate. In either case, given time, a steady state will be established where

$$\mu = D \tag{3.16}$$

Such a steady state can be achieved and maintained as long as the dilution rate does not exceed a critical rate, $D_c$. The critical dilution rate can be determined by combining Eqs. 3.9 and 3.16:

$$D_c = \mu_{max}\left(\frac{S}{K_s + S}\right) \tag{3.17}$$

Looking at Eq. 3.17, it can be seen that the operation efficiency of a chemostat can be optimized under conditions in which $S \gg K_s$, and therefore $D_c \approx \mu_{max}$. But it must be remembered that when a chemostat is operating at $D_c$, if the dilution rate is increased further, the growth rate will not be able to increase (since it is already at $\mu_{max}$) to offset the increase in dilution rate. The result will be washing out of cells and a decline in the operating efficiency of the chemostat. Thus, $D_c$ is an important parameter because if the chemostat is run at dilution rates less than $D_c$, operation efficiency is not optimized, whereas if dilution rates exceed $D_c$, washout of cells will occur as shown in Figure 3.10.

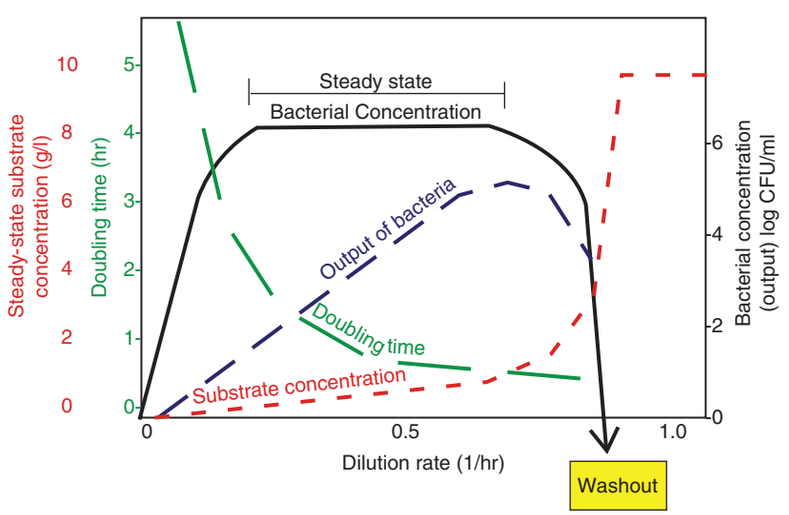
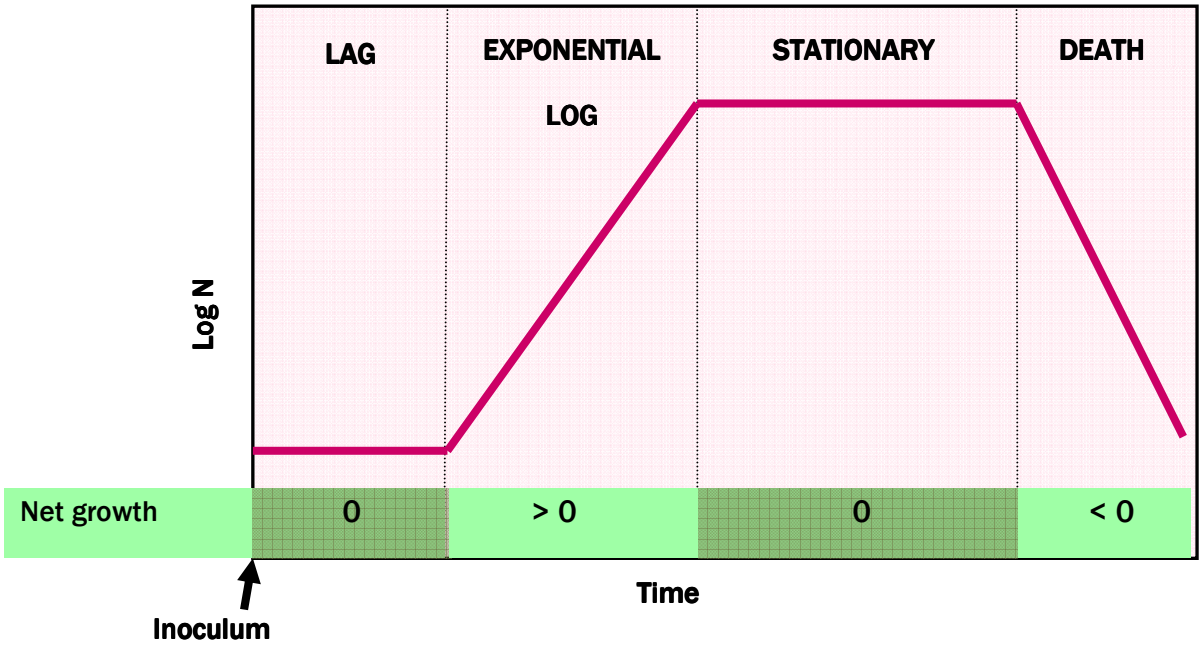



**FIGURE 3.10** Steady-state relationships in the chemostat. The dilution rate is determined from the flow rate and the volume of the culture vessel. Thus, with a vessel of 1000 ml and a flow rate through the vessel of 500 ml/h, the dilution rate would be 0.5 1/h. Note that at high dilution rates, growth cannot balance dilution and the population washes out. Thus, the substrate concentration rises to that in the medium reservoir (because there are no bacteria to use the inflowing substrate). However, throughout most of the range of dilution rates shown, the population density remains constant and the substrate concentration remains at a very low value (i.e., steady state). Note that although the population density remains constant, the growth rate (doubling time) varies over a wide range. Thus, the experimenter can obtain populations with widely varying growth rates without affecting population density. Adapted with permission from Madigan *et al.*, 1997.

# Mathematical modeling of bacterial growth curve/fermentation

Under controlled conditions, in the laboratory, the evolution of the number of cells over time can be followed in a batch culture. Bacteria growth can be represented with a growth curve, which consists of four phases: lag phase, exponential or log phase, stationary phase and death phase.




The concept of microbial growth and the mathematical expressions defining it, can be found in any textbook of Microbiology (Brock Biology of Microorganisms 2012, Prescott's Microbiology 2011).

**Exponential phase** is the growth phase itself and simplifying the mathematical development,

$$dN/dt = \mu N$$

$$N = N_0 e^{\mu(t-t_0)}$$

It is characterized by the following equation:

$$\ln N - \ln N_0 = \mu (t-t_0) \quad \text{or} \quad \log_{10} N - \log_{10} N_0 = \mu \times (t-t_0)/ 2.303$$

$$\mu = \{ (\log_{10} N - \log_{10} N_0) \times 2.303\} / (t - t_0)$$

Where t = time, N = cfu $ml^{-1}$ at time t, $N_0$ = cfu $ml^{-1}$ at time $t_0$, $\mu$ = specific growth rate constant ($h^{-1}$).

It is possible to use other parameters such as the time required to double the population or **generation time, g**.

$$\mathbf{g = 0.693/\mu}$$

The inverse of the generation time is called **growth rate (K),** and it is expressed as generations / hour.

$$\mathbf{K = 1/g}$$

It can be calculated the $\mu_{max}$ (maximum specific growth rate) value for a given microorganism and substrate. For that, growth curves are performed with increasing concentrations of the substrate and the $\mu$ values are calculated for each concentration.

$$\mathbf{\mu = \mu_{max}\, S/(K_S + S)}$$

Where $K_S$ = saturation constant for the substrate, the concentration where specific growth rate is half of $\mu_{max}$ ($\mu = ½\ \mu_{max}$).

It can be also used the following transformation of the equation:

$$\mathbf{1/\mu = 1/\mu_{max} + (K_s/\mu_{max})\ (1/S)}$$

In the **stationary phase** two interesting parameters can be determined: **maximum biomass** (cells or other parameter) **and yield coefficient.**

**M** is calculated by the following expression:

$$\mathbf{M = M_t - M_0}$$

Where $M_t$ = biomass (cells, etc) at time t (it is calculated in the stationary phase, where the number of cells is maximum) and $M_0$ = inoculum biomass (cells, etc). The result is expressed in grams, milligrams, cells/ml, etc.

The **maximum yield coefficient** is the amount of biomass, cells or other parameter, produced per substrate consumed and is given by:

$$\mathbf{Y = Biomass\ produced/Substrate\ consumed = (M_t - M_0)/(S_0 - S_t)}$$

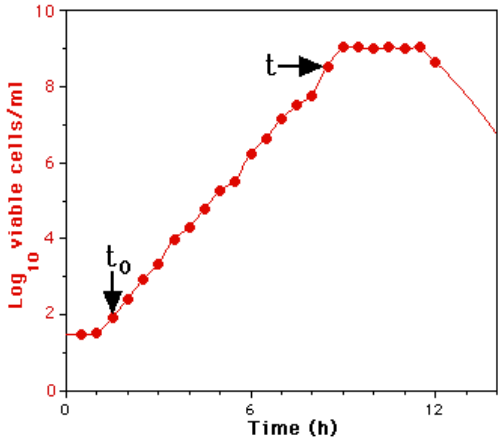
Where $S_0$ = substrate at the beginning of the culture and $S_t$ = substrate at time (t) when the number of cells is maximum. The result is expressed as g cells/g substrate or Nº cells/g substrate.

So much for the theoretical aspects of the study of microbial growth, but how can the results be analyzed? We will see below how to work with the data.

The simplest problems are those in which various terms are known (i.e. initial density of microorganisms, $\mu$ value and time), and we must determine an unknown term (in this example, final density of microorganisms). In these problems we must just replace the known terms in the equations described above and solve it.

These problems are very simple, but we must be careful with the units or the need to transform some data for being possible to use in them in the formula (i.e. the μ value is unknown but it is known the g value).

By measuring the increase in the number of cells during a certain time period, the growth rate constant (μ) can be calculated. In the experiment you have just done:




**$t_0$ = 1.5h:**
**N = $8.4 \times 10^1$,**
**$\log_{10}$ N = 1.92**

**t = 8.5h:**
**N = $3.39 \times 10^8$,**
**$\log_{10}$ N = 8.53**

Therefore in this case:

$\mu = ( (\log_{10} N - \log_{10} N_0) 2.303) / (t - t_0)$

$= ( (8.53 - 1.92) 2.303) / (8.5 - 1.5)$

$= (6.61 \times 2.303) / 7$

$= 15.22 / 7$

**$= 2.18\ hour^{-1}$**

Note that **μ** and **g** are related to each other: **$\mu = \ln 2 / g = 0.693/g$**

On this basis, solutions must be proposed for the following problems:

Q1: How many bacteria are present after 4 hours if a culture that doubles every 2 hours is inoculated with $10^4$ CFU/ml? and after 24 and 48 hours?

Q2 : If a culture in exponential phase has 100,00 cells/ml at a given time, and after 4 hours, the population is 100,000,000 cells/ml, which would the μ and g values be?

Sources: HOW TO SOLVE PRACTICAL ASPECTS OF MICROBIOLOGY, DETERMINATION OF THE PARAMETERS DEFINING THE BACTERIAL GROWTH. Inés Arana, Maite Orruño & Isabel Barcina, Department of Immunology, Microbiology and Parasitology University of the Basque Country Universidad del País Vasco (UPV/EHU)

Madigan, M.T., J.M. Martinko, D.A. Stahl, D.P. Clark. 2012. Brock Biology of Microorganisms, 13th ed. Benjamin Cummings.

Willey, J.M., L.M. Sherwood, C.J. Woolverton. 2011. Prescott's Microbiology Companion Site, 8th ed. McGraw-Hill Ryerson Ltd.