12. The kinetic parameters describing the growth characteristics of the different types of anaerobic microorganisms are difficult to assess because of the strong interactions within the microbial communities. Nevertheless, it can be stated that the aceticlastic methanogens have the lowest maximum specific growth rate coefficient, and thus represent the weak link in the chain.
13. Two approaches have been used to model the loss of viability and biomass in biochemical operations: the traditional decay approach and the lysis:regrowth approach. In the traditional approach, loss of active biomass leads directly to the use of electron acceptor and the production of biomass debris, which accumulates and acts to reduce the viability. In the lysis:regrowth approach, active biomass is lost by lysis, which releases particulate substrate and biomass debris. Electron acceptor consumption occurs only after soluble substrate, which is formed by hydrolysis of the particulate substrate, is used for new biomass growth. Because the yield is always less than one, the amount of new biomass formed is always less than the biomass lost by lysis, leading to a loss of biomass in the bioreactor.
14. Both the traditional and the lysis:regrowth approaches to modeling decay and loss of viability depict the rate of active biomass loss as being first order with respect to the active biomass concentration, as is the generation of biomass debris. However, the decay coefficient in the traditional approach is smaller than the coefficient in the lysis:regrowth approach, although the fraction of the biomass leading to debris is larger.
15. Although soluble microbial product formation is known to occur in biochemical operations, there has been insufficient research on the subject to allow consensus concerning the rate expressions to be used.
16. For modeling purposes, solubilization of particulate and high molecular weight organic matter is assumed to occur by hydrolysis, with conservation of COD. The rate expression adopted to describe hydrolysis is similar to the Monod equation, except that it is controlled by the particulate substrate to biomass ratio rather than by the particulate substrate concentration:

$$r_{XS} = -k_h \left[ \frac{X_S/X_{B,H}}{K_X + (X_S/X_{B,H})} \right] X_{B,H}$$

    This is necessary because the reaction is thought to be surface mediated.
17. Even though organic nitrogen may be used directly in biomass synthesis, it is simpler to model the flow of nitrogen in biochemical operations by assuming that nitrogen is released to the medium as ammonia and then taken up for biomass synthesis as needed. The release as ammonia, called ammonification, is assumed to be first order with respect to both the biomass and soluble, biodegradable organic nitrogen concentrations. The uptake of ammonia for growth is assumed to be proportional to the rate of growth.
18. During biological phosphorus removal, the uptake of acetic acid, the formation of PHB, and the release of soluble phosphate by PAOs under

anaerobic conditions are all coupled. The rate of acetic acid uptake is controlled by both the acetic acid concentration in solution, $S_A$, and the polyphosphate concentration in the biomass, $X_P$:

$$r_{SA} = -\hat{q}_A \left(\frac{S_A}{K_A + S_A}\right)\left[\frac{X_{PP}/X_{B,P}}{K_{PP} + (X_{PP}/X_{B,P})}\right] X_{B,P}$$

Under aerobic conditions, the PAOs grow by using the stored PHB as a carbon and energy source, storing polyphosphate in the process:

$$r_{XBP} = \hat{\mu}_P \left[\frac{X_{PHB}/X_{B,P}}{K_{PHB} + (X_{PHB}/X_{B,P})}\right]\left(\frac{S_P}{K_P + S_P}\right)\left(\frac{S_O}{K_O + S_O}\right) X_{B,P}$$

The rate of phosphorus storage is coupled to the rate of biomass growth and thus is expressed by a similar equation. An additional term is required, however, to reflect the fact that there is a limit to the amount of polyphosphate that the PAOs can accumulate.

19. The COD-based stoichiometric equation states that the COD removed by a biological reaction must equal the oxygen equivalents of the terminal electron acceptor used plus $\alpha$ times the COD of the biomass formed. The value of $\alpha$ depends on the nature of the nitrogen source. It is 1.0 when ammonia is the source and 1.4 when nitrate is.
20. If $C_5H_7O_2N$ can be considered to be representative of the elemental composition of biomass, then 0.087 mg of nitrogen is required to synthesize a mg of biomass COD. Conversely, each time a mg of biomass COD is destroyed by decay, 0.087 mg of nitrogen is released. Although not shown in the empirical equation for biomass, approximately 0.017 mg of phosphorus will be required (released) each time a mg of biomass COD is formed (destroyed).
21. Within a relatively narrow physiological range, the maximum specific growth rate coefficient, $\hat{\mu}$, increases as the temperature is increased. Furthermore, for rapidly growing cultures the effect of temperature on the traditional decay coefficient, b, appears to be closely correlated with the effect on $\hat{\mu}$. No conclusions can be drawn about the effects of temperature on the half-saturation coefficient, $K_S$.
22. Three expressions are commonly used to relate the rate coefficients in biological operations (k) at different temperatures (T):

$$k = A \cdot e^{-u/RT}$$
$$k_1 = k_2 \cdot e^{C(T_1 - T_2)}$$
$$k_1 = k_2 \cdot \theta^{(T_1 - T_2)}$$

The temperature coefficients for the three equations may be interconverted by:

$$\ln(\theta) = C \approx 0.0015\ u$$

## 3.11 STUDY QUESTIONS

1. Why must the yield be known before the stoichiometric equation for microbial growth can be written? Which type of yield, the true growth yield or the observed yield, is most appropriate for doing this? Why? How is knowledge of the yield used to write the stoichiometric equation using McCarty's half-reaction approach?
2. Using the half-reaction-technique, write the molar stoichiometric equation for microbial growth for each of the following situations:
   a. Aerobic growth on domestic wastewater with ammonia nitrogen as the nitrogen source. The yield is 0.60 mg biomass COD formed/mg substrate COD removed.
   b. Growth on a carbohydrate with nitrate as the terminal electron acceptor and ammonia as the nitrogen source. The yield is 0.50 mg biomass COD formed/mg substrate COD used.
   c. Growth on a carbohydrate with nitrate as the terminal electron acceptor and nitrogen source. The yield is 0.40 mg biomass COD formed/mg substrate COD used.
   d. Normalize them with respect to the electron donor.
3. Convert the molar stoichiometric equation from Study Question 2a into a mass based equation with the electron donor as the reference component.
4. Convert the molar stoichiometric equation from Study Question 2a into a COD based equation with the electron donor as the reference component.
5. Write the rate expression for bacterial growth and relate it to the rates of substrate and oxygen utilization for heterotrophic biomass growth on an organic substrate. Then state the Monod and Andrews equations relating the specific growth rate coefficient to the substrate concentration. Finally, draw sketches depicting the effects represented by both equations and use them to define the parameters in the equations.
6. State the zero- and first-order approximations of the Monod equation. Under what circumstances may they be used?
7. Explain the difference between complementary and substitutable nutrients. Then differentiate between interactive and noninteractive models for describing the effects of two complementary nutrients. Finally, state why the interactive approach was adopted herein.
8. Draw a sketch depicting the effects of two interactive, complementary nutrients on the specific growth rate of biomass and use it to explain why it is easier to design a biochemical operation to achieve a desired concentration of a given nutrient if that nutrient serves as the sole growth limiting nutrient for the biomass.
9. Even though it is best to characterize the kinetic parameters in the Monod and Andrews equations by ranges rather than by unique values, it is possible to state several generalities about the sizes of those parameters. Use such generalities to contrast and compare the growth characteristics of heterotrophic and autotrophic biomass.

10. Discuss the effects that organic compounds and heterotrophic biomass can have on the growth of nitrifying bacteria.
11. Describe the major groups of microorganisms participating in anaerobic operations and contrast their growth characteristics as described by their kinetic parameters.
12. Describe in detail the traditional and lysis:regrowth approaches to modeling the loss of biomass and viability observed in biochemical operations. In your description, contrast the routes of carbon, nitrogen and electron flow, and explain how they influence the magnitudes of the kinetic parameters used to characterize the events.
13. Write the rate equations for loss of active biomass as depicted by the traditional and lysis:regrowth approaches. Then explain the relationships between the kinetic and stoichiometric parameters used in the two approaches.
14. Write the rate equation for the hydrolysis of particulate substrate, compare it to the Monod equation, and explain any differences.
15. Discuss the fate of nitrogen in biochemical operations and state the rate equations used to model that fate.
16. State the rate equations that have been proposed to represent acetic acid uptake, PHB formation, and phosphorus release by PAOs under anaerobic conditions in a biological phosphorus removal system. Then state the rate equations depicting PAO growth, soluble phosphorus uptake, and polyphosphate formation under aerobic conditions. Use those equations in a discussion of the events occurring in such systems and explain why the various terms were included in the rate expressions.
17. An aerobic culture is growing on a mixture of organic matter, such as that found in domestic wastewater, with ammonia as the nitrogen source. How many mg of nitrogen (N), phosphorus (P) and oxygen ($O_2$) must be provided per mg of COD removed for each of the following situations? What quantities of micronutrients will be required? Do not derive the stoichiometric equations. Rather, answer the question using generalizations presented in the text.
    a. $Y_{Hobs}$ = 0.70 mg biomass COD formed/mg substrate COD removed.
    b. $Y_{Hobs}$ = 0.57 mg biomass COD formed/mg substrate COD removed.
    c. $Y_{Hobs}$ = 0.36 mg biomass COD formed/mg substrate COD removed.
18. Demonstrate why the value of $\alpha_N$ in Eq. 3.90 is 1.40 when nitrate serves as the nitrogen source and biomass is represented by $C_5H_7O_2N$.
19. Three techniques are often used to describe the effects of temperature on microbial cultures. Describe each of them and tell how you would plot data to determine the values of the temperature coefficients in the equations.
20. The data on the following page describe the effects of temperature on the traditional decay coefficient, b. Use that data to determine the temperature coefficient by each of the three techniques. Use 20°C as the reference temperature. Discuss the utility of each technique for describing the effects of temperature on this parameter.

| T | b |
|---|---|
| °C | $hr^{-1}$ |
| 10 | 0.0037 |
| 20 | 0.0095 |
| 30 | 0.0229 |
| 40 | 0.0372 |

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