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## Chapter 4

# Prokaryotes and Viruses

### Bacterial cell structure

01. Which statement is *true* about both prokaryotic and eukaryotic cells?
- a. Prokaryotic cells are generally much larger than eukaryotic cells.
  - b. Eukaryotic cells have ribosomes and prokaryotic cells do not.
  - c. Both have DNA as their primary genetic material.
  - d. Eukaryotic cells have plasma membranes and prokaryotic cells do not.
02. Which of the following statements about bacteria are *correct*?
- P. They are microscopic cellular organisms.
  - Q. They lack a defined nucleus.
  - R. They are typically single-celled organisms.
  - S. They have a simple cellular structure without membrane-bound organelles.
- a. P and Q
  - b. Q, R and S
  - c. P, Q and R
  - d. P, Q, R and S
03. Which of the following statements about acid-fast staining are *correct*?
- P. It is a differential staining technique.
  - Q. It distinguishes acid-fast bacteria from non-acid-fast bacteria.
  - R. It is commonly used to identify members of the genus *Mycobacterium*.
  - S. Differentiation is based on the presence of mycolic acid, a branched-chain hydroxy fatty acid.
- a. P and Q
  - b. Q, R and S
  - c. P, Q and R
  - d. P, Q, R and S
04. Which of the following statement is *correct*?
- a. Gram staining is a differential staining technique.
  - b. Action of lysozyme in Gram-negative bacteria results in formation of protoplast.
  - c. Gram staining is due to chemical nature of cell wall.
  - d. Cell wall peptide in eubacteria is made up of only L-amino acid residues.
05. Which of the following statements about carbohydrate present in peptidoglycan cell wall are *correct*?
- P. It is a structural polysaccharide providing rigidity and strength to the bacterial cell wall.
  - Q. It is classified as a heteropolysaccharide.

21. A rapidly growing bacterial species such as *E. coli* exhibits a typical growth phases in liquid nutrient broth. If a bacterial culture has a starting density of  $10^3$  cells/ml has a lag time of 10 minutes and a generation time of 10 minutes, what will the cell density (cells/ml) be at 30 minutes?
- $6.0 \times 10^3$
  - $2.0 \times 10^3$
  - $3.0 \times 10^3$
  - $4.0 \times 10^3$
22. Initial density of a culture of bacteria with a generation time of 30 minutes was  $1 \times 10^5$  cells/ml. After 5 hours of incubation, what serial dilution will you have to plate out to get ~100 colonies per ml?
- $10^3$
  - $10^4$
  - $10^5$
  - $10^6$
- [GATE]
23. A bacterial culture was diluted 1000 fold and 0.1 ml of this diluted sample was spread per plate on nutrient agar. In a triplicate run, the number of colonies formed is 121, 93 and 86, the number of colony forming units/ml in the original bacterial culture is
- $10^6$
  - $10^5$
  - $10^3$
  - $10^2$
- [GATE]
24. In an experiment designed to determine the number of phage particles in a sample 56 plaques were recorded when 100  $\mu$ l of a  $10^{-4}$  dilution was plated. How many phages are there per ml in the original?
- $5.6 \times 10^3$
  - $5.6 \times 10^4$
  - $5.6 \times 10^5$
  - $5.6 \times 10^6$
- [GATE]
25. A culture of bacteria has been diluted to  $1 \times 10^{-6}$  using a 10-fold serial dilution protocol. Then, 0.1 ml of each of the  $1 \times 10^{-3}$ ,  $1 \times 10^{-4}$ ,  $1 \times 10^{-5}$  and  $1 \times 10^{-6}$  dilutions was plated onto nutrient agar and 36 hours later the colonies that had formed were counted. The following data was generated:
- $1 \times 10^{-3}$  = Too numerous to count  
 $1 \times 10^{-4}$  = Too numerous to count  
 $1 \times 10^{-5}$  = 237 colonies  
 $1 \times 10^{-6}$  = 21 colonies
- Calculate the estimated number of bacteria as colony forming units (CFU) per milliliter of the original culture.
- $2.37 \times 10^5$
  - $2.37 \times 10^8$
  - $2.37 \times 10^7$
  - $2.37 \times 10^6$
26. *E. coli* is inoculated in a shake flask containing nutrient rich medium. The initial number of viable cells in the medium is  $10^2$ . After few hours, the number of viable cells is  $10^6$ . Assuming the cell divides by binary fission, how many generations have occurred?
- 13.20
  - 10.12
  - 20.10
  - 26.42
- [GATE]
27. *Escherichia coli* growing under favorable conditions doubles in every 20 minutes. If the initial number of *Escherichia coli* cells is 100, what will be the logarithmic number of cells at 17th generation?
- 5.1
  - 2.5
  - 7.1
  - 9.0
- [GATE]
28. A bacterial strain is grown in nutrient medium at  $37^\circ\text{C}$  under aerobic conditions. The medium is inoculated with  $10^2$  cells from a seed culture. If the number of cells in the culture reaches  $10^5$  after 10 hours of growth, what is the doubling time of the strain (in hours)?
- 1
  - 2
  - 3
  - 4
- [GATE]

29. A 0.1 mL aliquot of a bacteriophage stock having a concentration of  $4 \times 10^9$  phages  $\text{mL}^{-1}$  is added to 0.5 mL of *E. coli* culture having a concentration of  $2 \times 10^8$  cells  $\text{mL}^{-1}$ . What is the multiplicity of infection?
- a. 4  
b. 8  
c. 12  
d. 16
- [GATE]
30. Which of the following events occur during the stationary phase of bacterial growth?
1. Rise in cell number stops.  
2. Spore formation in some Gram-positive bacteria such as *Bacillus subtilis*.  
3. Cell size increases in some Gram-negative bacteria such as *Escherichia coli*.  
4. Growth rate of bacterial cells nearly equals their death rate.  
5. Decrease in peptidoglycan crosslinking.
- a. 1, 2 and 4 only  
b. 1, 4 and 5 only  
c. 2, 3 and 4 only  
d. 1, 3 and 5 only
- [GATE]
31. In balanced growth phase of a cell
- P. all components of a cell grow at the same rate.  
Q. specific growth determined by cell number or cell mass would be the same.  
R. the growth rate is independent of substrate concentration.  
S. the growth rate decreases with decreasing substrate concentration.
- a. P, Q and S only  
b. Q, R and S only  
c. P, Q and R only  
d. P only
- [GATE]
32. Substrate consumption in *lag* phase of microbial growth is primarily used for
- P. turnover of the cell material  
Q. maintenance of intracellular pH  
R. cell motility  
S. increase in cell number
- a. P, Q and S only  
b. Q, R and S only  
c. P, Q and R only  
d. S only
- [GATE]

## Bacterial groups

01. Carl Woese used the gene sequence of which one of the following for phylogenetic taxonomy of prokaryotes?
  - a. A ribosomal RNA of large ribosomal subunit.
  - b. A ribosomal RNA of small ribosomal subunit.
  - c. A ribosomal protein of large ribosomal subunit.
  - d. A ribosomal protein of small ribosomal subunit.
02. Which of the following molecular markers is most commonly used for bacterial phylogenetic analysis?
  - a. 16S rRNA gene
  - b. Cytochrome c oxidase gene
  - c. Histone H3 gene
  - d. Ribosomal 5S RNA gene
03. In bacterial phylogenetics, horizontal gene transfer complicates evolutionary relationships because:
  - a. It increases the rate of genetic drift.
  - b. It allows genes to be transferred between distantly related species.
  - c. It prevents bacteria from forming phylogenetic trees.
  - d. It reduces genetic diversity within bacterial populations.

# Answers

## Bacterial cell structure

01. c      02. d      03. d      04. a      05. c      06. d      07. b      08. d      09. c      10. a  
11. c      12. d      13. d      14. b      15. c      16. d      17. a      18. b,d      19. a      20. b  
21. a      22. a

## Explanations

10. Encapsulation helps bacteria evade the host immune system, making them more resistant to phagocytosis and increasing their virulence.
14. The rotation of the bacterial flagellum is powered by the proton motive force (PMF), which is generated by the transmembrane electrochemical gradient of protons. As protons flow back into the bacterial cell through the Mot protein complex, they drive the rotation of the flagellum, enabling bacterial movement. ATP hydrolysis is not directly involved in flagellar motion.

## Bacterial nutrition and growth

01. b      02. c      03. b      04. d      05. a      06. b      07. d      08. a      09. a      10. a  
11. d      12. b      13. a,b      14. d      15. d      16. a      17. a      18. b      19. d      20. b  
21. d      22. d      23. a      24. d      25. b      26. a      27. c      28. a      29. a      30. a  
31. a      32. c

## Explanations

04. Auxotrophs are mutant organisms that require additional nutrients (e.g., amino acids or vitamins) because they cannot synthesize them. They cannot grow on minimal media unless supplements are provided. Prototrophs are wild-type organisms that can synthesize all essential biomolecules from minimal media components. They do not require supplementary nutrients.
14. *Methanococcus*: A genus of methanogenic archaea; adapted to high pressure (barophilic).  
*Dunaliella*: A green alga that thrives in high salt concentrations (e.g., salt lakes).  
*Sulfolobus*: A thermoacidophilic archaeon that lives in acidic hot springs.  
*Escherichia* (e.g., *E. coli*): Grows best at moderate temperatures, typically around 37°C.
18. We use the formula:  $N_t = N_0 \times 2^n$   
Where:  $N_t$  = Final cell density =  $10^6$  cells/ml  
 $N_0$  = Initial cell density =  $10^3$  cells/ml  
 $n$  = Number of generations  
Putting the given values:  $10^6 = 10^3 \times 2^n$   
 $2^n = 10^3$   
Value of  $n$  will be approximately 10.

## Chapter 10

# Biochemical and Bioprocess engineering

### Bioenergetics

01. Which thermodynamic quantity remains unchanged during a phase transition at constant pressure & temperature?
  - a. Enthalpy
  - b. Entropy
  - c. Temperature
  - d. Gibbs free energy
02. Which one of the following statements is *correct* in the context of thermodynamics?
  - a. In a closed system, neither mass nor energy is transferred across the system boundary.
  - b. In a closed system, both mass and energy can be transferred across the system boundary.
  - c. Total energy of the system is the sum of kinetic and potential energies.
  - d. In a closed system, only energy can be transferred across the system boundary and not mass. [GATE]
03. Which of the following is a direct outcome of the *first law of thermodynamics* in biological systems?
  - a. Entropy of an organism always increases over time.
  - b. Energy can be freely converted from one form to another with 100% efficiency.
  - c. Total energy of an organism and its surroundings remains constant.
  - d. Biological systems tend towards a state of maximum disorder.
04. Which of the following is a direct outcome of the *second law of thermodynamics* in biological systems?
  - a. The total energy of a living organism remains constant throughout its life.
  - b. Living organisms can decrease their internal entropy without affecting the entropy of their surroundings.
  - c. To stay highly ordered, living organisms must continuously increase the entropy of their surroundings.
  - d. Spontaneous processes always lead to a decrease in the total entropy of the system and its surroundings.
05. Which of the following statements *correctly* describes the second law of thermodynamics?
  - a. The energy of the universe is constant.
  - b. Entropy of a system always decreases over time.
  - c. In any spontaneous process, the total entropy of the universe increases.
  - d. Heat cannot be converted into work.
06. The Gibbs free energy change ( $\Delta G$ ) of a reaction determines its spontaneity at constant temperature and pressure. A reaction with a negative  $\Delta G$  is:
  - a. At equilibrium.
  - b. Endergonic and requires energy input.

04. The elemental composition of dry biomass of a yeast species is  $\text{CH}_{1.6}\text{O}_{0.4}\text{N}_{0.2}\text{S}_{0.0024}\text{P}_{0.017}$ . What will be the contribution of carbon to the dry biomass? [Given: atomic weights of H, C, N, O, P and S are 1, 12, 14, 16, 31 and 32, respectively]
- 51.28
  - 13.60
  - 18.80
  - 36.24
- [GATE]
05. The following reaction represents biomass synthesis from hexadecane
- $$\text{C}_{16}\text{H}_{34} + 12.5 \text{O}_2 + 2.13 \text{NH}_3 \longrightarrow 10.6 \text{CH}_{1.66}\text{O}_{0.27}\text{N}_{0.27} + 5.37 \text{CO}_2 + 11.4 \text{H}_2\text{O}$$
- where,  $\text{CH}_{1.66}\text{O}_{0.27}\text{N}_{0.27}$  represents the biomass. What will be the value of respiratory quotient?
- 0.36
  - 0.01
  - 0.42
  - 0.94
- [GATE]
06. The most plausible explanation for a sudden increase of the respiratory quotient of a microbial culture is that
- cells are dying.
  - yield of biomass is increasing.
  - fermentation rate is increasing relative to respiration rate.
  - the maintenance rate is decreasing.
- [GATE]
07. If the chemical composition of proteins in an organism is  $\text{CH}_{1.5}\text{O}_{0.3}\text{N}_{0.3}\text{S}_{0.004}$ , what is the mass percentage of carbon in the proteins? [Given: Atomic weights of C = 12, H = 1, O = 16, N = 14 and S = 32].
- 53.03
  - 99.01
  - 25.04
  - 75.85
- [GATE]
08. Yeast biomass ( $\text{C}_6\text{H}_{10}\text{O}_3\text{N}$ ) grown on glucose is described by the stoichiometric equation given below:
- $$\text{C}_6\text{H}_{12}\text{O}_6 + 0.48 \text{NH}_3 + 3 \text{O}_2 \longrightarrow 0.48 \text{C}_6\text{H}_{10}\text{O}_3\text{N} + 3.12 \text{CO}_2 + 4.32 \text{H}_2\text{O}$$
- Calculate the amount of glucose (in kg) required to produce 50 g/L of yeast biomass in a 100,000 L batch reactor.
- 10000
  - 13000
  - 11000
  - 14500
- [GATE]
09. In microbial fermentation, the degree of reduction of a compound primarily reflects:
- Amount of ATP generated during metabolism.
  - Quantity of oxygen required for its complete oxidation.
  - Absorbance in colorimetric assays.
  - Volume of carbon dioxide produced during catabolism.
10. The degree of reduction of a substrate provides an indication of:
- Its molecular weight.
  - Number of electrons available for transfer during oxidation.
  - Its solubility in water.
  - Rate at which it can be metabolized.
11. What will be the degree of reduction for acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ )?
- 2
  - 4
  - 3
  - 6
- [GATE]
12. What will be the mass of one kmol of oxygen molecules in gram?
- 28000
  - 3200
  - 32000
  - 320
- [GATE]



31. The decimal reduction time of a bacterial strain is 20 minutes. What is the *specific death rate constant* (in  $\text{min}^{-1}$ )?  
a. 0.115  
b. 0.500  
c. 0.310  
d. 0.750  
[GATE]
32. The specific growth rate of a mold during the exponential phase in a batch culture is  $0.15 \text{ h}^{-1}$ . If the cell concentration at 30 hours is 33 g/L, what was the cell concentration (in g/L) at 24 hours?  
a. 9  
b. 10  
c. 15  
d. 13  
[GATE]
33. In a batch culture of *Penicillium chrysogenum*, the maximum penicillin synthesis occurs during the  
a. lag phase  
b. exponential phase  
c. stationary phase  
d. death phase  
[GATE]
34. The specific growth rate ( $\mu$ ) of a microbial culture in a batch bioreactor during the exponential growth phase is:  
a. Constant and maximal for the given conditions.  
b. Decreasing over time due to nutrient depletion.  
c. Increasing over time as the cell density rises.  
d. Zero, as there is no net increase in cell mass.
35. According to the Monod equation, the specific growth rate of a microorganism is most sensitive to changes in substrate concentration when:  
a. Substrate concentration is much greater than the saturation constant.  
b. Substrate concentration is much smaller than the saturation constant.  
c. Substrate concentration is equal to the maximum specific growth rate.  
d. Substrate concentration is equal to the saturation constant.
36. In the Monod equation for microbial growth kinetics, the term *saturation constant* represents:  
a. The maximum specific growth rate.  
b. The substrate concentration at which the specific growth rate is half of the maximum.  
c. The specific growth rate at infinite substrate concentration.  
d. The minimum substrate concentration required for growth.
37. The maximum specific growth rate of a microorganism is determined using the following method (where, X is biomass concentration):  
a. Slope of  $\ln X$  versus t for the entire growth curve.  
b. Slope of  $\ln X$  versus t during the exponential phase.  
c. Slope of X versus t.  
d. Slope of X versus t during the exponential phase.  
[GATE]
38. Match the microbial growth characteristics in group I with the corresponding features in group II.

Group I	Group II
P. Growth associated	1. Specific growth rate decreases with product formation increasing product concentration
Q. Non growth associated	2. Specific product formation rate is product formation constant
R. Product inhibition	3. Specific product formation rate is proportional to specific growth
S. Substrate inhibition	4. Specific growth rate decreases with increasing substrate concentration
a. P-1, Q-2, R-4, S-3	b. P-3, Q-2, R-1, S-4
c. P-2, Q-1, R-3, S-4	d. P-2, Q-3, R-4, S-1

**[GATE]**

# Answers

## Bioenergetics

01. d    02. d    03. c    04. c    05. c    06. c    07. c    08. c    09. c    10. b

## Explanations

02. In a closed system, only energy can be transferred across the system boundary and not mass.

## Biochemical engineering principles

01. b    02. c    03. d    04. a    05. c    06. c    07. a    08. b    09. b    10. b  
11. b    12. c    13. c    14. d    15. b    16. c    17. d    18. c    19. c    20. c  
21. c    22. b    23. b    24. c    25. c    26. d    27. b    28. b    29. a    30. c  
31. a    32. d    33. c    34. a    35. d    36. b    37. b    38. b    39. c    40. b  
41. d    42. a    43. b    44. c    45. b    46. b    47. d    48. a    49. b

## Explanations

01. In a biochemical reaction: total mass is conserved due to the law of conservation of mass. Number of atoms of each element is conserved, as atoms are neither created nor destroyed. Total energy is conserved, according to the first law of thermodynamics.

02.  $C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$   
Molar mass of  $C_6H_{12}O_6 = 6 \times 12 + 12 \times 1 + 6 \times 16 = 180$  g  
Molar mass of  $C_2H_5OH = 2 \times 12 + 5 \times 1 + 1 \times 16 + 1 \times 1 = 46$   
Total molar mass of  $C_2H_5OH = 2 \times 46 = 92$  g  
180 g of  $C_6H_{12}O_6$  produces 92 g of  $C_2H_5OH$   
200 g of  $C_6H_{12}O_6$  will produce  $(92 \times 200)/180 = 102.2$  g  $C_2H_5OH$

03. Equating the stoichiometric coefficients and we have  
Carbon balance:  $3 \times 6 = 1.5a + 3$  or,  $1.5a = 15$   
 $a = 15/1.5 = 10$   
Hydrogen balance:  $3 \times 12 + 2.5 \times 3 = 1.5b + 5 \times 2$   
 $36 + 7.5 = 1.5b + 10$   
 $b = 33.5/1.5 = 22.33$   
Oxygen balance:  $3 \times 6 + 2 = 1.5c + 3 \times 2 + 5 \times 1$   
 $20 - 11 = 1.5c$  or,  $c = 9/1.5 = 6$   
Nitrogen balance:  $2.5 \times 1 = 1.5d$   
 $d = 2.5/1.5 = 1.667$

Putting the values of a, b, c, d in the biomass  $C_a H_b O_c N_d$  and we get,  $C_{10} H_{22.33} O_6 N_{1.667}$ .

$$04. \text{ Atomic weights of } \text{CH}_{1.6}\text{O}_{0.4}\text{N}_{0.2}\text{S}_{0.0024}\text{P}_{0.017} = 12 \times 1 + 1 \times 1.6 + 16 \times 0.4 + 14 \times 0.2 + 32 \times 0.0024 + 31 \times 0.017$$

$$= 12 + 1.6 + 6.4 + 2.8 + 0.0768 + 0.527 = 23.40$$

$$\text{Percentage of carbon} = (\text{Atomic weight of carbon} / \text{Total atomic weight}) \times 100$$

$$= (12/23.40) \times 100 = 51.28$$

$$05. \text{ Respiratory quotient (RQ)} = \text{Volume of carbon dioxide produced} / \text{Volume of oxygen consumed}$$

$$= 5.37/12.5 = 0.42$$

$$07. \text{ Total molar mass of } \text{CH}_{1.5}\text{O}_{0.3}\text{N}_{0.3}\text{S}_{0.004} = (1 \times 12) + (1.5 \times 1) + (0.3 \times 16) + (0.3 \times 14) + (0.004 \times 32)$$

$$= 12 + 1.5 + 4.8 + 4.2 + 0.128 = 22.628 \text{ g/mol}$$

$$\text{Mass percentage of carbon} = \frac{\text{Mass of carbon}}{\text{Total molar mass}} \times 100 = \frac{12}{22.628} \times 100 = 53.03$$

$$08. \text{ Molecular weight of glucose} = 180$$

$$\text{Molecular weight of biomass} = 144$$

$$\text{Concentration of glucose needed for } 50 \text{ g L}^{-1} \text{ yeast/litre}$$

$$= (\text{MW of glucose}) / (\text{MW of yeast}) \times (1 \text{ mole glucose}) / 0.48 \text{ mole yeast} \times 50 \text{ g L}^{-1}$$

$$= (180) / (144) \times (1) / 0.48 \times 50 = 130 \text{ g glucose/l}$$

$$\text{Total amount of glucose for } 100,000 \text{ litres} = (130 \text{ g glucose/l}) \times 100,000 \text{ l}$$

$$= 1.3 \times 10^7 \text{ g glucose} = 13,000 \text{ kg glucose}$$

$$11. \text{ Degree of reduction for acetic acid } (\text{C}_2\text{H}_4\text{O}_2) = \frac{\text{Total number of available electrons}}{\text{Number of carbon}} = \frac{4 \times 2 + 1 \times 4 + (-2 \times 2)}{2} = 4$$

$$12. \text{ As we know that mass of oxygen molecule in 1 mole is 32 gram.}$$

$$\text{Hence, mass in 1 kilomole} = 32 \times 1000 = 32000 \text{ gram.}$$

$$17. \text{ Oxygen demand} = \frac{\text{Biomass generated}}{\text{Biomass with respect to oxygen}} = \frac{10 \text{ g / L}}{0.5 \text{ g biomass / gO}_2} = 20 \text{ g O / L}$$

$$26. \text{ Monod's growth kinetics describes how the specific growth rate } (\mu) \text{ of microorganisms depends on the concentration of a limiting extracellular substrate.}$$

$$30. \text{ } t = \frac{1}{\mu} \ln \frac{X}{X_0} = \frac{1}{0.00417} \ln \frac{8}{1} = 498.54$$

$$\text{Time} = 498.54 + 30 = 528.54 \text{ min}$$

$$31. \text{ Given that, } t_d = 20 \text{ minutes}$$

$$X_i = 100, X_f = 100 - 90 = 10$$

$$\text{As we know that, } \ln \left( \frac{X_f}{X_i} \right) = -k_d t$$

$$\ln \left( \frac{0.1}{1} \right) = -k_d (20) \text{ or, } -2.302 = -k_d \times 20$$

$$\text{Hence, } k_d = 0.115 \text{ minutes.}$$

$$33. \text{ Primary metabolites (e.g., amino acids, nucleotides) are produced during the exponential phase for growth. Secondary metabolites (e.g., penicillin) are typically synthesized during the stationary phase.}$$

$$41. \text{ Yield coefficient} = \text{Mass of new cells formed} / \text{Mass of substrate consumed.}$$

$$\text{New cells mass formed} = \text{Cell density} \times \text{Specific growth rate} = 20 \times 0.4 = 8$$

$$\text{Substrate consumed} = 16 \text{ g/l/h}$$

$$\text{Yield coefficient} = \text{mass of product formed} / \text{substrate consumed} = 8/16 = 0.50.$$

$$42. \mu = (\mu_{\max} S) / (K_s + S) \text{ and } \mu = D$$

$$0.1 = 0.4 (S) / 0.3 + S \Rightarrow S = 0.1$$