4.6 Culturing microorganisms and growth curves

Name: _____ Class: _____ Date: _____ **Total: 18 marks**

KEY WORDS aseptic technique • lag phase • stationary phase • colony-forming unit • turbidity

Objective

Practise statements 6.1-6.4 with Core Practical 13: media and aseptic technique, ways of measuring microbial growth, the growth curve's phases, and the exponential growth-rate constant k.

1 Key knowledge and worked examples

■ Aseptic technique protects both ways

Flame the inoculating loop to red heat; flame bottle and tube necks; open plates briefly; work in an updraught near a Bunsen; secure plates and use approved teaching cultures under the school's incubation and disposal procedures. Lower incubation temperatures reduce some risks but do not guarantee that pathogens cannot multiply. Explain the contamination route each precaution addresses.

■ Measuring growth

Total counts (haemocytometer under the microscope), mass/turbidity in broth, and viable counts by serial dilution + plating (each colony = one colony-forming unit from one cell or a clump).

■ The curve and k

Lag (adjusting, synthesising enzymes), log (constant doubling), stationary (deaths balance divisions as nutrients run out and waste builds), death.

$$k = \frac{\log_{10} N_t - \log_{10} N_0}{0.301 \times t}$$

■ Worked example: doubling time

From 1×10^5 to 4×10^6 in 4 hours: log difference $= \log 40 = 1.602$; $k = 1.602/(0.301 \times 4) = 1.33$ generations per hour; doubling time $= 1/k \approx 0.75$ h ≈ 45 minutes.

2 Practice

2.1 Name the four phases of a bacterial growth curve in order. [2]

2.2 State why plates are incubated at 25 °C, not 37 °C, in school laboratories. [1]

2.3 Give one advantage of viable counts over direct microscope counts. [1]

3 Exam-style questions

3.1 A student estimates bacterial concentration by spreading 0.1 cm^3 of a 10^{-4} dilution onto a plate; 46 colonies grow. Calculate the concentration in the original culture, showing your working, and explain why a second plate at the same dilution might give a different count. [4]

3.2 A culture in nutrient broth is followed over 24 hours. Optical density rises slowly for 2 hours, exponentially for 5, then flattens. Name the three phases, explain what limits growth in the last phase, and state why optical density counts dead cells too. [5]

3.3 A liquid culture grows from 8×10^3 to 2×10^7 cells per cm^3 between 2 and 8 hours. Calculate k and the doubling time, and predict the count at 10 hours if exponential growth continued. [5]

Solutions

Original-task marking: M1 method, A1 accuracy, B1 reasoning.

2.1 Lag, log (exponential), stationary, death.

2.2 Human pathogens multiply best at body temperature (37 °C); 25 °C keeps any accident from growing dangerously.

2.3 Viable counts count only living, dividing cells; microscope counts include dead cells.

3.1 Concentration in the diluted sample = 46/0.1 = 460 cm^{-3}; original = $460 \times 10^4 = 4.6 \times 10^6$ cm^{-3}. Different count: colonies arise from clumps as well as single cells, and pipetting/dilution errors plus randomness of distribution vary the plate count.

3.2 Lag, log, stationary. Stationary: nutrients exhausted and metabolic waste accumulates, so death rate rises to equal division rate. Optical density is scattering from ALL cells present, dead or alive.

3.3 Log difference = $\log_{10}(2 \times 10^7/8 \times 10^3) = \log_{10} 2500 = 3.398$; $k = 3.398/(0.301 \times 6) = 1.88\ h^{-1}$; doubling = $1/1.88 \approx 0.53$ h ≈ 32 min. At 10 h (2 more hours): $2 \times 10^7 \times 2^{2 \times 1.88} \approx 2 \times 10^7 \times 13.8 \approx 2.8 \times 10^8$ cm^{-3}.