Oxford Cambridge and RSA

A Level Biology A

H420/02 Biological Diversity

Question Set 10

1 Two students investigated the growth of bacteria at different temperatures.

Three flasks containing identical solutions of nutrient broth were used.

- Flask 1: inoculated with 1cm^3 of broth containing the bacterium *Bacillus subtilis* andincubated at 20 °C.
- Flask 2: inoculated with 1cm^3 of broth containing *B. subtilis* and incubated at 30 °C.
- Flask 3: inoculated with 1cm^3 of broth containing no bacteria and incubated at 30 °C.

Aseptic techniques were used throughout.

At set times over the next 3 days the students removed samples from each flask and measured the number of viable bacteria present.

(a) State one further variable the students should have controlled in their investigation in order to produce **valid** results. **[1]**

pH of broth

(b) The students used the following procedure to determine the number of viable bacteria in eachflask at a given time.

From each flask, 0.1 cm^3 was removed and mixed with 9.9 cm^3 of sterile water in a test tube.This was labelled **Tube A**. A serial dilution then proceeded, as shown in Table 19.1.

Tube	Contents	
B	1 cm^3 of Tube A mixture	9 cm^3 of sterile water
C	1 cm^3 of Tube B mixture	9 cm^3 of sterile water
D	1 cm^3 of Tube C mixture	9 cm^3 of sterile water
E	1 cm^3 of Tube D mixture	9 cm^3 of sterile water
F	1 cm^3 of Tube E mixture	9 cm^3 of sterile water

Table 19.1

From each tube, A–F, 0.1 cm^3 of mixture was cultured on nutrient agar for 24 hours at 30 °C.

The results from Flask 2 after 7 hours of incubation are shown in Fig. 19.

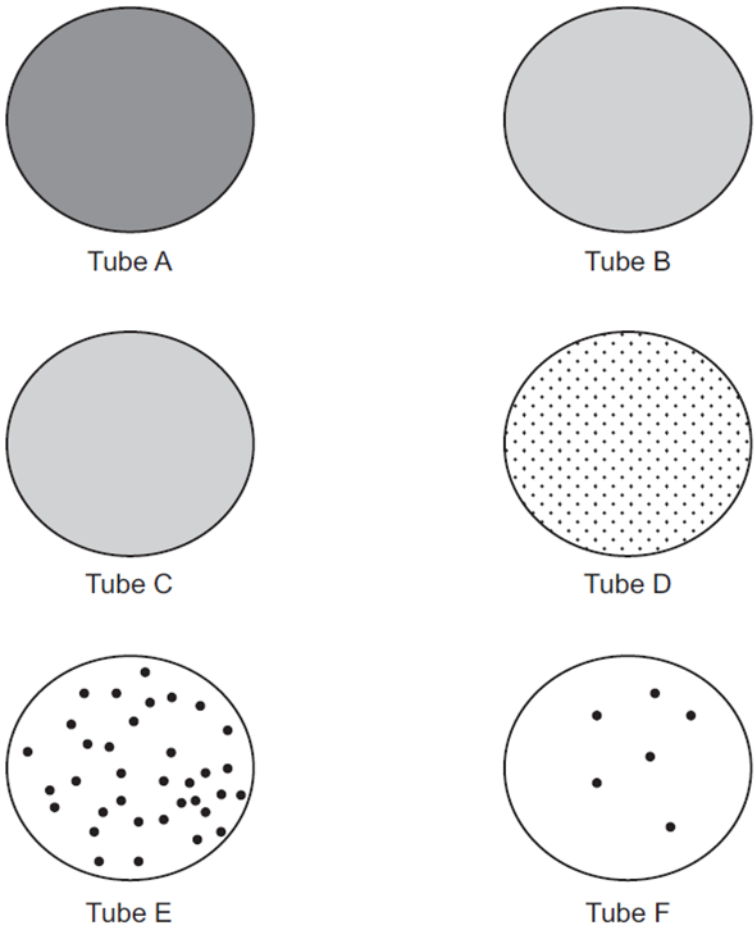

Fig. 19

The students used Tube F to calculate the number of viable bacteria present in the original sample.

(i) Use Tube F to calculate the number of viable bacteria present in the original 0.1 cm^3 sample from Flask 2 after 7 hours of incubation.

Give your answer in standard form.

Answer 6.0×10^7 **[2]**

(ii) The students disagreed about which tube's result to use as a starting point for their calculation.

Discuss whether the petri dish resulting from Tube F was the most appropriate for them to use. **[3]**

E should have been used because it has the most countable colonies. Due to large number of colonies, the anomalies will have smaller effect and the sample would be more representative. Fewer serial dilutions compared to F means the chance of error is reduced.

(c) The processed results from the students' investigation are shown in Table 19.2.

Time after incubation started (hours)	Number of viable bacteria present in Flask 1 at 20 °C	Number of viable bacteria present in Flask 2 at 30 °C
0	7.0×10^2	7.1×10^2
2	6.8×10^2	7.4×10^2
4	4.7×10^4	2.5×10^6
8	6.5×10^7	9.2×10^{10}
12	2.4×10^9	1.8×10^{11}
18	7.8×10^{10}	1.8×10^{11}
24	9.2×10^{10}	5.5×10^8
36	8.6×10^{10}	4.2×10^4
48	6.0×10^9	6.7×10^2
60	5.7×10^7	5.2×10^2
72	1.3×10^5	3.1×10^2

Table 19.2

(i)* Using the information in Table 19.2, compare and explain the patterns of growth seen at 20 °C and at 30 °C. **[6]**

Both temperatures have similar lag phase but it's quicker overall at 30°C. This is because the molecules have more kinetic energy and bacterial enzymes are closer to optimum temperature. More kinetic energy means faster enzyme activity thus exponential phase rises faster at 30°C and stationary phase at 30 °C is shorter by 6 hours. As the population rises, more competition for nutrients and other resources (e.g. carbon source, minerals) occurs but quicker at 30°C. Nutrients become limiting factor and toxic metabolites build up causing reduction in population. Resources reduce and metabolites build up faster at 30°C thus death phase is faster and it reaches lower population at 30°C.

(ii) No bacteria were detected at any time in the flask that was inoculated with nutrient broth that did not contain bacteria.

Explain the purpose of this flask. control to check for contamination **[2]**

(iii) The teacher told the students they should not investigate the growth of bacteria at 35 °C.

Suggest why the teacher told them not to grow bacteria at 35 °C. **[1]**

Because it could encourage the growth of human pathogens.

(iv) The teacher also suggested that the students should have carried out the investigationusing three flasks at each temperature.

Explain how this suggestion would have improved the students' investigation. **[3]**

repeat and calculate the mean to increase reliability and accuracy respectively. Statistical test can be also conducted.

Total Marks for Question Set 10: 18