

Cloning and Biotechnology

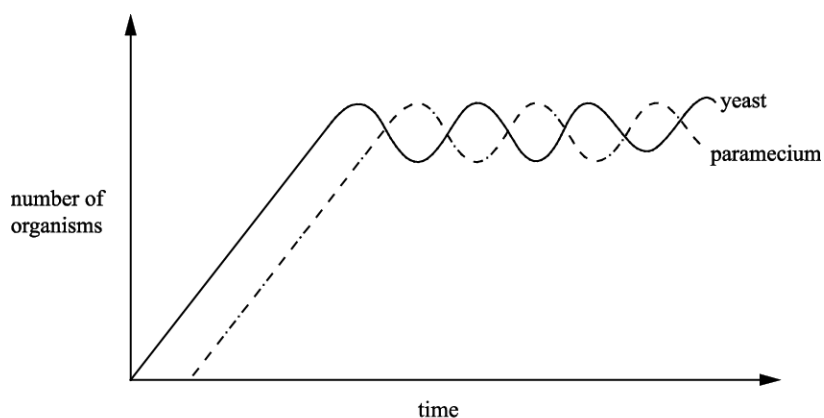
1. Immobilised enzymes can be produced by which of the following methods?

- A. binding enzyme to a soluble matrix
- B. intermolecular hydrogen bonding of enzymes
- C. absorbing enzymes onto the surface of a gel
- D. enclosing enzymes within a partially permeable membrane

Your answer

[1]

2. The graph shows a population of yeast and a unicellular organism, *Paramecium*, grown in a fermentation chamber.



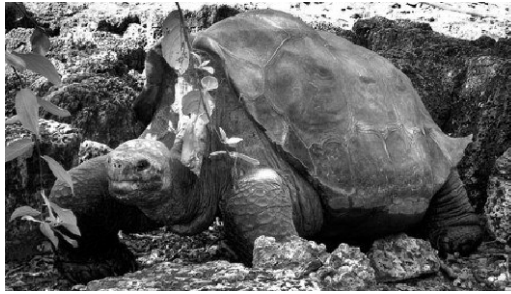
Which **one** of the following statements best describes the relationship between the two organisms?

- A. The *Paramecium* and yeast populations are complementary to each other.
- B. The yeast thrives in the relationship at the expense of the *Paramecium* population.
- C. The *Paramecium* feeds on the yeast and reduces the number in the yeast population.
- D. The two populations are in equilibrium and stable due to a type of negative feedback.

Your answer

[1]

3. The last giant Galapagos tortoise died in 2012. Scientists froze some of the tortoise's cells.



The following statements describe processes involved in potential cloning of the giant Galapagos tortoise using the cells. They are **not** in the correct order.

- 1 A donor egg is enucleated.
- 2 The embryo develops into a mature egg, which is incubated.
- 3 A somatic cell from the tortoise is defrosted and the nucleus is removed.
- 4 Electrofusion of the host cell and new nucleus.
- 5 The somatic cell nucleus is inserted into the enucleated oocyte.
- 6 The transformed egg divides *in vitro*.

Which option states the correct order for producing a clone of the giant Galapagos tortoise?

- A. 1, 3, 4, 5, 6, 2
- B. 3, 5, 1, 4, 2, 6
- C. 1, 6, 3, 5, 4, 2
- D. 3, 1, 5, 4, 6, 2

Your answer

[1]

4. A student investigated the effect of different sugars on the growth of bacteria.

The student found that the bacteria grew well when provided with glucose, sucrose and fructose, but did not grow well when provided with lactose.

Which statement, **A** to **D**, provides the best explanation for these results?

- A. lactose was too large to be absorbed
- B. the bacteria could respire only monosaccharides
- C. the bacteria did not possess the enzyme to digest lactose
- D. the bacteria were inhibited by lactose

Your answer

[1]

5. The table shows the growth of a population of microorganisms.

Time (h)	Estimated population size (cells per mm ³)
0	1.0×10^3
4	4.0×10^3
8	9.0×10^3
12	1.8×10^4
16	3.1×10^4
20	5.8×10^4
24	1.4×10^5

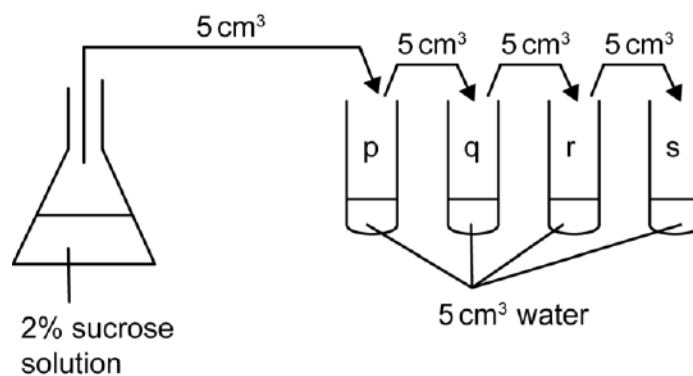
During which time period is the maximum (absolute) growth rate?

- A. 0 – 4 hours
- B. 8 – 12 hours
- C. 16 – 20 hours
- D. 20 – 24 hours

Your answer

[1]

6. The diagram shows a serial dilution.



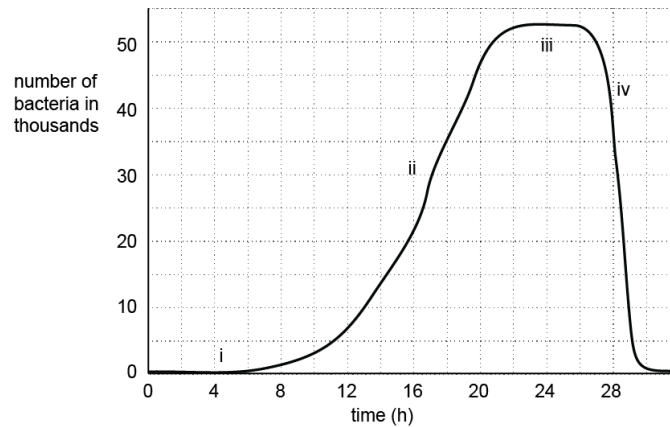
Which of the options, **A** to **D**, shows the correct concentrations of sucrose in tubes p – s?

- A. p = 0.2% q = 0.02% r = 0.002% s = 0.0002%
- B. p = 1% q = 0.5% r = 0.2% s = 0.1%
- C. p = 1% q = 0.5% r = 0.25% s = 0.125%
- D. p = 0.2% q = 0.1% r = 0.05% s = 0.025%

Your answer

[1]

7. The graph shows the growth of a population of bacteria in a closed culture.



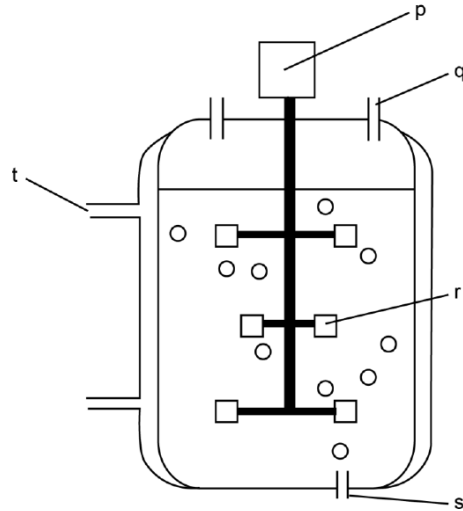
Which of the rows, **A** to **D**, correctly identifies the stages in the growth curve where primary and secondary metabolites are produced?

Row	No metabolites	Mainly primary metabolites	Mainly secondary metabolites
A	i	ii	iii & iv
B	-	i & ii	iii & iv
C	i & iv	ii	iii
D	iv	i & iii	ii

Your answer

[1]

8. The diagram shows a simple fermenter.



Which row, **A** to **D**, correctly identifies the labelled components?

Row	P	q	r	s	t
A	motor	air inlet	stirring paddle	gas outlet	water outlet
B	stirring paddle	gas outlet	nutrient block	air inlet	water inlet
C	motor	gas outlet	stirring paddle	air inlet	water outlet
D	stirring paddle	gas outlet	nutrient block	gas outlet	water inlet

Your answer

[1]

9. Bacteria are used in many areas of biotechnology.

In which of the following processes, **A** to **D**, do bacteria **not** play an active role?

- A** bioinformatics
- B** bioremediation
- C** cheese-making
- D** manufacturing human insulin

Your answer

[1]

10. Mycoprotein is a food produced using the fungus *Fusarium venenatum*.

Which statement about mycoprotein is correct?

- A production of protein is slower than in animals and plants
- B production is dependent on seasons
- C waste products can be used as a substrate
- D there are no ethical issues associated with production

Your answer

[1]

11. Corals are a group of animals that usually live on the sea bed close to the surface of the water.

Many corals can reproduce both sexually and asexually.

Which of the following statements about asexually-produced coral offspring is **not** true?

- A All offspring produced from an individual organism will be genetically identical.
- B If a change in the environment harms one of the offspring produced from an individual organism it will probably harm them all.
- C Meiosis occurred in order to produce the offspring.
- D The offspring will tend to thrive if conditions are similar to those present when the parent organism reproduced.

Your answer

[1]

12(a). Plants have the ability to propagate themselves naturally by cloning. This ability is used by humans commercially when carrying out tissue culture.

Strawberry plants produce clones using runners. This is an example of natural cloning.

State **one** other method of natural cloning in plants.

----- [1]

(b). Name the type of plant tissue from which natural clones are produced.

----- [1]

13. Scientists are able to clone desirable plants that show a high rate of photosynthesis. The following passage describes how plants are cloned.

Complete the passage using the most appropriate words or phrases.

Cells are removed from the meristem tissue in axial buds or tips. The tissue sample that is removed is called the Ethanol can be used to the plant tissue. Hormones are used to stimulate mitosis, which produces a mass of cells called a

[4]

14. The potato plant, *Solanum tuberosum*, is a staple food plant in many parts of the world. Potatoes are susceptible to infection by a pathogen called *Phytophthora infestans*, which causes a disease known as potato late blight. The most visible sign of the disease is a brown discolouration of the leaves. Some varieties of potato are resistant to infection by *P. infestans*.

The resistance of different varieties of *S. tuberosum* to infection by *P. infestans* was investigated.

- Three different clones, A, B and C, of *S. tuberosum* were used.
- The clones were grown in adjacent fields over the same time period.
- The percentage of leaf area affected by the disease was estimated at regular intervals.

The results are shown in Fig. 18.

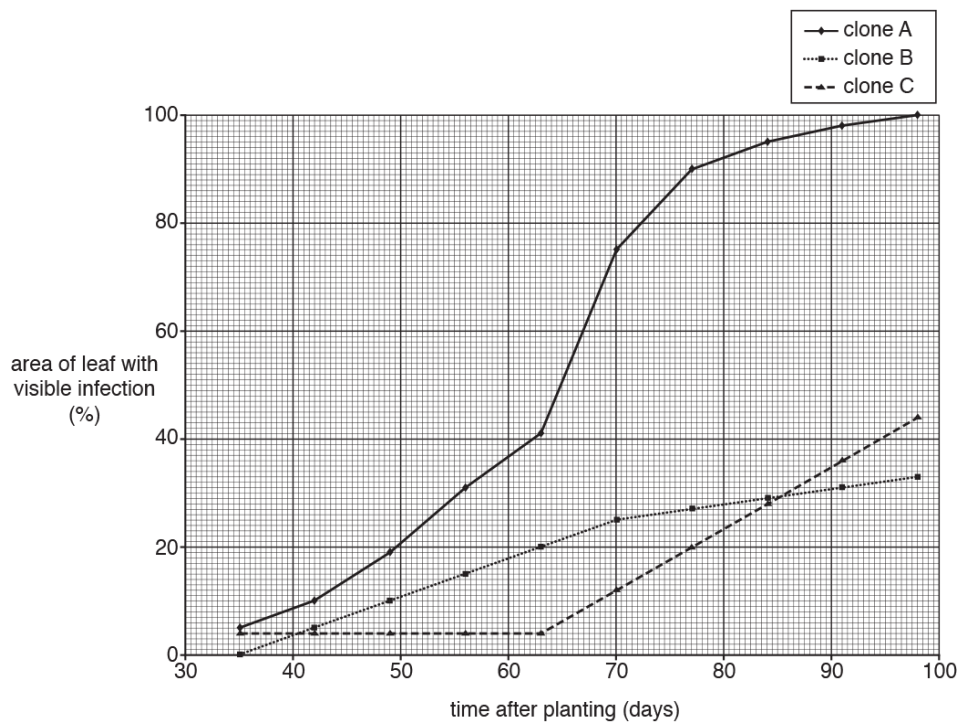


Fig. 18

i. Suggest why it is important to use clones in an investigation such as this.

.....

.....

.....

.....

[2]

- ii. State how a clone of potatoes could be produced for this investigation and explain why it is important to carry out this procedure under aseptic conditions.
procedure

Asepsis is important because

[2]

- iii. The extent of infection is estimated by comparing the area under the curve from the graph. The area under the curve for clone **B** is 1250. (Units can be ignored in this instance.)
Using Fig. 18, calculate the approximate area under the curve, between day 35 and day 98, for clone **C**.

Answer

----- [3]

- iv. Calculate the area under the curve for clone **C** as a proportion of the area under the curve for clone **B**.

Answer

----- [1]

- v. **Using Fig. 18**, suggest why the area under the curve is used as a measure of infection rather than the area of leaf that is visibly affected on a given day.

[2]

- vi. The clones were planted in adjacent fields in order to control variables such as temperature, wind speed and rainfall. Suggest two other abiotic variables that this precaution was intended to control.

1 -----

2 -----

[2]

15. Many plants can produce natural clones of themselves. Gardeners and farmers take advantage of this natural process by taking cuttings.

When a genetically modified plant is created, it may be cloned into many plantlets in the process called micropropagation.

Compare the equipment and techniques of taking cuttings with those used for micropropagation.

[2]

16(a). Plant cloning is often used by farmers to produce new plants.

Cloning plants is also known as vegetative propagation.

Identify **three** advantages of vegetative propagation in agriculture.

1

2

3

[3]

(b). A farmer had two fertiliser solutions, solution **A** and solution **B**, and wanted to investigate which one to use on lavender plants. In order to ensure the investigation would be valid, two cuttings were needed from the same parent plant.

i. Describe how to clone a plant by taking a cutting.

[4]

[6]

17(a). Tissue culture greatly increases the number of cloned plants that can be produced from a single parent plant.

Outline how it is possible to produce many clones from a single original parent plant.

[2]

(b). Pineapples are plants that can be cloned by tissue culture.

Plant hormones are used during the tissue culture process. One of these plant hormones is known as BAP.

The table below shows the effect of the concentration of BAP on the length of pineapple shoots.

BAP concentration (mg dm ⁻³)	Mean shoot length (mm)
0	33.8
1	27.5
2	23.6
3	30.8
4	37.0
5	49.9
6	26.4
7	22.3

i. Calculate the percentage increase in mean shoot length caused by adding 5 mg dm⁻³ BAP.

Give your answer to **2** significant figures.

Percentage increase = % **[2]**

ii. The valid investigation that generated the results shown above featured an independent variable, a dependent variable and several controlled variables.

State the independent and dependent variables and suggest **two** appropriate controlled variables.

Independent variable

Dependent variable

Controlled variables

1

2

[3]

18(a). It is possible to clone animals using a technique called somatic cell nuclear transfer (SCNT).

The most well-known example of this was the cloning of Dolly the sheep in 1996.

Thirty years before Dolly the sheep, successful cloning of an animal was carried out using a frog, *Xenopus laevis*.

Frogs lay eggs in water. These eggs then develop and hatch into swimming tadpoles. When the tadpoles grow to a certain size they develop into adult frogs.

The cloning process is outlined in Fig. 19.1.

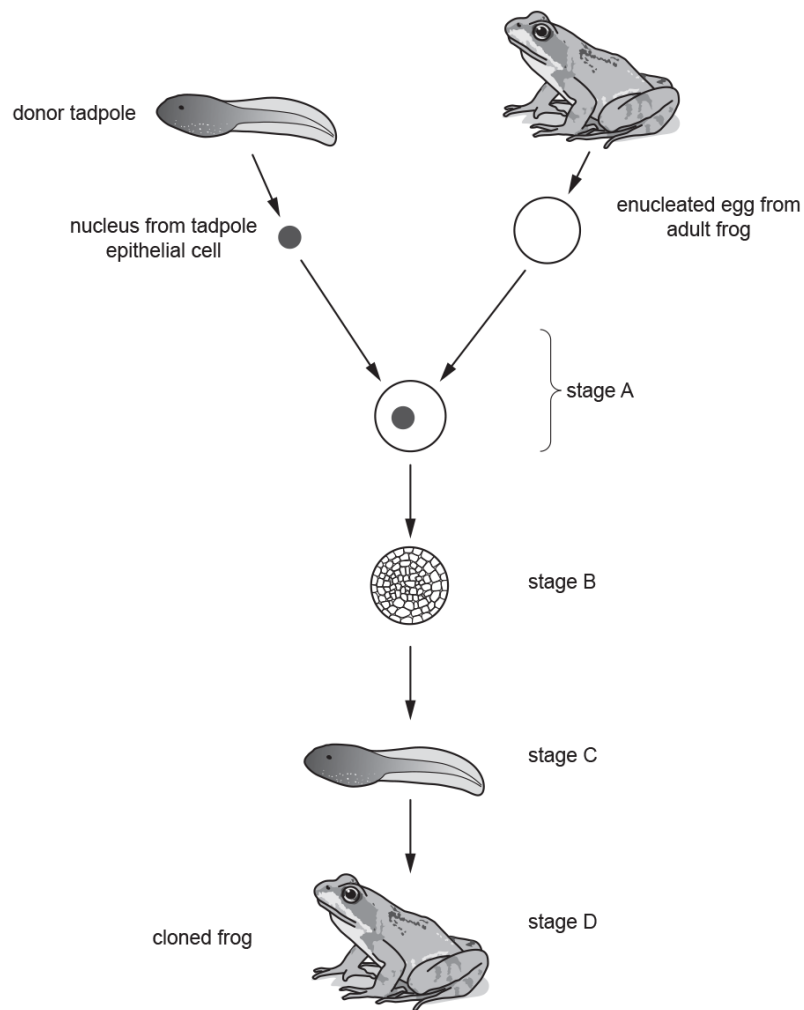


Fig. 19.1

- i. Describe what is happening at stage A **and** suggest a practical procedure that could allow this to occur.

- ii. Identify a key difference between the processes between stages A and C and the cloning of Dolly the sheep.

[1]

- iii. The frog produced by the process in Fig. 19.1 is not a complete clone of the donor tadpole.

Suggest why the cloned frog might not be considered a complete clone of the donor tadpole.

[1]

(b). The success of SCNT has been investigated in many species.

Sheep are more closely related to mice than they are to *Xenopus* frogs.

Fig. 19.2 shows the percentage of SCNT procedures that were successful in mice and *Xenopus* when the donor nucleus was taken from cells at different stages of development.

- The *Xenopus* data were published in 1962.
- The mouse data were published in 1998.

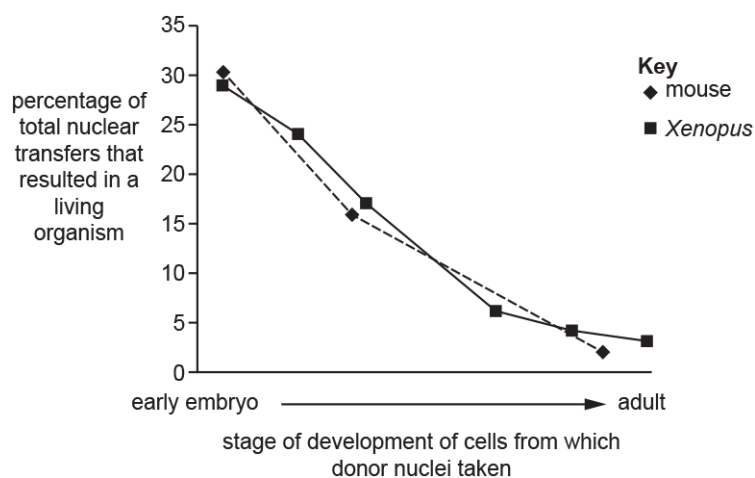


Fig. 19.2

- i. Suggest why the x-axis does not show the age of the donor nuclei.

[2]

- ii. Dolly the sheep suffered health problems throughout her life and died at an early age.

The donor nucleus that was used to create Dolly came from a sheep that was already five years old. The normal lifespan of a domestic sheep is ten years.

A student concluded that Dolly's health problems were caused by the stage of development of the sheep that provided the donor nucleus.

List three reasons why the information in Fig. 19.2 does **not** support the student's conclusion.

1 _____

2 _____

3 _____

[3]

- (c). One measure of the success of cloning procedures is the number of pregnancies that result in live births.

Table 19 shows information from the work of many scientists about the success of SCNT in four different species.

Species	Number of pregnancies	Number of live births
Goat	26	8
Monkey	3	2
Mouse	438	56
Sheep	110	48

Table 19

- i. Calculate the percentage of pregnancies that resulted in live births in goats and mice.

live births in goats = %

live births in mice = %

[2]

- ii. Compiling results from different scientists can have problems as their investigations may not have been controlled in the same way.

List **three** factors that should have been controlled when compiling the data to include in Table 19.

1

2

3

[3]

19. Mice are often used in laboratory studies to research treatments for heart conditions.

These mice are often clones.

Suggest one reason why clones are used in these studies.

[1]

20. Crude oil contains hydrocarbons.

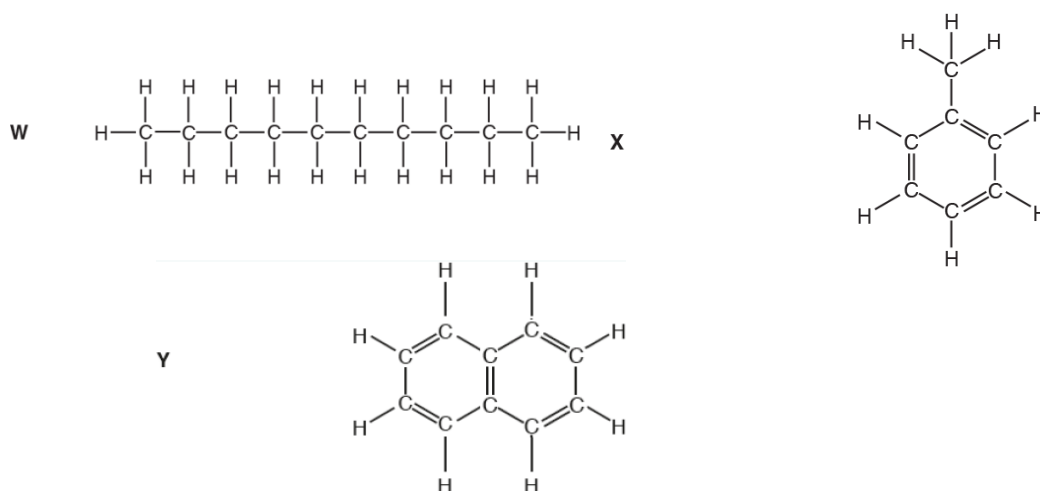


Fig. 22

Crude oil is often spilled from ships into the sea causing great damage to wildlife. The chemicals in crude oil are harmful to many species and do not break down quickly in the environment.

Some bacteria can break down the hydrocarbons in crude oil. These bacteria have been used by conservationists at sites where oil has been spilled.

- i. The rate of hydrocarbon breakdown by bacteria can be increased by spraying the oil with detergent. Detergents break up oil into droplets, thereby increasing their surface area.
 - Student A concluded that the detergent speeded up the rate of hydrocarbon breakdown **only** because it increased the surface area of hydrocarbon upon which the bacteria could grow.
 - Student B concluded that the detergents **also** increased the growth of the bacterial population by an alternative mechanism.

Use the information in Fig. 22 and your knowledge of bacterial growth requirements to provide support for student B's conclusion.

[3]

- ii. Suggest **one** piece of evidence that would further support student B's conclusion.

[1]

[6]

- ii. On an industrial scale, microorganisms can be cultured using either batch fermentation or continuous fermentation.

The table below lists statements about industrial culturing of microorganisms.

Place ticks (✓) in the table to indicate whether each statement applies to batch or continuous fermentation.

Statement	Batch	Continuous
Waste is removed during the fermentation process		
A fixed volume of nutrient medium is used		
Secondary metabolites are more likely to be produced		
The growth rate tends to be faster		
The culture is grown for a fixed period of time		

[3]

23. Table 18.1 compares burgers made from fungal protein with burgers made from beef.

Protein source	Total fat (%)	Saturated fat (%)	Cholesterol (%)	Essential amino acids (%)	Energy (kJ 100g ⁻¹)
Fungus	4.8	0.5	0.000	4.98	611
Beef	24.7	10.7	0.076	7.92	1218

Table 18.1

Some people think that we should produce fungal protein rather than beef for human consumption. Use Table 18.1 and your own knowledge to discuss this claim.

[4]

24. Laboratory techniques are used by workers in various professions, and by scientists.

A patient has been coughing blood, and it is suspected that bacteria will be found in the blood.

A medical technician cultures the blood on an agar plate. What measures should the technician take to keep the agar plate culture sterile?

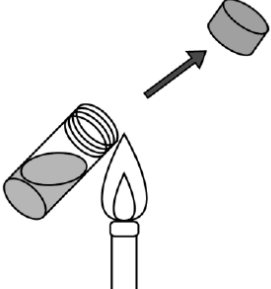
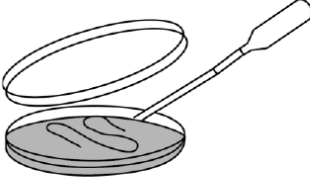
[2]

25(a). A student was asked to measure the population density of bacteria in a broth. The student was supplied with a broth culture of the bacterium *Bacillus subtilis*. The teacher suggested that the student should measure the population by transferring a sample of the broth to an agar plate then incubating the plate for 24 hours. The bacterial colonies could then be counted.

The student took certain precautions to avoid contaminating the cultures.

Explain how each precaution shown in the table below helped to avoid contamination.

Write your answers in the spaces provided on the table.

Precaution	Explanation
	
	

[2]

(b). After incubation for 24 hours, the student studied the agar plate. The plate was completely covered by a film of bacteria and it was not possible to count colonies.

Describe a modification to the procedure that would enable the student to estimate the population size.

[2]

(c). Microorganisms such as the single-cell fungus *Fusarium* can be cultured to grow food for the human population. In order to scale up cultures of microorganisms scientists use large fermenters. A study was carried out to determine which of two species of *Fusarium* would be better for production of fungal protein.

Fig. 18.1 shows the results of the study.

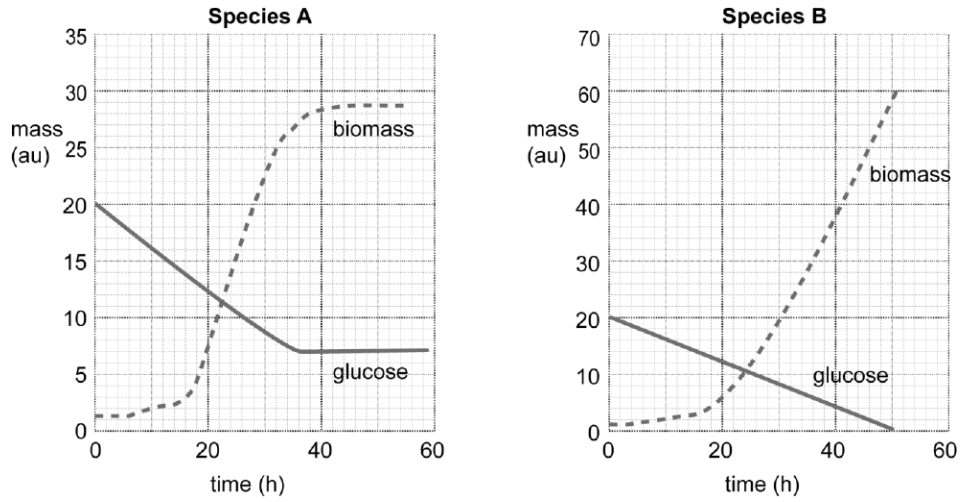


Fig. 18.1

- i. Calculate the percentage of glucose used by species A.

Answer = % [2]

- ii. Using the information in Fig. 18.1 suggest which species would be better for use in production of fungal protein for human consumption.

Explain your choice.

[2]

- ii. Describe **two** practical considerations to ensure the *S. cerevisiae* population grows successfully when the initial culture is established.

----- [2]

- iii. Scientists wanted to estimate the number of yeast cells in a 25 cm³ solution of *S. cerevisiae*. They carried out the following two dilutions:
- 1 cm³ of the original solution was mixed with 9 cm³ of nutrient solution to make solution 2.
 - 1 cm³ of solution 2 was mixed with 9 cm³ of nutrient solution to make solution 3.

The scientists transferred 0.1 cm³ of solution 3 onto an agar plate. 15 separate colonies grew on the plate.

Calculate the number of yeast cells in the original 25 cm³ solution.

Express your answer in standard form to **three** significant figures. Show your working.

Answer: [2]

27(a). Two students investigated the growth of bacteria at different temperatures.

Three flasks containing identical solutions of nutrient broth were used.

- Flask 1: inoculated with 1 cm³ of broth containing the bacterium *Bacillus subtilis* and incubated at 20 °C.
- Flask 2: inoculated with 1 cm³ of broth containing *B. subtilis* and incubated at 30 °C.
- Flask 3: inoculated with 1 cm³ 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.

State one further variable the students should have controlled in their investigation in order to produce **valid** results.

----- [1]

(b). The students used the following procedure to determine the number of viable bacteria in each flask 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 $30 \text{ }^\circ\text{C}$. The results from Flask 2 after 7 hours of incubation are shown in Fig. 19.

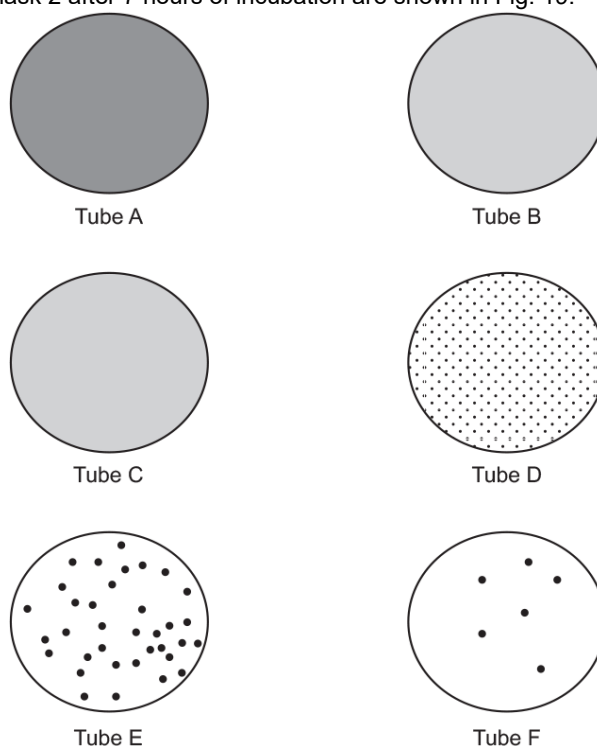


Fig. 19

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 [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]

(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

- 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.

[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]

- iv. The teacher also suggested that the students should have carried out the investigation using three flasks at each temperature.

Explain how this suggestion would have improved the students' investigation.

[3]

28. A student was asked to measure the population density of bacteria in a broth. The student was supplied with a broth culture of the bacterium *Bacillus subtilis*. The teacher suggested that the student should measure the population by transferring a sample of the broth to an agar plate then incubating the plate for 24 hours. The bacterial colonies could then be counted.

* Once the student had mastered the procedure to estimate population size, they decided to investigate the effect of temperature change on the rate of population growth.

The student used the following procedure:

- A broth culture was incubated at 20°C.
- Every four hours a pipette was used to transfer a sample of the culture to agar growth medium in a petri dish.
- The sample was spread over the surface of the agar by tilting and swirling the dish.
- The petri dish was incubated at 30°C for 24 hours.
- After 24 hours the petri dish was labelled and stored in a refrigerator until all results were complete.
- The procedure was repeated with broth cultures incubated at 10°C, 30°C and 40°C.
- Once all the agar plates had been collected the student removed them from the refrigerator and estimated population size by counting the visible colonies.
- Finally the student converted the data into a growth rate.

Describe and explain modifications that the student should make to improve the investigation and ensure the data collected are valid. (You may assume that full aseptic technique was used.)

- ii. Calculate the total number of bacterial cells that would have been present in a 50 cm³ container on day 1.

Write your answer in standard form.

Number of bacterial cells = [3]

- iii. Describe a laboratory procedure that the scientists might have used to estimate the bacterial population.

----- [2]

30. Some humans are lactose intolerant. Milk can be treated with lactase to reduce the concentration of lactose present. Fresh milk is passed over lactase molecules immobilised on a suitable matrix.

Give two **economic** advantages of immobilising enzymes for large-scale production.

1 -----
2 ----- [2]

31(a). Tannase is an enzyme produced by some microorganisms. Tannase is useful in many industrial applications including food production.

The tannase used in food production can be free in solution or immobilised.

State one method by which tannase could be immobilised.

----- [1]

- iv. The results suggested that immobilised tannase was more stable over a range of pH values than free tannase.

Explain why immobilised tannase is more active at pH8 than free tannase.

[2]

(c). Enzymes used in food production can be free in solution or immobilised.

Immobilised enzymes are often active over a greater pH and temperature range than free enzymes.

Using immobilised enzymes can be cheaper than using free enzymes.

- i. Suggest **two** reasons why using immobilised enzymes in industrial processes could be cheaper than using free enzymes.

1

2

[2]

- ii. State **one** disadvantage of using immobilised enzymes in industrial processes.

[1]