

Q1.

- 3 (a) Three different strains, **A**, **B** and **C**, of a species of bacterium were grown on nutrient agar in a divided petri dish until they formed 'lawns' covering the agar.

Three discs of filter paper were soaked in a solution of a penicillin antibiotic and one disc placed in contact with each of the bacterial strains for 10 minutes.

After 24 hours, zones of clearance, indicating bacterial cell death, were seen in the 'lawns' of strains **A** and **B**.

The appearance of the petri dish 24 hours after addition of antibiotic is shown in Fig. 3.1.

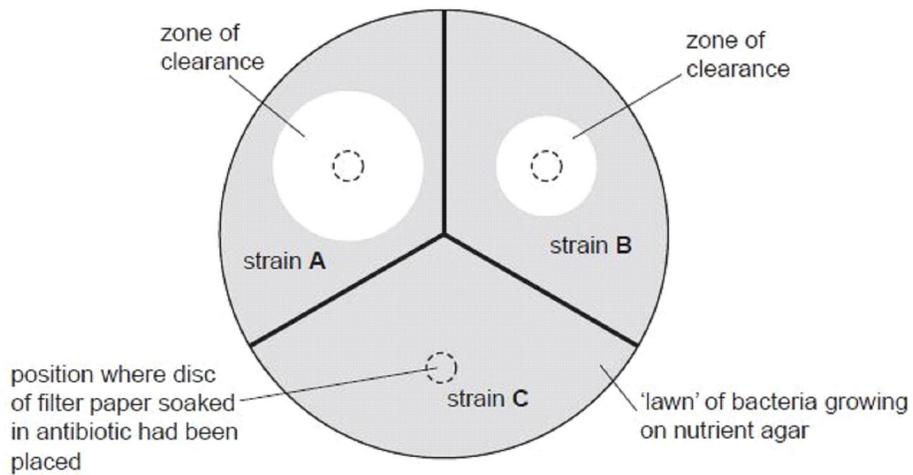


Fig. 3.1

- (i) The effectiveness of the antibiotic is proportional to the area of the zone of clearance.

Measure the diameters (**d**) of each of the zones of clearance of bacterial strains A and B and record them to the nearest mm in Table 3.1.

Using πr^2 , calculate the **area** in mm^2 of the zone of clearance for each strain of bacterium and record them in Table 3.1.

Calculate the ratio of the **area** for strain **A** to the **area** for strain **B** and record the ratio in Table 3.1.

Table 3.1

bacterial strain	A	B
diameter (d) of zone of clearance / mm
area of the zone of clearance / mm^2
area for strain A : area for strain B	

[3]

Q2.

- 5 A significant quantity of the metallic copper produced in some countries is obtained using the chemoautotrophic bacterium, *Thiobacillus ferro-oxidans*, which is present in rocks in many countries.

The process, known as bioleaching, is shown in Fig. 5.1.

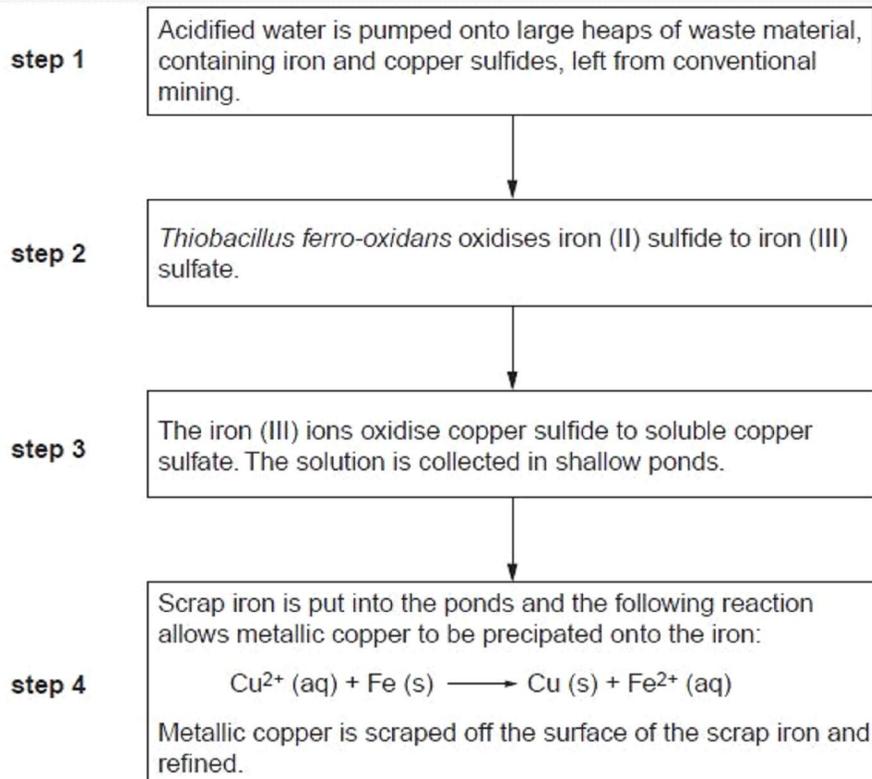


Fig. 5.1

- (a) Suggest the benefit to the bacterium of **step 2**.

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(b) State two environmental disadvantages of extracting metals by bioleaching.

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(c) Explain why the production of metallic copper by bioleaching can be cheaper than using conventional mining methods.

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[Total: 8]

Q3.

5 The fungus *Penicillium chrysogenum* is grown in fermenters on an industrial scale to produce penicillin, using a batch culture system.

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(a) Explain why batch culture, rather than continuous culture, is used for the production of penicillin.

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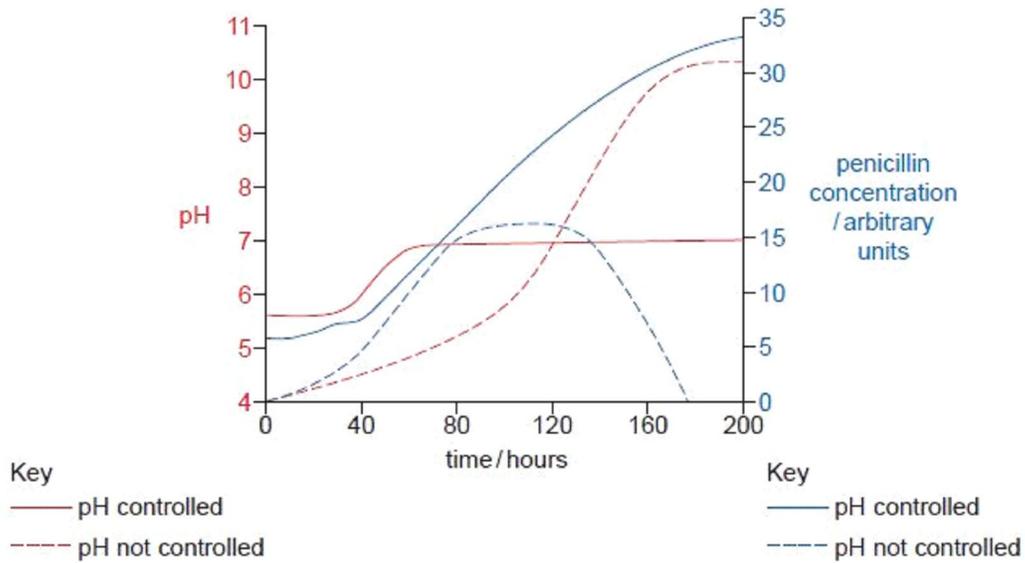
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(b) Temperature and pH are normally controlled in the fermenter. Temperature is kept constant, while pH is held at a value of 5.5 for the first stage of the fermentation and then raised to 6.8 and kept constant for the remainder of the fermentation period.

Fig. 5.1 shows how the pH and the concentration of penicillin in the culture change over time, when the pH is controlled and when the pH is not controlled.



With reference to Fig. 5.1, describe and explain the differences in the concentration of penicillin in the culture when the pH is controlled and when the pH is not controlled.

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(c) Explain why penicillin affects bacteria but not viruses.

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[Total: 9]

Q4.

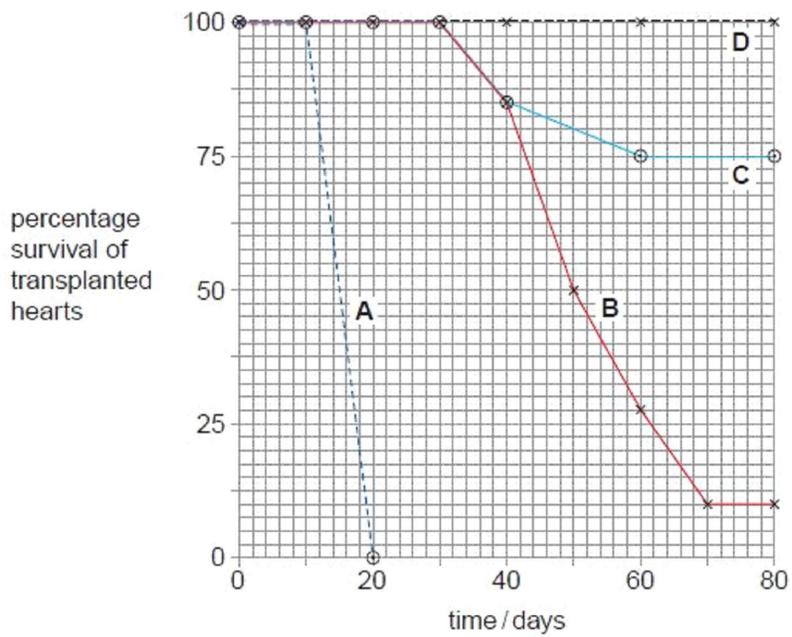


Fig. 2.1

With reference to Fig. 2.1

- (i) describe the effectiveness of the four different treatments

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(ii) suggest an explanation for the differences in survival of the transplanted hearts in groups **B** and **D**.

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(c) Examination of the transplanted hearts showed that the hearts in group **A** had significant damage to their coronary arteries, whereas in group **D** these blood vessels appeared normal.

Explain the importance of the coronary arteries of the heart.

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(d) State two uses of mAbs in humans, other than preventing rejection of transplanted tissue.

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[Total: 15]

Q5.

Bacteria **A** and **B** have different outer layers, as shown in Fig. 3.2.

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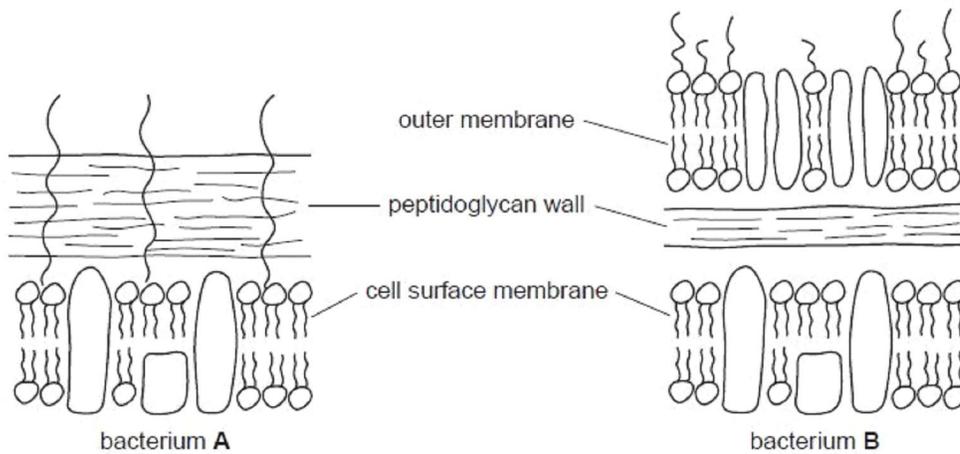


Fig. 3.2

(b) With reference to Fig. 3.1 and Fig. 3.2

(i) describe how the outer layers of bacterium **B** differ from those of bacterium **A**

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(ii) explain the different effects of penicillin V on bacteria **A** and **B**

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(iii) suggest how the synthetic penicillin, carboxypenicillin, is able to affect the growth of bacterium **B**.

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(c) Distinguish between batch culture and continuous culture of microorganisms.

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(d) Explain why batch culture rather than continuous culture is used in the production of penicillin.

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[Total: 15]

Q6.

- 2 When gold is associated with mineral ores such as iron sulfide, the sulfides must be oxidised to release the gold particles. Since the mid 1990s, gold has been extracted from such ores by bioleaching.

Suitable bacteria oxidise iron sulfide to soluble iron sulfate, releasing Fe^{3+} and SO_4^{2-} ions. The reaction releases heat energy and temperatures within a heap of ore that is being bioleached (a bioheap) can reach 70°C or higher.

Examples of bacteria used in this bioleaching are shown in Table 2.1.

Table 2.1

example of bacterium	temperature range for growth / $^\circ\text{C}$	activity	natural habitat
<i>Acidithiobacillus ferrooxidans</i>	35 – 45	oxidise iron and sulfur compounds	acid springs
<i>Sulfobacillus thermosulfidooxidans</i>	45 – 65		
<i>Sulfolobus metallicus</i>	65 – 95		

- (a) With reference to Table 2.1, suggest

- (i) a natural habitat for organisms such as *S. thermosulfidooxidans* and *S. metallicus*

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- (ii) why all three species of bacteria, rather than just one species, are mixed with ore in a bioheap.

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(ii) Explain why bioleaching is now used on a large scale throughout the world.

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[3]

(c) Gold-bearing sulfide ores often contain arsenic, which is potentially toxic to the bacteria used in bioleaching. However, arsenic-resistant strains of *A. ferrooxidans* have been found in some mines.

The activity of two strains of the bacterium, in the presence and absence of arsenic ions, is shown in Table 2.2.

Table 2.2

strain of <i>A. ferrooxidans</i>	oxidation rate of iron in the ore / $\text{mg dm}^{-3}\text{h}^{-1}$	
	arsenic ions absent	arsenic ions present
1	16	15
2	48	47

Describe the results shown in Table 2.2 **and** explain the role of natural selection in the evolution of arsenic-resistant bacteria.

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[4]

[Total: 14]

Q7.

2 (a) Outline how an enzyme can be immobilised in alginate.

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(b) State two advantages, other than stability, of using an immobilised enzyme in an industrial process compared with the same enzyme that has not been immobilised.

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Q8.

- 5 In the USA, about 35% of all maize that is grown has been genetically modified to produce a toxin, called Bt toxin, derived from the bacterium *Bacillus thuringiensis*. The genetically modified plants are known as Bt maize.

(a) Explain the advantages of growing Bt maize.

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- (b) An investigation was carried out into the potential effects of dead leaves from Bt maize on organisms living in streams that flow through areas where the maize is grown.

The researchers conducted a laboratory-based experiment in which larvae of one species of aquatic caddis fly, *Lepidostoma liba*, were fed on non-Bt maize leaves, or on leaves from Bt maize. The growth rates of the larvae were measured.

The results are summarised in Fig. 5.1.

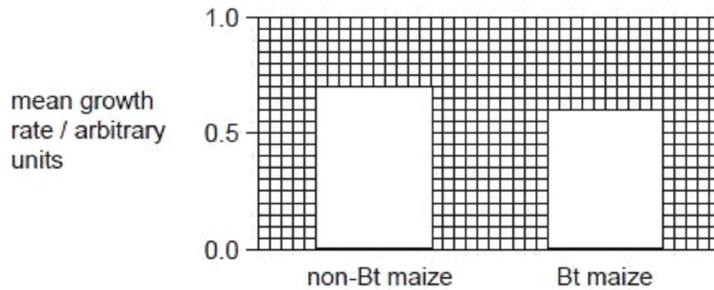


Fig. 5.1

Describe the effect of eating leaves from Bt maize on the growth rate of *L. liba* larvae.

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(c) In a second laboratory-based experiment, three groups of larvae of a different species of caddis fly, *Helicopsyche borealis*, were fed on pollen containing:

- A no Bt toxin
- B Bt toxin at concentrations found in streams in maize-growing areas
- C Bt toxin at concentrations twice as high as found in those streams.

The researchers measured the mortality rates of the caddis fly larvae.

Their results are summarised in Table 5.1.

Table 5.1

groups compared	difference in mortality rate
groups A and B	no significant difference
groups A and C	significantly greater mortality in C than in A

The researchers were careful to state that their results showed the 'potential ecological effects' on the caddis fly larvae of growing Bt maize.

Suggest **two** reasons why 'potential ecological effects' is a suitable description of any conclusions that could be drawn from the results of this experiment.

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(d) When the results of the experiments described in (b) and (c) were published, many other scientists criticised the research very strongly.

Suggest why some scientists might wish to suppress results such as these, even if there is no fault with the investigation itself.

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[Total: 7]

Q9.

2 Some of the steps in the production of monoclonal antibodies are shown in Fig. 2.1.

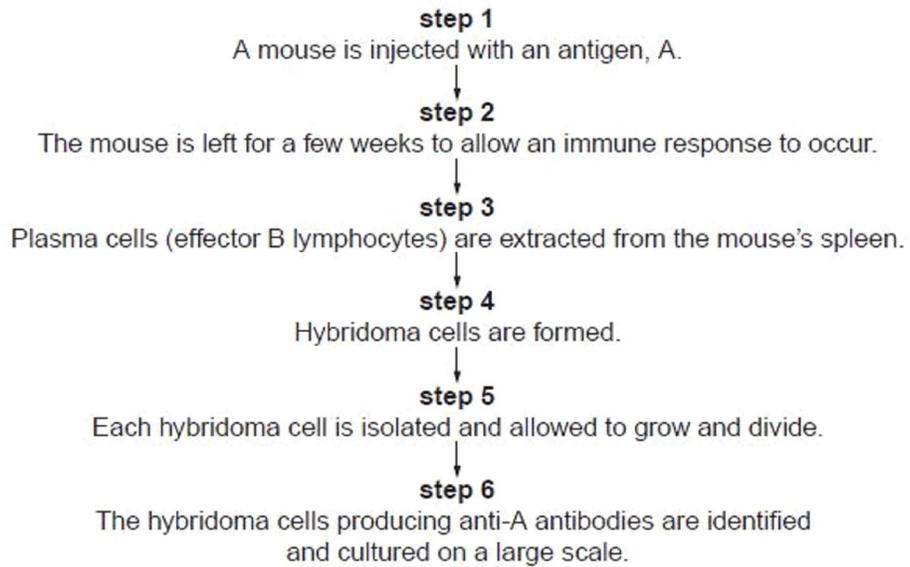


Fig. 2.1

(a) With reference to Fig. 2.1, explain:

(i) what happens during an immune response (**step 2**)

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(ii) what is meant by a hybridoma cell (**step 4**)

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(iii) why hybridoma cells need to be formed (**step 4**)

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(iv) how hybridoma cells producing anti-A antibody can be identified.

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(b) Rheumatoid arthritis (RA) is an autoimmune disease in which T lymphocytes attack the cartilage of joints by secreting a protein, $TNF\alpha$. When RA is untreated, joint damage increases considerably.

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The monoclonal antibody, infliximab, is used to treat RA. Infliximab specifically binds to $TNF\alpha$.

A trial was set up to compare the effectiveness of infliximab and a standard treatment for RA, the anti-inflammatory drug, MTX.

Five groups of people with RA received the following treatments for one year:

- group **P** – MTX only
- group **Q** – MTX plus low dosage of infliximab at intervals of eight weeks
- group **R** – MTX plus low dosage of infliximab at intervals of four weeks
- group **S** – MTX plus high dosage of infliximab at intervals of eight weeks
- group **T** – MTX plus high dosage of infliximab at intervals of four weeks.

At the end of the year's treatment, the proportion of people in each group with increased joint damage was determined.

The results are shown in Fig. 2.2.

The number of people in each group is shown in brackets.

- (d) The accuracy of the diagnosis of infection by *T. pallidum* using H9-1 was compared with that using dark-field microscopy and with blood testing. The results are shown in Table 2.1.

A positive test result indicated that *T. pallidum* is present and a negative test result that it is absent.

Table 2.1

test	test results of 30 people later confirmed to have the infection	test results of 31 people later confirmed not to have the infection
H9-1	all positive	all negative
dark-field microscopy	one negative	two positive
blood test	three negative	two positive

With reference to Table 2.1:

- (i) compare the accuracy of diagnosis of the presence of *T. pallidum* using the different tests

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- (ii) suggest why blood testing for anti-treponemal antibodies gave two positive results in patients later found not to have the infection.

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Exa

(e) Describe briefly **one** use of a monoclonal antibody in the **treatment** of disease.

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[Total: 15]

Q11.

3 The filamentous fungus, *Fusarium venenatum*, can be grown in a fermenter and harvested as mycoprotein. It is sold as a food in a number of different countries.

The fungus is grown in continuous culture in 150 000 dm³ airlift fermenters, in which the introduction of bubbles of compressed air both oxygenates and stirs the contents. The fungus grows as narrow, branched filaments, giving the harvested mycoprotein a naturally chewy, fibrous texture. Approximately 300 kg of fungus can be harvested per hour.

(a) Explain what is meant by the term *continuous culture*.

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- (b) After about six weeks, mutants may appear in the fungal population, for example, a more highly-branched form of the fungus.

The fermenter is emptied, cleaned and repopulated with the original strain of *F. venenatum* every six weeks.

Explain why the fermentation process should be stopped before mutants appear.

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- (c) Approximately 12% of the harvested fungus is protein.

Calculate the approximate mass of protein harvested in one day during continuous culture.

Show your working.

answer [1]

[Total:7]

Q12.

3 The filamentous fungus, *Fusarium venenatum*, is grown in continuous culture in large fermenters to provide mycoprotein for human consumption.

(a) Explain what is meant by the term *continuous culture*.

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 [2]

(b) In an investigation into the growth of the fungus in culture, several factors were varied including:

- temperature
- concentration of the carbon source
- concentration of the nitrogen source.

Some of the results are shown in Table 3.1.

Table 3.1

temperature /°C	concentration of carbon source /g dm ⁻³	concentration of nitrogen source /g dm ⁻³	dry mass of fungus /g dm ⁻³
25	7.0	2.9	3.1
25	14.0	3.5	4.3
30	7.0	3.5	4.8
30	14.0	2.9	4.2

(i) Describe the effect of temperature on the growth of the fungus at the different concentrations of the carbon source.

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(ii) Explain why the fungus needs sources of carbon and nitrogen.

carbon

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nitrogen

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[Total: 8]

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Q13.

4 (a) Outline the hybridoma method for the production of a monoclonal antibody.

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(b) Herceptin is a monoclonal antibody used in the treatment of some breast cancers. It binds strongly to molecules of a receptor protein, HER2, that is produced in abnormally large quantities in the plasma (cell surface) membranes of about 30% of human breast cancers.

Investigations have been made into the most effective way to use Herceptin to treat breast cancer.

One experiment investigated the ability of different treatments to induce cell death in breast cancer cells.

Herceptin and X-ray treatment were used both separately and together. The results are shown in Fig. 4.1.

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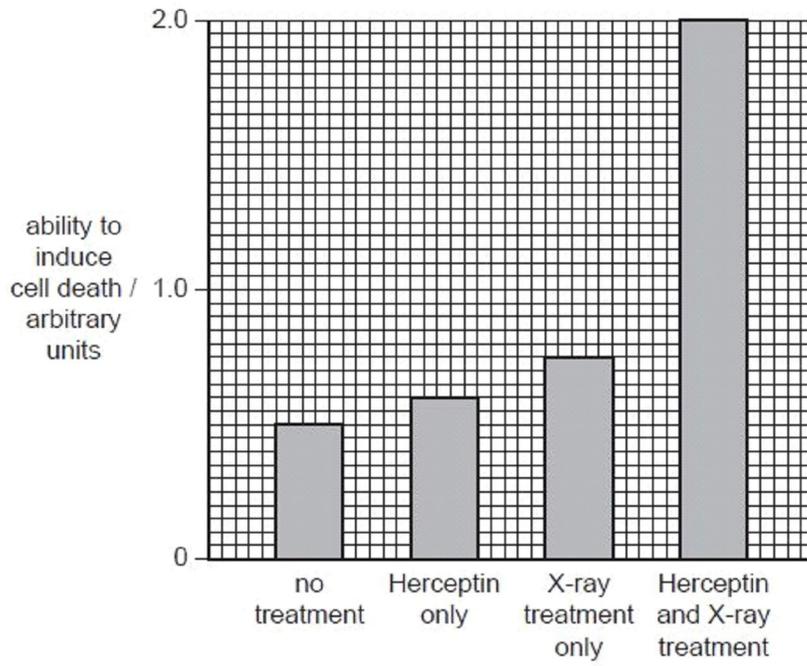


Fig. 4.1

With reference to Fig. 4.1,

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- (i) compare the effects on breast cancer cells of the different treatments

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- (ii) calculate the percentage increase in the ability to induce cell death of using Herceptin **and** X-ray treatment compared with using Herceptin only.

Show your working.

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Q14.

- 3** Proteases that work in alkaline conditions are made in large quantities for use in the detergent industry. The microorganism that is generally used for this is the bacterium *Bacillus subtilis*.

An investigation was carried out to compare three potential production methods:

- using free cells of *B. subtilis*
- using *B. subtilis* cells immobilised in cubes of agar
- using *B. subtilis* cells immobilised in beads of sodium alginate.

To immobilise the cells in agar, the agar was dissolved and cooled. A suspension of *B. subtilis* was then added. The agar-bacterium mixture was poured into sterile dishes and allowed to solidify. It was then cut into cubes with sides of 2 mm.

- (a) (i)** Explain why the agar was cooled before the suspension of *B. subtilis* was added.

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- (ii)** Describe how cells of *B. subtilis* could be immobilised in beads of alginate.

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- (b)** A liquid medium containing glucose, a nitrogen source and various mineral ions was made up, and 50 cm³ placed into each of three flasks.

Samples of a culture of free cells of *B. subtilis*, agar cubes containing immobilised *B. subtilis* and alginate beads containing *B. subtilis* were placed in the three flasks. Each flask contained the same number of bacteria. All the flasks were incubated at 37 °C for 48 hours.

Samples of the liquid medium in each flask were taken at six hourly intervals and the concentration of protease measured.

The results are shown in Fig. 3.1.

- (ii) Suggest why lower concentrations of protease were produced by *B. subtilis* immobilised in agar cubes than *B. subtilis* immobilised in alginate beads.

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- (c) Two new cultures of immobilised *B. subtilis* were set up as described in (b). However, this time a repeat batch fermentation method was used, in which the liquid medium was replaced every 24 hours. This was continued until the cubes or beads had begun to disintegrate.

The results are shown in Table 3.1.

Table 3.1

	number of batches before cubes or beads disintegrated	total fermentation time / hours	total protease produced / arbitrary units	mean productivity of protease / arbitrary units per hour
agar cubes	6	144	1792	12.44
alginate beads	9	216	3264	15.11

With reference to Table 3.1

- (i) calculate the percentage increase in the total protease produced when the bacteria were immobilised in alginate rather than agar.

Show your working.

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- (ii) explain why using bacteria immobilised in alginate rather than agar would be a more cost-effective production of protease.

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[3]

[Total: 15]

Q15.

- 3 (a) Cell walls of bacteria contain peptidoglycans. Peptidoglycans are long chains of the sugars N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) which alternate along the chain. A short peptide chain of three to five amino acids is attached to each NAM and these form cross-links with similar peptide chains from adjacent strands.

Fig. 3.1 shows a diagram representing part of a peptidoglycan structure.

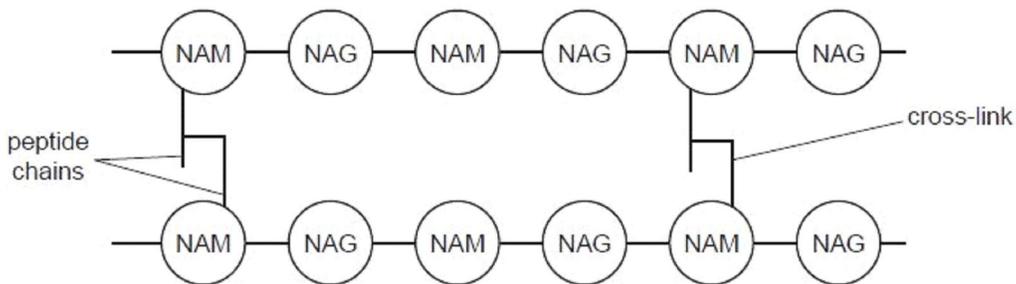


Fig. 3.1

- (i) Name the type of reaction that takes place to assemble the peptide chains that form the cross-links.

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(ii) Describe the mode of action of antibiotics, such as penicillin, on bacteria.

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(iii) Suggest the name of the type of enzyme that assembles the peptide chains that form the cross-links in peptidoglycans.

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(b) State why antibiotics, such as penicillin, have no effect on viruses.

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.....[1]

(c) Bacteria may be Gram-positive or Gram-negative.

Fig. 3.2 shows a diagram of part of the cell walls of both Gram-positive and Gram-negative bacteria.



Gram-positive bacteria cell walls have a peptidoglycan content of 50%

Gram-negative bacteria cell walls have a peptidoglycan content of 10 – 20%

Fig. 3.2

Suggest why Gram-positive bacteria are more susceptible to the action of penicillin than Gram-negative bacteria.

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(d) There is evidence that some bacteria have developed resistance to antibiotics.

One form of pneumonia, a serious lung disease, is caused by the bacterium *Streptococcus pneumoniae*. The Canadian Health Service has carried out a survey to show how the resistance of *S. pneumoniae* to penicillin has changed over the last 20 years.

Fig. 3.3 shows the results of this survey.

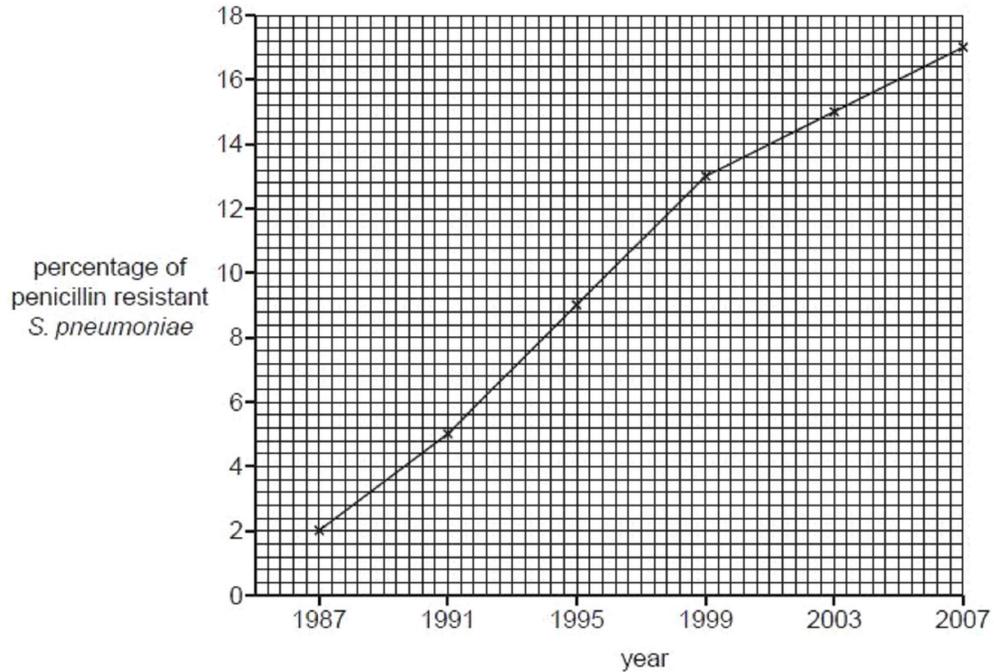


Fig. 3.3

(ii) Explain why penicillin does not affect viruses.

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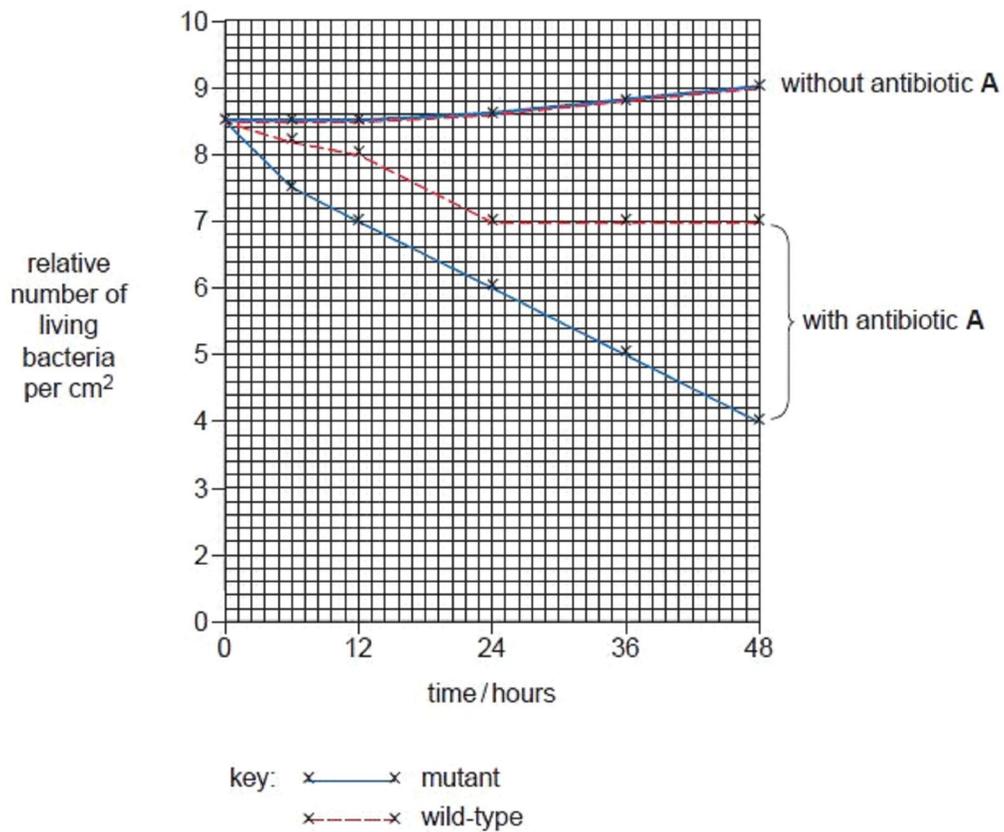
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(b) Wild-type and mutant bacteria were grown on solid culture media both with antibiotic A and without antibiotic A.

The subsequent change in numbers of living bacteria is shown in Fig. 2.1.



With reference to Fig. 2.1, describe the changes in numbers of the wild-type and mutant bacteria on culture media with antibiotic **A** and without antibiotic **A**.

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(c) The wild-type and mutant strains of this bacterium have different DNA sequences in part of a gene coding for an enzyme which is needed to produce polymers of glucose, called glucans. Glucans are secreted by bacteria and can bind to various molecules, including those of antibiotic **A**.

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Explain

(i) how a mutation of a gene coding for an enzyme may result in an enzyme with reduced activity,

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(ii) the different effects of antibiotic **A**, shown in Fig. 2.1, on the wild-type and mutant strains of bacteria.

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(d) Explain the role of natural selection in the evolution of antibiotic resistance in bacteria.

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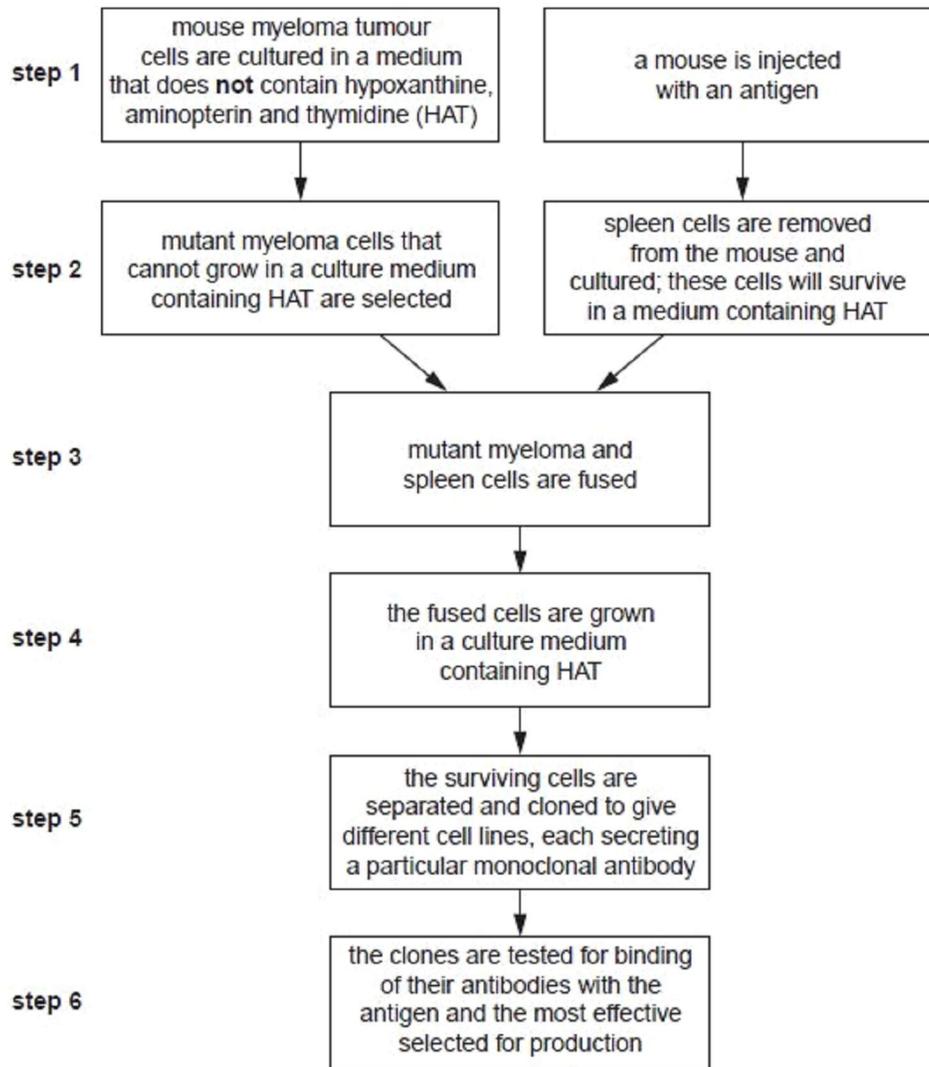
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[Total: 16]

Q17.

3 Fig. 3.1 shows a method of producing monoclonal antibodies.

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(a) (i) Name this method of producing monoclonal antibodies.

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(ii) Describe what is meant by the term *monoclonal antibody*.

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- (iii) Describe, in detail, the molecular structure of one of the antibodies produced in **step 6**. You may wish to use an annotated diagram to answer the question.

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- (b) The mutant myeloma cells used in **step 2** are myeloma cells with a gene mutation that prevents them from growing in a culture medium containing hypoxanthine, aminopterin and thymidine (HAT).

- (i) Suggest why cells with this gene mutation **cannot** grow in a culture medium containing HAT.

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- (ii) Explain why the mutant myeloma cells **can** grow in a culture medium containing HAT after they have been fused with mouse spleen cells (**steps 3 and 4**).

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(iii) Suggest why growing the fused cells in a culture medium containing HAT (**step 4**) is an important part of the procedure shown in Fig. 3.1.

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(c) Suggest advantages of using monoclonal antibodies for pregnancy testing.

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Exa

[Total: 16]

Q18.

2 (a) In the small intestine, the enzyme lactase hydrolyses the disaccharide lactose into the monosaccharides glucose and galactose. A deficiency of lactase can lead to a condition known as lactose intolerance. The lactose passes undigested into the large intestine resulting in diarrhoea. Some babies are born with congenital lactase deficiency, which is an inherited condition, and require lactose-free milk from birth.

Suggest how two parents, who can digest lactose, can have a child with congenital lactase deficiency.

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(b) The enzyme lactase can be produced by biotechnology and then used to produce lactose-free dairy products. Lactase is frequently used immobilised in alginate beads.

Fig. 2.1 shows a comparison between the activity of lactase free in solution and lactase immobilised in alginate beads, over a range of temperatures. Equal concentrations of free lactase and immobilised lactase were used.

(ii) suggest explanations for the differences between the activity of immobilised lactase and free lactase up to 40 °C.

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(c) State the advantages of using immobilised enzymes instead of free enzymes.

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[Total: 10]

Q19.

- (c) A strain of the bacterium *Pseudomonas aeruginosa*, strain **R**, has a gene coding for an efflux pump and is resistant to a β lactam antibiotic.

The minimum inhibitory concentration (MIC) of the β lactam for strain **R** was determined. The MIC is the lowest concentration of antibiotic that prevents a colony of the bacterium from growing.

The MICs were also determined for two mutant strains derived from strain **R**, mutant strain **1** and mutant strain **2**. Each of these strains differs from strain **R** in the expression of the gene coding for the efflux pump.

The MICs for the three strains of *P. aeruginosa* are shown in Table 2.1.

Table 2.1

strain of <i>P. aeruginosa</i>	MIC of β lactam / $\mu\text{g cm}^{-3}$
resistant strain R	64
mutant strain 1	0.5
mutant strain 2	256

With reference to Table 2.1, suggest:

- (i) why the MICs for mutant strains **1** and **2** differ from that for strain **R**

mutant strain 1

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mutant strain 2

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..... [4]

2 Many tumours release a protein growth factor called VEGF. This is a chemical signal that causes nearby blood vessels to grow new branches into the tumour.

The monoclonal antibody, bevacizumab (Avastin®), specifically binds to VEGF.

(a) Suggest how Avastin® can prevent the growth and spread of a tumour.

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(b) Avastin® is made by the hybridoma method.

State:

(i) the antigen that is injected into a mouse to produce this monoclonal antibody

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(ii) what is meant by a *hybridoma*.

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(c) The monoclonal antibody made by the hybridoma method is modified to obtain humanised mouse antibody. This type of antibody molecule resembles those produced by humans.

Suggest advantages of using humanised mouse antibody rather than mouse antibody.

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.....[3]

- (d) A second monoclonal antibody, ranibizumab (Lucentis[®]) is used to treat eye diseases. Lucentis[®] is a fragment of Avastin[®] and is shown in Fig. 2.1.

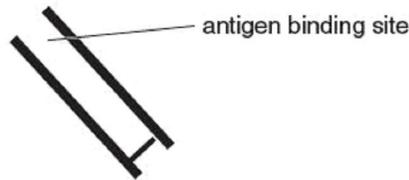


Fig. 2.1

Complete Fig. 2.1 to show a molecule of Avastin[®].

Labels are **not** required.

[2]

[Total: 9]

Q22.

- 5 (a) The pollutants in waste water from the textile industry may include azo-dyes, which give colour to textiles. Azo-dyes are organic pollutants that can be carcinogenic.

White-rot fungi are useful for the treatment of environmental pollution as they produce extracellular enzymes that are able to break down a number of organic pollutants, such as azo-dyes.

The extracellular enzymes produced by white-rot fungi are primary metabolites that are mass produced by continuous culture.

- (i) Outline the main operating conditions of continuous culture.

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.....[2]

- (ii) When a similar experiment was performed using the free enzymes from the white-rot fungal cells, all concentrations of azo-dye shown in Table 5.1 were broken down within 12 hours.

Suggest why free enzymes break down the azo-dye more quickly than intact white-rot fungal cells.

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.....[2]

- (c) These extracellular enzymes may be immobilised on an inert support.

Outline the advantages of using immobilised enzymes in the treatment of textile waste water.

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.....
.....[3]

[Total: 14]

Q23.

1 (a) Define the term *biotechnology*.

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..... [2]

(b) The extent to which the potential of biotechnology will be realised depends on public attitudes towards the technology involved.

Describe the factors that influence public opinion in determining the acceptability of the technology.

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..... [4]

(c) Fig. 1.1 shows the ethene production of a normal tomato and a 'Flavr Savr' genetically modified tomato left on a plant for three weeks prior to picking. Naturally produced ethene in fruits speeds up the ripening process.

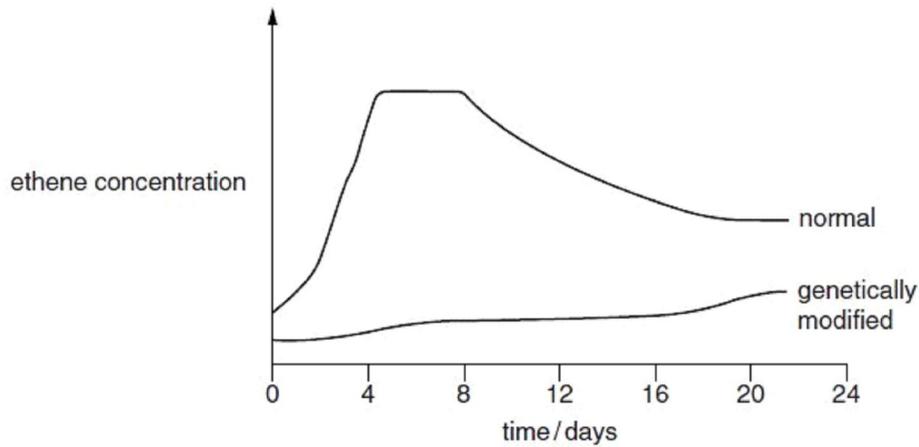


Fig. 1.1

- (i) Compare the production of ethene in normal tomatoes with that in genetically modified tomatoes.

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..... [3]

- (ii) Suggest what effect this genetic modification will have on the ripening of the tomatoes.

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..... [2]

- (iii) Explain the benefits that these genetically modified tomatoes might have for the grower and for the shopkeeper.

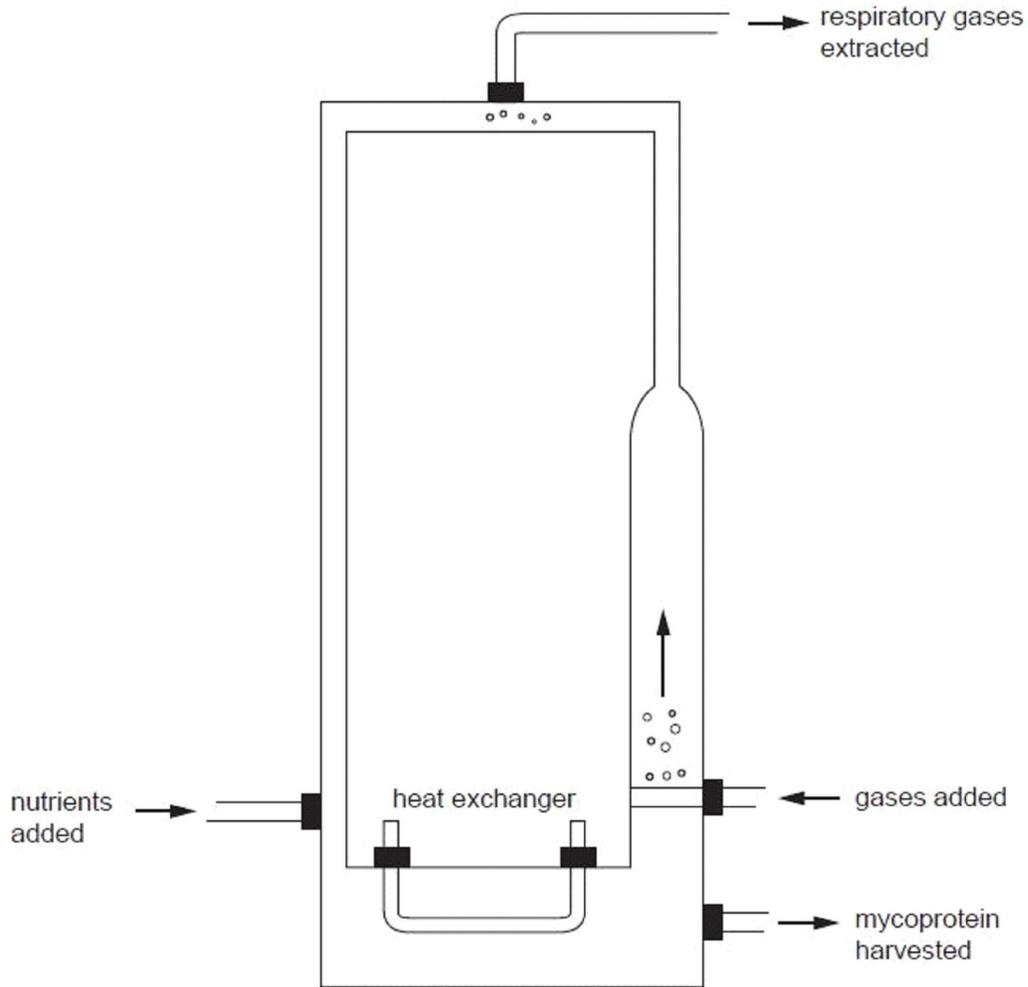
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.....
..... [2]

- (iv) Outline the ethical implications of consuming transgenic food products.

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.....
..... [2]

Q24.

- 2 Mycoprotein, an alternative to meat, is produced by growing the fungus, *Fusarium graminearum*, in an air-lift fermenter, similar to the one shown in Fig. 2.1.



(a) (i) With reference to Fig. 2.1, explain how an air-lift fermenter works.

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.....[3]

- (ii) List three nutrient growth requirements of *Fusarium*.
1.
 2.
 3. [3]

(b) Describe two ways in which *Fusarium* must be processed **after** fermentation to make it acceptable as a human food product.

1.
.....
2.
..... [2]

[Total : 8]

Section-B

1.

9 (a) Bacteria are members of the kingdom *Prokaryota*. Describe the main features of a bacterial cell. [8]

(b) Outline the use of bacteria in the extraction of metals from ores. [7]

[Total:15]

2.

10 (a) Explain how the *lac* operon is involved in the metabolism of lactose in *Escherichia coli*. [9]

(b) Describe the role of gibberellin in the germination of barley. [6]

[Total: 15]

3.

10 (a) Describe the action of penicillin on bacteria. [8]

(b) Outline the use of microorganisms in the extraction of heavy metals from their ores. [7]

[Total: 15]

4.

10 (a) Describe the production of penicillin using the batch culture method. [8]

(b) Mycoprotein is produced using a continuous culture method.

Describe the advantages of the batch culture method **and** the continuous culture method. [7]

[Total: 15]

