1. Immobilised enzymes can be used in bioreactors that attach to space suits. The bioreactors recover water from the astronauts' urine. The bioreactors use immobilised urease enzyme which catalyses the hydrolysis of urea, forming carbon dioxide and ammonia. These products react to form ions, which are then removed by the bioreactor.

(i) State the meaning of the term immobilised enzyme *and describe how immobilisation can be achieve*d.

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[3]

(ii) Suggest three practical advantages of using an immobilised urease bioreactor in a spaceship.

1 .....

2 .....

3 .....

[3]

[Total 6 marks]

2. An investigation was carried out to compare lipase in soluble and immobilised forms. Palm oil was hydrolysed to produce fatty acids and glycerol.

- The two forms of lipase showed optimal activity at the same pH and temperature (pH 7.5 and 35°C).
- At that pH and temperature, 100% of the oil was hydrolysed in two minutes.
- If the temperature was increased to 45°C, the immobilised enzyme hydrolysed 100% of the oil but the soluble enzyme hydrolysed only 80% of the oil in two minutes.

(i) Define the term *hydrolysis*.

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[1]

(ii) Explain, **using the information in the passage**, the advantages of using an immobilised enzyme to hydrolyse palm oil.

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[4]

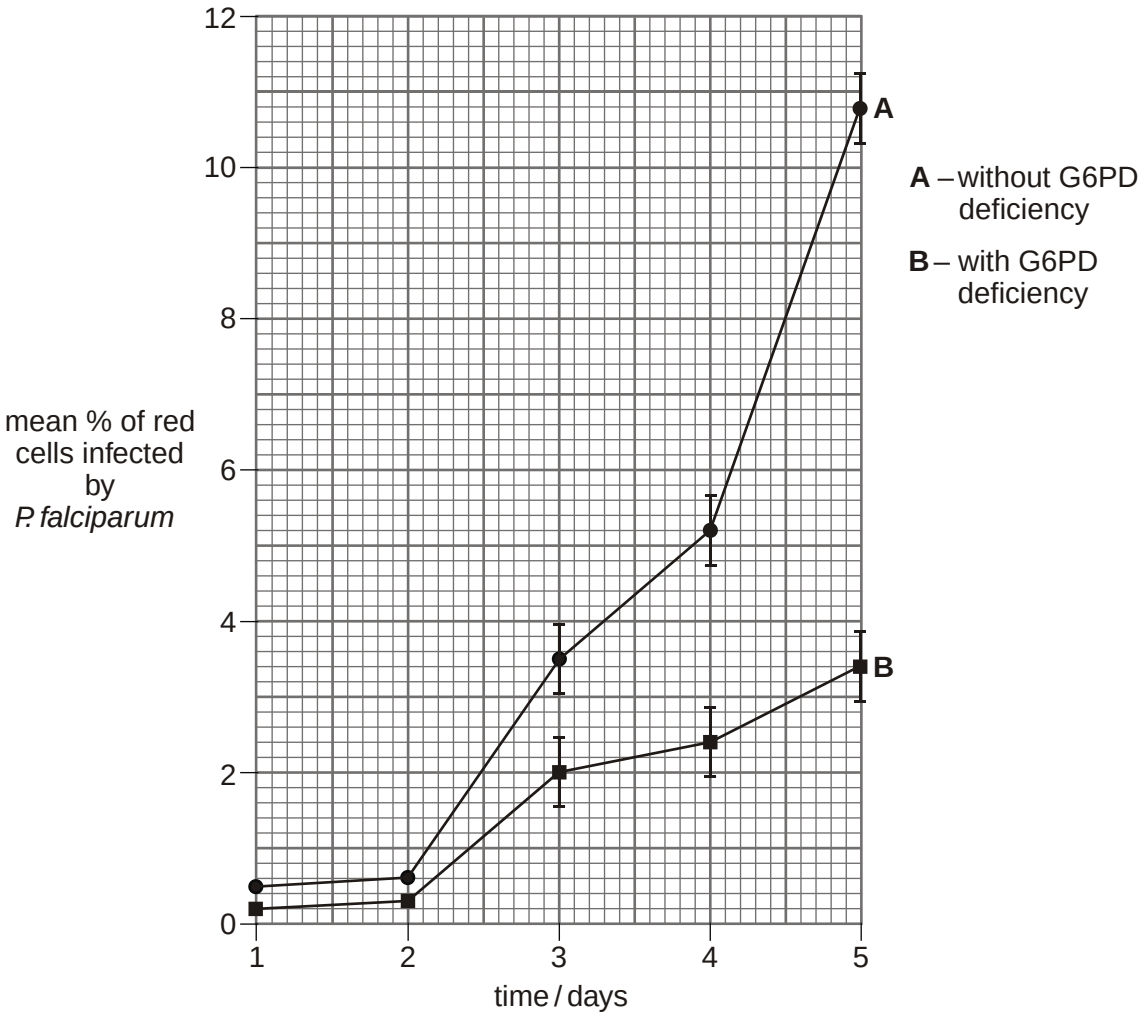
[Total 5 marks]

3. The information below refers to the deficiency of the enzyme, glucose-6-phosphate dehydrogenase (G6PD) in humans:

- a deficiency of G6PD is an inherited condition
- G6PD is necessary for the production of ribose
- ribose is a nutrient needed by *Plasmodium falciparum*
- individuals with G6PD deficiency may be resistant to the parasite *P. falciparum*
- G6PD deficiency is more common in areas where malaria occurs regularly.

In an experiment, red blood cells were collected from individuals deficient in G6PD and from individuals without this deficiency. The cells were collected in a solution containing an anticoagulant, as well as solutes used to maintain a suitable water potential. The red blood cells were used as a growth medium for *P. falciparum*.

The percentage of red blood cells infected by *P. falciparum* was determined over a five day period and the mean calculated. The results obtained are shown in the figure below.



Using the information in the figure above,

(i) suggest why error bars have been included;

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[2]

(ii) describe **and** explain the results obtained between day 1 and day 2;

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[2]

(iii) describe **and** explain the differences between the results for **A** and **B** between days 2 and 5.

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[4]

[Total 8 marks]

4. The synthesis of caffeine in coffee plants involves enzymes which add methyl groups (CH_3) to convert xanthosine to caffeine:

In an attempt to produce caffeine-free coffee, cells of a coffee plant, *Coffea canephora*, were grown in tissue culture and genetically modified to suppress expression of the gene for theobromine synthase.

DNA was constructed to code either for short or for long lengths of RNA with the **complementary** base sequences to parts of the messenger RNA (mRNA) produced by the gene for theobromine synthase.

(a) Explain how lengths of RNA that are complementary to mRNA may suppress the expression of a gene.

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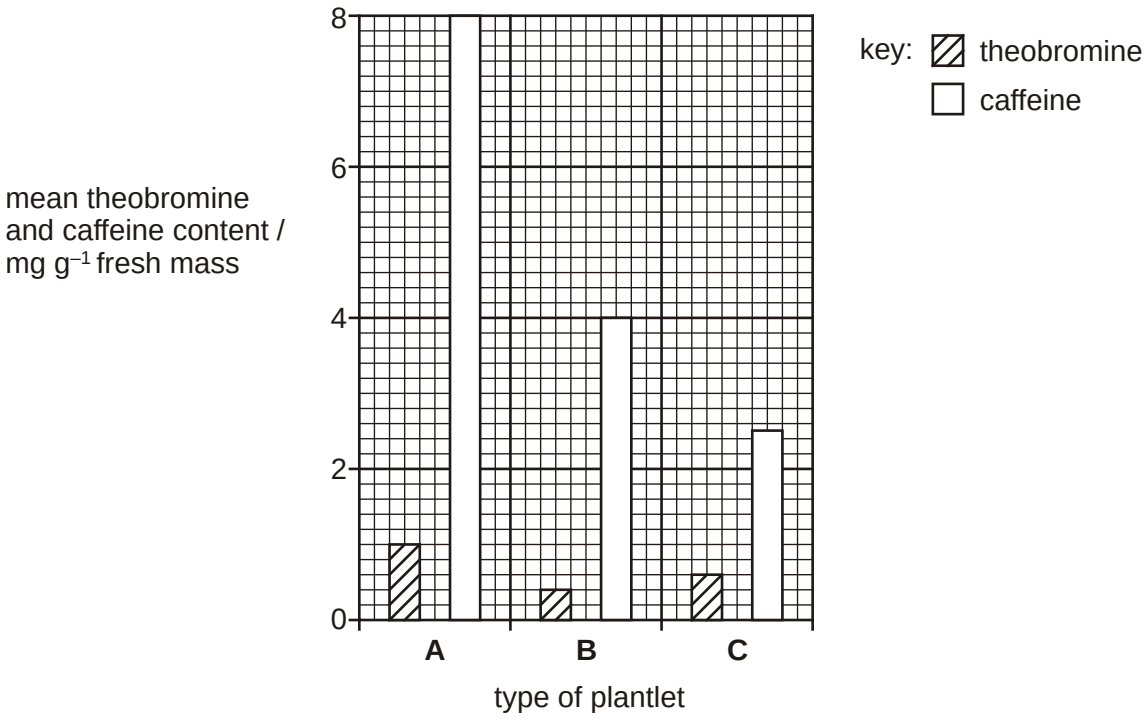
[4]

(b) Three types of cell were then cloned in tissue culture into plantlets:

A - unmodified (control) cells
B - genetically modified cells with the DNA code for short lengths of RNA complementary to mRNA for theobromine synthase
C - genetically modified cells with the DNA code for long lengths of RNA complementary to mRNA for theobromine synthase.

Samples of each of the three types of plantlet were analysed to measure their theobromine and caffeine content. The results of the analysis are shown below.



(i) Describe the results shown in the figure above.

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[3]

(ii) Suggest an explanation for the difference in the results of the two experimental treatments, **B** and **C**.

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[1]

(iii) Describe briefly how plants are cloned by tissue culture.

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[4]

(iv) Explain the advantages of using cloned plants in experiments such as this.

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[3]

[Total 15 marks]

5. Penicillin is an antibiotic that is used to treat bacterial diseases caused by Gram-positive bacteria. It can be produced commercially in large fermenters by a fed-batch culture method.

(i) Explain why a continuous culture method would **not** be suitable for the manufacture of penicillin.

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[2]

(ii) Suggest why limited amounts of glucose are added at regular intervals to the culture medium during the fed-batch process.

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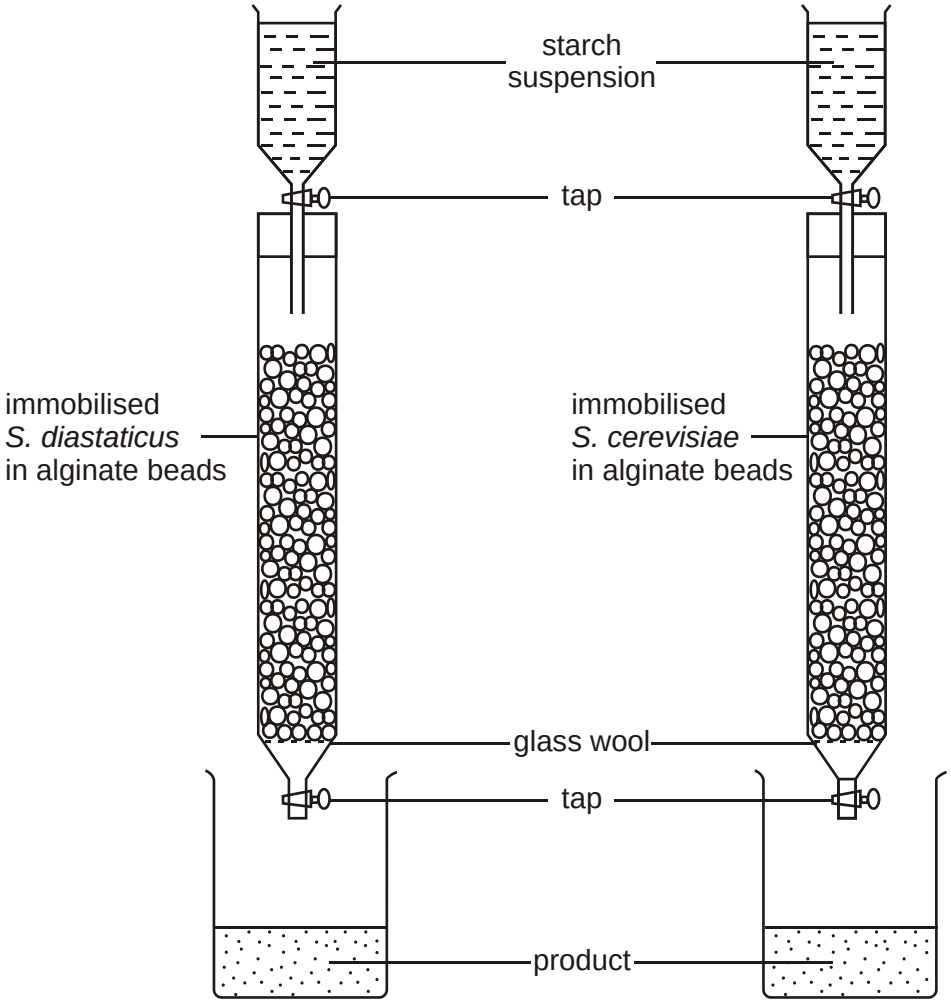
[2]

[Total 4 marks]

6. Yeast cells can be entrapped in alginate beads using the same methods as used for immobilising enzymes. A student performed an investigation to compare the glucoamylase activity of *S. diastaticus* with that of the genetically modified *S. cerevisiae*.

The figure below is a diagram of the experiment.



(i) List **three** factors that would need to be controlled in this experiment in order to make valid comparisons.

1 .....

2 .....

3 .....

[3]

(ii) Describe **one** method of measuring the concentration of reducing sugars in the products.

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[2]

(iii) The student expressed concerns that live yeast cells may be present in the product and that these cells would affect the results of the experiment.

Explain whether or not you agree with these concerns.

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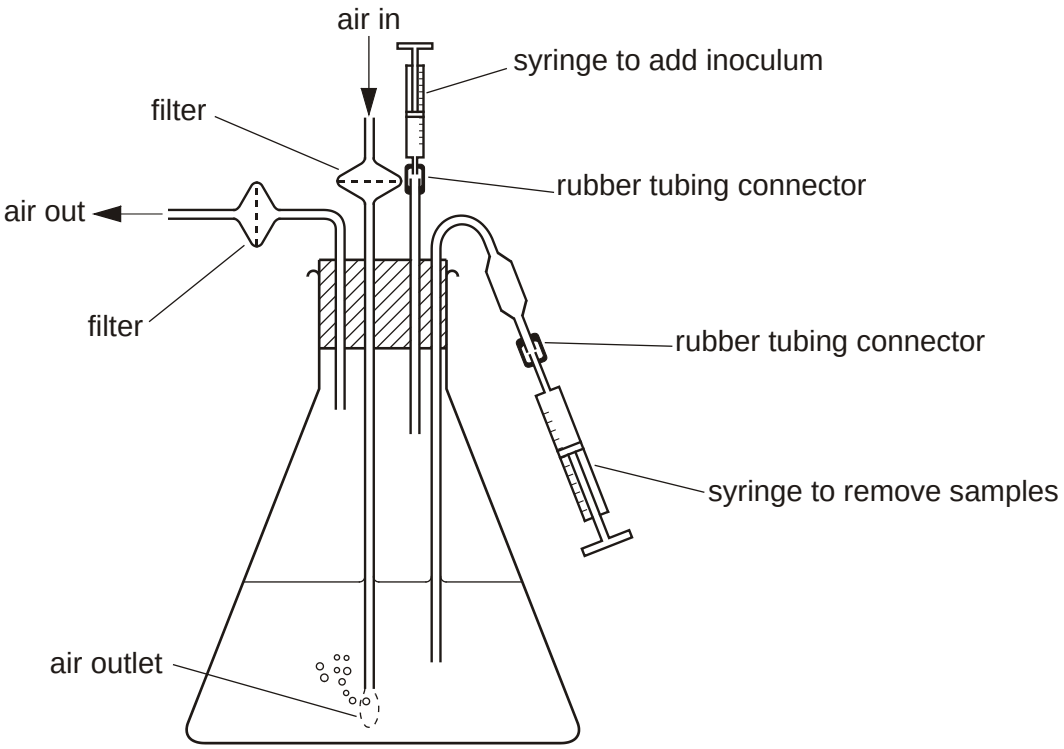
[2]

[Total 7 marks]

7. The figure below shows a laboratory fermenter (bioreactor) used by a student to **batch** culture microorganisms.



Explain how the student could modify the fermenter for **continuous fermentation**.

If you wish, you may add annotations to the figure to help you in your answer.

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[Total 4 marks]

8. (a) A number of organic chemicals are produced commercially using microorganisms.

Citric acid is produced by certain fungi and is a secondary metabolite.

(i) Name **one other** secondary metabolite produced commercially from a fungus.

.....

[1]

(ii) State what is meant by the term *secondary metabolite* .

.....

.....

[1]

(iii) State which method of fermentation would be used to produce a secondary metabolite and explain your answer.

method .....

explanation .....

.....

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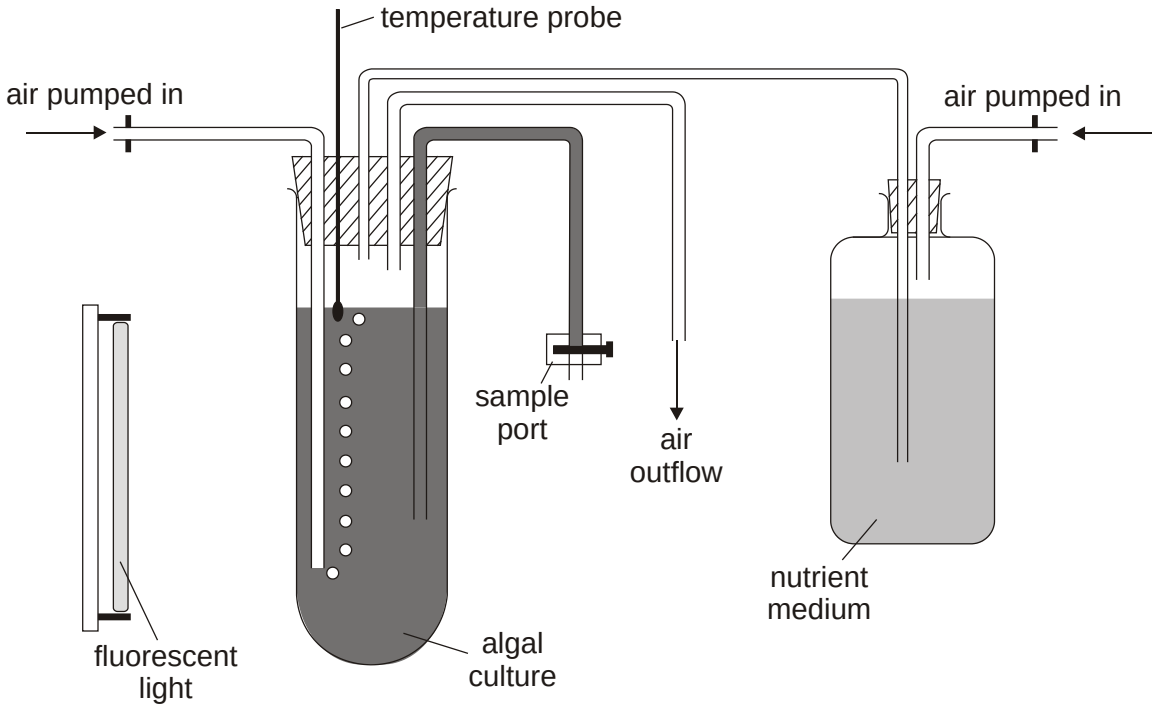
.....

.....

[3]

The figure below shows a 'pilot plant' assembled by a student in a school laboratory.



(b) The student has undertaken a project to culture an alga called *Chlorella* to feed brine shrimps for use as fish food. If it works, the student hopes to produce a **continuous culture** of algae.

Explain how the apparatus shown in the figure above allows a continuous culture of *Chlorella*.

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[6]

(c) Describe the major problems of developing this project to enable the large-scale production of *Chlorella*.

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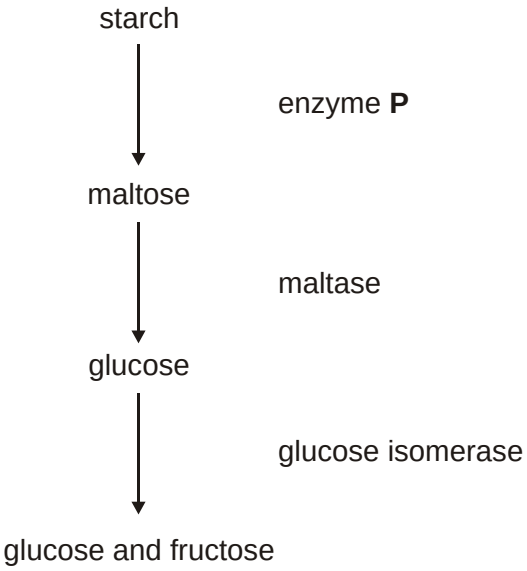
[4]

[Total 15 marks]

9. Immobilised glucose isomerase is used for the production of high-fructose syrups. Starch is used as a source of glucose, which is then treated by glucose isomerase to form a mixture of glucose and fructose.

Fructose is sweeter than glucose and the syrup formed is used in sweets and soft drinks.

The figure below shows the stages in this process.



(a) (i) Name enzyme **P**.

.....

[1]

(ii) Name the type of bond that is broken when maltose is converted to glucose.

.....

[1]

(iii) Name the form of glucose produced when maltose is broken down.

.....

[1]

(b) The enzyme glucose isomerase is immobilised by being attached to an insoluble material.

(i) State **two** ways in which glucose isomerase could be immobilised.

1 .....

2 .....

[2]

(ii) Explain **two** advantages of using immobilised glucose isomerase rather than the enzyme in solution.

1 .....

.....

.....

.....

.....

2 .....

.....

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.....

[4]

(c) Nitrogenase is an enzyme found in some bacteria that converts nitrogen gas into ammonia in a process known as nitrogen fixation. The enzyme is inactivated when exposed to oxygen. Commercial methods of fixing nitrogen are being developed but whole cells rather than the isolated enzyme are immobilised.

Suggest advantages of immobilising the whole cell rather than the enzyme.

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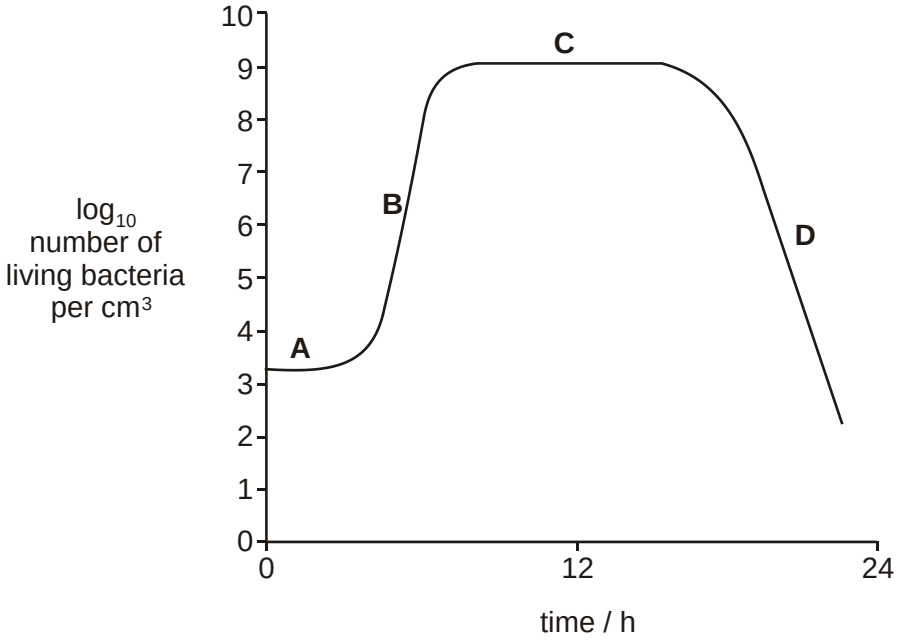
[2]

[Total 11 marks]

10. The figure below shows a typical bacterial growth curve for a closed system, such as a test tube or conical flask.



From The Control of Growth and Differentiation in Plants, p.123, by P. Waring & I. Phillips, published by Pergamon Press Ltd., 1970 (ISBN 0-08-015500-6).

(a) Complete the table below by writing the appropriate letter from the figure in the spaces provided.

description of stage	letter
cells divide at a constant rate depending upon the composition of the growth medium and the conditions of the incubation	
some cells are dividing and an equal number are dying	
number of living cells is decreasing	
time required for synthesis of inducible enzymes and factors involved in cell division	

[4]

(b) Generation time **(G)** is defined as the length of time **(t)** from one generation to the next.

The mean generation time is calculated using the following formula:

$$\mathbf{G = \frac{t}{n}}$$ where **t** = time and **n** = number of generations

(i) The bacterium *Streptococcus lactis* has been shown to divide 55 times during 24 hours.

Calculate the mean generation time of this bacterium in minutes. Show your working.

Generation time = ... minutes

[2]

(ii) The generation time for *Escherichia coli* in a laboratory can be 20 minutes, but in the intestinal tract it can be as much as 24 hours. Suggest **three** reasons for this difference.

1 .....

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2 .....

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3 .....

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[3]

[Total 9 marks]

11. Enzymes are used in many commercial processes, either in a free, soluble form or immobilised.

Immobilised enzymes are being used in a bioreactor that attaches to spacesuits. The bioreactor was developed during 'Water Recovery Tests'. This immobilised enzyme bioreactor removes the urea from an astronaut's urine. The bioreactor uses immobilized urease enzyme, which catalyses the hydrolysis of urea, forming carbon dioxide and ammonia. These products react to form ions, which are then removed by the bioreactor.

(i) State the meaning of the term *immobilised enzyme*.

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[1]

(ii) State **two** different methods of immobilising an enzyme.

1 .....

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2 .....

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[2]

(iii) Suggest **three** practical advantages of using an immobilised urease bioreactor in a space ship.

1 .....

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2 .....

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3 .....

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[3]

[Total 6 marks]

12. Soluble and immobilised lipases were tested for their ability to hydrolyse palm oil. When oil is hydrolysed, it produces fatty acids and glycerol.

The two forms of lipase showed **optimal** activity at the same pH and temperature (pH 7.5 and 35 °C). At that pH and temperature, 100% of the oil was hydrolysed in two minutes.

If the temperature was increased to 45 °C, 100% of the oil was hydrolysed using immobilised lipase but when soluble lipase was used, only 80% was hydrolysed within the two-minute period.

(i) Define the term *hydrolysis*.

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[2]

(ii) Using the information from the passage and your knowledge of the products of the reaction, explain the advantages of using an immobilised enzyme to hydrolyse palm oil.

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[4]

[Total 6 marks]

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[3]

[Total 6 marks]

13. A student investigated the fermentation of two sugars, glucose and maltose, by yeast cells.

Two fermentation tubes were prepared containing equal volumes of a yeast suspension and the respective sugar solutions.

Each fermentation tube was placed inside a test tube, as shown in Fig. 1.

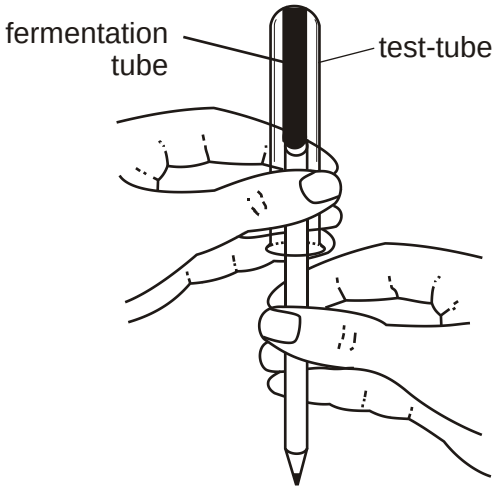

Fig. 1

The test-tubes were turned through 180° and placed in a test-tube rack. The yeast suspensions were left to ferment for 80 minutes. During this time, gas collected as shown in Fig. 2.

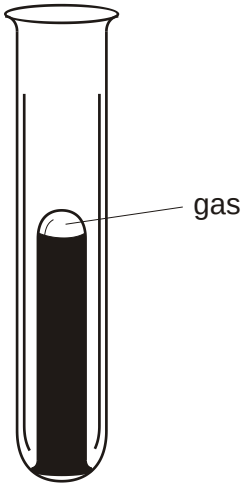

Fig. 2

The student determined the volume of gas collected in each tube at intervals of ten minutes.

The results are shown in Fig. 3.

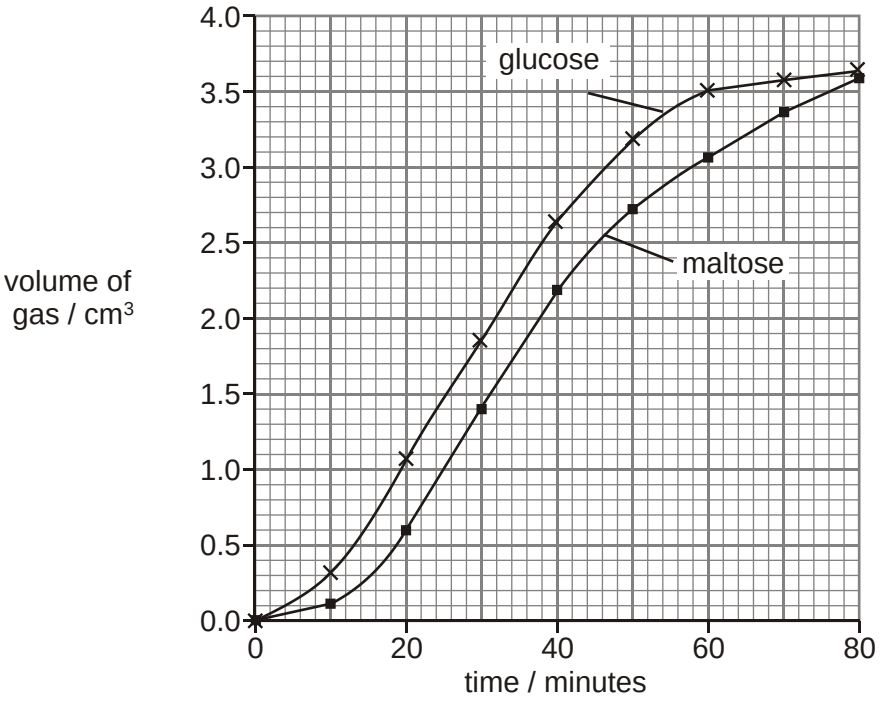

Fig. 3

(a) (i) Suggest **three** variables, **other than type of sugar**, that could affect the results of this investigation.

1 .....

2 .....

3 .....

[3]

(ii) Name the gas that is produced by fermentation.

.....

[1]

(b) (i) Using the data in Fig. 3, describe the results obtained with glucose.

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[4]

(ii) Suggest reasons for the results you have described in (b) (i).

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[4]

(c) Suggest why there is a difference between the results for maltose and the results for glucose.

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[2]

[Total 14 marks]

14. Fig. 1 shows a batch fermenter used to produce penicillin.

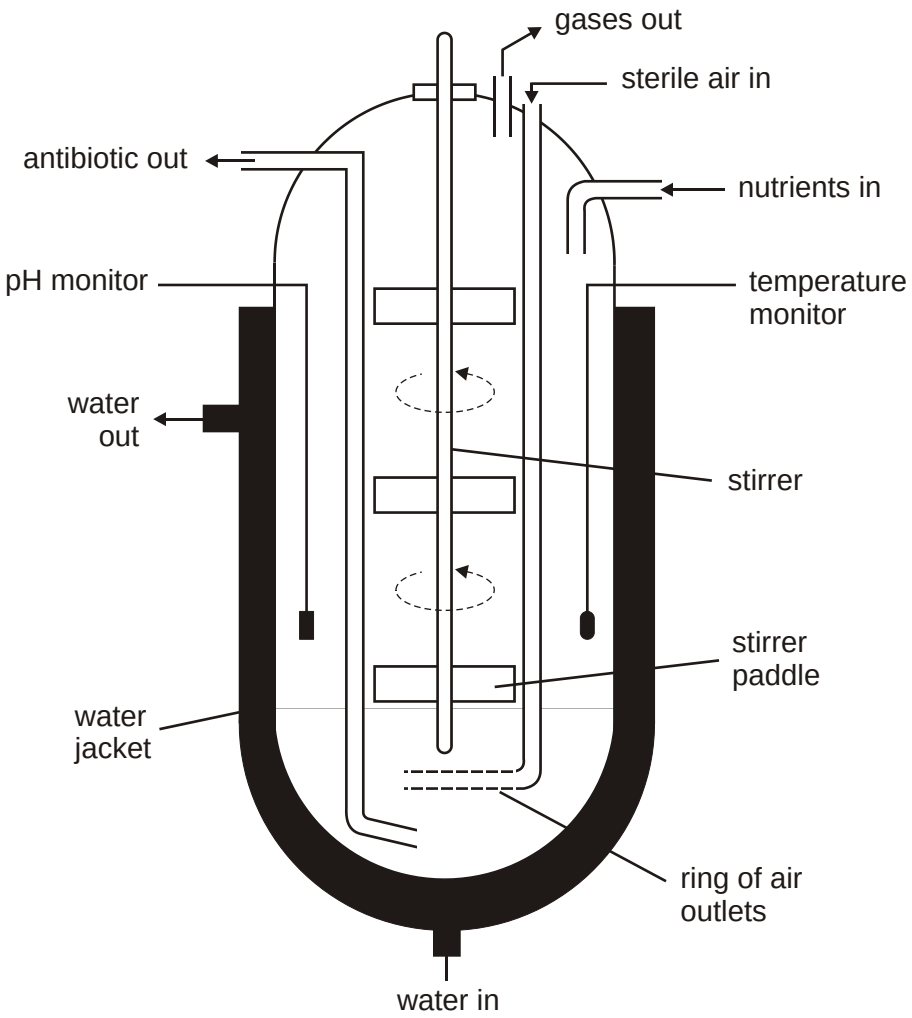

Fig. 1

(a) Explain why sterile air is pumped into the fermenter.

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[2]

(b) (i) The fungus that produces penicillin needs a supply of carbon and nitrogen.

Give the form in which these elements are added to the culture.

carbon .....

nitrogen .....

[2]

(ii) Explain why it is necessary to pump water into the jacket surrounding the culture.

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[3]

(iii) State why pH is monitored **and** describe how it is controlled.

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[2]

(c) Fig. 2 is a graph showing the production of penicillin and the growth of the fungus, *Penicillium*, in the fermenter shown in Fig. 1.

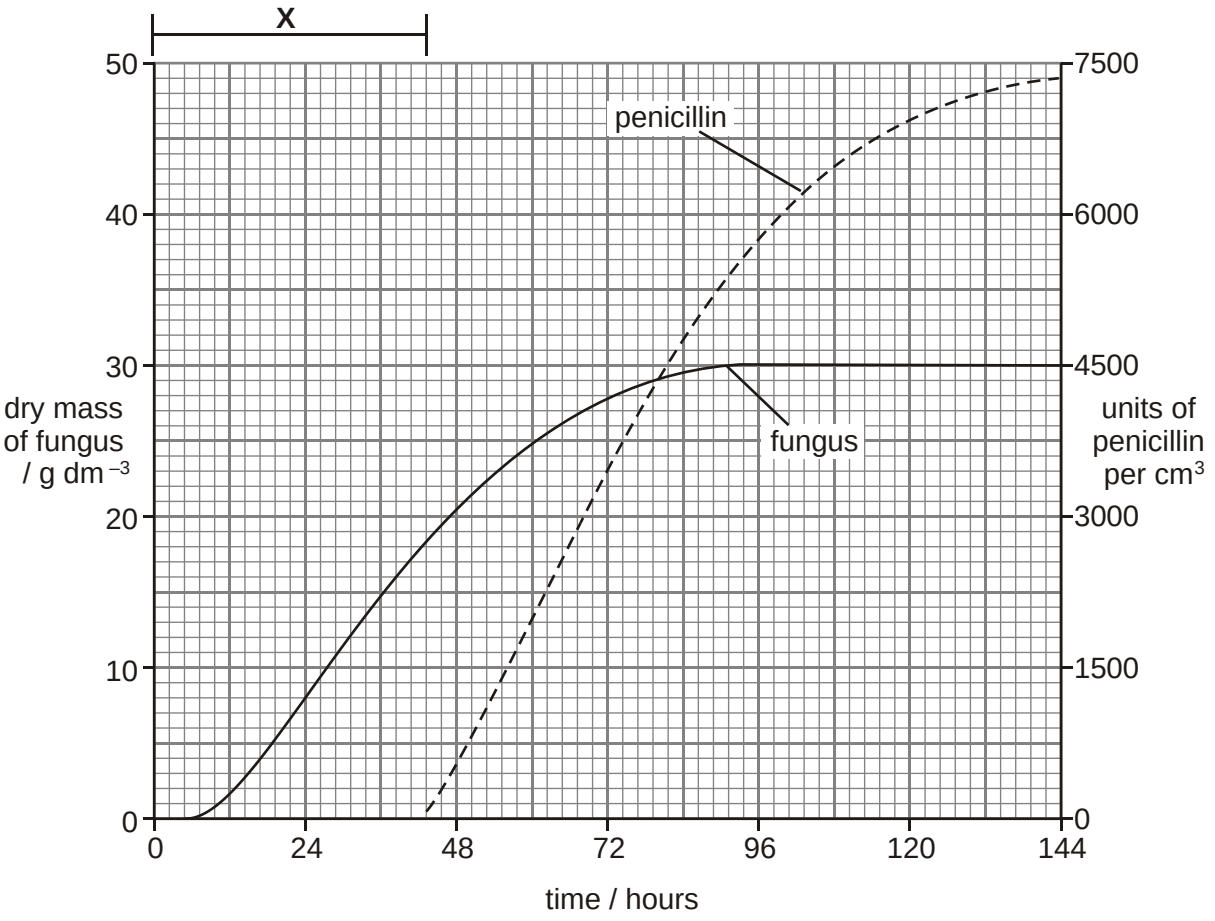

Fig. 2

(i) Using the data in Fig. 2, state the time when *Penicillium* enters its stationary phase.

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[1]

(ii) Explain why there is no antibiotic produced during phase **X**.

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[3]

(d) Penicillin is removed from the fermenter for downstream processing.

Describe what happens during downstream processing.

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[3]

(e) Other medically important products, such as insulin and growth hormone, are produced on a large scale using microorganisms.

Give reasons for using microorganisms in the production of insulin and growth hormone.

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[4]

[Total 20 marks]