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A2 Biology OCR

Unit F215: Control, genomes and environment

Module 2.2 Biotechnology

Notes & Questions

State that biotechnology is the industrial use of living organisms (or parts of living organisms) to produce food, drugs or other products.

- **Biotechnology**
 - Technology based on biology
 - Involves the exploitation of living organisms or their biological processes
 - Production of food, drugs and other products for human benefit.

 - Food production
 - Brewing – yeast
 - Mycoproteins – fungal mycelium used to make SCP
 - Soya Sauce – fermented soya beans with yeast
 - Cheese & Yogurt – Lactobacillus

 - Drugs & Pharmaceuticals
 - Penicillin – Penicillium
 - Insulin – GM *E.coli*

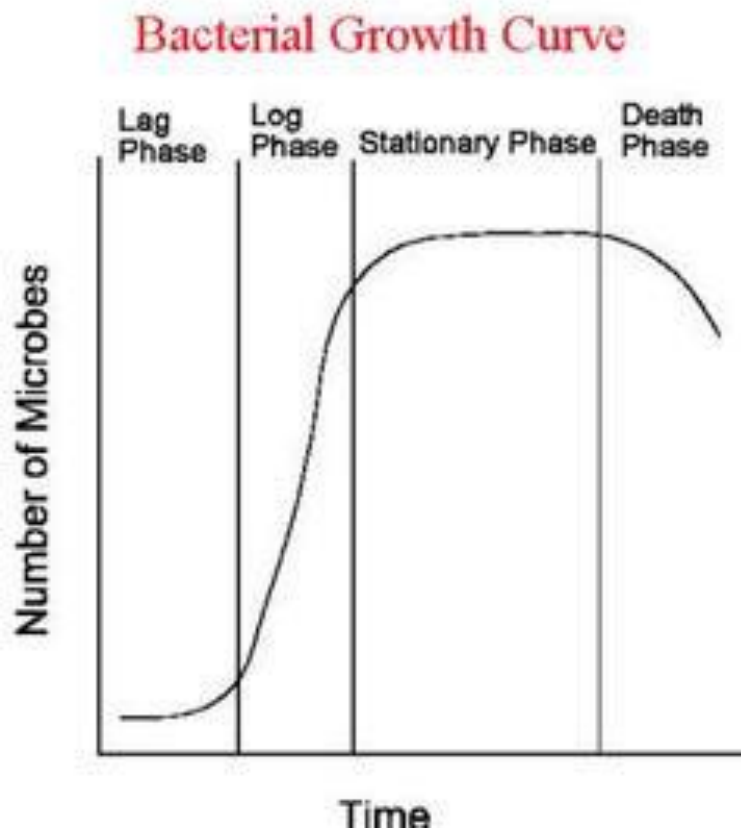
 - Enzymes & Chemicals
 - Pectinase – Fungi *Aspergillus niger*, used for extracting juice from puree
 - Calcium citrate
 - Bio-gas

 - Bioremediation
 - Waste treatment and oil spills

Explain why microorganisms are often used in biotechnological processes.

- Grow rapidly = large quantities of product in a short time
- Produce pure products (often more pure than from chemical processes) = less down stream processing
- Not dependant on climate so can grow anywhere
- Easy to genetically engineer = make many products/human products
- Do not require high temperatures = low costs
- Easy to harvest products = low costs
- Can grow on waste materials = low costs & environmental advantages

Describe, with the aid of diagrams, and explain the standard growth curve of a microorganism in a closed culture.

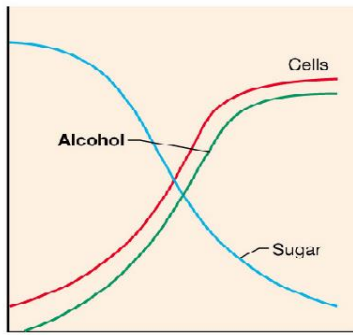


- **Lag Phase**
 - Organisms are adjusting to the surrounding conditions
 - Cells active but not dividing so population fairly constant
 - Synthesis of inducible enzymes and factors involved in cell division
- **Log Phase**
 - Population doubles with every generation
 - High levels of nutrients
 - Low levels of waste
 - low levels of competition
 - few limiting conditions
- **Stationary Phase**
 - Birth rate = death rate so population is stable
 - Nutrient levels are dropping
 - Waste levels are rising
 - Competition is rising

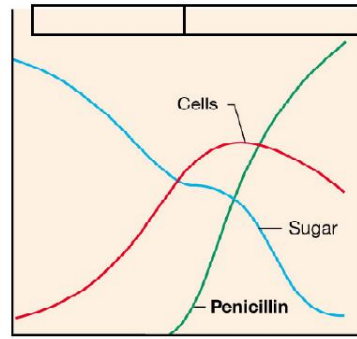
- **Death/Decline Phase**
 - Greater number dying than being produced
 - Nutrient levels are low
 - Waste levels are high
 - Competition is high

Describe the differences between primary and secondary metabolites.

- **Metabolism**
 - Sum total of all chemical reactions within an organism
- **Metabolites**
 - Chemicals that are produced as a result of metabolic reaction
 - Include
 - Hormones
 - Enzymes
 - Waste products
 - Cells and cell components
- **Primary Metabolites**
 - Produced as part of normal growth
 - Essential for life
 - Produced in line with growth curve
 - Example: insulin / amino acids / fatty acids / lipids / enzymes
 - Produced using a continuous culture
 - Highest production in the Log phase.
- **Secondary Metabolites**
 - Not produced as part of the normal growth
 - Not essential for life
 - Only start to be produced in the stationary phase when nutrients are in short supply and competition is high
 - Example: penicillin
 - Produced using a batch culture
 - Highest production in stationary phase.



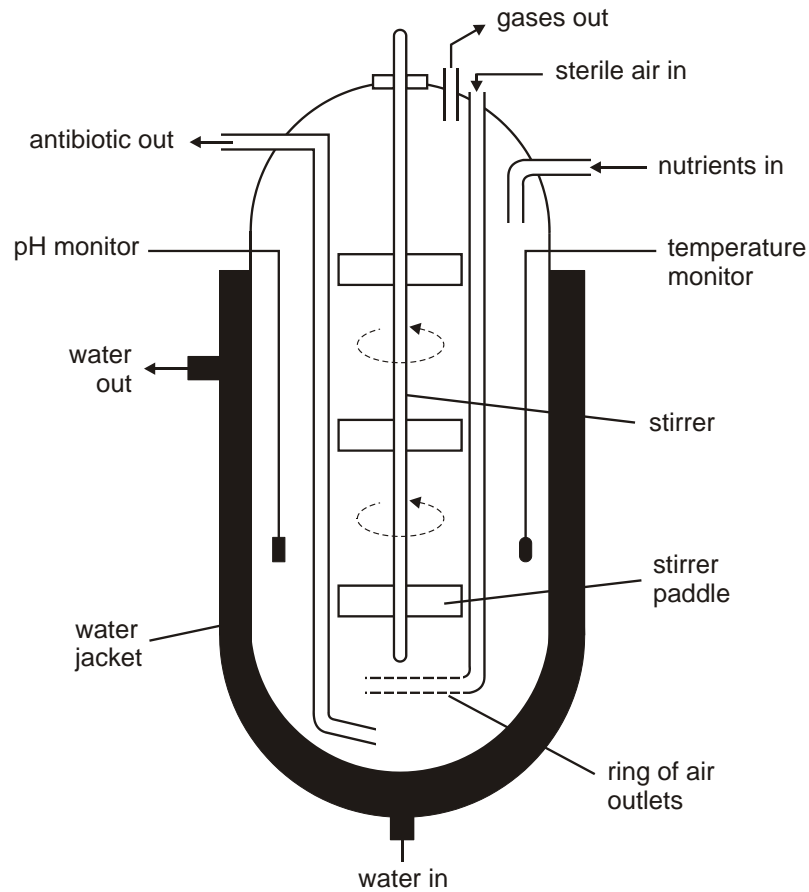
Alcohol or sugar concentration or cell number



Penicillin or sugar concentration or cell number

Compare and contrast the processes of continuous culture and batch culture.

- **Biofermenter**



- **Sterile air in**
 - provides oxygen for aerobic respiration;
 - any detail, e.g. oxidative phosphorylation;
 - sterile to prevent contamination;
 - mixes microorganism with substrate / prevents settling / bubbles help stirring / AW;
- **Air out**
 - To release the pressure that builds up from carbon dioxide build up from aerobic and anaerobic respiration
- **Water Jacket**
 - water is for, cooling / removing excess heat;
 - maintains, constant / optimum, temperature;
 - respiration produces heat; which would, denature enzymes / kill cells;
 - heat also produced by, stirrer / motor;
- **Inoculants**
 - Microorganisms we want to grow
- **Harvesting tap**
 - To allow the product / waste / microorganism to be removed
- **Probes**
 - Monitor the conditions inside the biofermenter
 - Conditions can affect the type, amount and quality of the product produced
 - pH
 - Temperature
 - Oxygen levels
 - Nutrient levels
- **Impeller/stirrer**
 - mixes microorganism with substrate / prevents settling

- **Batch Culture**
 - Used to produce secondary metabolites
 - Everything sterilised and added at the start then left.
 - Small quantities of nutrients are added throughout to maintain the stationary phase.
 - Not too much = log phase
 - Not too little = death/decline phase

- **Continuous culture**

- Used to produce primary metabolites
- Everything sterilised and added continuously to maintain the log phase.
- Oxygen, p.H and temperature are constantly monitored

	Batch Culture	Continuous Culture
Growth rate	Slower due to nutrients decreasing and waste increasing	Faster due to nutrients added and waste removed continuously
Efficiency	Less as not in operation all the time	More as in operation all the time
Contamination	Only 1 batch is lost	Huge volumes of product is lost
Setup	Easy to setup and maintain	Difficult to setup and maintain
Metabolites	Secondary	Primary

Explain the importance of manipulating the growing conditions in a fermentation vessel in order to maximise the yield of product required.

- **Limit contamination**

- Occurs because the biofermenter has the perfect conditions for microorganism growth and so will allow unwanted microorganisms just as well as desired microorganisms

- **Monitor pH**

- pH needs to be monitored as it will affect enzyme activity – denaturation
- pH buffers maintain the pH concentration of the nutrient medium

- **Monitor temperature**

- temperature needs to be monitored as it will affect enzyme activity – denaturation
- temperature is maintained by the use of a water jacket

- **Monitor oxygen levels**

- Oxygen levels need to be monitored as it will affect type of respiration.
- Aerobic respiration provides lots of energy and faster growth
- Anaerobic respiration provides less energy and slower growth
- Oxygen can be added to the biofermenter but it must be sterile.

- **Monitor nutrient levels**
 - Nutrient levels can maintain the growth of the microorganisms in different phases of their growth curve
 - Very important for the production of primary and secondary metabolites

Explain the importance of asepsis in the manipulation of microorganisms.

- **Contamination**
 - Kills culture
 - Spoils product
 - Produce toxins harmful to health
 - Competes with inoculants for space, nutrients etc
 - Decreases the yield of the product
- **Asepsis**
 - The absence of contamination from unwanted foreign microorganisms or pathogens
- **Aseptic techniques**
 - Any technique/manipulation of equipment or materials that are designed to prevent contamination by foreign and unwanted microorganisms
 - Includes
 - Autoclave – steamed at 121°C for 15 minutes
 - Gloves
 - Disinfectant
 - Fume cupboard
 - Stainless steel tables
 - Flaming equipment
 - Cultures are kept covered
 - Sterilised nutrient mediums

Describe how enzymes can be immobilised.

- **Enzymes are useful because**
 - They are specific to one reaction
 - They lower the activation energy of a reaction
 - They reduce the chemical requirements
 - Work at low temperatures
 - Not used up in the reaction

- **Immobilisation**
 - Enzymes are attached to an insoluble material
- **Immobilisation is carried out by**
 - **Adsorption**
 - Attached to an insoluble material such as clay, resin
 - Held by ionic bonds / hydrophobic associations
 - Doesn't affect reaction rate
 - Can be easily lost by leakage
 - **Covalent bonding**
 - Attached using a cross-linking agent using covalent bonds
 - Little leakage of the enzyme
 - Can have a large loss of function through immobilisation
 - **Entrapment**
 - Inside gel beads using alginate / silica
 - Behind a cellulose fibre network
 - Enzyme trapped in natural state
 - Can reduce reaction rate due to diffusion of substrates through immobilising agent
 - Can support enzymes natural state
 - **Membrane separation**
 - Trapped behind a partially permeable membrane / microspheres
 - Allows substrates and products to pass through it but not the enzyme

Explain why immobilised enzymes are used in large-scale production.

- **Advantages**
 - does not mix with / does not contaminate / stays separate from, the product; ref to, no / less / easier, downstream processing
 - recoverable / not lost during processing
 - reusable / cost effective = less downstream processing
 - matrix stabilises / protects the enzyme
 - so activity not affected by changes in, temperature / pH or run at a high temperature / wider range of pH
 - longer, use / shelf-life
 - so suitable for continuous culture / cost effective / greater yield

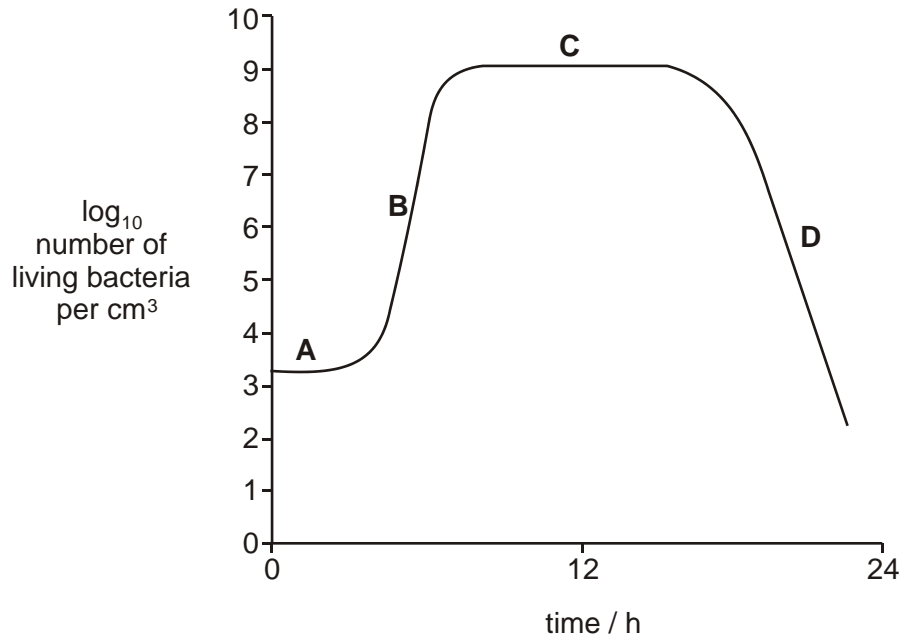
- **Disadvantages**

- Can reduce the reaction rate due to reduced ability to form enzyme-substrate complexes
- Difficult and costly to immobilise
- If contamination occurs you must destroy all the enzymes
- Can lose enzymes by leakage

Questions

1

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:

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

- (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
-
- 2
-
- 3
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[3]

[Total 9 marks]

5.2.2

2

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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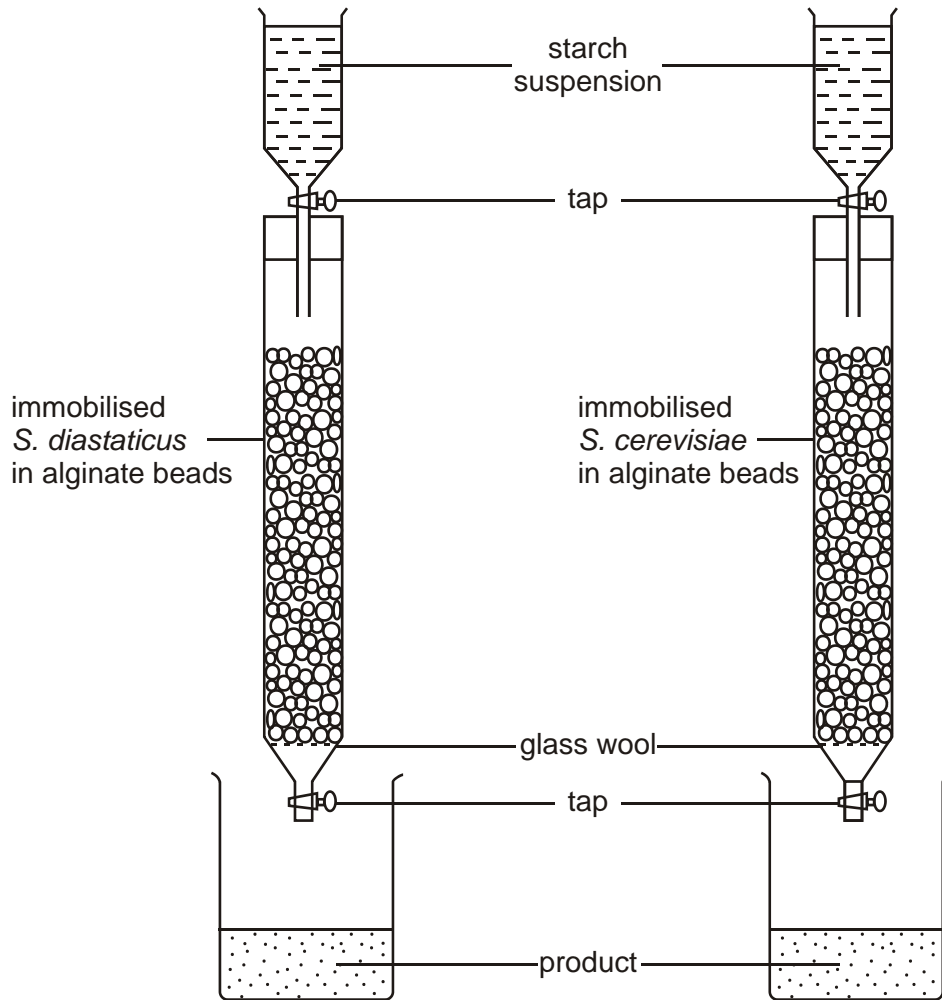
[2]

[Total 4 marks]

3

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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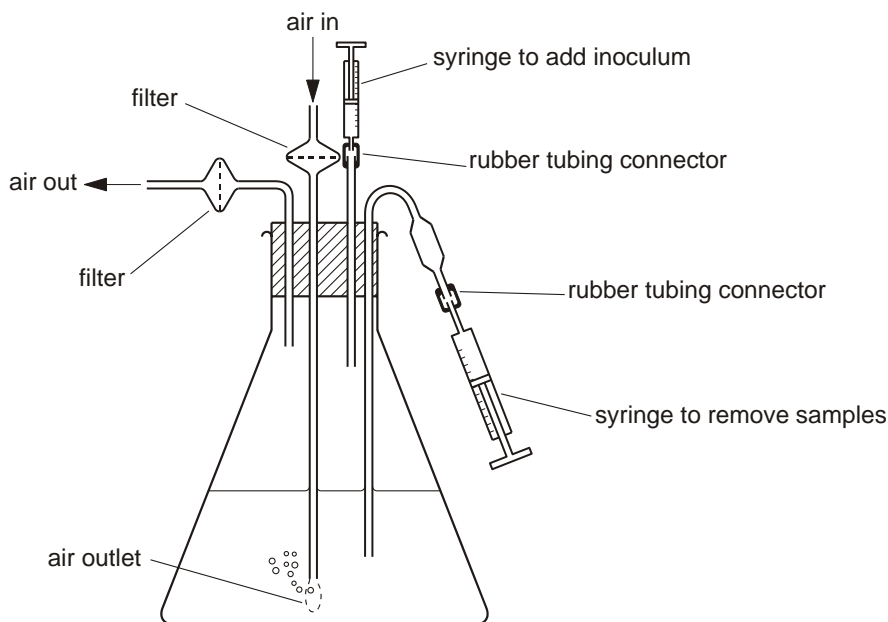
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[2]

[Total 7 marks]

4

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]

5

(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.

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[1]

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

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[1]

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

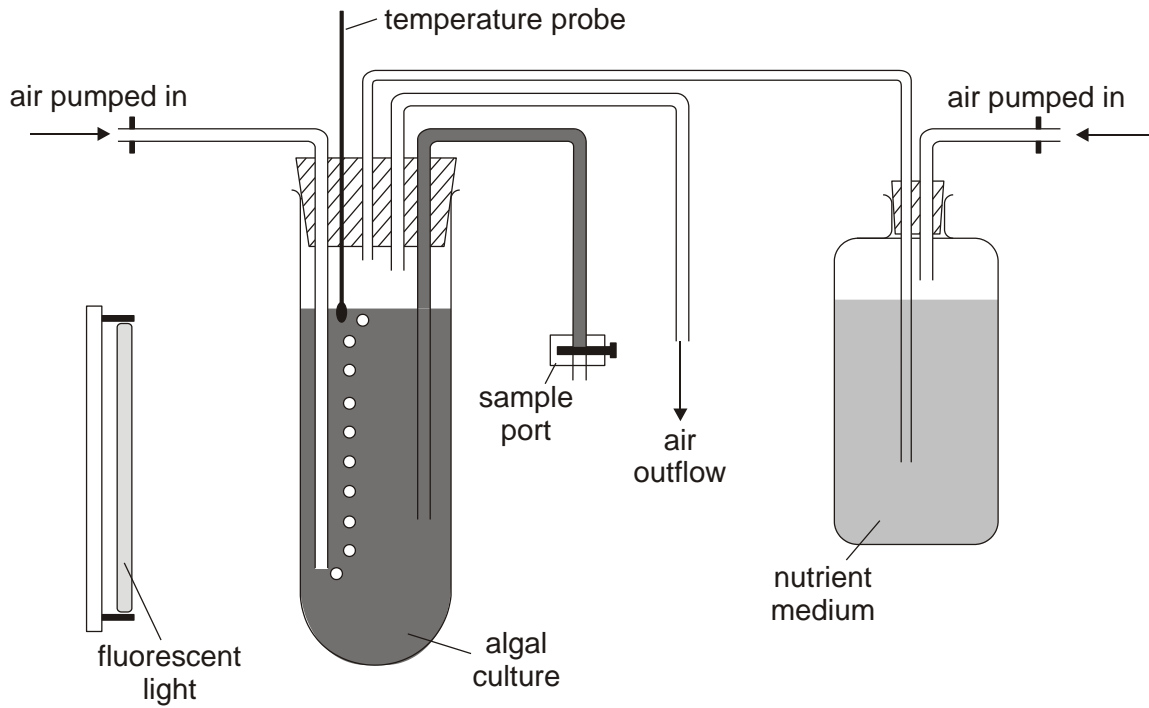
method

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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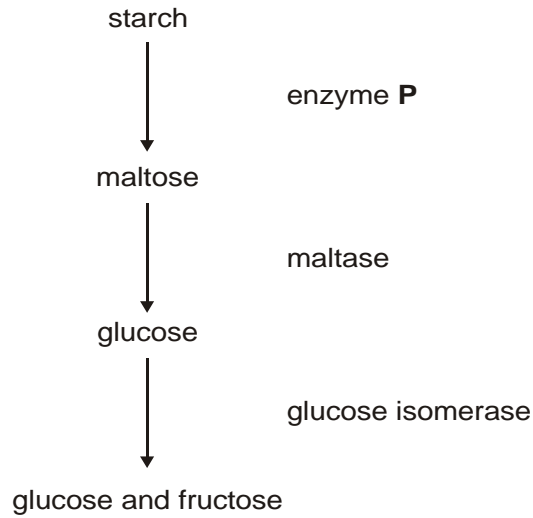
[Total 15 marks]

6

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**.

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[1]

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

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[1]

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

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[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.

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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]

7

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 achieved.*

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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]