Name	Date started	Target end date

GCE A LEVEL — BIOLOGY UNIT 3 QUESTION PACK

1074 (Legacy BY4) + 1075 (Legacy BY5) · New spec Unit 3 Topic 6 · A2 unit, first sat 2017, 90 marks, 2h paper

REVISE
.wales

BIOLOGY — UNIT 3 · MICROBIOLOGY — BACTERIAL GROWTH & ASEPTIC TECHNIQUE

3.4 Microbiology — bacterial growth curves, aseptic technique, fermenters and industrial applications

Bacterial cell structure (Gram-positive vs Gram-negative cell walls), the four phases of bacterial growth (lag, log, stationary, death), aseptic technique, viable vs total counts, batch and continuous fermenters, and industrial microbiology examples including penicillin production.

LEGACY 2008 SPECIFICATION

Estimated time for entire question pack: ~2 h 30 min

Derived from the legacy BY4 / BY5 papers' pace of ~1.3 min/mark, padded for long-prose answers (94 marks over 10 questions).

*You are advised to **not** attempt to complete all of this in one sitting.*

ABOUT THIS QUESTION PACK

This is a **comprehensive practice question pack**, not a single mock paper. It contains every question from the legacy WJEC BY4 (and BY5, where relevant) papers (2008 modular spec, 2011–2017) that maps onto new-spec A2 Unit 3 Topic 6 (3.4).

Questions are ordered by source paper date.

INSTRUCTIONS

Use black ink or black ball-point pen. Show all working — quality of written communication will affect marks. A calculator is allowed. Diagrams included in answers must be fully annotated.

For Examiner's use only

Q	Source	Max	Mark
1	BY4 Jun 11 Q5	9	
2	BY4 Jun 12 Q1	14	
3	BY4 Jun 13 Q4	9	
4	BY4 Jun 14 Q2	7	
5	BY4 Jun 15 Q3	11	

Q	Source	Max	Mark
6	BY4 Jun 15 Q5	12	
7	BY4 Jun 16 Q1	7	
8	BY4 Jan 12 Q6	8	
9	BY4 Jan 13 Q2	14	
10	BY4 Jan 14 Q1	3	
Total		**94**	

Microbiology — Bacterial Growth & Aseptic Technique — what the new spec asks

WJEC GCE A Level Biology (from 2015) · Unit 3: Energy, Homeostasis & the Environment · Topic 3.4.

Bacterial cell walls

- Gram-positive: thick peptidoglycan — retains violet stain.
- Gram-negative: thin peptidoglycan + outer membrane (LPS) — counterstained pink.
- Gram status affects antibiotic susceptibility (e.g. penicillin).

Growth curve phases

- Lag: adaptation, enzyme synthesis, no division.
- Log: exponential division; nutrients abundant.
- Stationary: birth = death rate; nutrients limiting / wastes accumulate.
- Death: cells lyse faster than they divide.

Counting cells

- Total count: haemocytometer / turbidity — counts living + dead.
- Viable count: serial dilution + plate count — only living cells.
- Both expressed per cm^3 (or cfu/cm^3 for viable).

Aseptic technique & fermenters

- Flame loops / Bunsen near cultures to prevent contamination.
- Batch fermenter (closed) for penicillin; continuous fermenter for biomass.
- Sterile media, controlled pH, temperature, O_2, stirring.

Crib sheet »

Microbiology — Bacterial Growth & Aseptic Technique in one page

Quick-reference notes — revisit before each question.

Bacterial structure

Cell wall (peptidoglycan), plasma membrane, cytoplasm.

Ribosomes (70S); single circular chromosome; plasmids.

No nucleus or membrane-bound organelles.

Gram staining

Crystal violet → iodine → alcohol wash → counterstain (safranin).

Gram+ retain violet (thick peptidoglycan).

Gram− lose violet, stain pink.

Growth phases

Lag: adaptation, no division.

Log: exponential growth, doubling time.

Stationary: nutrient limit / waste buildup.

Death: lysis exceeds division.

Aseptic technique

Flame loop / Bunsen.

Sterile media (autoclave 121 °C, 15 min).

Work near flame, lid lifted briefly only.

Fermenters

Batch: closed, used for penicillin (secondary metabolite).

Continuous: feed in, draw off — for biomass.

Both stirred, aerated, pH and temperature controlled.

Counting

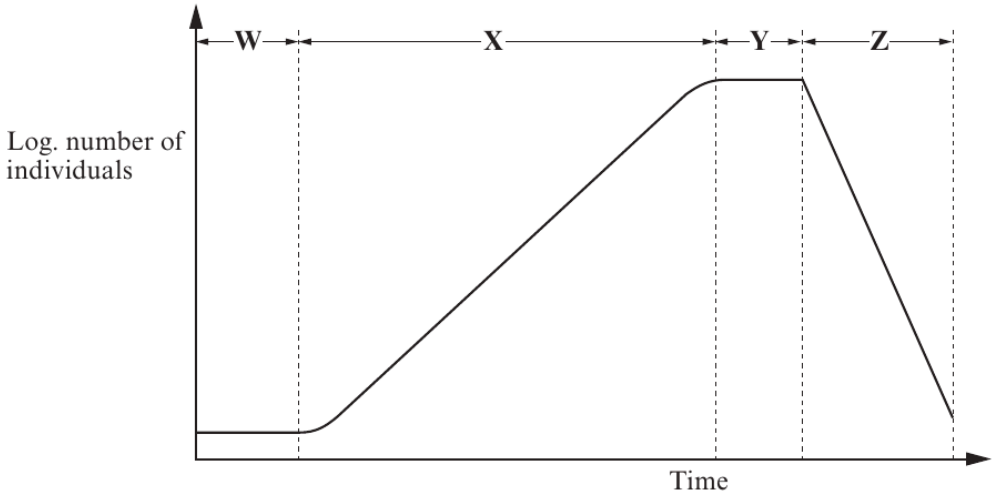
Total: haemocytometer / turbidity.

Viable: serial dilution & plate count (cfu/cm^3).

Viable count lags total during death phase.

Examiner only

5. The diagram below shows the growth curve for a population of a simple organism such as yeast.



(a) Name the phases of growth **W-Z**. [2]

W .....

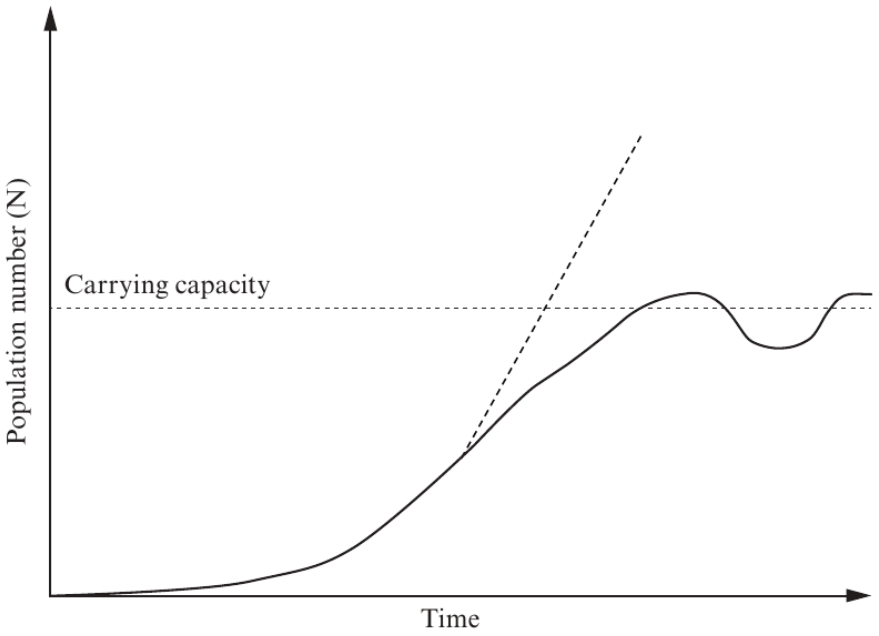
X .....

Y .....

Z .....

Examiner only

(b) The growth for more complex organisms may be given by the formula;
Population growth = (Births + Immigrations) – (Deaths + Emigrations)
A growth curve for such a population is shown with a solid line below.



(i) Draw an arrow labelled D on the solid line to show **one** phase where (Deaths + Emigrations) exceeds (Births + Immigrations). [1]

(ii) Explain the term *carrying capacity*. [2]

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(iii) State **two** examples of **density dependent** factors that can affect the carrying capacity of an ecosystem. [2]

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(iv) State **one** example of a density independent factor. [1]

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(v) On the graph, extend the dashed line to show what is likely to happen to a population whose size substantially exceeds the carrying capacity. [1]

(Total 9 marks)

Examiner only

1. A student introduced a pure culture of anaerobic bacteria into a nutrient medium and recorded the numbers of bacteria per cm^3. The results are shown in the table.

Time / hours	*Numbers of bacteria / millions per cm^3*
0	1.0
1	1.0
2	1.0
3	1.2
4	1.8
5	3.5
6	6.9
7	13.8
8	28.0
9	57.0
10	113.8
11	225.0
12	375.0
13	440.0
14	460.0
15	482.0
16	484.0
17	486.0
18	488.0
19	488.0
20	488.0

(a) (i) Calculate the percentage increase in population size between 7 and 8 hours after introduction of the bacteria into the nutrient. Show your working. [2]

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Examiner only

(ii) How do you account for the low rate of population growth in the first three hours of the experiment? [2]

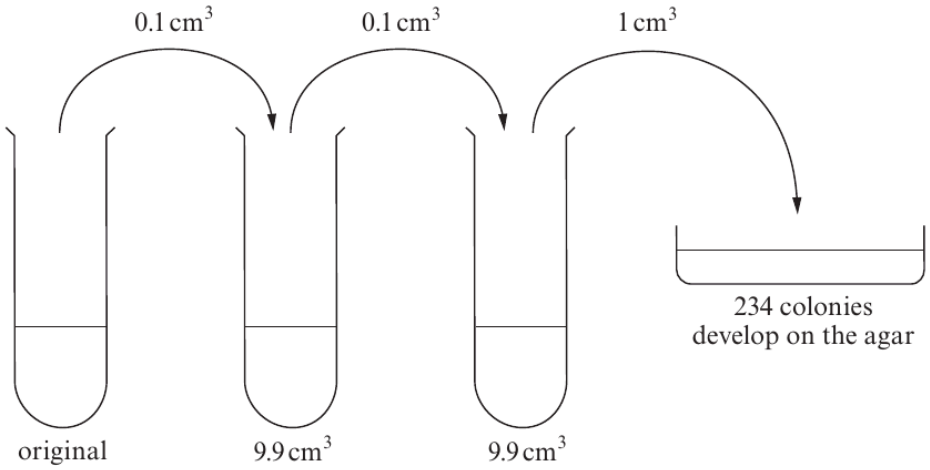
(iii) The stage of rapid growth in population size is described as being exponential. What is meant by the term exponential growth? [1]

(iv) Give **two** reasons which could lead to a decline in population growth in this culture. [2]

(b) What conditions would be needed for the growth of the bacteria in the experiment? [3]

Examiner only

(c) The numbers of bacteria in sea water are commonly monitored. Small samples of the water are taken, diluted and plated onto nutrient agar. The diagram represents the stages of serial dilution to assess the numbers of bacteria in an original sample.



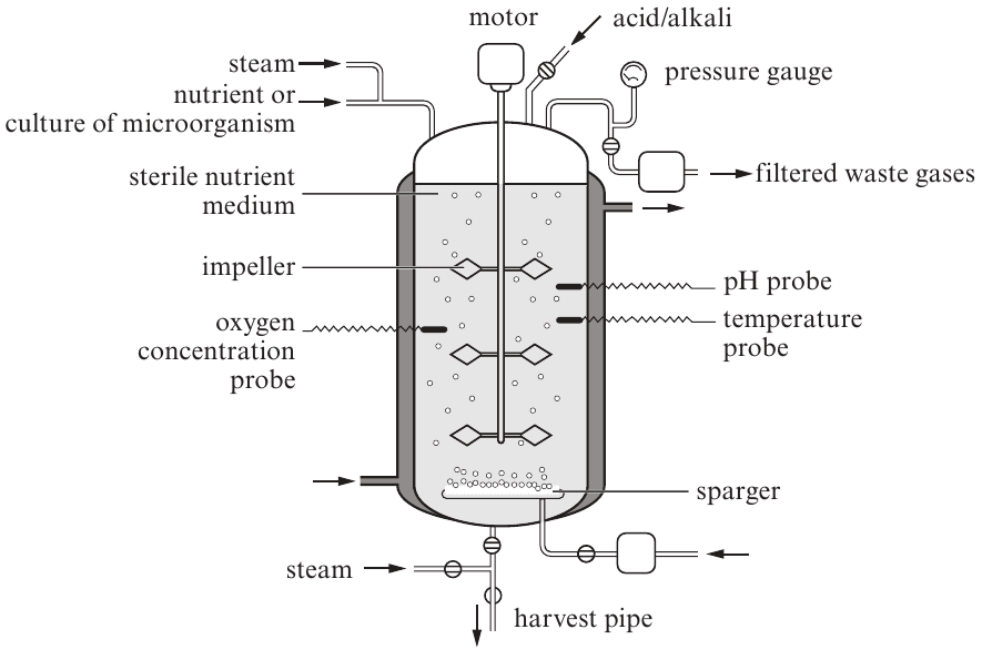
Calculate the number of bacteria per cm^3 in the original sample. Show your working. [2]

(d) Describe **two** precautions which should be carried out to ensure aseptic conditions in **this** experiment. [2]

(Total 14 marks)

Examiner only

4. The diagram below shows a fermenter that has been set up to culture a microorganism and harvest a product from it.



(a) Suggest **two** reasons for the use of a sparger in fermenters. [2]

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Examiner only

(b) (i) Using information in the diagram opposite, suggest why the pH probe is needed. [2]

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(ii) If the microorganism in the fermenter is an obligate aerobe, state **one** waste gas that will need to be removed. [1]

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(iii) In the early stages of fermentation by batch culture it may be necessary to warm the contents of the fermenter, but cooling is often needed towards the end. Suggest reasons for this difference. [2]

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(c) Suggest reasons for preventing the fermenter becoming contaminated with other microorganisms. [2]

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Examiner only

2. *(a)* Define the following terms with reference to bacterial growth. [3]

(i) obligate aerobe

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(ii) obligate anaerobe

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(iii) facultative anaerobe

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(b) Describe and explain the appearance of Gram positive and Gram negative bacteria following Gram staining. [3]

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(c) Most pathogens in humans are Gram-positive organisms. Six Gram-positive genera are typically pathogenic in humans. Two of these, *Streptococcus* and *Staphylococcus*, are cocci. The remaining organisms are bacilli.

What **three dimensional shape** would the cocci and bacilli be? [1]

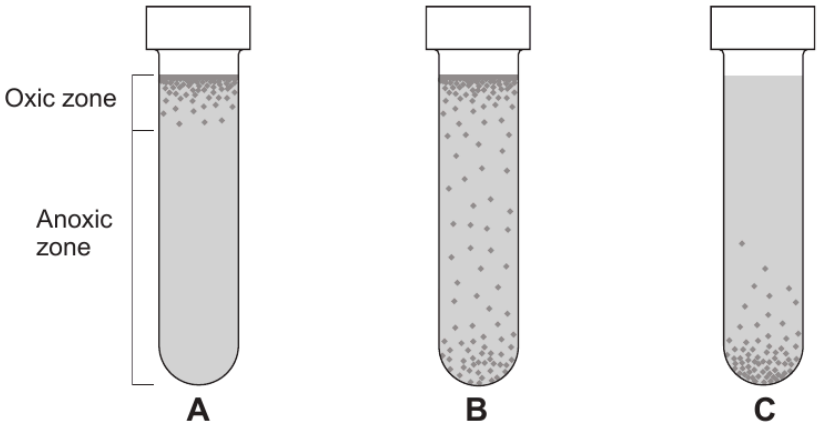
cocci .....

bacilli .....

7

Examiner only

3. The diagrams below show the results of a number of experiments investigating bacterial growth with different levels of oxygen.
Bacteria were mixed with agar in bacteriology tubes and the agar was allowed to set. The tubes were incubated for 24 hours at 37°C.



Each dot represents an individual bacterial colony within the agar or on its surface. The surface which is directly exposed to atmospheric oxygen contains oxygen (oxic zone). The oxygen content of the agar decreases with depth until it does not contain oxygen (anoxic zone) towards the bottom of the tube.

(a) Using the results above, what terms are given to the types of bacteria which grow in tubes **A**, **B** and **C**? [2]

A .....

B .....

C .....

(b) Some bacteria will not grow at all in the presence of oxygen. These bacteria cannot generate ATP through aerobic respiration. Using your knowledge of respiratory pathways describe how they are able to produce ATP in the absence of oxygen. [4]

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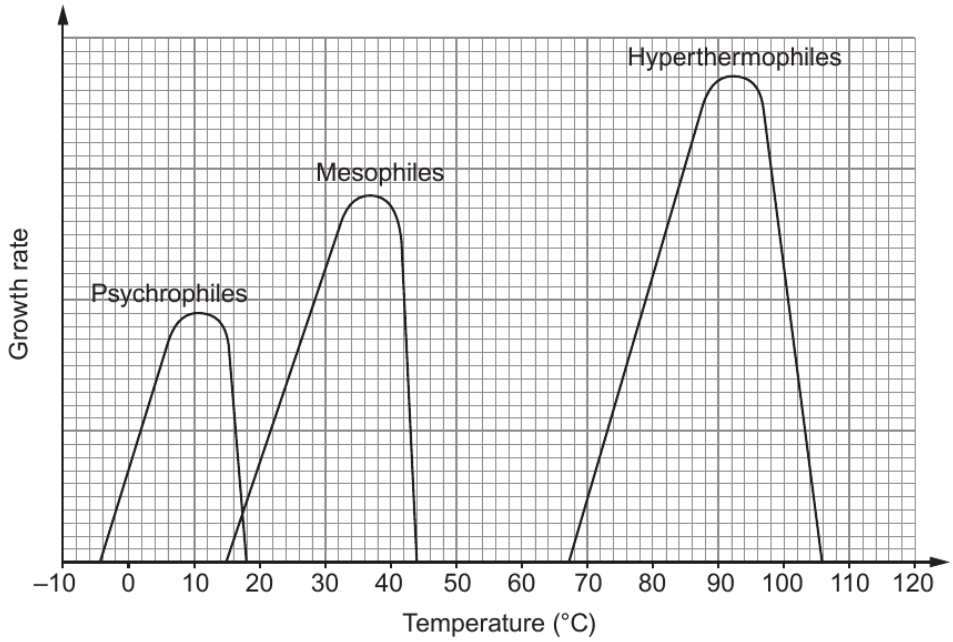
Examiner only

(c) Microorganisms can be grouped based on the temperatures in which they actively grow, as shown below.



(i) Using the graphs, state the optimum temperature for each group and suggest an environment where they can actively grow. [3]

	Optimum temperature (°C)	Environment
psychrophiles		
mesophiles		
hyperthermophiles		

(ii) Using your knowledge of cell biology, suggest **two** biological problems which psychrophiles must overcome in order to carry out cellular processes. [2]

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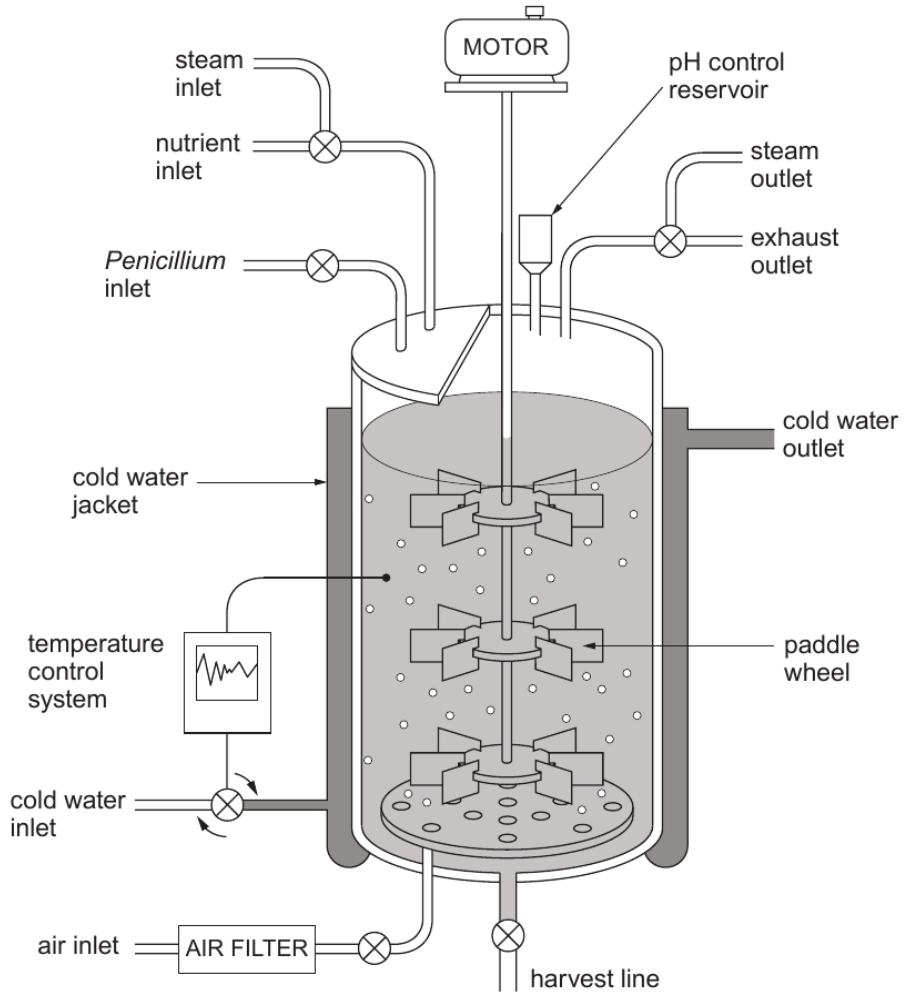
Examiner only

5. The diagram below shows a type of batch fermenter used to produce penicillin.



(a) (i) Apart from helping with the mixing, explain why sterile air is pumped into the fermenter. [2]

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(ii) The fungus that produces penicillin requires a supply of carbon and nitrogen. In what form might these elements be supplied to the fungus? [2]

Carbon source .....

Nitrogen source .....

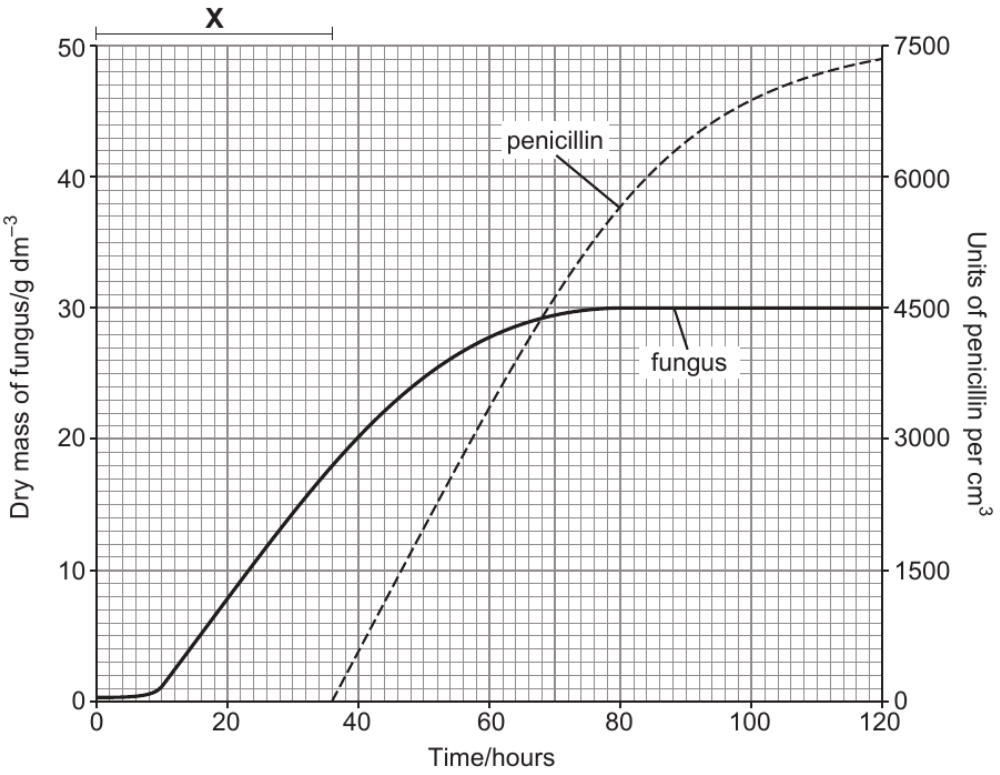
Examiner only

(iii) How would the pH be controlled? [1]

(iv) What is the purpose of the *temperature control system*? [2]

Examiner only

(b) Below is a graph showing the production of penicillin and the growth of the fungus, *Penicillium*, in the fermenter shown on page 10.



(i) Using the graph, state the time that *Penicillium* enters the stationary phase. [1]

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(ii) Why is there a high level of protein synthesis in the first ten hours of the *Penicillium* culture? [1]

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Examiner only

(iii) Explain why no penicillin is produced during time period **X**. [3]

12

Examiner only

Answer **all** *questions.*

1. *(a)* Gram-positive and Gram-negative bacterial cell walls have different structures. Explain how the structure of Gram-negative bacteria allows them to be resistant to certain antibiotics. [2]

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(b) Microorganisms require certain factors to be supplied in the culture medium to allow them to grow. These are molecules such as amino acids, purines and pyrimidines. State the functions of the following growth factors in microorganisms. [2]

Amino acids

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Purines and pyrimidines

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(c) It is not possible to culture viruses on sterile agar plates. Explain why. [1]

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(d) Describe how bacterial culture plates are safely disposed of. [2]

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Examiner only

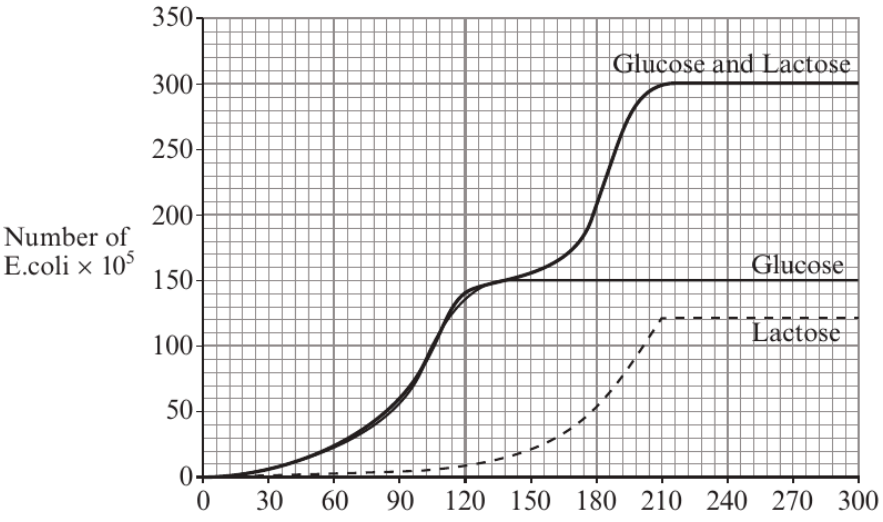
5. Fill in the missing blanks with appropriate scientific terms. [7]

Proteins in dead organisms are decayed by ..... into ..... . The bacterium called ..... converts these into ..... and finally the bacterium ..... converts the waste products of these bacteria into nitrate ions. Bacteria called ..... which live in the root nodules of legumes can fix atmospheric nitrogen.

The bacterium ..... is a free living bacterium in the soil which can also fix nitrogen. It does this by adding atmospheric nitrogen to a carbon source from sugars.

(Total 7 marks)

6. Three fermenters were set up in order to study the population growth of *E. Coli* in different sugar solutions, 0.001 M glucose, 0.001 M lactose and a mixture of glucose and lactose both at 0.001 M. Samples were removed from the fermenter at timed intervals. The population size in each fermenter was estimated. The results are shown in the graph below.



Examiner only

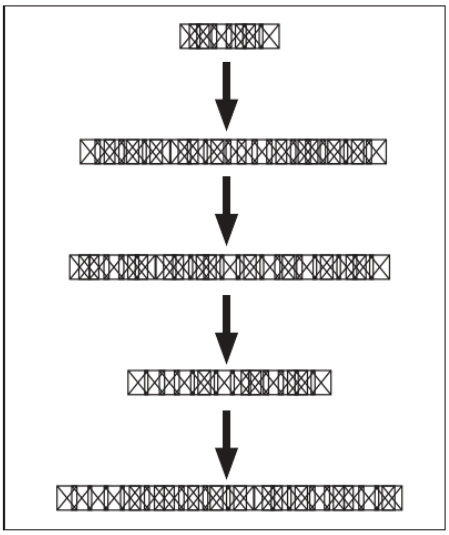
(a) Explain why there is a difference in population growth between the glucose and lactose. [3]

(b) Describe and explain the shape of the curve when the bacteria are grown in lactose and glucose together. [5]

(Total 8 marks)

Examiner only

2. The diagram below shows the process of Gram staining to identify Gram positive (+) and Gram negative (-) bacteria.



(a) State the colour of the bacteria following the application of the counter stain: [1]

Gram positive; .....

Gram negative. .....

(b) Use your knowledge of the structure of the bacterial cell wall to explain the differences in the appearance of the two types of bacteria when stained with the Gram staining technique. [3]

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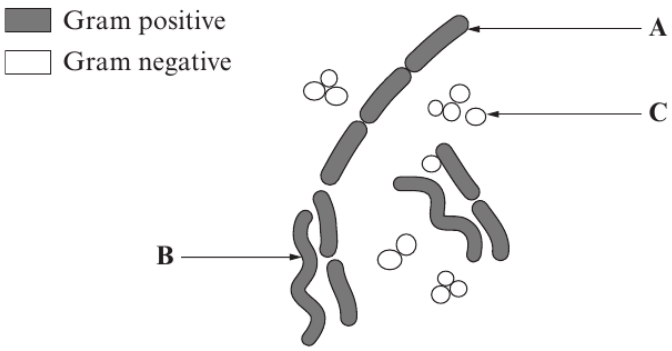
Examiner only

(c) Following an outbreak of food poisoning in a school, samples were taken from infected patients. The Gram staining technique was used, in conjunction with the shape of bacterial cells, to identify potentially pathogenic bacteria in the samples.

The diagram below shows part of a bacterial smear stained using the Gram staining technique.



(i) State the name given to the shapes of the bacteria labelled **A**, **B** and **C**. [3]

A .....

B .....

C .....

(ii) Suggest why the bacteria labelled **C** in the diagram might be the possible cause of the food poisoning. [1]

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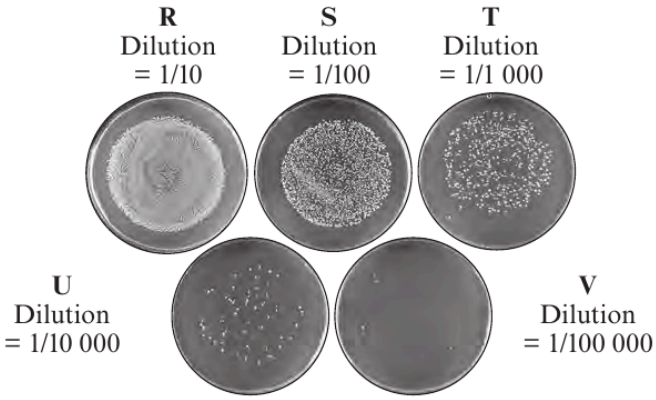
Examiner only

(d) Using the viable count method Environmental Health Officers made an estimate of the number of bacteria in various foods in the school canteen. Dilutions of 1/10, 1/100, 1/1 000, 1/10 000 and 1/100 000 were prepared and 0.5 cm^3 of each dilution were spread evenly over the surface of agar plates. The plates were incubated at 35°C for 24 hours. A photograph of the results for one of the samples is shown below.



They decided to use Plate **U** to estimate the number of bacteria in the food sample.

(i) With reference to the plates shown above explain why they decided to use Plate **U** and **not** any of the other plates. [2]

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(ii) On plate **U** 69 bacterial colonies were counted. Estimate the number of bacteria present in 1 cm^3 of the original food sample. Show your working. [2]

Estimated number of bacteria = ... per cm^3

(iii) Suggest why this number is likely to be an underestimate of the actual number of bacteria present. [1]

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(iv) Suggest why the bacteria were cultured at 35°C and not at 25°C. [1]

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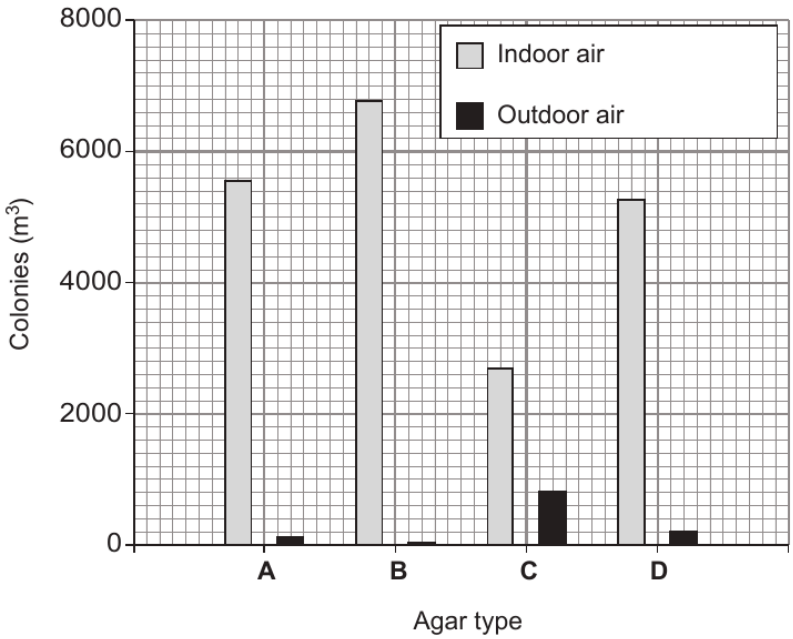
14

Examiner only

1. 1 m^3 of air was filtered from two different environments.
The microorganisms collected were grown, using aseptic technique, on four different types of agar plates (**A**, **B**, **C** and **D**) at the same temperature and for the same length of time.
The number of colonies grown from each sample is shown.



(a) What conclusion can you draw from the graph above about the numbers of microbes in the **two** air samples? [1]

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(b) The four agar types have resulted in different colony numbers because they contain different nutrients.
State **four** ways that the **agar types** could differ in composition. [2]

i. .....

ii. .....

iii. .....

iv. .....

3

END OF QUESTION PACK

10 questions · 94 marks · ~2 h 30 min
Source: WJEC BY4 + BY5 (2008 modular spec, 2011–2017)
Curated for WJEC Biology 2015 spec A2 Unit 3 — Topic 6 (3.4)