Bio Factsheet

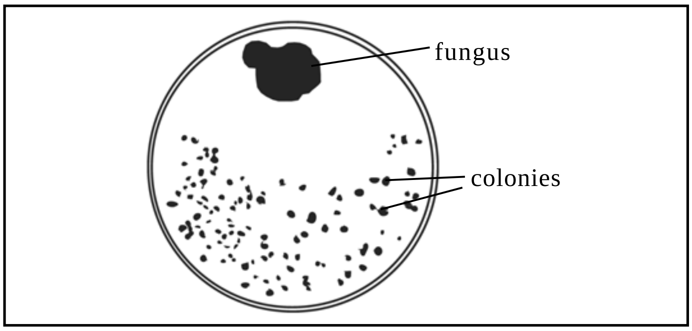
www.curriculum-press.co.uk Number 176

Penicillin Production and Use

In the beginning

In 1928, Alexander Fleming discovered that one of his agar plates, containing a culture of the bacterium *Staphylococcus aureus*, was contaminated by the fungus *Penicillium (Fig1)*

Fig 1 Bacterial agar plate contaminated with Penicillium fungus



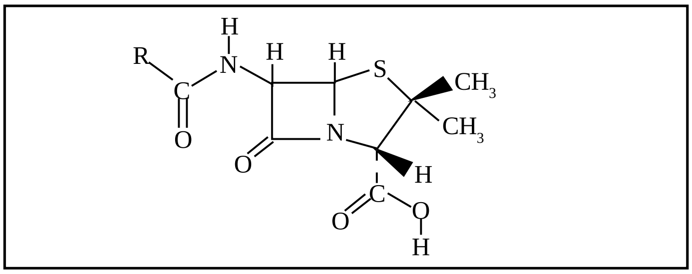
Fleming realized that no colonies of bacteria were growing near the fungus. He had discovered antibiotics!

Penicillin

Penicillin is an antibiotic. The term “antibiotic” was originally used to describe one of the many different substances produced by microorganisms, which kill other microorganisms or stop them from multiplying. All antibiotics work by interfering with specific cell components or by disrupting cell metabolism.

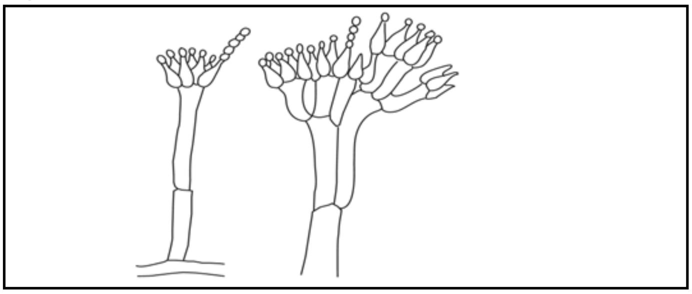
Penicillinis wers first used to treat infections in the 1940s and are now the most widely used antibiotics. They are all based on a ring-shaped nucleus to which various side-chains are attached (Fig 2).

Fig 2. Structure of a penicillin



Penicillins are derived from the fungus Penicillium chrysogenum (Fig 3).

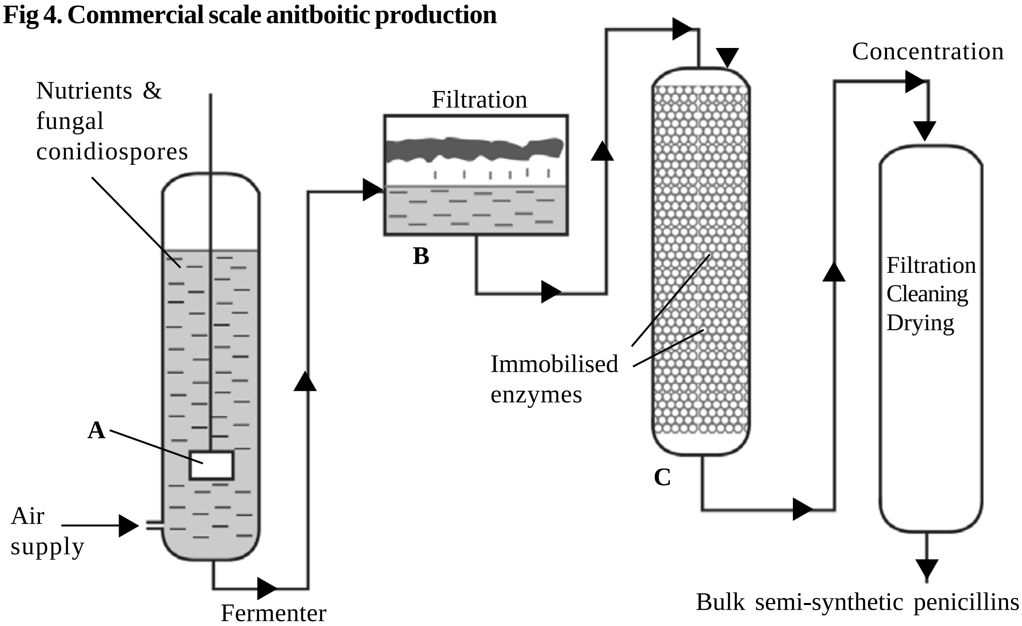
Fig 3 *Penicillium chrysogenum*



Penicillin Production

The antibiotic is produced on a commercial scale as shown in Fig 4.

Fig 4. Commercial scale anitboitic production



Typical Exam Questions

1. What nutrients will be added to the culture?
2. What is the purpose of structure A?
3. Why is air supplied to the fermenter?
4. What other conditions need to be controlled?
5. Explain the purpose of the filtration carried out at B.
6. Why are immobilized enzymes used?
7. Penicillium has a filamentous growth form. What problems does this pose for culture in a fermenter and how are they overcome?

***Exam Hint :-** Labelling and explaining this diagram is a commonly asked question.*

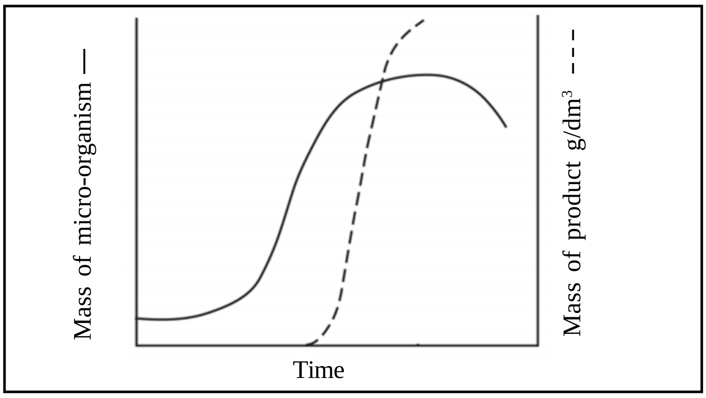
Penicillin Production Answers
1. The growth medium is often corn steep liquor which contains a high concentration of sugars and also a precursor, ie. a chemical which makes penicillin-G or benzylpenicillin.
   Glucose often provides the carbon source, lactose encourages greater production of penicillin and yeast extract may provide a nitrogen source for protein synthesis
2. The nutrients and the fungal culture are thoroughly mixed by the impellor (paddle) A
3. Air is added to provide oxygen so that the fungi can respire aerobically. Remember, the whole point of this is that we want them to produce a useful substance for us, so we have to try to keep them in optimum conditions.
4 Temperature is maintained at 24°C and the pH is kept slightly alkaline (6.5) via a monitor or probe which adds buffer or adds/removes H^+ ions
5 Filtration separates the penicillin from the fungal hyphae/mycelium
6 The enzymes can then be used to modify the penicillin.You should know four advantages of using immobilized enzymes – they are more stable, the enzyme can be reused, it is easier to separate the enzyme from the product and less enzyme is required – for more detail see Factsheet 148 Industrial Uses of Enzymes.
7 A viscous tangle of cells forms and it is difficult to get oxygen to the cells. To overcome this, small quantities may be produced at a time. Aeration is by a blower controlled by a sensor, rather than by stirrer (which might damage the cells).

Note: Even if penicillin isn't explicitly on your specification, you may be expected to apply your knowledge of biotechnology, growth requirements and enzyme activity in this type of diagram

Penicillin is a secondary metabolite
A secondary metabolite is a substance which is not essential for the life of the micro-organisms and which is not produced during growth.

Secondary metabolites are produced **after** the exponential growth phase has stopped (Fig 5). This is crucial because it means that secondary metabolites such as penicillin cannot be produced in continuous fermenters – which deliberately maintain the micro-organism in the exponential growth stage. They are grown in **batch culture or fed-batch culture**. In **fed-batch culture**, additional nutrients are added when the secondary metabolite is being synthesized – this enhances the yield of secondary metabolite. However, the extra nutrients have to be added very carefully and some of the culture is removed during the process.

Fig 5. Growth and production curves



Batch culture is when all the ingredients are added at the beginning. It is then left until the reaction forming the secondary metabolite has taken place, after which the products can be harvested.

Typical Exam Question
Explain why batch rather than continuous culture is used in the production of the antibiotic penicillin.

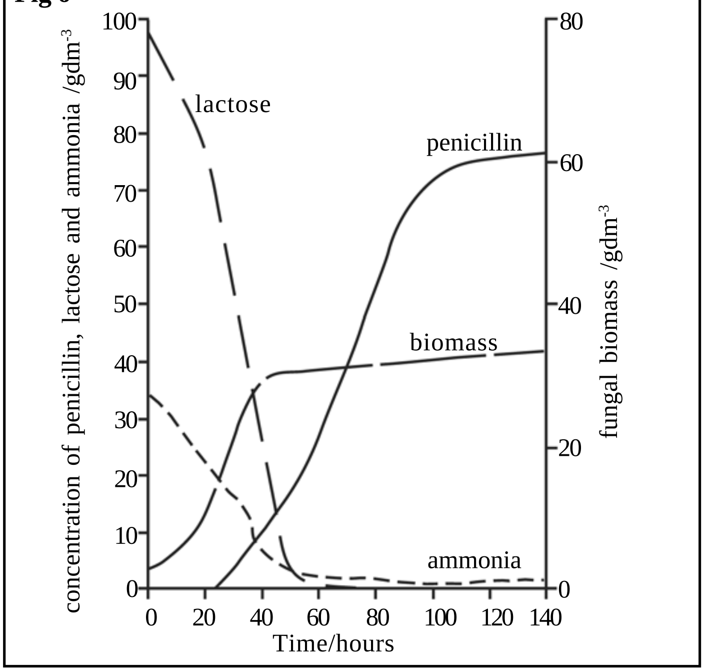
Answer Penicillin is a secondary metabolite;
Growth must not be in log phase/ must be in stationary phase;

Extract: Chief Examiners Report - Few realised that penicillin is a secondary metabolite and that it is produced during the stationary phase in the life cycle. As continuous culture maintains optimum conditions for growth, the organism remains in its exponential phase and thus the stationary phase is only produced in a batch culture.

Sometimes, you will be asked to interpret graphs showing what is happening inside a batch fermenter.

The fungus *Penicillium chrysogenum was* grown in a fermenter to produce penicillin. Fig 6 shows some of the changes which occur during this process.

Fig 6



Questions on these types of graph are just common sense –you can work out the answers from GCSE Biology.

Typical Exam Questions
1. Suggest why the concentration of ammonia decreases.
2. Why is a water jacket needed?
3. What evidence is there that penicillin is a secondary metabolite?
4 Suggest why X-rays might produce strains of *Penicillium chrysogenum* which give greater yields of penicillin.

Answers

1. Ammonia is NH_3
   So it is a source of nitrogen. Why would a growing fungus need nitrogen? To make proteins etc
2. The fungus is respiring, releasing heat. The water jacket cools the culture down, preventing enzyme damage.
3. The concentration of penicillin increases rapidly (look at the steepest part of the line) when the biomass of the fungus has stopped increasing ie when it is in the stationary phase
4. They may cause a gene mutation;
   That results in an enzyme/metabolite becoming more efficient

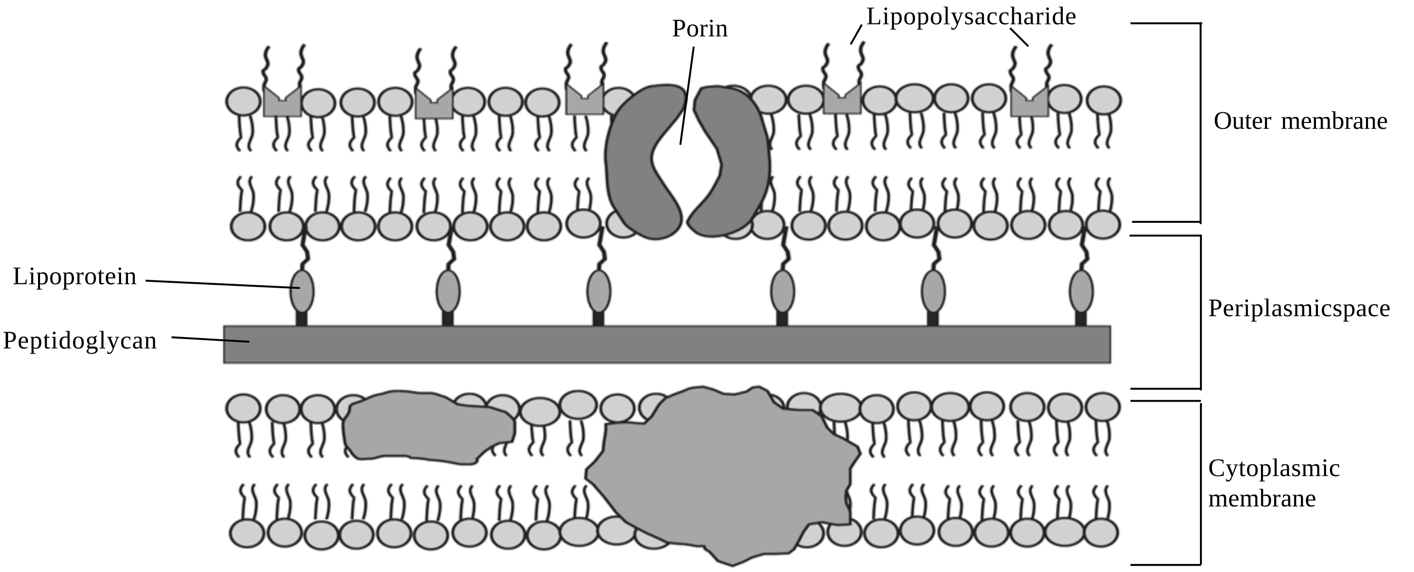
Most candidates gained a mark for stating that X-rays might produce gene mutations. However, only the most able related a gene mutation to the production of an enzyme which could result in greater yields of penicillin.

How do penicillins kill bacteria?

- The main component of bacterial cell walls is **peptidoglycan**. (Fig 7)
- Penicillins inhibit the last stage in the synthesis of peptidoglycan, so the bacteria cannot make their walls
- The water potential inside the bacterial cell is lower/more negative than outside
- Water therefore enters by osmosis
- The weakened cell wall cannot withstand the increased pressure and bursts

Extract from Chief Examiner's Report
Weaker candidates often thought that penicillin inhibited protein synthesis

Fig 7. Bacterial wall



Typical Question and student answer

Explain why bacterial cells burst when they are treated with penicillin (4).

The penicillin damages the bacterial wall. The pressure inside is too great and it busts.

The answer lacks detail. Learn and use the technical terms – peptidoglycan, water potential etc. There are 4 marks but the student has only told us 2 things – that it damages the wall, (which is imprecise) and that the pressure inside is too great (but they don't tell us why). So, they would get 1 mark at most, even though they probably thought "they knew it".

The interaction between penicillin and peptidoglycan is highly specific. Even though plants have cell walls, penicillin wouldn't affect these walls because they are made of cellulose, not peptidoglycan.

Bacteria fight back?

A growing number of bacteria are becoming resistant to penicillin. That is, the penicillin doesn't work anymore on these bacteria.
The bacterium *Staphylococcus aureus* is a common cause of life-threatening infections. By the 1960s it had already become resistant to penicillin.

Resistance mechanisms

There are a variety of mechanisms:

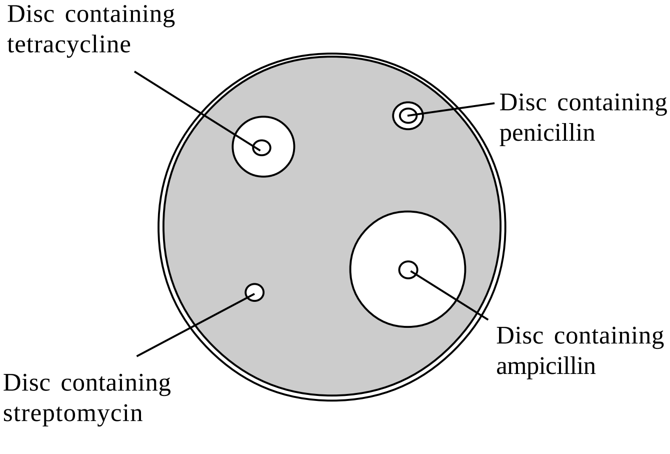
- Some bacteria produce penicillinase, an enzyme that breaks down penicillin
- Some bacteria have a capsule/wall and membrane complex that does not allow penicillin to penetrate or that pumps the penicillin straight out again

How does resistance develop?

This is a key question and one that trips up many students. The subtitle that I used (Bacteria fight back?) didn't help. Bacteria don't fight back. They don't as an individual or colony decide "enough is enough" and come up with a cunning penicillinase idea. All that happens is that one solitary bacteria has a mutation - a chance error in DNA replication - that changes the way this bacteria metabolises/builds its wall etc. This bacteria survives the course of antibiotics because of the selective advantage that its mutation gives it. Dividing mitotically, all of the daughter bacteria are resistant, whilst all those without the mutation are being wiped out. Soon, hundreds of thousands of individuals are resistant.

Practice Questions

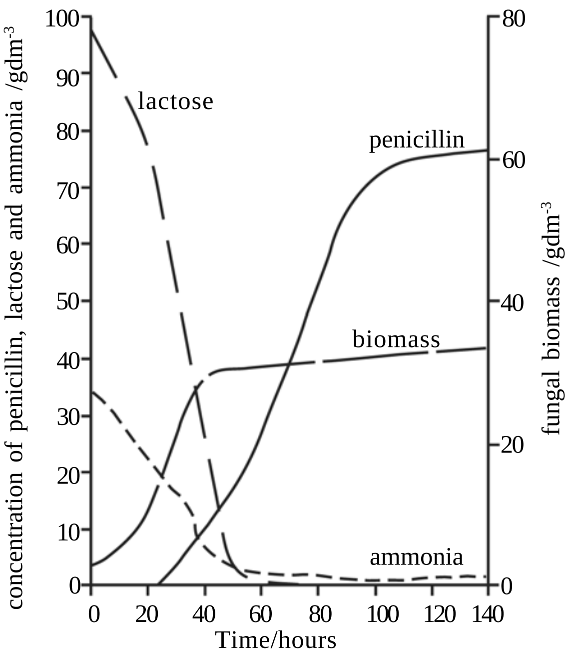
1. A sterile Petri dish of nutrient agar was inoculated with bacteria from a patient with a throat infection. Four discs, each of which had been soaked in a different antibiotic, were placed on top of the bacteria. The dish was incubated at 37 °C. The diagram shows the appearance of the dish after incubation.



(a) Explain the presence of clear zones around some of the discs containing antibiotic.

(b) Which antibiotic should be used to treat the patient's throat infection?

2. The graph shows the conditions inside a fermenter containing a culture of *Penicillium chrysogenum*.



(a) Why is lactose added to the culture?
(b) Why is ammonia added to the culture?
(c) What evidence is there that penicillin is a secondary metabolite?

.

Answers

1. (a) antibiotic has diffused/spread/moved into agar;
killed/inhibited bacteria; 2

(b) Ampicillin;
largest clear area/inhibition zone/killed the most bacteria; 1

2. (a) It is a source of carbon;
(b) It is a source of nitrogen;
(c) Rapid production is when penicillium has reached stationary phase/ when biomass has stopped increasing;

Acknowledgements:
This Factsheet was researched and written by Kevin Byrne.
Curriculum Press, Bank House, 105 King Street, Wellington, Shropshire, TF1 1NU.