

BIOTECHNOLOGY

Key Objectives

- To be able to describe the use of microorganisms in the extraction of heavy metals from low grade ores
- To understand the terms *Batch Culture* and *Continuous Culture* and to be able to compare the advantages and disadvantages of each type of culture with reference to the production of penicillin, protease enzymes and mycoprotein
- To be able to describe for penicillin (as an example of an antibiotic) its mode of action on bacteria and why it has no effect on viruses. Also, the causes and effects of antibiotic resistance.
- To immobilise an enzyme in alginate and compare this with the use of an enzyme that has not been immobilised
- To be able to explain the use of dip sticks and biosensors in the quantitative measurement of glucose
- To be able to outline the hybridoma method in the production of monoclonal antibodies
- To evaluate the use of monoclonal antibodies in the diagnosis and treatment of disease and testing for pregnancy

Key Definitions

- *Batch Culture* – when fermentation is carried out in a closed or batch fermenter. Microorganisms and nutrient medium are added to the fermenter and left for a period of time. During the process, nothing is added to or removed from the fermenter (except for the venting of waste gases). The product is separated from the mixture at the end. Temperature is controlled and nutrients are usually depleted at the end.
- *Continuous Culture* – when fermentation is carried out in an open fermenter. Nutrients are added and product removed at a steady rate throughout the process. This will maintain the microorganisms at the exponential phase of growth. It is very important to monitor pH, temperature and oxygen concentration as well as levels of nutrients and product. All of these should be kept constant.
- *Antibiotic* – a chemical substance, produced by a microorganism (bacterium or fungus) which will inhibit the growth or replication of other microorganisms.
- *Enzyme immobilisation* – the attachment of enzymes to insoluble materials, which then provide support for the enzymes. This allows enzymes to be held

in place throughout the reaction, following which they are easily separated from the products and may be used again.

- *Biosensor* - a device which makes use of a biological molecule (or sometimes a cell) to detect and measure a chemical compound.
- *Monoclonal antibodies* – these are identical antibodies, produced to be effective against a single, specific antigen.

Key Ideas

The use of microorganisms to extract heavy metals from low grade ores

Some bacteria are described as chemoautotrophic i.e. they derive their energy via the breakdown of inorganic chemicals. In some cases, this involves the breakdown of ores of heavy metals, as a result of which the metal itself is released.

Metals which can be extracted in this way include copper, uranium, cobalt, lead, nickel and gold.

Bacteria of the genus *Thiobacillus* are commonly used in this process e.g. *Thiobacillus ferrooxidans* is used in the extraction of copper and uranium.

Some species of bacteria actually accumulate metals. This property can be made use of, both in the extraction of metals and in the detoxifying of waste. For example, some species of *Pseudomonas* are known to accumulate mercury and uranium, while some species of *Thiobacillus* accumulate silver.

<http://en.wikipedia.org/wiki/Bioleaching> <http://www.spaceship-earth.org/REM/BRIERLEY.htm>

Compare the advantages and disadvantages of batch and continuous cultures in the production of secondary metabolites (such as penicillin), enzymes (such as proteases) and biomass (such as mycoprotein)

In general, batch culture methods have certain advantages over continuous culture. These are:

- the culture is easy to set up
- the environmental conditions are relatively easy to control
- the types of vessels used can be used for different processes at different times
- if the culture becomes contaminated, it is only one batch that is lost
- the level of nutrients drops, which can create the conditions necessary for the microorganism to manufacture secondary metabolite such as penicillin

Some advantages of continuous culture are:

- it can be carried out in smaller vessels, given that the microorganisms are maintained in the exponential phase and productivity is therefore high
- the high productivity for biomass and intra- and extra-cellular enzymes is more cost effective

However, some disadvantages of continuous culture are:

- microbial growth, clumping of cells and foaming can tend to block up inlet pipes
- it can be difficult to control all the environmental factors – if they are not controlled adequately, there can be a considerable amount of waste
- it is not possible to create the low-nutrient, high-stress conditions under which secondary metabolites such as penicillin are produced

<http://textbookofbacteriology.net/growth.html>

<http://www.biologymad.com/master.html?http://www.biologymad.com/Biotechnology/Biotechnology.htm>

The mode of action of penicillin on bacteria (and why it does not affect viruses)

In general, antibiotics bring about their effects in a number of ways :

- inhibiting protein synthesis – interfering with transcription or translation.
- interfering with the synthesis of bacterial cell walls – such antibiotics are only effective when the bacteria are growing.
- interfering with the functioning of the cell membrane – the bacteria will lose it's ability to control the uptake or removal of water and other molecules.
- inhibiting enzyme activity – this will disrupt metabolism.

Penicillin is described as a *broad spectrum* antibiotic i.e. it is effective against a wide range of bacteria. It is one of the antibiotics which work by interfering with the synthesis of new cell walls. Specifically, it inhibits the enzymes involved in the synthesis of cross-links between the peptidoglycan polymers in bacterial cell walls.

Some bacteria possess enzymes that break down penicillin (penicillinases) – this makes them resistant to such antibiotics e.g. all strains of *Mycobacterium tuberculosis* are resistant to penicillin.

Viruses do not have any form of cell structure or metabolism – hence, antibiotics are ineffective against viruses. Viruses replicate only within the living host cells, and make use of the living host cell's transcription and translation mechanisms. These are eukaryotic mechanisms and thus are not affected by any antibiotics, which affect only prokaryotic mechanisms. The absence of any sort of cell wall means that penicillin has no effect on viruses.

<http://www.elmhurst.edu/~chm/vchembook/652penicillin.html>

http://en.wikipedia.org/wiki/Beta-lactam_antibiotic

The causes and effects of antibiotic resistance

The development of antibiotic resistance in bacteria is an example of the process of natural selection.

Bacteria, like all organisms, are genetically variable. These variations occur by natural mutation giving rise to new alleles of genes. Natural selection can change the frequency of these alleles in the population so that most of the bacteria in the population are resistant, by the following steps:

- Within natural populations of bacteria, some individuals have alleles of genes which give resistance to a particular antibiotic
- bacteria cause an infection, leading to treatment of the infected person with antibiotic
- the antibiotic will kill susceptible bacteria, but resistant bacteria will survive
- only the resistant bacteria will reproduce, resulting in an increase in the frequency of the bacteria that are resistant to that particular antibiotic
- there will be an increase in allele frequency for the allele of the gene that gives resistance in the population of bacteria
- people infected in the future are infected by bacteria more likely to carry the alleles for resistance

During the second half of the twentieth century, there was an increasing and widespread use of a whole range of antibiotics. The result has been that many strains of bacteria have been established which are resistant to a variety of antibiotics.

The alleles of genes that cause resistance arise for the first time by mutation. Such genes are often located on plasmids, which means that they can rapidly spread from one bacterial species to another since plasmids are naturally exchanged between species. The plasmids may also contain a number of different antibiotic resistance genes so that species can suddenly acquire resistance to a number of antibiotics when before they had none. Such populations of bacteria that are now resistant to a number of different antibiotics, may cause infections that are extremely difficult to treat.

This has meant that it is constantly necessary to try and create new types of antibiotics, to which the bacteria are not resistant. However, once these new antibiotics are in use, it is often not long before resistant strains of bacteria are established. It is proving to be almost impossible to keep ahead of the development of antibiotic resistance by bacteria.

http://en.wikipedia.org/wiki/Antibiotic_resistance

<http://www.biologymad.com/master.html?http://www.biologymad.com/Evolution/Evolution.htm>

The immobilisation of an enzyme in alginate. The ease of recovering the enzyme and purifying the product compared with using an enzyme that has not been immobilised

Enzymes can be immobilised in a variety of ways. One way is to trap the enzyme molecules in alginate beads – amylase is often immobilised in this way. The process of immobilisation involves the following stages:

- amylase enzyme is mixed with a solution of sodium alginate
- this mixture is dripped (usually through a syringe) into a solution of calcium chloride
- the sodium ions are displaced by the calcium ions, resulting in the formation of hard, insoluble beads of calcium alginate, in which are trapped the molecules of amylase

- the alginate beads are left to harden further, following which they are rinsed

Following the formation of the beads in this way, they are normally placed in a suitable container to create a column of beads. A suspension of starch can then be trickled down the column and collected in a beaker. If the contents of the beaker are analysed, it is found to be a solution of maltose, without any starch being present i.e. the starch has been hydrolysed by the amylase as it passed through the beads.

By hydrolysing the starch in this way, there is no contamination of the product with enzyme – and the amylase remains in the beads, which can then be used again. This is the case whenever it is possible to use immobilised enzymes.

If the enzyme is not immobilised in this way, the product will contain enzyme molecules and a further stage will be required to recover them.

advantages of immobilisation:

- enzyme can be recovered after use using a very coarse filter rather than a molecular filter
- enzyme does not contaminate product
- immobilisation may enhance stability (thermostability or pH-stability) of the enzyme molecule as it is supported
- substrate can be easily passed through the enzyme several times

There is a useful table of advantages and disadvantages of immobilisation near the bottom of the document at <http://www.biologymad.com/master.html?http://www.biologymad.com/Biotechnology/Biotechnology.htm> and a useful summary at <http://www.rohmhaas.com/ionexchange/Pharmaceuticals/enzymes.htm> and <http://www.lsbu.ac.uk/biology/enztech/imeconom.html>

The use of dipsticks and biosensors in the quantitative measurement of glucose

Glucose is not usually detectable in the urine of healthy mammals – although it is filtered out of the blood in the kidney, it is reabsorbed in the proximal tubules. Humans with diabetes are unable to control the levels of glucose in their blood and their urine will usually contain variable amounts. One way to determine the quantity of insulin needed is to test a sample of urine with a glucose dipstick.

The dipstick has the enzyme glucose oxidase on its surface – this oxidises the glucose into hydrogen peroxide and gluconic acid. The hydrogen peroxide will oxidise an indicator chemical on the dipstick, which changes colour. The exact colour change is related to the concentration of glucose in the urine sample. The colour can be compared with a colour chart to give a reading for the glucose concentration.

There is a nicely illustrated summary of this in <http://www.irvingcrowley.com/cls/urin.htm>

It is now more common to measure the glucose concentration of the blood, using a glucose biosensor. A biosensor is a device which uses a biological material, such as an enzyme, a cell or an antibody to detect or measure a chemical compound. The reaction between the biological material and the chemical being measured brings about a change which is converted to an electrical signal by an appropriate

transducer. The electrical signal is then amplified to give a read-out on a digital display.

The glucose biosensor works as follows :

- It contains a layer of immobilised glucose oxidase enzyme.
- This enzyme binds with any glucose in the blood, which is oxidised with dissolved oxygen from the solution to form hydrogen peroxide and gluconic acid.
- An electrode (usually platinum) measures the drop in oxygen concentration as it is used to make hydrogen peroxide. The electrode generates an electrical signal.
- The size of the electrical signal is proportional to the concentration of glucose in the blood.
- A digital readout gives the user a figure for the glucose concentration

<http://www.ul.ie/elements/Issue6/Biosensors-%20Elements.htm>

The first part of this is about glucose biosensors <http://www.chemsoc.org/chembytes/ezone/1998/palmer.htm>

This university practical protocol gives interesting insights into the practicalities of using biosensors to measure glucose concentrations <http://www.lsbu.ac.uk/biology/enzyme/practical5.html>

The production of monoclonal antibodies, using the hybridoma method

Monoclonal antibodies are obtained from clones of single B cells. Unfortunately, B cells will not grow in culture and this problem has to be got round by fusing them with malignant B myeloma cells i.e. cancerous B cells. Myeloma cells will continue to grow and divide indefinitely, though they do not produce antibodies. The fused cells produced from myeloma cells with B cells are known as *hybridomas*. The hybridoma cells will also continue to grow and divide (given suitable and adequate nutrients) and they do secrete antibodies. The antibodies that they secrete are the specific antibodies that were produced by the original clone of B cells.

The production of monoclonal antibodies involves the following stages :

- A mouse is injected with antigen for which the antibodies are required
- An immune response takes place and the mouse plasma cells start to make the antibody
- Plasma cells are extracted from the mouse
- The plasma cells are fused with B cell myeloma cells
- The resulting hybridoma cells are separated individually and allowed to grow, divide and produce antibodies
- Some antibodies are removed and tested with the relevant antigen, to make sure they are the correct monoclons
- Those hybridoma cells which are producing the required antibodies are cultured in a large fermenter
- The monoclonal antibodies are harvested and purified

http://www.accessexcellence.org/RC/AB/IE/Monoclonal_Antibody.html (follow link for illustration in Graphics Gallery)

http://www.vetmed.ucdavis.edu/Animal_Alternatives/mabs.htm

Here is a virtual lab simulation of using monoclonal antibodies in diagnosis – click to enter lab

<http://www.hhmi.org/biointeractive/immunology/vlab.html>

The use of monoclonal antibodies in the diagnosis and treatment of disease and pregnancy testing

Diagnosis – because the monoclonal antibodies produced from a clone of B cells are all identical, they can be used to identify macromolecules with a very high degree of specificity. For example, they are now routinely used for the following:

- Blood typing before transfusions
- Tissue typing before transplants
- Identification of pathogens – using monoclonals, it is now possible to distinguish between different strains of certain pathogens, which would, otherwise, be every difficult
- Identification and location of tumours
- Detection of HIV
- Distinguishing between different types of leukaemia

This website gives a clue as the variety of monoclonal antibodies available for various applications

<http://www.antibodyresource.com/findantibody.html>

Treatment – principally, there are two ways in which monoclonal antibodies are used in the treatment of disease:

- Production of passive vaccines – monoclonal antibodies can be injected directly into the blood to attack a particular pathogen
- ‘Magic bullets’ – monoclonal antibodies can be produced which will combine specifically with cancer cells. It is now possible to bond cancer drugs to such antibodies. In this way, the drugs can be delivered directly to the tumour, thereby reducing the risk of damaging healthy cells.

http://en.wikipedia.org/wiki/Monoclonal_antibodies

Pregnancy testing – soon after becoming pregnant, women produce a hormone, called *human chorionic gonadotrophin (HGC)*. This hormone is produced by the placenta, so can only be present during pregnancy. Monoclonal antibodies are now used to detect the presence of this hormone in the urine – such a pregnancy test can be done very quickly and easily.

This type of pregnancy testing kits work as follows:

- The kit consists of a ‘sampler’, which is a type of dipstick with an absorbent pad
- On the surface of the pad are monoclonal antibodies, specific to HGC and to which coloured latex particles are attached – when the pad is moistened, the molecules of the antibody begin to move
- The sampler is dipped into urine – if HGC is present, it will bind to the monoclonal antibodies and will be drawn up the pad
- Further up the pad is an area at which there is a line of immobilised HGC antibodies

- Any HCG molecules drawn up the pad will bind with these antibodies and the latex particles will create a coloured line. This is a positive result.
- Further along the pad is a second line of immobilised antibodies, to which will bind any HCG antibodies without HCG. A coloured line in this second area (but no coloured line in the first area) will confirm that the HCG antibodies have moved up the pad, but that the result is negative.

General information about pregnancy test kits <http://www.madehow.com/Volume-4/Home-Pregnancy-Test.html>

Information about one particular pregnancy test kit <http://www.xpressdrugtest.com/preg.html>

Biotechnology Self Assessment Questions

SAQ 1 Outline the properties of some bacteria which make them suitable for use in the extraction of heavy metals from their ores.

SAQ 2 In the following table, list some of the advantages and disadvantages of batch culture when compared with continuous culture.

advantages of batch culture	disadvantages of batch culture

SAQ 3 Explain how a population of bacteria might develop antibiotic resistance.

SAQ 4 With reference to enzyme immobilisation :

- Describe how an enzyme is immobilised in alginate.
- Outline the advantages of using immobilised enzymes, rather than enzymes that have not been immobilised.

SAQ 5 Explain what is meant by a biosensor and outline the working of a glucose biosensor.

SAQ 6 With reference to Monoclonal Antibodies :

- Outline the use of hybridomas in their production.
- Explain their use in the diagnosis and treatment of disease.