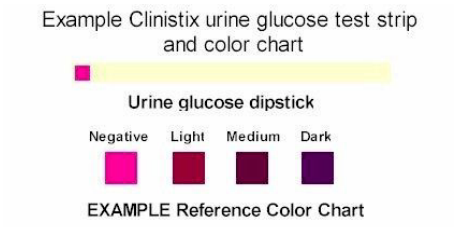
Bioprocessing

Bioprocessing is the use of biological materials (organisms, cells, organelles, enzymes) to carry out a process for commercial, medical or scientific reasons.
Some industries have a long tradition of enzyme use:

- In leather tanning, hides are softened and hair removed using the **proteases** in faeces.
- In brewing, **amylases** in germinating barley are used to convert starch to maltose
    - Maltose is then used by yeast for growth and ethanol production.
- In cheese-making, the proteins in milk are coagulated, using **rennin** from calf stomachs.

Other uses are much more recent developments, e.g.:



- **Glucose Isomerase**: production of fructose from glucose (sweeter – use in confectionary).
- **Sucrase**: production of glucose and fructose from sucrose (as above).
- **Glucose oxidase**: used in the detection of glucose in blood/urine by diabetics

The exam concentrates on 4 areas:

- **Why** use biotechnology;
- **How** to grow/use the organisms;
- **Extracting** the enzymes;
- **Using** the enzymes

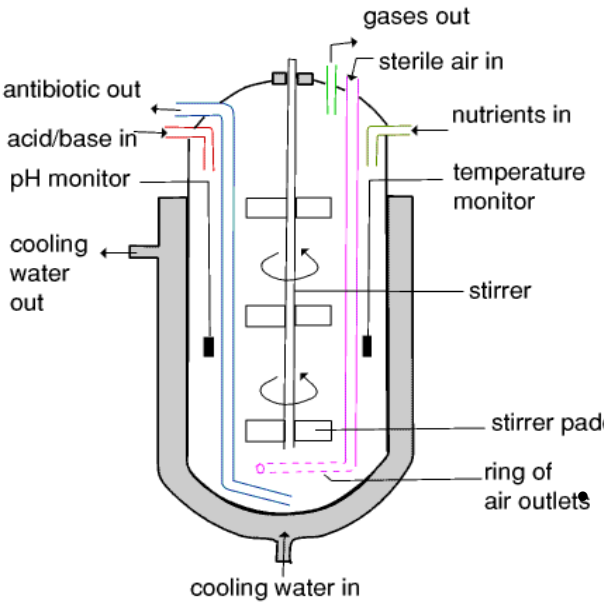
WHY use Bioprocessing?

Many of the reactions catalysed by enzymes have commercial uses. Previously, these reactions used heat and/or strong acids but enzymes offer the following advantages:

They are **specific** in their action and therefore **produce a pure product**.
They are extremely **efficient**, so a little enzyme **quickly** makes **a lot** of product
They are **biodegradable** and so cause **less environmental pollution** (£!)
Safer, since any contamination with an enzyme or known microbe is harmless
They work in mild conditions *i.e. low temperatures, neutral pH and normal atmospheric pressure*, and are **therefore energy saving**.
Some products (wine, cheese) are **virtually impossible to create** using chemicals alone
Some foods **rely on microbial by-products** to create/enhance flavour and **so add value**.

HOW do we produce the enzyme?

The microbes are grown in a fermenter *(below)* – an example of a **continuous-flow process**



Details:

- Sterilised (with steam), since other microbes would:
  - Reduce yield (compete for food)
  - Contaminate product (needing removal)
  - May produce toxins, ↓ value of product
- Sterile air, to aerate (respiration) and stir
- Constant temperature (cooling - enzyme denaturing)
- Stirrer – maximise yield, contents mixed
- pH buffered (enzyme denaturing)

Nutrients – change during process:

- High energy and N (protein) at first (rapid growth)

Very precise conditions later, to maximise yield

EXTRACTING the enzymes: 'Downstream Processing'

Enzymes that are stable and work quickly needed. For this reason, enzymes extracted from bacteria living in hot springs are preferred, since they are thermostable and can tolerate wide variation in pH.

Two types of enzyme exist:
Intracellular enzymes - work inside the cell, in a stable environment (cytoplasm) or
Extracellular enzymes – which are secreted and work in the surrounding environment

Extracellular enzymes are better because they are:

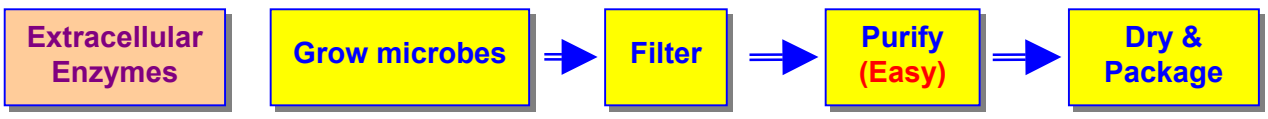
- More robust and can cope with a wider range of environmental conditions (pH, temperature)
- Easier to extract, and so cheaper to buy/use
- Have a longer functional 'life' in the commercial application

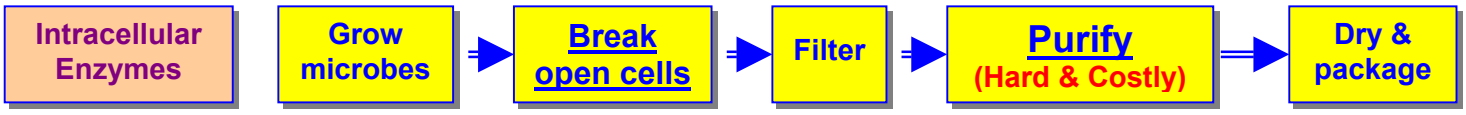
Steps (extracellular enzyme):

- The microbes are removed by filtering or centrifuging
- The filtrate is concentrated (evaporation)
- The desired enzyme/product is purified (molecular filtration)- a relatively easy step
- It is dried/packaged for sale

Steps (intracellular enzyme)
As above, but with the following additional steps:

- The cells are broken open, using ultrasound or other mechanical means
- The protein component is extracted, after removing the cell debris
- The **individual** enzyme is purified from the hundreds of others – by electrophoresis
- This makes them **much** more expensive and difficult to obtain





USING the enzymes

Industrial use of free enzymes

Protease in biological washing powders:

- Helps to break down protein stains such as blood, food and grass
- At lower washing temperatures - thus saving energy and are gentler on clothes.
- NB Some people are allergic to the enzymes
    - The enzymes are encapsulated in wax and only released during the wash.
    - Introduce extra rinse cycles to ensure enzymes are all removed before wearing

Pectinases in food/juice modification.

- Pectin forms part of cell walls and holds plant cells together – digested by pectinase.
- Used to digest fruit and vegetables in baby food and
- to extract fruit/vegetable juices e.g. for cider – it makes the juice clear, not cloudy.

The disadvantage of using free enzymes is that they **cannot be re-used** and therefore:

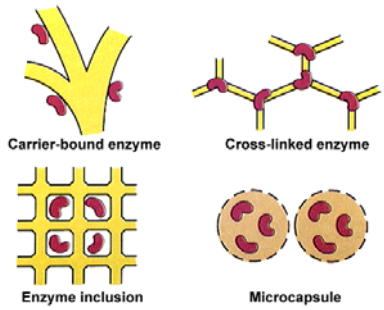
- Contaminate the end-product and
- Are more expensive to use

For these reasons, **immobilised enzymes** have been used for centuries

- Yeast in wine and beer production
- Traditional 'filter-bed' sewage works

Advantages of Immobilised Enzymes

- Immobilised enzymes are not free in solution – e.g. they can be held in a bead of soft permeable gel or coat the internal surface of a porous solid (*see right)*.
- **Easier purification of the product** as the separation of the enzyme beads is not a problem (thus cheaper).
- **Easy to recover and recycle** the enzymes (thus cheaper).
- **The enzymes are protected** in the beads and so remain functional for longer (thus cheaper)



Bioprocessing:

- Bioprocessing with immobilised enzymes is carried out in a **bioreactor**.
- The **bioreactor is sterile** – micro-organisms would badly contaminate the product.
- The **immobilised enzymes** are held **in beads in suspension** in the nutrient medium.
- **Temperature, pH, and substrate and product concentrations** are constantly monitored.
- The product can be produced by **continuous flow or batch processing**.