Unit 2 Revision Notes

BRIGHT MINDS
MENTORING

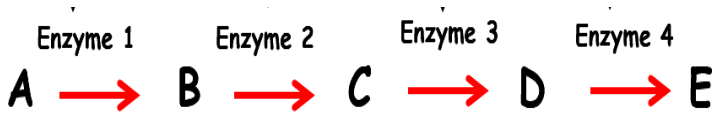
Unit 1 Metabolic Pathways, Enzymes & Respiration Revision

Metabolic Pathways

Integrated and **controlled** pathways of **enzyme-catalysed reactions** within a cell.

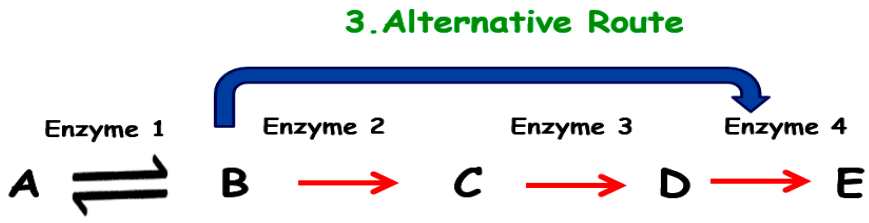
Metabolic Pathways

Each **step** in a metabolic pathway requires a **specific enzyme.**



Three Steps in a metabolic Pathway

1. **Reversible** Step (2 way arrow allowing forward and back ward reaction)
2. **Irreversible** Step (1 way conversion)
3. **Alternative route** (skips certain steps but produces same molecule at end regardless of route)



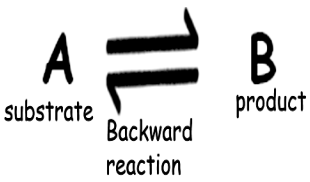
Substrate & Product Concentration in Reversible reactions

Forward Reaction

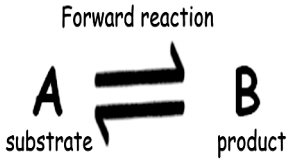
High substrate concentrations (A) or **low product** concentration (B) promotes the forward reaction A to B.

Backward Reaction

High product concentrations (B) or the backward reaction B to A.



low substrate concentration A promotes



Metabolism and Enzymes

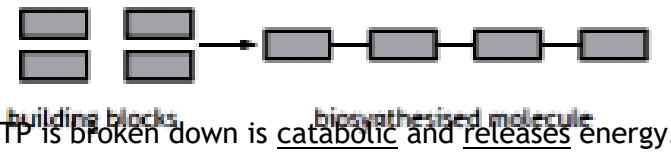
Types of Metabolic Reactions

1. **Catabolic** reactions **BREAK larger** molecules into **smaller** ones **RELEASING** energy.

Example Glycogen/starch → glucose protein → amino acids

2. **Anabolic** reactions involve the **BIOSYNTHESIS** of smaller molecules into larger ones **REQUIRES** energy to undertake this process.

Example Glucose → Starch amino acids → polypeptides (proteins)



ATP Example

Reaction 1 where ATP is broken down is catabolic and releases energy.

Reaction 2 where ATP is synthesised by the enzyme ATP synthase is anabolic and requires energy.

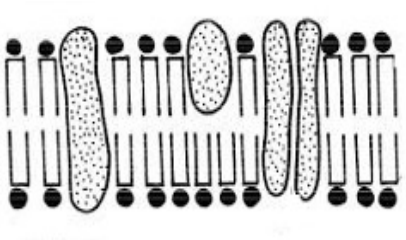
ATP synthase

Metabolism and Enzymes

Structure of Membrane

3 types of protein randomly embedded in the membrane.

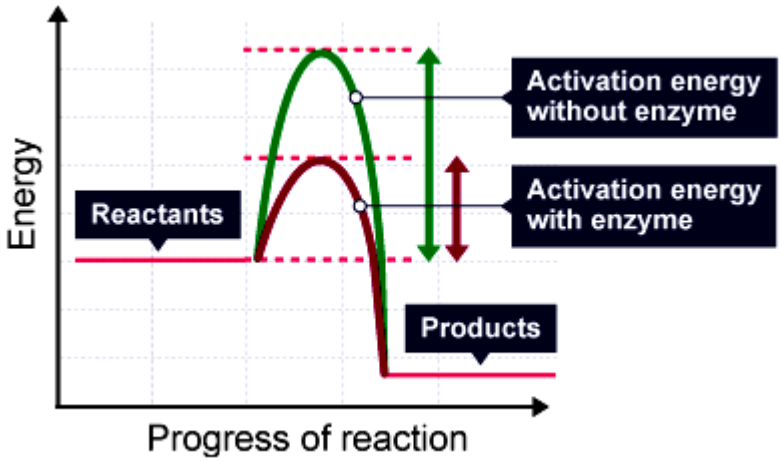
1. Protein pumps (active transport)
2. Protein pores (diffusion)
3. Enzymes (ATP Synthase in electron transport chain)



Activation Energy

The energy required to BREAK chemical bonds in the reactants to allow products to be made.

Enzymes speed up reactions as they **lower the activation energy** required to form products .



Induced Fit model

After the **substrate has bound** to the active site, the **ACTIVE site changes shape** to better fit the substrate.

Affinity for Active site

Substrate—**high affinity** for active site

Product—**low affinity** for active site

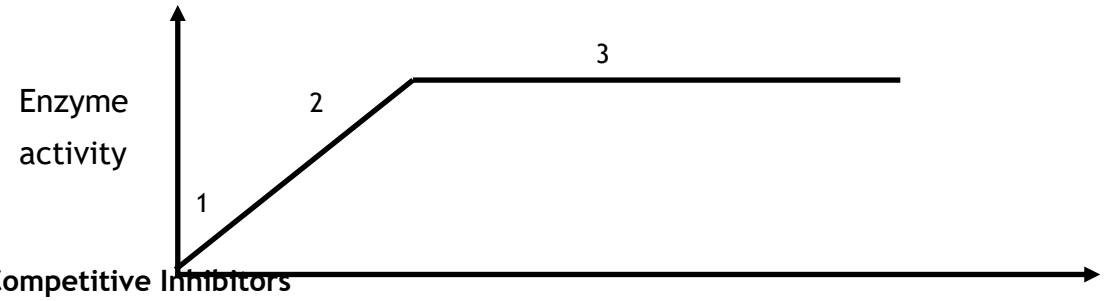
Metabolism and Enzymes

Substrate Concentration

1. **Low substrate concentration = Low enzyme activity**
   There are not enough substrate molecules to fill all the active sites.

2. **High substrate concentration = Higher enzyme activity**
   All active sites are filled by substrates due to increased concentration of substrates.

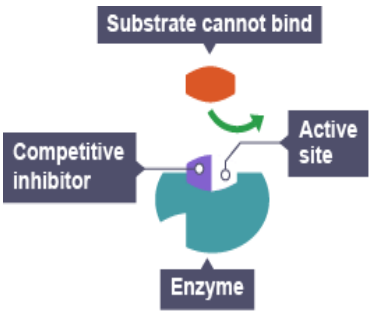
3. **Very High substrate concentrations = No further increase in enzyme activity**
   Enzyme working at maximum and all active sites are filled by substrates.
   No further increase in reaction rate.



Competitive Inhibitors

High Affinity for active site (resemble substrate)

Bind at the **active site** and prevent substrate from binding.



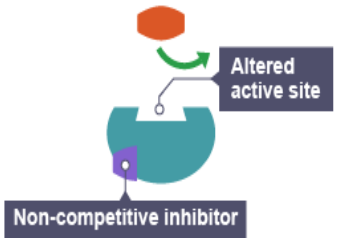
Inhibition reversed with **<u>increasing substrate concentration</u>**.

Non competitive Inhibitors

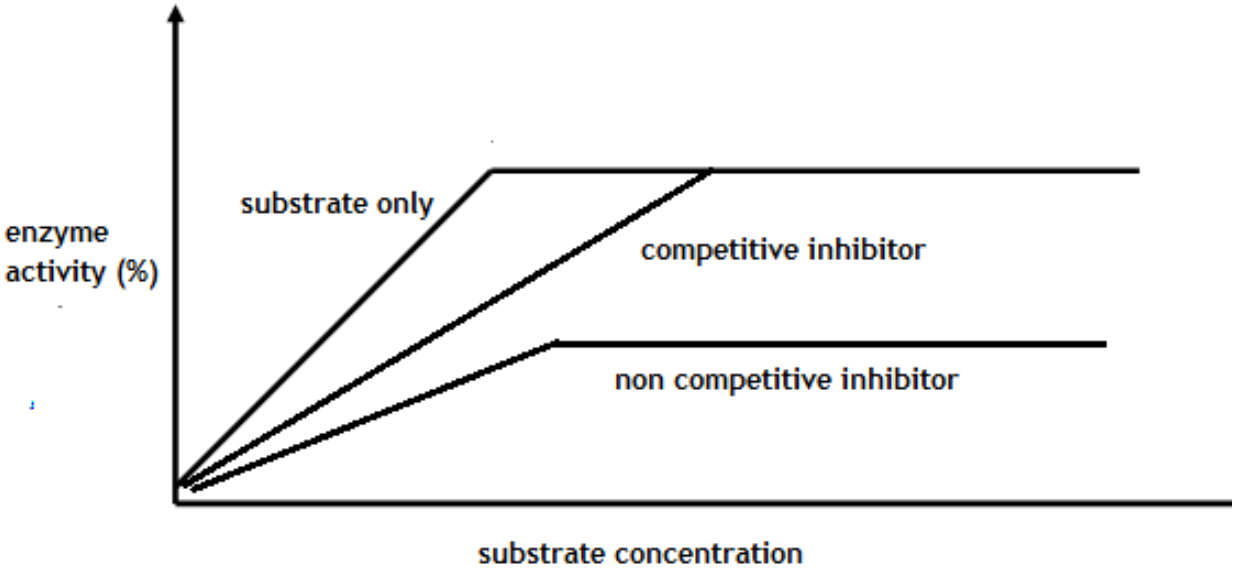
Low affinity for active site

Binds AWAY from active site but change shape of active site preventing substrate from binding.

Action irreversible—<u>no effect</u> when increasing substrate concentration.



Enzyme Inhibitor Graph

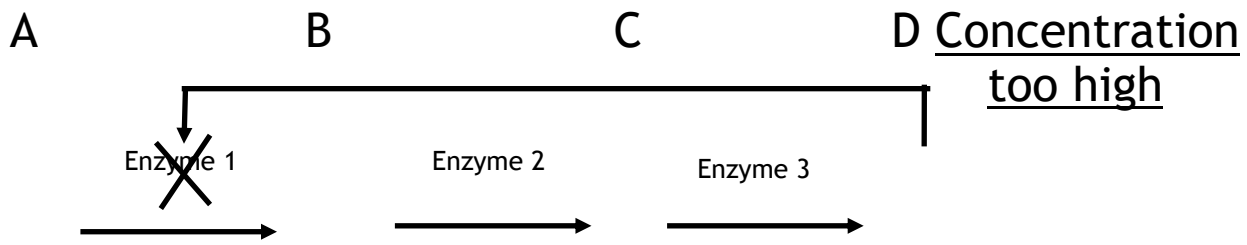


Feedback Inhibition

When the end product concentration reaches a **critical concentration** (too high).

End product binds to an **EARLIER ENZYME** in the pathway, preventing its own synthesis.

This is a form of **negative feedback**.



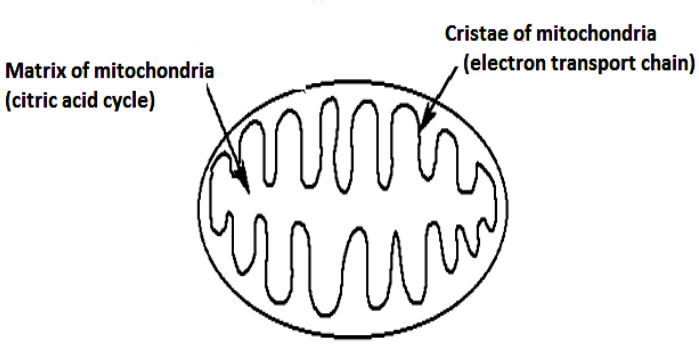
Respiration

Stages of respiration

1. **Glycolysis (**cytoplasm)

2. **Citric Acid Cycle** (Kreb Cycle) (matrix of mitochondria)

3. **Electron Transport Chain** (cristae/inner mitochondria membrane)

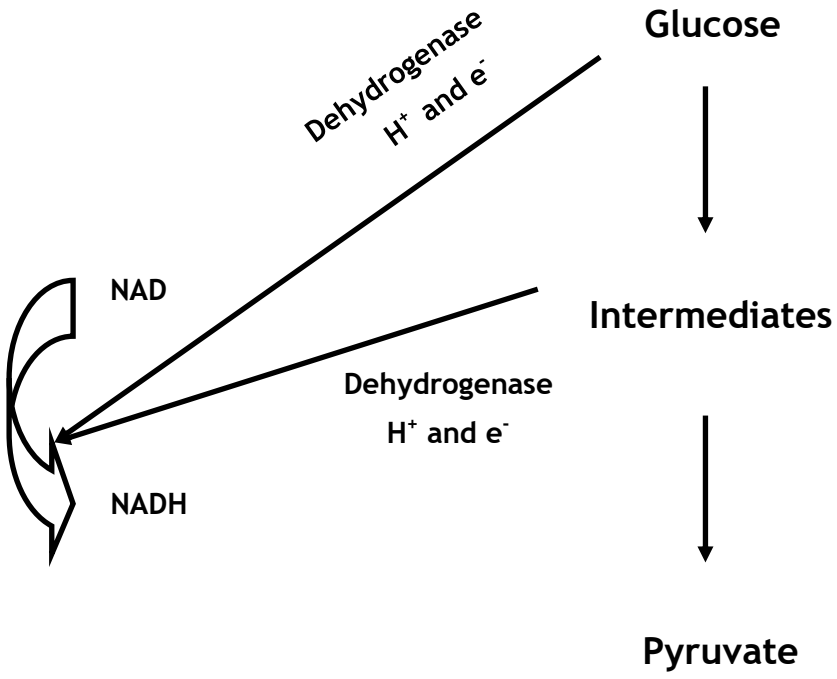


ATP production

The **majority** of the **ATP** is produced during the **electron transport chain** although **ALL stages** generate some ATP.

Glycolysis

Location—cytoplasm Process does not require oxygen Net gain of 2ATP



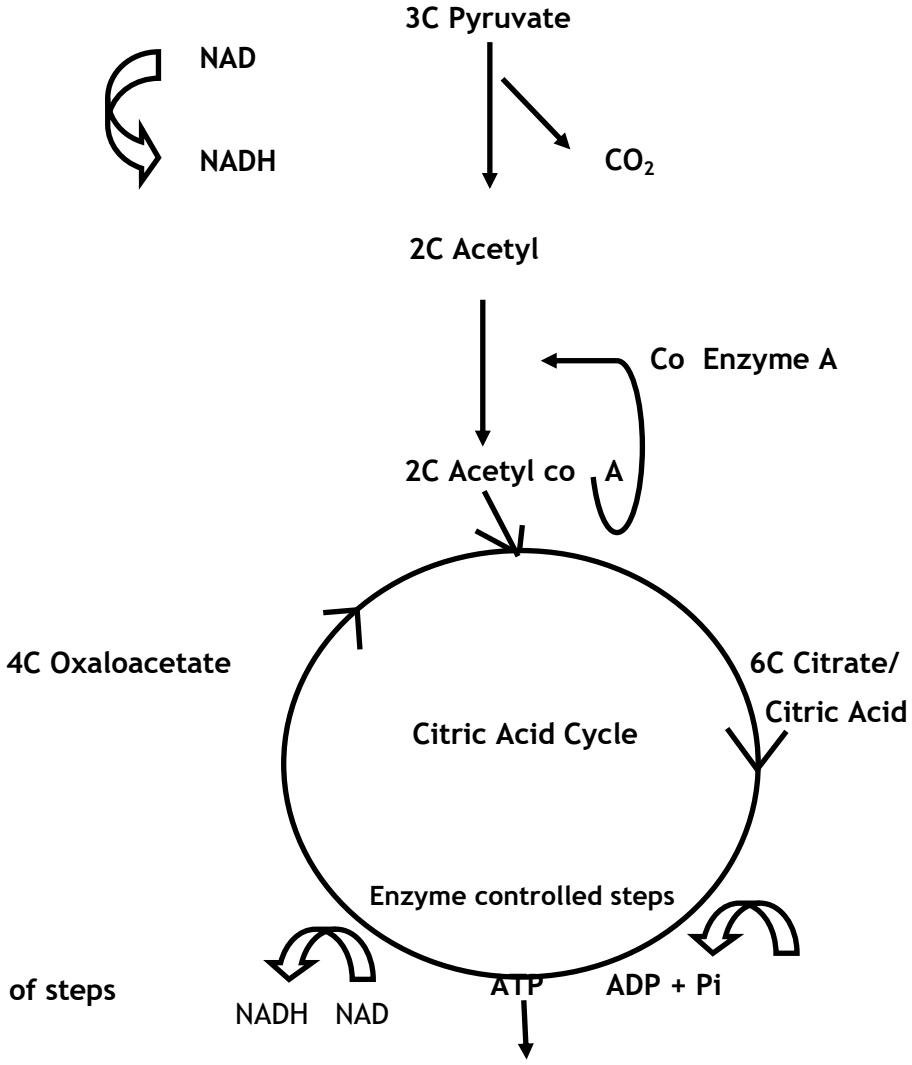
1. **Energy Investment Stage**
   2 ATP IN to phosphorylate glucose & intermediates

2. **Energy Payoff stage**
   4 ATP produced after 2 ATP in
   = Net gain of 2 ATP

Respiration

Aerobic Stage of Respiration

Process **requires oxygen** to move beyond pyruvate and is controlled by **enzymes** called dehydrogenases.



Summary of steps

1. In aerobic conditions, pyruvate is broken down to an acetyl group.
2. Acetyl combines with recycled coenzyme A forming acetyl coenzyme A.
3. In the citric acid cycle, the acetyl group from acetyl coenzyme A combines with oxaloacetate to form citrate.
4. During a series of **enzyme-controlled** steps, citrate is gradually regenerated back into oxaloacetate.
5. ATP, NADH and CO_2 are all produced during these enzyme-controlled steps.

Respiration

Role of dehydrogenase

Removes hydrogen ions and electrons from substances and passes to coenzyme NAD to form NADH.

Location—Glycolysis and Citric Acid Cycle.

Role of Co enzymes

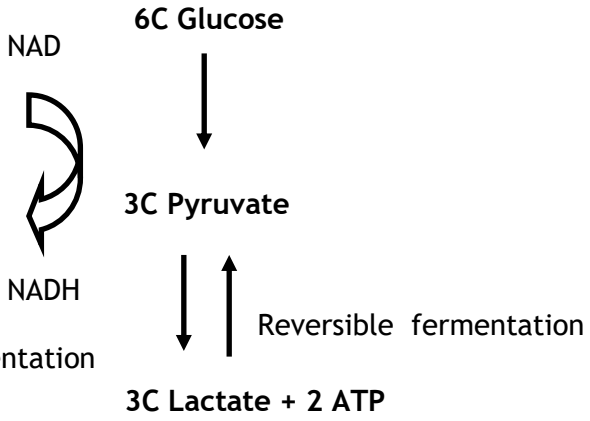
Accept hydrogen ions and electrons and pass to electron transport chain on inner mitochondrial membrane.

Location—Glycolysis, Citric Acid Cycle and Electron transport chain

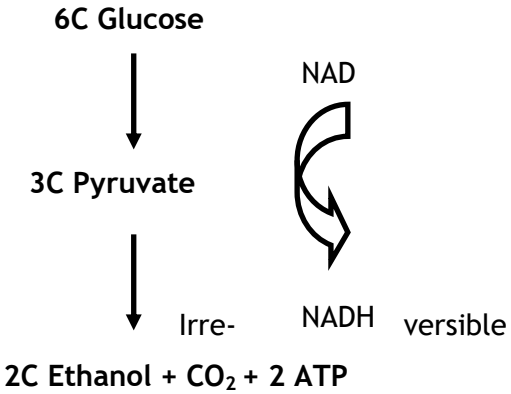
Fermentation

In absence of oxygen **fermentation** occurs in the cytoplasm.

Animal cell e.g. muscle



Plant/Yeast cells



Fermenta- tion in **animals** **cells** is **reversible**. Once oxygen is present(repaying the oxygen debt) lactate is broken down into pyruvate which can now be made into acetyl etc.

Fermentation in **yeast/plants** is **irreversible** due to loss of CO_2 and ethanol builds up and kills plant.

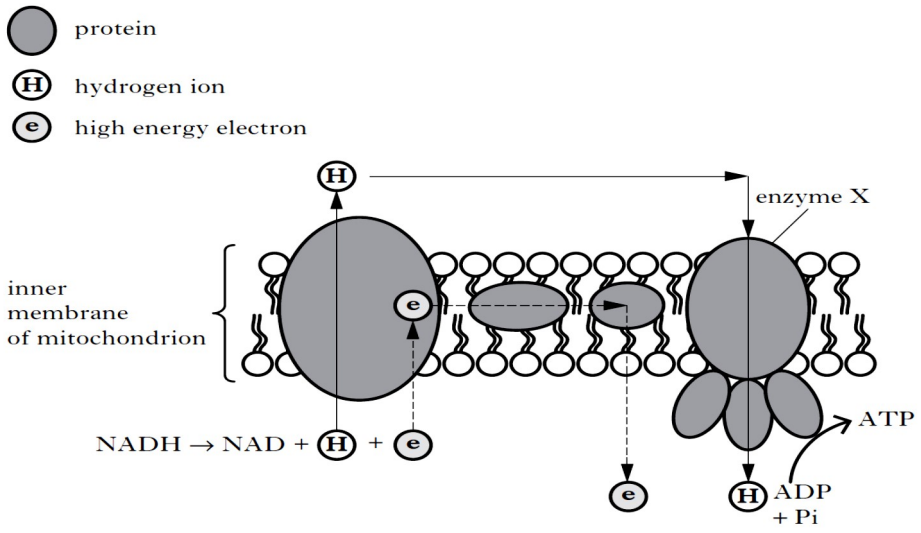
Fermentation results in much less ATP being produced than in aerobic respiration.

Electron Transport Chain

Electron Transport Chain

The electron transport is a series of **carrier proteins** attached to the **inner mitochondrial membrane**.

Location—Inner membrane of mitochondria (Cristae).



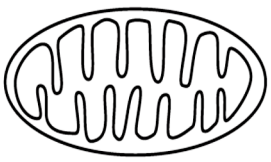
Summary of Process

1. The co enzyme NADH releases electrons and hydrogen ions in the inner mitochondrial membrane.
2. Electrons pass along the electron transport chain and release energy.
3. The energy released by electrons pumps H ions across the membrane by active transport.
4. H diffuses back across ATP synthase causing it to rotate to make ATP from ADP + Pi.
5. Oxygen is the final hydrogen ions and electron acceptor forming water.
6. Most of the ATP produced occurs during this stage.

High/Low Cristae

Lots of folds in the inner mitochondrial membrane are needed for active cells as they need lots of ATP e.g. muscle/brain/sperm cells.

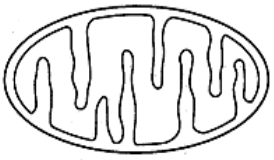
More folds/cristae mean more electrons pass down chain/more H is released & more ATP is produced by ATP synthase.



Lots Cristae/ High surface area

Lots ATP produced

Damaged



Less Cristae/ Less surface area

Less ATP produced

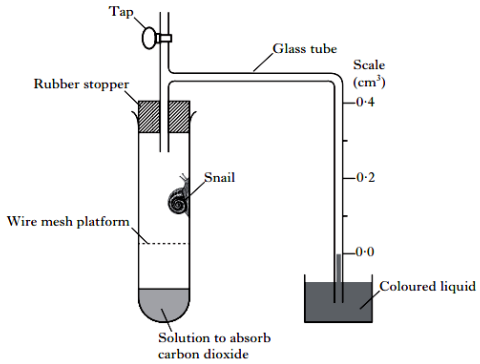
Measuring Metabolic Rate

Measurement of metabolic rate

1. Oxygen consumption (apparatus—respirometer OR O_2 probe)
2. Carbon dioxide production (apparatus—CO_2 probe)
3. Heat production (apparatus Calorimeter)

Respirometer

Used to measure oxygen consumption as an indirect measurement of the dependent variable of respiration rate/metabolic rate.



Variables kept constant for VALID results

1. Diameter of tubing
2. Volume of solution to absorb CO_2

The solution at the bottom of the test tube is critical to the success of the experiment as the animal uses up O_2 and the CO_2 is absorbed by the solution. This causes liquid to be forced up the tubing to replace the volume of gas lost to the solution.

Time (minutes)	Distance dye moved up tube (cm^3 per minute)	Metabolic Rate
0	0.0	↑
2	0.15	
4	0.20	
6	0.25	
8	0.30	
10	0.35	

Conclusion

As time increases, metabolic rate increases

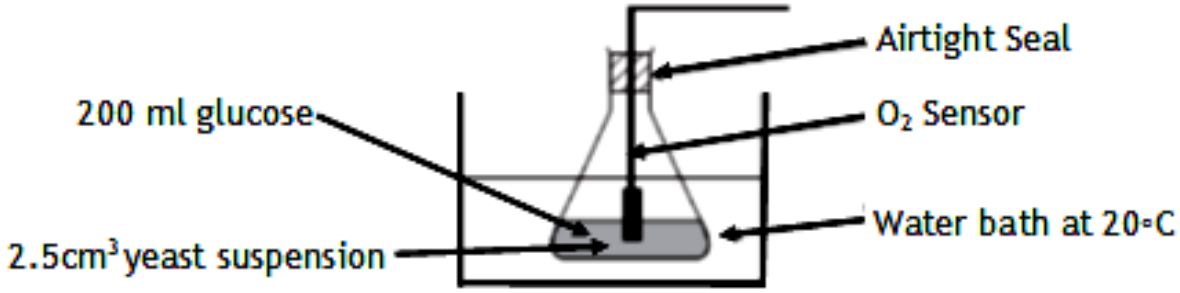
Hint: Remember the further the distance travelled = higher metabolic rate

Measuring Metabolic Rate

Oxygen/Carbon Dioxide Probes

Another piece of apparatus to measure dependant variable is metabolic rate indirectly is oxygen probes/sensors.

This measure O_2 consumption in a sealed container.



Time (minutes)	Oxygen Concentration in airtight flask (mg per litre)	Metabolic Rate
0	10.8	↑
2	8.5	
4	6.2	
6	4.1	
8	2.8	
10	0.0	

Conclusion

As time increases, metabolic rate increases

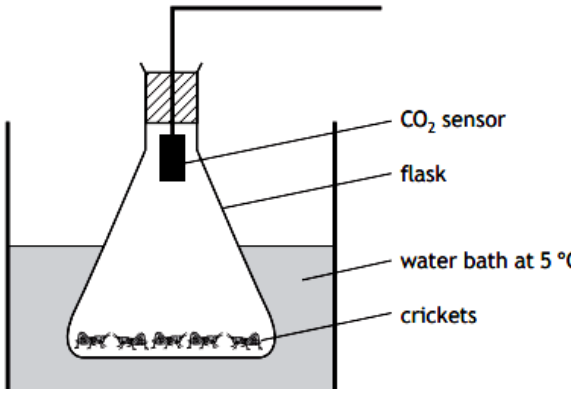
Hint

Remember lower oxygen concentration = higher metabolic rate

Measuring Metabolic Rate

Oxygen/Carbon Dioxide Probes

Another piece of apparatus to measure dependant variable of metabolic rate indirectly is CO_2 probes/ sensors which measures CO_2 production in a sealed container.



Time (minutes)	CO_2 Concentration in airtight flask (mg per litre)	Metabolic Rate ↑
0	0.0	
2	2.8	
4	4.1	
6	6.9	
8	7.3	
10	10.1	

Con- clusion

As time increases, metabolic rate increases

Hint

Remember higher CO_2 concentration = higher metabolic rate

Measuring Metabolic Rate

Calorimeter

Apparatus used to measure the dependent variable of metabolic rate indirectly via **heat production by a subject** in a sealed container.

Measurements

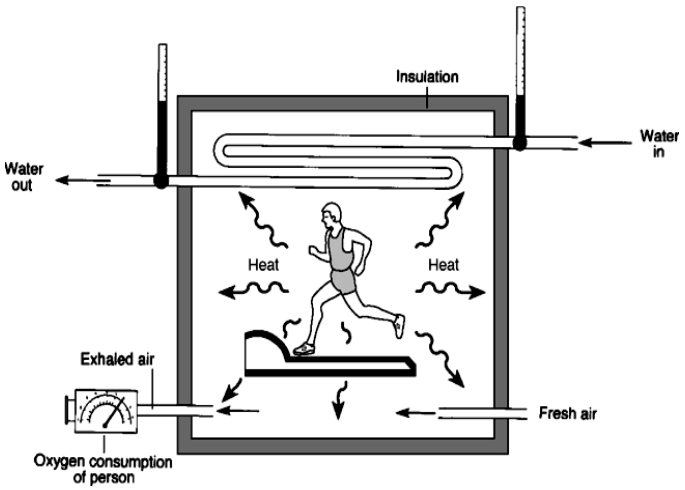
1. Measure temperature of water at start of experiment by probe/thermometer.
2. Measure temperature of water at end of experiment by probe/thermometer.

Metabolic rate measured indirectly from heat produced by subject via a specific formula.

Features of calorimeters

To maximize absorption of heat produced by subject into water pipe.

1. Copper tube coiled to increase surface area .
2. Insulated container to keep heat in/prevent heat loss to surroundings.



Time (minutes)	Heat released (◦C per minute)	Metabolic Rate
0	0.0	↑
2	2.8	
4	4.1	
6	6.9	
8	7.3	
10	10.1	

Conclusion

As time increases, metabolic rate increases

Hint: Remember the more heat produced = the higher the metabolic rate

Metabolic Rate Calculations

Metabolic Rate Calculations

As organisms all have **DIFFERENT** starting masses , when calculating different animal's metabolic rate it is important to divide by their weight.

Four athletes were weighed then given a fitness test during which their maximum oxygen uptake and body mass was measured.

Maximum oxygen uptake **per kg of** body mass can be used as a measure of fitness.

The **fitter** an individual, the **higher** their maximum oxygen uptake,

Athlete	*Body mass* (kg)	*Maximum oxygen uptake* (litres per minute)
A	60	3·6
B	55	3·6
C	60	3·7
D	55	3·7

To calculate the fitness level of Athlete C's the individual's maximum oxygen uptake is divided by the body mass.

3.7 litres per minute ÷ 60kg = **0.062** litres/minute/kg

Metabolic Rate Calculations

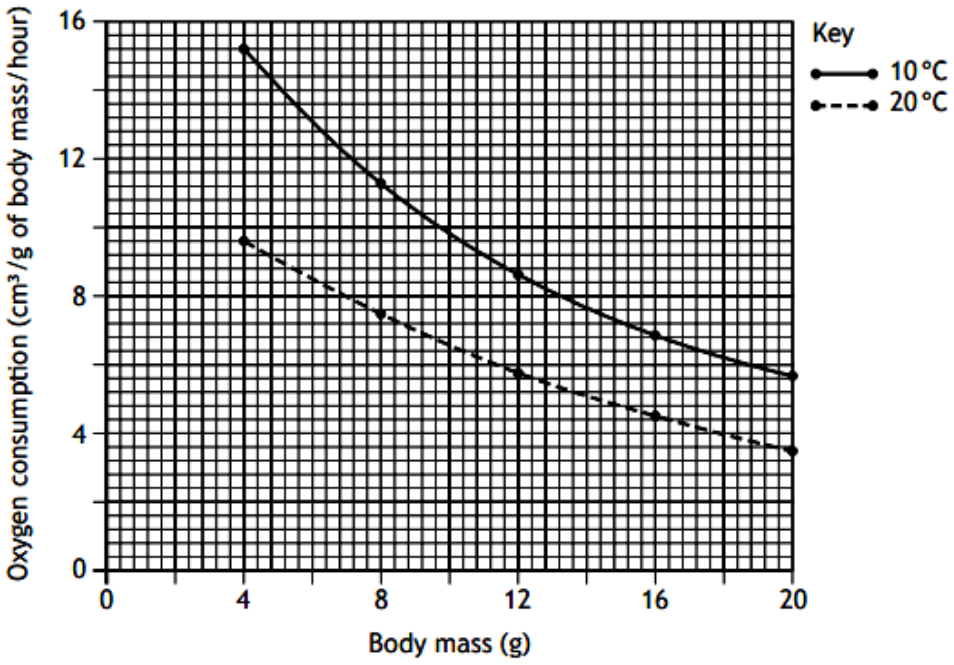
Metabolic Rate Calculations

Graphs and tables display metabolic rate **per gram** of body mass **per hour**.

Therefore when given information about an organism's metabolic rate, calculations will involve **MULTIPLYING** by body mass and OR hours.

Calculation One Example

The graph shows the relationship between body mass and oxygen consumption of different masses of shrews at two environmental temperatures.



The metabolic rate of the shrew at 10°C at 4g of body mass is 15.2 cm^3/g of body mass/hour.

To calculate the TOTAL metabolic rate of the 4g shrew over 1 day is

15.2 x 4g x 24 = 1459.20 cm^3/g of body mass/hour

Circulatory Systems

Metabolic Rates

Birds and mammals	Highest metabolic rates
Amphibians and reptiles	Lower metabolic rates
Fish	Lowest metabolic rate

Organisms with high metabolic rates require **more efficient oxygen delivery** to cells.

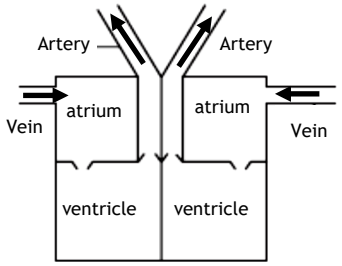
Birds and mammals Complete Double Circulatory System

Highest metabolic rate.

2 atria and 2 ventricles.

Prevents mixing of oxygenated and deoxygenated blood.

More efficient oxygen delivered to cells for respiration.



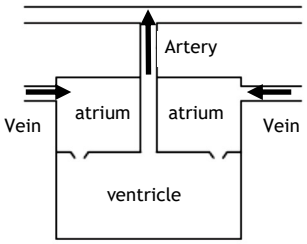
Amphibians and Reptiles Incomplete Double Circulatory System

Lower metabolic rates.

2 atria and 1 ventricle.

Allows mixing of oxygenated and deoxygenated blood.

Less oxygen delivered to cells for respiration.



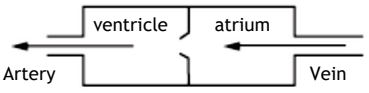
Fish Single Circulatory System

Lowest metabolic rates.

1 atria and 1 ventricle.

Allows mixing of oxygenated and deoxygenated blood.

Less oxygen delivered to cells for respiration.



Circulatory Systems

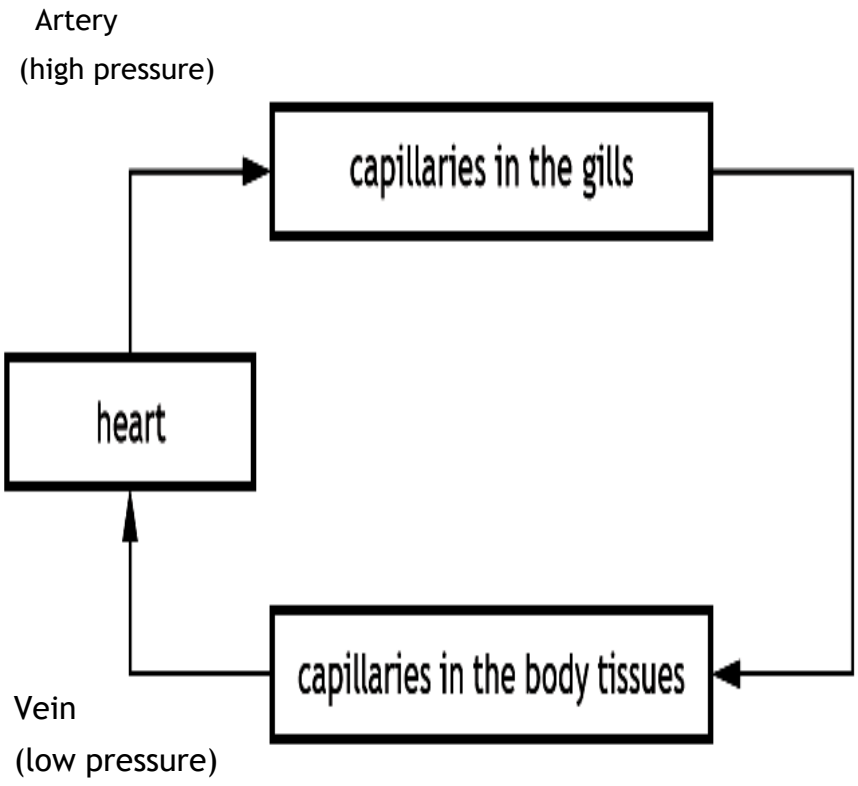
Explain why complete double circulatory systems **enable higher metabolic rates.**

1. There is **no mixing** of oxygenated and deoxygenated blood.
2. **Oxygenated blood** can be pumped out at a **higher pressure** enabling more efficient oxygen delivery to cells.

High & Low pressure

Veins carry blood at low pressure into the heart.

Arteries carry blood at high pressure away from the heart .



Thermoregulation

Thermoregulation Definition

Maintenance of the internal environment within tolerable limits despite changes to the external environment.

The **hypothalamus** is the temperature monitoring centre.

Information is communicated by **electrical impulses** through **nerves** to the effectors which bring about **corrective responses** to return temperature to normal

Hypothalamus (corrective responses) Nerve impulses → **Effectors**

Negative feedback

Corrective responses return system back to normal via **negative feedback.**

Too hot: make individual cooler

Three corrective responses

1. **Sweating increases**
   To increase evaporation of WATER to cool down skin
2. **Vasodilation of blood vessels (arterioles)**
   Increased blood flow to skin
   Increased heat loss
3. **Metabolic rate decreases**
   Less heat produced

Importance of regulating body temperature / thermoregulation in mammals

1. For optimal enzyme activity
2. To maintain high diffusion rates

Too Cold: make individual hotter

Four corrective responses

1. **Shivering increases**
   Generate heat by muscle contraction
2. **Vasoconstriction of blood vessels**
   Decreased blood flow to skin
   Decreased heat loss
3. **Hair erector muscles contract**
   Layer of insulating air trapped
4. **Metabolic rate increases**
   More heat produced

Conformers & Regulators

The ability of an organism to maintain its metabolic rate is affected by **external abiotic factors.**

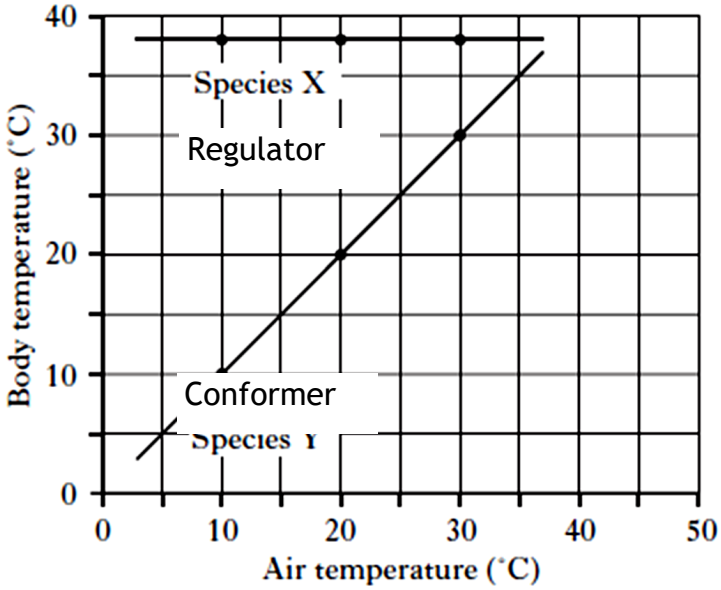
1. pH
2. Salinity (Salmon able to move from fresh to sea water via pumps in their gills to remove Na)
3. Temperature

Regulators

Internal environment is kept constant despite changes to external environment.
High metabolic costs.
Wider range of ecological niches
Regulators use metabolism (thermoregulation) to control their internal environment
This regulation costs a lot of energy to achieve homeostasis.

Conformers

Internal environment (metabolism) is dependent on the external environment.
Low metabolic costs.
Narrow range of ecological niches.
Behavioural responses by conformers allow them to tolerate SOME variation in their external environment to maintain optimum metabolic rate.



Surviving and Avoiding Adverse Conditions

Surviving Adverse Conditions

Many environments vary beyond tolerable limits for normal (high) metabolic activity.

Two strategies

1. Survive adverse conditions
2. Avoid adverse conditions

1. Surviving Adverse Conditions

Organisms survive by **reducing metabolic rate (dormancy**) during a period when the costs of normal metabolic activity would be too high.

Dormancy **saves energy** for the organism .

Dormancy is visible through lower heart rate, breathing and body temperature.

Three types of dormancy

1. **Hibernation**

Reduced metabolic rate when **temperatures are too low /winter** .

2. **Aestivation**

Reduced metabolic rate during **droughts**/very high temperatures

3. **Daily torpor**

Period of reduced metabolic activity in some animals with high metabolic rates.

Predictive/Consequential Dormancy

1. Predictive

Dormancy occurs **before** onset of adverse conditions.

2. Consequential

Dormancy occurs **after** onset of adverse conditions.

2. Avoiding Adverse conditions Strategy

Migration **avoids metabolic adversity by expending energy to relocate** to a more suitable environment.

Disadvantage of Migration

Costs energy to relocate**.**

Migration: Innate & learned components

1. Innate—instinctive ability to migrate/born with ability to migrate.
2. Learned component—gained by previous experiences such as direction of travel/where to stop flying etc.

Specialised techniques are used to study long distance migration such as **satellite tracking** and **leg rings.**

Microbe Growth in Fermenter

Types of Micro-organisms

Microbes are found across all 3 domains of life.

1. Bacteria
2. Archaea
3. Eukaryotes

Why use Microbes in culture

1. Adaptability
2. Ease of cultivation
3. Speed of growth.

Microbes use a **wide variety of substrates** for metabolism and produce a **range of products** from their metabolic pathways.

Growth Media Components

1. **Energy source** (light for photosynthetic organisms/ chemical substrates e.g. glucose).

2. **Raw materials** for biosynthesis

   Some micro-organisms produce all the complex molecules required for biosynthesis

   Others requires these raw materials to be added.

   Fatty Acids **Amino Acids** **Vitamins**

Culture Conditions

1. Sterility to prevent contamination by microbes.

   Why prevent contamination?

   1. This reduces competition with the desired micro-organisms for nutrients.
   2. Reduce the risk of spoilage of the product.

      HOW? Steam and filters.

2. Temperature to keep enzymes at optimum

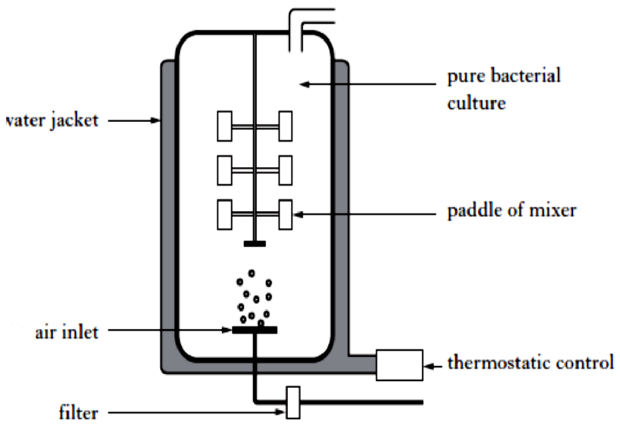
   HOW? Water jacket and thermostat.

3. Oxygen concentration for aerobic respiration

   HOW? Air inlet and paddles for aeration.

4. pH to keep enzymes at optimum

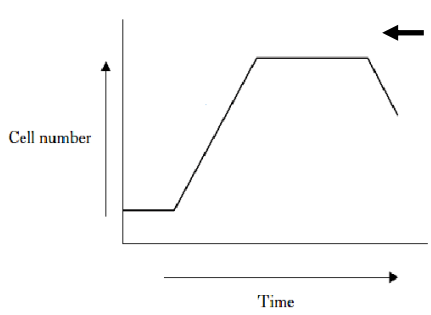
   HOW? Use of buffers.



Stages of Microbe Growth

Viable cell count

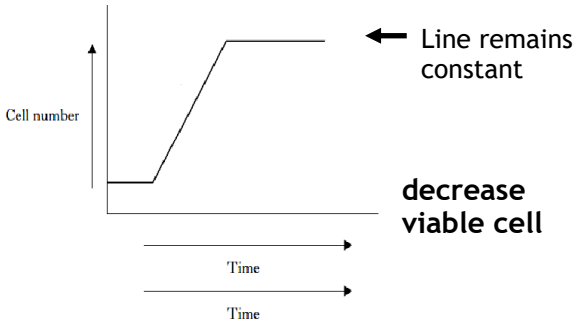
Living cells



If **Cell numbers overall (death phase) this is a count.**

Total Cell Count

Living and dead cells included



decrease viable cell

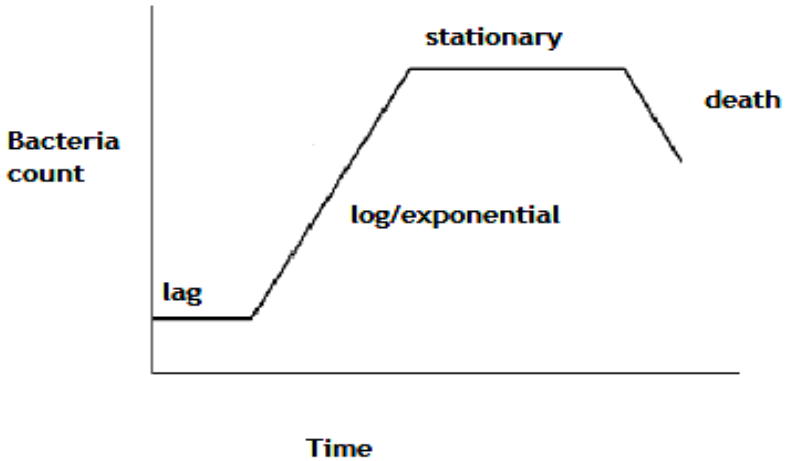
Phases of Viable Cell Microbe growth

1. **Lag** (no cell growth)
   Enzymes are being induced to metabolise substrate.

2. **Log/Exponential** (rapid growth)
   Most rapid growth due to plentiful nutrients.

3. **Stationary**
   Nutrients running out in the culture media and toxic metabolites start to be **produced**
   Secondary metabolites are produced e.g. antibiotics are produced to outcompete other bacteria which confers an ecological advantage to microbes in the wild.

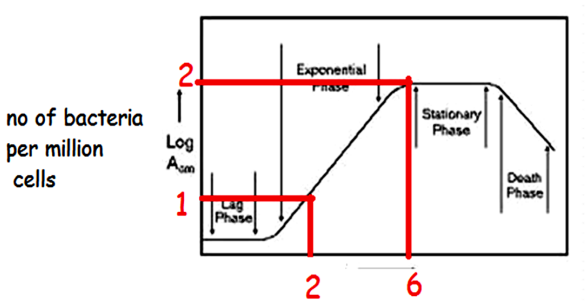
4. **Death phase**
   Toxic metabolites **accumulate** OR **lack of nutrients** in the culture
   The prove that cells are viable is that a death phase can occur.



Generation Time/ Semi-logarithmic Scales

Calculating Mean Doubling Time from graphs

The mean doubling time of bacteria aka the mean generation time occurs during the rapid growth of the log/exponential phase.



1 million cells - 2 hours
2 million cells - 6 hrs
Doubling time = 4 hrs

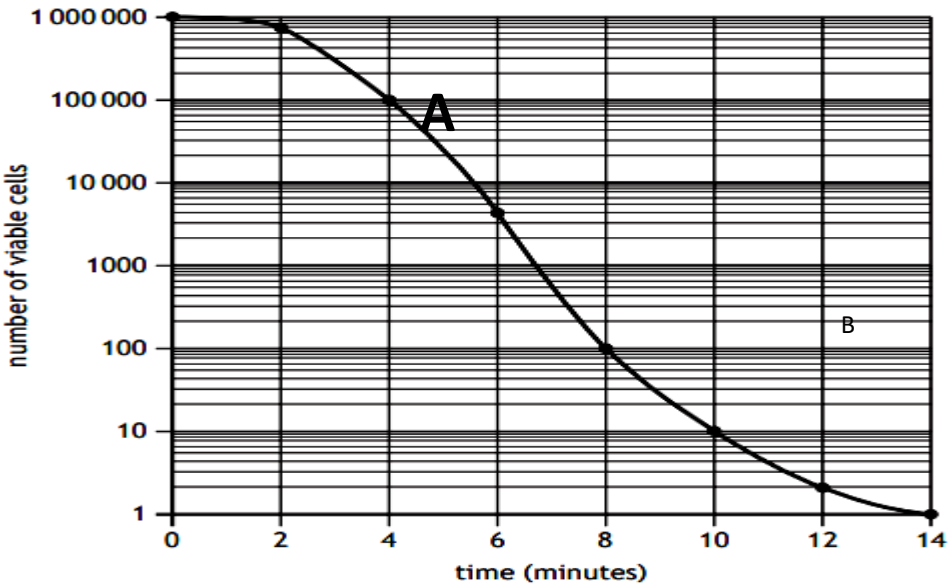
Semi Log Graph Paper

In Semi Log graph paper, the Y axis starts at 1 and the lines do not go up evenly.

Semi Log paper is needed as the growth is to big to fit on normal graph paper.

Example

The following diagram shows semi log paper and the viable cell count every 2 minutes after exposure to a disinfectant designed to kill bacteria.



Point A = 4000 viable cells

Point B = 2 viable cells

Recombinant DNA technology

Improving Wild strains of microorganisms

1. **Mutagenesis**

   Exposure to UV light and other forms of radiation or mutagenic chemicals results in mutations which may produce an improved strain of micro-organism.

2 **Recombinant DNA technology**

plant/animal genes transferred to microbes to make desired animal/plant protein.

Two key enzymes in Recombinant DNA technology

1. Endonuclease

   Same endonuclease is used to cut open the plasmid and cut the gene out of the chromosome to produce COMPLEMENTARY sticky ends.

2. Ligase

   Seals genes into plasmid.

Vector

A vector is a DNA molecule used to **carry foreign genetic information** into another cell.

Types of Vectors

1. Plasmids
2. Artificial chromosomes

   Artificial chromosomes are preferable to plasmids as vectors when larger fragments of foreign DNA are required to be inserted

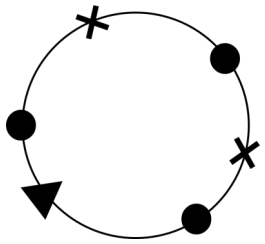
Bacteria vs Yeast Plasmids

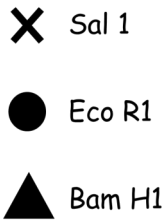
In bacteria the protein **cannot fold the polypeptide properly** so often the protein is inactive.

Yeast cells avoid this problem as they can fold the polypeptide correctly and the protein in active.

Restriction Endonuclease Puzzles

Circle Plasmid Endonuclease Puzzles





Rule

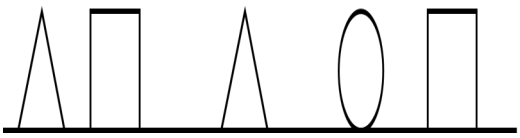
Number of restriction sites = Number of fragments produced

Worked Example

Sal 1 = 2 restriction sites = 2 DNA fragments

Sal 1 + Eco R1 = 4 restriction sites = 4 DNA fragments

Linear DNA Endonuclease Puzzles



Name of enzyme	Shape
Eco R1	Triangle
Bam H1	Square
Sal 1	Circle

Rule

Number of restriction sites = Number of fragments produced PLUS ONE

Worked Example

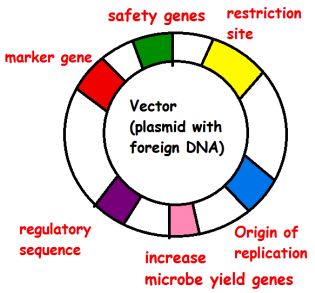
Sal 1 = 1 circle restriction site = 2 DNA fragments

Sal 1 + Eco R1 = 3 restriction sites (1 circle & 2 triangle) = 4 DNA fragments

Genes on Vector

Genes on Vectors

1. Selective marker gene (Antibiotic resistance)
2. Regulatory sequence
3. Restriction site
4. ORI sequence
5. Safety genes



Restriction Site

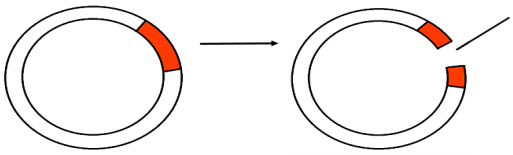
Contain target sequences of DNA where specific restriction endonucleases cut .

ORI sequence

Self replication of plasmid/ artificial chromosome.

Regulatory sequences

Controls gene expression (turn genes ON or OFF).



Safety genes

Introducing genes to prevent microbes surviving in external environment

Selectable marker (Antibiotic Resistance)

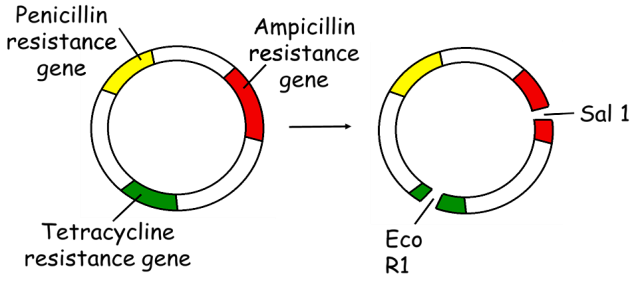
Expose bacteria to selectable marker (antibiotics)

Only transformed bacteria/those with plasmid survive/grow as they have antibiotic resistance.

Interrupting genes on a vector

Restriction sites can often cut through genes on a vector, interrupting the gene expression.

Restriction enzymes Eco R1 and Sal 1 have interrupted the Ampicllin and tetracycline resistance genes which result in these genes coming inactive.



Penicillin is unaffected therefore the resistance gene will still be expressed.

Genes on Vector

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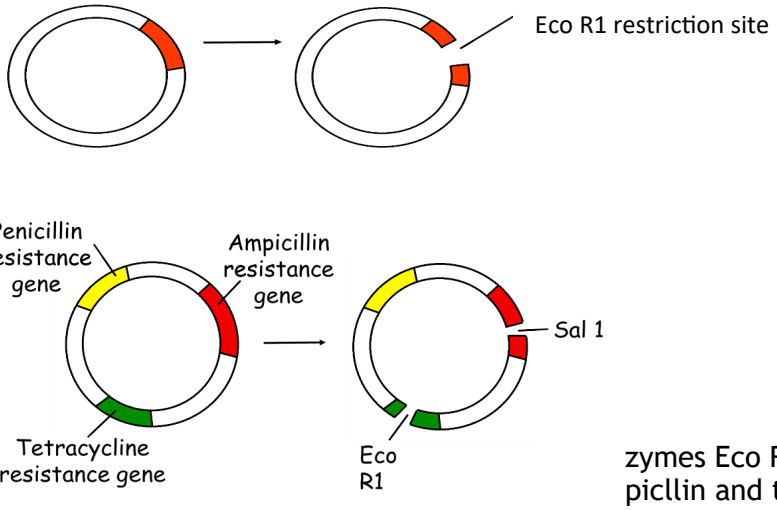

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