St Ninian's High School



Biology Department

Higher Biology

Unit 1: Metabolism and Survival Revision Notes

Name: _____

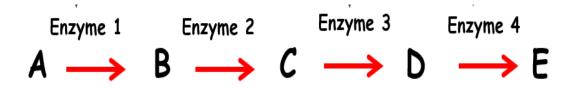
Unit 1 Metabolic Pathways, Enzymes & Respiration Revision

Metabolic Pathways

Metabolic pathways are integrated and controlled pathways of enzyme-catalysed reactions within a cell.

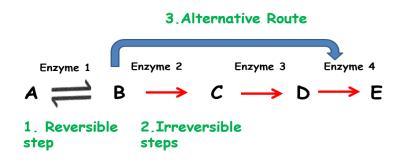
Metabolic Pathways

Each step in a metabolic pathway requires a specific enzyme.



Three Steps in a metabolic Pathway

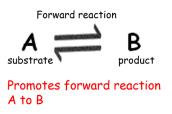
- 1. Reversible Step (2 way arrow allowing forward and back ward reaction)
- 2. Irreversible Step (1 way conversion)
- 3. Alternative route (skips certain steps but produces same molecule at end regardless)



Substrate & Product Concentration in Reversible reactions

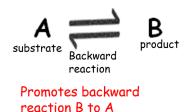
Forward Reaction

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



Backward Reaction

High product concentrations (B) or low substrate concentration A promotes the backward reaction.



Unit 1 Metabolic Pathways, Enzymes & Respiration Revision

Mutation on a metabolic pathway

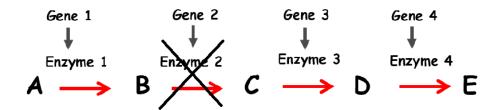
A gene mutation leads to an non functional enzyme affecting the concentration of most substances in the pathway.

Rules in an irreversible step

- 1. The molecule directly before the enzyme mutation increases.
- 2. ALL substance directly after the enzyme mutation decrease.
- 3. Molecules indirectly before the specific enzyme remain constant.

Worked Example

Enzyme 2 non functional due to gene mutation in gene 2



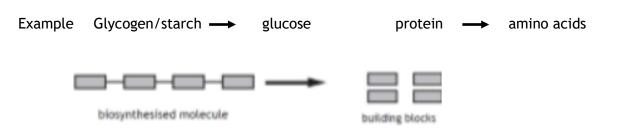
Molecule B increases in concentration.

Molecule C, D & E decreases in concentration.

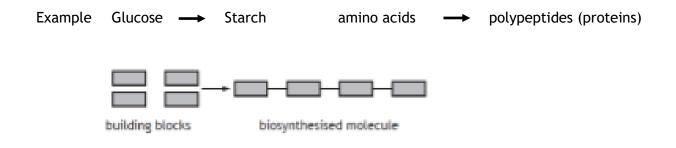
The concentration of molecule A is <u>unaffected</u>.

Types of Metabolic Reactions

1. Catabolic reactions break larger molecules into smaller ones RELEASING energy.



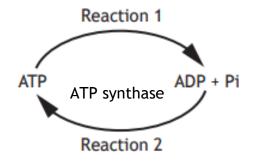
2. Anabolic reactions involve the **BIOSYNTHESIS** of larger molecules into smaller ones **REQUIRING** energy to undertake this process.



ATP Example

Reaction 1 where ATP is broken down is <u>catabolic</u> and <u>releases</u> energy.

Reaction 2 where ATP is synthesized by the enzyme ATP synthase is <u>anabolic</u> and <u>requires</u> energy.



Structure of Membrane

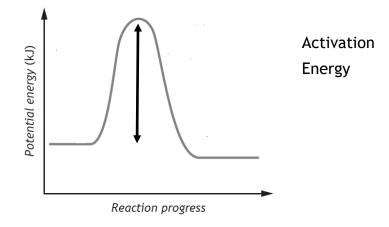
3 types of <u>protein</u> randomly embedded in the membrane.

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

Enzymes

Activation Energy

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



What is Activation Energy?

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

Induced Fit model

After the <u>substrate has bound</u> to the active site, the <u>ACTIVE site changes shape</u> to better fit the substrate.

Affinity for Active site

Substrate-high affinity for active site

Product-low affinity for active site

Substrate Concentration

1. Low substrate concentration Low enzyme activity

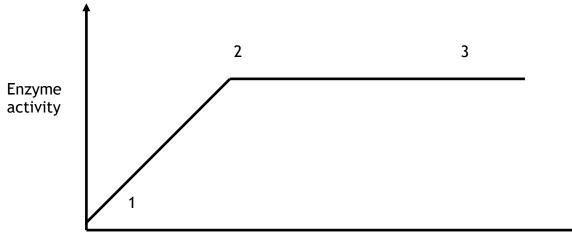
There are not enough substrate molecules to fill all the active sites.

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.



Substrate concentration

Inhibitors

Inhibitors <u>reduce</u> enzyme activity compared to a control.

3 types of enzyme inhibitors

- 1. Competitive Inhibitors
- 2. Non Competitive Inhibitors
- 3. Feedback Inhibition (end product inhibition)

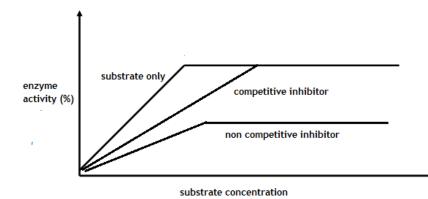
Competitive Inhibitors

Bind at the active site and prevent substrate from binding. Competitive inhibitor molecule resembles substrate. Inhibition reversed with <u>increasing substrate concentration</u>.

Non competitive Inhibitors

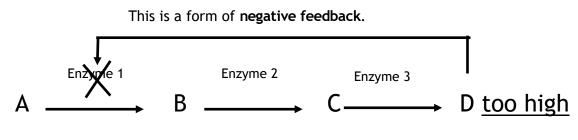
Bind AWAY from active site and change shape of active site preventing substrate from binding. Action irreversible—<u>no effect</u> when increasing substrate concentration.

Enzyme Inhibitor Graph



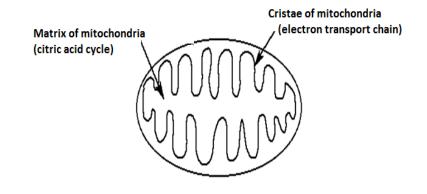
Feedback Inhibition

When the end product concentration reaches a critical concentration (too high), it binds to an <u>EARLIER ENZYME</u> in the pathway, preventing its own synthesis.



Stages of respiration

- 1. Glycolysis (cytoplasm)
- 2. Citric Acid/Kreb Cycle (matrix of mitochondria)
- 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

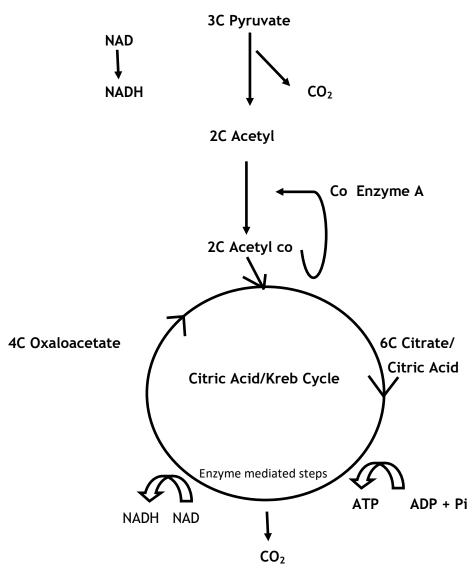
Location-cytoplasm Process does not require oxygen Net gain of 2ATP Dehydrogenase H^{*} and e **Energy Investment Stage** 1. Glucose 2 ATP IN to phosphorylate glucose & intermediates NAD Intermediates 2. **Energy Payoff stage** Dehydrogenase 4 ATP produced after 2 ATP in H^+ and e^- = Net gain of 2 ATP NADH

Pyruvate

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.
- 1. ATP, NADH and CO₂ 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.

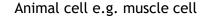
Role of Co enzymes

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

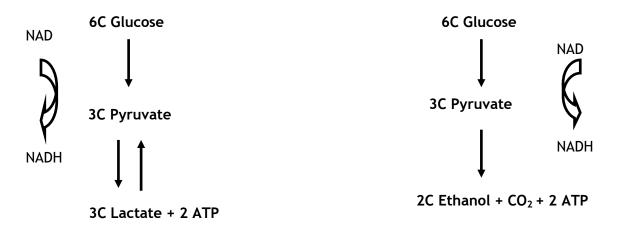
Location-Glycolysis, Citric Acid Cycle and Electron transport chain

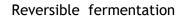
Fermentation

In absence of oxygen fermentation pathways occur in the cytoplasm



Plant/Yeast cells





Irreversible fermentation

Fermentation 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 CO₂ and ethanol builds up and kills plant.

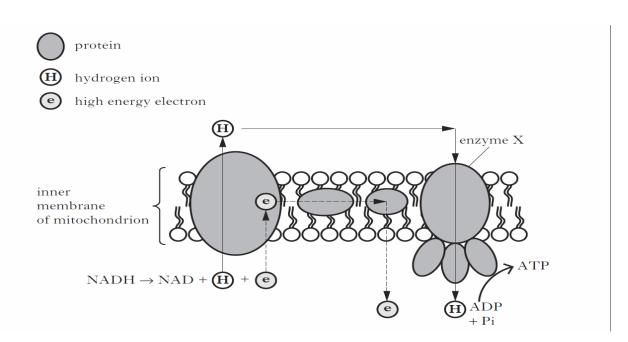
Fermentation results in much <u>less ATP</u> being produced than in aerobic respiration.

Electron Transport Chain

Electron Transport Chain

The electron transport is a collection of membrane proteins in the inner mitochondrial membrane.

Location-Cristae/Inner membrane of mitochondria



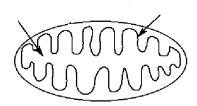
Summary of Process

- 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 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.

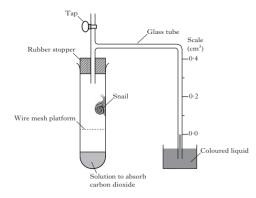


Measurement of metabolic rate

- 1. Oxygen consumption (apparatus-respirometer OR O₂ probe)
- 2. Carbon dioxide production (apparatus–CO₂ probe)
- 3. Heat production (apparatus Calorimeter)

Respirometer

Used to measure <u>oxygen consumption</u> as an indirect measurement of the dependant variable of respiration rate/metabolic rate.



Variables kept constant for VALID results

- 1. Diameter of tubing
- 2. Volume of solution to absorb CO₂

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	Î Î Î
4	0.20	
6	0.25	
8	0.30	
10	0.35	I

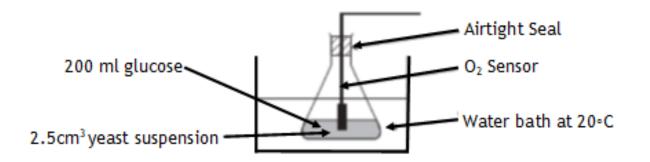
Conclusion

As time increases, metabolic rate increases

Hint: Remember the <u>further</u> the distance travelled = <u>higher</u> metabolic rate

Oxygen/Carbon Dioxide Probes

Another piece of apparatus to measure 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

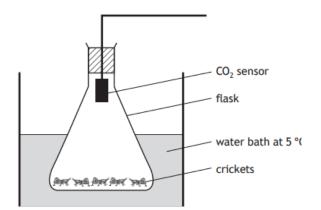
As time increases, metabolic rate increases

Hint

Remember <u>lower</u> oxygen concentration = <u>higher</u> metabolic rate

Oxygen/Carbon Dioxide Probes

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



Time (minutes)	CO2 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	

Conclusion

As time increases, metabolic rate increases

Hint

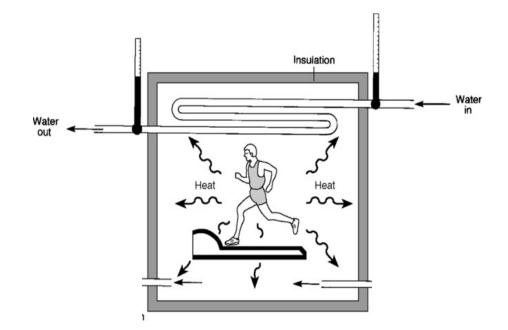
Remember <u>higher</u> CO₂ concentration = <u>higher</u> metabolic rate

Calorimeter

Another piece of apparatus to measure metabolic rate indirectly is a calorimeter which measures **heat production** in a sealed container.

Measurements

- 1. Measure temperature of water at start of experiment.
- 2. Measure temperature of water at end of experiment.
- 3. Use formula to equate difference in temperature to metabolic rate.



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 <u>more heat</u> produced = the <u>higher</u> the metabolic rate

Metabolic Rate Calculations

Metabolic Rate Calculations

As organisms all have DIFFERENT starting masses , 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)
А	60	3.6
В	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 = <u>0.062</u> litres/minute/kg

Metabolic Rate Calculations

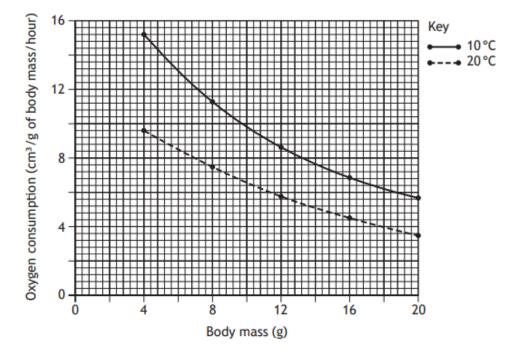
Metabolic Rate Calculations

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 cm³/g of body mass/hour.

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

 $15.2 \times 4g \times 24 = \frac{1459.20}{100} \text{ cm}^3/\text{g of body mass/hour}$

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.

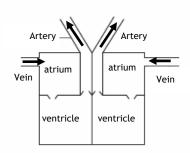
Birds and mammals Complete Double Circulatory System

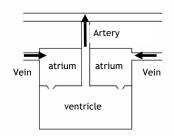
Highest metabolic rate.

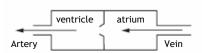
2 atria and 2 ventricles.

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

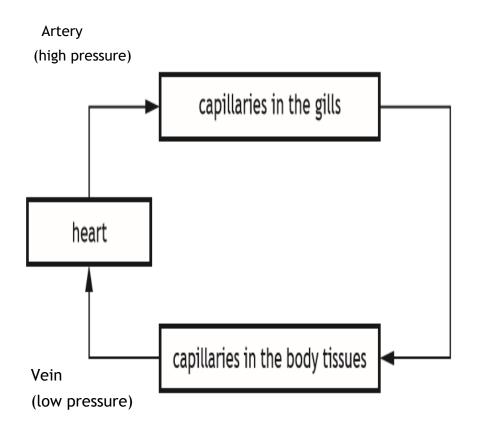
Explain why complete double circulatory systems enable higher metabolic rates.

- 1. There is **no mixing** of <u>oxygenated and deoxygenated blood</u>.
- 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.

<u>Arteries</u> carry blood at <u>high</u> pressure.



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 <u>effectors</u> which bring about **corrective responses** to return temperature to normal

Hypothalamus -

Effectors (corrective responses)

Negative feedback

Corrective responses return system back to normal via negative feedback.

Nerve impulses

Too hot: make individual cooler

Three corrective responses

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

Too Cold: make individual hotter

Four corrective responses

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

Importance of regulating body temperature /thermoregulation in mammals

- 1. To keep enzymes at their optimum temperature
- 2. To maintain high diffusion rates

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

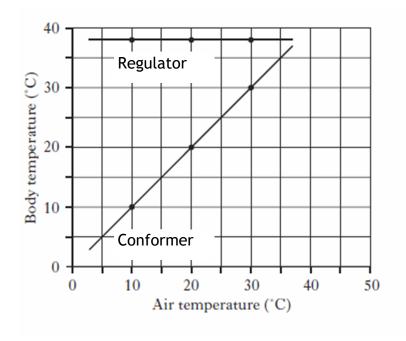
Internal environment (metabolism) is dependent on the external environment.

Low metabolic costs.

Narrow range of ecological niches.

Conformers use **behavioural responses** to maintain optimum metabolic rate.

Behavioural responses by conformers allow them to tolerate <u>SOME</u> variation in their external environment to maintain optimum metabolic rate.



Surviving and Avoiding Adverse Conditions

Surviving Adverse Conditions

Many environments vary beyond <u>tolerable</u> 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**) when condition make normal metabolic activity too high.

This saves energy for the organism involved.

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 aestivation/hibernation Dormancy occurs before onset of adverse conditions. 2. Consequential aestivation only Dormancy occurs after onset of adverse conditions.

2. Avoiding Adverse conditions Strategy

Migration avoids metabolic adversity by relocating 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 (BAE)

- 1. Bacteria
- 2. Archaea
- 3. Eukaryotes

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

Why use Micro-organisms?

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

Growth Media

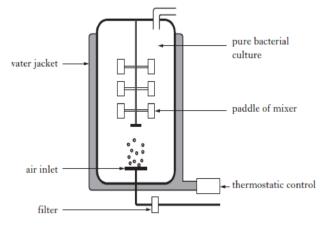
When culturing micro-organisms they require:

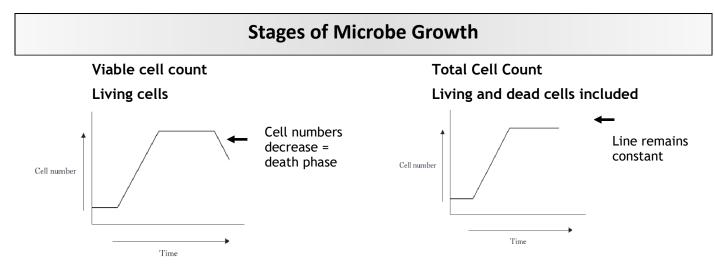
- 1. Energy source (light for photosynthetic organisms/ chemical substrates e.g. glucose)
- 2. Raw materials for biosynthesis

Many micro-organisms produce all the complex molecules required for biosynthesis e.g. amino acids, vitamins and fatty acids.

Culture Conditions

- <u>Sterility</u> 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.
- <u>Temperature</u> to keep enzymes at optimum HOW? Water jacket and thermostat.
- Oxygen concentration for aerobic respiration HOW? Air inlet and paddles for aeration.
- <u>pH</u> to keep enzymes at optimum HOW? Use of buffers.





Phases of Viable Cell Microbe growth

1. Lag (no cell growth)

Enzymes are being induced to metabolise substrate.

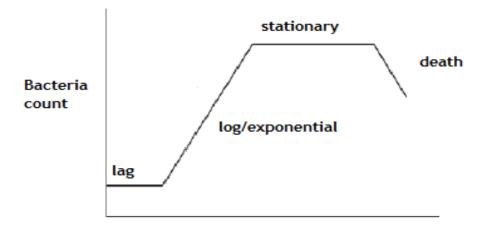
Log/Exponential (rapid growth)
 Most rapid growth due to plentiful nutrients.

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

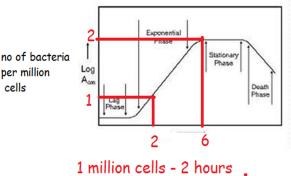
Toxic metabolites **accumulate** OR **lack of nutrients** in the culture The prove that cells are viable is that a death phase can occur.





Generation Time/ Semi-logarithmic Scales

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

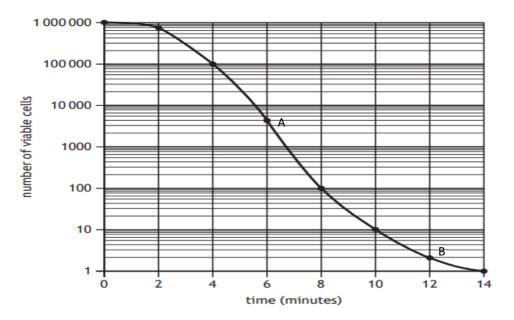


2 million cells - 2 nours 2 million cells - 6 hrs Doubling time = 4 hrs

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, some of 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 cuts open the plasmid and cuts gene out of chromosome Leaving 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

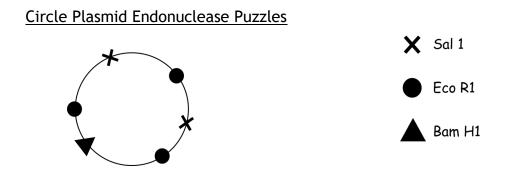
Artificial chromosomes are preferable to plasmids as vectors when larger fragments of foreign DNA are required to be inserted

Bacteria vs Yeast Plasmids

In <u>bacteria</u> the protein cannot fold the polypeptide properly so often the protein is <u>inactive</u>.

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

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 <u>PLUS ONE</u>

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.

Regulatory sequences

Controls gene expression (turn genes ON or OFF).

Safety genes

Introducing genes to prevent microbes surviving in external environment

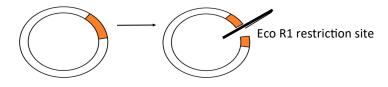
Selectable marker (Antibiotic Resistance)

Expose bacteria to antibiotics and only transformed bacteria survive/grow.

This is because only transformed bacteria have genes that have resistance to selectable marker

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 becoming inactive.

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

