12 Energy and respiration

12.1 Energy

a) The need for energy in living organisms

All living organisms need a continuous supply of energy to maintain their metabolism. They either absorb light energy (autotrophs) or chemical potential energy (heterotrophs). This is to do the work necessary to live. Work includes:

1) **anabolic reactions**
2) **active transport** (transport of substances against concentration gradient)
3) **movement** e.g., muscle contraction, cilia movement, locomotion
4) bioluminescence, electrical discharge, temperature regulation, etc.

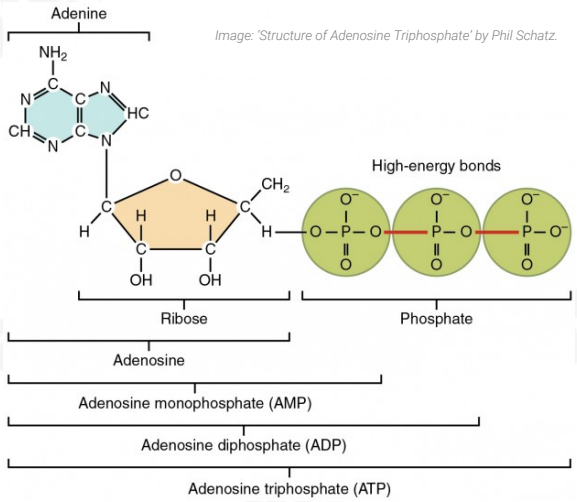
➢ **anabolic reactions** - synthesis of complex substances from simpler ones

Examples of anabolic reactions

1) DNA replication
2) protein synthesis
3) active transport
4) movement
5) the maintenance of body temperature
   making polysaccharides, triglycerides, polynucleotides (DNA/RNA)
6) phosphorylation

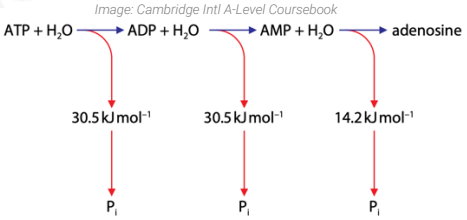
b) ATP

Features of ATP that make it suitable as the universal energy currency



1) small
2) water-soluble
3) easily transported around the cell
4) easily hydrolysed to release energy
5) relatively large quantity of energy released

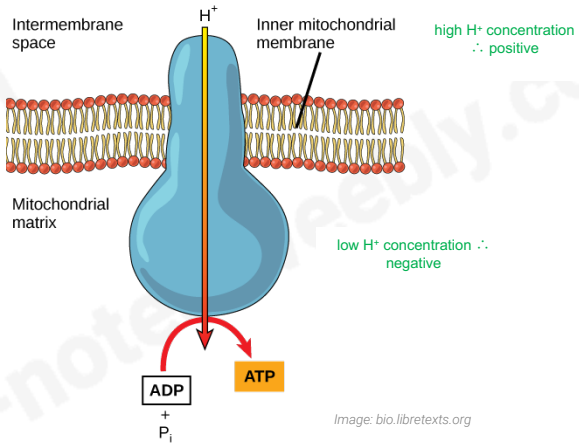
ATP + H_2O ⇌ ADP + P_i (inorganic phosphate)



6) rapid turnover rate
7) readily available in cells upon demand as stores are released in manageable amounts, meaning no energy is wasted

c) ATP synthesis

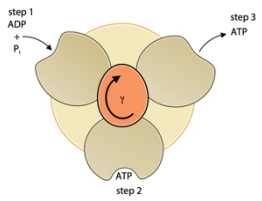
ATP is synthesised in substrate-linked reactions in glycolysis and in the Krebs cycle.



1) the process by which ATP is synthesised is called chemiosmosis
2) it occurs in the inner mitochondrial membrane (or the thylakoid membrane of a chloroplast)
3) ATP is generated using electrical potential energy (this is energy from the transfer of electrons by electron carriers like NADH and FADH in the electron transport chain)
4) the electrons cause conformation changes in the shapes of the proteins and cause them to pump H^+ across a selectively permeable cell membrane
5) the uneven distribution of H^+ ions across the membrane establishes both concentration and electrical gradients (thus, an electrochemical gradient) due to their positive charge as well as them collecting at one side of the membrane
6) H^+ are allowed to flow down their concentration gradient via facilitated diffusion through ATP synthase
7) the transfer of 3 H^+ allows the production of 1 ATP molecule provided that ADP and P_i are available inside the mitochondrion
8) as H^+ travel through the ATP synthase complex, it drives the down part by a rotation force provided by the electrochemical gradient that produces ATP

Structure of ATP synthase
Has a total of 6 binding sites; three of them are catalytically inactive and they bind ADP. The three other subunits catalyze the ATP synthesis.

Image: Cambridge Intl A-Level Coursebook



Transverse section of ATP synthase

d) Roles of coenzymes in respiration

1) NAD (nicotinamide adenine dinucleotide)

$$NAD^+ + H^+ + 2e^- \underset{\text{oxidation}}{\overset{\text{reduction}}{\rightleftharpoons}} NADH$$

- a hydrogen carrier molecule, accepts a hydrogen from one reaction (getting reduced) and donates it to another
- NADP (NAD + phosphate group) is used a hydrogen carrier molecule in photosynthesis

2) FAD (flavin adenine dinucleotide)

$$FAD + 2H^+ + 2e^- \underset{\text{oxidation}}{\overset{\text{reduction}}{\rightleftharpoons}} FADH_2$$

- similar in function to NAD
- used in respiration in the Krebs cycle

3) coenzyme A

pyruvate + CoA + NAD ⇌ acetyl COA + CO_2 + reduced NAD

- carries acetyl groups made from pyruvate during the link reaction to Krebs cycle
- can also carry acetyl groups made from fatty acids

e) Explain that the synthesis of ATP is associated with the electron transport chain on the membranes of mitochondria and chloroplasts *(refer to 12.2g)*

f) Relative energy values of carbohydrates, lipids & proteins

- energy in aerobic respiration comes from the oxidation of hydrogen to water when reduced NAD and FAD are passed onto the electron transport chain (ETC)
- therefore, greater the number of hydrogens, greater the energy value per unit mass (energy density)
- typical energy values can be determined by burning a known mass of a substrate in a calorimeter

TYPICAL ENERGY VALUES	
respiratory substrate	**energy density / kJ g^{-1}**
carbohydrate	15.8
lipid	39.4
protein	17.0

Why more ATP can be synthesised in aerobic respiration from one gram of lipid

1) lipids contain relatively more hydrogen atoms
2) more reduced NAD and FAD are produced
3) more electrons are passed along ETC
4) more hydrogens pumped across inner mitochondrial membrane

g) Respiratory quotient (RQ)

- the ratio of oxygen taken in and carbon dioxide released is 1:1, however when other substrates are being respired, this ratio differs
- measuring this ratio therefore can show what substrate is being in respiration
- change in RQ indicates that the substrate being respired has changed
- the ratio can also show whether or not anaerobic respiration is occurring

➢ **respiratory quotient** - the ratio of carbon dioxide molecules produced to oxygen molecules taken in during respiration

$$\mathbf{RQ = \frac{CO_2 \text{ produced}}{O_2 \text{ consumed}}}$$

Typical RQs for the aerobic respiration of different substrates:

respiratory substrate	**energy density / kJ g^{-1}**
carbohydrate	1.0
lipid	0.7
protein	0.9

RQ values when respiration is anaerobic

Example: yeast

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + \text{energy}$$

$$RQ = \frac{CO_2}{O_2} = \frac{2}{0} = \infty$$

- in reality, some respiration in the yeast will be aerobic so a small volume of oxygen will be taken up and the RQ will be less than infinity
- high values of RQ indicate that alcoholic fermentation is occurring

- no RQ can be calculated for muscle cells using the lactate pathway as no carbon dioxide is produced

h) using respirometers to determine RQ

using a respirometer to determine the RQ of germinating seeds or small invertebrates (e.g., blowfly larvae)

1) set up a respirometer
   - control tube must contain an equal amount of inert material (e.g., glass beads) to the volume of organism being used

2) use the manometer reading to calculate change in gas volume within a given time (x cm^3 min^{-1})

x – oxygen consumed (distance moved by fluid in experimental tube)
y – distance moved by fluid in control tube
x - y – carbon dioxide given out

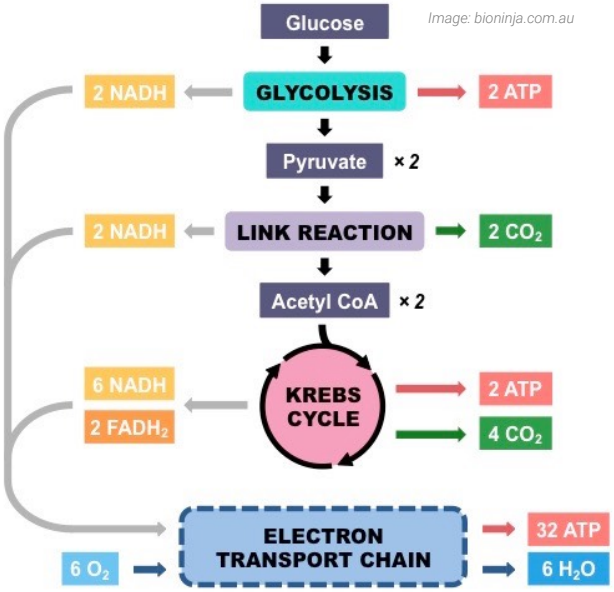
$$RQ = \frac{x-y}{x}$$

12.2 Respiration

a) The 4 stages of aerobic respiration

➢ **respiration** – the process whereby chemical potential energy from the breakdown of organic molecules is used to synthesise ATP

PROCESS	LOCATION	NET ATP PRODUCED
glycolysis	cytoplasm	2
link reaction	mitochondrial matrix	0
Krebs cycle		2 (one for every acetyl group)
oxidative phosphorylation	mitochondrial membrane	32



b) Glycolysis

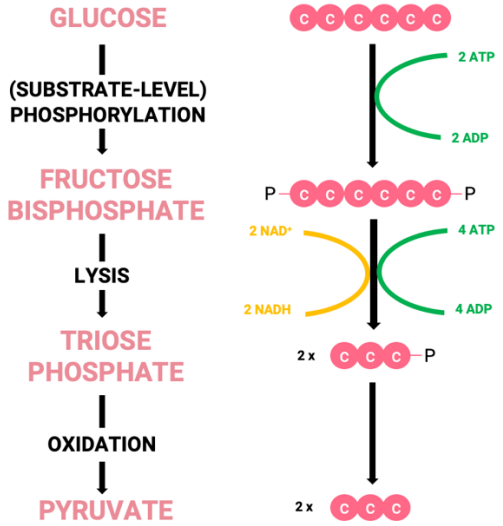
- phosphorylation of glucose
- the subsequent splitting of fructose 1,6-bisphosphate (6C) into two triose phosphate molecules
- these are further oxidised to pyruvate with a small yield of ATP and reduced NAD

1) glucose is phosphorylated (substrate-level phosphorylation) using 2 ATP molecules
2) this results in the formation of fructose bisphosphate (6C) (also known as hexose bisphosphate)
3) this raises the energy level of glycose / lowers the activation energy of the reaction
4) fructose bisphosphate is lysed to produce 2 triose phosphate molecules (3C)
5) hydrogen atoms and phosphate groups are removed from triose phosphate by coenzyme NAD to produce 2 molecules of pyruvate and reduced NAD

- since removal of hydrogen is oxidation:

TRIOSE PHOSPHATE —OXIDATION→ **2 PYRUVATE**

- removed phosphate groups are added to ADP to 4 ATP, however since 2 were used, there's a net gain of 2 ATP

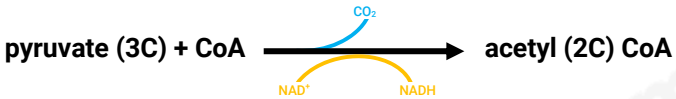


- overall, 2 molecules of ATP are used
- 4 are made during the glycolysis of one glucose molecule
- ∴ net gain of 2 ATP per glucose

c) Link reaction

- when oxygen is available, pyruvate is converted into acetyl (2C) coenzyme A in the link reaction
- pyruvate enters by active transport from the cytoplasm into the mitochondrial matrix

1) pyruvate is decarboxylated (CO_2 is removed)
2) then it is dehydrogenated (hydrogen is removed and picked up by NAD)
   - this converts pyruvate into a 2C compound
3) then it's combined with coenzyme A to produce acetyl coenzyme A (ACoA)



4) coenzyme A transfers the acetyl group with 2C to the Krebs cycle

d, e) Krebs cycle

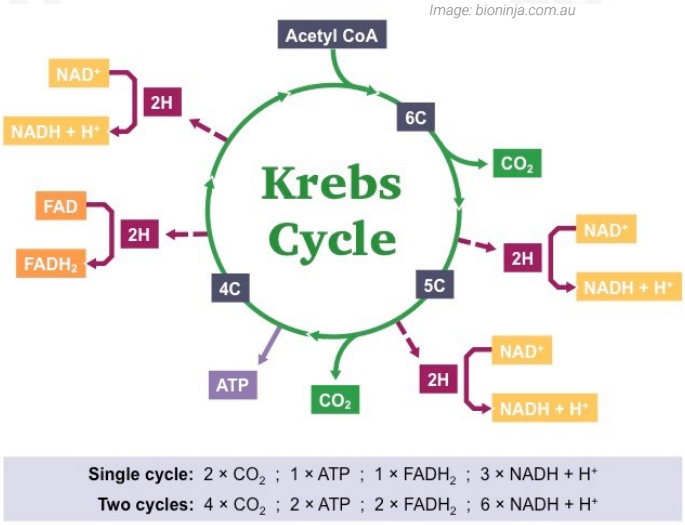
- oxaloacetate (a 4C compound) acts as an acceptor of the 2C fragment from acetyl coenzyme A to form citrate (a 6C compound), which is reconverted to oxaloacetate in a series of small steps
- reactions in the Krebs cycle involve decarboxylation and dehydrogenation and the reduction of NAD and FAD

1) **acetyl coenzyme A combines with a 4C compound called oxaloacetate to form citrate (6C)**
   - CoA is reformed, the cycle turns twice for each original glucose molecule

2) **citrate is decarboxylated and dehydrogenated in a series of steps**
   - this produces CO_2 (waste gas) and hydrogens (which are accepted by NAD and FAD)
   - 1 FAD and 3 NAD molecules are reduced each turn of the cycle (hydrogen in reduced NAD/FAD will be released in oxidative phosphorylation)
   - main role of the Krebs cycle in respiration is to generate many reduced hydrogen carriers to pass onto the next stage

3) **oxaloacetate is regenerated and can recombine with ACoA**

Image: bioninja.com.au



Single cycle: 2 × CO_2 ; 1 × ATP ; 1 × $FADH_2$; 3 × NADH + H^+
Two cycles: 4 × CO_2 ; 2 × ATP ; 2 × $FADH_2$; 6 × NADH + H^+

Summary

- for each turn of the cycle, 2CO_2 and 1 ATP produced, 1 FAD and 3 NAD reduced
- for every glucose molecule, 4CO_2 and 2 ATP produced, 2 FAD and 6 NAD reduced

f, g) Oxidative phosphorylation

- energetic electrons release energy as they pass through the electron transport system
- the released energy is used to transfer protons across the inner mitochondrial membrane
- protons return to the mitochondrial matrix by facilitated diffusion through ATP synthase providing energy for ATP synthesis (details of ATP synthase are not required)
- energy for phosphorylation of ADP to ATP comes from the activity of the electron transport chain (ETC)

1) reduced NAD and FAD are passed onto the ETC in the inner mitochondrial membrane (cristae)
2) hydrogen released from reduced NAD and FAD splits into electrons and protons
3) as these electrons pass along electron carriers from a higher to lower energy level, energy is released
4) energy released pumps H^+ into the intermembrane space creating a proton gradient across the crista
5) protons diffuse back through channel proteins which have ATP synthase associated with them into the matrix
6) 1 ATP molecule is produced from ADP and P_i when 3 H^+ pass down via the electrochemical gradient
7) oxygen is the final electron acceptor at the end of the ETC in the matrix
8) protons combine with electrons and oxygen atoms to form water

h) Effect of factors such as temperature and substrate concentration on the rate of respiration of yeast using a redox indicator (e.g., DCPIP or methylene blue)

Mechanism of redox indicators when determining respiration rates

1) dehydrogenation happens regularly throughout the different stages of aerobic respiration
2) the hydrogens that are removed from substrate molecules are used in oxidative phosphorylation and are transferred by NAD and FAD
3) when DCPIP and methylene blue are present, they can also take up hydrogens and get reduced (blue → colourless)
4) faster the rate of respiration, the faster the rate of hydrogen release and faster the dyes get reduced and turn colourless
5) therefore the rate of colour change can correspond to the rate of respiration in yeast
6) rate of respiration (sec^{-1}) = 1 / time (sec)

Effect of temperature on the rate of respiration of yeast using a redox indicator

1) add a redox dye such as DCPIP or methylene blue to a suspension of yeast cells
2) add the test tubes to a temperature-controlled water bath
3) record the time taken for a colour change to occur once the dye is added (& repeat across a range of temperatures)
4) when reduced, the blue dyes become colourless (rate of change from blue to colourless is a measure of the rate of respiration of the yeast)

Effect of substrate concentration on the rate of respiration of yeast using a redox indicator

1) add different concentrations of a substrate to the suspension of yeast cells (e.g. 0.1%, 0.5% glucose)
2) record the time taken for a colour change to occur once the dye is added (& repeat across a range of temperatures)
3) when reduced, the blue dyes become colourless (rate of change from blue to colourless is a measure of the rate of respiration of the yeast)

Variables to be controlled in redox indicator experiment to investigate respiration rate in yeast

1) volume of dye added
2) volume of yeast suspension
3) type of substrate
4) *concentration of substrate*
5) *temperature*

i) structure and function of the mitochondrion

Image: https://teachmephysiology.com/

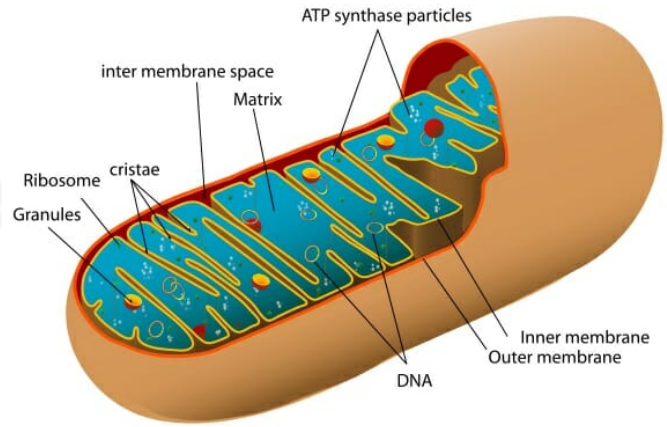
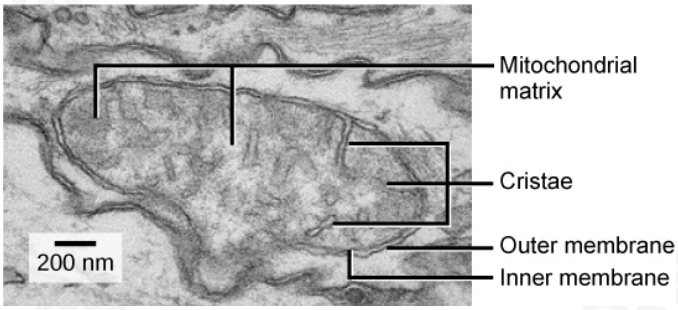


Image: http://kolibri.teacherinabox.org.au/



Structure

1) 0.5-1.0 μm
2) double membrane: inner one is folded inwards to form cristae
3) cristae increase surface area for reactions (mitochondria from more active cells have more densely packed cristae)
4) outer membrane is more permeable than the inner one
5) inner membrane has a lower pH than the matrix due to H^+ released due to the ETC
6) matrix contains enzymes for the link reaction and Krebs cycle, 70S ribosomes are circular DNA are also present

Function

1) ATP synthesis
2) aerobic respiration

j, k) anaerobic respiration

ALCOHOLIC FERMENTATION
PURVATE → ETHANOL
(by yeast)

*BOTH OCCUR IN THE CYTOPLASM

LACTIC FERMENTATION
PURVATE → LACTATE
(by mammalian muscle)

- when free oxygen is not present, hydrogen cannot be removed by combining with O_2 so reduced NAD is not recycled
- the ETC stops working
- oxidative phosphorylation cannot take place as there's nothing to accept e^- (& H^+)
- the mitochondrion then runs out of NAD or FAD that can accept hydrogen from the Krebs cycle
- Krebs cycle and link reaction pause
- glycolysis can still continue as long as pyruvate can be removed and reduced NAD → NAD

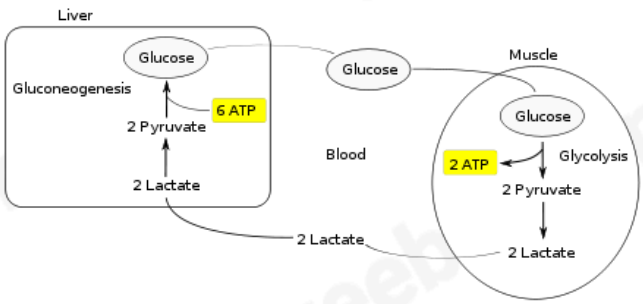
Ethanol pathway

1) pyruvate and reduced NAD formed from glycolysis
2) pyruvate is decarboxylated by *pyruvate decarboxylase* producing ethanal
3) ethanal is the hydrogen acceptor from reduced NAD
4) ethanol is formed – this reduction is catalysed by alcohol dehydrogenase
5) irreversible (so chemical potential energy of ethanol is wasted)
6) NAD is regenerated so it can accept more hydrogen atoms
7) therefore, glycolysis can continue

Lactate pathway

Pyruvate itself accepts the hydrogen and is converted to lactate by *lactate dehydrogenase*. Again, NAD is released and allows glycolysis to continue.

Image: https://en.wikipedia.org/wiki/Cori_cycle



The lactate pathway is reversible (in the liver).

Why less ATP can be synthesised from the same mass of glucose in anaerobic respiration than aerobic respiration

1) glucose is not completely broken down and only glycolysis occurs
2) pyruvate still contains energy
3) ETC stops as there's no oxygen to act as the final electron acceptor
4) so oxidative phosphorylation stops, Krebs cycle (no NAD or FAD to accept hydrogen) and link reaction pause

Both reactions 'buy time' by allowing some *(2 molecules)* ATP to be produced although oxygen is not available as a hydrogen acceptor. They, however, cannot continue as the products are toxic.

Oxygen debt

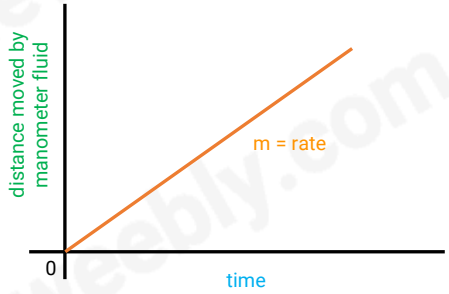
- during exercise, it takes time for the heart and lungs to meet demand, so anaerobic respiration occurs
- after exercise, the person continues deep breathing to absorb more oxygen
- the extra oxygen is needed for:
  1) converting lactate into glycogen
  2) reoxygenation of haemoglobin
  3) high metabolic rate

l) adaptations of rice for wet environments

Feature	**How it helps the plant to survive when roots are submerged**
cells are tolerant of high concentrations of ethanol	When roots are submerged in water, less oxygen is available than when the soil contains air spaces. Cells therefore respire anaerobically, producing ethanol.
stems have tissues called aerenchyma, containing large air spaces	Aerenchyma allows oxygen from the air to diffuse down to the roots
some types of rice are able to grow elongated stems to keep their leaves above the water as its level rises	The leaves remain exposed to air, which facilitates gas exchange for photosynthesis and respiration.

m) Using simple respirometers to measure the effect of temperature on the respiration rate of germinating seeds or small invertebrates

1) respirometer is placed in water baths at different temperatures
2) rate of respiration is represented by gradient of the graph



Using a respirometer to investigate the rate of uptake of oxygen

1) organisms to be investigated are placed in one tube and non-living material (glass beads) of the same mass in the other tube
2) soda lime is placed in both tubes to absorb carbon dioxide
3) coloured fluid is poured into the manometer reservoir and allowed to flow into the capillary tube (ensure that there are no air bubbles and volume of liquid is the same in both tubes)
4) rubber bungs are fitted on both tubes; spring clips are closed, and the manometer is then attached to the bent glass tubing (ensure that it's airtight)
5) open spring clips (to allow pressure throughout the apparatus to equilibrate with atmospheric pressure)
6) as organisms respire, oxygen is taken from air in the tube, reducing the volume and pressure, causing the manometer fluid to flow towards the organisms
7) carbon dioxide is removed by the soda lime which ensures that distance moved by the fluid is only affected by oxygen uptake
8) distance moved by the manometer fluid can be calculated using $\pi r^2 h$
9) volume of oxygen taken up can be calculated if the diameter of the tube is known