Biomass – Cell Biochemistry

2.1 METABOLISM

Now that we have an understanding of the basic chemistry and structure of a cell it is necessary to investigate the life-sustaining chemical processes that occur within a cell.

The term **metabolism** is used to collectively refer to all the chemical processes that take place within a cell. It is derived form the Greek "metabole", which means, "change". This serves to remind us that, although we study cells as fixed and stable structures, a cell is actually a dynamic entity that is constantly undergoing change as a result of the chemical reactions that take place.

All the metabolic processes can be divided into two types, i.e. **anabolic** processes (building processes) and **catabolic** processes (breaking down processes).   A simplified cell showing the anabolic and catabolic processes is shown below in Figure 1. Referring to **Figure 1**, the processes involved in anabolism are shown in black and those involved in catabolism are in white.
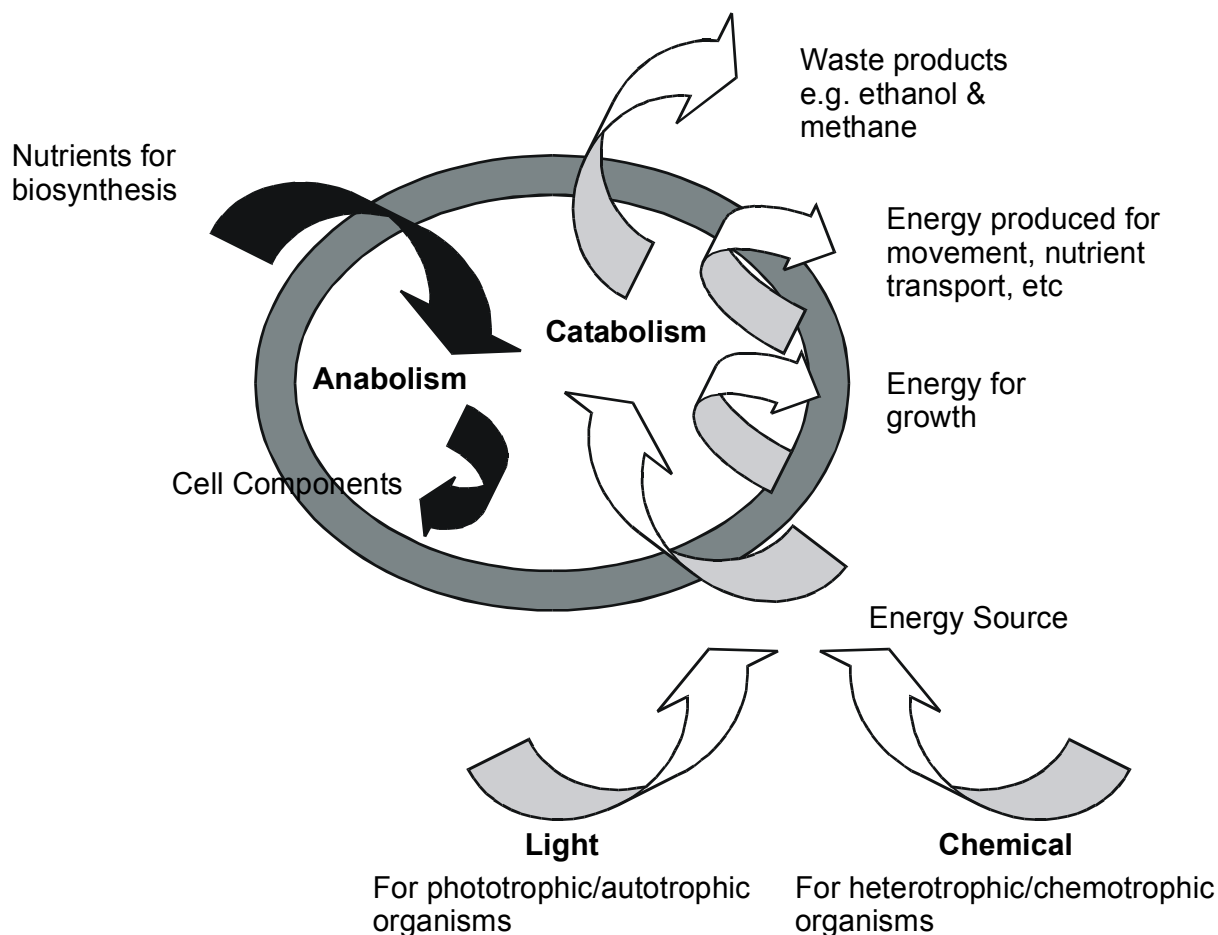

Figure 1. A simplified cell showing anabolic and catabolic processes.

Anabolism.   During anabolism nutrients are taken from the external environment and transported into the cell. Here, the nutrients are changed into cell components to be used for growth and reproduction. Anabolic reactions are always energy requiring reactions.   It takes an input of energy to transport nutrients and "build" new cell components, consequently, anabolism results in the consumption of energy. Some organisms obtain the energy for anabolism directly from light – this is the conversion of solar energy to chemical energy, which is, of course, photosynthesis, which we shall be looking at in Section 5.

Catabolism.   However, most organisms, apart from plants and algae, require a chemical energy source to fuel their anabolic reactions. This chemical energy source is simply what we refer to as "food". This "food" is transported into a cell where it must be "broken down" (oxidised) to release the chemical energy for use by the cell. This energy is then available to fuel anabolic reactions and cell functions such as movement, nutrient transport etc.

Probably the most important feature of catabolism (from a biofuels point of view) is that some catabolic processes are very "inefficient" at extracting all the energy from a particular food source. This means that the "waste products" excreted from the cell, for example ethanol and methane, still contain a lot of potential, useable chemical energy. However, these compounds cannot be used by the micro-organism and are therefore excreted back into the environment as waste.

The main features of metabolism are summarised below in **Table 1**.

Table 1. A summary of Metabolism.

Anabolism	**Catabolism**
Consumption of energy	Release of energy
Production (biosynthesis) of cell components for growth from external nutrients	Degradation of external chemical energy source and production of waste product

2.2 BIOENERGETICS

As we can see from the previous discussion on cellular metabolism, all non-photosynthetic organisms must release chemical energy from various compounds in order to fuel life-sustaining processes.

> *The release, storage, transport and use of this energy is collectively known as the bioenergetics of cellular metabolism.*

The following areas of bioenergetics will be briefly covered:

• Free energy.

• Activation energy, catalysts and enzymes.

- Oxidation – Reduction.

- Electron carriers.

- High energy phosphate compounds (Adenosine Triphosphate (ATP)).

These will give a good basis for understanding the processes of photosynthesis (Section 5), and anaerobic digestion for methane production (Unit 2).

2.2.1   Free Energy

The chemical reactions of a cell are accompanied by changes (gain or loss) of energy. There are two expressions of the amount of energy released during a chemical reaction, abbreviated H and G.

H, called **enthalpy**, expresses the total amount of energy released during a chemical reaction. However, some of this energy released is not available to do useful work, but instead is lost as heat energy. G, called the **free energy**, is used to express the energy released that is available to do useful work. The change in free energy that occurs during a reaction is expressed as ΔG. A chemical reaction can occur with the release of free energy. This is known as an **exergonic** reaction, it is expressed by a –ve ΔG and will proceed spontaneously. A chemical reaction that occurs with a net consumption of free energy is an **endergonic** reaction. This is expressed with a +ve ΔG and these reactions will not proceed spontaneously.

In an exergonic reaction, the reaction proceeds until the concentration of products builds up, and then the reverse reaction, the conversion of products back to reactants, increases. An equilibrium is eventually reached in which the forward and reverse reactions are equally balanced. However, this balanced condition does not mean that reactant and product occur in equal concentrations. The concentrations of product and reactant at equilibrium are related to the free energy of the reaction. If the reaction proceeds with a large negative ΔG, the equilibrium is pushed towards the products and very little of the reactant will remain. However, if the reaction proceeds with a small ΔG then, at equilibrium, there are nearly equal amounts of products and reactants.

2.2.2   Activation Energy, Catalysis and Enzymes

Free energy calculations tell us which conditions will prevail when the reaction or system is at equilibrium, but not how long it will take for equilibrium to be reached.

The formation of water from gaseous oxygen and hydrogen is a good example. The energetics of the reaction are favourable with a free energy of formation of –231.17. However, if we mix O_2 and H_2 together no measurable formation of water will occur during our lifetime. This is because the rearrangement of oxygen and hydrogen atoms to form water requires that chemical bonds must be broken. Breaking of these bonds requires an input of energy, which is know as activation energy.

Activation energy is defined as: *the amount of energy required to bring all the molecules in a chemical reaction to a reactive state.*

This means that, even though a reaction is exergonic, it may not proceed spontaneously because the reactants must first be activated. Once activation has occurred, the reaction then proceeds spontaneously.

This leads us to the concept of catalysis. A **catalyst** is a substance which serves to lower the activation energy of a reaction. A catalyst serves to increase the rate of a reaction but is itself not changed. Catalysts do not effect the energetics of a reaction, only the **speed** at which reactions proceed. A catalyst forms an intermediary complex with a particular substrate, the result of which is a reduction in the activation energy required to make the reaction proceed. A schematic showing the effects of catalysis on the activation energy of a reaction is shown below in **Figure 2**.
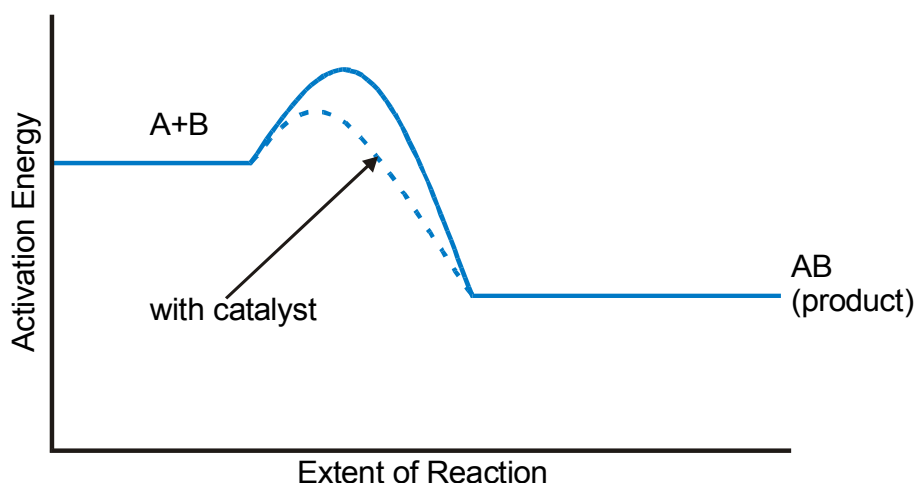

Figure 2. A schematic showing the effects of catalysis on the activation energy of a reaction.

Most reactions in living organisms would not occur at appreciable rates without catalysis. The catalysts of biological reactions are proteins called **enzymes**. Enzymes are highly specific in the reactions they catalyse, i.e. each enzyme catalyses only a single type of chemical reaction. This specificity is determined by the precise three-dimensional structure of an enzyme molecule, which can only combine with the corresponding substrate molecule.

The structural nature of enzyme specificity is illustrated with the "lock and key" model illustrated overleaf in **Figure 3**. In an enzyme catalysed reaction the enzyme temporarily combines with the substrate at the active site of the enzyme to form an enzyme-substrate complex. This serves to align reactive groups and places strain on specific bonds in the substrate, which means that less activation energy is required to make the reaction proceed. As the reaction proceeds the product is released and the enzyme is returned to its original state.
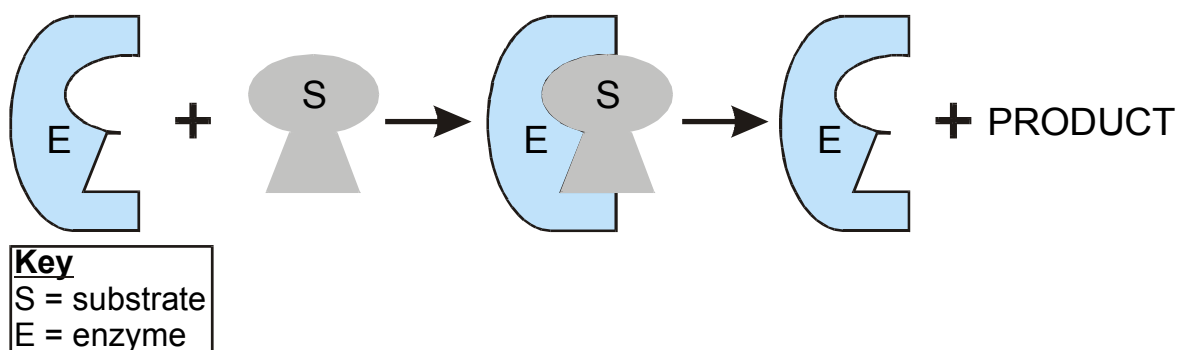
Key
S = substrate
E = enzyme

Figure 3. A schematic illustrating the "Lock and Key" model.

2.2.3   Oxidation – Reduction (REDOX)

The utilisation of chemical energy in living organisms involves oxidation – reduction (often abbreviated to REDOX) reactions. Chemically, an **oxidation** is defined as the removal of an electron or electrons from a substance. A **reduction** is defined as the addition of an electron (or electrons) to a substance.

A useful way of remembering this is **OIL RIG**, which stands for **Oxidation Is Loss** (of electrons) and **Reduction Is Gain** (of electrons).

However, in biochemistry, oxidations and reductions frequently involve the transfer of not just electrons, but whole hydrogen atoms. A hydrogen atom (H) consists of an electron plus a proton. When the electron is removed the hydrogen atom becomes a proton (or hydrogen ion, H^+).

$$H_2 \rightarrow 2e^- + 2H^+ \qquad \text{(half reaction 1 – oxidation)}$$

However, electrons cannot exist alone in solution; they must be part of atoms or molecules. The equation, as drawn above, does not itself represent a real reaction. This is because, for any oxidation to occur, a subsequent reaction must also occur. For example, the oxidation of H_2 could be coupled to the reduction of many different substrates, including O_2.

$$\tfrac{1}{2}O_2 + 2e^- + 2H^+ \rightarrow H_2O \qquad \text{(half reaction 2 – reduction)}.$$

The half reaction No. 2 (shown above), when coupled to the oxidation of H_2 (half reaction No. 1), yields the following overall balanced reaction:

$$H_2 + \tfrac{1}{2}O_2 \rightarrow H_2O \qquad \text{(whole balanced reaction)}.$$

The substance that is oxidised is referred to as the **electron donor** and the substance reduced as the **electron acceptor**. In the above case, hydrogen was the electron donor and oxygen the electron acceptor. Most molecules can serve either as electron donors or acceptors under different circumstances, depending on which other substance they react with.

Substances vary in their tendency to give up or accept electrons. This tendency is expressed as the **reduction potential** of the substance. This potential is measured electrically in reference to a standard substance, H_2. A compound with a negative reduction potential is more likely to donate electrons, i.e. become oxidised. A compound with a positive reduction potential is more likely to accept electrons, i.e. become reduced. One of the most common electron acceptors of living organisms is molecular oxygen.

In catabolism, the electron donor is often referred to as the energy source. However, it is necessary to remember that it is the *coupled* oxidation-reduction (O-R) reaction which actually releases energy. The amount of energy released in a O-R reaction depends on both the nature of the electron donor and acceptor: the greater the difference between reduction potentials of the two half reactions, the more energy their will be released upon their coupling.

This can be illustrated by imagining a vertical tower, which represents the range of reduction potentials for the O-R pairs, from the most negative at the top to the most positive at the bottom (see **Figure 4** overleaf).

Referring to **Figure 4**, each box contains a half reaction. The reduced substance in the pair (i.e. that on the right hand side of the half reaction) at the top of the tower (in this case, glucose) has the greatest amount of potential energy. The reduced substance at the bottom of the tower (in this case, H_2O) has the least amount of potential energy.

The oxidised substance on the oxidation-reduction (O-R) pair at the top of the tower (CO_2) has the least tendency to accept electrons. Whereas, the oxidised substance in the pair at the bottom of the tower (oxygen) has the greatest tendency to accept electrons, i.e. is the strongest oxidising agent.

During catabolic reactions an electron donor (e.g. glucose) is oxidised, the released electrons being 'cascaded' down a series of intermediate electron accepting compounds, enabling the generation of chemical energy (ATP) (see **Figure 5** page 2.9). The released electrons eventually come to rest at the 'final or terminal electron acceptor', which accepts these electrons and is irreversibly reduced (e.g. oxygen being reduced to water).

In terms of the 'electron tower', the further the electrons drop before they are caught by the final electron acceptor, the greater the difference in the reduction potential between the electron donor and the electron acceptor, and the greater the amount of energy released from the catabolic reaction. We can see from this that the greatest amount of energy that can be generated from oxidation of glucose is when molecular oxygen is used as the final electron acceptor, and is reduced to water.
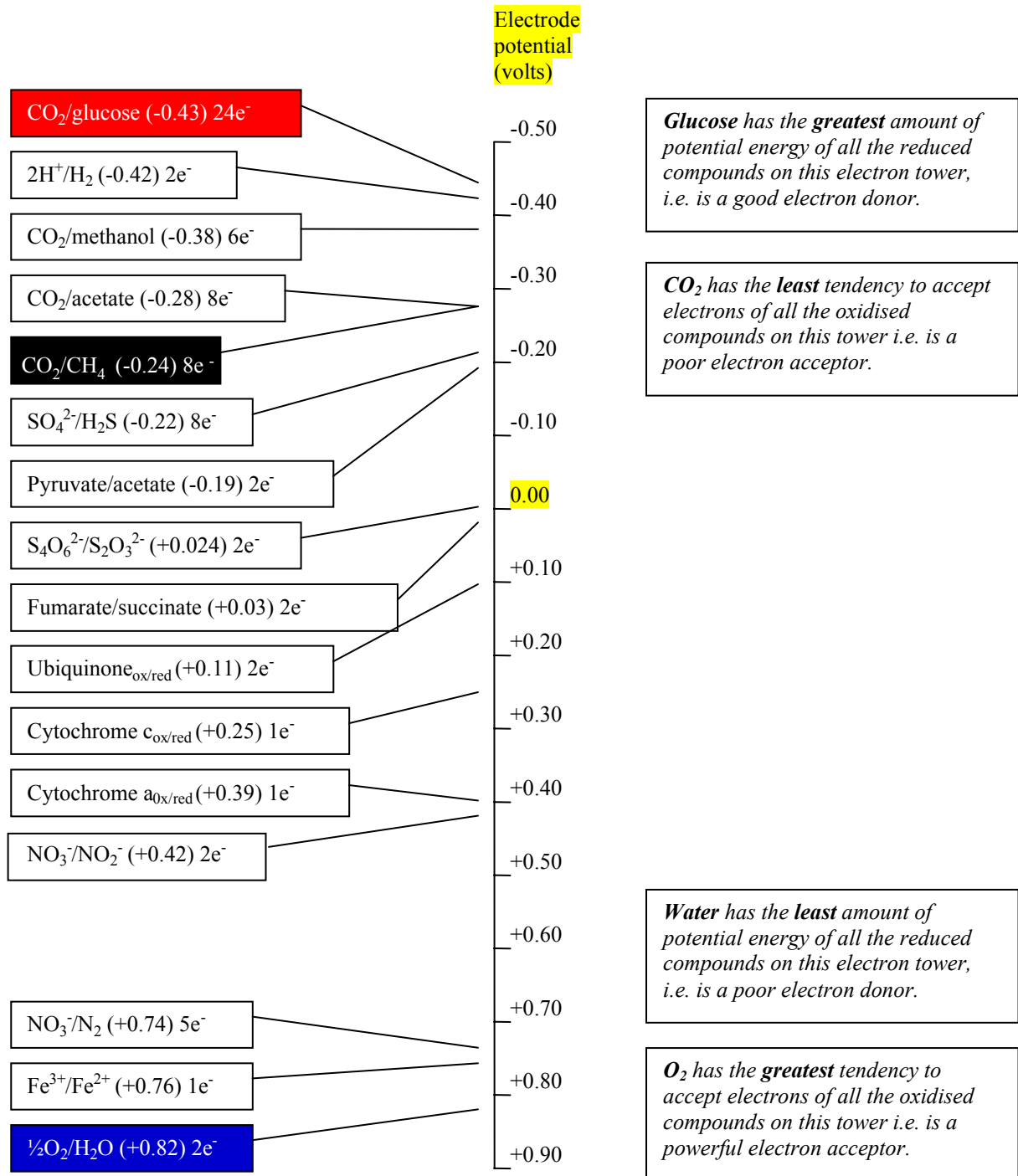
Electrode potential (volts)

Couple	Potential
CO_2/glucose (-0.43) 24e$^-$	-0.50
$2H^+$/H_2 (-0.42) 2e$^-$	-0.40
CO_2/methanol (-0.38) 6e$^-$	-0.30
CO_2/acetate (-0.28) 8e$^-$	-0.20
CO_2/CH_4 (-0.24) 8e$^-$	-0.10
SO_4^{2-}/H_2S (-0.22) 8e$^-$	0.00
Pyruvate/acetate (-0.19) 2e$^-$	+0.10
$S_4O_6^{2-}$/$S_2O_3^{2-}$ (+0.024) 2e$^-$	+0.20
Fumarate/succinate (+0.03) 2e$^-$	+0.30
Ubiquinone$_{ox/red}$ (+0.11) 2e$^-$	+0.40
Cytochrome c$_{ox/red}$ (+0.25) 1e$^-$	+0.50
Cytochrome a$_{ox/red}$ (+0.39) 1e$^-$	+0.60
NO_3^-/NO_2^- (+0.42) 2e$^-$	+0.70
NO_3^-/N_2 (+0.74) 5e$^-$	+0.80
Fe^{3+}/Fe^{2+} (+0.76) 1e$^-$	+0.90
½O_2/H_2O (+0.82) 2e$^-$	

Glucose has the **greatest** amount of potential energy of all the reduced compounds on this electron tower, i.e. is a good electron donor.

CO₂ has the **least** tendency to accept electrons of all the oxidised compounds on this tower i.e. is a poor electron acceptor.

Water has the **least** amount of potential energy of all the reduced compounds on this electron tower, i.e. is a poor electron donor.

O₂ has the **greatest** tendency to accept electrons of all the oxidised compounds on this tower i.e. is a powerful electron acceptor.
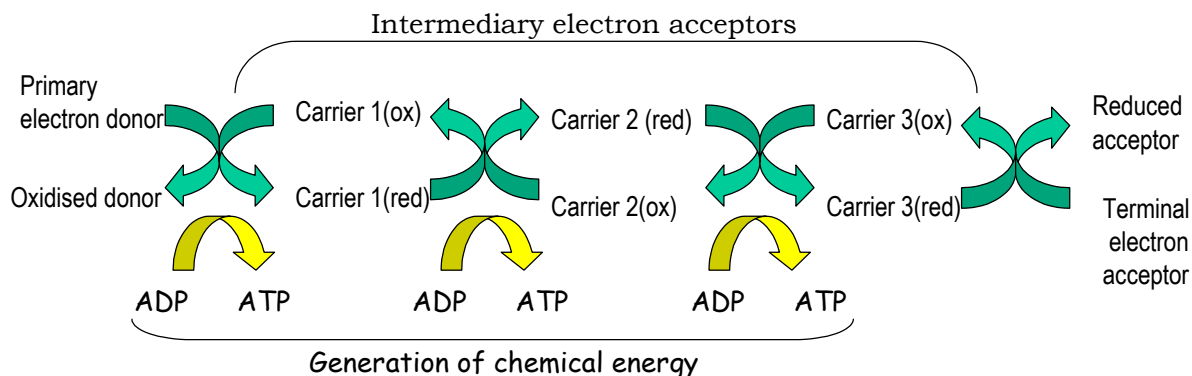
Figure 4. The Electron Tower.

Figure 5. Generation of Chemical Energy (ATP) by Electron Transport.

2.2.4   The Concept of Aerobic and Anaerobic Catabolism

This is a good point at which to introduce the concept of aerobic versus anaerobic catabolism.

In **aerobic** catabolism (referred to as respiration), molecular oxygen is always used at the final electron acceptor.   Therefore, degradation of a compound such as glucose results in complete destruction of the molecule to CO_2 and H_2O, with a large release of energy.   **Anaerobic** means in the absence of air and describes catabolic reactions that occur in the absence of molecular oxygen.

Anaerobic catabolism can be divide into two types, viz. anaerobic respiration and anaerobic fermentation.

Anaerobic respiration is essentially identical to aerobic respiration, except that compounds other than molecular oxygen are used as the final electron acceptor. Common anaerobic electron acceptors are nitrate, sulphate and acetate.   It is obvious from their position on the 'electron tower' that catabolism of glucose via anaerobic respiration is not as energy efficient as aerobic respiration.

Anaerobic fermentation involves the internal balancing of O-R reactions, with the same organic compound playing the role of electron donor and final electron acceptor. Under fermentative conditions, only partial oxidation of the carbon atoms of the electron donor occurs, therefore, only a small amount of the potential energy available is released. The rest of the energy is conserved in the end product. For example, the fermentation of fructose (fruit sugar) to alcohol releases a small amount of energy to be utilised by the yeast cell, but results in a high-energy waste product (ethanol).

2.2.5 Electron Carriers

We have been discussing the oxidation of a primary electron donor (e.g. glucose) and the coupling of this reaction to a terminal electron acceptor (e.g. oxygen). In practice, this reaction is further divided into a number of smaller steps and the transfer of electrons from one step to another is mediated by <u>intermediate</u> electron accepting compounds, or <u>electron carriers</u>. Electron carriers can be divided into two types:

1.   Those that are free in the cytoplasm.

2.   Those that are attached to enzymes in the cytoplasmic membrane.

We are concerned mainly with the freely-diffusable electron carriers, two of the most common being Nicotinamide-Adenine Dinucleotide (NAD^+) and Nicotinamide-Adenine Dinucleotide Phosphate ($NADP^+$). Generally speaking, NAD^+ is involved in catabolic reactions (e.g. alcohol production, anaerobic digestion), whereas $NADP^+$ is involved in anabolic reactions (e.g. photosynthesis). They are both hydrogen atom carriers and always transfer two hydrogen atoms to the next carrier in the chain. NAD^+ and $NADP^+$ are the oxidised forms; NADH[1] and NADPH are the reduced forms.

2.2.6 High Energy Compounds: ATP

It has been seen that energy is released during catabolism as a result of O-R reactions, and that this energy is used to fuel anabolic reactions. However, it stands to reason that these reactions do not always occur simultaneously. Therefore, the energy needs to be conserved in a chemical form, from which it will be readily available when required.

The energy released in O-R reactions is usually transferred to a variety of phosphate compounds in the form of high-energy phosphate bonds. These compounds then serve as intermediaries in the conversion of energy into useful work. That is, these compounds allow energy to be transferred from catabolic to anabolic reactions.

The most common of the high-energy phosphate compounds is Adenosine Triphosphate (ATP). The basis of the compound consists of the nucleotide, adenine and a sugar compound ribose. Phosphate groups are linked to this base compound to form Adenosine <u>Mono</u>phosphate (AMP), Adenosine <u>Di</u>phosphate (ADP) and ATP. The energy changes occur as a result of coupling and uncoupling of the phosphate groups. A large amount of energy (approx. 30 KJ/mol) is required to couple one phosphate group. Consequently, when one of these phosphate groups is removed, the energy is available to be utilised in a reaction. ATP is the high-energy form of the compound (containing three phosphate groups) (see **Figure ?** in the Photosynthesis section). As ATP provides a common energy link between catabolic and anabolic reactions it is sometimes referred to as the **common intermediate**.

---

[1] **NOTE**. NADH and NADPH can also be written as $NADH_2$ and $NADPH_2$. The latter method is more correct, as each carrier always transports *two* H atoms, however, the first form (as written in your notes) is more commonly used. Either is acceptable.

A common intermediate is a compound that is the product of an initial chemical reaction but forms the substrate of another reaction:

A + B $\rightarrow$ C + "ATP" (i.e. an energy-yielding reaction, exergonic).

"ATP" + E $\rightarrow$ F + G (i.e. an energy-requiring reaction, endergonic).

The principle of a common intermediate is the basis of all biological energy transfers.

Energy in the form of ATP can be stored in the cell for short periods of time. For long term storage of energy, high energy compounds such as fats or cellulose are used.