## Microbial fuel cell

Fuel cells are a novel addition to the inventory of alternate energy sources having minimal or no net-$CO_2$ emission. Electricity production using microbial cultures was first reported early in the last century [1]. Microbial Fuel Cells (MFCs) have been described as "bioreactors that convert the energy in the chemical bonds of organic compounds into electrical energy through catalytic activity of micro-organisms under anaerobic conditions" [2]. MFC technology represents a novel approach of using bacteria for generation of bioelectricity by oxidation of organic waste and renewable biomass [3].

The operational and functional advantages of MFCs are:

- MFCs use organic waste matter as fuels and readily available microbes as catalysts.
- MFCs do not require highly regulated distribution systems like the ones needed for Hydrogen Fuel Cells.
- MFCs have high conversion efficiency as compared to Enzymatic Fuel Cells, in harvesting up to 90% of the electrons from the bacterial electron transport system.

MFC technology represents a multi-disciplinary approach to the quest for alternate sources of energy. It symbolizes the confluence of the chemical-, physical- and life-sciences and is a meeting point of basic and applied research. The principle of working of MFCs is based on the tenets of microbial physiology coupled with electrochemistry. Structural design of MFCs brings the nuances of electrical and materials engineering to the fore. The applications of this technology come under the ambit of environmental engineering and bioremediation. MFC technology is thus multi-disciplinary, in the true sense of the term and provides scope for strengthening research across disciplines.

### Biochemical basis

How do microbes derive nutrition for their sustenance? Chemotrophic microbes utilize organic and other biodegradable compounds, under diverse conditions. The electrons resulting from the oxidation are conveyed to an electron transport chain, across appropriate electron carriers depending on the terminal electron acceptor molecule. In aerobic organisms, this terminal acceptor is oxygen which takes up the electrons and gets reduced to water.

The chemiosmotic hypothesis states that electron transfer chains of bacteria are coupled to the translocation of protons across the membranes which is in turn linked to ATP synthesis by the proton electrochemical potential across the energy transducing membrane [4].

The bacterial cell membrane functions as an energy transducing membrane operating according to the chemiosmotic principle. The translocation of protons towards the outside of the membrane results in the establishment of a proton electrochemical gradient. The pH gradient adds up to this membrane potential and results in the proton motive force. The re-entry of these protons across the ATP-synthase enzyme is accompanied by ATP synthesis. The ATP synthesised thus is used by the bacteria for their survival [5].

Keeping this theoretical background in mind let us now see how an MFC functions. A typical MFC (see Fig. 1) consists of two compartments - the anodic and cathodic half-cells - which are separated by a selectively permeable, cation-specific membrane or a salt-bridge. The anodic chamber consists of microbes suspended under anaerobic conditions in the anolyte and the

cathodic chamber contains the electron acceptor (oxygen). In essence, the electron donor is physically separated from the terminal electron acceptor across the two chambers. Most of the electrons released from the process of oxidation are conveyed to the anode. Electron transfer to the anode can be accomplished by electron mediators or shuttling agents [6], directly by the cell [7] or by means of 'nanowires' [8]. These electrons are directed to the cathode across an external circuit and for every electron conducted, a proton is transported across the membrane to the cathode for completing the reaction and sustaining the electric current [9].

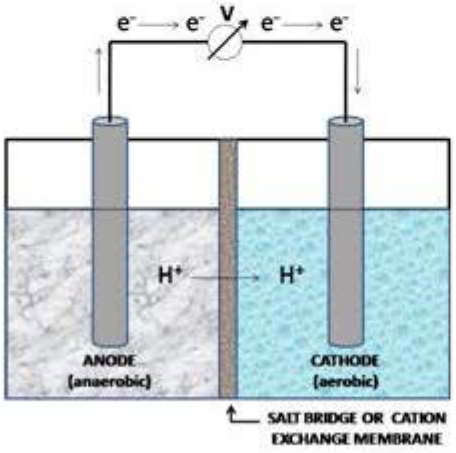


**Fig. 1:** Schematic of a simple two-chambered Microbial Fuel Cell
It must however be noted at this juncture, that MFCs can be constructed in different shapes and designs, a description of which is beyond the scope of this article.

## Applications of microbial fuel cell technology

Although MFCs have been studied as an alternative energy source, their application is presently limited to certain niche areas. With further improvements in design, cost effectiveness and performance efficiency based on these near-term applications, it would be possible to scale-up and use MFCs as a renewable energy resource.

*Wastewater treatment*

Micro-organisms can perform the dual duty of degrading effluents and generating power. MFCs are presently under serious consideration as devices to produce electrical power in the course of treatment of industrial, agricultural, and municipal wastewater. When micro-organisms oxidize organic compounds present in waste water, electrons are released yielding a steady source of electrical current. If power generation in these systems can be increased, MFCs may provide a new method to offset operating costs of waste water treatment plants, making advanced waste water treatment more affordable in both developing and industrialized nations [10]. In addition, MFCs are also known to generate less excess sludge as compared to the aerobic treatment process [11].

***Powering underwater monitoring devices***

Data on the natural environment can be helpful in understanding and modelling ecosystem responses, but sensors distributed in the natural environment require power for operation. MFCs can possibly be used to power such devices, particularly in river and deep-water

environments where it is difficult to routinely access the system to replace batteries. Sediment fuel cells are being developed to monitor environmental systems such as creeks, rivers, and oceans [12]. Power densities are low in sediment fuel cells because of both the low organic matter concentrations and their high intrinsic internal resistance. However, the low power density can be offset by energy storage systems that release data in bursts to central sensors [13].

***Power supply to remote sensors***

With the development of micro-electronics and related disciplines the power requirement for electronic devices has drastically reduced. Typically, batteries are used to power chemical sensors and telemetry systems, but in some applications replacing batteries on a regular basis can be costly, time-consuming, and impractical. A possible solution to this problem is to use self-renewable power supplies, such as MFCs, which can operate for a long time using local resources. Extensive research toward developing reliable MFCs to this effect, is focused mostly on selecting suitable organic and inorganic substances that could be used as sources of energy [14].

***BOD sensing***

Another potential application of the MFC technology is to use it as a sensor for pollutant analysis and *in situ* process monitoring and control. Biological Oxygen Demand (BOD) is the amount of dissolved oxygen required to meet the metabolic needs of aerobic organisms in water rich in organic matter, such as sewage. The proportional correlation between the columbic yield of MFCs and the concentration of assailable organic contaminants in wastewater make MFCs possible usable as BOD sensors. An MFC-type BOD sensor can be kept operational for over 5 years without extra maintenance, far longer in service life span than other types of BOD sensors reported in the literature [3].

***Hydrogen production***

Hydrogen production by modified MFCs operating on organic waste may be an interesting alternative. In such devices, anaerobic conditions are maintained in the cathode chamber and additional voltage of around 0.25 V is applied to the cathode. Under such conditions, protons are reduced to hydrogen on the cathode. Such modified MFCs are termed bio-electrochemically assisted microbial reactors (BEAMR) [15].

**Conclusion**

Though this technology is quite promising as a source of renewable energy, it will be some time before large-scale, highly efficient MFCs enter the commercial scene. The different research groups working around the world will definitely overcome the shortcomings being faced today, enthused and motivated by the immediate need for alternate energy.

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# Membrane potential

A resting membrane potential is the difference between the electric potential in the intracellular and extracellular matrices of the cell when it isn't excited. Every cell of the body has its own membrane potential, but only excitable cells - nerves and muscles - are capable to change it and generate an action potential.

For this reason, membrane potential for excitable cells when they are not excited is called the resting membrane potential, while its changes are associated with an action potential.

## Definition

Resting membrane potential (EM) originates from the different concentrations of ions (expressed in mmol/l) at the inner and outer surface of the cell membrane. There are four excitable tissues in our body, and all of them have different EM values:

- Skeletal muscle cell = -90 millivolts (mV)
- Smooth muscle cell = -55mV
- Cardiac muscle cell = -80mV
- Neuron = -65mV

The negative values indicate that the cytoplasm is more electronegative than the extracellular space. The values of EM depend on several factors:

- Concentration of ions inside and outside the cell. Ions that contribute the most are the sodium, potassium, calcium, and chloride ions.
- Activity of the sodium-potassium pump.
- Variable permeability of the cell membrane for ions.

## Ions

There are many ions in the cell and extracellular space, but not all of them can pass through the cell membrane. Those who can, are called diffusible ions (sodium, potassium, calcium, and chloride), and those who can't are non-diffusible ions (proteins). Nonetheless, both groups of ions contribute to membrane potential. Why? Ions are chemical elements that carry electricity, some positive (+) and some negative (-). Usually, there are more negative ions inside the cell than outside, which is why the EM has the negative values. This negativity is mostly due to non-diffusible proteins (-).

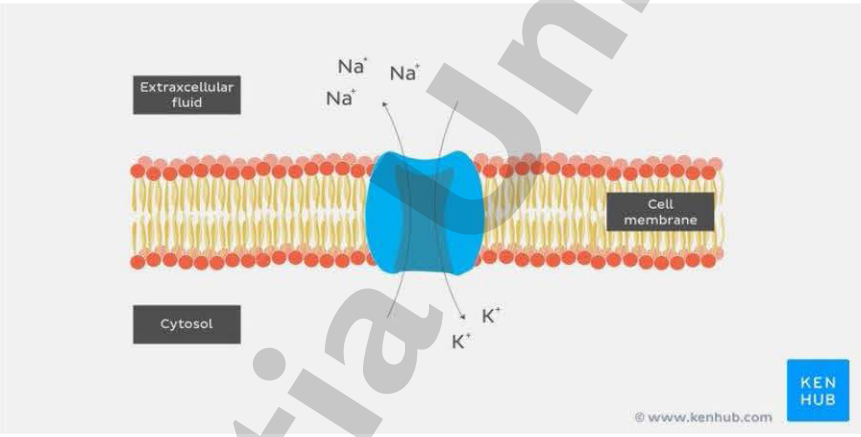
Diffusible ions are responsible for the change of the membrane potential. During action potential, a redistribution of the ions occurs, where large amounts of sodium (+) enter the cell, making the membrane potential less negative and closer to the threshold for the action potential.

| Distribution of ions | |
|---|---|
| Intracellular space | Sodium = 14 mmol/l<br>Potassium = 140 mmol/l |

| | |
|---|---|
| | Calcium = 0.0001 mmol/l<br>Chloride = 5 mmol/l |
| Extracellular space | Sodium = 142 mmol/l<br>Potassium = 4-5 mmol/l<br>Calcium = 2.5 mmol/l<br>Chloride = 103 mmol/l |

**Sodium-potassium pump (Na-K pump)**

Another factor that controls membrane potential is the Na(+)-K(+) pump. This pump uses energy to expel 3 molecules of sodium in exchange for 2 molecules of potassium. This is important because this pump creates concentration gradients for sodium and potassium, allowing more sodium in the extracellular space, and more potassium in the intracellular space.




The concentration gradient will later contribute to generating an action potential, because of one of the laws of physics. By concentration gradient definition, every element modifies its concentration gradient to seek equilibrium. For example, ions will diffuse from a place of higher concentration to a place of lower concentration until the concentration of the element is equal on both sides. This means that the sodium will diffuse from extra- to intracellular space, and the potassium will do the opposite. More about this process can be found in the action potential article.

**Cell membrane permeability**

The third factor that affects the membrane potential is the permeability of the membrane for the sodium and potassium, which depends on the ion channels. Ion channels are specialized proteins of the cell membrane that enable migration of the ions. There are two types of ion channels:

- Passive channels – which are the pores within the cell membrane, through which the molecules pass depending on their concentration gradient.
- Active channels – which open and allow the ion transport either depending on the change of the membrane potential (potential-gated channels), or after binding of some other protein (ligand-gated channels), or after mechanical stimulation.

Pores contribute to establishing resting membrane potential, and they are found along the entire excitable cell membrane. When the cell isn't excited, diffusion of ions occurs only through the pores. Note that during rest, a lot more potassium pores are open than for the sodium. For this, the potassium efflux is larger than the sodium influx, which contributes to maintaining the negativity of the intracellular space and EM.

Ligand-gated channels are located near the synapses and are responsible for local hypo- or hyperpolarization of the cell after the neurotransmitter binds to them. Potential-gated channels are responsible for generation and propagation of an action potential, which eventually causes the release of a neurotransmitter. They are found in the membranes of axons and axon terminals.

**Equilibrium potential**

From the aspect of concentration gradient, we would expect that all diffusible ions pass through the cell membrane until their concentrations are equal from both sides. But still, that doesn't happen. Why? There is another physical component in this entire process which opposes to the concentration gradient, called the electric gradient, that works similar to a magnet.

Let's take *potassium* as an example. Intracellular concentration of potassium is 140 mmol/l, while the extracellular is 4-5 mmol/l. We would expect that the potassium diffuses outside of the cell until there are around 70 mmol/l of potassium from both sides of the membrane. But, since potassium is a positive ion (+), its efflux increases the positivity of the extracellular space, and increases the negativity of the intracellular space. This leads to the point where the extracellular space is positive enough to repel the potassium, and the intracellular space becomes negative enough to attract the positive potassium. This point is called the electrochemical equilibrium. Physiologists calculated the value of the EM when the potassium cannot diffuse out of the cell anymore, and it is -94 mV.

Now, let's look at the *sodium*, which is also a positive ion. Because of the concentration gradient, the sodium tends to influx into the cell. At some point, the cell becomes electropositive enough to repel the new sodium ions, and thus opposes the sodium concentration gradient, reaching the electrochemical equilibrium. The value of electropositivity that stops the sodium influx is +61 mV.

As we mentioned earlier, potassium diffusion mostly affects the resting membrane potential. On the other hand, the sodium diffusion is massive during an action potential. This implicates two things:

- Membrane potential cannot be more negative than -94 mV
- Membrane potential cannot be more positive than +61 mV

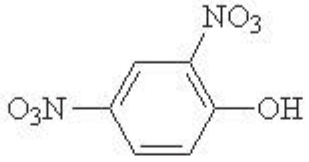
## The Chemiosmotic Hypothesis

The chemiosmotic hypothesis was proposed by *Peter Mitchell*. This hypothesis stated that a proton-motive force was responsible for driving the synthesis of ATP. In this hypothesis, protons would be pumped across the inner mitochondrial membrane as electrons went through the electron transfer chain. This would result in a *proton gradient* with an lower pH in the intermembrane space and a elevated pH in the matrix of the mitochondria. An intact inner mitochondrial membrane, impermeable to protons, is a requirement of such a model. The proton gradient and membrane potential are the proton-motive force that is used to drive ATP synthesis. In effect, the pH gradient acts as a *"battery"* which stores energy to produce ATP. Over the past several years, Mitchell's chemiosmotic hypothesis has been widely accepted as the mechanism of coupling of electron transport and ATP synthesis. He was awarded the Nobel Prize in Chemistry in 1978. This acceptance by the scientific community is a result of accumulating experimental evidence supporting the hypothesis.

Some of the evidence supporting Mitchell's chemiosmotic hypothesis is as follows.

1. Electron transport generates a proton gradient. The pH measured on the outside is lower than that measured inside the mitochondria.
2. Only a proton gradient is needed to synthesize ATP. Electron transport is *not* required as long as there is another mechanism for generating a pH gradient.
3. A reconstitution experiment carried out by Racker & Stoeckenius (J Biol Chem 1974 Jan 25;249(2):662-3, Reconstitution of purple membrane vesicles catalyzing light-driven proton uptake and adenosine triphosphate formation) showed that the generation of a proton gradient can result in ATP synthesis in a totally artificial system. In their experiment, a mitochondrial ATPase complex from beef heart was inserted into an artificial lipid bilayer. Also inserted in this bilayer was a membrane fragment containing the protein, bacteriorhodopsin* [pic], from the purple bacteria *Halobacterium*, so called because the bacteriorhodopsin gives the membrane a purple color. Bacteriorhodopsin is a light-driven proton pump. Therefore, shining light on this artificial "purple membrane" formed a proton gradient, which was used by the beef heart mitochondrial ATPase to synthesize ATP.

The electron transfers chains and the ATPases are asymmetrically oriented in the inner mitochondrial membrane. An asymmetric orientation is a requirement to establish a pH gradient. A random arrangement would not result in a net gradient of protons and therefore, no proton-motive force for the synthesis of ATP. Compounds called *uncouplers* were found to collapse the pH gradient by shuttling protons back across the membrane through the compounds. One such uncoupler, *dinitophenol* is shown below. In the presence of the uncoupler electron transport continues, but no ATP synthesis occurs. Such uncoupling can generate an energetically wasteful byproduct, heat. This occurs normally in many in hibernating animals, in new-born humans, and in mammals adapted to the cold. It occurs in a specialized tissue known as brown adipose tissue. An uncoupling protein called thermogenin can accomplish this uncoupling and thus allow heat to be generated.




2,4-dinitrophenol
DNP

## Subcellular fractionation methods

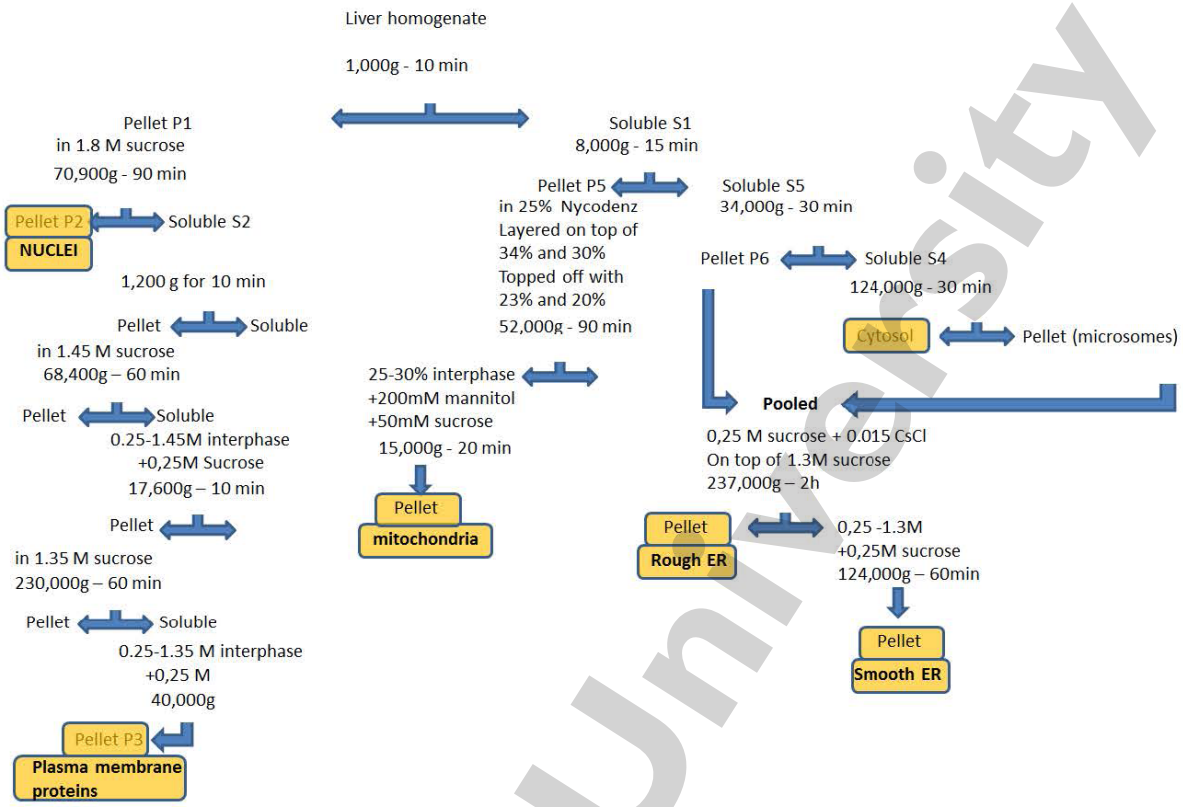
Separation of cellular compartments from one another is an important step for studying a specific intracellular structure or organelle or protein, or to assess possible associations between these macromolecular structures. Subcellular fractionation uses one or more of the properties of each compartment, such as buoyant density, surface charge density, size and shape, and is mainly based on differential centrifugation in media of high viscosity at 4°C. Media used for differential centrifugation are mainly sucrose, mannitol, glycerol, Ficoll 400 (a polymer of sucrose), Percoll (a type of colloidal silica) and iodixanol (OptiPrep, e.g., Hela or THP1 cell fractionation for cGAS activity assay). Sucrose is widely used because it is inexpensive. Mainly these methods will be discussed here, with a preference for the ones that are easily accessible to most labs and are less time-consuming, as speedy recovery is vital. Gel filtration, affinity chromatography, electrophoresis or selective density-shift perturbation can also be used. Variations in the conditions of the available protocols are dependent on the organelle, tissue or cell type and equipment used, and it is highly recommended to read the cited references for full details of each procedure. In the end, the purity and the yield of the fractionation should be assessed by detection of distinct markers in each collected fraction during the entire procedure.

**Isolation of mitochondria by differential centrifugation.** The procedure is as follows

1. Cell pellets, grown as a monolayer or in suspension, are homogenized in a homogenization buffer containing $MgCl_2$ and KCl. Later, sucrose is added up to 0.25 M and the nuclei are pelleted by low-speed centrifugation (1000 g). Second centrifugation of the supernatant at 5000 g will sediment the mitochondria. The pellet is re-suspended in a medium containing sucrose and $Mg^{+2}$ and is subjected to a few gentle strokes in a Dounce homogenizer. The last centrifugation step at 5000 g will enrich mitochondria which can be re-suspended in Tris buffer containing 0.25 M sucrose or in the buffer of preference for subsequent analyses (e.g., Laemmli).

2. Yeast cells are treated with zymolase to break the hard outer wall and produce spheroplasts, which are washed in a sorbitol buffer. The pellet is re-suspended in homogenization buffer containing 0.6 M mannitol and cells are lysed by a few strokes in a Dounce homogenizer. Nuclei are removed by low-speed centrifugation, while the cytoplasm-containing supernatant is centrifuged in a fixed-angle rotor at 6500 g to pellet mitochondria.

Additionally, there are protocols that utilize density-gradient separations which provide purer mitochondrial fractions, but they are more time-consuming and are avoided. Despite the "contamination" by lysosomes and peroxisomes in fractions obtained by the differential centrifugations, they are the method of choice. Therefore, the desired purity determines which the most suitable method is. For example, if metabolic studies are of interest, differential centrifugation is preferred; alternatively, if the exact localization of a protein is under investigation or samples of the purest form are a necessity, e.g., in proteomics, the density-gradient preparations are more suitable.



# Buffer Solution

Buffer Solution is a water solvent based solution which consists of a mixture containing a weak acid and the conjugate base of the weak acid, or a weak base and the conjugate acid of the weak base. They resist a change in pH upon dilution or upon the addition of small amounts of acid/alkali to them.

The pH of Buffer Solutions shows minimal change upon the addition of a very small quantity of strong acid or strong base. They are therefore used to keep the pH at a constant value.

## What is Buffer Solution?

The buffer solution is a solution able to maintain its Hydrogen ion concentration (pH) with only minor changes on the dilution or addition of a small amount of either acid or base. Buffer Solutions are used in fermentation, food preservatives, drug delivery, electroplating, printing, the activity of enzymes, blood oxygen carrying capacity need specific hydrogen ion concentration (pH).

Solutions of a weak acid and its conjugate base or weak base and its conjugate acid are able to maintain pH and are buffer solutions.

## Types of Buffer Solution

The two primary types into which buffer solutions are broadly classified into are acidic and alkaline buffers.

### Acidic Buffers

As the name suggests, these solutions are used to maintain acidic environments. Acid buffer has acidic pH and is prepared by mixing a weak acid and its salt with a strong base. An aqueous solution of an equal concentration of acetic acid and sodium acetate has a pH of 4.74.

- pH of these solutions is below seven
- These solutions consist of a weak acid and a salt of a weak acid.
- An example of an acidic buffer solution is a mixture of sodium acetate and acetic acid (pH = 4.75).

### Alkaline Buffers

These buffer solutions are used to maintain basic conditions. Basic buffer has a basic pH and is prepared by mixing a weak base and its salt with strong acid. The aqueous solution of an equal concentration of ammonium hydroxide and ammonium chloride has a pH of 9.25.

- The pH of these solutions is above seven
- They contain a weak base and a salt of the weak base.
- An example of an alkaline buffer solution is a mixture of ammonium hydroxide and ammonium chloride (pH = 9.25).

## Mechanism of Buffering Action

In solution, the salt is completely ionized and the weak acid is partly ionized.

- $CH_3COONa \rightleftharpoons Na^+ + CH_3COO^-$
- $CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$

## On Addition of Acid and Base

**1.** On addition of acid, the released protons of acid will be removed by the acetate ions to form an acetic acid molecule.

$$H^+ + CH_3COO^- \text{ (from added acid)} \rightleftharpoons CH_3COOH \text{ (from buffer solution)}$$

**2.** On addition of the base, the hydroxide released by the base will be removed by the hydrogen ions to form water.

$$HO^- + H^+ \text{ (from added base)} \rightleftharpoons H_2O \text{ (from buffer solution)}$$

## Preparation of Buffer Solution

If the dissociation constant of the acid ($pK_a$) and of the base ($pK_b$) are known, a buffer solution can be prepared by controlling the salt-acid or the salt-base ratio.

As discussed earlier, these solutions are prepared by mixing the weak bases with their corresponding conjugate acids, or by mixing weak acids with their corresponding conjugate bases.

An example of this method of preparing buffer solutions can be given by the preparation of a phosphate buffer by mixing $HPO_4^{2-}$ and $H_2PO^{4-}$. The pH maintained by this solution is 7.4.

Henderson-Hasselbalch equation may be used to prepare the buffer solution.

# USE OF OXYGEN ELECTRODE IN MEASUREMENTS OF PHOTOSYNTHESIS AND RESPIRATION

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## INTRODUCTION

In order to grow, plants must be able to convert the energy from the sun into a useful form (photosynthetic process). In higher plants the final products of photosynthesis are sucrose and starch or fructans. Sucrose is the major form into which carbon from carbon dioxide is assimilated for transport throughout the plant, while starch and fructans are the major forms in which carbon is stored. Oxygen is produced during photochemistry process in photosynthesis. The oxidation of photosynthates is converted to pyruvate or malate to produce energy or carbon skeletons for biosynthesis coupled with oxygen consumption (Bryce and Hill, 1993).

Quantification of oxygen release in presence of light and oxygen consumption in absence of light by a plant is a good approach to measure photosynthesis and respiration (Hall and Rao, 1999).

Photosynthesis and respiration constitute two coupled physiological processes (Figure 1). Photosynthesis is essential to maintain all the life forms in the Earth and respiration is an anabolic process that consumes the products of the photosynthesis. So that, it is very important in plant ecophysiology to know the relationship between photosynthesis and respiration, because these parameters are frequently affected by changes in the plant environment (Bazzaz, 1996).

The measurement of oxygen evolution, uptake and liberation, during a reaction in a closed system is one of the easiest (and cheapest!) ways of following the processes of photosynthesis and respiration in plants. The oxygen electrode works based on the principle of Clark; that is, using a polarographic measurement of the electricity that flows between an anode and a cathode and it is sensitive enough to detect oxygen concentrations in the order of 0.01 mmol.

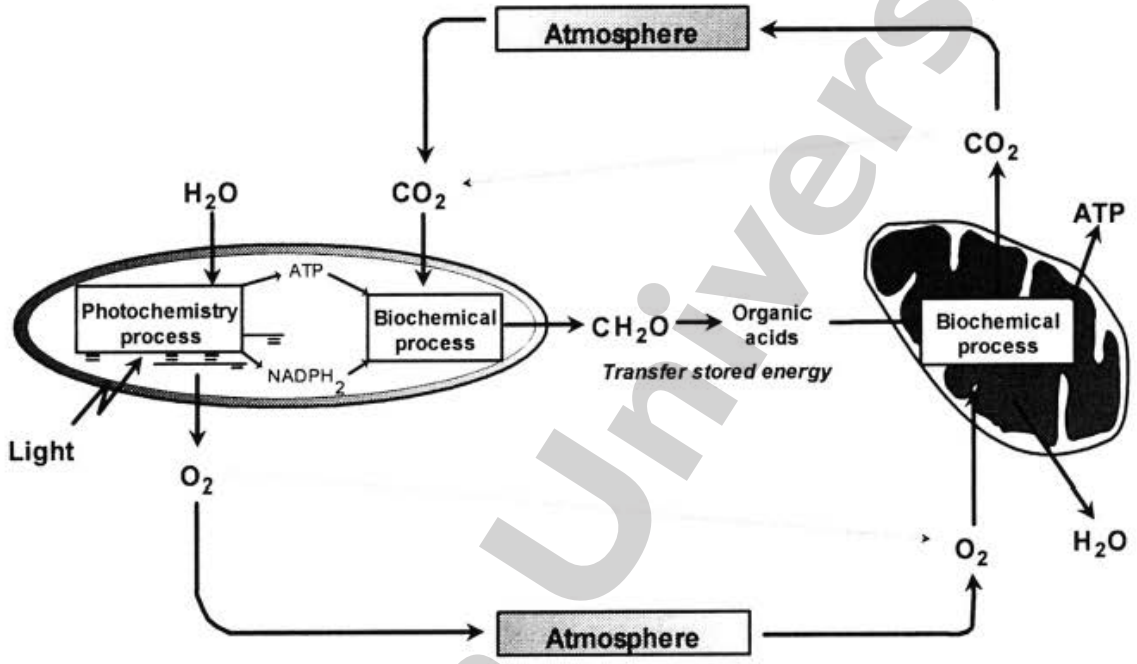


*Figure 1.* Coupling of the physiological processes photosynthesis and respiration.

This electrode consists on a cell which contains a circular silver wire anode and a platinum wire cathode, bathed in a saturated potassium chloride (KCI) solution. The electrodes are separated from the reaction mixture by an 02 gas-permeable teflon membrane. The reaction mixture in the appropriate container is stirred constantly with a small magnetic stirring rod (liffle flea). A voltage applied across the two electrodes undergoes oxygen electrolytic reduction in the solution (Figure 2).

The sample inside the reaction chamber maintains a balance between photosynthesis and respiration releasing and taking oxygen. Any variation in concentration is reflected in the electrolyte, as a consequence of this and related directly, the electric current between cathode and anode also changes.

Variation in the oxygen concentration inside the chamber is measured by connecting the electrode to a recorder throughout a control box. Taking into account that oxygen solubility is temperature dependent, the whole electrode and reaction chamber is kept to a constant temperature by mean of a water jacket attached to a temperature controlled water flow (Figure 3).

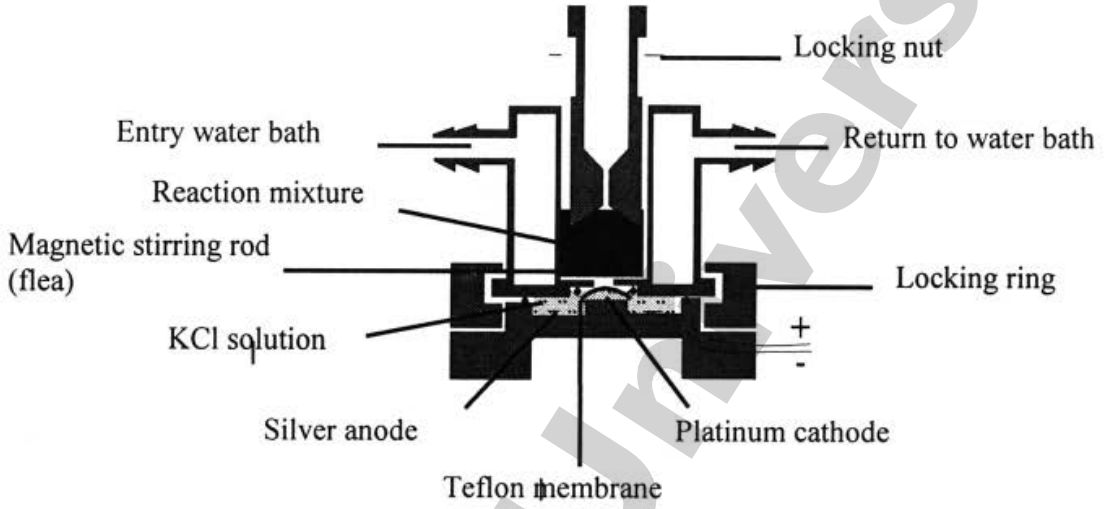


*Figure 2*. Oxygen electrode components

The basic Clark-type electrode is generally used to measure oxygen exchanges activities of isolated chloroplasts and of algal and cyanobacterial cells in the laboratory. Modified versions of the electrode can be used to measure oxygen evolution from seedlings and leaves and linked with other apparatus like a light controlled source or a pulse modulated chlorophyll fluorescence instrument, it will allow us more detailed study about photosynthesis.

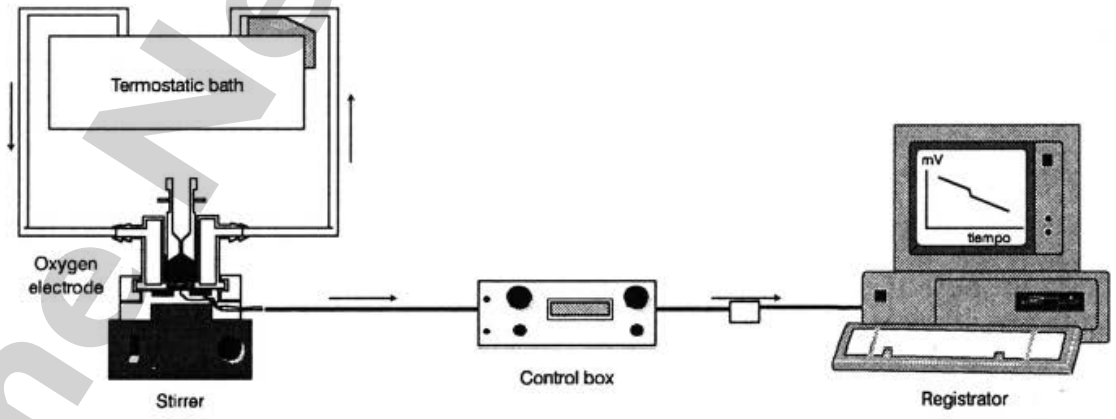


*Figure 3.* Measurement equipment associated to the oxygen electrode

# PHYSIOLOGICAL ASPECTS

Photosynthesis, and respiration are two physiological processes that we can measure by oxygen quantification. Biological oxidation can be seen as a reversal of the photosynthesis process. (Figure 4).

The photosynthesis pigment (P680) of photosystem II (PSII) is a pair of two chlorophyll molecules ([Chl a]$_2$) Their excitation by excitons results in a charge separation. An electron is transferred from the chlorophyll pair to plastoquinone (PQ). This plastoquinone accepts two electrons one after the other and is thus reduced to hydroquinone ($PQH_2$) (Heldt, 1997).

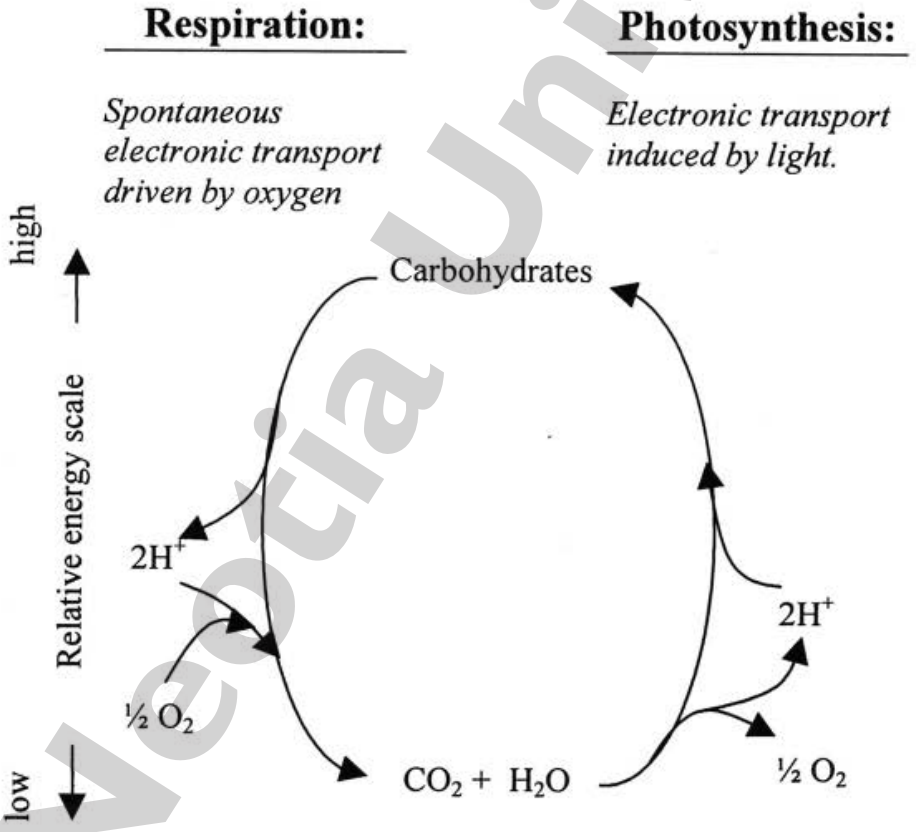


*Figure 4.* Whole electronic circulation in the metabolic process.

This molecule may be regarded as the final product of PS II. The electron deficit in [Chl $a$]$_2^+$ is compensated for by a cluster of four manganese atoms involved in the oxidation of water and they are derived to the positively charged chlorophyll radical by mean of a tyrosine residue (Figure 5).

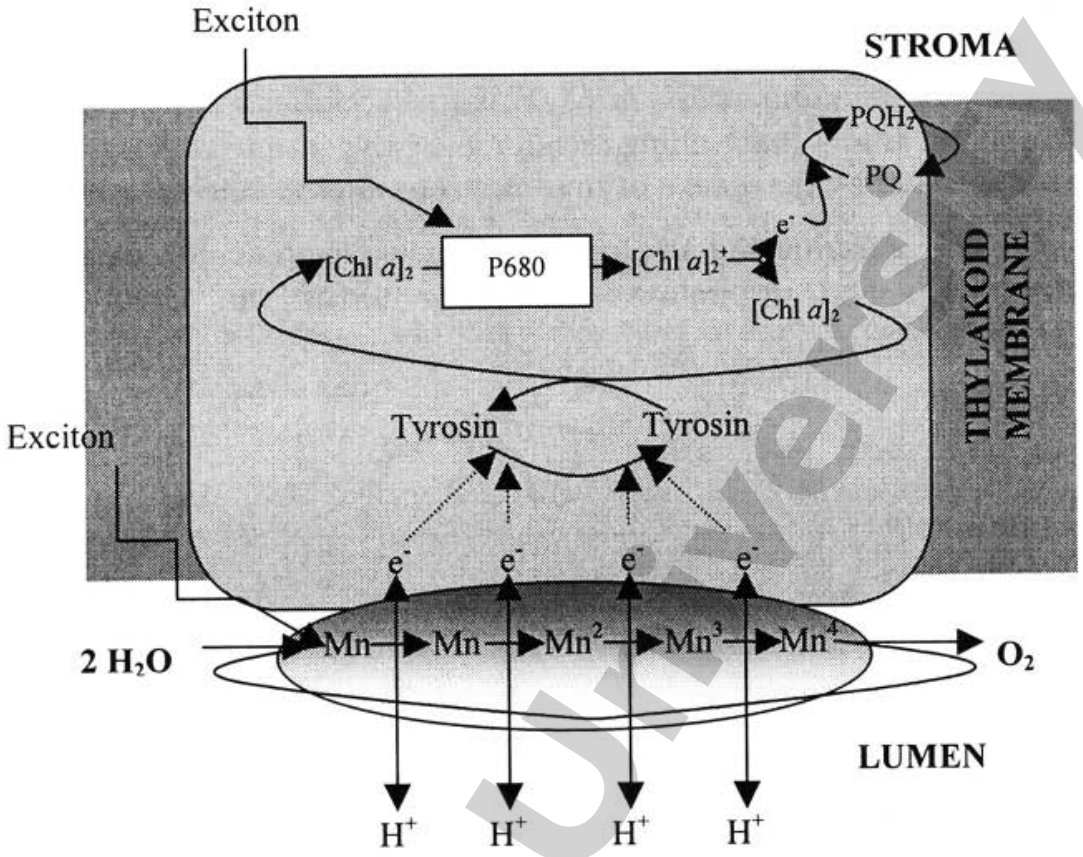


*Figure 5* Scheme of photosynthetic electron transport in the photosystem II complex where the oxygen site production is shown.

To liberate one molecule of oxygen from water the reaction centres must withdraw four electrons and thus capture four excitons. The watersplitting machinery of the Mn clusters minimizes the formation of oxygen radical intermediates, especially at low light intensities (Heldt, 1997).

The oxidation of the NADH formed from degradation of substrates in the mitochondria matrix to NAD+ provides two protons and two electrons to complex I and feeds the respiratory chain with the electrons. Via ubiquinone the electrons are passed to complex III (the cytochrome-b/c complex). Ubiquinone reduced by the NADH dehydrogenase complex or succinate dehydrogenase is oxidised by the cytochrome-b/c complex. Cytochrome-c is a mobile electron carrier. Due to its positive charge, reduced cyt-c diffuses along the negatively charged surface of the inner membrane to the complex IV (cytochrome-c oxidase, or cytochrome-a/a3 complex) (Buchanan et al., 2000).

This is the terminal complex of the electron transport chain and reduces oxygen to water on the matrix side of the membrane. Cytochrome-a, in the complex, receives electrons from cytochrome-c and passes them to cytochrome $a_3$ with a Fe atom and a Cu atom bound to histidine.

Sixth coordination position of the Fe atom is not saturated by an amino acid of the protein (Figure 6). This free coordination position, and the Cu atom, forms the binding site for the oxygen molecule, which is reduced to water by the uptake of four electrons (Heldt, 1997).

We will describe the use of the oxygen electrode for measuring photosynthesis and respiration in *Lactuca sativa* var. Great Lakes seedlings.

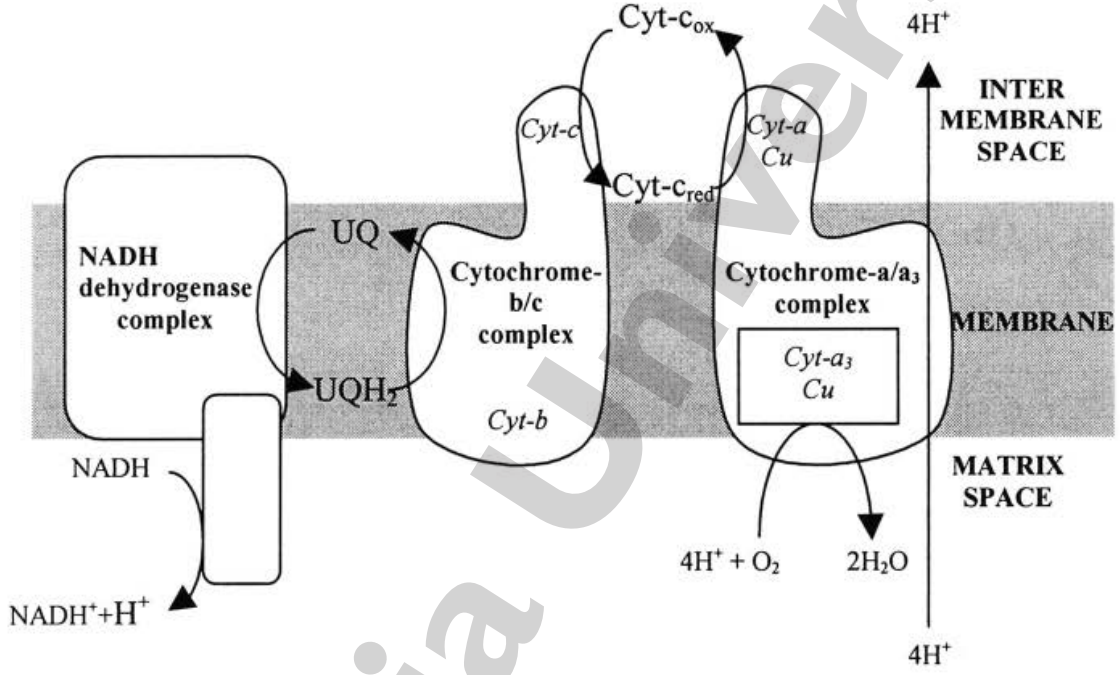


*Figure 6* Scheme of the respiratory chain complexes in the mitochondrial inner membrane where the oxygen site consumption is shown.

## MEASURE OF OXYGEN EVOLUTION

During photosynthesis, light energy is absorbed by chlorophyll with oxygen production while, during respiration, this molecule is consumed.

Accordingly, if a leaf, seedling or an algae suspension is enclosed in a chamber, provided with carbon dioxide (or bicarbonate as a source of $GO_2$) and then, illuminated or not, oxygen concentration will change. A 'Clark-type' electrode is able to detect polarographically the oxygen changes due to accumulation or consumption during photosynthesis or respiration in the chamber. Usually, Clark-type electrodes comprise a platinum cathode and a silver anode immersed in, and linked by an electrolyte (potassium chloride).

A thin teflon or polythene membrane which is permeable to oxygen and a piece of paper (beneath the membrane) are usually placed over the electrodes surface (Figure 7) in order to protect them and to provide a uniform layer of electrolyte between both anode and cathode.

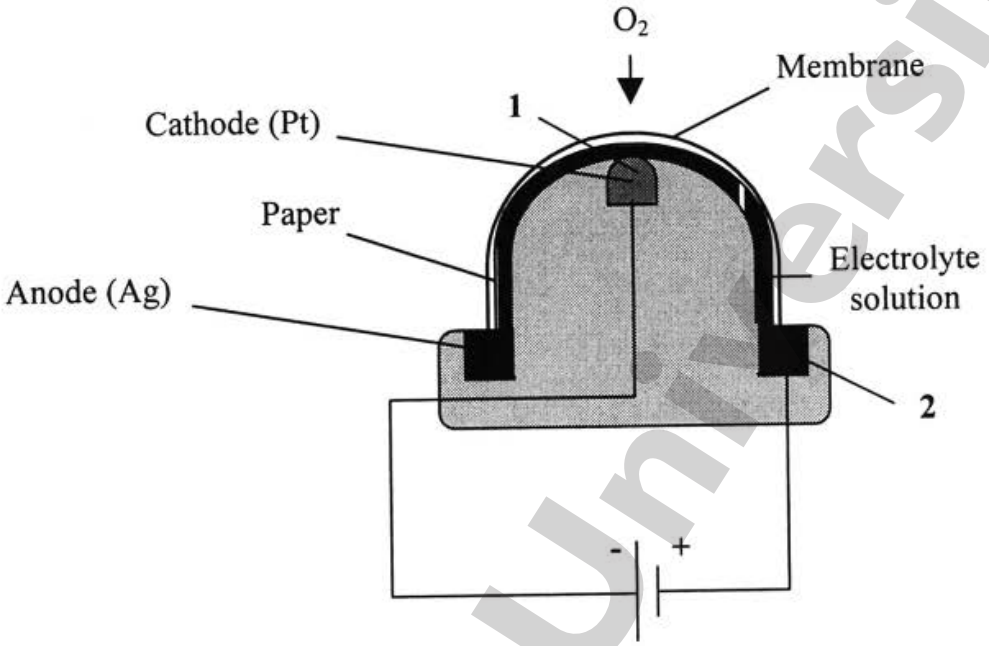


*Figure 7* Scheme of the electrodes dome and place of oxygen electrode reactions.

1.- $O_2+H_2O + 2e^- \rightarrow H_2O_2 + 2OH^-$; $H_2O_2 + 2e^- \rightarrow 2OH^-$

2.- $4Ag \rightarrow 4Ag^+ + 4e^-$; $4Ag^+ + 4Cl^- \rightarrow 4AgCl$

When a small voltage is applied across these electrodes (0.6-0.7 V), so that the platinum is made negative with respect to the silver, the current which flows is at first negligible and the platinum becomes polarised. Oxygen diffuses through the membrane and is reduced at the platinum surface, initially to hydrogen peroxide ($H_2O_2$). A thin layer of KCl solution closes the current circuit. The silver is oxidised and silver chloride deposited on the anode. The electrical current generated, by the reduction of oxygen at the cathode, is stoichiometrically related to the oxygen consumed and converted to a voltage output signal by a control box. The output signal is large enough to be monitored on a computer where the data are transformed in µmol $O_2$ min 1 g $(DW)^{-1}$.

As explained above oxygen solubility is temperature dependent and then the reaction chamber may be kept to constant temperature during the experiment and the final results corrected in function of this temperature.

Thirty Celsius degrees is the most usual temperature used in biological studies. Table 1 shows the oxygen content of air saturated water.

*Table 1.* Oxygen content of air saturated water

| Temperature °C | $[O_2]$ ($\mu moles \cdot ml^{-1}$) |
|---|---|
| 0 | 0.442 |
| 5 | 0.386 |
| 10 | 0.341 |
| 15 | 0.305 |
| 20 | 0.276 |
| 25 | 0.253 |
| 30 | 0.230 |
| 35 | 0.219 |

In this system, photosynthesis and respiration are often expressed as a rate of oxygen uptake or release per unit of weight. This is convenient if it is used as a basis for comparison between different treatments. There are another possibilities such as to express the results as a function of chlorophyll or protein amount or number of cells in the suspension.

Electrochemical reactions in the electrodes always generate a residual current that is detected by the system and recorded in the computer even in the absence of oxygen. Discrepancies between zero oxygen and the electrical zero should be identified for accurate detection of variations in oxygen.

To know the electrical current generated by the system in absence of oxygen it would be necessary to remove the initial oxygen dissolved in the reaction chamber. To do this it is usual to add sodium dithionite to the solution in the chamber that consumes oxygen according to the following reaction:

$$Na_2S_2O_4 + O_2 + H_2O \longrightarrow NaHSO_4 + NaHSO_3$$

Dithionite is corrosive and it could cause damage to the membrane. It should be removed from the membrane as quickly as possible.

# PRACTICAL MEASUREMENT

In order to describe the measure with more detail, we have selected the procedures used with a Hansatech D.W unit containing a Clark-type oxygen electrode based on a design by Delieu & Walker.

## Electrode assembly (DW 1/AD)

1. Switch on the temperature-controller bath fixing the temperature to 30 $^{o}$C (temperature and flow key).

2. Put a 50 ml flask with distilled water in a magnetic stirrer to get oxygen saturated water.

**Electrode disc preparation**

1.The dome, which bears the platinum cathode, should be moisten with some drops of electrolyte (5% saturated KCl) flooding the metacrylate well which bears the silver anode.

2. Cut approximately 2.5 $cm^2$ of cigarette paper (avoid touching it with fingers to prevent finger marks and waste the gummed part) and place it over the cathode dome.

3. Cut approximately 2.5 $cm^2$ of teflon membrane and place it over the paper (avoid touching it with fingers and getting folds).

4. Ensure the membrane and paper in position, over the dome, with the O-ring provided for this purpose. Fix the membrane smoothly over the surface of the electrode dome avoiding wrinkle formation with the help of the applicator.

5. Ensure for smoothness and that there are not wrinkles. Otherwise it would be rejected and the procedure should be repeated.

6. Check that the paper is wet with the electrolyte securing an electric continuity between anode and cathode.

7. Aspire the electrolyte excess.

8. Fit a larger O-ring to the channel surrounding the electrode dome.

9. Place the electrode disc, dome upwards, on the bottom section of the reaction chamber and thread the assembly section over the

electrode on to the base (avoid over-tightening because this can cause damage to the membrane).

3. Connect the electrode to the circuit by plugging it through the control box to the computer.

4. With the computer switched on, click over the 'minirec' icon to get the recorder software.

5. Add two millilitres of oxygen saturated distilled water to the reaction chamber.

6. Switch on the magnetic stiffer *(main power on)* and introduce a little magnetic rod in the reaction chamber.

7. Ensure that *back-off* controls are setting to cancel, the gain control *(output)* to the xl position and the variable gain control *(output)* should be set to the minimum position. Then switch on the control box, this should result in an output voltage of about 600-800 mV in a few minutes.

## Electrode calibration (zero adjust)

1. When the recorder shows a constant and smooth slope in the graphic, add a spatula tip of sodium dithionite to the reaction vessel.

2. Insert the plunger and adjust it so that the water in the reaction vessel just enters the conical section (Figure 8). Conductivity will decrease very fast before reaching a stable plateau around *30-50* mV (background or residual signal) in some minutes.

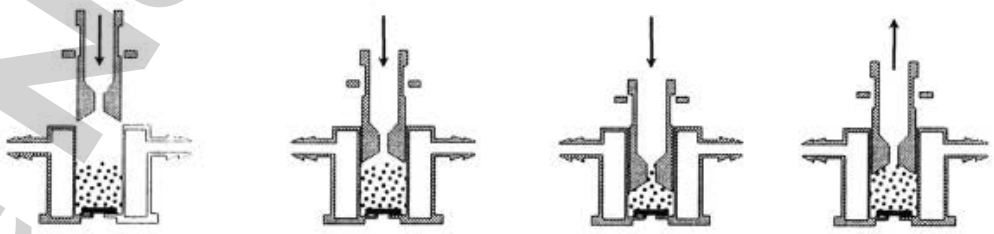

*Figure 8.* Scheme of the correct way to insert the plunger in the reaction chamber.

3. Set the electric difference between zero oxygen and the residual conductivity switching on the back-off key.

4. If the signal do not go to zero value, adjust it using the fine back-off key.

*5.* The content of the reaction chamber should be removed as quickly as possible by using an aspirator with a soft tip.

6. Clean the reaction vessel with distilled water for five or six times (remember that sodium dithionite is corrosive) to avoid damage to the membrane. Do not forget to clean the cover of the reaction vessel with distilled water as well.

## Measuring

1. Put in the reaction vessel 1.5 ml of oxygen saturated water.

2. Introduce a small net in the reaction vessel to protect the sample from the magnetic stirring rod. Wait *5* minutes to get a stable reading and write down the water conductivity *(wc)*.

3. Choose samples with homogeneous sizes for all the replicates. Introduce it in the reaction vessel with the help of the pincers.

4. Close the reaction vessel and tight with the locking nut adjusting the solution volume at the end of the dome of the cover.

*5.* Maintain the camera in darkness.

6. Wait 10 minutes by setting the electronic timer. Write down the second measurement of conductivity *(icond)*.

7. Wait *5* minutes by setting the electronic timer. Write down the third measurement of conductivity *(feond)*.

8. Switch on the source of light, wait for one minute to avoid variations in the signal because of changes in physical conditions and write down the conductivity again *(icond)*.

9. Wait 5 minutes by setting the electronic timer. Write down the new value of conductivity *(fcond)*.

10. Move away the sample from the chamber. Remove the outer water excess over soft paper and weigh the sample to know the fresh weight (FW). Dry in an oven (100 oC, 24 h), and weigh it again (DW).

11. Transform the data of conductivity to μmol $O_2$ $min^{-1}$ g $(DW)^{-1}$ consumed or produced using the following equation:

$$\mu\text{mol } O_2 \text{ min}^{-1} \text{ g (DW)}^{-1} = \frac{WOC \cdot Vol \cdot (fcond - icond)}{wc \cdot t^{-1} \cdot DW}$$

where:

*WOC*: Oxygen content of air saturated water at 30 °C (0.230 $\mu$mol·ml$^{-1}$)
*Vol*: water volume in the reaction vessel
*fcond*: final reading of conductivity
*icond*: initial reading of conductivity
*wc*: water conductivity
*t*: minutes between readings
*DW*: dry weight of the sample

| S.N° | Weight | | *wc* | Respiration | | | Photosynthesis | | | NP |
|---|---|---|---|---|---|---|---|---|---|---|
| | FW | DW | | *Icond* | *fcond* | $\Delta O_2$ | *icond* | *fcond* | $\Delta O_2$ | |
| 1 | | | | | | | | | | |
| 2 | | | | | | | | | | |
| 3 | | | | | | | | | | |
| 4 | | | | | | | | | | |
| 5 | | | | | | | | | | |

S.N°: Sample number; FW: fresh weight; DW: dry weight; $\Delta O_2$: µmol/min·g (DW); NP: net photosynthesis ($P\Delta O_2$- $R\Delta O_2$)

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