Algal Hydrogen Production using Electrochemical and Fermentative Electron Provision

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Abstract

The increasing concern regarding the depleting conventional energy resources has sparked an interest in the development of renewable energy resources. Among these resources, solar technologies have gained the most interest since it is the greatest energy source for the planet. Along with energy acquisition, energy storage is also an important issue and requires a reliable solution if solar technologies are to become the prime source of energy for humanity. One of the solutions gaining momentum nowadays is to switch to Hydrogen and use it as the primary energy carrier. Traditional methods of hydrogen production are not sustainable and efforts are being made to improve the reliability and performance of innovative and inspired technologies. One of these technologies is algal hydrogen production which relies on the natural mechanisms many microorganism have evolved in order to survive harsh conditions. Hydrogenase systems present in some algae allow them to carry out life sustaining reactions and produce hydrogen as a by-product. This process is linked strongly with the electron transport chain and relies on a constant supply of electrons and protons to continue. Here the focus has been on the provision of electrons in order to facilitate this process. Fermentative reactions can be a source of electrons which are released as a result of dissociation of any number of substrates. One of the well-known species, Saccharomyces Cerevisiae has been used to dissociate a simple sugar, glucose, and hence become a source of electrons for the algae to continue the hydrogen evolution process. The provision of electrons has been tackled in two different ways one where the yeast and the algae are in indirect contact i.e. in an MFC and the other where they are directly mixed together. Experimentation showed that direct mixing yielded higher amounts of hydrogen (276 μl/g.hr) owing to the interaction of algae and yeast in the mixture. The indirect contact in the MFC was enhanced to create an MEC, where external potential was provided, but the results were unfavourable. This led to the conclusion that direct mixing is a better and easier way of achieving improved hydrogen production. Along with this, Hematite based photoanodes were also developed in Arizona State University. The photoanodes were tested for their ability to split water under simulated sunlight and contained additives like Copper and Zinc oxides along with MWCNTs and Graphene nano platelets to improve electrical properties. The highest photocurrent achieved with the developed anodes was approximately 10 mA/cm^2. By comparing these biological and inorganic systems, it can be clearly seen that the Hematite based Hydrogen production system is much more efficient and produces higher amounts of Hydrogen.

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Chapter # 1

Introduction

1.1 Hydrogen as fuel

Mankind has relied on fire to be a source of energy that to not only sustain him but also propel him forward. Initially the use of wood as a source of fuel sufficed the day to day needs for humans but over the years as the lifestyles changed so did the energy requirements. The most famous boom in the primary energy consumption occurred in the 18^{th} century which the industrial revolution when huge amounts of oil coal and gas started to be consumed. Today we rely heavily on these fossil fuels which have led to the degradation of the environment and climate change. Besides the harmful effects of the fossil fuels, their limited resources are another crucial factor driving the adoption of alternative and renewable technologies [1].

Some of the factors that determine the quality of a fuel are energy density, heating value, ease of storage etc. Hydrogen gas is a simple diatomic molecule which has the highest energy density when compared to any other fuel. Hydrogen gas is not easy to store because of its low density and therefore the traditional fuel system, involving centralised production, is not a suitable option for this fuel. For hydrogen fuel to become an efficient large scale energy carrier the difficulties faced regarding its transportation and storage need to be overcome and one of the ways this can be achieved is by decentralised production and prompt consumption.

Being a very flexible fuel hydrogen can not only be used in traditional combustion engines but is also a main contender for fuelling fuel cell based systems. In fact it is much more effective in fuel cells and if the production means are renewable and efficient, the combination of hydrogen fuel and fuel cells will be the future of energy consumption [2].

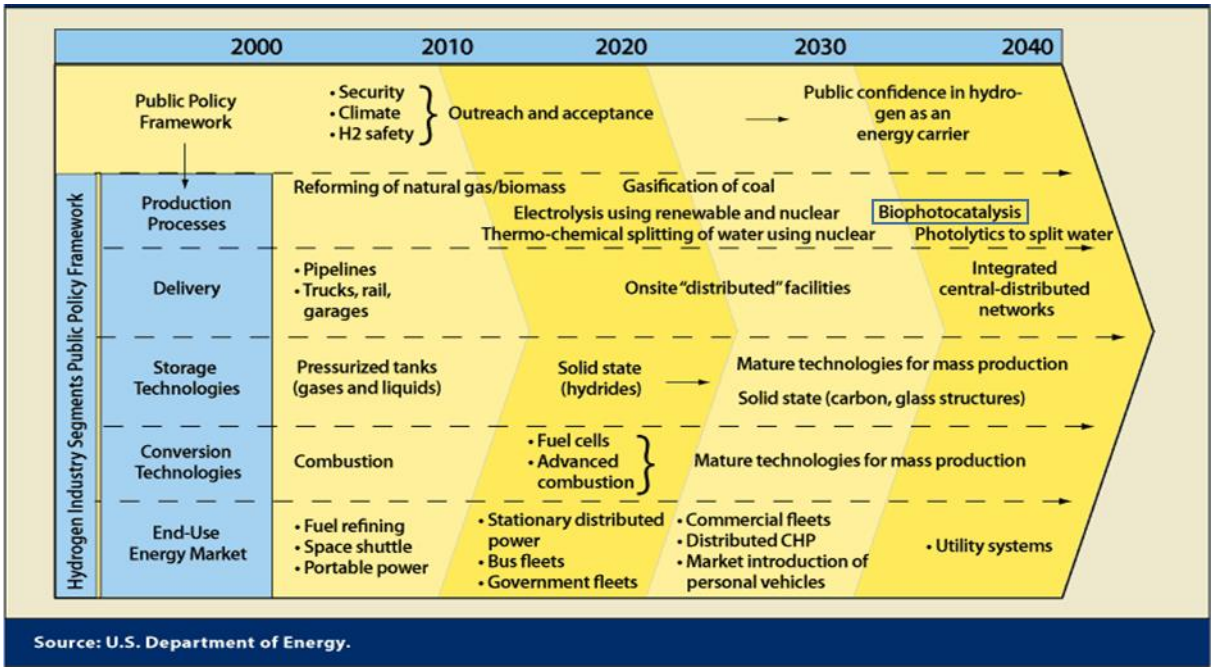


Figure 1 Transition to Hydrogen Economy

1.2 Hydrogen production technologies

Traditional hydrogen production has been driven by the easy access to fossil fuels and their higher hydrogen content. Most of the hydrogen produced has been used for fertilizer manufacturing and is traditionally produced using natural gas. The process of producing hydrogen using fuels is known as reforming which relies on pyrolysis of hydrocarbons or ammonia [3].

These sources of hydrogen, however, are not reliable and renewable and will eventually need to be replaced by other technologies. Most of the renewable hydrogen production technologies are non-reforming and use two main hydrogen resources; biomass and water [4].

Table 1 Hydrogen production technologies [5]

Reforming hydrogen production technologies	**Non reforming hydrogen production technologies**	
	Hydrogen from biomass	*Hydrogen from water*
Steam reforming	Biomass gasification	Electrolysis
Autothermal reforming	Biological hydrogen	Thermochemical water splitting
Partial oxidation		Photoelectrolysis

The technologies relying on reforming usually utilize fossil fuels as a source of hydrogen which renders them unsustainable. Of the non-reforming technologies, biomass gasification is the only one that does not utilize water as a source of water. Water is one of the most abundant compounds on earth and is also the final product of hydrogen fuel utilization either by direct combustion or by electrochemical oxidation. Utilization of water is therefore a sensible step forward in the establishment of hydrogen economy because it is not only economically viable as crude but will also set up a sustainable energy loop. Using solar energy for splitting water will provide a long term and sustainable energy resource [6],[7].

1.2.1 Hydrogen from water (Water splitting)

Energy is required in order to break the molecules of water and release hydrogen to be used as a fuel. The most commonly used method used for achieving this is electrolysis which relies on high power densities for bond lysis. Other methods used for water splitting supply the required energy in the form of heat or light and use catalysts to speed up the process and ease the energetics of the reaction. The amount of energy required for water splitting is 237.178 kJ/mol. It also requires energy to overcome the change in entropy of the reaction. Therefore, the process cannot proceed below 286 kJ per mol if no external heat/energy is added [8]. A brief description of the predominant water splitting technologies is given below.

1.2.1.1 Electrolysis

Electrolysis is the process of using electricity to split water into hydrogen and oxygen. This reaction takes place in a unit called an electrolyzer. Electrolysers can range in size from small, appliance-size equipment that is well-suited for small-scale distributed hydrogen production to large-scale, central production facilities that could be tied directly to renewable or other non-greenhouse-gas-emitting forms of electricity production [9]. They consist of two electrodes on which following reactions take place:

Anode (oxidation): $2\ H_2O_{(l)} \rightarrow O_{2\,(g)} + 4\ H^+_{(aq)} + 4e^-$ $\quad E^o_{ox} = -1.23$ V

Cathode (reduction): $2\ H^+_{(aq)} + 2e^- \rightarrow H_{2\,(g)}$ $\quad E^o_{red} = 0.00$ V

Different electrolysers function in slightly different ways, mainly due to the different type of electrolyte material involved. Three important types of electrolysers are:

- Polymer electrolyte membrane electrolysers
- Alkaline electrolysers

- Solid oxide electrolysers

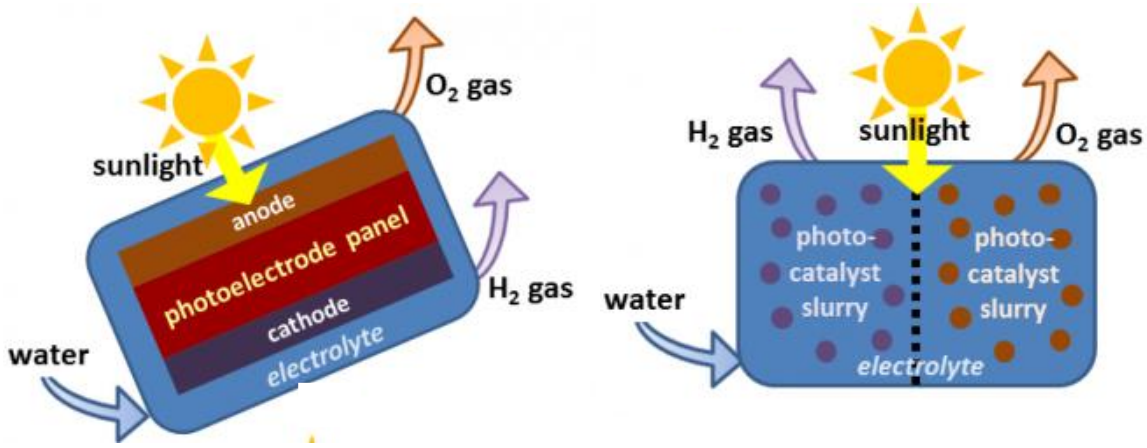
1.2.1.2 Thermochemical water splitting

Thermochemical water splitting processes use high-temperature heat (500°–2,000°C) to drive a series of chemical reactions that produce hydrogen. The chemicals used in the process are reused within each cycle, creating a closed loop that consumes only water and produces hydrogen and oxygen [10],[11]. The heat energy may be provided by:

- Concentrated sunlight
- Nuclear reactors

1.2.1.3 Photoelectrolysis

In photoelectrochemical (PEC) water splitting, hydrogen is produced from water using sunlight and specialized semiconductors called photoelectrochemical materials, which use light energy to directly dissociate water molecules into hydrogen and oxygen. The semiconductor materials used in the PEC process are similar to those used in photovoltaic solar electricity generation, but for PEC applications the semiconductor is immersed in a water-based electrolyte, where sunlight energizes the water-splitting process [12]. The photoelectrochemical systems can either be based on a plate or a slurry system



(a) Electrode system (b) Particle system

Figure 2 Different types of photoelectrochemical reactors

1.2.2 Thermodynamics of water splitting

Oxidation of hydrogen is the reaction which provides us energy in the form of heat in internal combustion engines or electricity in fuel cells. The heat of combustion of hydrogen is

286 kJ/mol but more energy is required for breaking a water molecule known as the activation energy. The activation energy can be reduced by changing the reaction conditions. Catalyzing the water splitting process involves creating pathways whereby minimum energy is required.

The redox potential is an indicator of the energetics of reactions. Positive redox potential indicates an exothermic process while a negative redox potential represents an endothermic process. Reduction of protons is used as a standard to calculate the potentials of all other reactions. A normal hydrogen electrode (NHE) is therefore used to meter reaction energies. The meter shows the redox potential of hydrogen to be 0V which in no way implies that no energy is required for the reaction.

The other half of water splitting reaction is the dissociation of water to produce oxygen. This half reaction has a potential of 1.229V and collectively gives a collectively gives the redox potential of water splitting to be 1.229V.

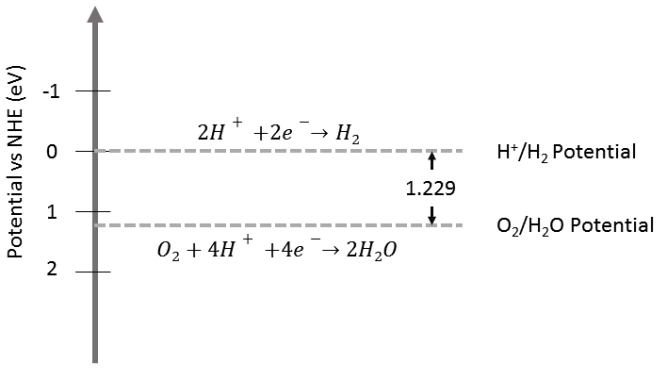
$$\boldsymbol{E_{water\ splitting} = E_{\frac{H_2}{H^+}} + + E_{\frac{2H_2O}{O_2}} = 0 + 1.229 = 1.229V}$$

This value is also reaffirmed by the use of ΔG for water splitting reaction which is 237.178 kJ/mol and yields the minimum voltage required for water splitting to be:

$$\boldsymbol{\Delta G = nFV_{rev}\ ;\ V_{rev} = 1.229\ V}$$

This value is true for standard temperature, pressure conditions of 25°C and 1 bar respectively. It is an endothermic reaction and hence, at isothermal conditions, heat energy ($T\Delta S$) must be applied [13].

a)



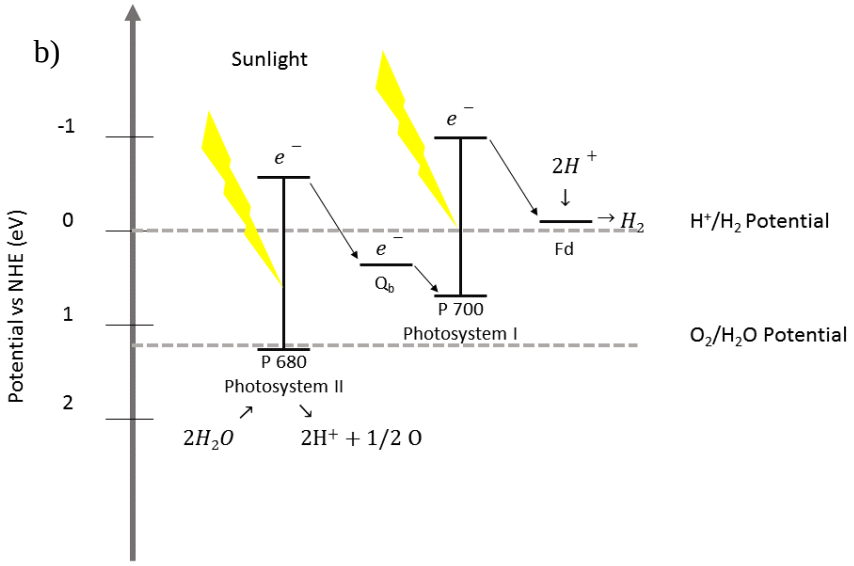


Figure 3 a) Graphical depiction of water splitting redox reactions. b) Water splitting energetics for biological systems

1.2.3 Hydrogen from biomass

Life on earth is based on hydrocarbons and as a result stores a vast amount of hydrogen along with major elements like oxygen, carbon, nitrogen and sulphur to name a few. The most common type of biomass available for hydrogen harvesting is crop residue while other sources like sewage waste are more difficult to handle [14]. The available biomass sources are divided into four major categories:

1. Energy crops
2. Agricultural residues and waste
3. Forestry waste and residues
4. Industry and municipal waste

The methods of obtaining energy from these sources are divided into two main categories:

a) Thermochemical processes
   i. Combustion
   ii. Pyrolysis

b) Biological processes
   i. Direct biophotolysis
   ii. Indirect biophotolysis

iii. Liquefaction

iv. Gasification

iii. Biological water-gas shift reaction

iv. Photo fermentation

v. Dark fermentation

Thermochemical process are inherently complex and involve mechanical means along with heat energy for breaking down the complex biological compounds. On the other hand biological processes are leaner in the sense that they involve less mechanical intensity and instead rely on efficient enzymatic pathways for breaking down complex molecules. This can result in the production of high purity products and eliminate the production of hazardous products like tar, ash etc.

1.2.3.1 Biological processes

Although the biological means of hydrogen production had been discovered over a century ago, they started receiving attention after the oil crisis. Among the biological processes the photosynthetic processes received the most attention because of the promise of being able to effectively, efficiently and directly converting solar energy into a marketable energy carrier like hydrogen.

The biological processes depend on enzymes like Hydrogenase and Nitrogenase which catalyse the reduction of protons. Hydrogenase is the enzyme commonly found in photosynthetic organisms and are classified as uptake hydrogenases and reversible hydrogenases [15]. Biological hydrogen production processes can be classified as follows:

- Biophotolysis of water by algae and cyanobacteria
- Photodecomposition of organic compounds using photosynthetic bacteria
- Fermentative hydrogen production from organic compounds
- Hybrid system using photosynthetic and fermentative bacteria

1.2.3.1.1 Biophotolysis of water using algae and cyanobacteria

This process involves the same photosynthetic processes found in plants but is capable of producing hydrogen gas by reduction of protons instead of reduction of carbon dioxide and generation of carbon containing compounds. Higher plants are not capable of performing hydrogen evolution because of the absence of required enzymes. The process involves the absorption of light by two distinct photosynthetic systems operating in series: a water splitting and O_2 evolving system (PSII) and a second photosystem (PSI), which generates the reductant

used for CO_2 reduction. In this coupled process, two photons used for each electron removed from water and used in CO_2 reduction or H_2 formation. In green plants only CO_2 reduction takes place, as the enzymes that catalyse hydrogen formation, the hydrogenases, are absent. Microalgae, both eukaryotic (such as the green algae) and prokaryotes, have hydrogenase enzymes, and can produce hydrogen under certain conditions [16].

One important factor that inhibits this method of hydrogen production is O_2 inhibition. Under dark anaerobic conditions Hydrogenase enzyme is synthesised and activated and when such cultures are exposed to light the hydrogen generation activity increases sharply before decreasing due to the continuation of normal photosynthesis which leads to oxygen production and uptake of hydrogen instead of its production [17]. The flow of electrons from the splitting of water to hydrogen producing enzyme via electron carrier i.e. Ferredoxin Fd is as follows:

$$H_2O \rightarrow PSII \rightarrow PSI \rightarrow Fd \rightarrow Hydrogenase \rightarrow H_2$$

$$\downarrow$$

$$O_2$$

Cyanobacteria on the other hand are capable of hydrogen production using nitrogenase enzyme as well.

1.2.3.1.2 Photodecomposition of organic compounds using photosynthetic bacteria

Phototrophic bacteria are indicated in the current literature as the most promising microbial system for the biological production of hydrogen. The major benefits include high theoretical conversion yields, lack of oxygen evolving activity, which causes problem of oxygen inactivation of different biological systems, and ability to consume organic substrates derivable from wastes and then, for their potential to be used in association with wastewater treatment [18].

The overall biochemical pathways for the photo fermentation process can be expressed as follows:

$$(CH_2O)_2 \rightarrow \text{Ferredoxin} \rightarrow \text{Nitrogenase} \rightarrow H2:$$

$$\uparrow ATP \qquad \uparrow ATP$$

Carbon monoxide can also be used for the production of hydrogen using microbial shift reaction by the photosynthetic bacteria as follows:

$$CO + H_2O \rightarrow CO_2 + H_2$$

1.2.3.1.3 Fermentative hydrogen production from organic compounds

A lot of attention has been given to the hydrogen production by photosynthetic organisms than to fermentative hydrogen production. Fermentative hydrogen production can prove to be industrially favourable due to:

a) Fermentative bacteria have very high evolution rate of hydrogen.
b) They can produce hydrogen constantly through day and night from organic substrates.
c) They can have growth rate good for supply of microorganisms to the production system.

Therefore, the fermentative evolution is more advantageous than photochemical evolution for mass production of hydrogen by microorganisms. Fermentative hydrogen production can be maximized through the effective coupling of an accessible and rich source of electron and biochemical electron pump with an active hydrogenase [19].

1.2.3.1.4 Hybrid system using photosynthetic and fermentative bacteria

These systems comprise of both photosynthetic and fermentative microbes. Various carbohydrates can be digested by fermentative bacteria and converted to hydrogen without the use of light. The resulting organic compounds can be used by photosynthetic bacteria to produce hydrogen.

Anaerobic bacteria decompose organic matter to obtain energy and electrons and since reactions with negative energy can be allowed, further decomposition of these acids cannot take place. Complete degradation of glucose to hydrogen and carbon dioxide is impossible by anaerobic digestion. Photosynthetic bacteria could use light energy to overcome the positive free energy reaction (bacteria can utilize organic acids for hydrogen production). The combination of the both kinds of bacteria not only reduces the light energy demand of photosynthetic bacteria but also increases hydrogen production [20].

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Chapter # 2

Algal hydrogen production

2.1 Introduction

Algal hydrogen production is divided into two types of processes which are direct and indirect biophotolysis. Direct biophotolysis occurs in the presence of light and is also termed photoautotrophic hydrogen production which involves the utilization of incident energy from the sum [17].

$$2H_2O \xrightarrow{Light\ energy} 2H_2 + O_2\ (\Delta G = +1498kJ)$$

Direct biophotolysis converts the protons produced form water splitting in the photosynthetic proteins directly into hydrogen while indirect biophotolysis involves firstly the conversion of these protons into substrate and its subsequent conversion to hydrogen by fermentation [21]. In the presence of light, direct photochemical water splitting or biophotolysis results in hydrogen production while under dark conditions the fermentative pathway for hydrogen production is more prevalent as it therefore becomes the main source of protons for hydrogen producing enzymes [19]. The products of the fermentative pathway depend on the availability of light energy and therefore fermentative pathways are classified as being either dark fermentation or photo fermentation. Dark fermentation results in the production of energy and releases organic acids like acetic acid while for light fermentation the by-product is carbon dioxide [22].

Dark fermentation:

$$C_6H_{12}O_6 + 2\,H_2O \rightarrow 2\,CH_3COOH + 4\,H_2 + 2\,CO_2\ (\Delta G = -206\,kJ)$$

Photo fermentation:

$$CH_3COOH + 2\,H_2O \rightarrow 4\,H_2 + 2\,CO_2\ (\Delta G = +104\,kJ)$$

On its own algal hydrogen production is a very inefficient process and converts only about 1% - 10% of the absorbed incident radiation into hydrogen fuel and requires a hard to maintain anaerobic environment [17].

Table 2 Hydrogen production capabilities of well-known algal species

Microorganism	Hydrogen production capability
Scenedesmus D_3	$126 - 181\ nmol/hr$
Chlamydomonas reinhardii	$44 - 104\ nmol/hr$
C. moewusii	$253 - 337\ nmol/hr$

The biological processes involved in the production of hydrogen using algae are described as under:

a) Bio-photolysis of water by algae.
b) Dark-fermentative hydrogen production during acidogenic phase of anaerobic digestion of organic matter.

2.1.1 Hydrogen gas production from water by algae

Algae split water molecules to hydrogen ion and oxygen via photosynthesis. The generated hydrogen ions are converted into hydrogen gas by hydrogenase enzyme. Chlamydomonas reinhardtii is one of the well-known hydrogen producing algae [23]. This process is also known as direct biophotolysis.

The algal hydrogen production could be considered as an economical and sustainable method in terms ofwater utilization as a renewable resource and CO2 consumption as one of the air pollutants. However, strong inhibition effect of generated oxygen on hydrogenase enzyme is the major limitation for the process. Inhibition of the hydrogenase enzyme by oxygen can be alleviated by cultivation of algae under sulfur deprivation for 2–3 days to provide anaerobic conditions in the light. Low hydrogen production potential and no waste utilization are the other disadvantages of hydrogen production by algae. Therefore, dark and photo-fermentations are considered to be more advantageous due to simultaneous waste treatment and hydrogen gas production.

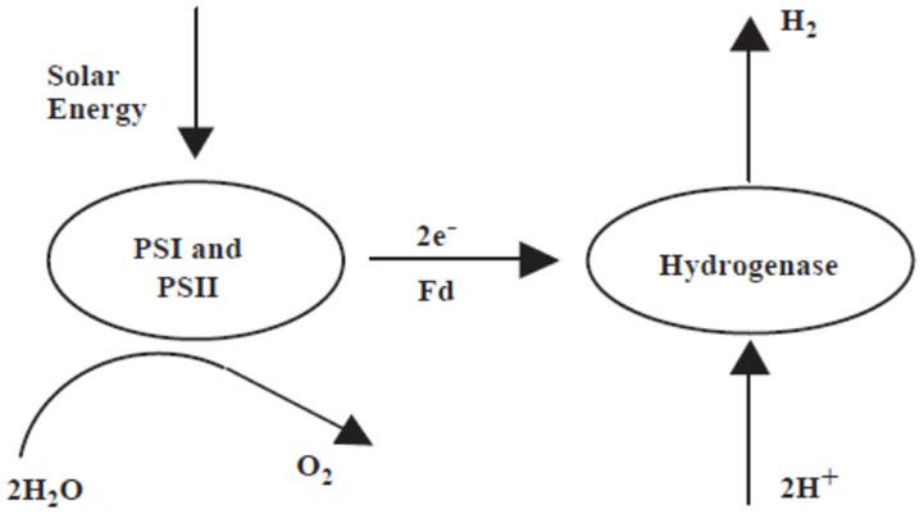

Figure 4 Direct biophotolysis

2.1.2 Hydrogen gas production by dark fermentation

Many anaerobic organisms can produce hydrogen from carbohydrate containing organic wastes. The organisms belonging to genus Clostridium such as C. buytricum, C. thermolacticum, C. pasteurianum, C. paraputrificum M-21 and C. bifermentants are obligate anaerobes and spore forming organisms. The major products in hydrogen production by anaerobic dark fermentation of carbohydrates are acetic, butyric and propionic acids. The hydrogenase enzyme present in anaerobic organisms oxidizes reduced ferrodoxin to produce molecular hydrogen [24].

The concept of indirect biophotolysis involves the following four steps:

(i) Biomass production by photosynthesis

(ii) Biomass concentration

(iii) Aerobic dark fermentation yielding 4 mol hydrogen/mol glucose in the algae cell, along with 2 mol of acetates

(iv) Conversion of 2 mol of acetates into hydrogen.

In a typical indirect biophotolysis, Cyanobacteria are used to produce hydrogen via the following reactions:

$$12\ H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2$$

$$C_6H_{12}O_6 + 12\ H_2O \rightarrow 12H_2 + 6CO_2$$

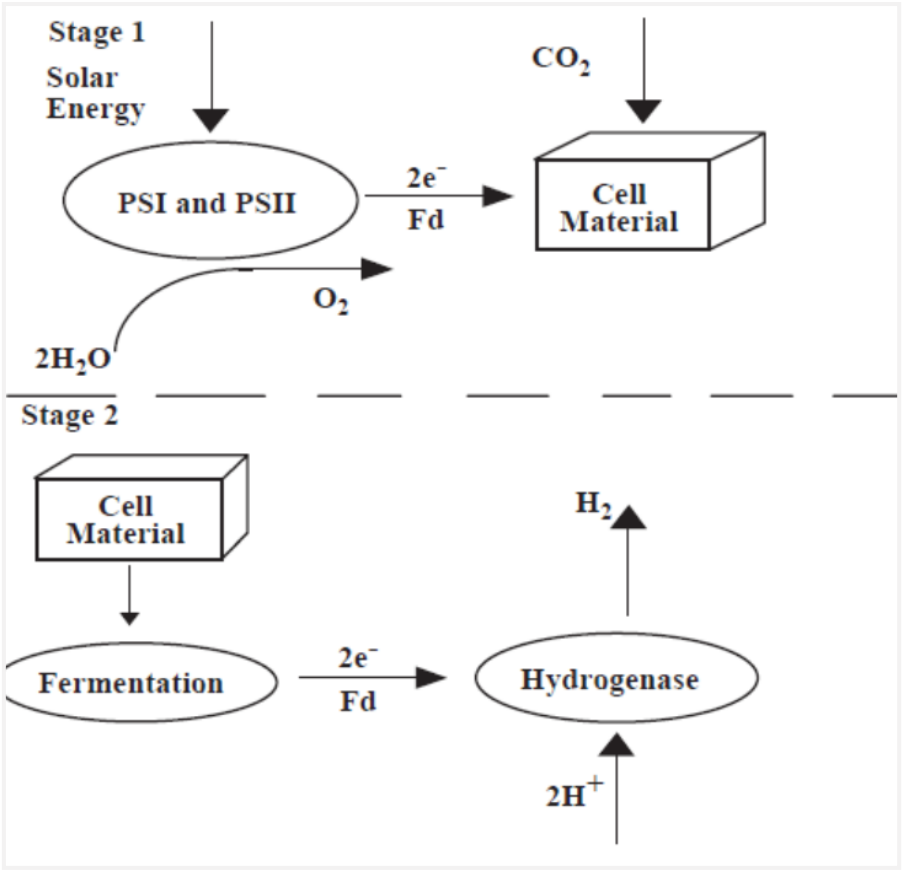

Figure 5 Indirect biophotolysis

2.2 Physiology of Hydrogen production in green algae

Historically, Hans Gaffron made the first observation of hydrogen metabolism in green algae. Upon exposure to hydrogen of anaerobically adapted cells, he observed uptake of molecular H2 by the algae and a concomitant CO2 reduction in the dark. The reverse reaction, e.g. hydrogen production in the light, was first reported with the green alga Scenedesmus obliquus.

High rates of H2 evolution could be measured in the light for short periods of time (from several seconds to a few minutes). Electrons were generated either upon:

- The photochemical oxidation of water by PSII, which results in the simultaneous production of O2 and H2
- Upon the oxidation of endogenous substrate, feeding electrons into the thylakoid membrane with the simultaneous release of CO2 to the medium.

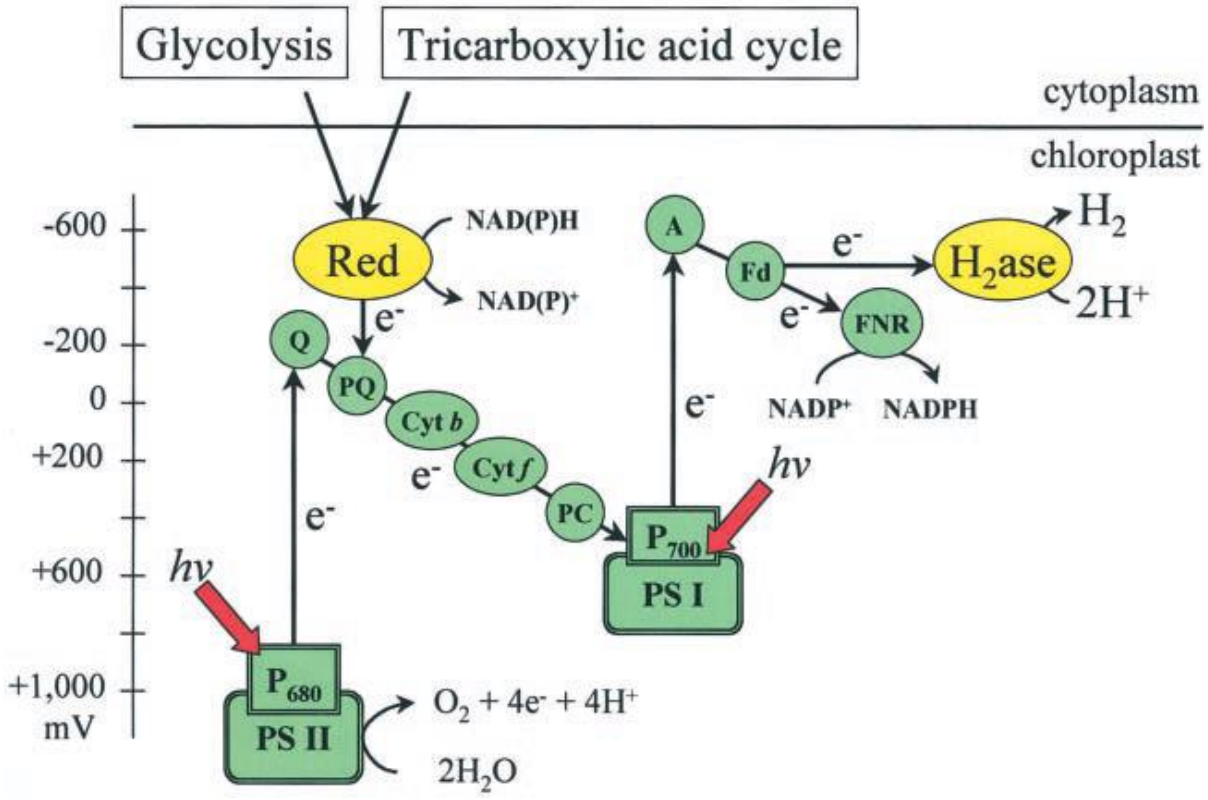

Figure 6 Hydrogenase related electron transport chain

Electrons may originate either at PSII upon photooxidation of water, or at the plastoquinone pool upon oxidation of cellular endogenous substrate (e.g. via glycolysis and the tricarboxylic acid cycle). Electrons in the electron transport chain are transported via PSI to ferredoxin, which serves as the physiological electron donor to the Fe hydrogenase. P680, Reaction center of PSII; P700, reaction center of PSI; Q, primary electron acceptor of PS II; A, primary electron acceptor of PSI; PQ, plastoquinone; Cyt, cytochrome; PC, plastocyanin; Fd, ferredoxin; Red, NAD(P)H oxido-reductase; H2ase, hydrogenase; FNR, ferredoxin-NADP reductase [25].

The process of hydrogen production can be sequentially explained as follows:

2.2.1.1 Oxygenic photosynthesis

Oxygenic photosynthesis, i.e. the residual PSII activity for the generation of electrons upon oxidation of water. These electrons are transported through the photosynthetic electron transport chain and eventually feed into the Fe hydrogenase, thereby contributing to H2 production.

2.2.1.2 Mitochondrial respiration

Mitochondrial respiration scavenges all oxygen generated by the residual photosynthesis and, thus, maintains anaerobiosis in the culture.

2.2.1.3 Catabolism of endogenous substrate

Endogenous substrate catabolism, including starch, protein, and probably lipid catabolism, yields substrate suitable for the operation of oxidative phosphorylation in mitochondria, and possibly for an NAD(P)H-dependent electron transport in the chloroplast, both of which contribute to the generation of much-needed ATP.

2.2.1.4 Electron transport via the hydrogenase pathway leading to H2 production

Electron transport via the hydrogenase pathway and the ensuing release of H2gas by the algae sustains a baseline level of photosynthesis and, therefore, of respiratory electron transport for the generation of ATP and thus ensures the survival of the organism under protracted stress conditions.

2.3 Behaviour of algae in electrochemical devices

The purpose of using microorganisms in electrochemical devices is to use the available substrate efficiently on the anode and to produce electrons. Algae, being phototrophs, are capable of being used both on the anode for provision of substrate and on the cathode to aid reduction. They can produce substrates by photosynthesis which is then used by other microorganisms like bacteria. Entire cells can be fed to the anode chamber while another option is to have algae produce extracellular compounds which are oxidized. When the use of heterotrophic organisms is avoided, the system becomes much simpler and the only problem which remains is the absence of direct electron transfer to the anode. Mediators are therefore used to aid the transfer of electrons while using phototrophs like the cyanobacterium Anabaena variabilis. There is however another way of using only phototrophs on the anode and produce electrons. This involves the production of hydrogen gas by algae and then the oxidation of this gas in the presence of noble metal catalysts on the surface of the electrode. Cyanobacteria have been observed to display direct electron transfer to the anode which is a trait exclusive to them. Discovering such a direct route of electron transfer in algae will allow them to be used much more effectively on the anodes. Algal exoelectrogenic activity has been observed and promises to produce low cost bio photovoltaic devices [26], [27].

Using algae on the cathode side provides a constant supply of oxygen under light conditions. This oxygen is used to reduce protons to water. Chlorella vulgaris has been used as the active species on the cathode side and has proven to be an effective reducing agent, either because of oxygen production or because of mediated electron transfer.

2.3.1 Algal biofilms

In order to be used in an electrochemical system, the algae need to transfer their electron or receive electrons from a solid electrode which can happed either with the help of mediators or by the development of a biofilm. Biofilms are a well understood phenomenon and vary in their characteristics on the basis of constituent microbes and can grow on a multitude of surfaces living and non-living. The result of formation of microbial biofilms may be disastrous because of the production of toxins or beneficial because of their influence on their environment. Algal cells also have the capability to form biofilms which brings about advantageous attributes including reduction of water and energy requirement and stimulation for lipid accumulation, thus resulting in better harvest in photo bioreactors. In electrochemical cells, algal biofilms on the cathode can act as a means of absorbing carbon dioxide produced at the anode because of the oxidation of any substrate. Such algae enriched electrodes can be used as anodes for power production as well [28].

2.3.2 Catalysis

An integral part of electrochemical devices is catalysis because of the need to lower activation energies of reacting species. Noble metal catalysis is extremely popular and has been developed over the years for devices like PEMFCs. These catalysts are, however, extremely expensive and therefore not suitable for use on a very large scale. Other non-noble metal based enzymes are also being developed which are much more beneficial on an economic basis [29].

For bio electrochemical cells, catalysis is mostly required on the cathode for the reduction of protons and platinum catalyzed carbon based electrodes are used extensively. A biological alternative to these catalysts are enzymes which can be easily replenished and have comparable activities. In the presence of oxygen, algae have been used as a microorganism to aid oxygen reduction. One such instance is found in the use of the species Chlamydomonas reinhardtii which performed oxygen reduction catalysis on the cathode even in the absence of mediators. This activity of algae is useful in MFC technologies where the primary purpose is power production, however for hydrogen production, a different catalytic activity needs to be

exploited. Hydrogen production from algae is attributed to the activity of hydrogenase enzymes which utilize the available protons for hydrogen generation. The source of protons can either be water or substrate which produce protons by photocatalytic water splitting and fermentation respectively. The activation of hydrogenase occurs under anaerobic environment and is brought on by Sulphur deprivation in the algal culture. When such cultures are grown on electrodes and maintained under anaerobic conditions, hydrogen production is observed.

2.3.3 Hydrogenases

Hydrogenases are metalloenzymes that reversible catalyze the production of hydrogen from protons. Algal hydrogenase has been investigated as a source of bio hydrogen. Hydrogenases are a classified on the basis of the type of active metal present in them. Iron and Nickel are the two metals that make the active center of the enzyme and thus result in three types of enzymes, iron hydrogenase, iron-iron hydrogenase and nickel-iron hydrogenase. Iron hydrogenase is a more active enzyme and results in larger yields of hydrogen but is easily inactivated by oxygen, while nickel-iron hydrogenase has comparatively lower yields but can resist oxygen deactivation to a certain degree. The function of the enzyme is linked with the presence of Ferredoxin which acts as a reducing agent. Ferredoxin is primarily involved in the NADP synthesis process and serves a secondary purpose in hydrogen production. Since the presence of electrons is just as important as the availability of protons for hydrogen production, therefore, ferredoxin is of utmost importance [30], [31].

The use of this class of enzymes is utilized in enzymatic fuel cells. Hydrogenase from the bacterium Clostridium acetobutylicum, specifically [FeFe]-hydrogenase HydA, was used on the cathode of a TiO2 based photo electrochemical cell for hydrogen production. This shows that hydrogenase obtained from algae may also possess the ability to perform as effective cathode catalysts. The advantage to be gained from isolation of the enzyme is its isolation from oxygen, produced otherwise because of photosynthesis, and thus a decrease in its oxygen deactivation. Hydrogenase being a reversible can also be used on the anode for oxidation of hydrogen gas and displayed the advantage of withstanding carbon monoxide poising when the hydrogenase form the bacterium Thiocapsa roseopersicina was tested as part of an enzymatic electrode.

2.4 Microbial electrolysis cells

These devices are a classification of Microbial Fuel Cells (MFCs). MFCs oxidize different substrates on the anode side and produce protons and electrons that travel through the cation exchange membrane and the external circuit respectively to the cathode side. On the cathode side, in the presence of oxygen, these protons form water. Specific voltage produced by an MFC depends on the redox reactions on either electrode. Acetate and glucose are mostly used as substrates where glucose is a better candidate for hydrogen production since it produces a higher potential upon oxidation. The architecture of these devices is very much similar to the MFCs with the difference being additional gas capture mechanism. These devices replace the oxygen reduction reaction with hydrogen reduction reaction which results in the requirement of an external potential as well. Microbial electrolysis cells can be thought of as an electrochemical device with two bio electrodes, one of which releases protons from any substrate and the other one combines them to form hydrogen [32].

MFCs are steered towards the production of electrical power which may not be the best use of such a technology because the power production is low and is justified only for the treatment of waste water. Microalgae have been suggested as a better working material in the MFCs for waste water treatment because of their ability to naturally produce oxygen and resultant detoxification of the water. The limitation of low power output led to the development of the idea of using the power output of MFCs as a means of energy storage in the form of hydrogen gas. Thus the energy from the oxidation of substrate on the anode is used to dissociate water directly or indirectly which is why these electrochemical cells were called microbially assisted electrolysis cells.

Since the environment in MECs is different from MFCs the microorganisms also have different behavior and develop different traits on electrodes. Studies have been carried out which highlight the changes that occur in the electrode cultures when the MFCs are converted to MECs. In one case when the switch was made, the diversity of the microbial cultures decreased and more Geobacter sulfurreducens were found in the system. These studies were carried out on bacterial communities and give a clue as to how different the conditions can be and how only a handful of microbial species are able to withstand the stresses of anaerobic environments [33].

a) b)

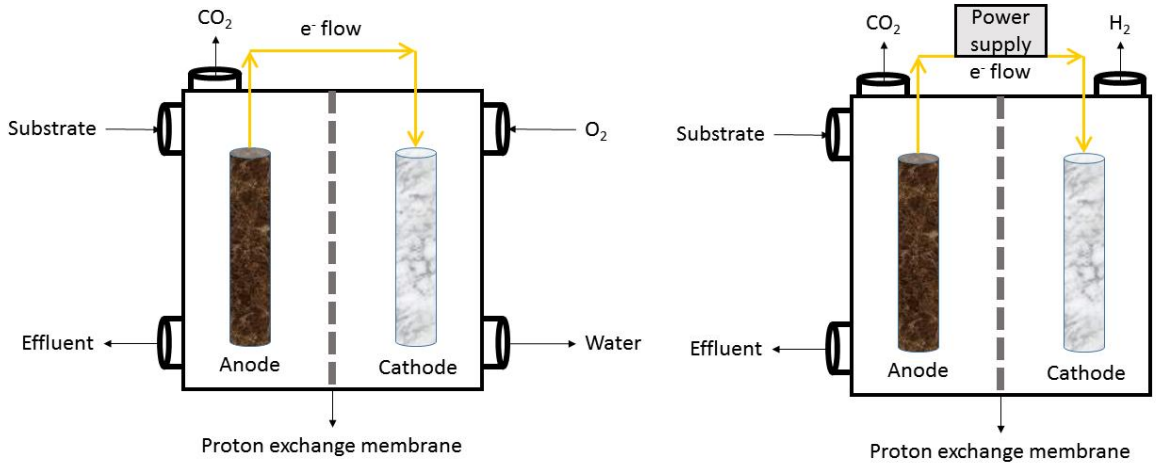

Figure 7 a) Microbial fuel cell which produces water on the cathode side b) Microbial electrolysis cell which produces hydrogen on the cathode side and requires external power to overcome the energy barrier for proton reduction

2.4.1 MEC Anodes

The materials used for the anodes need to act the electron sink for the microbial communities in the electrochemical system and are the same as those used in MFCs. Commonly carbon based electrodes like carbon paper, carbon cloth and carbon brushes have been used. Single and dual chamber cells can be built and in single chamber cells, it has been shown that the hydrogen produced on the cathode is recycled at the anode thus lowering hydrogen harvest. However such a system is beneficial for lowering the COD of waste water by using high surface area anode. For the anodes to perform well the losses related to mass transport need to be minimized because it ultimately effects the rate of electron transfer. An enhanced mass flow rate can be created by providing artificial flow through the anode or by using buffers that speed up and enhance the transfer of electrons to the electrode surface. The study regarding these effects resulted in a hydrogen production rate of 5.6 m^3 m^{-3} d^{-1} due to enhanced current densities.

In order to use microalgae on the anodes of MECs they need to be able to transfer their electrons directly to the electrode. One of the ways in which this has been done is by harvesting the electrons directly from the electron transport chain of the algae Chlamydomonas reinhardtii. Further improvements in this pathway can lead to the development of bioanodes that can harvest sunlight and turn it into hydrogen in electrochemical devices [34].

2.4.2 MEC Cathodes

Traditionally precious metal catalysis has been the prime means of carrying out proton reduction on the cathode surface. Platinum on carbon cathodes made into cloth or brush shapes have been tested for electrochemical activity with the brush electrodes having the advantage of providing a larger surface area and thus better activity. Since the carbon based cathodes provided lower current densities, different materials have since been tested for being cheap and effective cathodes, one of which is stainless steel. Cathodes of plate stainless steel or brush architecture have shown hydrogen production rates similar to those of platinum catalyzed ones. They are non-reactive and provide a large reactive surface with an economic advantage [35].

Considering the economic disadvantage that precious metals inherently possess, the use of readily available active materials paved the way forward for the use of microorganisms on the MEC cathodes and one of the early consortium studied showed hydrogen production at the rate of 0.63 m^3H_2/m^3. Much work has been done on biocathodes in the recent years and different ways of developing them have been shown which result in either a single chamber or a dual chamber device. In the dual chamber devices the anodes can either be biotic or abiotic. Because of the anaerobic environment, obligate anaerobes tend to thrive in MECs and along with them fermentative, methanogenic microbes can also be found.

Most of the recent work utilizes external work required in the form of applied electrical potential either from the grid or from another MFC. But as of yet no biocathodes has been developed which can utilize the incident photonic energy in order produce hydrogen. Inorganic photocathodes, however, have been developed and one of them has been shown to work using TiO2 along with Ru based dye in order to provide the external energy the electrons need in order to be able to reduce protons [36]. The hydrogen yield from this setup was comparable to the Pt loaded electrodes. This information should therefore be extended to study of biocathodes in order to avoid the tedious task of preparing sensitive photoactive, semiconductor based electrodes.

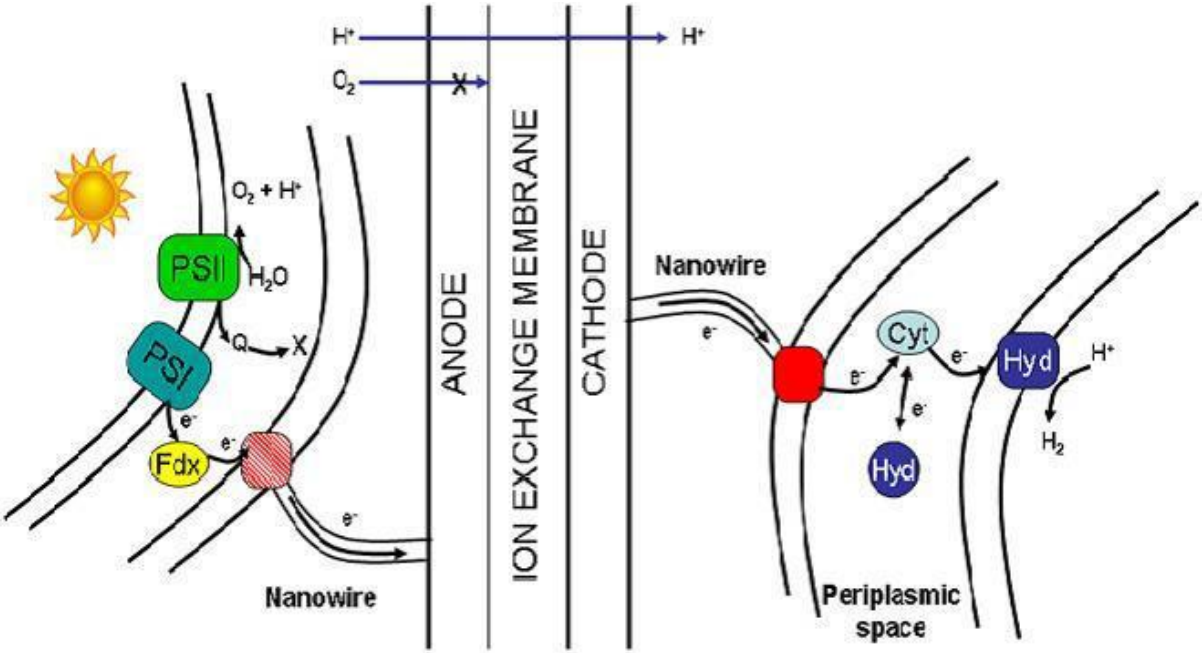

Figure 8 Electron transport in a Microbial Electrolysis Cell

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Chapter # 3

Photoelectrochemical Water Splitting

3.1 Introduction

Hydrogen production using solar energy from direct photoelectrochemical water splitting will provide a basis for a sustainable and renewable hydrogen economy. In pursuit of this goal, many different solar powered water splitting cells have been tested. The end use of any photocell will be inside a purpose built photo reactor which will allow for maintained of favourable conditions under which the photocell can perform [37]. Most work on photocells is carried out on small scale where controlled areas are tested with different materials acting as a base material for water splitting. This base material is usually a semiconductor which absorbs solar radiation and thus produces electron hole pairs that take part in water splitting reactions. The normal configuration used for tests is as follows:

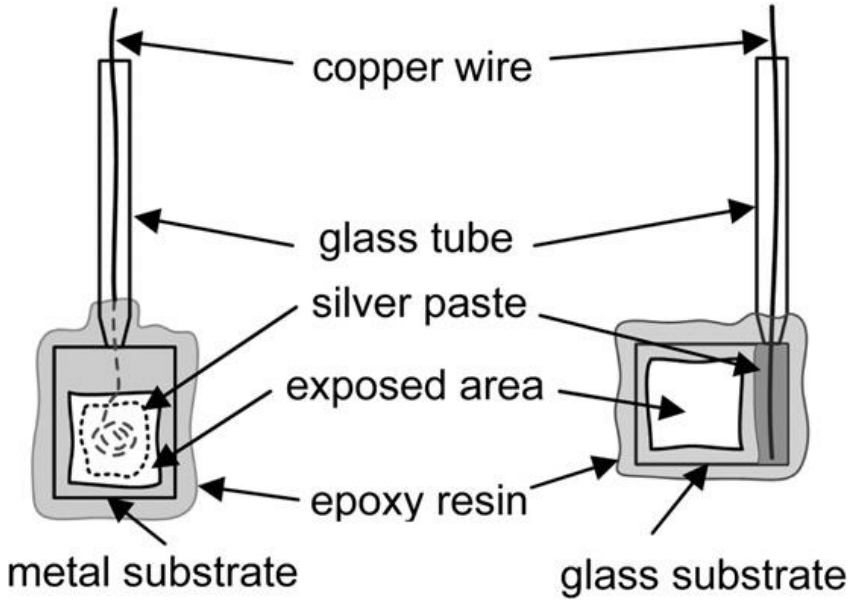

Figure 9 Configuration of photo electrode

The electrodes containing active material can be used in electrolysers where an external bias is provided in order to allow for water splitting reactions to occur. The benefit of using photosensitive materials is that they can allow for the water splitting reaction to occur spontaneously. There are however certain contraints imposed on these systems in order

for them to function spontaneously. One of the basic requirement is for the band gap of the material used to be around 2 eV with a conduction band energy higher and valance band energy lower than that of the reduction and oxidation potential of water respectively [38]. The electrochemical setup required for testing the performance of water splitting cells is shown here.

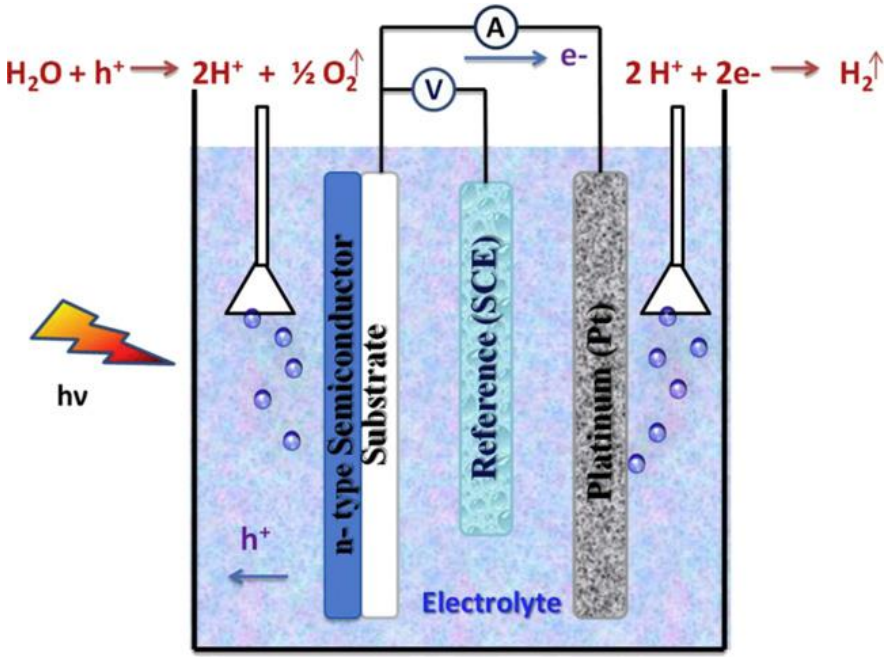

Figure 10 Schematic of a PEC cell consisting of a n-type photo anode, a reference electrode and a counter metal electrode for water splitting

3.1.1 Semiconductor photoelectrodes

An effective PEC system will have the following characteristics:

- A sufficient visible light absorption capability which means that they need to have a band gap in the range of 1.8 – 2.2 eV.
- The ability to efficiently separate and quickly transport photo-generated electron hone pairs to prevent recombination losses.
- Favourable conduction and valance band edge positions with respect to redox potential of water.
- They must be non-corrosive and have high chemical stability in the electrolyte
- The material must be abundant and low cost.

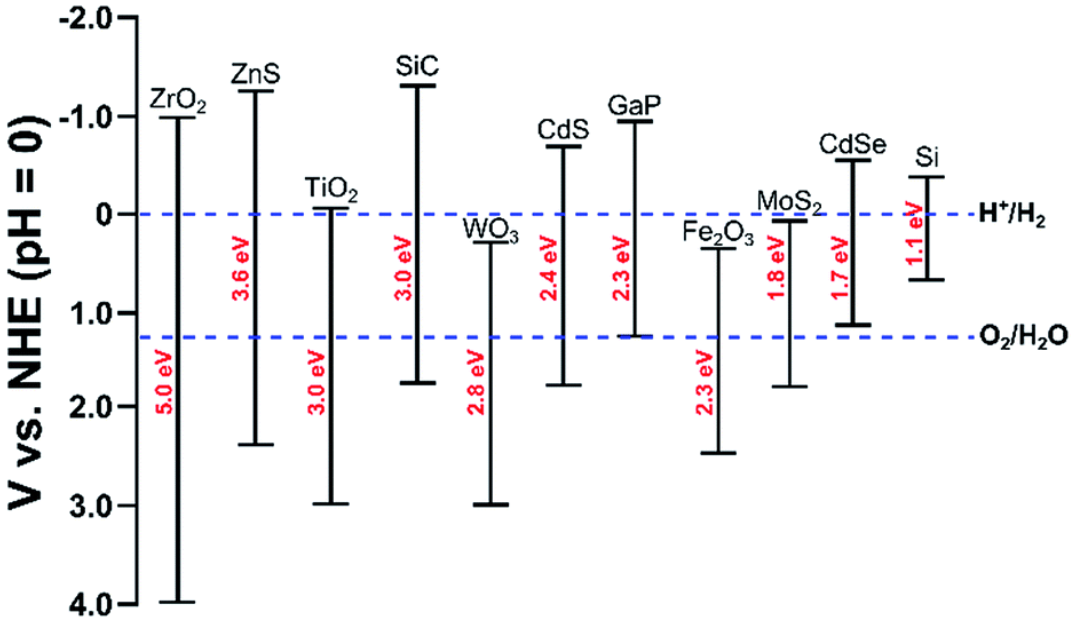

Figure 11 Candidate semiconductor materials for use in water splitting cells

3.2 Performance enhancement

The most important factors influencing the performance of the cells are electron transfer processes, the band gap energy and band structure of the semiconductors. Semiconductors can be modified using the following processes.

- Doping
- Swift heavy ion irradiation (SHI)
- Metal ion loading
- Composites of mixed oxides
- Bilayered systems
- Dye-sensitization

3.2.1 Bilayered systems

The bilayered systems consist of two semiconductor materials, both of which have complementary band gaps. One of the materials usually has a large band gap and the other has a small one. The small semiconductor is mainly responsible for sensitizing the large band gap semiconductor through electron hole injection by visible light absorption. The electrons transfer between the two semiconductors may enhance the charge separation and inhibit the recombination rate by forming a potential gradient at the interface [39].

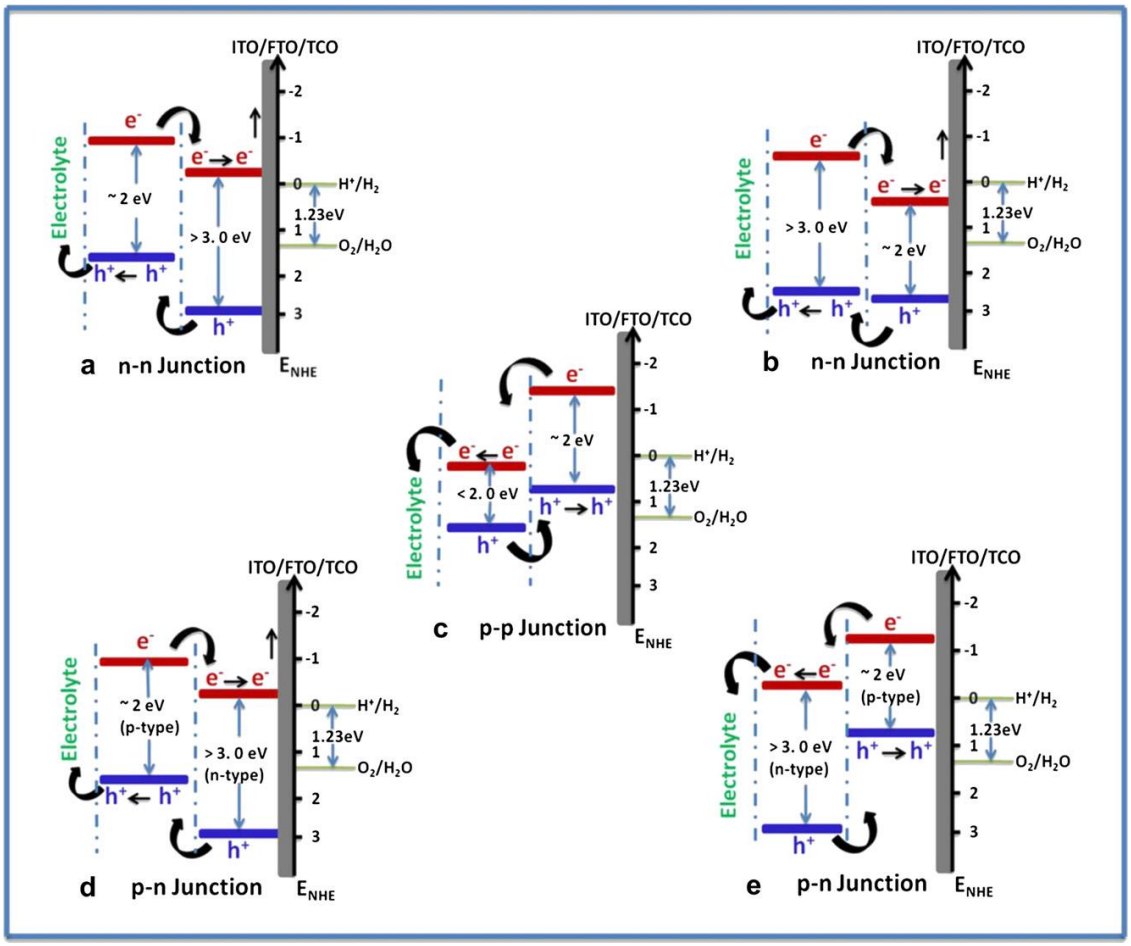

Figure 12 Favorable band edge alignment of wide/mid/narrow band gap semiconductors with redox potential

The significant reasons why bilayered systems show enhanced photocurrent are as follows :

- This allows for absorbance of large band gap material to be shifted towards visible region which is called red shift.
- The charge separation capabilities of both the materials is enhanced which results in decrease of recombination of charge carriers.
- The lifetime of charge carriers is also increased.

3.3 Hematite photoanodes

Iron oxide is a ubiquitous material because iron readily oxidizes to ferrous (+2) and ferric (+3) states, this along with iron being the fourth most common element (6.3 wt.%) make iron oxide an economical material for use in PEC systems. The band gap energy of hematite is reported to be between 1.9 and 2.2 corresponding of wavelength of 650 to 560 nm [40]. Some

other reasons why Hematite is considered as a leading photoanode material include its stability in alkaline environment and ability to be used in tandem with other small band gap materials like Si [41].

According to the U.S. Department of Energy, in order to be commercially viable, a PEC system needs to have a conversion efficiency of 10%, current density (JPC) of between 10 – 15 mA/cm2, be durable for up to 2000 hrs. and be economically feasible [42]. In order for Hematite to become a commercial material, it needs to follow the aforementioned guidelines. Altering both the structure and the composition of the material has been used to improve performance, which involves use of different deposition techniques like sputtering and spray pyrolysis. Concerning the changes in composition the best photocurrents have been observed by doping Hematite with Zn [43]. Another method for improving the performance of Hematite photoanodes is to use bilayered structure which allows for efficient utilization of incident radiation. The incorporation of high conductivity materials like MWCNTs and Graphene in the structure allows for better electron transport thus improving overall performance [38].

In the current study Hematite photoanodes have been prepared using spin coating and doped with Zn and Cu oxides (5 and 10%) in order to investigate the effect of their concentration on anode performance. The effect of using different conductive materials on the anode performance was checked by using MWCNTs and Graphene nanoplatelets.

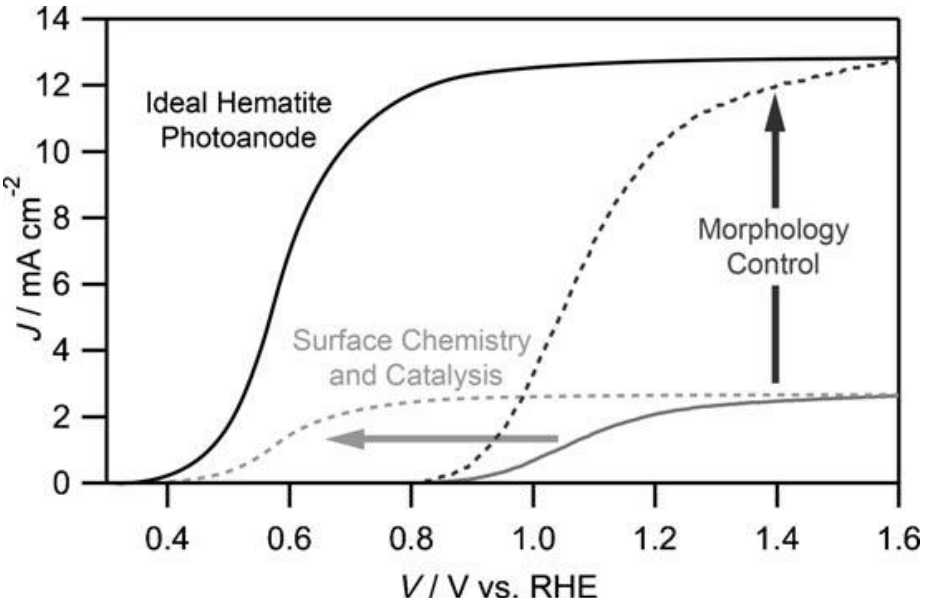

Figure 13 Improvement in the performance of Hematite photoanodes

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Chapter # 4

Materials and Methods

In order to access the hydrogen production capabilities of algae a suitable cultivation method needed to be set up after which subsequent experiments could be carried out using the algae harvested. The methods adopted are described under:

4.1 Algae cultivation

In order to study the algal hydrogen production, a sufficient supply of algae was needed. In this study the algal specie under consideration was Dictospherium iso 8-6. This is a non-motile algal specie and was cultivated under fluorescent light (800 lux).

4.1.1 Continuous culture

The continuous culture method, permits the maintenance of cultures very close to the maximum growth rate. Two categories of continuous cultures can be distinguished:

- Turbidostat culture, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system.
- Chemostat culture, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (e.g. nitrate) at a fixed rate and in this way the growth rate and not the cell density is kept constant.

4.2 Baseline hydrogen production

In order to enhance the algal hydrogen production, the baseline hydrogen production capacity of the specie under consideration needed to be known. Therefore, the algal cultures were subjected to experimentation in order to find out their capacities for producing hydrogen under different conditions. The main factors under consideration were light, substrate availability and oxygen concentration. The biological processes under consideration were:

- Direct biophotolysis

- Indirect biophotolysis

4.2.1 Direct Biophotolysis

a. In this process the splitting of water in the PS II because of incident solar radiation results in hydrogen production
b. Oxygen needs to be removed and anaerobic conditions need to be established in order for the algae to start hydrogen production by induction of Hydrogenase
    i. Dark adaptation (of up to 5 hr) is carried out in order to limit photosynthesis and to allow respiration to consume the oxygen present, thus allowing hydrogen production
        1. The rate of photosynthetic oxygen production falls below the rate of respiration and effectively caused anaebriosis
        2. It can take cells up to 40 hrs to reach hydrogen production state which can last up to 60 to 70 hrs.
    ii. As soon as light is provided photosynthetic activity is resumed and oxygen starts to form which can be removed either by:
        1. Physical removal using inert gas purging
        2. Addition of oxygen scavengers like Sodium dithionite (10 mg / 3.5 ml of 10^7 cell density culture)
c. Experimental setup required oxygen removal and thus addition of Sodium dithionite before exposure to light.

4.2.2 Indirect Biophotolysis

a. This process involves fermentative hydrogen production where the starch stored in the light time is converted to fermentative products like acids, carbon dioxide and hydrogen. Two steps are therefore involved:
    i. Photosynthetic assimilation of carbohydrate
    ii. Dark fermentation of the carbon reserve
b. This utilizes the temporal separation of hydrogen and oxygen production and thus utilizes the night times effectively as well. Other benefits include:
    iii. Avoids enzyme deactivation due to no oxygen production.
    iv. The formation of an explosive mixture of hydrogen and oxygen is avoided.

v. The purification of hydrogen produced this way is easier because carbon dioxide is easily removed.

c. Up to 0.43 moles of hydrogen are produced per mole of glucose utilized
d. 5 to 24 hr of induction time is required with 0.02 to 1.4 g/L glucose
e. Experimental design will be simple and addition of glucose may result in improved hydrogen production.

4.2.3 Experiments

Taking into consideration the above parameters multiple experiments had to be performed. The parameters which were kept constant were:

- Algal concentration (0.005g / 5ml)
- Volume of algal culture (60ml)
- Time (92hrs)

Following experiments were carried out:

4.2.3.1 Investigation of biophotolysis

a. Simple biophotolysis
b. Biophotolysis in the presence of Sodium dithionite
c. Biophotolysis in the presence of glucose
d. Biophotolysis in the presence of both Sodium dithionite and glucose

4.2.3.2 Investigation of indirect biophotolysis

e. Simple indirect biophotolysis
f. Indirect biophotolysis in the presence of Sodium dithionite
g. Indirect biophotolysis in the presence of glucose
h. Indirect biophotolysis in the presence of both Sodium dithionite and glucose

One gram of sodium dithionite and glucose were used in the tests.

4.2.4 Analysis of product gas

The amount of hydrogen produced was studied using a Gas Chromatograph. The testing conditions maintained in the GC are as follows:

Table 3 Conditions for gas analysis

Sample size	1ml
Injector conditions	
Temperature	200 °C
Pressure	98.8 kPa
Flow rate	78 ml/min
Column conditions	
Temperature	35 °C
Detector conditions	
Temperature	200 °C
Current	50 mA

4.2.5 Biodiesel production

Current research is focused on the use of algal biomass as a source of replacement hydrocarbon based fuel. One particularly important fuel that can be produced by algae is biodiesel. The amount of biodiesel that can be produced from algae depends on the amount of lipid content and the conversion technique used.

Since hydrogen production from algae accounts for a small amount of fuel production capability of the microbe a useful addition to the hydrogen production process could be the biodiesel production. Algal hydrogen production can be thought of as an additional step in the biodiesel production process. The lipid content and biodiesel production capacity of the strain under consideration has been found. A wet lipid extraction technique was used to firstly extract the lipid from the algae and then was converted to biodiesel before being analysed in the GC.

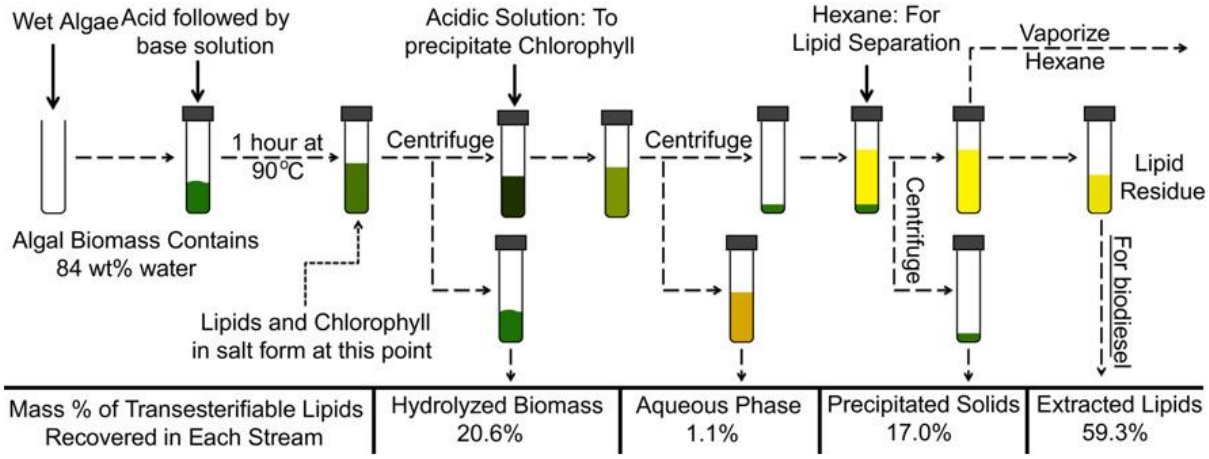


Figure 14 Biodiesel production scheme used utilizing wet lipid extraction

Two experiments were performed in order to compare the lipid content of the algae before and after the hydrogen production under dark anaerobic conditions. The process involved multiple steps which are described as follows:

4.2.5.1 Acid and base hydrolysis of algal biomass

Acid hydrolysis is accomplished by adding wet algal biomass (100 mg dry mass equivalent) to separate glass tubes with 1 mL of a 1 M sulfuric acid solution. The tubes are sealed using screw caps, mixed, and heated to 90 °C for 30 min with mixing provided at 15 min. These conditions allowed for the disruption of the algal cells in order to hydrolyze complex algal lipids to free fatty acids.

Following acid hydrolysis, 1.0 mL of a 5 M sodium hydroxide solution is added to each sample. The samples are subjected to heating at 90 °C for 30 min. The addition of sodium hydroxide neutralizes and transformed free fatty acids to their salt forms and saponifies any remaining complex lipids. The samples are cooled and centrifuged to pellet the residual algal biomass. The lipids remaining in their salt form dissolve in the aqueous phase by maintaining a high pH during centrifugation, thereby isolating them from association with the digested algal biomass.

The resulting supernatant phases are removed and collected from each sample in separate tubes, and the residual hydrolyzed biomass pellet is vigorously mixed with 1 mL of deionized water. The resulting suspension is re-centrifuged and the liquid phase again removed and added to the corresponding tubes containing the original supernatant. The residual hydrolyzed algal biomass is removed as a side stream.

4.2.5.2 Chlorophyll precipitation followed by lipid extraction from the precipitated solids

To the supernatant phases collected in the previous step, 3.0 mL of a 0.5 M sulfuric acid solution are added to form a solid precipitate. The addition of the acidic solution lowers the pH below seven, allowing for the salts of the free fatty acids to revert back to their free fatty acid form. In addition, the decrease in pH produces a solid precipitate. Due to the free fatty acids' insolubility in an aqueous solution, the lipids associate with the precipitating solids.

The resulting solid–liquid suspension is centrifuged, the supernatant removed as the aqueous phase and the precipitated solids are collected. To the tubes containing the collected solid precipitate, 5 mL of hexane is added. The samples are sealed, mixed, and heated to 90 °C for 15 min with mixing provided every 5 min. Heating the collected precipitate in the presence of hexane allows for the partitioning, or separation, of lipids from the solid phase into the solvent phase, while the chlorophyll remains within the solid phase.

After heating, the samples are cooled and centrifuged. The hexane phases are collected and transferred to separate tubes. Gentle heating is applied to the hexane phase to vaporize the solvent, leaving behind the extracted lipid residue. The precipitated solids are removed from the procedure as solid phase precipitate.

4.2.5.3 Direct transesterification of algal biomass

Direct transesterification is a procedure commonly cited and used in the literature to convert lipids within algal biomass to methyl esters for quantification, and is considered a candidate procedure for large scale biodiesel production from microalgae. Previously lyophilized algal biomass is directly transesterified. Results obtained from the direct transesterification of lyophilized algal biomass serve to both quantify the lipids present in the algal biomass used, and to serve as a positive control for comparison to the wet lipid extraction procedure. 100 mg samples of lyophilized algal biomass were directly transesterified using 1 mL of a 5% (v/v) solution of sulfuric acid in methanol..

4.2.5.4 Gas chromatography

Collected hexane phases, containing FAMEs, are analyzed using an FID detector. A chromatographic column is used to separate individual FAME compounds. Helium is used as the carrier gas at a constant flow rate of 2 mL/min. The oven is held at 100 °C for 1 min then ramped to 235 °C at a rate of 10 °C/min and held for 10 min. The front inlet is operated in splitless mode, with an initial temperature of 100 °C for 0.1 min and then increased to 235 °C

at a rate of 720 °C/min and held for 5 min. Injection volume is set at 1µl. FID temperature is maintained at 240 °C.

The total mass of FAMEs generated is based on the volume of hexane used to extract the FAMEs, after the esterification or transesterification reaction, and the concentration of FAMEs measured in the hexane phase.

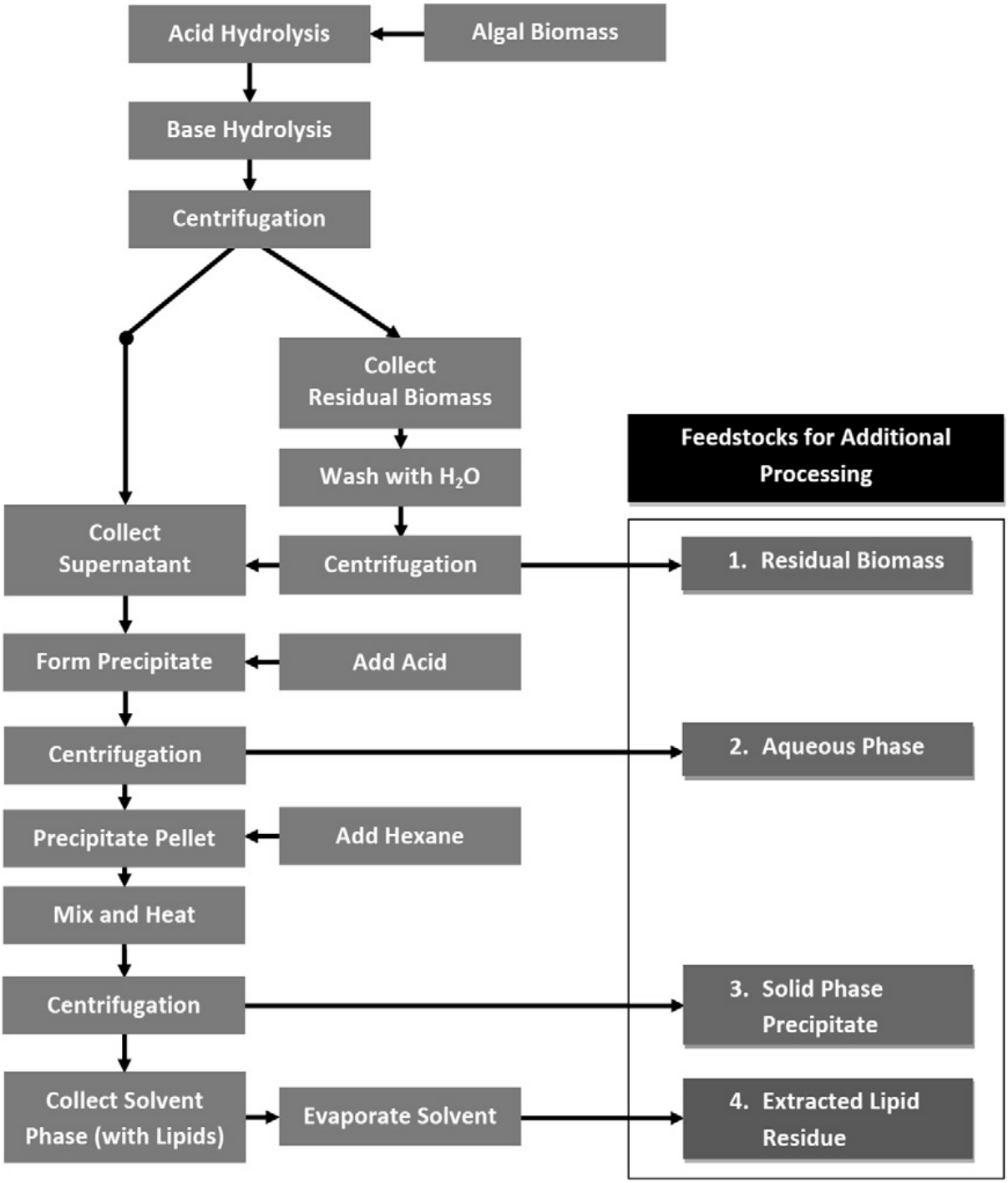


Figure 15 Wet lipid extraction procedure (Block Diagram)

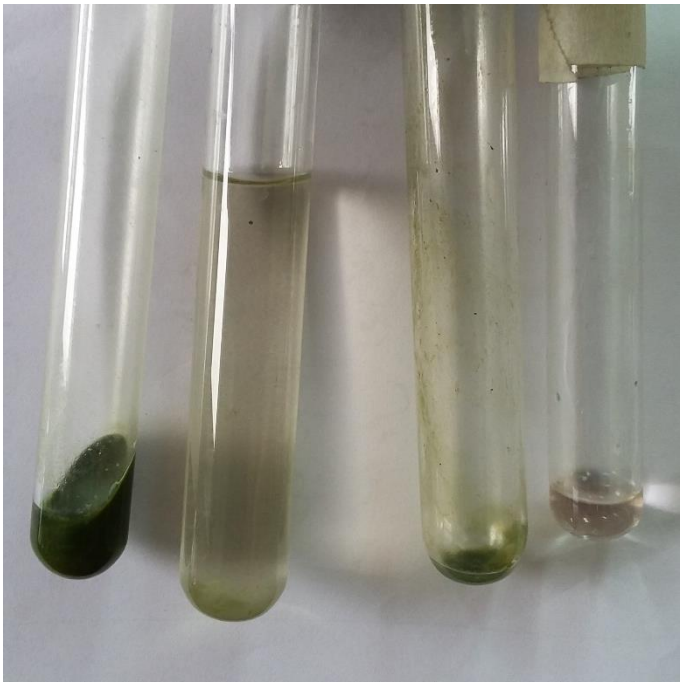


Figure 16 Sequential production of biodiesel from algae before Hydrogen production (Left to Right)

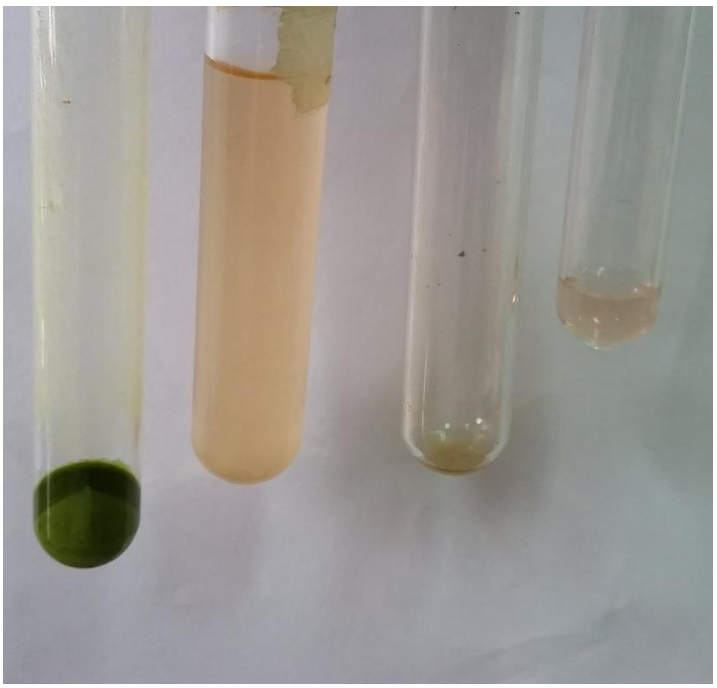


Figure 17 Sequential production of biodiesel from algae after Hydrogen production (Left to Right)

4.3 Microbial fuel cell

The fuel cell was a simple salt bridge based cell that contained Montmorillonite clay as electrolyte. The electrolyte was wrapped in filter paper and dipped in the anode and cathode mediums. Cathode was made of simple carbon paper with active surface area of $6 \times 9 = 54$ cm^2 while the anode was composed of copper plates with an area of 90 cm^2. The electrodes were connected to copper wire and an external resistance of 3KΩ was connected between the two electrodes in order to measure power. The cell was run for a month while its performance was monitored.

Two 500 ml beakers were used as containers for anode and cathode mediums. The yeast culture contained 2 g of S.cerevisiae along with 3 g of Granulated Yeast Extract to enhance electron transfer. Sugar molasses were used as the substrate with the final solution brix of 3%. This initial culture was fed with fresh molasses in order to maintain the brix. On the cathode side, Dictyosphaerium iso 8-6 culture was introduced with a cell count of 1.31×10^4 cells/ml. The algal culture was continuously aerated to avoid the algae from settling down.

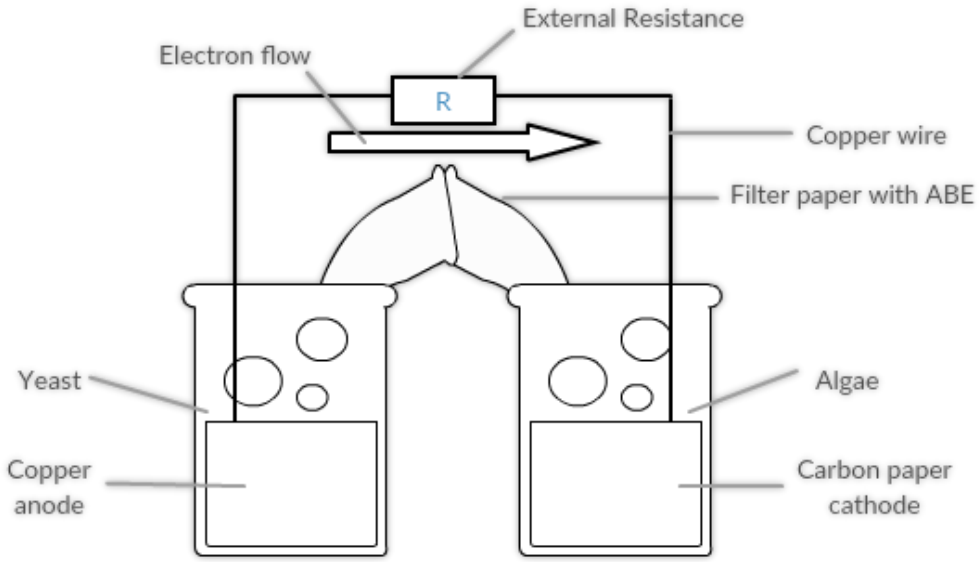


Figure 18 Microbial fuel cell with yeast based anode and an algal biocathode

4.3.1 Analytics

The voltage of the system was monitored using a digital multimeter. The readings were taken in mV. In order to find out the current, a 3kOhm resistor was connected to the system. Using the value of voltage, the current was found out following Ohm's law.

$$I=V/R$$

The polarization and power curve were plotted by manually varying the external resistance of the cell and calculating the voltage. The value of voltage was then used to find the current and subsequently the power density using the equation:

$$P=V\times I$$

Internal resistance of the cell was calculated using the Ohm's law.

$$\varepsilon=I(R+r)$$

$$V\_R=-rI+\varepsilon$$

Table 4 Chemical composition of Montmorillonite clay

SiO_2 %	55 ~ 80
Al_2O_3 %	5 ~ 20
Fe_2O_3 %	2 ~ 10
MgO %	0 ~ 8
CaO %	0 ~ 5
Na_2O %	0 ~ 2
K_2O %	0 ~ 2

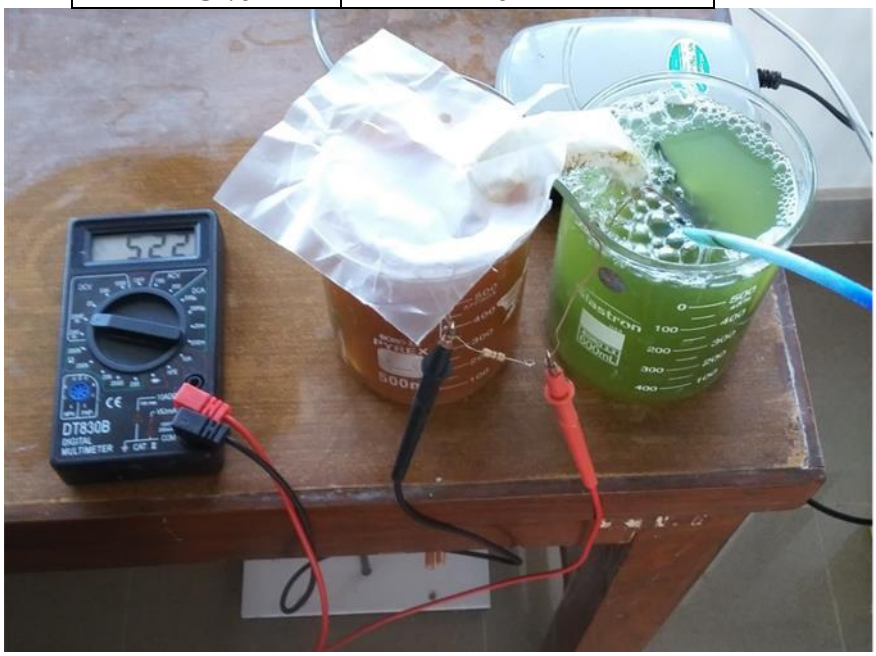


Figure 19 The yeast based MFC showing 522 mV of Open Circuit Voltage

4.4 Hydrogen production using yeast and algae mixture

The use of yeast Saccromyces Cerviaces with the algal culture allows for increased amounts of hydrogen production. In order to find out the concentration for which maximum

effect is observed, several experiments were performed in which the ration of algae to yeast was varied.

Table 5 Yeast to algae ratio studied for combined hydrogen production

Volume of algal culture	Volume of yeast culture	Ratio (Yeast to algae culture)
60	0	0
58	2	0.03
55	5	0.09
50	10	0.2
40	20	0.5

4.4.1 Algal culture

The algal culture used was the same as the one used for baseline studies. The concentration of algae was kept (0.005g / 5ml). The algae was kept under dark conditions in order to avoid photosynthesis and thus oxygen production which could disrupt the activity of Hydrogenase enzyme.

4.4.2 Yeast culture

The preparation of yeast culture involved mixing together 1g of dried yeast granules with 2g of yeast extract and 5g of glucose. This mixture was then left to incubate for at least half an hour to allow the yeast to activate before it was mixed with the algal culture.

4.5 Hematite photoanodes

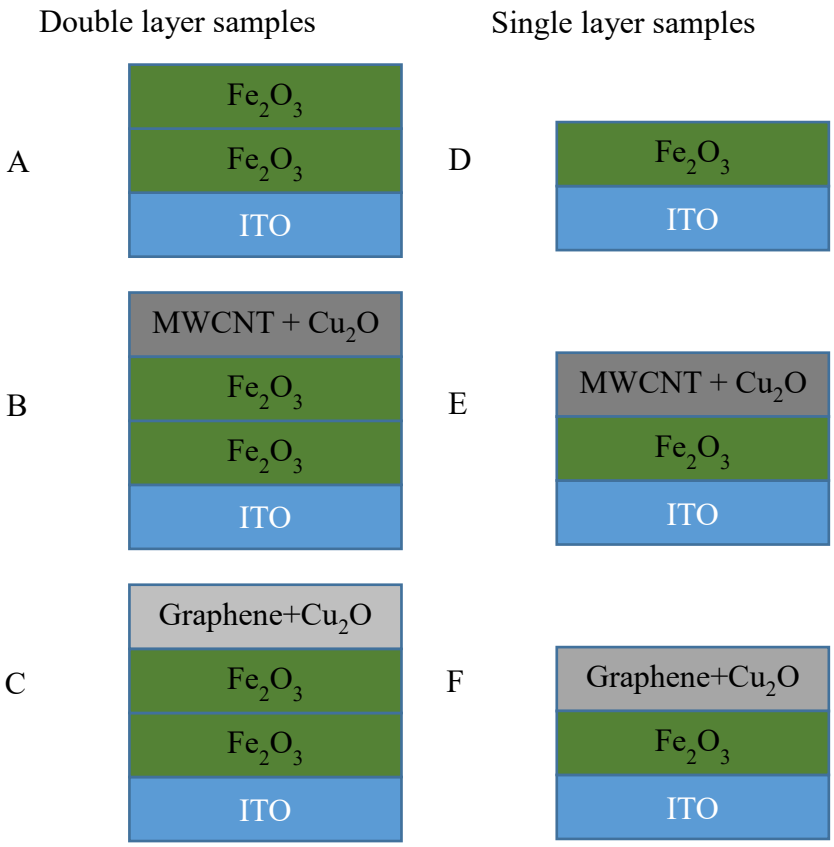
4.5.1 Synthesis of thin films

Iron (III) nitrate nonahydrate (99%, Aldrich), Zinc nitrate hexahydrate (98%, STREM Chemicals Inc.) & Copper II nitrate trihydrate (99%, Sigma Aldrich) were used to prepare solutions, using Acetlyacetone (1mol% compared to Iron (III) nitrate nonahydrate) as the solvent and PVP (Aldrich) (20wt% compared to Iron (III) nitrate nonahydrate) as the binder, in three concentrations (0%, 5% & 10% by weight) that were spin coated. The solutions were stirred at 80C for 4 hrs. before the layers were deposited on Indium tin oxide glass at 2500 rpm. Multiple layers were achieved by depositing on previously deposited layers which were all

heated at 100C for removing the solvent. The films were further treated at 350C for half an hour and at 500C for two hours to sequentially remove the binder and thermally drive the oxidation of Iron (III) nitrate nonahydrate.

For the secondary copper catalyst layers stock solutions of MWCNTs and Graphene nanoplatelets were prepared with 0.2mg of the conductive material with 1ml of IPA and 0.1ml of Nafion as solvent and binder respectively. The solutions were sonicated and stirred for four hours alternatively to allow homogenization. The Cu_2O nanopowder was then incorporated into the conductive solution resulting in the final solution containing 3mg of Cu_2O mixed with 0.1ml Nafion, 0.8ml IPA and 0.1ml of the original conductive solution. The layers were spin coated on to the Hematite layers and sintered at 450C for an hour in Argon atmosphere.

In order to use the deposited ITO glass as photoanode ohmic contacts were made using conductive silver paint and copper wire from the uncovered area. The contacts were then covered with non-transparent epoxy.



Set of samples with no addition of Cu_2O and ZnO

The sample set shown above contains undoped hematite layers. The same sample set was tested with 5% and 10% doped hematite layers.

1.1 Characterization

The crystalline phases of Hematite were characterized by X-ray diffactrometer using Cu Kα radiation (λ=1.5418 Å). The surface morphology of the films was examined using scanning electron microscopy.

The photoelectrochemical measurements were carried out using a potentiostat (PARSTAT® 2273, Advanced Electrochemical System) and Visible light source. The photoelectrochemical measurements involved current – voltage (I-V) characteristics of PEC cell consisting of three electrodes: the semiconductor electrode (anode), counter electrode (Platinum electrode) and reference electrode (saturated calomel electrode) immersed in 13 pH NaOH solution.

Chapter # 5

Results and Discussion

5.1 Algae cultivation

In order to find out the best conditions possible for the growth and easy harvesting of algae, two different cultures were maintained. One culture of 16L was compared with another of 50L both of which were inoculated with the same media and algae.

The cultures were started with BBM at first and in order to create sulphur deprived conditions, the culture was replenished with tap water each time it was harvested.

5.1.1 16L culture

The 16L culture was easier to maintain and to harvest algae from. The two 8L bottles were easier to clean and allowed for an easy way to harvest the algal culture by settling (decantation). The effect of temperature is apparent on the growth of algae in the continuous culture. With the increase in temperature as well as the age of the culture, the rate of algal production slowed. The optimum temperature of 26 °C for the growth of the specie had little effect on the overall growth pattern of the algae throughout the growth period.

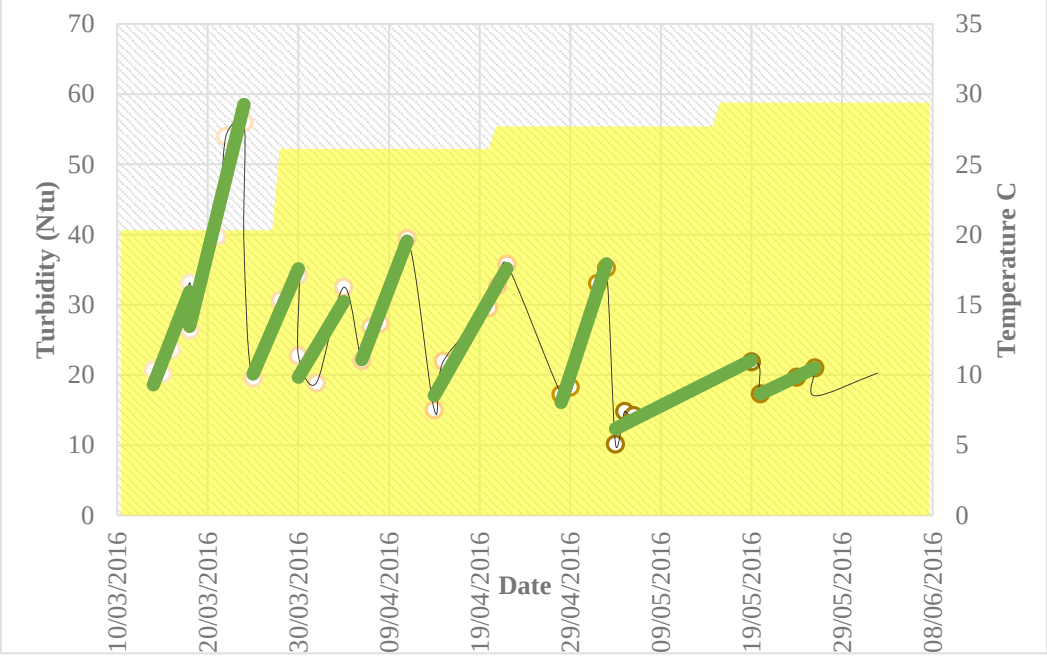


Figure 20 Continuous culture of 16 L

5.1.2 50L culture

The 50L culture was much harder to maintain, since the large volume was exposed to the environment and thus to contamination much more than the 16L culture. The effect of temperature was the same on the 50L culture as on the 16L culture. The growth rates were increasingly varied because of the large volume.

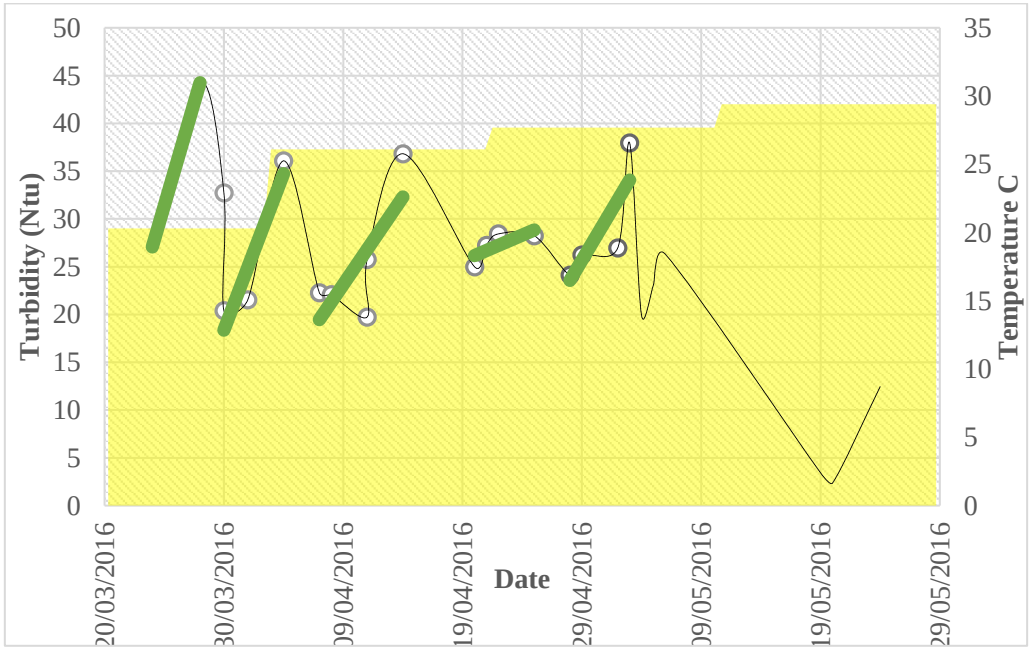


Figure 21 Continuous culture of 50 L

5.2 Baseline hydrogen production

5.2.1 Hydrogen production using indirect biophotolysis

These experiments were performed under dark conditions where the reaction vessels were covered with aluminum foil and left for 92hrs. The highest volume and rate of hydrogen production was observed when glucose was added. This is because of the availability of a substrate that can be broken down in order to release both protons and electrons that can then enter the electron transport chain and subsequently be reduced by the Hydrogenase enzyme. The catalytic activity of Hydrogenase is important here because without it other metabolic products instead of hydrogen will be produced.

The addition of sodium dithionite in order to eliminate the dissolved oxygen resulted in the death of the algae in the reaction vessel and therefore no gas was produced. While in conjunction with glucose, sodium dithionite did allow for production of some hydrogen because the absence of oxygen induced by it activated the hydrogenase and the substrate present was effectively fermented to hydrogen. This process can be a viable option for increased fermentative hydrogen production if the death of algae can be avoided.

It can be said that the additives result in enhancement of baseline hydrogen production by indirect biophotolysis. The effect of these additives is as follows:

- Glucose increases the availability of electrons because the breakdown of this substrate by algae releases electrons.
- Sodium dithionite induces Hydrogenase activity because it allows for anaerobic environment to be created which is a key factor.

Table 6 Algal hydrogen production under indirect biophotolysis

Experiment	Gas Volume (ml)	Moles of Hydrogen (nmol)	Total Hydrogen volume (µl)	Hydrogen conc. (%)	Production rates (nmol/l.h)
Indirect Biophotolysis	0.4	19.01	0.43	0.107%	3.30
Imdirect Biophotolysis (Na dithionite)	0	0.00	0.00	0.000%	0.00
Indirect Biophotolysis (Glucose)	3	23.72	0.53	0.018%	4.30
Indirect Biophotolysis (Glucose + Na dithionite)	2.9	19.44	0.44	0.015%	3.37

5.2.2 Hydrogen production by direct biophotolysis

As is the case with indirect biophotolysis, in direct biophotolysis the addition of glucose allows for greater yields of hydrogen. All of the experiments performed here involve a 24hr dark incubation period where the algae is subjected to anaerobic environment and thus the activity of Hydrogenase is induced. After this incubation period the reaction vessels are exposed to light and the process of light driven hydrogen production is allowed to begin.

In the baseline test for direct biophotolysis, hydrogen production is not observed mainly because of the presence of a large amount of oxygen. The results show significantly high peak for oxygen and this oxygen is produced because of the continuation of photosynthesis. Because of the presence of this oxygen the Hydrogenase enzyme is not activated and thus no hydrogen production is observed.

The highest amount of hydrogen is observed when glucose is added and the reason for this the availability of a substrate that can be used as an additional source of electrons. A point to be noted here is that this amount and rate of hydrogen production is higher than that of indirect biophotolysis under the presence of glucose. This is important because it assures us that the presence of light allows the algae to effectively use the substrate and at the same time split water to produce hydrogen.

Under the presence of sodium dithionite, hydrogen production is observed and is higher than that observed under indirect biophotolysis. This is a key result as it shows that not only can sodium dithionite induce hydrogenase activity but it also clarifies the effect of light on the ability of algae to produce hydrogen. The presence of light can aid in the hydrogen production process in two ways:

- The presence of light splits the water molecules with then allow for the availability of both electrons and protons for hydrogen production.
- The availability of light to the algae allows it to maintain remedial levels of photosynthesis, thus preventing its death due to the anaerobic environment created by the addition of sodium dithionite.

When both glucose and sodium dithionite are added, the production of hydrogen decreases. This can be attributed to the fact that addition of sodium dithionite caused anaerobic conditions which are unfavorable for the survival of algae, thus causes the algae to utilize the glucose for sustaining basic life functions instead of hydrogen production.

Table 7 Algal hydrogen production under direct biophotolysis

Experiment	Gas Volume (ml)	Moles of Hydrogen (nmol)	Total Hydrogen volume (µl)	Hydrogen conc. (%)	Production rates (nmol/L.h)
Biophotolysis (Control)	4.4	0.00	0.00	0.000%	0.00
Biophotolysis (Na dithionite)	0.45	4.86	0.11	0.024%	1.25
Biophotolysis (Glucose)	1.1	35.96	0.81	0.073%	6.52
Biophotolysis (Glucose + Na dithionite)	1.3	3.20	0.07	0.006%	0.56

5.2.3 Comparison of direct and indirect biophotolysis

The highest rate and amount and rate of hydrogen production is observed when glucose is added under direct biophotolysis while the minimum hydrogen production is observed when sodium dithionite is added under indirect biophotolysis.

The inferences drawn from the comparison of these two distinct modes of hydrogen production are as follows:

- The availability of a substrate increase the rate of hydrogen production because it not only sustains the microbe but provides additional electrons as well.
- This particular specie of algae is not able to produce hydrogen via biophotolysis. The conditions provided are not favourable for the induction and subsequent activity of Hydrogenase.

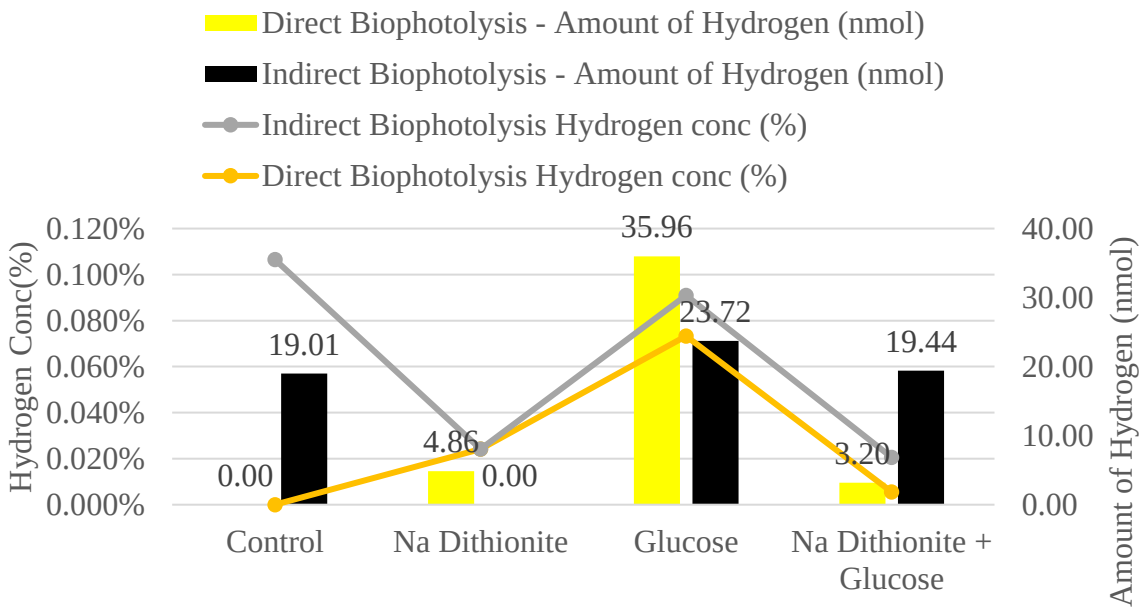


Figure 22 Comparison of product gas volume and Hydrogen concentration

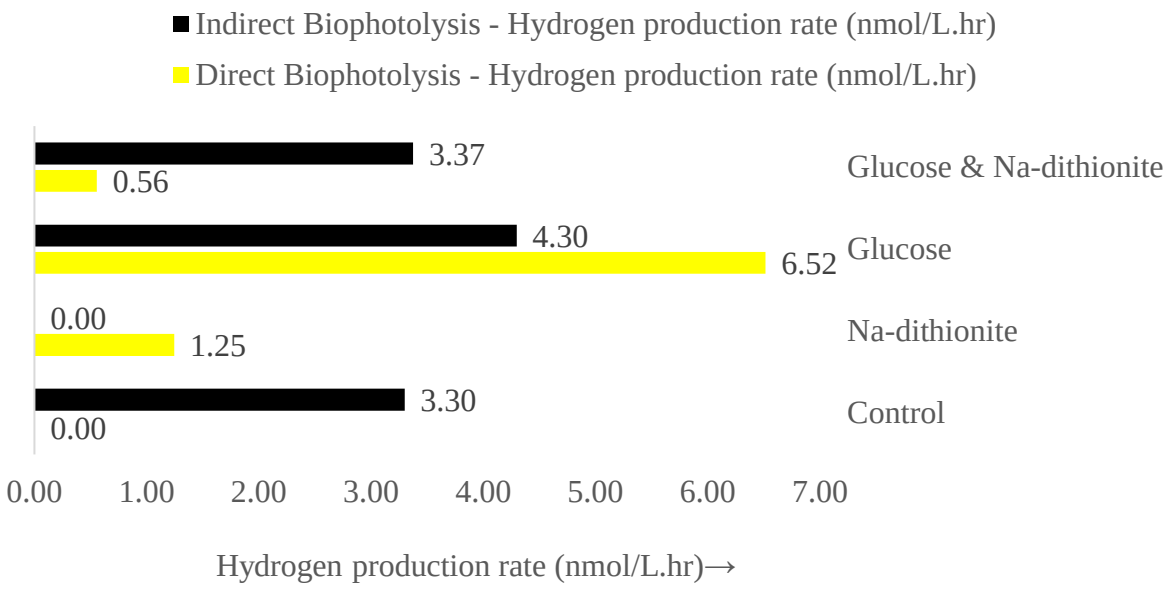


Figure 23 Graphical comparison of direct and indirect biophotolysis hydrogen production

The other main component in the product gas is oxygen which varies according to the conditions in the reaction vessel. The maximum amount of oxygen is found in the direct biophotolysis in the algal culture in the absence of any additive, which is because of the photosynthetic activity in the culture. All direct biophotolysis experiments produce oxygen.

On the other hand, the indirect biophotolysis experiments show lower amounts of oxygen production. This is advantageous because the lower amounts of oxygen in the product gas allow for the purity of the gas to increase.

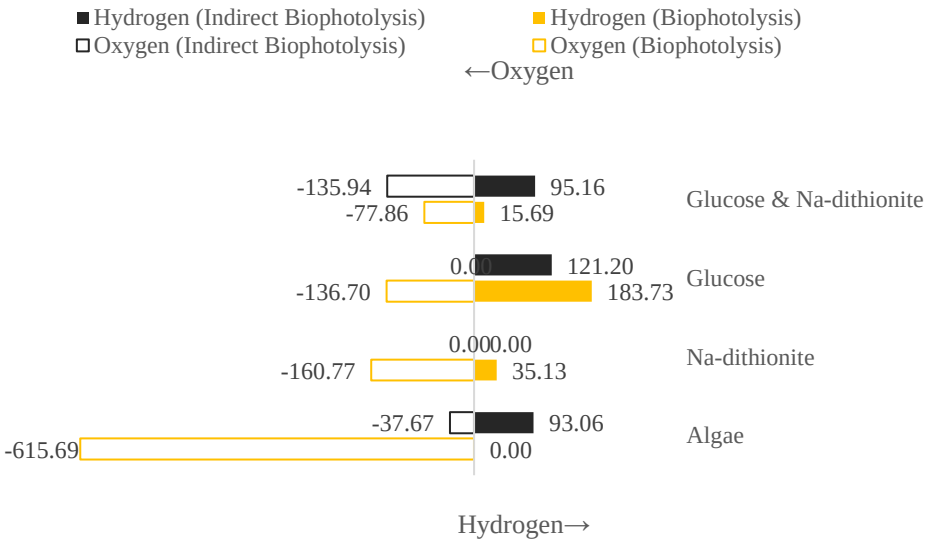


Figure 24 Comparison of hydrogen to oxygen production

5.2.4 Biodiesel production

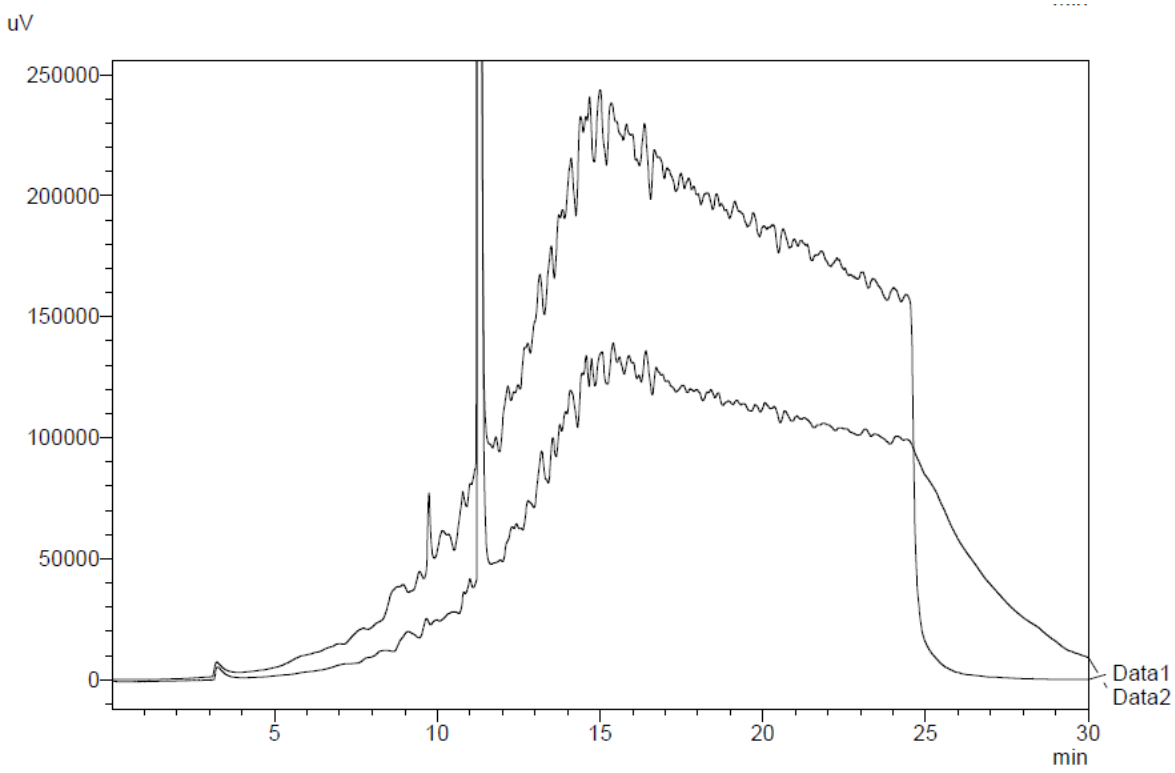


Figure 25 Comparison of biodiesel content of algae before and after hydrogen production (Data 1: Before Hydrogen Production & Data 2: After Hydrogen Production)

5.3 Microbial fuel cell

5.3.1 Effect of time on the open circuit voltage

The cell was observed to mature over a period of a month as the biofilm started to develop on the carbon paper electrode. After 30 days the cell obtained a maximum OCV and then the value started to decrease. This decrease in the OCV can be because of the deterioration of the biofilm deposited on the electrodes after which a constant OCV was achieved (0.6 V). This change in the OCV is demonstrated in Figure 2. This gradual increase in the voltage can be attributed to the development of biofilm on top of the electrode. The copper electrode gained a layer of rust over time which may have contributed in the creation of favorable conditions for the yeast to transfer electrons.

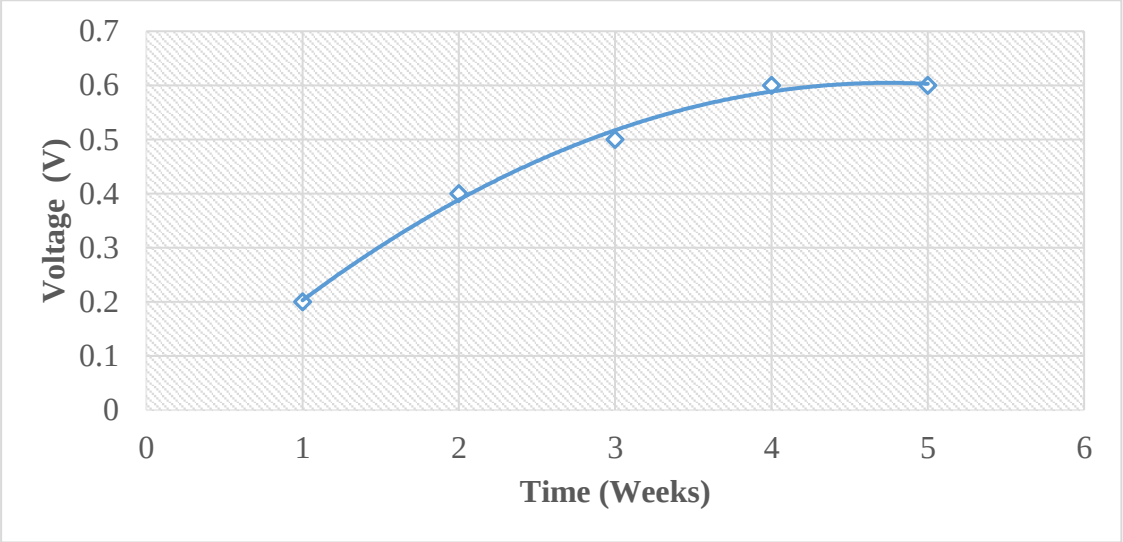


Figure 26 Development of the open circuit voltage of the MFC over a month

5.3.2 Charge transport

The electrons need to be transported from the medium to the anode in order to create potential. This transfer of electrons is carried out with the help of addition of mediators. The mediator added here was yeast extract. Still there is a lag between charge creation in the medium and the transfer of the charge to the surface of the anode. This results in a slow response form the cell. A long time is required in order to achieve the maximum potential which is roughly 5 min. The rise in the voltage is shown in Figure 3. About 98% of the

maximum voltage (490 mV) is achieved within 2 min after which the charge transfer is slowed down. This slowing down of charge transfer is because of increase in the negative potential of the anode which repels electrons. Because of this phenomenon, all the readings of voltage taken required a settling time of 2 min.

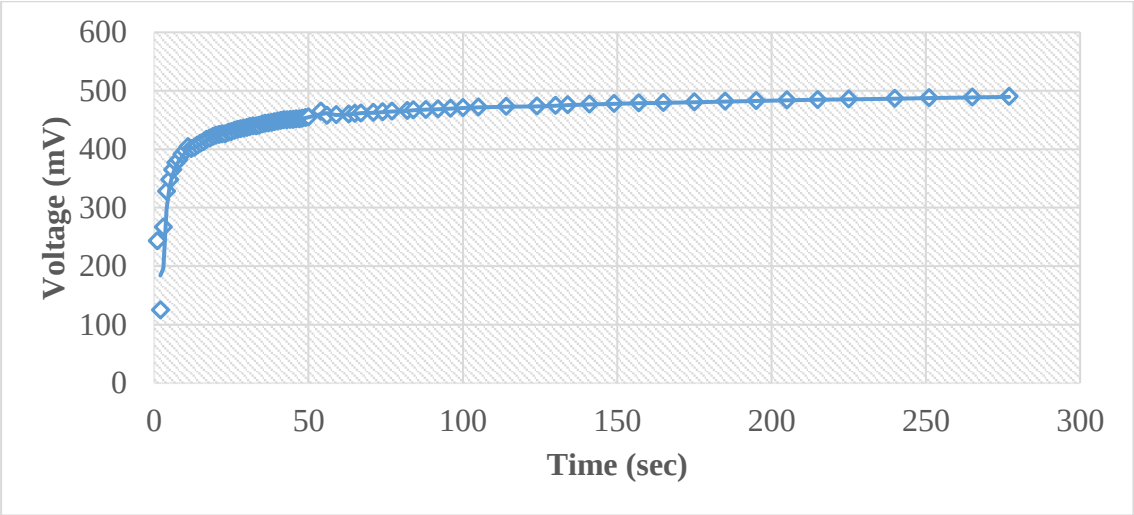


Figure 27 Time required for the MFC to achieve maximum open circuit voltage

5.3.3 Performance curves

The power curve of for the fuel cell was plotted and gave a peak of 1.04 mW/m^2 at a current density of 5.75 mA/m^2. A linear trend was observed in the V-I curves at lower values of resistances. This response is in coherence with the performance of previously demonstrated fuel cells.

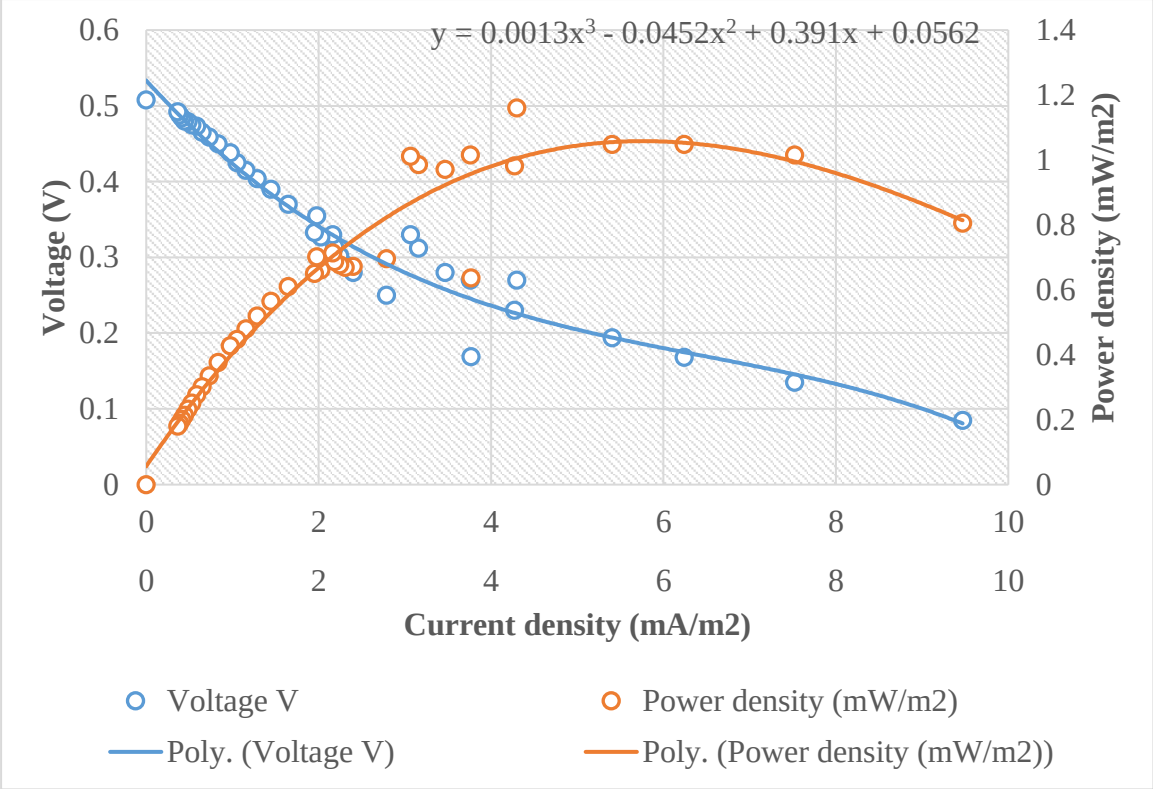


Figure 28 Performance curve of the MFC

5.3.4 V-R Curve

A second order quadratic equation represents the relation between the external resistance and the output voltage of the cell. The relation between voltage and current is not linear and this lag in the increase of voltage can be explained by the mass transport restrictions. Microbes are unable to create the potential with a pace fast enough to keep up with discharge.

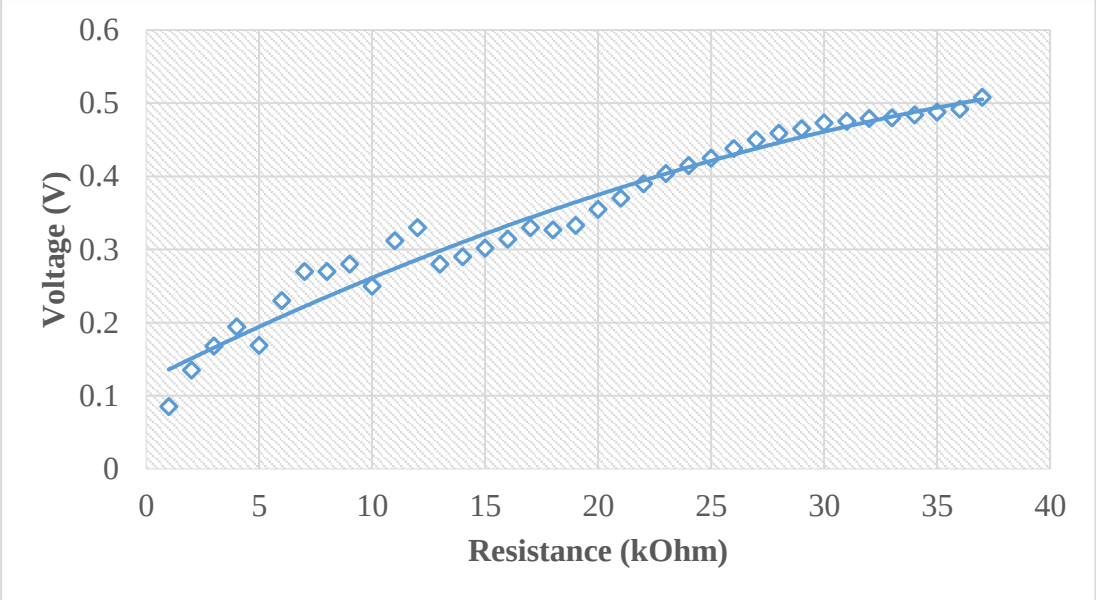


Figure 29 Voltage response of the MFC with change in the external resistance

5.3.5 Internal resistance

The internal resistance of the system needs to be high for the electronic current to be small. The relation between voltage and current of the cells shows the internal resistance to have a value of 5500 Ohm. The same equation also gave the value of the emf of the cell, by projecting the graph to the point where current is 0, which was around -464.8 mV. This value of emf can provide us with the cathode potential by using the standard potential for glucose i.e. -428 mV.

$$E_{emf} = E_{Cat} - E_{An}$$

$$E_{Cat} = E_{emf} + E_{An} = 464.8 + (-428) = 36.8\, mV$$

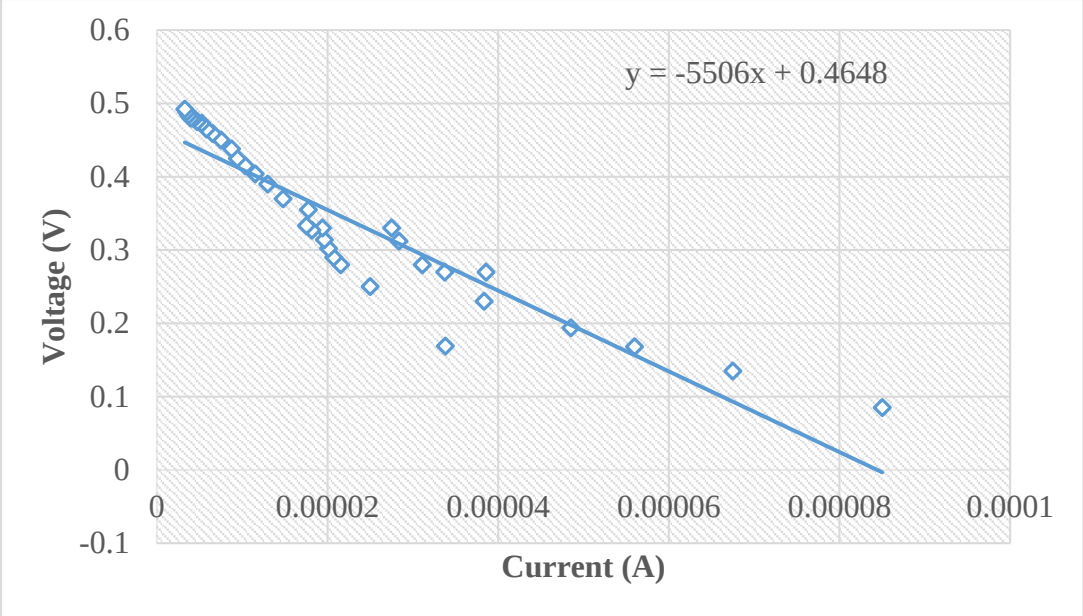


Figure 30 Current-Voltage plot for the MFC used for calculating the internal resistance

5.3.6 Evaluation of the MEC

The experiment for the evaluation of the capability of the algal culture to produce hydrogen by obtaining electrons from electrons is shown in figure. The yeast culture in the conical flask has copper anode present in it while the cathode is a carbon cathode. External potential is provided with a 1.5V cell. The experimental setup is established for about 92hrs before the gas produced is analyzed.

Figure 31 Experimental setup for Microbial Electrolysis Cell

5.3.6.1.1 Comparison of results with baseline

The algal culture was subjected to anaerobic dark conditions during the experiment which was to ensure the activity of Hydrogenase enzyme. The total volume of the algal culture used was 50ml the rest of the volume was occupied by the carbon cathode. The increase in hydrogen is about 3% which is marginal. This however is crucial to consider that the amount of oxygen is also higher in the MEC experiment which should impede the production of Hydrogen by deactivating Hydrogenase enzyme. The increase in the amount of hydrogen observed can be attributed to the availability of electrons by an electrode.

The MEC setup has its advantages and drawbacks which need to be considered while comparing it to more direct methods of improving hydrogen generation. The advantages include:

- Ability to keep the two cultures separate which results in longevity of the algal culture.
- The algal culture can be recycled after being used in the MEC.
- This setup can become a sequential part in the treatment of algal biomass whereby after being used in the MEC, the algae can be subjected to biodiesel production.
- The substrate on the anode side can be waste matter which can be treated separately in a different chamber.

Disadvantages of the MEC include:

- The setup for such a system needs to be extremely leakage proof in order to avoid deactivation of Hydrogenase.
- The proton transport through the Montmorillonite clay is relatively slow.

Table 8 Comparison of MEC with indirect biophotolysis

Experiment	Gas Volume (ml)	Moles of Hydrogen (nmol)	Total Hydrogen volume (µl)	Hydrogen conc. (%)	Production rates (nmol/l.h)
Indirect Biophotolysis	0.4	19.01	0.43	0.107%	3.30
MEC	7.8	19.64	0.44	0.006%	4.36

5.4 Hydrogen production using yeast and algae mixture

The addition of yeast culture in the algal culture allows the hydrogen production to increase manifold. Different ratios of algal to yeast culture were tested to find the optimum ratio for increased hydrogen production. The volume of gas produced increases as the ratio is increased which is because of the increase in fermentative gas production by yeast. The major portion of this increased gas production is composed of gases other than hydrogen.

The hydrogen gas volume starts to increase at even very small ratios as can be seen when compared to the baseline. The trend continues till the ratio of around 0.1 after which the amount of hydrogen decreases. The decrease in the amount of hydrogen is because of the decrease in the amount of algal culture which results in decrease of Hydrogenase enzyme and thus the reaction activity.

The trend of increase in the hydrogen production is due to the fact that the amount of Hydrogenase is high.

Table 9 Alage and Yeast mixture hydrogen production

Experiment	Gas Volume (ml)	Moles of Hydrogen (nmol)	Total Hydrogen volume (ul)	Hydrogen conc. (%)	Production rates (nmol/g.h)
Indirect Biophotolysis	0.4	19.01	0.43	0.107%	3.30
58ml algae with 2ml yeast sol.	16	5251.42	117.71	0.736%	963.21
55ml algae with 5ml yeast sol.	17	63716.14	1428.13	8.401%	12324.21
50ml algae with 10ml yeast sol.	31	16123.76	361.40	1.166%	3583.06
40ml algae with 20ml yeast sol.	24	8294.14	185.90	0.775%	2383.37

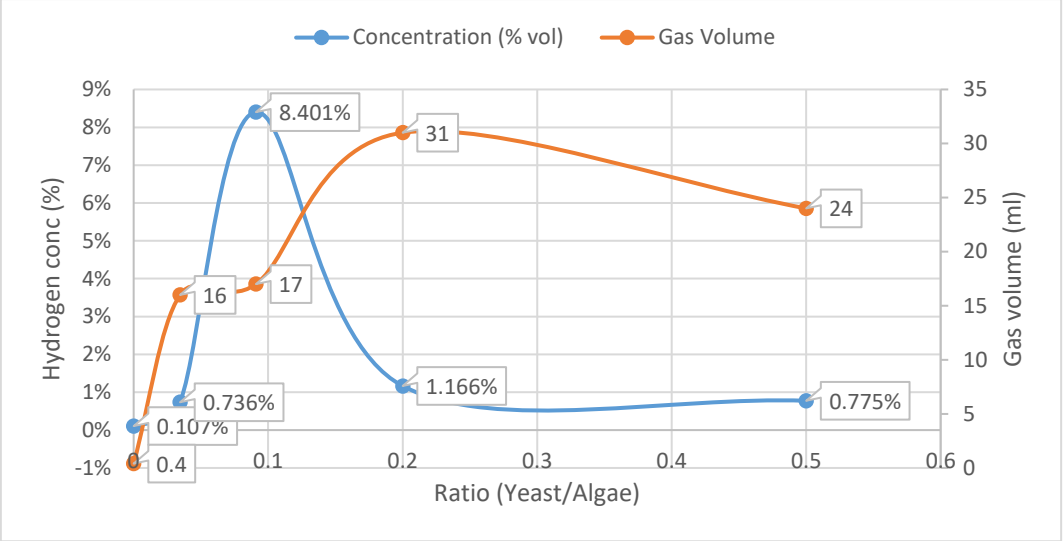


Figure 32 Comparison of total gas to Hydrogen volume

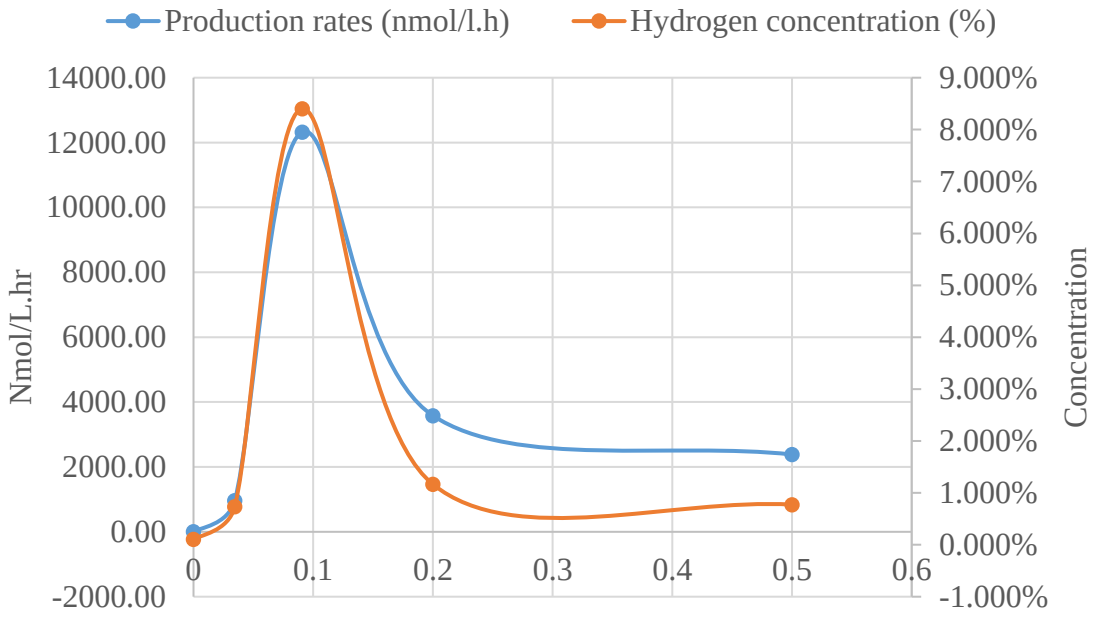


Figure 33 Hydrogen production from mixture of algae and yeast

5.5 Hematite based photoanodes

5.5.1 Electrochemical characterization

The effect of catalyst layer is most prominent and Table 10 shows that the current density increases with the introduction of a catalyst layer. Among the catalysts used MWCNT based catalyst layers show better performance as compared to Graphene based catalyst layers. The enhancement of performance can be attributed to the surface morphology of the electrodes

deposited with MWCNT based catalyst. The surface is more porous which allows for an increase in active surface area and thus improves overall performance.

Table 10 Average current density (mA/cm^2) of Hematite photoanodes of varying composition

Dopant concentration	No. of Hematite layers	Type of catalyst layer		
		No catalyst layer	Graphene Nano platelets catalyst layer	MWCNT catalyst layer
0%	Single layer	6.11	8.28	8.47
	Double layer	6.27	8.43	8.56
5%	Single layer	4.91	9.80	10.49
	Double layer	5.53	10.08	10.70
10%	Single layer	5.37	9.38	9.57
	Double layer	5.23	9.40	9.60

The best results were obtained with the double layered hematite layered anodes with MWCNT based catalysts. The increase in number of layers does increase the performance but not in all cases. The reason behind this effect is the increase in the thickness of the Hematite layer which results in absorption of more light which can be converted to chemical energy. This effect however is expected to disappear if the thickness of the layers is further increased because of the inevitable losses due to recombination.

The use of MWCNT increases the active surface area of the anode and allows for an increase in the reaction rate. When the surface morphology of MWCNT is compared to Graphene nanoplatelets, the difference in porosity is clearly visible. The Graphene based catalyst has low active surface area and thus the performance is better for MWCNT catalyst containing anodes.

The third and final factor tested here was the concentration of dopant in the Hematite layer. The increase of dopant upto 5% results in enhancement of anode performance, while further increase in the concentration to 10% reduces the performance possible due to increase in distortions caused because of the presence of impurities in the Hematite structure.

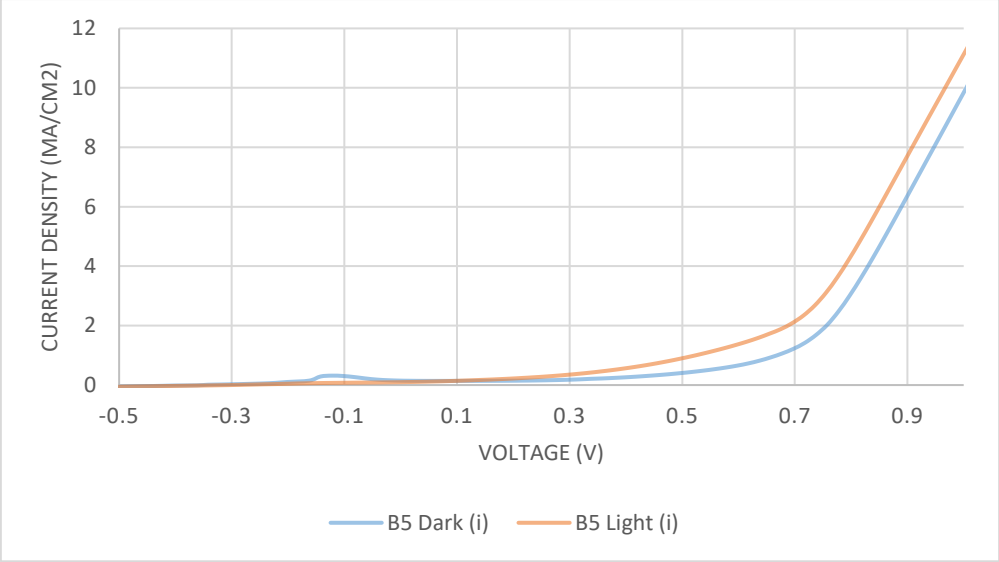


Figure 34 Photocurrent density - voltage (mV/SCE) characteristics for Hematite (2 layers) / (Cu2O + ZnO - 5%) + (MWCNT + Cu2O)

5.5.2 Morphological study

The morphology of the anodes gives a clue to the perfoemannce of the anode. The surface of Hematite layers is rough and has cracks that increase the active surface area. The Hematite layer alone is not capable of performing well in the electrochemical conditions. In order to enhance the activity, catalyst layers are introduced.

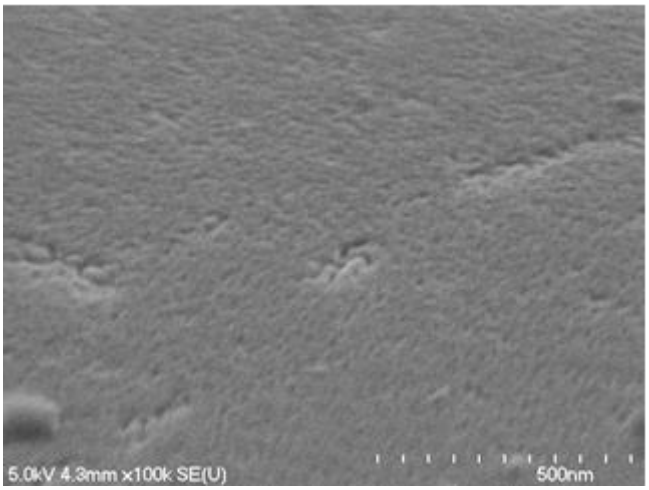


Figure 35 Surface morphology of Single hematite layer

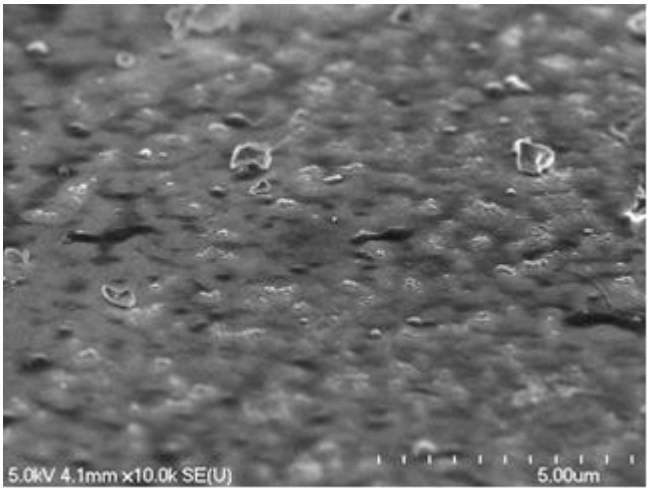


Figure 36 Surface morphology of anode deposited with Graphe nano platelet catalyst

Carbon based catalysts are used to increase the conductivity of the anode and subsequently enhance its performance. The use of Graphene nano platelets does increase the conductivity but the active area is still low.

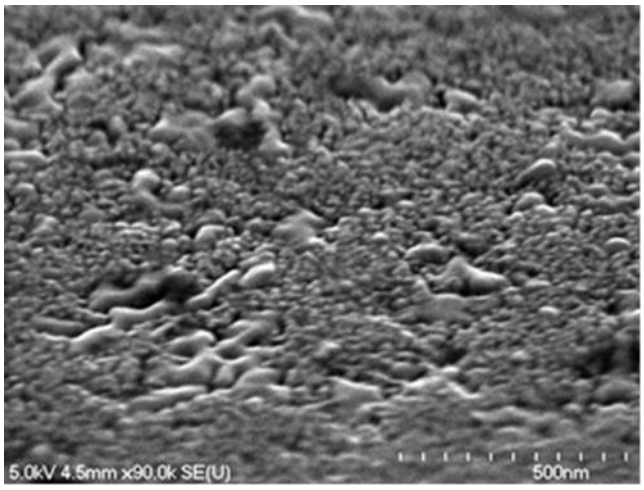


Figure 37 Surface morphology of photoanode doposited with MWCNT catalyst

Therefore, with the use of MWCNT catalyst, both the active area and the conductivity is improved which allows for a better overall performance.

Conclusion

- The amount of hydrogen produced increased significantly when Algae and Yeast were mixed i.e. 0.01 mmol/L.h or 12324.21 **nmol/L.h**
- The rate of H2 production by *C. reinhardtii* is 0:07 mmol/L.h *
- The difference is due to difference in the species used for Hydrogen production.
- The significant improvement in Hydrogen production due to algal and yeast mixing is of higher importance

Experiment	Production rates (nmol/L.h)
Indirect Biophotolysis	3.30
Biophotolysis (Glucose)	6.52
55ml algae with 5ml yeast sol.	12324.21

- Sample B5% [Hematite (2 layers) / (Cu_2O + ZnO - 5%) + (MWCNT + Cu_2O)] showed significantly high current density of about 10 $mA.cm^{-2}$
- The observed current density is an improvement compared to past work on such photoanodes (Ti -Fe_2O_3 + MWCNTs + Cu_2O – 6.4 $mA.cm^{-2}$) and is close to the maximum theoretically allowed for hematire (12 $mA.cm^{-2}$)*
- It appears that lowering the amount of Cu_2O and ZnO enhances the performance of the cell
- The best results are obtained with lower doping in the presence of a catalyst layer.

Proposed Future Work

Hydrogenase are a naturally occurring solution for the catalysis of Hydrogen production process. They can be harnessed better in conjunction with semiconductor anodes which can split water and produce free protons which can be converted to Hydrogen in the presence of Hydrogenase.

Future work can focus on the use of these two technologies together where Hematite based anodes can work with Algal bio cathodes in order to economically harness the water splitting properties of Hematite and the Hydrogen production capability of Algae. This will not only reduce the cost of the process but can also become part of more complex systems where Hydrogen can be stored for use in fuel cells and the algae can be harnessed for multiple uses.

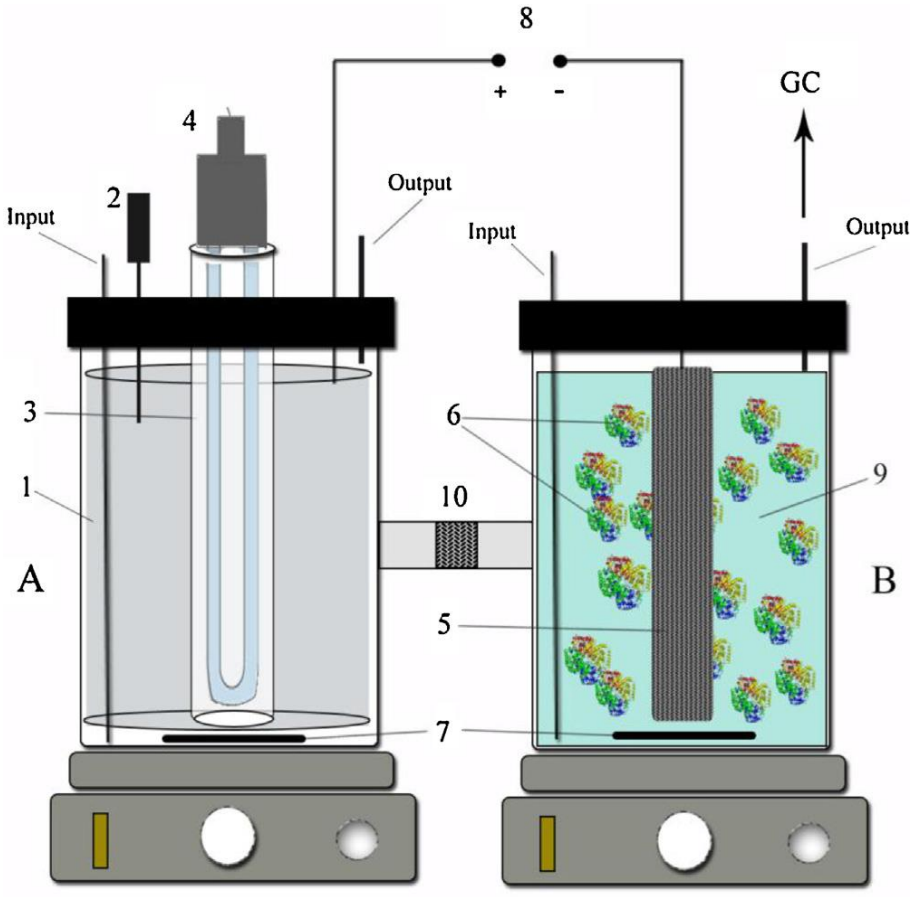


Figure 38 Schematic representation of the algae photoelectrocatalytic reactor*

(A)anode; (B) cathode

1: working electrode (TiO2/Ti cylinder)

2: Ag/AgCl referenceelectrode

3: cylindrical sleeve where lamp (4) is inserted

4: UV-A lamp

5: counterelectrode (Carbon rod)

6: hydrogenase enzyme

7: magnetic stirrer

8: potentio-stat

9: algae solution in Sueoka SF medium (contains MV+2and Triton X-100)

10:electrolytic junction (Nafion membrane).

Electrochemical integration of algal biodiesel production and yeast based fermentation for power production

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Abstract

Bio-refineries as a concept are focused on the production of chemical energy carriers and, in certain cases, electrical power as well. The production of electrical power, however, is done via thermochemical processing which an inherently inefficient process. Here an electrochemical means of electrical power production is proposed alongside the production of ethanol and biodiesel using yeast and algae respectively. The microbes interact with each other in a microbial fuel cell (MFC) consisting of acid treated Montmorillonite clay (Activated Bleaching Earth) as electrolyte instead of expensive proton exchange membranes. The breakdown of substrate on the anode by yeast provides protons to the algae on the cathode. Experimental evidence proving the efficacy of the concept is provided using an electrochemical cell with salt bridge architecture.

Key words

Microbial Fuel Cell; Bio-cathode; Fermentation; Activated Bleaching Earth

Microbial fuel cell (MFC)
Proton exchange membrane fuel cell (PEMFC)
Microbial electrolysis cell (MEC)
trans Plasma Membrane Electron Transport frameworks (tPMET)
Plasma Membrane Oxido-Reductase frameworks (PMOR)
Cation exchange capacity (CEC)
Open circuit voltage (OCV)

Introduction

Biodiesel and bio-ethanol are two of the most common fuels produced from biomass technologies. Anaerobic fermentation is a crucial technology which allows economic transformation of cellulosic biomass to valuable fuel and other valuable products [1]. Similarly, biodiesel production from algae is a promising source of renewable higher hydrocarbon based fuels [2]. Algae are capable of yielding higher amounts of lipids as compared to vascular plants. Ethanol is also utilized in the transesterification of raw lipids in order to convert them into fatty acid esters. Utilizing the ethanol from biomass fermentation will allow the process of biodiesel production to become more sustainable. Moreover, the carbon footprint of the process can be reduced by scrubbing the carbon dioxide produced during fermentation by the algae.

In order to ensure uninterrupted supply of bio-ethanol, the fermentation process should be carried out as close to the photobioreactors as possible. By doing so, the two processes, anaerobic fermentation and algal biodiesel production can be integrated together, thus allowing the sharing of resources and betterment of process economy. Another opportunity is also presented by the proposed integration which can allow us to harness electrical power. The fermentation broth is capable of providing a potential gradient compared to the algal culture growing under aerobic conditions. This potential gradient can be harnessed in microbial fuel cells.

A microbial fuel cell is a device that converts the chemical energy of organic matter into electricity. Most MFCs use anaerobic bacteria at the anode for the oxidation of the organic matter and transfer the electrons thus produced to the anode from where they are transferred to the cathode and are used to reduce protons. Baker's yeast can also be used as the active media in the anode chamber. While on the cathode side, the reduction of the protons is facilitated by the presence of noble metal catalysts such as platinum. Addition of yeast extract increases the performance of the cell as it acts as a mediator for electron transfer [3]. Inorganic catalysts are extremely expensive and their use can only be justified for extremely rigorous applications. An alternative approach for these inorganic catalysts is biological catalysts such as hydrogenase and nitrogenase. These enzymes are present in many different microbes including algae [4]. The cathode can therefore be submerged in algal medium to provide catalytic activity. Such bio cathodes therefore hold the key to an economical MFC based system.

An important component of the fuel cell is the electrolyte which provides a path for the protons to pass from the anode chamber to the cathode chamber. The most commonly used electrolyte for PEMFC and MFCs is Nafion. Nafion is an excellent conductor of protons and provides a reliable functioning of the fuel cell [5]. But the cost of the membrane is a hindrance to the large scale application of the fuel cell technology. Among other alternatives, soil has also been utilized as an effective electrolyte for the construction of soil based MFCs. The cation capacity of soil is much lower than that of Nafion which makes it unsuitable for replacing it. Here we suggest the use of a semantic clay, Montmorillonite, as a possible replacement of Nafion. The clay is low cost and can achieve higher proton conductivities than normal soil [6]. By treating with acid, the clay can be activated thus resulting in an enhanced cation exchange activity.

MFCs have as of yet been able to achieve very low power densities which has resulted in limitation of the usage of the technology. Here we suggest a simple application for the low power density system which can be coupled with anaerobic fermentation to increase its efficiency.

The use of yeast on an industrial scale can be enhanced by accompanying electricity production. Ethanol is a predominant product of yeast fermentation and electricity production will enhance the productivity of the process. Furthermore, many algal strains are used for the production of lipids that are converted to biodiesel. Growth of algae on a microbial cathode can result in increased growth due to dependence on the proton availability. Coupling Yeast fermentation with algal harvesting in photo bioreactors will enhance the fruitfulness of both the processes.

When the biomass for fermentation is replaced by the algal biomass remaining after lipid extraction, the process will be more optimized and sustainable. This will make it a microbial solar cell that will produce not only chemical products, but can provide electricity as well [7]. The main components of the cell include a Yeast based anodic compartment and an Algal based cathodic compartment.

Materials and Methods

The fuel cell was a simple salt bridge based cell that contained Montmorillonite clay (Ittehad Chemicals Ltd., Pakistan) as electrolyte. The electrolyte was wrapped in filter paper and dipped in the

anode and cathode mediums. Cathode was made of Carbon Paper (Paper 120 - TGP-H-120, Toray Industries Inc., Fuel cell store, College Station, Texas, USA) with active surface area of $6 \times 9 = 54\ cm^2$ while the anode was composed of copper plates with an area of $90\ cm^2$. The electrodes were connected to copper wire and an external resistance of $3K\Omega$ was connected between the two electrodes in order to measure power. The cell was run for a month while its performance was monitored.

Two 500 ml beakers were used as containers for anode and cathode mediums. The yeast culture contained 2 g of *S.cerevisiae* along with 3 g of Granulated Yeast Extract to enhance electron transfer. Sugar molasses were used as the substrate with the final solution brix of 3%. This initial culture was fed with fresh molasses in order to maintain the brix. On the cathode side, *Dictyosphaerium iso 8-6* culture was introduced with a cell count of $1.31 \times 10^4\ cells/ml$. The indigenous algal specie was isolated characterized locally. The algal culture was continuously aerated to avoid the algae from settling down.

The voltage of the system was monitored using a digital multimeter. The readings were taken in mV. In order to find out the current, a 3kOhm resistor was connected to the system. Using the value of voltage, the current was found out following Ohm's law.

$$I = V/R \text{----------} \quad (1$$

The polarization and power curve were plotted by manually varying the external resistance of the cell and calculating the voltage. The value of voltage was then used to find the current and subsequently the power density using the equation:

$$P = V \times I \text{----------} \quad (2$$

Internal resistance of the cell was calculated using the Ohm's law.

$$\varepsilon = I(R + r) \text{----------} \quad (3$$

$$V_R = -rI + \varepsilon \text{----------} \quad (4$$

Results

Effect of time on the open circuit voltage

The cell was observed to mature over a period of a month as the biofilm started to develop on the carbon paper electrode. After 30 days the cell obtained a maximum OCV and then the value started to decrease. This decrease in the OCV can be because of the deterioration of the biofilm deposited on the electrodes after which a constant OCV was achieved (0.6 V). This change in the OCV is demonstrated in Figure 2. This gradual increase in the voltage can be attributed to the development of biofilm on top of the electrode. The copper electrode gained a layer of rust over time which may have contributed in the creation of favorable conditions for the yeast to transfer electrons.

Charge transport

The electrons need to be transported from the medium to the anode in order to create potential. This transfer of electrons is carried out with the help of addition of mediators. The mediator added here was yeast extract. Still there is a lag between charge creation in the medium and the transfer of the charge to the surface of the anode. This results in a slow response form the cell. A long time is required in order to achieve the maximum potential which is roughly 5 min. The rise in the voltage is shown in Figure 3. About 98% of the maximum voltage (490 mV) is achieved within 2 min after which the charge transfer is slowed down. This slowing down of charge transfer is because of increase in the negative potential of the anode which repels electrons. Because of this phenomenon, all the readings of voltage taken required a settling time of 2 min.

Performance curves

The power curve of for the fuel cell was plotted and gave a peak of 1.04 mW/m^2 at a current density of 5.75 mA/m^2. A linear trend was observed in the V-I curves at lower values of resistances. This response is in coherence with the performance of previously demonstrated fuel cells (Figure 4).

V-R Curve

A second order quadratic equation represents the relation between the external resistance and the output voltage of the cell (Figure 5). The relation between voltage and current is not linear and this lag in the increase of voltage can be explained by the mass transport restrictions. Microbes are unable to create the potential with a pace fast enough to keep up with discharge.

Internal resistance

The internal resistance of the system needs to be high for the electronic current to be small. The relation between voltage and current of the cells (Figure 6) shows the internal resistance to have a value of 5500 Ohm. The same equation also gave the value of the emf of the cell, by projecting the graph to the point where current is 0, which was around -464.8 mV. This value of emf can provide us with the cathode potential by using the standard potential for glucose i.e. -428 mV [20].

$$E_{emf} = E_{Cat} - E_{An} \text{ ---------- (5}$$

$$E_{Cat} = E_{emf} + E_{An} = 464.8 + (-428) = 36.8\, mV \text{ ---------- (6}$$

Discussion

a. Algal bio cathode

Replacement of platinum catalyst on the cathode side is a reason for the usage of bio cathode. Algal cultures have been used as cathodic catalysts after enrichment in the anodic compartment of MFCs. A fuel cell catalyzed by the culture of *Desulfovibrio vulgaris* produced 1.1 A/m^2. Hydrogen production was also demonstrated with a bio cathode. The molasses fed bio electrode was successively fed with bicarbonate and hydrogen and after a few days the hydrogen supply was stopped and the potential of the electrode was lowered which resulted in electron consumption and hydrogen production [8].

In an MEC the sole electron acceptor are protons which reduce to form hydrogen gas. The microbial communities present on the cathode contain hydrogenases that can reversibly reduce protons. Purified hydrogenases have also been used as a catalyst [9].

Bio cathodes make it possible for materials other than oxygen to act as the electron acceptor. They contain bacterial colonies that can catalyze the reduction of multiple compounds. The added benefit of bio cathodes is the possibility of achieving the production of value added products through electrochemical reduction. The electron transfer from the electrode to the microbe can take place either directly or indirectly. The indirect transfer of electrons depends on the use of some mediator. The electron acceptors in the cells are mainly complexes like cytochromes. The development of a biofilm on the electrode with the use of oxygen as the electron acceptor is beneficial. The increase in the thickness of the biofilm was observed to reduce the electrical production. Increase in the electrode area is also shown to increase the catalytic activity of the microbes and hence increase the utilization of the electrons coming from the anode [10].

The anode of acetate oxidizing tubular microbial fuel cell was combined with an open air bio cathode for electricity production. Increasing the size of the cell reduced the volumetric power production [11].

The function of algae on the bio cathode in an MFC is to reduce the oxygen present and combine it with the protons flowing from the anode side. In this way the anode side reactions can be summarized as

$$Substrate \rightarrow Carbon\,dioxide + Protons + Electrons \text{ ---------- (7}$$

Meanwhile on the cathode side

$$Protons + Oxygen + Electrons \xrightarrow{Algae} Water \text{ ---------- (8}$$

In such a system, algae are used as a source of oxygen for the cathode. The level of dissolved oxygen therefore matter a lot and will vary throughout the day. The power output of such a system is very small and only a few micro watts are produced per meter sq. [12].

b. Yeast microbial fuel cell

Saccharomyces cerevisiae has been used as the biocatalyst to oxidize glucose. The catalytic activity came from the cells adhered to the anode and the dispersed cells did not seem to take part in electron transfer. There are many advantages to using Baker's yeast including its low cost, availability, easy dry storage and mass cultivation [13]. On the other hand this easy to grow yeast has also been used to ferment molasses. The main product of this fermentation process is ethanol which can be purified to be used as fuel [14]. The experiment that we have devised shows that this fermentation process can be coupled with algal bio cathode to produce additional power.

Yeast is thought to be perfect biocatalysts for microbial energy units which can utilize a wide range of substrates. Along with this, they are mostly nonpathogenic which can help in creating less hazardous cells. Yeasts are said to have two mechanisms for electron transfer i.e. trans Plasma Membrane Electron Transport frameworks (tPMET) & Plasma Membrane Oxido-Reductase frameworks (PMOR). Collectively these mechanisms result in enhanced electron transfer [15].

Copper is considered to be a toxic metal for bacteria. Even small traces of the metal can harm them [16]. But the toxicity of copper is irrelevant in bio electrochemical devices and copper can produce much higher current densities owing to its high conductivity [17].

c. Electrolyte

The electrolyte used was Activated Bleaching Earth which is a semantic mineral namely Montmorillonite. This mineral was used as a proton exchange medium because of its inherent cation exchange capacity (CEC) [18]. Montmorillonite is classified as a Phyllosilicate because it contains two tetrahedral sheets of silica which sandwich an octahedral sheet of alumina. Along with alumina, MgO is also found in the middle layer which can impart cation exchange capacity to the clay by isomorphous substitution. The composition of the clay is shown in Table 1. When these ions move a net negative charge remains on the clay because of oxygen atoms. This allows positive charge to flow through the clay [19].

d. Application

The yeast is used to oxidize molasses and produce ethanol. Our experiment has shown that electricity can also be produced during the fermentation process. Algae is grown in photo bioreactors and harvested to produce biodiesel. This algae can also be used in the bio cathode of a microbial fuel cell.

A configuration that allows these two microbes to work together is described here. It involves the flow of the liquids in microporous tubes. These tubes are embedded in ABE which acts as the ion exchange medium and transfers protons from the yeast to the algae. Copper wires are to be passed in the middle of the tubes carrying yeast while carbon paper electrodes will be present on the algae side. The large amount of clay present in the container will ensure the separation among the fluids.

A constant flow rate is to be maintained for the algal culture and the fermentation broth as it passes through the MFC chamber. Simultaneously the algal culture can be harvested for lipid extraction and the fermentation broth is subjected to ethanol purification. The ethanol is used for transesterification and production of biodiesel. Figure 8 shows the flow diagram of the algal and yeast cultures starting from the major input of a substrate to eventual production of biodiesel as a primary product.

The cation exchange capacity of Montmorillonite is between 70 – 100 meq/100g [6]. Nafion has an equivalent weight of 1100, which translates into an ion exchange capacity of 900 meq/100g. But up to 25% of the sites are unassailable which reduces the capacity of Nafion which is still significantly higher than that of ABE [21]. This difference in capacity is overcome by the difference in cost of the two materials.

The results show that with some additional refinement this fuel cell can be used on a large scale to produce power along with industrial fermentation. The anode area needs to be sufficient in order to collect as many electrons as possible. This will ensure an increase in the energy recovery from the fermentation process whereby substrate utilization will be maximized. For the algal bio cathode, this system can provide an additional usage of the algal biomass before being converted to biodiesel.

The most energy intensive step involved in the biodiesel production process is of algae separation from the culture. This process is mostly carried out by flocculation brought upon by increase in the pH of the culture which, along with sedimentation are the two least energy intensive processes, whit the appended disadvantage of low efficiencies and higher time consumption. On the other hand, centrifugation and ultrafiltration are efficient technologies which require massive amounts of energy input. Increase in the efficiencies of passive processes and decreasing the time requirement can allow the process to become economically feasible for implementation on a grander scale. This problem has been addressed by the introduction of electrocoagulation and electroflotation which relies on metallic electrodes to stimulate and enhance the formation of flocs and subsequently better separation [22],[23].

The proposed electrochemical is primarily tested as a MFC for power production but the available potential gradient can be put to another use to enhance the overall process of the biorefinery. Optimization of the voltage for bringing about electrocoagulation or electroflotation of the culture media can cause the economics of the process to shift and warrant its physical implementation. The concept is pictorially represented in Figure 9.

Conclusion

The possibility of using fermentation broth along with algal culture in an MFC has been experimentally tested here in a simple electrochemical cell configuration. In order to avoid the use of expensive components, mainly the proton exchange membrane, activated bleaching earth has been introduced as an electrolyte. The experiment shows that the potential gradient between the algal and yeast cultures can be harnessed even with a low quality and low cost electrolyte. The proposed MFC chamber, with further modifications, can become a valuable addition to the integrated approach towards the production of algal based biodiesel.

Further research looking in to the feasibility and effectiveness of integrating electrochemical technologies into bioprocessing plants is needed to warrant widespread use of renewable biological resources.

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