

Renewable Hydrogen Production from Algae and Efficient Utilization in PEMFCs



By

Salman Shahid Kunwar

Reg. # NUST201361495MCES64113F

Session 2013-16

Supervised by

Assistant Prof. Dr. Naseem Iqbal

**A Thesis Submitted to the U.S. Pakistan Center for
Advanced Studies in Energy in partial fulfillment of the
requirements for the degree of
MASTERS of SCIENCE in
ENERGY SYSTEMS ENGINEERING**

**U.S. Pakistan Center for Advanced Studies in Energy
(USPCAS-E)**

**National University of Sciences and Technology (NUST)
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Certificate

This is to certify that work in this thesis has been carried out by **Mr. Salman Shahid Kunwar** and completed under my supervision in Biofuels laboratory, U.S. Pakistan Center for Advanced Studies in Energy, National University of Sciences and Technology, H-12, Islamabad, Pakistan.

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Abstract

Photobiological hydrogen production from algae is considered as clean and promising energy source to substitute the fossil fuel with zero greenhouse gases emissions. Photoproduction of hydrogen by green algae has limiting factors because of oxygen inhibition and electron competing moieties like FNR (EC 1.18.1.2) and Rubisco (EC 4.1.1.39) to hydrogenase (EC 1.12.7.2). These biological regulatory mechanisms under anaerobic conditions inhibit hydrogen producing enzyme "hydrogenase" by oxygen produced at PSII through water splitting and at the same time compete for electrons with hydrogenase. Here, an innovative approach, bypassing all reported inhibitions to hydrogenase using crude enzyme extracts of algae leading towards hydrogen production has been presented. Zeolitic Imidazolate Frameworks (ZIFs) are one of the potential candidates as highly conducting networks with surface area with a possibility to be used as catalyst support. In the present study, highly active state-of-the-art Pt-NCNTFs catalyst was synthesized by pyrolyzing ZIF-67 along with Pt precursor under flowing Ar-H₂ (90-10 %) gas at 700 °C. XRD analysis indicated the formation of Pt-Co alloy on the surface of the nanostructured catalyst support. The high resolution TEM examination showed the particle size range of 7 to 10 nm. Proton exchange membrane fuel cell performance was evaluated by fabricating membrane electrode assemblies using Nafion-212 electrolyte using H₂/O₂ gases (100 % RH) at various temperatures. The peak power density of 630 mW.cm² was obtained with Pt-NCNTFs cathode catalyst and commercial Pt/C anode catalyst at 70 °C at ambient pressure.

Keywords: Photobiological; Hydrogenase; ZIF-67; ORR catalysts; PEM Fuel Cell

Table of Contents

Chapter 1 Introduction	1
1.1. Background	1
1.1.1. Energy Crisis and Hydrogen	1
1.1.2. Hydrogen Production from Algae	1
1.1.3. Hydrogen Utilization in PEMFCs	2
1.2. Problem Statement	2
1.3. Objectives	2
1.4. Overview of Thesis	3
Summary	5
References	6
Chapter 2 Literature Review	8
2.1. Microalgae: A Green Source of Renewable H₂	8
2.1.1. Photobiological H₂ Production in Microalgae	8
2.1.2. H₂ Production Pathway in Green Algae	9
2.1.3. Biological H₂ Production Inhibitory Mechanisms in Green Algae	9
2.1.4. Current Status and Advancements in H₂ Production from Algae	10
2.2. Proton Exchange Membrane Fuel Cells (PEMFCs): Most Promising Energy Conversion Technology	11
2.2.1. Commercialization Barriers of PEMFCs	11
2.2.2. Current Status of Oxidation Reduction Reaction Catalysts	11
2.2.3. Zeolite Imidazole Framework Derived Catalysts	12

Summary	13
References	14
Chapter 3 Experimentation	21
3.1. Isolation, Selection and Identification of Local Algal Strains	21
3.2. Harvesting of Dictyosphaerium	21
3.2.1. Synthetic Growth Medium Preparation	21
3.2.2. Cultivation of Dictyosphaerium in Standard Growth Medium (BBM)	22
3.3. Crude Enzymes and PS II Extraction	23
3.3.1. Algal Cells Collection	23
3.3.2. Cells Lysis and Enzymes Extraction	23
3.4. Bypassing Hydrogenase Inhibition for Hydrogen Production from Crude Enzymes Extract	24
3.5. Study of Heparin and 2-Carboxy-D-arabinitol-1-phosphatase to Regulate NADPH formation and utilization	25
3.5.1. Ferredoxin NADP Reductase (FNR) Inhibition	25
3.5.2. Rubisco Inhibition	26
3.6. Synthesis and Characterization of Platinum and Nitrogen Doped Carbon Nanotubes Framework (Pt-NCNTFs) Cathode Catalyst Using ZIF-67 for Proton Exchange Membrane Fuel Cell	27
3.6.1. Synthesis of ZIF-67 Particles	27
3.6.2. Synthesis of Pt-NCNTFs	27
3.6.3. Catalyst Characterization	27
3.6.4. Electrochemical Single Cell Measurements	28
3.6.4.1. Catalyst Coated Membranes	28

3.6.4.2. Gas Diffusion Layer	28
3.6.4.3. Fuel Cell Assembly and Testing	28
Summary	30
References	31
Chapter 4 Results and Discussion	32
4.1. Bypassing Hydrogenase Inhibition for Hydrogen Production from Crude Enzyme Extract	32
4.1.1. Gasses Collection and Analysis	32
4.1.2. Composition of Gasses	33
4.1.3. Electrons flow from PSII to Crude Enzymes	33
4.1.4. Mechanism for Bypassing Hydrogenase Inhibition for Hydrogen Production from Crude Enzyme Extract	34
4.2. Study of Heparin and 2-Carboxy-D-arabinitol-1-phosphatase to Regulate NADPH Formation and Utilization	35
4.3. Synthesis and Characterization of Platinum and Nitrogen doped Carbon Nanotubes Framework (Pt-NCNTFs) Cathode Catalyst using ZIF-67 for Proton Exchange Membrane Fuel Cell	35
Summary	39
References	40
Conclusions & Recommendations	41

List of Figures

Fig. #	Caption	Page #
Fig. 2.1	Z-Scheme Supporting H ₂ Production in Algae	9
Fig. 2.2	Biological H ₂ inhibitory mechanisms in Algae	10
Fig. 3.1	Cultivation of Dictyosphaerium under lab condition	22
Fig. 3.2	(a) Eppendorf centrifuge 5810 R (b) Algal Cells in BBM (c) Collected Algal Cells	23
Fig. 3.3	(a) Cell lysis in pestle mortar (b) Lyzed Cells in Eppendorf (c) Hielscher UP400S (d) Isolated Crude Enzymes and PS II	24
Fig. 3.4	(a) Hydrogen production bypassing Oxygen inhibition for Hydrogenase using Crude Enzymes Extracts (b) Hydrogen Collected in Syringe	25
Fig. 3.5	Effect of FNR and Rubisco Inhibitors on Hydrogen Production from algae under anaerobic conditions	26
Fig. 4.1	Gas Chromatograph of Gasses produced by Crude Enzymes with external electrons from PSII	32
Fig. 4.2	Gas Chromatograph of Gasses produced by Crude Enzymes	33
Fig. 4.3	Schematics for Hydrogen production bypassing Oxygen inhibition for Hydrogenase using Crude Enzymes Extracts.	34
Fig. 4.4	Transfer of electrons from isolated PSII to isolated hydrogenase for hydrogen production	35
Fig. 4.5	Scanning Electron Micrographs of (a,b) Pt-NCNTFs, and Transmission Electron Micrographs of (c) NCNTFs and (d) Pt-NCNTFs	36
Fig. 4.6	X-Ray diffractograms of (a) NCNTFs and (b) Pt-NCNTFs.	37
Fig. 4.7	Fuel Cell Performance using Pt-NCNTFs cathode catalysts at various temperatures with H ₂ and O ₂ gases, 100 % RH at ambient pressure. The open symbols represent the power density values	38

List of Tables

Table #	Caption	Page #
Table 3.1	Composition of BBM	21
Table 4.1	Hydrogen production from experimental and control vessel	33

List of Abbreviations

PEMFC	Proton Exchange Membrane Fuel Cell
ORR	Oxygen Reduction Reaction
MOFs	Metal Organic Frameworks
ZIFs	Zeolitic imidazolate frameworks
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
FNR	Ferredoxin NADP Reductase
NCNTFs	Nitrogen doped Carbon Nanotubes Frameworks
Pt-NCNTFs	Platinum & Nitrogen doped Carbon Nanotubes Frameworks
MEA	Membrane Electrolyte Assembly
GDL	Gas Diffusion Layer
LEF	Linear Electron Flow
PSI	Photosystem I
PSII	Photosystem II
PQ	Plastoquinone
PC	Plastocyanin
Cytb ₆ f	Cytochrome b ₆ f
Fd	Ferredoxin
ATP	Adenosine Triphosphate
BBM	Bold Basal Media
TCD	Thermal Conductivity Detector
CCM	Catalyst Coated Membrane
VGCF	Vapor Grown Carbon Fiber
PTFE	Polytetrafluoroethylene
SCCM	Standard Cubic Centimeters per Minute
CVD	Chemical Vapor Deposition
TEM	Transmission Electron Microscopy

Chapter 1

Introduction

1.1. Background

1.1.1. Energy Crisis and Hydrogen

World energy consumption in 2013 was 12730.4 million tons of oil equivalent and primary energy demand is expected to increase by 41% till 2035. In 2013, world energy mix was dominated by fossil fuels which contributed by 86.59% (Oil, natural gas and coal contributed by 32.87%, 23.72% and 30% respectively) [1,2]. Usage of these fossil fuels have some inherited problems mainly the CO₂ emissions, price oscillation and depleting reservoirs.

The motivation behind hydrogen production and utilization is due to the premise that hydrogen is a clean energy fuel having potential to replace fossil fuels, possesses three times the energy contents of gasoline on mass basis, abundantly available on earth and Proton Exchange Membrane Fuel Cell (PEMFC) is a proven H₂ based technology [3-5]. H₂ production from algae requires solar energy and its utilization in PEMFCs can be a viable solution to increasing world energy insecurity and global warming [6].

1.1.2. Hydrogen Production from Algae

Unfortunately, H₂ is not found free on earth, most of the H₂ on earth is fully oxidized into water and remaining is chemically incorporated into fossil fuels and biomass. Release of H₂ from these sources for use as a fuel requires energy. Currently H₂ gas is being produced 30%, 18%, 48% and 4% from heavy oils, coal, natural gas and electrolysis respectively [7]. In Pakistan, 15.6 % of total natural gas reserves is consumed in H₂ production by fertilizer industry for the production of anhydrous ammonia [8].

Biological H₂ production processes are less energy intensive and more environmental friendly in contrast to electrochemical and thermochemical processes. Microalgae hydrogen production is promising as it uses direct photosynthesis so requires small land footprint, it can grow on waste water, it can recycle waste CO₂ produced by industrial

emission sources. Moreover, it produces value added products along with fuels and fuel precursors [9-11].

1.1.3. Hydrogen Utilization in PEMFCs

Proton exchange membrane fuel cell (PEMFC) is the most promising energy conversion technology for stationary as well as automotive applications due to its advantages such as lower operating temperature, higher power density as compared to other types of fuel cells and ability to respond quickly to changing power demand [12]. PEMFCs not only operate at higher efficiency than internal combustion engines, they only produce H₂O as a byproduct. Hence, H₂ utilization in PEMFCs can be a viable solution to encounter global warming.

1.2. Problem Statement

World energy mix is dominated by fossil fuels which contributed by 86.59% in 2013. Usage of these fossil fuels have some inherited problems mainly the CO₂ emissions, price oscillation and depleting reservoirs. Hence, there is an urgency to produce and utilize alternate fuels to reduce dependency from finite fossil fuel reserves.

The motivation behind hydrogen production and utilization is due to the premise that hydrogen is a clean energy fuel having potential to replace fossil fuels, possesses three times the energy contents of gasoline on mass basis, abundantly available on earth and Proton Exchange Membrane Fuel Cell (PEMFC) is a proven H₂ based technology. H₂ production from algae requires solar energy and its utilization in PEMFCs can be a viable solution to increasing world energy insecurity and global warming.

Hydrogen production from algae is limited by biological inhibitory mechanisms and utilization of H₂ in PEMFCs is limited due to higher cost, less durability and higher cost of Oxygen Reduction Reaction (ORR) catalyst.

1.3. Objectives

The objectives of this research were:

- i. To encounter biological regulatory mechanisms to establish hydrogen production from algae.

- ii. To synthesize highly active and state of art ORR catalyst for PEMFCs.

1.4. Overview of Thesis

In this thesis two different approaches were developed to establish hydrogen production from algae overcoming the biological inhibitory mechanisms and highly active state-of-the-art ORR catalyst was synthesized and evaluated. Overall the research work is divided into five chapters:

Chapter 1 focuses on the issues associated with the domination of fossil fuels in the world energy mix and proposes H₂ production from algae and its utilization in PEMFCs as a viable option. This chapter presents the concise problem statement and objectives related to the proposed solution.

Chapter 2 describes the literature review for Photobiological hydrogen production pathways and explains in detail the biological inhibitory mechanism limiting H₂ production in green algae. It explains the strategies adopted to encounter these biological mechanisms to establish hydrogen production. It throws light on commercialization barriers of PEMFCs, current status of ORR catalyst for PEMFCs and discusses emerging novel class of Metal Organic Frameworks (MOFs) derived catalysts. It goes on explaining Zeolitic imidazolate frameworks (ZIFs) as a subclass of MOFs as excellent materials for the synthesis of nanocarbon electrocatalysts with abundant carbon and nitrogen.

Chapter 3 describes different experimental procedures performed to achieve the research objectives. It explains the harvesting of local algal species *Dictyosphaerium* under lab conditions and methodology adopted to isolate crude Hydrogenase from Photosystem II in algae and bypassing O₂ inhibition of Hydrogenase to establish H₂ production, it goes on explaining the attempts to regulate the Nicotinamide adenine dinucleotide phosphate (NADPH) using Rubisco and Ferredoxins NADP Reductase (FNR) inhibitors. It further explains the synthesis of ZIF-67 and Platinum & Nitrogen doped Carbon Nanotubes Framework (Pt-NCNTFs) cathode catalyst. It describes different exsitu and insitu characterization techniques applied for evaluation of Pt-NCNTFs catalyst along with the fabrication of Membrane Electrolyte Assembly (MEA) and Gas Diffusion Layer (GDL).

Chapter 4 describes and discusses the obtained results. It explains the Gas Chromatographs of gases produced bypassing O₂ inhibition of hydrogenase and elaborates the enhanced hydrogen production at expense of electrons from Photosystem (II) PSII to hydrogenase. It explains the study of Heparin and 2-Carboxy-D-arabinitol-1-phosphatase to regulate formation of NADPH formation and utilization for enhanced hydrogen production from algae. It shares and explains the Scanning Electron Micrographs, Transmission Electron Micrographs and X-Ray Diffractograms of NCNTFs and Pt-NCNTFs. At the end it shows the Fuel Cell Polarization Data and explains Fuel Cell Performance using Pt-NCNTFs cathode catalysts at various temperatures with H₂ and O₂ gases, 100 % RH at ambient pressure.

Finally chapter 5 highlights the major findings of research and possible recommendations for further research.

Summary

World energy mix is dominated by fossil fuels which contributed by 86.59% in 2013. Usage of these fossil fuels have some inherited problems mainly the CO₂ emissions, price oscillation and depleting reservoirs. The motivation behind hydrogen production and utilization is due to the premise that hydrogen is a clean energy fuel having potential to replace fossil fuels, possesses three times the energy contents of gasoline on mass basis, abundantly available on earth and Proton Exchange Membrane Fuel Cell (PEMFC) is a proven H₂ based technology. PEMFCs operate at higher efficiency than internal combustion engines and only H₂O is produced as a byproduct. H₂ production from algae requires solar energy and its utilization in PEMFCs can be a viable solution to increasing world energy requirements and global warming.

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Chapter 2

Literature Review

2.1. Microalgae: A Green Source of Renewable H₂

2.1.1. Photobiological H₂ Production in Microalgae

Biological H₂ production processes are less energy intensive and more environmental friendly in contrast to electrochemical and thermochemical processes. Microalgae hydrogen production is promising as it uses direct photosynthesis so requires small land footprint, it can grow on waste water, it can recycle waste CO₂ produced by industrial emission sources. Moreover, it produces value added products along with fuels and fuel precursors [1-3].

The ability of algae to produce hydrogen gas in the presence of light was discovered many decades before [4,5]. The succeeding studies were made to elaborate the factors involved in production of hydrogen from algae. Hydrogen production was found to be induced by subjecting the algal cells to anaerobic conditions [6,7]. Algae need carbon dioxide to carry out photosynthesis for biomass and hydrogen production. Algae possesses “Hydrogenase”, an enzyme which catalyzes the reaction:



Hydrogenases are divided into three groups based upon the structure of metal-sulphur cluster forming the active center. First group comprises of [Fe]-hydrogenases lacking iron-sulphur cluster and they are only found in methanogenic archaea [8]. Second group comprises [NiFe]-hydrogenases containing dinuclear nickel-iron center in the active site. They are distributed among prokaryotic organisms[9]. In the third group are more efficient [FeFe]-hydrogenases comprising of a dinuclear [FeFe]-cluster linked to a [4Fe-4S]-cubane [10-11]. Upon exposure to anaerobic conditions, a hydrogenase enzyme (EC 1.12.7.2) is expressed to utilize the available electrons for hydrogen production. The hydrogenase involved in hydrogen production is reported as a member of the class representing Fe Hydrogenases [12-13].

2.1.2. H₂ Production Pathway in Green Algae

Photobiological hydrogen production include the use of photosynthesis and water splitting in which microalgae utilizes the absorbed energy from Sun and evolve H₂ directly from water without the formation of intermediate products. Microalgae possesses the potential of producing H₂ at higher efficiency of 10% so land area can be utilized efficiently. Green algae and Cynobacteria contains enzymes [Fe-Fe]-hydrogenases and [Ni-Fe]-hydrogenases respectively which are responsible for H₂ production [14]. In green algae, photosynthetic electron transport pathways support the production of Nicotinamide adenine dinucleotide phosphate (NADPH) and hydrogen. In linear electron flow (LEF), light-activated Photosystem II (PSII) extracts electrons by water splitting and transfers them in a Z-scheme pattern to Plastoquinone (PQ), these electrons are then transferred to Plastocyanin (PC) through Cytochrome b6f, (Cytb6f), PC feeds electrons to light-oxidized Photosystem I (PSI). PSI-derived electrons are used to reduce Ferredoxins (Fd), which then transfers reductant to either Ferredoxins NADP Reductase (FNR) (EC 1.18.1.2), for NADPH production, or to Hydrogenase, for hydrogen production [15]. Eukaryotic green algae requires a period of several minutes to few hours in dark under anaerobic conditions to produce H₂ [16-18].

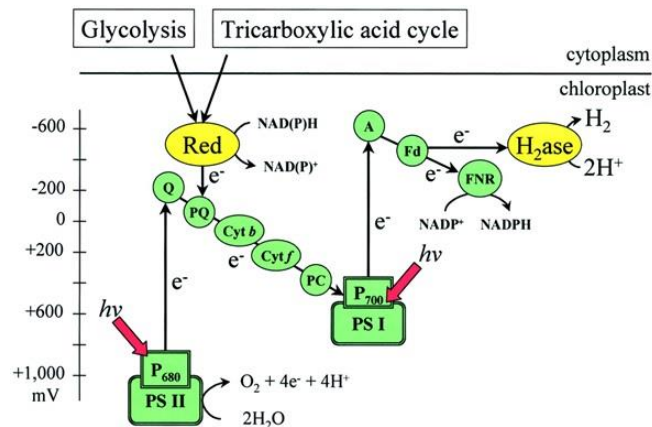


Fig. 2.1 Z-Scheme Supporting H₂ Production in Algae [19]

2.1.3. Biological H₂ Production Inhibitory Mechanisms in Green Algae

The process of illumination of water molecules split the water into protons, electron and molecular oxygen. Oxygen is proved to be a powerful inhibitor of the Fe hydrogenase [20]. Utilization of electrons by FNR (which leads to NADPH) is another barrier in

hydrogen production. Photoproduction of hydrogen by green algae has limiting factors because of oxygen inhibition and electron competing moieties like NADPH and Rubisco (EC 4.1.1.39) to hydrogenase. These biological regulatory mechanisms under anaerobic conditions inhibit hydrogen producing enzyme “hydrogenase” by oxygen produced at PSII through water splitting and at the same time compete for electrons with hydrogenase. Under anaerobic conditions, when CO₂ is absent and Adenosine triphosphate (ATP) is in abundance, Rubisco can act as Oxygenase and can reduce O₂, in this way it competes for electrons with Hydrogenase and can reduce the H₂ production [21] and also according to classic Mehler reaction, Fd can also serve as electron donor to O₂ [22].

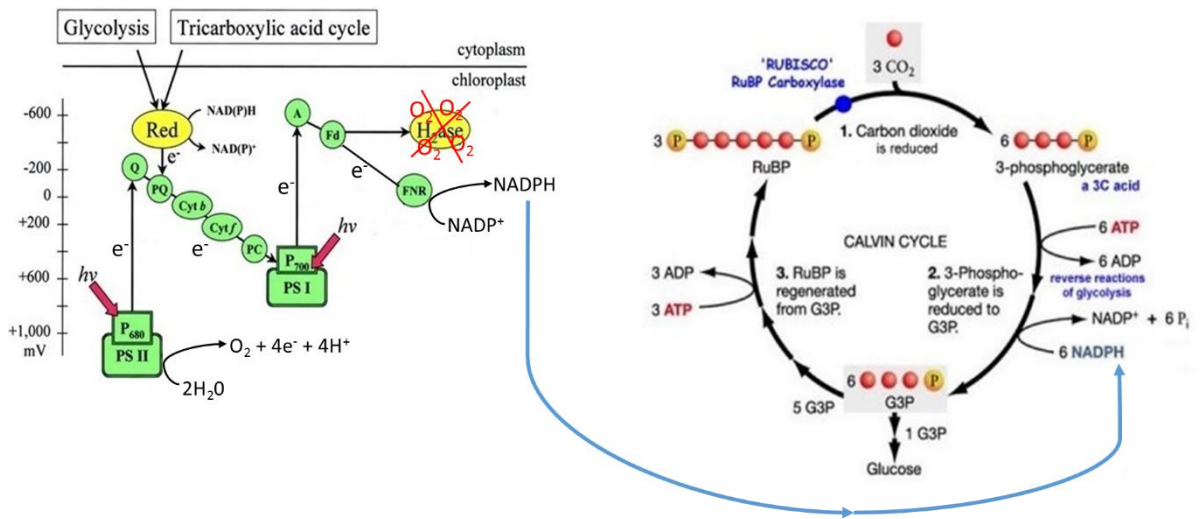


Fig. 2.2 Biological H₂ inhibitory mechanisms in Algae

2.1.4. Current Status and Advancements in H₂ production from Algae

Major breakthrough in H₂ production was reported under Sulfur deprived conditions, in which PSII system in green alga gradually diminishes and less oxygen is produced than required for respiration encountering the O₂ inhibition of Hydrogenase, the most significant barrier in H₂. In this condition Hydrogenase becomes active and attracts the electrons partially from PSII and endogenous substrate through electron acceptors like cytochrome b6-f and PS I in the Z-scheme producing H₂ as it is the only way to generate ATP for algal survival. The entire electron-transport capacity of photosynthetic equipment is not directed towards hydrogen production due to partial consumption of

electrons in Calvin Cycle through FNR, and H₂/O₂ ratio (mol/mol) of 0.17/1 is achieved [23]. Chlamydomonas mutant strains with reduced levels of FNR were produced by RNAi approach resulting in reduced electrons consumption in NADP⁺ reduction and increased availability of electrons to Hydrogenase producing 2.5 folds more H₂ than Sulfur deprivation[24].

2.2. Proton Exchange Membrane Fuel Cells (PEMFCs): Most Promising Energy Conversion Technology

2.2.1. Commercialization Barriers of PEMFCs

Proton exchange membrane fuel cell (PEMFC) is the most promising energy conversion technology for stationary as well as automotive applications due to its advantages such as lower operating temperature and higher power density as compared to other types of fuel cells. However, for wide-scale commercialization of PEMFCs, it should overcome several challenges including reducing the cost, maximizing the utilization of platinum catalyst, improving the performance and durability of the membrane electrode assembly. The higher cost of Membrane Electrolyte Assembly (MEA) due to noble metal catalysts is the major barrier in commercialization of PEMFCs as cost of MEA majorly attributes the total cost of fuel cell system [25-27]. Oxygen reduction reaction (ORR) is the most important one due to the potentially lower exchange current density value in the PEMFC [28-33].

2.2.2. Current Status of Oxidation Reduction Reaction Catalysts

Extensive research has been carried out to lower MEA cost by reducing Pt loading, developing transition metal-noble metal alloys or non-noble catalysts [31]. A large variety of materials has been investigated as possible replacement of noble metal catalysts for ORR including nanocarbons [32], metal oxides [33], carbides/nitrides and their composites [34-37]. Incorporation and functionalization of heteroatoms has been found effective to modify the electronic structure for enhancing catalyst ORR activity [38-40]. It has been reported that the introduction of N increases the defects in carbon structure and hence the electrochemical activity [41]. Nitrogen atom draws electron from adjacent carbon in matrix due to higher electronegativity producing positive charge on the carbon thus facilitating electroreduction of oxygen [42]. These active carbon-

nitrogen moieties are considered as anchoring sites for deposition of metal nanoparticles [43-44]. Furthermore carbon support with nitrogen doping are reported to show improved durability and catalyst activity towards ORR [45]. Carbon-supported transition metal/nitrogen (M/N/C) catalysts are reported as highly electrochemically active for ORR in both alkaline and acidic media [46-49]. Co and Fe have been found to exhibit highest ORR catalytic activity among different transition metals [46] and in some cases they are found to show enhanced activity than the commercial Pt/C catalyst [49]. In order to have reasonable reaction kinetics, noble metals based nanocomposites are the most efficient electrocatalysts towards ORR. However the noble metal ORR catalysts are highly expensive and are the leading barriers for the PEMFC commercialization [50-51]. In this context, it is important to develop highly efficient and durable electrocatalysts for ORR [52].

2.2.3. Zeolite Imidazole Framework Derived Catalysts

Recently, metal-organic frameworks (MOFs) have emerged as a novel class of porous crystalline materials both as template and precursor to produce nanoporous carbons for gas storage [53], catalyst support [54], and electrode materials for lithium batteries [55], sensors [56], and supercapacitors [57]. Zeolitic imidazolate frameworks (ZIFs) as a subclass of MOFs are excellent materials for the synthesis of nanocarbon electrocatalysts with abundant carbon and nitrogen [58-59]. MOF-derived nanocomposites reported as poor ion and electron transport materials due to poor graphitic degree and microporous structures [60-62]. Recently it is reported that this constraint can be overcome by carbonization under H₂/Ar atmosphere instead of inert atmosphere, which results in the formation of hollow hierarchical structure of carbon nanotubes followed by formation of metal nanoparticles [63].

Summary

Photobiological hydrogen production in algae include the use of photosynthesis and water splitting in which microalgae utilizes the absorbed energy from Sun and evolve H_2 directly from water without the formation of intermediate products. Photoproduction of hydrogen by green algae has limiting factors because of oxygen inhibition and electron competing moieties like NADPH and Rubisco to Hydrogenase. These biological regulatory mechanisms under anaerobic conditions inhibit hydrogen producing enzyme “Hydrogenase” by oxygen produced at PSII through water splitting and at the same time compete for electrons with Hydrogenase. Under anaerobic conditions, when CO_2 is absent and Adenosine triphosphate (ATP) is in abundance, Rubisco can act as Oxygenase and can reduce O_2 , in this way it competes for electrons with Hydrogenase and can reduce the H_2 production. When algal growth media is deprived of Sulfur, PSII starts to diminish, consequently less O_2 is produced than required for respiration. In this condition Hydrogenase becomes active and attracts the electrons partially from PSII and endogenous substrate through electron acceptors like cytochrome b6-f and PS I in the Z-scheme producing H_2 as it is the only way to generate ATP for algal survival. Proton exchange membrane fuel cell (PEMFC) is the most promising energy conversion technology for stationary as well as automotive applications due to its advantages such as lower operating temperature and higher power density as compared to other types of fuel cells. The higher cost of Membrane Electrolyte Assembly (MEA) due to noble metal catalysts is the major barrier in commercialization of PEMFCs as cost of MEA majorly attributes the total cost of fuel cell system. Extensive research has been carried out to lower MEA cost by reducing Pt loading, developing transition metal-noble metal alloys or non-noble catalysts. A large variety of materials has been investigated as possible replacement of noble metal catalysts for ORR including nanocarbons, metal oxides, carbides/nitrides and their composites. Zeolitic imidazolate frameworks (ZIFs) as a subclass of Metal Organic Frameworks (MOFs) are excellent materials for the synthesis of nanocarbon electrocatalysts with abundant carbon and nitrogen. MOF-derived nanocomposites are poor ion and electron transport materials due to poor graphitic degree and microporous structures.

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Chapter 3

Experimentation

3.1. Isolation, Selection and Identification of Local Algal Strains

Different local algal strains were collected and isolated by serial dilution method in the previous research work titled “Potential of local algae strains for possible hydrogen production and phyto-remediation of wastewater” conducted under supervision of Dr. Ehsan Ali at Biofuels Lab. US-Pakistan Center for Advanced Studies in Energy, NUST. The isolated strains were then grown in Bold Basal Media (BBM) and growth kinetics were studied on the basis of Chlorophyll Contents and No. of cells. The algal strain showing the better growth under local conditions was selected for the further research. This algal species was identified as *Dictyosphaerium* by Macrogen Korea.

3.2. Harvesting of *Dictyosphaerium*

3.2.1. Synthetic Growth Medium Preparation

Dictyosphaerium was grown on Bold’s Basal Medium which was prepared using standard recipe [1]. The initial pH was 6.6 which was brought to 7 before commencement of experimentation. BBM is a highly enriched medium with low salinity ideally used for culturing a wide variety of freshwater algal strains. This growth medium is similar to the standard microalgae media 3N-Basal Bold medium except that 3N-BBM has higher nitrate concentrations[2]. An overall composition of BBM is provided below:

Table 3.1: Composition of BBM[3]

Macro nutrients	Compound	Concentration (mmol/L)
Sodium	NaCl, NaNO ₃	3.37
Nitrogen	NaNO ₃	2.94
Phosphorus	KH ₂ PO ₄ , K ₂ HPO ₄	1.73
Calcium	CaCl ₂ .2H ₂ O	0.17
Magnesium	MgSO ₄ .7H ₂ O	0.30

Iron	FeSO ₄ .7H ₂ O	0.02
EDTA (chelating agent)	Na ₂ EDTA	0.03
Boron	H ₃ BO ₃	0.13
Micro nutrients	Compound	Concentration (mmol/L)
Manganese	MnCl ₂ .4H ₂ O	0.5
Zinc	ZnSO ₄ .7H ₂ O	0.05
Copper	CuSO ₄ .5H ₂ O	0.02
Cobalt	Co(NO ₃) ₂ .6H ₂ O	0.02
Molybdenum	Na ₂ MoO ₄ .2H ₂ O	0.02

3.2.2. Cultivation of Dictyosphaerium in Standard Growth Medium (BBM)

The selected algal strains were cultured (10% inoculum) indoors in reagent bottle containing autoclaved bold basal medium (BBM) which provided required nutrients. The flasks were covered with aluminum foils (to avoid contamination) and continuous supply of CO₂ to the cultures was ensured via filtered aeration. Cultures were grown at alternative light and dark period (14:10 hours) by placing them near window to provide natural sunlight and the lab's temperature was in the range of 20°C (±3°C) for all strains.



Fig. 3.1 Cultivation of Dictyosphaerium under lab condition

3.3. Crude Enzymes and PS II Extraction

3.3.1. Algal Cells Collection

Algal cells were collected by centrifugation of grown algal cells in BBM AT 25 °C for 10 minutes at 4000 RPM in falcon tubes using “Eppendorf centrifuge 5810 R” at ASAB NUST. The supernatant was removed and pellet was dissolved in Tris HCl and collected.

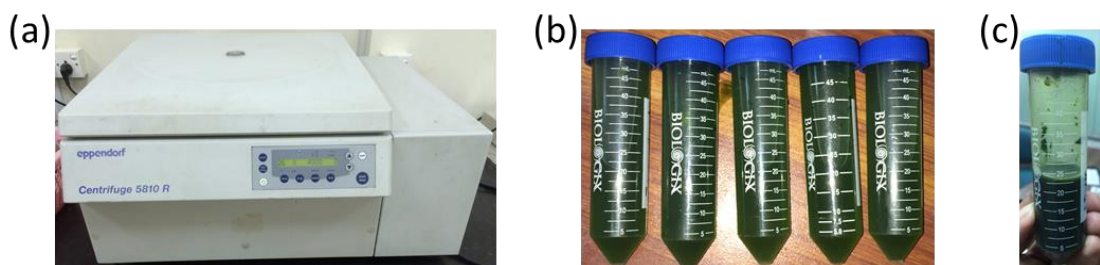


Fig. 3.2 (a) Eppendorf centrifuge 5810 R (b) Algal Cells in BBM (c) Collected Algal Cells

3.3.2. Cells Lysis and Enzymes Extraction

The collected cells were subjected to two step lysis process. Cells were first crushed by pestle mortar and then collected in Eppendorf Tubes and were lyzed by Ultrasonication (Hielscher UP400S) for 20 cycles of 20 seconds with gap of 20 seconds at 60% amplitude. Lyzed algae cells were subjected to centrifugation at 12000 RPM for 20 minutes to get the crude enzymes in supernatant and photosynthetic material in pellet.

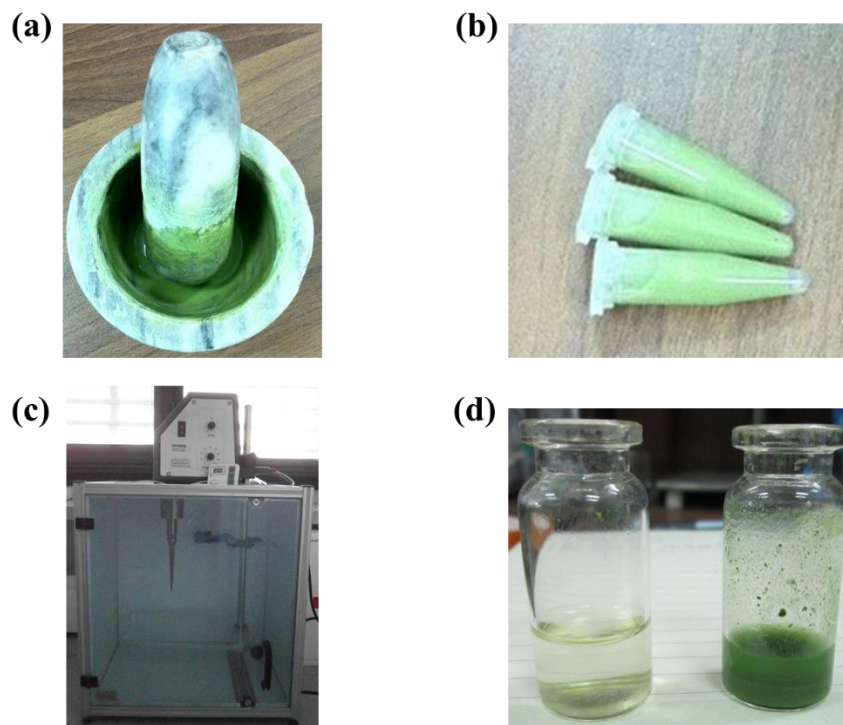


Fig. 3.3 (a) Cell lysis in pestle mortar (b) Lyzed Cells in Eppendorf (c) Hielscher UP400S (d) Isolated Crude Enzymes and PS II

3.4. Bypassing Hydrogenase Inhibition for Hydrogen Production from Crude Enzymes Extract

The crude extract, 11 ml each was poured in two 20ml vials and sealed with rubber stopper and aluminum seal. A Copper wire as electrodes was inserted in rubber stopper and injection syringe was used for increasing overhead/collection of gas. Pellet/debris was diluted with 75 ml of 1 mM Tris HCl and 45 ml of this mixture was poured in vial of 50 ml, it was sealed with rubber stopper and aluminum seal, and inserted with two copper wires and syringe. A potential difference of 0.015 V was measured using digital multimeter (UNI-T UT33D) across the two electrodes in the presence of sun light in the vial containing pellet, showing production of electrons as a result of water splitting at PSII. Electrodes were short circuited which lead to potential difference of 0.00 V, but it gradually raised up to 0.015 V in the sunlight. After short circuit when it was left in dark and potential difference was measured, it remained same 0V, which indicates that the potential difference is due to photolysis of water at PS II and one Cu wire from the vial containing pellet was removed. Vials were filled with water before sealing, after sealing

water was removed with the help of syringe to create vacuum in the vials. One crude enzyme vial was connected with vial containing Pellet through Cu wires (inserted in each vial) to allow flow of electrons from photosynthetic pathway to hydrogenase in crude enzyme. This circuit was broken and variable light dependent potential difference was observed using digital multimeter (UNI-T UT33D) which indicates the electron flow from pellet to crude enzymes extract. Other vial containing crude enzyme was used as control. Gases produced in each vial were collected in inserted syringes and after 24 hours and 0.5 ml of these gases was subjected to gas analysis using GC (Shimadzu GC-2010 Plus) at 25 °C using thermal conductivity detector (TCD) with N₂ as a carrier gas.

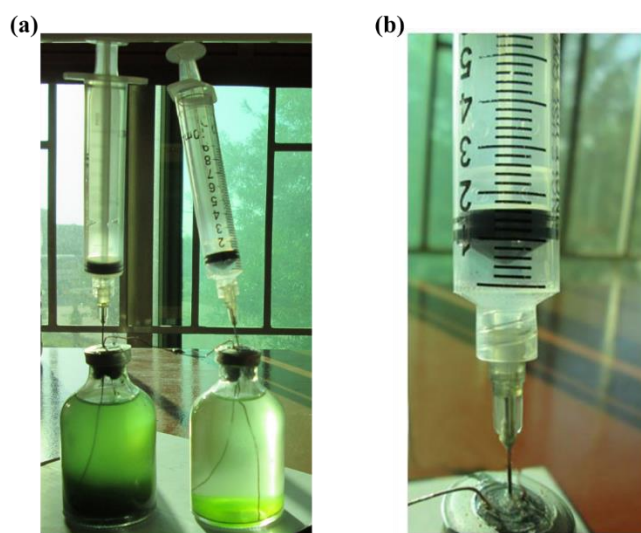


Fig. 3.4 (a) Hydrogen production bypassing Oxygen inhibition for Hydrogenase using Crude Enzymes Extracts (b) Hydrogen Collected in Syringe

3.5. Study of Heparin and 2-Carboxy-D-arabinitol-1-phosphatase to Regulate NADPH formation and utilization

3.5.1. Ferredoxin NADP Reductase (FNR) Inhibition

Heparin is an anticoagulant and has been reported as FNR inhibitor [4]. In the proceeding experiments the influence of heparin on algae under anaerobic conditions was studied to decrease electrons utilization by FNR and hence NADPH production so that the electrons can be directed towards the hydrogenase for enhanced hydrogen production.

Cultivated algae (1000 ml) was taken in reagent bottle with carbon cork inserted at the mouth. A gas syringe (50 ml) needle was inserted in it for collecting the produced gases and it was sealed with silicon to ensure the anaerobic conditions. 1 g sodium heparin was added in the algae after 48 hours of anaerobic conditions. Gas was collected for another 48 hours and was analyzed through Gas Chromatography.

3.5.2. Rubisco Inhibition

2-Carboxy-D-arabinitol-1-phosphatase has been reported as Rubisco inhibitor [5]. In the proceeding experiments its influence on algae under anaerobic conditions was studied to decrease electrons utilization by FNR by inhibiting Rubisco and hence NADPH utilization so that the electrons can be directed towards the hydrogenase for enhanced hydrogen production.



Fig. 3.5. Effect of FNR and Rubisco Inhibitors on Hydrogen Production from algae under anaerobic conditions

3.6. Synthesis and Characterization of Platinum and Nitrogen Doped Carbon Nanotubes Framework (Pt-NCNTFs) Cathode Catalyst Using ZIF-67 for Proton Exchange Membrane Fuel Cell

3.6.1. Synthesis of ZIF-67 Particles

ZIF-67 samples were synthesized as described in the published literature [3]. In a typical synthesis, 2-methylimidazole (1.97 g) was dissolved in a mixed solution of 20 ml of methanol and 20 ml of ethanol. $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (1.746 g) was dissolved in another mixed solution of 20 ml of methanol and 20 ml of ethanol. The above two solutions were then mixed under continuous stirring for few minutes and held for 20 h at room temperature. The purple precipitate was collected by centrifuging the solution, washed in ethanol several times and dried at 80 °C overnight.

3.6.2. Synthesis of Pt-NCNTFs

The ZIF-67 particles were soaked with $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ solution (5 wt % solution in DI water) and the resulting material was dried at 80 °C for 1h. The dried powder was heated at 350 °C for 1.5 h then raised to 700 °C at a ramp rate of 2 °C per minute and pyrolyzed for 3.5 h under flowing Ar/H_2 (90%/10% in volume ratio) atmosphere (see Figure 1). The as-prepared black powder product after cooling down to room temperature naturally was treated in 0.5M H_2SO_4 solution for 6 h. The resulting sample was collected by centrifugation, repeatedly washed with DI water, and then dried at 80 °C. NCNTFs were synthesized by following same protocol without adding Pt precursor. The synthesis process reported here used only single precursor as carbon, nitrogen and Co source.

3.6.3. Catalyst Characterization

The morphology and structure of the Pt-NCNTFs were characterized by Scanning Electron Microscopy (SEM, Hitachi S-3500 N) and Transmission Electron Microscopy (TEM, Philips CM200, 200 kV). Powder X-Ray diffraction (XRD) are recorded using SIEMENS D5000 X-Ray Diffractometer with $\text{Cu K}\alpha$ radiation ($\text{Cu K}\alpha$, $\lambda = 1.54$, 40 kV and 30 mA).

3.6.4. Electrochemical Single Cell Measurements

3.6.4.1. Catalyst Coated Membranes

For PEMFC single cell tests, membrane electrode assemblies (MEAs) with active area of 5.0 cm² were fabricated as described below. Commercial Pt/C (Tanaka TKK, Japan) and the Pt-NCNTF were used as anode and cathode catalysts, respectively. The cathode catalyst ink was prepared by dispersing Pt-NCNTFs (or Pt/C for anode) in Nafion solution (Ion Power, Inc., LQ-1005-1000 EW, 5% wt.) and isopropyl alcohol. The catalyst coated membrane (CCM) was fabricated coating the catalyst ink by micro-spray method on Nafion-212 (Ion Power Inc., USA) membrane and vacuum-dried at 70 °C. The catalyst loading was 0.2 and 0.12 mg Pt per cm² on the anode and cathode sides of the CCM, respectively.

3.6.4.2. Gas Diffusion Layer

Teflonized non-woven carbon paper (GD07508G, Hollingsworth & Vose Company) was used as a substrate for fabricating gas diffusion layers (GDLs). Nano-chain Pureblack carbon (grade 205-110) from Superior Graphite Co., VGCF from Showa Denka, Teflon dispersion (TE-3859) from DuPont Fluoroproducts and SDS (C₁₂H₂₅SO₄Na) from Fisher Scientific were used for carbon slurry preparation. 0.5 g of carbon powder (73.16 wt.% Pure black carbon powder and 26.84 wt.% VGCF) was dispersed in 8 ml DI water containing 120 mg of SDS by stirring the mixture for 10 min and sonicated for 30 min. PTFE (34 wt.%) dispersion was added into the mixture and followed with the magnetic stirring for 10 min. The carbon slurry (micro-porous layer) was coated onto the non-woven carbon paper substrates using Easycoater equipment (EC26, Coatema) at 3 m per min speed. After coating the micro-porous layer, the GDL samples were dried at room temperature overnight followed by sintering at 350 °C for 30 min in air. GDL sample was washed to remove the SDS impurities by immersing in warm DI water for 30 min. The carbon loading on the micro-porous layer was about 3 mg cm⁻², controlled by the wire thickness on the wire rod.

3.6.4.3. Fuel Cell Assembly and Testing

The MEAs were assembled by sandwiching the CCM with the gas diffusion layers on both sides in the PEMFC test cell (Fuel Cell Technologies Inc., USA) of the CCM. Silicone coated fabric (Product # CF1007, Saint-Gobain Performance Plastics, USA)

gasket used and the cell was tightened with a uniform torque of 40 lb-in. Single cell fuel cell performance was evaluated using Greenlight Test Station (G50 Fuel Cell Test Station, Hydrogenics, Canada) at 50, 60 and, 70 °C with H₂/O₂ at ambient pressure by galvanostatic polarization. The relative humidity of the reactant gases were maintained at 100 % by controlling the humidity bottle temperatures (both at 400 SCCM, respectively).

Summary

The algal species *Dictyosphaerium* was grown on Bold's Basal Medium (BBM) under lab conditions. Harvested algal cells were lysed and centrifuged to isolate the hydrogenase in supernatant and PSII in pellet. Electrons flow was established from isolated PSII to crude Hydrogenase through Cu wire, thus avoiding O₂ inhibition. Heparin and 2-Carboxy-D-arabinitol-1-phosphatase are reported as FNR and Rubisco inhibitors. The ZIF-67 particles were soaked with H₂PtCl₆.6H₂O solution and pyrolyzed under Ar/H₂ (90%/10% in volume ratio) atmosphere. The as-prepared black powder product after cooling down to room temperature naturally was treated in 0.5M H₂SO₄ solution for 6 h. The resulting sample was collected by centrifugation, repeatedly washed with DI water, and then dried at 80 °C. For PEMFC single cell tests, membrane electrode assemblies (MEAs) with active area of 5.0 cm² were fabricated. Commercial Pt/C and the Pt-NCNTF were used as anode and cathode catalysts, respectively. Single cell fuel cell performance was evaluated using Greenlight Test Station at 50, 60 and, 70 °C with H₂/O₂ at ambient pressure by galvanostatic polarization. The relative humidity of the reactant gases were maintained at 100 % by controlling the humidity bottle temperatures.

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Chapter 4

Results and Discussion

4.1. Bypassing Hydrogenase Inhibition for Hydrogen Production from Crude Enzyme Extract

4.1.1. Gasses Collection and Analysis

Gasses were collected in the syringes inserted in the vials containing crude enzymes extract and PS II. PSII, crude enzymes connected with PS II and crude enzymes (control) produced 9 ml, 1 ml and 0.9 ml of gasses respectively. These gasses (0.5 ml) were analyzed using Gas Chromatograph (GC) (Shimadzu GC-2010 Plus) at Quaid-e-Azam University, Islamabad.

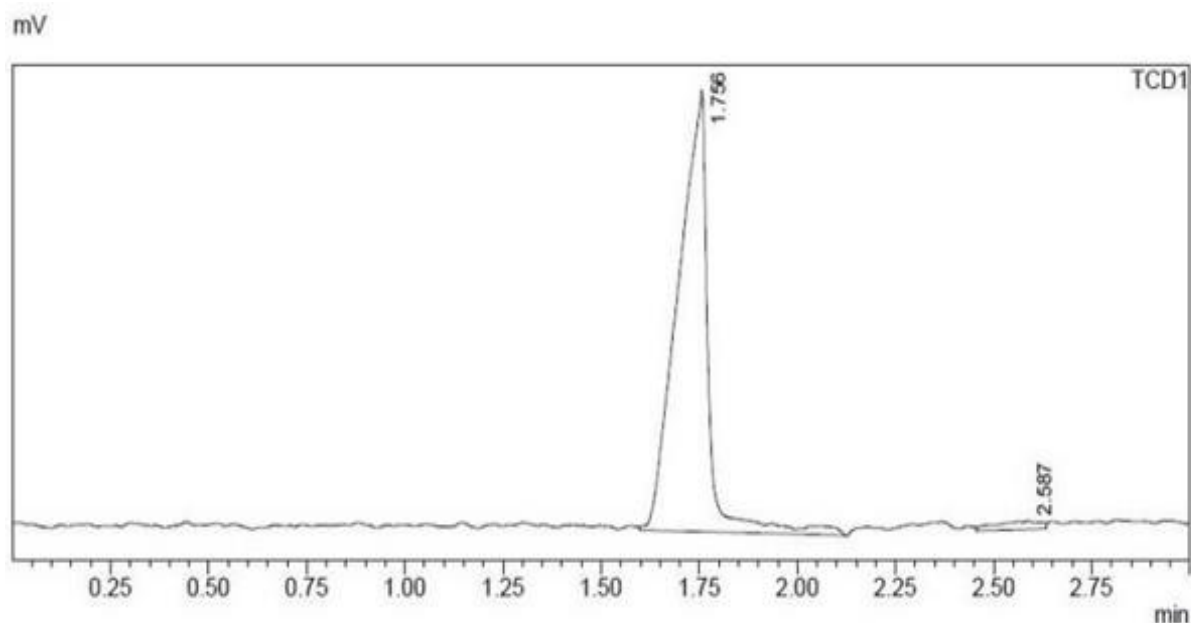


Fig. 4.1. Gas Chromatograph of Gasses produced by Crude Enzymes with external electrons from PSII

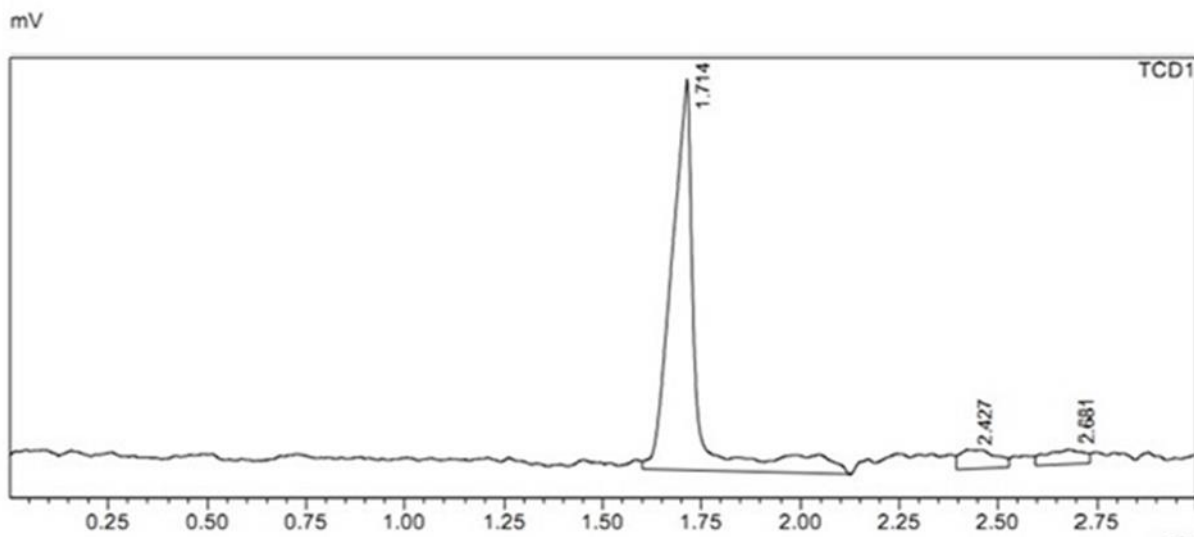


Fig. 4.2. Gas Chromatograph of Gasses produced by Crude Enzymes

4.1.2. Composition of Gasses

GC analysis of gas produced by pellet did not showed any evidence of hydrogen, instead it produced oxygen. Lack of hydrogen corresponds to the absence of hydrogenase. Hydrogen was detected at retention time of 1.714 minutes in vials containing crude enzymes, as shown in Table No.4.1.

Table 4.1 Hydrogen production from experimental and control vessel

S No.	Sample	*Total Gas Volume, ml	GC H ₂ conc. ml	H ₂ conc. %	Total H ₂ produced
1	Crude Enzymes with external electrons from pellet	10	0.106	21.2	2.12 ml
2	Crude Enzymes	9	0.05	10	0.9 ml

*Total Gas Volume = Total Vial Overhead + Rise in injection

Results indicate that hydrogen production enhanced by more than 2 folds by connecting the crude enzymes with pellet containing PS II through Cu wires.

4.1.3. Electrons flow from PSII to Crude Enzymes

Cu wire connecting crude enzymes and pellet was broken and light dependent potential difference was measured using digital multimeter (UNI-T UT33D) which indicates the electron flow from pellet to crude enzymes extract. Moreover potential difference

between two wires inserted in pellet showed the potential difference after short-circuiting, which confirms that PSII continue to work when extracted from the cell.

4.1.4. Mechanism for Bypassing hydrogenase inhibition for hydrogen production from crude enzyme extract

As crude enzymes dissolved in Tris HCl were used, so other photosynthetic material was also be present with hydrogenase, which contributed towards hydrogen production in control i.e. only crude enzymes. Moreover, enhanced hydrogen production by crude enzymes connected with pellet is attributed by external electrons as shown in Fig No. 4.3.

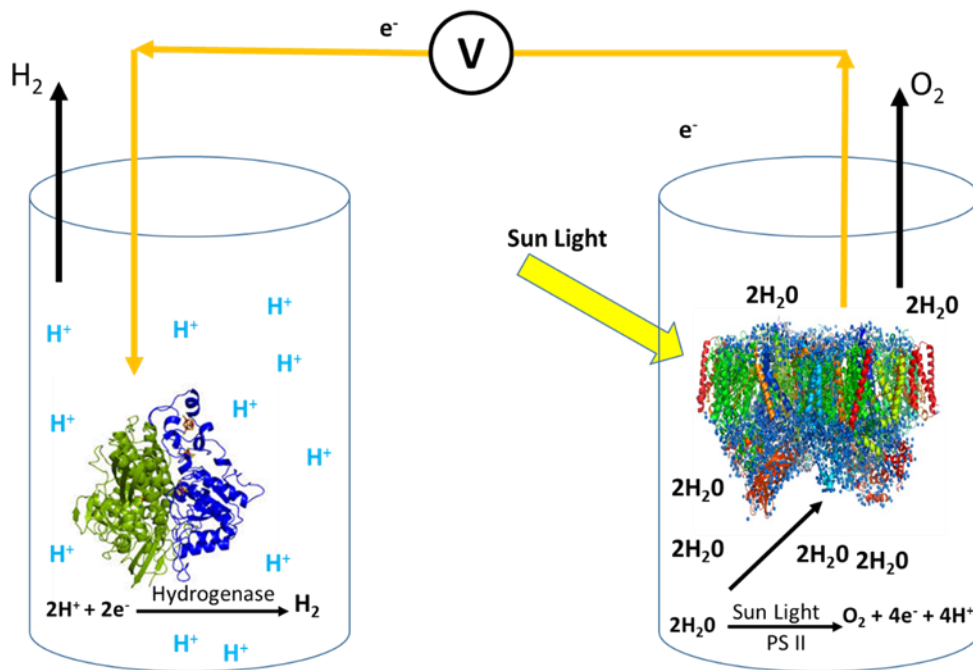


Fig. 4.3. Schematics for Hydrogen production bypassing Oxygen inhibition for Hydrogenase using Crude Enzymes Extracts.

As similar Cu wire was used as electrodes and making connections, so light dependent potential difference across the two electrodes in vial containing the pellet indicates that it is due to splitting of water at PS II, it also indicates that the PS II continues to work when separated from the cell. Application of PS II in biosolar cells is another evidence that the PS II continues to work after getting extracted from the cell. Presence of the

potential difference confirmed the flow of electrons from PS II to Hydrogenase and increased hydrogen production due to external electrons shows that the hydrogenase continues to work when separated from the cell and can utilize electrons from external source.

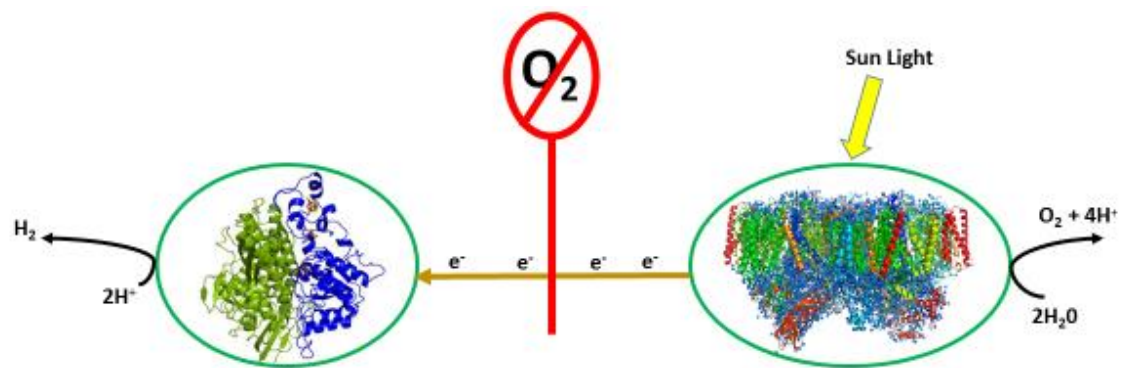


Fig. 4.4. Transfer of electrons from isolated PSII to isolated hydrogenase for hydrogen production

4.2. Study of Heparin and 2-Carboxy-D-arabinitol-1-phosphatase to regulate formation of NADPH formation and utilization

Gas Chromatographic analysis of gasses produced did not give any evidence of hydrogen. Heparin and 2-Carboxy-D-arabinitol-1-phosphatase have been reported as isolated FNR and Rubisco inhibitor. Lack of hydrogen indicates that these inhibitors were unable to contact FNR and Rubisco due to their inability to pass through different intercellular species.

4.3. Synthesis and Characterization of Platinum and Nitrogen doped Carbon Nanotubes Framework (Pt-NCNTFs) cathode catalyst using ZIF-67 for Proton Exchange Membrane Fuel Cell

The surface morphology as well as the physical dimension of the NCNTFs and Pt-NCNTFs cathode catalysts as examined under SEM and TEM are shown in Figure 4.5 (a and c) and 2(b and d), respectively. As can be seen clearly from the SEM images, the carbon nanotubes are grown on the surface of ZIF-67, which is slightly different from the published literature [1]. The possible reason for the growth of carbon nanotubes is due to the more reducing environment under flowing Ar gas with 10 % H₂ during the

pyrolysis, similar to a Chemical vapor deposition (CVD) process. As evident from the TEM image shown in Figure 2c, the presence of well-defined multiwall carbon nanotubes is confirmed, as observed in the surface examination by SEM.

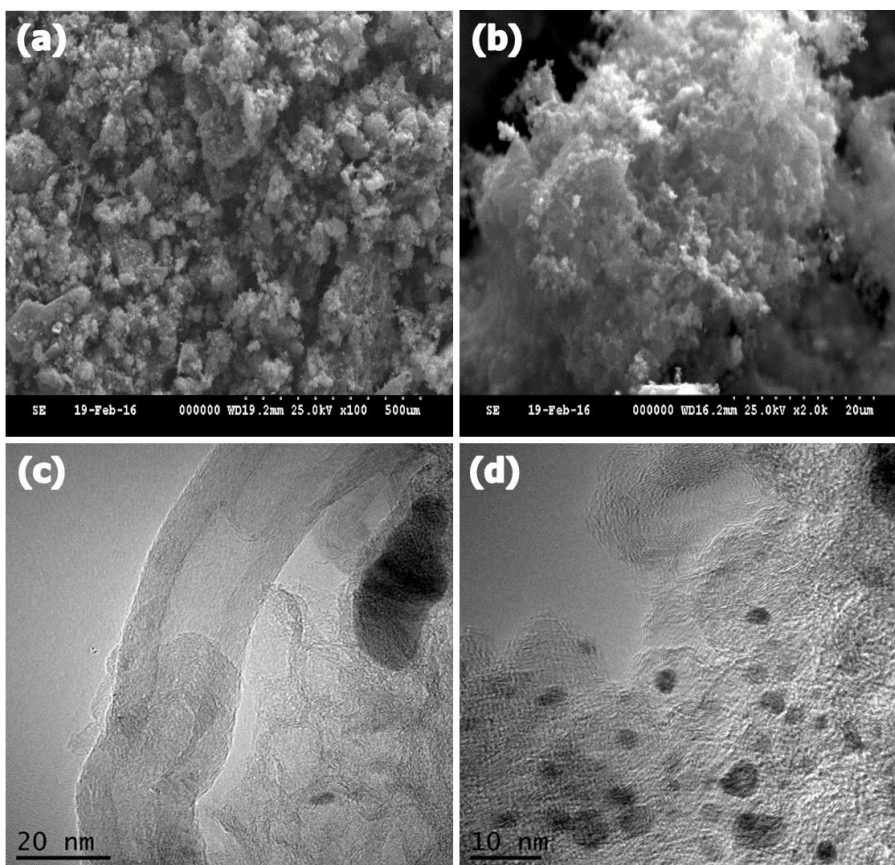


Fig. 4.5. Scanning Electron Micrographs of (a,b) Pt-NCNTFs, and Transmission Electron Micrographs of (c) NCNTFs and (d) Pt-NCNTFs.

The darker spot located at upper right is Co particle as measured by EDS analysis with ~15 nm in diameter. As seen in Figure 2d, the average Pt particle sizes are in the range of 7-10 nm, which could lead to fairly high performance and longevity. The catalyst particles are in the range of 4 to 8 nm yielding good performance and improved durability [2]. In addition, graphitic lattice fringes (with 0.34 nm) are also evidenced for providing superior conducting network as well as electrochemical stability. XRD patterns for NCNTFs and Pt-NCNTFs are given in Figure 3. As clearly observed, both the NCNTFs and Pt-NCNTFs show the presence of graphitic carbon at 2θ value of ~26.3

(hkl value: 002), confirming the graphitized CNTs under TEM examination. Other 3 diffraction peaks in the NCNTFs (Figure 3a) are identified as Co (111), Co (200) and Co (220) planes at the 2Θ values of 44.36, 51.67 and 75.98, respectively. In the case of Pt-NCNTFs, the presence of Pt-Co alloy is identified and is expected to exhibit enhanced electrochemical performance towards ORR [3]. Based on the lattice parameters of Pt (3.92 Å) and Co (3.53 Å), the composition of the Pt-NCNTFs (3.86 Å) is estimated to be 84 - 16 atomic wt. % Pt-Co alloy using Vegard's law [4]. From the Scherrer Equation the average crystallite size was calculated by FWHM, for the Pt-NCNTFs as 10.5 nm which is nearly identical to the values estimated from TEM images.

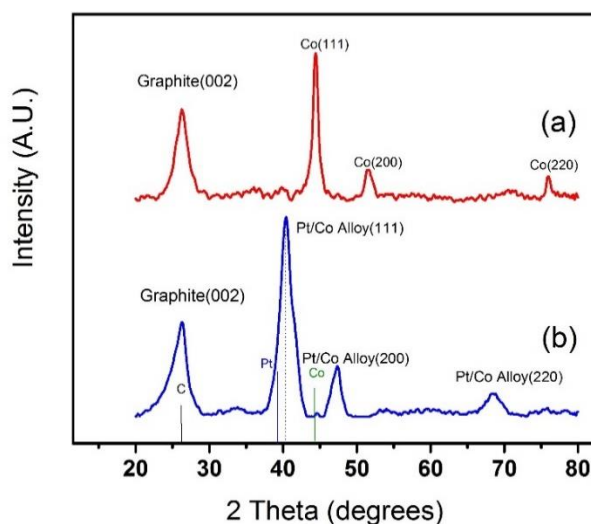


Fig. 4.6. X-Ray diffractograms of (a) NCNTFs and (b) Pt-NCNTFs.

Figure 4.7 shows the PEM fuel cell performance of the MEAs with Pt-NCNTFs cathode and commercial Pt/C anode catalysts using H_2 and O_2 at various temperatures up to 70 °C. It is very encouraging to observe that the cathode with extremely low loading of 0.12 mg Pt per cm^2 showed a peak power density of 630 mW per cm^2 at 70 °C with H_2 and O_2 gases at ambient pressure at 100 % RH.

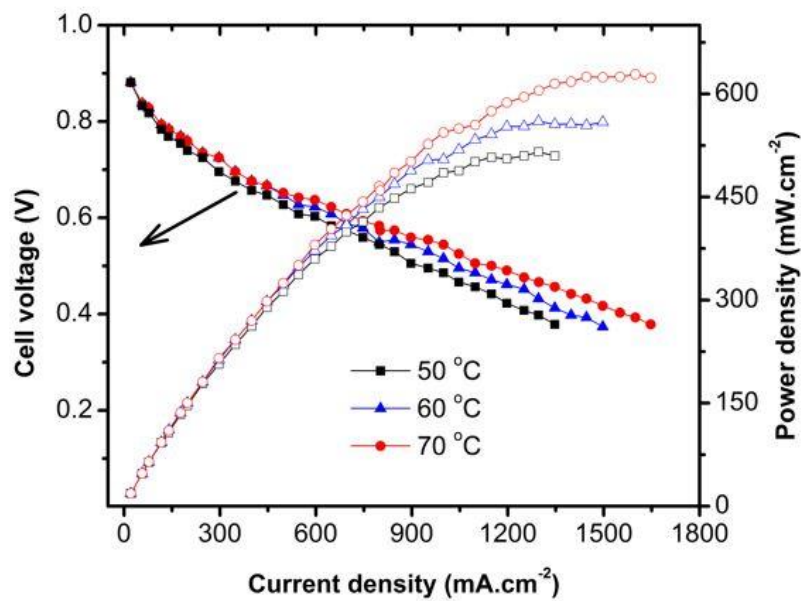


Fig. 4.7 Fuel Cell Performance using Pt-NCNTFs cathode catalysts at various temperatures with H₂ and O₂ gases, 100 % RH at ambient pressure. The open symbols represent the power density values.

Summary

Hydrogen production enhanced by more than 2 folds by connecting the Hydrogenase with pellet containing PS II through Cu wires as compared to the Hydrogenase without external electrons.. Heparin and 2-Carboxy-D-arabinitol-1-phosphatase have been reported as isolated FNR and Rubisco inhibitor. Gas Chromatographic analysis of gasses produced did not give any evidence of hydrogen. Lack of hydrogen indicates that these inhibitors were unable to contact FNR and Rubisco due to their inability to pass through different intercellular species. Pt nanoparticles of size 10 nm were supported on nitrogen doped carbon nanotubes framework prepared from pyrolysis of ZIF-67 at 700 °C in an Ar/H₂ (90/10 %) environment. ZIF-67 served as single source for Co, C and N. The MEA with Pt-NCNFs nanocatalyst showed excellent PEMFC performance with a peak power density of 630 mW.cm⁻² using Nafion-212 membrane at 70 °C with H₂ and O₂ gases at ambient operating pressure. The fuel cell performance with low Pt loading towards ORR could be attributed to the synergistic effect from Pt, Co, Pt-Co alloy, nitrogen and carbon nanotubes framework. It was interesting that the composition of the nannocatalyst Pt-NCNTFs estimated using Vegard's law was 84 - 16 atomic wt. % Pt-Co alloy.

References:

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Conclusions & Recommendations

Hydrogen production can be enhanced at expense of electrons from PSII to isolated Hydrogenase. Hydrogenase and PSII continue to work for several hours after they are isolated from the cell. An innovative approach has been established to separate the Hydrogenase in form of crude enzymes and PSII from the cell. Sodium Heparin and 2-Carboxy-D-arabinitol-1-phosphatase have been reported as isolated FNR and Rubisco inhibitor respectively but they did not affect the metabolic pathway of algae indicating that they can only inhibit the extracted FNR and Rubisco. It might be due to their inability to pass through different intercellular species. Pt nanoparticles of size 10 nm were supported on nitrogen doped carbon nanotubes framework prepared from pyrolysis of ZIF-67 at 700 °C in an Ar/H₂ (90/10 %) environment. ZIF-67 served as single source for Co, C and N. The MEA with Pt-NCNFs nanocatalyst showed excellent PEMFC performance with a peak power density of 630 mW.cm⁻² using Nafion-212 membrane at 70 °C with H₂ and O₂ gases at ambient operating pressure. The fuel cell performance with low Pt loading towards ORR could be attributed to the synergistic effect from Pt, Co, Pt-Co alloy, nitrogen and carbon nanotubes framework. It was interesting that the composition of the nanocatalyst Pt-NCNTFs estimated using Vegard's law was 84 - 16 atomic wt. % Pt-Co alloy. The composition analysis of isolated PSII and crude enzymes extract is recommended to further understand the mechanism of enhanced hydrogen production. Study with extracted PSII and crude hydrogenase isolated by proton exchange membrane i.e. Nafion and interconnected by Cu wire is also recommended. Efforts should be put together to provide the external electrons to hydrogenase from eukaryotic plants, if hydrogen production can be established then hydrogenase can be produced by bacteria through genetic engineering and it would revolutionize the hydrogen production. Optimization of external conditions is recommended to enhance the life of hydrogenase and PSII. Further evaluation of the Pt-NCNTFs nanoelectrocatalyst is recommended for extensive characterization including durability

Recommendations