Studies on efficacy of *Nannochloropsis granulata* Karlson et Potter for using in preparation of microbial photovoltaic cell by harnessing solar energy

Dissertation submitted in partial fulfillment of the requirements for the award of degree of Master of Science in

BOTANY

By

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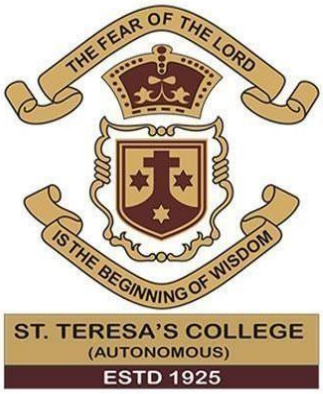

DEPARTMENT OF BOTANY

ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

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ABBREVIATIONS

µl	Microlitre
BLAST	Basic Local Alignment Search Tool
bp	Base Pairs
BPV	Biophotovoltaic Cell
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
FC	Fuel Cell
GHG	Greenhouse Gases
g	Gram
I	Current
ITO	Indium Tin Oxide
M	Molar
MFC	Microbial Fuel Cells
ml	Millilitre
mm	Millimetre
mMPV	Microalgal based Microbial Photovoltaic Cells
NCBI	National Centre for Biotechnology Information
nm	Nanometer
OD	Optical density
PCR	Polymerase Chain Reaction
PVP	Photovoltaic Panels
R	Resistance

rpm	Rotations per minute
rRNA	Ribosomal Ribonucleic Acid
SAC	Surface Area Coverage
srRNA	Smaller Subunit Ribosomal RNA
UV	Ultraviolet
V	Voltage

ABSTRACT

Microbial Photovoltaic cells are a new photo-bio-electrochemical technology for harnessing solar energy using the photosynthetic activity of autotrophic organisms. This is a new technology for the production of sustainable and clean energy. A better understanding of the electrochemical interactions between the autotrophic microorganisms and conductive materials will be likely to lead to increased power yields. In the current study, the microalgae Nannochloropsis granulata Karlson et Potter was investigated for microbial photovoltaic cell preparation. The most important element is represented by the electrode configuration, based on inexpensive materials, with the anode immersed in the cultural broth and the cathode coated with carbon. This configuration represents a very interesting simplification for the cell design, furthermore allowing a simple illumination of the algal culture via a light source positioned above the cell, perpendicular to the electrode surface. This device was then characterized by measuring the electrical performance of the microbial photovoltaic cell. A power density of 23.152 W/m2 was recorded; revealing interesting potentialities for green unicellular algae fuelled microbial photovoltaic cells

CHAPTER ONE

INTRODUCTION

INTRODUCTION

Global energy demand is rising as a result of an expanding population. Oil is consumed now 105 times quicker than it can be made by nature. Humankind currently depends inescapably on energy. Its quantity, convenience, and possibilities have become essential to our modern way of life on a personal and social level.

Because of the high energy demands of the expanding population, energy needs are likewise rising at an alarming rate. Energy is necessary for human life, and modern societies cannot be sustained without a reliable and accessible energy supply. The sun is the primary source of most of the energy used by people. The use of non-renewable energy sources is not new. These sources have been the sole source of energy for humans for many years. In terms of the calibre of the power generated, these fuels are highly effective, but they are not long-term advantageous.

About 80% of the world's energy needs are met by fossil fuels, which are also one of the leading contributors to environmental pollution and current global climate change (Chen *et al.*, 2011). These fossil fuels also pose a serious threat to ecological security and the delicate balance of the ecosystem. Concern over rising atmospheric carbon dioxide concentrations and their significant effects on global warming has grown over the past few decades (Mohsenpour, 2014).

The rapid depletion of finite oil reserves and the population boom both contribute to an increase in atmospheric carbon dioxide, which causes global warming. Today, it is acknowledged that one of the biggest challenges to humanity is climate change. The need for energy and its effects on the environment compels us to use additional renewable resources and search for fossil fuel substitutes.

Renewable energy is the solution to the growing energy challenge. Since they might theoretically supply much more energy than the world needs, renewable energy sources have immense promise. Based on the usage of commonly accessible, indigenous resources, renewable energy sources like biomass, wind, sun, hydropower, and geothermal can deliver sustainable energy services. But each of these renewable energy sources has their own environmental, social, and economic impacts.

Providing enough energy for a growing global population is currently one of our most pressing global challenges. The development of new technologies to exploit renewable energy sources is critical for meeting future power demands. Several non-biological technologies have been developed in the context of electricity production, two of which are closely related to BPV technology.

Photovoltaic panels (PVs) convert solar light into electrical energy. Fuel cells (FCs) can be used to generate electricity more efficiently from fossil fuels.

Microbial fuel cells are gaining popularity as potential sources of renewable energy. They rely, in principle, on heterotrophic microorganisms metabolising exogenous organic molecules and supplying electrons to an electrode, thereby providing a source of electrical energy.

Because of the requirement for exogenous metabolites (which can be obtained from waste materials), there is growing interest in using photosynthetic microorganisms in similar fuel cells. Photosynthetic microbial fuel cells, biophotovoltaic devices, or BPVs are fuel cells that contain photosynthetic microorganisms.

These organisms do not require an organic substrate and can use light energy to oxidise water and provide electrons, some of which may be lost from the cell to an external anode eventually (and perhaps very indirectly). Microorganisms may also provide electrons under dark conditions by metabolising substrates within the cell.

Although PVs have high light energy conversion efficiencies (up to 50% in the laboratory and around 10% for the majority of commercial silicon PVs), the fabrication and installation of PV solar farms are generally very expensive.

Similarly, FCs have high fuel utilisation (up to 80% of energy conversion), but they must frequently operate at high temperatures, require high-purity fuels, and use expensive precious metal catalysts. Thus, high costs are a major impediment to the widespread use of large-scale PVs and FCs for power generation.

Recently, biological components were introduced into FCs, resulting in microbial fuel cells (MFCs). These bio-electrochemical devices can operate at room temperature, use organic compounds (including biological waste materials) as fuel, and catalyse the fuel oxidation process with low-cost microbial organisms.

Recent advances aim to use MFCs as solar cells, integrating phototrophic biological components into the electrochemical apparatus to create a new generation of solar cells: biological solar panels. These are called biological photovoltaic (or biophotovoltaic) devices (BPV).

Although BPVs function similarly to MFCs, electrical energy is transduced from light energy by photosynthetic organisms' light-harvesting apparatus rather than being produced by metabolising organic fuels.

BPV technology was developed in the beginning by combining photosynthetic hydrogen-evolving microbes with hydrogen FCs. This two-stage process began with algae producing hydrogen gas, which was then used to generate electrical power in an FC device.

Successive efforts have focused on the direct generation of photocurrent, and BPV devices incorporating various combinations of photosynthesis components, such as photosynthetic pigments, photosystem complexes, and thylakoid membranes, have been developed.

Early BPV research revealed a direct light-driven power output from whole photosynthetic organisms using intact cyanobacterial cells. Although these studies showed promise, the requirements of anaerobic conditions in the anode, oxygen enrichment at the cathode, and an applied bias potential limited their further use.

This research study investigates the efficacy of microalgae *Nannochloropsis granulata* species for its usage in preparing microbial photovoltaic cell by harnessing solar energy. *Nannochloropsis* a genus of very small (less than 5 mm) coccoid unicells, is primarily recognised from the marine environment and is a marine ultraplankter. Both pyrenoid and the lamellate vesicles present in chloroplasts are absent in *N. granulata*. Electron-dense bodies in the cytoplasm of *N. granulata* were distinct from the structures seen in other species.

Biologists classify algae on the basis of their life cycle, pigmentation and cellular structure. Because of the constant changes in the revision of new genetic and ultrastructural evidence, there is no definitive classification system that could include all algae species. Table 1.1 shows the latest classification and systematic position of the *Nannochloropsis granulata* species based on the work of (Guiry & Guiry, 2018).

Classification	
Kingdom	Chromista
Phylum	Gyrista
Subphylum	Ochrophytina
Class	Eustigmatophyceae
Order	Eustigmatales
Family	Monodopsidaceae
Genus	*Nannochloropsis*
Species	*Granulata*

TABLE 1: Classification of algae

The whole cells of microalgae *Nannochloropsis* has more potential in generating power in a photovoltaic cell. Such cells also achieves more robust performance. BPVs based on multiple-cell systems, have shown improved electrogenic activity.

Although the BPV field has seen significant advancements, there are currently few quantitative data sources in the literature, and examples of how the principles of operation work are typically constrained to specific contexts. Additionally, the biological mechanisms responsible for the light-dependent electron flow from the water photolysis site to the outside of the cell have not yet been molecularly characterised. For the efficiency of BPV devices to be optimised in the future, this characterisation will be necessary.

AIM AND OBJECTIVES

AIM

- To study the efficacy of *Nannochloropsis granulata* in preparing microbial photovoltaic cells by harnessing solar energy.

OBJECTIVES

- To culture the organism and analyse its growth.
- To isolate DNA with PCR amplification and identification of the organism.
- To develop biofilms from *Nannochloropsis granulata* and to assess its photovoltaic efficiency.
- To generate high power output microbial photovoltaic cells.

CHAPTER TWO

REVIEW OF LITERATURE

REVIEW OF LITERATURE

This chapter provides a critical analysis of the literature on microbial photovoltaic cells from microalgae *Nannochloropsis* and provides the background knowledge needed for this research investigation. It begins with an introduction to the energy needs of the current world and the energy crisis that has to be faced shortly and the limitations of non-renewable sources of energy and then goes on to discuss the prospects of renewable sources of energy mainly, solar energy. The final section of the chapter examines the potential of microbial fuel cells and biophotovoltaic cells in power generation and the efficiency of microalgae in its preparation.

2.1 Energy needs and crisis

Without energy, modern life will be impossible. Imagining our lives without energy for even two minutes is impossible in this current world. Our modern life, both personally and socially, has become dependent on its abundance, convenience, and possibilities. The energy needs are also increasing at an alarming rate due to the high energy demands of the growing population (Satyanarayana *et al*., 2011).

Energy has always been a necessity for humanity, and while its source and methods of use have evolved, some patterns have not. Food served as the main energy source for people and their cattle in former times. Globally, societies have concentrated on creating new, sustainable food sources. How societies learned to organise themselves communally, survive times of scarcity, and also profit from sporadic abundance was influenced by the storage and distribution of food.

The development of food processing and preservation techniques allowed for the more effective and waste-free utilisation of new food sources (Coyle & Simmons, 2014). People moved across continents, seas, and oceans in response to sometimes complex social constraints, but undoubtedly, a common denominator in their journeys was the need for food and dependable supplies of sustenance.

There is no doubt that similar patterns have been present for thousands of years, although there may be a greater urgency now than there has ever been to identify sustainable sources of energy, improve the efficiency of energy use, and discover new sources of energy due to the expanding global population, the depletion of energy resources, and the rise in environmental concerns. The fact that

modern society is still raising crops that were formerly considered food but are now just grown to make biofuels has a timeless and circular quality.

The history of energy consumption demonstrates how resource exploitation is dictated by competing uses, frequently to the expense of the original but less powerful first adopters. The use of charcoal as a cooking fuel has a long history and is still popular for use in barbecues today. However, more than 5,000 years ago, people discovered that it was helpful in the smelting of iron and used it in the Bronze Age to produce copper and, more valuable, bronze. Woodlands were cleared as a result of these and other initiatives, and land that had previously been reserved primarily for agriculture was now competing with it. The retention of some trees, which were later coppiced to produce a source of charcoal, was made easier by the use of banks to divide the area.

By the thirteenth century, Europeans were aware of the powerful Chinese gunpowder, which rekindled interest in charcoal. Gunpowder was used for military purposes, which required the casting of cannons, which used a lot of charcoal. These factors put pressure on the availability of wood that may be used to produce charcoal, which causes limits to be put in place in some nations. A substitute was desired, and one was developed in the form of coke by the eighteenth century due to the enormous demand for charcoal to support the iron industry (Coyle & Simmons, 2014). In addition to replacing charcoal for a variety of industrial uses, coke also produced a combustible gas that could be utilised in homes.

Naturally, companies that make coal and coke promoted the use of their goods, which further decreased the demand for charcoal. The historical connections between coke and charcoal show how a single energy source may be exploited for a variety of purposes that interact with one another, leading to intricate relationships between buyers and sellers.

The world saw the horror of nuclear energy's deadly force during World Wars I and II and its aftermath, as well as the potential promise of an effective, dependable, and clean source of electrical energy. To address issues like nuclear waste disposal, nuclear power plant accidents and their effects on the environment and society, and the continued development and dependence on nuclear energy from an armaments perspective, the debate on the future mix of nuclear power in the provision of global energy continues today.

Additionally, the basic claim that environmental issues are not the only ones that affect choices regarding energy generation also holds true for what are sometimes referred to as "green" or "clean"

technology. The positions taken by lobbying organisations to advance their own agendas have not always been supported by reliable economic and environmental statistics. The result is that the informed public is now more vocal in challenging the most recent initiatives to extract energy from renewable and sustainable sources, including estuary barrages, wave power, offshore wind, solar power, and biofuels (Coyle & Simmons, 2014). Energy engineers have a clear obligation to assist in educating policy makers and the general public about the benefits and drawbacks of each energy production method in addition to looking for new solutions and creating new production techniques.

Energy is now an inevitable component of humankind. Energy is the ability of a physical system to do work (Khan *et al.*, 2018). Energy exists in multiple forms such as heat energy, mechanical energy, light energy, potential energy, electricity, or any other form. According to the law of conservation of energy, energy can be converted to another form, but the total energy of the system remains constant.

Energy is essential to human life, and a safe and accessible energy supply is essential for the sustainability of modern societies. Most of the energy consumed by humans comes directly or indirectly from the sun. Non-renewable energy sources have been around for a long time. Humans for decades are solely dependent on these sources for their energy needs (Shahzad, 2012). These fuels are quite efficient in terms of the quality of the power produced, but they are not beneficial over the long term.

Fossil fuels are non-renewable resources since their formation takes millions of years and their reserves are being used up much more quickly than they can be replenished. Over 22 billion tonnes of carbon dioxide are produced per year by burning fossil fuels, but only approximately half of that is thought to be absorbed by natural processes. Eleven billion tonnes of atmospheric carbon dioxide are increased annually as a result of this (Coyle & Simmons, 2014). In addition, these fossil fuels constitute a major danger to ecological safety and the environmental balance.

More than a quarter of the world's primary energy demand is met by coal, which is the most plentiful and commonly used fossil fuel. It continues to be one of the most significant sources of energy for the globe, notably for power generation, with global proven reserves totalling close to one trillion tonnes (Curtin & Maguire, 2011). The primary disadvantage of using traditional coal is that modern coal-fired power plants have low efficiencies and emit large amounts of pollutants. Petroleum, sometimes referred to as crude oil is another widely distributed fossil fuel. It can be found in the

Earth's crust in natural formations. It comes from prehistoric biological substances like zooplankton and algae. Oil drilling is the primary method of recovering petroleum.

Natural gas can be found as methane clathrates, in coal beds, or association with other hydrocarbon reserves deep down in natural rock formations. Petroleum can be found near and with natural gas (Serra, 2013). The production of most natural gas over time has either been biogenic or thermogenic. In marshes, bogs, landfills, and shallow sediments, methanogenic organisms (microorganisms that make methane as a metabolic byproduct in anoxic environments) produce biogenic gas. Organic material buried deeper in the soil produces thermogenic gas at higher temperatures and pressures (Serra, 2013).

Continued use of fossil fuels faces several other challenges. These include the depletion of fossil fuel reserves, global warming and other environmental issues, geopolitical and military conflicts, and the recent, sustained, and significant rise in fuel prices. These issues represent an unacceptable situation. Renewable energy is the solution to the growing energy challenge. Renewable energy sources such as solar, wind, biomass, wave, and tidal power are abundant, inexhaustible, and environmentally friendly (Asif & Muneer, 2007).

2.2 Renewable Energy Sources

Renewable energy sources have the potential to meet the world's energy demands. Sources like biomass, wind, hydropower, geothermal and solar can provide sustainable energy services (Herzog *et al.,* 2001). The abundance of renewable energy is its most important characteristic. Compared to traditional fossil fuel energy technology, renewable energy sources are hygienic energy sources with far less of an adverse environmental impact. The clean and sustainable nature of these resources has also made it the need of the hour. Researches by scientists and researchers are going on in the field to find out new ways to utilise these resources effectively.

As the cost of solar and wind power systems has decreased significantly over the past 30 years and has continued to diminish, while the price of oil and gas has continued to vary, a switch to renewable energy systems is becoming more and more possible (Herzog *et al.,* 2001). The economic and political frameworks required to promote the wide adoption and long-term viability of markets for renewable energy systems have also advanced quickly.

It is becoming increasingly obvious that the new regime of renewable energy sources—and to a lesser extent, natural gas-based systems—will drive future expansion in the energy sector, rather than traditional oil and coal sources.

Financial markets are starting to see the potential for future growth of renewable and other new energy technologies, and this is most likely a sign of what will happen when renewable energy systems become competitive from an economic perspective. The expenses of social and environmental issues are growing in opposite directions for fossil fuel and renewable energy pricing.

Moreover renewable sources of energy have less carbon emissions compared to fossil fuel burning. The climate change caused by non-renewable sources of energy can also be reduced. The Renewable energy sources can also create a new employment sector and thus can provide jobs for the unemployed (Shahzad, 2012). This can bring down the unemployment scales of the developing countries. Renewable energy sources can also stabilise the electricity prices by reducing the consumption. The initial investment for the renewable energy sources are the only cost needed for their use and is free from the fluctuating costs of the non-renewable energy sources.

Applications of renewable energy are broadly divided into on-grid and off-grid. A grid is an integration of generation, transmission, and distribution systems that supply energy to multiple consumers (Shahzad, 2012). On-grid and off-grid are terms that describe how electricity is derived. On-grid deals with power plants that are directly connected to the grid**,** such as wind farms and solar panels**.** Off-grid applications typically handle only one load like a small house or village house. Off-grid applications can take many forms, from photovoltaic panels for a single village house to centralised wind turbines that power village water pumps and commercial battery chargers. These off-grid applications are most commonly used in remote or rural environments. One of the important grid- connected applications is large- scale power generation.

2.2.1 Wind Energy

For many centuries, humans have relied on wind energy as one of their main energy sources to move commodities, process food, and pump water (Herzog *et al.*, 2001). It is widely accessible and doesn't emit any pollution while creating electricity, wind has a lot of potential as a clean energy source for the entire world.

The most important application of wind energy is wind turbines. Wind turbines can convert wind energy into mechanical energy and supply it to generators to generate large amounts of

electricity. This electricity can be used to charge batteries or pump water. Wind energy can also be used in windmills. This can save a lot of fuel and provide more power and efficiency.

Despite being seen as an environmentally friendly energy source, using wind energy has several adverse environmental effects. These include acoustic noise output, visual effects on the environment, effects on bird populations, the rotor's shadow, and electromagnetic interference that affects the reception of radio, TV, and radar signals. The most challenging aspects of the project in practice seem to be the noise and visual impacts (Herzog *et al.,* 2001). Aero-acoustic research advancements have produced design tools and blade configurations that have successfully made blades significantly quieter, which have helped to alleviate noise issues.

2.2.2 Hydropower

Hydropower is the most important renewable energy source for generating electricity. It is vital in many parts of the world, with more than 150 countries producing hydroelectric power (Herzog *et al.,* 2001). A common application of hydropower is in a compressor. Hydropower is a significant source of electricity worldwide, and it is expected to grow further, particularly in developing countries. While large dams have become a much riskier investment, there is still a lot of untapped potential for small hydro projects all over the world.

Although hydroelectricity is widely regarded as a clean energy source, it does not produce zero greenhouse gas emissions (GHG) and can frequently have significant negative socioeconomic consequences. Large-scale dams, some argue, do not reduce overall GHG emissions when compared to fossil-fuel power plants (Herzog *et al.,* 2001). If hydropower's role as a clean renewable energy source is to be maintained, improvements and efficiency measures in dam structures, turbines, generators, substations, transmission lines, and environmental mitigation technology are required.

2.2.3 Biomass Energy

All organic material derived from plants, including algae, trees, and crops are referred to as biomass, which is essentially the storage of solar energy through photosynthesis (Herzog *et al.,* 2001). The process of transforming biomass into usable energy sources like heat, electricity, and liquid fuels is known as bioenergy, also known as biomass energy. The future of the world's energy supply is expected to be significantly influenced by modernised biomass energy. Solid biomass can be burned

in an incinerator to generate heat and steam to generate electricity. Biomass can also be converted into biofuels such as ethanol for transportation purposes (Shahzad, 2012).

The recognised threat of global climate change, which is primarily brought on by the burning of fossil fuels, is what is driving this, rather than the depletion of fossil fuels, which has ceased to be a defining issue with the discovery of new oil and gas reserves and the substantial amount of existing coal resources (Herzog *et al.,* 2001). It is a promising energy source for the 21st century in many parts of the world due to its carbon neutrality (when produced sustainably) and relatively even geographic distribution, as well as the anticipated increase in energy demand in 12 developing nations where affordable alternatives are not frequently available.

Renewable energy sources are typically referred to as "green" energy since they generate electricity with a minimal impact on the environment, limited resource depletion, and minimal emissions.

However, even while biomass is theoretically renewable and can benefit the environment if handled properly, it also has many negative and beneficial traits in common with fossil fuels. Modernized bioenergy systems can have severe environmental effects related to both the growth of the biomass and its conversion to energy carriers, even though it can be transported and stored allowing for the creation of heat and power on demand.

The environmental effects of biomass production must be weighed against the alternative effects that would presumably occur (locally, regionally, and globally) in the absence of the bioenergy system. For instance, the relative effects of creating bioenergy feed stocks at the local or regional level will rely not only on how the biomass is generated but also on what would have occurred otherwise.

According to life cycle analysis (LCA) studies, where biomass replaces fossil energy systems, there will be a decrease in overall greenhouse gas emissions, which will reduce the impact on the planet's climate (Herzog *et al.,* 2001). However, for other types of emissions, such as NO_2, SO_2, and N_2O, the situation is less clear and is highly dependent on the source of the biomass, technical details of the conversion process, and other factors. While converting biomass into electricity, strict air quality regulations must be met in addition to environmental concerns about the condition of the land and water used in the process.

2.2.4 Geothermal Energy

Geothermal energy, or natural heat within the earth, is generated by the ancient heat that remains in the Earth's core, friction as continental plates slide beneath each other, and radioactive element decay, which occurs naturally in small amounts in all rocks (Herzog *et al.*, 2001). People have used hot springs and steam vents for bathing, cooking, and heating for thousands of years.

Throughout the twentieth century, technological advancements have made it feasible and cost-effective to locate and drill into hydrothermal reservoirs, pipe the steam or hot water to the surface, and use the heat directly (for space heating, aquaculture, and industrial processes) or convert the heat to electricity (Herzog *et al.*, 2001). Geothermal energy is most popular among farmers. They use this energy to heat greenhouses where they can grow different types of fruits and vegetables all year round.

Geothermal energy is not available everywhere, especially since the resources required for industrial-scale electrical energy production are not available. Nonetheless, geothermal energy is generally less expensive than conventional energy sources and is produced using well-established conventional technology.

2.2.5 Solar energy

Among the renewable energy sources, solar energy is a promising and freely available source of energy to address long-term problems in the energy crisis. Solar energy is obtained by various approaches such as direct solar energy-electrical conversion, solar energy-chemical energy conversion, and solar energy-thermal energy conversion (Kishore Ravi & Ching Tan, 2015) .

The solar industry is developing steadily due to high energy demand around the world. Solar energy offers advantages in terms of availability, cost-effectiveness, accessibility, capacity, and efficiency compared to other renewable energy sources (Kannan & Vakeesan, 2016). Similarly, solar energy can be used to power photovoltaic panels. This is a great option for small-scale generation, especially in rural and remote areas where power lines do not reach. Their low maintenance requirements and high reliability make them ideal for use in remote and remote locations.

In offices, glass PV modules can be used for reliable power supply. Solar energy is also commonly used in solar water heaters, solar thermal calculators, and photovoltaics. They work on the principle of storing solar energy during the day and using it at night.

2.3 Microbial Fuel Cells

The discovery that bacteria can be used to generate electricity from waste has received a lot of attention. Microbial fuel cells (MFCs) are a prominent example in this area. This is a new technology that converts the chemical energy stored in organic matter into electricity using heterotrophic bacteria (Logan *et al.,* 2006). Microbial fuel cells have recently been created as a result of the addition of biological components to FCs (MFCs). Recent developments in the study of microbial fuel cells (MFC) and fuel cells (FC) have shown that both electrochemical technologies are efficient ways to produce electricity from chemical fuels and organic chemicals (Bombelli *et al.,* 2011). Consequently, MFC-inspired photovoltaic (BPV) devices were created by biologically harnessing solar energy through the processes of photosynthetic organisms, producing electrical power.

Microbial fuel cells are gaining attention as a potential source of renewable energy. They rely on heterotrophic microbes to metabolize exogenous organic molecules and supply electrons to electrodes to provide a source of power.

Because of the need for exogenous metabolites (although these can be provided from waste materials) photosynthetic microorganisms have been used increasingly in similar fuel cells. These organisms don't need organic matter (Bombelli *et al*., 2011). Light energy can be used to oxidize water and donate electrons, some of which are ultimately (and possibly very indirectly) lost from the cell to the external anode. Under dark conditions, microorganisms can also donate electrons through the metabolism substrate in the cell.

Microbial photoelectrochemical cells use the photosynthetic activity of photosynthetic reaction centres of photosynthetic microorganisms like green plants, algae, etc. to convert light into electricity.

Cyanobacteria are nanoscale photovoltaic systems that use photosynthetically active radiation (PAR) to oxidize (photodegrade) water. The resulting electrons are used to synthesize adenosine triphosphate (ATP) and β-nicotinamide adenine dinucleotide phosphate (NADPH) (Bombelli *et al.,* 2011). The ability to harvest electrons and generate electricity in the MFC directly from the photosynthetic electron transport chain in photoautotrophic microorganisms has been largely unknown until now (Zou *et al.,* 2009).

2.4 Biophotovoltaic Cells

Fuel cells containing photosynthetic microorganisms are known as photosynthetic microbial fuel cells or Biophotovoltaic devices or BPV. The power output of these devices is low, and we need a better understanding of how they work to improve their power output. PVs, or photovoltaic panels, capture solar energy and convert it to electrical power (Bombelli *et al.,* 2011). Fuel cells (FCs) can be used to produce power more effectively from still-available conventional energy sources, or they can be combined with hydrogen to produce electricity without emitting greenhouse gases.

Similarly in the case of microbial fuel cells, self-sustainable microbial fuel cells that rely on light instead of organics as an energy source are being developed. Considering that sunlight offers an unlimited source of energy, this has become an increasingly popular area of research in recent years (De Caprariis *et al.*, 2014).

In more recent research, microbial fuel cells are being used as solar cells by incorporating phototrophic biological elements into the electrochemical system to create biological solar panels, a new generation of solar cells (Rosenbaum *et al.,* 2010). These are referred to as biological photovoltaic (or biophotovoltaic) devices.

There are two different types of microbial photoelectrochemical cells. Type one uses light as its sole energy source through photosynthesis. These microbial solar cells are called fuel cells because they do not have a continuous fuel supply. This type of cell is also called the bio-photovoltaic cell (BPV). Biophotovoltaics (BPV) is a novel photoelectrochemical technology for harnessing the photosynthetic activity of autotrophs to harness solar energy. This is a new technology for generating sustainable and clean energy(De Caprariis *et al.*, 2014). Type two photo microbial fuel cells (PMFC) use light that promotes power generation from anaerobic microbial oxidation of organic matter (Rosenbaum *et al.,* 2010).

Biophotovoltaics (BPVs) exhibit excellent light energy conversion efficiency (up to 50% in the lab and around 10% for the majority of commercial silicon PVs) (Mason, 2008). However, the creation and setup of PV solar farms are typically highly expensive (Breeze, 2005). The same is true for FCs, which have good fuel utilization (up to 80% of energy conversion), but frequently need to operate at high temperatures, demand highly pure fuels, and use pricey precious metal catalysts. Therefore, a major barrier to the widespread use of large-scale PVs and FCs for power generation is their high cost.

BPVs work similarly to MFCs, but instead of producing electrical energy by metabolising organic fuels, it is obtained from light energy via the light-harvesting system of photosynthetic organisms (He *et al.,* 2009). In the beginning, hydrogen FCs and photosynthetic hydrogen-evolving microorganisms were used to create BPV technology (Berk & Canfield, 1964). Algae's photo production of hydrogen gas served as the catalyst for this two-stage process, which was used to power an FC device (Strik *et al.,* 2008). To achieve this goal, BPV devices have been created that incorporate different arrangements of photosynthesis's constituent parts, including photosynthetic pigments, photosystem complexes, and thylakoid membranes (Maly *et al.,* 2005). Successive efforts have concentrated on the direct generation of photocurrent.

As there are fewer membrane barriers to obstruct electron transmission, sub-cellular photosynthetic components are anticipated to be favourable in terms of power output per unit volume compared to complete organisms (Ciesielski *et al.,* 2010). But when taken out of their natural physiological environment, sub-cellular systems can only supply energy for so long until the efficiency of the photosynthetic material deteriorates (Badura *et al.,* 2008). It is possible to extend working durations, but only by using methods that minimise energy generation while also protecting the material.

Early BPV research used entire cyanobacterial cells to show direct light-driven power output from full photosynthetic organisms (Martens & Hall, 1994). The need of anaerobic conditions at the anode, oxygen enrichment at the cathode, and an applied bias potential hindered the future exploitation of these devices even if these tests showed promise (Tsujimura *et al.,* 2001). To collect electrons from the thylakoid membrane, a nano-anode was most recently directly introduced into the chloroplast stroma of a single, immobilised cell of the unicellular green alga *Chlamydomonas reinhardtii* (Ryu *et al.,* 2010). Although this single-cell approach cannot be scaled up, the experiment showed a high current density and showed how whole cells might behave in BPV devices.

BPVs based on multiple-cell systems, such as cyanobacteria and more sophisticated plants, have been demonstrated to be capable of increased electrogenic activity with a focus on obtaining long-lasting and robust performance (Strik et al., 2008). Recent studies have demonstrated that some bacterial species biofilms have the ability to self-mediate extracellular electron transport. The potential for utilising this characteristic in photoautotrophic microorganisms that do not require an organic substrate could have significant ramifications for the advancement of solar energy

technology in the future (McCormick *et al.,* 2011). Even though the BPV sector has seen tremendous advancements, the current literature often lacks quantitative data. Principles of functioning can only be demonstrated in a specific environment.

Molecular characterization is also included. There is still a lack of biological mechanisms that are engaged in light-dependent electron transport from the location of water photolysis to the outside of the cell. For the efficiency of BPV devices to be optimised in the future, this characterization will be necessary.

A specific bio-photovoltaic cell was created and constructed to measure the exoelectrogenic activity of *C. vulgaris* (De Caprariis *et al.,* 2014). The electrode arrangement, based on low-cost materials, with the anode submerged in the culture broth and the cathode exposed to the atmosphere, is the most significant component.

In addition to providing for a straightforward illumination of the algal culture using a light source positioned above the cell, perpendicular to the electrode surface, this configuration represents a highly intriguing simplification for the cell design (De Caprariis *et al.,* 2014). There is no net carbon dioxide production in this novel type of bioelectrochemical system since it lacks an organic substrate and mediators.

2.5 Microalgae in Biophotovoltaics

Microalgae are photosynthetic organisms capable of converting carbon dioxide into food, biofuels, and high-value bioactives (Walker *et al.,* 2005). Microalgae's diverse evolutionary origins are reflected in their ecology and colonised habitats, cell size and cellular structure, morphology, photosynthetic pigments, storage, structural polysaccharides, and life history type (Barsanti & Gualtieri, 2005). Biofuel applications and fuel cell production research are mainly focused on the genera *Chlorella* and *Nannochloropis* of microalgae.

Halin and co- workers (Halin *et al.,* 2019) thoroughly explained in their study the design and test outcomes of an ultra-thin Biophotovoltaic (BPV) cell powered by *Nannochloropsis* SP. A thickness of less than 5 cm is a feature of the BPV cell design. When the BPV cell is illuminated with a 7800-lux full spectrum light source, a power output of 14.6 W/cm2 is seen, which is comparable to the output from prior research (Halin *et al.,* 2019). The suggested BPV cell is physically thin, sturdy, and economical. The structural design also makes it possible to link and arrange the device in a grid

for greater power output, much similar to how photovoltaic cells are arranged to produce solar panels.

Nannochloropsis is a genus of very small (less than 5 mm) coccoid unicells that is mostly known from the marine environment (Fawley & Fawley, 2007). *Nannochloropsis granulata* sp. nov. (Eustigmatophyceae) is a marine ultraplankter.

N. granulata lacked both a pyrenoid and the lamellate vesicles found in the chloroplasts. The cytoplasm of *N. granulata* contained electron-dense bodies that did not resemble structures found in other species (Karlson *et al.,* 1996). The ultrastructure, pigment composition, and 18S rRNA gene sequence of *N. oculata (Droop) Hibberd* and *N. salina Hibberd* were studied and compared. The pigment composition was very similar, with violaxanthin and a vaucheriaxanthin-like pigment dominating with some amount of chlorophyll (Karlson *et al.,* 1996).

Research is heavily biased toward a single lineage that includes the genera *Nannochloropsis* and *Microchloropsis* of class Eustigmatophyceae. They are extensively studied for their biotechnology applications, in microbial fuel cell preparations, and biofuel production (Elias *et al.,* 2017). There are only about 30 species of the unique lineage of ochrophyte (stramenopile) algae known as the Eustigmatophyceae (eustigmatophytes). However, there is evidence of a significant taxonomic diversity that has not yet been fully uncovered.

All unicellular coccoid algae (eustigmatophytes) have a spherical or ovoid shape, although occasionally have a more recognisable shape (e.g., stipitate, tetrahedral, or with branched projections) (Hibberd, 1981). Most frequently, eustigmatophytes have been treated as the class Eustigmatophyceae within the broadly defined phylum (division) Ochrophyta, although they have occasionally been considered as a separate division or phylum Eustigmatophyta or as a taxon (named Eustigmatales) with no explicitly assigned taxonomic rank (Heterokontophyta).

A single order, Eustigmatales, divided into four families (Eustigmataceae, Chlorobotrydaceae, Pseudocharaciopsidaceae, and Monodopsidaceae), was acknowledged as the official taxonomic classification for eustigmatophytes (Hibberd, 1981).

The presence or absence of zoospores, the number of flagella, the presence or absence of mucilage, and cell shape all played distinct roles in defining each family (Hegewald *et al.,* 2007). To accommodate the recently described alga *Pseudotetradriella kamillae*, the Loboceae family was added (Hegewald *et al.,* 2007).

The phylogenetic relationships within the Eustigmatophyceae, however, are incongruent with the "one order/five families" concept, and it is unable to accommodate the variety, as shown by current sampling, which has produced a high number of uncharacterized or unidentified isolates (Hegewald *et al.*, 2007). Most importantly, the separation of eustigmatophytes into two phylogenetically deeply divergent lineages is not included in the standard categorization.

The first branch corresponds to Hibberd's order of Eustigmatales because it contains all known eustigmatophyte taxa (Hibberd, 1981). The second lineage naturally represents a new candidate eustigmatophyte order because it includes organisms that have only recently been classified as eustigmatophytes. The two main eustigmatophyte groupings have yet to be given a thorough classification that is in line with the evolutionary relationships found by molecular phylogenetic analysis.

Since some of the already recognised families and genera have shown to be para- or polyphyletic, taxa must be created to account for some recently discovered or reported lineages (May *et al.*, 2019). However, the absence of cultures that correlate to type species hinders thorough revisions of many taxa. However, the family-level categorization of this group needs to be settled to formally establish the order based on the International Code of Nomenclature for algae, fungi, and plants (May *et al.*, 2019).

The genus *Nannochloropsis* is widely distributed and can be found in both fresh and brackish water as well as open oceans (Zhang *et al.*, 2015). The maximum dimension of the spherical, ovoid, ellipsoid or cylindrical cells is 5 m. No zoospores were found. Previously comprised two species that have been separated into their genus, *Microchloropsis*.

The five officially recognised species can be found in freshwater (N. limnetica, with various variants known) or marine settings (*N. oculata*, *N. granulata*, *N. oceanica*, and *N. australis*) (Fawley & Fawley, 2007). There is one more species, *Nannochloropsis maritima*, which has not yet been formally described but is represented by an 18S rDNA sequence in GenBank with the accession number AY680703 (Hu *et al.*, 2013).

The *Nannochloropsis* genus of algae differs from other closely related microalgae in that it contains chlorophyll a and completely lacks chlorophyll b and c. Additionally, they can accumulate large quantities of a variety of pigments, including canthaxanthin, zeaxanthin, and astaxanthin. (Lubián *et al.*, 2000).

Because of its capacity to accumulate significant amounts of polyunsaturated fatty acids, *nannochloropsis* is regarded as a promising alga for industrial uses (Sukenik *et al.,* 1989). Additionally, it exhibits intriguing traits that could enable genetic modification targeted at genetically enhancing the current oleaginous strains. *Nannochloropsis* of different species can be transfected, and there is evidence that some strains are capable of homologous recombination (Kilian *et al.,* 2011). Currently, rotifers and fish larvae utilise it primarily as an energy-rich dietary source. However, it has sparked an increasing amount of interest in the study of photosynthesis-based biofuel production.

CHAPTER THREE
MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Microalgal culture

Microalgal species selected for the current study was a strain of *Nannochloropsis granulata* which was previously isolated and preserved in Scire science R & D laboratory, KINFRA kalamassery, Kerala, India. The microalgal species were chosen due to their faster cell division, higher biomass index and the commercial importance of strain produced by the species compared to other strains available in the laboratory.

3.2 Culture media and composition and culture parameter

The algal culture medium F/2 was prepared (Ryther & Guillard, 1962). The culture tubes were incubated under optimum conditions as given in the table 2.

Sl.no	Parameter	Value
1	Working volume	250 ml
2	Temperature	24±1^{o}C
3	Light intensity	700-800 lux
4	Photo period	16/18h (light/dark)
5	Time	15 days
6	Salinity	21
7	pH	8.0

Table 2: Microalgal culture parameters

3.3 Microscopic observation

After 5 days of incubation, the culture were observed microscopically under 40X magnification using a light microscope (ZEISS prio star) on a daily basis in order to examine growth and multiplication of microalgal cells.

3.4 Maximum absorbance determination

The efficiency of biomass growth was controlled by measuring the optical density (OD) which is defined as the absorption of visible radiation. The optical absorbance was measured at various

wavelengths mainly 620 nm and 700 nm in order to determine maximum absorbance using a spectrophotometer (LAB India).

3.5 Cell counting using Neubauer Haemocytometer

The microalgae concentration in the mixed culture was counted using the improved Neubauer haemocytometer counting chamber. A clean Neubauer cover glass was attached to the counting chamber by pressing it carefully in place. Then the sample was collected using a pipette and gently allowed to run quickly through the leading edge of the coverslip into the chamber, exactly fitting to it. After that, the counting chamber was allowed to stand on the bench for 2 minutes before counting using the light microscope (ZEISS primo star). The grid under the microscope was examined using 10X objective for distribution of the cells and refocused at 40X objective before counting cell in the four squares.

$$\text{Cell number of Cell density} = \frac{\text{Counted cells}}{\text{Volume of square} \times \text{Dilution factor}}$$

3.6 Determination of chlorophyll content

The chlorophyll content of the microalgal cells was determined by using spectrophotometric technique. Sample of the microalgal suspension was centrifuged for 10 minutes at 13000rpm (Centrifuge HERMLE-Z 3242). The supernatant was decanted and the pellet was resuspended in 90% methanol. Chlorophyll was then extracted from the sample after incubation in a water broth (Rotek) at 50 ^{o} C.The sample was again centrifuged for 10 minutes at the same speed. For the spectrophotometric determination of chlorophyll, the absorbance of light green supernatant was measured at two wavelengths, 450 nm (A 450) and 405 nm (A 405), using the UV spectrophotometer and was blanked with methanol.

3.7 DNA isolation

DNA isolation method (Doyle & Doyle, 1987) , using CTAB yielded good quality DNA for PCR.

3.7.1 DNA isolation using CTAB

Freshly prepared CTAB (Cetyl Trimethyl Ammonium Bromide) buffer was preheated at 65^0C. 1gm of the microalgae sample was ground in 16 ml of CTAB buffer and homogenized. The ground tissue incubated at 65^0C in water bath for 30 minutes followed by incubation at the room temperature.

Equal volume of Phenol: Chloroform: Isoamylalcohol (25:24:1) was added and mixed thoroughly to form an emulsion. The aqueous layer was collected after centrifugation at 10,000 rpm for 10 minutes and transferred to a new 50 ml centrifuge tube using a cut tip. Phenol: Chloroform: Isoamylalcohol extraction was repeated and the aqueous layer was collected in Eppendorff tubes (1 ml in each tubes). 3M sodium acetate (pH 5.2) was added to the aqueous phase and DNA was precipitated by the addition of 2/3rd volume of ice cold isopropanol and thoroughly mixed by inverting. The sample was kept for overnight incubation at -20^{0}C. The supernatant was decanted off and the pellets were washed with cold 70% ethanol. The DNA was further pelleted by centrifugation at 12,000 rpm for 10 minutes. The ethanol was decanted off and the pellet was air dried to remove all traces of ethanol and resuspended in 100µl TE buffer.

3.7.2 Amount and purity of DNA

The yield of DNA extracted was measured using a UV spectrophotometer (Lab India) at 260 nm.The purity of DNA was also determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. DNA concentration and purity was also determined by running the sample in 1.0% Agarose gel. The intensities of band obtained by staining with (0.5µg/mL) Ethidium bromide wascompared with 250bp DNA marker from Chromous Biotech. The gel documentation system (BIORAD- Molecular image) was used for DNA visualization on the gel.

3.8 PCR amplification

18s region was amplified by polymerase chain reaction (PCR) with specific primers (forward and reverse primers). Amplification of the conserved regions of the 18s rRNA gene was conducted ina reaction mixture with a final volume of 20µl that contained about 20mg of template DNA and primers using the PR Master Mix (Fermentas, USA) and a thermal cycler. The reaction consistedof initial denaturation at 94ºC for 3 minutes followed by 40 cycles of denaturation at 94ºC for 30s, annealing at 55ºC for 30s, and extension at 72ºC for 1 minute, with a final extension at 72ºC for 7 minutes. Amplicons were checked for appropriate size by 2% agarose gel visualization. Amplicons were gel purified using commercial column-based purification kit (Invitrogen, USA) and sequencing was performed with forward and reverse primers in ABI 3730 XL cycle Sequencer. Forward and reverse sequences were assembled and contig was generated after trimming the low-quality bases.

3.9 Identification of microalgae using molecular sequencing

At the molecular level, rRNA genes are the most widely used markers for the identification of algae due to their conserved function and universal presence. Several researches have exploited the conserved regions of the 18s rRNA genes for the phylogenetic analysis. Here we explored the possibility of 18s forward and reverse primer for amplification. Sequence analysis was performed using online tool BLAST of NCBI database and based on maximum identity score E value top most sequences was utilized for multiple sequence alignment.

3.10 Development of microbial biofilm and assessment of its photovoltaic efficiency

A prototype microalgal photovoltaic cell consist of a photo electrode, counter electrode, and electrolyte in between the other 2 electrodes. These were constructed for studying. ITO (Indium Tin Oxide) glass used in the study was a conductive glass slide with conductivity on one side. Algal biofilm got attached to these ITO glass slide.

3.10.1 Preparation of electrode and electrolyte for microalgal photovoltaic cells

Microalgal photovoltaic cell consisted of electrolyte and electrode. Algal biofilm on the glass slidewas the anode and carbon cathode prepared was the counter electrode. Between these electrodes, electrolyte was prepared which serve as medium for the transfer of the electrodes between the two electrodes.

3.10.2 Development of biofilm on the ITO glass slide

Strains of *Nannochloropsis granulata* were used as the photosynthetic organisms for the development of biofilm in the ITO coated anode. 100 mL of logarithmically growing microalgae of optical density= 0.38 measured at 620 nm was used for the experiment. Normal glass slide of size same as the ITO glass slide was used as the control. ITO coated glass slide and control glass slide was dipped in the exponential phase culture of microalgae and placed in the glass bottle selected specifically for this experimental purpose. Then it is kept for 15 days at 24^0C illuminated by cool white light fluorescent lamps on a 16: 8 –hour's light-dark cycle for the development of micro algal biofilm on the surface of the both sides.

3.10.3 Determination of surface area percentage of biofilm (SAC %)

Biofilm coated on the ITO was determined by calculating the surface area of the biofilm at an interval of three days. The growth of biofilm was evaluated by photographing the surface of the slide and then the surface area coverage, SAC% of the biofilm captured in the photograph, was calculated using Image J software.

3.10.4 Preparation of electrolyte

0.5g of agar (HI Media) was dispersed in 30mL of water and heated under magnetic stirring for a few minutes up to 100^0 C for its complete dissolution. Then 0.5g of glycerol, 0.5g of formaldehyde and 0.5g of acetic acid were added to this solution under stirring. The resulted viscous solution was then cooled down to 30^0C and poured on petridishes and let to dry up for 48 h at 40^0C. The resulting transparent, freestanding films were stored in a dry box (Raphael *et al*., 2010).

3.10.5 Counter electrode

The conductive side of the ITO glass slide was shown on to the flame and carbon was coated on it.

3.11 Assembling of the microbial photovoltaic cells

The sandwiched type micro algal photovoltaic cell was created by placing a counter electrode on the photo electrode or the working electrode and the electrolyte was placed in between them in the form of a cell in such a way that the conductive side of both electrodes is facing each other. The alligator wires were clipped to the ends of each electrode and connected to the multimeter for the measurement of voltage.

3.12 Photovoltaic measurement

3.12.1 Voltage-current and power output

The voltage of the cell was measured using the multimeter by applying a 1kΩ resistance load to the external circuit. Current values were calculated according to Ohm's law, I=V/R. The power output of each biofilm was then calculated using equation $P=V^2/R$.

3.12.2 Current density and power density

The current density was determined by dividing the current output by the area of ITO glass slides. Length of square-shaped ITO glass slide =25mm

Therefore, the area of ITO glass slide=25×25=625mm^2.

Therefore, current density= current output /area of ITO

The power density was calculated by using the area of the ITO glass slide (62.5cm^2) and power outputs. The maximum power density was noted.

CHAPTER FOUR

RESULT AND DISCUSSION

RESULT AND DISCUSSION

4.1.1 Microalgal culture

The microalgal species that was chosen for the current study was *Nannochloropsis granulata*. The species was inoculated on the first day in a conical flask under sterile conditions and the optimum conditions were provided.

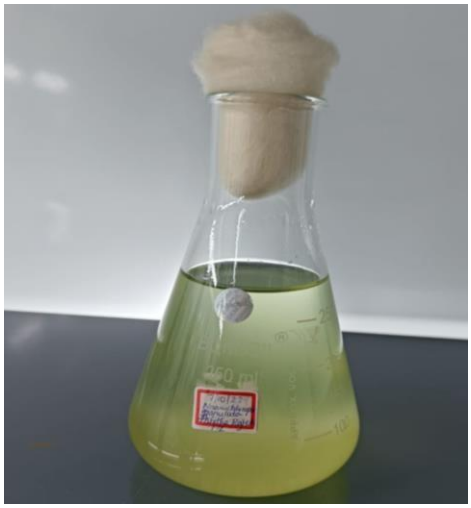

Figure 1: Microalgal culture on first day of inoculation

The culture in the conical flask showed a gradual colour change from the first day of inoculation up to the 15th day. The colour change from pale green to dark green was an indication of the increase in cell count and chlorophyll content.

Figure 2: Culture on maximum (15^{th}) day of growth

4.1.2 Maximum Absorbance Determination

The optical absorbance was measured using a UV Spectrophotometer at two wavelengths 620 nm and 700 nm. The maximum absorbance was observed at 620 nm.

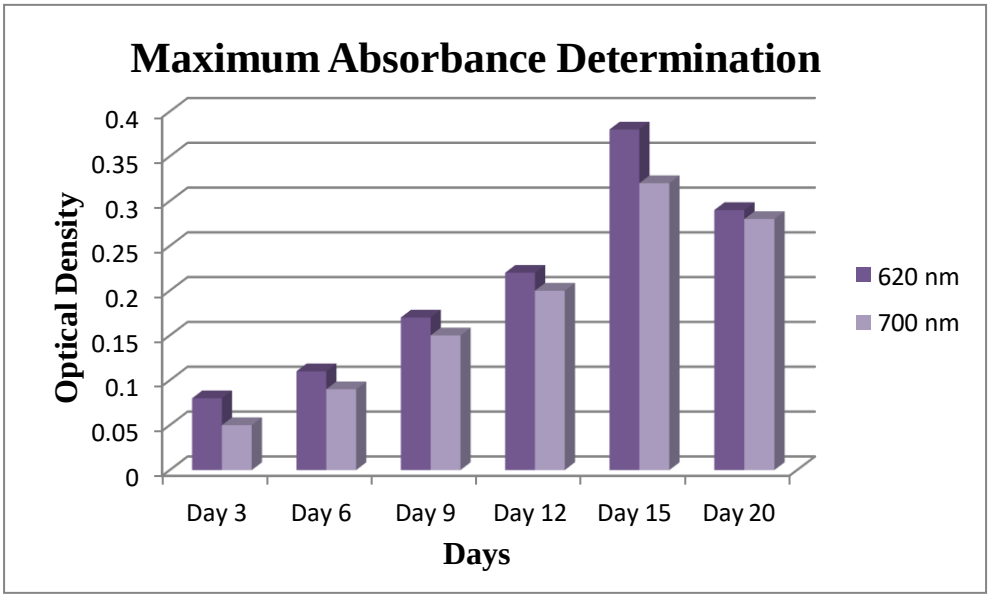

Figure 3: Determination of maximum absorbance at 620 nm and 700 nm.

4.1.3 Cell counting using Neubauer Haemocytometer

After 3 days of inoculation the cells in the culture were counted using Neubauer haemocytometer. The grids under the microscope were examined at 40X objective.

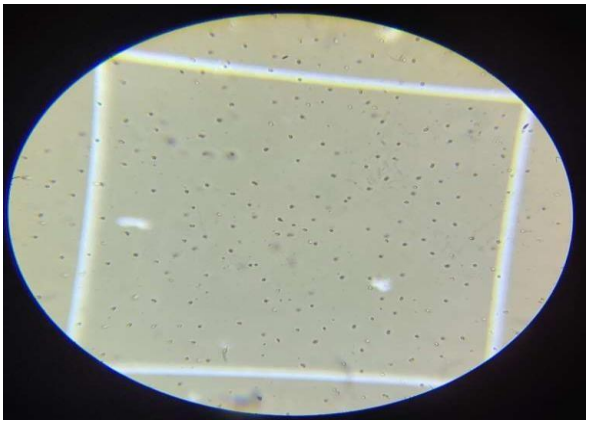

Figure 4: Cell counting under light microscope using Neubauer Haemocytometer

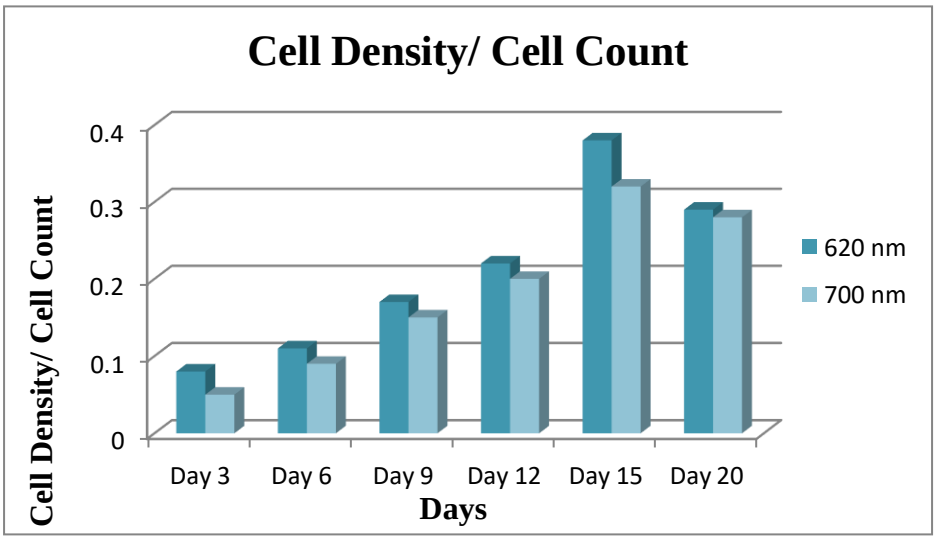

Figure 5: Cell density at different intervals

The cell count was gradually increasing and on the 15th day of incubation the culture attained the maximum cell count.

4.1.4 Determination of chlorophyll content

From the 3rd day of incubation the chlorophyll content of the micro algal cells was determined using UV Spectrophotometer. The absorbance of light green supernatant was measured at wavelengths 450 nm and 405 nm respectively. The maximum chlorophyll content was determined during the 15th day of incubation.

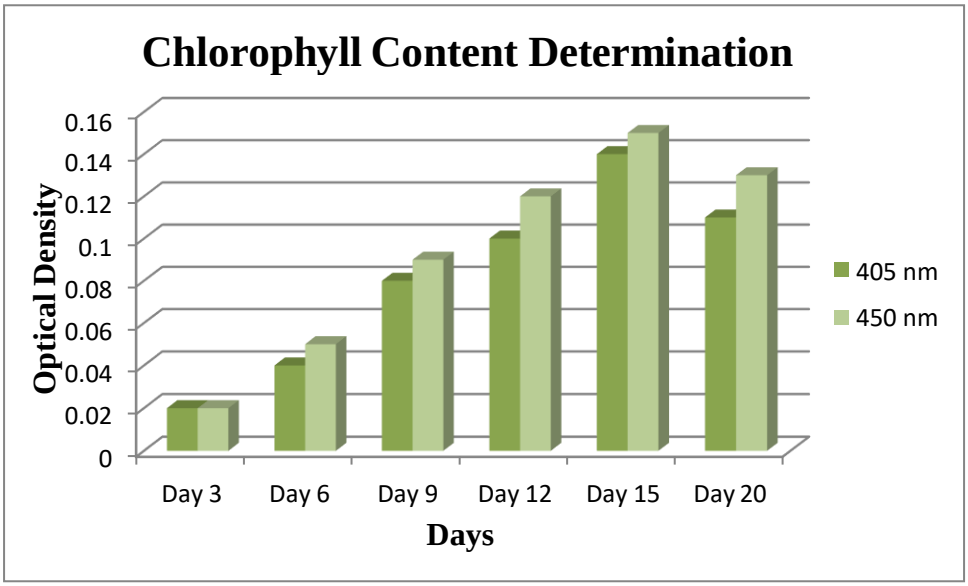

Figure 6: Determination of chlorophyll content

4.1.5 DNA isolation and band visualization

The DNA was isolated by Doyle and Doyle CTAB method. DNA concentration and purity was also determined by running the sample in 1.0% Agarose gel and PCR was conducted. After Gel electrophoresis, fragments of DNA got separated. Shorter fragments of the DNA moved more quickly than the longer fragments. The separated fragments of DNA can be observed as band and the band visualisation was done by gel documentation system (Image Lab Software).

Figure 7**:** An ethidium bromide stained 2% agarose gel showing 18s rRNA PCR amplification of DNA.

Lane (MBTO51): 100 bp marker.

Lane (S): 18s rRNA PCR amplicon of DNA.

4.1.6 Identification of microalgae using molecular sequencing

The microalga was identified by molecular sequencing, utilising the 18s forward and reverse primer for amplification. Sequencing was performed with forward and reverse primers in ABI 3730 XL cycle Sequencer.

>U38903.1:1-1788 Nannochloropsis granulata 18S ribosomal RNA gene
CCTGGTTGATCCTGCCAGTAGTCATACGCTTGTCTCAAAGATTAAGCCATGCACGTCTGAGAATAAAGAGTTTTCTCTGA
ATCTGCGAATGGCTCATTATATCAGTTATAGTTTATTTGATAGTCCTTTACTACTTGGATAACCGTAGTAATTCTAGAGC
TAATACATGCATCAACTCCCAACCGCTCGACGGACGGGATGTATTTATTAGATAGAAACCAATGCGGGGCAACCCGGTAT
TGTGGTGAATCATGATAACTTTGCGGATCGCCGGCTTCGGCCAGCGACGAATCATTCAAGTTTCTGCCCTATCAGCTTTG
GATGGTAGGGTATTGGCCTACCATGGCTCTAACGGGTAACGGAGAATTGGGGTTCGATTCCGGAGAGGGAGCCTGAGAGA
CGGCTACCACATCCAAGGAAGGCAGCAGGCGCGTAAATTACCCAATCCTGACACAGGGAGGTAGTGACAATAAATAACAA
TGCCGGGGTTTAACTCTGGCAATTGGAATGAGAACAATTTAAATCCCTTATCGAGGATCAATTGGAGGGCAAGTCTGGTG
CCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATACTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCTGGC
AGGGACGGCTGGTCGGTTCCGATAAGGGGCCGTACTGTTGTTTGTTCCTGTCATCCTTGGGGAGAGCGATTCTGGCATTA
AGTTGTTGGGGTCGGGATCCCTATCTTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCTTAGGCCCTGAATACATTA
GCATGGAATAATAAGATACGACCTTGGTGGTCTATTTTGTTGGTTTGCACGCCAAGGTAATGATTAATAGGGATAGTTGG
GGGTATTCGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATGGAAGACGAACTACTGCGAAAGCATTTACCAAGGAT
GTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGATGATTAGATACCATCGTAGTCTTAACCATAAACTATGCCGA
CTAGGGATCGGTGGGTGCATTTTAAGGCCCCATCGGCACCTTATGAGAAATCAAAGTCTTTGGGTTCCGGGGGGAGTATG
GTCGAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGG
GGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGAGAGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGC
CGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCTAAATAGTACTGGGAAT
GCTTAGCATTGCCAGAGACTTCTTAGAGGGACTTTCGGCGCTAGGCCGAAGGAAGTTGGGGGCAATAACAGGTCTGTGAT
GCCCTTAGATGTCCTGGGCCGCACGCGCGCTACACTGATGCGTTCAACGAGTTTATAACCTTGTCCGGAAGGACGGGTAA
TCTTGAAATGCGCATCGTGATAGGGATAGATTATTGCAACTATTAATCTTGAACGAGGAATTCCTAGTAAACGCGAGTCA
TCAGCTCGCATTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGATTCGGTGAAGCTTT
CGGATTGCGCCACTGGCCTCGGTCGGCAGCGTGAGAAGTTATCTAAACCTCATCATTTAGAGGAAGGTGAAGTCGTAACA
AGGTTTCCGTAGGTGAACCTGCAGAAGG

Figure 8: Sequencing of *Nannochloropsis granulata*

4.1.7 Sequence analysis

The obtained sequence was then compared to sequence database with BLAST (Basic Local Alignment Search Tool) to find the regions of similarity. The sequence compared showed similarity with *Nannochloropsis granulata* nuclear 18S ribosomal RNA gene.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Nannochloropsis granulata nuclear 18S ribosomal RNA gene	Nannochloropsis...	3302	3302	100%	0.0	100.00%	1788	U38903.1
Nannochloropsis CCMP529 18S ribosomal RNA gene, complete sequence	Nannochloropsis...	3293	3293	100%	0.0	99.89%	1792	U41092.1
Nannochloropsis sp. JL2/4-1 18S ribosomal RNA gene, partial sequence	Nannochloropsis...	3265	3265	100%	0.0	99.61%	1796	DQ977727.1
Nannochloropsis limnetica isolate AS3-9 18S ribosomal RNA gene, partial sequence	Nannochloropsis...	3254	3254	100%	0.0	99.50%	1796	DQ977726.1
Nannochloropsis sp. Tow 2/24 P-1w 18S ribosomal RNA gene, partial sequence	Nannochloropsis...	3243	3243	100%	0.0	99.33%	1796	DQ977728.1
Nannochloropsis CCMP505 18S ribosomal RNA gene, complete sequence	Nannochloropsis...	3243	3243	100%	0.0	99.39%	1792	U41050.1
Nannochloropsis limnetica culture SAG:18.99 strain SAG 18.99 18S ribosomal RNA gene, partial sequence	Nannochloropsis...	3241	3241	99%	0.0	99.50%	1783	AF251496.1
Nannochloropsis CCMP531 18S ribosomal RNA gene, complete sequence	Nannochloropsis...	3241	3241	100%	0.0	99.33%	1792	U41094.1
Uncultured eukaryote clone WS074.005 18S ribosomal RNA gene, partial sequence	uncultured eukar...	3238	3238	100%	0.0	99.33%	1795	KP404875.1
Nannochloropsis sp. strain IOLR 18S ribosomal RNA gene, complete sequence	Nannochloropsis...	3238	3238	100%	0.0	99.33%	1799	AF067956.1
Nannochloropsis oceanica strain LAMB2011 chromosome 14	Nannochloropsis...	3236	6467	99%	0.0	99.33%	961379	CP038111.1
Nannochloropsis oculata strain CCMP225 18S ribosomal RNA gene, partial sequence	Nannochloropsis...	3236	3236	100%	0.0	99.33%	1794	KU900229.1
Nannochloropsis sp. CSIRO P74 gene for 18S rRNA	Nannochloropsis...	3236	3236	100%	0.0	99.27%	1794	AB025532.1
Nannochloropsis oculata CCMP525 18S ribosomal RNA gene, complete sequence	Nannochloropsis...	3236	3236	100%	0.0	99.33%	1791	AF045044.1
Nannochloropsis oculata nuclear 18S ribosomal RNA gene	Nannochloropsis...	3236	3236	100%	0.0	99.33%	1789	U38902.1
Nannochloropsis maritima 18S ribosomal RNA gene, partial sequence	Nannochloropsis...	3234	3234	99%	0.0	99.33%	1788	AY680703.1
Nannochloropsis sp. NANNO-IOLR gene for 18S rRNA	Nannochloropsis...	3234	3234	99%	0.0	99.33%	1788	AB025533.1
Nannochloropsis oceanica strain LAMB2011 chromosome 6	Nannochloropsis...	3230	3230	99%	0.0	99.27%	1340162	CP038132.1
Nannochloropsis oceanica strain LAMB2011 chromosome 2	Nannochloropsis...	3230	3230	99%	0.0	99.27%	1596162	CP038117.1
Nannochloropsis oceanica strain KB1 chromosome 14	Nannochloropsis...	3227	3227	99%	0.0	99.22%	878997	CP044557.1

Figure 9: Significant alignments for the provided sequence

The Graphic summary displayed alignments of database matches to the query sequence as coloured boxes. The colour of the boxes reflected the alignment score (S). The red colour indicated the highest alignment scores.

Figure 10: Graphic summary

The Phylogenetic tree was constructed on the basis of the aligned sequences and MEGA X, a tool for building phylogenetic trees, was used to create the phylogenetic tree.

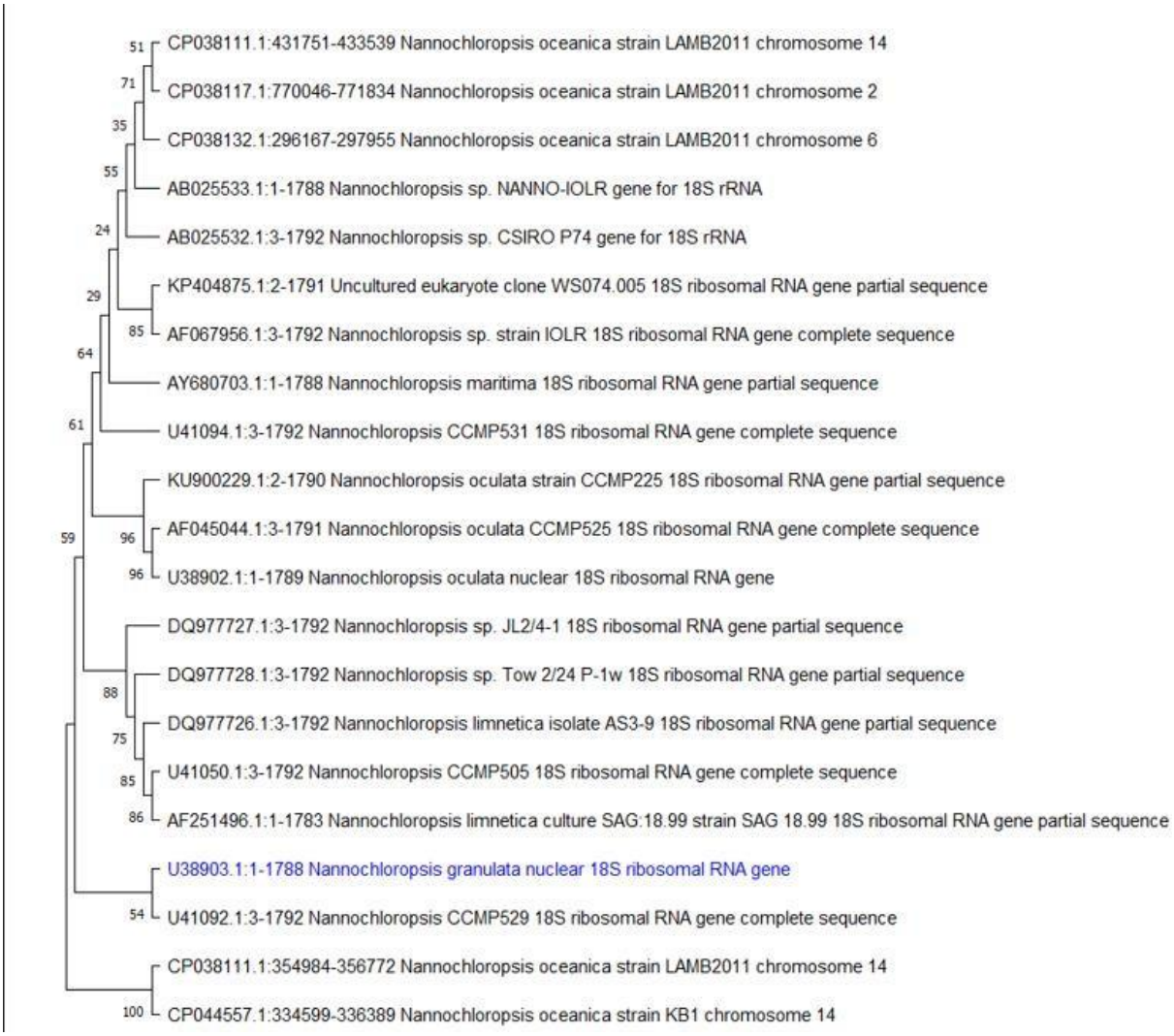

Figure 11: Phylogenetic tree

4.1.8 Development of microbial biofilm and assessment of its photovoltaic efficiency

A Prototype microalgal photovoltaic cell consists of a photo electrode, counter electrode and electrolyte in between the other 2 electrodes .These were constructed for the study. ITO glass slide were used as substrate for the attachment of the micro algal biofilm. This ITO glass slide was immersed in the algal culture in the glass bottle as shown in figure 12.

Figure 12: ITO glass slide dipped in glass jar containing algal culture

4.1.9 Development of biofilm on ITO glass slide

ITO glass slide were dipped in the glass bottle containing microalgal culture. As the day passes the algal biofilm started to develop on the ITO glass slide. On the 3rd day of incubation biofilm started to see visibly on the ITO. After 15th day formation of biofilm attained its maximum as shown in Figure 14.

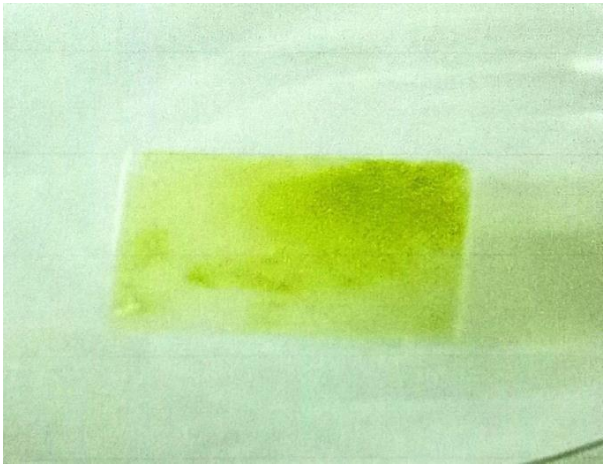

Figure 13: Development of biofilm on the surface of ITO on the 3rd day of inoculation.

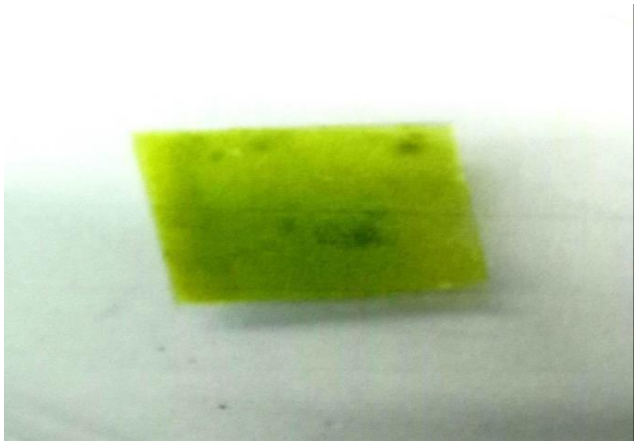

Figure 14: Development of Biofilm on the ITO glass slide on the 15th day of inoculation.

4.1.10 Determination of surface area percentage of biofilm (SAC %)

The surface area of the biofilm developed on the ITO glass slide was calculated at an interval of 3 days. The lowest percentage was observed on the 3rd day and the highest percentage was observed on the 15th day as shown in Figure 15.

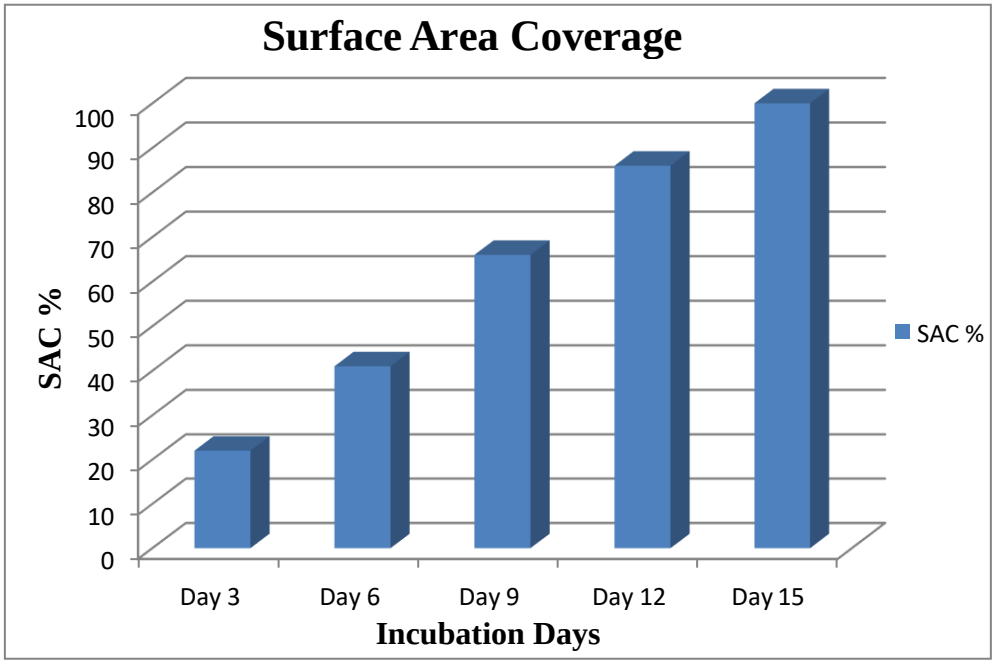

Figure 15: Surface area coverage of biofilm on ITO glass slide.

4.1.11 Counter Electrode

The carbon coated ITO glass slide was used as carbon cathode as shown in Figure 16.

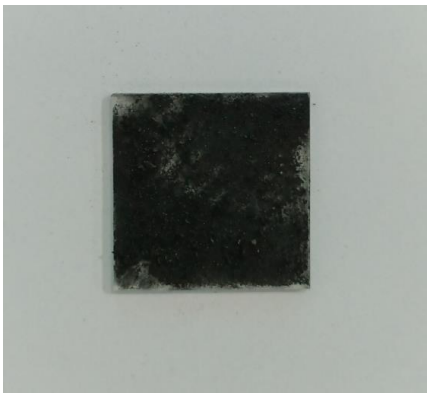

Figure 16: Carbon coated cathode

4.1.12 Assembling of the Microbial Photovoltaic cells

Microalgal photovoltaic cell was created. The cell consists of a photo electrode (anode), counter electrode (cathode) and an electrolyte was placed between these electrodes in a sandwich type manner. Alligator wires are clipped to the ends of the electrodes. Then the voltage was measured using a multimeter as shown in the Figure 17 below.

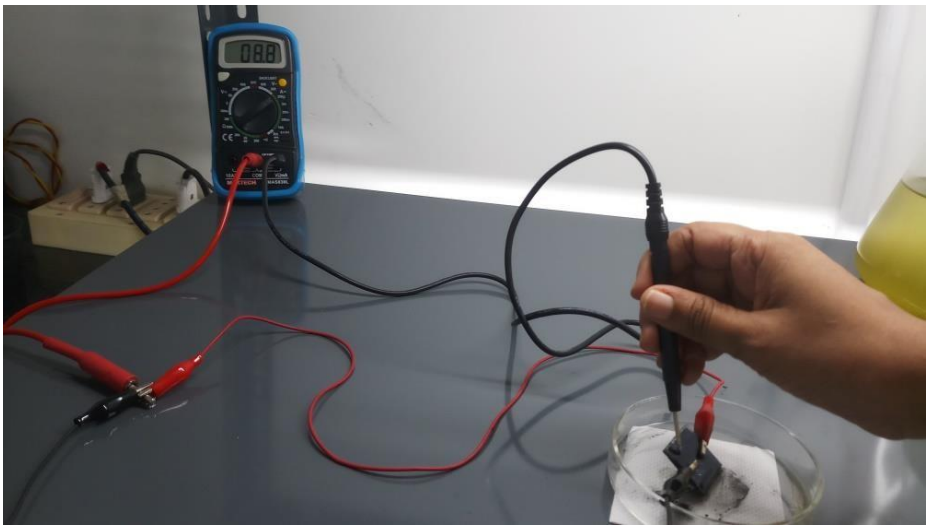

Figure 17: Voltage measurement from assembled microbial photovoltaic cell

4.1.13 Current Density

As the incubation days progressed, an increase in the value of current density was shown. On the 3rd day of incubation there was only a slight increase was observed, that is 1.51 × 10 $^{-2}$ A/m^2. The maximum current density was observed on 15th day of incubation that is 16.28 × 10^{-2} A/m².The variation in current density at different incubation days was shown in Figure 18.

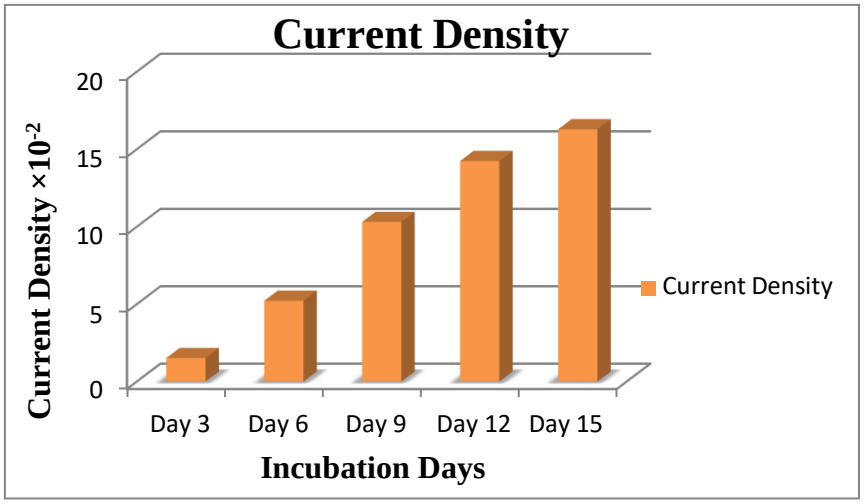

Figure 18: Variation in current density at various days of incubation.

4.1.14 Power Density

As the incubation days progressed, an increase in the value of power density was shown. The maximum power density was observed on 15th day of incubation that is 23.152 W/m^2.The variation in power density at different incubation days was shown in Figure 19.

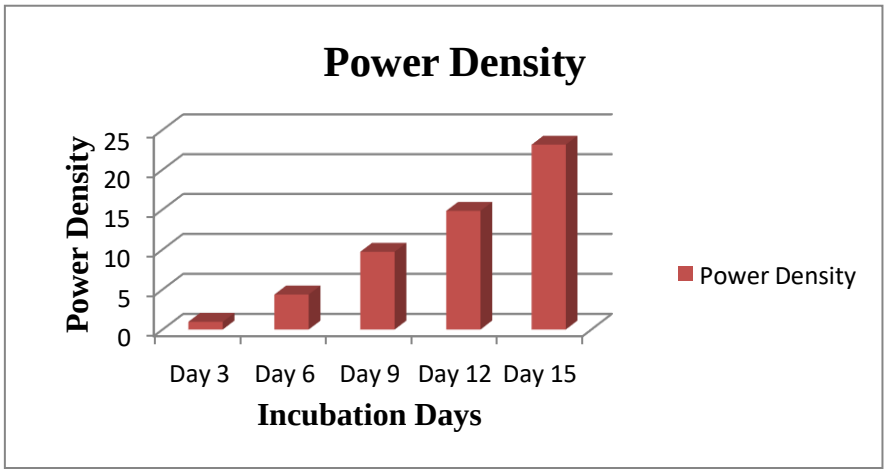

Figure 19: Variation in power density at various days of incubation.

4.2 Discussion

The present work was conducted to analyse the efficacy of *Nannochloropsis granulata* in preparing microbial photovoltaic cells. An approach to produce electricity through biocatalytic activity along with harnessing solar energy was analysed in this study. In this study the microalgal strain of *Nannochloropsis granulata* was exploited. This strain has commercial importance and also has higher cell division and faster growth.

The marine unicellular algae *Nannochloropsis granulata* was incubated in F/2 medium (Ryther & Guillard, 1962). The culture parameters were provided. The temperature was maintained at 24-25^{o}C.The light intensity was provided at 700- 800 lux. The photoperiod was provided for 16- 18 h.The salinity of the medium was measured using Abbe's refractometer and was noted as 21. A pH of 8.0 was also maintained in the culture medium. All these culture parameters were analysed during definite intervals of days. These growth parameters were examined at definite intervals as these parameters at its peak are necessary for the preparation of highly efficient microbial photovoltaic cells.

On three days interval the growth parameters were analysed. The maximum absorbance of the culture was determined using spectrophotometer at 620nm and 700 nm. The maximum absorbance was exhibited on fifteenth day and is shown in Figure 3. The cell density was also counted using Neuber's Haemocytometer and the maximum cell count was observed during the fifteenth day and is shown in Figure 5. The chlorophyll content was also determined by measuring the optical densities at 405 nm and 459 nm using a spectrophotometer and the maximum chlorophyll content was observed on fifteenth day and is shown in Figure 6. The DNA was isolated and PCR amplification was carried out. Molecular sequencing was also done for identifying the species.

On fifteenth day, when the growth parameters attained the maximum, the development of biofilm was done. The ITO coated glass slide was used for coating algal biofilm. The ITO glass slide mainly composed of Indium Tin Oxide. It was preferred as an excellent option mainly due to its optimum conductivity. These ITO glass slides also have low resistance and are highly transparent also. A control experiment was also set in which normal glass slide was used as the control.

The glass slides were kept immersed in the algal culture for about fifteen days at optimal conditions. The biofilm coated on the surface of the ITO slide was determined by calculating the surface area of biofilm at an interval of three days using Image J software and Figure 15 represents it. The surface

area coverage of the ITO slide attained the maximum percentage of 100% on the fifteenth day.

Microbial photovoltaic cell preparation mainly consists of an electrolyte and an electrode. Algal biofilm on the ITO glass slide with 100% surface area coverage was considered as the anode and the carbon coating prepared on another ITO glass slide as the cathode. The anode has major importance as it serves as a platform for algal adhesion and growth and both of its composition and surface pattern are crucial to MPVs ability to produce energy. Therefore, anode surface optimization is important to boost MPVs performance. ITO slide with Indium Tin Oxide serves as a surface modification for the anode with functional material nano- metal coating of Indium Tin Oxide (Liang *et al.*, 2022).The visual representation of the property improvement for MFC fitted with modified anode is illustrated in Figure 20.

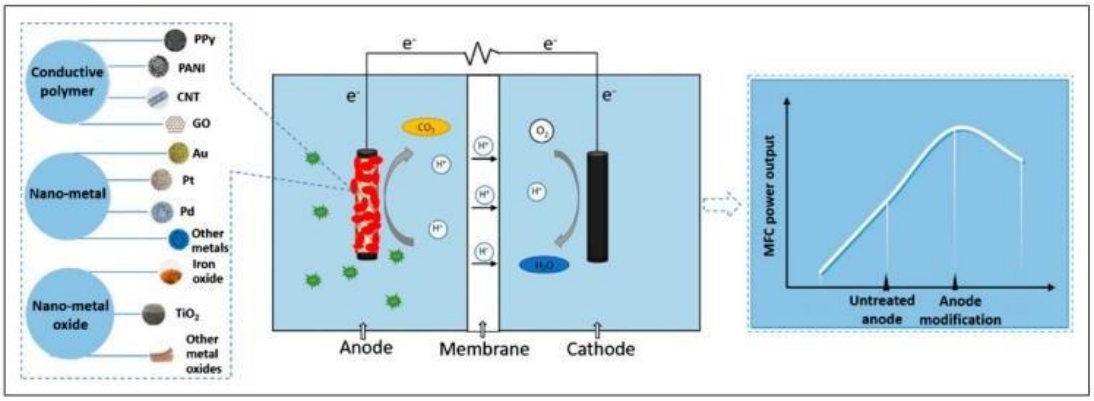

Figure 20: A schematic diagram of the modified anode to improve microbial photovoltaic cell power output.(Liang *et al.*, 2022)

A sandwiched microalgal photovoltaic cell was created by placing a counter electrode (cathode) on the photo electrode (anode) in which the conductive sides of both electrodes face each other. The alligator wires were clipped to ends of each electrode and were connected to the multimeter for measurement.

The voltage and power output of the microbial photovoltaic cells were noted at an interval of three days. The current density was calculated by dividing the current output of each day by the area of the glass slide. It was calculated at an interval of three days and the maximum current density was observed on fifteenth day and attained the value of 16.28×10^{-2} A/m² and is shown in Figure 18.

The power density was also calculated by dividing the power output obtained by the area of the ITO glass slide which was found as 6.25 cm^2. The power density obtained a value of 23.152 W/m^2 on the

fifteenth day. Although this power value is comparably higher to what was reported for the first generation of MPVs, and significantly feasible to that reported for MPV in recent years (Logan & Regan, 2006), the MPVs used in the current study differ significantly from traditional MPVs in a number of crucial ways. First off, MPVs are entirely powered by light and don't need an organic substrate to function. Second, a buffer or external electron shuttles are not necessary for the operation of the MPVs. This high power generation makes MPVs a most preferable alternative for the traditional energy sources and also proves the efficiency of *Nannochloropsis granulata* in preparing high power output microbial photovoltaic cells.

CHAPTER FIVE

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

In modern world of depleting non- renewable energy sources, biomass is considered as a better alternative for energy generation. Major researches are focused on utilizing algal biomass. Microalgal based microbial photovoltaic cells (mMPVs) prepared from algal biomass have successfully treated effluents and simultaneously generated electricity, and algae are a readily available biomass for power generation.

Biomass production and solar energy combined with electricity generation is an appealing solution for the modern energy problems. In the study conducted, Microbial photovoltaic cell with high power output was generated. The power density output of fifteen days was calculated as 23.152 W/m^2. The current density was also calculated and obtained a value of 16.28×10^{-2} A/m^2. It was graphically plotted and the highest power density was observed on fifteenth day. The current density was also plotted graphically and the highest density was observed on fifteenth day.

The prepared microbial photovoltaic cell was found to be cost effective and efficient through the analysis of power and current density. The main problems regarding MPVs, the expensive membrane and pt- implemented cathode can be solved by the implementation of these MPVs. MPVs can thus be regarded as the power source of future as these could be a possible and better alternative for power generation. The threat raised by the depletion of the fossil fuels can be solved in the near future by further researches in this area. This can also be considered as a green alternative as it is mainly dependent on microalgae and its photovoltaic efficiency.

Microbial fuel cells are also an alternative in this area of energy generation. Microbial fuel cells, on the other hand, can be replaced or linked with microalgae-based fuel technology to reduce costs, and the resulting algal biomass can be used to produce biofuels and other high-value products. But the power output generated in them now is also feasible.

The performance of cathodic and anodic reactions is also crucial to the technology's long-term viability. The anode used in this study involves algal biofilm coated in ITO glass slide and carbon coated ITO glass slide as the cathode. An electrolyte was also prepared as it is important for efficient voltage generation. A sandwich model of microbial photovoltaic cell was prepared and power was generated.

But much research and experimental proof is needed to overcome the barriers to economically

efficient and viable MPVs. Even though fuel cell costs are being reduced through research much more elaborate studies and findings are needed in this area. Further researches in this area could pave way for the production of more power generating MPVs and fuel cells with less capital and maintenance cost.

Furthermore, future MPV research can concentrate on technologies for developing low-cost energy input in mMPVs, as well as the utilisation of wet algal biomass for MPV production. As a result, mMPVs represent a novel, promising, environmentally friendly, and cost-effective technology for producing sustainable energy.

To summarize, this study demonstrated a first proof of concept for a promising, novel, potentially cost-effective, renewable, and sustainable electricity generation system from microalgae especially from *Nannochloropsis granulata* by harnessing solar energy. Additional research on improving culture photosynthetic efficiency, improving chemical energy transfer from algae to electrochemical energy, and producing electricity in the MPV cell resulting in higher yields and in developing a more sophisticated and practical technology in MPV production for large scale power production is needed.

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APPENDIX

APPENDIX 1

CULTURE MEDIUM : F/2 MEDIA

$NaNO_3$: 1ml

NaH_2PO_4 : 1ml

Na_2CO_3 : 1ml

Vitamin Solution : 0.5ml

Trace metal solution : 1ml

APPENDIX 2

CTAB BUFFER

1M Tris HCl : 1.576 g

4M NaCl : 2.3376 g

0.5 M EDTA : 1.8612 g

2% CTAB : 0.2 g

APPENDIX 3

PREPARATION OF MASTER MIX

Molecular biology grade water: 15µl

10X assay buffer : 2.5µl

Template DNA : 1µl

Forward primer : 0.5µl

Reverse primer : 0.5µl

$MgCl_2$: 2.5µl

DNTPS : 2.5µl

Taq DNA polymerase : 0.5µl