Studies on the potential of chlorophyll from *Chlorella vulgaris* Beijerinck as a photosensitizer in dye sensitized solar cell

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By

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CERTIFICATE

This is to certify that Dissertation entitled "**Studies on the potential of chlorophyll from *Chlorella vulgaris* Beijerinck as a photosensitizer in dye sensitized solar cell** "is an authentic record of work carried out by **Ms. Malavika K.** under the supervision and guidance in the partial fulfillment of the requirement of the MASTER'S DEGREE OF MAHATMA GANDHI UNIVERSITY, KOTTAYM. I, further certify that no part of the work embodied in this dissertation work has been submitted for the award of any other degree or diploma.

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ABBREVATION

BLAST – Basic local alignment search tool

bp – Base pair

CTAB- Cetyl trimethyl ammonium bromide

DSSC- Dye sensitized solar cell

FF- Fill Factor

FTO- Fluorine doped tin oxide

ITO- Indium tin oxide

NCBI- National centre for biotechnological information

PAN – Polyacrylonitrile

PCR – Polymerase chain reaction

rpm – Rotation per minute

r RNA- Ribosomal RNA

SAC – Surface area coverage

TCO -Transparent conducting oxide

TE Buffer -Tris EDTA buffer

TiO_2-Titanium dioxide

TPAI- Tetra propylammonium iodide

ZnO- Zinc oxide

CHAPTER ONE

INTRODUCTION

INTRODUCTION

The most plentiful and environmentally friendly form of free energy is sunlight. The process of photosynthesis transforms solar energy in to usable form of energy and is stored in photosynthetic plants and algae as biomolecules mainly as carbohydrate molecules such as sugars and starch. The majority of the energy required for life on Earth is produced and maintained through photosynthesis, which also produces and maintains the oxygen concentration of the atmosphere. Even though different kinds of plants and algae undertake photosynthesis in different ways, the process always starts with the absorption of light energy by reaction centers, which are proteins containing chromophores like the green pigment chlorophyll. These pigments are found in chloroplast. In plants they are primarily found in green plant parts. In bacteria they are embedded in the plasma membrane. In algae there are variation in size and shapes of chloroplast. In these light dependent processes, a small amount of energy is required to remove electrons from appropriate materials like water, producing oxygen gas. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP), known as" energy currency," are two additional compounds that are created. These compounds act as short-term energy stores that allow the transfer of hydrogen to power the reaction. Since sunlight is constantly being replenished it is a renewable source of energy. Wind energy, Geothermal energy, Hydropower, Tidal energy etc. are other renewable sources. On the other hand, non-renewable fossil fuels like coal, oil, and gas require hundreds of millions of years to create. When fossil fuels are used to create energy, they emit dangerous gases like carbon dioxide. As compared to burning fossil fuels, renewable sources produce fewer emissions. So, the most effective way to combat the climate catastrophe is to move away from fossil fuels. Also, continuous use of fossil fuels causes its depletion and permanent loss. So, the usage of renewable sources of energy is always preferred since it causes less emission and can be effectively replenished.

The most sustainable and cost-free energy source is sunlight. The rate at which the Earth absorbs energy from the sun is about 12×1017 J/S. This has topped the global energy consumption rate of 1.5×1013 J/S (Blankenship *et al*., 2011). Since fossil resources like oil and gas will eventually run out, it is difficult to come up with a strategy for the efficient capture and storage of solar energy for human consumption. Solar cell is a best way to utilize and store solar energy. Solar cell is key

device in photovoltaic energy conversion, which convert light energy in to electrical energy. Different types of solar cells have been developed. For various industrial and residential applications, crystalline, polycrystalline and amorphous silicon solar cells have been widely used. The world record efficiency for multijunction semiconductor solar cell is 46%. Solar cell materials are typically made of semi-conductors. The process of converting energy entails the separation of charge carriers after electron -hole pairs produced by the absorption of light (photon)energy in a semiconductor. For charge carrier separation, a p-n junction is typically utilized. One important type of solar cell is Dye sensitized solar cell (DSSCs). It is developed by Gratzel and coworkers, in order to imitate photosynthesis process. However, one key distinction between photosynthesis in plants and that in DSSCs is that whereas plants may store energy for later use, DSSCs cannot. The three important factors that are thought to have the greatest impact on cell stability are electrolyte leakage, dye desorption, and dye degradation.

A dye sensitized solar cell (DSSC, DSC) also called Gratzel cell is thin film solar cell. It is cost effective which is based on photoelectrochemical system that produces a semiconductor by combining an electrolyte with a photosensitized anode. A substance known as semiconductor may conduct electrons. DSSC semiconductors should have a large band gap. In DSSC, materials with band gaps of 3.2e V, 3.e V, 3.4e V, 3.6e V and 2.5e V are used as semiconductors. These materials include TiO_2, ZnO, Nb_2O_5, SnO_2 and CdO. Even though band gaps of TiO_2 and ZnO are same, ZnO has a lower energy than TiO_2. TiO_2 is favored mainly because it is inexpensive, non-toxic, chemically stable, and has high refractive index. A transparent-conducting oxide (TCO)glass substrate with a semiconductor put on top is the basis of a DSSC. A dye solution will be applied to semiconductor. The other components are a cathode, a reduction -oxidation(redox) mediator, and an electrolyte. The photoanode is an assembly of semiconductor, a fluorine-doped tin (FTO), and a dye. One of the widely utilized semiconductors for DSSC is titanium dioxide (TiO_2) since it is inexpensive, non -toxic, and has a wide bandgap. Indium-doped tin oxide (ITO) and FTO are widely used TCO in DSSCs. To expand the coverage area for the sensitizing dye, TiO_2 is coated on the TCO substrate as a network of nano porous TiO_2 particles. Another TCO serves as the cathode, which is then covered with platinum. Additionally, carbon and conducting polymers can be used as counter electrodes. There are mainly two types of dye: synthetic and natural. The most popular synthetic dyes are those based on Ruthenium (Ru), however because Ru is a heavy metal,

they are not ecofriendly (Fernando & Senadeera, 2008). Osmium metal-organic complexes are also used as dyes. Such dyes are costly. Natural dyes on the other hand, are commonly available, affordable, non-toxic, environmentally benign, quickly extracted and can be used without any purification. But they are less efficient as compared to synthetic dyes. Natural pigments like chlorophyll, carotenoids, phycocyanin etc. are commonly used natural dyes. Chlorophyll can also serve as a photosensitizer for DSSC because it imitates photosynthesis.

When radiation is first absorbed by molecular entity known as a photosensitizer, photochemical or physiochemical transformation to occur. This process is called photosensitization. The sensitizer injects electrons into the semiconductor and redox mediator in the electrolyte. Synthetic and natural dyes are utilized as photosensitizers since they are essential for determining the overall effectiveness of the cell. A good dye have characters like; absorb visible light in the spectrum ,Fast electron transport is ensured by a good attachment at the photoelectrode surface , high stability and superior interfacial characteristics allow for efficient absorption of TiO_2, electrolyte readily accepting replacement electron, the dye's excited state must be just above the TiO_2 conduction band and its ground-state level must be below the electrolyte's redox potential, sufficient to withstand 20 years of exposure to natural light. Synthetic dyes based on Ruthenium are more effective for photosensitization. Natural dyes are preferred because synthetic dyes can cause allergies, cancer, and other negative effects. The electrolyte triiode/iodide is frequently employed in DSSCs. The electrolyte has an impact on DSSC conversion efficiency. It is highly thermally and chemically stable, non-volatile, and conducts electricity. Additionally, the counter electrode must be highly efficient at photoconversion and be able to catalyse the redox process with electrolyte. Typically, carbon or platinum are employed as counter electrodes. Mediator is reduced in the counter electrode. In order to create a counter electrode, FTO glass is coated with platinum or carbon electron.

This study is using Chlorophyll of algae *Chlorella vulgaris* Beijerinck as photosensitizer of DSSC. *Chlorella vulgaris,* a green eukaryotic microalga that has existed on earth since the Precambrian era. As the first microalgae with a clearly defined nucleus, this unicellular alga was found by Martinus Willem Beijerinck in 1890.

Its size ranges from 1 to 10 microns, and its form is spherical. These microalgae are commonly used for the production of cosmetics, medical treatments, and even detoxification of heavy metals

in waste water. It is known for its nutritional values so widely used as food supplement. *Chlorella* contains significant amounts of intracellular proteins, carbohydrates, lipids, vitamin C, Beta - carotenes and B vitamins (B1, B2 and B12) in addition to chlorophyll. The rich green color of *Chlorella* comes from high concentration of chlorophyll. Chlorophyll is a pigment which is important for photosynthesis and energy production in plants and algae. This microalga has highest chlorophyll content found in nature (Burlew., 1953). *Chlorella vulgaris* can be grown both autotrophically and heterotrophically or in combination of both, mixotrophically. In addition to nutritional benefits, they are also useful in waste water treatments.

Kingdom	Plantae
Division	Chlorophyta
Class	Trebouxiophyceae
Order	Chlorellales
Family	Chlorellaceae
Genus	*Chlorella*

Table 1. Classification of *Chlorella*

Typically, the electrolyte is a liquid or a quasi-solid state. The I^-/I_3^- redox pair is a common mediator and platinum (Pt) or carbon was selected as the counter electrode. The molecules of the chlorophyll dye (D) will be activated (D*) when the light is shone on the cell, after absorbing photons(hv), and inject electrons in to the semiconductor band. The excited dye will subsequently be oxidized or ionized by the excited chlorophyll molecules, which will inject electrons into the TiO_2 conduction band. The oxidized chlorophyll dye molecules will take electrons from an iodide ion (I^-) in the electrolyte when the I ions were released to the oxidized molecules and in turn oxidized to triiodide ions. The electron in the TiO_2 conduction band exits the device through the load and reduces the triiodide at counter electrode. Now that the Iodide ion has been recovered, the electron circuit has been finished, and the entire system is in its initial configuration to begin a new cycle. As long as there is light there is constant current production in the external circuit (Arof & Ping, 2017).

There are different methods to enhance the efficiency of DSSCs. The oxidized dye must be securely reduced to its initial ground state following electron injection to maximize the efficiency

of DSSCs. In comparison to the process of dye oxidation, the regeneration process ought to be quick. By increasing the porosity of TiO_2 nanoparticle dye adsorption will be increased. Creating a homogeneous thin layer or underlayer of TiO_2 nanoparticles on top of the conduction plate to reduce the creation of the dark current. So, the electrolyte does not directly or indirectly make contact with the FTO and as a result, it is not reduced by the collector electrons. Co-sensitization also optimize the performance of DSSCs. The performance and efficiency of these cells can be improved by promoting the use of various electrode materials, such as nanotubes, nanowires of carbon, and graphene; using varied electrolytes instead of a liquid one, such as gel electrolyte and quasi-solid electrolytes, giving the working electrode various pre-post treatments, such as anodization pre-treatment and $TiCl_4$ treatment; using various types of CEs and by developing hydrophobic sensitizers. Placing luminescent or phosphorescent chromophores, such as rare-earth doped oxides in the DSSC, or adding a luminescent layer to the photoanode's glass, can produce phosphorescence.

Dye sensitized solar cells are highly relevant and useful form of solar cell. It helps to store the solar energy and convert it to electrical energy which can be utilized for various day today purpose. The large-scale popularization and utilization of dye sensitized solar cells helps to reduce the cost of energy production and also reduces the depletion of non-renewable sources of energies like fossil fuels.

AIM AND OBJECTIVES

AIM

Studies on potential of chlorophyll from *Chlorella vulgaris* as the photosensitizer in dye sensitized solar cell.

OBJECTIVES

- To culture organism and check its growth
- To isolate DNA, PCR amplification of DNA, then sequencing and identification of organism
- To study the potential of chlorophyll from *Chlorella vulgaris* as the photosensitizer in dye sensitized solar cell.

CHAPTER TWO

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Dye sensitized solar cell has become an attractive and cheap device for the conversion of solar light into electrical energy since Gratzel and O'Regan firstly reported the prototype of this solar cell in 1991(Gratzel & O'Regan ,1991). A conductive glass anode coated with platinum serves as the DSSCs anode, while porous TiO_2 film serves as the cathode and is anchored to a monolayer of dyes. The electrolyte is an organic solvent that contains a redox pair such as iodide or Triiodide. Sensitizer dyes have received a lot of attention since they are essential for capturing sunlight and converting it into electricity. To date, a variety of organic dyes and organic metal complexes have been used to sensitize nanocrystalline TiO_2 semiconductors, with transition metal coordination compound being one of the most effective sensitizers (Ruthenium polypyridyl complex).

A dye sensitized solar cell is made up of metal counter electrode, a dye capped nanocrystalline porous semiconductor electrode, and a redox electrolyte that mediates the electron transfer process taking place in the cell. The quality and composition of semiconductor electrode, and the sensitizer dye employed in the cell 's construction have significant impact on the performance of the device. Numerous wide band gap metal oxide semiconductors have been investigated for their use in DSSCs, although TiO_2 and ZnO are the semiconductors that are most frequently used (Kanmani& Ramachandran, 2012). Long-term thermal and photostability are just two benefits of Titanium dioxide(TiO_2). By employing various methods for their deposition on the substrate, semiconductor's fundamental properties can be considerably altered (Krebs, 2009).

Due to their low manufacturing costs and environmental friendliness, bio-inspired dye-sensitized solar cells (DSSCs) made from natural plant dyes have gained relevance. As an electron transporter in DSSCs, Titanium oxide nanoparticles are crucial. Comparing the efficiency to conventional silicon-based solar cells, it is still poor. The photodegradation of the dye, the electrolyte's long-term stability, and the dye's adherence to titanium oxide nanoparticles are just a few of the difficulties in increasing efficiency (Ganta *et al.*, 2019).

It was also investigated if plant derived natural and organic colors and dyes could be used as photosensitizers. Natural dyes that can be extracted through a straight forward process can be found in the fruit, flower and leaf of plants, which exhibit a range of colours from red to purple.

Numerous studies have stressed the importance of using natural dyes as photosensitizers (Tennakone *et al.,* 1997). According to the reports, organic dyes can achieve an efficiency of up to 9.8%. But there have been issues with these dyes, like difficult synthesis processes and poor yields. (Zhang *et al*., 2009). Many pigments including anthocyanin, carotenoid, chlorophyll, and flavonoid, that are taken from various plant parts, including leaves, fruits, and flowers have been studied for their ability to increase sensitivity.

There are several studies that checks the efficiency of chlorophyll over other natural pigments as photosensitizer. Several chlorophyll derivatives can also be used as photosensitizer with efficiency. Chlorine -6(Chl e6) a chlorophyll derivative, immobilized on TiO2 film show visible light sensitization. Based on fluorescence spectrum of Chl e6 immobilized it can be deduced that TiO2 effectively injected electron in to conduction band of TiO2 from excited singlet state of Chl-e6(Amao *et al*., 2003). The natural dyes derived from black rice, *Capsicum*, *Erythrina variegata* flower, *Rosa xanthina*, and kelp were used as sensitizers to assemble the dye-sensitized solar cells (DSC). The DSC sensitised with natural dye extracts yielded the ISC from 1.142 mA to 0.225 mA, the VOC from 0.551 V to 0.412 V, the fill factor from 0.52 to 0.63, and Pmax from 58 W to 327 W. The black rice extract had the best photosensitized effect among the natural fruit, leaf, and flower extracts, which was attributed to improved interaction between the carbonyl and hydroxyl groups of the anthocyanin molecule on the black rice extract and the surface of TiO2 porous film. The black rice extract in ethanol solution exhibits a blue-shift in its absorption wavelength (Hao *et al.,* 2006). The efficiency of pigments like Betalin and Chlorophyll pigments extracted from *Amaranthus caudatus* and *Bougainvillea spectabilis* using different solvents they demonstrated the performance and concluded that chlorophyll from flowers of *Bougainvillea* and *Amaranthus* show better performance compared to DSSCs with Betalin from same flower. A variety of natural anthocyanin colours derived from the leaves and petals of *Ixora coccinea* have been researched in a study. When compared to the conversion effectiveness of the individual dye sensitizers, the cocktail that was blended in a 1:4 ratio showed the highest conversion efficiency (0.80%). This is due to increased light absorption capacity (Zolkepli., 2015). The efficiency of Betalin and Chlorophyll from red amaranth was investigated by other workers also. In accordance with the findings, the chlorophyll dye exhibits a maximum photocurrent density of 1.3 mA/cm^2 and an energy conversion efficiency of 0.53%. The photocurrent density of the Betalin dye was 0.87

mA/cm^2 with an energy conversion efficiency of 0.23%. Electrochemical impedance spectroscopy and bode plot tests indicated the improved sensitizer performance of chlorophyll dye for dye sensitized solar cells (Ramanarayanan *et al.,* 2017). Such cell displayed a broad spectral responsiveness in the visible light spectrum, which is similar to the photosynthetic action spectrum. Under simulated sunshine with an intensity of 100 mW cm^2, the dye adsorption process was optimised by adding co-adsorbing surfactant agents that suppress intermolecular aggregation of the dye. This resulted in a high energy conversion efficiency of up to 4.3 (Ikegami *et al*., 2008). The efficiency of natural pigments from many plants were tested. Using the solvent extraction approach, the natural colours were taken out of the following plants: *Crocus sativus* (saffron), *Allium cepa* L (red onion), *Malva sylvestris* (mallow), and Oregano (*Origanum vulgare*). The extracted solutions have been found to include anthocyanin or chlorophyll pigments, which are excellent pigments needed for the formation of charge-carriers in the energy harvesting process from sunlight, according to the UV-vis data (Jalali *et al.,* 2020).

Dyes are important component of DSSCs which determine the performance of DSSCs and effect the efficiency and working. Due the toxicity of synthetic dyes, inexpensive and safe natural dyes are most preferred. They are biocompatible and extracted from plants and algae. Chlorophyll, the main pigment from algae and plants has greater importance in energy production through photosynthesis. Different types of chlorophyll are there but the most important are chlorophyll a and b. These chlorophyll molecules contain alternating single and double bond and orbitals can delocalize which make them stable and effective photoreceptors. They have long chain length due to which steric hindrance is caused which leads to low transferability of electrons. Also, it adsorbs weakly to the semiconductor (Pandey *et al*., 2019). The environment in which the pigment molecule exists typically affects the absorption properties. Chlorophylls' absorption spectra can be altered by 10-15 nm in a protein environment, frequently toward longer wavelengths (Evens *et al*., 1987).

Electron transfer between layers and their dynamics play an important role in the overall performance and efficiency of the device. Resistance encountered by electrons at layer interfaces and defects between layers leads to charge trapping and recombination. The working principle of DSSC devices is simple in that charges move between different adjacent layers, but charge

trapping and recombination reactions occur to lower the charge density and lower the current density (Ahmed & Anwar, 2022).

Microalgae have high photosynthetic capacity due to the large amount of chlorophyll when compared with plants. They have high growth rate, biomass productivity and high efficiency in photosynthesis (Aghdam & Janfaza, 2014). Chlorophyll absorbs light of the red, blue, and violet spectrums; however, it only reflects the green spectrum, which gives its color. Strong absorption peaks at 420nm and 660 nm in the visible area can act as a natural sensitizer in the visible light spectrum (Alwani *et al*., 2016).

In additions to microalgae and plants, pigments can also be extracted from Yeast, Molds, Bacteria and Cyanobacteria which can be cultivated in large scale in bioreactors. The pigments from them have maximum absorption near UV and IR region. Some bacteria (such as purple and green bacteria) use bacteriochlorophyll, a chlorophyll analogue, to capture light for anoxygenic photosynthesis. Archaea, on the other hand, use retinal proteins for the same function, with the added benefit of having adapted to low light, as well as extremely high temperatures and salinity levels. Despite not being photosynthesis, fungi are known to produce a number of pigments and secondary metabolites that have the ability to increase sensitivity in DSSCs. Sensitizers isolated from algae and microalgae range in reported photoconversion efficiency from 0.001% to 4.6%; bacteria range from 0.004 to 1.67%; cyanobacteria range from 0.07 to 0.23 percent; archaea range from 0.09 to 0.04 percent; and fungal pigments range from 0.26-2.3 percent (Orona-Navar *et al.*, 2021). The efficiency of algal pigments is also studied by different scientists. Pigments like xanthophyll and chlorophyll from algae *Cladophora* were used as sensitizer in DSSCs and found out mixture of these dyes gives better result than when they are singly used (Lim *et a*l.,). Carotenes like Beta-carotene also exhibit the property of photosensitizer. In tropical marine *Chlorella* sp.PP1, Beta–carotene extracted shown better results when applied to TiO_2 semiconductor of DSSCs (Nurachman *et al*., 2015).

Dye-sensitized solar cells fabricated with red pigments extracted from Antarctic algae were evaluated. Among all algae collected, samples from *Plocamium hookeri, Delesseria lancifolia,* and *Iridaea obovata* performed the best. Cells were evaluated using conventional electrochemical techniques and electrochemical impedance spectroscopy (Enciso & Cerdá, 2016). The pigments used as photosensitizers were natural pigments extracted as chlorophyll, β-carotene, and

phycocyanin from streak leaves, carrots, and blue-green algae (*Spirulina platensis*). Optimization was performed by altering chlorophyll added by β-carotene and phycocyanin (a cocktail dye) at a ratio of 1:1 and 1:2. Using electrophoresis, the dye coated onto TiO_2 nanotubes at 20 volts for 12 minutes. The strongest visible absorption of light was shown in electrophoresis samples using natural 1:1 Chlorophyll and Phycocyanin dye (Kezia, 2017). The efficiency of Chlorophyll of algae *Enteromorpha intestinalis* as photosensitizer on TiO_2 electrode was also investigated. Studies were done using by two methods, by successive adsorption of dyes, using cocktail of dyes. It was proven that co sensitization by cocktail method is best method than using individual dyes to enhance light absorption by natural dyes (Dumbrava *et al*., 2016). Brown algal pigments were also utilized in studies. Chlorophyll from algae *Undaria pinnatifida* good as photosensitizer in DSSCs (Calogero *et al*., 2014).

Anthocyanin pigment from pomegranate seed extract, betalin pigment from beet root extract, chlorophyll pigment from *Tridax procumbens* leaves. The dyes anthocyanins and betalins, anthocyanins and chlorophyll, betalins and chlorophyll are mixed in a cocktail in equal volume ratios. This study, investigate the effects of different extract concentrations and different plant pigments on the energy gap when applied to DSSCs. Test results show that the cocktail dye is mixed with extracts from pomegranate seeds, beet roots and *Tridax procumbens* leaves in a volume ratio of 1:1(Rajkumar & Suguna., 2016). Chlorophyll from parsley, lemon, and spinach leaves; *Hibiscus-rosa sinensis*; pomegranate juice; and beetroot betalin as single colours. The dyes were combined using both chlorophyll and betalin and chlorophyll and anthocyanin. UV-VIS spectrophotometry has been used to examine the absorption spectra of various dyes. The dyes exhibit significant adsorption onto the surface of the semiconductor (TiO_2) and broad-spectrum absorption in the visible region (400–700 nm) of the solar spectrum. The conversion efficiency of the solar cell was increased by the dye mixture's adsorption onto the TiO_2 electrode, which has a greater absorbance than single dye (Sakshi *et al*., 2022).

Pigments extracted from *Acanthus senni chiovenda* flower and *Euphorbia cotinifolia* leaf was used as natural dyes in DSSCs in which quasi solid electrolyte is sandwitched between electrodes. Best conversion efficiency is exhibited by them (Ayalew & Ayele., 2016). The dye sensitizer is derived from anthocyanin pigments found in plants like red cabbage, black rice, and dragon fruit. Compared to dragon, red cabbage sensitizer exhibits a reduced absorbance value in the visible

region (450–580 nm). Both black rice and fruit Each dye molecule has a R group (carbonyl and hydroxyl) that interacts chemically with the oxide layer to form a bond. The effectiveness of a red cabbage dye cell is the highest, at 0.06%, followed by dragon fruit and black rice, at 0.02% and 0.03% respectively (Ahliha *et al*., 2018).

Investigations have been made on the performance of quasi-solid-state polyacrylonitrile (PAN)-based iodide electrolytes in chlorophyll dye-sensitized solar cells (DSSCs). Tetra propylammonium iodide is the salt used to provide iodide ions (TPAI). A kind of moss bryophyte that grows on rocky surfaces is used to make chlorophyll. The highest efficiency of 1.97% is produced by the DSSC with an electrolyte composed of 9.9 weight percent PAN, 39 weight percent ethylene carbonate, 39.7 weight percent propylene carbonate, 9.9 weight percent TPAI, and 0.8 weight percent iodine, with short-circuit current density of 5.78 mA cm^2, open circuit voltage (Voc) of 0.60 V, and fill factor (FF) of 0.57 With the DSSC using the aforementioned electrolyte demonstrating the maximum power conversion at 339 nm, the incident photon-to-current efficiency curves follow the current density-voltage characteristics (Hassan *et al*., 2014). Using natural dyes taken from the red (*Gracilaria*) and green *(Ulva*) algae as photosensitizers, dye-sensitized solar cells (DSSC) have been constructed, and the impact of adding Graphene quantum dot (GQD) has been explored. Natural dyes from *Gracilaria*, *Gracilaria* + GQD, *Ulva*, and *Ulva* + GQD had open-circuit voltage (VOC) values of 0.64, 0.73, 0.70, and 0.75, respectively. For the forementioned samples, the short circuit current density (JSC) ranges from 0.96 to 2.26 mA cm2, and the fill factor (FF) ranges from 52 to 56%. For DSSC with *Gracilaria* + GQD, the greatest energy conversion efficiency of roughly 0.94% has been attained (Saedi *et al*., 2020).

In algae *Spirulina* phycobiliprotein is important component which play important role. Phycobilin extracted and its efficiency as photosensitizer on nanocrystalline TiO_2 photoanode and photoelectric effects are studied in combination with squaraine dye was investigated (Wang *et al*., 2014).

Metal doping on natural dyes influence its efficiency. Effect of Nickel (II) chloride as metal doping was studied. Characterization of dye adsorption on TiO_2 electrodes and adsorption of dye solution was done by UV-Vis spectroscopy. Electrical characteristics measured using I-V meter. And found that absorbance of dye solution increases at region of 700nm. This is the region of absorption for Nickel. Increase in concentration increases conductivity. The efficiency of DSSC

can be increased up to 135% as compared to pure chlorophyll dye (Pratiwi *et al*., 2018). Effect of Fe (III) on addition with Chlorophyll compound is also studied. By the phenomenon of metal ligand charge transfer Fe (III)-chlorophyll compound form. The complex formation can enhance the efficiency of Chlorophyll dye sensitizer (Setyawaty *et al*., 2017). The quick decomposition of chlorophyll (Mg-Chl) when used as a dye photosensitizer in DSSC is one of the major problems. Theoretically, increasing the stability of chlorophyll might be accomplished by modifying the alkali metal utilising transition metal ion chlorophyll structure extracted from *Gliricidia sepium* leaves had Mg^{2}+ ions (n = 2 and 4) replaced with Mn^{n}+ ions (n = 2 and 4). The separation of the chlorophyll using the column chromatography method was followed by the modification of the metal center using two different manganese-containing compounds, namely $MnCl_2$ and MnO_2. The outcomes demonstrate that the Rf values following substitutions [0.90 (Mg-Chl), 0.81 (Mn^{2}+-Chl), and 0.96 (Mn^{4}+-Chl)] differ.

The resulting products' IR absorption patterns are comparable to those of Mg-Chl, with the difference being only in their transmittance at somewhat larger wavenumber shifts. A change in UV-Visible and infrared properties indicates that the produced compounds have different molecular electronic natures. This suggests that the metal center might be successfully replaced without impairing the structure of the chlorophyll (Selan *et al*., 2021).

Natural dyes are more preferred over synthetic dyes but they are not efficient as synthetic dyes. The efficiency of natural dyes can be increased by various methods. Application of two-layer algal byproducts buffer can be done. Sodium alginate and *Spirulina* can be used in buffer. The application of Sodium alginate on photoelectrode enhance the dye concentration on film by the introduction of more hydroxyl groups. The TiO_2 film (with sodium alginate) was sensitized with spirulina prior to the sensitization procedure with anthocyanin colour. The anthocyanins' ability to effectively connect with TiO_2 is helped by the chlorophylls, xanthophylls, phycocyanin, and amino acids found in spirulina. Major drawback of natural dye is photo instability which causes photodegradation on exposure to sunlight. Titanium dioxide have photocatalytic activity that generates high energy free electrons on the surface which generate free radical ions that can degrade organic molecules like natural dyes. Thus, by reducing the photocatalytic activity of electrode stability od dyes can be improved (Prabavathi *et al*., 2018). By enhancing the absorptivity of dyes, the performance of DSSCs can be enhanced. Studying the influence of various factors like

difference in extraction approaches, extraction solvent acidity, different components of solvents and found that the optical absorptivity of dyes and performance of DSSCs improved by using proper ethanol and water mixture for extracting solvent and acidity of dye solution (Hemmatzadeh & Mohammadi., 2013).

Using clay from Timor to alter TiO_2 semiconducting material with chlorophyll dyes as photosensitizers, dye sensitised solar cell (DSSC) efficiency was improved. In this study, chlorophyll from three different plant leaves—*Leucaena leucoceph, Lannea coromandelica* and *Cromoleana odorata*—was used to develop and evaluate photoelectrochemical cells (C). The efficiency of cells using these three various sources of chlorophylls was improved by adding clay to TiO_2(Selan *et al.,* 2021).

A well- liked method for increasing the effectiveness and stability of dye sensitized solar cells is co-sensitization. In this context, with the inclusion of additives, co-adsorbents, and co sensitizers that lessen the aggregation and charge recombination in the device, the power conversion efficiency values of DSSCs containing Ru-and porphyrin-based dyes can be enhanced from 8-11% to 11-14%. Co-sensitizers enhance photovoltaic performance of devices (Krishna *et al.,* 2017).

DSSCs are highly relevant in present scenario as there is greater depletion of fossil fuels. This is a better and cost-effective strategy to follow. It can also make use of natural sensitizers from plant parts like pigments. Various pigments from Plants, and from microorganisms like Yeast, Bacteria and Microalgae can be used to sensitize solar cell. Since these microorganisms requires only limited space to grow and having high growth rate they can be easily cultured and maintained. Algae are better known for their Chlorophyll contents which can be utilized. In anyway DSSCs are better alternatives for non-renewable sources of energy.

CHAPTER THREE
MATERIALS AND METHODS

MATERIALS AND METHODS

Microalgal culture

Microalgal species selected for the current study was a strain of *Chlorella vulgaris* 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 species compared to other strains available in the laboratory

Culture media and composition and culture parameter

The algal culture broth was prepared. BG 11 medium is prepared and 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	$24 \pm 1^{o}C$
3	Light intensity	700-800 lux
4	Photo period	16/8h (light/dark)
5	Time	15 days

Table 2: Microalgal growth Culture Parameter

Microscopic observation

After 3 days of incubation, the culture was 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

Maximum absorbance determination

The efficiency of biomass growth was controlled by measuring the optical density , which is defined as the absorption of visible radiation . The optical absorbance was measured at various wavelengths such as 680nm and 750nm in order to determine maximum absorbance using a spectrophotometer (LAB India).

Cell counting using Neubauer Haemocytometer

The microalgae concentration in the culture was counted using the improved Neubauer hemocytometer 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 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 were examined using 10X objective for distribution of the cells and refocused at 40X objective before counting cell in the four squares.

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

Determination of chlorophyll content

The chlorophyll content of the micro algal cells was determined by using spectrophotometric technique. Sample of the micro algal suspension was centrifuged for 10 minutes at 13000rpm (Centrifuge HERMLE-Z 3242). The supernatant was decanted and the pellet resuspended in 90% methanol. Chlorophyll was then extracted from the sample during one hour of incubation in a water broth (Rotek) at 50^{0} 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 and 405 nm, using the UV spectrophotometer was blanked with methanol.

Identification of microalgae using molecular sequencing

At the molecular level, r RNA 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 16s r RNA genes for the phylogenetic analysis. Here we explored the possibility of 16s forward and reverse primer for amplification.

DNA isolation

DNA isolation method by Doyle and Doyle (1990), Using CTAB yielded good quality DNA for PCR.

DNA isolation using CTAB

Freshly prepared CTAB (Cetyl Trimethyl Ammonium Bromide) buffer was preheated at 65^0C, 1 gm. 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: Isoamyl alcohol (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: Isoamyl alcohol extraction was repeated and the aqueous layer was collected in Eppendorf tubes (1 ml in each tube). 3M sodium acetate (p H5.2) was added to the aqueous phase and DNA was precipitated by the addition of $2/3^{rd}$ volume of ice-cold isopropanol and thoroughly mixed by inverting. The sample were kept for overnight incubation at -20^0C. The supernatant was decanted off and the pellets was 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.

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 was compared with 250bp DNA marker from Chromous Biotech. The gel documentation system (BIORAD- Molecular image) was used for DNA visualization on the gel.

PCR amplification

16s region was amplified by polymerase chain reaction (PCR) with specific primers (forward and reverse primers). Amplification of the conserved regions of the 16s rRNA gene was conducted in a 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 consisted of initial denaturation at 94 º C for 3 minutes followed by 40 cycles of denaturation at 94 º C for 30s, annealing at 55 ºC for 30s, and extension at 72 º C for 1 minute, with a final extension at 72º C 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 aftertr imming the low-quality bases. 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.

DEVELOPMENT OF MICROBIAL BIOFILM AND ASSESSMENT OFITS PHOTOVOLTAIC EFFICIENCY

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

Preparation of electrode and electrolyte for microalgal photovoltaic cells

Microalgal photovoltaic cell consisted of electrolyte and electrode. Algal biofilm on the glass slide was 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.

Development of biofilm on the ITO glass slide

Strains of *Chlorella vulgaris 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 620nm was used for the experiment. ITO coated 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, the experiments were done in triplicates.

Determination of surface area percentage of biofilm (SAC %)

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

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 0.5 g of glycerol, 0.5g of formaldehyde and 1.5g of acetic acid were added to this solution under stirring. The resulted viscous solution was then cooled down to 30^0C and poured on petri dishes 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.*, 2012).

Counter electrode

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

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 electrode is facing each other. The alligators wire was clipped to the ends of each electrode and connected to the multimeter for the measurement.

PHOTOVOLTAIC MEASUREMENTS

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 $P=V^2/R$. These values were used to plotgraph and the maximum power output was noted.

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=$25\times25=625mm^2$

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 poweroutputs. The maximum power density was noted.

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CHAPTER FOUR
RESULTS AND DISCUSSION

The first step in the work is inoculating and culturing the algae *Chlorella vulgaris* in suitable medium . First we inoculated the sample and then incubated in sterile condition.

Fig.1.Micro algal culture -First day of inoculation

During culturing the growth of the micro algae can be observed day by day .There was a gradual colour change in culture from day one to fifteenth day .The colour of culture changes from light green to dark green towards the end .This change in colour indicates the increase in cell count as well as chlorophyll content.

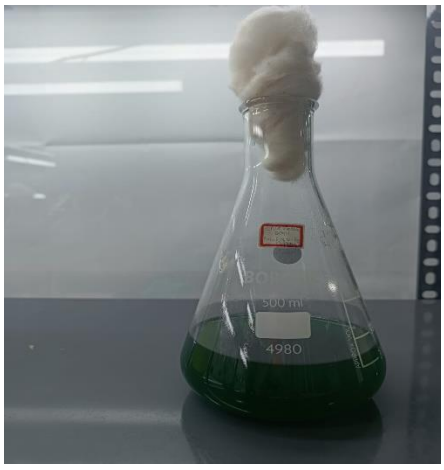

Fig.2.Micro algal culture -15th day

Absorbance is determined at two wavelengths, 620 nm and 700 nm using UV spectrophotometer at an interval of five days . As the number of days increases the OD value also increases gradually which indicate growth .

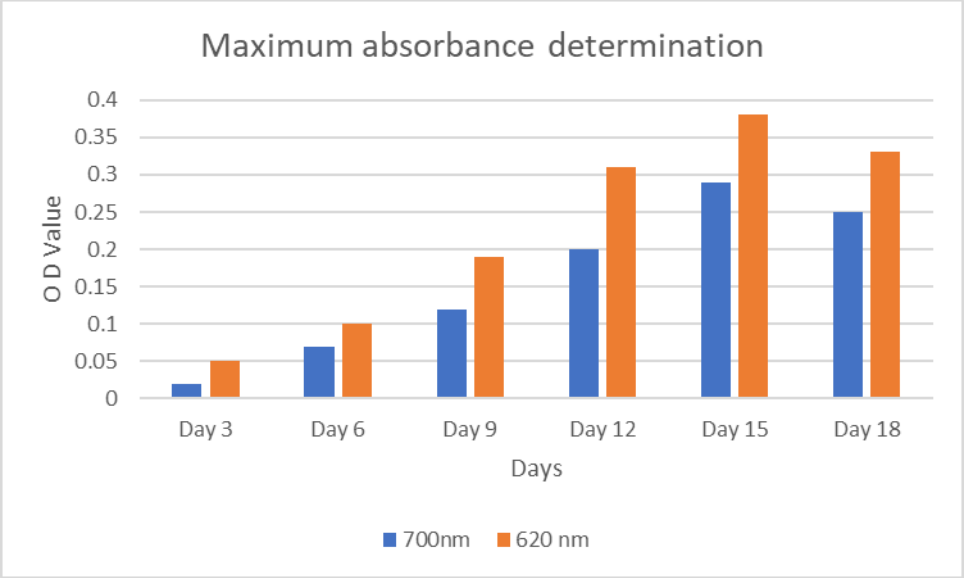

Figure .3. Maximum absorbance determination

The maximum OD value is obtained at 620 nm. OD value indicates the speed at which the light passes through the sample. Thus, higher OD value is an indication increment in growth with days. Maximum growth is observed during 15th day. After 15 th day there is slight decline in OD value.

The increase in cell count can be observed by microscopic observation. Using haemocytometer, the count of the cells are taken.

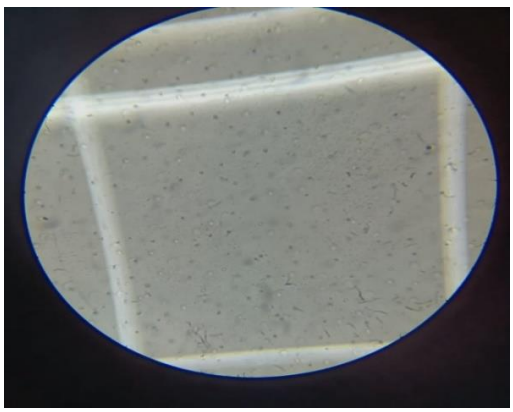

Figure .4. Microscopic observation of *Chlorella* under light microscope

The green dots under microscope are single *Chlorella*. The microscopic observation indicates that there is increase in growth of algae. The cell count increases gradually. The cell count at different intervals is calculated. The microalgae concentration counted using improved Neubauer haemocytometer counting chamber. The cell number density is calculated.

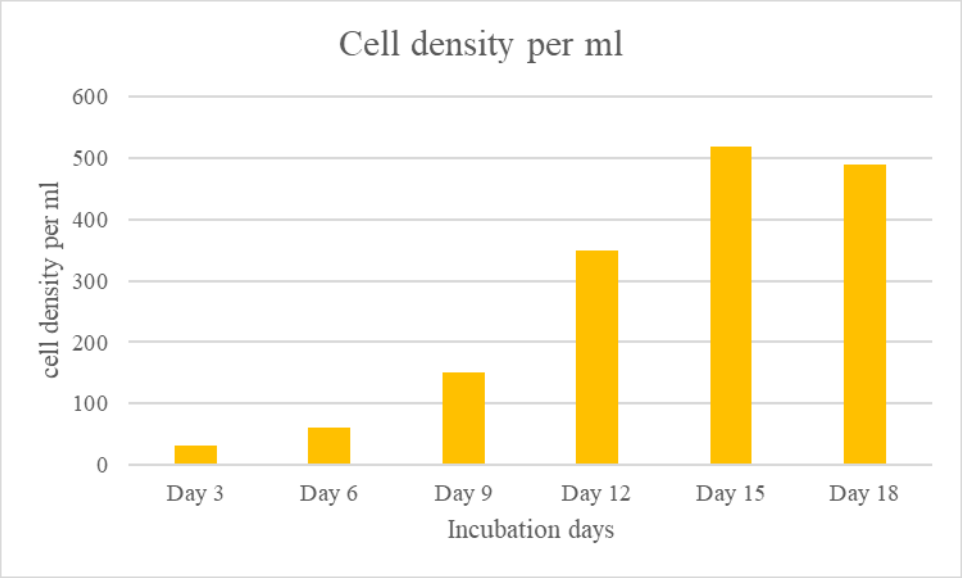

Figure 5. Variation of Cell number density in different incubation days

Gradual cell growth is observed from day 1 to day15 which is indicated by increase in cell number density per ml. This is due to the fast multiplication of cell in optimal conditions. After the attainment of maximum cell number density there occur cell death and so successive reduction in number of cells and cell number density. Here maximal growth is obtained at 15th day.

Chlorophyll content of the sample is determined using spectrophotometer at two wavelengths and O D value is calculated

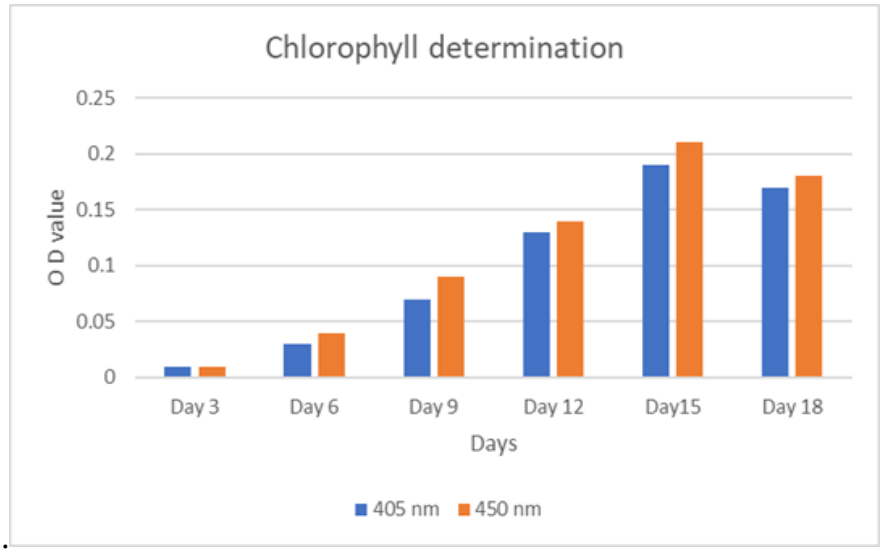

Figure.6. Chlorophyll content determination

The graph (Figure .6) shows the chlorophyll content of the culture during time interval. There is a gradual increase in chlorophyll content which indicate the growth. The chlorophyll content is maximum at 15th day. After 15th day there is a slight decrease in chlorophyll content.

Identification of organism using molecular sequencing method involves various steps. The very first step is the isolation of DNA . Isolation is done by Doyle and Doyle (1987), using CTAB yielded good quality DNA for PCR.

Figure.7.DNA Banding pattern

Lane (MBT051);100bp marker

Lane (s); 16s r RNA PCR amplicon of DNA

After gel electrophoresis the DNA get separated according to their molecular size . Small fragments will move faster than the larger fragments. The band pattern is specific for every organism thus this can be used for identification and confirmation of selected organism. The obtained band is viewed under gel documentation system.

>AF350260.1:1-586 Chlorella vulgaris 16S ribosomal RNA gene, partial sequence
AGGGACAACCATTGGAAACGATGGCTAATACCTCATAATACTGAGTAAGTTAAATGATGAATAATCG
CCAAGAGATGGGC
TTGCGGCTGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGATGATCAGTATCTGGTCTGAC
CAGGATGATCACCC
ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAACAGTGAGGAATTTTCCGCAATGGG
CGAAAGCCTGACGG
AGCAATGCCGCGTGAAGGATGAAGGCCTATGGGTTGTAAACTTCTTTTCTCAGAGAAGAAATTTTGA
CGGTATCTGAGGA
ATAAGCATCGGCTAACTCTGTGCCAGCAGCCGCGGTAAGACAGAGGATGCAAGCGTTATCCGGAAT
GATTGGGCGTAAAG
CGTCTGTAGGTGGCTTAAAAAGTCTCCTGTCAAAGATCAGGGCTTAACCCTGGGCCGGCAGGAGAA
ACTCTTAGGCTAGA
GTTTGGTAGGGGCAGAGGGAATTCCCGGTGGAGCGGTGAAATGCGTAGAGATCGGGAGGAACACCA
AAGGCGAAAGCACT
CTGCTGGGCCACAACTGACACTGAGA

Figure.8. *Chlorella vulgaris* 16s rRNA gene partial sequence

After sequencing the obtained sequence is compared with the database to identify the similarity. BLAST is used to identify the matching sequence from the database by word by word similarity search.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Chlorella vulgaris 16S ribosomal RNA gene, partial sequence; chloroplast gene for chloroplast product	Chlorella vulgaris	1083	1083	100%	0.0	100.00%	586	AF350260.1
Chlorella vulgaris strain MSU-AGM 14 16S ribosomal RNA gene, partial sequence; chloroplast	Chlorella vulgaris	1050	1050	100%	0.0	98.98%	609	KM189121.1
Chlorella sp. ArM0029B chloroplast, complete genome	Chlorella sp. Ar...	1042	1042	100%	0.0	98.81%	119989	KF554427.1
Micractinium singularis strain MM0003 plastid, complete genome	Micractinium sin...	1037	1037	100%	0.0	98.63%	139597	MN894287.1
Micractinium pusillum strain CCAP 232/1 chloroplast, complete genome	Micractinium pus...	1031	1031	100%	0.0	98.46%	115638	MN649872.1
Micractinium sp. LBA 32 chloroplast, complete genome	Micractinium sp. ...	1026	1026	100%	0.0	98.29%	109688	MH983006.1
Uncultured Streptophyta clone UVmas1_53 16S ribosomal RNA gene, partial sequence; chloroplast	uncultured Strep...	1026	1026	100%	0.0	98.29%	701	JQ701246.1
Uncultured bacterium clone AD05 16S ribosomal RNA gene, partial sequence	uncultured bacte...	1024	1024	100%	0.0	98.29%	990	KC009751.1
Uncultured Streptophyta clone UV-2_3 16S ribosomal RNA gene, partial sequence; chloroplast	uncultured Strep...	1020	1020	100%	0.0	98.13%	706	JQ700677.1
Uncultured cyanobacterium clone Gap-2-18 16S ribosomal RNA gene, partial sequence	uncultured cyan...	1018	1018	100%	0.0	98.12%	870	EU642172.1
Chlorella sp. SUN-2 16S ribosomal RNA gene, partial sequence; plastid	Chlorella sp. SU...	1014	1014	100%	0.0	97.96%	1454	EF114678.1
Auxenochlorella pyrenoidosa isolate FACHB-5 chloroplast, complete genome	Auxenochlorella ...	1009	1009	100%	0.0	97.78%	107442	MN128434.1
Uncultured phototrophic eukaryote clone NV1_CYA_1_29 16S ribosomal RNA gene, partial sequence; plastid	uncultured photo...	1007	1007	100%	0.0	97.78%	666	FJ204892.1
Uncultured bacterium clone CK-86 16S ribosomal RNA gene, partial sequence	uncultured bacte...	1003	1003	100%	0.0	97.61%	1453	KM200526.1
Uncultured bacterium gene for 16S ribosomal RNA, partial sequence, clone: Sa75_M_3	uncultured bacte...	1002	1002	100%	0.0	97.61%	1415	LC065717.1
Uncultured bacterium clone N06Jun-31 16S ribosomal RNA gene, partial sequence	uncultured bacte...	996	996	100%	0.0	97.44%	803	EU442895.1
Uncultured bacterium clone P1O-64 16S ribosomal RNA gene, partial sequence	uncultured bacte...	996	996	100%	0.0	97.44%	809	EU375419.1
Uncultured cyanobacterium clone XZNMC45 16S ribosomal RNA gene, partial sequence	uncultured cyan...	990	990	100%	0.0	97.27%	1371	EU703214.1
Chlorella variabilis clone DT025 chloroplast, complete genome	Chlorella variabilis	985	985	100%	0.0	97.10%	118106	MZ647689.1
Chlorella sp. ATCC 30562 plastid, complete genome	Chlorella sp. AT...	983	983	100%	0.0	97.10%	124881	KY629617.1
Chlorella variabilis isolate NC64A chloroplast, complete genome	Chlorella variabilis	983	983	100%	0.0	97.10%	124793	KJ718922.1
Chlorella variabilis plastid, complete genome	Chlorella variabilis	983	983	100%	0.0	97.10%	124579	HQ914635.1

Figure .9 BLAST search

The sequence show similarity with *Chlorella vulgaris* 16s ribosomal RNA gene , partial sequence ;chloroplast gene for chloroplast product .

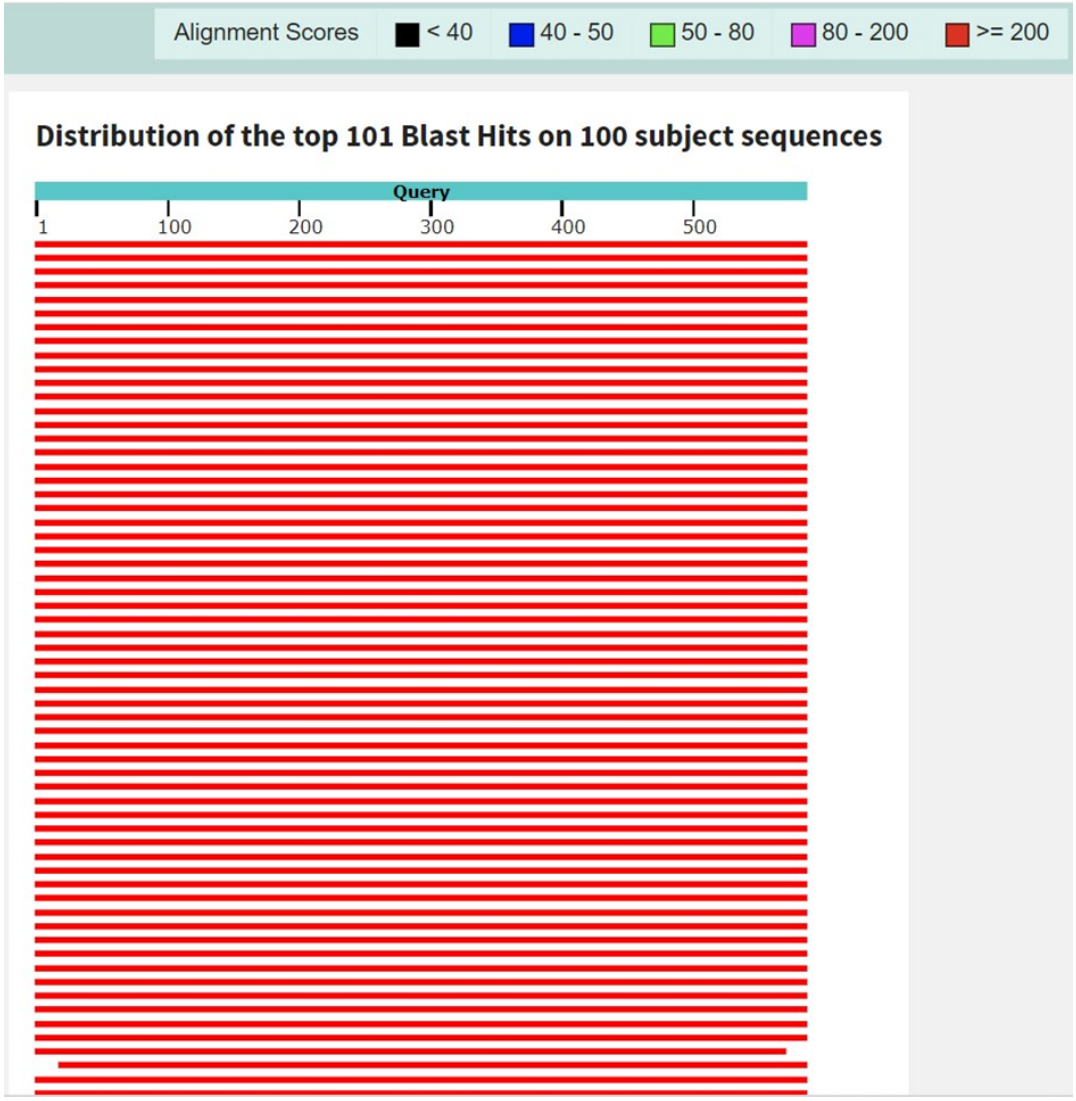

Figure .10. Graphic summary of alignment score

The graphic summary of alignment represents sequence similarity of query sequence with that of sequences in the databases . The query length is greater than 500 nucleotides. Each of the horizontal lines are representation of sequence that is in the database matches. Here the alignment score is greater than 200 which is represented by red lines which indicates that there is a great quality or good matches.

Phylogenetic analysis of algae is carried out using Mega X , a highly efficient tool in evolutionary studies.

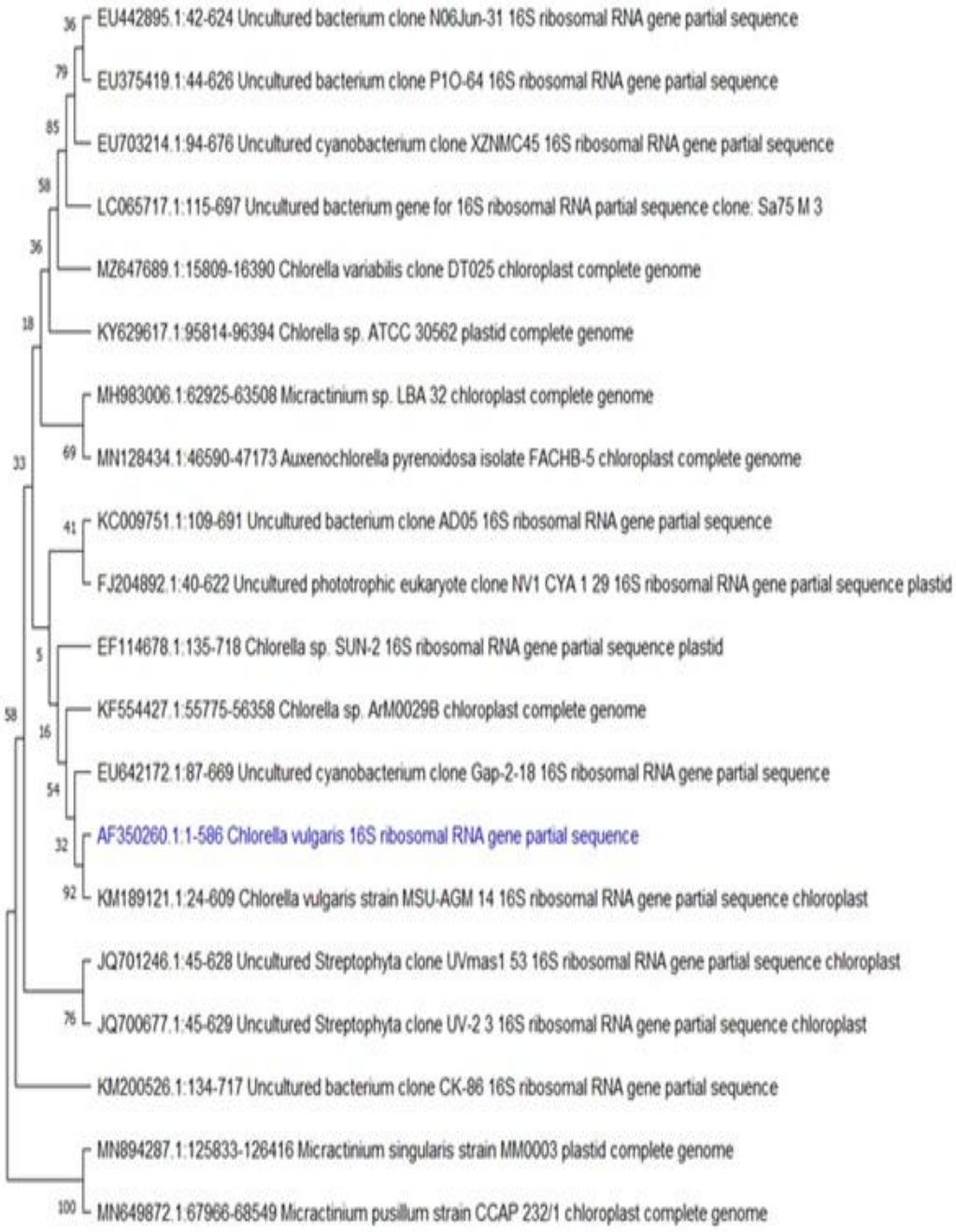

Figure .11.Phylogenetic tree

The phylogenetic tree shows that the *Chlorella vulgaris* 16s ribosomal RNA gene partial sequence show close relationship with *Chlorella vulgaris* strain MSU-AGM 14 16s Ribosomal RNA gene partial sequence chloroplast and with uncultured Cyanobacterium clone Gap -2-18 16s Ribosomal RNA gene partial sequence.

A prototype of microalgal photovoltaic cell comprises of an electrolyte between the other two electrode , a counter electrode, and a photoelectrode. These are constructed for the study. For the adhesion of the microalgal biofilm, ITO glass slides were employed as the substrate. This ITO glass slide was submerged in the glass bottle containing algal culture , as depicted in figure .

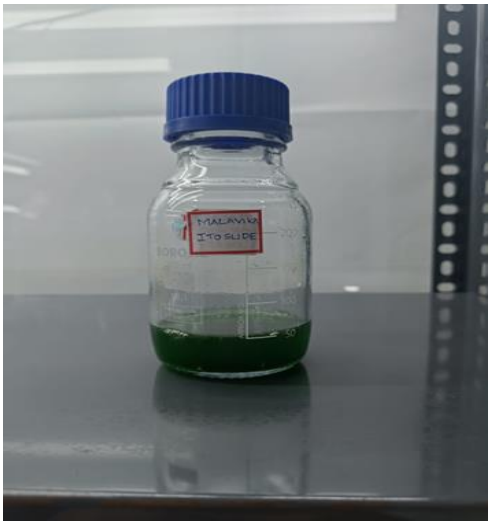

Figure 12. Culture containing ITO glass slide

ITO glass slides dipped in microalgal culture , algal biofilm begins to form on the ITO glass slide as the day passes. Biofilm will appear visible on the ITO slide on the third day of incubation. The highest biofilm production occurs after day 15 , as shown in the figure

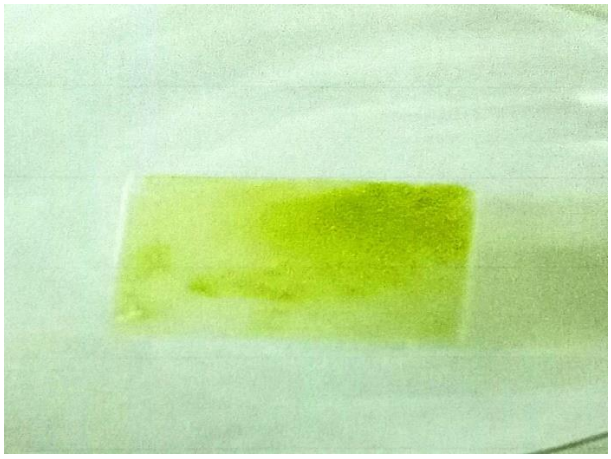

Figure.13. Development of biofilm on the surface of ITO at 3 rd day of inoculation

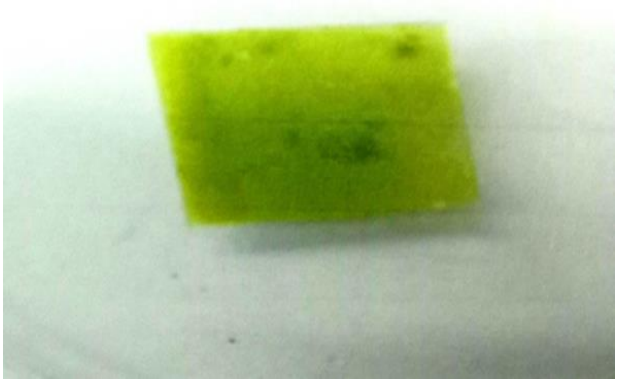

Figure .14 . Development of biofilm on the ITO glass slide at the 15 th day of inoculation

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

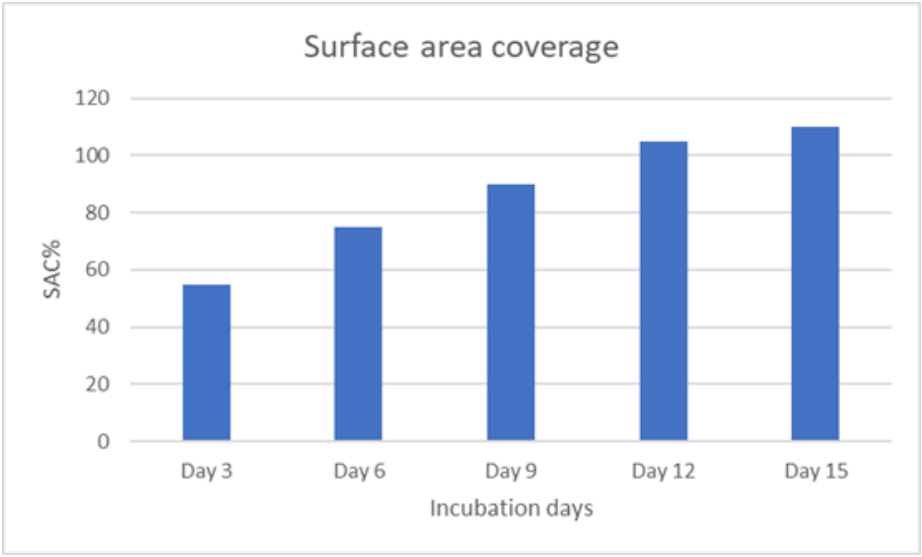

Figure . 15. Surface area coverage of ITO glass slide

Carbon coated glass slide was used as carbon cathode as shown in figure.16

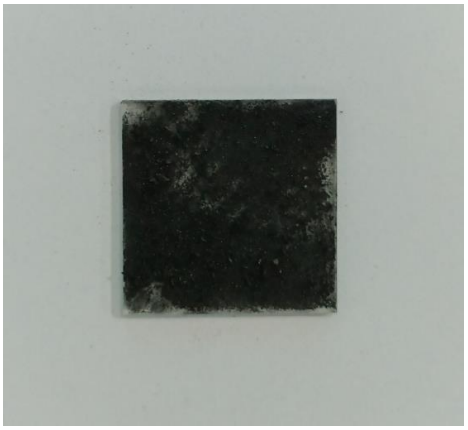

Figure.16 . Carbon coated cathode

Microalgal photovoltaic cell was created . The cell consists of a photoelectrode (anode) , counter electrode(cathode) and an electrolyte is placed between these electrodes in a sandwitch type manner, alligator wires are clipped to the ends of the electrodes . Then the current was measured using a multimeter .

Figure.17. Circuit

As the incubation days progress , there shown an increase in the value of current density . At the third day of incubation there is only a slight increase observed that is 1.12×10^{-2} A/m^2 . The maximum current density was observed at 15th day of incubation that is 17.28×10^{-2} A/m^2. The variation in current density at different incubation days is shown below

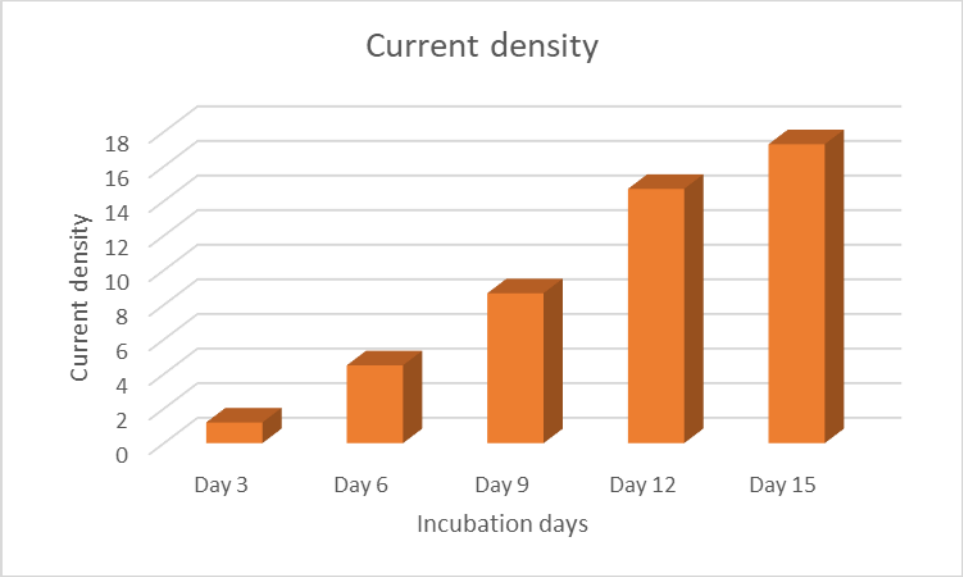

Figure.18 .variation in current density at various days of incubation

As the incubation days progress , there shown an increase in the value of power density . At the third day of incubation there is only a slight increase observed that is 0.08 W/m^2 . The maximum current density was observed at 15th day of incubation that is 18.65 W/m^2 The variation in power density at different incubation days is shown below

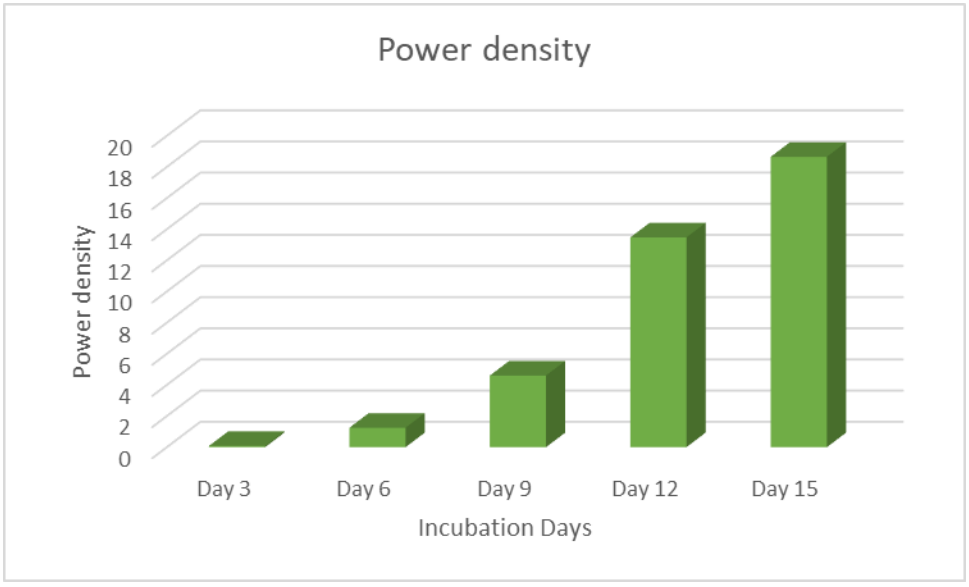

Figure.19. Variation in power density at various days of incubation

DISCUSSION

A dye sensitized solar cell (DSSC, DSC) also called Gratzel cell is thin film solar cell. It is cost effective which is based on photoelectrochemical system that produces a semiconductor by combining an electrolyte with a photosensitized anode. It's construction is successful using chlorophyll from *Chlorella vulgaris* as photosensitizer which is shown by this work.

Photosensitizer is an important component of dye sensitized solar cell which should be care fully choosesn. They can be natural or artificial. Natural is usually the pigments from plants. Thus the study is relevent in checking whether the algal pigments like chlorophyll is also usefull as photosensitizer. *Chlorella vulgaris* a unicellular microscopic algae is easily multiply and grow in laboratory condition day by day in a geometrical proportion. This is also a added advantage in the usage of algae as a source of photosensitizer. The growth conditions for the algae is provided and its growth parameters like Cell number density , Maximum absorbance and chlorophyll contents are checked periodically at an interval of three days and there is a visible increase upto 15 th day and afterward there occur slight decreament they are represented graphically. The maximum growth is observed at 15th day of incubation thus algal growth at this stage is taken for the further steps. The algae taken is Chlorella and is confirmed by the procedures like isolating DNA , Amplifying , gel electrophoresis, DNA sequencing and finally by the BLAST similarity search. A pylogenetic tree is constructed using MEGA X and evolutionary relationships also studied

In the construction of microalgal photovolataic cell a prototype is constructed between photoelecrode(figure.14), counter electrode (figure. 16) and electrolyte between the two. ITO glass slide act as a substarte for the adhesion of microalgal biofilm. For this aadhesion slide is dipped in microalgal culture.

Surface area coverage of biofilm is checked at an interval of 3 days . Maximum suraface area coverage is shown at 15th day of incubation. The changes in surface area coverage gradually at an interval of three days is represented graphically (Figure .15) Counter electrode which is a cathode is constructed by coating the carbon over glass slide. Electrolyte is sandwitched between the anode and cathode. Alligator wires are used for the circuit construction. They are clipped at the ends .A multimeter is used for measuring current.(Figure.17).

As the incubation days passes there is an increasing biofilm coating thereby increases the function as a photosensitizer. When radiation is first absorbed by molecular entity known as a photosensitizer, photochemical or physiochemical transformation to occur. This process is called photosensitization. The sensitizer injects electrons into the semiconductor and redox mediator in the electrolyte. The light passes through the anode and excites the chlorophyll molecule . The excited dye molecules inject electrones in to the semiconductor layer .Then the electrones flow through the external circuit to the cathode and then flow to the electrolyte. This is usefull in electricity production if the construction and operations are large scale after checking the stability at indoor and outdoor conditions. The usage of natural dyes makes the DSSC less harmfull. But the natural dyes have very low efficiency because of poor connection between the semiconductor and dyes. Also dye aggregation is important factor. Also some recommandations are there to improve the efficiency of DSSC which includes development of low volatile and less viscous electrolyte to improve the charge transfer rate , the improvement of mechanical contact or adhesion between the two electrodes, the improvement in morphology of semiconductor to reduce dark current and also the use of dye and electrolyte additives to improve their properties. (Umer Mehmood .,*et al*,2014).

Flexible ,thin ,light weight DSSC modules can be constructed and can be used in large scale. Works were also there to study the use of plastic as a flexible substrate. Due to distinctive qualities such as light weight ,flexibility and viability of roll to roll quick mass production plastic substrate based DSSCs are showing greater potential for commercialization (Hasitha C.Weerasinghe *et al*., 2013).

The works involving construction of DSSCs using dyes that are from different types of algae including sea weeds was done and is found to be very effective . The DSSCs using Chlorophyll of *Chlorella vulgaris* also give good out put power which can be utilized . DSSCs using natural dyes are safe but its production process require good knowledge . If it is popularised like any other solar cell it will be more beneficial for overcoming energy crisis.

CHAPTER FIVE
SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Dye sensitized solar cell (DSSC) also known as Gratzel cell. It is developed by Gratzel and coworkers, in order to imitate photosynthesis process. Due to increasing energy requirement and the depletion of fossil fuels and other non renewable energy resources a alternative method, that is the development of DSSC is a great breakthrough. It is cost effective which is based on photoelectrochemical system that produces a semiconductor by combining an electrolyte with a photosensitized anode. A substance known as semiconductor may conduct electrons. When radiation is first absorbed by molecular entity known as a photosensitizer, photochemical or physiochemical transformation to occur. This process is called photosensitization. The sensitizer injects electrons into the semiconductor and redox mediator in the electrolyte. Synthetic and natural dyes are utilized as photosensitizers since they are essential for determining the overall effectiveness of the cell.

Both natural and synthetic dyes can be used as photosensitizer. Natural dyes are usually pigments from plants like chlorophyll, anthocyanin, carotein etc. This study is using Chlorophyll of algae *Chlorella vulgaris* as photosensitizer of DSSC. Chlorella vulgaris, a green eukaryotic microalga that has existed on earth since the Precambrian era. As the first microalgae with a clearly defined nucleus.

The first step in the work is the inoculation and culturing of Chlorella in suitable culture medium under optimal conditions. The periodic checking of parameters like Cell nummber density , absorbance , chlorophyll content is done at an interval of 3 days . As the day passes the colour of culture changes from light green (Figure.1) to dark green (Figure.2) which is an indication of growth. Cell number density show increase gradually from day 3 to 15 after that there occur decrease in growth which indicate 15 th day shows the maximum growth of culture. Absorbance determination at two wavelengths 620nm and 700 nm also show same trend. The culture shows greater optical density at 620 nm as compared to 700nm . Chlorophyll content is also maximum at 15th day due to the cell growth. So the culture at 15 th day is suitable for construction of DSSC. Also the confirmation of organism is done using sequencing method after gel electrophoresis of isolated DNA of *Chlorella* . After sequence is obtained the it is compared with sequences of database for similarity search using BLAST. The sequence show 100 percent match with *Chlorella vulgaris* 16s r RNA gene .

The construction of DSSC is the important step in the work. It involves component like photo electrode which acts as the anode contains biofilm over ITO glass slide , a counter electrode which is constructed by coating carbon over glass slide acts as cathode and electrolyte. The development of biofilm over ITO glass slide is done by dipping the glass slide in culture medium for days. The development is measured by surface area coverage .The results shows more surface area coverage is observed in 15thday than the 3rd day. So the biofilm at 15 th day used in cell construction. Circuit is constructed using alligator wires by clipping at the ends of anode and cathode .They are connected to a multimeter for measuring the current . The curent density increases gradually day by day and reaches maximum at 15 th day. This indicate that the algal biofilm formation contribute to the production of the current thus the effiency of chlorophyll of *Chlorella vulgaris* as photosensitizer in dye sensitized solar cell is confirmed. So the chlorophyll of Chlorella is a good photosensitizer and can be used commercially.

So the conclusion is that DSSC's are best alternative of conventional energy resources and its production should be encouraged greatly. The usuage of natural dyes over synthetic dyes are more prefered because of less cost and toxicity. There are various options for natural dyes. Algae is best source of pigments which can also used as photosensitizer. Pigments from the algae are more easy to obtaion from that of plants. Also algae have high growth rate and needs less space for the growth which can also cultured in the laboratary conditions. Thus Algae have added adavantage. *Chlorella* is known to produce high amount of chorophyll than any other algae so it is suitable for the construction DSSCs.

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APPENDIX

APPENDIX 1

BG 11 medium

BG 11 : 0.3254 g

Distilled water: 200 ml

APPENDIX 2

CTAB Buffer

1 M Tris HCl: 1.576 g

4M NaCl : 2.3376 g

05 M EDTA: 1.8612 g

2% CTAB : 0.2 g

APPENDIX 3

PREPARATION OF MASTER MIX

Molecular biology grade water :15 µl

10X assay buffer : 2.5 µl

Template DNA : 1 µl

Forward primer :0.5 µl

Reverse primer :0.5 µl

$MgCl_2$:2.5 µl

DNTPs :2.5 µl

Taq DNA polymerase :0.5 µl