



Photosynthetic Efficiency of Microalgae and Cyanobacteria on Reduced Graphene Oxide (RGO) Anode Surface for Utilization in Bio-Photovoltaics (BPV)

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Article History	Abstract:
Received: 20 June 2023 Revised: 10 Sept 2023 Accepted: 12 Oct 2023	<i>Bio-photovoltaics (BPV) is sustainable energy production technology that utilize photosynthetic organisms and convert it into electricity. This Study has been carried out to study the photosynthetic efficiency of three microalgae on a Reduced Graphene Oxide (RGO) anode surface. RGO, with its exceptional electrical conductivity and large surface area, presents an attractive platform for enhancing the performance of BPV systems. The work aims to investigate the combined effect of microalgae and RGO anodes for use in BPV technology. RGO was synthesized and characterized on which Chlorella vulgaris, Gloeocapsa and Synechocystis were allowed to grow. A model BPV system was assembled, incorporating the microalgae and cyanobacteria as photoactive agents and RGO as the anode surface. The system was subjected to different experimental condition and photosynthetic efficiency, current generation, and power output were collected and analysed. Results demonstrated a significant improvement in the photosynthetic activity of microalgae when cultured on the RGO anode surface. Chlorella Shows maximum Efficiency in terms of growth and current generation. Statistical analysis confirmed the reliability and significance of these findings. Our finding bridges a crucial knowledge gap in the field of BPV, highlighting the potential of microalgae-RGO systems for cleaner energy production.</i>
CC License CC-BY-NC-SA 4.0	Keywords: Cyanobacteria, Bio photovoltaic Device, Sustainable energy, Reduced Graphene, RGO Anode

Introduction:

Global energy demand is increasing continuously to fulfil the growing human population needs, and advancement of industrial activities in both developed and developing countries (Asdrubali, & Desideri, 2019). Today major requirement of energy is satisfied using conventional sources of energy like petroleum oil, coal and natural gas have caused adverse effect to the environment by raising atmosphere CO₂ level (Bhui.,2021., Su et al., 2023), causing a range of global climatic events, like global warming (Al-Ghussain., 2019; Watts, 2022). Sustainable and environmentally friendly energy solutions have become an urgent global need due to the limited fossil fuels (Watt 2022; Singh, 2021; Shamooun et al., 2022) and the environmental consequences of their use. Among the various innovative approaches like hydropower (Siri et al., 2021), solar power (Nwaigwe 2019, Hayat et al., 2019), wind power (Lehtola, & Zahedi, A. 2019), wave power, geothermal power (Kulasekara, & Seynulabdeen, 2019, Zhang et al.,2019), artificial photosynthesis (Nguyen et al., 2020; Yoshino et al., 2022), tidal power (Lv et al., 2021) biodiesel (Deora et al., 2022; Anerao et al., 2022), Bio-Photovoltaics (BPV) has emerged as a promising technology that use combined principles of photosynthesis with photovoltaics (Elhadad, & Choi, 2023; Jawre, & Center 2018). This renewable and carbon-neutral energy generation uses photosynthetic prowess of microorganisms, such as microalgae and cyanobacteria, to convert sunlight into electricity (Elhadad, & Choi, S. 2023).

Microalgae and Cyanobacteria are photosynthetic organisms, they are present in single to multicellular form. They are generally found in moist places and all types of water bodies hence algae are common in aquatic (Deep et al., 2013) and terrestrial environments (Wagner, 2007; Bhattacharyya et al., 2013; Pradhan et al.,

2018). Algae are also known as kelp (macroalgae) and phytoplankton (microalgae). Most of the algae are eukaryotic except cyanobacteria (earlier known as Blue green algae) (Packer, 2009; Metting 1994; Castenholz 1992). Algae are like plants because they also require sunlight, water, and carbon dioxide for growth (Bruton et al., 2009). The photosynthetic organisms efficiently capture solar energy and convert it into chemical energy via the process of photosynthesis (Nayak et al., 2012; Kruse et al., 2005, Moore, & Brudvig 2011). Thus, they are considered a promising feedstock for the investigation and advancement of alternative energy resources (Powar et al., 2022; Anto et al., 2020; Shuba, & Kifle, 2018). The organisms under consideration have notable characteristics such as accelerated rates of growth, the ability to generate a diverse array of products, and a remarkable capacity to withstand and recover from adverse circumstances (Chandra et al., 2019; Bhattacharyya et al., 2011). The biomass productivity of microalgae has been found to be 50 times higher than that of Land Plants (Chisti 2008; Benedetti et al., 2018). Thus, Microalgae and cyanobacteria have captured the attention of next generation bioenergy researchers in the BPV. Research on cyanobacteria in BPV applications indicated their significance in the transition towards clean and renewable energy sources (Hwang et al., 2020; Liu et al., 2019; Wu et al., 2018).

In recent decades, there has been a significant body of literature documenting the use of carbon-based electrodes in electrochemical sensing across various biological systems (Liu et al., 2022; Wring and Hart 1992). These applications have encompassed investigations into enzyme metabolism as well as the detection of proteins for the purpose of biosensor development (Joshi, et al., 2021; Janssen, et al., 2019). Moreover, the utilization of electrode materials based on graphene has exhibited distinctive characteristics in the electrical sensing of biological systems, owing to the numerous functional groups that are inherent to graphene (Mathew, et al., 2020; Liu, et al., 2012). Moreover, there has been a significant focus on the study of graphene and graphene oxide (GO) layers in recent years, with active research efforts aimed towards their utilization in the development of novel composite materials. This interest stems mostly from their notable biocompatibility properties. The comprehensive reporting of the behaviour observed in reduced graphene oxide (RGO) or functionalized graphene sheets, which is attributed to the presence of oxygen-containing functional groups on their surface, has highlighted their significance in the development of electrochemical devices (Muthoosamy, et al., 2015; Pimenta et al., 2007). This manuscript aims to investigate synthesis of RGO anode for growth of Microalgae and cyanobacteria and compare the photosynthetic performance and bioenergy potential of *Chlorella vulgaris*, *Gloeocapsa*, and *Synechocystis* by analyzing chlorophyll content, carotenoids, DCPIP reduction, and current power density, with the goal of selection of microorganisms for sustainable Bio-Photovoltaics (BPV) applications.

Material and Methods

Graphene oxide (GO) was synthesized in our laboratory utilizing a modified Hummer's approach. The raw materials used for the synthesis, namely natural graphite, sodium nitrate (NaNO_3), potassium permanganate (KMnO_4), sulphuric acid (H_2SO_4), and hydrogen peroxide (H_2O_2), were obtained from Himedia Pvt. Ltd. in India. Ascorbic acid was purchased from Spectrochem. Pvt. Ltd. India. Further, the double distilled water was used throughout the experiment and all chemicals were used as received.

Preparation of Graphene Oxide

The modified Hummers method, which is commonly employed, was utilized to extract graphene oxide from natural graphite particles. The utilization of this technique is attributed to the observable intercalation capability of layered graphite. The interstices of crystalline graphite enable the passage of molecules of reactive metals and a limited quantity of oxidizing chemicals. The presence of chemical interaction between functional groups on the surface of graphitic crystal layers was found to result in surface alteration, accompanied by an observed increase in the interlayer distance. The prescribed protocol involved the gradual addition of 30 milliliters of sulphuric acid (H_2SO_4) in a dropwise manner, with simultaneous stirring of a blend of 1.0 gram of natural graphite powder, 0.5 gram of sodium nitrate, and 3.0 grams of potassium permanganate (KMnO_4). Following a duration of 120 minutes of agitation at a temperature of 300°C , a substantial quantity of graphene oxide (GO) was successfully produced from the resultant mixture (Zaaba et al., 2017).

Synthesis of Reduced Graphene Oxide (RGO)

The reduction of graphene oxide was achieved through the utilisation of ascorbic acid as a reducing agent (De Silva et al., 2018). A dispersion was prepared by adding 400 mg of graphene oxide (GO) powder to 400 mL of distilled water, resulting in a concentration of $0.1 \text{ mg} \cdot \text{mL}^{-1}$. Subsequently, a quantity of 4 grams of ascorbic acid (AA) was introduced into the solution and agitated using a magnetic stirrer for a duration of 30 minutes at a temperature of 60°C . The resulting reduced product was then subjected to centrifugation at a rate of 4000 revolutions per second for a period of 40 minutes to eliminate the supernatant. Subsequently, a surplus of

hydrogen peroxide (H_2O_2) with a weight percentage of 30% was introduced to the dark-coloured paste in order to facilitate the oxidation of the residual ascorbic acid. This process involved stirring the mixture for a duration of 30 minutes at a temperature of 60°C . Following agitation, the resulting dark substance was obtained through centrifugation at a speed of 4,000 revolutions per second. It was next subjected to three rounds of washing using ethanol and distilled water, respectively. Finally, the material was dried for a duration of 24 hours at a temperature of 120°C .

Characterization

The structure and Surface Chemistry of the GO and RGO were characterized by X-ray diffractometer (Rigaku Miniflex, Japan) and Fourier infrared spectroscopy (FTIR) ((Bruker, Vector 22)) analysis in the region from $400\text{--}4000\text{ cm}^{-1}$.

Cultivation of Microalgae and Cyanobacteria on RGO Anode

Three Microalgae and Cyanobacterial strains were allowed to grow in RGO anode surface for 10 days with BG11 medium (Ripka et al., 1979; Bhattacharya et al., 2011; Sahu et al., 2015) for Cyanobacteria and bold basal Medium (Preisig, & Andersen, 2005) for *Chlorella vulgaris*.

Photosynthetic Efficiency of Microalgae and Cyanobacteria on RGO Anode

Photosynthetic efficiency of the test organisms was measured in terms of DCPIP dye reduction (Bhattacharyya et al., 2011) assay using UV visible Spectrophotometer. Total Photosynthetic pigment like Chlorophyll a (Mackinney, 1941) and Carotenoid content were measured (Jaiswal et al., 2018) using 80% chilled acetone as a solvent to know the growth status of the Microalgae and cyanobacteria on RGO Anode surface.

Results and Discussion

FTIR Spectroscopy

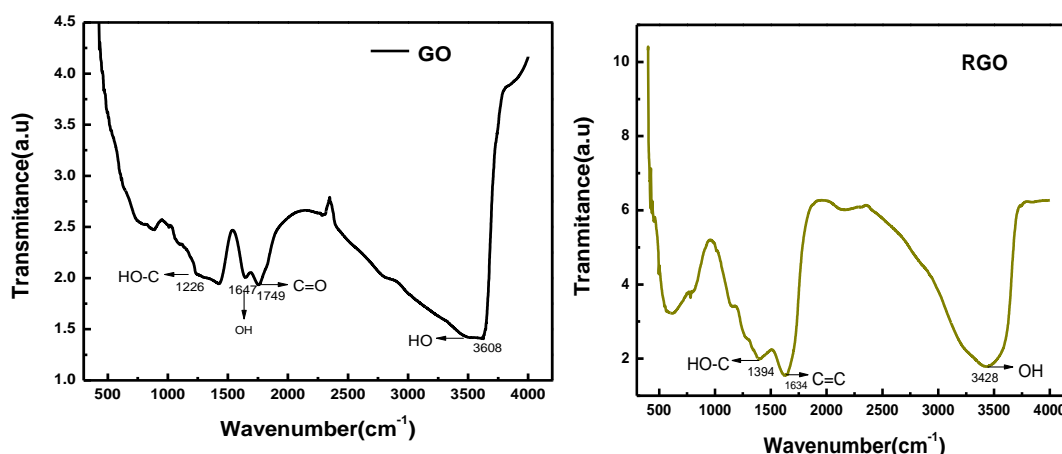


Figure 1. FTIR Spectra of (a) Graphene Oxide and (b) Reduced Graphene Oxide

The investigation focused on evaluating the efficacy of integrating oxygen-containing functional groups into the carbon lattice through the utilization of Fourier transform infrared spectroscopy. In figure 1(a) and (b), it was observed that multiple characteristic modes, each corresponding to a certain functional group such as oxygen, were present. The manifestation of stretching vibration modes of C-O bonds occurs at a wavelength of $1,078\text{ cm}^{-1}$. The peaks observed between $1,190$ and $1,382\text{ cm}^{-1}$ are attributed to the stretching vibration of the C-OH bond (Habte, & Ayele, 2019). The deformation vibration modes of the O-H group were observed at a wavenumber of $1,431\text{ cm}^{-1}$. The hydrophilic properties of graphene oxide are evidenced by the presence of spectral bands observed at a wavenumber of $1,880\text{ cm}^{-1}$. The vibrational frequency of the carboxyl group's C=O stretching was determined to be approximately $1,622\text{ cm}^{-1}$. The presence of peaks at approximately $3,400\text{ cm}^{-1}$ can be attributed to the O-H stretching vibration. The peak corresponding to the C-H stretching vibration was seen at around 2854 cm^{-1} .

The reduction process of graphene oxide to graphene resulted in the elimination of functional groups. The lack of discernible peaks in the Fourier Transform Infrared (FTIR) spectrum (Figure 1(b)) that correspond to specific functional groups. However, the identification of oxygen-containing functional groups, such as C=O and C-O, provides additional evidence supporting the oxidation of graphite into graphene oxide (GO). Graphite

undergoes oxidation, resulting in the formation of graphene oxide (GO), which is supported by the identification of C=C functional groups. However, the fundamental structure of graphite remains intact within the layer.

X-ray diffraction (XRD) Study

The X-ray diffraction patterns of graphene oxide (GO) powder, and reduced graphene oxide (rGO) powder were obtained. The interlayer spacing of graphene oxide (GO) is higher than that of graphite layers due to the formation and introduction of oxygen-containing functional groups between the GO layers (Suhaimin et al., 2022). The presence of these functional groups facilitated the water absorption process and initiated the exfoliation of graphene oxide (GO). Moreover, the X-ray diffraction (XRD) pattern of graphene oxide (GO) (as depicted in Figure 2(a)) exhibits a distinct reflection peak at around 43° , indicating the presence of turbostratic disorder (Kokmat et al., 2023). This disorder is commonly associated with incomplete oxidation processes. Furthermore, the design lacked uniformity. One potential explanation is that the GO layers exhibit a high degree of proximity, resulting in a densely packed arrangement. The decrease in the concentration of GO was additionally verified using X-ray diffraction (XRD) research. In accordance with the findings presented in Fig 2(b), it is evident that the interlayer spacing for reduced graphene oxide (rGO) measures a mere 0.37 nm, but for graphene oxide (GO), it was determined to be 210° (as indicated by the diffraction peak of GO) (Wen et al., 2020). The reduction process of graphene oxide (GO) involves the removal of oxygen-containing functional groups and the subsequent aggregation of reduced graphene oxide (rGO) sheets. This reduction process is responsible for the observed decrease in the interlayer distance of rGO compared to GO. The X-ray diffraction (XRD) pattern of reduced graphene oxide (rGO) exhibits the presence of minor peaks, as visually observed in the accompanying image. The potential modification of the rGO sheets' structure can be attributed to the existence of residual functional groups.

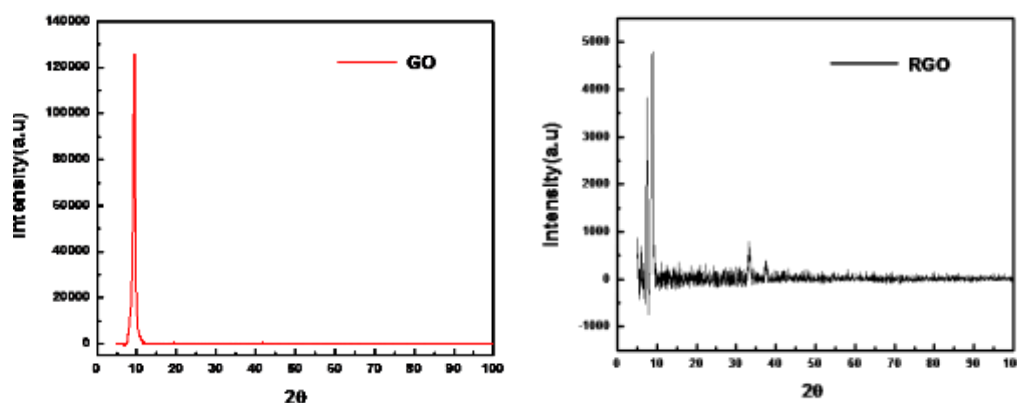


Figure 2. X-ray diffraction (XRD) pattern of (a) GO and (b) RGO

Estimation of Major photosynthetic Pigments

Photosynthesis is the fundamental biological process through which these organisms harness the energy of sunlight to convert carbon dioxide and water into glucose and oxygen (Brown and Zeiler 1993), with the help of sunlight. Chlorophyll *a* (Chl *a*) is the primary pigment responsible for photosynthesis in microalgae and cyanobacteria (Björn et al., 2009). Chlorophyll *a* play a critical role in capturing and transferring light energy for this process. Chlorophyll *a* accumulation patterns observed in *Chlorella vulgaris*, *Gloeocapsa*, and *Synechocystis* reveal varying responses of these microorganisms over the 32-day period (Figure3.). *Chlorella vulgaris* shows a steady increase over the 32-day period. This pigment accumulation appears to be relatively consistent with a slight decrease between 28th and 32nd. *Synechocystis* also shows a continuous growth pattern over the 32-day period. However, a less pronounced increase of Chlorophyll *a* was observed in *Gloeocapsa*. This pigment absorbs light most effectively in the red and blue regions of the electromagnetic spectrum (Shevela et al., 2023; Wasielewski et al., 1989), making it a key molecule for efficient light harvesting in Photosystem II (PSII) photochemistry, specifically in capturing light energy and initiating the electron transport chain. The electron transport chain in PSII is critical for the conversion of light energy into chemical energy and the production of adenosine triphosphate (ATP) and oxygen (Qi et al., 2023; Messinger, & Shevela, 2012). Kinetic of Chl *a* in BPV is pivotal because it is the primary pigment responsible for capturing light energy and initiating the photosynthetic electron transport chain (Tschörtner et al., 2019; Thong et al., 2023). This allows for the conversion of sunlight into electrical energy (Sekar, & Ramasamy, 2015), making BPV a

sustainable and environmentally friendly approach to energy production. Chlorophyll a and *Synechocystis* shows better photosynthesis during growth in RGO Anode.

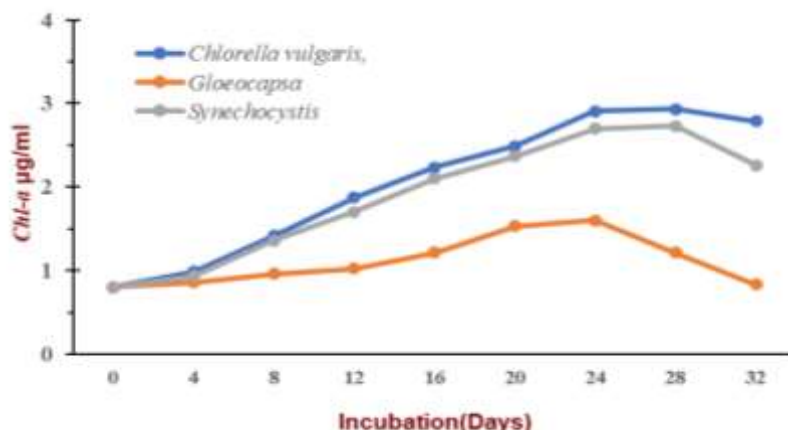


Figure 3. Changes in Chl-a content in three Microalgae and Cyanobacteria grown on RGO Anode during experimental conditions

Carotenoids are accessory pigments in the photosynthetic machinery, and they are particularly important for their photoprotective and structural functions in the photosynthetic apparatus. Carotenoids play several essential biochemical roles in photosynthesis (Hashimoto et al., 2016), including their involvement in photoprotection (Britton 2008) and facilitating the process of photosynthetic electron transport, which ultimately contributes to water splitting in photosystem II (PSII). The Carotenoid (car) content of *Chlorella vulgaris*, *Gloeocapsa*, and *Synechocystis* over a 32-day period on RGO anode surface were measured (Figure 4). Total car of all the alga exhibited a steady increase over the 32-day period. *Gloeocapsa* and *Synechocystis*, shows less pronounced pigment accumulation as compared to *Chlorella vulgaris*.

In a Bio-Photovoltaics (BPV) experiment using cyanobacteria, carotenoids play a multifaceted role in enhancing the overall efficiency and performance of the BPV system (Zhang, & Reisner, 2020). Microalgae and Cyanobacteria are photosynthetic microorganisms, and their ability to harness light energy for photosynthesis is a key component of BPV. Being a major antenna pigment, this can indirectly contribute to the flow of electrons from the photosystems to the electrode, thus facilitating the generation of electrical energy in BPV. Carotenoids also help protect photosynthetic organism from photodamage, thereby increasing their long-term viability in BPV systems. This is essential for maintaining consistent energy production over extended periods. It can enhance the tolerance of cyanobacteria to various stressors, including environmental factors, which can improve the strength and efficiency of the BPV system.

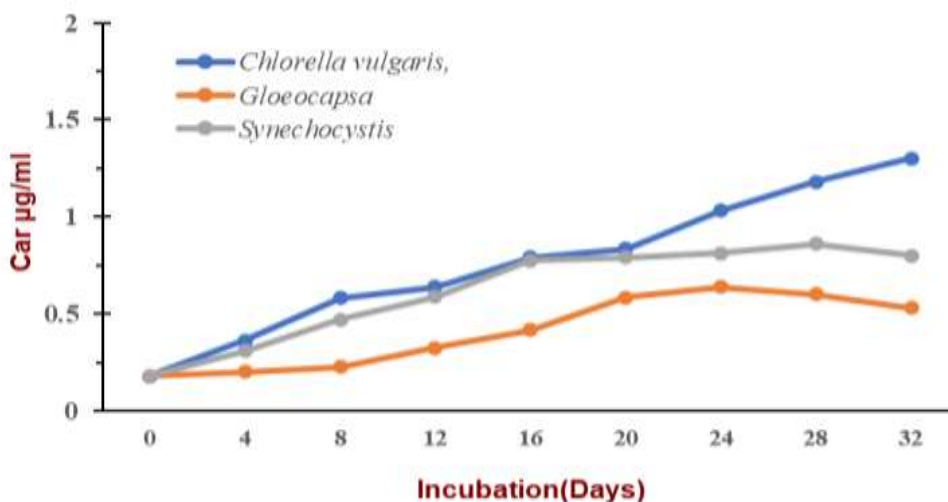


Figure 4. Changes in total Carotenoid in three Microalgae and Cyanobacteria grown on RGO Anode during experimental conditions

Photosynthetic Electron Transport Ability

The measurement of DCPIP reduction is pivotal in Bio-Photovoltaics (BPV) as it serves as a fundamental tool for quantifying electron transfer and photosynthetic efficiency within microorganisms employed in BPV systems. This assay allows for the quantitative assessment of the capacity of photosynthetic organisms to convert light energy into chemical energy, facilitating the comparison of different species and the optimization of growth conditions (Bennett. Et al., 1982). Fig 5 represents electron transport ability of PS-II in terms of 2,6-dichlorophenolindophenol (DCPIP) dye reduction among three different microorganisms, *Chlorella vulgaris*, *Gloeocapsa*, and *Synechocystis*, over a 32-day period on RGO anode. The reduction of DCPIP by *Chlorella vulgaris* shows a substantial increase over the 32-day period. The rate of DCPIP reduction accelerates significantly, indicating heightened electron transfer activity. A consistent electron transfer activity was observed in *Synechocystis*. However, DCPIP reduction of *Gloeocapsa* was comparatively lower than other two alga. A significant declines of electron transport activity of PS-II was observed after day 28th. DCPIP reduction provides insights into the electron transfer activity associated with the photosynthetic processes of *Chlorella vulgaris*, *Gloeocapsa*, and *Synechocystis*. *Chlorella vulgaris* and *Synechocystis* appear to have optimal conditions, which is reflected in their sustained and even accelerating DCPIP reduction rates. *Gloeocapsa* also shows initial growth but experiences a decline, may be indicative of photoinhibition.

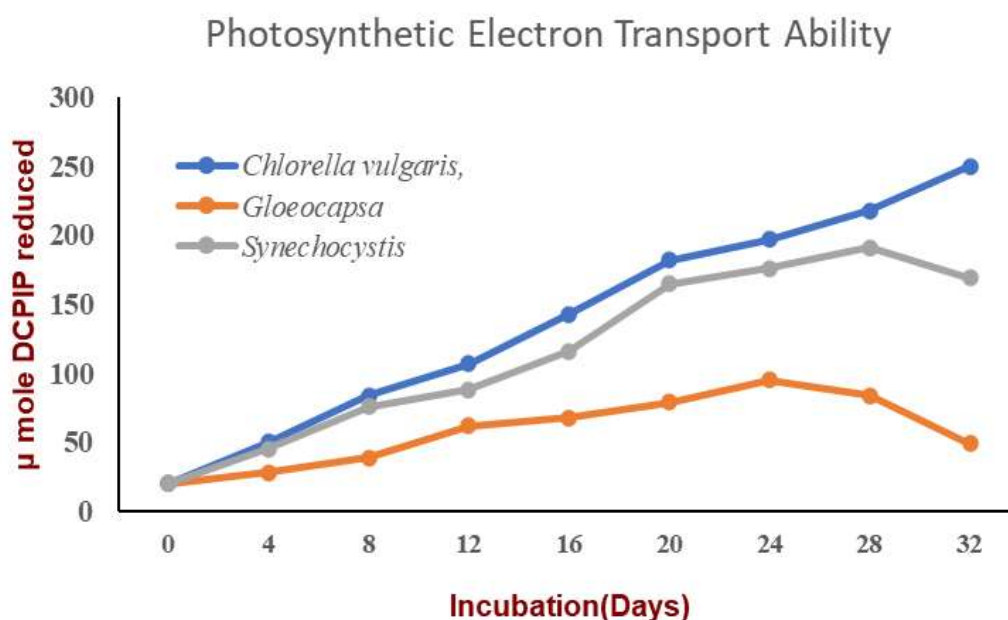


Figure 5. Changes in Photosynthetic Electron transport ability of PS-II of three Microalgae and Cyanobacteria grown on RGO Anode during experimental conditions

The measurement of DCPIP (2,6-dichlorophenolindophenol) reduction holds substantial scientific significance in Bio-Photovoltaics (BPV) research, primarily due to its role in quantifying photosynthetic electron transfer and the overall efficiency of energy conversion. In BPV systems, the reduction of DCPIP serves as a robust proxy for assessing the functionality and health of photosynthetic microorganisms, as it directly correlates with their ability to transfer electrons during the light-dependent reactions. Notably, DCPIP reduction assays have been widely adopted in photosynthesis research Joliot and Joliot 1999. DCPIP reduction measurements have broader applications in tracking redox reactions (Allakhverdiev, & Klimov, 1992), emphasizing their reliability and value in BPV investigations.

The results from the DCPIP reduction measurements in the context of Bio-Photovoltaics (BPV) indicate the photosynthetic electron transfer efficiency of three microorganisms *Chlorella vulgaris*, *Gloeocapsa*, and *Synechocystis*. These findings have direct implications for their suitability in BPV applications. *Chlorella vulgaris* demonstrates a significant increase in DCPIP reduction over the 32-day period, suggesting better and sustained electron transfer, making it a promising candidate for long-term energy generation. *Gloeocapsa* exhibits efficient electron transfer in the initial stages, but a subsequent decline may necessitate further optimization for extended BPV applications. *Synechocystis* maintains steady electron transfer throughout the experiment, rendering it a stable choice for consistent energy production. Among the three Organisms

Chlorella vulgaris has efficient and consistent photosynthetic electron transfer capabilities in BPV systems to maximize electrical energy generation.

Power density measurements

The power density measurements, expressed in milliwatts per square meter (mW/m^2), reveal the energy-generating potential of *Chlorella vulgaris*, *Gloeocapsa*, and *Synechocystis* under varying light conditions, offering insights into their suitability for Bio-Photovoltaics (BPV) and bioenergy applications. *Chlorella vulgaris* shows more energy conversion under light, with a notable power density of 0.258 mW/m^2 (Figure 6.), and minimum energy production in the dark, shows its metabolic adaptability (Chisti, 2008). *Gloeocapsa* and *Synechocystis* also displayed efficient energy output under light, but with varying capabilities in the dark. These results indicate the performance of *C. vulgaris* having optimal energy output hence can be utilize for BPV applications. They also highlight the potential of these microorganisms to contribute to sustainable energy generation, both in the presence and absence of light, which has practical implications for BPV system design and operation. The power density measurements indicate the ability of *Chlorella vulgaris*, *Gloeocapsa* and *Synechocystis* to convert light energy into electrical energy, demonstrating their suitability for sustainable energy generation. *Chlorella vulgaris*, known for its better performance under both light and dark conditions, presents a versatile choice for BPV (Chisti, 2007, Larkum et al., 2016). Our findings contribute to the advancement and enhanced viability of BPV technology by selecting suitable microorganisms with the highest energy output efficiency, (Dismukes et al., 2008).

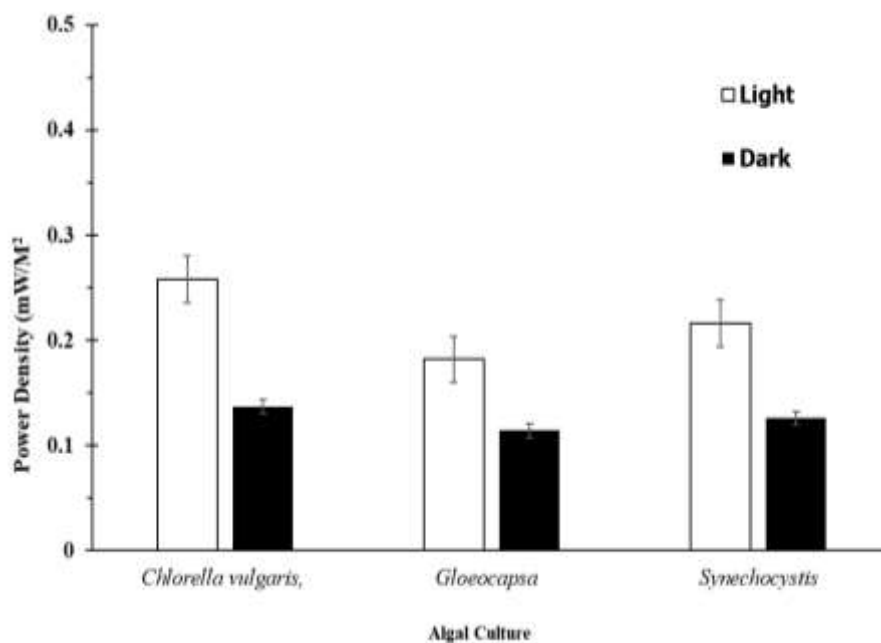


Figure 6. Power Density (mW/m^2) of *Chlorella vulgaris*, *Gloeocapsa*, and *Synechocystis* under light and Dark conditions.

Conclusion

Our study encompassing chlorophyll content, carotenoid content, DCPIP reduction, and current power density measurements, collectively shed light on the photosynthetic performance of three microorganisms, *Chlorella vulgaris*, *Gloeocapsa*, and *Synechocystis*. These findings are instrumental for comprehending the capabilities of these microorganisms in the context of Bio-Photovoltaics (BPV) and renewable energy production. Firstly, the chlorophyll content data provides insights into the photosynthetic pigment composition of the microorganisms. Chlorophyll, as the primary pigment of photosynthesis, plays a pivotal role in capturing light energy and initiating electron transport. The variation in chlorophyll content among the three species reflects their adaptation to different environmental conditions, with *Chlorella vulgaris* exhibiting the highest chlorophyll content. This suggests its potential for efficient light absorption and utilization, making it a promising candidate for BPV applications. Carotenoids, as accessory pigments, have multifaceted roles in photosynthesis. They aid in light absorption, photoprotection, and structural support for photosynthetic complexes. Result shows significant variation in carotenoid contents among the microorganisms, with *Gloeocapsa* showing the highest carotenoid content. This suggests its potential for enhanced photoprotection

and stability in BPV systems. The presence of abundant carotenoids may help mitigate the harmful effects of excess light, safeguarding the photosynthetic apparatus during periods of high irradiance. Electron transport activity of PS-II in terms of DCPIP reduction data reveals the electron transfer activity and photosynthetic efficiency of these microorganisms. *Chlorella vulgaris* demonstrates a significant increase in DCPIP reduction over the experimental period, showcasing its excellent electron transfer capacity under varying light conditions. The current power density measurements provide a direct assessment of the energy generation capacity of these microorganisms under light and dark conditions. *Chlorella vulgaris* demonstrates a commendable ability to convert light energy into electrical energy, as evidenced by its high-power density.

In conclusion, our findings encompassing chlorophyll content, carotenoid content, DCPIP reduction, and current power density measurements, provides a holistic view of the photosynthetic and energy production potential of *Chlorella vulgaris*, *Gloeocapsa* and *Synechocystis*. These findings not only advance our understanding of their photosynthetic capabilities but also offer valuable insights into their suitability for BPV and renewable energy applications. These findings show the way for further research to harness the full energy-producing potential of these microorganisms, thereby advancing the development of environmentally sustainable bioenergy systems, including BPV, to meet our growing energy needs.

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Conflict of Interest: Authors do not have any conflict of interest

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