

Microbial Fuel Cell as Microalgae Bioreactors for Carotenoids and Power Generation

MFCs have gained significant prominence as bio-electrochemical systems, finding applications in power generation, wastewater treatment, CO₂ capture, biosensors, and resource recovery [Meylani *et al.*, 2023; Sharma and Chhabra, 2023; Sharma *et al.*, 2023]. Recently, a significant interest has been shifted to employing PMFCs for microalgae biomass production, and extraction of valuable pigments and lipids, as well as the efficient reduction of oxygen at the cathode [Ahirwar *et al.*, 2023]. Various microalgae species, including *C. vulgaris*, *C. reinhardtii*, and *D. salina* have been investigated within the PMFC anode/cathode chambers, showcasing their potential contributions [Sharma and Chhabra, 2021; Mishra and Chhabra, 2022]. In our previous study, *C. reinhardtii* demonstrated remarkable performance in power generation and the production of valuable microalgae biomass at the cathode [Sharma and Chhabra, 2021]. However, despite their considerable potential, the practical implementation of MFCs on a large scale faces substantial challenges [Tong *et al.*, 2023]. MFC performance can be augmented by different means, which are broadly categorized into two domains: electrochemical modifications and biochemical advancements. Biochemical advancements in PMFCs can improve their applications through microalgae engineering increased by their biotechnological applications.

GM strains have been created through algal transformation to yield value-added compounds such as lipids, carotenoids, and vaccines [Cao *et al.*, 2023]. *C. reinhardtii* is a promising candidate for genetic manipulation due to its haploid genotype, well-annotated genome, and available genetic toolkits [Kong *et al.*, 2023]. For instance, a GM strain of *C. reinhardtii*, designed to overproduce ferredoxins, was introduced into the MFC anode chamber, improving electron transfer efficiency, starch synthesis, and lipid production [Huang *et al.*, 2015]. Our previous study showcases the augmentation of β -carotene production through the nuclear transformation of *C. reinhardtii* [Sharma *et al.*, 2024a].

More studies must be conducted to compare GM microalgae and their WT counterparts within PMFC systems. Hence, it is essential to investigate the performance of PMFCs using these modified strains to potentially integrate them into a biorefinery framework, thereby enhancing resource recovery. Consequently, this study aims to establish a proof of concept for globally scalable GM microalgae-based PMFCs for bioproduct and power generation applications. In this order, the present study incorporates *C. reinhardtii* GM and WT strains at the PMFC cathode and compares their performance. The primary objective of this study is to assess bioproducts and bioelectricity generation using PMFC. The study will evaluate various MFC performance metrics, including power generation and wastewater treatment efficiency. Factors such as microalgae growth rate, biomass productivity, and β -carotene production will also be investigated.

6.1 EXPERIMENTAL

In this study, we used dual-chamber acrylic MFC configurations, with both the anode and cathode chambers having a working volume of 100 ml and a total capacity of 120 ml each). A fixed resistor of 1000 Ω was used to close the circuit and enable the current flow. A 10 ml of pre-treated cow manure was added as an inoculum into anode chamber [Vijay *et al.*, 2016], while the cathode was inoculated with different microalgae strains as specified previously [Sharma and

Chhabra, 2021]. The cathode chamber was supplied with TAP media with/without hygromycin B as per the dose in the antibiotic selection medium. The anodic off gases from anode chamber were supplied to cathode chamber which may attribute to high growth of microalgae [Khandelwal *et al.*, 2018; Das *et al.*, 2023]. The organic substrates at the anode chamber were supplied with grey water, which also functions as an anolyte. The grey water supplementation in the anode chamber was implemented to introduce complex substrates, aiming to replicate realistic experimental conditions. A total of four MFC reactors having different cathodic cultures were prepared where Systems 1, 2, 3, and 4 consist of *C. reinhardtii* WT CC-503, WT CC-125, GM 1 (G4), and GM 2 (E1), respectively at the cathode chamber. The GM microalgae strains G4 and E1 had higher β -carotene productivity due to nuclear transformation as described in Chapter 5. All the reactors were inoculated with similar cell densities and later operated in a fed-batch mode by replacing half of the cathodic culture with fresh media. The anode cycle was maintained for 4-5 days while the cathode media changed over a 10 to 15-day period (with the supplementation of hygromycin for maintaining GM microalgae). Steam autoclaving/sterilization was followed for decontamination of all biological samples. All the GM microalgae cultivation was carried out in closed systems.

The MFC systems were maintained in an algal incubator (Waiometra) at $25 \pm 2^\circ\text{C}$ under a light intensity of 2200-2300 lx, following a 16:8 h light-dark cycle. The study was conducted for 85 days, during which the systems operated in open circuit conditions for the first 15 days before resistors were introduced to close the circuit. For control experiments, microalgae were grown in transparent Erlenmeyer flasks with a 250 ml capacity and a working volume of 100 ml where the atmospheric CO_2 was available. All the flasks and MFC systems were manually shaken intermittently thrice a day. The outline of the present study is shown in Figure 6.1.

The soluble COD, nitrates, and pH were monitored regularly, and variations in the different batches were reported in this study. The COD measurement was done using photometric method while nitrates in greywater were measured using a Jal TARA water testing kit. For MFC performance analysis, the OCV and CCV of each system were recorded manually three times a day. Electrochemical characterization was done using the CV technique. In addition, the microalgae growth rate and biomass productivity of different systems were estimated and compared with flask-grown microalgae cultures under similar conditions. Pigment analysis was done using the HPLC.

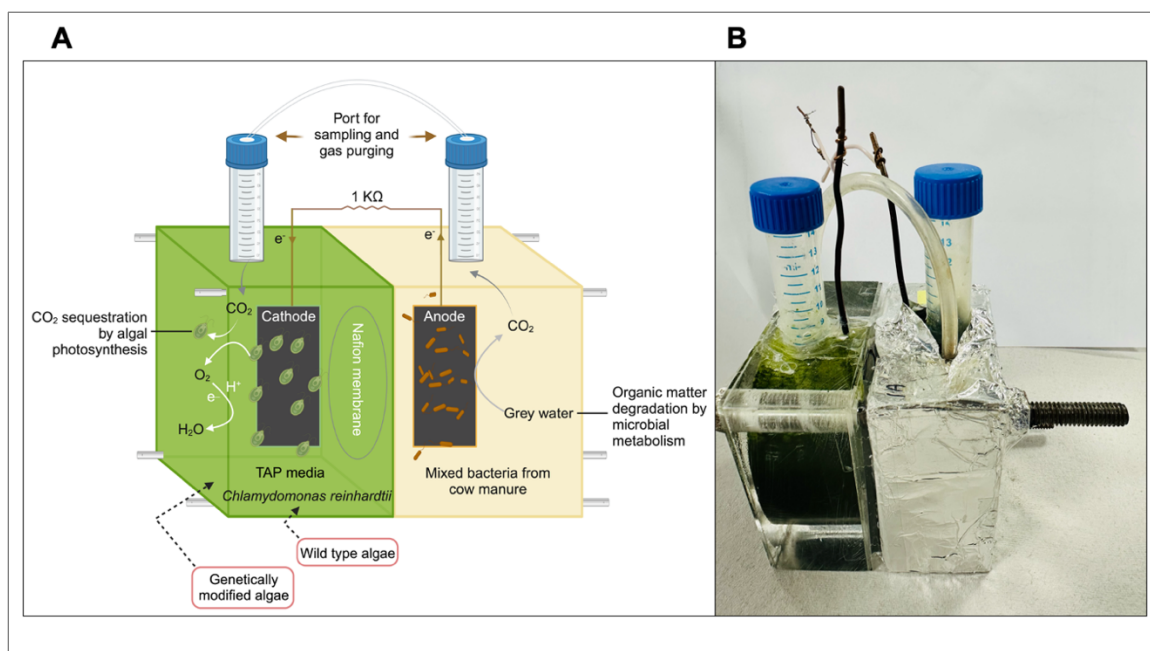


Figure 6.1 : The schematic shows A) reactor design and B) real image of PMFC used in this study

6.2 RESULTS AND DISCUSSION

6.2.1 Voltage Generation

The PMFC performance was characterized in terms of voltage, power, and current generation. All the systems were kept in an open circuit for 15 days before connecting to an external resistance of 1000 Ω . The OCVs for systems 1, 2, 3, and 4 are 622.5 ± 24.75 mV, 511 ± 31.11 mV, 473 ± 16.97 mV, and 508 ± 67.15 mV, respectively. The total operation time for this study was 85 days. The closed-circuit voltage at 1000 Ω for each system is depicted in Figure 6.2. The study suggests that the system voltage output was affected by batch-to-batch variation in wastewater composition. The maximum closed-circuit voltage reached 460.5 ± 9.19 mV, 441.5 ± 19.08 mV, 411 ± 50.2 mV, and 452 ± 43.14 mV, for systems 1, 2, 3, and 4, respectively. This data suggests that there were no statistically significant differences (p-values > 0.05) in maximum voltage output from different systems irrespective of the *C. reinhardtii* strains used. Therefore, it is crucial that GM microalgae perform similarly to WT microalgae with similar electrode pre-treatment, and this could strengthen their application in PMFCs for biorefinery applications.

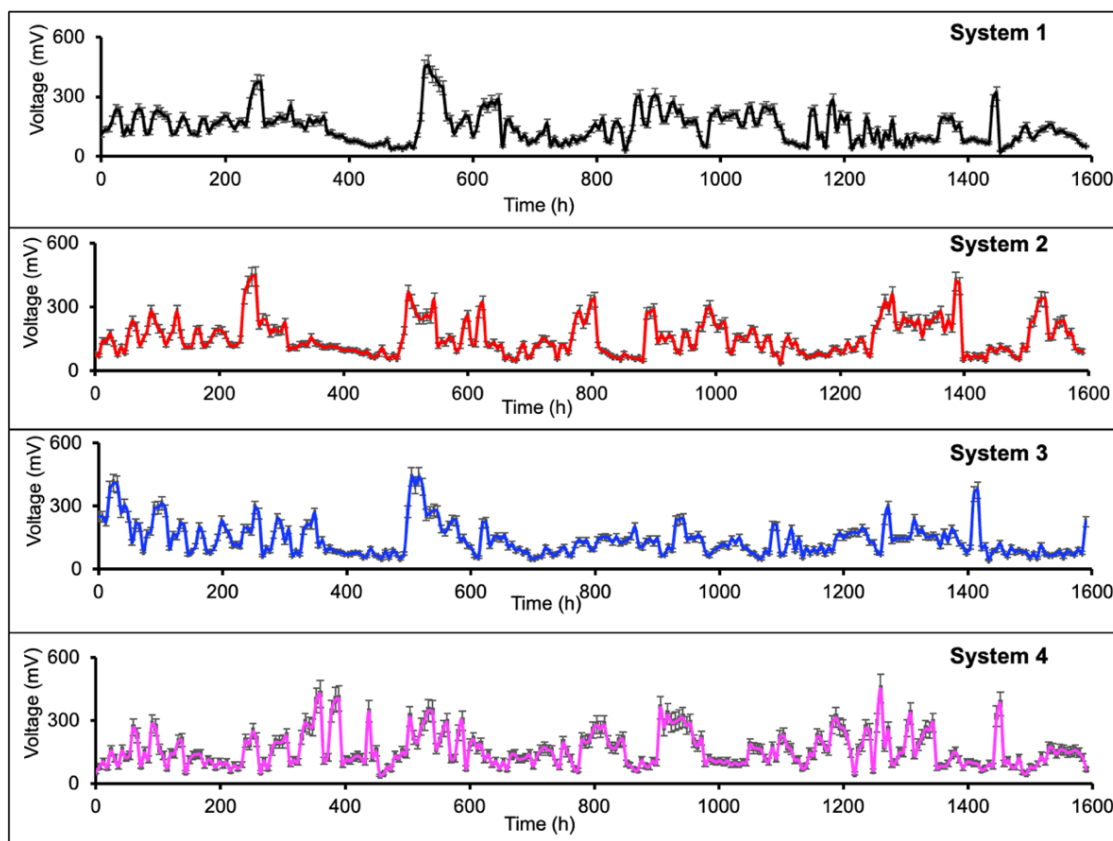


Figure 6.2 : The schematic shows the closed-circuit voltage (mV) vs. time (h) pattern during 85 days of operation for different MFC systems

6.2.2 COD Removal and Coulombic Efficiency

MFCs are compelling systems for wastewater treatment with high COD removal rates [Logan *et al.*, 2006]. To check the COD removal efficiency at the anode chamber, all the systems were supplied with grey water without prior treatment having a COD of 650-1100 mg/l (pH: 7.2 - 7.3). The grey water was collected fresh on the day of the next batch cycle. All the systems were efficient in wastewater treatment, and COD removal for each system is shown in Table 6.1. The maximum COD removal rate for different MFC systems including System 1, System 2, System 3, and System 4 are 161.49 ± 52.79 Kg/m³/d ($71.07 \pm 21.95\%$), 164.34 ± 32.35 Kg/m³/d ($73.67 \pm 19.88\%$), 160.24 ± 32.52 Kg/m³/d ($75.88 \pm 15.56\%$), and 152.19 ± 22.85 Kg/m³/d ($78.95 \pm 11.77\%$), respectively as shown in Figure 6.3. Therefore, this study has recommended that all *C. reinhardtii* strains, including WT and GM microalgae, are found suitable for cultivation at the cathode chamber of MFC in terms of COD removal through anodic degradation and stand well with the

existing systems [Sharma and Chhabra, 2021; Ahirwar *et al.*, 2023]. The fluctuations in different fed-batch cycle may occur due to the real wastewater application in long-term operation as well as microbial diversity of the anodic bacteria in complex substrates, which may be investigated in detail.

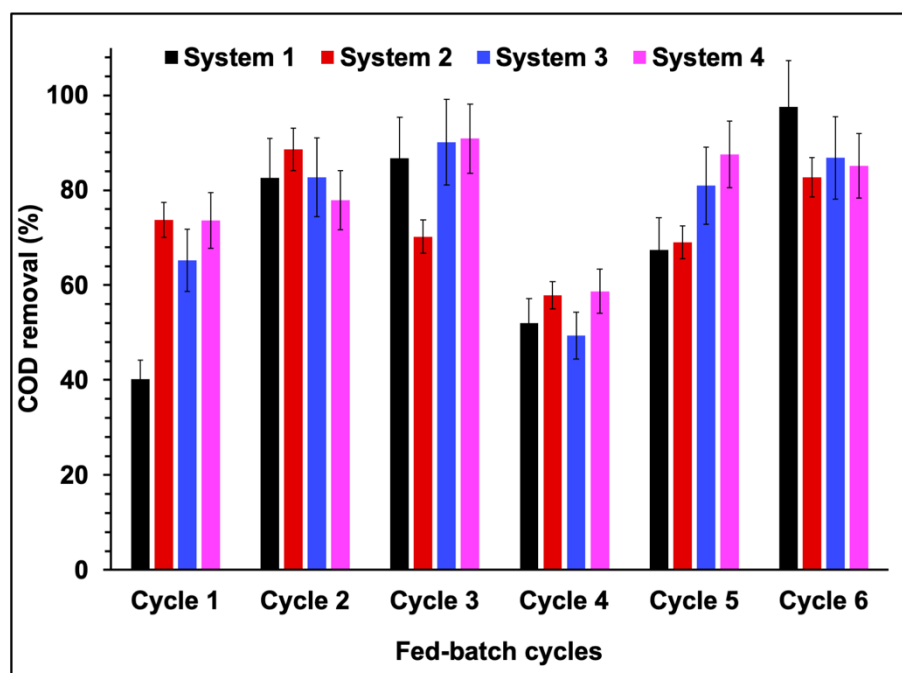


Figure 6.3 : The schematic shows the COD removal (%) in different fed-batch cycles in Systems 1-4

The CE for System 1, System 2, System 3, and System 4 are $9.77 \pm 0.19\%$, $9.05 \pm 1.11\%$, $10.84 \pm 0.11\%$, and $8.59 \pm 0.27\%$, respectively. These results have suggested the effective performance of System 3 consisting of GM 1 microalgae. The CE of MFC can be improved further by electrode modification with catalyst coating, reducing the internal resistance, and altering the system configuration [Logan *et al.*, 2006; Santoro *et al.*, 2017]. The nitrate levels were below 10 ppm in the collected greywater. The PMFCs have shown high efficiencies for nitrate removal from wastewater through algae cultivation in previous studies [Vijay *et al.*, 2022]. In PMFCs, nitrate and nitrite are not only assimilated by algae but can also be eliminated as electron acceptors at the cathode. However, the present study has utilized a microalgae cultivation medium in the cathode chamber; therefore, the study has only reported the anodic COD removal.

6.2.3 Microalgae Growth at Cathode Chamber- WT v/s GM Microalgae

The present study analyzes the impact of GM microalgae cultivation on MFC performance and vice versa. To examine the microalgae growth in different systems, all the systems were inoculated with the same initial cell density, and further growth was monitored using absorbance at 750 nm, which is reciprocal of the cell number for microalgae species [Ahmad *et al.*, 2015] as shown in Figure 6.4A. Literature studies have reported enhanced microalgae growth when cultivated in an MFC cathode chamber [Das *et al.*, 2023]. Therefore, the microalgae growth in different MFC systems was compared with the microalgae grown in a 250 ml Erlenmeyer flask, with intermittent shaking. The results were in line with the previous PMFC studies that the cathode-grown microalgae have higher growth rate, and productivity as can be seen in Figure 6.4B as well [Shukla and Kumar, 2018; Sharma *et al.*, 2023]. The specific growth rate achieved through different systems is $0.246 \pm 0.043 \text{ d}^{-1}$, $0.202 \pm 0.012 \text{ d}^{-1}$, $0.205 \pm 0.008 \text{ d}^{-1}$, and $0.174 \pm 0.007 \text{ d}^{-1}$ for System 1, System 2, System 3, and System 4, respectively. The microalgae biomass achieved through the cultivation in the MFC cathode for System 1, System 2, System 3, and System 4 is $0.33 \pm 0.06 \text{ Kg/m}^3/\text{d}$, $0.23 \pm 0.08 \text{ Kg/m}^3/\text{d}$, $0.26 \pm 0.07 \text{ Kg/m}^3/\text{d}$, and $0.18 \pm 0.02 \text{ Kg/m}^3/\text{d}$, respectively.

Various researchers are altering the microalgae through genetic/metabolic engineering to improve the yield of target metabolites such as lipids, proteins, and other high-value products, including pigments. While these strains produce a high titer of the desired end product, the productivity declines due to slower cell growth [Chen *et al.*, 2023; Sun *et al.*, 2023]. PMFCs enhance algae growth by providing more suitable conditions, such as low DO and continuous CO₂ supply. The movement of CO₂/H⁺ into the cathode chamber also helps keep the pH static, which would otherwise turn alkaline upon algae growth [Daneshvar *et al.*, 2022]. Therefore, PMFC cathodes favor algae cell growth and compensate for the drop in non-MFC cultures. The MFCs' environment is helping microalgae grow faster than the flask-grown cultures by supplying anodic-off gases as well as removing the DO, which support their application as microalgae bioreactors.

Also, the closed cultivation in the MFC cathode chamber helps in the biocontainment of GM microalgae [Sebesta *et al.*, 2022.] Currently, biosafety guidelines recommend cultivating GM microalgae in closed systems due to the presence of antibiotic-resistance genes as selection markers. Consequently, to mitigate the potential escape of foreign genes via horizontal gene transfer, our study employs PMFC-based closed cultivation systems. Nonetheless, measures can be implemented within open cultivation systems to mitigate risks associated with these GM microalgae. For instance, a constructed wetland MFC system could be encircled by a water buffer zone, reducing direct contact with the surrounding environment [Kumar, 2015; Nadzri *et al.*, 2023].

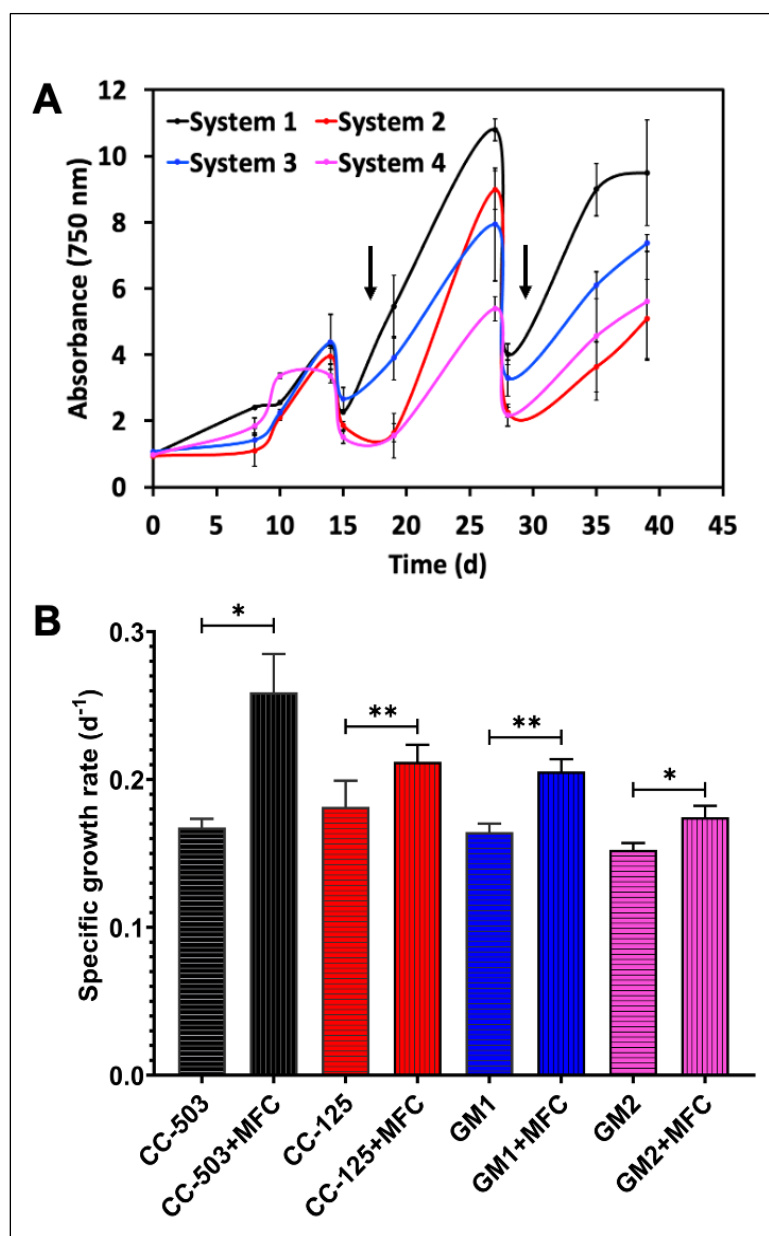


Figure 6.4 : The schematic shows A) the microalgae growth curve for different MFC systems and B) the specific growth rate compared for flask and MFC cathode chamber grown *C. reinhardtii*. Here, * means p-value < 0.05, ** means p-value < 0.01

6.2.4 Dissolved Oxygen at Cathode Chamber

The DO plays an important role in MFC function as anaerobic conditions are required at the anode, while the cathode chamber may/may not have oxygen. DO functions as an electron acceptor in the cathode, facilitating electron transfer from the anode and stabilizing electrogenesis. Therefore, it reduces the system's reliance on mechanical aeration or chemical electron acceptors and helps in sustainable operations at scaled-up applications. The DO concentration in all the MFC systems was monitored, and minor changes were observed in daytime DO at the cathode chamber. The daytime DO for MFC Systems 1 to System 4 are 10.17 ± 3.28 mg/l, 9.32 ± 2.54 mg/l, 11.32 ± 1.42 mg/l, 8.13 ± 2.36 mg/l, respectively, which indicates the ideal behavior of catholyte and resulted in the high bioelectricity generation. However, the nighttime DO levels were dropped below 6 mg/l at the MFC cathode chamber, lowering of operational voltage. A similar trend was observed in the DO when *Chlamydomonas* sp. and *Scenedesmus* sp. were introduced into the MFC cathode chamber to generate bioelectricity [Nadzri *et al.*, 2023]. While the anolyte was consistent across all reactors, variations in biological systems may occur, given the erratic flow of electron donors influenced by biotic and abiotic factors.

In contrast, the flask-grown microalgae cultures have shown DO levels above 15 mg/l in the daytime. Previous studies have shown that DO in high-density algae cultures reach supersaturated levels due to photosynthesis [Gao *et al.*, 2022]. Therefore, photobioreactors are equipped with deoxygenation accessories, or degasification is done through nitrogen gas to maintain safe DO levels in high-density algae cultures. Hence, MFC-based cultivation offers unique deoxygenation to achieve high-density microalgal cultures [Li *et al.*, 2019]. Therefore, microalgae derived DO is a viable electron acceptor due to its high oxidation potential, availability, sustainability, and affordability.

6.2.5 β -carotene Production

For the synthesis of β -carotene from microalgae, various strategies have been established, including media engineering and stress induction. It is still necessary to select a suitable bioreactor type, strain development, and trophic mode for high biomass and pigment productivity, as batch culture production is not yet adequate for commercial scale [Zittelli *et al.*, 2023]. Previous studies have reported the influence of the fuel cell environment on higher carotenoid production [Gouveia *et al.*, 2014]. Our previous study has also demonstrated that cathode-grown microalgae have high antioxidant, high phenolic activity, and antibacterial properties [Sharma and Chhabra, 2021]. Therefore, this study has built a microalgae MFC-based bioreactor to accelerate β -carotene production.

Previous studies have reported the limitations to use cell wall-less microalgae strains (*C. reinhardtii* WT CC-503) at scaled-up cultivation due to shear stresses or mechanical abruptions [Wang and Lan, 2018]. Hence, a cell-walled *C. reinhardtii* CC-125 (WT) was engineered for carotenoid production as carotenoid content in the *C. reinhardtii* CC-503 (WT) strain was comparatively lesser which is a wall-less strain. This study has also compared the β -carotene production in different cultivation systems, which is in the MFC cathode chamber and Erlenmeyer flask, with each having a 100 ml working volume. The GM and WT microalgae biomass from both cultivation systems were harvested, and their pigment concentration was examined. A concentration range of 2-50 μ g/ml β -carotene was used to prepare the standard regression equation, which is as follows: The linear regression equation obtained was: $y=31895x - 48263$, $R^2 = 0.9973$, where x is retention time, and y is peak area.

The β -carotene productivities in different MFC systems 1- 4 are 1.42 ± 0.07 mg/g DCW, 1.55 ± 0.05 mg/g DCW, 3.26 ± 0.20 mg/g DCW, 3.88 ± 0.14 mg/g DCW, respectively while the flask-grown microalgae cultures have shown a productivity of 0.43 ± 0.05 , 0.57 ± 0.02 , 1.53 ± 0.08 , and 1.58 ± 0.24 , for *C. reinhardtii* WT CC-503, WT CC-125, GM 1, and GM 2, respectively. It was observed that all the systems containing *C. reinhardtii* WT CC-503, WT CC-125, GM 1, and GM 2, have shown high β -carotene production in MFC when compared with the flask-cultivated microalgae under similar conditions. The HPLC chromatograms for various conditions are shown in Figures 6.5 – 6.12. The maximum β -carotene production was found in MFCs with GM 1 and GM 2 at the cathode in Systems 3 and 4, respectively (Figure 6.13). These results were aligned with our previous study which has shown high total carotenoids in MFC-grown biomass [Angioni *et al.*, 2018; Sharma and Chhabra, 2021].

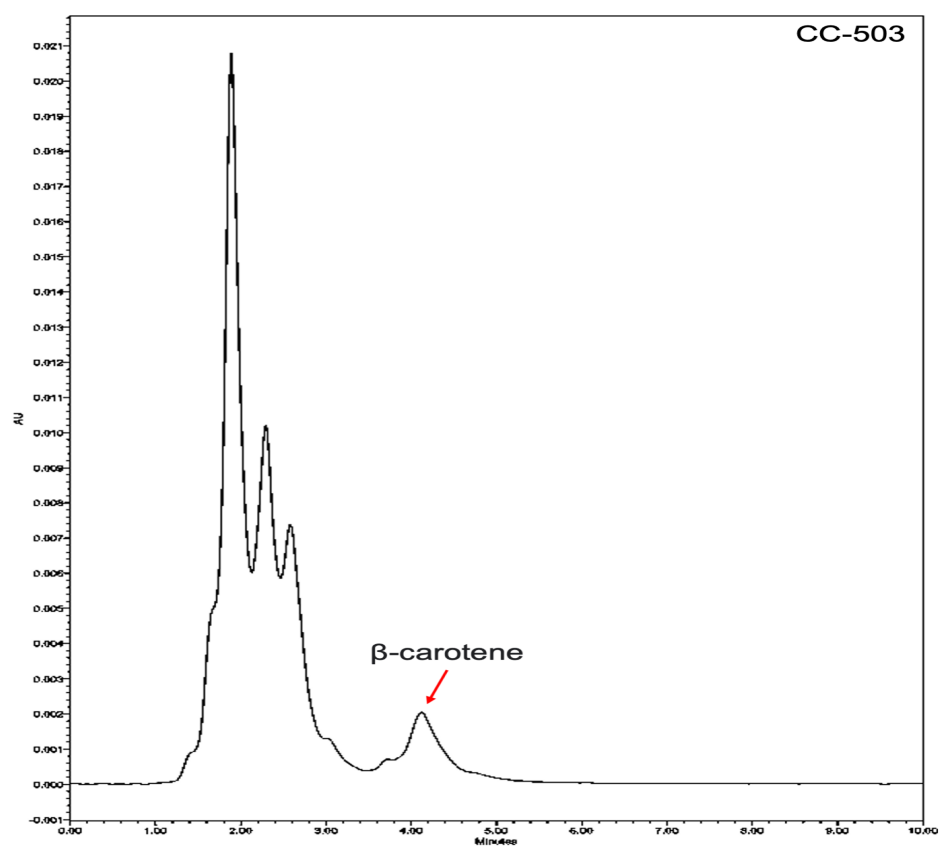


Figure 6.5 : HPLC chromatogram of *C. reinhardtii* CC-503 grown in Erlenmeyer flask showing peak arbitrary values and retention time (min)

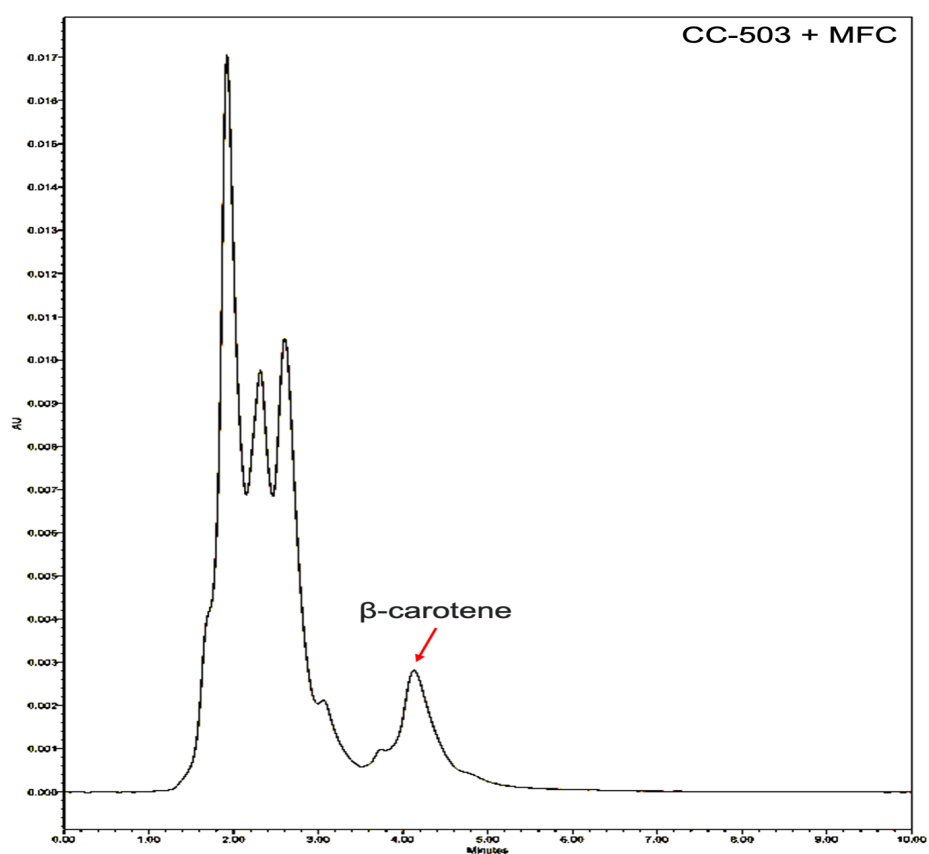


Figure 6.6 : HPLC chromatogram of *C. reinhardtii* CC-503 grown in MFC cathode chamber showing peak arbitrary values and retention time (min)

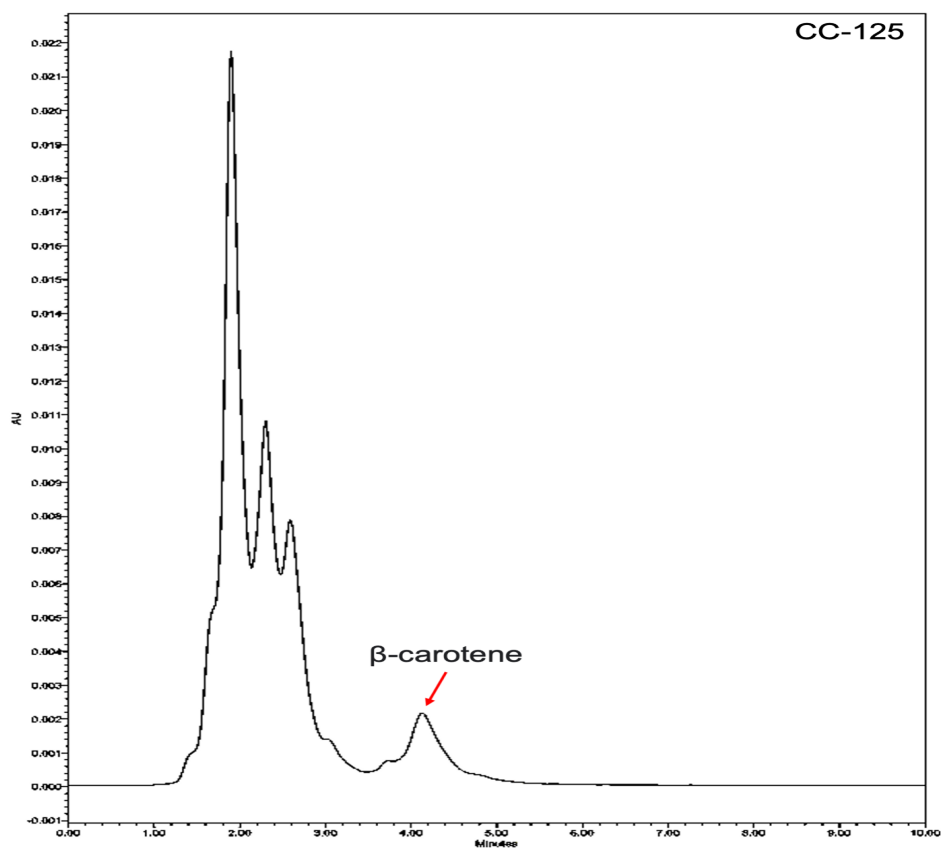


Figure 6.7 : HPLC chromatogram of *C. reinhardtii* CC-125 grown in Erlenmeyer flask showing peak arbitrary values and retention time (min)

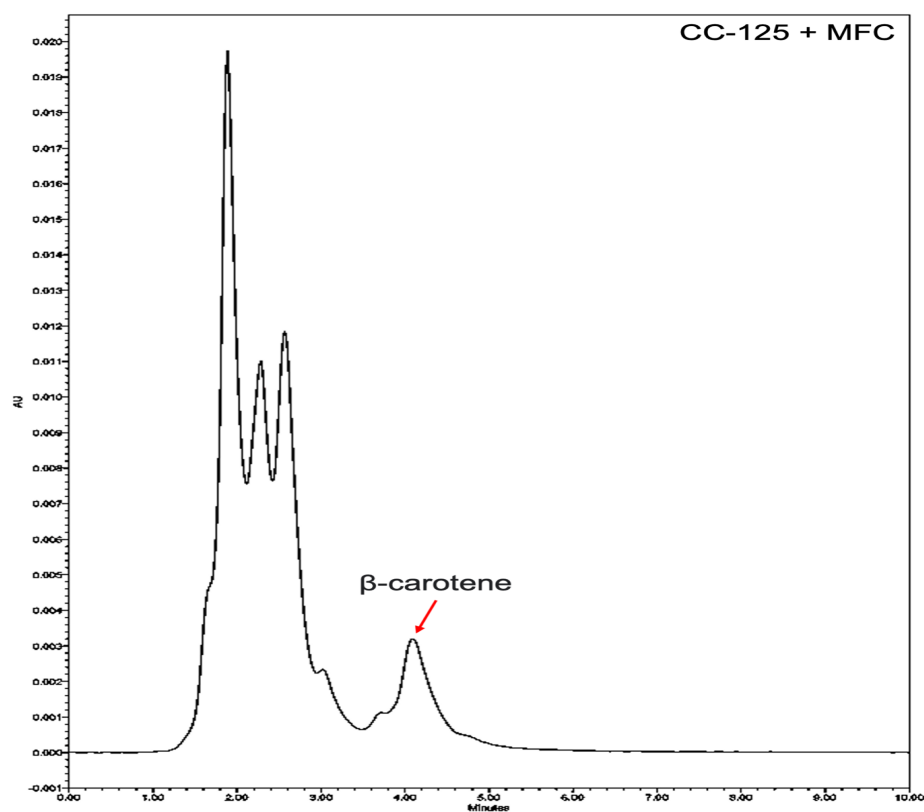


Figure 6.8 : HPLC chromatogram of *C. reinhardtii* CC-125 grown in MFC cathode chamber showing peak arbitrary values and retention time (min)

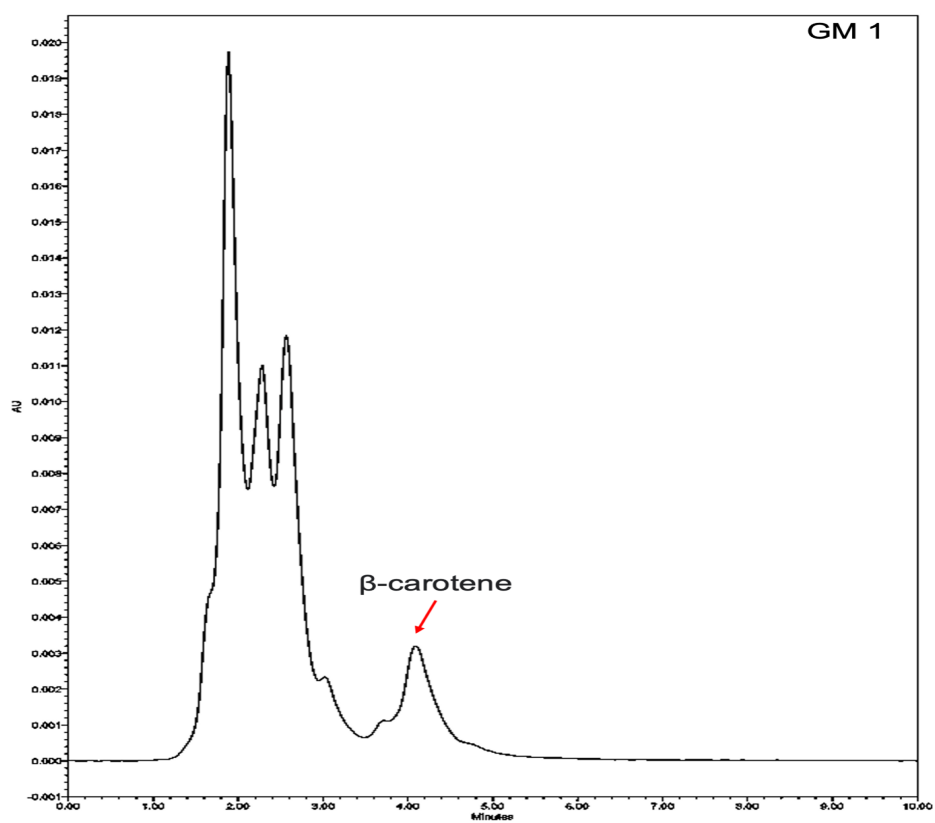


Figure 6.9 : HPLC chromatogram of *C. reinhardtii* GM 1 grown in Erlenmeyer flask showing peak arbitrary values and retention time (min)

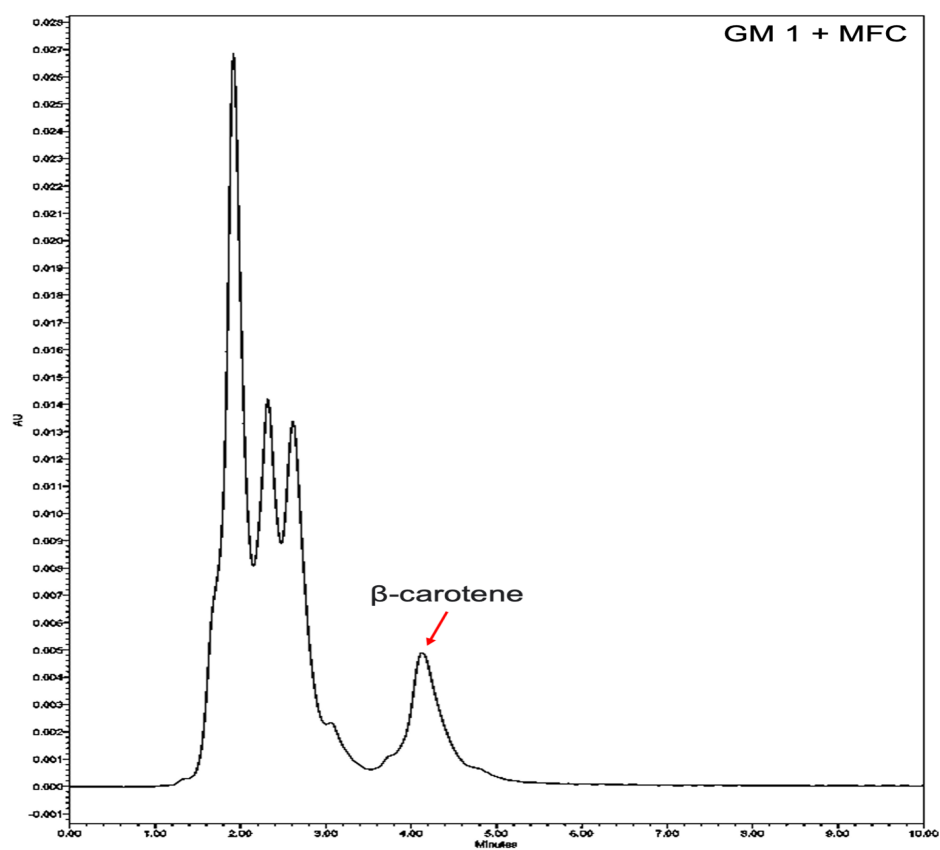


Figure 6.10 : HPLC chromatogram of *C. reinhardtii* GM 1 grown in MFC cathode chamber showing peak arbitrary values and retention time (min)

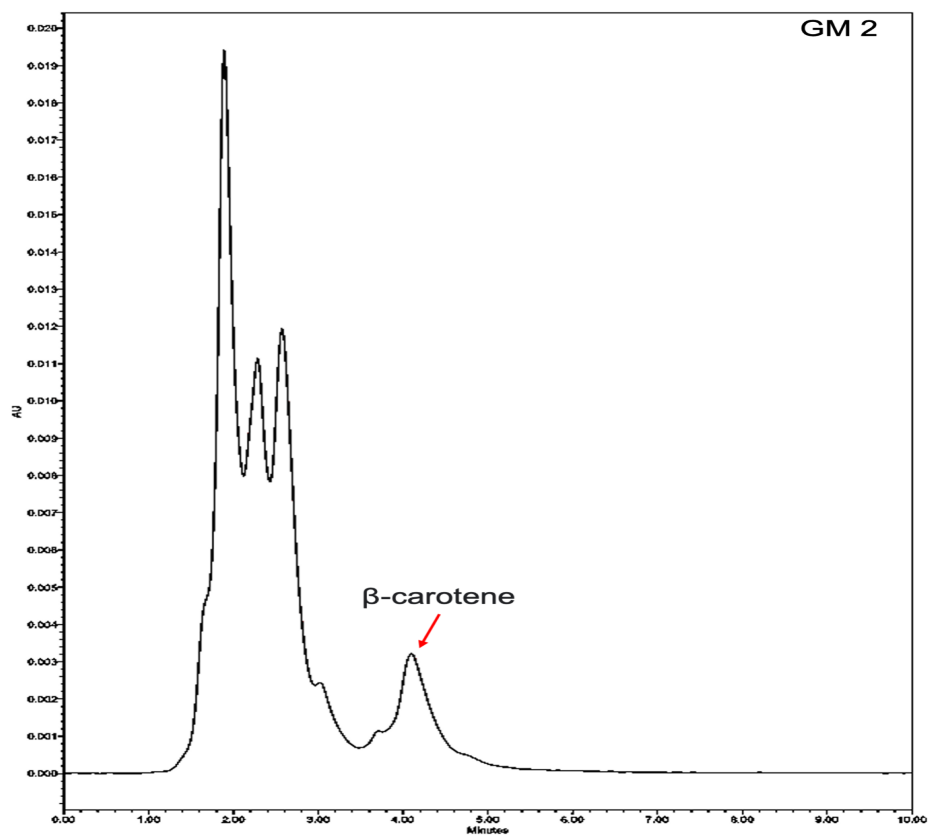


Figure 6.11 : HPLC chromatogram of *C. reinhardtii* GM 2 grown in Erlenmeyer flask showing peak arbitrary values and retention time (min)

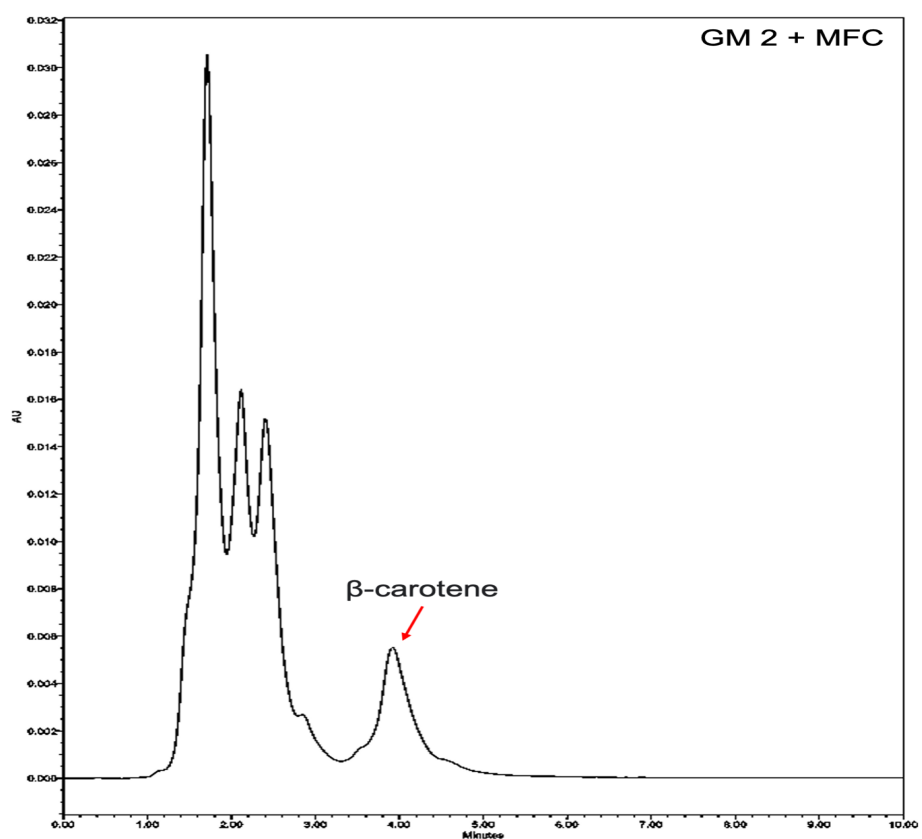


Figure 6.12 : HPLC chromatogram of *C. reinhardtii* GM 2 grown in MFC cathode chamber showing peak arbitrary values and retention time (min)

It is also crucial to consider that β -carotene is a primary carotenoid produced constitutively under exponential growth. It does not require stress conditions to enhance its production, which is a critical consideration for the present study. The stress-induced carotenoid production may slow down the microalgae biomass production which may lead to affect the cathodic ORR and the MFC performance. Therefore, it is advantageous to utilize GM microalgae at the MFC cathode for large-scale mixotrophic cultivation to produce high-value metabolites, achieve high microalgae growth, and efficient power output [Vinayak *et al.*, 2021].

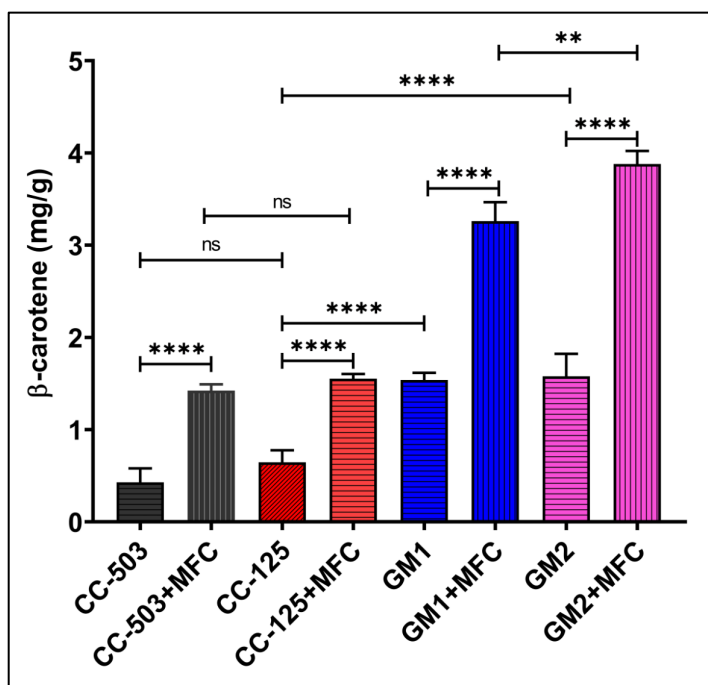


Figure 6.13 : The bar graph shows β -carotene concentration for flask and MFC cathode chamber grown *C. reinhardtii*. Here, ns means no significant change, ** means p -value < 0.01, **** means p -value < 0.0001

6.2.6 Power Output

The maximum current and power densities were determined using the polarization curve as shown in Figure 6.14. These curves help to determine the system's fate in the scaled-up applications. In this study, the maximum current density (normalized by anode chamber volume) achieved by different reactors for System 1, System 2, System 3, and System 4 are 10.36 ± 0.36 A/m³, 9.58 ± 1.77 A/m³, 9.03 ± 0.98 A/m³, and 8.33 ± 0.77 A/m³, respectively. The maximum power densities for System 1, System 2, System 3, and System 4 are 1.26 ± 0.27 W/m³, 1.39 ± 0.52 W/m³, and 0.89 ± 0.21 W/m³, 0.99 ± 0.15 W/m³, respectively. **The GM microalgae grew slower than its WT counterpart, even in the MFC. This led to the differences in the power production. However, the effect is compensated by GM algae's higher production of carotenoids.** Also, the energy produced from one batch cycle from System 1 to System 4 is 0.111 kWh/m³, 0.073 kWh/m³, 0.112 kWh/m³, and 0.069 kWh/m³, respectively. Also, the energy produced from one batch cycle from System 1 to System 4 is 0.111 kWh/m³, 0.073 kWh/m³, 0.112 kWh/m³, and 0.069 kWh/m³, respectively. The TAP (high-salt concentrations) media was used at the cathode chamber, which may improve the solution conductivity and enhance the power production in the present study for both WT and GM microalgae-driven MFCs.

These results have shown that WT and GM microalgae-driven MFCs do not show significant differences in power generation. **Moreover, the power recovery through microalgae MFC can be increased by altering the light intensity, as it may enhance the photosynthetic efficiency to release the oxygen for cathodic ORR [Gouveia *et al.*, 2014]. Therefore, the GM microalgal lines of *C. reinhardtii* can also be applied for MFC-based biorefinery frameworks for higher productivity of carotenoid compounds [Ahirwar *et al.*, 2023].**

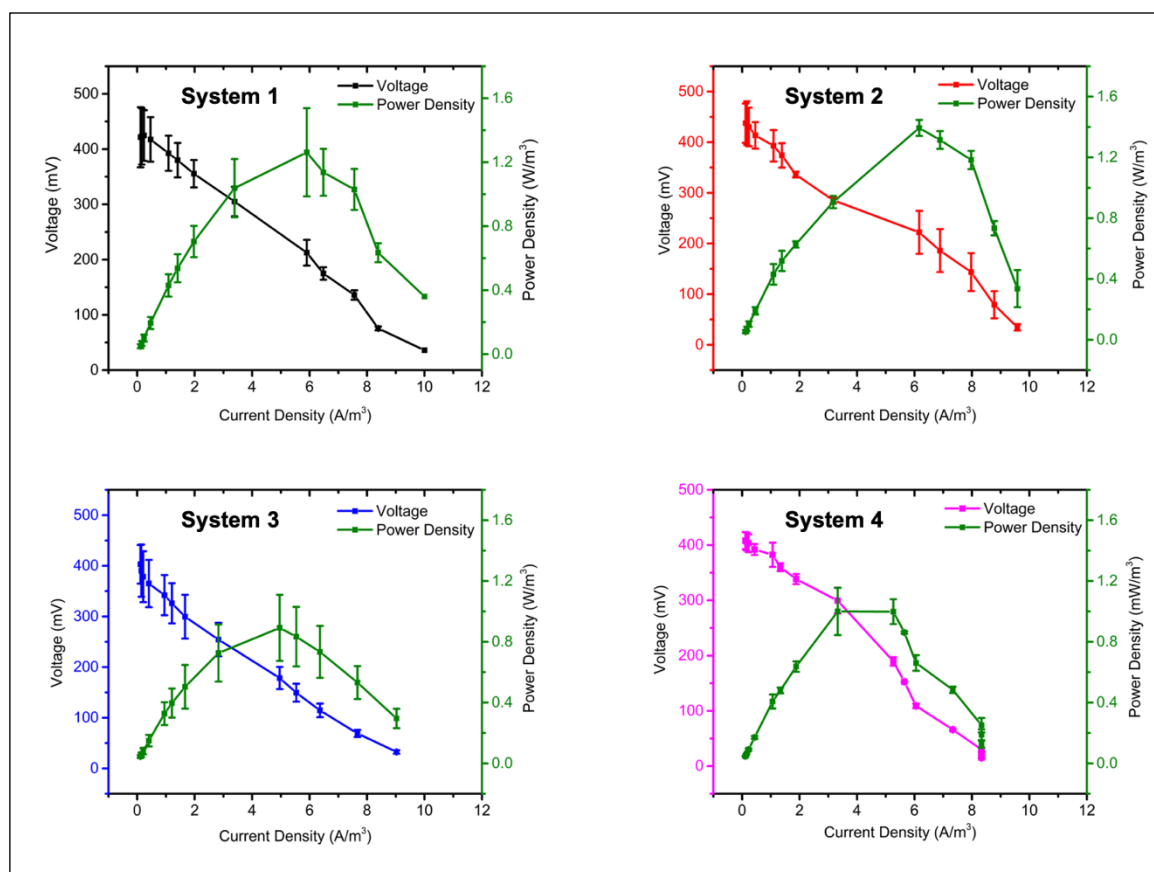


Figure 6.14 : Polarization curve and power curve for different MFC systems

6.2.7 Electrochemical Analysis

The CV is a non-destructive tool for analyzing bio-electrochemical processes. The CV curve also details the redox potential, reaction kinetics, and electrochemical stability of the MFC, including the presence of electron mediators for electron transfer processes [Logan *et al.*, 2006]. The CV curves have shown that the WT species, including *C. reinhardtii* CC-503 (WT) and CC-125 (WT) had produced higher voltage in MFC than the GM strains, contributing to high cathodic currents (Figure 6.15). For system 1 anodic CV, the current peaks were observed at 0.700 V (64.49 mA), another peak was observed at -0.683 V (14.76 mA), and for cathodic CV, current peaks were observed at 0.499 V (32.3 mA) and -0.501 V (17.09 mA). For system 2, anodic CV peaks were observed at 0.698 V (34.28 mA), -0.371 V (4.4646 mA), and -0.326 V (2.385 mA), and cathodic CV showed peaks at 0.499 V (-70.61 mA) and 0.500 V (30.41 mA). For system 3 anodic CV, the peaks were observed at -0.698 V (6.659 mA), -0.514 V (7.205 mA), -0.626 V (7.36 mA), -0.393 V (6.905 mA), 0.381 V (19.3 mA), -0.171 V (4.969 mA), and 0.699 V (36 mA) and for cathodic CV 0.2016 V (46.15 mA) and 0.499 V (40.86 mA). For system 4, anodic CV peaks are 0.699 V (17.76 mA), 0.2445 V (7.441 mA), and 0.700 V (5.954 mA), and for cathodic CV, the peaks were at 0.350 V (80.99 mA), 0.380 V (77.65 mA), and -0.2116 V (33.45 mA) (Figures 6.15A and 6.15B). These oxidation and reduction peaks indicate the electrochemical activity and substrate utilization of the respective anodic and cathodic species in the PMFC. The highest current density obtained through CV can be directly correlated to the maximum current density achieved through the system, such as the highest oxidation current observed for System 1, which could improve the current and power density through the system, as evident by the polarization curve in Figure 6.14.

Anodic oxidation depicts the substrate conversion to carbon dioxide, electrons, and protons [Kumar and Jujjavarapu, 2023]. With these results, it was evident that the use of different cathodic inoculum can affect the system's overall performance. It was apparent from the CV curves that GM 2 microalgae performance was enhanced in MFC as compared with *C. reinhardtii* WT CC-125 and GM 1 microalgae, as shown in Figure 6.4B. Other factors that may hinder the

efficiency of PMFCs are operational temperature, pH, mass transfer limitation, and the presence of toxic chemical species when employing real wastewater that needs further exploration [López Zavala *et al.*, 2019].

Table 6.1 : A Comparative Performance Analysis for MFC Systems used in this Study

Study Parameters	System 1	System 2	System 3	System 4
Closed-circuit voltage (mV)	474 ± 53.74	471 ± 21.21	445 ± 38.18	468 ± 5.66
Maximum current density (A/m ³)	10.36 ± 0.36	9.58 ± 1.77	9.03 ± 0.98	8.33 ± 0.79
Maximum power density (W/m ³)	1.26 ± 0.27	1.39 ± 0.52	0.89 ± 0.22	0.999 ± 0.15
COD degradation rate (Kg/m ³ /d)	161.49 ± 52.79	164.34 ± 32.35	160.24 ± 32.52	152.19 ± 22.85
Coulombic efficiency (%)	9.77 ± 0.19	9.05 ± 1.11	10.84 ± 0.11	8.59 ± 0.27
DO Concentration (mg/l)	10.17 ± 3.28	9.32 ± 2.54	11.33 ± 1.42	8.13 ± 2.36
Specific growth rate (d ⁻¹)	0.246 ± 0.04	0.202 ± 0.01	0.205 ± 0.008	0.174 ± 0.007
β-carotene concentration (mg/g DCW)	1.42 ± 0.06	1.56 ± 0.051	3.26 ± 0.20	3.88 ± 0.14

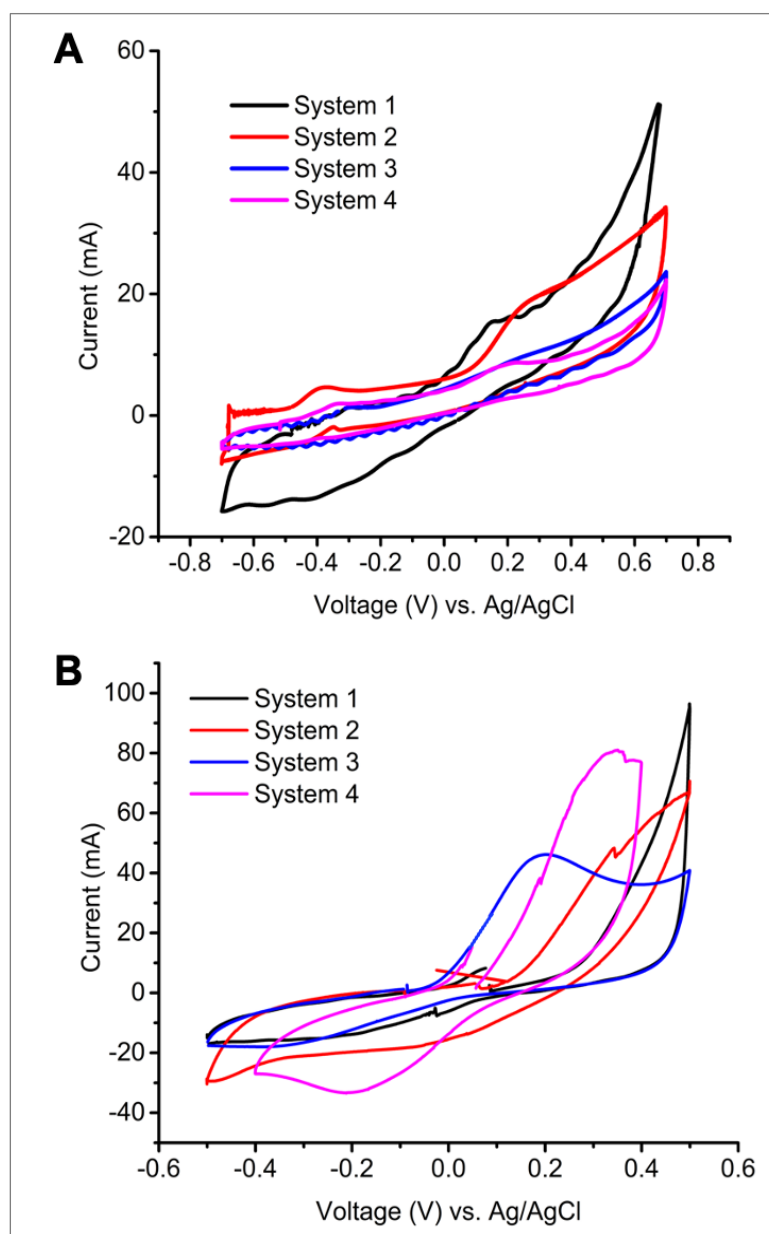


Figure 6.15 : CV curves for A) anodes, and B) cathodes for different MFC systems

6.3 CONCLUSIONS

Challenges in energy production, and treatment efficiency through PMFCs have prompted their integration into biorefinery frameworks, aiming to produce value-added compounds to enhance resource recovery. This study has compared GM and WT microalgae species for MFC applications. Both GM and WT *C. reinhardtii* strains demonstrated effectiveness in MFC, with WT yielding slightly higher power generation, while GM strains excelled in carotenoid production. This innovative approach establishes a foundation for an MFC-based microalgal biorefinery. Future investigations should delve into characterizing biomolecular profiles via these GM microalgae-integrated MFCs, especially in enclosed cultivation using scaled-up reactors. **More strategies need to be developed for biocontainment of GM microalgae-based PMFCs for bioprocess development.**

