7
Summary and Conclusions

7.1 SUMMARY

The study aims to advance MFCs as an economical alternative for wastewater treatment and bioenergy applications. The study was motivated by the existing challenges within MFC technology for biorefinery applications. Previous efforts have explored the utilization of PMFCs to cultivate microalgae within cathode chambers. However, their substantial capital and operational expenses hindered the feasibility of scaling up PMFCs. These included the costs of constructing reactors, maintaining axenic microalgae cultures for biofuel applications, expensive microalgae cultivation processes, and limited yields of valuable microalgal compounds. These constraints impeded the practical transition of PMFCs from laboratory to field applications. In response, the study aimed to mitigate these issues by implementing a two-pronged approach: employing a strategically selected microorganism within the cathode chamber to enhance power generation and facilitate the recovery of valuable microalgae biomass. Additionally, the economic potential of MFC systems was augmented through genetic modifications of microalgae to synthesize the high-value carotenoid β-carotene, which was subsequently cultivated at the MFC cathode.

7.2 CONCLUSIONS

Initially, the investigation commenced with a dual-chamber PMFC configuration. Synthetic wastewater, with acetate as the substrate, was employed. Concurrently, a specific microalgae species, *C reinhardtii*, was cultivated within the cathode chamber using TAP media. This approach yielded a high power density and current density. The harvested microalgae biomass from the cathode chamber exhibited noteworthy attributes, including antioxidant activity, antibacterial properties against known pathogens, elevated total phenol activity, and heightened pigment content. These favorable outcomes also played a crucial role in establishing and maintaining axenic algal cultures within the MFC system.

The study then progressed to genetically modifying the microalgae species *C. reinhardtii* to produce the high-value pigment β-carotene and astaxanthin. The increased production was accomplished by co-expressing two crucial enzymes responsible for carotenoid biosynthesis- β-carotene ketolase and β-carotene hydroxylase from *H. pluvialis*-under robust constitutive promoters. These genetic modifications, seamlessly integrated into the *C. reinhardtii* nuclear genome, resulted in GM microalgae strains exhibiting a remarkable 5.39-fold increase in β-carotene content and a two-fold enhancement in total carotenoid production compared to the WT strain. These findings unveiled a valuable strategy for elevating carotenoid production in microalgae by stable co-expression of specific genes within the GM *C. reinhardtii* nuclear genome.

Subsequently, the applicability of GM *C. reinhardtii* within the MFC cathode chamber was systematically evaluated to assess its influence on both MFC performance and β-carotene production. Moreover, the study explored the impact of electrode treatment by comparing the performance of two WT *C. reinhardtii* strains with two GM strains. Results indicated negligible alterations in the MFC performance in terms of power generation and wastewater treatment efficiencies. Moreover, the production of β-carotene was notably boosted by up to 2.49 times within the MFC environment. Consequently, the study positioned PMFCs as a promising biorefinery tool capable of concurrently delivering power output, wastewater treatment, and β-carotene recovery through GM microalgae cultivation at the cathode as shown in Figure 7.1.

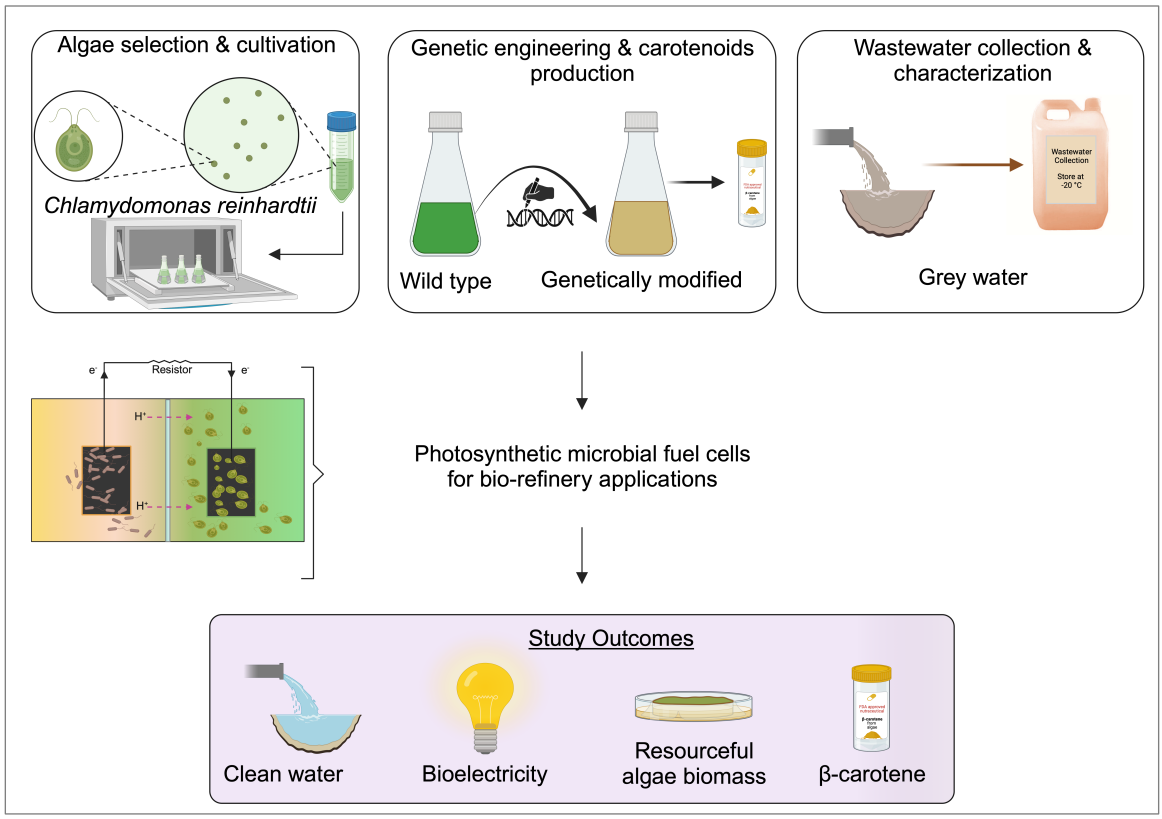


Figure 7.1 : The outcome of the present study

7.3 CLOSING REMARKS

While the study yielded promising outcomes at lab-scale fed-batch operation, there remain avenues for refinement. More research is required on the development of pilot scale continuous process using the presented lab-scale proof-of-concept. The LCA and technoeconomic assessment data from pilot scale study will be more reliable and applicable for bioprocess development-based on GM microalgae. This necessitates further investigations into MFC design and the enhancement of biofilms on both anodic and cathodic surfaces through genetic engineering methodologies. The recently developed MoClo toolkit, renowned for its streamlined and highly efficient gene expression in *Chlamydomonas*, offers a promising approach for enhancing the ORR and bio H_2 production via genetic modifications in algal genomes. Moreover, the augmentation of electrical energy could be achieved by stacking multiple MFC reactors and integrating power management circuits to optimize charge amplification. Also, the MFC performance can be influenced by factors such as increased system complexity, mass transfer limitations, and overall system stability. While larger-scale MFCs have the potential to generate higher power outputs, challenges may arise in maintaining optimal conditions for microbial activity and electron transfer. Therefore, it is essential to critically consider the reactor design, substrate distribution, and microbial community dynamics to ensure effective scaling and sustained performance in practical applications. In tandem, a thorough assessment of sustainability, and LCA is imperative to validate the process's applicability in industrial settings. The culmination of these efforts underscores the technology's commercial potential. By seamlessly integrating microalgae cultivation, this technology could evolve into a multifaceted bio-refinery catering to energy and associated industries. Such viability can be corroborated through rigorous sustainability and techno-economic analyses. Future studies may include the GM microalgae cultivation risk assessment by scaling up the MFC-based closed PBRs.