1
Introduction

The current global energy demands heavily rely on finite fossil fuels, which constitute 90% of the total energy consumption. However, these fossil fuels are rapidly depleting. In response to this, bioenergy, which includes biofuels and bioelectricity, is emerging as a potential substitute for conventional energy sources. A notable advancement is seen in third-generation biofuels, such as biofuels derived from microalgae. These microalgae-based biofuels have the distinct advantage of simultaneously producing Value-added Products (VAPs), giving them a competitive edge in a comprehensive biorefinery framework. The cultivation of microalgae requires light, carbon inputs, water, and inorganic nutrients like N and P [Chowdhury and Loganathan, 2019]. High-density biomass production is often achieved through closed cultivation systems. However, these systems can impede microalgae growth due to the accumulation of high oxygen levels, leading to auto-oxidation of cells and hindering overall biomass productivity and VAP synthesis.

To address this issue, a promising approach involves integrating microalgae cultivation with Microbial Fuel Cells (MFCs). In these systems, microalgae cultivation occurs in the cathode chamber, while wastewater is simultaneously treated at both the anode and cathode chambers. MFC-based microalgae cultivation offers several advantages over traditional methods [Ahirwar *et al.*, 2023].

MFCs are emerging as wastewater treatment systems and renewable energy sources. MFCs convert chemical energy in wastes into electrical energy through Extracellular Electron Transfer (EET) carried out by exo-electrogenic bacteria [Logan, 2009]. By harnessing the redox potential differences between electron donors and acceptors, MFCs produce electrical energy. The MFC-coupled microalgae cultivation system is particularly promising compared to conventional Photobioreactors (PBRs) because it reduces reliance on external de-gasifiers. The CO_2 generated during anodic oxidation reactions can enhance overall biomass productivity. The energy produced can offset the total energy requirements of the process. Therefore, the photosynthetic MFC offers power generation, and algae biomass accompanied by wastewater remediation and CO_2 mitigation in a single unit.

1.1 PURPOSE AND OBJECTIVES OF THE STUDY

Several studies have reported microalgae cultivation at Photosynthetic MFC (PMFC) cathode chamber for biofuel and oxygenation applications. This amalgamation of MFC and microalgae cultivation holds promise by optimizing the performance of each system [Ahirwar *et al.*, 2023; Das *et al.*, 2023]. However, process economics arises as the major concern as microalgae biofuels have relatively higher prices than conventional natural petroleum oils. While capital and operational outlays for PMFC components (electrodes, membranes, substrates, and maintenance) tend to be steep, energy recovery from electricity generation and cost recovery through microalgal products remains limited [Kannan and Donnellan, 2021]. Microalgal pigments are alternative means of resource recovery, which are naturally synthesized therapeutic products. Therefore, microalgae species can be redirected to overproduce these high VAPs using biochemical/genetic engineering approaches.

Among available microalgae cultivation systems, the PMFC cathode chamber is very promising for achieving high-density microalgae cultures [Sharma *et al.*, 2023]. The production and recovery of these VAPs from the PMFC cathode chamber may help reduce the economic burden of the process. This can be achieved by identifying suitable cathodic cultures, cathodic media optimization, or engineering cathodic species for VAP generation. The goal is to attain optimal treatment efficacy, power recovery, and the production of the VAPs in a sustainable manner.

Keeping all these points in consideration, and after reviewing the state of the art, the following objectives are defined for this study:

1. Assessing the suitability of *Chlamydomonas* for the generation of bioenergy and bio-products within a PMFC framework.

2. Genetic engineering of *Chlamydomonas* strain to amplify carotenoid production.

3. Performance assessment of the genetically modified *Chlamydomonas* in the PMFCs.

1.2 BRIEF RESULTS, SCOPE, AND FUTURE PROSPECTS OF THE WORK

The thesis is divided into seven chapters. The second chapter provides a literature review in the area, summarizing the significance of microalgae cultivation strategies, microalgae-based VAPs, integration of microalgae cultivation with MFCs, MFCs principles, and applications, and microalgae-assisted MFCs-related studies. The next chapter provides the experimental methodology followed in this study. The subsequent three chapters talk about the detailed experimental results, which are outlined as follows:

Performance evaluation of a photosynthetic microbial fuel cell using *Chlamydomonas reinhardtii* at the cathode (Chapter 4: published as Sharma and Chhabra, 2021; *Bioresource Technology*, 338, 125499)

This study reports the application of microalgae species *C. reinhardtii* at the cathode in a PMFC. The PMFCs were operated using synthetic wastewater and sodium acetate as a substrate in the anode chamber, and tris-acetate phosphate media was fed at the cathode chamber to support high microalgae growth. The reactor working volume was kept at 100 ml and operation was carried out in fed-batch mode. The application of *C. reinhardtii* at the cathode resulted in high current and power densities of 15.21 W/m^3 and 39 A/m^3, respectively. Moreover, the microalgae growth was also enhanced by cultivating at the cathode chamber 0.284 d^{-1}. In addition, the usability of microalgae biomass was assessed in terms of its total phenol content (157.69 mg GAE/g DCW), antioxidant activity (IC_{50} = 67.07 µg/ml), total Chlorophyll (18.95 mg/g DCW), total Carotenoids (2.40 mg/g DCW), and antibacterial properties against known pathogens including *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus licheniformis*. The COD removal efficiency of 73.30% was achieved with a CE of 9.068% at the anode chamber. In conclusion, *C. reinhardtii* supports high power output from a PMFC and is highly resourceful regarding VAPs.

Genetic engineering of the *Chlamydomonas* strain to amplify carotenoid production (Chapter 5: published as Sharma *et al.*, 2024a; *Systems Microbiology and Biomanufacturing*, 4, 983-995)

Microalgae biorefineries are emerging as significant sources of therapeutic agents, including pigments and proteins. To promote a thriving circular bioeconomy, it's crucial to enhance pigment synthesis in conjunction with microalgae biofuel production while considering the economic aspects. However, naturally fast-growing strains of microalgae, such as *C. reinhardtii*, have limitations in synthesizing ketocarotenoids. Two essential enzymes in the carotenoid biosynthetic pathway, β-carotene hydroxylase (*crt*) and β-carotene ketolase (*bkt*), were overexpressed in *C. reinhardtii* using strong promoters (35Sde and *Hsp*70A/*Rbc*S2) to enhance carotenoid production. Genetically Modified (GM) microalgae strains were verified through PCR, Western blotting, and Southern hybridization techniques, which confirmed the presence and expression of both genes in the GM *C. reinhardtii* strains. The present study suggests that the GM microalgae significantly outperformed Wild Type (WT) microalgae, exhibiting a remarkable 5.39-fold increase in β-carotene concentration and a 2-fold boost in total carotenoids compared to WT microalgae. Notably, the GM microalgae produced astaxanthin up to 1.47 ± 0.063 mg/g which was absent in WT *C. reinhardtii*. These results suggest that the exogenous genes from *Hematococcus pluvialis* were successfully expressed through a nuclear transformation in *C. reinhardtii* to produce ketocarotenoids.

Performance assessment of the genetically modified *Chlamydomonas* in the PMFCs (Chapter 6: published as Sharma *et al.*, 2024b; *Journal of Environmental Chemical Engineering*, 12(3), 112751)

The potential of PMFCs as sustainable bioprocesses for bioenergy and bioproduct generation is significant. However, the exploration of genetically engineered microalgae as cathodic species in PMFCs remains limited. This study systematically compares the performance of WT and GM *C. reinhardtii* in PMFCs for bioelectricity and bioproduct generation using a reactor of 100 ml working volume operated in fed-batch mode. β-carotene productivity in MFCs with WT and GM strains ranged from 1.42 ± 0.07 to 1.55 ± 0.05 mg/g DCW and 3.26 ± 0.20 to 3.88 ± 0.14 mg/g DCW, respectively. The MFC cathode environment significantly enhanced β-carotene production (2.49-fold) in microalgae. Furthermore, power generation in MFCs using GM strains (0.89 ± 0.21 to 0.99 ± 0.15 W/m^3) proved comparable to that of WT species (1.26 ± 0.27 to 1.39 ± 0.52 W/m^3). The COD removal of up to 79% was achieved in PMFC systems while treating greywater at the anode chamber.

This study demonstrates the applicability of *Chlamydomonas*-based PMFCs for simultaneous wastewater treatment and carotenoid production while producing power (Figure 1.1). The process was carried out on a small scale and needs to be scaled up for large-scale applications. The long-term stability of the GM microalgae for carotenoid production must also be assessed. Also, the anodic consortia can be improved further to improve the COD removal.

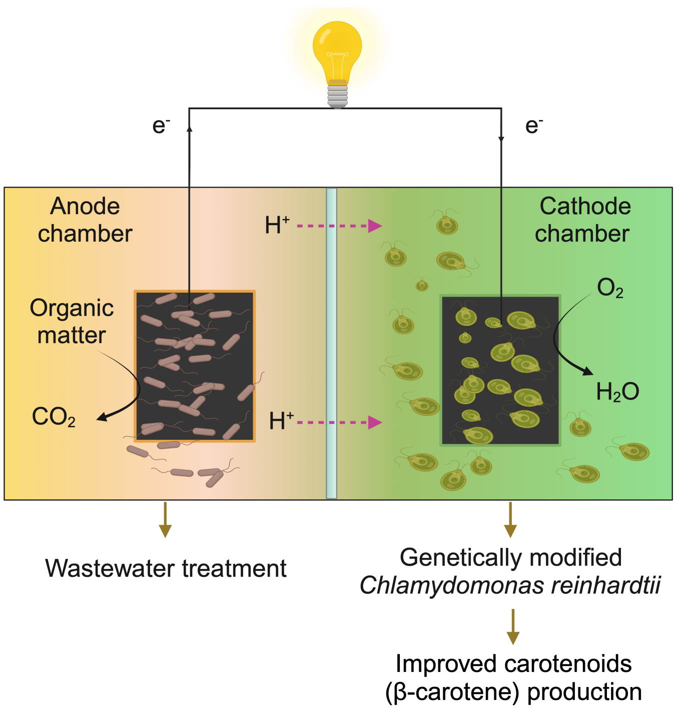

Figure 1.1 : A schematic representation of the theme employed in this thesis work