Conclusion & Future Research Prospective

Chapter-9

CHAPTER 9

9.1 CONCLUSIONS

The main outcome of the present study is the isolation of novel bacterial strains from previously acclimatized MFC and evaluate their degradation potential for two model dyes: Reactive orange 16 (RO16) and Reactive red 120 (RR120). Isolated bacteria LO showed high dye degradation potential for RR120 dye and DO for RO16 dye. A very high concentration (up to 500 ppm for RO16 and 300 ppm for RR120) of dye can be degraded efficiently in a microbial fuel cell (MFC). The effect of different process parameters affecting the decolorization efficiency of MFC for both species was investigated and optimized using response surface methodology (RSM) combined with central composite design (CCD). A bacterial kinetic study was also carried out and very useful information such as substrate inhibition was confirmed experimentally as well as results of the Kinetic study. From preliminary experimental study and literature review, Nafion as proton exchange membrane and Carbon cloth as an electrode (current collector) had been chosen for all the dye degradation experiments in MFC. The novelty of the present work is the enhanced dye degradation using strains obtained from previously acclimatized MFC along with the energy production. Here MFC proves its utility for bioremediation of dyes and simultaneously electricity is generated. Different analytical techniques such as UV-Vis spectrophotometer, COD, FTIR and, CO_2 determination have been used to establish biodegradation phenomena in MFC, and the LCMS technique was used to identify the dye degradation metabolites formed during the degradation of dye at the end of the experiment with isolated strains. The toxicity of the effluent after degradation of both the model dyes has been evaluated using phytotoxicity analysis. The results in the present study indicate that MFCs can effectively be used for the remediation of color waste along with electricity production. The major contributions of this thesis work are summarized as follows:

- The two most dominant bacterial strains having higher dye degradation tendency were identified using 16s rRNA analysis as *Staphylococcus equorum (LO)*, *Acinetobacter pitii* (DO).
- All species were found to have the capability of degrading both the dyes RO 16 and RR120. But LO has more potential for degrading RR120 and DO for RO16 dye.
- An inhibition study reveals that a high concentration of more than 400 ppm is detrimental to bacteria.
- A wide range concentration (up to 500 ppm for RO 16 and 300 ppm for RR120) can be degraded efficiently in MFC within 24 h.
- Ambient temperature (25 ± 5 ºC), neutral to slightly alkaline pH (7 to 7.5), anaerobic or facultative environment, 72 h of time, and static incubation condition were found to be well suited to achieve maximum decolorization for all the isolates for both the dyes.

- For *Staphylococcus equorum (LO)* strain:

  - ✓ The degradation of reactive red 120 dye was successfully demonstrated by *Staphylococcus equorum* in MFC.
  - ✓ Almost 90 % dye (100ppm) was removed in 72 h of operation at pH 7.
  - ✓ The highest voltage of 0.48 V was obtained at 200 ppm at pH 7.0 after 72 h of operation of MFC with a maximum power density of 0.52 W/m^3 (0.02W/m^2).

- ✓ Predicted R^2 values for dye degradation were found to be 0.99 and for current density, R^2 was found to be 0.94 with P-value <0.05. A quadratic model was found to be the best fit for the experiment. Experimental results demonstrated approximately the same condition for the removal of dye as predicted by Center Composite design methodology.

- For *Acinetobacter pitii* (DO) strain:
  - ✓ 90% color removal was obtained in the MFC inoculated with *Acinetobacter pitii* at 500 ppm at the 20^{th} of operation.
  - ✓ The highest voltage of 0.47 V was obtained at 300 ppm at pH 7.0 after 72 h of operation of MFC with the maximum power density of 0.49 W/m^3 (0.02 W/m^2) for isolated species.
  - ✓ The quadratic model was found to be best suited to the experiment with a p-value <0.05. For dye degradation, the "Predicted R-Squared" of 0.91 is in reasonable agreement with the "Adjusted R-Squared" of 0.98. For current density, the "Predicted R-Squared" of 0.89 is in reasonable agreement with the "Adjusted R-Squared" of 0.97.

- UV-Vis, FTIR, and LC-MS analysis establish the biodegradation phenomena in MFC, and metabolites present at the end of the experiment were used to predicate the biodegradation pathway of both the dyes.

- At the end of the experiment %, CE was calculated and ranges between 2.29 - 3 %. A very low value indicated that most of the electron released during dye degradation was not only utilized for electricity production, rather utilized for biomass growth and other metabolites.

- A phytotoxicity study using wheat and mung dal seed also reveals the conversion of toxic reactive dye to less toxic compounds in the MFC.

The above studies suggest that organisms isolated from previously acclimatized MFC running with wastewater have the potential for the bioremediation of azo dyes and simultaneous electricity can be produced. Although the power output is low when MFC utilization was combined with bioremediation for the treatment of such a high concentration of dye, the overall viability of MFC can be explored considering it as green and clean technology.

9.2 FUTURE RESEARCH PROSPECTIVE

The present study reveals the enhanced decolorization when microorganisms were isolated from MFC running for six months or more. An improvement in power is not much observed this may be due to high internal resistance, electrode material, and design of MFC. Hence, the outcome of the present work highlights several areas for future studies that can be used to increase power output and decolorization simultaneously.

- A single unit of MFC produces low power output. So, there is a need for a Multi-stack MFC that can be used to increase power density.
- Power and current densities significantly decrease with the enlargement of the physical (geometrical) size of the reactor because of the increased internal resistance. Miniaturization of MFCs needs to be implemented using several small size MFC in series or multiple electrode MFC.
- Voltage fluctuation is another major drawback.

- Low columbic efficiency and costly electrode materials are still the bottlenecks restricting its large-scale application. Finally, the existing problems limit the practical application of MFC. It's believed that the application of MFC will have a great prospect if these problems are solved.
- To decrease the overall cost of MFC, low and efficient proton exchange membrane development is another area that needs attention. The development of biocathode may also decrease the cost of MFC.
- A better understanding of the factors which affect MFC performance is the behavior of electrochemically active bacteria, Their detailed study will also increase the efficiency of the process.
- Use of enzyme may also improve degradation rate in MFC which ultimately improve power.
- A better understanding of the enzymatic pathway of dye degradation is required for achieving enhanced bioremediation efficiency.