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VARANASI - 221005

CERTIFICATE

It is certified that the work contained in the thesis titled "**A study on bioremediation of azo dyes in microbial fuel cell and its feasibility for simultaneous electricity generation"** by **Ms. Amrita Shahi** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy and SOTA for the award of Ph.D. Degree.

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DECLARATION BY THE CANDIDATE

I, Amrita Shahi, certify that the work embodied in this thesis is my own bonafide work and carried out by me under the supervision of Prof. R.S. Singh from July 2014 to March 2021, at the Department of Chemical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not willfully copied any other's work, paragraphs, text, data, results, etc., reported in journals, books, magazines, reports, dissertations, thesis, etc., or available at websites and have not included them in this thesis and have not cited as my own work.

Date:

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CERTIFICATE BY THE SUPERVISOR

It is certified that the above statement made by the student is correct to the best of our knowledge.

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TO

MY BELOVED FATHER

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(AMRITA SHAHI)

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Mo_2	Molar Mass of Oxygen (gm/mol),
V	Volume of Anode Chamber (L)
F	F is the Faraday constant (96485 C/mole)
I	I is the current (A),
Δ COD	Changed in COD over time t (gm/L)
μ	Specific Growth Rate (1/day),
μ_{max}	Maximum Specific Growth Rate (1/day)
Ks	Half-Saturation Constant (mg/L).
K_i	Substrate Inhibition Constant (mg/L).
S	Substrate Concentration (mg/L)

List of Abbreviations

MFC	Microbial Fuel Cell
PEM	Proton Exchange Membrane
MSM	Mineral Salt medium
COD	Chemical Oxygen Demand
TCA	Tricarboxylic Acid Cycle
HRT	Hydraulic Retention Time
LDH	Layered Double Hydroxide
TBP	Tributyl phosphate
ORP	oxidation-reduction potential
UV-Vis	Ultraviolet-Visible
FTIR	Fourier-transform infrared spectroscopy
LCMS	Liquid chromatography and mass spectroscopy
LC-ESI	liquid chromatography-electrospray ionization
FESEM	Field Emission Scanning Electron Microscopy
SAIF	Sophisticated Analytical Instrumentation Facility
CDRI	Central Drug Research Institute
DET	Direct electron transfer
MET	Mediated electron transfer
RO16	Reactive Orange 16
RR120	Reactive Red 120
rDNA	Ribosomal DNA
NCBI-	National Center for Biotechnology - Basic Local Alignment Search Tool
CFU	Colony Forming Unit
CE	Coulombic efficiency
EC	Elimination Capacity
RSM	Response surface methodology

DX	Design Expert
CCD	Central composite design
FCC	Face Centered Central
ANOVA	Analysis of Variance
LO	Light Orange
DO	Dark Orange
PW	Pure White
LP	Light Pink
DP	Dark Pink
PW	Peach White
LY	Light Yellow
WO	White Orange
WY	White-Yellow
WP	White Pink
WW1	White White 1
WW2	White White 2
WW3	White White 3

Preface

Extensive use of Azo dyes by various industries and discharge of colored wastewater directly to the aquatic system leads to deterioration of our environment. Toxicity and aesthetic effects of these dye wastewater make them necessary to be treated before discharge. There are many efficient physical and chemical technologies for the treatment of dye-containing wastewater, but they have the disadvantage of high operating costs and secondary waste generation. Nowadays, biological methods seek attention for the treatment of dyes due to their low cost, eco-friendly nature and versatility, and their capability to degrade a variety of dyes to partially or wholly to a stable and non-toxic compound. Generally, dyes degraded easily in an anaerobic environment but they lead to the formation of aromatic amines. Therefore, further treatment is recommended for complete mineralization of amines into non -toxic compounds. Several combinations of anaerobic and aerobic biological treatments have been suggested for enhanced dye degradation. Most of the Azo dyes are degraded under anaerobic conditions. Developments of the toxic intermediates during anaerobic degradation and lower decolorization are some of the operational limitations of biological methods. Hence the study is being diverted in search of new technology that increases the overall biological process efficiency. Microbial Fuel Cell (MFC) is a new emerging green and non-combustion based degradation technology that utilizes the potential of microbes for the degradation of waste along with the production of electricity. It has the dual advantage of producing electricity, and simultaneously it degraded waste.It has proven its utility for treating many industrial wastes such as food industry waste, pharmaceuticals waste, paint industry waste, and sewage wastewater along with electricity production. The MFC technology has already been well established for electricity production, but it has the only disadvantage of low power output, which limits its large scale use commercialization. If this MFC technology is combined with the bioremediation of dyes, then the overall performance of MFC can be increased. Avery small amount of work in the area of dye degradation with electricity production has been reported to date. Therefore taking all these research gaps, the present work was planned which emphasizes decolorization and degradation of two different Azo dyes, namely Reactive Orange 16 and Reactive Red 120, using mixed and isolated strain. Then the degradation of both the dyes is optimized using response surface methodology (RSM) combined with central composite design (CCD). The bacterial kinetic study was also carried out for inhibition study in MFC. Before this study, a preliminary setup (MFC1 and MFC2) was constructed, and sewage wastewater from a primary clarifier fed with glucose as a carbon source was used. From the literature review, Nafion and agar salt bridge were taken as proton exchange membrane (PEM), and the most suitable membrane was chosen for further studies. It was concluded from different preliminary experiments that Nafion as PEM and carbon cloth as a current collector would be suitable for dye degradation and electricity generation. Experimental results are analyzed, discussed, and compared against results from similar investigations and correlations. An extensive literature survey has been conducted before all experimental studies.

Thesis Overview

Chapter 1of the thesis contains the general introduction of dyes, their use, toxic effects, different treatment technologies, and treatment of these dyes using MFCs along with the production of electricity. Problem statements and the significance of researches have also been discussed here.

Chapter 2 represents detailed reviews of relevant literature related to synthetic dyes, classification, potential threats to the environment, and current treatment technologies. It also contains a review on MFC, its working principle and metabolism, recent researches, and parameters affecting its performance. It also highlights the significant research gap for the treatment of dyes and in MFC technologies. Research objectives of the present studies have also been discussed here.

Chapter 3 describes the details of materials and methods involved in the present study, which includes sampling, enrichment, and acclimatization, isolation and identification of dye degrading bacterial strain, optimization of process parameters in MFC for dye degradation. Also, different analytical techniques used for the analysis of the performance of MFC have been discussed in detail.

The result and discussion part are divided into five chapters (4,5,6,7,8). In **Chapter 4**, isolation and identification of bacterial strain for dye degradation have been carried out for finding out potent dye degrading bacterial strain. **Chapter 5** contains a comparative study of two MFCs with different proton exchange membranes. The **Chapter 6** describes the behavior of MFCfor Azo dye degradation using mixed culture and their microbial kinetics study. The**Chapter 7** contains process optimization using RSM for dye degradation using a single strain in MFC. The **Chapter 8** of this thesis describes the comparative study of mixed and isolated strain in MFC for enhanced dye degradation and its process optimization.

An effort has been made to conclude the thesis in **chapter 9**. In the concluding chapter, valuable experiences gained as a result of the work done for this thesis are discussed. From the lessons learned, recommendations are made to carry forward the present work more efficiently and systematically. References used in this thesis have been compiled at the end of the thesis. Three papers from the work have already been published in the referred journals and one paper has been communicated for the publication and is under review.