Development and Performance Evaluation of Microbial Fuel Cells for Simultaneous Treatment of Organic Waste and Bioelectricity Generation.

Thesis submitted in partial fulfilment for the

Award of Degree

DOCTOR OF PHILOSOPHY

By

MANISHA VERMA

School of Biochemical Engineering
Indian Institute of Technology
(Banaras Hindu University)
Varanasi – 221005
India

Roll No. 18011010 **2023**

CERTIFICATE

It is certified that the work contained in the thesis titled " Development and performance evaluation of microbial fuel cells for simultaneous treatment of organic waste and bioelectricity generation." by Manisha Verma 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.

Vmishra
17/07/2023

Dr. Vishal Mishra

(Supervisor)

School of Biochemical Engineering

Indian Institute of Technology

(Banaras Hindu University)

Varanasi – 221005, India

DECLARATION BY THE CANDIDATE

I, Manisha Verma, certify that the work embodied in this thesis is my own bona fide work and carried out by me under the supervision of Dr. Vishal Mishra from July, 2019 to June, 2023, at the School of Biochemical Engineering, Indian Institute of Technology (BHU), 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 wilfully 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: 17/07/2023

Signature of the Student

Manisha

Manisha Verma

Place: IIT-BHU, Varanasi

CERTIFICATE BY THE SUPERVISOR

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

Vmishra 17/07/2023

Dr. Vishal Mishra

(Supervisor)

School of Biochemical Engineering

Indian Institute of Technology

(Banaras Hindu University)

Varanasi – 221005, India

Forwarded

Signature of Head of Department/Coordinator of School

समन्वयक
Coordinator
जैव रासायनिक अभियांत्रिकी स्कूल
School of Biochemical Engg
भा ौद्योगिकी संस्थान
Inc ute of Technology
(व o) वाराणसी-221005
aranasi-221005

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis:

Development and Performance Evaluation of Microbial Fuel cells for Simultaneous Treatment of Organic waste and Bioelectricity generation

Name of the Student: Manisha Verma

Copyright Transfer

The undersigned hereby assigns to the Institute of Technology (Banaras Hindu University) Varanasi all rights under copyright that may exist in and for the above thesis submitted for the award of the PhD.

Date: 17/07/2023

Signature of the Student

Manisha

Place: IIT-BHU, Varanasi

Manisha Verma

Note: However, the author may reproduce or authorize others to reproduce material extracted verbatim from the thesis or derivative of the thesis for author's personal use provided that the source and the Institute's copyright notice are indicated.

Acknowledgements

- Through this page, I offer my salutation, to the creator of this pious seat of learning Bharat Ratna Mahamana Pt. Madan Mohan Malviya Ji.

- It is indeed my proud privilege to express my deep sense of gratitude and indebtedness to my supervisor, Dr. Vishal Mishra, Assistant Professor, School of Biochemical Engineering, Indian Institute of Technology (BHU), Varanasi for his immense help, cooperation and valuable guidance that he has extended to me for the successful completion of this investigation. I am grateful for his constant encouragement, sustained interest and parental care throughout the research period.

- I am obliged very much to express my sincere thanks to Coordinator, Prof. Vikas Kumar Dubey, School of Biochemical Engineering, Indian Institute of Technology (BHU) for providing necessary facilities and constant motivation throughout my research work.

- It is my privilege to express my thanks to all RPEC members Prof. Vikas Kumar Dubey, School of Biochemical Engineering, IIT (BHU) and Dr. Sweta, Department of Chemical Engineering and Technology, IIT (BHU) for appropriate suggestions and kind cooperation.

- I am obliged very much to express my sincere thanks to all the faculty members, School of Biochemical Engineering, IIT (BHU) for their support and encouragement.

- I reserve special thanks for all the non-teaching staff of the School of Biochemical Engineering, IIT (BHU) as this work would have never been completed without their technical support.

- I also gratefully acknowledge to the Ministry of Human Resource and Development (MHRD), Government of India, New Delhi, and Director, IIT (BHU) for the financial support in the form of teaching assistantship.
- My heartfelt thanks go to my dear labmates, I would like to say special thanks to Dr. Vishal Singh, Dr. Veer Singh, Dr. Jyoti Singh, Manoj Kumar Verma, Bhola Prasad, Alok Das, Mahesh Sanjay Chivate, Rashmi Kamal, Shivangi Kesarwani and Sonam Mishra for their valuable support, encouragement towards the successful completion of my research work and making the journey happy.
- It's been possible with the blessings of my father, mother, and brothers that provided me all the wisdom, strength and guidance to carry on this journey which at times became very tough and tedious. Without their care, love, sacrifice, prayers, wishes, patience and motivation I can’t even imagine of my present position in life.

Table of Contents

List of Figures

List of Tables

Abbreviations

A	Surface area
APHA	American Public Health Association
ASTM	American Society for Testing and Materials methods
BBM	Bold basal medium
BDP	Dried banana peel powder
BDW	Dried banana peel wastewater
BDY	Dried banana peel with *S. cerevisiae*
BPW	Banana peel waste
BS	Banana slurry
BSW	Banana slurry wastewater
BSY	Banana slurry with *S. cerevisiae*
BW	Banana peel waste
CB	Cellulolytic bacteria
CCD	Central composite design
CE	Coulombic efficiency
COD	Chemical oxygen demand
COD_f	Final chemical oxygen demand
COD_i	Initial chemical oxygen demand
DNSA	3,5-Dinitrosalicylic acid
E_{Cell}	The electromotive force of the MFC
F	Faraday's constant
F-MFC	Fruit peel powder based MFC
FP	Mixed fruit peels

HPTCL	High pressure and temperature pretreated sweet lime peel slurry
I	Current
J	Current density
MB	Dried microalgae
MFC	Microbial fuel cell
ML	Machine Learning
M-MFC	Dried microalgae powder based MFC
MO_2	The molecular weight of oxygen
N-MFC	Newspaper powder based MFC
NP	Non-Pretreated
OCV	Open circuit voltage
OG	Oil and grease
P	Pretreated
PCL	Pretreated peels
PCLY	Pretreated peel with yeast
PCLYB	Pretreated peel with yeast and bacteria
PD	Power density
R	External resistance
R_{ext}	External resistance
R_{int}	Internal resistance
RSM	Response surface methodology
SC	Slurry Concentration
SEO	Spent engine oil
SSM	Stainless steel mesh
T	Temperature

TDS	Total dissolved solids
TE	Treatment efficiency
TFS	Total Fixed Solids
TS	Total solids
TSS	Total suspended solids
TVS	Total Volatile Solids
V	Voltage
V_{anode}	The volume of the anode chamber
YMFC	*S. cerevisiae* -Microbial fuel cell
YPD	Yeast Extract-Peptone-Dextrose
ΔCOD	Change in the COD over time (t)

Preface

Organic waste generation, particularly food waste, is a significant global issue with substantial environmental consequences. According to the Food and Agriculture Organization (FAO) of the United Nations, approximately one-third of the food produced for human consumption worldwide is lost or wasted each year, amounting to about 1.3 billion metric tons of food waste annually. The improper management of organic waste leads to greenhouse gas emissions, water and soil pollution, and air pollution. Leachate, a liquid byproduct of organic waste decomposition, can contain pollutants that seep into the soil and contaminate water sources, leading to water pollution. Moreover, the decomposition of organic waste can generate odorous gases, contributing to air pollution and creating unpleasant living conditions for nearby communities. Microbial Fuel Cells (MFCs) have shown promise as a technology for organic wastewater treatment. One of the primary advantages of MFC is the production of electricity during the wastewater treatment process. As microorganisms break down organic substrate, and release electrons as part of their metabolic processes. The first objective explores the potential of pretreated sweet lime peel slurry as an anolyte with yeast and bacteria anode biocatalyst for bioenergy generation in MFCs. Sterilised cow urine was used as catholyte and Chlorella pyrenoidosa strain as cathode biocatalyst. Three H-shaped dual-chamber MFCs were fabricated using two plastic containers operating with no inoculum, Saccharomyces cerevisiae as only inoculum and co-culture of Saccharomyces cerevisiae with isolated cellulolytic bacteria, respectively, in the anode chamber. The anode was prepared using a rectangular stainless-steel mesh; the cathode was a cylindrical graphite rod. The maximum open-circuit voltages achieved by co-culture of bacteria and S. cerevisiae were 792.33 ± 1.53 mV and 481.33 ± 3.51 mV, respectively. The maximum power densities in these two MFCs were found to be 22.20 ± 1.28 mW/m2 (210.66 ± 6.11 mA/m2) and 204.80 ± 1.28 mW/m2 (640.0±2.0 mA/m2) correspondingly. Results noticeably disclosed that microorganisms consumed the

carbon source available in sweet lime peel. Thus, the sweet lime peel can be an inexpensive alternative for operating MFCs. In the second objective, newspaper powder, dried dead microalgae biomass and mixed fruit peel powder were separately targeted as substrate for the growth of an isolated cellulolytic bacteria inside the anode chamber of three different MFCs. At cathode, bold basal medium with spent engine oil was used as catholyte. An isolated and acclimatized microalgae was used as cathode biocatalyst in order to perform bioremediation of spent engine oil wastewater at cathode chamber. The MFC with newspaper powder showed highest maximum power density of 34.88 ± 1.00 mW/m^2. Both fruit peel powder and dried microalgae powder-based MFCs achieved almost parallel maximum power densities of 15.57 ± 0.49 mW/m^2 (at 161.1 ± 2.54 mA/m^2) and 16.00 ± 0.32 (at 163.3±1.66 mA/m^2), respectively. The anode chambers of all MFCs reflected chemical oxygen demand (COD) removal efficiencies in range of 65 - 86 %. At anode chamber, maximum COD removal of 86.25% was achieved by microalgae powder based MFC. The removal percentage of oil and grease were 37.7%, 42.3% and 29.1% for fruit peel, newspaper powder and microalgae powder based MFCs respectively. Up to 76-80% nitrate-nitrogen, 96-97% ammonium-nitrogen, 90-94% phosphate-phosphorous removal was observed in all the MFCs. Microbial community analysis revealed that Proteobacteria, Firmicutes and Bacteroidetes were the dominant phyla present in all the three anodic biofilms. The third objective evaluates the performance of *S. cerevisiae*-based H-shaped microbial fuel cell with banana peel waste as substrate, operated for 30 days in three cycles. Dried banana peel and banana slurry substrates were prepared with initial COD of 1126±41 mg. L^{-1} and 1366± 64 mg. L^{-1} respectively. Dried banana peel powder was fed into two MFCs, one with no inoculant and the other with *S. cerevisiae*. Dried banana peel powder without inoculant yielded negligible power output, whereas dried banana peel powder with *S. cerevisiae* generated a maximum power density of 2.2±0.1 mW.m^{-2}. The banana peel slurry was fed into two different MFCs one was with *S. cerevisiae* and another was without *S.*

cerevisiae. Banana slurry with *S. cerevisiae* generated a maximum power output of 86.9±0.4 mW. m^{-2}. Banana peel slurry without inoculation, generated a maximum power output of 44.6±0.8 mW. m^{-2}. Microbial community analysis indicated that the high-power output obtained from banana slurry-based MFCs was due to the presence of indigenous microbial consortia. Up to 70-88% COD removal was recorded in MFCs with banana slurry, however, 18-44% of COD removal was observed in MFCs with dried banana peel powder. It was also observed that the simple saccharides available in banana peel waste were consumed by *S. cerevisiae* and other indigenous microbes in the anode chamber. The microbial community released electrons in the anode chamber, which were responsible for voltage generation in MFC. In the fourth objective, MFC was operated using banana peel slurry as substrate with baker's yeast as external inoculum. Decision tree algorithms were applied for optimizing the input parameters of MFC. Input variables including temperature, pH, resistance, pretreatment of slurry and slurry concentration were optimized for achieving the maximum power density in MFC. Total five combination were obtained by the decision tree model that led to high power density. All five combinations were also tested for validation. Experimental validation of decision tree models showed accuracy in range of 77 to 99%. In order to obtain high power density, the best combination was determined by accuracy level and experimental validation. The best set of rules for high power density was 41.47 mL/L < slurry concentration < 87.5 mL/L, 22.44 °C ≤ temperature < 36.25 °C, $5.23 \leq \text{pH} < 7.25$, slurry was pretreated and resistance < 285 Ω. It was also observed that temperature, resistance and pretreatment were the most influential input parameters to achieve high power density. Results obtained in this work can be directly implemented at pilot and industrial scale without further experimental trails.